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Timing of hormonal therapy in prostate cancer

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The optimum timing for starting hormonal treatment in prostate cancer is still a matter of discussion.

Low-grade Clinically Confined Prostate Cancer: There is a growing experience in the deferred treatment of patients with low grade clinically localized prostate cancer indicating a high rate of local progression, a moderate rate of distant progression and low mortality due to tumour up to 10 years observation.

Prior To Radical Prostatectomy: Hormonal treatment prior to radical prostatectomy is even more controversial. The few randomized trials currently in progress will eventually show whether this approach can lead to improved survival.

Positive lymphnodes at or after radical prostatectomy: Should endocrine therapy be started immediately at the time of diagnosis, or should treatment be deferred until evidence is gained of progression? The ongoing FORTC Trail 30864, still in progress, will hopefully answer this question.

Advances Stages of Prostate Cancer: There are sufficient medical and ethical reasons to advise and employ early endocrine therapy in patients with metastatic prostate cancer.

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Is micrometastasis the target of preference for monoclonal antibody therapy?

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Purpose: A review of the clinical trials of antibody-based cancer therapies reveals that this approach can, in rare cases, induce complete remissions in individual cancers. Since these trials have usually involved patients with large tumor masses, tumor cell inaccessibility in probably a major reason for the prevailing failures. Minimal residual disease, the stage when tumor cells are few and dispersed, should therefore be a more promising target for the therapeutic antibodies.

Results: In breast cancer patients infusion of monoclonal Lewis Y antibody as well as monoclonal antibody 17-1A, directed against an epithelial adhesion molecule, led to a reduction of disseminated tumor cells in bone marrow. These observations were supported by a prospective randomized adjuvant trial, using monoclonal antibody 17-1A in patients with resected Dukes'C colorectal carcinoma, which resulted in increased survival and prolonged recurrence-free intervals.

Conclusion: Thus, in addition to strategies designed to produce more effective human-derived reagents, efforts need to be concentrated on directing passive antibody therapy towards the appropriate target.

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Antibody therapy of lympoid malignancies: Experience with CD52 (CAMPATH-1) antibodies

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Although monoclonal antibodies (MAbs) have the specificity to deliver targeted therapy, several barriers may preclude their routine use in lymphoid malignancy. Notably, only certain target antigens expressed on specific cell types are permissive for cell lysis or induce cell death by other methods: eg CD20 MAbs induce apoptosis in some malignant B-cells. CD52 MAbs were originally isolated for their ability to lyse lymphocytes with human complement. Subsequent work showed that rat and human CD52 MAbs also able to elicit antibody-dependent cellular cytotoxicity consistently depleted lymphocytes from blood, marrow and spleen whereas lymph nodes and extra-nodal masses were usually resistant: since activity of CD52 MAbs depends on the activation of cellular effectors, resistance may reflect their absence in these sites. Clinically useful activity of CD52 MAbs has therefore been demonstrated primarily in the chronic lymphoid leukemias. T-cell prolymphocytic leukemia (T-PLL) is remarkably sensitive to CD52 MAbs with most patients entering durable remissions. The biological basis of this sensitivity is not known. Secondly, patients with fludarabine-resistant B-CLL may usefully respond: in particular, clearance of the blood and marrow has allowed harvesting of uncontaminated stem cells. A similar approach of "*in vivo* purging" may be of value in patients with non-Hodgkin lymphoma undergoing high-dose chemotherapy.

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Monoclonal antibodies in combination with growth factors and chemotherapeutics

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Introduction: Unconjugated monoclonal antibodies (MAb) targeted to tumor cells mediate tumor lysis by activating various immune functions and/or induce apoptosis. Immune functions include antibody dependent cellular cytotoxicity (ADCC), complement dependent cytolysis (CDC) and induction of a tumor specific humoral and cellular idiotypic network response. Thus, it may be plausible to assume that augmentation of immunological effector mechanisms may increase the antitumor effect of MAb.

Results: In various clinical trials GM-CSF and IL-2 have been added to the MAb with the aim to augment ADCC as well as the idiotypic network response. α-interferon has been given to enhance unspecific killer functions and increase the expression of tumor antigens. Cytostatics have been administered also with the goal to decrease tumor volume. Combinations have mainly been given in colorectal carcinoma, melanoma, NHL and ovarian carcinoma. Improved effects might be seen during certain conditions but also impaired clinical results as well as decreased immune effector functions. The effects might be dose-schedule dependent. A survey of clinical studies with emphasis on clinical results and immune effector functions will be presented.

