

Predictive assays of tumor response to chemo and radiotherapy

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Currently used predictive assays of treatment outcome of tumors treated by chemo- or radiotherapy and radiation damage of normal tissues are presented. This review is focused on the assays where tumor cells derived from the human tumors need to be cultured in *in vitro* conditions to perform the test. In addition, an overview of the clinical studies dealing with the correlation between predictive assays and treatment outcome or radiation damage to the normal tissues is given.

Key words: neoplasms-drug therapy-radiotherapy; radiation injuries; treatment outcome

Introduction

The best treatment for a particular patient is based on a variety of factors predictive of the outcome of the therapy. In radiotherapy, these factors include tumour- and host- related factors, technical aspects of treatment and knowledge of the dose response relationship for tumour control and normal tissue injury. At present, the treatment plan is usually based on parameters such as tumour site, histology, stage, size, morphology, patterns of invasion of anatomical structures, location with regard to vulnerable normal tissues, and patient's performance status. Within these categories, some tumours show greater response to radiotherapy than others. If these

were identified before treatment, alternative therapies offering a better chance of cure than the standard conventional therapy might be selected. Therefore, there is a need for additional (other, more specific) predictive assays, which will provide the information that can be useful in the selection of an optimal treatment protocol for each patient.¹⁻³

An ideal predictive assay should,

- (a) correlate specifically with local tumour control, independently of other prognostic parameters,
- (b) be measurable precisely,
- (c) be relatively insensitive to sampling error,
- (d) be measurable quickly with regard to the initiation of treatment,
- (e) have low probability of falsely predicting resistance to conventional treatment,
- (f) be relatively harmless.

The research in the field of predictive assays started almost 30 years ago with the investigation of the relationship between clinical and tissue culture response to chemotherapeutic agents of human cancer.⁴ After this first report, a lot of researchers started to

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work on the development of tests that would predict the response of tumors to a particular treatment. Current research in predictive assays can be divided into three categories;

- (a) Studies of intrinsic cellular radio- and chemo- sensitivity;
- (b) Detection and quantification of hypoxic cells in human tumours;
- (c) Tumour cell proliferation kinetics and ploidy (repopulation).

Intrinsic radio- and chemo-sensitivity

The research in predicting the outcome of treatment started with the development of methods which enable to grow *in vitro* human tumor cells.³ The described assays can be used for predicting the treatment outcome either after radiotherapy or after treatment with chemotherapeutic drugs. The differences in responses of particular tumors to treatment with drugs are usually larger than to treatment with radiation. Therefore, the use of predictive assays in chemotherapy would be highly beneficial for a particular patient. If the patients with a resistant disease could be identified before the initiation of treatment, the toxicity of ineffective treatment would be spared to them.

Intrinsic radio- and chemo-sensitivity can be measured by survival, growth of cells, DNA damage and chromosome damage after treatment. Only colony forming assay which measures out the cell kill after a particular treatment, is a direct assay. All others tests, which measure either growth, DNA or chromosome damage, are indirect and measure the parameters which should correlate with cell kill.

Survival

The survival of cells is measured by colony forming, i.e. clonogenic assay. This assay is the gold standard for determination of treat-

ment efficiency, since, with this test, the ability of tumor cells to proliferate is measured directly. From the theoretical point of view, the principle of this test is very simple. The cells have to be removed from the tumors, prepared as single cell suspension, placed into appropriate growth environment, and exposed to radiation or drugs. After certain period of time, depending on the growth rate of the tumor cells, the formed colonies are fixed, stained and counted. By comparing the number of colonies in the treated group with the number in the control group, the surviving fraction can be calculated. As a predictor of treatment outcome after radiotherapy, a surviving fraction at 2 Gy is commonly used, as this is a usual daily dose in clinical radiotherapy.⁵

Growth

Since there are several practical problems associated with clonogenic assay, such as that not all human tumors can be grown *in vitro* and long duration of this test, alternative tests that measure the growth of the cells have been developed. The growth of the cells can be measured simply by counting the cells after certain period of time by means of dye-exclusion technique or by means of automated colorimetric assays. One of the examples of these assays is methyl tetrazolium test (MTT test), which estimates cell survival based upon the capacity of living cells to reduce a tetrazolium compound to a formazan crystals, a colored product that can be measured spectrophotometrically.^{6,7} The principle of this test is very similar to that of clonogenic assay. Cells are plated in microtiter plates and subjected to treatment. The difference between these tests is at the end of growth period, which is usually shorter in MTT assay than in the clonogenic assay. In the case of MTT assay, a substrate, methyl tetrazolium compound, is added to the cells which are further incubated for approx. 3-4 hours.

