and NCI-H460 (NSCLC) xenograft models, compared with DC101 alone ($p < 0.0001$). In addition, 2C5 antibody showed additive antitumor effects with DC101 in several other models, including MIA-PaCa-2 (pancreatic), Detroit-562 (head and neck), HCT-116 (colon) and NCI-H292 (NSCLC). ELISA analysis of NCI-H460 tumor homogenates showed that 2C5, either alone or with DC101, increased the expression of PDGF-BB, and also significantly reduced the level of PDGFRβ in the tumors. 2C5 inhibited DC101 induced increase in both tumor bFGF and VEGF expression. No overt toxicities were observed in mice treated with high doses of 2C5/DC101 for up to 8 weeks. Taken together, these results support the use of PDGFRβ antagonists in combination with VEGF targeted agents in the treatment of a broad range of human cancers.

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In vivo profiles of a novel compound, TAK-593, a highly potent and selective inhibitor against VEGFR and PDGFR tyrosine kinases

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TAK-593 is a novel small molecule compound that potently and selectively inhibits VEGFR and PDGFR tyrosine kinases. TAK-593 uniquely shows potent pseudo-irreversibility against VEGFR2 and PDGFRβ.
In vivo pharmacological and pharmacodynamic profiles of TAK-593 were investigated in this study. Twice-daily (BID), oral administration of TAK-593 potently inhibited the tumor growth of A549 human lung carcinoma xenograft in athymic mouse with a T/C value of 34, 7.8, and −8.1% (T/C: treated per control on tumor volume growth over treatment period) at 0.25, 1, and 4 mg/kg, respectively. When the treatment was initiated at a larger tumor volume (430 mm^3 of the average volume), TAK-593 (1.5 and 3 mg/kg, BID) clearly exhibited the tumor regression in A549 xenograft nude mouse model. Four weeks treatment with TAK-593 at a high dose (10 mg/kg, p.o., BID) did not affect body weight of nude mouse. In the nude rat xenograft model, TAK-593 (0.1 and 0.2 mg/kg, BID) showed more potent antitumor activity against A549 with a T/C value of 34 and 26%, respectively. TAK-593 (0.25 to 4 mg/kg, BID, p.o.) also showed potent antitumor activities in xenograft models generated from various human cancer cell lines (HT-29 colon, CFPAC-1 pancreas, MDA-MB-231 breast, SK-OV-3 ovary, DU145 prostate, and MKN45 gastric carcinoma and U87 MG glioblastoma).
PK/PD study of TAK-593 was investigated to clarify the contribution of pseudo-irreversibility to the strong *in vivo* antitumor activity. When the plasma concentration of TAK-593 in nude mice was below the detection limit at 8 hours after the administration, the phosphorylation of VEGFR2 (PD marker of VEGF signaling) was still potently suppressed. Furthermore, the antitumor activity of once-daily treatment with TAK-593 against A549 lung carcinoma was approximately equivalent to that of twice-daily treatment of TAK-593, indicating that efficacy is not driven by C_{trough}. These results indicate a unique profile of TAK-593 with the potent pseudo-irreversibility profile on VEGFR and PDGFR kinases might greatly contribute to the long duration of its antitumor activity *in vivo*.

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Calixarene-based angiogenesis inhibitor 0118 attenuates endothelial cell anergy and promotes a cytotoxic T-cell-mediated anti-tumor response