Conclusion: Improvement of MAb therapy might be possible by adding other biologicals and/or cytostatics. However, the dose-effect relation seems to be complex. Careful clinical and immunological analyses have to be done in man to be able to optimize the concept.

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Is there a future for clinical use of bispecific monoclonal antibodies?

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A review will be presented on the current state of therapies with bispecific monoclonal antibodies (biMabs).

BiMabs are very effective *in vitro* in mediating destruction of tumor cells. Either T-cells or F_c receptor bearing effector cells can be recruited.

In animal studies both localized (in traperitoneal) and systemic tumors can be effectively treated with biMabs and effector cells.

In patients some encouraging results – including complete responses – have been obtained in advanced glioma as well as in ovarian cancer by local application of the biMab. Despite these results no real breakthrough has been reported.

Issues are: the difficulties of purifying biMabs, the need for supplying activated effector cells, toxicity related to release of cytokines and the immune response against biMabs of murine origin.

Various approaches to overcome these issues will be discussed.

Conclusion: BiMabs have a future in combating advanced disease, however considerable hurdles still remain.

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Antibody-targeted activation of cellular immunity

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Altering the tumor microenvironment by cytokine therapy and the induction of cytotoxicity by cellular components of the innate and adaptive immune system have long been goals of cancer therapy. Relatively rare, but intriguing clinical responses have been observed in clinical trials of these strategies. One obstacle to the successful implementation of these strategies is the absence of selective targeting of cellular cytotoxicity to tumor sites; this obstacle is compounded by the limitations of cell trafficking to tumor sites. Multifunctional antibody binding site-based proteins can provide cellular activators with tumor-targeting properties, and have been extensively evaluated in preclinical models and clinical trials. Conventional antibodies with appropriate Fc domains can mediate antibody-dependent cellular cytotoxicity (ADCC), but this property has dubious clinical relevance to cancer therapy. Bispecific antibodies (Bsab) targeting tumor antigens and the defined cellular trigger molecule FcγRIII expressed by human natural killer cells exhibit improved anti-tumor properties in preclinical models, and one such Bsab has completed Phase I evaluation at our center. We also have investigated the novel immunoconjugate PNU 21-4565, a recombinant protein composed of an antibody Fab fragment and the bacterial super-

antigen, staphylococcal enterotoxin A. This agent is capable of inducing exceedingly potent superantigen-dependent cellular cytotoxicity mediated by T-cells. Phase I trials designed to define the optimal dose and schedule of administration are in progress; multiple variables contribute to the identification of the proper dose for each patient. These new strategies are examples of contemporary approaches to antibody-promoted induction of cellular immunity.

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Targeted cytokine delivery with recombinant antibody fusion proteins for therapeutic intervention

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Purpose: A major goal of tumor immunotherapy is a cell-mediated antitumor response effective in eradicating disseminated metastasis followed by a persistent tumor protective immunity. We tested the hypothesis that this can be achieved by targeted cytokine therapy with genetically engineered antibody fusion proteins.

Methods: Syngeneic animal models of murine melanoma, neuroblastoma and colon carcinoma were established and treated with tumor specific recombinant antibody cytokine fusion proteins compared to mixtures of antibody and cytokine at equivalent dose levels.

Results: We demonstrate that the fusion proteins can eradicate experimental and spontaneous metastases and prolong the animals' life span in contrast to equivalent mixtures of antibody and cytokine. Effector mechanisms involved included natural killer cell mediated tumor cell eradication and CD8+ T-cell responses. This was demonstrated in vivo with immunodeficient animals and by depletions of CD8+ T cells or NK cells, followed by anti tumor cytotoxicity assays in vitro with purified T or NK cells.

Conclusion: These data demonstrate that targeted delivery of cytokines to the tumor microenvironement offers a new strategy to elicit an effective cellular immune response against metastasized tumors.

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Radiation induced anomalies in control of signal transduction in Ataxia telangiectasia and Fanconi anemia

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Ataxia telagiectasia (AT) and Fanconi anemia (FA) are recessive genetic diseases featuring increased predisposition to cancer, chromosomal instability and hypersensitivity to DNA damaging agents. In both syndromes, altered induction of the tumor suppressor protein p53 as well as that of p53-target genes (bax, gadd45 and wafl) after gamma-irradiation is observed. Moreover cells from AT and Fa display a deregulation of the apoptotic process spontaneously, after a gamma-rays exposure or following Fas activation. The recently observed alteration of Poly ADP ribose polymerase (PARP) and of DNA-PK cleavage might explain the altered response to ionizing radiations and suggests a deregulation of the ICE-like proteases. Our current investigation of the Bcl-2-like proteins and of the ICE-like proteases should give insights about the functions of the proteins altered in AT and FA. Our results support the contention that a) the AT and FA genes play a major role in regulating apoptosis; b) the hypersensitivity to genetoxic agents is related in both syndromes to necrosis rather than apoptosis.

