new biomarkers of response as well as novel therapeutic targets. Functional CRISPR screens have proven to be an important tool to identify key genes in complex biological processes. We use an in vitro and in vivo CRISPR screening strategy to better understand how tumours evade the immune system.

**Material and methods** We collected pre and post treatment biopsies from melanoma and lung cancer patients treated with an anti PD-1 inhibitor. Upon performing whole exome sequencing on biopsies from patients who progressed on therapy, we identified a list of candidate genes. We included genes that fell into one of the three categories: were mutated in more than one patient, were mutated only at relapse and had mutations in both alleles or had a heterozygous mutation at baseline and homozygous on progression.

In addition, we also generated an adenovirus transformed mouse embryonic fibroblast cell line, which does not form tumours in the immunocompetent mice due to T-cell killing. We use this system do perform an in vivo CRISPR screen, investigating which genes are crucial for immune evasion.

**Results and discussions** We have successfully generated a target gene list from sequences of patient biopsies. We have constructed a focused CRISPR library consisting of multiple gRNAs targeting each of those genes. This library will be used in a genetic screen in melanoma cells co-cultured with matched T-cells that recognise and eliminate the melanoma cells. Moreover, we will also use our in vivo adenoviral system to perform genome wide screens.

**Conclusion** After the screening, we can cross validate hits from both platforms to identify robust hits. Furthermore, we can validate them in a bigger patient cohort to determine their potential as predictive biomarkers for immunotherapy response.

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**Tumour Antigens and Immune Effectors**

**PO-406 INVESTIGATION OF THE REPERTOIRE OF PEPTIDES BOUND TO MHC CLASS I MOLECULES IN TASMANIAN DEVIL TRANSMISSIBLE CANCERS FOR THE DEVELOPMENT OF A PEPTIDE VACCINE.**

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**Introduction** The marsupial species Tasmanian devil harbours two contagious cancers, Devil Facial Tumour 1 and 2 (DFT1 and DFT2); both are passed between individuals as an allogeneic graft, initiating tumours around the neck and face. DFT1 emerged 20 years ago, causes 100% mortality and has decimated the devil population. In contrast, DFT2 emerged only recently and is still being characterised. As allografts, both tumours should be recognised by T cells interacting with non-self Major Histocompatibility Complex (MHC) molecules and associated peptides. We aim to isolate tumour-specific MHC-bound peptides in order to determine MHC/peptide complexes breaking or inducing tolerance and design a vaccine.

**Material and methods** We have characterised the immunopeptidomes of DFT1-IFNγ, DFT2 and devil fibroblast cell lines. The DFT1 cell line was stimulated with devil interferon-gamma to up-regulate MHC class I expression. MHC class I molecule-peptide complexes were isolated by reverse-phase HPLC and analysed by Liquid Chromatography tandem mass spectrometry (LC-MS/MS). All experiments were performed in triplicate. Peptides were identified by searching spectra against custom Tasmanian devil databases using the PEAKS software and data visualised in R studio and GraphPad Prism.
Results and discussions Between 6373 and 2243 peptides were identified for each cell line, but only peptides found in all replicates for each cell line analysed further. 1806 peptides were identified in all three replicates of the fibroblast cell line, while 455 and 379 peptides were common to all three replicates for the DFT1-IFNg and DFT2 cell lines respectively. Analysis of peptide length shows a preference for 8mers and 9mers in devil fibroblasts and DFT2 cells and a preference for 9mers in DFT1-IFNg. We next searched for binding motifs for the 8mers and 9mers across all cell lines and found potential anchor residues at position 3 and position 8/9, where there was a preference for hydrophobic amino acids (in particular Leucine). We then identified 61 and 55 peptides unique to DFT1-IFNg and DFT2 respectively.

Conclusion This is the first study to characterise the repertoire of peptides bound to MHC molecules in contagious cancers and represents a pivotal step for understanding the immunological features of transmissible cancers.