NORTHWESTERN UNIVERSITY

Immune approach to Primary Graft Dysfunction

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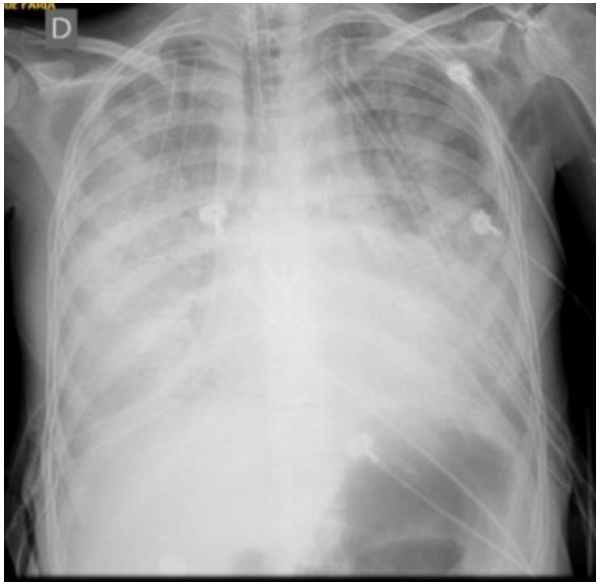
Northwestern Memorial Hospital

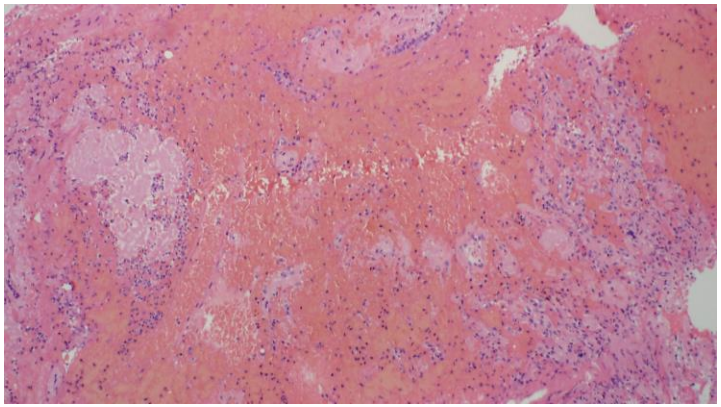
Disclosures

None

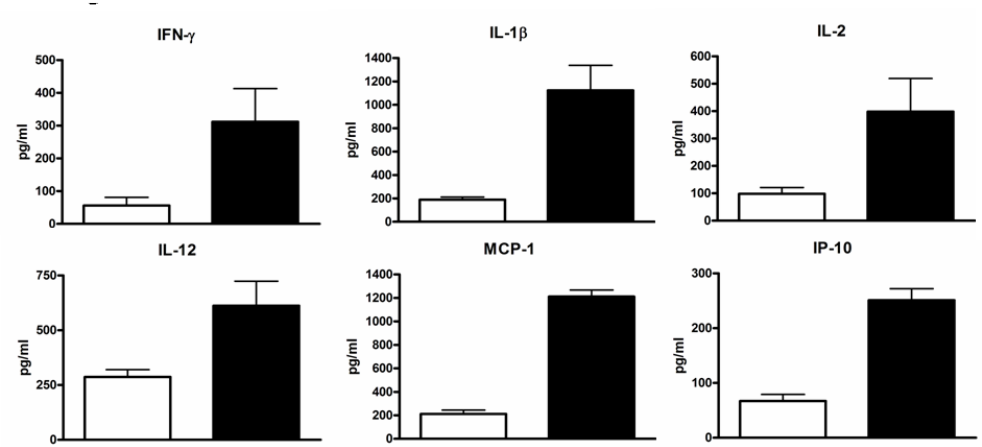
PRIMARY GRAFT DYSFUNCTION

- Incidence >50-70%
- Occurs within first 24 hours following transplant
- Characterized by respiratory failure
- Leading cause of short-term mortality
- Predominant risk factor for chronic rejection

