Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Nang Kham Su[1], Oksana Zavidij[1], Sabrin Tahri[1], Gad Getz[2], Irene Ghobrial[1]

[1]Dana-Farber Cancer Institute; [2]Broad Institute of MIT & Harvard

Introduction: Patients with Smoldering Multiple Myeloma (SMM) already exhibit hallmarks of immune dysregulation, as well as suboptimal response to immune challenges, such as vaccination for SARS-CoV-2. Preliminary evidence suggests that early therapeutic intervention may prolong progression-free survival in patients with high-risk SMM, however it is unclear how underlying immune dysregulation may impact risk and outcomes. Immune profiling of large cohorts of patients may help to independently identify subsets at risk of progression and/or suboptimal response to therapy. **Methods:** Here, we performed single-cell RNA-sequencing (scRNA-seq) on 149 samples, drawn from 34 patients with high-risk SMM who were enrolled on a Phase II clinical trial of Elotuzumab, Lenalidomide, and Dexamethasone (E-PRISM), and 32 healthy donors. Specifically, we profiled 117 patient samples, including 57 bone marrow (BM), and 60 peripheral blood (PB) samples, drawn at baseline, during, or at the end of treatment (EOT). For 24 patients and 40 samples, data was generated from matched BM and PB immune cells to enable head-to-head comparisons. Furthermore, we profiled BM immune cells from 22 healthy donors and PB immune cells from 10 healthy donors. **Results:** In patients with high-risk SMM, we observed increased abundance of: naïve and memory CD4+ T-cells, and in particular, regulatory T-cells, Th2, Th17, and Th1 cells, GZMB+ effector CD8+ T-cells and cytotoxic NK cells, memory B-cells, marginal-zone B-cells, CD16+ monocytes, macrophages expressing the complement component C1q, and canonical type 2 dendritic cells (DCs). Furthermore, we observed decreased abundance of: progenitor cells, central memory and GZMK+ effector memory CD8+ T-cells, mucosa-associated invariant T-cells, CD160+ GZMK+ NK cells, CD14+ monocytes expressing L-Selectin, pro-inflammatory cytokines and chemokines, and plasmacytoid DCs. Immune composition in the PB strongly correlated with that of matched BM samples (median Pearson's r=0.73). In principal component (PC) analysis, diagnostic BM and PB patient samples clearly separated from normal BM and PB samples (Wilcoxon p=3.9e-10, and p=3.2e-4, respectively), demonstrating that both BM and PB immune cells reflect the presence of SMM. Moreover, BM samples drawn at EOT were more closer to normal BM in PC space compared to diagnostic samples, providing evidence of post-therapy immune normalization, which could have prognostic implications. **Conclusions:** To our knowledge, this is the largest to date study of immune scRNA-seq profiling in patients with SMM, and the first systematic attempt of this scale to assess the feasibility of using PB for immune profiling in patients with myeloma. Here, we present a comprehensive dissection of alterations in the composition of the BM immune microenvironment, demonstrate that PB mirrors the composition of the BM immune compartment, and provide evidence of post-therapy immune normalization, which could have prognostic implications.

OAB-029

Single-cell transcriptomic analysis reveals reduction of cytotoxic NK cells in a subset of newly diagnosed multiple myeloma patients impacting outcome after daratumumab therapy

Sabrin Tahri[1], Madelon de Jong[1], Cathelijne Fokkema[1], Natalie Papazian[1], Zoltan Kellermayer[1], Chelsea Den Hollander[1], Michael Vermeulen[1], Mark van Duin[1], Pieter van de Woestijne[1], Kazem Nasserinejad[2], Elona Saraci[3], Mattia D'Agostino[4], Francesca Gay[5], Vincent Van der Velden[1], Sonja Zweegman[6], Niels W.C.J. van de Donk[7], Annemiek Broijl[1], Pieter Sonneveld[1], Tom Cupedo[1]

[1]Erasmus MC Cancer Institute, Rotterdam, The Netherlands; [2]Erasmus Medical Centre; [3]Flow cytometry laboratory- Division of Hematology University of Turin, A.O.U. Città della Salute e della Scienza di Torino; [4]Department of Molecular Biotechnology and Health Sciences, Division of Hematology, University of Torino, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino, Torino, Italy; [5]University of Torino; [6]Department of Hematology, Amsterdam University Medical Center, VU University Amsterdam, Cancer Center Amsterdam, Amsterdam, Netherlands; [7]Amsterdam University Medical Center, Vrije Universiteit Amsterdam, Department of Hematology, Cancer Center Amsterdam, Amsterdam, Netherlands

