The most dramatic telomere shortening was seen in PC3, the TRF length (3.5 kb) decreased within 28 days to a critical length of 2.3 kb. Telomere attrition was accompanied by end-to-end chromosome fusions and replicative senescence. PC3 cells were highly β-galactosidase positive and ceased growth under treatment with 1 μM drug after 4 weeks. UXF 1138L were able to proliferate in presence of 5 μM KML001 for more than 7 weeks; MCF-7 cells did not senesce. KML001 caused chromosomal abnormalities, with chromosome end-to-end fusions seen in UXF 1138L and PC3 cells. The end-to-end fusions increased e.g. in metaphases of UXF1138L to an extent of 69% compared to controls.
Telomerase activity, however, was not inhibited. Even at supra-toxic drug levels of 1000 μM, KML001 did not inhibit telomerase or the polymerase activity in the PCR reaction, suggesting telomere poisoning by this drug. Thus, it is most conceivable that KML001 directly targets the telomeres by specific or unspecific DNA-damage in telomeric sequence regions. Senescence and genomic instability occurs and will lead to cancer cell death foremost in cells with short telomeres.
Our findings indicate that KML001 can target telomeres and that this effect should be considered in clinical trials design.

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Targeting telomere maintenance in childhood neuroblastoma and primitive neuroectodermal brain tumors

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Background: Primitive neuroectodermal brain tumors (PNET) and neuroblastoma are the most common intra- and extra-cranial malignant tumors in childhood. These embryonal tumors share important biological similarities. Unlimited replicative potential is an important acquired capacity of cancer. Mechanisms of telomere maintenance in cancer cells include upregulated expression of the enzyme telomerase (85–90%), or different mechanisms known as alternative lengthening of telomeres (ALT). Epigallocatechin gallate (EGCG), the major polyphenol in green tea, is a telomerase inhibitor with antiproliferative and anti-carcinogenic effects against different types of cancer. Telomestatin is a G-quadruplex intercalating drug specific for telomeric sequences.
Methods: mRNA expression of human telomerase reverse transcriptase (hTERT) was measured in 12 human neuroblastoma cell lines, 6 PNET cell lines, 50 primary PNET samples, and 14 normal human brain samples by real time RT-PCR. In cell lines, telomerase activity was determined by a quantitative telomeric repeat amplification protocol (TRAP). Telomere length was quantified using terminal restriction fragment analysis. Cell viability was quantified using the colorimetric MTS assay.
Results: Compared to normal human cerebellum, 38/50 (76%) primary PNET samples had >5-fold upregulated hTERT mRNA expression. While a positive correlation between hTERT mRNA expression and telomerase activity was detected in both PNET and neuroblastoma cell lines, no correlation was found between telomerase activity and telomere length in PNET cell lines. Both EGCG and telomestatin inhibited telomerase activity in TRAP-positive neuroblastoma and PNET cell lines. Although EGCG displayed strong proliferation inhibitory effects against TRAP-positive PNET cells, it had no significant effect against TRAP-negative D425 cells. In contrast, telomestatin inhibited proliferation in all neuroblastoma and PNET cells tested.
Conclusions: These results provide evidence for a possible role of telomerase in the pathogenesis of PNET and neuroblastoma and indicate the presence of ALT in subsets of PNET. Successful telomere-targeted anti-cancer therapy for PNET might therefore require a combination of telomerase and ALT inhibitors, such as telomestatin.

Gene therapy and antisense approaches

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Mesenchymal progenitor cells as gene delivery systems for cancer and leukemia therapy

