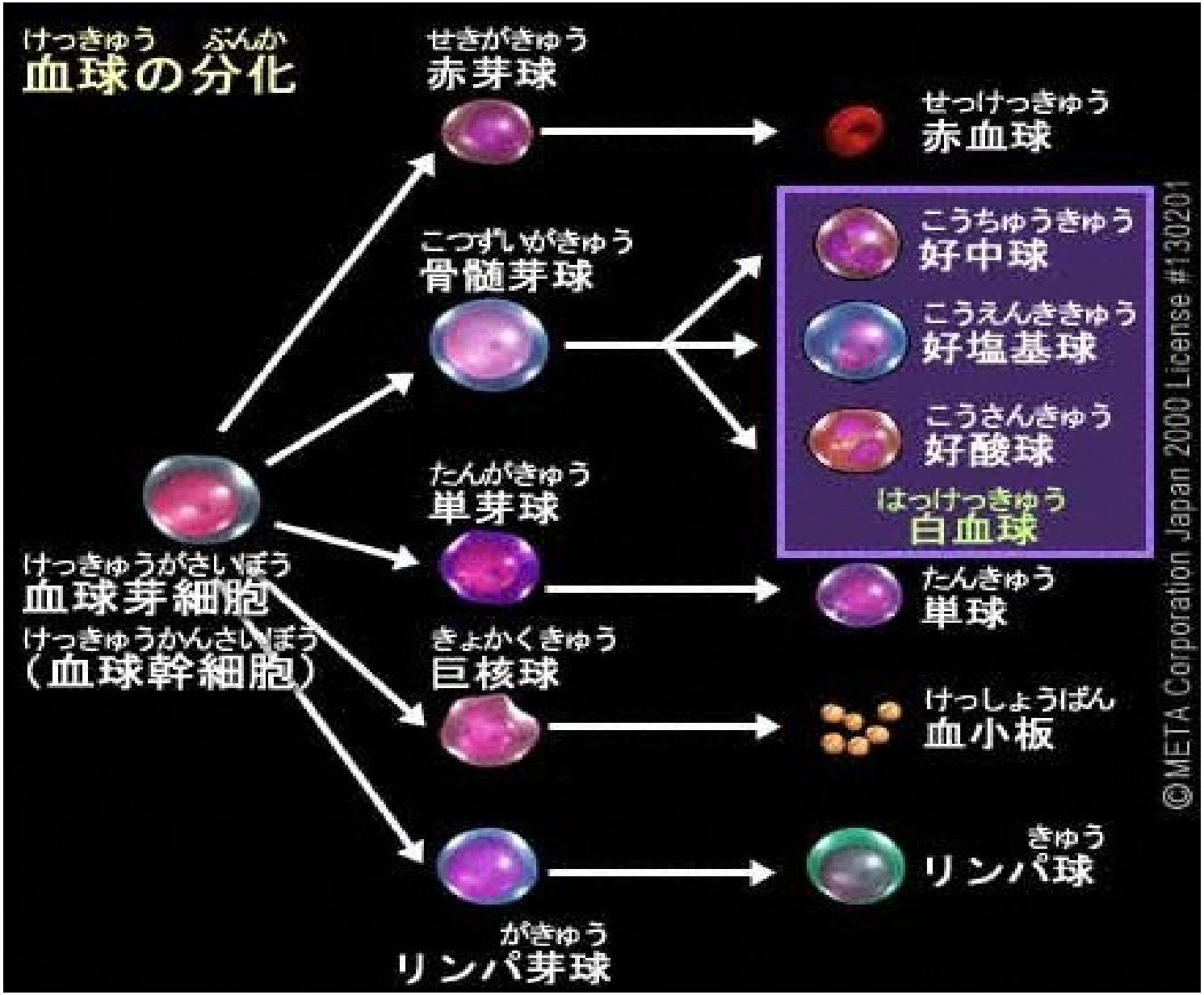
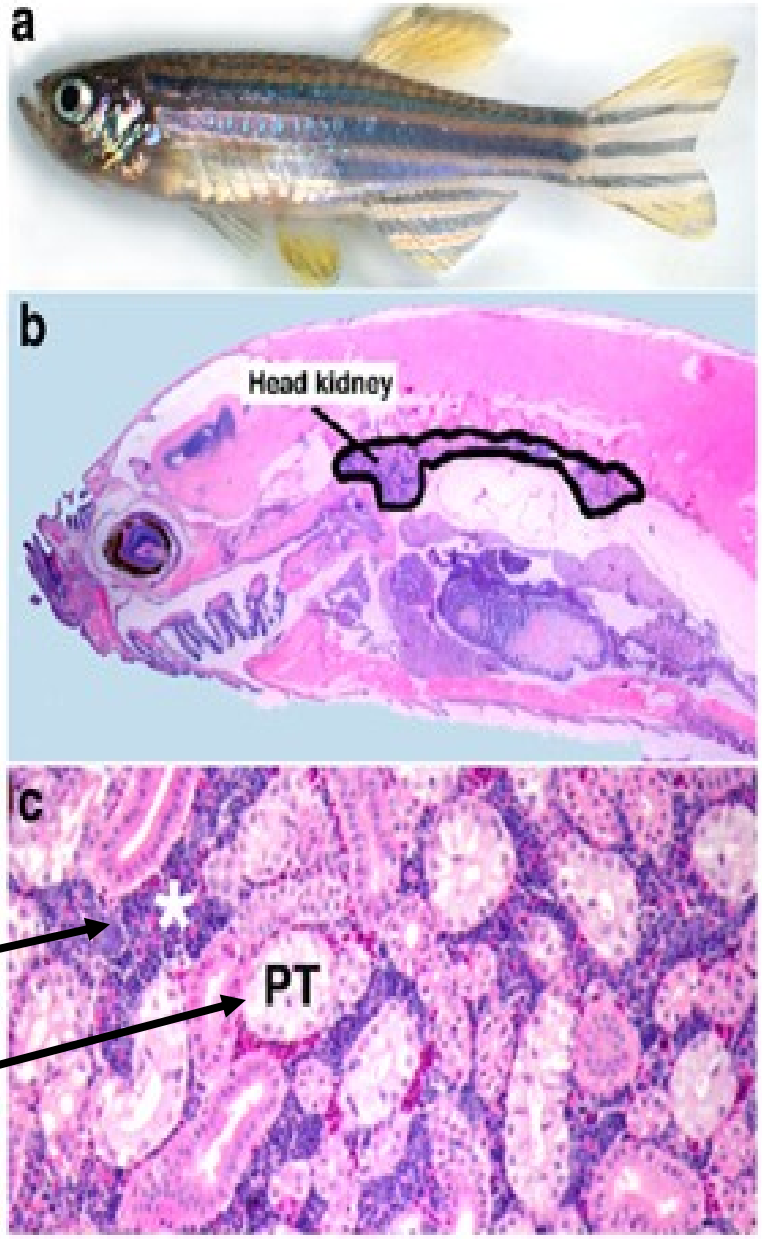
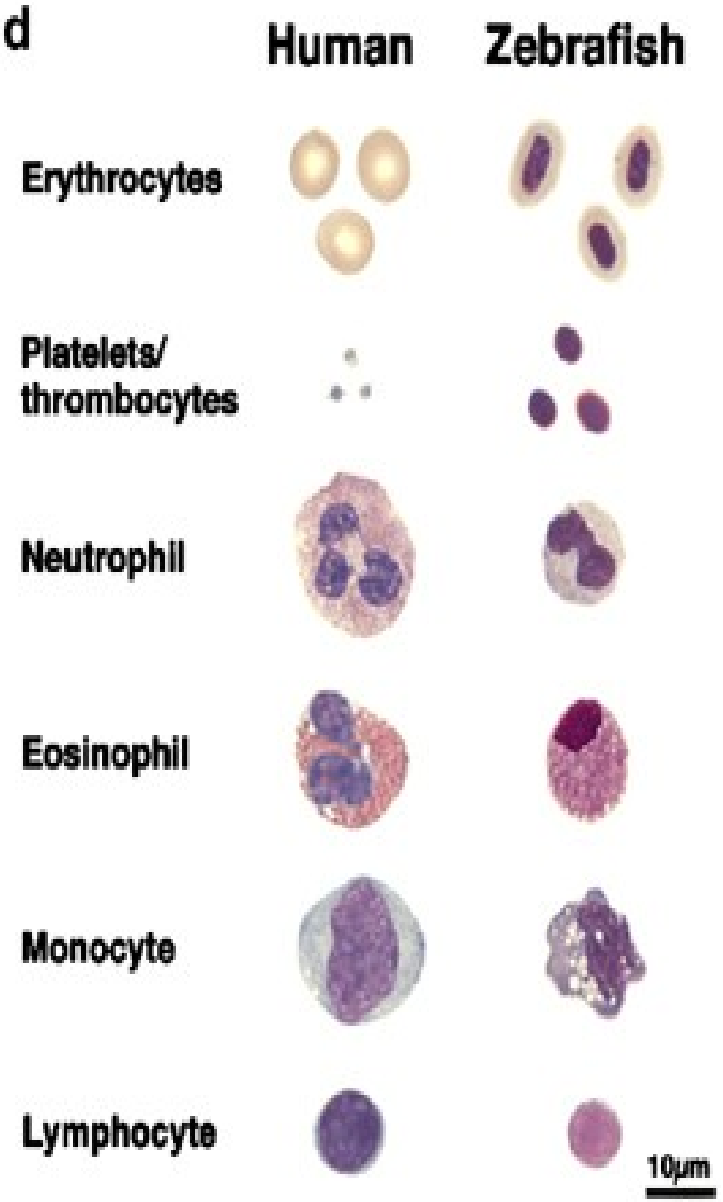
5. DEVELOPMENT OF BLOOD

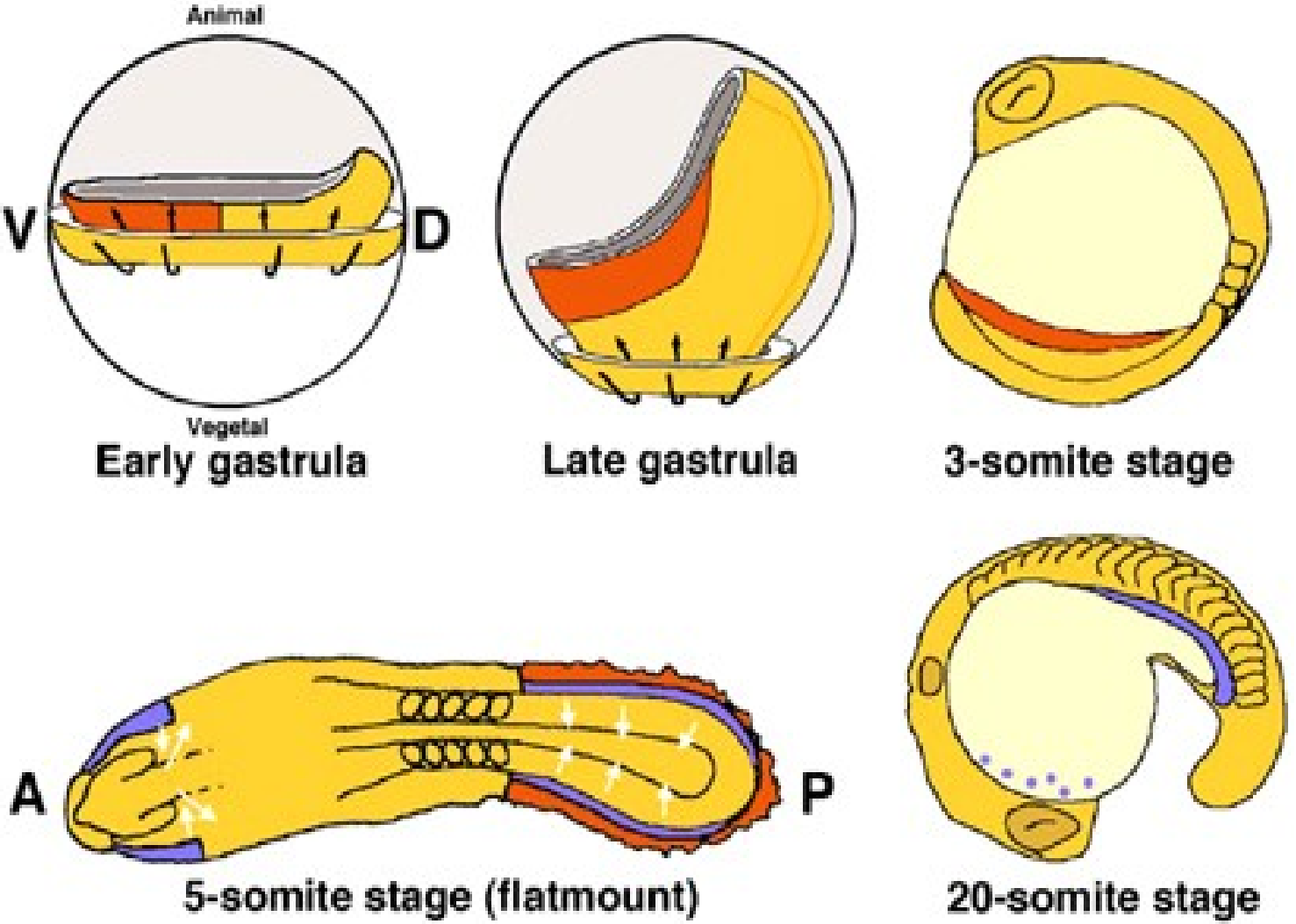




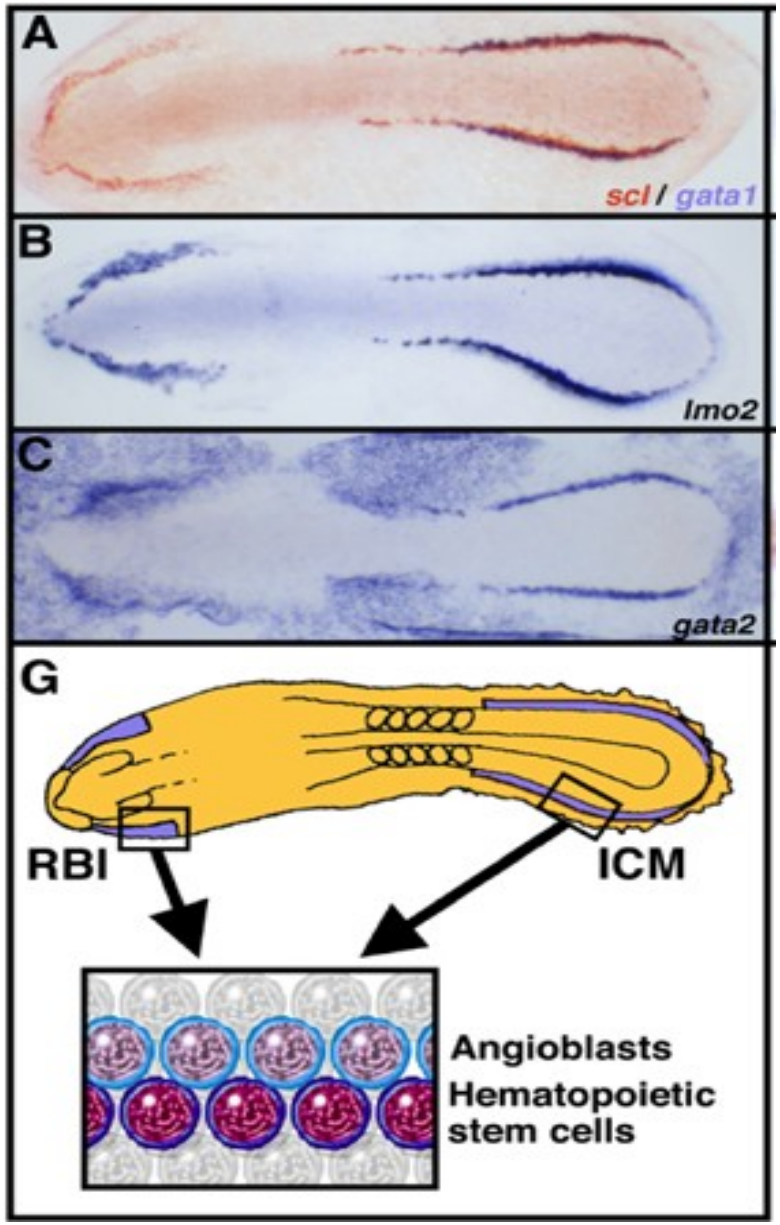
Blood cells

Mesonephric tubules



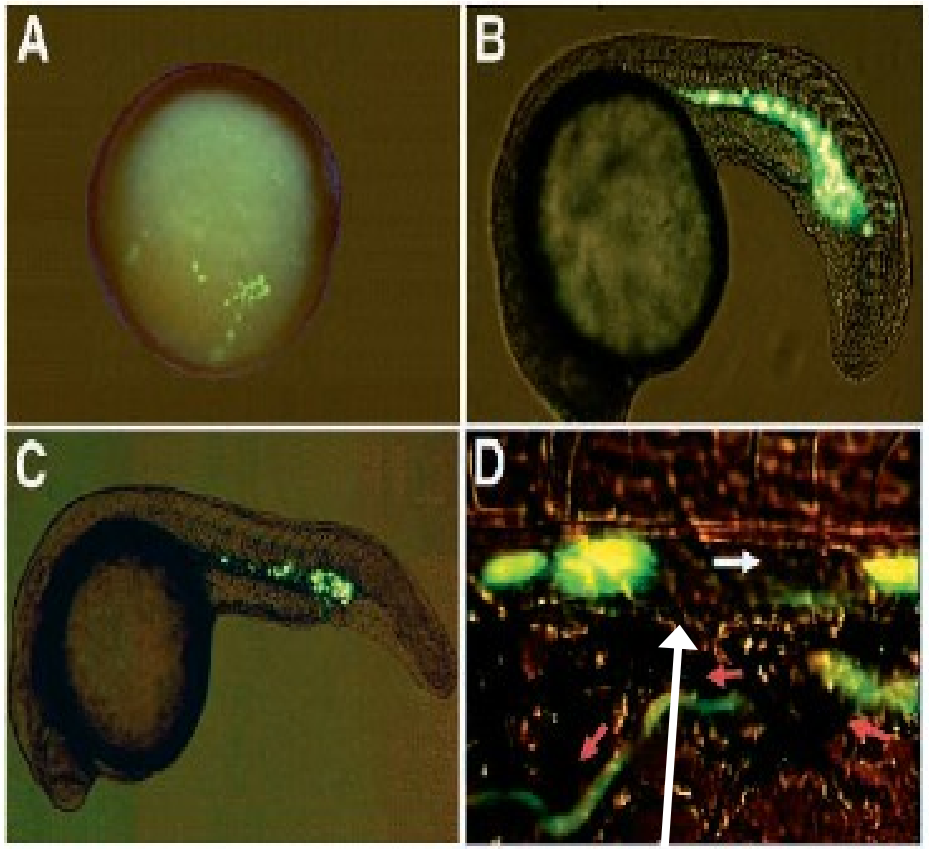


Fish gastrulation movie



scl, gata1/2, lmo2 - transcriptional factors that specify early hematopoietic tissue (gata2 expressed also in ectoderm)
RBI – rostral blood islands
ICM – inner cell mass

