Heterogeneity of Abnormal *RUNX1* Leading to Clinicopathological Variations in Childhood B-Lymphoblastic Leukemia

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Background

- **RUNX1**
  - **Runt-related transcription factor 1**
  - **Also known as:**

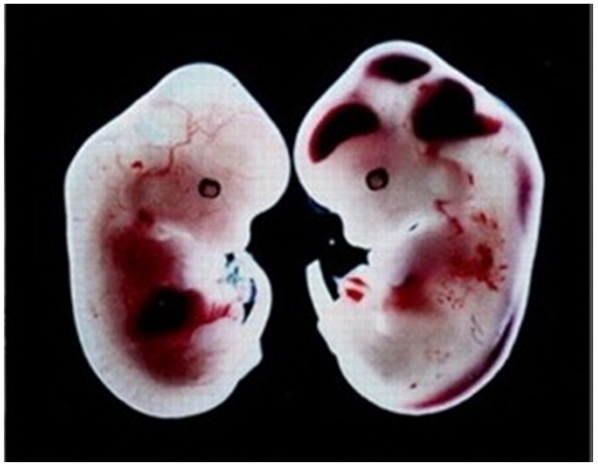
    Acute myeloid leukemia 1 protein (AML1)

    Core-binding factor subunit alpha-2 (CBFA2)
- ***RUNX1* gene – chromosome 21q22**
- **Function – participation in hematopoiesis**

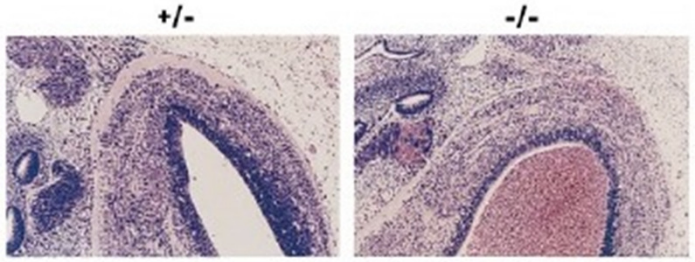
RUNX1 Function
Participation in Hematopoiesis

+/-
(control, E12.5)

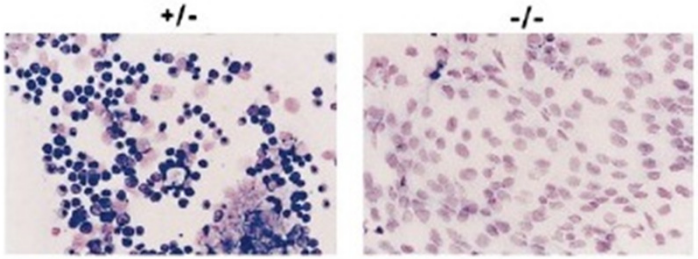
-/-
(mutant embryos, E12.5)



Hemohrrage within the ventricles of the brain and vertebral canal in the mutant embryo.



Hemorrhage within the ganglia of the cranial nerves with extension into the ventricles in mutant embryo.



Liver TP. Control: numerous erythroid precursors. Mutant: primarily hepatocytes.

