Tumor-infiltrating T cells: Complete workflows allow faster and improved analysis

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Introduction

Immunotherapies based on T cells have proven clinical efficacy and tremendous potential. However, responses are often suboptimal. Therefore, further research is required to gain a deeper understanding of tumor-infiltrating leukocytes' (TILs) biology and enhance outcomes. TIL analysis is technically challenging and labor intensive. Cell numbers can be very low and small subpopulations might escape analysis as they are lost in the background noise. Importantly, tumor-infiltrating T cells are embedded in a highly immunomodulatory environment such that unbiased cell-intrinsic functional characterization is impeded. Therefore, it is fundamental to use effective tools to streamline the workflow and to generate reliable data.

Material and methods

1 Complete workflow combining tissue storage, dissociation, T cell isolation, and flow cytometric phenotyping

In this study we used a workflow based completely on Miltenyi Biotec products, from tissue storage and sample preparation through to cell isolation and analysis (fig. 1).

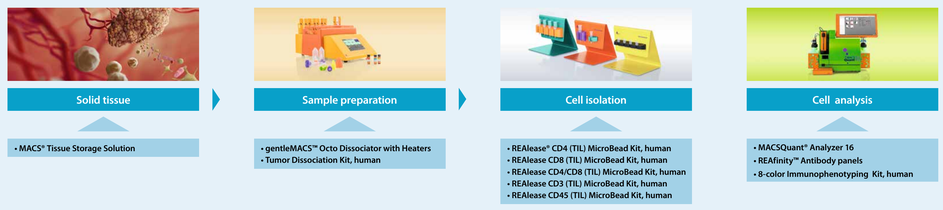

Figure 1

2 REAlease® Technology enables isolation of label-free cells

REAlease® Technology relies on recombinantly engineered antibody fragments instead of antibodies to label specific cell surface markers. The antibody fragments are engineered to have a low affinity for markers when present as monomers. However, when the fragments are multimerized as a complex they bind markers with high avidity. REAlease Technology can control the multimer/monomer state of the fragments and thus allows for a controlled release where monomerized antibody fragments dissociate from the cell surface. Therefore, the technology enables users to obtain cells that are free from antibody fragments and magnetic label (fig. 2).

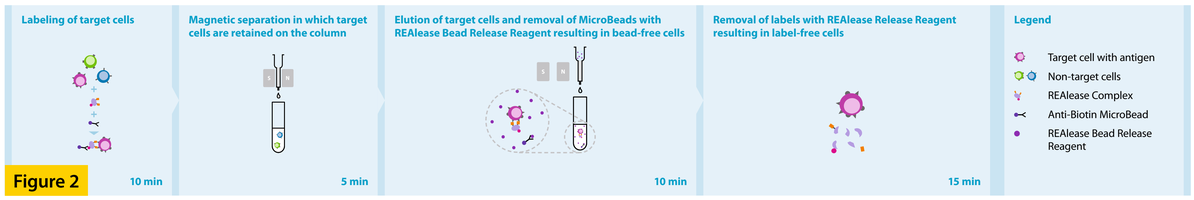

Figure 2

Results

1 Effective isolation of tumor-infiltrating T cells by REAlease® Technology enables improved analysis

Colon or ovarian tumor samples were shipped overnight in Tissue Storage Solution and dissociated using the gentleMACS Octo Dissociator with Heaters and the Tumor Dissociation Kit, human (based on a new protocol using 20% Enzyme R). Subsequently, T cells were isolated using the indicated REAlease® MicroBead Kits. Frequencies of tumor-infiltrating T cells among living cells before or after isolation are shown, as assessed by flow cytometry (fig. 3A). Magnetic isolation resulted in significant enrichment of target cell populations (fig. 3B; n = 2 to 4), thus facilitating clear identification of distinct target populations for improved analysis.

