RADIOLOGY AND ONCOLOGY

Special section

Biomedical applications of electroporation

Guest Editors: Richard Heller, Maja Čemažar

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Halaven®
eribulin

NOVA SMER DO PODALJŠANJA **CELOKUPNEGA PREŽIVETJA**

Eisai

Prva in edina samostojna kemoterapija,
ki v primerjavi z ostalimi možnostmi zdravljenja
z enim zdravilom, pri bolnicah s predhodno že
večkratno zdravljenim metastatskim rakom dojke,
dokazano značilno podaljša celokupno preživetje.[1,2]

- **Halaven** (eribulin): ne-taksanski zaviralec dinamike mikrotubulov, prvo zdravilo iz nove skupine kemoterapevtikov, imenovanih *halihondrini*.
- Zdravilo HALAVEN je indicirano za zdravljenje bolnic z lokalno napredovalim ali metastatskim rakom dojke, ki je napredoval po vsaj enem režimu kemoterapije za napredovalo bolezen. Predhodna zdravljenja morajo vključevati antraciklin in taksan, bodisi kot adjuvantno zdravljenje ali za zdravljenje metastatskega raka dojke, razen če to zdravljenje za bolnice ni bilo primerno.[1]
- Priporočeni odmerek 1,23 mg/m^2, intravensko, v obliki 2- do 5-minutne infuzije, 1. in 8. dan vsakega 21-dnevnega cikla.
- Ena 2 ml viala vsebuje 0,88 mg eribulina.
- Raztopina, pripravljena za uporabo, redčenje ni potrebno.

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

HALAVEN 0,44 mg/ml raztopina za injiciranje (eribulin)
TERAPEVTSKE INDIKACIJE: Zdravljenje lokalno napredovalega ali metastatskega raka dojke, ki je napredoval po vsaj enem režimu kemoterapije za napredovalo bolezen vključno z antraciklinom in taksanom (adjuvantno zdravljenje ali zdravljenje metastatskega raka dojke), razen če to ni bilo primerno. **ODMERJANJE IN NAČIN UPORABE:** Halaven se daje v enotah, specializiranih za dajanje citotoksične kemoterapije, in le pod nadzorom usposobljenega zdravnika z izkušnjami v uporabi citotoksičnih zdravil. Odmerjanje: Priporočeni odmerek eribulina v obliki raztopine je 1,23 mg/m^2 i.v. v obliki 2- do 5-minutne infuzije 1. in 8. dan vsakega 21-dnevnega cikla. Bolnikom je lahko slabo ali bruhajo. Treba je razmisliti o antiemetični profilaksi, vključno s kortikosteroidi. *Preložitev odmerka med zdravljenjem*: Dajanje Halavena je treba preložiti, če se pojavi kaj od naslednjega: absolutno število nevtrofilcev (ANC) < 1 x 10^9/l, trombociti < 75 x 10^9/l ali nehematološki neželeni učinki 3. ali 4. stopnje. *Zmanjšanje odmerka med zdravljenjem*: Za priporočila za zmanjšanje odmerka ob pojavu hematoloških ali nehematoloških neželenih učinkov glejte celoten povzetek glavnih značilnosti zdravila. Okvara jeter zaradi zasevkov: Priporočeni odmerek pri blagi okvari jeter (stopnje A po Child-Pughu) je 0,97 mg/m^2 v obliki 2- do 5-minutne i.v. infuzije 1. in 8. dan 21-dnevnega cikla. Priporočeni odmerek pri zmerni okvari jeter (stopnje B po Child-Pughu) je 0,62 mg/m^2 v obliki 2- do 5-minutne i.v. infuzije 1. in 8. dan 21-dnevnega cikla. Pri hudi okvari jeter (stopnje C po Child-Pughu) se pričakuje, da je treba dati še manjši odmerek eribulina. Okvara jeter zaradi ciroze: Zgornje odmerke se lahko uporabi za blago do zmerno okvaro, vendar se priporoča skrbno nadziranje, saj bo odmerke morda treba ponovno prilagoditi. Okvara ledvic: Pri hudi okvari ledvic (očistek kreatinina < 40 ml/min) bo morda treba odmerek zmanjšati. Priporoča se skrbno nadziranje varnosti. Način uporabe: Odmerek se lahko razredči z do 100 ml 0,9 % raztopine natrijevega klorida (9 mg/ml) za injiciranje. Ne sme se ga redčiti v 5 % infuzijski raztopini glukoze. Pred dajanjem glejte navodila glede redčenja zdravila v celotnem povzetku glavnih značilnosti zdravila ter se prepričajte, da obstaja dober periferni venski dostop ali prehodna centralna linija. Ni znakov, da bi eribulin povzročal mehurje ali dražil. V primeru ekstravazacije mora biti zdravljenje simptomatsko. **KONTRAINDIKACIJE:** Preobčutljivost na zdravilno učinkovino ali katerokoli pomožno snov. Dojenje. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** Mielosupresija je odvisna od odmerka in se kaže kot nevtropenija. Pred vsakim odmerkom eribulina je treba opraviti pregled celotne krvne slike. Zdravljenje z eribulinom se lahko uvede le pri bolnikih z vrednostmi ANC ≥ 1,5 x 10^9/l in s trombociti > 100 x 10^9/l. Bolnike, pri katerih se pojavijo febrilna nevtropenija, huda nevtropenija ali trombocitopenija, je treba zdraviti v skladu s priporočili v celotnem povzetku glavnih značilnosti zdravila. Hudo nevtropenijo se lahko zdravi z uporabo G-CSF ali enakovrednim zdravilom v skladu s smernicami. Bolnike je treba skrbno nadzirati za znake periferne motorične in senzorične nevropatije. Pri razvoju hude periferne nevrotoksičnosti je treba odmerek prestaviti ali zmanjšati. Če začnemo zdravljenje pri bolnikih s kongestivnim srčnim popuščanjem, z bradiaritmijami ali sočasno z zdravili, za katera je znano, da podaljšujejo interval QT, vključno z antiaritmiki razreda Ia in III, in z elektrolitskimi motnjami, je priporočljivo spremljanje EKG. Pred začetkom zdravljenja s Halavenom je treba popraviti hipokaliemijo in hipomagneziemijo in te elektrolite je treba občasno kontrolirati med zdravljenjem. Eribulina ne smemo dajati bolnikom s prirojenim sindromom dolgega intervala QT. To zdravilo vsebuje majhne količine etanola (alkohola), manj kot 100 mg na odmerek. Eribulin je pri podganah embriotoksičen, fetotoksičen in teratogen. Halavena se ne sme uporabljati med nosečnostjo, razen kadar je to nujno potrebno. Ženske v rodni dobi naj ne zanosijo v času, ko same ali njihov moški partner dobivajo Halaven, in naj med zdravljenjem in še do 3 mesece po njem uporabljajo učinkovito kontracepcijo. Moški naj se pred zdravljenjem posvetujejo o shranjevanju sperme zaradi možnosti nepopravljive neplodnosti. **INTERAKCIJE:** Eribulin se izloča do 70 % prek žolča. Sočasna uporaba učinkovin, ki zavirajo jetrne transportne beljakovine, kot so beljakovine za prenos organskih anionov in beljakovine, odporne na številna zdravila, z eribulinom se ne priporoča (npr. ciklosporin, ritonavir, sakvinavir, lopinavir in nekateri drugi zaviralci proteaze, efavirenz, emtricitabin, verapamil, klaritromicin, kinin, kinidin, dizopiramid itd). Sočasno zdravljenje z indukcijskimi učinkovinami, kot so rifampicin, karbamazepin, fenitoin, šentjanževka, lahko povzroči znižanje koncentracij eribulina v plazmi, zato je ob sočasni uporabi induktorjev potrebna previdnost. Eribulin je blag inhibitor encima CYP3A4. Priporočljiva je previdnost in spremljanje glede neželenih učinkov pri sočasni uporabi snovi, ki imajo ozko terapevtsko okno in se odstranjujejo iz telesa predvsem preko CYP3A4 (npr. alfentanil, ciklosporin, ergotamin, fentanil, pimozid, kinidin, sirolimus, takrolimus). **NEŽELENI UČINKI:** Povzetek varnostnega profila Neželeni učinek, o katerem najpogosteje poročajo v zvezi s Halavenom, je supresija kostnega mozga, ki se kaže kot nevtropenija, levkopenija, anemija, trombocitopenija s pridruženimi okužbami. Poročali so tudi o novem začetku ali poslabšanju že obstoječe periferne nevropatije. Med neželenimi učinki, o katerih poročajo, je toksičnost za prebavila, ki se kaže kot anoreksija, navzea, bruhanje, driska, zaprtost in stomatitis. Med drugimi neželenimi učinki so utrujenost, alopecija, zvečani jetrni encimi, sepsa in mišičnoskeletni bolečinski sindrom. Seznam neželenih učinkov: *Zelo pogosti (≥ 1/10):* nevtropenija (57,0 %) (3./4. stopnje: 49,7 %), levkopenija (29,3 %) (3./4. stopnje: 17,3 %), anemija (20,6 %) (3./4. stopnje: 2,0 %), zmanjšan apetit (21,9 %) (3./4. stopnje: 0,7 %), periferna nevropatija (35,6 %) (3./4. stopnje: 7,6 %), glavobol (17,2 %) (3./4. stopnje: 0,8 %), dispnea (13,9 %) (3./4. stopnje: 3,1 %), kašelj (13,6 %) (3./4. stopnje: 0,6 %), navzea (33,8 %) (3./4. stopnje: 1,1 %), zaprtost (19,6 %) (3./4. stopnje: 0,6 %), driska (17,9 %) (3./4. stopnje: 0,8 %), bruhanje (17,6 %) (3./4. stopnje: 0,9 %), alopecija, artralgija in mialgija (19,4 %) (3./4. stopnje: 1,1 %), bolečina v hrbtu (13,0 %) (3./4. stopnje: 1,5 %), bolečina v udu (10,0 %) (3./4. stopnje: 0,7 %), utrujenost/astenija (47,9 %) (3./4. stopnje: 7,8 %), pireksija (20,4 %) (3./4. stopnje: 0,6 %), zmanjšanje telesne mase (11,3 %) (3./4. stopnje: 0,3 %). *Pogosti (≥ 1/100 do < 1/10):* okužba sečil (8 %) (3./4. stopnje: 0,5 %), pljučnica (1,2 %) (3./4. stopnje: 0,8 %), ustna kandidiaza, ustni herpes, okužba zgornjih dihal, nazofaringitis, rinitis, limfopenija (4,9 %) (3./4. stopnje: 1,4 %), febrilna nevtropenija (4,7 %) (3./4. stopnje: 4,5 %), trombocitopenija (4,3 %) (3./4. stopnje: 0,7 %), hipokaliemija (6,1 %) (3./4. stopnje: 1,7 %), hipomagneziemija (2,9 %) (3./4. stopnje: 0,2 %), dehidracija (2,8 %) (3./4. stopnje: 0,5 %), hiperglikemija, hipofosfatemija, nespečnost, depresija, disgevzija, omotičnost (7,9 %) (3./4. stopnje: 0,5 %), hipoestezija, letargija, nevrotoksičnost, oslinjenje solzenje (6,0 %) (3./4. stopnje: 0,1 %), konjunktivitis, vrtoglavica, tahikardija, vročinski valovi, orofaringealna bolečina, epistaksa, rinoreja, bolečina v trebuhu, stomatitis (9,3 %) (3./4. stopnje: 0,8 %), suha usta, dispepsija (5,9 %) (3./4. stopnje: 0,2 %), gastroezofagealna refluksna bolezen, razjede v ustih, distenzija trebuha, zvišanje alanin-aminotransferaze (7,6 %) (3./4. stopnje: 2,1 %), zvišanje aspartat-aminotransferaze (7,4 %) (3./4. stopnje: 1,5 %), zvišanje gama-glutamiltransferaze (1,8 %) (3./4. stopnje: 0,9 %), hiperbilirubinemija (1,5 %) (3./4. stopnje: 0,3 %), izpuščaj, pruritus (3,9 %) (3./4. stopnje: 0,1 %), bolezni nohtov, nočno potenje, suha koža, eritem, hiperhidroza, bolečina v kosteh (9,6 %) (3./4. stopnje: 1,7 %), mišični spazmi (5,1 %) (3./4. stopnje: 0,1 %), mišično-skeletna bolečina in mišično-skeletna bolečina v prsih, mišična oslabelost, disurija, vnetje sluznice (8,3 %) (3./4. stopnje: 1,1 %), periferni edem, bolečina, mrzlica, bolečina v prsih, gripi podobna bolezen. *Občasni (≥ 1/1.000 do < 1/100):* sepsa (0,5 %) (3./4. stopnje: 0,2 %), nevtropenična sepsa (0,1 %) (3./4. stopnje: 0,1 %), herpes zoster, tinitus, globoka venska tromboza, pljučna embolija, hepatotoksičnost (1,0 %) (3./4. stopnje: 0.6 %), palmarno-plantarna eritrodisestezija, hematurija, proteinurija, odpoved ledvic. *Redki (≥ 1/10.000 do < 1/1.000):* diseminirana intravaskularna koagulacija, intersticijska pljučna bolezen, pankreatitis, angioedem. Za popoln opis neželenih učinkov glejte celoten povzetek glavnih značilnosti zdravila. Vrsta ovojnine in vsebina: viala z 2 ml raztopine. **Režim izdaje: H Imetnik dovoljenja za promet:** Eisai Europe Ltd, European Knowledge Centre, Mosquito Way, Hatfield, Hertfordshire, AL10 9SN, Velika Britanija
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Viri: (1) Povzetek glavnih značilnosti zdravila Halaven, junij 2014; (2) Cortes J *et al. Lancet* 2011; 377: 914–23.

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editorial

How to increase the quality and visibility of Radiology and Oncology?

Dear friends of Radiology and Oncology. First of all we would like to thank authors and reviewers for their valuable work that significantly contributes to the quality of our journal. Last year we published 58 articles in four issues. The internationality of our journal is evident through the participation of the authors from many European and other countries throughout the world. Furthermore, in 2015 our Impact factor increased to 1.912. We regret, but according to the limited place, we were unable to accept many manuscripts for publication. Currently we have been able to publish only part of submissions. The rejecting rate was about 78% (58/262). Thus, many otherwise worthwhile papers were not accepted relative to their publication priority assigned by the reviewers and editors.

The published articles are well cited. Many authors from the United States and from China quoted papers from Radiology and Oncology. Furthermore, they were quoted also in some prestigious journals, such as Nature Reviews Cancer. This trend we would like to continue in the future.

Our journal is general in the fields of oncology, radiology and nuclear medicine. We publish works from basic research to clinical studies. In relation to that we decided this year to devote part of the first issue to a theme of biomedical applications of electroporation. Our guest editors, Prof. Richard Heller from USA and Prof. Maja Čemažar from Slovenia have prepared several interesting manuscripts that are published in this issue of Radiology and Oncology. The results were in major part presented at the 1st World congress on electroporation that was held in Portorož in September 2015.

Our intended next step in development of Radiology and Oncology is to apply for the MEDLINE. They adhere to specific requirements that journals have to follow. Among them are ethical issues that we have to comply with. Therefore we require that all clinical and animal data have clearance of the respectful bodies. We will also start to enforce that the prospective clinical studies - beside ethical consideration - are registered either in European or American clinical studies repositories. We would like to inform the authors and also the reviewers to be very strict in this respect.

In the end we would like to thank you again for the support of our efforts for continuous development of Radiology and Oncology.

With best regards,

Prof. Gregor Serša, Ph.D.
Editor in Chief

Assoc. Prof. Viljem Kovač, M.D., Ph.D.
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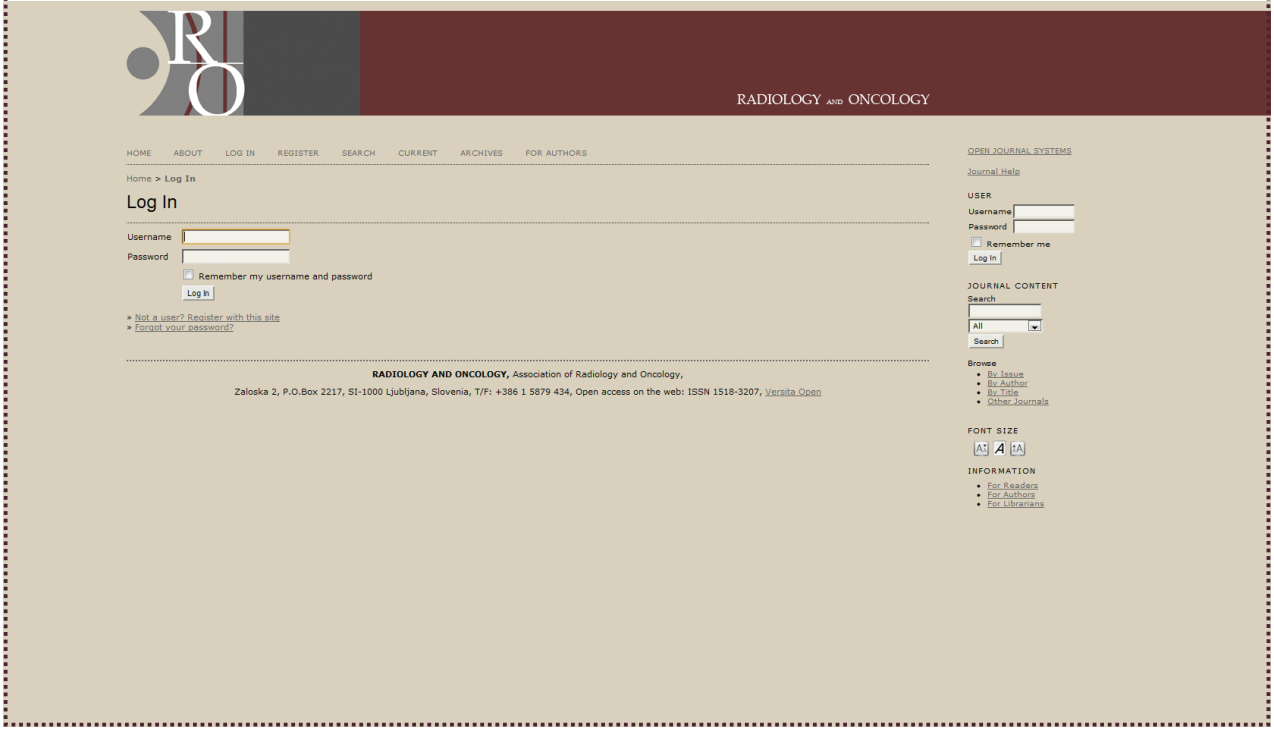

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I *slovenian abstracts*

review

Recommendations for improving the quality of reporting clinical electrochemotherapy studies based on qualitative systematic review

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Disclosure: DM holds patents on electrochemotherapy that have been licensed to IGEA S.p.a. and is also a consultant to IGEA. The other co-authors have nothing to disclose.

Background. Electrochemotherapy is becoming a well-established treatment for malignancies of skin and non-skin origin and its use is widening across Europe. The technique was developed and optimized from solid experimental and clinical evidence. A consensus document is now warranted to formalize reporting results, which should strengthen evidence-based practice recommendations. This consensus should be derived from high quality clinical data collection, clinical expertise and summarizing patient feedback. The first step, which is addressed in this paper, aims to critically analyze the quality of published studies and to provide the recommendations for reporting clinical trials on electrochemotherapy.

Methods. The quality of reporting in published studies on electrochemotherapy was analyzed in order to produce procedure specific reporting recommendations. A comprehensive literature search of studies published from 2006 to 2015 was performed followed by qualitative analysis of manuscripts assessing for 47 quality criteria grouped into four major clusters: (1) trial design, (2) description of patient population, (3) description of treatment delivery and patient outcome, (4) analysis of results and their interpretation. The summary measure during literature assessment was the proportion of studies fulfilling each manuscript quality criteria.

Results. A total of 56 studies were screened, from the period 2006 to 2015, of which 33 were included in the qualitative analysis, with a total of 1215 patients. Overall, the quality of reporting was highly variable. Twenty-four reports (73%) were single-center, non-comparative studies, and only 15 (45%) were prospective in nature (only 2 of them were entered into a clinical trials registry). Electrochemotherapy technique was consistently reported, with most studies (31/33) adhering closely to published standard operating procedures. The quality of reporting the patient population was variable among the analyzed studies, with only between 45% and 100% achieving dedicated quality criteria. Reporting of treatment delivery and patient outcome was also highly variable with studies only fulfilling between 3% and 100%. Finally, reporting study results critically varied, fulfilling from 27% to 100% of the quality criteria. Based on the critical issues emerging from this analysis, recommendations and minimal requirements for reporting clinical data on electrochemotherapy were prepared and summarized into a checklist.

Conclusions. There is an increasing body of published clinical data on electrochemotherapy, but more high quality clinical data are needed. Published papers often lack accurate description of study population, treatment delivery as well as patient outcome. Our recommendations, provided in the form of a summary checklist, are intended to ameliorate data reporting in future studies on electrochemotherapy and help researchers to provide a solid evidence basis for clinical practice.

Key words: electrochemotherapy; clinical trials, recommendations

Introduction

Electrochemotherapy is becoming a well-established non-thermal ablative technique for malignancies of skin and non-skin origin.[1,2] The medical applications of electrochemotherapy are based on the principle of electroporation, which dates back to 1982, when sequences of electric pulses were applied to deliver naked DNA molecules within mouse lyoma cells.[3] Preclinical studies carried out by several research groups, coupled with technical developments, culminated in the clinical application of electroporation during the early 1990s.[4-13] These initial data on electroporative uptake of molecules are viewed as seminal for various biotechnological and medical applications.[14,15] The principle of electrochemotherapy is the use of electroporation to enhance chemotherapeutic drug delivery. Two agents, bleomycin or cisplatin, can achieve a several fold increase in their intracellular availability, and consequently cytotoxicity, when the tumor tissue is exposed to reversible electroporation and transient cell membrane permeabilization, thus achieving an optimal intratumor drug distribution.[7,16-18] Electrochemotherapy has proven effective for the treatment of different tumor histotypes, including both skin and non-skin cancers, as well as for the palliation of metastases involving cutaneous and subcutaneous tissues.[19-22] The treatment of primary skin tumors is largely restricted to multifocal cutaneous tumors, most notably some selected cases of basal cell carcinoma, when tumor anatomical location and patient medical conditions contraindicate more aggressive treatments.[23]

The publication of the European Standard Operating Procedures of Electrochemotherapy (ESOPE) in 2006 facilitated a broad acceptance of electrochemotherapy for treatment of cutaneous tumors and metastases.[24] Over a number of years, several clinical reports have confirmed its effectiveness. Interestingly, the vast majority of studies used the Standard Operating Procedure (SOP) as a guideline for electrochemotherapy. The availability of SOP allowed for reproducibility and improvement of results in the clinical practice. Several large follow up series confirmed the efficiency of electrochemotherapy. A recent meta-analysis of the use of electrochemotherapy in the treatment of cutaneous metastasis places it well amongst other, more established, treatment options.[2] Recently, electrochemotherapy has also been recognized by the National Institute for Health and Care Excellence (NICE) as an integral part of the multidisciplinary treatment for patients with skin metastases of non-skin origin and melanoma (NICE interventional procedure guidance IPG 446, http://www.nice.org.uk/guidance/ipg446). More recently, electrochemotherapy has been introduced into the treatment of deep-seated and endoluminal tumors.[25-28] The first clinical report on visceral metastases indicates its effectiveness, and suggests a possible role of electrochemotherapy for the treatment of liver metastases, especially when located close to major blood vessels and when not manageable with surgery or other ablative techniques.[29]

Overall, literature data from Web of Science database indicate a steady increase in number of publications and their citations under the key word "electrochemotherapy" (Figure 1A,B) and "clinical electrochemotherapy" (Figure 1C,D). Despite a steady increase in the number of published reports, a higher quality and standardization of reported studies is needed to improve and support a truly evidence based practice. In our study we only included papers published after 2006, specifically only to include reports published after the Standard Operating Procedures (SOP).[24]

The purpose of this recommendation paper is to provide practical recommendations in order to improve the precision of reported clinical studies on electrochemotherapy (a summary checklist is provided as Supplementary file). This, in turn, we hope will stimulate the scientific community to report research using these guidelines to give comprehensive reports on areas including study design, definition of study endpoints, patient selection criteria, treatment plan and outcome assessment. The adoption of more precision in reporting will enable researchers and clinicians to perform more meaningful outcome comparisons with other ablative techniques, to clarify the direction for future research, and to produce more evidence-based practice. It is our hope that these advancements may improve patient selection, resource allocation, and ultimately patient outcome.

This report was prepared based on initiative of the Steering Committee of the COST TD 1104 Action (www.electroporation.net) and in response to a general call for increased awareness and concern for low quality reporting practice[30]; moreover, it has been prepared by the committee within the Working group of Medical applications of electroporation, in COST action TD 1104 EP4Bio^{2}Med, and is included in the series of publications addressing the same topic in preclinical research in electroporation as well as in the pulsed electric fields for industrial purposes.[31]

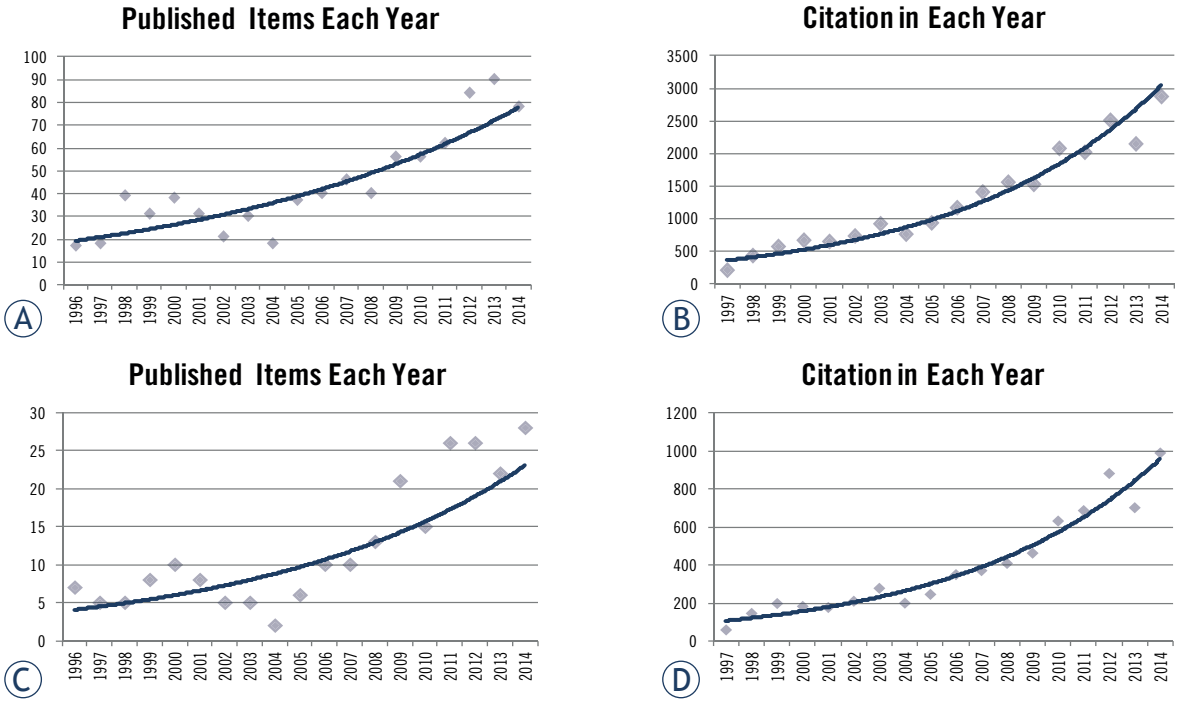

FIGURE 1. Search in Web of Science demonstrates a steady increase in number of publications under the key word "electrochemotherapy" **(A,B)** as well under the "electrochemotherapy, clinical" **(C,D)**. The Meta data indicate the expanding field.

Several guidelines exist with the aim of assuring sound research practices, and improving the quality of clinical trials and, ultimately, allow for generalizable results. At a basic level, Good Clinical Practice (GCP) represents an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. At a higher level, dedicated guidelines and recommendations have been developed according to the specific type of study performed. For instance, the STROBE statement (www.strobe-statement.org) indicates a checklist for details that should be reported in observational trials; the Consolidated Standards of Reporting Trials (CONSORT) statement (www.consort-statement.org/consort-statement/) provides guidance for reporting the aim, methods, results and implications of randomized controlled trials; the PRISMA statement (www.prisma-statement.org) indicates preferred reporting for systematic reviews; finally, the REporting recommendations for tumor MARKer prognostic studies (REMARK, www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumour-marker-prognostic-studies-remark/) suggest guidelines to provide relevant information about study design, preplanned hypotheses, patient and specimen characteristics, assay methods, and statistical analyses when evaluating tumor markers in oncology. In addition, these guidelines provide helpful suggestions on how to present data and important elements to include in discussions. Although these guidelines provide a fundamental guidance for conducting a valid clinical trial and reporting generalizable findings, nonetheless it is recognized that there is a need for specialty-specific guidelines and that these guidelines will lead to improvement in the quality of reports and to higher impact publications.[32,33] In the field of electrochemotherapy, comprehensive meta-analyses or Cochrane style reviews of efficiency are hampered by the lack of some relevant clinical data in published reports. Therefore, we evaluated the published papers on clinical electrochemotherapy and identified possible pitfalls in data reporting. On this basis, we prepared recommendations for improving the quality of future studies and fostering further rational development of electrochemotherapy.

Systematic review and qualitative analysis of publications

Methods

The initial step was to identify and access all published trials evaluating the efficacy of electrochem-

TABLE 1. Manuscript quality criteria

Manuscript quality criteria			
Trial design	**Description of Patient population**	**Treatment delivery and outcome assessment**	**Analysis of results and interpretation**
1. Prospective trial	1. Setting (curative / palliative)	1. Type of anaesthesia	1. Summary of trial endpoints
2. Trial registration		2. Drug route and dosages	
3. Comparative trial	2. Demographic data (in tabular form)	3. Pulse generator	2. Predictive factors
4. Mention of trial design		4. EP parameters	
5. Multicenter study	3. No of tumors	5. Electrode description	3. Other patient outcome parameters
6. Mention of sponsor		6. Tumor safety margins indicated	
7. Trail hypothesis and sample size	4. Tumor location	7. Deviation from SOPs	4. Results interpretation
8. Informed consent	5. Tumor histotype	9. Criteria for retreatment	5. Comparison to historical controls
9. EC approval		8. Tumor coverage with EP	
10. Structured abstract		10. Total No of ECT sessions	
11. Rationale of the trial	6. Tumor size	11. ECT sessions required[a]	6. Future directions
12. A priori inclusion criteria		12. Toxicity criteria	
13. Follow-up dates	7. Visceral mts indicated	13. Response criteria	7. COI statement
14. Statistical methods		14. Evaluation of tumor control	
15. Software used	8. Concomitant treatments	15. ECT success[b]	
16. C.I., p-values		16. Keep track of patients lost to follow-up	

C.I. = confidence intervals; COI = conflict of interest statement; EC = Ethic Committee; EP = electric pulses (including number, duration and amplitude); mts = metastases; SOPs = Standard Operating Procedures.

[a] Number of electrochemotherapy (ECT) sessions required for achieving response (either complete or partial) on baseline tumors

[b] Decision rule for determining ECT success

otherapy in the treatment of tumors including skin cancers, cutaneous/subcutaneous metastases from other histotypes, deep-seated tumors or visceral metastases.

From October 4 to 10, 2015, we conducted a comprehensive literature assessment that included searches of Medline (EBSCO), Pubmed (NLM), Web of Science and Embase. The search terms used were "electrochemotherapy", "electrochemotherapy" AND "clinical trial". We limited our search to humans. Articles published from January 2006 to September 30, 2015 were retrieved. We included studies on the clinical application of electrochemotherapy regardless of study design (both prospective and retrospective) patient population, tumors histotype and anatomical location or electrochemotherapy treatment protocol. However, treatment outcome had to include tumor response and follow-up tumor control evaluation, procedural morbidity and toxicity or patient quality of life. Two of the authors (LGC and SV) and an external collaborator with experience in clinical trials independently screened the retrieved studies based on the title, key words, and abstract to exclude non-relevant and non-English written studies. After completion of all searches, duplicates were removed and only the most recent report from follow-up series was included in order to avoid overlapping series. Both retrospective and prospective studies were included, while case reports and small series were excluded because of their intrinsic lower level of evidence (the minimum number of patients was arbitrarily set at 9). Published reviews on electrochemotherapy were similarly excluded, but their reference list was reviewed in order to identify possible additional studies. Studies whose main purpose was unrelated to electrochemotherapy efficacy and biological studies (*i.e.*, those exploring immune effects of treatment) were also excluded, unless clear and standardized description of patient outcome was retrievable from the manuscript. Studies that did not meet the inclusion criteria were discarded during the initial review. When uncertainty existed in the abstract evalu-

ation, we retrieved and assessed the full text. A third author (GS) resolved differing opinions. Full text of the included articles was independently reviewed by two of the authors using a predefined checklist quality criteria. These quality criteria were discussed and agreed among the authors in a series of operative meetings which were hold during the 1st World Congress on Electroporation in Portoroz, Slovenia, between September 6 to 10 2015 and were also based on deliberations at the Recommendation paper workshop organized by COST TD1104 on 28th March 2014 in Copenhagen, Denmark. The checklist was also adapted from similar reporting standard guidelines in the field of neuro-oncology, isolated limb perfusion and in phase II cancer trials.[34-36] As a result, we had a final count of 47 quality criteria that were clustered into four domains: trial design, description of patient population, treatment delivery and outcome assessment, and analysis of results and their interpretation (Table 1). The summary measure during literature assessment was the proportion of studies fulfilling each manuscript quality criteria.

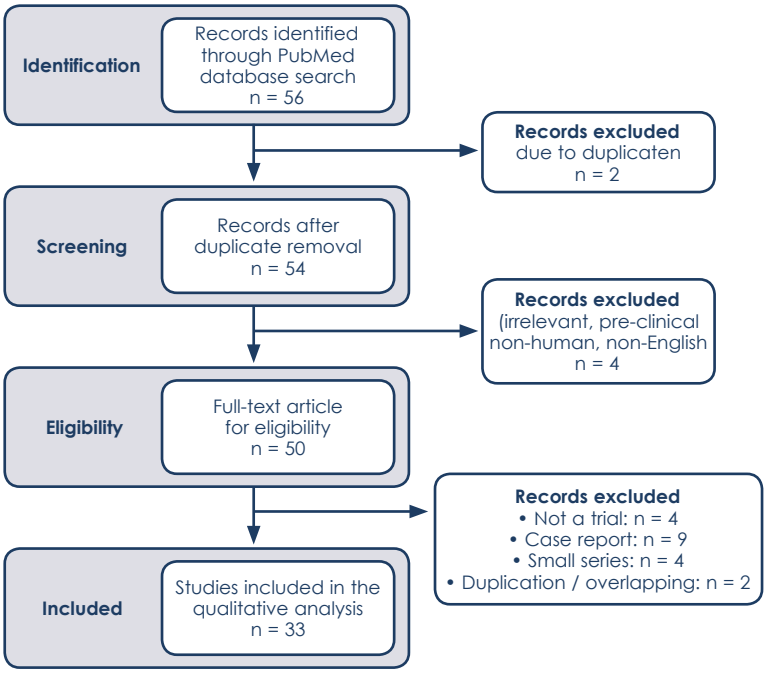

FIGURE 2. PRISMA flow diagram of identification, screening, eligibility and inclusion of studies.

Results

A total of 56 papers were initially identified. Of these, only 33 reports were finally retained in the qualitative synthesis; the reasons for exclusion of the remaining reports are listed in Figure 2.

A summary of the studies included in the final analysis is presented in Table 2.[20-22,29,37-65] The total number of patients across all studies was 1215. Electrochemotherapy protocol was following the SOP as defined in ESOPE study in all but two cases.[40,65]

The majority (24/33) of reports were single-center studies. There were 24 tumor-specific studies (melanoma, n=8; breast cancer, n=5; head and neck squamous cell carcinoma, n=4; Kaposi sarcoma, n=3; pancreatic cancer, n=1; colorectal cancer, n=1; soft tissue sarcomas, n=1; vaginal squamous cell cancer, n=1) and 9 studies including heterogeneous histologies. Response assessment was based on clinical evaluation in all except 3 studies on pancreatic cancer[41], liver metastases from colorectal cancer[29], and chest wall recurrence from breast cancer[57], where response assessment was radiological (ultrasound scan, magnetic resonance imaging, computed tomography, or fluorine-18-deoxyglucose PET-CT scan). Details of the quality criteria used to assess trial design are presented in Figure 3. Less than half (15/33, 45%) of studies

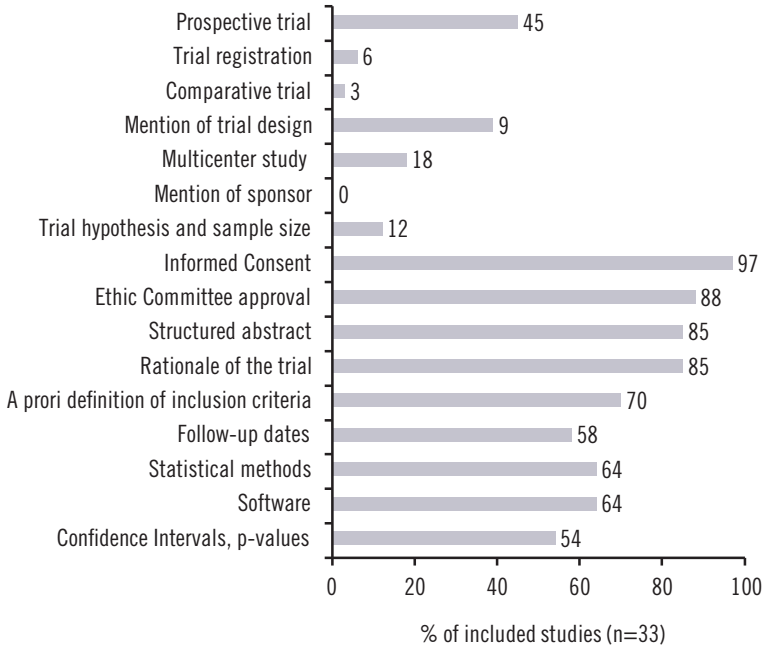

FIGURE 3. Assessment of published studies according to **quality criteria concerning trial design**.

were prospective and only two of them (6%) were entered into a publicly accessible clinical trials registry.[29,57] Eighteen percent (6/33) of papers represented the report of a multicenter study. There was a single comparative trial (an internally controlled

TABLE 2. Trials identified included in the qualitative analysis

Study, year	Setting	No of pts	Tumor histotype	ECT protocol
Rotunno, 2015 [37]	Two-center, Italy	55	non-melanoma SC	ESOPE
Cabula, 2015 [38]	Multi-center, Italy	125	BC	ESOPE
Mozzillo, 2015 [39]	Single-center, Italy	15	melanoma	ESOPE
Landstrom, 2015 [40]	Single-center, Sweden	19	HNSCC	Other [a]
Granata, 2015 [41]	Single-center, Italy	13	pancreatic cancer	ESOPE
Kreuter, 2015 [42]	Multi-center, Germany	56	various	ESOPE
Quaglino, 2015 [43]	Multi-center, Europe	121	various	ESOPE
Mir-Bonafé, 2015 [44]	Single-center, Spain	31	melanoma	ESOPE
Campana, 2014 [45]	Single-center, Italy	39	HNSCC	ESOPE
Ricotti, 2014 [46]	Single-center, Italy	30	melanoma	ESOPE
Campana, 2014 [47]	Single-center, Italy	55	BC	ESOPE
Edhemovic, 2014 [29]	Single-center, Slovenia	16	CRC-liver mts	ESOPE [b]
Seccia, 2014 [48]	Single-center, Italy	9	HNSCC	ESOPE
Campana, 2014 [50]	Two-center, Italy	34	STS	ESOPE
Solari, 2014 [51]	Single-center, Italy	39	various	ESOPE
Di Monta, 2014 [52]	Single-center, Italy	19	KS	ESOPE
Caracò, 2013 [49]	Single-center, Italy	60	melanoma	ESOPE
Perrone, 2013 [53]	Single-center, Italy	9	V-SCC	ESOPE
Benevento, 2012 [54]	Single-center, Italy	12	BC	ESOPE
Mevio, 2012 [55]	Single-center, Italy	15	HNSCC	ESOPE
Campana, 2012 [20]	Single-center, Italy	35	BC	ESOPE
Latini, 2012 [56]	Single-center, Italy	18	KS	ESOPE
Matthiessen, 2012 [57]	Single-center, Denmark	12	BC	ESOPE
Gargiulo, 2012 [58]	Single-center, Italy	52	non-melanoma SC	ESOPE
Campana, 2012 [21]	Single-center, Italy	85	melanoma	ESOPE
Curatolo, 2012 [59]	Two-center, Italy	23	KS	ESOPE
Kis, 2011 [60]	Single-center, Hungary	9	melanoma	ESOPE
Matthiessen, 2011 [22]	Two-center, Denmark-UK	52	various	ESOPE
Skarlatos I, 2011 [61]	Multi-center, Greece	52	various	ESOPE
Campana, 2009 [62]	Single-center, Italy	52	various	ESOPE
Quaglino, 2008 [63]	Single-center, Italy	14	melanoma	ESOPE
Larkin, 2007 [64]	Single-center, Ireland	30	various	ESOPE
Gaudy, 2006 [65]	Single-center, France	12	melanoma	Other [c]

BC = breast cancer; ECT = electrochemotherapy; CRC-liver mts = colorectal cancer liver metastases; HNSCC = head and neck squamous cell cancer; KS = Kaposi's sarcoma; SC = skin cancer; STS = soft tissue sarcomas; V-SCC = vaginal squamous cell cancer

[a] Intratumoral BLM injection (1000 IU/cm^3 and tumor electroporation by means of six 1100 V/cm square wave pulses with 0.1 ms duration

[b] In this trial, the ESOPE protocol was integrated by the application of variable geometry electrodes for the treatment of deep visceral metastases.

[c] Intratumoral BLM injection (concentration, 4 mg/mL; dose, 1 mg/cm^3 of tumor volume was followed, after 10 minutes, by the application of electric pulses (six 100 µsec-long pulses, 4 pulses/sec, electric field >600V/cm

study with intrapatient randomization of melanoma metastases to intralesional bleomycin versus intralesional bleomycin followed by electric pulses)[65]; a formal sample size calculation or analysis of "intent-to-treat" population was found in only 4/33 (12%) studies.[20,50,57,65]

Details of the quality criteria used to assess the description of patient population are presented in Figure 4. Treated tumors were described in detail in most reports: number of tumors, 94%; tumor location, 100%; tumor histotype, 100%; tumor size, 91%. On the other hand, additional clinical information was less frequently reported: study setting -palliative/curative-, 54%; presence of visceral metastases, 54%; concomitant oncologic treatments, 45%.

Details of the manuscript quality criteria used to assess the description of treatment delivery and response assessment are presented in Figure 5. Treatment details were accurately described in most reports: type of anaesthesia, 32/33 (97%); drugs, 33/33 (100%); pulse generator, 33/33 (100%); electrode types, 31/33 (93%); electric pulse parameters, 32/33 (97%). The criteria for response assessment were clearly stated in 29/33 (88%) of studies, while toxicity criteria were indicated in only 14/33 (42%) of papers.

Details of the quality criteria used to assess the analysis of results and their interpretation are presented in Figure 6. The majority of reports included a critical analysis: interpretation of results, 33/33 (100%); comparison to historical control, 25/33 (76%); indication of possible future directions, 33/33 (100%); conflict of interest statement, 27/33 (82%). On the contrary, only a minority of them fulfilled other specific quality criteria: summary of primary and secondary endpoints, 13/33 (39%); indication of predictive factors, 9/33 (27%); additional patient outcome parameters, 9/33 (27%).

Based on the results of this analysis, the consensus between authors was to recommend some minimal requirements for reporting clinical data in future studies.

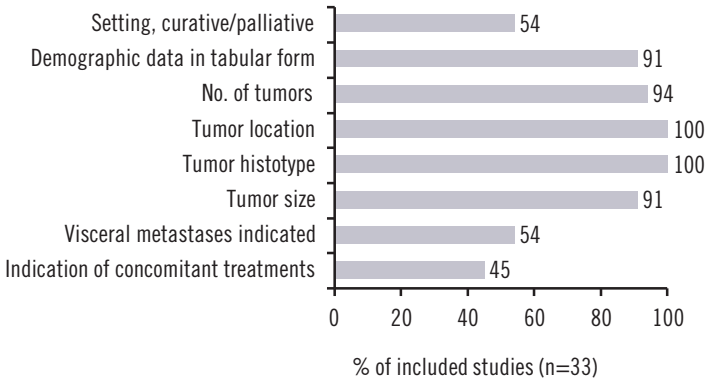

FIGURE 4. Assessment of published studies according to **quality criteria concerning description of patient population**.

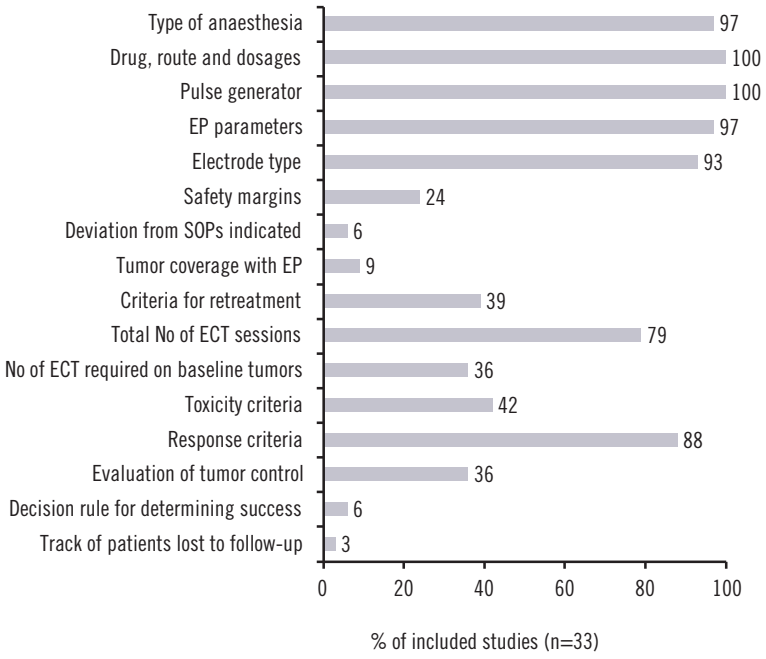

FIGURE 5. Assessment of published studies according to **quality criteria concerning treatment delivery and outcome assessment**.

ECT = electrochemotherapy; EP = electric pulses.

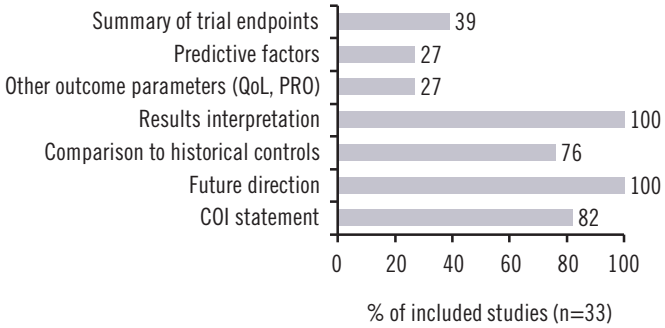

FIGURE 6. Assessment of published studies according to **quality criteria concerning analysis of results and interpretation.**

COI = conflict of interest statement; PRO = patient reported outcomes; QoL = quality of life.

Recommendations and minimal requirements for reporting clinical trial results on electrochemotherapy

Trial design

Any consolidation of the evidence base of electrochemotherapy requires that reports adhere strictly to research reporting standards and are the result of well-designed clinical trials. Much of these

topics are covered by STROBE (STrengthening the Reporting of Observational studies in Epidemiology, http://www.strobe-statement.org/) checklist and CONSORT (CONsolidated Standards of Reporting Trials, http://www.consort-statement.org/checklists/view/32-consort/66-title) guidelines which should be adhered to as much as possible when reporting observational studies and randomized controlled trials, respectively. Incorporation of these electrochemotherapy guidelines will further improve the quality of the reports. So far, only phase I-II single-arm trials have been reported, with the exception of a single small-sized study, which included an intra-patient randomization of tumors to direct bleomycin injection or bleomycin injection followed by electroporation.[65] It is likely that improving the evidence base will involve conducting properly designed, prospective comparative - possibly randomized - clinical trials in order to perform accurate analyses of the advantages of electrochemotherapy against other ablative procedures or alternative local treatments. Of utmost importance, future trials should aim to be prospective and preferably multicentric, with clearly defined endpoints and inclusion criteria. It is also advisable that all trials should be registered at publicly accessible clinical trials registries, (*e.g.*, clinicaltrials.gov, ISRCTN registry at http://www.isrctn.com, WHO registry at www.apps.who.int/trialsearch, or similar) and approved by institutional review boards or respective national bodies. Finally, according to the current requirements of most scientific journals – which refer to the recommendation of the International Committee of the Medical Journal Editors (ICMJE, http://www.icmje.org/), manuscripts should conform to well-defined general principles and include, for example, a statement about patient informed consent, modalities of study conduct, as well as authors conflicts of interest.

Key elements of trial design:

- Explanation of the rationale of the study
- Description of trial design and sponsorship
- Indication of trial endpoints
- Indication of inclusion and exclusion criteria
- Trial approval and registration
- Informed consent statement

Description of patient population

Electrochemotherapy was initially used with palliative intent. First trials demonstrated remarkable efficiency in the treatment of skin metastases from malignant melanoma.[21,60,63] Subsequently, electrochemotherapy was also evaluated for the treatment of other tumor histotypes (*e.g.*, non-melanoma skin cancers and cutaneous metastases from other tumor histotypes) with equally high success.[20,37-38,47,57-58] Reports of small series indicate also its possible usefulness in the treatment of primary basal cell carcinomas[23] and a clinical trial is currently ongoing comparing the effectiveness of electrochemotherapy to standard surgical resection and is due to report 5 year follow up data next year (EudraCT Number: 2010-019260-37). A particular advantage of electrochemotherapy is that it is a reliable alternative treatment option for patients who have exhausted more conventional oncological treatments or are judged unfit for or refuse repetitive surgical interventions.[47] Therefore, future reports need to include detailed description of patient's demographic and clinical data including detailed description of previous treatments. A detailed description of tumor location, histotype as well as number and size of the electrochemotherapy target and non-target lesions is paramount. Authors should also specify whether targeted lesions had previously received irradiation or not, whether visceral metastases are present and whether the treatment is intended as palliative or curative. Additionally, since electrochemotherapy is finding its place among other oncologic treatments, and will be increasingly used also in combination with them, an accurate record of concomitant treatments is also advisable.[66]

Key elements of patient population:

- Patient demographic data (in tabular form)
- Setting - palliative or curative
- Tumor histology
- Disease stage (lymph node or visceral metastases)
- Description of target lesions treated with electrochemotherapy (anatomical location, number and size)
- Previous local treatments
- Concomitant oncological treatments
- Adjuvant and / or following oncological treatments

Treatment information

The treatment is applied by performing a procedure conjugating the administration of a drug and local application of electric pulses. In one "session"

or "cycle" a single or several tumor nodules can be treated. Since the procedures can be repeated on the same and also on newly emerged tumor nodules, patient treatment may require one or more sessions of electrochemotherapy. Therefore, reports should clearly indicate how many sessions (or cycles) were needed for the treatment of baseline tumors and, overall, for patient management. If retreatment is necessary, the indication should be clear, detailing previous response and disease status in target and non-target tumors. In order to ensure the maximum efficacy, electrochemotherapy needs two key elements: the presence of a cytotoxic agent within tumor tissue and the adequate coverage of tumor with electric pulses above the threshold of reversible membrane electroporation.[67] The results of the ESOPE study and the adoption of SOP that were prepared within the ESOPE project (QLK-2002-02003) were of great importance for the development of electrochemotherapy.[68,24] In fact, they provided practical guidelines and standardization of the procedure. The clinical data evaluation demonstrated that the use of guidelines and a standardized protocol enabled to reach the same level of effectiveness also in the centers without previous experience with electrochemotherapy.[17] The ESOPE study provided evidence for electrochemotherapy in the treatment of skin metastases of different histotypes.[68] It included use of bleomycin or cisplatin as chemotherapeutics, different routes of administration (intravenous or intratumoral) and the use of either local or general anaesthesia. The pulse parameters (number, sequence and amplitudes) for different electrodes were however well defined within the Cliniporator project.[45] A specific electric pulse generator has been consistently used with different electrodes, according to the size, depth and anatomical location of treated tumors.

As confidence with the procedure has developed, treatment indications have also widened. The first studies were based on patients with tumors less than 3 cm, however lesions greater than 3 cm are now routinely treated[57,62,69], representing a natural development of the field based on success with smaller tumors for which the ESOPE guidelines were prepared. As such, there is a need to adapt and revise the SOP and this is already underway. Furthermore, new producers of electric pulse generators are coming to the market, and new electrodes with different design for different treatment settings are emerging. All these changes will make the clinical data evaluation even more challenging. First of all, to address this topic, future reports will need to state the type of anesthesia used (local or general; drugs and doses), the chemotherapeutic agent, drug concentration and dose used, which both depend on the route of administration. The duration of bolus injection, as well as time interval between the drug administration and application of electric pulses, should be specified. The type of electric pulse generator as well as the type of electrodes and their manufacturers should be reported. Additional information should include if the pulse generator is under software control and the specification of the version of that software. If new types of electrodes are used, a detailed description of the design and the sequence and amplitude of pulses is needed. It must be clearly stated whether applied electrodes are needle or plate, the distance between the electrodes, their shape and size, the amplitude of applied electric pulses, their duration, number and repetition frequency. Furthermore, the total number of pulse deliveries, as well as the time interval required for electrode applications after drug injection, should be specified. Additionally, the report of adequate or inadequate coverage of the tumor as well as the way the pulses are applied (e.g., from the margins to the tumor centre or if the pulses were applied in 4+4 (perpendicular) configuration each time) would also be advisable, when possible (Figure 7).[70]

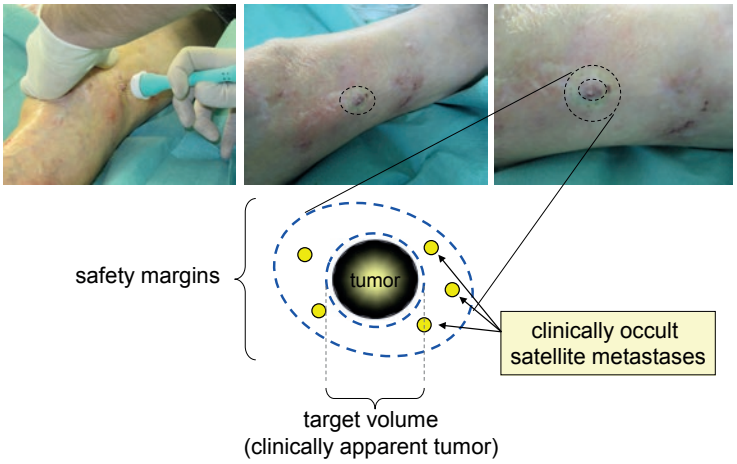

FIGURE 7. Importance of covering whole tumor area along with safety margins. Reporting of the type of electrode applied is essential.

Electrochemotherapy has a high therapeutic index, therefore after successful treatment minimal damage is observed on normal surrounding tissues. During treatment, it is also possible for the treating physician to include a safety margin around the target tumor, depending on tumor size, biologic aggressiveness and propensity for

developing satellite lesions such as in the case of malignant melanoma or soft tissue sarcomas. In order to improve reporting, the information about the safety margins and their extent should also be reported. In addition, electrochemotherapy can be repeated several times (however there is a ceiling for the total lifetime dose of bleomycin), according to tumor response and disease behavior.[62,63] This fundamental aspect is not covered by the currently available SOP. For providing a more informative report, data regarding repetitive treatments should be included, along with the description on what basis the retreatment was performed and at what time interval.

Key elements on treatment information:

- Indication of electroporation protocol (adherence to SOP or other)
- Type of anesthesia
- Drug (producer)
- Drug details (dose, concentration and route of administration
- Time interval between drug administration and application of electric pulses
- Technical details of the electric pulse generator, including type, manufacturer and version of software, if applicable
- Information about the electrodes used, for respective tumor(s)
- Number of electric pulses application per tumor
- Inclusion of a report on electrical parameters (n, T, U, I, f)*
- Adequacy of tumor treatment (treatment application success rate)
- Extent of the safety margins treated
- Number of treatment sessions (with interval between sessions)

* Legend: n = number; T = duration of pulses; U = voltage amplitude applied; I = measured current; f = pulse repetition frequency

Outcome assessment

The early studies on electrochemotherapy antitumor activity have carefully evaluated the response of treated tumors. Response assessment was initially performed by the bidimensional WHO criteria.[71] According to these criteria, baseline and post-treatment tumor size is determined by bidimensional measurements *e.g.* the sum of the two longest diameters in the perpendicular dimensions. The tumor response to treatment is divided into four categories (complete response, partial response, stable disease, progressive disease, according to the change from baseline tumor measurement).

Indeed, most past studies were focused on tumor response and on patient early outcomes. Nevertheless, a number of reports indicate that the disease locally relapsed or progressed elsewhere, but only few reports indicated the value of electrochemotherapy in the local management of patient symptoms. Hence, the clinical benefit for patients, especially in the palliative setting, where preservation of quality of life (QoL) and evaluation of patient reported outcome (PRO) are crucial, should be based on dedicated assessments and described.

The new RECIST (Response Evaluation Criteria in Solid Tumors) version 1.1 criteria, with some adaptations, have proven a suitable tool for response assessment of superficial tumors[21,72], whereas for the setting of treatment of deep-seated tumors (*i.e.*, electrochemotherapy application on liver metastases) the modified RECIST criteria represent the most appropriate and standardized method for the evaluation of tumor response.[73] In general, for standard electrochemotherapy on superficial tumors, the RECIST 1.1 criteria, which are based on one-dimensional measurements, seem even more practical and offer highly concordant response assessment compared with the bidimensional WHO criteria.[74]

So far, most of the published papers do not report on any serious treatment related adverse event after electrochemotherapy. Nevertheless, the process surrounding the determination, recording and reporting of adverse events remains moderately challenging especially for the clinician who may not be involved in drug or device-related research. Nevertheless, it is important to understand the basic definitions of adverse events reporting in order to ensure that the proper information is collected in clinical protocols. Moreover, a comprehensive patient observation and a detailed report of all types and grades of toxicities are essential for providing a comprehensive report of treatment outcome, not only in the early, but also in the long-term follow-up. In this way, only large cohorts of patients will enable in-deep view of long-term toxicity and more detailed analyses of treatment-related adverse events according to different patients subgroup, as demonstrated by a recently published report on electrochemotherapy-related pain.[43] For this purpose, Common Terminology Criteria for Adverse Events (CTCAE v4.0) is widely accepted throughout the oncology community as the standard classification and severity grading scale for

adverse events. Unfortunately, most of the studies conducted so far do not report consistently on this crucial aspect.

Key elements of treatment outcome assessment:

- Time of response assessment
- Standardized response evaluation criteria (e.g. WHO, RECIST 1.1, mRECIST)
- Time to local and systemic disease progression
- Standardized toxicity criteria (e.g. CTCAE v4.0)
- Quality of Life (QoL), patient reported outcomes (PRO)
- Track of patients lost to follow-up

Analysis and interpretation of the results

A clear summary of the trial endpoints is essential. In fact, the field is moving beyond simply reporting on tumor control, as treatment now includes, in some instances, also primary tumors. Here it is important to report and discuss other parameters, such as time to local/systemic progression and, if possible, also the patient survival time and QoL as well. Such data will increase the evidence level of electrochemotherapy effectiveness, and consolidate a role for electrochemotherapy outside the palliative setting and into a confirmed primary treatment modality.

It has been clearly demonstrated that tumor size is the most reliable predictive factor for response in patients who underwent electrochemotherapy.[21,22,69] In future, detailed reports including data on previous local therapies (*e.g.*, radiation) as well as on local (within electrochemotherapy field) tissue status (*e.g.*, presence of lymphedema or fibrosis) and concomitant/adjuvant oncologic treatments would allow for the identification of other reliable predictive indicators for response.

Key elements for analysis and interpretation of the results:

- Summary of trial endpoints
- Additional patient outcome parameters (e.g., QoL, PRO)
- Predictive factors
- Results interpretation
- Future research directions

Conclusions

Electrochemotherapy represents an effective treatment option for an increasing number of cancer patients with superficial tumors. Nevertheless, to further improve its evidence basis, it will be crucial to raise the quality of future reports.

In this study, we have highlighted some relevant aspects of clinical data reporting, with the aim of improving the quality of future studies in the field of electrochemotherapy. Although a large amount of data are published so far, clinical research needs to adopt detailed and accurate reporting as well as moving from small, non-comparative series to well-designed, possibly randomized, clinical trials. Despite the encouraging results indicated, the vast majority of included reports are case series from single institutions. Although there was a wide consensus to use previously published SOP for the treatment protocol, these studies often present a variety of designs and reporting methods, thus limiting the understanding of patient selection, treatment effect, toxicity and overall patient outcome. Of note, published studies often lack sufficient procedural as well as patient data. These shortcomings represent a major hurdle to performing systematic reviews or meta-analysis, which may provide a more robust evaluation of treatment effectiveness and, ultimately, encourage wider acceptance of electrochemotherapy in the clinical practice.

Our study has some limitations. We identified a set of manuscript quality criteria from available literature and we have expanded this list by including additional, procedure-specific criteria that were discussed and agreed among the authors. The list of 47 quality criteria that were used for reviewing published reports represents an arbitrary selection of criteria performed by a relatively small number of authors. There is potential for selection bias in the inclusion of papers for analysis, as the initial screen was based on broad, non-selective inclusion criteria. However, we feel that these were widely inclusive and fitting in order to develop the proposed recommendations. Nevertheless, we believe that our suggestions largely cover the most crucial aspects, which are required to improve the quality of clinical practice and future research: trial design and conduction, definition of study endpoints, patient selection, treatment delivery, patient management and follow-up, standardization of outcome assessment. Our recommendations are open to a broader discussion with the community users of electrochemotherapy and, possibly, to further improvements in line with other interventional

oncology procedures.[75,76] Electrochemotherapy requires standardization of terminology and reporting criteria to facilitate effective communication among researchers and appropriate comparison between different treatment technologies. As such, investigators involved in this field should be familiar with these recommendations and use them for future study design and conduction, treatment application as well as data reporting. We envision that the adoption of these recommendations will further improve the quality of future studies and allow more meaningful comparisons of outcome data of patients treated with electrochemotherapy (Supplementary file).

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review

Electrochemotherapy in pancreatic adenocarcinoma treatment: pre-clinical and clinical studies

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Background. Pancreatic adenocarcinoma is currently one of the deadliest cancers with high mortality rate. This disease leads to an aggressive local invasion and early metastases, and is poorly responsive to treatment with chemotherapy or chemo-radiotherapy. Radical resection is still the only curative treatment for pancreatic cancer, but it is generally accepted that a multimodality strategy is necessary for its management. Therefore, new alternative therapies have been considered for local treatment.

Conclusions. Chemotherapeutic resistance in pancreatic cancer is associated to a low penetration of drugs into tumour cells due to the presence of fibrotic stroma surrounding cells. In order to increase the uptake of chemotherapeutic drugs into tumour cells, electrochemotherapy can be used for treatment of pancreatic adenocarcinoma leading to an increased tumour response rate. This review will summarize the published papers reported in literature on the efficacy and safety of electrochemotherapy in pre-clinical and clinical studies on pancreatic cancer.

Key words: electrochemotherapy; pancreatic carcinoma; bleomycin; pre-clinical study; clinical study; safety; efficacy

Introduction

Pancreatic adenocarcinoma, despite extensive research, remains one of the deadliest cancers with high mortality rate. Surgical resection represents the only curative treatment for this pathology, but the majority of the patients are incurable at initial presentation with metastatic (stage IV) or surgically non-resectable disease (stage III disease).[1-3] At present gemcitabine and paclitaxel (Abraxane) are the best chemotherapeutic agents used for treatment of pancreatic cancer, however, patients develop drug resistance over the time. Thus, new alternative strategies, involving less toxic agents have been considered for local treatment of pancreatic cancer.[4-6] It is of note that chemotherapeutic

TABLE 1. Electrochemotherapy treatment in pancreatic cancer cell lines

Reference	Cell lines	Drugs	IC50 (P value)	Methods	EP parameters
Girelli et al., 2015[17]	PANC1 MiaPaCa2	Bleomycin Cisplatin	< 0.0001 ≤ 0.0001	MTS assay FACS	8 pulses, 100 µs of duration, 5 Hz
Sundararajan, 2014[26]	PANC1 PANC28	Gemcitabine	< 0.0001 ≤ 0.0001	MTS assay	high intensity, low duration (microseconds) pulses; low intensity and long duration pulses (milliseconds).

resistance in pancreatic cancer is associated with low penetration of drugs into tumour cells due to the presence of fibrotic stroma.

Reversible electroporation (EP) is a physical method used to overcome the barrier of the cell membranes by applying short and intense electric field, depending by cell characteristics as shape, size, and cytoskeleton structure and membrane composition. Applications of these pulses create rapid voltage changes that usually reach values between 0.5-1 V. This local polarization, deforms mechanically the membrane by forming a hydrophilic pore with a radius of ≈ 1 nm. It was hypothesized, that primary hydrophobic pores induced the transition to hydrophilic based precisely on a strong and nonlinear local transmembrane voltage. This treatment enables access for extracellular agents into the cells. For this reason EP may be combined with chemotherapeutic agents to improve their uptake, in particular hydrophilic drugs that, differently from lipophilic, are poorly or not permeant; this new cancer treatment modality is named electrochemotherapy (ECT).[7-10] The use of ECT for tumour treatments leads to a local potentiation of chemotherapy by reducing the doses of the drugs, minimizing the side effects, and increasing the efficacy of chemotherapy. In the perspective of pancreatic tumour treatment with ECT, several pre-clinical and clinical studies have been performed. This review will summarize the published papers reported in literature on the efficacy and safety of ECT in preclinical and clinical studies on pancreatic cancer.[11-17]

ECT in treatment of pancreatic cancer: *in vitro* studies

Several studies have been conducted in order to identify the optimal electric field characteristics (amplitude, duration and number of electric pulses and repetition frequency) and the appropriate antineoplastic drugs whose cytotoxicity could be increased by combination with EP.[18,19]

MTT assay allowing to define cellular cytotoxicity, is the method commonly used to screen drugs for feasibility and safety on both electro-permeabilized and not electro-permeabilized cells. It has been shown that, cell death due to the EP procedure was less than 4%, and that more than 90% of cells were permeabilized. Among several substances evaluated in association to EP some of them, such as daunorubicin, doxorubicin, etoposide and paclitaxel, did no show increased cytotoxicity. For carboplatin and cisplatin (CDDP) the efficacy of EP was indexed with a factor 3 and 2.3, respectively, on the IC50 (inhibitory concentration 50%), while bleomycin (BLM) was indexed with a value of 300.[20-22] These data suggest that BLM, by inducing apoptosis of several cancer cell lines, is considered the drug of choice for ECT, while the use of CDDP still remains to be fully explored.[23, 24]

Few studies have been performed on the use of ECT in pancreatic cancer treatment, but the emerging results are very encouraging. In particular, in a recent publication, were reported the feasibility and the safety of ECT for the treatment of pancreatic ductal adenocarcinoma (PDAC), a highly aggressive disease which normally is diagnosed in advanced stage. In this study it was demonstrated that EP represents a safe procedure in the treatment of PDAC and that can potentiate the effect on cytotoxicity of bleomycin and cisplatin in pancreatic tumour cell lines, PANC-1 and Miapaca-2 (Table 1).[17]

New applications of ECT protocol on pancreatic cancer cells have been developed. These techniques combined the use of natural compounds and chemotherapeutics drugs to ECT procedure, in order to reduce cytotoxic drug effects. In particular, two different studies have demonstrated that nanocurcumin, a polymeric nanoparticle-encapsulated curcumin, has better efficacy on pancreatic or breast tumour cell lines respect to normal curcumin and it is able to activate the same molecular pathways.[25,26] Another natural compound that could be used in association with ECT is the epi-

gallocatechin-3-gallate (EGCG), the most abundant catechin found in green tea, in particular combined with BLM. Recently, we showed the efficacy and synergism of EGCG and BLM on the inhibition of pancreatic cancer MiaPaCa-2 cell proliferation by inducing apoptosis.[27]

Several studies are ongoing in our laboratory, to demonstrate that ECT treatment can potentiate the efficacy and synergism of EGCG and BLM in pancreatic cancer cells and in pancreatic cancer mouse model.