The formed formazan crystals are then dissolved in dimethyl sulfoxid and absorbance measured using microplate reader. The results of these assays show very good correlation with the clonogenic assay, therefore these assays represent a promising alternative to the clonogenic assay (Figure 1).^{6,7}

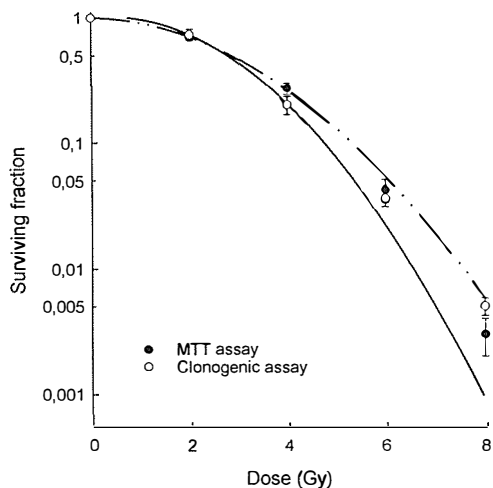


Figure 1. Survival curves for mouse fibrosarcoma cells SA-1 using MTT and clonogenic assay as a function of irradiation dose. Cells were irradiated in Petri dishes using Darpac 230 kV irradiation machine at a dose rate 2 Gy/min. For MTT test cells were transfer to microtiter plates and incubated for 7 days. After that period MTT test was performed. In clonogenic assay, colonies were fixed and stained 10 days after the irradiation (Čemažar unpublished data).

DNA damage

Another method used to determine intrinsic cellular sensitivity is based on measuring DNA damage. To assess DNA damage several test can be employed such as sucrose velocity sedimentation, filter elution, alkaline gel electrophoresis, membrane filtration, DNA precipitation, nucleoid sedimentation, alkaline unwinding, Halo, end tailing, viscoelastic and high performance liquid chromatography (HPLC), pulsed-field gel electrophoresis and single cell electrophoresis. The last two listed tests are the most promising test to be employed as a predictive one.⁸⁻¹³ However,

the predictive value of these two assays has to be confirmed, since the results of several studies are controversial, some showing very strong correlation with the colony forming assay, and others the lack of it.¹¹⁻¹³ The advantage of pulsed field gel electrophoresis is high sensitivity and specificity for measurement of DNA double strand breaks. The principle of pulsed field gel electrophoresis is as follows: the cells that have been taken from human tumor and treated are either radiolabelled before lysis and electrophoresis or stained with ethidium bromide after the electrophoresis. The advantage of pulsed field electrophoresis over the conventional one is that, by alternation of electric field the separation of DNA fragments is improved. This technique is therefore especially suitable for separation of large DNA fragments up to 12Mbp. If the DNA molecule of a known molecular weight is used as calibration, the separation of DNA from irradiated cells can be subsequently translated into a measure of strand breaks.⁹⁻¹³

Single cell electrophoresis (comet assay) is also widely studied for potential use as a predictive assay. The advantage of this assay is that we can monitor the response of a single cell to treatment and thus the problem of tumor or normal cell specificity may be overcome. The basis of this test is first to embed the cells into low-density agarose gel on a microscope slide. Then, the cells are lysed and subjected to electrophoresis. The broken DNA molecules migrate away from the general mass of DNA towards to anode and produce a typical feature which is called "comet". Variations in lysis conditions allow us to detect single and double DNA strand breaks, cross links and base damage.^{8,12,13}

Chromosome damage

One of the most obvious effects of radiation is chromosome damage. It has been demonstrated that certain chromosome changes

such as deletion of substantial part of chromosome lead to cell death. Therefore, the measurements of chromosome damage are another possible approach to measure intrinsic cellular sensitivity. The conventional technique to assess chromosome damage is the preparation of the karyotype of cells that have been exposed to radiation in *ex vivo* conditions and count the aberrations.¹⁴ Chromosomes are conventionally examined during metaphase. When chromosomal samples (karyotype) are prepared the colchicine or related agents that disrupt the formation of mitotic spindle fibres are added to arrest the cells in metaphase. The cells are then further exposed to hypotonic solution, fixed, placed on microscope slide and stained.

