Preclinical data in prostate cancer shows upregulation of a wide variety of growth factors and their receptors such as PDGF, EGF, IGF, FGF, and VEGF suggesting efficacy of agents targeting these pathways. Data on the use of growth signal targeting in prostate cancer by tyrosine kinase inhibitors and monoclonal antibodies alone, and in combination with chemotherapy and/or radiotherapy seem to be promising in prostate cancer.

Antisense oligonucleotide therapy: One of the most important pathways that bypasses the androgen receptor involves the deregulation of apoptotic genes. Bcl-2, which regulates apoptosis (programmed cell death), is expressed in most human cancers and is an important contributor of resistance to therapy. Bcl-2 protein is a critical regulator of apoptosis in many tissues and is over expressed in the majority of patients with HRPC. Bcl-2 may mediate resistance to androgen ablation and chemotherapy and appears to have a critical role in the transition from androgen-dependent to androgen-independent growth. Antisense oligonucleotide therapy is currently under evaluation.

Bone targeted therapy: HRPC is often associated with the development of painful bone metastases. Newer generation bisphosphonates may relieve pain caused by bone metastases, prevent treatment-related loss of bone mineral density, possibly slow the growth of metastases, and reduce skeletal complications. They are effective for the treatment of both osteolytic and osteoblastic metastases.

Targeting the Endothelin Receptor: Endothelin A plays a role by inhibition of apoptosis, stimulation of proliferation, stimulation of osteoblasts and has pain nociceptive effects. In HRPC there are increased plasma concentrations of Endothelin-1 (ET-1), decreased clearance of endothelin and increased endothelin A expression. Endothelin axis deregulation triggers a series of events that lead to a profound deregulation in cancer cells, including key tumorigenic cellular events such as proliferation, invasion, escape from programmed cell death, new vessel formation, abnormal osteogenesis and the alteration of nociceptive stimuli. Atrasentan is a potent, oral, selective endothelin-A receptor antagonist. Two large randomized studies of atrasentan have been performed in metastatic HRPC. A meta-analysis demonstrated a reduction in the risk associated with disease progression, attenuation of the rise of biomarkers, delay in time to biochemical progression, decrease in time to bone pain and incidence of bone pain, and disease-specific quality of life benefit.

Targeting Angiogenesis: Vascular endothelial growth factor (VEGF) is a growth factor that is essential for pathological neoplastic angiogenesis, tumor growth and metastasis. Antiangiogenesis is a relatively new antitumor strategy that has been employed in the treatment of many malignancies. As prostate cancer is likely dependent on angiogenesis for its growth and progression, it would logically serve as a good target for this modality. Initially met with great enthusiasm, antiangiogenic drugs have seen only limited success when used as single agents. This has been attributed to many possible etiologies including lack of cytotoxicity and use in situations of large tumor burden. In order to overcome these problems, antiangiogenic agents are also being used in combination with more traditional cytotoxic chemotherapy regimens.

The tumor-associated stroma may also produce significant amounts of VEGF via tumor-associated induction of the VEGF gene promoter. Inhibition of VEGF signaling with several strategies, including monoclonal antibodies to VEGF and tyrosine kinase inhibition are currently under clinical development. The combination of docetaxel and thalidomide an anti-angiogenic agent has demonstrated activity in HRPC.

Other drugs inhibit several raf kinases and other tyrosine kinase targets including VEGFR-2, PDGFR-b, FLT-3 (flit 3) and c-KIT, which may inhibit both angiogenesis and cell signaling.

The outline of our present and future focus in the study of HRPC is becoming clearer with an increasing understanding of therapeutic targeting based on the biologic sub setting and novel therapeutics targeting several different hallmarks of cancer.

