Molecular Analysis of del(5q) t-MN: Identification of Haploinsufficient Tumor Suppressor Genes

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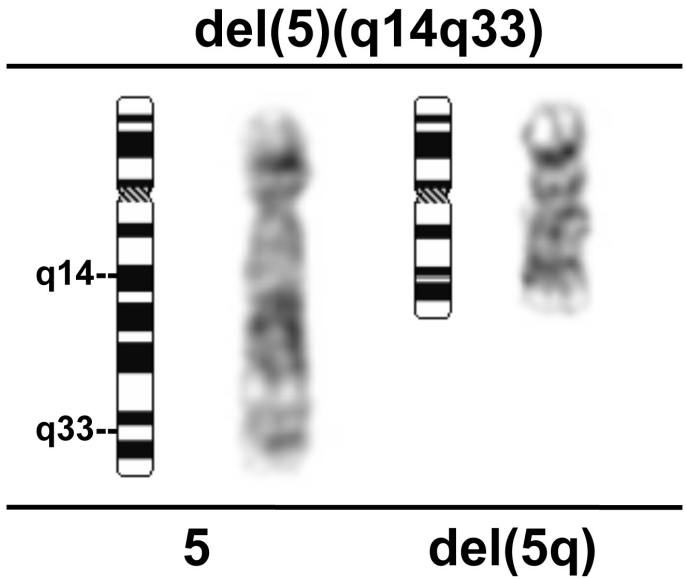


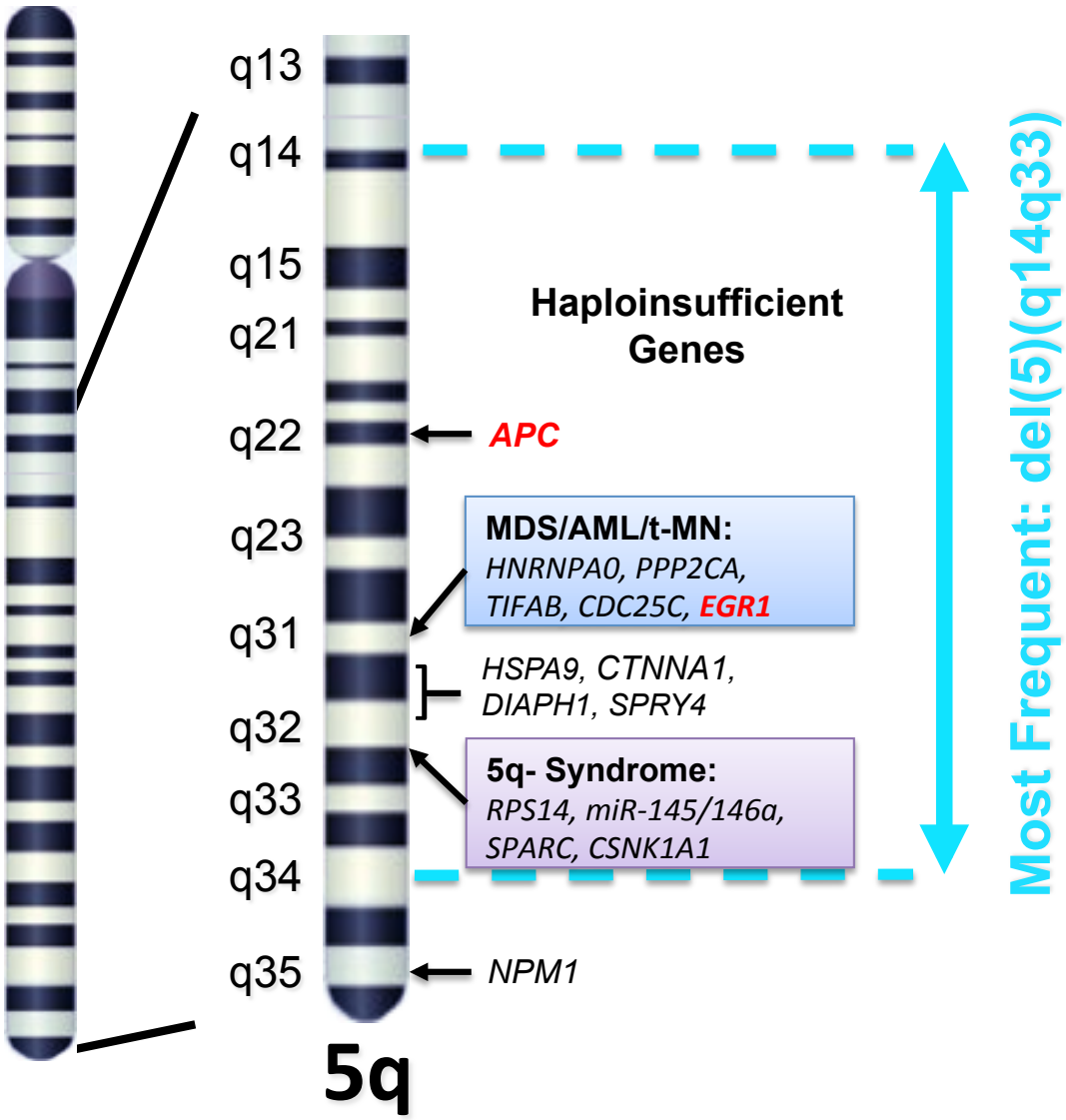
THE UNIVERSITY OF CHICAGO MEDICINE
Comprehensive Cancer Center

NCI Designated Comprehensive Cancer Center

Outline of Talk

- Review of Tumor Suppressor Genes on 5q – haploinsufficiency of multiple genes
- Modeling t-MN in mice
- Role of aberrant WNT signaling in both the BM niche and HSCs in driving myeloid leukemogenesis
- Therapeutic targeting of Wnt Signaling
- Role of cytotoxic therapy - alters both the BM niche and HSCs

Haploinsufficiency Drives del(5q) Disorders



- Two Commonly Deleted Regions (CDRs):
  - 5q31.2
  - 5q33.1
- No homozygous mutations
- Many genes in CDRs are expressed at ~50% levels (Haploinsufficient)
- Loss of multiple genes contribute to disease:

Phenotype	Gene(s)
Anemia	*RPS14, APC*
Megakaryocytic Dysplasia	*miR145/146a*
HSC expansion	*EGR1, APC, CSNK1A1*
Clonal Dominance	*CSNK1A1*

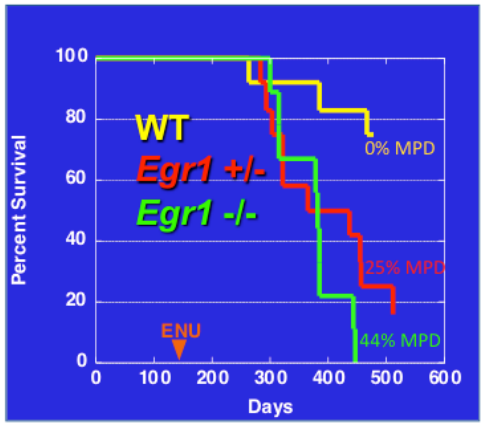
Critical Genes in del(5q) t-MNs

EGR1 (5q31.2)

Transcriptional regulator of *CDKN1A* (*p21*), *TP53*

HSC quiescence and retention in BM niche

***Egr1*$^{+/-}$ mice, treated with ENU, develop a MPD with ineffective erythropoiesis.**



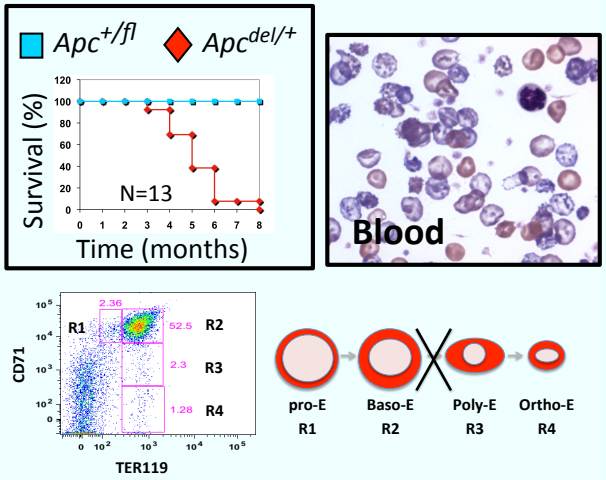
Joslin et al., Blood 110: 719, '07

APC (5q22.2)

WNT signaling cascade

Regulates mitosis and cell migration

***Mx1-Cre+, Apc*$^{del/+}$ mice develop MDS, fatal macrocytic anemia**

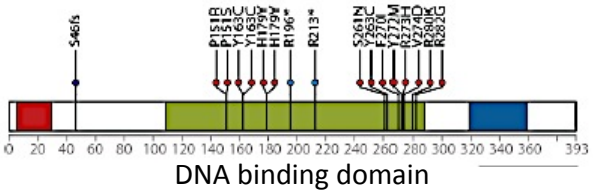


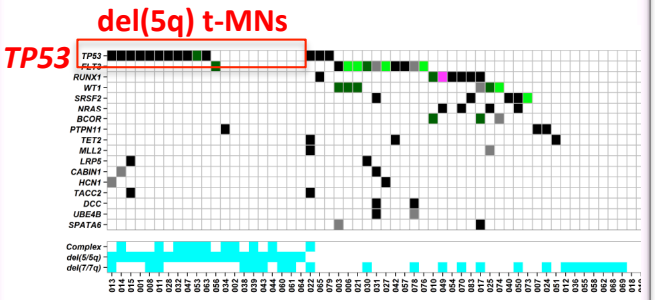
Wang et al. Blood 115:3481, 2010

TP53 (17p13.1)

Cell cycle arrest, DNA repair and apoptosis

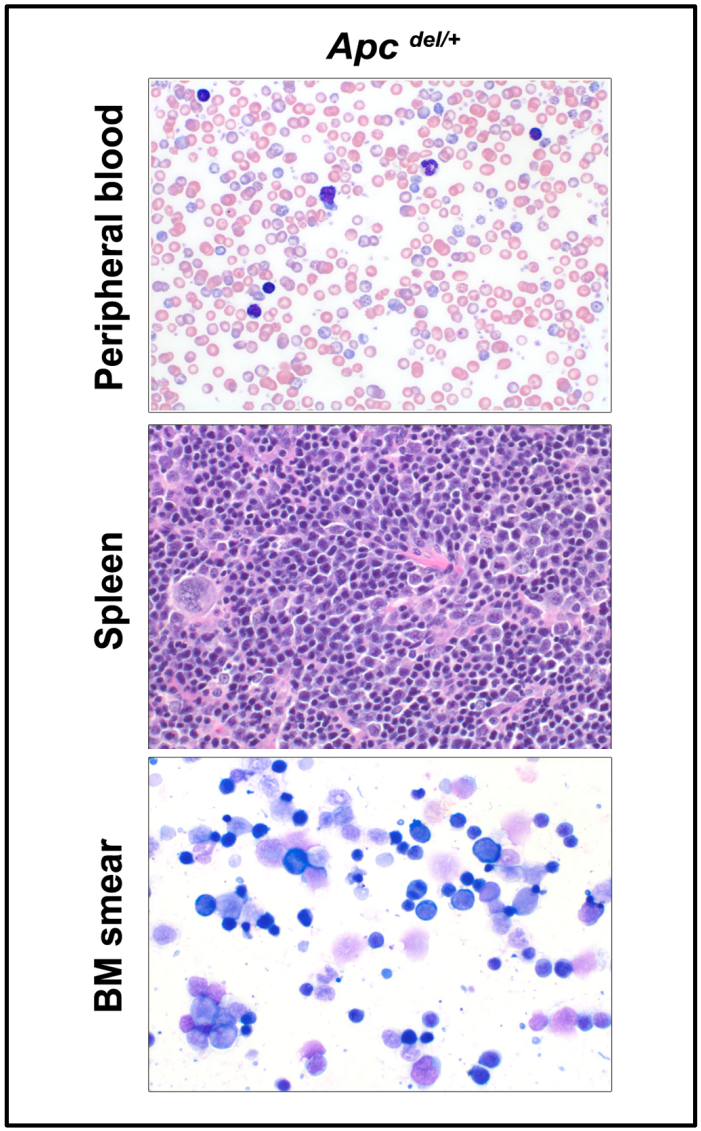
Loss of *TP53* activity observed in up to 80% of t-MN with a del(5q).