Another test used for the measurement of chromosome damage is micronucleus assay.¹⁵⁻¹⁹ Micronuclei arise from acentric chromatid or chromosome fragments induced by drugs or irradiation. In diploid cells, the presence of micronuclei signals cell death. The basis of this test is, first, to culture the cells after their exposure to drugs or radiation in the presence of cytochalasin B, the drug which in appropriate concentration allows karyokinesis, but inhibits cytokinesis. After that, the cells are fixed on the microscope slide and stained. Micronuclei can be counted by means of microscope (Figure 2). Some studies have shown very good correlation of the micronucleus assay with the cell kill measured by colony forming assay, and some have not.^{15,20} Therefore, the use of this assay as a possible predictor of tumor response have to be validated in further *in vitro* studies and also correlated with treatment outcome in clinical studies.

The newer techniques employed for measuring chromosome damage are premature chromosome condensation and fluorescence *in situ* hybridization (FISH technique).^{14, 21-23} When the interphase cell is fused with a cell in mitosis, it undergoes a process of premature chromosome condensation in which

chromosomes become visible. The mitotic cell can be of different type and its chromatin can be labelled with BrUdR so that, in binucleated fusion product, it is possible to identify the chromosome of target cell. The advantage of this technique is that it is very quick. It enables the scoring of breaks in chromatin within 10-15 minutes after irradiation and also the speed of their rejoining.^{21, 22}

The analysis of chromosome damage has been greatly facilitated by the development of specific probes (chromosome-specific lengths of DNA) that can be used in FISH. In this technique the chromosomes of target cell are fixed on microscope slide after exposure of cells to irradiation and heated to the level that much of their DNA becomes single stranded and incubated in the presence of labelled probes. The probes bind to the regions of chromosome DNA with which they are homologous. The bound probe is then detected with a fluorescent ligand which binds to the probe and which can be seen under fluorescence microscope.^{14,21-23} FISH technique has the following advantages over other techniques of measuring chromosomal damage: it is highly sensitive and requires small samples of tissue.

Gene expression after drug or radiation treatment

Cell death after therapy occurs by at least three mechanisms: apoptosis, necrosis and reproductive cell death. There are numerous genes that are associated with the cell response to agents and radiation. The development of techniques in molecular biology, which enables rapid assessment of gene expression and mutation, have stimulated an increasing number of reports dealing with correlation of molecular parameters with treatment outcome and prognosis.²⁴⁻²⁶ The screening of mutations in genes that are involved in radio and chemo resistance, cell

