Myeloid-Derived Suppressor Cells & Altered Innate Immunity in MDS Pathogenesis

Alan List, MD
H. Lee Moffitt Cancer Center & Research Institute
Tampa, FL, USA

Disclosures

- **Consultant**

  - Celgene, Tetralogics, Boehringer-Ingelheim

- **Research Funding**

  - Celgene

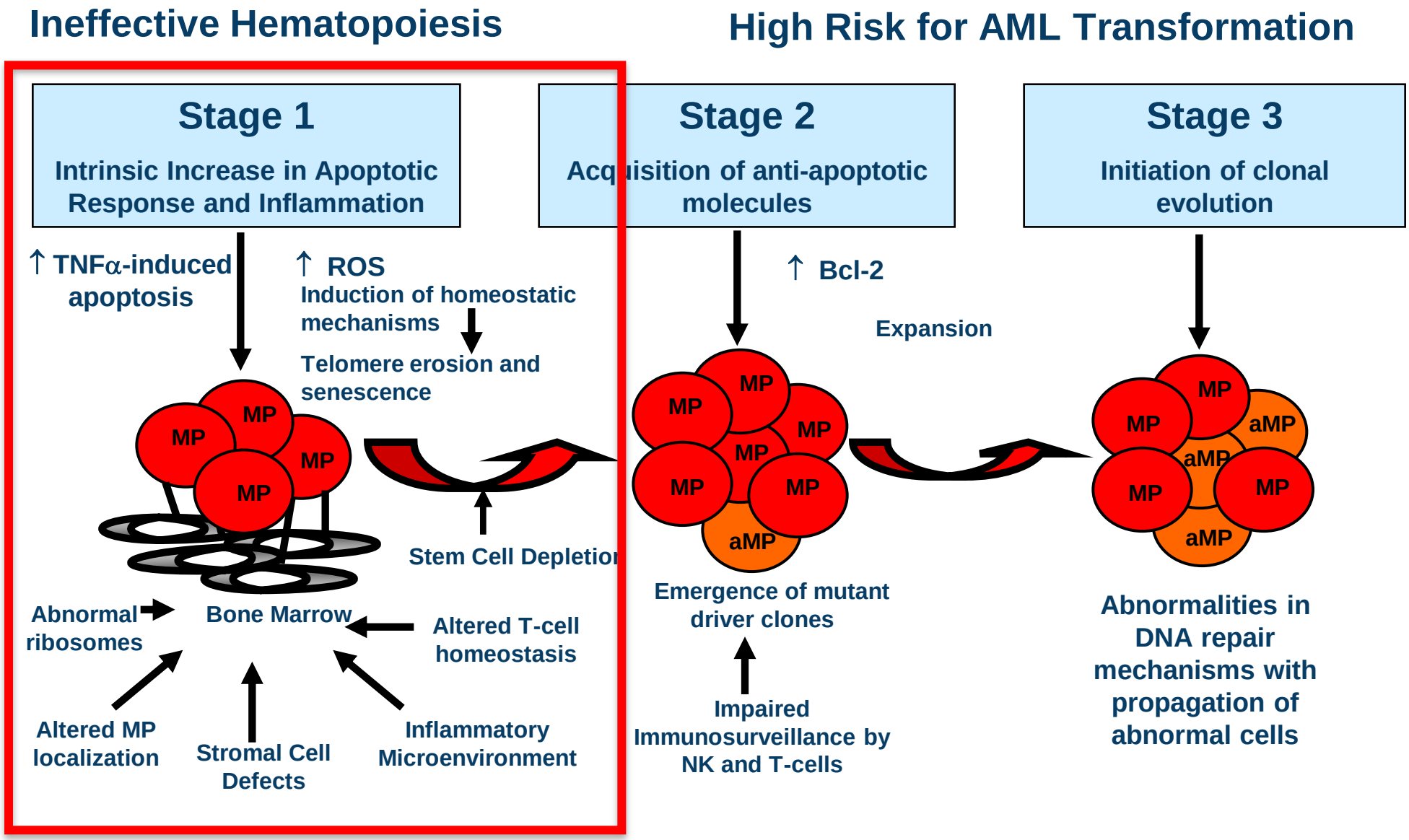
- **Data Safety & Montoring Committee**

  - Amgen

- **Scientific Advisor**

  - Cell Therapeutics Inc., Trillium, Amphimed, Amphivena

Traditional Model of MDS Pathogenesis

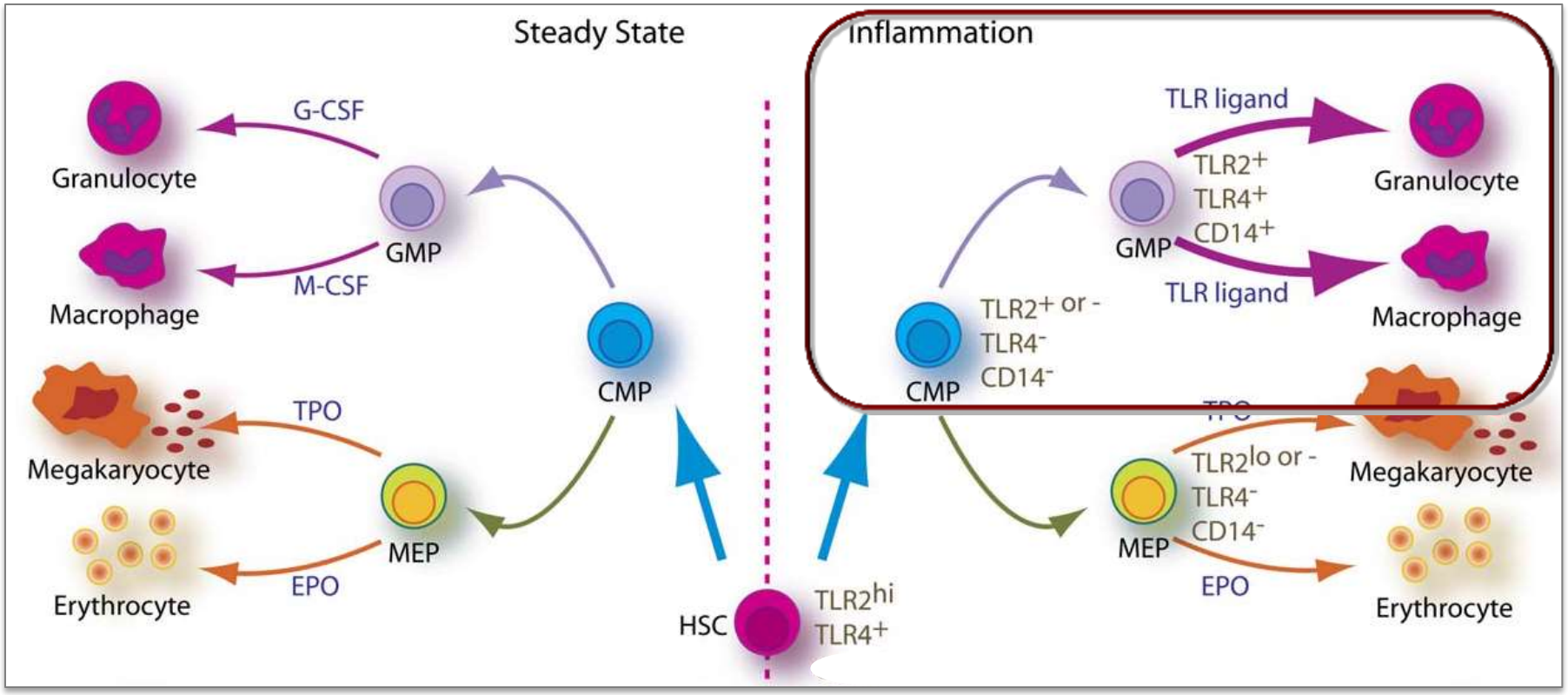


Innate Immunity
An Emerging Pathogenetic Driver in MDS

- Chronic inflammation & activation of innate immunity are linked to hematopoietic senescence & MDS pathobiology
  - TLR-2, -4 & -9 are overexpressed in MDS HSPC, with TLR4 implicated in progenitor apoptosis & cytopenias (Hoffman W, Blood 2002; Wei Y, Leukemia 2013)
  - TRAF6 is up-regulated in MDS CD34+ cells, with amplification of the TLR4 signaling intermediates,TRAF6 & TIRAP* (Gondek LP; Starczynowski DT, Blood 2008)
  - TLR signaling is constitutively active in del5q MDS d/t *miR-145 & miR-146* allelic deletion and TIRAP & TRAF6 de-repression (Starczynowski DT, Nat Med 2010;16:49)
  - The TLR4 adaptor kinase IRAK is overexpressed & hyperactive in MDS, whereas IRAK1 inhibition impairs MDS HPC expansion (Rhyasen, Cancer Cell 2013)
- Our recent work implicates expansion of Myeloid Derived Suppressor Cells (MDSC) as key innate immune effectors in MDS pathogenesis (Wei S, JCI 2013)

*TRAF6: tumor necrosis factor receptor- associated factor-6;
TIRAP:Toll-interleukin-1 receptor domain-containing adaptor protein.

TLR Signaling Skews HSPC Toward Myelopoiesis



- TLR ligation drives GMP expansion in the absence of myeloid GFs, while reducing lymphocyte production by CLPs similar to normal senescence
- Chronic TLR activated HSC lose quiescence causing HSC depletion

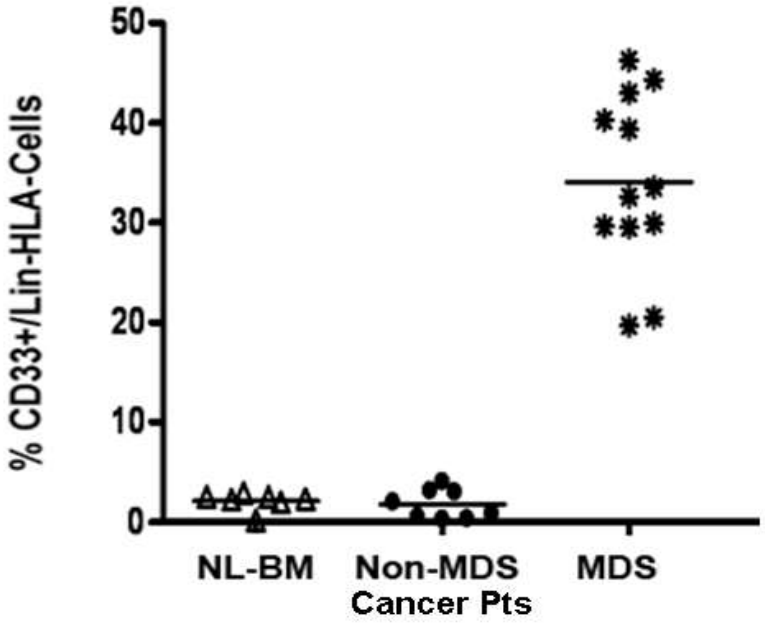
Holl TM, e. al. Immunity 2006; Esplin BL, et. al. J Immunol 2011.

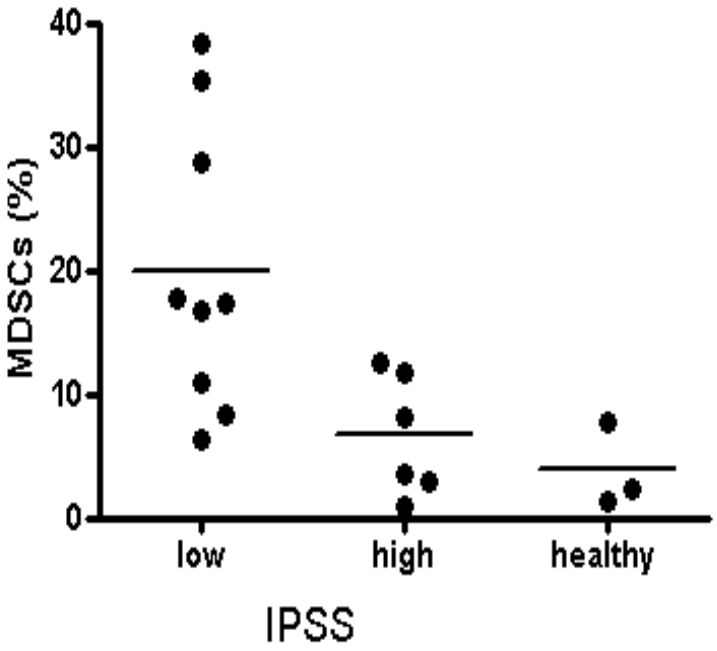
Myeloid-Derived Suppressor Cells

- Immature myeloid cells with distinct function & phenotype
  - Mouse MDSC: $CD11b^{+}Gr^{-}1^{+}$ ($\pm$B220, CD31)
  - Human MDSC: $Lin^{-}HLA^{-}DR^{-}CD33^{+}$
- MDSC expand with age, infection, inflammation, and neoplastic diseases.
- MDSC induce tumor immune tolerance & T-reg cell expansion.
- Mechanisms of inhibition: elaboration of ROS, NO, and Arginase, VEGF, TGF-β, IFN, IL-6, IL-10 & others
- MDSC expansion and activation driven by TLR ligands (e.g., DAMP signals)

*DAMP: danger-associated molecular pattern.

