Analysis of Macrophage Polarization via qPCR

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Myeloid Cell Polarization

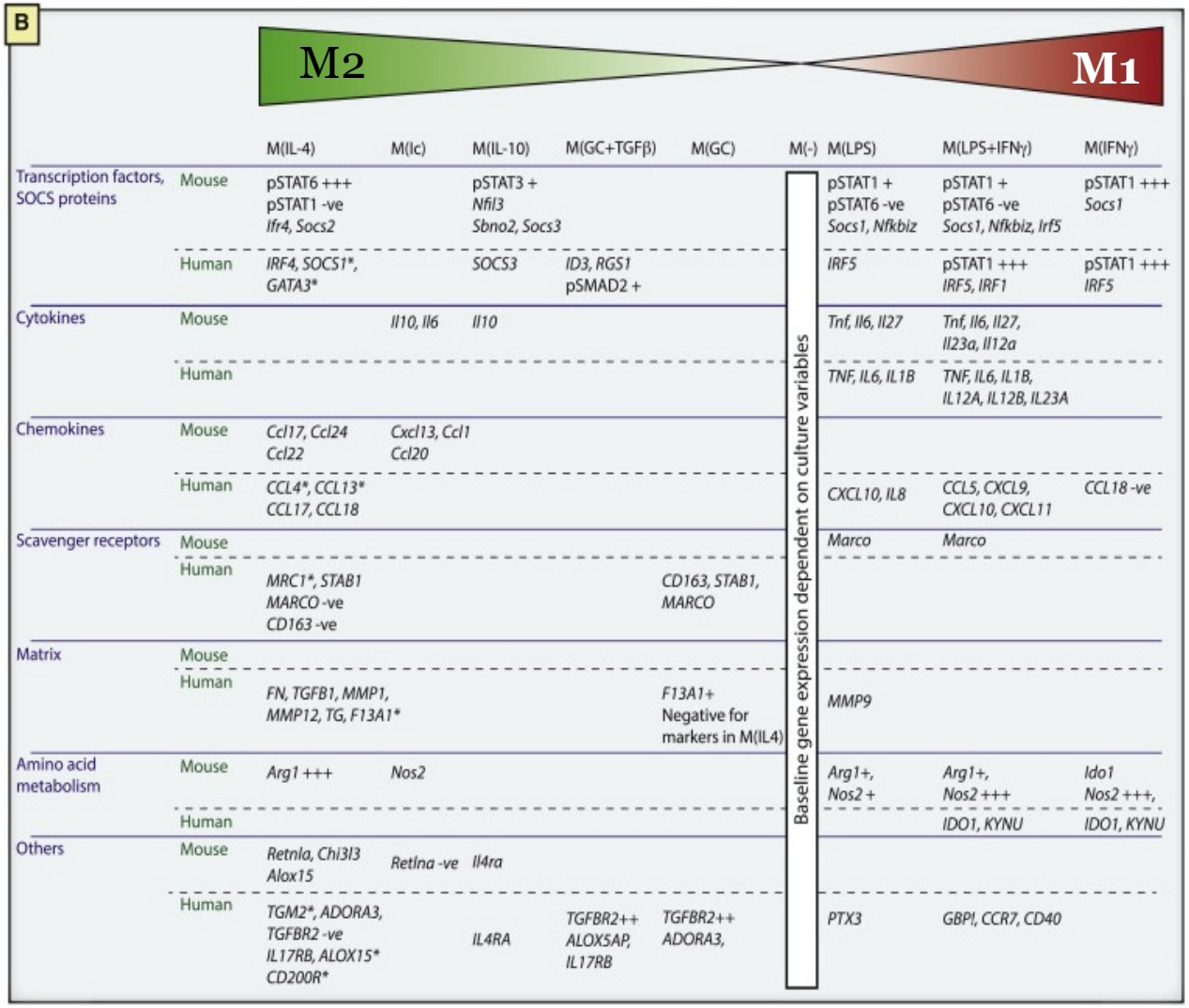
M1	M2	MDSC
• Classical Macrophage	• Alternative Macrophage • Tumor-associated Macrophage (TAM)	• Myeloid-derived suppressor cell
• Pro-inflammatory	• Anti-inflammatory	• Anti-inflammatory
• Anti-tumor	• Pro-tumor	• Pro-tumor • Suppress T cells

- Distinction between M2 and MDSCs still poorly understood

Purpose

- Macrophage gene expression poorly understood
  - Identify protocols for reliable macrophage differentiation
  - Classify gene expression profiles of M1, M2, MDSC
- Genetically engineered macrophages (GEMs)
  - Help T cells fight solid tumors, such as glioblastoma (GBM)

