**Introduction**

Metabolic reprogramming is one of the hallmarks of cancer, to support their needs for massive growth and proliferation. One major metabolic reprogramming is from oxidative phosphorylation to aerobic glycolysis, a well-documented phenomenon known as the Warburg effect. A key enzyme in this process is hexokinase 2 (HK2), which catalyses the first step of glucose metabolism and is overexpressed in many cancer types. Unlike HK1, which is ubiquitously expressed in normal cells, HK2 is required for cancer initiation and transformation even though their catalytic activity is highly similar. HK2 in cancer cells is attached to the outer mitochondrial membrane via the VDAC1 channel. VDAC1/HK2 association blocks pro-apoptotic signals, is less sensitive to feedback inhibition by the HK product, glucose-6-phosphate, as well as allows a continuous flux of mitochondrial ATP to HK, leading to apoptosis prevention and a high rate of glycolysis.

Temporal high HK2 expression, and binding to VDAC, is also found in a variety of activated immune cells to support their changing metabolic needs. Detachment of HK2 from VDAC1 in activated immune cells leads to a range of responses ranging from glycolysis inhibition, NLRP3-mediated inflammasome activation, and metabolic reprogramming to activate immune pathways.

**Material and methods**

A novel small molecule VDAC/HK2 modulator, VDA-1102, is being developed as a bi-functional drug for the treatment of solid tumours – triggering apoptosis in cancer cells while simultaneously enhancing an immune-mediated anti-tumour response by regulating immune cell metabolism.

**Results and discussions**

*In vitro* studies established that VDA-1102 selectively detaches HK2, but not HK1, from VDAC1 leading to cancer cell apoptosis, glycolysis inhibition, and prevention of cancer cell proliferation. *In vivo* efficacy studies demonstrated significant tumour growth delay and prolongation of survival in syngeneic solid tumour models. Analysis of tumor-associated macrophages *in vivo* indicated a treatment-induced change in these macrophage phenotype from M2 to M1.

**Conclusion**

This data supports the notion that VDA-1102 is a bi-functional drug that targets both cancer and the innate immune system. In cancer cells it induces apoptosis, whereas in macrophages it stimulates an anti-tumour immune response. Our findings support further development of VDA-1102 to evaluate its potential as an anti-cancer therapy, either as a monotherapy or in combination with checkpoint inhibitors in high HK2-expressing solid tumours.
Establishment of KRAS (G12D)/TRP53 NULL/PDX1-CRE (KPC) Mouse Homograft Tumour Models to Facilitate Preclinical Efficacy Evaluation of Combinatory Immunotherapies

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Introduction Pancreatic ductal adenocarcinoma (PDAC) has a poor 5 year survival rate of 6% and remains one of the most difficult to treat cancer types, with very few standard of care options. Fueled by the clinical success of immune checkpoint inhibitors in other solid tumours, several clinical trials have been conducted in PDAC patients. Unfortunately, these attempts have achieved limited clinical benefit as single agent treatments. Now investigators are eager to test combinatorial regimens of chemotherapies, small molecule immunomodulators, and immune checkpoint antibodies. Therefore, highly relevant preclinical models are much needed for proof of principle efficacy evaluation studies.

KPC models, first described by Taiwesn et al. (Cancer Res 2016;76:242–47) such as LSL-Kras<sup>G12D/+</sup>, LSL-Trp53<sup>R172H/+</sup>, Pdx-Cre, and later established as a variety of derivatives with Trp53 heterozygous or homozygous knockouts, recapitulate human PDAC tumours in many aspects including: morphological small ductal tumours with enriched stromal contents, key features of the PDAC immune microenvironment representing robust inflammatory reactions, e.g. high levels of B cells and macrophages, and exclusion of effector T cells. Most importantly, no response to a series of chemotherapies, including cytokine releasing, cell proliferation and specific target cell cytotoxicity. A human cancer xenograft animal model was used to evaluate the efficacy of these CAR-T cells.

Results and discussions T cells expressing the VEGFR-2 CARs mediated antigen-specific immune responses as revealed by IFN-γ production, cell proliferation and target cell lysis. A single dose of VEGFR-2 antigen-redirected T cells was potent in inhibiting the well-established and vascularized breast tumour growth.

Conclusion These VEGFR2 CAR-T cells exhibited the antigen-specific immune responses and the MHC-independent cell killing of target cells. This approach may provide an opportunity for the cellular gene therapy of various solid tumours.

Radiotherapy, Immunocytokines and Immune Checkpoint Inhibitors: Finding the Optimal Combination

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Introduction Although immunotherapy is currently changing cancer treatment practice, primary resistance is still seen partly due to insufficient immunogenic priming. Radiotherapy (RT) induces the release of tumour antigens and thereby increases tumour immunogenicity. Therefore, combination of these therapies has high potential. Here, we investigated the therapeutic outcome of combining a single dose RT with either immunocytokines (L19-IL2) or immune checkpoints inhibitors (ICIs) (aCTLA-4, aPD-L1 and aPD-1), aiming to further activate and/or prolong the RT-elicited immune response.

Material and methods Balb/c or C57BL/6 mice were injected in the right flank with either C51 and CT26 colon carcinoma or Lewis lung carcinoma (LLC) cells. Upon an average volume of 200mm³, animals were randomised in different treatment groups: RT +vehicle/L19-IL2 +IgG or RT +vehicle/L19-IL2 +aPD-L1/aPD-1/aCTLA-4. Tumours were irradiated with 5Gy (C51 and CT26) or 10Gy (LLC), as the latter is known to be poorly immunogenic. Vehicle/L19-IL2 (1 mg/kg), aCTLA-4 and IgG (both 10 mg/kg) were given i.v. on day 1, 3 and 5 after RT, aPD-1, aPD-L1 and IgG (all 10 mg/kg) were given i.p. 1, 3, 5, 7 and 9 days after RT. Blood was collected before and after treatment for immune-profiling. Tumour response was quantified as time to reach 4 times starting tumour volume (T4xSV).

Results and discussions In the CT26 model, RT +L19-IL2, RT +aCTLA-4 and RT +aPD L1 resulted in higher T4xSV compared to RT only (p<0.001, p<0.01 and p<0.05). In the C51 model solely RT +L19-IL2 was better (p<0.001) compared with RT only. RT +L19-IL2 efficacy was significantly (p<0.001) better compared to RT +ICIs in the C51, but not in the CT26 and LLC models. Adding ICIs to 5Gy+L19-IL2 did not result in improved outcome in either of the two models. Conversely, in the LLC model, the triple combination of RT +L19-IL2+aPD L1 yielded a better outcome compared to RT +L19-IL2 (p<0.05) and RT +aPD L1 (p<0.001). Similar results were observed for aCTLA-4 and aPD-1. Overall, we found an IC1-driven increase and decrease of circulating CD4 and CD8 +T cells respectively and an increase of PD-L1 associated with L19-IL2 therapy in all three models.

Conclusion Our results show a larger therapeutic effect when RT is combined with L19-IL2 as compared to ICIs in the majority of the models, yet trimodal therapy was also beneficial in one model.