Research Meeting on Advances in Cancer Therapy

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Program:

13:00 – 13:10	Opening
13:10 – 13:30	**Paul Keall** (MCV/VCU, Richmond, USA): *Potential Therapeutic Gains with Gated Radiotherapy*
13:30 – 13:50	**Martin Ebert** (Sir Charles Gairdner Hospital, Perth, Australia): *Dose-Area Analysis for Endovascular Brachytherapy*
13:50 – 14:10	**Vlado Robar** (Institute of Oncology, Ljubljana): *BEAM: Radiation Therapy Modelling Tool*
14:10 – 14:30	**Božidar Casar** (Institute of Oncology, Ljubljana): *Polystyrene Phantom for Simulation of Stereotactic Radiosurgery using Radiochromic Films*
14:30 – 14:50	**Robert Jeraj** (Jožef Stefan Institute, Ljubljana): *Importance of Accurate Dose Calculation in Inverse Treatment Planning*
14:50 – 15:10	Coffee break
15:10 – 15:30	**Hotimir Lešničar** (Institute of Oncology, Ljubljana): *Effect of thermoradiotherapy: a consequence of direct or indirect radiosensitization*
15:30 – 15:50	**Marjan Budihna** (Institute of Oncology, Ljubljana): *The treatment of advanced, inoperable sqamous cell carcinoma of the oropharynx with radiotherapy and concomitant Mitomycin C and Bleomycin and some prognostic factors*
15:50 – 16:10	**Srdjan Novakovič** (Institute of Oncology, Ljubljana): *Tumour Vaccines against Malignant Melanoma*
16:10 – 16:30	**Damijan Miklavčič** (Faculty of Electrical Engineering, Ljubljana): *Engineering Perspective of in vivo Electrochemotherapy*
16:30 – 16:50	**Gregor Serša** (Institute of Oncology, Ljubljana): Improvement of *Combined Modality Therapy with Cisplatin and Radiation using Electroporation of Tumours*
16:50 – 17:30	General discussion

Dear participants

We are very happy to welcome you at the *Research Meeting on Advances in Cancer Therapy*. The meeting was initially intended to be just a small informal meeting of the radiotherapy group from Institute of Oncology, Ljubljana and medical physics group from Jožef Stefan Insitute, Ljubljana. However, because of general interest, which we are very pleased about, we decided to organise a larger one. The high interest is also indication that more similar meetings should be organised and hopefully they would become regular practice of exchanging ideas.

The meeting is organised in two large sections: medical physics part and combined radiobiological/electroengineering/clinical part. Hopefully the variety of the presentations would stimulate even more fertile discussions and brainstorming. Even though very different in their approach both sections share a common goal – presenting ways to fight cancer more efficiently. Fighting cancer more efficiently is possible only if we understand it better, if we understand current methods of treatment and are familiar with the current research. If we manage to add just a small stone to the huge pile of knowledge on cancer treatment today, this goal will be achieved.

Enjoy the meeting!

Robert Jeraj
Božidar Casar

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Potential Therapeutic Gains with Gated Radiotherapy

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Respiration motion in radiotherapy during imaging reduces the contrast, causes artifacts, and leads to less well-defined tumour and critical structure delineation. The size and position of the defined gross tumour volume (GTV) is uncertain, and therefore larger margins need to be added to construct the PTV.

Gated radiotherapy uses a respiration-related measurement, such as chest wall motion, which is correlated with internal anatomy motion. During both CT imaging and treatment, the beam is only on during a certain part of the breathing cycle, and therefore the position and size of the GTV is more reproducible.

Gated radiotherapy can potentially reduce the size of the PTV without compromising the treatment. If a smaller volume is treated, treatment related complications will be reduced, and/or the dose can be increased to achieve greater tumour control.

This talk will discuss both the potential gains with gated radiotherapy, and the gated imaging experience at the Medical College of Virginia.

Dose –Area Analysis for Endovascular Brachytherapy

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Introduction The optimal choice of isotope and form (i.e., wire, liquid etc) for endovascular brachytherapy depends on many factors including dose uniformity across arterial structures, the influence of source centering, cost/availability, half-life and consideration of radiation protection. The centering issue was considered by calculating dose distributions due to several sources, and examining the dose-area relationship for artery wall as sources are moved off-centre.

Material and methods Several sources currently being used in clinical trials were considered - ^{32}P (wire), ^{192}Ir (wire) and ^{188}Re (in saline solution). EGS4 Monte Carlo was used with a cylindrical user-code in calculation of dose distributions around the various sources (assuming in a water-equivalent medium), and to obtain dose fall-off curves with distance from the centre of each source.

