RPI-1 was associated with Ret downregulation and apoptosis characterized by early caspase-8 activation and relocalization of CD95 death receptor into lipid rafts. This event was allowed by the downregulation of Fap-1, an inhibitor of CD95 trafficking to the cell membrane. Ret was found associated with both Fap-1 and procaspase-8 which were tyrosine dephosphorylated upon drug treatment. We propose a model in which tyrosine dephosphorylation of procaspase-8 induces its local activation initiating Ret proteolytic processing and destabilizing Fap-1. The following caspase-dependent degradation of Fap-1 may thus allow releasing the negative constraint on CD95. Accordingly, we found that drug-induced cell growth inhibition and apoptosis were enhanced in the presence of the CD95 agonist antibody CH11. Moreover, exogenous expression of the RET-M918T mutant in HEK293 cells upregulated Fap-1 and analysis of MTC specimens showed high levels of Fap-1 in RET-MEN2B type as compared to RET-wild type MTCs.
Overall, these findings reveal a functional interplay between Ret-MEN2B proteins and the extrinsic apoptosis pathway mediated by Fap-1 and caspase-8. The ability of Ret-MEN2B to exert a control on CD95 cell surface expression may contribute to MTC malignant phenotype and provide a rational basis for novel treatment strategies combining Ret inhibitors and CD95 agonists.

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Glioblastoma multiforme is characterized by high incidence of PDGFRalpha expression and susceptibility to the PDGFRalpha-specific antibody MEDI-575 in mouse tumor models

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Background: The platelet-derived growth factor receptor alpha (PDGFRα) acts not only as a receptor tyrosine kinase in tumor cells but also as a mediator of stromal support for cancer growth and survival. In glioblastoma multiforme (GBM), PDGFRα is activated in an autocrine fashion which is reminiscent of PDGFRα functions during development of mesenchymal-derived tissues. Since GBM is a malignant tumor derived from the mesenchyme, we hypothesized that blockade of the tumoral PDGFRα signaling cascade would result in anti-tumor activity.
Material and Methods: Human tumor microarrays with primary and recurrent GBM were stained for PDGFRα. Human GBM cell lines were grown as subcutaneous xenografts in nude mice and treated with MEDI-575, a fully human IgG2 monoclonal antibody that selectively targets PDGFRα without blocking PDGFRβ. Efficacy of MEDI-575 with temozolomide (TMZ) was measured by tumor growth inhibition (dTGI) and by delay in tumor regrowth after cessation of single and combination treatments.
Results: Tumor microarray analysis demonstrated PDGFRα expression in 41% of primary (n = 59) and 38% of recurrent (n = 53) GBM. The human GBM cell lines U118-MG, SNB-19 and U251-MG displayed high PDGFRα protein levels. MEDI-575 at ~2 nM inhibited ligand-induced phosphorylation of PDGFRα in all three GBM lines and PDGF-AA ligand was readily detected in tissue culture medium from SNB-19 cells. Mice with U118-MG or SNB-19 GBM xenograft tumors were dosed with MEDI-575 at 1 mg/kg (2×/wk) which resulted in anti-tumor efficacy of 118% or 78% dTGI, respectively. In U251-MG xenografts, 3 mg/kg (2×/wk) of MEDI-575 produced 71% dTGI. Efficacy in the U118-MG and U251-MG tumor xenografts was achieved at an exposure level of MEDI-575 in mouse serum of 34 ug/ml and 73 ug/ml of MEDI-575. Furthermore, combinations of 10 mg/kg of MEDI-575 with the GBM standard of care drug TMZ at 1 mg/kg or 15 mg/kg did not result in weight loss in mice. Both combination regimens delayed the regrowth of U251-MG xenograft tumors after stopping treatment when compared to treatment with MEDI-575 or TMZ alone.
Conclusions: High incidence of PDGFRα expression in primary and recurrent GBM together with high efficacy of MEDI-575 in GBM xenografts supports further testing of MEDI-575 with or without chemotherapy and radiation in preclinical and clinical settings to develop innovative medicines for GBM.

