Targeting critical immune cell populations in pulmonary metastasis using novel monocyte-focused therapy

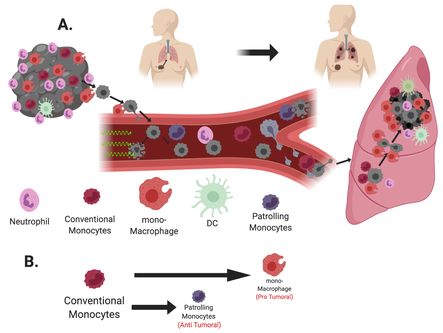
H. Ross[1,2], D. Tolstrup[1], M. Headley, PhD[1,3]

[1]Fred Hutchinson Cancer Research Center, Seattle, WA, [2]Clemson University, Clemson, SC, [3]University of Washington, Seattle, WA

BACKGROUND

Cancer research in the Headley Lab focuses on the immune system, as it pertains to the formation of metastasis and tumor survival. The immune system plays a dual role in tumor pathology with some elements recognizing and eliminating cancer while others support tumor growth. Pulmonary metastasis is a lethal development for many cancer types, made possible in part by pro-tumor monocyte populations within the pre-metastatic niche. I am investigating therapeutic targeting of conventional monocytes in pulmonary metastatic cancer prevention. Conventional monocytes can differentiate into distinct populations with either anti or pro-tumor function.

Figure 1: Role of The Immune System in Tumor Metastasis

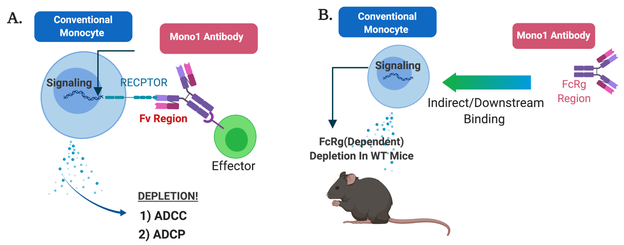


A) Basic Schematic for Immune Cells in Tumor Environment
B) Conventional Monocyte Differentiation

We have recently developed a novel Monoclonal antibody (**Mono1**) that depletes conventional monocytes but not other cell populations. This reagent allows us to interrogate the function of conventional monocytes in vivo in ways that were previously impossible. In order to effectively this tool it is critical that we first understand the mechanisms by which it depletes conventional monocytes so we can properly interpret results in future experiments.

Known pathways for immune cell depletion include antibody dependent cellular cytotoxicity (ADCC) and antibody dependent cellular phagocytosis (ADCP). In the studies presented here we explored mechanism behind Mono1 mediated depletion of conventional monocytes. Intriguely, despite robust depletion of only conventional monocytes we show that Mono1 does not in fact bind to conventional monocytes directly. Based on this unexpected result we next investigated the role of the other functional element on antibodies, the Fc region in Mono1-mediated depletion of conventional monocytes. Canonical ADCC and ADCP require recognition of the Fc region of the depleting antibody by activating Fc Receptors on effector cells, thus we have explored the requirement for Fc receptor signaling in mediating the monocyte-depleting effect of Mono1 in vivo. My work has played a crucial role in the advancement of preventative immunotherapy and provided useful data moving forward with Mono1 research, though more studies are required to define the mode of action of this intriguing new tool.

Figure 2: Proposed Model of Conventional Monocyte Depletion



A) Canonical mechanism of antibody-mediated cell depletion in vivo
B) Hypothetical mechanism of Mono1-mediated depletion of conventional monocytes

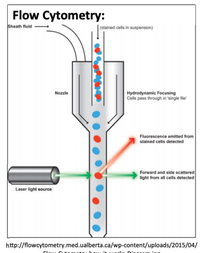
METHODS

Antibody Injections

WT, FcRgKO, and FcRg het mice (as indicated for given experiments) were injected *intraperitoneally* with 15mg/kg of Mono1 or Isotype Control (mIgG2a) antibody at time zero

Lung Cell Harvesting and Flow Cytometry Protocol

1. At **24:00** hours all mice treatments were injected *intraperitoneally* with **1ml** of **10% Avertin** (in PBS).
2. Lung tissue was collected from each mouse and digested using a **DNAse/Liberase** enzyme solution and GentleMACs blender tube to create a single cell suspension.
3. Approximately 5 million cells were resuspended with FACs buffer in a 96 well plate.
4. Cells were stained with **eflour780 Live/Dead** antibody then treated with **FcBlock** solution to prevent non-specific binding to the **FcRg** activating receptors.
5. Cells stained with 22 fluorescent antibody markers and **50ul** of *countbrite beads* for flow cytometry on the BD FACSymphony.
6. Critical immune cell populations were analyzed for all 31 mice lung samples using 15 designated laser excitation channels.

