Seqalis

Séminaire FHU Care

"Seqalis' qTCR seq platform, an unbiased and quantitative enabling platform to unlock the immune repertoire diversity"

Dr. Javier Carrasco

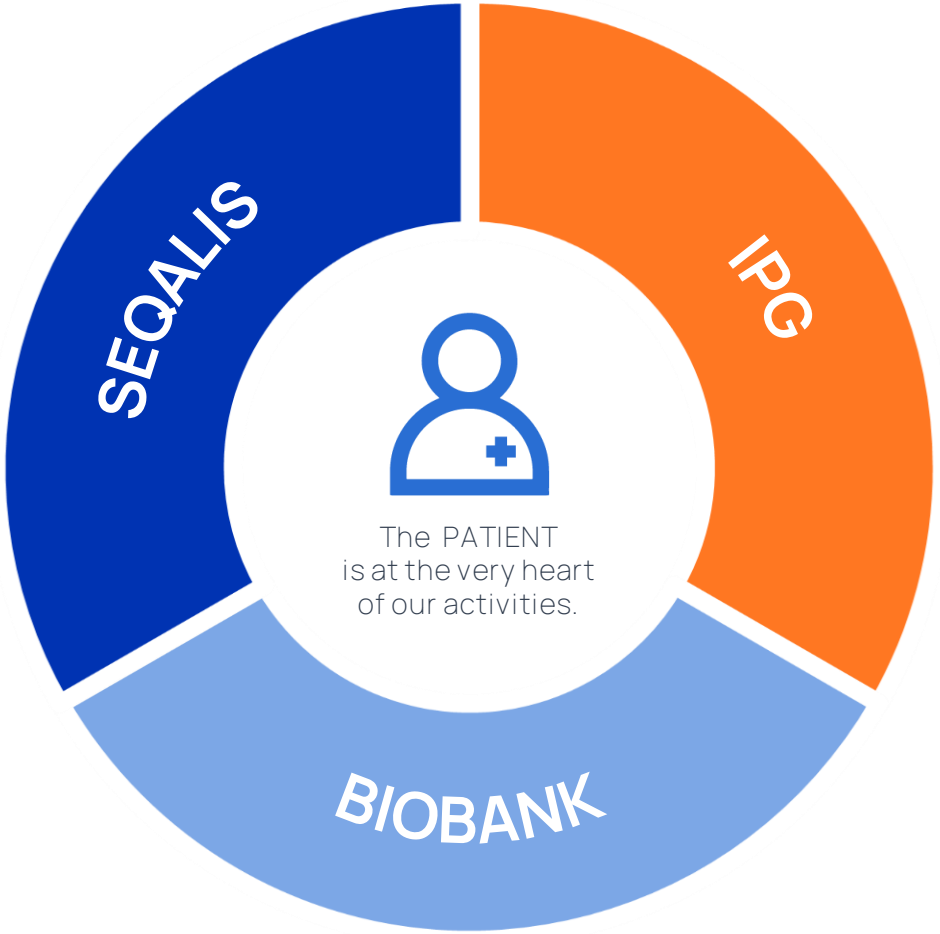
Medical Advisor

Seqalis

GOSSELIES — SEPT. 20 , 2024

IPG Group
Structure

J-F Ghidetti
CEO IPG & Seqalis™



Seqalis

10+ 21 years

JL Henrioul
COO Seqalis

Institut de Pathologie
et de Génétique

350+ 63 years

Dr J Gras
Medical Director IPG

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Our 3 Key Markets

1. Immunotherapy (Immuno-oncology, auto-immune diseases)

- qTCR Seq – « Flagship Service » associated to Anatomo-Pathology, Cytogenomics and Spatial Omics

2. ATMPs - Cell and Gene Therapies, Regenerative Medicine – QC Genomic stability

- Cytogenomics + NGS/PCR + Digital PCR, Optical Genome Mapping (Bionano)

3. Micro-organisms & Microbiomes

- Total RNA-seq
- Whole Genome Sequencing
- Custom services

qTCR-seq platform

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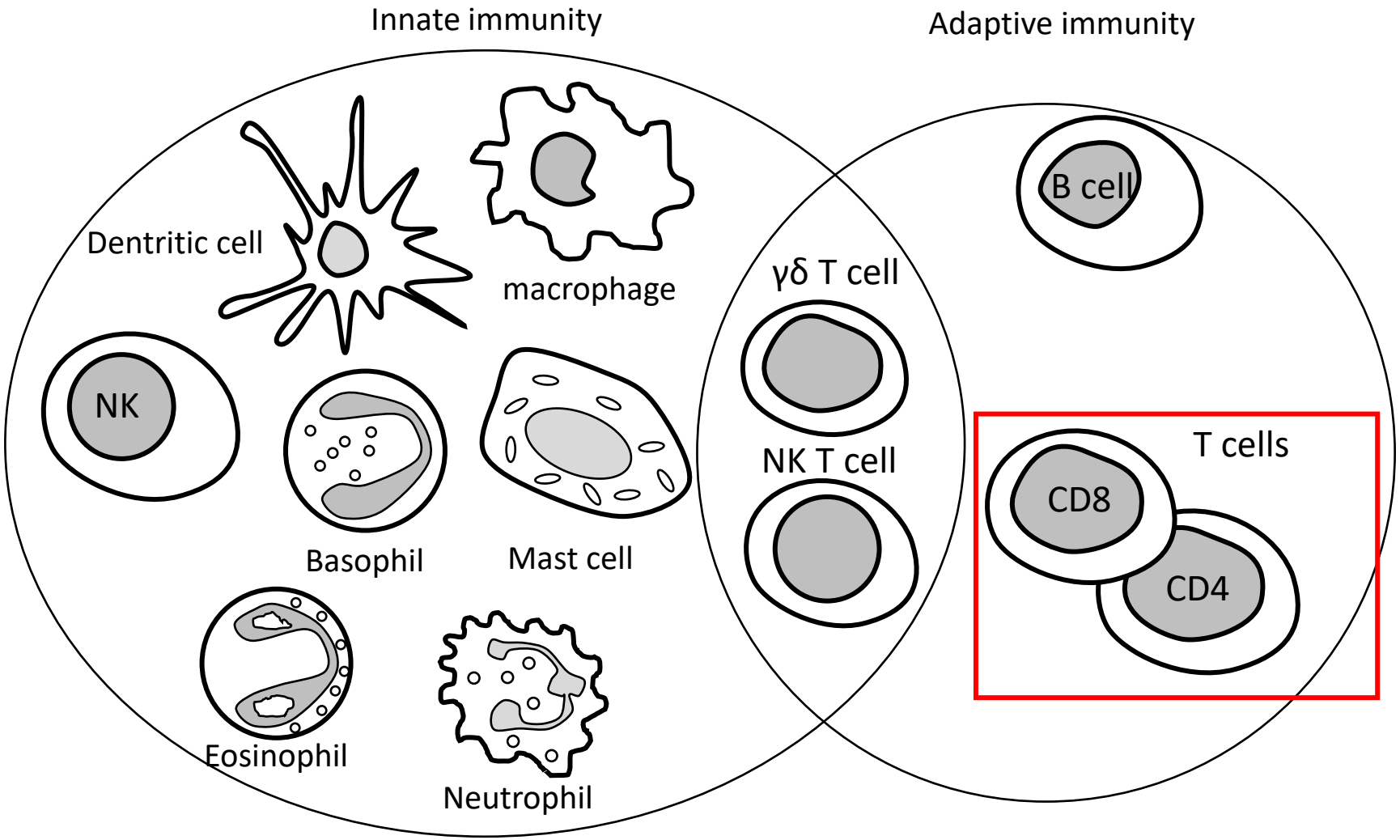
TCR sequencing rationale



