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Antisense strategies for induction of tumor cell apoptosis

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Conventional cytotoxic therapies are still lagging behind the rapid strides that have been made in our understanding of cancer biology. As the biology of neoplastic transformation and malignant progression of tumors becomes deciphered and the critical genes identified, direct genetic approaches for the treatment of cancer have become possible. Antisense technology takes advantage of available gene sequencing data and has opened new avenues to specifically target the myriad of genes involved in tumor development by use of exogeneously delivered oligonucleotides. The most promising candidate genes are those that are functionally linked to growth signaling, cell cycle control, regulation of apoptosis or angiogenesis. Several first generation oligonucleotides have entered clinical trials and provided encouraging results. Recently, advanced chemistry second generation oligonucleotides have been developed which show more favorable biochemical properties including increased metabolic stability, nuclease resistance and RNA binding affinity, as well as improved therapeutic and safety profiles. In our laboratory we have developed high affinity binding 2'-ribose modified mixed-backbone oligonucleotides targeting major inhibitors of apoptosis signaling, and have tested their ability to induce tumor cell apoptosis and sensitize tumor cells to chemotherapy. Recent developments and achievements in the field as well as the results of our own preclinical antisense program and future directions will be discussed.

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ANTISENSE APPROACHES FOR THE TREATMENT OF PROSTATE CANCER

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Failure of androgen ablation therapy in prostate cancer is associated with hyperactive androgen receptor signaling and expression of androgen-regulated genes. Currently there is no efficient treatment available for this stage of the disease. One of the strategies intensively investigated for the development of new treatment methods is the use of antisense technology to inhibit crucial signaling and survival pathways.

We have identified antisense oligonucleotides inhibiting expression of human androgen receptor. Oligonucleotide treatment resulted in a significant inhibition of LNCaP prostate cancer cell proliferation, inhibition of secretion of the androgen-regulated PSA gene, reduction of EGF receptor levels and induction of apoptosis. In LNCaP tumor-bearing nude mice the antisense AR oligonucleotides also showed significant growth inhibition. Tumor weight was reduced to 43% of control. Treatment was well tolerated by all animals and a scrambled control ODN did not affect tumor growth. AR expression in the tumors and serum PSA levels were found to be correlated with tumor size.

In other approaches followed worldwide by several research groups antisense oligonucleotides are used for inhibition of expression of antiapoptotic proteins such as Bcl-2, insulin-like growth factor binding protein-2, growth factor receptors or clusterin. These provided promising results in models systems and some clinical studies have been initiated and are under way.

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Modulation of the apoptotic threshold by BCL-2 antisense therapy of melanoma

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Treatment resistance in malignant melanoma has been linked to expression of the proto-oncogene BCL-2. We recently demonstrated that a combination treatment of antisense oligonucleotides (ASO) targeted against BCL-2 mRNA and dacarbazine (DTIC) decreased BCL-2 protein, enhanced tumor cell apoptosis and led to major tumor responses in a SCID mouse xenotransplantation model. In the present Phase I-II clinical study evaluating BCL-2 ASO (Genasense™, formerly known as G3139, Genta Inc.) 33 patients with advanced malignant melanoma have been treated by systemic administration of BCL-2 ASO combined with standard DTIC treatment. Using an intra-patient dose-escalation protocol, the dose limiting toxicity was reached at 12 mg/kg/d in form of thrombocytopenia. Further side effects included leucopenia and transient transaminases elevations. Steady-state plasma levels of BCL-2 ASO reached within 24 hours increase linear with the administered dose. By day five, doses $\geq$ 1.3 mg/kg/day lead to a median 40% reduction of BCL-2 protein in melanoma biopsies compared to baseline in 14 patients accessible for biopsy. This reduction coincides with increased tumor cell apoptosis, which is markedly enhanced after DTIC treatment. Repeat cyclic therapy resulted in durable responses over 1 year in some patients. This clinical study is the first to document that systemic treatment with an antisense molecule down-regulates its target in a solid tumor.