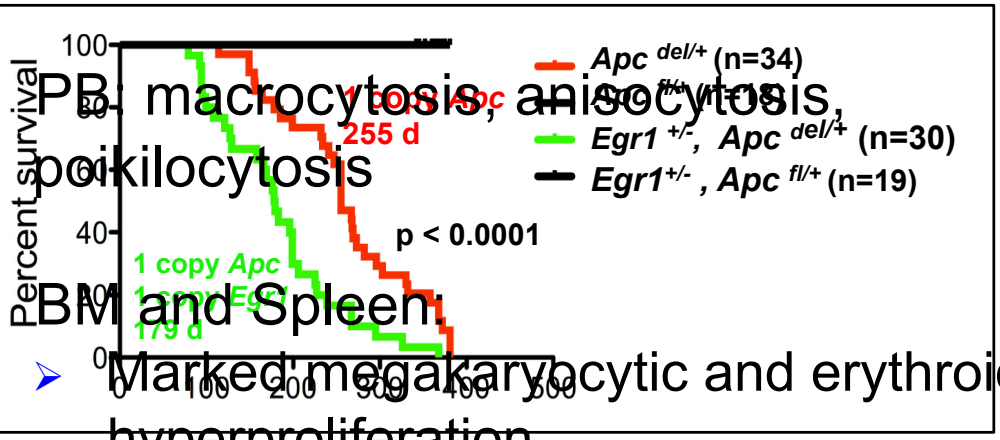
Unpublished data J. Nakitandwe, J. Zhang, M. Le Beau, J. Downing

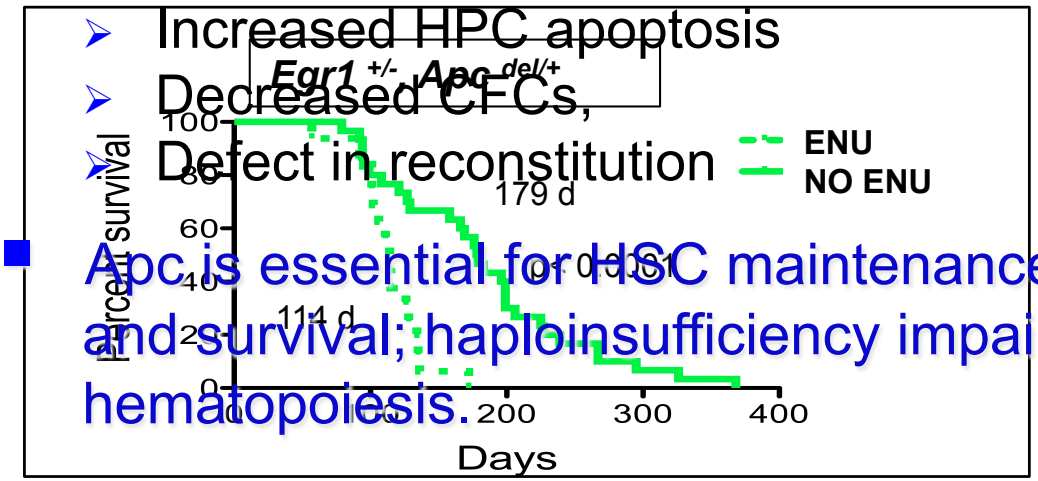
MDS in *Apc*$^{del/+}$ Mice



Wang et al. Blood 115:3481, 2010
Stoddart et al., Blood 123: 228, 2014
Stoddart et al., Blood 123:1069, 2014

- Haploinsufficiency for *Egr1* or *Tp53* accelerates MDS in *Apc*$^{del/+}$ mice
- Mice succumb to severe anemia
- PB: macrocytosis, anisocytosis, poikilocytosis
- BM and Spleen:
  - Marked megakaryocytic and erythroid hyperproliferation
  - Dyserythropoiesis, differentiation block and with partial loss of
- END: accelerates MDS in *Apc*$^{del/+}$ mice, and with partial loss of *Egr1*$^{+/-}$ and/or *Tp53*$^{+/-}$
  - Increased LSKs
  - Increased HPC apoptosis
  - Decreased CFCs,
  - Defect in reconstitution
- Apc is essential for HSC maintenance and survival; haploinsufficiency impairs hematopoiesis.





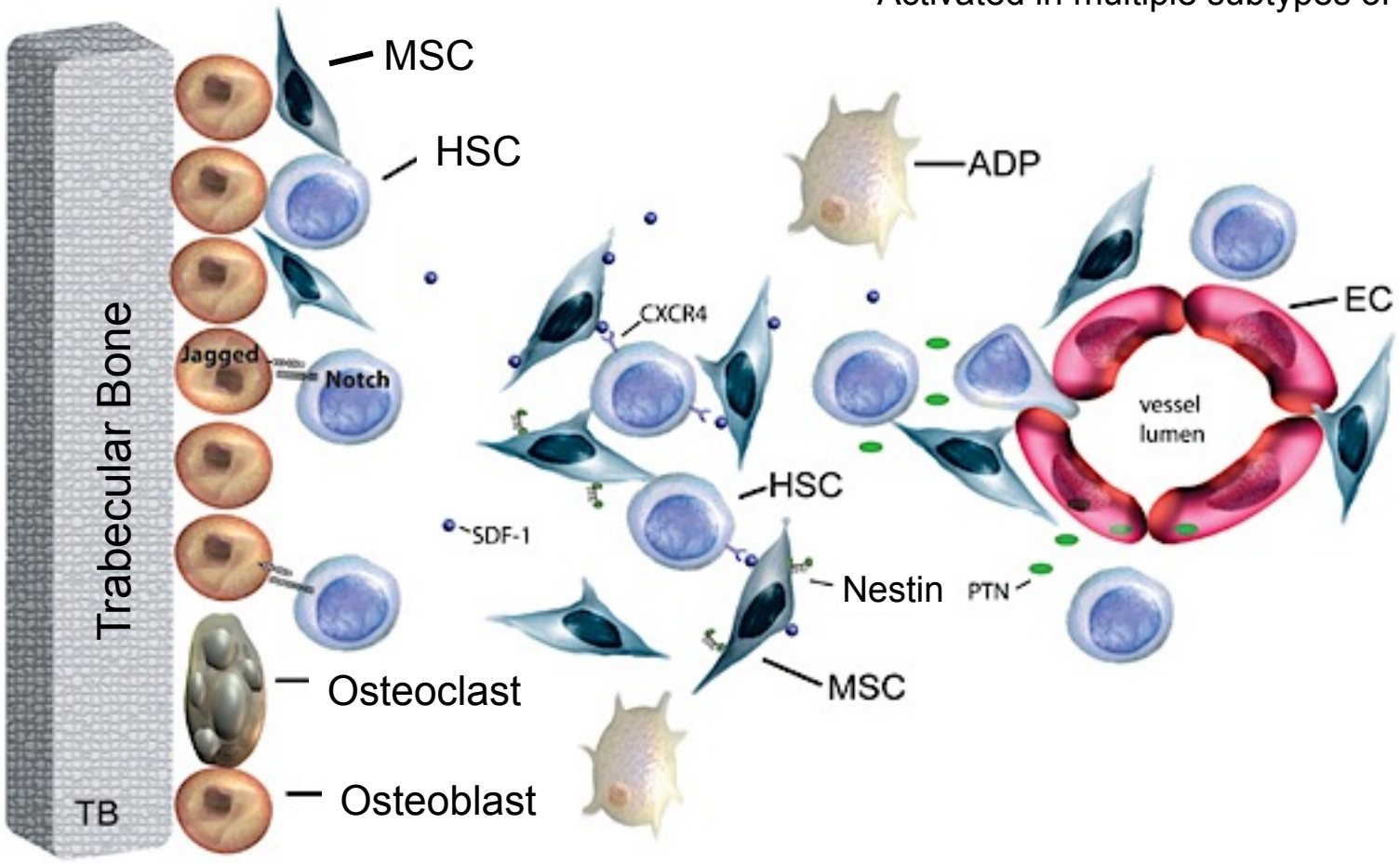
Role of WNT Signaling in Hematopoiesis

BM Microenvironment – WNT Signaling:

- Regulates differentiation/function of osteoblasts
- Constitutive: leads to AML in mice *(Ctnnb1 osb)*
- Activated in osteoblasts, MSCs in some MDS/AML

HSPCs – WNT Signaling:

- Essential for self-renewal and quiescence
- Exquisitely sensitive to levels of signaling
- Involved in development of LICs
- Activated in multiple subtypes of MDS/AML



MDS is Induced by an *Apc*-Haploinsufficient BM Microenvironment

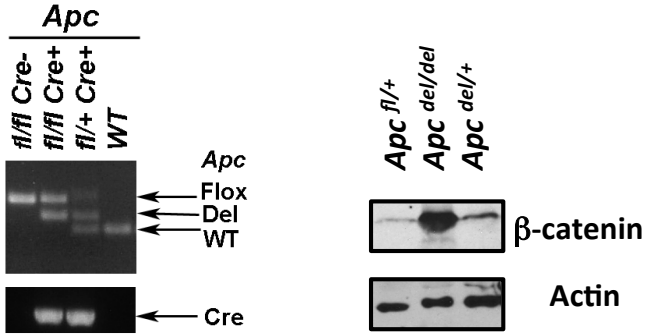
BM cells:

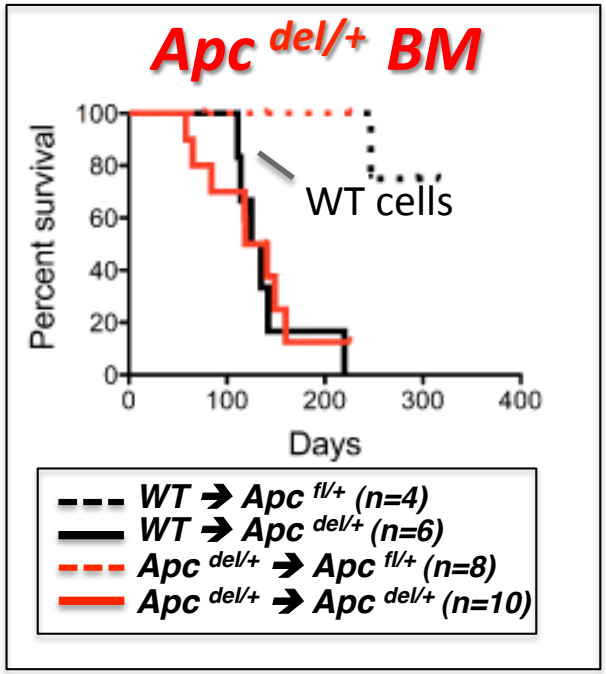
1. ***Apc$^{del/+}$***
2. ***Egr1$^{+/-}$***
3. ***Tp53$^{+/-}$***
4. ***WT***

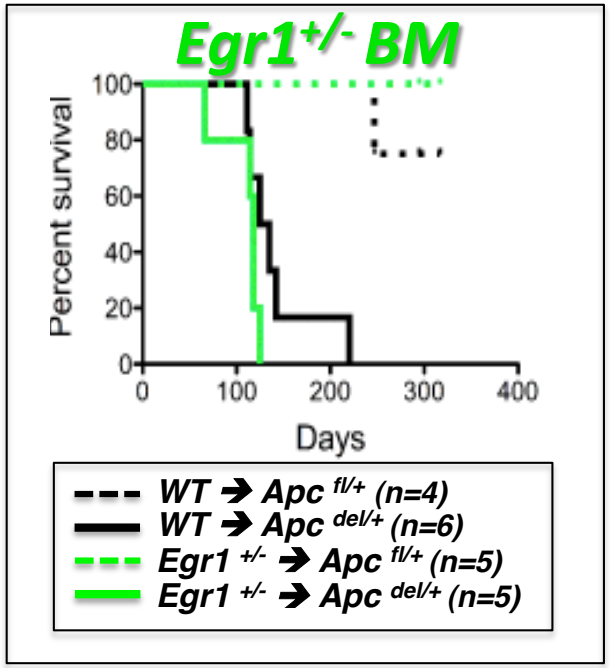
Recipients:

