Background Prostate cancer (PCa) is the second most frequent cancer in men and the fifth most frequent cause of cancer-related deaths in men worldwide. Current treatments for castrate (hormone)-resistant prostate cancer (CRPC) are limited and not curative, with a median survival from diagnosis of 23 months. Sipuleucel-T is the only FDA approved autologous cellular immunotherapy for PCa targeting prostatic acid phosphatase (PAP), showing a 4.1 month survival benefit for metastatic castration-resistant prostate cancer patients. However, its anti-neoplastic responses remain minimal and is cost prohibitive and while PAP is a good target for future prostate cancer vaccine, new, more affordable therapeutic approaches are therefore needed to treat advanced PCa. We have previously shown that a 15 amino acid (AA) PAP sequence-derived peptide could induce strong immune responses and delay the growth of murine TRAMP-C1 prostate tumours. We have now substituted one amino acid and elongated the sequence to include epitopes predicted to bind to several additional HLA haplotypes. Herein, we present the immunological properties of this 42mer-mutated PAP-derived sequence (MutPAP42mer) and the additional use of another PAP-derived sequence of 15 AA long to increase the CD4+ immune responses and delay the growth of murine TRAMP-C1 prostate cancer cells.

Materials and Methods The presence of PAP-135–143 epitope-specific CD8+ T cells in the blood of patients with prostate cancer (PCa) was assessed by flow cytometry using Dextramer™ technology. HHIDII/DR1 transgenic mice were immunized with mutated and non-mutated PAP-derived 42mer peptides in the presence of CAF®09 or CpG ODN1826 or 2395 (TLR-9 agonist) adjuvants. WT-hPAP42mer was also used to immunise syngeneic C57Bl/6 mice. Vaccine-induced immune responses were measured by assessing the proportion and functionality of splenic PAP-specific T cells in vitro.

Results PAP-135–143 epitope-specific CD8+ T cells were detected in the blood of patients with PCa and stimulation of PBMCs from patients with PCa with mutPAP42mer enhanced their capacity to kill human LNCaP PCa target cells expressing PAP. MutPAP42mer peptide was significantly more immunogenic in HHIDII/DR1 mice than the wild type sequence, and immunogenicity was further enhanced when combined with the CAF09b® adjuvant. The vaccine induced secretory (IFNy and TNFα) and cytotoxic CD8+ T cells and effector memory splenic T cells.

Conclusions The periphery of patients with PCa exhibits immunogenicity to the MutPAP42mer peptide and immunization of mice induces/expands T cell-driven, wild-type PAP immunity, and therefore, has the potential to drive protective anti-tumour immunity in patients with PCa.

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A MUTATED PROSTATIC ACID PHOSPHATASE (PAP) PEPTIDE-BASED VACCINE INDUCES PAP-SPECIFIC CD8+ T CELLS WITH EX Vivo CYTOTOXIC CAPACITIES IN HHIDII/DR1 TRANSGENIC MICE

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Background Human endogenous retroviruses constitute 8% of the genome and are distributed among viral families of which HERV-K is the most recently integrated. Endogenous retroviruses are well established, natural targets for immunotherapy. Previously, we observed that encoding an endogenous variant of the murine leukemia virus as a particle-forming transgene in adenoviral vectors, allowed for curative therapy against small established cancers. In addition, immunogenicity could be further improved by point mutations of an immune suppressive domain (ISD) (WO 2019/043127). In humans, HERV-K Gag and Env genes are structurally intact, and while expression is almost absent in healthy tissues, HERV-K proteins are detected in human cancers, including on cell surfaces and exosomes. Functionally, the HERV-K Env genes are implicated in oncogenic signaling pathways, Epithelial Mesenchymal Transition and immune evasion. Consequently, we developed a particle forming HERV-K vaccine incorporating ISD mutations for treatment of cancer with a combined T and B Cell response.