gata1 promoter-driven GFP = early erythroid lineage

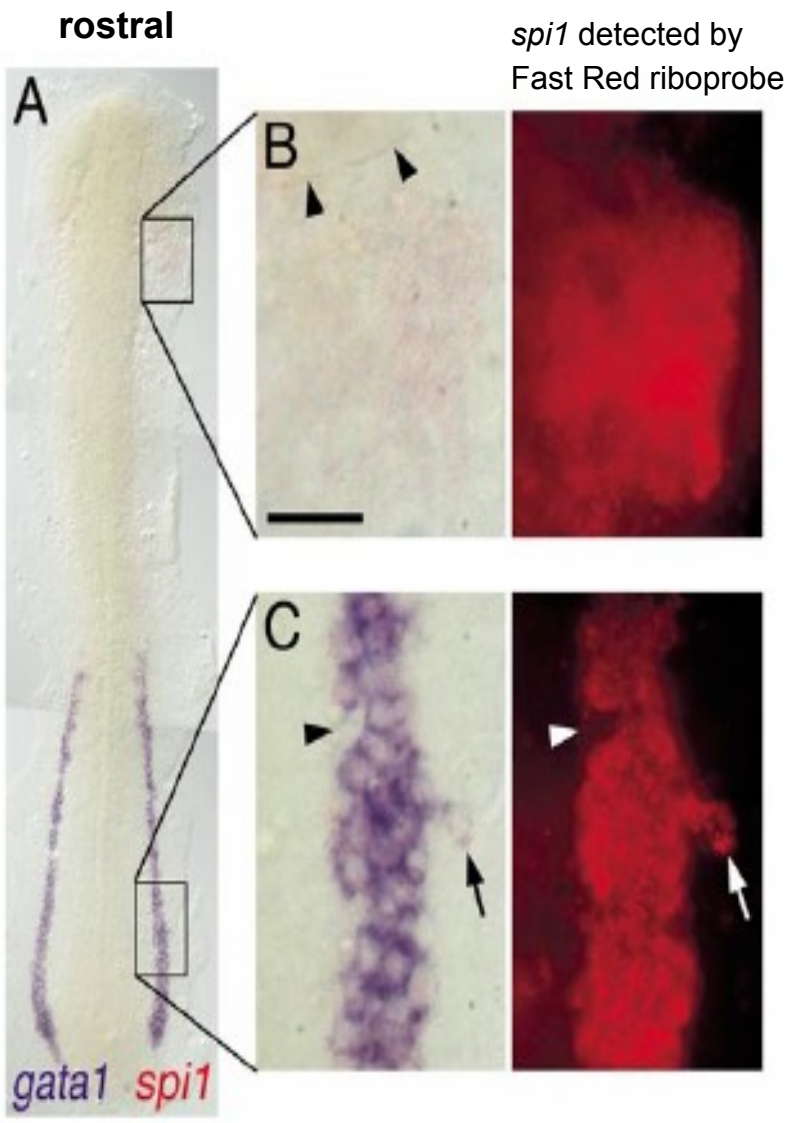


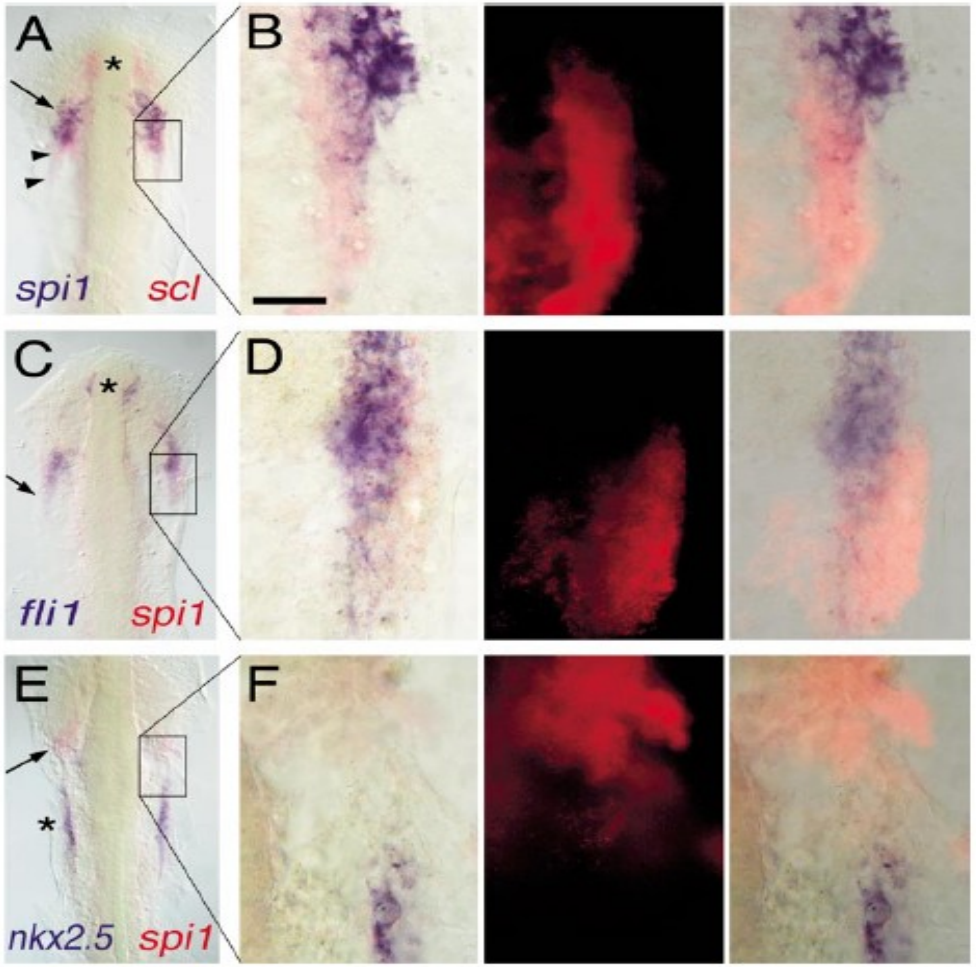
dorsal aorta

5-somite stage embryo – hematopoiesis starts officially

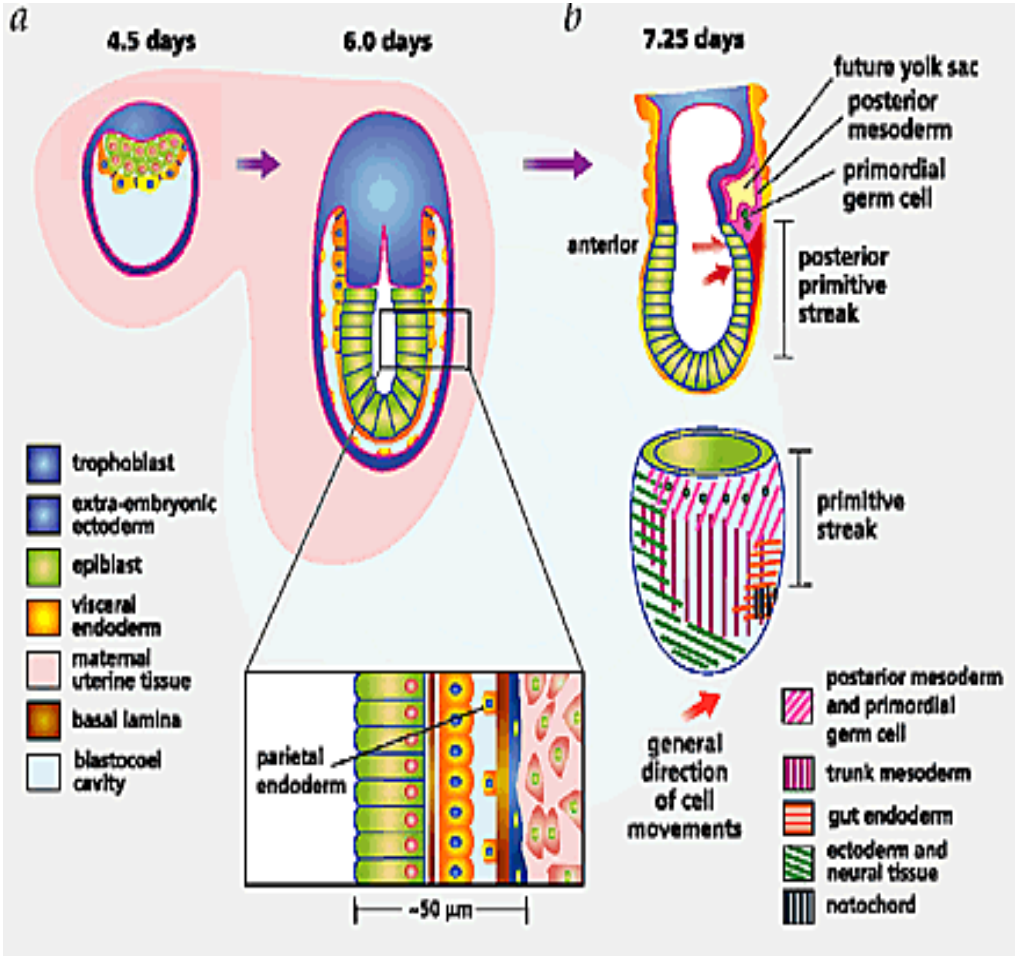
MYELOPOIESIS STARTS IN RBI @ 10-SOMITE STAGE

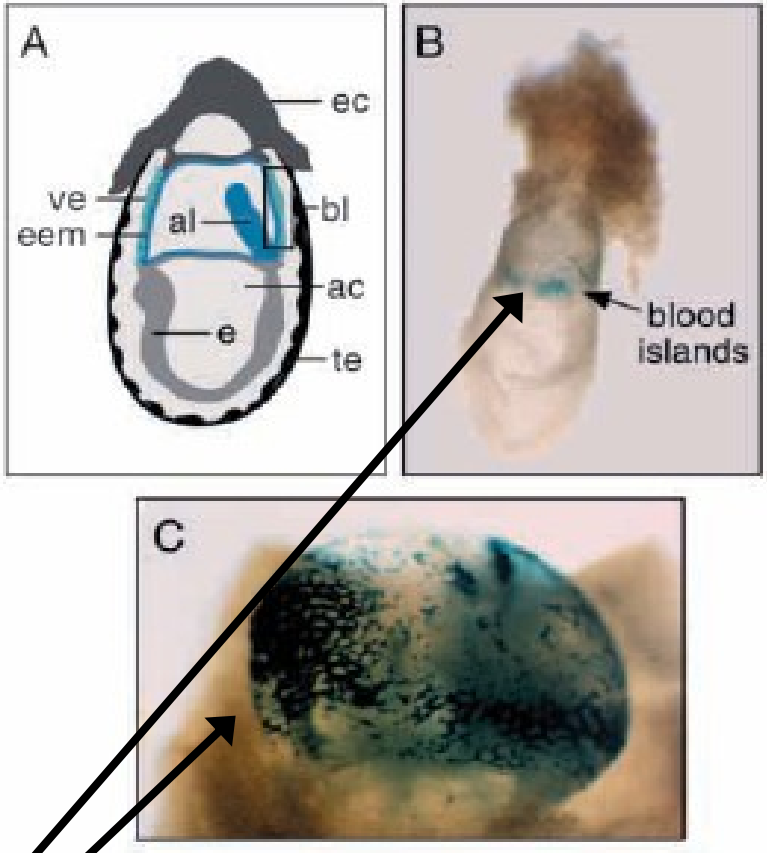
spi1 – marker of myeloid lineage





scl – early hematovascular cell fate
fli1 – early vascular fate
nkx2.5 – heart fate





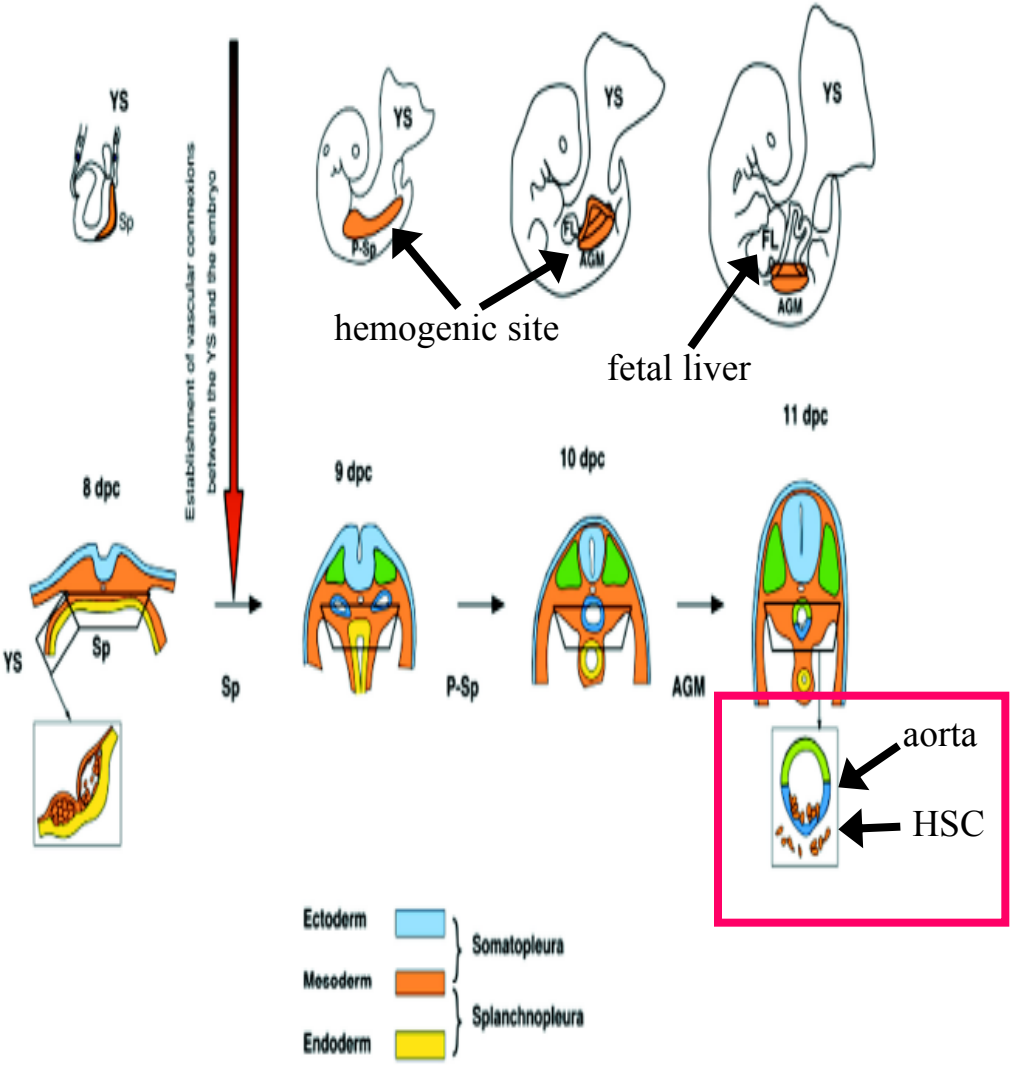
8.5 dpc – yolk sac encloses entire embryo
blood islands merge to form vascular channels

LacZ driven by β-globin promoter
(X-gal staining – primitive erythroblasts)

bl - blood islands
ec - ectoplacental cone
ac - amniotic cavity
te - trophectoderm
al - allantois
eem - extraembryonic mesoderm (blue)
ve - visceral endoderm
e - embryonic ectoderm

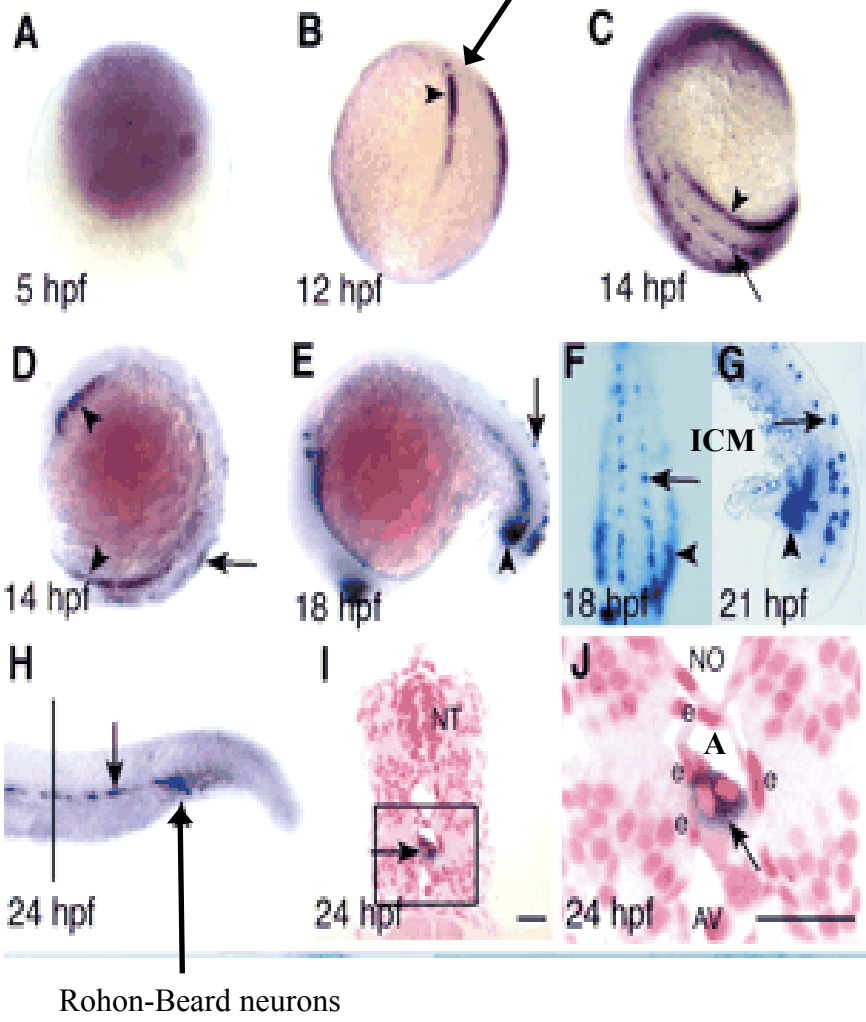
AORTA-GONAD MESONEPHROS (AGM)

Development of intra-embryonic hemogenic site



RUNX1 riboprobe

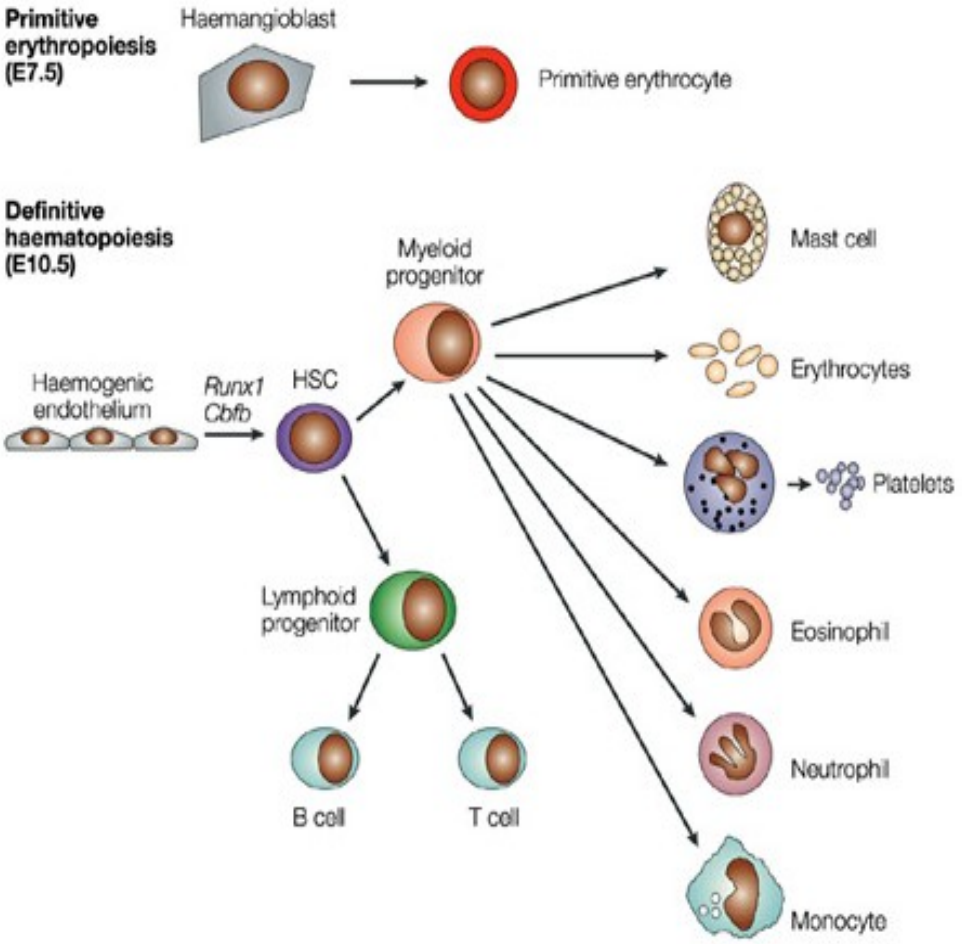
lateral plate mesoderm

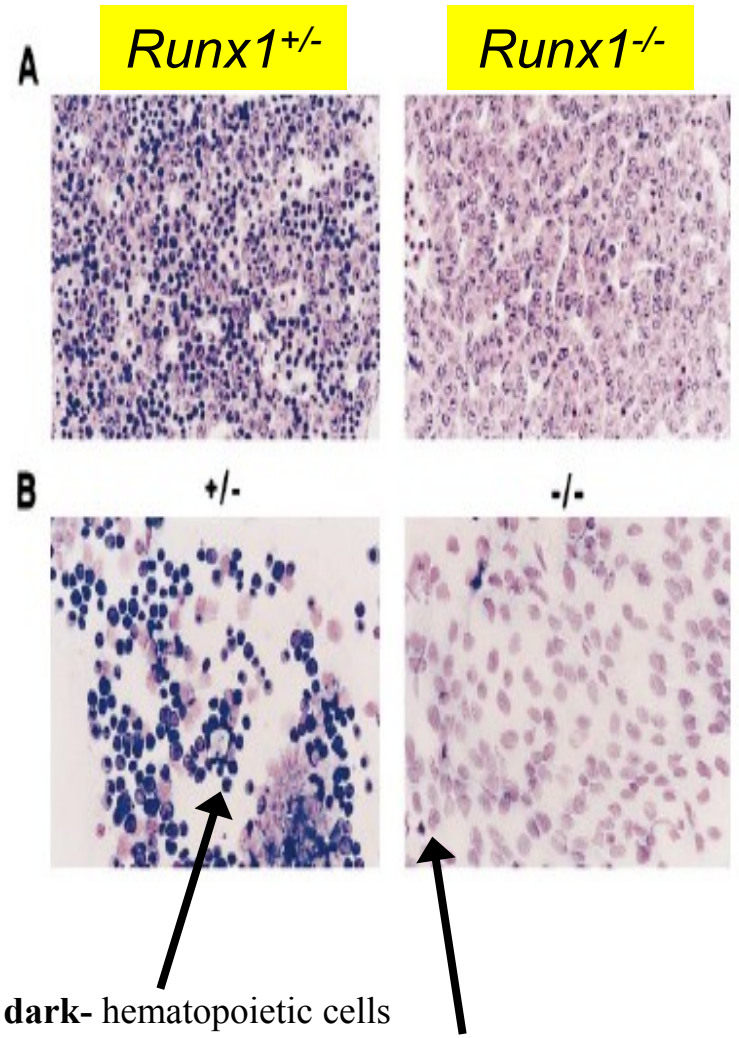


Rohon-Beard neurons

A- aorta
NO- notochord
AV- axial vein
e - endothelial cell

RUNX1 (CBFA2, AML1) is necessary for 'definitive' hematopoiesis to start at AGM

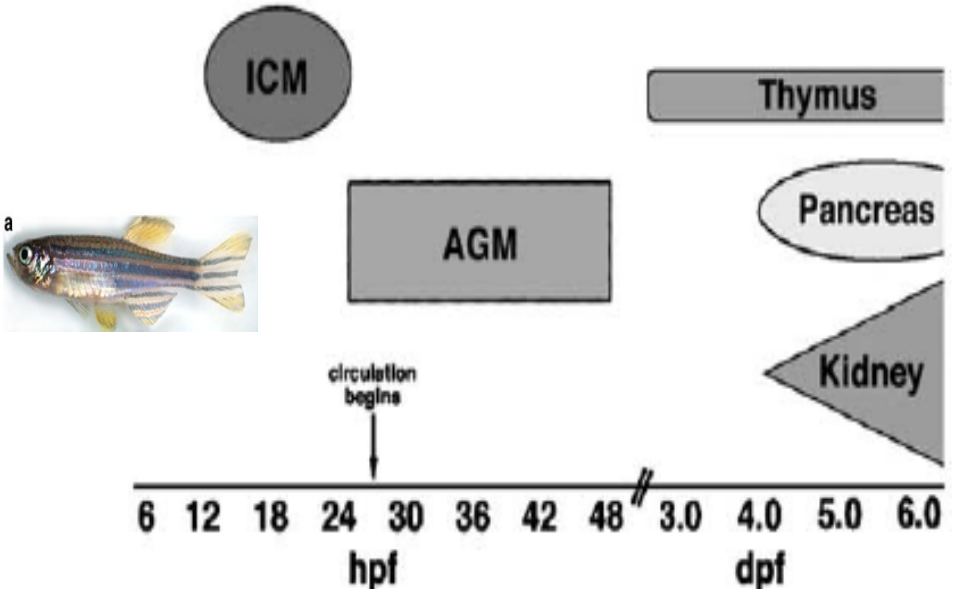


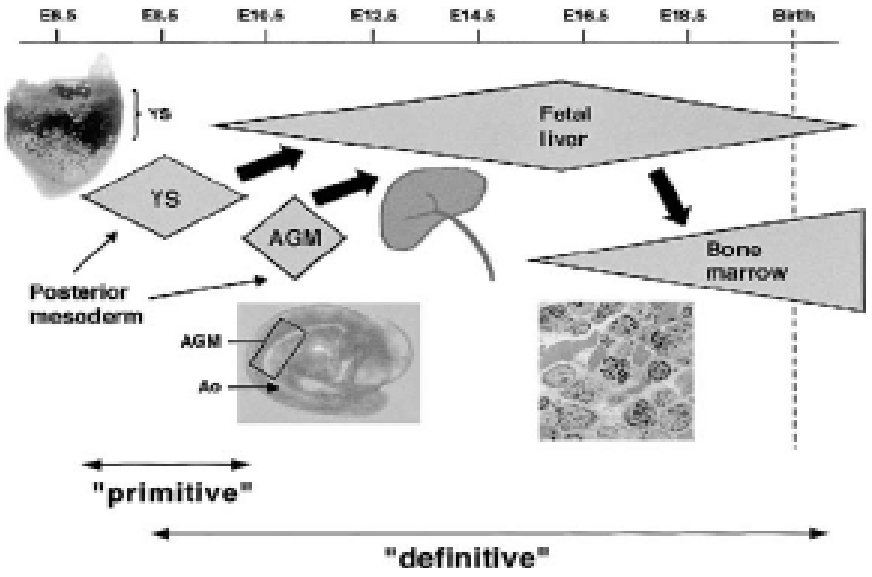


E11.5: **dark-** hematopoietic cells

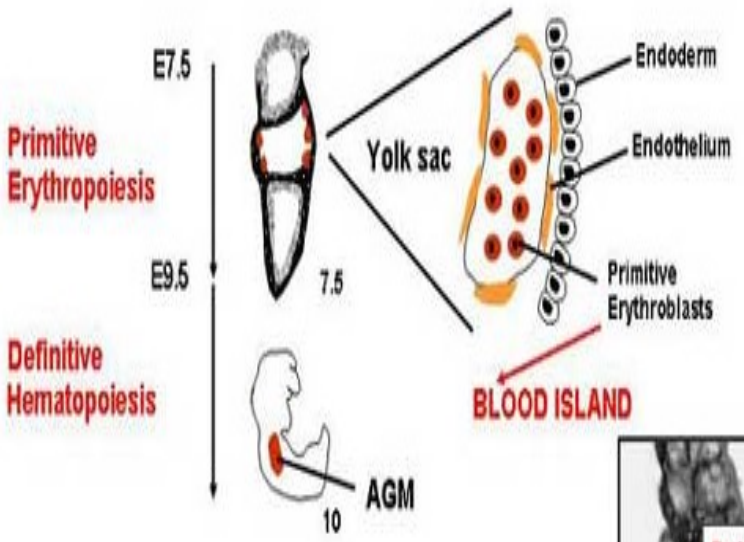
only a few primitive erythrocytes left – likely a carry-over from the 'primitive' hematopoiesis

