CENTER FOR CANCER RESEARCH

Connecting the Cancer Community

Pre-clinical models for MDS

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Disclosures

I receive royalties through the NIH Technology Transfer Office for the invention of NUP98-HOXD13 mice

Pre-clinical models of cancer

- *In vitro*
  - Purified components (eg, enzymes)
  - Cell culture (eg, organoids, co-culture)
  - MDS studies hampered by lack of cell lines
- *In vivo*
  - Model organisms (yeast, fly, fish, rodent, primate)
  - Mouse models
    - Xenograft of immunodeficient mice (MDS very difficult to engraft)
    - Genetic Engineered Mice (GEM)

MDS GEMs

ASXL1	*Abdel-Wahab, J Exp Med, 2013*
CREBBP	*Rebel, PNAS, 2002*
DICER	*Raaijmakers, Nature, 2010*
EVI1	*Buonamici, JCI, 2004*
NPM1	*Grisendi, Nature, 2005*
BCL2/NRAS	*Omidvar, Cancer Res, 2007*
RUNX1	*Watanabe, Blood, 2008*
SALL4B	*Ma, Blood, 2006*
S100A9	*Chen, JCI, 2013*
TRAF6	*Starczynowski, Nat Med, 2010*
TET2	*Moran-Crusio, Cancer Cell, 2011*
SRSF2	*Smeets, Blood, 2018*
U2AF1	*Shirai, Cancer Cell, 2015*
NUP98-HOXD13	*Lin, Blood, 2005*

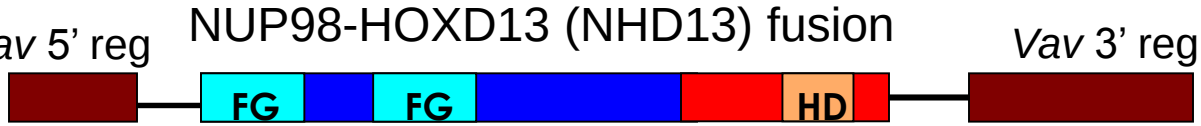
NUP98 Translocations and Hematologic Malignancy

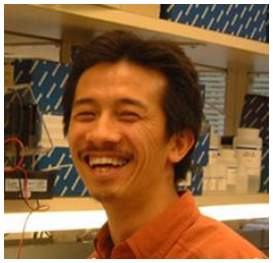
- NUP98 is fused to >30 different partner genes in patients with MDS, AML, CML, CMML, and T-ALL.
- Chimeric protein- NUP98 at amino terminus; partner at carboxy terminus.
- More common in children than adults (6-10% of pediatric AML pts have NUP98 fusions) (Bisio, Blood, 2014; Bolouri, Nat Med 2018).

Translocation	Partner gene	Homeo-domain?	Disease
t(7;11)(p15;p15)	*HOXA9, 11, 13*	Yes	MDS, AML, CML
t(11;12)(p15:q13)	*HOXC11,13*	Yes	MDS, AML
t(2;11)(q31;p15)	*HOXD11, 13*	Yes	MDS, AML, CML
t(1;11)(q23;p15)	*PMX1(PRRX1)*	Yes*	MDS, AML
t(9;11)(q34;p15)	*PRRX2*	Yes*	MDS, AML
t(11;20)(p15;q11)	*TOP1*	No	MDS, AML
inv11(p15q22)	*DDX10*	No	MDS, AML

Chris Slape

*vav*NHD13 mice develop MDS



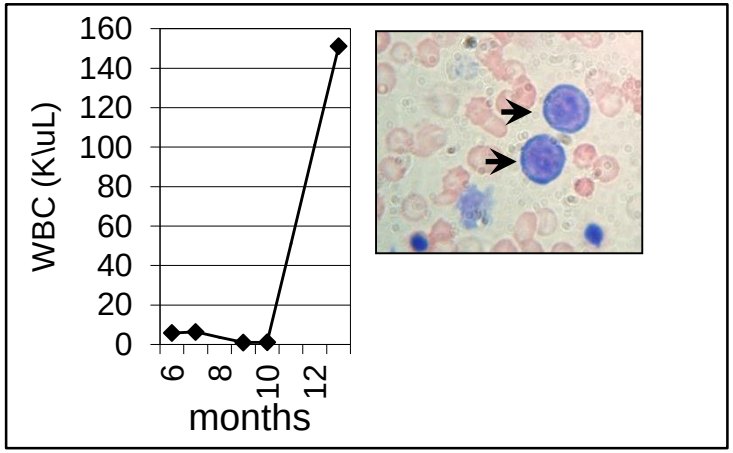


Ying-Wei Lin

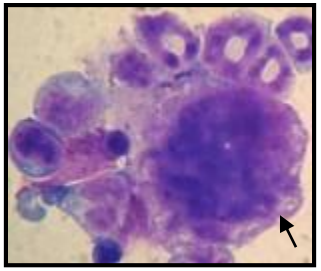
CBCs (age 4-7 mos)

	WBC (10^9/L)	NE (10^9/L)	Hb (g/dL)
NHD13 (n = 22)	1.8	0.44	11.9
Control (n = 7)	6.5	1.4	14.2
p value	<0.001	<0.001	<0.001

