Innate Immunity in MDS Pathogenesis

Alan List, MD

Regulation of Innate Immune Response

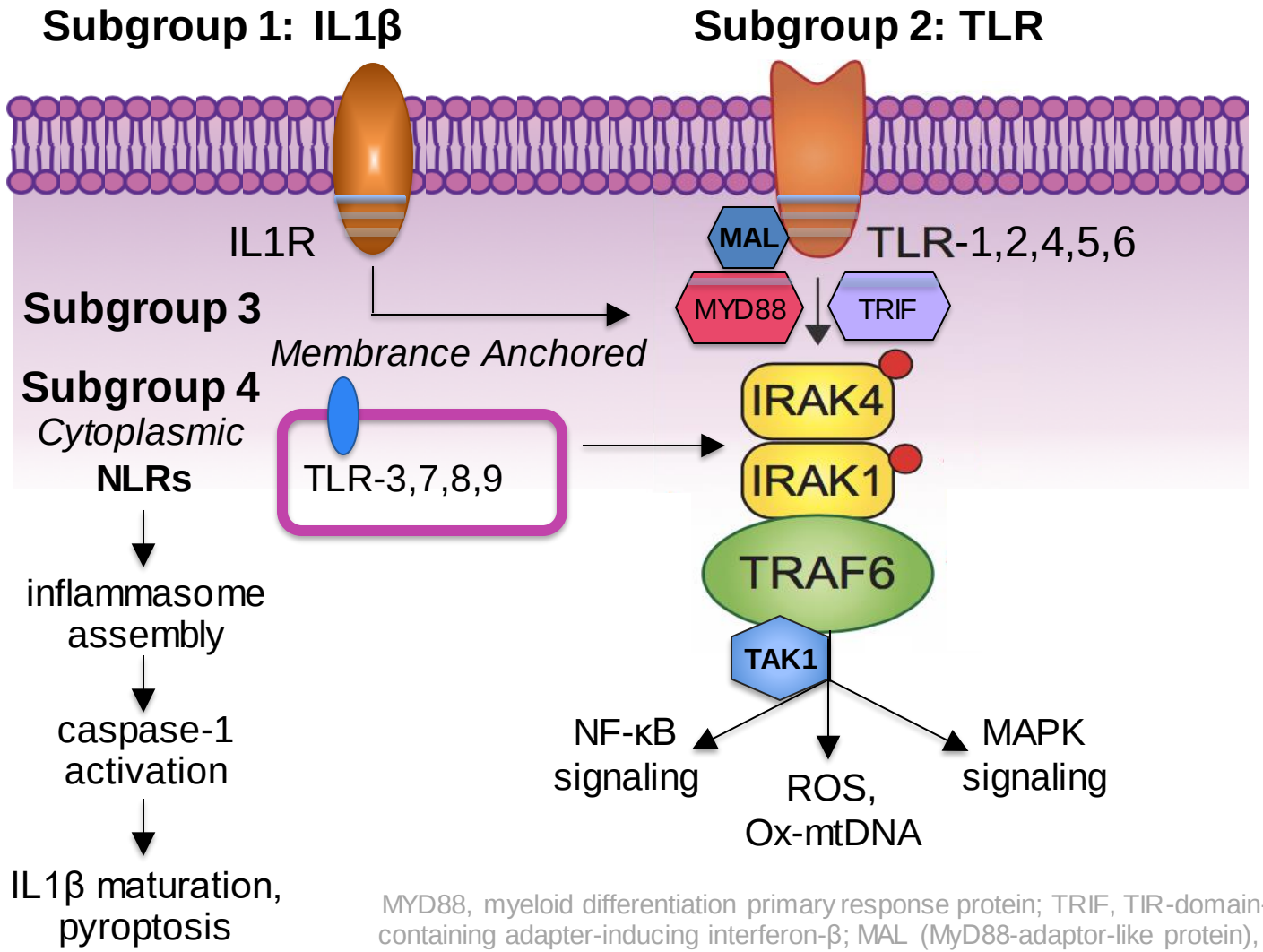
Nlrp3 Inflammasome & Pyroptosis

MDSC Effectors of Ineffective Hematopoiesis

Somatic Mutations License the Inflammasome

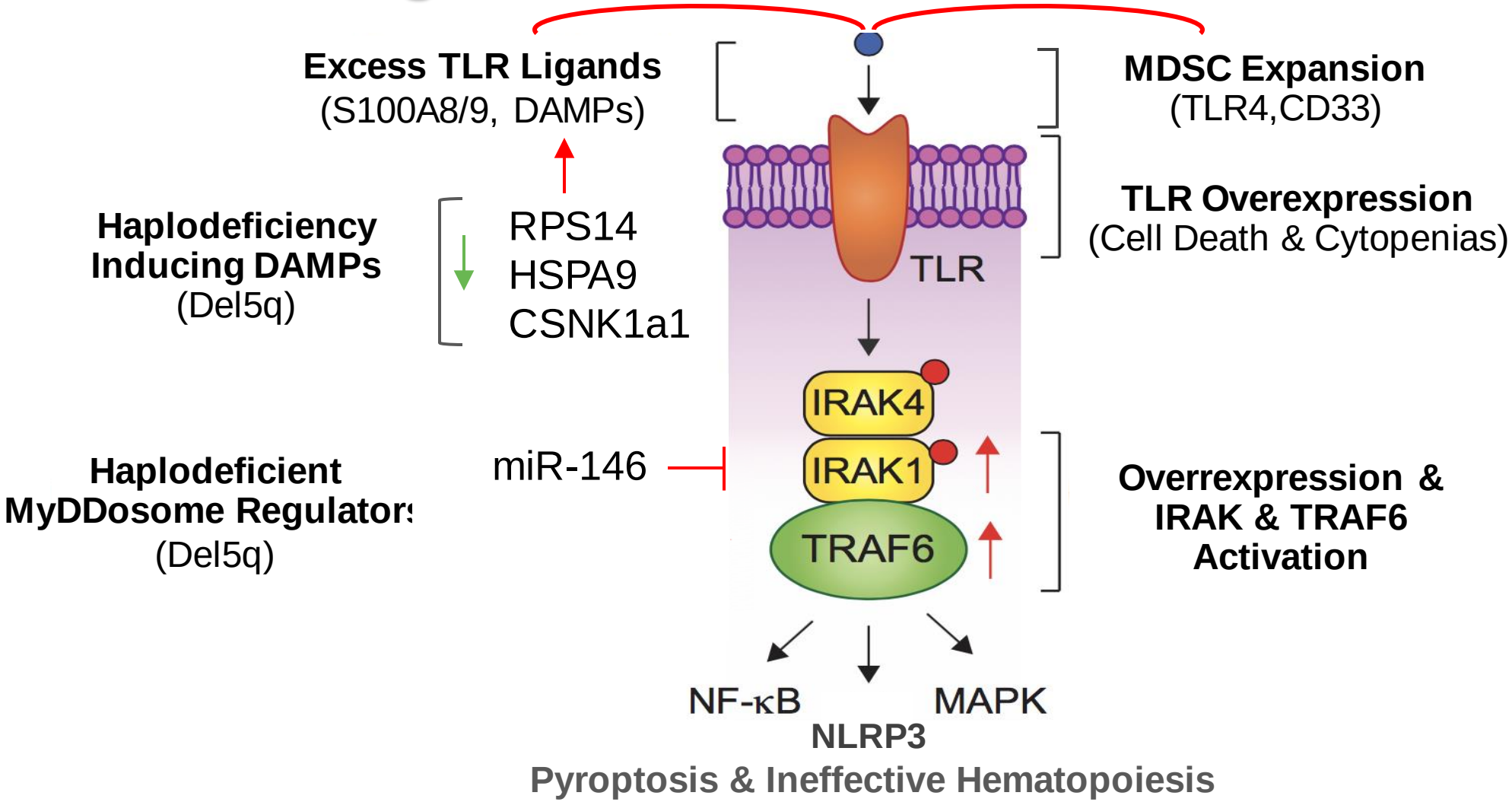
Pattern Recognition Receptors (PRR) Central to Innate Immune Response

Classical Membrane Anchored PRRs (PAMPs, DAMPs)



MYD88, myeloid differentiation primary response protein; TRIF, TIR-domain-containing adapter-inducing interferon-β; MAL (MyD88-adaptor-like protein), .

MDS HSPCs are Primed for Response to Innate Immune Signals



Adopted from Dan Starczynowski 2017; Xing Y, et. al. I Immunol 2017;199 .

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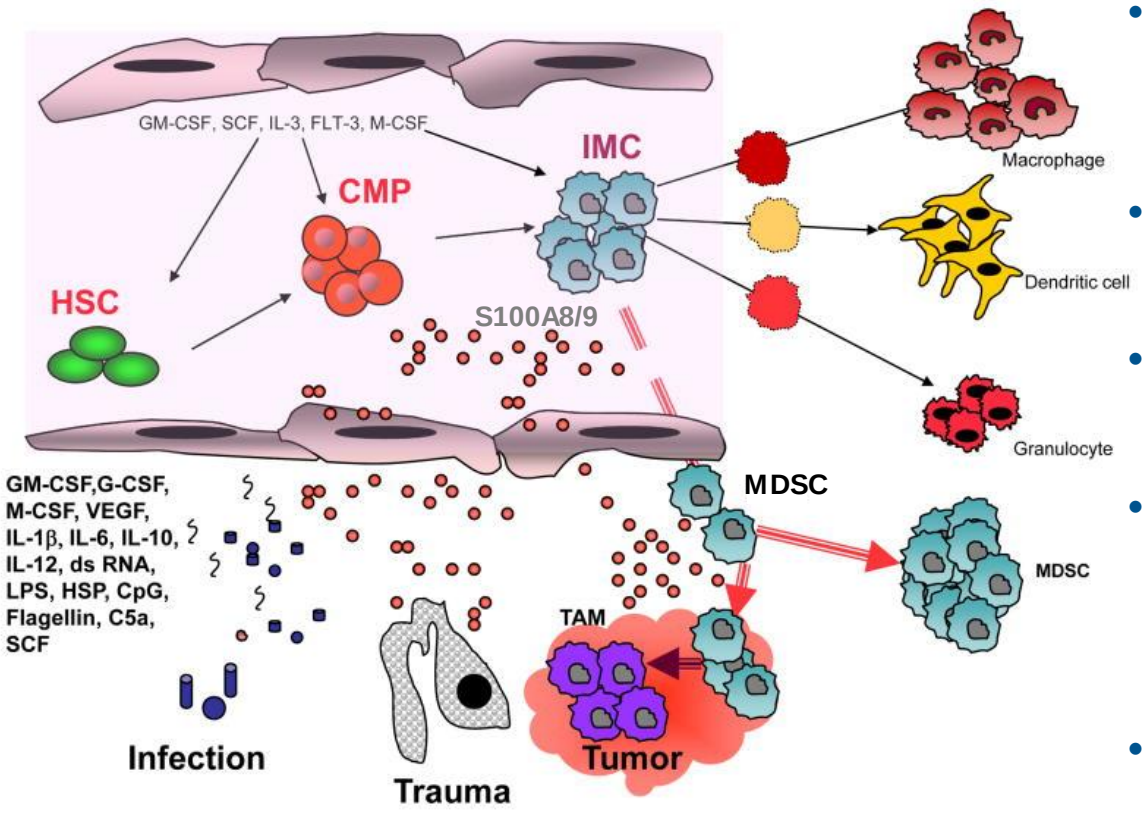
Myeloid-Derived Suppressor Cells (MDSC)



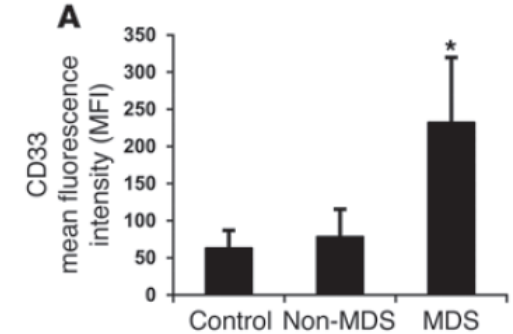
*DAMP: danger-associated molecular pattern.

