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

of signal transduction inhibitors which lead to tumor radiosensitization such as the EGFR inhibitor Iressa®, the Ras processing inhibitor FTI L744,832®, is associated with a decrease in the active form of AKT, direct pharmacological inhibition of the PI3K/AKT pathway by LY291002 and wortmanin cause radiosensitization. Therefore, pharmacological targeting of the PI3K/AKT pathway is a promising novel strategy to improve tumor response to ionizing irradiation.

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Modulators of apoptotic signaling

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In order to increase the efficacy of ionizing radiation or to reduce radiation mediated side effects research centers for translational radiation oncology head for a specific modulation of defined cellular death pathways. In this regard, several signaling systems proved to be of high potential value. It has previously been shown that apoptotic pathways induced by ionizing radiation are distinct from pathways triggered by death ligands (e.g.TRAIL). The combination of both was highly efficient in vitro and preclinical mouse models. However, several aspects of normal tissue toxicity have not been solved and no phase I data are available yet. Thus, up to now the use of TRAIL is limited to experimental settings. A second approach which is currently tested in a phase I trial is based on the observation that synthetic phospholipid derivatives strongly enhance apoptotic effects by modulating the balance between the mitogenic, antiapoptotic MAPK and phosphatidylinositol 3'-kinase (PI3K)/Akt, and the proapoptotic JNK signaling pathways. Furthermore, others provided evidence that an inhibition of anti-apoptotic signals by mitogenic signals increases radiation responses. In this context, controversial data are available regarding the influence of a pharmacological abrogation of MEK1, Erk1/2 signaling on apoptosis sensitivity. However, inhibition of the PI3K/Akt survival pathway using compounds like the PKC inhibitor PKC412 was shown to induce apoptosis or to increase the apoptosis sensitivity of tumor cells. Therefore, these drugs may be used alone or in combination with radiation in order to increase tumor control. Several other drugs including COX-2 inhibitors, betulinic acid and proteasome inhibitors were shown to interact with apoptosis signal transduction. Again, most of the drugs have not been tested in combination with radiation in vivo or – in the case of COX-2 inhibitors – exert pleiotropic effects. Although the examples presented above cannot be considered to reflect all available strategies, it becomes clear that several promising approaches targeting defined cell death pathways have been developed and entered clinical trials. The use of synthetic phospholipid derivatives in a phase I trial is one of the first important examples proving that basic research in radiation biology finally guides the development of new treatment strategies. This and other approaches will increase tumor control rates and reduce side effects in the future.

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Molecular determinants of glioma biology

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We have been studying a series of 190 astrocytic gliomas (136 glioblastomas (GB), 39 anaplastic astrocytomas (AA) and 15 astrocytomas (A)) for abnormalities of genes in the RB1 pathway (CDKN2A, CDKN2B, CDK4 and RB1), the p53 pathway (p14ARF, MDM2, and TP53), as well as PTEN and EGFR. A main finding was that 67% of A and AA had no wild-type TP53 or one mutated allele with a wild type allele. These were the main findings A and AA. Only 29% of the GB had no wild type TP53 and an additional 6% had one mutated allele. Loss of wild type p14ARF occurred in 38% of GBs and a further 8% had amplification and overexpression of MDM2. Thus 76% of GB (103/136), 72% of AA (28/39) and 67% of A (10/15) had a deregulated p53 pathway - almost a prerequisite for astrocytic tumors. All A had at least one wild type RB1 gene and no other abnormalities of this pathway. Abnormalities of the RB1 pathway occurred in 21% AA and 67% GB either by mutation/loss/ homozygous deletion of RB1, CDKN2A and CDKN2B, or amplification of CDK4, indicating that disruption of the RB1 pathway is involved in astrocytic tumour progression. Amplification of the EGFR gene was not observed in A, was unusual in AA (8%) but common in GB (33%). Loss of wild type PTEN occurred in one AA (3%) but in 47% of GB. Both EGFR amplification and loss of wild type PTEN were found with all combinations of the other genetic abnormalities. Survival of patients with GB is typically 11 to 12 months. We studied whether any of the genetic factors examined were related to survival in GBs. Abnormalities in any of the four genes (CDKN2A, CDKN2B, RB1, CDK4) coding for components of the Rb1 pathway were associated with shorter survival (p=0.002). When combined with loss of wild-type PTEN the association was stronger (p

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What next in low grade glioma therapy

Abstract not received.

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Does radiotherapy matter?

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Radiotherapy (RT) remains the principal treatment modality in patients with malignant glioma. Conventional treatment to 60 Gy provides median survival benefit of approximately 6 months with no further advantage for higher doses. RT should be tailored to prognosis with radical treatment reserved for favourable prognosis patients; those with adverse prognostic features (defined by age and performance status) should receive palliative treatment. Attempts at improving the results of RT have concentrated on altered dose and fractionation (hyperfractionation and/or acceleration), the use of modifiers of radiation response and particle irradiation. Most have shown little benefit in single arm or randomised studies. High dose localised irradiation in the form of brachytherapy or stereotactic radiosurgery/radiotherapy boost have also failed to demonstrate prolongation of survival while associated with increased toxicity. Present research strategies concentrate on biological methods to overcome tumour hypoxia, on combined chemoradiotherapy approaches and on the use of biological modifiers, which may in association with radiation improve therapeutic ratio. New agents under evaluation include modifiers of EGFR signalling pathway, COX 2 inhibitors, modifiers of Ras signalling pathway and angiogenesis inhibitors. Radiotherapy remains the most effective primary treatment modality in patients with malignant glioma. New approaches to modification of radiotherapy have a real chance to demonstrate improved therapeutic ratio over RT alone. Before introduction into clinical practice they need robust preclinical and clinical testing.

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Current status of malignant glioma chemotherapy - hype or hope?

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Brain tumors are among the most debilitating diseases. Treatment options are limited to surgery and radiation, the role of chemotherapy has been marginal. Nitrosourea-based chemotherapy has shown activity in selected patients, but failed to show a benefit as adjuvant therapy for malignant glioma in a large randomized trial. Higher response rates to PCV-chemotherapy have been demonstrated for oligodendroglioma, in particular when associated with deletions on chromosomes 1p and 19q. Recently temozolomide (TMZ), a novel alkylating agent has been approved. The low response rates of only 5-8% in glioblastoma (higher in anaplastic astrocytoma) and the absence of phase III data have cast doubts whether TMZ offers a clinically relevant benefit over older alkylating agents. Some benefit may simply be derived by the closer follow-up and better supportive care in patients receiving chemotherapy. More intensive TMZ schedules are being explored. Continuous administration of alkylating agents will deplete the cells of the DNA repair enzyme O6-alkyltransferase (AGT), and may thus have a theoretical advantage over the intermittent schedules. No comparative data are available. Combining X-irradiation with TMZ has been shown to be at least additive in vitro in some glioblastoma cell lines. Using chemotherapy with intrinsic activity immediately after diagnosis together with radiotherapy may allow eliminating microscopic infiltrating disease early in the disease course. Concomitant administration of chemoradiotherapy may increase the radiosensitivity. In a phase II trial we treated 64 patients with newly diagnosed glioblastoma multiforme with TMZ and concomitant radiotherapy. At a median follow-up of now over 3 years the median survival of 14.3 mo (95% c.i. 10.4-18.3) and in particular the 2-year survival of 28% (17-39%) are promising for this poor-prognosis group of patients. A large international randomized trial conducted by the EORTC and the NCI Canada has accrued over 550 patients. Conclusive resuts are expected in early 2004. Insights into gene expression and signaling pathways have