

Background Currently, most neoantigen pipelines often focus on the detection of neoantigens derived from mutations in the coding regions of the genome. However, in some cancer indications, the number of mutations detectable in tumours can be very low (low tumour mutational burden). This limits the number of actionable neoantigens and results in so-called ‘cold’ tumours. In these cases, non-canonical neoantigens resulting from alterations in non-coding regions of the human genome could represent a high potential alternative for treatment.

Indeed, recent research has revealed that previously presumed non-coding regions of the human genome, such as long non-coding RNAs (lncRNAs), can contain translatable small open reading frames (smORFs) generating micropeptides. Some of these micropeptides have already been shown to be involved in cancer development, but these small peptides could also represent a high potential source of non-canonical neoantigens for personalised therapy.

Materials and Methods Here, we present smORFin, a machine learning algorithm specifically trained to identify smORFs in transcripts and to assess their coding potential. While most tools are focused on longer sequences, smORFin is specifically developed to target small ORFs (<303 nucleotides). Furthermore, smORFin also accounts for smORFs with alternative initiation codons, thereby improving its sensitivity for the detection of novel unannotated smORFs.

In addition, the impact of mutations in allegedly non-coding regions of tumour genomes and its influence on the neoantigen repertoire, was evaluated through integration of smORFin in a neoantigen identification pipeline targeting lncRNA-derived mutated epitopes; lncRNeos.

Results The smORFin model reaches a precision of 0.98 and an accuracy of 0.95 on its testing dataset. Using this new prediction tool, a library of human smORFs was assembled, the so-called smORFeome. This library of smORFs, and their associated proteins, was evaluated as a reference for spectrum to peptide matching in mass spectrometry data (MS) analysis. Indeed, the evaluation of seven MS samples revealed and validated the presence of smORFeome-related micropeptides and HLA-I associated epitopes originating from smORFs.

Furthermore, it was observed that lncRNA-derived epitopes only represent a minor fraction of the total neoantigen load. Strikingly, when only focusing on tumours with a low neoantigen load, lncRNeos represented up to 27% of the total neoantigen load. This indicates that for tumours with a low TMB, and therefore with a low neoantigen load, lncRNeos allows to significantly expand the neoantigen repertoire. Biological *in vivo/in vitro* validation remains necessary to assess the existence, presentation, and actionability of lncRNeos.

Conclusions A novel random forest-based algorithm was developed to address the need for reliable identification of lncRNA-born smORFs. Furthermore, the integration of this prediction algorithm in a neoantigen pipeline allowed the identification of lncRNA-derived neoantigens and marks them as a potential novel source for personalised immunotherapy.

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P03.06 DECIPHERING VACCINE-INDUCED NEOEPITOPE-SPECIFIC T CELL RESPONSES IN A PATIENT WITH H3K27M-MUTANT MIDLINE GLIOMA WHO GRADE 4

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Background K27M-mutant histone-3 (H3K27M) defines a clinically and molecularly distinct entity of diffuse midline gliomas WHO grade 4 with an unfavorable prognosis. From an immunological perspective, H3K27M constitutes a cancer neoepitope: in a syngeneic MHC-humanized mouse model, an H3K27M-specific long peptide vaccine induced mutation-specific T cells responses capable of inhibiting growth of H3K27M-expressing tumors.

Materials and Methods Here, we describe clinical response of a patient diagnosed with H3K27M-mutant midline gliomas to H3K27M-specific peptide vaccination and exploit vaccine-induced T cell phenotypes.

Results Repeated peptide vaccinations were well tolerated and resulted in long-term response after pseudoprogression. Longitudinal immune monitoring showed induction of H3K27M-specific CD4-driven T cell responses in the peripheral blood. Within the cerebrospinal fluid, expansion of HLA-specific vaccine-induced T cell receptor (TCR) clones was observed and associated with distinct HLA types.

Conclusions Identification of vaccine-induced TCR clones within the peripheral blood and CSF of patients with H3K27M-mutant midline glioma may be used to prioritize TCRs for adoptive T cell therapy.

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P03.07 ANALYSIS OF SCRNAS FROM THE HUMAN THYMUS NOMINATES GENES POTENTIALLY MISSING FROM CENTRAL TOLERANCE OF CYTOTOXIC T CELLS

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Background During thymic development, cytotoxic T cells that can bind to and attack self antigens undergo negative selection thus preventing damage to the self tissues. The sparse medullary thymic epithelial cells (mTECs) present in the thymus are responsible for presenting self antigens to T cells so that they

can trigger apoptosis or differentiation into non-cytotoxic lineages if they bind too strongly.