Scientific Symposium
The challenge of implementing intensity modulated radiotherapy in the clinic

78 INVITED

Target volume definition and organ motion for IMRT

J.J. Nuyttens. *Erasmus University Medical Center, Radiation Oncology, Rotterdam, The Netherlands*

Intensity-modulated radiotherapy (IMRT) can shape the radiation dose distribution with high precision, such that we can treat irregularly shaped tumor target volumes with therapeutic doses while sparing the surrounding healthy tissues. However, setup errors and motion of the patient or the inner organs during the treatment can result in a geographical miss and have a negative effect on the outcome, if the dose is conformed too closely to the clinical target volume. For this reason, the target volume definition is very important and is described by the International Commission on Radiation Units and Measurements (ICRU) Reports 50 and 62. These recommendations clearly define the Gross Tumor Volume (GTV), the Clinical Target Volume (CTV), the Internal Target Volume (ITV), and the planning target volume (PTV). The GTV is the volume of known tumor. The CTV is the volume of suspected microscopic spread. The ITV encompasses the motion of the CTV and is formed by adding a margin [Internal Margin (IM)] to the CTV. The PTV is the volume necessary to account for patient motion and is formed by adding a Setup Margin (SM) to the ITV. Although these recommendations are widely used, they have some practical limitations because the ITV or IM is only defined for some organs like prostate, pancreas and rectum. However for prostate cancer, the IM was differently reported by several investigators (e.g.: the anterior-posterior motion of the prostate ranges from 2.7 to 4.5 mm [1 standard deviation (SD)]. The ITV has also been defined for lung cancer but the ITV is different according to the location of the tumor in the lung. Another problem is the deformation of the ITV caused by motion during the CT. Other pitfalls in the ITV definition are the bladder filling or the bowel motion. A full bladder during simulation can be requested but previous research showed that it was difficult for the patient to be treated with a full bladder. Also the variation of bowel inside the pelvis can be large and differ from day to day: an anterior-posterior motion of the rectum up to 10 mm (1SD) was found. Many techniques are developed to reduce the internal margin or setup margin because a smaller PTV combined with an IMRT technique often results in a dose reduction to the organs at risk. Examples of these techniques that reduce the set up margin are patient immobilization using molds, casts, or other restraining devices and on- or offline portal imaging protocols. Some of the techniques that reduce internal margin are abdominal compression, deep inspiration breathing training, active breathing control and target tracking with markers placed in the tumor. With this last technique the CTV to PTV margin (IM+SM) can be reduced from 10 mm to 1 mm.

79 INVITED

The challenge of implementing IMRT planning

W. De Neve. *University Hospital Gent, Department of Radiotherapy, Gent, Belgium*

Ideally, the IMRT planning system creates a desired dose distribution as a sequence of treatment machine-states and monitor unit values (often called control point sequence). In reality, the present IMRT planning systems are not yet capable to achieve this goal autonomously. The systems are interactive and many machine parameters have to be set upfront by a skilled planner. Also, the desired dose distribution may be impossible to achieve and the systems then need expert guidance to achieve an *acceptable dose distribution* as a realistic goal. We define as acceptable; a dose distribution that differs from the desired dose distribution 1) within preset limits of dose and 2) only in regions where the desired dose distribution cannot be physically achieved.

For IMRT planning, the dose provisional prescription guidelines in a protocol often need to be complemented by additional parameters to obtain suitable dose objectives for planning. Procedures to obtain these parameters will be discussed including i) PTV-fragmentation, relaxing the dose objectives and securing flash to deal with build-up and in-air PTV regions, ii) priority ranking, fragmentation and weighting of importance factors to deal with PTV and PRV/OAR overlap volumes and with conflicting dose objectives and iii) dose constraints to the UIV (Unspecified Imaged Volume); the creation of virtual critical structures, pseudo-OARs and shells and the use of stepwise constraints with distance from PTV to avoid dose littering and hot spots outside PTV and PRV/OAR.

Finally, future directions will be discussed including i) the use of probability distributions of the CTV and OAR location based on models for anatomical deformation that will make the concepts of PTV and PRV obsolete and will solve many of the problems associated with build-up, flash and overlap; ii) the incorporation of biological and functional imaging in IMRT planning; iii) voxel-based instead of contour-based IMRT planning and iv) the use of biological- instead of dose-objectives in IMRT planning.

