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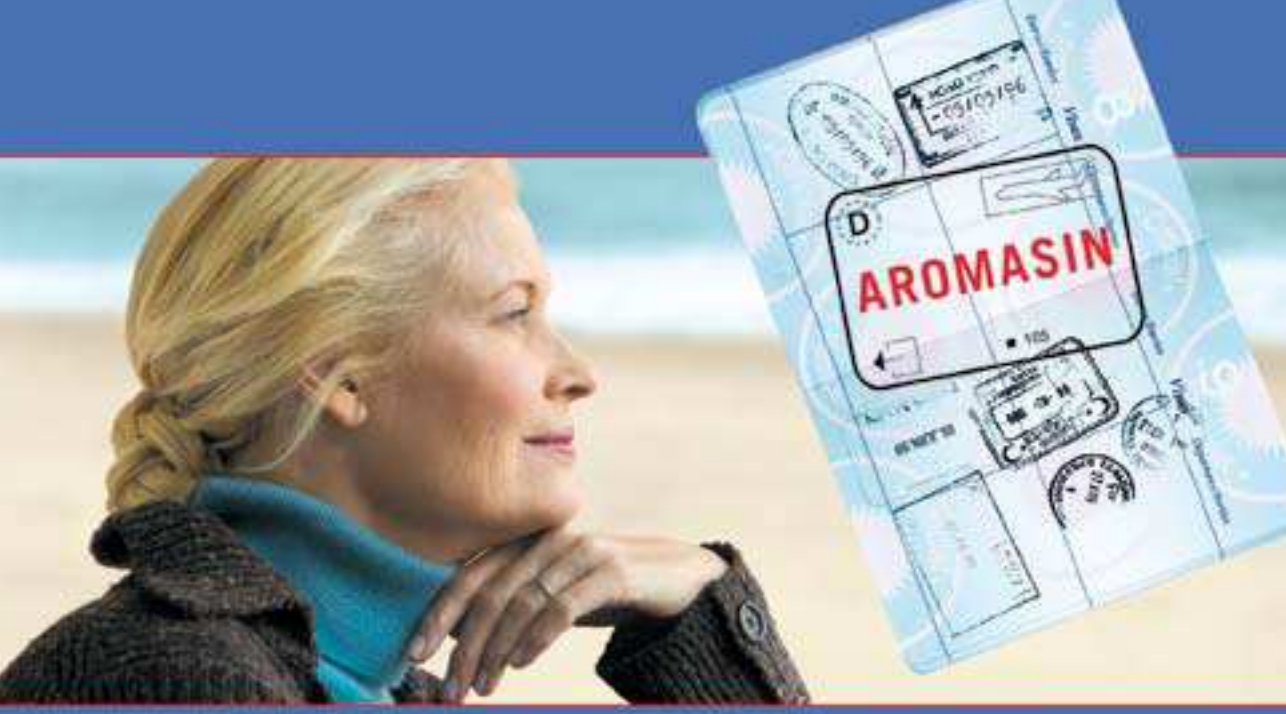

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review

Targeted gene therapy in radiotherapy

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The dramatic pace in development of gene therapy over the past decades has made it a realistic alternative for the treatment of cancer. Radiotherapy, on the other hand, is one of the most commonly used and well established cancer treatment modalities. The latest improvements in the physical targeting ability of radiotherapy and understanding of the molecular mechanism involved in the cellular response to ionizing radiation have presented an opportunity to combine radiotherapy with gene therapy. This review article will focus on gene therapy strategies that can be used to enhance the effectiveness of radiotherapy, with an emphasis on transcriptional targeting approaches.

Key words: gene therapy; radiotherapy; transcriptional targeting

Developments in radiotherapy

Radiotherapy is the use of ionizing radiation in the treatment of malignant tumors. It is one of the main treatment modalities for many forms of cancer, with more than half of all cancer patients receiving radiation therapy at some point in their treatment.[1]

Biological and technological advances have brought notable improvements in radiotherapy over the years. Biologically based advances include improvements in fractionation schedules, treatment planning and combining radiotherapy with other treatment modalities such as surgical tumor debulking, chemotherapy and, most recently, gene therapy.[1]

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Recent progress in diagnostic imaging, such as computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) and molecular imaging techniques, have led to the development of conformal (CRT) and intensity modulated radiotherapy (IMRT), which is the most advanced form of conformal radiotherapy. CRT and IMRT have enabled more precise dose delivery, conforming closely to the shape of the tumor and thus improving the therapeutic index;[2] namely better local tumor control without compromising normal tissue. Although higher doses of radiation can produce better tumor control, the dose is limited by the possibility of normal tissue damage surrounding the tumor *i.e.* in the irradiation field.

Despite a marked progression in the efficacy of radiotherapy, there is still a need for improvement of this treatment modality. Because radiotherapy is a local treatment, tumor cells outside the immediate field of radiation and those that have metastasized

Table 1. Strategies of gene therapy

Gene therapy strategy		Genes
Genetic replacement or correction therapy		*p53, CTS1, MDA7, Bcl-2-, BclXL-, survivin-* antisense...
Suicide gene therapy (gene chemotherapy)	Endogenous precursors	*HSV-tk, CD, CD/HSV-tk fusion, HRP, IAA*
	Exogenous precursors	*iNOS*
Gene based immunotherapy		*TNF-α, IFN-γ, IL-12..., tumor associated antigens (PSA)*
Vascular-targeted gene therapy		*VEGF*-antisense, *soluble Flt-1, endostatin, angiostatin, vazostatin, TNF-α, IL-12*

CTS1, Chimeric Tumor Suppressor 1 (synthetic variant of wild-type *p53*); *HSV-tk, Herpes Simplex Virus thymidine kinase*; *CD, Cytosine Deaminase*; *HRP, Horseradish Peroxidase*; *IAA, Indol-3-Acetic Acid*; *iNOS, inducible Nitric Oxide Synthase*; *TNF- α, Tumor Necrosis Factor-α; IL-12, Interleukin-12*; *PSA, Prostate Specific Antigen*; *VEGF, Vascular Endothelial Growth Factor*.

out of the primary tumor are not destroyed. In addition, there are some radio-resistant cells within a single tumor mass that may survive despite a relatively high radiation dose. The efficacy of radiotherapy is also limited by chronic and intermittent hypoxia in the tumors.

To increase the efficacy of radiotherapy, while minimizing its side effects, developments have been made in combining radiotherapy with chemotherapy and, lately, gene therapy.

Gene therapy and its combination with radiotherapy

Gene therapy consists of the transfer of exogenous genes, called transgenes, into human somatic cells and the expression of these genes in transfected cells for a therapeutic purpose. In cancer treatment, this means either correction of genetic defects, characteristic of cancer cells, or induction of targeted tumor cell death.[3] In gene correction or replacement approach, a defective or inactivated tumor suppressor gene is replaced, for example to increase radiation-induced apoptosis (wild-type *p53* replacement therapy) or high oncogene expression levels are repressed with the use of antisense, ribozymes or siRNA technology. However, because cancer is a consequence of countless genetic mutations, most anti-tumor therapies aim to destroy cancer cells, rather than correct these complex defects. Strategies to destroy cancer cells can be exerted in different ways; by gene directed chemotherapy, potentiation of immune response and targeting of the tumor vasculature (Table 1).

Recent advances in gene therapy approaches have allowed researchers to successfully combine gene therapy with radiotherapy.[4,5] There are many potential benefits of combining radiotherapy with gene therapy:

- Gene therapy and radiotherapy techniques have different mechanisms of action and they target best at different parts of the cell cycle, which may result in an additive effect (Figure 1).
- Gene therapy can cause radiosensitization, which means that a synergistic (supra-additive) anti-tumor effect is possible (Figure 1).
- Radiation can enhance the "bystander effect" of gene therapy, meaning that more

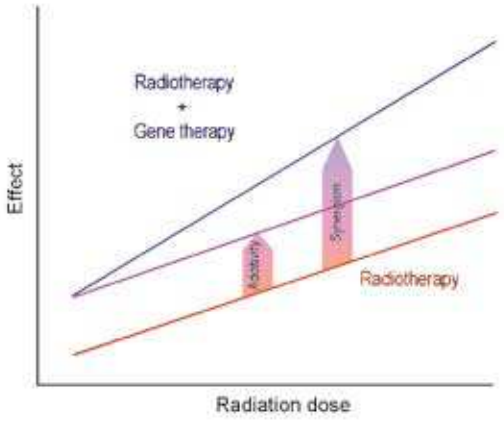

Figure 1. The effect of combined radio- and gene therapy can be either the same as the sum of individual monotherapies (additive) or greater (synergistic).

cells are affected by gene therapy than are transfected initially. This is probably because of the release of products of therapeutic genes from radiation damaged cells and the activation of an anti-tumor immune response.[6]

- Radiation can increase the efficacy of gene delivery and expression.[7-9]
- The targeting ability of radiotherapy is exploited in an approach that uses radiation-inducible promoters to control the timing and location of gene expression within the irradiated tumor volume.[10]

This last approach, called transcriptional targeting, is especially promising since any therapeutic gene with radio-sensitizing properties can be chosen, and will be the main focus of this review. Therapeutic genes used in combination with radiation-inducible promoters can be alienated in the last three categories of gene therapy shown in Table 1: suicide gene therapy, gene based immunotherapy and vascular-targeted therapy.

Suicide gene therapy

Radiosensitizers are chemical or biological agents that increase the sensitivity of tumor cells to radiotherapy. In the past, attempts have been made with radiosensitizers mimicking oxygen effect, with hyperbaric oxygen breathing, with the administration of carbogene and nicotinamid and, most successfully, with chemotherapeutic agents.[11,12] However, the dose of chemotherapy agents required to give a sufficient anti-tumor effect often results in severe systemic toxicity.[13]

The quest to find non-toxic agents that selectively sensitize tumors to radiotherapy has led to innovations in so called suicide gene therapy or gene chemotherapy. The basic principle in suicide gene therapy involves the transfer of a gene encoding an enzyme that converts an otherwise none or mildly toxic substance of either exogenous (pro-drugs) or endogenous origin into a cytotoxic agent that kills cancer cells.[14]

Exogenous precursors. In the first suicide gene therapy strategy, called gene directed enzyme pro-drug therapy (GDEPT), a gene encoding a drug metabolizing enzyme is delivered to a tumor, followed by systemic administration of the pro-drug, which is then metabolized and converted by the expressed enzyme into a cytotoxic substance specifically within the site of transfection.[14,15] Genes used in this strategy originate from viruses, bacteria, or fungi and are foreign to the transfected mammalian cells.

There is a range of enzyme pro-drug combinations available for effective GDEPT, many have also been combined with radiotherapy.[16] The most widely investigated radio-GDEPT combination, involves *herpes simplex virus thymidine kinase* (*HSV-tk*) and the pro-drug gancyclovir (GCV). The viral thymidin kinase enzyme phosphorylates gancyclovir into a nucleoside analog, which is then incorporated into a newly synthesized strand of DNA[4] resulting in cell death in rapidly proliferating cells, which are also targeted with radiotherapy. A supra-additive cytotoxic effect can thus be

expected when this approach is combined with radiotherapy. Furthermore, these nucleoside analogs increase radiation induced DNA breaks and interfere with DNA repair mechanisms.[4] A similar GDEPT system, efficient in radiosensitizing tumor cells in oxic, as well as hypoxic conditions, consists of the plant enzyme horseradish peroxidase (HRP) and the non-toxic plant hormone indol-3-acetic acid (IAA).[17] The next GDEPT strategy to be also tested with concomitant radiotherapy was the combination of bacterial or yeast cytosine deaminase (CD) and pro-drug 5-fluorocytosine (5-FC). CD converts 5-FC to 5-fluorouracil (5-FU), which is a widely used cancer chemotherapy agent with well-known radiosensitizing effects.[18] These strategies were tested in preclinical studies, which showed added benefits of the combined radio-GDEPT compared with either therapy alone. Both enhanced local tumor growth control and systemic effects were observed. Preclinical studies led to clinical trials, which all involve the *HSV-tk*/GCV combination. Early results from the phase I-II clinical trial using *HSV-tk*/GCV gene therapy combined with radiotherapy for the treatment of previously untreated prostate cancer confirm the safety and feasibility of this approach. In the following clinical trials, this combined therapy proved to be safe, but no significant tumor growth control was detected. [4,5,19]

In order to further increase the therapeutic index, chimeric fusion genes combining *CD* and *HSV-tk*, were designed.[20] This so called double suicide gene therapy approach has been evaluated in combination with radiotherapy in preclinical and also phase I clinical studies.[20-23] Yeast *CD/HSV-tk* fusion gene was tested in combination with radiation and pro-drugs in CNE-2 nasopharyngeal carcinoma xenografts model, demonstrating a synergistic anti-tumor effect.[21] In another study, using the bacterial *CDglyTK* fusion gene, the addition of pro-drugs 5-FC and gancyclovir increased the radiosensitivity of prostate cancer and glioma cells *in vitro* and showed a significant anti-tumor effect in a preclinical model of prostate cancer.[22] Results from phase I clinical trial in patients with locally recurrent prostate cancer, indicated that this double suicide therapy is a relatively safe and effective method for increasing the therapeutic index of radiation.[23]

Endogenous precursors. Next therapeutic gene, attractive for use in combined radiogene therapy, is *inducible nitric oxide synthase iNOS*. iNOS is an enzyme that generates nitric oxide (NO), which has many anticancer properties, including cytotoxicity in hypoxic conditions, anti-angiogenic effects and radiosensitization.[24-26] Although NO is a potent chemical radiosensitizer, its clinical use was limited by systemic side effects.[27] By means of gene therapy NO production can be activated at the site of transfection with *iNOS* gene, where it can synergize with radiation.[28] An additional advantage of *iNOS* gene therapy is pronounced bystander effect: namely, because NO is an easily diffusible gas it can exert its effects deep within the tumor mass, resulting in large tumoricidal effects even when only a small portion of the tumor cells are transfected with the *iNOS* gene.[29] The first *in vitro* study using a gene transfer strategy in a murine sarcoma model demonstrated that genetically produced iNOS can increase the radiosensitivity of hypoxic tumor cells.[28] In a subsequent study, evident tumor growth delay was reported after combined radio-gene therapy with *iNOS* in a human colorectal cancer model.[30] The efficacy and safety of this approach has been confirmed in other studies, which are so far still at the preclinical stage.[31,32]

Gene based immunotherapy

Immunotherapy is a promising strategy for cancer treatment because it has the poten-

tial to fight both the primary tumors and metastases[33], which are the major cause of treatment failure in most cancer types. Recent advances in immunology and radiobiology indicate that radiation can modify the tumor microenvironment and generate an antigen specific immune response.[34] Radiation creates inflammation by the induction of cell death and upregulation of immunomodulatory cell surface molecules and secretory molecules in tumor, stromal and vascular endothelial cells. This radiation induced "danger" microenvironment can then lead to breaking of the tolerance to otherwise weakly immunogenic tumor antigens and the generation of an antigen specific cell-mediated antitumor immune response.

These newly discovered immunomodulatory properties of ionizing radiation have given rise to the idea of combining immunotherapy with radiation therapy.[35] The main gain of combining immunotherapy with local radiotherapy could be the elimination of the radio-resistant fraction of cells in the primary tumor and the prevention of shedding of metastatic cells from the tumor. Results from preclinical studies using different non-gene based immunotherapeutic strategies have shown synergistic effects when combined with radiotherapy. The most promising of these combined strategies are now being tested in clinical trials.[35]

An alternative method for execution of immunotherapy is gene therapy.[36,37] There are two major forms of gene based immunotherapy: genetic vaccination and gene-based immunomodulation.

Genetic vaccination. The first form of gene based immunotherapy for the treatment of cancer involves transfection or vaccination with recombinant viruses expressing tumor associated antigens and usually also costimmulatory molecules (CD80, CD54, and CD58, cytokines).[38-40] An improved therapeutic efficacy of combined vaccination and radiotherapy was reported in a mouse adenocarcinoma tumor model.[41] Vaccines were able to induce an anti-tumor immune response and act synergistically with local tumor irradiation. Furthermore, the development of T cells directed against tumor associated antigens, which were not present in the vaccine, was observed, resulting in broadening of the immune response. This phenomenon, also called antigen spread or antigen cascade, was also indicated in a phase II clinical study in patients with localized prostate cancer.[42] In this study, vaccination with poxvirus encoding prostate specific antigen (PSA) combined with standard external beam radiotherapy was well tolerated and induced a PSA-specific immune response to vaccine in the majority of patients.

Gene-based immunomodulation. In contrast to vaccination, gene-based immunomodulation or cytokine gene therapy is a form of nonspecific immunotherapy. In this strategy, immunostimulatory genes such as cytokines are utilized to boost the immune system.[36,43] Early treatment strategies using systemic administration of recombinant immunostimulatory cytokines were associated with dose limiting normal tissue toxicities.[44,45] Gene therapy approaches significantly improved the prospects for the use of cytokine cancer therapy.[37] Two important cytokine genes, which have been tested in combination with radiotherapy, are *interleukin 12 (IL-12)* and *tumor necrosis factor-α (TNF-α).*

IL-12 is a heterodimeric pro-inflammatory cytokine with multiple functions, including the induction of interferon-γ (IFN-γ), activation of T helper and NK cells[46,47] and anti-angiogenic activity.[48,49] IL-12 has been proved to have potent antitumor and antimetastatic effects against murine tumors.[50] A combination of genetically produced IL-12 and local radia-

tion was tested in a mouse fibrosarcoma model.[51] Intratumoral injection of adenoviral vector with *IL-12* combined with radiotherapy improved both local and systemic tumor control compared to either treatment alone. Enhanced local tumor control could be partially attributed to the anti-angiogenic effects of IL-12, while the systemic, antimetastatic effect on microscopic metastases distant from the primary irradiated site was clearly due to an IL-12 induced anti-tumor immune response. In another *in vivo* study, adenovirus mediated local *B7/IL-12* immunotherapy combined with radiotherapy was tested in two murine tumor models.[52] In both tumors, growth delay was significantly longer when radiotherapy was combined with immunotherapy. The therapeutic effect was explained by IL-12 mediated activation of T- and NK-cells and inhibition of angiogenesis. Similar results were obtained in subsequent studies combining IL-12 gene therapy and radiotherapy.[53-55]

TNF-α is another attractive candidate for cancer gene therapy since it encodes for secretory protein with a broad range of potent anti-tumor properties, which include induction of the immune system, enhancement of radiosensitivity, direct cytotoxicity and disruption of the tumor vasculature.[56] An additive killing effect of systemic recombinant TNF-α administration and radiation was reported in a MCA-K mouse tumor model.[57] A phase I trial combining systemic TNF-α administration and radiation demonstrated that the systemic toxicities from TNF-α limit the efficacy of treatment.[45] To overcome this problem and at the same time preserve the potent anticancer activity of TNF-α, a new form of gene therapy was designed, in which the *TNF-α* gene is placed under the control of radiation inducible promoter.[10] This form of gene therapy is called transcriptional targeting and will be discussed in more detail later in this article. Briefly, in pre-clinical tests treatment with genetically produced TNF-α was shown to synergize with local radiation to produce an increased anti-tumor effect and was not associated with increased local and systemic toxicity.[13] The same approach was further developed for clinical studies as TNFerade and is now in phase II/III clinical trials.[58] A combination of intra-tumoral injections of TNFerade and concomitant radiation was well tolerated in clinical trials. In addition, substantial anti-tumor responses were reported. However, no systemic effects were observed although TNF-α has the potential to induce an immune response. It seems that the therapeutic effectiveness of this combination cannot be attributed to the immune system, and that some other mechanisms are involved. Most probably antitumor effect of TNF-α is mediated by direct cytotoxicity on the tumor vessels.[59] TNF-α should perhaps therefore be placed in the next group of therapeutic genes, classified as vascular-targeted therapies, which will be discussed in the next chapter.

Vascular-targeted gene therapy

Growth of new blood vessels from pre-existing vessels or angiogenesis is necessary for solid tumor progression and metastasis[60] and is thought to be one of six hallmarks of cancer;[61] it was therefore proposed as a new target in cancer treatment.[62] According to the angiogenic "switch" hypothesis, a shift in the balance between pro- and anti-angiogenic factors toward pro-angiogenic allows the tumor to expand.[63-65] Among many pro-angiogenic factors, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are widely considered to be the most important in the angiogenic process. They are normally opposed by endogenous inhibitors of angiogenesis such as thrombospondin-1 (TSP-1), platelet factor 4 (PF4), soluble Flt-1 (sFlt-1), angiostatin, and endostatin. Vascular-targeted therapies

target different parts of the angiogenesis process, from inhibition of pro-angiogenic factors, or augmentation of anti-angiogenic factors, to direct targeting of tumor endothelial cells. There are several advantages of vascular-targeted therapies over other cancer therapies: the first is that they target endothelial cells, which are more easily accessed and are considered to be relatively genetically stable compared to tumor cells, therefore lower risk of acquired drug resistance is expected;[66] secondly, angiogenesis is very limited in normal physiology, so normal tissue toxicities can be mostly avoided;[67] and lastly, since a large number of cancer cells depend on a small number of endothelial cells for their metabolic supplies, targeting the tumor vasculature should lead to an enhanced antitumor effect.[62,68,69] Vascular targeted therapy can be differentiated into two groups: anti-angiogenic and vascular-disrupting approaches.[70,71] Anti-angiogenic agents inhibit the formation of new blood vessels, consequently they often require chronic administration, and are predominantly beneficial for the treatment of early-stage or metastatic cancers. Vascular disrupting agents, in contrast, destroy the existing tumor vasculature and are thus suitable for acute treatment of advanced disease. In reality, the boundaries between anti-angiogenic and vascular-disrupting agents are not so evident and many vascular targeted agents may exhibit both an anti-angiogenic and a vascular-disrupting action.

A range of vascular targeted approaches, including recombinant proteins, monoclonal antibodies and small molecules, has already been tested for their anti-tumor properties; many are already in clinical trials.[72] Although most proved to be safe and effective in suppressing tumor growth, they were not tumoricidal,[73] indicating the need for prolonged administration to maintain tumor suppression.[72] That is especially true for the anti-angiogenic group of agents. Gene therapy was therefore adopted for the delivery of these agents.[74,72] In addition to being more efficient in the persistent production of therapeutic proteins at therapeutic levels, a further advantage supporting this form of delivery, is the selective expression of the vascular targeting gene only in targeted organs containing tumors, minimizing systemic toxicity, which could become a problem after prolonged treatment. Anti-angiogenic gene therapy strategies can be categorized into those that suppress the pro-angiogenic factors, either by inhibiting the expression of angiogenic genes (antisense and siRNA against *VEGF*) or interfering with angiogenic signaling pathways using decoy receptors (*e.g.* soluble Flt-1 that can sequester VEGF or inactivates its receptors), and those that enhance the inhibition of angiogenesis using genes encoding endogenous angiogenesis inhibitors (*e.g.* endostatin, angiostatin).[72,75] Preclinical studies demonstrate that this type of gene therapy can be effective in controlling or even eradicating tumor growth in animal models, but vascular targeting strategies in the form of gene therapy remain for the moment at the preclinical stage.