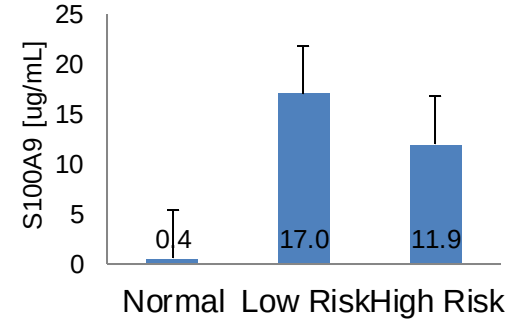
- Immature myeloid cells (IMC)
  - Human: $Lin^{-}HLA^{-}DR^{-}CD33^{+}$;
    Mouse: $CD11b^{+}Gr^{-}1^{+}$ ($\pm$B220, CD31)
- Expand with age, infection,chronic inflammation, and neoplasia.
- Induce tumor immune tolerance & T-reg expansion.
- Elaborate multiple soluble effectors: ROS, NO, and arginase; VEGF, TNFα, TGF-β, IFN, IL-6, IL-10 IL-1β & granzyme granules
- MDSC expansion and activation driven by TLR ligands (e.g., DAMP signals)

Gabrilovich D. Nat Rev Immunol. 2009 Mar; 9(3): 162–174.

MDSC Direct Ineffective Hematopoiesis in MDS

- Medullary MDSC are markedly expanded in LR-MDS and genetically distinct from the malignant clone
- MDS MDSC suppress autologous hematopoiesis
- The CD33-SIGLEC3 ITIM signaling receptor is over-expressed by MDS-MDSC & is indispensable for S100A9 induction of inflammatory cytokines
- S100A9 is a Ca^{++} binding, proinflammatory myeloid-related protein that binds CD33 & heterodimerizes with S100A8 to engage TLR4 & CD33
- S100A9 is overexpressed in MDS progenitors with high concentration in MDS BM plasma
- S100A9-Tg mice develop trilineage dysplasia and pancytopenia that phenocopies human MDS



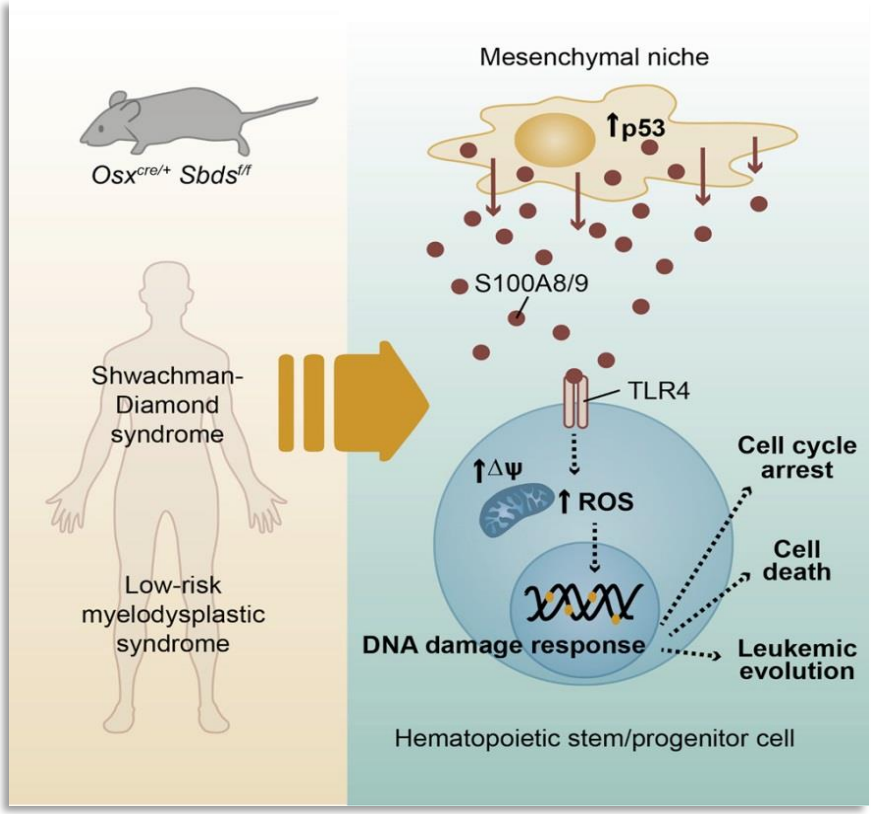


Chen X, et. al. J Clin Invest 2013; 123(11):4595-4611

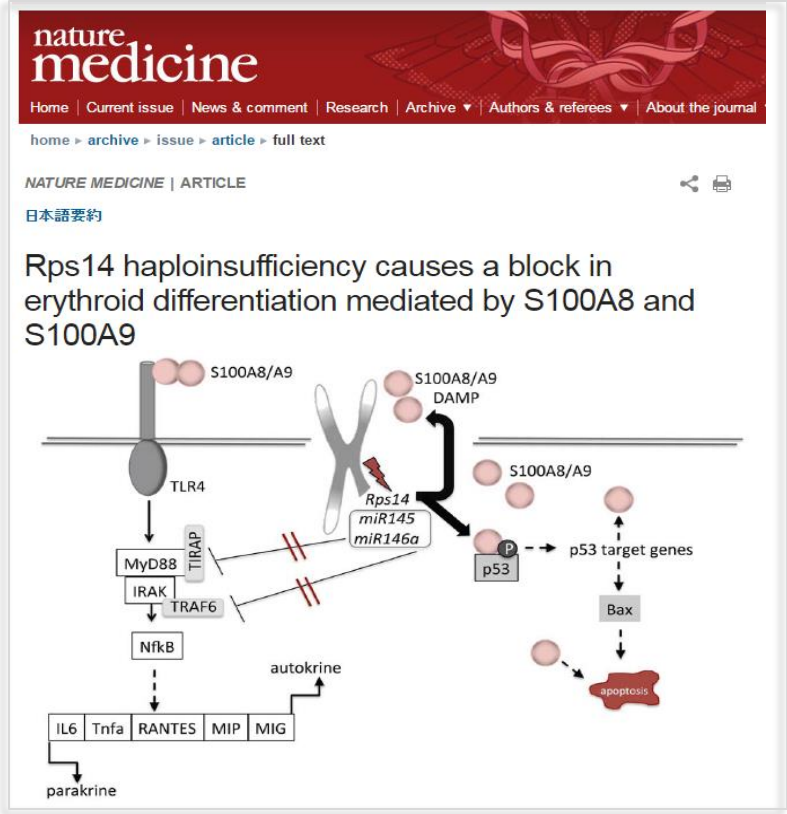
*Immunoreceptor tyrosine-based inhibition motif (ITIM); Sialic Acid-binding Ig-Type Lectin

S100A8/9-TLR4 Signaling Drives Mesenchymal Inflammation-induced Genotoxic Stress & Erythroid Death

Cell Extrinsic



Cell Intrinsic



Schneider R, et. al. Nat Med 2016; 22: 288
Zambetti & Raaijmakers. Cell Stem Cell 2016;19: 613–627.

Regulation of Innate Immune Response

Nlrp3 Inflammasome & Pyroptosis

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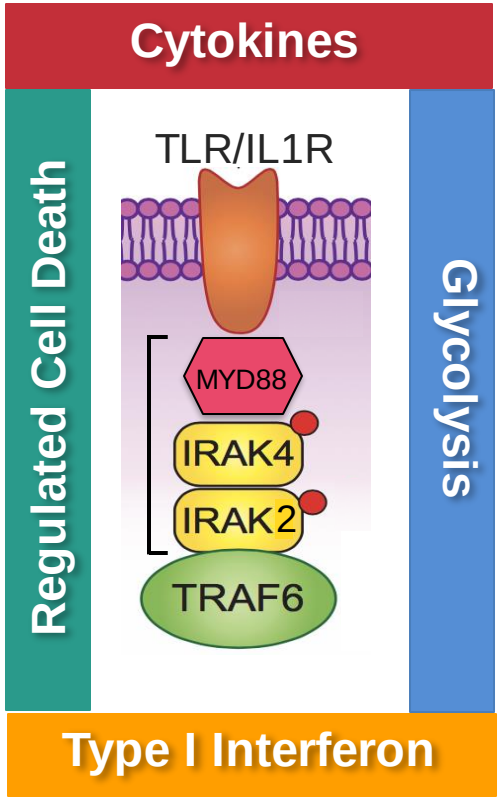
Somatic Mutations License the Inflammasome

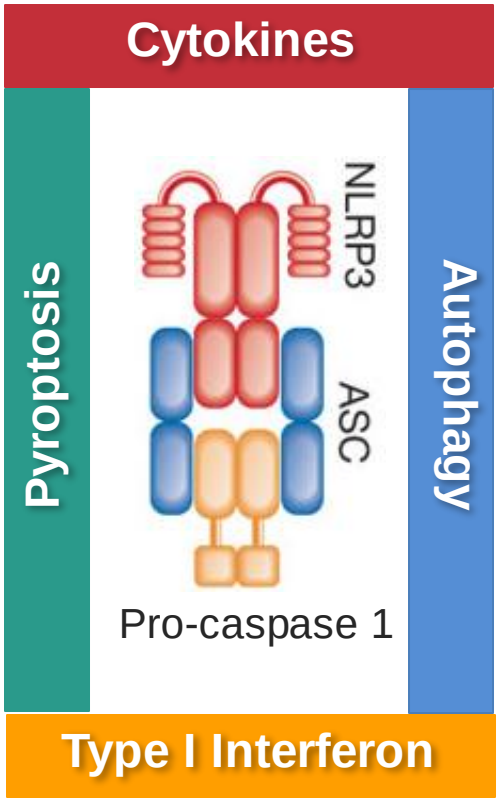
Supramolecular Organizing Centers (SMOCs)

Innate Immune Signaling Modules

MyDDosome



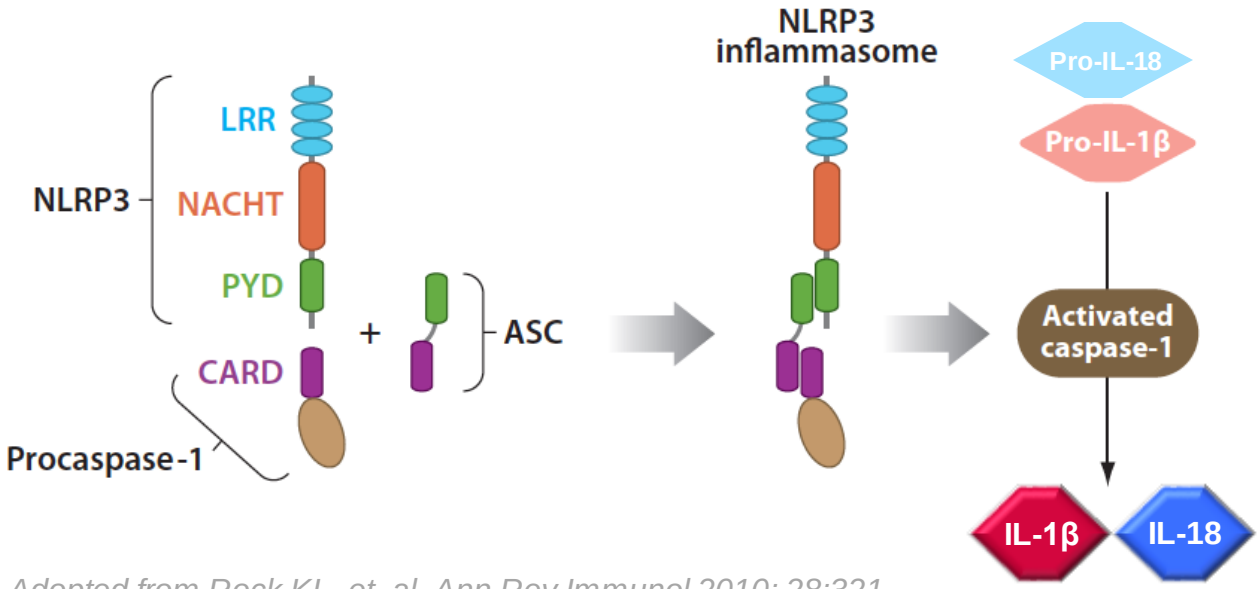
Inflammasome



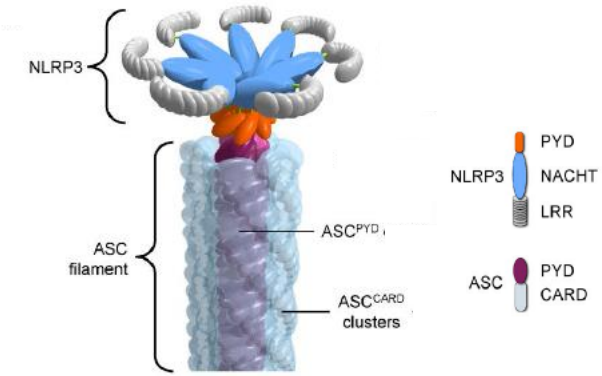
Tan Y, Kagan J. Cell 2019; 177:1-15.