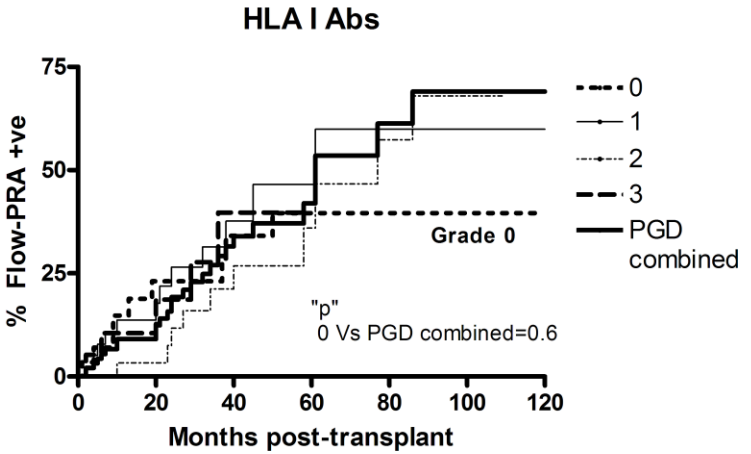


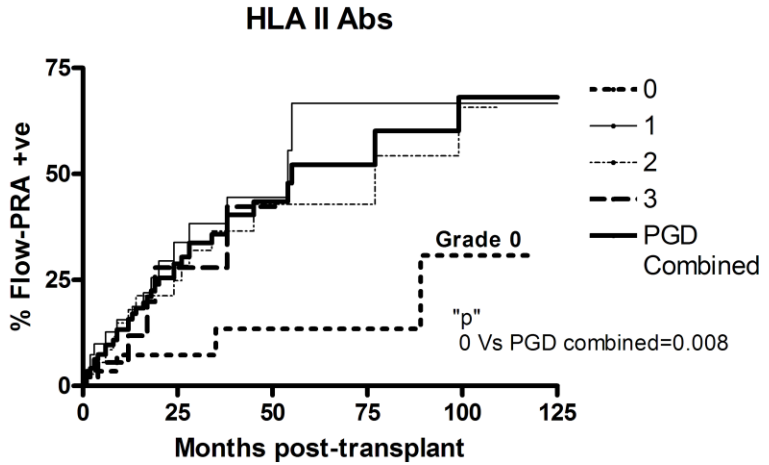


PGD INDUCES CYTOKINE STORM AND ALLOIMMUNITY

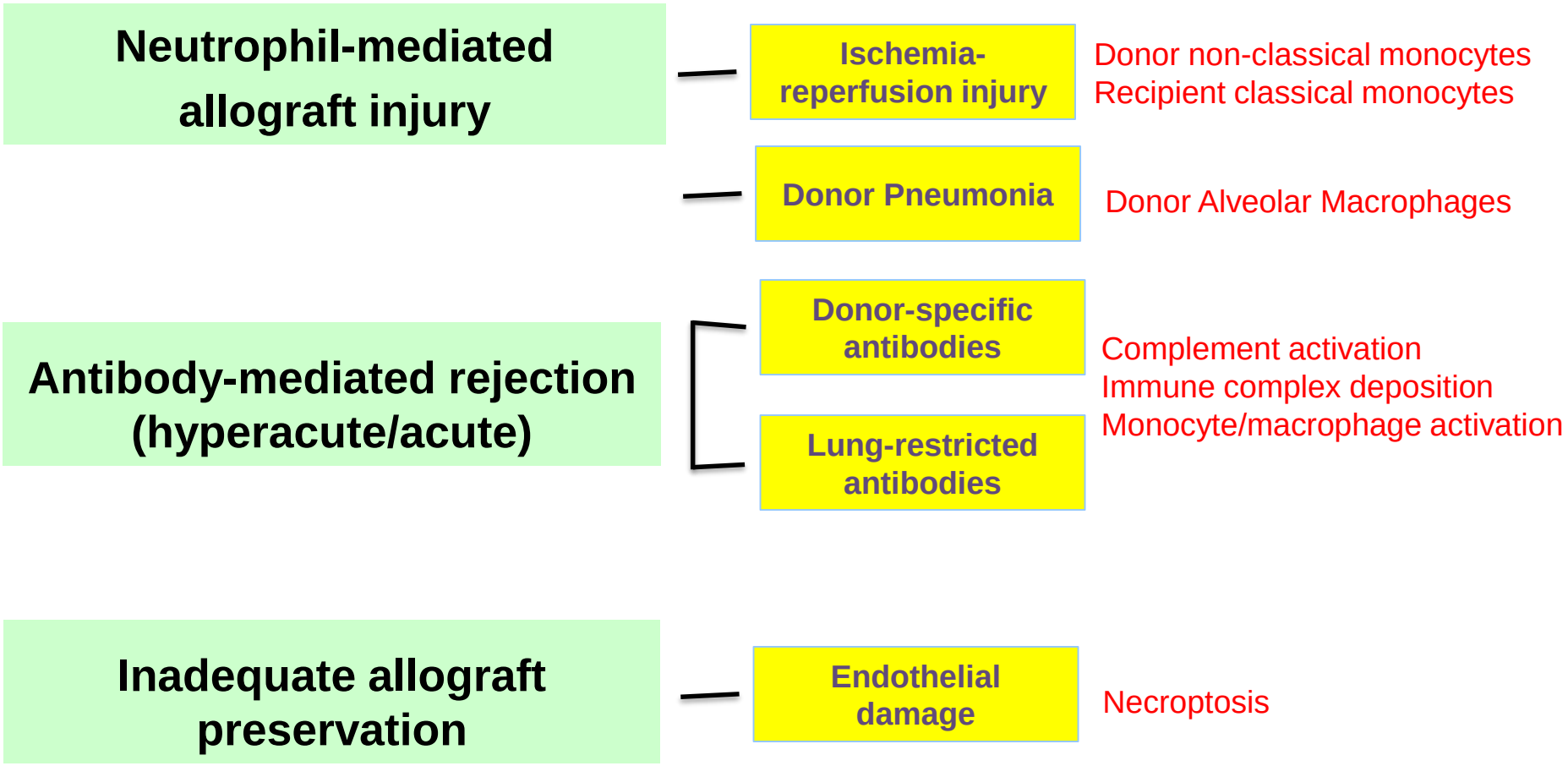


Bharat et al, Annals Thor Surg, 2011

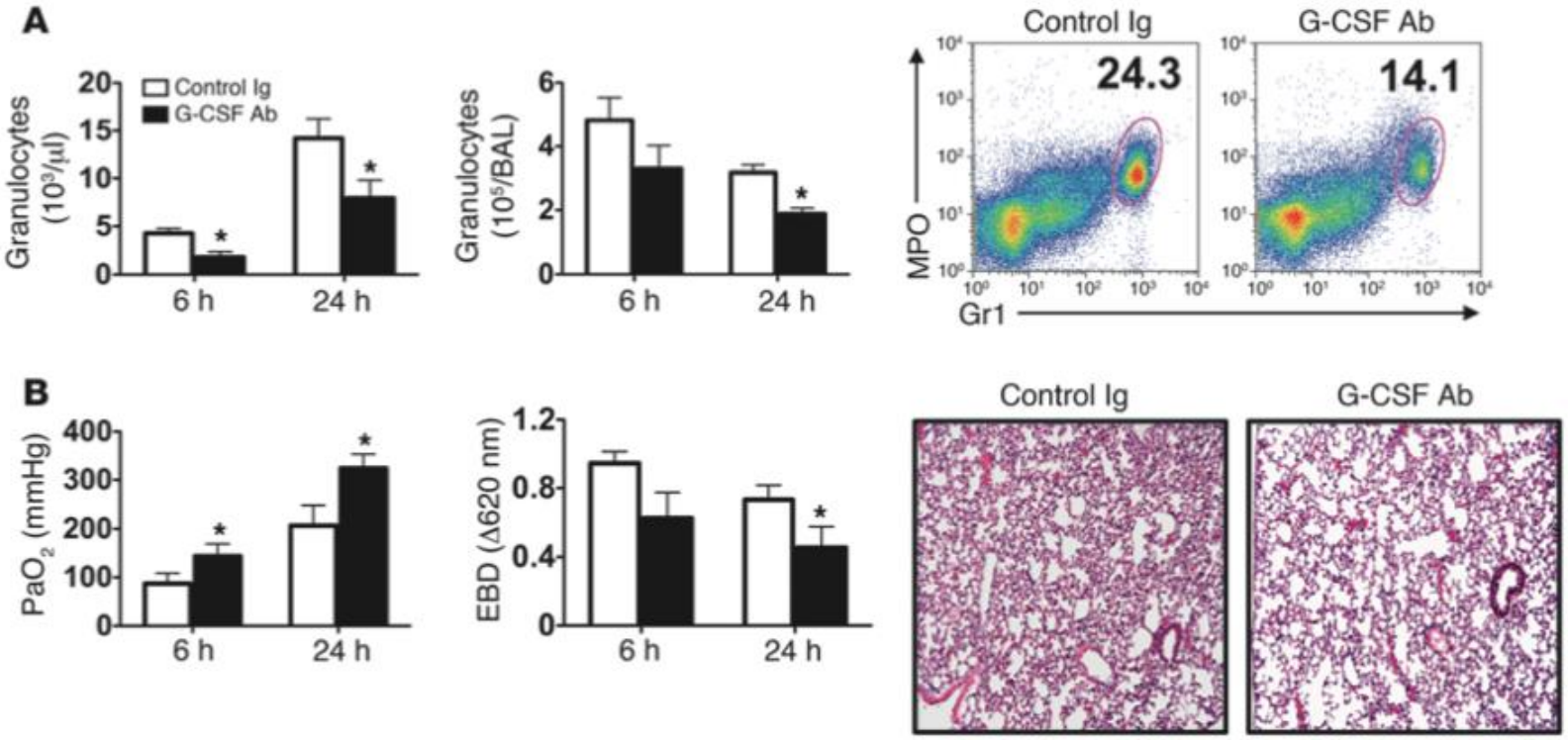




Spectrum of PGD

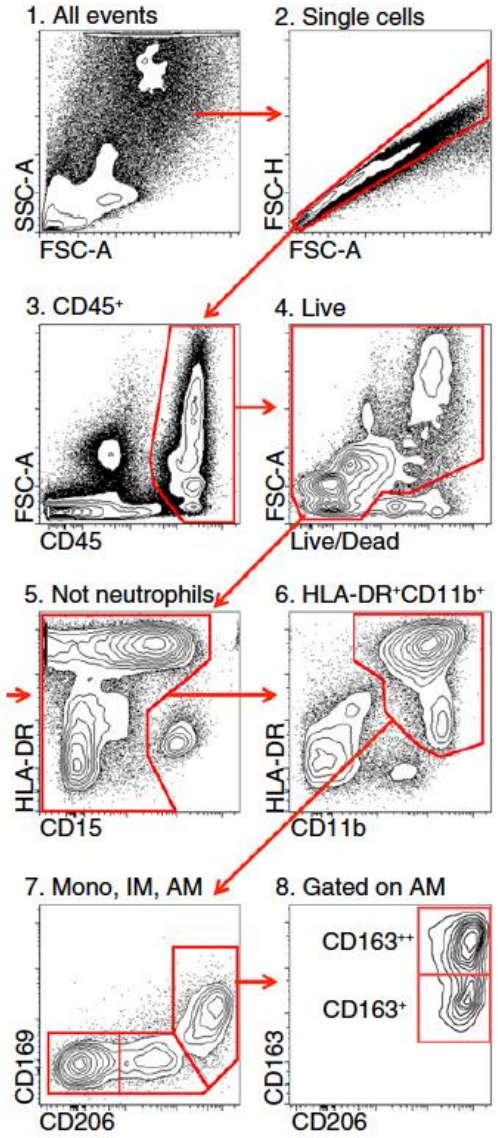


NEUTROPHILS MEDIATE PGD

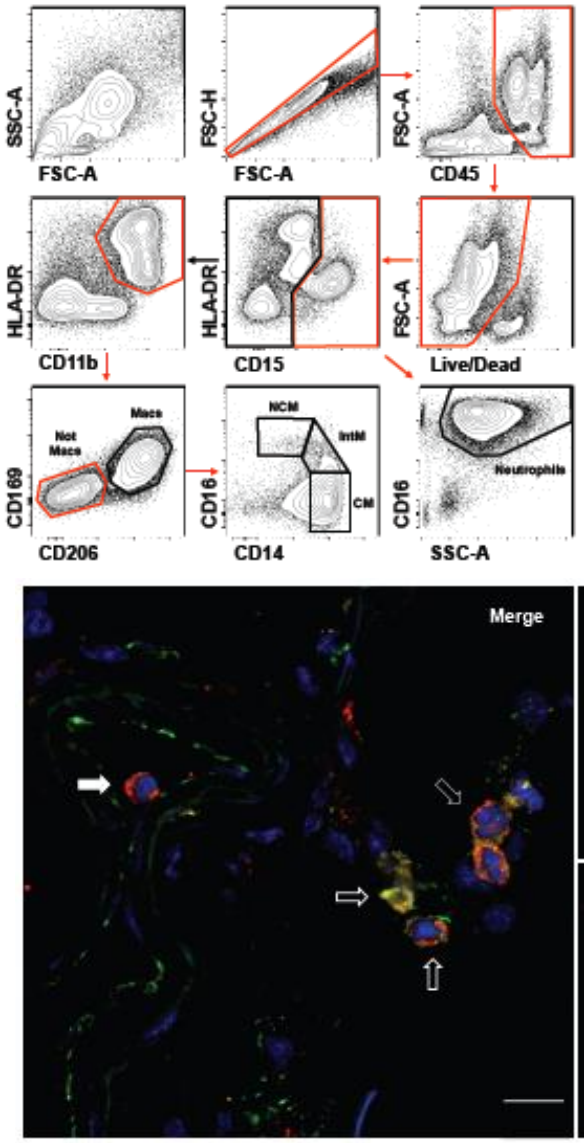


Kreisel D et al. *J Clin Invest* 2011;121:265–276.

PERFUSED HUMAN DONOR LUNGS CONTAIN MONOCYTES

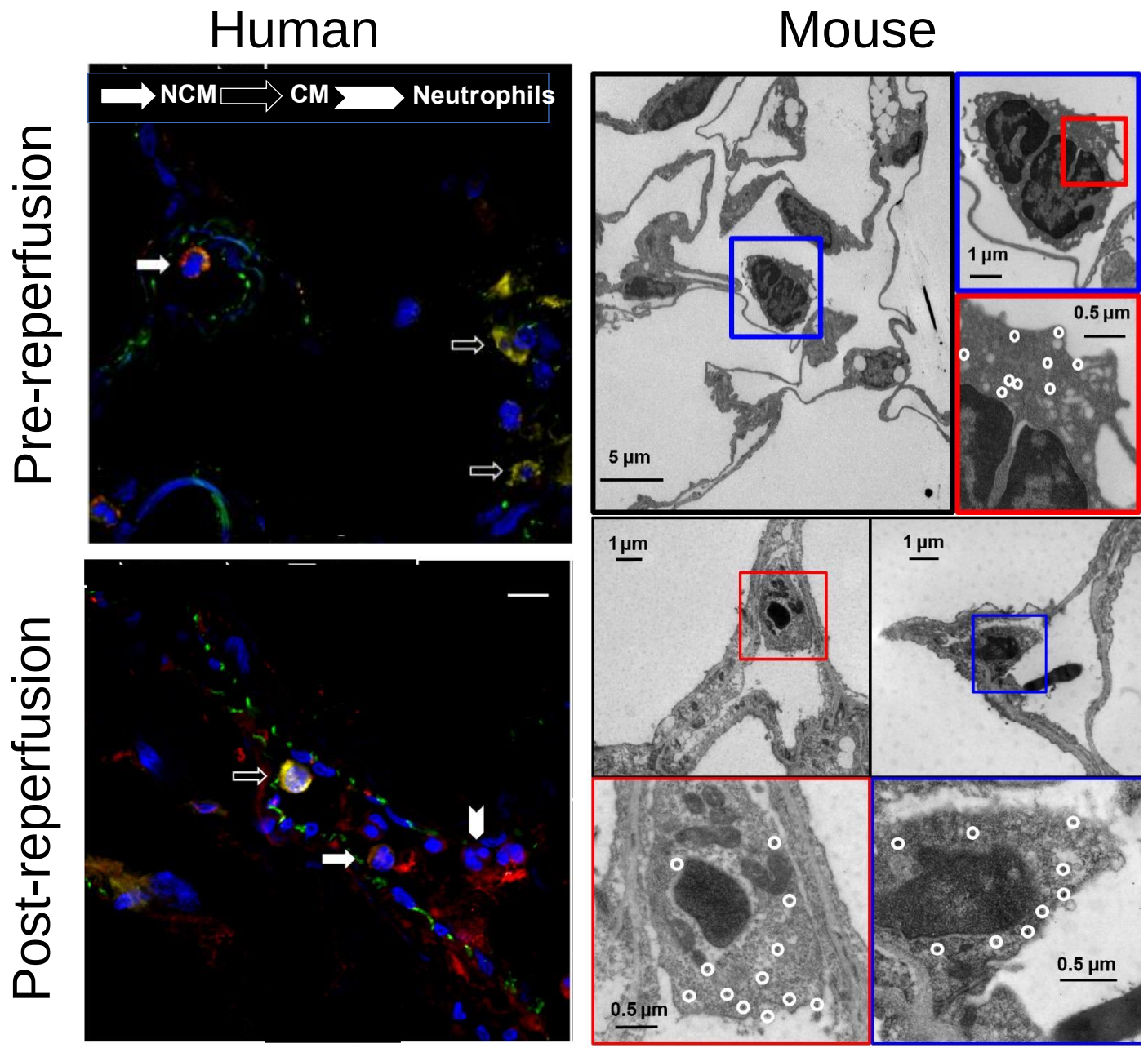


Bharat et al, AJRCMB, Jan 2016



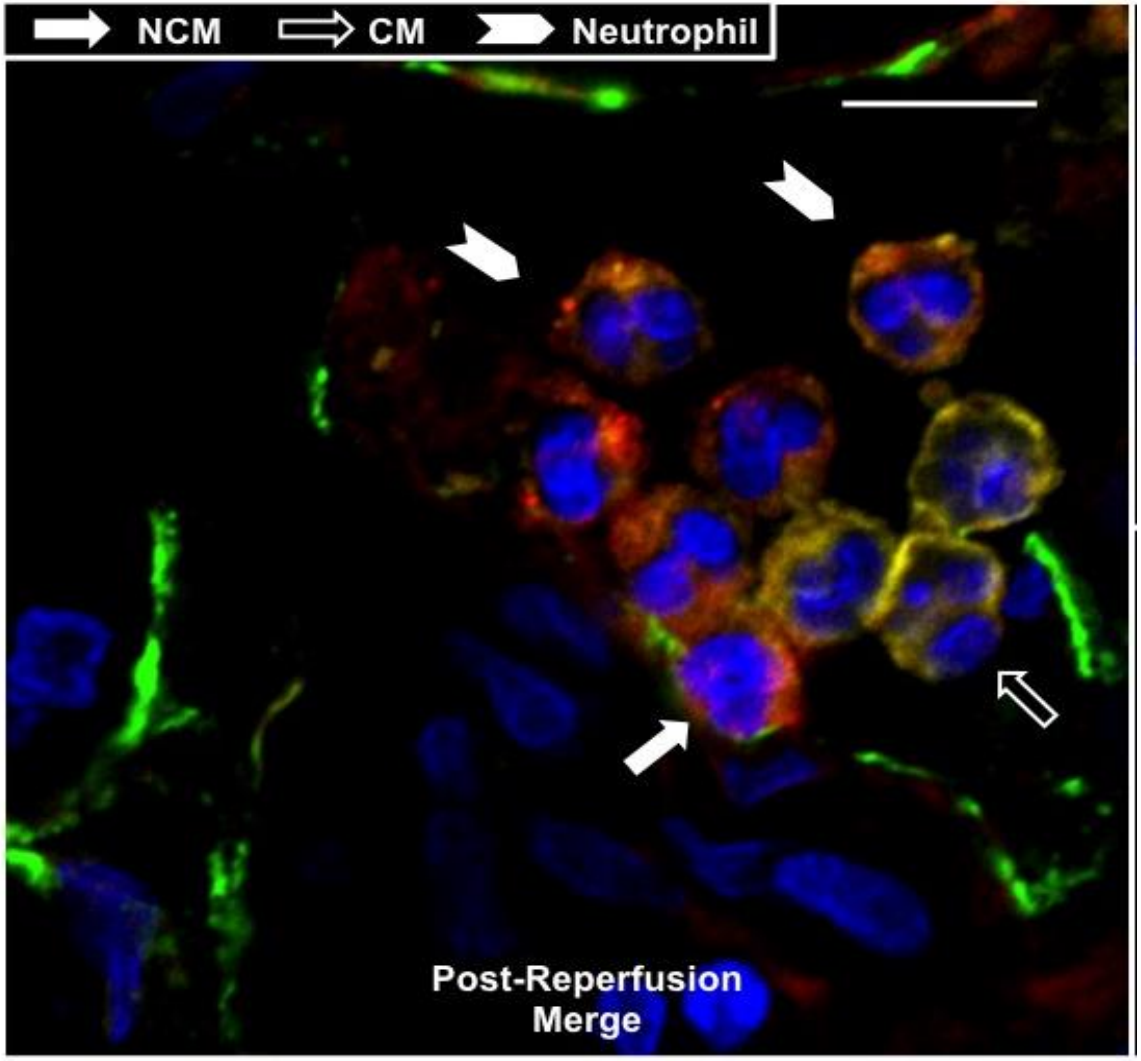
Zhikun et al, Science Transl Med, 2017

Demonstration of non-classical monocytes in the intravascular space of donor lungs