Taken together, these studies suggest that ECT could be considered as a valid technique for treatment of pancreatic cancer, although more studies will be needed in order to refine the ECT protocols.

Electrochemotherapy in pancreatic cancer: *in vivo* animal models

It is has been reported that (EP), has been used for different types of clinical applications in treatment of cancer: irreversible (IRE) or reversible electroporation combined with chemotherapeutic agents (ECT). IRE is an ablative non-thermal technique which uses a high voltage (maximum 3,000 V) small microsecond pulse lengths (70 to 90 μs) to induce cell membrane permeability which leads to slow/protracted cell death over time. Pre-clinical data supporting both the safety and effectiveness of IRE in treatment of pancreatic cancer have been published. In particular, Bower *et al*. performed an in *vivo* study demonstrating no adverse events of IRE around the portal veins in a large porcine animal model. All pigs have been exposed to a pulse field generated with maximum 3,000 V for 70-90 μs and revealed only mild adhesions, no ascites, and no pancreatic necrosis. This study demonstrates that IRE protocol of the pancreas performed at an optimal voltage is well tolerated, with rapid resolution of pancreatic inflammation and preservation of vascular structures.[28] Similar results were confirmed by Charpentier *et al*. who generated an acute animal model (2 hours survival) and also demonstrated no vascular thrombosis as well as effectiveness with complete ablation in pancreas and liver.[29-31] Another group, showed the feasibility of IRE against pancreatic ductal adenocarcinoma (PDAC). This study demonstrated that IRE treatment had significant antitumour effects and prolonged survival in mice with orthotopic xenografts. Extensive tumour necrosis, reduced tumour cell proliferation and disruption of microvessels, were observed at different days post-IRE.[32] Recently, it has been demonstrated the efficacy of irreversible electroporation in human pancreatic adenocarcinoma by using heterotopic murine model. In this paper, authors optimized IRE parameters and evaluated the effects of IRE on surrounding tissues, recurrence, and biomarker expression changes in recurrent/incompletely electroporated mice tumours.[33,34]

In alternative to use of IRE in treatment of cancer, some pre-clinical studies using different animal models have been performed to investigate the local and systemic effects of ECT in cancer. Sersa *et al.*, in mice models of murine fibrosarcoma SA-1 treated with bleomycin-ECT, described mice tumour destruction due to the immune system activity highly stimulated by ECT, an increased apoptosis of endothelial cells surrounding the tumour, and a reduction of blood flow in the vessels supplying the lesion.[35] Roux *et al.*, by analyzing two tumour mouse models (sarcoma and melanoma) treated with bleomycin-ECT, have demonstrated an increase of local T-dependent response due to a massive recruitment of CD11c and CD11b positive cells in the tumours depending on tumour-associated antigen (TAA) release.[36] However, the parameters and safety of ECT are well calibrated for the treatment of cutaneous and subcutaneous lesions, but not for deep-seated tumours as the pancreatic cancer. So, in order to perform a standardization of ECT protocol for pancreatic tumours, other studies on animal models will be needed. One of the first works on the use of ECT in pre-clinical pancreatic cancer treatment was published in 1998 by Nanda *et al.* In this study, human pancreatic tumours (Pan-4-JCK) implanted subcutaneously in nude mice, were treated with ECT using BLM, mitomycin C or carboplatin. Tumours were monitored for a period of 89 days after the therapy and showed a significant regression (Table 2).[12] Similar results were obtained in another study in which nude mice, xenografted with pancreatic adenocarcinoma cells, were subjected to a different scheme of EP with new electrodes for drugs (doxorubicin, fluorouracil or cisplatin) delivery. Tumour growth analysis performed after 28 days of ECT treatment, revealed a significant regression (Table 2).[37] Another study investigated the use of electrically mediated drug delivery for the treatment of pancreatic adenocarcinoma in a hamster model. Authors showed that treatment of subcutaneous tumours with bleomycin and electric fields resulted in a 100% complete response rate while treatment of tumours induced in the gland, resulted in a 25% complete response rate (Table 2).[13]

TABLE 2. Electrochemotherapy (ECT) in animal models of pancreatic cancer

Reference	Animal models	Cell lines	Methods	Drugs	Effects
Nanda et al., 1998[12]	Nude mice	Pan-4JCK	ECT	Bleomycin Carboplatin Mitomicin C	Tumour regression after 89 days
Dev et al., 2000[37]	Nude mice	BxPc3	ECT	Cisplatin Doxorubicin Fluruoracil	Tumour regression after 28 days
Jaroszeski et al., 1999[13]	Golden Syrian hamster	PC-1	ECT	Bleomycin	100% complete response rate in subcutaneous tumours, 25% response rate in orthotopic tumours

As reported previously, recently Girelli *et al.* have demonstrated the feasibility and the safety of ECT for the treatment of pancreatic ductal adenocarcinoma. In this study, New Zealand non pathological rabbits were subjected to open surgery EP of pancreas and duodenum, according to the ESOPE pulse protocol. Neither systemic nor local toxic effects due to the electroporation procedure were observed, demonstrating the safety of the optimized electric parameters in the treatment of the pancreas *in vivo*.[17]

Taken together these studies suggest that ECT can be used for the local control of non-resectable pancreatic cancer adenocarcinoma (PDAC).

Electrochemotherapy in clinical studies of pancreatic cancer

Recently, different experiences showed the clinical approach of ECT for the treatment of deep-seated tumours as pancreatic cancer diseases[15,38] and liver metastases from colorectal cancer.[39] Specifically for pancreatic cancer, a clinical phase I/II study on patients with locally advanced disease, is ongoing at the National Cancer Institute, "*G. Pascale Foundation*" of Naples.[38] Patients are enrolled in this study by using the following inclusion criteria: age between 18 and 80 years; good mental health; life expectancy ≥ 3 months; diagnosis of pancreatic adenocarcinoma or pancreatic neuroendocrine tumours, confirmed by histological analysis; locally advanced disease [stage III]. In this study, were not included patients with one or more of the following conditions: pregnancy positive test for women, significant heart disease, coagulation disturbances, and allergy to bleomycin, lung and kidney dysfunction, concomitant presence of distant metastases. It is important to underline that all patients received systemic chemotherapy (GEMOX or FOLFIRINOX). Subsequently, to choose the patients suitable to receive ECT treatment, were performed clinical and radiological examinations (CT, MRI and PET). By using functional MRI parameters, it was observed a significant reduction of viable tumour tissue in ECT treated target area. Results from PET analysis, indicated that the uptake of ^{18}FDG during post-operative PET examination was lower in respect to pre-operative evaluations. No serious side effects for the patients were observed. In addition, pain reduction of patients (evaluated by VAS-score) was reported immediately after the ECT treatment compared to pre-operative status. Preliminary data on feasibility and safety of the ECT treatment on patients with locally advanced cancer were reported by Granata et *al.*[15] For a significant number of patients, a reduced diameter and tumourigenicity of the lesions associated with good clinical parameters were reported.

These data suggest that ECT can be safely performed in locally advanced pancreatic tumours.

ECT *vs* IRE in treatment of patients with unresectable pancreatic cancer

It is of note that multi-modality therapy, including chemotherapy, surgery and/or radiation therapy represent the optimal treatment option for patients with pancreatic adenocarcinoma especially stage II disease. Since the incidence of more advanced staged disease (stage III and stage IV) is becoming higher over the time, only a small percentage of patients who are diagnosed with pancreatic adenocarcinoma are eligible for definitive surgical resection. Due to this high incidence, alternative techniques have been developed in order to improve quality-of-life especially in patients with stage III pancreatic adenocarcinoma. Radiofrequency ablation (RFA) has been studied as possible therapy centered on thermal techniques, but the reported morbidity rates were high in the majority of these

TABLE 3. Clinical studies on irreversible electroporation (IRE) in pancreatic cancer

Reference	No. of patients	Stage of pancreatic cancer	Results
Bagla *et al.*, 2012[54]	78	Stage III	No residual disease and a decreasing cancer antigen 19-9 level.
Mansson *et al.*, 2014[55]	5		No serious treatment-related adverse events were observed.
Paiella *et al.*,2015[56]	10	Stage III	Overall survival of 7.5 months
Martin *et al*, 2013[57]	54	Stage III	Improvement in local progression-free survival (14 vs. 6 months, p = 0.01), distant progression-free survival (15 vs. 9 months, p = 0.02), and overall survival (20 vs. 13 months, p = 0.03).
Martin *et al*, 2014[58]	48	Stage III	No significant vascular complications were seen, and of the high-grade complications, bleeding (2), biliary complications (3) and DVT/PE (3) were the most common.

DVT/PE = deep vein thrombosis and pulmonary embolism

published studies.[40-44] In addition, anatomy of pancreas represents a significant obstacle to other thermal ablation techniques including cryoablation, high intensity focal ultrasonography, and MWA which to date have not been as well studied as RFA. To bypass the problems relative to thermal techniques, irreversible electroporation (IRE) has been introduced to treat pancreatic cancer, since it does not use thermal energy and does not damage blood vessels and bile ducts.[45-49] Recent studies have demonstrated the safety and palliation with encouraging improvement in overall survival. It has also been demonstrated that for patients with LAPC (stage III), the addition of IRE to conventional chemotherapy and radiation therapy results in substantially prolonged survival compared with historical controls.[50] Table 3 summarizes clinical studies on IRE in pancreatic cancer.

It is important to underline, that recent studies have shown that a small area of thermal effect of IRE is likely present immediately adjacent to the probe.[51] In addition, treating deep seated tumours either during open surgery or percutaneously in liver or other organs due to high voltage (up to 3000 V) and consequently high currents (up to 50 A) delivered pulses could potentially interfere with cardiac activity.[52] Moreover, one limitation of IRE remains tissue heterogeneity and the unique settings based on tumour histology and prior induction therapy. For this reason, based on our knowledge, IRE could not be considered a standard-of-care practice for treatment of locally advanced pancreatic cancer. As previously described, preliminary studies indicate that ECT represents a feasible and safe treatment modality in patients with locally advanced pancreatic adenocarcinoma. Differently from IRE, ECT protocols for pancreatic cancer uses a lower voltage and lower currents of delivered pulses. In this way the risk of interference with cardiac activity of patients is lower than those induced by IRE protocols. A recent observational study on the effects of ECT in colorectal liver metastases treatment, demonstrated that in patients after ECT treatment, were found in ECG signals recorded during early post-operative care, no major changes in functioning of the heart or pathological morphological changes.[53]

Conclusions

IRE and ECT represent new non-thermal techniques with high interest in treatment of locally advanced pancreatic cancer. IRE applies a higher voltage leading to cell death by apoptosis rather than necrosis. Despite the exact mechanism by which IRE induces apoptosis is still unclear, it seems to induce permanent nanopore formation and consequent ion disruption. As previously reported, although IRE is known as non-thermal technique, studies provided evidence that induces a small area of thermal effect near the probe. One complication for patients treated with IRE is a significant musculature contraction as consequence of high voltage induced. On the other hand, ECT protocol for pancreatic cancer uses a lower voltage and lower currents of delivered pulses. No side effects or major complications have been recorded for ECT treatment of patients with pancreatic cancer, although clinical studies need to be improved.

Taken together, these data suggest that IRE and ECT are promising techniques for treatment of pancreatic cancer, although both require more investigation in the future.

Authors' contributions

BS and LM performed preparation of the manuscript; BA, DV, FM performed experimental support; AV, PM, AA, DR, NA, GV, PA, SL, SS, FR performed bibliographic research. AN and IF were responsible for coordination of this study. All authors read and approved the final manuscript.

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research article

Effectiveness of electrochemotherapy after IFN-α adjuvant therapy of melanoma patients

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Background. The combination of electrochemotherapy with immuno-modulatory treatments has already been explored and proven effective. However, the role of interferon alpha (IFN-a) adjuvant therapy of melanoma patients and implication on electrochemotherapy effectiveness has not been explored yet. Therefore, the aim of the study was to retrospectively evaluate the effectiveness and safety of electrochemotherapy after the previous adjuvant treatment with IFN-a in melanoma patients.

Patients and methods. The study was a retrospective single-center observational analysis of the patients with advanced melanoma, treated with electrochemotherapy after previous IFN-a adjuvant therapy. Five patients, treated between January 2008 and December 2014, were included into the study, regardless of the time point of IFN-a adjuvant therapy.

Results. Electrochemotherapy of recurrent melanoma after the IFN-a adjuvant therapy proved to be a safe and effective treatment. Patients with one or two metastases responded completely. Among patients with multiple metastases, there was a variable response rate. In one patient all 23 metastases responded completely, in second patient more than 85% of all together 80 metastases responded completely and in third patient all 5 metastases had partial response. Taking into account all metastases from all patients together there was an 85% complete response rate.

Conclusions. The study showed that electrochemotherapy of recurrent melanoma after the IFN-a adjuvant therapy is a safe and effective treatment modality, which results in a high complete response rate, not only in single metastasis, but also in multiple metastases. The high complete response rate might be due to an IFN-a immune-editing effect, however, further studies with a larger number of patients are needed to support this presumption.

Key words: electrochemotherapy; melanoma; IFN-a

Introduction

Melanoma is, due to the high risk of metastases development and its resistance to different treatment strategies, still the most lethal skin cancer. The most effective treatment for primary melanoma of stage I and II is its removal by radical surgical excision with the associated safety margin, usually followed by a sentinel lymph node biopsy. Although this approach is curative in many cases, relapse with disseminated disease occurs in some patients. Therefore, in patients with an increased risk for recurrent disease adjuvant immunotherapy might be applied.[1,2]

The sole recognized postsurgical adjuvant therapy still is interferon alpha (IFN-α). In several clinical studies both interferon types, the high-dose interferon alpha-2b (IFN-α2b) and low-dose interferon alpha-2a (IFN-α2a) were shown to have significant effect on progression-free survival.[3-5] Recent meta-analysis showed statistically significant improvement in disease free survival and overall survival in patients with high risk melanoma (stage IIb-IIIc according to The American

Joint Committee on Cancer [AJCC] TNM Cancer Staging Manual 7th edition[6]) treated with adjuvant IFN-α after surgery.[7,8]

When melanoma recurs, other treatment modalities are needed for local or systemic control of the disease. Most commonly used are systemic chemotherapy with dacarbazine, irradiation, isolated limb perfusion or electrochemotherapy and in recent years also new targeted therapies with BRAF and/or MEK inhibitors and antibodies against CTLA-4.[9,10]

Electrochemotherapy is one of the treatment modalities for local treatment of malignant melanoma, which is using electroporation as a delivery system for the chemotherapeutic drugs bleomycin or cisplatin into the tumor.[11-14] Under the high external electric field the plasma membrane becomes permeable, thus facilitating drug delivery into the tumors. Numerous clinical studies have demonstrated the effectiveness of electrochemotherapy on a variety of cutaneous and also deep seated tumors, such as liver metastases of colorectal cancer.[15] Among cutaneous tumors, electrochemotherapy is very effective in treatment of melanoma, with a complete response rate after a single treatment of 74%.[16] Some studies have recently reported on beneficial effect on melanoma treatment after combining electrochemotherapy with new targeted therapies such as dabrafenib or ipilimumab.[17,18] Furthermore, electrogene therapy with plasmids coding for interleukin-12 (IL-12) or antiangiogenic molecules, is also in clinical testing for melanoma treatment.[19-22]

In preclinical studies, it was shown that adjuvant therapy with TNF-α, IL-2, IL-12 and CpG oligonucleotides might boost electrochemotherapy response.[23-30] However, the role of adjuvant IFN-α has not been explored yet, neither on preclinical or clinical level. Therefore, the aim of the study was to evaluate the safety and effectiveness of electrochemotherapy on recurrent melanoma after IFN-α adjuvant therapy of melanoma patients.

Patients and methods

Study design and patient selection

The study was conducted as a retrospective single-center analysis of patients with advanced malignant melanoma treated with electrochemotherapy, who previously received IFN-α adjuvant therapy, after surgery of primary melanoma. All the patient files where electrochemotherapy was performed in the last 6 years, between January 2008 and December 2014, were reviewed regardless of the time point of IFN-α adjuvant therapy. Among all the (50) patients treated with electrochemotherapy in that time period only 5 of them met the requirements and were further investigated. The trial was approved by an Institutional Review Board and National Medical Ethic Committee 97/06/02. The patients signed the informed consent before the treatment.

Investigated entities

First the patient's general characteristics (gender, age), the site and TNM, pathological stage (AJCC TNM Cancer Staging Manual, 7th edition) and Breslow thickness of primary melanoma were recorded. Than therapeutic dose and duration of IFN-α adjuvant therapy were recorded for each patient. The treatment free-interval between the end of IFN-α adjuvant therapy and electrochemotherapy treatment was calculated. The site and number of melanoma nodules treated with electrochemotherapy were further recorded and afterwards effectiveness of electrochemotherapy was evaluated.

IFN-α adjuvant therapy

All five patients received IFN-α as a post-surgical adjuvant therapy. Patient 1 and 2 received low-dose IFN-α since the IFN-α adjuvant therapy was administered before 2010, when new guidelines for melanoma treatment at the Institute of Oncology Ljubljana were accepted. Patient 1 and 2 received Roferon-A® (interferon Alfa-2a) (Roche, Basel, Switzerland) subcutaneously at a dose of 3 or 6 million IU three times a week (Table 2) for 33 and 7 months, respectively, according to the instructions of an oncologist. Patient 3, 4 and 5 received high dose interferon Intron® A (interferon a- alpha 2b) (Merck, Kenilworth, New Jersey, USA) according to the Kirkwood scheme.[31] The exact schedule and the dose was adjusted for each patient by his oncologist (Table 2).

Electrochemotherapy

Patients were treated according to the Standard Operating Procedure (SOP) for electrochemotherapy.[16] Briefly, electrochemotherapy of cutaneous melanoma nodules was performed using either intravenous bleomycin (Bleomedac, Medac, Wedel, Germany) in a dose of 15,000 IU/m^2 or intratumoral cisplatin (Cisplatin, Medac) injection in a concentration of 2 mg/ml and dose is applied according

TABLE 1. Patients' characteristics

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Gender	Male	Male	Female	Female	Female
Birth year	1958	1935	1937	1953	1941
TNM*	T3aN0M0	T2aN0M0	T4bN1aM0	T3aN1aM0	T4bN1aM0
Patological stage*	Stage IIa	Stage Ib	Stage IIIb	Stage IIIa	Stage IIIb
Breslow	4 mm	1.5 mm	10 mm	2.8 mm	9.5 mm
Ulceration	/	/	10 mm	/	2.5 mm
Localisation of primary tumor	Right lower leg	Left foot	Right foot	Right lower leg	Back
Localisation of metastases treated with ECT	Right lower leg	Left foot (dorsum)	Left lower leg	Right lower leg	Breast, left side

* according to AJCC TNM Cancer Staging Manual 7 th edition (2010)[6]; ECT = electrochemotherapy

to ESOPE protocol.[16] Standard pulse parameters for electrochemotherapy (voltage to distance ratio 1300 V/cm, 8 pulses, 100 μs, 5000 Hz) were used.[16] Electric pulses were generated by Cliniporator pulse generator (IGEA, s.r.l., Carpi, Italy) and delivered by parallel stainless steel plate electrodes with 6 or 8 mm distance in between. Electric pulses were applied to the tumors nodules in a way so as to cover the whole tumor area, including the safety margin.

Response assessment

Antitumor efficacy was evaluated 4 weeks after electrochemotherapy, patient were then monitored monthly. Treatment response was defined either as complete response (CR), when the tumor was not palpable, partial response (PR), when the tumor decreased more than 50% of the measurable lesions; no change (NC), when tumor decreased less than 50% or increased up to 25%, or progressive disease (PD), when tumor increased for more than 25%. Determination was based on criteria of WHO Handbook for Reporting Results of Cancer Treatment where for all response definitions minimum 4-week duration was required for qualifying the response.

Results

Patients' characteristics

Only 5 patients fulfilled all the requirements for inclusion into this retrospective study. Among them there were two male and three female patients with a median age of electrochemotherapy treatment 71 years (range from 50–76 years). TNM and pathological stage were recorded for all five patients, as well as Breslow thickness (Table 1). The localizations of primary tumors were on the upper leg, foot or the back (Table 1). All the patients were identified as patients with high risk of recurrence and were therefore assigned for IFN-α adjuvant therapy. The relapse of melanoma occurred in all five patients. Recurrence time was variable among patients; from a few months to a few years (Table 1). Other comorbidities were also recorded for all 5 patient; only patient 2 had arterial hypertension and no other comorbidities were recorded.

IFN-α adjuvant therapy and disease progression

Adjuvant therapy with IFN-α in our investigated patients can be divided into two subtypes; low-dose treatment for patients 1 and 2 and high dose treatment for another three patients (Table 2). After the completed adjuvant therapy with IFN-α, in all five patients disease had progressed to a metastatic disease. Disease free interval, progression of the disease and treatment procedures vary for each patient (Table 2). Based on the decision of an institutional committee for melanoma treatment, electrochemotherapy was offered to the patients as another treatment option after several surgical excisions and in patient 1 and 2 also irradiation.

- In *patient 1* inguinal lymph node metastasis occurred after a disease free interval and inguinal and retroperitoneal lymph node dissection was performed thereafter. The patient was irradiated as well. When progression of the disease occurred 3 years later, two metastases were excised within 2 months and the patient was irradiated again. In 2 months new metastases,

TABLE 2. Treatment regime

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
INF-a dose	6 million IU 3 x weekly (s.c.)	3 million IU 3 x weekly (s.c.)	35 milion IU 20 x (i.v.) in 8 weeks/ 15 million IU 3x weekly (s.c.) for 13 weeks/ 10 milion IU 3 x weekly (s.c.) for 13 weeks*	30 milion IU 20 x (i.v.) in 4 weeks followed by 15 million IU 3x weekly (s.c.) for 35 weeks followd by 10 milion IU 3 x weekly (s.c.) for 12 weeks	30 milion IU 9 x (i.v.) in 2 weeks followed by 20 milion IU 11x (i.v.) in 3 weeks followed by 15 million IU 3x weekly (s.c.) for 13 weeks**
INF-a treatment period	2 years 9 months	7 months	8 months	12 months	4 months
Last date of INF-a treatment	March 2004	August 1998	July 2012	January 2012	June 2011
Disease free interval	4 months	5 years 6 months	6 months	3 months	2 months
Surgically excised metastases+	Yes	Yes	No	Yes	Yes
Interval between INF-a and ECT treatment	4 years 8 months	12 years 11 months	7 months	6 months	7 months
ECT treatment	November 2008	July 2011	February 2013	July 2013	January 2012
ECT drug	Cisplatin	Cisplatin	Bleomycin	Bleomycin	Bleomycin
Number of lesions	2	1	80	23	5
Size of the nodules	1.0 x 1.5 cm 1.5 x 1.5 cm	3 x 3 cm	0.3–1 cm	0.1–0.8 cm	0.7–1.5 cm
Effect after 4 weeks	CR	CR	> 85% CR	All CR	100% PR
LRD* or date of death (D)**	April 2010 (LRD)	December 2014 (LRD)	April 2014 (LRD)	December 2013 (D)	March 2013 (D)

CR = complete response; ECT = electrochemotherapy; i.v. = intravenous; PR = partial response; s.c. = subcutaneously; s.c = subcutaneous; *prematurelly terminated treatment due to ineffective treatment; ** intravenous dose (i.v. dose) was decreased to 20 million IU due to pathological liver tests - prematurelly terminated treatment due to side effects, *** after last record date at the Institute of Oncology Ljubljana patients were given only paliative care at their regional centers, +details on localization and number of excised metastates in paragraph Pacients' caracteristics.

which were treated with electrochemotherapy, occurred.

- In *patient 2* after a disease free interval a metastasis on the limb occurred and was immediately excised. Four years later, another excision with following inguinal dissection and irradiation was performed. In the following 2 years three in-transit metastases were excised from the dorsum of the left foot. A new metastasis occurred 1 year after the last in-transit metastasis excision, which was then treated with electrochemotherapy.
- Disease free interval for the *patient 3* was 6 months; thereafter multiple metastases occurred on the limb and were treated with electrochemotherapy.
- In *patient 4* a metastasis occurred on the scar of a primary excised melanoma only 3 months after completing adjuvant therapy with IFN-α. It was immediately excised, although PET/CT scan and thin needle biopsy showed multiple metastases in the same area, which were then treated with electrochemotherapy.
- In *patient 5* the disease-free interval was 2 months, followed by two excisions of metastases and shortly afterwards electrochemotherapy of 5 metastases on the trunk. Due to partial response of all 5 metastases, these metastases and newly formed metastases on the trunk were again treated with electrochemotherapy 6 weeks after the first treatment.

Electrochemotherapy following IFN-α adjuvant therapy

At the time of electrochemotherapy patient 2 was presented with a single metastasis on the limb, whereas patients 1, 3, 4 and 5 were presented with multiple metastases on the limb (patient 1, 3 and 4) or trunk (patient 5). All metastases present at the time of electrochemotherapy were treated. Electrochemotherapy was effective in all five patients, with a variable response rate (Table 2).

In patient 1 and 2 cisplatin was given intratumoraly due to previous irradiation of the patients. In some studies, it was reported that previous irradiation can cause lower effectiveness of i.v. electrochemotherapy.[32] Fibrosis can be one of the causes for lower effectiveness. Less fluid in the tissue results in less lymphatic infiltration and also lower

current in the nodule and can therefore contribute to the lower effectiveness of electrochemotherapy of pre-iradiated tissues. Intratumoral injection of chemotherapeutic drug can overcome those obstacles and can results in higher effectiveness.

Electrochemotherapy following IFN-α adjuvant therapy was effective treatment modality, regardless of drug used for electrochemotherapy, bleomycin or cisplatin. Single metastasis responded completely, while multiple metastases had a variable response rate. In patient 4 all 23 metastases responded completely, in patient 3 more than 85% of all together 80 metastases responded completely and in patient 5 all 5 metastases had partial response. Taking into account all metastases from all patients together there was an 85% complete response rate. After electrochemotherapy no side effects, such as local erythema, bleeding, infection on the site of electrochemotherapy, or muscle contractions, were reported. Nevertheless new metastases mostly occurred within 1 month (patients 1, 3 and 5) or 2 months (patient 4) after the treatment. In patient 2, with a single metastasis at the time of electrochemotherapy, new metastases occurred after 1 year and 10 months. In patient 1 additional electrochemotherapy of 14 new lesions was performed thereafter, which also resulted in 100% complete response. Nevertheless the disease progressed and although systemic chemotherapy with dacarbazine was administered, new distant metastases in the head and neck region occurred. Electrochemotherapy was then used for palliative care. In patient 2 disease also progressed and due to several metastases, isolated limb perfusion was performed 4 years after electrochemotherapy. No new metastasis occurred yet. Dacarbazine and later also ipilimumab were prescribed for patient 3 with progressive metastatic disease, with metastases present also in liver and lungs. In patient 4 new subcutaneous metastases were effectively irradiated and year after brain metastasis occurred. Electrochemotherapy response in all those patients (patient 1–4) remains the same during the whole observational period. Due to partial response of all 5 metastases in patient 5, these metastases and 12 newly formed metastases on the trunk were again treated with electrochemotherapy, 6 weeks after the first treatment. After the second electrochemotherapy treatment all 17 metastases (including 5 retreated metastases) responded completely. The patient was later treated with dacarbazine and vemurafenib due to soft tissue and lung metastases, but disease progressed with new metastases in lungs and brain.

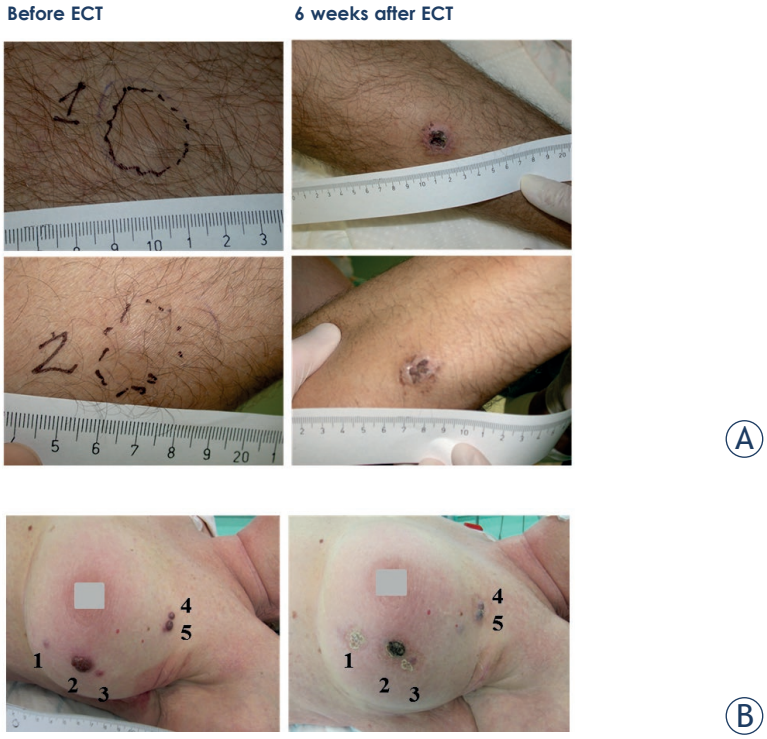

FIGURE 1. Melanoma lesions in patient 1 **(A)** and patient 5 **(B)** before and 6 weeks after electrochemotherapy (ECT). Both metastases in patient 1 responded completely, while in patient 5 although the crust is seen on the images there was nodule under it and response was evaluated as partial response.

Discussion

This is the first study to our knowledge which discusses the effectiveness of electrochemotherapy after IFN-α adjuvant therapy for treatment of melanoma metastases.

In recent years electrochemotherapy has been widely used in clinical studies for treatment of cutaneous and also deep seated tumors. Among skin cancers electrochemotherapy was very effective in the treatment of malignant melanoma, with a complete response rate after single treatment 74%, according to ESOPE study.[16] Although the electrochemotherapy response rate is quite high, and effective on most of tumor histologies, recently there is some evidence that there is a variability in the response rates of different tumor histologies. The meta-analysis by Mali *et al.* has shown that melanoma tumors are less responsive than non-melanoma tumors. The effectiveness of electrochemotherapy was tumor type dependent, namely the complete response rate was 57% of melanoma tumors and 67% of other, non-melanoma tumors. Among carcinomas, basal cell carcinoma was the most responsive tumor type, with up to 89% complete responses.[33]

Furthermore, the importance of the immune response elicited by electrochemotherapy locally was explored.[34,35] Immunogenic cell death of cancer cells was proposed to contribute to the curability of the treated metastases. The concept of immunogenic cell death, which is triggered by some cancer therapies, is initiated by damage-associated molecular patterns, which can further trigger an adaptive immune response against tumors.[34] Some pre-clinical studies have explored the possibility of adjuvant electrogene therapy with plasmid encoding IL-12, which greatly increase the response rate of the electrochemotherapy treated tumors.[30] The recent clinical study, investigating the combined treatment of ipilimumab and electrochemotherapy has shown a better response than ipilimumab alone.[17]

In this report we show that electrochemotherapy was safe and effective also after IFN-α therapy. IFN-α, although given to the patients in different periods before electrochemotherapy may also contribute to the response rate of the electrochemotherapy treated melanoma metastases. Namely, response rate in patients with electrochemotherapy after adjuvant IFN-α was 100% partial response (patient 5) or from 85% to 100% complete response (patient 1, 3 and 4) in patients with multiple metastases, which is an equal or even higher percentage than demonstrated in previous studies; 85% of metastases responded completely in the present study, while the results of meta-analysis showed that 57% of melanoma metastases responded completely.[16,33]

We might speculate that the effectiveness may be increased by the previous immunostimulatory IFN-α adjuvant therapy, which would be reflected in high response rate of the treated tumors. IFN-α is one of the type I interferons, an important interferon family, involved in immune-editing process. Their main importance is the effect on the hematopoietic cells; induction of bystander T cell proliferation, long-term survival and expression of anti-apoptotic genes.[36-37] Furthermore, interferons have also great impact on maturation and differentiation of dendritic cells, cells which are considered to be the most effective antigen presenting cells.[34] Taking all this findings into account, IFN-α might have a significant role in a link between the innate and adaptive immune system. Similarly, new targeted therapy with ipilimumab acts on dendritic cells - cytotoxic T lymphocytes (CTLs) interaction. Dendritic cells are presenting tumor antigens to CTLs, which can then destroy cancer cells. But along with tumor antigens the dendritic cells present also an inhibitory signal, which can bind to a receptor on the CTLs; cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and thereby block the cytotoxicity of CTL. Ipilimumab binds to CTLA-4 and block the inhibitory signal.[38,39]

One of the possible reasons for the very good efficiacy of electrochemotherapy, following IFN-α adjuvant therapy is immune system activation by electrochemotherapy, which was previously modulated by IFN-α.[40] Calvet *et al.* demonstrated in an *in vivo* preclinical study that electrochemotherapy not only has a cytotoxic effect towards cancer cells, but can also generate a systemic anticancer immune response, with imunogenic cell death.[33,40] Dying cancer cells then behave as a therapeutic vaccine, leading to a cytotoxic immune response against remaining tumor cells.[35,42] It was also demonstrated that electrochemotherapy is more effective in immunocompetent mice, causing complete tumor regression, whereas in immunodeficient mice the complete response was not obtained.[35]

The drawback of our study is that we have no data on the immune status of the patients and that this is an observational study. Nevertheless, although this group of patients is small it might indicate on the potential of combining immunostimulatory treatments with electrochemotherapy, which can be explored in different ways. One of the recent ideas is that electrochemotherapy can serve as a vaccination to adjuvant peritumoral immunostimulatory therapy that can boost the local effect as well it may have the systemic effect.[42]

Conclusions

The report demonstrates that combining electrochemotherapy with preceded IFN-α adjuvant therapy is a safe and effective treatment modality, which results in high complete response rate, not only in single metastasis, but also in multiple metastases. The high complete response rate might be due to IFN-α immune-editing effect, however further controlled studies on a larger number of patients are needed to support this presumption.

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research article

A statistical model describing combined irreversible electroporation and electroporation-induced blood-brain barrier disruption

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Disclosure: We declare that Damijan Miclavcic holds patents in the area of electroporation and is consulting for various companies with financial interest in the area of electroporation use in medicine. The other authors declare no competing financial interests.

Background. Electroporation-based therapies such as electrochemotherapy (ECT) and irreversible electroporation (IRE) are emerging as promising tools for treatment of tumors. When applied to the brain, electroporation can also induce transient blood-brain-barrier (BBB) disruption in volumes extending beyond IRE, thus enabling efficient drug penetration. The main objective of this study was to develop a statistical model predicting cell death and BBB disruption induced by electroporation. This model can be used for individual treatment planning.

Material and methods. Cell death and BBB disruption models were developed based on the Peleg-Fermi model in combination with numerical models of the electric field. The model calculates the electric field thresholds for cell kill and BBB disruption and describes the dependence on the number of treatment pulses. The model was validated using in vivo experimental data consisting of rats brains MRIs post electroporation treatments.

Results. Linear regression analysis confirmed that the model described the IRE and BBB disruption volumes as a function of treatment pulses number ($r^2 = 0.79$; $p < 0.008$, $r^2 = 0.91$; $p < 0.001$). The results presented a strong plateau effect as the pulse number increased. The ratio between complete cell death and no cell death thresholds was relatively narrow (between 0.88-0.91) even for small numbers of pulses and depended weakly on the number of pulses. For BBB disruption, the ratio increased with the number of pulses. BBB disruption radii were on average 67% ± 11% larger than IRE volumes.

Conclusions. The statistical model can be used to describe the dependence of treatment-effects on the number of pulses independent of the experimental setup.

Key words: electroporarion; blood brain barrier; Peleg-Fermi

Introduction

Electroporation (EP) is a physical phenomenon in which electric fields make cell membranes transiently permeable to ions and macromolecules which are otherwise deprived of or have limited trans-membrane transport mechanisms.[1-3] Electric pulses applied to the tissue induce an electric field which in turn induces a change in cell membrane potential. This change depends on various tissue related parameters such as tissue type and cell size as well as pulse parameters including pulse ampli-

tude, shape, duration, number of pulses, and pulse repetition frequency. As a function of the induced electrical field, electric pulses can either: reversibly permeabilize the cell membrane (reversible EP) or permeabilize the cell membrane in a manner that leads to cell death (irreversible EP).[4] It was recently demonstrated that when applying EP to brain tissue it also induces reversible disruption of the blood-brain barrier (BBB).[5-7]

Both irreversible EP (IRE), and reversible EP combined with chemotherapy, also known as electrochemotherapy (ECT), are emerging as new treatment techniques for solid tumors.[3,8-16] ECT uses EP to allow increased uptake of chemotherapeutic drugs into tumor cells[12] and IRE is a method aimed at inducing tumor ablation without thermal damage.[17,18] Brain tumors are excellent candidates for local EP treatment. Glioblastoma multiforme (GBM) is the most frequent and most aggressive primary brain tumor with an average survival of 14 months from diagnosis. Existing treatments offer poor prognosis for GBM mainly due to tumor infiltration into the surrounding brain, high resistance to therapeutic apoptotic stimuli and poor BBB penetration of most therapeutic agents.[19,20] A combined approach, consisting of inducing significant/rapid necrosis in the tumor mass and simultaneous delivery of high chemotherapy doses to the tumor and surrounding infiltrating zone is suggested as a treatment strategy. EP-induced tissue necrosis within the massive region of the tumor and surrounding BBB disruption, enabling efficient local delivery of systemically administered chemotherapy was recently demonstrated.[7,21]

Individual treatment planning is an important key for EP-based treatment success.[22] Treatment parameters should be chosen in such a manner that will induce maximal damage to the tumor while sparing surrounding healthy tissue. This is usually done by numerical models. Several numerical models describing the electric filed distribution in the tissue have been introduced, and are applied for predicting treatment outcome and planning the electrodes placement to ensure full tumor coverage by electrical fields higher than the EP threshold.[23-28] These models are usually based on experimental data. Treatment volumes calculated from MRI[29] or histological data[30,31] are incorporated into computerized models together with the organ characteristics and the electrodes configuration. These calculations traditionally use deterministic models, *i.e* all the cells exposed to electrical fields higher than a specific threshold, known in the literature, will be irreversibly/reversibly electroporated. Nevertheless, live tissues are more complex, especially malignant tissues which are inherently inhomogeneous, and therefore assuming a statistical effect of EP parameters maybe more appropriate.[32,33] For this reason we chose to apply a statistical model to describe reversible/irreversible effects *in vivo*.

The Peleg-Fermi model is the most widely used mathematical model for describing cell death as a consequence of IRE in medicine.[32-34] Although several other models have been proposed[35] the Peleg-Fermi model seems the most adequate since it includes dependency on the number of pulses as well as in electrical field. For this reason we decided to apply it on our experimental data and further extend it to irreversible and reversible EP effects *in vivo*.

The Peleg-Fermi statistical model was first introduced as a model describing the survival of bacteria after exposure to pulsed electrical fields.[36] Later on it was suggested that this model can be adapted to describe the effects of IRE.[32,34] Goldberg and Rubinsky[32] extrapolated experimental data obtained using prostate cancer cells and demonstrated the feasibility of applying this model to describe the effects of IRE for up to 10 treatment pulses. Garcia *et al*. extended the model up to 90 pulses, by theoretical analysis that is yet to be confirmed with experimental data.[34]

Treatment parameters such as pulse shape, amplitude, frequency, duration and number of pulses[37,38] affect treatment outcome. Here, we chose to study and model the effect of number of pulses while other pulse parameters remain fixed.

A numerical model describing electric field distribution in the brain tissue based on the applied voltage, tissue and electrodes electrical properties and electrodes configuration was constructed. The calculated electrical field was then implemented in the statistical model that was estimating the effect of the number of pulses on the outcome- irreversible damage and BBB disruption.

The first goal of our study presented below was to extend the Peleg-Fermi model to describe a wider range of the number of treatment pulses *in vivo* and to validate the extended model using experimental data obtained from naïve rats treated with EP in the brain.

The second goal was to adapt the statistical Peleg-Fermi model to describe the effects of pulse parameters on BBB disruption. BBB disruption is a vital key in treating brain tumors since it is important to disrupt a large enough volume surrounding the tumor mass to enable efficient drug penetration

into the infiltrating zone. Once established, models describing both IRE and BBB disruption can be implemented to provide a complete treatment planning for brain tumors with EP.

Materials and methods

Animal experiments

The study was approved by and performed in accordance with the guidelines of The Animal Care and Use Committee of the Sheba Medical Center, which is approved by the Israeli authorities for animal experimentation.

We have recently presented the results of an animal experiment designed to study both IRE and BBB disruption using the same experimental setup.[6,7] Here we describe in detail the aspects relevant to our statistical model which are based on that experimental data. Our unique electrode setup employs a single insulated intracranial needle electrode with an exposed tip placed in the target tissue and an external surface electrode pressed against the skin. The electric field produced by this electrode configuration is highest at the exposed tip of the intracranial electrode tissue interface and then decays with the square of the distance. Therefore, the electric fields surrounding the needle electrode tip induce nearly spherical IRE effects at the target tissue and gradually decrease further away to reversible EP effects which induce BBB disruption. Regions of interest (ROIs) plotted on MR images acquired post EP treatments with various pulse parameters were used for calculating the tissue damage and BBB disruption radii. We then studied the correlation between the experimental radii and the extended statistical model.

Animal model and procedure

The study was performed by treating 46 male Spring Dawly rats with 50 μs monopolar electric pulses at 1 Hz and 600 V, as previously described.[7] The rats were divided into seven groups of 5-7 rats each, treated with varying number of pulses (N = 10, 45, 90, 180, 270, 450 and 540).

MR imaging

Rats were scanned 30 minutes post treatment and periodically thereafter up to 2 weeks post treatment, using a 1.5 T GE Optima MR system (Optima MR450w, General Electric, Milwaukee). The MR sequences included contrast-enhanced T1-weighted MRI for depiction of BBB disruption and T2-weighted MRI for depiction of tissue response. Gradient echo (GE) MRI was acquired to assess possible procedure-related bleeding.

The damage radius induced by IRE (r_d) (in mm) for each rat was calculated from the hyper-intense regions on T2-weighted MR images acquired two weeks post treatment. This time point was previously determined by histology as adequate to describe IRE.[7] BBB disruption radius (r_b), referring to the maximal radius of tissue in which the BBB was breached, was calculated from enhancing regions on contrast-enhanced T1-weighted MR images acquired 30 minutes post EP treatment.

In both cases the radii were calculated by delineating ROIs over the entire enhancing region in each slice (excluding the ventricles). The number of pixels in the ROIs was then counted and multiplied by the volume of a single pixel to receive the ROI volume. The slice thickness was 2 mm and in-plane pixel size was 0.3 X 0.3 mm. Radii of each slice was then extracted by calculating the biggest radius based on the Euclidean distance transform of the corresponding slice. The biggest radii computed over all slices were chosen as IRE radius and BBB disruption radius.

The radii r_d and r_b where then plotted as a function of the number of treatment pulses (N) to determine the dependence of the radii on the number of treatment pulses.

Numerical modeling

The mathematical models were based on a two-dimensional finite element model (assuming spherical symmetry of the produced IRE lesions and BBB disruption) (Figure 1) that was implemented in the COMSOL software package (Comsol Multiphysics, v.4.2a; Stockholm, Sweden) as previously described.[7,34]

The rat head and chest were modeled as a 30 x 15 mm ellipse (Figure 1C) with an initial conductivity of 0.258 S/m to match the conductivity used by Sel *et al*.[37] The electric field was described by the Laplace equation for electric potential distribution in a volume conductor:

$$\nabla \cdot (\sigma(E)\nabla\varphi) = 0 \qquad [1]$$

where σ is the electric conductivity of the tissue, E is the applied electric field and ϕ is the potential. The $\sigma(E)$ dependence of brain tissue was described by an smoothed Heaviside function using 500 V/cm and 700 V/cm as reversible and irreversible

thresholds.[37,39] These values where used traditionally for 90 pulses and are recalculated using the Peleg-Fermi model for the different number of pulses in this paper.

Dirichlet boundary condition was applied to the surface of the electrode:

$$\varphi = \varphi_0 \quad [2]$$

and to the ground

$$\varphi = 0 \quad [3]$$

where ϕ_0 is the applied potential on the intracranial electrode.

The boundaries where the analyzed domain was not in contact with an electrode were treated as electrically isolative and Neumann boundary condition was set to zero on the outer border of the model:

$$\frac{\partial \varphi}{\partial n} = 0 \quad [4]$$

where n denotes the normal to the boundary.

Thermal modeling

Control of the temperature during EP treatments is important in order to avoid damage to unwanted regions. The goal is to achieve complete coverage of the targeted tissue with sufficiently high electric field while ensuring that the temperature increase during the procedure does not generate thermal damage. The thermal effects of EP were determined from solution of the modified Pennes' bioheat equation (equation [5]) in the 2D numerical model with the inclusion of the Joule heating source term. A duty-cycle approach was used, in which a time dependent solver for the duration of the treatment was applied and the thermal dissipation was multiplied by the pulse length.

$$\nabla \cdot (k \nabla T) + w_b c_b (T_a - T) + Q_{met} + q''' = \rho c_p \frac{\partial T}{\partial t} \quad [5]$$

$$Q_m et = \sigma |\nabla \varphi|^2 \quad [6]$$

where k is the thermal conductivity of the tissue, T is the temperature, w_b is the blood perfusion, c_b is the heat capacity of the blood, T_a is the arterial temperature, q''' is the metabolic heat generation, ϱ is the tissue density, c_p is the heat capacity of the tissue and is the local voltage amplitude. Q_{met} accounts for Joule heating, where φ is the electrical potential and σ is the electrical conductivity of the

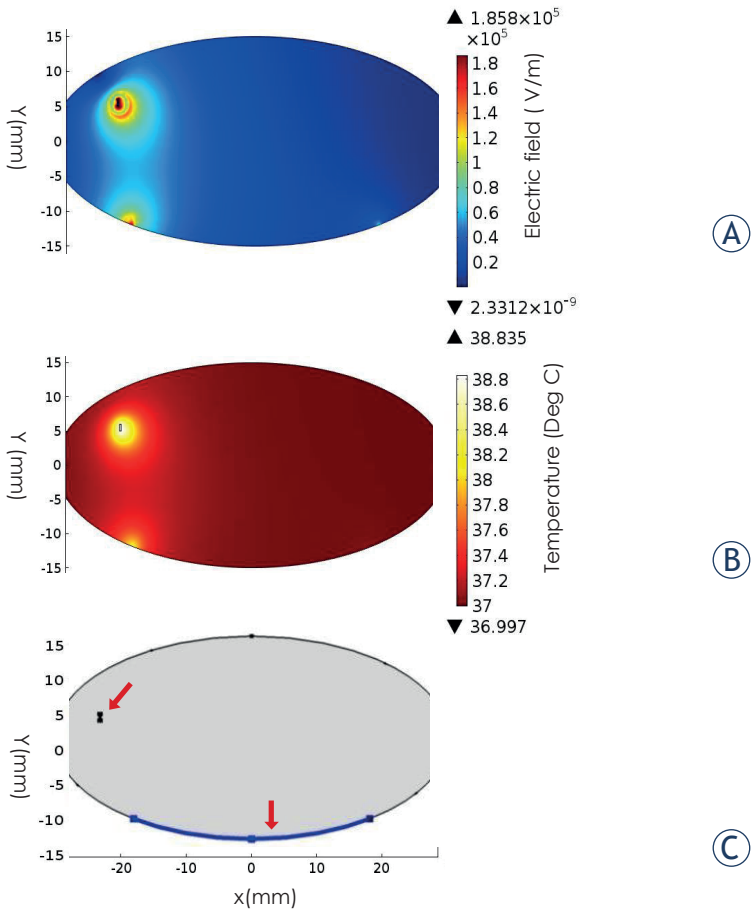

FIGURE 1. Simulation results. **(A)** Electric field distribution in the numerical model. The shape of the field assumes a nearly spherical shape. **(B)** Temperature distribution after 540 pulses. **(C)** Model geometry including the location of the electrodes (red arrows)

tissue. The initial brain temperature was set to 37°C to match human temperature although anesthesized rat temperature is around 32°C. The parameter values utilized in the bioheat equation were taken from the literature[40] and were used by others to follow/measure temperature increase and determine possible thermal damage due to EP treatments.[41,42] All parameters used in the simulations are summarized in Table 1. The thermal properties of the silver plating and copper were taken from the Comsol Multiphysics database.

Statistical modeling

The original Peleg-Fermi model computes the ratio (S) of surviving bacteria after EP. Here we extended this model to describe the effects of EP on brain tissue as following:

TABLE 1. Material properties used for numerical model

Brain	σ - basic conductivity	0.258[S/m]
	k - Thermal conductivity	0.0565[W/(m*K)]
	Cp - Heat capacity	3680 [J/(kg*K)]
	ρ - density	1039 [kg/m^3]
	Q'''- metabolic heat generation	10437 [W/m^3]
	T - temperature	37°C
Blood	*Cp*-heat capacity	3840 [J/(kg*K)]
	ρ density	1060 [kg/m^3]
	Wb-Perfusion rate	7.15E-3 [1/s]
copper	σ - basic conductivity	5.998E7 [S/m]
	k - thermal conductivity	400 [W/(m*K)]
	Cp heat capacity	385 [J/(kg*K)]
	ρ - Density	8700 [kg/m^3]
Silver	σ - basic conductivity	6.273E7 [W/m^3]
	k - thermal conductivity	429 [W/(m*K)]
	Cp heat capacity	234 [J/(kg*K)]
	ρ - Density	10500 [kg/m^3]

First, the model was adapted to predict tissue damage (cell death) probability induced by EP. In the Peleg-Fermi model the probability for cells survival is given by:

$$S(E,N) = \frac{1}{1+\exp\left[\frac{E-E_c(N)}{A(N)}\right]} \quad [7]$$

where E is the electrical field, N is the number of pulses, E_c is the critical electric field in which 50% of the cells are killed and A is a kinetic constant which defines the slope of the curve.

The electric field calculated using the numerical model was exported to Matlab (R2011a, Mathworks, USA) and was implemented in the Peleg-Fermi model.

We have previously shown that the hyperintense regions on T2-weighted MRI obtained 14 days post treatment were significantly correlated with rarified regions in histology, confirming that these regions represent damaged tissues.[7] Based on this, r_d was set as S(E,N) = 0, assuming over 99.99% of the cells were irreversibly electroporated.

An optimization based on A Nelder-Mead simplex algorithm[43] with added constrains was applied to Equation [7] for each group treated with N pulses, calculating a map of S(E), until r(S = 0) matched r_d. The coefficients E_c and A for the different number of pulses were extracted and behavior equations were fitted to $E_c(N)$ and A(N).

For each group treated with N pulses, Electric field distribution (E) was calculated using COMSOL Multiphisics and extracted to Matlab. The map E, along with the equation [7] allows to associate a map of S with any pair of the Fermi distribution (Ec,A). From the S map, the two iso-contours of S = 0.9999 and S = 0.0001 are fitted to circles. An optimization based on A Nelder-Mead simplex algorithm on Ec and A as variables is used to find the (Ec,A) pair of parameters best corresponding to rd/rb.

The process of extracting r from S is nonlinear as it is based on fitting S = 1 iso-contour to a circle. Therefor the global dependency between Ec, A and rb/rd is noisy. This noisiness could potentially cause problems with computation of derivatives. Additionally, since S is monotonous there is no risk of the simplex finding a local minimum.

In order to assess whether the goodness of the $E_{cd}(N)$ and $A_d(N)$ (Ec and A for IRE) fits to the experimental data, r(S = 0) for different number of treatment pulses was calculated and compared to r_d.

Although the Peleg-Fermi model was originally used to describe cell death, here we adapted it to describe BBB disruption as well and calculated the relevant coefficients. For this purpose the model was fitted to the radii calculated from contrast-enhanced T1-weighted MR Images. This time r_b was set as BBB(E,N) = 1, meaning less then 0.001% of the BBB was disrupted in radii larger than r_b.

After determining E_{cb} and A_b (Ec and A for BBB disruption) for each N and the behavior equations $E_c(N)$ and A(N), the goodness of the fit to the experimental data was evaluated by recalculating r(BBB = 1) and fitting it to r_b.

The electrical field threshold for cell kill, *i.e.* IRE extent and for BBB disruption, i.e. reversible EP extent, for different N values were then extracted from the results of the model and compared with thresholds reported in the literature.

Results

MR images of 46 rats that were previously treated with EP as described above were included in the current analysis. Treatment parameters were 600 V, 50 μs pulses at 1 Hz with varying number of treatment pulses from 10 to 540 pulses. The extent of tissue damage and BBB disruption, *i.e.* r_d- the irreversible damage radius and r_b – the BBB disruption radius were calculated from the MR images acquired 30 minutes post treatment and 2 weeks post treatment as described in the Methods section.

Dependence on the number of treatment pulses

The average radius of each treatment group as calculated from the MR images is presented in Table 2.

The dependence of r on N has been previously described by both logarithmic and power functions.[44] Here, by fitting the mean r_d of each treatment group to the number of electric pulses - N, we found the logarithmic function to provide a better fit to the data, resulting in the following dependence of r_d on N:

$$r_d(N) = 0.3267 \cdot \ln(0.8123 \cdot N) \quad [8]$$

$r^2 = 0.84$, $p < 0.005$.

Similarly, by fitting the mean r_b of each treatment group to N, the dependence of r_b on N was found to be:

$$r_b(N) = 0.4213 \cdot \ln(1.535 \cdot N) \quad [9]$$

$r^2 = 0.96$, $p < 0.0001$.

r_d and r_b can be seen in Figure 3. The average ratio between $r_b(N)$and $r_d(N)$ was found to be 1.67 ± 0.11 (s.e.m), confirming the coverage of significant volumes surrounding the IRE with BBB disruption. The small error suggests that the ratio between $r_d(N)$ and $r_b(N)$ is not affected by the number of applied pulses. The ratio between $r_d(N)$ and $r_b(N)$ plotted as a function of the number of treatment pulses supports this observation (Figure 3B). The coefficients of the empirical function for the BBB disruption are higher, because the BBB is disrupted by electric fields lower than those required for IRE ablation.

Irreversible damage model

The coefficients E_{cd} and A_d of equation [7] were calculated for each value of N as shown in Figure 4A-B. In order to find E_{cd} and A_d we used r_d values obtained from equation [8] rather than using the average values obtained from the experiments, as this equation describes the dependence of r_d on the number of treatment pulses based on the experimental data. Although $E_c(N)$ is traditionally described with an exponential function we chose to describe it here using a power function as it fitted the data considerably better (r^2 was considerably larger: 0.89 for the power function versus 0.5 for the exponential function), especially in the high N range. Still, when fitting the optimization results of $E_c(N)$ of only the first 90 pulses to an exponential function, r^2 increased to 0.83 (Figure 4C).

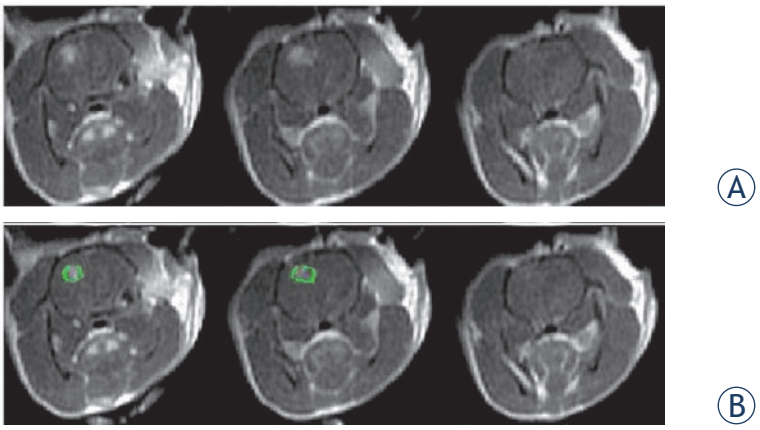

FIGURE 2. MRI example. **(A)** 3 slices of contrast-enhanced T1-weighted MR images of a rat treated with 45 electroporation pulses. The MR images was obtained 30 min post treatment. Each slice is 2mm thick. The enhancing region represents BBB disruption. **(B)** ROI (green) plotted in the MR image to mark the enhancing region.

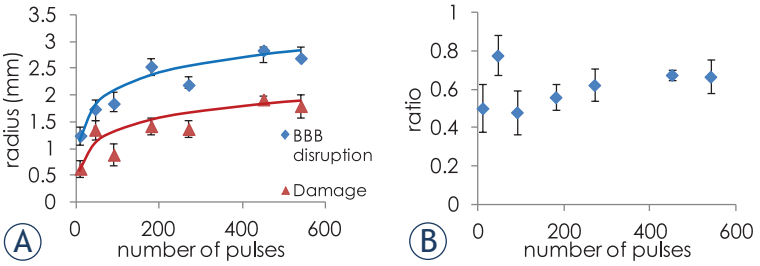

FIGURE 3. **(A)** radii of irreversible damage and BBB disruption calculated from the MRIs, as a function of the number of treatment pulses, and the logarithmic equations fits **(B)** ratio between rb(N) and rd(N) as a function of number of the number of treatment pulses.

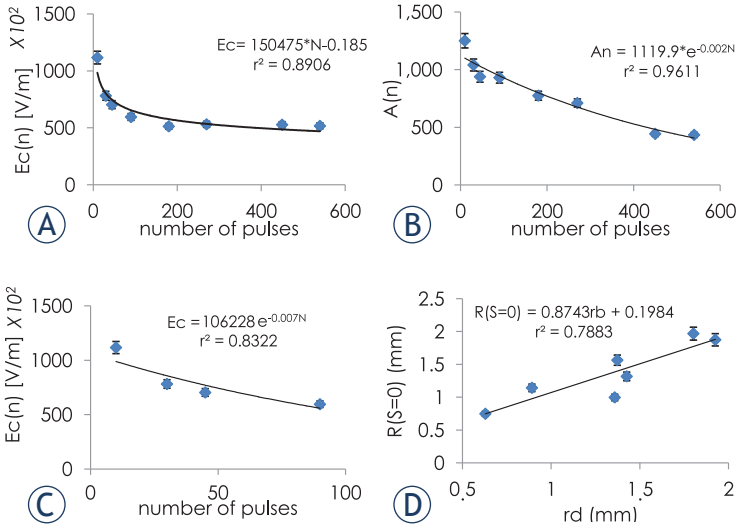

FIGURE 4. Dependence of Ecd **(A)** and Ad **(B)** on the number of treatment pulses. **(C)** Exponential dependence of E_{cd} on the number of treatment pulses with N limited to 90 pulses. **(D)** Correlation between radii obtained from experimental data and radii obtained from the statistical model for IRE. Error bars represent 95% confidence level.

TABLE 2. Average radii of IRE and BBB disruption for each treatment group. Each group of 5-7 rats was treated with different number of pulses (10-540) at 600V, 50μs pulses at 1Hz

# of pulses	10	45	90	180	270	450	540
IRE radius (mm)	0.62 ± 0.15	1.35 ± 0.18	0.89 ± 0.20	1.42 ± 0.15	1.37 ± 0.16	1.92 ± 0.07	1.80 ± 0.21
BBB disruption radius (mm)	1.25 ± 0.06	1.74 ± 0.04	1.84 ± 0.07	2.54 ± 0.15	2.19 ± 0.14	2.84 ± 0.04	2.69 ± 0.12

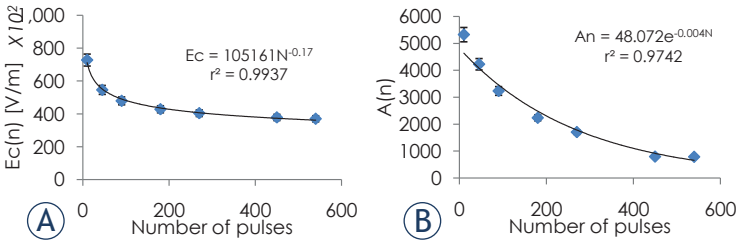

FIGURE 5. Dependence of E_{cb} **(A)** and A_b **(B)** on the number of treatment pulses for BBB disruption. Error bars represent 95% confidence level.

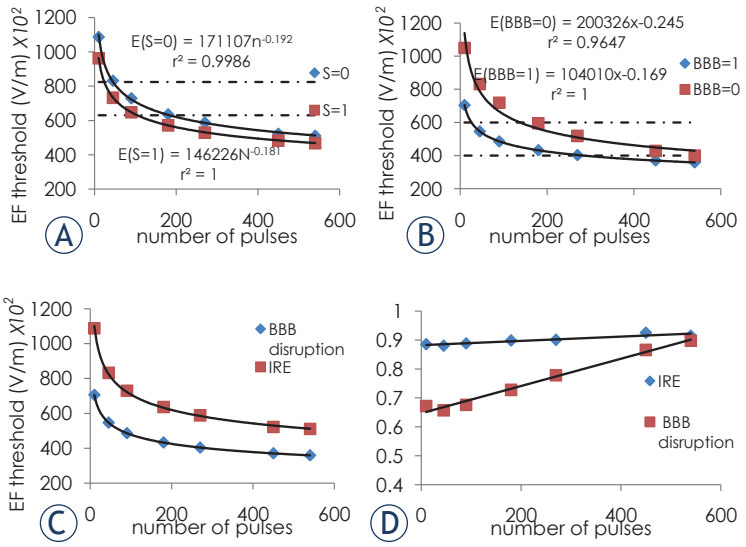

FIGURE 6. Electrical field thresholds. **(A)** IRE thresholds. Dashed line represents published IRE thresholds for white matter for 80 50 μs pulses at 4 Hz. **(B)** BBB disruption thresholds. Dashed line represent previously published threshold for 90 50 μs pulses at 4 Hz.[5] **(C)** Thresholds for E(S = 0) for the IRE and E(S = 1) for BBB disruption. **(D)** Ratio between E(S = 1) and E(S = 0) for IRE and E(BBB = 0) and E(BBB =1) for BBB disruption. Error bars are smaller than markers.

Linear regression analysis confirmed that r(S = 0), calculated from the Peleg-Fermi equation with $E_{cd}(N)$ and $A_d(N)$described well the r_d obtained from the experimental data: $F(1,5) = 45$, $p < 0.008$, $r^2 = 0.79$. The resulting regression equation was: $r_d = 0.19 + 0.87$ x, (x = r(S = 0)).

BBB disruption model

The same optimization method that was used to calculate E_{cd} and A_d was applied to the BBB disruption data with r_b set to BBB(E,N) = 1, meaning that for radii larger than r_b the BBB was not breeched(less than 0.001%). E_{cb} and A_b were calculated for each value of N as can be seen in Figure 5A-B.

As for the IRE models, the goodness of the fit to the experimental data was also evaluated. r(BBB = 1) and r(BBB = 0) were calculated from BBB(E,N) for each value of N using $A_b(N)$ and $E_{cb}(N)$.

Next we evaluated the correlation between $r_b(N)$ obtained from the experimental data, and r(BBB = 0) linear regression analysis confirmed that r(BBB = 1), calculated from the extended Peleg Fermi model with $E_{cb}(N)$ and $A_b(N)$ described well the behavior of r_b obtained from experimental data: $F(1,5) = 45$, $p < 0.001$, $r^2 = 0.91$. The regression equation was: $r_b = 0.19 + 0.87$ x (x = r(S = 0)).

Electric field thresholds

The electrical field thresholds for E(S = 0) and E(S = 1) were calculated from the model for cell death. E(BBB = 0) and E(BBB = 1) were calculated for BBB disruption. In the cell death model, E(S = 0) represents the threshold needed for over 99.99% cells death whereas electric field lower then E(S = 1) will cause cell death lower than 0.001%. In the BBB disruption model E(BBB = 0) represents the threshold needed for over 99.99% of the BBB to be breeched while electric field lower then E(BBB = 1) will not disrupt the BBB(BBB disruption lower than 0.001%). Thresholds are presented in Figure 6.

The ratio between S(E,N) = 0 and S(E,N) = 1 thresholds, representing the transition zone between over 99.99% cell death and no cell death (S(E,N) = 0 / S(E,N) = 1) thresholds was calculated. The ratio is relatively high (between 0.88 and 0.91) even for small numbers of pulses and depends only weakly on the number of treatment pulses. This means that the transition between 99.99% cell death threshold and no cell death threshold is narrow and gets even narrower for large numbers of

treatment pulses. This is not the case with BBB disruption, where the ratio between the thresholds increases with the number of treatment pulses eventually converging to one (Figure 6). The average ratio between BBB disruption ratio and damage ratio is 1.67 ± 0.11 (s.e.m).

Thermal model

The initial temperature of the rats' brain in the simulation was set to 37°C to show that the treatment should not induce thermal damage in clinical use. The maximum temperature reached in the tissue after treatment at 600 V was 38.9°C (Figure 1B). This temperature was reached using 540 pulses. Although temperature was not measured during EP treatment, histological analysis of brains extracted 60 min post treatment revealed no signs indicative of thermal damage such as coagulation, or extensive hemorrhages.[45] Connective tissue and blood vessels were preserved in the treated area suggesting damage induced only by IRE.[46]

Discussion

When treating tumors by EP, it is important to deliver the electric pulses so that the entire tumor volume will be treated to avoid recurrence. It is also vital to treat the infiltrating zone surrounding the tumor mass with high efficacy while preserving the healthy tissue. This is especially important in the case of brain tumors, where the infiltrative zone is relatively large[47] and the preservation of healthy brain tissue is of critical importance.

The electrode configuration in this experiment, which consists of a single intracranial insulated electrode with an exposed tip combined with an external surface ground electrode, provides an electric field distribution that is strongest at the intracranial needle tip tissue interface and decreases with the square of the radius. This setup provides a well-controlled region of permanent damage induced by IRE, further surrounded by significant BBB disruption zone. This combined response offers the potential of this configuration for the treatment of brain tumors combining IRE and chemotherapy. This setup induces rapid tissue damage in the tumor mass surrounded by significant BBB disruption, thus potentially enabling efficient drug delivery of systemically administered drugs to the infiltration zone surrounding the tumor. Since GBM cells are highly resistant to therapeutic apoptotic stimuli, however, they exhibit a paradoxical propensity for extensive cellular necrosis[19,20], IRE may be efficient for treating the tumor mass. Disrupting the BBB in the local vicinity of the tumor can also improve drug intake since peripheral administration of therapeutic agents is inefficient due to poor penetration of most drugs across the BBB. As clinical trials using IRE or ECT for the treatment of deep seated tumor are becoming more common[8-16], a model that can predict treatment outcome and enable individual treatment planning is increasingly being recognized as a need.

The statistical model of EP-induced cell death used in this manuscript was originally suggested by Golberg *et al*.[32] who validated the model using experimental data *in vitro*. Here, we present for the first time an experimentally validated statistical model for tissue IRE where the Peleg-Fermi model was extended to a wide range of number of treatment pulses r and to BBB disruption.

The results of this study demonstrate the feasibility of applying the Peleg-Fermi model for describing irreversible EP in the brain and for treatment planning. Furthermore, since our model is based on experiments with up to 540 pulses we were able to extend the model beyond the up to 90 traditional pulses used for IRE. This is important since protocols outside the traditional 100 pulses are being evaluated[48,49] and a tool to evaluate protocols with higher number of pulses is needed.

The results of the model indicate, as expected, that with increasing number of treatment pulses it is possible to treat larger volumes of tissue and that the IRE threshold decreases with the number of pulses. This is however true up to a limited extent since both $E_c(N)$ and the thresholds eventually plateau. This suggests that although increasing the number of pulses while lowering the treatment voltage may represent a safe way to avoid thermal damage while still achieving large enough treatment volumes, there is an upper limit for this effect. In addition, when using higher voltages, raising the number of pulses will eventually lead to increased damage induced by Joule heating but will not increase the damage induced by IRE. This plateau phenomenon does not only result from the logarithmic behavior of r(N),as can be seen in Equations [8], [9] (Figure 6) but also in $E_{cd}(N)$ (Figure 4B) and $E_{cb}(N)$ (Figure 5B).