NLRP Inflammasomes

- Nucleotide-binding & oligomerization domain (NOD)-like receptor proteins (NLRP) are cytosolic PRRs that respond to danger signals to trigger inflammasome (IFM) formation
- NLRP3 (NALP3 or cryopyrin) forms IFM complex by associating with ASC adaptor, which recruits Pro-Caspase-1 through its CARD domain
- Caspase-1 undergoes autocatalytic processing to yield two subunits that form the active caspase cleaving pro-IL-1 & -18, and gasdermin-D (pyroptosis)

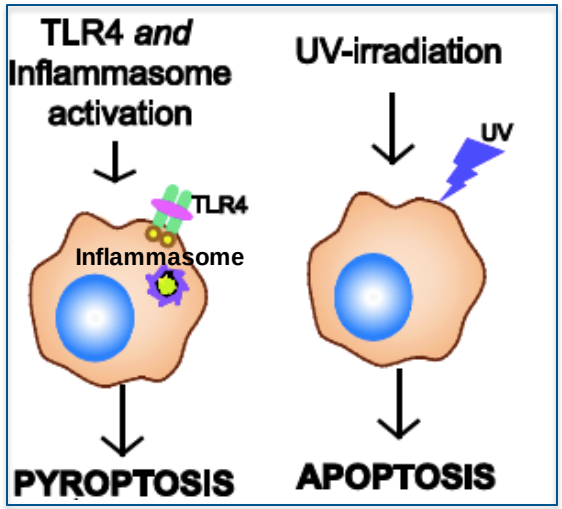


Adopted from Rock KL, et. al. Ann Rev Immunol 2010; 28:321.



Schmidt FI, et. al. JEM 2016;213:771.

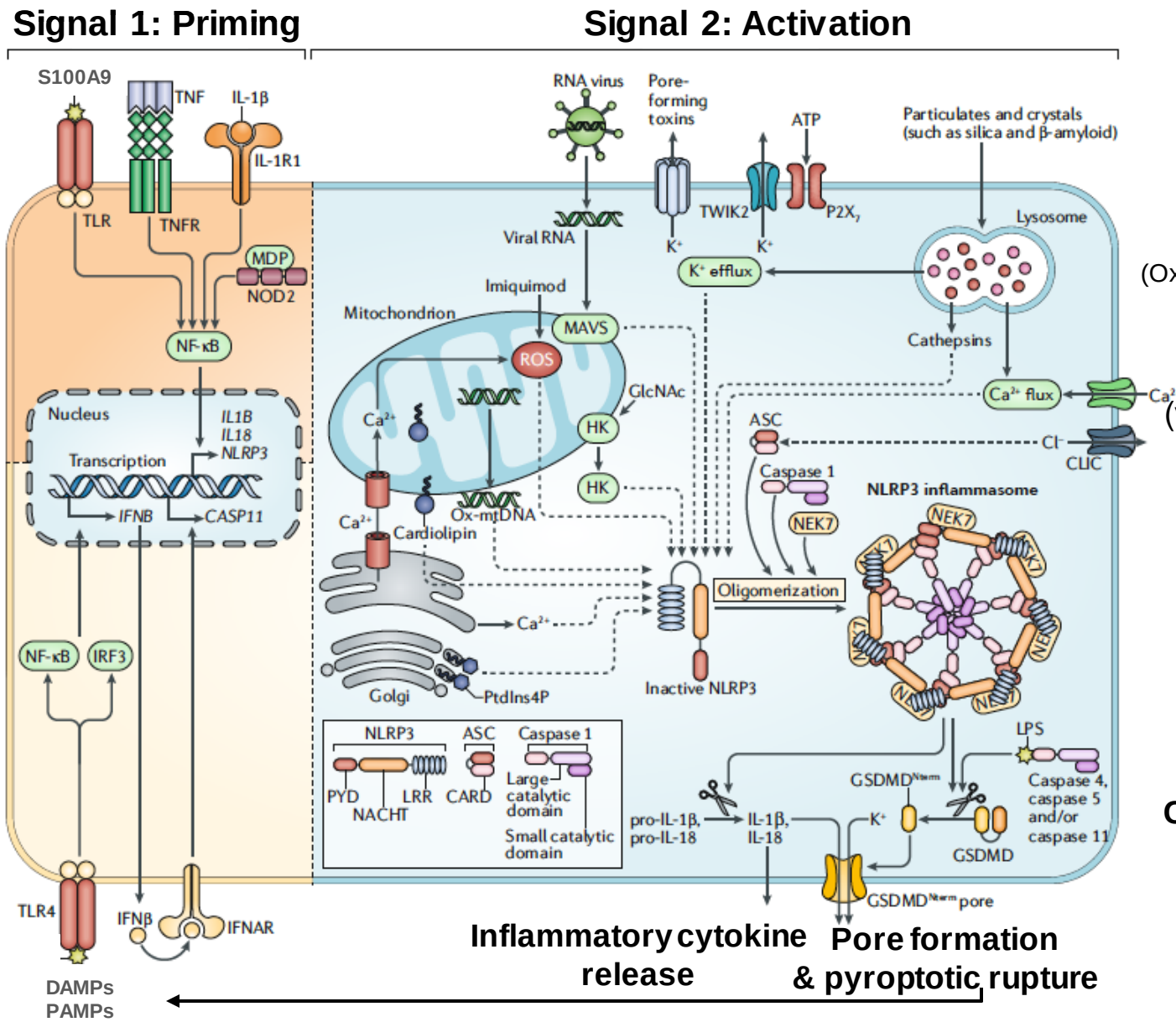
Pyroptosis: Caspase-1 Dependent Inflammatory Cell Death



Characteristic	Apoptosis	Pyroptosis
Cell lysis	-	+
Cation channel activation	-	+
Nuclear condensation	+	+
DNA fragmentation	+	+
PS externalization	+	+
Inflammasome assembly	-	+
Caspase-1 activation	-	+
Caspase-3 activation	+	late
Inflammatory cytokines	-	+

PS denotes Phosphatidylserine

NLRP3 Inflammasome (IFM) Priming & Activation



Signal sensing by NLRP3
(Ox-mtDNA, NEK7, PKR, TXNIP, cathepsin-B)

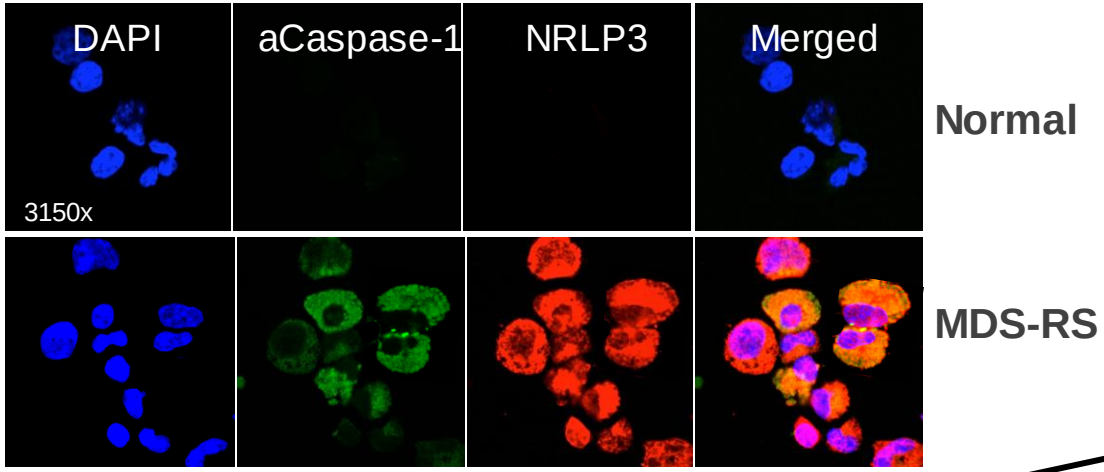
Cation/Ca^{2+} influx
(vimentin digestion → cytoskeletal collapse & osmotic swelling)

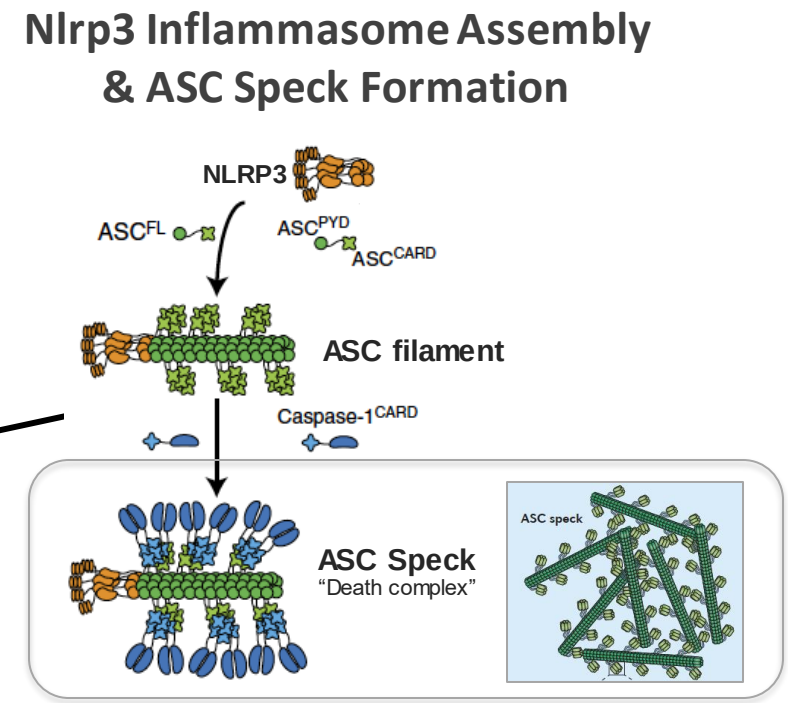
NLRP3 anchorage to mitochondrial MAVS

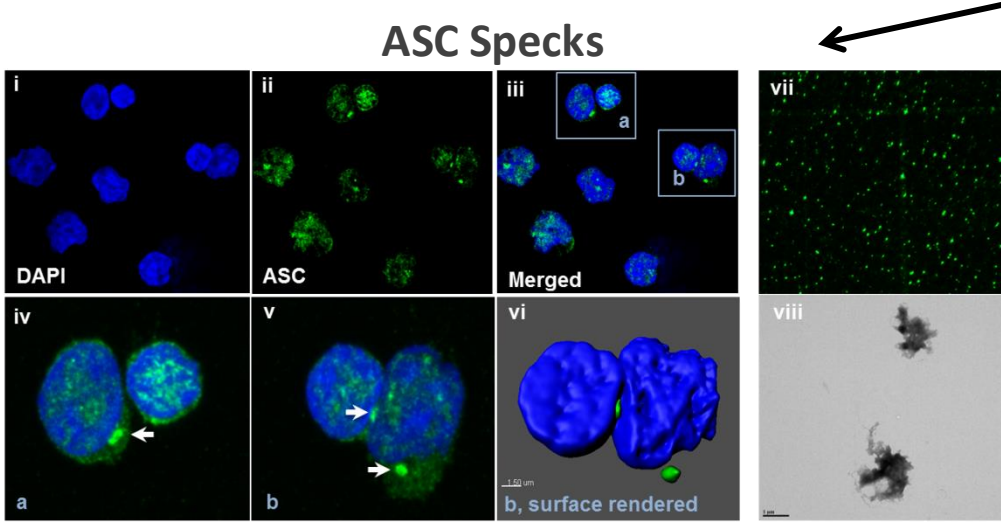
NLRP3 IFM assembly & caspase-1 activation

