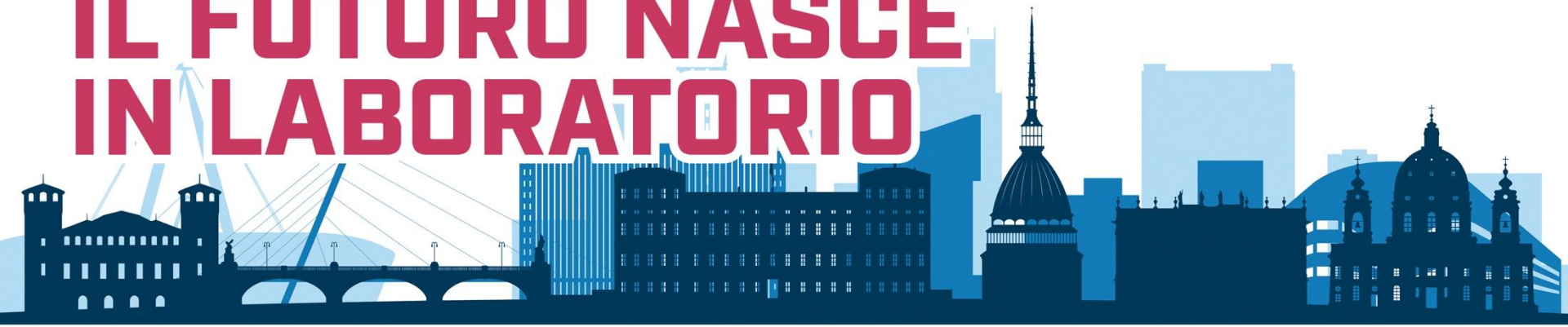
# YOUNG SCIENCE FORUM: IL FUTURO NASCE IN LABORATORIO



## Analisi funzionale delle cellule eritroidi di pazienti con talassemia non trasfusione-dipendente trattati con luspatercept

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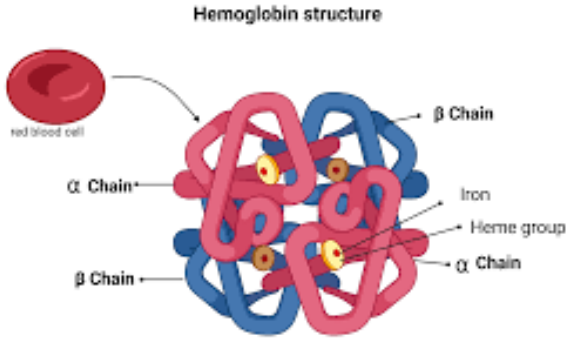
Conflict of Interest: I declare no conflict of Interest

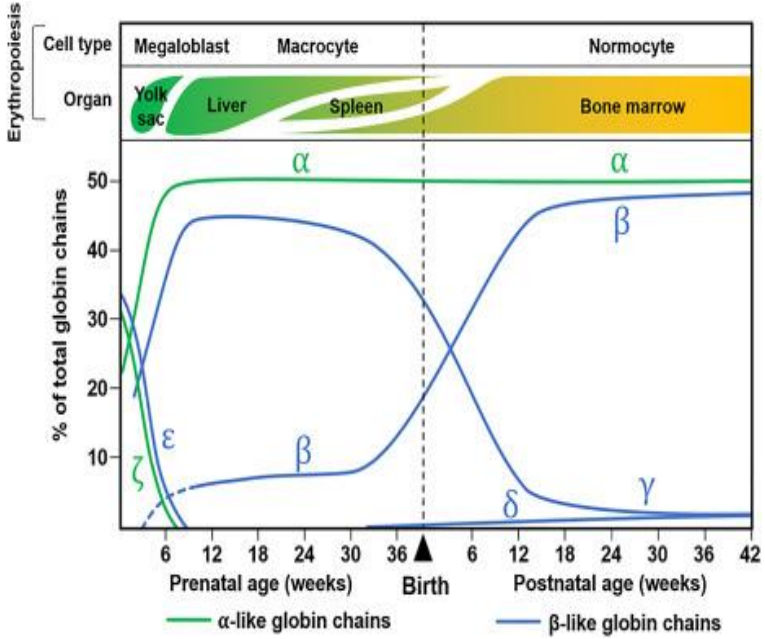
# β-Thalassemia: A Disorder of Globin Synthesis

- β-thalassemia is a hereditary hemoglobin disorder characterized by reduced or absent β-globin chain synthesis.
- Reduced β-globin synthesis disrupts the α/β-globin chain balance.