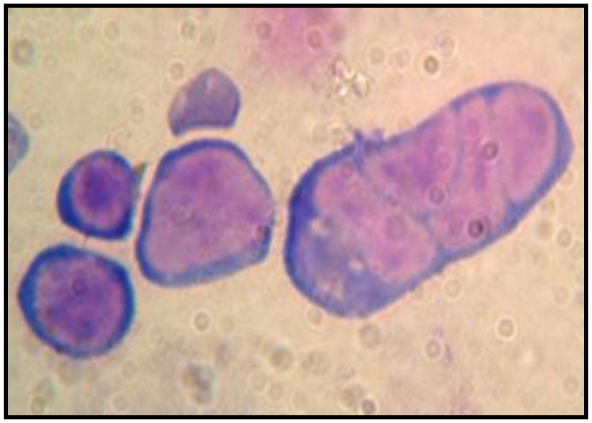
Transformation to AML



Micro-megakaryocytes



Multinucleate erythroblasts



Overexpressed genes

Oas2, Ifit1, Ifi44, Hoxa9, Hoxa7, Pbx, Hoxc6,

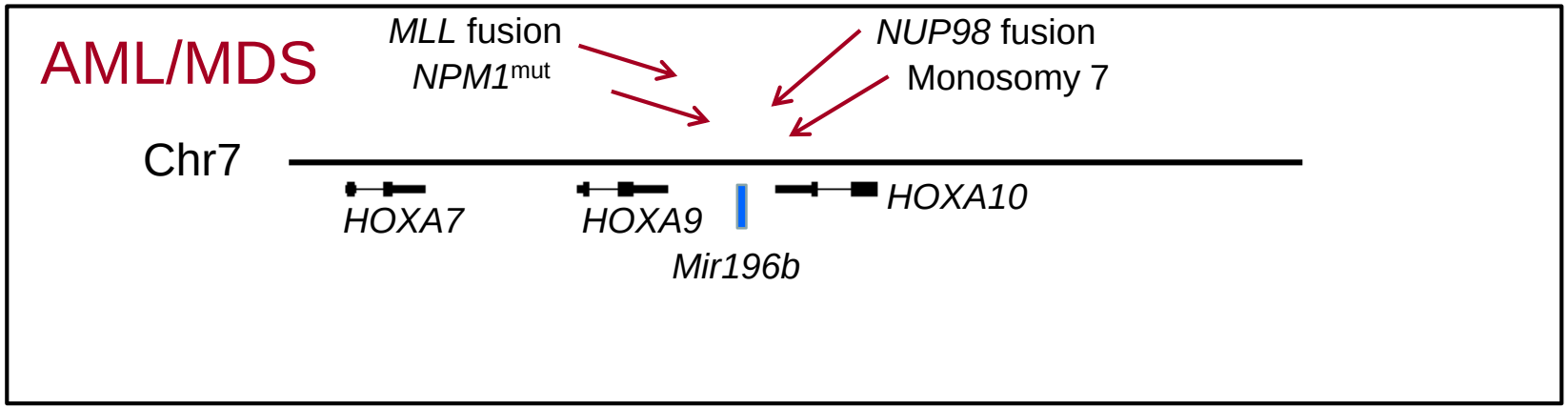
*Interferon induced *Homeodomain

Lin et al., Blood, 2005.
Slape et al., JNCI, 2008.
Novak et al., Exp. Hematol, 2013.

.

Overexpression of *HOXA* cluster genes is a common finding in MDS and AML

- *HOXA9* was the single most differentially expressed gene in CD34+ cells from MDS patients with monosomy7 (Chen, Blood, 2004).
- Over 50% of AML patients showed overexpression (>5X nl BM) of *HOXA5/7/9 and MEIS1*. (Palmqvist, PLOS One, 2007).
- Overexpression of HOXA9 associated with enforced stem cell self-renewal (Wu, Cell Stem Cell 2007; Ito, Nature, 2010)

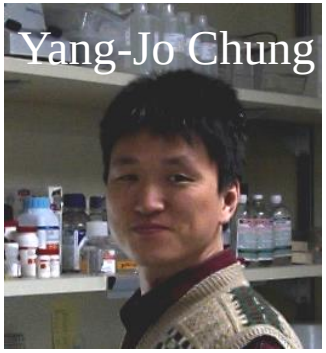


Suggests that enforced expression of *HOXA* cluster genes might be a common pathway for MDS/AML.

Is MDS transplantable?

NHD13 $^{+}$ Donor (CD45 Ly5.2) NHD13 $^{-}$ (WT)

± WT competitor BM (CD45 Ly5.1)



1000rad

Recipient mice
(CD45 Ly5.1)

1000rad

Assay for hematologic engraftment and differentiation.

Cell number

$1x10^6$ NHD13 or WT (Ly5.2) + $1x10^5$ WT competitor (Ly5.1)/ mouse

$1x10^5$ NHD13 or WT (Ly5.2) + $1x10^6$ WT competitor (Ly5.1)/ mouse

(Collaborator: T. Fry)

(Chung et al., PNAS, 2008)

Peripheral blood cytopenias and normocellular BM = ineffective hematopoiesis

Mice transplanted with 1x10^6 Donor (Ly5.2) and 1x10^5 WT competitor (Ly5.1) cells

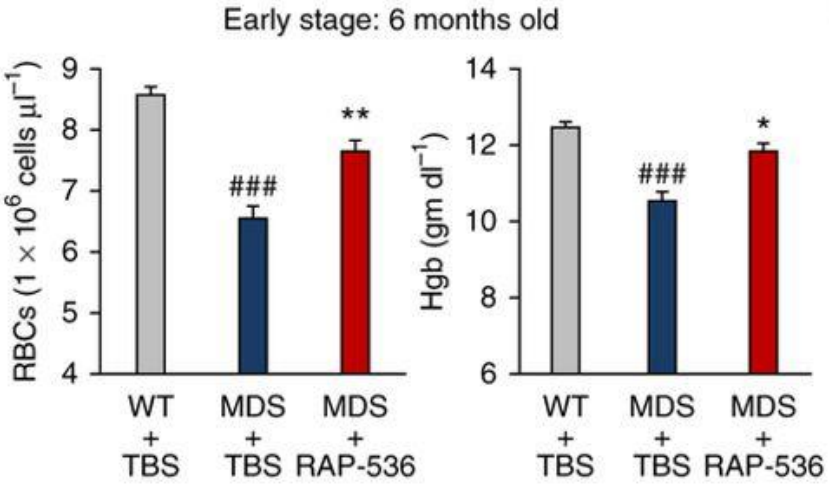
6 week	HGB (g/dL)	MCV (fL)	PLT (K/uL)	WBC (K/uL)	Polys (K/uL)	BM cellularity	% Ly5.2
WT e6/5	13.37 ± 0.29	48.43 ± 0.28	813.0 ± 34.9	8.27 ± 0.49	2.09 ± 0.16		
NHD13 e6/5	**11.53** ± 0.26	**55.70** ± 0.67	932.8 ± 89.9	2.36 ± 0.15	**0.51** ± 0.06		
16 week							
WT e6/5	13.27 ± 0.27	45.80 ± 0.12	864.3 ± 3.2	12.00 ± 1.55	1.80 ± 0.33	6.53X10^7 ± 0.7 X 10^7	80.49 ± 1.73
NHD13 e6/5	**8.40** ± 1.57	**57.13** ± 2.37	540.3 ± 259.1	3.70 ± 0.75	**0.53** ± 0.18	5.33X10^7 ± 0.5 X 10^7	82.87 ± 2.57

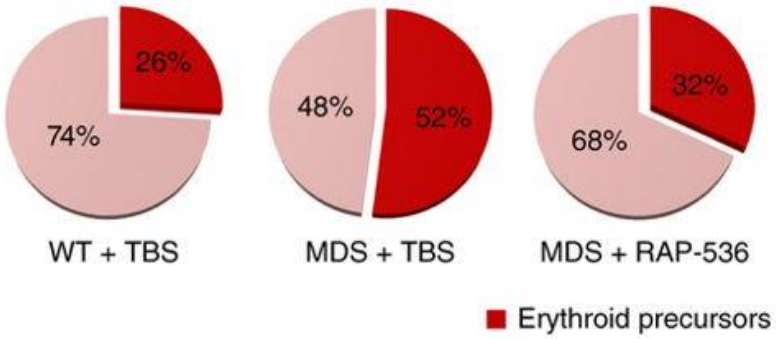
NHD13 mice as an *in vivo* model for MDS therapy

