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Highly reproducible, sensitive and early detection and characterization of antidrug responses using RNA-seq

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Niek de Vries, AMC, The Netherlands

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Background

To overcome existing hurdles in assessment of antidrug responses we aimed to develop a robust tool for analysis of B- and T-cell responses that allows:

- Reliable assessment of clonal B- and T-cell responses
- Linking of clonal responses to antigen specificity (epitopes)
- Linking *in vitro* to *in vivo* clonal responses

→ Quantitative Next Gen Sequencing (NGS) with single cell approaches

efpia
imi Innovative Medicines Initiative
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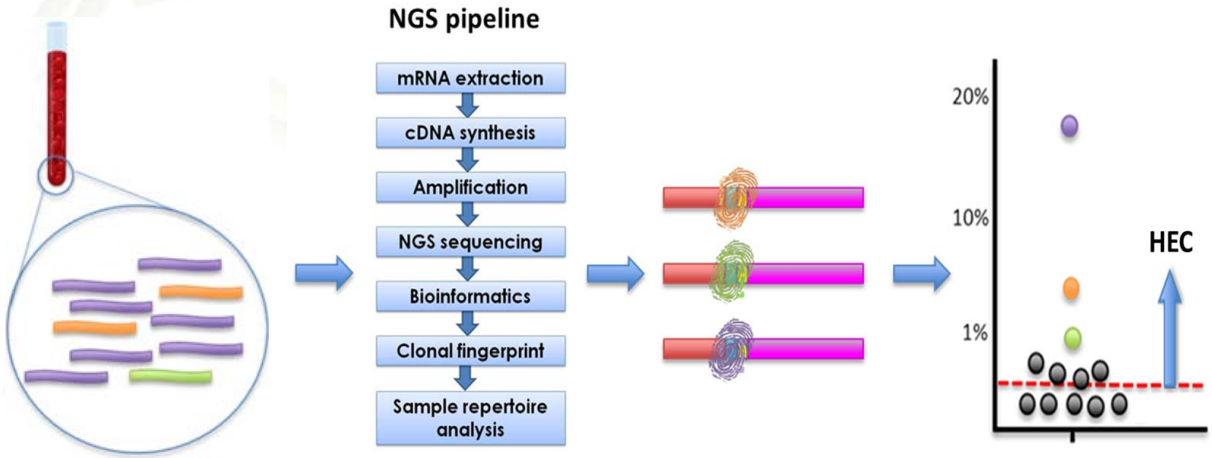
T cell receptor (TCR) and B cell receptor (BCR) repertoire analysis



Applications:

