VACCINIA VIRUS DELIVERED BY ISOLATED LIMB PERFUSION COMBINES WITH PD-1 BLOCKADE TO PREVENT LOCAL AND DISTANT RELAPSE IN SOFT-TISSUE SARCOMA

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Introduction Systemic relapse is the major cause of mortality in extremity soft tissue sarcoma (ESTS). Traditional systemic therapies for metastatic sarcoma have limited efficacy, as do novel agents such as immune checkpoint inhibitors (ICI). Oncolytic virotherapy is capable of improving the efficacy of ICI in some non-sarcomatous pathology. In a rat model of ESTS, we have previously found isolated limb perfusion (ILP) to be ideally suited to delivering a vaccinia virus (GLV-1h68), alongside melphalan and tumour necrosis factor-α (TNFα). Using this model, we sought to investigate the effects of GLV-1h68 delivered by ILP on the tumour microenvironment and determine whether viral ILP can sensitise sarcomas to subsequent immune checkpoint blockade.

Material and methods In vivo experiments were performed in Brown Norway rats bearing BN175 sarcoma in accordance with a Home Office animal license. Therapeutics included GLV-1h68, a PD-1 inhibitor (J43), melphalan and TNFα.

Results and discussions PD-1 inhibition had limited in vivo monotherapy efficacy in BN175 sarcoma. Pre-treatment with GLV-1h68 delivered by ILP prior to PD-1 blockade markedly improved therapy, with complete tumour regression in a third of animals. Without further treatment, resistant disease rapidly evolved leading to both local and distant relapse. However, when performed as a neoadjuvant treatment prior to surgery and radiotherapy, viral ILP and PD-1 blockade prevented local and distant relapse in all animals. Treatment with both GLV-1h68 and PD-1 blockade were found to be necessary for durable cure. In vitro, GLV-1h68 induced ATP, calreticulin and HMGB1, markers of immunogenic cell death. In vitro, viral ILP and PD-1 blockade significantly increased the number of intra-tumoural CD4+ and CD8+ effector cells, with an increased proportion of these cells expressing activation markers. Viral ILP and PD-1 blockade also altered the topography of intra-tumoural immune invasion; significantly increasing CD8+ cells within the tumour parenchyma relative to the invasive margin. The accumulation of effector cells within regional lymph nodes, located outside of the perfusion field, was also noted indicative of at least a locoregional, and perhaps, systemic immune-priming.

Conclusion Viral ILP and PD-1 blockade combine to prevent local and distant relapse in an animal model of high-grade ESTS. These data provide a strong rationale for clinical translation of this neoadjuvant combination immunotherapy.

PO-361 MODULATION OF CANCER CELL RESPONSE TO ETOPOSIDE BY M1 AND M2 POLARISED MACROPHAGES

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Introduction Tumour associated macrophages (TAMs) are an important part of the tumour microenvironment in most cancers. It has been shown that TAMs are mostly anti-inflammatory M2-like macrophages. It is clearly known that M2 macrophages in tumour can inhibit immune responses but the direct effect of these M2 macrophages on cancer cell response to chemotherapeutic drugs is still unknown.

Material and methods The aim on this work is to characterise for the first time the direct effect of TAMs on cancer cell chemoresistance. Here, we used THP-1 monocytes firstly differentiated into macrophages (M0) with phorbol 12-mystrate 13-acetate (PMA). Then, M0 macrophages were polarised in M1 or M2 macrophages using LPS and INFγ or IL-4 and IL-13 respectively. Using co-cultures with cancer cells, we investigated the effect of these polarised macrophages on the cancer cell resistance to etoposide.

Results and discussions The co-culture of macrophages and HepG2 cancer cells reveals that M2 macrophages decreased the etoposide-induced cell death whereas M1 macrophages increased the effects of chemotherapy. These results have been observed by western blots for cleaved Caspase-3 and cleaved PARP-1 and by caspase-3 activity assay in co-culture or by using M2 macrophage conditioned medium. Moreover, a slight chemoresistance was observed in co-culture with unpolarized M0 macrophages. Further investigations revealed that HepG2 cells induced a M0 to M2 polarisation, which seemed to be at the origin of this resistance. The resistance seems to occur by a decrease in DNA damage induction. Furthermore, we showed that M2 conditioned medium increased the expression of cancer stem cell markers by HepG2 cells.

Conclusion Taken together, these results show a direct protective effect of M2 macrophages on etoposide induced cell death of HepG2 cancer cells.

PO-362 RATIONAL DESIGNING COMBINATORIAL T-CELL BASED IMMUNOTHERAPY BY HIGH-DIMENSIONAL PROFILING

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Introduction Clinical benefit of immunotherapeutic approaches against cancer has been well established. However, the benefit of cancer patients was mostly noted as prolonged survival. The lack of full cancer eradication is linked to immunosuppressive and immune escape mechanisms. Combination immunotherapy to improve the efficacy and duration of the tumour-specific T cell response offers an attractive avenue to develop more effective cancer therapies.

Material and methods Here we aimed to decipher the mechanisms governing the PD-1/PD-L1 checkpoint blockade to rationally design combination immunotherapy to improve immunotherapeutic benefit. We established high-dimensional...
immune signatures of immunotherapy-specific of cell subsets utilising mass cytometry with 38 markers.