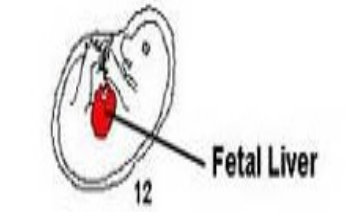
EXTRAEMBRYONAL vs EMBRYONAL

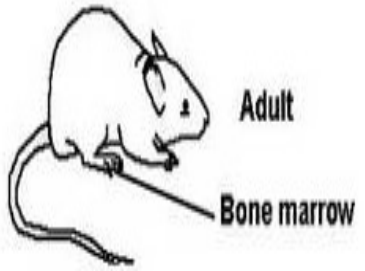


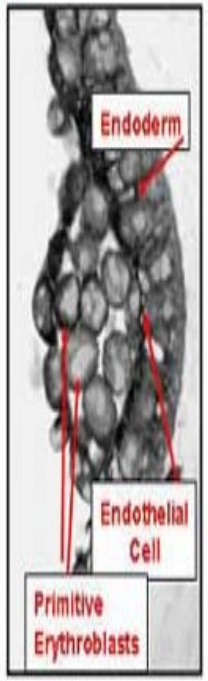


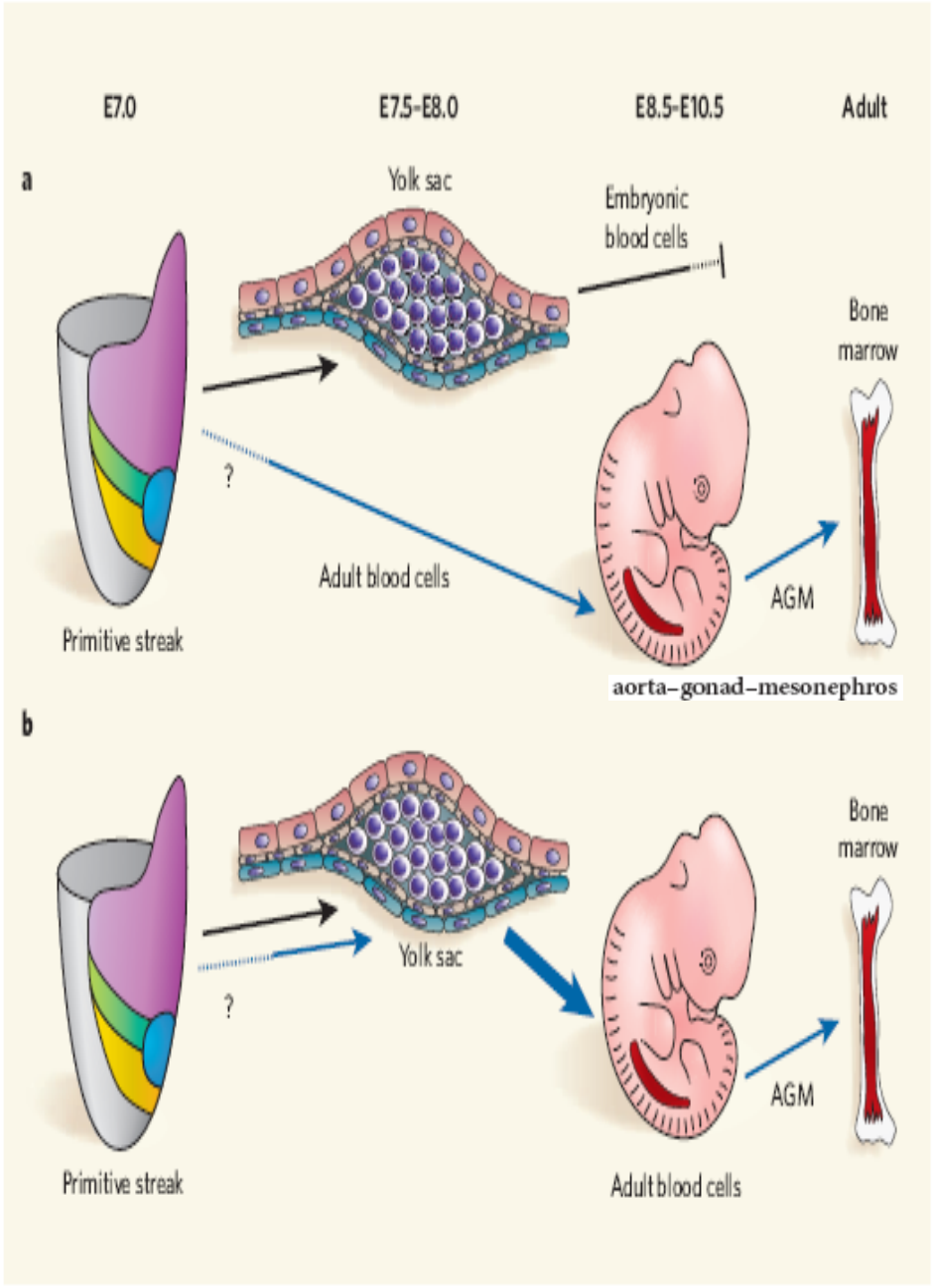
PRIMITIVE vs. DEFINITIVE

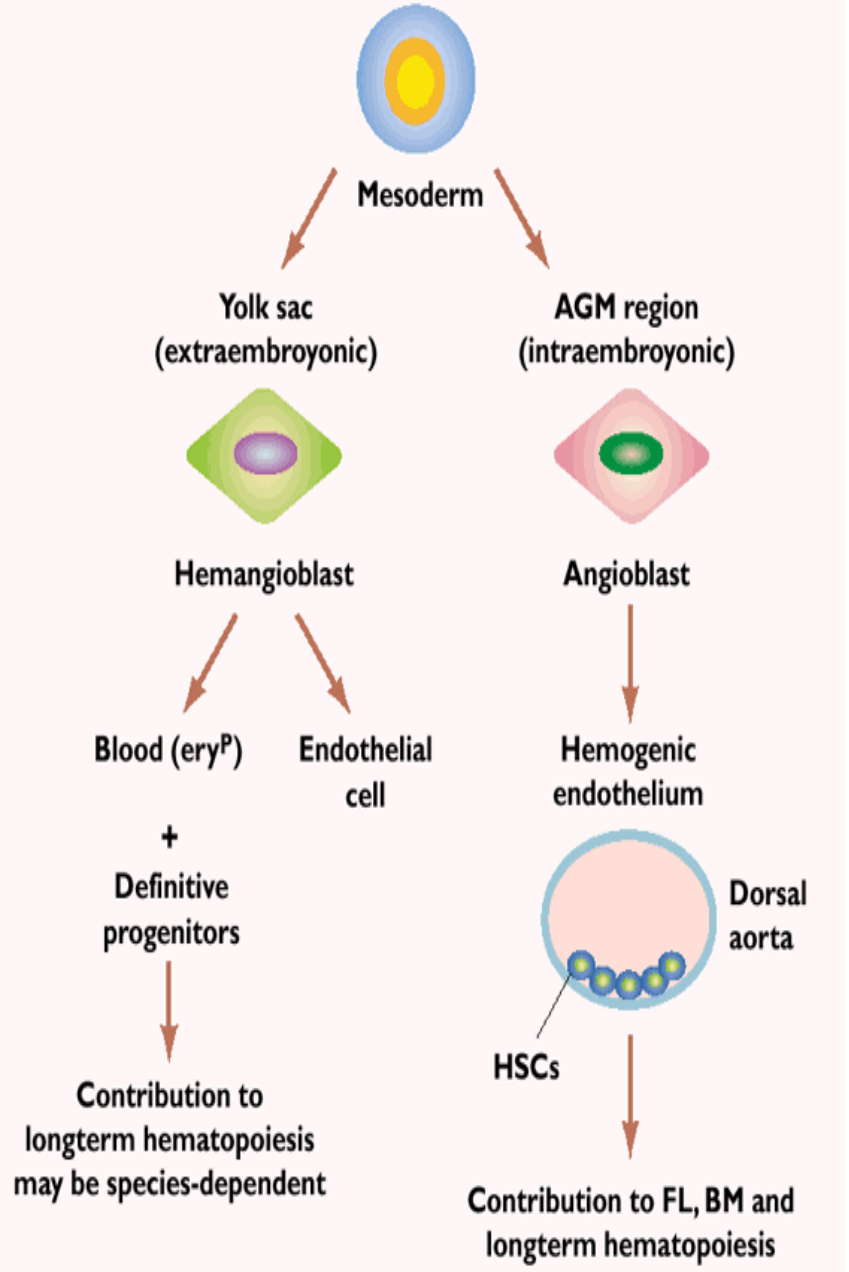




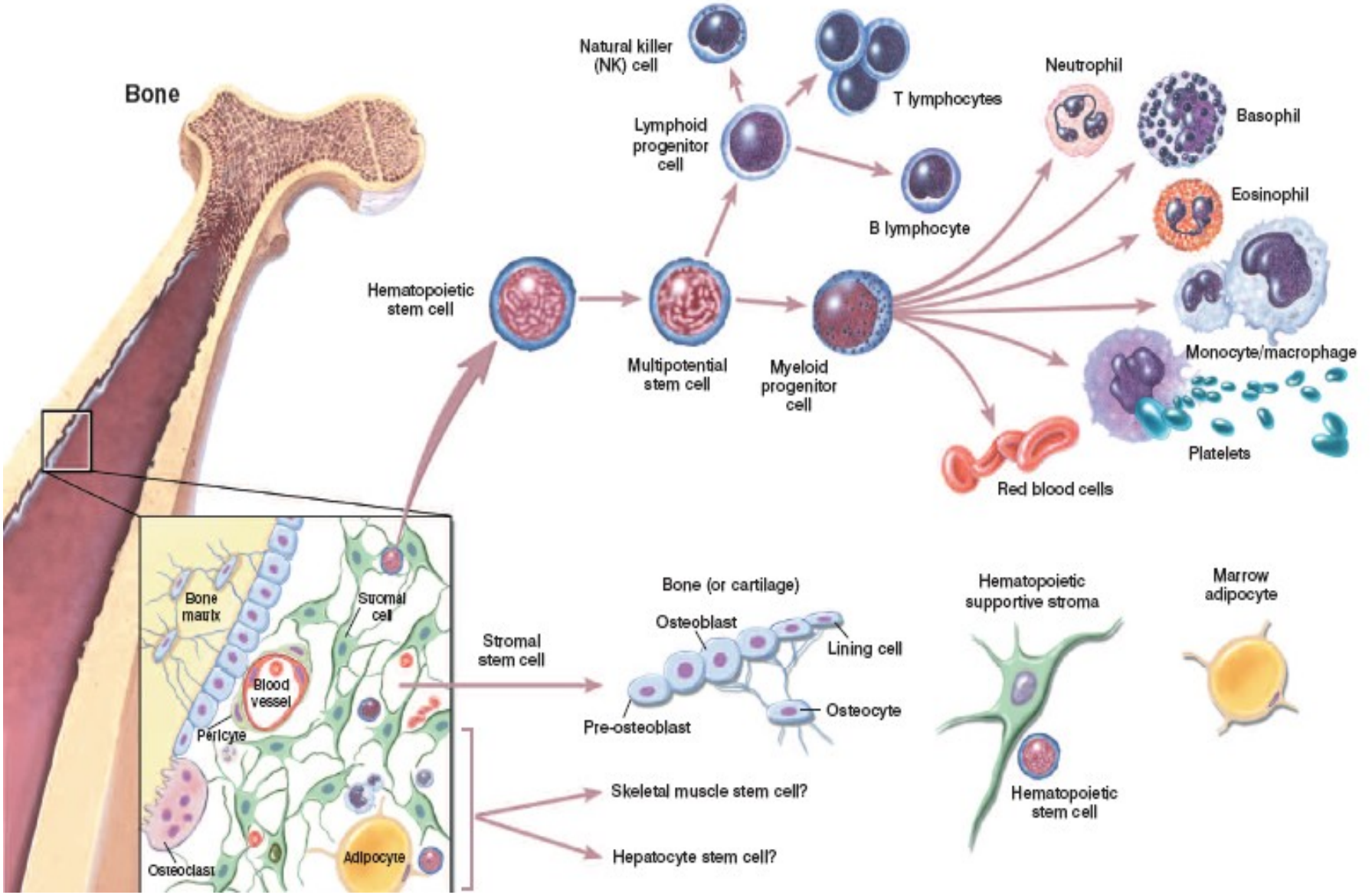




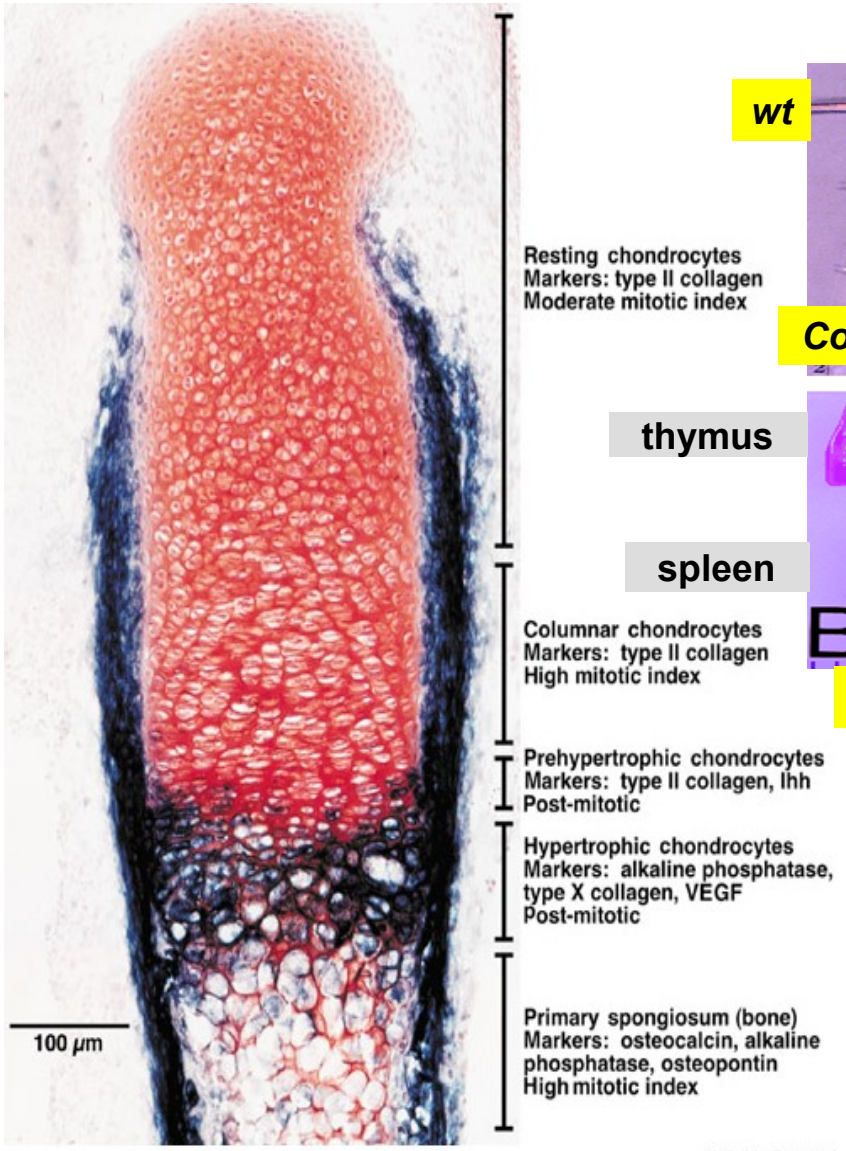

E7.0
E7.5–E8.0
E8.5–E10.5
Adult
a
Yolk sac
Embryonic blood cells
Bone marrow
?
Adult blood cells
AGM
Primitive streak
aorta–gonad–mesonephros
b
Bone marrow
Yolk sac
?
AGM
Primitive streak
Adult blood cells

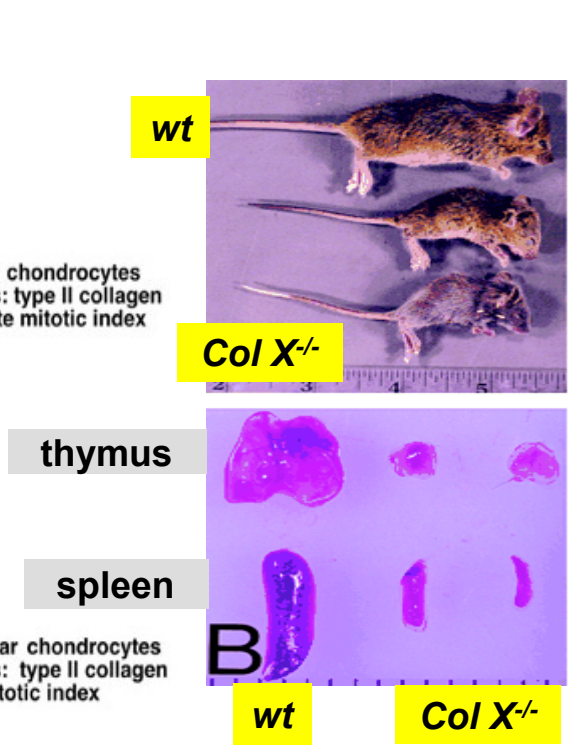

Mesoderm
Yolk sac (extraembryonic)
AGM region (intraembryonic)
Hemangioblast
Angioblast
Blood (eryP)
Endothelial cell
Hemogenic endothelium
+
Definitive progenitors
Dorsal aorta
HSCs
Contribution to longterm hematopoiesis may be species-dependent
Contribution to FL, BM and longterm hematopoiesis

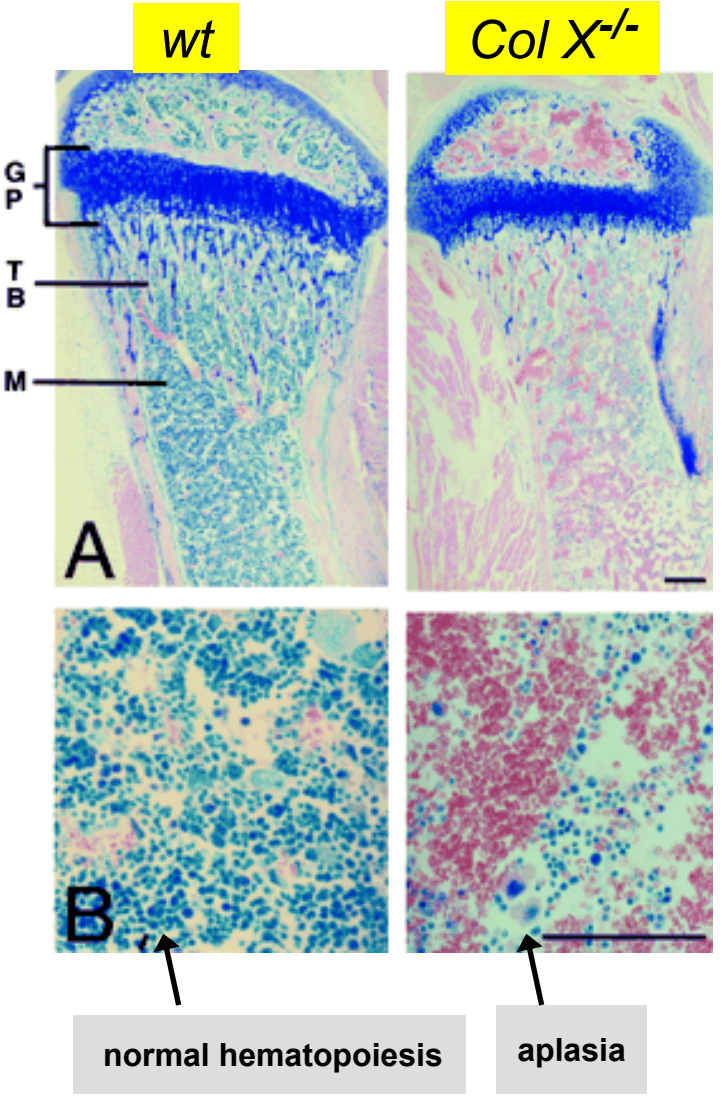
BONE MARROW NICHE IS CRITICAL FOR HEMATOPOIESIS



