

III Congresso Nacional Ciências Biomédicas Laboratoriais

Potencial da genisteína na reativação da expressão do gene da γ -globulina e indução da hemoglobina fetal

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25 a 27 de outubro de 2019

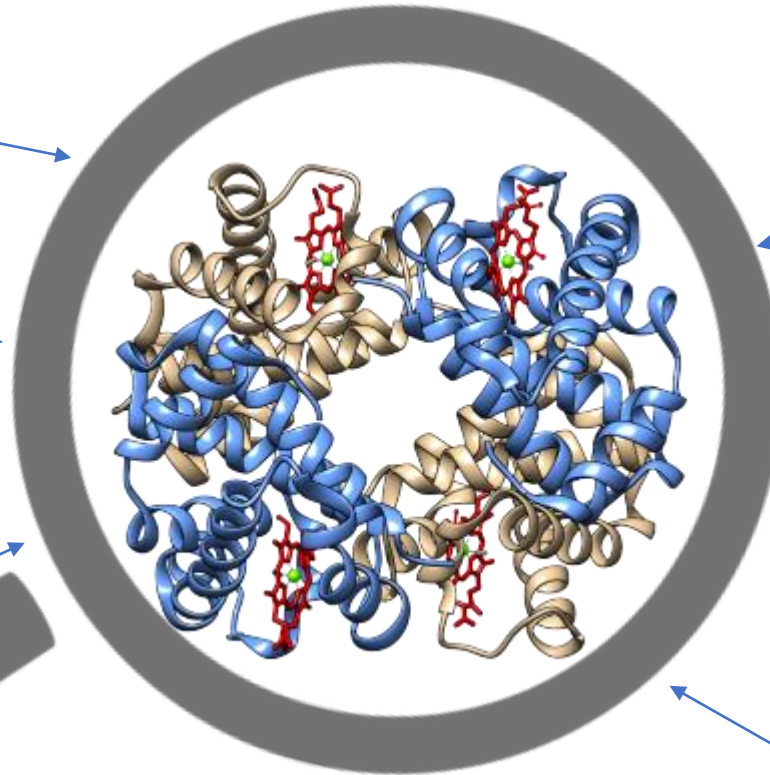


HEMOGLOBINOPATHIES

Hemoglobin (Hb) disorders

Most common monogenic disorders

7% of population are carriers of hemoglobinopathies (WHO)



Human hemoglobin structure

Adapted from: <https://www.sebia.com/en-US/produits/hemoglobin-disorders> - October, 10

Resulting from changes in genes responsible for the synthesis of globin chains

Structural globin chain variants: Sickle cell disease, hemoglobin C, D, and E disease

Disorders of synthesis of the globin chains (decreased or absent): thalassemias

Cavazzana & Mavilio, (2018). Hum Gene Ther. 29(10):1106-1113.

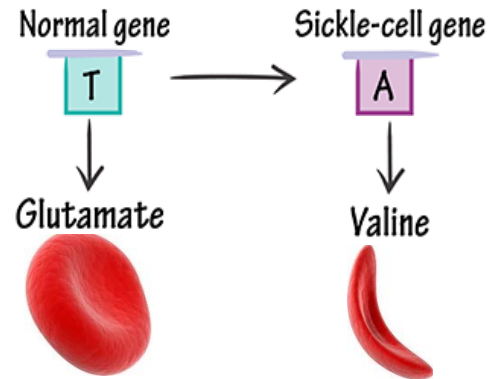
Diepstraten & Hart, (2019). Blood Rev. 33:11-23.

Goonasekera *et al.*, (2018). Annu Rev Genomics Hum Genet. 19:355-380.

