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Title:

NOVEL COMBINATION IMMUNOTHERAPY FOR BOOSTING AND PRIMING IMMUNE RESPONSES IN PANCREATIC CANCER: STRONG ANTI-TUMOUR EFFECTS WITH INTERLEUKIN-15 AND CD40 AGONIST TREATMENT

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interferon genes agonists (STINGa) were shown to induce a potent type I interferon response in preclinical studies. The intratumoral administration of STINGa, to promote tumor inflammation, was shown to result in a protective spontaneous immune response in several murine tumor models. However, the encouraging preclinical results are not supported by recent clinical data, challenging the efficacy of unspecific monotherapy. As it is more and more clear that an effective cancer immunotherapy will require the combination of different treatment strategies, we investigate here the efficacy of combining KISIMA™ cancer vaccine with STINGa treatment.

Methods Mice were vaccinated with subcutaneous (s.c.) injection of KISIMA™ vaccine combined with s.c. administration of STINGa. Safety and immunogenicity were assessed by measuring temperature, serum cytokines and the peripheral antigen-specific response. Anti-tumoral efficacy as well as in depth monitoring of TILs and tumor microenvironment modulation were assessed following therapeutic vaccination in a HPV16 E6 and E7 expressing TC-1 cold tumor model.

Results Combination treatment was well tolerated and promoted the development of circulating antigen-specific CD8 T cells. In TC-1 tumor bearing mice, KISIMA™ therapeutic vaccination resulted in the infiltration of both antigen-specific CD8 and CD4 T cells within the tumor, as well as a switch of tumor associated macrophages polarization toward the more inflammatory type 1. Combination therapy further increased the tumor microenvironment modulation induced by KISIMA™ vaccine, promoting the polarization of inflammatory Thelper 1 CD4 T cells and increasing the effector function of antigen-specific CD8 T cells. The profound modulation of the tumor microenvironment induced by combination therapy enhanced the beneficial effect of KISIMA™ vaccination, resulting in a prolonged tumor control.

Conclusions Combination of KISIMA™ cancer vaccine with systemic STINGa treatment induces the development of a potent, tumor-specific immune response resulting in a profound modulation of the TME. As check-point inhibitor (CPI) therapy is ineffective on poorly infiltrated tumors, combination with therapies able to highly enhance tumor infiltration by T cells could expand CPI indications.

Ethics Approval The study was approved by the Canton of Geneva Ethic Board, under the license number GE165/19

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NOVEL COMBINATION IMMUNOTHERAPY FOR BOOSTING AND PRIMING IMMUNE RESPONSES IN PANCREATIC CANCER: STRONG ANTI-TUMOUR EFFECTS WITH INTERLEUKIN-15 AND CD40 AGONIST TREATMENT

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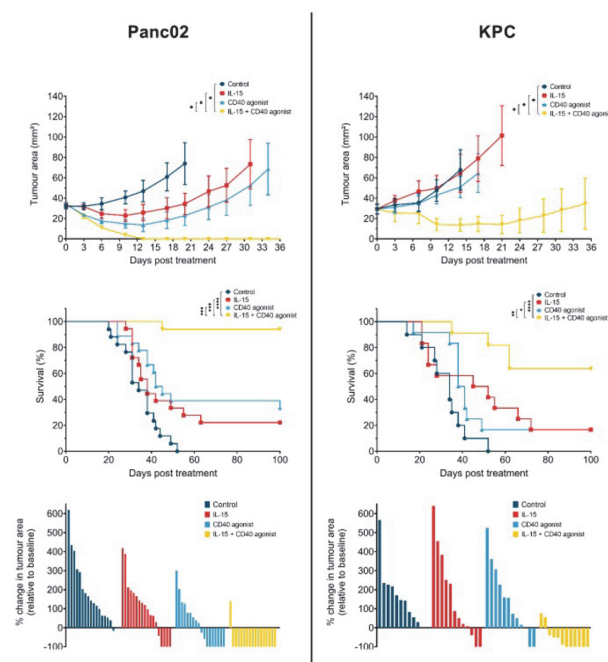
Background With the poorest 5-year survival of all cancers, improving treatment for pancreatic cancer is one of the biggest challenges in cancer research. In this era of combination

immunotherapies, we sought to explore the potential of combining both priming and activation of the immune system. To achieve this, we combined a CD40 agonist with interleukin-15 and tested its potential in pancreatic cancer.

Methods Two different mouse models of pancreatic cancer were used to assess the potential of this combination regimen. Therefore, effects on tumour growth kinetics and survival were charted. Differential effects on immune signatures was investigated using RNA sequencing. Functional immune subset involvement was tested using different immune depletion experiments and multicolour flow cytometry in different relevant immune sites. Immune memory was checked using re-challenge experiments.

Results We demonstrated profound reduction in tumour growth and increased survival of mice with the majority of mice being cured when both agents were combined, including an unprecedented dose reduction of CD40 agonist without losing any efficacy (fig 1). RNA sequencing analysis showed involvement of natural killer cell and T cell mediated anti-tumour responses and the importance of antigen-presenting cell pathways. This combination resulted in enhanced infiltration of tumours by both cytotoxic T cells and natural killer cells, as well as a striking increase in the ratio of CD8+ T cells over T regulatory cells. We also observed a significant increase in numbers of dendritic cells in tumour draining lymph nodes, particularly CD103+ dendritic cells with cross-presentation potential. A critical role for CD8+ T cells and involvement of natural killer cells in the anti-tumour effect was highlighted. Importantly, strong immune memory was established, with an increase in memory CD8+ T cells only when both interleukin-15 and the CD40 agonist were combined.