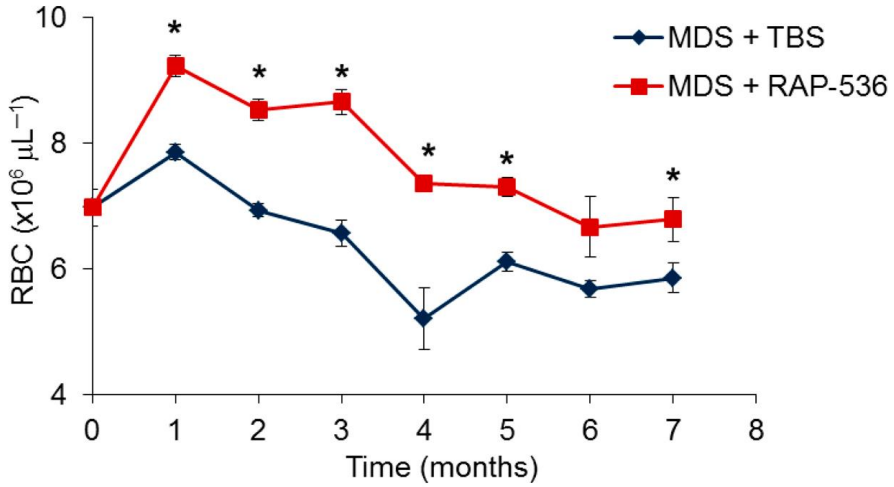
CENTRAL HYPOTHESIS: GEM models can provide data to inform clinical trials.

1. TGFβ trap—ACE536/Luspatercept
2. DNA methyltransferase inhibitors
3. Hematopoietic Stem Cell Transplant

NHD13 mice provide *in vivo* proof of principle for ACE-536 (Luspatercept)