Currently, therapies combining vascular targeting strategies with conventional therapies like radiotherapy are receiving great attention.[70,76] There are many possible mechanisms for enhanced tumor response to radiation with anti-angiogenic and vascular disrupting therapies.[68] The original justification for a combined therapy was that it targets two separate cell populations: endothelial cells and cancer cells.[77] Although there was initially some concern that vascular targeting agents would increase tumor hypoxia, and thus limit the effectiveness of radiotherapy, there is accumulating experimental evidence suggesting that these agents actually improve tumor oxygenation, leading to radio-sensitization.[68] This apparently paradoxical evidence could be

explained if the anti-angiogenic therapy was to cause normalization of the otherwise structurally and functionally abnormal tumor vasculature before its destruction.[77] During this brief normalization, the tumor oxygenation status would be improved, leading to an enhanced radiotherapy effect. Improved tumor oxygenation could also be the result of a reduced number of oxygen-consuming tumor and endothelial cells, caused by anti-angiogenic therapies.[68] In the case of vascular disrupting agents, an improved tumor response to radiation is probably the result of additive killing of two micro-regionally different populations of tumor cells. Namely, vascular disrupting agents selectively destroy the tumor vasculature, leading to centralized necrosis within the tumor, whereas the peripheral rim of tumor cells remains viable, probably because those areas are perfused by normal tissue vessels, which are not targeted by vascular disrupting genes. These remaining tumor cells are therefore well-oxygenated and, as such, present an excellent target for radiation therapy.[78] Genes used in combination with radiotherapy because of their vascular targeting properties include *angiostatin, endostatin, IL-12* and *TNF-α.*

Angiostatin and endostatin are both endogenous inhibitors of angiogenesis with confirmed anti-tumor and anti-metastatic activity in preclinical tumor models.[79,80] The anti-tumor efficacy of endostatin gene therapy with radiotherapy was evaluated in a human colorectal tumor model HT29. Intramuscular injection of virus vector expressing endostatin led to sustained endostatin serum levels and enhanced tumor growth delay of HT29 xenografts.[81] An enhanced anti-tumor efficacy of radiation therapy after intratumoral injections of liposome-endostatin complex was also demonstrated in human liver carcinoma BEL7402 xenograft models.[82] In a Lewis lung carcinoma (LLC) mouse tumor model, naked plasmid DNA encoding mouse endostatin gene was injected intratumorally as an adjuvant to radiation.[83] The anti-tumor efficacy of radiotherapy was significantly enhanced with the anti-tumor effect in the combination treatment being at least additive compared with either treatment alone. Gene therapy delivery of angiostatin was also shown to enhance the treatment efficacy of radiotherapy. Using adenovirus expressing a secretable angiostatin-like molecule (AdK3) in combination with radiotherapy in rat C6 gliomas subcutaneously pre-established into athymic mice, significantly higher and possibly synergistic, anti-tumor effects were observed that tightly correlated with an obvious decrease in vascularization of the tumor.[84]

TNF-α and IL-12 are two multifunctional proteins with vascular-disrupting and anti-angiogenic effects. The mechanism of the IL-12 anti-vascular effect is complex, including the induction of secondary cytokines such as IFN-γ or chemokines such as interferon-inducible protein 10 (IP-10), which may have direct cytotoxic and/or anti-angiogenic effects on tumor and endothelial cells.[48,49] The effect of TNF-α is even more complex; its anti-vascular effect can be either stimulatory or inhibitory depending on the amount, the site, the microenvironment, and the presence of other cytokines.[56,85] The anti-vascular effect of TNF-α is considered to be primarily vascular disrupting and not anti-angiogenic.[59,86] Studies involving IL-12 and TNF-α in combination with radiation have already been discussed in the previous chapter.[51-59]

Targeted expression of therapeutic genes

As with other cancer therapies, the major problem of gene therapy is poor therapeutic index caused by uncontrolled gene

expression, which can lead to normal tissue toxicity.[10,87,88] A tightly controlled regulation of transgene expression is required to increase the efficiency and safety of gene therapy. For the clinical success of gene therapy, gene regulation systems are especially desired, not only to maintain the therapeutic level of the transgene product without systemic toxicity but also to be able to adjust transgene expression in response to disease progression. Several strategies have been explored to control gene expression. These involve restricted or targeted vector delivery and transcriptional targeting with the use of tumor and tissue specific promoters and inducible promoter systems.[89,90] The latter are the most important for combination with radiotherapy and will therefore be discussed in more detail in the following chapters.

Inducible promoter systems

Expression of therapeutic genes can be controlled by locating them downstream of promoter regions that are induced in response to various signals.[88] The advantage of this kind of inducible promoter system is that not just the location, but also the level, timing and duration of transgene expression can be modulated. Optimal inducible promoters should have low basal activity and high inducibility, with a so-called "on switch". They should be dose dependent, safe, and reversible (off switch).

Several inducible promoters have already been utilized for use in cancer gene therapy. They can be controlled either by internal (endogenous) or external (exogenous) signals.[91] Internally controlled promoters take advantage of a tumor associated microenvironment such as hypoxia. Externally controlled promoters, on the other hand, can be induced by chemical signals (Tet-On, Tet-Off inducible systems),[92] heat *(heat shock protein 70* promoter*)*,[93] controlled electric stimuli such as administrated in electroporation protocols (*metallothionein* promoter)[94] and, most importantly, ionizing radiation (*Egr-1*, *p21*).[10]

Radio-inducible promoters. It is well known that exposure of cells to ionizing radiation induces DNA damage by direct interaction with DNA and through the generation of reactive oxygen species (ROS), which results in transcriptional activation of a variety of genes, leading to changes in their expression.[13,95] The initial signal for transcriptional activation of these genes is probably generation of ROS by radiation, rather than direct damage to DNA.

Numerous genes that are activated by radiation have so far been identified (*Egr-1*, multiple members of the *jun/fos* family, *NFκB, p21(WAF-1/Cip-1*), bacterial *RecA* gene...). Promoters of these radiation inducible genes can be exploited to drive the expression of therapeutic genes.[10,96] With the use of the excellent targeting properties of new stereotactic radiation techniques, the expression of downstream genes can be spatially and temporally controlled within the irradiated tumor tissue (Figure 2).

By far the most widely used and well characterized promoter for this purpose is that of the *Egr-1* gene, next in line is the promoter of the *p21* gene.

Egr-1 promoter. *Early growth response-1* gene (*Egr-1*) is a transcription factor for some cytokines and growth factors (TNF-α, IL-1, PDGF-β, bFGF) involved in repair or death of tissue after various kinds of stress, including irradiation. The radiation induced expression of *Egr-1* accurs in different cell types and is fast and transient.[97] Sequences responsive for radiation inducibility consist of 425 bp located upstream of the transcription start site of the *Egr-1* gene and contain six consensus motifs CC(A+T rich)GG, known as CArG elements. Their response is mediated by intracellular free radical formation caused by ionizing radiation.[97]

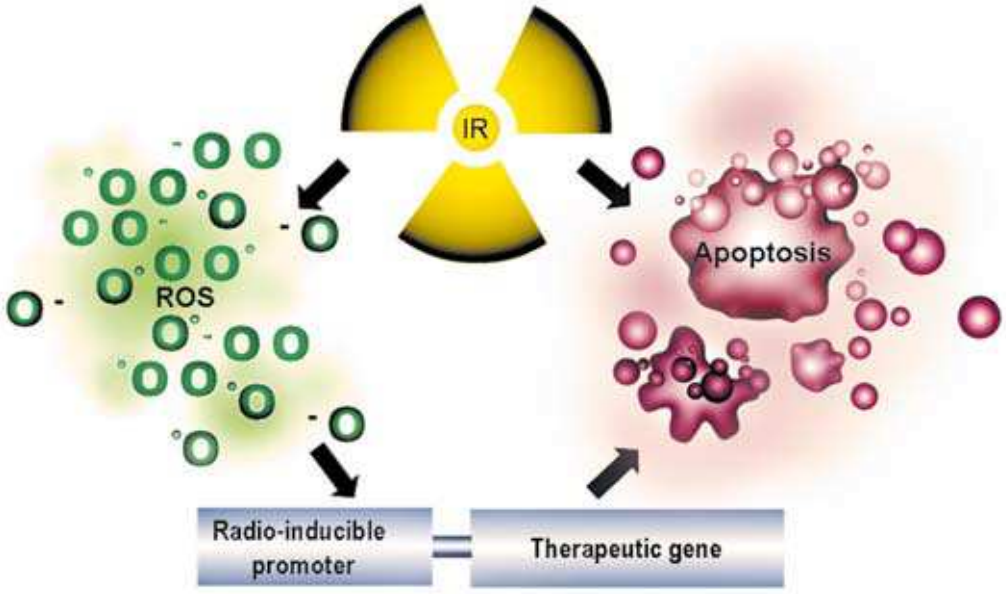

Figure 2. Ionizing radiation induces ROS generation, which causes transcriptional activation of radioinducible promoter leading to increased expression of the therapeutic gene. The combination of the damaging effects of irradiation and increased level of therapeutic protein results in increased tumor cell apoptosis and improved anti-tumor activity.

To check the potential of these radio-inducible sequences for use in radiation inducible gene therapy, *Egr-1* promoter was ligated upstream of the cDNA encoding the *TNF-α* gene. In the initial study, the *Egr-TNF-α* construct was transfected into human leukemia cell line HL525.[98] Induction of *TNF-α* expression was observed when cells were exposed to radiation. Stably transfected cells were then injected into human xenografts of the radio-resistant squamous carcinoma cell line SQ-20B. When animals were treated with radiation, increased TNF-α protein levels were detected in tumors and an increased anti-tumor effect was observed.

The *Egr-TNF-α* chimeric construct was later cloned into a replication-deficient adenoviral vector and termed Ad.Egr.TNF-α. Preclinical studies on human carcinoma, prostate and glioma xenografts showed that tumors transfected with Ad.Egr.TNF-α responded to radiation with 7-8 fold induction of *TNF-α* expression within the irradiated field and substantially increased tumor growth inhibition, compared to tumors treated with radiation alone.[59,99-102] Importantly, the combined treatment was not associated with increased systemic toxicity and also no TNF-α could be detected in the circulation of the experimental animals.

For clinical studies, the same construct containing human *TNF-α* and *Egr-1* radio-inducible promoter was incorporated into a second generation adenovector and called TNFerade.[103-105] Toxicology studies with TNFerade on nude mice showed that combined therapy with radiation is well tolerated and also associated with sub-

stantial anti-tumor activity. There was no systemic toxicity and serum TNF-α levels were not significantly increased.[103] In the first clinical study on patients with a range of advanced, treatment refractory solid tumors, TNFerade was administrated by intratumoral injection with concomitant radiotherapy.[104] The combined treatment was well tolerated and serum TNF-α levels were not significantly increased during the treatment. In addition, no adenovirus was detectable in patients' blood, urine and sputum samples. In the following phase I/II clinical trial on soft tissue sarcoma of the extremity, no dose limiting toxicities were observed, treatment was well tolerated and substantial responses, even in large tissue sarcomas, were reported, and therefore provide support for further evaluation of TNFerade.[13] Therapy has now successfully reached phase II/III clinical trials for treatment of pancreatic and esophageal carcinomas, rectal carcinomas, metastatic melanomas, soft tissue sarcoma and head and neck cancer.[58]

The radio-inducibility of CArG elements has also been demonstrated using reporter genes *β-galactosidase* (*β-gal*) and *green fluorescent protein* (*GFP*) inserted downstream of the *Egr-1* promoter. Expression of *β-gal* under the control of *Egr-1* promoter was enhanced 3-fold after irradiation with 2 Gy in glioma cells.[106] In another study, a construct with *GFP* responded to 5 Gy irradiation with increased GFP expression.[107]

For the use in radiation targeted suicide gene therapy, *Egr-1* promoter was inserted upstream to the *herpes simplex virus thymidine kinase (HSV-tk)* gene. Transfection of *Egr-HSV-tk* constructs into different tumor cells produced enhanced tumor cell killing in the presence of the prodrug gancyclovir following radiation treatment.[107-110]

After the success of *TNF-α* and *HSV-tk* radio-inducible constructs, a number of preclinical studies have successfully used CArG elements to drive the expression of several other cytotoxic or immune-modulatory therapeutic genes such as *IFN-γ,*[111] *iNOS,*[31,112] *mIL-12,*[55] *mIL-18,*[113] etc. using different gene delivery systems (liposomes, adenoviruses, naked DNA injection, cell carrier).

In order to further improve the performance of wild-type *Egr-1* promoter, CArG elements were isolated from *Egr-1* promoter and integrated into synthetic promoters.[107] These new promoters demonstrated greater inducibility and lower basal activity than the wild-type *Egr-1,* despite containing the same number of CArG elements. Furthermore, a cumulative effect was observed after fractionation, with five times 1 Gy doses being as effective as a 5 Gy dose. By increasing the number of CArG elements from four to nine, induction with clinically relevant doses (2-3 Gy) was further improved and a lower basal activity was achieved. Further *in vitro* studies showed that specific alternations of the core A/T sequence in the CArG elements caused an even greater induction after irradiation, while the spacing between the elements had no effect.[114] Using an *HSV-tk*/GCV system, synthetic CArG promoters were also shown to work *in vitro* and *in vivo,* with significant radio-sensitizing and anti-tumor effects.[114]

p21 promoter. Studies with *Egr-1* promoter have led to the investigation of other radiation-inducible promoters, such as the promoter of the *cyclin dependent kinase inhibitor p12*, also known as *WAF1* or *CIP-1.* Gene *p21* is an immediate-early response gene, mediating cell cycle G1 phase arrest in response to a variety of stresses.[95,115] Its expression is regulated mostly by tumor suppressor protein p53,[116] which is activated by DNA damage caused by irradiation and genotoxic agents. The *p21* promoter region contains at least two binding sites for the p53 transcriptional factor, and also specific DNA motifs responsive to a wide range of

other cell growth regulatory signals, indicating that p53- independent pathways for the *p21* gene transcriptional activation also exist.

The promoter of the *p21* gene has predominantly been studied in the context of suicide gene therapy with the *iNOS* gene. First, the response of the *p21* promoter to radiation was tested using a *p21/GFP* reporter gene construct in an *in vitro* model with human endothelial cells HMEC-1 and in an *ex vivo* rat tail arterial segment model.[117] Transfection of both models followed by irradiation with 4 Gy resulted in a significant increase (9.5 and 4.5-fold, respectively) in *GFP* expression. Similarly, when *p21* promoter was used to control expression of the therapeutic gene *iNOS*, a five-fold induction of *iNOS* gene was obtained after 4 Gy radiation in a rat tail arterial segment model. The radio-sensitizing properties of the *p21/iNOS* construct were next tested in murine fibrosarcoma cells RIF-1. After a large single dose of radiation, tumor cell radio-sensitization *in vitro* and tumor growth delay *in vivo* was achieved.[28]

In order to optimize the synergistic interaction between radiation and the transgene product, induction of transgene expression and radiation therapy should be temporally adjusted. An alternative therapeutic regime was therefore proposed using an initial priming dose of 4 Gy to induce transgene expression, followed by a subsequent treatment dose. This approach was tested *in vivo* on *p53* wild-type RIF-1 tumors and *p53* mutant HT29 human colorectal tumor xenografts.[30] Intra-tumoral injection of *p21/iNOS* construct, followed 16 h later by a 4 Gy priming dose and then, 8 h later, by treatment doses of 10 or 20 Gy, resulted in significant radio-sensitization in both tumor types, compared with radiation treatment alone. Furthermore, western blot analysis revealed that transgene protein levels were significantly increased only in tissue within the irradiated volume, even though vector sequences were detected in all the main organs tested, indicating that effective transcriptional targeting had been achieved. A similar approach with a priming radiation dose was later tested on the same tumor models using fractionated radiation schedules at clinically relevant doses per fraction.[118] Again, significant radio-sensitization was demonstrated for both *p35* normal and *p53* mutated tumor models.

Another study focused on the importance of integration of the *p21* promoter into chromatin[119], since there had been reports that the binding of *p53* to its recognition sequence in *p21* promoter depends on the chromatin structure.[120] Indeed, *p21* promoter transduced by recombinant adeno-associated virus vector, which can stably integrate transgenes into chromosomes, proved to be more responsive to low dose radiation than transiently transfected by electroporation. Significant induction of *p21* promoter by radiation doses as low as 0.2 Gy was demonstrated using *luciferase* reporter gene. Induction after 5 Gy reached a 6-fold induction, which was significantly higher than in transiently transfected cells (1.9-fold). Also when cells were stably transduced with suicide gene *HSV-tk* under regulation of the *p21* gene promoter, they were sensitized to repetitive treatment with low dose radiation (1 Gy).

Although p53 was shown to be important for the radiation inducibility of *p21* in some cases, there is plenty of evidence that *p21* can also be activated independently of p53. For instance, as mentioned before, *p21/iNOS* gene therapy was effective in radio-sensitizing both *p53*-wild type and mutant tumors to radiotherapy.[30,118] Characterization of the *p21* promoter in a range of normal and tumor cell lines with different *p53* status using the *GFP* reporter gene revealed that induction by radiation is independent of *p53* status.[118] In addition,

basal level activity of *p21* promoter proved to be high in tumor cells, but low in normal cells. So *p21* promoter is not only inducible by radiation but is also selectively inducible within the tumor environment and, as we will see in the next chapter, can also be induced in response to hypoxic conditions. All these characteristics make *p21* promoter a good candidate for use in cancer gene therapy, especially for the systemic treatment of disseminated disease. Systemic delivery could be used to target metastatic deposits, where tumor and hypoxia specific expression of the transgene would be attained in the absence of radiation.

Chimeric radiation and hypoxia inducible promoters. Hypoxia is a physiological feature of solid tumors that is a major hindrance to radiotherapy[121], since hypoxia leads to radiation resistance because of lack of oxygen to facilitate DNA damage by radiation-induced ROS.[122] Hypoxic conditions also create a microenvironment in which tumor cells become less angiogenesis dependent, more apoptosis resistant, and more malignant.[122,123] The presence of this physiological difference can, on the other hand, be exploited for selective cancer treatment.[124] One way to do that is by using hypoxia inducible promoters to drive the expression of therapeutic genes.[125-127] Namely, similar as radiation, hypoxia can activate the expression of numerous genes, important for angiogenesis, cell metabolism and cell growth. Their response to hypoxia is, in most cases, mediated by binding of hypoxia-inducible factor-1 (HIF-1) to specific hypoxia response elements (HREs) containing the consensus sequence (A/G)CGT(G/C)(G/C) within the promoter regions of these genes.[124]

To date, HRE derived from several hypoxia responsive genes, including *phosphoglycerate kinase 1 (PGK1)*, *vascular endothelial growth factor (VEGF)*, *erythropoietin (Epo)* and *lactate dehydrogenase A (LDH A)* have been successfully used for hypoxia specific targeting of gene expression.[128] Similarly to radio-inducible CArG elements, HREs have also been incorporated into synthetic promoters, tested for inducibility in hypoxic conditions using reporter genes and then used in experimental gene therapy with suicide therapeutic genes such as *HSP, HSV-tk* and *CD*.[128-130]

The oxygenation status of tumor tissue is highly heterogeneous, with areas of low and high oxygen levels indistinctly mixed together. Since hypoxia inducible gene therapy relies on a lack of oxygen and radio-inducible gene therapy needs the production of oxygen derived free radicals, neither approach is adequate for the treatment of an entire tumor. Vectors containing chimeric promoters responsive to both stimuli have therefore been developed.[131]

Chimeric promoters containing HREs derived from *Epo*, *PGK1* and *VEGF* genes and radio-inducible CArG elements were tested using *GFP* reporter assay on human T24 bladder and MCF-7 mammary carcinoma cells.[17] Treatment with 5 Gy irradiation under a 0.1% oxygen concentration resulted in the induction of all promoters, with the *Epo* HRE/CArG promoter being most responsive and robust. Subsequent promoter induction tests in a range of physiological oxygen concentrations characteristic of solid tumors showed that the *Epo* HRE/CArG promoter is most responsive in the radio-biologically significant levels of 0.1-0.5% O_2. *Epo* HRE/CArG promoter was next successfully used to control a *HRP* mediated GDEPT strategy following irradiation under hypoxic conditions *in vitro* and *in vivo*.[114] Similar results were reported when chimeric HRE/CArG promoter was used to control *HSV-tk* expression in human lung carcinoma A549 xenografts.[26] In another study, *Epo* HREs were ligated upstream of the *Egr-TNF-α* construct.[132] Combined treatment with *Epo-Egr-TNF-α* plasmid and radiation resulted in significant tumor growth delay in

human colon adenocarcinoma WIDR xenografts.

Another promoter that can be induced by both radiation and hypoxia is *p21* mentioned earlier.[118] This promoter lacks HRE so induction by hypoxia occurs by a novel mechanism involving the Myc transcriptional factor. In the already mentioned *in vitro* study, *p21* promoter was activated in hypoxic conditions by a factor of 5.4 in the RIF-1 cell line and 4.3 in the HT29 cell line.[118] These findings were extended to other cell lines with different *p53* status. Following exposure to hypoxia, all cell lines showed elevated levels of GFP compared to normoxic cells.

Cre/loxP molecular amplification switch. One problem associated with inducible promoters is that, ones they are induced, they are relatively weak compared to strong constitutive promoters. In addition, transgene expression is restricted only to the period of the associated stimulation. In order to generate sufficient concentrations of transgene product without compromising the specificity of the inducible promoters, the expression should be amplified and sustained. For this purpose, an inducible molecular switch was devised based on the Cre/loxP site specific recombination system of the P1 bacteriophage.[133] In this new approach, the inducible promoter controls the expression of *Cre recombinase* instead of the therapeutic transgene, which is transcriptionally silenced by the loxP "stop" cassette incorporated between the gene and the constitutive promoter (Figure 3).

In the evolution of this molecular switching device, the expression of *Cre recombinase* was first controlled by a radio-inducible promoter and two vectors were required: one containing *Cre recombinase* with an inducible promoter and the other with the therapeutic gene and constitutive promoter. The active components were next incorporated into a single vector and the radio-inducible promoter was replaced by chimeric radiation and a hypoxia inducible promoter.[131]

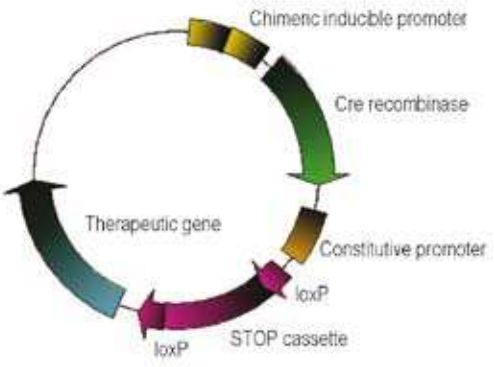

Figure 3. Radiation and/or hypoxia stimulation activates the chimeric promoter on the vector, leading to expression of the *Cre* gene. The produced Cre recombinase then recognizes the loxP sites and cuts out the "stop" cassette, bringing the therapeutic transgene under the control of the strong constitutive promoter.

The efficacy of the two vector system with radio-inducible promoter was tested on a MCF-7 breast cancer cell model using the *GFP* reporter gene and *HSV-tk*/GCV mediated tumor cell killing assay.[133] An increase in *GFP* expression and tumor cell killing was achieved following clinically relevant doses: a 3 Gy dose induced a 40-fold increase in radiation activated *GFP* expression, compared to a two- to three-fold increase when the reporter was controlled directly by the same promoter. Tumor cell growth inhibition equivalent to that of 3 Gy without the switch was achieved by the switch system after a single dose as low as 1 Gy. For testing of a single vector system containing chimeric promoter inducible by hypoxia and radiation, human mammary adenocarcinoma MCF-7 and glioma cells U87-MG, U373-MG were used. *In vitro* higher and more selective tumor cell killing was achieved using switch controlled *HSV-tk*/GCV GDEPT. The single vector switch was also tested in nude mouse xenograft models, in which it

induced significant growth delay and tumor eradication.[134]

Targeted gene therapy in radiotherapy, conclusions

The ultimate aim of gene therapy, common to all cancer therapies, is to selectively target tumor cells while minimizing normal tissue toxicity. Although gene therapy has the potential to provide sustained, high local concentrations of the therapeutic gene, poor tumor specificity is a major problem. Inducible promoters activated by ionizing radiation have the potential to limit gene expression to the irradiated tumor volume. To date, the only radiation-inducible promoter used in clinical trials is the *Egr-1* promoter. Considerable problems remain to be overcome for this radio-gene combined modality to achieve wider clinical application. Each modality of combined treatment has its own drawbacks. For instance, gene therapy still lacks safe and efficient delivery systems. Bystander cell killing can partially improve the efficiency of gene therapy but the quest to find a better delivery system continues. Two major problems of radiotherapy are metastases and radio-resistance. Using radiation induced transcriptional targeting, a high level of local control is achieved at the expense of poor systemic control. One way to solve this problem is to choose a secretory therapeutic gene that has local radio-sensitizing activity and can also induce an effective systemic immune response against tumor antigens or inhibit angiogenesis of metastases (*IL-12*). Another solution is to use a promoter or a combination of promoters that can be induced by radiation, to target the primary tumor, and tumor specific conditions like hypoxia to target the metastases (*p21* promoter, chimeric promoters). An additional advantage of hypoxia inducible promoters is that they target exactly the cell population resistant to radiotherapy. Since a transcriptional targeting approach allows for any therapeutic gene with radio-sensitizing properties to be chosen, careful selection of the best combination of inducible promoters and therapeutic genes is important for translation of this approach to the clinic.

