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Blood 142 (2023) 453–454

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Single Cell Multi-Omic Dissection of Response and Resistance to Chimeric Antigen Receptor T Cells Against BCMA in Relapsed Multiple Myeloma

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Background: Chimeric antigen receptor (CAR) T cell therapy has revolutionized treatment of relapsed/refractory multiple myeloma (RRMM). Robust variables that predict long-term response are currently missing. Limited data are especially available on the impact of bridging therapies on manufacturing and outcome. We conducted a longitudinal single-cell multi-omics study to identify factors that predict response to BCMA-directed CAR T cells. Changes in the immune microenvironment associated with response were analyzed as well as the impact of prior bridging therapy with bispecific antibodies on subsequent CAR T cell manufacturing and outcome.
Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from 29 consecutive MM patients treated with commercially available anti-BCMA CAR T cells on the day of leukapheresis as well as days 30 and 100 after CAR T cell infusion. PBMCs were subjected to single cell RNA, T-cell receptor (TCR) and B-cell receptor (BCR) sequencing. A custom panel of 57 oligonucleotide-coupled antibodies was used to study surface proteomics. Downstream analyses were performed with Seurat. Differences in cellular compositions at all three time points were analyzed with scCODA. To analyze CAR T cell functionality, CAR T cells from peripheral blood were isolated 7 days after infusion and subjected to an *in vitro* cytotoxicity assay

after expansion and stimulation. Patients were grouped based on their best response following CAR T cell infusion (CR: n=12, non CR: n=17).

Results: In total, 375,338 cells were sequenced (median 7246 cells/sample, range 1,569-10,972 cells) and 354,878 cells (94.5%) passed quality assessment. Quantitative and qualitative differences in the cellular composition of peripheral blood between CR and non CR patients were detected at the time of leukapheresis as well as on days 30 and 100 following infusion. CR patients harbored significantly more CD8+ effector memory T cells (TEM) at leukapheresis and less NK cells on day 30 after therapy compared to non CR patients. Regulatory T cells isolated at the time of leukapheresis from non CR patients exhibited significantly higher surface protein levels of CXCR3, CD40, CD95 and KLRG1 ($p<0.015$, respectively) that have been associated with T cell senescence and impaired tumor immunity. No significant differences in cell numbers between CR and non CR were detected on day 100 after CAR T cell infusion. However, single cell TCR analysis revealed an increasing diversity in the TCR repertoire over time in patients with CR, while Shannon diversity decreased from leukapheresis over day 30 to day 100 in non CR patients ($p=0.004$). The prior administration of the bispecific antibody teclistamab had no significant impact on the quantitative cellular composition at the time of leukapheresis. However, termination of manufacturing in the first attempt occurred in all patients with a close proximity of teclistimab administration and apheresis. Differential gene expression analysis showed that the application of teclistamab was associated with impaired T cell activation and exhaustion indicated by upregulation of e.g. *CTLA4, TIGIT, LAG3* and *GZMK*. After discontinuation of teclistamab (median 4 weeks) and successful manufacturing of CAR T cells, we found no significant differences for *in vitro* cytotoxicity and *in vivo* expansion of CAR T cells. CAR T cells isolated at day 7 post-infusion from patients in CR, non CR or with prior teclistamab exposure, effectively eliminated MM cells (U-266). Tracking of single CAR T cells over time showed that the majority of CAR+ cells were CD8+ TEMs regardless of remission achievement or prior teclistamab exposure.

Conclusion: We demonstrate that differences between MM patients achieving a CR and patients with suboptimal response upon anti-BCMA CAR T cell therapy can already be identified at the time of leukapheresis. Long-term changes associated with CR include a diversification of the TCR repertoire. Successful CAR T cell manufacturing is hampered by exposure to bispecific antibodies but can be successfully achieved by allowing for a wash-out phase of ca. 4 weeks.

Disclosures Merz: *AMGEN, TAKEDA, BMS, JANSSEN, STEMLINE, ROCHE:* Honoraria. **Fricke:** *Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V.:* Patents & Royalties, Research Funding; *MSGO:* Consultancy, Honoraria; *AstraZeneca:* Consultancy, Honoraria; *Vertex Pharmaceuticals:* Consultancy, Honoraria; *Kite/Gilead Sciences:* Consultancy, Honoraria; *Novartis:* Consultancy, Honoraria; *Janssen-Cilag:* Consultancy, Honoraria. **Scolnick:** *Singleron Biotechnologies:* Current Employment. **Xu:** *Singleron Biotechnologies:* Current Employment. **Kloetzer:** *Janssen:* Honoraria. **Heyn:** *Janssen:* Honoraria. **Ussmann:** *Sanofi:* Other: former travel support. **Herling:** *Abbvie, Beigene, Janssen, Stemline, Takeda:* Consultancy; *Mundipharma EDO, Janpix, Novartis, Roche:* Research Funding. **Jentzsch:** *Blueprint Medicine:* Honoraria; *Pfizer:* Honoraria; *Novartis:* Honoraria; *BMS:* Honoraria; *Amgen:* Honoraria; *JAZZ:* Honoraria. **Platzbecker:** *Syros:* Consultancy, Honoraria, Research Funding; *MDS Foundation:* Membership on an entity's Board of Directors or advisory committees; *Fibrogen:* Research Funding; *Silence Therapeutics:* Consultancy, Honoraria, Research Funding; *Servier:* Consultancy, Honoraria, Research Funding; *Bristol Myers Squibb:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Other: travel support; medical writing support, Research Funding; *BMS:* Research Funding; *BeiGene:* Research Funding; *Takeda:* Consultancy, Honoraria, Research Funding; *Celgene:* Honoraria; *Jazz:* Consultancy, Honoraria, Research Funding; *AbbVie:* Consultancy; *Curis:* Consultancy, Research Funding; *Merck:* Research Funding; *Janssen Biotech:* Consultancy, Research Funding; *Geron:* Consultancy, Research Funding; *Roche:* Research Funding; *Amgen:* Consultancy, Research Funding; *Novartis:* Consultancy, Honoraria, Research Funding. **Vucinic:** *Takeda:* Consultancy, Honoraria; *MSD:* Consultancy, Honoraria; *Sobi:* Honoraria, Other: Travel/Accommodations/Expenses; *Amgen:* Honoraria; *AstraZeneca:* Honoraria; *Janssen:* Honoraria; *Gilead/Kite:* Consultancy, Honoraria; *BMS/Celgene:* Consultancy, Honoraria, Other: Travel/Accommodations/Expenses; *Novartis:* Consultancy, Honoraria; *Abbvie:* Honoraria.

https://doi.org/10.1182/blood-2023-187371