SELECTED OPPORTUNITIES IN HAEMATOLOGY

Free α-hemoglobin as biomarker for diagnosing and stratifiying severity of β-thalassemia and other hemoglobin-related disorders (BIO08398)

FREE A-HEMOGLOBIN AS BIOMARKER FOR DIAGNOSING AND STRATIFIYING SEVERITY OF B-THALASSEMIA AND OTHER HEMOGLOBIN-RELATED DISORDERS (BIO08398)

Product factsheet

Stage:
Pre-Analytic
Validation

- **Biomarker:**
  - Free α-hemoglobin (α-Hb)
- **Technology:**
  - ELISA, HTRF
- **Sample:**
  - Blood
- **Information:**
  - Diagnostic
  - Patient Stratification
  - Prognosis

- **Scientific and Clinical Rationale:**
  - The normal development of red blood cells requires a coordinated synthesis of the hemoglobin (Hb) subunits, the α- and β-globins in the case of adult hemoglobin (Hb A). Unlike the β-hemoglobin chains (β-Hb) which are soluble and form homologous tetramers, the free α-hemoglobin chains (α-Hb) are highly instable, and when in excess, form precipitates and act as active oxidants causing apoptosis and inefficient erythropoiesis.
  - The molecular chaperone of α-Hb, the « Alpha-Hemoglobin Stabilizing Protein » (AHSP) specifically binds to α-Hb to form a stable soluble heterodimer but not to the β-Hb or to tetrameric Hb A.
- **POC:**
  - Ctrl healthy donor (n=50); vs β-thalassemic (n=58) or α-thalassemic (n=24) patients
  - Free α-Hb levels are higher in β-thalassemic patients (n=20) compared to α-thalassemic patients (n=6) or healthy subjects without Hb anomalies (n=28).
  - Additional prospective cohort ALPHAPOOL: β-thalassemic (n=58) or α-thalassemic patients (n=24) or healthy subjects (=50)

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Clinical State and Market Opportunity

- **Clinical State:**
  - Epidemiology:
    - Affects a prevalent population of around 440000 patients worldwide[1]
  - Treatment:
    - Blood transfusion, Iron Chelation therapy, hydrowycarbamide…
- **Opportunity:**
  - Variety of other potential indications:
    - Tracking extranumerous α genes: various duplicated or triplicated genotypes
    - Suspicion of α^0-thal trait in association with β-thalassaemia trait
    - Normocytic cobalamin or folate deficiency (masking thalassaemia trait)
    - Monitoring therapies (inducers of the synthesis of γ-globin, Hydroxycarbamide, Sotatercept…)
    - Diagnostic orientation needed as an emergency in genetic counselling
    - Identification of the risk of major β-thalassaemia disease during pregnancy and the diagnosis of difficult symptomatic γ-thalassaemias of newborn

