Regulation of Hb F production and fetal globin gene inducers

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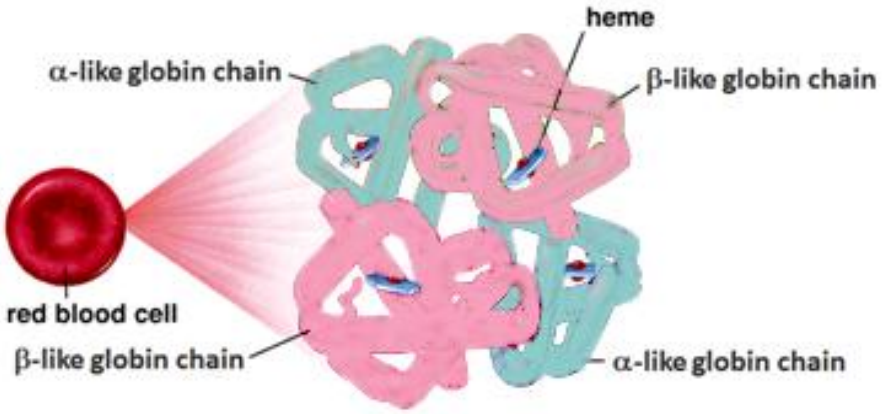
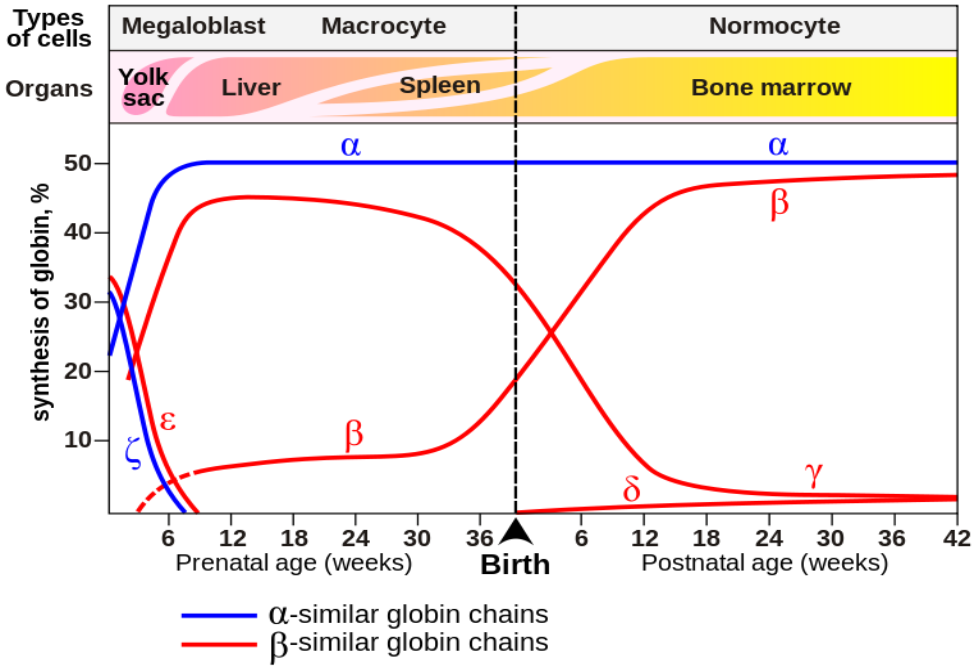
Hemoglobin switching

Hemoglobin (Hb)



Modified from
http://easypediatrics.com/physiology-of-hemoglobin



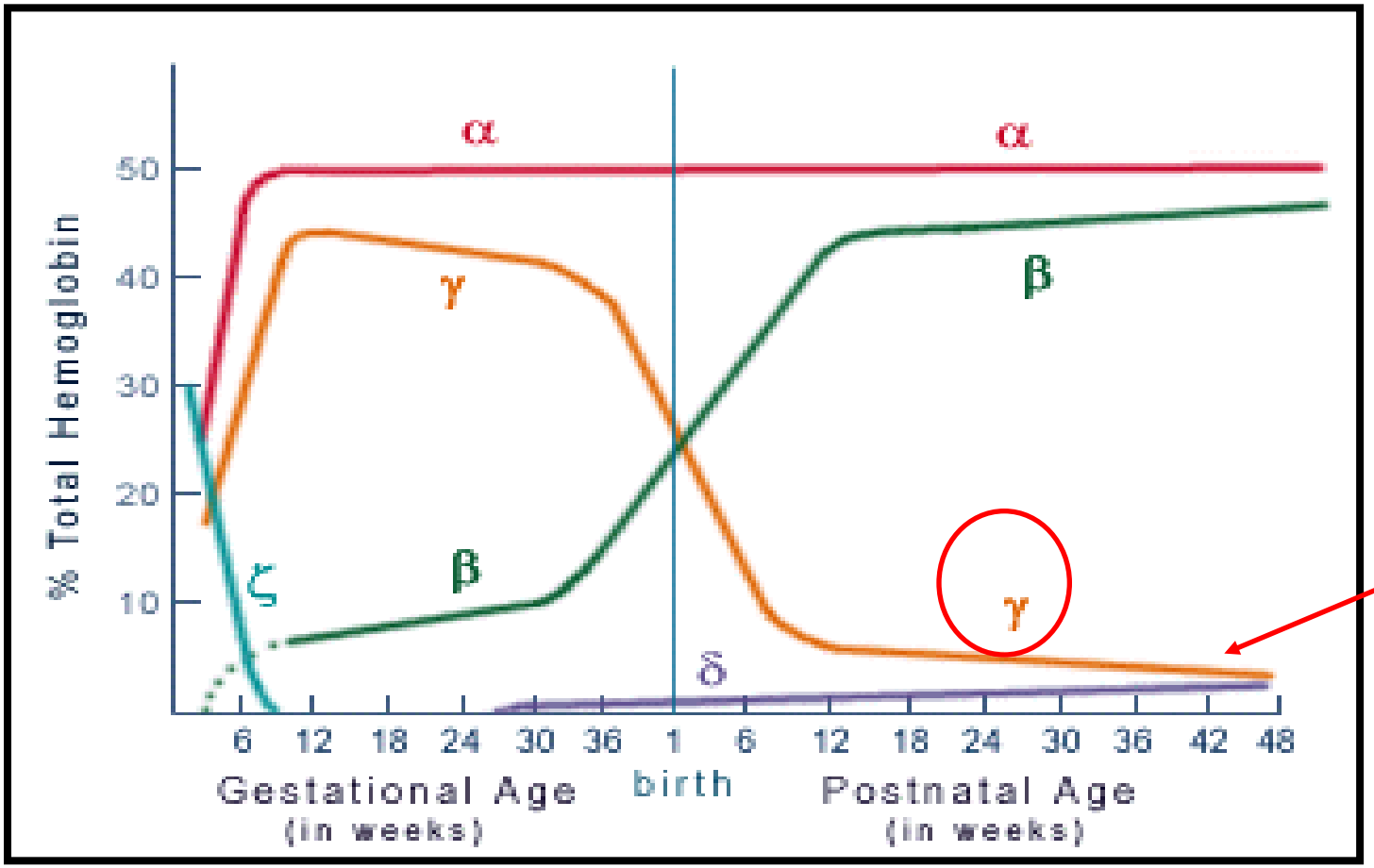
Embryonic hemoglobin	
$\zeta_2\varepsilon_2$	Hemoglobin Gower1
$\zeta_2\gamma_2$	Hemoglobin Portland
$\alpha_2\varepsilon_2$	Hemoglobin Gower2

Fetal hemoglobin	
$\alpha_2\gamma_2$	Hemoglobin F

Adult hemoglobin		
$\alpha_2\beta_2$	Hemoglobin A	≈ 97%
$\alpha_2\delta_2$	Hemoglobin A2	
$\alpha_2\gamma_2$	Hemoglobin F	< 1%

Modified from: Wood WG,1976 and Weatherall DJ, 2001

The fetal to adult globin gene switch – partial *reversal can correct the beta thalassemias*



Infants with beta thalassemia do not become ill until HbF is suppressed

Hereditary Persistence of Fetal Hemoglobin (HPFH)

