

Dissociated Tumor Cells

A Viable Alternative to Fresh Tumor Tissue

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INTRODUCTION

Primary tumor tissue is an integral part of therapeutic and diagnostic research, but the logistics and budgetary challenges of obtaining fresh tissues can often lead to significant delays for research projects. The purpose of Dissociated Tumor Cells (DTCs) is to serve as a viable alternative to acquiring fresh tumor tissue. DTCs can be acquired quickly from inventory, from a variety of different solid tumor indications and unique patient donors, and are more cost effective than acquiring fresh tissue and working through dissociation protocols in house. These viable single cell suspensions are useful for many of the same downstream applications as fresh tissue (**Box 1A**). DTCs contain all of the various cell populations present in tumor microenvironment: tumor, immune and support cells (**Box 1B**).

Dr. Shawn Fahl has led a thorough examination of Discovery Life Science's DTC samples, conducted on 26 post-thaw samples across five major oncology indications. The body of this whitepaper presents the data and analysis from this study. Some important highlights from the findings are listed below:

Key Points:

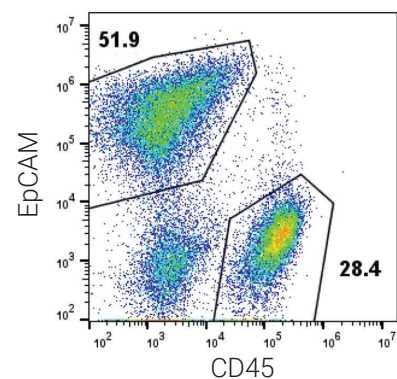
- DTCs contain high percentages of viable cells both pre-freeze (81.3%) and post-thaw (69.7%)
- DTCs consist of the full cellular composition of the tumor microenvironment (tumor, immune and support cells)
- All lymphoid subsets can be found in dissociated tumor cells (T cells, B cells and NK cells)
- Myeloid cells can be found in DTCs

Box 1.

A. Potential Applications:

- Cell isolation studies
- Flow Cytometry
- Cell Culturing
- Patient Derived Xenografts (PDX Models)
- Drug response and target discovery
- 3D Organoids
- Sequencing

B. Ovarian Cancer



Dissociated Tumor Cells (DTCs) Are Viable Following Dissociation and Cryopreservation

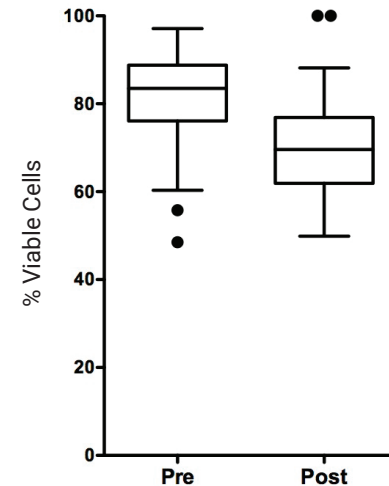
Dissociated Tumor Cells, or DTCs, are single cell suspensions generated from fresh primary tumors using both enzymatic and mechanical dissociation. Our tumor dissociation protocol has been validated for 5 cancer indications: colorectal cancer, kidney cancer, lung cancer, melanoma, and ovarian cancer. This gentle procedure results in an average viability of 81.3% across all indications (**Figure 1**). Following isolation, single cell suspensions are cryopreserved and stored in liquid nitrogen. Cells are thawed at 37°C, and following cryopreservation, an average viability of 69.7% was observed across all validated indications (**Figure 1**). Our dissociation protocol, therefore, provides excellent viability of DTCs and greater than 1 million viable cells per vial.

DTCs Recapitulate the Cellular Composition of the Tumor Microenvironment

The tumor microenvironment is composed of numerous cell populations in addition to tumor cells, including immune cells and tumor support cells such as fibroblasts and endothelial cells¹. To determine if our dissociated tumor cells accurately represented the cellular components of the tumor microenvironment, we performed flow cytometric analysis of each of the 5 validated indications for the presence of tumor and immune cells. Immune cells, identified using the pan-hematopoietic cell marker CD45, were present in all indications tested (**Figure 2A-B**). Unlike immune cells, which ubiquitously express CD45, tumor cells from different indications have differing cell surface expression. Epithelial cell adhesion molecule, or EpCAM, is expressed on many different tumors², and we were able to readily identify EpCAM+ tumor cells in colorectal, lung, and ovarian cancer DTCs (**Figure 2A**). Melanoma tumor cells, on the other hand, do not express EpCAM³, but do often express melanoma cell adhesion molecule, or CD146⁴. Indeed, melanoma tumor cells, as identified as CD146+, were observed in Melanoma DTCs (**Figure 2B**). Across all indications, we observed patient-specific variability in the percentage of tumor cells (**Figure 2C**) and immune cells (**Figure 2D**), highlighting the inherent differences between different patients and indications.

