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703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Utilizing Chemotherapy to Enhance the Anti-Tumor Properties of *Ex Vivo* Expanded Gamma Delta ($\gamma\delta$) T Cells Against Acute Myeloid Leukemia

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Background: Acute myeloid leukemia (AML) is the second most common type of blood cancer in children with a high rate of treatment failure and aggressive relapse. While allogeneic HSCT offers a cure, limitations arise in achieving remission prior to transplant. Immunotherapy-based approaches can induce remission, gaining interest in AML. Here, we explore the use of gamma delta ($\gamma\delta$) T cells as a promising tool to fulfill this unmet need. Unlike predominant alpha beta ($\alpha\beta$) T cells, $\gamma\delta$ T cells do not require antigen presentation and identify their targets in a major histocompatibility complex (MHC) independent manner. Allogeneic $\gamma\delta$ T cells thus have minimal risk of causing graft-versus-host disease (GvHD), creating 'off-the-shelf' cellular therapeutics. This would be ideal to target AML, especially given the aggressive nature of relapsed disease. Cytotoxic mechanisms used by $\gamma\delta$ T cells include recognition of cellular stress molecules such as NKG2D and DNAM-1 receptor ligands on target cells, activation of death receptor pathway through Fas-FasL and TRAIL-R interactions, as well as $\gamma\delta$ TCR mediated killing through recognition of butyrophilins (BTNs) on target cells. These stress molecules are expressed on AML cells and can be further upregulated through chemotherapeutic agents such as azacitidine (a hypomethylating agent) and venetoclax (BCL-2 antagonist), drugs currently in use to treat relapsed AML. Here, we tested these drugs in combination with $\gamma\delta$ T cells to target AML.
Methods Our studies explored four different AML cell lines (Kasumi-1, Nomo-1, MV4-11 and MOLM13). Each one was respectively treated at their corresponding inhibitory concentration 25 (IC_{25}) and 50 (IC_{50}) of azacitidine and/or venetoclax for 24 hours. After treatment, AML cells were assessed by flow cytometry for expression of NKG2D and DNAM-1 ligands, death receptor pathway molecules (CD95, TRAIL-R1/R2) as well as the butyrophillins (BTN2A and BTN3A1) to elucidate which pathways are over-expressed, aiding in $\gamma\delta$ T cell targeting and cytotoxicity. Next, we co-cultured AML cells pre-treated with azacitidine and/or venetoclax with $\gamma\delta$ T cells and measured cytotoxicity using a flow cytometry-based assay and a bioluminescence assay.
Results: Each cell line expressed a unique pattern of markers at baseline. Cell lines treated with azacitidine had a robust expression of the NKG2D ligands ULBP1, ULBP2/5/6 and ULBP3. Azacitidine also increased CD155 and TRAIL-R2 in Nomo-1 while CD112 increased in MOLM13. Most venetoclax-treated AML cells over-expressed BTN2A and BTN3A1 along with CD95 in MOLM13. Cell lines were then treated with a combination of both drugs. Dual treatment of cells resulted in a significant increase in expression of most NKG2D markers as well the death receptor Fas (CD95). Additionally, increase in CD155 as well as BTNs was seen in several cell lines. Importantly, azacitidine and venetoclax had minimal effects on the viability of $\gamma\delta$ T cells when treated for 24 hours. *In vitro* cytotoxicity studies indicate increased cell death across multiple AML cell lines when $\gamma\delta$ T cells were co-cultured with cells pretreated with azacitidine and venetoclax.
Conclusions: Azacitidine and venetoclax treatment resulted in upregulation of several markers involved in $\gamma\delta$ T cell-mediated cytotoxicity. Ex vivo expanded $\gamma\delta$ T cells combined with chemotherapy-treated AML cells resulted in increased overall cytotoxicity, which is likely both additive and synergistic. Our next steps include preclinical validation of this combinatorial approach in an in-vivo mouse study.

Disclosures No relevant conflicts of interest to declare.

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