Genetic conditions with increased HbF in adults

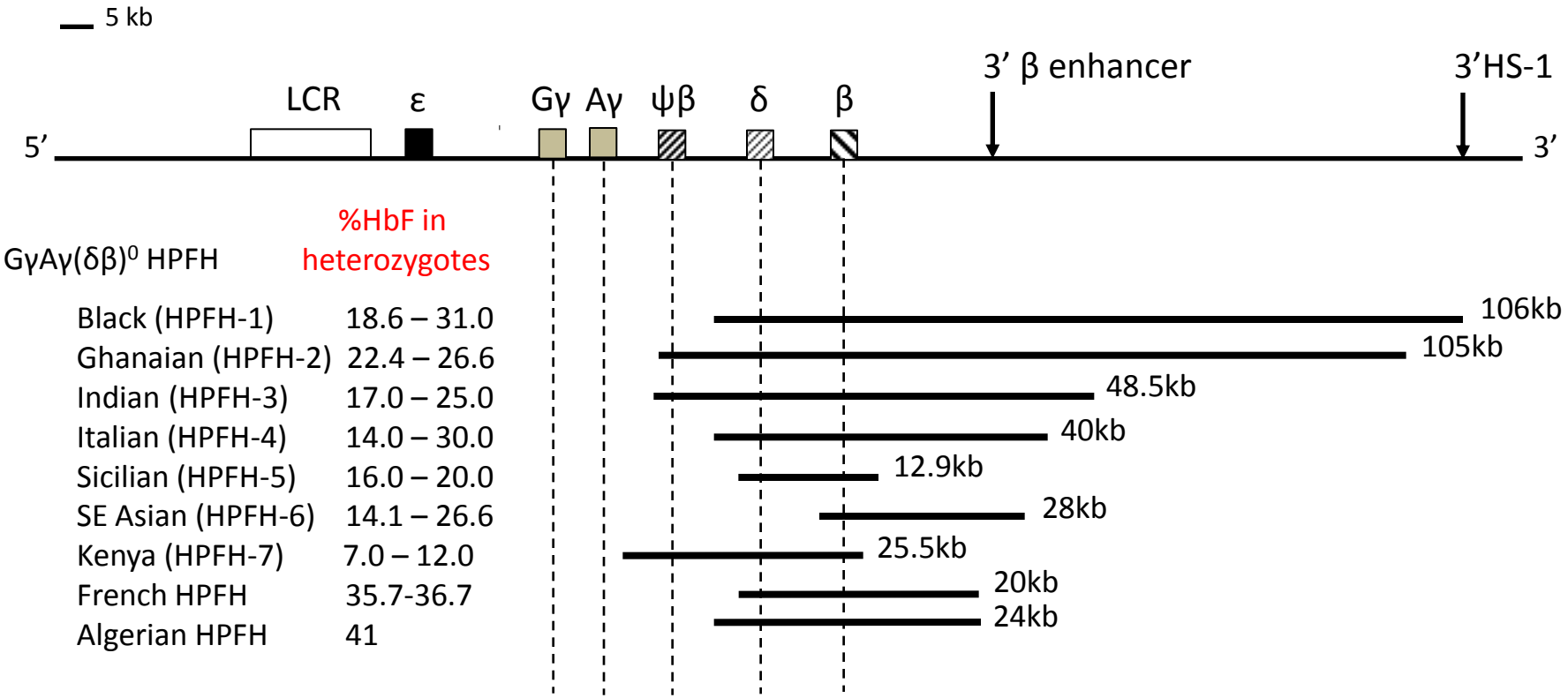
Deletions

- $G\gamma A\gamma(\delta\beta)^0$ HPFH
- δβ thalassemia

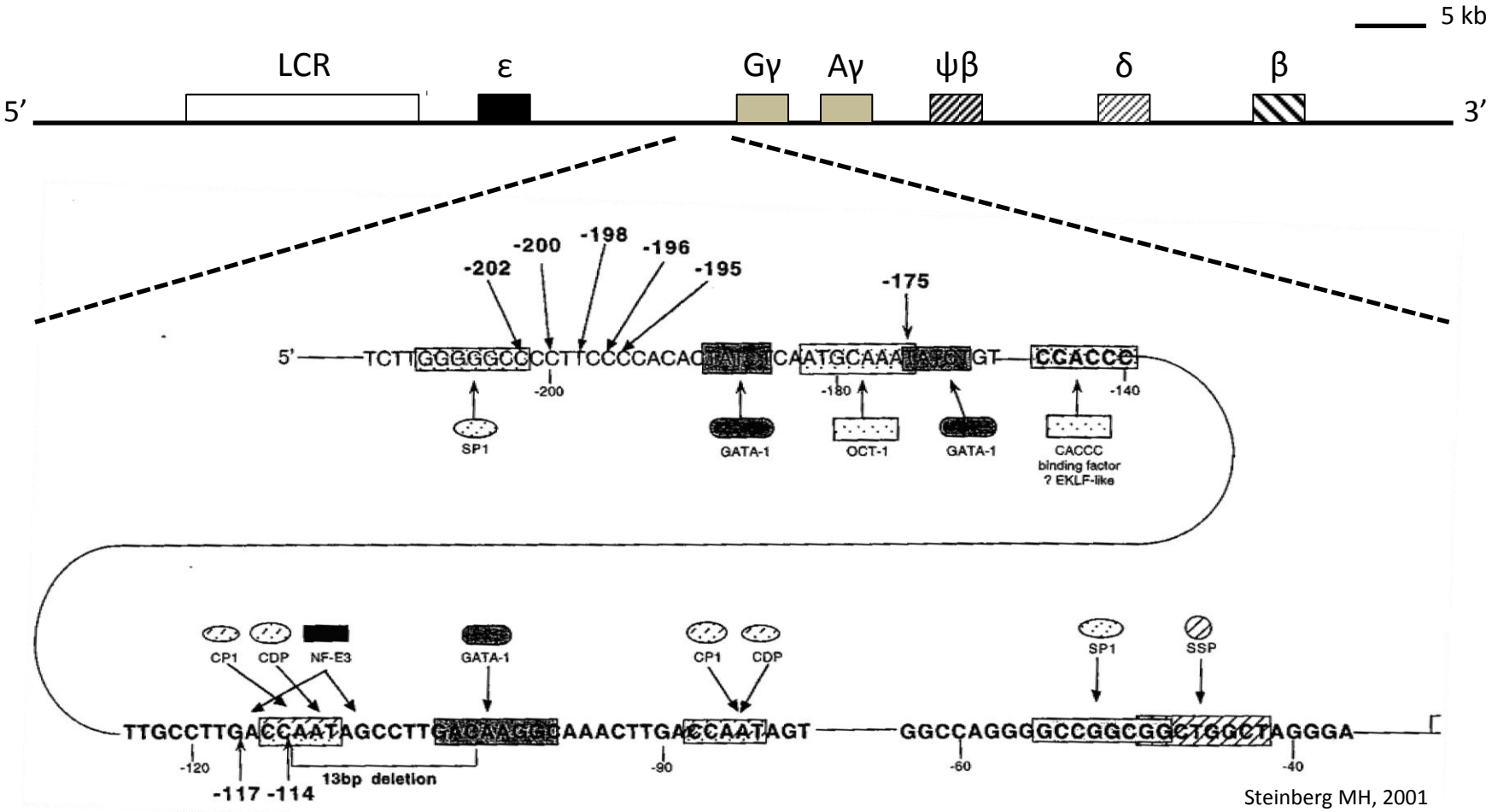
Non-deletions

- Linked to the β cluster
  - Mutations on *γ-globin* promoters
- Unlinked to the β cluster
  - Mutations on trans-regulators of *γ-globin*

Deletion causing HPFH



Non-deletion on *cis*-elements causing HPFH



Steinberg MH, 2001

Mutations on γ-globin promoters that result in non-deletion HPFH

Summary of hematological data (mean±SD) of non-deletional HPFH heterozygoytes

Condition	Mutation	Hb (g/dL)	HbF (%)	XmnI at -158 in *cis*
Gγ mutations				
Black	-202 C→G	13.3 ± 1.3	15.6 ± 1.2	-
Tunisian	-200 +C	13.1 ± 0.9	25.2 ± 4.1	+
Black/Sardinian/British	-175 T→C	12.7 ± 1.1	20.3 ± 2.8	-
Japanese	-114 C→T	ND	12.5 ± 2.1	+
Australian	-144 C→G	14.2	8.6	-
Aγ mutations				
Black	-202 C→T	12.9 ± 0.9	2.5 ± 0.9	-
British	-198 T→C	14.2 ± 1.2	6.9 ± 2.2	-
Italian/Chinese	-196 C→T	normal	13.7 ± 2.0	-
Brazilian	-195 C→G	normal	5.4 ± 1.4	ND
Black	-175 T→C	normal	37.4 ± 1.0	+
Greek/Black	-117 G→A	14.2 ± 1.1	12.1 ± 2.8	-
Black	-114 to -102 del	11.4 ± 2.9	31.0 ± 1.2	-
Georgia	-114 C→T	normal	3.8 ± 1.3	ND

Steinberg MH, 2001

Hematological data from Baltimore HPFH homozygote with 100% Hb F (Charache *et al.*, 1976)

Age (years)	Hb (g/dl)	MCV (fl)	MCH (pg)
1	13.3	71	22
4	15.2	71	27
6	15.0	75	24
7	14.3	70	23
11	14.1	68	23
13	15.3	69	23
14	15.9	69	24
15	15.9	68	25

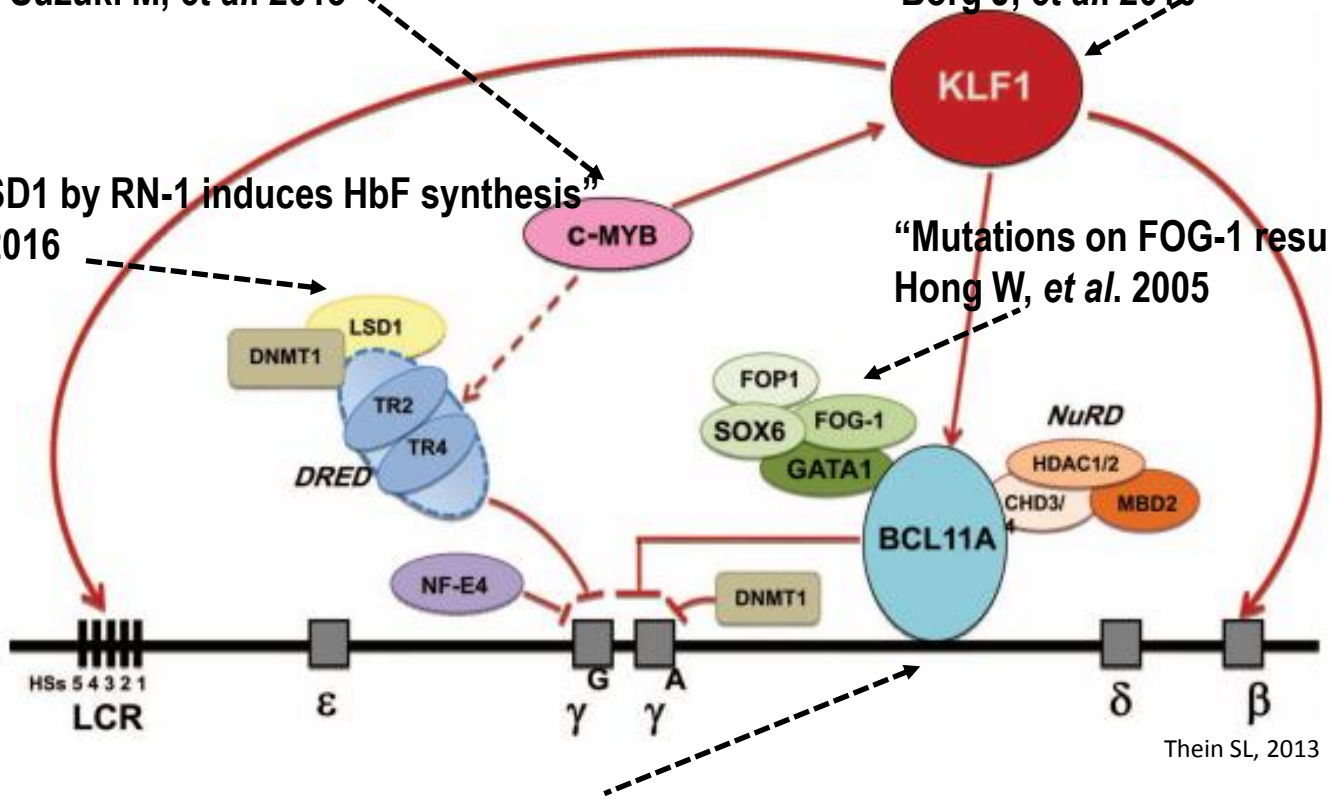
Non-deletion on *trans*-elements causing HPFH