Although the behavior of the equations describing Ec(N) was previously described as exponential[32,36], which supports the claim that larger number of pulses increases lethality, we found that Ec(N) is better described by a power function. As power functions plateau faster than exponential

functions it further supports the limited effect of increasing the number of treatment pulses. One explanation for the difference might be that previously the model was limited to 10-90 pulses[32,36], and therefore the plateau effect was not yet reached. In a paper published about evaluation of the Fermi equation as a model of dose-response curves on dose response the author described Ec as a Weibull function suggesting exponential function is just a simplification for limited range of pulses.[50]

Once we found that the Peleg-Fermi model can be used to describe IRE, we continued to further extend the model to describe BBB disruption induced by EP. For the model, we correlated radii calculated from contrast-enhanced T1-weighted MRI with BBB(E,N) = 1 since even a relatively small BBB disruption can be visible. We found that the extended Peleg-Fermi model describes well not only the behavior of IRE radii but also that of BBB disruption induced by EP with high statistical significance. This also indicates that there are possibly similar underlying mechanisms at play, which cause the effects.

The combination of the two models can be used for efficient treatment planning for brain tumors where IRE is used for ablating the tumor mass while BBB is disturbed in the rims and infiltrating zone thus allowing efficient access of therapeutic agents. The rims in this setup are on average 1.67 ÷ 0.11 mm wider than the damage, with no correlation to number of pulses. This suggests that the volume of BBB disruption is over 4 times larger than the volume of IRE.

Although both the IRE and the BBB disruption models were constructed separately, when planning a treatment protocol for brain, both should be used since BBB disruption with no irreversible damage only occurs in relatively low electric fields and when higher voltages or higher number of pulses are used, irreversible damage is difficult to avoid.

The electric field thresholds for IRE and reversible EP are mostly limited to the traditional treatment protocol, *i.e.* 90 pulses, but when using protocols that include a different number of pulses, different thresholds should be used.[51] In this study we calculated the thresholds needed for the different number of pulses and fitted them to a power function. The thresholds we found for 90 pulses fit well within the thresholds previously reported in the literature for IRE in white matter[52] and BBB disruption[5] as can be seen in Figure 6.

The ratio between the thresholds of BBB(E,N) = 0 and BBB(E,N) = 1 were found to increase with the number of pulses suggesting that the window between 99.99% of BBB disruption and no BBB disruption narrows with the number of pulses. This suggests that while increasing the number of pulses will eventually not lead to bigger radius of BBB disruption, larger percentage of the BBB will be disrupted thus improving drug penetration to the tissue. This is not the case for IRE where the ratio between the thresholds for S(E,N) = 0 and S(E,N) = 1 seems to be nearly independent on the number of treatment pulses. This is somewhat surprising but could be explained by the fact that the ratio is relatively high to begin with (between 0.88-0.91) and that our dataset starts with 10 pulses, however the range of pulse numbers in this study covers the most commonly used IRE protocols in clinical practice. This is also consistent with previous publications saying there is a sharp delineation of IRE treated and healthy tissue[53] and demonstrates that the sharp delineation is maintained even for high number of pulses.

The ratio between r_b and r_d was found to be nearly independent on the number of pulses. This is further supported by the relatively constant ratio between the thresholds that where calculated from the model. Thus, during treatment planning it might be sufficient to calculate one radius. It also indicates that BBB disruption may be used as a safety limit for irreversible EP. Though when using other electrode configurations, caution is needed. If thermal damage occurs, typically at high voltages or high number of pulses, it may influence the ratio between cell death and BBB disruption.

The thermal model showed only a mild increase in brain temperature. The maximal temperature at the end of 540 pulses reached 38.9°C. Since 42°C is often considered the thermal damage threshold if sustained for long durations[42], it is safe to assume that the tissue damage found in our experiments was induced solely by EP and not by thermal effects. This was also confirmed by histology[7] showing no signs of thermal damage, although a temperature assessment in real time is advisable.

Despite our understanding that this model may be used by physicians and researchers for the selection of treatment protocols, a model that also incorporates dependence on additional treatment parameters such as frequencies and pulse durations should be developed.[28] Such all-inclusive model would enable physicians to choose the safest and most efficient protocol on a per-patient bases. Another point to bear in mind is that although the electrode configuration suggested in this paper produces very low Joule heating, using other elec-

trode configurations with high number of pulses might induce thermal damage in addition to IRE.[54] Although this study indicates that the combination of IRE and BBB disruption may be applied for the treatment of brain tumors, experimental validation using animals bearing intracranial tumors is yet to be done.

In conclusion, the results of our study indicate that it is possible to apply high voltage electric pulses in a manner that induces localized focused irreversible damage in the brain surrounded by a larger volume of BBB disruption while using a single minimally invasive intracranial electrode. We used existing statistical models of cell kill by electric pulses that were based on theoretical cases and validated them using *in vivo* experimental data and extended the knowledge of EP thresholds beyond the traditional 90 pulses protocol used in IRE. We further extended the model to describe BBB disruption induced by EP. These models can assist physicians and researchers in selecting optimal treatment protocols allowing them to achieve the desired outcome in treating brain tumors. Although validation of the model in tumors is yet to be done, the results confirm that treatments outside the most commonly used protocols can achieve expected outcome.

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research article

Electrochemotherapy by pulsed electromagnetic field treatment (PEMF) in mouse melanoma B16F10 *in vivo*

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Introduction. Pulsed electromagnetic field (PEMF) induces pulsed electric field, which presumably increases membrane permeabilization of the exposed cells, similar to the conventional electroporation. Thus, contactless PEMF could represent a promising approach for drug delivery.

Materials and methods. Noninvasive electroporation was performed by magnetic field pulse generator connected to an applicator consisting of round coil. Subcutaneous mouse B16F10 melanoma tumors were treated with intravenously injection of cisplatin (CDDP) (4 mg/kg), PEMF (480 bipolar pulses, at frequency of 80 Hz, pulse duration of 340 μs) or with the combination of both therapies (electrochemotherapy – PEMF + CDDP). Antitumor effectiveness of treatments was evaluated by tumor growth delay assay. In addition, the platinum (Pt) uptake in tumors and serum, as well as Pt bound to the DNA in the cells and Pt in the extracellular fraction were measured by inductively coupled plasma mass spectrometry.

Results. The antitumor effectiveness of electrochemotherapy with CDDP mediated by PEMF was comparable to the conventional electrochemotherapy with CDDP, with the induction of 2.3 days and 3.0 days tumor growth delay, respectively. The exposure of tumors to PEMF only, had no effect on tumor growth, as well as the injection of CDDP only. The antitumor effect in combined treatment was related to increased drug uptake into the electroporated tumor cells, demonstrated by increased amount of Pt bound to the DNA. Approximately 2-fold increase in cellular uptake of Pt was measured.

Conclusions. The obtained results in mouse melanoma model *in vivo* demonstrate the possible use of PEMF induced electroporation for biomedical applications, such as electrochemotherapy. The main advantages of electroporation mediated by PEMF are contactless and painless application, as well as effective electroporation compared to conventional electroporation.

Key words: pulsed electromagnetic field; bipolar pulses; contactless electroporation; CDDP; electrochemotherapy; platinum determination; mouse melanoma

Introduction

Electroporation is a physical method enabling delivery of impermeable drugs, macromolecules, proteins and genetic material (plasmid DNA, siRNA, miRNA) into cells.[1] Electroporation is related to the induced transmembrane voltage which if sufficiently high, enables the formation of tempo-

rary structural changes in the plasma membrane and increases its permeability for molecules otherwise deprived of transmembrane transport mechanisms.[2-5] Electroporation of cells is predominantly induced by pulsed electric fields, which are generated with the train of square wave electric pulses of sufficient amplitude establishing local electric field (hundreds of V/cm).[1,3,6,7] The electric field intensity and duration of the pulses determine whether the structural changes in the plasma membrane are reversible, allowing cells to survive, or irreversible, leading to cell death, due to the loss of homeostasis.[8-11] Nowadays, reversible electroporation is used as a platform technology[12] and among others for drug delivery to various tissues, with therapeutic purposes for the treatment of cancer, known as electrochemotherapy.[7,13-18]

Electrochemotherapy is used in treatment of human cutaneous tumors of different histology, and has been translated also in treatment of deep seated tumors.[15,18-22] In parallel, electrochemotherapy is being used for treatment of tumors in veterinary oncology.[23-26] The main chemotherapeutics used in electrochemotherapy are nonpermeable bleomycin and poorly permeable cisplatin (CDDP), via systemic or intratumoral administration route. Electric pulses can be delivered to the tumors via noninvasive plate electrodes, which embrace the tissue, or invasive needle electrodes, which are inserted into the tumor.[7,27]

In the past the effects of externally applied pulsed electromagnetic fields (PEMF) on the cells were studied extensively. It was demonstrated that externally applied PEMF can influence intracellular signal transduction, affect the cytoskeletal proteins involved in cell shape modification, induce changes in mitochondrial membrane potential, and besides that increase transmembrane molecular transport (electroporation).[28-34] Since then, a few studies actually defined the PEMF parameters that enabled successful electropermeabilization of cells; *i.e.* large number of 25 up to 800 the μs long magnetic field pulses applied at frequencies from 25 Hz up to 40 Hz and strength from 725 V/m up to 160 kV/m.[30,35,36] Furthermore, its use as electroporation tool was shown in an approach for drug as well as for plasmid DNA delivery.[36,37] Thus, such electromagnetic induction with alternating currents has the potential for simple contactless tissue electroporation, used for electrochemotherapy and gene electrotransfer.

The majority of studies with PEMF induced electroporation were using bipolar pulses. Generally, shorter and larger number of pulses resulted in better membrane permeabilization.[30,34] In a recent study, time varying magnetic field of 6.1 T was shown as an interesting tool in drug delivery for antifungal treatment, as well as for irreversible electroporation.[36,38] Furthermore, the bipolar pulses generated by magnetic field (4 T) were used for gene electrotransfer of plasmid DNA into the skin.[37] Thus, as simple, noninvasive and contactless application of PEMF, which could enable electroporation of cells in the tissue, this approach showed the potential use for the clinical applications.

Due to only few studies in the field of electroporation induced by pulsed electromagnetic field we designed experiments and considered the use of such physical delivery technique in treatment of cancer, as a model for electroporation of tissues *in vivo* induced by PEMF. If feasible and effective, electroporation induced by PEMF would have the advantage over "conventional" electroporation, since it is noninvasive, contactless and does not induce pain during electroporation. We assessed the feasibility and antitumor effectiveness of electroporation induced with PEMF as drug delivery system for CDDP to murine melanoma B16F10 subcutaneous tumors. To prove the underlying mechanism of electroporation we measured the platinum (Pt) bound to DNA in tumors. Electroporation induced by PEMF proved to facilitate drug uptake in tumors, such as CDDP, thus providing evidence of its feasibility and effectiveness.

Materials and methods

Drug

CDDP, a chemotherapeutic drug used in electrochemotherapy protocol in human and veterinary clinic, was chosen in the study to test the application of induced electroporation mediated with magnetic field. The stock solution of the chemotherapeutic drug used in the study, CDDP (5 mg/mL, Cysplatyl, Aventis Laboratory, Paris, France) was dissolved in aqua pro injection and frozen in aliquots of 1 mL. In order that each animal received a dose of 80 μg of CDDP, a fresh solution at appropriate concentration of CDDP (1 mg/mL) was prepared in 0.9% sodium chloride solution daily before each experiment.

Mouse tumor model

Female C57Bl/6 mice were purchased from Charles River Laboratories Italy s.r.l. (Calco, Italy) and were maintained in an adaptation period for 14

days. They were kept at a constant room temperature with a 12 hours light cycle in a conventional animal facility. Eight-week old animals weighing 20–22 g were used in the experiments. Tumors in C57Bl/6 mice were implanted subcutaneously in the right flank of the mice by inoculation of suspension 1 × 10^6 B16F10 melanoma cells prepared in 100 μL of phosphate-buffered saline (PBS) for electrochemotherapy experiments. All animal experimental manipulations were conducted in accordance with the principles and procedures outlined with the guidelines for animal experiments of the EU directives and the permission from The administration of the Republic of Slovenia for food safety, veterinary and plant protection (permission No.: 34401–4/2012/2).

In vivo electrochemotherapy protocol using noninvasive electroporation induced by PEMF or conventional electroporation

Seven days after subcutaneously induction of B16F10 melanoma tumors (40 mm^3) mice were randomly divided into the experimental groups as follows: intravenously injection of saline solution alone (Control) or combined with electroporation induced pulsed electromagnetic field (PEMF), intravenously injection of CDDP (CDDP) or combined with electroporation induced PEMF (PEMF + CDDP). Noninvasive electroporation was performed 3 minutes after intravenous injection of chemotherapeutic drugs by magnetic field pulse generator (TESLA Stym, Iskramedical, Slovenia) connected to an applicator consisted of round coil with 72 turns. The generator supplied the applicator with pulses of electric current that generated time-varying magnetic field around the coil, which in turn induced an electric field in the treated tissue (Figure 1).

In order to obtain precise application of electroporation mice were initially anesthetized with inhalation anaesthesia in the induction chamber with 2% (v/v) of isoflurane (Isoflurane; Piramal Healthcare UK Limited, London, UK) and afterwards the mouse muzzle was placed under inhalation tube to remain anesthetized during experiment. The applicator for electroporation was positioned over the tumor so that the tumor was in the middle of the applicator (Figure 1A).

Based on the preliminary experiments, where four different sequences of bipolar pulses of electric current alone or in the combination with bleomycin were tested (Supplementary Table 1,

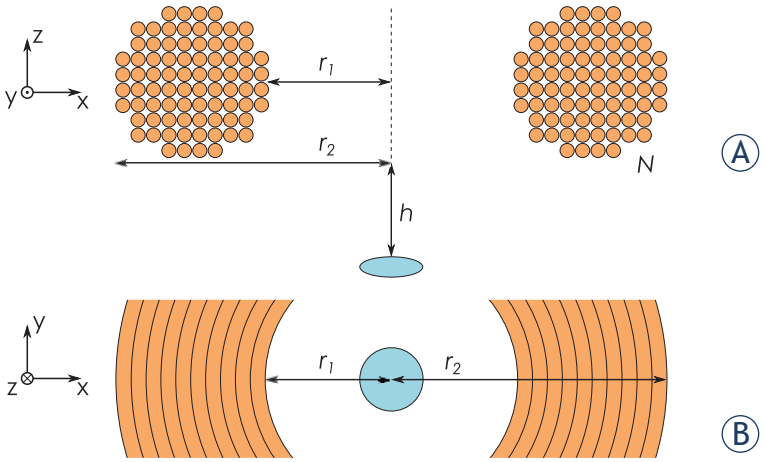

FIGURE 1. (A) Illustrated lateral view of multi-turn coil (colored orange) and the treated tumor (colored blue). Tumor was located in the center of the coil, *i.e.* 31 mm and 42 mm from the inner (r_1) and outer boundary (r_2) of the coil, respectively. The number of turns (N) in coil was 72. Due to the casing of the applicator the coil was placed 23 mm above the tumor (h). **(B)** Illustrated view from above.

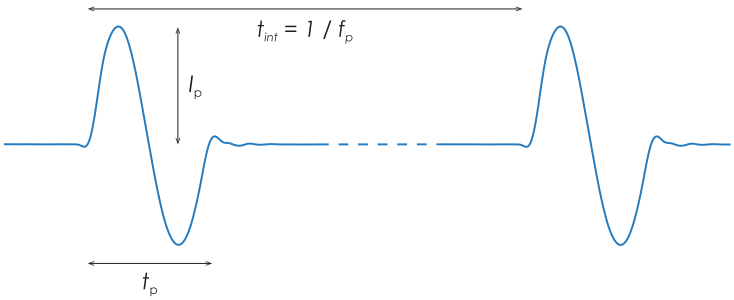

FIGURE 2. Sequence of bipolar electric pulses where t_p is a duration of the pulse, I_p is a pulse amplitude, t_{int} is an interval between pulses and f_p is a repetition frequency.

Supplementary Figure 1), the most promising sequence of bipolar pulses (Supplementary Figure 1) was used in the combination with CDDP. Briefly, the sequence had 480 bipolar pulses with duration of t_p = 340 μs, with a peak of I_p = 400 A, repetition frequency (f_p) of 80 Hz and duration of each sequence (t_s) of 6 s (Figure 2). Electric pulses were measured using an oscilloscope (WavePro 7300A, LeCroy, Chestnut Ridge, NY) and current probe CWT Rogowski Current Transducer (Powertek, UK).

In previous experiments, groups of positive controls, such as conventional electroporation (EP) and the combination of EP with CDDP (ECT), were obtained. The growth of untreated tumors (tumor doubling time (the time in which tumor reaches twice of the initial volume, DT) in control was 1.4 ± 0.2, n = 6) and of CDDP treated tumors alone (2.3 ± 0.1, n = 4) in that independent experiment (data previously not published) were comparable

to the experiment performed with PEMF treatment). In the conventional electrochemotherapy protocol three minutes after intravenously injection of CDDP eight square wave electric pulses at 1300 V/cm voltage to distance ratio, 100 μs long and 1 Hz (Cliniporator™, IGEA s.r.l., Carpi, Italy) were applied by plate electrodes (d = 8 mm) to the tumors. Electric pulses were delivered in perpendicular orientation (4 + 4) and good contact between the electrodes and tumor was assured using conductive gel.

Determination of magnetic and electric field in the tumor

Time-varying magnetic field and induced electric field of PEMF in the tumor was determined by means of numerical modelling. Numerical model of the applicator was modelled as multi-turn coil node which is a lumped model for tightly wound 72 wires separated by electrical insulator. Numerical model of the tumor was represented by an ellipsoid (Figure 1). Since volume of mice tumors varied from 30 to 40 mm^3 an average volume, *i.e.* 35 mm^3, was used in the numerical model of the tumor. Bipolar pulse (Figure 2) was used as electric current in the numerical model of the applicator. Calculations of time-varying magnetic field and induced electric field were performed using finite element method on a desktop PC (Windows 8.1, 3.50 GHz, 32 GB RAM) using commercial finite element software package COMSOL Multiphysics 5.1 (COMSOL AB, Stockholm, Sweden).

Treatment evaluation

The muscle contraction during PEMF treatment and conventional EP, tumor growth after therapy, skin area above the tumor and 2 cm in diameter around the tumor exposed to PEMF or conventional EP and the general well-being of animals (consumption of water and food, weight loss) were monitored during the experiment. Tumor growth was followed by measuring three mutually orthogonal tumor diameters (a, b, and c) with a Vernier caliper, every day. The tumor volumes were calculated by the formula:

$$V = \frac{\prod \times a \times b \times c}{6}.$$

The arithmetic mean of the tumor volumes and the standard error of the mean (SE) were calculated for each experimental group for each measurement day. The tumor growth delay was determined for each individual tumor by subtracting the average DT of the control group from the DT of each individual tumor.

Platinum determination in the serum and tumors

The measurements of platinum accumulation in the serum, tumors, platinum bound to the DNA in the cells and in extracellular fraction were performed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent Technologies, model 7700x, Tokyo, Japan). ^{195}Pt isotope was monitored. At optimized instrumental parameters, instrumental limit of detection (LOD) was 0.005 ng Pt/mL (3σ of the blanks). The linearity of the signal was confirmed from LOD to 10 μg Pt/mL. Repeatability of the measurements was better than 3%.

The platinum uptake in tumors and its total concentration in the serum were measured 1 hour after the treatment of mice with intravenously injection of CDDP, electroporation induced by PEMF or the combination of those therapies (PEMF + CDDP). The blood was collected with glass capillary from intraorbital sinus (3–8 samples per group) and was coagulated at room temperature for two hours. Thereafter the blood was centrifuged at 3000 rpm for 10 minutes and serum was collected and stored at the temperature -20°C. On the day of measurements total amount of serum samples were digested in 1 mL of 1 : 1 mixture of 65% nitric acid (MERCK KgaA, Dermstadt, Germany) and 30% hydrogen peroxide (MERCK KgaA, Dermstadt, Germany) by incubation at 90°C for 48 hours. Obtained clear solutions were diluted with Milli-Q water before analysis.

For platinum determination in tumors, animals were sacrificed after the blood collection. The tumors (3–8 tumors per group) were excised and removed from the overlying skin. Each tumor was weighed, and placed into a 15 mL graduated polyethylene tube. For tumors digestion, the same procedure as for serum was applied, with the exception that 2 mL instead of 1 mL of 1 : 1 mixture of 65% nitric and 30% hydrogen peroxide was used. Before analysis samples were diluted with Milli-Q water.

Determination of platinum bound to the DNA in the tumor cells and the extracellular fraction (fluid)

The tumors were obtained as described in the chapter above, weighed and immediately mechan-

ically disintegrated. The sample was washed with 3 mL of freshly prepared PBS and filtered through the cell strainer with pore size of 40 μm (Corning Incorporated, Life Sciences, Durham, USA). The obtained cells in suspension were centrifuged at 1500 rpm for 10 minutes. Collected cells were used for the fast DNA isolation by salting-out protocol. Briefly, cells were lysed with lysis buffer (10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid [EDTA], 1% sodium dodecyl sulfate [SDS]) with proteinase K (20 μg) for 30 minutes at 55°C by constant shaking. After the samples were cooled down proteins were precipitated by adding of 120 μL 4 M NaCl and shaken for 15 seconds. Precipitated proteins were centrifuged at 13000 rpm for 6 minutes. Supernatant was collected and centrifuged one times more at 13000 rpm for 6 minutes. In addition, DNA was precipitated with 1 mL of ethanol (70%) for 2 min by gentle mixing of tube and centrifuged at 13000 rpm for 2 minutes. Precipitated DNA was washed with additional 1 mL of ethanol (70%) and centrifuged at 13000 rpm for 2 minutes. The pellet of DNA was dried out, resuspended in 100 μL of distilled water, digested under the same procedure as serum and the concentration determined in diluted samples by ICP-MS.

The rest of two fractions, supernatant and the interstitial fraction on the top of the cell strainer, named as extracellular fraction, were collected and stored at -20°C till the digestion with the mixture of nitric acid and hydrogen peroxide (see the section above).

Statistical analysis

All data were tested for normal distribution with the Shapiro–Wilk test. A t-test and one-way analysis of variance followed by a Holm–Sidak test were used for evaluation of the differences between the experimental groups. A p value less than 0.05 was considered significant. SigmaPlot Software (Systat Software, Chicago, IL, USA) was used for statistical analysis and graphical representation.

Results

Antitumor effectives of electrochemotherapy mediated by PEMF

Exposure of tumors to PEMF or conventional EP, performed 3 minutes after intravenous injection of CDDP, resulted in significant tumor growth delay, up to 3 days compared to untreated tumors, as well as compared to monotherapies. Nevertheless significantly higher antitumor effect was obtained after conventional electrochemotherapy compared to electrochemotherapy mediated by PEMF. Treatment of tumors with CDDP alone or exposure to PEMF or conventional EP had no significant effect on tumor growth (Table 1, Figure 3).

The application of PEMF did not exert muscle contraction, indicating on painless treatment procedure. Additionally, all treatments were well tolerated by animals, since no body weight loss or any detectable changes in skin, exposed to PEMF treatment during observation period was obtained, indicating that there was no systemic toxicity after treatments.

TABLE 1 Tumor doubling times of melanoma B16F10 tumors after treatment with CDDP or combined with electroporation induced by PEMF.

Group	n	DT (Mean ± SE)	GD	P (<0.05)
Control*	12	1.5 ± 0.1		
CDDP* 4 mg/kg	12	2.2 ± 0.2	0.7	
PEMF	9	1.9 ± 0.1	0.4	
PEMF + CDDP	10	3.8 ± 0.1	2.3	<0.001 (to PEMF)
EP*	12	2.2 ± 0.3	0.7	
ECT CDDP*	8	4.5 ± 0.2	3.0	<0.009 (to PEMF + CDDP)

CDDP = intravenously injection of cisplatin (4 mg/kg); PEMF = pulsed electromagnetic field treatment; PEMF + CDDP = PEMF after intravenously injection of CDDP; EP = electric pulses treatment; ECT = electrochemotherapy, EP after intravenously injection of CDDP; DT = tumor doubling time; GD = tumor growth delay; $p < 0.05$ statistically significant difference; *Data pooled from separate experiments after checking that DT in control and CDDP treatment alone were comparable.

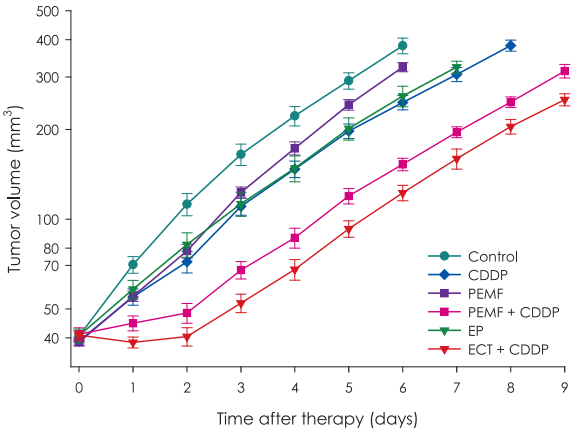

FIGURE 3. Antitumor effectiveness of electrochemotherapy with CDDP mediated by PEMF in mouse melanoma B16F10. Data were collected from two individual experiments and each point on graph represents mean and standard error of the mean (AM ± SE). Each group consisted at least of 8 animals.

CDDP = intravenously injection of cisplatin (4 mg/kg); ECT = electrochemotherapy, EP after intravenously injection of CDDP; EP = electric pulses treatment; PEMF = pulsed electromagnetic field treatment; PEMF + CDDP = PEMF after intravenously injection of CDDP

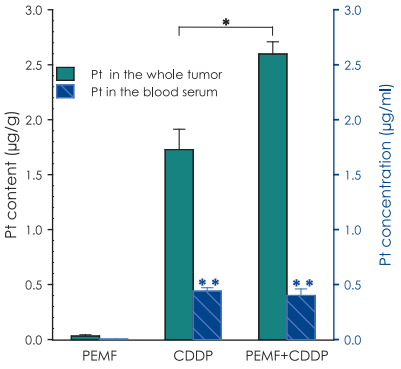

FIGURE 4. Platinum (Pt) accumulation in tumor and serum after electroporation induced by PEMF. Each group consisted from 3–8 animals. Data represent mean and standard error of the mean (AM ± SE).

CDDP = intravenously injection of cisplatin (4 mg/kg); PEMF = pulsed electromagnetic field treatment; PEMF + CDDP = PEMF after intravenously injection of CDDP. * = $p < 0.05$ statistically significant difference; ** = $p < 0.05$ statistically significant difference to measured Patinum (Pt) content in the whole tumor

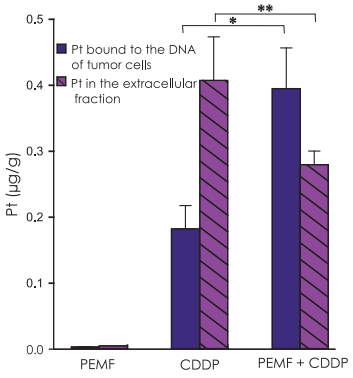

FIGURE 5. Platinum (Pt) bound to the DNA in tumor cells representing intracellular fraction and Pt content in extracellular fractions after electroporation induced by PEMF. Data represent mean and standard error of the mean (AM ± SE). Each group consisted from 3–8 animals.

CDDP = intravenously injection of cisplatin (4 mg/kg); PEMF = pulsed electromagnetic field treatment; PEMF + CDDP = PEMF after intravenously injection of CDDP. *, ** = $p < 0.05$ statistically significant difference

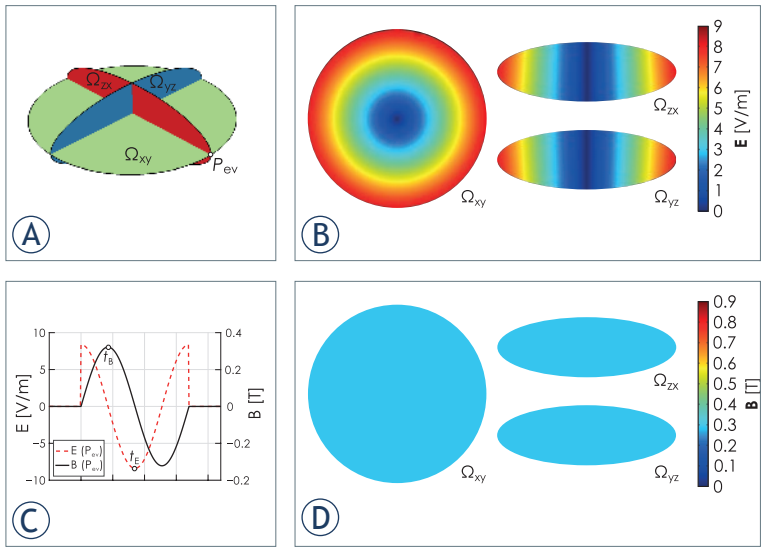

FIGURE 6. (A) Evaluation surfaces (Ω_{xy}, Ω_{yz}, Ω_{zx}) in three different planes (xy, yz, zx) where electric field distribution and magnetic flux density were simulated by means of numerical modelling. **(B)** Distribution of induced electric field in evaluation surfaces when it reached its peak at t_E. **(C)** Time course of magnetic flux density and induced electric field at evaluation point P_{ev}. Time points when magnetic flux density and induced electric field reached its maximum are marked with t_E and t_B, respectively. **(D)** Distribution of magnetic flux density in evaluation surfaces when it reached its peak at t_B.

Overall, the antitumor effect CDDP, *i.e.* the chemotherapeutic drug used, in the electrochemotherapy performed by PEMF was significantly increased in comparison to monotherapies, presumably due to facilitated transport of CDDP. However, electrochemotherapy after conventional EP was more effective than PEMF mediated electroporation.

Determination of platinum in the serum and tumor after electroporation induced by PEMF

In order to determine whether electroporation induced by PEMF facilitates drug delivery into the cells, as the underlying antitumor mechanism, Pt accumulation in the serum and tumors with plasma mass spectrometry was determined. First, Pt was measured as indicator of CDDP[39] in whole tumors and plasma of the blood in mice. Intravenous CDDP injection demonstrated the drug accumulation in tumors, and electroporation induced by PEMF as successful method for increasing Pt accumulation in whole tumors, one hour after the drug administration (Figure 4). A statistically significant increase in platinum content in the tumors treated by electroporation induced by PEMF was observed, however this measurement does not indicate whether the electroporation induced by PEMF in fact facilitated drug delivery into the cells.

To prove that electroporation induced by PEMF facilitates transmembrane transport of CDDP, the extracellular and intracellular Pt amounts were measured. After mechanically disintegration of tumors, the suspension of tumor cells and extracellular fractions were obtained, and Pt was measured in both fractions. The concentration of the Pt detected in extracellular fraction statistically significantly decreased after electroporation induced by PEMF (Figure 5). Moreover, the Pt bound to DNA as indicator of the drug bound to the intracellular target was significantly increased after the electroporation induced by PEMF (Figure 5). Approximately 2 times higher values of Pt bound to DNA were obtained.

Estimation of PEMF in the tumor

Simulation results of PEMF in the tumor are presented in Figure 6. Electric field distribution in the tumor was linearly decreasing from the boundary of the tumor towards the center with a peak value of 8.6 V/m on the tumor boundary. Magnetic flux density had a peak value of 0.3 T and its distribu-

tion in evaluation surfaces remained homogeneous through the whole surface of the tumor.

Discussion

This study demonstrated the use of contactless pulsed electromagnetic field (PEMF) treatment as an approach to achieve electroporation of melanoma tumor tissue, which increases drug uptake *in vivo*. We evaluated for the first time the antitumor effectiveness of electrochemotherapy obtained by PEMF after systemic injection of chemotherapeutic drug, CDDP, which is used in conventional electroporation protocol. Furthermore, we proved that the antitumor effect was related to increased drug uptake into the electroporated tumor cells, demonstrated by increased amount of Pt bound to the DNA. Thus PEMF treatment can be used (once optimized) for noninvasive drug delivery *in vivo,* which may be important for research where delicate tissues and organs needs to be avoided and for clinical applications, since it is noninvasive, contactless and painless compared to the classical electroporation using different electrodes.

Potential use of strong time-varying magnetic field which induced electric field to increase transmembrane molecular transport (*i.e.* electroporation), was already suggested before.[30,34-36,38] In order to expand the use of contactless PEMF induced electroporation as drug delivery method the melanoma B16F10 tumor model *in vivo* was chosen, as it represents a great challenge in the treatment of human melanoma.[1,14,40-43] We have shown that magnetic field generated by round coil which induced the 480 bipolar pulses, at frequency of 80 Hz, pulse duration of 340 μs, significantly improved the antitumor effectiveness of electrochemotherapy with CDDP. Our results are in accordance with *in vitro* study, where much stronger PEMF (6.1 T) was indicated as delivery method for therapeutic molecules in human pathogenic fungi. Namely, the synergistic effect of simultaneos treatment of 200 applied magnetic field pulses at frequency of 35 Hz and drug was observed.[36] However, the application of stronger magnetic field (up to 16.4 T) did not result in better membrane permeabilization.[35] The membrane permeabilization seems to be dependent more on the shape, number and frequency of generated pulses[34-36] in addition to the amplitude of induced electric field, similar to the conventionally generated bipolar pulses.[44] In addition, bipolar pulses were demonstrated two times more effective as monopolar and almost equally effective as conventional square wave pulses.[37] In fact, for the almost equal membrane permeabilization larger number of short duration induced bipolar electric pulses at higher frequency has to be delivered.[37] Similarly, in our study the obtained antitumor effectiveness of cisplatin by using short and larger number of induced electric pulses at higher frequency in comparison to conventional electric pulses was comparable. Furthermore, it is known that simple round coils induce less focused and lower peak electric field than figure-of-eight coils.[45,46] It was also demonstrated *in vitro* that by using figure-of-eight coils the increased transmembrane molecular transport could be obtained by pulses of lower frequencies and larger number.[34] However, we and others[34] have shown that by increasing the number of pulses at the same frequency the effect of electroporation can be improved (supplementary data).

Presently, in conventional electrochemotherapy protocols mainly square wave or monotonically decreasing electric pulses are delivered through plate or needle electrodes to the cells or tissues.[15,18,23,25] On the contrary, only a few studies were performed with bipolar electric pulses for the electroporation of cells *in vitro* and tissues *in vivo*.[44,47-55] In general, the lower pulse amplitudes were needed for effective electroporation of cells *in vitro* with respect to unipolar pulses.[48,51] Besides that, the pulse shape played important role in electroporation of cells as well.[34,51,56] It was shown that electroporation, cell death and the uptake of Lucifer Yellow occurred by using the rectangular bipolar pulses at lowest, the sine bipolar pulses at medium and the triangular bipolar pulses at the highest pulses amplitudes.[44] Bipolar pulses were already applied successfully in electrochemotherapy for human and veterinary clinic.[49,57,58]

In fact, the combination of PEMF induced electroporation with CDDP had significant antitumor effectiveness, whereas the application of PEMF or the drug alone had none. The antitumor effectiveness of electrochemotherapy of applied PEMF (480 bipolar pulses, at frequency of 80 Hz, pulse duration of 340 μs) was presumably due to improved membrane permeabilization of cells in the tissue, since the monotherapies alone had very little but no significant effect on tumor growth in comparison to control. Similarly, preclinical and clinical studies performed in conventional scheme of electrochemotherapy with CDDP have shown great antitumor effectiveness of electrochemotherapy on different tumor types[15,42,59-62], mostly due to direct cytotoxic effect on tumor cells.[60-62] It has been demonstrated

that after conventional electroporation the cytotoxicity of CDDP could be improved by 70-times.[61-63] Nevertheless, in our study sufficient antitumor effectiveness on melanoma B16F10 was obtained with electrochemotherapy after PEMF induced electroporation, despite the effect was significantly lower compared to that obtained after conventional electrochemotherapy with CDDP. However, calculations suggest that levels of electric field are 4 orders of magnitude lower than those associated to classical electroporation[64], but tend to be high enough to induce electroporation. On the other side, while using electric field bellow 0.09 V/cm at the position of tumor site, we suspect there was no possible occurrence of irreversible electroporation or thermal effect, as obtained at much higher electric field induced by PEMF (up to 40 V/cm).[38] Even more, we speculate that PEMF could be improved by positioning of tumor towards the edge of the coil, where based on calculations the highest strength of magnetic field could be obtained, which consequently could induce higher electric field strength and thus, even more cells could be successfully electroporated at deeper parts of tumor tissue. Therefore, further studies to optimize the PEMF are warranted.

Even though it was previously known that the time varying magnetic field could induce electroporation[34-37,65], affect the cytoskeleton and intracellular signal transduction[28,32,66,67], the mechanisms of its action in the combination with CDDP have not been studied yet. Therefore, to clarify the antitumor effectiveness of electrochemotherapy the measurements of platinum amount after electroporation induced by PEMF were performed. Observed significant 1.6-fold increased platinum uptake into melanoma B16F10 tumors after electroporation induced by PEMF indirectly confirmed membrane permeabilization of the tumor cells and thus, its correlation with the antitumor effectiveness of electrochemotherapy. Our results are in accordance with results reported in other studies, where up to two times higher platinum uptake was obtained in sarcoma SA-1 and fibrosarcoma LPB tumors after conventional electrochemotherapy with CDDP.[60,61] Even though lower increase of platinum amount in tumors was obtained after PEMF in comparison to conventional electrochemotherapy[60,61], the final amount of platinum in the tumors was comparable. The difference of platinum amount in tumors treated only with CDDP, might be tumor type dependent, since melanoma tumors are well vascularized and contained large spherical cells, with less surrounding extracellular matrix component in comparison to stiff SA-1 and LPB tumors with small spindle-shaped cells and high content of extracellular matrix component.[68,69] On the other hand, the amount of platinum in the serum was significantly up to 6-times lower compared to tumor tissue. Therefore we could assume that excess of the drug which was not entrapped in the tumors after electroporation was washed out with similar kinetic as in nonelectroporated tumors. Moreover, our results indicated that CDDP in the cells reached its main intracellular target DNA, in fact significantly two times higher amount of Pt was bounded to the DNA in PEMF and CDDP treated tumors than in CDDP only treated tumors. At the same time, as expected the pool of Pt amount in the extracellular fraction of these tumors was lowered, up to 1.4- times. Thus, the increased Pt uptake in the cells and its binding to the DNA could indicate the main reason for antitumor effectiveness of electrochemotherapy mediated by PEMF.

Presently, it is not clear if the membrane permeabilization obtained after application of time varying magnetic field occurs only due to induced electric field as in conventional electroporation or due to direct effect of magnetic field with the plasma membrane and surrounding ions. Thus, the precise mechanism of cisplatin uptake after electroporation mediated by PEMF into the cells remains unclear. Obtained Pt amount in the tumor cells after treatment with CDDP only could be ascribed to passive diffusion and active transport mechanisms of cisplatin through the membrane, which are carrier-mediated through formed pores or via endocytosis.[70,71] In addition, it has been demonstrated that exposure of cells to train of unipolar pulsed low electric fields at strength from 1.2 up to 20 V/cm can induce electro-endocytosis.[72,73] Thus, we suspect that generated bipolar electric field of just below 0.09 V/cm by PEMF might also trigger endocytosis besides membrane permeabilization which enables the internalization of cisplatin in the cell and contributes partially to the increase of platinum amount.

In conclusion, our results show that PEMF at magnetic field below of 1 T was sufficient to achieve membrane permeabilization of tumor cells, thus, small molecules such as drug (CDDP) improved delivery and cellular uptake in solid tumors was enabled. Due to simple, contactless, painless, focused local application of PEMF, better field distribution irrespective of tissue type and thus, achieving electric field strength for membrane permeabilization can be established in deeper parts of tissue. However this approach has the limitation that the strength of the magnetic field decreases rapidly

with distance from the coil which has to be taken into account by designing coils[74] in order to achieve successful permeabilization at a greater tissue depth. PEMF might thus represent an alternative to conventional electroporation with electric fields in electrochemotherapy. However further studies are needed to improve the equipment, to optimize and establish precise protocols of drug application and PEMF parameters, as well as to reveal the effects of PEMF on variety of normal and tumor tissues.

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research article

A prototype of a flexible grid electrode to treat widespread superficial tumors by means of Electrochemotherapy

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Background. In recent years, superficial chest wall recurrence from breast cancer can be effectively treated by means of electrochemotherapy, with the majority of patients achieving response to treatment. Nevertheless, tumor spread along superficial lymphatic vessels makes this peculiar type of tumor recurrence prone to involve large skin areas and difficult to treat. In these cases, electroporation with standard, small size needle electrodes can be time-consuming and produce an inhomogeneous coverage of the target area, ultimately resulting in patient under treatment.
Materials and methods. Authors designed and developed a prototype of a flexible grid electrode aimed at the treatment of large skin surfaces and manufactured a connection box to link the pulse applicator to a voltage pulse generator. Laboratory tests on potato tissue were performed in order to evaluate the electroporation effect, which was evaluated by observing color change of treated tissue.
Results. A device has been designed in order to treat chest wall recurrences from breast cancer. According to preliminary tests, the new flexible support of the electrode allows the adaptability to the surface to be treated. Moreover, the designed devices can be useful to treat a larger surface in 2–5 minutes.
Conclusions. Authors developed the prototype of a new pulse applicator aimed at the treatment of widespread superficial tumors. This flexible grid needle electrode was successfully tested on potato tissue and produced an electroporation effect. From a clinical point of view, the development of this device may shorten electrochemotherapy procedure thus allowing clinicians to administer electric pulses at the time of maximum tumor exposure to drugs. Moreover, since the treatment time is 2–5 min long, it could also reduce the time of anesthesia, thus improving patient recovery.

Key words: electrochemotherapy; electrode; flexible support; breast cancer recurrence

Introduction

Electrochemotherapy (ECT) is an effective local therapy in use for unresectable skin cancers as well as cutaneous metastases from different tumor histotypes such as melanoma, head and neck cancer, soft tissue sarcomas and breast cancer.[1-3] During the procedure, high voltage pulses are applied to a needle pair implanted into tumor tissue in order to generate an electric field aimed at increasing cell membrane permeability and the uptake of chemotherapeutic drugs (bleomycin or cisplatin). In fact, electric fields over a suitable threshold allow the opening of transient aqueous pores on the cell membrane, thus inducing a temporary permeabilization (reversible electroporation).[1,4-8]

In recent years, ECT has shown efficacy in several tumors types and has been adopted by several

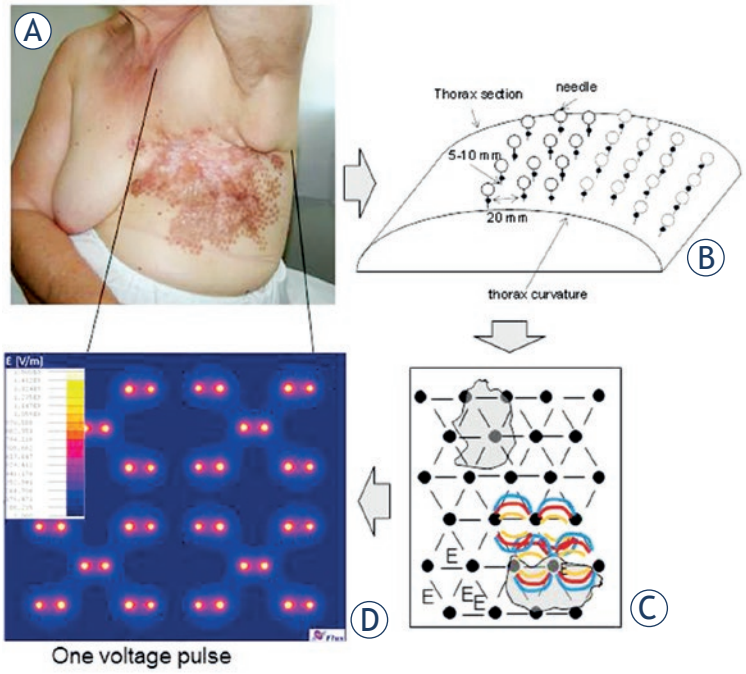


FIGURE 1. Chest wall recurrence from breast cancer and development of the grid electrode. **(A)** An example of a breast cancer patients who underwent repetitive ECT cycles to treat cutaneous metastases. The extension of electrode-induced skin marks highlights the extension of the treatment field and the need for more effective pulse delivery. **(B)** Sketch of the device, **(C)** resulting electric field lines of a grid electrode and (**D**) electric field color map.

centers for the treatment of skin tumors and, most of all, superficial metastases with the aim to improve local tumor control without discontinuation of concomitant systemic treatments.[4,8-13] Among ECT indications, breast cancer (BC) represents a promising, but challenging field. Chest wall recurrence (*i.e.* the occurrence of skin / soft tissue metastases on the chest wall after previous mastectomy) is an uncommon, but not negligible, pattern of recurrence observed in BC patients. It may occur also after optimal multidisciplinary management (*e.g.* mastectomy, radiation and systemic therapy). Its occurrence is more frequent (up to 45%) if the primary tumor was advanced in stage, while drops to 2–15% if adjuvant radiotherapy was applied after mastectomy. About 40–50% of chest wall recurrences occur around the mastectomy scar.[14-18]

ECT represents a promising treatment option in these patients and its efficacy in achieving an effective tumor control is particularly high when disease is limited in size.[10,19,20] Nevertheless, when chest wall recurrence is multifocal and widespread, it poses a therapeutic challenge to the treating oncologist.

Generally, during ECT procedure a 7-needle electrode, arranged in hexagonal geometry, is used to apply the electric fields to tumor tissue.[8,11] This type of electrode covers a surface close to 3 cm^2 at each single application.

Since it is well known that bleomycin maintains a sufficiently high concentration in tissue for a limited time interval (*i.e.*, 20 minutes according to Standard Operative Procedures[8,21]), the voltage pulses have to be applied within this interval. Consequently, large tumor-involved areas can be managed only by applying the 7-needle electrode several times during the 20-minute interval which follows the infusion of bleomycin. In theory, the standard hexagonal array needle electrode can be applied indicatively 100–120 times during a single ECT procedure, thus allowing for the coverage of approximately an area of 200–360 cm^2. In the clinical practice, this surface area may prove to be insufficient for effective treatment of patients with widespread skin tumor infiltration of the chest wall (Figure 1A).

As a consequence, a larger area can be managed only planning an additional ECT cycle, which inevitably will require a new anesthesia and an additional administration of drugs. As a consequence, many patients need to undergo repetitive ECT cycles in order to completely treat their chest wall recurrences or to treat newly occurred metastases outside treatment field (Figure 1A).

To avoid this problem, some electrodes designed to treat surfaces up to tens of cm^2 have been proposed.[22-24] Among these solutions, a composition of triangular configurations or arrays of parallel needles mounted on rigid support has been indicated[25-28]. An alternative strategy is represented by the use of planar antenna technology, thus avoiding needles insertion and developed in Nenzi *et al*.[29]

In this paper we present the prototype of a grid electrode suitable for not-plane surfaces such as chest wall and the treatment of recurrences from BC. The concept is based on a grid device including several electrodes arranged in a regular mesh (Figure 1). The new device is mounted on a flexible support that can be adapted to the skin surface and is equipped with removable needles that can be positioned one by one on the treating area.[44] As a result, the proposed device is a grid composed by a several needles that can be positioned before electric field application. For instance, the flexible version of the prototype tested in Ongaro *et al*. [30] has 13 electrodes and can cover an area of 50 cm^2. The treatment time in this case can be equivalent to one application of the 96 voltage pulses sequence prescribed by ECT Standard Operative Procedures[8,21] for hexagonal electrodes. Then, by using such de-

vice, the electric field could be applied in a shorter time interval compared with the currently used procedure which requires multiple, juxtaposed electrode placements (*e.g.* at least 16 applications for an area of 50 cm^2 considering the area covered by the standard electrode of 3 cm^2). In this paper, a prototype with 67 needles mounted in a flexible support covering an area of 225 cm^2 is proposed. The aim of this device is to apply the electric field more homogeneously in the treatment field and to respect the suggested time interval of 20 minutes after bleomycin infusion. Also the electric connection to the voltage pulse generator is here described.

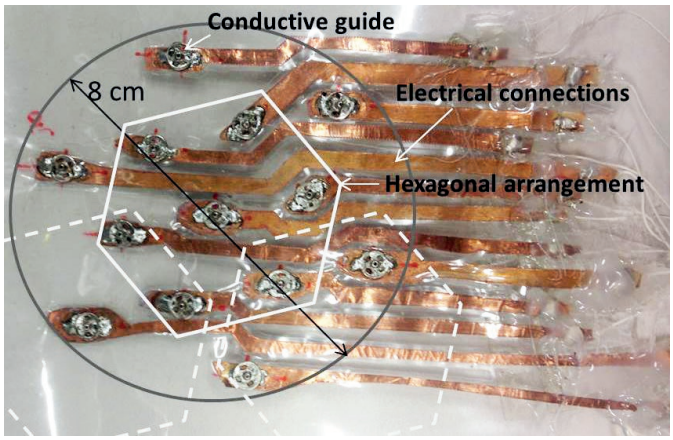
The prototype of the new flexible device consists of a grid of needles distant 2 cm apart, *i.e.* the needle configuration use hexagonal geometry of needle as in standard ECT needles, but their distance has been increased in order to reduce the number of needles per cm^2. The amplitude of the electric field generated by this needle configuration has been verified by simulation and compared with the one obtained with lower distance needles.[30] Moreover, the effect of the field in term of electroporated cells has been also verified using potato tissue tests and in vitro tests.[28,30,31] Finally, in order to verify the electroporation, the results of simple preliminary tests carried on vegetable tissue are presented.

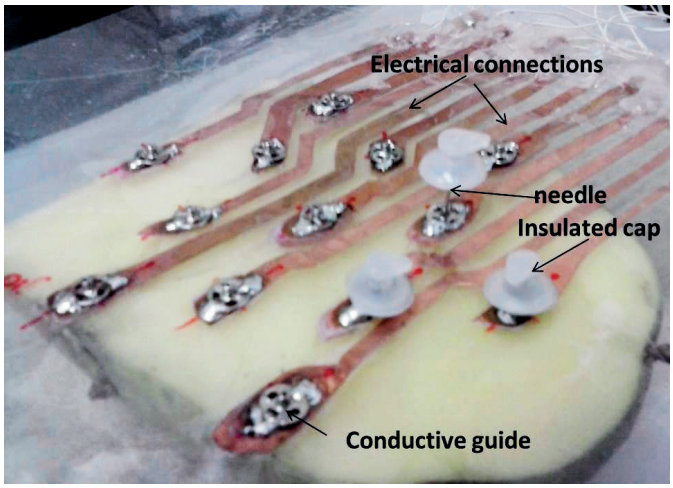
Materials and methods

The prototype of the grid electrode (Figure 2) is composed by a flexible support and 67 (5 or 10 mm-long) stainless steel needles that can be inserted one by one and linked to the electrical connection of the support.

The flexible support is equipped with some electrical conductive strips with some holes where the needles can be inserted. Each hole is provided with an insertion guide. The guides allow maintaining the perpendicularity of the needle with the surface where they are inserted. The guides center is positioned in points belonging to the vertex of adjacent equilateral triangles like in[28,30] with side 2 cm long.

The connection guides are arranged in hexagons as shown in Figure 2A in order to reproduce the standard hexagonal electrode geometry.[8,21,28,30] The guides allow both the insertion and electrical connection of the needle electrodes. The flexible plastic support is also provided with electric connections to the voltage pulses generator. The flexible electrode in Figure 2A has the electrical connections formed by copper strip, whereas in the electrode





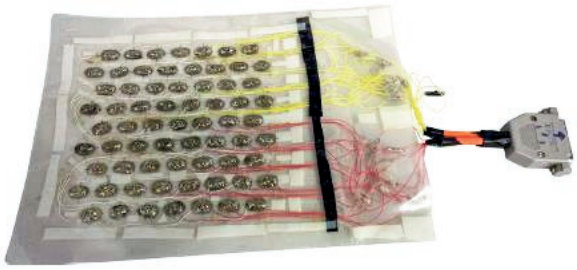


FIGURE 2. Prototypes of the flexible electrode: **(A)** device with diameter 8 cm with hexagonal electrode disposition, **(B)** removable needles inserted in electrical connection and **(C)** square device 15 x 15 cm with hexagonal arrangement of conductive guides highlighted.

of Figure 2C the electrical connections are made, for sake of simplicity, with copper wires. Figure 2B shows the insertion of the needles in the guides. The connection guides are realized in order to easily place and remove the needles. The needles are provided with an insulant cap (Figure 2B) and the flexible support by needle guides in order to allow the normal penetration of the tip. Moreover, for ensuring a user friendly, safe and effective appli-

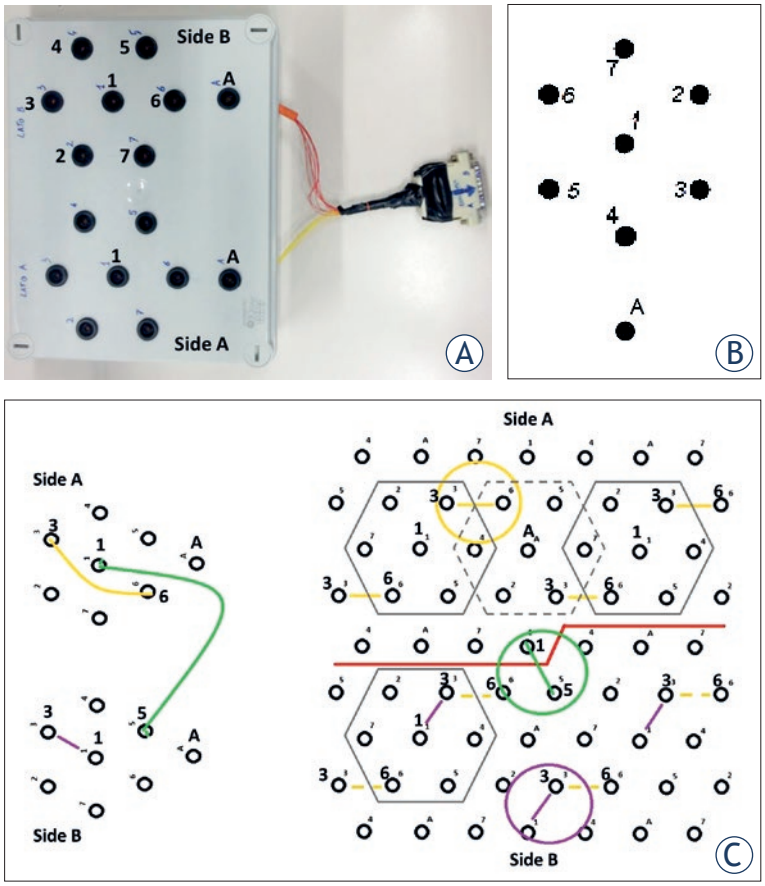


FIGURE 3. (A) Connection box to interface the flexible electrode to the voltage pulse generator. **(B)** Schema of the arrangement of clamps and **(C)** examples of connections.

TABLE 1. Example of supply needle pair sequence

#STEP	Needle pair	#STEP	Needle pair
#1	1–2	#14	A-3
#2	1–3	#15	A-4
#3	1–4	#16	A-5

cation on chest wall BC recurrences, needle length was limited to 5–10 mm. In practice, it is expected that, when the flexible plastic support is fixed on a curved surface (e.g. the thoracic wall), the short length of the needles and the 2 cm distance will limit the approach of their tips. Finally, the device is connected to the pulse generator by means of fast connectors.

The prototype of the grid electrode has been realized in two different sizes. The first has a diameter of 8 cm with 13 needles and the second one is a square of side 15 cm with 67 needles that covers an area of 225 cm^2.

Electrode supply

The new grid electrodes are supplied by a voltage pulse generator manufactured by Igea S.p.A. (Carpi (MO), Italy).[32,33] In each test performed the voltage generator delivers sequences of 10 rectangular pulses between 0 and 2000 V with pulse duration of 100 μs at 100 Hz (total time of 10 msec). In this work the voltage has been fixed to 2000 V for all test performed.

Figure 3A shows the connection device. It is a box equipped with 16 plug clamps that can be connected two by two to the voltage pulse generator. The 16 clamps are arranged in groups of 8 each one identified by a number except the eighth that is identified by the letter 'A' like in Figure 3B.

The needles in the flexible electrode are arranged in hexagon and numbered consequently following the scheme in Figure 3B. This schema is formed by a hexagonal structure of numbered points and a point named 'A' (corresponding to the clamping numbers). Continuous gray hexagons in Figure 3C highlight the hexagonal scheme in Figure 3B in which the central electrode is named '1'. Considering the dotted hexagons, the central point is named 'A', whereas the other points of the hexagon correspond to the points identified by numbers. Figure 3C shows also the connection scheme of the flexible grid electrode with 67 needles to the voltage pulse generator. Some examples of supplied electrode pairs are presented in the same Figure. For instance, Table 1 reports the supply sequence where the needle pairs selected involve needle '1' or needle 'A'.

The clamps are subdivided in two groups that supply a half of the needle pairs. In fact, since the average resistance at electrode ends, R_e, is 130–200 W (value measured at needle extremities during pulses application) considering a current of 40 A (maximum value deliverable by the voltage pulse generator, I_{max}) and an applied voltage, V_A, of 2000 V the maximum number of electrode pairs that can be parallel connected, N_m, is between 3 and 5.[28]

$$N_m = R_e \frac{I_{max}}{V_A} \quad [1]$$

For instance, like shown in Figure 3C for the pair 3–6, the number of electrode pairs between needles with the same number is up to 8. Consequently, the larger grid electrode is divided into two areas where there are up to 4 electrode pairs between needles with the same number.

Each group of eight clamps in the box in Figure 3A supplies half of the grid electrode, and

the electrode pairs on the boundary are supplied by connecting two clamps belonging to different group as it has been highlighted for pair '1–5' in Figure 3C. All the electrodes, both the activated and not-supplied ones, are inserted in the treating area before pulse delivery. They remain inserted during the entire treatment. In fact the influence of the not-supplied electrodes is irrelevant, as reported in a previous work, since they act as an 'open circuit'[30] and any current can flow.

Potato test procedure

The two models of the grid electrode have been tested on a phantom made of potato tissue. In fact, it is well known that after few hours of application of voltage pulses, the potato tissue appears dark if cell membranes have been electroporated.[28,30,34,35] For instance in Castiello *et al*.[28] and Ongaro *et al*.[30] have observed potato color after 24 h even if the darkening started after few hour after pulses application.

Voltage pulses have been applied to the potato tissue using the pulse generator and applying 2000V at each electrode pair following the pair-sequence described in Standard Operative Procedure.[8,21] A sequence of 10 pulses has been applied to each possible electrode pair. Potatoes tissue was preserved at room temperature for 24 hours after application of voltage pulses and then observed looking for the electroporation effect; pictures of the investigated tissue were also taken (Figure 4A,B). The electroporated potatoes appear dark only in the areas where the voltage pulses have been applied (Figure 4A), whereas any color change did not occur for the potato cut and maintained at room temperature (Figure 4B) as described in Ongaro *et al*.[30]. The intense dark coloration is due to the electroporation and not to the cut. Nevertheless, this technique cannot discriminate between reversible or irreversible electroporation and is a simple qualitative test to visualize the area where the electroporation was occurred.

The two prototypes of the device have been tested on two type of phantoms: a single potato tuber for the 13 needles electrode, whereas the larger device with 67 needles has been tested using more potatoes piece immerged in Meat Liver Agar gel (46379 Fluka Analytica) dissolved in hot water at 5% and cooled at room temperature. Agar gel allows electrical conduction between adjacent potato pieces. Potatoes are arranged as shown in Figure 5A.

The flexible electrode is positioned over the phantom, a single half of potato tuber for the 13 needle device or the arrangement in Figure 5A for the large electrode. The needles are positioned manually one by one in each contact as in Figure 5B. Figure 5C shows the flexible electrode with 67 needles ready to apply the voltage pulses to all possible needles pairs. Finally, Figure 5D shows the connection of larger flexible electrode to the connection box.

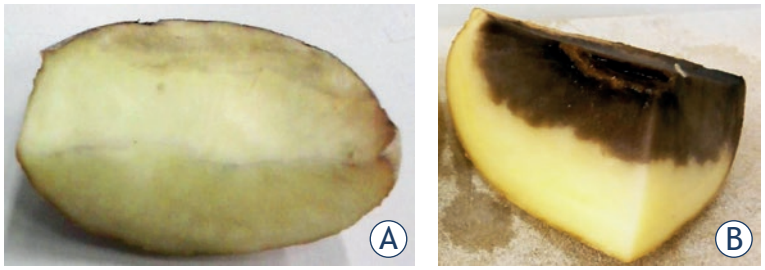


FIGURE 4. Potato tuber after 24 h preserved at room temperature: **(A)** no voltage pulses (control) and **(B)** treated with voltage pulses.

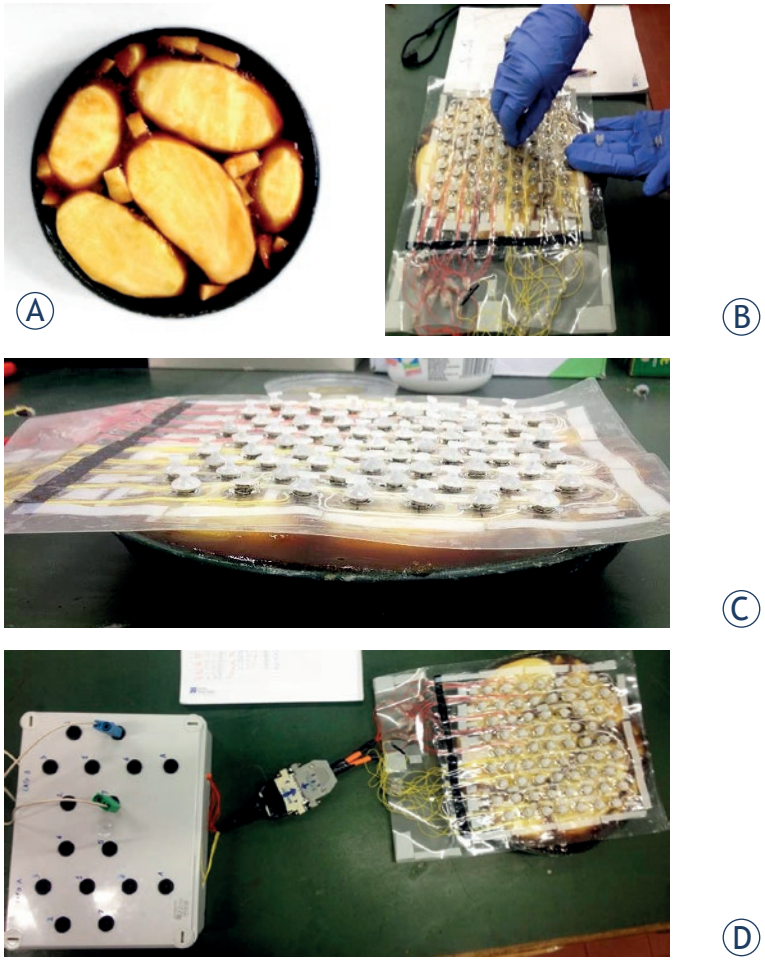


FIGURE 5. (A) Phantom used to test the square flexible electrode with side 15 cm. **(B)** Manual insertion of needles, **(C)** device ready for pulse application and **(D)** connection of the electrode with the connection box.

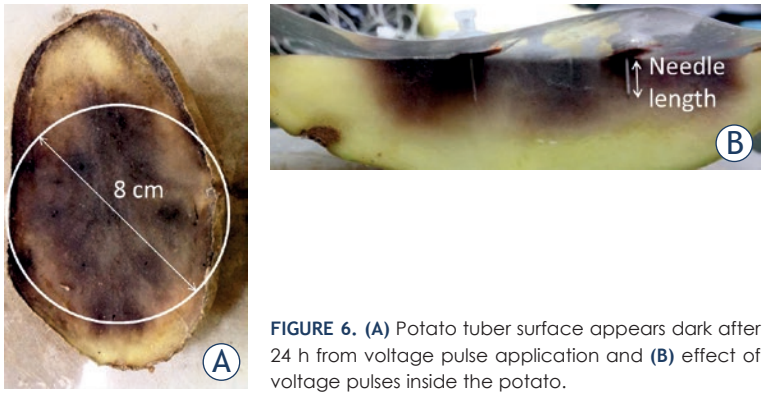


FIGURE 6. (A) Potato tuber surface appears dark after 24 h from voltage pulse application and **(B)** effect of voltage pulses inside the potato.

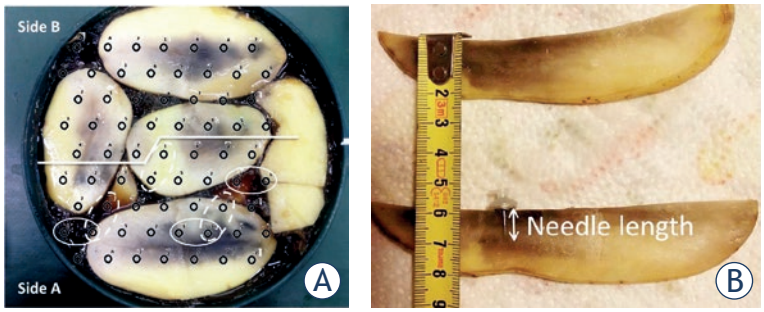


FIGURE 7. (A) Potato phantom surface appears dark after 24 h from voltage pulse application and **(B)** effect of voltage pulses inside the potato.

Results

Figure 6 shows the electroporation effect observed 24 hours after the application of voltage pulses by means of the 13-needles flexible electrode presented in Figure 2A. The 15 cm^2 area of potato surface appears dark (Figure 6). After 24 h the potato has been cut vertically in order to evaluate the effect of electroporation inside the phantom. The tissue appears dark for a depth larger than the electrode length as shown in Figure 6B. In this case, the needle is 10 cm long and the dark area has a depth double than the needle length (close to 20 mm). This effect has been described also in Castiello *et al*.[28] and in Ongaro *et al*.[30] In fact, the electrode distance affects the electric field distribution in the treated volume: using electrode with a larger distance the electric field can penetrate more deeply in the tissue as it has been demonstrated in Ongaro *et al*.[30] by means of numerical modeling by means of finite element method.[30,36-39]

Figure 7A shows the surface of phantom in Figure 5A used to test large electrode, captured 24 hours after voltage pulse application. It is evident the electroporation effect produced by the device from the dark color of the tissue surface. Nevertheless, in some areas the electroporation did not occur. Considering the pairs underlined with continuous or dotted line in Figure 7A, it appears that in these cases almost one needle pair is entirely immersed in Agar gel that is more conductive than potato tissue. In these cases, the pulse generator has not delivered the current to the load because the internal control of the device has measured a current greater than the maximum allowed (I_{max} = 40 A).

Finally, pieces of potato in the phantom have been vertically cut 24 h after the voltage pulse application showing the appearance of dark areas inside the tissue.

The electrode has 67 needles and can treat an area of 225 cm^2 (a square with side of 15 cm). Also in this case the electroporation depth is larger than the electrode length as shown in Figure 7B, where the needle length is 10 mm and the electroporation depth is 20 mm.

Tests on potato tissue are very easy and cheap. Nevertheless, they are not sufficient to explore if the electroporation is reversible or irreversible even though some authors have tried to couple simulation results and dark color gradation[40-42] and searched for the dark intensity for which the electric field overcame the irreversible electroporation threshold. The evidence whether electropoation effect is reversible or irreversible can be obtained by means of in-vitro tests using cell culture as reported in previous experiences.[30,31]

Discussion

Chest wall recurrence from BC represents a peculiar type of tumor recurrence, and, when superficial and widespread (due to lymphangitic diffusion), it is crucial, during ECT, to apply electric fields on a wide and thin surface instead of a target volume.

The preliminary experimental results show that the flexible device is able to electroporate larger tissue surfaces with respect to the standard hexagonal electrode. In fact, the designed device covers an area from 50 cm^2 (by using 13 needles) to 225 cm^2 (by using 67 needles). From a practical point of view, the plastic flexible support allows the adaptability to non-planar surfaces as the chest wall and can be applied to the skin as a plaster. Its main drawback is represented by the loss of parallelism between adjacent needles because of the curved surface. Nevertheless, this kind of device has been primarily conceived to treat superficial tumors of the chest wall, which has a limited radius of curvature; moreover, this an anatomical area, especially

in mastectomy patients, is characterized by the presence of a rigid, underlying plane represented by the rib cage, which limits the penetration of needle electrodes. Moreover, and importantly, tumor growth in these patients follows a superficial pattern of spread and tumor thickness is limited to 4–5 mm from the superficial skin layer; consequently, needle electrode should be inserted only few millimeters, thus limiting the convergence of their tips. With the proposed device, needle electrodes can penetrate only few millimeters (maximum 10 mm), and the distance between two points of insertion was fixed at 20 mm, so that the effect of parallelism loss is limited.