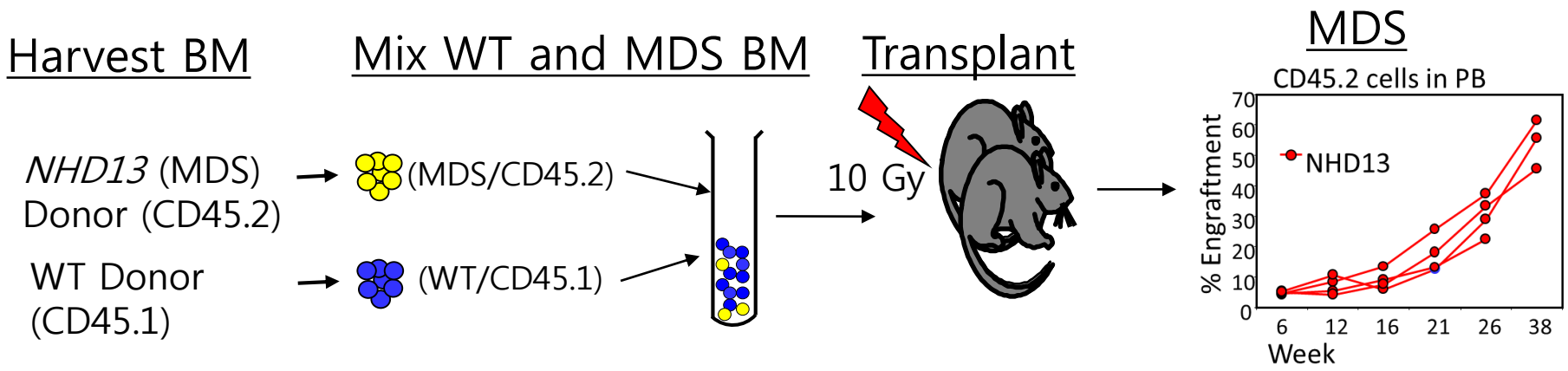


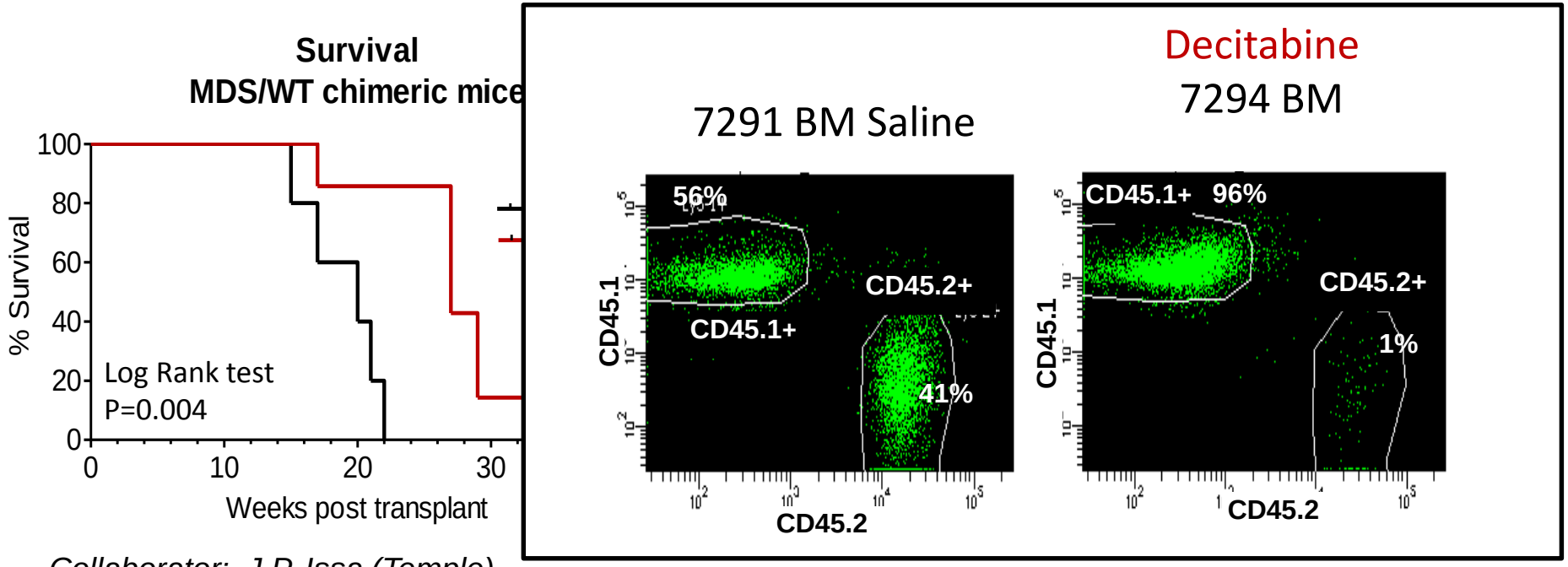




Suragani et al., Nat Med, 2014

Efficacy of DNMT1i decitabine (DAC) in MDS model



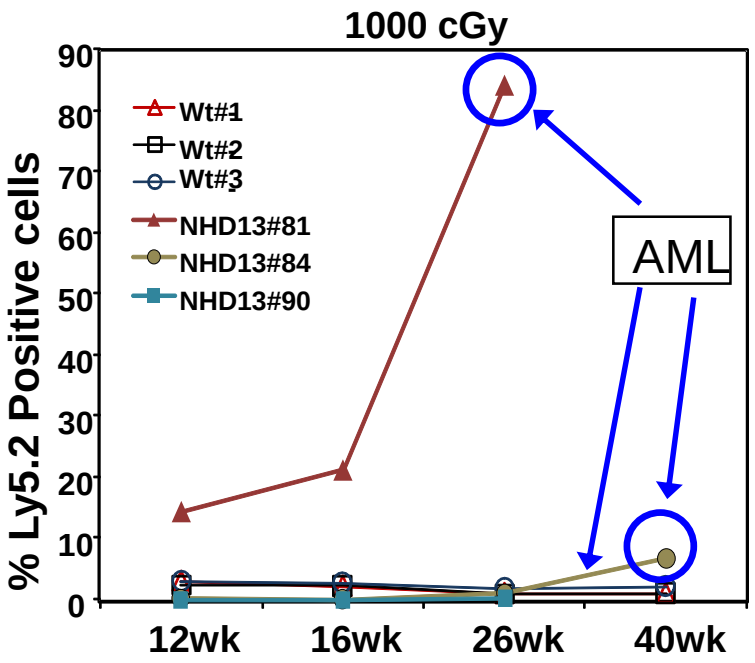


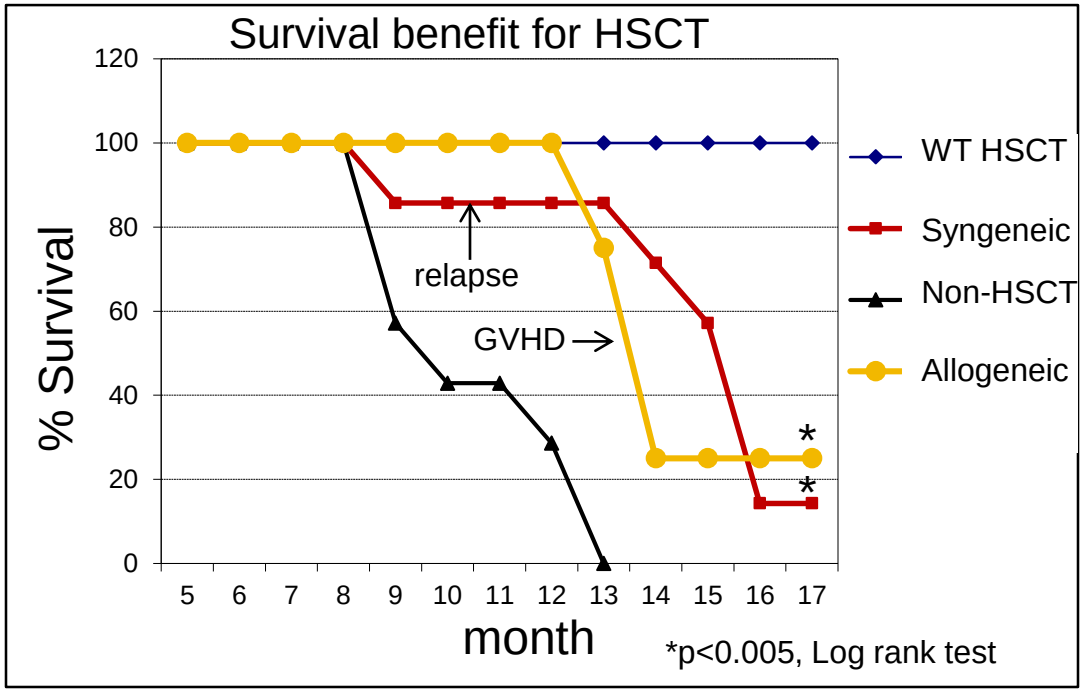
Collaborator: J.P. Issa (Temple)

Unpublished

Hematopoietic Stem Cell Transplant (HSCT) with syngeneic (C57Bl6) or allogeneic (C3H.SW) cells

Recipient: NHD13 (Ly5.2)
Donor: WT (Ly5.1)





Conclusion: 1000 cGy XRT (myeloablative in C57Bl6 mice) can induce long-term remission, but was not curative.

GVHD: graft versus host disease; GVT: graft versus tumor

Open Questions:

- Induce GVT with minimal GVHD
  - -Donor lymphocyte infusions (DLI) post-transplant
  - -Transplant with specific T-cell subsets (Tregs)

Collaborator: Terry Fry

Chung et al, PLoS One, 2018

Summary

- NHD13 mice develop a highly penetrant form of MDS which recapitulates the key features of human MDS.
- NHD13 hematopoietic cells outcompete WT cells *in vivo*.
- Treatment of NHD13 mice with DNMTi (DAC) leads to increased survival and normalization of blood counts.
- Myeloablative doses of IR lead to increased survival, but ultimately fail due to persistence of radio-resistant MDS.

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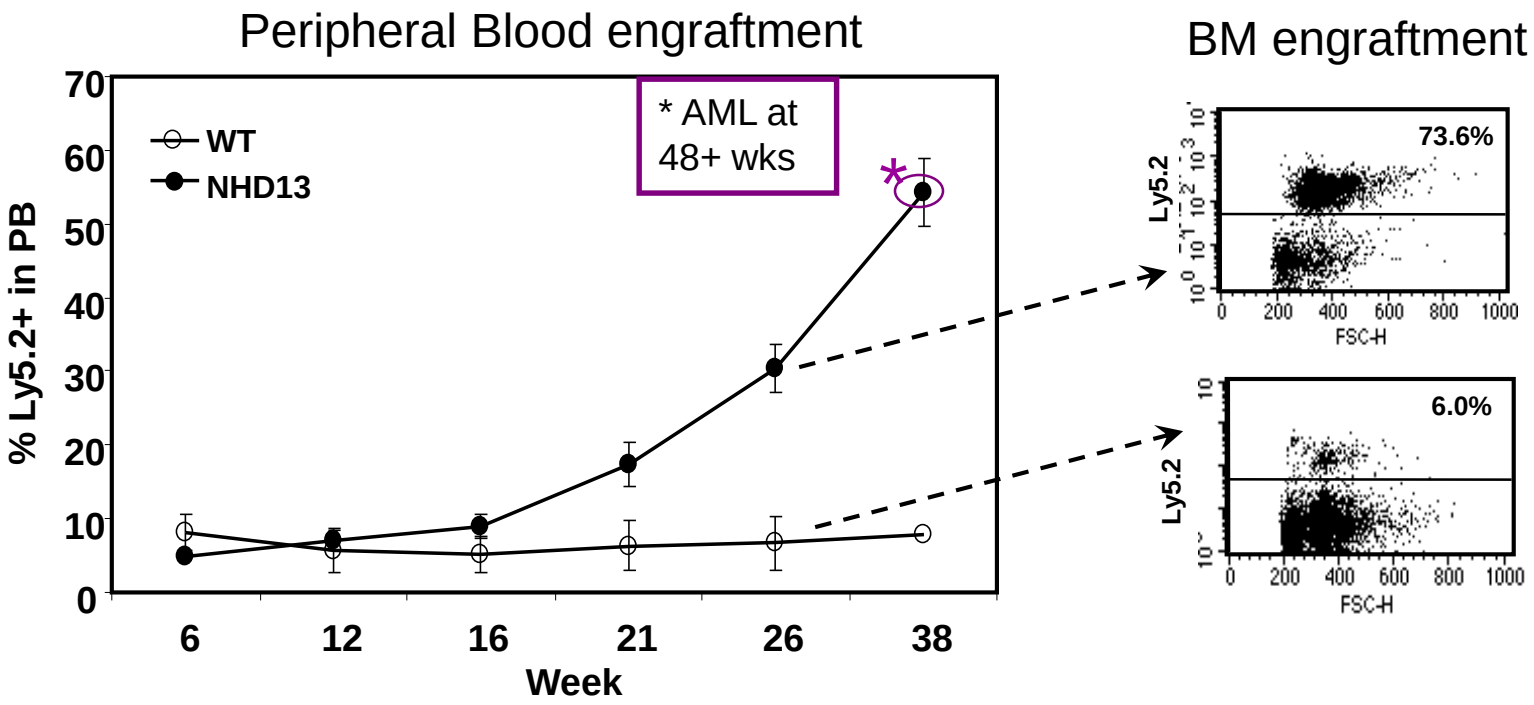
  - Li Li
- John Denu (U. Wisc)
- JP Issa (MD Anderson/Temple)