MDSC Expand in the BM of Lower Risk MDS Patients

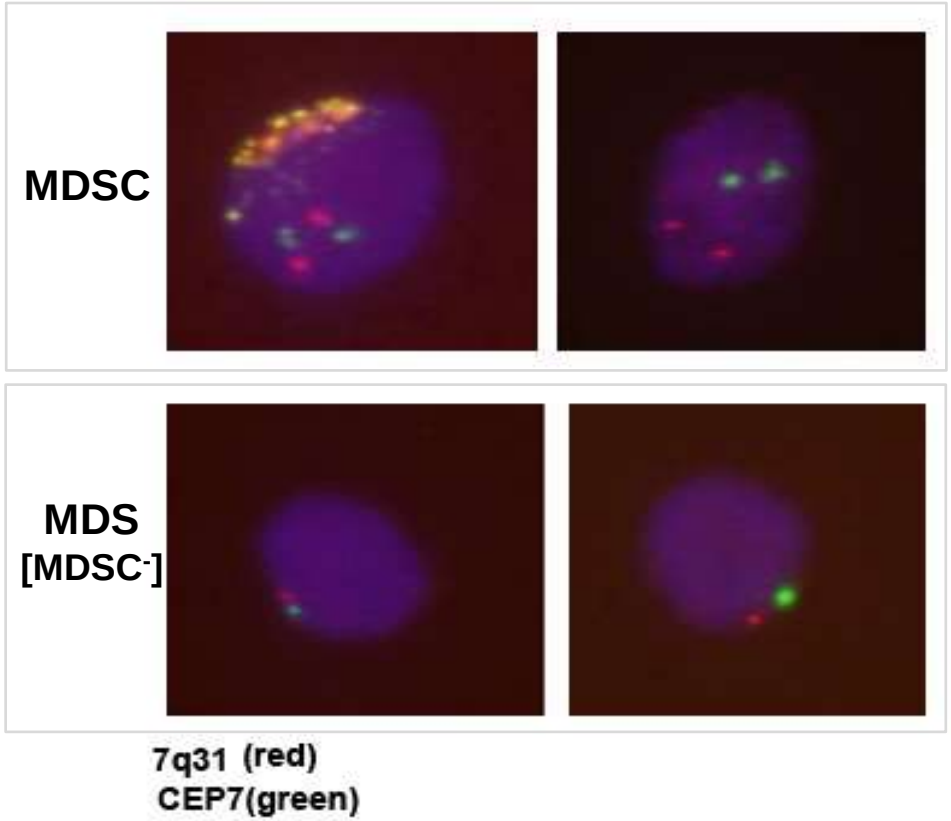




Chen X, et. al. J Clin Invest 2013; 123(11):4595-4611

MDS MDSC are Genetically Distinct from the MDS Clone

- MDSC lack both cytogenetic abnormalities & gene mutations intrinsic to the MDS clone
- Absence of genetic abnormalities indicates that MDS MDSC derive from non-neoplastic HSPC & precede emergence of MDS clones



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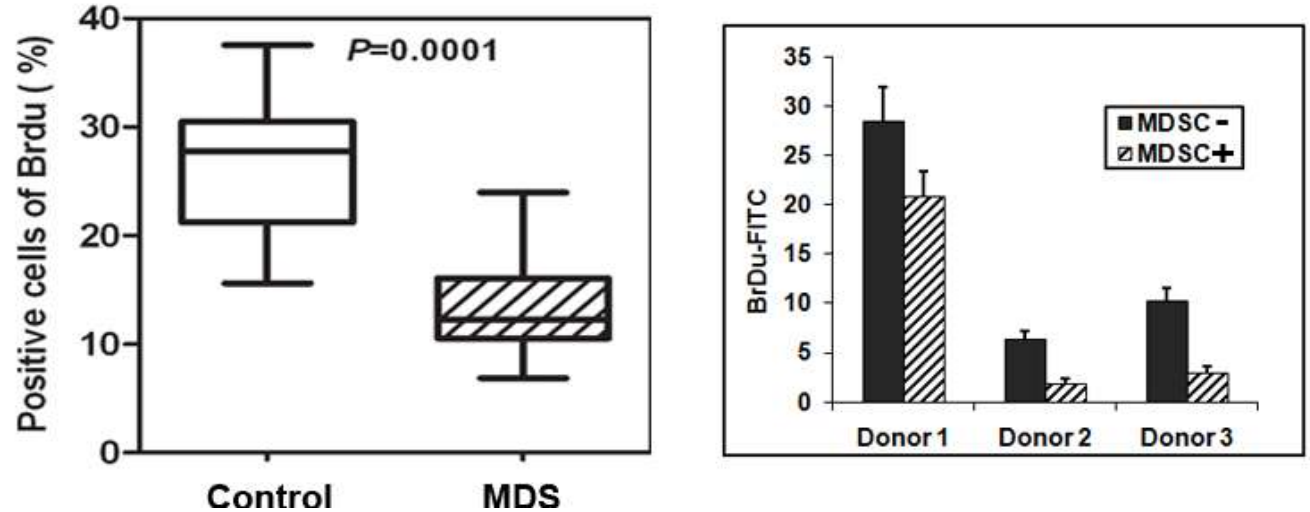
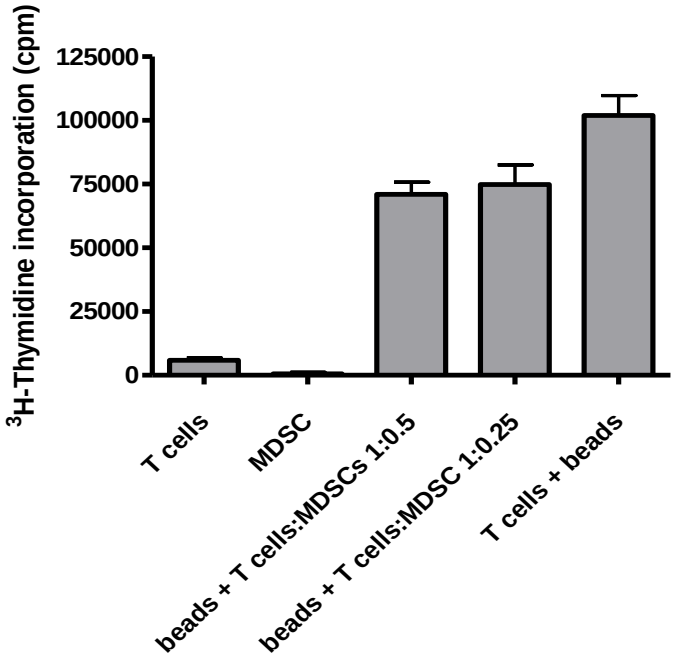
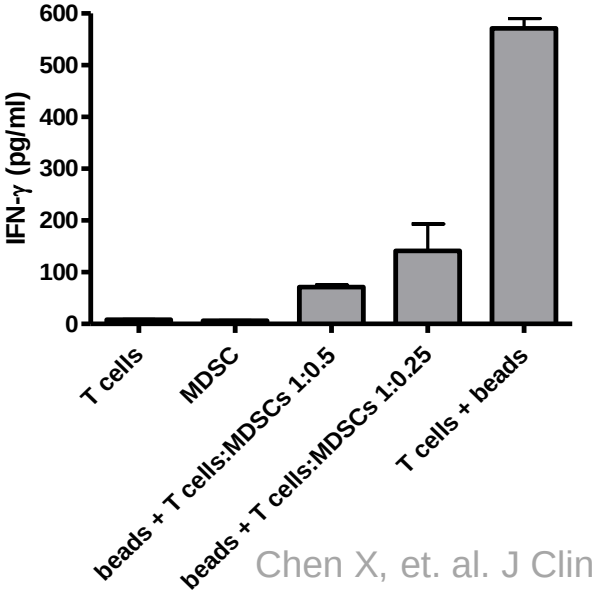
MDS-MDSC Suppress T cell Proliferation & Interferon-γ Elaboration







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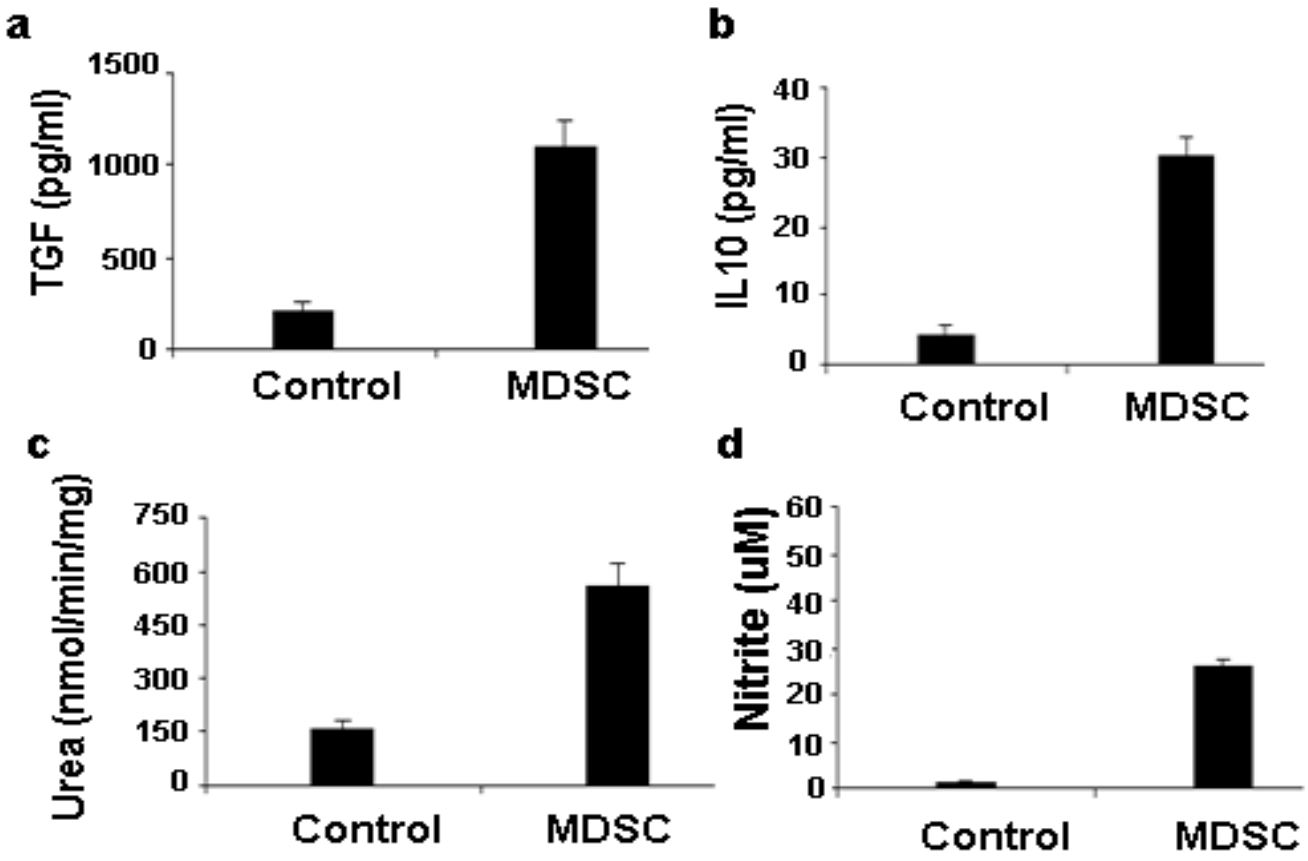
MDS-MDSC Generate Inflammatory Molecules



