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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

FT538, iPSC-Derived NK Cells Are Potent Inducers of Apoptosis in AML Cells and Their Effect Is Synergistic in Combination with Approved Therapeutic Strategies

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Background: Natural killer (NK) cell-based therapies have been increasingly studied with their advantages of less treatment-related toxicity and lack of HLA restriction. Induced pluripotent stem cell (iPSC)-derived NK cells offer an opportunity for a standardized, off-the-shelf treatment with the potential to treat a wider patient population. FT538 cells are triple gene-edited iPSC-derived NKs that express a high affinity non-cleavable CD16 maintaining constant activity, a CD38 knock out preventing CD38 antibody-induced fratricide, and an IL-15/IL-15R fusion preventing the need for cytokine administration, the main source of side effects in NK cell-based therapies. Several studies have shown success in treating AML with NK cell therapy, but the effect of iPSC-derived NK cell therapy in AML is unexplored. We hypothesized that FT538 cells may be able to target high-risk AML that is traditionally resistant to most chemotherapeutic agents and synergize with Venetoclax, 5-azacytidine, Cytarabine, and Gilteritinib.
Methods: To examine the effect of the FT538 cells on AML cells, we co-cultured FT538 cells at ratios of 2:1, 4:1, and 8:1 with 8 AML cell lines: OCI-AML2, OCI-AML3, THP1, U937, Molm-13, Molm-14, MV4-11, and Kasumi-1, and with cells from 11 different AML patients. We analyzed the NK cell-induced apoptosis through an Annexin-V binding assay using IncuCyte live cell imaging. As Molm-13 and MV4-11 cell lines are FLT3 mutated, to determine the combination effect of FLT3 inhibitors with FT538 cells, we treated Molm-13 and MV4-11 cells with Gilteritinib for 24 hours before seeding the FT538 cells and co-culturing overnight. The effects of the combination were then analyzed through flow cytometry with a Beckman Coulter CytoFlex. Finally, to determine the combinatorial effect of the FT538 cells with approved therapies, we treated OCI-AML3 and U937 cell lines with Venetoclax, Azacitidine, and Cytarabine. After treatment, we performed flow cytometry to measure the leukemic cell death following the combinatorial therapies.
Results: We observed FT538 NK-mediated killing in all the leukemic cell lines tested. A two-way ANOVA determined that an increase in the FT538 to AML cell ratio resulted in an increase in NK cell-mediated killing of AML cells ($p<0.0001$). Interestingly, the number of apoptotic AML cells was 1.5 to 6.1-fold higher in the OCI-AML2, OCI-AML3, U937, and THP1 cell lines than in the Molm-13, Molm-14, MV4-11, and Kasumi-1 cell lines ($p<0.0001$). This could be related to the FLT3 mutations in the Molm-13, Molm-14, and MV4-11 cell lines or to the TP53 mutation in the Kasumi-1 cell line. Further, to validate this finding in primary AML cells, we observed a ratio-dependent effect on FT538-mediated killing of AML cells. However, we did not observe any statistical difference by mutation, though this could be related to the limited sample size for specific mutations. Finally, flow cytometric analysis of the effects of combination treatment demonstrated that the FT538 cells synergize with Venetoclax, or Cytarabine, or Azacitidine, or Gilteritinib ($p<0.05$) in the killing of AML cells. This could be because NKG2DL upregulation has been shown in AML cells treated with Venetoclax and FLT3 inhibitors and in Cytarabine-resistant AML cells, and the NKG2D/NKG2DL pathway is a main mechanism of NK cell-mediated killing.
Conclusion: iPSC-derived NK therapy offers a standardized, off-the-shelf option for NK cell therapies. FT538 iPSC-derived NK cells induce apoptosis in AML cell lines and patient samples in a dose-dependent manner and show a synergistic effect with Venetoclax, Gilteritinib, Azacitidine, and Cytarabine. Therefore, iPSC-derived NK cell therapy may be a promising possibility for AML treatment, particularly for patients resistant to standard therapies.

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