On the other hand, single needle insertion may be more user friendly for the clinician in presence of fibrous tissue. In fact, in this case, it is simpler to insert one needle instead of 7 needles at the same time. Moreover, each needle is inserted using a rigid guide provided by the insertion mask in order to allow parallel penetration of tips. Moreover, the device has been designed to use needles with a length between 5 and 10 mm, so that the effect on electric field intensity due to tip convergence should be limited. During the procedure, when the needle tips are too close (*e.g*. by visual inspection or by resistance evaluation before pulse application), applied voltage can be accordingly reduced.

The device and its positioning have been designed in order to contain the time of ECT procedure and to permeabilise tumor cell membrane when drug concentration is higher. To make electrode application time-sparing, the needles of the new device can be pre-positioned on the flexible support, before pulse delivery. In this way, the repetitive, operator-dependent, placement and displacement of standard electrodes could be avoided and voltage pulse delivery may be performed within the optimal time interval from the infusion of bleomycin.

Authors are aware that the present study has several drawbacks. In fact, the phantom used to test the electrode shows areas with large difference in conductivity, likely due to Agar gel between potato pieces. These areas have caused the failure of the electroporation procedure since the pulse generator in some case has not delivered voltage pulses for its proper current limitation (internal control detected low impedance and blocked pulse delivering). Nevertheless, in previous works a lower scale device with 52 needles 1 cm apart has been already tested on a single potato tissue and experimental results are in Ongaro *et al*.[28]. These tests on single potato piece did not show any drawback due to high conductivity area.

Since, the designed electrode can be considered an extension of existing devices for clinical ECT treatments (*e.g*. hexagonal array electrodes), then current and electric field thresholds are borrowed from standard protocol for ECT.[4,8,20,21,43]

In future experiments, the new grid electrode will be tested in animal models in order to verify its efficacy. Moreover, it will be tested also a prototype with more distant needles (2 cm apart) with respect to standard electrode as well as the increment of electric field depth, up to two times the electrode length shown in Figure 7B.

The flexible device has the potential to reduce the time required for the application of electric pulses. For instance, the 13 needles device which, can treat an area of 50 cm^2, is supplied by means of two 96-pulse sequences for hexagonal electrode described in Standard Operative Procedures.[8,21] Therefore, the 50 cm^2 area can be treated in less than 2 min (one 96-pulse sequence has a time duration approximately of 20 ms) considering the time to arm the pulse generator. Moreover, the 67 needles electrode, which covers an area of 225 cm^2, can be supplied by means of 5 sequences of 96 pulses as described in the Electrode supply paragraph. Considering the time to change connections and arm the pulse generator, the treatment can be performed in approximately 5 min. This short time may assure a higher drug availability in tumor tissue during tumor electroporation. The new device is advantageous since the clinician can repeat the 96-pulse sequence up to 150 times moving the standard electrode several times to cover all the chest wall surface. Considering, for instance, an application time of 20 ms per sequence (*i.e*. 96 pulses) and the time required to move the electrode and arm the generator of approximately 15s it gives a treatment time of approximately 40 min.

Conclusions

A flexible device has been designed in order to manage chest wall recurrence from BC, which usually involves large skin areas in mastectomy patients. A prototype of a grid electrode aimed to treat widespread superficial tumors has been designed and tested in preclinical preliminary experiments. This report presents the results obtained with a flexible device that can be used to treat by means ECT skin areas between 50 cm^2 and 225 cm^2. The tip approach effect is limited by needle distance, which

was fixed to 2 cm, and by needle lenght, which was set between 5 and 10 mm. Moreover, the possibility to pre-insert all the needles before pulse delivery has several advantages: it may allow to reduce the duration of the procedure and anesthesia, to expose tumors to higher drug concentration and, hopefully, to increase the antitumor effectiveness of ECT in a challenging subgroup of BC patients.

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research article

Combined local and systemic bleomycin administration in electrochemotherapy to reduce the number of treatment sessions

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Background. Electrochemotherapy (ECT), a medical treatment widely used in human patients for tumor treatment, increases bleomycin toxicity by 1000 fold in the treated area with an objective response rate of around 80%. Despite its high response rate, there are still 20% of cases in which the patients are not responding. This could be ascribed to the fact that bleomycin, when administered systemically, is not reaching the whole tumor mass properly because of the characteristics of tumor vascularization, in which case local administration could cover areas that are unreachable by systemic administration.

Patients and methods. We propose combined bleomycin administration, both systemic and local, using companion animals as models. We selected 22 canine patients which failed to achieve a complete response after an ECT treatment session. Eleven underwent another standard ECT session (control group), while 11 received a combined local and systemic administration of bleomycin in the second treatment session.

Results. According to the WHO criteria, the response rates in the combined administration group were: complete response (CR) 54% (6), partial response (PR) 36% (4), stable disease (SD) 10% (1). In the control group, these were: CR 0% (0), PR 19% (2), SD 63% (7), progressive disease (PD) 18% (2). In the combined group 91% objective responses (CR+PR) were obtained. In the control group 19% objective responses were obtained. The difference in the response rate between the treatment groups was significant ($p < 0.01$).

Conclusions. Combined local and systemic bleomycin administration was effective in previously to ECT non responding canine patients. The results indicate that this approach could be useful and effective in specific population of patients and reduce the number of treatment sessions needed to obtain an objective response.

Key words: electrochemotherapy; combined treatment; systemic and local; bleomycin; resistant

Introduction

Electrochemotherapy (ECT) is an ablative approach that is rapidly growing, both in human and veterinary medicine. ECT is based on administration of bleomycin followed by application of an electric field on the tumor that enhances cell permeability to the drug. This technique can increase bleomycin cytotoxicity by 1000 fold. The effectiveness of ECT is approximately 80% objective response (OR) rate.[1,2]

A meta-analysis of ECT clinical studies in human oncology showed that the overall OR rates vary from 62.6% and 82.2% OR rate depending also on the route of the drug administration, being either intravenous or intratumoral.[3] Despite its

success related to its low cost and minimum side effects, ECT still has room for improvement. Even with such a high response rate there are 20% of cases on which attention must be focused in order to improve the outcome of the treatment.

The application of ECT in companion animals showed the same pattern of success as in humans, with many studies demonstrating its high efficiency, with a very similar response rate to that of human patients.[4,5]

The use of companion animals with spontaneous tumors as models for tumor treatment therapy became a generalized practice due to its many advantages. The most important is that these tumors behave similarly to human ones and are thus better preclinical models for testing new therapies. As these animals were exposed to environmental carcinogens, they developed the tumors in the context of an intact immune system that has the same tumor-host interactions.[6,7]

A study on melanomas in dogs conducted by Spugnini *et al*. reported 80% effectiveness.[8] Another study by Tamzali *et al*. on spontaneouslyoccurring tumors showed very high effectiveness when treating sarcoid tumors in equines using ECT with local cisplatin in up to 6 sessions of ECT.[9] A ganglioneuroblastoma case was published in which a cat with a very small tumor was treated with up to 3 sessions of ECT in order to obtain an OR.[10] In large tumors, however, it is often the case that no OR is possible with a single treatment session.[11,12]

Systemic bleomycin administration consists of injecting the drug into a vein, thus allowing the drug to reach the tumor through the bloodstream and diffuse from the vessels into the tumor.[13] On the other hand, local bleomycin administration consists of directly injecting the drug into the tumor tissue, thus allowing it to diffuse from the injecting point to the target. Multiple injections into the tumor can provide an adequate coverage in small tumors[13], but the case of large tumors is different where it is very difficult to homogeneously cover them. Tumor vasculature is structurally and functionally abnormal; blood vessels leak and are tortuous, dilated, and saccular and have a random pattern of interconnection.[14] In solid tumors, these aberrant vessels determine an increase in the liquid outlet out of these, together with the contribution of the compression caused by the proliferation of cancer cells, leading to an increase in interstitial hydrostatic pressure.[15] The heterogeneous flow of blood and interstitial hypertension pose a serious obstacle to the antineoplastic agents, especially in the case of large tumors with a broader vascular system that are more likely to have areas of tumor that cannot be reached by the systemic route.[16,17] This characteristic of tumor vessels could lead to an insufficient bleomycin distribution when administered systemically. Repeated ECT sessions could lead to modifications in the characteristics of the tumors, such as its size reduction and changes in its vasculature that improve treatment response after each session. For these reasons, performing many treatment sessions can improve the results obtained in the first session, increasing, however, the cost of the treatment and its risks related to multiple anesthetic procedures. To address this problem, here, we propose combined bleomycin administration, both systemic and local, using companion animals as models for ECT tumor treating.

The aim of this study was to determine whether it is possible to reduce the number of treatment sessions using a combined administration of bleomycin (both systemic and local) *vs.* systemic bleomycin administration alone in ECT. Accordingly, for the purpose of this work, we selected companion animals with spontaneous tumors.

Patients and methods

Patients

Consent was obtained from the dog's owner to use the dog's image in this scientific work and for the treatment of the other patients. In all cases, all recommendations from the Consejo Profesional de Medicos Veterinarios de Buenos Aires (Buenos Aires Veterinary Council) were observed, as well as the relevant local legislation in Argentina, Act No. 14072 which governs veterinary medicine practice.

Twenty-two patients from the oncology service from the Centro de Epecialidades Medicas Veterinarias (CEMV), Buenos Aires, Argentina, were selected. These patients had tumors of a varied histology and had failed to achieve a complete response after an ECT treatment session. We divided them into two groups: eleven received combined bleomycin administration in a second treatment session, and 11 underwent another standard ECT session (control group). The first ECT session in both groups and the second ECT session in the control group were performed in accordance with the Standard Operating Procedure for Electrochemotherapy.[13] The patients were allocated on a 'first come, first served' basis to the control group first, and from the eleventh patient onwards, they were allocated to the combined administration group. The size of their tumors was

calculated by multiplying their two diameters and their height.

The patients underwent a full clinical examination, blood samples were taken, and a biopsy for histological confirmation of the tumor was performed. The histological analysis of the biopsies was performed with hematoxilin-eosin staining. Most patients treated in the first session were expected to require further ECT sessions in order to obtain an objective response because of their tumor size.

Treatment procedure

General anesthesia procedure consisted of premedication with 0.5 mg/kg of xylazine (Xilacina 100®, Richmond, Buenos Aires, Argentina), 2 mg/kg of tramadol (Tramadol®, John Martin, Buenos Aires, Argentina) and induction with 3 mg/kg of propofol (Propofol Gemepe®, Gemepe, Buenos Aires, Argentina). Then maintenance was assured with 2–3% of isofluorane (Zuflax®, Richmond, Buenos Aires, Argentina) and 2 mcg/kg of fentanyl (Fentanilo Gemepe®, Gemepe, Buenos Aires, Argentina). Meloxicam (Meloxicam Denver Farma®, Denver Farma, Munro, Argentina) 0.2 mg/kg was administered for analgesia after the treatment. This scheme of anesthesia provided adequate comfort during the treatment. Prophylactic antibiotic amoxicillin/clavulanic acid (Clavamox® Zoetis®, San Isidro, Argentina) 12.5mg/kg/bid was administered.

ECT with systemic bleomycin administration alone was performed as follows: the patient was anesthetized using general anesthesia, after an intravenous bolus of bleomycin (Blocamicina®, Gador, Buenos Aires, Argentina) at a dose of 15 000 IU/m^2 BSA in 30–45 seconds was administered. Eight minutes after the intravenous injection, to allow drug distribution, the pulses were delivered covering the whole tumor surface.

ECT with systemic and local bleomycin administration was performed as follows: the patient was anesthetized using general anesthesia. An intravenous bolus of bleomycin (Blocamicina®) at a dose of 15 000 IU/m^2 BSA in 30–45 seconds was administered, after a local injection of bleomycin (Blocamicina®) at a dose of 125 IU/cm^3 of tumor was administered.[13] The drug was injected into the tumor using a 27G 2.5 cm needle (Terumo, Tokyo, Japan) in a 3 ml syringe (Darling, Korea), and for an even distribution of the drug, the injections were placed 5 mm apart in one plane and 2 or 3 planes of injections were placed 1 cm apart according to the size of the tumor. The injections started at the center of the tumor and continued at its periphery.[9] Healthy margins were not injected with bleomycin since they are covered by the systemic administration of the drug; there are no vascular abnormalities in healthy tissue to justify the additional administration.

The pulses were administered using a six needle electrode, consisting of three rows of two needles 2 cm long and 1 mm diameter, each row separated by 4 mm and each column separated by 8 mm. The pulse generator used was a BTX ECM 830 (Harvard Apparatus, Holliston, MA, USA). A train of 8 electric pulses (1000 V/cm, 100 microseconds, 10 Hz) was applied, covering the whole tumor[13], beginning at the periphery of the tumor in a circular fashion in order to have maximum drug concentration at the margins and prevent the spreading of tumor cells. The superposition of electric fields was avoided in order to prevent overtreatment of the lesions.

The response to each treatment was evaluated according to the WHO criteria for tumor response[18], 30 days after the treatment. A complete response (CR) is obtained when there is a complete disappearance of all known disease, a partial response (PR) when there is a 50% reduction of the tumor or more, a stable disease (SD) when PR or PD criteria are not met, and a progressive disease (PD) when there is a 25% or more increase in the size of the tumor, and no CR, PR or SD is documented before the increase of the disease or new lesions appear. All of this must be confirmed within 4 weeks after the treatment.

After the treatment, the patients returned to the veterinary clinic within 7, 15, 21, 30 and 60 days in order to evaluate response, toxicity and side effects by means of a full clinical examination and questions to their owners.

Results were compared and statistical significance was evaluated using the chi square test.

Results

The total dose of bleomycin in combined treatment was slightly higher than that of systemic administration alone; in both cases, no toxicity or side effects were reported. Table 1 shows the response of the patients in which combined treatment was performed in the second session. Table 2 shows the control group, for which patients the second session was a repetition of the first procedure.

The responses obtained with combined bleomycin administration were significantly different

TABLE 1. List of group 1 patients treated using combined systemic and local bleomycin administration in the second treatment session

Patient	Breed	Location of the tumor	Weight (kg)	Histology	Stage	Size (cm^3)	Response 1 (ECT)	Response 2 (S+L)
1	Labrador retriever	Oral	32	Mastocytoma	II	10.6	PR	CR
2	Cross-breed	Oral	21	Squamous cell carcinoma	II	36.2	SD	PR
3	Labrador retriever	Nasal	32	Squamous cell carcinoma	II	43.5	PR	CR
4	Yorkshire	Perianal	5	Solid differentiated carcinoma	IV	173.8	SD	SD
5	Cross-breed	Elbow	12	Schwannoma	I	67.6	SD	PR
6	Rottweiler	Oral	37	Fibrosarcoma	I	109.5	SD	CR
7	Labrador retriever	Nasal	38	Squamous cell carcinoma	III	42.4	SD	PR
8	Boxer	Oral	37	Fibrosarcoma	III	112.2	SD	PR
9	Cocker spaniel	Oral	15	Melanoma	II	8.7	PR	CR
10	Beagle	Oral	16	Melanoma	III	12.4	PR	CR
11	Cocker spaniel	Oral	16	Melanoma	III	26.64	PR	CR

CR = complete response; ECT = electrochemotherapy; PR = partial response; SD = stable disease; S+L = systemic + local

from those of systemic administration alone in selected cases ($p < 0.01$). In the combined administration group the following response were obtained: CR 54% (6), PR 36% (4), SD 10% (1). In the control group the obtained response were: CR 0% (0), PR 19% (2), SD 63% (7), PD 18% (2). Figure 1 shows a case treated using combined intravenous and intratumoral bleomycin administration in which a CR was obtained.

The OR rates obtained were significantly better when using combined treatment compared with the standard ECT treatment ($p < 0.01$). As seen in Figure 2, in the combined group, 91% (10) of OR (CR+PR) were obtained, and 19% (2) were obtained in the control group.

It is worth noting that no complete responses were obtained in the control group with two sessions of ECT, as opposed to 54% of CR obtained when applying combined treatment in the second session.

The average tumor size in the control group was 99.9 cm^3, while it was 58.5 cm^3 in the combined group. In general, the patients were at a lower stage of the disease in the control group compared with the combined group.

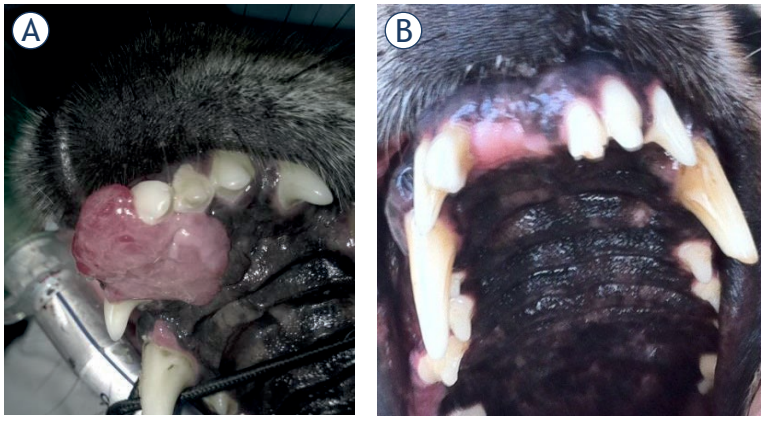

FIGURE 1. Case number 6. **(A)** before combined treatment, a fibrosarcoma which failed to respond to the first ECT treatment. **(B)** CR was obtained after combined treatment.

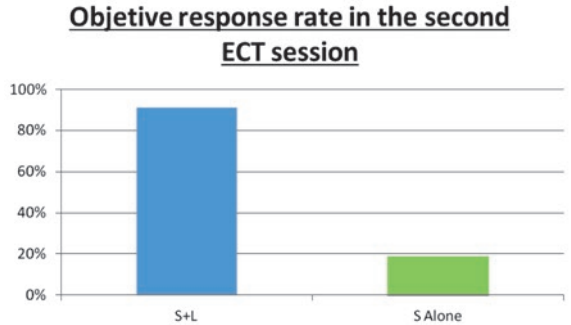

FIGURE 2. Graph shows the objective response rate obtained in the second session, in a comparison between combined bleomycin administration, both systemic and local (S+L), and systemic alone (S Alone).

ECT = electrochemotherapy

Discussion

ECT is based on a physical phenomenon, electroporation, which acts directly on cell membranes, which accounts for its effectiveness in practically all histological types of tumors. In our experience with veterinary patients, we found that large tu-

TABLE 2. List of group 2 patients (control) treated using a repetition of the first session

Patient	Breed	Location of the tumor	Weight (kg)	Histology	Stage	Size (cm^3)	Response 1 (ECT)	Response 2 (ECT)
12	Cross-breed	Oral	30	Melanoma	I	158.2	PR	SD
13	Cross-breed	Oral	21	Sarcoma	III	79.76	PR	SD
14	Cross-breed	Oral	20	Carcinoma	III	96.5	PR	SD
15	Toy Poodle	Oral	5	Fibrosarcoma	II	23.23	PD	PD
16	Cross-breed	Oral	11	Melanoma	II	73.8	PR	SD
17	Cross-breed	Oral	16	Schwannoma	II	467.02	PD	SD
18	Cross-breed	Oral	6	Squamous cell carcinoma	II	12.32	SD	SD
19	Labrador retriever	Oral	32	Fibrosarcoma	II	40	PR	SD
20	Rottweiler	Oral	34	Melanoma	II	33	PR	PR
21	German Shepherd	Oral	39	Fibrosarcoma	II	101.18	PR	PD
22	Cross-breed	Oral	14	Melanoma	II	14.4	PR	PR

CR = complete response; ECT = electrochemotherapy; PD = progressive disease; PR = partial response; SD = stable disease; S+L = systemic + local

mors have poorer responses and require further sessions to obtain an objective response. Our hypothesis was that the abnormal vasculature of large tumors impedes proper drug distribution when it is administered intravenously, even though this route of drug administration is prescribed for tumors of this size in standard operating procedure (SOP).[13]

Based on this hypothesis, we decided to make an approach by combining both systemic and local bleomycin administration to improve drug distribution in the tumor. In this way, local administration can cover areas where vasculature proves insufficient. There are many reasons against considering using a local injection alone to improve results. According to literature, in tumors above 2 cm in diameter, intravenous administration is recommended.[13] It is highly challenging to provide proper drug distribution in the tumor by using local administration only, because during its local application, it is easy to leave sections without the adequate drug concentration, and sometimes it is even impossible to reach the base of the lesion.

It is worth mentioning that some authors obtained good response rates with several treatment repetitions. These repetitions lead to changes in the tumor that can improve drug distribution in later applications.[12,19-22] Here, we obtained good results with only one repetition.

Tamzali *et al*. obtained very good results with a local injection of cisplatin in multiple applications treating sarcoids. It is important to take into account that this kind of tumors behave like benign tumors, thus giving a veterinarian time to perform multiple treatments. Our scenario is different since these kinds of tumors are significantly large, and the survival of the patients is compromised, so we need to reduce the tumor as fast as possible in order to improve their quality of life. Frequently, patients with large tumors are in bad clinical shape, so it is important to reduce the number of treatment sessions in order to reduce the risk of anesthetic procedures. On the other hand, costs are also a very important issue, as performing many sessions of treatment increases the cost of the procedure, and makes it rather impossible with our resources.

The fact that the tumors in the combined group were smaller could contribute to better responses achieved, but we also have to consider that the stages were higher. Tumor size rather than disease stage is likely to be a better prognostic factor in ECT, but this speculation is yet to be confirmed.

Further study is needed in order to determine in difficult cases whether practitioners should firstly try ECT with systemic bleomycin alone, or directly apply ECT with its combined systemic and local administration. Since the dose of bleomycin used is very low, the greatest risk of the ECT procedure lies in the application of anesthesia. Reducing anesthesia procedures outweighs the risk of adverse reactions related to the accumulated dose of bleomycin.[23,24]

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review

Medical physics in Europe following recommendations of the International Atomic Energy Agency

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Background. Medical physics is a health profession where principles of applied physics are mostly directed towards the application of ionizing radiation in medicine. The key role of the medical physics expert in safe and effective use of ionizing radiation in medicine was widely recognized in recent European reference documents like the European Union Council Directive 2013/59/EURATOM (2014), and European Commission Radiation Protection No. 174, European Guidelines on Medical Physics Expert (2014). Also the International Atomic Energy Agency (IAEA) has been outspoken in supporting and fostering the status of medical physics in radiation medicine through multiple initiatives as technical and cooperation projects and important documents like IAEA Human Health Series No. 25, Roles and Responsibilities, and Education and Training Requirements for Clinically Qualified Medical Physicists (2013) and the International Basic Safety Standards, General Safety Requirements Part 3 (2014). The significance of these documents and the recognition of the present insufficient fulfilment of the requirements and recommendations in many European countries have led the IAEA to organize in 2015 the Regional Meeting on Medical Physics in Europe, where major issues in medical physics in Europe were discussed. Most important outcomes of the meeting were the recommendations addressed to European member states and the survey on medical physics status in Europe conducted by the IAEA and European Federation of Organizations for Medical Physics.

Conclusions. Published recommendations of IAEA Regional Meeting on Medical Physics in Europe shall be followed and enforced in all European states. Appropriate qualification framework including education, clinical specialization, certification and registration of medical physicists shall be established and international recommendation regarding staffing levels in the field of medical physics shall be fulfilled in particular. European states have clear legal and moral responsibility to effectively transpose Basic Safety Standards into national legislation in order to ensure high quality and safety in patient healthcare.

Key words: medical physics; Europe; International Atomic Energy Agency; recommendations; basic safety standards

Introduction

Medical physics is a dynamic and constantly growing field of applied physics mainly directed towards the application of physics principle to health care in order to ensure safety and quality in diagnostic and therapeutic procedures involving the application of ionizing radiation. Medical physics traditionally covers four main areas of applied physics in medicine:

1. Diagnostic and interventional radiology physics
2. Radiation oncology/radiotherapy physics

3. Nuclear medicine physics
4. Radiation protection physics sometimes referred also as health physics

Within these four subspecialties, medical physicists are involved in four basic activities: clinical service, research and development, teaching and management/administration. Although mentioned specialties of medical physics cover almost completely the area of medical physics profession, medical physicists are and where appropriate should be involved in other applications of physics in medicine as well, such as ultrasound imaging, magnetic resonance imaging, bioelectrical investigation of the brain and heart (electroencephalography and electrocardiography), bio magnetic investigation of the brain (magneto encephalography) applications of lasers in medicine and medical informatics.[1] Within the present discussion we limit ourselves to the application of ionizing radiation to medicine.

Over hundred years ago three major events opened doors of medicine to applied radiation physics: discovery of x rays by Wilhelm Conrad Roentgen in 1895, discovery of natural radioactivity by Henry Becquerel in 1896 and discovery of radium by Pierre and Marie Curie in 1898, followed in 1934 by the discovery of artificial radioactivity by Irene Curie and Frederic Joliot, resulting from the creation of short-lived radioisotopes from the bombardment of stable nuclides and the advances in radar and radiofrequency technology during World War II that made linear accelerators development possible. Since then physics has started to play an important role in medicine for routine use of ionizing radiation in medical diagnostic and therapy. Over the last few decades we were witnessed of enormous development of radiation medicine, mainly through the technological development of the equipment which is used for accurate diagnostic or therapeutic procedures: optimization of image quality for computed tomography and magnetic resonance imaging, development of radiation therapy equipment (high energy linear accelerators with sophisticated options for dose delivery, computerized treatment planning systems, record and verify systems, etc.) and overall integration of computers into the routine clinical work. This is reflected in the huge increase of medical radiological procedures in the world; presently there are around 4 billion x-ray examinations, 35 million nuclear medicine examinations and 5 million radiotherapy courses undertaken annually.

Such tremendous development has triggered demands and need for more highly educated and well trained medical physicists. Introduction of formal systems for education and clinical training became crucial and many universities in Europe offer academic programmes in medical physics. However, there is still a lack of accredited clinical training programs in the majority of countries in Europe. Although international and European professional medical physics organizations together with European Commission (EC) and International Atomic Energy Agency (IAEA) have undertook efforts to raise awareness of national authorities across Europe regarding the role and the importance of medical physics in radiation medicine it seems, that these efforts have not been fully successful. There is still no harmonization and full recognition of medical physics profession in Europe, there is still shortage of well-educated and clinically trained medical physicists, there are still lack of educational frameworks and structured clinical training programmes in several European countries, there are difficulties to implement continuous professional development (CPD) systems and unfortunately, there are still reports and news about incidents and accidents in the field of radiation medicine.[2-8]

This review has no intention to cover current status of medical physics in Europe, neither has the ambition to discuss medical physics history or its future perspectives and importance in radiation medicine. The subject is far too broad and complex and it is described and discussed in depth in general medical physics textbooks and international literature.[9-11]

The main purpose of this paper is to present comments on most recent recommendations from the IAEA after the *"Regional Meeting on Medical Physics in Europe: Current Status and Future Perspectives"* held in Vienna from 7th to 8th May 2015. Invited representatives - over 60 from more than 30 European countries - from World Health Organization (WHO), international professional organizations and societies (International Organisation for Medical Physics – IOMP, European federation of Organisations for Medical Physics – EFOMP and European Society for Radiotherapy and Oncology – ESTRO) , national regulatory bodies and Health Ministries and representatives of medical physicists, were discussing the current status and future perspectives of medical physics in Europe.[12]

The recommendations of the IAEA Regional Meeting, serving as an outcome of the meeting, are presented in original form and are the bases of the paper, while notes and observation from the same document were omitted due to the journal space considerations.[13]

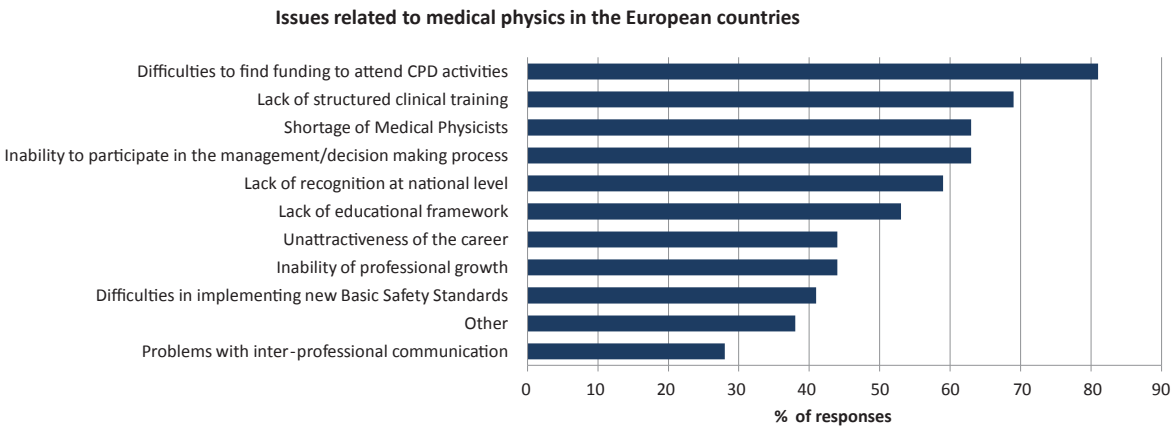

FIGURE 1. Issues/difficulties in medical physics identified in IAEA/EFOMP survey in 2015 (Damilakis J, Lopes M. C. Overview of medical physics status and future prospects: Results of survey in Europe. "Regional meeting on Medical Physics in Europe: Current Status and Future Perspectives", IAEA 7th -8th May 2015). Survey has revealed pronounced problems in several issues: difficulties to find funding to attend continuous professional development (CPD) activities, lack of structured clinical training, shortage of medical physicists, inability to participate in the management/decision making process, lack of recognition and lack of educational framework, were appointed by more than 50% of the 32 respondent countries to be felt problems concerning medical physics.

For each of seven IAEA recommendations, it has been tried to find justifications for and rationales behind the recommendations as well as to limited extent also legislative backgrounds, mostly within recently published international documents and basic safety standards (BSS) – European Union Council Directive 2013/59/EURATOM (EU BSS Directive), European Commission RP 174 *European Guidelines for Medical Physics Experts* (EC RP 174), IAEA Radiation Protection and Safety of Radiation Sources: International Basic Safety Standards (IAEA IBSS) and IAEA Human Health Series No. 25, *Roles and Responsibilities, and Education and Training Requirements for Clinically Qualified Medical Physicists* (IAEA HHS 25).[14-17] Although the citations from various documents are presented only fragmentally, they provide sufficient information about the solid background of presented recommendations of the IAEA Regional Meeting regarding the medical physics profession in the Europe region.

Recommendations have additional basis in the convincing and unambiguous results of the survey on medical physics status in Europe conducted by the IAEA and EFOMP in 2015 (Figure 1).[18]

Recommendations of the IAEA Regional Meeting on medical physics in Europe

"Recalling the provisions of "Radiation Protection and Safety of Radiation Sources: International Basic Safety Standards" (General Safety Requirements Part 3, IAEA 2014) regarding the role of medical physicists in ensuring safety in diagnostic and therapeutic procedures involving application of ionizing radiation, the Meeting recommended that Member States of the Europe Region should fully recognize Clinically Qualified Medical Physicist[A] (CQMP) as a health professional with specialist education and training in the concepts and techniques of applying physics in medicine and competent to practice independently in one or more of the subfields (specialties) of medical physics

The Meeting also recommended that Member States of the Europe Region should, in particular:

1. **Recognize** medical physics as an independent profession in health care with radiation protection responsibilities, as given in the "Joint position statement by the IAEA and WHO – Bonn call for action";
2. **Ensure** that medical physics aspects of therapeutic and diagnostic procedures, including patient and equipment related tasks and activities are performed by CQMPs or under their supervision;
3. **Establish** the appropriate qualification framework for CQMPs including education, specialized clinical training, certification, registration

[A] The term "**clinically qualified medical physicsts**" was defined in Roles and responsibilities and Education and training Requirements for Clinically Qualified Medical Physicists, IAEA Human Health Series No. 25, IAEA 2013 corresponds to "**qualified expert in medical physics**" defined in the IAEA International Basic Safety Standards and the "**medical physics expert**" defined in the European Council Directive 2013/59/EURATOM

and continuing professional development in the specializations of medical physics, i.e. diagnostic and interventional radiology, radiation oncology and nuclear medicine;
4. **Follow and fulfil** international recommendations regarding the staffing levels in the field of medical physics;
5. **Establish** mechanisms for medical physics services integration in all centres practicing radiation medicine, and establish, where appropriate, independent medical physics departments in which accredited clinical training can take place;
6. **Promote** involvement of CQMPs in hospital governance boards and relevant national health committees;
7. **Establish and enforce** the legislative and regulatory requirements related to radiation safety in medical imaging and therapy where medical physics is concerned, in accordance with the international and, where applicable, European basic safety standards."

Recognition of medical physics as independent health profession

In 2012 the IAEA, co-sponsored by WHO, held the "*International Conference on Radiation Protection in Medicine: Setting the Scene for the Next Decade*" in Bonn, Germany. The specific outcome of this conference was the published document *"Joint position statement by the IAEA and WHO – Bonn call for action"*[19], where some actions were identified as being essential for the strengthening of radiation protection in medicine over the next decade. Regarding the strengthening of radiation safety culture in health care, Action 8f: says the following: *"Work towards recognition of medical physics as an independent profession in health care, with radiation protection responsibilities"*.

Furthermore in the IAEA IBSS[16], medical physicist is defined as *"A health professional with specialist education and training in the concepts and techniques of applying physics in medicine and competent to practice independently in one or more of the subfields (specialties) of medical physics"*.

Through the committed efforts of the IOMP and other organizations, medical physicists have been included for the first time, in 2008 in "The international Standard Classification of Occupations (ISCO.08)".[20,21] Medical physicists are classified under the group 2111, "Physicists and Astronomers" but 5 out of 11 enumerated tasks concern explicitly medical physicists.[B]

There is also an explicit note of 2111 group stating *"… medical physicists are considered to be an integral part of the health work force alongside those occupations classified in sub-major group 22, Health professionals"*. On the other hand, under the group 22 of "Health professionals" also a specific note is included saying that "*it should be noted that a number of professions considered to be a part of the health work force are classified in groups other than sub-major group 22, Health professionals. Such occupations include but are not restricted to: addictions counsellors, biomedical engineers, clinical psychologists and* ***medical physicists.***"

Mentioned statements and definitions from quoted documents give unambiguous justification for the first recommendation of the IAEA Regional Meeting. The recognition of medical physicists as a health profession is of paramount importance and should be reflected at the national level (list of recognized professions, legal and fiscal environment, involvement in hospital governance etc.).

Roles and responsibilities of medical physics experts

Recommendation No. 2 clearly emphasizes the role and responsibilities of medical physics expert (MPE) in the fields of medical diagnostic and therapeutic procedures. In the new EU BSS Directive[14] from 2013, MPE is mentioned in 9 articles, while in the former EU BSS Directive[22] from 1997, MPE was mentioned only in 2 articles. New EU BSS Directive[14] thus recognizes the importance and growing role of medical physics profession in Europe. In Article 83 of the directive definitions are found of roles and responsibilities of MPE which are required to be implemented by the EU Member states:

"Member States shall ensure that depending on the medical radiological practice, the medical physics expert takes responsibility for dosimetry, including physical measurements for evaluation of the dose delivered to the patient and other individuals subject to medical exposure, give advice on medical radiological equipment, and contribute in particular to the following:

[B] "(e) ensuring the safe and effective delivery of radiation (ionising and non-ionising) to patients to achieve a diagnostic or therapeutic result as prescribed by a medical practitioner; (f) ensuring the accurate measurement and characterization of physical quantities used in medical applications; (g) testing, commissioning and evaluating equipment used in applications such as imaging, medical treatment and dosimetry; (h) advising and consulting with medical practitioners and other health care professionals in optimizing the balance between the beneficial and deleterious effects of radiation; … (j) developing, implementing and maintaining standards and protocols for the measurement of physical phenomena and for the use of nuclear technology in industrial and medical applications;"…

(a) optimisation of the radiation protection of patients and other individuals subject to medical exposure, including the application and use of diagnostic reference levels;

(b) the definition and performance of quality assurance of the medical radiological equipment;

(c) acceptance testing of medical radiological equipment;

(d) the preparation of technical specifications for medical radiological equipment and installation design;

(e) the surveillance of the medical radiological installations;

(f) the analysis of events involving, or potentially involving, accidental or unintended medical exposures;

(g) the selection of equipment required to perform radiation protection measurements;

(h) the training of practitioners and other staff in relevant aspects of radiation protection;"

It is evident that the tasks described in the EU BSS Directive[14] impose indispensable role and responsibility of medical physics experts and can only be performed by experienced medical physicists with high level of competence. Article 79 of the directive specifically requires from member states to ensure arrangements for the recognition of medical physics experts.

One of the most important requirements from the new EU BSS Directive[14] is that MPE shall be involved in all three major clinical fields of radiation medicine: radiotherapy, nuclear medicine and diagnostic and interventional radiology.

The document IAEA HHS 25[17], published by IAEA in 2013 and endorsed by IOMP and American Association of Physicists in Medicine (AAPM), defines appropriately and unequivocally the roles and responsibilities of CQMP in the different specialties of medical physics and recommends minimum requirements for their academic education and clinical training, including recommendations for their accreditation, certification and registration, along with continuing professional development.

The main goal of all these documents and recommendations is to establish criteria that support the harmonization of education and clinical training, as well as to promote the recognition of medical physics as a health profession.

Establishment of the appropriate qualification framework

European commission has recently published guidelines for medical physics expert – EC RP 174.[15] In this document detailed qualification framework (QF) for MPE in Europe is presented and discussed. QF for medical physicists in Europe should be referred to the European Qualification Framework (EQF) for lifelong learning, laid down by the European parliament and council of the European Union with learning outcomes expressed as inventories of Knowledge, Skills and Competences (KSC).[23] Education and clinical training requirements for medical physicists are discussed comprehensively in the IAEA HHS 25.[17] In depth description and guidance on clinical training of medical physicists specializing in radiation oncology, diagnostic and interventional radiology and nuclear medicine can be found in the IAEA *Training Course Series*.[24-26] Education and training of medical physicist in Europe is also covered in the EFOMP Policy Statement No. 12.[27] According to these documents, appropriate QF for medical physicists should consist of adequate education, accredited clinical training in hospitals and CPD programmes in place.

In the IAEA IBSS[16] similar accent is given already within the definition: "**medical physicists** is *a health professional with specialist education and training in the concepts and techniques of applying physics in medicine and competent to practise independently in one or more of the subfields (specialties) of medical physics*." and further defines that "**qualified expert** *is an individual who, by virtue of certification by appropriate boards or societies, professional licence or academic qualifications and experience, is duly recognized as having expertise in a relevant field of specialization, e.g. medical physics, radiation protection, occupational health, fire safety, quality management or any relevant engineering or safety specialty."*

From the definitions in IAEA IBSS[16] it can be deducted that **qualified expert in medical physics (i.e. MPE)** is a health professional having officially recognized specialization in one or more fields of medical physics.

Regarding the subject discussed in this section, new EU BSS Directive[14] requires from European Union member states in Article 14, point 2 the following: "*Member States shall ensure that arrangements are made for the establishment of education, training and retraining to allow the recognition of radiation protection experts and medical physics experts, as well as occupational health services and dosimetry services, in relation to the type of practice*." Also EC RP 174[15] presents as the first of the seven final recommendations that "*Each Member State should consider designating, through a legal instrument, a Competent Authority specifically for the recognition of the MPE*". And recom-

mendation No. 3 of EC RP 174[15] clearly links recognition to a proper qualification framework as stated: "*The Competent Authority designated for the recognition of the MPE, should use the Qualifications Framework and KSC of the MPE specified in the present document, for the recognition of the MPE to Level 8 of the EQF.*"

Within this frame, recognition of MPE encompasses also certification and registration of an individual professional. Certification is the formal process by which an authorized body evaluates and recognizes the knowledge and proficiency of an individual, which must satisfy pre-determined requirements or criteria. The process must thus always be based on a proper qualification framework involving both education and clinical training. Professional certification of medical physicists should be formally conducted by competent national boards - designated governmental body or alternatively national medical physics organization authorized by the government. In either case, members of such boards shall be predominantly senior MPEs in order to ensure competency in assessment and decision making procedures. The process of certification should be followed by formal registration of medical physics professionals and the register should be operated at the national level by an official authority (e.g. Ministries of health) or professional medical physics society/organization if an official authorization is given by the government. Re-certification system should be established as well in order to maintain high level of proficiency of medical physics experts (EQF level 8). This is usually achieved via formal CPD programme which should ensure up to date KSC of an individual professional. It is evident that without appropriate education, clinical training and CPD system, it cannot be expected medical physics service to play effective role in radiation medicine. However, in order to have a transparent system of certification and re-certification for MPEs, it needs to be consistent with the certification and recognition system of other health professionals/specialists (physicians, dentists) and Ministries of health have to play a key role in this process.

Situations where a formal QF system is not established yet, are mentioned in IAEA IBSS[16] (footnote under definition of medical physicist): "*Competence of persons is normally assessed by the State by having a formal mechanism for registration, accreditation or certification of medical physicists in the various specialties (e.g. diagnostic radiology, radiation therapy, nuclear medicine). States that have yet to develop such a mechanism would need to assess the education, training and competence of any individual proposed by the licensee to act as a medical physicist and to decide, on the basis of either international accreditation standards or standards of a State where such an accreditation system exists, whether such an individual could undertake the functions of a medical physicist, within the required specialty.*"

In countries where the desirable qualification system is not (completely) implemented yet, adequate mechanisms for transition period should be established in order to recognize and certify experienced professionals who have been already continuously employed in the field of medical physics for a specific period.[13] In such cases, the certification through an international or European instance may be a solution. In this concern EFOMP has given recognized steps to fostering education and training on a European level, encouraging the establishment of national training centres, networking and cooperative actions within European projects (e.g. EUTEMPE.RX) that may be taken as facilitators towards European certification process.[28]

Moreover, senior professionals who have been working for a longer period on active duty as medical physicists and are in possession of the core KSC of medical physics should be deemed to satisfy the requirements for recognition as an MPE. For these professionals a **"grandparenting clause"** might and shall be applied and they should be recognized/certified by competent authorization board as MPEs and not required to meet new legislative, educational or training (specialization) requirements.

Staffing levels in the field of medical physics

Fulfilment of the recommendation regarding the staffing levels in the field of medical physics is of major importance if high quality radiation health care service is to be ensured and the risk of radiological incidents and accidents reduced. Among many reports about incidents/accidents published within the last two decades, several of them can be attributed to shortage of experienced medical physicists.[3,4] Many national and international recommendations and other publications regarding the staffing levels in medical physics were published in the past.[29-35] Most recent documents about staffing levels for all subspecialties of medical physics have been published as Annex 2 of EC RP 174[15] and Staffing in Radiotherapy: An Activity Based Approach IAEA Human Health Reports No. 13 (2015).[36]

Despite all recommendations, there is still unacceptable understaffing in the field of medical physics in many European countries.[18] *Call for action* is addressed to the national authorities (e.g. Ministries of health) and hospital's management to incorporate recommendations regarding medical physics staffing levels into national legislations and standards in close cooperation with professional societies and organizations. Insufficient number of qualified and competent medical physicists – MPEs - will result in lower level of health care, even if requests, recommendations and standards from EU BSS Directive[14] and IAEA IBSS[16] will be formally transposed into national legislations.

Independent medical physics departments

EU BSS Directive[14] defines MPE as: "*medical physics expert means an individual or, if provided for in national legislation, a group of individuals, having the knowledge, training and experience to act or give advice on matters relating to radiation physics applied to medical exposure, whose competence in this respect is recognised by the competent authority.*" In this context "group of individuals" clearly means group of medical physics professionals (e.g. medical physics departments) with appropriate knowledge, skills and competencies in relevant medical physics specialization fields.

It seems reasonable that medical physics service is governed by the size, type and specific needs of the medical facility. In large hospitals medical physicists are often organized into an autonomous medical physics department which provides services to the various clinical departments e.g. diagnostic and interventional radiology, radiation oncology/radiotherapy and nuclear medicine.[17] If at least two major medical physics subspecialties are required for clinical work in hospitals, autonomous and independent medical physics departments shall be established as appropriate with well-defined safety and quality management system.[37] In many large European hospitals independent medical physics departments have been already established. Examples from developed countries are Institute Gustave Roussy in Paris and Royal Marsden Hospital in London and from less developed countries University Clinical Centre in Sarajevo, which offer services to various clinical departments. Such medical physics departments should competently cover also the field of radiation protection as the fourth major specialty where medical physicists have clear responsibilities, roles and competency.

The added value of a medical physics departments is multiple folded and can be shown through clinical and economic indicators in terms of efficiency and profitability, services quality, improved patient safety and patient satisfaction, increased patient throughput, improved communication and moral of professionals and reduce costs and liabilities. Accredited clinical training for medical physicists and other health professionals (clinicians, technologists, and nurses) is also promoted through such organizational structures that may be constituted as accredited clinical training centres by competent authorities. Importance of integrated medical physics departments was recognized by EFOMP already more than two decades ago in EFOMP Policy No. 5.[38]

Involvement of MPEs in hospital governance boards

EFOMP has recently published Policy statement no. 15, where guidelines on the role of the medical physicist within the hospital governance board are laid down.[39] Explicit recommendation is given regarding the involvement of medical physicists in hospital governance board: *"EFOMP recommends that National Member Organisations encourage their Medical Physicists to be closely involved in hospital governance and, where this has not already happened, to seek membership of their hospital's governance boards and its committees, emphasising the importance of such membership for the good of the patients and the hospital as a whole."*

Involvement of medical physicists in the hospital governance is presently very limited across Europe and often they are not officially included in management and decision making processes (Figure 1).

We have entered the era of fragile and sensitive economy with constantly growing demands for higher quality and safer health care system especially in the field of radiation medicine, where medical physicists are and should be strongly involved. The work of medical physicists in hospitals goes far beyond routine clinical and research tasks and reach demanding fields from radiation protection of patients, personnel and general public to the selection of expensive and complex equipment used in radiation medicine. Recalling the roles and responsibilities of MPE as defined and requested in the EU BSS Directive[14], it is clear that all mentioned

tasks cannot possibly be fulfilled, if MPE is not officially involved in the policy and decision making processes in the hospitals.

Legislative and regulative requirements

Throughout this paper the two most important recently published documents were quoted several times: EU BSS Directive[14] and IAEA IBSS.[16] In the foreword of the second document, the IAEA Director General Yukiya Amano among other said: *"Standards are only effective if they are properly applied in practice."* And continued: "*Regulating safety is a national responsibility, and many States have decided to adopt the IAEA's standards for use in their national regulations. For parties to the various international safety conventions, IAEA standards provide a consistent, reliable means of ensuring the effective fulfilment of obligations under the conventions. The standards are also applied by regulatory bodies and operators around the world to enhance safety in nuclear power generation and in nuclear applications in medicine, industry, agriculture and research."*

Any standard, if it is not implemented into national legislations and regulations, followed by a committed introduction into the clinical work, have a limited value. IAEA IBSS are important and extremely well prepared official recommendations from distinguished authority; however, adoption of these standards is, as said by Director General, a national responsibility. It is even binding for those IAEA Member States who are involved in Technical Cooperation (TC) activities with the IAEA.

EU BSS Directive[14] on the other hand is legally binding. In Article 106 the obligations for European Union member states are clearly stated: ***"Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive by 6 February 2018."***

The two above mentioned documents require from national authorities to transpose written standards and recommendations into local legislation. Concerning IAEA IBSS[16] national authorities have at least moral obligation to follow and implement recommended safety standards in order to optimize medical diagnosis and treatment of human diseases and to improve human health and well-being. Regarding the EU BSS Directive[14], there is a clear and firm legal obligation and responsibility for all European Union countries to adopt national legislation in order to comply with the requirements of the directive.

Conclusions

Work and devotion of medical physicists was nicely described by the esteemed medical physicist Prof. Ervin B. Podgorsak in his speech after acceptance of Coolidge award in 2006:

"A healthy man has a thousand wishes, a sick man has only one. Most of the work of medical physicists is indirectly related to people who have only one wish. We must not forget that, despite our scientific and technical training, our strongest guiding attributes must be compassion for patients and discipline toward our work."

Call for action is addressed to the national authorities, ministries of health and hospitals, to implement the latest international recommendations discussed in this paper without hesitation, completely, with great care and empathy in close cooperation with professional bodies, societies and organizations; it is their moral and legal responsibility. National authorities shall follow this road, above all for the benefit of millions of patients throughout the Europe and all over the world, otherwise "*compassion for patients and discipline toward our work*" might soon become an insufficient driving force.

Acknowledgments

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research article

Diagnostic accuracy of MRI to evaluate tumour response and residual tumour size after neoadjuvant chemotherapy in breast cancer patients

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Background. The aim, of the study was to estimate the accuracy of magnetic resonance imaging (MRI) in assessing residual disease in breast cancer patients receiving neoadjuvant chemotherapy (NAC) and to identify the clinicopathological factors that affect the diagnostic accuracy of breast MRI to determine residual tumour size following NAC.

Patients and methods. 91 breast cancer patients undergoing NAC (92 breast lesions) were included in the study. Breast MRI was performed at baseline and after completion of NAC. Treatment response was evaluated by MRI and histopathological examination to investigate the ability of MRI to predict tumour response. Residual tumour size was measured on post-treatment MRI and compared with pathology in 89 lesions. Clinicopathological factors were analyzed to compare MRI-pathologic size differences.

Results. The overall sensitivity, specificity, positive predictive value, negative predictive value, and accuracy for diagnosing invasive residual disease by using MRI were 75.00%, 78.57%, 88.89%, 57.89%, and 76.09% respectively. The Pearson´s correlation coefficient (r) between tumour sizes determined by MRI and pathology was $r = 0.648$ ($p < 0.001$). The size discrepancy was significantly lower in cancers with initial MRI size ≤ 5 cm ($p = 0.050$), in cancers with high tumour grade ($p < 0.001$), and in patients with hormonal receptor-negative cancer ($p = 0.033$).

Conclusions. MRI is an accurate tool for evaluating tumour response after NAC. The accuracy of MRI in estimating residual tumour size varies with the baseline MRI tumour size, the tumour grade and the hormonal receptor status.

Key words: breast cancer; MRI; residual tumour; neoadjuvant chemotherapy

Introduction

Neoadjuvant chemotherapy (NAC) has been used in the management of large operable breast tumours with the purpose of modifying the surgical planning and increase the rate of breast conservative surgery (BCS).[1-6] Currently, NAC has been extended to selected patients with early-stage disease to improve the cosmetic outcome of BCS, especially in women with small breast size.[7-9] NAC has proved to be equivalent to postoperative chemotherapy in terms of disease-free and overall surviv-

al.[10-12] However, in the neoadjuvant setting, there is evidence that patients who achieve a pathologic complete response (pCR) in the breast after NAC have a better prognosis than patients with a partial response or non-responders.[6,11,13-15] Based on the different behaviour of each tumour subtype, a molecular classification system identifies subgroups of breast invasive carcinoma patients who are most likely to achieve a pCR.[16]

An accurate imaging assessment of tumour response to NAC may facilitate the surgical planning. Magnetic resonance imaging (MRI) is more accurate and sensitive than conventional methods in assessing residual tumour extent after NAC.[17-23] In addition; there has been a positive correlation between MRI-determined and pathologic residual tumour size.[24-29] However, it is necessary to know the factors affecting the diagnostic accuracy of MRI in breast cancer treated with NAC.

The aims of the present study are to evaluate the diagnostic accuracy of MRI to detect residual disease and to predict the tumour extent in patients with breast cancer receiving NAC, and to identify the factors that influence the accuracy of MRI in predicting residual tumour size.

Patients and methods

Patients and tumour molecular characteristics

A total of 91 patients with invasive breast cancer (92 carcinomas) were included in this institutional retrospective study. All patients were diagnosed by core needle biopsy between October 2006 and June 2013. All patients were treated with NAC followed by surgical treatment and underwent MRI before and after NAC for monitoring tumour response to treatment. Considering the Helsinki Declaration principles, the Institutional Research Ethics Committee approved this study (No. 2015/059).

According to the results of the initial diagnostic biopsy, tumours were classified into 5 molecular subtypes based on immunohistochemical characteristics of breast cancer[30] (hormone receptor status, human epidermal growth factor receptor 2 (HER2) status and level of expression of ki-67). Estrogen receptor (ER) and progesterone receptor (PR) were considered positive if ≥ 10% of tumour cell nuclei stained positive. Hormone receptors (HR) were considered positive when the ER, PR, or both were positive. HER2 tumours scoring 3+ (intense homogeneous membranous staining in ≥ 10% of tumour cells) were considered positive. In case of 2+ scores (moderate complete membranous staining in ≥ 10% of tumour cells), the technique of fluorescent in situ hybridization was used to determine HER2 gene amplification. Samples with scores 0 to 1+ were considered negative. The cut-off of ki-67 expression level was set at 14%, to determine whether the cell proliferation index was high (> 14%) or low (≤ 14%). The five categories of molecular subtypes were: *luminal A-like* subtype (HR positive, HER2 negative, ki-67 ≤ 14%), *luminal B/HER2-negative-like* subtype (HR positive, HER2 negative, ki-67 > 14%), *luminal B/HER2-positive-like* subtype (HR positive, HER2 positive), *HER2-positive-like* subtype (HR negative, HER2 positive) and *triple negative* subtype (HR negative, HER2 negative).

Chemotherapeutic and MRI protocols

The treatment plan was chosen by oncologists and explained to every patient. The initial evaluation of patients before NAC included a complete medical history, physical examination, complete blood work, chest X-ray, CT scan and bone scan. Clip titanium was placed in the tumour bed in all patients before starting chemotherapy, to identify the area of the primary tumour at the time of surgery. All patients with *HER2-positive* cancers received trastuzumab-based regimen as neoadjuvant therapy.

Tumour size was measured on the last MRI performed after completing NAC. The studies were done on a 1.5 T MRI scanner (Best, The Netherlands). The patient was placed in a prone position. Protocol in space-occupying lesions in the breast includes an axial T1-weighted sequence (TR: 494 msec, TE: 8 msec, number of acquired signals: 2, slice thickness: 3 mm, interval: 0.3 mm) and T2-weighted sequence (TR: 5000 msec, TE: 120 msec, number of acquired signals: 2, slice thickness: 3 mm, interval: 0.3 mm), followed by 3D T1-weighted fast spoiled gradient-echo dynamic sequence, selective excitation of water, (TR: 23 msec, TE: 5.7 msec, angle: 20º, slice thickness: 2 mm) acquiring 6 series, one pre-contrast series and five consecutive post-contrast series at 90-second intervals. Contrast agent (Gd-DOTA, DOTAREM, Guerbet) was administered using a bolus injection (2 mL/s) at a dose of 0.1 mmol/kg followed by a bolus of saline solution (20 mL). All images are analysed at a workstation. Subtraction images between the without contrast stage and the 2nd-3rd-4th-5th post-contrast phase were obtained, which were interpreted with the help of specific programs for the analysis of contrast enhancement and time-signal intensity curves.

MRI assessment

Assessment of response was based on changes in tumour size in the MRI contrast sequences. Tumour size was calculated by summing the maximum diameters of tumour enhancement on axial slices of MRI, as the Response Evaluation Criteria in Solid Tumours (RECIST). The absence of a clear enhancement indicates no residual cancer. The final response was defined as the change in size between the pre-treatment and post-treatment MRI. Response categories, based on radiological examination with contrast MRI, were classified as: (1) imaging complete response on MRI (iCR: no evidence of residual disease on posttreatment MRI); and (2) non-iCR: residual disease on posttreatment MRI.

Histopathologic analysis

Pathologic measurement of the tumour size was used as the "gold standard" and compared to the MRI-measured residual tumour size. Samples for histopathological examination were cut into 5 mm slices, fixed in 10 % neutral-buffered formalin, trying to identify any lesion that corresponded with invasive carcinoma. If the tumour lesion was evident, it was included in its entirety for morphological study with hematoxylin and eosin (H&E). If no evident tumour was found, the clip marker was identified, and slides from the block containing the marker as well as the adjacent blocks were examined. Tumour response after NAC was classified as (1) pCR: no residual invasive tumour in the breast on final pathology; and (2) non-pCR: presence of residual invasive cancer on final pathology. If any residual invasive disease, pathologic tumour size was determined by measuring the longest dimension of a sample stained with H&E and the number of blocks in which invasive tumour was detected.

Statistical analysis

A descriptive analysis of the variables included in the study was performed. Continuous variables were expressed as mean and standard deviation, and categorical variables were expressed as absolute values and percentages with their estimated 95% confidence interval. Comparison of means was performed using Student´s t test or Mann-Whitney test and analysis of variance or Kruskal-Wallis test, as appropriate after checking normality with the Kolmogorov-Smirnov test. Association of qualitative variables was estimated using the Chi-square test. Pearson correlation analysis was used to compare the MRI-measured and pathological tumour sizes. Linear regression model was used to analyse the diagnostic accuracy of MRI. $P \leq 0.05$ was considered significant. Basic statistical indicators to assess the accuracy of MRI in detecting residual disease after NAC were calculated. The efficacy of MRI was measured by the predictive values. True negative (TN) was defined as negative in both MRI and pathology; true positive (TP) was defined as positive in both MRI and pathology; false negative (FN) was defined as negative on MRI and positive on pathology; and false positive (FP) was defined as positive on MRI and negative on pathology. To assess the accuracy of MRI in detecting residual disease, sensitivity: TP / (TP + FN), specificity: TN / (TN + FP), positive predictive value (PPV): TP / (TP + FP), negative predictive value (NPV): TN / (TN + FN), and overall accuracy: (VN + VP) / (TP +

TABLE 1. Clinical and tumour characteristics

CONTINUOUS VARIABLES	Mean	SD	Median	Range
AGE (years)	47.22	10.10	42.0	31.0-75.0
BASAL TUMOR SIZE (cm)	3.99	1.97	3.40	1.60-13.0
CATEGORICAL VARIABLES		**n**	**%**	**95% CI**
CLINICAL TUMOR STAGE	T1	7	7.6	1.6-13.6
	T2	69	75.0	65.6-84.4
	T3	13	14.1	6.5-21.8
	T4	3	3.3	0.7-9.2
HISTOLOGICAL TYPE	Ductal	85	92.4	86.4-98.4
	Lobular	7	7.6	1.6-13.6
HISTOLÓGICAL GRADE	G1	9	10.0	3.2-16.8
	G2	32	35.6	25.1-46.0
	G3	49	54.4	43.6-65.3
	NA	2		
HORMONAL RECEPTOR STATUS	Positive	60	65.2	54.9-75.5
	Negative	32	34.8	24.5-45.1
HER2 STATUS	Positive	25	27.2	17.5-36.8
	Negative	67	72.8	63.2-82.5
MOLECULAR SUBTYPE	Luminal A	11	12.0	4.8-19.1
	Luminal B/HER2-	35	38.0	27.6-48.5
	Luminal B/HER2+	16	17.4	9.1-25.7
	HER2+	9	9.8	3.2-16.4
	Triple negative	21	22.8	13.7-31.9
PRE-NAC AXILLARY STATUS	Positive	76	82.6	74.3-90.9
	Negative	16	17.4	9.1-25.7

CI = confidence interval; NA = not available; SD = standard deviation

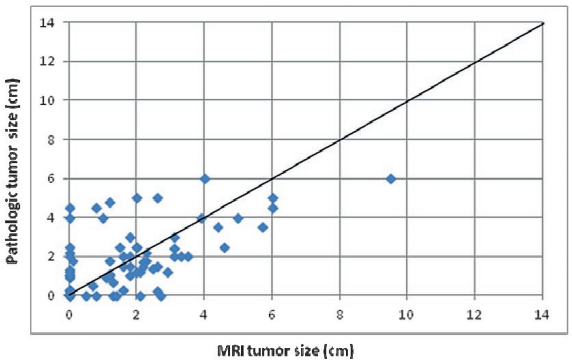


FIGURE 1. Dispersion graph of the correlation between residual tumour sizes (cm) calculated at preoperative MRI (on the X-axis) and at pathological examination (on the Y-axis). Each point represents a tumour; if the pairs of data coincide, the points overlap. The bisector corresponds to the equivalence line. On the graph, the closer the points are to the equivalence line, the greater the correlation between MRI and pathology.

TN + FP + FN) were calculated. Analyses were performed using IBM SPSS Statistics version 19.

Results

92 invasive breast tumours were analysed (1 patient with bilateral breast cancer was recorded). Initial clinicopathologic characteristics of patients and tumours of the study are shown in Table 1. Patients age ranged between 31 and 75 years (mean 47.22 years). Mean baseline tumour size determined by MRI was 3.99 cm. Most tumours were diagnosed as T2 stage (75%) and grade 3 (53.3%). The initial biopsy of the lesions revealed 85 cases of invasive ductal carcinoma and 7 cases of invasive lobular carcinoma. 11 tumours were classified as *luminal A*, 35 as *luminal B/HER2-*, 16 as *luminal B/HER2+*, 9 as *HER2+* and 21 as *triple negative*. Most patients (65.9%) received taxane-anthracycline based chemotherapy; *HER2-positive* patients (27.5%) were treated with a trastuzumab-based regimen. After NAC, mastectomy was performed in 21 cases (21.8%) and BCS was attempted in 71 cases (77.2%). Of the latter group, 16 cases were re-operated because tumour involvement or proximity to the resection margins.

Accuracy of MRI to detect residual disease after NAC

Tumour responses to NAC were compared based on the results obtained by MRI and pathological examination. MRI showed complete remission in 38 cases (41.3%) and residual disease in 54 cases (58.7%). The pathological study showed a pCR in 28 cases (30.4%) and invasive residual tumour was found in 64 samples (69.6%).The diagnostic performance of MRI for detecting residual tumour is summarized in Table 2. The sensitivity of MRI for detecting residual disease after NAC was 75% (48/64) and the specificity was 78.57% (22/28). The PPV (accuracy of MRI for detecting residual disease) was 88.89% (48/54). The NPV (accuracy of MRI in predicting pCR) was 57.89% (22/38). The overall accuracy of MRI was 76.09% (70/92). MRI showed FN diagnoses in 25% cases (16/64).

TABLE 2. MRI diagnostic performance in predicting pathologic response

		Pathology		Total
		No pCR	pCR	
MRI	No iCR	TP = 48	FP = 6	54 (58.70%)
	iCR	FN =16	TN = 22	38 (41.30%)
Total		64 (69.60%)	28 (30.40%)	92 (100%)

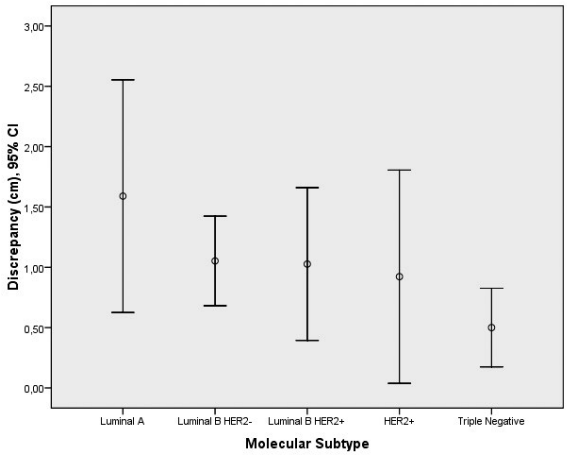
FN = false negative; FP = false positive; iCR = imaging complete response; pCR = pathologic complete response; TN = true negative; TP = true positive

Accuracy of MRI to predict the residual tumour size after NAC

It was possible to compare the residual tumour size determined by MRI and pathological examination in 89 of the 92 cases of the study. In 3 cases it was not possible to determine the pathologic tumour size due to the presence of scattered residual multifocal disease. The mean residual tumour size determined by MRI after NAC was 1.44 cm. The final pathologic tumour size was 1.53 cm. The two measurements are correlated forwardly significantly ($r = 0.648$, $p < 0.001$) (Figure 1). The mean discrepancy between the two measures was 0.96 cm. The discrepancy was less than 1 cm in 57 cases (64.04%).

Analysis of factors influencing the accuracy of MRI for predicting residual tumour size.

Linear regression models were performed to find clinicopathological predictors of the diagnostic accuracy of MRI based on the absolute difference between the MRI-measured and pathologic residual tumour size (Table 3). The strongest predictor was tumour grade ($p < 0.001$). The mean absolute



Discrepancy (cm) based on molecular subtypes [Mean±SD]: luminal A [1.59±1.34]; luminal B/HER2– [1.05±1.06]; luminal B/HER2+ [1.02±1.14]; luminal HER2+[0.92±1.14]; triple negative [0.50±0.70]

FIGURE 2. Discrepancy between MRI-measured and pathologic residual tumour size, based on molecular subtypes.

discrepancy was significantly lower in the group of high-grade tumours. In addition, the HR status was associated significantly with the diagnostic accuracy of MRI, observing a lower discrepancy in the group of *non-luminal* tumours (p = 0.033). Baseline tumour size was kept in the limit of significance, with a lower discrepancy in the group with baseline tumour size ≤ 5 cm. The age, histological type and HER2 status were not associated with the diagnostic accuracy of MRI. *Triple negative* subtype showed the smallest difference between the two measurements (Figure 2), although no statistically significant difference regarding the molecular phenotype of the tumour (p = 0.055). After a multivariate linear regression analysis, tumour grade (p = 0.001) and baseline tumour size (p = 0.030) remained significant independent predictors of MRI accuracy (Table 4).

TABLE 3. Factors affecting the MRI diagnostic accuracy based on the discrepancy between MRI and pathologic residual tumour size

Variable	No.	Discrepancy (mean ± SD)	p-value
Age (years)			
≤45	43	1.09 ±1.14	0.281
>45	46	0.84 ±1.01	
Baseline tumour size (cm)			
≤5	74	0.85 ±0.99	0.050
>5	15	1.53 ±1.33	
Histological type			0.818
ductal	83	0.97 ±1.09	
lobular	6	0.87 ±0.82	
Histological grade			<0.001
1 or 2	40	1.44 ±1.24	
3	47	0.56 ±0.71	
Hormonal receptor status			0.033
positive	59	1.14 ±1.13	
negative	30	0.63 ±0.87	
HER2 status			0.906
positive	24	0.99 ±1.12	
negative	65	0.96 ±1.07	
Molecular subtype			0.055
Luminal A	10	1.59 ±1.34	
Luminal B-HER2-	34	1.05 ±1.06	
Luminal B-HER2+	15	1.02 ±1.14	
HER2+	9	0.92 ±1.14	
Triple negative	21	0.50 ±0.70	

SD = standard deviation

TABLE 4. Results from the Multivariate Regression Analysis

Variable	Regression coefficient (B)	se	p	95% CI
Tumour grade	0.807	0.236	0.001	0.338-1.276
HR status	0.086	0.249	0.729	-0.408-0.581
BTS (MRI)	0.610	0.277	0.030	0.060-1.161

BTS = baseline tumour size; CI = confidence interval; se = standard error

Discussion

Several researchers have previously studied the diagnostic accuracy of MRI in detecting invasive breast carcinoma in patients undergoing NAC[31-34]; however, an accurate determination of residual tumour size is necessary to perform an optimal surgery and achieve negative margins. We conducted a comparative analysis of post-NAC MRI and pathological findings to describe the diagnostic accuracy of MRI to detect residual invasive disease and to estimate the residual tumour size after NAC. In the current study, the overall diagnostic accuracy of MRI for detecting residual invasive carcinoma in the breast was 76.09%. The PPV and NPV were 88.89% and 57.89%, respectively. These data suggest that breast MRI is an accurate tool for assessing tumour response after NAC, although it is more limited in predicting pCR, which may be due to the NAC antiangiogenic effect in the tumour bed.