"Disruption of Myb causes HPFH in mouse model"
Suzuki M, *et al*. 2013

"Haploinsufficiency of KLF1 causes HPFH"
Borg J, *et al*. 2010

"Inhibition of LSD1 by RN-1 induces HbF synthesis"
Rivers A, *et al*. 2016

"Mutations on FOG-1 result increased HbF"
Hong W, *et al*. 2005

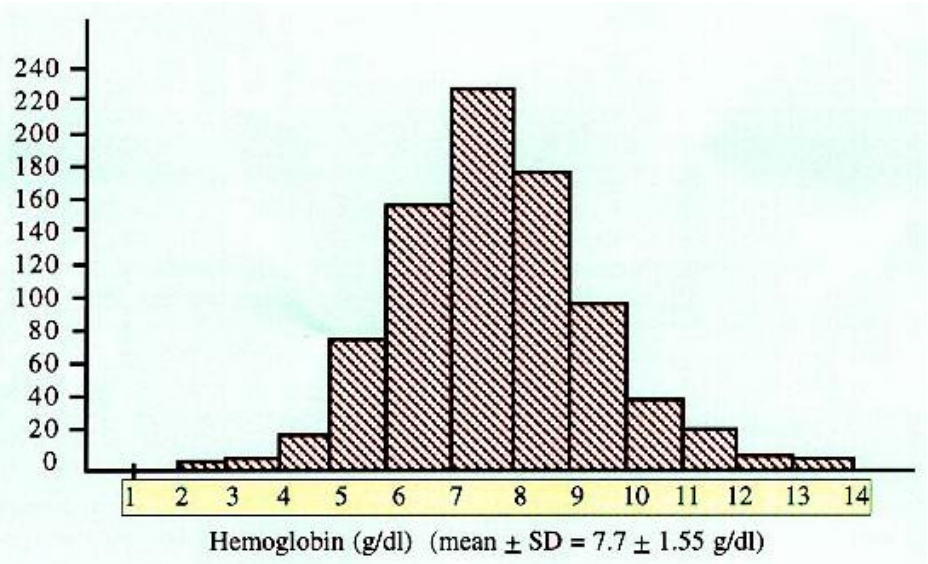


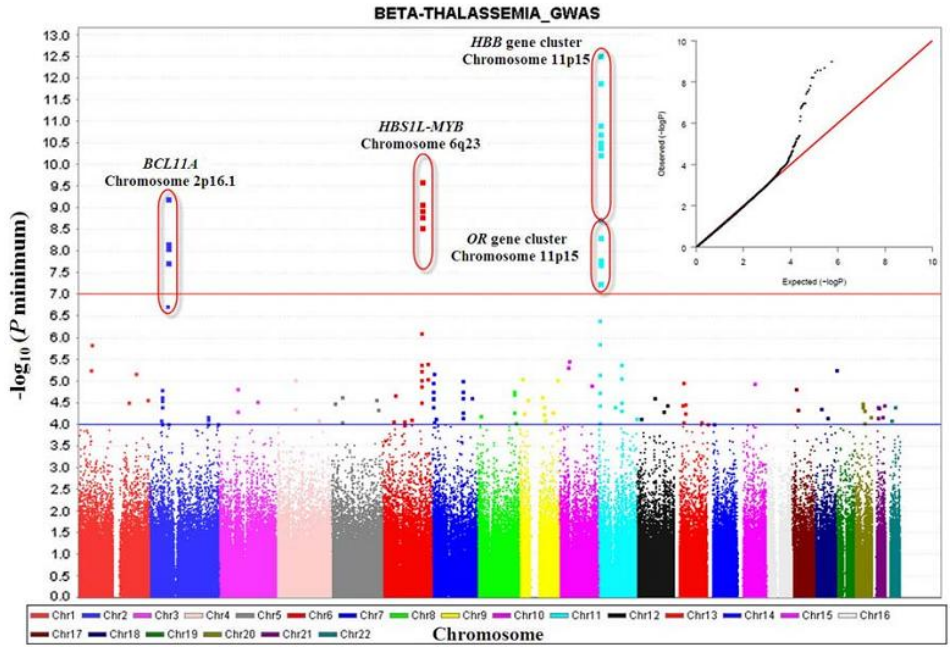
Thein SL, 2013

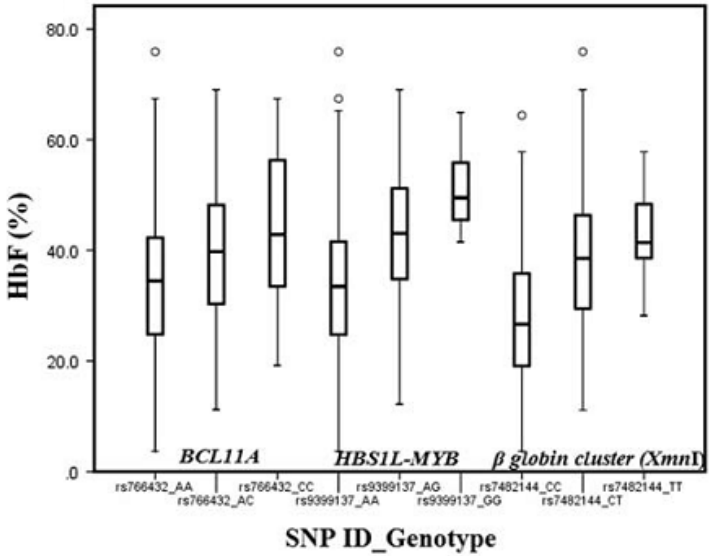
"Down-regulation of BCL11A increases HbF levels"
Sankaran VJ, *et al*. 2008

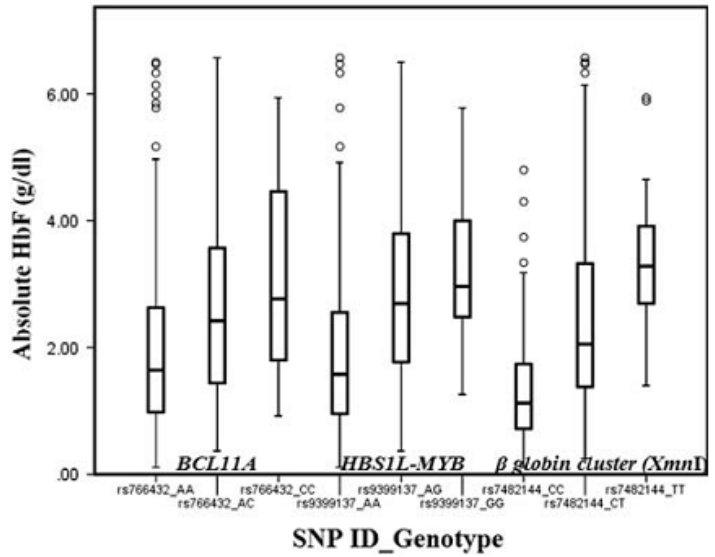
γ-globin regulators network

β-thalassemia/HbE Disease Severity and HbF levels





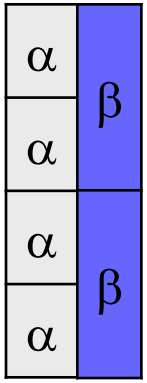




Fucharoen S, *et al. Birth Defects*. 1988.
Nuinoon M, *et al. Hum Genet*. 2010; 127: 303-14.

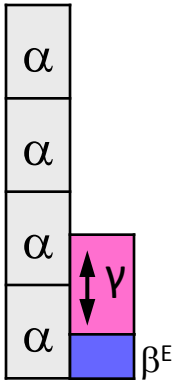
HbF is a modifying factor of β-thalassemia

αα/αα::β/β

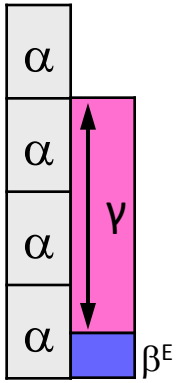


Normal

αα/αα::β^0/β^E with low HbF

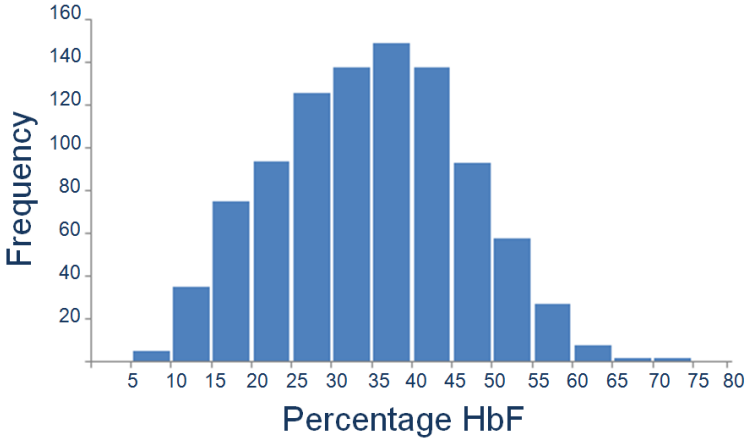


αα/αα::β^0/β^E with high HbF



Reduce the degree of imbalance through the production of HbF

Distribution of fetal hemoglobin (HbF; $\alpha_2\gamma_2$) in Thai β^0-thalassemia/HbE cohort (n = 950)



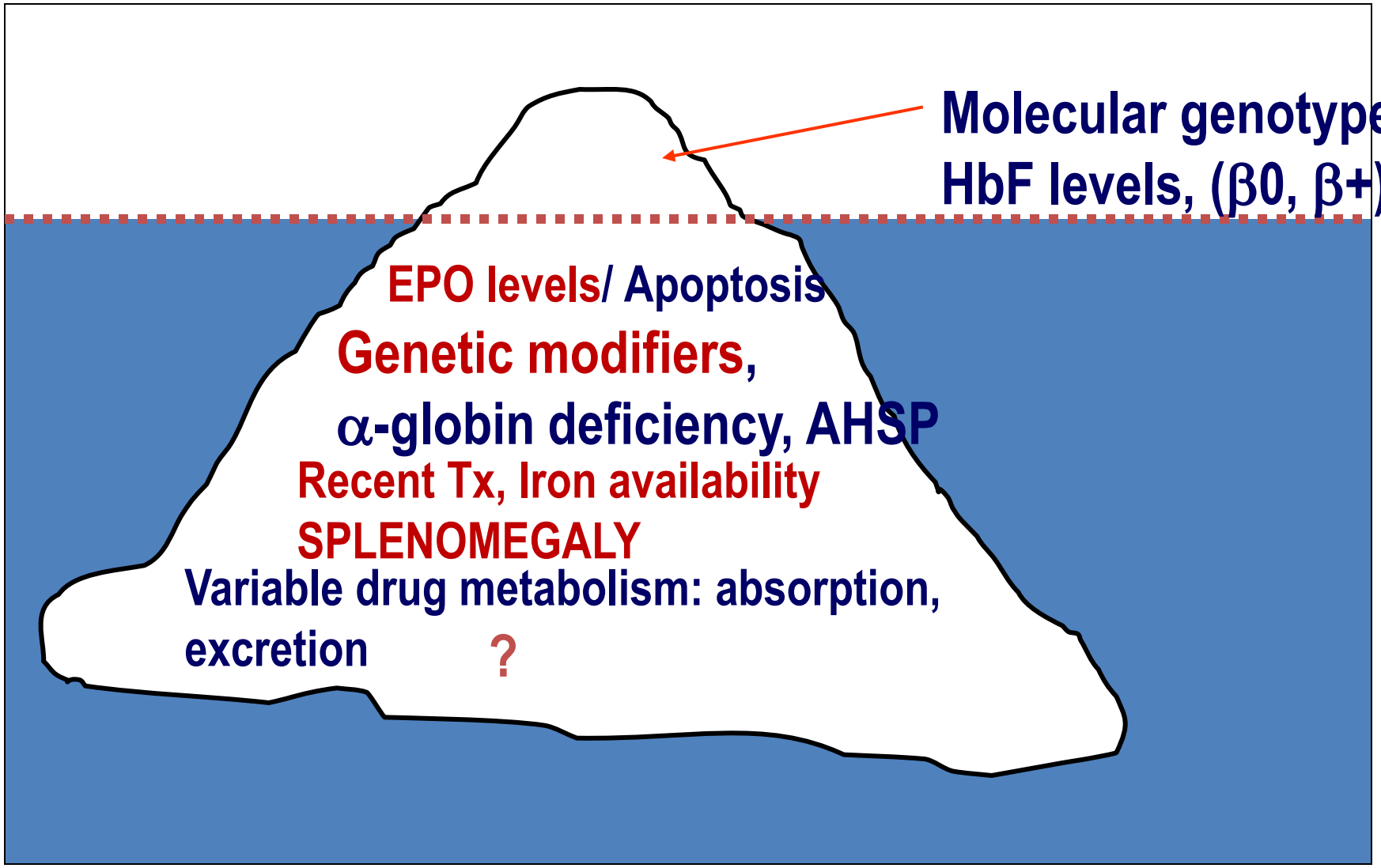
Severity	Percentage HbF	Absolute HbF (g/dL)
Mild (n = 233)	41.0 ± 11.2	3.1 ± 1.2
Moderate (n = 310)	35.3 ± 11.1	2.0 ± 0.9
Severe (n = 407)	31.2 ± 11.2	1.4 ± 0.7