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TCR sequencing applications

Sequencing immune receptors using NGS has been proposed to evaluate adaptive immune responses in several pathologies or therapeutic approaches:

- Cancer diseases
- Cancer immunotherapy (preventive and therapeutic vaccines)
- Auto-immune diseases
- Infectious diseases
- Endocrine diseases
- Neurological diseases
- Organ grafts and bone marrow grafts

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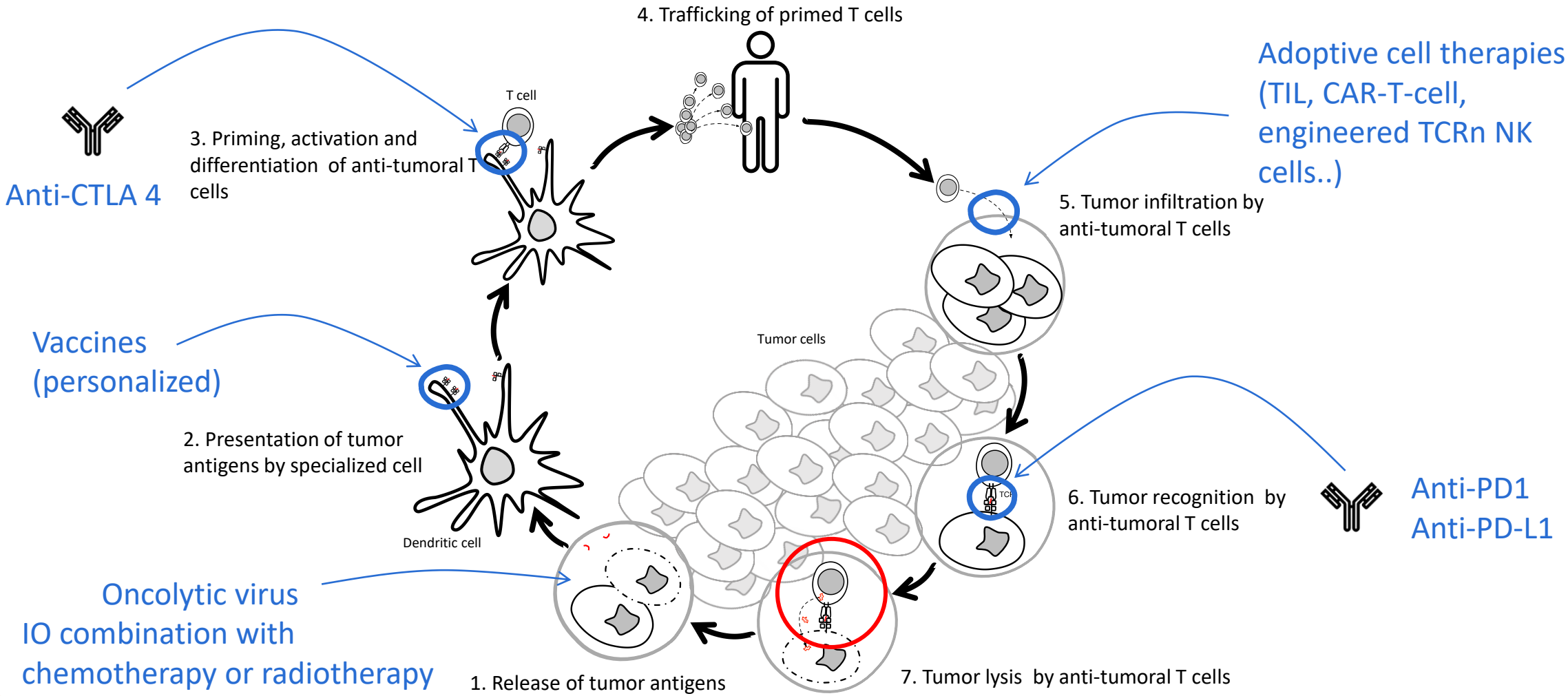
Immuno-oncology landscape



