IMPROVED ANTITUMORAL ACTIVITY OF ANTIGEN-SPECIFIC CD8+T CELLS IN COMPARISON TO UNSPECIFIC ACTIVATED CD8+T CELLS IN A 2D T CELL KILLING ASSAY TARGETING HER2+ BREAST CANCER

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Background
Breast cancer is one of the leading causes of death in women worldwide. Hence, it is important to develop and improve (immuno)therapies for breast cancer patients. Adoptive T-cell therapy with autologous CD8+T cells is a promising method that has already succeeded in melanoma patients. Unfortunately, there is almost no information about the use in breast cancer patients.

Methods
We developed an in vitro protocol to generate antigen-specific CD8+T cells by priming on the HER2+ breast cancer cell line JIMT-1. Subsequently, these primed CD8+T cells were tested in a 2D immune cell killing assay in a life cell imaging device in combination with an endpoint viability assay. The activity was compared with unspecific CD8+T cells activated by phytohemagglutinin (PHA) or αCD2/αCD3/αCD28 beads. All T cells were tested as monotherapy and in combination with pembrolizumab (anti-PD1 antibody).

Results
The antigen specific CD8+T cells displayed a significantly improved killing potential of JIMT-1 cells compared to unspecifically activated or non-activated CD8+T cells. Along these lines, those cells showed the highest TNF-alpha secretion and expression of CD69 (determined by flow cytometry, FC) among all T cell lines. The use of complete peripheral blood monocytes (PBMC) instead of isolated CD8+T cells did not influence the activity against JIMT-1 significantly. Combination treatment with pembrolizumab did not increase the antitumoral activity, which was in line with the fact that PD-1 expression did not increase across the different settings. Testing of different donors revealed a donor dependent degree of killing, however in all donors the activity pattern was the same with antigen specific CD8+T cells being the most active. A direct comparison of fresh vs frozen immune cells from the same donors indicated no significant differences in the killing activity of unspecifically activated CD8+T cells. However, there were substantial differences in antigen-specific immune cells: the killing potential of the freshly isolated immune cells was significantly higher than of the frozen.

Conclusions
In summary, we have developed a protocol to produce antigen-specific tumor-infiltrating CD8+T cells primed to the heterologous JIMT-1 breast cancer cell line. The antigen-specific CD8+T cells showed an increased killing potential over non-specific CD8+T cells. The 2D immune cell killing assay proved to be robust and amendable for mid throughput screening. The impact of donor variability and fresh vs frozen immune cells must be further evaluated. In the future, this assay will be used to screen novel drugs in combination with antigen-specific CD8+T cells to select the most promising candidates.