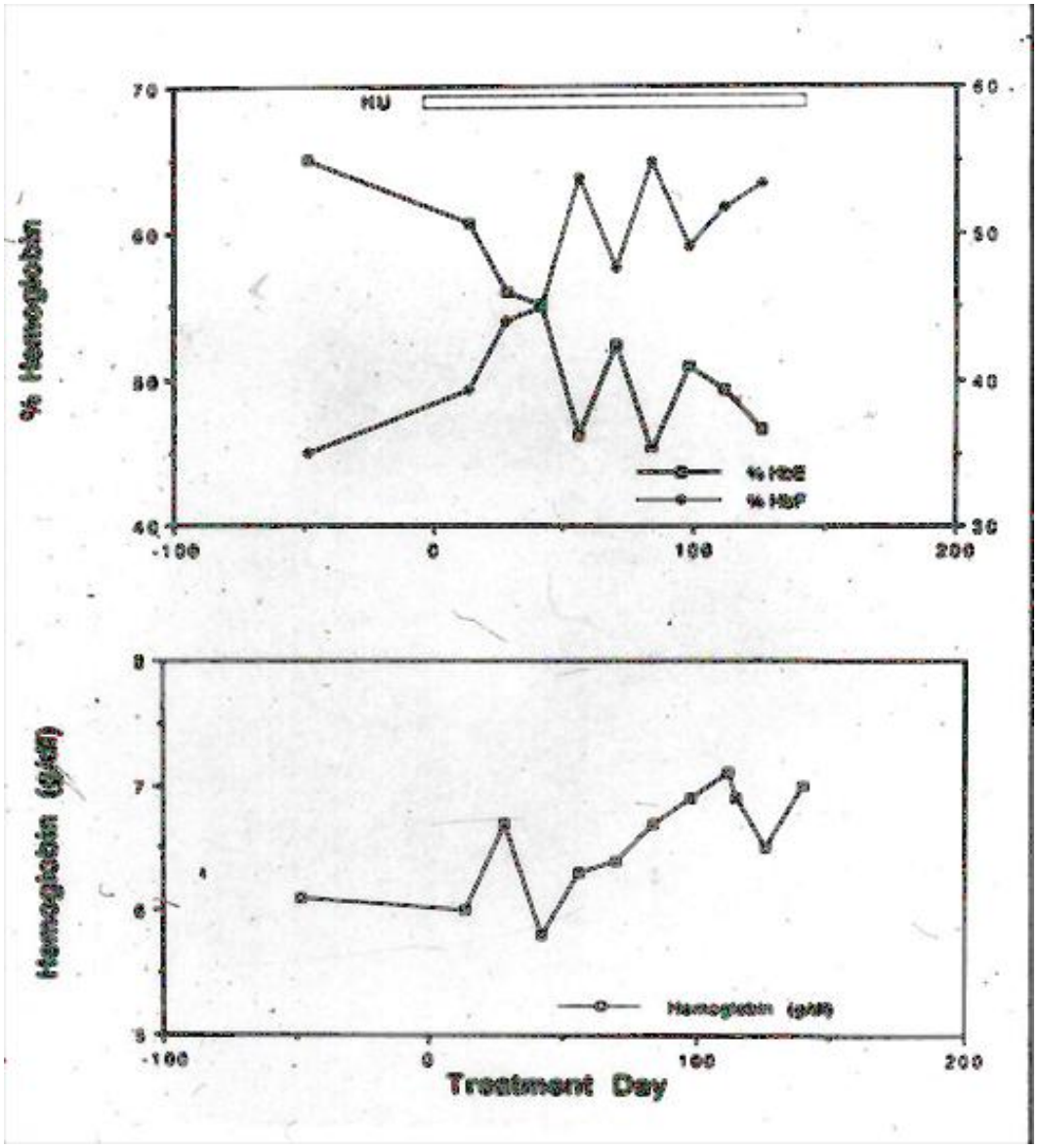
Sripichai O, 2009

Proof-of-Concept with first generation inducers: Hematologic (total Hgb) responses

- 5-Azacytidine, decitabine: 2.5 g/dl (1.5-4)
  (Ley, DeSimone, & Neinhuis, Dunbar, Lowrey, Koshy, Sauntharajah)
- Hydroxyurea: 0.6- 2.7 g, mean 0.9-1.5
  (Zeng, Hajjar, Fuchareon, Singer)
- A. Butyrate +/- EPO: Mean 2.7 g/dl (1 - 5 g/dl)
  Perrine et al
- Sodium Phenylbutyrate: 2 g, (1-2.5, mean 2 g)
  (*Collins, Giardina, Dover, Pearson1994*)
- EPO, Darbopoietin: 1.6-2 g/dl
  (Nisli, Rachmilevitz, Bourantas, Singer)
- Isobutyramide: HbF increased in 4-8 wks, Tx reduced
  (*Cappellini, Reich*, 2000)

Physiologic factors affecting γ globin induction





Changes in the hemoglobin levels, percent Hb E and Hb F after Hydroxyurea treatment

Fucharoen et al, Blood 1995

Hematologic Effects of Hydroxyurea

	Baseline	36 months
Hb (g/dL)	6.15 ± 0.9	6.71 ± 0.93**
MCV (fL)	62.3 ± 8.2	68.3 ± 8.2*
MCH (pg)	17.25 ± 2.38	18.01 ± 4.63**
Reticulocyte ($x10^6$)	0.11 ± 0.24	0.14 ± 0.31**
Hb E (%)	67.6 ± 16.1	62.6 ± 16.5**
Hb F (%)	24.2 ± 11.9	28.8 ± 16.7**
Absolute Hb F (g/dL)	1.88 ± 0.89	2.37 ± 0.97**
$^{G}\gamma:^{A}\gamma$ ratio	1.18 ± 0.18	1.65 ± 0.79*
Serum ferritin (ng/ml)	3,778 ± 2,413	2,047 ± 2,322*
Tf receptor (μg/ml)	45.3 ± 19	37.4 ± 15.1**

** = P-value < 0.001, ** = P-value < .05, N=17*

Pharmacologic induction of HbF

5-azacytidine	DNA methylation
Hydroxyurea **Erythropoietin**	Alter erythroid growth kinetic
Butyrate derivatives	HDAC inhibition (Histone Deacetylase)
Cisplatin **Mithramycin** **Tallimustine**	DNA-binding drugs

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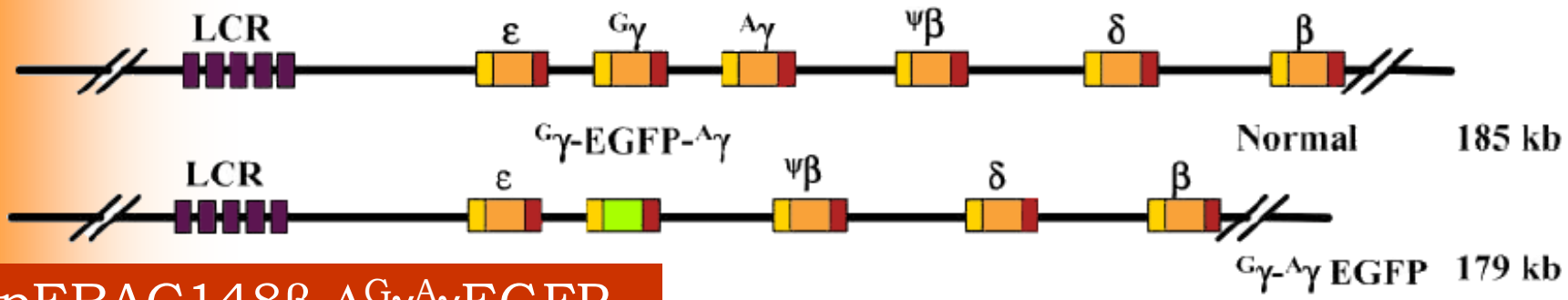
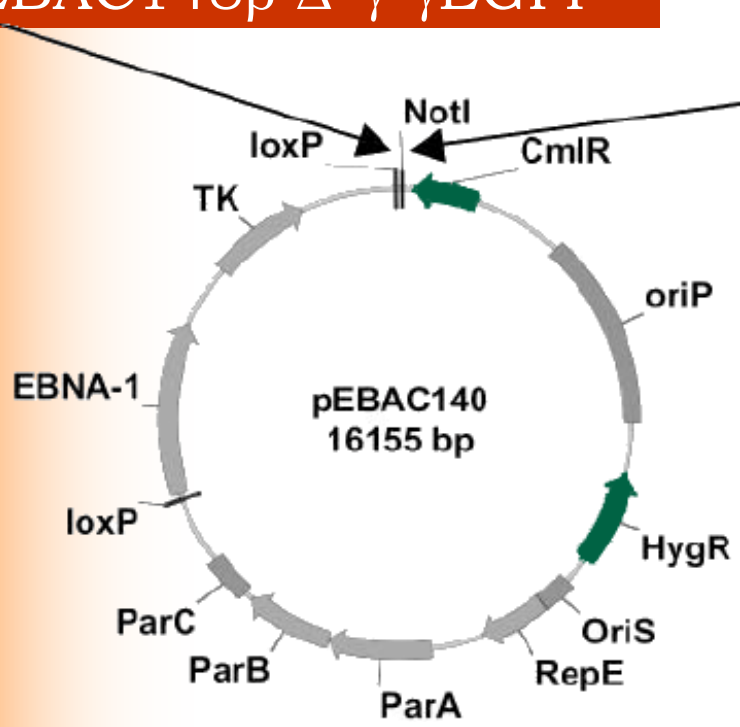
pEBAC148β



pEBAC148β $\Delta^{G\gamma A\gamma}$EGFP



RED CELLS

Development of sensitive fluorescent assays for embryonic and fetal hemoglobin inducers using the human β-globin locus in erythropoietic cells

