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Complete workflow to sort highly pure and viable sub-populations of TILs from a single mouse tumor sample

Evelyn Rodriguez-Mesa[1], Daryl Grummitt[1], Janina Brauner[2], David Agorku[2], Olaf Hardt[2]
[1]Owl Biomedical, Santa Barbara, USA,
[2]Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany

Background

Immunotherapy has proven its clinical efficacy and tremendous potential in a number of cancers, but significant clinical benefit has only been experienced by a subset of patients. One major goal is therefore to understand how the composition of tumor-infiltrating myeloid and lymphoid cells contributes to the patient-stratification. However, TIL numbers can be very low and small sub-populations can easily be lost in the background noise, particularly when dealing with single cell analysis. Magnetic enrichment of CD45$^+$ TILs from dissociated tumors, as well as traditional FACS sorting, are frequently used in the field. However, the options offered by various suppliers are not optimized for tumor material and therefore provide only low purity and low viability of target TILs. Furthermore, they do not allow the isolation of different cell sub-populations from the same sample, thus requiring more precious starting material and more rounds of analysis. Here, we applied a semi-automated complete workflow to dissociate tumor tissue of a mouse model into single-cell suspensions, magnetically pre-enrich CD45$^+$ TILs, and subsequently sequentially sort highly pure and viable TIL sub-populations (fig. 1).

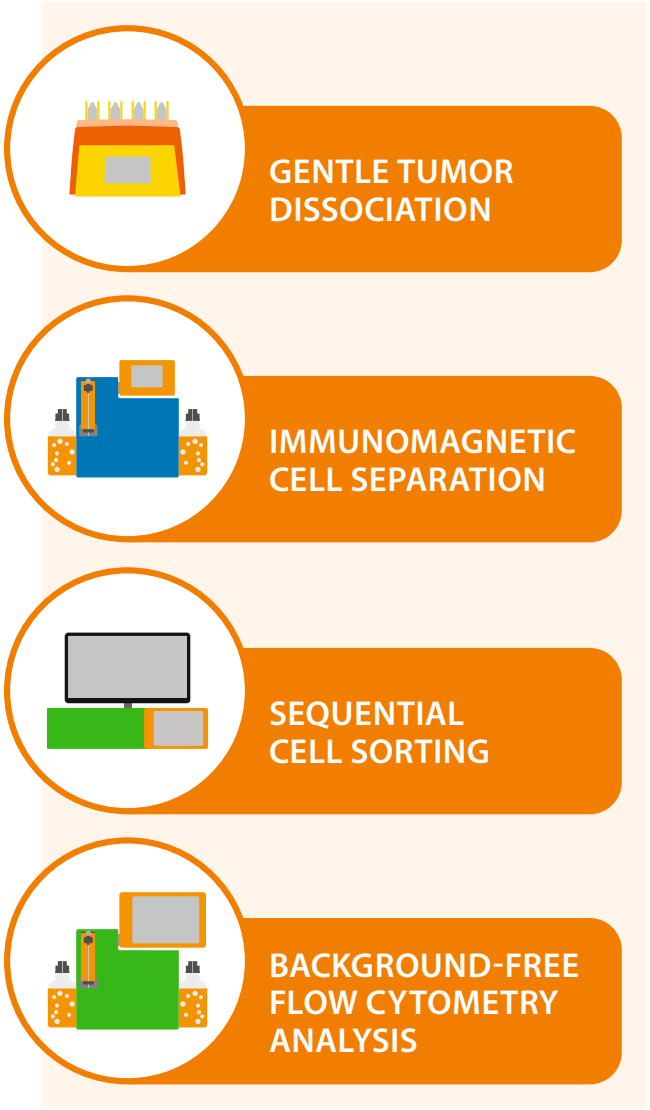

Figure 1: Semi-automated complete workflow to isolate highly pure and viable sub-populations of TILs.

Materials and methods

Tumor dissociation
An amount of 1g of CT26 mouse tumor was dissociated using the gentleMACS™ Octo Dissociator with Heaters and the Tumor Dissociation Kit, mouse, as per manufacturer's instructions.

Immunomagnetic pre-enrichment of CD45⁺ TILs
In order to increase the purity and the frequency of target TILs, dissociated cells were magnetically pre-enriched using the CD45 (TIL) Microbeads, mouse – specifically optimized for tumor tissue – on the automated separator autoMACS™ Pro, as per manufacturer´s instructions.

Sequential sorting of TIL sub-populations with the MACSQuant® Tyto®
The MACSQuant Tyto is a benchtop cell sorter equipped with three lasers to enable multiparameter sorting, which offers the flexibility needed to sort TIL sub-populations. The sorting process occurs exclusively in the fully closed and sterile cartridge, so there is no opportunity for sample-to-sample cross-contamination. The gentle sorting in the MACSQuant Tyto is based on a mechanical valve in a microchip sitting on the bottom of the cartridge. Compared to droplet-based sorting, microchip-based cell sorting occurs under low pressure and mild shear forces, and without any decompression or charge applied to the cells. Gentle microchip-based cell sorting preserves the full functionality of the sorted cells, allowing the best performance and reliability for any downstream application. In this setting, sequential sorting of different sub-populations of TILs from one tumor sample was made possible.

