Immunosequencing

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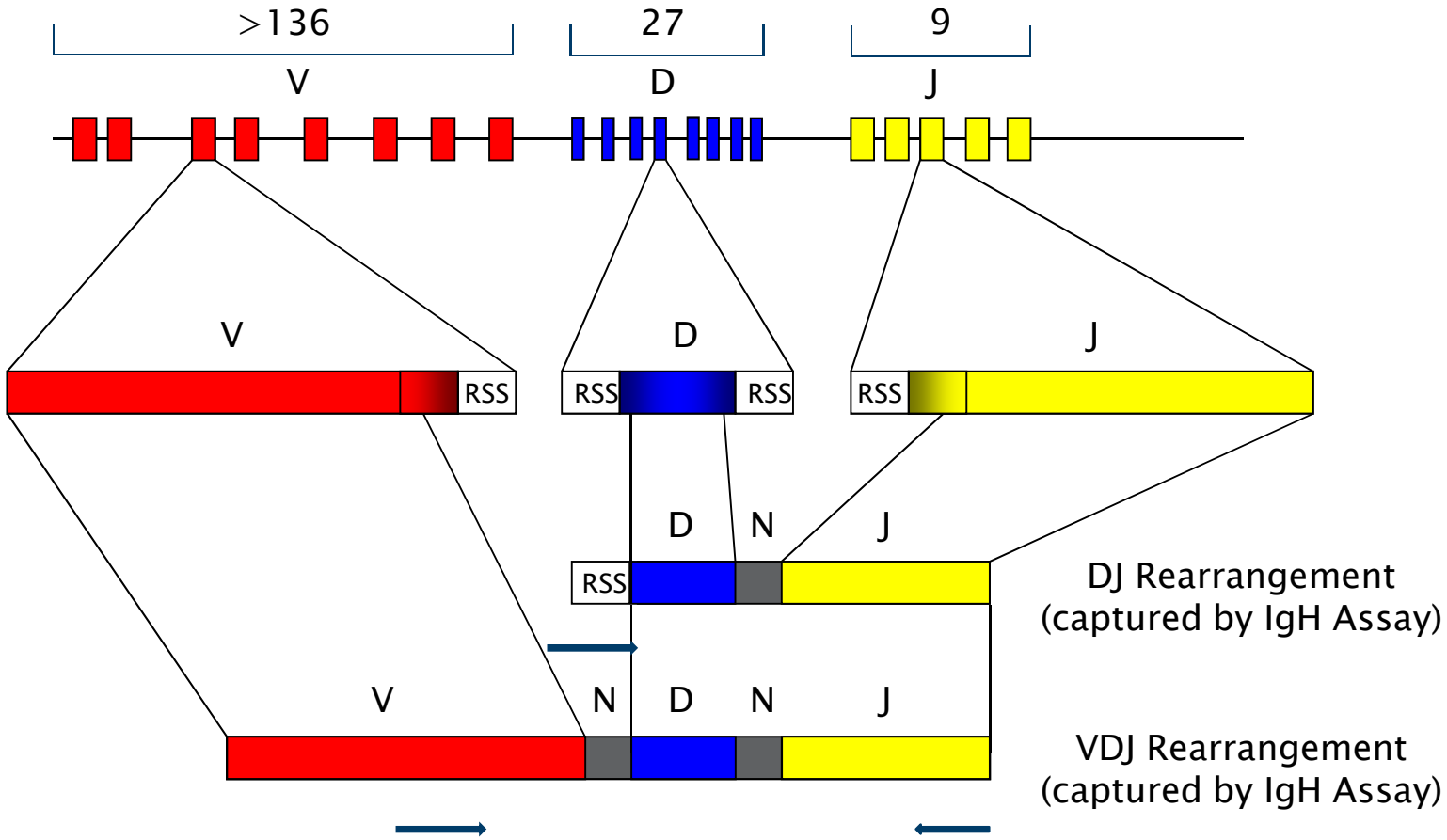
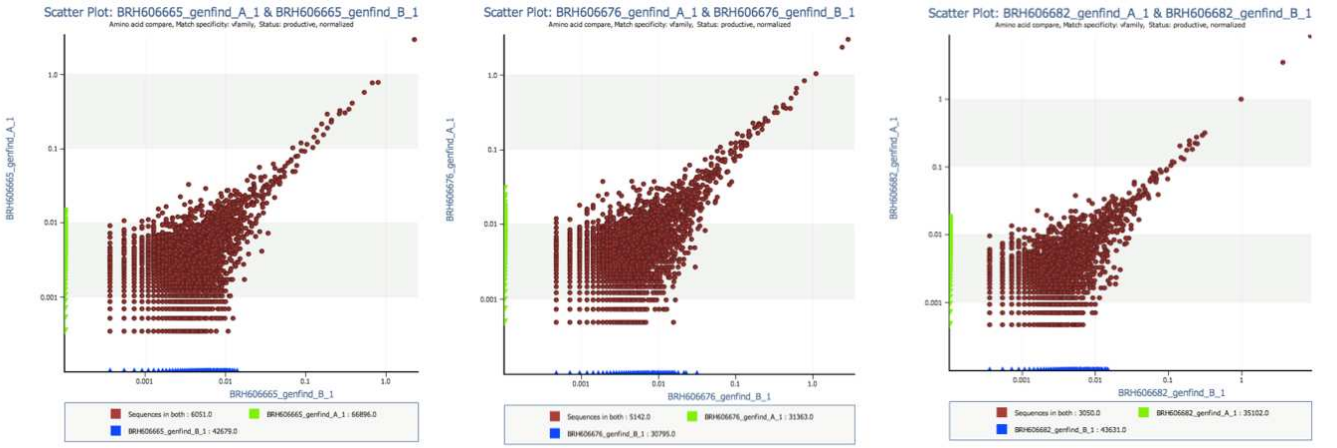
Immunoglobulin Heavy Chain Locus