Correlation coefficients of residual tumour size assessed by MRI and pathology were considered good. Lobbes *et al.*[35] reviewed 17 studies comparing MRI-measured and pathologic residual tumour size. In this review, the mean correlation coefficient was 0.698. In our series, the value of the correlation coefficient was 0.648, reflecting a moderate correlation. If we remove the *luminal A-like* cases from

the calculation, in which it has been observed that NAC is not the optimal therapeutic strategy, the value of global correlation coefficient rises to 0.706 ($p < 0.001$). Although correlation coefficients provide important information about the MRI´s ability to assess response to NAC, the determination of the absolute difference between MRI-measured and pathologic residual tumour size is necessary to evaluate the diagnostic accuracy of MRI. MRI overestimation of residual tumour size can increase the number of unnecessary mastectomies and alter the cosmetic outcome of BCS with wide resection margins, while MRI underestimation can increase the number of reoperations. In the current study, the mean discrepancy was 0.96 cm. Less than 1 cm difference in the calculation of residual tumour size between MRI and pathology was observed in 57 tumours (64.04%). Identifying factors that may affect the accuracy of MRI for predicting residual tumour size could help to interpret breast MRI findings. Three recent studies evaluated the accuracy of MRI to predict residual tumour size after NAC and investigated the factors that influence the accuracy of MRI. In a study conducted by Ko *et al*.[36], the Pearson´s correlation coefficient between the tumour sizes measured using MRI and pathology was 0.749 ($p < 0.001$) and the mean of size discrepancy was 1.26 cm. According to the molecular subtype, tumour grade and tumour morphology on initial MRI, statistically significant differences of size discrepancy between both measurements were observed. *Triple negative* subtypes were measured more accurately (mean, size discrepancy = 0.8 cm). In the study by Chen *et al*.[37], the mean discrepancy was 1.0 cm, and predictive factors found in the univariate analysis were histological type, tumour morphology, HR status, HER2 status and type of MRI. Multivariate analysis identified as independent predictors histological type, tumour morphology and the combination of HER2-HR status. Finally, in a study by Moon *et al*.[38], the Pearson´s correlation coefficient and the mean difference between MRI-measured and pathologic tumour size was 0.664 ($p < 0.001$) and 1.39 cm, respectively. The clinicopathological factors associated with MRI accuracy were the initial T stage, the age at the time of the diagnosis and the ER expression status. In addition, Moon *et al* observed increased accuracy of MRI in predicting the residual tumour extent after NAC in *triple negative* breast cancer. In our study, a statistically significant association was observed between the absolute discrepancy and each of covariates: baseline tumour size, tumour grade and HR status. A minor discrepancy was observed in tumours with an initial size ≤ 5 cm, in high-grade tumours and in *non-luminal* tumours. Despite a statistically significant association between molecular subtypes and the diagnostic accuracy of MRI was not observed ($p = 0.055$), a tendency to find better accuracy in *triple negative* tumours was found. The mean discrepancy in residual tumour size was lower in the group of *triple negative* tumours (0.50 cm). Multivariate analysis identified only as independent predictors baseline tumour size and tumour grade.

There are two important limitations to note in our study. The absence of ductal carcinoma in situ (DCIS) was not included in the definition of pCR, which can affect the accuracy of MRI. In addition, the current molecular classification includes a cut-off value of ki-67 expression level at 20% to define low or high level.[39]

Conclusions

In conclusion, MRI can accurately measure tumour response and residual tumour size in breast cancer patients treated with NAC. Both overestimation and underestimation of MRI-measured residual tumour size may cause an incorrect surgical planning so it´s important to consider the clinicopathological factors that can affect the diagnostic accuracy of breast MRI. In our series, evaluation of residual tumour size was more accurate in baseline tumour size ≤ 5 cm lesions, in high tumour grade lesions and in *non-luminal* breast cancer.

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research article

Antioxidant defence-related genetic variants are not associated with higher risk of secondary thyroid cancer after treatment of malignancy in childhood or adolescence

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Background. Thyroid cancer is one of the most common secondary cancers after treatment of malignancy in childhood or adolescence. Thyroid gland is very sensitive to the carcinogenic effect of ionizing radiation, especially in children. Imbalance between pro- and anti-oxidant factors may play a role in thyroid carcinogenesis. Our study aimed to assess the relationship between genetic variability of antioxidant defence-related genes and the risk of secondary thyroid cancer after treatment of malignancy in childhood or adolescence.

Patients and methods. In a retrospective study, we compared patients with childhood or adolescence primary malignancy between 1960 and 2006 that developed a secondary thyroid cancer (cases) with patients (controls), with the same primary malignancy but did not develop any secondary cancer. They were matched for age, gender, primary diagnosis and treatment (especially radiotherapy) of primary malignancy. They were all genotyped for *SOD2* p.Ala16Val, *CAT* c.-262C>T, *GPX1* p.Pro200Leu, *GSTP1* p.Ile105Val, *GSTP1* p.Ala114Val and *GSTM1* and *GSTT1* deletions. The influence of polymorphisms on occurrence of secondary cancer was examined by McNemar test and Cox proportional hazards model.

Results. Between 1960 and 2006 a total of 2641 patients were diagnosed with primary malignancy before the age of 21 years in Slovenia. Among them 155 developed a secondary cancer, 28 of which were secondary thyroid cancers. No significant differences in the genotype frequency distribution were observed between cases and controls. Additionally we observed no significant influence of investigated polymorphisms on time to the development of secondary thyroid cancer.

Conclusions. We observed no association of polymorphisms in antioxidant genes with the risk for secondary thyroid cancer after treatment of malignancy in childhood or adolescence. However, thyroid cancer is one of the most common secondary cancers in patients treated for malignancy in childhood or adolescence and the lifelong follow up of these patients is of utmost importance.

Key words: secondary thyroid cancer; antioxidant genes; genetic polymorphism

Introduction

Modern treatment modalities and better diagnostic techniques greatly improved the survival of children and adolescents with malignancies.[1,2] With increasing number of survivors and years of follow up late effects of treatment are encountered more frequently.[3,4]

The most detrimental late effects are secondary cancers. Several studies report on increased risk of subsequent secondary cancers even several decades after treatment of primary malignancy.[5] Increased incidence of secondary thyroid cancer was reported even up to 40 years after radiotherapy.[6-9]

The thyroid gland is very sensitive to the carcinogenetic effect of ionizing radiation, especially in children.[7] Ionizing radiation damages DNA directly or indirectly through production of free radicals and reactive oxygen species (ROS). It has been shown that gamma radiation and hydrogen peroxide (H_2O_2), which is one of the ROS, induce similar DNA damages in the thyroid.[10] Oxidative DNA damage involves single- or double DNA strand breaks, purine and pyrimidine or deoxyribose modifications as well as DNA cross links. ROS can also damage the cell through lipid peroxidation, protein modification, membrane disruption and mitochondrial damage.[11,12]

The thyroid cell is constantly exposed to ROS and an imbalance between pro- and anti-oxidative factors has been suggested as an important mechanism in thyroid carcinogenesis. The accumulation of oxidative DNA damage may drive genomic instability events and lead to somatic mutations. Many studies have shown that oxidants are increased and antioxidants are decreased in patients with thyroid cancer.[13-19]

The most important antioxidants in the thyroid are antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). Manganese superoxide dismutase (SOD2) is the major antioxidant in mitochondria, catalysing the dismutation of superoxide anion to H_2O_2, which is then reduced to water by CAT or GPX.[20,21] Many studies have investigated genetic variability in genes coding for antioxidant enzymes and their relationship to cancer risk, however the results were inconclusive[22] and the data on thyroid cancer risk are lacking.[23]

The most common polymorphism in the gene coding for SOD2 (*SOD2*) leads to substitution of alanine (Ala) with valine (Val) at codon 16 (p.Ala16Val) and affects transport of the enzyme into the mitochondria.[24] According to several studies the 16Ala allele of *SOD2* polymorphism is associated with an increased risk of prostate and oesophageal cancer.[21,25]

CAT activity is affected by functional single nucleotide polymorphism (SNP) *CAT* c.-262C>T in the promoter region of *CAT* gene, which leads to lower catalase activity. This polymorphism was implicated in increased susceptibility to breast and cervical cancer.[26,27]

The most common *GPX1* polymorphism results in the amino acid substitution of leucine with proline at codon 200 (p.Leu200Pro) and results in lower enzyme activation. As it may influence the balance between oxidative stress and antioxidant defence, it may therefore increase cancer risk.[28] Indeed several studies associate *GPX1* p.Pro200Leu polymorphism with increased susceptibility to prostate and breast cancer.[29,30]

Glutathione S-transferase (GSTs) enzymes, encoded by *GST* genes, are implicated in detoxification of xenobiotics and reactive products of ROS, so they may have a crucial role in protecting tissue from oxidative damage.[31] Deletions of the *GSTM1* and *GSTT1* genes result in null genotypes and lead to impaired enzyme activity.[32] In *GSTP1* two frequent SNPs resulting in an amino acid substitution have been reported. The *GSTP1* p.Ile105Val SNP results in Ile to Val substitution near the active site and leads to decreased enzyme activity. The functional role of the *GSTP1* p.Ala114Val polymorphism is not clear, however, reduced conjugation capacity was reported in the enzyme with both polymorphisms present.[30] Variants of these loci have been implicated in the aetiology of numerous cancers.[29,31-34]

There is some inconclusive data on *GST* polymorphisms in association to thyroid cancer risk. According to some studies individuals with homozygous deletions of *GSTM1* or *GSTT1* have an increased risk of thyroid cancer, whereas Lemos *et al*. found the opposite in his study.[33-35] Mertens *et al*. studied radiotherapy related malignancies in survivors of Hodgkin disease and found that individuals lacking *GSTM1* but not *GSTT1* were at increased risk of any subsequent SMN.[36] To our knowledge data on genetic variability of antioxidant enzymes in primary thyroid cancer are scarce, while no data have been published regarding the secondary thyroid cancer.

The aim of the present study was to investigate the relationship between genetic variability in antioxidant defence-related genes (*SOD2* p.Ala16Val, *CAT* c.-262C>T, *GPX1* p.Pro200Leu, *GSTM1* deletion, *GSTT1* deletion, *GSTP1* p.Ile105Val and *GSTP1* p.Ala114Val) and the risk of secondary thyroid cancer after treatment of malignancy in childhood or adolescence.

Patients and methods

Patients

A population based study of all patients known to have developed a secondary thyroid cancer after treatment of malignancy in childhood or adolescence was performed. A retrospective matched case-control study was designed. Individuals were eligible for inclusion in the study patients group (cases) if they were diagnosed with any kind of primary malignancy between 1960 and 2006 and before the age of 21 and were treated at the Department of Hematology and Oncology, University Children's Hospital, Ljubljana, or at the Institute of Oncology, Ljubljana and had a secondary thyroid cancer diagnosed 5 years or later after the primary malignancy. Study controls were patients with a primary malignancy in childhood or adolescence but free of secondary thyroid cancer. They were selected with a ratio of 1 control to 1 case matched for: type of the primary malignancy, treatment of primary malignancy, especially regarding the radiotherapy to the neck, head or mediastinal region, sex and age at the time of primary malignancy diagnosis (if possible not more than 2 years younger or older then the case). Study patients and study controls were identified from a search from the Cancer Registry of Slovenia.[37]

All patients with primary childhood or adolescent malignancy were followed up at the Department of Hematology and Oncology, University Children's Hospital, Ljubljana, or at the out-patient Clinic for Late Effects at the Institute of Oncology, Ljubljana.[38]

Thyroid follow-up included yearly thyroid stimulating hormone (TSH) and thyroglobulin level evaluation and occasional neck ultrasound. All patients with palpable nodules and/or elevated thyroglobulin levels underwent a neck ultrasound as a method commonly used in the work-up of thyroid diseases.[39] If malignancy was suspected, fine needle aspiration biopsy (FNAB) was performed. When papillary/follicular lesions were detected or were just suspected by cytology, thyroidectomy was performed at the Institute of Oncology, Ljubljana.

The follow up interval was defined as the time between primary malignancy and secondary thyroid cancer in the study group or between primary malignancy and the last appointment at the Out-Patient Clinic for Late Effects in the control group. All patient's data (demographic, clinical and treatment data) were collected from the patient's medical records.

Single experienced pathologist reviewed all the primary malignancies and corresponding thyroid cancers.

The study was approved by the Slovenian Ethics Committee for Research in Medicine (No.138/04/10) and was carried out according to the Declaration of Helsinki.

DNA isolation and genotyping

According to the presence of tumour or normal tissue on hematoxylin and eosin (HE) slides the pathologist chose one representative paraffin block from each biopsy and marked the tumour and normal tissue area on the block. From the marked area (if possible we chose normal tissue) two to three cores of 1 mm in diameter were obtained for DNA extraction using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.[40]

For two control patients genomic DNA was isolated from archived cytological smears of bone marrow specimens using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany).[41] For all other patients DNA was obtained from paraffin blocks contained tumour or normal tissue as described above.

Genotypes of *GPX1* p.Pro200Leu (rs1050450) and *SOD2* p.Val16Ala (rs4880) were determined by TaqMan genotyping method (Applied Biosystems, Foster City, CA, USA) as described previously.[42] Genotyping of *CAT* c.-262C>T (rs1001179), *GSTP1* p.Ile105Val (rs1695) and *GSTP1* p.Ala114Val (rs1138272) was carried out using a fluorescence-based competitive allele-specific (KASPar) assay (KBiosciences, Herts, UK).[43]

Multiplex polymerase chain reaction (PCR) was used for detection of *GSTM1* and *GSTT1* gene deletions. *GSTM1*, *GSTT1*, and *BGLO* genes were simultaneously amplified in a multiplex PCR reaction as previously described.[41] This approach allowed us to identify homozygous *GSTM1* or *GSTT1* gene deletion, but we could not distinguish between carriers of one or two copies of each gene.

Statistical analysis

Frequencies were used to describe the distribution of categorical variables and median and interquartile ranges were used for continuous variables. Standard chi-square test was used to assess deviation from Hardy-Weinberg equilibrium (HWE), comparing the distribution of genotype frequencies in the control group with the expected distribution within the population.[44]

To compare the genotype distribution, McNemar test for the analysis of matched samples based on binomial distribution was used. The influence of polymorphisms on the time to the occurrence of second cancer was examined by Cox proportional hazards model with stratification on the matched pairs to calculate relative risks (RRs) and their 95% confidence intervals (CIs).

All statistical analyses were carried out by IBM SPSS Statistics, version 19.0 (IBM Corporation, Armonk, NY, USA), except for odds ratios (OR) and 95% CIs in McNemar test that were calculated using GraphPad Software. A dominant genetic model was used in all statistical analyses and p values below 0.05 were considered statistically significant.

Results

Patients' characteristics

Based on data from The Cancer Registry of Slovenia, in the period between 1960 and 2006 a total of 2641 patients were diagnosed with primary cancer before the age of 21 years.[40] Among them 155 developed secondary cancer (5.9%), out of which 28 (18.1%) were secondary thyroid cancers.

Only 24 (85.7%) eligible cases were included in the study because we could not get the histopathological material for 4 controls. Therefore the study group included 8 (33.3%) males and 16 (66.7%) females with a median age of 12.7 years at diagnosis of primary cancer (range 7.1–6.7 years) and 29.2 years at diagnosis of secondary thyroid cancer (range 23.5–35.4 years). Six out of 24 patients (25.0%) were under 5 years old at the time of primary diagnosis. The control group included 8 (33.3%) males and 16 (66.7%) females with a median age of 12.9 years at diagnosis of primary cancer (range 5.0–15.4 years).

The most frequent primary cancer was Hodgkin's disease (HD) (15 pairs, 62.5%), then acute lymphoblastic leukemia (ALL) (2 pairs, 8.3%) and central nervous system (CNS) tumours (2 pairs, 8.3%). Non-Hodgkin lymphoma (NHL), neuroblastoma, rhabdomyosarcoma, nasopharyngeal carcinoma and ovarian tumours were observed in 1 pair each (4.2%). Most of the patients with secondary thyroid cancer received radiation therapy to the head, neck or mediastinum during the treatment for primary cancer (23 patients, 95.8%): 15 (62.5%) to the neck, 6 (25.0%) to the head and 2 (8.3%) to the mediastinum. The same distribution of irradiated sites was observed also in the control group.

The most frequent histology of secondary thyroid cancer was papillary carcinoma (23, 95.8%). Only 1 tumour (4.2%) was follicular neoplasm of undefined malignant potential. Using TNM classification for staging most of thyroid cancer were stage 1 with tumour localised to the thyroid and/or lymph nodes (23, 95.8%), 1 (4.2%) was stage 2 with lung metastasis (M1). Among 12 (50.0%) T1 tumours (tumour diameter ≤ 2cm), there were 9 (37.5%) microcarcinoma (T1a: tumour ≤ 10 mm). There were 2 (8.3%) T2 tumours (tumour > 2 cm but ≤ 4 cm in greatest dimension, limited to the thyroid); 6 (25.0%) were T3 (minimal extrathyroid extension) and 3 (12.5%) were T4 (extending beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, oesophagus, or recurrent laryngeal nerve) and 11 (45.8%) had regional lymph node metastasis (N1).

A total or near total thyroidectomy was carried out in all cases. Additional radioiodine treatment was applied to 19 (79.2%) patients. In 6 (25.0%) cases lymph node metastases were excised.

The follow up interval was comparable in both groups and was 19.6 (range 9.0–23.6) years in the primary group and 18.8 (range 12.80–27.6) years in the control group. Both groups did not differ significantly regarding the demographic data.

Genotype frequencies of the antioxidant defence-related genes are presented in the Table 1. All the investigated polymorphisms were in HWE in the control group.

To assess if the investigated polymorphisms influence the risk of secondary thyroid cancer, we performed a matched analysis. When all the cases were compared to controls, no significant differences in the genotype frequency distribution were observed (Table 2). There were also no differences in genotype distribution between microcarcinoma and other secondary thyroid cancers.

We also assessed the influence of investigated polymorphisms on time to development of secondary thyroid cancer using Cox regression with stratification on matched pairs. We have not observed any association between studied polymorphisms and the time pattern of occurrence of secondary thyroid cancers after treatment of malignancy in childhood or adolescence.

Discussion

In the present study we investigated if *SOD2* p.Ala16Val, *CAT* c.-262C>T, *GPX1* p.Pro200Leu, *GSTM1*, *GSTT1*, *GSTP1* p.Ile105Val and *GSTP1* p.Ala114Val polymorphisms influence the risk of secondary thyroid cancer after treatment of malig-

TABLE 1. Genotype frequencies of the antioxidant defence-related genes

SNP	Genotype	All patients N (%)	Cases N (%)	Controls N (%)	P_{HWE} controls
GPX1 rs1050450 p. Pro200Leu	CC	28 (58.3)	16 (66.7)	12 (50)	0.967
	CT	17 (35.4)	7 (29.2)	10 (41.7)	
	TT	3 (6.3)	1 (4.2)	2 (8.3)	
SOD2[a] rs4880 p.Val16Ala	GG	10 (21.7)	4 (16.7)	6 (27.3)	0.338
	GA	31 (67.4)	18 (75)	13 (59.1)	
	AA	5 (10.9)	2 (8.3)	3 (13.6)	
CAT rs1001179 c.-262G>A	GG	32 (66.7)	16 (66.7)	16 (66.7)	0.834
	GA	14 (29.2)	7 (29.2)	7 (29.2)	
	AA	2 (4.2)	1 (4.2)	1 (4.2)	
GSTP1 rs1695 p.Ile105Val	AA	22 (45.8)	10 (41.7)	12 (50)	0.432
	AG	23 (47.9)	12 (50)	11 (45.8)	
	GG	3 (6.3)	2 (8.3)	1 (4.2)	
GSTP1 rs1138272 p.Ala114Val	CC	39 (81.3)	19 (79.2)	20 (83.3)	0.106
	CT	8 (16.7)	5 (20.8)	3 (12.5)	
	TT	1 (2.1)	/	1 (4.2)	
GSTM1[b] gene deletion	non-null	23 (48.9)	14 (58.3)	9 (39.1)	
	null	24 (51.1)	10 (41.7)	14 (60.9)	
GSTT1[b] gene deletion	non-null	39 (83)	19 (79.2)	20 (87)	
	null	8 (17)	5 (20.8)	3 (13)	

CAT = catalase; *GPX* = glutathione peroxidase; *GSTM1* = glutathione S-transferase Mu 1; *GSTP1*= glutathione S-transferase pi gene; *GSTT1* = glutathione S-transferase theta 1; HWE= Hardy-Weinberg equilibrium; N = number; SNP = single nucleotide polymorphism; SOD2 = manganese superoxide dismutase; [a]data missing for 2 controls; [b]data missing for 1 control

nancy in childhood or adolescence. To the best of our knowledge no such study was yet performed in the secondary thyroid cancer, while data on the role of common functional polymorphisms in antioxidant defence-related genes in the primary thyroid cancer are scarce.[29]

In total 5.9% of patients diagnosed between 1960 and 2006 with primary malignancy before the age of 21 years has developed a secondary cancer. Secondary thyroid cancers represented 18% of secondary cancers. Our data are comparable to other studies and show that thyroid cancer is one of the most common secondary cancers after treatment of malignancy in childhood or adolescence. Similar to other studies the most frequent primary malignancy was Hodgkin's lymphoma and the median time to develop a secondary thyroid cancer was 19.6 years.[2-6]

According to several studies the *SOD2* 16Ala allele was associated with an increased risk of prostate and oesophageal cancer, but with decreased risk of lung cancer.[21,24] Never the less, the meta-analysis showed no significant effect of *SOD2* p.Val16Ala polymorphism on overall cancer risk.[21] This is also in concordance with our data that show no association between the *SOD2* polymorphism and the risk of secondary thyroid cancer.

TABLE 2. Influence of selected polymorphisms on the risk for secondary thyroid cancer

SNP	OR (95% CI)	p
GPX1 (rs1050450)	0.43 (0.07-1.88)	0.344
SOD2[a] (rs4880)	1.50 (0.36-7.23)	0.754
CAT (rs1001179)	1.00 (0.27-3.74)	1.000
GSTP1 (rs1695)	1.40 (0.38-5.59)	0.774
GSTP1 (rs1138272)	1.25 (0.27-6.30)	1.000
GSTM1 (gene deletion)[b]	0.43 (0.07-1.88)	0.344
GSTT1 (gene deletion)[b]	2.00 (0.29-22.11)	0.687

CAT = catalase; CI = confident interval; *GPX* = glutathione peroxidase; *GSTM1* = glutathione S-transferase Mu 1; *GSTP1*= glutathione S-transferase pi gene; *GSTT1* = glutathione S-transferase theta 1; OR = odd ratio; SNP = single nucleotide polymorphism; *SOD2* = manganese superoxide dismutase; [a]data missing for 2 controls; [b]data missing for 1 control

Recent studies suggested that the *GPX1* p.Pro200Leu polymorphism increased the susceptibility to bladder cancer[27], whereas a meta-analysis showed no significant association of *GPX1* p.Pro200Leu polymorphism with cancer risk in general.[45] Similar to the meta-analysis we observed no association between *GPX1* p.Pro200Leu polymorphism and the risk of secondary thyroid cancer.

CAT polymorphism was implicated in cancerogenesis of several tumours, including breast and cervical cancer[24], but again, meta-analysis has not confirmed these observations for the breast cancer risk.[46] Our results also showed no association between *CAT* polymorphism and risk of secondary thyroid cancer.

Genetic variability at the *GSTM1, GSTT1 and GSTP1* loci has been linked to increased susceptibility to several cancers, including thyroid cancer.[28,29,31] Our study was the first to analyse the association between the *GST* polymorphisms (*GSTM1, GSTT1, GSTP1* p.Ile105Val and *GSTP1* p.Ala114Val) and the risk of developing secondary thyroid cancer after treatment of malignancy in childhood or adolescence, however, we were not able to detect any significant association. Mertens *et al*. found only a non-significantly increased risk of thyroid cancer in *GSTM1* or *GSTT1* homozygous patients that had Hodgkin lymphoma as a primary cancer[36], but a meta-analysis concluded that GST polymorphisms are unlikely to be major determinants of susceptibility to primary thyroid cancer.[29]

Our results are comparable to the results of relevant studies on antioxidant defence-related polymorphisms and the risk of cancer in general. While the sample size is small and therefore in some instances lacks statistical power, its prime advantage is the homogeneity of data because we designed a population-based study, which included all patients diagnosed and treated for secondary thyroid cancer in Slovenia after treatment of malignancy in childhood or adolescence between 1960 and 2006.

In conclusion, we observed no association of common functional polymorphisms in antioxidant defence related genes with the risk for secondary thyroid cancer after treatment of malignancy in childhood or adolescence. However, thyroid cancer is one of the most common secondary cancers after treatment of malignancy in childhood or adolescence and it can develop several decades after the treatment. Hence the lifelong follow up of patients with childhood or adolescent malignancy is of utmost importance and further studies on genetic factors associated with thyroid cancer risk should be performed.

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case report

Cerebral toxoplasmosis in a diffuse large B cell lymphoma patient

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Background. Toxoplasmosis is an opportunistic protozoal infection that has, until now, probably been an underestimated cause of encephalitis in patients with hematological malignancies, independent of stem cell or bone marrow transplant. T and B cell depleting regimens are probably an important risk factor for reactivation of a latent toxoplasma infection in these patients.

Case report. We describe a 62-year-old HIV-negative right-handed Caucasian female with systemic diffuse large B cell lymphoma who presented with sudden onset of high fever, headache, altered mental status, ataxia and findings of pancytopenia, a few days after receiving her final, 8th cycle of rituximab, cyclophosphamide, vincristine, doxorubicin, prednisolone (R-CHOP) chemotherapy regimen. A progression of lymphoma to the central nervous system was suspected. MRI of the head revealed multiple on T2 and fluid attenuated inversion recovery (FLAIR) hyperintense parenchymal lesions with mild surrounding edema, located in both cerebral and cerebellar hemispheres that demonstrated moderate gadolinium enhancement. The polymerase chain reaction on cerebrospinal fluid (CSF PCR) was positive for Toxoplasma gondii. The patient was diagnosed with toxoplasmic encephalitis and successfully treated with sulfadiazine, pyrimethamine and folic acid. Due to the need for maintenance therapy with rituximab for lymphoma remission, the patient now continues with secondary prophylaxis of toxoplasmosis.

Conclusions. With this case report, we wish to emphasize the need to consider cerebral toxoplasmosis in patients with hematological malignancies on immunosuppressive therapy when presenting with new neurologic deficits. In such patients, there are numerous differential diagnoses for cerebral toxoplasmosis, and the CNS lymphoma is the most difficult among all to distinguish it from. If left untreated, cerebral toxoplasmosis has a high mortality rate; therefore early recognition and treatment are of essential importance.

Key words: toxoplasmosis;,cerebral; lymphoma, B-cell; rituximab; hosts, immunocompromised; magnetic resonance imaging; treatment

Introduction

Toxoplasmosis is caused by an infection with the obligate intracellular parasite *Toxoplasma gondii*. The general assumption is that approximately 25 to 30% of the world's human population is infected by *Toxoplasma*[1], which makes it one of the most common human infections in the world. The prevalences vary widely between countries, with the lowest seroprevalences observed in the countries of North America, South East Asia, Northern Europe and the Sahelian countries of Africa (between 10–30%). Toxoplasmosis is highly prevalent in Latin America and tropical African countries, while in the countries of Central and Southern Europe, including Slovenia, moderate seroprevalences have been found (30–50%).[1]

Toxoplasma gondii has a complex lifecycle involving felines, in which the sexual phase is completed, as it's definite host. Oocysts shed in feline

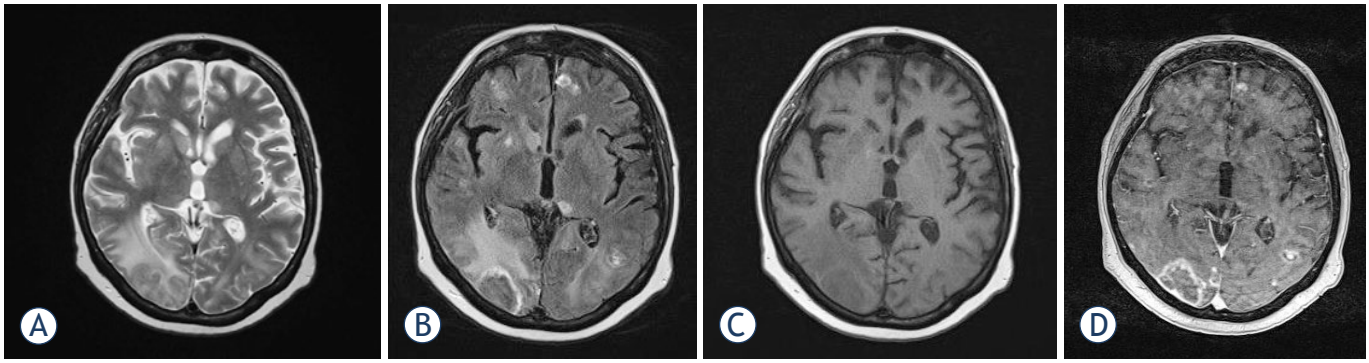

FIGURE 1. (A) MRI at time of diagnosis demonstrating multiple T2 and **(B)** fluid attenuated inversion recovery (FLAIR) hyperintense parenchymal lesions, located in both cerebral and cerebellar hemispheres, with mild surrounding edema. **(C)** On T1 sequences, these lesions were hypointense. (**D**) After contrast administration, only moderate rim enhancement was seen.

feces can infect a wide range of animals, including birds, rodents, grazing domestic animals, and humans.[2] Humans usually get infected either by the ingestion of tissue cysts in infected meat or by ingestion of soil, water, or food contaminated with sporulated oocysts derived from the environment or, less frequently, directly from feline feces.[1] In addition to oral transmission, direct transmission of the parasite by blood or organ products during transplantation takes place at a low rate. Apart from horizontal transmission, a vertical route of transmission is also recognized; parasite transmission to the fetus occurs in about one-third of pregnant women with primary toxoplasmic infection.[1,2]

In the immunocompetent host, the infection is usually rapidly cleared and only about 10-20% of infected individuals present with a self-limited and nonspecific illness, that only rarely requires therapy.[1-3] In the immunocompromised host, the immune factors necessary to control the spread of the infection are lacking and the disease can be life-threatening. In these individuals, toxoplasmosis almost always happens as a result of reactivation of a latent infection. The most common site affected by toxoplasmosis is the central nervous system (CNS). Besides CNS, multiple other organs may be involved, including the lungs, gastrointestinal tract, pancreas, skin, eyes, heart, and liver. With the advent of the human immunodeficiency virus (HIV) pandemic, toxoplasmic encephalitis has become one of the most frequent opportunistic infections. Other heavily immunocompromised patients like those after allogeneic stem cell transplantation (SCT) or previous T cell depleting treatment regimens (e.g. with fludarabine or alemtuzumab) are also at high risk for opportunistic infections. If left untreated, the infection with *Toxoplasma gondii* in such individuals is usually fatal.

Case report

A 62-year-old HIV-negative right-handed Caucasian female with systemic diffuse large B cell lymphoma (DLBCL) presented to our oncology clinic in the beginning of September 2013 with high fever, headache and altered mental status.

Her past medical history included arterial hypertension and type 2 diabetes mellitus, both well controlled with medications. Since 2010, she was treated for marginal zone B cell lymphoma stage IV A, involving the spleen, bone marrow and lymph nodes. A splenectomy was performed in July 2011. At that time, no other treatment was administered due to clinical remission. In August 2012, the disease progressed and she was started on chemotherapy with rituximab and chlorambucil (R-LP), but was switched after only two cycles of R-LP to fludarabine and cyclophosphamide in combination with rituximab (R-FC) due to further disease progression. During this time, she suffered an episode of cutaneous herpes zoster, so valacyclovir prophylaxis was initiated. In January 2013, a transformation into DLBCL was confirmed and rituximab, cyclophosphamide, vincristine, doxorubicin and prednisolone (R-CHOP) chemotherapy regimen introduced. After 4 cycles of R-CHOP, disease remission was achieved and chemotherapy continued until the beginning of August 2013, when a neutropenia was noted and prophylactic therapy with ciprofloxacin and fluconazole initiated. Only two days later she had to be hospitalized in a regional hospital because of febrile neutropenia due to urinary infection with *E. Coli*, which was successfully treated with amoxicilline/clavulanic acid. At the same time, a left posterior tibial vein thrombosis was discovered and therapy with low molecular weight heparin (LMWH) initiated.

By the end of August 2013 she had received her 8th, final cycle of R-CHOP. Only five days after, the patient started complaining of headache with photophobia and was admitted to a regional hospital a day later with high fever, confusion and pancytopenia. A head CT scan revealed diffuse hypodensities involving the right parieto-temporo-occipital region, spreading into the frontal lobe and across the corpus callosum into the left parieto-occipital lobe. A hypodensity in the central region of the cerebellum was present as well. There were no midline brain shifts or herniations and the ventricular system was normal. Progression of the lymphoma to the CNS was suspected and the patient transferred to our oncology clinic for further evaluation and management.

At the time of our first neurological consultation, the patient was conscious but apathetic, with elements of dysexecutive syndrome. She had poor orientation in time and situation, seemed to fill in the memory gaps with confabulations and exhibited finger agnosia. Her speech was normal. A mini mental state examination (MMSE) test could not be applied at that time due to the lack of attention needed to complete the task. A left homonymous hemianopsia, denser in the lower quadrant, was clinically suspected. A slight mask-like appearance of the face was noted. The rest of the cranial nerve examination revealed no abnormalities. Meningeal signs were absent. A mild pyramidal weakness of the right upper extremity was present and she exhibited a moderate symmetrical ataxia along with dysmetria of all four extremities. The plantar responses were flexor. There were no marked sensory deficits. The clinical picture indicated bilateral multifocal cerebral cortico-subcortical involvement along with damage to the cerebellum.

She was started on antiedematous therapy with 20% mannitol solution. A diagnostic lumbar puncture was performed and the cerebrospinal fluid (CSF) analysis revealed an elevated white cell count of 27 cells (1 neutrophil, 20 lymphocytes, 6 monocytes), with increased lactate, LDH and protein concentration, but normal glucose level. Cytological analysis of the CSF with Giemsa staining and flow cytometric immunophenotypisation revealed no malignant cells, but there were signs of reactive pleocytosis with T cell predominance.

Magnetic resonance imaging (MRI) of the head revealed multiple T2 and fluid attenuated inversion recovery (FLAIR) hyperintense parenchymal lesions with mild surrounding edema, located in both cerebral and cerebellar hemispheres, with the largest lesion located in the right occipital lobe.

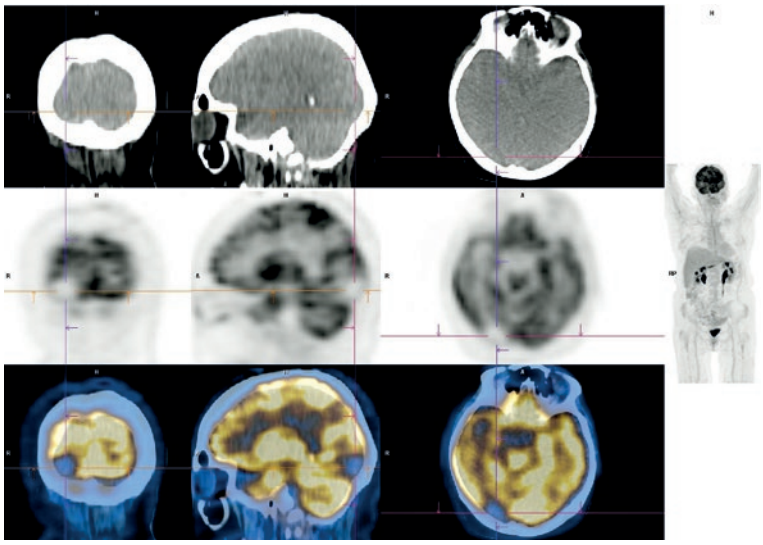

FIGURE 2. Full body 18F-FDG PET/CT revealing focal hypometabolism, corresponding to toxoplasma lesions. The largest lesion is seen in the right occipital lobe.

The lesions were hypointense on T1 sequences and demonstrated moderate rim enhancement after contrast administration (Figure 1).

The polymerase chain reaction on cerebrospinal fluid (CSF PCR) was positive for *Toxoplasma gondii* and serological analysis revealed borderline positive IgG and negative IgM antibodies. Microbiological assays for other bacteria, fungus or viruses, such as *Borrelia burgdorferi*, *Mycobacterium tuberculosis*, *Cryptococcus neoformans*, herpes simplex virus type 1 and 2, varicella zoster virus, enteroviruses, polioviruses, tick-borne encephalitis virus, JC virus, Epstein-Barr virus, and cytomegalovirus were negative.

There was no apparent evidence of toxoplasmosis in other organs. A full body 18F-FDG PET/CT showed remission of lymphoma and local hypometabolism in the right occipital lobe, corresponding to the location of the largest toxoplasma lesion (Figure 2).

Based on these findings, the patient was diagnosed with toxoplasmic encephalitis and therapy with sulfadiazine, pyrimethamine and folic acid was administered. Due to an underlying mild cardiomyopathy, caused by anthracycline therapy in the past, the patient suffered an acute heart failure episode in the beginning of treatment, which was managed conservatively. This condition was not directly associated with toxoplasma infection and was caused by fluid volume overload. After a few days of antibiotic therapy the patient slowly began improving. She became more alert and her cognitive status improved. A follow-up MRI of the head

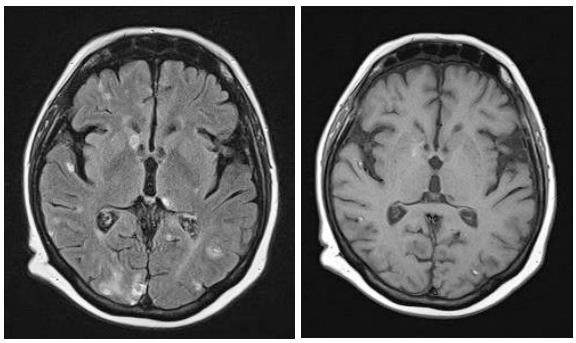

FIGURE 3. Lesion size and edema reduction after 6 weeks of intensive antibiotic therapy, as demonstrated by **(A)** fluid attenuated inversion recovery (FLAIR) and **(B)** T1 sequence.

6 weeks later showed reduction of edema and lesion size, with hemorrhagic inclusions in some of the lesions (Figure 3.) After 6 weeks of intensive antibiotic treatment we started with maintenance therapy with rituximab for lymphoma remission and continued with prophylactic doses of sulfadiazine, pyrimethamine and folic acid.

Three months after the first presentation, the patient was seen again in the neurooncology outpatient clinic for a follow-up. There were only mild cognitive deficits still present and she scored 28/30 points on MMSE. In January 2014, follow-up MRI of the head showed further reduction of brain lesions, this time without any visible hemorrhagic inclusions (Figure 4.)

Patient's written consent to publish this case report has been obtained.

Discussion

In patients with malignancies, especially those after previous allogeneic bone marrow or stem cell transplantation, the incidence of various CNS infections may be up to 15%.[4] There are several risk factors for CNS infection in these individuals, depending on the underlying malignancy, its treatment and various other factors. Among causative organisms, *Toxoplasma gondii* is the most prevalent.[4]

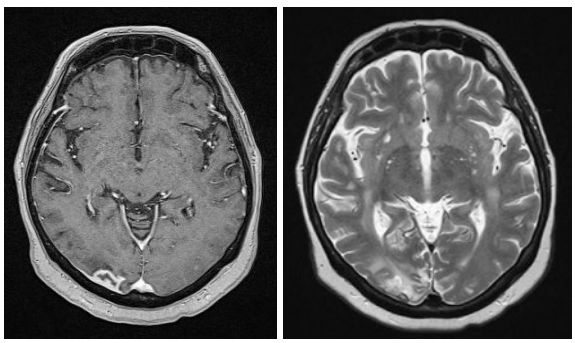

FIGURE 4. Follow-up MRI after 4 months reveals further reduction of lesion size. **(A)** T1 sequence + gadolinium, **(B)** T2 sequence.

The majority of clinical experience with cerebral toxoplasmosis was acquired in HIV/AIDS patients, in whom the risk of reactivation of latent infection with *T. gondii* is greatest when the CD4+ T-cell count decreases below 100 cells/uL.[5] Cerebral toxoplasmosis is also a rare, but well known opportunistic infection in bone marrow transplant (BMT) recipients[6], as well as in solid organ transplant recipients.[7]

Clinical presentation of cerebral toxoplasmosis is unspecific, ranging from altered mental status, fever, seizures and headache to focal neurologic deficits, including motor deficits, cranial nerve palsies, visual field defects, aphasia, movement disorders and cerebellar dysfunction.

The gray-white matter junction, basal ganglia and thalamus are the areas predisposed to cerebral toxoplasmosis; however, the brainstem and the corpus callosum may also be involved. The lesions are usually characterized by a central zone of necrosis containing only few organisms. The central zone is surrounded by a hypervascular intermediate zone, comprised of numerous inflammatory cells mixed with tachyzoites and encysted organisms, the latter being the predominating ingredient of the final, peripheral zone.[8] On nonenhanced CT scans, the lesions are usually hypo- to isodense to grey matter with surrounding vasogenic edema and mass effect, while solitary or multiple solid, nodular or ring enhancing lesions are demonstrated on contrast-enhanced CT scans.[8,9] The method of choice for evaluation of mass lesions in the brain remains MRI. On MRI scans, a so-called "target sign" on T2 and FLAIR images, consisting of at least three alternating zones, is usually found. The classic constellation consists of a hypointense core surrounded by an intermediate hyperintense region and a peripheral hypointense rim, delineated by surrounding edema. Contrast T1-weighted images show an inverse appearance with ring enhancement of the inflammatory zone.[8,9] A highly specific, but relatively insensitive finding for cerebral toxoplasmosis, seen in less than 30 % of cases, is an asymmetric target sign, exhibiting an off-centre nodular lesion along the wall of the enhancing rim.[8,10]

Our patient had multiple parenchymal lesions on MRI with only moderate enhancement and surrounding edema. In patients on immunosup-

pressant therapy, such as those after bone marrow transplant (BMT), cerebral toxoplasmosis may manifest differently than in patients with HIV/AIDS. Due to a more global loss of immune cells, these patients are usually unable to build a sufficient immune inflammatory response at the blood-brain barrier, therefore allowing for passage of gadolinium and vasogenic edema. However, due to a more global loss of the immune system, most of the parenchymal lesions are non-enhancing, although mass effect and edema are usually present, and there is only subtle enhancement along the meninges.[8,11] In post-BMT patients, parenchymal lesions may undergo hemorrhagic transformation[8], as had our patient's lesions.

There are numerous differential diagnoses for cerebral toxoplasmosis in immunocompromised patients, among which CNS lymphoma can often be the most difficult to distinguish it from. Features suggestive of cerebral toxoplasmosis are subcortical location, multiplicity, and asymmetric target sign.[8,10] In addition to standard MR imaging, MR spectroscopy, perfusion and diffusion MRI, thallium-201 SPECT or 18F-FDG PET/CT[8,12-14] can be of further aid in distinguishing cerebral toxoplasmosis from lymphoma.[8,12-14] On 18F-FDG/PET CT scan, which provides remarkable accuracy for detection, treatment monitoring and follow-up of patients with systemic lymphoma as well as of patients with primary central nervous system lymphoma[15], the standardised uptake for cerebral lesions is much higher than in toxoplasma lesions[8].

Cerebral toxoplasmosis in HIV-negative patients has been most commonly described in patients after BMT due to underlying lymphoma or leukemia. There are only rare reports of toxoplasmosis in patients with hematologic malignancies, independent of BMT.[16,17] Despite clinical advancements and good safety profiles, chemotherapeutics, especially T and B cell depleting regimens, present an important risk factor for infectious complications.

Rituximab is a chimeric human-murine monoclonal antibody, designed to specifically target the transmembrane protein CD20 of B cells. The mechanism of rituximab-induced B cell depletion includes antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and the direct induction of apoptosis, in addition to enhancing the sensitivity of B cells to chemotherapy and inducing cell cycle arrest.[18] Rituximab can deplete peripheral B cells while B cell precursors and mature plasma cells remain relatively unaffected, allowing for a new population of B cells to develop from lymphoid stem cells.[19] This remarkable activity allowed for its approval by the US Food and Drug Administration (FDA) in 1997 and European Medical Agency (EMA) in 1998 for treatment of CD20 positive cancers, including DLBCL, follicular lymphoma and chronic lymphocytic leukemia.[19,20] In addition, it is also widely used in therapy of autoimmune diseases, including FDA approved therapy of rheumatoid arthritis and various off-label uses.[21] In the pre-rituximab era, CHOP regimen was considered the best therapy for DLBCL patients. The addition of rituximab to such chemotherapy (R-CHOP) resulted in significant improvement of outcomes in DLBCL patients, therefore becoming the preferred treatment regimen for DLBCL patients.[18]

Rituximab shows a good safety profile with its main adverse event being infusion reactions, however concerns of increasing the risk of infections are being raised. In addition to a rapid depletion of B cells, which can remain at low or undetectable levels for 2-6 months before returning to baseline levels, rituximab may also cause immunosuppression through several other mechanisms. Growing evidence, supported by an increased incidence of viral infections with the use of rituximab, now suggests rituximab also influences T cell immunity and predisposes patients to opportunistic infections.[19,22] When administered for long periods, such as in maintenance therapy, it also causes delayed-onset neutropenia and hypogammaglobulinemia.[22] Several studies have implicated B cells and antibodies (Abs) in host survival and protozoan parasite clearance, especially the B1 cell subpopulation, which appears to be evolutionary selected and maintained to facilitate prompt Ab responses. The B1 cells also appear to modulate T cell response and are implicated in the pathogenesis of toxoplasmosis through fine tune regulation of the exacerbated Th1 response by secretion of IL-10, and production of Abs against heat shock protein 70 of *T. Gondii*.[23] Parasitic infections have rarely been described in association with rituximab use[22], although recently a case of reactivation of cerebral toxoplasmosis in a patient with cutaneous vasculitis was attributed to rituximab therapy.[24] On the other hand, a recent study by Lanini *et al.* found several factors influencing the risk for infections, independent of rituximab use, among them HIV sero-status, presence of graft-versus-host-disease, type of malignancy and lymphocyte count at nadir.[19]

In BMT patients, the risk of latent toxoplasmosis reactivation is highest within 2–4 months post-BMT, but can be prolonged to 6 months, especially

in cases of prolonged immune reconstitution.[25] In cases of toxoplasmosis in patients with hematological malignancies, the exact time relationship between immunosuppresive therapy and reactivation of latent toxoplasma infection is not clearly established. In addition to rituximab, other immunosuppressants (e.g. fludarabine) also influence T cell function and this impact may persist for 1-2 years after discontinuation of therapy.[16] Therefore, the emergence of febrile neutropenia in an immunosuppressed patient should be an important clinical sign, which prompts the physician to look for opportunistic infections.

Diagnosis of cerebral toxoplasmosis in immunocompromised patients is considered a medical emergency, since undiagnosed and untreated infection can rapidly be lethal. PCR testing has revolutionized the diagnosis of toxoplasmosis allowing for early detection of the parasite DNA in the CSF and blood, thereby reducing the need of direct demonstration of tachyzoites in body fluids or tissues by conventional methods, such as Giemsa staining.[1,3] Serologic testing has only a limited value in immunocompromised patients, nowadays being used mostly as (i) an exclusion criterion when negative for patients, except for haematopoietic stem cell transplant patients, with symptoms consistent of acute toxoplasmosis or (ii) as a monitoring indicator, mainly for solid-organ transplant patients, prompting further investigations in cases of strong increases of IgG titers.[1] An IgG avidity ratio can be helpful in establishing the diagnosis of recent toxoplasmic infection in immunocompetent patients[1,3], with questionable results in immunocompromised patients due to their lack of ability to mount a sufficient immune response.

Treatment of cerebral toxoplasmosis comprises either (i) a combination of pyrimethamine plus sulfadiazine plus leucovorin, (ii) a combination of pyrimethamine plus clindamycin plus leucovorin or (iii) trimethoprim-sulfametoxazole.[4,26] Prophylactic therapy is an important factor in toxoplasmosis prevention. In immunocompromised patients with high risk of reactivation of a latent *T. gondii* infection (eg. HIV patients with CD4+ T cell count less than 100 cells/uL or transplant patients), primary prophylaxis with trimethoprim-sulfametoxazole is usually initiated.[5,25,27] Secondary prophylaxis guidelines for patients with HIV/AIDS who have completed initial therapy for cerebral toxoplasmosis clearly state that such patients should be administered lifelong suppressive therapy (eg. secondary prophylaxis) unless immune reconstitution occurs as a consequence of antiretroviral therapy, in which case discontinuation of treatment is indicated.[26] To our knowledge, there are no such guidelines for other immunocompromised patients. Considering the fact that a combination of pyrimethamine plus sulfadiazine plus leucovorin is highly effective in HIV/AIDS patients[26], we decided to proceed with secondary prophylaxis for the duration of maintenance therapy with rituximab. If rituximab was to be discontinued in the future, we planned for an additional prolongation of prophylactic therapy until rituximab-mediated depletion of B cell population would be reversed.

Within 10 days of therapy initiation, the effect of medications can already be detected with MRI; there is a decrease in the number and size of the lesions, with a reduction of edema and mass effect. Complete resolution may take as long as 6 months, and healed foci may calcify or show changes consistent with leukomalacia.[8] Sometimes, a paradoxical worsening of the clinical and radiological picture is seen due to immune reconstitution inflammatory syndrome (IRIS) after initiation of therapy.[8] Our patient showed a good clinical and radiological response to therapy, but further follow-up will be needed.

With this case report, we wish to emphasize the need to consider cerebral toxoplasmosis in patients with malignancies on immunosuppressive therapy when presenting with new cognitive or neurologic deficits. Although to our knowledge, no guidelines for screening of patients for latent toxoplasmosis prior to administration of potent immunosuppressants, such as rituximab, exist, we would strongly recommend it. On the basis of serology results, the need for primary prophylaxis against toxoplasmosis should then be determined for every patient.

Conclusions

Despite the widespread prevalence of latent toxoplasmosis, cerebral toxoplasmosis is an opportunistic infection that has until now only rarely been reported in patients with hematological malignancies, independent of stem cell or bone marrow transplant.

Chemotherapeutics, such as rituximab and fludarabine, probably present an important risk factor for reactivation of a latent toxoplasma infection in patients with hematological malignancies.

There are numerous differential diagnoses for cerebral toxoplasmosis, among which CNS lymphoma is the most difficult to distinguish it from. Early recognition and confirmation of cerebral

toxoplasmosis is of essential importance, since an undiagnosed and untreated infection presents a life-threatening condition. Management of cerebral toxoplasmosis in immunocompromised patients with malignancies is currently based on guidelines for prevention and treatment of cerebral toxoplasmosis in patients with HIV/AIDS or BMT patients, for whom abundant epidemiological data exist. With the advent of new chemotherapeutic drugs there is an evolving need for recommendations for prevention and management of toxoplasmosis and other opportunistic infections in patients receiving these agents.

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research article

Obstructive urination problems after high-dose-rate brachytherapy boost treatment for prostate cancer are avoidable

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Background. Aiming at improving treatment individualization in patients with prostate cancer treated with combination of external beam radiotherapy and high-dose-rate brachytherapy to boost the dose to prostate (HDRB-B), the objective was to evaluate factors that have potential impact on obstructive urination problems (OUP) after HDRB-B.

Patients and methods. In the follow-up study 88 patients consecutively treated with HDRB-B at the Institute of Oncology Ljubljana in the period 2006-2011 were included. The observed outcome was deterioration of OUP (DOUP) during the follow-up period longer than 1 year. Univariate and multivariate relationship analysis between DOUP and potential risk factors (treatment factors, patients' characteristics) was carried out by using binary logistic regression. ROC curve was constructed on predicted values and the area under the curve (AUC) calculated to assess the performance of the multivariate model.

Results. Analysis was carried out on 71 patients who completed 3 years of follow-up. DOUP was noted in 13/71 (18.3%) of them. The results of multivariate analysis showed statistically significant relationship between DOUP and anticoagulation treatment (OR 4.86, 95% C.I. limits: 1.21-19.61, p = 0.026). Also minimal dose received by 90% of the urethra volume was close to statistical significance (OR = 1.23; 95% C.I. limits: 0.98-1.07, p = 0.099). The value of AUC was 0.755.

Conclusions. The study emphasized the relationship between DOUP and anticoagulation treatment, and suggested the multivariate model with fair predictive performance. This model potentially enables a reduction of DOUP after HDRB-B. It supports the belief that further research should be focused on urethral sphincter as a critical structure for OUP.

Key words: prostate cancer; high-dose-rate brachytherapy boost; late effects; urinary stricture; obstructive urination problems.

Introduction

Several modes of radical local treatment are on disposal for patients with localized or locally advanced prostate cancer. Beside radical prostatectomy, radical irradiation in the form of external beam radiotherapy (EBRT), and permanent brachytherapy (PB) or high-dose-rate brachytherapy (HDRB) are established ways of treatment. Both treatment modalities should be considered as equally effective in the absence of randomized trials. Similar consideration should also be given to different ways of radiation treatment.[1,2] When radiation therapy is applied, EBRT could be combined with either form of brachytherapy. The combination of EBRT and HDRB to boost the dose to prostate (HDRB-B) is effective treatment, according to some reports more effective than EBRT alone.[3-5]

Any of above mentioned treatments could expose patients to late side effects. Especially long-term consequences could be decisive for patients' determination for one or other treatment. Well

known long-term complications are bladder neck contractures after radical prostatectomy[6], and urethral strictures after PB[7,8] or HDRB-B.[9-11]

In HDRB-B obstructive urination problems (OUP), including urethral strictures, are the most frequent urinary severe late effect. According to results of studies in the past the frequency of only strictures was 1.5–9%.[9-12] Some recent studies report even considerably higher frequency.[4,13] Previous transurethral resection of the prostate (TURP)[14-16], fractionation schedule of HDRB-B with increased risk with higher fractional dose[13], older age[14], and hypertension[15], were considered as potential patient-related risk factors but their role was not clearly defined.[17] Furthermore, there were found no reliable normal tissue absolute dose constraints for urinary toxicity.[17]

This high stricture risk with problems that evolve with stricture formation should be factored into counselling all men who are considering HDRB-B and could curtail patient's decision for HDRB-B, since with refinement of radical prostatectomy, or PB, the expected frequency of these marked lower urinary tract OUP could be considerably lower.[18-20]

At the Ljubljana Institute of Oncology HDRB-B was started in October 2006, and up to now, late effects, including OUP and stricture formation, have not been evaluated yet.

Aiming at improving treatment individualization in patients with prostate cancer treated with HDRB-B, the objective of the present study was to evaluate factors that have potential impact on OUP after HDRB-B.

Patients and methods

Patients

In the follow-up study 88 patients, consecutively treated by the author with HDRB-B at the Institute of Oncology Ljubljana in the period 2006–2011, were included.

HDRB-B treatment was primarily offered to patients with intermediate- or high-risk clinically localized or locally advanced prostate cancer (according to D'Amico risk stratification of prostate cancer patients)[21] and to low risk patients refused to get radical prostatectomy, if feasible for brachytherapy. In general, patients were considered eligible for HDRB-B if (i) ultrasound showed no pubic arch interference, (ii) were eligible to undergo regional anaesthesia with spinal block, and (iii) were eligible to perform CT/MRI scan. TURP was considered as a contraindication for the procedure.

Study protocol was approved by the Protocol and Ethical Committee of the Institute of Oncology Ljubljana.

Treatment characteristics

Brachytherapy consisted of ultrasound guided transperineal insertion of 20 or 30 cm long close-end plastic needles (Varian, California, USA) into the prostate, and in selected patients also into the initial part of seminal vesicles. An in-house made template allowing needle fixation was used. Needles were typically placed into prostate periphery. Cystoscopy was performed to control for urinary bladder or urethral puncture. CT or MRI scan was used for planning purposes. Brachyvision planning system (Varian, California, USA) was used for image registration, contouring and dosimetry. Two planning target volumes (PTVs) were routinely defined: PTV1 and PTV2. PTV1 encircled the prostate with additional 3 mm margin around the zone of suspected capsular invasion, while PTV2 encircled peripheral part of the prostate. If visible on the MRI images, also gross tumour volume (GTV) was defined and included in the PTV2. Initially, prescribed dose was 21 Gy to PTV1 and 22.5 Gy to PTV2, with 7 Gy and 7.5 Gy per fraction, respectively. Later, the dose was reduced to 18 Gy to PTV1 and 19.5 Gy to PTV2, also given in 3 fractions in 2 consecutive days. Urethra was indentified with the urinary catheter. The contour also enclosed additional 1-2 mm margin around the catheter. Contouring of urethra started at bladder base and extended to genitourinary diaphragm inferiorly (always at least 0.5 cm caudally from the last slice of contoured apex of the prostate). Minimal dose received by 90% of the urethra volume ($D90_{urethra\ volume}$) was planned to be below 110%, while minimal dose received by 1% of the urethra volume ($D1_{urethra\ volume}$) was planned to be below 130% of the prescribed dose. Treatment was delivered with Gammamed plus device (Varian, California, USA) using ^{192}Iridium with the activity of 0.7-1.4 Ci.

EBRT was delivered as 3-dimensional conformal radiation. The details of technique have been described elsewhere.[22] The patients were simulated in supine position with knee and feet fixation device and urethrogram, to define prostatic apex. Clinical target volume (CTV) included prostate and distal 2/3 of seminal vesicles with lymph nodes along external, internal and common iliac vessels in patients with Gleason Score (GS) 8–10 or locally advanced tumours, or if the risk of positive lymph

TABLE 1. Questions addressing obstructive urination problems in Ljubljana Institute of Oncology questionnaire about adverse health effects of radiation therapy

Question	Possible answers
Did you have a sensation of not emptying your bladder in the previous month	Yes/No
Did you find stopping and starting again several times when you urinated in the previous month	Yes/No
Did you have weak urinary stream in the previous month	Yes/No
Did you have to strain to start urination in the previous month	Yes/No
If you had any of the problems, how often did they occur	Occasionally At least once a week Daily At every urination
How big were these problems for you	No problem Very small problem Small problem Moderate problem Big problem
Did you have to get urinary catheter in the last half of the year	Yes/No
Were you operated because of the mentioned problems	Yes/No
Did you still have urinary catheter	Yes/No

nodes ($Risk_{N+}$) exceeded 15% according to the equation of Roach *et al.* (Equation 1).[23]

$$Risk_{N+} = 2/3PSA + 10(GS - 6) \quad \text{[Equation 1]}$$

PSA = the pre-treatment prostate-specific antigen
GS = the pre-treatment Gleason score

Uniform 1 cm margin was added to CTV to form PTV. Prior to treatment planning three golden markers were implanted into the prostate. If treatment started with HDRB treatment, markers were implanted together with needle insertion. Prescribed dose was 50.4 Gy. The PTVs were required to be enclosed in 95% isodose relative to the prescribed dose. Basically, box technique was used, with additional small fields to homogenize the dose delivery. All patients were treated using 15 MV photons. During treatment prostate position was determined using MV portal imaging and implanted markers. Daily off-line position correction was used.

A constitutive part of treatment was also the androgen deprivation therapy. In principle 3 years of androgen deprivation was advised to high-risk patients and also to some intermediate-risk patients with cancer overgrowth in the majority of biopsy cores or with MRI evidenced infiltration of periprostatic tissue or seminal vesicles. One year of androgen deprivation was advised to the rest of intermediate-risk patients. In low-risk patients androgen deprivation therapy was given either to reduce the prostate volume, or was initiated by the referring urologist after prostate biopsy and continued afterwards until the end of radiation treatment. Patients on androgen deprivation were followed-up every 6 months. After the discontinuation of androgen deprivation, patients were seen yearly, with PSA testing every six months. After the first follow-up visit 3–6 months after treatment, the same way of annual check-ups was used in patients without adjuvant androgen deprivation.

Study instrument for assessment of obstructive urination problems

An in-house made questionnaire, used already for several years, was used as the study instrument. The questionnaire was discussed with patients at follow-up visits. The aim of the questionnaire was to detect late effects of treatment more precisely as would be by open questions, and to allow to grade late toxicity according to Radiation Therapy Oncology Group (RTOG)[24] subjective part of RTOG/EORTC Soma Scales[25] and Common Terminology Criteria for Adverse Events Ver. 3.0 (CTCAE). Late urinary toxicity was addressed with regard to dysuria, frequency, haematuria, incontinence and obstruction. In Table 1 the questions addressing OUP are presented. Patients were asked to complete the questionnaire just before the start of the HDRB-B treatment, at first follow-up visit and yearly thereafter.

Observed outcome

The observed outcome was deterioration of OUP (DOUP) during the follow-up period longer than 1 year, supposing to be a manifestation of late radiation urethral injury. It was defined in several steps.

Firstly, the presence of OUP just before the start of the HDRB-B treatment was established. The problems were graded according to the following scale: 1-occasional (less than weekly), 2-regular (about daily), 3-regular (daily) with at least one episode of urgent urethral catheter placement, 4-regular (daily) with at least one episode of urethral dilatation or endoscopic intervention, 5-refractory obstruction (permanent urinary catheter, supravesical urine derivation).

During the follow-up period the grade of OUP was checked-up in the 2nd, the 3rd, the 4th and the 5th year after the beginning of the HDRB-B treatment.

Finally, the difference in grade of OUP between OUP at the start of the HDRB-B treatment and latter follow-up visits was assessed. The following scale was used: 1-major improvement (decrease in OUP for two or more grades), 2-minor improvement (decrease in OUP for one grade), 3-no change, 4-minor deterioration (increase in OUP for one grade), 4-major deterioration (increase in OUP for two or more grades). Since minor and major deterioration were the categories of interest, these two categories were combined in the observed outcome - DOUP (0 = no, 1 = yes).

In order to achieve a sufficiently large number of observed persons, analysis of association between observed outcome and potential risk factors was carried out only in patients who completed 3 years of follow-up. The occurrence of DOUP in the 2nd or in the 3rd year of follow-up was considered.

Risk factors for deterioration of obstructive urination problems

Two groups of risk factors were observed. The first group consisted of HDRB-B and supportive treatment factors, while the second group consisted of patients' characteristics.

In the group of HDRB-B and supportive treatment factors following factors were observed: number of implanted needles ($N_{implanted\ needles}$), planning imaging (1 = CT, 2 = MRI), number of interventions ($N_{interventions}$) (0 = 1, 1 = 2+), and dosimetric factors, being PTV1, minimal dose received by 100% of the PTV1 ($D100_{PTV1}$), minimal dose received by 90% of the PTV1 ($D90_{PTV1}$), minimal dose received by 100% of the PTV2 ($D100_{PTV2}$), urethral volume ($V_{urethra}$), mean urethral dose ($D\text{-}MEAN_{urethra}$), $D90_{urethra\ volume}$, minimal dose received by 10% of the urethra volume ($D10_{urethra\ volume}$), and $D1_{urethra\ volume}$. All of dosimetric factors were retrospectively extracted from Dose-Volume Histograms stored in electronic patients' files. As supportive treatment factor the duration of androgen deprivation therapy was included (0 = < 12 months, 1 = ≥ 12 months).

In the group of patients' characteristics the following factors were observed: age, co-morbidity (hypertension: 0 = no, 1 = yes, diabetes: 0 = no, 1 = yes, coronary insufficiency: 0 = no, 1 = yes, hyperlipidemia: 0 = no, 1 = yes, history of cerebrovascular insult or peripheral deep venous thrombosis: 0 = no, 1 = yes), and anticoagulant treatment (vitamin K antagonist or antiplatelet drug) (0 = no, 1 = yes). All of them were extracted from patients' files.

Statistical analysis

All data were first statistically described. Parametric (mean ± standard deviation, minimum and maximum) or nonparametric methods (median, range) for numerical data, or percentages for attributable data were used.

Afterwards the univariate and multivariate relationship analysis between DOUP and potential risk factors (treatment factors, patients' characteristics) was carried out. Univariate analysis was carried out by using binary logistic regression or Fisher's exact test (in one variable logistic regression analysis could not be performed due to no observed outcome in one category). In multivariate analysis binary logistic regression was used. All variables that were meaningful for the observed outcome and univariately at least marginally statistically significantly associated with DOUP ($p < 0.250$) were included in the multivariate model.[26] On the basis of logistic regression model, the risk-score (logit) for each participant was calculated (Equation 2) and afterwards converted to the risk estimates (p(x)) for the observed outcome (Equation 3).[26]

$$\text{logit} = \ln\left[\frac{p(x)}{1-p(x)}\right] = a + b_1 x_1 + b_2 x_2 + \ldots + b_n x_n$$

[Equation 2]

$$p(x) = \frac{e^{\text{logit}}}{1+e^{\text{logit}}} = \frac{e^{a+b_1x_1+b_2x_2+\ldots+b_nx_n}}{1+e^{a+b_1x_1+b_2x_2+\ldots+b_nx_n}}$$

[Equation 3]

Finally, the risk estimate values were put in an ordered series from the lowest to the highest value.

TABLE 2. Characteristics of dosimetric parameters of high-dose-rate brachytherapy in Ljubljana Institute of Oncology study of late toxicity after high-dose-rate brachytherapy boost treatment for prostate cancer

Dosimetric parameter	Minimum	Maximum	Mean±SD
PTV1	18 ml	95 ml	37.6 ± 14.8 ml
$D100_{PTV1}$	8.3 Gy	17.1 Gy	11.8 ± 1.9 Gy
$D90_{PTV1}$	13.4 Gy	24.6 Gy	19.2 ± 2.0 Gy
$D100_{PTV2}$	12.8 Gy	23.7 Gy	17.3 ± 2.1 Gy
$V_{urethra}$	1.2 ml	4.0 ml	1.9 ± 0.8 ml
$D\text{-}MEAN_{urethra}$	14.4 Gy	26.1 Gy	19.0 ± 2.6 Gy
$D90_{urethra\ volume}$	7.5 Gy	18.9 Gy	13.2 ± 2.9 Gy
$D10_{urethra\ volume}$	17.8 Gy	31.1 Gy	23.1 ± 2.7 Gy
$D1_{urethra\ volume}$	17.3 Gy	36.0 Gy	24.5 ± 3.4 Gy

$D100_{PTV1}$ = minimal dose received by 100% of the PTV1; $D90_{PTV1}$ = minimal dose received by 90% of the PTV1; $D100_{PTV2}$ = minimal dose received by 100% of the PTV2; PTV1 = planning target volume 1; PTV2 = planning target volume 2; $D\text{-}MEAN_{urethra}$ = mean urethral dose; $D90_{urethra\ volume}$ = minimal dose received by 90% of the urethra volume; $D10_{urethra\ volume}$ = minimal dose received by 10% of the urethra volume; $D1_{urethra\ volume}$ = minimal dose received by 1% of the urethra volume; $V_{urethra}$ = urethral volume

At every value the cut-point was placed, a decision matrix defined taking into account the actual status of presence/absence of observed outcome, and nosological (true-positive, false-positive, true-negative and false-positive rates) as well diagnostic test validity measures (positive and negative predictive values) calculated.[27-29] Also the receiver operating characteristic (ROC) analysis was performed (ROC curve constructed and the area under the curve calculated).[29] Decision about the best possible cut-point (the cut-point with the highest true positive rate at the highest acceptable false positive rate) was supported by calculating Youden index (the maximum vertical distance between the ROC curve and the diagonal/chance line).[30]

In all statistical tests p-value 0.05 or less was considered significant. SPSS statistical package for Windows Ver. 21.0 was used for analysis.

Results

Description of the study group

Patients in the study group were aged 67.6 ± 6.1 years, with following tumour characteristics: Gleason score: £ 6 22/88 (25.0%), 7 37/88 (42.0%), ≥ 8 29/88 (33.0%); percent of positive cores: median 50 (range 10–100); PSA: median 10 ng/ml (range 4–60 ng/ml); stage: T1 18/88 (20.5%), T2 40/88 (45.5%), T3 30/88 (34.0%); risk category: low 13/88 (14.8%), intermediate 27/88 (30.7%), high 48/88 (54.5%). Androgen deprivation treatment was received by 81/88 (92.0%) patients (median duration 24 months (1–60 months); duration less than 12 months: 10/81 (12.3%) patients).

In most patients the comorbidities were present. The most frequent was hypertension (46/86, 53.5%), followed by hyperlipidemia (20/85, 23.5%), coronary insufficiency (17/86, 19.8%), history of cerebrovascular insult or peripheral deep venous thrombosis (11/86, 12.8%) and diabetes (10/85, 11.8%).

During the HDRB-B treatment 22/86 (25.6%) of patients were receiving anticoagulation therapy.

Description of the treatment

Regarding the dose to PTV1, 21 Gy was delivered to 27/88 (30.7%) patients, while 18 Gy to 61/88 (69.3%) patients. Target volume was restricted to prostate in 79/88 (89.8%), while in 9/88 (10.2%) of patients it was enlarged to enclose infiltrated parts of seminal vesicles. Dosimetric parameters of HDRB-B applied in the study are summarized in Table 2.

Mean $N_{implanted\ needles}$ was 15±3, while $N_{interventions}$ was one in 80/88 (90.9%), and two or more in 8/88 (9.1%) patients. CT based planning was used in 30/88 (34.1%), while MRI was used in 58/88 (65.9%) patients.

