safety profile, immunogenicity, pharmacokinetics, and monocyte saturation, as monocytes express the a5 ß1 receptor.
Results: To date, 16 pts have been enrolled and 15 pts have received M200. Baseline demographics for the 15 treated pts were median age=58 years (range 29–81 years), mean ECOG score=1 (range 0–2), and tumor types: colorectal (4), hepatoma (2), melanoma (2), bronchioalveolar carcinoma (1), thyroid (1), parotid (1), renal cell carcinoma (1) breast (1), esophageal (1) and neuroendocrine tumor of the pancreas (1). One pt received 0.5 mg/kg, 2 received 1 mg/kg, 3 received 2.5 mg/kg, 3 received 5 mg/kg, and 6 received 10 mg/kg. No dose-limiting toxicities have been observed. The adverse events that were possibly-related to study drug were mild to moderate nausea (5), fever (2), vomiting (2), headache (2), anorexia (2), and asthenia (2). No pts had an infusion reaction. Two of 16 (13%) pts developed HACA, but there were no apparent associated adverse events. A dose of 10 mg/kg was well-tolerated, achieved monocyte saturation, and a mean trough level of 82 mcg/mL two weeks after the 1st dose, which is above the minimum effective *in vitro* concentration of 2–3 mcg/mL. Thus, 10 mg/kg every 2 weeks is the recommended dosage regimen for subsequent clinical trials. The response outcomes were: SD (9) and PD (6), with 5 of 6 pts who received 10 mg/kg having SD.
Conclusions: M200 appears to be well tolerated at doses up to 10 mg/kg. As dose-limiting toxicity has not been observed, dose escalation is continuing with additional patients to be enrolled into the 15mg/kg cohort. Final data from this study will be presented.

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Combination with PI3 kinase inhibitors allows drastic dose reduction of tumor necrosis factor

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Both in patients treated using the isolated limb perfusion technique and in animal models, Tumor Necrosis Factor (TNF), alone or in combination treatment, displays a very potent antitumor effect. The severe shock-inducing effect resulting from a systemic application of the high doses of TNF needed to obtain the antitumor activity, however, limits its use to locoregional treatment. In mice, we previously could show that the antitumor and shock-inducing activities are not inevitably linked (Cauwels et al., Immunity 13:223, 2000). In order to allow systemic treatment, lowering of the dose of TNF required for tumor destruction is nevertheless necessary. Using transgenic mouse technology, we could show that TNF exerts its antitumor activity by a selective destruction of the tumor vasculature, rather than by a selective cytotoxic effect on transformed cells. This is triggered by an interaction of TNF with the TNF-R1 receptor on endothelial cells. Since PI3kinase (PI3K) is a central component of survival pathways in neovascular endothelial cells, we investigated whether they could be sensitised to the angiodestructive effects of TNF by PI3K inhibitors.
C57BL/6J mice bearing an established tumor (B16, LLC, EL4 or PG19) were treated with daily paralesional injections of murine TNF in combination with wortmannin, or either agent alone, for 10days. In the presence of the latter PI3K inhibitor (0.25 mg/kg, 1h before TNF), the dose of TNF required to obtain complete tumor destruction dropped from 0.35 mg/kg to 0.04 mg/kg. Also human TNF, that, in mice, is a selective TNF-R1 agonist, has a much faster clearance, and can not induce regression of the tumor, turned to be able to cause complete tumor destruction in mice in combination with wortmannin (as was previously shown for the combination of hTNF and IFN-gamma). No synergism was observed when TNF-R1−/− mice were used, while the synergism was retained when tumor cells were used that were rendered insensitive to TNF by a transfection with a dominant negative mutant of TNF-R1. This indicates again that the synergism targets the vasculature rather than the cancer cell in strict sense. Selected key experiments were repeated using the reversible PI3K inhibitor LY 294002, resulting in similar
Conclusions: In groups where higher doses of TNF were used, wortmannin but not LY294002 increased the toxicity of TNF. At lower doses of TNF, effective in the combination treatment, no increase of the toxicity was observed. The PI3K inhibitors used had no or only a marginal growth retarding effect on the tumors, when used alone at the same doses.
Together with some enhancement of the previously established maximal tolerated dose, that could be obtained by supportive measures and/or inhibitors of the toxicity, the order of magnitude of this dose reduction, when also present in human cancer patients, is likely to result in effective doses that are of the same order of magnitude as the tolerated ones. In this respect, it is worthwhile to refer to our recent results, showing that low doses of TNF could enhance the uptake and the effect of doxorubicin encapsulated in long-circulating liposomes (Brouckaert et al., Int. J. Cancer 109:442, 2004), indicating that further combinations with chemotherapeutic agents could result in additional synergisms.