Introduction: Anti-CD38 antibody-based therapies in multiple myeloma (MM) rely to a large degree on natural killer (NK) cell-mediated antibody-dependent-cellular cytotoxicity. Classically, NK cells are divided into cytotoxic CD56dim and cytokine-producing CD56bright subsets. However, accumulating evidence suggests a larger degree of heterogeneity in the NK cell compartment and we hypothesized that changes in NK cell subset composition would impact responses to NK cell-driven immunotherapies in MM. Here, we used single-cell RNA sequencing to investigate the heterogeneity of the NK cell compartment in the bone marrow of newly diagnosed MM (NDMM) patients, and the effects of altered NK cell subset composition on therapy response in patients undergoing first-line daratumumab-containing therapy. **Methods:** We performed single-cell RNA sequencing of the CD38+ immune cell compartment in 19 NDMM patients. NK cells were identified in silico resulting in a single-cell transcriptomic dataset of 24,664 BM NK cells. **Results:** UMAP dimensionality reduction identified 5 transcriptomic distinct NK cell clusters, present in all NDMM patients. MM bone marrow was characterized by heterogeneity in the ratio of cytotoxic vs cytokine-producing NK cell cluster composition, with a subset of patients (3/19) presenting with a relative decrease in cytotoxic NK cell clusters. The reduction in cytotoxic NK cell subsets in this patient subgroup was reflected in an altered overall transcriptome of the total BM NK cell population, with enrichment for inhibitory receptors such as KLRB1 and KLRC1, and a relative loss of activating receptors such as FCGR3A (CD16), NCR3 (NKp30) and CD226 (DNAM-1), and cytotoxic effector genes including NKG7 and GNLY. Flow cytometric analyses of 246 NDMM patients from 2 independent clinical cohorts revealed reduced frequencies of cytotoxic CD56dim

NK cells, determined as < 90% CD56dim NK cells, in 17.9 % of 162 transplant-eligible treatment-naïve NDMM and in 28.6% of 84 treatment-naïve frail or unfit transplant ineligible MM patients. To test whether such relative decrease in cytotoxic NK cells would negatively impact responses to daratumumab, we associated BM NK cell composition with clinical outcome in the context of first-line daratumumab therapy in a cohort of frail NDMM patients ineligible for transplant from the HO143 trial (n=43). Multivariate cox regression analyses revealed that reduced frequencies of CD56dim NK cells in the BM correlated with significantly shorter progression-free survival (PFS) (hazard ratio 1.38; 95% confidence interval: 1.057 – 1.803; P = 0.018). **Conclusions:** Here, we show that about 20% of NDMM patients have a relative reduction in cytotoxic NK cells, which was correlated with significantly shorter PFS following daratumumab-containing therapy in a frail cohort of NDMM patients. Our data suggest that defining BM NK cell composition could stratify patients and identify a subgroup less likely to benefit from therapies driven by NK cell-mediated ADCC.

OAB-030

Single-cell RNA-seq reveals XBP1-SLC38A2 axis as a player in immunosuppressive T lymphocytes in multiple myeloma

Yike Wan[1, 2], Mengping Chen[1, 2], Xin Li[1, 2], Xiaofeng Han[1, 2], Lu Zhong[1, 2], Fei Xiao[1, 2], Jia Liu[1, 2], Jing Xiang[1, 2], Jinxing Jiang[1, 2], Xiaotong Chen[1, 2], Junling Liu[3], Bin Li[4], Honghui Huang[1, 2], Jian Hou[1, 2]

[1]Department of Hematology, Ren Ji Hospital; [2]Shanghai Jiao Tong University School of Medicine; [3]Department of Biochemistry and Molecular Cell Biology, Shanghai Jiao Tong University School of Medicine; [4]Shanghai Institute of Immunology, Shanghai Jiao Tong University School of Medicine