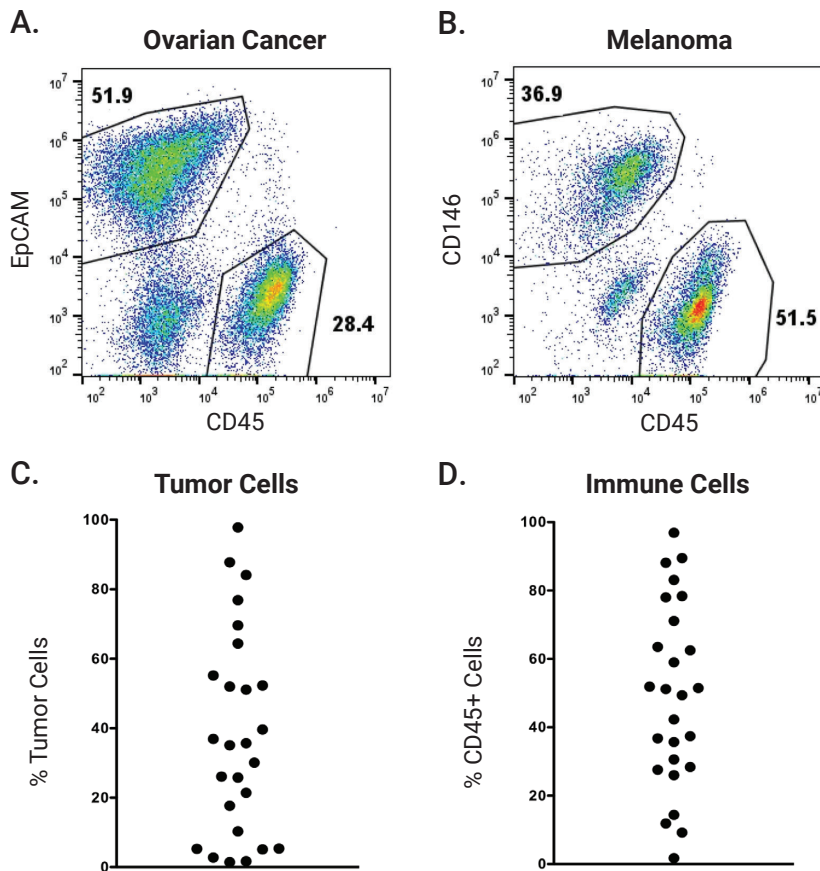
Figure 1.

Viability of DTCs in Validated Indications Prior to and Following Cryopreservation.



The viability of DTCs was measured directly following dissociation prior to cryopreservation and immediately following thawing. Viability was measured on a Nexcelom Cellometer by dual-fluorescence using acridine orange and propidium iodide. Data is compiled from 91 unique DTC samples across the 5 validated indications.

Figure 2.



Tumor and Immune Cells are Both Present in DTCS.

A. Ovarian Cancer DTCs were analyzed for the presence of tumor cells (EpCAM+), immune cells (CD45+), and tumor support cells (EpCAM- CD45-). Live cells were identified using propidium iodide and red blood cells were excluded by GlyA surface expression.

B. Melanoma DTCs were analyzed for the presence of tumor cells (CD146+), immune cells (CD45+) and tumor support cells (CD146- CD45-) after removal of red blood cells by GlyA and dead cells by propidium iodide.

C. Compilation of the percentage of tumor cells (defined as either EpCAM+ or CD146+ depending on indication) from 26 unique DTC samples across the 5 validated indications.