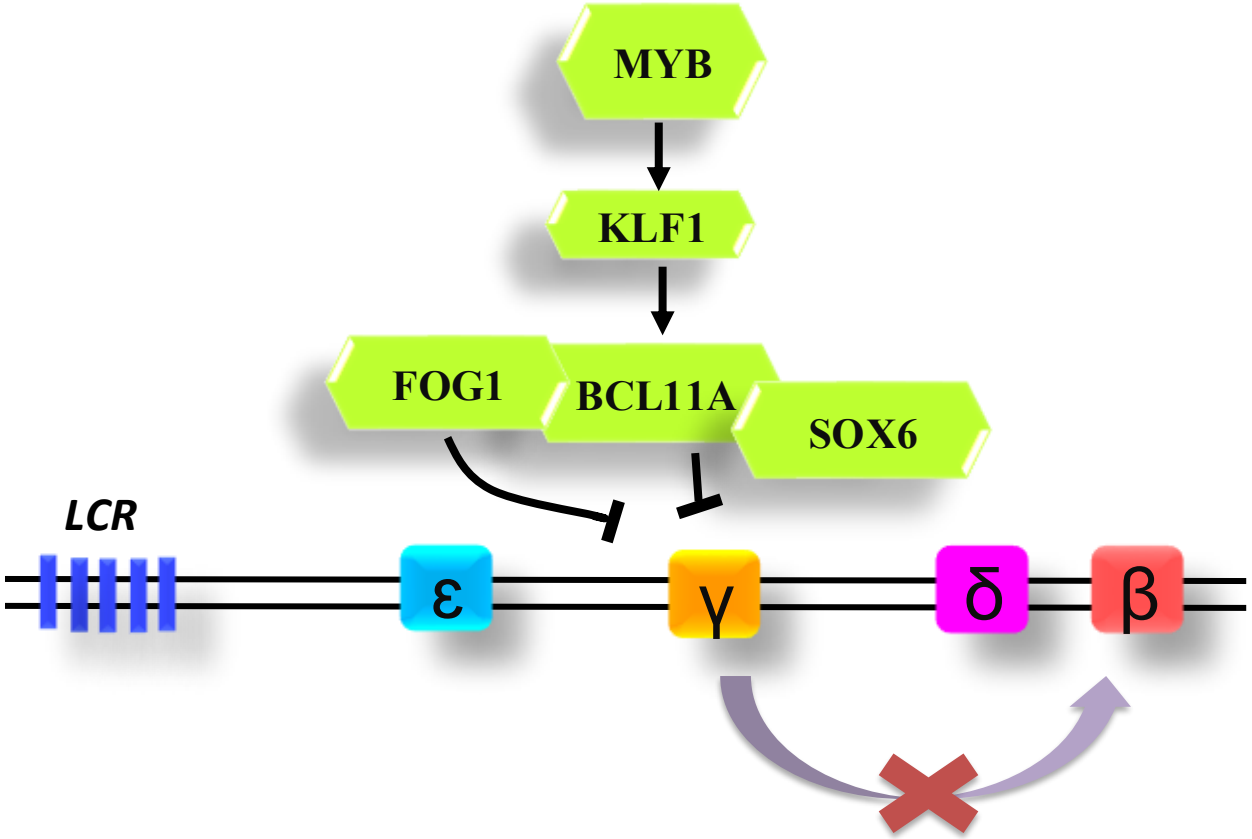

# Fetal Hemoglobin (HbF) and Disease Modulation

- HbF predominates during fetal life and decreases after birth.
- β-thalassemia shows delayed γ-to-β globin switching.
- Elevated HbF reduces globin chain imbalance and ineffective erythropoiesis

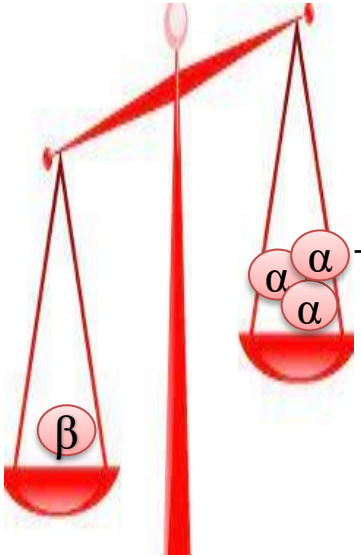



**Induction of γ-globin is therapeutically beneficial in β-thalassemia.**

# Schematic representation of γ globin gene modulation



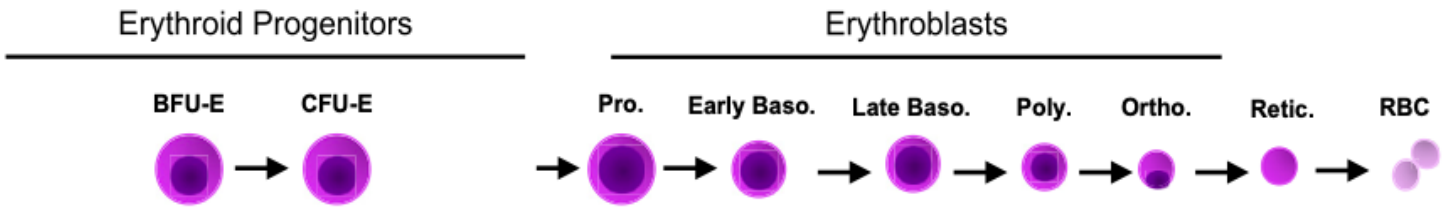
# Pathophysiology of β -thalassemia




Precipitation lead to premature destruction of erythroid precursor

Ineffective erythropoiesis

- Anemia
- Hypoxia
- Hemolysis
- Iron Overload




**NORMAL ERYTHROPOIESIS**

Positive regulation by EPO

**Proliferation Stage**

Positive regulation by GATA1

**Differentiation/Maturation Stage**

**RED BLOOD CELL HOMEOSTASIS**

**INEFFECTIVE ERYTHROPOIESIS**

- ↑ EPO levels
- ↑ ROS generation and apoptosis

**Clonal Expansion**

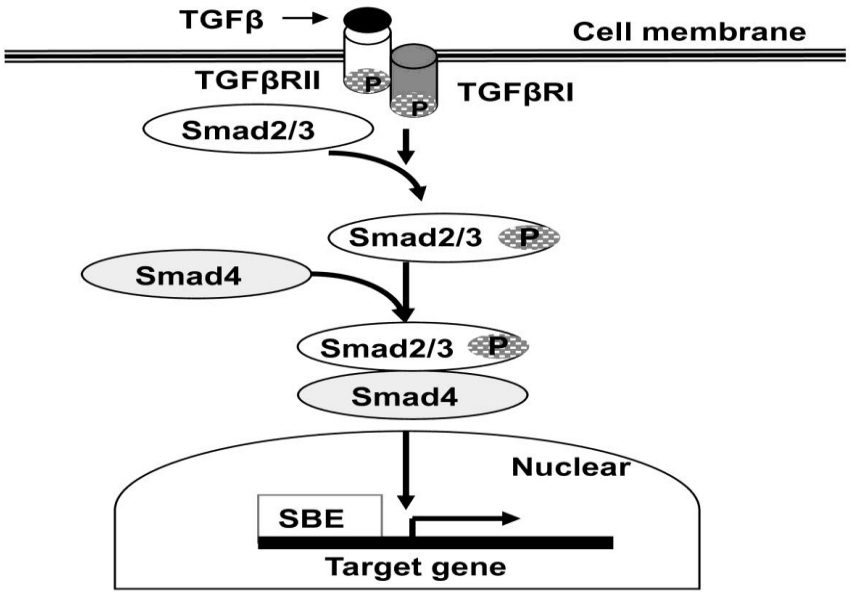
- GATA1 cleavage
- Activation of the SMAD2/3 signalling pathway

**Blocked Differentiation**

**ANEMIA**

# Ineffective erythropoiesis and SMAD Signalling

- TGF-β superfamily signalling contributes to ineffective erythropoiesis.
- Activin/GDF11-mediated SMAD2/3 activation suppresses late-stage erythroid maturation.