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case report

MR rectum imaging with ultra sound gel as instrumental contrast media in tubulovillous adenoma

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Bacground. *Colorectal polyps are frequent and can be found in 10% of adults, most common in elderly with prevalence of 20% in age group of 60. Over 90% cases of cancer are being developed from benign adenomas. Colorectal cancer (CRC) is a significantly large cause of death right after bronchial cancer in males, and breast cancer in women. Therefore, a standpoint was adopted that the removal of polyps as precursor will prevent the development of colorectal area cancer. Polyps can occur as peduncular or sessile. Adenomas are grouped in three subtypes based on histological criteria: tubular, tubulovillous and villous. Villous adenomas are larger than others and show a higher level of dysplasia. The prevalence of adenomas increases with the patient's age. Having in mind that the risk of malign adenoma transformation is 10 years average, and that small lesions have no clinical potential to turn into cancer, their removal would lead to unnecessary complications and additional costs. CRC risk grows both with the size and the number of adenomas. In patients who refuse polypectomy, we can expect cancer development in average of 5 years 4% and in 10 years 14%.*

Case report. *We present a patient with a years long history of rectal polyp. She has refused any treatment of polyp removal up so far. Due to stool problems, mostly constipation, occasional bleeding and falling out feeling, she has decided to remove the polyp. The polyp has been detected through colonoscopy and described as very risky for polypectomy due to its suspected malign appearance. We did rectum MR on 1.5T Siemens, so that the patient came with clean lumen into which we applied ultra sound gel with huge 60 ml syringe (no needle) simply and pain free with three fillings (total 180 ml of gel). We have concluded that the polyp was of uneven outline and stretched partially along the inner rectum wall without extra rectal infiltration into mesorectal area. After that, we performed endoscopic polypectomy according to peace meal method resection up to real muscular layer after adrenalin undermining. Pathohistological finding which was done in HE technique showed tubulovillous adenoma.*

Conclusions. *Rectal MR is a new, very reliable method of contemporary radiological imaging that gives better characterization of polyp tissue and of other tumours. It is currently the best imaging modality enabling very accurate evaluation and topographic ratio of tumour growth within the rectum wall and outside the wall, especially compared to mesorectal fascia. In addition, it is a very comfortable procedure without radiation. The application of ultra sound gel as intra luminal rectal contrast agent can distend the lumen and make an excellent contrast of lumen against the rectum wall and thus can better show polyps and tumours.*

Key words: colorectal polyps; colorectal cancer; tubulovillous adenoma; polypectomy; rectum MRI

Introduction

Colorectal polyps are frequent and can be found in 10% of adults, most common in elderly with prevalence of 20% in age group of 60. Over 90% cases of cancer are being developed from benign adenomas. Colorectal cancer (CRC) is a significantly large cause of death right after bronchial cancer in males, and breast cancer in women. Therefore, a standpoint was adopted that the removal of polyps as precursor will prevent the development of colorectal area cancer.[1,2] Polyps can occur as peduncular or sessile.

Adenomas are grouped in three subtypes based on tubulovillous histological criteria: tubular, tubulovillous and villous. Tubular adenomas are most frequent – 80-86%, tubulovillous are somewhat less frequent – 8-16%, and villous only 5%. Villous adenomas are larger than other adenomas and show a higher level of dysplasia. They can be found mostly in rectum and rectosigmoid area although they can appear anywhere. They are famous for their possibility of malignant transformation.[3,4] Adenomas malignly alterate in 40% of cases. Besides, they can bleed, obstruct, invaginate and make torsion. It is believed that the adenoma occurrence is linked both with the abnormal cell proliferation and the apoptosis process. Clinical, histopatological and epidemiological studies enable us to have an insight into adenoma progressing into cancer. Molecular genetic trials describe transit adenoma-cancer through the accumulation of multiplied genetic mutation resulting into the transit from normal adenoma mucosa into dysplasia and then into cancer. Minimal adenoma progression time into CRC is 4 years, and median is 10 years from the time of setting diagnosis.[5]

Numerous sources provide an insight into records indicating that CRC that were found during operations are made of one or several synchronic adenomas. The CRC risk grows both with the size and number of adenomas. In patients who refuse polypectomy, we can expect cancer development in average of 5 years 4%, and in 10 years 14%. The frequency of adenoma occurrence in USA indicates that the adenoma prevalence is closely connected to the frequency of CRC occurrence. Regions with law polyps prevalence – 12 % are Costa Rica and Columbia while countries with really high prevalence – 30-40% are USA, Canada, West Europe, Argentina, New Zealand, Australia. The race is not an insignificant factor for adenoma prevalence, although regional belonging is taken into account as a factor. We can cite example of African Americans in USA who suffer from the disease much more than the blacks in South Africa. A similar example is with the yellow race where Japanese suffer more from the disease than those on Hawaii. In general, the risk of adenoma occurrence does not depend on gender, although some authors suggest insignificant predominance in male population. The prevalence of adenoma increases with the patient's age. In fifties, the prevalence is 30%, in sixties 40-50%, and in seventies 50-65%. Distribution of polyps varies with age. 75% polyps of 10 mm and more are located distally. In patients of age 65 and more, 50% of polyps size 10 mm and more are located more proximally.[6]

Two thirds of polyps are asimptomatic and have insignificant lab findings. A polyp larger than 1 cm can show symptoms such as rectal bleedings and abdominal pain. Nonspecific symptoms are diarrhoea, constipation and flatulence. Changes in stool caliber are usually related to large distal polyps. French data from records in Côte-

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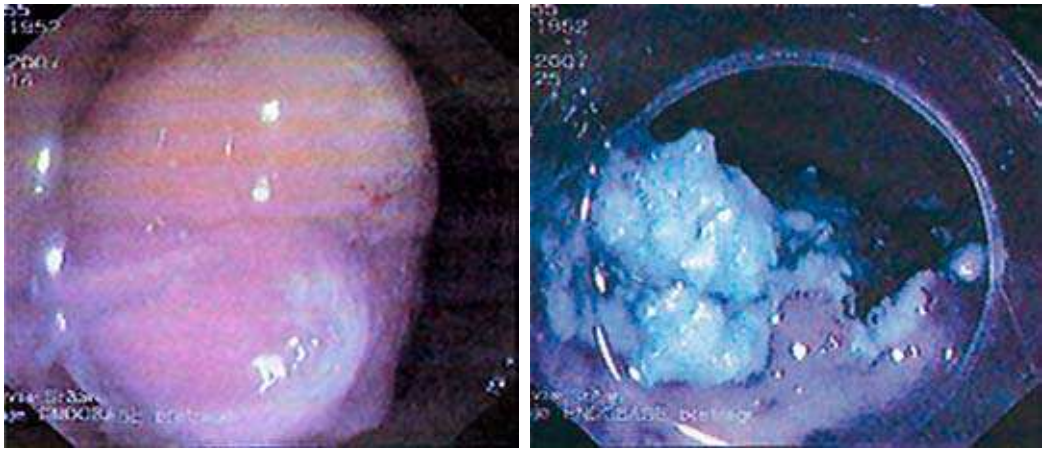


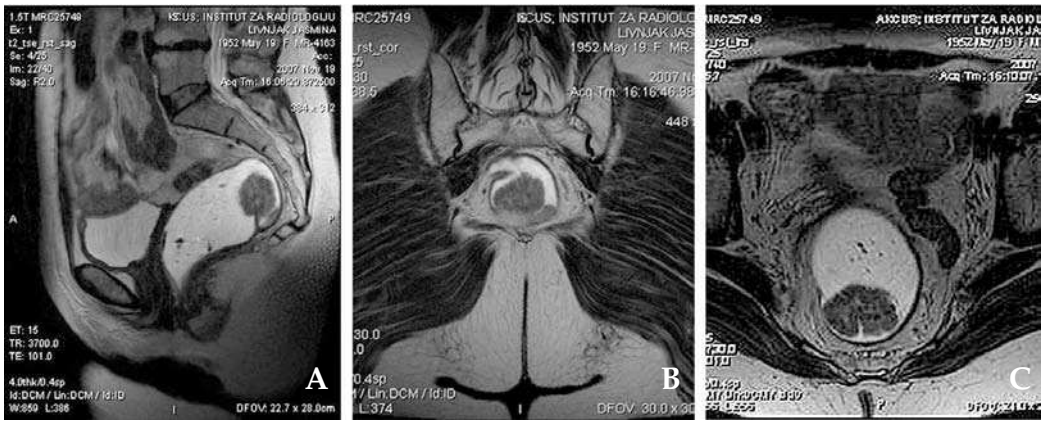
Figure 1. Colonoscopic appearance of tubovillous adenoma.

d'Or indicate that 70% of polyps detected during colonoscopy are smaller than 1 cm in diameter, which represents a strategic problem in prevention. In addition, most of these small polyps are not adenomas but mucosa hyperplasia without malign potential. Having in mind that the risk of malign adenoma transformation is 10 years average, and that small lesions have no clinical potential to turn into cancer, their removal would lead to unnecessary complications and additional costs.[5]

Around 40-50% of all cancers are in rectum. The question is what to do when we detect a polyp through MRI. Target groups are, in any case, polyps larger than 1 cm which could have a villous and displastic component. The standpoint that all polyps larger than 1 cm must be removed either endoscopically or surgically is generally accepted. There are no concrete agreements as to polyps smaller than 1 cm. The possibility of malign polyp transformation smaller than 5 mm is less than 1%, and of 6-9 mm exactly 1%. Thus, there are opinions that polyps smaller than 5 mm should be monitored in screening interval from 5-10 years since it is believed that such many years it takes for their malignant degeneration. There are also opposing opinions that any polyp, no matter of size, should be removed. Flat adenomas or non-polypoid adenomas are defined as lesions with flat morphology and are less than 2 mm of height. There are controversies about the significance of such lesions having in mind their frequency – 8.5-42%. The frequency of malign degeneration is not known. Optical colonoscopy can anticipate these lesions even when using enlargement and chromoscopy. It can be expected that both CT colonographically and MR colonographically anticipate these lesions.

Case report

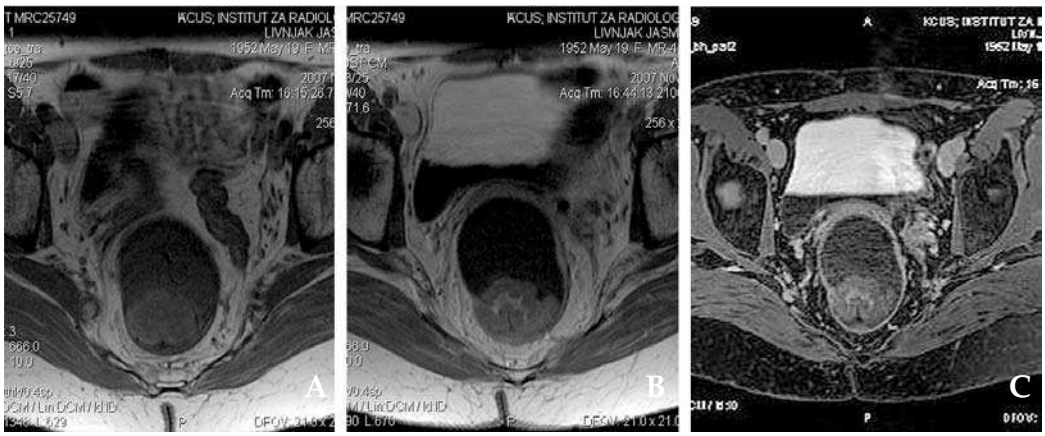
Our patient is 55 and has a year long history of rectal polyp. She has refused any treatment of polyp removal up so far. Due to stool problems, mostly constipation, occasional bleeding and falling out feeling, she has decided to remove the polyp. The polyp has been detected through colonoscopy and described as very risky for polypectomy due to its suspected malignant appearance (Figure 1). Colonoscopy operator could not give information about possible malignant alteration, infiltration of rectum wall or penetration into mesorectal tissue.



T2 tse sag T2 tse cor T2 tse tra

Figure 2a,b,c. MR rectum imaging. Lumen of rectum filled with the ultra sound gel appeared as water in T2 in intensive homogeneous hyper signal, and polyp in hypo signal.

We did rectum MRI on 1.5T Siemens, so that the patient came with clean lumen into which we applied ultra sound gel with huge 60 ml syringe (no needle) simply and pain free with three fillings (total 180 ml of gel). We took out the syringe and gel was left in lumen, it did not leak out. The patient felt very comfortable lying on her back. We did rectum MRI following the appropriate protocol and intravenous (IV) application of gadolinium contrast produced by Schering (Magnevista) in the amount of 10ml. We used Body matrix coil placed on pelvis so that the lower edge of coil was below the pubic bone. Coil was attached with a belt, and the patient entered the machine head forward. The protocol has the following sequences -T1fl3d cor fsFOV400 slice thickness 2 mmTR 3.25 ms PE 1.2 mls voxelsize 1.7 x 1.6 x 2 mm. T2 trufi 3d cor FOV 450, slth1 mm TR 4.09, TE 1.8 voxelsize 1.6 x 1.4 x 1, T2 tsesag FOV 280 slth4mmm TR 3700, TE 101 voxel size 0.7 x 0.7 x 4, Afterwards T2tse tra FOV 210, slth 4 mm TR 3730, Te



T1 tse tra T1 tse tra (post cm magnavist 10 ml) T1 vibe fs tra

Figure 3a,b,c. MR rectum imaging. The polyp itself appeared in T1 in hypo signal very clearly defined against rectum lumen.

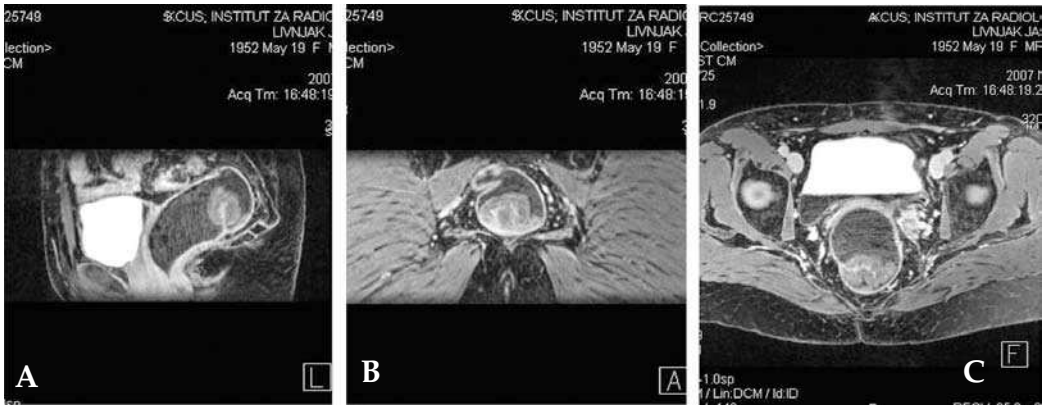


Figure 4a,b,c. MR rectum imaging. After IV application of gadolinium, the polyp as well as the rectum wall raised the signal intensity showing a clear polyp characterization.

101 voxelsize 0.8 x 0.8 x 4. T2 cor FOV 300 slth 4 mm, TR 5230 Te 99, voxelsize 0.7 x 0.7 x 4. Vibe T1 fs tra FOV 450, TR 4.99, Te 2.61, slth 2.5 mm voxelsize 2.7 x 1.8 x 2.5 T1 tsetra Fov210, slth4mm, TR 666, TE10, voxelsize 0.8 x 0.8 x 4. We got excellent polyp and rectum lumen images. Lumen of rectum filled with the ultra sound gel appeared as water in T2 in intensive homogeneous hyper signal, and polyp in hypo signal (Figures 2a-2c). The polyp itself appeared in T1 in hypo signal very clearly defined against rectum lumen (Figures 3a-3c). After IV application of gadolinium, the polyp as well as the rectum wall raised the signal intensity showing a clear polyp characterization (Figures 4a-4c).

Central part and stalk were of the same larger intensity compared to other parts of the polyp. We have concluded that the polyp was of uneven outline and stretched partially along the inner rectum wall without any extra rectal infiltration into mesorectal area. After that, we performed the endoscopic polypectomy according to peace meal method resection up to real muscular layer after adrenalin undermining (Figure 5). Substance was sent to pathohistological analysis in two containers, one with upper parts of polyp and the other with lower base parts. Pathohistological findings of both substances, which were done in HE technique, showed tubovillous adenomas. Pathohistological diagnose of base parts of substances in the first container was *Particulae adenomatis tubullovilosi cum dysplasia gradus gravis focalis colonis*. Pathohistological diagnose of the upper part of polyp reads *Partticula adenomatis tubullovilosi inflammati cum dysplasia gradus gravis focalis epithelii superfitialis et glandularum adenomatis colonis*.

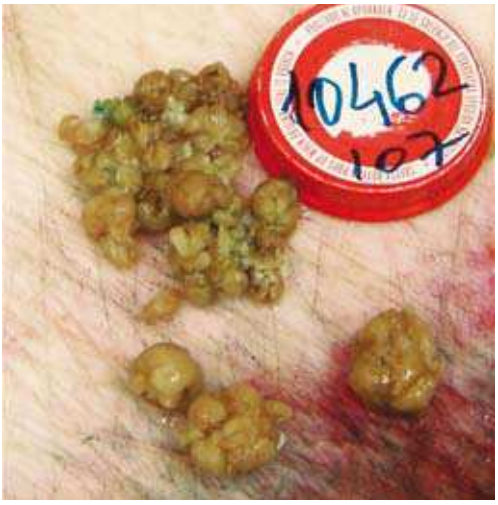


Figure 5. Macroscopic appearance of tubovillous adenoma.

Discussion

Irigography and classic colonoscopy have been used to diagnose polyps so far. We have recently started using CT colonography, MR colonography or targeted MR of rectum, as well as endoscopic ultra sound (EUS). The accuracy of pathological lesions detected through EUS is between 69-97%. It is the oldest and most appropriate widely used imaging technique. Unfortunately, it is not suitable for the evaluation of progressed tumour processes. EUS detects anatomical layers of the rectal wall, but not the relation of tumour and mesorectal fascia. The accuracy of CT colonography in detecting polyps is between 60% for polyps under 1 cm and 100% for those larger than 1 cm.[7] The accuracy of irigography for polyps is between 40%-70%.[8] The accuracy of classic colonoscopy for polyps is up to 85%.[9]

It is known that MRI represents imaging modality with the highest contrast between the soft tissues. This is the reason why the MRI is used for rectal cancer staging. The first initial results with MRI were disappointing. The initial results were so poor due to the use of only whole body-coil systems which at first made bad spatial resolution. Once we started using endorectal coil, we have achieved satisfactory results as well as with EUS.[10,11] However, endorectal coil shows several shortcomings. As in EUS field of view (FOV) is pretty small and enables the evaluation of non-progressed rectum processes but does not enable the depiction of other pelvic anatomic structures.[12] Besides, the insertion of endorectal coil can be very painful and uncomfortable in progressed pathological processes. Some authors fill rectum with positive or negative enteral contrast agent while some are of opinion that no preparation is necessary. The application of spasmolytic drug or IV administration of gadolinium contrast agent is also the subject to discussion. There are controversies regarding the need to apply IV administration of gadolinium. Many are of opinion that it is unnecessary since the tumour is well visualized in T2 sequences if the rectum is thoroughly distended with water. Wallengren *et al.* examined the patients using ferristene solution and IV administration of gadodiamide and achieved 100 % sensitivity. Lupo *et al.* compared the results of pathological process examination without enema and with water enema and proved that the accuracy is higher with water enema –up to 84%. Today, it has also been experimented with MRI of colorectal area without the prior enteral purification and the use of lactulose *per os*, and water *per rectum*. In that case, lactulose would mark faeces with hypo intense dark (faecal tagging) while polyps and tumours would remain light -i.e. hyper intense.[1]

Some studies suggest barium[13] or super paramagnetic iron oxide[14] as rectal MRI contrast agent. The use of ultra sound gel as endorectal contrast agent is cited in the work of Fletcher and Bharuche. While studying anorectal structures and functions, Berman *et al* examined the purposefulness of MRI in anorectal dysfunctions including fistulas, abscesses and tumors.[15-17] Halligan deals with pelvis MRI dynamics.[18] In future we can expect similar researches with ultra sound gel and filling of not only rectum but maybe even vagina in examinations of gynaecological illnesses.

Conclusions

Rectal MRI is a new, very reliable method of contemporary radiological imaging that gives better characterization of polyp tissue and of other tumours. It is currently the best imaging modality enabling a very accurate evaluation and topographic ratio of tumour growth and rectum wall and

outside the wall, especially compared to mesorectal fascia. In addition, it is a very comfortable procedure without radiation. The application of ultra sound gel as intra luminal rectal contrast agent can distend the lumen and make an excellent contrast of lumen against the rectum wall and thus better show polyps and tumours.

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research article

Influence of magnesium sulphate infusion before total thyroidectomy on transient hypocalcemia – a randomised study

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Background. *Transient hypocalcemia is the most common complication after thyroidectomy. Normomagnesemia is needed for normal secretion of PTH and end-organ responsiveness. Our aim was to determine the influence of infusion of magnesium sulphate before thyroidectomy on the incidence of laboratory and clinical transient hypocalcemia.*

Methods. *In our prospective study, 48 patients (5 men, 43 women; age 22-73 years, median 45 years), who underwent total or near-total thyroidectomy, were randomised preoperatively. Half of them received intravenously 4 ml of 1M magnesium sulphate at the beginning of the surgical procedure, the other half were the control group. Serum concentrations of calcium, ionised calcium, magnesium, phosphate, albumin and PTH were measured prior to surgery and on the first day after surgery.*

Results. *Laboratory postoperative hypocalcemia was present in 27% of patients and 23% of patients had clinical signs and/or symptoms of postoperative hypocalcemia. The concentration of total calcium (p=0.024) and of albumin (p=0.01) was lower in the group that received magnesium sulphate.*

Conclusions. *The patients who received infusion of magnesium sulphate before total thyroidectomy had lower concentration of total serum calcium and albumin in comparison to the control group. There was no statistical difference in the incidence of clinical transient hypocalcemia.*

Key words: magnesium sulphate; transient hypocalcemia; randomised study; total thyroidectomy

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Introduction

Transient hypocalcemia is the most common complication after total thyroidectomy (TT) and usually fades away in a few days.[1] The incidence of transient hypocalcemia after total thyroidectomy is 6.2 – 68%.[2,3] Transient hypocalcemia may be very unpleasant to the patient. It may entail a few

Table 1. Characteristics of 48 patients

Factor	Subgroup	Number of patients (%)
Gender	Male	5 (10)
	Female	43 (90)
Aplication of magnesium sulfate	Yes	24 (50)
	No	24 (50)
Ca lower (<2.1 mmol/L) postoperatively	Yes	13 (27)
	No	35 (73)
Ionised Ca lower (<1.12 mmol/L) postoperatively	Yes	23 (52)
	No	21 (48)
Mg lower (<0.7 mmol/L) Postoperatively	Yes	3 (6)
	No	45 (94)
PTH lower (<12 ng/L) postoperatively	Yes	6 (13)
	No	40 (78)
Albumin lower (<34 g/L) postoperatively	Yes	4 (9)
	No	40 (91)
Paresthesia postoperatively	Yes	9 (19)
	No	39 (81)
Muscular spasms postoperatively	Yes	2 (4)
	No	46 (96)
Chvostek's sign postoperatively	Yes	7 (15)
	No	41 (85)
Symptoms/signs of postoperative hypocalcemia	Yes	11 (23)
	No	37 (77)
Therapy of postoperative hypocalcemia	Yes	15 (31)
	No	33 (69)
Histologicaly parathyroid tissue in bioptic sample	Yes	5 (10)
	No	43 (90)
Permanent hypocalcemia	Yes	0 (0)
	No	100 (0)

more laboratory tests and sometimes longer hospitalisation, thus increasing treatment costs.

Many authors tried to find out how to prevent postoperative hypocalcemia. To our knowledge only three prospective randomised studies were done.[4-6] Bellantone *et al*.[5] and Tartaglia *et al*.[6] treated their patients with oral calcium salts and calcitriol. Uruno *et al*. proved that prophylactic infusion of diluted calcium after total thyroidectomy can effectively inhibit the development of symptomatic hypocalcemia.[4] But it should be noted that intravenous application of calcium may cause thrombophlebitis or even skin necrosis. On the other hand, magnesium is known to have an impact on the calcium homeostasis in the serum,[7] whereas parenetral application of magnesium sulphate is safe and without

side effects.[8] The aim of our study was to verify whether intravenous application of 4 ml of 1 M magnesium sulphate before thyroidectomy reduces the occurrence of laboratory and clinically transient postoperative hypocalcemia.

