Introduction

Immunotherapy against cancer has proven clinical efficacy and tremendous potential in multiple tumor entities. Syngeneic immune tumor models represent the gold standard to analyze effects of immunotherapy as they provide a fully controlled immune environment. However, the amount and diversity of infiltrating immune cells vary between tumor entities. The analysis of individual subpopulations in the context of tumor-specific TILs is particularly challenging. Therefore, pre-enrichment of TILs is highly desirable to increase the potential in multiple tumor entities. Syngeneic mouse tumor models have been used in various countries worldwide. Copyright © 2017 Miltenyi Biotec GmbH and/or its affiliates. All rights reserved. Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for ... VioBlue, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec GmbH and/or its affiliates in countries worldwide.

1 Reliable and fast isolation of TILs from syngeneic mouse tumors

We have developed a new CD45-specific enrichment reagent for magnetic cell sorting (MACS) Technology. Briefly, human dissociated tumor tissue (fig. 2A) or with (fig. 2B) prior CD45-specific TIL enrichment, we kept the minimal T cell numbers, and therefore acquisition time, constant. In light of the results, the overall TIL enrichment factor was determined to be 15-fold. This means that an acquisition of 4,800,000 events from the unseparated fraction would have been necessary to reach an equal level of detection as the acquisition of 300,000 events from the enriched fraction.

2 Immunophenotyping of TIL subpopulations

We used flow cytometry to detect and quantify TIL subpopulations in syngeneic immune tumors induced by subcutaneous transplantation of B16-F10 melanoma cells. The tumor model showed TCell Frequencies of only 2–4%, making it difficult to analyze single immune cell subpopulations in a whole tumor tissue sample. Therefore, we used the MultiMACS X to develop a panel of antibodies based on MACS Technology, directly from dissociated tumor tissue. The whole workflow was automated with a gentleMACS™ Octo Dissociator and optimized for epitope preservation to enhance the detection and quantification of infiltrating immune cell populations.

3 Automation and parallelization of TIL isolation

Automation and parallelization of tumor dissociation has been achieved previously by developing the gentleMACS Octo Dissociator. At TIL subpopulation analysis is a common method for pre-clinical or clinical studies in various countries worldwide. Copyright © 2017 Miltenyi Biotec GmbH and/or its affiliates. All rights reserved. Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for...