Two model arteries were considered, one with the lumen and artery wall concentric, and the other with assymetry present in both lumen and artery wall. The artery walls were broken up into multiple sectors, and dose to these sectors due to an off-centre wire calculated using the Monte Carlo-derived dose distributions. Dose-Area information was then derived from the resulting arterial dose distributions (only 2D information was considered). This calculation was repeated for several off-axis positions of the two wire-based sources (see Table 1) to yield a series of dose-area hisotgrams (DAHs). For the solution-based source (^{188}Re), only a single DAH was obtained since the balloon used for irradiation with ^{188}Re is self-centering.

Results and discussion The results indicate the benefits for uniformity of using a γ-emitting source, at the cost of increased dose to distal normal tissue. The γ-emitting source has a slower dose fall-off with resulting higher distal-doses. The β-emitters have a more rapid dose fall-off with a small bremsstrahlung tail past the maximum electron range. This more rapid dose fall-off leads to a potentially broader range of doses to the artery wall when sources are not centred .This should be considered in light of the general inaccuracy associated with dose-prescription in endovascular brachytherapy.

BEAM: Radiation Therapy Modeling Tool

Vlado Robar
Institute of Oncology

What is it? BEAM is a general purpose Monte Carlo code package that allows visual modeling of radiation therapy units, mostly linear accelerators. It is built on EGS4 code system and it runs on most Unix computers, with Linux being the platform of choice for low cost simulation. To run BEAM a Fortran F77 compiler is required (g77 recommended), whereas MORTRAN3, a preprocessor for Fortran is hidden in the code and average user is shielded from using it. Knowledge of MORTRAN3 is necessary only for development of new component modules. BEAM code can be efficiently run on networked computers or on parallel CPUs.

What it does? BEAM code simulates radiation beams from any radiation therapy source, including low-energy x-rays, ^{60}Co beam and electron and photon beams from linear accelerators. BEAM models any radiation therapy source in detail by means of component modules (CM). CM is a simple 3D geometric object that can be fully described by a few parameters (e.g. a cylinder, a cone, a stack of cones). User can build a radiation therapy unit by putting together existing CMs (collimating jaws, mirrors, target, applicators) modeled individually. If needed one can develop new CMs. At the therapy unit exit window, radiation beam is represented in 5 dimensional phase-space. The dosimetric phantom may also be made of component modules, but a set of CT images can be employed for calculation of dose distribution in a phantom or patient. Even at the present stage of development, BEAM can function as an experimental 3D treatment planning system.

How to get it? BEAM code is result of the OMEGA collaboration between National Research Centre (NRC) in Ottawa, Canada, and Madison University in Wisconsin. In order to get one's hands on the code, a course given at NRC has to be taken (http://www.irs.inms.nrc.ca/inms/irs/omega/brochure.html). All upcoming versions are Web-distributed free of charge for the course attendants.

What is its future? As computers becomes more powerful and cheaper, computing time for simulation is decreasing, which makes BEAM a capable tool for solving a wide range of dosimetric problems in radiation therapy. Calculations can be faster not only by using faster computers but also by improving computing algorithms, especially those for variance reduction. Future improvements of computer speed and further development of the code makes BEAM a firm ground for a 3D Monte Carlo treatment planning system.

Reference: **BEAM: A Monte Carlo code to simulate radiotherapy treatment units**, D.W.O. Rogers et al., *Med.Phys. 22 (5), May 1995*

Polystyrene phantom for simulation of stereotactic radiosurgery using radichromic films

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Purpose: The aim of the study was to design and build polystyrene phantom appropriate for simulation of stereotactic radiosurgery, and to measure absorbed does distribution in three orthogonal planes through isocenter using radiochromic films

Methods: A bullet shaped phantom contained 6 cm edge cube. A radiochromic film (Nuclear Associates, Div. of Victoreen) was placed in the middle of the cube in parallel to one of its planes. Phantom was scanned on the Siemens Somatom 4+ Ct in the sequence and the spiral mode. To mark the cube edges, angiographic catheters 5F (french) were glued in cranio-caudal direction orthogonal to transverse CT slices. After delineation of the target volume by neuroradiologist, a simple simulated treatment was performed with 5 MV philips SL-75/5 linac. After location the tumor center, a dedicated tertiary collimator system (1) was used during simulation. Absorbed dose was measured in orthogonal planes through isocenter for three cube orientations . After three days, films were scanned with Multidata film densitometer (Multidata. corp).

Results: Our phantom can be used for acquisition of quasi 3D information about dose distrubution.