Results and discussions PD-L1 blockade induced the expression of highly specific tumor-infiltrating CD4 and CD8 T cells, displaying both activating (ICOS) and inhibitory (PD-1, LAG-3) molecules. Expansion of these therapy-induced T cell subsets was observed three days after treatment and significantly expanded in time leading to tumour delay. By targeting the activating and inhibiting molecules on the T cells by agonistic and blocking antibodies, respectively, we were able to further restore the T cell dysfunction and thereby improving the therapeutic benefit of single immunotherapy.

Conclusion Thus, high-dimensional profiling is a powerful means for rational designing combinatorial T-cell based immunotherapies.

PO-363 ABSTRACT WITHDRAWN

PO-364 EFFECT OF EXERCISE AND IMMUNOTHERAPY ON TUMOUR IMMUNOGENICITY AND GROWTH

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Introduction Several preclinical studies have shown that exercise can reduce the tumour growth across a range of tumour models. We recently demonstrated that this exercise-dependent suppression of tumour growth was due to an epinephrine-dependent mobilisation and IL-6-driven activation of cytotoxic cells. In line with this, exercise markedly increased intratumoral immune cell infiltration, thus promoting an immunostimulatory intratumoral environment. Based on these original findings, we hypothesised that exercise may enhance the effect of immune checkpoint blockade treatment given the exercise-dependent increased intratumoral immune cell infiltration.

Material and methods We used C57Black/6 mice randomised to cages with or without running wheels (EX). After 4 weeks of running (with a running distance ranging from 1.8 km to 5.4 km per mice per day), the mice were inoculated with B16 melanoma cancer cells. In addition, a subset of mice was treated with αPD-L1 antibodies (100 μg per mouse three times per week starting on day 4 after tumour cell injection).

Results and discussions First, we observed that voluntary wheel running increased intratumoral expression of the immune checkpoint molecules, PD-L1, B7.1 and B7.2. In parallel, voluntary wheel running increased intratumoral infiltration of cytotoxic NK and T cells, and thus intratumoral expression of the receptors, PD-1 and CD28. Next, we tested the combination therapy of exercise and αPD-L1 antibody treatment. In this setup, wheel running had an overall suppressive effect (p=0.01, 2-way ANOVA). Post hoc analysis showed that αPD-L1 +EX reduced tumour growth by 82.6%, while EX alone reduced tumour growth by 72% with no significant difference between the two interventions. qPCR analysis of these tumours revealed that combination therapy further increased the expression of immune cell markers and immune checkpoint molecules.

Conclusion In conclusion, our results show that exercise increases the expression of immune checkpoint markers and therefore, could be a good combination partner for immune checkpoint blockade treatment. However, more studies are needed to show an effect of this combination therapy on tumour growth.

PO-365 DISSECTING THE SYNERGISTIC EFFECT OF CHEMOTHERAPY AND IMMUNOTHERAPY ON ANTITUMORAL T CELL FUNCTIONS IN BREAST CANCER.

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Introduction Immunotherapies that target immune checkpoint molecules proved effective for the treatment of several solid tumours. However, the majority of breast cancer patients does not respond to this kind of approach, underlining the need of new strategies able to elicit an effective anti-tumoral immune response. The combination of conventional and immuno-based therapies represents a promising strategy to elicit such response. Our aim is to evaluate the efficacy of immunotherapy and chemotherapy combination in breast cancer, to dissect its effect on the endogenous anti-tumoral cytotoxic CD8 +T cells, and finally to assess the impact of a spontaneously developing tumour on T-cell biology.

Material and methods The experiments are performed in K14cre;Cdh1fl/fl;Trp53f/f (KEP) mice, a spontaneous breast tumour model that closely resembles human invasive lobular carcinoma. Mice are treated with α-CTLA-4 and α-PD-1 antibodies, as a single treatment modality or in combination with MTD dosing Cisplatin or docetaxel. Therapy is initiated when the tumour has reached 50mm² and continues until it reached 225mm² or after a pre-defined time point, depending on the experiment.

Results and discussions Mammary tumours in KEP mice show an altered T-cell balance compared to tumour-free mammary glands, with a reduced infiltration of CD8+ T cells and conventional CD4+ T cells, and higher percentage of T regulatory cells. Tumor-infiltrating CD8+ T cells (TILs) have an increased expression of multiple inhibitory receptors, and reduced IFNγ production, compared to peripheral CD8+T cells of KEP mice. In vitro exposure of sorted CD8+ TILs to IL-15 or IL-2 augments their capacity to produce IFNγ, indicating that their suppressed state is reversible. Treatment of tumor-bearing KEP mice with a-CTLA-4 and a-PD-1 does not affect the tumour growth, but a synergistic therapeutic benefit is observed when α-CTLA-4 and α-PD-1 are combined with cisplatin, and not docetaxel, in a CD8+ T-cell dependent mechanism.

Conclusion CD8+ TILs display some features of dysfunctional T cells. However, ex vivo cytokine stimulation can restore part of their effector functions, indicating that they are not terminally dysfunctional. Nonetheless, only the combination with chemotherapy and immunotherapy is able to elicit an efficient CD8+ T cell response, in a drug-dependent manner. We are currently dissecting the mechanisms underlying the synergistic therapy response, in order to ultimately contribute to the rational design of new immunomodulatory treatment strategies for breast cancer.