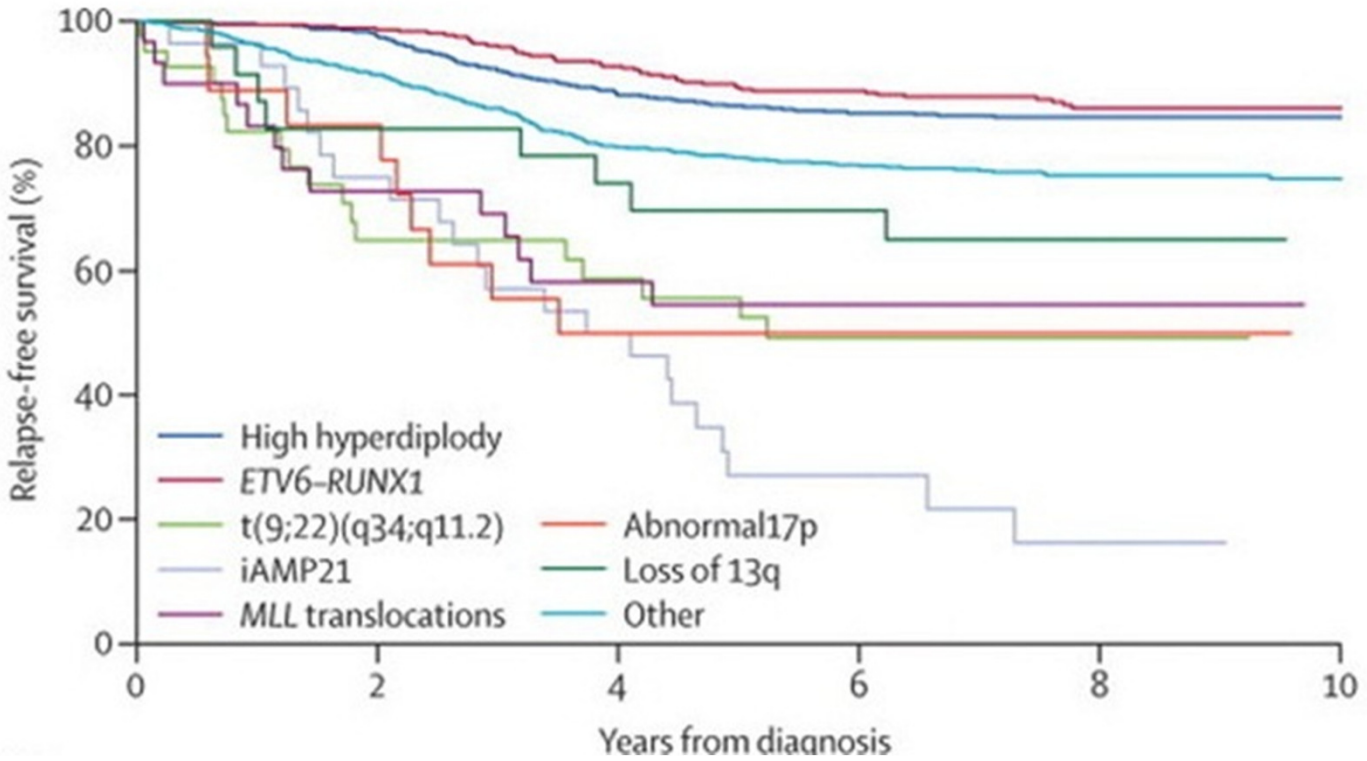
Okuda T1, van Deursen J, Hiebert SW, Grosveld G, Downing JR. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell*. 1996;84:321-330.

RUNX1 Abnormalities in Acute Leukemia

- **Translocations**
  - ***ETV6-RUNX1***/t(12;21)(p13;q22) → childhood B-ALL (25%) with good prognosis
  - ***RUNX1-RUNX1T1***/t(8;21)(q22;q22) → AML with good prognosis
  - ***RUNX1-MECOM***/t(3;21)(q26;q22) → MDS & blastic phase of CML
- **Amplifications** (≥ 4 *RUNX1*copies on a single chromosome 21) → childhood B-ALL (2%) with unfavorable prognosis
- **Point Mutations** → myeloid malignancies

Prognostic Significance of Chromosomal Abnormalities

UK ALL Trials



Study Objectives

Compare how abnormalities of *RUNX1* affect the clinicopathological expression in childhood B-ALL.

MATERIALS AND METHODS

- Case Selection
  - Newly diagnosed B-ALL with *RUNX1* amplification or *ETV6-RUNX1*
  - < 20 years of age
  - 1999-2013
  - Children's Hospital Colorado (CHC)
- Clinical Information – age, gender, WBC, CSF, relapse, mortality
- Flow Cytometry – immunophenotype (≥20%) and S-phase (≥ 10%)
- Cytogenetics
- FISH Analysis - Vysis LSI *ETV6(TEL)-RUNX1(AML1)* extra signal dual-color probe set (Abbott Molecular)