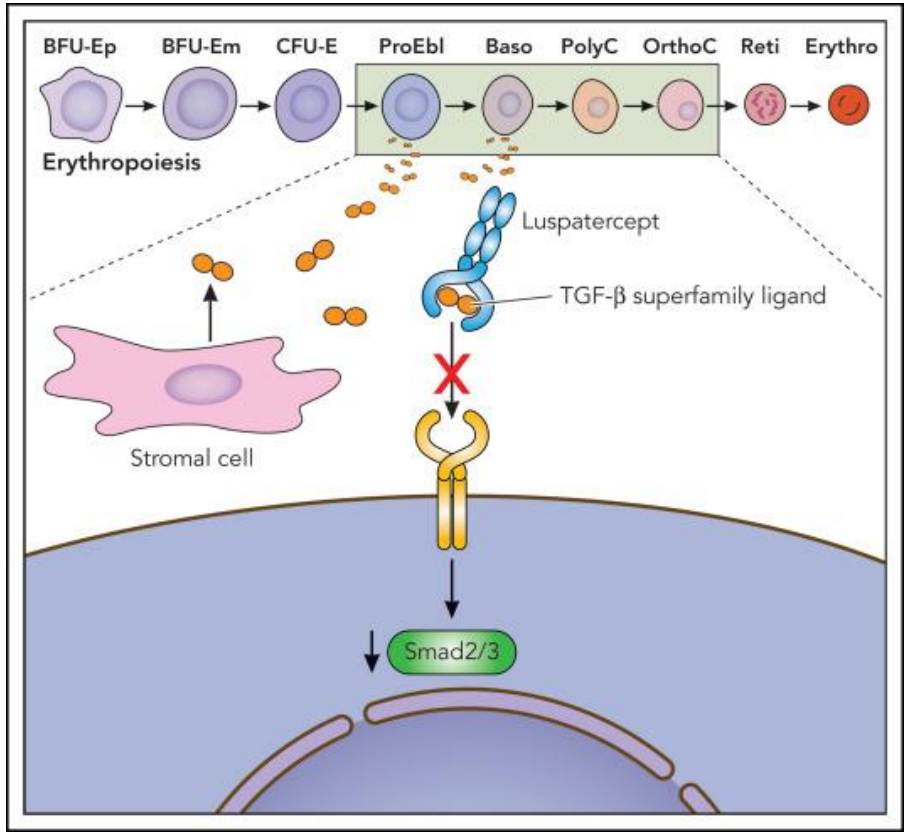

## Mechanism line

GDF11 → ActRIIB receptor →SMAD2/3 phosphorylation →impaired erythroid maturation

# Luspatercept: Mechanism of Action

- Luspatercept (ACE-536) is an activin receptor type IIB ligand trap.
- It binds selectively to TGF-β superfamily ligands.
- This inhibits SMAD2/3 signalling.

- Result:
  - ✓ Enhanced late-stage erythroid maturation
  - ✓ Improved erythropoiesis