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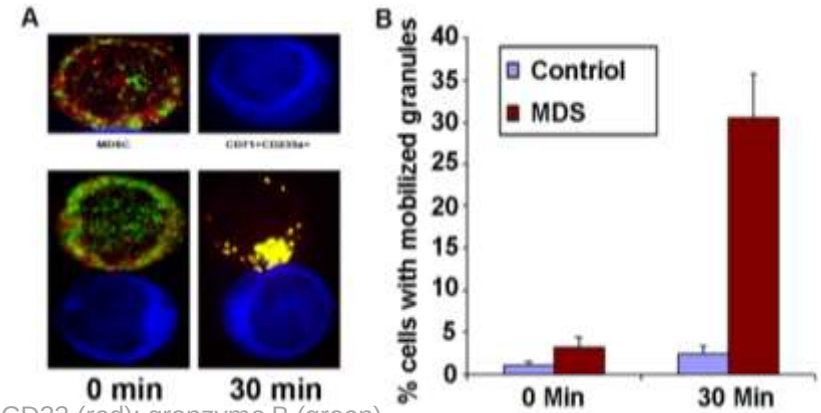
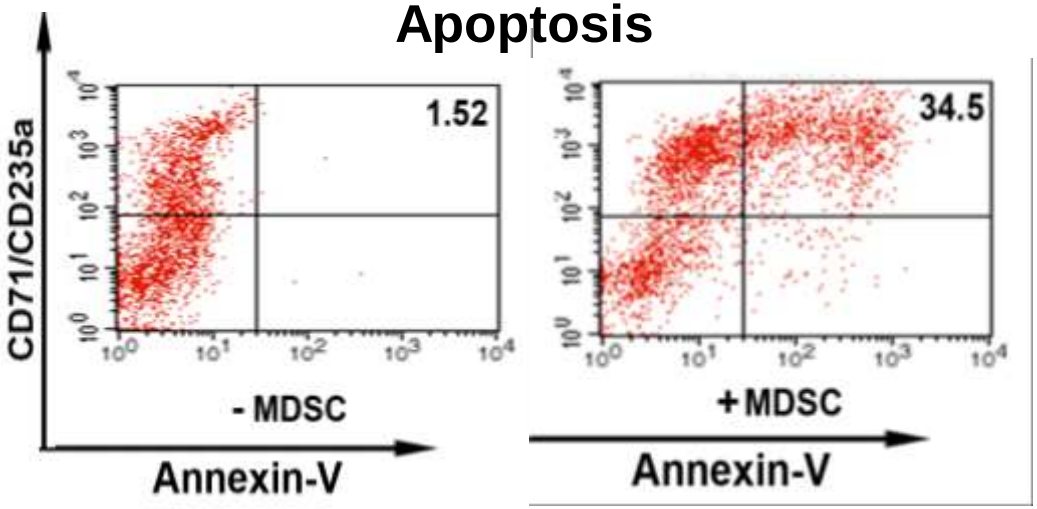
MDS-MDSC Suppress Autologous Hematopoiesis

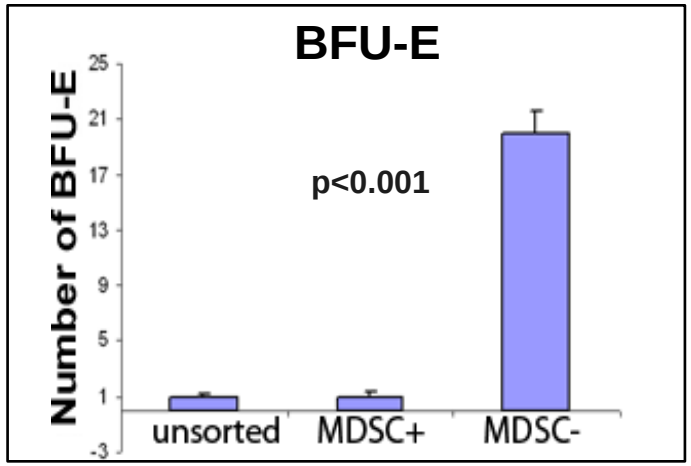
Granzyme Mobilization

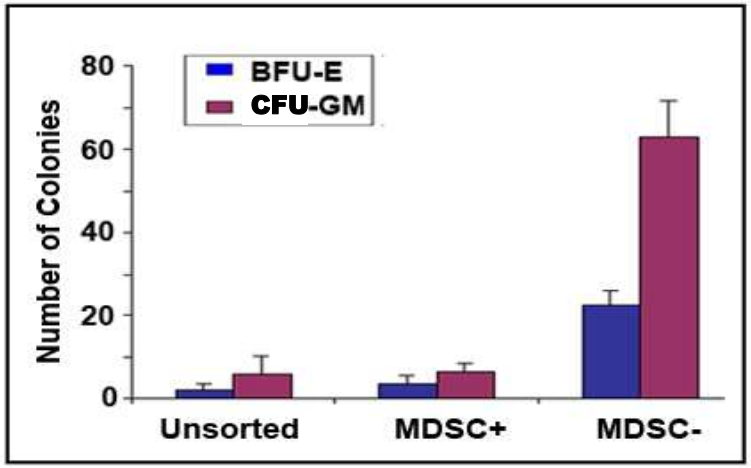


CD33 (red); granzyme B (green)

Apoptosis







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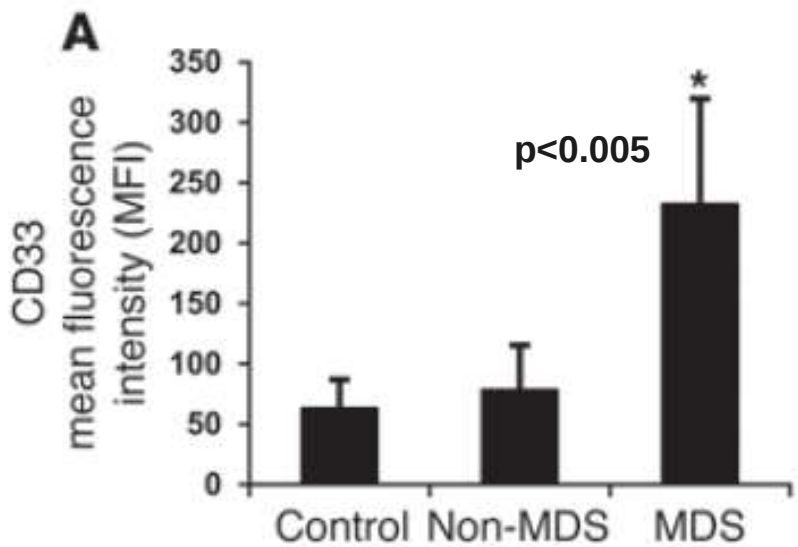
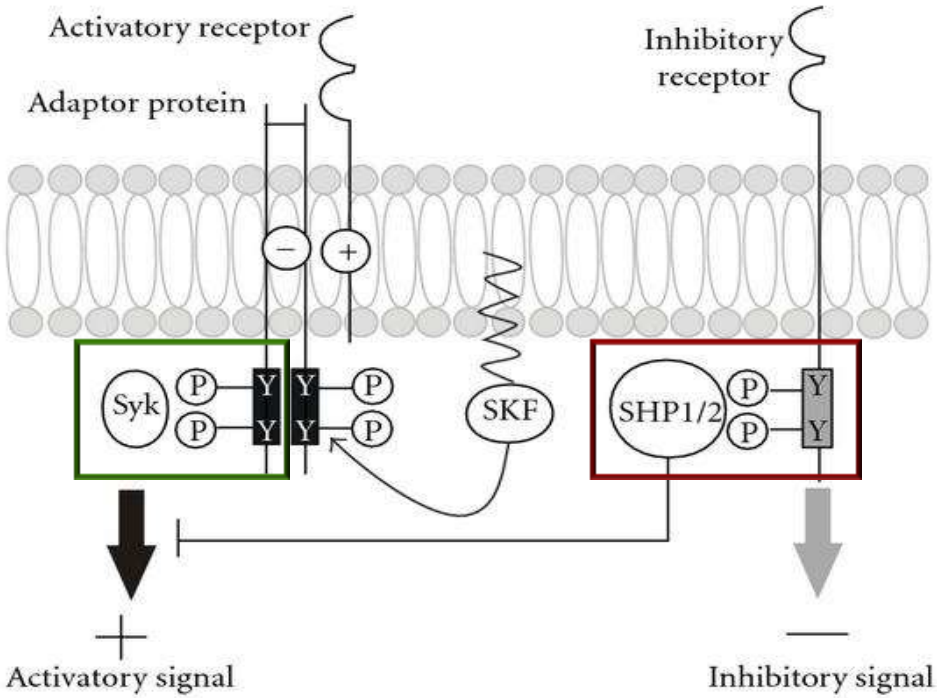
The ITIM Signaling Receptor CD33-SIGLEC3 is Over-expressed in MDS-MDSC

MDSC CD33 Surface Density



ITAM Signaling

ITIM SIgnaling



Promotes Myeloid Differentiation & Maturation

Blocks Differentiation & Maturation

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

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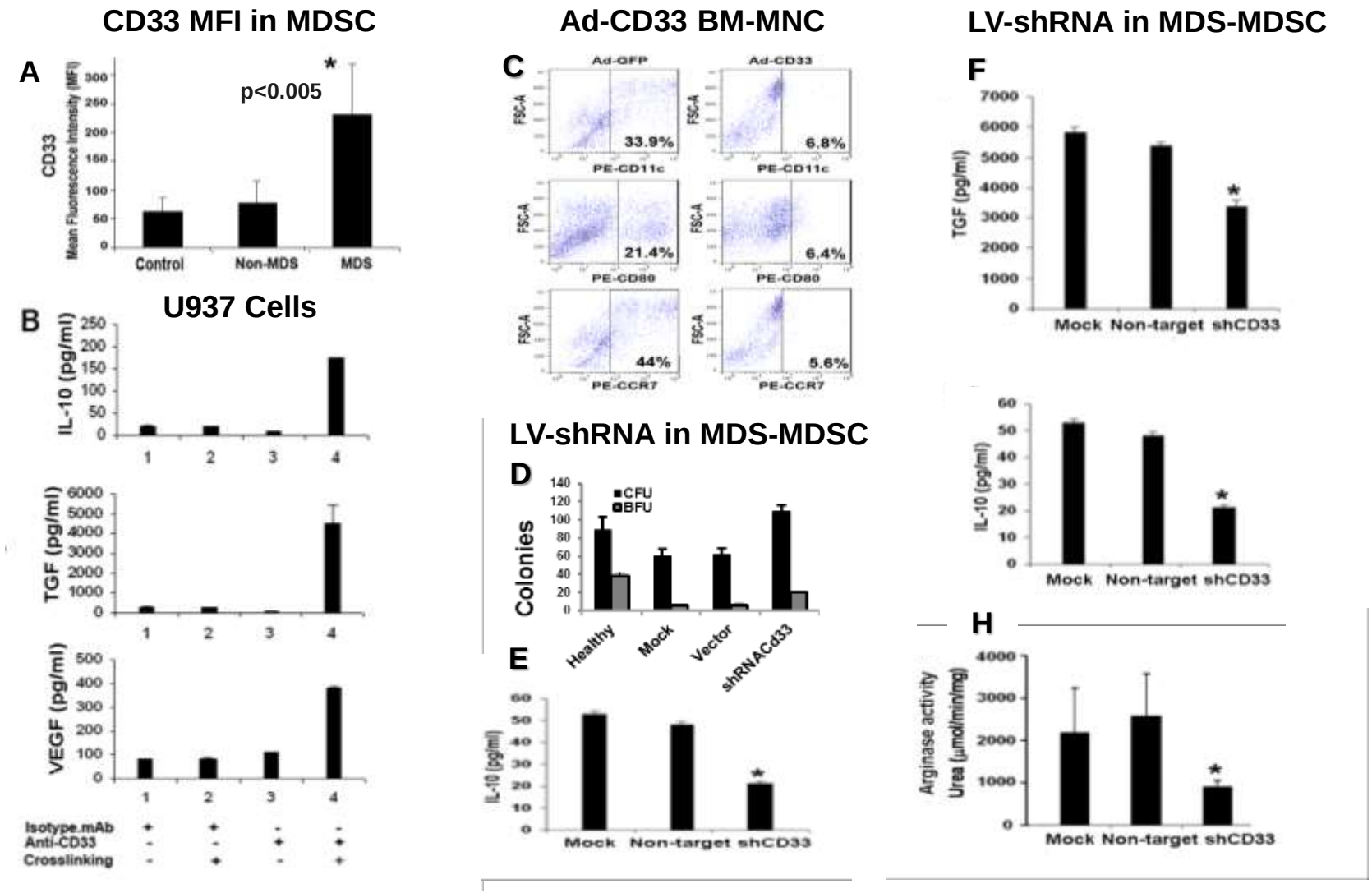
CD33-ITIM* Signaling Cooperates in MDSC Expansion & HPC Apoptosis