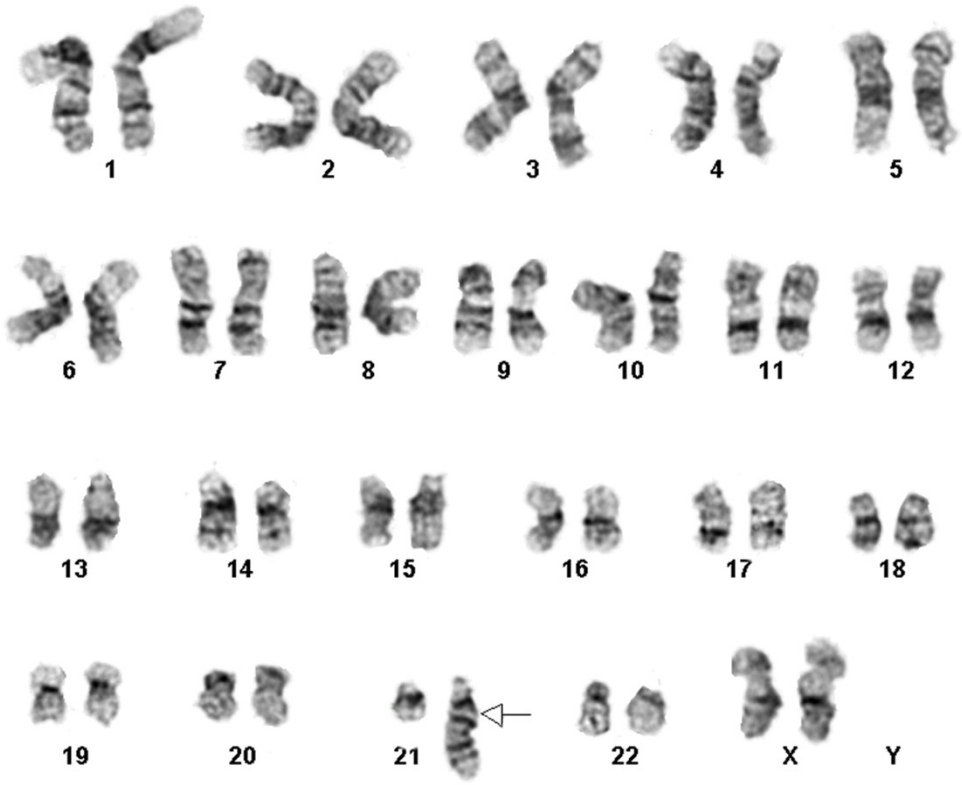
Results

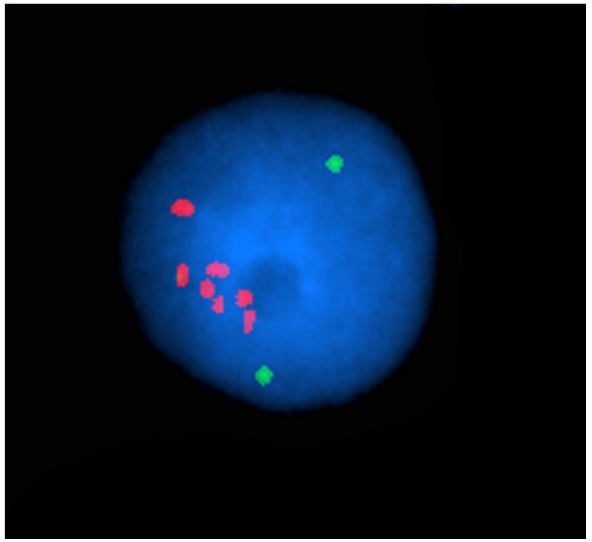
- ***RUNX1* amplification – 10 cases**
- ***ETV6-RUNX1* – 67 cases**

Results

RUNX1 amplification/*iAMP*

46,XX,add(21)(q22)(iAMP21)[14]





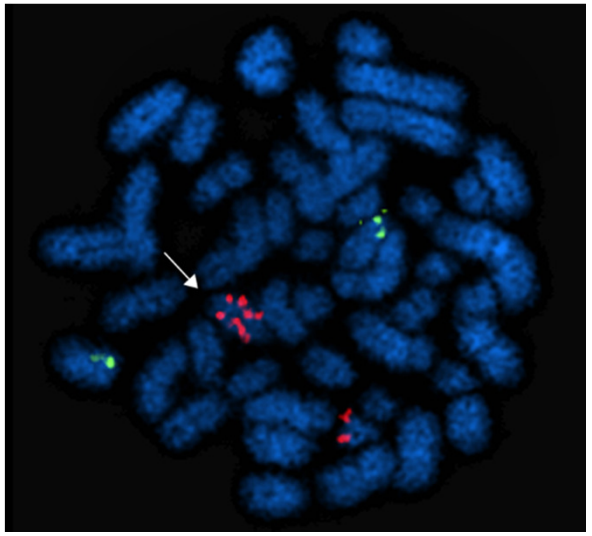
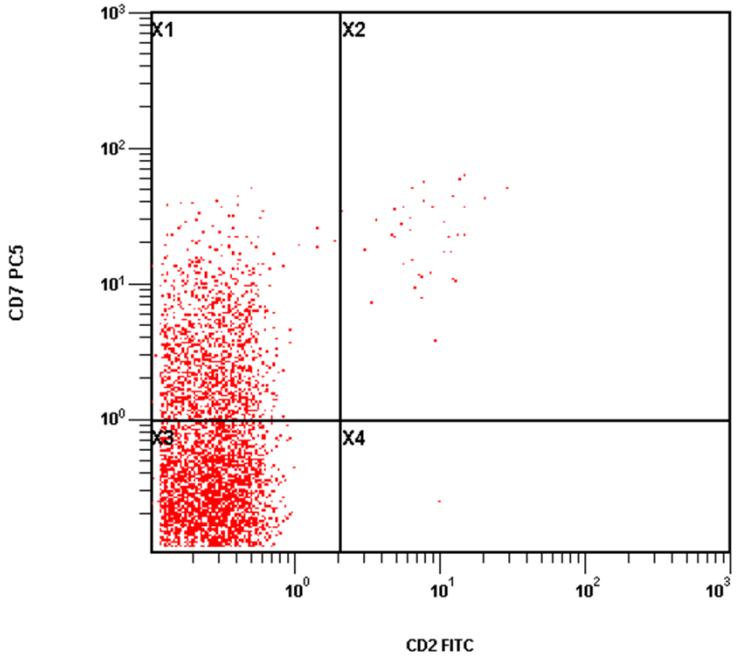


