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ORAL ABSTRACTS

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

Characterization of Multiple Myeloma Subpopulations Associated with Treatment Resistance By Single-Cell RNA Sequencing

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Multiple myeloma (MM) has extensive genomic and transcriptomic heterogeneity with identical variable-diversity-joining (VDJ) region recombination of immunoglobulin loci (called repertoire). Even though new treatment approaches have been developed, most cases of MM remain incurable. Recent evidence suggests that epigenetic cell plasticity and changes in cell state underlie the therapy resistance, but the mechanisms remain largely unknown.

We attempted to understand the comprehensive architecture of the entire tumor cell populations using repertoire clonality as a fingerprint. First, we subdivided bone marrow samples of primary MM at different stages (n=8) into 11 fractions and performed bulk RNA sequencing (bulk RNA-seq). Analysis of the repertoire revealed that tumor cells were present at very low frequencies in some minor fractions other than the main fraction (Lineage marker $^{-}$ (Lin $^{-}$)/CD19 $^{-}$/CD38 $^{+}$/CD138 $^{+}$) as the disease stage progressed. Importantly, MM cells were consistently present in CD138 negative fraction (Lin $^{-}$/CD19 $^{-}$/CD38 $^{+}$/CD138 $^{-}$) regardless of disease stage. Flow cytometry (FCM) analysis confirmed that CD138negative MM cells were present at a rate of less than 10% across the disease stages. Compared with CD138 positive cells, they were slowly cycling and showed unique morphology with large cytoplasm (n=59).

To achieve a selective and comprehensive evaluation of MM population, we performed single-cell RNA sequencing (scRNA-seq) coupled with VDJ targeted sequencing (scVDJ-seq) on Lin $^{-}$/CD19 $^{-}$/CD38 $^{+}$(without CD138 gating) samples from 11 MM patients, including those at diagnosis and in remission with minimal residual disease (MRD). This method allowed us to discriminate MM cells (20,084 cells) clearly from others by clonal repertoire sequence even in the post-treatment samples with extremely small populations of tumor cells. Because mRNA levels of CD138 did not correspond to the differential intensity in FCM, we defined gene signatures of CD138 positive and negative cells respectively by bulk RNA-seq (n=6). As a result of integration of these gene signatures using Gene Set Enrichment Analysis (GSEA), the heterogeneity on the CD138 axis was also unraveled in scRNA-seq data.

Interestingly, gene ontology (GO) analysis of bulk RNA-seq data revealed that several terms related to H3K4 methylation were positively enriched in CD138 negative fraction, which included *KMT2A*, *KMT2B*, *KMT2C* and *KMT2D*. Western blotting confirmed significant reductions in H3K4me1 and H3K4me3 levels in CD138 negative cells. Furthermore, primary MM cell culture indicated the plasticity between CD138 positive and negative cells, which was also suggested by trajectory/velocity analysis of scRNA-seq data. Collectively, it was suggested that epigenetic changes, including those in histone modifications, contribute to the heterogeneity along the CD138 axis.

We next compared the size of each cluster before and after treatment in scRNA-seq data to identify the MM populations associated with treatment resistance. Remarkably, we identified unique clusters which tended to remain at high proportions against treatment in the CD138 negative population. They were characterized by low expression of MHC class-I genes and

TNFRSF17/BCMA, as well as 198 significantly upregulated genes including *MCL1*, *MALAT1* and *NEAT1*, which have been previously implicated in treatment resistance of MM.
To systematically interrogate their contribution in MM cell survival, we performed CRISPR/Cas9 knockout screening of selected 100 genes, which were differentially up-regulated in the resistant clusters and associated with poor prognosis in a MMRF CoMMpass Study database. GO analysis of indispensable genes reproducibly identified among three MM cell lines revealed enrichment of several pathways related to mRNA splicing. These results suggested that the RNA splicing pathway is a promising therapeutic target to eliminate resistant populations. Indeed, we confirmed that inhibition of a splicing factor by CRISPR/Cas9-mediated knockout and pharmacological protein degradation suppresses MM cell proliferation effectively.
Taken together, we achieved a complete view of MM diversity by using repertoire clonality and confirmed heterogeneity along the CD138 axis, which may be characterized by epigenetic plasticity. Our results highlight a novel subpopulation as a candidate therapeutic target for cure.

Disclosures Doki: *Janssen Pharmaceutical K.K.:* Honoraria; *Novartis Pharma K.K.:* Honoraria. **Kataoka:** *Eisai:* Honoraria, Other: Scholarship; *Novartis:* Honoraria; *Chugai Pharmaceutical:* Honoraria, Other: Scholarship, Research Funding; *AstraZeneca:* Honoraria; *Sumitomo Pharma:* Honoraria, Other: Scholarship; *Kyowa Kirin:* Honoraria, Other: Scholarship; *Janssen Pharmaceutical:* Honoraria; *Takeda Pharmaceutical:* Honoraria, Other: Scholarship, Research Funding; *SymBio Pharmaceuticals:* Honoraria; *Bristol Myers Squibb:* Honoraria; *Pfizer:* Honoraria; *Ono Pharmaceutical:* Honoraria; *Nippon Shinyaku:* Honoraria, Other: Scholarship; *Daiichi Sankyo:* Honoraria, Other: Scholarship; *Alexion Pharmaceuticals:* Honoraria; *AbbVie:* Honoraria; *Meiji Seika Pharma:* Honoraria, Research Funding; *Sanofi:* Honoraria; *Sysmex:* Honoraria; *Mundipharma:* Honoraria; *Incyte Corporation:* Honoraria; *Kyorin Pharmaceutical:* Honoraria; *Asahi Kasei Pharma:* Other: Scholarship; *Otsuka Pharmaceutical:* Other: Scholarship, Research Funding; *Shionogi:* Other: Scholarship; *Teijin Pharma:* Other: Scholarship; *Japan Blood Products Organization:* Other: Scholarship; *Mochida Pharmaceutical:* Other: Scholarship; *JCR Pharmaceuticals:* Other: Scholarship; *Nippon Kayaku:* Other: Scholarship; *Chordia Therapeutics:* Research Funding. **Iwama:** *Daiichi-Sankyo Pharmatheutical:* Research Funding; *Nissan Chemical Indistries:* Research Funding.

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