Materials and Methods HERV-K Gag and Env consensus sequences were encoded in human adenovirus type 5 and 19a/64 adenoviral vectors. Expression analyses were performed on human and mouse DCs. Immune responses were analyzed by intracellular cytokine staining and tetramers. Murine colorectal cancer cells were engineered to express the HERV-K Gag and Env antigens. Immunotherapy experiments in tumor-bearing mice were performed by transplantation of selected immune cell populations obtained from vaccinated donor mice.
**Results** Expression of the HERV-K transgene from adenoviral vectors directed high levels of transcripts to the cellular surface and led to the formation of virus like particles. Mutations in the ISD resulted in increased expression of HERV-K in human but not in murine DCs. In addition, ISD mutations increased humoral immune responses to WT Env during recombinant protein immunizations. Adenoviral vectors expressing HERV-K Gag and Env with mutations in the ISD (HERV-K-ISDmut) were highly immunogenic with rapidly induced antibody and T cell responses in mice and break of tolerance in non-human primates. Following prime-boost immunization with selected combinations of checkpoint inhibitors, T cell responses were obtained in the range of 40–50% of circulating CD8+ T cells. As Gag and Env expressing cell lines were rejected in WT mice, we engrafted CT26 cells expressing HERV-K Gag and Env in nude mice and performed adoptive transfer immunotherapy. While initially effective, CD8+ T cells rapidly lost tumor control, whereas combinations of CD8+ T cells with CD4+ T cells and B cells exhibited rapid and sustained tumor control in most animals.

**Conclusions** The HERV-K ISDmut antigen holds promise for directing a broad and effective immune response to a large proportion of human cancers.

**Disclosure Information**

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**COMBINED SCIB1 DNA VACCINE AND PD-1 CHECKPOINT BLOCKADE FOR THE TREATMENT OF INTRACRANIAL TUMOURS**

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**Background** Intracranial tumours present a significant therapeutic challenge due to their physiological location. Immunotherapy approaches represent attractive treatments for targeting these intracranial tumours due to their tumour specificity relatively low toxicity. The SCIB1 ImmunoBody® is a DNA vaccine developed by Scancell Ltd. that encodes a human IgG1 antibody with one TRP-2 two gp100 epitopes engrafted into its complementarity determining regions. SCIB1 has been shown to induce a stronger immune response than peptide, whole antigen DNA vaccines and peptide pulsed dendritic cells due to Fc-receptor mediated cross presentation. Taking all of this into account, we decided to examine the efficacy of SCIB1 therapy in combination with anti-PD-1 immune checkpoint blockade for the treatment of intracranial tumours.

**Materials and Methods** C57BL/6 HHDII/DR1 mice were immunised with SCIB1 ImmunoBody® DNA vaccine and ex vivo ELISpot assays were used to assess the immune response while the frequency of TRP-2 specific T-cells was examined via pentamer staining and subsequent flow cytometry analysis. The efficacy of this vaccine was then tested in mice B16 HHDII/DR1 tumours implanted intracranially. These cells where knockouted for murine beta2m and transfected with the chimeric HLA-A2 (HHDII) construct, and naturally express both the TRP-2 and gp100 antigens making it the ideal target for SCIB1 therapy. These mice also received anti-PD-1 therapy, the survival of these mice was then monitored. Immunohistochemical staining for TRP-2 and gp100 was also performed on human GBM tissue micro arrays to study whether SCIB1 could be applicable to this type of cancer. Furthermore, the expression of PD-L1 on GBM tumour cell lines was measured before and after IFNγ treatment via flow cytometry. This was done to give an indication of the efficacy of combined anti-PD-1 with SCIB1 vaccination in the GBM setting.

**Results** Here we demonstrate that SCIB1 generates a strong TRP-2 specific immune response in humanised C57BL/6 HHDII/DR1 mice, and this method of vaccination increased the frequency of TRP-2 specific CD8+ T-cells. The survival of mice harbouring intracranial B16 HHDII/DR1 tumours was significantly prolonged when SCIB1 ImmunoBody® vaccination was combined with anti-PD-1 immune checkpoint blockade compared to mice that received sham vaccination combined with anti-PD-1 and mice that received sham vaccination combined with an isotype control antibody. Our analyses revealed that GBM patients could benefit from SCIB1 ImmunoBody® therapy due to the expression of TRP-2 witnessed in GBM tumour tissues. GBM cell lines were also shown to express PD-L1 on their surface and the cell lines studied were shown to upregulate PD-L1 on their surface when exposed to IFNγ, providing further evidence for the combination of anti-PD-1 with active immunotherapy such as SCIB1.

**Conclusions** Combinatorial SCIB1 and anti-PD-1 therapy represents an exciting therapeutic intervention for the treatment of intracranial tumours. Our analysis of GBM tumours reveals that this therapy may be applicable to these types of tumours due to their antigen expression profile, because of these findings the efficacy of this combined treatment is now being tested in the GL261 and CT-2A murine GBM models with the aim of moving this combinatorial therapy forward for the treatment of GBM.

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