NHD13 BM cells outcompete WT cells

Mice transplanted with 1x10^5 Donor (Ly5.2) and 1x10^6 WT competitor (Ly5.1) cells

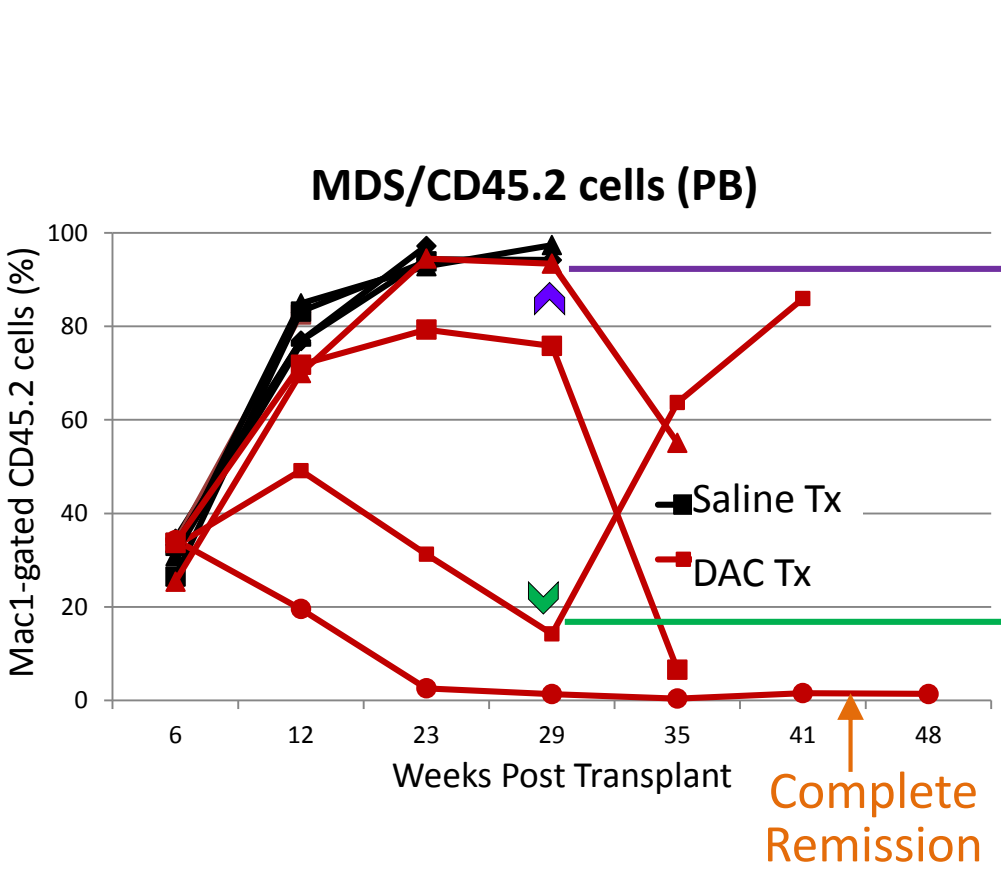
16 week	HGB (g/dL)	MCV (fL)	PLT (K/uL)	WBC (K/uL)	Polys (K/uL)
WT e5/6	**13.43 ± 0.22**	**44.97 ± 0.38**	899.0 ± 62.5	**10.39 ± 0.89**	**2.40 ± 0.25**
NHD13 e5/6	**12.05 ± 0.22**	**53.55 ± 0.26**	863.8 ± 57.7	**5.05 ± 0.44**	**0.87 ± 0.13**

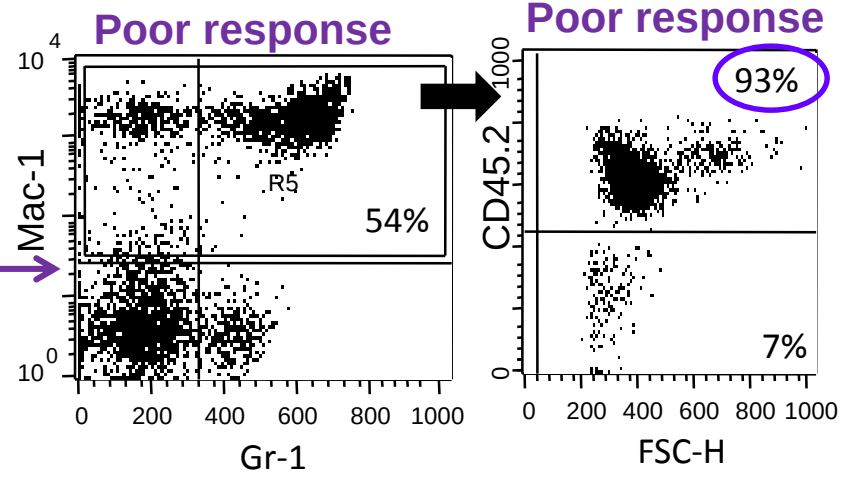


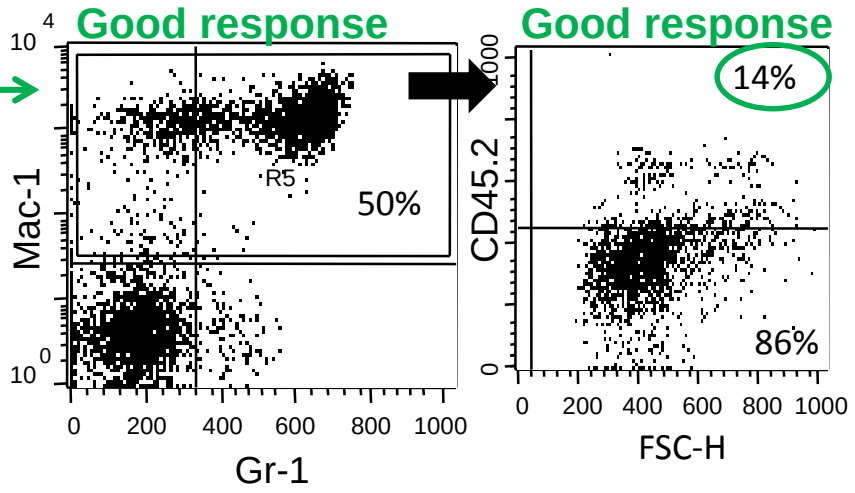
Case Report

- Initial dx of B-lineage ALL at 4 yrs of age , *TEL-AML1* fusion.
- Isolated CNS relapse 6 mos off rx
- Reinduction with HD ARA-C, VP-16, VCR, L-ASP, CTX, DOX and CNS XRT; continuation chemorx x 2 yrs.
- Thrombocytopenia 9 mos off rx, MDS->AML M6
- t(2;11)(q31;p15) (no *TEL/AML1* fusion)
- NUP98-HOXD13 fusion gene