Jim Vadolas, Hady Wardan, Michael Orford, Lucille Voullaire, Faten Zaibak, Robert Williamson, and Panayiotis A. Ioannou

Human Molecular Genetics, 2004, Vol. 13, No. 2
DOI: 10.1093/hmg/ddh023
Advance Access published on November 25, 2003

Cellular genomic reporter assays for screening and evaluation of inducers of fetal hemoglobin

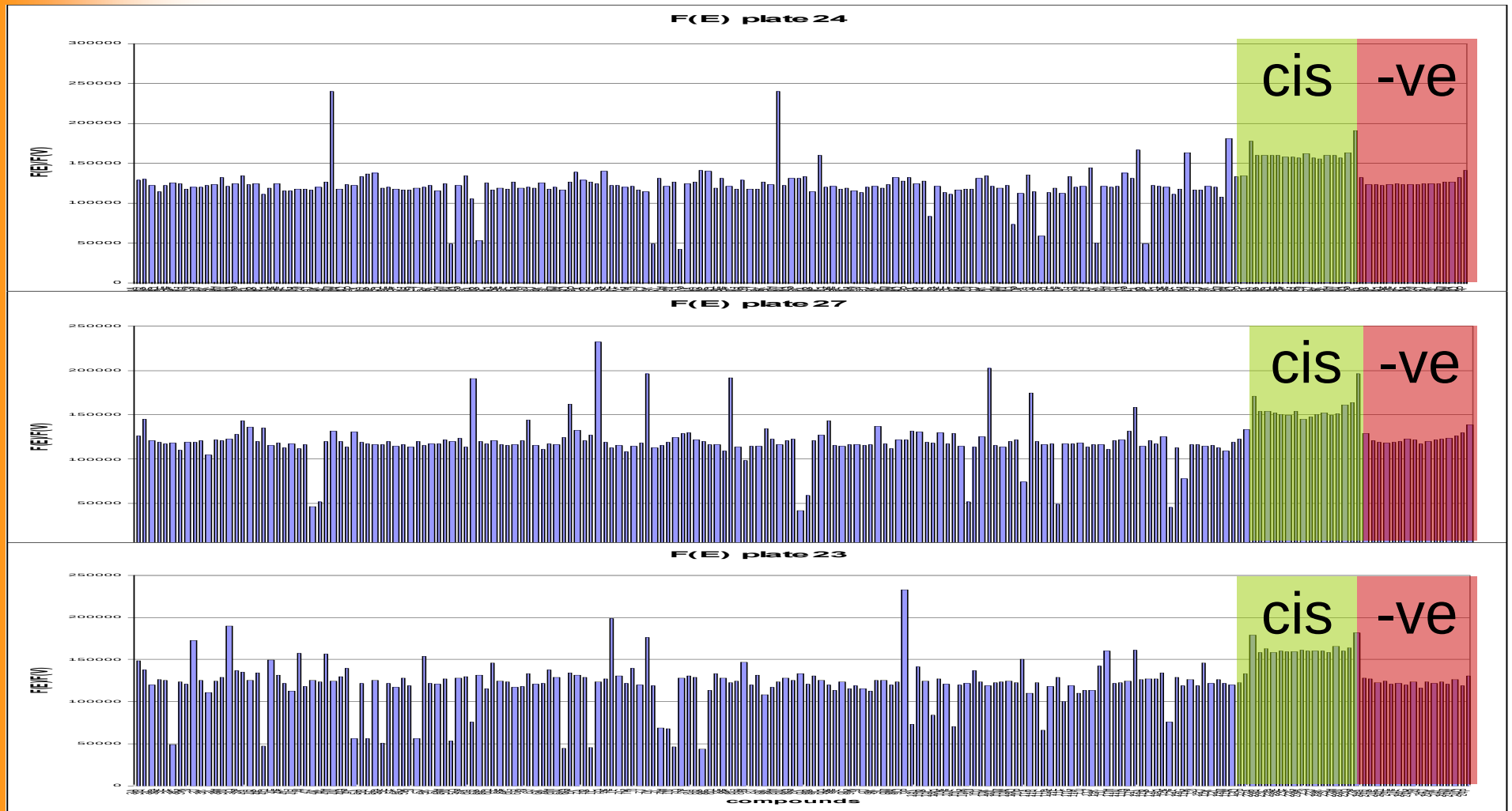
Jim Vadolas[1], Hady Wardan[1], Michael Orford[2], Robert Williamson[1] and Panayiotis A. Ioannou[1,2,*]

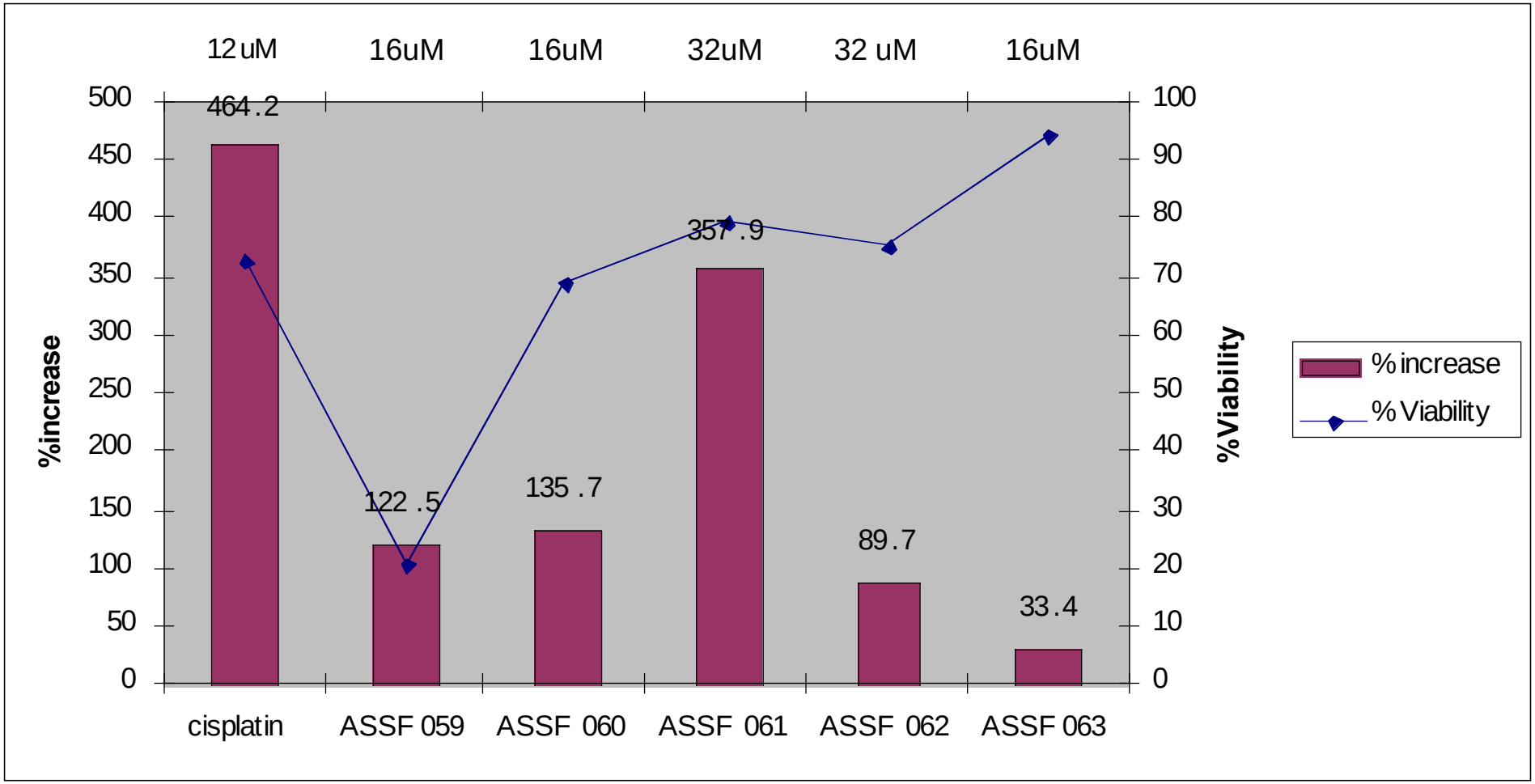
Stably transfected into K562-EBNA1 (KEB) erythroleukemia cell line (**M Clone)**

High Throughput Screening

PrimaryScreening Result

- 2000 compounds library
- Wheat Grass extract fractions
- Thailand natural products




12 uM
16uM
16uM
32uM
32 uM
16uM
464.2
122.5
135.7
357.9
89.7
33.4
500
450
400
350
300
250
200
150
100
50
0
100
90
80
70
60
50
40
30
20
10
0
%increase
%Viability
% increase
% Viability
cisplatin
ASSF 059
ASSF 060
ASSF 061
ASSF 062
ASSF 063

Compound Screening Summary

Bioactive Lib (2000)
Thai Natural cpd (92)
Wheat Grass Fraction (40)

High Throughput
Low Throughput

7 **High HTS Hits (8/18)**

3 **Low HTS Hits (6/8)**

6 **Nucleoside analog Antiviral Drugs(7/19)**

5 **Thai Natural Product (34) & Wheat Grass**

19 of 2172 compounds

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A Phase 2 Trial of HQK-1001, an Oral Fetal Globin Gene Inducer, in β-Thalassemia/HbE in Thailand

S Fucharoen, N Chaneiam, P Patthamalai, SP Perrine

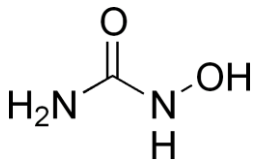
Thalassemia Research Center, Mahidol University

Boston University School of Medicine

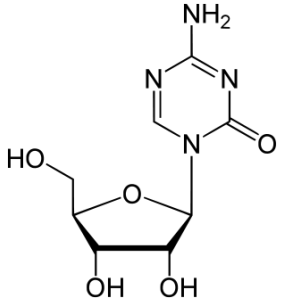
Supported by HemaQuest Pharmaceuticals, Inc.