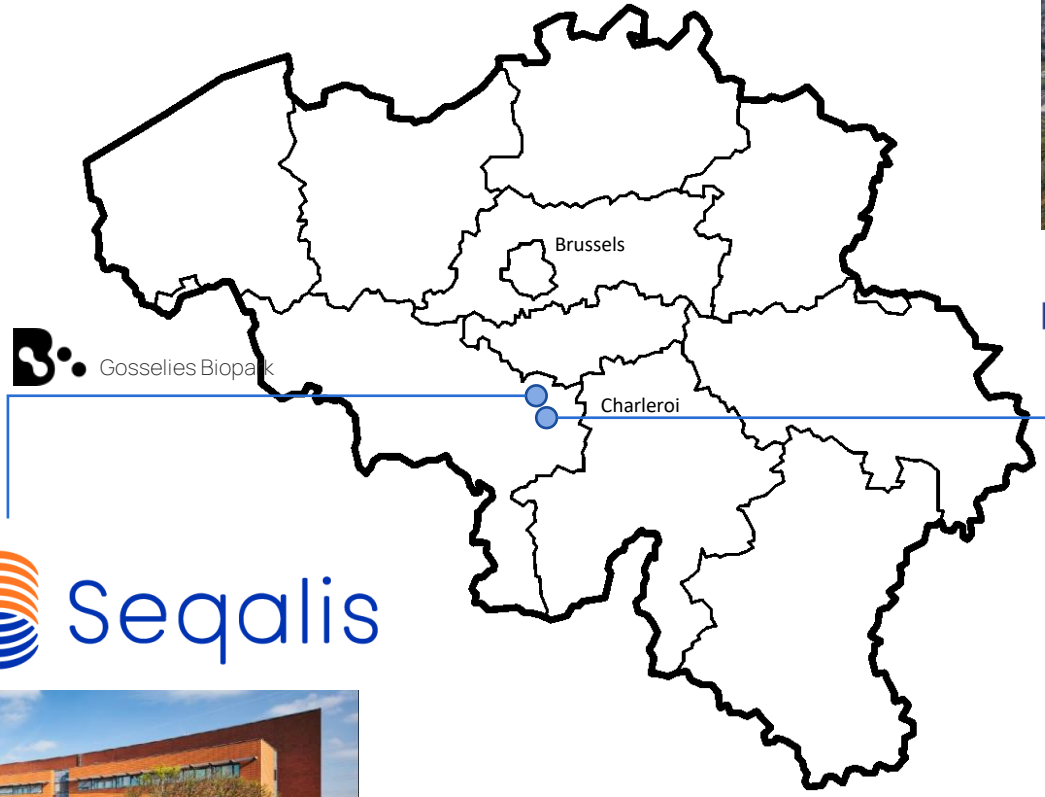
T cell adaptive response plays a crucial role

Adapted from Chen and Mellman 2013 Immunity

South of Brussels

A hub of clinical, scientific and technological expertise

Laboratory of Translational Oncology GHdC / IPG



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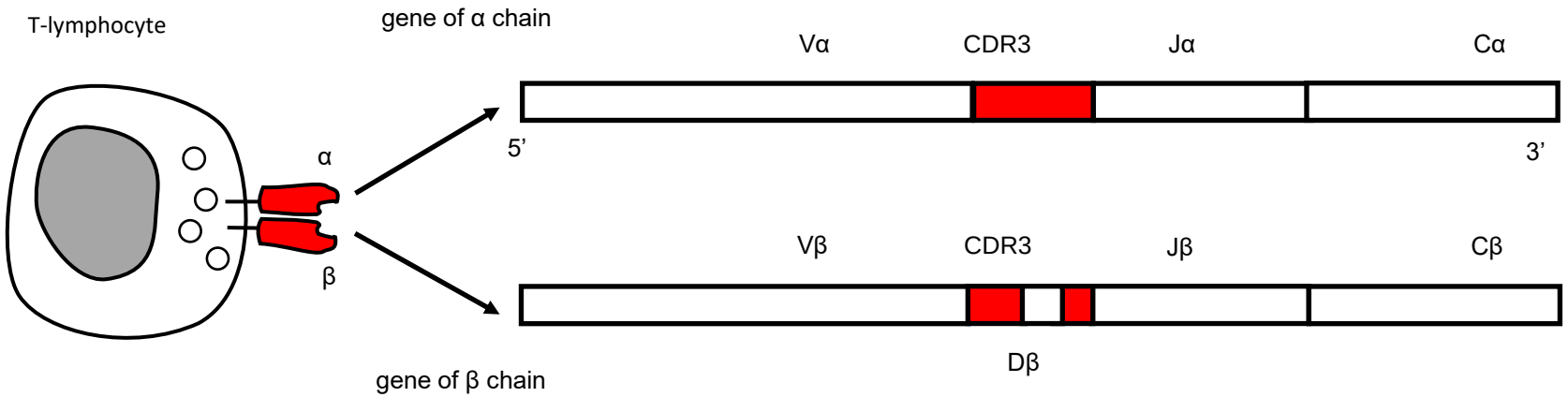
How to decipher the T cells adaptive response?

A proposed approach: sequencing of TCR receptors
(Illustration for CD8 or CD4 T cell lymphocytes)



TCR = T-cell fingerprint – ID card of the T-cell

TCR contains a highly variable sequence (CDR3 region) that recognizes a specific antigen and that characterizes T-cell clonotype

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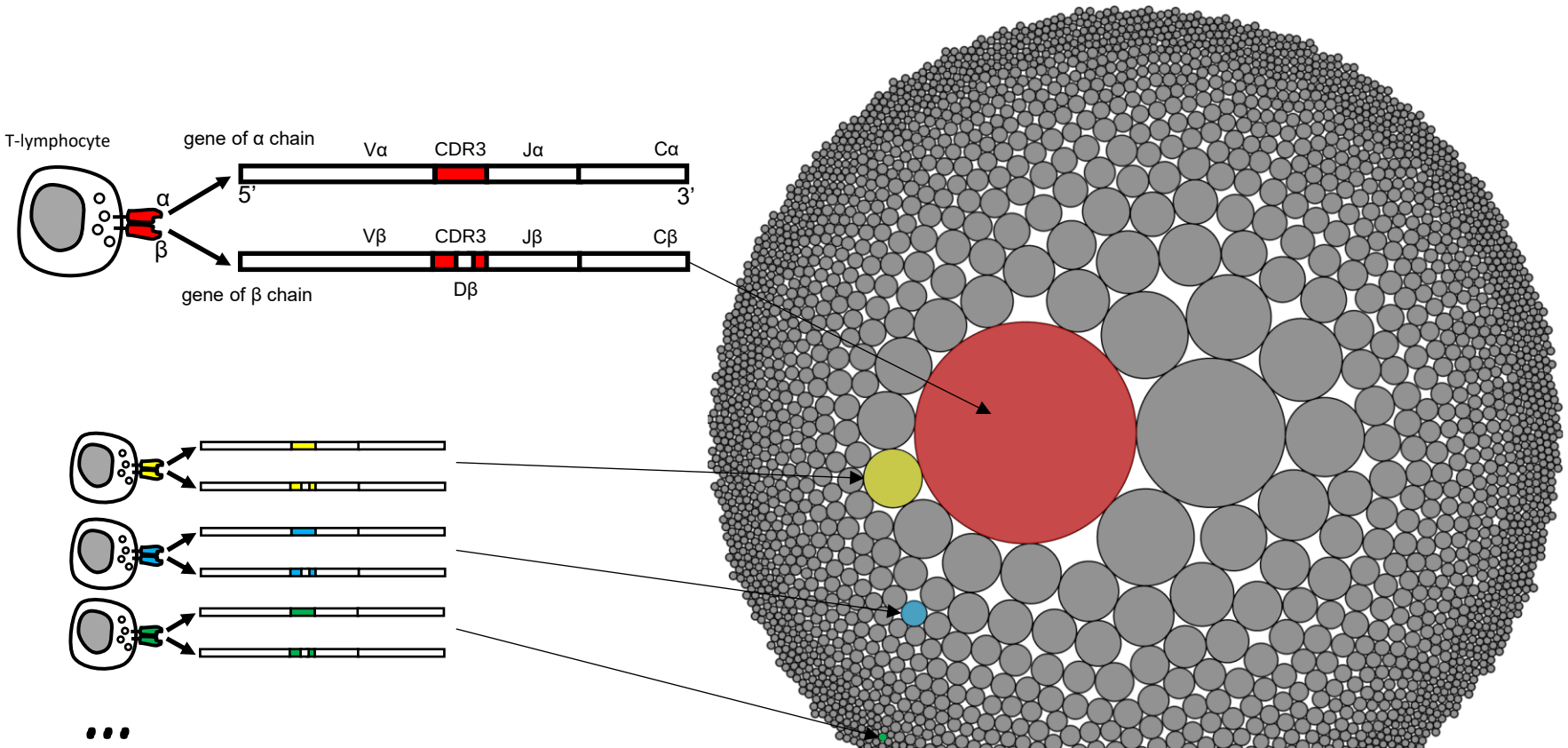
All the TCR rearrangements represent the T lymphocytes repertoire