Assay Development Process Leads to High Reproducibility

Within Sample Variability
(r^2=0.98)

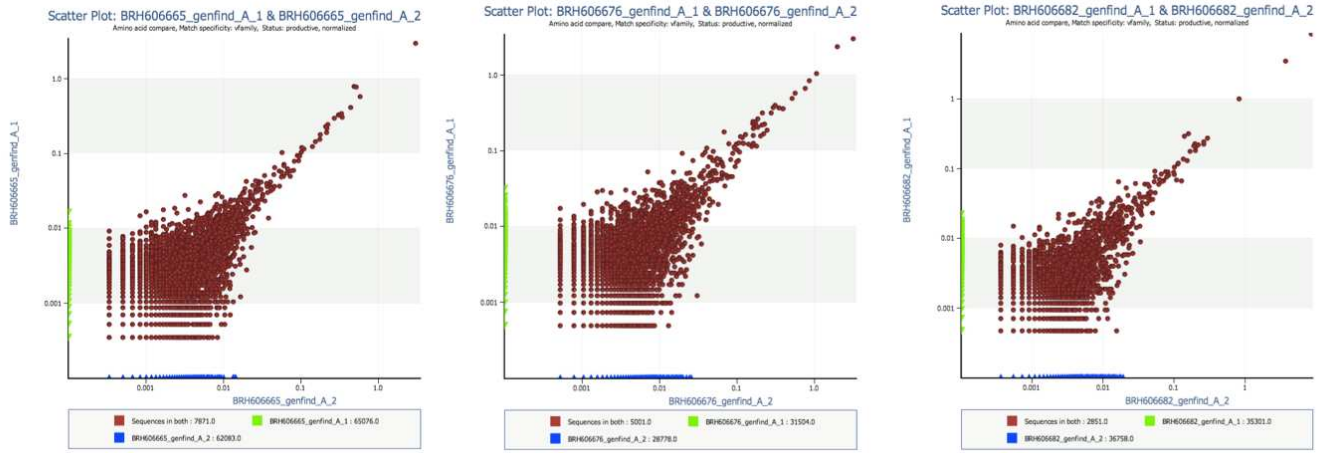


Sample #1 - 2.5% clone

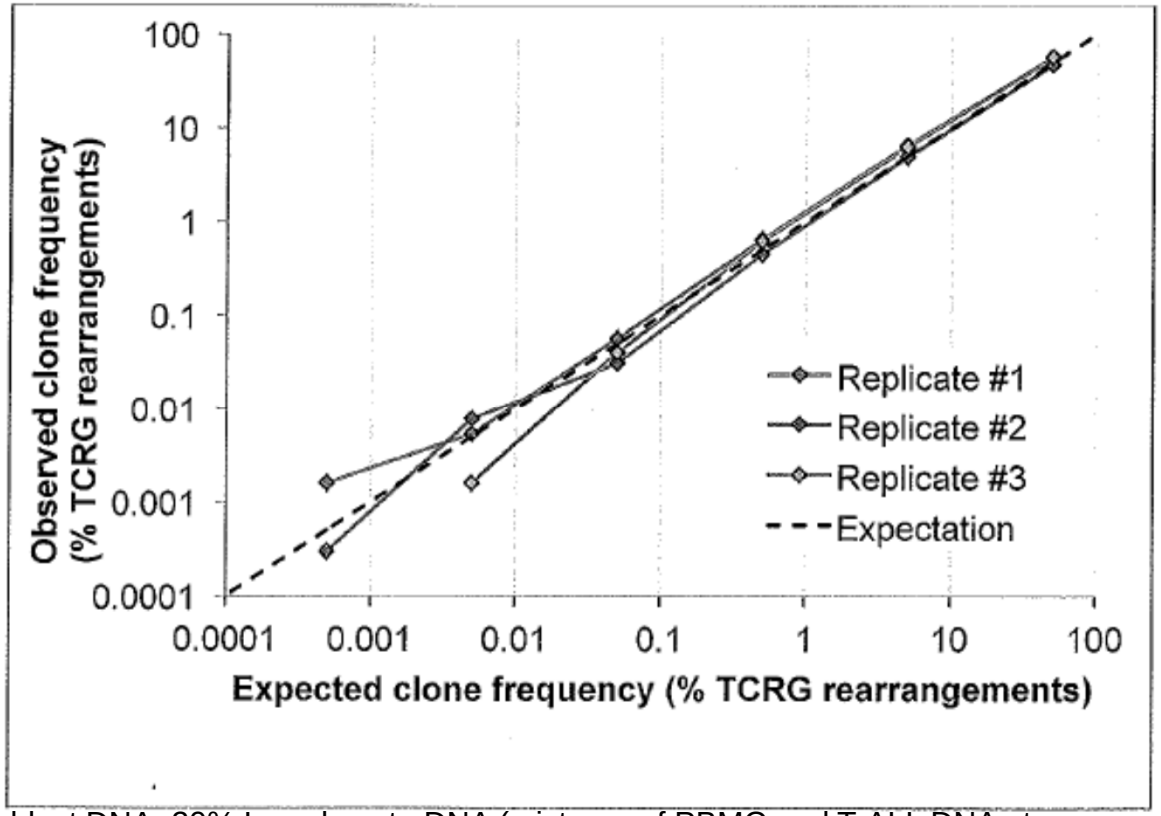
Sample #2 - 3% clone

Sample #3 - 8% clone

Between Experiment Variability
(r^2=0.98)