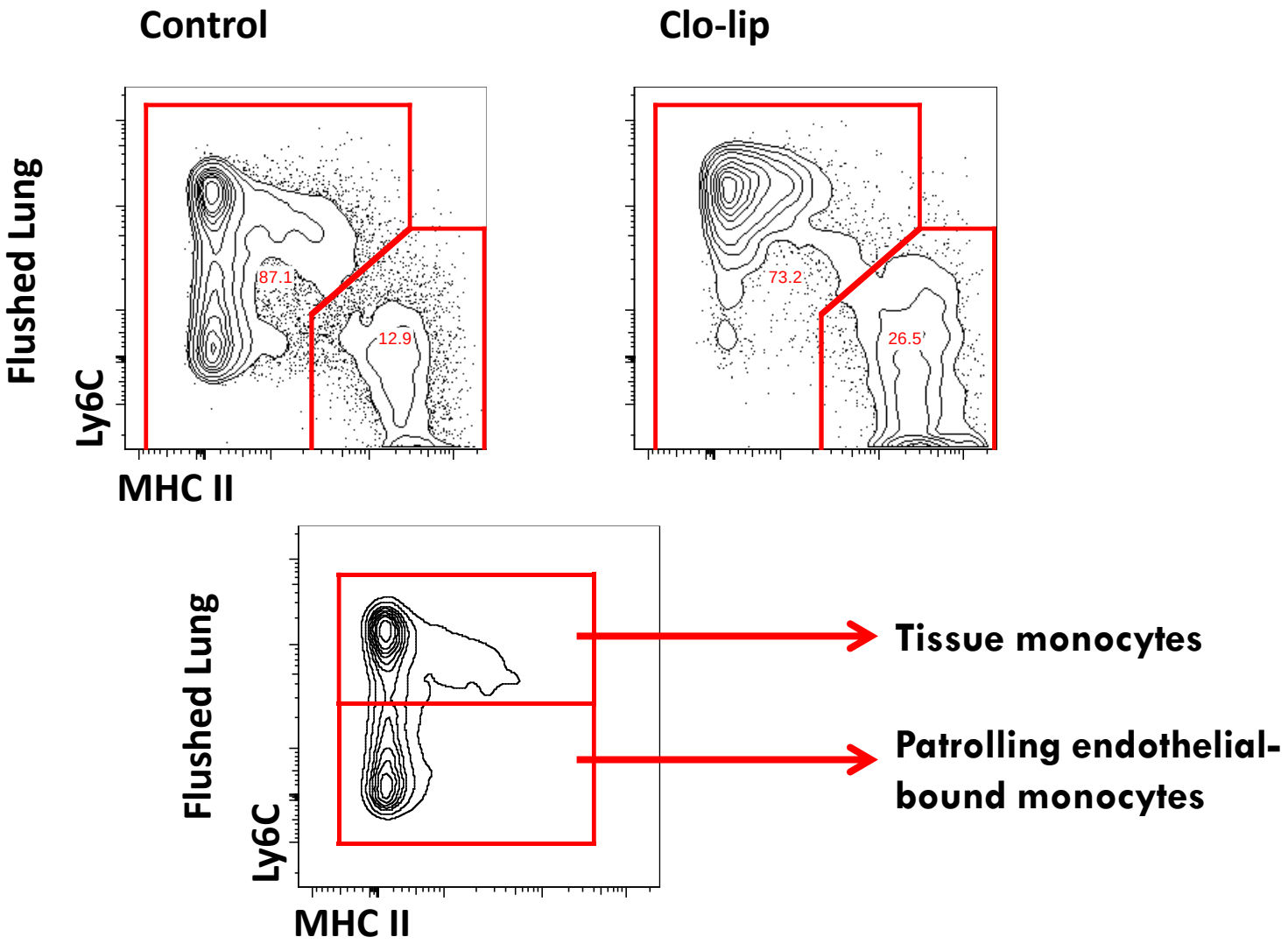


NCM are visualized at sites of neutrophil recruitment and endothelial injury

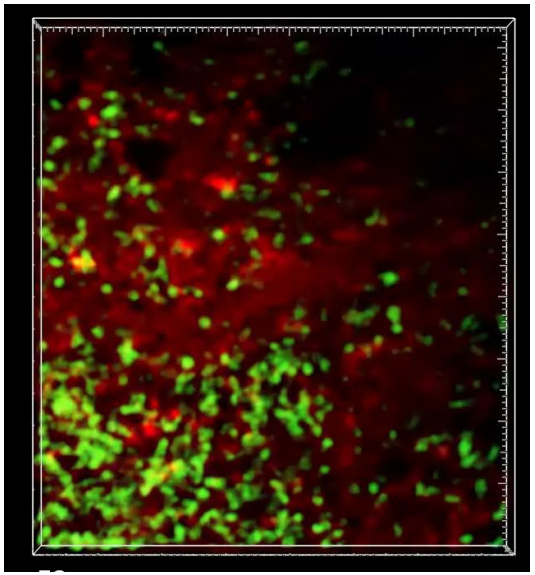
Human PGD

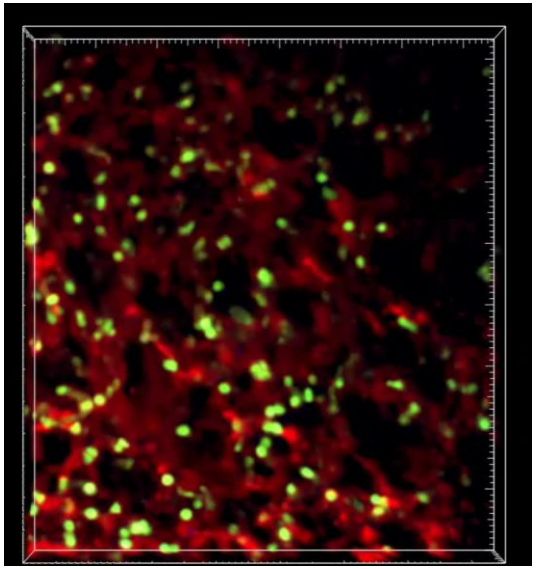


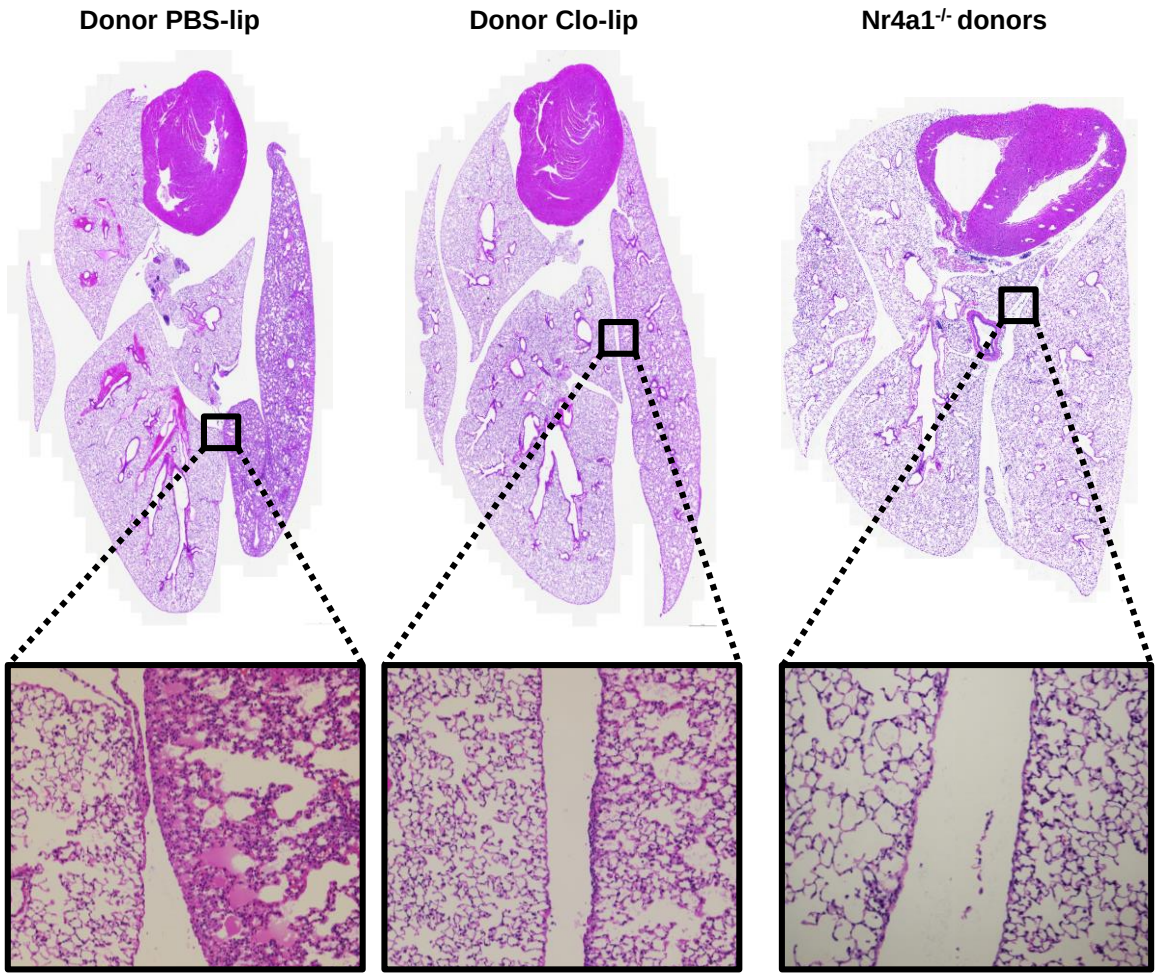
LIPOSOMAL CLODRONATE DEPLETES Ly6C^{low} MONOCYTES IN PERFUSED LUNGS



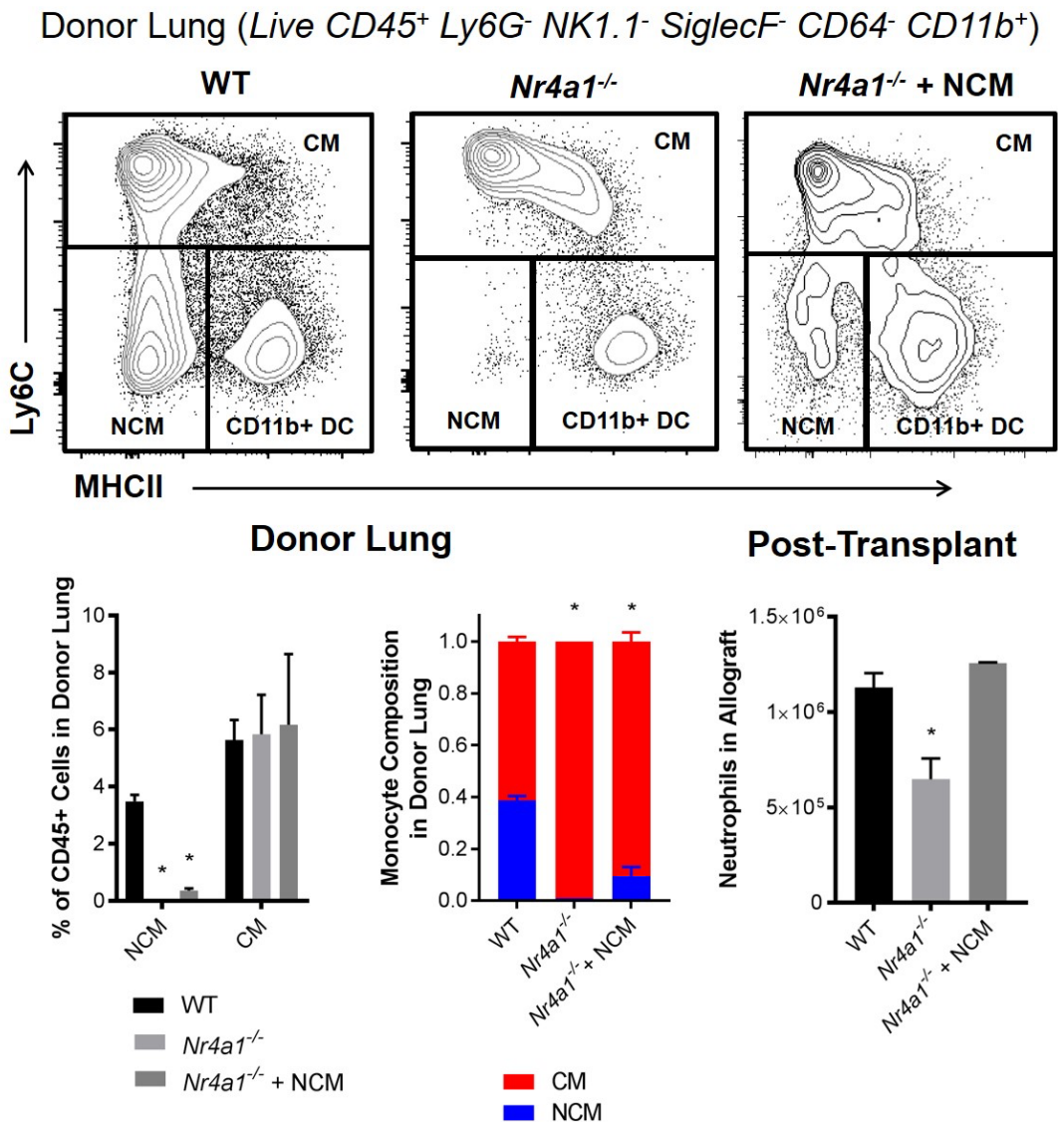
Depletion of donor NCM abrogates neutrophil recruitment and ameliorates PGD



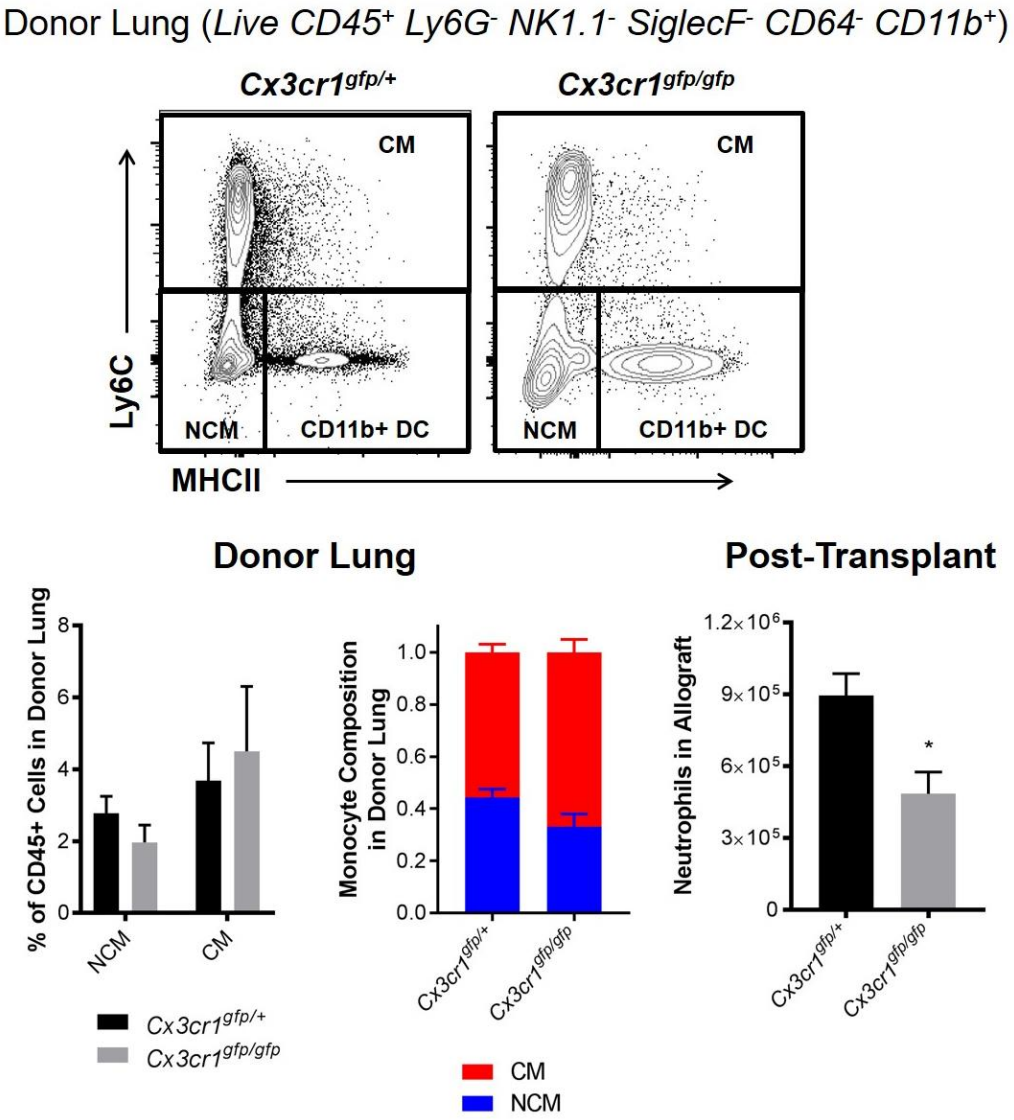




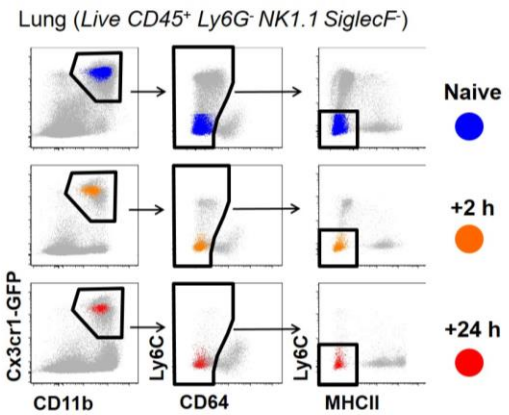
Genetic depletion of donor NCM abrogates neutrophil recruitment

