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Paclitaxel enhances therapeutic efficacy of F8-antibody mediated delivery of interleukin-2 to xenografted melanoma cancer

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Advanced melanoma remains a solid malignancy with limited therapeutic options. Dacarbazine (DTIC) is the benchmark treatment for advanced melanoma while paclitaxel (PTX) is a reasonable second line therapy. Interleukin-2 (IL2) appears the most active biologic therapy against melanoma. Several studies evaluating efficacy of regimens containing the combination of chemotherapy with IL2 have reported durable tumor response or increased progression-free survival, but no improved overall survival. Furthermore, chemo-immunotherapy regimen is generally associated with considerable toxicity.

The "antibody-based tumor targeting" strategy involves the selective delivery of bioactive agents to the tumor site by their conjugation to antibodies directed to a tumor-associated antigen. F8 is a recently cloned high-affinity human monoclonal antibody recognizing EDA domain containing fibronectin (EDA^+ Fn, a tumor stroma associated antigen) which displayed tumor targeting selectivity.

Aim of this study was to investigate the therapeutic performance of the scFv F8-IL2 immunocytokine (F8-IL2) in the WM1552/5pt human melanoma xenograft model growing in nude mice and expressing high levels of interstitial and vascular EDA^+ Fn.

In this model, F8-IL2 was found to have minimal but significant antitumor effect. Its therapeutic efficacy was significantly potentiated (60−80% complete responses) by the combination with PTX, but only marginally with DTIC. Accordingly, assessment of the immune effector cell infiltration showed a significant increase of NK cells in tumor specimens harvested from mice treated with PTX combined with F8-IL2, confirming the involvement of the host immune reaction in the tumor response. DCE-RMI and optical *in vivo* imaging analysis showed changes in tumor perfusion and permeability and enhanced delivery of the antibody to tumors treated with PTX. The relevance of these findings was investigated giving the combination of F8-IL2 plus PTX accordingly to different sequences of administration. PTX administered concomitantly or 24 h before F8-IL2 produced the best therapeutic outcome. The combination treatments were well tolerated, with no clinical signs of toxicity.

In conclusion, F8-IL2 shows therapeutic efficacy against a human melanoma xenograft model and its activity is potentiated by the addition of PTX.

Supported by 7th EU Framework Programme for Research and Technological Development (FP7 HEALTH-F2–2008–201342).

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Molecular imaging of Death Receptor 5 (DR5) occupancy in-vivo by humanized monoclonal antibody CS-1008

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Background: CS-1008 (Tigatuzumab), a humanised monoclonal IgG1 antibody agonistic to the human Death Receptor 5 (DR5), has shown anti-tumour efficacy in a wide range of preclinical models, and is currently in phase I/II trials. To assist in clinical development, this study aimed to determine the pharmacokinetics and quantitative tumour targeting properties of CS-1008 in a mouse model, and assess the effects of antibody dose on DR5 receptor saturation *in-vivo* through molecular imaging.

Materials and Methods: CS-1008, parental antibody mTRA-8, and an isotype control IgG1 antibody was radiolabelled with Indium-111 (^{111}In), and characterised for DR5 binding and labelling efficiency on DR5 +ve COLO205 cells. Pharmacokinetic and biodistribution studies were performed in BALB/c nude mice bearing COLO205 or DR5 −ve CT26 colon tumours, with CS-1008 and isotype control antibody. Dose levels of 0.2, 1 or 10 mg/kg of antibody were explored, with gamma camera/CT imaging also performed to allow quantitative dosimetry of whole body, liver and tumour regions up to 10 days post infusion.