Identification and tracking of each clonotype



Bubble chart

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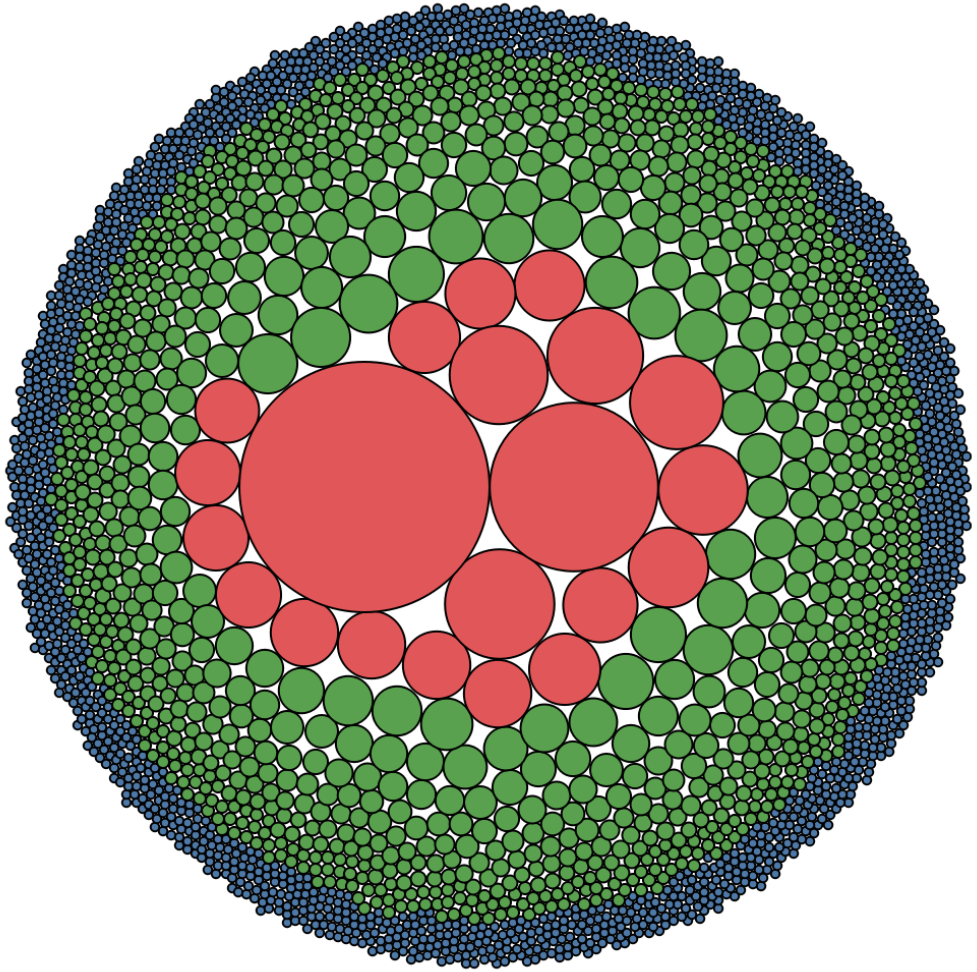
All the TCR rearrangements represent the T lymphocytes repertoire

Evaluate diversity



Bubble chart

Hill diversities:

Richness

Shannon diversity

Simpson diversity

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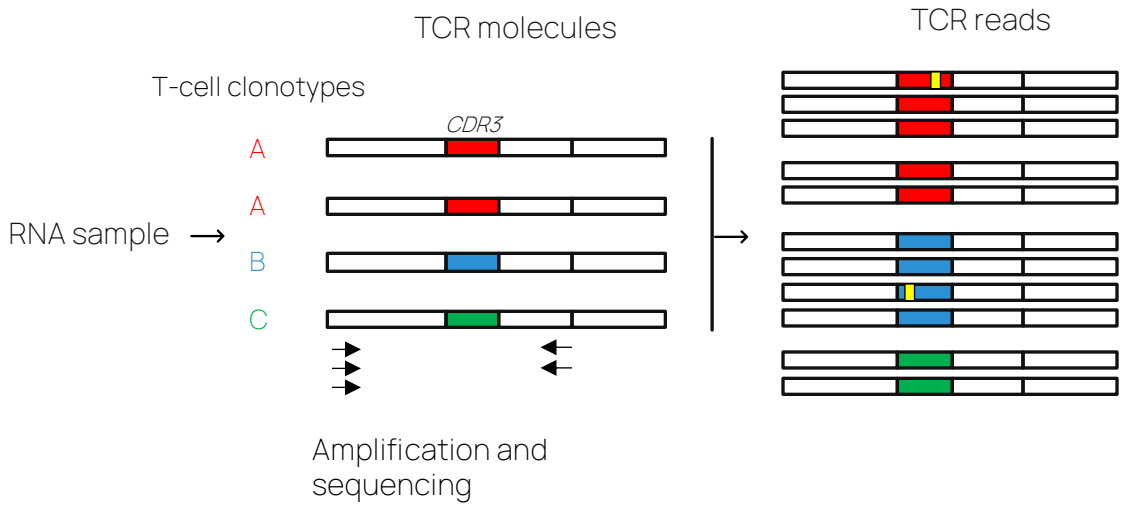
TCR-seq pitfalls