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Development and characterization of novel orally available Hypoxia-inducible factor (HIF) signaling inhibitors as dual-mechanism cancer therapeutics

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The HIF signaling pathway is crucial, in particular for solid tumors, to circumvent the constraints of low oxygen supply (hypoxia) to induce angiogenesis and maintain proliferation. The oxygen regulated subunit of the transcription factor hypoxia-inducible factor 1 (HIF1), HIF1alpha, is a key factor in tumor growth, and its expression has been correlated with poor patient prognosis in a number of settings.
Here we here present in vitro and in vivo data for a novel series of orally available small-molecule HIF signaling modulators that show nanomolar inhibition of the HIF signaling pathway, in addition to potent anti-proliferative activity against a large number of cell lines derived from solid and blood tumors (EC50 range: 1–100 nM). Phenotypically, the compounds elicit an initial G2/M arrest, followed by the induction of caspase-3/7 and the onset of apoptosis.
The in vitro results also translate into in vivo xenograft models. The lead compounds from the series demonstrate efficacy in such tumor models in mice, with dose-dependent tumor growth inhibition of >60% after oral dosing (breast, renal, and multiple myeloma models). Structural optimization has allowed us to improve the PK and physicochemical profile of the compounds, with a lead candidate having entered formal pre-clinical development. The objective is to commence Phase I clinical studies in Multiple Myeloma and other indications in the second half of 2011.
In conclusion, we believe that the development towards clinical Proof-of-Concept of this novel class of Dual-Mechanism Inhibitors (DMIs) impairing HIF signaling and cellular proliferation presents a promising new treatment option for cancer patients.

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Dual targeting of mTOR and HSP90 for therapy of pancreato-biliary carcinomas

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Background: Although mTOR has been identified as a therapeutic target in pancreato-biliary cancers, inhibitors to mTOR may lead to an undesired feed-back-loop activation via the TORC2 complex that *per se* exerts oncogenic activity, thus counteracting the antineoplastic potential. Interestingly, heat shock protein 90 (Hsp90) inhibitors harbor the potential to impair a broad range of oncogenic signaling molecules, including AKT, ERK and IGF-IR. Since activation of mTOR and IGF-IR signaling cascades represent driving oncogenic forces in pancreato-biliary carcinomas, we focused on elucidating the molecular effects of a dual Hsp90/mTOR inhibition in cholangiocarcinoma and pancreatic cancer cell lines, using 17DMAG, one novel synthetic Hsp90 inhibitor, and the mTOR inhibitor RAD001.
Materials/Methods: The effects of RAD001 and HSP90 were investigated in human cholangiocarcinoma cells (EGI-1, TFK1) and pancreatic cancer cells (L3.6pl), both K-ras mutated. Constitutive and IGF-I-induced signal transduction pathways were investigated by Western blotting. Cell proliferation was determined via an *in vitro* colorimetric BrdU-assay and incubation with RAD001 and/or Hsp90 inhibitors.
Results: Although effective inhibiting p-mTOR(Ser2448) and p-S6 (Ser240/244), the mTOR inhibitor RAD001 induced a positive feedback activation of p-AKT(Ser473), p-AKT(Thr308) and p-p44/42-MAPK(Thr 202/Tyr 204) in cancer cell lines. By adding the HSP90 inhibitor, this feedback was completely abrogated. However, inhibition of Hsp90 induced Hsp27 in all cancer cell lines except EGI-1. Inhibition of proliferation was achieved for up to 20% in L3.6pl and EGI-1 cells with RAD001. The HSP90 inhibitor dose-dependently reduced proliferation up to 25% in EGI-1 cells and up to 75% in L3.6pl cells. This effect was further increased in L3.6pl cells by adding RAD001, whereas no significant additive effect was observed in EGI-1 cells. However, the Hsp27 inducible cholangio cancer cell line TFK1 responded well to Hsp90 inhibition, suggesting Hsp27 as a potential marker for responsiveness. Moreover, IGF-I-induced signaling pathways were effectively blocked by HSP 90/mTOR inhibition.
Conclusion: Dual-targeting of mTOR/Hsp90 appears valuable for treating pancreato-biliary cancers through synergistic effects. However, despite achieving a robust signaling inhibition by mTOR/Hsp90 blockade, some cholangiocarcinomas are more susceptible towards Hsp90 inhibition, than a combinational therapy. Hsp90 serves as an interesting target for molecular therapy of pancreato-biliary carcinomas.