Table 1 **Clinicopathologic and Genetic Characteristics of Patients with *RUNX1* Amplification**

Case	Age/Sex	WBC	CSF	Immunophenotype	S-phase (%)	Karyotypes	FISH for *BUNX1*	Outcome
1	13y/M	3.7	+	CD34+, HLA-DR+, TdT wk+, CD10+, CD19+, CD20+ , CD22+, cIgM-, K-, λ-, CD2-, CD7+, CD13-, CD33-, CD45 moderate	1.0	46,XY,inv dup(21)(q22.13q22.3)del(21)(q22.3) ish inv dup(21)(q22.13q22.3)del(21)(q22.3) (D21S259,D21S341,D21S342x5->10, AML1x5->10,VIJ2yRM2185-)	*RUNX1* x 5-10	Moved to other state
2	15y/M	2.1	-	CD34-, HLA-DR+, TdT wk+, CD10+, CD19+, CD20+, CD22+, cIgM+, K-, λ-, CD2-, CD7-, CD13-, CD33-, CD45 moderate	23.4	46,XY,der(21)r(21)(p11.2q12)dup(21q)[8] /46,XY[3]	*RUNX1* x 4-8	CR & alive for 7 ys & moved to other state
3	12y/M	14.3	-	CD34+, HLA-DR+, TdT+, CD10+, CD19+, CD20+, CD22+, cIgM+, K-, λ-, CD2-, CD7+, CD13-, CD33-, CD45 dim to moderate	3.2	46,XY,del(7)(q22),t(9;22)(q34;q11.2),ider(21)(q10)hsr(21)(q22)add(21)(q22)	*RUNX1* x 5-10	CR and alive for 7 years
4	1y/F	8.5	-	CD34-, HLA-DR+, TdT+, CD10+, CD19+, CD20+, CD22+, cIgM-, K-, λ-, CD2-, CD7+, CD13-, CD33-, CD45 moderate	7.23	46,XX,del(7)(q32),der(21)r(21)(q11.2q22.3) qdp(21)(q11.2q22.3)[14]/46,XX[6]	*RUNX1* x 5-10	CR and alive for 6 years
5	5y/M	6.6	-	CD34+, HLA-DR+, TdT+, CD10+, CD19+, CD20-, CD22+, cCD79a+, cIgM-, K-, λ-, CD2-, CD7-, CD13-, CD33-, CD45 dim	9.88	47,XY,+X,del(16)(p12),add(21)(q22)[3]/ 47,sl,add(1)(q32)[5]/49,sl,+6,+14[2]/ 46,XY[10]	*RUNX1* x 4-10	CR and alive for 5 years
6	13y/F	3.0	-	CD34-, HLA-DR+, TdT+, CD10+, CD19+, CD20-, CD22+, K-, λ-, CD3-, CD13-, CD33+, CD45 dim	NA	47,XX,+X,add(21)(q22)[12]/46,XX[8]	*RUNX1* >5	CR and alive for 3 years
7	7y/F	6.9	-	CD34+, HLA-DR+, TdT+, CD10+, CD19+, CD20+, CD22+, cCD79a+, cIgM+, K-, λ-, CD2-, CD7-, CD13-, CD33-, CD45 moderate	5.8	46~47,XX,+X,add(3)(p24),add(9)(q22), add(12)(p13), del(13)(q12q22),-21,+1~5mar,inc[cp4]/ 46,XX[20]	*RUNX1* x 4-8	CR and alive for 3 years
8	6y/F	5.5	+	CD34+, HLA-DR+, TdT+, CD10+, CD19+, CD20-, CD22+, cCD79a+, cIgM-, K-, λ-, CD2-, CD7-, CD13-, CD33-, CD45 negative to dim	3.8	46,XX[20]	*RUNX1* x 6-8	CR and alive for 2 years
9	19y/F	5.3	+	CD34-, HLA-DR+, TdT+, CD10+, CD19+, CD20-, CD22+, cCD79a+, cIgM-, K-, λ-, CD2-, CD7-, CD13-, CD33+, CD45 dim	3.78	46,XY,dup(21)(q21q22)amp(AML1x5-10+)[14]/46,sl,del(7)(q11.2)[4] /46,XY[2]	*RUNX1* x 5-9	CR and alive for 2 years
10	2y/F	10.6	+	CD34+, HLA-DR+, TdT+, CD10+, CD19+, CD20-, CD22+, cCD79a+, cIgM-, K-, λ-, CD2-, CD7+, CD13-, CD33-, CD45 moderate	6.4	46,XX,add(21)(q22)(iAMP21)[14]/ 46,XX[6]	*RUNX1* x 4-7	CR and alive for 6 months