β -HEMOGLOBINOPATHIES

Sickle cell disease

Missense mutation in 17th nucleotide of β -globin gene

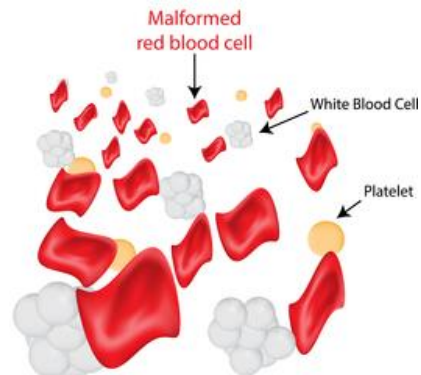


SCD mutation

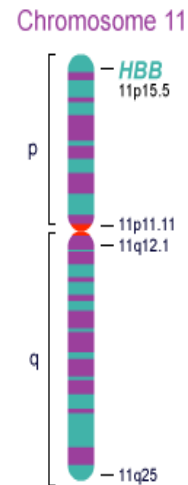
Adapted from: <http://img2018beauty.pw/point-mutation-deletion.html> - November, 24

B-thalassemia

+200 alleles have been described



Shutterstock.com/pt/g/joshya - October, 10



Significant worldwide burden

ANNUAL BIRTHS



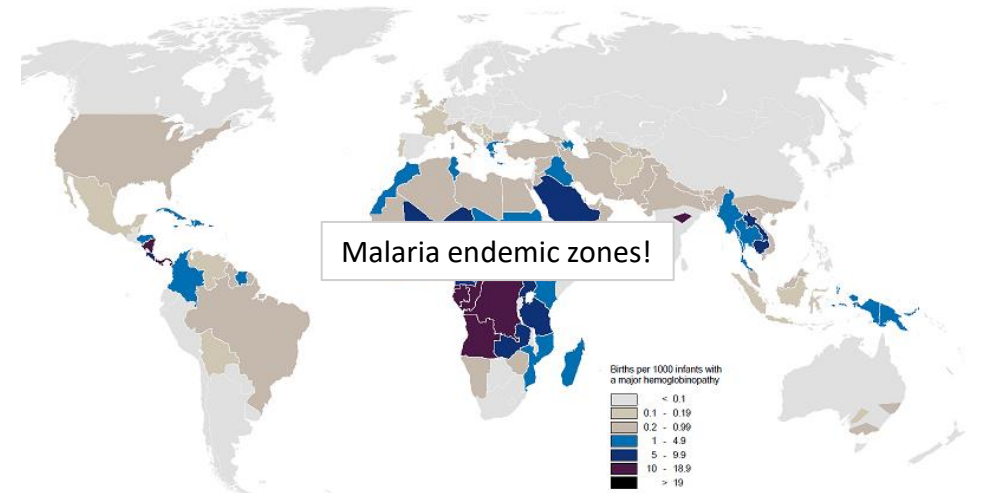
300k

Sickle cell disease

60k

β -thalassemia

<http://crispr.devurlonly.com/programs/hemoglobinopathies> - October, 10



Births with a pathological hemoglobin disorder (per 1,000 live births). Source: WHO

