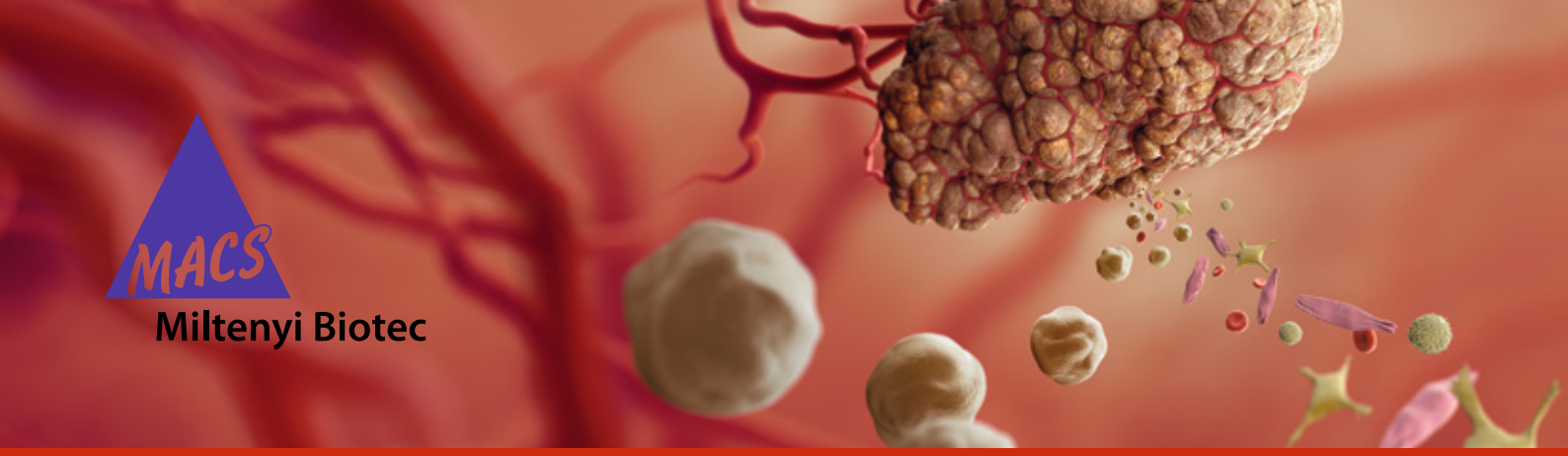

Miltenyi Biotec

Effective enrichment of human TILs

Effective enrichment of human tumor-infiltrating leukocytes from xenograft tumors

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Background

Adoptive T cell therapy is a potent option for the treatment of patients suffering from certain cancers.[1] In particular, autologous tumor-infiltrating lymphocytes obtained from resected metastatic tumor samples showed great promise in the eradication of metastatic melanoma.[2]

For comprehensive research on tumor-infiltrating leukocytes (TILs) including myeloid and lymphoid cells, it is desirable to have reliable methods that allow for a fast isolation of these cells from tumors. Various techniques for the enrichment of $CD45^+$ TILs from dissociated tumors are frequently used in the field. However, thus far all these options have major drawbacks. I) Density gradient centrifugation results in a non-specific enrichment and involves long centrifugation steps. II) Flow sorting or direct flow cytometry analysis are time consuming since many cells need to be acquired for effective isolation and accurate analysis. Moreover, in flow cytometry, non-enriched rare cell populations are often lost in the background noise and thus escape detection. III) Magnetic cell separation options offered by various suppliers are not optimized for tumor material and therefore provide only low TIL purities.

Here we used an automated workflow for the dissociation of human colon tumor xenografts into single-cell suspensions and the subsequent magnetic enrichment of TILs based on MACS® MicroBead Technology. CD45 (TIL) MicroBeads are optimized for use with dissociated tumor tissue and resulted in high purities of isolated TILs.

Materials and methods

Induction of tumors, adoptive transfer of T cells, and sample preparation

Xenograft tumors were induced by injection of human colon tumor cells into NOD/SCID/IL-2Rγnull (NSG™) mice. Additionally, human T cells were adoptively transferred into the mice and re-directed to the tumor by a bispecific antibody.[3]

After two weeks, tumors were resected and dissociated into single-cell suspensions using the Tumor Dissociation Kit, human and the gentleMACS™ Octo Dissociator with Heaters (program 37C_h_TDK_1) as per manufacturer's instructions.