Literature



B

M2 ⟵ ⟶ M1

		M(IL-4)	M(Ic)	M(IL-10)	M(GC+TGFβ)	M(GC)	M(-)	M(LPS)	M(LPS+IFNγ)	M(IFNγ)
Transcription factors, SOCS proteins	Mouse	pSTAT6 +++ pSTAT1 -ve *Ifr4, Socs2*		pSTAT3 + *Nfil3* *Sbno2, Socs3*				pSTAT1 + pSTAT6 -ve *Socs1, Nfkbiz*	pSTAT1 + pSTAT6 -ve *Socs1, Nfkbiz, Irf5*	pSTAT1 +++ *Socs1*
	Human	*IRF4, SOCS1*, GATA3**		*SOCS3*	*ID3, RGS1* pSMAD2 +			*IRF5*	pSTAT1 +++ *IRF5, IRF1*	pSTAT1 +++ *IRF5*
Cytokines	Mouse		*Il10, Il6*	*Il10*				*Tnf, Il6, Il27*	*Tnf, Il6, Il27, Il23a, Il12a*	
	Human							*TNF, IL6, IL1B*	*TNF, IL6, IL1B, IL12A, IL12B, IL23A*	
Chemokines	Mouse	*Ccl17, Ccl24* *Ccl22*	*Cxcl13, Ccl1* *Ccl20*							
	Human	*CCL4*, CCL13** *CCL17, CCL18*						*CXCL10, IL8*	*CCL5, CXCL9, CXCL10, CXCL11*	*CCL18 -ve*
Scavenger receptors	Mouse							*Marco*	*Marco*	
	Human	*MRC1*, STAB1* *MARCO -ve* *CD163 -ve*				*CD163, STAB1, MARCO*				
Matrix	Mouse									
	Human	*FN, TGFB1, MMP1, MMP12, TG, F13A1**				*F13A1+* Negative for markers in M(IL4)		*MMP9*		
Amino acid metabolism	Mouse	*Arg1 +++*	*Nos2*					*Arg1+, Nos2 +*	*Arg1+, Nos2 +++*	*Ido1* *Nos2 +++,*
	Human								*IDO1, KYNU*	*IDO1, KYNU*
Others	Mouse	*Retnla, Chi3l3* *Alox15*	*Retlna -ve*	*Il4ra*						
	Human	*TGM2*, ADORA3, TGFBR2 -ve* *IL17RB, ALOX15** *CD200R**		*IL4RA*	*TGFBR2++* *ALOXSAP, IL17RB*	*TGFBR2++* *ADORA3,*		*PTX3*	*GBP!, CCR7, CD40*	

Baseline gene expression dependent on culture variables

Canonical Findings

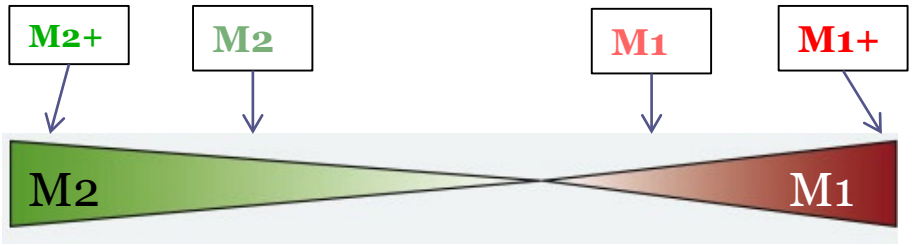
M2	M1
• IL10	• IFNg
• CD163	• IL8
• TGFb	• IL1b
	• IP-10
	• IL-12a
	• IL-12b

Hypothesis

Polarization Hypothesis		
M1	**M2**	**MDSC marker**
IFNg	MMP9	CD62L
TNFa	CD163	iNos
IL-8	TGFb	MMP9
IL-6	IL-10	Arginase
IP-10	VEGF	
IL-1b		
IL-12a		
IL-12b		
IFNa		
IFNb		