TREATMENTS

High morbidity and mortality



Anemia and transfusions



Pain



Early death

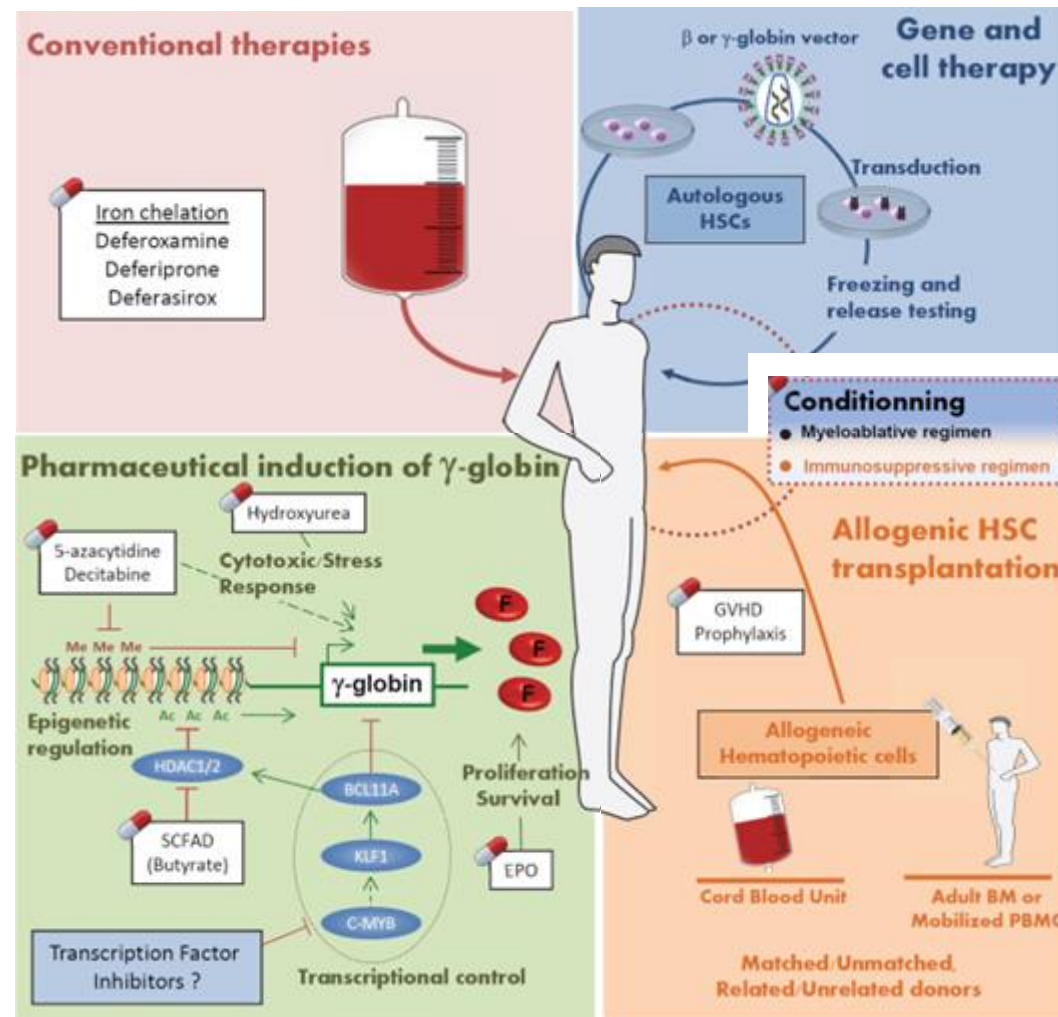
<http://crispr.devurlonly.com/programs/hemoglobinopathies> - October, 10

- Available treatments prevent or manage disease complications;
- Palliative management!

Dreuzy *et al.*, (2016). *Biomed J.* 39(1):24-38.

Schrier & Angelucci, (2005). *Annu Rev Med.* 56:157-71.

Ware *et al.*, (2017). *Lancet.* 390(10091):311-323.



Current and future therapies for β -hemoglobinopathies. Adapted from: Dreuzy, *et al.* (2016). *Biomed J.* 2016 Feb;39(1):24-38.

TREATMENTS

**Significant limitations
particularly in low income countries!!!**

PROJECT GOALS

Identification of **novel agents with higher HbF inducing activity and lower cytotoxicity, accessible in low income countries**, has been one of the major challenges over the past few years

Genistein (4',5,7-trihydroxyisoflavone)



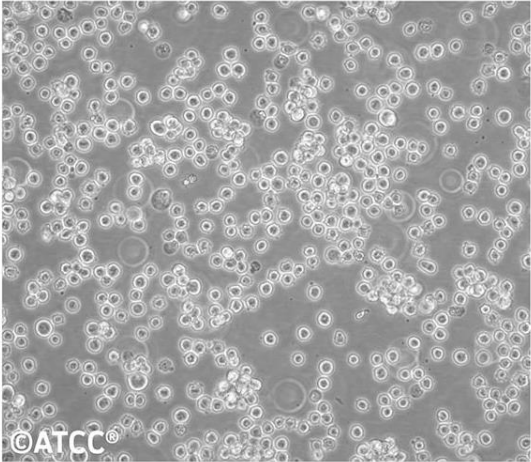
Credit: 1989studio/Shutterstock

- A naturally occurring isoflavone found in plant products (soybeans, lentils, and chickpeas)
- Potent inhibitor of the PTK and topoisomerase-II (\rightarrow G2/M cell cycle arrest); regulates MAPK pathways (p38 MAPK and ERK1/ERK2)
- Antioxidant, antineoplastic, antihelmintic, antiangiogenic and immunosuppressive properties
- Structurally similar to estrogen (estradiol-17 β)
- Potential to mimic, enhance, or impair the estradiol biosynthesis pathway with high reported binding selectivity for ER β isoform