Caspase-1 substrate cleavage
(IL1β, IL-18, gasdermin D, GATA1)

Primary MDS Bone Marrow Progenitors Display NLRP3 Inflammasome Activation

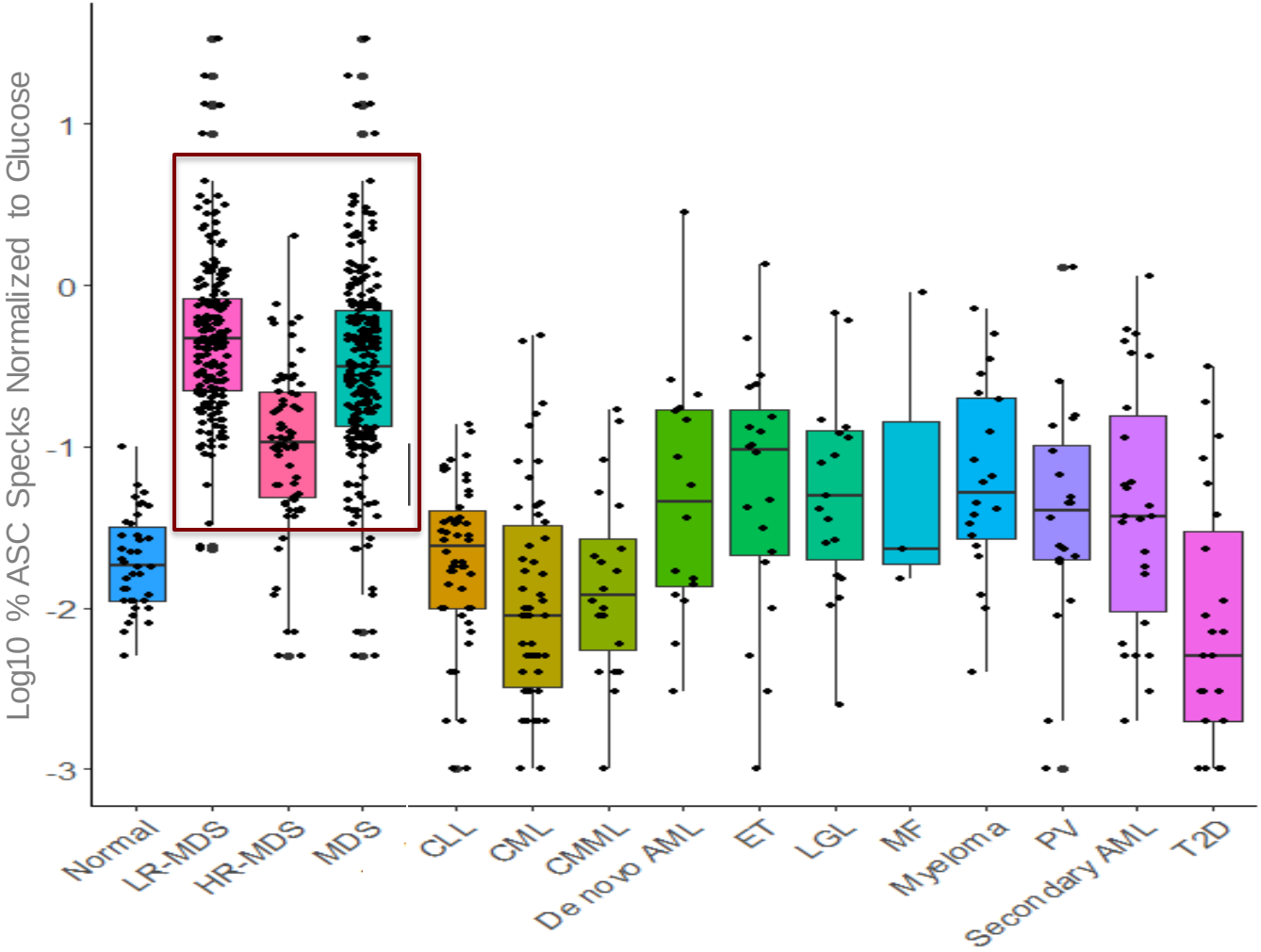




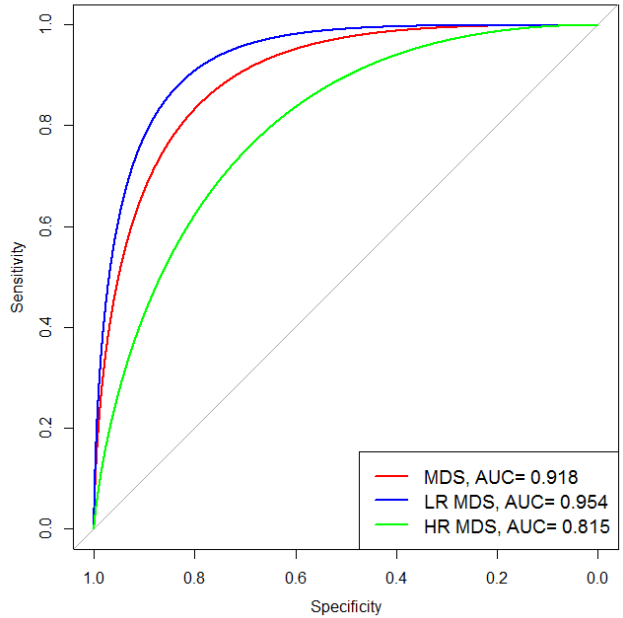


Basiorka A, et. al. Lancet Haematol 2018; Sep;5(9):e393-e402.

Plasma ASC Specks are a Pyroptosis Biomarker in MDS [n=249]



ROC/AUC = 0.918
Specificity 0.89,
Sensitivity 0.87 at 0.93 cutoff.



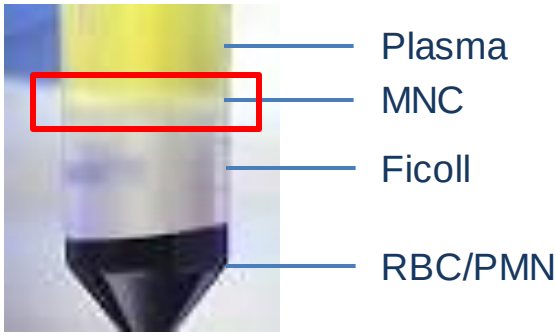
ROC: receiver operating characteristic (ROC), AUC, area under the curve.

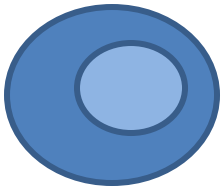
Basiorka A, et. al. Lancet Haematol 2018; Sep;5(9):e393-e402.

Flow Cytometric Assessment of Pyroptotic Versus Apoptotic Cell Death

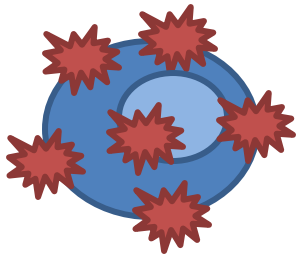
Heparinized BM Aspirate
MDS
Normal donors

Ficoll-Hypaque Plus Gradient Centrifugation

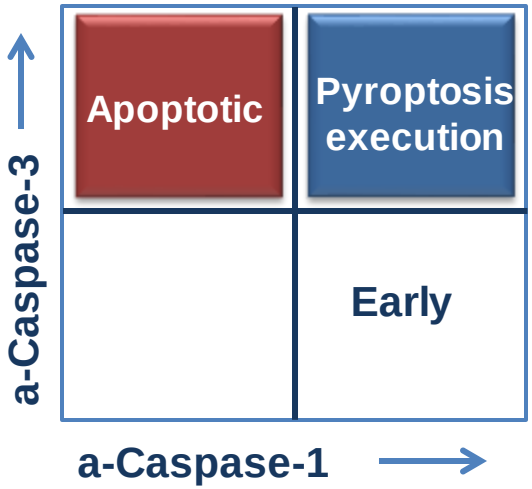




Lineage Gating:
MSC (CD45-CD105+)
Endothelial cells (CD31+)
Osteoblasts (CD34-OCN+)

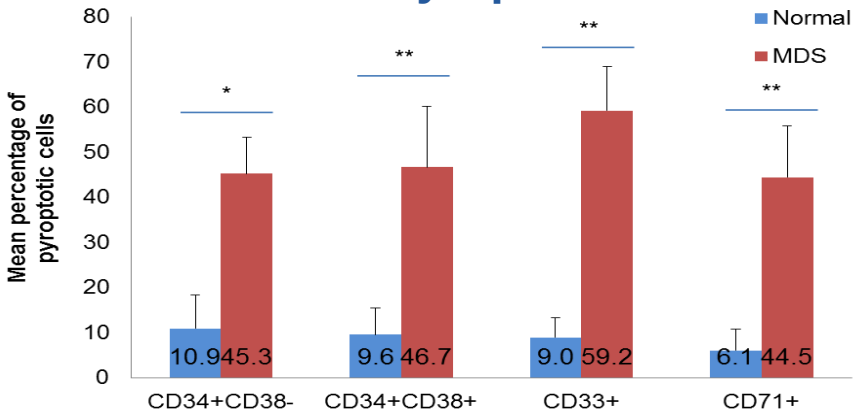


Annexin-V+

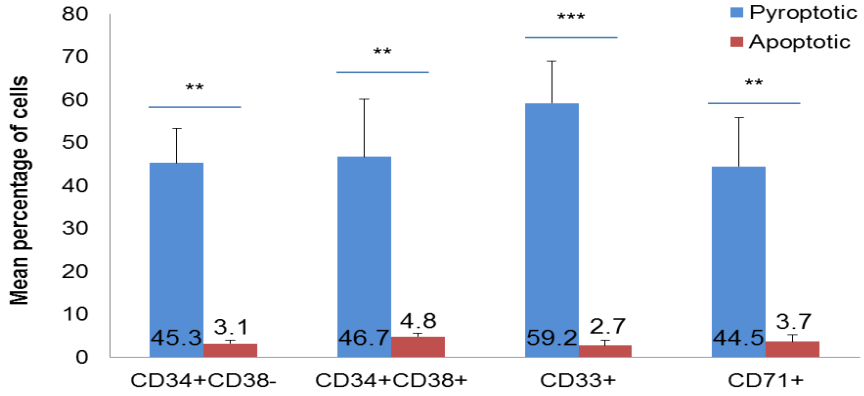


Functional Dependence on Pyroptosis vs. Apoptosis in MDS

Mean % Pyroptotic Cells

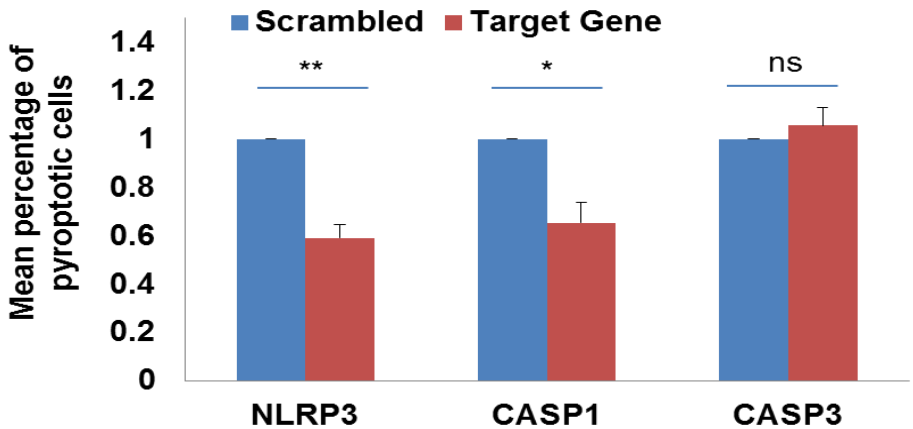


Pyroptotic vs. Apoptotic Fraction



Apoptotic: cleaved Caspase-3^{+}/1^{-}/Annexin V^{+}

CASP-1, NLRP3 vs. *CASP-3* shRNA



Basiorka A, et. al. Blood. 2016;128(25):2960-2975.