Variable responses to DAC







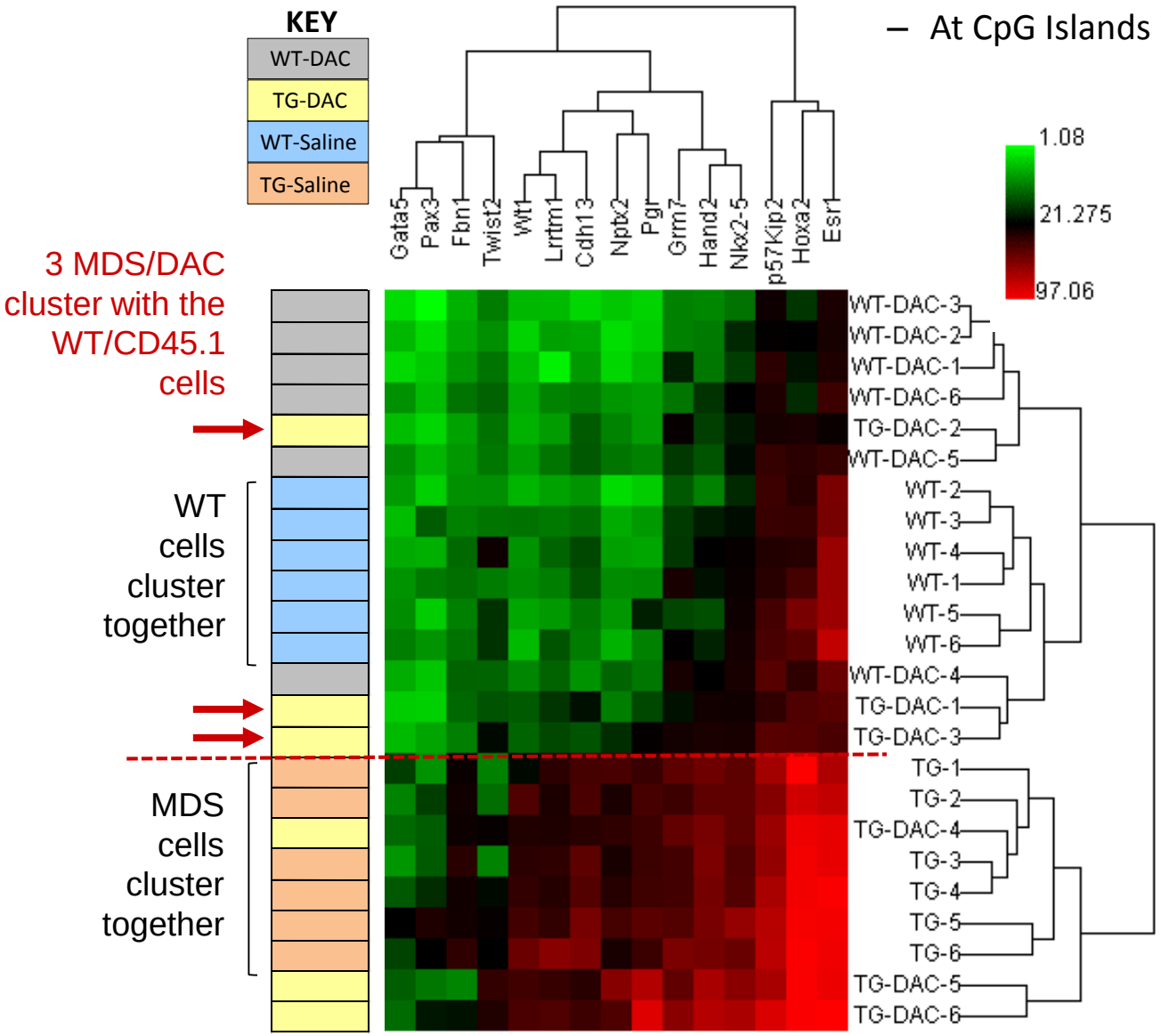
DNA methyltransferase 1 inhibitors (DNMT1i) and MDS

- 2 of 3 FDA approved agents for MDS are DNMT1i.
  - At diagnosis, irrespective of blast percentage, a large fraction (>80%) of hematopoiesis is derived from the MDS clone.
    - ➢ After successful therapy, majority of hematopoiesis derived from normal hematopoietic precursors.
    - ➢ Thus, pre/post treatment analysis of DNMT1i effect may be studying vastly different cell populations.

Walter, et al., NEJM, 2012
Saunthararajah, ASH, 2013

Demethylation of MDS gene set with DAC treatment

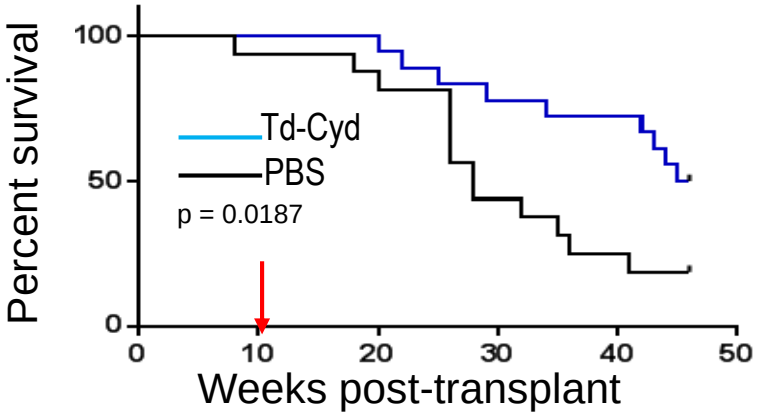
- Bisulfite pyrosequencing in sorted cells:
  - At CpG Islands in MDS-associated genes.