Immune receptors sequencing libraries construction is complex and subject to potential technical bias:



1. Amplification bias
   Distortion of clonotypes abundance during the amplification steps.

2. Artefactual diversity
   Introduction of an artificial repertoire diversity due to polymerase mistakes during DNA replication ( ).

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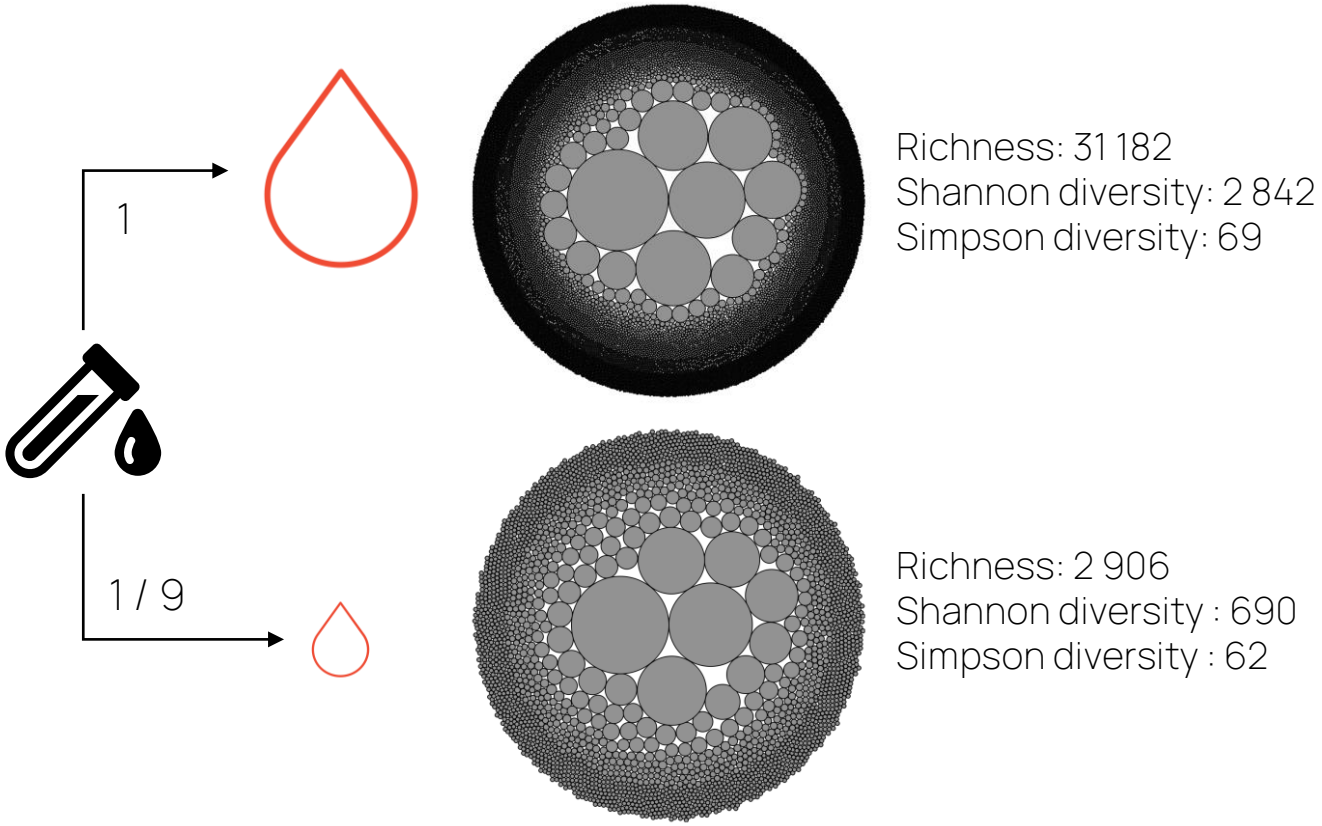
TCR-seq pitfalls

