Flow-Based Phenotyping of Tumor-Infiltrating Immune Cells in Different I/O Tumor Models

Abstract B75

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INTRODUCTION

Cancers are increasingly being recognized as abnormal immunological conditions, in addition to the longstanding knowledge of their genetic aberration. Immuno-oncology (I/O) treatments, e.g. checkpoint inhibitors, have achieved great success in treating cancers, reflective of this notion. However, not all patients respond to these treatments, likely due to patients' and/or tumors' genetic and immunological makeup, particularly of the tumor microenvironment (TME). The connections between specific immune compartments of the TME, and tumor genomic profiles, with response to I/O treatment remain to be elucidated, and experimental animal models could potentially be very useful (Li *et al*. *Pharmacol Ther* 2017;173:34-46).

By far the most commonly used I/O animal model has been mouse syngeneic tumors, but with certain limitations. We therefore set out to develop various novel I/O models and investigate the TME, and its relationship to treatment outcomes. We are testing three important I/O models that can provide unique I/O evaluation, as we have recently described, including: 1) MuPrime™, GEMM tumor-derived homografts; 2) syngeneic cell line homograft tumors in chimeric HuGEMM™ mice with human targets knocked in (KI); and 3) PDX in HuNSG™, humanized mice engrafted with human hematopoietic stem cells (CD34+, The Jackson Laboratory).

We first developed a plethora of unique tumor dissociation protocols, tailored to dissociate various model tumors. We then used multi-color flow cytometry (up to 16 colors) to systematically analyze the tumor-infiltrating immune cells, either mouse or human, to establish baselines for these model tumors in terms of tumor immune phenotypes characterized by specific immune compositions: total leukocytes (CD45+), T cell panels (CD3+) of different subtypes: T_{helper}, T_{reg}, CTL, naïve, memory, and fffector T cells; macrophage panels of subtype M1, M2, G-MDSC, M-MDSC, as well as NKs and NTK, etc. These immune baseline datasets set the stage for these tumor models to be used for comprehensive I/O research.

METHODS

Fig 1. Concept of MuPrime and HuGEMM. (A) MuPrime: mouse version of PDX within a fully functional mouse immune system; **(B)** HuGEMM: a chimeric mouse tumor model with fully functional murine immune system but a humanized drug target

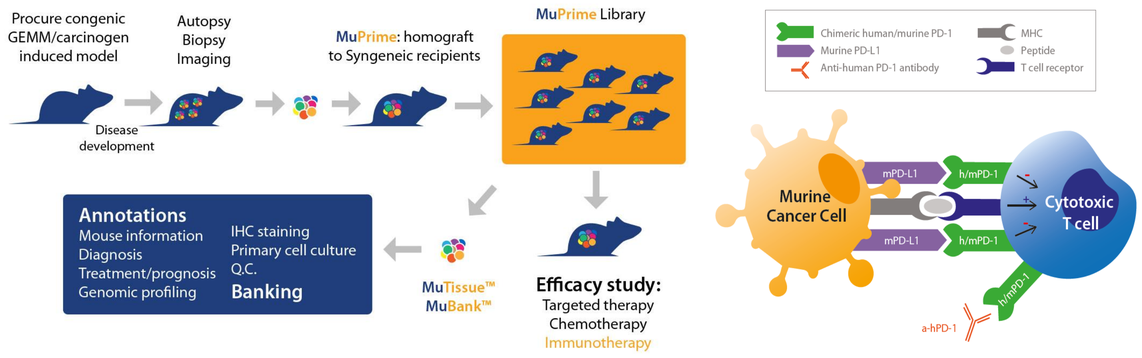


Table 1: Commonly used murine immunological markers. The most common immune cell lineages in blood, spleen, and tumor, as well as associated markers, respectively. In the study, 16 murine markers are used: CD45, CD3, CD4, CD8, FoxP3, CD11b, Ly6c, Ly6g, F4/80, CD206, IA/IE, CD19, PD-1, PD-L1, CD335, and live/dead