Aberrant Expression of CD7 Frequently Seen in B-ALL with *RUNX1* Amplification Than B-ALL with *ETV6-RUNX1*



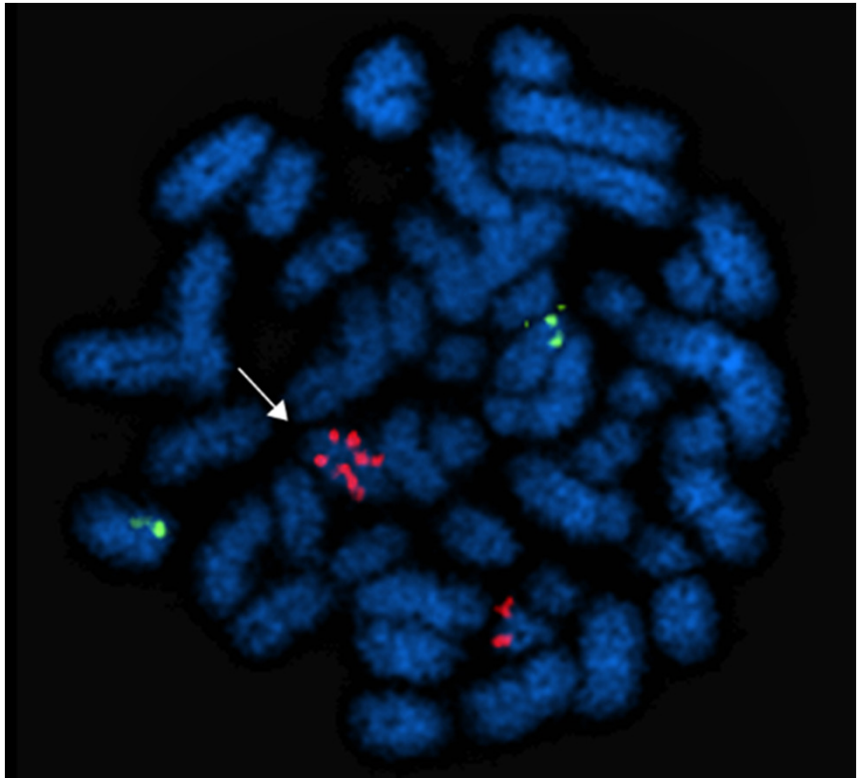
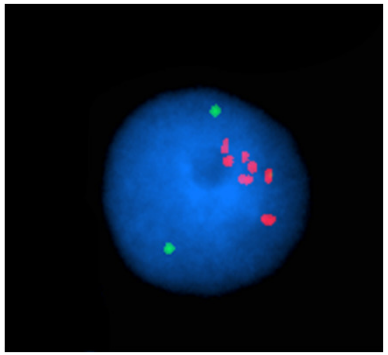
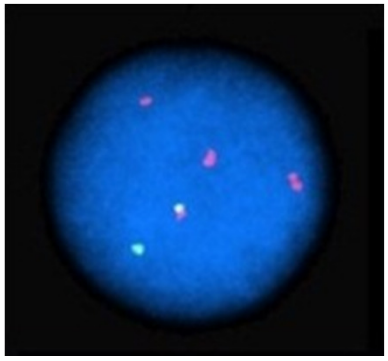