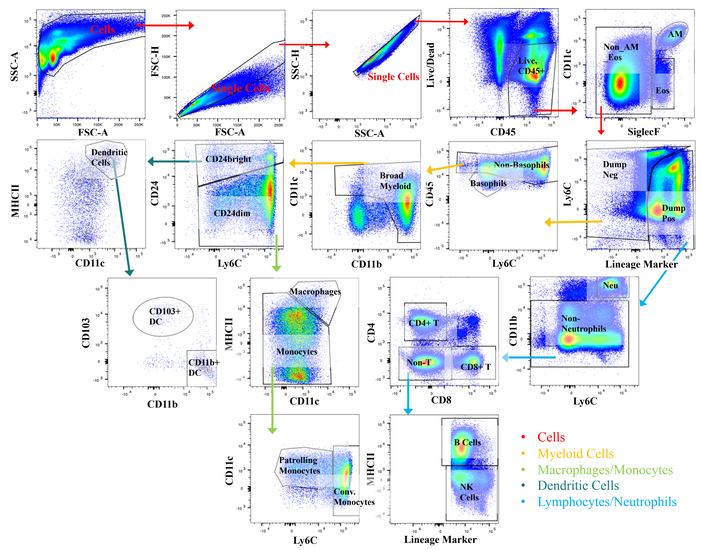


http://flowcytometry.med.ualberta.ca/wp-content/uploads/2015/04/Flow-Cytometry-how-it-works-Diagram.jpg

Antibody	Fluorochrome	Laser
CD4	BUV395	UV355-G
CD44	BUV737	UV355-B
CD8	BV421	V405-H
CD11c	BV510	V405-G
CD11b	BV605	V405-E
F4/80	BV650	V405-D
Ly6C	BV711	V405-C
Ly6G	BV785	V405A(Dump)
B220	BV785	Dump
CD19	BV785	Dump
CD3	BV785	Dump
CD90.2	BV785	Dump
NKp46	BV785	Dump
NK1.1	BV785	Dump
CD45	PerCP-Cy5-5	B488-B
CD103	PE	YG552-E
Siglec F	PE-CF594	YG552-D
CD24	PECy7	YG552-A
Clec12a	APC	R628-C
MHCII	AP700	R628-B

*all **WT mice** were bred from the Headley Lab Colony.
**all *Fcer1g*^{tm1Rav} mice were donated from the Koch Lab, originating from Jackson Labs

Figure 3: Current Gating Scheme for BD FACSymphony



CONCLUSIONS

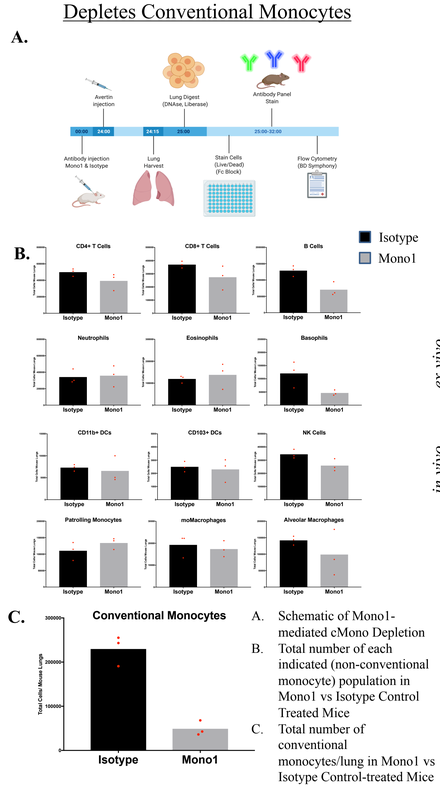
- **Mono1** specifically depletes conventional monocytes in-vivo
- **Mono1** does not bind conventional monocytes *in vivo* nor *ex vivo*.
- **Mono1** robustly labels neutrophils *in vivo* and *ex vivo*
- **Mono1**-mediated depletion of conventional monocytes is activating Fc Receptor dependent

FUTURE DIRECTIONS

- What is the effector cell population required for Mono1-mediated depletion of conventional Monocytes?
  - Do Neutrophils mediate conventional monocyte depletion via an indirect mechanism
  - Alternatively, is another effector cell population required (e.g. NK cells) for Mono1-mediated depletion of conventional monocytes
- Ascertain long term effect of monocyte depletion to track turnover rate in downstream populations
- Interrogate the role of conventional monocytes specifically in diseases such as metastasis of breast cancer to lungs.