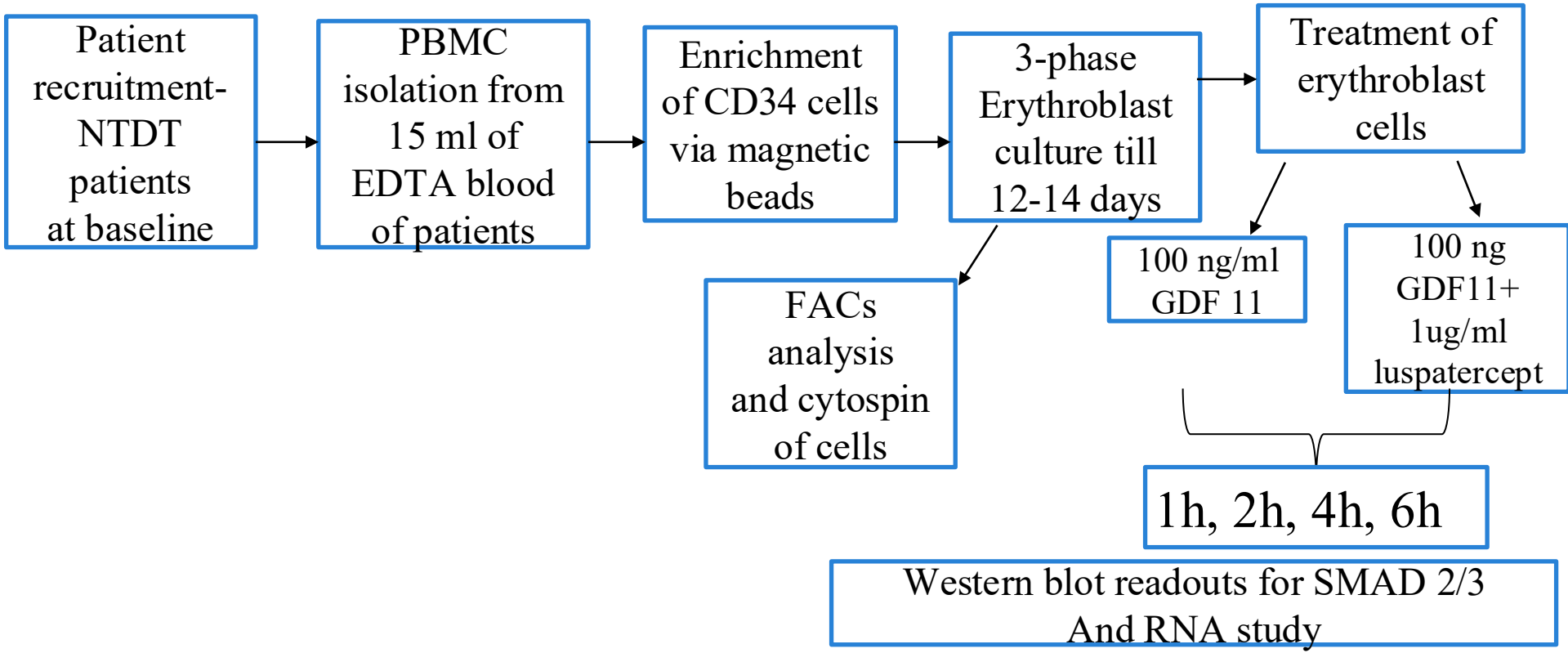
# Hypothesis

Could luspatercept-mediated inhibition of SMAD2/3 signalling alter erythroid transcriptional networks and enhance γ-globin expression in β-thalassemia erythroid cells?

# AIM of the Study

To investigate the effects of luspatercept on SMAD2/3 levels, erythroid transcription factors (GATA1, BCL11A) and γ globin gene expression in erythroid cells derived from non transfused dependent thalassemia patients

# Workflow


Patient recruitment- NTDT patients at baseline
PBMC isolation from 15 ml of EDTA blood of patients
Enrichment of CD34 cells via magnetic beads
3-phase Erythroblast culture till 12-14 days
Treatment of erythroblast cells
FACs analysis and cytospin of cells
100 ng/ml GDF 11
100 ng GDF11+ 1ug/ml luspatercept
1h, 2h, 4h, 6h
Western blot readouts for SMAD 2/3 And RNA study