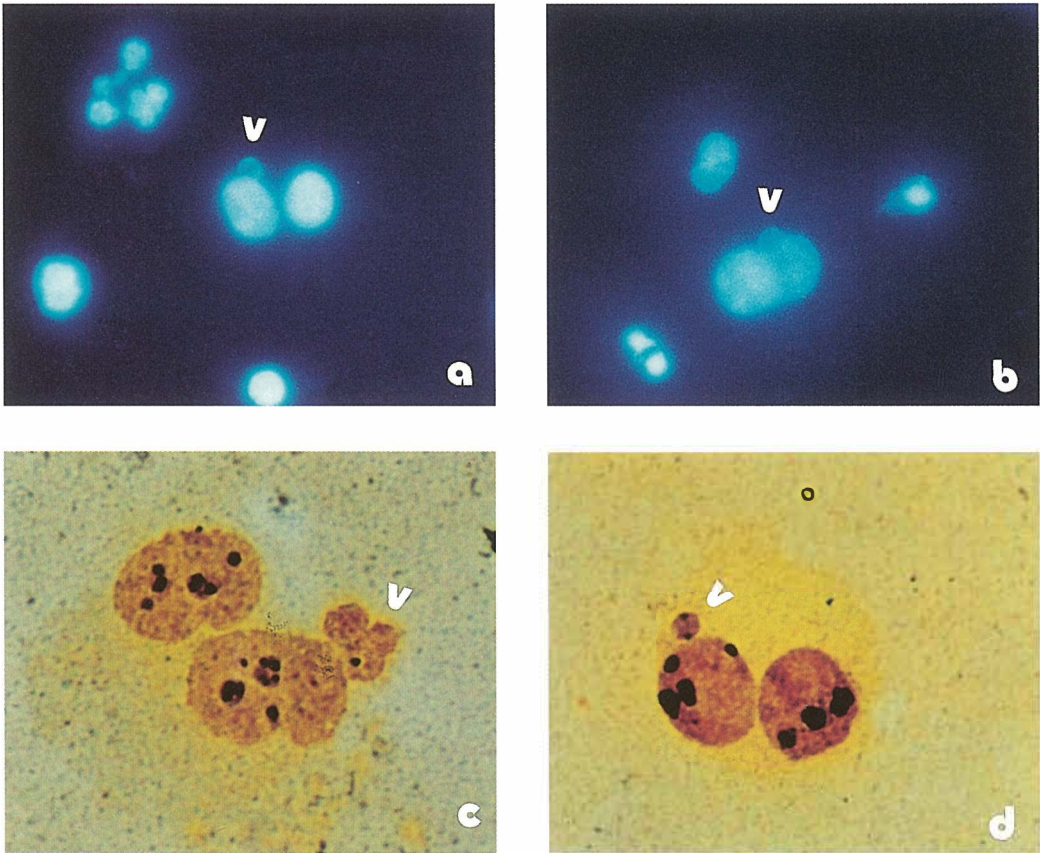


Figure 2. Presence of micronuclei in binucleated lymphocytes taken from nurses, occupationally exposed to chemotherapeutic drugs. (a) signal-positive micronucleus stained with DAPI, (b) signal-negative micronucleus stained with DAPI, (c) cell with two Ag-NOR- and one Ag-NOR+ micronuclei after silver staining, (d) Ag-NOR+ micronucleus with two signals after silver staining. From Garaj-Vrhovac *et al.* (1998) with permission.¹⁹

proliferation and repopulation, inflammatory response (growth factors), vascular damage, together with the cell cycle checkpoint and apoptosis genes could be, in future, one of the possibility to help predicting the sensitivity of human tumours to treatment with drugs or radiation therapy and also for predicting the radiation induced damage to normal tissues.²⁴ At present, at least five genes influencing radiation sensitivity were cloned, *viz.* *Ku70*, *Ku80*, *SCID*, *XRCC4* and *ATM* gene.²⁷ In the area of chemotherapy, expression of multidrug resistance *mdr* genes can

be measured and correlated with treatment outcome.²⁸

Hypoxia

The evidence that tumor hypoxia can reduce the efficiency of radiotherapy was provided a long time ago.²⁹ The identification of hypoxic tumors before the initiation of radiotherapy is now feasible with new quick and reliable techniques.^{1,30-34} The methods to detect hypoxia in human tumours are the following;

- Polarographic measurements using oxygen electrodes;
- Autoradiographic detection of radiolabelled misonidazole or its analogues which selectively bind to hypoxic cells;
- The detection of fluorine-labelled misonidazole or its derivatives using positron-emission tomography;
- The detection of ^{123}I -labelled nitroimidazoles by external scanning;
- Immunohistochemistry or detection by flow cytometry of nitroimidazole compounds;
- Non-invasive determination of oxygen distribution in tumours using magnetic resonance techniques;
- Invasive determination of oxygen using a fluorophore-tipped optical fibre (0.2 mm diameter) where the change in fluorescence lifetime, resulting from quenching of the fluorescence by oxygen, is measured in real time.

Tumor cell kinetics

The third category in predictive assays, that is subjected to extensive research, is tumor cell repopulation. The evidence for the importance of repopulation during radiotherapy has been obtained by the analysis of clinical data and measurements of the kinetics of cell proliferation in human tumour biopsies. Tumor cell proliferation can be measured by the use of radioactive precursors of DNA such as tritiated thymidine ($^3\text{HTdR}$) and autoradiographic detection of radiolabelled $^3\text{HTdR}$ in cells or tissue sections, by measuring total DNA content and iodo- or bromodeoxyuridine (IUdR or BrUdR) uptake using flow cytometry, or by detection of BrUdR-labelled cells on frozen sections or slides.³⁵⁻³⁷ In addition, proliferation status of tumors can be detected by staining tumor section with proliferation dependent antibodies, such as Ki67 (proliferation associated protein) and proliferating cell nuclear antigen (PCNA).^{38,39} When the cell proliferation is measured by

flow cytometry, labelling index (LI; proportion of cells within S phase) and potential doubling time (T_{pot} ; doubling time of clonogenic cells in the assumed absence of cell loss) can be determined.³⁷