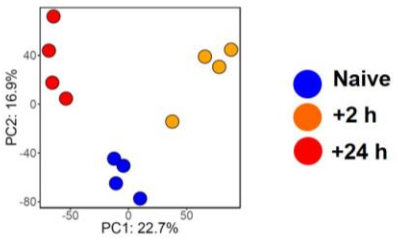


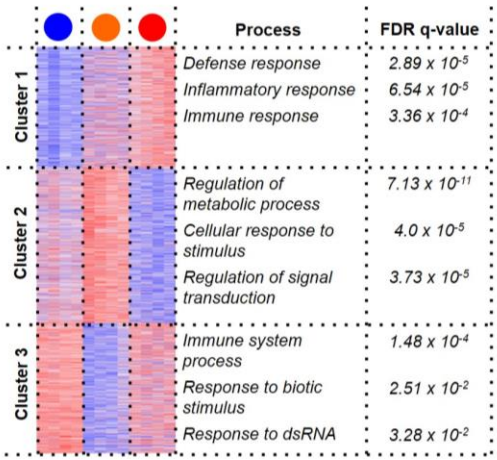
Genetic deletion of fractalkine receptors on NCM inhibits their function

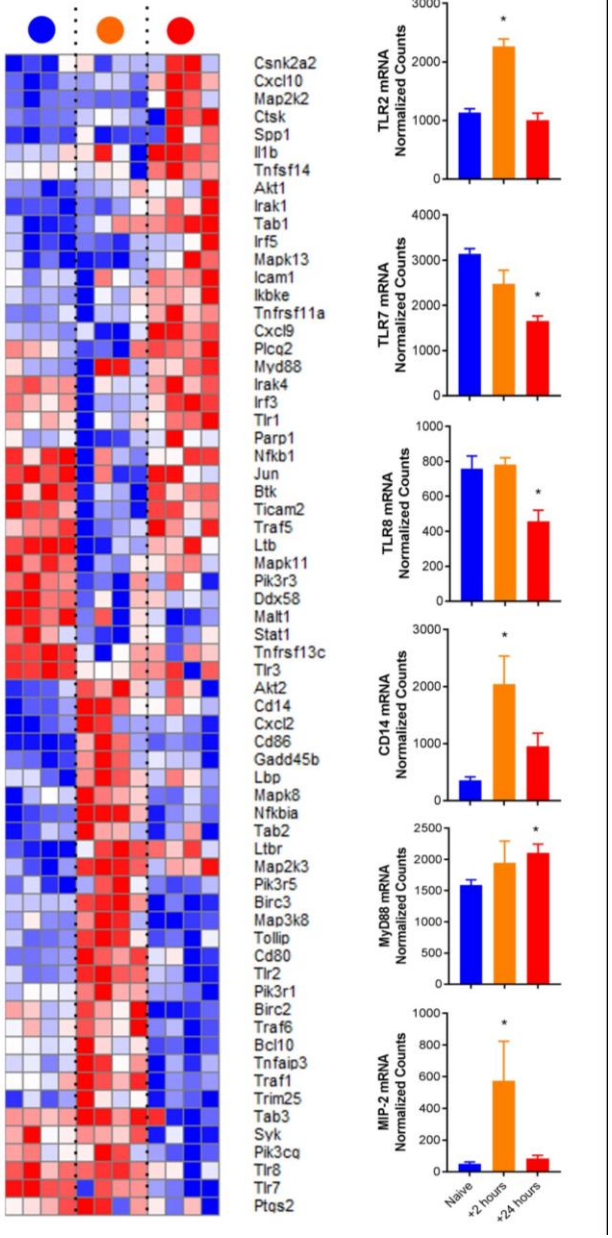


Unbiased transcriptomic profiling of NCM

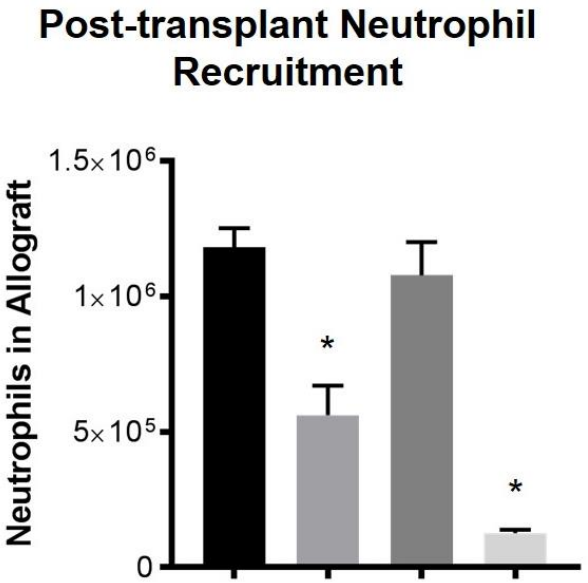




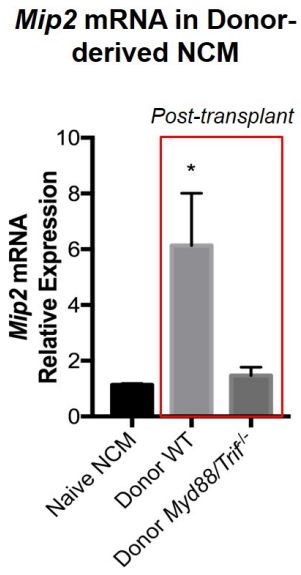


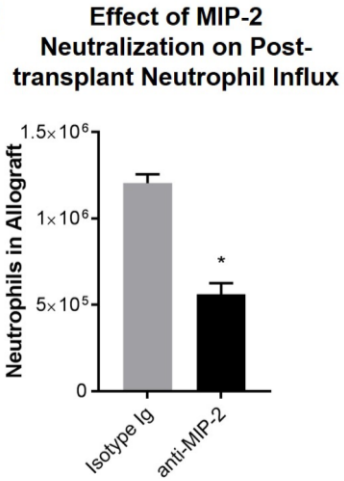


Donor NCM produce MIP-2 in a MyD88-dependent fashion to recruit recipient neutrophils

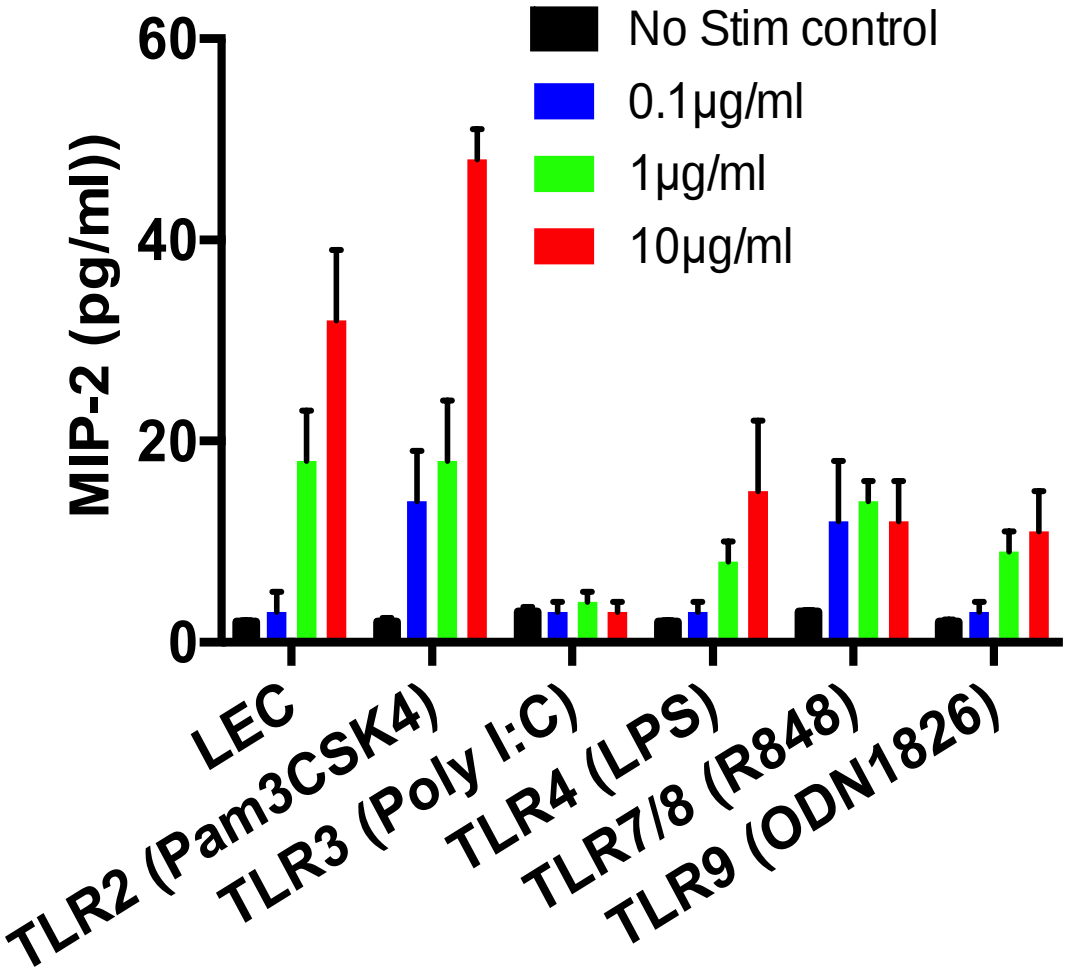


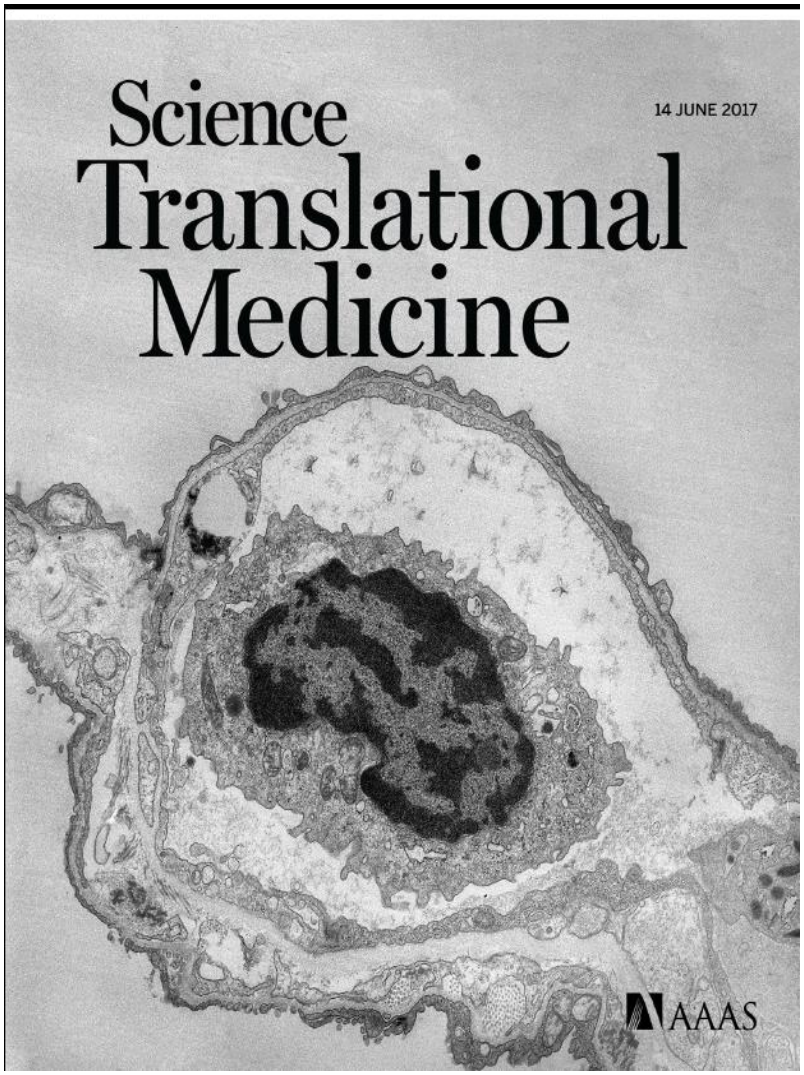
Experiment	I	II	III	IV
Donor WT NCM	+	-	+	-
Donor *Myd88/Trif*$^{-/-}$ NCM	-	+	-	+
WT Allograft Stroma	+	-	-	+
Myd88/Trif$^{-/-}$ Allograft Stroma	-	+	+	-

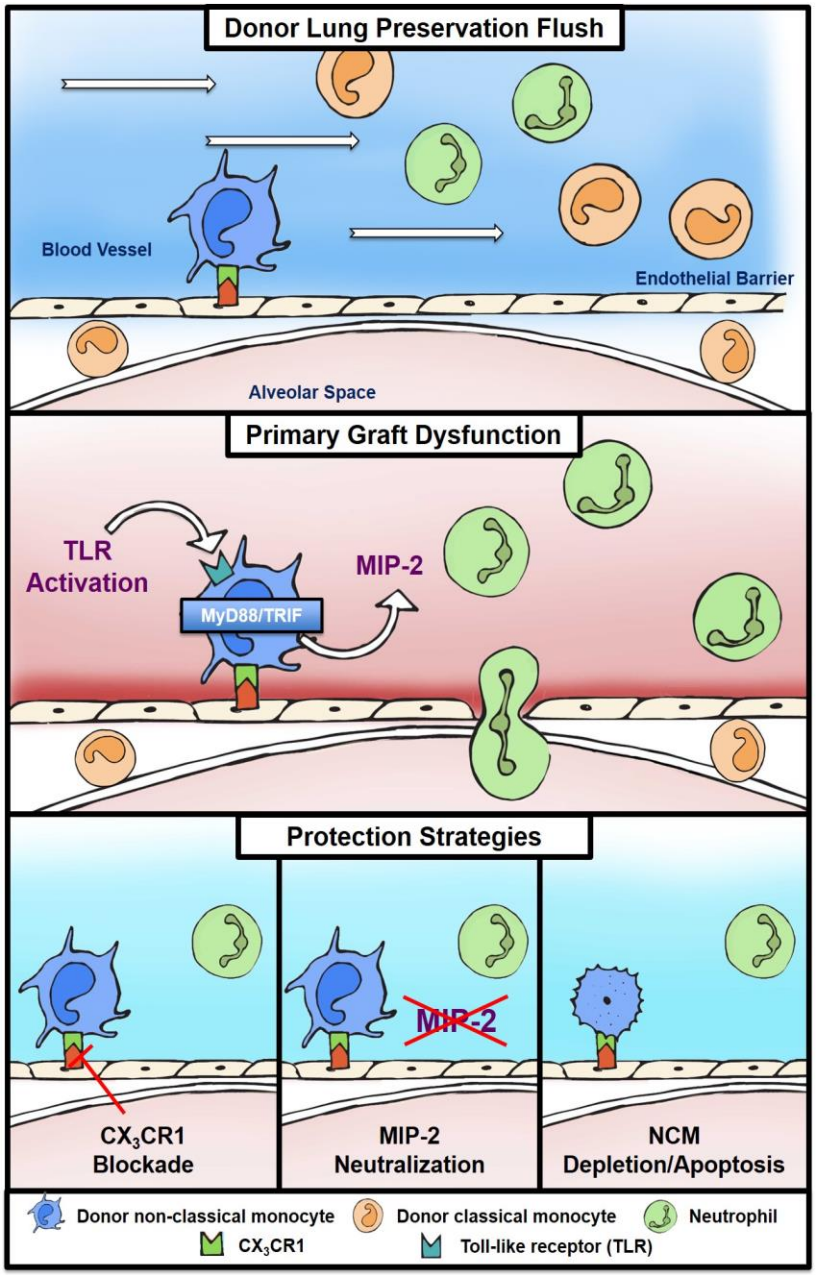




Donor NCM produce MIP-2 following TLR2 stimulation




Science
Translational
Medicine
14 JUNE 2017
AAAS


Donor Lung Preservation Flush
Blood Vessel
Endothelial Barrier
Alveolar Space
Primary Graft Dysfunction
TLR
Activation
MyD88/TRIF
MIP-2
Protection Strategies
MIP-2
CX3CR1
Blockade
MIP-2
Neutralization
NCM
Depletion/Apoptosis
Donor non-classical monocyte
Donor classical monocyte
Neutrophil
CX3CR1
Toll-like receptor (TLR)

