Radioterapie
&
Oncologie Medicală

ESTRO Course: Molecular Oncology for the Radiation Oncologist

Estoril, Portugal, April 29-May 3, 2007

Recent advances in our understanding of radiation biology, molecular biology, and imaging are allowing us to see and study tumors like never before. We can now develop unique treatment strategies for patients in ways only dreamed of by previous generations of physicians, scientists and patients. The possibilities for increasing cancer cure while at the same time minimizing the side effects of treatment are exciting and invigorating. This is the reason for the organization by ESTRO of such teaching courses like the one held in Estoril, Portugal, "*Molecular Oncology for the radiation oncologist",* the target audience being formed of radiation oncologists, radiobiologists and physicists. This course had an excellent teaching staff, prominent personalities in radiobiology, molecular biology and radiotherapy:

- Bradley G. Wouters, the course director, professor of molecular radiotherapy at the Research Institute Growth and Development, University of Maastricht, Netherlands
- Adrian Begg, Experimental Therapy Department, The Netherlands Cancer Institute, Amsterdam
- Kevin Harrington, Cellular and Molecular Biology Department of Oncology Research Institute, London
- Philippe Lambin, Radiotherapy Department, Maastro Clinic, Netherlands
- Martin Pruschy, Molecular Radiobiology Laboratory of the Universitary Hospital Zurich
- Marie-Catherine Vozenin, Institute Gustave Roussy.

The program of this 5 day course was abundant with interesting talks on very actual topics and also included daily tutorials and workshops, aiming to encourage the attendees to actively participate with questions and opinions.

First day was dedicated to the molecular basis of cancer:

- *The Hallmarks of cancer*. Acquired properties caused by changes that occur in the genome by different genetic routes: 1.Self- sufficiency in growth signals; 2. Insensitivity to anti-growth signals; 3.Evading apoptosis; 4.Limitless replicative potential; 5. Hypoxia tolerance/ angiogenesis; 6. Tissue invasion and metastasis. These "hallmarks" affect treatment and radiation sensitivity.
- *Molecular basis of radiation response*: *genome organization and regulation of gene expression:* flow of information from DNA to proteins, with all the mechanisms and factors which control gene expression; transcription factors, mRNA stability, translation factors, protein stability.
- Based on the central dogma of molecular biology: DNA↔ RNA ↔ proteins, the main *techniques and model systems used in molecular biology* were reviewed by Philippe Lambin, such as: DNA analysis (cloning, Southern Blot, PCR); RNA analysis (Northern Blot, RT-PCR, microarray); protein analysis (Western Blot, immunohistochemistry, immunofluorescence, bi-dimensional separation of proteins and mass spectrometry analysis).
- *Oncogenes/ Tumor suppressor genes:* two classes of genes play a fundamental role in cancer initiation and progression: the oncogenes stimulate cell survival, proliferation, spreading (functionally activated in cancer), while the tumor suppressor genes inhibit cell survival and proliferation, being functionally inhibited in cancer.
- *Cell signaling pathways*- the cell- cell and cell- microenvironment communication network. Modifications of either one of these pathways lead to modifications with important effects on the organism. Important concept: regulation occurs both when turning the signal on and when turning it off.
- *Molecular responses to hypoxia*- Hypoxia is an omnipresent factor in cancer, being an important prognostic factor, having a negative impact on cancer due to the biological responses to hypoxia. It changes the biology of

cell, activates HIF (hypoxia inducible factor) important in angiogenesis and energy production, inactivates translation (preserving ATP and activating other genes important for tumors). Tumors become hypoxia tolerant, allowing the use of hypoxia to provide angiogenesis, gene expression variation.

- *Tumor angiogenesis*- the "angiogenic balance", regulated by activators and inhibitors. Angiogenesis is a dynamic process and a prognostic/ predictive factor: high MVD and VEGF mean worse clinical outcome for most tumors.

