THE EFFECTS OF NEGATIVE ISOLATION AND AGGREGATE STRAINERS ON EXPANDED GAMMA/DELTA T CELLS AND THEIR CANCER CELL CYTOTOXICITY

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Background One of the most effective ways to purify gd T cells after their expansion, is through negative selection isolation kits. However, even though the target gd T cells are not stained themselves, they appear to suffer stress during the process. An alternative method is to use aggregate strainers which filter cell aggregates through a membrane. This is based on the concept that expanding activated gd T cells, aggregate together. In the context of gd T cells ability to kill cancer cells, the potential stress caused by these methods could affect their functionality.

To shed light on this topic we here analysed the viability, purity, and the functionality of 14 days expanded gd T cells with 24h killing assays on HER2 positive SKBR3 breast cancer cells.

Methods Gd T cells were expanded for 14 days with Zoledronate added on day 0 and IL-2 added every 3 days. Negative isolation was performed using EasySep™ Human Gamma/Delta T Cell Isolation Kit from StemCell Technologies. StemCell Technologies 37mm Reversible Strainers were used for the aggregate isolation. An MTS assay with 1:1 target to effector, with 24h incubation was performed to analyse the killing capacity.

Results The negative isolation showed the highest reliability in generating a near 100% purity of gd T cells (figure 1). The aggregate strainer increased the purity significantly with donors with low gd T cells yield before isolation from 40% to 70%, but could not purify donors with high purity before isolation over 92%. While not statistically significant, the CD69 expression was elevated on the aggregate strainer isolated gd T cells compared to the negative selection isolated gd T cells (figure 2). The viability was significantly reduced after negative selection isolation but was not affected by the aggregate strainer isolation. The killing capacity of gd T cells was significantly reduced for 2 donors after isolation via the negative selection isolation, compared to the gd T cells isolated via aggregate strainers (figure 3). The absolute number of cells isolated was significantly reduced in the aggregate strainer isolation.

Conclusions This indicates that negative selection isolation, while generating a high purity, has a negative impact on the functionality of gd T cells. Aggregate strainers on the other hand can purify gd T cells not to the same purity as negative selection isolation, however retain a high viability in the cell population and show higher killing capacity against SKBR3 cancer cells.

Abstract 293 Figure 1 Purity and absolute cell count
The left graph shows the percent of gd T cells before and after isolation with negative isolation kit and aggregate strainers. The right shows the absolute cell count before and after isolation with the methods mentioned above.

Abstract 293 Figure 2 The Viability and CD69 expression of gd T cells
The left graph shows the viability before isolation as well as after negative isolation and aggregate strainer isolation in percentage. The right graph shows the expression of CD69 on the same three conditions as the left graph. (* p<0.05; ** p<0.005; n=3)

Abstract 293 Figure 3 Percentage of killing of SKBR3 cells by gd T cells
The killing of SKBR3 cancer cells after 24h with a target to effector ratio of 1:1 is shown for two donors. The killing was analysed via MTS assay.