COLLAGEN TYPE-X DELETION



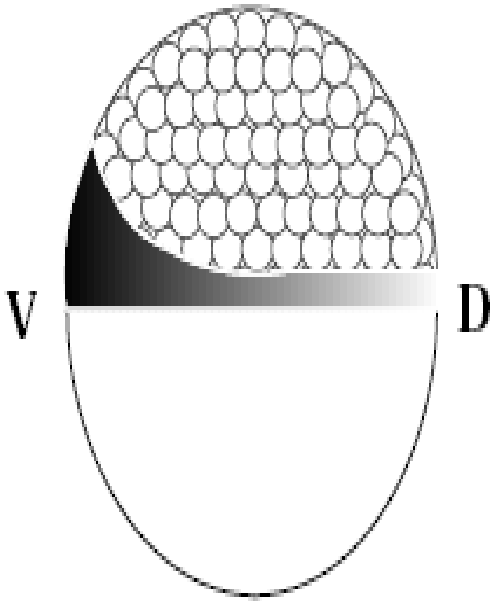


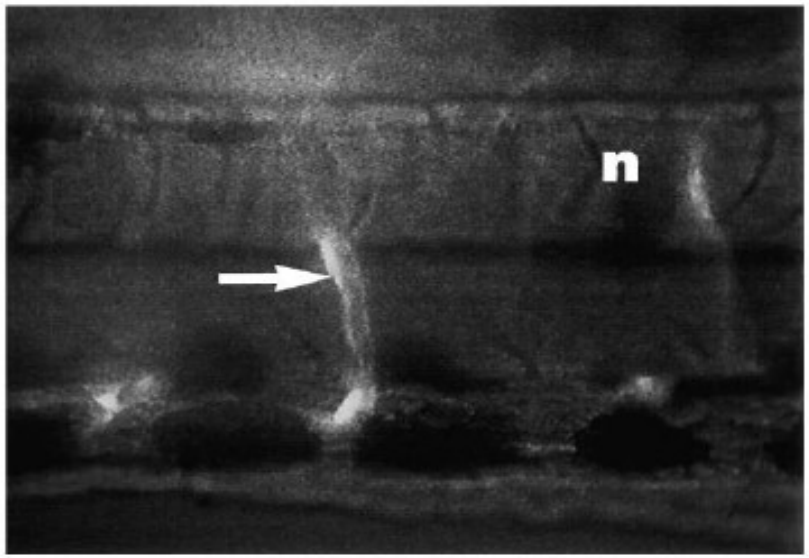


THE HUNT FOR HEMANGIOBLAST AND ITS HEM-ANGIO-DIFFERENTIATION

HEMANGIOBLAST

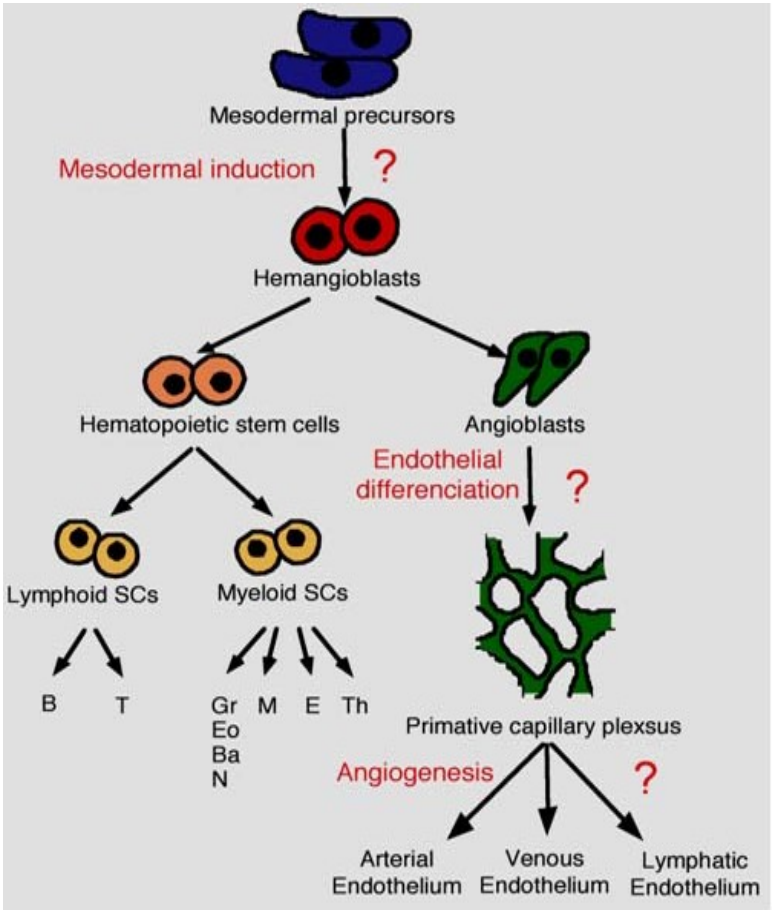
scattered among endothelial and HSC in heart field of the gastrula

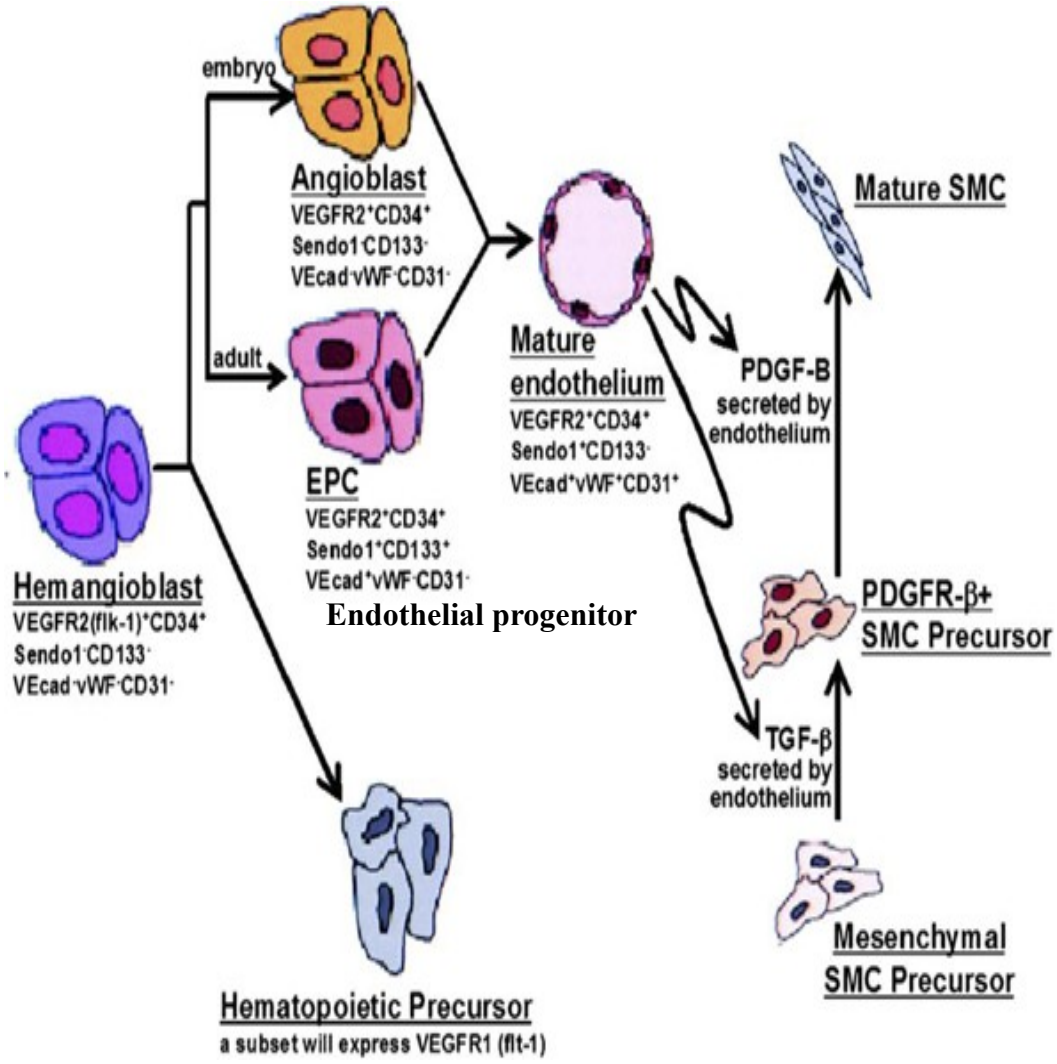




ventral marginal cells of the heart field, n- notochord

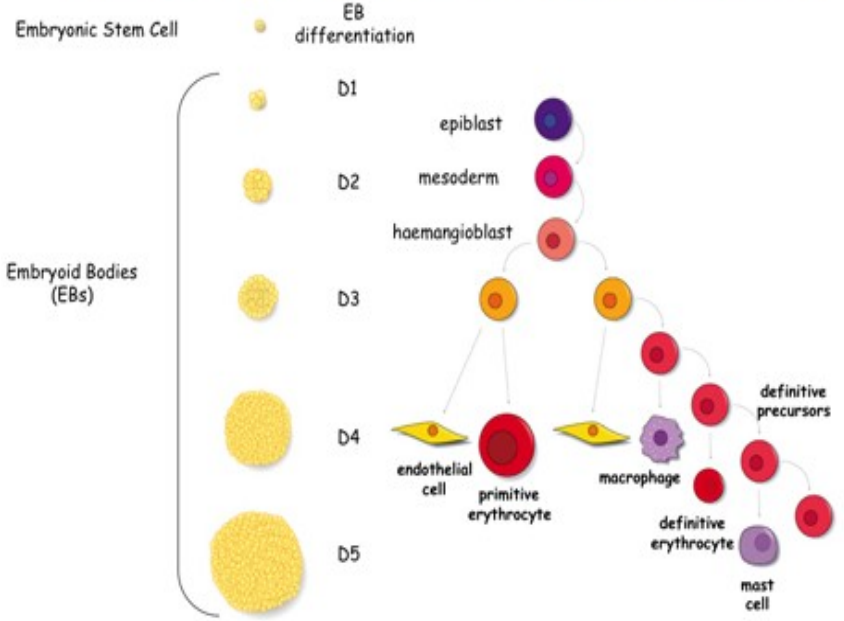
Fig. 5. The heart field. Diagrammatic representation of the heart field in the early blastula. The intensity of grey represents the propensity to form heart.



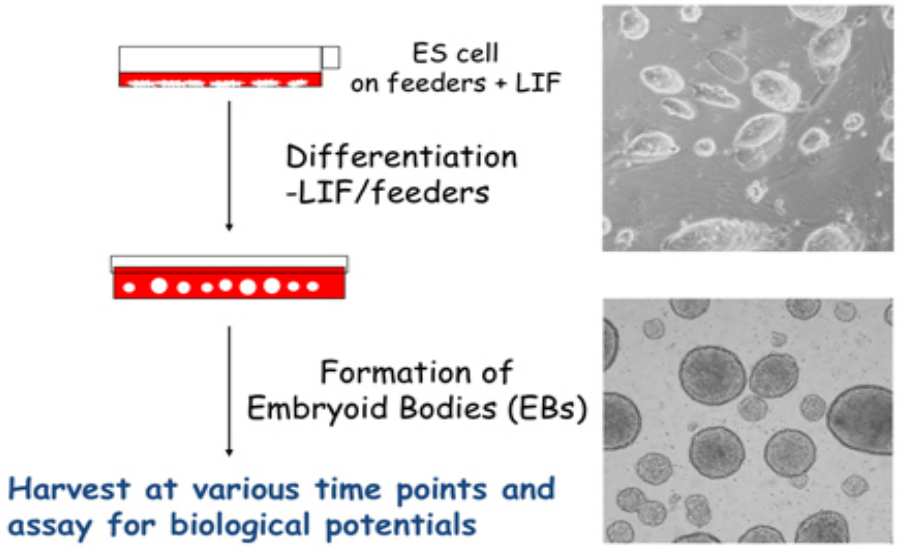


HEMANGIOBLAST or HAEMOGENIC EPITHELIA, or BOTH?

ES/EB as a Model of Yolk Sac Haematopoiesis

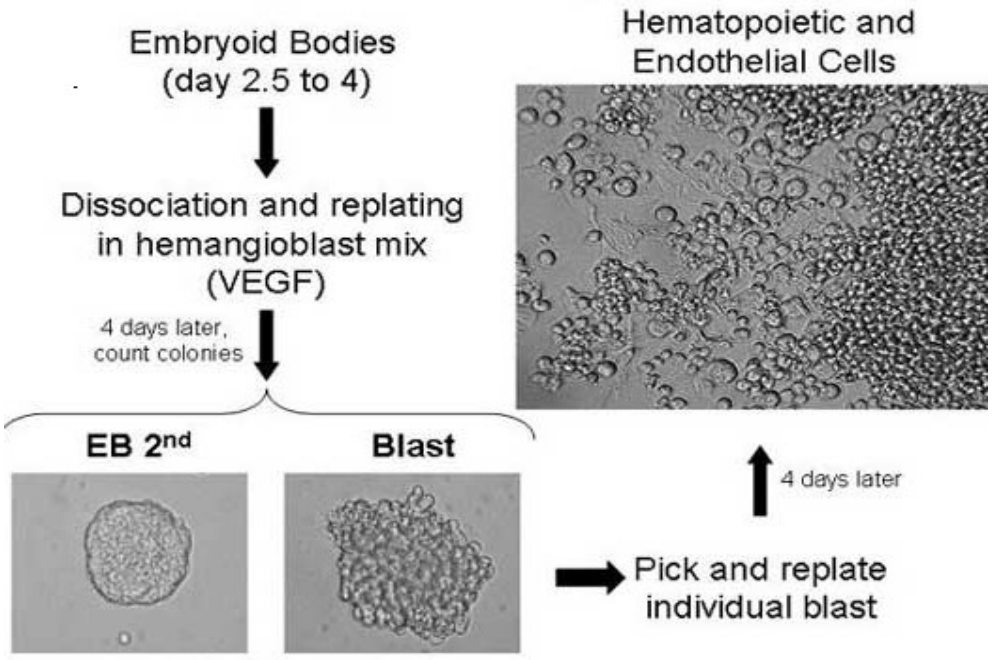


In vitro differentiation of ES cells



blast colony-forming cell

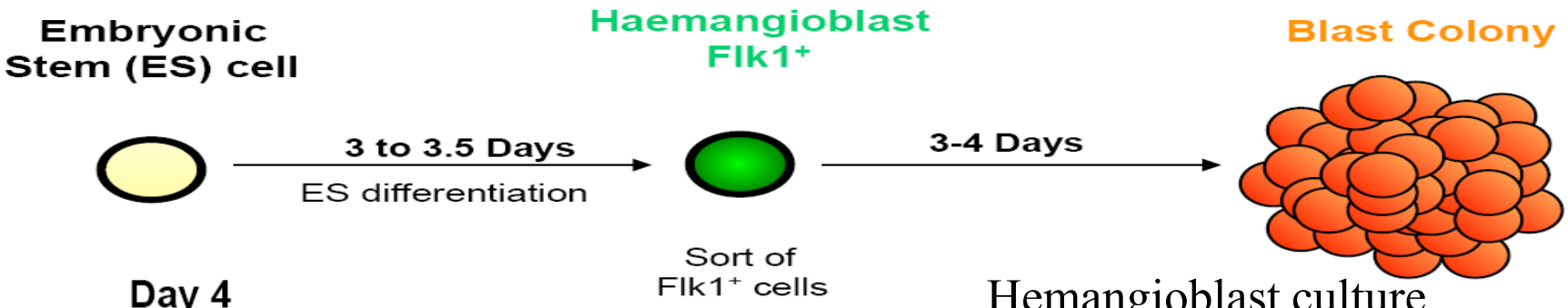
Hemangioblast FLK-1$^+$ (BL-CFC) Assay

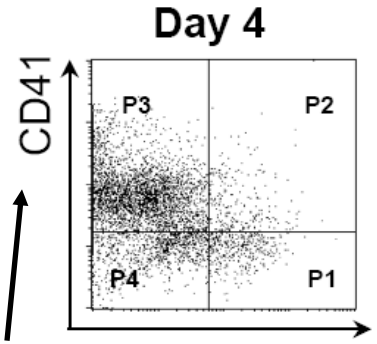


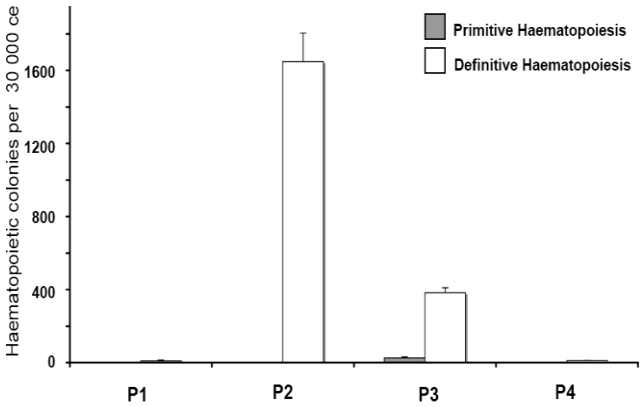
The haemangioblast generates haematopoietic cells through a haemogenic endothelium stage

NATURE | Vol 457 | 12 February 2009

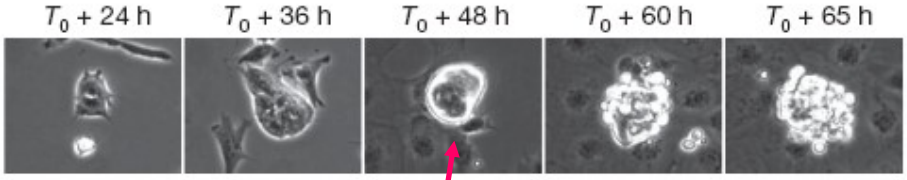
Christophe Lancrin[1], Patrycja Sroczynska[1], Catherine Stephenson[1], Terry Allen[2], Valerie Kouskoff[3] & Georges Lacaud[1]



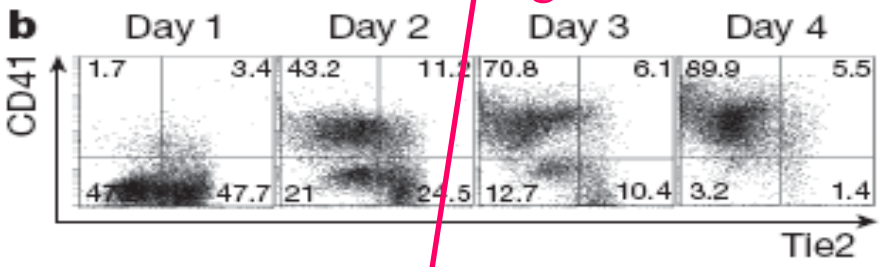


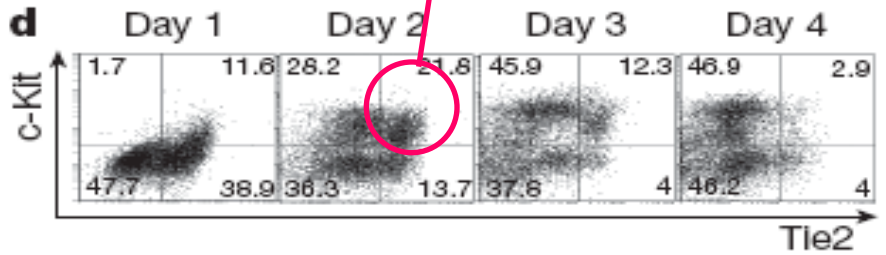


Hemangioblast culture



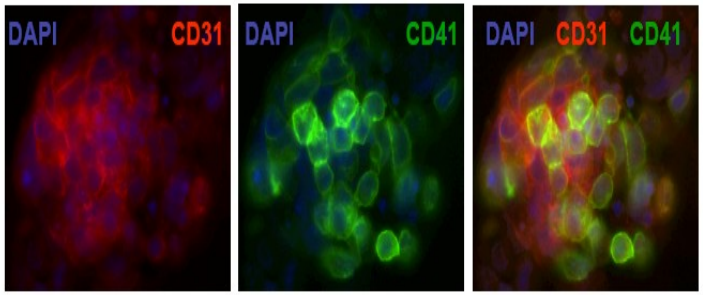
tight adherent structure



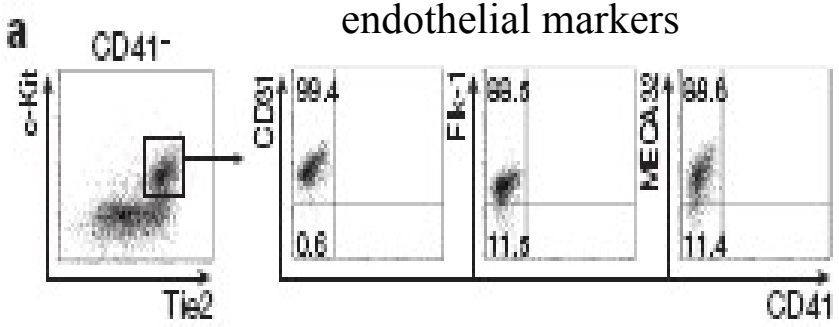


Tie2^{hi}c-KIT^{+}CD41^{-} can generate hematopoietic precursors

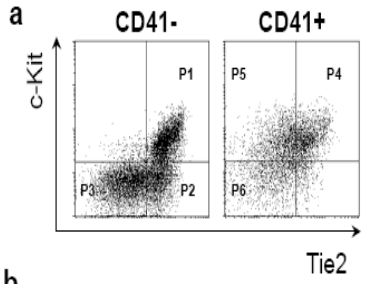
endothelia in matrigel

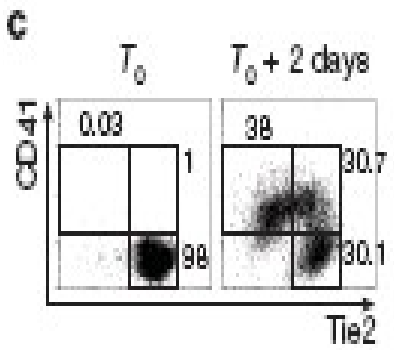


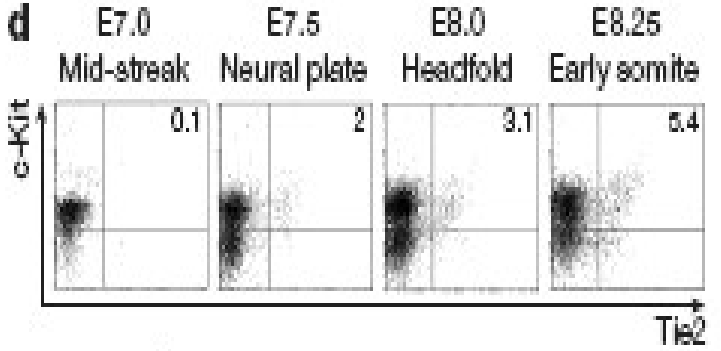
endothelial markers

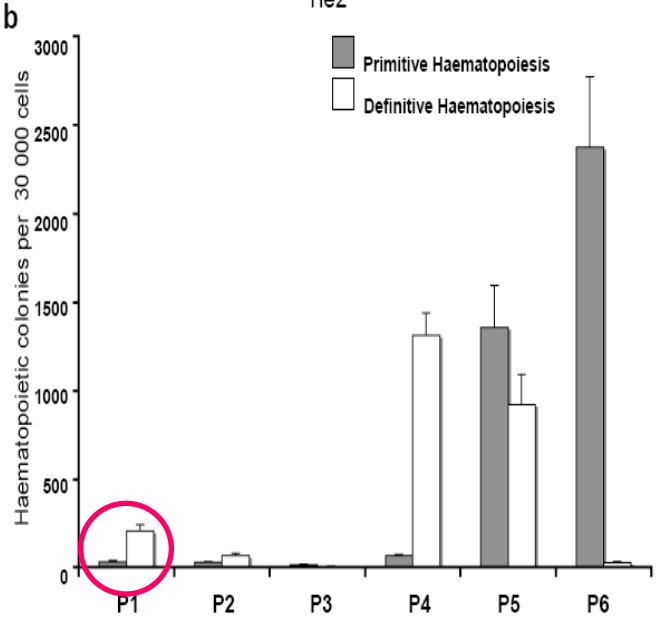


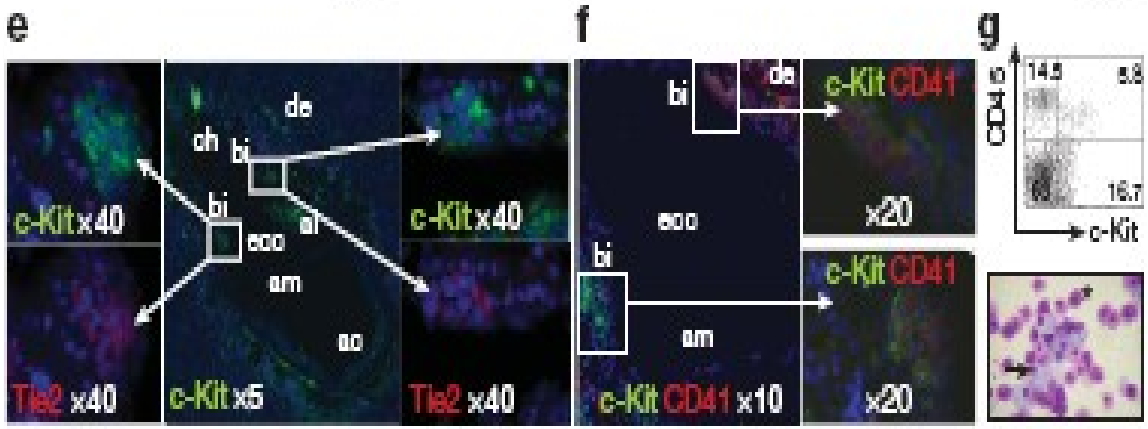
Tie2^{hi}c-KIT^{+}CD41^{-} are present in vivo by FACS