Correlation with treatment outcome

In general, the results of predictive assays showed a good correlation with treatment outcomes in different types of tumors. In the case of intrinsic radiosensitivity, the survival at 2 Gy (SF_2) of carcinoma of the cervix correlated with both, local tumor control and survival of patients after treatment with radiotherapy.⁴⁰ However, for head and neck squamous cells carcinoma, this correlation was not demonstrated.⁴¹⁻⁴³ Correlation of *in vitro* drug sensitivity testing with response to chemotherapy showed that, in the case of small and non-small cell lung cancer, there was no correlation.⁴⁴ On the other hand, strong correlation of ^3H -uridine uptake assay and clinical response in patients with metastatic breast cancer was noted.⁴⁵ In a study of Klumper *et al.*, MTT assay was used to assess the chemosensitivity in childhood acute non-lymphoblastic leukemia. Among the drugs tested, the only failures of chemotherapy in these patients were found to be due to the resistance to cytosine arabinoside.⁴⁶ The role of micronucleus assay in predicting response to radiotherapy was demonstrated on 11 tumors of different origin. The tumors that produced more micronuclei after irradiation of cells *in vitro* showed better response to radiotherapy.¹⁸

In cervical cancer, it has been demonstrated by several authors that oxygenation of tumors can predict radiation response and survival of patients.^{47,48} In a study of Fyler *et al.*, it was shown that pO_2 reading below 5 mmHg as well as tumor size are significant prognostic factors in a univariate analysis of disease-free survival of patients with cervical

cancer.⁴⁸ In head and neck tumors, the differences in pO₂ measurements were observed between tumors and, for the majority of 35 tumors included in the study, the values of pO₂ were lower than that of normal tissue. However, there was no correlation with the treatment outcome, probably due to the limited number of patients included in that study.⁴¹

The T_{pot} as a predictor of tumor response to therapy did not prove its usefulness. In a study of Begg *et al.* as well in the study of Eshwege *et al.*, it was demonstrated that T_{pot} did not predict the treatment outcome of patients with head and neck carcinoma. However, LI showed to be more promising as a predictor of tumor response in head and neck tumors.^{41,49-52} Proliferation marker Ki67 showed to be associated with recurrent disease and PCNA with prediction of survival in patients with laryngeal cancer.³⁸

Radiation induced damage to normal tissues

Besides measurement of intrinsic radiosensitivity of tumor cells, several studies were also dealing with the response of normal tissue to radiotherapy and its use as a predictor of normal tissue complications after radiotherapy. Currently, the doses used in a conventional treatment are determined primarily by the most sensitive patients. Therefore, if a predictive assay that would recognise sensitive patients prior to the treatment could be developed, the doses given to those patients could be reduced and, consequently, the risk of severe complications could also be reduced. On the other hand, the doses given to more resistant patients could be increased to achieve an improved tumor control. It has been already recognised that at identical treatment regimens, the reactions of the normal tissues to treatment are more severe in some patients than in others. This is not due only to the interpatient difference in tissue

physiology and biology or genetically based difference in radiosensitivity, but also to the physical parameters, such as dosimetry (differences in the actual radiation dose delivered to the target cells of the normal tissue), treatment volume (irradiation volume of normal tissue vary with tumour size) and Poisson statistics (critical levels of "Tissue rescuing unit").^{37,53}

To predict the susceptibility to radiation damage, the same predictive assays as for the treatment outcome can be applied. Most of the current studies involve the measurements of colony formation, chromosome damage, counting of micronuclei, measurement of differentiation and DNA damage in fibroblasts or lymphocytes. Some studies indicate that a significant therapeutic gain could be achieved for a subset of patients from the use of the predictive assay of normal tissue radiosensitivity.^{15, 54-59} However, further validations of these results are needed on larger groups of patients.

Conclusion

Despite numerous predictive assays available at the time, their use has neither been widely accepted nor integrated into at least some aspects of the care of patients with cancer. There are several problems associated with predictive assays: not all patients' tumours can be grown *in vitro*, quality control, mimicking the *in vivo* pharmacokinetics of drugs in *in vitro* cell cultures, and long duration of clonogenic assay. Most of the problems have been solved with new *in vitro* predictive assays such as FISH assay for intrinsic cellular sensitivity or use of compounds that binds selectively to hypoxic cells, which will probably lead into clinical practice, hopefully in near future.