TCR sequencing results depend on the size of the evaluated T cell population

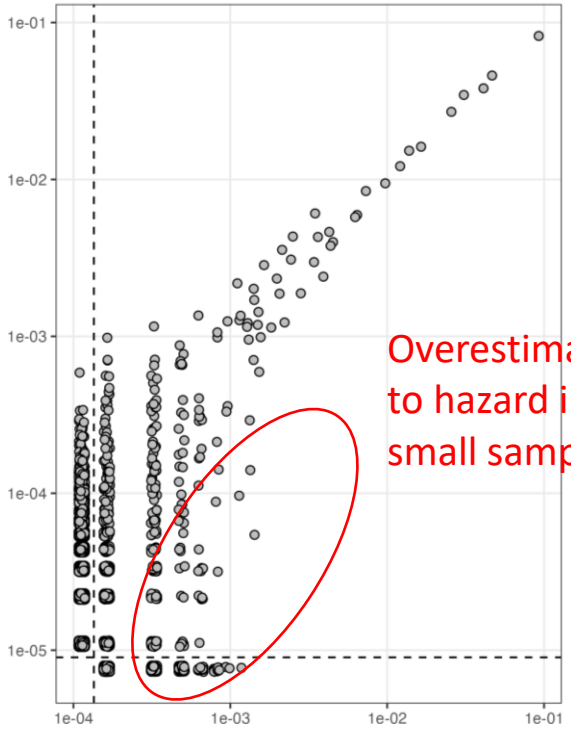
Influence on diversity assessment



Richness: 31 182
Shannon diversity: 2 842
Simpson diversity: 69

Richness: 2 906
Shannon diversity : 690
Simpson diversity : 62

Influence on individual clonotypes assessment



Overestimation due to hazard in the small sample

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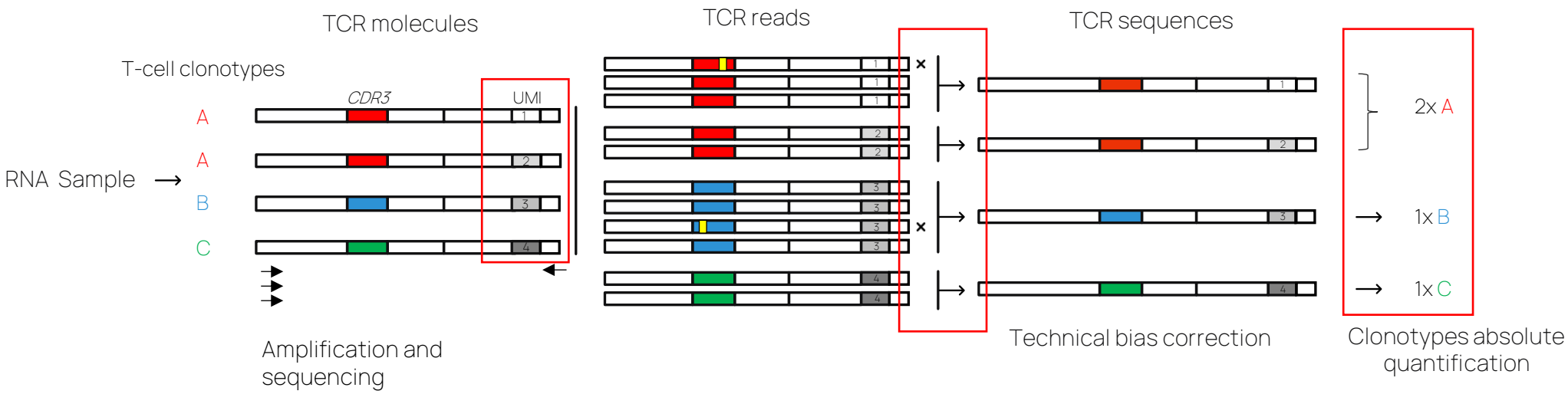
Our TCR sequencing solution

Seqalis has developed and validated a proprietary method for accurate quantitative TCR sequencing using molecular barcodes or UMIs (Unique Molecular Identifiers).



ABSOLUTE quantitative TCR sequencing = qTCR-seq

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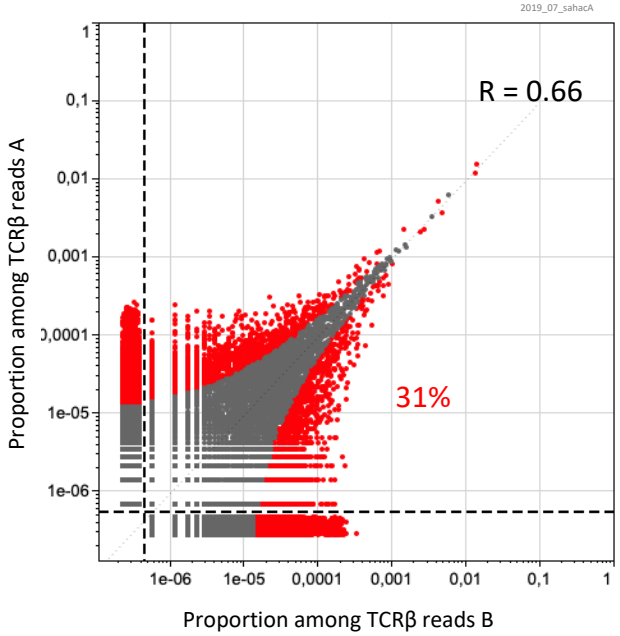
Our TCR sequencing solution

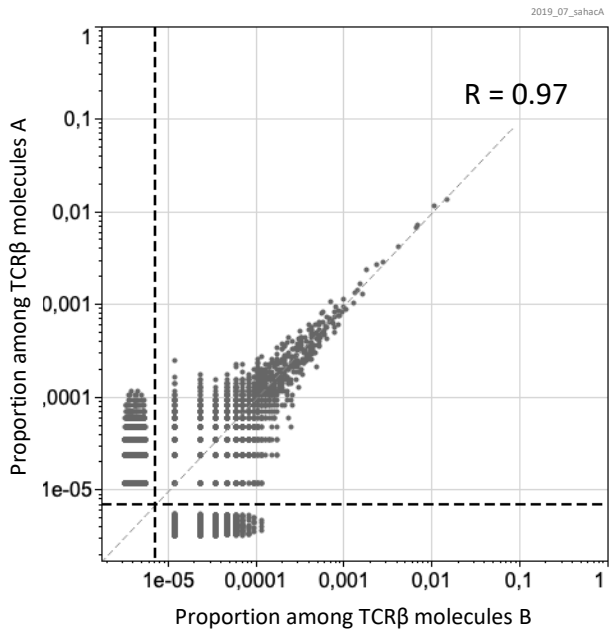
1. Amplification bias correction

Blood replicates

Uncorrected TCRseq(*)



qTCRseq with UMI



(*) Based on aligned reads count

● Significant difference ($p < 1x10^{6}$) between both replicates that are explained by the amplification bias

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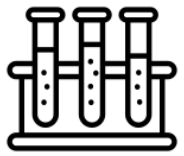
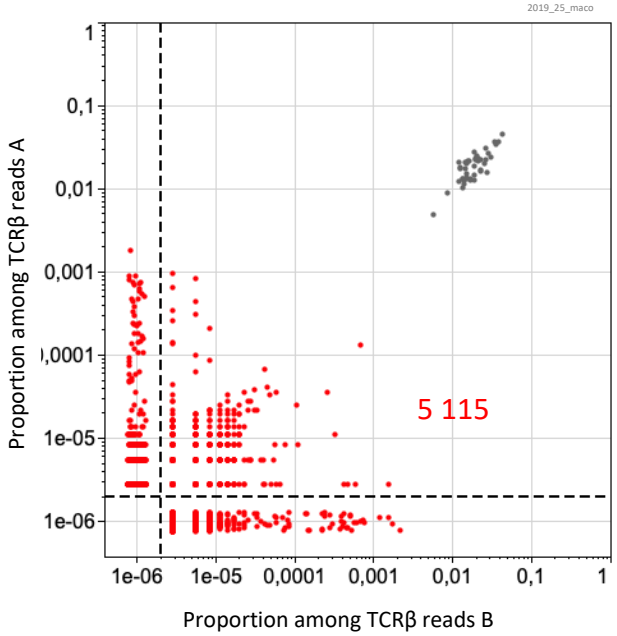
Our TCR sequencing solution