Obstructive urination problems at the beginning of the study

At the start of treatment OUP were declared in 52/82 (63.4%) patients that had complete entry data. In majority of them problems were not significant and were assessed as grade 1 in 46/52 (88.5%). In the rest of patients, problems were more pronounced and assessed as grade 2 in 5/52 (9.6%) and as grade 3 in 1/52 (1.9%) patients.

Analysis of deterioration of obstructive urination problems during the follow-up period

The course of OUP after treatment in relation to initial problems is presented as a prevalence rates during the 2nd, the 3rd, the 4th and the 5th year of observation (Table 3). In this time frame the proportion of patients with DOUP after initial increase remained stable. On the other hand it seemed that the proportion of patients that experienced improvement of initial obstructive problems increased (Table 3).

TABLE 3. Prevalence rates of alteration of obstructive urination problems in Ljubljana Institute of Oncology study of late toxicity after high-dose-rate brachytherapy boost treatment for prostate cancer

Year of follow-up	N	Major improvement	Minor improvement	No change	Minor deterioration	Major deterioration
2nd year	80	1 (1.3%)	10 (12.5%)	57 (71.3%)	12 (15.0%)	0 (0%)
3rd year	71	1 (1.4%)	11 (15.5%)	51 (71.8%)	8 (11.3%)	0 (0%)
4th year	45	2 (4.4%)	7 (15.6%)	31 (68.9%)	4 (8.9%)	1 (2.2%)
5th year	25	0 (0%)	6 (24.0%)	16 (64.0%)	3 (12.0%)	0 (0%)

Analysis of association between deterioration of obstructive urination problems and potential risk factors

Analysis of association between observed outcome and potential risk factors was carried out in 71 patients who completed 3 years of follow-up. DOUP occurred in the 2nd or in the 3rd year in 13/71 (18.3%) of patients.

In the group of HDRB-B and supportive treatment factors none of them was statistically significantly associated with the DOUP (Table 4). However, according to predefined criterion PTV1 and $D90_{urethra\ volume}$ were candidates for entering the multivariate analysis.

In the group of patients' characteristics also the vast majority of factors did not show statistically significant association with DOUP (Table 5). The only exception was anticoagulation treatment in which association with observed outcome was statistically significant, and thus was a candidate for entering the multivariate analysis. In the majority of patients receiving this treatment, it consisted of acetyl salicylic acid either alone (11/17 patients) or in the combination with warfarin (2/17 patients). Tidapidine was given to 2/17, warfarin to 1/17,

TABLE 4. Results of univariate logistic regression analysis of association between deterioration of obstructive urination problems and treatment factors in Ljubljana Institute of Oncology study of late toxicity after high-dose-rate brachytherapy boost treatment for prostate cancer

Risk factor		N_{tot}	N_{det}/N_{cat} (%)	OR	95 % C.I. limits for OR		p-value
					Lower	Upper	
$N_{implanted\ needles}$		71		0.89	0.71	1.11	0.305
Planning imaging	CT	71	3/25 (12.0%)	1.00			
	MRI		10/46 (21.7%)	2.04	0.51	8.22	0.317
$N_{interventions}$	1	71	10/63 (15.9%)	1.00			
	2+		3/8 (37.5%)	3.18	0.65	15.48	0.152
PTV1 (ml)		70		1.03	0.99	1.07	0.149
$D100_{PTV1}$ (Gy)		71		1.08	0.76	1.53	0.656
$D90_{PTV1}$ (Gy)		71		0.93	0.68	1.28	0.673
$D100_{PTV2}$ (Gy)		70		0.99	0.75	1.31	0.941
$V_{urethra}$ (ml)		70		1.22	0.55	2.74	0.626
$D\text{-}MEAN_{urethra}$ (Gy)		70		1.06	0.84	1.35	0.629
$D90_{urethra\ volume}$ (Gy)		71		1.18	0.95	1.47	0.145
$D10_{urethra\ volume}$ (Gy)		70		1.07	0.87	1.31	0.521
$D1_{urethra\ volume}$ (Gy)		71		1.03	0.86	1.22	0.780
Androgen deprivation	< 12 months	69	2/9 (22.2%)	1.00			
	≥ 12 months		11/60 (18.3%)	0.79	0.14	4.31	0.781

N^{tot}=total number of observations, N^{det}= number of patients with deterioration; N^{cat}= number of patients within the category; $N_{implanted\ needles}$ = number of implanted needles; $N_{interventions}$ = number of interventions; PTV1 = planning target volume 1; PTV2 = planning target volume 2; $D100_{PTV1}$ = minimal dose received by 100% of the PTV1; $D90_{PTV1}$ = minimal dose received by 90% of the PTV1; $D100_{PTV2}$ = minimal dose received by 100% of the PTV2; $V_{urethra}$ = urethral volume; $D\text{-}MEAN_{urethra}$ = mean urethral dose; $D90_{urethra\ volume}$ = minimal dose received by 90% of the urethra volume; $D10_{urethra\ volume}$ = minimal dose received by 10% of the urethra volume; $D1_{urethra\ volume}$ = minimal dose received by 1% of the urethra volume

TABLE 5. Results of univariate logistic regression analysis of association between deterioration of obstructive urination problems and patients' characteristics in Ljubljana Institute of Oncology study of late toxicity after high-dose-rate brachytherapy boost treatment for prostate cancer

Risk factor		N_{tot}	N_{det}/N_{cat} (%)	OR	95 % C.I. limits for OR		p-value
					Lower	Upper	
Age		70		1.05	0.94	1.17	0.372
Hypertension	No	69	5/32 (15.6%)	1.00			
	Yes		7/37 (18.9%)	1.26	0.36	4.44	0.719
Diabetes	No	68	12/62 (19.4%)	NA			
	Yes		0/6 (0.0%)	NA	NA	NA	0.581*
Hyperlipidemia	No	68	8/52 (15.4%)	1.00			
	Yes		4/16 (25.0%)	1.83	0.47	7.14	0.382
CVI	No	69	10/62 (16.1%)	1.00			
	Yes		2/7 (28.6%)	2.08	0.35	12.26	0.418
Coronary insufficiency	No	69	8/54 (14.8%)	1.00			
	Yes		4/15 (26.7)	2.09	0.53	8.22	0.291
Anticoagulation treatment	No	69	6/52 (11.5%)	1.00			
	Yes		6/17 (35.5%)	4.18	1.13	15.48	0.032

N^{tot}=total number of observations, N^{det}= number of patients with deterioration; N^{cat}= number of patients within the category; NA = not applicable; * = Fisher exact test results; C.I. = confidence interval; CVI = history of cerebrovascular insult or peripheral deep venous thrombosis; OR = odds ratio

TABLE 6. Results of multivariate logistic regression analysis of association between deterioration of obstructive urination problems and selected treatment factors and patients' characteristics in Ljubljana Institute of Oncology study of late toxicity after high-dose-rate brachytherapy boost treatment for prostate cancer (N = 68)

Risk factor		OR	95% C.I. limits for OR		p
			lower	upper	
PTV1 (ml)		1.02	0.98	1.07	0.292
$D90_{urethra\ volume}$ (Gy)		1.23	0.96	1.57	0.099
Anticoagulation treatment	No	1.00			
	Yes	4.86	1.21	19.61	0.026

$D90_{urethra\ volume}$ = minimal dose received by 90% of the urethra volume; OR = odds ratio; PTV1 = planning target volume 1; OR = odds ratio

while acenocoumarol to 1/17 patients. In the group of 6 patients with DOUP, anticoagulation treatment consisted of acetyl salicylic acid in 4 patients, a combination of acetyl salicylic acid and warfarin in one, and warfarin alone in one patient.

All data necessary to perform multivariate analysis were present in 68/71 patients (95.8%). The results of the logistic regression model showed that anticoagulation treatment not only remained statistically significantly associated with observed outcome but even increased (the odds for DOUP were about 4.9-times higher in patients on anticoagulation treatment). In addition, $D90_{urethra\ volume}$ came closer to the border of statistical significance (for a one-Gy increase in $D90_{urethra\ volume}$, about 23% increase in odds of experiencing observed outcome could be expected). All other results are presented in Table 6.

Finally, the risk of DOUP was estimated for each patient. The values varied between 0.02509 and 0.62421, with the median value 0.10973. The value of area under ROC curve was 0.755, indicating fair predictive performance of the model. The best cut-point was placed at value 0.16441 (true positive rate: 9/12 or 0.750; false positive rate: 16/56 or 0.286; true negative rate: 40/56 or 0.714; false negative rate: 3/12 or 0.250; positive predictive value: 9/25 or 0.360; negative predictive value: 40/43 or 0.930; Youden index: 0.464).

Discussion

The main results of the study

The most prominent result of present study was strong association of DOUP after HDRB-B with anticoagulation treatment. Based on the available literature this association has not been reported yet up to now. Generally, patient-related risk factors, with exception of age and prior TURP, were only

exceptionally considered in the studies of OUP after HDRB-B. In the available literature only one study that considered patients' characteristics could be found. In this study hypertension was identified as an independent predictor of urinary tract obstruction grade 2 or more according to CTCAE after HDRB-B, but anticoagulation treatment was not considered.[15] Anticoagulation treatment, however, was found to be, together with total dose, the most important predictive factor for 5-year risk of global urinary toxicity in a large study of 965 patients who received definitive EBRT. One of the conclusions of the study was that urinary toxicity might be more related to patients' risk factors than dose parameters.[31] A similar conclusion can be drawn on the basis of results of present study. They pointed out that perhaps higher dose sensitivity of urethral sphincter region in comparison to the urethra, as already suggested by Hindson[13], is further increased with anticoagulation treatment (with almost 5-times higher odds of DOUP in patients receiving either vitamin K antagonists or antiplatelet agents in present study). One can only speculate about exact aetiology of this increased radiosensitivity. Since anticoagulation treatment is as a rule considered in patients with vasculopathy, poor circulation could be the underlying cause. However, other parameters that implicate this mechanism and were considered in present study (hypertension, diabetes, hyperlipidemia, history of cerebrovascular insult and deep venous thrombosis) did not show significant association with the observed outcome.

Other important results of the study

Additionally, study offered other results that could be interesting. Urethra is, as suggested by Hsu[32], with regard to late urinary toxicity, a dose limiting structure. In present study $D90_{\text{urethra volume}}$ expressed the strongest, although statistically only marginal, association with observed outcome. This relation of dose applied to the major part of urethra to OUP seems to be reasonable as the location of stricture formation is at or beyond the prostatic apex, which is at the margin or beyond the contoured urethra.[13,15,33] Apparently, this is in the area of external urethral sphincter. This way it is more likely that the dose applied to the major part of urethra is a better representative of the actual sphincter dose than are dose parameters that represent high doses to small parts of (contoured) urethra. Some similarity can be found between the significance of the $D90_{\text{urethra volume}}$ and the minimum dose to bulbomembranous urethra which was found to be (with regard to maximum prostatic urethral dose, the mean, maximum and minimum bulbomembranous urethral doses, and bulbomembranous urethral doses 10 and 15 mm from apex) the stongest predictor of stricture formation in a large study of patients treated with PB.[18] However, in two studies that addressed whole range of dose-volume histogram urethral data, and related them to late urinary toxicity grade 2 or higher according to CTCAE[32], or late urinary toxicity grade 3 or higher according to RTOG[34], small urethral volumes and high doses were emphasized. In the study of Ischiyama this was the volume that received minimal 13 Gy per fraction with the prescribed dose of 5×7.5 Gy, and in the study of Hsu multiple dose levels above 110% of the prescribed 19 Gy in 2 fractions. Although in 8/12 patients with grade 3 toxicity in the study of Ischiyama was due to stricture formation, studies that focused only on strictures, failed to prove the value of high-dose urethral volumes. In the study of Hindson this was the minimal dose received by the »hottest« 10% of the urethral volume[13], and in the study of Ghadjar the minimal dose to the urethral volumes that received at least 100%, 120%, 125% of the prescribed target dose and the minimal dose received by the »hottest« 1% urethral volume.[35] Both were negative as no significant association was found with 5-year stricture-free survival in the study of Ghadjar, and no correlation was recorded within dose groups of 18 Gy in 3 fractions, 20 Gy in 4 fractions, 19 Gy in 2 fractions, or 16 Gy in 2 fractions between $D10_{\text{urethra volume}}$ and stricture risk in the study of Hindson. Nevertheless, low-dose urethral volumes were decisive for obstructive or any other urinary toxicity after HDRB-B. We can only speculate that with analogy to EBRT data, different dose-volume histogram parameters should be emphasized for different grades of toxicity. In all of patients that were included in the present study, OUP would either be missed if RTOG criteria were used, or graded as grade 1 morbidity according to CTCAE. As it is suggested by the results of present study, low-dose parameters may be crucial for this low-grade urinary toxicity.

Another parameter that was included in predictive model in present study was PTV1. It was also only marginally significantly associated with observed outcome and was included in the final model primarily due to results of other studies that reported positive correlation between late genitourinary toxicity and PTV.[32,36] Additionally, the study of Pinkawa *et al.* showed that PTV may relate to the length of prostatic urethra that is also predictive for late genitourinary toxicity.[37]

Limitations of the study

The presented study has some potential limitations. Firstly, one could argue that in the study the internationally validated International Prostate Symptom Score (I-PSS) Questionnaire was not used as a study instrument instead of in-house made questionnaire. This is certainly a limitation, however, the in-house made questionnaire was used already for several years at Ljubljana Institute of Oncology and replacing the questionnaire would affect the comparability of responses in time. Secondly, a shortcoming of the study was that patients with OUP did not undergo cystoscopic evaluation so it was unclear weather OUP were actually a consequence of urethral obstruction. The deduction from OUP to urethral stricture, at least if assessed by I-PSS, may not be so straightforward, as it has been already shown.[13] Thirdly, some caution may be posed also to two different prescribed target doses. However, as the technique and the fractionation remained the same and not prescribed doses but dose volume parameters obtained at planning were considered, this concern may be redundant. Perhaps more plausible may be the effect of either CT or MRI based planning. It may be expected that the dose that urethra was actually exposed is different when CT or MRI based planning is used. Delineated volume of urethra is different with different imaging technique - urethra with inserted urinary catheter is more clearly visible on MRI and impression is that urethral volumes are larger on MRI even if urethral sphincter is not considered, in contrast to other organs involved in the contouring of prostate cancer.[38] Finally, according to merely statistical criterion also $N_{interventions}$ could be considered in the model. However, as this factor primarily reflects excessive needle movement during treatment that could not be predicted in the phase of treatment planning, it was not treated as important in the present study. Actually, only one intervention was planned in all patients. Exact evaluation of needle movement with regard to apical fiducial marker using orthogonal x-ray or CT images was done only before third fraction was applied in the morning next day after needle placement. Separate intervention was considered only if needle movement could not be compensated with additional planning. This primarily caudal needle translocation after HDRB-B is well documented but potential detrimental effect, as perhaps suggested by the results of presented study, is less obvious.

The importance of the study results for clinical practice

The study stresses the importance of clinical patients' data in the evaluation of late toxicity after HDRB-B. Considering clinical patients' data together with treatment and dosimetric parameters it is possible to estimate toxicity of HDRB-B more precisely and also give better opportunity to alleviate side effects. The implementation of results of presented study can hopefully reduce OUP after HDRB-B, and can perhaps also reduce stricture formation that requires surgical intervention.

Possibilities for further research in the field

Further research in the field should be focused in improvement of safety of HDRB-B treatment. For improvement of safety in terms of late adverse effects it is vital to recognize structures which could be at risk for certain type of toxicity. It seems that the critical structure for OUP may be urethral sphincter. However, it is needed to confirm a relation between urethral sphincter, potential risk factors and OUP. Among potential risk factors also anticoagulation treatment should be considered.

Conclusions

Treatment factors as well as patients' characteristics that are associated with OUP, and can predict it, and eventually prevent overt stricture formation after HDRB-B, are not sufficiently known. The study emphasizes the relationship between DOUP and anticoagulation treatment and suggests a fair predictive performance of the model which includes its high negative predictive value. It supports the belief that further research should be focused on urethral sphincter as a critical structure for OUP.

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research article

Prognostic factors of choroidal melanoma in Slovenia, 1986–2008

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Introduction. Choroidal melanoma is the most common primary malignancy of the eye, which frequently metastasizes. The Cancer Registry of Slovenia reported the incidence of choroid melanoma from 1983 to 2009 as stable, at 7.8 cases/million for men and 7.4/million for women. The aim of the retrospective study was to determinate the prognostic factors of survival for choroidal melanoma patients in Slovenia.

Patients and methods. From January1986 to December 2008 we treated 288 patients with malignant choroidal melanoma; 127 patients were treated by brachytherapy with beta rays emitting ruthenium-106 applicators; 161 patients were treated by enucleation.

Results. Patients with tumours thickness < 7.2 mm and base diameter < 16 mm were treated by brachytherapy and had 5- and 10-year overall mortality 13% and 32%, respectively. In enucleated patients, 5- and 10-year mortality was higher, 46% and 69%, respectively, because their tumours were larger. Thirty patients treated by brachytherapy developed local recurrence. Twenty five of 127 patients treated by brachytherapy and 86 of 161 enucleated patients developed distant metastases. Patients of age ≥ 60 years had significantly lower survival in both treatment modalities. For patients treated by brachytherapy the diameter of the tumour base and treatment time were independent prognostic factors for overall survival, for patients treated by enucleation age and histological type of tumour were independent prognosticators. In first few years after either of treatments, the melanoma specific annual mortality rate increased, especially in older patients, and then slowly decreased.

Conclusions. It seems that particularly younger patients with early tumours can be cured, whereby preference should be given to eyesight preserving brachytherapy over enucleation.

Key words: choroid melanoma; therapy; brachytherapy; prognostic factors

Introduction

Uveal melanoma is the most common primary malignancy of the eye.[1] Approximately 90% of all uveal melanoma develop in the choroid, 7% in the ciliary body and 3% in the iris.[2] The disease is more common in older age, with the highest incidence at about 60 years of age.[1,2] For the period 1983–1994, the incidence of uveal melanoma in 16 European countries was analysed by the European Cancer Registry (EUROCARE).[3] The incidence in Europe was found ascend from South to North, being 2/million inhabitants in Spain and southern Italy and more than 8/million in Denmark and Norway. In Slovenia, the incidence of choroid melanoma between 1983-2009 was stable, at 7.8 cases/million for men and 7.4/million for women.[4]

In the majority of patients, the biopsy of tumour is not indicated because the accuracy of clinical diagnosis is reaching 99%.[5] However, there is no agreement

about the optimal therapy.[6-10] Until development of eye conserving therapies in 1960's, for more than 100 years, enucleation was the only mode of treatment. The first among eye conserving approaches was the plaque brachytherapy[9,11-14], followed by proton beam[15-17] and helium ion radiotherapy[18-20], stereotactic radiotherapy, transscleral or transretinal local resection[10,21,22], and phototherapy brachytherapy[23], several types of radioactive plaques with photon emitting isotopes were used, including cobalt-60, iodine-125, and iridium-192. Beta emitting plaques with ruthenium (106Ru/106Ro), however, were introduced in 1964 by Lommatzsch.[24-26]

In Slovenia, treatment of choroidal melanoma by brachytherapy with ruthenium plaques using the Lommatzsch method was introduced in 1985 by the Eye Clinic at the University Clinical Centre Ljubljana in collaboration with the Institute of Oncology Ljubljana.[27,28] Before that time, the only available treatment was enucleation of the diseased eye. The aim of this retrospective study was to evaluate these two modalities in the treatment of choroidal melanoma in Slovenia during the period from 1986 to 2008 and to determinate the prognostic factors of survival for choroidal melanoma patients in Slovenia.

Patients and methods

Patients

The database of the Cancer Registry of Slovenia was used for identification of patients with the diagnosis of choroidal melanoma in Slovenia in the years 1986–2008.[4] The medical records of identified patients from the Eye Hospital of the University Clinical Centre Ljubljana and from the Department of Ophthalmology of the University Medical Centre Maribor were reviewed for relevant information on clinical characteristics, treatment and outcome. The diagnosis of choroidal melanoma was based on clinical features and full ophthalmologic examination, indirect ophthalmoscopy, fundus photography, ultrasonography and fluorescein angiography. At the time of diagnosis, the patients were evaluated by chest radiography, lymph gland and liver ultrasonography[29] and routine blood tests. Genetic testing was not done because it was not available at the time of the study.

The study was approved by the institutional review board and was carried out according the Helsinki Declaration.

Treatment

Applicators manufactured by Bebig (Eckert& Ziegler BEBIG Gmbh, Berlin; later Amersham, GB) were used. The applicators were concave, shell-shaped, with Ru-106/Ro-106 isotope covering the concave surface as a thin, insoluble film and emitting beta rays with the energy of 3.54 MeV and half-life of 373 days. The tumours were localized by transillumination and indirect ophthalmoscopy, and the applicators were sutured to the sclera. The dose at the tumour apex was aimed to be about 120 Gy. The applicator was removed after expiration of appropriate time.

Treatment was selected according to the tumour size: patients with tumours ≤ 16 mm in diameter and ≤ 7.2 mm thick, with useful vision preserved, were treated by brachytherapy, patients with larger tumours had enucleation. The enucleation was performed in general anaesthesia.

First follow-up visits took place one month after the procedure, in 3-month intervals during the first year and once a year thereafter. At each follow up visit, patients underwent ophthalmologic examinations with indirect ophthalmoscopy, fundus photography and ultrasonography.

Statistical methods

For comparative analyses, the Fisher exact test for two proportions as well as t-test and Mann-Whitney test for data of two independent groups were used. Survival estimates were carried out using the Kaplan-Meier method and reported at 5 and 10 years follow up. The difference between the survival curves was evaluated by means of a log-rank comparison. Multivariate survival analysis for study of an independent effect of various parameters that appeared statistically significant on univariate analysis to treatment outcome and survival was performed according to Cox's proportional hazard models with backward stepwise selection. The end points of survival analysis were locoregional control (LRC, persistent disease or locoregional recurrence considered as an event), disease-free survival (DFS, appearance of loco-regional recurrence or systemic metastases considered as event), disease-specific survival (DSS, melanoma related death considered as event) and overall survival (OS, death from any cause considered as event) which were measured from the first day of therapy. These statistical analyses were performed by using SPSS version 18.0 (SPSS Inc., Chicago, IL)

TABLE 1. The characteristics of patients and tumours by treatment modality

	Treatment		
	Brachytherapy	**Enucleation**	**Total**
All patients	130	161	291
Excluded	3 palliations	-	3
Treated	127	161	288
Gender			
Man	58	84	142
Women	69	77	146
Age (median)			
Men	58 (29-74)	58 (19-86)	
Women	60 (22-89)	61 (23-92)	
T-stage (AJCC)			
1	38		
2	69		
3	8		
No data	12		
Thickness			
< 3 mm	11		
3.1-5.0 mm	64		
5.1-7.2 mm	49		
> 7.8 mm	3		
No data	0		
Basal diameter			
≤ 10 mm	52		
10,1-12,0 mm	38		
> 12 mm	25		
No data	12		
Histology		161	
Spindle cell		33	
Epithelioid		38	
Mixed		23	
No data		37	

AJCC = American Joint Committee on Cancer

and nonlinear regression Gaussian curve fitting was performed by GraphPad Prism version 5. All tests were two-sided and a P-value of 0.05 was considered statistically significant.

Results

Clinical records of 288 patients with choroidal melanoma treated from January 1986 to December 2008 at the Eye Hospital of the University Clinical Centre Ljubljana and from the Department of Ophthalmology of the University Medical Centre Maribor were reviewed. The follow-up close-out date was December 31, 2013. Median follow-up of all patients was 15 years (range, 4–27 years). In December 2013, 130 patients were alive. The cause of death was melanoma in 107 patients and 51 patients died of melanoma unrelated disease; 20 among them died of other malignant diseases. The characteristics of patients and tumours are shown in Table 1.

Survival

In univariate analysis of all patients, the LRC and DFS were better in enucleation than in brachytherapy group and better in females than in males. Patients < 60 years had better DFS, DSS and OS than older patients. In brachytherapy group, females had statistically better LRC and DFS than males; younger patients had better DSS and OS than older patients. Tumour thickness < 6 mm was associated with better LRC and DFS than thicker tumours, while the base diameter < 11 mm was a good prognostic sign for LRC; DFS, DSS and OS. The treatment time influenced LRC and DFS, while the dose-rate had no influence of the outcome of the treatment. In the enucleation group, age and histology influenced DFS, DSS and OS, while sex had no effect on survival. The detailed data of survival are presented in Tables 2–4.

In multivariate analysis for all patients, gender was independent prognostic factor for LRC, while first treatment and age were independent prognostic factors for DFS, DSS and OS. In the brachytherapy group, gender was independent prognostic factors for LRC; treatment time for LRC and DFS; base diameter for DFS and OS. The age was independent prognostic factor for DFS and OS. In enucleation group, age and histology were independent prognostic factors for DFS and DSS, while on OS influenced only age (Table 5).

Second treatment

In 30 patients treated by brachytherapy, a local recurrence of the tumour occurred. The second application of ruthenium plaque was performed in 13 of these patients, and in 17 patients had enucleation: 12 patients - because of extent of the recurrent tumour and 5 patients - because of the treatment-related side effects (glaucoma, cataract). The eyes were enucleated from 7 months to 18 years (median 24 months) after the first brachytherapy (Figure 1).

TABLE 2. Univariate analysis of survival: all patients (N = 288)

	n	LRC (%)			DFS (%)			DSS (%)			OS (%)		
		5 yrs	10 yrs	p	5 yrs	10 yrs	p	5 yrs	10 yrs	p	5 yrs	10 yrs	p
All	288	90	88	-	65	50	-	76	58		68	46	-
Ruthenium	127	78	75	0.000	71	60	0.014	92	79	0.000	87	68	0.000
Enucleation	161	100	100		60	42		64	42		54	31	
Men	142	85	82	0.026	61	51	0.673	74	61	0.647	66	47	0.952
Women	146	95	93		69	49		78	55		70	46	
< 60 years	150	89	86	0.648	74	58	0.002	86	68	0.000	84	64	0.000
≥ 60 years	138	90	90		56	40		65	47		52	28	

DFS = disease free survival; DSS = disease specific survival; LRC = loco-regional control; n = number of patients; OS = overall survival; yrs = years

TABLE 3. Univariate analysis of survival: patients treated by brachytherapy (N = 127)

		LRC (%)			DFS (%)			DSS (%)			OS (%)		
	n	5 yrs	10 yrs	p	5 yrs	10 yrs	p	5 yrs	10 yrs	p	5 yrs	10 yrs	p
Men	58	66	60	0.003	60	49	0.039	90	76	0.703	87	67	0.859
Women	69	89	87		81	69		93	81		88	70	
< 60 years	68	76	71	0.305	76	66	0.156	98	89	0.002	98	83	0.000
≥ 60 years	59	80	80		65	51		84	65		75	52	
T-stage													
1	38	79	79		73	67		97	84		94	71	
2	69	79	74	0.451	72	57	0.354	90	75	0.378	86	72	0.508
3	8	60	40		45	45		86	86		0	50	
Tumour thickness													
2-5.9 mm	97	83	82	0.003	74	66	0.021	92	79	0.489	86	68	0.724
6-.2 mm	29	64	54		64	39		96	80		96	70	
Base													
< 11 mm	61	83	83	0.043	80	72	0.002	96	84	0.024	96	77	0.002
≥ 11mm	54	70	64		60	45		87	72		78	64	
Dose rate Top (Gy/h)													
≥ 108 Gy	53	81	78	0.302	74	66	0.099	95	84	0.280	87	72	0.690
< 108 Gy	52	74	68		68	46		89	72		87	62	
Dose- rate base (Gy/h)													
≥ 532	53	82	74	0.708	74	57	0.804	95	81	0.665	87	69	0.862
< 532	52	74	71		68	55		89	75		87	65	
Treatment time													
≤ 96 hours	52	87	84	0.015	80	72	0.004	95	84	0.400	89	74	0.565
> 96 hours	53	68	62		62	40		89	71		85	60	

DFS = disease free survival; DSS = disease specific survival; LRC = loco-regional control; n = number of patients; OS = overall survival; yrs = years

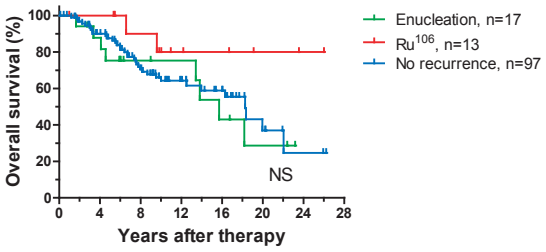

FIGURE 1. Overall survival of patients treated by brachytherapy after treatment of recurrence.

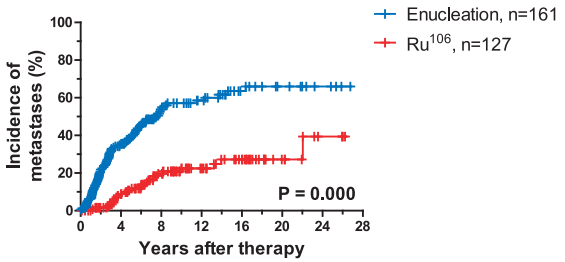

FIGURE 2. Incidence of distant metastases according to the type of treatment.

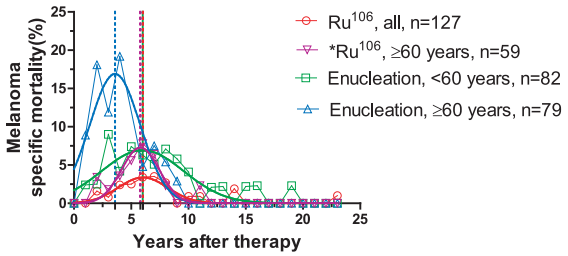

FIGURE 3. Percentage of annual post-treatment melanoma specific mortality according to the type of treatment. *There was no peak in ruthenium treated patients < 60 years.

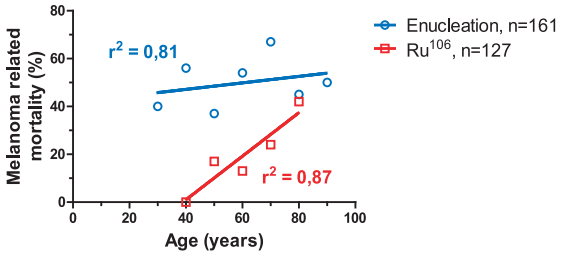

FIGURE 4. Linear regression analysis of melanoma related mortality per age decades, according to the type of treatment. Points represent percent mortality rate for the elapsed decade. No patient less than 40 years who was treated with Ru-106 died of melanoma.

Distant metastases

Twenty-five of 127 patients treated by brachytherapy and 86 of 161 those treated by enucleation developed systemic metastases. Seventy per cent of all metastases were localized in the liver. The actuarial rates of metastases by treatment modality are depicted in Figure 2. At 5 and 10 years, the incidences were 39% and 57%, respectively, for enucleated patients, and 11% and 21%, respectively, for irradiated patients ($P < 0.001$).

In patients treated by brachytherapy, half of the metastases developed in 5 years, and in those treated by enucleation in 2.6 years.

Annual melanoma specific mortality rate

The mortality of patients was increased in the first few years after treatment and then slowly returned to pre-treatment values. Melanoma specific mortality rate is displayed in Figure 3.

The peak percentage of annual melanoma specific mortality after treatment was achieved at 3.6 years for patients older than 60 years treated by enucleation and at approximately 6 years for younger enucleated patients and for all patients treated with brachytherapy. The irradiated patients below 60 years contributed little to the peak because of low mortality rate.

No patient from brachytherapy group aged below 40 years died of melanoma. In brachytherapy treated patients the mortality began to increase after the age of 40 and reached 40 % in 70–80 year's age group. In patients treated by enucleation, the mortality started to increase one decade earlier: the rise started with about 40% and reached about 70 % in patient's 80–90 years of age (Figure 4).

Complications

Because of retrospective character of the study, acute complications were not systematically recorded. For chronic complications patients were reviewed annually. Post-radiation retinopathy started to appear after two years and was documented in 18 patients (12 mild, 6 severe), neovascular glaucoma in 5 patients and cataract in 6 patients. None of the patients had optic neuropathy.

Vision after treatment

After brachytherapy, the eye was retained in all patients and the vision was assessed in 112 patients. Compared to pre-treatment status, 22 (19.6%) patients had better visual acuity; in 12 (10.7%) patients the vision was unchanged while in 78 (69.6%) patients the acuity of vision was worse. The majority of brachyradiotherapy patients retained vision which was better than counting fingers.

Discussion

Our retrospective study reports results of the treatment of patients with choroidal melanoma in Slovenia from 1986 to 2008. In our study, the overall and specific mortality rate in patients treated by enucleation was higher mainly because larger tumours were selected for enucleation as compared to those treated by brachytherapy. Brachytherapy could be used only for selected tumours, depending on their size, location and shape of applicators, for which a satisfactory dose distribution of dose can be achieved. Because no data about the dimensions of the enucleated tumours was available, comparison of results between the two treatment modalities by tumour stage could not be made.

The randomized as well as nonrandomized studies reported no difference in survival rates in patients treated either by enucleation or brachytherapy when matched by the stage, age and other prognostic parameters.[6-8,11,12,30-33] The largest of these studies was the COMS, which included 1317 patients and prospectively compared on randomized fashion enucleation and brachytherapy. There was no statistical difference in 5- and 10-year OS between the two treatment groups.[30] In the matched pairs study of Guthoff *et al.* melanoma specific survival at 12 years of follow-up was 77.9% in irradiated patients and 78.6% in enucleated patients (P > 0.05).[31] When the OS at 10 years of our patients treated by brachytherapy was compared with that from COMS study, no difference could be observed: 32% vs. 35%; similarly, the DSS at 10 years in our series was 79% and was comparable with DSS reported by Guthoff.

There are several prognostic factors for outcome of the choroidal melanoma, including age[30-33], gender[33], basal tumour diameter[34], tumour thickness[33-37], T-stage[35], cell morphology[1,7,33,38] and various genetic changes of the tumour, especially monosomy of chromosome 3.[33,39-41] Some of them appeared statistically significant also in the present study, although the strength of our results should be interpreted with caution. Namely, we only had complete information on age and gender of the patients, histology of the enucleated tumours, and data of tumour diameter, thickness, irradiation dose on the base and top of the tumour and the treatment time for brachytherapy patients, but not also on some other highly relevant prognosticators (e.g. genetic alterations), which limits the strength of statistical analysis.

In both treatment groups, the post-treatment annual mortality related to melanoma at first increased, as expected due to systemic metastases, but few years later it decreased to a few or zero percent. In patients of 60 years or more who were treated by enucleation, mortality reached its peak of 18% at 3.7 years after treatment, while in patients younger than 60 years the peak was reached at six years after treatment and was 7%. Patients treated by brachytherapy fared better: regardless of age, six years after treatment completion the peak mortality was 3%. However, the mortality of irradiated patients aged ≥ 60 years reached the peak of 7% at 6 years post-treatment, while no increase in mortality was noticed among younger patients, probably due to the small number of deaths.

The increase in annual mortality following enucleation was first observed by Zimmermann.[42,43] He re-analysed the data of Paul *et al.*[38] who monitored 2652 patients for 40 years and found a steep increase in mortality following enucleation. In this study, the peak of 8% was reached at 2 years after enucleation, slowly diminishing during the next few years to the "normal" 1%.[43,44] Similarly, Seddon *et al.* reported the increase in mortality to 6.5 % in the first 2–3 years after treatment and slowly return to »normal« 1% during the next 7 years.[45]

The post-treatment increase in melanoma related mortality can be attributed to the loss of antiangiogenic activity of the primary tumour after its removal or destruction. Uveal melanoma cells produce angiostatin, growth inhibitor of metastatic foci[46,47], which was found to be present in the circulation only up to five days after the removal of the primary tumour.[48,49]

Damato *et al.*[33] found that the probability of metastases was greater in older patients as their

TABLE 4. Univariate analysis of survival: patients treated by enucleation (N = 161)*

		DFS (%)			DSS (%)			OS (%)		
	n	5 yrs	10 yrs	p	5y	10 yrs	p	5 yrs	10 yrs	p
Men	84	62	51	0.154	63	51		53	34	0.775
Women	77	59	33		65	34	0,275	56	27	
< 60 years	82	73	52	0.001	76	52		74	51	0.000
≥ 60 years	79	49	30		50	30	0.000	34	10	
Spindle cell	33	74	70		81	72		66	49	
epithelioid	38	56	36	0.050	61	33	0.029	55	24	0.026
Mixed cell	23	62	28		67	24		52	15	

*None of enucleated patients had local recurrence; DFS = disease free survival; DSS = disease specific survival; n = number of patients; OS = overall survival; yrs = years

TABLE 5. Multivariate analysis of survival of all patients (N = 288)

			HR	95% CI		p
				lower	upper	
All patients	LRC	First treatment	40.842	5.565	299.717	0.000
		Gender	2.658	1.245	5.678	0.012
	DFS	First treatment	1.628	1.144	2.316	0.007
		Age < 60 years vs. ≥ 60 years	1.800	1.275	2.540	0.001
	DSS	First treatment	3.937	2.509	6.178	0.000
		Age < 60 years vs. ≥ 60 years	2.534	1.714	3.747	0.000
	OS	First treatment	3.153	2.218	4.480	0.000
		Age < 60 years vs. ≥ 60 years	3.818	2.710	5.377	0.000
Ruthenium	LRC	Gender	2.306	1.013	5.251	0.047
		Treatment time (≤ 96 h vs. > 96 h)	2.841	1.220	6.623	0.015
		Treatment time (≤ 96 h vs. > 96 h)	2.674	1.276	5.587	0.009
	DFS	Base (< 11 mm vs. ≥ 11 mm)	2.519	1.015	6.250	0.046
		T-stage	2.320	1.002	5.376	0.050
	DSS	Age (< 60 years vs. ≥ 60 years)	4.762	1.709	13.333	0.003
	OS	Base (< 11 mm vs v11 mm)	3.610	1.391	9.434	0.008
		Age (< 60 years vs. ≥ 60 years)	5.650	2.538	12.658	0.000
Nucleation	LRC	-	-	-	-	-
	DFS	Age (< 60 years vs. ≥ 60 years)	2.132	1.149	3.968	0.016
		Histology S *VS* E VS M	1.467	1.000	2.151	0.050
	DSS	Age (< 60 years vs. ≥ 60 years)	2.326	1.229	4.403	0.009
		Histology S vs. E vs. M	1.555	1.052	2.298	0.027
	OS	Age (< 60 years vs. ≥ 60 years)	3.876	2.222	6.757	0.000
		Histology (S vs. E vs. M)	1.444	1.051	1.983	0.023

CI = confident interval; DFS = disease free survival; DSS = disease specific survival; E = epitheloid; HR = hazard ratio; LRC = loco-regional control; M = mixed cell; n = number of patients; OS = overall survival; S = spindle cell

tumours grew longer and had more time for accumulation of chromosome instabilities, making the tumour more malignant and more prone to metastasize. Accordingly, the younger patients should have smaller and perhaps less malignant tumours, and the appearance of metastases is less likely. It is assumed that following primary tumour removal, metastases in younger patients reach the lethal tumour mass at a later time. The peak in melanoma-related mortality in younger enucleated patients from our series appeared 2.5 years later than in older counterpart, confirming this assumption. However, not all patients from advanced age group have advanced primary tumour and metastases. In our series, 59 patients ≥ 60 years had primary tumours small enough to be treated by brachytherapy. The annual melanoma related mortality curve suggests that the burden of their metastases was also smaller, and reached the lethal mass at a later time. The synchronous peaks of enucleated patients < 60 years and of irradiated patients ≥ 60 years suggests that the burden of metastases in enucleated group, was similar in these two groups (Figure 3).

There is no good scientific evidence that treatment can prolong patients' life.[33] The increase in annual post-treatment mortality rate implies that the life of some patients might be shortened due to

the therapy, particularly of older ones. This observation and the fact that some tumours and their metastases grow very slowly raise the question when the treatment of uveal melanoma can be withheld. The COMS study showed that the estimated risk of death at 5 years of follow-up in 42 untreated patients was 50%, and risk of 1317 patients treated by a standard method, was 18%.[50] It seems that treatment in older patients without eyesight problems, in spite of evident metastases, could be postponed until the problems eventually ensue. On the other hand, it may be assumed that some of the younger patients are without micrometastases at the time of therapy and can be cured by the early treatment. Indeed, in our study, none of the patients younger than 40 years from brachytherapy group died of metastases, while death of metastases in older patients steeply increased with age (Figure 4).

To conclude, treatment-specific and age-dependent pattern of -related mortality was confirmed in our study, confirming observation of other authors. For quality of life reasons we believe that preference should be given to eyesight preserving brachytherapy or other eye preserving treatments of choroidal melanoma over enucleation, if the size and location are suitable even though the definite opinion on the best treatment differed in the literature.[51,52]

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research article

The impact of anaemia on treatment outcome in patients with squamous cell carcinoma of anal canal and anal margin

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Background. Radiochemotherapy is the main treatment for patients with squamous cell carcinoma of the anal canal. Anaemia is reported to have adverse effect on survival in cancer patients. The aim of the study was to evaluate the influence of anaemia on radiochemotherapy treatment outcome in patients with squamous cell carcinoma of the anal canal.

Patients and methods. One hundred consecutive patients with histologically confirmed squamous cell carcinoma of the anal canal were treated radically with 3-dimensional conformal or intensity-modulated radiation therapy followed by brachytherapy or external beam radiotherapy boost and with concurrent mitomycin C and 5-fluorouracil. The influence on survival of pre-treatment, mean on-treatment and end-of-treatment haemoglobin (Hb) concentrations was studied.

Results. The 5-year locoregional control, disease free survival, disease specific survival and overall survival rates for all patients were 72%, 71%, 77% and 62%, respectively. In univariate analysis, patients with pre-treatment and end-of-treatment Hb > 120 g/L survived statistically significantly better compared to patients with Hb ≤ 120 g/L. Patients with mean on-treatment Hb > 120 g/L only had statistically significant better locoregional control and overall survival than patients with Hb ≤ 120 g/L. In multivariate analysis, independent prognostic factors were pre-treatment Hb (> 120 g/L vs. ≤ 120 g/L) for overall survival (hazard ratio [HR] = 0.419, 95% confidence interval [CI] = 0.190–0.927, p = 0.032) and stage (I & II vs. III) for disease specific (HR = 3.523, 95% CI = 1.375–9.026, p = 0.009) and overall survival (HR = 2.230, 95% CI = 1.167–4.264, p = 0.015).

Conclusions. The pre-treatment, mean on-treatment and end-of-treatment Hb concentration > 120 g/L carried better prognosis for patients for with squamous cell carcinoma of the anal canal treated with radiochemotherapy. The pre-treatment Hb > 120 g/L was an independent prognostic factor for overall survival of patients with anal canal cancer.

Key words: anaemia; anal canal squamous cell carcinoma; radiochemotherapy

Introduction

Squamous cell anal cancer is a rare tumour which represents 1.5% of gastrointestinal cancers, but in Slovenia only 0.5%.[1-5] Despite its infrequent occurrence its incidence is increasing.[4] Women are more commonly affected than men.[3-6] Causal factors in the anal canal cancer are usually associated with human papilloma virus (HPV) infection (being the most important risk factor), human immuno-

deficiency virus (HIV) infection, anal intercourse, higher lifetime number of sexual partners, genital warts and cigarette smoking.[3,6-8]

Anal canal cancer is predominantly a loco-regional disease, because it metastasizes in less than 10% of patients, mainly to lungs and liver.[6]

The management of anal canal cancer has undergone an interesting transformation over the course of the past three decades. With the report by Nigro *et al*. in 1974 it shifted from abdominoperineal resection with or without inguinal lymph node dissection to radical radiochemotherapy.[9,10] Radiochemotherapy with 5-fluorouracil and mitomycin C, nowadays being the main treatment, results in complete tumour response in 70–90% and has a 5-year survival rate of 60–70%, leaving surgery only as a salvage treatment for tumours that do not respond to radiochemotherapy or recur.[4,7] Anal margin cancers are classified as skin tumours and small tumours can be treated by surgery, while tumours T2 or larger should be treated with definitive radiochemotherapy.[11]

Radiotherapy as well as chemotherapy is known to be more efficacious in the presence of oxygen than in hypoxic conditions.[12-15] Tumours are more hypoxic than the surrounding normal tissue.[13] Anaemia, present in 75% of cancer patients, could increase the proportion of hypoxic tumour cells.[13] Hypoxia is widely recognized as a major factor leading to the resistance of tumour cells to radiotherapy, but several mechanisms may also cause cells in the hypoxic region to be resistant to anticancer drugs.[16] The influence of anaemia on the outcome of treatment was first recognized in 1940s in cervical cancer patients and later in patients with other tumours such as head and neck squamous cell carcinoma, carcinoma of the lungs, bladder, prostate and anus.[7,17,18] The purpose of present study was to evaluate the influence of anaemia on radiochemotherapy treatment outcome in patients with squamous cell carcinoma of the anal canal.

Patients and methods

One hundred consecutive patients (60 females and 40 males) with histologically confirmed squamous cell carcinoma of the anal canal were included in the retrospective study. They were treated at the Institute of Oncology Ljubljana from January 2003 till June 2013.

For performance status (PS) the scoring system of the World Health Organization (WHO) was used[19], and for TNM staging the criteria of the Union for International Cancer Control (UICC).[20]

Pre-treatment evaluation

Pre-treatment evaluation consisted of physical and digital rectal examination, rectoscopy with biopsy and fine needle aspiration biopsy of enlarged inguinal lymph nodes, also ultrasound-guided, like in other cancer patients.[21] Imaging included chest X-ray or computer tomography (CT) of chest, abdominal ultrasound (US) or CT and magnetic resonance imaging (MRI) of the pelvis. Laboratory tests included serum chemistry and complete blood count in all patients, and testing for HIV infection in high-risk patients. A multidisciplinary team consisting of a surgeon, a radiation oncologist and a medical oncologist decided the treatment for each patient.

Radiotherapy

Clinical target volume (CTV) consisted of the tumour volume with a safety margin of 2–2.5 cm and the regional lymph node areas. An additional margin of 1 cm was applied to the CTV for the planning target volume (PTV). Initial tumour borders were marked with tattoo. Positron emission tomography with computed tomography (PET-CT) was used as an aid in treatment planning. The treatment schedule for external beam radiotherapy (EBRT) consisted of 3-dimensional (3-D) conformal photon beam radiotherapy or intensity modulated radiotherapy (IMRT) with individual field arrangement. The total dose was 45 Gy in 25 fractions, 5-times weekly with 15 MV photon beam linear accelerator, plus a boost 10–15 Gy with interstitial pulsed-dose rate brachytherapy if tumour size was less than 5 cm. Metal needles were homogeneously implanted through a perineal template according to the rules of the Paris system. In tumours larger than 5 cm or in N2–3 disease, the boost was delivered with EBRT. CTV (brachytherapy/EBRT) of the boost corresponded to the initial gross tumour extension. In cases with positive inguinal lymph nodes, inguinal areas were boosted with electrons to a total dose of 59.4 Gy. When IMRT technique was used, inguinal lymph nodes were involved in CTV and PTV and irradiated to the same total dose of 59.4 Gy. If the tumour involved or crossed the external anal sphincter, this area was covered with a 1 cm thick gelatinous bolus to raise the dose at the surface to at least 95% of the planned dose.

Chemotherapy

Chemotherapy protocol consisted of 2 cycles of 96-hour continuous infusion of 5-fluorouracil with a daily dose of 1000 mg/m^2 of body surface in the first and fifth week of radiotherapy. On day 1 the patients also received a bolus of mitomycin C in a dose of 10 mg/m^2. Since 2006, we administered peroral cytostatic capecitabine in a dose of 825 mg/m^2, twice daily, to cooperative patients with good performance status and without important comorbidities. First dose of capecitabine was administered one hour before the irradiation and the second dose 12 hours after. In cases of severe treatment toxicity according to common toxicity criteria[22] radiotherapy and/or chemotherapy was modified according to the patient's general condition and laboratory findings or was even temporarily interrupted.

Follow-up

During treatment, the patients were examined weekly to assess acute toxicity and compliance with radiochemotherapy, and complete blood count and serum biochemistry were performed as well.

The first post treatment examination was performed six weeks after the completion of radiochemotherapy, and then every 2–3 months for the first 2 years and every 6 months in the following 3 years.

When tumour response was incomplete, patients were examined every 6 weeks over a period of 4 months after the end of the treatment. In this period we performed all necessary investigations to prove tumour viability or its progression and in such cases surgery (abdomino-perineal resection) was recommended.

Tumour response was evaluated according to the WHO criteria.[19]

TABLE 1. Patients' and tumours' characteristics

Characteristics					No. of patients
Gender					
female					60
male					40
Mean age (range)					63 (34–87)
Performance status (WHO)					
0					76
1					20
2					3
3					1
Tumour type					
Carcinoma of the anal canal					72
Carcinoma of the anal margin					28
Tumour histology					
Basaloid					12
Squamous					88
TNM	N0	N1	N2	N3	
T1	9	0	1	0	
T2	36	6	1	0	
T3	19	10	3	1	
T4	1	1	7	5	
Tumour stage					
I					9
II					55
IIIA					17
IIIB					19

WHO = World Health Organization

Statistical analysis

The survival estimates were carried out by using the Kaplan-Meier method[23] and a log rank test[24] was used to test the differences in survival between subgroups.

The end points of survival analysis were defined as follows: loco-regional control (LRC) as the time interval from the beginning of the treatment to the appearance of local and/or regional progression; disease-free survival (DFS) as the time interval from the beginning of the treatment to the appearance of local and/or regional progression and/or appearance of distant metastases; disease-specific survival (DSS) as the time interval from the beginning of the treatment to the death because of cancer; and overall survival (OS) as the time interval from the beginning of the treatment to the death due to any cause.

For multivariate analysis, Cox proportional hazard model (with "Enter method") was used.[25]

All statistical tests were two-sided and a P-value of $p \leq 0.05$ was considered statistically significant. Statistical analyses were performed by using SPSS version 22 (Chicago, IL).

TABLE 2. Haemoglobin (Hb) values in subgroups of patients

Hb (g/L)	No. of patients	Median Hb (g/L)	Hb range (g/L)
Pre-treatment Hb		128	86–169
> 120 g/L	69	136	122–169
≤ 120 g/L	31	107	86–120
Mean on-treatment Hb		127	96–157
> 120 g/L	67	134	121–157
≤ 120 g/L	33	113	96–119
End-of-treatment Hb		121	77–159
> 120 g/L	46	134	121–159
≤ 120 g/L	54	114	77–120

Ethical consideration

The study was carried out according to the Helsinki Declaration (1964, with later amendments) and according to the European Council Convention on Protection of Human Rights in Bio-Medicine (Oviedo, 1997). It was approved by the Institutional Review Board Committee and by the National Committee for Medical Ethics, Ministry of Health, of the Republic of Slovenia.

Results

The study was closed on February 15, 2014. Median follow-up time of all patients was 52 months (range: 1–129 months) and 72 months (range: 6–129 months) for the survivors. On the day of analysis, 59 patients were alive, 22 patients died of anal canal cancer, 15 patients died of other causes and in 4 patients the cause of death was unknown.

Characteristics of patients and tumours are shown in Table 1.

Characteristics of Hb values in subgroup of patients are shown in Table 2.

Ninety-two patients (92%) completed their treatment according to the protocol. In 8 patients the treatment was modified: three did not receive chemotherapy due to significant comorbidities (ischemic heart disease or significant hepatopathy); in 1 patient chemotherapy was terminated due to acute side effects (chest pain due to a suspected ischemic event) and in 1 patient due to febrile neutropenia. One patient refused further treatment after 45 Gy and 1 patient refused chemotherapy. One patient received concurrent chemotherapy with cisplatin due to simultaneous treatment of the synchronous oropharyngeal cancer.

Median duration of radiochemotherapy was 1.9 months (range: 1–3.7 months). Fifty-six patients received brachytherapy boost with medial dose of 18.5 Gy (range: 10–25 Gy) or EBRT boost with medial dose of 14.4 Gy (range: 9–14.4 Gy). Capecitabine was used instead of 5-fluorouracil in 25 patients.

Tumour response to treatment

Complete clinical remission of the disease was achieved in 80 patients. The tumour disappeared within six weeks after the treatment completion in 73 patients, and within 4 months in 7 patients. One of them was operated on because of presumed persistent disease, yet the pathologist did not find disease residues. Of the remaining 20 patients, in 1 patient the disease progressed during treatment, 9 patients had APR performed and 2 patients had inguinal lymphadenectomy due to recurrence in inguinal lymph nodes; 8 patients had inoperable residual disease.

Survival

The 5-year LRC, DFS, DSS and OS rates for all patients were 72%, 71%, 77% and 62%, respectively.

Univariate analysis for survival according to the Hb level and other parameters is shown in Table 3.

In multivariate analysis, pre-treatment Hb (> 120 g/L *vs.* ≤ 120 g/L) was an independent prognostic factor only for OS (hazard ratio [HR]= 0.419, 95% confidence interval [CI] = 0.190–0.927, p = 0.032) and stage (I & II *vs.* III) for DSS (HR = 3.523, 95% CI = 1.375–9.026, p = 0.009) and OS (HR = 2.230, 95% CI = 1.167–4.264, p = 0.015).

Patients' age, gender, tumour site, type of radiotherapy boost (tele- or brachytherapy) and type of chemotherapy (5-fluorouracil or capecitabine) did not have an influence on survival.

Haemoglobin concentration during treatment

In the group of patients with Hb > 120 g/L the mean Hb concentration during the treatment slightly but not significantly decreased (mean pre-treatment Hb = 139 g/L, mean end-of-treatment Hb = 125 g/L). However in the group of patients with Hb ≤ 120 g/L it slightly increased (mean pre-treatment Hb = 106 g/L, mean end-of-treatment Hb = 113 g/L). One third of patients had low iron levels and received iron preparations. Nine patients received blood transfusion due to a drop in their Hb concentration below 100 g/L.

Acute side effects

None of the patients died because of acute side effects. Most grade 3 side effects were caused by radiodermatitis. Serious, life-threatening infections were observed in 3 patients: 2 patients experienced severe pneumonia that requested transfer to the intensive care unit and 1 patient developed febrile neutropenia which required termination of radiochemotherapy. One patient developed severe stomatitis and needed parenteral nutrition. In 1 patient, serious diarrhoea developed, which required hospitalization. Frequency and intensity of acute side effects are shown in Table 4.

Discussion

Survival rates of our patients and the profile and frequency of acute side effects are similar to the results of other researchers.[2,7,26-29] There was no difference in survival of anal canal and anal margin cancer patients. The survival rate of patients with higher pre-treatment and end-of treatment Hb concentrations was generally better, compared to those patients with lower Hb concentrations, yet only pre-treatment Hb concentration was an independent prognostic factor for OS. Patients with mean on-treatment Hb > 120 g/L only had statistically significant better LRC and OS than patients with Hb ≤ 120 g/L. Many authors found that anaemic patients respond worse to radiotherapy and/or chemotherapy and have worse survival rates.[2,8,12,13,15-18,30-41] There is convincing evidence of a correlation between Hb concentration and tumour oxygenation in various kinds of tumours.[42] Nordsmark's *et al*. comparison of pre-treatment Hb with pre-treatment tumour pO_2 measurements in head and neck cancer showed a quadratic regression correlation between Hb concentration and median pO_2.[43] Tumours of anaemic patients are consequently more hypoxic and more resistant to radiotherapy (and chemotherapy).[16] The *National Comprehensive Cancer Network* (NCCN) guidelines recommend the use of blood transfusion in symptomatic patients with Hb concentration <100 g/L to improve oxygen delivery to the tumour.[44] Nine patients in our study received blood transfusion. They had statistically significant worse OS than other patients. The conclusions about beneficial effect of transfusion in our study cannot be made because the patients who received transfusion were few. The contribution to low survival of other un-

TABLE 3. Univariate analysis of survival of patients at 5 years by Hb level, tumour-, patients-, and treatment characteristics

Characteristics	n	LRC	DFS	DSS	OS
Pre-treatment Hb					
> 120 g/L	69	79%	77%	85%	73%
≤ 120 g/L	31	57%	57%	56%	39%
		P = 0.011	**P = 0.017**	**P = 0.003**	**P = 0.000**
Mean on-treatment Hb					
> 120 g/L	67	78%	76%	82%	68%
≤ 120 g/L	33	60%	60%	67%	50%
		P = 0.037	**P = 0.054**	**P = 0.081**	**P = 0.007**
End-of-treatment Hb					
> 120 g/L	46	82%	80%	89%	75%
≤ 120 g/L	54	63%	63%	65%	49%
		P = 0.022	**P = 0.037**	**P = 0.011**	**P = 0.003**
Performance status					
PS 0	76	73%	73%	80%	72%
PS 1–3	24	69%	64%	66%	34%
		P = 0.480	P = 0.283	P = 0.231	**P = 0.000**
Tumour stage					
T1–3	86	75%	75%	84%	68%
T4	14	50%	44%	38%	25%
		P = 0.054	**P = 0.015**	**P = 0.000**	**P = 0.001**
Lymph node involvement					
no	65	79%	79%	87%	70%
yes	35	59%	56%	60%	48%
		P = 0.032	**P = 0.017**	**P = 0.000**	**P = 0.000**
Overall disease stage					
I / II	64	79%	79%	87%	70%
IIIA / IIIB	36	59%	57%	61%	49%
		P = 0.044	**P = 0.025**	**P = 0.000**	**P = 0.000**
Histologic tumour type					
basaloid	12	100%	100%	100%	100%
squamous	88	68%	67%	74%	57%
		P = 0.030	**P = 0.026**	P = 0.051	**P = 0.016**
Tumour site					
anal canal	72	69%	68%	78%	62%
anal margin	28	81%	81%	73%	61%
		P = 0.250	**P = 0.212**	**P = 0.994**	**P = 0.738**
Blood transfusion					
no	91	72%	71%	78%	64%
yes	9	0%	0%	0%	0%
		P = 0.993	P = 0.950	P = 0.333	**P = 0.044**
Overall radiation time					
≤ 1,08 months	29	89%	89%	93%	83%
> 1,08 months	71	64%	63%	69%	51%
		P = 0.015	**P = 0.011**	**P = 0.012**	**P = 0.012**
Operation					
no	73	89%	88%	88%	69%
yes	27	29%	29%	52%	45%
		P = 0.000	**P < 0.000**	**P = 0.001**	**P = 0.018**

DFS = disease-free survival; DSS = disease-specific survival; Hb = haemoglobin; LRC = loco-regional control; N = number of patients; OS = overall survival

TABLE 4. Acute treatment toxicities

Toxicity	Grade					
	0	1	2	3	4	Total
Stomatitis	68	12	10	9	1	**100**
Nausea, vomiting	79	9	9	3	0	**100**
Diarrhoea	57	17	12	13	1	**100**
Hand-foot syndrome*	22	0	1	2	0	**25**
Radiodermatitis	10	12	13	64	1	**100**
Infection	51	14	23	9	3	**100**
Leucocyte count	37	31	20	10	2	**100**
Haemoglobin level	43	44	11	2	0	**100**
Platelet count	58	36	3	3	0	**100**

* Only in patients treated with capecitabine

favourable factors, which are often combined with anaemia, was not possible to assess.

The reports in the literature of the influence of transfusions on the outcome are not consistent. Some authors found favourable effect[45], some found none[46,47] and some found unfavourable effect.[2,32] It is possible that a better oxygen delivery is not sufficient to improve oxygenation of a tumour with high oxygen consumption.[30,35] Moreover, anaemic patients are assumed to have a more aggressive disease from the start.[35,46] Immune suppression in patients could also play a part (7, 35).[7,35]

The use of erythropoetin is controversial due to the possible effect on tumour growth[14,33,48], however, only in the subpopulation of patients whose tumours expressed erythropoetin receptors.[49] Another potential mechanism by which erythropoetin therapy may result in negative outcomes in cancer patients is through promotion of thrombovascular events.[50] Therefore, it was not used in our patients. De Los Santos *et al*. believe the connection between anaemia and hypoxia is complex; therefore, it is not clear whether transfusion or erythropoetin do patients any favour.[51]

The Hb concentration during treatment progressively decreased, which is in agreement with other reports.[2,7,17,18,30-33,46] At the beginning of treatment, 31% of our patients were anaemic, and at the end 54%. That should cause more hypoxia in the tumour. It is possible that a decreased delivery of oxygen to the tumour due to of Hb drop during the treatment is partially counterbalanced by the reoxygenation due to shrinkage of the tumour and does not influence very much the outcome. In some patients with Hb ≤ 120 g/L it was possible to raise the mean Hb level by the blood transfusion or by iron preparations.

The significance of mean on-treatment Hb concentration and end-of-treatment Hb concentration is less clear. Some authors found a positive effect of higher mean on-treatment Hb concentration on treatment outcome[2,15,18,32,33,35] and some found a positive effect of higher end-of-treatment Hb concentration on treatment outcome[35,36], while others found no influence on outcome of either mean- or end- of-treatment Hb level.[31] In our patients, the mean- or end- of treatment Hb levels had less influence on survival compared to the pre-treatment values of Hb concentration.

Our study showed that pre-treatment Hb was an important independent prognostic factor for overall survival in patients with squamous cell carcinoma of the anal canal and anal margin treated with radiochemotherapy, which is in agreement with findings of most other authors. Mean on-treatment Hb and end-of-treatment Hb do not seem to have much influence on survival.

Because of a small number of patients who needed blood transfusion its influence on survival could not be assessed in our study.

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research article

Evaluation of dosimetric effect caused by slowing with multi-leaf collimator (MLC) leaves for volumetric modulated arc therapy (VMAT)

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Background. This study is to report 1) the sensitivity of intensity modulated radiation therapy (IMRT) QA method for clinical volumetric modulated arc therapy (VMAT) plans with multi-leaf collimator (MLC) leaf errors that will not trigger MLC interlock during beam delivery; 2) the effect of non-beam-hold MLC leaf errors on the quality of VMAT plan dose delivery.

Materials and methods. Eleven VMAT plans were selected and modified using an in-house developed software. For each control point of a VMAT arc, MLC leaves with the highest speed (1.87-1.95 cm/s) were set to move at the maximal allowable speed (2.3 cm/s), which resulted in a leaf position difference of less than 2 mm. The modified plans were considered as 'standard' plans, and the original plans were treated as the 'slowing MLC' plans for simulating 'standard' plans with leaves moving at relatively lower speed. The measurement of each 'slowing MLC' plan using MapCHECK®2 was compared with calculated planar dose of the 'standard' plan with respect to absolute dose Van Dyk distance-to-agreement (DTA) comparisons using 3%/3 mm and 2%/2 mm criteria.

Results. All 'slowing MLC' plans passed the 90% pass rate threshold using 3%/3 mm criteria while one brain and three anal VMAT cases were below 90% with 2%/2 mm criteria. For ten out of eleven cases, DVH comparisons between 'standard' and 'slowing MLC' plans demonstrated minimal dosimetric changes in targets and organs-at-risk.

Conclusions. For highly modulated VMAT plans, pass rate threshold (90%) using 3%/3mm criteria is not sensitive in detecting MLC leaf errors that will not trigger the MLC leaf interlock. However, the consequential effects of non-beam hold MLC errors on target and OAR doses are negligible, which supports the reliability of current patient-specific IMRT quality assurance (QA) method for VMAT plans.

Key words: VMAT; MLC; MapCHECK2; quality assurance; DVH

Introduction

Volumetric modulated arc therapy (VMAT) demands high level of precision and reliability from the linear accelerator (LINAC) control system because the gantry rotation is synchronized with multi-leaf collimator (MLC) movement for accurate dose delivery.[1,2] Highly modulated dose distribution commonly requires MLC leaves of high speed moving along the whole arc.[3-5] However, fast leaf motion during gantry rotation may be affected by interleaf friction or MLC motor problems that result in leaf position errors.[6] Wijesooriya et al and Ling *et al* reported an increase in MLC leaf position errors due to fast moving leaves.[3,7]

Detecting dosimetric variation caused by MLC leaf errors is one important concern in patient-specific quality assurance (QA). Currently, several dose measuring systems are available for patient-specific QA.[8-11] Fredh *et al.* evaluated the dosimetric effect of MLC position error on four single arc plans using Delta4®, OCTAVIUS®, COMPASS® and

TABLE 1. Volumetric modulated arc therapy (VMAT) plan parameters (dose, gantry speed, Leaf travel and modulation complexity score [LTMCS] and arcs), ranges of the maximal leaf speed, multi-leaf collimator (MLC) leaf position changes along 178 control points (CPs), and total modified leaves percentage of each VMAT arc

Case[a]	Dose Prescription (Gy/fx × fx)	Gantry speed (deg/s)	LTMCS[b]	Arc	Maximal MLC leaf speed range along 178 CPs (cm/s)	Range of Leaf Position Changes along 178 CPs (mm)	Total Modified MLC leaves %
P1	2.0 x 33	4.8	0.164	1	1.87-1.92	-1.90-1.88	1.5
P2	2.0 x 33	4.8	0.262	1	1.87-1.90	-1.90-2.00	1.6
				2	1.87-1.90	-1.90-2.00	1.6
P3	2.0 x 33	4.8	0.163	1	1.87-1.90	-1.90-2.00	1.5
				2	1.87-1.90	-1.90-2.00	1.6
B1	1.8 x 33	4.8	0.204	1	1.87-1.92	-1.88-1.90	1.6
B2	1.8 x 33	4.8	0.199	1	1.87-1.92	-1.88-1.90	1.5
				2	1.87-1.92	-1.88-1.90	1.7
B3	1.8 x 33	4.8	0.217	1	1.85-1.90	-1.88-2.00	1.5
				2	1.85-1.90	-1.88-2.00	1.6
A1	1.8 x 33	4.8	0.081	1	1.90-1.92	-1.80-1.80	1.7
				2	1.90-1.92	-1.80-1.80	1.7
				3	1.90-1.92	-1.80-1.80	1.7
				4	1.90-1.92	-1.80-1.80	1.8
A2	1.8 x 33	4.8	0.083	1	1.92-1.95	-1.87-1.87	1.5
				2	1.92-1.95	-1.87-1.87	1.6
				3	1.92-1.95	-1.87-1.87	1.6
				4	1.92-1.95	-1.87-1.87	1.5
A3	1.8 x 33	4.8	0.105	1	1.92-1.95	-1.87-1.87	1.7
				2	1.92-1.95	-1.87-1.87	1.7
				3	1.92-1.95	-1.87-1.87	1.7
A4	1.8 x 33	4.8	0.076	1	1.87-1.90	-1.80-1.93	2.2
				2	1.87-1.90	-1.80-1.93	2.2
				3	1.87-1.90	-1.80-1.93	2.3
				4	1.87-1.90	-1.80-1.93	2.3
A5	1.8 x 33	4.8	0.084	1	1.90-1.92	-1.80-1.80	1.7
				2	1.90-1.92	-1.80-1.80	1.7
				3	1.90-1.92	-1.80-1.80	1.8

[a] P: Prostate VMAT cases; B: Brain VMAT cases; A: Anal VMAT cases.
[b] Leaf travel and modulation complexity score (LTMCS) for a VMAT plan. LTMCS ranges from 0 to 1. Low LTMCS indicates high modulation complexity.

Epiqa™.[11] All detectors demonstrated low pass rate failure to the 2 mm widening of both MLC banks, and this error has the least dosimetric impact on the plans. In addition, several studies have stated poor correlation between the gamma index pass rates of QA procedure and DVH deviations.[12, 13]

Although previous studies have studied both systematic and random MLC leaf errors in VMAT plans, in actual beam delivery, if the difference between actual leaf position and planned one is larger than 2 mm, the LINAC will trigger an MLC interlock which invokes a "beam hold-off".[14] Therefore, the purpose of this study is to evaluate the dosimetric error of clinical VMAT plans caused by MLC leaf position errors that will not trigger MLC interlock (*i.e.* beam hold-off). In addition, we evaluated the sensitivity of patient-specific IMRT QA method using MapCHECK®2 for VMAT plans with non-beam-hold MLC errors.

Methods

A. Patient selection and plan complexity evaluation

We selected 11 VMAT plans (Table 1) on three types of targets: anus, brain and prostate. Leaf travel and modulation complexity score (LTMCS) was used to characterize the modulation complexity of each VMAT plan.[15] LTMCS ranges from 0 to 1 and

it approaches 0 for increasing degree of modulation and increasing total leaf travel distance. In this study, anal VMAT plans had low LTMCS while brain and prostate VMAT plans had moderate to high LTMCS (Table 1).

