CRC with full clinico-pathological data. Furthermore, gene expression levels were assessed on CRC tissues by quantitative PCR.
Finally, in order to characterize the phenotype of IL-17-positive cells, expression of IL-17, in combination with that of specific surface molecules, was analyzed on freshly excised CRC specimens by flow cytometry.
Results: Frequencies of IL-17-producing cells, as well as IL-17 gene expression levels were significantly increased in tumour tissues as compared to autologous normal mucosa. IL-17-producing cells isolated from clinical specimens were exclusively comprised within the lymphocyte population and expressed CD4, but not CD8, and surprisingly,Foxp3 molecules. Accordingly, mRNA levels of genes encoding for cytokines favouring IL-17 acquisition by Foxp3+ T cells, including IL-6, IL-1beta, TGF-beta and IL-23, were found more elevated in CRC tissues as comparing to corresponding healthy mucosa.
High infiltration by IL-17 producing cells significantly correlated with low T and N stages, and, most importantly, with prolonged survival time in mismatch repair (MMR)- proficient, but not-deficient CRC. Moreover, the simultaneous CRC-infiltration by IL-17+ and Foxp3+ cells was significantly associated with improved survival in both MMR-proficient and -deficient tumours.
Conclusions: Our data suggest that IL-17 produced by tumour-infiltrating either CD4+ or Foxp3+ cells may promote a benign clinical outcome in CRC.

659 POSTER

A novel mechanism of action of platinum-drugs: breaking STAT6-mediated suppression of immune responses against cancer

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Tumor micro-environments feature inhibitory mechanisms that prevent T cells from generating effective immune responses. Therapeutic interventions aimed at disrupting these inhibitory mechanisms have been shown to result in enhanced anti-tumor immunity, but lack direct cytotoxic effects. We investigated the effect of cytotoxic chemotherapeutics on dendritic cell function and on tumor cell immunogenicity.
Using allogeneic and antigen-specific in vitro models, we found that when dendritic cells (major regulators of cellular immune responses) were activated in the presence of platinum-based chemotherapy, their T cell stimulatory capacity was strongly enhanced. Expression of the immune-inhibitory molecule *Programmed death receptor-ligand* 2 (PD-L2) by dendritic cells was markedly reduced upon platinum exposure. The enhanced T cell stimulatory capacity by dendritic cells upon platinum exposure was abrogated in the presence of PD-L2 blocking antibodies. This was also observed when the regulator of PD-L2 expression, *signal transducer and activator of transcription* 6 (STAT6), was knocked down using siRNA.
In addition, we also found in tumor cells that STAT6 is dephosphorylated by platinum compounds, leading to marked downregulation of PD-L2 and resulting in enhanced recognition by tumor-specific T cells.
In line with these in vitro findings, we observed in a retrospective study that patients with STAT6-expressing head and neck cancer displayed significantly enhanced recurrence-free survival upon treatment with cisplatin-based chemoradiation compared to patients with STAT6-negative tumors, demonstrating the clinical relevance of platinum-induced STAT6 modulation.
The PD-L2/STAT6 pathway is known as a major immunosuppressive network that paralyzes the immune system and builds an immune-evasive tumor microenvironment. Our findings demonstrate that platinum compounds not only directly kill tumor cells but also enhance T cell stimulation by dendritic cells. At the same time tumor cells are also sensitized to lysis by cytotoxic T cells through inactivation of this pathway. This novel action of platinum compounds, which are part of the standard treatment of many cancer types, may extend their therapeutic application and provides a rationale for their use in combination with other immunostimulatory compounds to increase the clinical efficacy of cancer treatment.

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Discovery of a novel series of indoleamine 2,3-dioxygenase 2 (IDO2) selective inhibitors for probing IDO2 function in cancer

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Indoleamine 2,3-dioxygenase (IDO or IDO1) is a tryptophan (trp)-catabolizing enzyme implicated in immune suppression during pregnancy, transplantation and in diseases such as infection and cancer. The role of IDO in cancer has been supported by studies with the IDO inhibitor, 1-methyl-tryptophan (1MT), which has been shown to improve the antitumor effects of chemo- or immunotherapeutic agents in tumor models by reversing IDO-mediated T cell suppression. A related protein, IDO2, was recently identified, but its role in cancer is unclear. Studies suggest that the L stereoisomer of 1MT inhibits trp to kynurenine (kyn) conversion by IDO1 *in vitro*, whereas the D isomer of 1MT is more selective for inhibiting IDO2 and also exhibits better activity than L-1MT in murine tumor models. D-1MT has since been advanced into clinical trials for cancer. Although both IDO1 and 2 can be detected in human tumors, emerging data indicate that only murine IDO2 can efficiently convert trp to kyn and that human IDO2 may not do so effectively. Given the conflicting data, a potent IDO2-selective inhibitor would provide a valuable tool to study IDO2 function and explore the potential utility of IDO2 inhibition in cancer therapy. Here we describe a novel series of IDO2 inhibitors. Due to the ineffectiveness of human IDO2 in catabolizing trp, we screened compounds in assays measuring trp to kyn conversion using mouse IDO1 or IDO2-transfected HEK293 cells. Representative lead compounds potently inhibited IDO2-mediated trp conversion and exhibited selectivity (up to 100-fold) over mouse IDO1. These inhibitors exhibited significantly weaker activity against human IDO1 compared to their activity against mouse IDO2. L- and D-1MT were >1000-fold less active against mouse IDO2 in these assays. Using IDO1 and IDO2 selective inhibitors, we find that IDO2 activity is not responsible for trp $\rightarrow$ kyn conversion in either human dendritic cells (DCs) or tumor cells that were induced to express IDO2. Further, in co-cultures of human allogeneic lymphocytes with IDO1/2-positive DCs, IDO2 selective inhibitors did not reverse T cell suppression at doses that significantly inhibit murine IDO2 activity, supporting that IDO2 is not involved in T cell suppression via this particular mechanism. In summary, we have identified a novel series of IDO2 selective inhibitors and our preliminary data suggest that, unlike IDO1, in man IDO2 may lack activity in catabolizing trp and consequently in regulating immune responses.