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References

1. Peters LJ, Brock WA, Chapman JD, Wilson G. Predictive assay of tumor radiosensitivity. *Am J Clin Oncol*; 1988; **11**: 275-87.
2. West CML. Invited review: Intrinsic radiosensitivity as a predictor of patient response to radiotherapy. *Br J Radiol* 1995; **68**: 827-37.
3. Von Hoff DD. He's not going to talk about *in vitro* predictive assays again, is he? *J Natl Cancer Inst* 1990; **82**: 96-101.
4. Wright JL, Cobb JP, Gumpert SL et al. Investigation of the relation between clinical and tissue culture response to chemotherapeutic agents of human cancer. *N Engl J Med* 1957; **252**: 1207-11.
5. Steel GG. Clonogenic cells and the concept of cell survival. In: Steel GG, editor. *Basic Clinical Radiobiology*. New York: Arnold, London & Oxford University Press; 1997. p. 40-51.
6. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of radiosensitivity. *Cancer Res* 1987; **47**: 943-6.
7. Wasserman TH, Twentyman P. Use of colorimetric microtiter (MTT) assay in determining the radiosensitivity of the cells from a murine tumours. *Int J Radiat Oncol* 1988; **15**: 699-702.
8. Poller F, Bauch T, Sauerwein W, Bocker W, Wittig A, Streffer C. Comet assay study of DNA damage and repair of tumour cells following boron neutron capture irradiation with fast d(14)+Be neutrons. *Int J Radiat Biol* 1996; **70**: 593-602.
9. Whitaker SJ, Ung YC, McMillan TJ. DNA double strand breaks induction and rejoining as determinants of human tumour cell radiosensitivity. A pulsed field gel electrophoresis study. *Int J Radiat Biol* 1995; **67**: 7-18.
10. Whitaker SJ, Powell SN, McMillan TJ. Molecular assays of radiation induced DNA damage. *Eur J Cancer* 1991; **27**: 922-8.
11. Wurm R, Burnet NG, Duggal N, Yarnold JR, Peacock JH. Cellular radiosensitivity and DNA damage in primary human fibroblasts. *Int J Radiat Oncol Biol Phys* 1994; **30**: 625-33.
12. Sarkaria JN, Bush C, Eady JJ, Peacock JH, Steel GG, Yarnold JR. Comparison between pulsed-field gel electrophoresis and the comet assay as a predictive assays for radiosensitivity in fibroblasts. *Radiat Res* 1998; **150**: 17-22.
13. Olive PL, Banath JP. Multicell spheroid response to drugs predicted with the comet assay. *Cancer Res* 1997; **57**: 5528-33.
14. Sheer D. Chromosomes and cancer. In: Franks LM, Teich NM, editors. *Introduction to the Cellular and Molecular Biology of Cancer*. New York: Oxford University Press; 1997. p 202-29.
15. Johansen J, Streffer C, Furmann C, Bentzen SM, Strasbol-Gron B, Overgaard M, et al. Radiosensitivity of normal fibroblasts from breast cancer patients assessed by the micronucleus and colony assays. *Int J Radiat Biol* 1998; **73**: 671-8.
16. Masunaga S, Ono K, Hori H, Shibata T, Suzuki M, Kinashi Y, et al. Effects of bioreductive agents, tirapazamine and mitomycin C, on quiescent cell populations in solid tumors, evaluated by micronucleus assay. *Jpn J Cancer Res* 1997; **88**: 907-14.
17. Muller WU, Nusse M, Miller BM, Slavotinek A, Viaggi S, Streffer C. Micronuclei: a biological indicator of radiation damage. *Mutat Res* 1996; **366**: 163-9.
18. Shibamoto Y, Shibata T, Miyatake S, Oda Y, Manabe T, Ohshio G, et al. Assessment of the proliferative activity and radiosensitivity of human tumours using the cytokinesis-block micronucleus-assay. *Br J Cancer* 1994; **70**: 67-71.
19. Garaj-Vrhovac V, Kopjar N. Micronuclei in cytokinesis-blocked lymphocytes as an index of occupational exposure to antineoplastic drugs. *Radiol Oncol* 1998; **32**: 385-92.
20. Abend M, Rhein A, Gilbertz KP, Blakely WF, Van Beuningen D. Correlation of micronucleus and apoptosis assay with reproductive cell death. *Int J Radiat Biol* 1995; **67**: 315-26.
21. Coco-Martin JM, Begg AC. detection of radiation-induced chromosome aberrations using fluorescence in situ hybridization in drug-induced premature chromosome condensations of tumour cell lines with different radiosensitivities. *Int J Radiat Biol* 1997; **71**: 265-73.
22. Coco-Martin JM, Ottenheim CPE, Bartelink H,