		Markers	Immune Cell Populations
Tumor	Blood/spleen	CD45	Total leukocytes
		CD3	Total T cells
		CD3+CD4+CD8-	CD4+ T helper cells
		CD3+CD4-CD8+	CD8+ cytotoxic T cells
		CD44/CD62L	Naïve, memory, and effector T cells
		CD69/CD44/OX40/CD25 etc.	T cell activation markers
		CD4+CD25+FoxP3+	Regulatory T cells
		CD19	B cells
		CD3-CD335+	NK
		CD3+CD335+	NKT
		CD11b+Ly6c/Ly6g	MDSCs, M&G MDSCs
		CD11b+F4/80+	Total macrophages
		IA/IE/CD206	M1 and M2 macrophages
	Others	TNF-a/IFN-r/IL-17/IL-3 etc.	Cytokines
		PD-1/PD-L1/CTLA-4 etc.	Checkpoints
		Granzym B	Activation markers
		Ki-67/BrdU/PCNA etc.	Proliferation
		Live/dead (fixable)	Live/dead

RESULTS

Fig 2. 16-color panel immune cell population hierarchy, the principle of gating

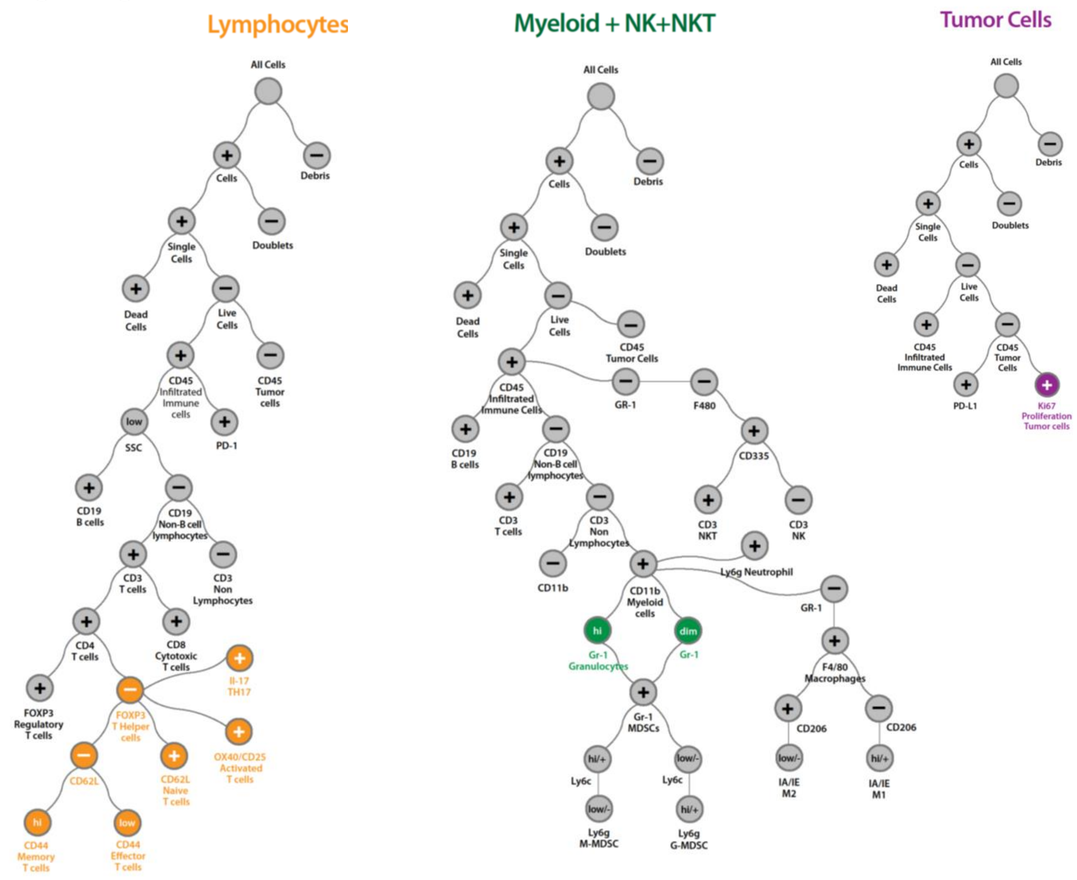


Fig 3. Representative gating strategy for I/O models (murine). Tumor tissue was harvested from animals and stained with a 16 color panel. The panel was designed to enumerate T cell subtypes, MDSCs, macrophages, NK, and NKT cells