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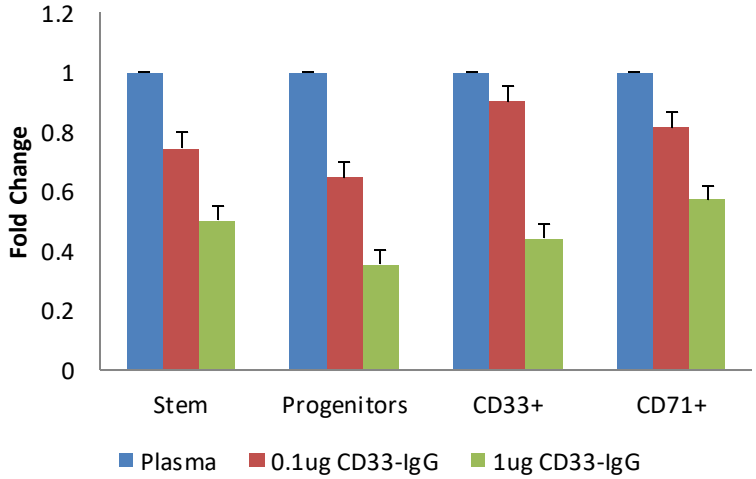
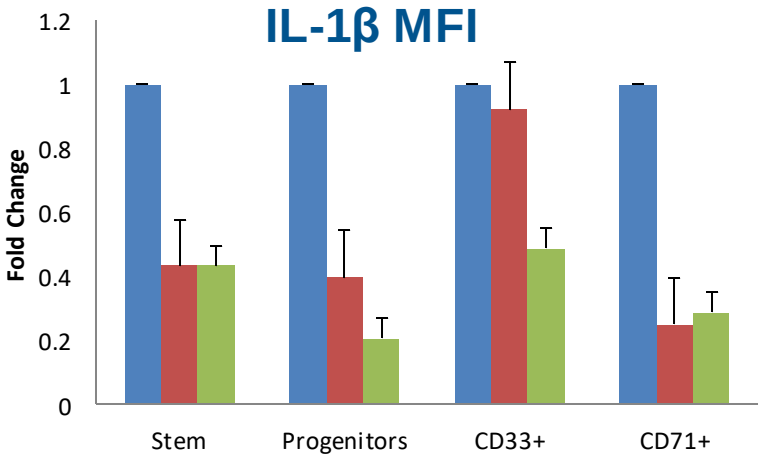
S100A9 Neutralization Suppresses Pyroptosis & Improves CFC in LR-MDS BM Specimens

aCaspase-1 MFI

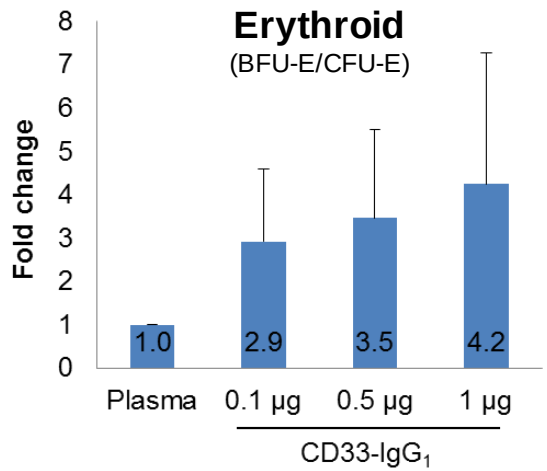
Normalized to Plasma Treated Control

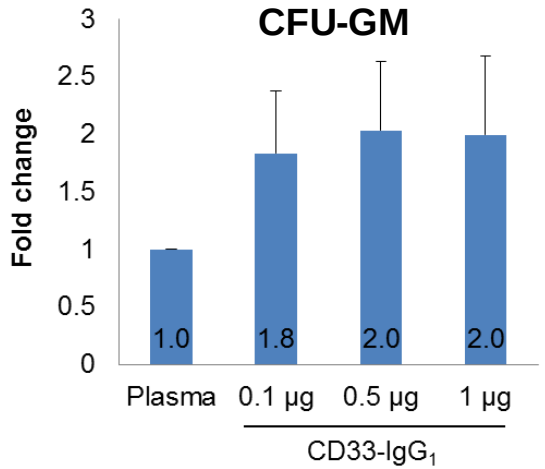


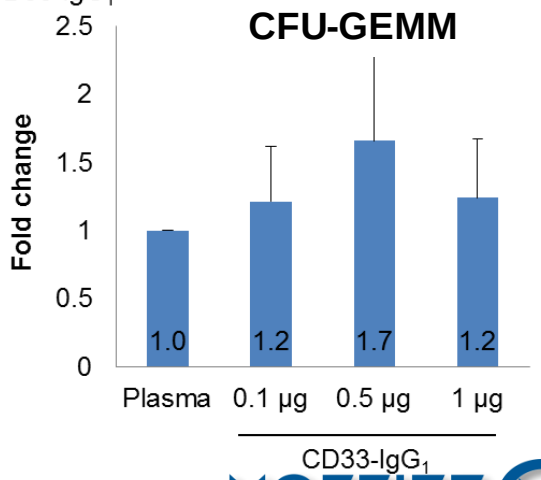
IL-1β MFI



Colony-Forming Capacity







Basiorka A, et. al. Blood. 2016;128(25):2960-2975.

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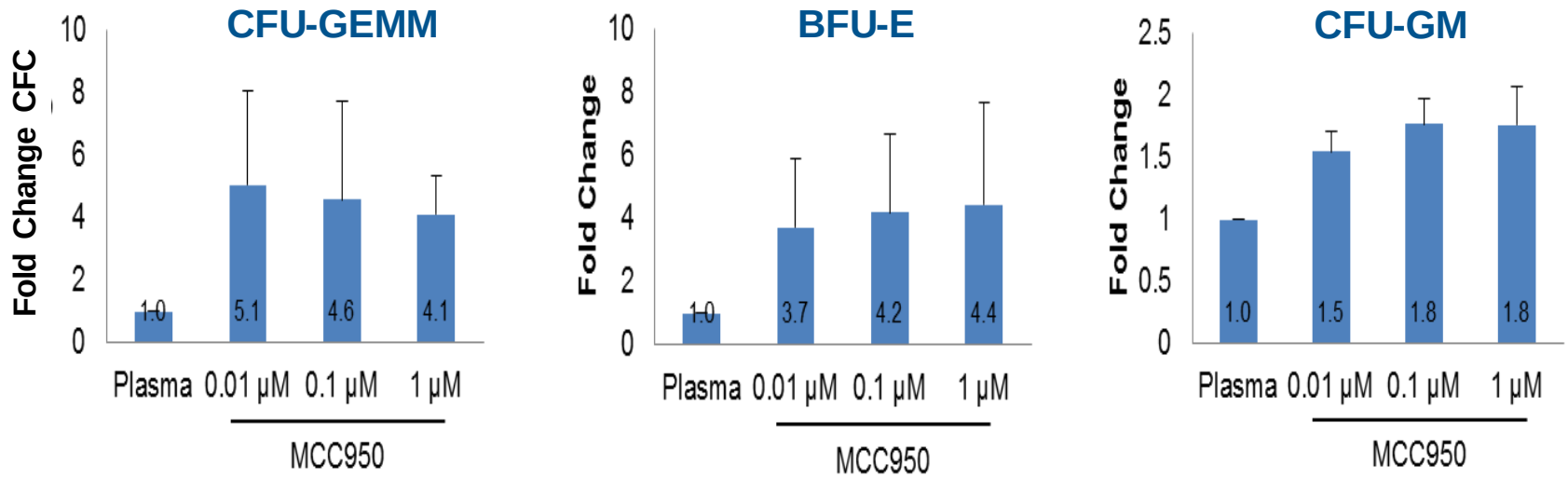
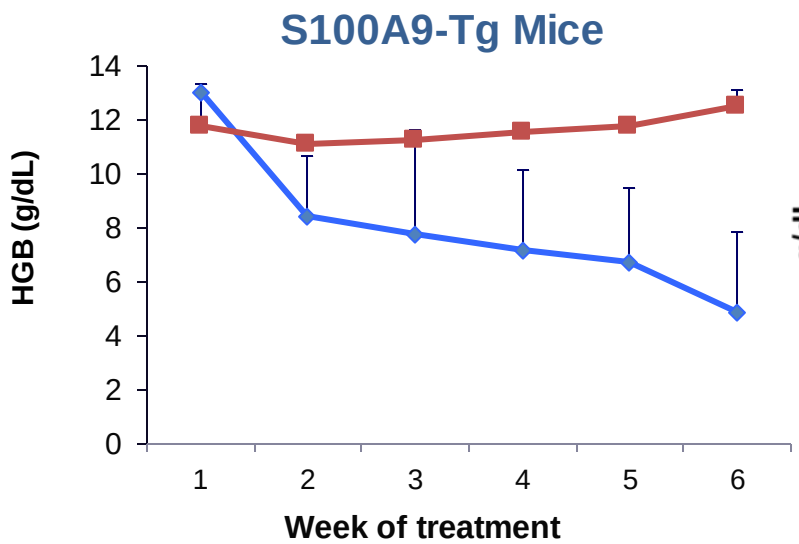
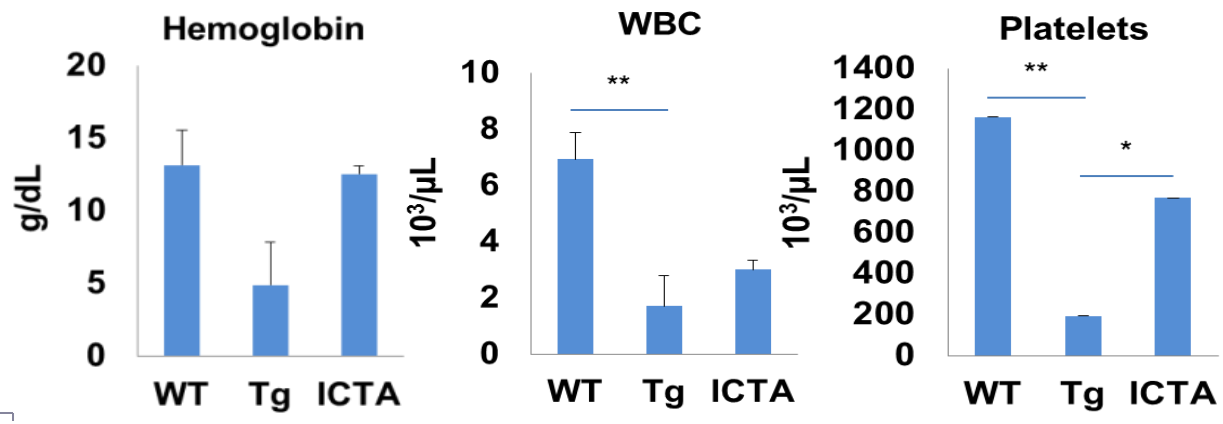
NLRP3 IFM Inhibition Improves Hematopoiesis in LR-Risk MDS & S100A9-Tg Mice