Unique Selling Points

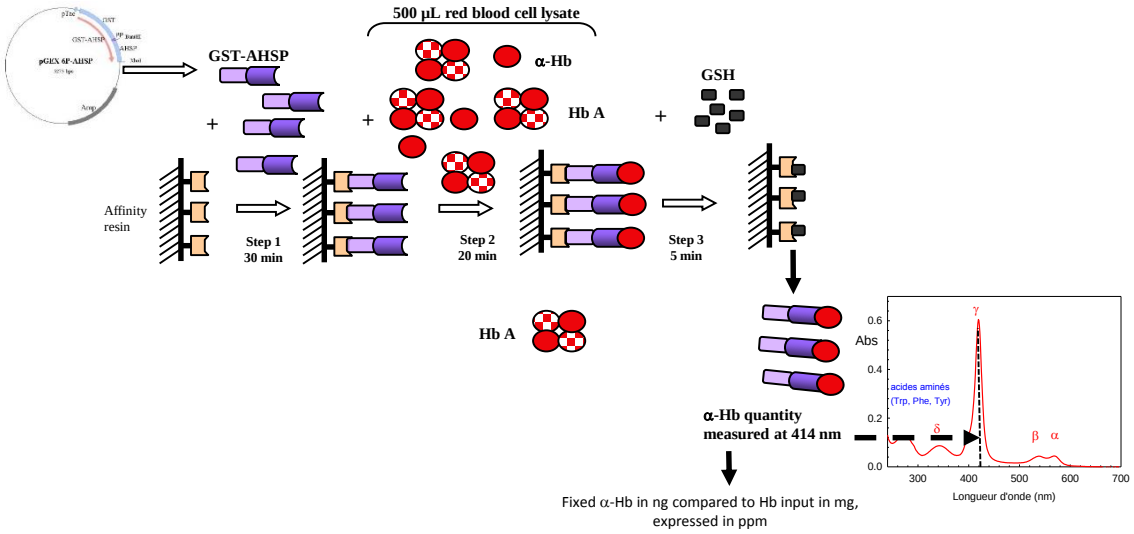
- **Priority or Patent:**
  - EP09 305 352.8 on 2009/04/24
  - PCT/EP2010/055479 on 2010/04/23
  - Granted in US, EP(FR, ES, DE, GB, IT)
- **Scientific Publication(s):**
  - Am J Hematol., 2017 Oct, *Vasseur C. et al.*, doi: 10.1002/ajh.24835
  - BJH, 2017 Apr, *Vasseur C. et al.*, doi: 10.1111/bjh.14800
  - Am J Hematol., 2011 Feb, *Vasseur C. et al.*, doi: 10.1002/ajh.21918
- **Development opportunities**
  - Ongoing research open for partnering

[1] Worldwide: 2015 prevalence of thalassemias; Lancet; V.388; No.10053; 10/8/16; DOI:10.1016/S0140-6736(16)31678-6)

Proof of concept

<u>Biomarker detection:</u> Use of GST-tagged AHSP for specific measurement of free α-Hb

- After expression and purification, 400 µg of GST-AHSP was bound to the microspin column containing 50 µL of Glutathione Sepharose 4B and was then incubated with hemolysates of patients to capture the free α-Hb. After a 30 minute incubation at 4°C under gentle agitation, the microspin column was washed five times with PBS (150 mM NaCl, 10 mM Na2HPO4, pH 7.4) and the bound proteins were eluted by 200 µL glutathione buffer (10 mM reduced glutathione in 50 mM Tris-HCl buffer at pH 8.0). The a-Hb containing in the elution fraction was quantified by spectrophotometry at 414 nm (e = 125 mM-1.cm-1) with a HP 8453 spectrophotometer. The total quantity of subunits of Hb in 1 mL of hemolysate was also determined at 414 nm after a 400 fold dilution. The fraction of free a-subunits is thus simply the ratio of absorption of eluted a-subunits from the column on the absorption of total subunits of Hb and is reported in ng/mg equivalent to ppm.



