PO-422 DEVELOPMENT OF AN ALK LYMPHOMA-DERIVED AUTOPHAGOSOMAL AND DENDRITIC CELLS VACCINE.
1D Sorrentino*, 2R Chaire, 3S Manenti, 4S Giuriato. 1Cancer Research Center of Toulouse CRCT, Haute-garonne, Toulouse, France; 2Harvard Medical School, Department of Pathology- Children’s Hospital Boston, Boston, USA; 3Cancer Research Center of Toulouse CRCT, Haute Garonne, Toulouse, France

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Introduction ALK-positive Anaplastic Large Cell Lymphoma (ALCCL) accounts for 10% to 15% of paediatric lymphomas. Their current treatments, based on chemotherapy or targeted therapy, are not optimal since relapses are invariably observed in 30% of the cases. Facing these therapeutic failures, the development of ALK lymphoma immune-therapies represents a promising research field. Indeed, the ALK oncogene has been proposed as an effective antigen for vaccination.

Autophagy, a process of cell self-digestion, has important roles in immunity, notably through its participation in antigen processing and presentation through both MHC I and II molecules. Therefore, our project aims at isolating pure autophagosomes from ALK +lymphoma cells, to incubate them with dendritic cells specialised for cross-presentation, and to use this preparation as a vaccine to stimulate anti-ALK immune responses.

Material and methods
1. Isolation of autophagosomes from murine ALK +tumour cells.

The cell line (VAC) was treated with Chloroquine a well-known blocker of Autophagosome-Lysosome fusion. The goal of the treatment is to keep intact the autophagosomal fraction (AF). The AF purity will then be assessed by western-blot and by electron microscopy.

2. Production of dendritic cells (DC) specialised for crosspresentation.

After extracting the mice bone marrow, we decided to use mouse FLT3 ligand to generate preferentially CD8 alpha like DC cells, which are the most prone for antigen crosspresentation.

3. In-vitro immunological assays to assess the effective ability of the DCs to crosspresent ALK tumour specific antigens (in progress).

4. In-vivo immunological assays to evaluate the efficiency of the vaccine (in progress).

Results and discussions
1. Our preliminary results indicate that an autophagosome rich fraction (as assessed by LC3-II and p62 enrichment) could be recovered from the tumour cell culture medium since autophagosome exocytosis has been reported. Of utmost importance, NPM-ALK could be detected in this fraction. However, we are concerned by the quantity and also the purity of this ‘autophagosome preparation’ and we expect to improve this step using cells (VAC) homogeneous lysates, followed by centrifugation on a Nycodenz gradient.

2. Our preliminary results are encouraging but the percentage of CD8 DC cells need to be improved.

Conclusion The use of autophagosomes for a new formulation of anti-ALK vaccine may represent new weapons to improve the therapy of ALK lymphoma, and possibly other ALK oncogene-associated cancers.

PO-423 INVESTIGATION OF COMBINED IMMUNE CHECKPOINT BLOCKADE IN HUMAN MALIGNANT PLEURAL MESOTHELIOMA
1E Marco*, 2D De Vaele, 3IRM Van Audenaerde, 4J Jacobs, 5J Van Loenhout, 6P Pauwels, 7IP Van Meerbeeck, 8ELJ Smits. 1University of Antwerp, Center for Oncological Research CORE, Antwerp, Belgium; 2Antwerp University Hospital, Department of Pathology, Antwerp, Belgium; 3Antwerp University Hospital, Thoracic Oncology/MOCA, Antwerp University Hospital, Belgium

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Introduction Till today, human malignant pleural mesothelioma (MPM) remains an aggressive cancer with a poor prognosis due to the limited impact on overall survival of the current treatments. Data from us and others about the presence of the immune checkpoint-related molecules PD-1, PD-L1, TIM-3 and LAG-3 in MPM lay the basis to evaluate their suitability as immunotherapeutic targets. Two clinical trials that investigated PD-1 and PD-L1 inhibition in mesothelioma (KEYNOTE-28, JAVELIN trial) have shown promising results with room for improvement. It is of great interest to investigate the effect of combined treatments and compare them to stand-alone treatment to select the best therapeutic strategy for MPM.

Material and methods Human cell lines representative for the epithelioid (NCI-H2818 and NCI-H2795) and sarcomatoid (NCI-H2731) subtypes of MPM were placed in allogeneic co-cultures with healthy donor peripheral blood mononuclear cells. The co-cultures were treated with the following immune checkpoint blocking antibodies: anti PD-1 (Nivolumab , BMS) or anti PD-L1 (Durvalumab , AstraZeneca) in combination with anti TIM-3 or anti LAG-3. Supernatant was collected and enzyme-linked immunosorbent assays and multiplex electro-chemo-luminescence were used to look at the secretion of 7 cytokines, being IFNg, IL-2/5/6/10, IL-1b and TNF-a, as well as the enzyme granzyme B. Statistical analysis was done to investigate the differences between the treatment conditions.

Results and discussions Treatment with immune checkpoint blockers as monotherapy or in combination resulted in a significant increase in the secretion of granzyme B and the cytokines IFNg, IL-2, IL-5 and IL-10. Although the increased secretion was not always statistically significant for all 3 MPM cell lines of the two subtypes, the same trends were observed among them. Interestingly, highest concentrations of granzyme B and these 4 cytokines were noticed for monotherapy treatment with anti PD-1, anti PD-L1 or either of these antibodies with anti TIM-3. In vivo investigation of PD-1 or PD-L1 blockade in combination with TIM-3 or LAG-3 blockade is currently ongoing to validate our in vitro results.

Conclusion Our data show that treatment with anti PD-1, anti PD-L1 or their respective combination with anti TIM-3 resulted in the highest secretion of cytokines and granzyme B, suggesting that these treatments stimulate the antitumor response the most. In vivo experiments are currently ongoing for validation.

PO-424 MODULATING HEXOKINASE 2 (HK2) AS A NOVEL APPROACH TO TARGET METABOLIC IMMUNO-Oncology

V Behar*, R Yosef, E Dor-On, N Amsaleh, Y Horev, OM Becker. Vidac Pharma, Discovery, Jerusalem, Israel

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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.