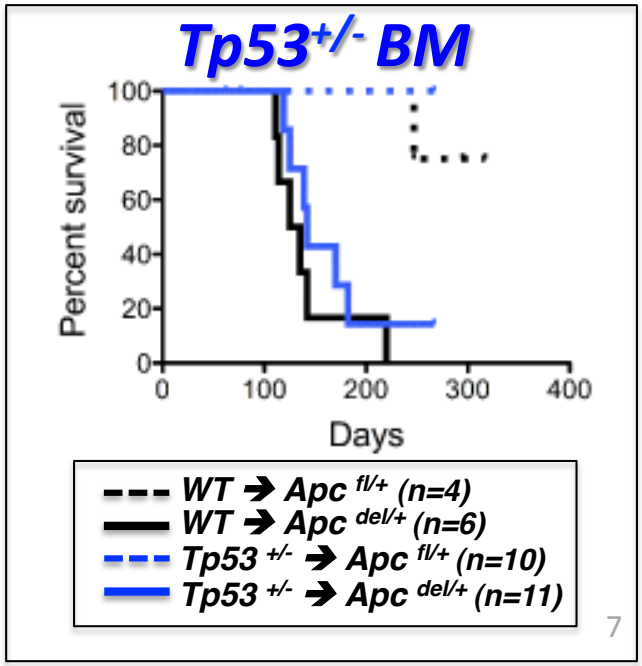
1. ***Apc$^{del/+}$***
2. **WT**

***Apc* is deleted and β-catenin levels are increased in mesenchymal stromal cells**





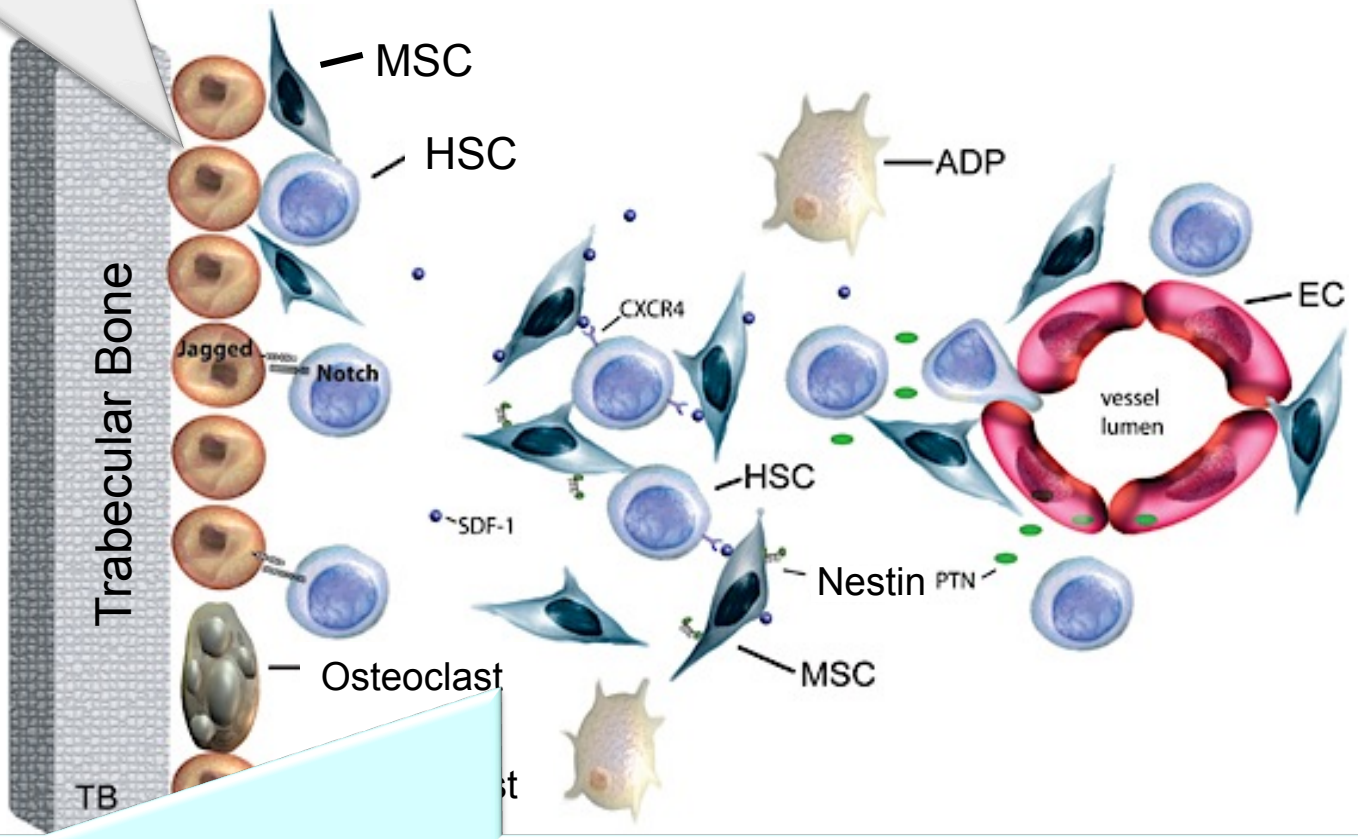




Conclusions-1

Niche:

1. *Apc*$^{del/+}$ -induced MDS is HSPC extrinsic, implicating aberrant WNT signaling in the niche in myeloid disorders.



2. Cytotoxic therapy accelerates the onset of myeloid diseases, likely impacting the niche and HSPCs.

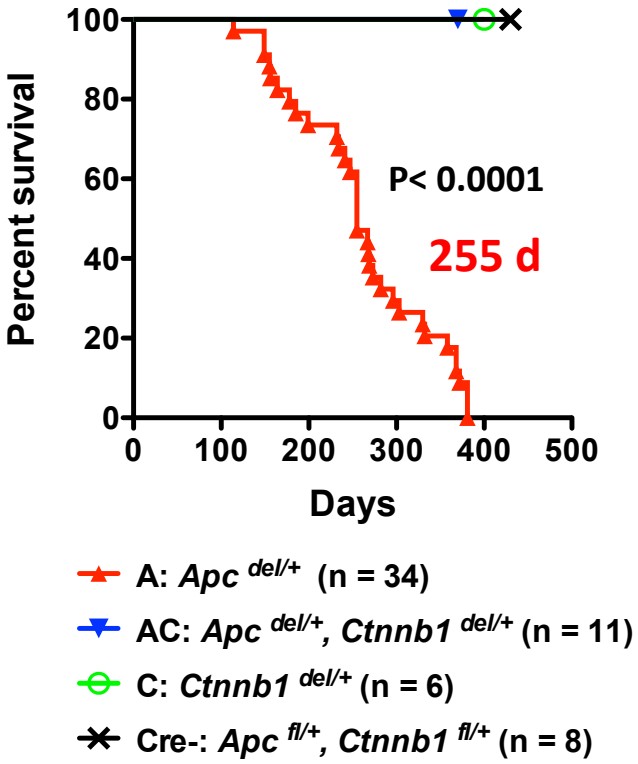
Is *Apc* loss Mediated by the WNT Pathway:

Heterozygous loss of β-catenin gene (*Ctnnb1*) is sufficient to prevent development of fatal MDS in $Apc^{del/+}$ mice

A

$Apc^{del/+}$, $Ctnnb1^{del/+}$ *Cre+* heterozygote

B



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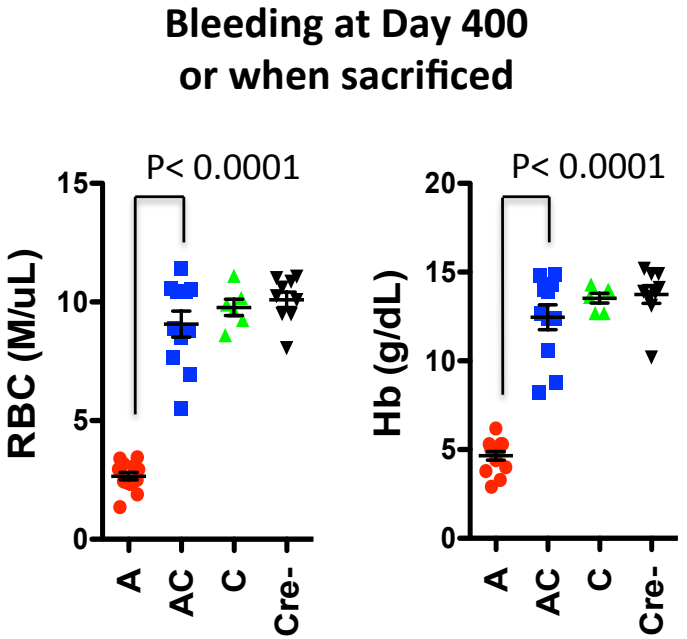
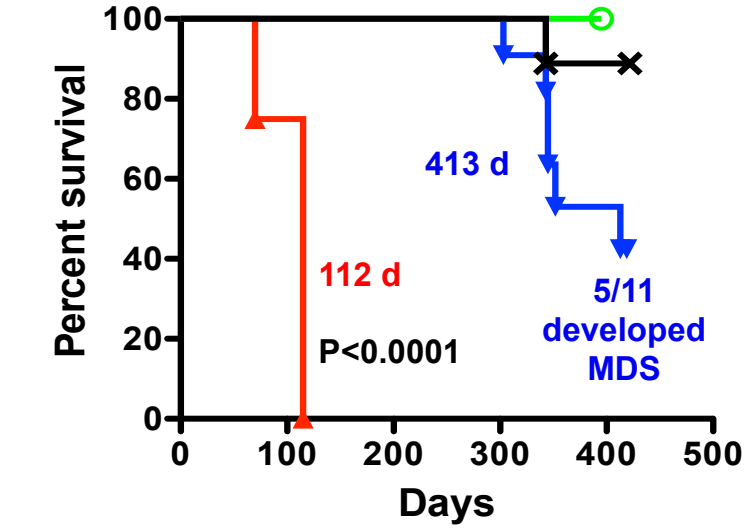


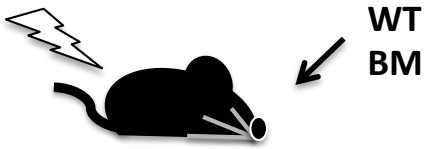
Transplantation of WT Bone Marrow:

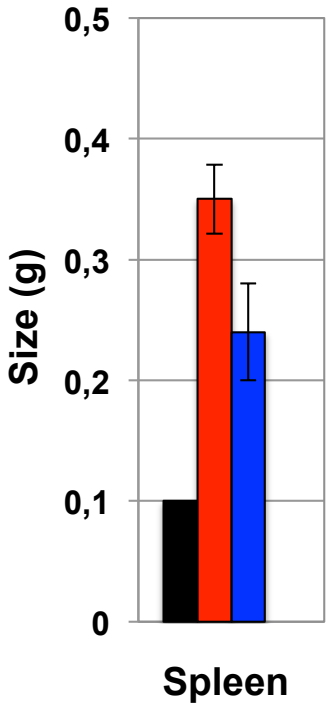
Cell extrinsic loss of one copy of *Ctnnb1* delays disease development in $Apc^{del/+}$ mice

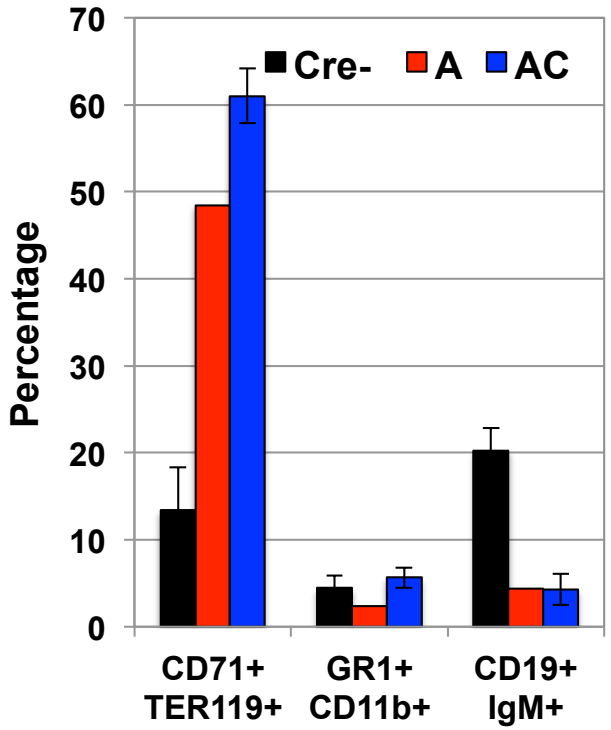


recipients

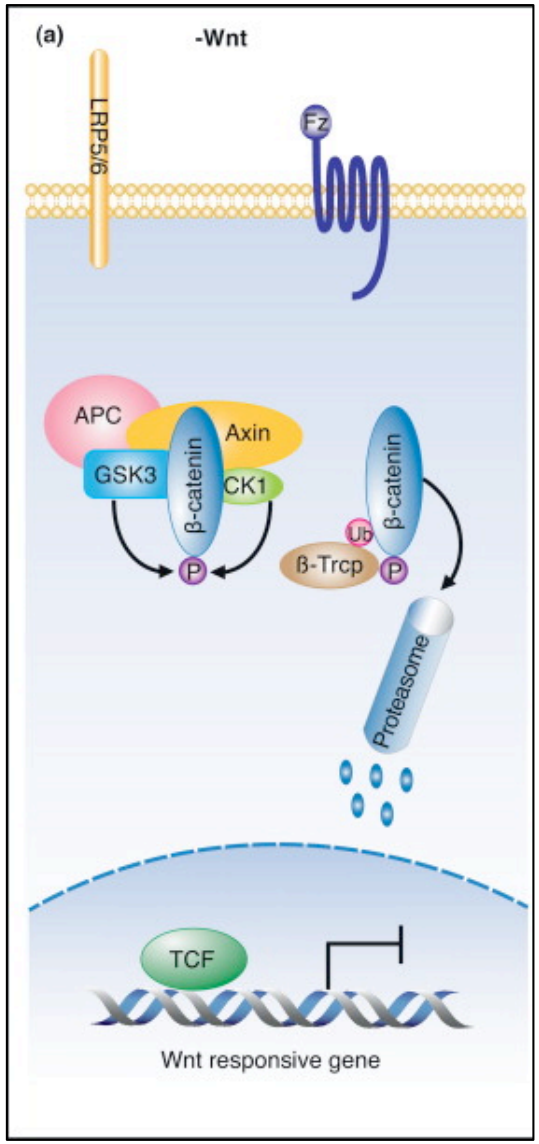
- A: *Apc* $^{del/+}$ (n = 4)
- AC: *Apc* $^{del/+}$, *Ctnnb1* $^{del/+}$ (n = 11)
- C: *Ctnnb1* $^{del/+}$ (n = 3)
- Cre- : *Apc* $^{fl/+}$ *Ctnnb1* $^{fl/+}$ (n = 9)



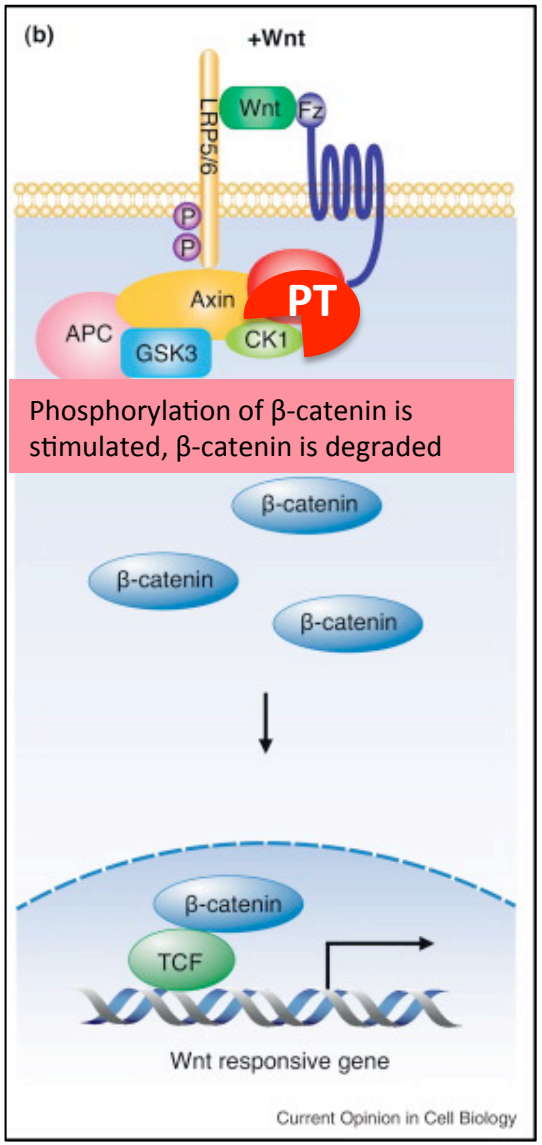




Pyrvinium Tosylate inhibits WNT activity by activating CK1α (CSNK1A1)



