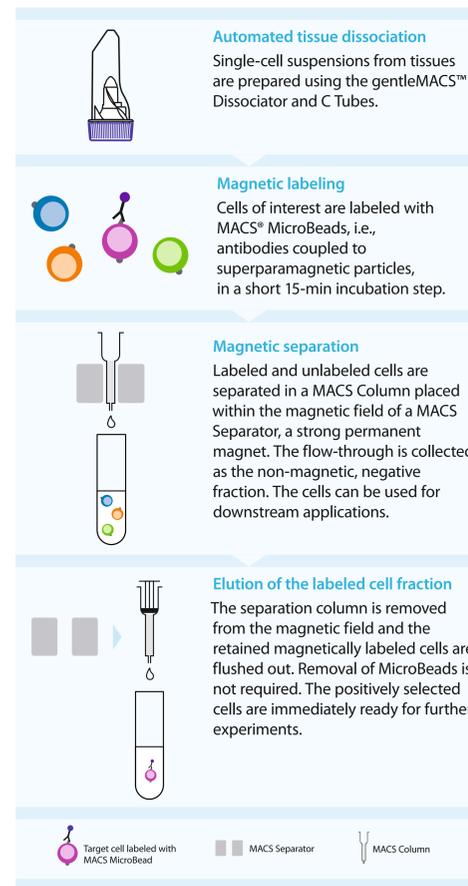


## Introduction

The analysis of tumor-resident cell and stem cell populations requires proper methods for tissue dissociation and cell purification. Over the past few years, there has been a particular focus on the study of tumor-infiltrating lymphocytes (TIL) and cancer stem cells (CSCs). TIL can be isolated and characterized using conventional lymphocyte markers (1) whereas CSC-specific cell surface markers are currently being established (2). Two important markers, namely CD24 and CD44, have been shown to define CSC in various neoplasms such as breast cancer (3) or colon cancer (4).

We have established methods for the enzymatic and mechanical dissociation of solid tumors and optimized them according to the specific needs of a given tissue or cell type. The major goal was to disrupt the extracellular matrix and cell adhesion components without harming the integrity of the cell membrane. This was achieved by a combination of varying enzyme mixes, mechanical forces, incubation periods, and temperatures. The automation of all mechanical steps, using the gentleMACS™ Dissociator, led to reproducible and moreover user-independent results with reduced overall processing times. In addition, we have developed novel methods for the isolation of CD44<sup>+</sup> and CD24<sup>+</sup>CD44<sup>+</sup> cell subpopulations by magnetic cell sorting (MACS® Technology).



## Conclusion

1. We have developed a semi-automated protocol for the dissociation of melanoma tissue leading to single-cell suspensions with good yields and viabilities.
2. The isolated TIL can be efficiently expanded in culture and are functional *in vitro* and *in vivo*.
3. A subpopulation of CD44<sup>+</sup> human teratocarcinoma cells was enriched from a starting frequency of 9% to a final purity of 92%.
4. Combining depletion of CD24<sup>+</sup> cells with subsequent enrichment of CD44<sup>+</sup> cells, CD24<sup>+</sup>CD44<sup>+</sup> cell subpopulations can be easily isolated.

## Outlook

The separation of pure tumor cell populations is necessary for their further characterization and targeted drug screening approaches. The methods presented here are suitable for a wide variety of applications as they can be easily adapted to

other cancer tissues and cell types and may help to optimize and standardize cell separation procedures in future basic and clinical cancer research.

## Results

### 1 Dissociation of melanoma tissue and generation of TIL cultures

Human melanoma metastases were dissociated using a standardized, semi-automated protocol based on a combination of mechanical tissue disruption and Collagenase IV digestion (5). The results of this protocol were compared to the classical protocol, in which the tumor tissue is dissociated by overnight incubation in Collagenase IV-containing medium under continuous agitation (1). After dissociation, the single-cell suspensions are composed mainly of tumor cells (TC), tumor-infiltrating lymphocytes (TIL), and erythrocytes (ERY) (A). When compared to the overnight digest, no significant difference was observed concerning the cellular composition (data not shown), the yield of viable cells (B), or the overall viability (C). However, the isolated TIL from the standardized, semi-automated protocol expanded significantly faster than those from manually dissociated tumors (D). Further analysis of these expanded TIL showed full functionality of the cells *in vitro* and *in vivo* (data not shown).

