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PO-413

IMMUNE MODULATING PROPERTIES OF CYCLOPHOSPHAMIDE SYNERGISE WITH IMMUNOTHERAPY IN PRECLINICAL MODELS OF NEUROBLASTOMA

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Introduction Neuroblastoma (NB) is one of the most common childhood cancers accounting for 15% of paediatric cancer deaths. Current therapies, which include chemotherapy, are highly toxic with significant treatment related mortality. There is little scope for further intensification therefore alternatives strategies such as immunotherapy, are a priority for improving patient outcomes. Classically, chemotherapy is regarded as immunosuppressive but recent work has highlighted that some may illicit an immunogenic form of cell death. This work sets out to identify the immunomodulating effects of cyclophosphamide (CPM) on tumour infiltrating immune cells and whether these properties can synergise with immunomodulatory antibodies in preclinical models of NB.

Material and methods The effects of CPM and doxorubicin on tumour cells were investigated *in vitro* by analyses of immunogenic cell death (ICD) markers. Two different *in vivo* subcutaneous murine NB models (NXS2 +NB9464D), were treated i.p with different doses of CPM. Tissues were harvested and detailed immunophenotyping performed. Combination of CPM and anti-PD-1 therapy was investigated in both models using tumour growth and survival as end points. Combination therapy was also assessed in TH-MYCN mice which develop spontaneous neuroblastoma.

Results and discussions Chemotherapy application *in vitro* led to an increase in expression of the ICD markers, ecto-calreticulin and Hsp-70. CPM was found to have numerous immune modulating activities *in vivo*, including the reduction of intratumoural Treg cells, even at low doses (20 mg/kg), in both NB models. Combination therapy was shown to increase CD8⁺ and CD4⁺ percentages within tumours, along with an increase CD4⁺ effector and memory cell proportions. Anti-PD-1 therapy synergised with CPM to improve median survival and slow tumour growth. Metronomic dosing of CPM and anti-PD-1 antibody led to tumour regression in TH-MYCN tumour bearing mice.

Conclusion This work supports combining low dose CPM to enhance immunomodulatory antibody therapy, to generate therapeutic anti-neuroblastoma immunity. Ongoing work is focused on elucidating the mechanism of action of the CPM and anti-PD-1 combination therapy, along with identifying the optimal chemotherapy and antibody dosing strategies.

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PO-414

STAGE IV MELANOMA PATIENTS WITH TUMOURAL MHC CLASS I LOSS ONLY RESPOND TO ANTI-PD-1 THERAPY IN THE PRESENCE OF HIGH NK CELL DENSITY

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Introduction Prior to 2011 there were no effective systemic therapies for advanced stage melanoma patients and the overall survival was 7.5 months. Recently, immune checkpoint inhibitors, anti-PD-1 and anti-CTLA-4 inhibitors, have produced response rates as high as 50% and doubled overall survival, but only 30% of patients have a durable response. One important resistance mechanism to immunotherapies is the downregulation of MHC Class I, resulting in the expansion of melanoma cells resistant to CD8 +T cell killing. Natural Killer (NK) cells monitor MHC Class I expression and eliminate cells that fail to express it; thus, NK cells are likely critical in preventing resistance to anti-PD-1 therapy. This study sought to investigate whether tumour-infiltrating NK cells in the presence of MHC class I loss improved survival outcome of patients treated with anti-PD-1.

Material and methods Twenty-five stage IV metastatic melanoma patients treated with anti-PD-1 therapy were categorised into responders (CR/PR/SD >6 mo, n=13) and non-responders (SD <6 mo/PD, n=12) based on RECIST response. Whole transcriptome sequencing and multiplex immunofluorescent staining were performed on formalin-fixed-paraffin embedded pre-treatment tumour samples. Flow cytometry was used to confirm novel NK phenotypes in melanoma lymph node metastases of treatment naïve stage IV melanoma patients (n=5).

Results and discussions Differential expression analysis identified nine up-regulated NK cell specific genes in responders when compared to non-responders (adjusted p<0.05). Immunofluorescent staining of biopsies confirmed a significantly higher density of intratumoural and peritumoural CD16+ (intratumoural p=0.0015 and peritumoural p=0.0039) and granzyme B+ (intratumoural p=0.019 and peritumoural p=0.011) NK cells in responding patients. Flow cytometry demonstrated 46%±8% of the NK cells were identified as expressing PD-1. When stage IV melanoma patients were further stratified by MHC class I loss, responding patients with MHC class I loss, had a higher NK cell density and better survival outcome, compared to non-responders (p=0.012).

Conclusion This study showed that the presence of higher numbers of NK cells are associated with improved responses to anti-PD-1 therapy. Most importantly, responding patients with MHC class I loss had higher NK cell densities, suggesting that NK cells play an important role in mediating response to anti-PD-1 in patients whose tumour down regulates MHC class I expression.

PO-415

MULTIVALENT POLYMERIC NANOPARTICLES AS AN INNOVATIVE CANCER IMMUNOTHERAPY FOR COLORECTAL CANCER

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Introduction Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the fourth cause of cancer death worldwide. It is responsible for approximately 7 00 000 deaths