Methods: Cytotoxic activity of control non transduced T cells (NT) and CAR^+ T cells was measured using a standard ^{51}Chromium release assay. Co-culture experiments of NT and CAR^+ T cells (1×10^6 cells/well) with viable LCLs (ratio 1:1) in the presence or in the absence of serial dilutions of B-CLL patients plasma enriched in soluble CD23 (sCD23) have been performed to assess sCD23 mediated inhibition of the CD23.CAR^+ T lymphocytes cytotoxic activity. The expansion of CAR^+ T lymphocytes in response to $CD23^+$ targets has been proved by weekly stimulation with allogeneic, g-irradiated (30 rads) LCLs (ratio 1:1), without addition of exogenous cytokines. IFN-g, TNF-a and TNF-b release was measured with a Flow Cytomix Assay, while IL-2 production was measured using a specific Enzyme-Linked Immunosorbent Assay. Soluble CD23 levels of B-CLL patients-derived plasma samples have been detected using a human CD23 ELISA kit.
Results: CD23.CAR^+ T cells showed specific cytotoxic activity against $CD23^+$ tumour cell lines (average lysis 54%, at Effector:Target (E:T) ratio 40:1) and primary $CD23^+$ B-CLL cells (average lysis 58%, at E:T ratio 20:1). This effect was obtained without any toxicity against normal B lymphocytes, differently from other CARs that target CD19 or CD20 antigens expressed by leukemic cells, but physiologically also by normal B lymphocytes. Moreover, CD23.CAR^+ T cells released inflammatory cytokines (4-fold more IFN-g, ??157-fold more TNF-a and 1445-fold more TNF-b) in response to $CD23^+$ target cells. IL-2 was also released (average release 2.681 pg/mL) and sustained the antigen-dependent proliferation of CD23.CAR^+ T cells.
Conclusions: Altogether these data suggest that gene modification of T cells to express the CD23.CAR represents a selective immunotherapy approach to eliminate CD23+ leukemic cells, while sparing normal B lymphocytes, in patients with B-chronic lymphocytic leukemia.

301 Combined immunogene therapy and Treg inactivation in treatment of weakly immunogenic solid tumours

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Background: The majority of cancers occur against a background of normal immune function and evidence suggests that host tumour antigen-specific effector and memory T cells should eliminate the neoplastic cells. Cancers avoid immune elimination by a number of interrelated mechanisms, which occur predominantly within the region of the tumour itself. Anti-tumour immune responses are subjugated by tumour-mediated immunosuppressive mechanisms that render the host tolerant to the cancer and thus create obstacles to immune therapies. This compartmentalised immune evasion does however provide an opportunity for the design of a regionally-based immunotherapy. Establishing an immune responsiveness at the level of the primary tumour would also inhibit progression of metastatic disease, as the antigen spectrum is similar on the primary and metastatic cancer cell.
We present an effective immune-based therapy of weakly immunogenic tumours using locally delivered immunogene therapy and systemic T regulatory (Treg) cells inactivation. The aims are obtained by promoting the development of immune effector responses in the tumour environment, and potentiate these responses by elimination of tolerogenic or immune suppressor influences.
Material and Methods: We investigated the tumour models murine fibrosarcoma (JBS) and colon carcinoma (CT26). Plasmids encoding GM-CSF and B7−1 were electrically delivered into tumours and Treg inactivation was accomplished by systemic administration of anti-CD25 antibody.
Results: Complete eradication of tumours was achieved at a level of 60% by immunogene therapy, 25% by Treg inactivation and 90% by the combined therapies. Cured animals by Treg inactivation, were resistant to re-challenge using the Winn assay. Cured mice displayed no signs of autoimmune disease after one year follow up and the antitumour responses were non cross reactive with normal tissues.
Conclusions: Combination of immunogene therapy and Treg inactivation constitutes an effective treatment which results in the eradication of weakly antigenic solid tumours. The combination augments the total effect of either treatment. The therapy was immune specific, tumour specific, transferable, safe and effective. This therapeutic model should be considered for clinical development as a primary or neoadjuvant therapy.