Collaborator: JP Issa

Novel DNMT1 inhibitors

- T-dCyd and 5’-aza-T-dCyd—thiol substituted cytosine analogs
- Potent DNMT1i *in vitro*
- Effective in *NHD13* MDS model



Ghanwa Khawaja

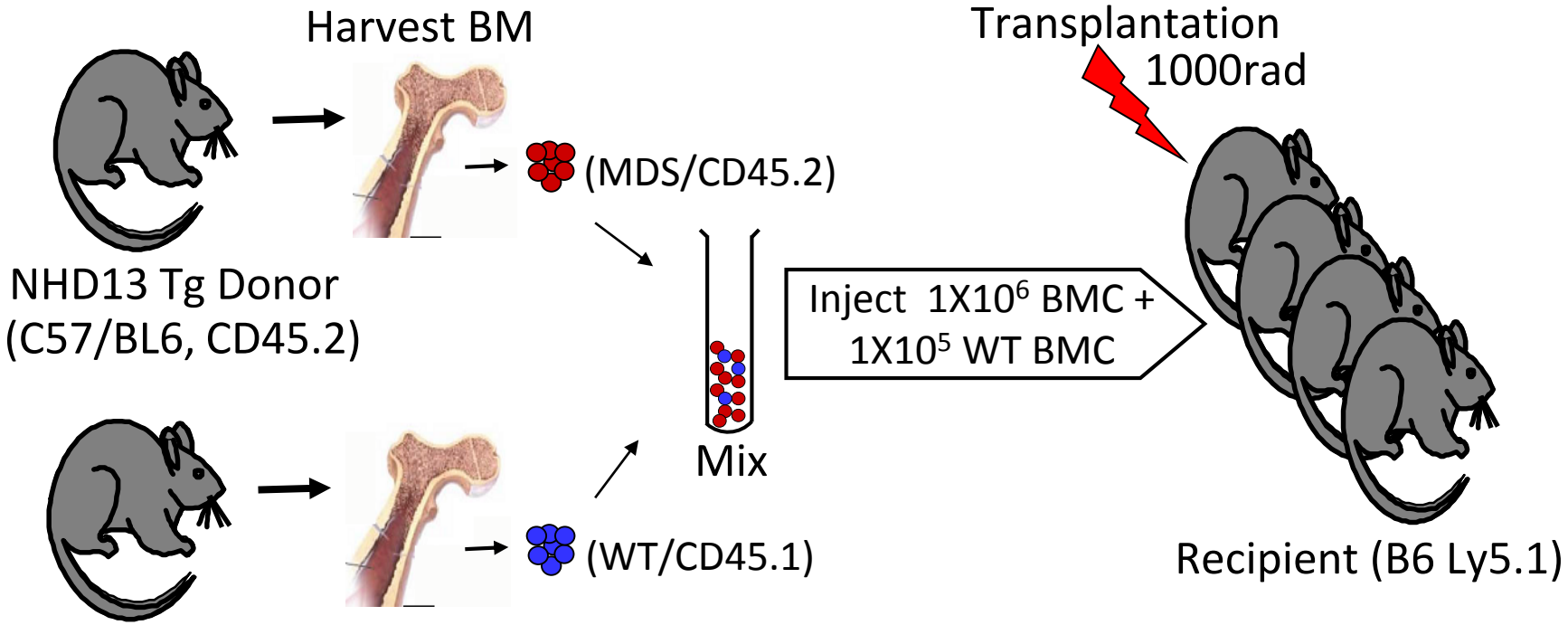
- Both agents now in phase I clinical trial

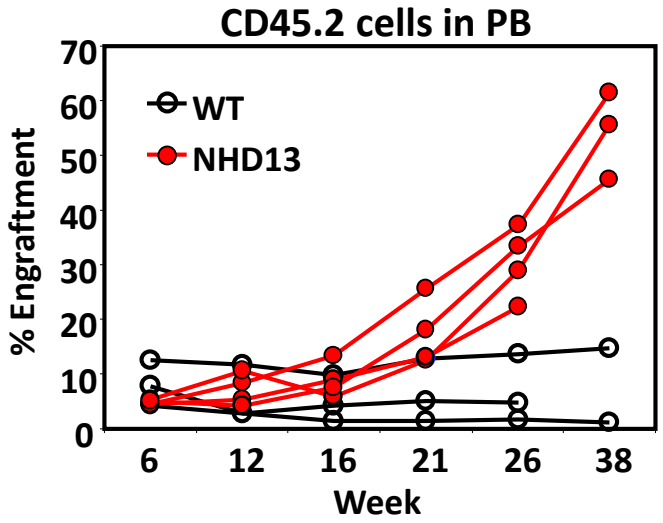
Collaborators: Michael Difilipantonio, Jim Doroshow, NCI NExT

