the appropriate sequence and use of radiation and chemotherapy in both respectable and locally advanced disease. These refinements have great potential to improve disease control.

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How do we choose new molecular targets for clinical exploitation?

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The recent expansion of our knowledge of new molecular targets for cancer therapy provides an exciting opportunity to introduce a new generation of molecular biological response modifiers (MBRM) in clinical studies. Encouraging clinical results using for example epidermal growth factor receptor blockers, angiogenesis inhibitors and signal transduction modulators are the product of basic science translating this increased understanding of molecular biology to an improved clinical care for cancer patients. However, many of these inhibitors of growth factors and signal transduction are cytostatic and, as single agent, not sufficient to eradicate all malignant cells. The advantage of combining these MBRM with radiation lies in the interaction between both treatment modalities, leading to increased and sometimes synergistic cytotoxicity. Moreover, high-dose high-precision radiotherapy will add another dimension to this approach by enhancing cytotoxicity selectively at the tumor site while sparing normal tissues. *How do we choose new molecular targets for clinical exploitation?* There are many aspects that need to be considered to guide a promising MBRM from its molecular identification through preclinical models into phase I-III trials. For example, in the first *in vitro phase* of this process, characterization of the *type of interaction* between a candidate MBRM and radiation is crucial, since it may provide a first indication about the chance of a successful application *in vivo*. Additive cytotoxicity may simply be not enough to improve therapeutic results. The choice of the *appropriate in vitro assays* should be dictated by properly predefined endpoints/read-outs, because almost no compound exhibits absolute target specificity and biological effects develop over time. As most *in vitro* assays focus on one particular cell type, they fail to take into account relevant *microenvironmental influences*, like survival signals, hypoxia and normal cell interactions. The next *in vivo phase* of the process provides a better approximation of the physiological state and habitat of primary tumors. In this context, transgenic animal models for *spontaneous tumorigenesis* offer many advantages over xenograft tumor systems since they allow studying the impact of specific genes on treatment sensitivity. Collectively, these preclinical *in vitro* and *in vivo* results should form the basis for the rational design of new clinical trials for combined modality treatment.

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p53 signaling and novel mechanisms for targeted radiosensitizers

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Objective: p53 protein can induce growth arrest, apoptosis and cell senescence upon stress stimulation including ionizing radiation. P53 mutations are common and occur in more than 50% of human malignancies. P53 mutations, abnormal sub-cellular localization, subsequent dysfunction (e.g. abnormal cytoplasmatic sequestration) and downstream defects in the p53 signaling pathway have been reported in radio-resistant human cancer. Therefore both better mechanistic insight into tissue specific p53 signaling and tumour specific defects in p53 signaling are needed. P53 independent radiosensitizers are of clinical interest if they re-sensitize radio-resistant human tumours with a known intrinsic/acquired defect in p53 signaling and if they maintain a large therapeutic index given concurrently with low dose fractionated ionizing radiation (IR). Our group is focusing on various strategies to overcome clinically relevant tumor specific defects in p53 signaling.

Results: Overall the screening for p53 "independent" radiosensitizers lead to the following preclinical results: 1)Taxol was among the first compounds identified in a p53 mouse sarcoma system but with a small therapeutic window 2) The PKC inhibitor, PKC-412, did no longer induce apoptotic cell death if combined with IR in p53 dysfunctional tumours but induced a G2 cell cycle arrest in combination with IR. This effect was supra-additive and well tolerated in vivo. An intact PI3K/AKT pathway is required for this combination. 3) The transcription factor E2F1 and specific genetically engineered mutants of E2F1 are potent radiosensitizers if combined with IR in tumour cells lacking p53. However "gene replacement" is still far from clinical application. 4) Anti-angiogenic agents like inhibitors of the VEGF receptor tyrosine kinase (e.g. PTK 787/ ZK222548) are of interest, because combined treatment with IR primarily targets the p53 wildtype angiogenic tumour system. 5) Recent screening identified recombinant Lectin I (one of the main compounds of mistletoe) as a novel radiosensitizer in p53-mutated tumour cell lines.

Conclusion: Both better mechanistical understanding of p53 tissue- and tumour specific signaling and novel radiosensitizers with a broad therapeutic index targeting the p53 signaling pathways are required.

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EGF-receptor inhibition and radiotherapy

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Background: The epidermal growth factor receptor (EGFR) is overexpressed in many human tumours and plays a major regulative role in cell proliferation. The EGFR may be activated by irradiation leading to a proliferative response. This might be an important component of accelerated repopulation of clonogenic tumour cells during fractionated radiotherapy. Furthermore activation of the EGFR may increase intrinsic cellular radioresistance. From these findings combination of radiotherapy with EGFR inhibition appears to be a promising strategy in cancer treatment.

Materials and methods: Preclinical experiments on combination of radiotherapy and EGFR inhibition are reviewed.

Results: Investigations in vitro showed an antiproliferative effect and increased radiosensitivity in several tumour cell lines after inhibition of the EGFR. Experiments on tumour models in vivo demonstrated that EGFR inhibitors can prolong growth delay (GD) compared to irradiation alone. GD and local tumour control (TCD50 assay) after single dose irradiation were evaluated in one tumour model. The enhancement ratio was significantly lower in the TCD50 than in the GD assay. In own experiments GD and TCD50 were investigated for fractionated irradiation combined with the selective EGFR-TK-inhibitor BIBX1382BS. As in the experiments reported by others GD was significantly enhanced after simultaneous combined treatment, however, this did not translate into improved local tumour control. In a further experiment the same finding was obtained for adjuvant EGFR inhibition after radiotherapy.

Conclusions: To fully utilize the potential of combining EGFR-inhibitors with irradiation, further investigations are necessary that explore the mechanisms of action and the efficacy of the approach.

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The PI3K/AKT pathway: a target for new chemo-radiation approches?

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Phosphoinositide 3-Kinase (PI3K) catalyze the production of specific inositol lipids that have been implicated in a plethora of cell functions. One of the best-characterized targets of PI3K lipid products is the serine/threonine protein kinase AKT (PKB). Activation of the PI3K/AKT pathway can suppress the apoptotic response, undermine cell cycle control, enhance cell survival and proliferation. The PI3K/Akt signal transduction cascade has been investigated extensively for its roles in oncogenic transformation. Compelling evidence suggests that members of PI3K family can be considered as oncogenes because they control cell cycle progression, differentiation, survival, invasion and metastasis as well as angiogenesis. The activity of fundamental growth factor receptors like PDGFR, EGFR and IGFR are blocked by the specific PI3Ks inhibitor wortmannin, leading to the conclusion that the PI3Ks/AKT pathway is critical for cell signaling. Response to ionizing radiation is also regulated through the PI3K/AKT signaling pathway by distinct mechanisms. When transiently expressed a constitutively active PI3K gene can induce radioresistance. It has been suggested that the phosphorylated active form of AKT could be a significant predictor for local control in head and neck cancer patients after radiation therapy. PTEN, which is a lipid phosphatase frequently inactivated in cancers, acts as an inhibitor of the PI3K/AKT pathway. Restoration of the PTEN gene can sensitize malignant cells to irradiation. The PI3K/AKT pathway is involved in cell cycle control, activated AKT overrides G2/M. checkpoint induced by irradiation. This pathway is also regulating survival of vascular endothelial cells after irradiation. Enhancement of endothelial cell viability after irradiation. occurs through the PI3K/Akt signal transduction pathway. Interestingly, the use