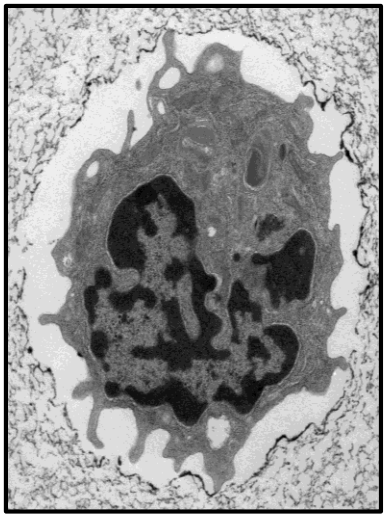
Monocyte subsets in mice and humans

Classical Monocyte (CM)

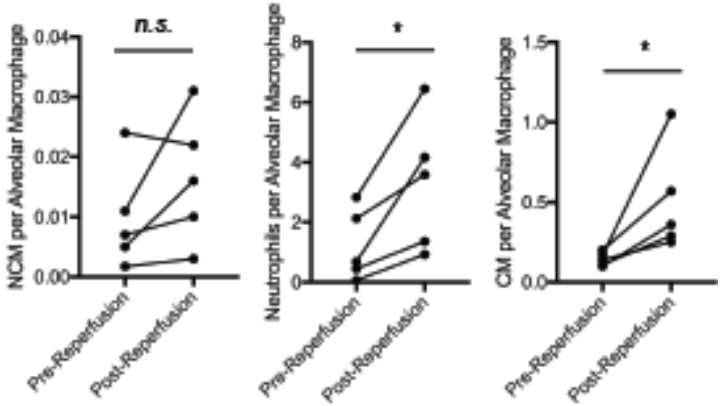
$CCR2^{+}Ly6C^{high}CX_3CR1^{low}$

Nonclassical Monocyte (NCM)

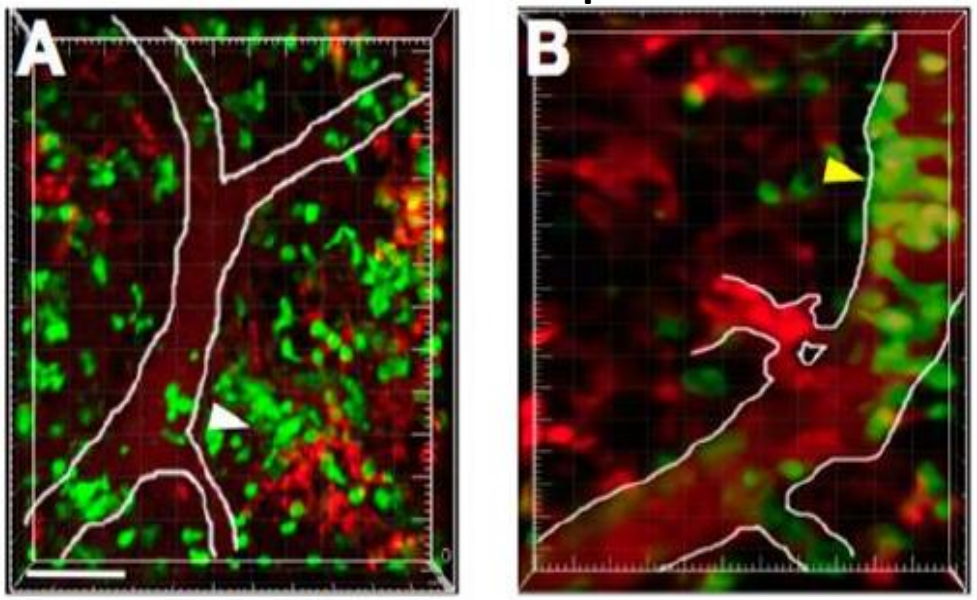
$Ly6C^{low}CX_3CR1^{High}CCR2^{-}$



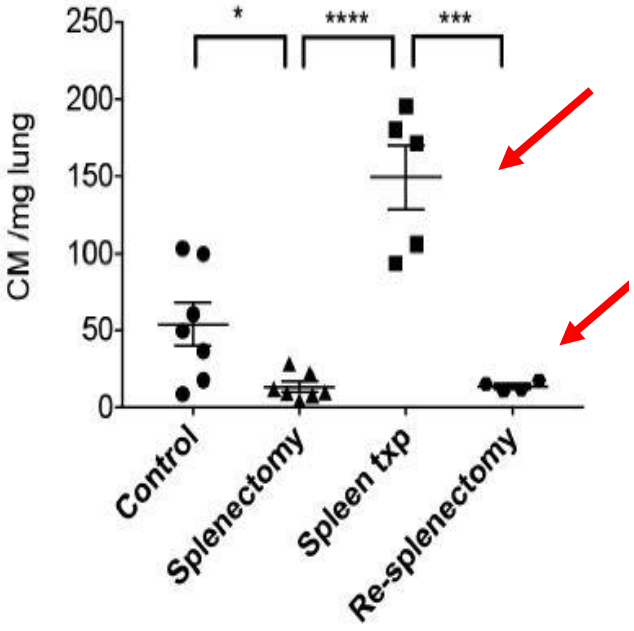
Depletion of recipient classical monocytes impairs neutrophil extravasation

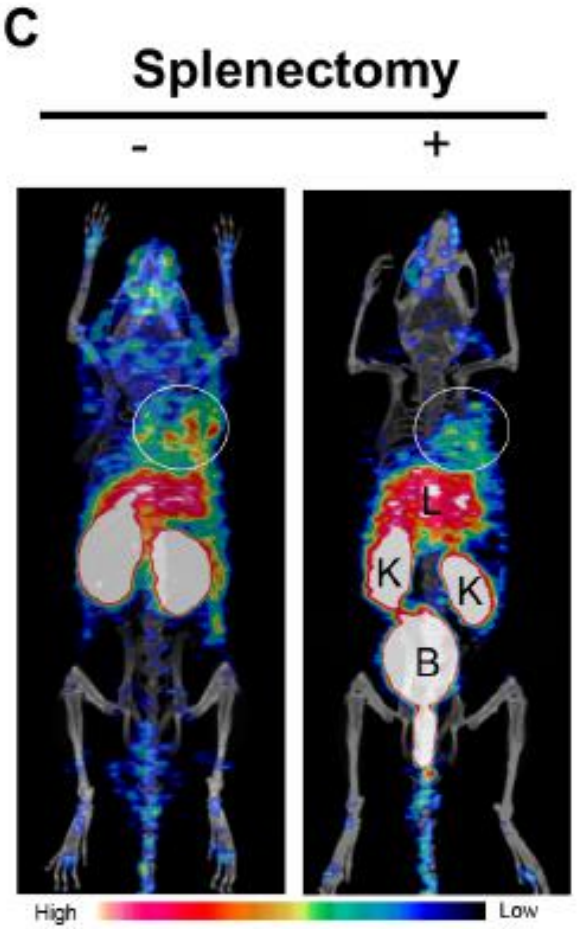


Control **Depletion of host CM**

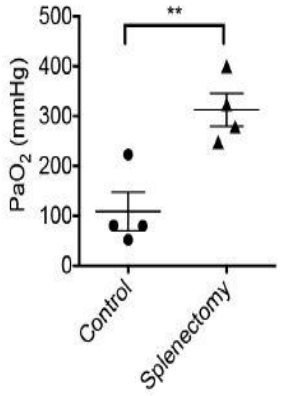


Inflammatory host-derived classical monocytes are recruited from the spleen

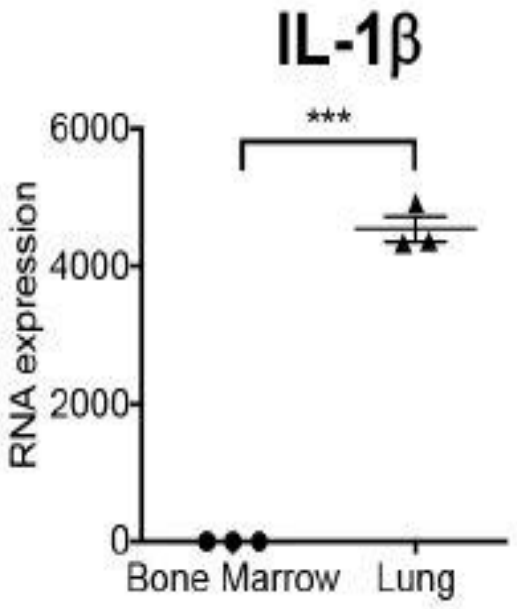




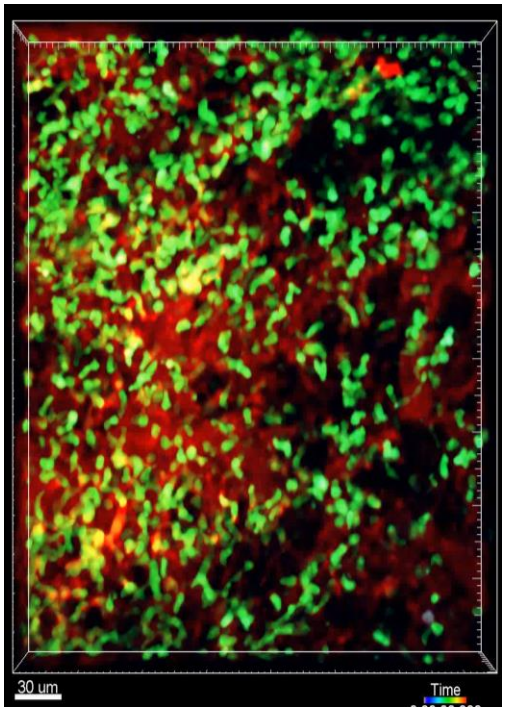
Gas exchange



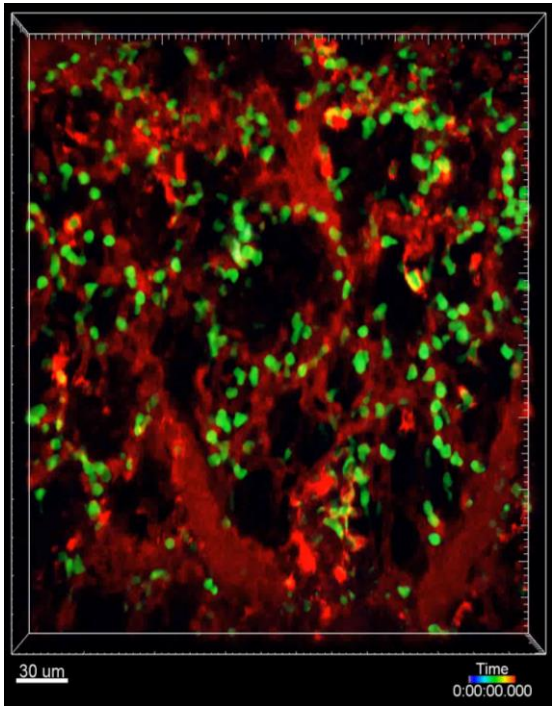
IL-1β production by host classical monocyte is necessary for neutrophil extravasation