80 INVITED

Treatment delivery

T. Knöös. *Lund University Hospital, Department of Radiation Physics, Lund, Sweden*

This review will be about the developments during the last 10 years in delivery of intensity modulated radiation therapy a.k.a. IMRT. The techniques can in short be divided in

- tomotherapy or fan based and
- multi-leaf collimator, MLC or cone based methods.

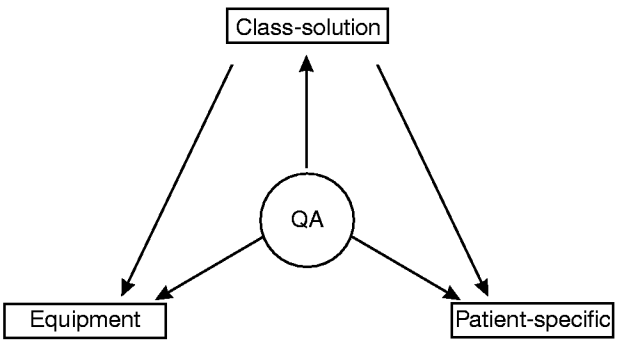
In tomotherapy you use a fan beam (line dose delivery) which either treat the volume in a slice-by-slice mode similar to the earlier CT scanner or you use the same helical scanning that today is the standard technology in diagnostic CT scanning. The fan beam is intensity modulated by a kind of MLC technique. MLC methods (area dose delivery) uses beams which are either static or dynamic where the first type consists of a series of sub-beams delivered in sequence. The radiation is turned off in-between each sub-beam while the leaves move to the next position. Once the leaves are in position the dose for that segment is delivered. This delivery technique has been named Segmented MLC (SMLC). An earlier name has been step and shoot. The dynamic method can be described as a sweeping technique of each leaf-pair according to a predetermined pattern. This technique is named Dynamic MLC (DMLC). The pros and cons of the different techniques will be discussed during this session.

81 INVITED

Towards a realistic and effective Quality Assurance paradigm for IMRT

C. De Wagter. *University Hospital Gent, Department of Radiotherapy, Gent, Belgium*

Quality Assurance (QA) of radiation therapy should ideally extend to the therapeutic outcome. Fortunately, the quality of IMRT is closely related to the absorbed dose distribution which can be elegantly expressed and tested quantitatively. The concept of a distribution inherently combines the positional and intrinsically dosimetric endpoints of IMRT.
Apart from IMRT-dedicated delivery systems, which are still scarce, the majority of IMRT treatments are delivered using traditional linear accelerators, equipped with multileaf collimators that were at the onset basically designed to replace shielding blocks. A great deal of QA activities should therefore be focused on machine performance characteristics that are rarely rigorously specified and usually receive little attention during maintenance and periodic quality control. This is the domain of **equipment QA**, which continues the commissioning phase of the radiation delivery equipment and the treatment planning system.
Class-solution QA comprises the experimental assessment of the total dose distribution delivered to an anthropomorphic phantom. Class-solution QA is the ultimate pre-treatment test for the planning and delivery chain of IMRT, including the human links. Dosimetric verification is achieved by comparing the delivered (measured in the phantom) dose distribution to the aimed (computed for the phantom) distribution. All critical parts of the dose distributions should be involved in the comparison, e.g., dose to PTV, dose to OARs and dose gradients. The clinical class solution itself, the delivery equipment, the planning approach followed within the class solution, and the experience gained by the IMRT team will determine the number of clinical cases required within a given class solution.
Patient specific QA should focus on the verification of patient positioning (during-treatment with image guided radiation therapy) and on the detection of possible gross errors (pre-treatment), e.g., of more than 5% in absolute dose (relative to the prescribed dose).



A pyramid-shaped conceptual approach will be presented that optimises and streamlines QA procedures within class solutions, rather than proliferating redundant QA checks. Then, as illustrated in the figure below, an IMRT QA program can be considered as a triad of three categories of QA. This paradigm stimulates familiarization with IMRT and keeps the level of alertness high.