The second day was dedicated to the molecular basis of radiation response:

- *DNA repair*- Based on the idea that cell survival need DNA lesions to be repaired, there are several mechanisms involved in this process: base excision repair for base damages or single-strand breaks and recombination for double-strand breaks (homologous recombination –HR and non-homologous end-joining- NHEJ). Inhibition of DNA repair can increase radiosensitivity. Double-strand breaks are the most lethal type of damage produced by radiation.
- *Checkpoints*- cell cycle consists of two major phases: the interphase (90%), during which the chromosomes duplicate, cell components are made and normal cell functions are carried out and the mitotic phase- cell division. A cell that moves through the cell cycle has to pass important checkpoints when the cell "checks" whether to continue progressing through the cycle, being of great importance for preventing errors. Alterations of these checkpoints lead to mutations, cancer, genetic instability. Cell progression through the cell cycle is regulated by the cyclin dependent kinases (CDKs), their activity being regulated by cyclin levels, CDK phosphorylation, CDK inhibitors. Radiation induces four distinct checkpoints, each having different implications in the radiosensitivity of the cell.
- *Cell death- Apoptosis*- apoptosis is one of the five modes of cell death= programmed death. The importance of this process in tumor radiosensitivity was discussed: is apoptosis important? The conclusion would be that in carcinomas the prognostic and therapeutic value is limited, being more important in lymphomas and other tumors predisposed to apoptosis. It could also be important in normal tissue protection. The other modes of cell death: mitotic death, necrosis, senescence, and autophagy were also discussed.
- *Gene expression and normal tissue effects*- normal tissue response to radiation is dependent on the individual gene expression.

Third day: combining radiotherapy with targeted therapies. Reasons: to overcome resistance mechanisms, for spatial co-operation, radiosensitisation, favorable alteration of tumor biology: reoxygenation, cell cycle redistribution, inhibition of DNA repair, accelerated repopulation. The final goal is to increase the therapeutic window, by decreasing the therapeutic effect dose and increasing the dose that gives normal tissue toxicity.

- Association of radiotherapy with chemotherapy showed limited effects in numerous clinical trials.
- The perspective would be the selection of target following the six "hallmarks" of cancer

1. Targeting hypoxia/angiogenesis. There were several approaches: increasing tumor oxygenation leads to increase in radiosensitivity; specific killing of hypoxic cells using Tyrapazemine (TPZ), a bioreductive drug which in hypoxic conditions forms TPZ radicals, which cause DNA breaks. In aerobic conditions, TPZ radicals are reoxidized towards the parent compound with the production of superoxide radicals which are mildly cytotoxic; targeting hypoxic molecular pathways- HIF-1 (hypoxia inducible factor) using small molecule inhibitors of HIF-1 activity; inhibiting angiogenesis, normalizing tumor vasculature, with anti-VEGF compounds- Bevacizumab; inducing severe hypoxia by destroying tumor vessels= vascular targeting
2. Targeting proliferation. The two main pathways involved in cell proliferation are the MAPK (mitogen activated protein kinase) pathway and the PI3K (phosphatidyl inozitol 3 kinase) pathway, both being involved in growth stimulation, the last also in inhibition of apoptosis. The cell cycle also controls cell proliferation, being regulated by cyclins and cyclin-dependant kinases (CDKs). Alterations of the cell cycle regulation affects cell proliferation, CDK inhibitors protect non-mitotic cells from apoptosis, while on the mitotic cells they have antiproliferative effects: G1arrest or G2 arrest.
3. Targeting DNA repair. The problem is how therapeutic effect can be obtained, by targeting tumor tissue and sparing normal tissues. So when should we target DNA repair? When the tumor uses a repair pathway more than normal tissues (homologous recombination-HR), when a tumor has already weakened repair capacity

(reduced expression, deletion, mutation- BRCA) or when a drug can be targeted preferentially or exclusively to the tumor (difficult or impossible in most situations at present)

4. Targeting apoptosis. Apoptosis is involved in physiological and pathological processes. Radiation and most chemotherapeutic drugs induce apoptosis. Insensitivity to apoptosis contributes to tumorigenesis and may contribute to treatment resistance. Modulation of the apoptotic threshold may increase the efficacy of conventional anti-cancer regimens

– *Targeting approaches*:
  - Antibodies (high specificity): anti VEGF, anti EGFR- inhibit the signals mediated through receptors. Also the combination of antibodies with radiotherapy showed promising results in clinical studies.
  - Small molecules (kinase inhibitors)- have a series of advantages: less toxic than cytotoxic agents, they target the genetic alterations in the tumor cells;
  - Stem cells and radiotherapy- stem cells represent a target for radiation toxicity. They can also be a therapeutic option for tissue regeneration after irradiation accidents.