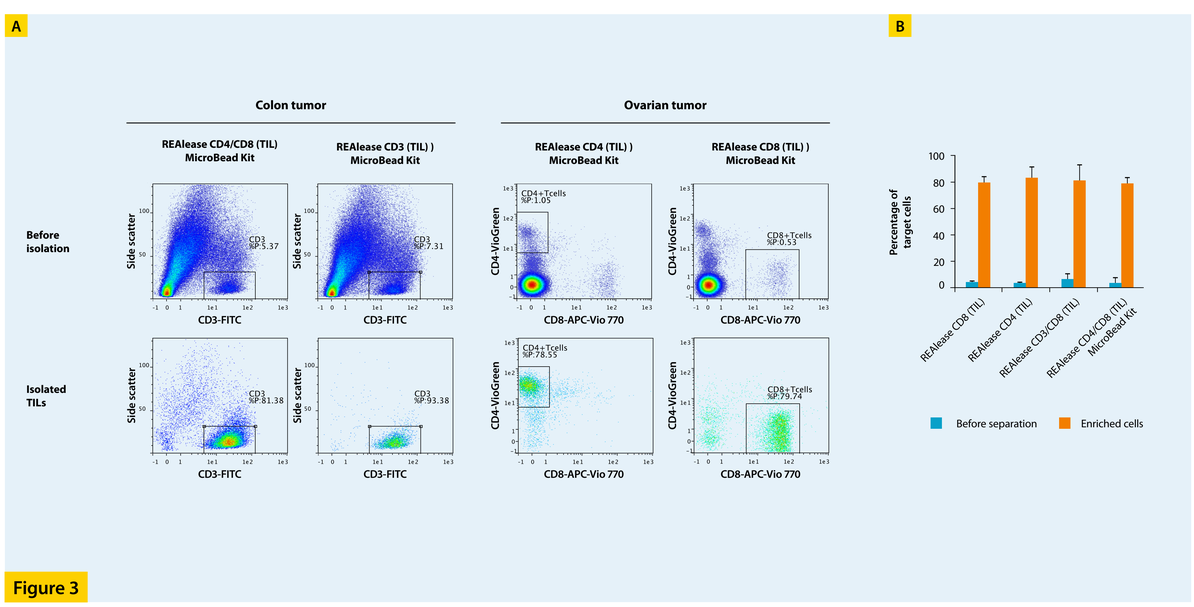

Figure 3

2 Isolation with REAlease® Technology maintains T cell phenotype

As a model system to test the T cell activation status, T cells were enriched from PBMCs by the REAlease® CD3 MicroBead Kit, human (fig. 4). Isolated cells were cultured in the absence or presence of plate-bound anti-CD3 antibody (OKT3) for 20 h. Activation of the cells was assessed by immunofluorescent staining of activation marker CD69. Unstimulated isolated CD4+ and CD8+ cells were not activated, comparable to unstimulated cells before separation. In contrast, stimulated cells showed increased expression of CD69. T cells isolated by the REAlease CD8 MicroBead Kit or REAlease CD4 MicroBead Kit showed similar results.

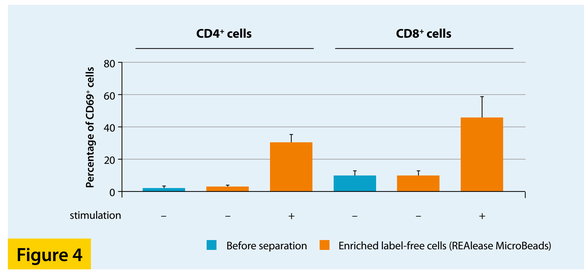

Figure 4

3 Dead cell removal downstream of isolation with the REAlease® CD4/CD8 (TIL) MicroBead Kit increases purity of live T cells

A sample of dissociated breast cancer tissue (fig. 5A, left) underwent T cell isolation with the REAlease® CD4/CD8 (TIL) MicroBead Kit, human (fig. 5A, center), followed by release of the magnetic label and enrichment of live cells by the Dead Cell Removal Kit (fig. 5A, right). The frequency of live T cells and dead cells in the different samples was assessed by flow cytometry. Figure 5B shows a summary of frequency of live cells after isolation of T cells with the REAlease CD4/CD8 (TIL) MicroBead Kit, before or after dead cell removal (means±SD; n = 5).

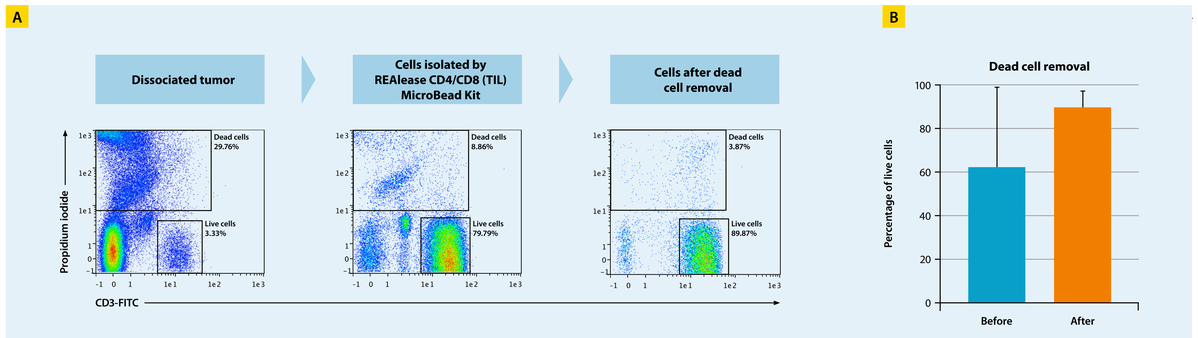

Figure 5

4 TIL isolation reduces time required for analysis

An ovarian tumor sample was dissociated using the gentleMACS Octo Dissociator with Heaters and the Tumor Dissociation Kit, human. CD4+ and CD8+ T cells were isolated by the REAlease CD4/CD8 (TIL) MicroBead Kit, human. Within the isolated population, critical tumor-specific subpopulations such as CD279 (PD1)^{hi}CD366 (TIM-3)+CD39+ CD8+T cells and CD279(PD1)+CD39+CD137+CD4+ T cells could be identified easily. Using enriched target cells, the number of events that needed to be acquired for proper flow cytometry analysis was 10-fold lower than with unseparated bulk tumor cells. This greatly reduced the time required for analysis.

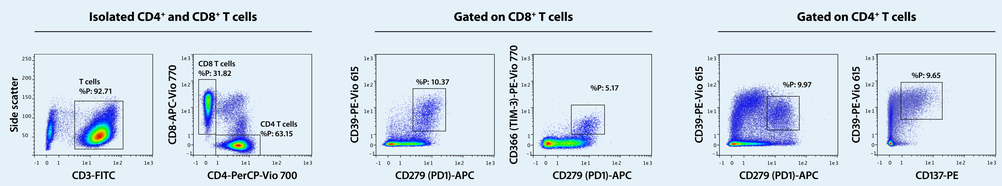

Figure 6

Conclusion

- The optimized workflow includes newly developed tools for magnetic isolation of tumor-infiltrating T cells using the novel REAlease Technology.
- REAlease Technology combines two key features, i.e., highly specific cell isolation by positive selection and removal of any labels.
- Importantly, this technology provides a strategy for isolating complex cell subpopulations from heterogeneous tissues (such as tumor) where negative selection is not possible or efficient.

We believe the use of these tools and workflows can significantly increase the quality of the data obtained in immuno-oncology and immunotherapy research.