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 immunphenotype 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 (cm^3). 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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