Patients and methods

Only the patients who gave their informed consent were included in our study. The study was approved by the Protocol Review Board and Committee for Medical Ethics at the Institute of Oncology Ljubljana and by the Medical Ethics Committee of the Republic of Slovenia (Ref. No. 29/02/05) and was performed in accordance with the medical ethics standards laid down in Declaration of Helsinki of 1975, as revised in 1983.

In our prospective study, 48 adult patients (5 men, 43 women; age range 22-73 years, median 45 years) who underwent total or near-total thyroidectomy were preoperatively randomised into two groups. Half of the patients received intravenously 4 ml of 1 M magnesium sulphate after induction of anaesthesia and immediately after skin incision (before thyroid part of surgical procedure); the other half of the patients were in the control group. Total or near-total thyroidectomies were performed from May 2005 to June 2006 at the Institute of Oncology Ljubljana, by two surgeons, both experienced in thyroid surgery. The patients with lymph node dissection of the central neck compartment, hyperthyroidism, hypercalcemia or renal insufficiency were not included in the study. None of our patients was preoperatively treated with antireabsorbtive drugs, diuretics, antiepileptics or with supplementation of calcium, vitamin D or magnesium.

Total or near-total thyroidectomy was performed because of benign goiter and follicular neoplasm in 14 and 34 patients, respectively. Among patients wilth follicular neoplasm, a definitive histology showed follicular variant of papillary carcinoma, follicular carcinoma and follicular adenoma in 19, 4 and 11 patients, respectively.

Serum concentrations of calcium, ionised calcium, magnesium, phosphate, albumin and PTH were measured prior to surgery and on the first day after surgery. When hypocalcemia was detected postoperatively, the serum concentration of calcium, magnesium, phosphate, albumin and PTH was measured till the normalisation of laboratory results (daily during the first week after the operation and once in the second week).

In order to detect a statistical difference between the groups and 10% change in serum calcium concentration (*i.e.* 0.2 mmol/L) with our sample size (*i.e.* 24 patients in each group), the power calculation for our trial showed that it was 90%.

Laboratory hypocalcemia

Hypocalcemia was defined as transient if: (1) serum calcium concentration dropped to the value lower than 2.1 mmol/L and (2) hypocalcemia faded away in six months. A cut-point concentration value of low serum ionized calcium was 1.12 mmol/L, and of low PTH, it was 12 ng/L.

If hypocalcemia is present after 6 months, it is permanent. Permanent hypocalcemia was present if vitamin D supplementation was still required six months after thyroidectomy.

Clinical hypocalcemia

The presence of the symptoms and/or signs of transient hypocalcemia was followed by the two surgeons and their team and registered into a special questionnaire. In the first four days after the operation

Table 2. Comparison of preoperative and postoperative laboratory values in the group of patients having received the magnesium sulphate infusion (Group 1) and the other that had not (Group 2)

Factor	Group	Preoperative median value	P-value	Postoperative median value	P-value
Calcium (mmol/L)	1	2.40	0.316	2.11	0.024
	2	2.42		2.20	
Ionised calcium (mmol/L)	1	1.22	0.437	1.10	0.235
	2	1.23		1.13	
Magnesium (mmol/L)	1	0.87	0.636	0.82	0.670
	2	0.88		0.81	
Phosphate (mmol/L)	1	1.10	0.103	1.36	0.288
	2	1.17		1.29	
Albumin (g/L)	1	43.9	0.144	37.3	0.010
	2	45.1		40.4	
PTH (ng/L)	1	14.4	0.619	27.6	0.331
	2	17.5		32.3	

the following symptoms and/or signs of transient hypocalcemia were registered: paresthesias, spasm, carpopedal spasm, and Chvostek's sign. Chvostek's sign was regarded as positive when a facial muscle spasm was induced by the percussion of the facial nerve. Clinical transient hypocalcemia was defined as the occurrence of any of its symptoms (limb or perioral parasthesia, cramps) and/or signs (carpopedal spasm, Chvostek's sign) that developed after thyroidectomy and faded away in six months.

The data about patients' age and gender, pathological diagnosis, presence of parathyroid tissue in bioptic specimen, laboratory results, presence of clinical signs and symptoms of hypocalcemia, duration of hospitalisation and therapy of hypocalcemia were collected. Hospitalisation was defined as the time interval from surgery to the day of release from the hospital. Any therapy (i.v. or oral administration of calcitriol, calcium salts or magnesium salts) applied postoperatively was specified as postoperative treatment of hypocalcemia.

Statistical analysis

The patients were divided into two groups: the first one that received an intraoperative infusion of magnesium sulphate and the other that did not. We compared the preoperative and postoperative data of the first group to those of the other one. Analysis of variance was used for the evaluation of the effect of magnesium sulphate infusion on the continuous laboratory variables (calcium, magnesium, phosphate, albumins and PTH), whereas the effect of magnesium sulphate infusion on nonparametric variables (clinical hypocalcemia) was analysed by Fischer's exact test. The analysis of variance was also used for the comparison of duration of hospitalisations of both groups, while Fisher's exact test was used for the comparison of the rate of patients in both groups requiring postoperative treatment of hypocalcemia. Statistical analysis was made by the computer program SPSS for Windows, version 13.0 (SPSS Inc., 2004).

Results

Characteristics of the patients are presented in Table 1. Before thyroidectomy, the concentration of calcium, ionised calcium, magnesium, phosphate, albumin and PTH of the first group did not significantly differ from those of the second group (Table 2). The first day after the operation (Table 2), serum calcium concentration was lower in the group having received the magnesium sulphate infusion in comparison to the control group (p=0.024). Postoperative serum albumin concentration was lower in the group having received the magnesium sulphate infusion in comparison to the control group (p=0.01).

The comparison of the occurrence of symptoms and/or signs of postoperative hypocalcemia did not show any statistically significant difference between the patients who received the magnesium sulphate infusion and those who did not (Table 3).

The duration of hospitalisation of the group that received magnesium sulphate did not significantly differ from that of the control group (p=0.370). The proportion of patients who were treated for hypocalcemia was not significantly different in the patients who received magnesium sulphate in comparison to the control group (p =0.755).

Pathological examination confirmed the presence of parathyroid gland tissue in the bioptic material of five patients, while in the bioptic material of the remaining 43 patients, no parathyroid tissue was detected. The concentrations of calcium (p=0.013), ionised calcium (p=0.06), and PTH (p=0.007), measured postoperatively, were lower in the group of patients with histologically confirmed presence of parathyroid tissue, whereas the phosphate concentration in this group of patients was higher than in other patients.

Discussion

Pathophysiological mechanism of postoperative hypocalcemia after total thyroidectomy has not been fully explained; but, it has been agreed that it is definitely multifactorial. Potential mechanisms causing transient hypocalcemia after total thyroidectomy are: transient perioperative hemodilution,[9,10] increased calcitonin release,[11,12] abnormal functioning of the parathyroid glands due to edematous gland or transient insufficiency in blood supply,[13] and impairment or unintentional excision of the parathyroid gland.[9,14,15] Some parathyroid glands may be anatomically intact; however, their function may nevertheless be physiologically impaired because of parathyroid arteria occlusion or edematous gland.[16,17] Intraoperative change of the colour of parathyroid is believed to be an indicator of insufficient blood supply; however, Ander *et al.* proved that the disorders in the microcirculation of parathyroid glands are not related to their macroscopic appearance.[18] We claim that, in our case, the experience of the surgeon could not significantly affect the outcome of surgery because both surgeons applied similar surgical procedures and were also equally skilled. The incidence of an incidentally detected parathyroid tissue in histologic material (10.5%) is comparable to that in other similar studies, yet still at lower level of the incidence range reported in the literature (9-15%).[19-21]

The incidence of transient hypocalcemia after thyroidectomy reported in the literature differs from study to study[14,22-26] due to the differences in the reference calcium values of laboratory test, definitions of laboratory transient hypocalcemia and clinical transient hypocalcemia. In our study, the rate of the patients with laboratory transient hypocalcemia (27%) and those with clinically transient hypocalcemia (23%) was in the interval of 6.2-68%, which was simi-

Table 3. Comparison of symptomps and/or signs of hypocalcemia in the group of patients having received the magnesium sulphate infusion (Group 1) and the other that had not (Group 2)

Symptom / sign of hypocalcemia	Group	Patients with Symptom/sign	P-value (fisher's exact test)
Paresthesia	1	6	0.46
	2	3	
Muscular spasms	1	1	1.000
	2	1	
Chvostek's sign	1	4	1.000
	2	3	
Any symptom or sign	1	6	1.000
	2	5	

lar to that reported in the literature.[2,3,14,22-26] In the study by Wilson *et al.*, in which the definitions of laboratory and clinical hypocalcemia are similar to the definitions in our study, 68% of patients were diagnosed with laboratory transient hypocalcemia and 36% of patients had symptoms of hypocalcemia.[3] They defined hypomagnesemia as serum concentration of magnesium lower than 0.7 mmol/L and the same definition was used in our study. They reported that the incidence of postoperative hypomagnesemia was considerably higher (72%) than that in our patients who did not receive infusion of magnesium sulphate (2/24 patients, *i.e* 8%). It is established that hypomagnesemia may well contribute to postoperative tetany after total thyroidectomy, especially with concomitant hypocalcemia.[3] Possibly, the cause of hipomagnesemia in the study by Wilson *et al.* [3] is the volume of fluids that were given. The volume of fluids correlated with hypomagnesemia (p=0.027). Namely, the patients with severe hypomagnesemia were given on average of 4.93 liters during the 24-hour perioperative period, compared to 4.69 liters in mild hypomagnesemia and 3.64 liters in normomagnesemia patients.[3] Our patients during the 24-hour perioperative period were given only 1.5 to 2.5 liters of fluids. There was no statistical difference of the given volume of fluids in the group of our patients who received intraoperative infusion of magnesium sulphate in comparison to the control group.

The results of our study demonstrate that intraoperative infusion of magnesium sulphate does not reduce the incidence of laboratory hypocalcemia. On the contrary, the first day after operation, the total serum concentration of calcium was statistically significantly lower (p=0.032) in the group of patients who received intraoperative infusion of magnesium sulphate in comparison to the control group. A possible mechanism which could be the reason of the decreased total serum calcium concentration is the drop of PTH serum concentration caused by the infusion of magnesium sulphate. This assumption was supported by the study in which magnesium was infused to healthy subjects;[27] the PTH serum concentration dropped significantly in five minutes after the infusion.[27] But, the two groups of patients in our study do not differ significantly between each other either in the PTH serum concentration or in ionised calcium concentration the first day after the operation; therefore, the above mechanism does not provide a satisfactory explanation of the drop of calcium concentration in the group of patients who received magnesium sulphate infusion.

Another mechanism which could be the cause of the decreased total serum calcium concentration is the drop of albumin concentration. The total serum calcium concentration depends, in fact, on the albumin concentration. One gram of albumins binds approximately 0.7 mg of calcium. Falk *et al*. observed that a significant drop of albumin concentration occured the first and the second day after thyroidectomy.[28] They assumed that the decreased concentration of the albumin induced by non-specific release of antidiuretic hormone (ADH) and hemodilution.[28] This might have been the most probable mechanism that decreased the total calcium concentration in our patients who were treated with magnesium sulphate infusion. Significantly lower total serum calcium concentration ($p = 0.024$) and lower serum albumin concentration ($p = 0.01$) was observed in the group of patients with application of magnesion sulphate in comparison to the control group. The mechanism of a more intense drop of albumin as a consequence of magnesium sulphate application has so far not been clarified and needs further investigation.[29,30]

In terms of the incidence of postoperative clinical simptoms and/or signs of hypocalcemia, no statistically significant difference was observed between the two groups of patients. This was predictable from the laboratory test results. In the group treated with magnesium sulphate infusion, a statistically significantly lower total calcium concentration was observed in comparison to the control group, whereas the concentration of ionised calcium as well as PTH was not significantly different. As ionised calcium is a more reliable indicator of laboratory and clinical hypocalcemia than the total calcium concentration,[28] it is understandable that the proportion of the patients with clinical hypocalcemia was the same in both groups of patients. Furthermore, it is also logical that there were no statistically significant differences between the two groups in the rate of the patients who were treated for hypocalcemia. In addition, there were no statistically significant differences between the two groups in the duration of hospitalisation.

Conclusions

The patients who are treated with intraoperative magnesium sulphate infusion have statistically significantly lower total calcium and albumine concentration than the patients in the control group. The two groups of patients does not differ significantly in the incidence of the transient clinical hypocalcemia, the duration of hospitalisation or the rate of patients who were treated for hypocalcemia.

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research article

Prognostic value of immunohistochemical expression of HER-2/neu in patients with lung carcinoma

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Background. *The amplification and the overexpression of the Her-2/neu gene have been shown in certain human tumours and are postulated to be important in human carcinogenesis. In this study we evaluated the expression of HER-2/neu gene in patients with lung carcinoma (LC) and assessed its prognostic significance.*

Patients and methods. *HER-2/neu expression was determined in 127 LC patients using immunohistochemistry (IHC) performed on paraffin-embedded section – Hercep Test™ (DAKO).*

Results. *The overall HER-2/neu expression was seen in 36 (28.35%) of 127 LC patients. According to the histological type, HER-2/neu overexpression was detected in 12 patients with adenocarcinomas (60%), in 19 patients with squamous cell carcinomas (31.14%), in 4 patients with small cell-lung carcinomas (10%) and in 1 patient with other carcinomas (16.66%). Only in patients with small cell-lung carcinomas HER-2/neu overexpression was in correlation with the stage of the disease ($p<0.001$). The patients with HER-2/neu positive expression were associated with a significantly shorter survival compared with those who were HER-2/neu negative (log rank, $p<0.002$).*

Conclusions. *These observations suggest that HER-2/neu positivity may serve as a prognostic indicator in patients with LC. HER-2/neu plays a role in identifying patients at risk for the shortened survival, who may benefit from a more aggressive therapy.*

Key words: HER-2/neu, lung cancer; immunohistochemisty; survival

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Introduction

Lung cancer is the leading cause of cancer death worldwide with a still increasing incidence.[1,2] Despite new chemotherapeutic

drugs, improvement in surgical techniques, histological classification, and staging procedures, lung cancer survival has not greatly improved over the past 20 years. The cure rate remains less than 15%.[3] Recent advances in biology and molecular biology identified the relationship between specific gene alterations and clinical behaviour of lung cancers. A number of studies have been performed to assess the prognostic role of tumour-suppressor genes and proto-oncogenes.

HER-2/neu (also known as c-erbB2) oncogene is the second member of the epidermal growth factor of the receptor family.[4] The HER-2/neu dominant gene is localized in normal human cells as a singular copy on the long arm (q21) of chromosome 17.[5] This gene codes for a 185-kDa receptor-type tyrosine protein kinases (p185neu or c-erbB2), similar to EGF-R.[6-8] This 1255 aminoacid transmembrane glycoprotein is composed of there domains: extracellular factor-binding domain, transmembrane domain and intracellular domain with a tyrosine kinase activity.[9] When an EGF-like ligand (there is no known specific ligand for HER-2/neu) binds to a receptor of the EGF-R family, (HER-1, 3 or 4) there is a heterodimerisation of this receptor with HER-2.[10] HER-2 is necessary for the regulation of the normal cell growth and differentiation, and can be associated with multiple signal transduction pathways.[11] The amplification of the HER-2 gene leads to an overexpression of the receptor and protein product p 185, which could be implicated in the development of many types of tumours. HER-2/neu is expressed in a wide variety of human epithelial malignancies, including breast, ovary, salivary gland, gastrointestinal tract, prostate, lung, kidney, liver.[12-18] This suggests that HER-2/neu overexpression probably plays a critical role in the development and progression of human cancers.

The aim of this study was to determine the correlation between HER-2/neu expression and clinicopathological features in patients with lung carcinoma and to assess its relation to the survival.

Patients and methods

Patients and tissue samples

Our study population consisted of 127 patients with lung carcinoma (LC) diagnosed between January 2004 and December 2006 in the Institute for Lung Diseases and Tuberculosis, Skopje, Macedonia. Patients included 115 men and 12 women. Their age ranged from 37 to 87 years old (mean, 58.49 ± 8.15); 80.10% of them were smokers.

The diagnosis of LC was established by the histological examination of tissue samples obtained during the bronchoscopy in 120 patients (94.48%) or by surgery in 7 patients (5.51%). All histological analysis of the tumour tissue and immunohistochemistry (ICH) were performed at the Institute for Pathology, Medical Faculty, Skopje. The histological type and degree of differentiation were assigned according to World Health Organization criteria.[19] Tumours included 40 small-cell lung carcinomas (SCLC) and 87 non-small-cell lung carcinomas (NSCLC) – 20 adenocarcinomas, 61 squamous cell carcinomas and 6 tumours assigned as "others" (bronchoalveolare, large cell carcinomas, nondifferentiated carcinomas). For the histological differentiation, well moderately and poorly differentiated tumours were graded as grade 1, 2 and 3, respectively.

According to the International Staging System for Lung Cancer,[20] 87 NSCLC patients were divided in: 3 patients with IB stage (3.44%), 1 patient with IIA stage (1.14%), 11 patients with stage IIB (12.64%), 22 patients with stage IIIA (25.28%), 34 patients with stage IIIB (39.08%) and 16 pa-

tients with stage IV (18.39%). The patients with SCLC were staging in 23 patients with limited diseases (57.5%) and 17 patients with extensive diseases (42.5%).

One hundred and twenty patients were treated with chemotherapy and/or radiotherapy at the Institute for Radiotherapy and Oncology, Medical Faculty, Skopje. Seven patients underwent surgery first, and after that they were treated with radiotherapy. All patients were followed up regularly in a time frame of 2 to 3 months. The patients were followed up from 1 to 48 months (median 24 months). At the time of the last follow-up 114 patients (89.76%) had died and 13 patients (10.23%) were still alive. The survival time was calculated from the date of histological diagnosis to the date of death or last follow-up.

Immunohistochemistry

HER-2/neu oncogene expression was determined by immnunohistochemical staining with Hercep Test^{tm} (DAKO, Copenhagen, Denmark). After bronchoscopy or surgery, fresh tumour tissue specimens were immediately formalin fixed. Section 4 μm thick were cut from tissue blocks, placed on glass slides and exposed to xylene 3 times for 5 min each. Tissues were hydrated in decreasing concentrations of ethanol (100%, 95% and 70% for 2min each) and rinsed in distilled water. Slides were washed in PBS (Phosphate Buffer Saline) for 20 min. After excess liquid was blotted off, slides were heated at 120°C in 0.01 M citrate buffer (pH 6.0) to expose the HER2 protein antigen. The endogene peroxidase activity was blocked by Peroxidase Blocking Reagent for 5 min. The rabbit antihuman HER2 protein polyclonal antibody was applied for 2 h at room temperature. Slides were rinsed with PBS, and peroxidase-conjugated antirabbit antibody was added for 30 min at room temperature. Diaminobenzidine solution was added to achieve specific staining. Slides were counterstained with hematoxylin.

The evaluation of HER-2/neu immunoreactivity was performed according to the DAKO protocol for the Hercept Test^{tm}, with minor modifications. Only membrane staining was considered positive, whereas cytoplasmatic staining was considered nonspecific. Immunostaging was classified as follows: score 0: no staining at all or membrane staining in <10% neoplastic cells; score 1+: a faint/barely appreciable membrane staining is detected in >10% of the tumour cells; the cells are only stained in part of their membrane; score 2+: a with weak to moderate complete membrane staining is observed in >10% of the tumour cells; score 3+: a strong immunoreactivity of the entire membrane is observed in >10% of the tumours cells. Tumours classified as 0 or 1+ were considered "negative", and those scored 2+ or 3+ were classified as "positive".

Statistical Analysis

Statistical significances of the relationship between clinicopathological data and IHC were assessed by a chi-square test. The Fisher's exact test was used when the frequency of a cell in a 2 x 2 table was <5. The overall survival rate was calculated using the Kaplan-Meier method. The difference was considered to be statistically significant at $p<0.05$.

Results

Figure 1 shows the staining of HER-2/neu in adenocarcinoma using immunohistochemical methods. The dark brown colour denotes positive staining located predominantly at the cell membrane.

Quantitative data are shown in Table 1, which summarizes expressions of HER-2/neu

Table 1. The relationship between the expression of the HER-2/neu and clinicopathological features in patients with lung cancer

Features (n)	HER-2/neu + n (%)	p Value
Sex		
Male (115)	34 (29.56%)	
Female (12)	2 (16.66%)	NS
Age		
≤ 65 (90)	25 (27.77%)	
> 65 (37)	11 (29.72%)	NS
Smoking history		
Smoker (103)	27 (26.21%)	
Nonsmoker (24)	9 (37.5%)	NS
Performans status		
WHO 0 (27)	2 (7.40%)	
WHO 1 (79)	26 (32.91%)	
WHO 2 (11)	2 (18.18%)	
WHO 3 (9)	6 (66.66%)	
WHO 4 (1)	0 (0%)	p<0.006
Tumour type		
SCLC (40)	4 (10.0 %)	
NSCLC (87)	32 (36.78 %)	p<0.001
Differentiation		
Well (3)	2 (66.66 %)	
Moderate (55)	14 (25.45 %)	
Poorly (69)	20 (28.98 %)	NS
Stage (NSCLC)		
I B (3)	0 (0 %)	
II A (1)	0 (0%)	
II B (11)	4 (36.36 %)	
III A (22)	7 (31.81 %)	
III B (34)	11 (32.35 %)	
IV (16)	10 (62.5 %)	NS
Stage (SCLC)		
Limited (23)	0 (0 %)	
Extensive (17)	4 (23.52 %)	p<0.001
Total (127)	36 (28.35%)	

Note: statistical analysis was performed using x^2 test and Fisher exact test

in 127 LC patients with different clinicopathological features.

HER-2/neu immunoreactivity (3+/2+) was detected in 36 of 127 lung cancers cases (28.35%). Fifteen tumours (11.81%) showed strong HER-2/neu immunoreactivity (3+), while twenty one (16.54%) tumours were moderately immunoreactive (2+). Ninety-one cases (71.65 %) were considered negative; 81 cases were classified as score 0 (63.78 %) and 10 cases as score 1+ (7.87%).

As seen in Table 1, no statistically significant correlation was found between the frequency of HER-2/neu protein product expression and some clinicopathological

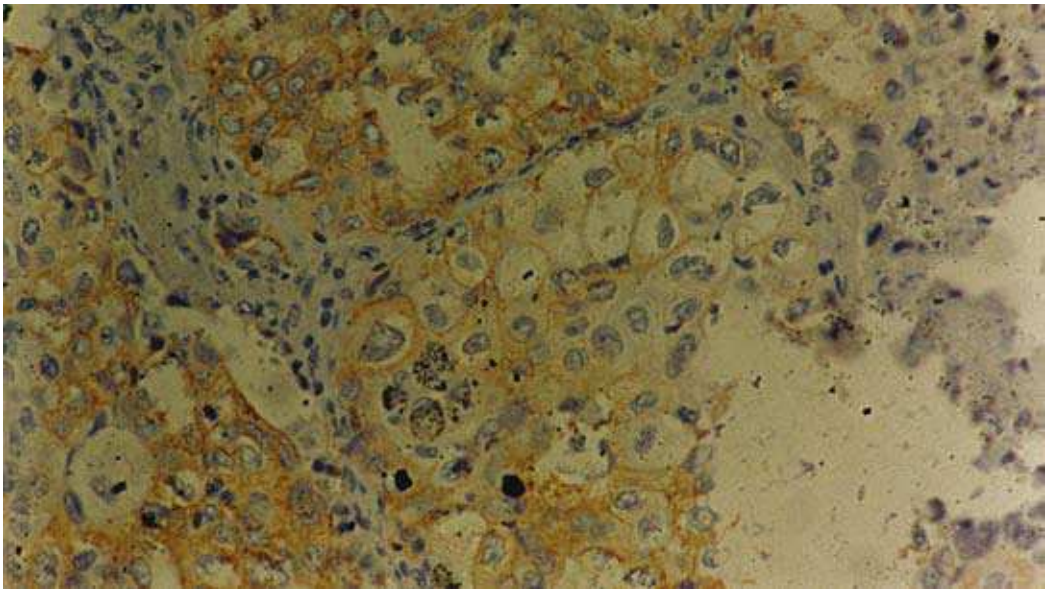

Figure 1. Immunohistochemical staining of HER-2/neu overexpression in lung adenocarcinoma (strong staining 3+).

features under the study including sex, age, smoking history and tumour differentiation (well, moderately and poorly). We found the correlation between HER-2/neu expression and performance status (x^2=14.1; p<0.006). We also obtained the significant differences in HER-2/neu expression between SCLC and NSCLC (p<0.001). Among the NSCLC's HER-2/neu expression was found in 12 of 20 (60%) adenocarcinomas, in 19 of 61 (31.14%) squamous cell carcinomas, and in 1 of 6 (16.66%) "other" carcinomas (Table 2). The difference in the expression rate between the adenocarcinomas and the other groups was highly significant (x^2=17.13; p<0.0001). Eight patients (66%) with HER-2/neu positive adenocarcinomas showed strong immunoreactivity (3+).