HbF Enhancers

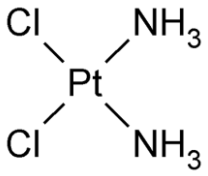
Example



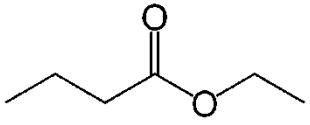
Hydroxyurea



5-azacytidine (decitabine)



Cisplatin



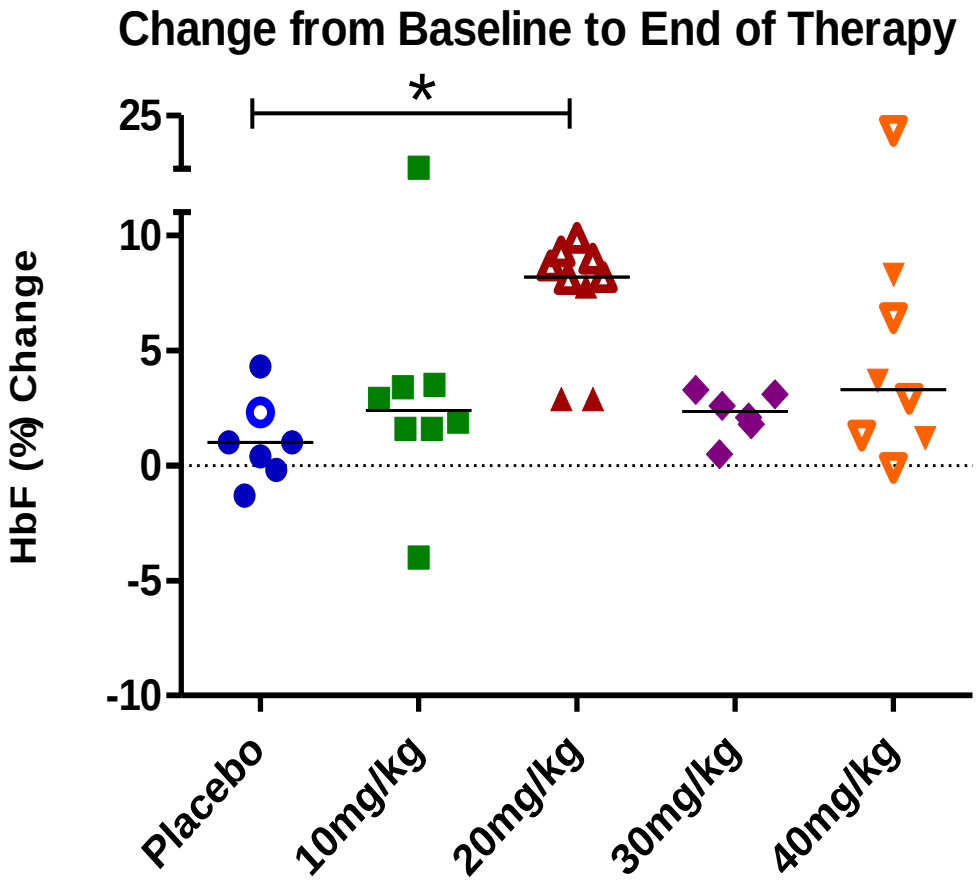
Butyrate and derivatives

Sodium 2,2-Dimethylbutyrate (HQK-1001)

- **Targeted inducer**, activates the *γ-globin* gene promoter without epigenetic effects
- **Favorable cellular actions**: prolongs expression of erythroid survival protein BCL-XL
- Active in transgenic and primate animal models
- Favorable safety profile in preclinical studies & normal human subjects
- Non-mutagenic, not cytotoxic
- Favorable PK profiles for long-term use ($t_{1/2}$ =11 hrs)

Sodium 2,2-Dimethylbutyrate (HQK-1001)

HQK-1001 in β-thalassemia Intermedia in a dose- ranging trial: active dose induces HbF in 8 wks



* *p* value = 0.003

8/9 subjects responded to 20 mg/kg

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Patients Demographic Data

10 β-Thal/HbE intermedia patients

↓

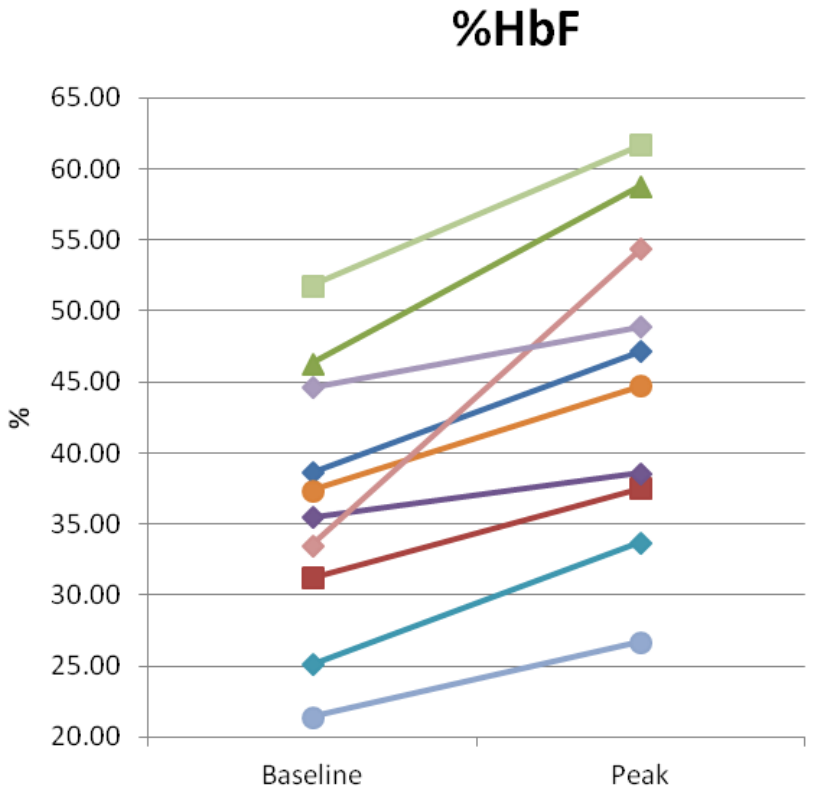
treated with 20 mg/kg/d HQK-1001 for 26 weeks

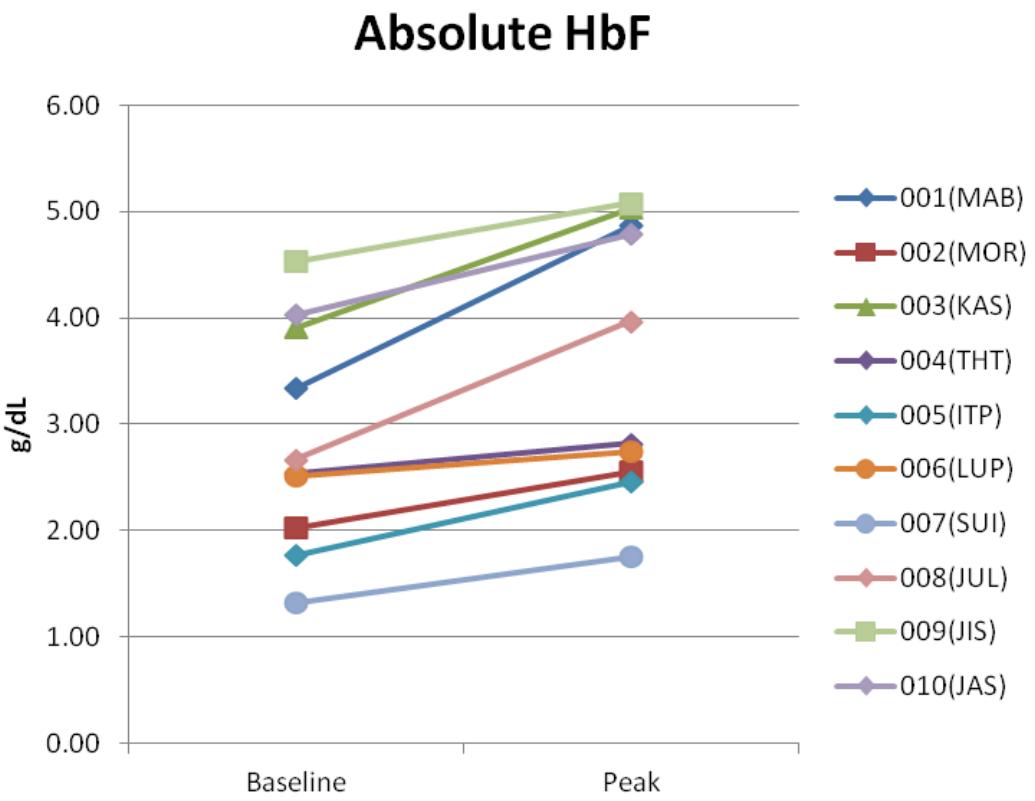
↓

Collect blood every 4 weeks for CBC, Hb Typing monitoring

Code	Age/ Sex	Spleen	Basal Hb (g/dL)	Basal HbF %)	Basal HbF (g/dL)	β-globin mutation (with β^E)
001	33 / F	NS	8.6	38.6	3.32	IVS I-1 (G>T)
002	28 / M	S	6.5	31.2	2.03	Cod17 (A>T)
003	34 / F	NS	8.4	46.3	3.92	Cod110 (T>C)
004	23 / F	NS	7.1	35.5	2.54	Cod41/42 (-TTCT)
005	31 / M	NS	7.0	23.9	1.67	Cod17 (A>T)
006	36 / F	S	6.7	37.4	2.51	Cod17 (A>T)
007	39 / F	S	6.1	21.4	1.31	Cod17 (A>T)
008	37 / F	S	8.0	33.5	2.68	Cod41/42 (-TTCT)
009	36 / F	NS	8.7	51.8	4.54	Cod41/42 (-TTCT)
010	20 / M	S	9.0	44.6	4.01	Cod41/42 (-TTCT)