302 The effect of differential in vitro regulation of NKG2D and CD161 NK cell receptors by IL-2 or IFN-a on activation of NK cells in metastatic melanoma patients

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Background: In metastatic melanoma (MM) immunomodulating agents such as IL-2 and IFN-α have shown therapeutic response. Considering that first line of antitumour immune defense is mediated by natural killer (NK) cells, and that NK cell activity is often impaired in MM it was of interest to study the effect of these cytokines on the less investigated CD16-defined NK cells and their dim and bright subsets.
Material and Methods: Peripheral blood lymphocytes (PBL) obtained from 45 MM patients in clinical stage IV prior to therapy treated for 18 h with 200 IU/ml IL-2 and 250 IU/ml IFN-α were evaluated for NK cell cytotoxicity. Expression of NKG2D, CD161, CD158a, CD158b receptors was analyzed on $CD3^-CD16^+$ NK cells by FACS flow, pSTAT1 and pSTAT5 protein expression by Western blotting, and mRNA for IFN regulatory factor-1 (IRF-1) after 4 h by rt-PCR.
Results: Both cytokines induced significant in vitro enhancement of NK cell cytotoxic activity. IL-2 induced NKG2D, while IFN-α induced NKG2D and CD161 receptor expression on NK cells and $CD16^{bright}$ subset, with no effect on the expression of investigated KIRs. Furthermore our results show that in MM patients only the induction of NKG2D with IL-2 on CD3-CD16+ NK cells and on $CD16^{bright}$ subset correlates with its enhancement of NK activity. Contrary to this, the induction of NKG2D by IL-2 on the regulatory $CD16^{dim}$ subset does not correlate with augmented NK activity. We found substantial specific inducibility of pSTAT1 and pSTAT5, as well as induction of IRF-1 transcription by IFN-α in PBL of investigated patients.
Conclusions: Although NK cell-killing of tumour cells depends on the balance of stimulatory and inhibitory signaling there is no data on IL-2 and IFN-α mediated NK cell activating receptor induction, especially on $CD3^-CD16^+$-defined NK cells or their cytotoxic $CD3^-CD16^{bright}$ subset. By showing differential induction of activating receptors with IL-2 and IFN-α, we found for the first time that IL-2 and IFN-α in vitro enhanced NK cell activity of MM patients would be in favor of IL-2, as it has more extensive correlation with NKG2D induction on $CD3^-CD16^+$ or cytotoxic $CD3^-CD16^{bright}$ NK cells. The obtained IL- enhanced NK cell cytotoxicity may result, as opposed to IFN-α, from its better up-regulation of numerous cytotoxic mediators. The results obtained in this study support the shown therapeutic effects in MM of these cytokines, applied in immunotherapy alone, or in combination with chemotherapeutic agents.

303 Cytotoxic treatment for rectal cancer reveals different innate immunity in responder and non-responder patients

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Background: Neo-adjuvant radio-chemo therapy for rectal cancer has dissimilar outcomes, with about 70% of partial/complete responses and 30% of no responses at surgery. Little is known on the role that innate immune responses play in the outcome of conventional therapies.
Material and Methods: We performed a prospective study in which we analyzed serial tumour and blood samples of 32 consecutive rectal cancer patients who underwent neo-adjuvant therapy. We characterized *in-situ* cell death, circulating monocytes, infiltrating macrophages and inflammatory molecules in the blood.
Results: 10 underwent complete pathological remissions, 10 had partial responses and 12 had no responses. In the responders we observed: after an initial expansion, a decrease in the number of circulating monocytes; an up-regulation of monocytes CD16 expression and a clear expansion of CD14+CD86+ monocytes at the earlier time points. The latter event was transient as it abated at the later time point. The therapy caused a decrease of hemoglobin concentration in all the pts. This was related to an expansion of CD14+CD163+ monocytes in responder pts only. In non-responder pts we observed: higher plasma concentration of C1q, C3 and C4; and a significant increase of CRP concentration at the end of the therapy and an expansion of Tie2 expressing monocytes.
Conclusions: The preliminary characterization of the macrophage infiltrate suggests a possible bias toward an alternative M2 polarization. These data suggest that neo-adjuvant therapy modulates the cellular and the humoral innate immune responses and that this response correlates with clinical outcomes. It could either reflect an heterogeneity in the response to primary inflammation signals, elicited as a consequence of cell death, or different patterns in the anti-inflammatory clearance of cell debris, that influence the long term outcome of the treatment.

304 Myeloid response and macrophage polarization in mouse melanoma lung metastasis

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Myeloid derived cells contribute to tumour growth by suppressing immune responses and providing the tumour cells with inflammatory cytokines. Monocytes acquire different functional traits during polarization to the classical