SELECTED OPPORTUNITIES IN IMMUNOLOGY

A 7 protein signature for Stratifying patients with Rheumatoid Arthritis for TNFa blocking agent treatment. (BIO12348)

A 7 PROTEIN SIGNATURE FOR STRATIFYING PATIENTS WITH RHEUMATOID ARTHRITIS FOR TNFA BLOCKING AGENT TREATMENT. (BIO12348)

Product factsheet

Stage:
Pre-Analytic
Validation

- **Biomarker:**
  - A 7 protein signature
- **Technology:**
  - ELISA
- **Sample:**
  - Serum
- **Information:**
  - Patient Stratification

- **Scientific and Clinical Rationale:**
  - Several anti-cytokines targeted against TNFα, IL-1b, IL-6 or IL-6 receptor and several cellular immunotherapies (anti-CD20 or CTLA-4Ig) have been successfully introduced for RA treatment.
  - Five TNFα blocking agents (TBAs) are currently used for RA treatment, one corresponding to a recombinant soluble form of TNF receptor, TNFRSF1B (etanercept), four others corresponding to an anti- TNFα monoclonal antibody: infliximab, adalimumab (ADA), certolizumab and golimumab.
  - However, clinicians observe that around 30 to 40% of treated patients fail to respond to TBAs. Moreover, TNFα blocking agents may have side effects, they are costly and the efficacy of any given TBA in a given patient is unpredictable.
  - Taking into account the risk of these treatments, the increasing number of available therapeutic molecules in RA, the variability of the response to the various treatment, and to optimize the drug prescription, identification of predictive markers of TBA / methotrexate combination may be highly desirable.
- **POC:**
  - Tested on two independent cohort of RA patient treated by subcutaneous injection of ETA in combination with MTX
  - Discovery cohort: 22 RA patients
  - Validation cohort: 16 RA patients

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Clinical State and Market Opportunity

- **Clinical State:**
  - Epidemiology
    - A reported worldwide prevalence of 24,5 M patients affected with RA, with at least 700 000 new incident cases each year
- **Clinical needs:**
  - Not all patients respond to TNFα blocking treatment, and non-responders are exposed to increased risk of infection
  - Circulating biomarker for easier patient stratification

Unique Selling Points

- **Priority or Patent:**
  - EP13 305 778.6 on 2013/06/10
  - PCT/EP2014/062035 on 2014/06/10
- **Scientific Publication(s):**
  - Theranostics, 2015 Aug 9, *Obry A. et al.*, doi: 10.7150/thno
- **Development opportunities**
  - Ongoing research open for partnering

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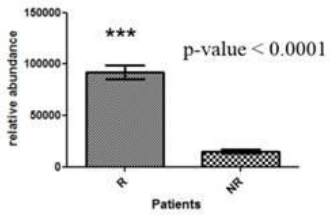
Proof of concept

- **<u>Pre-Analytic Validation:</u> Discovery of a proteic signature for TNFα responding RA patients**
  - (A) Table shows the 12 proteins over-expressed in R serum samples and the unique protein (transferrin, TRFE) over-expressed in NR serum samples identified by mass spectrometry. Np is the number of peptides above significance threshold allowing protein quantitation and the associated protein sequence coverages. Confident score is the value extracted from Progenesis LCMS calculating after importing Mascot search results and restricted to the peptides used for quantitation. Fold change refers to the ratio of protein abundance in responders samples divided by the one of non-responders samples. The associated p-value, given in the last column is significant if p-value is < 0.05 (Mann-Whitney test).
  - (B,C) Illustration of relative quantification for proteins PROS (B) and CO7 (C) at baseline between R and NR samples.

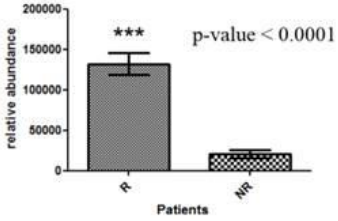
A

Accession	# peptides	Sequence coverage, %	Confidence score	Fold change	p-value
CO7	8	10	434	5.87	< 0.0001
PROS	5	11	485	5.84	< 0.0001
C1R	4	5	198	4.86	< 0.0001
CPN2	2	6	110	3.18	0.0001
CERU	31	41	2383	2.25	0.0003
ITIH1	15	3	1199	4.15	0.0003
ITIH3	2	3	173	5.45	0.0003
IC1	5	11	301	3.03	0.0004
S100A9	3	31	91	1.89	0.0022
ZA2G	2	14	158	1.46	0.0022
TRFE	2	7	144	0.72	0.0033
PLMN	3	5	138	1.61	0.0111