1. Quantify clonal expansion
2. Follow clones over time
3. Compare clonal distributions between different samples

Klarenbeek P.L. et al., *Immunol Lett* 2010

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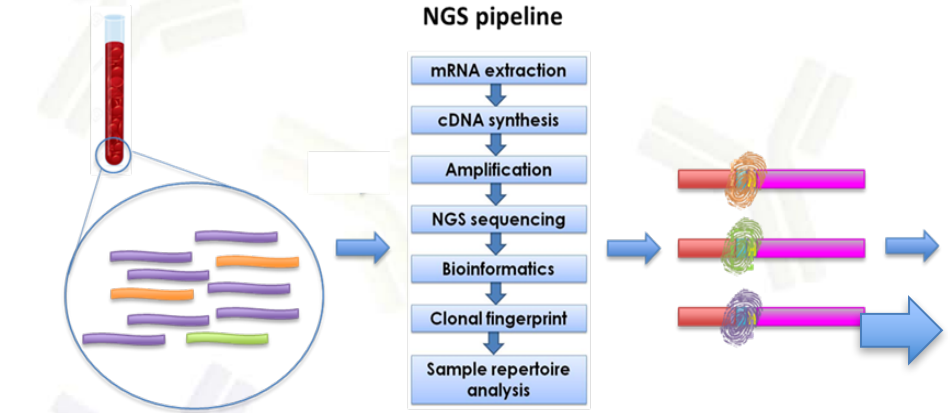
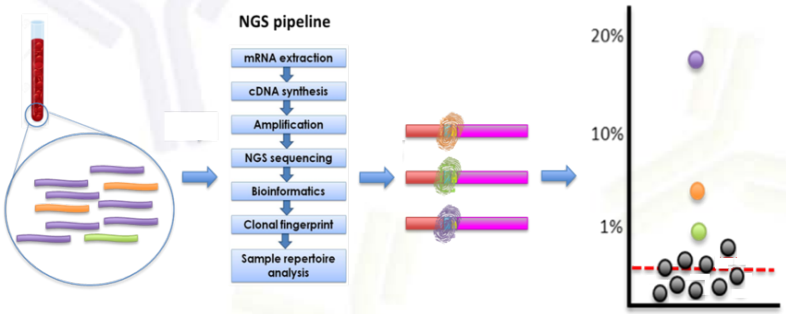
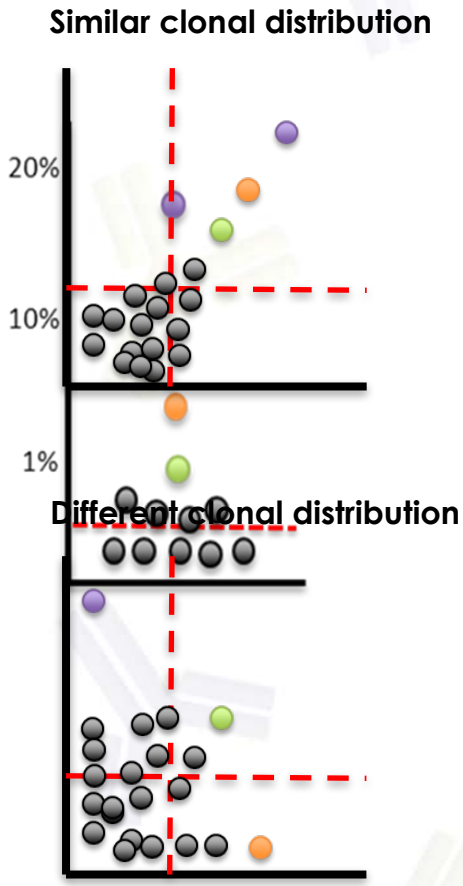
Comparing two samples







- Chao-Sorensen similarity index
- Top clonal overlap analysis

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IgG4-related disease

Tumor formation in different organs, e.g. biliary, retroperitoneum, retro-orbital, salivary glands, thyroid, lungs …

2/3 have increased serum IgG4

Characteristic histopathological appearance[1]

- dense lymphoplasmacytic infiltrate
- storiform pattern of fibrosis
- obliterative phlebitis
- increased numbers of IgG4+ plasma cells

However: Value of each criterion is variable and tissue specific …

Diagnosis is problematic

[1] Consensus statement on the pathology of IgG4-related disease: Deshpande V, et al. Mod Pathol. 2012; 25:1181.