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Suppressing the expression of endothelial adhesion molecules (EAM, like ICAM and VCAM) by release of pro-angiogenic factors like VEGF and bFGF, is one way in which tumors avoid immuno-surveillance and prevent leukocyte extravasation. Here, we demonstrate that the novel calixarene-based angiogenesis inhibitor 0118 restores tumor EAM expression levels on endothelial cells and enhances T-cell infiltration in tumors. ELISA, immunohistochemistry, and multi-color flow cytometry were used to monitor the time-dependence of EAM expression and leukocyte infiltration in B16F10 melanoma progression. We found that tumor progression correlates with reduced levels of EAMs and CD8 and CD4 cells in untreated animals, while treatment with 0118 normalizes EAM expression and increases the T-cell population in the tumor. To further elucidate the adaptive immune system in tumor progression, we used two tumor models (B16F10 melanoma and Lewis Lung Carcinoma (LLC)) to compare tumor incidence and progression in wild type B6 mice vs. CD8 (and CD4) null mice. To differentiate anti-angiogenic and immunomodulatory effects, we compared tumor growth inhibition in wild type mice with that in CD8 and CD4 null mice, in both the B16F10 and LLC models. In either tumor model, 0118 inhibited tumor growth significantly less in null mice than in wild type mice, indicating that increased leukocyte infiltration into tumors promoted by reduced tumor endothelial cell anergy accounts for at least one-third of the tumor growth inhibitory effect from angiogenesis therapy. Because cellular immunotherapy (adoptive transfers or dendritic cell vaccinations) relies on leukocyte extravasation into the tumor, our results open a novel line of investigation and strongly suggest that combination therapy with angiogenesis inhibitors may hold great promise for the future of immunotherapy.

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Novel approaches to breast cancer therapy – simultaneous targeting to tumor and endothelial cells of tumor blood vessels

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Background: The present work is aimed at investigating the ability of a tumor vasculature-homing peptide to function as a targeting agent of poly(ethylene glycol) (PEG) sterically stabilized liposomes to human breast tumor cells.
Materials and Methods: Cellular association studies of rhodamine or calcein-labelled PEG-grafted liposomes with tumor and endothelial cells were performed by fluorimetry, flow cytometry and confocal microscopy at 4 or 37ºC. Competitive inhibition experiments with the free targeting peptide were also performed. Aiming at investigating the cell entry pathway of the targeted liposomes, cells were pre-incubated with drugs that selectively compromise macropinocytosis, caveolae- and clathrin-mediated endocytosis. The cellular content of rhodamine was compared with that for an internalization inhibitor-free control. Cytotoxicity of free doxorubicin (DXR) and DXR-containing targeted and non-targeted liposomes was determined at different time points using the MTT assay.
Results: The extent and rate of cellular association were dramatically higher for peptide-targeted liposomes as compared to non-targeted liposomes, increasing with the lipid concentration and as the temperature rose from 4 to 37ºC. These results were in agreement with the intracellular fluorescence observed by confocal microscopy. Pre-incubation of the target cells with the free peptide inhibited the cellular association of targeted liposomes. Studies performed with endocytosis inhibitors indicated that peptide-targeted liposomes were internalized by a receptor-mediated mechanism, most likely through clathrin-mediated endocytosis. Treatment of both tumor and endothelial cell lines with peptide-targeted liposomes containing DXR induced a faster and stronger inhibition of cell growth than the other tested formulations.
Conclusions: The results provide evidence that the vasculature-homing peptide tested has the ability to target PEG sterically stabilized liposomes to human breast tumor and endothelial cells, on a peptide- and cell-specific manner, resulting in a dramatic improvement of the cytotoxic activity of the encapsulated drug. Such targeted nanosystem provides an important therapeutic advantage as compared to existent treatments for breast cancer.
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Antiangiogenic and tumor growth inhibitory effects of heparin-taurocholate conjugate

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Background: Though low molecular weight heparin has been known to regulate angiogenesis, the administration of heparin for treating cancer is limited in clinical applications due to its unsatisfactory therapeutic effects and a strong anticoagulant activity, which induces hemorrhages.
Materials and Methods: Heparin-taurocholate conjugate (HT10), was prepared by using low molecular weight heparin which was purchased from Sanofi-Synthelabo (Gentilly, France), taurocholic acid sodium salt, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimidehydrochloride, 4-nitrophenyl chloroformate, and n-hydroxysuccinimide from Sigma Chemical Co. (St. Louis, MO). Circular dichroism method was used to evaluate a structural property of heparin derivatives. Binding constants and thermodynamic parameters in binding between vascular endothelial growth factor 165 (VEGF165) and HT10 were obtained using isothermal titration calorimetry.