“Spike-in” Experiment Using a T-ALL Cell Line



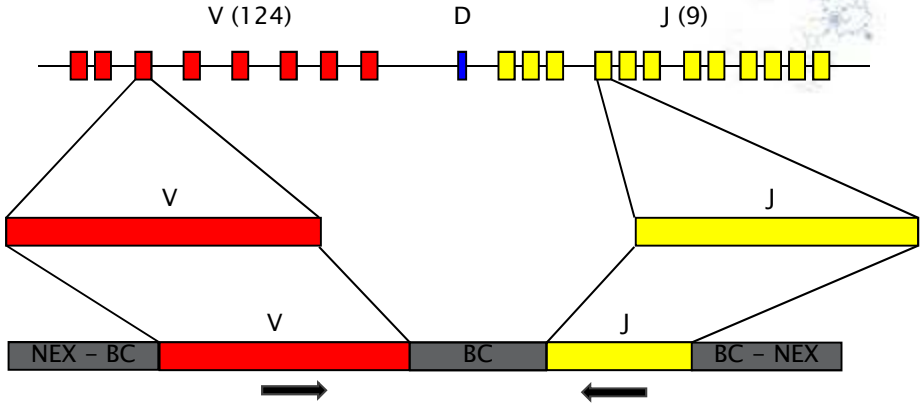
80% fibroblast DNA; 20% Lymphocyte DNA (mixtures of PBMC and T-ALL DNA at different ratios). Two of three replicates + at 1/1,000,000 (=0.0005% of TCRG sequences)

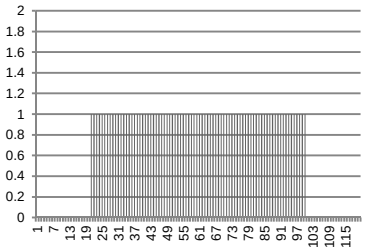
Process Validation / Optimization

Synthetic Immune System Standard
(Measurement of PCR and sequencing bias)

- Synthesized 500 base molecules
- Terminal/internal identifying barcodes
- PCR primer hybridization sequences

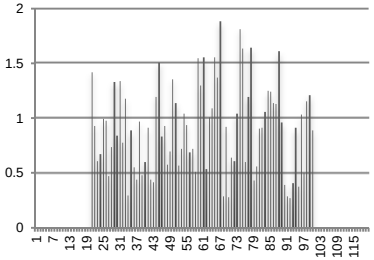
hsTCRB: ~860 molecules
hsIGH: ~1000 molecules
hsTCRG: ~70 molecules



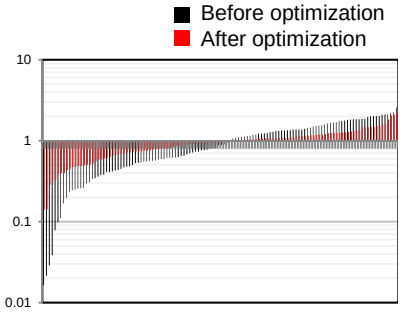


Equalized Template Input
Synthetic Immune
Molecules

PCR +
SEQUENCING



Measure Bias
(due to PCR & Sequencing)



Quantify and remix primers
to correct bias

Optimize Primer Mix

The immunoSEQ Kit

Now amplifying discoveries locally

Control
You determine where and when samples are sequenced

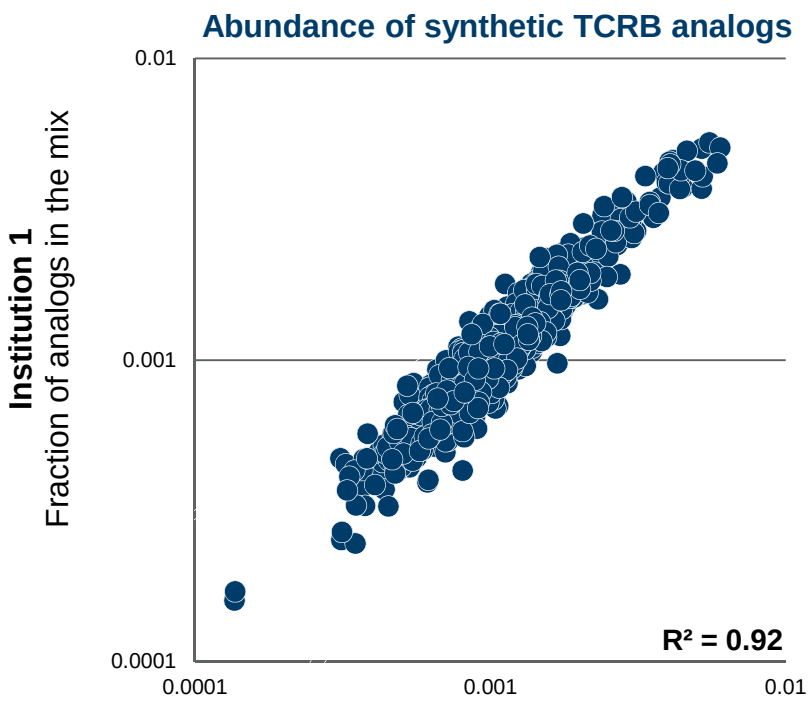
Consistency
Unmatched accuracy and reproducibility from lab to lab

Convenience
Only 4 hours of hands-on time

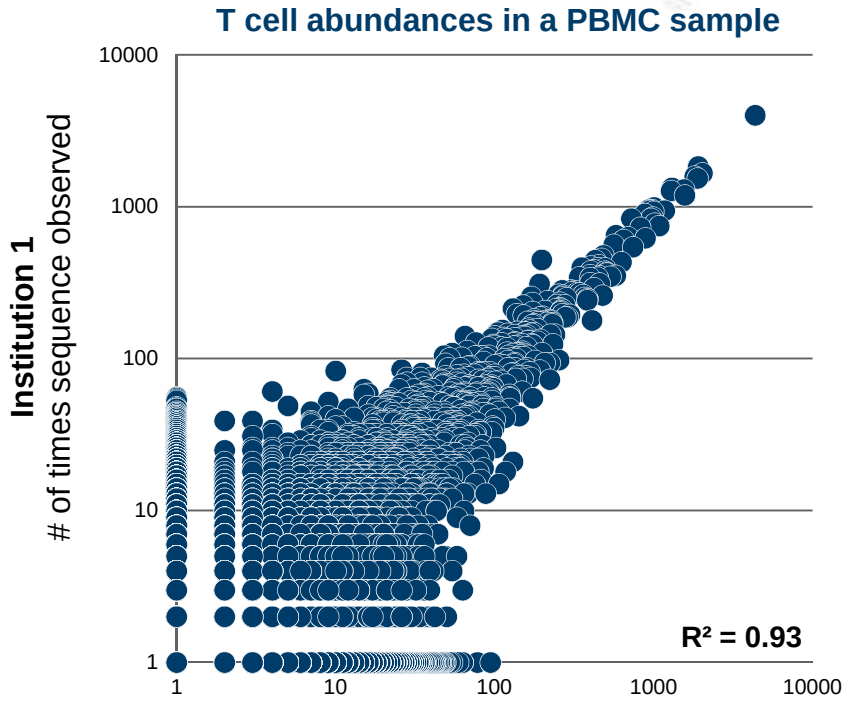
Customization
Scalable barcode setup facilitates flexibility in design