*Immunoreceptor tyrosine-based inhibition motif (ITIM)

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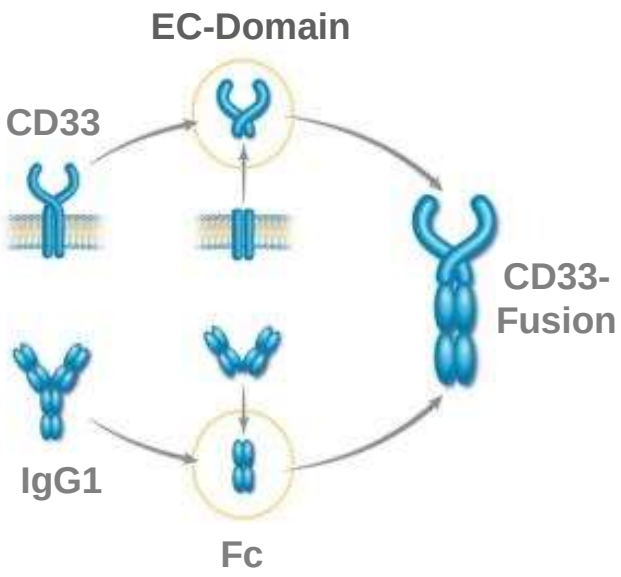
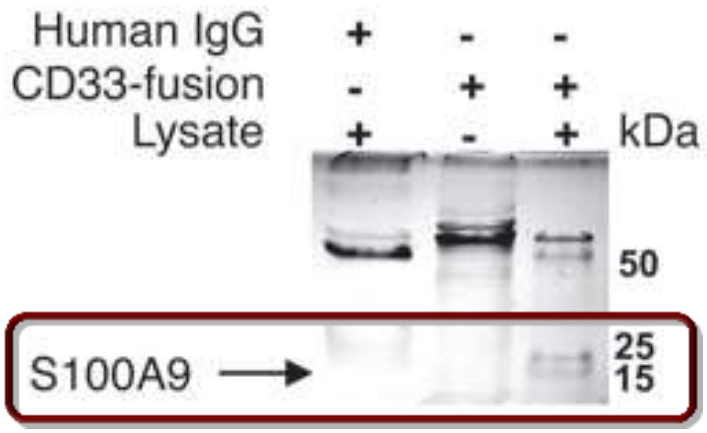
S100A9 is the Native Ligand for CD33

CD33-IgG Fc Fusion



CD33 Binds S100A9



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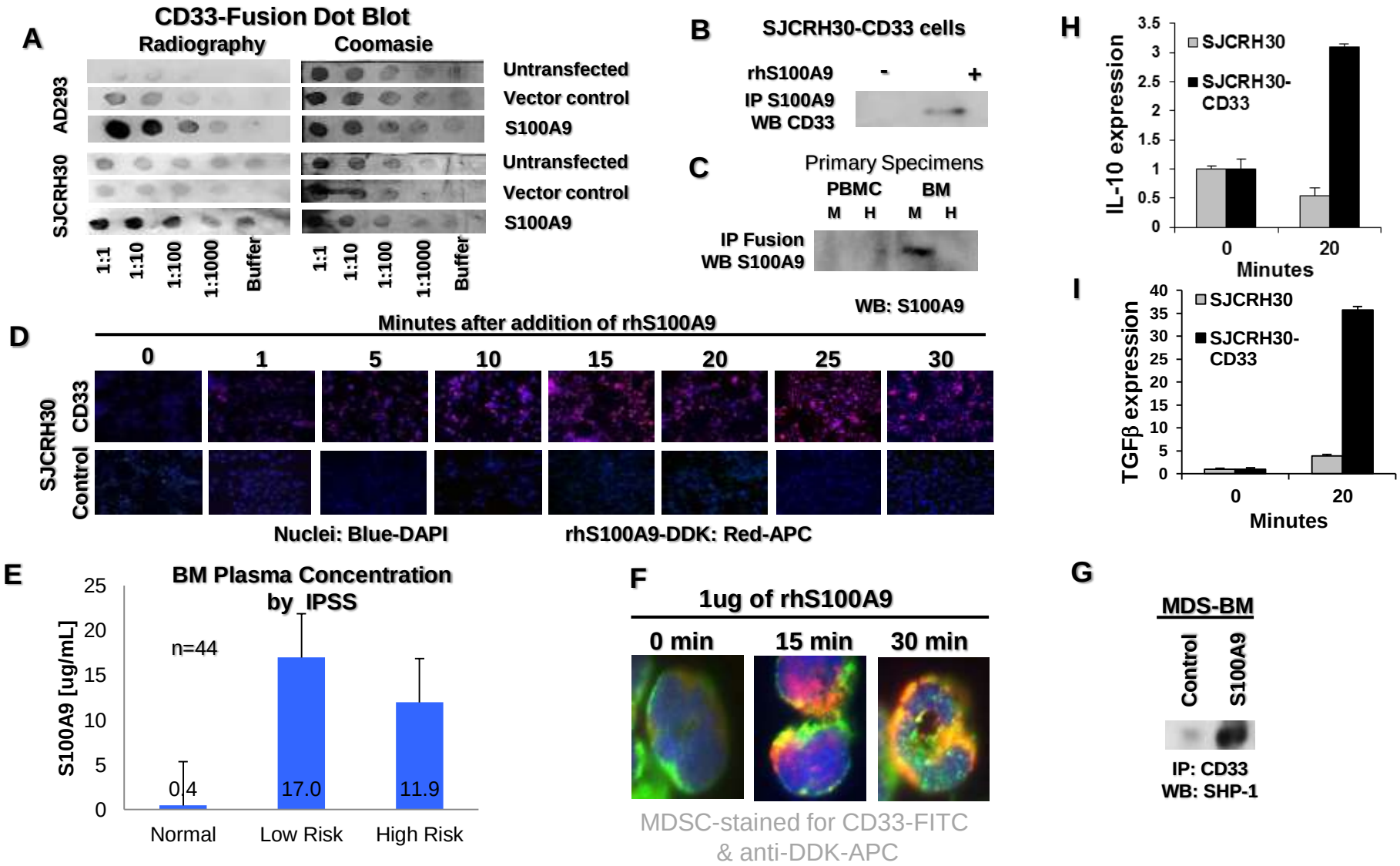
S100A9/CD33 Engagement Induces MDSC Activation



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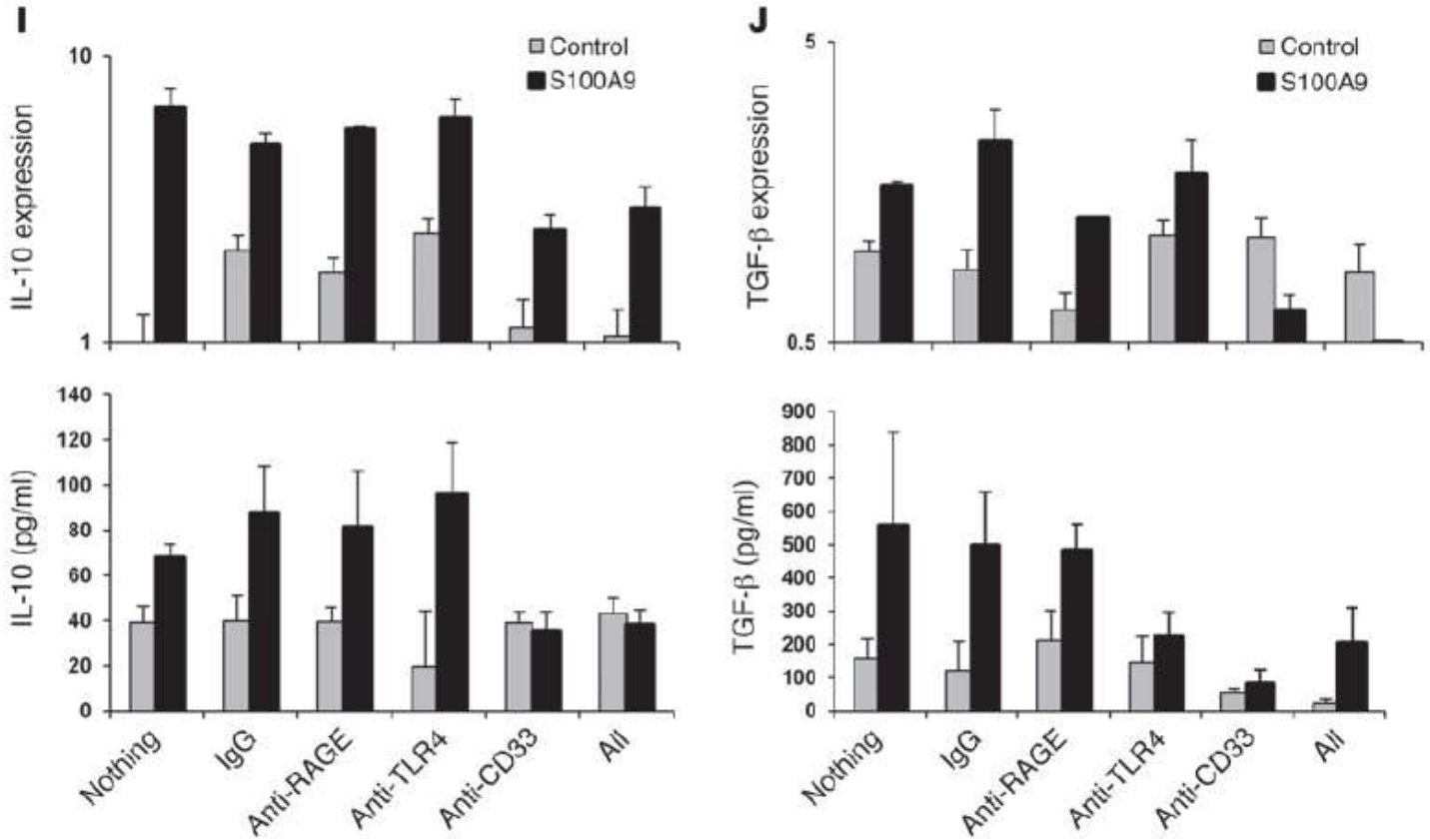
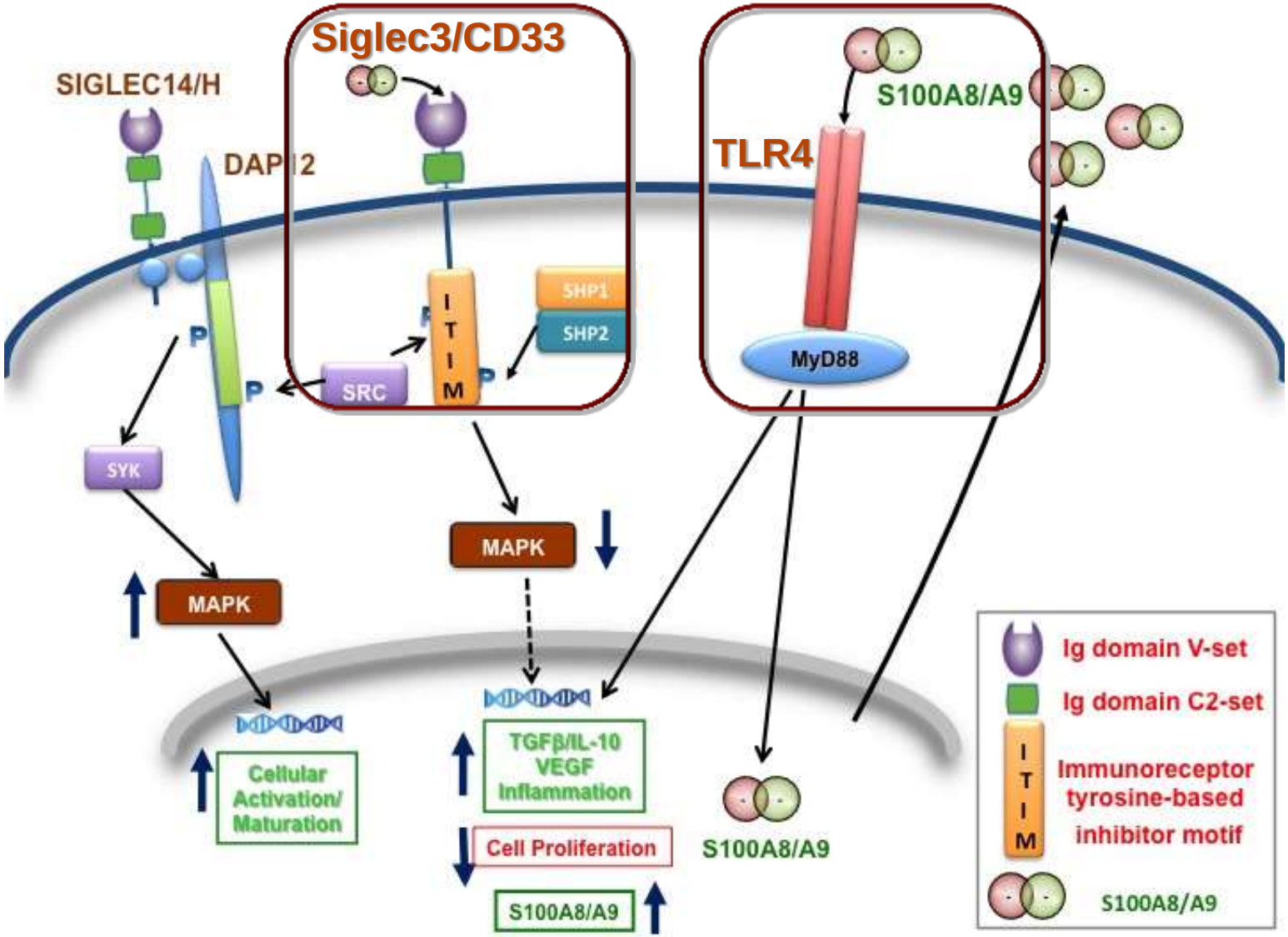
CD33 is Indispensible for S100A9 Inflammatory Cytokine Induction