Hematological Data: Peak HbF Response

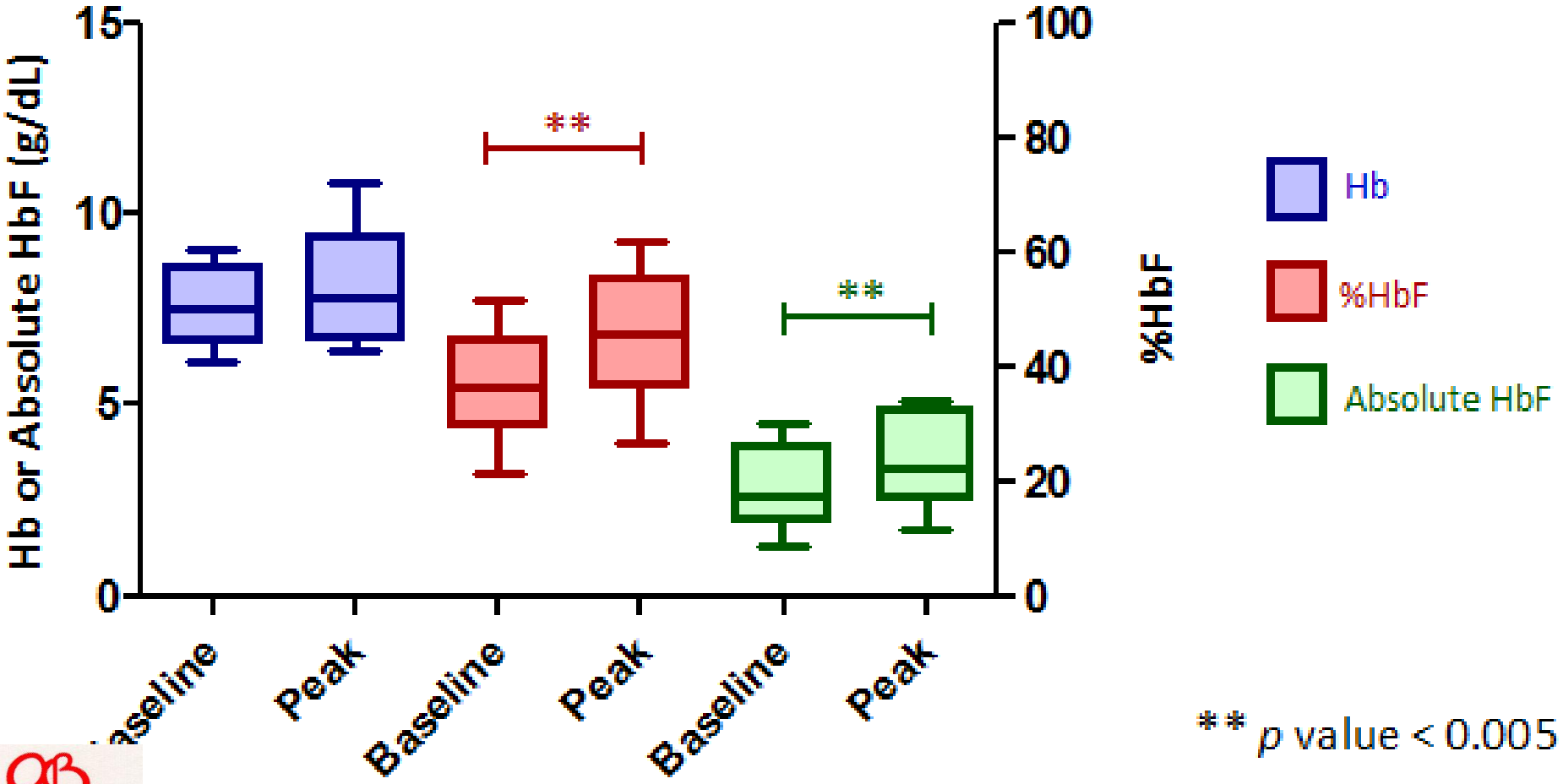




$p < 0.001$
mean increase = 8.68%

$p < 0.001$
mean difference = 0.74 g/dL

Hematological Data: Cumulative Hb, %HbF, Ab HbF



** *p* value < 0.005

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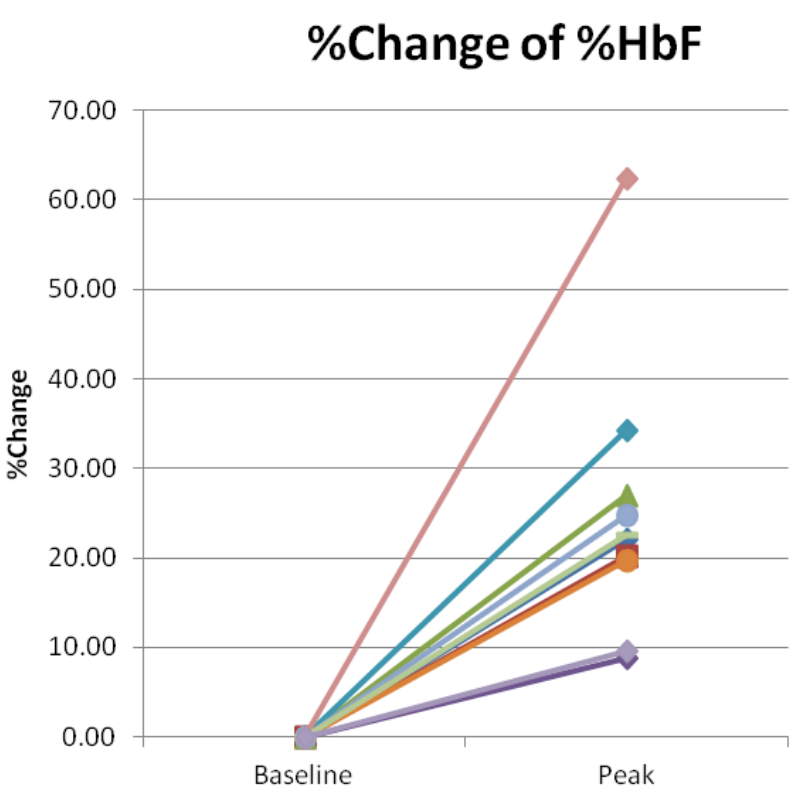
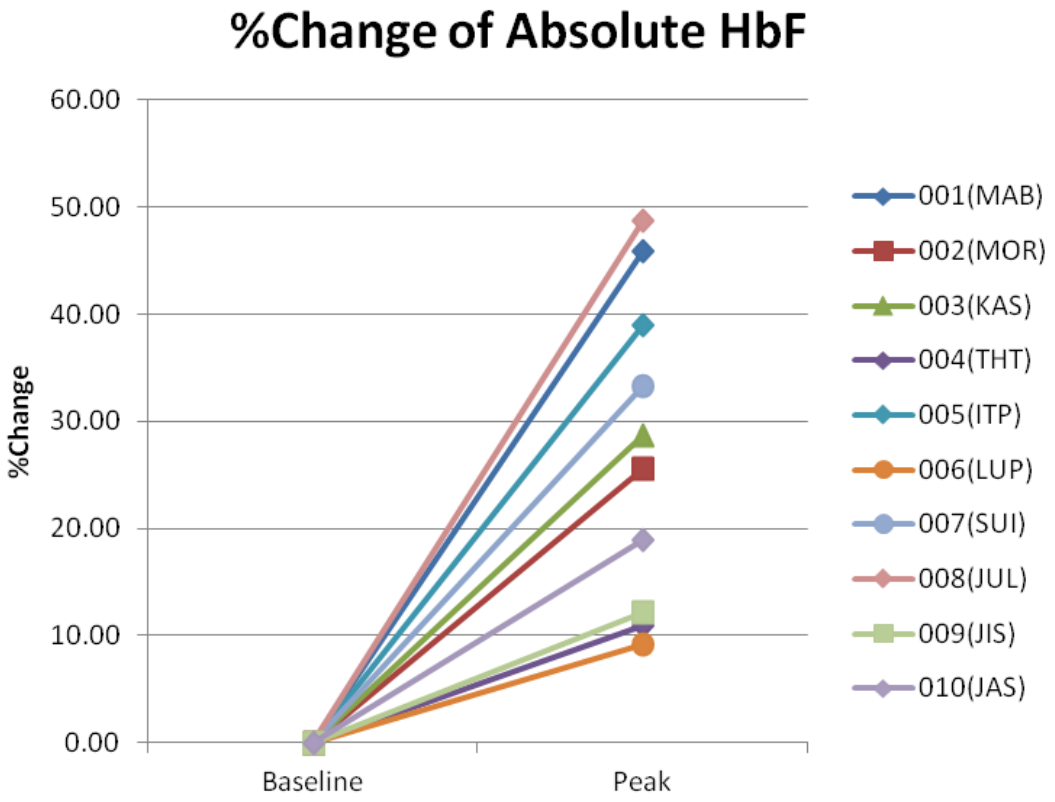
Hematological Data: %Change Peak HbF Response



p < 0.001
mean increase = 24.79 %



p < 0.001
mean difference = 27.22 %

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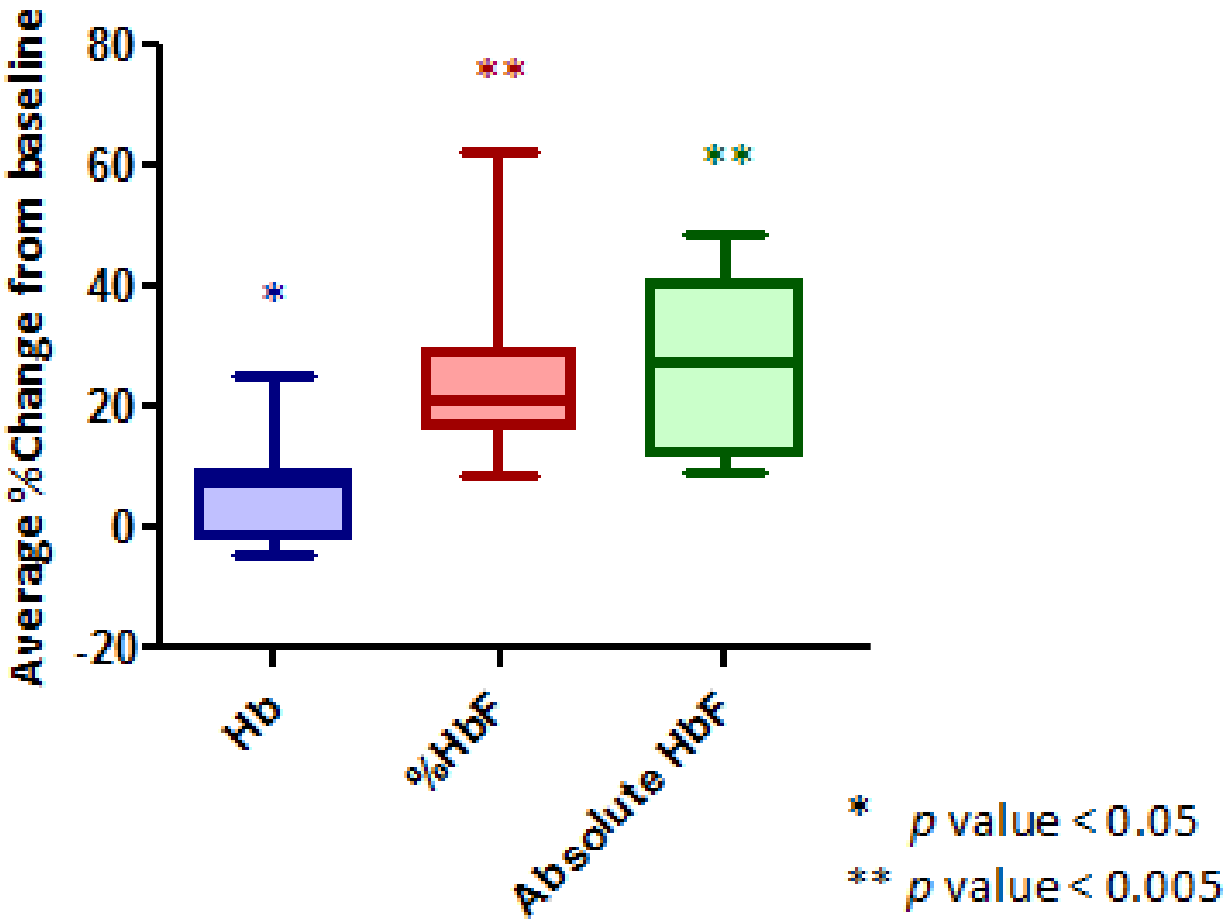
Hematological Data: %Change of Average data