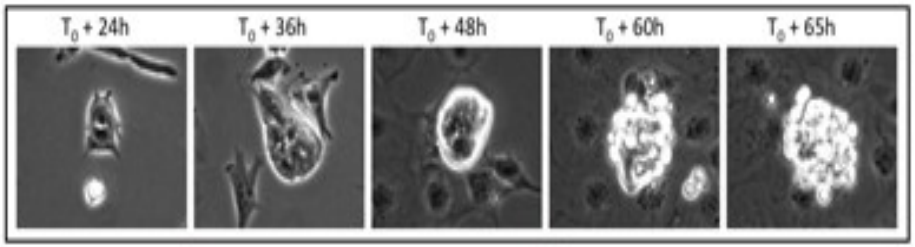






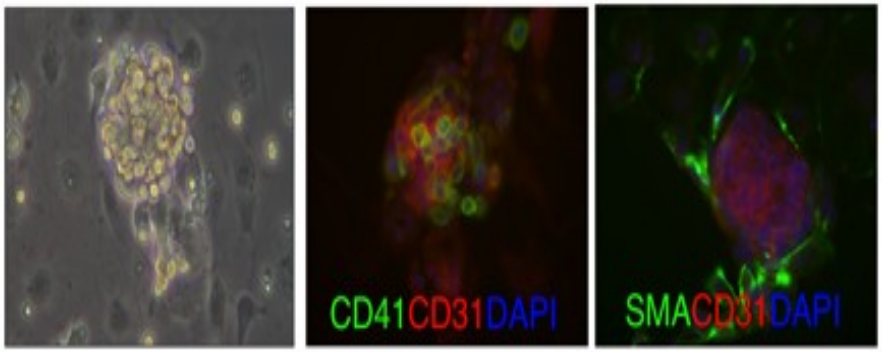


Tie2^{hi}c-KIT^{+}CD41^{-} are present in vivo by IHC

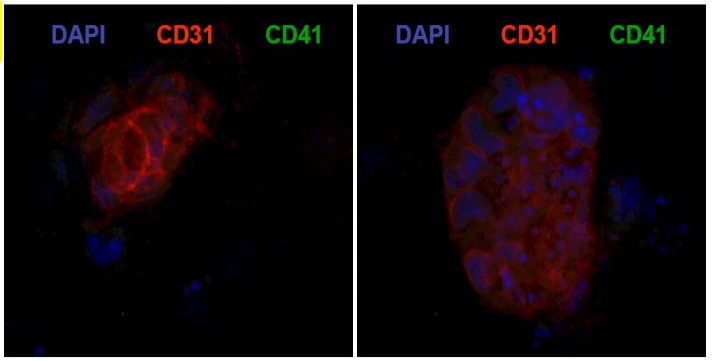


Generation of structure of tightly associated cells

Generation of round cells



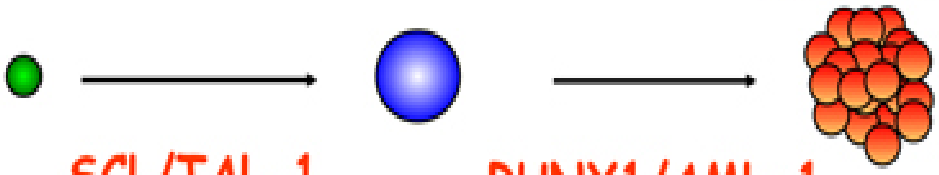
Runx1-/-



Haemangioblast

Haemogenic endothelium

Definitive Haematopoiet progenitors



SCL/TAL-1

RUNX1/AML-1

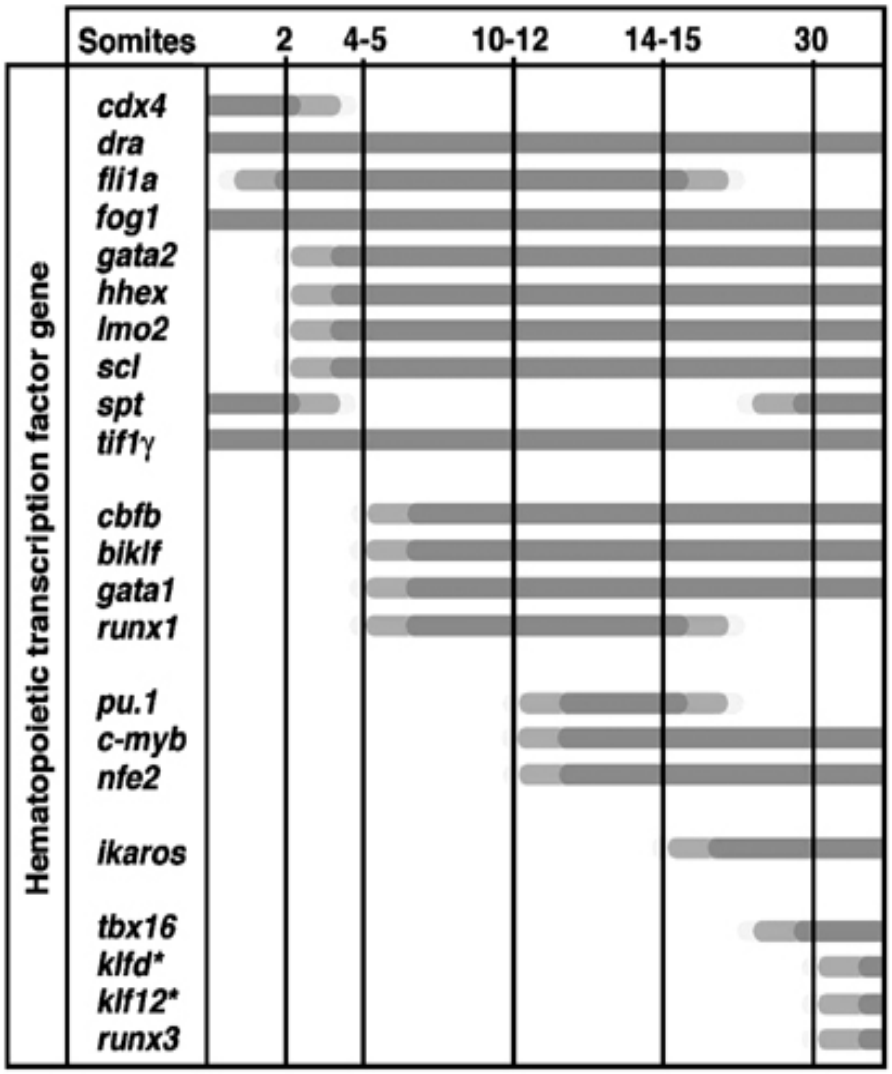
Flk1+
Bry-GFP+

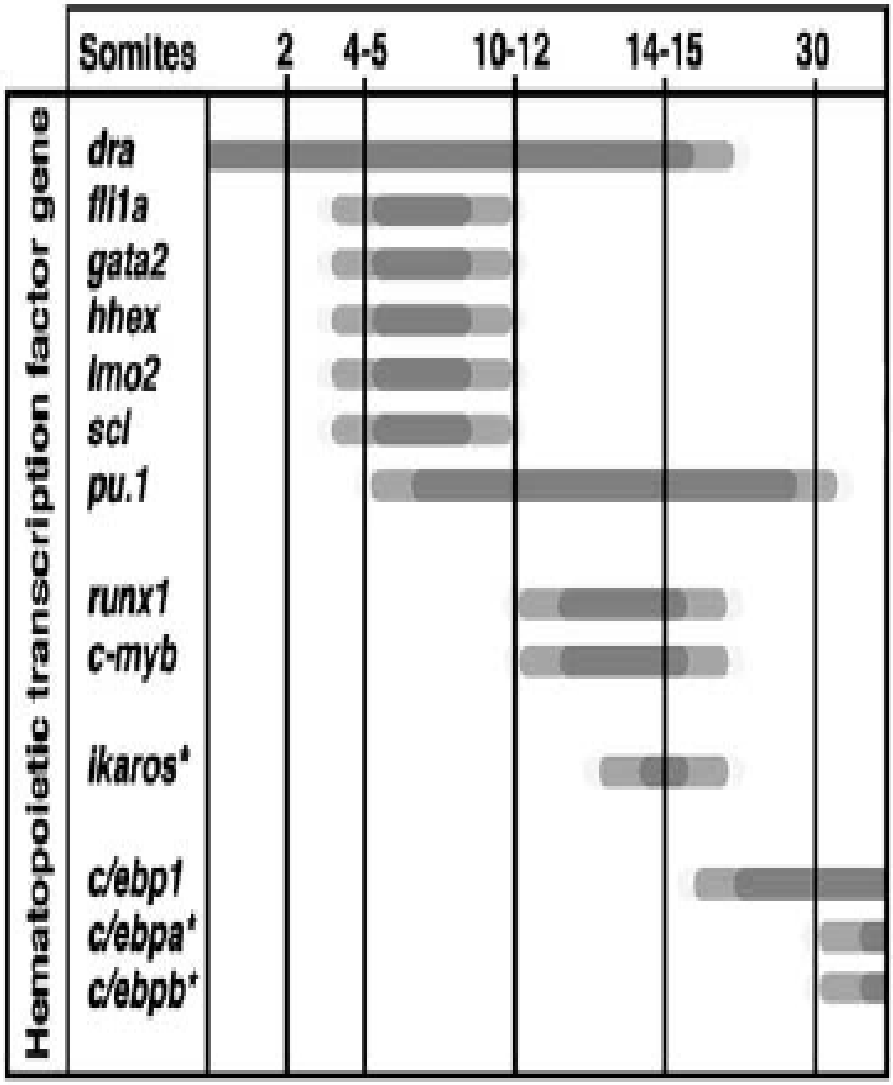
Tie2Hi
c-Kit+

CD41+
CD45+

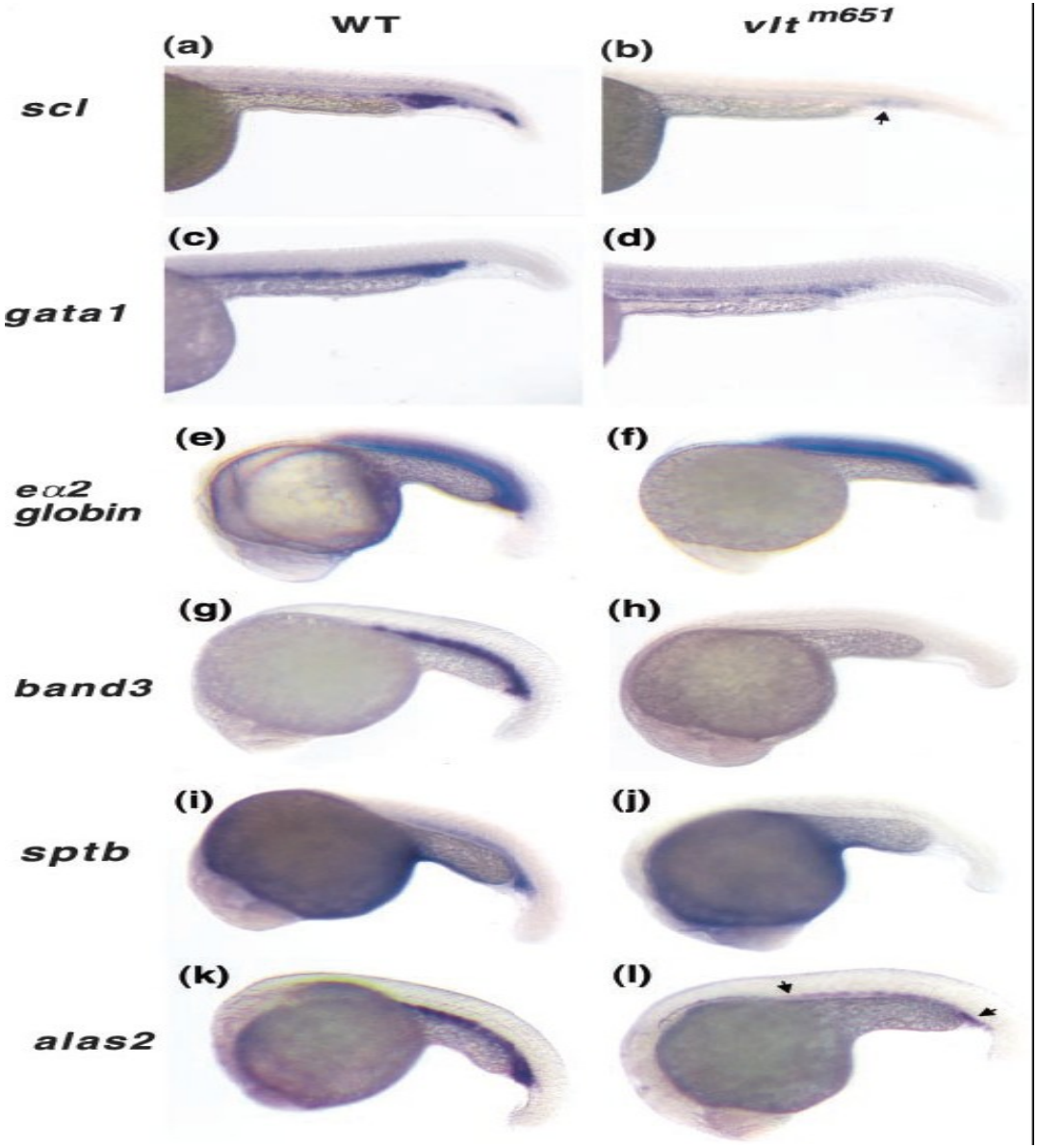
Blast movies 3, 4
Runx-/- movie

TRANSCRIPTIONAL FACTORS IN BLOOD DEVELOPMENT





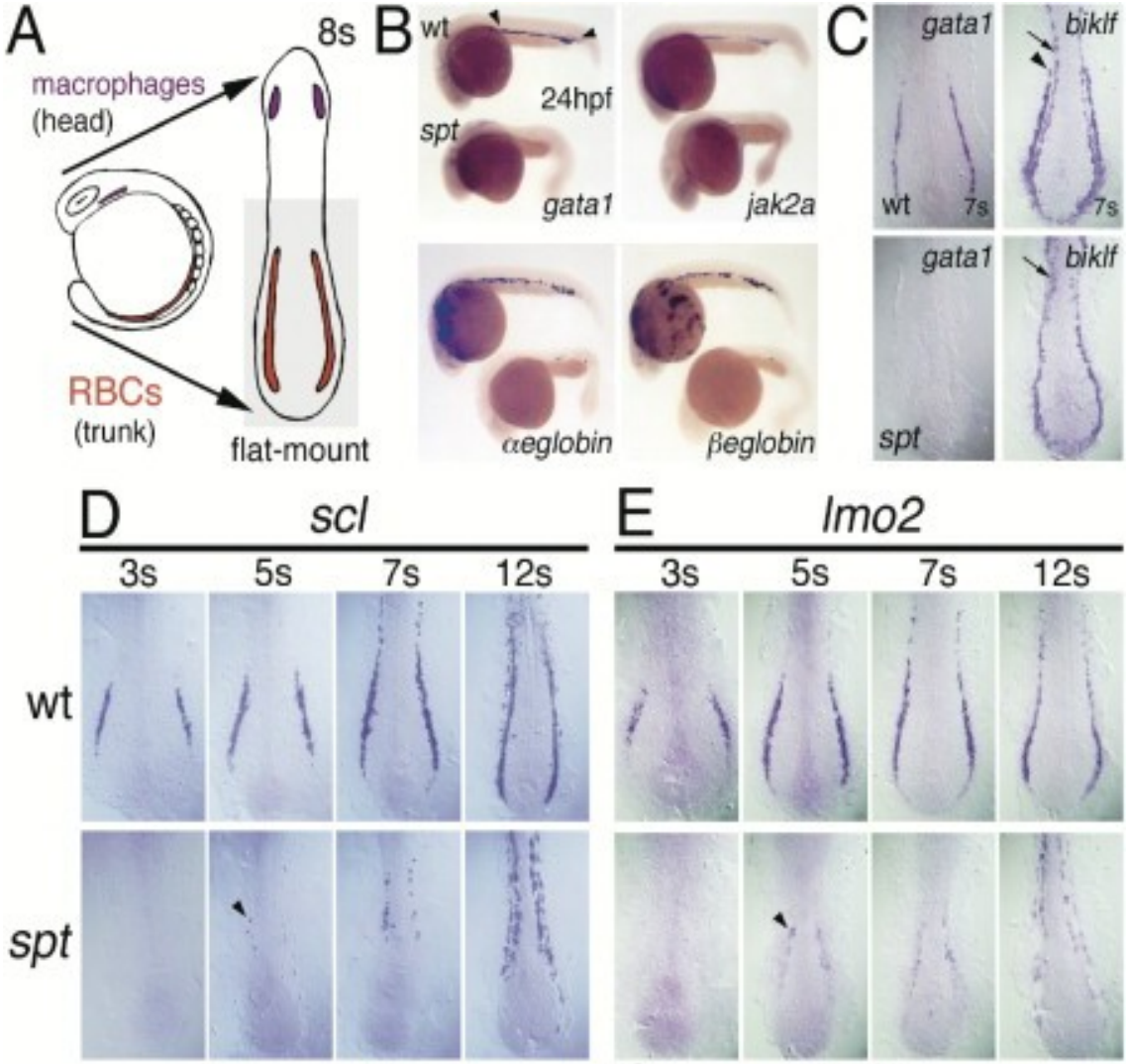
GATA-1 - binds DNA via a zinc finger motif



Dracula fish – loss-of-function mutation in GATA1 – impaired erythroid differentiation

Spadetail/TBX16

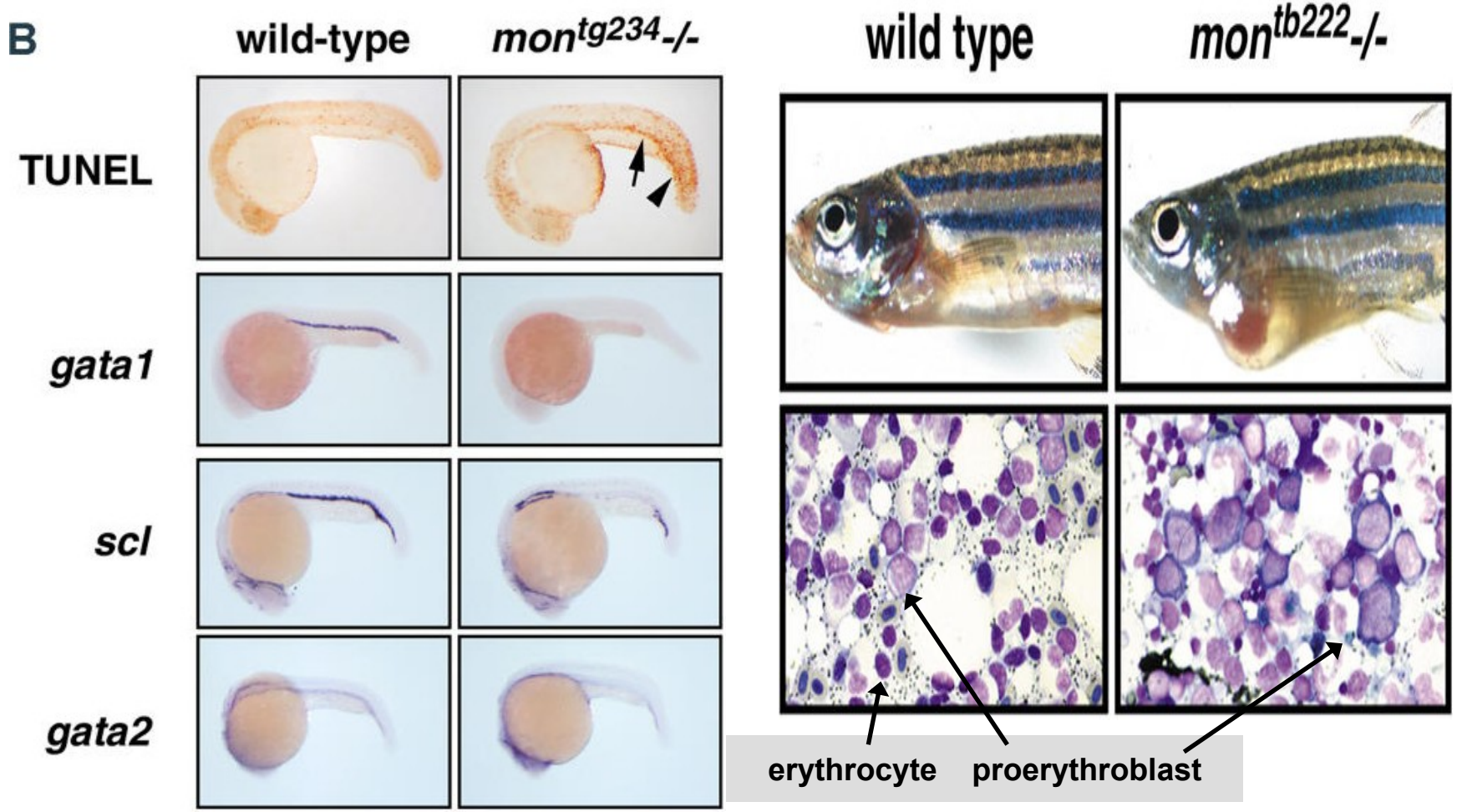
DNA binding domain derived from the prototype gene called transcription factor T