METHODS

Objective: Evaluate the potential of GN in the induction of γ -globin mRNA and HbF expression and potential effects on the transcriptional levels of the gene regulators

K562 Cell line




GATCC®
High Density

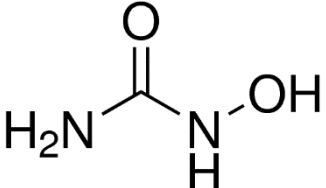
Cell Line Origin
Human Caucasian chronic myelogenous leukaemia

Morphology
Lymphoblast

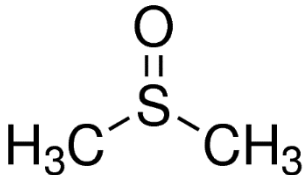
Genistein
100, 250 and 500 ng/ml



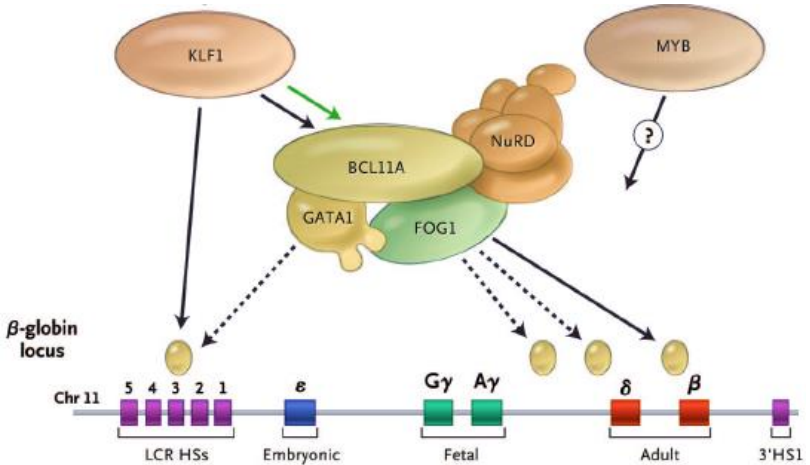
Hydroxyurea
25 ug/ml



DMSO
500 ng/ml




Genes	
Housekeeping	GAPDH
Globins	α -globin
	β -globin
	γ -globin
Signaling	KLF1
	BCL11A

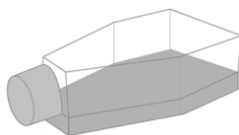


Molecular regulation of the fetal-to-adult hemoglobin switch. Adapted from: Sankaran, V. G. (2011). *Hematology Am Soc Hematol Educ Program*. 2011:459-65.

METHODS

(Six biological replicates)

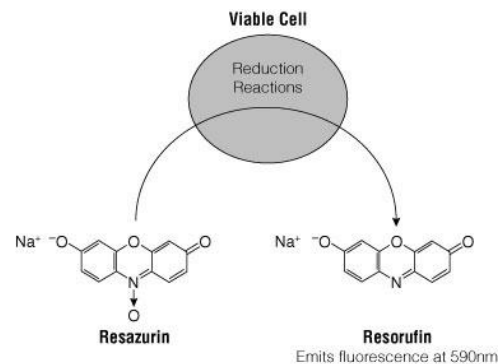
- RPMI 1640



Cell culture 72-96h

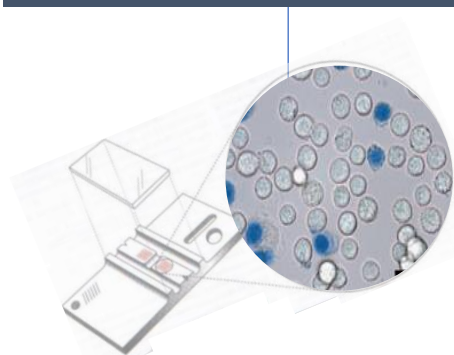
- K562 cells [1×10^4 cells/ml]
- K562 cells + Genistein (100, 250 and 500 ng/ml)
- K562 cells + HU (25 μ g/ml)
- K562 cells + DMSO (500 ng/ml)

- Cell Titer-Blue Cell Viability



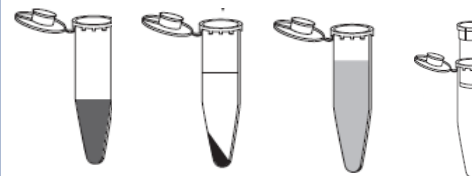
Normal Conversion of resazurin to resorufin by metabolically active cells.
CellTiter-Blue® Cell Viability Assay protocol.