LTMCS is derived by the product of leaf travel index (LTi) and modulation complexity score (MCSv).[15,16] MCSv was derived by Masi et al to characterize the modulation degree of the MLC leaves of a VMAT plan. MCSv value of 1 indicates no modulation by MLC leaves (*i.e.* a plan of the least complexity), and the value decreases as modulation complexity increases. Total travel distance of all in-field moving MLC leaves was calculated and normalized to acquire LTi. When LTi approaches 1, it indicates short travel distance of all in-field moving MLC leaves. LTi decreases to zero as total leaf travel distance increases.

B. MLC leaf speed modifications

All original treatment plan DICOM files were exported from the Eclipse™ (version 10.0, Varian Medical Systems, Inc., Palo Alto, USA) treatment planning system (TPS). The DICOM plans were modified through an in-house developed software. For each arc in the VMAT plan, in-field moving MLC leaves of 178 control points (CPs) on both banks were selected for leaf speed modifications. Speed of each moving MLC leaf per CP was calculated based on MLC leaf position, gantry rotation angle and gantry speed as shown in Equation [1].

$$V_{leaf}(m,n) = \frac{LP(m,n+1) - LP(m,n)}{\Delta t(n)} \quad [1]$$

where $\Delta t(n) = \frac{\theta(n+1) - \theta(n)}{u(n)}$

and m: MLC leaf ϵ [1,120]; n: CP ϵ [0,177].

Here Δt(n) is the gantry rotation time between two adjacent CPs, θ(n) is the gantry angle of CP 'n', u(n) is the gantry speed of CP 'n', V_{leaf} (m, n) is the speed of mth leaf of CP 'n' and LP(m, n) is the position of mth leaf of CP 'n'. MLC leaves on bank 'A' were marked from 1 to 60 while those on bank 'B' were marked from 61 to 120.

For each CP of the arc, leaves on both banks (MLC leaf: 1-120) with the highest speed were set to move at 2.3 cm/s, resulting in a leaf position difference at a maximum of 2 mm (Table 1: *Range of leaf position chages along 178 CPs*). New leaf position of CP 'n' is:

$$LP_{new}(m,n) = LP(m,n-1) + 2.3(cm/s) \times \Delta t(n-1). \quad [2]$$

If one leaf was moving with the highest speed at two consecutive CPs, leaf motion direction of each CP was further considered as shown below:

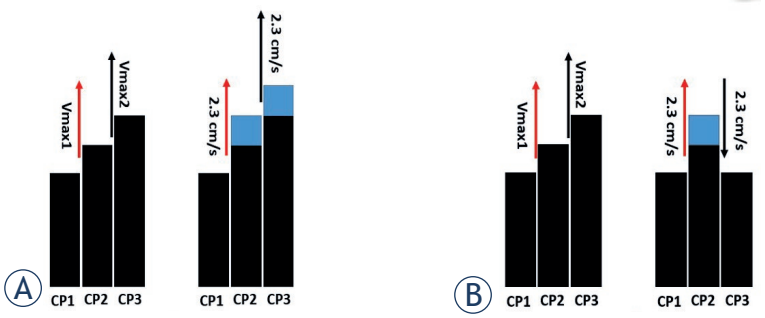

FIGURE 1. Illustration of multi-leaf collimator (MLC) leaf position modifications of one leaf when it is moving with the highest speed at two consecutive control points (CPs) (i.e. CP2 and CP3). **(A)** The leaf is moving in the same direction. **(B)** The leaf is moving back and forth. Red arrow (Vmax1 or 2.3cm/s) represents leaf speed from CP1 to CP2; Black arrow (Vmax2 or 2.3cm/s) represents leaf speed from CP2 to CP3. Black bars represent original leaf positions. Blue bars represent new leaf positions after modification.

1. If the motion directions of the next two CPs remained the same, then both leaf positions of corresponding CP were subject to modification (Figure 1A).

2. If the motion directions of the next two CPs were different, we only modified the leaf position of the middle CP so that both leaf speed values would be increased (Figure 1B).

The *total modified MLC leaves percentage* (Equation 3 and Table 1) was an indicator of the amount of MLC leaves that had been changed to the maximal speed in each arc;

$$Total\ modified\ MLC\ leaves\% = 100\% \times \frac{\sum_{i=1}^{178} Modified\ in\ field\ leaves\ on\ both\ banks\ of\ CPi}{\sum_{i=1}^{178} Total\ in\ field\ moving\ leaves\ on\ both\ banks\ of\ CPi} \quad [3]$$

where *modified in field leaves on both banks of CPi* is the total modified MLC leaves that moving with the highest speed of current CP. Leaf speed modification would not be applied if it caused any leaf pair collision (Gap between leaf pair should be no less than 0.5 mm in actual delivery).

In this study, modified plans were considered as 'standard' plans where MLC leaves were allowed to move at the maximal speed (2.3 cm/s). The original plans were considered as 'slowing MLC' plans where the highest MLC speed was lower than 2.3 cm/s. There were no changes in monitor unit (MU) and gantry speed per CP in all modified VMAT plans.

C. MLC leaf speed change evaluation

Having increased the leaf speed of one CP to the maximal limit without triggering the MLC error interlock (*i.e.* MLC leaf position difference was less than 2 mm), leaf speed of the next CP would be

TABLE 2. Demonstration of the impact on leaf speed of adjacent control points (CPs) due to leaf modifications

Scenario[a]	LP1 (cm)	LP2 (cm)	LP3 (cm)	LP1-2 Speed (cm/s)	LP 2-3 Speed (cm/s)
A (ori)	4.6	5.4	5.9	1.8	1.1
A (mod)	4.6	5.6	5.9	**2.3**	**0.7**
B (ori)	4.6	5.4	4.9	1.8	-1.1
B (mod)	4.6	5.6	4.9	**2.3**	**-1.6**

[a] Scenario A: Leaf moved forward from LP1 to LP2, then moved forward from LP2 to LP3. Scenario B: Leaf moved forward from LP1 to LP2, then moved backward from LP2 to LP3.

In the table, '**LPn**': leaf position at CP 'n' =1,2,3; '**ori**': original leaf positions; '**mod**': leaf positions after modification; **positive speed**: leaf moved forward; **negative speed**: leaf moved backward. The speed was the distance between LP1,2,3 divided by Δ**t =0.435s**. For both scenarios A and B, we only modified **LP2** from 5.4 to 5.6 to increase LP1-2 speed from 1.8 cm/s to 2.3 cm/s.

affected by this modification. With Δt(n) of each CP remained unchanged, increasing leaf speed by modifying leaf position of current CP while keeping the leaf position of the next CP unchanged resulted in consequential change of leaf speed of the next CP. As a result, total number of leaf speed changes in one arc is twice as many as the total number of MLC leaves that were set to the maximal leaf speed (see Equation [4] below). This accompanied effect caused by the MLC leaf modification either increases or decreases the MLC leaf speed of the next CP according to the leaf motion direction (Table 2). We have taken this accompanied leaf speed modification into account when evaluating dosimetric changes.

Because of the high complexity (i.e. low LTMCS) of the anal VMAT plans, we further analyzed MLC leaf changes in these VMAT plans. The *average percentage of modified MLC leaves* (Table 3) was the summation of total percentage of modified MLC leaves for all arcs (Table 1: *Total Modified MLC leaves %)* divided by number of total arcs in the plan. The *average percentage of faster moving leaves* ($\overline{Faster\ Moving\ Leaves}$ %) depends on MLC leaves that were set to the maximal speed and total faster moving leaves after modifications including those modified leaves of current CP and affected leaves of the next CP (Equation [4]);

$$\overline{Faster\ Moving\ Leaves}\% = \frac{\sum_{arc=1}^{n} leaves\ of\ higher\ speed\ after\ modifications}{\sum_{arc=1}^{n} 2\times MLC\ leaves\ set\ to\ the\ maximal\ speed} \times 100\% \quad [4]$$

where n is the number of total arcs.

D. Planar dose measuring system

In this study, we used MapCHECK®2 2D diode array system (Model 1177, Sun Nuclear Co., Melbourne, FL) for evaluating the effect of slowing MLC leaves on planar dose delivery accuracy. MapCHECK®2 along with its software have been widely used as the clinical implementation for patient-specific verification of VMAT plans due to its compact diode size (0.8 mm×0.8 mm), dose linearity, real-time measurement, reproducibility and sensitivity.[17-21]

E. Dosimetric evaluation

E.1 Measurement and uncertainty evaluation

All the VMAT plans ('standard' and 'slowing MLC' plans) were delivered using a Varian Trilogy® LINAC on the same day. Measurement of each arc was then compared with the corresponding calculated planar dose from the TPS with respect to absolute dose Van Dyk distance-to-agreement (DTA) comparison (dose difference is normalized to global maximum) using 3%/3 mm criteria.[22] All measurements were repeated on two consecutive days. The uncertainty was then obtained by evaluating the variation in repeated measurements.

E.2 Dosimetric evaluation of 'slowing MLC' plans

According to current pre-treatment IMRT QA method for VMAT plans with MapCHECK®2, measurement of each arc in the 'standard' plan was compared with calculated planar dose of the 'standard' plan with respect to absolute dose Van Dyk DTA comparison using 3%/3 mm and 2%/2 mm criteria. Pass rate ($PS_{standard}$) of the comparison was demonstrated in percentage. $PS_{standard}$ of each arc using 3%/3 mm criteria was used as a baseline to verify that all the plan parameters had been correctly transferred from control console computer to LINAC for delivery.

Each 'slowing MLC' plan was considered as a 'standard' plan with MLC leaf errors that would not trigger any MLC interlock to interrupt the beam delivery. In order to evaluate the sensitivity of the IMRT QA method for VMAT plans with non-beam-hold leaf errors, we delivered each 'slowing MLC' plan and compared the measurement with calcuated planar dose of the 'standard' plan with respect to absolute dose Van Dyk DTA comparison using 3%/3 mm and 2%/2 mm criteria to acquire the pass rate ($PS_{slowing\ MLC}$) in percentage. Because of the MLC leaf errors in each 'slowing MLC' plan, there was a decrease in pass rate of each arc (Equation [5]).

$$Decrease\ in\ pass\ rate\ of\ specific\ arc(\%) = |PS_{standard}(\%) - PS_{slowng\ MLC}(\%)| \quad [5]$$

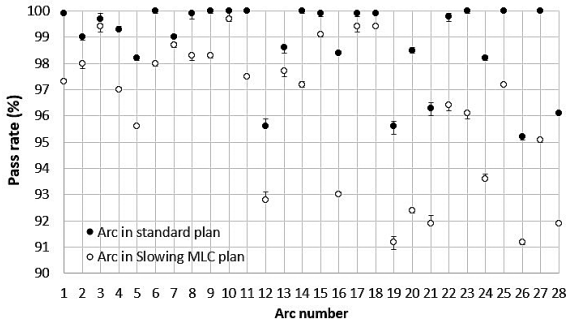

FIGURE 2. Variation of pass rates (3%/3mm) of each volumetric modulated arc therapy (VMAT) arc. Solid dots: pass rates of arcs in 'standard' plans ($PS_{standard}$). Soft dots: pass rates of arcs in 'slowing multi-leaf collimator (MLC)' plans ($PS_{slowing\ MLC}$). Error bars are pass rates variation based on repeated measurements of each arc on two consecutive days.

TABLE 3. Target dose differences between 'standard' and 'slowing multi-leaf collimator (MLC)' anal volumetric modulated arc therapy (VMAT) plans, total leave states, and average percentages of modified leaves and faster moving leaves of anal cases

Case	ΔDmean(Gy)[a]	ΔD95(Gy)[a]	Total leave states per arc (leaves on both banks /CP)×(CP)	Average modified MLC leaves (%)	Average faster moving leaves (%)
A1	-0.8	-0.2	120 × 178	1.7	56.5
A2	-0.9	-0.3	120 × 178	1.6	53.6
A3	-1.2	-0.5	120 × 178	1.7	51.3
A4	**-2.2**	**-1.0**	120 × 178	**2.3**	**69.0**
A5	-1.1	-0.3	120 × 178	1.7	52.7

[a] Negative sign means dose of 'standard' plan is lower than that of 'slowing MLC' plan
CP = control point

The correlations between the decreases in pass rates using 3%/3mm and 2%/2mm criteria and LTMCS were analyzed through Spearman's correlation coefficient.[23]

Finally, the 3D dose distribution of each plan was calculated in the TPS and dose-volume histogram (DVH) for targets and organs-at-risk (OAR) were obtained. For clinical dosimetric evaluation, mean target dose (Dmean), dose that covers 95% (D_{95}) of the planning target volume, and Normal Tissue Complication Probability (NTCP) using Lyman Kutcher Burman (LKB) model[23] were calculated for all the plans. Clinical dosimetric parameters of 'standard' and 'slowing MLC' plans were compared using the Wilcoxon signed-rank test.[24]

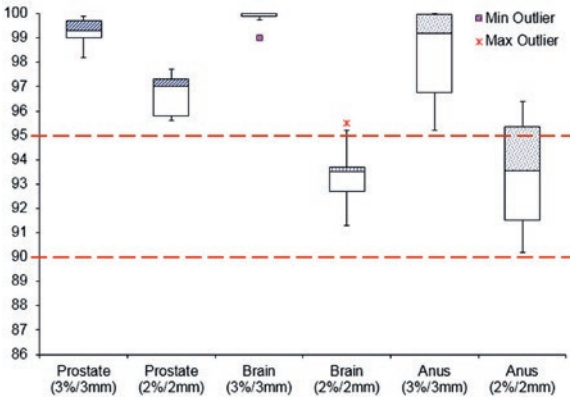

FIGURE 3. Pass rates of 'standard' volumetric modulated arc therapy (VMAT) plans with respect to absolute dose Van Dyk distance-to-agreement (DTA) comparisons using 3%/ 3 mm and 2%/2 mm criteria.

Results

A. Pass rate and uncertainty evaluation

Figure 2 demonstrated pass rate of each arc and variation of measurements based on repeated measurements on two consecutive days. Among all the arcs in both 'standard' and 'slowing MLC' plans, the maximal variation found was 0.3% with respect to the 91.5% pass rate.

B. Prostate cases

For all three prostate cases, $PS_{standard}$ and $PS_{slowing\ MLC}$ using 3%/ 3 mm and 2%/2 mm criteria were all higher than 90% (Figure 3 and 4: *Prostate*). Dosimetric differences of Dmean, D95, NTCP (bladder, rectum, leaf and right femoral heads) between 'slowing MLC' plans and 'standard' plans were: 0.47 ± 0.17 Gy ($p > 0.05$), 0.33 ± 0.13 Gy ($p > 0.05$), 1% ± $1\%_{bladder}$ ($p > 0.05$), 3% ± $2\%_{rectum}$ ($p > 0.05$), 2% ± $1\%_{left\ fem}$ ($p > 0.05$), 2% ± $1\%_{right\ fem}$ ($p > 0.05$), respectively.

C. Brain cases

For all three brain cases, $PS_{standard}$ and $PS_{slowing\ MLC}$ using 3%/ 3 mm and 2%/2 mm criteria were all higher than 90% (Figure 3 and 4: *Brain*) except for arc 2 of brain case B2. Dosimetric differences of Dmean, D95, NTCP (brain stem, cerebellum, spinal cord, left and right cochlea) between 'slowing MLC' plans and 'standard' plans were: 0.13 ± 0.05 Gy ($p > 0.05$), 0.17 ± 0.09 Gy ($p > 0.05$), 1% ± $1\%_{brain\ stem}$ ($p > 0.05$), 1% ± $1\%_{cerebellum}$ ($p > 0.05$), 0% ± $1\%_{spinal\ cord}$ ($p > 0.05$), 1% ± $2\%_{left\ cochlea}$ ($p > 0.05$), 1% ± $1\%_{right\ cochlea}$ ($p > 0.05$), respectively.

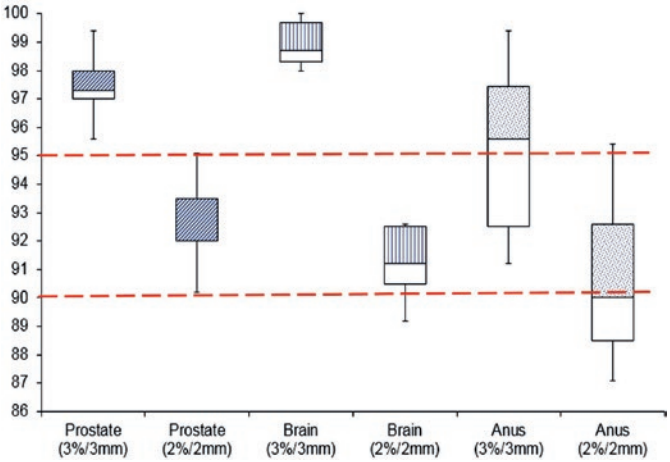

FIGURE 4. Pass rates of 'slowing multi-leaf collimator (MLC)' volumetric modulated arc therapy (VMAT) plans with respect to absolute dose Van Dyk distance-to-agreement (DTA) comparisons using 3%/ 3 mm and 2%/2 mm criteria.

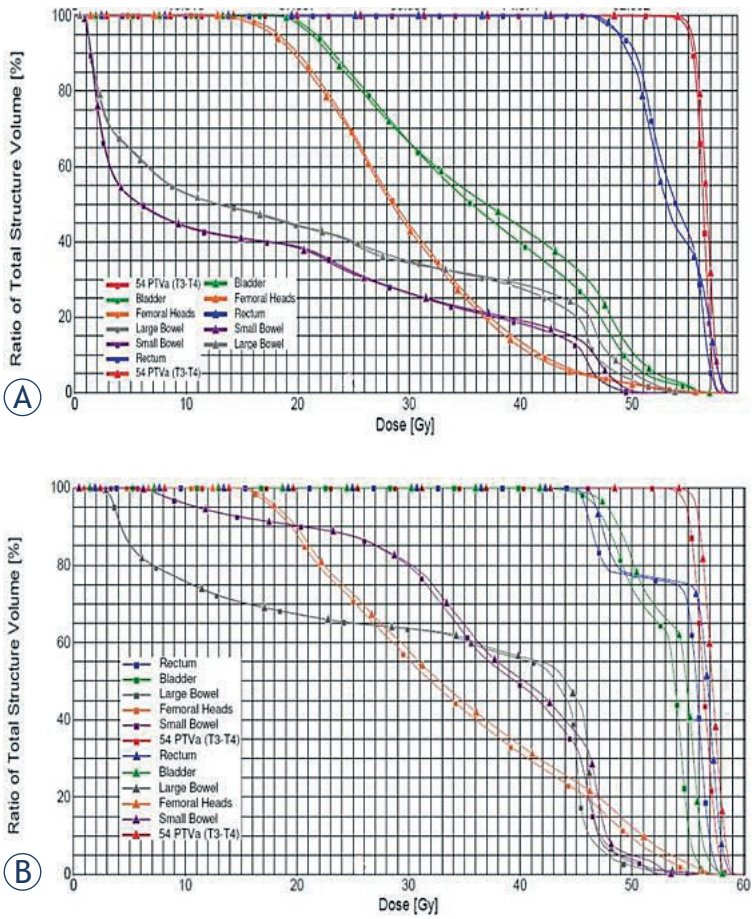

FIGURE 5 (A) DVH comparison between 'standard' and 'slowing MLC' VMAT plans of **anal case A3**. **(B)** DVH comparison between 'standard' and 'slowing MLC' VMAT plans of **anal case A4**. ▲:'slowing MLC' plan; ■ :'standard' plan. Red: PTV; Blue: Rectum; Green: Bladder; Grey: Large bowel; Purple: Small bowel; Orange: Femoral heads

D. Anal cases

The LTMCS scores of Anal VMAT plans were smaller than both brain and prostate VMAT plans (Table 1: *LTMCS*) indicating higher modulation by MLC leaves. For anal VMAT cases, $PS_{standard}$ and $PS_{slowing\ MLC}$ using 3%/3 mm criteria were all higher than 90% while 'slowing MLC' plans of cases A3, A4 and A5 demonstrated less than 90% pass rates using 2%/2 mm criteria (Figure 4: *Anus*). Dosimetric differences of NTCP (bladder, rectum, large bowel and femoral heads) between 'slowing MLC' plans and 'standard' plans were: $2\% \pm 2\%_{bladder}$ ($p > 0.05$), $3\% \pm 1\%_{rectum}$ ($p > 0.05$), $2\% \pm 1\%_{large\ bowel}$ ($p > 0.05$), $1\% \pm 1\%_{femheads}$ ($p > 0.05$), respectively. Compared with anal case A3 and A5, case A4 demonstrated substantial dosimetric differences between the 'standard' and 'slowing MLC' plans where ΔD_{mean} and ΔD_{95} were 2.2 Gy and 1.0 Gy respectively (Figure 5 and Table 3).

E. Correlation between LTMCS and dosimetric parameters

The correlation between decreases in pass rates of VMAT arcs using 2%/2 mm criteria and LTMCS is moderate to strong ($r_s = 0.597$, Figure 6A). When using 3%/3 mm criteria, the correlation is weak to moderate ($r_s = 0.453$, Figure 6B).

Discussion

A. Measurement uncertainty

By using lasers and front pointer for device positioning, the measurement setup was of high consistency. Absolute dose calibration for MapCHECK®2 was performed every day before dose measurement.[25,26] Therefore, the source of the uncertainty is mainly due to variability of MLC leaf motion. The small error bars in Figure 2 indicate that the measurement variability is very small.

B. Anal case A4 results

For anal case A4, since the MU of each control point remained unchanged, and 'slowing MLC' plan had more slowly moving MLC leaves, more area were being irradiated that resulted in higher dose. The *average percentage of faster moving leaves* indicates the amount of MLC leaves moving back-and-forth. The *average percentages of modified MLC leaves* (2.3%) and *average percentage of faster moving leaves* (69%) of anal case A4 are higher compared with other anal

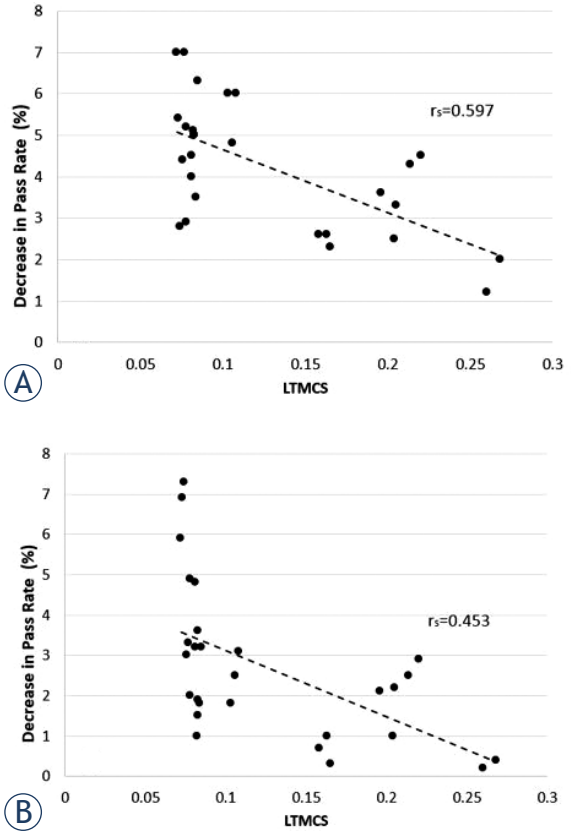

FIGURE 6 Correlation between absolute pass rate difference ($|PS_{standard}$ (%) – $PS_{slowng\ MLC}$ (%)|) of each arc and LTMCS of each arc. **(A)** LTMCS vs decrease in pass rate (%) using 2%/2mm criteria; **(B)** LTMCS vs decrease in pass rate (%) using 3%/3mm criteria

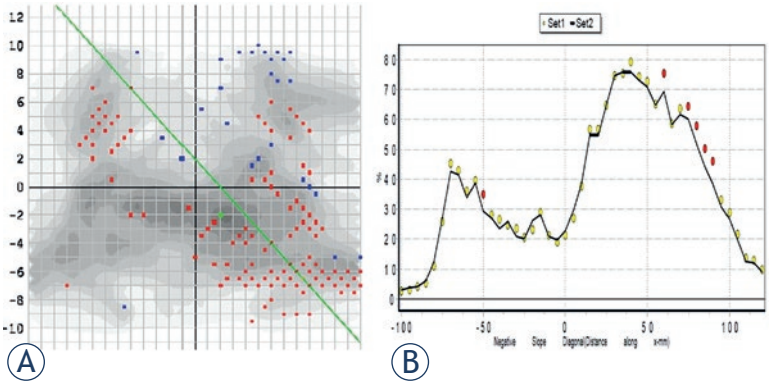

FIGURE 7 MapCHECK®2 measurements of single arc of case A4. **(A)** Red dots are MapCHECK®2 measurements of 'slowing MLC' plan showing delivered dose is higher than planed dose ('standard' plan); **(B)** Dotted line represents dose profile of 'slowing MLC' plan; Black solid line represents dose profile of 'standard' plan.

cases (Table 3), indicating that more high speed MLC leaves were moving back and forth to create a highly modulated VMAT plan. Accordingly, the anal case A4 has the minimal LTMCS among all anal cases studied.

Moreover, all four arcs of anal case A4 have large fields (e.g. 14 cm ×30 cm, 14 cm ×29 cm, 30 cm ×14 cm, 30 cm ×14 cm for arc 1, 2, 3, 4 respectively). Wijesooriya et al reported the accuracy of RapidArc delivery holds for leaf velocities with small dosimetric uncertainties for 5 mm width MLC leaves which are in the central 20 cm of field.[7] They found that three VMAT plans with large MLC leaves with 1cm width at high speed (2.1–2.4 cm/s) demonstrated higher leaf position inaccuracy. Therefore, large MLC leaves in the VMAT plan of anal case A4 have more effect on dose delivery inaccuracy.

C. Pass rates and dosimetric parameters

When using 3%/3 mm criteria, all 11 cases including 'standard' and 'slowing MLC' plans passed the institutional 90% acceptance threshold of absolute dose DTA comparison. Dosimetric differences (e.g. ΔD_{mean}, ΔD_{95} and NTCP) between 'standard' and 'slowing MLC' plans in targets and normal tissues were minimal indicating that VMAT plans with non-beam-hold MLC leaf errors (leaf position difference ≤ 2 mm) remain the planned dose coverage except for anal case A4. Using 2%/2 mm criteria, decrease in pass rates of VMAT arcs demonstrated stronger correlation with VMAT modulation complexity which is characterized by LTMCS (Figure 6A).

Some arcs in 'slowing MLC' plans of anal cases A3 and A5 showed less than 90% pass rates using 2%/2mm criteria although differences in dosimetric parameters are small (e.g. ΔD_{mean} and ΔD_{95}). However, anal case A4 showed a consistent decrease in pass rates and dose conformity. Compared with the 'standard' plan, 'slowing MLC' plan of anal case A4 delivered higher planar dose (Figure 7) which is consistent with the changes in DVH curves in Figure 5B. To further ensure the dosimetric quality of VMAT plans like anal case A4 that have the following features: 1. Highly modulated multiple arc VMAT plan (e.g. LTMCS < 0.1); and 2. Arc has large field size that involves more thick MLC leaves, we recommend 2%/2mm criteria for absolute dose Van Dyk DTA comparison which is more sensitive to non-beam-hold leaf position errors.

Conclusions

For ten out of eleven cases, DVH comparisons between 'standard' and 'slowing MLC' VMAT plans

demonstrated minimal dosimetric changes in targets and OAR. Pass rate threshold (90%) using 3%/3 mm criteria is not sensitive in detecting MLC leaf errors that will not trigger the MLC leaf interlock. However, the consequential effects on target and OAR are negligible, which supports the reliability of current IMRT QA method for VMAT plan verification.

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Priporočila za izboljšanje kvalitete poročanja pri kliničnih raziskavah o elektrokemoterapiji, ki so utemeljena na kvantitativnem sistematičnem pregledu

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Izhodišča. Elektrokemoterapija postaja uveljavljena metoda za zdravljenje malignomov kože in ostalih lokalizacij. Njena uporaba v Evropi narašča. Izvedba je razvita iz čvrstih eksperimentalnih in kliničnih dokazov. S predlaganim konsenzom želimo formalizirati poročanje in okrepiti z dokazi utemeljena priporočila za prakso. Konsenz naj nastane na podlagi visoko kvalitetnih kliničnih podatkov, kliničnega znanja in odgovora bolnikov. Prvi korak je objavljen v pričujočem članku, katerega namen je kritično ovrednotiti kvaliteto poročanja o elektrokemoterapiji in podati priporočila za nadaljne poročanje.

Metode. Analizirali smo kvaliteto poročanja v objavljenih raziskavah o elektrokemoterapiji, da bi ustvarili posebna, za metodo specifična priporočila za poročanje. Opravili smo vseobsežen pregled literature, ki je bila objavljena med leti 2006 in 2015. Nato smo kvantitativno analizirali tekste s 47 kriteriji za kvaliteto, med katere so sodili zasnova raziskave, opis obravnavanih bolnikov, opis izvedbe zdravljenja in izida zdravljenja ter tudi analiza rezultatov. Končna ocena je temeljila na deležu raziskav, ki so izpolnjevale posamezen kriterij za kvaliteto.

Rezultati. Pregledali smo 56 raziskav, ki so jih objavili v obdobju med leti 2006 in 2015, in jih 33 analizirali. Skupaj smo analizirali 1215 bolnikov. Kvaliteta poročanja je bila zelo raznolika. 24 (73 %) raziskav so izvedli v enem centru, brez primerjave z drugimi centri, in samo 15 (45 %) raziskav je bilo prospektivnih, samo dve od teh pa sta bili uvrščeni v register raziskav. Tehnični način izvedbe elektrokemoterapije so vedno opisali, pri večini raziskav (31/33) glede na standardne pogoje izvedbe. Kakovost poročanja o značilnostih bolnikov je bila raznolika, med 45 % in 100 % izpolnjevanja kakovostnih meril za opis populacije bolnikov. Poročanje o načinu in izidu zdravljenja je bilo prav tako zelo raznoliko; izpolnjenih je bilo med 3 % in 10 0% meril. Na koncu lahko ugotovimo, da poročanje o rezutatih raziskav variira, med izpolnitvijo 27 % do 100 % meril za kakovost. Na osnovi teh rezultatov smo pripravili priporočila za izboljšanje kvalitete poročanja pri kliničnih raziskavah o elektrokemoterapiji.

Zaključki. Obseg kliničnih podatkov o elektrokemoterapiji narašča, vendar potrebujemo kakovostneješe podatke. Objavljeni članki velikokrat ne navajajo primernega opisa preučevane populacije, načina zdravljenja in izida zdravljenja. Naša priporočila so namenjena izboljšanju kakovosti podatkov v prihodnjih raziskavah o elektrokemoterapiji, želimo, da bi bila v pomoč raziskovalcem, da bi zagotovili večjo enovitost publikacij, ki bi omogočila utemeljitve na dokazih.

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Elektrokemoterapija žleznega raka trebušne slinavke

Bimonte S, Leongito M, Granata V, Barbieri A, del Vecchio V, Falco M, Nasto A, Albino V, Piccirillo M, Palaia R, Amore A, di Giacomo R, Lastoria S, Setola SV, Fusco R, Petrillo A, Izzo F

Izhodišča. Žlezni rak trebušne slinavke je eden od najbolj agresivnih rakov z visoko umrljivostjo. Bolezen se širi agresivno infiltrativno lokalno in zgodaj metastazira. Hkrati ni odzivna na kemoterapijo ali kemo-radioterapijo. Radikalna resekcija je še vedno edino kurativno zdravljenje raka trebušne slinavke, vendar smernice priporočajo multimodalno zdravljenje. Zato iščemo druge terapevtske modalitete za lokalno zdravljenje.

Zaključki. Kemorezistenca raka trebušne slinavke je pogojena s slabim privzemom citostatikov v tumorske celice zaradi prostega fibrotičnega tkiva. Če bi povečati privzem citostatikov v tumorske celice z elektrokemoterapijo, bi lahko povečali terapevtski odgovor. Povzemamo do sedaj objavljene raziskave o učinkovitosti in varnosti elektrokemoterapije na predkliničnem nivoju kot tudi v objavljenih kliničnih raziskavah.

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Učinkovitost elektrokemoterapije pri bolnikih z malignim melanomom po predhodnem zdravljenju z IFN-α

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Izhodišča. Kombinacija elektokemoterapije z imunomodulatorji je do sedaj že povečala uspešnost zdravljenja malignega melanoma. Vendar pa učinkovitost elektrokemoterapije pri bolnikih z malignim melanomom po predhodnem zdravljenju z interferonom alfa (IFN-a) še ni bila ovrednotena. Cilj naše raziskave je bil retrospektivno ovrednotiti varnost in učinkovitost elekrokemoterapije po predhodnem zdravljenju z IFN-a.

Bolniki in metode. Z retrospektivno opazovalno raziskavo smo analizirali bolnike z napredovalim malignim melanomom, ki smo jih po predhodnem pooperativnem dopolnilnem zdravljenju z IFN-a zdravili z elektokemoterapijo. V raziskavo smo lahko vključili pet bolnikov, ki smo jih zdravili z elektrokemoterapijo med januarjem 2008 in decembrom 2014, ne glede na čas predhodnega zdravljenja z IFN-a.

Rezultati. Elektrokemoterapija se je pri bolnikih z malignim melanomom, po predhodnem zdravljenju z IFN-a, pokazala kot varna in učinkovita metoda zdravljenja. Pri bolnikih z eno ali dvema metastazama je bil odgovor na zdravljenje popoln, bolniki z multiplimi metastazami pa so na zdravljenje odgovorili različno. Pri prvem bolniku z 23 metastazami so vse metastaze na zdravljenje odgovorile popolnoma, pri drugem bolniku je več kot 85 % izmed 80 zdravljenih metastaz odgovorilo popolnoma, pri tretjem bolniku z multiplimi metastazami pa je vseh 5 zdravljenih metastaz na zdravljenje odgovorilo delno. Če upoštevamo vse metastaze vseh pacientov, je kar 85 % metastaz na zdravljenje odgovorilo popolnoma.

Zaključki. Elektrokemoterapija po predhodnem zdravljenju z IFN-a se je pokazala kot varna in učinkovita metoda zdravljenja, z visoko stopnjo popolnega odgovora tako pri posameznih, kot tudi pri multiplih metastazah. Razlog za visoko stopnjo popolnega odgovora je morda imunomodulatorni učinek IFN-a, vendar so za potrditev te domneve potrebne nadaljnje klinične raziskave.

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Statistični model za opis istočasne ireverzibilne elektroporacije in poškodb krvno-možganske pregrade z elektroporacijo

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Izhodišča. Na elektroporaciji temelječe terapije kot sta elektrokemoterapija in ireverzibilna elektroporacija predstavljajo obetajoča orodja za zdravljenje tumorjev. Kadar jih uporabljamo v možganih, lahko elektroporacija povzroči začasno poškodbo krvno-možganske pregrade, ki je večja od prostornine ireverzibilno elektroporiranega tkiva in s tem lahko omogoči učinkovito prehajanje zdravil. Glavni namen raziskave je bil razvoj statističnega modela, ki bi napovedal celično smrt in poškodbo krvno-možganske pregrade po elektroporaciji. Model bi lahko uporabljali za individualno načrtovanje zdravljenja.

Materiali in metode. Modele celične smrti in poškodbe krvno-možganske pregrade smo razvili na osnovi Peleg-Fermijevega modela v kombinaciji z numeričnimi modeli električnega polja. Model lahko računa pražne vrednosti električnega polja za celično smrt in poškodbo krvno-možganske pregrade ter razloži odvisnost od števila električnih pulzov. Validirali smo ga z uporabo posnetkov MRI možganov podgan po elektroporaciji.

Rezultati. Analiza z uporabo linearne regresije je potrdila, da model dobro razloži prostornine ireverzibilno elektroporiranega tkiva in poškodbi krvno-možganske pregrade kot funkcijo števila električnih pulzov (r^2 = 0,79; p < 0,008, r^2 = 0.91; p < 0.001). Rezultati so pokazali, da je naraščanje učinka z naraščanjem števila pulzov omejeno. Razmerje med pražnimi vrednostmi električnega polja za celično smrt in preživetje celic je bilo relativno ozko (med 0,88−0,91) tudi za majhno število električnih pulzov in je bilo šibko odvisno od števila električnih pulzov. Za poškodbo krvno-možganske pregrade je razmerje med volumnom poškodovane in nepoškodovane krvno-možganske pregrade naraščalo s številom električnih pulzov. Premeri poškodbe krvno-možganske pregrade so bili v povprečju večji za 67 % ± 11 % kot premeri ireverzibilno elektroporiranega tkiva.

Zaključki. Statistični model se lahko uporablja za razlago odvisnosti učinkov zdravljenja od števila električnih pulzov, ne glede na zasnovo poskusa.

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Zdravljenje mišjega melanoma B16F10 z elektrokemoterapijo s pulznim magnetnim poljem (PEMF) *in vivo*

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Izhodišča. S pulznim magnetnim poljem (PEMF) inducirano električno polje domnevno poveča prepustnost membrane magnetnemu polju izpostavljenih celic, podobno kot pri običajni elektroporaciji. Takšno nekontaktno PEMF predstavlja obetaven pristop vnosa zdravilnih učinkovin v celico.

Materiali in metode. Neinvazivno elektroporacijo smo izvedli s pulznim generatorjem magnetnega polja, ki smo ga dovedli preko aplikatorja s tuljavo. Podkožne mišje tumorje melanoma B16F10 smo zdravili z intravenoznim vbrizganjem cisplatina (CDDP) (4 mg/kg), lokalno aplikacijo PEMF (480 bipolarnih pulzov pri frekvenci 80 Hz, posamezni pulz je trajal 340 µs) in kombinacijo obeh zdravljenj (elektrokemoterapija = PEMF + CDDP). Protitumorsko delovanje zdravljenja smo vrednotili s testom zaostanka rasti tumorjev. Nadalje smo določili vnos platine (Pt) v tumorje, Pt v serumu kot tudi vezavo Pt na DNK v celicah in vsebino Pt v medceličnini z induktivno sklopljeno plazmo in masno spektrometrijo.

Rezultati. Protitumorsko delovanje elektrokemoterapije s CDDP posredovano s PEMF je bilo primerljivo z običajno elektrokemoterapijo s CDDP. Zaostanek rasti tumorjev je bil 2,3 dneva in pri slednji 3,0 dni. Zdravljenje tumorjev samo s PEMF ali samo s CDDP ni zakasnilo rasti tumorjev. Učinek kombiniranega zdravljenja tumorjev je posledica povečanega vnosa Pt v tumorje po izpostavitvi PEMF, kot tudi njene vezave na DNK. Dokazali smo približno dvakratno povečanje vnosa Pt v celice.

Zaključki. Rezultati zdravljenja mišjega melanoma *in vivo* nakazujejo možno uporabo elektroporacije s pulznim magnetnim poljem v biomedicini, npr. pri elektrokemoterapiji. Prednosti takšne elektroporacije so nekontaktna, neboleča aplikacija ob primerljivi elektroporaciji z običajno elektroporacijo.

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Prototip fleksibilnih in prilegajočih elektrod za zdravljenje velikih površinskih tumorjev z elektrokemoterapijo

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Izhodišča. Kožne recidive raka dojke na prsnem košu lahko učinkovito zdravimo z elektrokemoterapijo. Zaradi limfogenega razsoja pa so te lezije večkrat velike in jih je težko zdravimo z ustaljeno obliko elektrokemoterapije. Metoda zdravljenja je s komercialno dostopnimi elektrodami lahko zamudna in je možno, da nehomogeno pokrije zdravljeno področje.

Materiali in metode. Namen raziskave je bil izdelati prototip elektrod s fleksibilnim vzorcem elektrod za izvajanje elektrokemoterapije velikih kožnih tumorjev. Naredili smo konektor za priključitev na generator električnih pulzov. Izvedli smo laboratorijske teste na krompirju, da bi ocenili učinkovitost elektroporacije.

Rezultati. Razvili smo nov tip elektrod za elektrokemoterapijo, ki je primeren za zdravljenje kožnih recidivov raka dojke na prsnem košu. Fleksibilnost vzorca namestitve elektrod smo preizkusili na krompirju, kjer se spremeni barva ob učinkoviti elektroporaciji. Preliminarni rezultati nakazujejo da fleksibilna podlaga elektrod omogoča prilagajanje površini prsnega koša in omogoča zdravljenje večjih površinskih tumorjev v kratkem času 2–5 minut.

Zaključki. Naredili smo prototip novih elektrod za zdravljenje večjih površinskih tumorjev. Nove elektrode so klinično uporabne, saj je njihova prednost v kratkem času dovajanje večjega števila električnih pulzov, kar tudi omogoča izvedbo elektrokemoterapije v dovoljenem časovnem oknu. Tako smo tudi skrajšali čas anestezije bolnika.

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Kombinacija lokalne in sistemske aplikacije bleomicina pri elektrokemoterapiji za zmanjšanje ponovitev zdravljenja

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Izhodišča. Elektrokemoterapija, ki je pogost način zdravljenja tumorjev v humani in veterinarski medicini, poveča toksičnost bleomicina 1000-krat in dosega 80 % objektivnih odgovorov tumorjev. Kljub temu visokemu deležu objektivnih odgovor pa pri 20 % bolnikov zdravljenje ni uspešno. Razlog za to je morda, ker se pri sistemski aplikaciji bleomicina ta ne razporedi po celotnem tumorju zaradi nezadostnega tumorskega krvožilja. Lokalna aplikacija bleomicina bi lahko pokrila dele tumorja, ki jih sistemska ne doseže.

Bolniki in metode. Predlagamo kombinacijo sistemske in lokalne aplikacije bleomicina na modelu tumorjev domačih živali. V raziskavo smo vključili 22 pasjih bolnikov, pri katerih nismo dosegli popolni odgovor na elektrokemoterapijo. Enajst psov je prejelo še eno standardno elektrokemoterapijo (kontrolna skupina), drugih enajst pa smo zdravili s kombinacijo sistemske in lokalne aplikacije bleomicina pri ponovitvi zdravljenja z elektrokemoterapijo.

Rezultati. Po kriterijih Svetovne zdravstvene organizacije smo pri skupini, ki smo jo zdravili s kombinirano elektrokemoterapijo, dosegli popoln odgovor (CR) pri 54 % (6), delni odgovor (PR) pri 36 % in stabilno bolezen (SD) pri 10 % (1) bolnikov. Pri kontrolni skupini smo CR dosegli pri 0 %, PR pri 19 % (2), SD pri 63 % (7) in pri 18 % (2) bolnikov je bolezen napredovala. Objektivni odgovor na zdravljenje smo dosegli pri 91 % (CR+PR) bolnikov, ki smo jih zdravili s kombinacijo sistemske in lokalne aplikacije bleomicina, medtem ko je bil pri kontrolni skupini ta odstotek nižji, 19 %. Razlika med obema skupinama je bila statistično značilna ($p < 0,01$).

Zaključki. Kombinirana sistemska in lokalna aplikacija bleomicina je bila učinkovita pri pasjih bolnikih, ki smo jih predhodno neučinkovito zdravili z elektrokemoterapijo. Ti rezultati nakazujejo, da bi lahko bil tak pristop uporaben in učinkovit pri specifični populaciji bolnikov in da bi se lahko zmanjšalo število ponovitev zdravljenja z elektrokemoterapijo, ki so potrebni za dosego objektivnih odgovorov.

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Medicinska fizika v Evropi po priporočilih Mednarodne agencije za atomsko energijo

Casar B, Lopes MC, Drljević A, Gershkevitsh E, Pesznyak C

Izhodišča. Medicinska fizika je zdravstveni poklic, kjer fizikalna načela uporabne fizike služijo pretežno uporabi ionizirajočega sevanja v medicini. Ključno vlogo specialista medicinske fizike pri varni in učinkoviti uporabi ionizirajočega sevanja v medicini so določili v nedavnih uradnih evropskih smernicah, imenovanih "Direktiva Sveta Evropske unije 2013/59/EURATOM (2014)" in v "Smernicah za specialista medicinske fizike Evropske komisije (2014)". Tudi Mednarodna agencija za atomsko energijo (*International Atomic Energy Agency*, IAEA) je jasno izrazila svoja stališča glede podpore in spodbujanja pri ureditvi statusa medicinske fizike na področju medicine v okviru tehničnih projektov in objavljenih dokumentov kot sta *"IAEA Human Heath Series No. 25*: Vloga, odgovornost in zahteve po izobrazbi in usposabljanju klinično kvalificiranih medicinskih fizikov (2013)" ter "Mednarodni temeljni varnostni standardi (2014)". Pomembnost omenjenih dokumentov ter ugotovitev o neizpolnjevanju zahtev in priporočil v več evropskih državah je vodilo IAEA do organizacije Regionalnega srečanja o medicinski fiziki v Evropi, kjer so udeleženci razpravljali o ključnih vprašanjih glede medicinske fizike v Evropi. Najpomembnejši rezultat srečanja so priporočila naslovljena na evropske države in raziskava o statusu medinske fizike v Evropi, ki sta jo izvedli IAEA in Evropska zveza organizacij za medicinsko fiziko (European Federation of Organizations for Medical Physics).

Zaključki. Objavljena priporočila IAEA z regionalnega srečanja o medicinski fiziki v Evropi morajo biti upoštevana in uveljavljena v vseh evropskih državah. Vzpostaviti moramo primerne okvirje kvalifikacij, ki vključujejo izobrazbo, klinično specializacijo, certifikacijo in registracijo medicinskih fizikov, s posebnim poudarkom na izpolnjevanju mednarodnih priporočil po kadrovskih normativih. Evropske države imajo jasno pravno in moralno odgovornost, da učinkovito prenesejo temeljne varnostne standarde v lokalno zakonodajo in s tem zagotavljajo visoko kakovost in varnost pri zdravljenju bolnikov.

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Natančnost slikanja z magnetno resonanco za opredelitev odgovora raka dojk na neoadjuvantno zdravljenje in za ugotavljanje velikosti rezidualnega tumorja

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Izhodišča. Namen raziskave je bil oceniti natančnost slikanja z magnetno resonanco (MRI) za ugotavljanje rezidualnega tumorja pri bolnicah z rakom dojk, ki so dobivale neoadjuvantno kemoterapijo. Poleg tega smo želeli opredeliti kliničnopatološke dejavnike, ki vplivajo na diagnostično natančnost MRI, za določanje velikosti rezidualnega tumorja po neoadjuvantnem zdravljenju.

Bolnice in metode. V raziskavo smo vključili 91 bolnic z rakom dojk (92 tumorjev dojk), ki so dobivale neoadjuvantno kemoterapijo. MRI dojke smo izvedli na začetku in po končani neoadjuvantni kemoterapiji. Odziv na zdravljenje smo ovrednotili s pomočjo MRI in s pomočjo histopatološke analize. Ovrednotili smo možnost MRI, da opredeli odgovor tumorja na zdravljenje. Pri 89 tumorjih smo s pomočjo MRI določili velikost rezidualnega tumorja po koncu neoadjuvantne kemoterapije ter jo primerjali z velikostjo rezidulanega tumorja, ugotovljeno s patološko analizo. Analizirali smo kliničnopatološke dejavnike, ki vplivajo na velikost razlike v velikosti rezidualnega tumorja med MRI in patološko analizo.

Rezultati. Občutljivost za diagnosticiranje rezidualnega tumorja s pomočjo MRI je bilo 75,00 %, specifičnost 78,57 %, pozitivna napovedna vrednost 88,89 %, negativna napovedna vrednost 57,89 % in natančnost 76,09 %. Pearsonov korelacijski koeficient (r) med velikostjo rezidualnega tumorja, ugotovljeno s pomočjo MRI, in velikostjo, izmerjeno s patološko analizo, je bil 0,648 ($p < 0,001$). Razlika v velikosti rezidualnega tumorja je bila značilno manjša pri tumorjih, pri katerih je bila z MRI ugotovljena velikost ≤ 5 cm ($p = 0,050$), pri tumorjih visokega gradusa ($p < 0,001$), in pri tumorjih, ki so bili negativni na hormonske receptorje ($p = 0,033$).

Zaključki. MRI je natančno orodje za opredelitev odziva tumorja po neoadjuvantni kemoterapiji. Natančnost MRI pri oceni velikosti rezidualnega tumorja se razlikuje glede na velikosti tumorjev pred zdravljenjem, glede na gradus ter glede na njihov status hormonskih receptorjev.

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Polimorfizmi antioksidantnih genov niso povezani s povišanim tveganjem za nastanek sekundarnega raka ščitnice po zdravljenju zaradi raka v otroštvu ali mladostništvu

Vodušek AL, Goričar K, Gazić B, Dolžan V, Jazbec J

Izhodišča. Sekundarni rak ščitnice je eden izmed najpogostejših rakov, ki nastanejo kot posledica zdravljenja raka v otroštvu ali mladostništvu. Ščitnica je predvsem v otroštvu zelo občutljiva na kancerogene učinke ionizirajočega sevanja. Nesorazmerje med pro- in antioksidantnimi dejavniki je eden izmed možnih mehanizmov za nastanek raka ščitnice. Z raziskavo smo želeli oceniti vpliv polimorfizmov antioksidantnih genov na nastanek sekundarnega raka ščitnice po zdravljenju raka v otroštvu ali mladostništvu.

Bolniki in metode. V retrospektivno raziskavo smo vključili bolnike, ki smo jih med letoma 1960 in 2006 zdravili zaradi primarnega raka v starosti manj ali enako 21 let, in ki so nato zboleli zaradi sekundarnega raka ščitnice. Naredili smo raziskavo s kontrolnimi primeri in zato dodatno poiskali primerljive bolnike po starosti, spolu, diagnozi in zdravljenju primarnega raka (predvsem obsevanju), ki pa niso zboleli zaradi sekundarnega raka. Vsem smo določili polimorfizme *SOD2* p.Ala16Val, *CAT* c.-262C>T, *GPX1* p.Pro200Leu, *GSTP1* p.Ile105Val, *GSTP1* p.Ala114Val in *GSTM1* in *GSTT1*. Njihov vpliv na pojav sekundarnega raka smo opredelili z McNemarovim testom in Coxovo regresijsko analizo.

Rezultati. Med letoma 1960 in 2006 je v Sloveniji zbolelo zaradi raka 2641 bolnikov mlajših od 21 let. Sekundarni rak smo ugotovili pri 155 bolnikih, od katerih je 28 zbolelo zaradi ščitničnega raka. Med primeri in kontrolami ni bilo statistično pomembnih razlik v porazdelitvi genotipov preiskovanih polimorfizmov, kakor tudi ne statistično pomembnega vpliva polimorfizmov na čas nastanka sekundarnega raka ščitnice.

Zaključki. Nismo ugotovili vpliva polimorfiizmov antioksidantnih genov na tveganje za nastanek sekundarnega raka ščitnice pri bolnikih, ki smo jih v otroštvu ali mladostništvu zdravili zaradi raka. Ker je rak ščitnice eden izmed najbolj pogostih sekundarnih rakov po zdravljenju zaradi primarnega raka v otroštvu ali mladostništvu in lahko nastane tudi več deset let po zdravljenju, je potrebno doživljenjsko spremljanje teh bolnikov.

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Toksoplazmoza osrednjega živčevja pri bolniku z limfomom B

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Izhodišča. Oportunistična okužba s protozojem *Toxoplasmo gondii* je doslej najverjetneje slabše prepoznana pri bolnikih s hematološkimi malignimi obolenji, ki niso bili zdravljeni s presaditvijo zarodnih celic ali kostnega mozga. Sodobne metode zdravljenja rakavih bolezni vključujejo načine, ki povzročijo zmanjšanje števila limfocitov B in T ter najverjetneje predstavljajo pomemben dejavnik tveganja za reaktivacijo latentne okužbe s *Toxoplasmo gondii*.

Prikaz primera. Opisujemo 62-letno HIV-negativno desnoročno bolnico z difuznim velikoceličnim limfomom B, ki je nekaj dni po zadnjem (osmem) krogu zdravljenja z rituksimabom, ciklofosfamidom, vinkristinom, doksrubicinom in prednisolonom (R-CHOP) akutno zbolela s povišano telesno temperaturo, glavobolom, motnjo zavesti, ataksijo in pancitopenijo. Postavili smo sum na napredovanje limfoma v osrednje živčevje. MR glave je na sekvencah T2 in sekvencah z izločanjem signalov tekočin (FLAIR) pokazala številne hiperintenzivne spremembe obojestransko v možganovini malih in velikih možgan z okolnim edemom. Zmerno so se obarvale z gadolinijem. Polimerazna verižna reakcija (PCR) likvorja je bila pozitivna za DNK *Toxoplasme gondii*. Postavili smo diagnozo toksoplazmoze osrednjega živčevja. Bolnico smo uspešno zdravili s kombinacijo sulfadiazina, pirimetamina in folne kisline. Zaradi vzdrževalne terapije z rituksimabom ob remisiji limfoma je bolnica nato nadaljevala s sekundarno profilakso toksoplazmoze.

Zaključki. S prikazom tega primera želimo poudariti pomembnost vključitve toksoplazmoze osrednjega živčevja v diferencialno diagnostiko bolnikov s hematološkimi malignimi obolenji, ki zbolijo z novo nevrološko simptomatiko. Izmed številnih diferencialno diagnostičnih možnosti je namreč najtežje razločevanje limfoma in toksoplazmoze osrednjega živčevja. Nezdravljena toksoplazmoza osrednjega živčevja ima visoko smrtnost, zato je zgodnje prepoznavanje in zdravljenje tovrstnih bolnikov ključnega pomena.

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Obstruktivne težave z uriniranjem po kombinaciji zunanjega obsevanja in brahiradioterapije raka prostate

Kragelj B

Izhodišča. Cilj raziskave je bil opredeliti dejavnike, ki vplivajo na nastanek obstruktivnih težav z uriniranjem po kombiniranem zdravljenja z zunanjim obsevanjem in brahiradioterapijo pri bolnikih z rakom prostate. Ob upoštevanju teh dejavnikov bi lahko zdravljenje prilagajali posameznemu bolniku.

Bolniki in metode. V raziskavo smo vključili 88 bolnikov, ki smo jih na Onkološkem inštitutu v Ljubljani zdravili z zunanjim obsevanjem in brahiradioterapijo v letih 2006-2011. Opazovani izid je bilo poslabšanje obstruktivnih težav z uriniranjem eno ali več let po zaključenem zdravljenju. Univariatno in multivariatno analizo povezanosti poslabšanja uriniranja z morebitnimi dejavniki tveganja smo naredili z metodo binarne logistične regresije. Pri tem smo upoštevali značilnosti bolnikov ter značilnosti zunanjega obsevanja in brahiradioterapije. S pomočjo analize ROC, pri kateri smo ocenili površino pod krivuljo ROC (AUC), smo nato ocenili še učinkovitost multivariatnega modela pri napovedovanju poslabšanja obstruktivnih težav z uriniranjem.

Rezultati. V končno analizo smo zajeli 71 bolnikov, ki smo jih sledili vsaj 3-leta po zdravljenju. Napovedovanju poslabšanja obstruktivnih težav z uriniranjem smo ugotovili pri 13/71 (18,3 %) bolnikov. Rezultati multivariatne analize so pokazali statistično značilno povezanost poslabšanja obstruktivnih težav z uriniranjem z antikoagulantnim zdravljenjem (razmerje obetov [OR] = 4,86; 95 % interval zaupanja [C.I.]: 1,21−19,61; p = 0,026) in mejno značilno povezanost z dozo ki jo prejme 90 % volumna uretre (OR = 1,23; 95 % C.I.: 0,98−1,07; p = 0,099). Vrednost AUC je bila 0,755 .

Zaključki. Raziskava je izpostavila vpliv antikoagulantnega zdravljenja na napovedovanje poslabšanja obstruktivnih težav z uriniranjem. Izdelali smo model s sprejemljivo napovedno vrednostjo, ki bi lahko omogočil zmanjšanje obstruktivnih težav z uriniranjem. Izsledki raziskave podpirajo tudi pomen uretralnega sfinktra kot rizične strukture za poslabšanja obstruktivnih težav z uriniranjem.

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doi:10.1515/raon-2015-0009

Napovedni dejavniki pri zdravljenju malignih melanomov žilnice v Sloveniji, 1986–2008

Jančar B, Budihna M, Drnovšek-Olup B, Novak Andrejčič K, Brovet Zupančič I, Pahor D

Izhodišča. Melanom žilnice je najpogostejši primarni tumor v očesu. Incidenca bolezni je bila v Sloveniji stabilna od 1983 do 2009 s 7,8 bolnikov/milijon za moške in 7,4 bolnikov/milijon za ženske. V retrospektivni raziskavi smo želeli ugotoviti napovedne dejavnike preživetja pri bolnikih z malignim melanomom žilnice, ki smo jih zdravili v Sloveniji.

Bolniki in metode. Od januarja 1986 do decembra 2008 smo zdravili 288 bolnikov z malanomom žilnice. 127 bolnikov smo zdravili z brahiterapijo z rutenijevimi (Ru-106) aplikatorji in 161 bolnikov z enukleacijo.

Rezultati. Pri bolnikih s tumorji višine < 7 mm in premera < 16 mm, ki smo jih zdravili z brahiterapijo, je bila 5- in 10-letna splošna smrtnost 13% in 32%, pri bolnikih zdravljenih z enukleacijo pa 46% in 69%. Razlika v preživetju je bila posledica izbora: bolnike z večjimi tumorji smo zdravili z enukleacijo. Pri 30 bolnikih smo ugotovili ponovno rast tumorja. Pri 25 bolnikih od 127, ki smo jih zdravili z brahiterapijo ter pri 86 od 161, ki smo jih zdravili z enukleacijo, pa smo po zdravljenju ugotovili oddaljene zasevke. Preživetje bolnikov starih ≥ 60 let, ki smo jih zdravili tako z brahiterapijo kot z enuklacijo, je bilo krajše. V multivariatni analizi sta bila neodvisna dejavnika za preživetje bolnikov zdravljenih z brahiterapijo premer tumorja in čas implantacije, za bolnike zdravljene z enukleacijo pa samo histološki tip tumorja. V prvih letih po zdravljenju smo opazili začasen porast specifične smrtnosti, zlasti pri starejših bolnikih.

Zaključki. Verjetnost ozdravitve bolnikov z malignim melanom žilnice je večja pri mlajših bolnikih in pri tistih z manjšimi tumorji. Brahiterapija je prednostna metoda zdravljenja glede na enukleacijo zaradi ohranitve očesa.

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doi:10.1515/raon-2015-0015

Vpliv anemije na izid zdravljenja bolnikov s ploščatoceličnim rakom analnega kanala

Oblak I, Češnjevar M, Anžič M, But Hadžič J, Šečerov Ermenc A, Anderluh F, Velenik V, Jeromen A, Peter Korošec

Izhodišča. Temeljno zdravljenje bolnikov s ploščatoceličnim rakom analnega kanala je radiokemoterapija. Anemija pa je lahko eden od dejavnikov, ki poslabša učinkovitost zdravljenja. Namen raziskave je bil ugotoviti, kakšen je vpliv anemije na izid zdravljenja bolnikov z rakom analnega kanala.

Bolniki in metode. V retrospektivno raziskavo smo vključili 100 bolnikov s histološko potrjenim ploščatoceličnim rakom, ki smo jih zdravili s 3-dimenzionalno (3-D) konformalno planiranim obsevanjem ali intenzitetno modulirano radioterapijo (IMRT). Sočasno z obsevanjem so bolniki prejemali kemoterapijo z derivati fluoropirimidinov in mitomicina C. Kasneje so prejeli še dodatno obsevanje na tumor z brahi- ali teleradioterapijo. Analizirali smo vpliv koncentracij hemoglobina (Hb) pred, med in ob zaključku zdravljenja na izid poteka bolezni.

Rezultati. 5-letno preživetje brez ponovitve bolezni lokalno in/ali področno je bilo 72 %, preživetje brez ponovitve bolezni 71 %, bolezensko specifično preživetje 77 % in celokupno preživetje 62 %. V univariatni analizi so bolniki s koncentracijo Hb >120 g/L pred začetkom zdravljenja in ob zaključku zdravljenja imeli statistično pomembno boljše preživetje kot bolniki, ki so imeli Hb ≤ 120 g/L. Bolniki, ki smo jim med zdravljenjem ugotovili Hb > 120 g/L, so imeli statistično pomemben vpliv le na boljše preživetje brez ponovitve bolezni lokalno in/ali področno in celokupno preživetje. V multivariatni analizi se je koncentracija Hb pred zdravljenjem (> 120 g/L *vs.* ≤ 120 g/L) pokazala kot neodvisni napovedni dejavnik za celokupno preživetje (razmerje ogroženosti [HR] = 0,419; 95 % interval zaupanja [CI] = 0,190–0,927; p = 0,032). Prav tako se je pokazal stadij bolezni (stadij I in II *vs.* III) kot neodvisni napovedni dejavnik za bolezensko specifično preživetje (HR = 3,523; 95 % CI = 1,375–9,026; p = 0,009) in celokupno preživetje (HR = 2,230; 95% CI = 1,1675–4,264; p = 0,015).

Zaključki. Koncentracija Hb > 120 g/L pred, med in po zdravljenju z radiokemoterapijo je bila ugoden napovedni dejavnik preživetja pri bolnikih z rakom analnega kanala. Najpomembnejša je bila koncentracija Hb > 120 g/L pred začetkom zdravljenja, ki se je pokazala kot neodvisni napovedni dejavnik za celokupno preživetje.

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doi:10.1515/raon-2016-0008

Ocena dozimetričnega učinka povzročenega z upočasnitvijo lističev večlistnega kolimatarja na volumetrično modulirano ločno terapijo (VMAT)

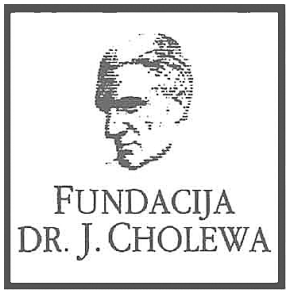
Xu ZZ, Wang IZ, Kumaraswamy LK, Podgorsak MB

Izhodišča. Poročamo o občutljivosti metode zagotavljanja kakovosti načrtov intenzitetnega moduliranega obsevanja (IMRT) za napake lamel večlistnega kolimatorja (MLC) pri volumetrično moduliranem ločnem obsevanju (VMAT), ki ne sproži blokade MLC med postopkom obsevanja. Prav tako poročamo o učinku napak na kakovost obsevanja z VMAT zaradi neustavitve žarka z lističi.

Materiali in metode. S pomočjo internega računalniškega programa smo izbrali in spremenili 11 planov VMAT. Za vsako kontrolno točko na loku VMAT so bili lističi MLC največje hitrosti (1,87–1,95 cm/s) nastavljeni tako, da so se premikali z največjo dovoljeno hitrostjo (2,3 cm/s), kar je povzročilo razliko položaja lističa za manj kot 2 mm. Spremenjene načrte smo obravnavali kot ‚standardne' načrte in prvotne načrte kot ‚upočasnjene' načrte MLC za simulacijo ‚standardnih' načrtov s premikanjem lističev pri relativno nižji hitrosti. Meritve vsakega ‚upočasnjenega' načrta MLC z uporabo MapCHECK®2 smo primerjali z izračunano planirano dozo ‚standardnega' načrta ob upoštevanju primerjave absolutne doze dogovorne razdalje Van Dyk (DTA) z meriloma 3 % / 3 mm in 2 % / 2 mm.

Rezultati. Vsi ‚upočasnjeni' načrti MLC so uspešno prešli prag 90 % ob uporabi merila 3 % / 3 mm, medtem ko je bil en primer načrta VMAT za možgane in trije primeri za analni kanal pod pragom 90 % ob uporabi merila 2 % / 2 mm. Primerjava dozno-volumnih histogramov (DHV) ‚standardnih' načrtov in ‚upočasnjenih' načrtov MLC je pri desetih od enajstih primerov pokazala minimalne dozimetrične spremembe na tarčnih in rizičnih organih.

Zaključki. Za visoko modulirane načrte VMAT prag merila 3 % / 3 mm ni dovolj občutljiv za odkrivanje napake lističev MLC, ki ne sprožijo njihove blokade. Vendar pa so učinki doze na tarčne in kritične organe kot posledica napak MLC zaradi ne ustavitve žarka zanemarljive, kar podpira zanesljivost trenutne metode bolniku specifičnega prilagojenega zagotavljanja kakovosti IMRT za načrte VMAT.



FUNDACIJA DOC. DR. JOSIP CHOLEWA

RAZPISUJE

DENARNO POMOČ ZA SOFINANCIRANJE MATERIALNIH STROŠKOV PRI ZNANSTVENO-RAZISKOVALNIH DELIH S PODROČJA ONKOLOGIJE.

PRIJAVA NAJ VSEBUJE:

1. KRATKO OBRAZLOŽITEV ZNANSTVENO-RAZISKOVALNEGA DELA S FINANČNO KONSTRUKCIJO

2. KRATKO BIOGRAFIJO IN BIBLIOGRAFIJO PROSILCA/PROSILCEV

PRIJAVE, PROSIMO, POŠLJITE DO **30. 4. 2016** NA NASLOV
ZDRUŽENJE FUNDACIJA DOC.DR. JOSIP CHOLEWA,
DUNAJSKA CESTA 106, 1000 LJUBLJANA

Uporabljena moška slovnična oblika se enakovredno nanaša na oba spola.

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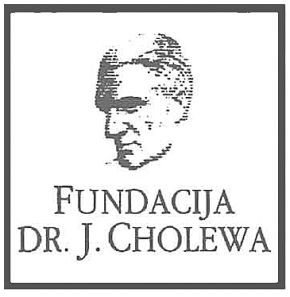

Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education – a report for the first quarter of 2016

The “Docent Dr. J. Cholewa Foundation for Cancer Research and Education” is named after Dr. Josip Cholewa, one of the first researchers in cancer in Slovenia and the founder of the “Banovinski Inštitut za raziskovanje in zdravljenje novotvorb” in 1937, that later became the Institute of Oncology in Ljubljana, Slovenia. His laboratory and clinical research work was based on an innovative and far-reaching multidisciplinary approach that included studies on prevention, detection and treatment of cancer. This pioneering approach facilitated the understanding of the complexities of all the problems and troubles experienced by cancer patients, their doctors and other medical staff when facing this disease. It could also be regarded as a harbinger of the progress observed in a large part of the world in the last half of the previous century. Therefore, the Foundation is a non-profit, non-political and non-government organisation that helps professionals, institutions and individuals obtaining financial help for cancer research and education in the Republic of Slovenia with the goal of continuing and expanding the great work and efforts of Dr. Josip Cholewa.

The “Docent Dr. J. Cholewa Foundation for Cancer Research and Education” hopes and strives to provide at least part of the financial support needed by qualified individuals and organisations interested in cancer research in the Republic of Slovenia. One of the objectives of the Foundation is to facilitate the transmission of the latest diagnostic and therapy procedures to the clinical environment in Slovenia, thus benefiting the ever increasing number of patients with various types of cancer in Slovenia. With this in mind, it is important to note that the incidence rates of many cancer, like colon, prostate and breast cancer have kept rising in recent decades in Slovenia.

The Foundation continues to provide financial support to "Radiology and Oncology”, an international scientific journal that is edited and published in Ljubljana, Slovenia. It publishes scientific research articles, reviews, and letters to the editor about research and studies in experimental and clinical oncology, supportive therapy, radiology, radiophyics, prevention and early diagnostics of different types of cancer. It is an open access journal freely available in pdf format and with a respectable Science Citation Index Impact factor. All the abstracts in “Radiology and Oncology” are available in Slovenian and the journal can thus provide sufficient scientific information from various fields of high quality cancer research to interested lay public in Slovenia.

The “Docent Dr. J. Cholewa Foundation for Cancer Research and Education” has thus an important role in support of cancer research, cancer education and many of the related fields in the Republic of Slovenia.

Tomaž Benulič, M.D.
Viljem Kovač, M.D., Ph.D.
A Borut Štabuc, M.D., Ph.D.
Andrej Plesničar, M.D., M.Sc.

NOVA INDIKACIJA[1]

Vectibix® + FOLFIRI v 1. liniji zdravljenja bolnikov z mKRR in nemutiranim genom *RAS*

Zdravilo Vectibix® je sedaj indicirano za zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom (mKRR) in nemutiranim genom RAS:

- v 1. liniji zdravljenja v kombinaciji s FOLFOX ali FOLFIRI
- v 2. liniji zdravljenja v kombinaciji s FOLFIRI pri bolnikih, ki so v prvi liniji zdravljenja prejemali kemoterapijo, ki je vključevala fluoropirimidin (vendar ni vključevala irinotekana)
- kot monoterapija po neuspehu shem kemoterapije, ki so vključevale fluoropirimidin, oksaliplatin in irinotekan.