# Results

# Isolation and Enrichment of CD34 cells

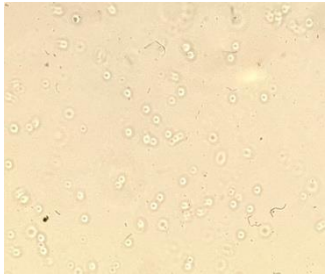
15 ml EDTA blood : PBMC: 3.2 X $10^7$ cells

↓

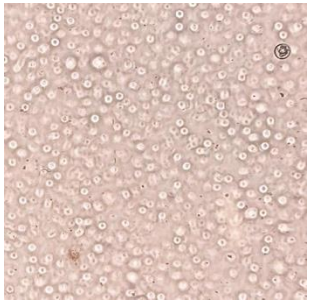
CD34+: 5.44 X $10^5$ cells

↓ 3 phase liquid culture 12 day

Erythroid cells: 3.67 X $10^7$ cells

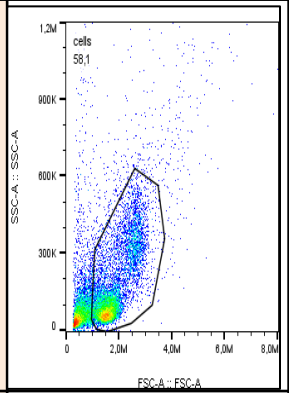
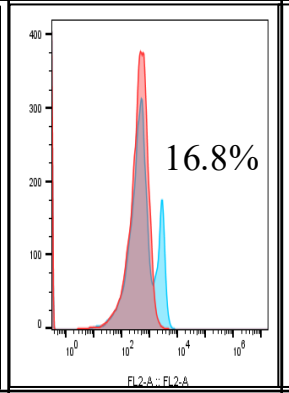
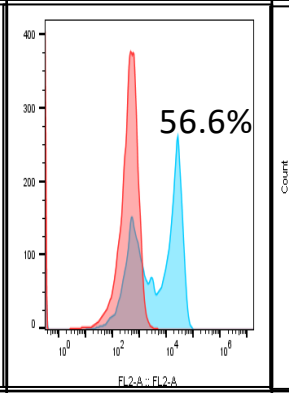
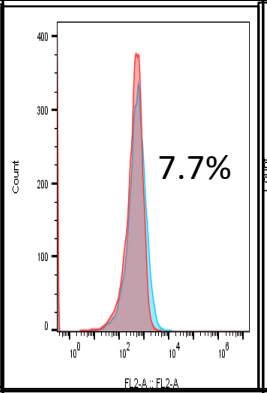
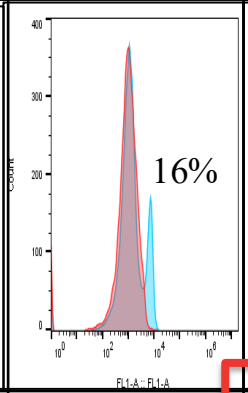
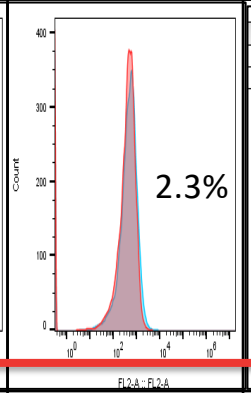
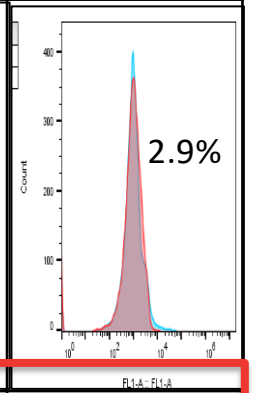
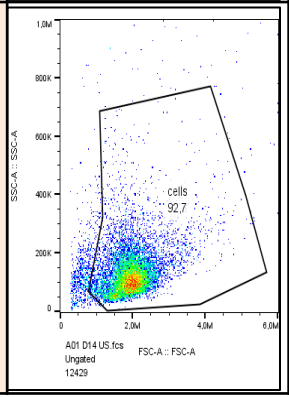
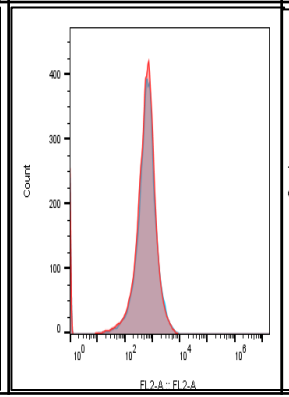
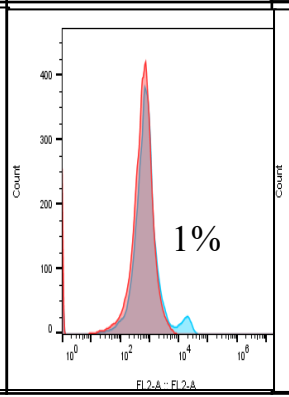
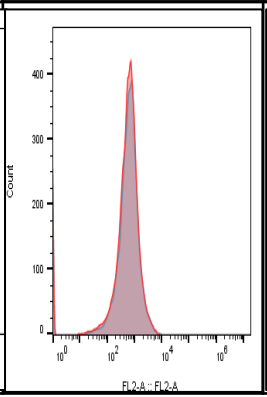
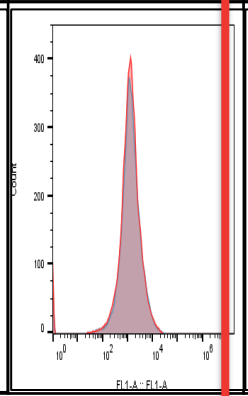
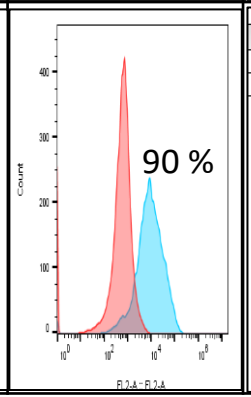
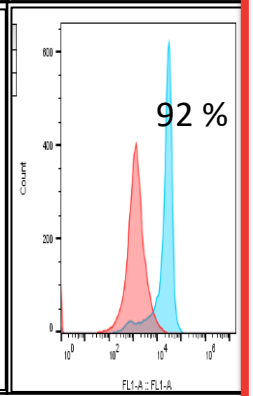