- **In the absence of WNT signaling, APC destruction complex proteins, CK1α (casein kinase)** and GSK3, phosphorylate (P) β-catenin in a coordinated fashion.
- β-catenin is then recognized by β-Trcp, an E3 ubiquitin ligase subunit and targeted for proteasomal degradation.
- **Pyrvinium binds to and activates CK1α, leading to β-catenin degradation and inhibition of WNT activity**



Pyrvinium Tosylate (PT), an Inhibitor of Wnt Signaling, Prevents Disease in *Apc$^{del/+}$* Mice

Apc $^{fl/+}$ Cre- (WT)

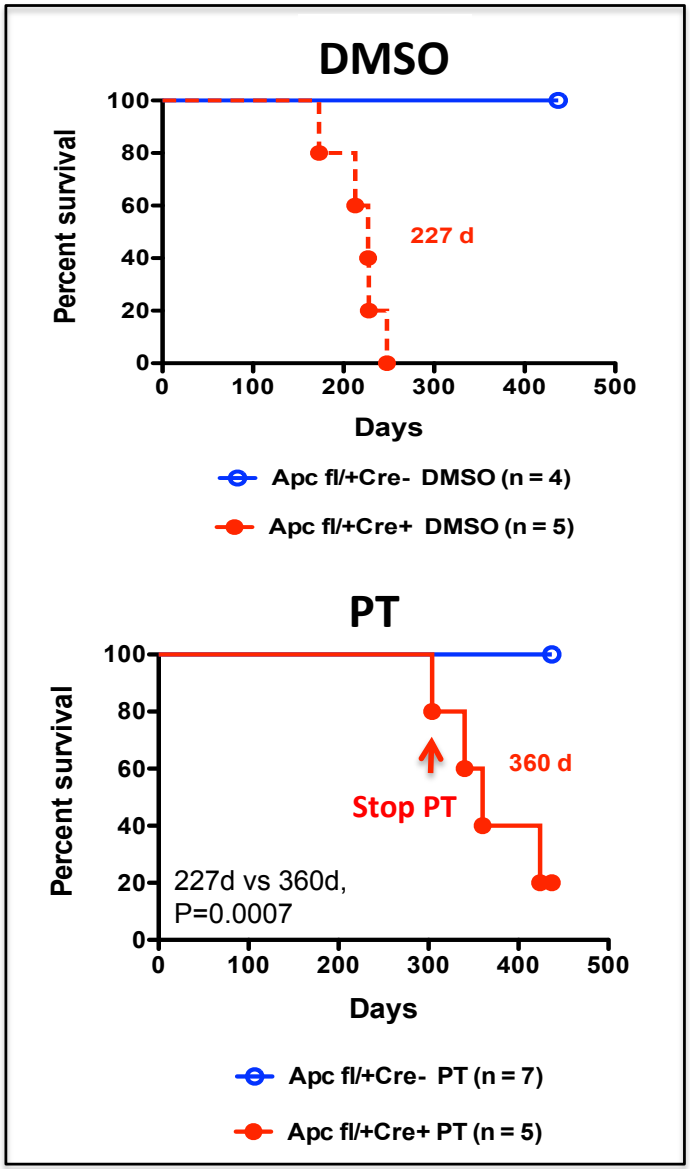
DMSO, 2x per week

PT (0.1 mg/kg),
2x per week, 30 wks

Apc $^{del/+}$ Cre+ (Het)

DMSO, 2x per week

PT (0.1 mg/kg),
2x per week, 30 wks

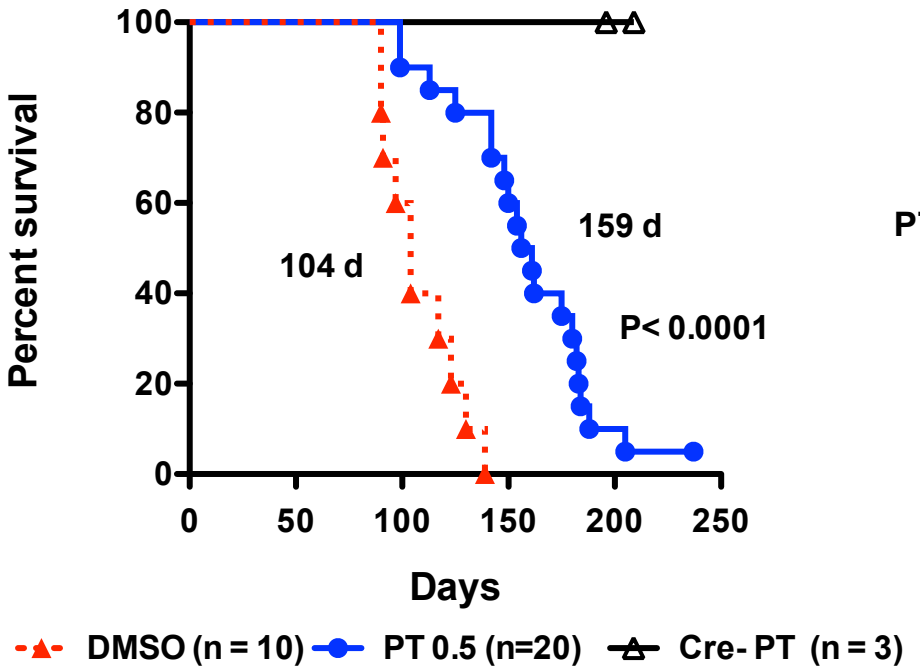


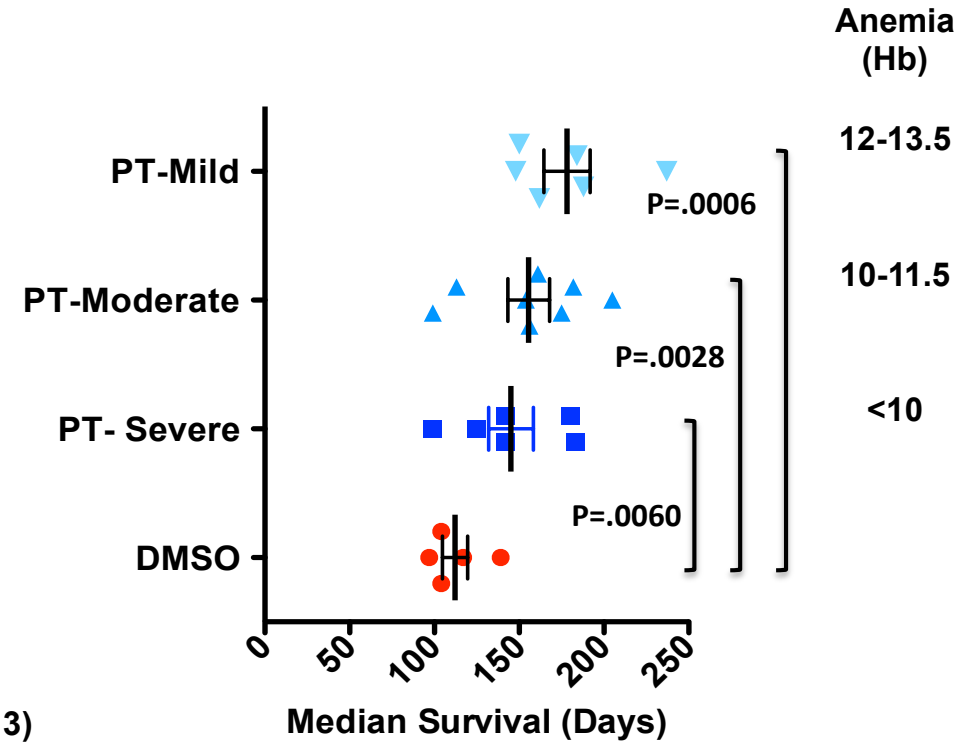
Pyrvinium Tosylate Prolongs Survival in *Apc*$^{del/+}$ Mice That Have Developed Anemia

Apc $^{del/+}$ Cre+ (Het)
with anemia

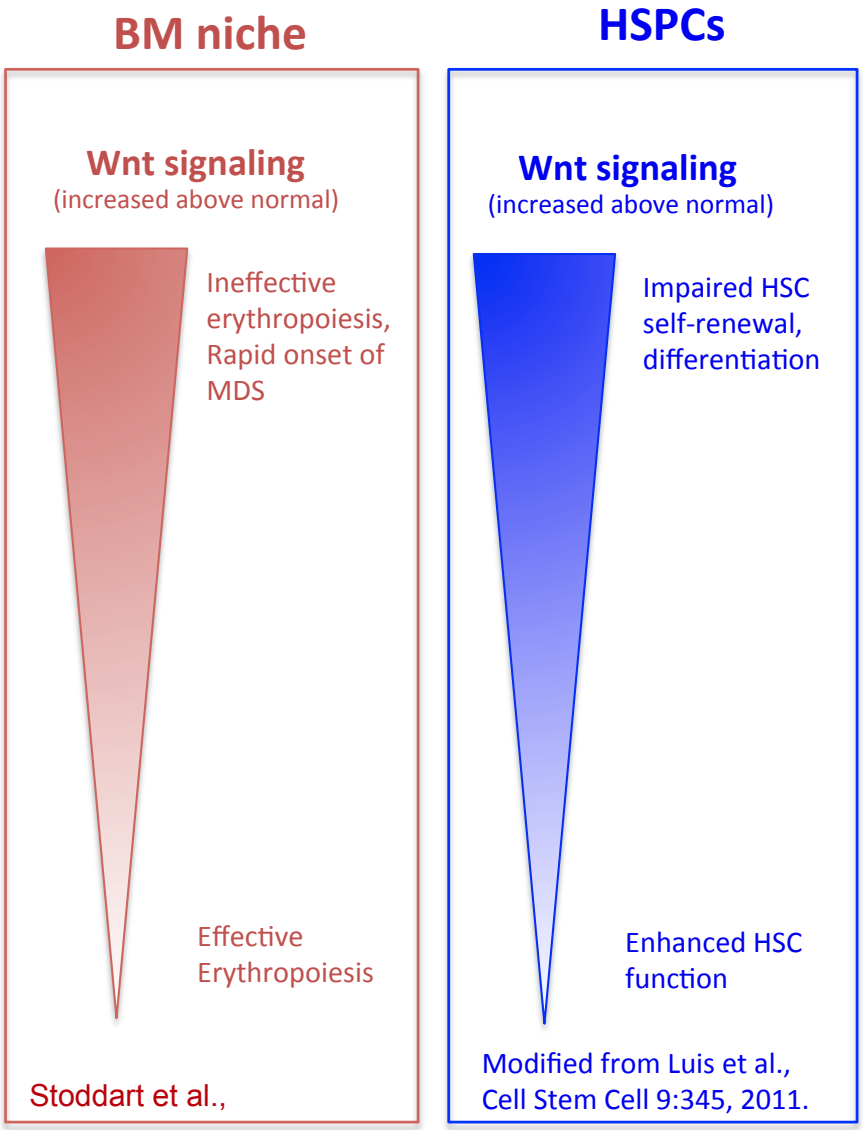
DMSO, 2x per week

PT (0.5 mg/kg),
2x per week, 30 wks





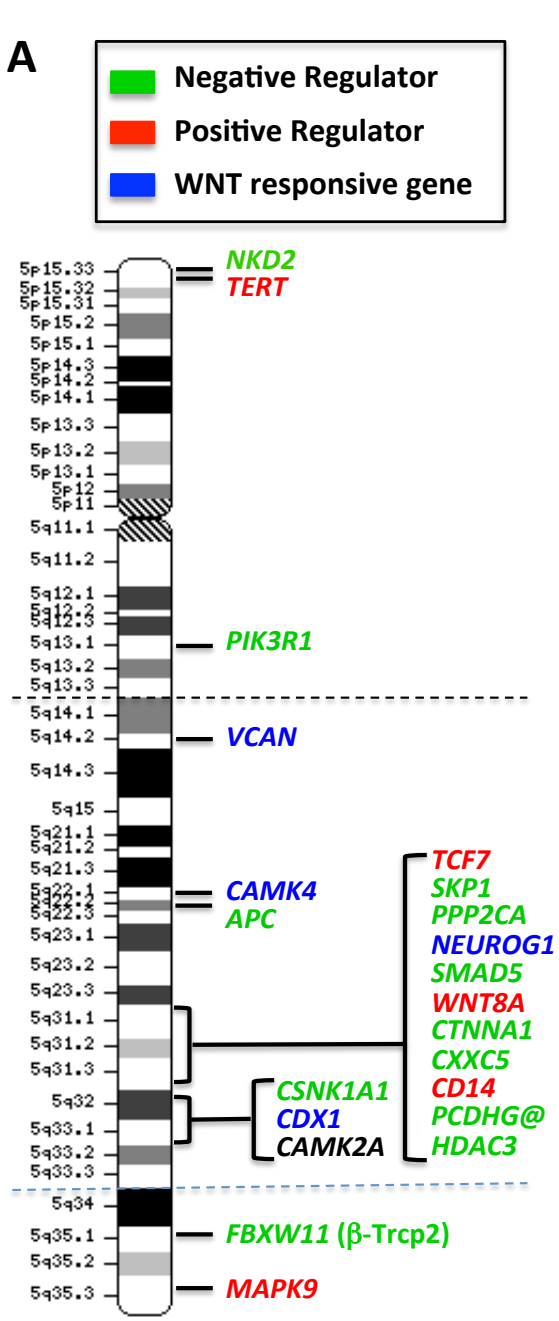
Canonical WNT Regulates Hematopoiesis in a Dosage-Dependent Fashion