Table 2 Clinicopathologic Variations in Patients with Abnormal *RUNX1*

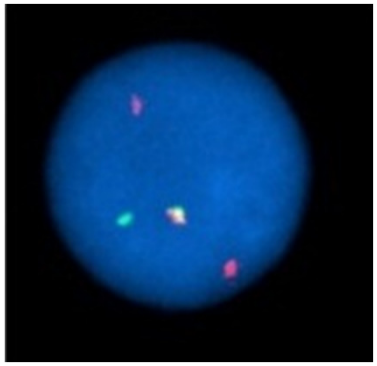
		RUNX1 amplification (group 1)	*ETV6-RUNX1* with *RUNX1* gain (group 2)	*ETV6-RUNX1* without *RUNX1* gain (group 3)	*P* (G1 vs G2)	*P* (G1 vs G3)	*P* (G2 vs G3)	*P* (G1 vs G2 & G3)
# of Cases		10	34	33				
Mean Age (years) (range)		10.1 (2 - 19)	5.1 (1 - 14)	3.5 (1 - 8)	0.0002	0.0001	0.0051	0.0001
M:F		5:5	21:13	21:12	0.7161	0.4809	1.0000	0.4990
WBC ≥50,000/mm^3		0/10 (0%)	4/34 (12%)	4/33 (12%)	0.5592	0.5579	1.0000	0.5867
CSF+		3/10 (30%)	7/34 (21%)	2/33 (6%)	0.6707	0.0733	0.1497	0.1835
Phenotype	CD2+	0/9 (0%)	0/30 (0%)	0/33 (0%)	1.0000	1.0000	1.0000	1.0000
	CD7+	4/9 (44%)	0/30 (0%)	0/33 (0%)	0.0015	0.0011	1.0000	0.0001
	CD13+	0/10 (0%)	13/34 (38%)	8/32 (25%)	0.0212	0.1646	0.2972	0.0536
	CD33+	2/10 (20%)	8/34 (24%)	9/31 (29%)	1.0000	0.7004	0.7785	1.0000
S-phase ≥10%		0/8 (0%)	1/31 (3%)	5/31 (16%)	1.0000	0.5628	0.1953	1.0000
Outcome	Relapse	1/10 (10%)	1/33 (3%)	4/31 (13%)	0.4153	1.0000	0.1968	1.0000
	Mortality	0/8 (0%)	2/33 (6%)	1/31 (3%)	1.0000	1.0000	1.0000	1.0000



***RUNX1* amplification**
Group 1



***ETV6-RUNX1* with *RUNX1* gain**
Group 2

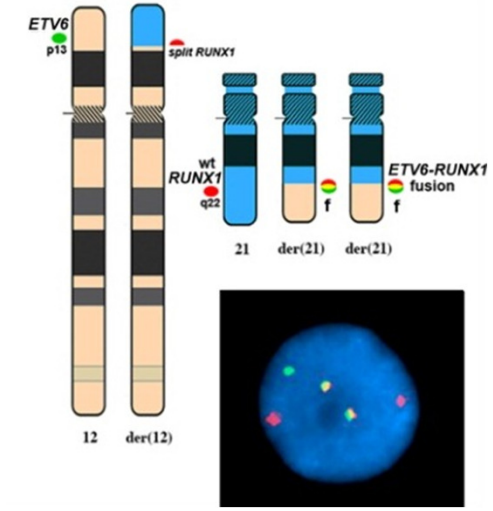


***ETV6-RUNX*1 without *RUNX1* gain**
Group 3

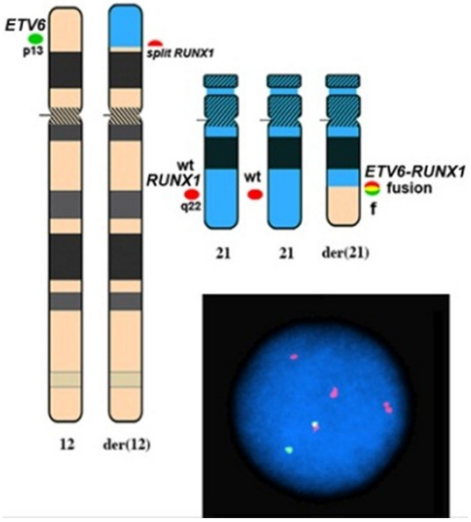
Table 3 **Aberrant Expression Myeloid-Associated Antigens in Subgroups of Patients with *ETV6-RUNX1* Translocation**