impaired erythroid but not myelopoietic differentiation

Moonshine

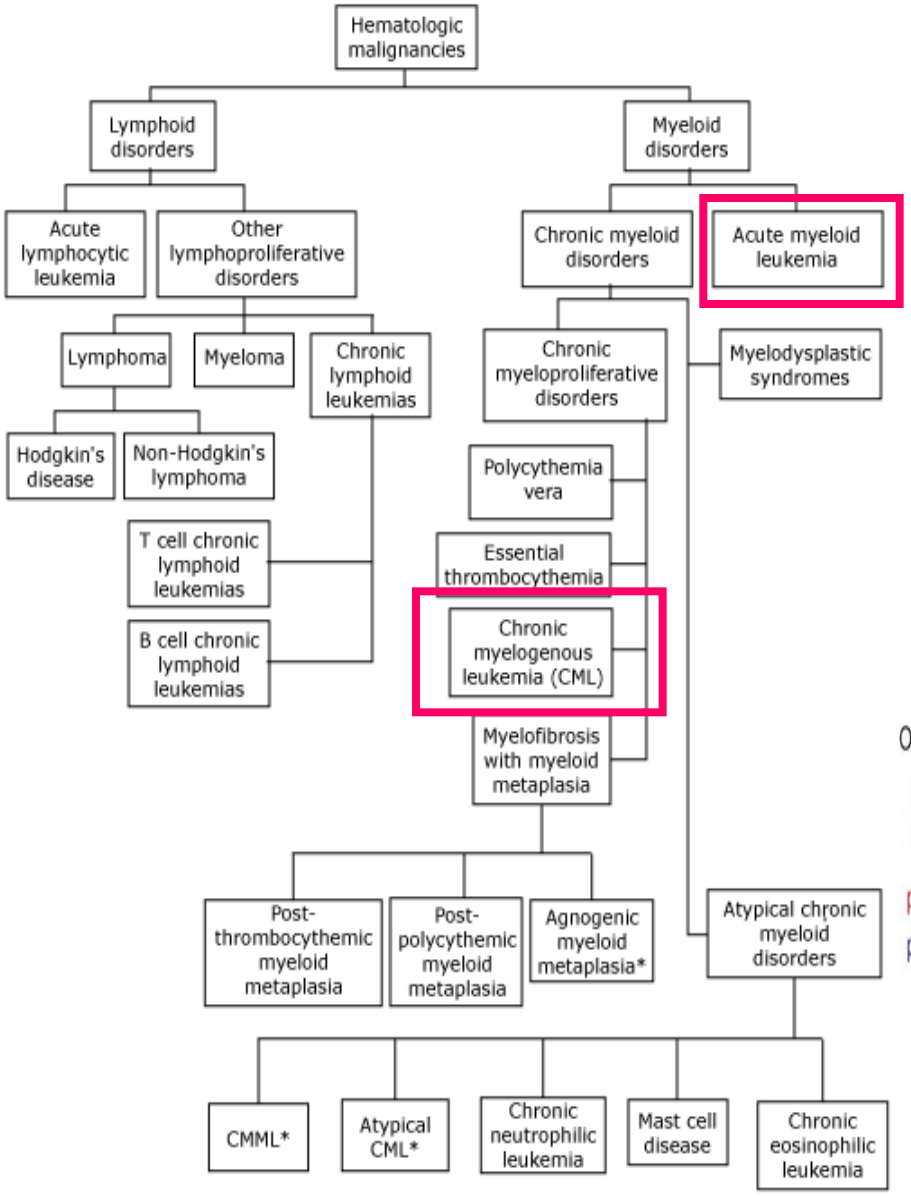
TIM-family of transcriptional factors

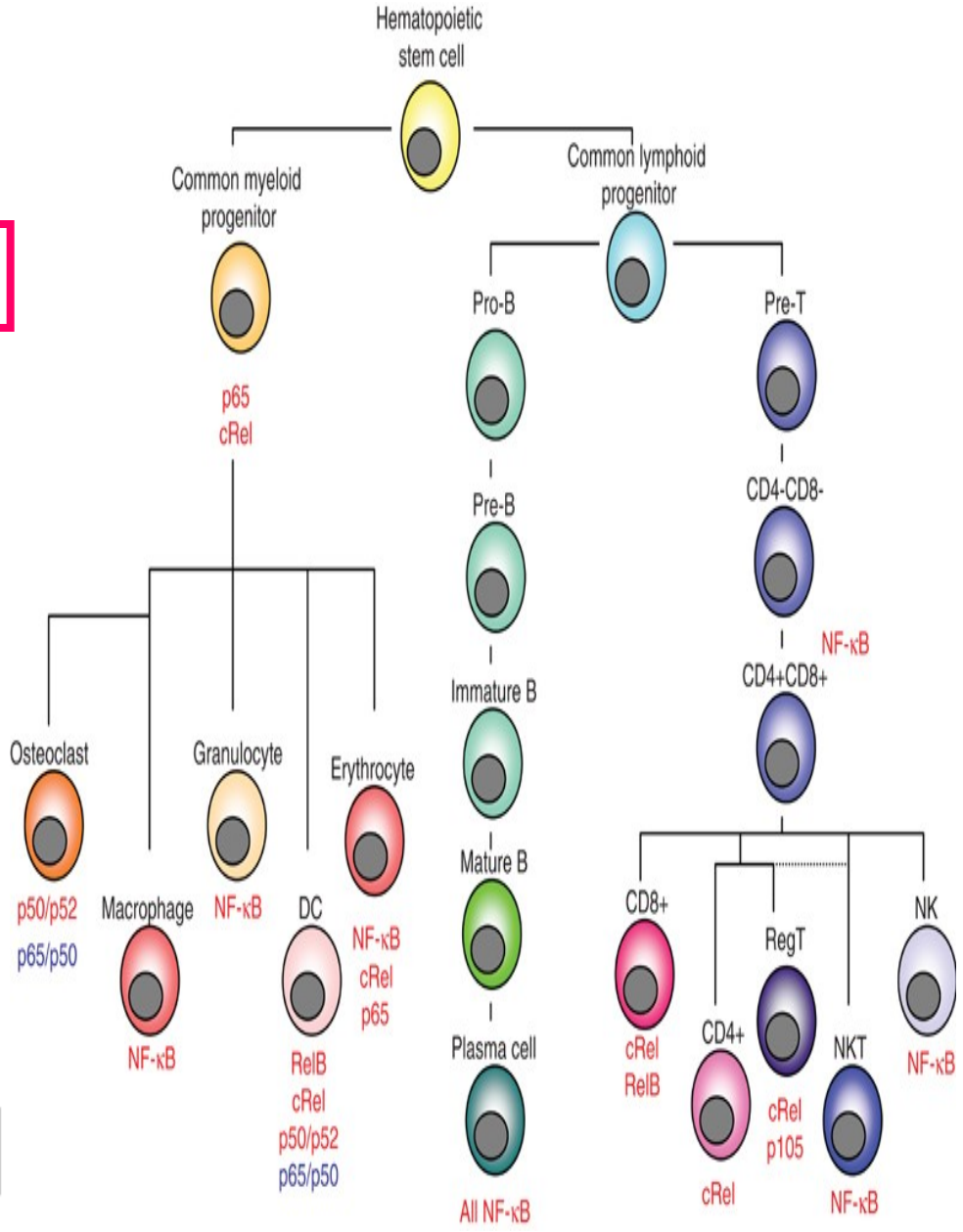


‘primitive’ – survival of HSC

‘definitive’ – cardiomegaly and impaired red cell differentiation

WHEN SOMETHING GOES WRONG WITH BLOOD





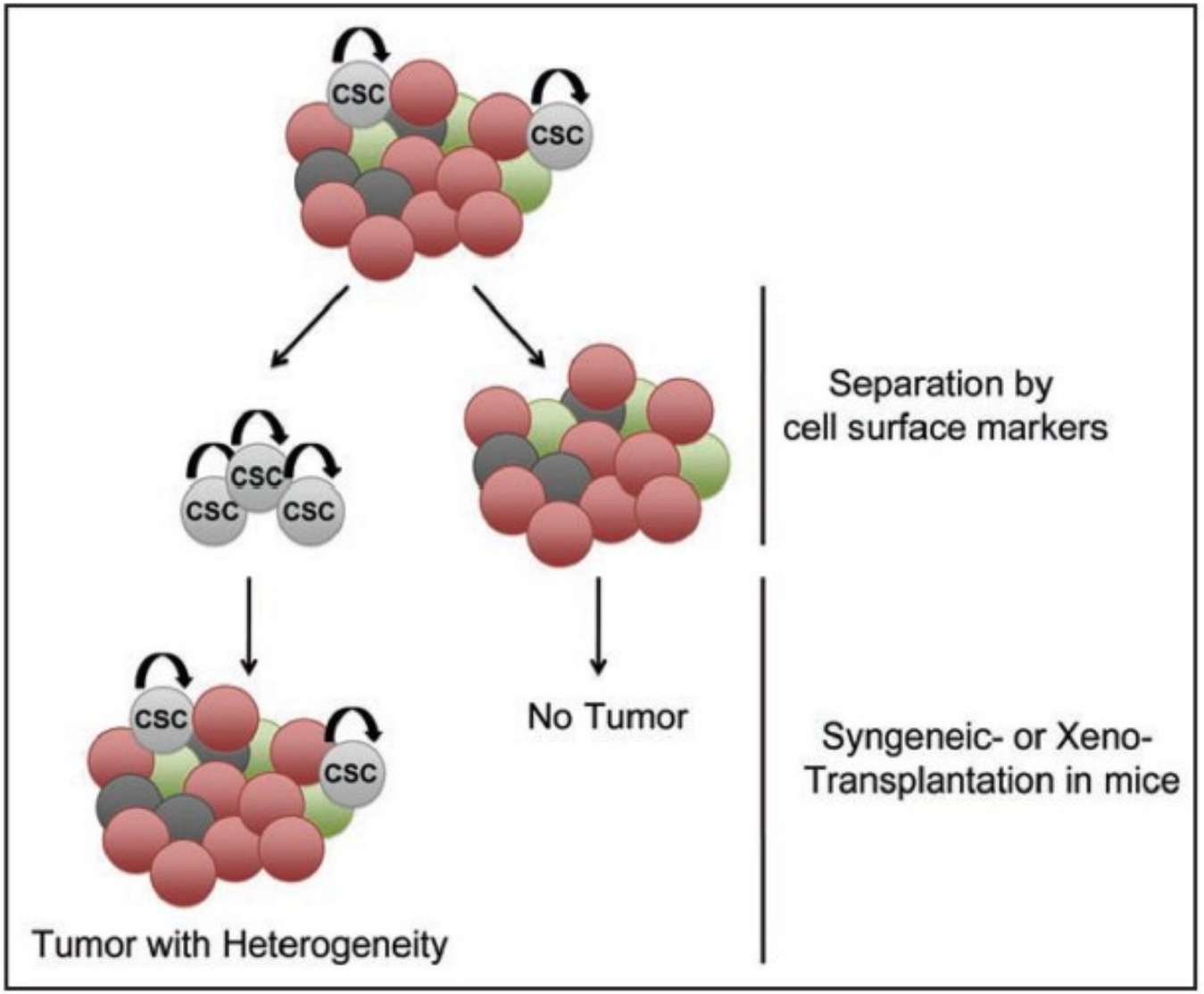
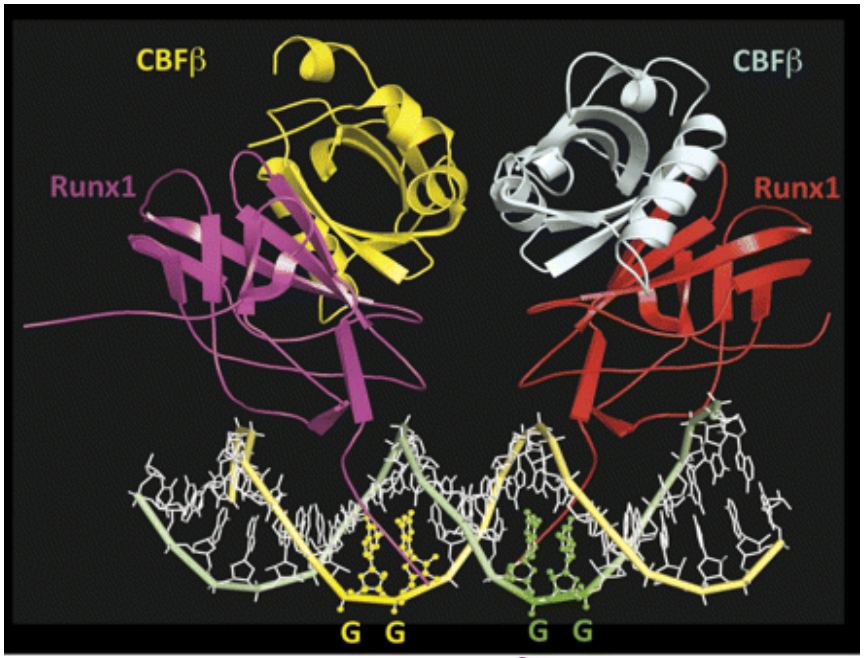
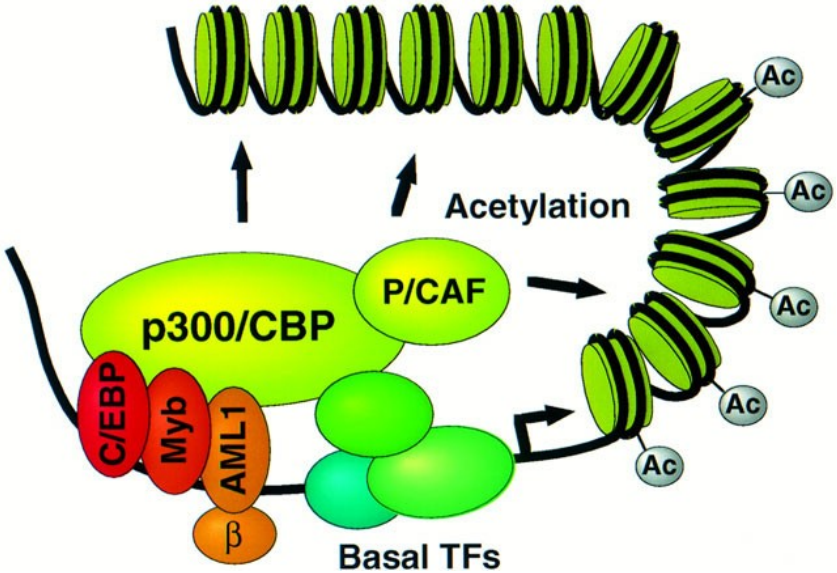


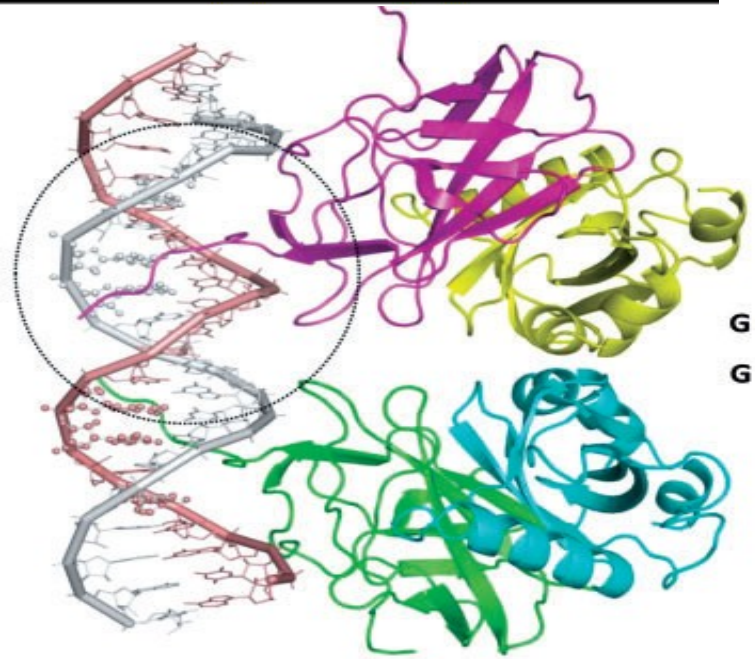
Figure 1.
The Cancer Stem Cell Hypothesis. Only a specific subset of tumor cells, i.e., the cancer stem cells (CSC), is capable of forming tumors and generating the heterogeneous population of cells in a tumor. Arrows indicate self-renewal potential that is unique to CSC.

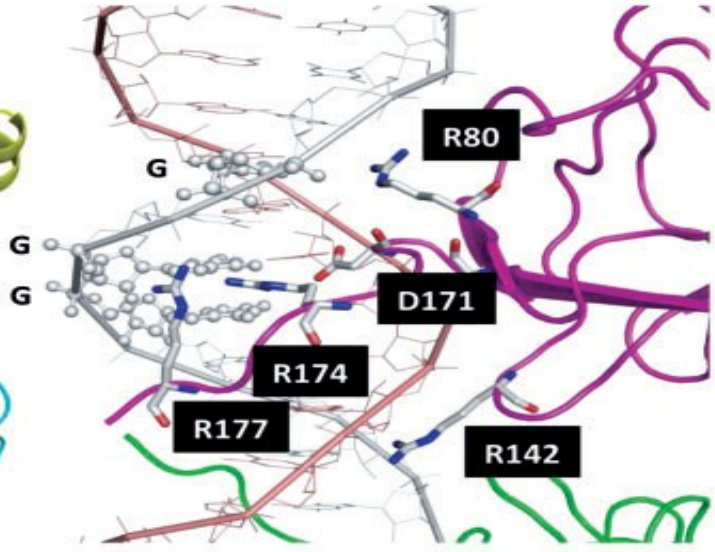
AML1 (RUNX1)


CBFβ
CBFβ
Runx1
Runx1
G G
G G


Ac
Ac
Ac
Ac
Ac
Acetylation
P/CAF
p300/CBP
C/EBP
Myb
AML1
β
Basal TFs

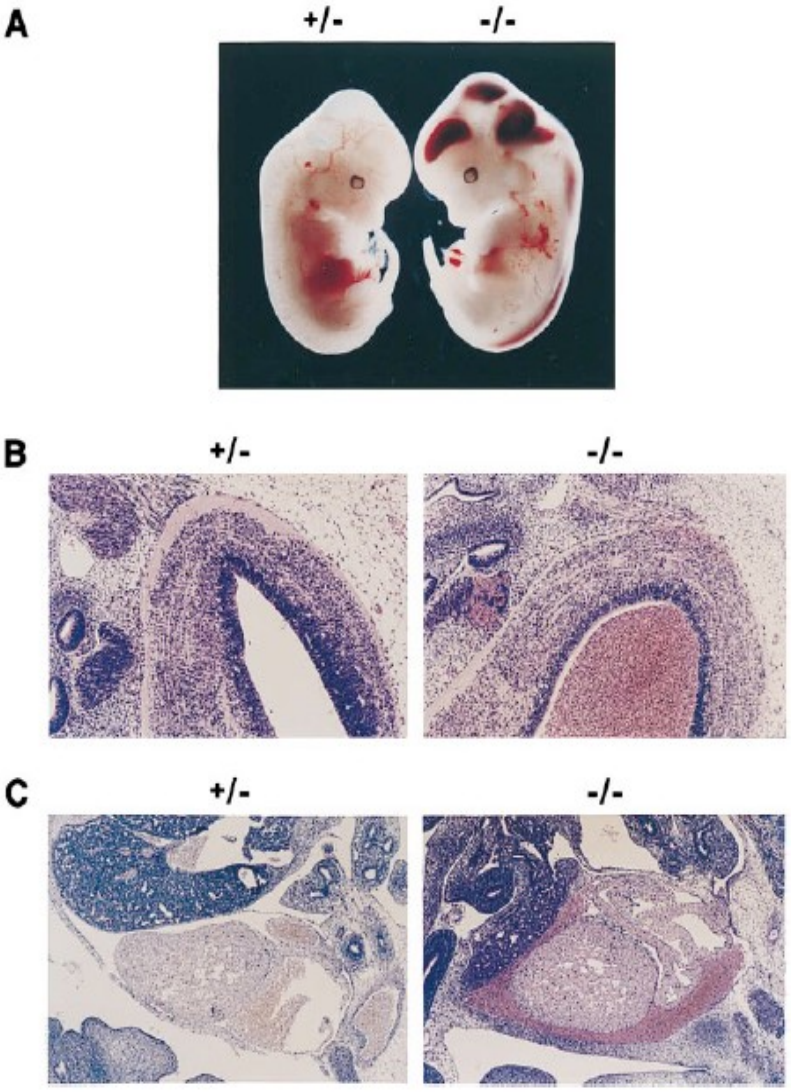
B

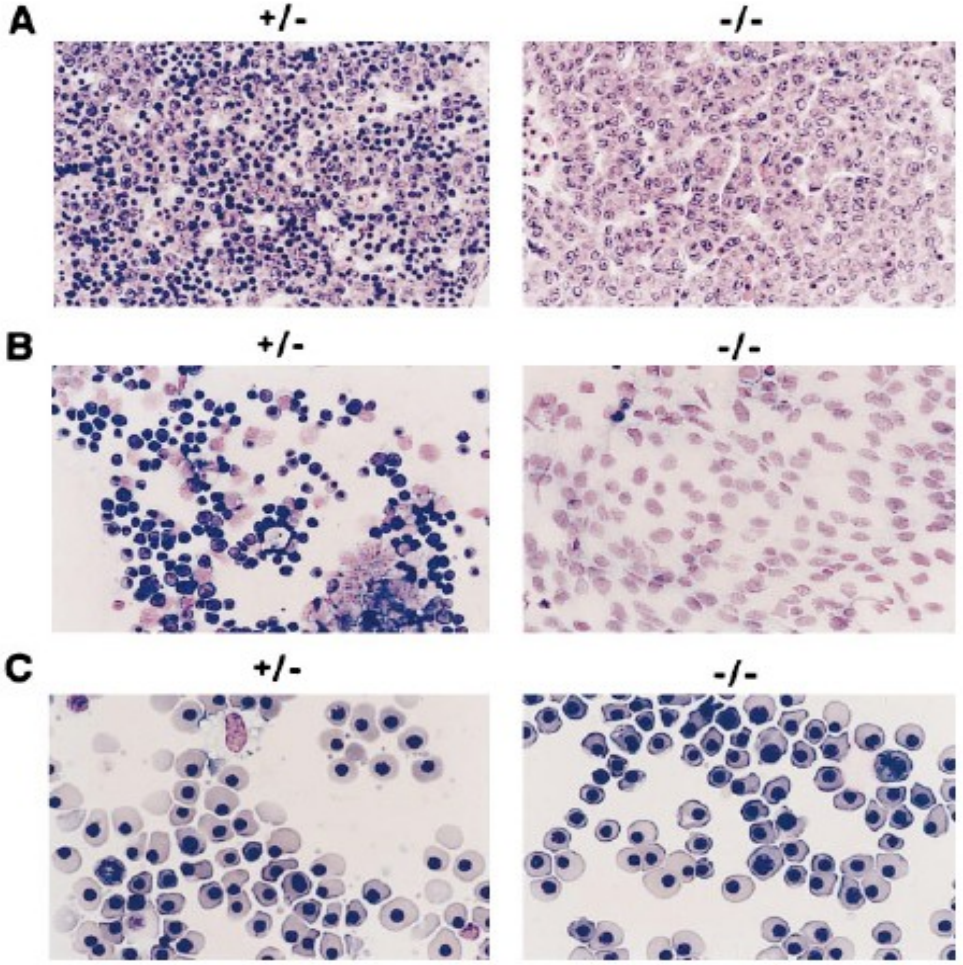



G
G
G
R80
D171
R174
R177
R142

AML1, the Target of Multiple Chromosomal Translocations in Human Leukemia, Is Essential for Normal Fetal Liver Hematopoiesis