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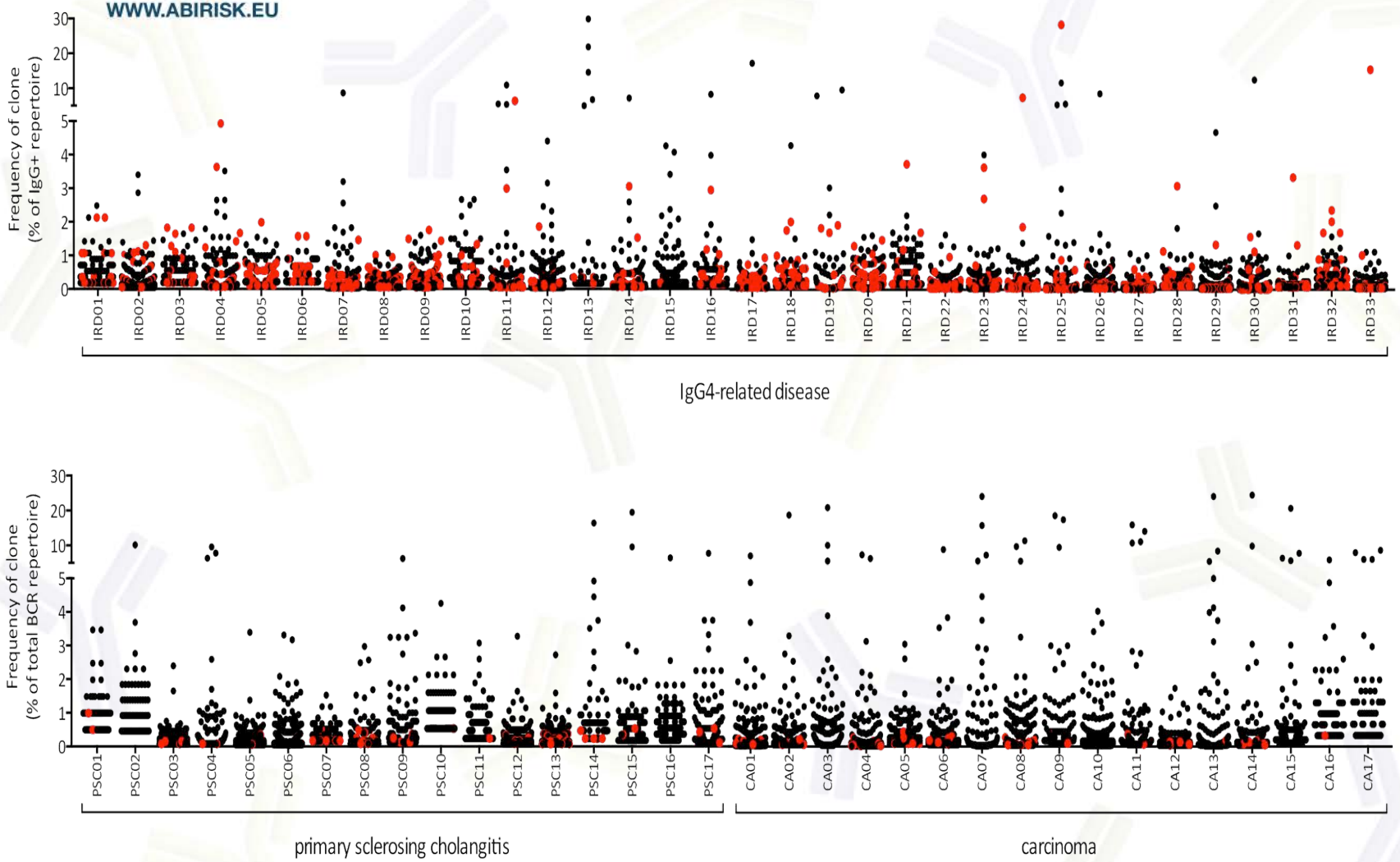
IgG4-RD : PB BCR (red = IgG4)