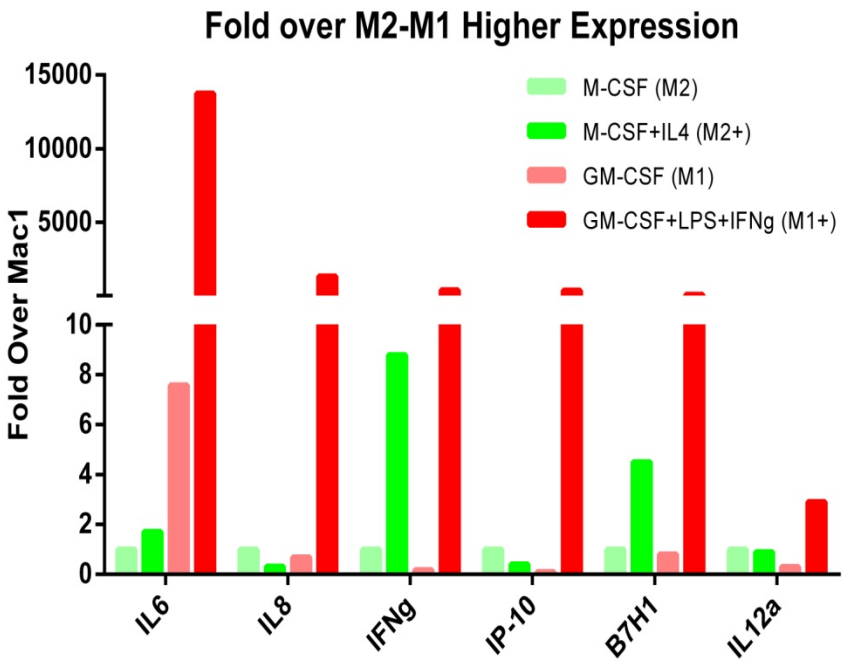
qPCR Analysis

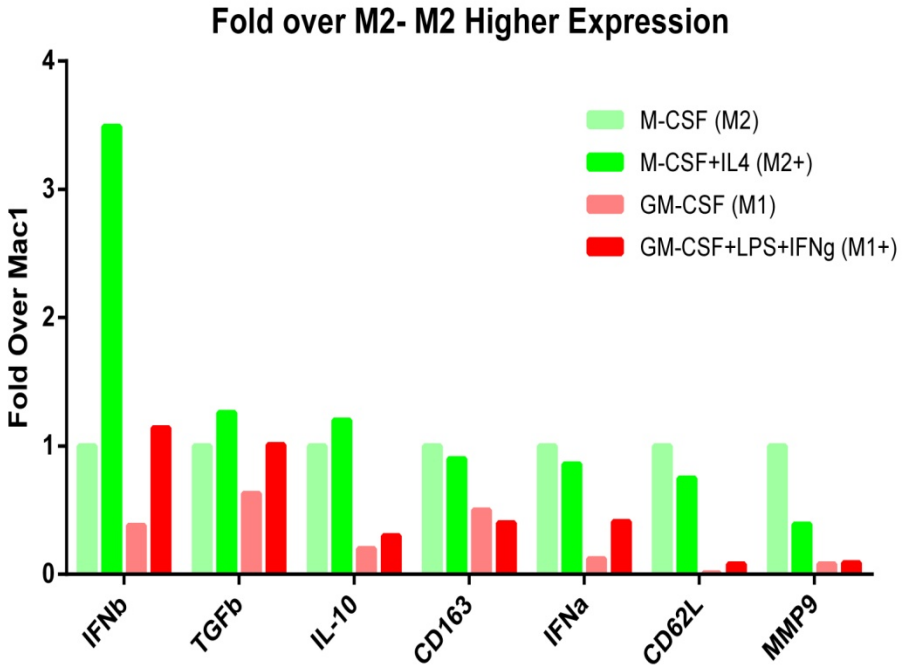
- Sample Treatments
  - **Panel 1**
    - M2: M-CSF
    - M2+ : M-CSF + IL4
    - M1: GM-CSF
    - M1+ : GM-CSF + LPS + IFNg
  - **Panel 2**
    - M2+ : M-CSF + IL-4
    - **MDSC**: Tumor cell-conditioned media



Results (Panel 1)