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We have previously demonstrated that bone marrow-derived non-hematopoietic stem cells (MSC) integrate into solid tumors as stromal fibroblasts following intravenous injection (Cancer Res 62:3603–3608, 2002). This finding suggests the development of novel anti-cancer therapies based on the local production of biological agents by gene-manipulated MSC. We examined whether human MSC producing human interferon-beta (IFNβ-MSC) can inhibit the growth of metastatic tumors in the lungs of SCID mice. MSC were transduced with an IFNβ expressing adenoviral vector. These IFNβ-MSC produced 40–50,000 I. U. of IFNβ /10^6cells/24 hours. IFNβ-MSC, but not vector-control transduced MSC, directly inhibited the growth of both A375 melanoma and MDA 231 breast carcinoma cells in co-culture experiments *in vitro*, and when injected intravenously (IV) (four doses of 10^6 MSC/week) into SCID mice bearing pulmonary metastases of carcinomas or melanomas, tumor growth was inhibited as compared to untreated or vector-control MSC controls (p=0.0073). Recombinant IFNβ protein (50,000 IU every other day) injected subcutaneously was ineffective (p=0.14). IV injected IFNβ-MSC prolonged the survival of mice bearing metastatic breast carcinomas or melanomas (p=0.001). MSC marked with β-gal were found only in tumors, where they proliferated and incorporated BudR, but not in normal tissues. Intraperitoneal injections of IFNβ-MSC in mice carrying ovarian carcinomas resulted in doubling of survival (SKOV-3) and cures of 70% of mice carrying OVAR-3 tumors. MSC injected into the carotid artery (IA) of mice selectively proliferated in human glioma xenografts, but not in normal brain tissues, and significantly prolonged survival of these animals. In a model of chronic myelogenous leukemia in blast crisis (KBM5), mifepristone (RU486) regulated production of interferon α (IFNα (in AAV infected MSC induced tumor regressions and doubled survival. MSC delivering tumor selective replicating adenovirus (delta24) exerted anti-tumor effects in ovarian cancer after I. P. injection also prolonged survival.
Data suggest that IV, IP or IA administered gene-modified MSC can inhibit the growth of leukemias, metastatic tumors of the lungs, ovarian and brain tumors. Importantly, the anti-tumor effects were only observed when MSC were integrated into the tumor microenvironment. Mechanisms responsible for MSC tropism in tumors are under investigation and will be discussed. Results suggest the use of gene-manipulated MSC for cancer and leukemia therapy.

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TGF-beta2 suppression by the antisense oligonucleotide AP 12009 as therapy for high-grade glioma: safety and efficacy results of phase I/II clinical studies

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Background: Tumor derived transforming growth factor beta (TGF-beta) is a pivotal factor for malignant progression by inducing metastasis, angiogenesis, proliferation and immunosuppression. High-grade gliomas are highly aggressive tumors showing marked overexpression of the TGF-beta2 isoform which is correlated with advanced tumor stage.
Methods: In 3 phase I/II dose escalation studies adult high-grade glioma patients (WHO grades III/IV) with recurrent tumor and evidence of tumor progression on MRI were treated with AP 12009, a TGF-beta2 specific phosphorothioate antisense oligonucleotide. AP 12009 was administered intratumorally by convection enhanced delivery (CED) in up to 12 cycles. In the 3rd study, an indwelling pump system was used allowing repeated treatment cycles with a single catheter placement on an out-patient basis. Safety and tolerability were primary endpoints. Secondary endpoint was clinical efficacy.
Results: In 5 of the total of 24 patients "possibly" related adverse events were observed, mostly of grade 1 or 2, one was classified as serious. Alternatively, this event could also be related to rapid reduction of steroids performed in this patient prior to study entry. There were no relevant changes in laboratory values, including hematology. Application system and CED were well tolerated without problems. Median overall survival after recurrence was 138.4 weeks for anaplastic astrocytoma (AA) and 44.0 weeks for glioblastoma (GBM) patients as compared to the published data from start of temozolomide therapy of 42.0 (AA) and 32.0 weeks (GBM), respectively. One AA patient had a complete response in all tumor sites after one cycle of AP 12009 experiencing an overall survival of 195 weeks after first recurrence. A further tumor remission with similar time course was documented for a second AA patient receiving 12 cycles of AP 12009. The remaining enhancing lesion was considered to be most likely scar tissue by the responsible neuroradiologist. Additionally, one GBM patient showed a strong reduction in tumor size.
Conclusions: AP 12009 application was safe and well tolerated. These results show AP 12009 mediated TGF-beta2 suppression to be a highly promising therapeutic approach for TGF-beta2 overexpressing tumors such