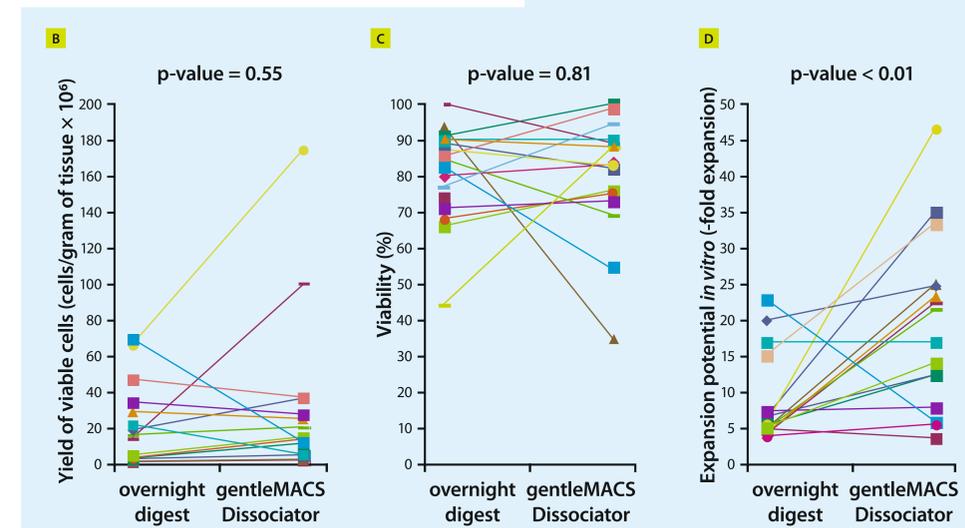
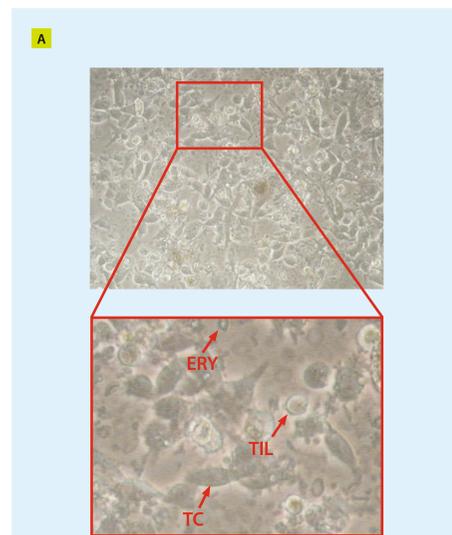


Figure 1

### 2 Isolation of CD44<sup>+</sup> human teratocarcinoma cells

CD44 is an important marker for cancer stem cells in various neoplasms (2). To provide a novel tool for the isolation of CD44<sup>+</sup> cell populations, a monoclonal antibody specific for CD44 was coupled to superparamagnetic MACS MicroBeads. A minor subpopulation (approximately 9%) of the human teratocarcinoma cell line NTERA-2cl.D1 expresses the CD44 antigen (A and B). After trypsin-based dissociation, the cells were incubated with CD44 MicroBeads for 15 minutes, washed, separated using an LS Column, and analyzed by flow cytometry using the MACSQuant® Analyzer. Dead cells were identified by PI fluorescence and excluded from the analysis. Using this approach, the CD44<sup>+</sup> cell population was enriched to 92% purity (C).

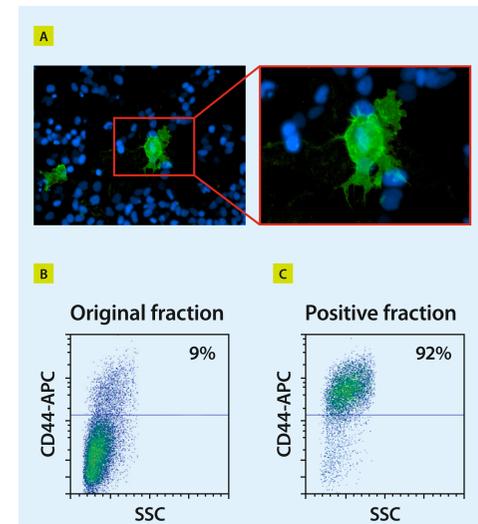


Figure 2

### 3 Isolation of CD24<sup>+</sup>/CD44<sup>+</sup> human chronic myeloid leukemia (CML) cells

Human mammary carcinoma stem cells are defined by the expression of CD44 and the absence of CD24 (3). To allow for the isolation of CD24<sup>+</sup>CD44<sup>+</sup> cell populations, we combined depletion of CD24<sup>+</sup> cells with subsequent enrichment of CD44<sup>+</sup> cells. K562 human CML cells show heterogeneity for CD24 as well as CD44 expression similar to human breast cancer cells (A and reference 3) and were used as a model system. The cells were incubated with CD24-Biotin, washed, labeled with Anti-Biotin-MicroBeads, and CD24<sup>+</sup> cells were depleted using an LD Column. The flow-through was then labeled with CD44 MicroBeads for 15 minutes, washed, separated using an LS Column, and analyzed by flow cytometry using the MACSQuant Analyzer. Dead cells were identified by PI fluorescence and excluded from the analysis. Using this approach, a CD24<sup>+</sup>CD44<sup>+</sup> cell population was isolated with a final purity of 95% (B).

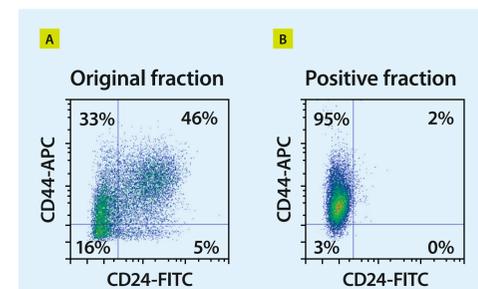


Figure 3

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