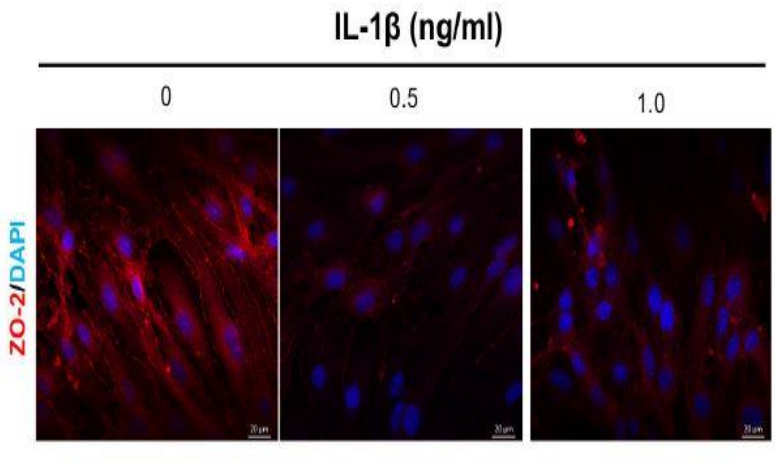
WT donor

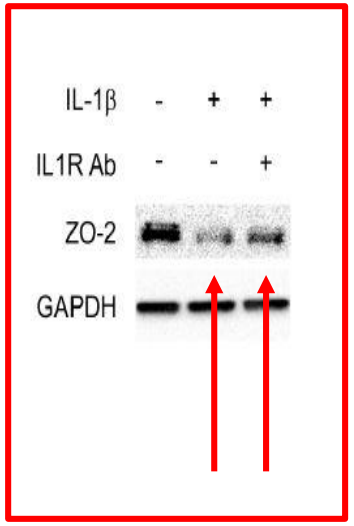


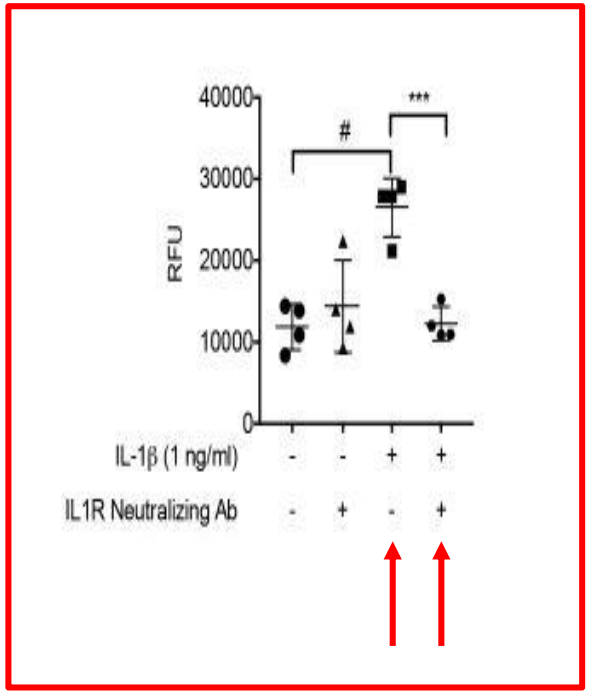
IL1-R KO donor



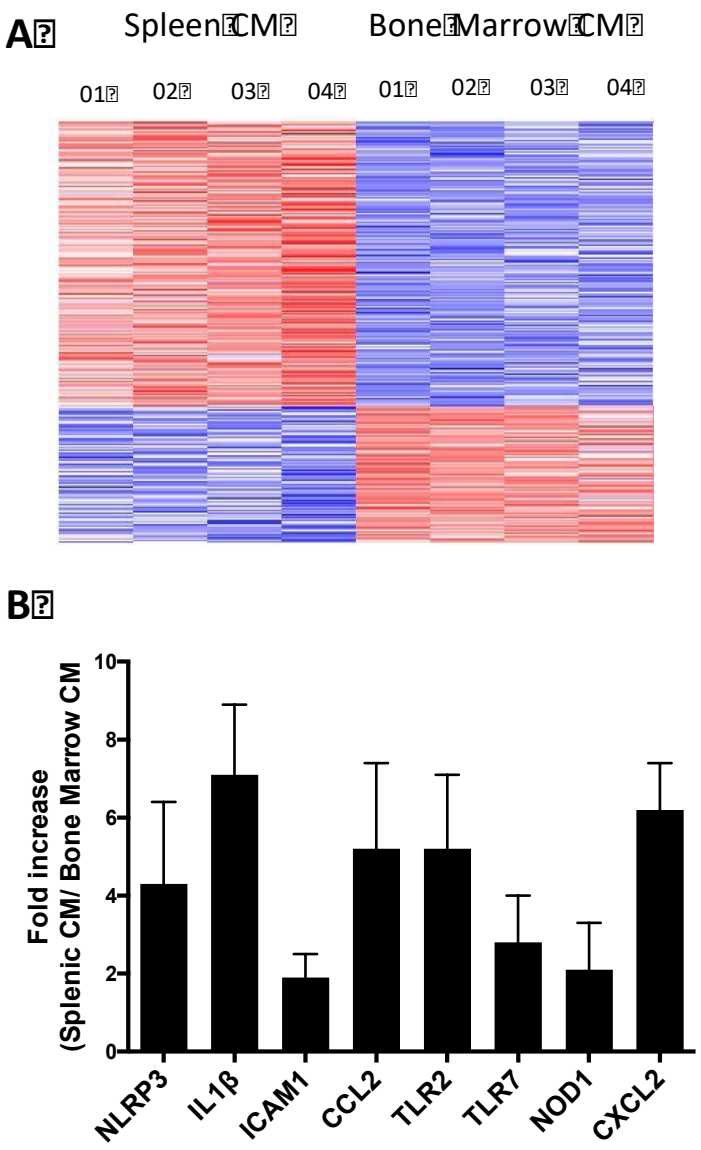
IL-1β downregulates ZO-2 in endothelial cells disrupting endothelial barrier



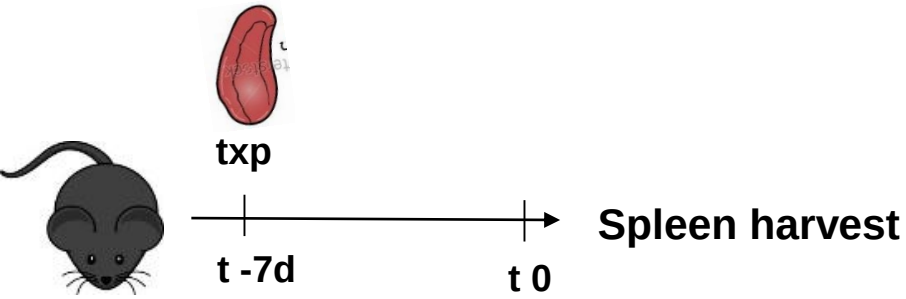


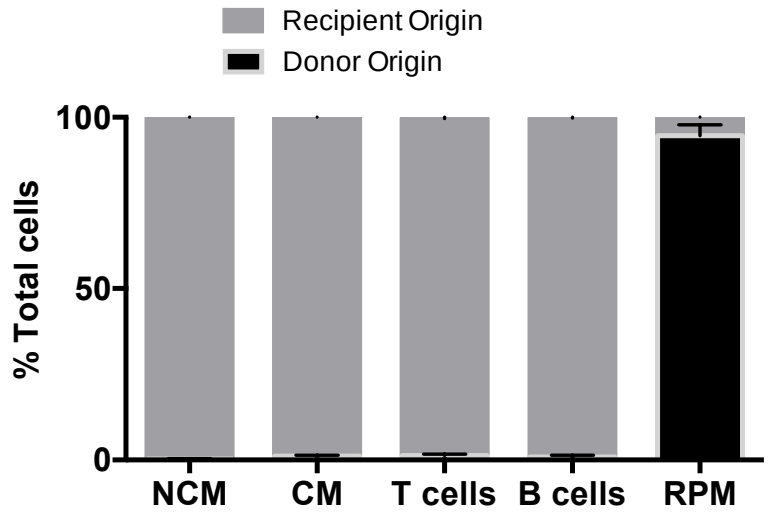


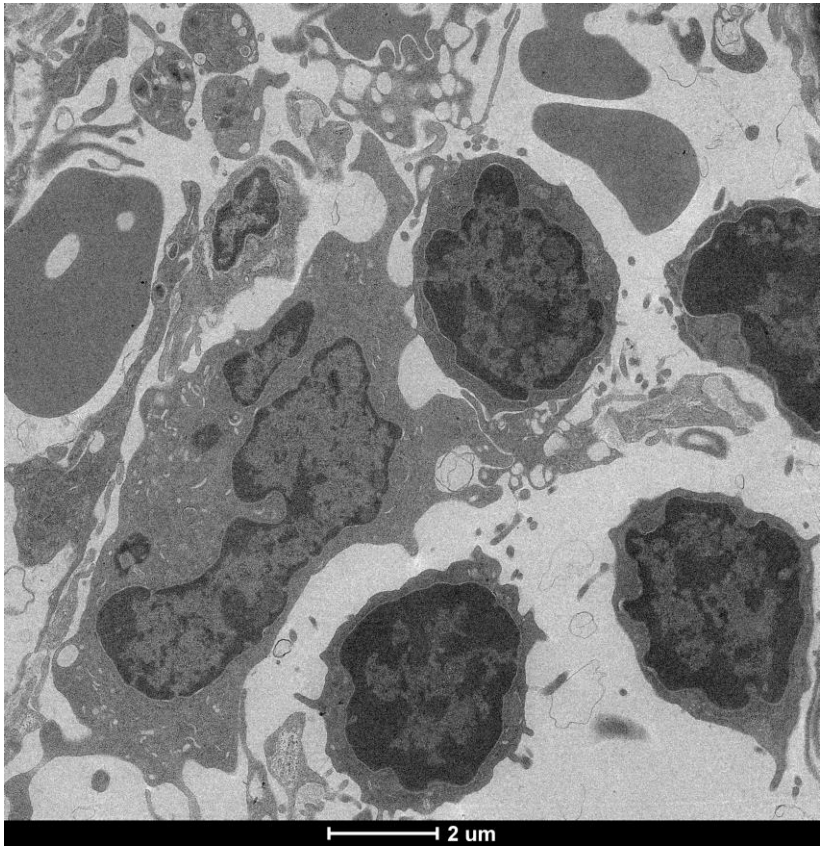
Spleen not merely a monocyte reservoir – A new paradigm for splenic education of monocytes