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Molecular radiation biology at the clinical interface

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The last decade has been characterized by the development of an increased understanding of the role of signal transduction in carcinogenesis. We will review signal transduction models of tumor development with particular emphasis on the role of the ras oncogene in carcinogenesis and its role in signal transduction. We will also discuss the control of the cell cycle and the impact of oncogenes and tumor suppressor genes on the control of the cell cycle. Finally we will review the control of apoptosis and its integration with cell cycle control. This lecture will review the clinical evidence that radiosensitivity is a determinant of outcome in the radiotherapy of cancer and will also examine some of the methods that have been used to try to determine the impact of this factor on cancer management. We will also review the current data on the molecular mechanisms which underlie radioresistance to attempt to define targets for manipulation of radiosensitivity in the clinic. In particular we will show that the ras oncogene can be directly targeted with prenyl transferase inhibitors to sensitize human cell lines carrying naturally occurring ras mutations to the killing effects of ionizing radiation.

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Genetic determinants of radiosensitivity: Potential for therapeutic modulation

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Elucidation of the key components of the signal transduction pathways involved in the cellular response to DNA damage is fundamental to understanding mechanisms of therapeutic resistance. It is also critical to the development of novel strategies for modulation of radiosensitivity, p53 is an inducible regulator of the response to DNA damage; it is activated by DNA strand breaks, inducing G1 arrest via transcriptional regulation of the cyclin kinase inhibitor, p21. We have evaluated the role of p53 in the processing of DNA damage induced by ionising radiation in murine and human cells of defiend p53 status (wild type; knockout p53 −/−, mutant p53 expression systems) using endpoints of clonogenic survival, DNA repair, and mutability at the hprt locus.

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Pathways and time effect of radiation-induced apoptosis

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The dominant cause of radiation-induced cell death is loss of clonogenic capacity due to unrepaired DNA damage. The recent (re)emergence of apoptosis may provide an alternative to this conventional cell kill model. One of the signalling mechanisms initiated by radiation that transduces cell membrane-derived death signals to the nucleus, and does not require DNA damage as a triggering mechanism, is the sphingomyelin (SM) pathway. This pathway is initiated by hydrolysis of the plasma membrane lipid SM, generating ceramide, a potent inducer of apoptosis. We have recently shown that ceramide activates a cascade of kinases that leads to stimulation of *stress-activated protein kinase (SAPK),* a critical event in radiation-induced apoptosis. In bovine aortic endothelial cells (BAEC) radiation induced a biphasic pattern of ceramide generation and SAPK activation. The first, immediate phase of SAPK activation occurred independently of *de novo* protein synthesis, while the second, starting around 4 h, was abolished by cycloheximide. Which of both radiation-induced signals is essential to cause apoptosis, remains to be established. Recent studies have implicated an important role of the interleukin-1β-converting enzyme (ICE)-like proteases in TNF- and Fas-mediated apoptosis. CPP32 has been identified as the protease that cleaves poly (ADP-ribose) polymerase (PARP) during apoptotic DNA degradation. In BAEC, radiation-induced CPP32 cleavage products were identified around 10 h after exposure, but inhibition of this protease by the tetrapeptide DEVD did not affect radiation-induced apoptosis. Currently, studies are conducted to further evaluate the role of the ICE-like protease cascade in radiation-induced apoptosis, and to establish its relation with the ceramide-SAPK signalling pathway.

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Induced radioresistance: Possible mechanisms and impact in the clinic

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Stress responses are upregulated following exposure to radiation and other DNA-damaging agents. Therefore response may be dose-dependent so that small acute radiation exposures, or exposures at very low dose rates, are more effective per unit dose than larger exposures above a threshold where induced radioprotection is triggered. This is termed low-dose hypersensitivity (HRS) and induced radioresistance (IRR) as the dose increases. HRS/IRR has been recorded in studies with yeast, bacteria, protozoa, algae, higher plant cells, insect cells, mammalian and human cells *in vitro*, and in studies on animal models *in vivo*. There is indirect evidence that HRS/IRR in response to single doses is a manifestation of the same underlying mech-