The power of the immunoSEQ Assay is now available as a Research Use Only (RUO) Kit

Lab-to-Lab Consistency



Pool of synthetic templates run at different institutions



Sequencing reads for each T cell clone, from PBMC samples run at different institutions

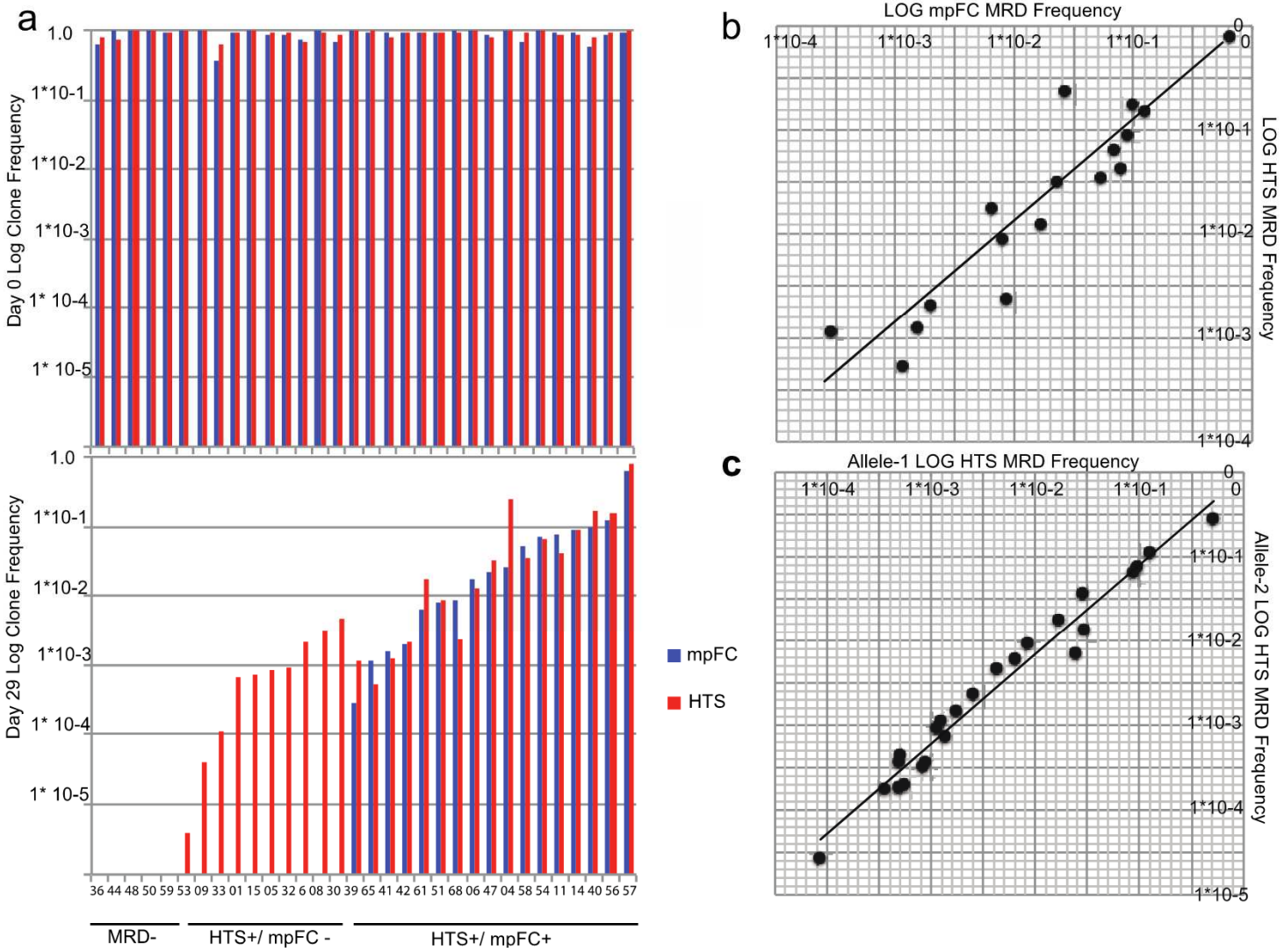
The Three Pillars of Immunosequencing

- Clonality assessment
  - Define the number and diversity of T- and/or B-cells
  - Monoclonal? More likely to be malignant
  - More diverse? More likely to be inflammatory
  - More clonal? More likely to represent an immune response to cancer

- Clone tracking
  - Track a malignant lymphocyte in response to therapy
  - Track clones from tissue to tissue
  - Track “public” responses from one person to another

