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### Identification of target tissue for glioma gene therapy by multi-tracer PET imaging

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**Objective:** To identify viable target tissue in heterogenous gliomas amenable for biological treatment strategies such as gene therapy.

**Methods:** Rat F98 and RG2 glioma as well as human Gli36dEGFR and U87dEGFR glioma cells were grown as subcutaneous tumors in nude rats (n=12) and nude mice (n=8). After tumors had grown to at least 200 mm<sup>3</sup>, DNA-, protein- and glucose-metabolism were determined by means of 3-deoxy-3-[18F]-fluoro-L-thymidine (FLT), methyl-[11C]-L-methionine (MET) and [18F]-2-fluoro-2-deoxy-D-glucose (FDG) PET after intravenous administration of FLT (250-300  $\mu$ Ci/rat; 50-100  $\mu$ Ci/mouse), MET (600  $\mu$ Ci/rat; 200  $\mu$ Ci/mouse) and FDG (250-300  $\mu$ Ci/rat; 50-100  $\mu$ Ci/mouse) using new generation ECAT HRRT (Siemens, CTI) and microPET scanners (Concord). **Results:** In small tumors (< 500 mm<sup>3</sup>), homogenous uptake of all three tracers indicated actively proliferating tumor tissue as potential target tissue for gene therapy. In larger tumors (> 500 mm<sup>3</sup>), heterogenous tracer uptake was observed with a rim of high FLT-, MET- and FDG-uptake immediately adjacent to metabolically inactive or necrotic tumor, where no specific tracer accumulation occurred. The metabolically active tumor tissue presented as a narrow band indicating that application and targeting of gene therapy vectors into the active proliferating tissue compartment in large tumors might be difficult.

**Conclusions:** Identification of target tissue for gene therapy is possible by multi-tracer PET in experimental animals at high spatial resolution. Multi-tracer PET imaging of tumor metabolism and gene expression shall contribute to the development of standardized gene therapy protocols and of efficient and safe vector applications in humans.

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### Antitumor activity of g3139 (Genasense™) plus dacarbazine against human melanoma is superior to G3139 alone

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Bcl-2 inhibits apoptosis and is expressed in major tumor types, including melanoma. Bcl-2 maintains viability and contributes to chemo- and radiotherapy resistance of cancer cells. Dacarbazine (DTIC) is the standard single chemotherapeutic agent for melanoma; however, response rates and duration are low. Previously, we reported that Bcl-2 antisense oligonucleotide G3139 (oblimersen sodium, Genasense™, Genta Inc.) dramatically enhances DTIC effectiveness in a human melanoma xenograft model (Nat. Med. 4:232-4, 1998). In this study, we compare the antitumor activity of G3139 as a single agent versus the combination treatment with DTIC against human melanoma cells in a xenograft model. Spleen weights and IL-6 levels were also measured. **METHODS:** SCID mice were inoculated s.c. into the left lower flank with 518A2 cells. After developing palpable tumors (day 9), mice were randomized into 3 groups (n=8), implanted with miniosmotic pumps filled with saline or G3139 in saline. Mice were treated for 14 days with saline, G3139 (5mg/kg/d), or G3139 (5mg/kg/d) plus DTIC (80 mg/kg/d i.p. for 5 days on days 5-9 after pump implantation). Blood samples were collected for plasma IL-6 and IL-12 analysis. After 14 days of treatment, mice were sacrificed and tumors and spleens were evaluated by excision and weighing. **RESULTS:** The tumor weights were analyzed for between-group comparisons using an analysis of variance (ANOVA) model. G3139 as a single agent resulted in moderate reduction in tumor weights relative to saline-treated controls (P=0.08), whereas tumor weight reduction by G3139 + DTIC combined treatment was highly significant relative to either saline or G3139 only groups (P<0.001). Spleen weights and IL-12 levels were elevated 2-3 fold in both the G3139 and G3139 + DTIC groups versus saline controls, while IL-6 values were in the normal range and similar among the 3 treatment groups. **CONCLUSION:** These data confirm that G3139 markedly enhances the efficacy of dacarbazine in human melanoma xenograft, while the antitumor effects of G3139 as a single agent are minimal and support the continuing evaluation of G3139 combined with dacarbazine in patients with malignant melanoma. Further analysis by gene array and proteomics is ongoing.