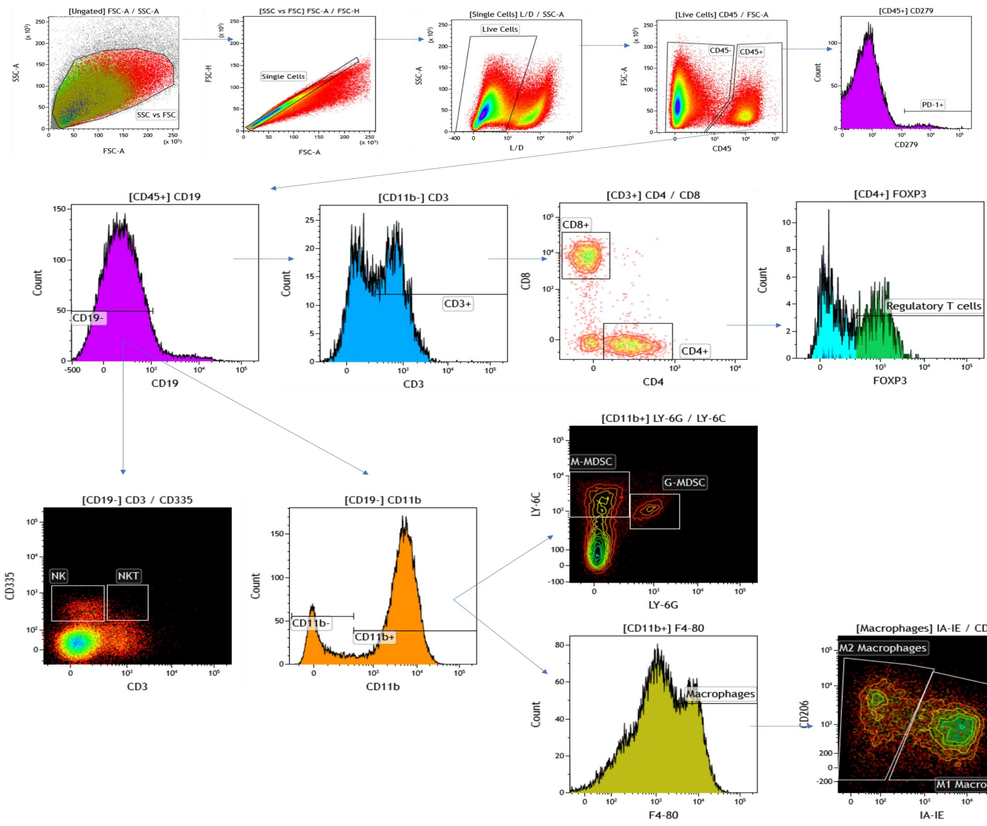
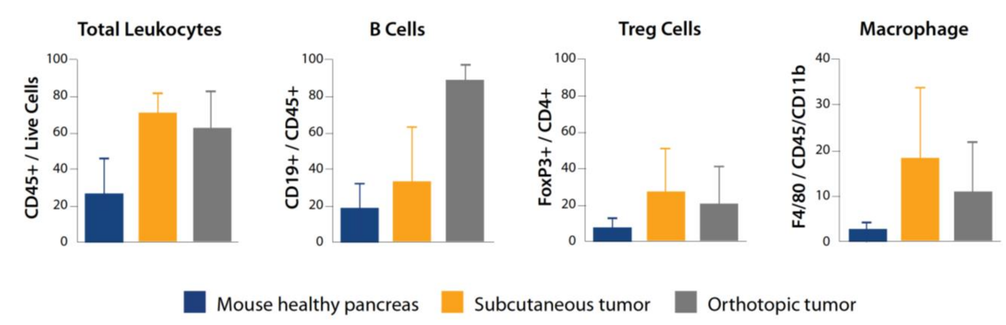


Fig 4. Representative FACS data on syngeneic mouse tumor panel Data from MuPrime models



SUMMARY

- We have explored methods to effectively dissect and delineate detailed immunophenotypes within the TME using flow cytometry, including specially customized tumor dissociation for each tumor, large 16-color panel design, and unique gating strategies.
- This study comprehensively profiled tumor-infiltrating immune cells of a large panel of mouse homograft tumors (baseline tumor volume between 100~500mm^3: syngeneic cell line derived, GEMM tumor derived, syngeneic in the context of chimeric humanized GEMM) using multi-color flow cytometry.

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