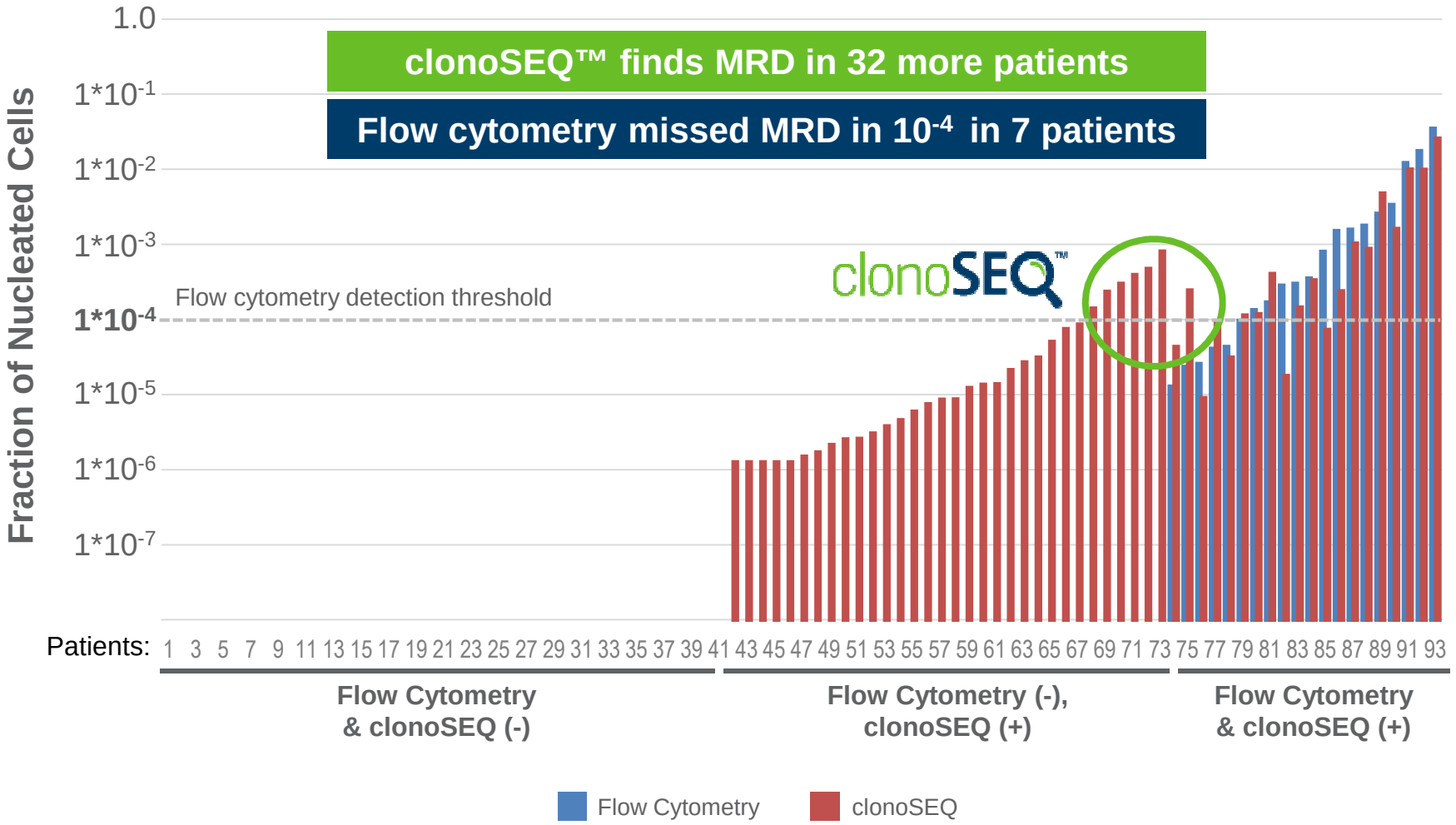
- Mutation detection
  - Follow affinity maturation over time as immune response improves
  - Identify stages of healthy and malignant lymphocyte development