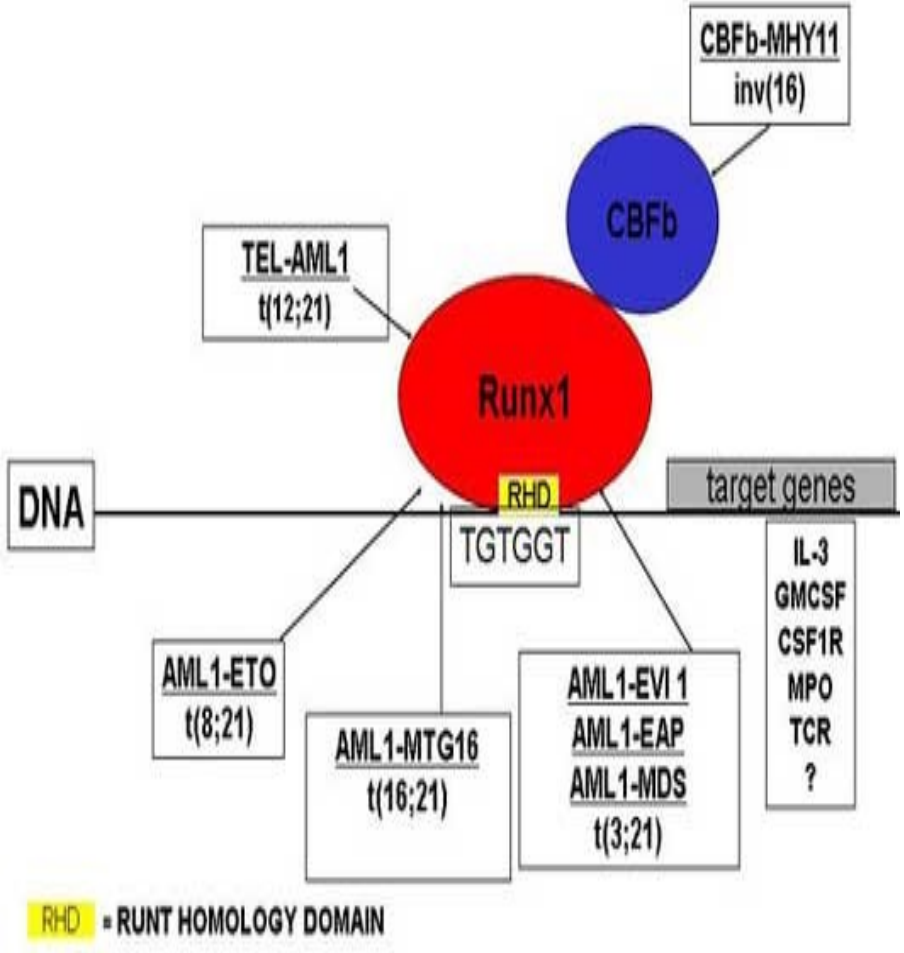
Cell, Vol. 84, 321–330, January 26, 1996,

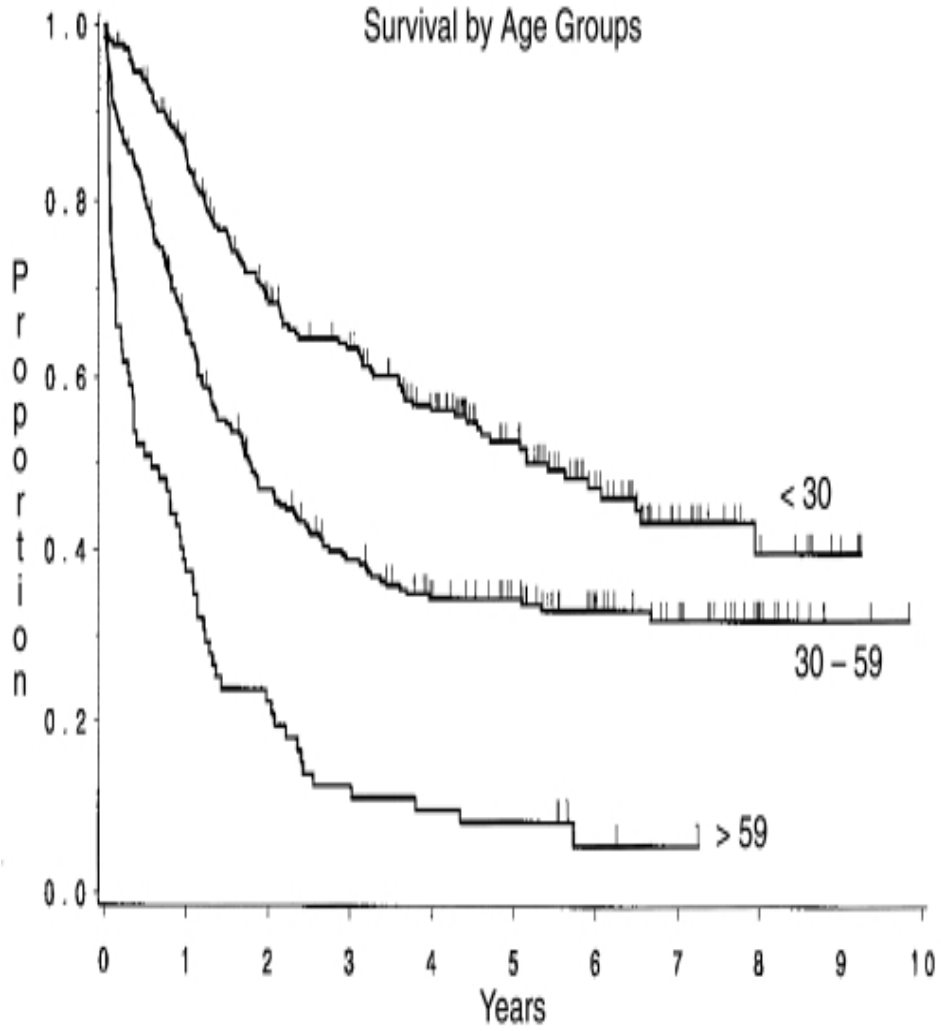




-no hematopoiesis in liver
-only primitive erythrocytes in peripheral blood, no platelets

ACUTE MYELOID LEUKEMIA





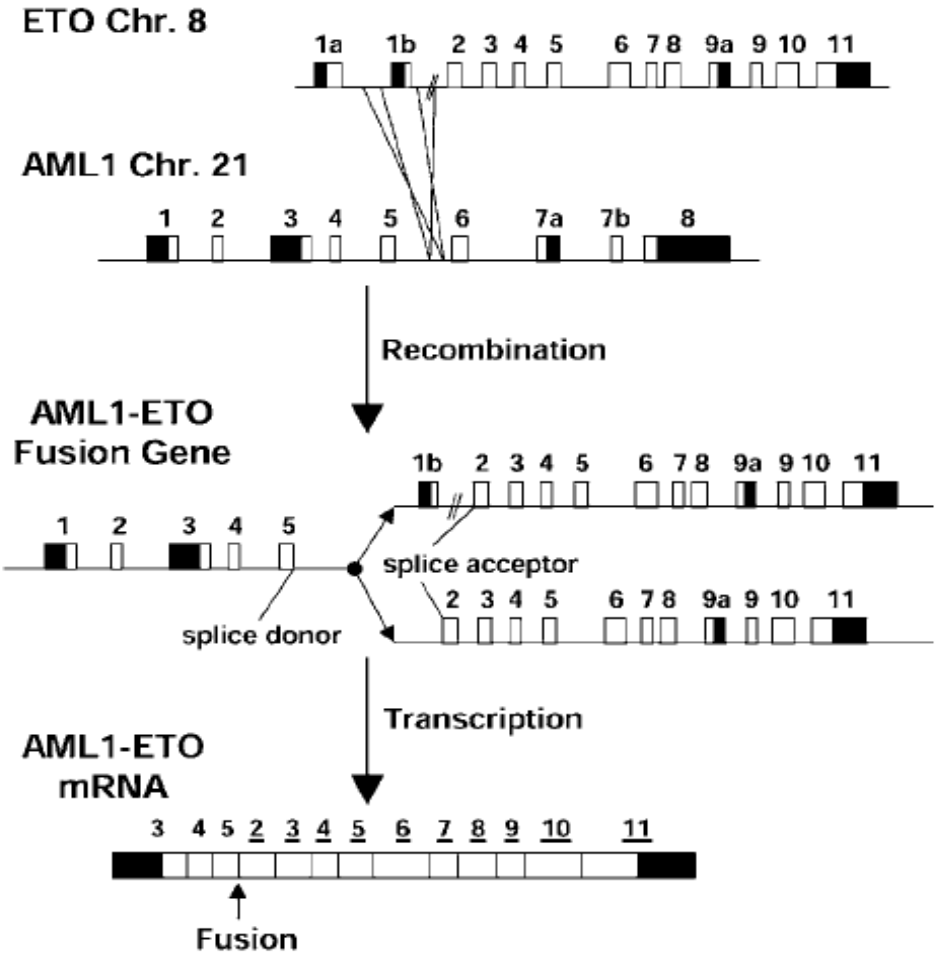


Figure 1 Genomic structure of t(8;21). Chromosome 8 containing the *ETO* gene is made up of 13 exons spanning approximately 87 kb, which can give four alternative splice forms and is regulated by two promoters. Chromosome 21 contains the *AML1* gene with nine exons that give various alternative splice forms and is regulated by two promoters and spans 260 kb. The breakpoint cluster areas are denoted by the crossing lines between ETO and AML1. Owing to the absence of a splice acceptor in exon 1b of ETO, the mRNA of the fusion transcripts does not include this exon. White boxes and black boxes indicate translated and untranslated exon sequences, respectively. Underlined numbers in the AML1-ETO mRNA denote exons contributed by the *ETO* gene

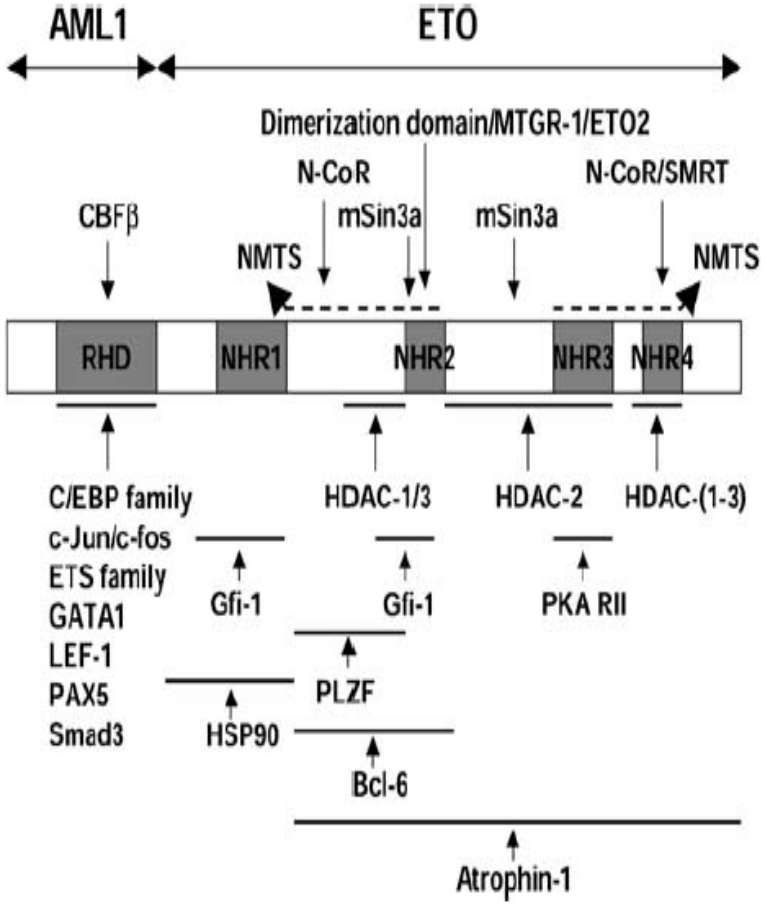


Figure 2 AML1-ETO-interacting proteins. AML1-ETO contains the N-terminal sequences of AML1, including the RHD. Subsequent a.a.'s are of ETO, containing the four NHR1–4. Indicated as well are the regions containing the NMTS of ETO (broken line arrows). Known RHD and ETO/AML1-ETO-interacting proteins (or family of proteins) are shown. The dimerization domain of AML1-ETO and ETO family members is located in NHR2

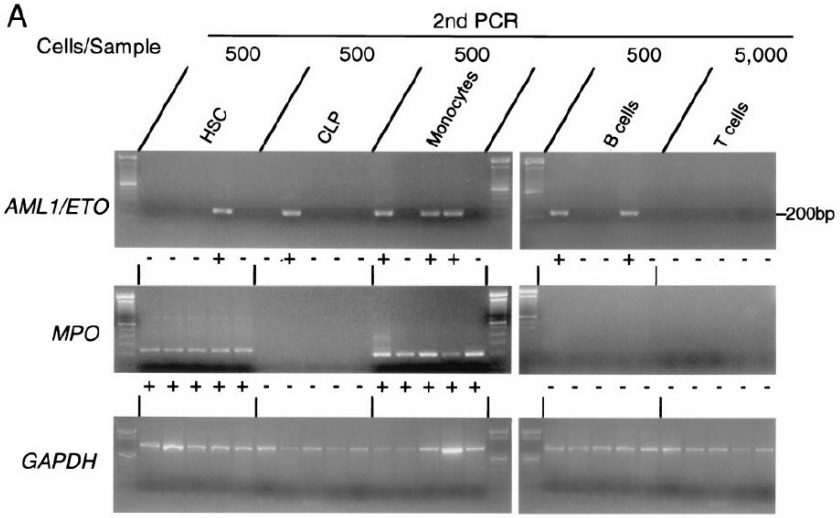
AML1/ETO-expressing nonleukemic stem cells in acute myelogenous leukemia with 8;21 chromosomal translocation

PNAS | June 20, 2000 | vol. 97 | no. 13 | 7521–7526

Table 3. *AML1/ETO* mRNA in FACS-purified cells in leukemic phase

	No. of AML1/ETO$^+$ samples/no. of samples tested			
Case	$CD34^+CD38^{-/lo}$ (500 cells)	$CD34^+CD38^+$ (500 cells)	B cells (500 cells)	T cells (5,000 cells)
1	11/11	5/5	4/14	0/10
3	64/64	5/5	2/2	ND
4	10/10	5/5	3/15	0/9
5a	4/4	5/5	5/75	0/20
6a	30/30	5/5	5/40	0/21
Total	119/119 (100)	25/25 (100)	19/146 (13)	0/60 (0)

Pooled 500 $CD34^+$ (Thy-1^-)$CD38^{-/lo}$ cells, $CD34^+$(Thy-1^-) $CD38^+$ cells and B cells or 5,000 T cells were analyzed by RT-PCR for *AML1/ETO* mRNA. Representative data are shown in Fig. 2*B*.



		No. of AML1/ETO$^+$ samples/no. of samples tested				
Case	*AML1/ETO* mRNA (10^5 BM cells)	HSC (500 cells)	CLP (500 cells)	Monocytes (500 cells)	B cells (500 cells)	T cells (5,000 cells)
5b	+	0/3	0/8	4/29	4/205	0/24
6b	+	0/8	0/3	3/32	3/52	0/20
12	+	1/12	0/23	2/11	1/82	0/25
13	+	1/20	0/5	ND	ND	0/10
15	+	3/26	0/8	1/7	2/81	0/6
16	+	1/20	0/30	2/9	2/37	0/13
17	+	0/4	0/2	ND	1/64	0/2
18	+	0/3	0/2	1/3	0/16	0/5
20	+	1/9	0/7	2/17	2/140	0/6
22	+	1/27	1/30	3/13	3/149	0/6
23	+	2/17	1/8	2/10	6/230	0/10
24	+	0/11	0/4	3/16	2/41	0/9
25	+	0/12	0/3	ND	0/26	0/9
	Total	10/172 (5.8)	2/133 (1.5)	23/147 (15.6)	26/1123 (2.3)	0/145 (0)

Each population was purified by a five-color FACS sorting and pooled either 500 cells (HSC, CLP, monocytes, and B cells) or 5,000 cells (T cells) were analyzed by RT-PCR for *AML1/ETO* mRNA. Representative data are shown in Fig. 2*A*. ND, not done.

Stem cell expression of the AML1/ETO fusion protein induces a myeloproliferative disorder in mice

Sca1 – locus active in hematopoietic stem cells in adult mice

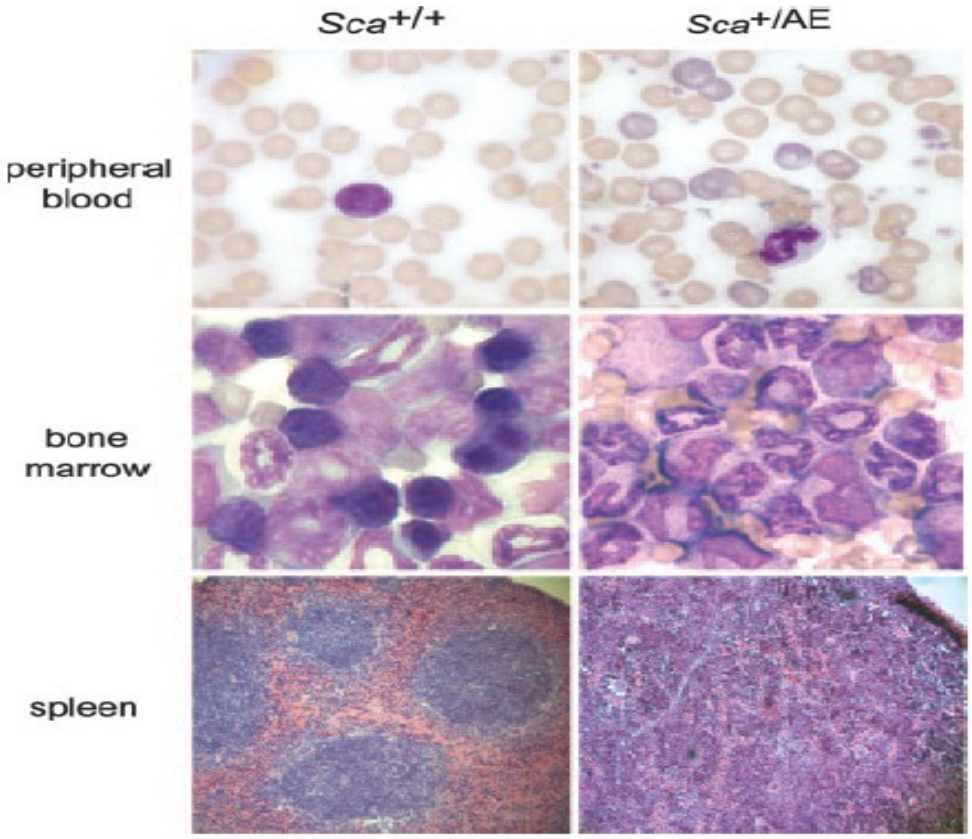


Fig. 4. Morphologic analysis of MPD. Peripheral blood and bone marrow slides show evidence of myeloid hyperplasia with loss of erythroid and lymphoid precursors in the bone marrow and polychromasia in peripheral blood of $Sca^{+/AE}$ mice compared with WT littermate mice. Increased extramedullary hematopoiesis with disruption of follicular architecture is evident in the spleens of $Sca^{+/AE}$ mice.

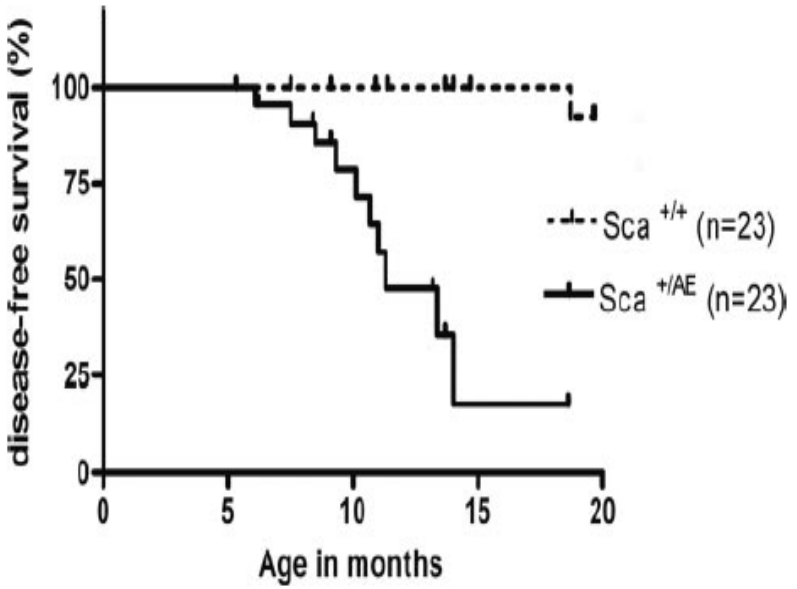
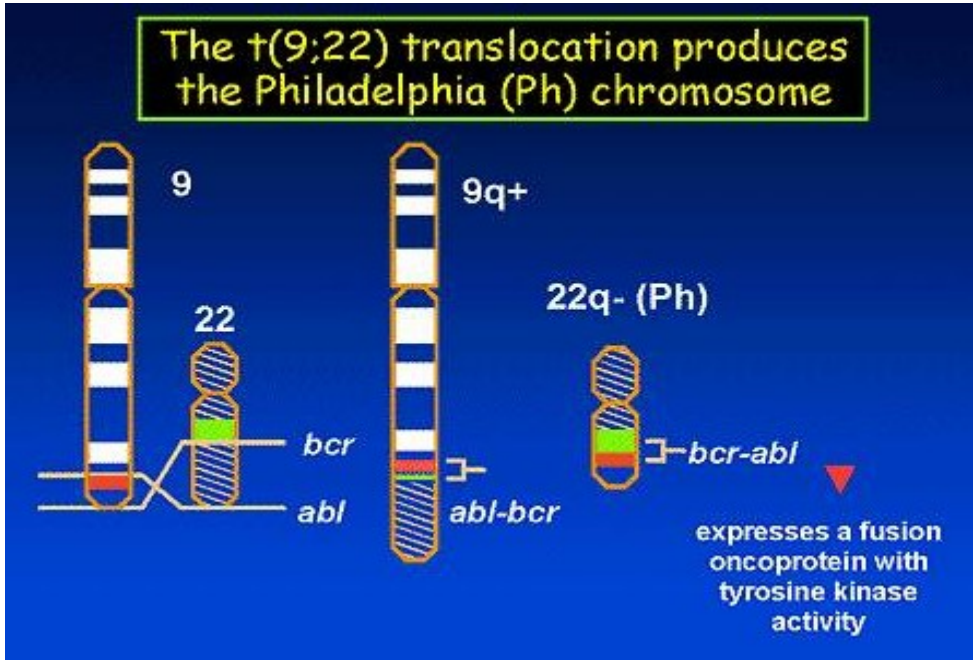
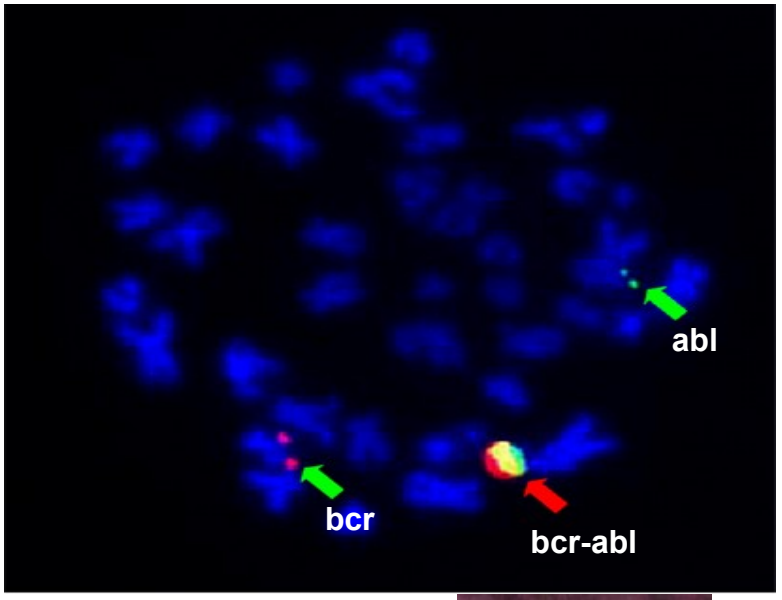
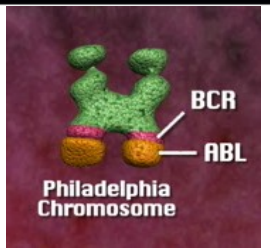


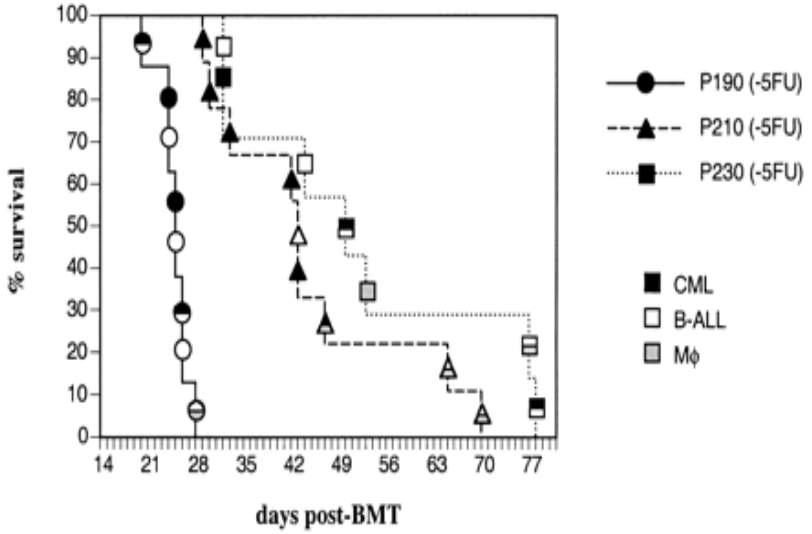
Fig. 5. Survival analysis. Kaplan–Meier plot demonstrates high penetrance and long latency of a nonlethal MPD in $Sca^{+/AE}$ mice compared with WT littermates ($P < 0.0001$).