661 POSTER

A pharmacokinetic, pharmacodynamic and electrocardiographic study of L-MTP-PE in healthy volunteers

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Background: L-MTP-PE (liposomal muramyl tripeptide phosphatidyl-ethanolamine; mifamurtide; MEPACT®) is an activator of monocytes and macrophages. In Europe, L-MTP-PE is indicated for treatment of high-grade resectable non-metastatic osteosarcoma in children, adolescents, and young adults after macroscopically complete surgical resection in combination with postoperative chemotherapy. The recommended mifamurtide dose is 2 mg/m^2. This study aimed to characterize the pharmacokinetics (PK) and pharmacodynamics (PD) of single-dose L-MTP-PE, and evaluate effects on QTc interval in healthy adults.
Materials and Methods: Adults with normal baseline cardiac function and no risk factors for cardiac arrhythmias received a single 4 mg intravenous (IV) infusion of L-MTP-PE over 30 mins. Blood samples were collected pre-dose and serially post-dose for PK (serum MTP-PE) and PD (serum IL-6, TNF-α and CRP) measurements for noncompartmental data analysis. Continuous Holter ECG monitoring was performed over 48 hr, starting 24 hr pre-dose, to analyze changes in QTc (ΔQTc) relative to time-matched baseline values.
Results: 21 adults were enrolled (median age 31 years [range 20−58], 57% male, 71% African American). Maximum serum MTP-PE concentration (mean $\pm$ SD, 15.7$\pm$3.72 nM) was reached at the end of the infusion. Mean $\pm$ SD MTP-PE PK parameters were: clearance 3,409$\pm$928 mL/min (1,747$\pm$390 mL/min/m^2), terminal phase volume of distribution 589$\pm$138 L (305$\pm$69.9 L/m^2), steady-state volume of distribution 406$\pm$120 L

($212 \pm 66.0\ L/m^2$). Serum MTP-PE concentrations declined rapidly in a biphasic manner, with a mean terminal half-life of 2.05 ± 0.40 hrs. PK variability was low (%CV for $AUC_{(0-inf)}$ and C_{max} <30%). Serum IL-6 and TNF-α concentrations increased after dosing and peaked at 4 and 2 hrs, respectively, after infusion start. CRP was elevated in all subjects at 24 hr post-infusion. Heart rate increased following L-MTP-PE infusion with a mean maximum increase of ~31 bpm, sustained 4−8 hrs postdose. Mean changes from baseline in QTcF or QTcI were negative at all time points except 0.5 hrs. Maximum negative values for ΔQTcF and ΔQTcI were −25 and −22 msec, respectively, at 6 hrs postdose. Upper bounds of the 90% two-sided confidence intervals for ΔQTcF and ΔQTcI were <10 msec, with maximal values of 6.9 and 8.6 msec 0.5 hrs postdose. Most frequent adverse events (AEs) were headache (86%), chills (71%), tachycardia (67%), nausea (52%), and pyrexia (43%), all mild-to-moderate in severity. No serious AEs or deaths were reported.

Conclusions: The PK of IV L-MTP-PE was characterized by low variability and a short serum half-life of MTP-PE. PD effects included increases in serum concentrations of IL-6, TNF-α, and CRP. The ECG effects of L-MTP-PE infusion were characterized by an increase in heart rate without prolongation of QTc interval, supporting lack of a clinically relevant effect on cardiovascular repolarization.

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Neuropilin 2 expression on T CD4+ lymphocytes phenotypic study

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Background: The immune system is the only body protection against tumor development. There are 3 cell types involved: dendritic cells (DC), effector T cells and regulatory T cells. As cancer symptoms are detected, tumor immunosurveillance already failed. Neuropilins (Nrp)1 and 2 are transmembrane glycoproteins with no tyrosin kinase activity. They are involved in both axonal and vascular guidance. Nrp2 has been described on DC, but not T cells, unlike Nrp1. Nrp2 has ongenic properties. It enhances cell proliferation and survival through the Akt pathway. Nrp2 interacts with cytoskeleton connectors such as plexins. It may also be involved in TGFβ1 conversion. Those properties suggest Nrp2 as a potential interesting molecule regarding immunity. We were interested in Nrp2 expression on T cells and its involvement in anti-tumor immune response.