Normal donor BM-MNC's RAGE, TLR4, CD33, or their combination were blocked prior to culturing cells by with or without 1 µg of S100A9 for 48 hours followed by assessment of IL-10 gene and protein expression (qPCR – top, ELISA on the bottom).

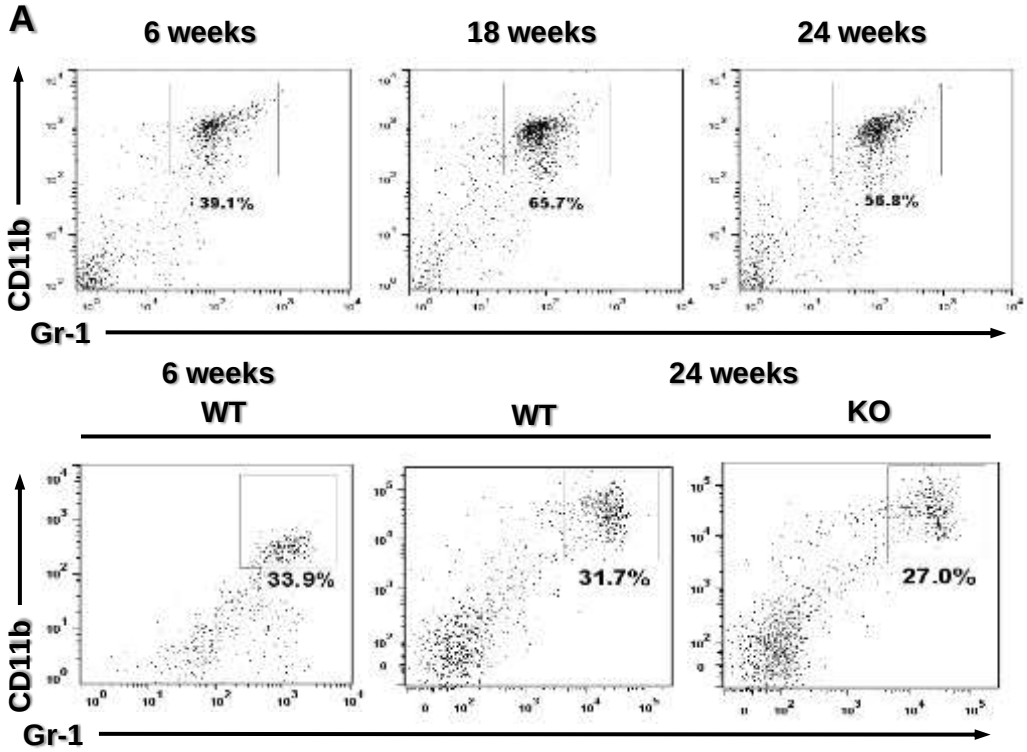
S100A9 Interacts with TLR4 & CD33 (SIGLEC-3)



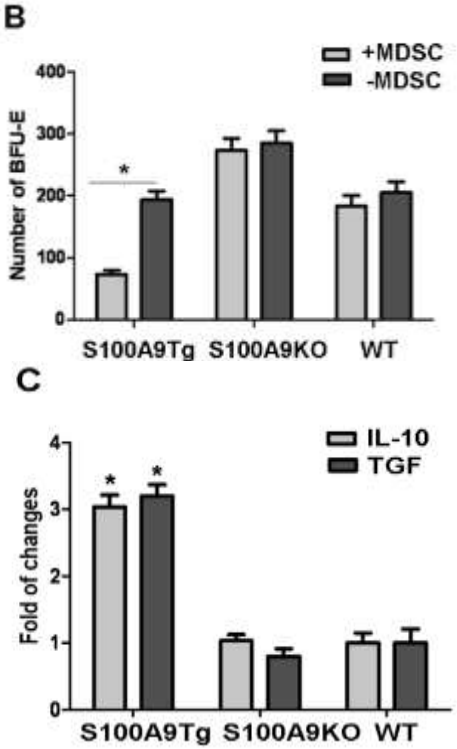
Siglecs (Sialic acid-binding immunoglobulin-type lectins)

S100A9-Tg Mice Display Age-related MDSC Expansion & Ineffective Hematopoiesis

MDSC Expansion



BFU-E & Cytokines



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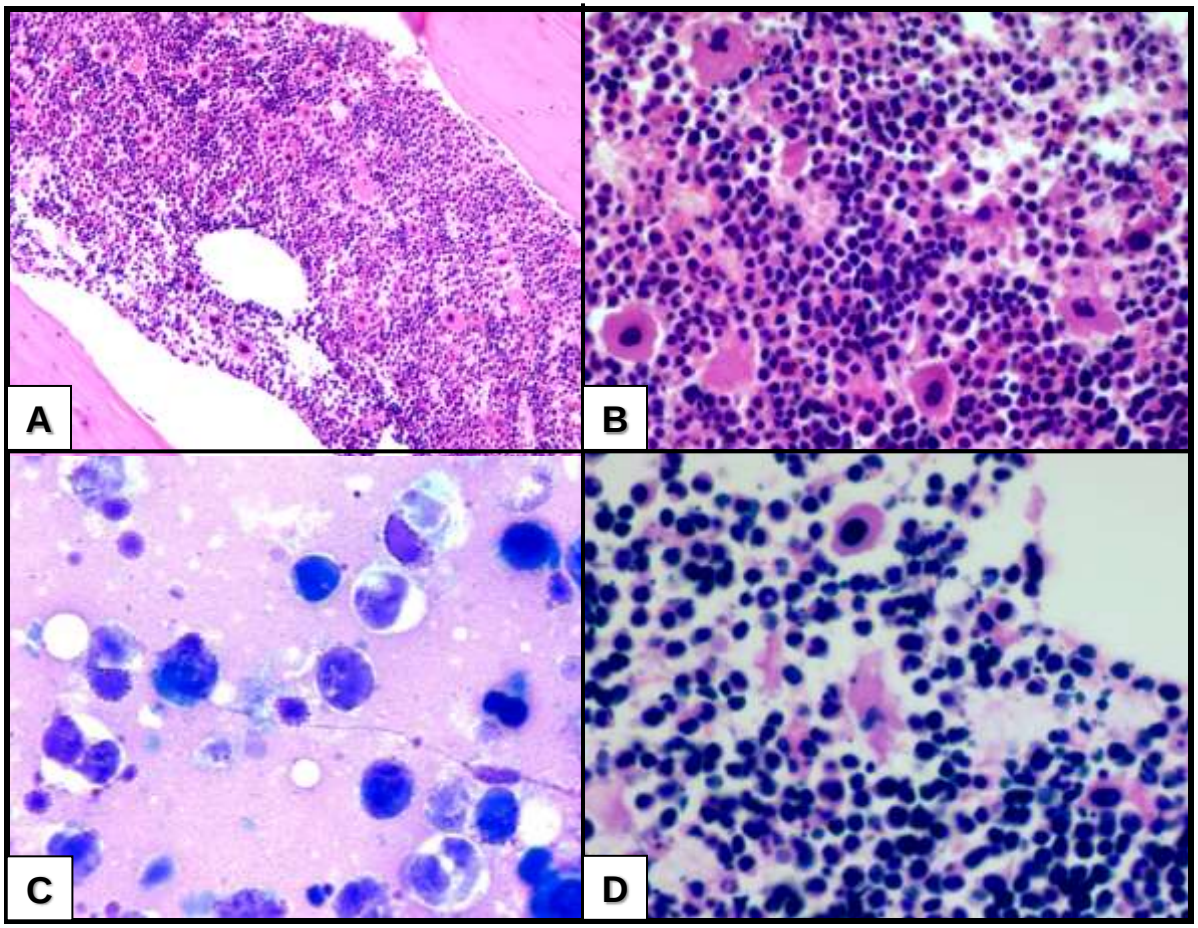
S100A9-Tg Mice Develop Trilineage Cytological Dysplasia Phenocopying MDS



A. Hypercellular marrow with megakaryocytic hyperplasia

B. Dysplastic megakaryocytes showing single or hypolobation and increased micro-megakaryocytes (dwarf megakaryocytes)

C. Hypogranulated and hyposegmented PMNs (pseudo-Pelger-Huet changes) and nuclear budding in erythroid precursors. (All cells are partially degenerated)

D. PAS stain highlights erythroid predominance

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S100A9-Tg Mice Develop Multilineage Cytopenias with Age

Test	Units	6 weeks		18 weeks		24 weeks	
		WT	S100A9-Tg	WT	S100A9-Tg	WT	S100A9-Tg
WBC	$10^3/\mu l$	5.4 ± 0.5	4.2 ± 1.5	6.0 ± 1.4	2.9 ± 0.2*	6.3 ± 0.8	3.0 ± 0.4*
LYM	$10^3/\mu l$	3.9 ± 0.5	2.6 ± 1.2	4.7 ± 1.1	2.4 ± 0.1*	4.8 ± 0.7	2.5 ± 0.3*
MONO	$10^3/\mu l$	0.4 ± 0.1	0.3 ± 0.2	0.4 ± 0.1	0.2 ± 0.1*	0.4 ± 0.1	0.2 ± 0.1*
GRAN	$10^3/\mu l$	1.1 ± 0.6	1.2 ± 1.2	0.9 ± 0.3	0.3 ± 0.2*	1.1 ± 0.4	0.3 ± 0.1**
HCT	%	48.1 ± 3.2	42.4 ± 2.1	45.7 ± 0.7	35.5 ± 3.0**	45.4 ± 3.5	32.1 ± 2.7**
MCV	fl	51.4 ± 1.6	49.8 ± 2.0	50.3 ± 0.5	50.0 ± 1.2	50.0 ± 1.3	50.0 ± 1.6
RDWa	fl	35 ± 0.7	33.2 ± 2.2	34.0 ± 1.3	32.5 ± 1.7	33.3 ± 1.4	32.2 ± 1.5
RDW%	%	16.3 ± 0.7	15.9 ± 0.1	16.3 ± 0.5	15.5 ± 0.5	16.0 ± 0.5	15.4 ± 0.6
HGB	g/dl	14.2 ± 0.8	13.0 ± 0.7	13.9 ± 0.3	11.1 ± 0.7**	13.7 ± 0.6	10.3 ± 0.5**
MCHC	g/dl	29.5 ± 1.1	30.6 ± 1.3	30.4 ± 0.4	31.5 ± 0.9	30.3 ± 1.0	32.3 ± 1.1
MCH	pg	15.1 ± 0.3	15.2 ± 0.1	15.3 ± 0.1	15.8 ± 0.3	15.2 ± 0.2	16.2 ± 0.4
RBC	$10^6/\mu l$	9.4 ± 0.7	8.5 ± 0.4	9.1 ± 0.1	7.1 ± 0.6**	9.1 ± 0.3	6.4 ± 0.2***
PLT	$10^3/\mu l$	555.7 ± 96.6	412.0 ± 124.0	431.3 ± 33.9	95.7 ± 35.0***	437.0 ± 41.9	61.0 ± 23.5***

