IDENTIFICATION OF UNIQUE ANTIGENS ON PROSTATE CANCER STEM CELLS FOR CYTOTOXIC T CELL RECOGNITION

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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-IFNγ 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-IFNγ. 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-IFNγ 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.

PO-408 NEW TARGETS FOR THE IMMUNOTHERAPY OF ADULT B-CELL ACUTE LYMPHOCYTIC LEUKAEMIA

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Introduction B-cell acute lymphocytic leukaemia (B-ALL) is a rare heterogeneous disease characterised by excess lymphoblasts of the B-lineage in the bone marrow. The most effective treatment to date is allogeneic stem cell transplant which can improve overall survival rates. This may in part be due to a ‘graft-versus-leukaemia’ effect via antigens that are are specifically expressed by leukaemic cells. However, few of the cancer antigens have been identified in adult B-ALL which could act as targets for immunotherapy.

Material and methods Through literature evaluation we identified potential target antigens for the immunotherapy of adult B-ALL. We wanted to determine which known antigens would act as comparators for novel antigens we are identifying through the use of antibody specific profiling on sera samples. We used an existing microarray dataset (GSE38403) to examine whether the expression of the antigens we had identified as promising, by proteo-array and in silico methods, were frequently expressed in 215 B-ALL patient samples and correlated with survival. Real-time quantitative (RQ)-PCR and immunocytochemistry were used to confirm the expression of the most promising antigens in our patient samples.

Results and discussions By real-time PCR we examined a total of 12 different antigens in adult B-ALL patient samples and healthy volunteers. We found that only survivin and WT1 were expressed in B-ALL patient samples (7/11 and 6/11, respectively) but not normal donor control samples (0/8). RQ-PCR showed that survivin was the only antigen whose transcript exhibited significantly higher expression in the B-ALL samples (n=10) compared with healthy controls (n=4) (p=0.015). Immunolabelling detected SSX2, SSX2IP, survivin and WT1 protein expression in all 10 B-ALL samples examined, but survivin was not detectable in healthy volunteer samples. To determine whether these findings were supported by the analyses of a larger cohort of patient samples, we performed metadata analysis on an already published microarray dataset. By microarray analyses, survivin (p=0.013, ANOVA)
was found to be frequently over-expressed in B-ALL patient samples versus normal pre-B cells. In addition, we also found that having HAGE and SSX2IP gene expression were poor prognostic markers for the probability of overall survival, although neither reached statistical significance.

Conclusion Further analysis of patient samples and healthy donor lymphoblasts will determine whether survivin remains the most promising target for the immunotherapy of adult B-ALL.

REFERENCES
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Discovery of Immunogenic Neoantigens for Peptide Vaccination Approaches in Murine Colorectal Cancer.

Introduction Recent developments have shown that effectiveness of therapy with checkpoint-blocking antibodies correlates with the expansion and invigoration of neo-antigen specific T cells. Alongside, peptide-based vaccines targeting onco-viral antigens have shown to be effective inducers of T cell responses related to reduction of HPV-induced pre-malignant. This suggests that peptide-based vaccination targeting neoantigens is a viable immunotherapeutic strategy.

A major limitation for broad application of peptide-based vaccinations is the characterisation of cancer-specific epitopes. Where virus-induced cancers have foreign antigens of which epitope identification is relatively straight-forward, cancers driven through mutations are patient-specific and require personalised approaches. The process of epitope identification for such patients is yet not trivial. Here we describe the process in a murine colorectal cancer model to identify immunogenic epitopes for peptide vaccination.

Material and methods Expressed mutations on a murine colorectal cancer cell-line where determined through DNA and RNA analysis by comparison with WT databases. Next, we used NetMHC4.0 for the prediction and ranking of MHC class I binding peptides. Immunogenicity was determined by vaccination, and relevance was asserted by analysis of splenocytes of PD-L1 antibody-mediated tumor-protected mice. Expression was confirmed through mass spectrometric analysis of MHC class I eluted peptides.

Results and discussions DNA and RNA analysis revealed the expression of several thousand mutations. Prediction of MHC class I binding peptides containing mutations limited this number to several hundred high- and moderate-affinity peptides. Selection of 57 high-affinity peptides for further analysis was based on the type of amino-acid substitution. Vaccination with pooled groups of peptides and \textit{ex vivo} stimulation with single peptides indicated a large percentages of peptides induced CD8- and CD4-specific responses. However, \textit{ex vivo} restimulation and subsequent readout of splenocytes from tumor-protected mice, showed specific responses to a limited number of three peptides, with strong responses to a novel peptide sequence. Mass spectrometry could confirm the expression and presentation of this epitope, but not the other two.

Conclusion Our approach was successful in the characterisation of immunogenic and relevant epitopes. Further research is now needed to improve effectivity in therapeutic vaccinations.