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 alternative 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 (NXX2 +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 to an increase in expression of the ICD markers, ecto-calreticulin and Hsp-70. CPM was found to have numerous immune in vivo, including the reduction of intra-tumoural 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.

PO-415
MULTIVALENT POLYMERIC NANOPARTICLES AS AN INNOVATIVE CANCER IMMUNOTHERAPY FOR COLORECTAL CANCER

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-peraffin 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.
per year. This work focused on the development of a combinatorial multivalent nanoparticle for CRC immunotherapy and immunomodulation based on the design of polymeric nanoparticles (NP) able to deliver a combination of CRC-associated antigen, adjuvants and gene regulators according to targeted cells, dendritic cell (DC) and CRC cells.

**Material and methods** Poly(lactic-co-glycolic) (PLGA)-based NP were prepared by the double emulsion (w/o/w) solvent evaporation method. NP were physicochemically characterised in terms of size, zeta potential and surface morphology. CRC antigen loadings were quantified by fluorescence. Immature DC (ATCC® CRL-11904™) were used to evaluate the *in vitro* cytotoxicity by the propidium iodide assay, as well as NP cellular uptake profile by flow cytometry. *In vivo* biodistribution assay of plain NP was also performed using the IVIS Lumina® Bioimaging system. NP uptake *in vivo* by myeloid antigen presenting cells and the expression of maturation and co-stimulatory molecules at the surface of these cells sorted within draining lymph nodes, were also evaluated by flow cytometry.

**Results and discussions** PLGA-based NP presented a mean size diameter close to 200 nm, with low polydispersity index (Pdi) (<0.200), a surface charge close to neutrality, as well as, a spherical shape and smooth surface. These multivalent delivery systems presented high loadings for antigen and adjuvants. No cytotoxic effect was observed on immature DC up to 48 hour incubation. NP were extensively internalised by immature DC *in vitro* after 48 hour incubation, and by migratory DC *in vivo* 17 hour after animal immunisation. *In vivo* real-time monitoring of NP accumulation in mice whole bodies and dissected organs showed a fluorescent signal at 17 hour close to the site of immunisation and in the lymph nodes. No significant differences in the expression of the co-stimulatory CD80, CD86 and MHC class I markers on CD11b+CD11c+MHCII+ population at lymph nodes were observed among different polymeric combinations upon mice immunisation with NP carrying CRC antigen and adjuvant.

**Conclusion** According to NP physicochemical characteristics, internalisation and biodistribution patterns, this innovative nanoparticle can lead to a safe multivalent nanomedicine able to modulate dendritic cell activity and T cell expansion against tumour cells expressing entrapped antigens.

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**PO-416**

**A NOVEL MULTIFUNCTIONAL POLYPEPTIDE-BASED PLATFORM AS AN IMMUNOTHERAPEUTIC APPROACH FOR MELANOMA**

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**Introduction** Melanoma is the most dangerous type of skin cancer and novel treatments are needed. Alternative therapeutics should be devised isolated or in combination with targeted immunotherapies, to efficiently stimulate specific anti-tumour responses. Branched polypeptides exhibit advanced engineered complexity and unique structural properties inaccessible to linear polymers that make them ideal drug delivery systems with enhanced biological performance. Branched nanosystems have the ability to activate immune cells, as dendritic cells (DC) and natural killer (NK) cells, constituting potential platforms to modulate the release profile of loaded molecules, including tumour associated antigens (TAA), adjuvants and drugs. This work aims to evaluate the *in vivo* anti-tumour efficacy of peptide-1 –conjugated polypeptide (pept-1-BP), with special emphasis on their impact on the modulation of the immune cell function.

**Material and methods** BP were synthesised and conjugated with the peptide-1 (pept-1-BP) via reductive-sensitive disulfide linker. To address *in vitro* and *in vivo* studies, Cy5.5 was conjugated to platform. To evaluate the effect of the conjugate on melanoma tumour growth, B16.F10 cells were implanted subcutaneously into C57BL/6 mice. At day 7, animals were injected with two doses (1 week apart) of 100 µL of PBS, Toll-like receptor ligands CpG (20 µg/dose) and Poly I:C (40 µg/dose) in solution, BP backbone (575 µg/dose) and pept-1-BP (575 µg/dose) mixed with adjuvants. Every 2 days, weight of the mice and tumour growth was followed. At day 21, mice were sacrificed and tumour and lymph nodes were collected. A cell suspension from tumour cells and lymph nodes of each animal was prepared and analysed for infiltrated lymphocytes (CD45.1, CD3ε, CD8α, CD4, CD107, PD-1, CTLA-4) by flow cytometry.

**Results and discussions** The BP presented a size of 81.86±1.63 nm and a zeta potential of −45.10±1.72 mV, while pept-1-BP showed a mean average diameter of 104.1±2.21 nm and a zeta potential of −24.8±0.64 mV, with a pept-1 loading efficiency of 8.7% (w/w).

*In vivo* results showed a significant reduction of tumour size in conjugate treated mice compared with the other groups. In addition, the FACS analysis of infiltrating lymphocytes within tumour site evidenced an increased expression for CD4, CD8α and NK cells.

**Conclusion** Overall, our results support the promising use of this novel conjugate for the delivery of TAA, as an effective anti-tumour immune therapeutic strategy able to decrease and control of tumour growth.

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**PO-417**

ANTI-TUMOURAL EFFECTS OF IL-15 AND CD40 STIMULATION AS A NOVEL COMBINATION IMMUNOTHERAPY FOR PANCREATIC CANCER

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**Introduction** Pancreatic cancer (PC) is the 3rd deadliest cancer worldwide with the lowest 5 year survival of all cancers. Despite all efforts, therapeutic improvements have barely been made over the last decade. Even recent highly promising targeted and immunotherapeutic approaches did not live up to expectations. Within the tumour microenvironment, a strong desmoplastic reaction occurs and is held responsible for the formation of a protective shield. Tackling this stromal shield is needed to overcome treatment resistance. CD40 stimulation has already demonstrated moderate anti-tumour responses in PC, including some anti-stroma effects. We have shown that interleukin (IL)–15 stimulated NK cells are capable of tackling both tumour as well as the surrounding desmoplastic stroma.