per year. This work focused on the development of a combinatorial multivalent nanoplatform 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 NP 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. This multivalent delivery systems presented high loadings for antigen and adjuvants. No cytotoxic effect was observed on immature DC up to 48 hour of 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+MHCIIT 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 nanoplatorm can lead to a safe multivalent nanomedicine able to modulate dendritic cell activity and T cell expansion against tumour cells expressing entrapped antigens.

**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, B16F10 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 peptide-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).

**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.

**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.
Therefore, we explored a novel combination immunotherapy consisting of an agonistic anti-CD40 monoclonal antibody and IL-15 in two mouse models of PC.

**Material and methods** C57BL/6 mice bearing subcutaneous Panc02 or KPC tumours were treated over a two-week period with IL-15 and anti-CD40, either as stand-alone or combination therapies. Tumour kinetics and survival were monitored. To investigate therapeutic mechanisms, experiments depleting different immune cell populations were performed. Re-challenge experiments were executed to check for immune memory induction. Tumour infiltrating lymphocytes are being characterised using flow cytometry and immunohistochemistry.

**Results and discussions** The combination treatment of IL-15 and anti-CD40 caused a distinct reduction of tumour growth rates in comparison with single agent treatments. Moreover, mice receiving the combination treatment showed significantly increased survival, with 60%–80% of the mice being completely tumour free. Depletion studies revealed both CD8⁺ T cells and NK cells are mechanistically involved in the anti-tumour effect of this novel treatment. Re-challenge experiments also showed that immune memory was induced. Flow cytometry experiments and immunohistochemistry experiments are being performed to provide more details on the phenotype of the tumour infiltrating lymphocytes and their spatial distribution within the tumour.

**Conclusion** To our knowledge, this is the first study demonstrating that combination of IL-15 and anti-CD40 exhibits a profound anti-tumour response in two mouse models of PC resulting in prolonged survival and even total eradication of >60% of PCs. These data provide a solid proof of principle to advance with this combination strategy to a phase I clinical trial.

**INTRODUCTION**

Nanotechnologies based on superparamagnetic iron oxide nanoparticles (SPIONs) have emerged as a promising tool for the theranostics of advanced and metastatic melanoma. Decoration of the nanoparticle surface with specific ligands to target Hsp70-positive melanoma cells. Therefore, we investigated how poly(I:C) affects immune modulation by GBM cells as well as potential to potentiate immune checkpoint blocking therapy, in particular programmed death ligand (PD-L) blockade.

**Material and methods** Primary human GBM cells were cultured from residual tumour tissue obtained from standard surgery of GBM patients. Their phenotype following poly(I:C) treatment was investigated by flow cytometry, immunohistochemistry and multiplex electrochemiluminescence. Mechanistic studies were performed using qRT-PCR, blocking antibodies and chloroquine (inhibitor of Toll-like receptor 3 or TLR3 signalling). Cocultures with peripheral blood mononuclear cells were studied for lymphocyte migration using a transwell assay, and immune activation using flow cytometry and ELISA. Additional PD-L1 or PD-L2 blockade was evaluated using ELISA and CFSE-labelled CD8⁺ T-cell proliferation.

**Results and discussions** Poly(I:C) stimulated a pro-inflammatory secretome by GBM cells, including type I interferons (IFN), interleukin-15, reduced transforming growth factor-beta, and immune activation using flow cytometry and ELISA. Additional PD-L1 or PD-L2 blockade was evaluated using ELISA and CFSE-labelled CD8⁺ T-cell proliferation.