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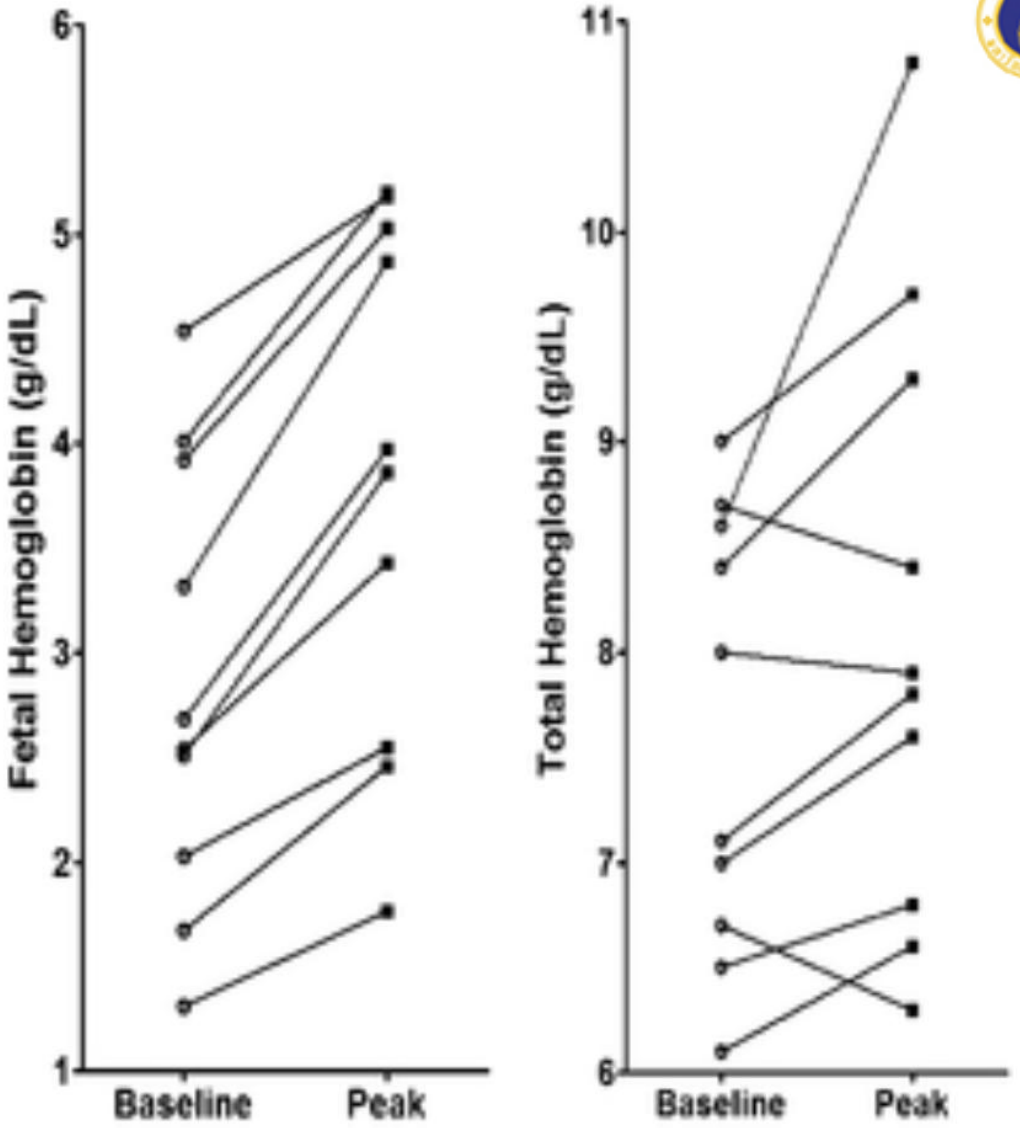


Figure 1. Baseline and peak HbF and total Hb in HbE-β^0 thalassemia subjects treated with HQK-1001.

. *(Blood 2014; 123: 1956)*

Conclusions

HQK-1001, a non-cytotoxic, non-mutagenic oral therapeutic, was well-tolerated with no drug-related adverse events

Responses

- All %HbF of patients increased in different degree.
- Total Hemoglobin increased in 7/10.

- Hb increases began after several months of treatment in some, suggesting that maximal responses were not reached in this trial
- The changes are higher than previously observed with 8 weeks of treatment

- Almost patients increase quality of life.

Conclusions

Safety Findings

- The drug was well-tolerated
- No serious adverse events
  (Possible drug-related AEs: mild nausea in 2 pts)

QTLs Analysis

- *Xmn*I, *BCL11A,* and HMIP minor alleles were commonly observed.
- The homozygote of on *BCL11A* and *Xmn*I polymorphism on -158 $^{G}\gamma$ promoter suggest the correlation of drug response of %Change of HbF.

The findings suggest that further longer trials are warranted to determine hematologic potential in diverse thalassemia subjects

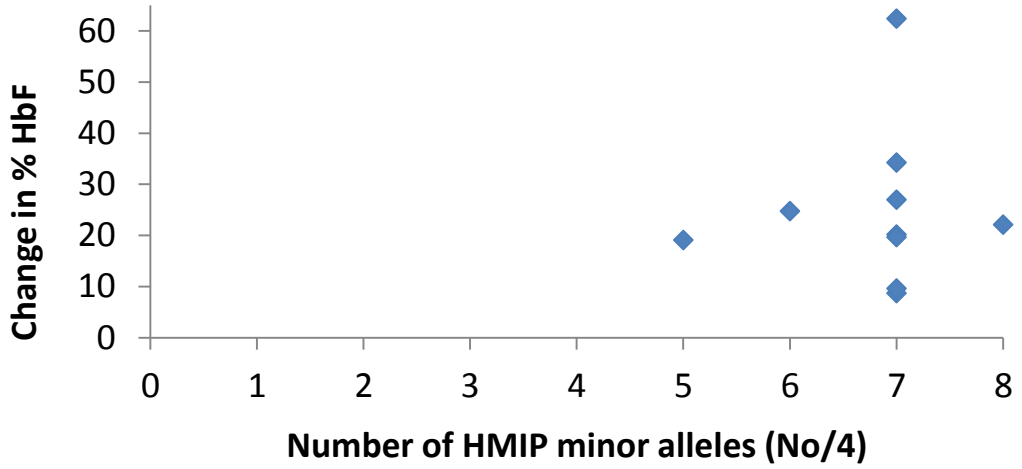
Thalassemia Research Center

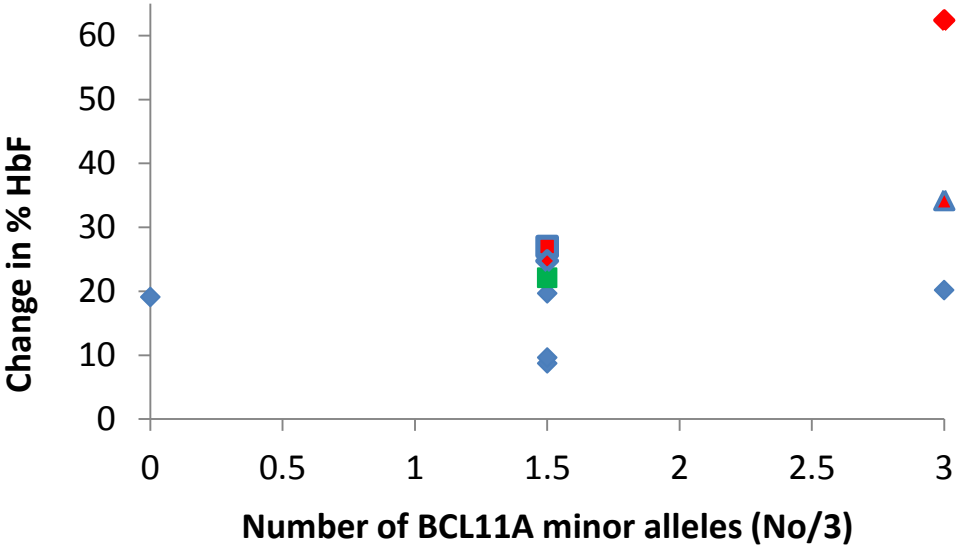
QTLs Analysis

MAHIDOL UNIVERSITY
Wisdom of the Land

ST20 Code	%Change of %HbF	BCL11A						HBB cluster		Intergenic HBS1L-MYB region							
		rs6545816		rs766432		rs6729815		rs7482144/ XmnI		rs9376092		rs7775698 (-3bp)		rs9399137		rs9494145	
008	62.39	C	C	A	A	G	G	C	T	T	G	G	-	A	G	C	C
005	34.26	C	C	A	A	G	G	C	T	G	G	G	G	A	A	T	T
003	27.00	A	C	A	C	A	G	T	T	G	G	G	G	A	A	T	T
007	24.77	A	C	A	C	A	G	C	C	G	G	G	G	A	A	T	T
001	22.12	A	C	A	C	A	G	C	T	T	G	G	-	A	G	C	T
002	20.19	C	C	A	A	G	G	C	T	G	G	G	G	A	A	T	T
006	19.68	A	C	A	C	A	G	C	T	G	G	G	G	A	A	T	T
009	19.11	A	A	C	C	A	A	C	T	T	G	G	-	A	G	C	T
010	9.64	A	C	A	C	A	G	C	T	G	G	G	G	A	A	T	T
004	8.73	A	C	A	C	A	G	C	T	G	G	G	G	A	A	T	T

QTL minor alleles and HbF responses





To analyze further,
select most influential allele

Rationale for inducing γ-globin gene expression for treatment of β thalassemias

- γ globin genes are endogenous and functional in all humans
- Continued expression is safe (HPFH)
- Normally integrated in hematopoietic stem cells
- Genotypes with higher γ globin levels are less anemic than counterparts with lower levels of γ globin

- *An approach feasible for many patients world-wide*