All data are means ±SEM (n=3-5 mice). Peripheral blood samples were prepared from both S100A9Tg and control (wt) mice in ages of 6, 18 and 24 weeks and analyzed on a Hema True Hematology Analyzer (Heska). *p<0.05; **p<0.01; ***p<0.001 vs wt-mice

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

Candidate Therapeutics Targeting Innate Immune Activation in MDS

Sites of Target Inhibition

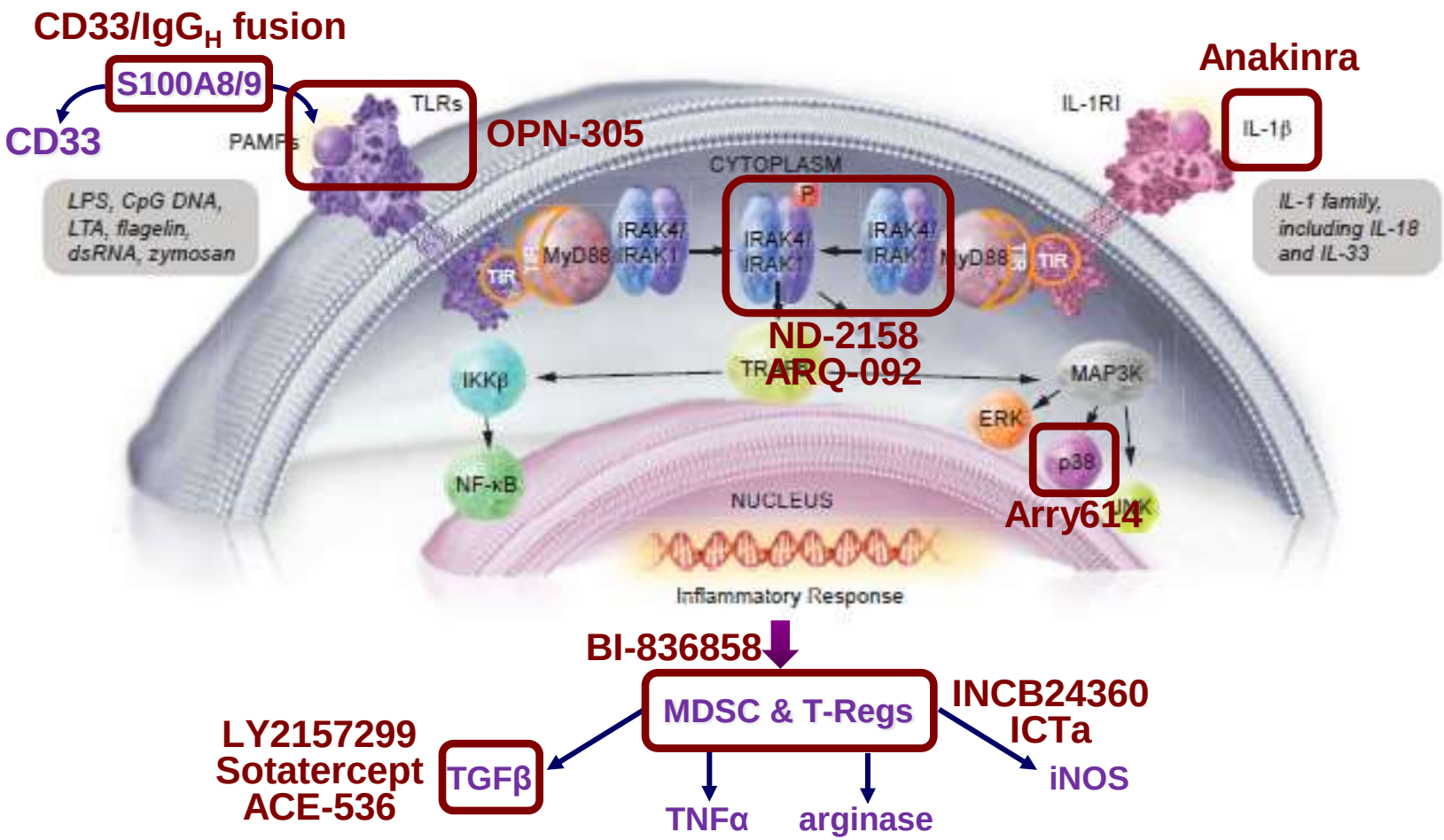


Figure modified from www.nimbusdiscovery.com

IL-1 Receptor-Associated Kinase [IRAK]
A Candidate Therapeutic Target in MDS

IRAK4 Inhibition (ND-2158)

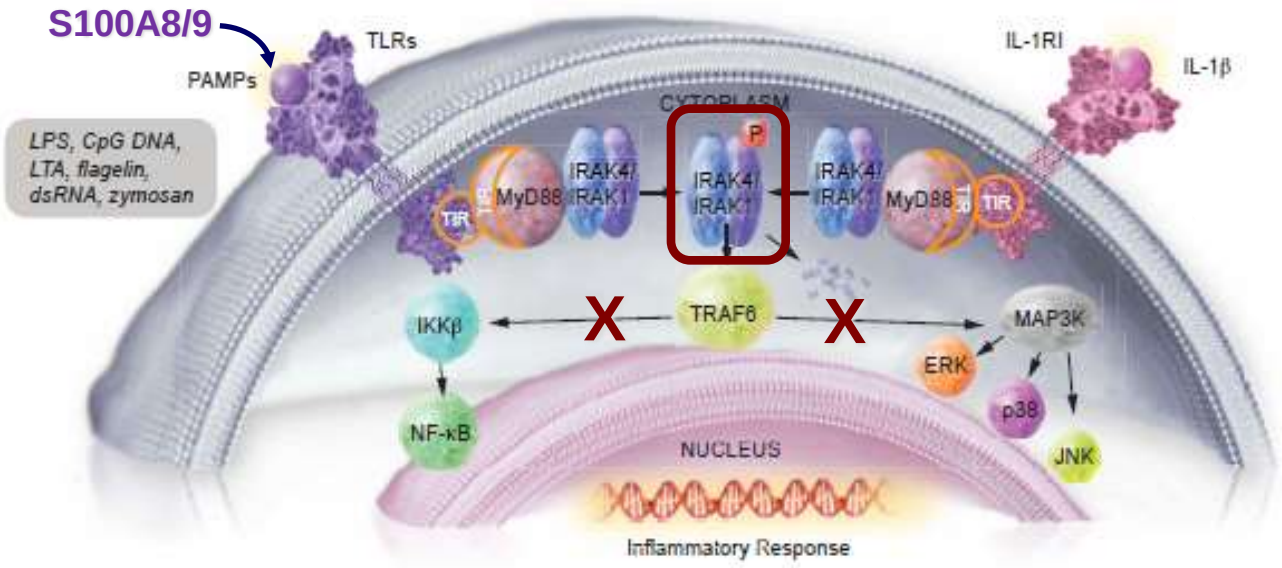
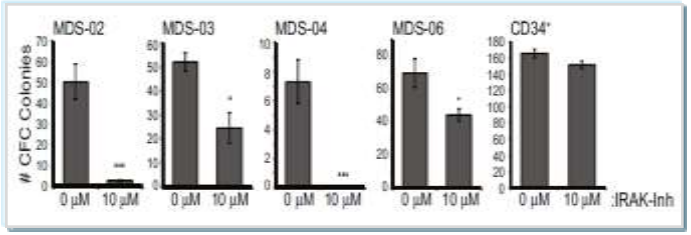
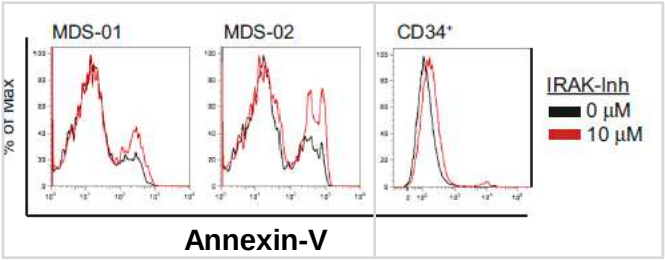


Figure adopted from www.nimbusdiscovery.com

Selective Suppression of MDS CFC with IRAK Inhibition



Selective Apoptotic Response to IRAK Inhibition



Rhyasen GW & Starczynowski D. Cancer Cell 2013.