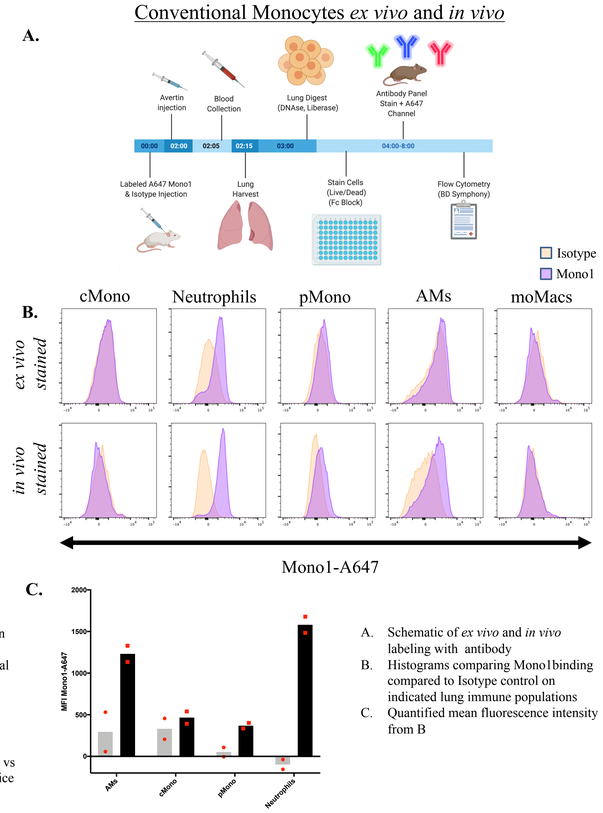
RESULTS

Figure 4: In-Vivo Mono1 Antibody Specifically Depletes Conventional Monocytes



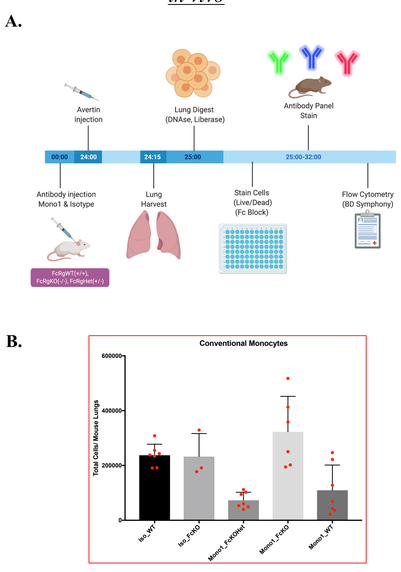
A. Schematic of Mono1-mediated cMono Depletion
B. Total number of each indicated (non-conventional monocyte) population in Mono1 vs Isotype Control Treated Mice
C. Total number of conventional monocytes/lung in Mono1 vs Isotype Control-treated Mice

Figure 5: Mono1 Selectively Binds to Neutrophils but not Conventional Monocytes *ex vivo* and *in vivo*



A. Schematic of *ex vivo* and *in vivo* labeling with antibody
B. Histograms comparing Mono1binding compared to Isotype control on indicated lung immune populations
C. Quantified mean fluorescence intensity from B

Figure 6: Mono1-depletion is FcRg-dependent *in vivo*



A) Schematic for experimental design to test requirement for FcR-dependent signaling in Mono1-mediated depletion of conventional monocytes.
B) Total Numbers of conventional monocytes in lungs of indicated mice.

FUNDING

This work is funded by grants from the Andy Hill CARE foundation, The METAvivor Early Career Investigator Fellowship, and the Roberta Robinson Fede Foundation. Additional funds were provided by The Summer Undergraduate Research Program supported in parts by the Cancer Center Support Grant (CCSG) CURE Supplement: NCI 3P30CA015704, the Fred Hutch Internship Program, and individual labs/research groups. Thank you to Pionyr Immunotherapeutics for Mono1 antibody and to Fred Hutch CRC for Flow Cytometry Shared Resource and Comparitive Medicine for Animal Housing and Resources.