Conclusions: Relative simple yet precise absorbed dose measurements confirm that radiochromic films and our phantom can be used as a succesful tool for absorbed dose verification.

References: (1) Casar B, Tertiary collimator system for stereotactic radiosurgery with linear accelerator, Radiol Oncol 1998; 32(1): 125-128

Importance of accurate dose calculation in inverse treatment planning

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Most of the current inverse treatment planning algorithms use fast, but inaccurate dose calculation algorithms. As in conventional treatment planning, the use of such algorithms may lead into systematic errors in dose predictions of the order of a few percent. In inverse treatment planning a convergence error also appears because the inverse planning algorithm converges to the optimal solution for the inaccurate dose calculation, which is different from the optimal solution for the accurate dose calculation. In this work, both errors were identified and examined for three typical treatment sites: head and neck, lung and prostate. In addition, the biological significance of the errors was assessed.

Three different dose calculation methods were used: Monte Carlo, convolution/ superposition and pencil beam. Monte Carlo-based inverse treatment planning algorithm (MCI) was modified to incorporate non-Monte Carlo dose calculation methods as well. The effect of different types of objective functions and hard constraints on the errors were examined.

It was found that the systematic errors are not dependent on the objective functions, while the convergence errors express strong dependence. Typical standard deviations of systematic errors for the collapsed cone convolution dose calculations are 1-3% of D_{max} (highest for head and neck) and standard deviations for the convergence errors are 3-8% of D_{max}. This results in systematic tumour control probability (TCP) errors up to 4%, and convergence TCP errors up to 2%. Values and biological significance of errors for pencil beam-based inverse treatment planning were significantly higher.

Effect of thermoradiotherapy: a consequence of direct or indirect radiosensitization

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Introduction: Although, there are evidences supporting the direct radiosensitizing effect of hyperthermia (1), lately, some reports claimed that indirect radiosensitization (via enhanced oxygenation) is probably more important (2). The latter statement is based on randomized clinical trials data in which thermoradiotherapy showed definite advantage over radiotherapy alone, though, cumulative minimum intratumoral temperatures did not exceed 41° C. On the other hand, it was shown in controlled animal experiments that the enhancement of tumor oxygenation caused by "mild hyperthermia" can not last more than 10 minutes after the heating (3). It is obvious that only the sequencing of the two therapies in which hyperthermia (HT) precedes radiotherapy (RT) should support the hypothesis of indirect radiosensitization. It was our aim to overview our clinical records of patients treated by thermoradiotherapy and to find some prognostic parameters which support one or another of above hypotheses.

Material and methods: At the Institute of Oncology, Ljubljana, Slovenia 52 patients with locoregionaly inoperable malignant tumors were treated by thermoradiotherapy (TRT). Majority of patients were preiradiated. The cumulative total tumor dose did not exceed 100 Gy. There were 29 patients in whom RT preceded HT, while in 23 patients RT immediately followed HT. Total tumor dose of RT ranged from 20-70 Gy (median 60 Gy). In 28 patients an immediate fraction size >3 Gy was used concurrent with HT. Complete response (CR) rates and toxicity were evaluated according to internationally established criteria.

Results: In all 52 treated patients a CR rate of 60% was observed. Histology, tumor volume, minimum temperature, total tumor dose, immediate fraction size of RT, and sequence of RT and HT were found to have a significant prognostic value. For 28 patients in whom minimum intratumoral temperature of 42.5°C was achieved and a fraction size of ≥ 3 Gy was applied immediately before HT a CR rate of 75% was observed. Late toxicity was more pronounced in patients in whom a cumulative tumor dose of ≥ 85 Gy was applied.

Conclusions: From our data it is obvious that only minimum intratumoral temperatures of ≥ 42.5°C enabled an acceptable final treatment outcome. The effect of TRT was significantly pronounced in those treatments where heat was applied immediately after irradiation. With such a sequencing a hypothetical improvement of intratumoral oxygenation could not play a beneficial role. Therefore, our own clinical data speaks in favor of direct radiosensitizing effect of hyperthermia.

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The treatment of advanced, inoperable sqamous cell carcinoma of the oropaharynx with radiotherapy and concomitant Mitomycin C and Bleomycin and some prognostic factors.

M. Budihna, E. Šoba, L. Šmid, B. Zakotnik, H. Lešničar

Background. The incidence of the oropharyngeal squamous cell carcinoma in Slovenia is rapidly increasing, almost as fast as lung cancer. More than 2/3 of patients referred to our institute are in an advanced stage. The results of the radiotherapy as a sole treatment were very poor: only 10% were cured. Therefore, concomitant chemotherapy was added to the conventional irradiation treatment.
The aim of this study is to show the recurrence-free survival (RFS) according to some patient, tumour or chemoradiotherapy related parameters.