Novel Strategies to Abrogate Aberrant Innate Immune Activation

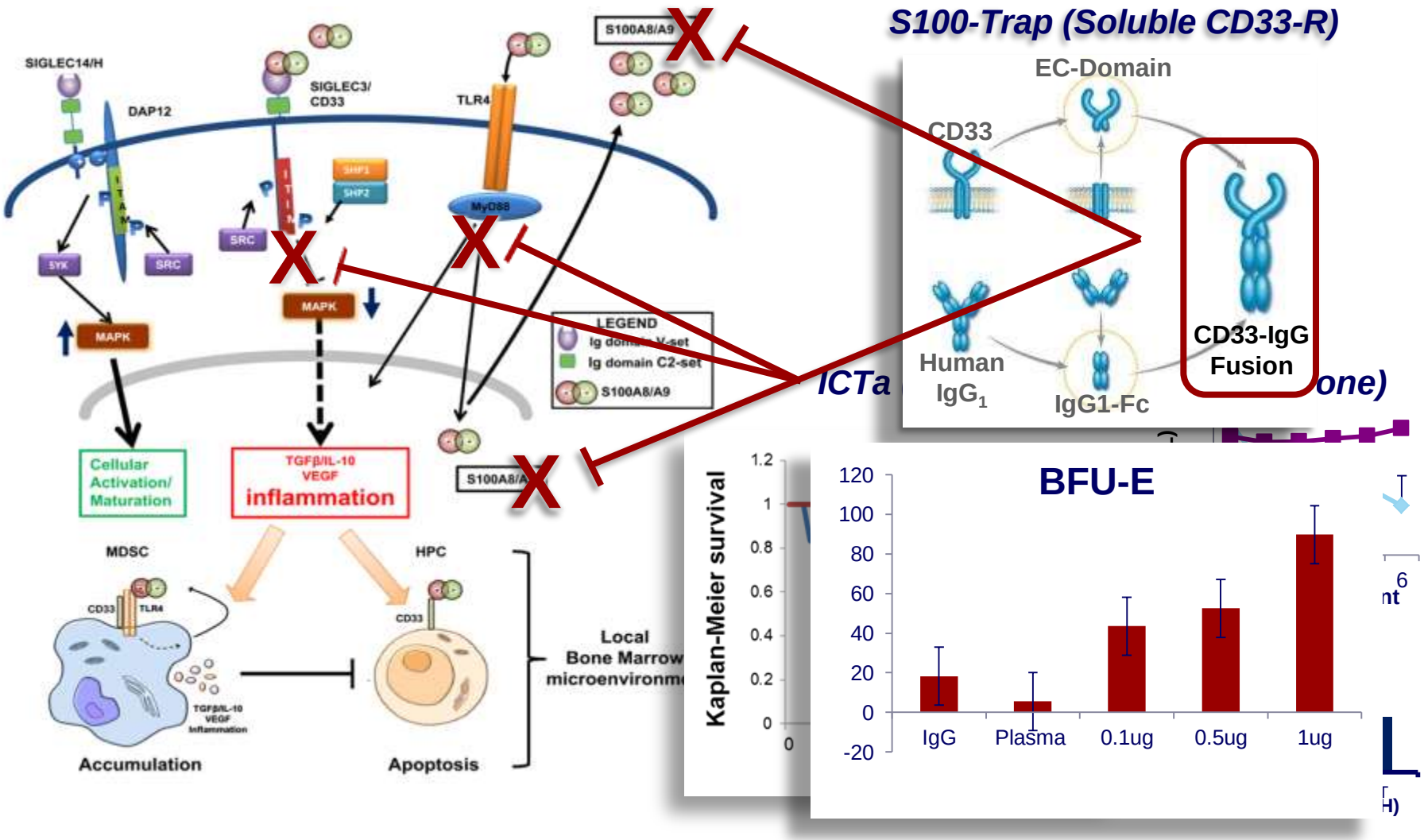
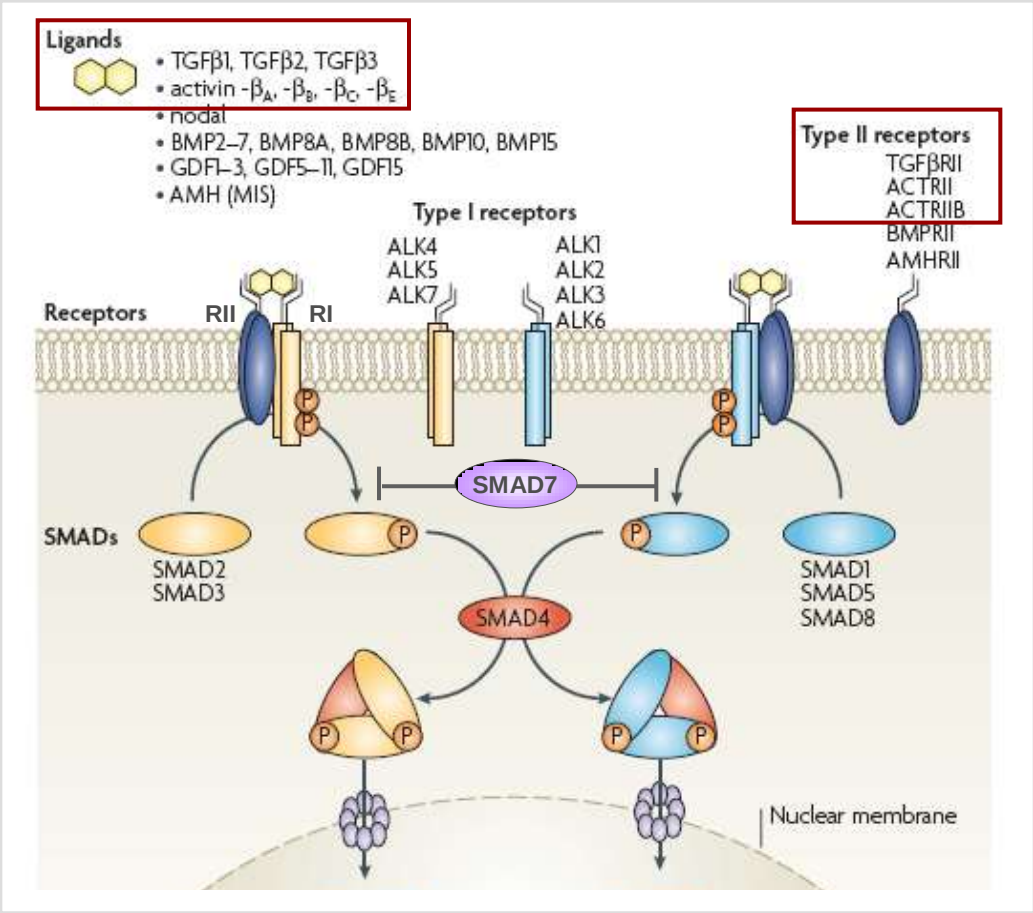
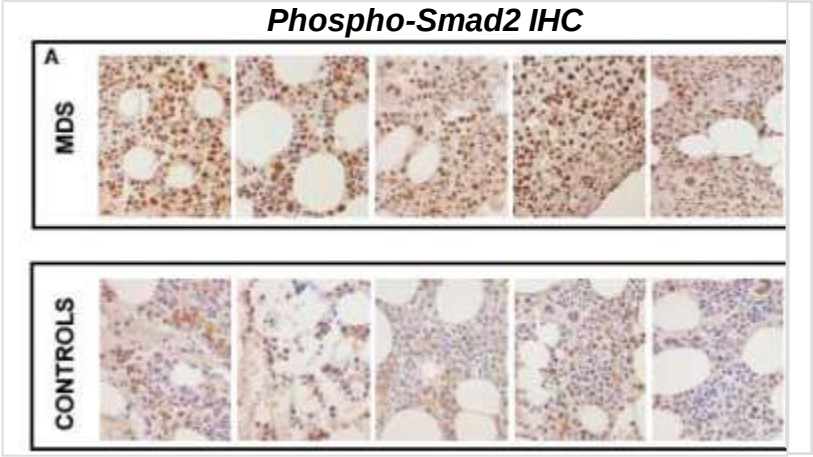


Figure adapted from Chen X, et. al. J Clin Invest 2013; 123(11):4595-4611

Constitutive Activation of TGF-β Signaling Suppresses Hematopoiesis in MDS



- TGFβ Type I receptor kinase phosphorylates Smad2 & 3 forming transcriptional complexes, whereas the inhibitory Smad7 extinguishes TGFβ-R1 activity
- miR-21 upregulation significantly reduces Smad7 in MDS BM progenitors
- R1 kinase is constitutively activated in MDS with sustained Smad2 phosphorylation
- Suppression of R1 kinase improves MDS progenitor CFC *in vitro*

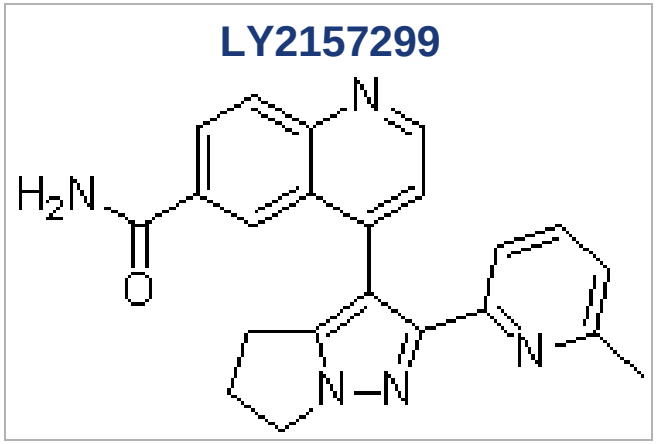


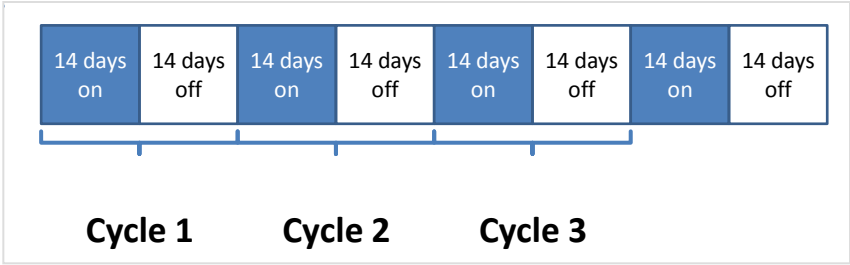
Schmierer B. et. al. Nat Rev Molec Cell Biol 2007; 8(12):970-82

Zhou L, et al. Blood 2008; 112(8):3434-3443
Bhagat TD, et. al. Blood 2013; 121(15):2875-2881

Phase 2a Study of TGF-□ Receptor I Kinase Inhibitor LY2157299 (galunisertib)

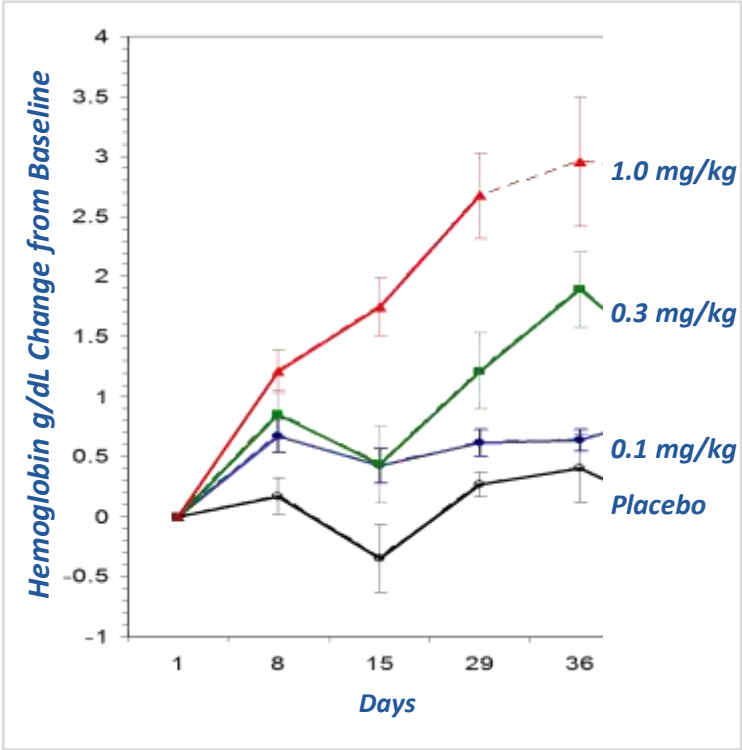
- Selective a novel oral TGF-βRI/II dual kinase inhibitor
- Dihydropyrolopyrazole ATP binding pocket binder with RI IC_{50}86 nM
- Phase I activity in GBM
- p-SMAD2/3 inhibition
- Eligibility: Low/Int-1 MDS, Hgb<9.5
- Dose: 300mg/d po x14d,q4wks
- Primary endpoint: HI_E@24 wks
- N=40





ACE-011 (sotatercept) Targets Stromal-Mediated Inhibition of Erythropoiesis

- High affinity Activin-A receptor (RIIA)/IgG1 fusion protein
- Sustained neutralization of activin-A & GDF11 ligands for up to 32 days
- Relieves GDF11 and activin-A suppression of erythropoiesis to restore differentiation
- Inhibits osteoclasts & promotes osteoblast survival
- MTD in normal volunteers: erythrocytosis



Dussiot M, et. al. Nat Med 2014 Apr;20(4):398-407.
Carrancio S, et. al. Br J Haematol 2014 Jun;165(6):870-82.