B



C



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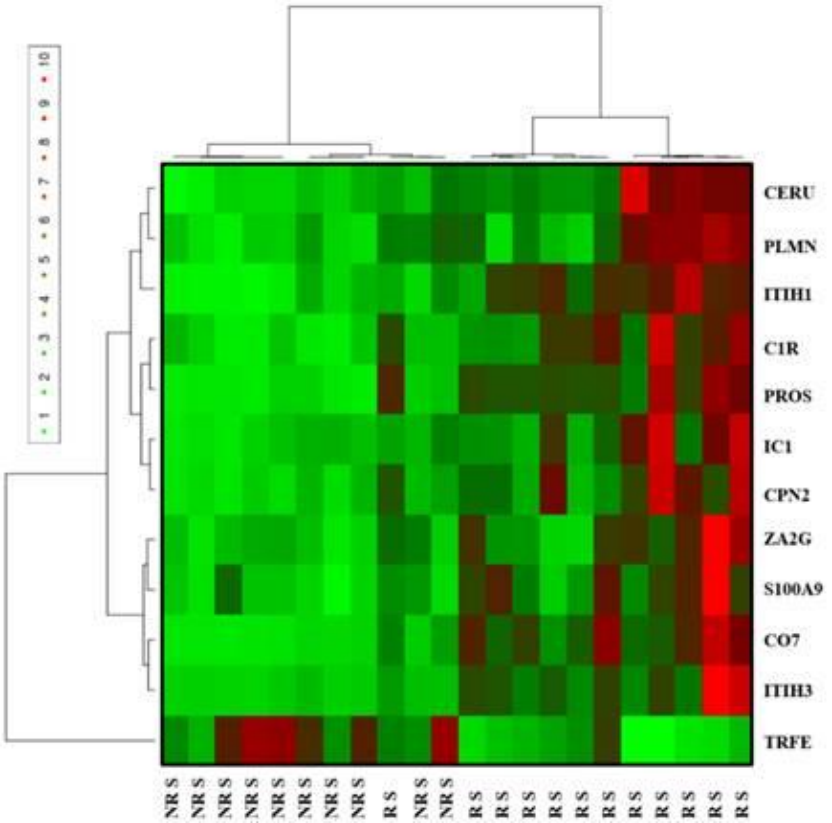
Proof of concept

- **<u>Pre-Analytic Validation:</u> Discovery of a proteic signature for TNFα responding RA patients**
  - The heat map was built from the 12 proteins that were differentially expressed between both groups (R and NR). Each row represents a protein and each column represents an individual patient serum sample (the name below the cluster indicates patient response status to ETA/MTX combination at six months according to the EULAR criteria: NR, non-responder and R: responder). Relative abundance levels are colored green for lower intensities and red for higher intensities in R patient samples.



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Proof of concept

- **Pre-Analytic Validation: Cross validation of the proteic signature in a second RA cohort**
  - Cross validation was performed for relative quantification in RA patient sera.
  - Receiver Operating Characteristic (ROC) curve analysis of potential theranostic biomarkers in population 2 (from targeted label free analysis on the proteins CERU, CO7, ITIH1, PLMN, PROS, S100A9 and ZA2G). The AUCs and associated standard errors, the 95% confidence interval as well as sensitivities and specificities are reported.

	Responders vs no Responders				
Protein biomarkers	Area	Std. Error [a]	95% confidence interval	Sensitivity	Specificity
CERU	0.92	0.07	0.78-1.00	87.50%	87.50%
CO7	0.89	0.09	0.72-1.00	87.50%	87.50%
ITIH1	0.86	0.10	0.67-1.00	75.00%	62.50%
PLMN	0.88	0.09	0.69-1.00	87.50%	87.50%
PROS	1.00	0.00	1.00-1.00	100.00%	100.00%
S100A9	0.98	0.02	0.94-1.00	100.00%	87.50%
ZA2G	0.91	0.08	0.76-1.00	75.00%	100.00%

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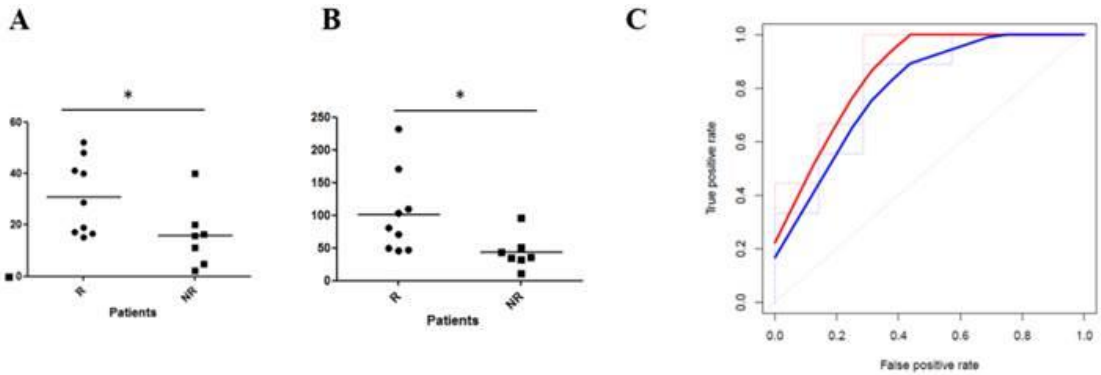
- **<u>Pre-Analytic Validation:</u> Validation in absolute quantification by ELISA**
  - Absolute quantification by ELISA of serum proteins PROS (A) and CO7 (B) at baseline in responders versus non-responders. Significant difference is noted by asterisk ($p < 0.05$, Mann- Whitney test). The horizontal bars correspond to the means.
  - (C) Receiver Operating Characteristic (ROC) curves averaging of CO7 (red line), PROS (blue line) were built.
  - (D) Table showing the different parameters resulting from ROC curve analysis from each individual protein and for their combination.



D

Proteins Markers (ELISA)	Area	Std. Error[a]	95% confidence interval	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PROS	0.81	0.39	0.59-1.00	88.9	71.4	80.0	83.3
CO7	0.87	0.13	0.69-1.00	100	71.4	81.8	100
[Pros]>16.5µ g+ [CO7]>44.5µg	-	-	-	**88.9**	**100**	**100**	**87.5**

a. Under the nonparametric assumption