LEARN MORE

If you want to learn more about this unique sorting principle watch this video:

- **miltenyibiotec.com/products/macs-flow-cytometry/cell-sorter/macsquant-tyto-sorting-principle.html**

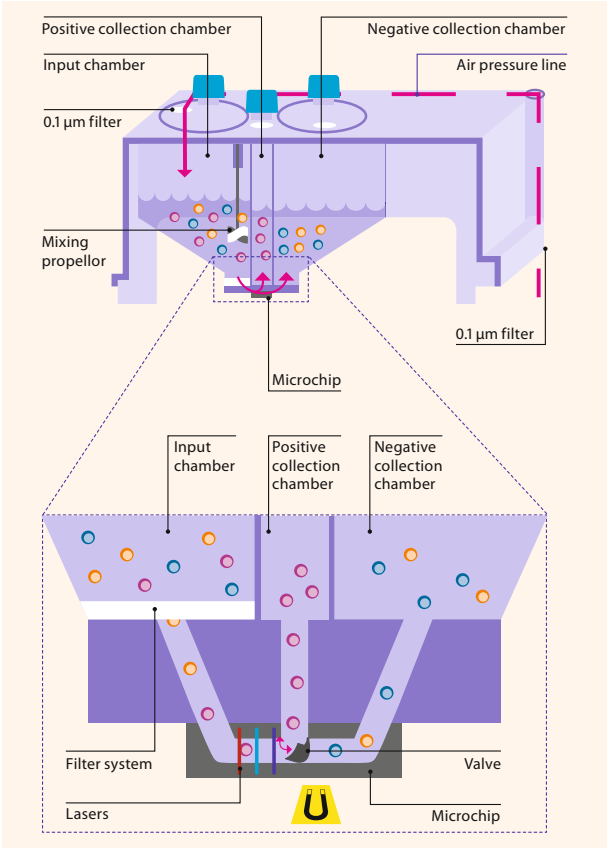

Figure 2: The sorting mechanism of the MACSQuant® Tyto®.

Cells come from the input chamber and cross a filter system before entering the microchip through a microchannel, where they are interrogated by the three lasers. When a target cell (pink) is identified, a magnetic pulse coming from the solenoid opens the microvalve, which then redirects the target cell into the positive collection chamber. In the default state, the valve is closed, allowing non-selected cells (blue and orange) to flow through into the negative collection chamber.

CD45⁺ (TIL)-enriched fraction was used to sort two sub-populations of TILs using the MACSQuant Tyto Cell Sorter. Cells were sorted sequentially in two sorts, from the same starting material. A high-speed version of the cartridge was used (MACSQuant Tyto Cartridge HS), facilitating a significantly reduced sorting time while increasing sort performance through inertial focusing. First, the CD45⁺-enriched fraction was used as the input to sort NK cells. Non-NK cells from the negative collection chamber were then used to sort B cells.
First sort (NK cells): $CD45^+CD11b^-$, Anti-$NKp46^+$.
Second sort (B cells): $CD45^+$, $CD11b^-$, Anti-$NKp46^-$, $CD19^+$.

Background-free flow-cytometric analysis of target cells
Before and after the TIL sorting, cells were phenotyped by flow cytometry on the MACSQuant® Analyzer 10 to assess the expression of lineage markers, and define cell purity, as well as cell viability. Analyses were performed using REAfinity™ Recombinant Antibody clones, which guarantee unbiased, reproducible cell phenotyping without the need to utilize FcR blocking[1].

Results

Tumor dissociation followed by automated cell separation enables the pre-enrichment of highly pure and viable CD45⁺ TILs

Thanks to the gentleMACS™ Octo Dissociator with Heathers and the Tumor Dissociation Kit, mouse, 1g of CT26 mouse tumor was effectively dissociated, exhibiting an average of ~21.5% CD45⁺ cells (fig. 3A). By virtue of TIL-optimized CD45 enrichment, the respective cell purity was increased to up to 81% (fig. 3B). Using the appropriate gating strategy, the NK and B cell sub-populations could be further identified with frequencies of 4% and 26%, respectively. Furthermore, the tumor dissociation followed by the TIL enrichment process demonstrated its gentleness to cells by preserving their viability (figs. 4 and 5 lower dot plots).

