Array Screening Reveals Autoantigenicity Patterns Predicting Anti-TNF alpha Therapy Response in Rheumatoid Arthritis Patients

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Current status for therapy response prediction I

- Markers for RA diagnosis or prognosis, such as C-reactive protein (CRP), autoantibodies (e.g. rheumatoid factors and ACPA), erythrocyte sedimentation rate, metalloproteinases and bone proteins cannot predict the responsiveness to anti-TNF treatments (TBA).
- High levels of IgA rheumatoid factor in sera of patients with RA have been suggested to identify a sub-group of patients at risk of poor clinical response to treatment with TBA.

- So far, only reduction of all isotype levels of rheumatoid factor during and after treatment is associated with a positive response and outcome of TBA treatment.

Burning questions

- Is it possible to identify therapy response prior therapy?
- Can we obtain markers predicting the response to TNFalpha blocking agents before therapy?
- Can we predict therapy success with autoantibodies?
  - Preferably with a small set of autoantigens?
- Proteome-wide screening with protein arrays
  - Screening patient IgA and IgG, classified as TBA Responder versus Non-Responder

Current status for therapy response prediction II

- Lack of replication of 16 single nucleotide polymorphisms (SNPs) genetic predictors for anti-TNF treatment response in RA.
- No evidence of genetic variants associated with response to TBA in RA.
- A few informative transcripts for TBA response have been identified by gene profiling, which rely predominantly on differences in transcript levels.
- Expression markers not predictive for combination treatment MTX/ TBA.

Until now,

- all discussed biomarkers show insufficient differentiation (OR <3, spec / Sens. <75%)
- no reliable and good markers for routine use established

Protein array technology: human Expression 1 (hEx1)

- cDNA library from human foetal brain tissue
  - initially 193,500 clones
  - 37,200 (19%) positive with RGS-His antibody
    - re-arrayed and spotted onto protein macroarrays
  - clones represent ~ 15,000 human genes
    - The identity of all clones is known / DNA is sequenced
    - average insert size of ~1.5 kb
    - ~ 40% in the correct reading-frame

Screening of protein arrays with sera from RA patients before and after TBA treatment

Detection of autoantigens before treatment

Detection of autoantigens after treatment (8 months)

Pre-treatment array

Post-treatment array

Computational scoring of positive clones

- Software recognises 5x5 spotting grid
- Automatic scoring of positive signals due to a reference spot
- Spot intensities are recorded
- Direct correlation to 384-well coordinates
- Allows user to cross check all positions manually

Patients used for screening hEx1 Protein arrays

- RA patients treated with Adalimumab (Humira®), Etanercept (Enbrel®) or Infliximab (Remicade®) in combination with MTX
- Cohort 1: TBA therapy Responder patients
- Cohort 2: TBA therapy Non-Responder patients seen during the same period, age- and sex-matched

- Clinical assessment of patients:
  - DAS 28 scores in Responder group before treatment was from 4.4 – 6 (mean 4.83) and in the Non-Responder group 4.1 – 8.6 (mean 6.2).
  - Responder had a mean change of 2.36 during therapy, no mean change in the DAS 28 scores in the Non-Responder group.

Correlation of serum autoantibody patterns and clinical response

<table>
<thead>
<tr>
<th></th>
<th>Responder</th>
<th>Non-Responder</th>
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</thead>
<tbody>
<tr>
<td>IgA reactivity</td>
<td>6 clones</td>
<td>221 clones</td>
</tr>
<tr>
<td>IgG reactivity</td>
<td>40 clones</td>
<td>70 clones</td>
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</tbody>
</table>

221 clones from IgA screens represent ~ 30 Proteins, of which five antigens selected as predictor for Non-Response

Expression and purification of five predictive Biomarkers for ELISA development

- Expression in E. coli
- Multi-dimensional purification strategy
- Stability testing
- Immunological assessment
- ELISA development

TBA Non-Responder ELISA

Non-Responder have high titre IgA against 5 biomarkers prior, throughout and after therapy

- Set of 5 biomarkers
- Measuring IgA Autoantibodies
- Still in single tests (one ELISA per Marker)
- Easily combinable, no need for multiplexing
- Easily transferable to routine test-systems
TBA Non-Responder ELISA evaluation for Humira, Enbrel, Remicade

<table>
<thead>
<tr>
<th></th>
<th>No. of Responders</th>
<th>No. of Non-Responder</th>
<th>No. tested</th>
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</thead>
<tbody>
<tr>
<td>Humira</td>
<td>25</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>Enbrel</td>
<td>34</td>
<td>9</td>
<td>43</td>
</tr>
<tr>
<td>Remicade</td>
<td>61</td>
<td>8</td>
<td>69</td>
</tr>
<tr>
<td>Total</td>
<td>144</td>
<td></td>
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</tbody>
</table>

- Mean rate of Non-Responder in cohort: 17%
- No false positives detected

Specificity: 100%
Sensitivity: 91.7%
ppv = 0.92

Conclusions

First Biomarker-set predicting TBA response prior treatment

- measuring IgA autoantibodies against five autoantigens
  - antigens are available in robust format
- identification of Non-Responders for:
  - Adalimumab (Humira®), Etanercept (Enbrel®), Infliximab (Remicade®)
- high positive predictive value: 0.92
  - 100% specificity and 92% sensitivity
- accessible in routine-format (e.g. ELISA)
  - under further validation with several clinical study cohorts
- plausible hypothesis for pathomechanism

Pathomechanism of TBA Non-Response

- Bacteria modify the balance of Treg and Th17 cells
- Tissue destruction: high IgA and IgG IC induce IL-17 production
- TBA Non-Responders might be treated by IL-17 blockage


Induction of intestinal Th17 cells by segmented filamentous bacteria

TLR4

TGF-β → IgA autoantibodies

Th17 cell

IL-17 over-rules TBA inhibition

Bacteria induce immune activation and IgA production

In the early phase, IL-17 effect is dependent on the presence of TNF!
In the later stage, the disease becomes mostly IL-17 driven, and TNF independent!

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