Methods. From September 1990 to December 1997, 95 males and 3 females aged 37 – 70 years (median 52,5 years) were treated for Stage III (21 pts.), IVa (64 pts.), and IVb (13 pts) squmous cell carcinoma of the oropharynx. The daily irradiation dose was 1.8 Gy – 2 Gy 5 times weekly, up to
36 Gy – 78 Gy (median 68 Gy), the dose of Mitomycin 15 mg / m^2 was given on day 5 or 6, and 5 mg of Bleomycin 2 times weekly from the start of the treatment. The irradiation treatment was the same in both groups. Patients were randomized in the arm without chemotherapy and the arm with chemotherapy only in the first 2 years. After 2 years of study the randomization was stopped because of far better results in chemotherapy arm.
Three patients received only paliative radiotherapy and were excluded from the study.
The curves for RFS were done according to Kaplan–Mayer, the difference in RFS was calculated with logrank test.

Results. Table 1 shows results according to some parameters related to the tumour patient or the treatment

Table 1.

Observed parameter	N	Probability RFS (%) at 7 years	p
All patients	95	53	-
T3	49	61	0.03
T4	45	46	
N0-2a	43	71	
N2b-N2c	40	51	0.003
N3	12	0	
Stage III	21	68	
IVa	61	58	0.006
IVb	12	0	
Performance status (WHO) 0	67	66	<0.0001
1 or 2	28	22	
Treatment time <48 days	44	70	0.06
≥48 days	51	44	
Tumour dose ≥68 Gy	56	62	0.06
<68 Gy	39	42	
BED* (Gy) >67	65	60	0.02
<67	30	38	
Mitomycin C ≥28 mg	43	68	0.11
<28mg	52	43	
Bleomycin ≥25 mg	49	62	0.06
<25 mg	46	46	

*BED = biological effective dose

Toxic effects: Radiomucositis was more severe in patients in chemotherapy arm. In few patients the preplanned irradiation tumour dose and/or Bleomycin dose had to be reduced. There was no serious leukopenia or thrombopenia. Late effects were comparable in both groups.

Conclusion

The irradiation treatment with concomitant Mitomycin C and Bleomycin is an effective treatment for advanced oropharyngeal carcinoma.

Tumor vaccines against malignant melanoma

Srdjan Novaković

It has been widely recognized that current adjuvant (post-surgical) therapies of cancer are not fully satisfactory mainly owing to the two global reasons: insufficient effectiveness and severe toxic side effects. In the past few years we have witnessed a rapid development of different biological and genetic therapies created to fight malignant diseases. These therapies have been designed to be more effective, more specific for tumor cells, and cause no or negligible toxic side effects. The most attractive current biological approach seems to be the creation of specific tumor vaccines. Several distinct ideas how to design a tumor vaccine were formed predominately to achieve: 1) enhanced production of various cytokines that participate in immune processes (IL-2, GM-CSF, IFN-γ, TNF-α); 2) expression of allogeneic HLA antigens; 3) replacement of defective p53 genes, or 4) introduction of "suicide genes" into target cells - genes that sensitize cells to drugs that are normally non-toxic for mammalian cells. Most of the mentioned propositions include a genetic manipulation (that favors some characteristics or processes capable of inducing the antitumor immune response) of autologous or homologous cells and usage of different non-viral or viral vectors for gene transfer. However, there is still some hesitation present when genetically manipulated constructs should be applied in humans. So our idea was to prepare and compare the effectiveness of two different vaccines against malignant melanoma: one prepared without utilizing foreign genetic material and another by inserting the gene of interest into syngeneic (autologous) tumor cells. The first approach proposes the creation of tumor vaccine by simple mixing of autologous - syngeneic sublethally irradiated B-16 melanoma tumor cells with pleiotropic biological response modifier - MVE-2 (maleic anhydride divinyl ether). The irradiated tumor cells are supposed to provide a sufficient quantity of tumor antigens, while the immunomodulator MVE-2 should at least multiply the number of cytotoxic macrophages that play a crucial role in the antitumor activity of the immune system together with CTL. By the second approach the HuTNF-α gene was ligated into the expression vector - pcDNA3 plazmid and transferred into the syngeneic B-16 melanoma tumor cells. The transfected cells were then sublethally irradiated and used for vaccination of experimental animals. From the results it could be concluded that the effectiveness (i.e. tumor preventing potential) of the vaccine prepared without genetic manipulation is at least comparable (if not superior) to the one of genetically manipulated vaccine. Namely, the genetically manipulated tumor vaccine only prolonged the survival of vaccinated animals, while the vaccine containing irradiated syngeneic tumor cells and MVE-2, completely prevented tumor development in more than 40% of prevaccinated animals challenged intraperitoneally with $5x10^5$ viable tumor cells. Moreover, the percentage of tumor-free animals (survivors) rose to as much as 90% when the application of this vaccine was repeated two weeks after the first vaccination. However, it has to be mentioned that the potentials of the genetically manipulated vaccine have not been fully determined so far since there are still some essential experiments that remain to be carried out.

