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ORAL ABSTRACTS

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

Discovery of Tumor Reactive T Cell Receptors in Newly Diagnosed Multiple Myeloma

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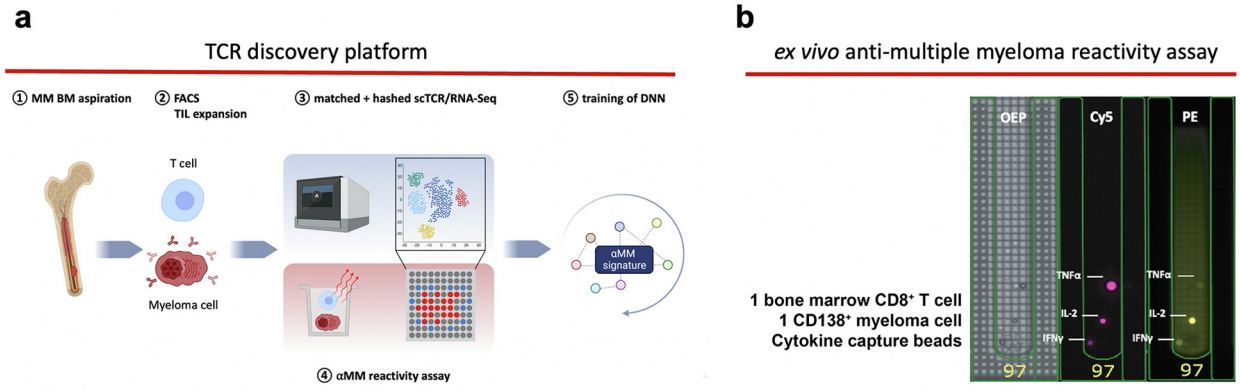
Abstract Introduction: Immunotherapeutic approaches including cell therapy are believed to be the next generation of paradigm-changing treatment options in hematological malignancies. Cell therapy using CAR-T cells or expanded autologous T cells (TILs) has shown promising results in non-solid and solid malignancies. However, acquired resistance to immunotherapy or rapid disease progression represent major clinical challenges in hematological cancers such as multiple myeloma and tumor-infiltrating T cells are often dysfunctional and inert to reactivation. Transgenic T cell receptors (TCRs) redirect patient-autologous lymphocytes to tumor antigens and can induce regression of refractory solid tumors. However, a *bone fide* population of myeloma reactive TCRs has yet to be identified.
Methods: We performed single-cell profiling coupled with functional TCR testing of tumor-associated T cells in multiple myeloma patients to understand diversity in the diseased TCR repertoire and tumor reactivity of T cells and translated these findings into a workflow suited for the development of personalized adoptive T cell therapy. To do so, we utilized samples from n = 18 patients with newly diagnosed multiple myeloma. From these patients, we matched combined single-cell RNA/TCR/CITE sequencing data with mutational load estimates and MHC class I and II neoepitope prediction of corresponding tumor cells. We performed validation of TCR reactivity against malignant plasma cells and have linked these functional data to single-cell transcriptional states using the CDR3 sequence as clone-specific barcode. Furthermore, we have established a multiplexed optical barcoding assay that allows for instant *ex vivo* profiling of single bone marrow-resident T cells in order to identify sequences and transcriptional signatures of patient-individual tumor-reactive lymphocytes.
Results: By integrating antigen specificity and the transcriptional profile of myeloma-associated T cells at single-cell resolution, we found that tumor-reactivity results in a specific gene expression state of bone marrow-resident $CD8^+$ T cells. Non-tumor-reactive clones were either $CD4^+$ or enriched for viral specificities, while myeloma reactive TCRs demonstrated an increased clonality within the overall lymphocyte repertoire. TCR repertoire investigation further revealed that the patients exhibiting the highest tumor mutational load acquired hyperexpanded T cell clones in both bone marrow and peripheral blood. Single-cell B cell receptor (BCR) sequencing of malignant plasma cells revealed several point mutations in the immunglobulin heavy chain (IgH) gene that were not immunogenic, whereas several somatically mutated transcripts of

malignant plasma cells formed putative neoepitopes on the cell surface. By performing downstream neoepitope prediction and functional *in vitro* testing of neoepitope- and anti-tumor reactivity in primary patient lymphocytes, we provide proof of principle of bone marrow-resident tumor reactive T cells in multiple myeloma. We furthermore identified a previously unknown subset of *Thymocyte selection-associated high mobility group box protein (TOX)*-expressing dysfunctional T cells that is associated with limited response to induction immunochemotherapy.

Conclusions: Our results demonstrate antitumor reactivity in a subset of bone marrow-resident T cells against malignant plasma cells and provide the rationale for future personalized TCR-transgenic cell therapy approaches in newly diagnosed multiple myeloma patients. More broadly, the generated resources from this project might contribute to identifying and monitoring tumor reactive T cell responses targeting hematological neoplasias.



a. Multiple Myeloma TCR discovery platform. ① Newly diagnosed patients with multiple myeloma were prospectively included and bone marrow (BM) biopsies acquired. ② BM-derived myeloma cells and tumor-infiltrating T cells (TILs) were isolated. ③ TILs were subjected to combined scTCR/RNA-Seq and ④ functionally tested against myeloma cells (see also panel b). ⑤ Matched data points from ③ and ④ will be used for training of a Deep Neural Network (DNN) to identify tumor reactive TCRs based on their transcriptional profile. **b. Identification of tumor-reactive T cells from a patient with multiple myeloma.** Multiplexed fluorescence-based *ex vivo* detection of cytokine production by a primary patient T cell recognizing CD138+ multiple myeloma cells via interferon-gamma (IFNg), interleukin-2 (IL-2) and tumor necrosis factor alpha (TNF-a) cytokine capture beads. Imaging was acquired 48h after loading.

Figure 1.

Disclosures Neri: *Janssen:* Consultancy, Honoraria; *BMS:* Consultancy, Honoraria; *Sanofi-Aventis:* Consultancy, Honoraria; *Pfizer:* Consultancy, Honoraria. **Bahlis:** *Forus:* Consultancy, Honoraria; *GSK:* Consultancy, Other; *Sanofi:* Consultancy, Honoraria; *Janssen:* Consultancy, Honoraria, Research Funding; *Karyopharm Therapeutics:* Consultancy, Honoraria; *Takeda:* Consultancy; *Amgen:* Consultancy, Honoraria; *Genentech:* Consultancy; *Pfizer:* Consultancy, Honoraria, Research Funding; *AbbVie:* Consultancy, Honoraria; *Celgene:* Consultancy, Honoraria. **Goldschmidt:** *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, BMS, Chugai, GlaxoSmithKline, Janssen, Novartis, Sanofi, Pfizer:* Honoraria; *Amgen, BMS, GlaxoSmithKline, Janssen, Novartis, Sanofi, Pfizer:* Other: Support for attending meetings and/or travel; *Array Biopharma:* Research Funding; *BMS:* Consultancy, Honoraria, Other: Grants, Research Funding; *Celgene:* Consultancy, Honoraria, Other: Grants, Research Funding; *AMGEN:* 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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