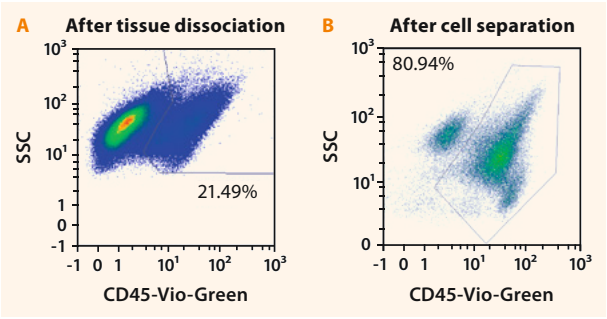

Figure 3: Purity of CD45⁺ TILs before (A) and after (B) immunomagnetic enrichment. The CD45⁺ cell purity was assessed by flow cytometry after tumor-optimized tissue dissociation (A, exemplary dot plot), as well as after TIL-specific immunomagnetic enrichment (B).

The MACSQuant® Tyto® Cell Sorter enables the sequential isolation of NK and B cells from the same tumor sample without affecting the cell viability

Sort results demonstrated a selective isolation of highly pure TIL sub-populations, starting from the same sample, while preserving high cell viability.

In the first sorting, the purity of isolated NK cells (CD45⁺/CD11b⁻/Anti-NKp46⁺) could be increased from 4–97% with a cell viability of 98% (fig. 4).

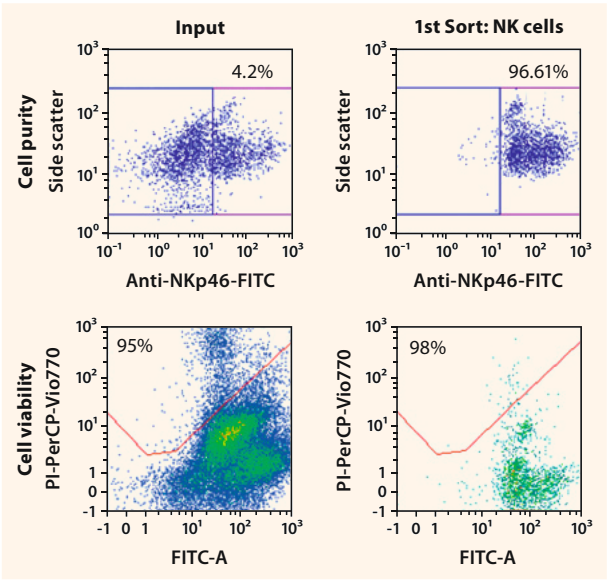

Figure 4: First sorting: tumor-infiltrating NK cells. The MACSQuant Tyto allowed the isolation of highly pure CD45⁺/CD11b⁻/Anti-NKp46⁺ NK cells (96.6%) while preserving cell viability (98%).

In the consecutive sorting from the negative fraction of the first isolation, the purity of sorted B cells (CD45⁺/CD11b⁻/Anti-NKp46⁻/CD19⁺) was increased from 26–97%, with a cell viability of 99% (fig. 5).

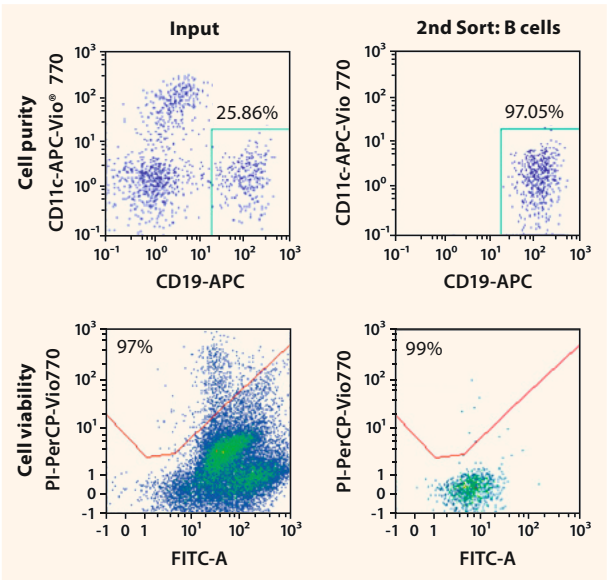

Figure 5: Second sorting: tumor-infiltrating B cells. Starting from the negative fraction of the first sort, the MACSQuant® Tyto® allowed a second consecutive isolation of highly pure CD45⁺/CD11b⁻/Anti-NKp46⁻/CD19⁺ B cells (97%) while preserving their viability (99%).

Conclusions

- We have developed an innovative workflow to isolate viable TIL sub-populations starting from the same fresh tumor tissue, thereby maximizing analysis potential from limited material.
- With this workflow, highly pure B and NK cells can be obtained in only six hours, without affecting cell viability, thus increasing the quality of data obtained in immuno-oncological research.
- The sorting technology of the MACSQuant Tyto allows for TIL purities >95% while being gentle to the cells.

References

1. Application Note by Miltenyi Biotec: Background-free analysis of mouse TILs.

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Miltenyi Biotec B.V. & Co. KG | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macsde@miltenyi.com | **www.miltenyibiotec.com**

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