of the treatment was given monthly. Serum Anti-NGcGM3 antibody titers were determined by ELISA. Patients were regularly submitted to CT scans and US during the study. WHO's toxicity criteria were applied.
Results: Vaccination induced specific antibody titers (IgM; range 640–2560 and IgG; range 80–2560) at both dose levels. Though anti-NGcGM3 serum antibodies increased with the higher dose of vaccine clinical outcome was more significant for the lower one. It was encouraging to observe objective responses in 3 out of 10 valuable patients of the 0, 2 mg dose level (regression of cutaneous metastases and stabilization of lung lesions for 22 months). Other patients showed mixed responses with elimination, stabilization or progression of cutaneous tumours. Main toxicities included erythema, mild local pain, and low grade fever. Most noteworthy vitiligo was developed by 4 patients after being injected at least 6 times with the lower dose of the vaccine.
Conclusion: For the first time evidences of the antigen spreading phenomena have been clinically observed (the appearance of vitiligo in patients only can be explained over the spreading basis because NGcGM3 epitopes are absent from normal melanocites but present in melanoma). The vaccine was safe and immunogenic also in advanced melanoma patients. These encouraging first clinical evidences of the NGcGM3/VSSP/Montanide ISA 51 vaccine effectiveness in stage IV (without surgical resection) melanoma patients guarantee further investigation.

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Biological characterisation of CD40-activated B cells as cellular adjuvant in cancer vaccines

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Cellular immunotherapy is a promising approach to specific treatment of cancer. Dendritic cells (DC) are the best-studied antigen presenting cells (APC) and have been tested in multiple clinical trials over the last years. To extend this strategy to clinical situations in which DC therapy might be challenging, e.g. pediatric patients or frequent vaccinations, we have established CD40-activated B cells (CD40-B cells) as a complementary autologous APC, due to the simple generation of large amounts of highly efficient CD40-B cells from small amounts of peripheral blood and efficient presentation of antigen to $CD8^+$ T-cells.
However, the induction of naïve and the amplification of memory $CD4^+$ T cell responses is most likely a prerequisite of potent cellular adjuvants. Antigen uptake and presentation by B-cells is highly epitope-specific. It therefore remains unclear whether CD40-B, have the capacity to take up, process and present a broad range of antigens in the context of MHC class II.
Here we address, whether CD40-B process antigens in the context of MHC class II and induce secondary and primary $CD4^+$ T-cell responses: We developed a T-cell expansion system that uses CD40-B cells as sole APC to induce antigen-specific responses of purified $CD4^+$ T-cells: 1) tetanus toxoid and KLH, as model protein antigens, 2) the artificial promiscuous MHC class II binding peptides PADRE-AKF and PADRE-AKX as model peptide-neoantigens. While specific cells were successfully expanded for all antigens studied, INF-γ and IL-4 ELISPOT profiles did not indicate a dominant TH_1 or TH_2 bias.
Similarly important, we addressed, if CD40-B cells have the potential to home to lymph nodes and induce T-cell chemotaxis: CD40-B lack receptors important for relocating to peripheral tissue but do express CD62L, LFA-1, CCR7 and CXCR4, receptors implied in homing to secondary lymphoid organs. Migration experiments using their cognate ligands CXCL12, CCL19 and CCL21 demonstrated that these receptors are fully functional. Furthermore, CD40-B cells express several important T-cell attractants including IP-10, Rantes, MCP-1 and ENA-78. Correspondingly supernatant from CD40-B cultures induces strong chemotaxis of T-cells.
Taken together, CD40-B cells efficiently induce primary MHC class I and II restricted T-cell responses, have potential to home to secondary lymphoid organs and induce T-cell chemotaxis. These data further support the potential of these autologous APC as adjuvants in cancer immunotherapy.