https://clinicaltrials.gov/ct2/show/NCT02423057

Unpublished

Generation of chimeric mice with MDS

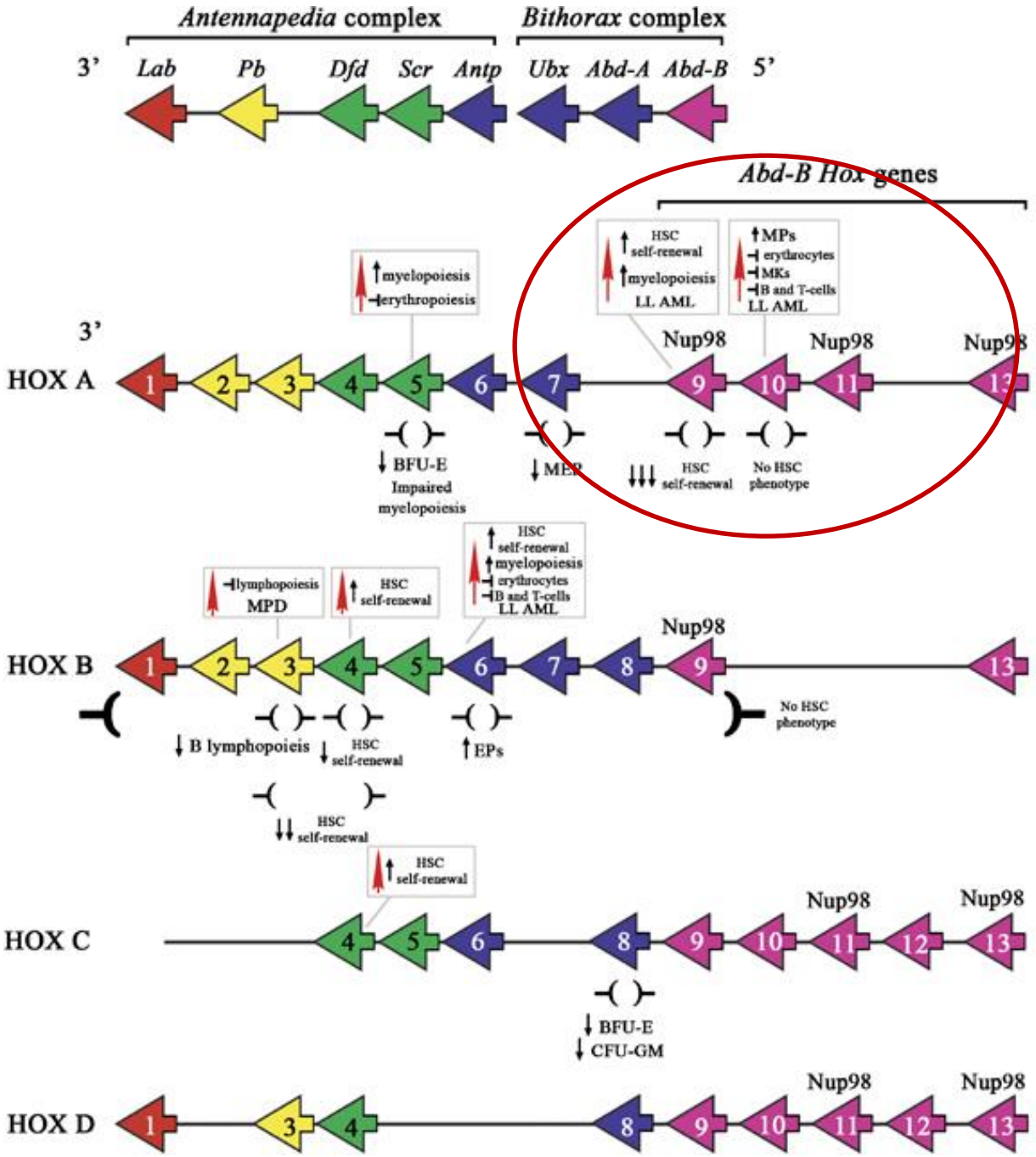




CBCs

- -Macrocytosis
- -Anemia
- -Neutropenia

HOX genes and hematopoiesis

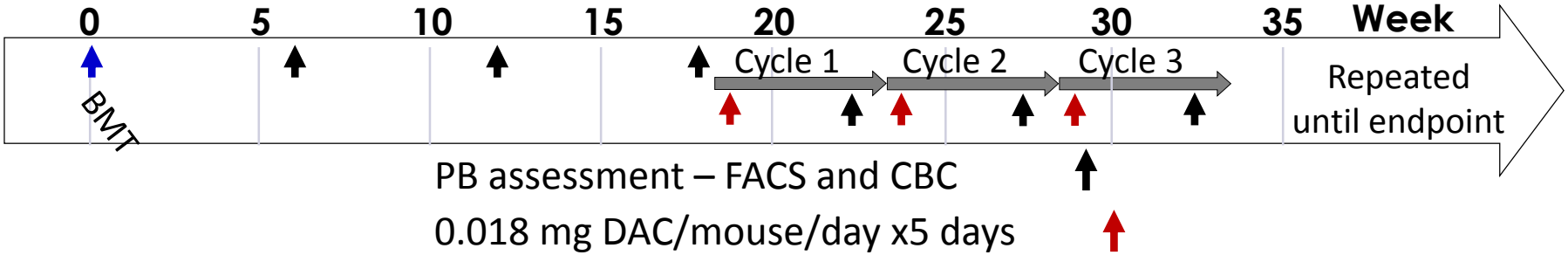


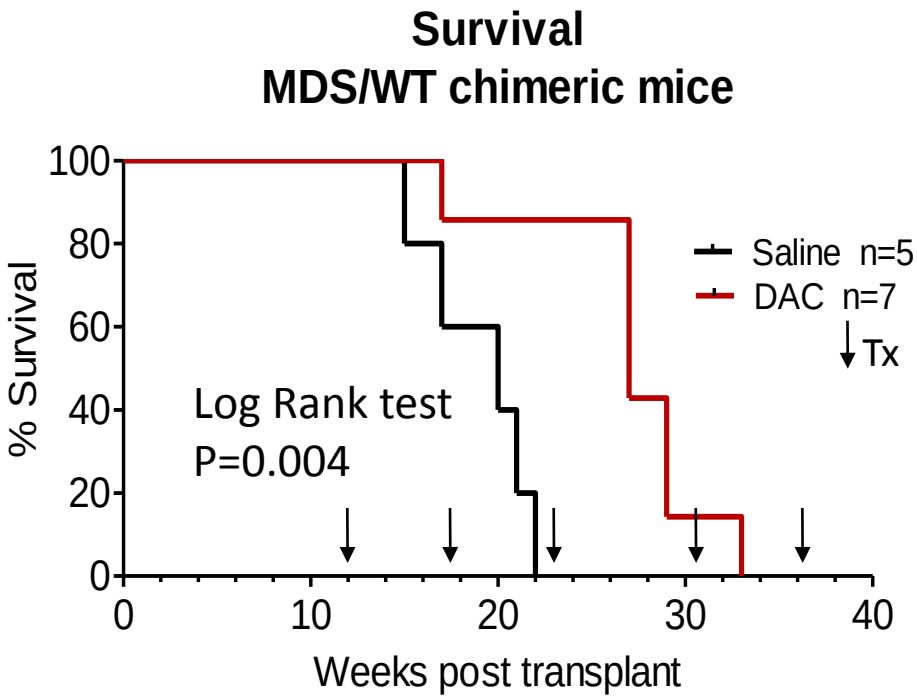
1) *HOXA7-11* often co-regulated="*HOXA* cluster".
2) "*HOXA* cluster" genes expressed in HSPCs, and down-regulated as cells mature.

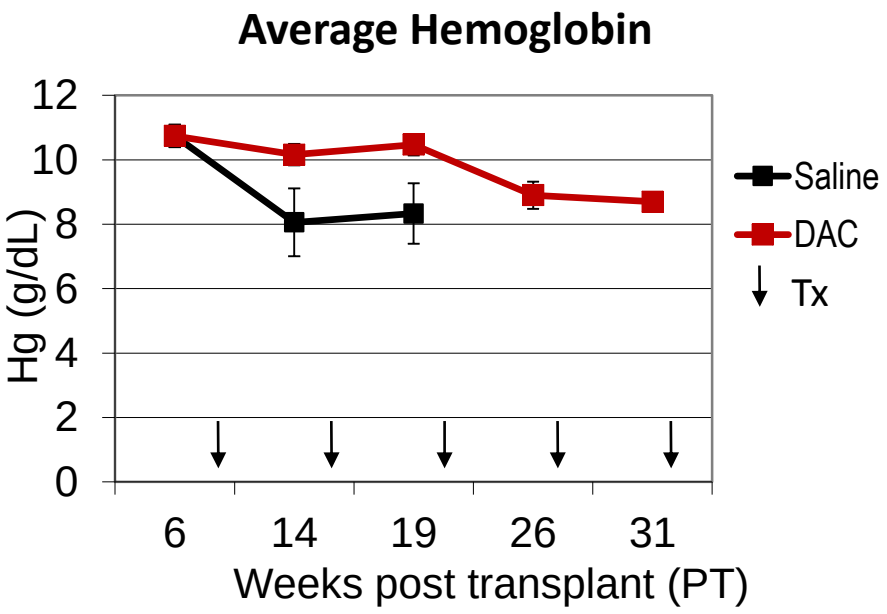
(Argiropoulos and Humphries, Oncogene, 2007)

Increased survival and hematologic improvement in chimeric MDS mice treated with DAC

Treatment schedule







Increased survival and hematologic improvement in chimeric MDS mice treated with DAC

Treatment schedule