	ETV6-RUNX1 with *RUNX1* gain (group 2)		*ETV6-RUNX1* without *RUNX1* gain	p	p	p
	Double *ETV6-RUNX1* fusions (group 2A)	Single *ETV6-RUNX1* with wild type *RUNX1* gain (group 2B)	(group 3)	(G2A vs G2B)	(G2A vs G3)	(G2B vs G3)
# of Cases	13	21	33			
CD13+	10/13 (77%)	3/21 (14%)	8/32 (25%)	0.0006	0.0022	0.4938
CD13-	3/13 (23%)	18/21 (86%)	24/32 (75%)			
CD33+	5/13 (38%)	3/21 (14%)	9/31 (29%)	0.2106	0.7241	0.3184
CD33-	8/13 (62%)	18/21 (86%)	22/31 (71%)			

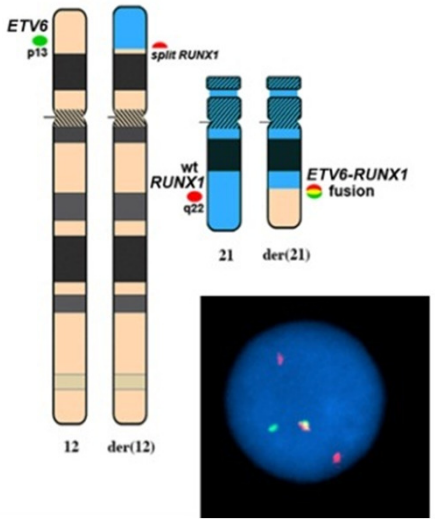
Group 2A ***ETV6-RUNX1*** **with gain of fusion**



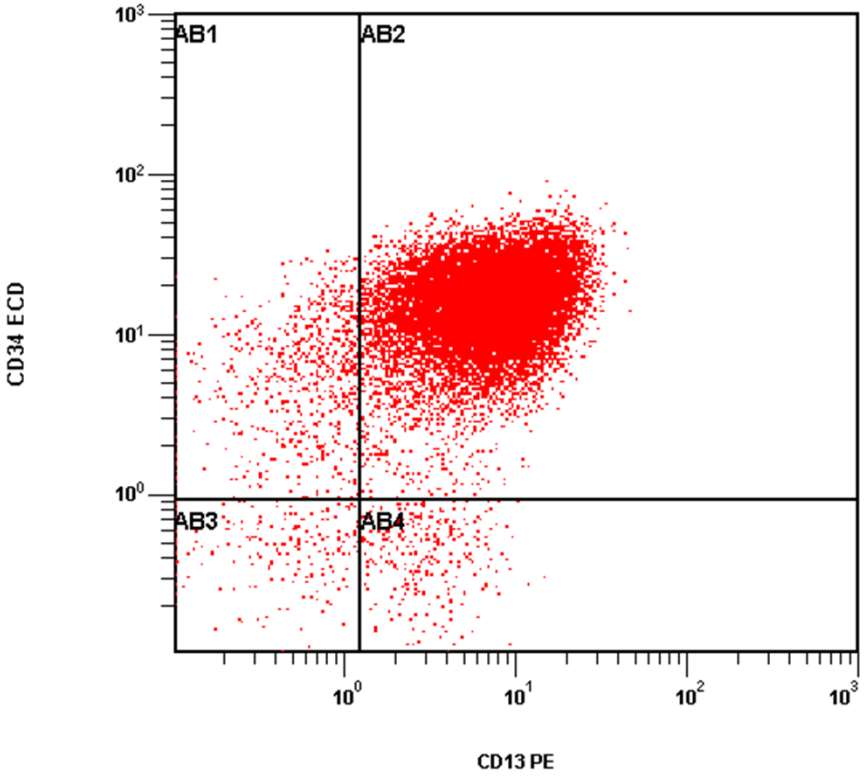
Group 2B ***ETV6-RUNX1*** **with gain of wild type (wt) *RUNX1***