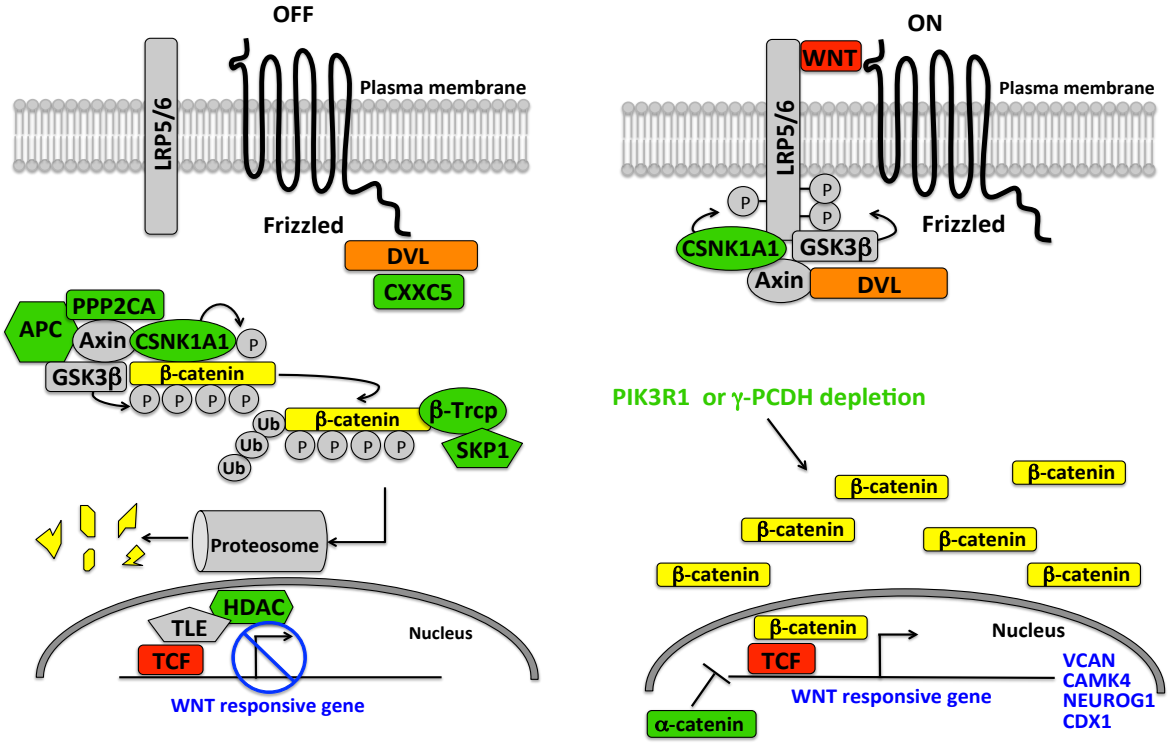


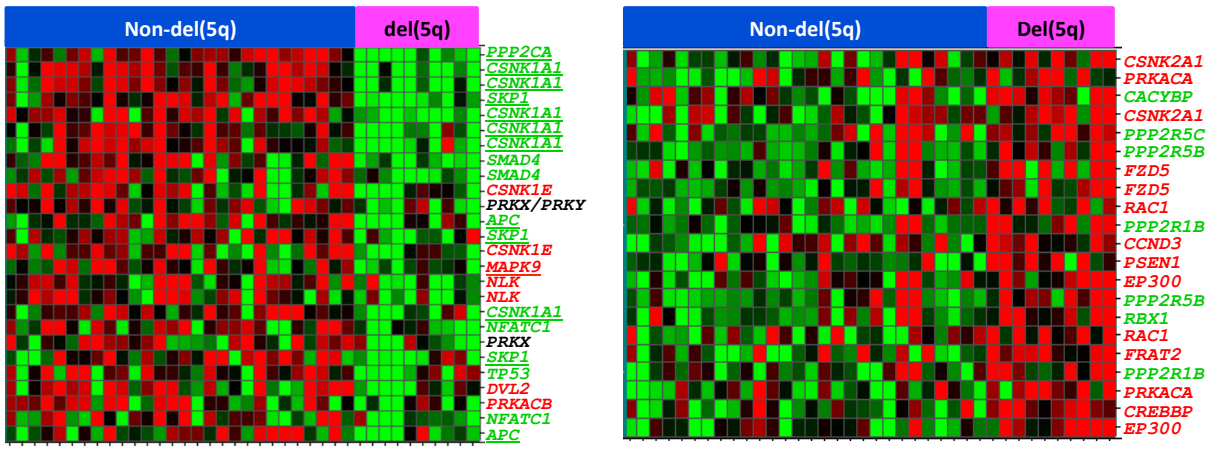
Conclusions-2

- Apc function in BM niche and HSPCs is through the WNT signaling pathways. Inhibition of WNT signaling using genetic models (*Ctnnb1*$^{+/-}$) rescues the MDS phenotype.
- Pharmacological inhibition of the WNT pathway (Pyrvinium tosylate) appears to prevent the development of MDS and anemia.
- Targeting the WNT pathway may be an effective therapeutic approach in human MDS, AML, and t-MN.

WNT Signaling Signature in del(5q) t-MNs





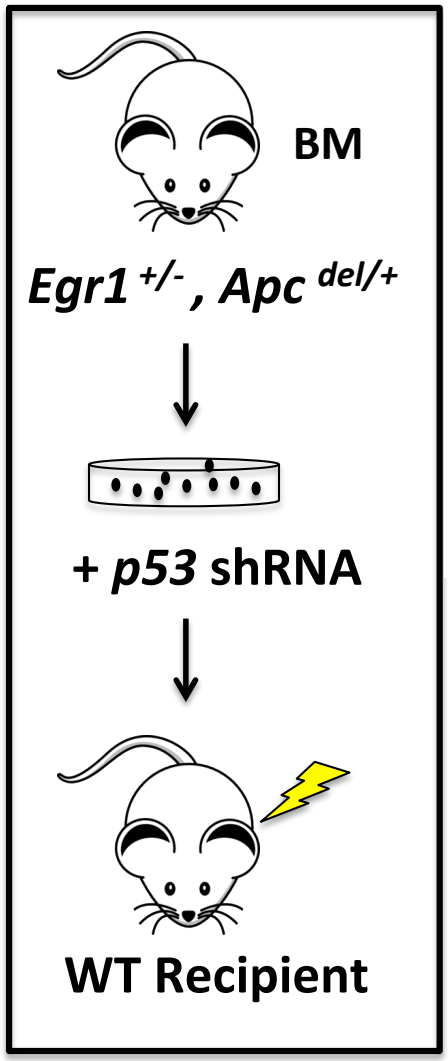


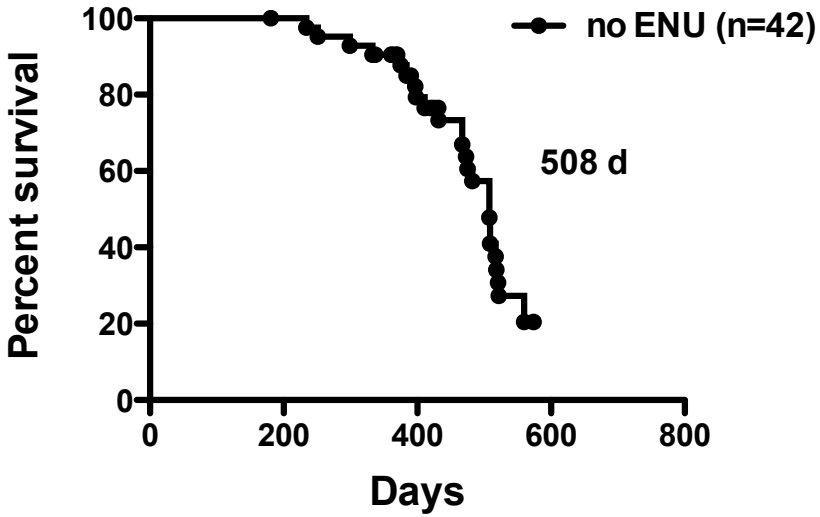
Negative regulators are DOWN, e.g., SMAD4, CSNK1A1, PP2A

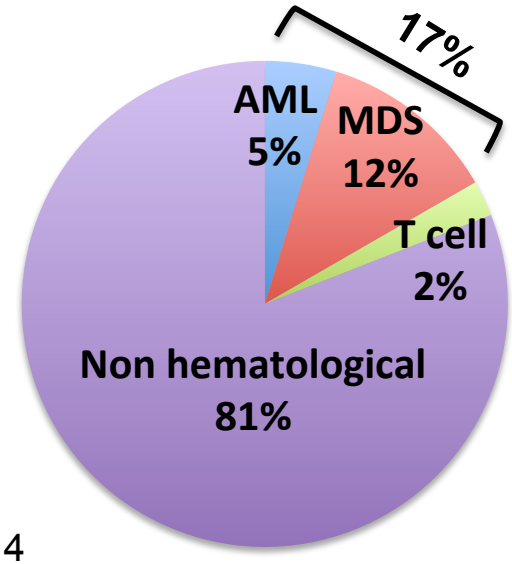
Positive regulators are UP, e.g., CREBBP, FZD5, CCND1

Stoddart et al., Blood 126:2899. 2015

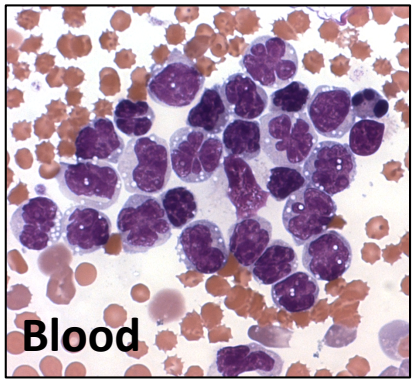
Loss of *p53*, in the Context of *Egr1* and *Apc* Haploinsufficiency, Promotes AML Development





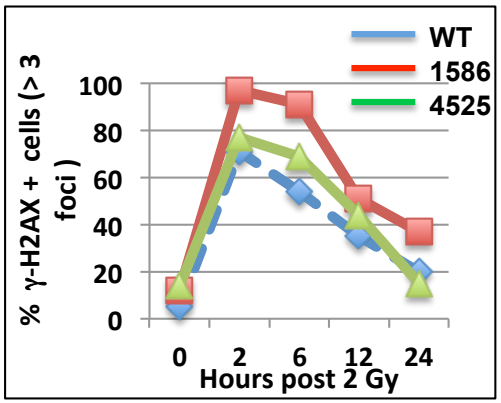


AMML, KIT+, MPO+, (234 days, #1586)



Genetic Instability:

- Complex karyotype
- Aberrant DSB response

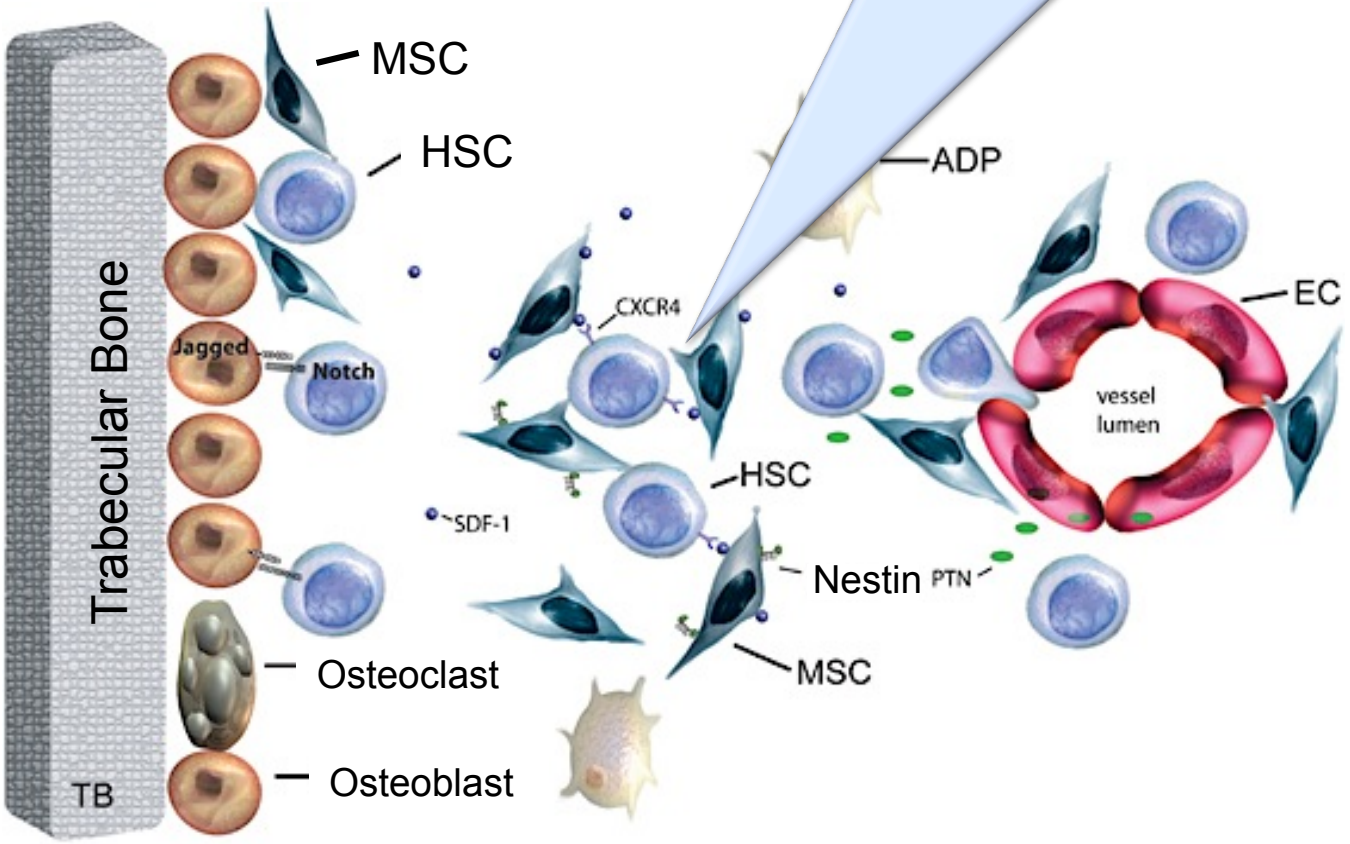


Stoddart et al., Blood 123:1069, 2014

Conclusions-3

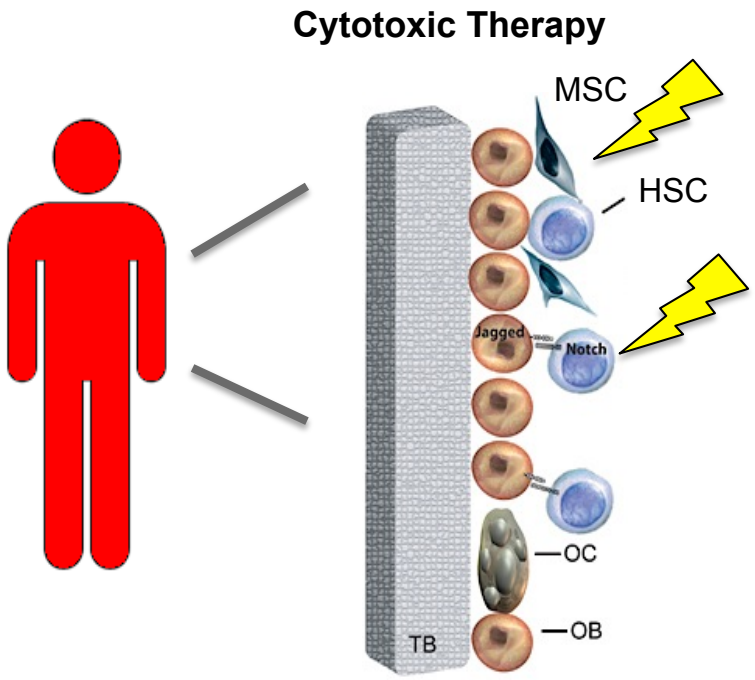
HSPCs:

1. Active WNT signature in del(5q) HSPCs
2. Haploinsufficiency of *Egr1* and *Apc* cooperate with loss of *Tp53* in HSPCs to induce myeloid disorders.



Effect of Cytotoxic Therapy

A



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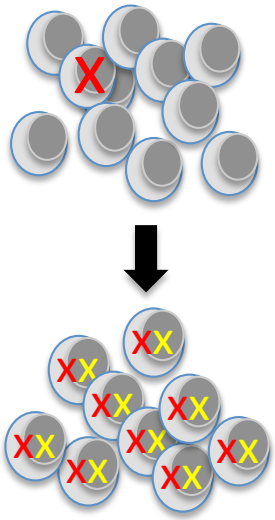


HSCs:

- Induces mutation(s) in HSCs.
- Setting of pre-existing mutations in HSCs, e.g., *TP53 (Wong TN et al. Nature 518:552, 2015)*
- Eliminates HSCs, but rare mutant stem cells survive.
- Permits acquisition of 2º mutations -> leukemogenesis.

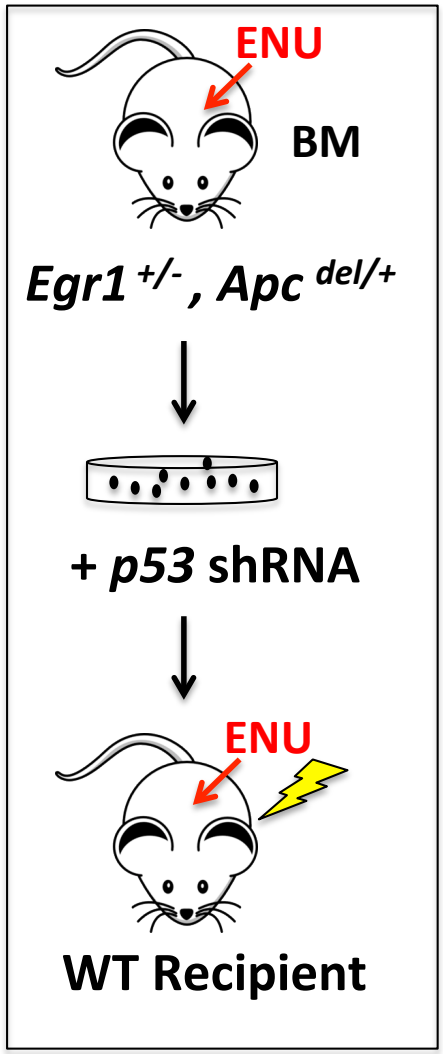
and/or

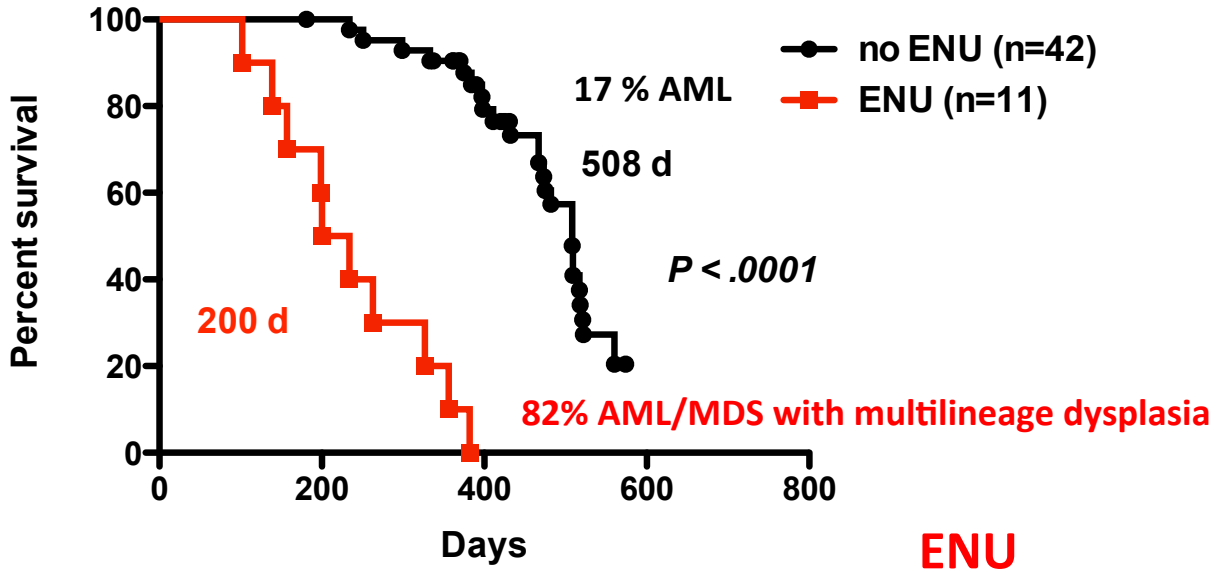
C

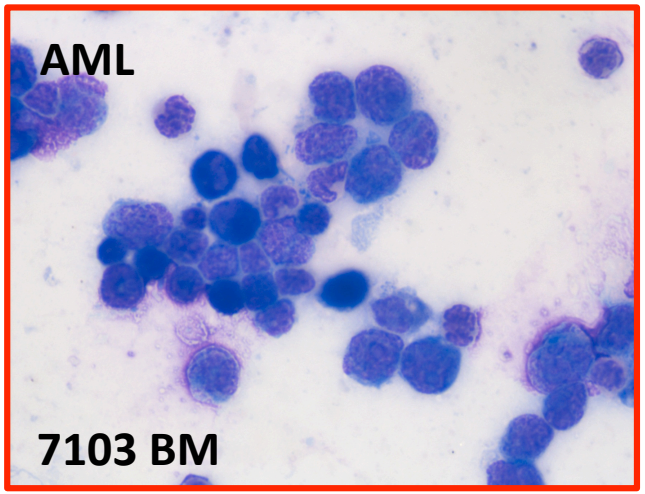
BM Niche:

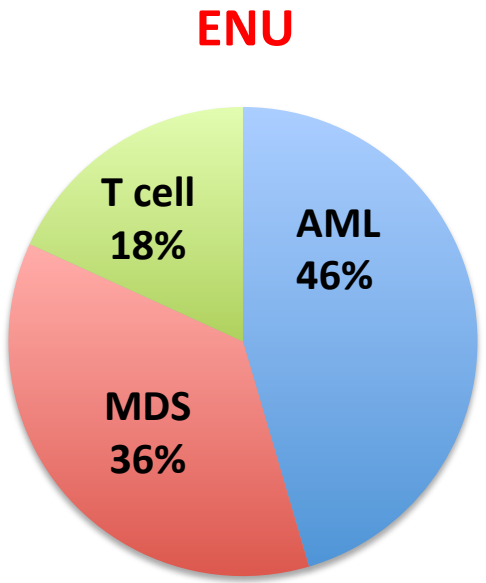
- Creates a permissive stromal cell niche enabling the survival and expansion of the rare mutant HSC clone
  - Epigenetic alterations?
  - Cytokine secretion?
  - Altered adhesion?
  - Changes in oxidative stress?