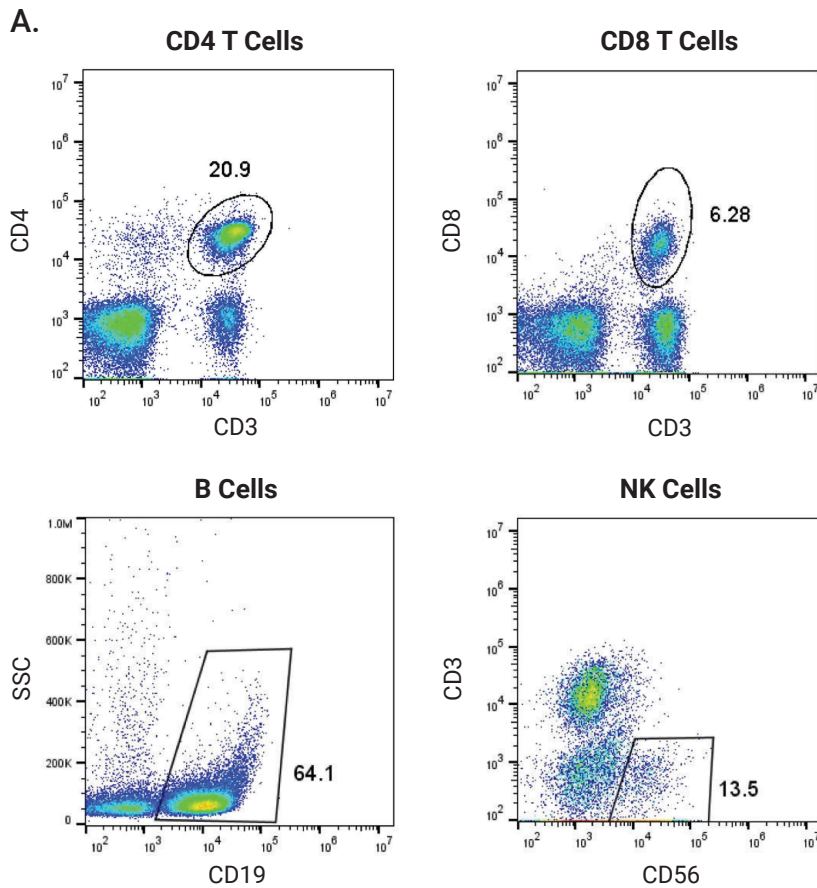
D. Compilation of the percentage of immune cells (defined as CD45+) from 26 unique DTC samples across the 5 validated indications.

All Major Immune Cell Subsets are Present in DTCs

The immune cell filtration into the tumor microenvironment is a heterogeneous mixture of cells including lymphoid cells, such as T cells and B cells⁵, as well as various myeloid cell populations⁶. As the percentage of CD45+ immune cells was maintained following dissociation and cryopreservation, we next evaluated what immune cell subsets constituted the CD45+ cells present in our DTC samples by flow cytometry. We were readily able to detect CD4 T cells, CD8 T Cells, B cells, and NK cells from the dissociated tumor samples (Figure 3A). Furthermore, there was patient-specific variability in the percentage of each lymphocyte subset across the 5 validated indications (Figure 3B). The major lymphoid subsets, therefore, are easily identified within our DTC samples.

Tumor-infiltrating myeloid cells have recently become a very active area of research, as they play a potentially vital role during tumor pathogenesis⁶. Examination of the CD45+ immune cells present within our dissociated tumor cells revealed that myeloid cells were maintained during the dissociation and cryopreservation process. Indeed, we were able to detect CD11b+ myeloid cells, as well as CD14+ monocytic and CD15+ polymorphonuclear cells, within our DTC samples (Figure 4A). Similar to the lymphocyte subsets, the myeloid subsets demonstrated patient-specific variability across our 5 validated indications (Figure 4B). Collectively, the immune cell profiling of our DTC samples demonstrated that the major lymphoid and myeloid cells are maintained and viable following dissociation and cryopreservation.

Figure 3.



Lymphocyte Subsets are Present in DTCs.

A. Lung cancer DTCs were analyzed for the presence of CD4 T cells (CD3+ CD4+), CD8 T cells (CD3+ CD8+), and B cells (CD19+). Ovarian cancer DTCs were analyzed for the presence of NK cells (CD3- CD56+). Live cells were identified by propidium iodide and immune cells were identified by CD45.

B. The percentage of each lymphocyte population of CD45+ cells from a representative DTC sample for each validated indication of 5-6 unique DTC samples per indication analyzed.