Cellular therapies and cytokines

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Pharmacokinetics and pharmacodynamics of recombinant human IL-18 (rhIL-18) in patients with solid tumors

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Background: rhIL-18, a Th-1 inducing cytokine, has demonstrated anti-tumor activity in a variety of preclinical models. Exposure and biomarker response data from the first human trial are described below.
Materials and Methods: Patients with solid tumors received doses of rhIL-18 (SB485232) ranging from 3–1000 mcg/kg administered as 2h infusions daily for 5 consecutive days. Assessments included tolerability, immunogenicity, anti-tumor activity, pharmacokinetics, and biomarkers of immunomodulatory activity to define a potentially biologically effective dose range. Plasma drug concentrations were measured by ELISA. Cytokine/protein biomarkers were measured by ELISA and cytokine biochip. Cell activation biomarkers were measured by flow cytometry.
Results: Twenty-six patients were evaluable for these analyses, including 21 with metastatic renal cell carcinoma, 4 with melanoma, and 1 with Hodgkin's lymphoma. Plasma concentrations of rhIL-18 exhibited 2.5-fold accumulation with daily dosing, a dose-independent accumulation half-life of 35h, and a tri-phasic nonlinear dose-concentration relationship. Clearance and volume of distribution were dose-dependent, largely due to saturable binding to IL-18 binding protein, a high-affinity circulating modulator of rhIL-18 activity induced through INF-gamma. Dose-related induction of INF-gamma and GM-CSF were observed, as well as increased levels of downstream chemokines. Lymphopenia was maximal by 8h after the first infusion at all dose levels. Drug related increases were observed in expression of FasL on CD4, CD8, and NK cells, expression of CD69 on $CD8^+$ cells, and expression of CD11b on NK cells and monocytes. Evidence of clinical activity was observed in two patients at the 100 mcg/kg dose, and associations with biomarkers were explored.
Conclusions: These data demonstrate the complex pharmacokinetic behavior, immunomodulatory activity, and therapeutic potential of rhIL-18.

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P43/Endothelial monocyte-activating polypeptide-II: protein exaptation provides a novel counterattack mechanism in colorectal tumours

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P43 is an auxiliary component of the mammalian multisynthase complex, playing a central role in protein synthesis. This 34kDa protein is normally retained intracellularly; however, in what appears to be an example of protein exaptation (appropriation of a molecule with one function for a different purpose), tumour cells may express this protein in a soluble extracellular form, known as Endothelial Monocyte-Activating Polypeptide II. EMAP-II has multiple cytokine-like activities, inducing procoagulant activity on the surface of endothelial cells, increasing expression of E-and P-selectins and tumour necrosis factor (TNF) receptor-1, and directing migration of monocytes and neutrophils. Since it is not biologically advantageous to a tumour to attract lymphocytes, we hypothesized that EMAP-II might simultaneously attract phagocytic cells while suppressing the activity of lymphocytes. We therefore investigated the effects of p43/EMAP-II on lymphocytes. Recombinant protein induced apoptosis in mitogen-activated peripheral blood lymphocytes, and in Jurkat T-cells. We then examined EMAP-II expression in the HT29 and DLD-1 colorectal cancer cell lines. Both express extracellular p43/EMAP-II; however HT29 retain it on the external cell surface, while DLD-1 cells release a soluble, biologically active 20kDa fragment of the molecule. Co-culture of Jurkat cells with HT29 cells induced activation of caspase 8 and apoptosis in the Jurkat cells, which was partially blocked by addition of neutralizing antibodies against p43/EMAP-II. Conditioned medium from DLD-1, but not HT29 cells, had similar effects, suggesting that both membrane-bound and soluble forms of p43/EMAP-II can induce lymphocyte apoptosis. We conclude that p43/EMAP-II in its extracellular form plays multiple roles in the tumour micro-environment, one of which is to assist in immune evasion by providing a counterattack against cytotoxic T-cells. Tumour-associated exaptation may provide a new source of therapeutic targets.