

Workflow automation and parallelization improves the isolation and analysis of tumor-infiltrating immune cell subpopulations

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Introduction

Immunotherapy against cancer has proven clinical efficacy and tremendous tion with specific TIL isolation. Tumor dissociation was automated using the potential in multiple tumor entities. Syngeneic mouse tumor models gentleMACS™ Octo Dissociator and optimized for epitope preservation to represent the gold standard to analyze effects of immunotherapy, as they overcome bias in immunophenotyping caused by dissociation with aggrespossess a fully competent immune repertoire. However, the amount and sive or impure enzymes. Moreover, isolation of TILs was improved by develcomposition of tumor-infiltrating leukocytes (TILs) is highly variable, compli- oping a new CD45-specific enrichment reagent for magnetic cell sorting, cating the analysis of individual subpopulations. In particular, small subpopu- based on MACS[®] Technology, directly from dissociated tumor tissue. The lations might escape analysis as they could get lost in the background noise. whole workflow takes only about 90 min to complete. To fulfill the need for When working with large cohort sizes, even immunophenotyping of TILs parallelization and automation of sample processing in large cohort sizes by flow cytometry is time consuming and data processing highly work intensive. Therefore, pre-enrichment of TILs is highly desirable to increase the sensitivity of analysis and save time and effort during flow cytometry. To this handling platform. This new system, the MultiMACS X, can perform 24 cell end, we have established an automated workflow combining tissue dissocia-

used in *in vivo* studies, a fully automated version of the MultiMACS[™] Cell24 Separator was developed by integrating the instrument into a robotic liquid separations in parallel with minimal hands-on time.

Reliable and fast isolation of TILs from syngeneic mouse tumors

by injection of three different murine tumor cell lines. Tumors derived from 95% for B16-F10 tumors (fig. 1B and C). B16-F10 melanoma cells showed TIL frequencies of 2–4% among total viable

We have developed a new CD45-specific enrichment reagent for magnetic cells after dissociation. CT26.WT cell-derived colon carcinoma contained cell sorting (MACS Technology) directly from dissociated tumor tissue 15–21%, and 4T1 cell–derived breast carcinoma 32–37% TILs. Using a manual (fig. 1A). To validate this method for starting material showing variable separation system, TILs were enriched to purities above 90% at yields above frequencies of TIL infiltration, we used syngeneic mouse tumors induced 70% for CT26.WT and 4T1 tumors, and purities above 80% at high yields above





Immunophenotyping of TIL subpopulations

We used flow cytometry to detect and quantify TIL subpopulations in such as plasmacytoid dendritic cells (pDCs), which could be detected reliably syngeneic mouse tumors induced by subcutaneous transplantation of only in the pre-enriched sample. Importantly, the composition of infil-B16-F10 melanoma cells. This tumor model showed TIL frequencies of only trating immune cell populations was not affected, excluding the risk of 2–4%, making it difficult to analyze smaller immune cell subpopulations in a introducing a bias by this method (fig. 2C). An alternative approach to increase reliable way. To directly compare the results from samples without sensitivity would be to acquire more events during flow cytometric (fig. 2A) or with (fig. 2B) prior CD45-specific TIL enrichment, we kept the analysis. However, for this tumor, the overall TIL enrichment factor was number of acquired events, and therefore acquisition time, constant. In 48-fold. This means that an acquisition of 4,800,000 events from the both cases, 100,000 events were acquired for subsequent analysis. In the unseparated fraction would have been necessary to reach an equal level of sample without TIL enrichment, it was hard to detect and quantify immune detection as the acquisition of 100,000 events from the enriched fraction. cell subpopulations. This was particularly obvious for small subpopulations,





Automation and parallelization of TIL isolation

Automation and parallelization of tumor dissociation has been achieved instrument into a robotic liquid handling platform. This new system, the previously by developing the gentleMACS Octo Dissociator. As TIL MultiMACS X (fig. 3A), can process 24 cell separations in parallel with minimal subpopulation analysis is a common readout for pre-clinical *in vivo* studies in hands-on time. When compared to the manual system, equal purities were the immuno-oncology field, the method for TIL isolation had to be adapted achieved, while the overall yield of target cells was increased from 70% to to large cohort sizes as well. To address this need, a fully automated version 90% as shown for TILs from CT26.WT tumors (fig. 3B). of the MultiMACS Cell24 Separator was developed by integrating the





• We have developed an automated workflow for the isolation of TILs from mouse tumors, streamlining downstream analysis while standardizing and enhancing the detection and quantification of immune cell subpopulations.

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Importantly, while the TIL enrichment significantly reduced time and reagent costs for immune cell subset analysis, the composition of infiltrating immune cell populations was not affected, excluding the risk of introducing a bias by this method.