In NSCLC patients HER-2/neu expression was not in correlation with the stage of the diseases. But, there was a significant difference in HER-2/neu expression between SCLC patients with limited and extensive diseases (Fisher exact test p<0.001).

We found that the expression of HER-2/neu protein was associated with a significant short survival in LC patients. The 24-months survival rates of patients with HER-2/neu overexpresssion and those without were 21.97% and 2.77%, respectively (Figure 2) with a statistical significant difference (log-rank Test = – 3.06; p=0.002).

Discussion

Since HER-2/neu proto-oncogene has been originally identified, its overexpression was detected in various types of cancers, first of all in those with epithelial origin.[21] HER-2/neu expression is associated mainly with high-grade breast carcinoma and considered an important prognostic factor for adverse outcome in node positive breast cancer.[13] In lung cancer studies, HER-2/neu expression varies depending on the histological classification of the tumours.[4,10,22-24] Her-2/neu expression was reported in 13-80% in adenocarcinomas,[17,22,24] in 2-45% in squamous cell carcinomas,[15,16,18] in 0-20% in large-cell carcinomas[23] and in 13-31% in small cell lung carcinomas.[4,25,26]

Table 2. HER-2/neu expression in different histological type of lung cancer

Histological type	No of cases	**Level of expression***				
		+++	**++**	**+**	**–**	**% positive**
SCLC	40	2	2	2	34	10.0
Squamous cell cacinoma	61	5	14	3	39	31.14
Adenocarcinoma	20	8	4	5	3	60.0
Others	6	0	1	0	5	16.6

* –: no detectable HER-2/neu expression; +, ++, +++: faint, weak and strong expression, respectively

We observed HER-2/neu overexpression in 36 of 127 patients with lung carcinoma (28.25%). The overexpression was more frequent in NSCLC (36.78 %) than in the SCLC (10.0%). The patients with adenocarcinoma showed the highest incidence of positive findings (60%), with the biggest number of strong reactivity patterns (53.33%). This observation concurs with the findings of Shi *et al.*, Harpole *et al.*, Pellegriniet *et al.*, Hsieh *et al.* and others.[8,17,27,28]

The techniques used to detect HER-2/neu expression might be one of the potential sources of biases. HER-2/neu protein overexpression is most often measured by IHC using one of several monoclonal antibodies, and the gene expression is measured most often by fluorescence in situ hybridization (FISH) for clinical studies.[5] Immunohistochemical results can vary according to the primary used antibody, dilution of the antibody and tissue conservation. Moreover, this technique is semiquantitative and standards for positive or negative specimen vary between studies making difficult the direct comparison of studies.

In this study we did not find any correlation between HER-2/neu overexpression and the pathologic stage of disease in patients with NSCLC. Even our results showed very low HER-2/neu overexpression in patients with SCLC, we found significant differences of HER-2/neu overexpression between SCLC patients with limited and extensive diseases: HER-2/neu overexpression was seen only in patients with the extensive disease (Fisher exact test $p<0.05$). Tateishi *et al.*[22] reported a higher positivity for HER-2/neu in Stage III-IV disease with a poor influence on the prognosis. Similarly, Osaki *et al.*[29] reported increased serum levels of HER-2/neu in Stage IIIB cases. However, the comparison among different studies is difficult due to a consistent lack of balance in the histotype and the stage distribution from one study to another.

Although HER-2/neu protein expression is widely studied in lung carcinoma, its prognostic role remains uncertain. Even several investigators reported that HER-2/neu immunostaining adversely affects the prognosis and the survival in LC patients, especially NSCLC patients,[17,18,22,23,30] some large studies did not find any prognostic implication for HER-2/neu overexpression.[31-33] There are many studies showing adverse outcomes among patients with breast and ovarian cancers overexpressing this oncogene. Besides, the overall survival rate and time to relapse for those patients with HER-2/neu overexpression is shorter than those lacking the overexpression.[13,21,34] Our results show that patients with HER-2/neu positive tumours had a significantly decreased survival opposite to patients with HER-2/neu negative tumours. After 24 months, only one patient from the group who were HER-2/neu positive was still alive, in comparison with 20 patients from the group who were HER-2/neu negative.

There are studies which have shown that HER-2/neu overexpression is associated with intrinsic chemoresistance of NSCLC.[35]

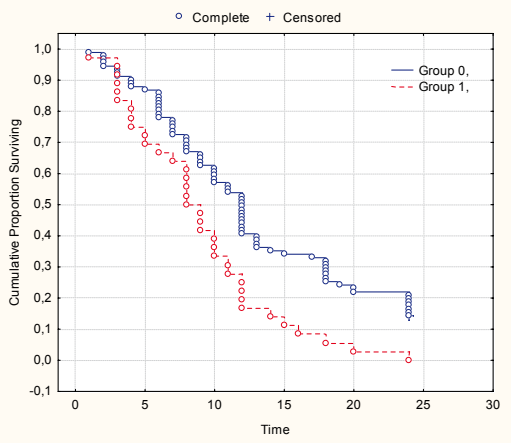

Figure 2. 24-month survival of a patient with lung cancer as a function of absence (solid line) or presence (dashed line) of HER-2/neu overexpression.

Thus, HER-2/neu overexpression may play a role as a predictive factor of response to therapy in patients with LC, who may be candidates for adjuvant therapeutic modalities and the gene therapy possibly improving the overall survival.

Conclusions

In conclusion, HER-2/neu expression detected in LC is a negative predictor of the survival. Immunohistochemical stainig of Her-2/neu may aid in defining a subpopulation of patients with LC whose tumours may behave more aggressively. In addition, focused adjuvant therapeutic modalities and gene therapies might improve the overall survival in these patients expressing the poor prognostic immunohistochemical marker.

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research article

Numerical modeling in electroporation-based biomedical applications

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Background. *Numerous experiments have to be performed before a biomedical application is put to practical use in clinical environment. As a complementary work to in vitro, in vivo and medical experiments, we can use analytical and numerical models to represent, as realistically as possible, real biological phenomena of, in our case, electroporation. In this way we can evaluate different electrical parameters in advance, such as pulse amplitude, duration, number of pulses, or different electrode geometries. Such numerical models can contribute significantly to the understanding of an experiment and treatment planning as well as to the design of new electroporation devices and electrodes.*

Methods. *We used commercially available modeling software, based on finite element method. We constructed a model of a subcutaneous tumor during electrochemotherapy (EMAS) and a model of skin during gene electrotransfer (COMSOL Multiphysics). Tissue-electrode geometries, pulse parameters and current-voltage measurements from in vivo experiments were used to develop and validate the models.*

Results. *To describe adequately our in vivo observations, a tissue conductivity increase during electroporation was included in our numerical models. The output currents of the models were compared to the currents and the voltages measured during in vivo experiments and a good agreement was obtained. Also, when comparing the voltages needed for a successful electropermeabilization as suggested by the models, to voltages applied in experiments and achieving a successful electrochemotherapy or in vivo gene electrotransfer, good agreement can be observed.*

Conclusions. *Modeling of electric current and electric field distribution during cell and tissue electroporation proves to be helpful in describing different aspects of the process and allowing us to design electrodes and electroporation protocols as a part of treatment planning.*

Key words: electroporation; electropermeabilization; electrochemotherapy; subcutaneous tumor; gene electrotransfer; skin; numerical modeling; finite element method

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Introduction

A cell membrane is, in general, impermeable for molecules; however, the application of electric pulses to cells, either in suspension or in tissue, causes structural changes in the cell membrane.[1-3] Cell

membrane is transiently permeabilized due to increased transmembrane voltage caused by external electric field. The phenomenon is also termed electroporation. Even a short electric pulse of a sufficiently high voltage causes an increased permeability of the cell membrane. If the pulse is of adequate amplitude, the electric field and consequently the transmembrane potential are high enough to cause cell membrane permeabilization. The increase in permeability of the cell membrane makes it possible for molecules that otherwise can not cross the membrane, such as drug molecules or DNA, to enter the cell. After exposure to electric pulses, cell membrane reseals provided the applied voltage was not too high to cause permanent damage. Currently, the most widely used applications of electroporation are electrochemotherapy, gene electrotransfer and transdermal drug delivery. The outcome of the electroporation depends on cell and tissue parameters and, most of all, electric pulse parameters.

Electrochemotherapy is one of the most advanced and efficient biomedical applications of electroporation. It is a combination of chemotherapy and electric pulses aimed at temporarily permeabilizing tumor cell membranes to introduce drug molecules more efficiently into the cells. The results of clinical studies show a highly increased efficiency of bleomycin and cisplatin when used in combination with electric pulses.[4-6] Another promising application of electroporation is gene electrotransfer into cells. It is a method using electric pulses to temporarily and reversibly permeabilize the cell membrane and to drive the DNA into the cell electrophoretically.[7] This method can be used both *in vivo* and *in vitro* and when a transient (*e.g.* skin[8,9]) or long-term (*e.g.* muscle[10]) transfection is needed. Electroporation can also be used to create aqueous pathways across the skin's outermost layer, the *stratum corneum* to enhance transdermal drug delivery.[11-13]

Numerous experiments, both *in vitro* and *in vivo*, have to be performed before a biomedical application is put to practical use in clinical environment. As a complementary work to *in vivo* experimenting, we can use analytical and numerical models to represent, as realistically as possible, real biological phenomena of, in our case, electroporation.[14-18] In this way we can better understand some of the processes involved and analyze and explain some experimental results. We can evaluate different electrical parameters in advance, such as pulse amplitude, duration, number of pulses. All of that can help us plan new protocols, design electroporation devices, plan new experiments and treatments. Of course, models must always be validated by experiments, and if necessary, improved. Although a model can not replace experimental work entirely, it can show us another aspect of the same problem. Both, experimental work and numerical modeling combined give us valuable information and help us understand the underlying mechanisms. In the present paper, we will show two examples of numerical models of *in vivo* electroporation; a subcutaneous tumor during electrochemotherapy and skin during gene electrotransfer.

Materials and methods

Numerical modeling of the electric field and the electric current distributions inside the biological systems represent an important field in the study of the effects of the electromagnetic fields on cells, tissues and organs. It is a relatively simple yet powerful tool for analysis and explanation of intricate processes taking place inside biological systems. Various electrical parameters (current and voltage amplitude, field strength and

orientation, electrode geometries...) can be evaluated by means of numerical modeling. Namely, experimenting with such models is easier and sometimes the only possible or ethically acceptable alternative to experimenting on real biological systems. Our models are based on the finite elements method (FEM). The essence of the method is the discretization of the geometry into smaller elements – finite elements – where the quantity of interest is approximated with a simple function. Mathematically, the finite element method is used for finding an approximate solution of partial differential equations (PDE). For our work, we were using commercially available modeling software EMAS (Ansoft, Pittsburgh, PA, USA) – for the tumor model and COMSOL Multiphysics, (COMSOL, Los Angeles, CA, USA) for the skin fold model, both based on finite element method.

Theoretical background

The bulk properties of biological materials are important in many applied problems of electrical stimulation. They dictate the current densities and pathways that result from an applied stimulus and are thus very important in the analysis of a wide range of biomedical applications.[19] To analyze the response of a tissue to electric excitation with direct current, we need data on the conductivities of the tissues or organs. Electrical conductivity is a measure of a material's ability to conduct electric current. When an electrical potential difference exists on a conductor, its free charges start moving, which results in an electric current. Electrical conductivity (σ) is defined as the ratio of the current density to the electric field strength ($\sigma=J/E$) and has the units of Siemens per meter (S/m). Material's ability to conduct electric current can also be given by its electrical resistivity (ρ). Electrical resistivity is the inverse of the electrical conductivity and is a measure of how strongly a material opposes to the flow of electric current. A low resistivity indicates a material that readily allows the movement of electrical charge. The unit of electrical resistivity is the Ohm meter (Ωm).

It is very important not to confuse the electrical conductivity and the electrical resistivity with the conductance and the resistance. Similarly to the definitions above, we can state that electrical conductance (G) is a measure of an object's (not material's) ability to conduct electric current, and electrical resistance (R) is a measure of how strongly an object (not a material) opposes to the flow of electric current. An object's electrical conductance (electrical resistance) is thus a function of both its physical geometry and the conductivity (resistivity) of the material it is made from:

$$G = \sigma \frac{A}{l} \qquad (1)$$

$$R = \rho \frac{l}{A} \qquad (2)$$

Where l is the object's length, A is its cross sectional area, σ and ρ are the conductivity and the resistivity of the material, respectively.

If we know the electric potential difference (the voltage U) and the electrical conductance (or electrical resistance) of the object, we can calculate the electric current I:

$$I = G \cdot U \qquad (3)$$

$$I = \frac{U}{R} \qquad (4)$$

Upon applying electric pulses on a setup of more materials (tissues) with different dimensions and electrical conductivities (electrical resistivities), connected in a serial configuration (*e.g.* skin), the voltage is divided among them proportionally to their electrical resistances, as in a voltage divider represented in Figure 1a. Similarly, where

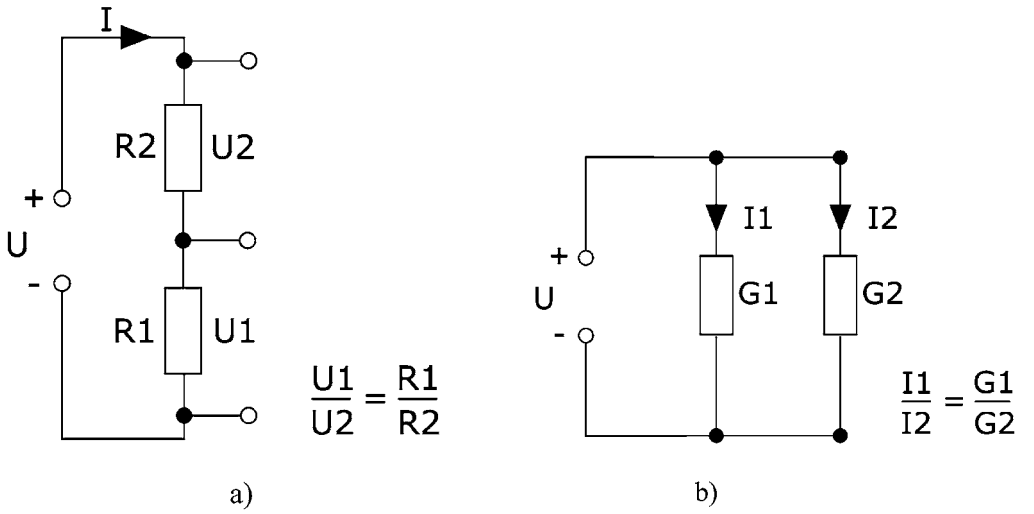

Figure 1. a) voltage divider, b) electric current divider

objects of different electrical conductances are in parallel configuration, the current is divided among them proportionally to their electrical conductances, as in a current divider represented in Figure 1b.

Numerical models – geometry

Experimental results show a successful electrochemotherapy of a subcutaneous tumor when pulses are delivered through external plate electrodes.[4] Also, a successful gene electrotransfer into rat skin cells was achieved when skin fold was formed and placed between plate electrodes delivering electric pulses.[9] Numerical models were made in order to describe theoretically the

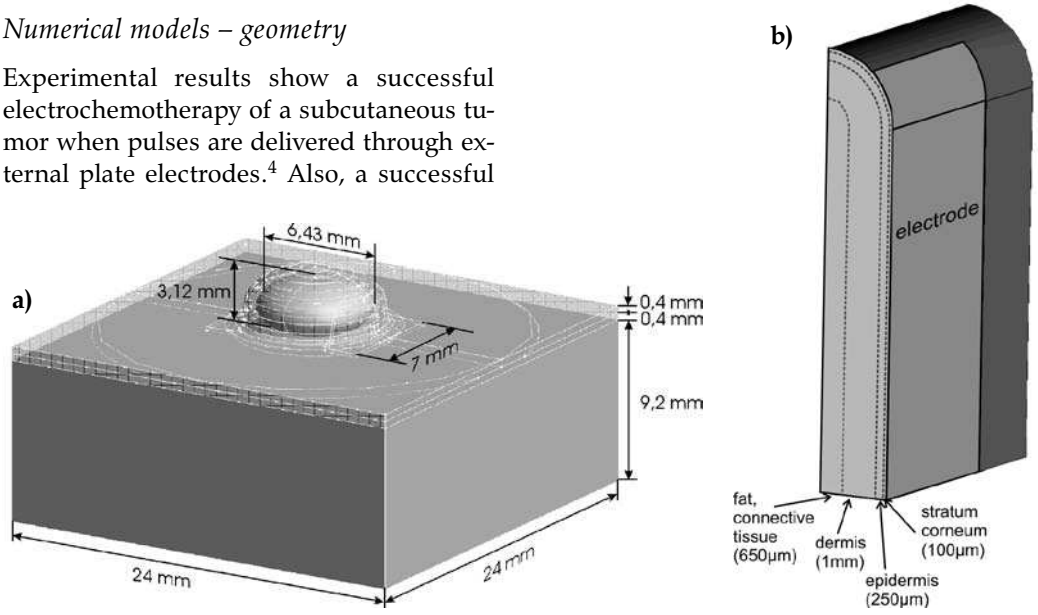

Figure 2. Geometries of finite element numerical models. a) The subcutaneous tumor model made in EMAS. b) One quarter of the skin fold model made in COMSOL.

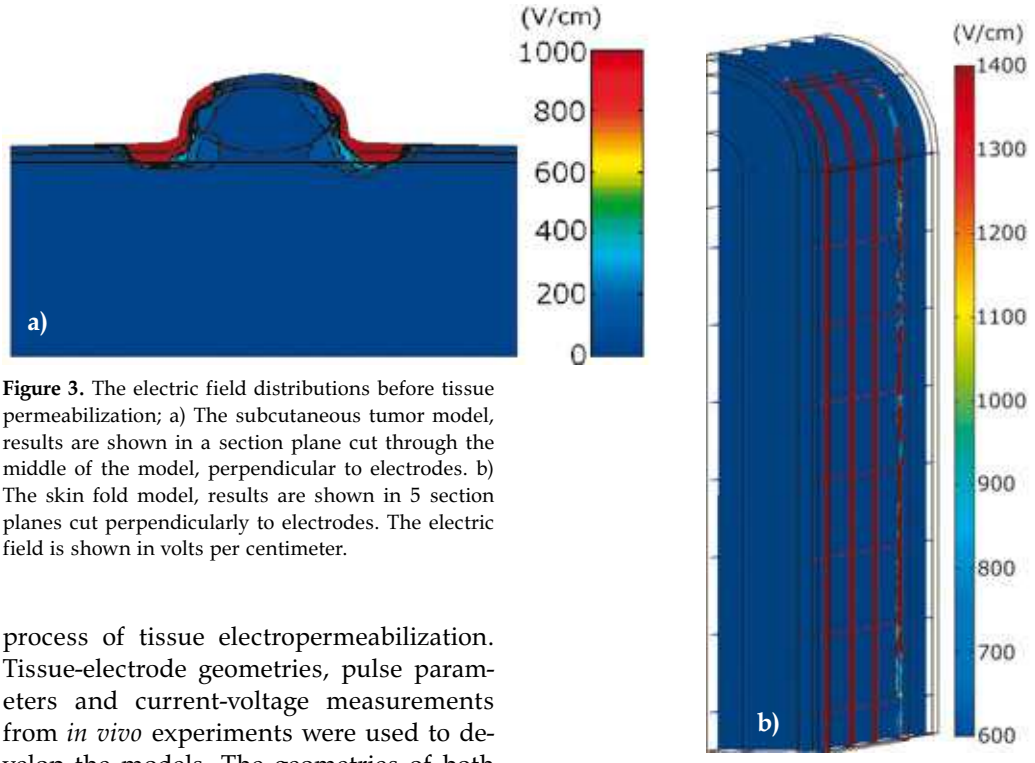

Figure 3. The electric field distributions before tissue permeabilization; a) The subcutaneous tumor model, results are shown in a section plane cut through the middle of the model, perpendicular to electrodes. b) The skin fold model, results are shown in 5 section planes cut perpendicularly to electrodes. The electric field is shown in volts per centimeter.

process of tissue electropermeabilization. Tissue-electrode geometries, pulse parameters and current-voltage measurements from *in vivo* experiments were used to develop the models. The geometries of both models are shown in Figure 2 and were made as close to the *in vivo* experimental tissue-electrode set-ups as possible. In the case of the subcutaneous tumor four different tissues were modeled: skin, subcutaneous fat, tumor and the underlying muscle.[18] In the case of the skin fold, skin's layered structure was modeled: stratum corneum, epidermis, dermis and the subcutaneous layer of fat and connective tissue.[16]

Numerical models – the electroporation process

In tissue, the voltage is the highest in the layer with the highest resistivity (the lowest conductivity). This leads to a certain electric field distribution (as in voltage divider), meaning that different layers are exposed to different electric field strengths. The electric field is the highest in the layer with the highest resistivity (lowest conductivity). In the case of the subcutaneous tumor this is the skin, which has the lowest electrical conductivity, and in the case of the skin fold, the highest electric field is in the non-conductive outermost skin layer, the stratum corneum. This can be clearly seen in Figure 3, showing the electric field distribution in the two models at 1000 V and 400 V between the plates, respectively. We can observe a very high electric field in the tissues with the highest electrical resistivity, while the electric field in the target tissues (tumor and viable skin layers) stays too low for successful electropermeabilization.

This fact raises the question of how is the experimentally confirmed successful permeabilization of the target tissues theoretically possible when external plate electrodes are used. The answer lies in the increase in bulk conductivities of the permeabilized tissues, a phenomenon that

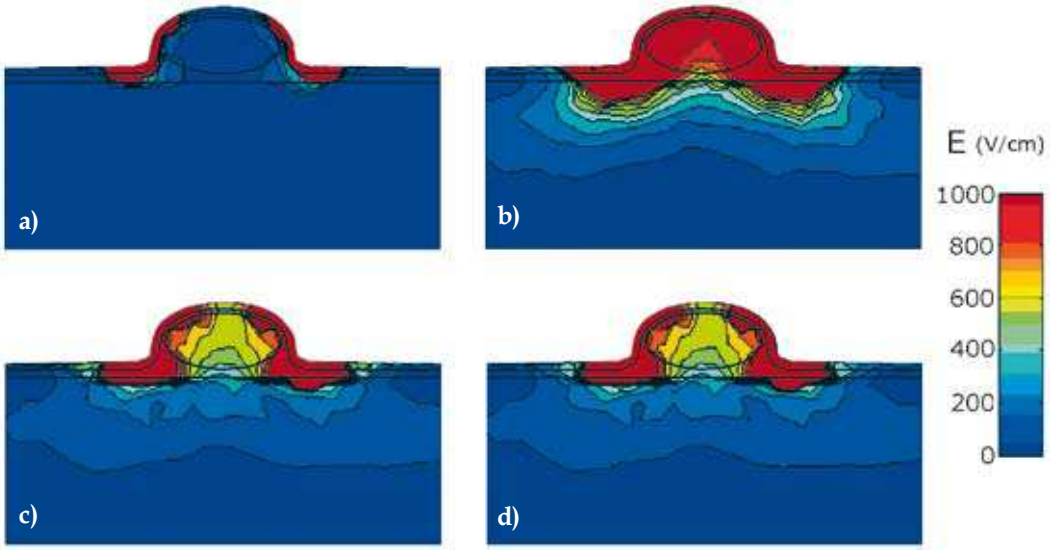

Figure 4. Electric field distributions in subcutaneous tumor, results are shown in a section plane cut through the middle of the model, perpendicular to electrodes for 1000 V between two external plate electrodes of 8 mm distance. Electric field distributions are shown in four time steps, from the non-permeabilized state (a), to the tissues being fully permeabilized (d). The electric field distribution is shown in V/cm.

was also observed *in vivo*. Namely, the high electric field in skin / stratum corneum is above the permeabilization threshold, which causes the electropermeabilization of the two tissues. As a consequence, the conductivity of skin / stratum corneum increases, and the electric field distribution is changed. In this way, the electric field high enough reaches the target tissues below skin and the stratum corneum. Therefore, our numerical models have to reflect this nonlinear dependence of conductivity on electric field. The electric field distribution (the model output) depends on the changes in the electrical conductivity of the tissues involved (model input parameters), the numerical analysis needs to be performed in iterations.