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PRL-3 promotes invasion in tumor epithelial cells and tube formation in normal endothelial cells

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Phosphatase of Regenerating Liver 3 (PRL-3) has become a drug target in cancer since it was first identified as a marker of colon tumor endothelium (St. Croix et al., Science, 2000) and subsequently recognized as a marker of colon tumor metastasis (Saha et al, Science, 2001; Bardelli et al, Science, 2003). Because of the presence of PRL-3 in two cellular compartments of the tumor, the endothelial and epithelial compartments, future inhibitors of PRL-3 promise a two-pronged attack against tumors. Anti-PRL-3 compounds are expected simultaneously to induce a regression of the tumor vasculature and to interfere directly with epithelial malignancy. We sought to identify the function of PRL-3 in cultured endothelial cells and tumor epithelial cells. We have previously reported that the phorbol ester PMA stimulated PRL-3 mRNA expression and induced proliferation, invasion and tube formation in human microvascular endothelial cells (HMVECs). Given the known upregulation of multiple genes by PMA, it was unclear at the time whether PRL-3 was a major driver of any of these three phenotypes. We now report that HMVECs infected with a PRL-3-expressing adenoviral vector express high levels of the PRL-3 protein and show increased tube formation. These data suggest that PRL-3 was a major driver of the PMA-stimulated tube formation and that it can on its own increase tube formation. These results implicate PRL-3 directly in tumor angiogenesis.
We have previously reported that PRL-3 exogenous expression promoted invasion in the DLD-1 human colorectal adenocarcinoma cell line. We also reported earlier that endogenous PRL-3 mRNA expression correlated positively with invasiveness in the MCF-7 and SKBR3 human breast cancer cell lines and in the SKNAS and IMR-32 human neuroblastoma cell lines in the Matrigel invasion assay. We now report that a PRL-3 siRNA reduces invasion down to 20% of control in the transfected DLD-1 cell model. These data provide additional evidence that PRL-3 stimulates tumor epithelial cell invasion. We also report that the expression of MMP-3 enzyme correlates positively with invasiveness and with PRL-3 protein expression in the transfected DLD-1 cell model as well as in the MCF-7, SKBR3, SKNAS and IMR-32 cell lines, which express PRL-3 endogenously. These data strengthen the evidence indicating that PRL-3 promotes tumor epithelial cell invasion. In addition, these results implicate a possible coregulation of PRL-3 and MMP-3 as a mechanism by which PRL-3 stimulates invasion.
Our studies confirm the involvement of PRL-3 in both tumor angiogenesis and epithelial malignancy. Specifically, our results shed light upon two different functions for PRL-3, depending on which cell type expresses the protein. PRL-3 seems to promote tube formation in endothelial cells, while stimulating invasion in tumor epithelial cells.

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Maximizing the anti-tumor and anti-proliferative effects of 2ME2 by maintaining levels above a threshold concentration for a defined period of time

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2-Methoxyestradiol (2ME2), an endogenous metabolite of estradiol, is an inhibitor of both tumor and endothelial cell proliferation. 2ME2 is currently in oncology clinical trials under the name Panzem®. Various new formulations of Panzem® have been developed recently to increase absorption. These studies were undertaken in an effort to understand the pharmacodynamics of 2ME2, by defining the critical pharmacokinetic parameters associated with inhibition of tumor growth.
MDA-MB-231 human breast carcinoma or Lewis lung carcinoma cell lines were incubated with media alone or increasing concentrations of 2ME2 and inhibition of proliferation was determined by cell counts. Following 48 hr exposure the IC50 values of 2ME2 in MDA-MB-231 and LLC cells are 0.8 μM and 3 μM, respectively. Further studies in which MDA-MB-231 cells were exposed to 2ME2 for limited periods of time each 24 hours demonstrated that a 6 hr incubation with 0.6 μM 2ME2 most closely approached the level of growth inhibition observed following continuous exposure. The level of growth inhibition following exposure of MDA-MB-231 cells to 0.6 μM 2 ME2 for 6 hr could not be mimicked by comparable exposures (AUCs) obtained by altering 2ME2 concentration and incubation time. Moreover, incubation of MDA-MB-231 cells with high concentration