maintain exponential growth. All experiments were conducted with cells in exponential growth phase. Cells were free of mycoplasma contamination.
Results: 17-AAG was toxic to A549 cells and synergistic toxicity was observed during simultaneous exposures with each of the four TKIs. Flow cytometry analysis of cell surface expression of EGFR using monoclonal anti-EGFR antibodies showed 50% decrease upon pre-treatment with 17-AAG for 24 h. Uridine transport in A549 cells mediated by human equilibrative nucleoside transporter 1 (hENT1) was inhibited by 17-AAG with an IC_{50} value of 15 μM.
Conclusions: Combination therapy with 17-AAG and several clinically used TKIs looks promising and should lead to the design of future trials based on these combinations. The inhibition by 17-AAG of hENT-1 mediated uridine transport suggests an explanation for the observed antagonism between cytarabine with 17-AAG in leukemic cells since cellular uptake of cytarabine is mediated primarily by hENT1. These results caution against combination of nucleoside analogs with 17-AAG in patients.

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Investigating the role of Hedgehog signaling in tumor models

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Background: Hedgehog (Hh) signaling is activated in medulloblastoma (MB) and basal cell carcinoma, either through loss of the inhibitory protein, Patched (Ptch), or through genetic activation of Smoothened (SMO), a protein that transduces Hh signals. In some colon and pancreatic tumor xenograft models, antagonism of Hh signaling has been reported to attenuate tumor growth, and inhibition of growth correlated with reduced stromal expression of Gli1, a marker of Hh signaling. Here we describe the effects of antagonizing Hh signaling a Ptch-deficient model of MB. We also describe the effects of antagonizing Hh signaling in models of pancreatic, prostate, lung, and bile duct cancers, including models with expression of Hh in the tumor and Gli1 in the stroma.
Materials and Methods: Tumor bearing nude mice were treated with an antagonistic anti-Hh antibody or with specific SMO small molecule antagonists. Effects of these treatments on Gli1 RNA expression and tumor growth rate were assessed.
Results: Mice bearing tumor allografts derived from a Ptch+/− p53−/− mouse model of MB were treated with SMO antagonists. These treatments caused regression of the allografts and robust reduction in tumor Gli1 expression. Mice bearing tumors of ten different xenograft models were also treated with Smoothened antagonists or with anti-Hh antibody. The xenograft models were patient-derived pancreatic or lung tumors or were cell line models of pancreatic, prostate, or bile duct cancers. In these models, antibody and compound treatment significantly reduced Gli1 expression in the stroma, but neither treatment affected tumor growth. All treatments were well tolerated, and no significant weight loss was observed.
Conclusions: The SMO antagonists used in these studies are efficacious in a preclinical model of MB and are ineffective in other tumor models. Although antagonism of Hh signaling in the stroma has previously been correlated with attenuated growth of some tumor models, our data indicate antagonism of Hh signaling in the stroma is insufficient to inhibit growth of all tumor models.

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The treatment of breast cancer tumor growth and metastasis with an anti-MMP9 deoxyribozyme