Enrichment of TILs

TILs were enriched from dissociated xenograft tumors using CD45 (TIL) MicroBeads, human and the autoMACS® Pro Separator. Regular CD45 MicroBeads, human were used for comparison. Experiments were performed according to the manufacturer's instructions. Figure 1 shows the basic principle of magnetic separation by MACS MicroBead Technology.

Flow cytometry

Cells were stained with a CD45-PE antibody (clone 5B1; Miltenyi Biotec) and analyzed by flow cytometry.

Results

Generation of single-cell suspensions from human xenograft tumors

Isolation of TILs from solid tumors requires effective, yet gentle, dissociation of the tumor tissue into single-cell suspensions. Combined mechanical and enzymatic dissociation with the gentleMACS Octo Dissociator with Heaters and the Tumor Dissociation Kit, human resulted in cell samples that were appropriate for subsequent TIL isolation based on the CD45 epitope (fig. 1).

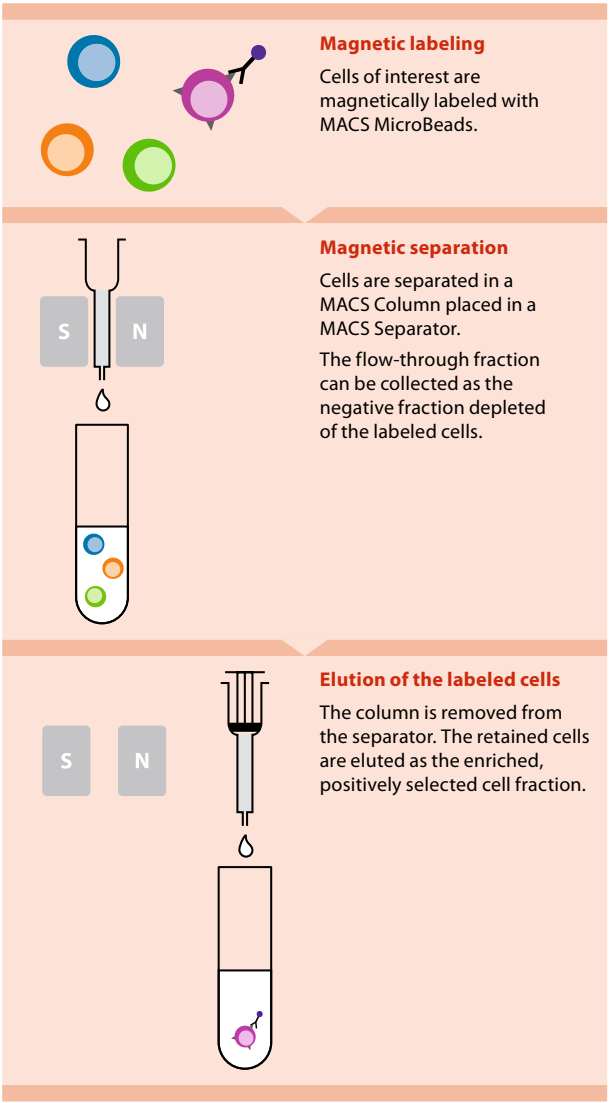

Figure 1: Principle of MACS MicroBead Technology. For the isolation of TILs from dissociated tumor tissue, cells are labeled with CD45 (TIL) MicroBeads and applied to the column. Positively selected TILs are eluted after removal of the column from the magnetic field. The autoMACS Pro Separator performs all three steps fully automatically.

Isolation of TILs from dissociated tumor tissue

CD45 (TIL) MicroBeads are specifically designed for the separation of TILs from dissociated tumor tissue. We used this reagent in combination with the autoMACS Pro Separator to isolate TILs from human xenograft tumors (fig. 2). Starting frequencies of CD45$^+$ cells in the tumor-derived single-cell suspensions amounted to about 30%. TILs were then isolated to frequencies of about 90% in relation to the entire tumor cell population (table 1).