VECTIBIX® 20 mg/ml koncentrat za raztopino za infundiranje (sterilni koncentrat) (panitumumab) – SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA
Samo za strokovno javnost. Pred predpisovanjem si preberite celoten Povzetek glavnih značilnosti zdravila (SmPC).

SESTAVA ZDRAVILA: 1 ml koncentrata vsebuje 20 mg panitumumaba. 1 viala vsebuje 100 mg panitumumaba v 5 ml, 200 mg panitumumaba v 10 ml ali 400 mg panitumumaba v 20 ml koncentrata. **TERAPEVTSKE INDIKACIJE:** Zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom (mKRR) z divjim tipom *RAS* in sicer v prvi liniji zdravljenja v kombinaciji s FOLFOX ali FOLFIRI, v drugi liniji zdravljenja v kombinaciji s FOLFIRI pri bolnikih, ki so v prvi liniji zdravljenja prejemali kemoterapijo, ki je vključevala fluoropirimidin (vendar ni vključevala irinotekana), ter kot monoterapija po neuspehu shem kemoterapije, ki so vključevale fluoropirimidin, oksaliplatin in irinotekan. **ODMERJANJE IN NAČIN UPORABE:** Zdravljenje z zdravilom Vectibix® mora nadzirati zdravnik z izkušnjami pri uporabi terapije proti raku. Pred začetkom zdravljenja z zdravilom Vectibix® mora biti potrjeno, da gre za stanje divjega tipa *RAS (KRAS* in *NRAS)*. Mutacijsko stanje mora ugotoviti izkušen laboratorij z uporabo validiranih testnih metod za detekcijo mutacij *KRAS* (eksoni 2, 3 in 4) in *NRAS* (eksoni 2, 3 in 4). Priporočeni odmerek zdravila Vectibix® je 6 mg/kg telesne mase enkrat na dva tedna. V primeru hudih (≥ 3. stopnja) dermatoloških reakcij je lahko potrebna prilagoditev odmerka zdravila Vectibix®. *Bolniki z okvaro ledvic ali jeter:* Varnost in učinkovitost zdravila Vectibix® nista raziskani. *Starejši bolniki:* Ni kliničnih podatkov, ki bi podprli prilagoditev odmerka. *Pediatrična populacija:* Zdravilo Vectibix® nima relevantne uporabe za indikacijo zdravljenja kolorektalnega raka. Zdravilo Vectibix® morate aplicirati v intravenski (i.v.) infuziji z infuzijsko črpalko. Če se pojavijo z infundiranjem povezane reakcije, je lahko potrebna upočasnitev infundiranja zdravila Vectibix®. Zdravila Vectibix® ne smete injicirati z intravenskim vbrizgavanjem ali v bolusu. **KONTRAINDIKACIJE:** Anamneza hude ali smrtno nevarne preobčutljivosti na zdravilno učinkovino ali katero koli pomožno snov, intersticijski pnevmonitis, pljučna fibroza. Pri bolnikih z mKRR z mutantnim *RAS* in tistih bolnikih z mKRR, pri katerih stanje *RAS* ni znano, je kontraindicirana kombinacija zdravila Vectibix® in kemoterapije, ki vključuje oksaliplatin. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** ***Dermatološke reakcije in toksičnost mehkih tkiv:*** Skoraj pri vseh bolnikih (približno 90%), zdravljenih z zdravilom Vectibix®, se pojavijo dermatološke reakcije, ki so farmakološki učinek, opažen pri zaviralcih EGFR. Če se pri bolniku pojavijo dermatološke reakcije 3. ali višje stopnje (po CTCAE verzija 4.0) ali dermatološke reakcije, ocenjene kot neznosne, je priporočljiva naslednja prilagoditev odmerka: *Prvi pojav kožnih simptomov ≥ 3. stopnje:* Zadržite 1 ali 2 odmerka zdravila Vectibix®. Če se po tem simptomi izboljšajo (< 3. stopnja), nadaljujte infundiranje s 100 % odmerka, ki ste ga aplicirali pred pojavom kožnih simptomov; če izboljšanja ni, prekinite uporabo zdravila Vectibix®. *Ob drugem pojavu kožnih simptomov ≥ 3. stopnje:* Zadržite 1 ali 2 odmerka zdravila Vectibix®. Če se simptomi izboljšajo (< 3. stopnja), nadaljujte infundiranje z 80 % odmerka, ki ste ga aplicirali pred pojavom kožnih simptomov; če izboljšanja ni, prekinite uporabo zdravila Vectibix®. *Ob tretjem pojavu kožnih simptomov ≥ 3. stopnje:* Zadržite 1 ali 2 odmerka zdravila Vectibix®. Če se simptomi izboljšajo (< 3. stopnja), nadaljujte infundiranje s 60 % odmerka, ki ste ga aplicirali pred pojavom kožnih simptomov; če izboljšanja ni, prekinite uporabo zdravila Vectibix®. *Ob četrtem pojavu kožnih simptomov ≥ 3. stopnje:* Prekinite uporabo. Bolnike s hudimi dermatološkimi reakcijami ali toksičnostjo mehkih tkiv ali poslabšanjem reakcij med uporabo zdravila Vectibix® morate nadzirati zaradi možnih vnetnih ali infekcijskih posledic (vključno s celulitisom in nekrotizirajočim fasciitisom) ter jim nemudoma uvesti ustrezno zdravljenje, če se pojavijo. Če se pojavi dermatološka toksičnost ali toksičnost mehkih tkiv, povezana s hudimi ali življenjsko ogrožujočimi vnetnimi ali infekcijskimi zapleti, zadržite ali prekinite zdravljenje z zdravilom Vectibix®. ***Zapleti na pljučih:*** V primeru akutnega nastanka ali poslabšanja pljučnih simptomov morate zdravljenje z zdravilom Vectibix® prekiniti in simptome takoj raziskati. Če diagnosticirate intersticijsko pljučno bolezen, morate zdravljenje z zdravilom Vectibix® za stalno prekiniti in bolnika ustrezno zdraviti. Pri bolnikih z anamnezo intersticijskega pnevmonitisa ali pljučne fibroze je potrebno skrbno razmisliti o koristih zdravljenja s panitumumabom v primerjavi s tveganjem za zaplete na pljučih. ***Elektrolitske motnje:*** Bolnike je treba pred uvedbo zdravljenja, med zdravljenjem in še 8 tednov po končanem zdravljenju z zdravilom Vectibix®, redno spremljati glede hipomagneziemije in spremljajoče hipokalciemije. Priporočljivo je dodajanje magnezija, kot je ustrezno. Opažali so tudi druge elektrolitske motnje, vključno s hipokaliemijo. Priporočljivo je spremljanje, kot opisano zgoraj in dodajanje teh elektrolitov, kot je ustrezno. ***Z infundiranjem povezane reakcije:*** Če se kadarkoli med ali po infundiranju pojavi huda ali smrtno nevarna reakcija (npr. z bronhospazmom, angioedemom, hipotenzijo, potrebo po parenteralnem zdravljenju ali anafilaksijo), je treba uporabo zdravila Vectibix® za stalno prekiniti. Bolnikom, ki se jim pojavi blaga ali zmerna (1. in 2. stopnja po CTCAE verzija 4.0) z infundiranjem povezana reakcija, je treba hitrost infundiranja za čas infuzije zmanjšati. To manjšo hitrost infundiranja je priporočljivo ohraniti tudi pri vseh nadaljnjih infuzijah. Opisane so preobčutljivostne reakcije, ki so se pojavile več kot 24 ur po infundiranju, vključno s smrtnimi primeri angioedema, ki so se pojavili več kot 24 ur po infundiranju. Bolnike je potrebno opozoriti na možnost reakcij s poznim nastankom in jim naročiti, naj se obrnejo na svojega zdravnika, če se jim pojavijo simptomi preobčutljivostne reakcije. ***Akutna odpoved ledvic:*** Opisana je akutna odpoved ledvic pri bolnikih, ki se jim pojavi huda driska in dehidracija. Bolnikom, ki se jim pojavi huda driska, je treba naročiti, da se takoj posvetujejo z zdravnikom. ***Zdravilo Vectibix® v kombinaciji s kemoterapijo na podlagi oksaliplatina pri bolnikih z mKRR z mutantnim RAS oz. bolnikih z mKRR, pri katerih stanje RAS tumorja ni znano:*** Kombinacija zdravila Vectibix® s kemoterapijo, ki vključuje oksaliplatin, je kontraindicirana pri bolnikih z mKRR z mutantnim *RAS* ali pri katerih stanje *RAS* ni znano. Če je predvidena uporaba zdravila Vectibix® v kombinaciji s FOLFOX, je priporočljivo, da mutacijsko stanje določi laboratorij, ki sodeluje v programu Eksterno zagotavljanje kakovosti *RAS*, ali da se stanje divjega tipa potrdi z dupliciranjem preiskave. ***Očesni toksični učinki:*** Bolnike, ki se jim med prejemanjem zdravila Vectibix® pojavijo znaki in simptomi, ki kažejo na keratitis, kot so akutni pojav ali poslabšanje: vnetja očesa, solzenja, občutljivosti na svetlobo, zamegljenega vida, bolečine v očesu in/ali rdečih oči, je priporočljivo takoj napotiti k specialistu oftalmologu. Če je potrjena diagnoza ulcerativnega keratitisa, je treba zdravljenje z zdravilom Vectibix® začasno ali trajno prekiniti. Če je diagnosticiran keratitis, je treba skrbno pretehtati koristi in tveganja nadaljevanja zdravljenja. Zdravilo Vectibix® morate uporabljati previdno pri bolnikih z anamnezo keratitisa, ulcerativnega keratitisa ali zelo suhih oči. ***Bolniki z zmogljivostnim stanjem 2 po ECOG, zdravljeni z zdravilom Vectibix® v kombinaciji s kemoterapijo:*** Pri teh bolnikih je pred uvedbo zdravila Vectibix® v kombinaciji s kemoterapijo za zdravljenje mKRR priporočljivo oceniti koristi in tveganje, saj pri njih pozitivno razmerje med koristnostjo in tveganjem ni bilo zabeleženo. ***Starejši bolniki:*** V celoti niso ugotovili razlik v varnosti ali učinkovitosti med starejšimi bolniki (starimi ≥ 65 let), ki so prejemali monoterapijo z zdravilom Vectibix®. ***Drugi previdnostni ukrepi:*** Zdravilo vsebuje manj kot 0,150 mmol natrija (kar je 3,45 mg natrija) na mililiter koncentrata. To je treba upoštevati pri bolnikih, ki potrebujejo prehrano z nadzorovano količino natrija. **MEDSEBOJNO DELOVANJE Z DRUGIMI ZDRAVILI IN DRUGE OBLIKE INTERAKCIJ:** Podatki študije o medsebojnem delovanju zdravil, ki je vključevala zdravili Vectibix® in irinotekan, pri bolnikih z mKRR kažejo, da se med sočasno uporabo zdravil farmakokinetika irinotekana in njegovega aktivnega metabolita SN-38 ne spremeni. Rezultati primerjave v navzkrižni študiji so pokazali, da sheme z irinotekanom (IFL ali FOLFIRI) ne vplivajo na farmakokinetiko panitumumaba. Zdravila Vectibix® se ne sme uporabljati v kombinaciji s kemoterapijo IFL ali kemoterapijo, ki vključuje bevacizumab. Kombinacija zdravila Vectibix® s kemoterapijo, ki vključuje oksaliplatin, je kontraindicirana pri bolnikih z mKRR z mutantnim *RAS* ali pri katerih stanje *RAS* ni znano. **NEŽELENI UČINKI:** Neželeni učinki, zabeleženi v kliničnih študijah in v spontanih poročilih v obdobju trženja zdravila pri bolnikih z mKRR, ki so dobivali panitumumab kot edino zdravilo ali v kombinaciji s kemoterapijo, so: Zelo pogosti (≥ 1/10): paronihija, anemija, hipokaliemija, anoreksija, hipomagneziemija, nespečnost, konjunktivitis, dispneja, kašelj, driska, navzea, bruhanje, bolečine v trebuhu, stomatitis, zaprtost, akneiformni dermatitis, izpuščaj (vključuje splošne termine kožne toksičnosti, eksfoliacije kože, eksfoliativnega izpuščaja, papuloznega izpuščaja, pruritičnega izpuščaja, eritematoznega izpuščaja, generaliziranega izpuščaja, makularnega izpuščaja, makulo-papuloznega izpuščaja, kožne lezije), eritem, srbenje, suha koža, fisure na koži, akne, alopecija, bolečine v hrbtu, utrujenost, pireksija, astenija, vnetje sluznice, periferni edemi, paronihija, zmanjšanje telesne mase; Pogosti (≥ 1/100 do < 1/10): pustulozen izpuščaj, celulitis, okužba sečil, folikulitis, lokalizirana okužba, levkopenija, preobčutljivost, hipokalciemija, dehidracija, hiperglikemija, hipofosfatemija, anksioznost, glavobol, omotica, blefaritis, rast trepalnic, močnejše solzenje, očesna hiperemija, suho oko, srbenje oči, draženje oči, tahikardija, globoka venska tromboza, hipotenzija, hipertenzija, zardevanje, pljučna embolija, epistaksa, krvavitev iz danke, suha usta, dispepsia, aftozni stomatitis, heilitis, gastroezofagealna refluksna bolezen, sindrom palmarno-plantarne eritrodizestezije, kožni ulkus, krasta, hipertrihoza, lomljenje nohtov, bolezni nohtov, hiperhidroza, dermatitis, bolečine v okončini, bolečine v prsih, bolečina, mrzlica, znižanje magnezija v krvi; Občasni (≥ 1/1.000 do < 1/100): okužba oči, okužba vek, draženje vek, keratitis, cianoza, bronhospazem, suhost nosu, razpokane ustnice, suhe ustnice, angioedem, hirzutizem, vraščanje nohta, onioholiza, z infundiranjem povezane reakcije; Redki (≥ 1/10.000 do < 1/1.000): anafilaktična reakcija, ulcerativni keratitis, nekroza kože, Stevens-Johnsonov sindrom, toksična epidermalna nekroliza; Pogostnost neznana (pogostnosti ni mogoče oceniti iz razpoložljivih podatkov): intersticijska pljučna bolezen. **FARMACEVTSKI PODATKI:** Shranjujte v hladilniku (2 °C - 8 °C). Ne zamrzujte. Shranjujte v originalni ovojnini za zagotovitev zaščite pred svetlobo. Zdravilo Vectibix® ne vsebuje nobenih antimikrobnih konzervansov ali bakteriostatikov. Zdravilo je treba uporabiti takoj po razredčenju. Če ni uporabljeno takoj, so čas shranjevanja med uporabo in pogoji pred uporabo odgovornost uporabnika; čas običajno ne sme presegati 24 ur pri temperaturi od 2 °C do 8 °C. Razredčene raztopine ne smete zamrzniti. Zdravilo Vectibix® je namenjeno za enkratno uporabo. Inkompatibilnosti med zdravilom Vectibix® in natrijevim kloridom 9 mg/ml (0,9 %) raztopino za injiciranje v polivinilkloridnih ali poliolefinskih vrečkah niso ugotovili. **NAČIN IN REŽIM PREDPISOVANJA TER IZDAJE ZDRAVILA:** Predpisovanje in izdaja zdravila je le na recept s posebnim režimom – H. **IMETNIK DOVOLJENJA ZA PROMET:** Amgen Europe B.V., Minervum 7061, NL-4817 ZK Breda, Nizozemska. **Dodatna pojasnila lahko dobite v lokalni pisarni:** Amgen zdravila d.o.o., Šmartinska 140, SI-1000 Ljubljana. **DATUM ZADNJE REVIZIJE BESEDILA:** Marec 2015. **DATUM PRPRAVE INFORMACIJE:** Avgust 2015. Podrobne informacije o zdravilu so objavljene na spletni strani Evropske agencije za zdravila http://www.ema.europa.eu.

Literatura: 1. Povzetek glavnih značilnosti zdravila Vectibix®, Amgen.

SI-HR-SL-P-954-0815-112854 (1)

AMGEN®

Vectibix® panitumumab

INDIVIDUALIZIRANO TARČNO ZDRAVLJENJE.

TANTUM VERDE®

Lajšanje bolečine in oteklin pri vnetju v ustni votlini in žrelu, ki nastanejo zaradi okužb in stanj po operaciji in kot posledica radioterapije (t.i. radiomukozitis).

ANGELINI

Predstavnik:
Angelini Pharma d.o.o.
Koprska ulica 108 A, Ljubljana

www.tantum-verde.si

Tantum Verde 1,5 mg/ml oralno pršilo, raztopina

Kakovostna in količinska sestava
1 ml raztopine vsebuje 1,5 mg benzidaminijevega klorida, kar ustreza 1,34 mg benzidamina. V enem razpršku je 0,17 ml raztopine. En razpršek vsebuje 0,255 mg benzidaminijevega klorida, kar ustreza 0,2278 mg benzidamina. En razpršek vsebuje 13,6 mg 96 odstotnega etanola, kar ustreza 12,728 mg 100 odstotnega etanola, in 0,17 mg metilparahidroksibenzoata (E218).

Terapevtske indikacije
Samozdravljenje: lajšanje bolečine in oteklin pri vnetju v ustni votlini in žrelu, ki so lahko posledica okužb in stanj po operaciji. Po nasvetu in navodilu zdravnika: lajšanje bolečine in oteklin v ustni votlini in žrelu, ki so posledica radiomukozitisa.

Odmerjanje in način uporabe
Uporaba 2- do 6-krat na dan (vsake 1,5 do 3 ure). Odrasli: 4 do 8 razprškov 2- do 6-krat na dan. Otroci od 6 do 12 let: 4 razprški 2- do 6-krat na dan. Otroci, mlajši od 6 let: 1 razpršek na 4 kg telesne mase; do največ 4 razprške 2 do 6-krat na dan.

Kontraindikacije
Znana preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov.

Posebna opozorila in previdnostni ukrepi
Pri manjšini bolnikov lahko resne bolezni povzročijo ustne/žrelne ulceracije. Če se simptomi v treh dneh ne izboljšajo, se mora bolnik posvetovati z zdravnikom ali zobozdravnikom, kot je primerno. Zdravilo vsebuje aspartam (E951) (vir fenilalanina), ki je lahko škodljiv za bolnike s fenilketonurijo. Zdravilo vsebuje izomalt (E953) (sinonim: izomaltitol (E953)). Bolniki z redko dedno intoleranco za fruktozo ne smejo jemati tega zdravila. Uporaba benzidamina ni priporočljiva za bolnike s preobčutljivostjo za salicilno kislino ali druga nesteroidna protivnetna zdravila. Pri bolnikih, ki imajo ali so imeli bronhialno astmo, lahko pride do bronhospazma. Pri takih bolnikih je potrebna previdnost.

Medsebojno delovanje z drugimi zdravili in druge oblike interakcij
Pri ljudeh raziskav o interakcijah niso opravljali.

Nosečnost in dojenje
Tantum Verde z okusom mentola 3 mg pastile se med nosečnostjo in dojenjem ne smejo uporabljati.

Vpliv na sposobnost vožnje in upravljanja s stroji
Uporaba benzidamina lokalno v priporočenem odmerku ne vpliva na sposobnost vožnje in upravljanja s stroji.

Neželeni učinki
Bolezni prebavil Redki: pekoč občutek v ustih, suha usta.
Bolezni imunskega sistema Redki: preobčutljivostna reakcija.
Bolezni dihal, prsnega koša in mediastinalnega prostora Zelo redki: laringospazem.
Bolezni kože in podkožja Občasni: fotosenzitivnost. Zelo redki: angioedem.

Rok uporabnosti
4 leta. Zdravila ne smete uporabljati po datumu izteka roka uporabnosti, ki je naveden na ovojnini. Posebna navodila za shranjevanje Za shranjevanje pastil niso potrebna posebna navodila. Plastenko z raztopino shranjujte v zunanji ovojnini za zagotovitev zaščite pred svetlobo. Shranjujte pri temperaturi do 25°C. Shranjujte v originalni ovojnini in nedosegljivo otrokom.

PRVA REGISTRIRANA TERAPIJA V 2. LINIJI ZA ZDRAVLJENJE ADENOKARCINOMA ŽELODCA ALI GASTRO-EZOFAGEALNEGA PREHODA[1]

CYRAMZA™
(ramucirumab)

UKREPAJTE ZDAJ

USPOSOBLJENI ZA SPREMEMBE, ZA NEPRIMERLJIVE IZKUŠNJE

Skrajšan povzetek glavnih značilnosti zdravila

▼ Za to zdravilo se izvaja dodatno spremljanje varnosti. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o katerem koli domnevnem neželenem učinku zdravila.

Cyramza 10 mg/ml koncentrat za raztopino za infundiranje

En mililiter koncentrata za raztopino za infundiranje vsebuje 10 mg ramucirumaba. Ena 10-mililitrska viala vsebuje 100 mg ramucirumaba. **Terapevtske indikacije** Zdravilo Cyramza je v kombinaciji s paklitakselom indicirano za zdravljenje odraslih bolnikov z napredovalim rakom želodca ali adenokarcinomom gastro-ezofagealnega prehoda z napredovalo boleznijo po predhodni kemoterapiji, ki je vključevala platino in fluoropirimidin. Monoterapija z zdravilom Cyramza je indicirana za zdravljenje odraslih bolnikov z napredovalim rakom želodca ali adenokarcinomom gastro-ezofagealnega prehoda z napredovalo boleznijo po predhodni kemoterapiji s platino ali fluoropirimidinom, za katere zdravljenje v kombinaciji s paklitakselom ni primerno. Zdravilo Cyramza je v kombinaciji s shemo FOLFIRI indicirano za zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom (mCRC), z napredovanjem bolezni ob ali po predhodnem zdravljenju z bevacizumabom, oksaliplatinom in fluoropirimidinom. Zdravilo Cyramza je v kombinaciji z docetakselom indicirano za zdravljenje odraslih bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim pljučnim rakom, z napredovanjem bolezni po kemoterapiji na osnovi platine. **Odmerjanje in način uporabe** Zdravljenje z ramucirumabom morajo uvesti in nadzirati zdravniki z izkušnjami v onkologiji. Odmerjanje *Rak želodca in adenokarcinom gastro-ezofagealnega prehoda* Priporočeni odmerek ramucirumaba je 8 mg/kg 1. in 15. dan 28-dnevnega cikla, pred infuzijo paklitaksela. Priporočeni odmerek paklitaksela je 80 mg/m^2 in se daje z intravenskim infundiranjem, ki traja približno 60 minut, 1., 8. in 15. dan 28-dnevnega cikla. Pred vsakim infundiranjem paklitaksela je treba pri bolnikih pregledati celotno krvno sliko in izvide kemičnih preiskav krvi, da se oceni delovanje jeter. Priporočeni odmerek ramucirumaba kot monoterapije je 8 mg/kg vsaka 2 tedna. *Kolorektalni rak* Priporočeni odmerek ramucirumaba je 8 mg/kg vsaka 2 tedna, dan z intravensko infuzijo pred dajanjem sheme FOLFIRI. Pred kemoterapijo je treba bolnikom odvzeti kri za popolno krvno sliko. *Nedrobnocelični pljučni rak (NSCLC)* Priporočeni odmerek ramucirumaba je 10 mg/kg na 1. dan 21-dnevnega cikla, pred infuzijo docetaksela. Priporočeni odmerek docetaksela je 75 mg/m^2, dan z intravensko infuzijo v približno 60 minutah na 1. dan 21-dnevnega cikla. *Premedikacija* Pred infundiranjem ramucirumaba je priporočljiva premedikacija z antagonistom histaminskih receptorjev H1. Način uporabe Po redčenju se zdravilo Cyramza daje kot intravenska infuzija v približno 60 minutah. Zdravila ne dajajte v obliki intravenskega bolusa ali hitre intravenske injekcije. Da boste dosegli zahtevano trajanje infundiranja približno 60 minut, največja hitrost infundiranja ne sme preseči 25 mg/minuto, saj morate sicer podaljšati trajanje infundiranja. Bolnika je med infundiranjem treba spremljati glede znakov reakcij, povezanih z infuzijo, zagotoviti pa je treba tudi razpoložljivost ustrezne opreme za oživljanje. **Kontraindikacije** Pri bolnikih z NSCLC je ramucirumab kontraindiciran, kjer gre za kavitacijo tumorja ali prepletenost tumorja z glavnimi žilami. **Posebna opozorila in previdnostni ukrepi** Trajno prekinite zdravljenje z ramucirumabom pri bolnikih, pri katerih se pojavijo resni arterijski trombembolični dogodki, gastrointestinalne perforacije, krvavitev stopnje 3 ali 4, če zdravstveno pomembne hipertenzije ni mogoče nadzirati z antihipertenzivnim zdravljenjem ali če se pojavi fistula, raven beljakovin v urinu > 3 g/24 ur ali v primeru nefrotskega sindroma. Pri bolnikih z neuravnano hipertenzijo zdravljenja z ramucirumabom ne smete uvesti, dokler oziroma v kolikor obstoječa hipertenzija ni uravnana. Pri bolnikih s ploščatocelično histologijo obstaja večje tveganje za razvoj resnih pljučnih krvavitev. Če se pri bolniku med zdravljenjem razvijejo zapleti v zvezi s celjenjem rane, prekinite zdravljenje z ramucirumabom, dokler rana ni povsem zaceljena. V primeru pojava stomatitisa je treba takoj uvesti simptomatsko zdravljenje. Pri bolnikih, ki so prejemali ramucirumab in docetaksel za zdravljenje napredovalega NSCLC z napredovanjem bolezni po kemoterapiji na osnovi platine, so opazili trend manjše učinkovitosti z naraščajočo starostjo. **Plodnost, nosečnost in dojenje** Ženskam v rodni dobi je treba svetovati, naj se izognejo zanositvi med zdravljenjem z zdravilom Cyramza in jih je treba seznaniti z možnim tveganjem za nosečnost in plod. Ni znano, ali se ramucirumab izloča v materino mleko. **Neželeni učinki** *Zelo pogosti (≥ 1/10)* nevtropenija, levkopenija, trombocitopenija, hipoalbuminemija, hipertenzija, epistaksa, gastrointestinalne krvavitve, stomatitis, driska, proteinurija, utrujenost/astenija, periferni edem, bolečina v trebuhu. *Pogosti (≥ 1/100 do < 1/10)* hipokaliemija, hiponatriemija, glavobol. **Rok uporabnosti** 3 leta **Posebna navodila za shranjevanje** Shranjujte v hladilniku (2 °C–8 °C). Ne zamrzujte. Vialo shranjujte v zunanji ovojnini, da zagotovite zaščito pred svetlobo. **Pakiranje** 2 viali z 10 ml **IMETNIK DOVOLJENJA ZA PROMET Z ZDRAVILOM** Eli Lilly Nederland B.V., Papendorpseweg 83, 3528 BJ Utrecht, Nizozemska **DATUM ZADNJE REVIZIJE BESEDILA** 25.01.2016

Režim izdaje: Predpisovanje in izdaja zdravila je le na recept, zdravilo pa se uporablja samo v bolnišnicah.

Pomembno obvestilo:

Pričujoče gradivo je namenjeno **samo za strokovno javnost**. Zdravilo Cyramza se izdaja le na recept. Pred predpisovanjem zdravila Cyramza vas vljudno prosimo, da preberete celotni Povzetek glavnih značilnosti zdravila Cyramza. Podrobnejše informacije o zdravilu Cyramza in o zadnji reviziji besedila Povzetka glavnih značilnosti zdravila so na voljo na sedežu podjetja Eli Lilly (naslov podjetja in kontaktni podatki spodaj) in na spletni strani European Medicines Agency (EMA): www.ema.europa.eu. in na spletni strani European Commission http://ec.europa.eu/health/documents/community-register/html/alfregister.htm.

Eli Lilly farmacevtska družba, d.o.o., Dunajska cesta 167, 1000 Ljubljana, telefon: (01) 5800 010, faks: (01) 5691 705

Referenca: 1. Cyramza, Povzetek glavnih značilnosti zdravila, zadnja odobrena verzija.

EERAM00010a, 12.02.2016.

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Individualizirano zdravljenje za bolnike z metastatskim kolorektalnim rakom

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Skrajšan povzetek glavnih značilnosti zdravila

Sestava: En ml raztopine za infundiranje vsebuje 5 mg cetuksimaba in pomožne snovi. Cetuksimab je himerno monoklonsko IgG_1 protitelo. **Terapevtske indikacije:** Zdravilo Erbitux je indicirano za zdravljenje bolnikov z metastatskim kolorektalnim rakom z ekspresijo receptorjev EGFR in nemutiranim tipom RAS v kombinaciji s kemoterapijo na osnovi irinotekana, kot primarno zdravljenje v kombinaciji s FOLFOX in kot samostojno zdravilo pri bolnikih, pri katerih zdravljenje z oksaliplatinom in zdravljenje na osnovi irinotekana ni bilo uspešno in pri bolnikih, ki ne prenašajo irinotekana. Zdravilo Erbitux je indicirano za zdravljenje bolnikov z rakom skvamoznih celic glave in vratu v kombinaciji z radioterapijo za lokalno napredovalo bolezen in v kombinaciji s kemoterapijo na osnovi platine za ponavljajočo se in/ali metastatsko bolezen. **Odmerjanje in način uporabe:** Zdravilo Erbitux pri vseh indikacijah infundirajte enkrat na teden. Pred prvo infuzijo mora bolnik prejeti premedikacijo z antihistaminikom in kortikosteroidom najmanj 1 uro pred uporabo cetuksimaba. Začetni odmerek je 400 mg cetuksimaba na m^2 telesne površine. Vsi naslednji tedenski odmerki so vsak po 250 mg/m^2. **Kontraindikacije:** Zdravilo Erbitux je kontraindicirano pri bolnikih z znano hudo preobčutljivostno reakcijo (3. ali 4. stopnje) na cetuksimab. Kombinacija zdravila Erbitux s kemoterapijo, ki vsebuje oksaliplatin, je kontraindicirana pri bolnikih z metastatskim kolorektalnim rakom z mutiranim tipom RAS ali kadar status RAS ni znan. **Posebna opozorila in previdnostni ukrepi:** Pojav hude reakcije, povezane z infundiranjem, zahteva takojšnjo in stalno ukinitev terapije s cetuksimabom. Če pri bolniku nastopi blaga ali zmerna reakcija, povezana z infundiranjem, lahko zmanjšate hitrost infundiranja. Priporočljivo je, da ostane hitrost infundiranja na nižji vrednosti tudi pri vseh naslednjih infuzijah. Če se pri bolniku pojavi kožna reakcija, ki je ne more prenašati, ali huda kožna reakcija (≥ 3. stopnje po kriterijih CTCAE), morate prekiniti terapijo s cetuksimabom. Z zdravljenjem smete nadaljevati le, če se je reakcija izboljšala do 2. stopnje. Če ugotovite intersticijsko bolezen pljuč, morate zdravljenje s cetuksimabom prekiniti, in bolnika ustrezno zdraviti. Zaradi možnosti pojava znižanja nivoja elektrolitov v serumu se pred in periodično med zdravljenjem s cetuksimabom priporoča določanje koncentracije elektrolitov v serumu. Pri bolnikih, ki prejemajo cetuksimab v kombinaciji s kemoterapijo na osnovi platine, obstaja večje tveganje za pojav hude nevtropenije. Takšne bolnike je potrebno skrbno nadzorovati. Pri predpisovanju cetuksimaba je treba upoštevati kardiovaskularno stanje in indeks zmogljivosti bolnika in sočasno dajanje kardiotoksičnih učinkovin kot so fluoropirimidini. Če je diagnoza ulcerativnega keratitisa potrjena, je treba zdravljenje s cetuksimabom prekiniti ali ukiniti. Cetuksimab je treba uporabljati previdno pri bolnikih z anamnezo keratitisa, ulcerativnega keratitisa ali zelo suhih oči. Cetuksimaba ne uporabljajte za zdravljenje bolnikov s kolorektalnim rakom, če imajo tumorje z mutacijo RAS ali pri katerih je tumorski status RAS neznan. **Interakcije:** Pri kombinaciji s fluoropirimidini se je v primerjavi z uporabo fluoropirimidinov, kot monoterapije, povečala pogostnost srčne ishemije, vključno z miokardnim infarktom in kongestivno srčno odpovedjo ter pogostnost sindroma dlani in stopal. V kombinaciji s kemoterapijo na osnovi platine se lahko poveča pogostnost hude levkopenije ali hude nevtropenije. V kombinaciji s kapecitabinom in oksaliplatinom (XELOX) se lahko poveča pogostnost hude driske. **Neželeni učinki:** Zelo pogosti (≥ 1/10): hipomagneziemija, povečanje ravni jetrnih encimov, kožne reakcije, blage ali zmerne reakcije povezane z infundiranjem, mukozitis, v nekaterih primerih resen. Pogosti (≥ 1/100 do < 1/10): dehidracija, hipokalciemija, anoreksija, glavobol, konjunktivitis, driska, navzeja, bruhanje, hude reakcije povezane z infundiranjem, utrujenost. **Posebna navodila za shranjevanje:** Shranjujte v hladilniku (2 °C - 8 °C). **Pakiranje:** 1 viala z 20 ml ali 100 ml raztopine. **Način in režim izdaje:** Izdaja zdravila je le na recept-H. **Imetnik dovoljenja za promet:** Merck KGaA, 64271 Darmstadt, Nemčija.

Datum zadnje revizije besedila: november 2014.

Pred predpisovanjem zdravila natančno preberite celoten Povzetek glavnih značilnosti zdravila.

Samo za strokovno javnost.

Podrobnejše informacije so na voljo pri predstavniku imetnika dovoljenja za promet z zdravilom:
Merck d.o.o., Ameriška ulica 8, 1000 Ljubljana, tel.: 01 560 3810, faks: 01 560 3830, el. pošta: info@merck.si
www.merckserono.net
www.Erbitux-international.com

PM-ONC-05/2015 /13.10.2015

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Prva linija zdravljenja lokalno napredovalega ali metastatskega nedrobnoceličnega raka pljuč z EGFR-aktivirajočimi mutacijami[1]

ČAS ZA ŽIVLJENJE

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SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Ime zdravila: Tarceva 25 mg/100 mg/150 mg filmsko obložene tablete. **Kakovostna in količinska sestava:** Ena filmsko obložena tableta vsebuje 25 mg, 100 mg ali 150 mg erlotiniba (v obliki erlotinibijevega klorida). **Terapevtske indikacije:** Nedrobnocelični rak pljuč: Zdravilo Tarceva je indicirano za prvo linijo zdravljenja bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč z EGFR-aktivirajočimi mutacijami. Zdravilo Tarceva je indicirano tudi za vzdrževalno zdravljenje bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč z EGFR-aktivirajočimi mutacijami in stabilno boleznijo po kemoterapiji v prvi liniji zdravljenja. Zdravilo Tarceva je indicirano tudi za zdravljenje bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč po neuspehu vsaj ene predhodne kemoterapije. Pri predpisovanju zdravila Tarceva je treba upoštevati dejavnike, povezane s podaljšanim preživetjem. Koristnega vpliva na podaljšanje preživetja ali drugih klinično pomembnih učinkov zdravljenja niso dokazali pri bolnikih z EGFR-negativnimi tumorji (glede na rezultat imunohistokemije). Rak trebušne slinavke: Zdravilo Tarceva je v kombinaciji z gemcitabinom indicirano za zdravljenje bolnikov z metastatskim rakom trebušne slinavke. Pri predpisovanju zdravila Tarceva je treba upoštevati dejavnike, povezane s podaljšanim preživetjem. Koristnega vpliva na podaljšanje preživetja niso dokazali za bolnike z lokalno napredovalo boleznijo. **Odmerjanje in način uporabe:** Zdravljenje z zdravilom Tarceva mora nadzorovati zdravnik z izkušnjami pri zdravljenju raka. Pri bolnikih z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč, ki še niso prejeli kemoterapije, je treba testiranje za določanje mutacij EGFR opraviti pred začetkom zdravljenja z zdravilom Tarceva. Zdravilo Tarceva vzamemo najmanj eno uro pred zaužitjem hrane ali dve uri po tem. Kadar je potrebno odmerek prilagoditi, ga je treba zmanjševati v korakih po 50 mg. Pri sočasnem jemanju substratov in modulatorjev CYP3A4 bo morda potrebna prilagoditev odmerka. Pri dajanju zdravila Tarceva bolnikom z jetrno okvaro je potrebna previdnost. Če se pojavijo hudi neželeni učinki, pride v poštev zmanjšanje odmerka ali prekinitev zdravljenja z zdravilom Tarceva. Uporaba zdravila Tarceva pri bolnikih s hudo jetrno ali ledvično okvaro ter pri otrocih ni priporočljiva. Bolnikom kadilcem je treba svetovati, naj prenehajo kaditi, saj so plazemske koncentracije erlotiniba pri kadilcih manjše kot pri nekadilcih. Nedrobnocelični rak pljuč: Priporočeni dnevni odmerek zdravila Tarceva je 150 mg. Rak trebušne slinavke: Priporočeni dnevni odmerek zdravila Tarceva je 100 mg, v kombinaciji z gemcitabinom. Pri bolnikih, pri katerih se kožni izpuščaj v prvih 4 do 8 tednih zdravljenja ne pojavi, je treba ponovno pretehtati nadaljnje zdravljenje z zdravilom Tarceva. **Kontraindikacije:** Preobčutljivost na erlotinib ali katero koli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** Pri določanju bolnikovega statusa mutacij EGFR je pomembno izbrati dobro validirano in robustno metodologijo, da se izognemo lažno negativnim ali lažno pozitivnim rezultatom. *Kadilci:* Bolnikom, ki kadijo, je treba svetovati, naj prenehajo kaditi, saj so plazemske koncentracije erlotiniba pri kadilcih zmanjšane v primerjavi s plazemskimi koncentracijami pri nekadilcih. Verjetno je, da je velikost zmanjšanja klinično pomembna. *Intersticijska bolezen pljuč:* Pri bolnikih, pri katerih se akutno pojavijo novi in/ali poslabšajo nepojasnjeni pljučni simptomi, kot so dispneja, kašelj in vročina, je treba zdravljenje z zdravilom Tarceva prekiniti, dokler ni znana diagnoza. Bolnike, ki se sočasno zdravijo z erlotinibom in gemcitabinom, je treba skrbno spremljati zaradi možnosti pojava toksičnosti, podobni intersticijski bolezni pljuč. Če je ugotovljena intersticijska bolezen pljuč, zdravilo Tarceva ukinemo in uvedemo ustrezno zdravljenje. *Driska, dehidracija, neravnovesje elektrolitov in ledvična odpoved:* Pri približno polovici bolnikov, ki so se zdravili z zdravilom Tarceva, se je pojavila driska (vključno z zelo redkimi primeri, ki so se končali s smrtnim izidom). Zmerno do hudo drisko zdravimo z loperamidom. V nekaterih primerih bo morda potrebno zmanjšanje odmerka. V primeru hude ali dolgotrajne driske, navzeje, anoreksije ali bruhanja, povezanih z dehidracijo, je treba zdravljenje z zdravilom Tarceva prekiniti in dehidracijo ustrezno zdraviti. O hipokaliemiji in ledvični odpovedi so poročali redko. Posebno pri bolnikih z dejavniki tveganja (zlasti sočasnim jemanjem kemoterapevtikov in drugih zdravil, simptomi ali boleznimi ali drugimi dejavniki, vključno z visoko starostjo) moramo, če je driska huda ali dolgotrajna oziroma vodi v dehidracijo, zdravljenje z zdravilom Tarceva prekiniti in bolnikom zagotoviti intenzivno intravensko rehidracijo. Dodatno je treba pri bolnikih s prisotnim tveganjem za razvoj dehidracije spremljati ledvično delovanje in serumske elektrolite, vključno s kalijem. *Hepatitis, jetrna odpoved:* Pri uporabi zdravila Tarceva so poročali o redkih primerih jetrne odpovedi (vključno s smrtnimi). K njenemu nastanku je lahko pripomogla predhodno obstoječa jetrna bolezen ali sočasno jemanje hepatotoksičnih zdravil. Pri teh bolnikih je treba zato premisliti o rednem spremljanju jetrnega delovanja. Dajanje zdravila Tarceva je treba prekiniti, če so spremembe jetrnega delovanja hude. *Perforacije v prebavilih:* Bolniki, ki prejemajo zdravilo Tarceva, imajo večje tveganje za razvoj perforacij v prebavilih, ki so jih opazili občasno (vključno z nekaterimi primeri, ki so se končali s smrtnim izidom). Pri bolnikih, ki sočasno prejemajo zdravila, ki zavirajo angiogenezo, kortikosteroide, nesteroidna protivnetna zdravila (NSAID) in/ali kemoterapijo na osnovi taksanov, ali so v preteklosti imeli peptični ulkus ali divertikularno bolezen, je tveganje večje. Če pride do tega, je treba zdravljenje z zdravilom Tarceva dokončno ukiniti. *Kožne bolezni, pri katerih so prisotni mehurji in luščenje kože:* Poročali so o primerih kožnih bolezni z mehurji in luščenjem kože, vključno z zelo redkimi primeri, ki so nakazovali na Stevens-Johnsonov sindrom/toksično epidermalno nekrolizo in so bili v nekaterih primerih smrtni. Zdravljenje z zdravilom Tarceva je treba prekiniti ali ukiniti, če se pri bolniku pojavijo hude oblike mehurjev ali luščenja kože. Pri bolnikih s kožnimi boleznimi z mehurji in luščenjem kože je treba preveriti prisotnost okužbe kože in jih zdraviti v skladu z lokalnimi smernicami. *Očesne bolezni:* Bolniki, pri katerih se pojavijo znaki in simptomi, ki nakazujejo na keratitis in so lahko akutni ali se poslabšujejo: vnetje očesa, solzenje, občutljivost na svetlobo, zamegljen vid, bolečine v očesu in/ali rdeče oči, se morajo takoj obrniti na specialista oftalmologije. V primeru, da je diagnoza ulcerativnega keratitisa potrjena, je treba zdravljenje z zdravilom Tarceva prekiniti ali ukiniti. V primeru, da se postavi diagnoza keratitisa, je treba skrbno razmisliti o koristih in tveganjih nadaljnega zdravljenja. Zdravilo Tarceva je treba pri bolnikih, ki so v preteklosti imeli keratitis, ulcerativni keratitis ali zelo suhe oči, uporabljati previdno. Uporaba kontaktnih leč je prav tako dejavnik tveganja za keratitits in ulceracijo. Med uporabo zdravila Tarceva so zelo redko poročali o primerih perforacije ali ulceracije roženice. *Medsebojno delovanje z drugimi zdravili:* Močni induktorji CYP3A4 lahko zmanjšajo učinkovitost erlotiniba, močni zaviralci CYP3A4 pa lahko povečajo toksičnost. Sočasnemu zdravljenju s temi zdravili se je treba izogibati. Tablete vsebujejo laktozo in jih ne smemo dajati bolnikom z redkimi dednimi stanji: intoleranco za galaktozo, laponsko obliko zmanjšane aktivnosti laktaze ali malabsorpcijo glukoze/galaktoze. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Erlotinib se pri ljudeh presnavlja v jetrih z jetrnimi citokromi, primarno s CYP3A4 in v manjši meri s CYP1A2. Presnova erlotiniba zunaj jeter poteka s CYP3A4 v črevesju, CYP1A1 v pljučih in CYP1B1 v tumorskih tkivih. Z zdravilnimi učinkovinami, ki se presnavljajo s temi encimi, jih zavirajo ali pa so njihovi induktorji, lahko pride do interakcij. Erlotinib je srednje močan zaviralec CYP3A4 in CYP2C8, kot tudi močan zaviralec glukuronidacije z UGT1A1 *in vitro*. Pri kombinaciji ciprofloksacina ali močnega *zaviralca CYP1A2* (npr. fluvoksamina) z erlotinibom je potrebna previdnost. V primeru pojava neželenih učinkov, povezanih z erlotinibom, lahko odmerek erlotiniba zmanjšamo. Predhodno ali sočasno zdravljenje z zdravilom Tarceva ni spremenilo očistka prototipov *substratov CYP3A4*, midazolama in eritromicina. Inhibicija glukoronidacije lahko povzroči interakcije z zdravili, ki so *substrati UGT1A1* in se izločajo samo po tej poti. Močni *zaviralci aktivnosti CYP3A4* zmanjšajo presnovo erlotiniba in zvečajo koncentracije erlotiniba v plazmi. Pri sočasnem jemanju erlotiniba in močnih zaviralcev CYP3A4 je zato potrebna previdnost. Če je treba, odmerek erlotiniba zmanjšamo, še posebno pri pojavu toksičnosti. Močni *induktorji aktivnosti CYP3A4* zvečajo presnovo erlotiniba in pomembno zmanjšajo plazemske koncentracije erlotiniba. Sočasnemu dajanju zdravila Tarceva in induktorjev CYP3A4 se je treba izogibati. Pri bolnikih, ki potrebujejo sočasno zdravljenje z zdravilom Tarceva in močnim induktorjem CYP3A4, je treba premisliti o povečanju odmerka do 300 mg ob skrbnem spremljanju njihove varnosti. Zmanjšana izpostavljenost se lahko pojavi tudi z drugimi induktorji, kot so fenitoin, karbamazepin, barbiturati ali šentjanževka. Če te zdravilne učinkovine kombiniramo z erlotinibom, je potrebna previdnost. Kadar je mogoče, je treba razmisliti o drugih načinih zdravljenja, ki ne vključujejo močnega spodbujanja aktivnosti CYP3A4. Bolnikom, ki jemljejo *kumarinske antikoagulante*, je treba redno kontrolirati protrombinski čas ali INR. Sočasno zdravljenje z zdravilom Tarceva in *statinom* lahko poveča tveganje za miopatijo, povzročeno s statini, vključno z rabdomiolizo; to so opazili redko. Sočasna uporaba *zaviralcev P-glikoproteina*, kot sta ciklosporin in verapamil, lahko vodi v spremenjeno porazdelitev in/ali spremenjeno izločanje erlotiniba. Za erlotinib je značilno zmanjšanje topnosti pri pH nad 5. *Zdravila, ki spremenijo pH v zgornjem delu prebavil,* lahko spremenijo topnost erlotiniba in posledično njegovo biološko uporabnost. Učinka antacidov na absorpcijo erlotiniba niso proučevali, vendar je ta lahko zmanjšana, kar vodi v nižje plazemske koncentracije. Kombinaciji erlotiniba in zaviralca protonske črpalke se je treba izogibati. Če menimo, da je uporaba antacidov med zdravljenjem z zdravilom Tarceva potrebna, jih je treba jemati najmanj 4 ure pred ali 2 uri po dnevnem odmerku zdravila Tarceva. Če razmišljamo o uporabi ranitidina, moramo zdravili jemati ločeno: zdravilo Tarceva je treba vzeti najmanj 2 uri pred ali 10 ur po odmerku ranitidina. V študiji faze Ib ni bilo pomembnih učinkov *gemcitabina* na farmakokinetiko erlotiniba, prav tako ni bilo pomembnih učinkov erlotiniba na farmakokinetiko gemcitabina. Erlotinib poveča koncentracijo platine. Pomembnih učinkov *karboplatina* ali paklitaksela na farmakokinetiko erlotiniba ni bilo. *Kapecitabin* lahko poveča koncentracijo erlotiniba. Pomembnih učinkov erlotiniba na farmakokinetiko kapecitabina ni bilo. Zaradi mehanizma delovanja lahko od *zaviralcev proteasomov*, vključno z bortezomibom, pričakujemo, da vplivajo na učinek zaviralcev EGFR, vključno z erlotinibom. **Neželeni učinki:** *Zelo pogosti neželeni učinki* so kožni izpuščaj in driska, kot tudi utrujenost, anoreksija, dispneja, kašelj, okužba, navzea, bruhanje, stomatitis, bolečina v trebuhu, pruritus, suha koža, suhi keratokonjunktivitis, konjunktivitis, zmanjšanje telesne mase, depresija, glavobol, nevropatija, dispepsija, flatulenca, alopecija, okorelost, pireksija, nenormalnosti testov jetrne funkcije. *Pogosti neželeni učinki* so krvavitve v prebavilih, epistaksa, resna intersticijska bolezen pljuč, keratitis, paronihija, folikulitis, akne/akneiformni dermatitis, fisure na koži in ledvična insuficienca. *Občasno* so poročali o perforacijah v prebavilih, hirzutizmu, spremembah obrvi, krhkih nohtih, odstopanju nohtov od kože, blagih reakcijah na koži (npr. hiperpigmentaciji), spremembah trepalnic, nefritisu in proteinuriji. *Redko* pa so poročali o jetrni odpovedi in sindromu palmarno-plantarne eritrodisestezije. *Zelo redko* so poročali o Stevens-Johnsonovem sindromu/toksični epidermalni nekrolizi ter o ulceracijah in perforacijah roženice. Poročanje o domnevnih neželenih učinkih: Poročanje o domnevnih neželenih učinkih zdravila po izdaji dovoljenja za promet je pomembno. Omogoča namreč stalno spremljanje razmerja med koristmi in tveganji zdravila. Od zdravstvenih delavcev se zahteva, da poročajo o katerem koli domnevnem neželenem učinku zdravila na: Univerzitetni klinični center Ljubljana, Interna klinika, Center za zastrupitve, Zaloška cesta 2, SI-1000 Ljubljana, Faks: + 386 (0)1 434 76 46, e-pošta: farmakovigilanca@kclj.si. **Režim izdaje zdravila:** H/Rp. **Imetnik dovoljenja za promet:** Roche Registration Limited, 6 Falcon Way, Shire Park, Welwyn Garden City, AL7 1TW, Velika Britanija. **Verzija:** 1.0/16. **Informacija pripravljena:** februar 2016.
Samo za strokovno javnost.

PG-03-0216-TAR-TG

[1]Povzetek glavnih značilnosti zdravila Tarceva. Dostopano februar 2016 na:
http://www.ema.europa.eu/docs/sl_SI/document_library/EPAR_-_Product_Information/human/000618/WC500033994.pdf

DODATNE INFORMACIJE SO NA VOLJO PRI: Roche farmacevtska družba d.o.o., Vodovodna cesta 109, 1000 Ljubljana.

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The Editorial Board requires that the paper has not been published or submitted for publication elsewhere; the authors are responsible for all statements in their papers. Accepted articles become the property of the journal and, therefore cannot be published elsewhere without the written permission of the editors.

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Preparation of manuscripts

Radiology and Oncology will consider manuscripts prepared according to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals by International Committee of Medical Journal Editors (www.icmje.org). The manuscript should be written in grammatically and stylistically correct language. Abbreviations should be avoided. If their use is necessary, they should be explained at the first time mentioned. The technical data should conform to the SI system. The manuscript, excluding the references, tables, figures and figure legends, must not exceed 5000 words, and the number of figures and tables is limited to 8. Organize the text so that it includes: Introduction, Materials and methods, Results and Discussion. Exceptionally, the results and discussion can be combined in a single section. Start each section on a new page, and number each page consecutively with Arabic numerals.

The Title page should include a concise and informative title, followed by the full name(s) of the author(s); the institutional affiliation of each author; the name and address of the corresponding author (including telephone, fax and E-mail), and an abbreviated title (not exceeding 60 characters). This should be followed by the abstract page, summarizing in less than 250 words the reasons for the study, experimental approach, the major findings (with specific data if possible), and the principal conclusions, and providing 3-6 key words for indexing purposes. Structured abstracts are preferred. Slovene authors are requested to provide title and the abstract in Slovene language in a separate file. The text of the research article should then proceed as follows:

Introduction should summarize the rationale for the study or observation, citing only the essential references and stating the aim of the study.

Materials and methods should provide enough information to enable experiments to be repeated. New methods should be described in details.

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Discussion should explain the results rather than simply repeating them and interpret their significance and draw conclusions. It should discuss the results of the study in the light of previously published work.

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References must be numbered in the order in which they appear in the text and their corresponding numbers quoted in the text. Authors are responsible for the accuracy of their references. References to the Abstracts and Letters to the Editor must be identified as such. Citation of papers in preparation or submitted for publication, unpublished observations, and personal communications should not be included in the reference list. If essential, such material may be incorporated in the appropriate place in the text. References follow the style of Index Medicus. All authors should be listed when their number does not exceed six; when there are seven or more authors, the first six listed are followed by "et al.". The following are some examples of references from articles, books and book chapters:

Dent RAG, Cole P. In vitro maturation of monocytes in squamous carcinoma of the lung. *Br J Cancer* 1981; **43:** 486-95.

Chapman S, Nakielny R. *A guide to radiological procedures*. London: Bailliere Tindall; 1986.

Evans R, Alexander P. Mechanisms of extracellular killing of nucleated mammalian cells by

macrophages. In: Nelson DS, editor. *Immunobiology of macrophage.* New York: Academic Press; 1976. p. 45-74.

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The research using animal subjects should be conducted according to the EU Directive 2010/63/EU and following the Guidelines for the welfare and use of animals in cancer research (Br J Cancer 2010; 102: 1555 – 77). Authors must state the committee approving the experiments, and must confirm that all experiments were performed in accordance with relevant regulations.

These statements should appear in the Materials and methods section (or for contributions without this section, within the main text or in the captions of relevant figures or tables).

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BISTVENI PODATKI IZ POVZETKA GLAVNIH ZNAČILNOSTI ZDRAVILA

SUTENT 12,5 mg, 25 mg, 37,5 mg, 50 mg trde kapsule
Sestava in oblika zdravila: Ena kapsula vsebuje 12,5 mg, 25 mg, 37,5 mg ali 50 mg sunitiniba (v obliki sunitinibijevega malata). **Indikacije:** Zdravljenje neizrezljivega in/ali metastatskega malignega gastrointestinalnega stromalnega tumorja (GIST) pri odraslih, če zdravljenje z imatinibom zaradi odpornosti ali neprenašanja ni bilo uspešno. Zdravljenje napredovalega/metastatskega karcinoma ledvičnih celic (MRCC) pri odraslih. Zdravljenje neizrezljivih ali metastatskih, dobro diferenciranih nevroendokrinih tumorjev trebušne slinavke (pNET), kadar gre za napredovanje bolezni pri odraslih (izkušnje z zdravilom Sutent kot zdravilom prve izbire so omejene). **Odmerjanje in način uporabe:** Terapijo mora uvesti zdravnik, ki ima izkušnje z uporabo zdravil za zdravljenje rakavih bolezni. *GIST in MRCC:* Priporočeni odmerek je 50 mg peroralno enkrat na dan, 4 tedne zapored; temu sledi 2-tedenski premor (Shema 4/2), tako da celotni ciklus traja 6 tednov. *pNET:* Priporočeni odmerek je 37,5 mg peroralno enkrat na dan, brez načrtovanega premora. *Prilagajanje odmerka*: Odmerek je mogoče prilagajati v povečanjih po 12,5 mg, upoštevaje individualno varnost in prenašanje. Pri GIST in MRCC dnevni odmerek ne sme preseči 75 mg in ne sme biti manjši od 25 mg; pri pNET je največji odmerek 50 mg na dan, z možnimi prekinitvami zdravljenja. Pri sočasni uporabi z močnimi zaviralci ali induktorji CYP3A4 je treba odmerek ustrezno prilagoditi. *Pediatrična populacija:* Uporaba sunitiniba ni priporočljiva. *Starejši bolniki (≥ 65 let):* Med starejšimi in mlajšimi bolniki niso opazili pomembnih razlik v varnosti in učinkovitosti. *Okvara jeter:* Pri bolnikih z jetrno okvaro razreda A in B po Child-Pughu prilagoditev odmerka ni potrebna; pri bolnikih z okvaro razreda C sunitinib ni bil preizkušen, zato njegova uporaba ni priporočljiva. *Okvara ledvic:* Prilagajanje začetnega odmerka ni potrebno, nadaljnje prilagajanje odmerka naj temelji na varnosti in prenašanju pri posameznem bolniku. *Način uporabe:* Zdravilo Sutent se uporablja peroralno, bolnik ga lahko vzame s hrano ali brez nje. Če pozabi vzeti odmerek, ne sme dobiti dodatnega, temveč naj vzame običajni predpisani odmerek naslednji dan. **Kontraindikacije:** Preobčutljivost na zdravilno učinkovino ali katerokoli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** *Bolezni kože in tkiv:* obarvanje kože, gangrenozna pioderma (običajno izgine po prekinitvi zdravljenja), hude kožne reakcije (multiformni eritem (EM), Stevens-Johnsonov sindrom (SJS) in toksična epidermalna nekroliza (TEN)). Če so prisotni znaki EM, SJS ali TEN, je treba zdravljenje prekiniti. *Krvavitve* v prebavilih, dihalih, sečilih, možganih; najpogosteje epistaksa; krvavitve tumorja, včasih s smrtnim izidom. Pri bolnikih, ki se sočasno zdravijo z antikoagulanti, se lahko redno spremlja celotna krvna slika (trombociti), koagulacijski faktorji (PT / INR) in opravi telesni pregled. *Bolezni prebavil:* poleg diareje, navzee/bruhanja, bolečine v trebuhu, dispepsije, stomatitisa/bolečine v ustih in ezofagitisa tudi hudi zapleti (včasih s smrtnim izidom), vključno z gastrointestinalno perforacijo. *Hipertenzija:* pri bolnikih s hudo hipertenzijo, ki je ni mogoče urediti z zdravili, je priporočljivo začasno prenehanje zdravljenja. *Hematološke bolezni:* zmanjšanje števila nevtrofilcev, trombocitov, anemija. *Bolezni srca in ožilja:* srčno-žilni dogodki, vključno s srčnim popuščanjem, kardiomiopatijo, miokardno ishemijo in miokardnim infarktom, v nekaterih primerih s smrtnim izidom; sunitinib povečuje tveganje za pojav kardiomiopatije; previdna uporaba pri bolnikih s tveganjem za te dogodke, ali ki so te dogodke imeli v preteklosti. *Podaljšanje intervala QT:* previdna uporaba pri bolnikih z znano anamnezo podaljšanja intervala QT, tistih, ki jemljejo antiaritmike ali zdravila, ki lahko podaljšajo interval QT, in tistih z relevantno, že obstoječo srčno boleznijo, bradikardijo ali elektrolitskimi motnjami. *Venski in arterijski trombembolični dogodki*; arterijski včasih s smrtnim izidom. *Trombotična mikroangiopatija (TMA)*: TMA, vključno s trombotično trombocitopenično purpuro in hemolitično-uremičnim sindromom, v nekaterih primerih z odpovedjo ledvic ali smrtnim izidom. *Dogodki na dihalih:* dispneja, plevralni izliv, pljučna embolija ali pljučni edem; redki primeri s smrtnim izidom. *Moteno delovanje ščitnice:* bolnike je treba med zdravljenjem rutinsko spremljati glede delovanja ščitnice vsake 3 mesece. *Pankreatitis*, tudi resni primeri s smrtnim izidom. *Hepatotoksičnost*, nekateri primeri s smrtnim izidom. *Holecistitis*, vključno z akalkuloznim in emfizemskim holecistitisom. *Delovanje ledvic:* primeri zmanjšanega delovanja ledvic, odpovedi ledvic in/ali akutne odpovedi ledvic, v nekaterih primerih s smrtnim izidom. *Fistula:* če nastane fistula, je treba zdravljenje s sunitinibom prekiniti. *Oteženo celjenje ran:* pri bolnikih, pri katerih naj bi bil opravljen večji kirurški poseg, je priporočljiva začasna prekinitev zdravljenja s sunitinibom. *Osteonekroza čeljustnic:* pri sočasnem ali zaporednem dajanju zdravila Sutent in intravenskih bisfosfonatov je potrebna previdnost; invazivni zobozdravstveni posegi predstavljajo dodatni dejavnik tveganja. *Preobčutljivost/angioedem*. *Motnje okušanja*. *Konvulzije:* obstajajo poročila, nekatera s smrtnim izidom, o preiskovancih s konvulzijami in radiološkimi znaki sindroma reverzibilne posteriorne levkoencefalopatije. *Sindrom lize tumorja*, v nekaterih primerih s smrtnim izidom. *Okužbe:* hude okužbe z ali brez nevtropenije (okužbe dihal, sečil, kože in sepsa), vključno z nekaterimi s smrtnim izidom; redki primeri nekrotizitajočega fasciitisa, vključno s prizadetostjo presredka, ki so bili včasih smrtni. *Hipoglikemija*: če se pojavi simptomatska hipoglikemija, je treba zdravljenje s sunitinibom začasno prekiniti. Pri sladkornih bolnikih je treba redno preverjati raven glukoze v krvi in, če je treba, prilagoditi odmerek antidiabetika. **Medsebojno delovanje z drugimi zdravili:** (Študije so izvedli le pri odraslih.) Zdravila, ki lahko zvečajo koncentracijo sunitiniba v plazmi (ketokonazol, ritonavir, itrakonazol, eritromicin, klaritromicin ali sok grenivke). Zdravila, ki lahko zmanjšajo koncentracijo sunitiniba v plazmi (deksametazon, fenitoin, karbamazepin, rifampin, fenobarbital, *Hypericum perforatum* oz. šentjanževka). **Plodnost, nosečnost in dojenje:** Zdravila Sutent ne smemo uporabljati med nosečnostjo in tudi ne pri ženskah, ki ne uporabljajo ustrezne kontracepcije, razen če možna korist odtehta možno tveganje za plod. Ženske v rodni dobi naj med zdravljenjem z zdravilom Sutent ne zanosijo. Ženske, ki jemljejo zdravilo Sutent, ne smejo dojiti. Neklinični izsledki kažejo, da lahko zdravljenje s sunitinibom poslabša plodnost samcev in samic. **Vpliv na sposobnost vožnje in upravljanja s stroji:** Sutent lahko povzroči omotico. **Neželeni učinki:** Najbolj resni neželeni učinki (nekateri s smrtnim izidom) so: odpoved ledvic, srčno popuščanje, pljučna embolija, gastrointestinalna perforacija in krvavitve (npr. v dihalih, prebavilih, tumorju, sečilih in možganih). Najpogostejši neželeni učinki (ki so se pojavili pri vsaj 20 % bolnikov v registracijskih preskušanjih) so: zmanjšan tek, motnje okušanja, hipertenzija, utrujenost, prebavne motnje (npr. driska, navzea, stomatitis, dispepsija in bruhanje), sprememba barve kože in sindrom palmarno-plantarne eritrodisestezije. Med najbolj pogostimi neželenimi učinki so tudi hematološke motnje (nevtropenija, trombocitopenija, anemija in levkopenija). Ostali zelo pogosti (≥ 1/10) neželeni učinki so: hipotiroidizem, nespečnost, omotica, glavobol, dispneja, epistaksa, kašelj, bolečina v trebuhu, zaprtje, obarvanje kože, izpuščaj, spremembe barve las, suha koža, bolečine v udih, artralgija, bolečine v hrbtu, vnetje sluznice, edem, pireksija. **Način in režim izdaje:** Predpisovanje in izdaja zdravila je le na recept, zdravilo pa se uporablja samo v bolnišnicah. Izjemoma se lahko uporablja pri nadaljevanju zdravljenja na domu ob odpustu iz bolnišnice in nadaljnjem zdravljenju. **Imetnik dovoljenja za promet:** Pfizer Limited, Ramsgate Road, Sandwich, Kent, CT13 9NJ, Velika Britanija. **Datum zadnje revizije besedila:** 25.06.2015
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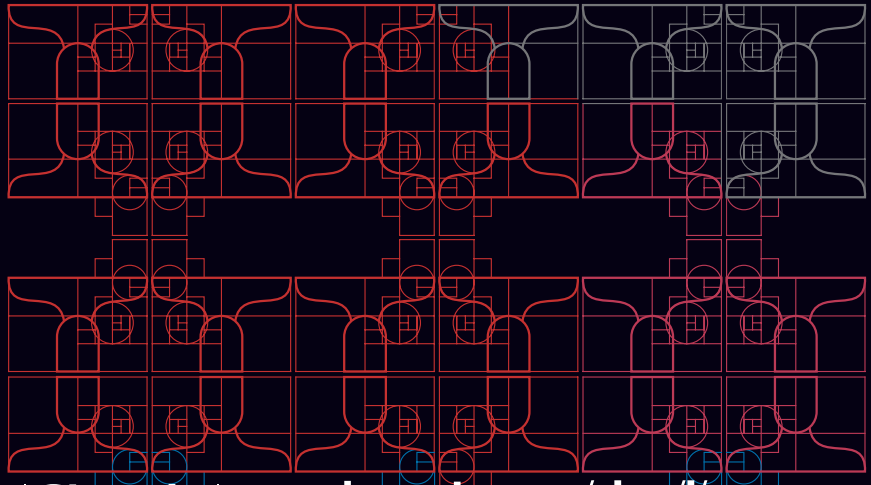

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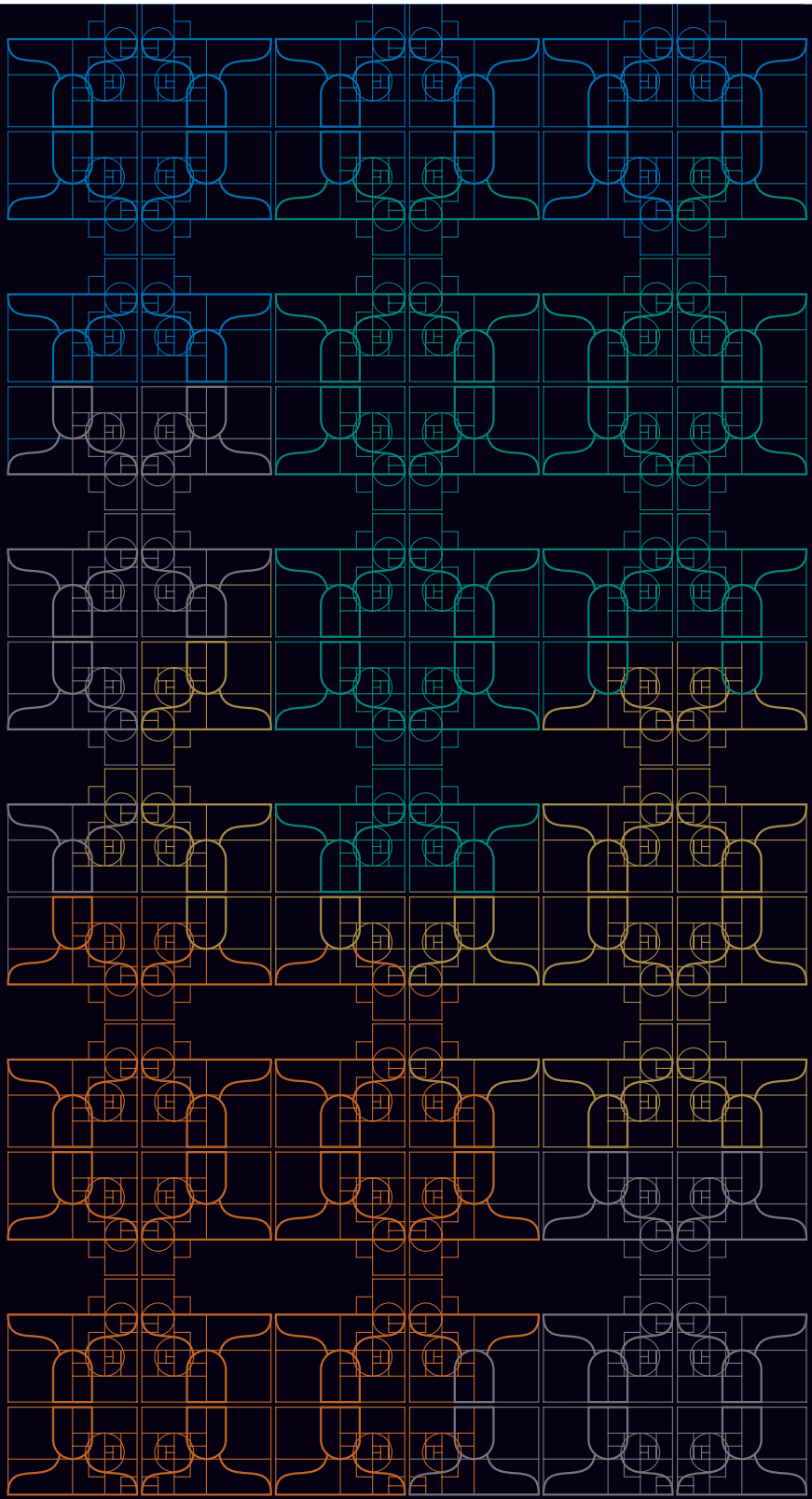

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