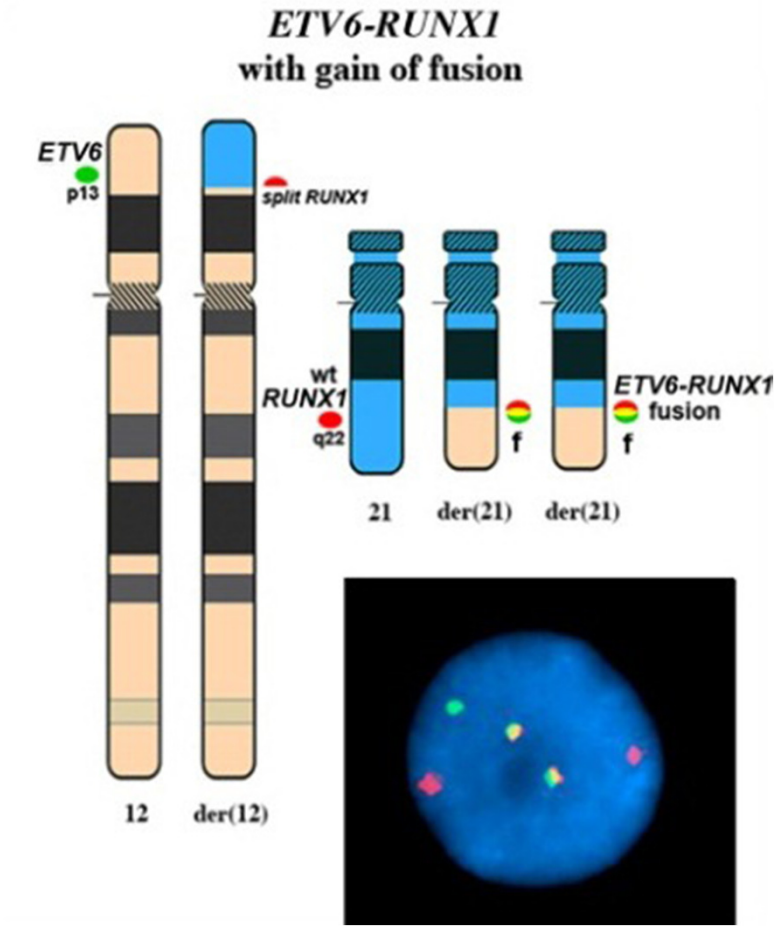


Group 3 ***ETV6-RUNX1*** **"Classic" t(12;21)**



Aberrant Expression of Myeloid Antigens Is More Common in Double *ETV6-RUNX1* Fusion Group Than a Single *ETV6-RUNX1* with a Wild *Type RUNX1* Gain Group





Result Summary

- Mean age
  - amplification group (10.1 y) older than translocation group (5.1 y)
- Genders
  - equal distribution in amplification group (M:F = 5:5)
  - male predominant in translocation group (M:F = 21:13)
- Hyperleukocytosis
  - translocation group (12%) > amplification group (0%)
- CSF+
  - amplification group (30%) > translocation group (13%)
- Phenotype
  - amplification group – **CD7**
  - translocation group – **CD13** and CD33

    double translocations > single translocation with *RUNX1* gain
- Outcomes
  - amplification group with high risk treatment = translocation group

Conclusions

- Patients with *RUNX1* amplification are older than patients with *ETV6-RUNX1* suggesting that the factors driving amplification of *RUNX1* may require longer time to develop or operate than those driving translocation of *RUNX1*.

- B-ALLs with *RUNX1* amplification more frequently show aberrant expression of CD7, suggesting amplification of *RUNX1* may prevent silencing of T-cell phenotype in B-lymphoblasts.

- B-ALLs with *ETV6-RUNX1* carry aberrant myeloid markers more often than those with *RUNX1* amplification suggesting that *RUNX1* at 21q22 likely is a myeloid associated breakpoint as seen in AML with t(8;21)(q22;q22)/*RUNX1-RUNX1T1*.

- Increased number of *ETV6-RUNX1* translocation, rather than gain of wild type *RUNX1* promotes more frequent expression of myeloid-associated antigens in B-ALL.

- More frequent CNS involvement may be partially responsible for more aggressive clinical behavior in patients with *RUNX1* amplification, although the differences are not statistically significant.

- Similar clinical outcome between *RUNX1* amplification and *ETV6-RUNX1* groups is attributed to different risk stratification treatments.

Contributors

- Virginia Knez, MD: Unversity of Colorado Hospital
- Billie Carstens : Colorado Cytogenetic Laboratory
- Karen Swisshelm, PhD: Colorado Cytogenetic Laboratory
- Amy McGranahan: Children's Hospital Colorado