2. Artefactual diversity correction comparisons with no UMI TCRseq methods

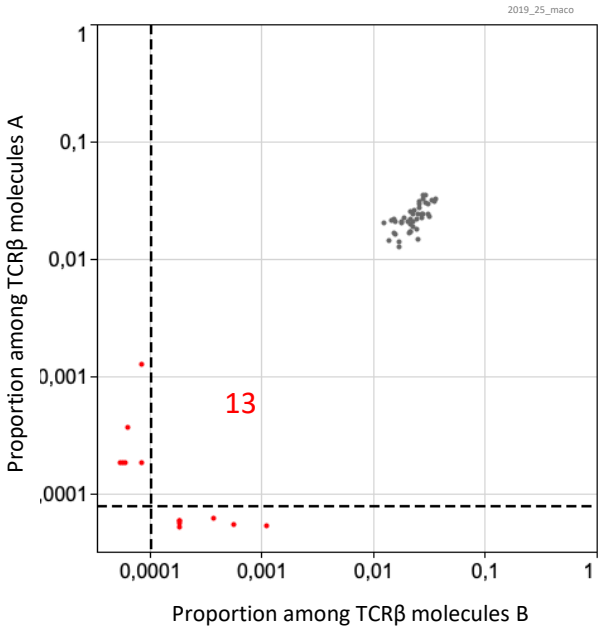


Synthetic repertoire replicates (1)

Uncorrected TCRseq(*)



qTCRseq with UMI



(*) Based on aligned reads count

● Unexpected generated sequences

(1) a mix of TCR Beta chains for which we have previously identified the sequences

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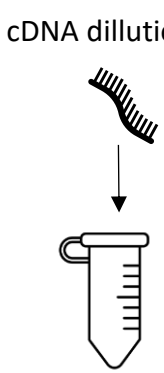
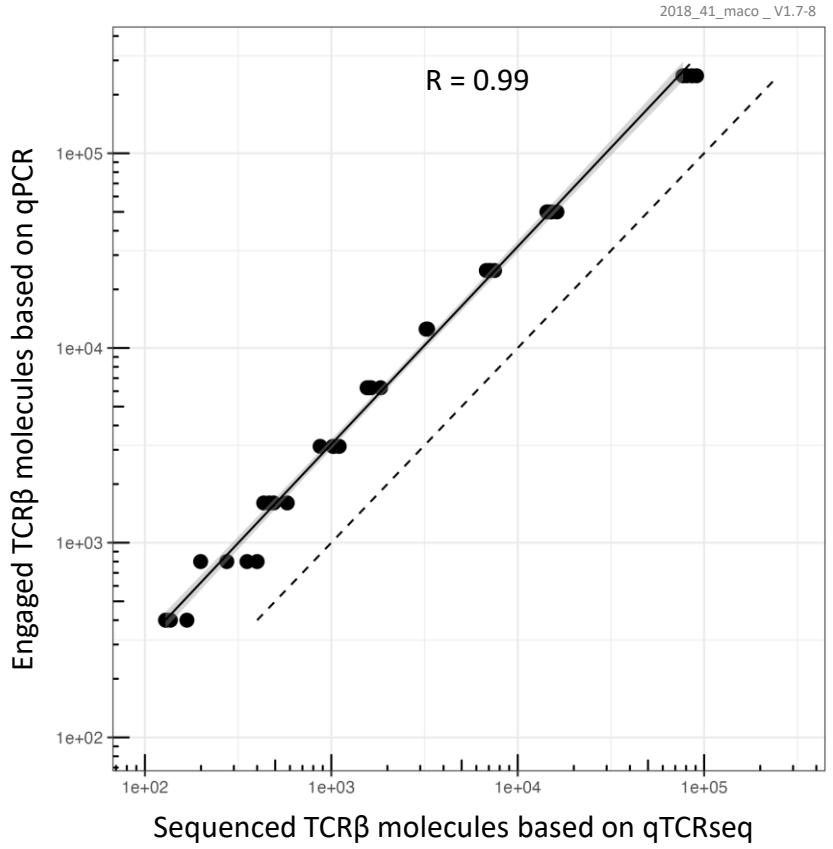
Our TCR sequencing solution

3. absolute quantification of sequenced molecules





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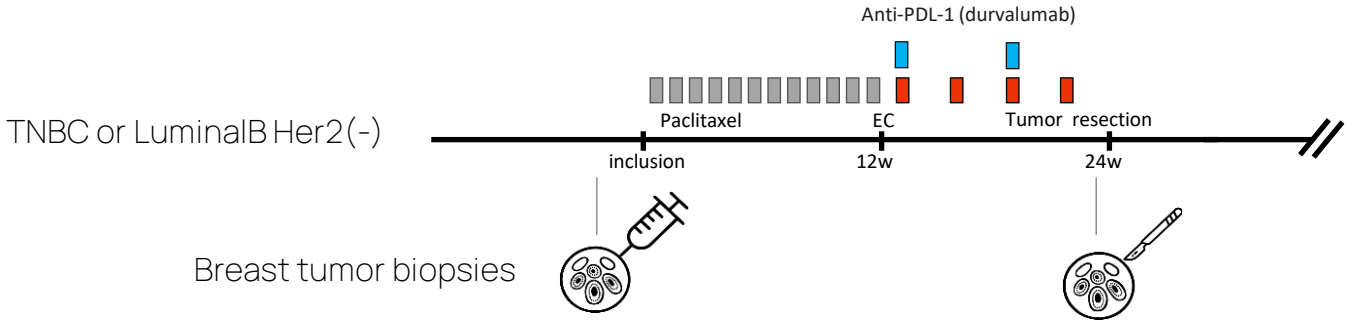
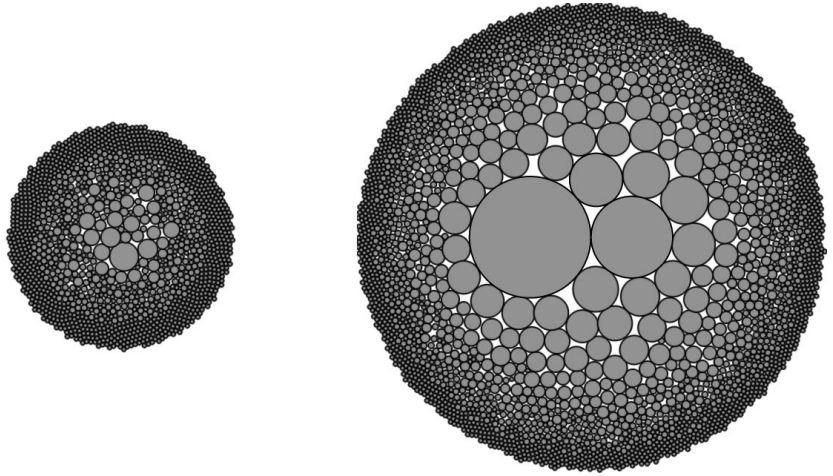
Case study: Evaluation of T repertoire diversity