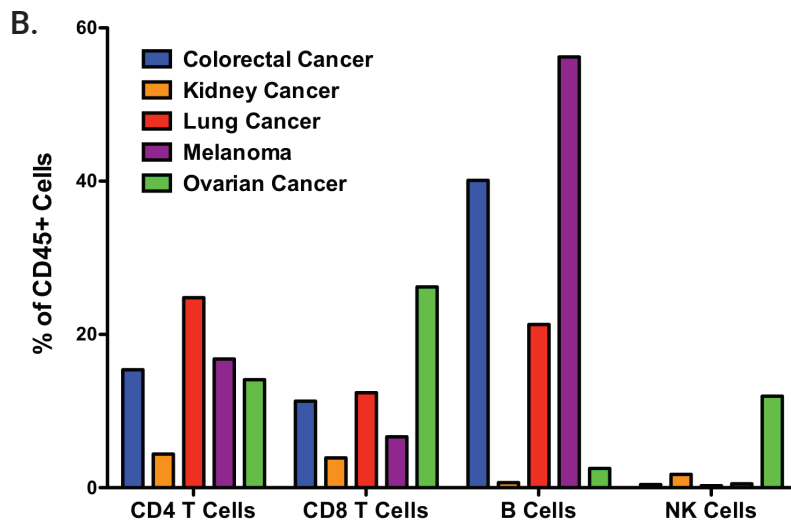
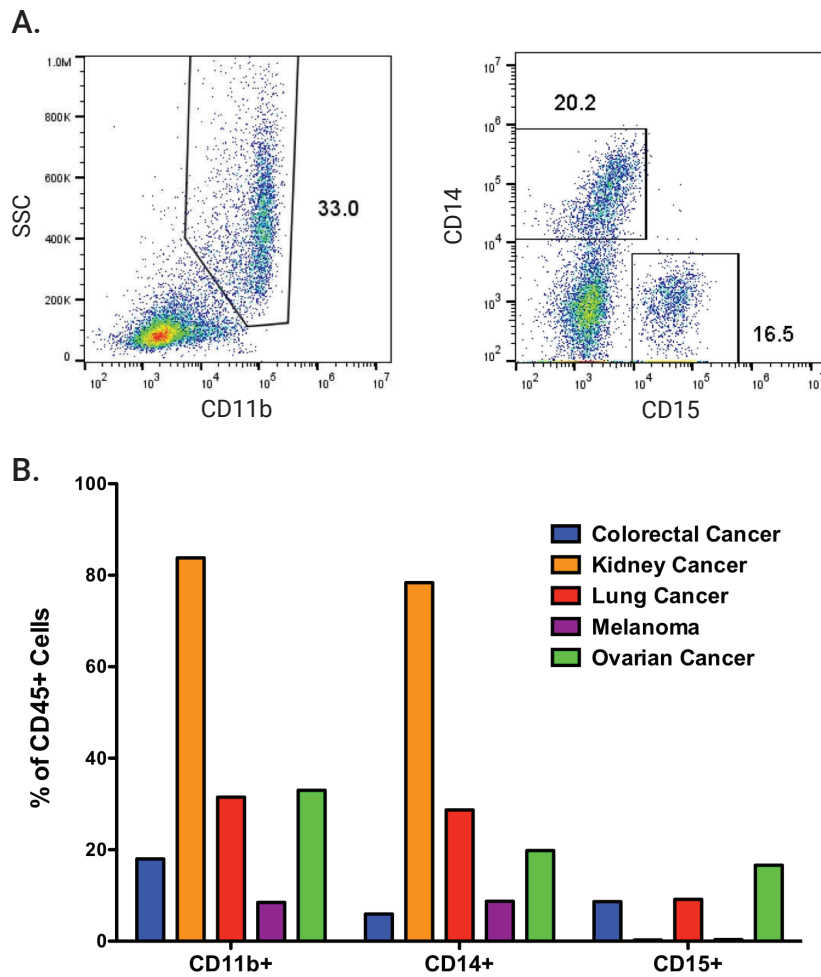


Figure 4.



Myeloid Cells are Present in DTCs.

A. Ovarian DTCs were analyzed for the presence of myeloid cells (CD11b+), monocytes (CD14+), and polymorphonuclear cells (CD15+). Live cells were identified by propidium iodide and immune cells were identified by CD45.

B. The percentage of each myeloid population of CD45+ cells from a representative DTC sample for each validated indication of 5-6 unique DTC samples per indication analyzed.

References:

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