Conclusions We demonstrated profound synergistic anti-tumour effects upon combination of CD40 agonist and interleukin-15 treatment in mouse models of pancreatic cancer. This preclinical data supports initiation of a first-in-human clinical trial



Abstract 453 Figure 1 Tumour kinetics and survival in Panc02 (left) and KPC (right) pancreatic cancer mouse models

with this combination immunotherapy strategy in pancreatic cancer.

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454 ONCOLYTIC PARAINFLUENZA VIRUS 5 VECTOR ENHANCES NATURAL KILLER CELL KILLING OF LUNG TUMOR CELLS IN 2D AND 3D SPHEROID CULTURES

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Background Natural killer (NK) cells are innate immune cells with natural cytotoxicity towards both tumor cells and virus infected cells. We have developed a particle-based method for in vitro specific expansion of NK cells that yields highly cytotoxic NK cells (PM21-NK cells). There is intense interest in the use of novel oncolytic viruses with the potential to synergize with immune cells to kill tumor cells. Here we have tested the hypothesis that infection with a tumor-selective cytopathic Parainfluenza virus 5 (PIV5-P/V) vector will enhance PM21-NK cell-mediated killing of lung cancer cells in both 2-dimensional (2D) and 3-dimensional (3D) cultures.

Methods In 2D cultures, live cell time-lapse imaging, flow cytometry and luminescence-based methods were used to assess the killing efficiency of PM21-NK cells against A549 lung tumor cells infected with PIV5-P/V. Blocking antibodies were used to evaluate different NK cell activating receptors involved in recognition of infected tumor cells. IncuCyte live cell imaging system was used to assess real time killing of 3D lung spheroids by a combination of NK cells and PIV5-P/V virus. Z-stack spheroid images were captured using Keyence microscope.

Results In 2D cultures, PM21 NK cells efficiently kill A549 cells that have been infected with P/V CPI- virus and enhance the overall rate of killing compared to uninfected cell targets. Antibody blocking showed that the viral Hemagglutinin-Neuraminidase (HN) glycoprotein and NK cell receptors NKp30, NKp46 and NKG2D were involved in PM21-NK cell recognition of PIV5-P/V infected A549 cells. In 3D cultures of A549 tumor spheroids, PIV5-P/V infection was limited to the outer layer of the spheroid, with restricted spread of the infection to inner compartments. However, addition of PM21-NK cells to PIV5-P/V-infected spheroids resulted in killing of not only the infected surface of the spheroid but continued to the uninfected cells located at the center of the spheroid.

Conclusions Our data support the potential of combining oncolytic virotherapy along with PM21-NK cell adoptive therapy against lung cancer.

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455 IMPACT OF EPHB4 AND PD-1 TREATMENT ON IMMUNE INFILTRATE IN ADVANCED BLADDER CANCER

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Background Bladder cancer is the fourth most common cancer in American men with chances of 1 in 27 developing this form of cancer. Despite the progress in treating these patients with immunomodulatory agents, the vast majority of patients remain refractory to therapeutic intervention. EphB4 and

EphrinB2 are induced in the tumor vasculature and modulate immune response within the tumor microenvironment. Intervention blocking Ephrin and PD-1/PD-L1 pathway has shown promising data in preclinical models. These data form the basis of clinical investigation of combined therapy in bladder cancer and other tumor types.

Methods Preclinical mouse models were treated with decoy soluble EphB4 and tumor infiltrating immune cells were profiled by RNA expression analysis post-treatment and compared to control treated mice. Next, patients were treated with soluble Eph4B in combination with anti-PD1 therapy, biopsies were obtained prior to and during the course of treatment. Biopsies were used for analysis of localized protein and RNA expression by GeoMx Digital Spatial Profiling (DSP). DSP analysis focused on tumor rich regions of interest (ROIs), adjacent stromal immune populations and microniches around vascular sites, with emphasis on sites where CD45+ T-cells were observed to be surrounding capillaries within and surrounding the tumor, presumably from extravasation.

Results In preclinical mouse models, EphB4 was found to induce several inflammatory pathways as a monotherapy including key immunomodulatory checkpoints such as PD1, PDL1, PDL2. Similarly, patients enrolled in this study were observed to have elevated T-cell infiltration in primary and secondary tumor sites, resulting in tumor mass reduction in post-treatment observations. DSP between matched samples discovered interesting differences in T-cell populations between both protein and mRNA expression. We observed evidence of tumor-debulking by decreased expression of epithelial markers such as Pan-cytokeratin and S100B within tumor ROIs, and increased infiltration within these ROIs measured by immune cell markers such as CD3 and CD163. Additionally, we observed increased GZMA expression post-treatment in perivascular regions suggestive of higher ongoing response by cells entering the tumor microenvironment. Additional analysis of localized RNA expression provided further support for activation of inflammatory cascades in post-treatment samples.

Conclusions These discoveries provide insights into the mechanism of action of EphB4 combination therapy in bladder cancer, providing support for a role of EphB4 acting as an adjuvant for PD1 therapy. Our results highlight the ability of EphB4 to activate the immune system both in preclinical models and in key structures within the tumor microenvironment during combination therapy.

Trial Registration NA

Ethics Approval The studies were approved by USC IRB Protocol 4B 15-11 and IACUC Protocol 20570.

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456 IMPACT OF ANGIOTENSIN II PATHWAY INHIBITION ON TUMOR RESPONSE TO ANTI PD(L)1 BASED THERAPY

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Background Angiotensin II (Ang II) has been shown preclinically to increase VEGF and TGF- β expression through AT1 receptor signaling but to decrease VEGF and TGF- β through AT2. Thus, we hypothesized that the ang II pathway might have a role in carcinogenesis and immune evasion and selectively inhibiting AT1 via angiotensin receptor blockers (ARBs)