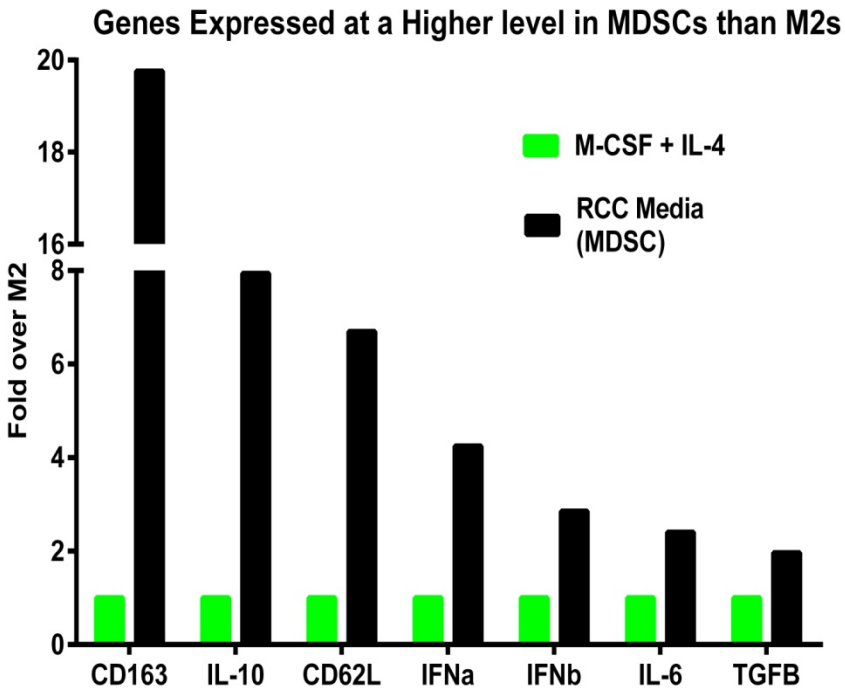
Differential Gene Expression Profiles

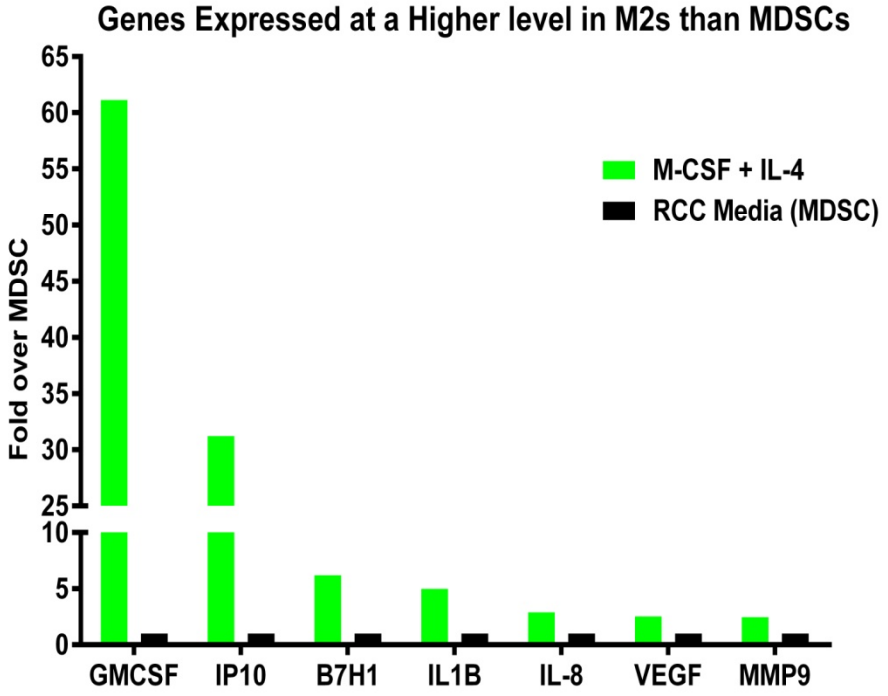




Results (Panel 2)

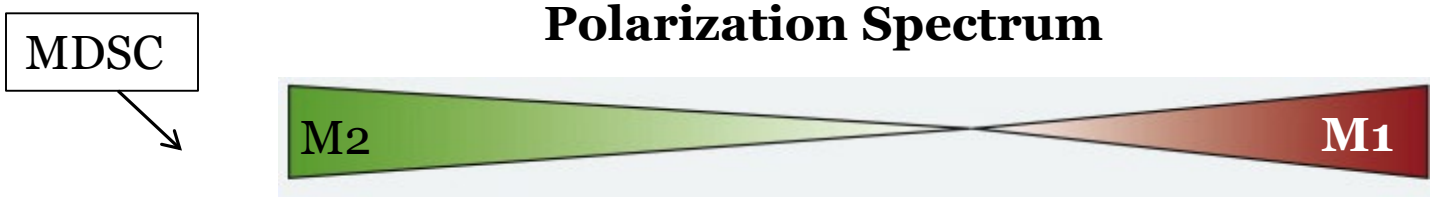
Differential Gene Expression Profiles





Conclusions

- **Panel 1**
  - **M1+** (GM-CSF + LPS + IFNg) treatment presents strongest pro-inflammatory gene expression vs. M2
    - IFNg, IL-8, IL-6, IP-10, IL-12a, IL-12b: Confirmed
    - PDL-1 also expressed at high levels
  - **M2+** (M-CSF + IL-4) treatment presents strongest anti-inflammatory gene expression vs. M1
    - TGFb, IL-10, CD163: Confirmed
    - IFNa/IFNb : Unexpected upregulation
- **Panel 2**
  - **M2+** treatment (M-CSF + IL-4) presents higher expression pro-inflammatory signals vs. MDSC
  - MDSC cells expressing higher levels of anti-inflammatory signals vs. M2



Thank you

- Crane Lab
  - Courtney Crane, Principal Investigator
  - Kara White Moyes, Postdoctoral Fellow
  - Nicole Perry, Postdoctoral Fellow
  - Kristen Haberthur, Postdoctoral Fellow
  - Shannon Kreuser
  - Amira Davis
  - Harrison Chinn
  - Katie Hoon
- UW Neurosciences Summer Student Program
  - Dr. and Mrs. Ellenbogen
  - Jim Pridgeon
  - Christina Buckman