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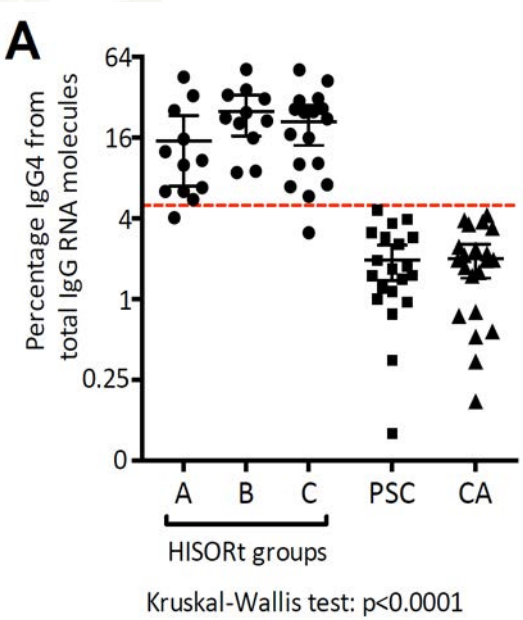
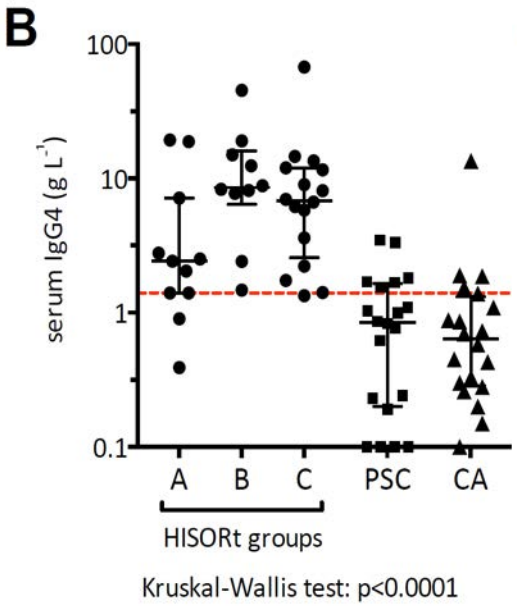
To summarize IgG4-RD