Alkylating Agent (ENU) Exposure Significantly Increases the Incidence of Disease

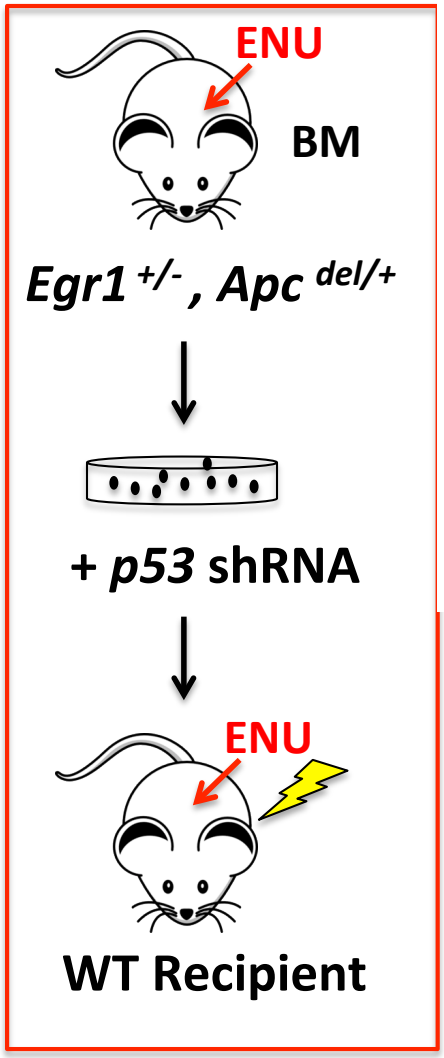


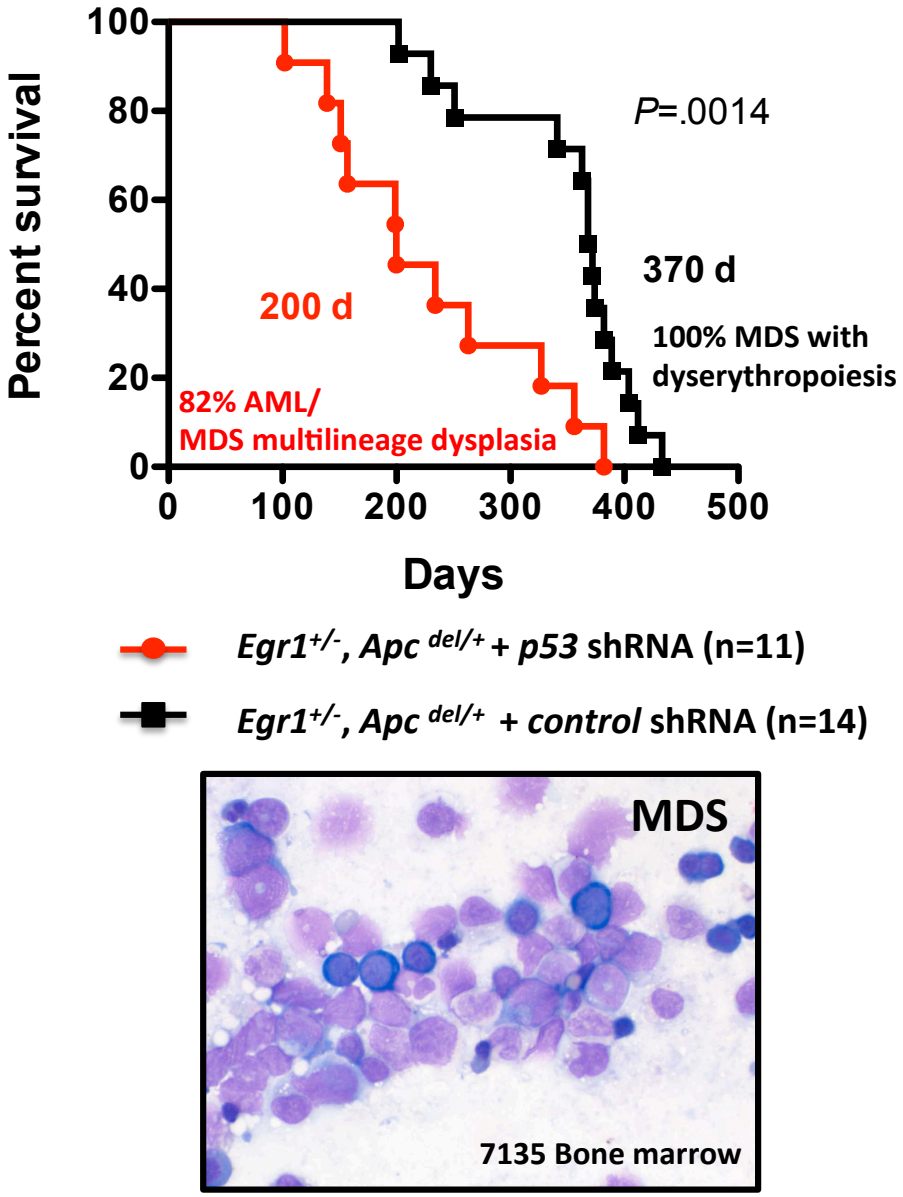


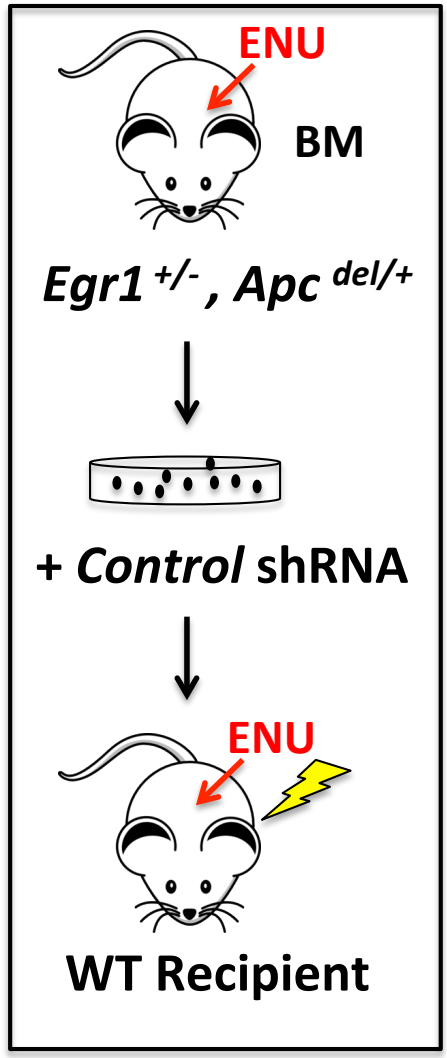




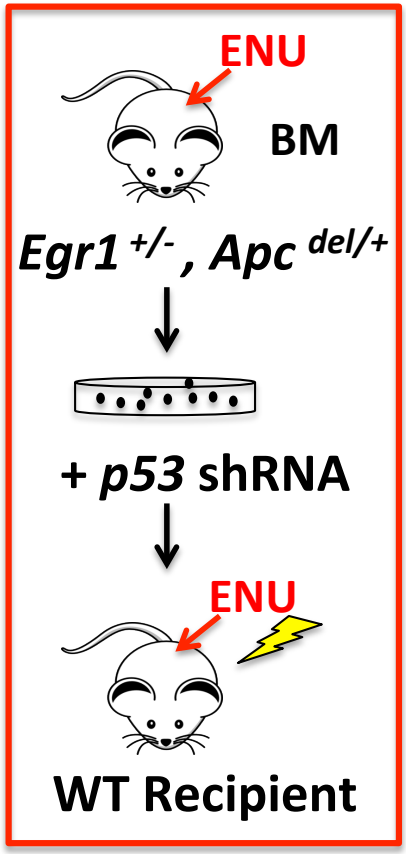
Loss of p53 is Critical for the Development of AML

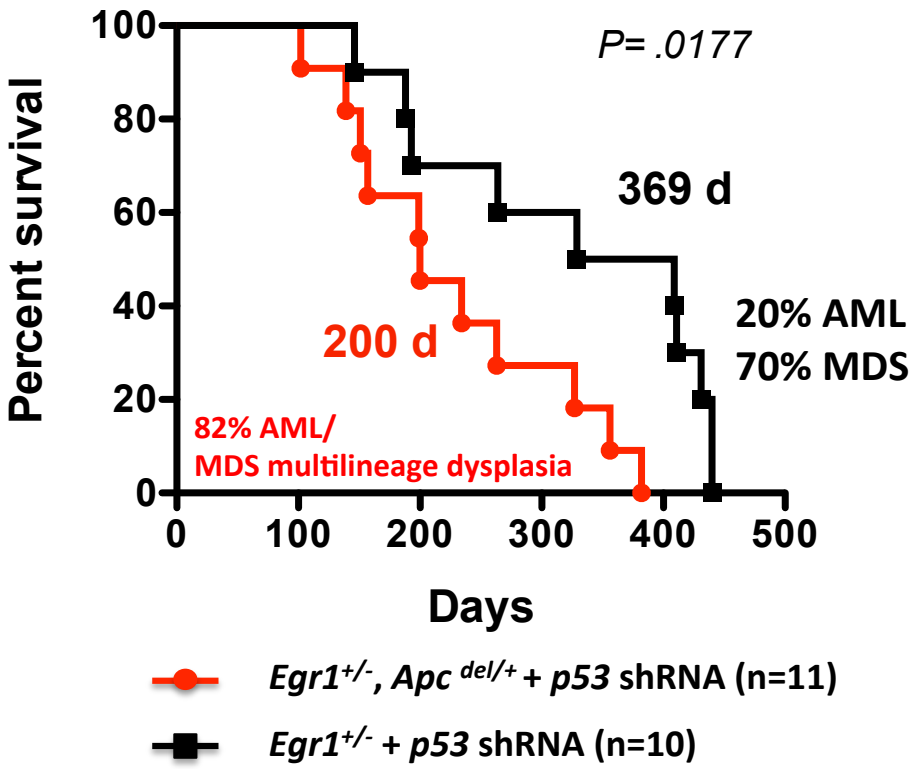


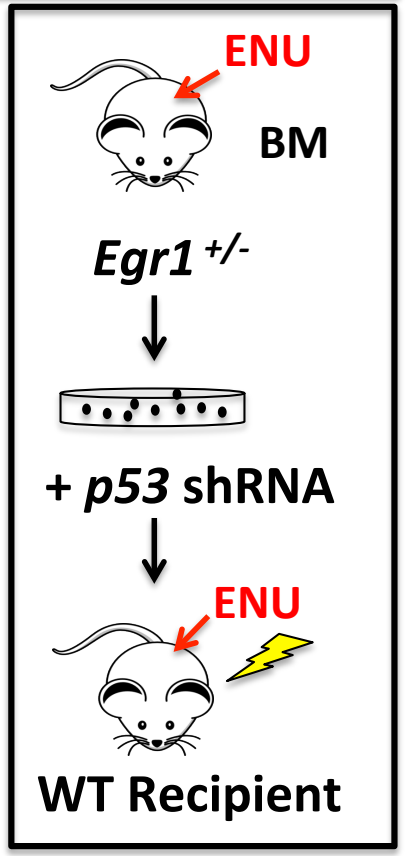


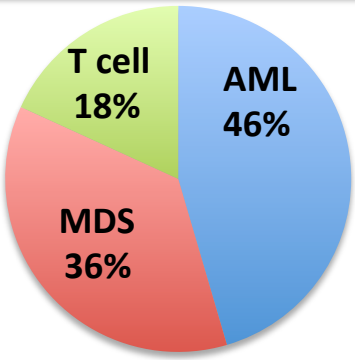


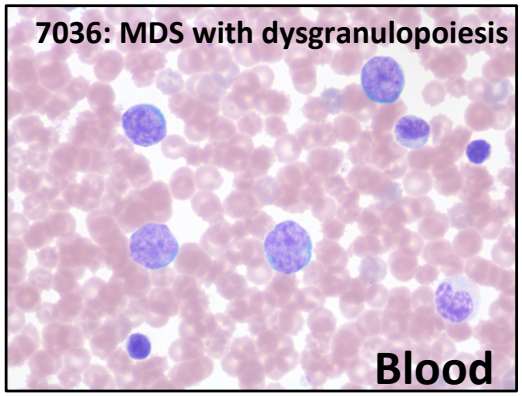
Increased Severity and Earlier Onset of Disease with Loss of More than one del(5q) Gene