Cell viability assays



- Trypan Blue Cell Viability

- Silica spin column method
- SV Total RNA Isolation System (Promega)



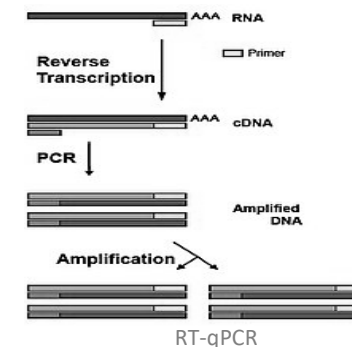
Schematic representation of the SV Total RNA Isolation System.
www.promega.com/protocols/ - October, 10

RNA isolation/quantification

- RNA concentration and integrity
- Qubit™ 3.0 fluorometer



- iTaq™ Universal SYBR® Green Supermix kit (Bio-Rad)



https://en.wikipedia.org/wiki/Reverse_transcription_polymerase_chain_reaction - December, 1

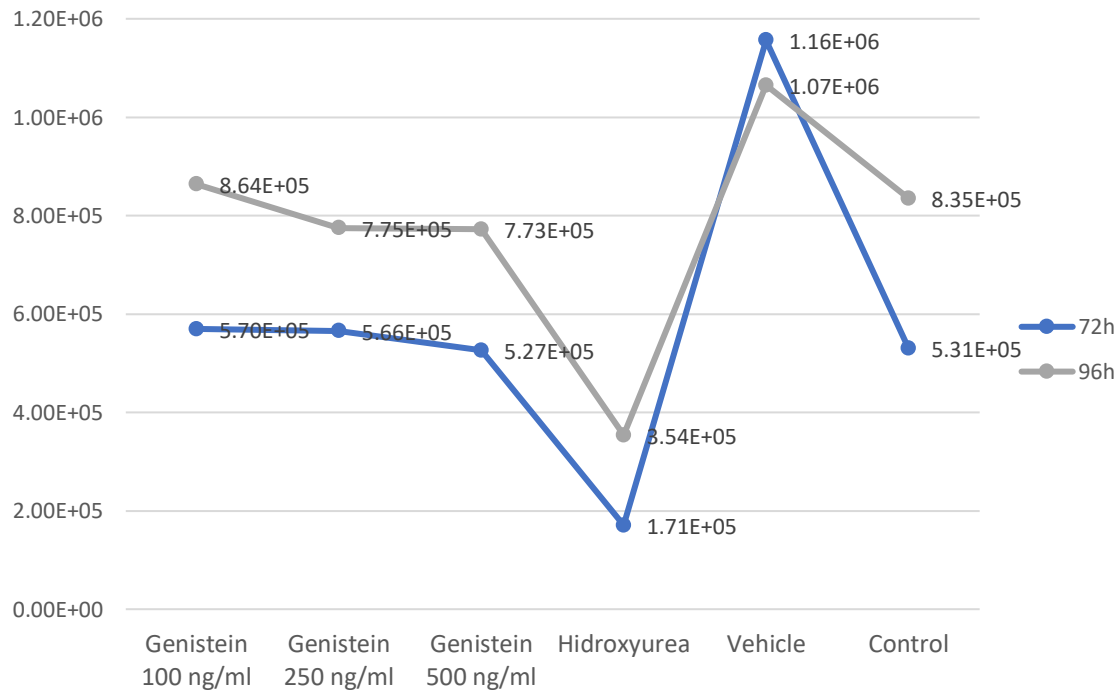
qRT-PCR/analysis

- CT method ($2^{-\Delta\Delta CT}$)
- Student's t test and ANOVA
- SPSS® Statistics 25