CHRONIC MYELOID LEUKEMIA



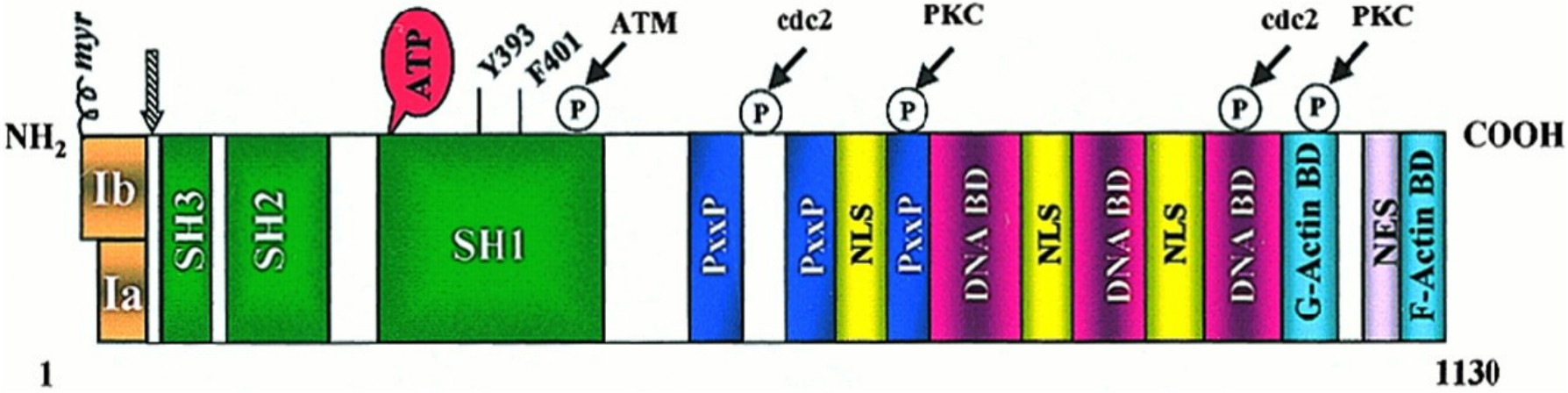




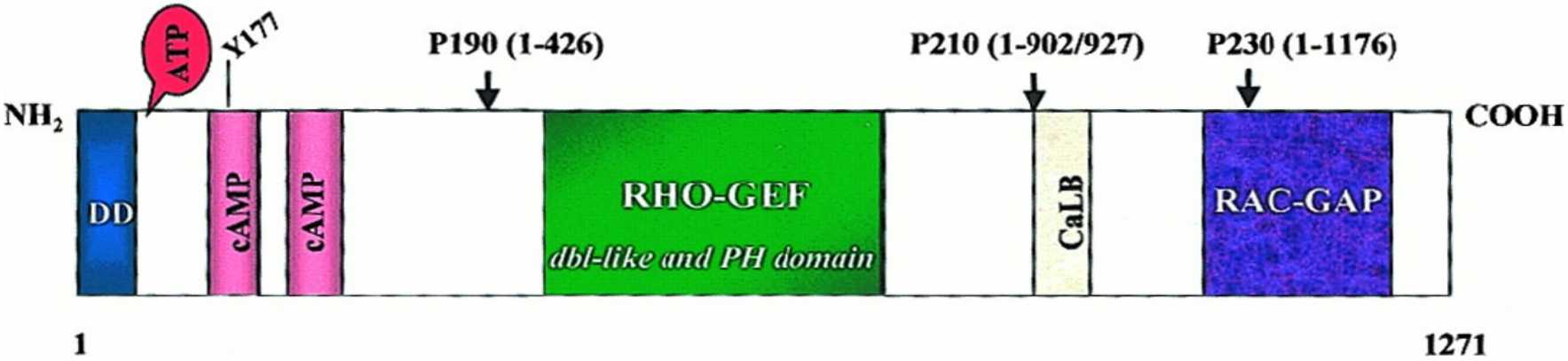


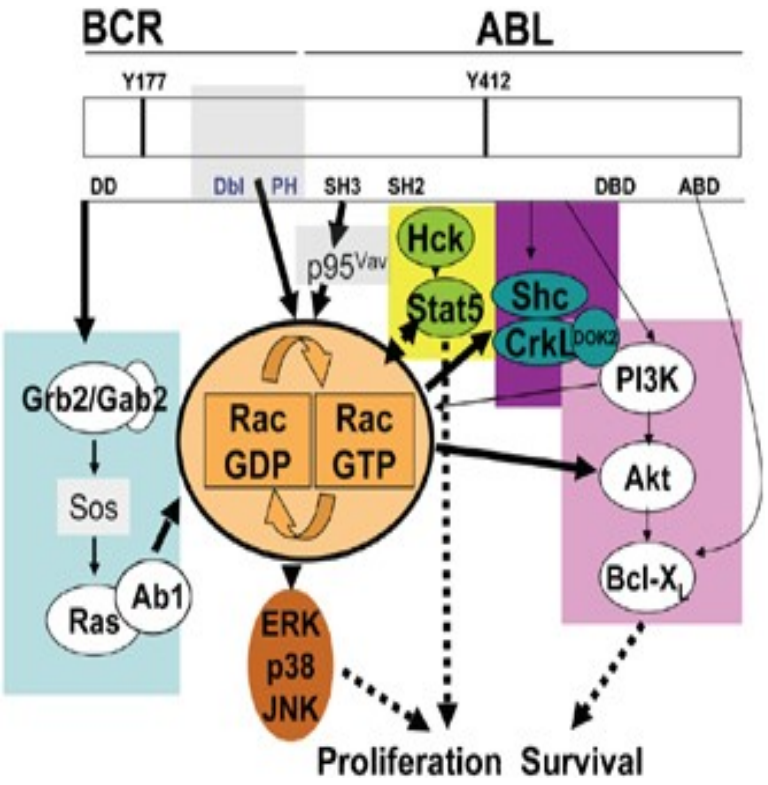
mice transplanted with patient bone marrow

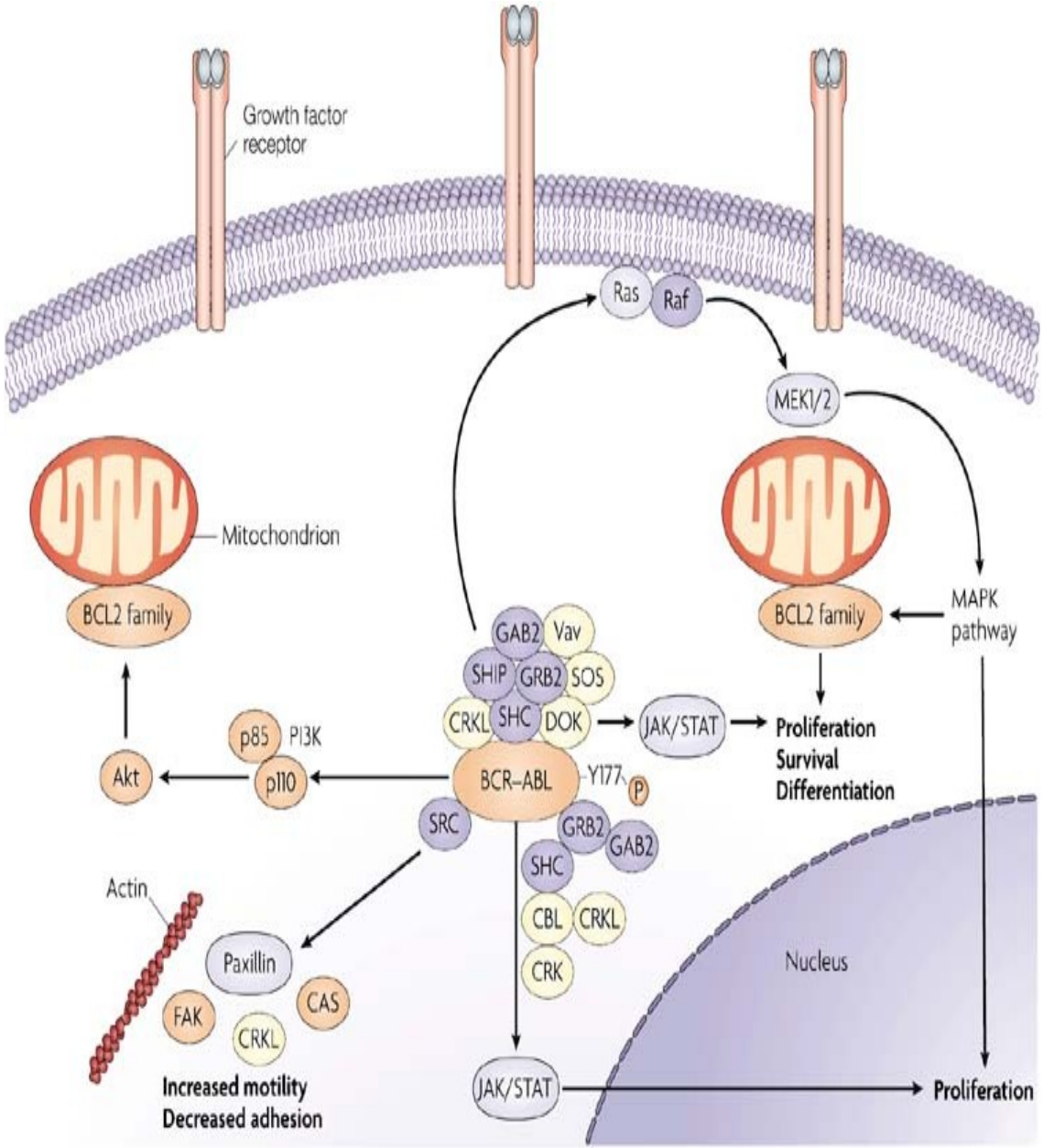
p145 *ABL*



p160 *BCR*




BCR
ABL
Y177
Y412
DD
Dbl
PH
SH3
SH2
DBD
ABD
p95Vav
Hck
Stat5
Shc
CrkL
DOK2
PI3K
Akt
Bcl-XL
Grb2/Gab2
Sos
Ras
Ab1
Rac GDP
Rac GTP
ERK
p38
JNK
Proliferation
Survival


Growth factor receptor
Ras
Raf
MEK1/2
Mitochondrion
BCL2 family
BCL2 family
MAPK pathway
GAB2
Vav
SHIP
GRB2
SOS
CRKL
SHC
DOK
JAK/STAT
Proliferation
Survival
Differentiation
p85
PI3K
p110
Akt
BCR–ABL
Y177
P
SRC
GRB2
GAB2
SHC
CBL
CRKL
CRK
Actin
Paxillin
FAK
CRKL
CAS
Increased motility
Decreased adhesion
Nucleus
JAK/STAT
Proliferation

Am J Med. 1977 Jul;63(1):125-30. **Chronic myelocytic leukemia: clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage.** Fialkow PJ, Jacobson RJ, Papayannopoulou T.

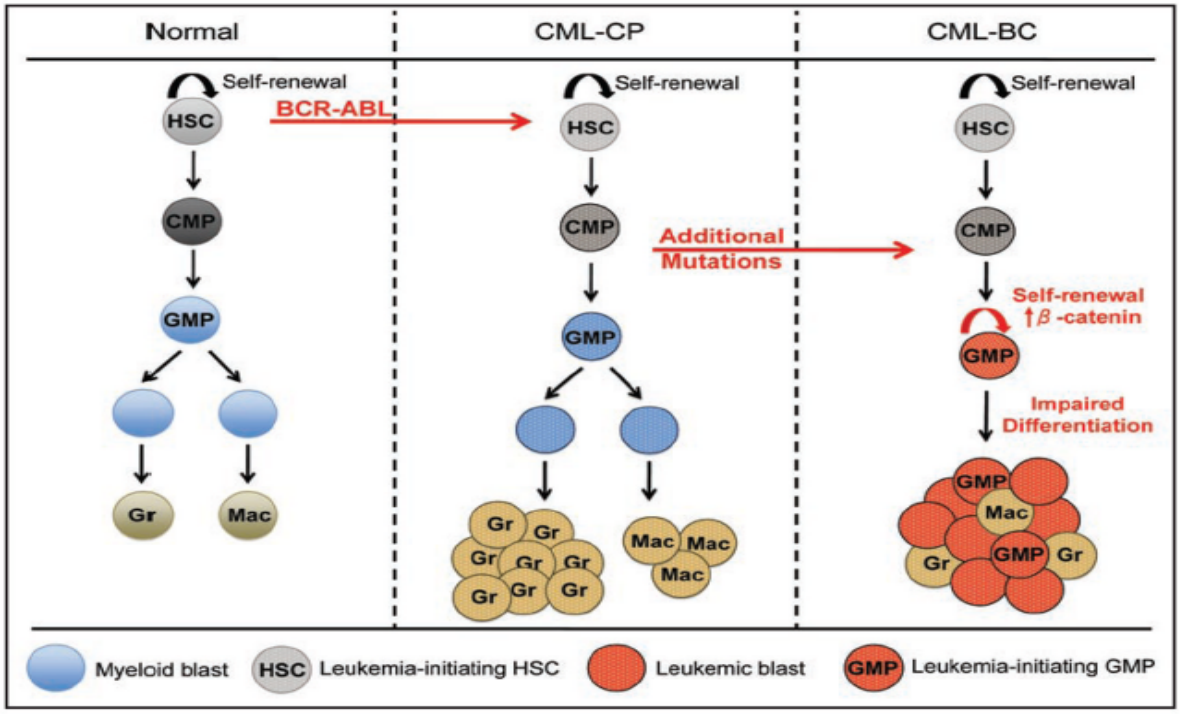


Figure 2.
Evolution of Leukemic Stem Cells in CML Disease Progression. In normal hematopoiesis, the hematopoietic stem cell (HSCs) gives rise to all other lineages of hematopoietic cells. In CML chronic phase (CP) patients, expression of BCR-ABL in the HSC compartment leads to an expansion of the myeloid lineage resulting in an abnormal number of mature granulocytes. The HSC likely functions as the leukemia-initiating cell during CML-CP. Transition to blast crisis (BC) involves additional genetic and epigenetic alterations leading to the accumulation of immature blasts. Progression to blast crisis also results in the acquisition of self-renewal potential by a GMP population with elevated β-catenin activity. In a mouse model, as few as fifty cells from the BCR-ABL-transformed GMP compartment can initiate a CML-like disease. GMP from CML-BC patients also initiate leukemia when transplanted into immunocompromised mice. Arrows mark critical events in disease progression and self-renewal potential. Dotted circles indicate BCR-ABL-positive cells. (HSC, hematopoietic stem cell; CMP, common myeloid progenitor; GMP, granulocyte-macrophage progenitor; Gr, granulocyte; Mac, macrophage).

Cell Cycle. 2009 May 1; 8(9): 1338–1343.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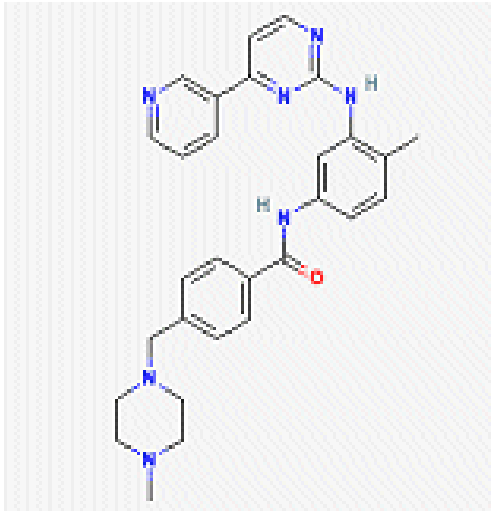
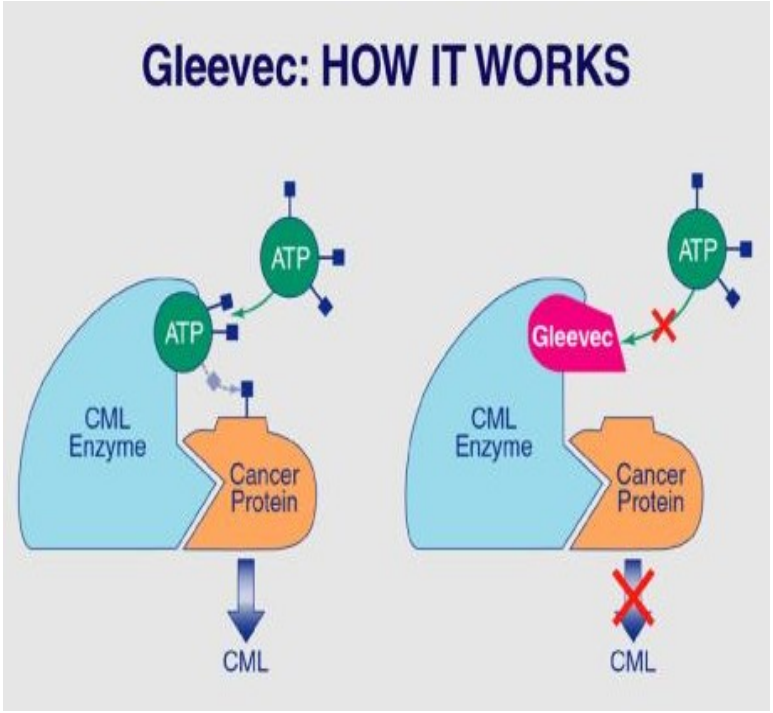
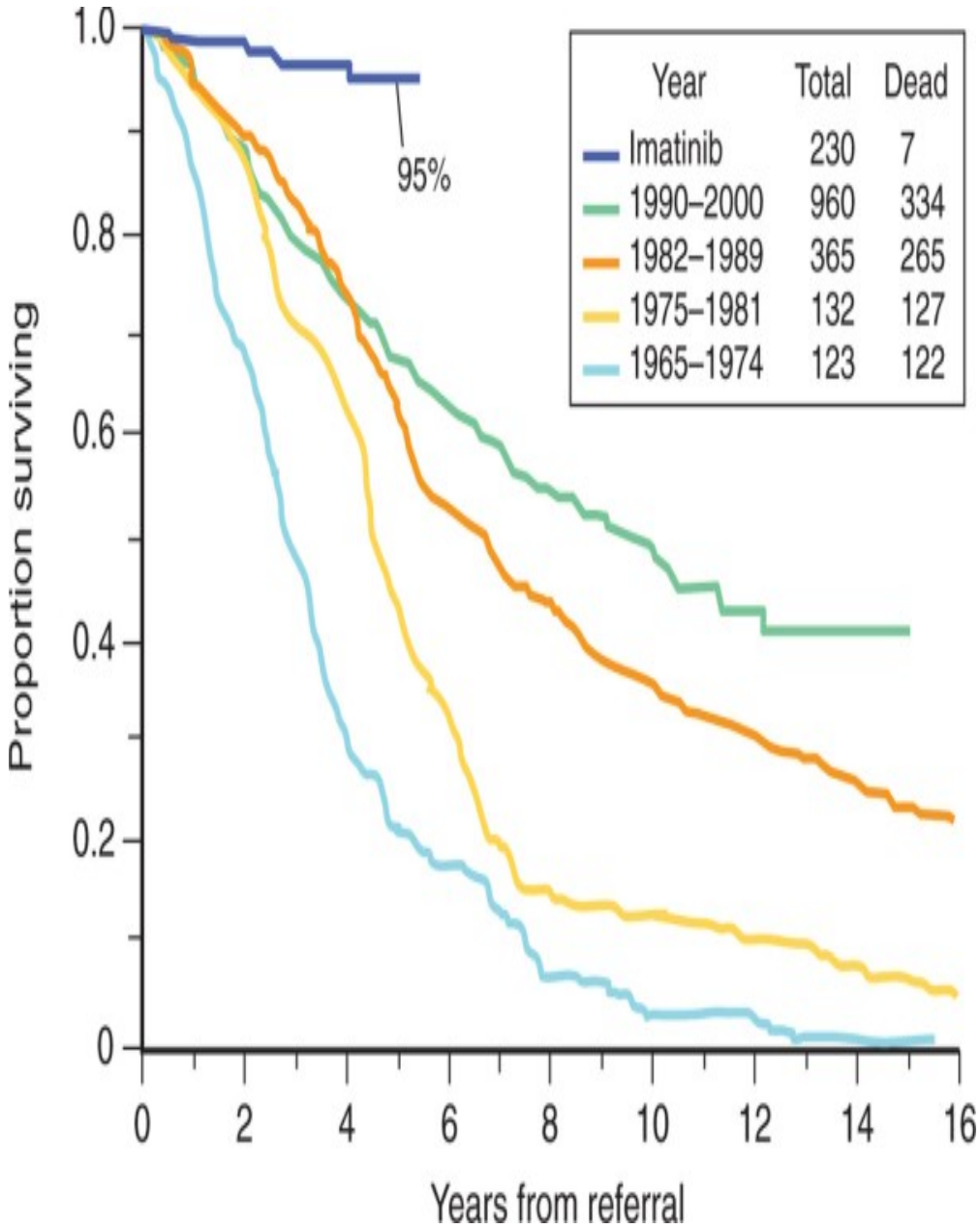



Gleevec: HOW IT WORKS
ATP
ATP
CML Enzyme
Cancer Protein
CML
ATP
Gleevec
CML Enzyme
Cancer Protein
CML


Year Total Dead
Imatinib 230 7
1990–2000 960 334
1982–1989 365 265
1975–1981 132 127
1965–1974 123 122
95%
Proportion surviving
1.0
0.8
0.6
0.4
0.2
0
0 2 4 6 8 10 12 14 16
Years from referral