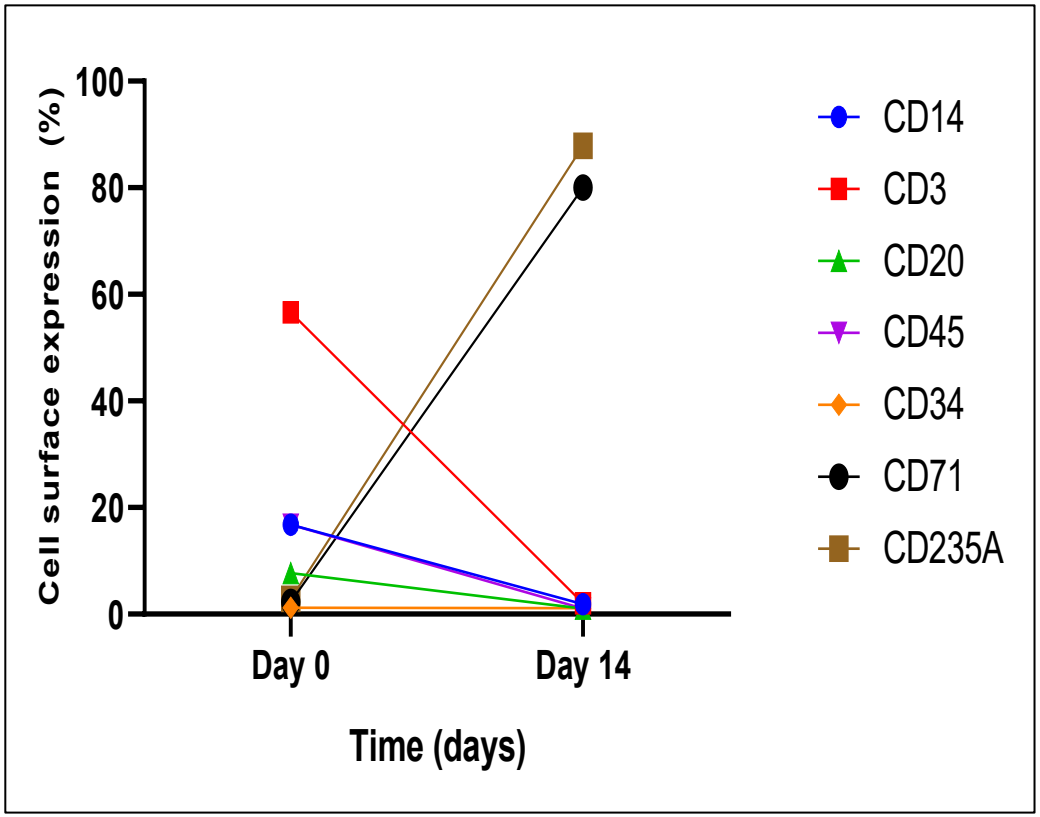
Day 0



Day 12

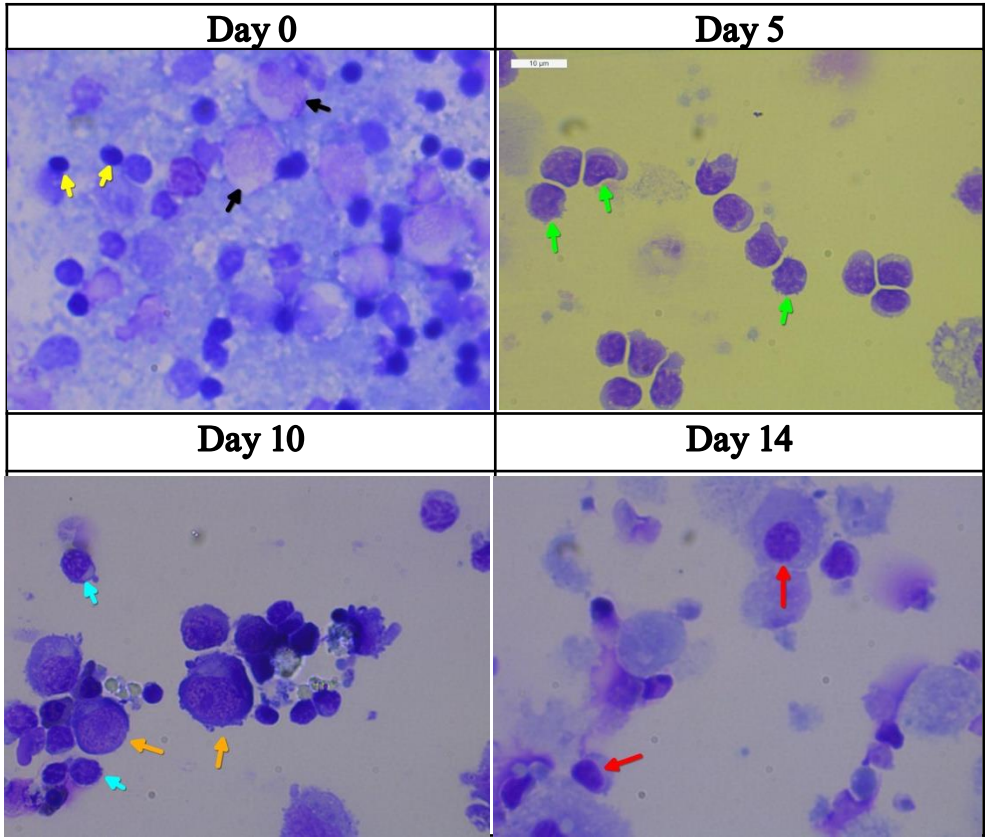
# Validation of erythroid differentiation (FACS)

| Day | Cell population | Lymphocytes marker | | | | Erythroid marker | |
|---|---|---|---|---|---|---|---|
| | | CD 14 | CD 3 | CD 20 | CD 45 | CD 71 | CD 235A |
| DAY 0 |  |  |  |  |  |  |  |
| DAY 12 |  |  |  |  |  |  |  |




Efficient terminal erythroid differentiation achieved

# Cell morphology assessment




Day 0: lymphocytes (yellow arrows) and monocytes (black arrows)

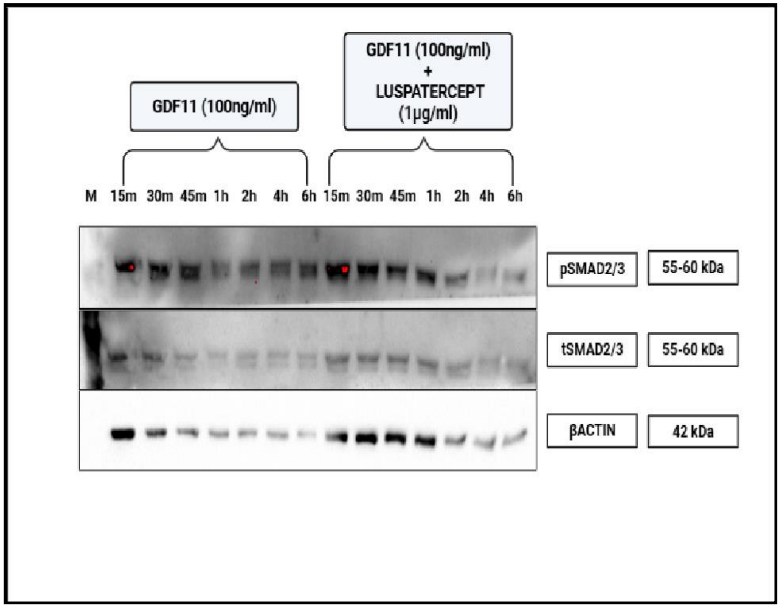
Day 5: proerythroblast stage (green arrows)

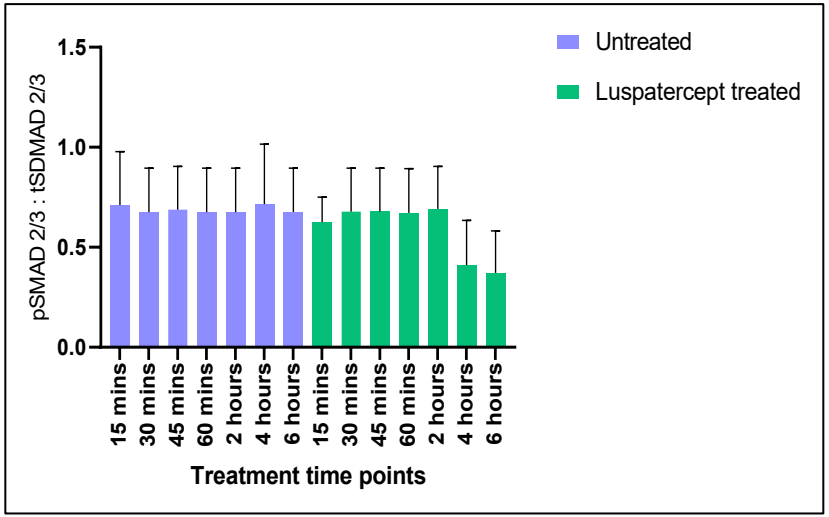
Day10: basophilic erythroblasts, (blue arrows); polychromatic erythroblasts, (orange arrows)

Day 14: orthochromatic erythroblasts (red arrows)