Scientific Symposium
Apoptosis in drug and radiotherapy resistance

82 INVITED

Apoptosis: no life without death

P.H. Krammer. *German Cancer Research Center, Abteilung Immungenetik, Heidelberg, Germany*

CD95, a member of the tumor necrosis factor (TNF) receptor superfamily induces apoptosis upon receptor oligomerization. The receptor and its ligand are important for apoptosis of peripheral T cells, for downregulation of an immune response and most likely, at least in part, also for peripheral T cell tolerance. In AIDS, apoptosis mediated by this system might contribute to the depletion of T helper lymphocytes. Likewise, in diseases in which liver cells are destroyed the CD95 system might play a major role. In a search to identify the intracellular signalling pathway of CD95 several molecules coupling to oligomerized CD95 were immunoprecipitated from apoptosis-sensitive human leukemic T cell and lymphoblastoid B cell lines. The following binding molecules were only associated with aggregated and not with monomeric CD95: phosphorylated FADD (MORT1) and caspase 8. Thus, caspase 8 was identified as the most CD95 receptor proximal protease which starts the cascade of protease reactions important for CD95-mediated apoptosis. Association of FADD and caspase 8 with CD95 was not observed with C-terminally truncated non-signalling CD95. FADD and FLICE did also not associate with a CD95 cytoplasmic tail carrying the lprcg amino acid replacement. FADD and caspase 8 form a death-inducing signalling complex (DISC) with the CD95 receptor and are, thus, the first CD95 associating proteins of a signalling cascade mediating apoptosis. The function of the DISC is discussed in detail, particularly with respect to its role in sensitivity and resistance to apoptosis.
The CD95 death system plays a role in destruction of liver tissue. In hepatitis cytotoxic T lymphocytes might use the CD95 system to kill infected hepatocytes. In M. Wilson copper overload leads to upregulation of the CD95 ligand that may finally contribute to acute liver failure. In HCC from patients treated with chemotherapeutic drugs the CD95 receptor and ligand are upregulated and may contribute to apoptosis of the tumor or, dependent on the drug sensitivity of the tumor, to the status of the tumor as an immunoprivileged site.

References

[1] Krammer, P.H. CD95(APO-1/Fas)-Mediated Apoptosis: Live And Let Die (ed. Frank J. Dixon). Advances in Immunology, 163–210, 1998.
[2] Peter, M.E. and Krammer, P.H. Mechanisms of CD95(APO-1/Fas)-mediated Apoptosis. Current Opinion in Immunology 10, 545–51, 1998.

83 INVITED

Chemoresistance and apoptosis: shifting from the apoptotic default pathway to the adaptive stress response

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Chemotherapy resistancle has often been related to disabled apoptosis with deficient caspase activation. We have recently found that, even in the absence of any adjuvant, tumor cells dying in response to anthracyclins can elicit an effective anti-tumor immune response that precludes the growth of inoculated tumors or leads to the regression of established neoplasia. Caspase inhibition by Z-VAD-fmk or transfection with the baculovirus inhibitor p35 did not inhibit doxorubicin-induced cell death (as measured as clonogenic survival), yet suppressed the immunogenicity of dying tumor cells in a variety of different rodent models of neoplasia. Depletion of DC in vivo curtailed the immune response against doxorubicin-treated apoptotic tumor cells. Caspase inhibition suppressed the capacity of doxorubicin-killed cells to be phagocytosed by dendritic cells (DC), yet had no effect on their capacity to elicit DC maturation. Freshly excised tumors became immunogenic upon doxorubicin treatment in vitro, and intratumoral inoculation of doxorubicin could trigger the regression of established tumors in immunocompetent mice. These results delineate a procedure for the generation of cancer vaccines and the stimulation of anti-neoplastic immune responses in vivo.
Moreover, they suggest that inhibition of caspase activation (which leads to a shift from apoptotic to non-apoptotic death modalities) can abrogate the immunogenic nature of cell death, thus favoring the escape of tumors from immune surveillance. In addition, I will discuss the role of HSP70 as an apoptosis inhibitor as well as an inhibitor of immunogenic cell death.