Results: Labelling efficiency of 99% was obtained for the radioconjugate, with ^{111}In-CS-1008 specific activity in the range 2.7−3.4 mCi/mg, and stable in serum for up to 11 days. Scatchard analysis showed high and low affinity binding sites for ^{111}In-CS-1008 ($Ka = 3.3 \times 10^8\ M^{-1}$ and $2.0 \times 10^7\ M^{-1}$). ^{111}In-CS-1008 showed high, specific uptake in COLO205 tumours at 0.2 mg/kg dose (up to 26%ID/g), with prolonged retention at >20%ID/g over 14 days. Tumour uptake at 48 hrs post injection of ^{111}In-CS-1008 at 0.2 mg/kg was significantly higher than 1 mg/kg and 10 mg/kg ($p = 0.001$). No differences in serum clearance were observed between dose levels. Gamma camera/CT imaging demonstrated no normal tissue uptake of ^{111}In-CS-1008, and excellent uptake in COLO205 tumours which continued up to 10 days. No specific uptake of ^{111}In-CS-1008 was observed in control CT26 tumours. Dosimetry calculations of quantitative imaging datasets revealed saturable DR5 receptor occupancy in tumour by ^{111}In-CS-1008 occurring above 1 mg/kg, reaching 40% at 10 mg/kg.

Conclusions: ^{111}In-CS-1008 provides optimal information on the *in-vivo* behaviour of systemically injected CS-1008 in mouse DR5 +ve tumour models, which is specifically taken upin DR5 +ve tumours with prolonged retention. Importantly, DR5 receptor saturation can be demonstrated *in-vivo* with molecular imaging of ^{111}In-CS-1008. These results have direct implications for clinical development and optimal dose and patient selection for trials of DR5 targeting antibodies.

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515H7, a novel anti-CXCR4 antibody − Part I: in vitro efficacy on CXCR4-associated signaling pathways

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Background: CXCR4 is a widely expressed chemokine receptor of CXCL12/stromal cell-derived factor (SDF)-1; it plays a central role in various physiological and pathological processes, including cancer. CXCR4 expression by tumor cells plays a critical role in cell metastasis by a chemotactic gradient towards organs expressing SDF-1 and promotes angiogenesis. CXCR4 over-expression has been correlated with poor prognosis in many types of cancer. As a member of the GPCR superfamily, CXCR4 signaling is mainly driven by G proteins and downstream effectors: adenylate cyclase, phospholipase C, b-arrestin and GRK. Modulation of downstream mediators like RAF, PI3K, MAPK, JNK has also been described.

A novel mouse monoclonal antibody (Mab 515H7) was raised against the human CXCR4. It displayed efficacious antagonist properties for all major pathways associated with SDF-1-induced CXCR4 signaling. The influence of 515H7 Mab on CXCR4 homodimers was also demonstrated by using a bioluminescence resonance energy transfer (BRET) assay.

Materials and Methods: CHOK1 and NIH3T3 cells were stably transfected with a human CXCR4 cloned into a pcDNA3.1 vector. [^{125}I]SDF1 and [^{35}S]GTPgS binding assays were performed on cell membranes containing CXCR4, using SPA-WGA beads. Calcium mobilization was monitored using Fluo-4NW dye. Intracellular cAMP dosage was performed upon forskolin (10 μM) stimulation by using an AlphaScreen cAMP assay kit. BRET assays were developed upon genetic fusion of CXCR4 to Rluc and b-arrestin to YFP and transient co-expression in HEK293 cells.

Results: Series of monoclonal antibodies were generated upon immunization of mice with various combinations of NIH3T3/CXCR4 cells and peptides derived from the receptor extracellular loops. They were able to efficiently label CXCR4 on both transfected cells and human tumor cell lines. Several hit-candidates were identified and one, Mab 515H7, was further characterized for its pharmacological properties. It was found to strongly inhibit SDF-1 binding, Ga protein activation and beta-arrestin recruitment. It also blocked second messenger release (calcium, inositol phosphates, cAMP) and constrained by itself the formation of CXCR4 homodimers. A chimeric form of the mouse Mab was produced and yielded similar antagonist activities.

Conclusion: The herewith data demonstrate that mAb 515H7 in both its murine and chimeric formats, behaved as a potent and efficacious antagonist of all major CXCR4-controlled signaling pathways. These efficient inhibitory properties are likely to participate in its *in vivo* anti-tumoral activities in mouse models as shown in the companion poster. These pharmacological properties suggest that antibodies targeting CXCR4 are of interest for therapeutic applications in Oncology.