# Western blot Readouts SMAD2/3 signaling

# Effect of Luspatercept on pSMAD 2/3 levels

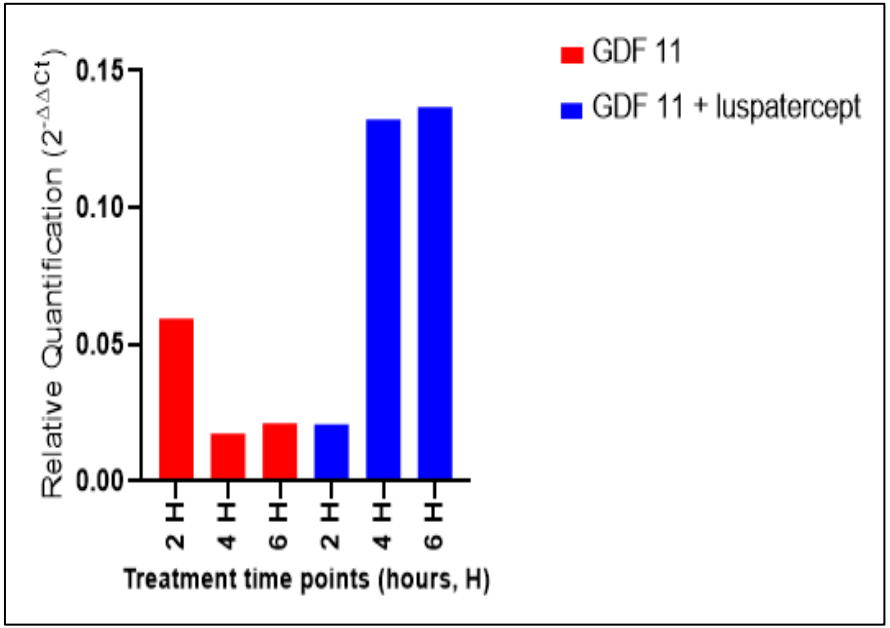


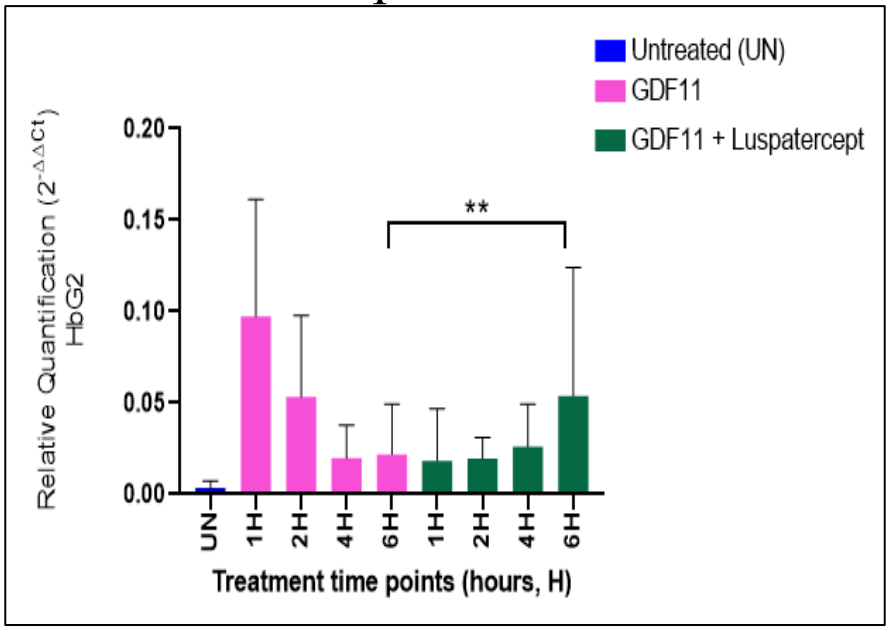

Luspatercept treatment led maximal decrease in pSMAD2/3 levels by 25% at 6 hours in erythroid cells of patients