- Begg AC. Lethality of radiation-induced chromosome aberrations in human tumour cell lines with different radiosensitivities. *Int J Radiat Biol* 1996; **69**: 337-44.
23. Begg AC, Russell NS, Ottenheim CPE, Mooren E, Balm AF, Bartelink H, et al. What can we learn from FISH and chromosome damage that other techniques won't tell us? In: Kogelnik HD, Sedlmayer F, editors. *Progress in Radio-Oncology VI*, Bologna: Monduzzi Editore; 1998. p. 491-500.
 24. Hallahan DE. Radiation-mediated gene expression in the pathogenesis of the clinical radiation response. *Sem Radiat Oncol* 1996; **6**: 250-267.
 25. Franks LM, Teich NM. *Introduction to the Cellular and Molecular Biology of Cancer*. New York: Oxford University Press; 1997.
 26. Rocques PJ. Techniques of molecular biology. In: Yarnold J, Stratton M, McMillan T, editors. *Molecular Biology for Oncologists*. Amsterdam: Elsevier Science Publishers B.V.; 1993. p. 307-16.
 27. McMillan TJ. Genetic control of the cellular response to ionizing radiation. In: Steel GG, editor. *Basic Clinical Radiobiology*. New York: Arnold, London & Oxford University Press; 1997. p. 70-7.
 28. Hochhauser D. The molecular biology of drug resistance. In: Yarnold J, Stratton M, McMillan T, editors. *Molecular Biology for Oncologists*. Amsterdam: Elsevier Science Publishers B.V.; 1993. p. 239-48.
 29. Thomlinson RH, Gray LH. The histological structure of some human lung cancers and the possible implications for radiotherapy. *Br J Cancer* 1955; **9**: 539-49.
 30. Kavanagh MC, Sun A, Hu Q, Hill RP. Comparing techniques of measuring tumor hypoxia in different murine tumors: Eppendorf pO₂ histogram, [³H] Misonidazole binding and paired survival assay. *Radiation Res* 1996; **145**: 491-500.
 31. Rofstad EK, DeMuth P, Fenton BM, Ceckler TL, Sutherland RM. ³¹P NMR spectroscopy and HbO₂ cryospectrophotometry in prediction of tumor radioresistance caused by hypoxia. *Int J Radiat Oncol Biol Phys* 1989; **16**: 919-23.
 32. Hodgkiss RJ, Webster L, Wilson GD. Development of bioreductive markers for tumour hypoxia. *Acta Oncol* 1995; **34**: 351-5.
 33. Collingridge DR, Young WK, Vojnovic B, Wardman P, Lynch EM, Hill SA, et al. Measurement of tumor oxygenation: A comparison between polarographic needle electrodes and a time-resolved luminescence-based optical sensor. *Radiat Res* 1997; **147**: 329-34.
 34. Chapman JD, Engelhardt EL, Stobbe CC, Schneider RF, Hanks GE. Measuring hypoxia and predicting tumor radioresistance with nuclear medicine assays. *Radiother Oncol* 1998; **46**: 229-37.
 35. Werner BE, Ran S. The 5-Bromo-2'-deoxy-uridine labelling and detection kit III: A non-radioactive ELISA measuring DNA synthesis in endothelial cells. *Biochimica* 1995; **4**: 37-9.
 36. Begg AC. Cell proliferation in tumours. In: Steel GG, editor. *Basic Clinical Radiobiology*. New York: Arnold, London & Oxford University Press; 1997. p. 14-23.
 37. Begg AC. Individualization of radiotherapy. In: Steel GG, editor. *Basic Clinical Radiobiology*. New York: Arnold, London & Oxford University Press; 1997. p. 234-45.
 38. Welkoborsky HJ, Hinni M, Dienes HP, Mann WJ. Predicting recurrence and survival in patients with laryngeal cancer by means of DNA cytometry, tumor front grading, and proliferation markers. *Ann Otol Rhinol Laryngol* 1995; **104**: 503-10.
 39. Iatropulus MJ, Williams GM. Proliferation markers. *Exp Toxicol Pathol* 1996; **48**: 175-81.
 40. West CM, Davidson SE, Roberts SA, Hunter RD. The independence of intrinsic radiosensitivity as a prognostic factor for patient response to radiotherapy of carcinoma of the cervix. *Br J Cancer* 1997; **76**: 1184-90.
 41. Eschwege F, Bourhis J, Girinski T, Lartigau E, Guichard M, Deble D, et al. Predictive assays of radiation response in patients with head and neck squamous cell carcinoma: A review of the Institute Gustave Roussy experience. *Int J Radiat Oncol Biol Phys* 1997; **39**: 849-53.
 42. Brock WA, Baker FL, Peters LJ. Radiosensitivity of human head and neck squamous cell carcinomas in primary culture and its potential as a predictive assay of tumor radiocurability. *Int J Radiat Biol* 1989; **56**: 751-60.
 43. Brock WA, Baker FL, Wike JL, Sivon SL, Peters LJ. Cellular radiosensitivity of primary head and neck squamous cell carcinomas and local tumor control. *Int J Radiat Oncol Biol Phys* 1990; **18**: 1283-6.
 44. Shaw GL, Gazdar AF, Phelps R, Steinberg SM, Linnoila RI, Johnson BE, et al. Correlation of the *in vitro* drug sensitivity testing results with response to chemotherapy and survival: Comparison of