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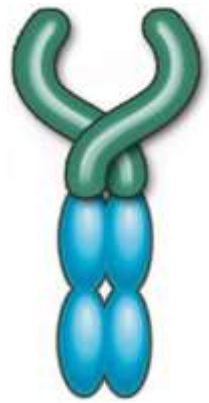
ACE-011 (Sotatercept) and ACE-536

Novel Ligand Traps for TGFβ Superfamily Ligands

Fusion protein with ligand trap activity toward the activin type 2 receptors

Drug does not bind EPO receptors

ACE-011 (Sotatercept)



Extracellular Domain of ActRIIA

Fc Domain of human IgG_1 Antibody

ACE-536

Modified Extracellular Domain of ActRIIB

Fc Domain of human IgG_1 Antibody

	ACE-011 (Sotatercept)	ACE-536
Heme effect	+	+
Bone effect	+	−

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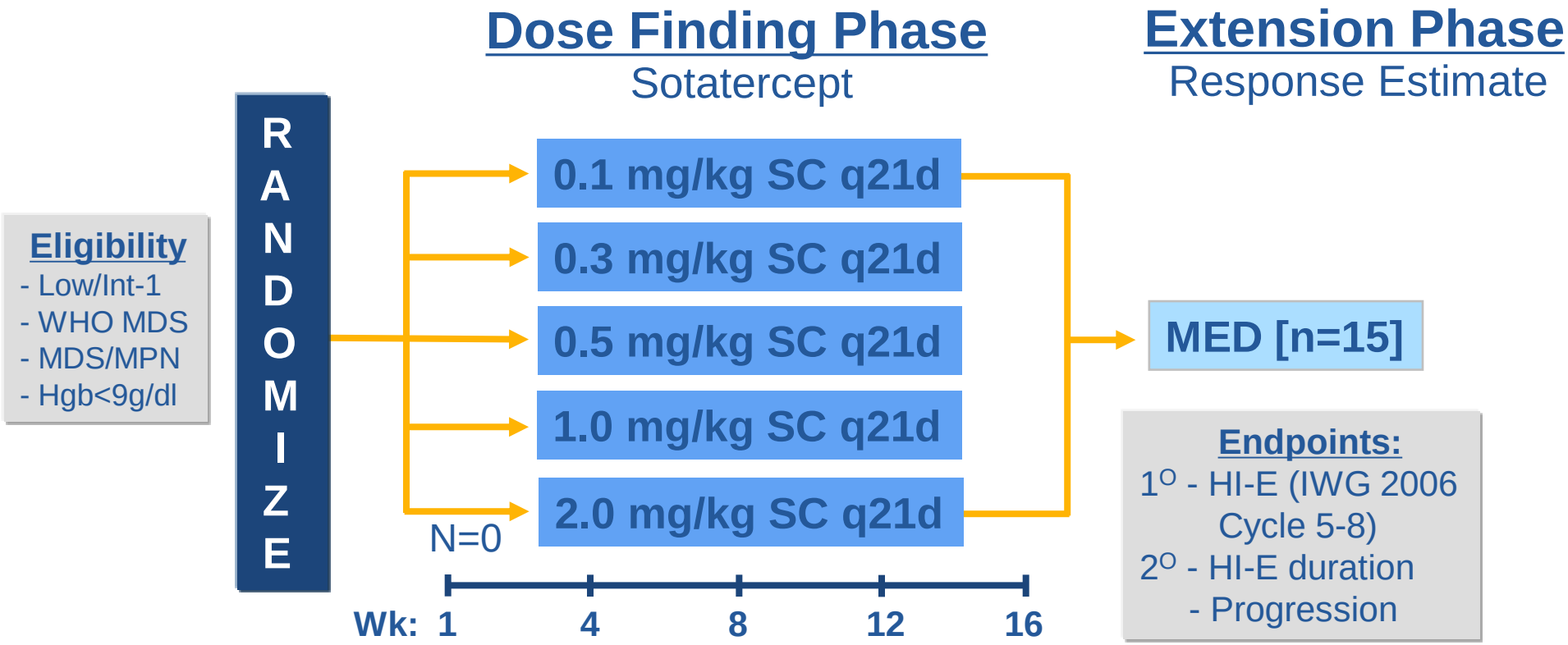
Randomized Phase II Study of Sotatercept in Transfusion-Dependent LR-MDS Epo Failures



MED, Maximally Effective Dose.

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ARRAY-614-112 Phase 1 Study in LR-MDS

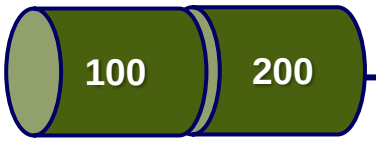
Enabled ARRY-614 Formulation

p38 MAPK Inhibitor

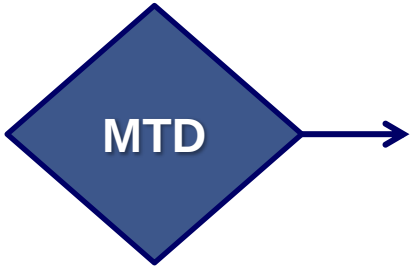
Dose Escalation Phase

Daily dosing

BID dosing



- 3 + 3 dose escalation design with expansion
- Cycle = 28 days



Expansion Phase

Daily Dosing

Primary Objective

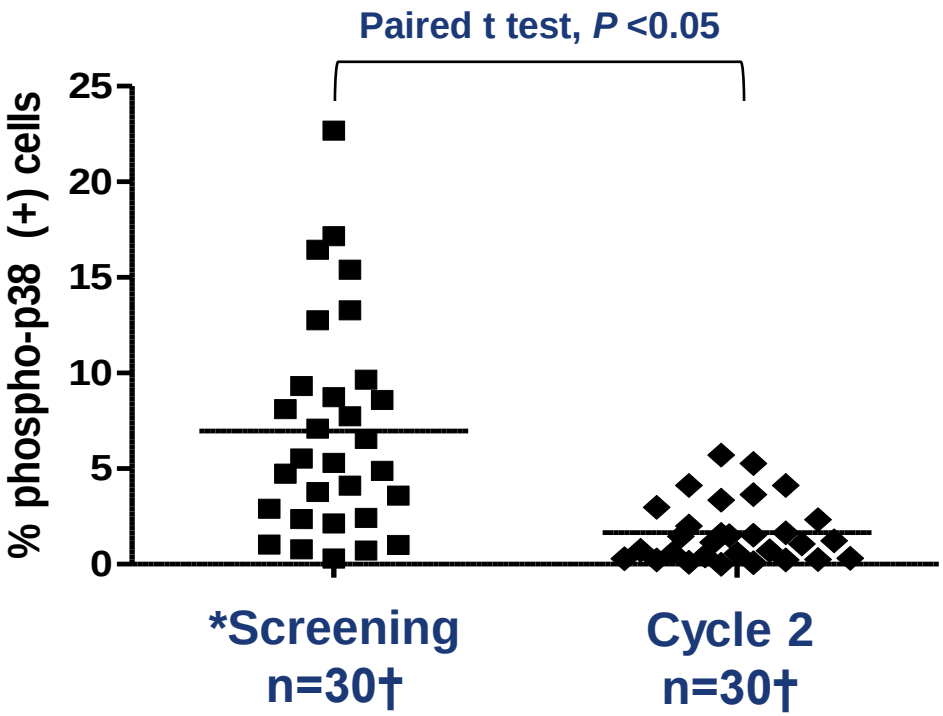
- Safety, tolerability & MTD.
- PK

Secondary

- IWG 2006 response
- Explore PD profile

Garcia-Manero G, et. al. ASH 2013.

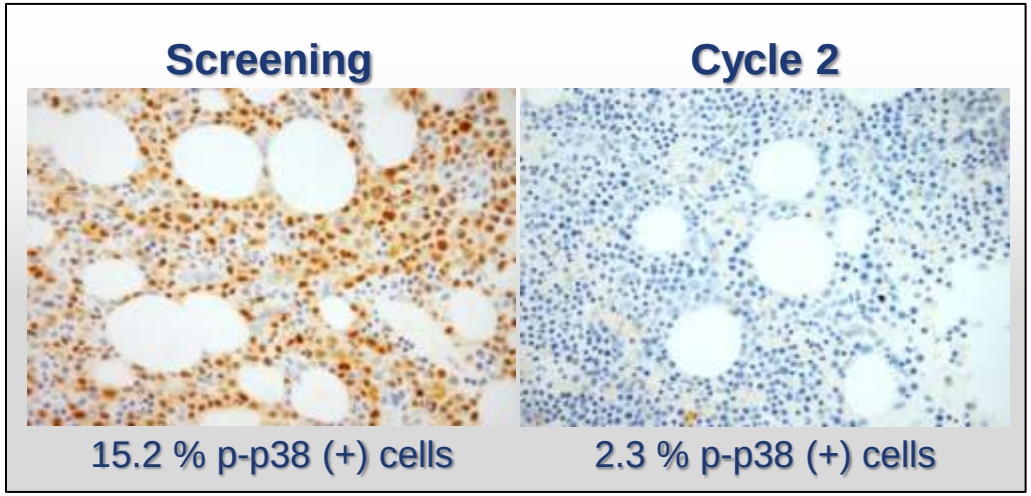
ARRY-614 Reduces BM-MNC phospho-p38



*Sample collected prior to the first dose of ARRY-614

†Number of pts for whom bone marrow samples available at screening and cycle 2

Representative Image



Aperio whole slide scanning and scoring performed by Flagship Biosciences

Garcia-Manero G, et. al. ASH 2013.

ARRY-614 Hematologic Response

All responses (HI and platelet transfusion)	HI-E	HI-P-Any	HI-N	Total pts with HI
	n = 66	n = 42	n = 22	N = 71
Total (%)	5 (7.6)	8 (19)	6 (27)	14 (20)
Median duration, weeks (range)	11 (9-29)	30.1 (10.4-91)	17.6 (8.7-67.4)	

Transfusion improvement	RBC	Median Duration (range)	platelets	Median Duration (range)
	n=41		n=16	
Transfusion reduction n (%)	5 (12)	11 (9.0-28.6)	7 (44)	18 (10.4–91.1)
Transfusion indepnt n (%)	2 (5)	19.0 (9.3-28.6)	5 (31)	14.1 (10.4–90.7)

Garcia-Manero G, et. al. ASH 2013.

Conclusions

- MDSCs (LIN-HLA-DR-CD33+) are activated & profoundly expanded in the bone marrow of MDS patients.
- MDS-MDSCs are distinct from the MDS clone, display a $CD33^{Hi}$/$lineage^{-}$ phenotype, produce inflammatory/suppressive molecules & serve as cellular effectors of ineffective hematopoiesis via direct cytotoxicity to autologous progenitors.
- S100A9 is a myeloid-derived peptide & TLR4/CD33 ligand that promotes both autocrine-reinforced MDSC activation, & paracrine mediated myeloid progenitor apoptosis.
- Strategies that neutralize S100A9, or inhibit TLR & CD33 ITIM signaling offer therapeutic potential in the treatment of patients with MDS.

Acknowledgements

List Lab

Kathy McGraw
Ashley Basiorka

Wei Lab

Xianhong Chen
Erika Eksioglu
Nicole R. Fortenbery

Collaborators

Dmitry I. Gabrilovich
P. K. Epling-Burnette
Eric Padron
Rami Komrokji

Post-Test

1. Which factors determine primarily the incidence of relapse after HCT for MDS?
   a. Transfusions given before HCT
   b. Marrow myeloblast count
   c. Cytogenetics
   d. Pre-transplant therapy
   e. b and c

2. Which would be your order of priority in selecting a transplant donor?
   a. HLA-matched (HLA=) sibling > HLA= unrelated donor (URD) >HLA haplo-identical relative > cord blood
   b. HLA= sibling > HLA= URD > cord blood >HLA haplo-identical relative
   c. HLA= sibling > HLA haplo-identical relative >HLA= URD > cord blood
   d. HLA= sibling > cord blood > HLA=URD >HLA haplo-identical relative

Post-Test

3. Iron overload in MDS is prognostic and:
    a. Correlates with a poor overall survival
    b. Correlates with certain comorbidities
    c. Should be corrected before stem cell transplantation
    d. a, b and c

4. In the context of MDS, fatigue is:
    a. Rarely seen
    b. Frequently recorded
    c. Often found in those who have comorbidities
    d. b and c

Post-Test

5. The presence of TET2 mutations predicts for:
    a. Worse survival in MDS patients
    b. A worse response to hypomethylating agents
    c. A lower than normal platelet count
    d. None of the above

6. DNA methylation patterns predict for:
    a. A worse survival in patients with RAEB-I
    b. Response to decitabine or 5-azacytidine
    c. The presence of specific mutations within the MDS genome
    d. Clonal diversity at diagnosis in MDS patients

Post-Test

7. Myeloid-derived suppressor cells (MDSC) are a phenotypically distinct innate immune effector cell that displays high expression of which of the following antigens?
    a. CD34
    b. CD33
    c. CD14

8. Bone marrow-MDSC are markedly expanded in MDS and are responsible for which of the following?
    a. Cell death of hematopoietic progenitors
    b. Suppression of anti-tumor immune response
    c. Elaboration of inflammatory cytokines
    d. All of the above

Post-Test

9. Somatic mutations in one of the following genes of RNA splicing machinery are associated with an MDS subtype with distinct phenotype and indolent clinical course. Which is the gene?
   a. SF3B1
   b. SRSF2
   c. U2AF1

10. More than 90% of patients with chronic myelomonocytic leukemia carry somatic mutations of genes of various biologic pathways. Many of them have concomitant mutations in 2 genes: which is the typical co-mutation of CMML?
   a. SF3B1-JAK2
   b. TET2-SRSF2
   c. CSF3R-SETBP1