Material and method: We used C57Bl/6 ($H\text{-}2^b$) male mice for *in vivo* experiments and to get splenocytes, B16 cells that are human melanoma cells, and EL4 cells that are murin thymoma cells. Humain lymphocytes were obtained from healthy volunteers or humain blood cord. Anti-CD3/CD28 beads, conavaline A and PMA ionomycin were used to activate lymphocytes. Cellular sort kits provided sorted lymphocytes. Cells and molecules were identified thanks to flow cytometry and/or western blotting, and confocal microscopy. si RNA were used via transfection and transduction (lentiviral systems) to obtain no Nrp2 expressing or 100% Nrp2 expressing cells.

Results: We observed that blood cord resting CD4+CD25+ T cells express more Nrp2 than other resting T cells. It was also the case of activated T cells. Tumor environment effect on Nrp2 expression was assessed *in vivo*. Nrp2+ T cells were present in draining lymph node and within the tumor. To develop the previous observation, we cocultured murine splenic T cells with B16 or B16 supernatant. B16 supernatant, even diluted, was efficient enough to increase Nrp2 expression by T cells. In the next stage we used EL4 CD3+CD4+ tumor T cell line to assess Nrp2 repression and its consequences with siRNA technology. We first followed immunological synapse between T cells and DC. We demonstrated that DC-EL4 junctions and actin relocalisation were thiner if EL4 expressed Nrp2. Our next step will focus on hematopoïetic reconstitution with or without Nrp2, and tumor growth in Nrp2 T cells depleted mice. Nrp2 is implicated in TGFβ1 conversion on tumor cell lines, as suggested by its homology with Nrp1, and TGFβ1 ELISA tests are ongoing in different conditions of activated lymphocytes. Nrp2 PCR are ongoing to confirm those results too.

Conclusions: Finally, Nrp2 is an interesting molecule regarding T cells regulation, and anti-tumor immunosurveillance. Its implication in DC-EL4 junction, and possibly in TGFβ1 conversion could make Nrp2 a good tool for immunomodulation.

663 POSTER

Intratumoral and serum interleukin-4 levels in prostate adenocarcinoma

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Background: Interleukin-4 (IL-4) is a pleiotropic cytokine that has been implicated in the aetiopathogenesis of several cancers. Pre-clinical studies in prostate cancer (PC) strongly suggest that IL-4 plays a key role in transition to androgen independence. To test clinical correlation, we have analysed IL-4 levels in serum and in prostate tissue.

Material and Methods: Serum samples were taken from patients with radically-treatable ($n = 30$), androgen sensitive (AS; $n = 29$) and androgen resistant (AR; $n = 30$) PC. Control patients had confirmed benign prostatic hypertrophy ($n = 23$). Serum IL-4 was measured using an ultra-sensitive enzyme-linked immunosorbent assay in a single setting, to minimise inter-assay variation. Since data were not normally distributed, comparison was made using the Kruskal Wallis Test. Frozen blocks from 14 radical prostatectomies performed for PC were sectioned and stained using haematoxylin and eosin. Immunohistochemical (IHC) staining of validated 5μm sections was performed with rabbit anti-human IL-4 IgG, using an Envision™ System. Staining protocols were optimised using IL-4 transfected cells and tonsil. The study protocol was approved by the Guy's and St Thomas' Research Ethics committee.

Results: Median serum IL-4 concentrations were 0.16pg/ml for radical patients, 0.20pg/ml for AS patients, 0.32pg/ml for AR patients and 0.31pg/ml for benign patients. Serum IL-4 was significantly lower in radical patients compared to others combined ($p = 0.009$). Immunohistochemistry revealed greater intensity of IL-4 expression within malignant compared to benign prostate tissue (Figure). Furthermore, IL-4 staining was diffusely found throughout the cytoplasm in malignant epithelium but focally in the apical/peri-nuclear cytoplasm of benign luminal epithelium.

Conclusion: In our series (the largest reported to date), consistent differences in intensity and distribution of IHC staining between malignant and benign tissue were observed. However, these differences were not reflected in serum IL-4 levels, at variance with smaller published data sets. These data emphasize the importance of analysing low level and labile cytokines such as IL-4 in the primary site of disease and are consistent with a role for IL-4 in prostate cancer.

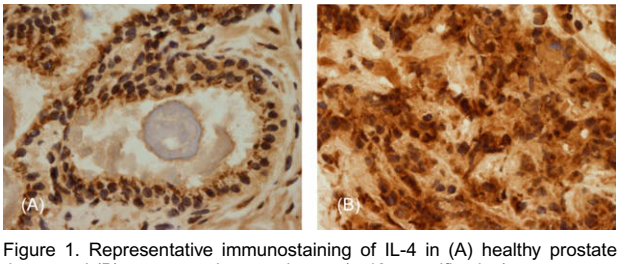

Figure 1. Representative immunostaining of IL-4 in (A) healthy prostate tissue and (B) prostate adenocarcinoma (×40 magnification).