Introduction: Functional impairment of T lymphocytes results in immune escape of multiple myeloma (MM). How to correct the immunosuppressive state of T cells in MM and enable them to restore normal immune function becomes the principal focus of MM research. The growing field of immune metabolism has provided new insights into anti-myeloma immunity. The fate and function of T cells are intrinsically related to metabolism, through which cells need to produce bioenergy intermediates to support proliferation and effector functions. The mechanisms of immunosuppression and metabolic reprogramming of T lymphocytes in MM have not been fully elucidated. **Methods:** We applied single-cell RNA sequencing to mononuclear cells of bone marrow and peripheral blood samples from 3 healthy volunteers and 10 newly-diagnosed MM patients before and after two cycles of bortezomib/cyclophosphamide/dexamethasone treatment, and analyzed the differential expressed genes, enriched signaling pathways and developmental trajectories of T cell subsets. Flow cytometry was used to validate the immune suppressive profiles of T cells in MM. Spearman correlation analysis was performed to evaluate the correlation among SLC38A2, XBP1 and markers of T cell dysfunction. Luciferase reporter assay was used to detect the direct binding of XBP1s and the promoter of SLC38A2. XBP1 shRNA and overexpression vector were transfected into CD8+ T cells to investigate the regulation of XBP1 on SLC38A2. **Results:** We identified 15 T cell clusters through single-cell data. Cytotoxic T (Tc) cell clusters are extensively characterized by senescence, while some cells in each cluster concurrently show exhaustion features. The senescence markers KLRG1/CTSW and exhaustion markers LAG3/TIGIT were representatively expressed in Tc cells in MM. The upregulation of GZMK and CXCR4 in Tc cells further supported T cell dysfunction. Impaired metabolism and unfolded protein response (UPR) pathway enrichment in Tc cell clusters in MM were revealed, along with decreased and increased expressions of glutamine transporter gene SLC38A2 and UPR hallmark XBP1 respectively. Meanwhile, SLC38A2 expression negatively correlated with XBP1, exhaustion markers HAVCR2/TNFRSF14 and senescence markers FGL2/CTSW. The transcription factor XBP1s could directly bind to the promoter of SLC38A2. Overexpressing XBP1s in healthy CD8+ T cells downregulated expressions of SLC38A2 and effector marker GZMA, while silencing XBP1 upregulated expression of SLC38A2 and downregulated expression of senescence marker KLRG1. **Conclusions:** Our integrative single-cell transcriptome analysis dissects the immunosuppression profiles of Tc cells in MM and the underlying dysfunction mechanism. We have demonstrated that UPR hallmark XBP1 directly binds to SLC38A2 promoter and inhibits its gene expression, leading to T cell dysfunction. This study reveals a novel metabolic regulating mechanism in Tc cells and provides new insights to the immunotherapy for MM.

OAB-031

PHF19 promotes multiple myeloma cell resistant to daratumumab/isatuximab via upregulation in immunosuppressive microenvironment and reduced CD38 target expression

Tengteng Yu[1], Hailin Chen[1], Kenneth Wen[1], Tingjian Wang[1], Phillip Hsieh[1], Thomas Smiths[1], Mehmet Samur[1], Lijie Xing[1], Liang Lin[1], Mu Hao[2, 3, 4, 5, 6], Lugui Qiu[2, 3, 4, 5, 6], Yu-Tzu Tai[1], Kenneth Anderson[1, 7, 8]

[1]Dana Farber Cancer Institute; [2]State Key Laboratory of Experimental Hematology; [3]National Clinical Research Center for Blood Diseases; [4]Haihe Laboratory of Cell Ecosystem; [5]Institute of Hematology & Blood Diseases Hospital; [6]Chinese Academy of Medical Sciences & Peking Union Medical College; [7]Jerome Lipper Multiple Myeloma Center; [8]Harvard Medical School, Boston, MA, USA

Introduction: We here investigated whether Polycomb-like protein (PHF19), an epigenetic gene recently identified in high-risk multiple myeloma (MM), influences MM cell response to anti-CD38 immunotherapies and further defined cellular and molecular mechanisms underlying these processes. **Methods:** Ex vivo cocultures were used to determine the percentage, viability, proliferation, and function of PHF19-overexpressing (PHF19-OE) vs control (ctrl) MM cell-induced regulatory T (iTreg), in parallel with immune checkpoint markers on conventional T cells (Tcon). Immunosuppressive cytokines and MM antigens (BCMA