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### Heat shock protein 27 prevents gamma radiation-induced apoptosis

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The ability of heat shock proteins (HSP) to protect cells from stressful stimuli, including hyperthermia, oxidative stress or cytotoxic drugs and their unusually high levels in a wide range of tumours, suggest that these proteins could limit the efficiency of radiation therapy. Among them, HSP27 acts as molecular chaperones, modulates redox parameters and prevents the activation of the apoptosome-dependent apoptotic pathway. To our knowledge, no previous study has considered its role in g-radiation induced apoptosis. This work focuses on the mechanisms by which HSP27 could modulate the three levels of radiation-induced apoptosis: the induction phase (ceramide release), the regulation phase (mitochondrial events), and the executive phase (apoptosome assembly and caspases activation). The radiosensitive human leukemic Jurkat cell line, which does not express the protein HSP27 at the basal state or in response to g-ray, was transfected with a NeoPCl plasmid containing either the full-length human HSP27 cDNA or an insertless plasmid. The kinetics (4 to 48h) and sequence of apoptotic events triggered in response to 10 Gy irradiation were investigated in both HSP27-transfected and insertless control cell lines. HSP27 did not interfere with the inductive phase of apoptosis since the increasing release of ceramide observed in Jurkat control cells from 4 to 48h post-irradiation, was not prevented by HSP27 overexpression. At the mitochondrial level, HSP27 overexpression did not influence the progressive fall in mitochondrial transmembrane potential. In contrast, the generation of reactive oxygen species appeared significantly lower 24h after irradiation in the HSP27 transfected cells. Moreover, the cytochrome c release was also significantly delayed by HSP27 overexpression. In the course of the executive phase, caspase-3 and caspase-9 activations were significantly decreased 24h and 48h post-irradiation in transfected cells. Finally, the delayed cytochrome c release combined to the decrease of caspases activation led to a significant decrease of apoptotic death in HSP27 overexpressing cells (18% of transfected cells in the sub-G1 phase 48h post-irradiation, compared to 43% in the time-matched control cells). We conclude that HSP27 prevents gamma radiation induced apoptosis by delaying cytochrome c release and reducing effector caspase activation. Thus, it could be implicated in the mechanisms of radioresistance in tumours overexpressing HSP27.

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### Biomarkers of *in vivo* response to BCL-2 antisense in human prostate cancer xenografts

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Bcl-2 anti-apoptotic gene expression is targeted with antisense oligonucleotide G3139 (Genasense™, oblimersen, Genta Inc.), which currently is in clinical trials. To test a hypothesis that G3139 can decrease bcl-2 expression in androgen-refractory prostate cancer *in vivo*, PC-3 cells were implanted in 15 mice, allowed to establish for 10 days, then continuous infusions with G3139 at 10 mg/kg/day was started on Day 0. A control mouse (no drug) was sacrificed on Day 0, followed by one G3139-treated mouse sacrificed daily through Day 14. Global gene expression in xenografts at Day 1 and Day 14 was compared with the control using Operon microarray with 1,154 human cancer genes. The expression of anti-apoptotic [BCL-2 and BCL-X(L)] and pro-apoptotic (BAX) proteins was evaluated in all tumor homogenates (n=15) by western blots, quantified by densitometry and normalized for b-actin levels. Microarray analysis revealed that G3139 significantly (>10-fold) downregulated 19 genes, including gravin and plasminogen activator inhibitor, and >5-fold upregulated 28 genes, including mitochondrial aminotransferase and cyclin-dependent kinase inhibitor. The levels of bcl-2-like mRNA (D87461) decreased from 1.41±0.16 at Day 1 to 0.57±0.05 at Day 14. The corresponding values for bax (U66879) were 0.85±0.02 and 1.68±0.019, respectively. The bcl-2/bax ratios predict therapeutic response in patients with prostate cancer (Urology 1998, 52:1085). In this study, bcl-2-like/bax decreased from 1.65 at Day 1 to 0.34 at Day 14. BCL-2 increased expression by 80% from Day 0 to Day 14 in comparison with the control. BCL-X(L) and BAX levels increased by 30% and 100%, respectively, however, BCL-2/BAX decreased about two-fold by Day14. Net increase in BCL-2 could be due to downregulation of low bcl-2 expressing