Table 1. Conductivity values used for tissues/skin layers represented in our models, where σ_0 denotes initial tissue conductivity, and σ_1 is the conductivity of permeabilized tissue.

	Tissue	**σ_0 (S/m)**	**σ_1 (S/m)**
Subcutaneous tumor	Subcutaneous layer	0.03	0.09
	Skeletal muscle (longitudinal/transverse)	0.735/0.11	2.94/0.44
	Tumor	0.3	0.8
	Skin	0.002	0.16
Skin fold	Subcutaneous layer	0.05	0.2
	Dermis, viable epidermis	0.2	0.8
	Stratum corneum	0.0005	0.5

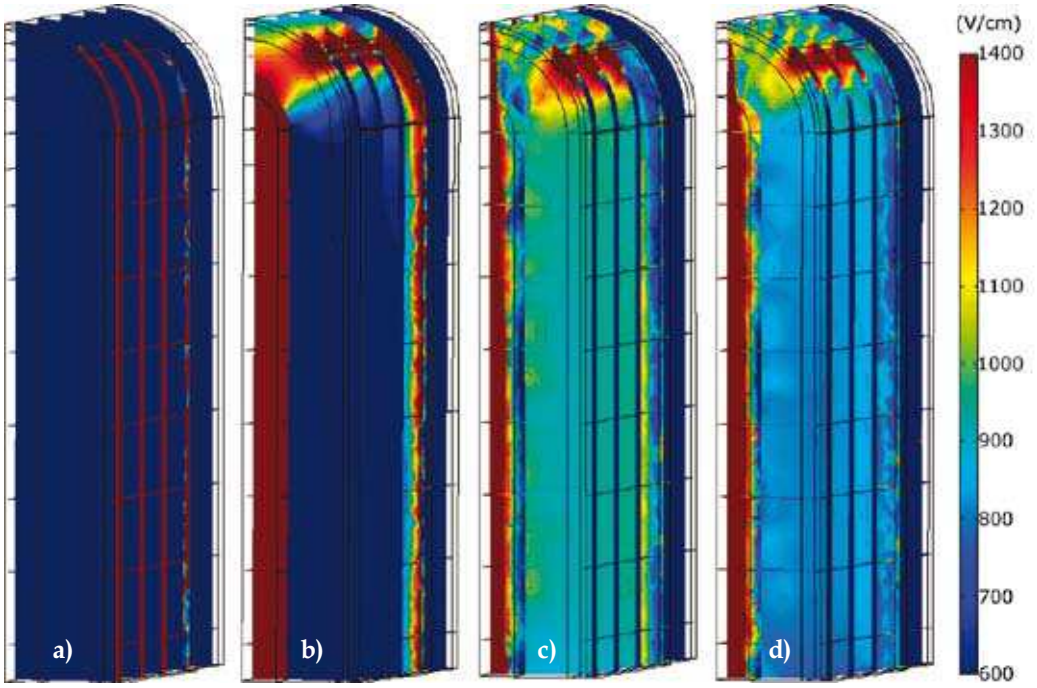

Figure 5. Three dimensional slice plots of the electric field distributions during the electropermeabilization process in the skin fold for the applied voltage 400 V between two plate electrodes of 4 mm distance. Electric field distributions are shown in four time steps, from the non-permeabilized state (a), to the skin layers being fully permeabilized (d). The electric field distribution is shown in V/cm.

Exactly how tissue conductivities change with electric field is another unknown or poorly known parameter. By using our own experiments and literature data[20-35], we set the initial and the permeabilized conductivity values of the tissues in both models as given in Table 1.

Results and discussion

The improved, nonlinear models where tissue conductivities change according to the current electric field were solved for different electric pulse amplitudes. The subcutaneous tumor model was solved for 500 V, 1000 V and 1500 V, while the skin model was solved for 160 V, 280 V, 400 V, 520 V and 700 V.

For the case of the subcutaneous tumor, the electric field distributions at 1000 V are shown at 4 time steps (Figure 4). The first step is the same as shown before (Figure 3a), with the highest electric field in the skin, while the electric field in other tissues is very low. But due to the conductivity changes of the permeabilized tissues, the next time step shows a different picture. The high electric field reaches the tissues below, permeabilizing them, thus changing the electric field distribution again. This process is repeated until we reach the steady state. The last step shows a high enough electric field for its permeabilization throughout the tumor, which is what we also observed *in vivo* – a successful electrochemotherapy at this voltage and electrode-tissue geometry.

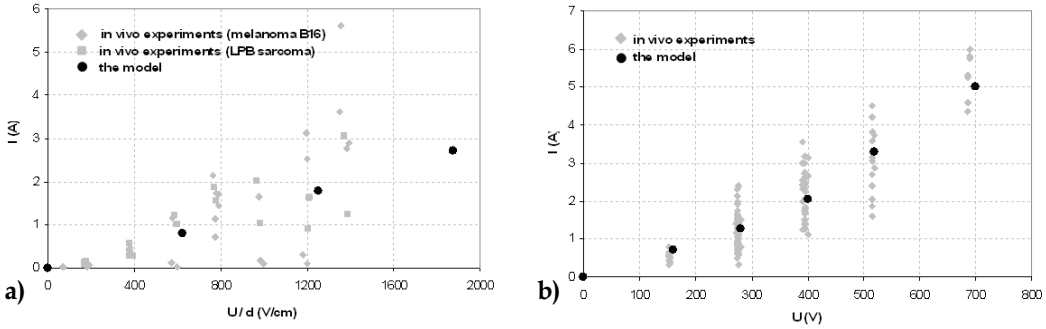

Figure 6. Currents measured during the pulse, compared to the currents given by the models, with respect to applied voltages for a) Subcutaneous tumor: The distance between plate electrodes was not uniform, hence the applied voltage is given in volts per centimeter; b) Skin fold

Further, the electric field distribution in skin fold shows similar progression (Figure 5). The first step is again the same as shown in Figure 3b, with the highest electric field in the stratum corneum, and a very low electric field in the target tissues below. Again, due to the conductivity changes, a high enough electric field moves to the tissues below stratum corneum, thus permeabilizing the viable epidermis and the dermis. Again, this agrees with our *in vivo* results. Namely, a high-level gene expression was observed at this voltage.

For further comparison of the models with the *in vivo* results the currents computed with the model were compared to the stationary currents flowing through the tissue, measured *in vivo* during the pulse (Figure 6). A good agreement can be observed for both models. Further, the current/voltage dependence given by our models exerts the nonlinearity observed in the *in vivo* data, suggesting that the approach we used to describe the process explains well the nonlinear nature of tissue electropermeabilization.

Conclusions

Numerical modeling of the electric field and the electric current distributions inside the biological systems represent an important field in the study of the effects of the electromagnetic fields on cells, tissues and organs. It is a powerful tool for the analysis of various electrical parameters and the explanation of the intricate processes taking place inside the biological systems. We have shown examples of numerical modeling on two electroporation-based applications: electrochemotherapy of subcutaneous tumors and skin gene electrotransfer. The regression of tumor growth after electrochemotherapy, proven also in clinical environments, and a successful gene electrotransfer to skin cells had shown that deeper target tissues (tumor, the dermis and the viable epidermis) can be permeabilized when external plate electrodes are used. The electropermeabilization of these tissues was possible even though the ratios of the nonpermeabilized tissue conductivities suggest that the electric field in the target tissues will be too low for a successful electropermeabilization. However, a phenomenon we can observe in the *in vivo* experiments is the increase in tissue conductivity due to cell membrane electroporation. This conductivity increase of the permeabilized tissues was included in our numerical models. The output currents of the models were compared to the stationary currents and the voltages

measured during *in vivo* experiments and a good agreement was obtained. Also, based on already published *in vivo* experiments and comparing the voltages needed for a successful electropermeabilization as suggested by the models, with voltages achieving a successful electrochemotherapy or *in vivo* gene electrotransfer, good agreement can be observed.[4,9,16]

In conclusion, with the models presented in this paper we used the available data in order to explain the mechanism of tissue electropermeabilization. Our models serve as a proof of principle and proved useful for describing different aspects of the observed process. Furthermore, such numerical models can help us design electrode geometries and electroporation protocols as a part of treatment planning.

Acknowledgements

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images in clinical medicine

Extensive squamous cell carcinoma of the lower lid

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An 88-year-old patient was referred to our unit for the treatment of extensive squamous cell carcinoma of the lower lid of the right eye, extending to the lid and bulbar conjuctiva (Figures 1, 2).

Treatment proposed by ophthalmologist was resection of the lower lid with exenteration of the right eye. The patient was treated with the combination of teletherapy and brachitherapy. The lower lid and conjunctiva were treated by an ortovoltage unit with the dose of 70 Gy, with the shielding of the globe. The bulbar conjunctiva was treated by an ophthalmic applicator with 90Sr, with the dose of 60 Gy in four fractions. Complete regression of the tumour was achieved.

At the follow-up three years after the completed treatment, there was no local recurrence, distant metastases, or other evidence of disease. (Figures 3, 4).

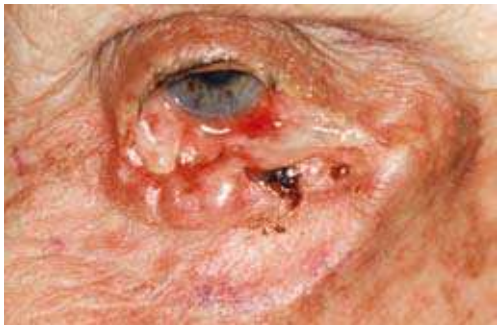

Figure 1. An extensive squamous cell carcinoma of the lower lid of the right eye.

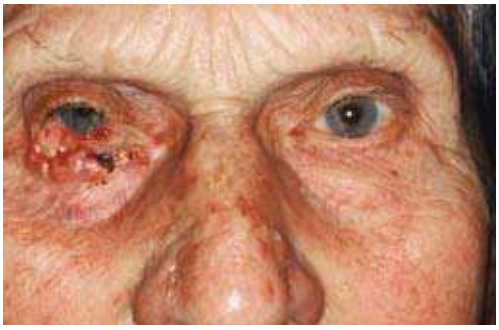

Figure 2. An extensive squamous cell carcinoma of the lower lid of the right eye.

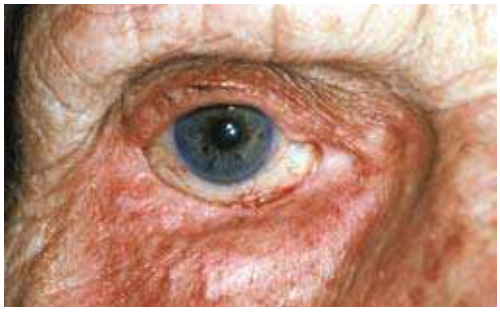

Figure 3. Three years after the completed treatment there was no evidence of disease.

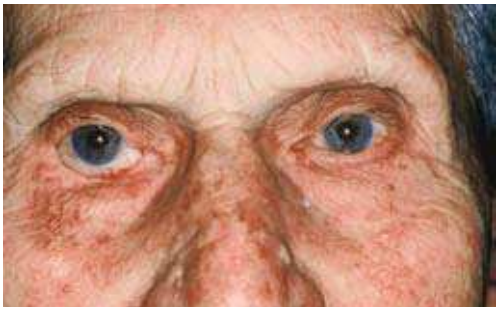

Figure 4. Three years after the completed treatment there was no local recurrence.

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professional paper

In search of the shortest regimen: fractionation of a fully isoeffective combination of hyperfractionated and hypofractionated treatment

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Purpose. *To analyze the possibility of reducing the number of fractions but maintaining the full biological effect of radiotherapy by varying the dose per fraction.*

Methods. *An arbitrary treatment with a constant dose per fraction is substituted for a fully isoeffective combination of a hyperfractionated and hypofractionated treatment. The number of fractions of the combined treatment is derived. All calculations are based on the linear-quadratic model.*

Conclusions. *Standard uniform fractionation requires the fewest fractions. Any variation in dose per fraction increases the number of fractions of a fully isoeffective treatment.*

Key words: radiotherapy; fractionation; LQ model

Introduction

The primary goal of radiotherapy is to deliver sufficient radiation dose to destroy the clonogenic cancer cells while causing as little damage as possible to the normal cells. A contribution towards this objective is made by dividing the total therapeutic dose into fractions allowing radiation damage repair and recovery in healthy tissues and possibly reoxygenation and reassortment in tumors between consecutive treatment sessions.[1-3] The fractionation prolongs the therapy making it also a logistic issue. Most treatment regimens prescribe a constant dose per fraction throughout the treatment. In this article we look at the possibility of reducing the number of treatment fractions by replacing the constant dose per fraction schedule with an isoeffective combination of hyperfractionated and hypofractionated treatments.

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Methods and results

Using the linear-quadratic (LQ) model Joiner illustrated how an incorrect dose delivered in the first few fractions can be counterbalanced by modifying the dose per fraction in the rest of the treatment to achieve the same biological effect as with the intended fractionation.[4] In a few examples he calculated that the cumulative number of fractions exceeded that of the initially prescribed treatment. Bortfeld and

Paganetti suspected this to always be the case[5], but did not prove it. In this paper we analyze the number of fractions in an isoeffective treatment where we deliberately deliver different doses per fraction without any other changes to the treatment plan, *i.e.* we vary the absolute dose but preserve the relative dose distribution.

Joiner derived his equations assuming all the tissues received equal absorbed dose, *i.e.* they all lay on the 100% isodose. We start by generalizing the validity of these equations to all isodoses. Let d be the dose per fraction and let D be the total dose in a treatment that consists of $n = D / d$ fractions. Also let the subscripts H and h denote the same quantities belonging to the *hyper*fractionated and *hypo*fractionated part of the isoeffective treatment respectively, so that $d_H < d < d_h$. Further let k be the relative isodose of interest. Now we request the biologically effective dose (*BED*) of the constant dose per fraction treatment be equal to that of the mixed regime

$$BED = BED_H + BED_h$$

In the LQ model this translates to (after multiplying with α/β and relating to the k isodose)

$$k\,D\,(\alpha/\beta + k\,d) = k\,D_H\,(\alpha/\beta + k\,d_H) + k\,D_h\,(\alpha/\beta + k\,d_h)$$

Dividing the equation by $k \neq 0$ and separating the terms with α/β and k we get

$$\alpha/\beta\,(D - D_H - D_h) = k\,(D_H\,d_H + D_h\,d_h - D\,d)$$

For the equation to be valid for all the values of α/β and k, the following has to hold

$$D_H + D_h = D \qquad [1]$$

$$D_H\,d_H + D_h\,d_h = D\,d \qquad [2]$$

The above equations are the same as derived by Joiner, but now enjoy broader validity.

Next we look at the number of fractions in such a treatment

$$n_H + n_h = (D_H\,d_h + D_h\,d_H) / d_H\,d_h$$

The expression on the right calls for some maneuvering and application of equations [1] and [2]

$$n_H + n_h = [(D_H + D_h)\,(d_H + d_h) - D_H\,d_H - D_h\,d_h] / d_H\,d_h = D\,(d_H + d_h - d) / d_H\,d_h$$

after which we arrive to

$$n_H + n_h = n\,[1 + (d_h - d)\,(d - d_H) / d_H\,d_h]$$

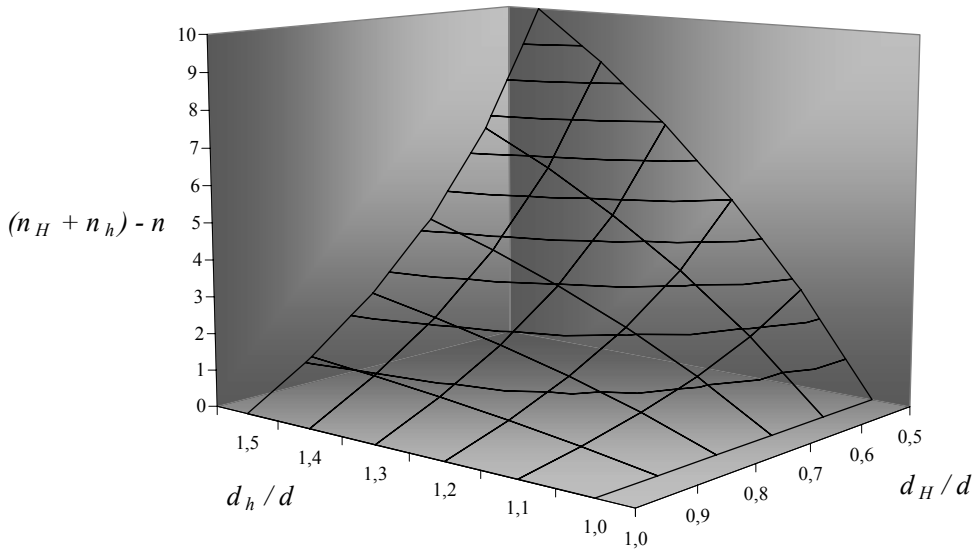

Figure 1. The chart shows the increase in number of fractions when moving from the standard to the combined regimen as a continuous function of relative doses per fraction d_H / d and d_h / d. The number of fractions in the standard regimen n is in this example assumed to be 30.

Reminding ourselves that $d_H < d < d_h$, we clearly observe that the value in the square brackets is greater than 1. This brings us to the conclusion

$$n_H + n_h > n$$

An example of this inequality is given in Figure 1.

Conclusions

Not only is a treatment with a constant dose per fraction the most practical and the easiest to manage among all the isoeffective schedules, it also involves the fewest fractions: any deviation from the uniform fractionation increases their number. If this also prolongs the duration of the radiation therapy, undesired consequences, such as cancer cell proliferation, may require delivery of additional dose. This, however, is beyond the scope of this paper.

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Radiol Oncol 2008; 42(3): 115-35.

Ciljana genska terapija v radioterapiji

Kamenšek U in Serša G

Izhodišča. Genska terapija postaja s hitrim razvojem v zadnjih desetletjih alternativa za zdravljenje raka, medtem ko je radioterapija ena najbolj uveljavljenih in najpogosteje uporabljenih terapij v onkologiji.
Zaključki. Najnovejše izboljšave v ciljanosti radioterapije in razumevanju molekularnih mehanizmov vključenih v celični odziv na ionizirajoče sevanje predstavljajo možnost za kombiniranje radioterapije z gensko terapijo. V članku so predstavljene strategije genske terapije, ki jih lahko uporabimo za povišanje učinkovitosti radioterapije, s posebnim poudarkom na trankripcijskem ciljanju.

Radiol Oncol 2008; 42(3): 136-42.

Magnetnoresononančna preiskava danke z ultrazvočnim gelom kot kontrastnim sredstvom pri bolniku z tubuloviloznim adenomom

Sofić A, Šehović N, Bešlić Š, Prnjavorac B, Bilalović N, Čaluk J, Sofić D

Izhodišča. Kolorektalni polipi so pogosti in jih najdemo pri 10% odraslih oseb. Najpogosteje pa jih najdemo pri bolnikih nad 60 let, pri katerih se pojavljajo v 20%. Polipi so lahko pendikularne in sesilne oblike. Histopatološko jih razdelimo na 3 podtipe: tubularni, tubulovilozni in vilozni adenom. Vilozni adenomi so večji in imajo tudi bolj izrazito displazijo. 90% primerov črevesnega raka nastane iz benignih oblik polipov, adenomov. Ker je črevesni rak drugi najpogostejši vzrok smrti zaradi raka, skušajo z odstranitvijo polipov preprečiti nastanek raka. V povprečju se polipi maligno spremenijo v 10 letih, in iz manjših polipov redko nastane črevesni rak, zato drugi menijo, da polipov ni potrebno odstranjevati v vsakem primeru. Bolnikih, ki so odklonili polipektomijo, bodo v 5 letih povprečno v 4% dobili črevesnega raka, v 10 letih pa v 14%.

Prikaz primera. Prikazujemo primer bolnice, ki je nekaj let imela polip v danki in ni želela polipektomije. Zaradi težav z odvajanjem blata, zlasti zaprtosti in tudi občasne krvavitve, se je odločila za odstanitev polipa. Ob kolonoskopiji se preiskovalec ni odločil za polipektomijo, ker je menil, da je preveč rizična. Po čiščenju smo naredili magnetno resonančno preiskavo (MR) z aparatom 1,5T in uporabili ultrazvočni gel kot kontrastno sredstvo. Ugotovili smo, da je bil polip neravnih površin in se je širil v steno danke, ni pa se širil izven črevesa oz. v mezorektum. Nato smo naredili polipektomijo in resecirali del mišične stene danke. Patohistološka preiskava je pokazala tubulovilozni adenom.

Zaključki. MR danke je novejša preiskava, ki natančneje prikaže značilnosti polipa in tudi drugih tumorjev. Pomemben je predvsem prikaz morebitne tumorske rašče v steno in izven stene črevesa, zlasti v mezorektum. Preiskava za bolnika ni težavna in ga ne izpostavlja sevanju. Intraluminalna uporaba ultrazvočnega gela kot kontrastnega sredstva širi lumen in omogoča boljši prikaz polipov in drugih tumorjev v danki.

Radiol Oncol 2008; 42(3): 143-50.

Vpliv infuzije magnezijevega sulfata pred totalno tiroidektomijo na prehodno hipokalcemijo – randomizirana raziskava

Besic N, Zagar S, Pilko G, Peric B, Hocevar M

Izhodišče in namen. Prehodna hipokalcemija je najpogostejši zaplet po operaciji ščitnice. Za normalno izločanje parathormona (PTH) in periferno odzivnost tkiv na PTH je potrebna normalna serumska koncentracija magnezija. Namen naše raziskave je bil preveriti, če medoperativna intravenozna aplikacija magnezijevega sulfata pred totalno tiroidektomijo zmanjša pogostost prehodne laboratorijske in klinične hipokalcemije.

Bolniki in metode. V prospektivni klinični raziskavi smo randomizirali 48 polnoletnih bolnikov (5 moških, 43 žensk; starost 22-73 let, mediana 45 let), pri katerih je bila napravljena skoraj totalna ali totalna tiroidektomija. Polovica bolnikov je ob začetku operacije prejela intravenozno 4 ml 1M magnezijevega sulfata, druga polovica bolnikov je predstavljala kontrolno skupino. Predoperativno in prvi dan po operaciji smo izmerili serumsko koncentracijo kalcija, ioniziranega kalcija, magnezija, fosfata, albumina in PTH.

Rezultati. Bolnikov z laboratorijsko pooperativno hipokalcemijo je bilo 27%, s klinično pooperativno hipokalcemijo pa 23%. Prvi dan po operaciji je bila v skupini, ki je prejela magnezijev sulfat, nižja koncentracija celokupnega kalcija (p=0,024) in albumina (p=0,01).

Zaključki. Bolniki, ki so dobili intravenozno infuzijo magnezijevega sulfata pred totalno tiroidektomijo, so imeli po operaciji nižjo koncentracijo celokupnega serumskega kalcija in albumina kot tisti iz kontrolne skupine. Infuzija magnezijevega sulfata ni imela vpliva na pogostost klinične prehodne hipokalcemije.

Radiol Oncol 2008; 42(3): 151-8.

Napovedna vrednost imunohistokemično določenega HER-2/neu pri bolnikih s pljučnim rakom

Ilievska Poposka B, Smickova S, Jovanovska Crvenkovska S, Zafirova Ivanovska B, Stefanovski T, Petrusevska G

IIzhodišča. Pri različnih rakih so ugotovili histokemično povečano izražanje Her-2/neu gena, zato menijo, da ima ta gen poseben pomen pri nastanku raka. V raziskavi smo želeli določiti povečano izražanje HER-2/neu gena pri bolnikih s pljučnim rakom in oceniti njegovo napovedno vrednost.

Bolniki in metode. Za določitev nivoja izražanja HER-2/neu smo pri 127 bolnikih s pljučnim rakom uporabili imunohistokemični test na parafinskih rezih, imenovan Hercep Test™ (DAKO).