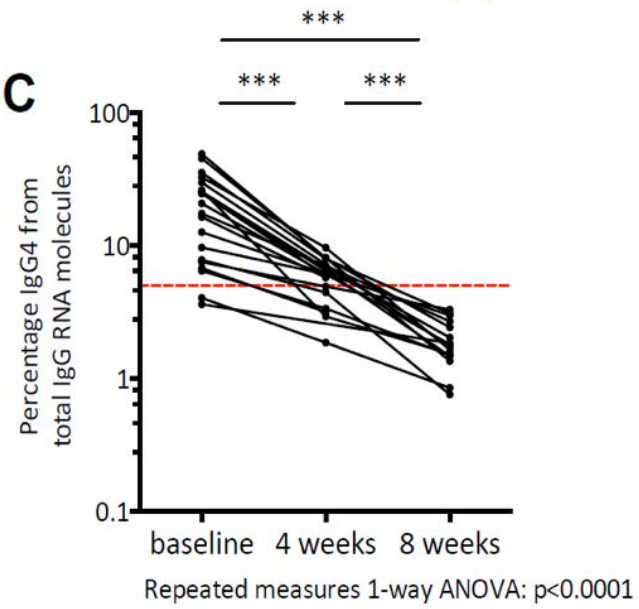
Excellent diagnostic marker



Outperforms IgG4 serum protein



Reflects disease activity



Doorenspleet et al. Hepatology 2016

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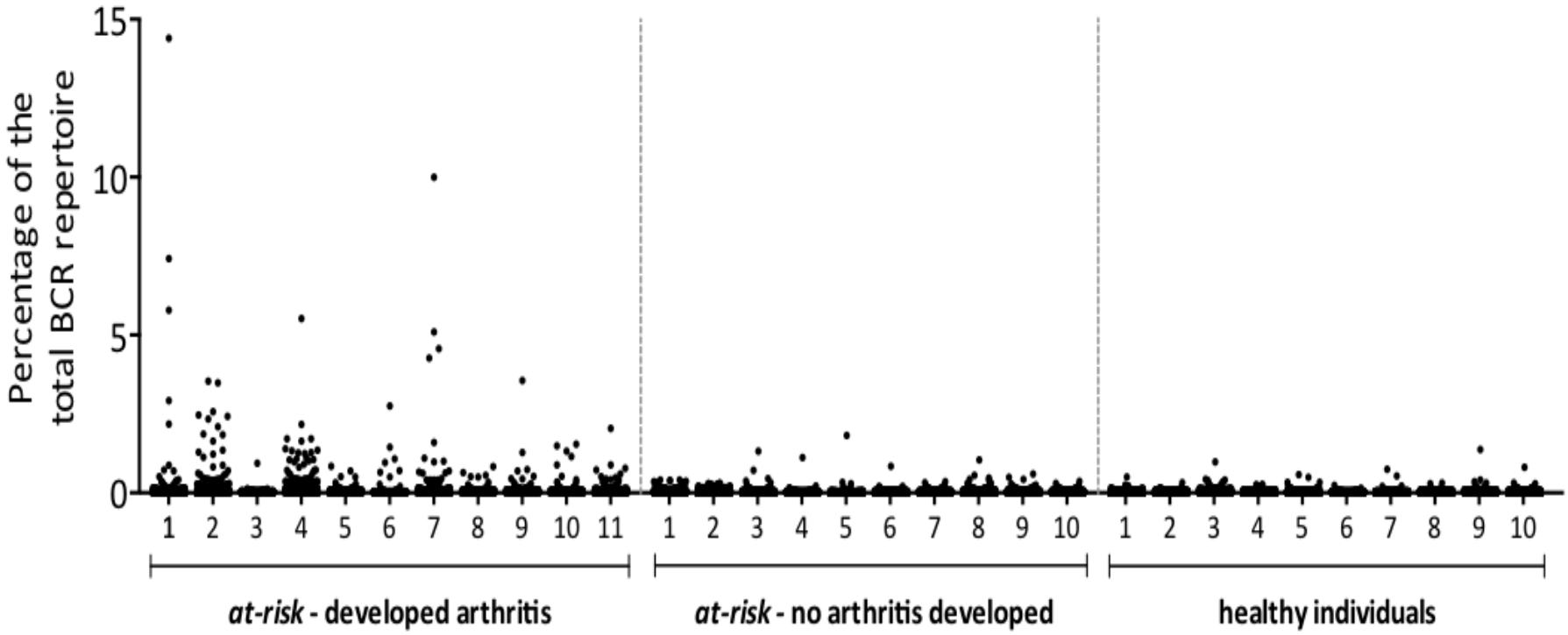
PB BCR in arthralgia with IgM-RF or anti-CCP2 antibodies



Increased number of dominant BCR clones heralds imminent onset of (rheumatoid) arthritis

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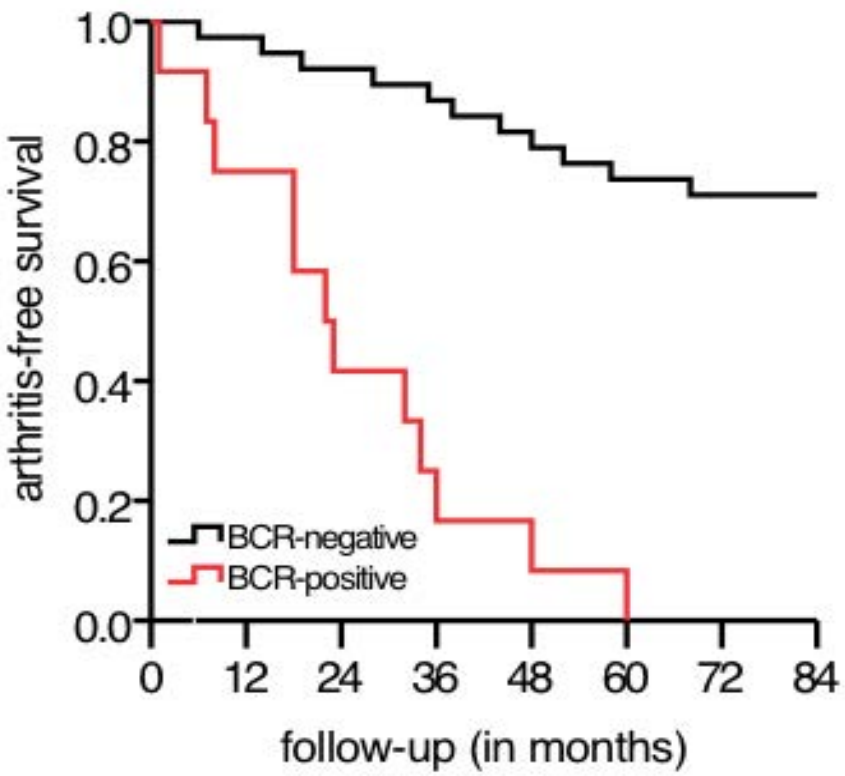
Validation : Kaplan Meier curve



