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POSTER ABSTRACTS

711.CELL COLLECTION AND PROCESSING

Comparing Phenotyping, Gene Expression, and Function of T Lymphocytes before Mobilization Chemotherapy Versus after Stem Cell Harvesting in Patients with B Cell Lymphoma and Multiple Myeloma

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Chimeric Antigen Receptor T cell (CAR-T) therapy is an effective treatment for relapsed and refractory hematological diseases. However, a significant medical unmet need persists, particularly for patients at the relapse and refractory (R/R) status, who often experience leukopenia or lymphopenia during lymphocyte collection. This limitation poses considerable challenges to the successful implementation of CAR-T treatment, primarily due to the impaired function of T cells resulting from multiple lines of treatment and disease progression. As a consequence, the quality of the CAR-T cell product may be compromised.
Before introducing CAR-T therapy, most patients with B cell lymphoma or multiple myeloma (MM) experienced mobilization chemotherapy and high-dose granulocyte colony-stimulating factor (G-CSF) administration for stem cell harvesting, followed by autologous stem cell transplantation as a salvage or consolidation treatment.
Given that the procedure of lymphocyte collection for CAR T cell manufacturing is quite similar to stem cell harvesting, it becomes reasonable to explore the possibility of collecting lymphocytes immediately after the stem cell harvesting process. The advantage of this strategy is that patients are typically in a healthier condition with better T cell function compared to their status during R/R status. However, the effects of mobilization chemotherapy and high-dose G-CSF on T lymphocytes remain inadequately understood. To assess the feasibility of lymphocyte collection, it is essential to delve into the function of T lymphocytes at two critical time points: before mobilization chemotherapy compared to after stem cell harvesting.
In this study, we recruited 19 patients with B cell lymphoma or MM. Paired peripheral blood samples were obtained before administering mobilization chemotherapy and after stem cell harvesting. Flow cytometry analysis using CCR7 and CD45RA markers was applied to classify T cell subsets, including T naive or T stem cell-like (TN/SCM, CCR7+/CD45RA+), T central memory (TCM, CCR7+/CD45RA-), T effector memory (TEM, CCR7-/C5RA-), and T effector (Teff, CCR7-/CD45RA+) (Figure A). The data indicated no significant difference in T cell phenotype between pre-mobilization chemotherapy and after stem cell harvesting. CD4and PD-1 expression were also analyzed and showed no significant difference between the two time points.
To gain further insights, we performed RNA sequencing on 3 paired samples from patients with large B cell lymphoma (UPN#3, UPN#11, UPN#16). Through our observations, we noticed that these 3 paired samples could be clustered into 2 groups based on differential gene expression (Figure B), which corresponds to pre-mobilization chemotherapy versus post-stem cell harvesting status. Our findings strongly indicate that T cells at the pre-mobilization chemotherapy status exhibit significant differences in gene expression compared to those at the post-stem cell harvesting status. Specifically, T cells from post-stem cell harvesting show significantly higher expression of *NIBANI, ALOX5AP*, and *GLUL* compared to those from pre-mobilization. *NIBANI* plays a vital role in down-regulating apoptosis by interacting with the MDM2/p53/Bcl2/Bax pathway, promoting cell survival under stress-dependent conditions. *GLUL* is responsible for glutamine synthetase, and glutamine is an important source of biosynthetic precursors in active T cells. On the other hand, T cells from pre-mobilization show higher expression of *TOX, DUSP4*, and *TGFBR3* compared to those from post-stem cell harvesting. *TOX* is a transcription factor involved in T cell exhaustion and senescence, while *DUSP4* expression is associated with defective TCR signaling and accelerated T cell senescence.
Our data indicate that the proportion of T naive or T stem cell-like phenotypes remains unchanged after mobilization chemotherapy and G-CSF injections during stem cell harvesting. RNA sequencing data further reveal that T cells at the post-stem cell harvesting status are associated with down-regulated apoptosis, favorable survival and activation, and reduced

exhaustion and senescence compared to those at the pre-mobilization stage. This study suggests that instead of collecting lymphocytes during relapse or refractory status, collecting them right after stem cell harvesting may be a viable alternative.

Disclosures No relevant conflicts of interest to declare.

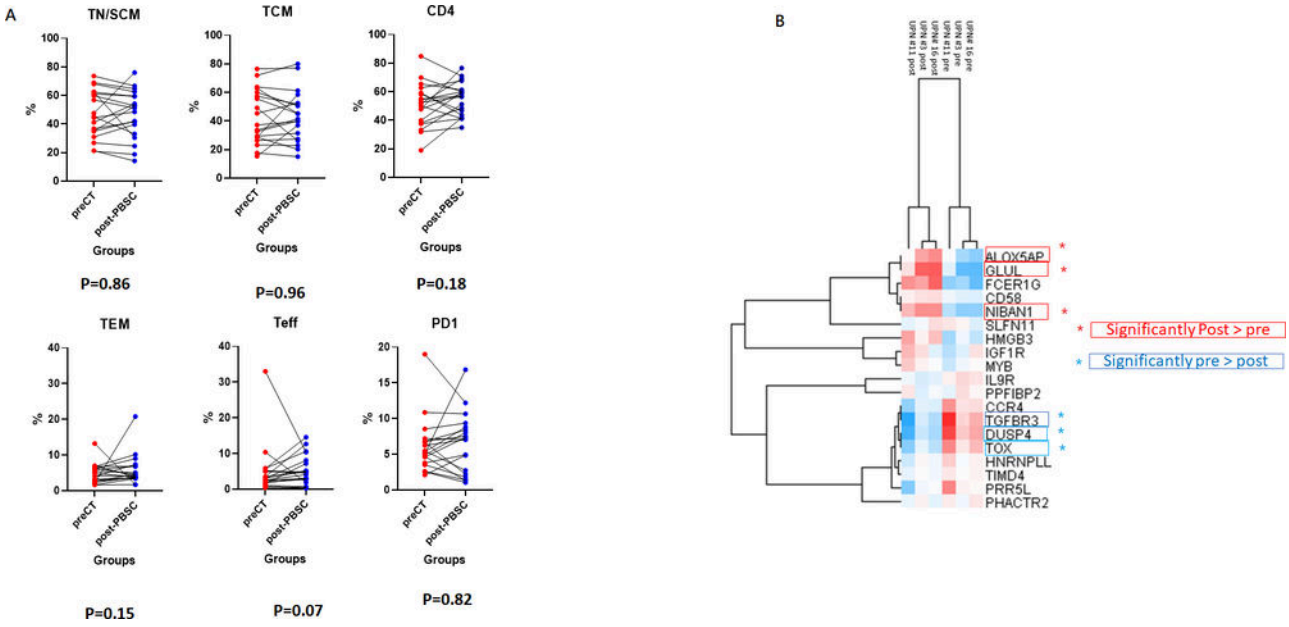

Figure 1

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