Bone marrow derived CM receive maturation signals from red pulp macrophages

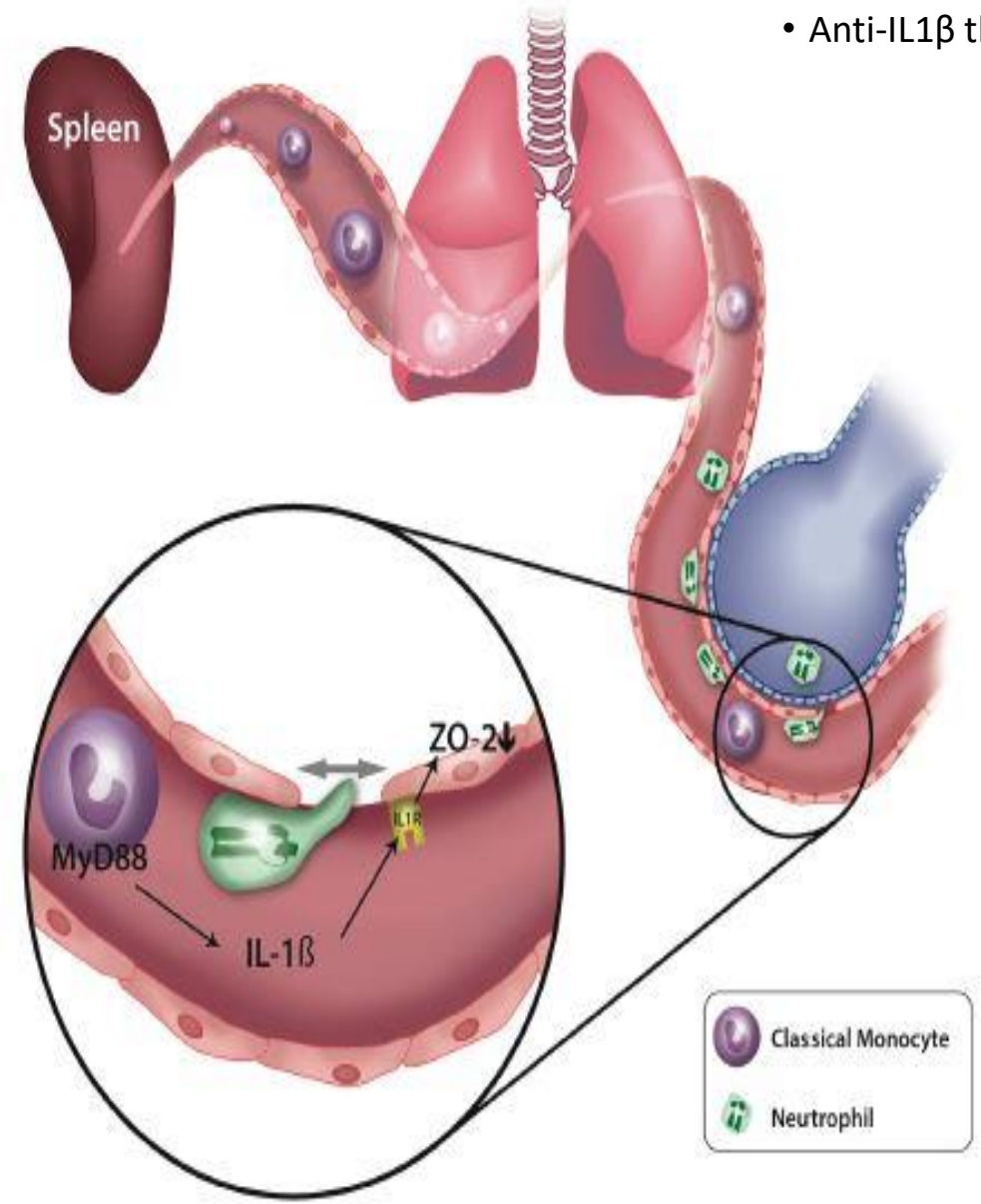




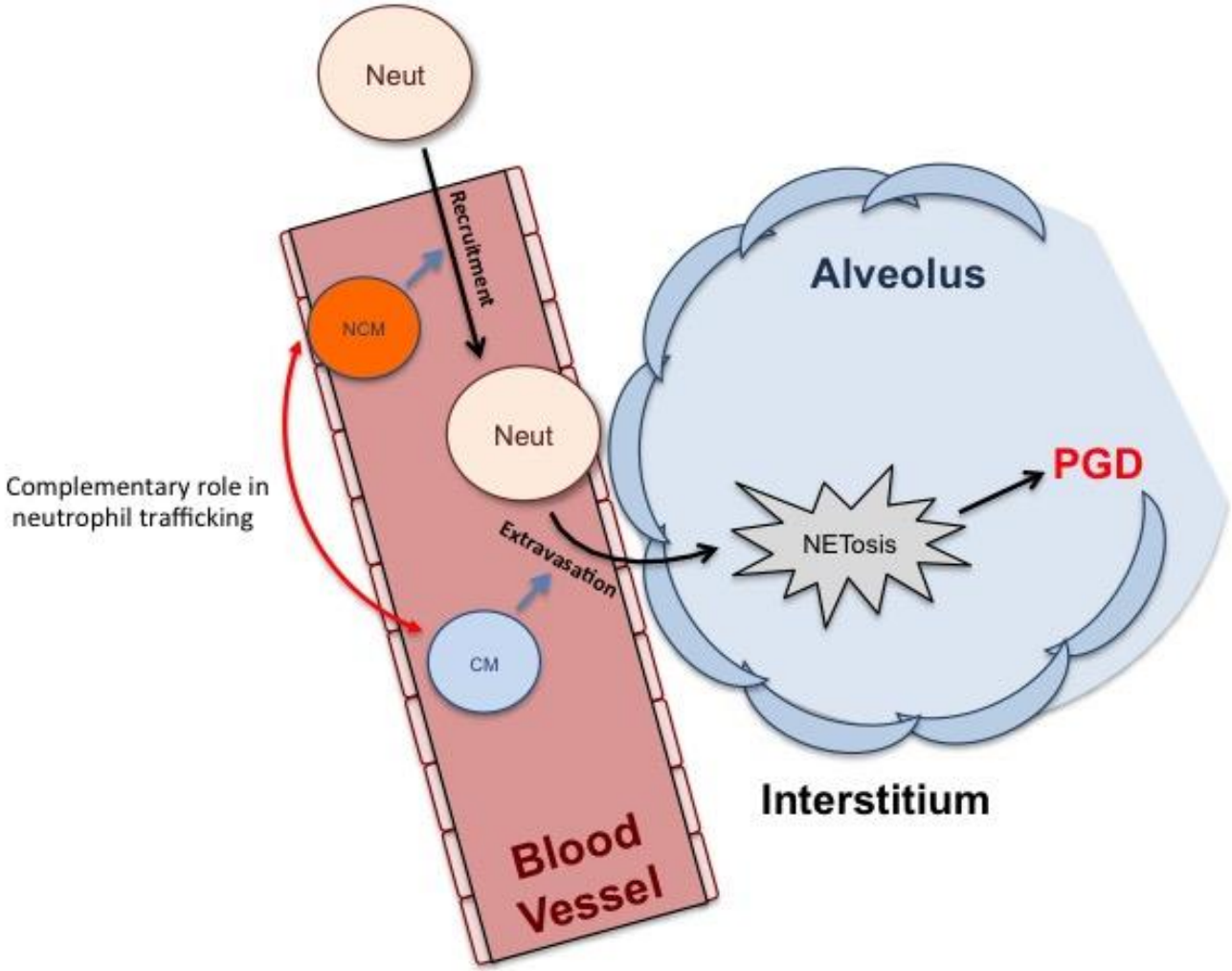


Potential therapeutic targets

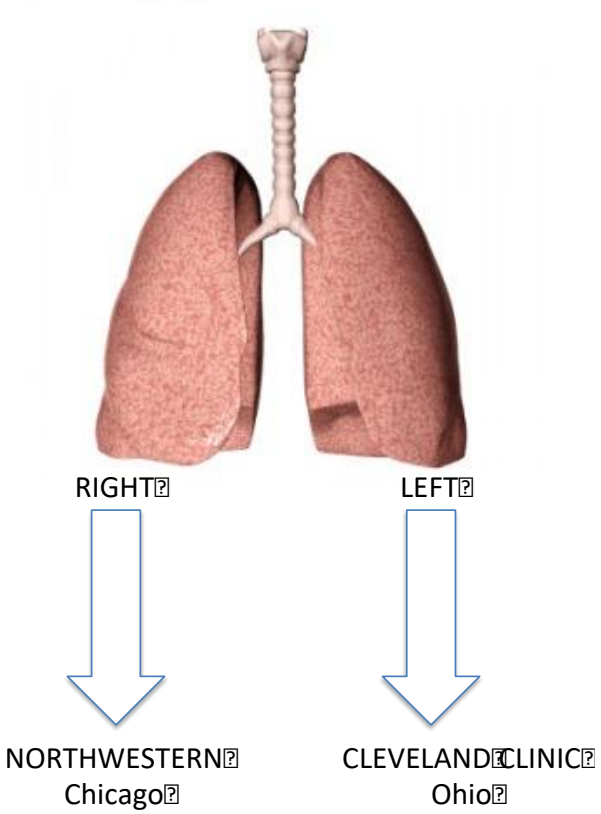
- Anti-IL1β therapy



Hsiao et al, J Clin Invest, 2018



Bharat & Kreisel, Ann Thor Surg, 2018



NORTHWESTERN

62-yr female Emphysema 6L/min O_2

No traditional risks for PGD

CLEVELAND CLINIC

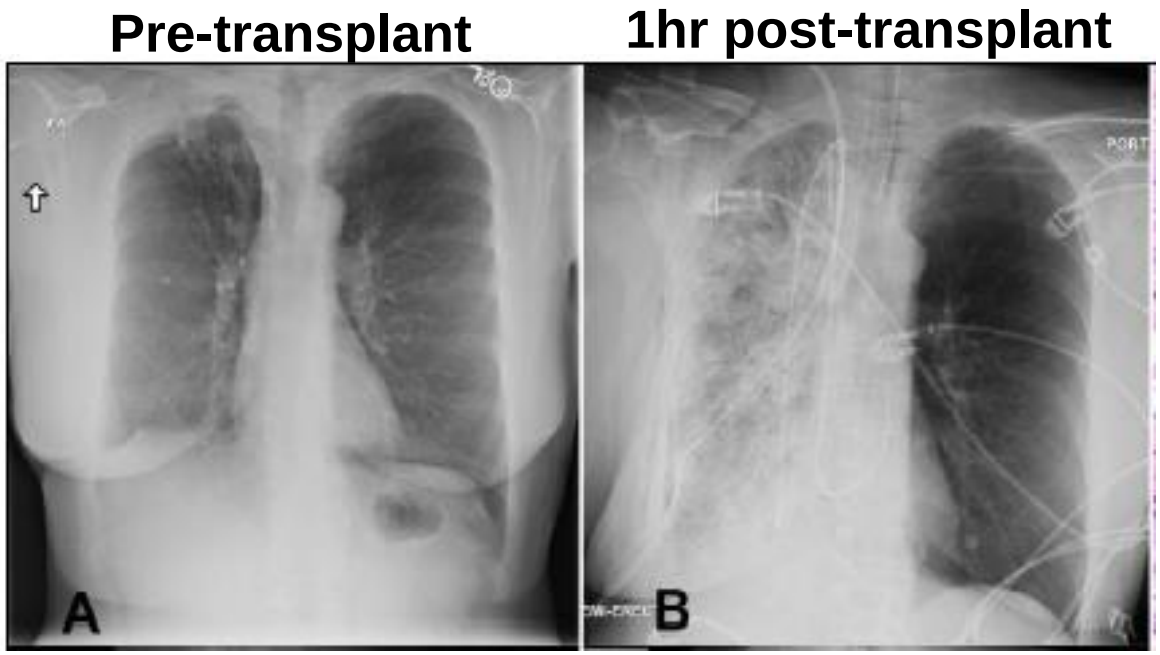
66-yr male, IPF, Pulmonary hypertension, Prior LIMA graft, Cardiopulmonary Bypass

Ischemia time <3 hours for both

All Donor/ Recipient Cultures Negative

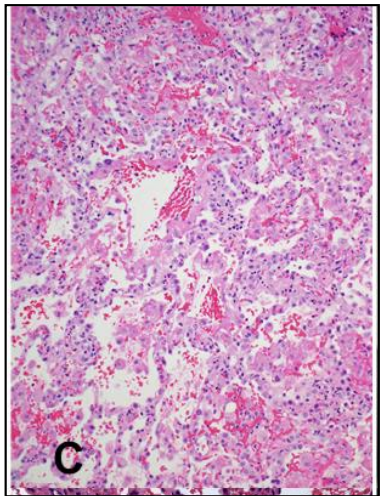
No DSA and Cross Match negative

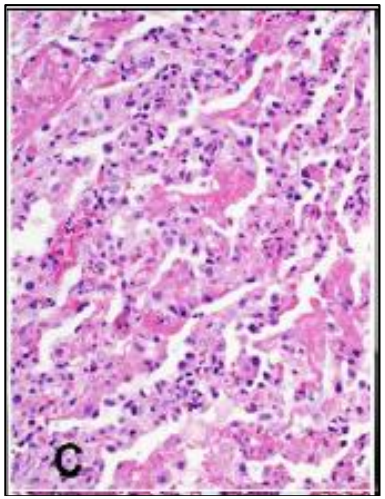
	Pre-reperfusion	Post-reperfusion			
		10m	15m	30m	45m
FiO_2(%)	100	30	30	100	100
O_2 sat(%)	90	100	100	92	93
PaO_2(mmHg)	80	150	155	78	82



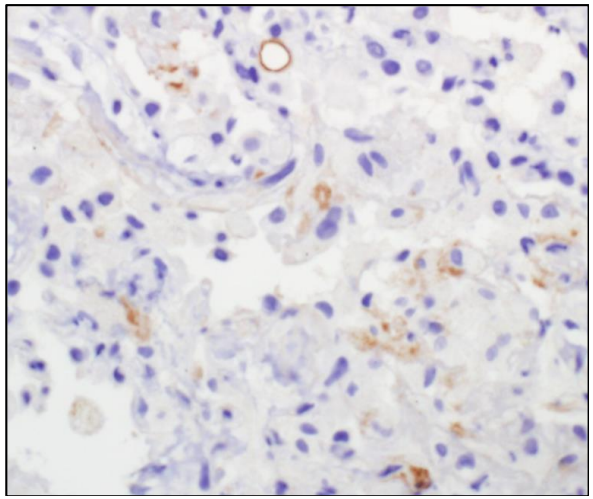
Autoantibody mediated rejection can mimic PGD

H & E STAINING



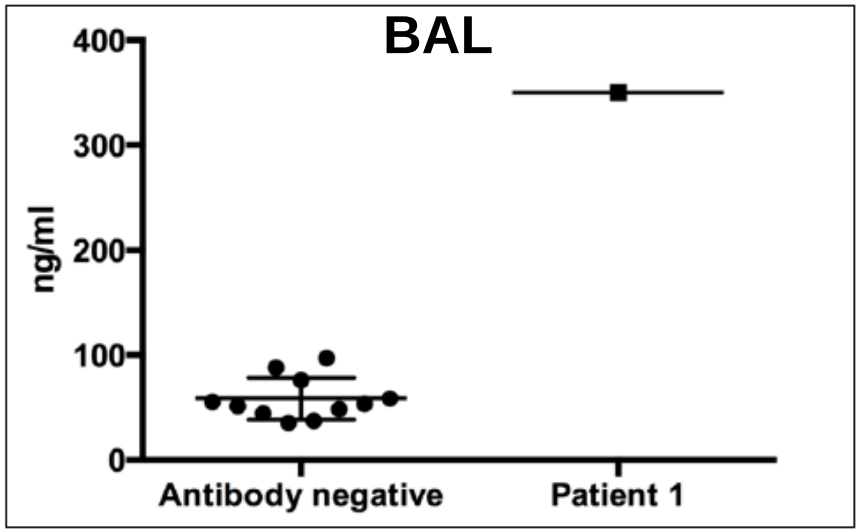


COMPLEMENT STAINING

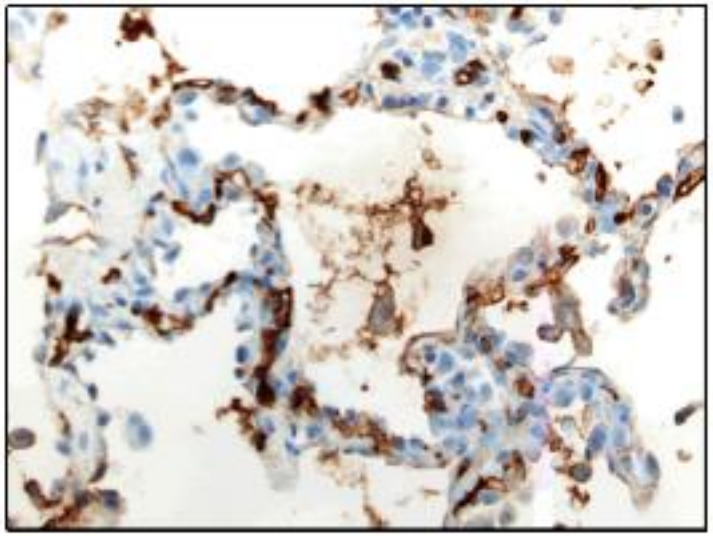


Septal Neutrophils
Hyaline membrane
Alveolar Damage

Elevated soluble C4D in BAL



Peri-capillary IgG staining



	Serum Autoantibodies	Pre-Transplant	Day of Transplant
Lung-restricted antigens	**Col V**	**Strong Positive**	**Strong Positive**
	K-α1 Tubulin (KAT)	**Moderate Positive**	**Moderate Positive**
	Col I	**Mild Positive**	**Mild Positive**
	Col IV	Negative	Negative

TREATMENT

IVIG (1g/kg)

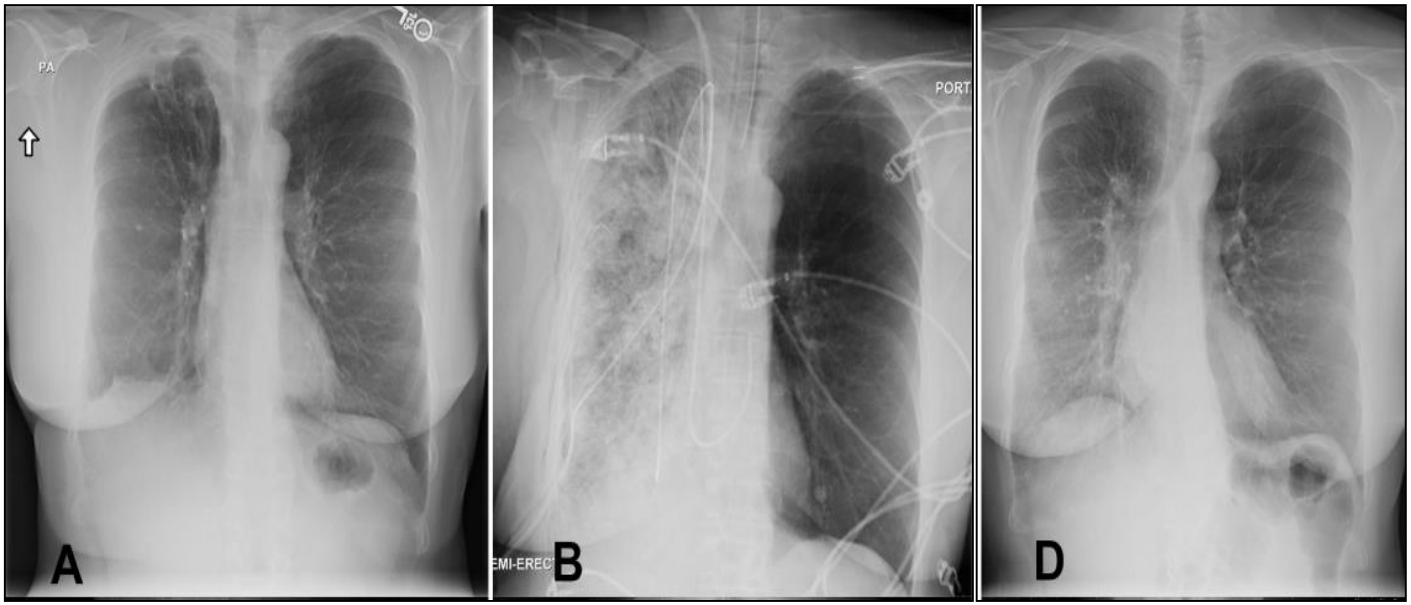
PLASMAPHERESIS

Eculizumab

Rituxamab (375mg/m^2)