Accuracy: HTS TCRG Assay detects T-ALL MRD

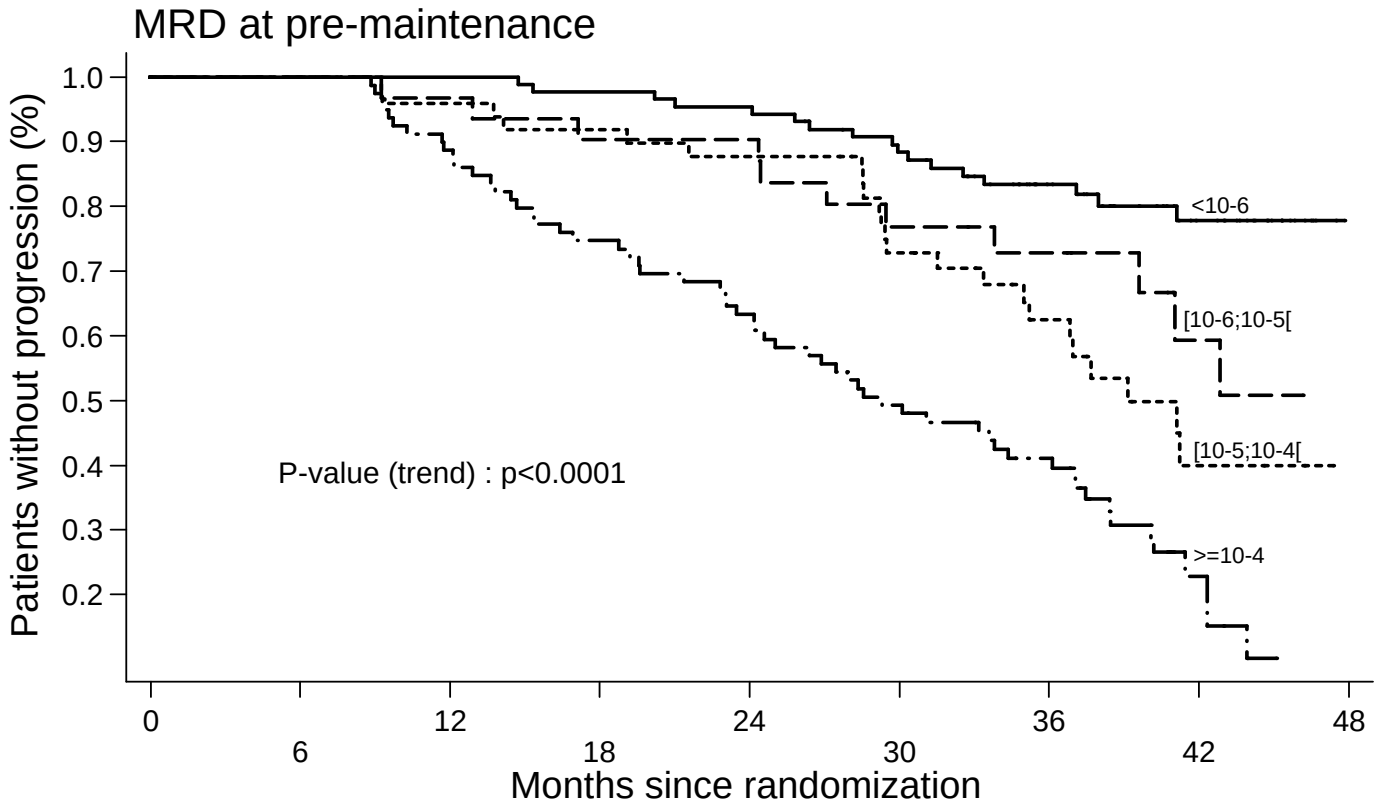


clonoSEQ™ Demonstrates Superiority Over Flow Cytometry

Children's Oncology Group Study (Wu/Wood): 93 Patients with B-ALL



Sensitivity is Key to Prognostic Value in MM



N at risk (events)

$<10^{-6}$	87	(0)	87	(0)	87	(2)	85	(2)	83	(6)	74	(4)	54	(3)	31	(0)	8
$[10^{-6};10^{-5}[$	31	(0)	31	(1)	30	(2)	28	(0)	27	(4)	22	(1)	17	(2)	8	(1)	4
$[10^{-5};10^{-4}[$	49	(0)	49	(2)	47	(2)	45	(2)	43	(7)	34	(4)	22	(6)	8	(0)	2
$[10^{-4};10^{-3}[$	79	(0)	79	(9)	70	(11)	59	(9)	50	(11)	38	(6)	28	(9)	6	(3)	0

tilSEQ — A New Class of Immune Molecular Diagnostics

What is tilSEQ?

tilSEQ **Accurate and reliable TIL quantitation**

TIL Frequency — Absolute TIL counts

TIL Density — Sum total # of clonal TCR rearrangements per total cell #

TIL Clonality — Evenness of clone frequency distribution; inverse of Shannon's Entropy (scale of 0 → 1)

TIL Tracking — Track tumor-specific TCR clones in the blood; subtract blood TCR clones not in the tumor ('noise')

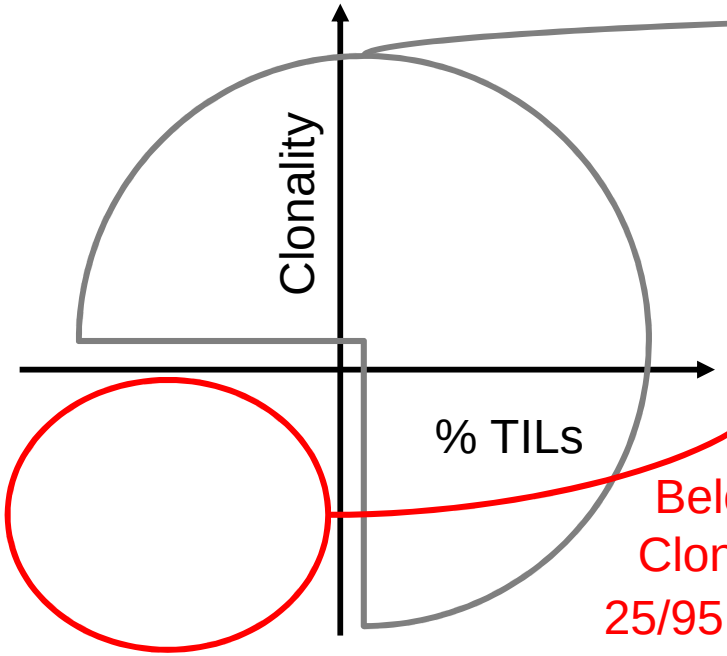
Key Applications

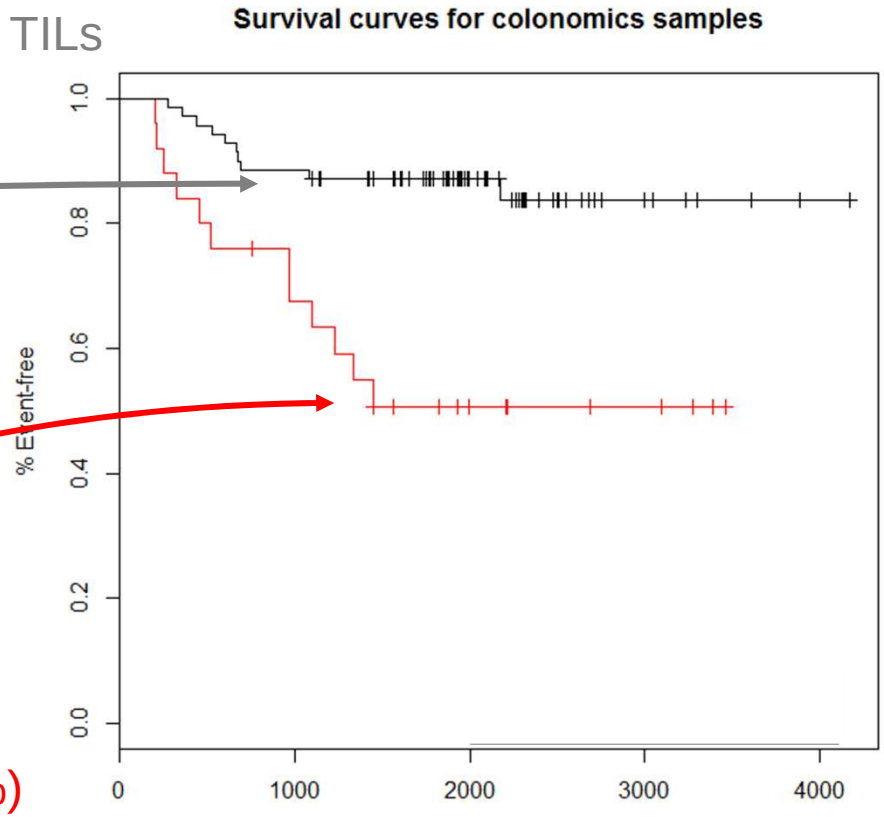
- Understand **drug MOA**
- Measure immune system **dynamics**
- Better **stratify patients**
- **Predict response** to treatments
- Develop smart **drug combinations**, **sequential** therapies