Engineering Perspective of *in vivo* Electroporation

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In the last two years, promising results for a new non-viral efficient gene therapy have been obtained in *in vivo* DNA electrotransfer studies [1-8]. It is also important to note that, recently, drug delivery using electric pulses has entered an active period of clinical trials [9-13]. These two new therapeutical approaches are based on cell electropermeabilization, also termed electroporation, a phenomenon where a transiently increased plasma membrane permeability is obtained after the cells were exposed to short and intense electrical pulses. Electropermeabilization thus allows otherwise nonpermeant molecules to enter the cytosol [14,15].

For effective drug delivery and gene transfection *in vivo*, the knowledge of electric field distribution is of utmost importance, to obtain an effective permeabilization as well as to maintain the viability of the electropermeabilized cells. Indeed, in order to achieve electropermeabilization in the tissue of interest, the magnitude of electric field intensity has to be above a critical threshold value [14,16,17], i.e. the reversible threshold. Furthermore, the magnitude of electric field intensity should not exceed the value which would produce irreversible damages to the plasma membrane, i.e. the irreversible threshold. Thus, the magnitude of electric field intensity should be high enough to cause reversible electropermeabilization but lower than the value causing irreversible damage [2,18]. The latter is the most critical for *in vivo* gene transfer but is also desirable in electrochemotherapy in order not to produce large instantaneous necrosis, which would result in massive tumour necrosis and possible exulceration and wound appearance. Moreover, for gene therapy, it has been recently reported [19] that, under relatively homogeneous exposure conditions [20], the optimal conditions for gene transfer correspond to the use of long pulses (20 milliseconds) at a voltage just necessary to obtain cell electropermeabilization, i.e. just above the reversible permeabilization threshold. Above the irreversible permeabilization threshold, when permanent damages are inflicted to the plasma membrane, viability is lost and efficacy of the DNA transfer is severely impaired [2]. Therefore it is necessary to determine (i) the electric field distribution in the target tissues, and (ii) the reversible as well as (iii) the irreversible permeabilization thresholds in order to use voltages and electrode geometries resulting in optimal exposure of the targeted tissue to electric fields intensities comprised between the two thresholds.

Needle electrodes seem to be the most practical type of electrodes both for electrochemotherapy and gene therapy. However, the field generated by two or more needles is very inhomogeneous, as compared to the field distribution between two parallel plates. Nevertheless, it has been previously demonstrated that electric field distribution in the tissue can be controlled by the position of the electrodes in the tissue [21-23]. Therefore, control of electrode geometry and of pulse shape should allow to get the goals described here above, making electroporation quite safe and efficient both for electrochemotherapy and DNA electrotransfer.

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Improvement of combined modality therapy with cisplatin and radiation using electroporation of tumors

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Purpose: To evaluate whether local drug delivery method, *i.e.* electroporation of tumors, increases radiosensitizing effect of cisplatin.

Materials and Methods: Subcutaneous EAT tumors in CBA mice were treated either by cisplatin, electric pulses or ionizing radiation. In electrochemotherapy protocol electric pulses were given to the tumor 3 min after intravenous injection of cisplatin. The interval between electrochemotherapy and irradiation was 20 min. Treatment effectiveness was evaluated by tumor growth delay and local tumor curability.

Results: Electrochemotherapy of EAT tumors proved to be effective treatment, resulting in 12% tumor cures, whereas treatment with cisplatin or electric pulses alone did not yield any tumor cures. As expected, injection of cisplatin 20 min prior to irradiation, increased radioresponse of tumors from 27% to 73% tumor cures. Electroporation of tumors also increased radiation response of tumors to 54% tumor cures. Electrochemotherapy given prior to irradiation increased radioresponsiveness of tumors, resulting in 92% tumor cures.

Conclusions: This study shows that delivery of cisplatin into the cells by electroporation of tumors increases radiosensitizing effect of cisplatin. However, some effect may also be ascribed to application of electric pulses to the tumors that in our study also predisposed tumor cells to radiation damage.

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