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POSTER ABSTRACTS

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

Single-Cell Multi-Omics Reveal Longitudinal Dynamics of Clonal Architecture and Microenvironment Interactions in Relapsed-Refractory Myeloma

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Abstract Introduction: Subclonal dynamics in multiple myeloma (MM) have been demonstrated to be strongly influenced by treatment. Moreover, the bone marrow (BM) microenvironment (BME) plays an important role in mediating treatment-associated drug resistance. However, the underlying transcriptional and epigenetic changes across genetically distinct subclones and their interaction with the BME remain poorly characterized.
Methods: Single-cell and whole genome sequencing (WGS) was performed in 15 relapsed/refractory MM patients with samples collected prior to the respective salvage therapy (T1) and at the time of subsequent relapse (T2). Using 10X Genomics scATAC- and scRNA-seq protocols, we analysed 44,637 and 37,280 BM CD138-enriched plasma cells after quality control with ArchR and Seurat, respectively. Copy number aberrations (CNAs) were called with inferCNV (scRNA-seq), an approach by Lareau and co-workers (scATAC-seq) and ACESeq (WGS). For mitochondrial DNA (mtDNA) mutations mgatk was used. Cell-cell interactions between subclones and the BME were predicted using CellChat.
Results: We developed a WGS-guided clustering strategy to identify individual CNA-based subclones in scRNA- and scATAC-seqdata. Next, this subclone definition was further refined by integrating mtDNA mutations. We found unique mtDNA mutations in 23/53 (43%) CNA-defined subclones. These mutations allowed us to discriminate between subclones with identical CNA profiles or to assign subclones based on CNAs that were otherwise below the WGS detection limit. Furthermore, some mtDNA mutations were jointly enriched in multiple subclones, defining subclonal branches. With our integrative analysis of CNAs and mtDNA mutations, we detected on average 4 (range 1-11) subclones per patient with very similar proportions between the two sc modalities (correlation $\rho = 0.97$). This allowed us to map the transcriptional profile of each subclone to its epigenetic profile. In patients with multiple subclones and a stable subclonal composition between T1 and T2, we found that subclones similarly adapted their transcriptomic and epigenomic profiles concordantly to the respective treatment. For instance, in a carfilzomib-treated patient, >85% of gene expression changes were shared between both subclones including several heat shock proteins, as well as TNFa signaling via NFkB, apoptosis, hypoxia and the P53 pathway. On the epigenomic level, the transcription factor (TF) motifs of ZNF384 and MEF2 family members were upregulated in both subclones at T2. Looking at patients with subclonal changes between T1 and T2 (n=7), we found a patient in

which a subclone carrying bi-allelic *TP53* inactivation was depleted by MCL-1 inhibitor treatment. But in a validation experiment, AMO-1 wild-type cell lines were significantly more sensitive to MCL-1 inhibition than bi-allelic TP53 altered AMO-1 cell lines (TP53 del/mut (R175H), $p<0.05$). However, several surface markers such as CD44 were differentially expressed between the competing subclones. Therefore, we inferred cellular interactions between the respective subclones and BME cells. We identified a strong interaction between CD44 and LGALS9 on monocytes and dendritic cells that was specific for the TP53-inactivated subclone. Extending the interaction analysis to all patients with matched scRNA-seq of the BME (n=9), an average of 32 ligand-receptor MM-BME interactions were predicted per patient. Interestingly, 19% (range 2-12) were subclone specific and primarily driven by Visfatin, ICAM, BAFF, CD23 and Galectin pathways. In addition, we observed shared longitudinal changes in expression of surface markers, including increased ICAM1 expression as well as chromatin co-accessibility at the ICAM1 promotor in the majority of patients. Lastly, known TFs of ICAM1, including IRF1/4, and STAT1/2 demonstrated higher motif activity at T2.

Conclusion: Overall, we demonstrate the power of paired bulk and single-cell multi-omics approaches for deciphering changes in the subclonal architecture and cell-cell interactions in the MM ecosystem. Treatment can induce concordant transcriptional and epigenetic changes even in genetically distinct subclones, generating a repertoire of shared targets, including ICAM1. Furthermore, our analysis shows that subclones can interact differently with their BME, which could be one explanation for differential treatment response.

Disclosures Rasche: *GSK:* Honoraria; *Pfizer:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *Sanofi:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *BMS:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *Amgen:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *Janssen:* Honoraria, Membership on an entity's Board of Directors or advisory committees. **Kortuem:** *Janssen:* Research Funding; *Janssen, BMS, GSK, Abbvie, Pfizer:* Consultancy. **Giesen:** *Pfizer:* Membership on an entity's Board of Directors or advisory committees; *Hexal:* Honoraria; *GSK:* Honoraria; *Abbvie:* Honoraria; *MSD:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *AstraZeneca:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *Janssen:* Other: Travel support. **Mueller-Tidow:** *BiolineRX:* Research Funding; *Pfizer:* Research Funding. **Goldschmidt:** *Amgen, BMS, Chugai, GlaxoSmithKline, Janssen, Novartis, Sanofi, Pfizer:* Honoraria; *BMS:* Consultancy, Honoraria, Other: Grants, Research Funding; *Chugai:* Honoraria, Other: grants, Research Funding; *Janssen:* Consultancy, Honoraria, Other: Grants, Research Funding; *SANOFI:* Consultancy, Honoraria, Other: Grants, Research Funding; *Incyte:* Research Funding; *Molecular Partners:* Research Funding; *Merck Sharp and Dohme (MSD):* Research Funding; *Mundipharma GmbH:* Research Funding; *Takeda:* Research Funding; *Novartis:* Honoraria, Research Funding; *Adaptive Biotechnology:* Consultancy; *GlaxoSmithKline (GSK):* Honoraria; *Amgen, BMS, Celgene, Chugai, Dietmar-Hopp-Foundation, Janssen, Johns Hopkins University, Sanofi:* Other: Grants and/or provision of Investigational Medicinal Product; *Amgen, BMS, Celgene, Chugai, Janssen, Incyte, Molecular Partners, Merck Sharp and Dohme, Sanofi, Mundipharma GmbH, Takeda, Novartis:* Research Funding; *Amgen, BMS, Janssen, Sanofi, Takeda:* Membership on an entity's Board of Directors or advisory committees; *AMGEN:* Consultancy, Honoraria, Other: Grants, Research Funding; *Array Biopharma:* Research Funding; *Amgen, BMS, GlaxoSmithKline, Janssen, Novartis, Sanofi, Pfizer:* Other: Support for attending meetings and/or travel; *Celgene:* Consultancy, Honoraria, Other: Grants, Research Funding; *Dietmar-Hopp-Foundation:* Research Funding. **Raab:** *Takeda:* Membership on an entity's Board of Directors or advisory committees; *Sanofi:* Membership on an entity's Board of Directors or advisory committees; *Novartis:* Membership on an entity's Board of Directors or advisory committees; *Heidelberg Pharma:* Research Funding; *BMS:* Membership on an entity's Board of Directors or advisory committees; *Amgen:* Membership on an entity's Board of Directors or advisory committees.

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