Rezultati. HER-2/neu je bil prekomerno izražen pri 36 bolnikih od 127 (28,35%). 12 bolnikov je imelo žlezni rak (60%), 19 bolnikov skvamoznocelični rak (31,14%), 4 bolniki drobnocelični rak (10%) in 1 bolnik druge vrste rak (16,66%). Samo pri bolnikih z drobnoceličnim rakom pljuč je bilo prekomerno izražanje HER-2/neu sorazmerno s stadijem bolezni ($p<0,001$). Sicer pa je imelo 36 bolnikov s prekomerno izraženim HER-2/neu značilno krajše preživetje glede na preostale bolnike ($p<0,002$).

Zaključki. Raziskava kaže, da bi lahko pozitivna vrednost izražanja HER-2/neu predstavljala negativni napovedni dejavnik tudi pri bolnikih s pljučnim rakom. Pri teh bolnikih bi se zato lahko odločili za bolj agresivno zdravljenje.

Radiol Oncol 2008; 42(3): 159-68.

Numerično modeliranje elektroporacije v tkivih

Pavšelj N in Miklavčič D

Izhodišča. Pred vključitvijo neke metode v klinične namene je potrebno opraviti veliko število poskusov *in vitro* in tudi *in vivo*. Kot nadomestilo in dopolnilo k eksperimentalnemu delu so nam na voljo analitične in numerične metode, s pomočjo katerih realne biološke procese predstavimo z modeli. Tako lahko tudi za biomedicinske metode, ki temeljijo na elektroporaciji celične membrane, s pomočjo modelov ovrednotimo vpliv parametrov terapije (amplituda, trajanje, število električnih pulzov, oblike elektrod) na njen izid, še preden le-to uporabimo v eksperimentalnem ali kliničnem okolju. Takšni modeli v veliki meri pripomorejo k izboljšanju razumevanja metode in s tem k načrtovanju poskusov in terapij ter elektrod in ostale opreme, ki jo pri tem potrebujemo.

Metode. Zgradili smo numerični model podkožnega tumorja med elektrokemoterapijo in model kože med vnosom genov z elektroporacijo. Pri tem smo uporabljali komercialno dostopna programa EMAS (model tumorja) in COMSOL Multiphysics (model kože), oba temeljita na numerični metodi končnih elementov.

Rezultati. V numeričnih modelih smo upoštevali porast prevodnosti tkiva med elektroporacijo, na kar kažejo tudi poskusi *in vivo*. Električne tokove, ki smo jih izračunali s pomočjo modelov, smo primerjali s tokovi, ki smo jih izmerili med poskusi *in vivo*. Ugotovili smo dobro ujemanje. Prav tako se ujemajo s pomočjo modelov predlagane amplitude električnih pulzov z amplitudami, s katerimi smo dosegli uspešno elektrokemoterapijo ali vnos genov *in vivo*.

Zaključki. Numerični modeli so dobro orodje za opis in analizo dogajanja med elektroporacijo in nam hkrati omogočajo načrtovanje elektrod, novih protokolov in terapij.

Radiol Oncol 2008; 42(3): 170-2.

Iskanje najkrajšega režima: frakcionacija biološko enakovredne kombinacije hiper- in hipofrakcioniranega zdravljenja

Strojnik A

Izhodišča. Zdravljenje z obsevanjem je običajno razdeljeno na več frakcij, kar ga podaljša in s tem otežuje obravnavo bolnikov. V članku smo preučili možnost, da bi s spreminjanjem doze na frakcijo zmanjšali število frakcij, a hkrati ohranili celoten biološki učinek zdravljenja.

Metode. Poljubno zdravljenje s konstantno dozo na frakcijo smo zamenjali za biološko enakovredno kombinacijo hiperfrakcioniranega in hipofrakcioniranega zdravljenja. Izpeljali smo število frakcij tako sestavljenega zdravljenja. Vsi izračuni so bili zasnovani na linearno-kvadratnem modelu.

Zaključki. Standardna frakcionacija s konstantno dozo na frakcijo zahteva najmanj frakcij. Vsakršno spreminjanje doze na frakcijo poveča število frakcij biološko enakovrednega zdravljenja.

Notices

*Notices submitted for publication should contain a mailing address, phone and/or fax number and/or e-mail of a **Contact** person or department.*

Thoracic oncology

October 1-5, 2008
The "International Thoracic Oncology Congress" will be offered in Dresden, Germany.
E-mail: prof.manegold@t-online.de

Paediatric oncology

October 2-6, 2008
The "40th Congress of the International Society of Paediatric Oncology (SIOP)" will be offered in Berlin, Germany.
See http://www.ecco-org.eu

Oncology

October 9-10, 2008
The ''3rd Latin American Cancer Conference'' will take place in Vina del Mar, Chile.
E mail nisehy@uol.com.br or rodrigo.arriagada@ki.se

Cancer therapy

October 21-24, 2008
The 20th EORTC-NCI-AACR Symposium on "Molecular Targets and Cancer Therapeutics" will be offered in Geneva, Switzerland.
Contact EORTC-NCI-AACR 2008 Secretariat; C/o ECCO-European CanCer Organisation; Avenue E. Mounier, 83; B - 1200 Brussels Belgium; or phone +32 2 775 02 47; or fax +32 2 775 02 00; or e-mail: barbara.vanbelle@ecco-org.eu

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The ESO/ESTRO masterclass in radiation oncology will be offered in ST. Julians, Malta.
Contact Chatrina Melcher, European School of Oncology, ESO Bellinzona Office, Ospedale Regionale Bellinzona e Valli, CH-6500 Bellinzona, Switzerland; or phone +41 91 811 8050; or fax +41 91 811 8051; or e-mail cmelcher@eso.net; or see www.cancerworld.org

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Lung cancer

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Contact by e-mail Dr AA. Ranade draaranade@yahoo.com; or see http://www.aplcc.cpm

Head and neck oncology

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The "2nd International Conference on Innovative Approaches in Head and Neck Oncoloy" will take place in Barcelona, Spain.
See http:// www.estro.be

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E-mail: pia.hirsch@uchsc.edu

Clinical oncology

May 29 – June 2, 2009
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E mail enews@asco.org; or see http://www/asco.org

Clinical trial statistics

June 9-12, 2009
The EORTC course "Clinical Trial Statistics for Non Statisticians" will be held in Brussels, Belgium.
See http://www.eortc.be/Seminar/Educationpgm/Stats2009/Default.htm

Lung cancer

July 31 - August 4, 2009
The "13th World Conference on Lung Cancer" will be offered in San Francisco, USA.
Contact Conference Secretariat; e-mail WCLC2007@ncc.re.kr; or see http://www.iaslc.orgIumages/12worldconfannounce.pdf

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September 20-24, 2009
The "15th ECCO and 34th ESMO Multidisciplinary Congress" will be offered in Berlin, Germany.
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Therapeutic radiology and oncology

November 1-5, 2009
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Contact ASTRO, 8280 Willow Oaks Corporate Dr., Suite 500, Fairfax, VA 22031; or call +1 703 502-1550; or see http://www.astro.org

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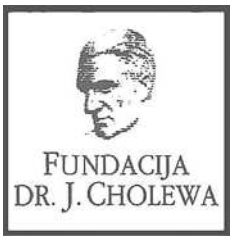

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Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education – a report for the third quarter of 2008

The "Docent Dr. L. Cholewa Foundation for Cancer Research and Education" continues to support cancer research and education in Slovenia. It thus continues to assess carefully the requests and proposals for research grants and scholarships submitted by Slovenian experts in oncology and other associated scientific activities.

High quality research relies heavily on steady and generous financial support. Government supported activities have an important role in the advancement of oncology related sciences, however, in a number of countries the researchers can also count on financial assistance in the form of grants provided by a variety of funds and foundations. The "Docent Dr. L. Cholewa Foundation for Cancer Research and Education« is of opinion that many excellent and unorthodox ideas must not be prevented to succeed for the simple lack of funding.

The "Docent Dr. L. Cholewa Foundation for Cancer Research and Education« continues to support the regular publication of "Radiology and Oncology" international medical scientific journal, that is edited, published and printed in Ljubljana, Slovenia. The support for "Radiology and Oncology" emphasizes the need for the spread of information and knowledge about advances in cancer among professionals and to many interested individuals in public in Slovenia and elsewhere. "Radiology and Oncology" is an open access journal, available on its own website, thus allowing its users and readers to access it freely.

The Foundation considers the support of the publication of the results from cancer research in Slovenia and from Slovenian authors in international scientific journals and other means of communication worldwide as one of its main activities.

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Cetuksimab je monoklonsko IgG_1 protitelo, usmerjeno proti receptorju za epidermalni rastni faktor (EGFR). **Terapevtske indikacije:** Zdravilo Erbitux je indicirano za zdravljenje bolnikov z metastatskim kolorektalnim rakom in nemutiranim tipom KRAS; v kombinaciji s kemoterapijo in kot samostojno zdravilo pri bolnikih, pri katerih zdravljenje z oksaliplatinom in irinotekanom ni bilo uspešno. Zdravilo Erbitux je v kombinaciji z radioterapijo indicirano za zdravljenje bolnikov z lokalno napredovalim rakom skvamoznih celic glave in vratu. **Odmerjanje in način uporabe:** Zdravilo Erbitux pri vseh indikacijah infundirajte enkrat na teden. 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. **Posebna opozorila in previdnostni ukrepi:** 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 huda kožna reakcija (≥ 3. stopnje po kriterijih *US National Cancer Institute, Common Toxicity Criteria;* NCI-CTC), morate prekiniti terapijo s cetuksimabom. Z zdravljenjem smete nadaljevati le, če se je reakcija pomirila do 2. stopnje. Priporoča se določanje koncentracije elektrolitov v serumu pred zdravljenjem in periodično med zdravljenjem s cetuksimabom. Po potrebi se priporoča nadomeščanje elektrolitov. Posebna previdnost je potrebna pri oslabljenih bolnikih in pri tistih z obstoječo srčno-pljučno boleznijo. Neželeni učinki: Zelo pogosti (≥ 1/10): dispneja, blago do zmerno povečanje jetrnih encimov, kožne reakcije, blage ali zmerne reakcije povezane z infundiranjem, blag do zmeren mukozitis. Pogosti (≥ 1/100, < 1/10): konjunktivitis, hude reakcije povezane z infundiranjem. Pogostost ni znana: Opazili so progresivno zniževanje nivoja magnezija v serumu, ki pri nekaterih bolnikih povzroča hudo hipomagneziemijo. Glede na resnost so opazili tudi druge elektrolitske motnje, večinoma hipokalciemijo ali hipokaliemijo. **Posebna navodila za shranjevanje:** Shranjujte v hladilniku (2 °C - 8 °C). Ne zamrzujte**. Vrsta ovojnine in vsebina:** 1 viala po 20 ml ali 100 ml. Imetnik dovoljenja za promet: Merck KGaA, 64271 Darmstadt, Nemčija. Podrobne informacije o zdravilu so objavljene na spletni strani Evropske agencije za zdravila (EMEA) http://www.emea.europa.eu.

Dodatne informacije so vam na voljo pri: Merck d.o.o., Dunajska cesta 119, 1000 Ljubljana, tel.: 01 560 3810, faks: 01 560 3831, el. pošta: info@merck.si

www.oncology.merck.de

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SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Epufen 12,5, 25, 50 in 100 mikrogramov/uro transdermalni obliži SESTAVA: 1 transdermalni obliž vsebuje 2,89 mg, 5,78 mg 11,56 mg ali 23,12 mg fentanila. **TERAPEVTSKE INDIKACIJE**: Huda kronična bolečina, ki se lahko ustrezno zdravi le z opioidnimi analgetiki. **ODMERJANJE IN NAČIN UPORABE**: Odmerjanje je treba individualno prilagoditi ter ga po vsaki uporabi redno oceniti. Izbira začetnega odmerka: velikost odmerka fentanila je odvisna od predhod-ne uporabe opioidov, kjer se upošteva možnost pojava tolerance, sočasnega zdravljenja, bolnikovega splošnega zdravstvenega stanja in stopnje resnosti obolenja. Pri bolnikih, ki pred tem niso dobivali močnih opioidov, začetni odmerek ne sme presečі 12,5-25 mikrogramov na uro. Zamenjava opioidnega zdravljenja: pri zamenjavi peroralnih ali parenteralnih opioidov s fentanilom je treba začetni odmerek izračunati na osnovi količine analgetika, ki je bila potrebna v zadnjih 24 urah, jo pretvoriti v odgovarjajoči odmerek morfina s pomočjo razpredelnice in nato preračunati ustrezen odmerek fentanila, spet s pomočjo razpredelnice (glejte SmPC). Prvih 12 ur po prehodu na transdermalni obliž Epufen bolnik še vedno dobiva predhodni analgetik v enakem odmerku kot prej; v naslednjih 12 urah se ta analgetik daje po potrebi. Titracija odmerka in vzdrževalno zdravljenje: obliž je treba zamenjati vsakih 72 ur. Odmerek je treba titrirati individualno, dokler ni dosežen analgetični učinek. Odmerek 12,5 mikrogramov/uro je primeren za titriranje odmerka v manjšem odmernem območju. Če analgezija na koncu začetnega obdobja nošenja obliža ni zadostna, se lahko odmerek po 3 dneh zveča. Možno je, da bodo bolniki potrebovali občasne dodatne odmerke kratko delujočih analgetikov (npr. morfina) za prekinitev bole-čine. Sprememba ali prekinitev zdravljenja: vsaka zamenjava z drugim opioidom mora potekati postopoma, z majhnim začetnim odmerkom in počasnim zvečevanjem. Splošno veljavno pravilo je postopna ustavitev opioidne analgezije, da bi preprečili odtegnitvene simptome, kot so navzeja, bruhanje, diareja, anksioznost in mišični tremor. Uporaba pri starejših bolnikih: starejše in oslabljene bolnike je treba skrbno opazovati zaradi simptomov prevelikega odmerjanja ter odmerek po potrebi zmanjšati. Uporaba pri otrocih: transdermalni obliži Epufen se lahko uporabljajo le pri pediatričnih bolnikih (starih od 2 do 16 let), ki tolerirajo opioide in peroralno že dobivajo opioide v odmerku, enakovrednemu najmanj 30 mg morfina na dan. Bolnik mora prvih 12 ur po prehodu na Epufen še vedno dobivati predhodni analgetik v enakem odmerku kot prej. V naslednjih 12 urah je treba ta analgetik dajati odvisno od kliničnih potreb. Titracija odmerka in vzdrževalno zdravljenje: če je analgetični učinek Epufena prešibak, je treba bolniku dodati morfin ali drugi opioid s kratkim delovanjem. Odvisno od dodatnih potreb po analgeziji in jakosti bolečine pri otroku se lahko uporabi več obližev. Odmerek je treba prilagajati korakoma, po 12,5 mikrogramov/uro. Uporaba pri bolnikih z jetrno ali ledvično okvaro: Zaradi možnosti pojava simptomov prevelikega odmerjanja je treba te bolnike skrbno spremljati in odmerek ustrezno zmanjšati. Uporaba pri bolnikih s povečano telesno temperaturo: Pri teh bolnikih bo morda treba prilagoditi odmerek. **Način uporabe**: transdermalni obliž Epufen je treba takoj po odprtju vrečke nalepiti na nerazdraženo, neobsevano kožo, na ravno površino prsnega koša, zgornjega dela hrbta ali nadlakti. Po odstranitvi zaščitne plasti je treba obliž trdno pritrditi na izbrano mesto in z dlanjo pritiskati približno 30 sekund, da se obliž popolnoma nalepi, še zlasti na robovih. Uporaba pri otrocih: pri mlajših otrocih je obliž priporočljivo nalepiti na zgornji del hrbta, ker je manjša verjetnost, da bi otrok odstranil obliž. Transdermalnega obliža se ne sme deliti, ker podatkov o tem ni na voljo. **KONTRAINDIKACIJE**: Preobčutljivost za zdravilno učinkovino, hidrogenirano kolofonijo, sojo, arašide ali katerokoli pomožno snov. Akutna ali pooperativna bolečina, ko v kratkem časovnem obdobju ni možno titriranje odmerka in obstaja verjetnost za življenjsko ogrožajočo respiratorno depresijo. Huda okvara osrednjega živčnega sistema. Sočasna uporaba MAO ali v obdobju 14 dni po prekinitvi jemanja zaviralcev MAO. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI**: Zaradi razpolovne dobe fentanila je treba bolnika v primeru pojava neželenega učinka opazovati še 24 ur po odstranitvi obliža. Pri nekaterih bolnikih, ki uporabljajo transdermalni obliž Epufen, se lahko pojavi respiratorna depresija. Epufen je treba previdno dajati: bolnikom s kronično pljučno boleznijo, zvišanim intrakranialnim tlakom, možganskim tumorjem, boleznimi srca, jeter in ledvic, tistim z zvišano telesno temperaturo, pri starejših bolnikih in otrocih, bolnikih z miastenijo gravis. Odvisnost od zdravila: kot posledica ponavljajoče se uporabe se lahko razvijeta toleranca na učinkovino ter psihična in/ali fizična odvisnost od nje. Ostali: lahko se pojavijo neepileptične (mio)kloničnе reakcije. **MEDSEBOJNO DELOVANJE Z DRUGIMI ZDRAVILI IN DRUGE OBLIKE INTERAKCIJ**: Derivati barbiturne kisline, opioidi, anksiolitiki in pomirjevala, hipnotiki, splošni anestetiki, fenotiazini, mišični relaksanti, sedativni antihistaminiki in alkoholne pijače, zaviralci MAO, itrakonazol, ritonavir, ketokonazol, nekateri makrolidni antibiotiki, pentazocin, buprenorfin. **VPLIV NA SPOSOBNOST VOŽNJE IN UPRAVLJANJA S STROJI**: Zdravilo ima močan vpliv na sposobnost vožnje in upravljanja s stroji. **NEŽELENI UČINKI**: Najbolj resen neželen učinek fentanila je respiratorna depresija. Zelo pogosti (≥ 1/10): dremavost, glavobol, navzeja, bruhanje, zaprtje, znojenje, srbenje, somnolenca. Pogosti (≥ 1/100 do < 1/10): kserostomija, dispepsija, reakcije na koži na mestu aplikacije, sedacija, zmedenost, depresija, tesnoba, živčna napetost, halucinacije, zmanjšan apetit. Občasni (≥ 1/1000 do < 1/100): tahikardija, bradikardija, tremor, parestezija, motnje govora, dispneja, hipoventilacija, diareja, zastajanje urina, izpuščaj, rdečina, hipertenzija, hipotenzija, evforija, amnezija, nespečnost, vznemirljivost. Nekateri od naštetih neželenih učinkov so lahko posledica osnovne bolezni ali drugih zdravljenj. Drugi neželeni učinki: odpornost, fizična in psihična odvisnost se lahko razvijejo med dolgotrajno uporabo fentanila. Pri nekaterih bolnikih se lahko pojavijo odtegnitveni simptomi, ko zamenjajo prejšnje opiodne analgetike s transdermalnim obližem s fentanilom ali po nenadni prekinitvi zdravljenja. **NAČIN IZDAJE**: Samo na zdravniški recept. **OPREMA**: Škatle s 5 transdermalnimi obliži. **IMETNIK DOVOLJENJA ZA PROMET**: Lek farmacevtska družba, d.d., Verovškova 57, Ljubljana, Slovenija **INFORMACIJA PRIPRAVLJENA**: november 2007

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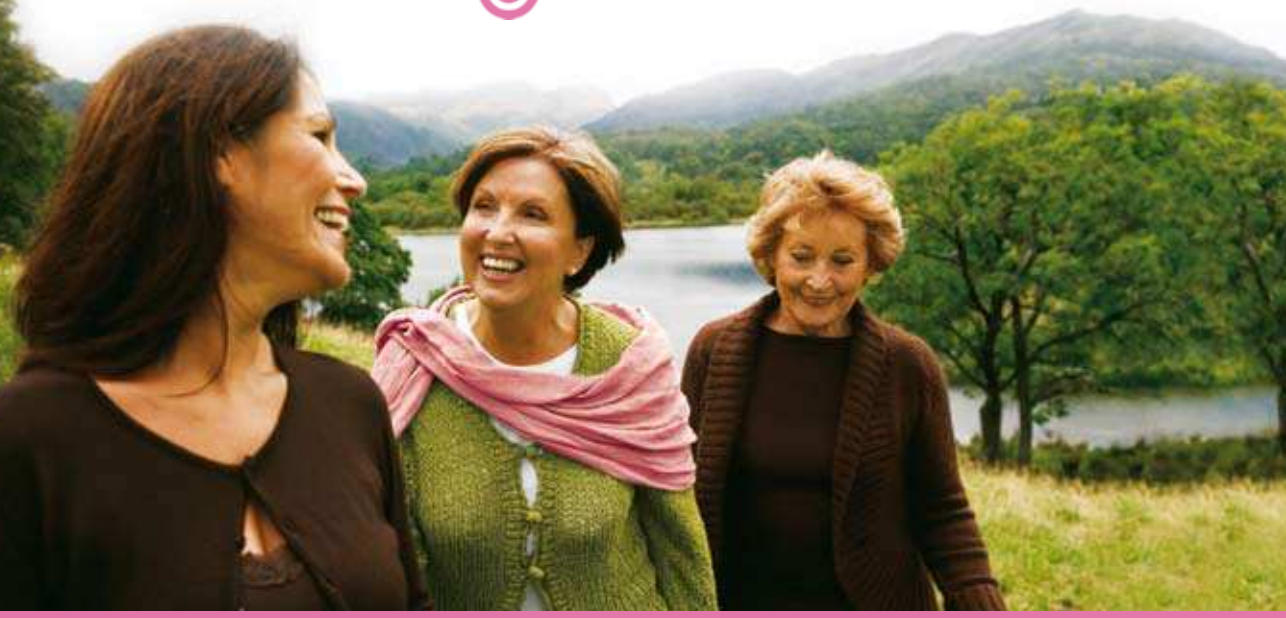
Arimidex®
anastrozol



Posodobili smo slovar

Skrajšan povzetek glavnih značilnosti zdravila Arimidex® 1 mg filmsko obložene tablete

Sestava zdravila: Ena tableta vsebuje 1 mg anastrozola.
Indikacije: Adjuvantno zdravljenje žensk po menopavzi, ki imajo zgodnji invazivni rak dojke s pozitivnimi estrogenskimi receptorji. Adjuvantno zdravljenje zgodnjega raka dojke s pozitivnimi estrogenskimi receptorji pri ženskah po menopavzi, ki so se dve do tri leta adjuvantno zdravile s tamoksifenom. Zdravljenje napredovalega raka dojke pri ženskah po menopavzi. Učinkovitost pri bolnicah z negativnimi estrogenskimi receptorji ni bila dokazana razen pri tistih, ki so imele predhodno pozitiven klinični odgovor na tamoksifen.
Odmerjanje in način uporabe: Odrasle (tudi starejše) bolnice: 1 tableta po 1 mg peroralno, enkrat na dan. Odmerka zdravila ni treba prilagajati pri bolnicah z blago ali zmerno ledvično odpovedjo ali blagim jetrnim odpovedovanjem. Pri zgodnjem raku je priporočljivo trajanje zdravljenja 5 let.
Glavni neželeni učinki: Zelo pogosti (≥ 10 %): navali vročine, običajno blagi do zmerni. Pogosti (≥ 1 % in < 10 %): astenija, bolečine/okorelost v sklepih, suhost vagine, razredčenje las, izpuščaji, slabost, diareja, glavobol (vsi običajno blagi do zmerni)
Posebna opozorila in previdnostni ukrepi: Uporabe Arimidexa ne priporočamo pri otrocih, ker njegova varnost in učinkovitost pri njih še nista raziskani. Menopavzo je potrebno biokemično določiti pri vseh bolnicah, kjer obstaja dvom o hormonskem statusu. Ni podatkov o varni uporabi Arimidexa pri bolnicah z zmerno ali hudo jetrno okvaro ali hujšo ledvično odpovedjo (očistek kreatinina manj kakor 20 ml/min (oziroma 0,33 ml/s)). Pri ženskah z osteoporozo ali pri ženskah s povečanim tveganjem za razvoj osteoporoze je treba določiti njihovo mineralno gostoto kosti z denzitometrijo, na primer s slikanjem DEXA na začetku zdravljenja, pozneje pa v rednih intervalih. Po potrebi je treba začeti z zdravljenjem ali preprečevanjem osteoporoze in to skrbno nadzorovati. Ni podatkov o uporabi anastrozola z analogi LHRH. Arimidex znižuje nivo estrogena v obtoku, zato lahko povzroči zmanjšanje mineralne kostne gostote. Trenutno ni na voljo ustreznih podatkov o učinku bifosfonatov na izgubo mineralne kostne gostote, povzročene z anastrozolom, ali njihovi koristi, če se uporabijo preventivno. Zdravilo vsebuje laktozo.
Kontraindikacije: Arimidex je kontraindiciran pri: ženskah pred menopavzo, nosečnicah in doječih materah, bolnicah s hujšo ledvično odpovedjo (očistek kreatinina manj kot 20 ml/min (oziroma 0,33 ml/s)), bolnicah z zmernim do hudim jetrnim obolenjem, bolnicah, ki imajo znano preobčutljivost za anastrozol ali za katerokoli pomožno snov. Zdravila, ki vsebujejo estrogen, ne smete dajati sočasno z Arimidexom, ker bi se njegovo farmakološko delovanje izničilo. Sočasno zdravljenje s tamoksifenom.
Medsebojno delovanje z drugimi zdravili in druge oblike interakcij: Klinične raziskave o interakcijah z antipirinom in cimetidinom kažejo, da pri sočasni uporabi Arimidexa in drugih zdravil klinično pomembne interakcije, posredovane s citokromom P450, niso verjetne. Pregled baze podatkov o varnosti v kliničnih preskušanjih pri bolnicah, ki so se zdravile z Arimidexom in sočasno jemale druga pogosto predpisana zdravila, ni pokazal klinično pomembnih interakcij.
Imetnik dovoljenja za promet: AstraZeneca UK Limited, 15 Stanhope Gate, London, W1K 1LN, Velika Britanija
Režim predpisovanja zdravila: Rp/Spec
Datum priprave informacije: april 2007

Pred predpisovanjem, prosimo, preberite celoten povzetek glavnih značilnosti zdravila.