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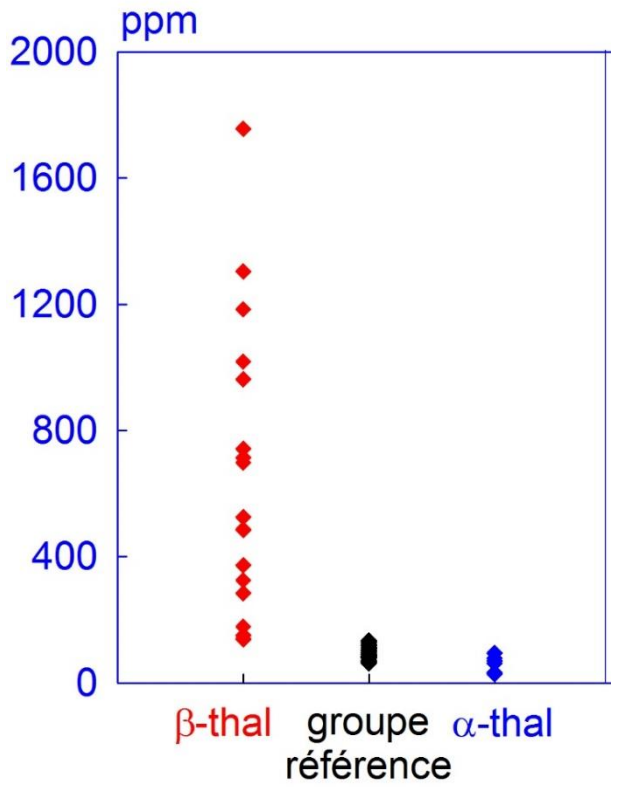
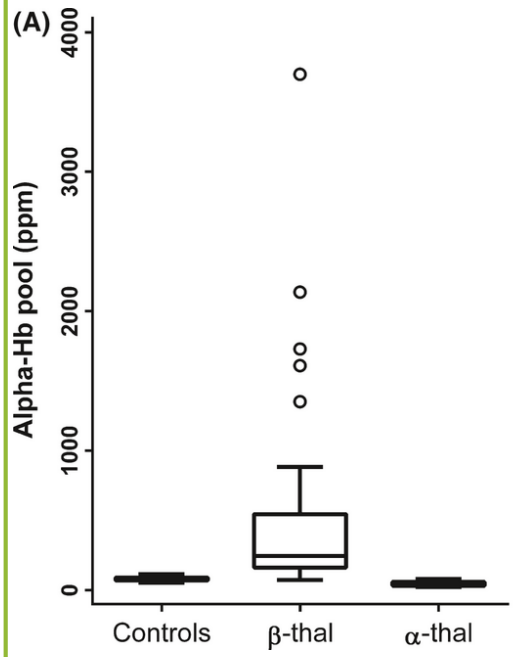
Proof of concept

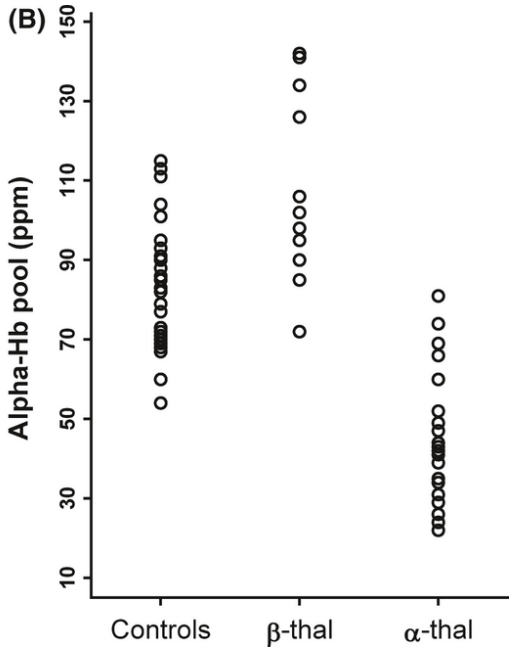
- **<u>Biomarker Discovery:</u> α-Hb levels are higher in β-thalassemic patients compared to α-thalassemic patients or healthy subjects without Hb anomalies.**
  - Using the described method, levels of α-Hb relative to total Hb A input are measured
  - β-thalassemic patients (n=20) compared to α-thalassemic patients (n=6) or healthy subjects without Hb anomalies (n=28)



Proof of concept

- **Pre-Analytic Validation: A prospective study in α-thalassemic and β-thalassemic patients.**
  - Comparison of α-Hb pool between controls (n = 38) and patients with β-thal (n = 57) and α-thal (n = 23).
  - (A) The α-Hb dosing applied to β- and α-thal patients compared with controls without Hb abnormality.
  - (B) Enlargement of the area between 10 and 150 ppm.
  - (C) Sensitivity, specificity and accuracy of the test for predicting individual carrying β-thal comparing of using direct sequencing as gold standard. α-Hb pool is expressed in ppm, equivalent to ng α-Hb/mg of total Hb subunits per ml of haemolysate.





(C)

α-Hb pool	Direct sequencing of genes for β-thal		Total
	Positive	Negative	
ppm > 120	49	0	49
ppm < 120	8	61	69
Total	57	61	118
Sensitivity	86% (49/57)		
Specificity	100% (61/61)		
Accuracy	93% (110/118)		