These dominant clones:

- Are not present in the synovium in the arthralgia stage
- But are present in the synovium during onset of arthritis

Tak PP, et al. Ann Rheum Dis. 2017 Aug 8

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ADA : Potential implications

Main points to emphasize:

1. **Cells are not static :** they differentiate and migrate
2. **Cells ≠ antibodies :** Different kinetics, give different representations of the same immunological response
3. **Cellular B cell responses correlate with immunological activity**
4. **Antibodies ≠ active disease**

NGS-based cellular analysis can help:

1. Direct assessment of cellular ADR
2. Identification of drug characteristics contributing to cellular B/T-cell responses and subsequent (humoral) immunogenicity
3. Sensitive assessment of active antidrug responses & epitope spreading in patients

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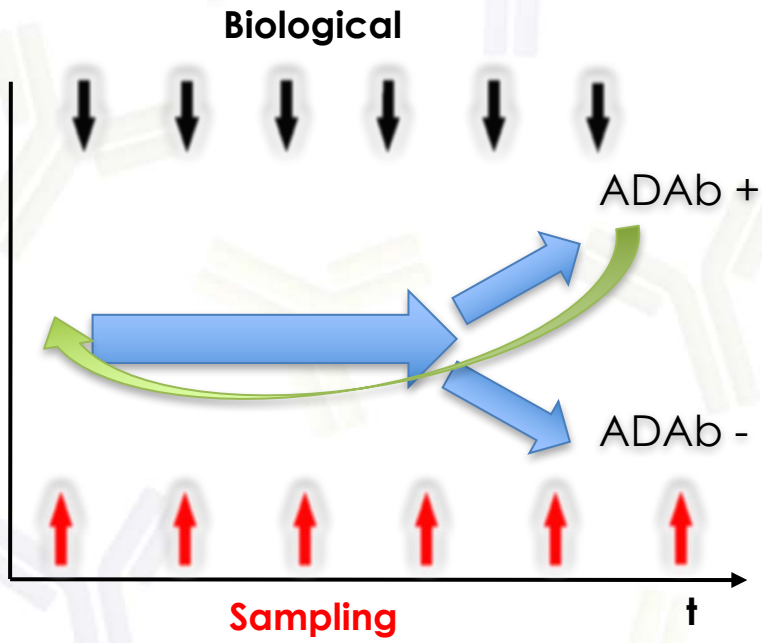
Molecular fingerprinting of anti-drug responses



1. Identify and fingerprint anti-drug T- and B-cell responses
2. Show development of anti-drug responses over time
3. Phenotype/Genotype drug-responsive cells early on

Predictive tools for anti-drug immunogenicity
Novel targets for preventing anti-drug responses

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- Technological advances and preliminary results on NGS-based analysis of B- and T-cell receptor repertoires were discussed
- Manuscripts in preparation

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Overall conclusions

- Reliable sensitive quantitative assessment of clonal B- and T-cell responses introduces statistical evaluation of immune responses at the clonal level

- Allows:
  - Linking of clonal responses to antigen specificity (epitopes)
  - Linking *in vitro* to *in vivo* clonal responses & phenotypes

- Can be used to
  - detect and monitor active immune responses resulting in immunogenicity
  - Characterize cells involved using single cell approaches

- May find application in:
  - Sensitive detection of immunogenicity issues in preclinical research
  - Rapid epitope mapping

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