tilSEQ Prognostic Potential

Tumor DNA from 95 stage II colon cancer patients with outcomes*

Above-median Clonality or TILs
70/95 patients (74%)
10/22 events (45%)



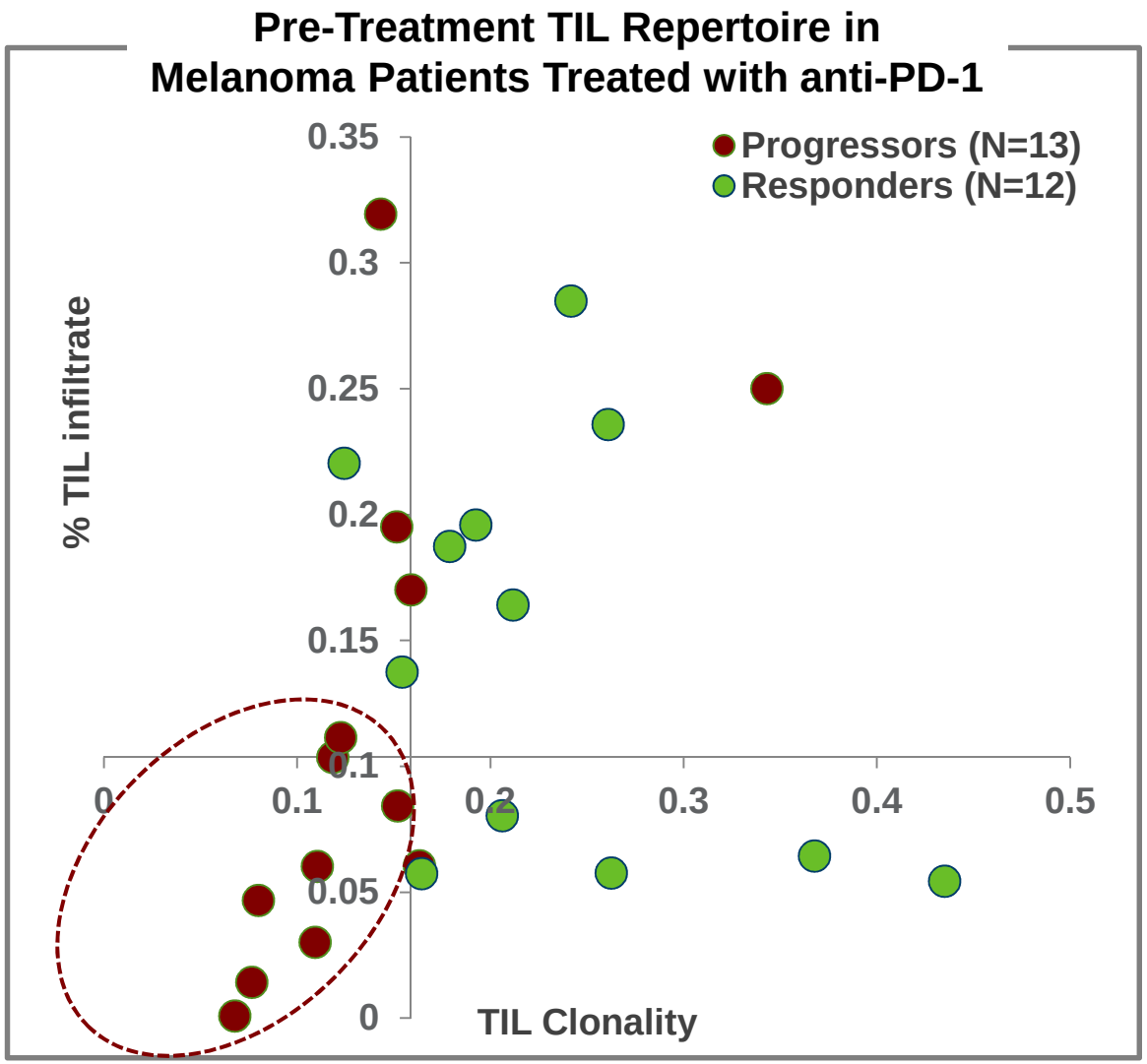


Below-median
Clonality & TILs
25/95 patients (26%)
12/22 events (55%)

Patients with below-median TILs & clonality are at higher risk (p = 0.00095)