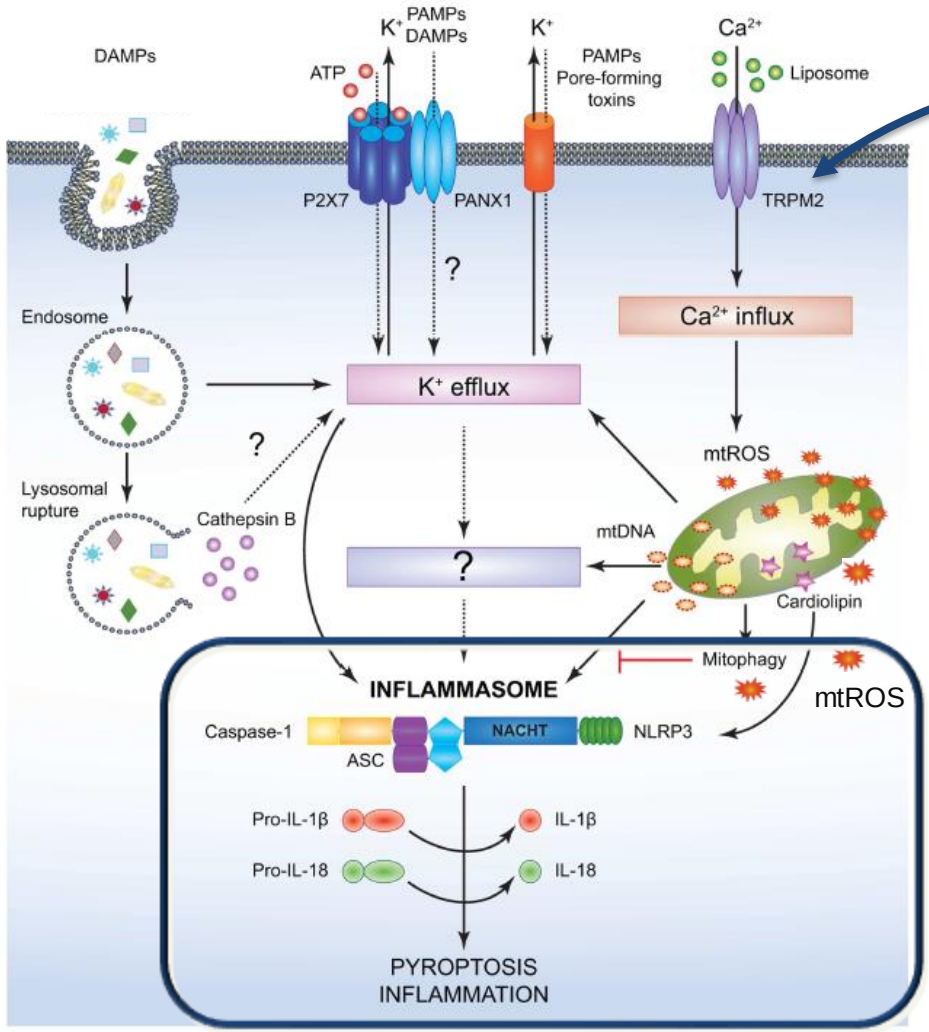


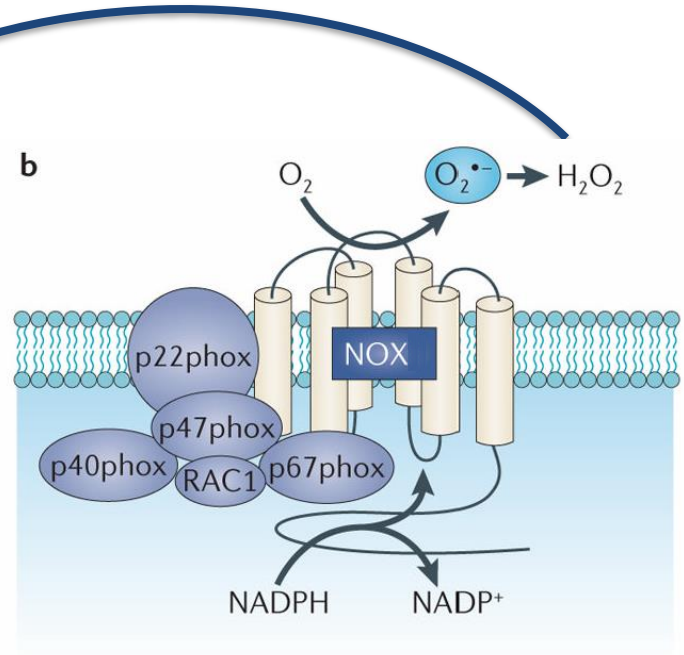




Basiorka A, et. al. Blood. 2016;128(25):2960.

Cation Channel Activation Triggers Cell Swelling & NLRP3 Inflammasome Assembly



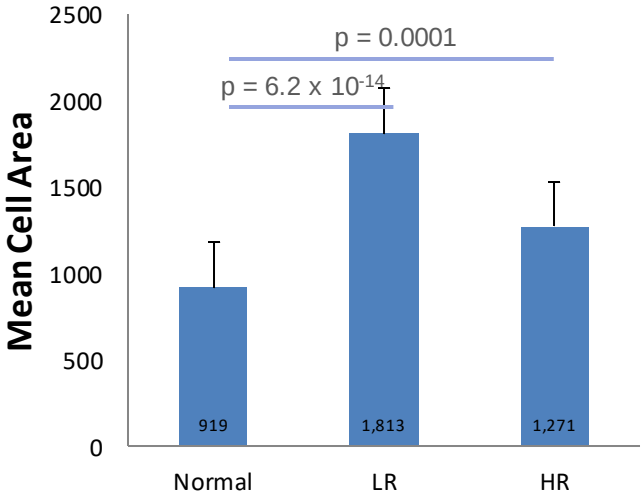


S100A9

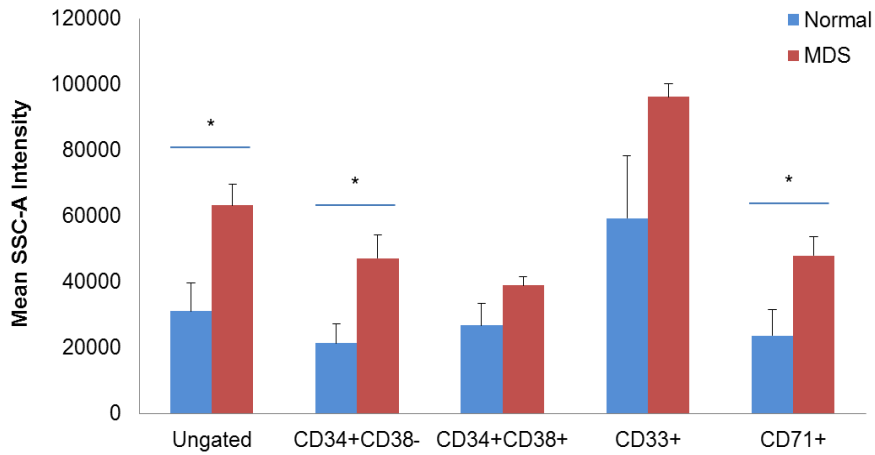
Adopted from Saxena M, et. al. Front Immunol 2014; Block K, et. al. Nat Rev Cancer 2012 .

ROS-sensitive Ion Channels Promote Cation Influx & Cell Volume Expansion in MDS Precursors

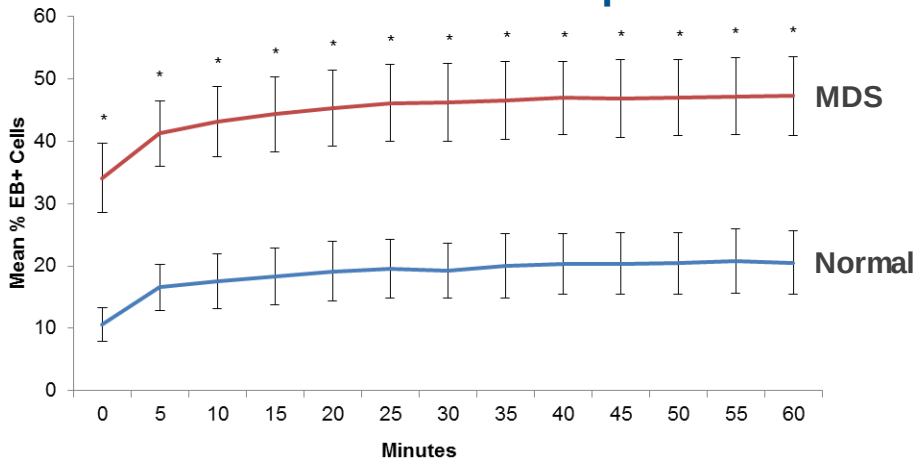
Cell Size



Cell Size by Hematopoietic Lineage

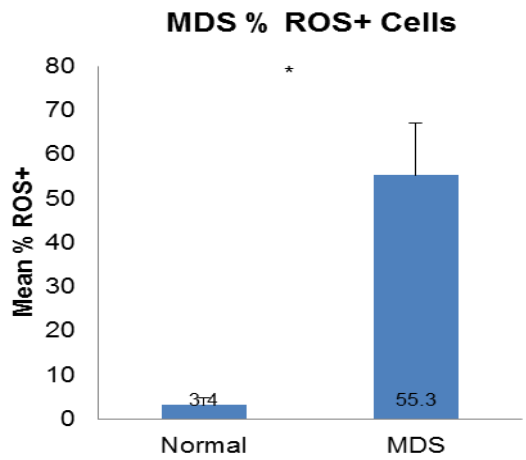


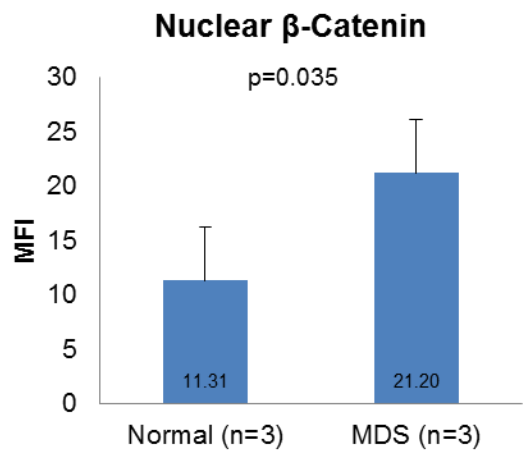
Ethidium Bromide Update

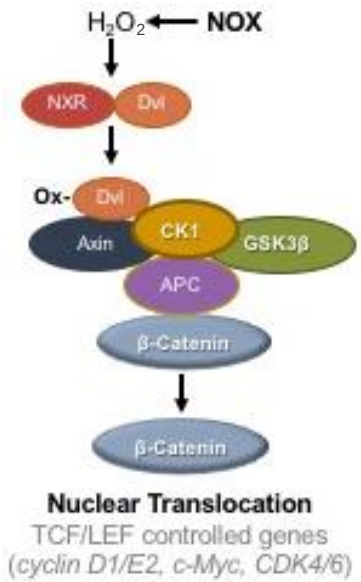


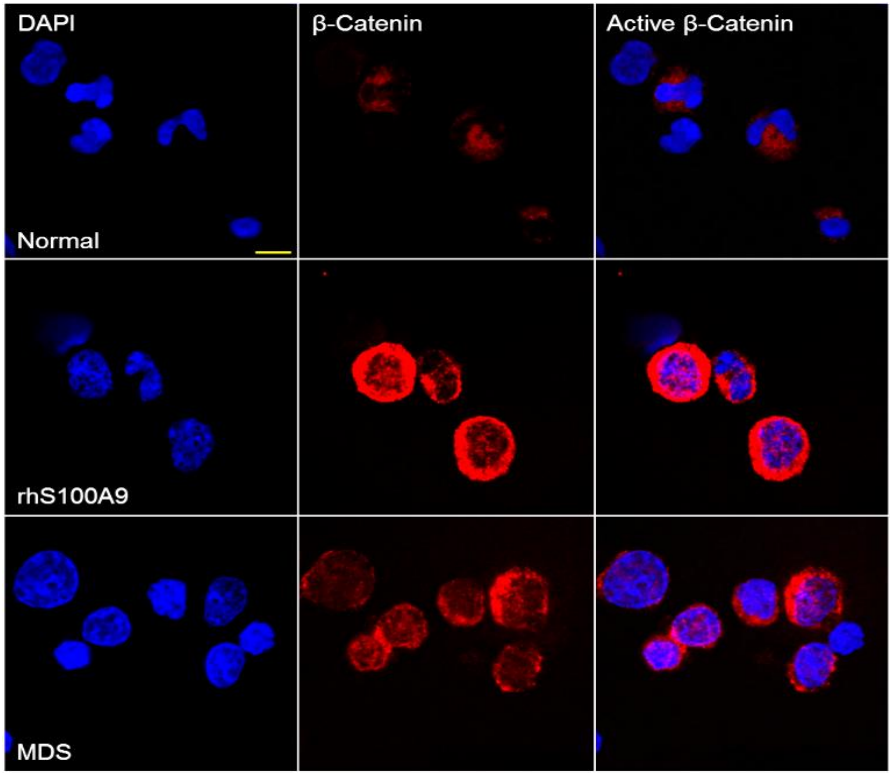
Basiorka A, et. al. Blood. 2016;128(25):2960-2975