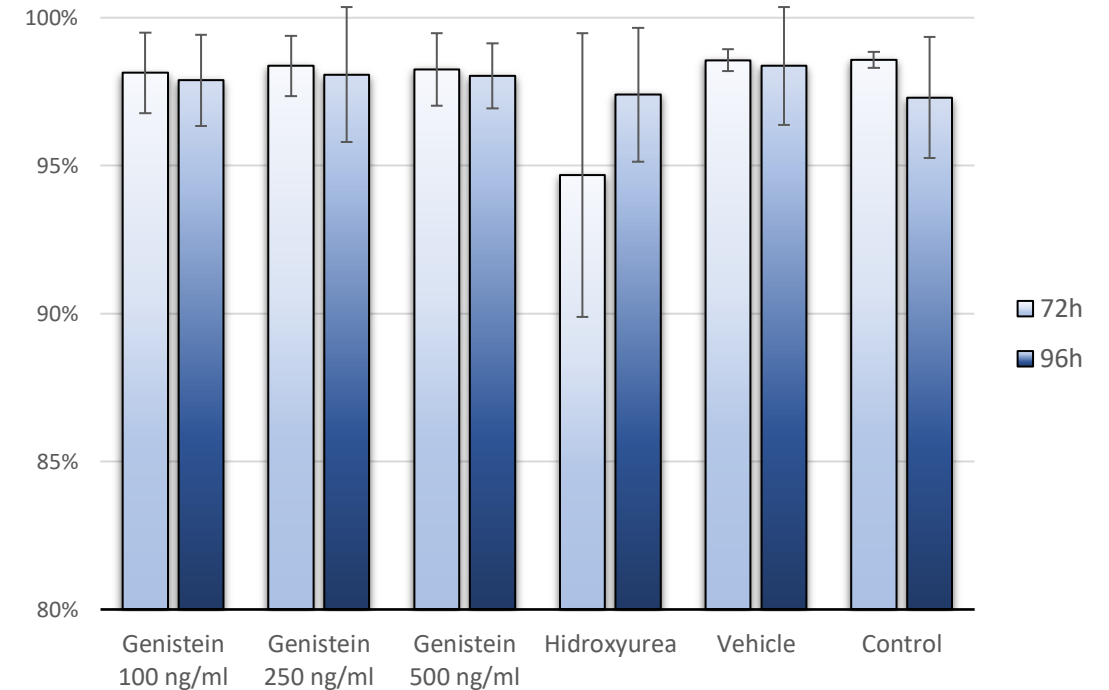


RESULTS: CELL CONCENTRATION AND VIABILITY

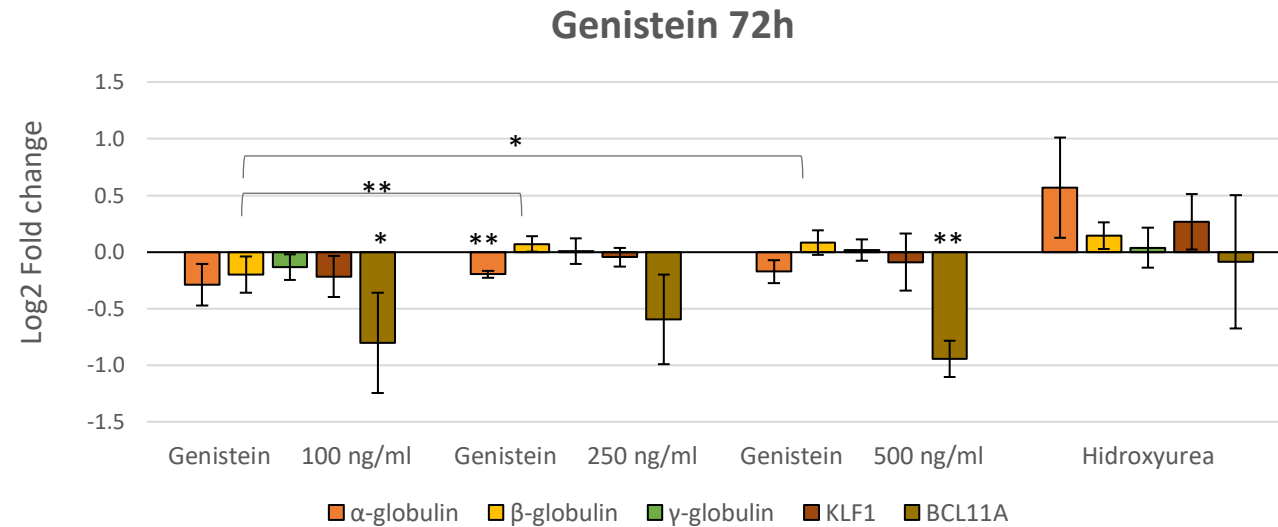
Cell Concentration



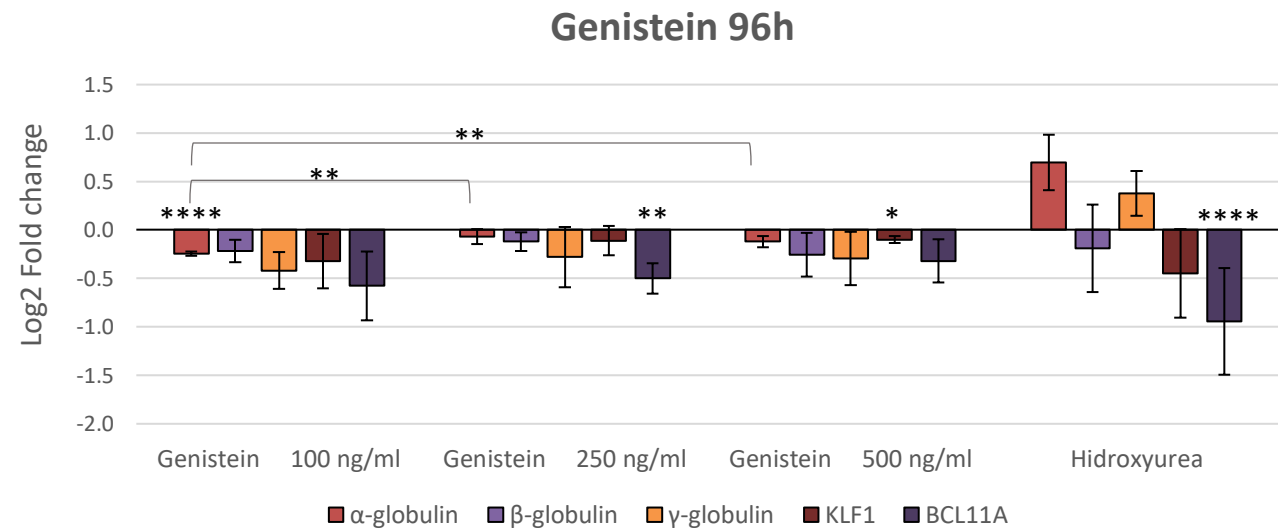
Cell Viability



RESULTS: TRANSCRIPTIONAL EFFECTS ON HB GENES



Symbol	Meaning
ns	$P > 0.05$
*	$P \leq 0.05$
**	$P \leq 0.01$
***	$P \leq 0.001$
****	$P \leq 0.0001$

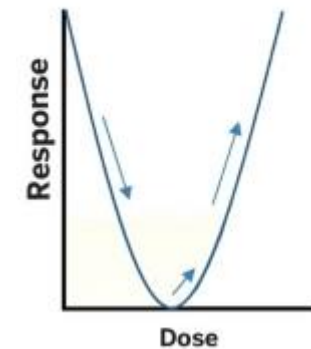


CONCLUSIONS / FUTURE PERSPECTIVES

- GN is not cytotoxic (> 95% viability)
- GN showed a non-monotonic response in KLF1 and BCL11A genes
- Effects in globin genes were only significant for α -globin



Nonmonotonic Curve



A pilot study for project continuation

- Increase exposure time to 120 hours
- Increase GN dose to 800 ng/ml
- Culture with human erythroid progenitor cells
- Protein expression
- Involvement of MAPK signaling pathways in γ -globin mRNA
- GN epigenetic mechanisms associated with γ -globin reactivation (DNMT1, DNMT3A and DNMT3B)

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