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 expansion 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.

XAV939-MEDIATED INHIBITION OF WNT/β-CATENIN SIGNALLING IN BOTH LNCAP PROSTATE CANCER CELLS AND PROSTATE CANCER PATIENT’S LYMPHOCYTES ENABLES A SUSTAINED ELIMINATION OF LNCAP CELLS BY THE LYMPHOCYTES

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Introduction Wnt/β-catenin signalling plays an important role in several cancers and regulation of immune cell development. Whereas in cancer cells its upregulation is associated with increased cancer cell resistance, in immune cells it is associated with either immunosuppression or immunostimulation. What impact an inhibition of this signalling may have on immune cell-mediated elimination of cancer cells is therefore largely unknown.

Material and methods Inhibition of Wnt/β-catenin in LNCaP prostate cancer (PCa) cell line and lymphocytes was determined by β-catenin translocation to the nucleus using Image-stream. LNCaP cells were stably transfected with red fluorescent protein TagFP635 to monitor their numbers. LNCaP cells and lymphocytes from 12 patients with biochemically recurrent PCa were pretreated for 2 days with Wnt/β-catenin inhibitor XAV939. The cells were then mixed at a ratio 1:4 (LNCaP: lymphocytes) and co-cultured for 5 days.

The co-cultures were then transferred to fresh LNCaP cells and co-cultured for additional 10 days. The numbers of LNCaP cells were evaluated by fluorescence microscopy.

Results and discussions We determined that 5 µM XAV939 did not affect proliferation of LNCaP cells but yet still inhibited the signalling of both LNCaP cells and the lymphocytes by 50% as determined by the inhibition of β-catenin translocation to the nucleus. When co-culturing LNCaP cells with the lymphocytes, we revealed that 2 day pre-treatment of the lymphocytes with 5 µM XAV939 accelerated elimination of LNCaP cells by 54% after 3 day co-culturing. No significant acceleration was observed when LNCaP cells were pretreated with the inhibitor or the inhibitor was present in the co-cultures. Following a transfer of 5 day co-cultures to fresh LNCaP cells, we found that, regardless of the XAV939 pretreatment, the lymphocyte-mediated elimination of LNCaP cells ceased and only their expansion was abrogated. However, the co-cultures supplemented with XAV939 still showed a sustained elimination of LNCaP cells leading to elimination of 86% of LNCaP cells in 10 days. In addition, this elimination was associated with 61% decrease of PD1+CD8+ T cell population in the co-culture.

Conclusion Data in this study indicate that whereas a short-term inhibition of Wnt/β-catenin in lymphocytes licenses these cells to mediate an accelerated elimination of cancer cells, a continuous inhibition of the signalling in both the lymphocytes and the cancer cells is necessary for sustained lymphocyte-mediated cancer cell elimination.

IMMUNE RECOGNITION OF TUMOURS BY VOLUNTARY WHEEL RUNNING MAY BE TRIGGERED BY INDUCTION OF ZBP1

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Introduction Epidemiological studies have shown that regular exercise reduces the risk of developing cancer; however, only a few studies have addressed the mechanistic effects of exercise on cancer-related outcomes. We recently demonstrated that exercise leads to reduced tumour growth due to a mobilisation of cytotoxic immune cells dependent on epinephrine and IL-6 in murine tumour models. Nonetheless, the signalling pathways initiating this exercise-dependent immune-mediated tumour growth inhibition remain to be elucidated.

Material and methods We randomly assigned female C57BL/6 mice to cages with or without running wheels as a model of voluntary exercise. After 4 weeks of voluntary exercise, the mice were subcutaneously inoculated with B16F10 melanoma cells. Proteomic and microarray analysis were carried out on the tumours to identify a differential regulation of signalling pathways in exercised mice. In parallel, tumour-free female BALB/c mice were subjected to 1 hour of swimming as a model of acute exercise, and we evaluated gene expression by qPCR in several tissues.

Results and discussions Proteomic and microarray analysis uncovered a possible player in exercise-mediated induction of immune recognition, namely Zbp1 (Z-DNA Binding Protein 1). This protein is a cytosolic DNA sensor that via IRF3 (Interferon Regulatory Factor 3) and NF-kB initiates...
necroptosis, as well as the innate immunity via type I interferons and cytokines.

Next, we validated Zbp1 expression in B16 tumours from mice randomised to wheel running or not, and showed increased Zbp1 expression in tumours from exercising mice. This increased expression strongly correlates with enhanced cytotoxic immune cell infiltration within the tumour (CD68 and NKG2D). As Zbp1 has not been previously linked to exercise performance, we investigated if acute exercise had any effect on Zbp1 expression. We evaluated Zbp1 expression in 8 different tissues of the mice subjected to 1 hour of swimming and found that Zbp1 expression increased by 85% in visceral fat. Additionally, 2 hours into the recovery phase Zbp1 was suppressed in liver by 63% and in quadriceps muscle by 70%.

Conclusion Exercising mice inoculated with B16F10 cells showed an upregulation of the innate immunity activator Zbp1, which correlated with increased cytotoxic immune cell infiltration. Moreover, acute exercise showed to regulate Zbp1 in a tissue dependent manner. These data suggest that initial exercise-dependent induction of Zbp1 could be involved in eliciting an innate immune response to tumours during exercise.

PO-378 EXERCISE-DEPENDENT REGULATION OF IMMUNE CELL FUNCTION IN A CLINICAL CANCER PERSPECTIVE
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Introduction Exercise is used as supportive therapy for cancer patients, aiming to improve patients’ physical functioning, quality-of-life and cancer-related fatigue. Emerging evidence suggests that exercise may also have direct anti-oncogenic effects on disease progression. Yet, in order to pursue exercise as cancer medicine, detailed insight into the underlying mechanisms is warranted. In murine cancer models immune cell mobilisation leads to control of tumour growth through increased infiltration by immune cells in the tumour tissue in a manner dependent on release of adrenalin and IL-6. To further explore these mechanisms in humans, we studied the NK cell mobilisation and characteristics in healthy subjects or cancer patients undergoing neo-adjuvant chemotherapy.

Material and methods Blood immune cell profiles were evaluated in 5 healthy subjects performing either 2 hours of interval-based cycling or 2 hours of cycling at constant Watt intensity, and in 4 patients with cancer of the gastro-esophageal junction (GEJ) performing an acute bout of interval-biking following by resistance training. Blood was collected before training, after warm-up, during training, and after a resting period. The samples were analysed by flow cytometry staining for CD3, CD19, CD14, CD16, CD56, and HLA-DR for differentiation of T cells, B cells, NK cells, and monocytes. The abundance of Granzyme B and Ki67 reflected cytotoxic profile and proliferation status, respectively.

Results and discussions In healthy subjects, we observed a rapid up to 4-fold mobilisation of NK cells to the circulation, as well as increased Granzyme B content, decreased Ki-67 expression, and increased cellular size in the mobilised NK cells. These findings were particularly evident with interval-based training. In the 4 GEJ cancer patients we found up to 8-fold increase in circulating NK-cells after 35 min of interval-based cycling, along with increases in Granzyme B abundance and decreased Ki67 expression.

Conclusion An acute bout of interval-based exercise mediates recruitment of cytotoxic NK cells to the circulation in both healthy young men and GEJ cancer patients undergoing neo-adjuvant chemotherapy. Furthermore, immune cell mobilisation in the cancer patients was comparable to (if not better than) the recruitment observed in healthy subjects.