- non-small cell lung cancer and small cell lung cancer. *J Cell Biochem Suppl* 1996; **24**: 173-85.
45. Elledge RM, Clark GM, Hon J, Thant M, Belt R, Maguire YP, et al. Rapid *in vitro* assay for predicting response to fluorouracil in patients with metastatic breast cancer. *J Clin Oncol* 1995; **13**: 419-23.
 46. Klumper E, Pieters R, Kaspers GJ, Huismans DR, Loonen AH, Rottier MM, et al. *In vitro* chemosensitivity assessed with the MTT assay in childhood acute non-lymphoblastic leukemia. *Leukemia* 1995; **9**: 1864-9.
 47. Hockel M, Vormdran B, Schlenger K, Baubmann E, Knapstein PG. Tumor oxygenation: A new predictive parameter in locally advanced cancer of the uterine cervix. *Gynecol Oncol* 1993; **51**: 141-50.
 48. Fyles AW, Milosevic M, Wong R, Kavanagh MC, Pintilie M, Sun A, et al. Oxygenation predicts radiation response and survival in patients with cervix cancer. *Radiother Oncol* 1998; **48**: 149-56.
 49. Begg AC, Hofland I, Moonen L, Bartelink H, Schraub S, Bontemps P, et al. The predictive value of cell kinetic measurements in a European trial of accelerated fractionation in advanced head and neck tumours: and interim report. *Int J Radiat Oncol Biol Phys* 1990; **19**: 1149-53.
 50. Begg AC. The clinical status of T_{pot} as a predictor? or why no tempest in the T_{pot} ? *Int J Radiat Oncol Biol Phys* 1995; **32**: 1539-41.
 51. Begg AC. Prediction of repopulation rates and radiosensitivity in human tumours. *Int J Radiat Biol* 1994; **65**: 103-8.
 52. Antognoni P, Terry NHA, Richetti A, Lauraghi R, Tordiglione M, Danova M. The predictive role of flow-cytometry derived tumor potential doubling time (T_{pot}) in radiotherapy: Open questions and future perspectives. *Int J Oncol* 1998; **12**: 245-56.
 53. Tucker SL, Geara FB, Peters LJ, Brock WA. How much could the radiotherapy dose be altered for individual patients based on a predictive assay of normal-tissue radiosensitivity? *Radiother Oncol* 1996; **38**: 103-13.
 54. Burnet NG, Nyman J, Turesson I, Wurm R, Yarnold JR, Peacock JH. The relationship between cellular radiation sensitivity and tissue response may provide the basis for individualising radiotherapy schedules. *Radiother Oncol* 1994; **33**: 228-38.
 55. Burnet NG, Wurm R, Peacock JH. Low dose-rate fibroblast radiosensitivity and the prediction of patient response to radiotherapy. *Int J Radiat Biol* 1996; **70**: 289-300.
 56. Russell NS, Grummels A, Hart AM, Smolders IJH, Borger J, Bartelink H, et al. Low predictive value of intrinsic fibroblast sensitivity for fibrosis development following radiotherapy for breast cancer. *Int J Radiat Biol* 1998; **73**: 661-70.
 57. Geara FB, Peters LJ, Ang KK, Wike JL, Brock WA. Prospective comparison of *in vitro* normal cell radiosensitivity and normal tissue reactions in radiotherapy patients. *Int J Radiat Oncol Biol Phys* 1993; **27**: 1173-9.
 58. Begg AC, Russell NS, Knaken H, Lebesque JV. Lack of correlation of human fibroblast radiosensitivity *in vitro* with early skin reactions in patients undergoing radiotherapy. *Int J Radiat Biol* 1993; **64**: 393-405.
 59. Brock WA, Tucker SL, Geara FB, Turesson I, Wike J, Nyman J, et al. Fibroblast radiosensitivity versus acute and late normal skin responses in patients treated for breast cancer. *Int J Oncol Biol Phys* 1995; **32**: 1371-9.