Case study*:
Biomarkers for anti-TNFα non-responders

Cell-centred meta-analysis reveals baseline predictors of anti-TNFα non-response in biopsy and blood of patients with IBD. *GUT, 2018*
Identification Of Clinical Biomarkers For Infliximab non-responders

3 Public datasets of IBD colon biopsies mRNA

Analysis to identify Infliximab responders vs non-responders

Conventional Vs CytoReason’s approach
The conventional analysis vs CytoReason workflow

Conventional Analysis

CytoReason’s Workflow

Responder / Non-Responder Data Set

Cell Proportion Differential Expression

Identify differentially expressed cell types

Adjust data to account for cell difference

Differential expression (adjusted)

Significant Post-Adjustment

Disease genes masked by infiltration

Build Cell Centered Model & Integrate with CR Model

Meta Analysis

Immune modulators

Map to specific cell types

Strong signals not explained by infiltration

Conventional Analysis

Differential expression (Non-adjusted)

Pre-Adjustment

Significant Pre-Adjustment

Responder / Non-Responder Data Set

Cell Proportion Differential Expression

Identify differentially expressed cell types

Adjust data to account for cell difference

Differential expression (adjusted)

Significant Post-Adjustment

Disease genes masked by infiltration

Build Cell Centered Model & Integrate with CR Model
The conventional workflow
Differential expression between responders and non-responders

Meta-Analysis

Many of these differences are typically explained by a different immune cell composition
For Example: OSM in 2 of 3 data sets
The CytoReason workflow:
Identifying differential cell types and rebuilding cellular composition

Mean Cell Proportions Within Each Cohort

- **Macrophages**
  - Crohn's Disease
  - Ulcerative Colitis - A
  - Ulcerative Colitis - B

- **Plasma cells**
  - Crohn's Disease
  - Ulcerative Colitis - A
  - Ulcerative Colitis - B

Responder
Non-Responder

Meta Analysis Of Differential Cells Between Responders and Non-Responders

- **Log(FDR)**
  - 0.000
  - 4.730

- **Effect Size**
  - Activated cytotoxic T cells
  - BCR-igated B cells
  - Resting NK cells

- **Macrophages**
- **Plasma cells**
- **Neutrophils**
- **IgM memory B cells**

CytoReason's Workflow
- Cell Proportion Estimation
- Differential cell types
- Adjust data
- Gene Differential expression
- Cell Specific Expression
Correcting for enriched cell types unmasks disease genes

- Enriched for disease genes
- Other immune modulators or disease genes
- Enriched for potential modulators of macrophages and/or plasma cells
TREM1, CCR2 - CCL7 identified as important axis in Infliximab response

Analysis of upstream regulators of genes differentially expressed in infliximab responder/non-responder post macrophage and plasma cell signal adjustment

Additional network analysis pointed to CCR2-CCL7 pathway
Validation:
Plasma Cells as tissue biomarker for Infliximab response at baseline

Validation Study on 52 Patients

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<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<tbody>
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<td>Responder</td>
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<tr>
<td>Non-Responder</td>
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CD138 (Plasma Cells)

Respender

Non-Responder

Validation study by Prof. Chowers (Rambam Hospital) & Prof. Dotan (Ichilov Hospital)
* 82% AUC refers to patients w/ inflammation score>2.5
Validation:
TREM1 identified as blood biomarker for Infliximab response at baseline

In addition to TREM1, CCR2-CCL7 axis was found up-regulated in non-responders

Validation study by Prof. Yehuda Chowers (Rambam Hospital)