ROS & Nuclear β-Catenin Expression is Increased in LR MDS & Induced by S100A9









Basiorka A, et. al. Blood. 2016;128(25):2960-2975

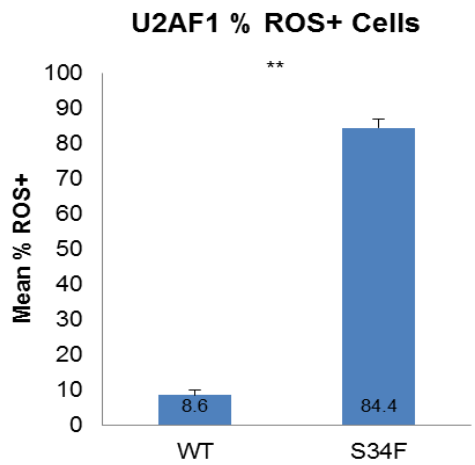
Regulation of Innate Immune Response

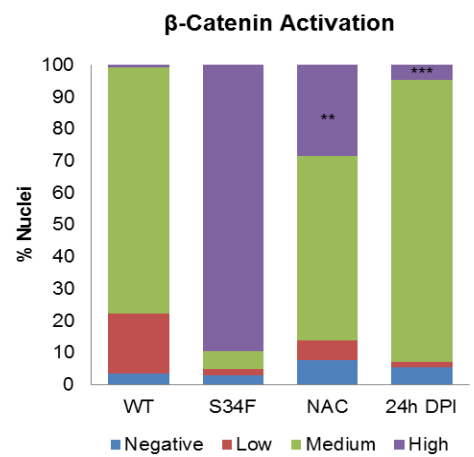
Nlrp3 Inflammasome & Pyroptosis

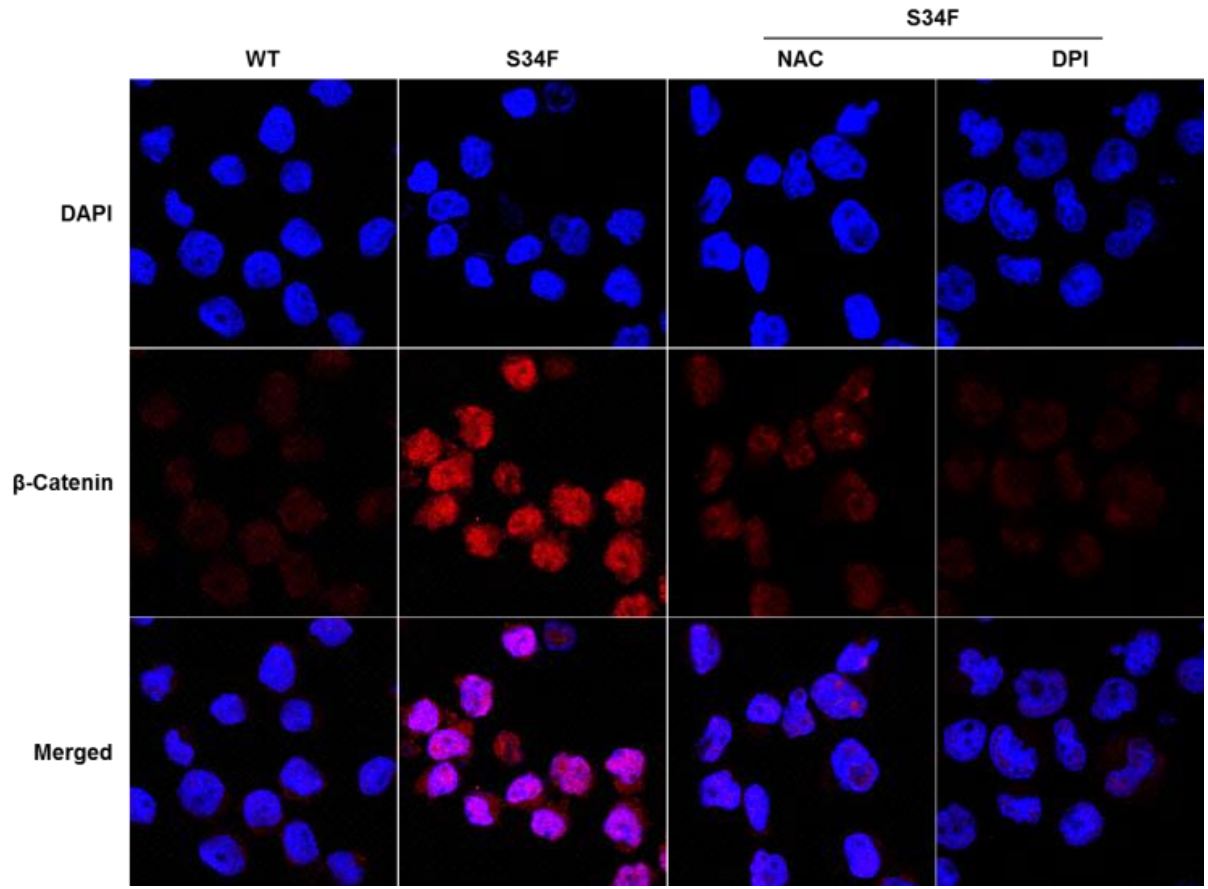
MDSC Effectors of Ineffective Hematopoiesis

Somatic Mutations License the Inflammasome

U2AF1 Splicing Gene Mutations Induce Nuclear β-Catenin Localization via NOX-generated ROS

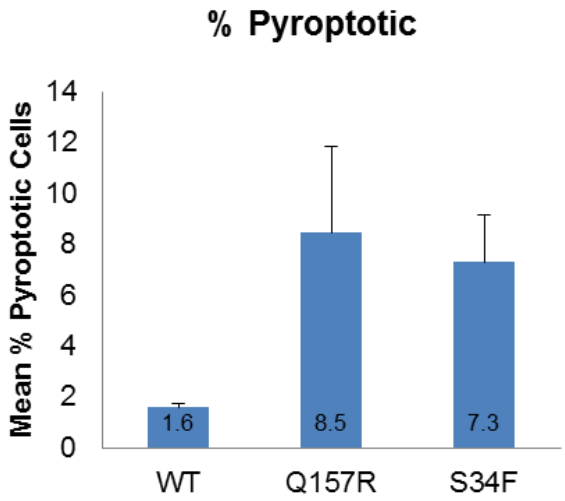






Basiorka A, et. al. Blood. 2016;128(25):2960-2975.

U2AF1 Mutant Cells Display Increased Pyroptosis & Cation Channel Activation

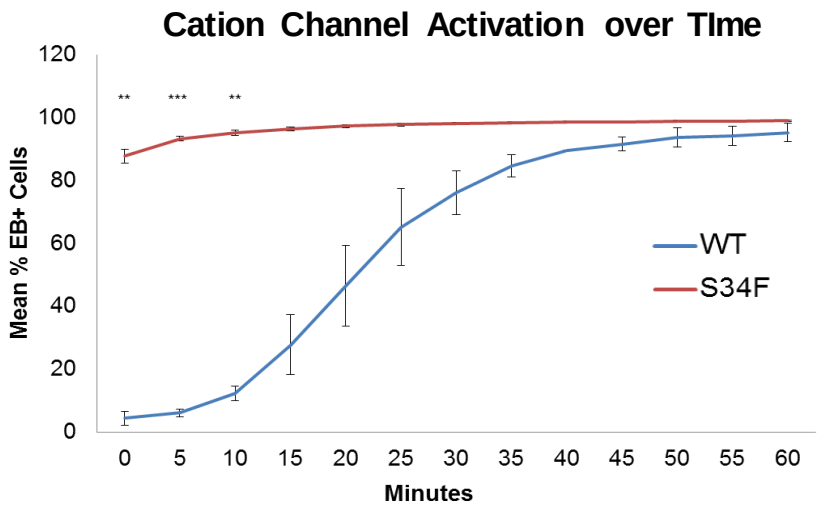


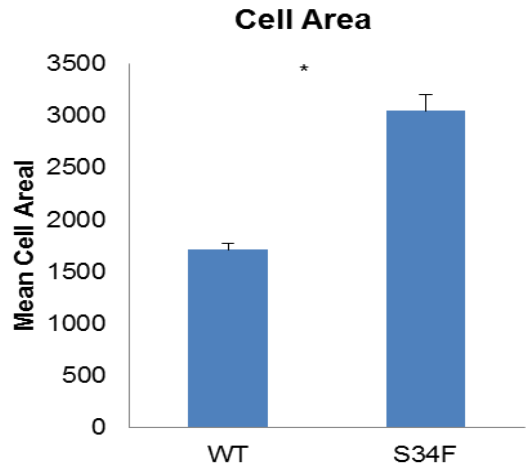
Splicing mutations [U2AF1, SF3B1, SRSF2]

Chromatin remodeling – ASXL1

DNA methylation - TET2

Ribosomopathy – RPS14$^{+/-}$

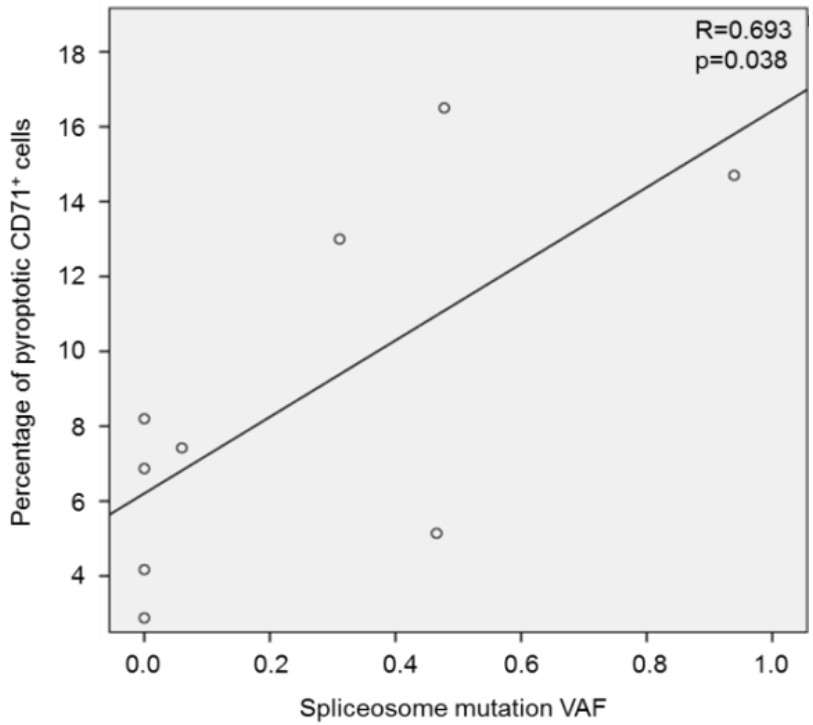




Basiorka A, et. al. Blood. 2016;128(25):2960-2975.