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Background: Despite continued improvements in diagnosis, surgical techniques, and chemotherapy, breast cancer patients are still overcome by cancer metastasis. Tumor cell proliferation, invasion and metastasis are known to be mediated, at least in part, through degradation of basement membrane by neutral MMPs produced by tumor and stromal cells. Evidence suggests that MMP-9 plays a significant role in breast tumor cell proliferation, invasion and metastasis. Our novel catalytic DNA molecule (DNAzyme) based strategy is capable of specifically down regulating MMP9 expression without affecting other MMPs.
Materials & methods: DNAzymes are catalytic enzymes that bind to and cleave specific mRNA, resulting in a decreased protein expression. The application of anti-MMP-9 DNAzyme (AM9D) for the treatment of metastatic breast cancer was evaluated *in vitro* and *in vivo* using MDA-MB-231 human breast cancer cells and a MMTV-PyMT transgenic breast cancer mouse model, respectively. AM9D was intratumorally injected into the mammary tumors of the transgenic mice, once a week for 4 weeks. Tumor sizes were monitored bi-weekly and final tumor size was measured by weighing tumors. The role of AM9D on MMP-9 protein production and blood vessel formation were determined by immunohistochemistry. To determine safety and efficacy of AM9D systemically delivered to animals, AM9D was labeled with ^{35}S and injected intravenously into the tail vein of mice. Distribution and clearance rates were determined by excising tissues and quantizing the amount of radioactivity in each tissue.
Results: The DNAzyme studied in these experiments represent a novel application of this nucleic acid. Treatment with AM9D in vitro lead to reduced expression of MMP-9 mRNA and in vivo resulted in delayed rate of tumor growth, retarded final tumor volume by up to 70%, and a reduction in lung macrometastases. This decrease in tumor growth and lung metastasis was correlated with decreased MMP-9 protein production within the treated tumor tissues. Tumors treated with AM9D were also less vascular compared to control and untreated tumors. Furthermore, DNAzyme is distributed to major organs including lung through intravenous injection, thus, breaking ground for a clinical treatment strategy.
Conclusion: These results show that targeting and down regulation of MMP-9 by a novel DNAzyme molecule could prove useful as a therapy against breast carcinoma tumor growth and invasion.

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N3-Substituted temozolomide analogs overcome methylguanine DNA methyltransferase and mismatch repair

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Glioblastoma multiforme (GBM) is the most prevalent and aggressive malignant adult CNS tumor. Treatment includes radiotherapy and temozolomide (TMZ) alkylating agent chemotherapy. TMZ methylates purine residues of DNA: *N7*-guanine, *N3*-adenine and *O6*-guanine. *O6*-methylguanine, the primary cytotoxic lesion, is a substrate for direct repair by methylguanine DNA methyltransferase (MGMT). Response to TMZ requires low MGMT levels and functional DNA mismatch repair (MMR). Resistance to TMZ (inherent or acquired), conferred by MGMT expression or MMR deficiency (a consequence of mutation(s) in MMR proteins), represents a huge barrier to successful treatment of GBM.
To address this problem, analogs of TMZ have been synthesized, substituting the N3 methyl moiety with substituents which may lead to DNA lesions able to evade MGMT and DNA MMR.
MTT assays were conducted to compare *in vitro* antitumor activity of TMZ and novel analogs in SNB19 and U373 isogenic glioma cell line pairs: (V = vector control; M = MGMT transfected). TMZ potency reduced 13- and 5.4-fold in SNB19 and U373 cells expressing MGMT; in contrast SNB19M and U373M cells were equi-sensitive as SNB19V and U373V cells to analogs **1** and **2** (Table).

N3-substituent	Analog	GI_{50} (μM)			
		SNB19V	SNB19M	U373V	U373M
CH_3	TMZ	35.7	469.9	68.0	368.7
$CH_2C{=}CH$	**1**	35.6	37.8	37.6	36.1
CH_2SOCH_3	**2**	28.9	14.3	8.2	7.3

In addition, analogs **1** and **2** inhibit growth of vector control glioma cells generated to possess acquired resistance to TMZ. GI_{50} values <50 μM were observed in SNB19VR (MMR deficient; hMSH6 loss) and U373VR (MGMT up-regulation) following analog **1** or **2** challenge.
Analogs **1** and **2** cause G2/M cell cycle arrest in glioma cells irrespective of MGMT status and MMR deficient HCT116 colorectal carcinoma cells. Comet assays demonstrate DNA single strand breaks following SNB19V cell treatment with TMZ and novel analogs, formation of γH2AX foci infer conversion to DNA double strand breaks preceding death by autophagy and apoptosis.
Taq polymerase stop assays reveal that N3 propargyl imidazotetrazine **1** and ring opened N3 propargyl imidazotriazene preferentially alkylate guanine rich DNA sequences. *N-7* guanine alkylation by analog **1** was detected by piperidine cleavage assay.
We conclude that novel imidazotetrazines **1** and **2** elicit *in vitro* antitumor activity irrespective of MGMT and MMR status. Such molecules may offer