Fourth day was dedicated to the actual goal of individualized treatment.

– *Molecular functional imaging-tumor biology/phenotype* – this approach aims the exact localization of the tumor 1. Physical conformality (where is the tumor?) (PET-CT-posythron emission tomography); 2. Biological conformality (what is the tumor like?)- imaging combined with different markers of the tumor-receptors, hypoxia. (PET-FDG-FDG uptake of the primary tumor- prognostic factor after radical surgery). An innovation is represented by radiochemistry: PET imaging of EGFR, etc.; 3. Optical imaging in preclinical research- tagging with luminescence or fluorescence. 4. The future would be molecular imaging during treatment (imaging apoptosis with annexin V, imaging EGFR)
– *Profiling techniques: genotype*- Genomic profiling can be conducted on single gene, several genes or the whole genome. The comparative genomic hybridization (CGH) identifies genes and signaling pathways involved in cancer and is also used to identify predictive profiles of therapy outcome. There are several types of DNA sequence variations: differences in copy numbers of repeated sequences (CNV), insertions or deletions and single nucleotid polymorphysms (SNP). SNP are responsible of 90% of genetic variations. They can occur both in introns and exons and can affect protein function. Applications of SNPs: predicting risk of getting specific malignancies, predicting toxicity to drugs and radiation, predicting tumor response to therapy. There is no link between SNPs and CNVs. CNVs are important in genetic diversity and evolution, they could affect radiation and drug response of normal tissues. The future in this field is represented by pharmaco-genomics- determination of drug sensitivity by SNPs in drug metabolizing genes and radio-genomics- determination of radiosensitivity by SNPs and CNVs in cytokine, DNA repair and other genes.
– *Profiling techniques: microarrays*- potential applications of microarray: gene discovery and sub-typing complex genetic diseases, gene signatures as prognostic factors. There are different types of microarray data analysis: 1. Gene selection: find genes for therapeutic targets, find new response pathways; 2. Classification: identifying diseases, predicting outcome, selecting best treatment; 3. Clustering: finding new biological classes/ refine existing ones and exploration.
– *Profiling techniques: proteomics*- the goals of this technique are: identification of all proteins expressed in a cell or tissue, identification of their properties, functional linkages (activity, interactions with other proteins, networks). This approach would permit an earlier and more accurate clinical diagnosis, complementing gene and tissue microarrays.

The last day- *A vision for the future: a timeline for biological integration*. In this talk, the possibility of integration of new technology and new biology in clinic was evaluated. The conclusions would be that technical advances have significantly improved radiation dose delivery to tumors, with the concomitant sparing of normal tissues, and in the meantime the advances in biology and technology have made possible to understand individual cancers at molecular level. "Theranostics" will be used in the future to classify tumors based on biology and direct therapy. New targeted therapies are being developed which can exploit defects in tumors and target the biological limitations of radiotherapy. The knowledge of biological pathways can be exploited to improve radiotherapy in novel ways.

The take-home message would be: the future is individualized radiation oncology. There is a need for more research to be done in three directions:

1. Decrease the irradiation of normal tissues in order to allow dose escalation
2. Make the tumor radiosensitive
3. Individualize treatment

Treatment decision- Hypothesis: **prediction of outcome** (survival and complications) **will improve outcome.**
Predictive factors allow treatment individualization.

The participation of trainees from the entire world, with very diverse scientific background (most of them radiation oncologists, but also radiobiologists and physicists) demonstrated the increasing interest for this very challenging domain which is molecular biology. Treating cancer nowadays claims a better understanding of the molecular mechanisms of this complex disease, the future in cancer treatment being an individualized treatment based on the characteristics of each patient and each tumor. ESTRO does an excellent job in further improving the training of the specialists involved in cancer therapy by providing these outstanding teaching courses.

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