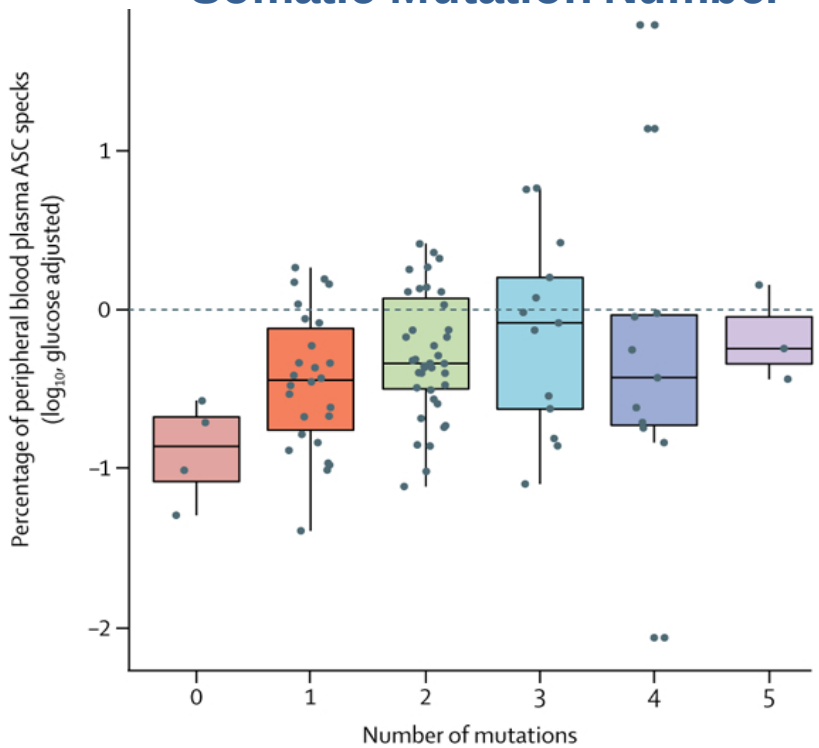
Pyroptotic Fraction Increases with Somatic Mutant Clone Size & Complexity

% Pyroptotic Erythroids vs. Splicing Mutation VAF



Basiorka A, et. al. Blood. 2016;128(25):2960.

Plasma ASC Specks by Somatic Mutation Number



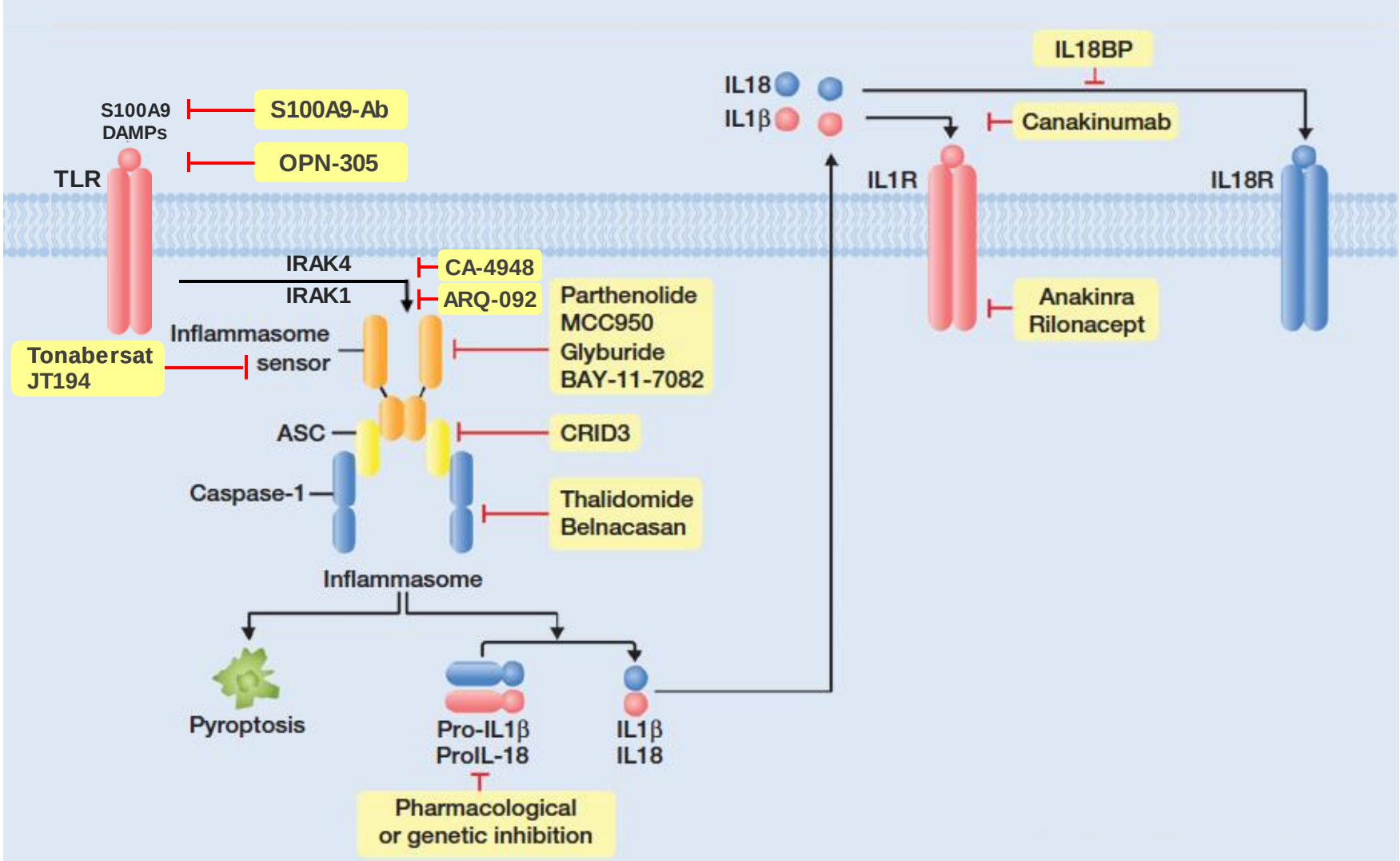
Basiorka A, et. al. Lancet Haematol 2018; Sep;5(9):e393-e402.

Genetic Priming of TLR-Signaling in MDS

Genetic Abnomality	Gene Class	Mutant Gene or Chromosome Alteration	Innate Immune Signaling Effect	Reference
Somatic Mutations	Epigenetic Modifiers	*TET2*	↓HDAC2 recruitment: ↑IL-6, NF-κB, ↑IL-1β	23, 25, 34
		DNMT3A	↑HDAC9;↑Type 1 IFN	24
		ASXL1	↑NADPH oxidase ROS; ↑TLR4, TICAM2	34
		EZH2	↑ S100A8/A9	20-21
	Spliceosomal	*SF3B1*	↑ Degradation of TLR negative regulator MyD88S	26, 34
		SRSF2	↑ S100A8/9, DNA-RNA hybrids	20-21; 34, 35
		U2AF1	↑ DNA-RNA hybrids, *ATG7* alternate splicing impairing autophagy; IRAK4-L mydosome activation	34-37
Chromosomal Abnormality	N/A	Deletion 5q	Allelic deletion *RPS14*: ↑S100A8/A9; *miR-145/146:+ TIFAB*: ↑TRAF6, IRAK1	8-10; 19

Sallman & List. BLOOD 2019.

Strategies for Therapeutic Intervention



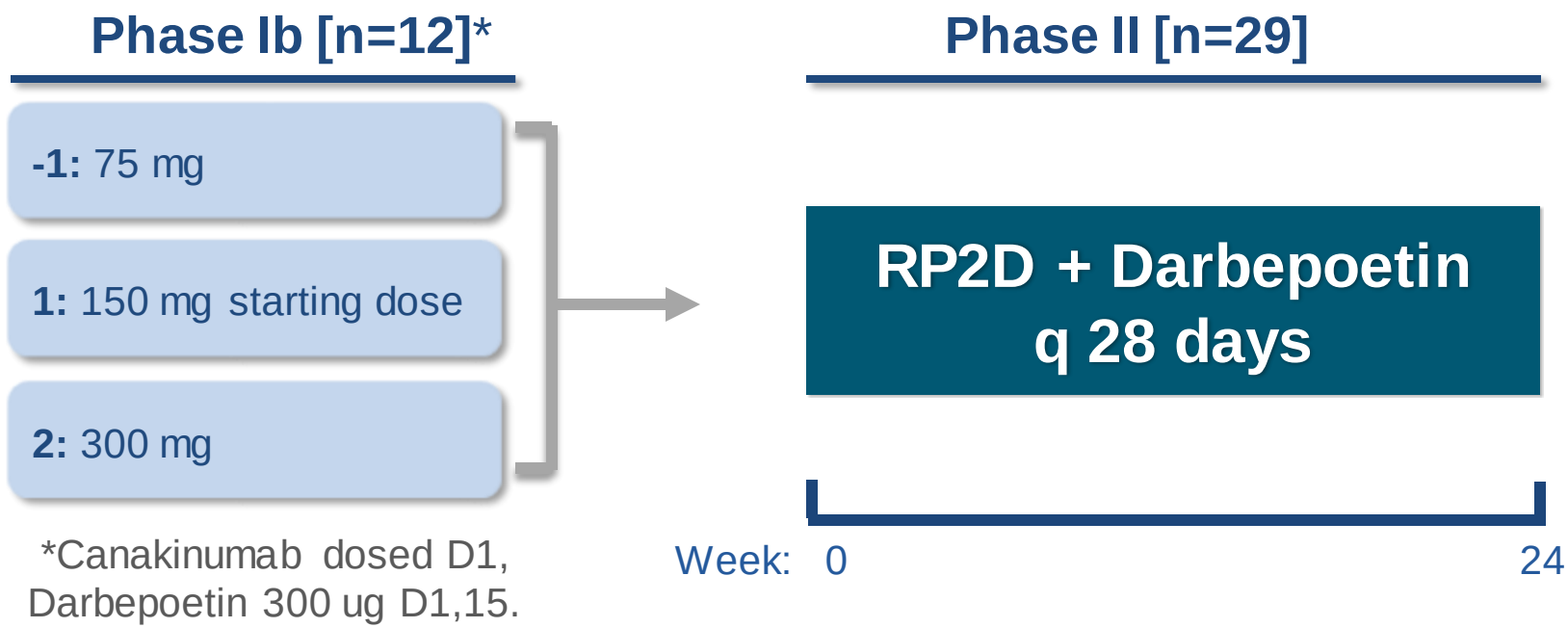
Adopted from Karki R, et. al. Cancer Immunol Res 2017; 5(2); 94–99.

Targeting the IL-1β Signaling Axis in MDS

- IL-1β is the principal inflammatory cytokine generated by the Nlrp3 inflammasome that has broad biological activities:
  - TLR/myddosome signaling induces **inflammatory cytokines** (S100A9, TNFα, IL-6), *PDL-1, SPY1 (PU.1)* and Nlrp3 **inflammasome activation**
  - activates **β-catenin** to induce MYC and **MDSC expansion**, and **chromatin remodeling**
  - active caspase 1 cleaves **GATA1** to raise the Spi1/GATA1 ratio favoring myeloid commitment, maturation arrest & anemia
  - directs **myeloid skewing** & immuno-senescence, suppresses late stage erythropoiesis, and decreases release of **erythropoietin**
- Canakinumab is a fully humanized monoclonal antibody of the IgG1/k isotype that neutralizes IL-1β
- FDA approved & effective in autoinflammatory disorders including CAPS syndromes with activating NLRP3 mutations

Cluzeau T, et. al. Haematologica 2017 Dec;102:2015; Kennedy DE. J Immunol 2017, 198:3471-79; Tyrkalska SD, et. al. Immunity 2019; 51:1-14.; Tu, S. et al. Cancer Cell 2008;14: 408.; Song, X, et al. J Immunol 2005.

Phase Ib/II Study of Canakinumab with Darbepoetin in Patients with LR-MDS who Failed ESAs



*Canakinumab dosed D1, Darbepoetin 300 ug D1,15.

Eligibility: VL, LR, IR- IPSS-R; ≥1unit RBC x8 wks prior to randomization, ESA failure
Exclusions: prior HMA or allo-HCT
Design: Phase 1b: 3+3 dose escalation; Phase 2: Simon's two-stage (Stage 1: enroll 10 at MTD, if > 2 achieve HI-E, Stage 2: enroll additional 19 pts). >6 HI-E, merits further study.
Primary end-point: *Phase 1b* – MTD & RP2D; *Phase 2* – IWG 2006 HI-E.

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