Materials and Methods Understanding gene expression in mTECs is essential for understanding the shape of the human T cell receptor repertoire, which is key for current and emerging cancer immunotherapies. Recent availability of human thymus single cell RNAseq (scRNAseq) data provides an extremely high-resolution view into the pattern of expression within this critical cell type. To determine which epitopes have had to opportunity to be presented during T cell negative selection, we analyzed the human thymus scRNAseq dataset to establish which genes are expressed in mTECs and therefore subject to central tolerance.

Results The coverage of the whole transcriptome of a particular cell is generally sparse. It is therefore difficult to understand basic features of individual cells or cell types such as how many genes are expressed. We used cell- and read-level subsampling to estimate whether a sufficient number of cells and reads had been captured to support categorizing a gene as non-expressed in mTECs. We also examined the expression of the genes not expressed in mTECs in other healthy tissues, and found their expression was almost exclusively restricted to the testis (an immune-privileged site) and the liver (a site of peripheral tolerance)

Conclusions Altogether, these analyses establish a strategy for determining if a data set has sufficient depth to estimate the total number of genes expressed and secondly define a key list of genes that are not expressed during central tolerization of T cells, which represent a compelling list of possible cancer immunotherapy targets.

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P04 Precision Medicine Meets Immunotherapy (Immuno-Monitoring)

P04.01 IMMUNOMONITORING OF CD19. CAR T-CELLS IN LARGE B-CELL LYMPHOMA- A TWO-CENTER EXPERIENCE

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Background CD19. CAR T-cells for the treatment of relapsed and refractory (r/r) Diffuse Large B-Cell Lymphoma (DLBCL) demonstrated complete responses in 40%-58% of

the patients. Recently, others could associate high tumor volume and low CAR T-cell expansion *in vivo* with poor outcome. We hypothesize, that the expansion and immunophenotype of (CAR) T cells *in vivo* determine treatment response and depend on patient- and disease associated factors.

Materials and Methods Patients with r/r DLBCL (n=34) were treated with either Axi-cel or Tisa-cel at the University Hospitals of Erlangen and Munich (LMU). The CAR T-cell product and peripheral blood were collected on day 0, 4, 7, 14, 30, 60 and 90 post transfusion. CAR T-cells were detected through flow cytometry utilizing a two step-staining with a biotinylated CD19 protein. Effector:Target (E:T) Ratios were estimated as absolute peak expansion of CAR T-cells (/ul) per tumorvolume (cm³). Responder (R, complete or partial remission) were compared to Non-Responder (NR, stable or progressive disease) according to response assessment with PET-CT three months after transfusion.

Results CAR T-cell expansion peaked between day 7 and day 14 after transfusion with a greater expansion of CD8⁺ compared to CD8⁻ CAR T-cells on day 14 (59.27% vs 37.42%, p=0.021). The ratio of CD8⁺ and CD8⁻ CAR T-cells did not differ between R and NR, however R exhibited higher E:T ratios of CD3⁺ CAR T-cells compared to NR (20.94 vs 12.81, p=0.015) and an increased E:T ratio of CD8⁺ CAR T-cells correlated with better progression-free survival (p=0.033). Interestingly, high CRP and ferritin levels at baseline were inversely associated with the E:T ratio (p=0.048 and p=0.017). CD3⁺ CAR T-cells of R showed earlier peak expression of PD-1 than NR (day 7 vs day 21). Further, peak expansion of CD3⁺ CAR T-cells correlated with higher PD-1 expression in R but not in NR (p=0.003 vs p=0.12). In addition, R revealed an increased relative frequency of effector memory differentiated CD3⁺ CAR T-cells (CCR7⁻CD45RA⁻, p=0.02), whereas CAR T-cells in NR showed an increased relative frequency of a naïve phenotype (CCR7⁺CD45RA⁺, p=0.001) on day 7 post infusion.

Conclusions Flow-based immunomonitoring with longitudinal characterization of CAR T-cells demonstrated a correlation of the E:T ratio with treatment response and survival. Increased inflammatory conditions at baseline correlated with diminished E:T ratios. Notably, in R CAR peak expansion was positively associated with higher PD-1 expression suggestive for superior CAR T-cell activation. In addition, greater memory differentiation was associated with efficacy during the time of peak expansion. Multiparameter analysis with other clinical covariates will show, whether CAR T-cell expansion and immunphenotypes can predict patient outcome.

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