# Effect of luspatercept on HbG2 (γ) gene expression

Non transfused thalassemia

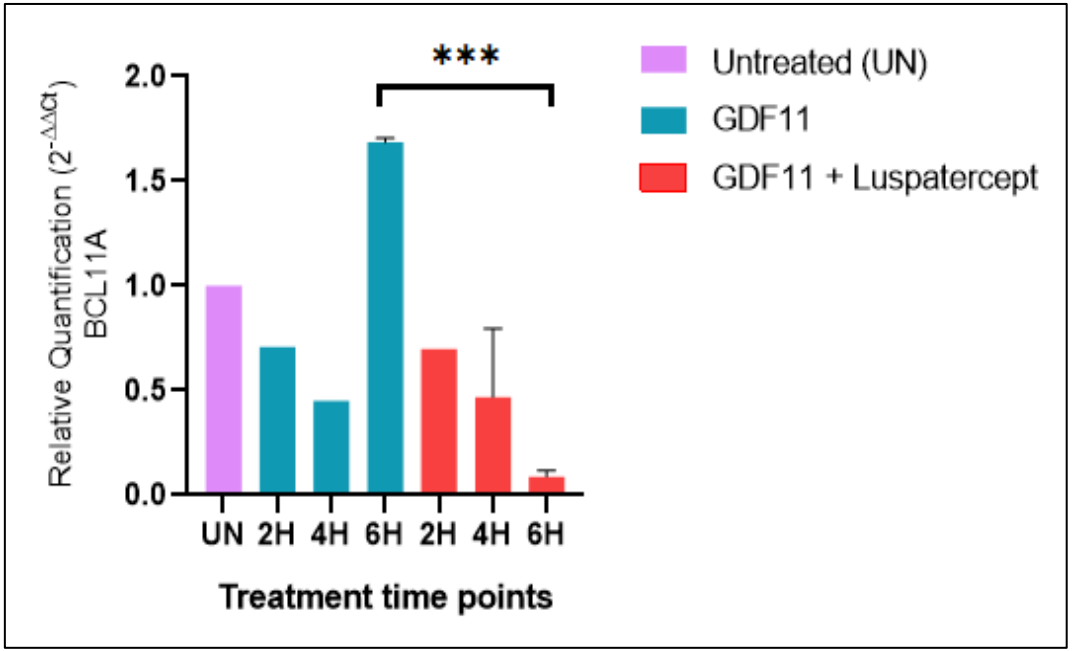



Transfused dependent thalassemia



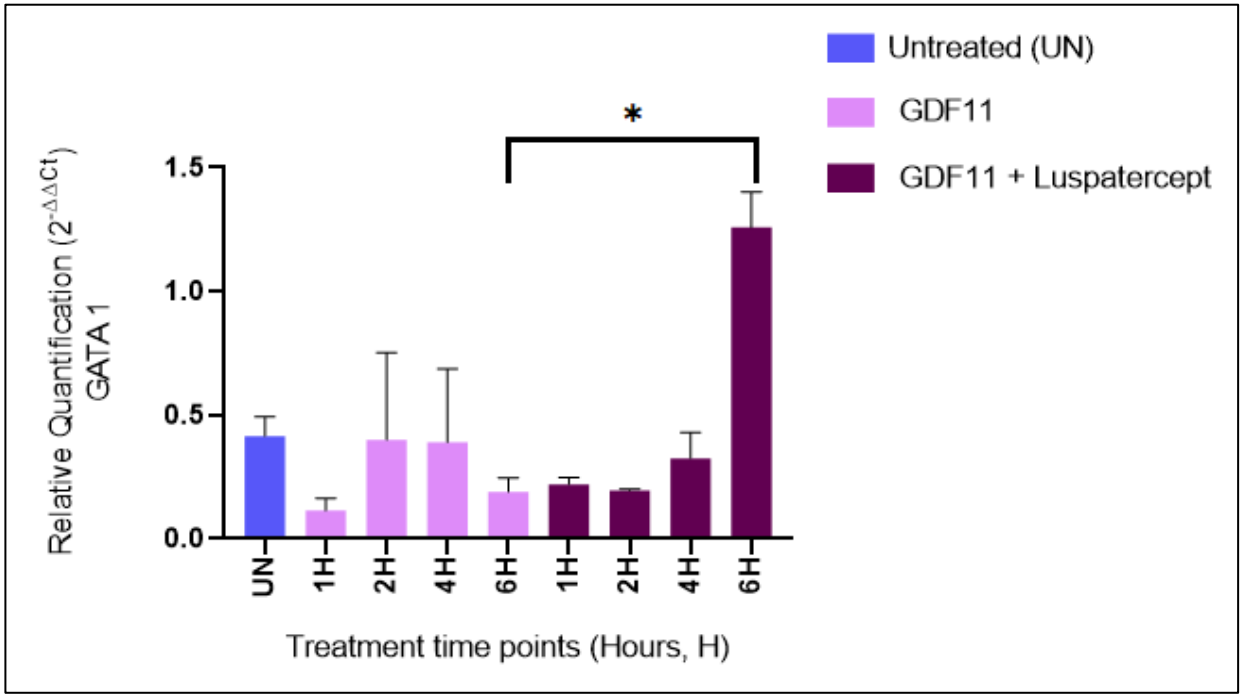

Luspatercept induced a time-dependent increase in γ-globin expression, reaching a significant 6.1-fold upregulation at 6 h compared with GDF11 alone.

# Effect of Luspatercept on BCL11A expression



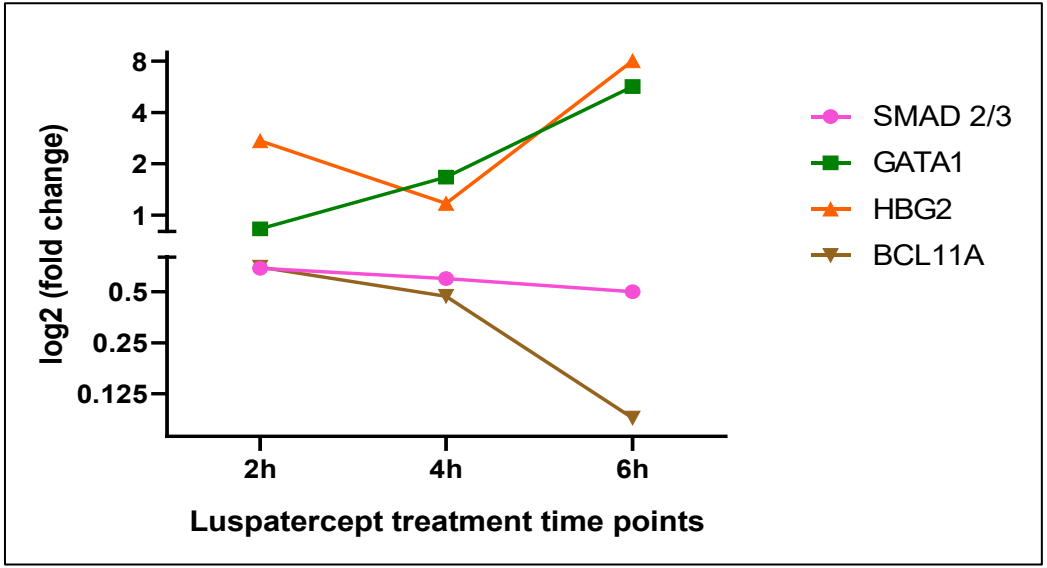

BCL11A expression decreased significantly following luspatercept treatment, reaching a 9.3-fold reduction at 6 h compared with GDF11 alone ($p < 0.0001$)

# Effect of Luspatercept on GATA1 expression




GATA1 gene expression was significantly elevated (5.7-fold, $p < 0.05$) after 6 h of luspatercept treatment compared to GDF11 alone.

# Integrated response of luspatercept




Luspatercept appears to coordinate signalling and transcriptional pathways associated with erythroid maturation

# Conclusion

- Luspatercept reduces GDF11-induced SMAD2/3 signalling and is associated with increased GATA1 and γ-globin expression along with decreased BCL11A expression in β-thalassemia patient-derived erythroid cells.
- Overall, these findings suggest that luspatercept promotes a fetal hemoglobin–favourable erythroid transcriptional program through modulation of the SMAD–GATA1–BCL11A axis.

# Plan for Future

- Future studies will use time-resolved RNA-seq (2h, 4h, 6h, 8 h) in untreated, GDF11, and GDF11 + luspatercept conditions to identify key transcripts and regulatory networks involved in its mechanism.
- Candidate genes will be functionally validated to define their role in luspatercept-mediated erythroid reprogramming.

# Acknowledgment

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# THANK YOU

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