* *Catalan Institute, Dr. Moreno, Spain.*

TIL sequencing may predict anti-PD-1 response

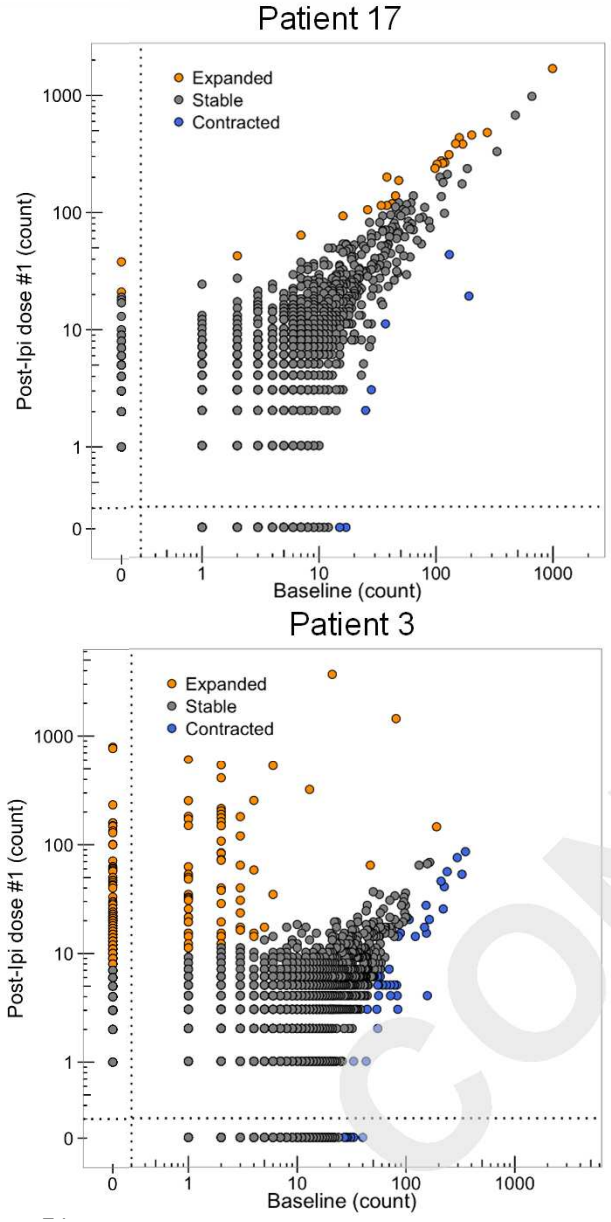


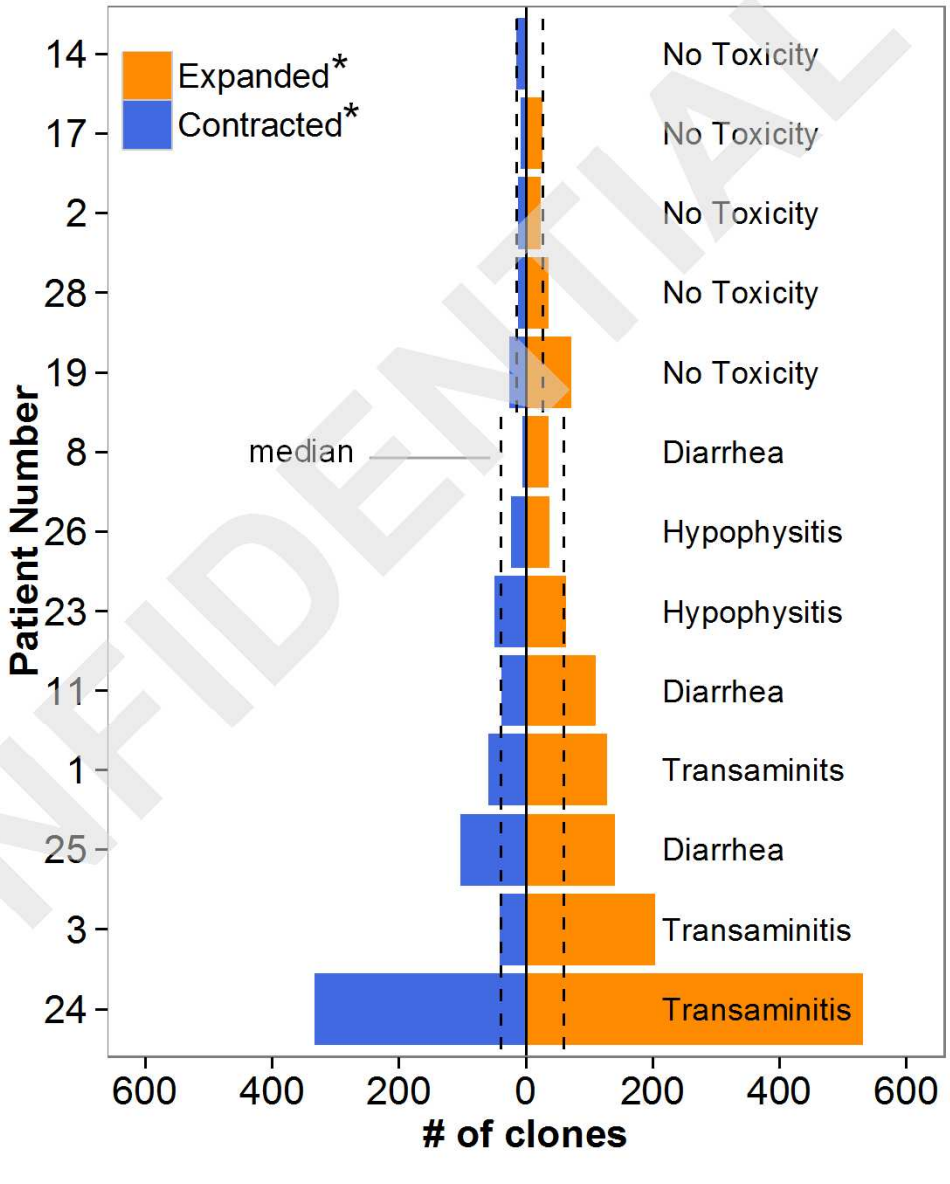
Conclusions

- Progressors (●) were associated with:
  - Lower levels of TILs
  - Lower TIL clonality (a more diverse TIL repertoire)
- TIL profiling has predictive value in evaluating response to anti-PD-1 therapy

Source: Tumeh…Ribas et. al., Nature, 2014.

Increased repertoire turnover through clonal expansion and contraction of CD8+ PBMCs precedes irAEs as a result of anti-CTLA4





*Toxicity vs. no toxicity:
p = 0.019 and 0.045

THANK YOU!