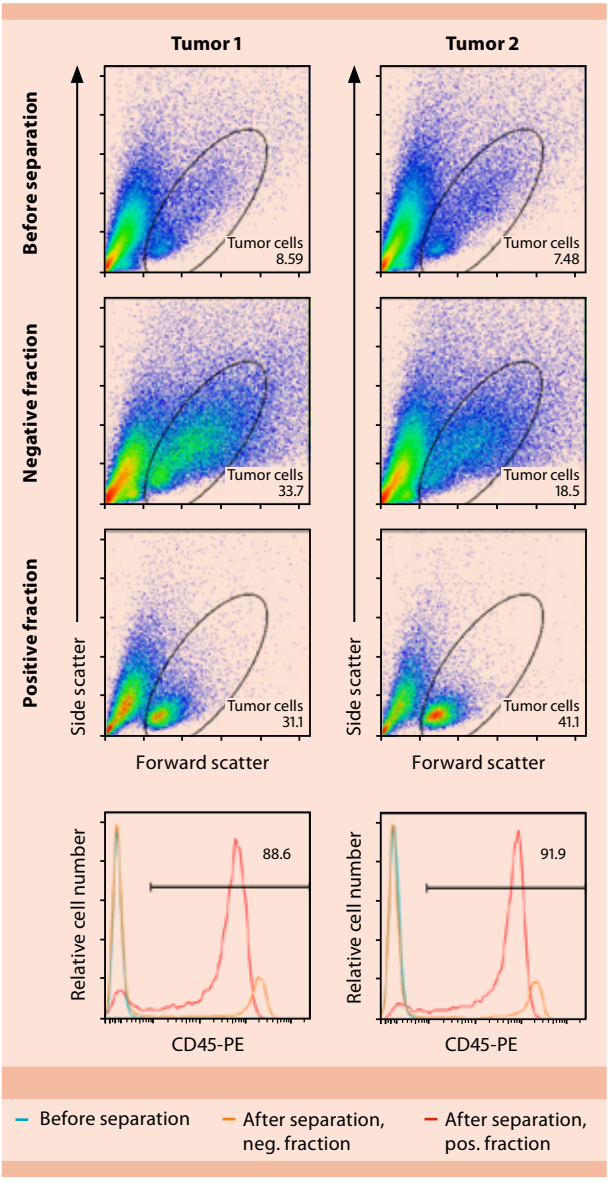

Figure 2: Isolation of TILs from human xenograft tumors using CD45 (TIL) MicroBeads. Tumor tissue samples from two independent experiments were dissociated using the gentleMACS Octo Dissociator with Heaters and the Tumor Dissociation Kit, human. Subsequently, CD45$^+$ TILs were isolated using CD45 (TIL) MicroBeads, human in combination with the autoMACS Pro Separator. Cells were stained with a PE-conjugated CD45 antibody and analyzed by flow cytometry. The negative fraction consisted of cells that were not magnetically labeled with CD45 (TIL) MicroBeads, i.e., CD45$^-$ non-target cells. The positive fraction contained the magnetically labeled CD45$^+$ TILs. Gates denote the tumor cell populations.

	Tumor 1	Tumor 2
Before separation	29.6%	29.3%
After separation, negative fraction	0.7%	0.8%
After separation, positive fraction	88.6%	91.9%

Table 1: Isolation of TILs from human xenograft tumors. Numbers indicate the percentages of CD45$^+$ cells in relation to the entire tumor cell population, before and after separation with CD45 (TIL) MicroBeads. Data correspond to the experiments shown in figure 2.