Dodatne informacije in literatura so na voljo pri:
AstraZeneca UK Limited
Podružnica v Sloveniji
Verovškova ulica 55
1000 Ljubljana

in na spletnih straneh:
www.arimidex.net
www.bco.org
www.breastcancersource.com

AstraZeneca
ONKOLOGIJA

ARI 03/07 P

*adjuvant [ae´džuv*nt]*

1. *adjective* pomagljiv, koristen; *~ treatment with Arimidex:* Adjuvantno zdravljenje žensk po menopavzi, ki imajo zgodnji invazivni rak dojke s pozitivnimi estrogenskimi receptorji.

*advanced [*dva:nst]*

1. *adjective* napreden; zvišan (cene); to be ~ napredovati; ~ in years visoke starosti; *treatment of ~ breast cancer with Arimidex:* Zdravljenje napredovalega raka dojke pri ženskah po menopavzi. Učinkovitost pri bolnicah z negativnimi estrogenskimi receptorji ni bila dokazana razen pri tistih, ki so imele predhodno pozitiven klinični odgovor na tamoksifen.

switch [swič]

1. *transitive verb* udariti, bičati s šibo (z repom); šibati z, hitro mahati z; naglo pograbiti; railway ranžirati, zapeljati (usmeriti) (vlak) na drug tir; electrical vključiti, vklopiti; spremeniti (pogovor), obrniti drugam (tok misli); to ~ back to figuratively (v mislih) vrniti se na;
~ to Arimidex: Adjuvantno zdravljenje zgodnjega raka dojke s pozitivnimi estrogenskimi receptorji pri ženskah po menopavzi, ki so se dve do tri leta adjuvantno zdravile s tamoksifenom.

Temodal 20 mg, 100 mg, 140mg, 180 mg, 250 mg.

Sestava zdravila: Vsaka kapsula zdravila Temodal vsebuje 20 mg, 100 mg, 140 mg, 180 mg ali 250 mg temozolomida.

Terapevtske indikacije Temodal kapsule so indicirane za zdravljenje bolnikov z:

- za zdravljenje novo diagnosticiranega glioblastoma multiforme, sočasno z radioterapijo in kasneje kot monoterapija
- malignim gliomom, na primer multiformnim glioblastomom ali anaplastičnim astrocitomom, ki se po standardnem zdravljenju ponovi ali napreduje.

Odmerjanje in način uporabe Temodal smejo predpisati le zdravniki, ki imajo izkušnje z zdravljenjem možganskih tumorjev. **Odrasli bolniki z novo diagnosticiranim glioblastomom multiforme** Temodal se uporablja v kombinaciji z žariščno radioterapijo (faza sočasne terapije), temu pa sledi do 6 ciklov monoterapije z temozolomidom. **Faza sočasne terapije** Zdravilo Temodal naj bolnik jemlje peroralno v odmerku 75 mg/m^2 na dan 42 dni, sočasno z žariščno radioterapijo (60 Gy, danih v 30 delnih odmerkih). Odmerka ne boste zmanjševali, vendar se boste vsak teden odločili o morebitni odložitvi jemanja temozolomida ali njegovi ukinitvi na podlagi kriterijev hematološke in nehematološke toksičnosti. Zdravilo Temodal lahko bolnik jemlje ves čas 42-dnevnega obdobja sočasne terapije do 49 dni, če so izpolnjeni vsi od naslednjih pogojev: absolutno število nevtrofilcev ≥ 1,5 x 109/l, število trombocitov ≥ 100 x 109/l, skupni kriteriji toksičnosti (SKT) za nehematološko toksičnost ≤ 1. stopnje (z izjemo alopecije, slabosti in bruhanja). Med zdravljenjem morate pri bolniku enkrat na teden pregledati celotno krvno sliko. **Faza monoterapije** Štiri tedne po zaključku faze sočasnega zdravljenja z zdravilom Temodal in radioterapijo naj bolnik jemlje zdravilo Temodal do 6 ciklov monoterapije. V 1. ciklu (monoterapija) je odmerek zdravila 150 mg/m^2 enkrat na dan 5 dni, temu pa naj sledi 23 dni brez terapije. Na začetku 2. cikla odmerek povečajte na 200 mg/m^2, če je SKT za nehematološko toksičnost za 1. cikel stopnje ≤ 2 (z izjemo alopecije, slabosti in bruhanja), absolutno število nevtrofilcev (AŠN) ≥ 1,5 x 109/l in število trombocitov ≥ 100 x 109/l. Če odmerka niste povečali v 2. ciklusu, ga v naslednjih ciklusih ne smete povečevati. Ko pa odmerek enkrat povečate, naj ostane na ravni 200 mg/m^2 na dan v prvih 5 dneh vsakega naslednjega ciklusa, razen če nastopi toksičnost. Med zdravljenjem morate pregledati celotno krvno sliko na 22. dan (21 dni po prvem odmerku zdravila Temodal). **Ponavljajoči se ali napredujoči maligni gliom Odrasli bolniki** Posamezen ciklus zdravljenja traja 28 dni. Bolniki, ki še niso bili zdravljeni s kemoterapijo, naj jemljejo Temodal peroralno v odmerku 200 mg/m^2 enkrat na dan prvih 5 dni, temu pa naj sledi 23-dnevni premor (skupaj 28 dni). Pri bolnikih, ki so že bili zdravljeni s kemoterapijo, je začetni odmerek 150 mg/m^2 enkrat na dan, v drugem ciklusu pa se poveča na 200 mg/m^2 enkrat na dan 5 dni, če ni bilo hematoloških toksičnih učinkov (glejte poglavje 4.4). **Pediatrični bolniki** Pri bolnikih starih 3 leta ali starejših, posamezen ciklus zdravljenja traja 28 dni. Temodal naj jemljejo peroralno v odmerku 200 mg/m^2 enkrat na dan prvih 5 dni, potem pa naj sledi 23-dnevni premor (skupaj 28 dni). Otroci, ki so že bili zdravljeni s kemoterapijo, naj prejmejo začetni odmerek 150 mg/m^2 enkrat na dan 5 dni, s povečanjem na 200 mg/m^2 enkrat na dan 5 dni v naslednjem ciklusu, če ni bilo hematoloških toksičnih učinkov (glejte poglavje 4.4). **Bolniki z motnjami v delovanju jeter ali ledvic** Pri bolnikih z blagimi ali zmernimi motnjami v delovanju jeter je farmakokinetika temozolomida podobna kot pri tistih z normalnim delovanjem jeter. Podatki o uporabi zdravila Temodal pri bolnikih s hudimi motnjami v delovanju jeter (razred III po Child-u) ali motnjami v delovanju ledvic niso na voljo. Na podlagi farmakokinetičnih lastnosti temozolomida obstaja majhna verjetnost, da bo pri bolnikih s hudimi motnjami v delovanju jeter ali ledvic potrebno zmanjšanje odmerka zdravila. Kljub temu je potrebna previdnost pri uporabi zdravila Temodal pri teh bolnikih. **Starejši bolniki:** Analiza farmakokinetike je pokazala, da starost ne vpliva na očistek temozolomida. Kljub temu je potrebna posebna previdnost pri uporabi zdravila Temodal pri starejših bolnikih. **Način uporabe** Temodal mora bolnik jemati na tešče. Temodal kapsule mora bolnik pogoltniti cele s kozarcem vode in jih ne sme odpirati ali žvečiti. Predpisani odmerek mora vzeti v obliki najmanjšega možnega števila kapsul. Pred jemanjem zdravila Temodal ali po njem lahko bolnik vzame antiemetik Če po zaužitju odmerka bruha, ne sme še isti dan vzeti drugega odmerka. **Kontraindikacije** Temodal je kontraindiciran pri bolnikih, ki imajo v anamnezi preobčutljivostne reakcije na sestavine zdravila ali na dakarbazin (DTIC). Temodal je kontraindiciran tudi pri bolnikih s hudo mielosupresijo. Temodal je kontraindiciran pri ženskah, ki so noseče ali dojijo. **Posebna opozorila in previdnostni ukrepi** Pilotno preskušanje podaljšane 42-dnevne sheme zdravljenja je pokazalo, da imajo bolniki, ki so sočasno prejemali zdravilo Temodal in radioterapijo, še posebej veliko tveganje za nastanek pljučnice zaradi okužbe s Pneumocystis carinii (PCP). Profilaksa proti tovrstni pljučnici je torej potrebna pri vseh bolnikih, ki sočasno prejemajo zdravilo Temodal in radioterapijo v okviru 42-dnevne sheme zdravljenja (do največ 49 dni), ne glede na število limfocitov. Če nastopi limfopenija, mora bolnik nadaljevati s profilakso, dokler se limfopenija ne povrne na stopnjo ≤ 1. Antiemetična terapija: Z jemanjem zdravila Temodal sta zelo pogosto povezana slabost in bruhanje. **Laboratorijske vrednosti**: Pred jemanjem zdravila morata biti izpolnjena naslednja pogoja za laboratorijske izvide: ANC mora biti ≥ 1,5 x 109/l in število trombocitov ≥ 100 x 109/l. Na 22. dan (21 dni po prvem odmerku) ali v roku 48 ur od navedenega dne, morate pregledati celotno krvno sliko in jo nato spremljati vsak teden, dokler ni ANC nad 1,5 x 109/l in število trombocitov nad 100 x 109/l. Če med katerimkoli ciklusom ANC pade na < 1,0 x 109/l ali število trombocitov na < 50 x 109/l, morate odmerek zdravila v naslednjem ciklusu zmanjšati za eno odmerno stopnjo. Odmerne stopnje so 100 mg/m^2, 150 mg/m^2 in 200 mg/m^2. Najmanjši priporočeni odmerek je 100 mg/m^2. **Moški bolniki** Temozolomid lahko deluje genotoksično, zato morate moškim, ki se zdravijo z temozolomidom svetovati, da naj ne zaplodijo otroka še šest mesecev po zdravljenju. **Interakcije** Sočasna uporaba zdravila Temodal in ranitidina ni povzročila spremembe obsega absorpcije temozolomida ali monometiltriazenoimidazol karboksamida (MTIC). Jemanje zdravila Temodal s hrano je povzročilo 33 % zmanjšanje Cmax in 9 % zmanjšanje površino pod krivuljo (AUC). Ker ne moremo izključiti možnosti, da bi bila sprememba Cmax lahko klinično pomembna, naj bolniki jemljejo zdravilo Temodal brez hrane. Analiza populacijske farmakokinetike v preskušanjih druge faze je pokazala, da sočasna uporaba deksametazona, proklorperazina, fenitoina, karbamazepina, ondansetrona, antagonistov receptorjev H2 ali fenobarbitala ne spremeni očistka temozolomida. Sočasno jemanje z valprojsko kislino je bilo povezano z majhnim, a statistično značilnim zmanjšanjem očistka temozolomida. Uporaba zdravila Temodal v kombinaciji z drugimi mielosupresivnimi učinkovinami la hko poveča verjetnost mielosupresije. **Nosečnost** Študij na nosečih ženskah ni bilo. Predklinične študije na podganah in kuncih z odmerkom 150 mg/m^2 so pokazale teratogenost in/ali toksičnost za plod. Zato naj noseče ženske načeloma ne bi jemale zdravila Temodal. Če pa je uporaba v času nosečnosti nujna, morate bolnico opozoriti na možne nevarnosti zdravila za plod. Ženskam v rodni dobi svetujte, naj med zdravljenjem z zdravilom Temodal preprečijo zanositev. **Dojenje** Ni znano, ali se temozolomid izloča v materino mleko, zato ženske, ki dojijo ne smejo jemati zdravila Temodal. **Neželeni učinki** V kliničnih preskušanjih so bili najpogostnejši neželeni učinki, povezani z zdravljenjem, prebavne motnje, natančneje slabost (43 %) in bruhanje (36 %). Oba učinka sta bila ponavadi 1. ali 2. stopnje (od 0 do 5 epizod bruhanja v 24 urah) in sta prenehala sama, ali pa ju je bilo mogoče hitro obvladati s standardnim antiemetičnim zdravljenjem. Incidenca hude slabosti in bruhanja je bila 4 %. Laboratorijski izvidi: Trombocitopenija in. nevtropenija 3. in. 4. stopnje sta se pojavili pri 19 % in. 17 % bolnikov, zdravljenih zaradi malignega glioma. Zaradi njiju je bila potrebna hospitalizacija in/ali prekinitev zdravljenja z zdravilom Temodal pri 8 % in. 4 % bolnikov. Mielosupresija je bila predvidljiva (ponavadi se je pojavila v prvih nekaj ciklusih in je bila najizrazitejša med 21. in 28. dnem), okrevanje pa je bilo hitro, ponavadi v 1 do 2 tednih. Opazili niso nobenih dokazov kumulativne mielosupresije. Trombocitopenija lahko poveča tveganje za pojav krvavitev, nevtropenija ali levkopenija pa tveganje za okužbe. **Imetnik dovoljenja za promet** SP Europe 73, rue de Stalle B-1180 Bruxelles Belgija. **Način in režim izdaje** Zdravilo se izdaja samo na recept, uporablja pa se pod posebnim nadzorom zdravnika specialista ali od njega pooblaščenega zdravnika. **Datum priprave informacije** oktober 2007.

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ing telephone, fax and e-mail), and an abbreviated title. This should be followed by the *abstract page*, summarising in less than 200 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. The text of the report should then proceed as follows:

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Material and methods should provide enough information to enable experiments to be repeated. New methods should be described in detail. Reports on human and animal subjects should include a statement that ethical approval of the study was obtained.

Results should be presented clearly and concisely without repeating the data in the tables and figures. Emphasis should be on clear and precise presentation of results and their significance in relation to the aim of the investigation.

Discussion should explain the results rather than simply repeating them and interpret their significance and draw conclusions. It should review the results of the study in the light of previously published work.

Illustrations and tables must be numbered and referred to in the text, with appropriate location indicated in the text margin. Illustrations must be labelled on the back with the author's name, figure number and orientation, and should be accompanied by a descriptive legend on a separate page. Line drawings should be supplied in a form suitable for high-quality reproduction. Photographs should be glossy prints of high quality with as much contrast as the subject allows. They should be cropped as close as possible to the area of interest. In photographs mask the identities of the patients. Tables should be typed double spaced, with descriptive title and, if appropriate, units of numerical measurements included in column heading.

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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.

Page proofs will be faxed or sent by E-mail to the corresponding author. It is their responsibility to check the proofs carefully and fax a list of essential corrections to the editorial office within 48 hours of receipt. If corrections are not received by the stated deadline, proof-reading will be carried out by the editors.

- *ALIMTA je v kombinaciji s cisplatinom indicirana za zdravljenje bolnikov z neresektabilnim malignim plevralnim mezoteliomom, ki jih še nismo zdravili s kemoterapijo.*[1,2]
- *ALIMTA je indicirana kot monoterapija za zdravljenje bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim pljučnim karcinomom po predhodni kemoterapiji.*[1,3]

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SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA
ALIMTA 500 mg prašek za koncentrat za raztopino za infundiranje **Sestava** Vsaka viala vsebuje 500 mg pemetrekseda (v obliki dinatrijevega pemetrekseda). Pomožne snovi: manitol, klorovodikova kislina, natrijev hidroksid. **Indikacije** ALIMTA je v kombinaciji s cisplatinom indicirana za zdravljenje bolnikov z neresektabilnim malignim plevralnim mezoteliomom, ki jih še nismo zdravili s kemoterapijo. ALIMTA je indicirana kot monoterapija za zdravljenje bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim pljučnim karcinomom po predhodni kemoterapiji. **Odmerjanje in način uporabe** ALIMTO smemo dajati le pod nadzorom zdravnika, usposobljenega za uporabo kemoterapije za zdravljenje raka. Maligni plevralni mezotelion Priporočeni odmerek ALIMTE je 500 mg/m² telesne površine (TP), dan kot intravenska infuzija v 10 minutah prvi dan vsakega 21-dnevnega ciklusa. Nedrobnocelični pljučni karcinom Priporočeni odmerek ALIMTE je 500 mg/m² TP, dan kot intravenska infuzija v 10 minutah prvi dan vsakega 21-dnevnega ciklusa. **Kontraindikacije** Preobčutljivost za pemetreksed ali katerokoli pomožno snov. Med zdravljenjem s pemetreksedom je treba dojenje prekiniti. Sočasno cepljenje proti rumeni mrzlici. **Opozorila** Pri bolnikih moramo biti med zdravljenjem pozorni na morebitni pojav mielosupresije, pemetrekseda pa bolnikom se smemo dajati, dokler se absolutno število nevtrofilcev (ANC) ne povzne na ≥ 1500 celic/mm³ ter število trombocitov na ≥ 100.000 celic/mm³. Bolnikom, zdravljenim s pemetreksedom, moramo naročiti, naj jemljejo folno kislino in vitamin B12 kot preprečevalni ukrep za zmanjšanje toksičnosti, povezane z zdravljenjem. Predhodno zdravljenje z deksametazonom (ali drugim ustreznim kortikosteroidom) lahko zmanjša incidenco in resnost kožnih reakcij. Uporabe pemetrekseda pri bolnikih z očistkom kreatinina < 45 ml/min ne priporočamo. Bolniki z blagim do zmernim popuščanjem delovanja ledvic (očistek kreatinina od 45 do 79 ml/min) naj se izogibajo jemanju nesteroidnih protivnetnih zdravil (NSAID), denimo, ibuprofena in acetilsalicilne kisline (> 1,3 g dnevno) 2 dni pred dajanjem pemetrekseda, na dan dajanja in še 2 dni po dajanju pemetrekseda. Vsi bolniki, ki jih lahko zdravimo s pemetreksedom, naj se izogibajo jemanju NSAID-ov z dolgimi razpolovnimi časi izločanja vsaj 5 dni pred dajanjem pemetrekseda, na dan dajanja in še vsaj 2 dni po dajanju pemetrekseda. Pri bolnikih s klinično pomembno tekočino tretjega prostora moramo razmisliti o drenaži telesa pred dajanjem pemetrekseda. Bolnike moramo pred projekcijami terapije (vsaki po njej) ustrezno hidrirati ter skrbeti za zadostno antiemetično zdravljenje. Občasno so v kliničnih študijah pemetrekseda, obžarjajo ob sočasnem dajanju z drugo citotoksično učinkovino, poročali o resnih neželenih dogodkih, vključno z miokardnim infarktom in možganskožilnimi dogodki. Odsvetujemo uporabo živih oslabljenih cepiv (razen za rumeno mrzlico). Spolno zrelim moškim odsvetujemo zapiditev otroka v času zdravljenja in še 6 mesecev zatem. Zaradi možnosti, da zdravljenje s pemetreksedom povzroči trajno neplodnost, naj se moški pred začetkom zdravljenja posvetujejo o shranjevanju semena. Ženske v rodni dobi moramo v času zdravljenja s pemetreksedom uporabljati učinkovito kontracepcijo. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij** Sočasno dajanje nefrotoksičnih zdravil (denimo, aminoglikozidov, diuretikov zanke, spojin platine, ciklosporina) lahko potencialno povzroči zakasnjeni očistek pemetrekseda. Sočasno dajanje snovi, ki se naši izločajo s tubulno sekrecijo (denimo, probenecid, penicilin), lahko potencialno povzroči zakasnjeni očistek pemetrekseda. Pri bolnikih z normalnim delovanjem ledvic (očistek kreatinina ≥ 80 ml/min) lahko visoki odmerki nesteroidnih protivnetnih zdravil (NSAID-i, denimo, ibuprofen > 1600 mg dnevno) in acetilsalicilna kislina v visokih odmerkih (≥ 1,3 g dnevno) zmanjšajo eliminacijo pemetreksida in tako lahko povečajo pojavnost neželenih učinkov pemetreksida. Pri bolnikih z blagim do zmernim popuščanjem delovanja ledvic (očistek kreatinina 45 - 79 ml/min) se moramo izogibati sočasnemu dajanju pemetreksida z NSAID-i (denimo, ibuprofenom) ali acetilsalicilno kislino v visokih odmerkih 2 dni pred dajanjem pemetreksida, na dan dajanja in še 2 dni po dajanju pemetreksida. Sočasnemu dajanju NSAID-ov z daljšimi razpolovnimi časi s pemetreksedom se moramo izogibati vsaj 5 dni pred dajanjem pemetreksida, na dan dajanja in še vsaj 2 dni po dajanju pemetreksida. Velika različnost med posamezniki v koagulacijskem statusu v časi bolezni ter možnost medsebojnega delovanja med peroralnimi antikoagulacijskimi učinkovinami ter kemoterapijo proti raku zahtevata povečano pogostost spremljanja INR. Kontraindicirana sočasna uporaba: Cepivo proti rumeni mrzlici: tveganje za smrtno generalizirano bolezen po cepljenju. Odsvetovana sočasna uporaba: Živa oslabljena cepiva (razen proti rumeni mrzlici): tveganje za sistemsko, potencialno smrtno bolezen. **Neželeni učinki** Klinične študije malignega plevralnega mezotelioma Zelo pogosti: znižani nevtrofilci/granulociti, znižani levkociti, znižan hemoglobin, znižani trombociti, slabost, bruhanje, stomatitis/faringitis, anoreksija, diareja, zaprtje, utrujenost, nevropatija-senzorična, povišan kreatinin, znižan očistek kreatinina, izpuščaj, alopecija Pogosti: konjuktivitis, dispepsija, dehidracija, disgevzija Klinične študije nedrobnoceličnega pljučnega karcinoma Zelo pogosti: znižan hemoglobin, znižani levkociti, znižani nevtrofilci/granulociti, slabost, anoreksija, bruhanje, stomatitis/faringitis, diareja, utrujenost, izpuščaj/luščenje Pogosti: znižani trombociti, zaprtje, povišana telesna temperatura, povišanje SGPT (ALT), povišanje SGOT (AST), srbenje, alopecija Občasni: neni srčnožilni in možganskožilni dogodki, vključno z miokardnim infarktom, angino pektoris, cerebrovaskularnim insultom in predvidoma inhibicijskimi atakami Redki: primeri potencialno resnega hepatitisa, pankreatopatija Po uvedbi zdravila na trg so pri bolnikih poročali o redkih primerih kolitisa. **Imetnik dovoljenja za promet** Eli Lilly Nederland B.V., Grootslag 1-5, NL 3991 RA, Houten, Nizozemska **Datum zadnje revizije besedila** 30.01.2006 Podrobnejše informacije o zdravilu ALIMTA, so na voljo na lokalnem predstavništvu:

1. Povzetek glavnih značilnosti zdravila ALIMTA; 2. Hanna N et al. J Clin Oncol 2004;221589-1597; 3. Vogelzang NJ et al. J Clin Oncol 2003;21:2636-2644

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