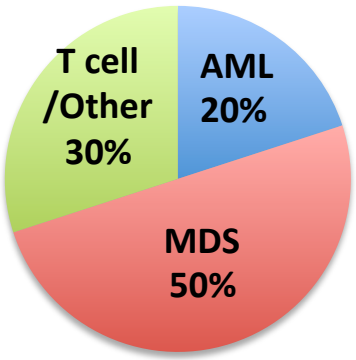




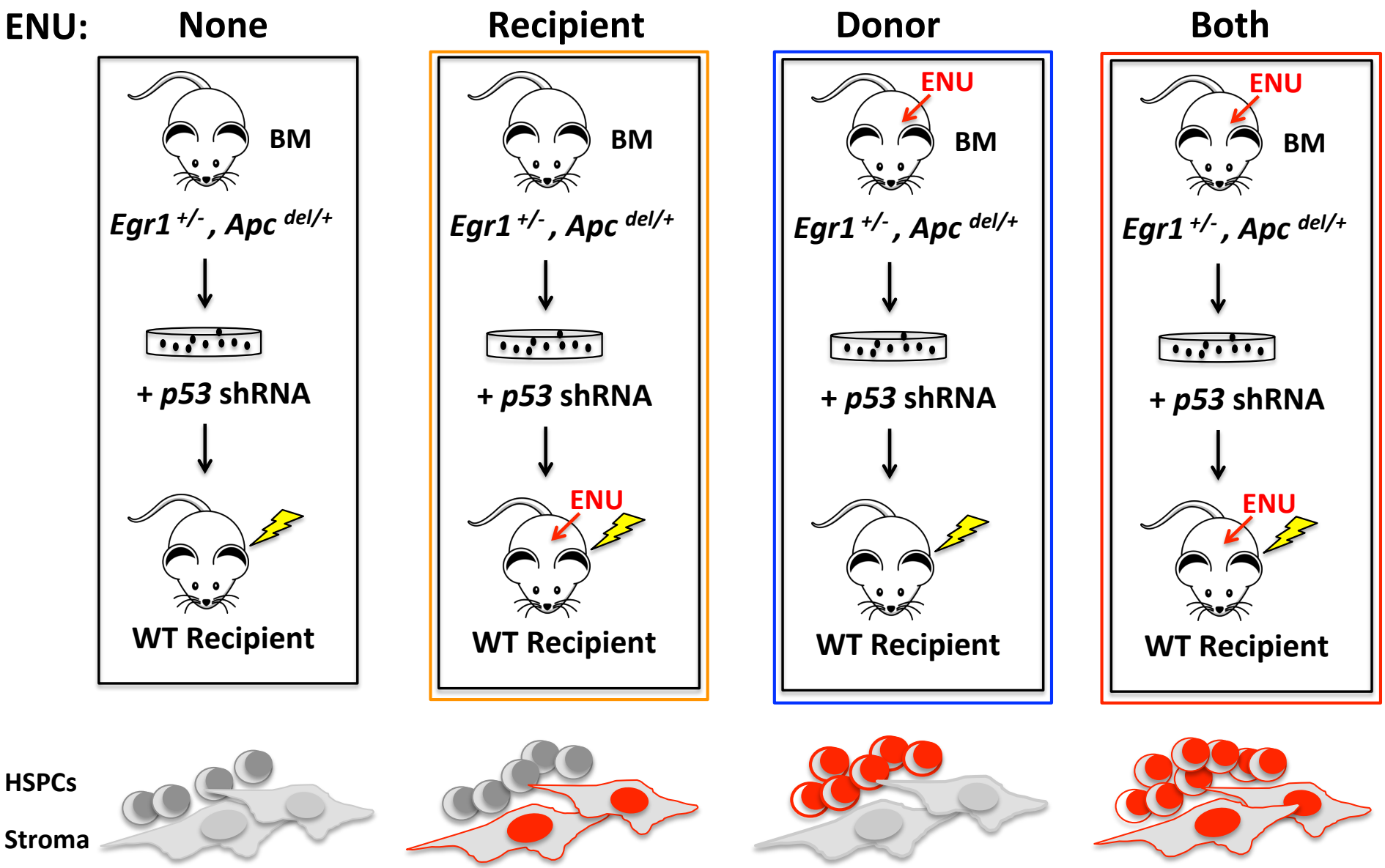




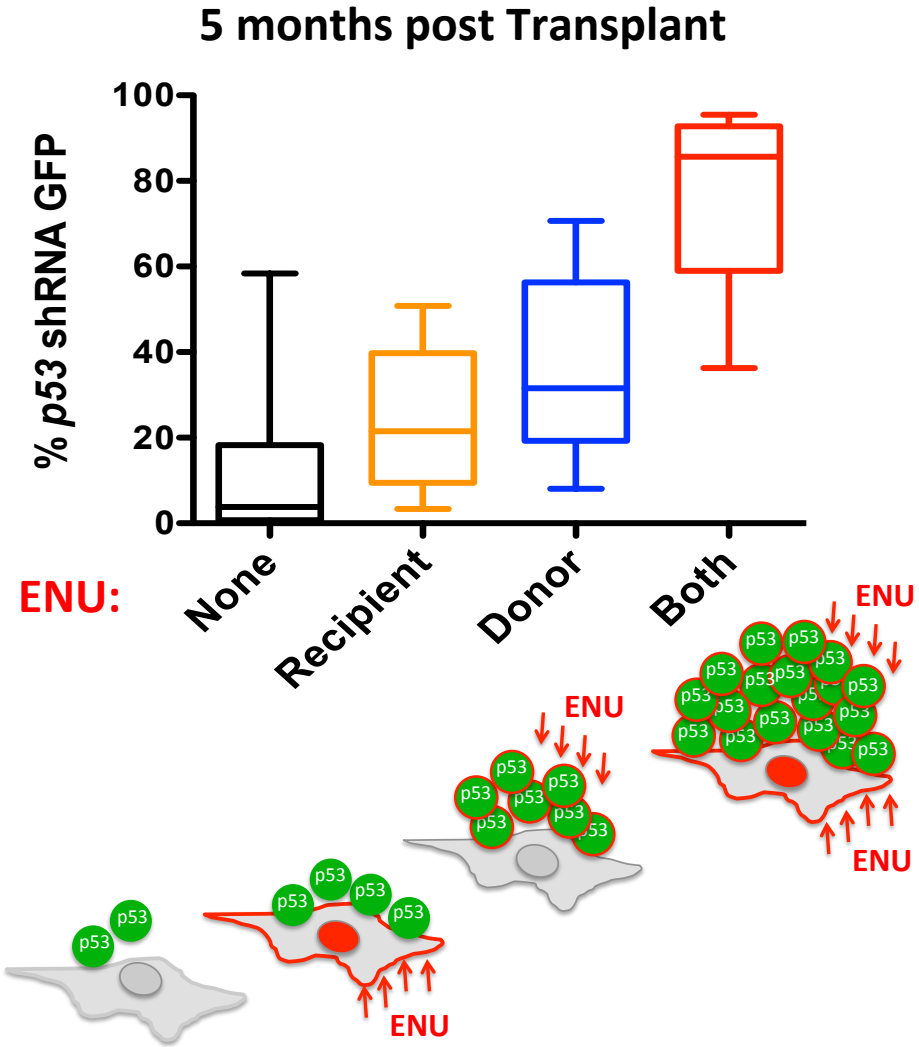


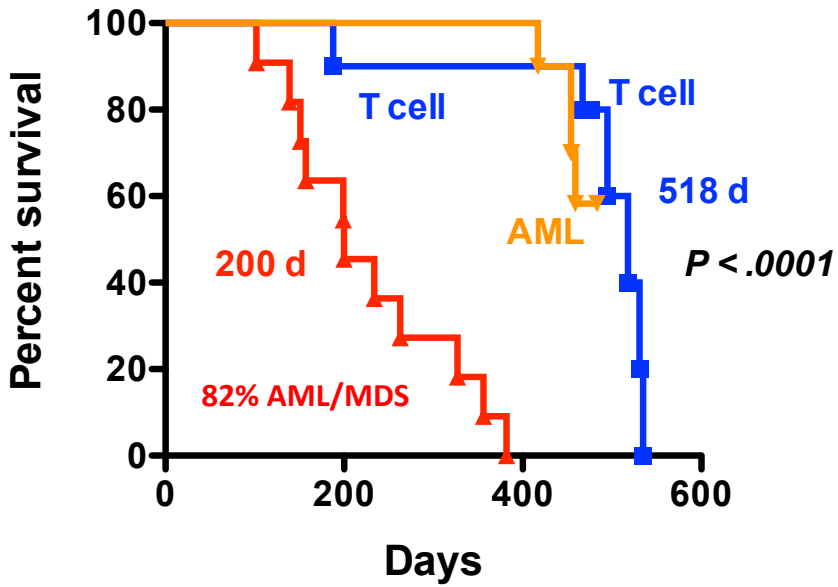


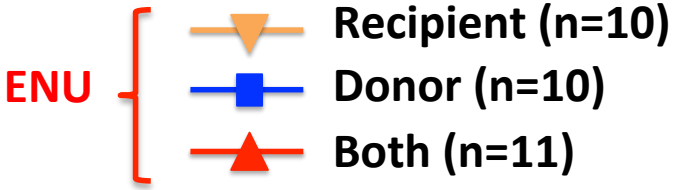
Effects of Alkylating Agents on HSPCs and the BM microenvironment



ENU Treatment of HSPCs and BM niche Promotes Expansion of *p53* shRNA-GFP^{+} Cells



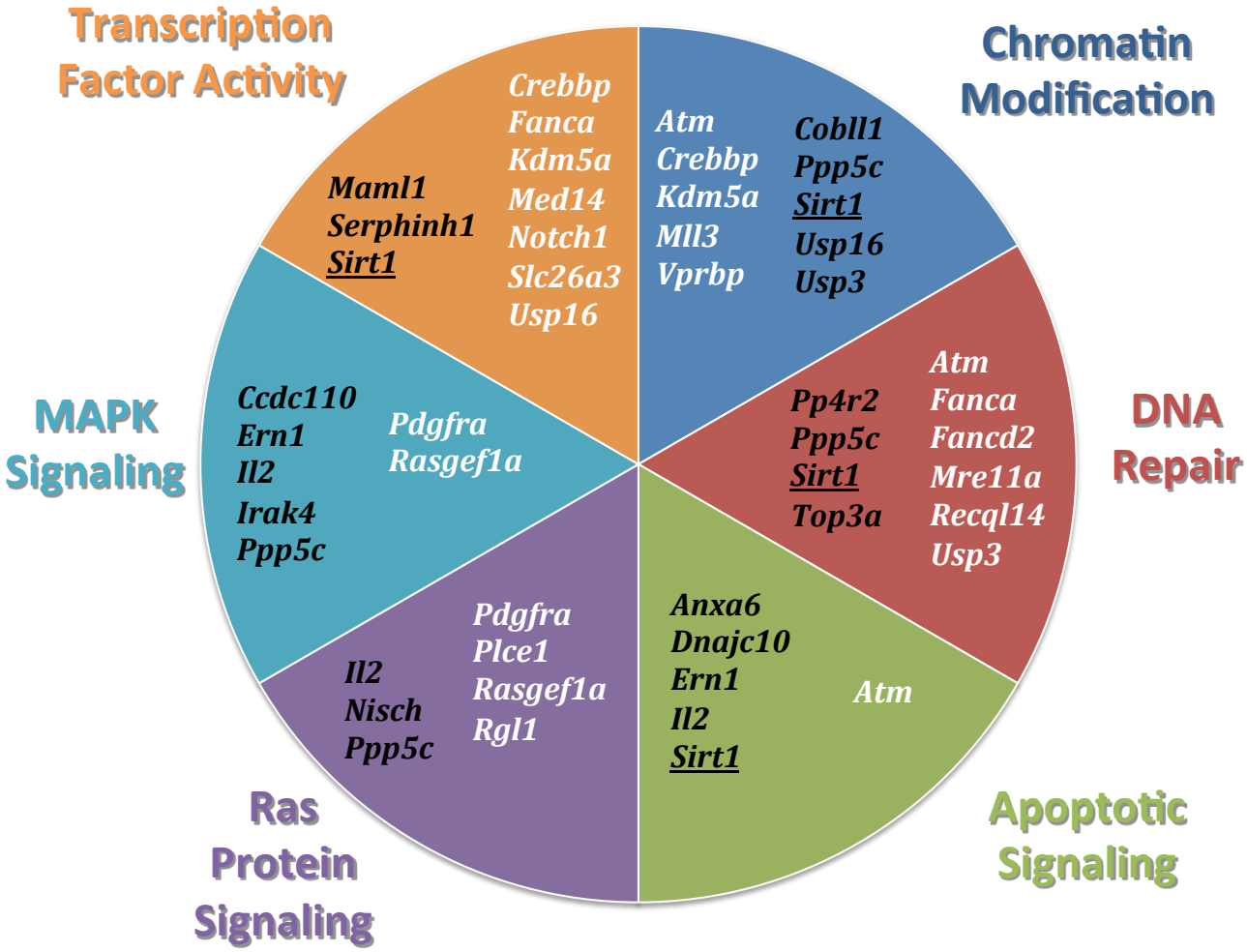




AML/MDS multilineage dysplasia

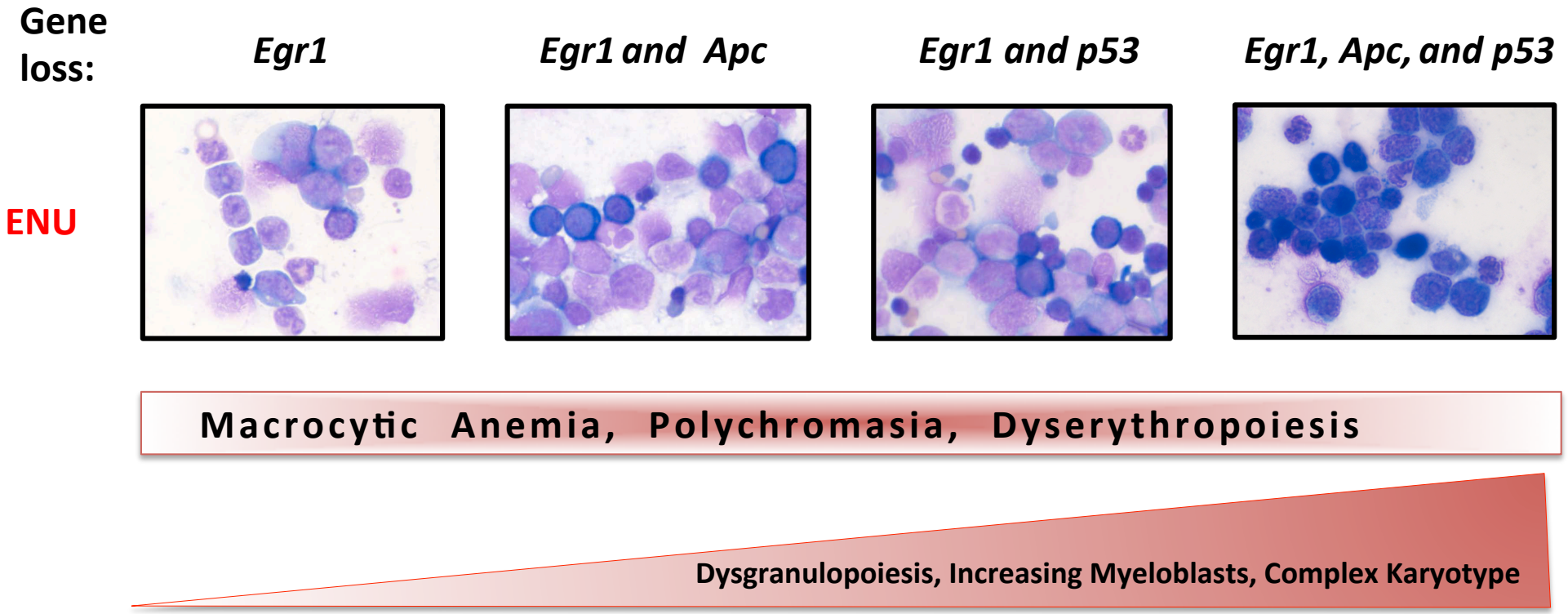
Major Gene Ontology Categories Over-Represented in List of Mutated Mouse Genes

- **139** — 120 genes with NS mutations and 19 genes with Indels
- **125** — No. corresponding human genes in COSMIC Database
- **102** — No. genes mutated in hematopoietic malignancies
- **58** — No. genes mutated in MDS/AML



White text identifies genes mutated in human MDS/AML

CONCLUSIONS-4

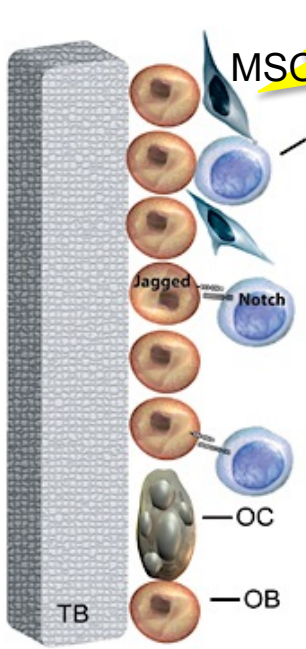


- *Egr1* and *Apc* haploinsufficiency promotes the development of MDS and AML
- Severity of disease increases with loss of >1 5q gene and loss of *p53*
- Loss of *p53* is critical for leukemic transformation
- t-MN development is likely promoted by the effects of alkylating agent therapy on both the HSPCs and the BM niche

Pathways to t-MN

~5 years → **t-MDS** → ~5 mos → **t-AML**

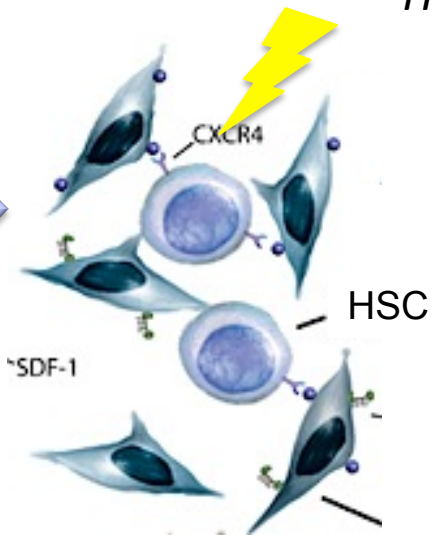
Alkyating Agents, IR



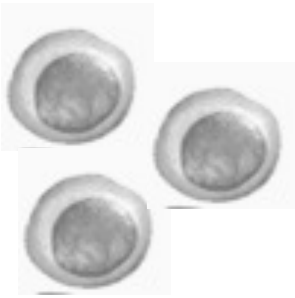
Permissive environment

HSC

TP53/del(5q)



Genetic instability



Stroma:
Aberrant Wnt Signaling

---→ **Cytokines, adhesion, receptors**

Apoptosis, cytopenias

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