Maintenance: Tacrolimus, Mycophenolate, Prednisone

Pre-Transplant | 1-Hr Post-Transplant | 2 weeks



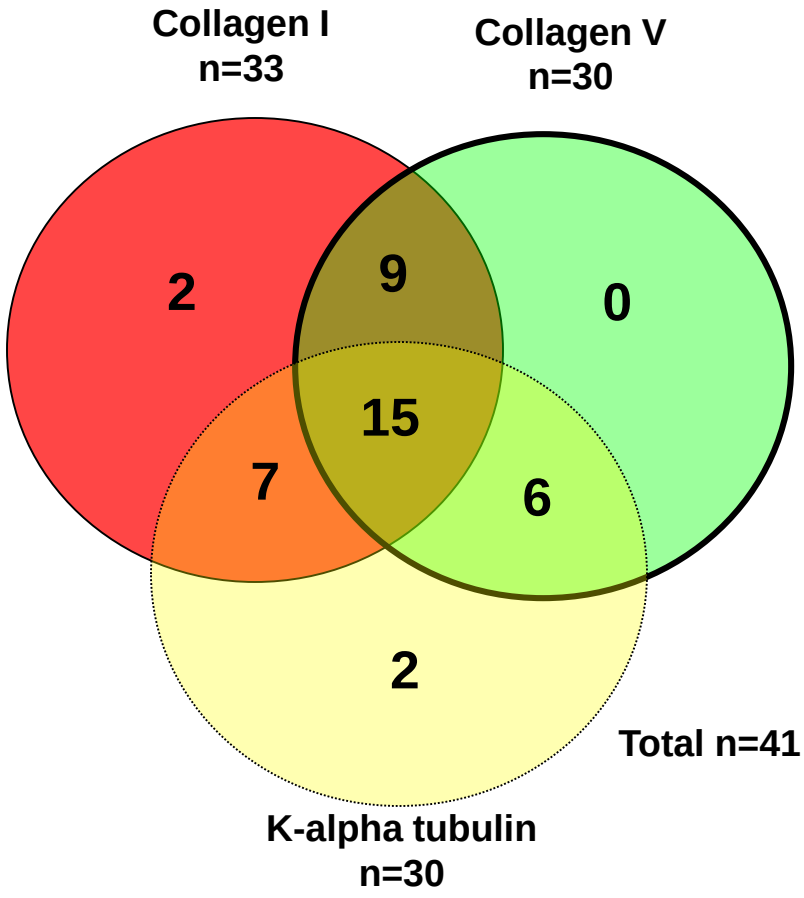
Fernandez et al, Ann Thor Surgery, Oct 2016

6-MONTH FEV1

71%

High incidence of pre-existing lung-specific autoantibodies in transplant recipients

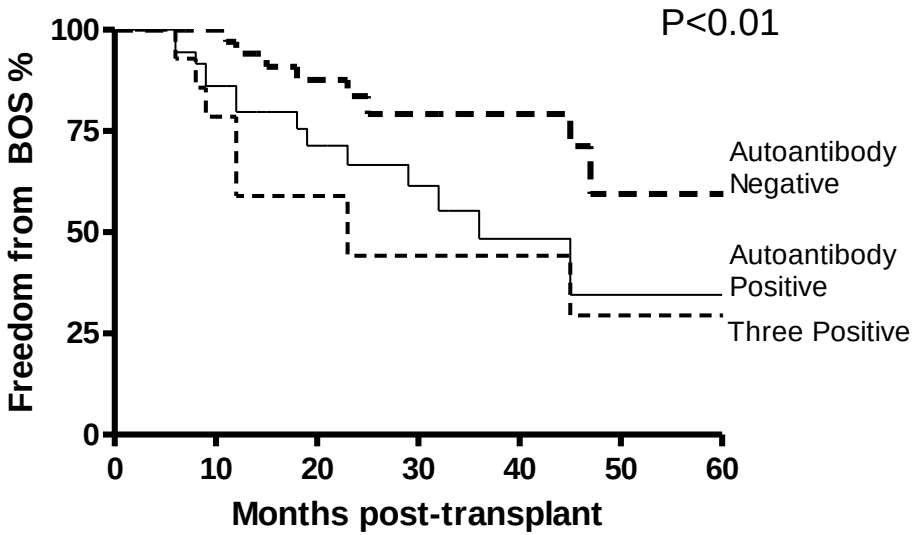
Total study subjects: 142
Antibody positive: 41 (28.9%)
One: 4 (2.8%)
Two: 22 (15.5%)
Three: 15 (10.6%)



Bharat A et al, Ann Thor Surg, 2012

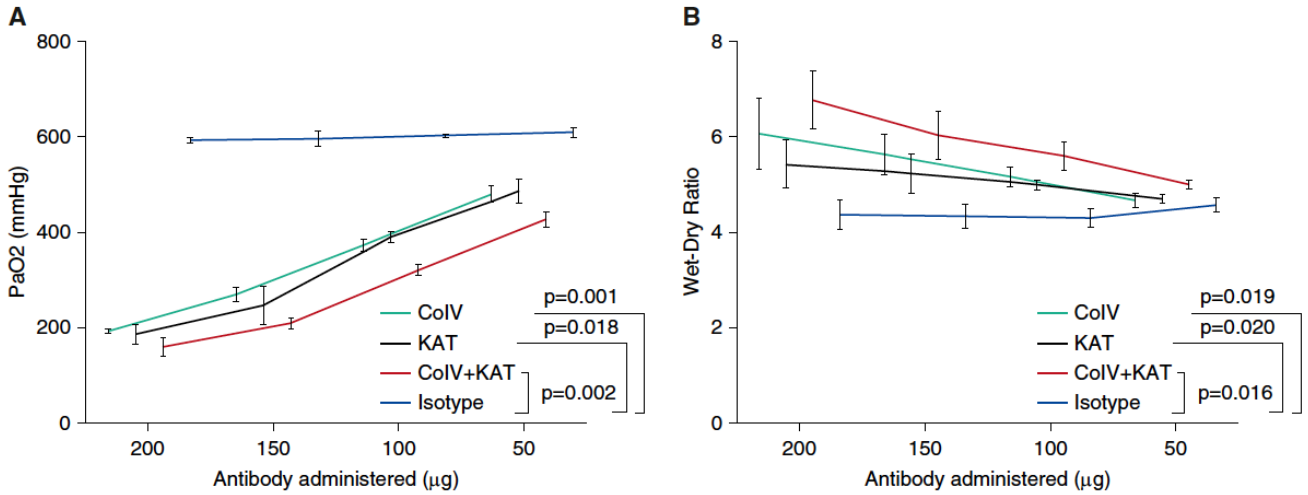
LRA predispose to PGD and chronic rejection

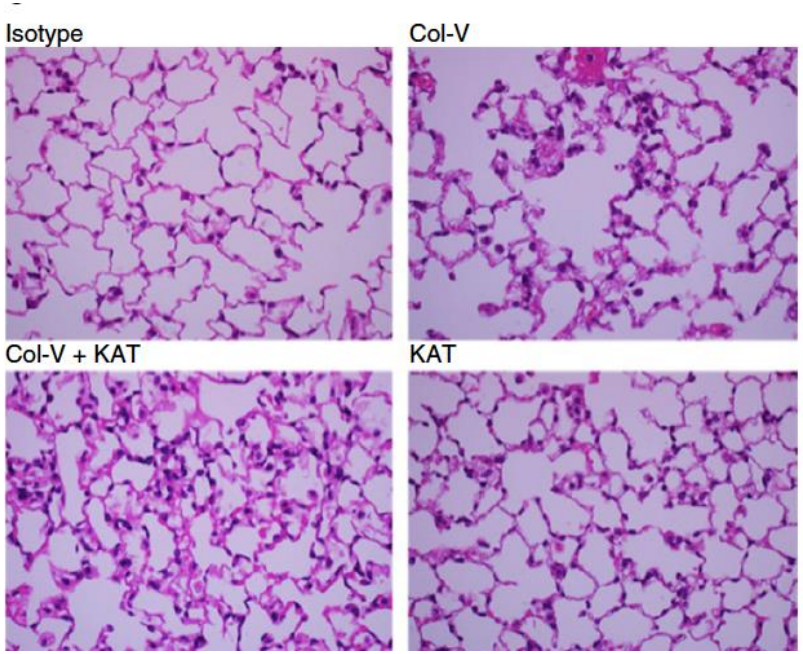
	PGD -ve	PGD +ve	Odds Ratio	CI	p
All (n=142)	41(28.9%)	101(71.1%)			
Antibody –ve	35 (34.5%)	66(65.5%)			
Antibody +ve					
All	6(19.4%)	35(80.6%)	3.09	1.2–8.1	0.02
Two Positive	3(13.6%)	19(86.4%)	0.07	0.9–12.1	0.07
Three Positive	1(6.7%)	14(93.3%)	7.4	0.9–58.9	0.03



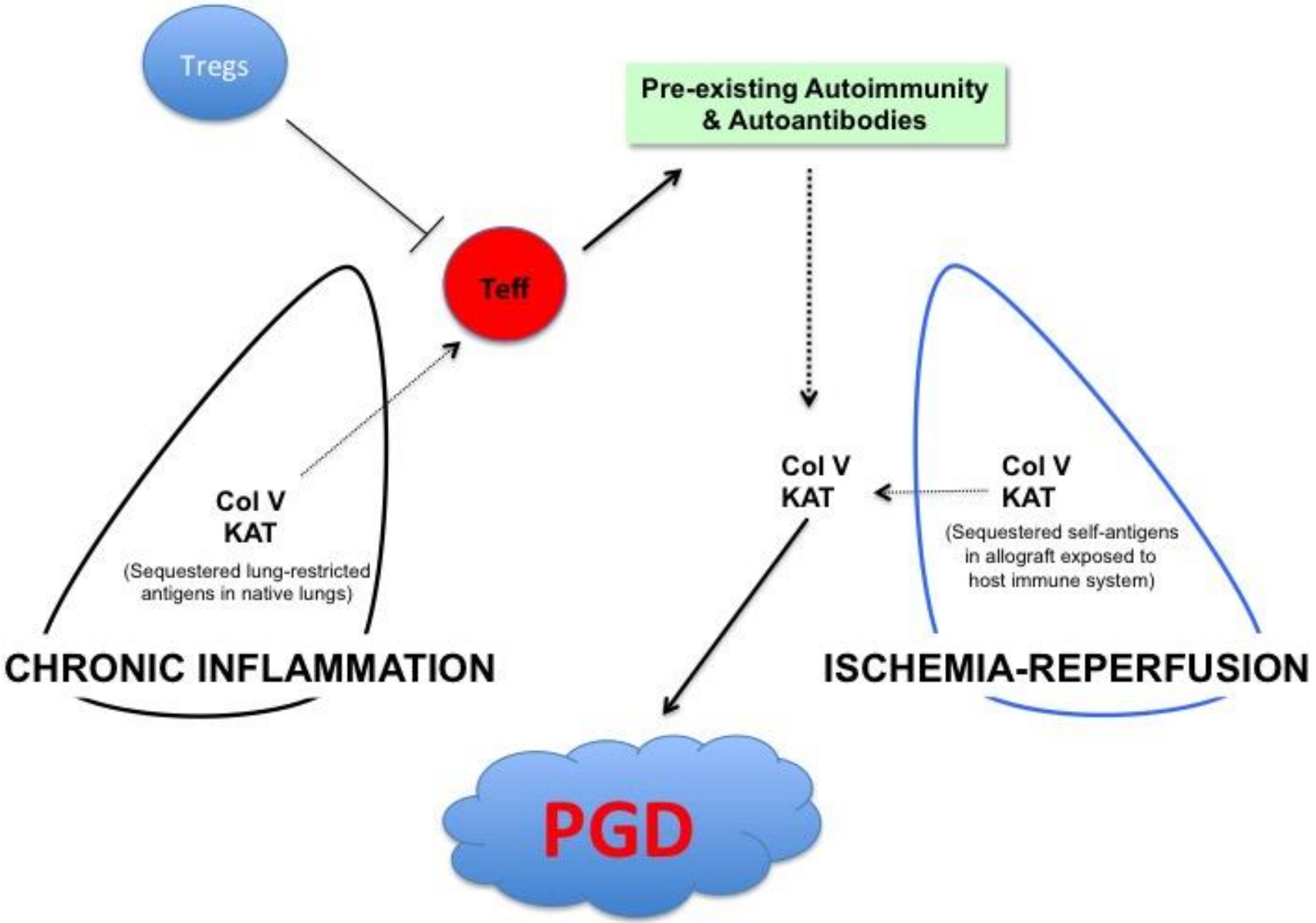
Bharat A et al, Ann Thor Surg, 2012

Lung autoantibodies induce rejection of murine lung grafts





Bharat et al, AJRCMB, Nov 2016



Bharat & Kreisel, Ann Thor Surg, 2018

Severe PGD

Lung biopsy

Antibody-mediated
LRA or DSA

Complement staining

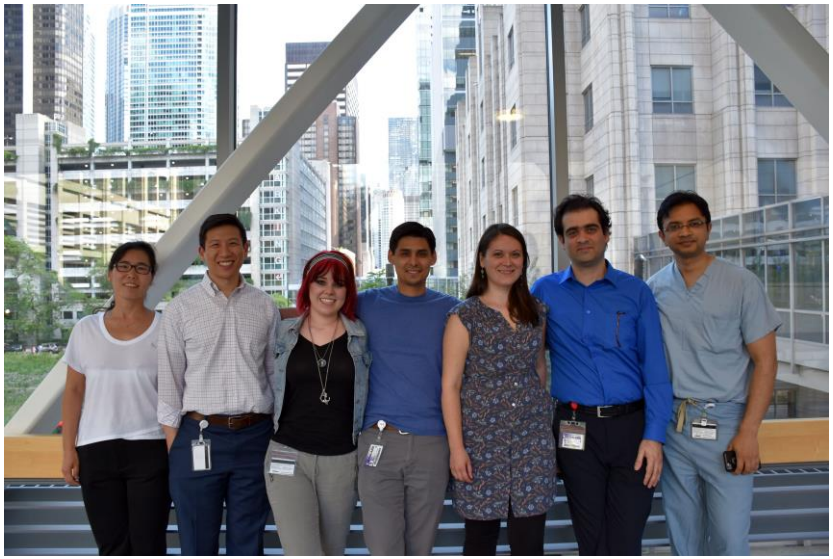
Complement inhibition

Antibody-directed therapy
- Plasmapheresis
- IVIG
- Rituximab
- Bortezomib

Monocyte/Macrophage mediated
Or DAD

Supportive therapy
- ECMO
- Novel targets
  Anti-IL1β
  Bisphosphonates

Acknowledgements



Funding

R01 HL487967
R01 HL757667

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