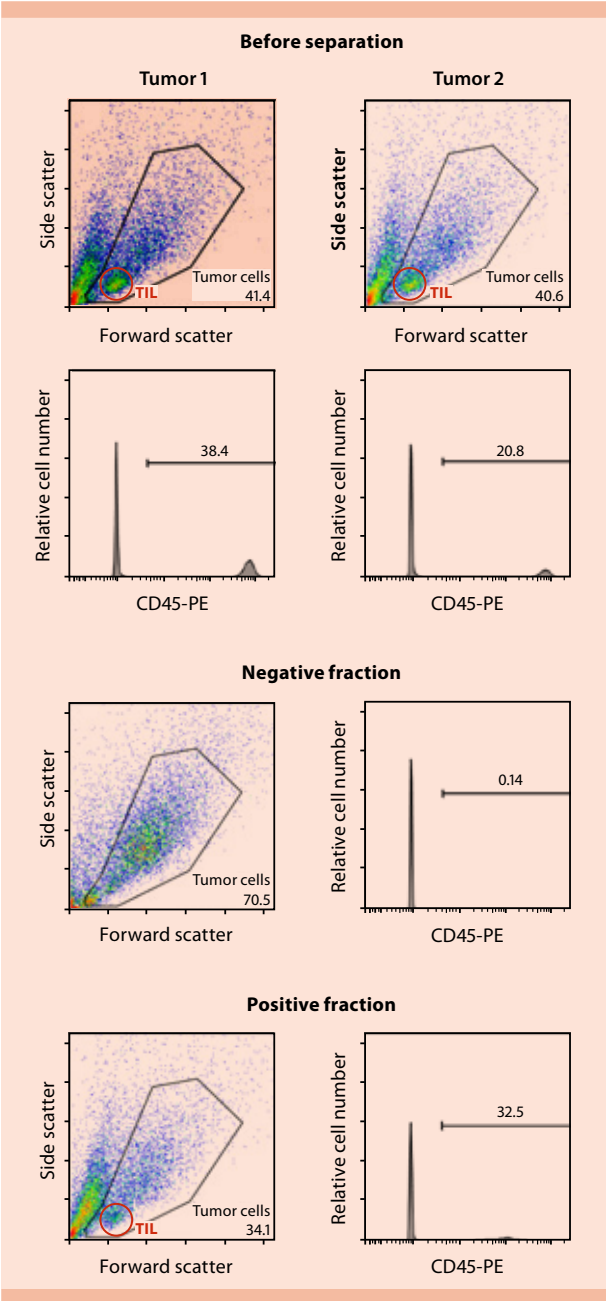

Figure 3: Separation of cells from human xenograft tumors using CD45 MicroBeads. Tumor tissue samples from two independent experiments were dissociated into single-cell suspensions using the gentleMACS Octo Dissociator with Heaters and Tumor Dissociation Kit, human. Prior to cell separation, the two samples were pooled and labeled with CD45 MicroBeads, human. The combined samples were processed using the autoMACS Pro Separator. Cells were stained with a PE-conjugated CD45 antibody and analyzed by flow cytometry. Gates denote the tumor cell populations.

For comparison, we also used regular CD45 MicroBeads, which are designed for positive selection or depletion of CD45$^+$ cells from peripheral blood. We found no significant enrichment of TILs from dissociated tumor tissue with this reagent (fig. 3, table 2), indicating that CD45 (TIL) MicroBeads are the reagent of choice when using tumor tissue as starting material for the enrichment of CD45$^+$ cells.

	Tumor 1	Tumor 2
Before separation	38.4%	20.8%
After separation, negative fraction	0.14%	
After separation, positive fraction	32.5%	

Table 2: Separation of cells from human xenograft tumors using CD45 MicroBeads. Numbers indicate the percentages of CD45$^+$ cells in relation to the entire tumor cell population before and after separation with CD45 MicroBeads. Data correspond to the experiment shown in figure 3.

Conclusion

- gentleMACS™ Octo Dissociator with Heaters and Tumor Dissociation Kit, human provide an automated solution for combined mechanical and enzymatic dissociation of tumors into single-cell suspensions. The dissociated tumors are suitable for subsequent isolation of TILs based on the CD45 antigen.
- CD45 (TIL) MicroBeads, which are optimized for use with tumor tissue, and the autoMACS Pro Separator enable reliable automated isolation of TILs.
- Purities of isolated TILs amount to approximately 90%.

References

1. Hinrichs, C. S. and Rosenberg, S. A. (2014) Exploiting the curative potential of adoptive T-cell therapy for cancer. Immunol. Rev. 257: 56–71.
2. Rosenberg, S. A. *et al.* (2011) Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin. Cancer Res. 17: 4550–4557.
3. Fisher, T. S. *et al.* (2017) A CD3-bispecific molecule targeting P-cadherin demonstrates T cell-mediated regression of established solid tumors in mice. Cancer Immunol. Immunother. https://doi.org/10.1007/s00262-017-2081-0.

All procedures performed on animals were in accordance with regulations and established guidelines and were reviewed and approved by Pfizer's Institutional Animal Care and Use Committee. Research was conducted on human samples acquired in accordance with all applicable Pfizer policies including IRB approval.

MACS Product	Order no.
gentleMACS Octo Dissociator with Heaters	130-096-427
Tumor Dissociation Kit, human	130-095-929
autoMACS Pro Separator – Starter Kit	130-092-545
CD45 (TIL) MicroBeads, human	130-118-780
CD45-PE, human (clone 5B1)*	
CD45-PE, human (clone REA747)*	

* Order numbers for different sizes and additional conjugates are available at **www.miltenyibiotec.com/antibodies**