IIS AstraZeneca

Locally advanced breast cancer treated with Neoadjuvant chemotherapy + immune checkpoint inhibitor (anti PDL-1 mAb)
B-Immune trial (GHdC)



T cell repertoire diversity assessment:	qTCR-seq	qTCR-seq
Richness	1 506	3 657
Shannon diversity	1 092	632
Simpson diversity	545	158
Sample size (TCRbeta transcripts)	2 347	17 649



Devaux, A. *et al.* cancers. *Cancer Research* (2023).

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Case study: Evaluation of T repertoire diversity

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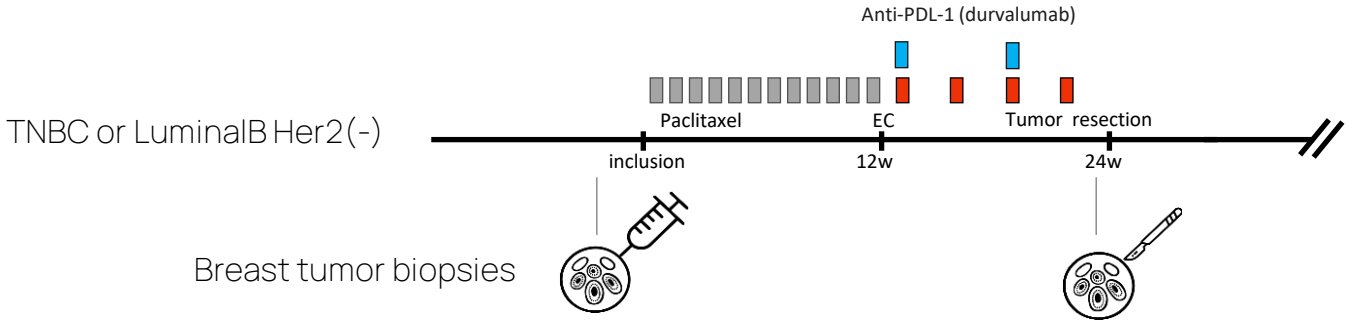

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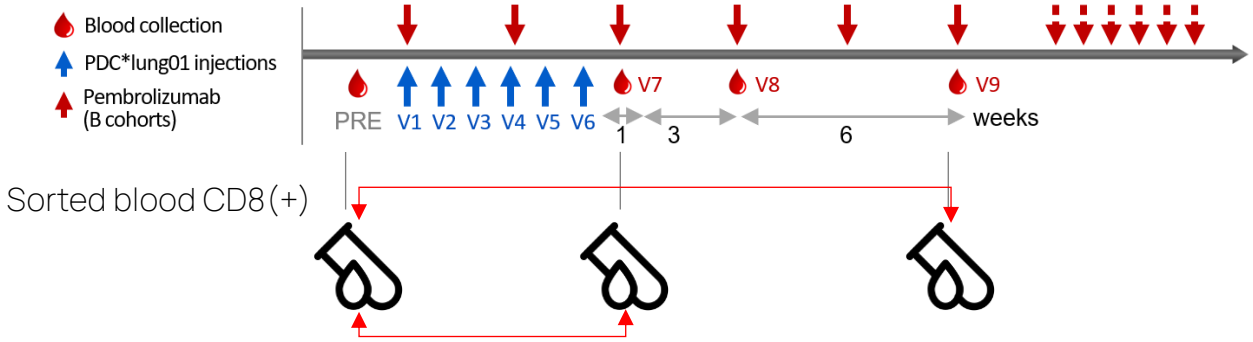
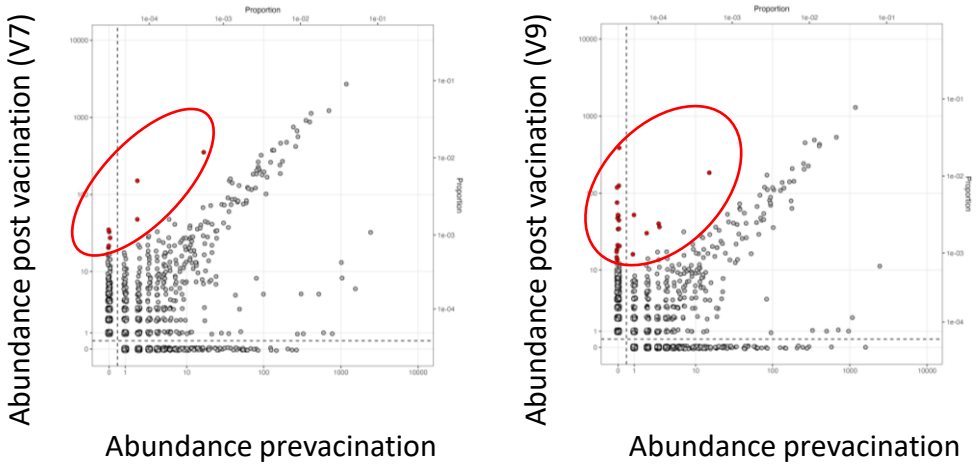
Case study: monitoring of targeted T cell clonotypes

Monitoring T cell repertoire in anti-tumoral vaccines trial

PDC-Lung01 trial
Cohort B1
NSCLC Stage IV



T cell clonotypes monitoring:



S. Michel et al _ presentation CIMT 2024

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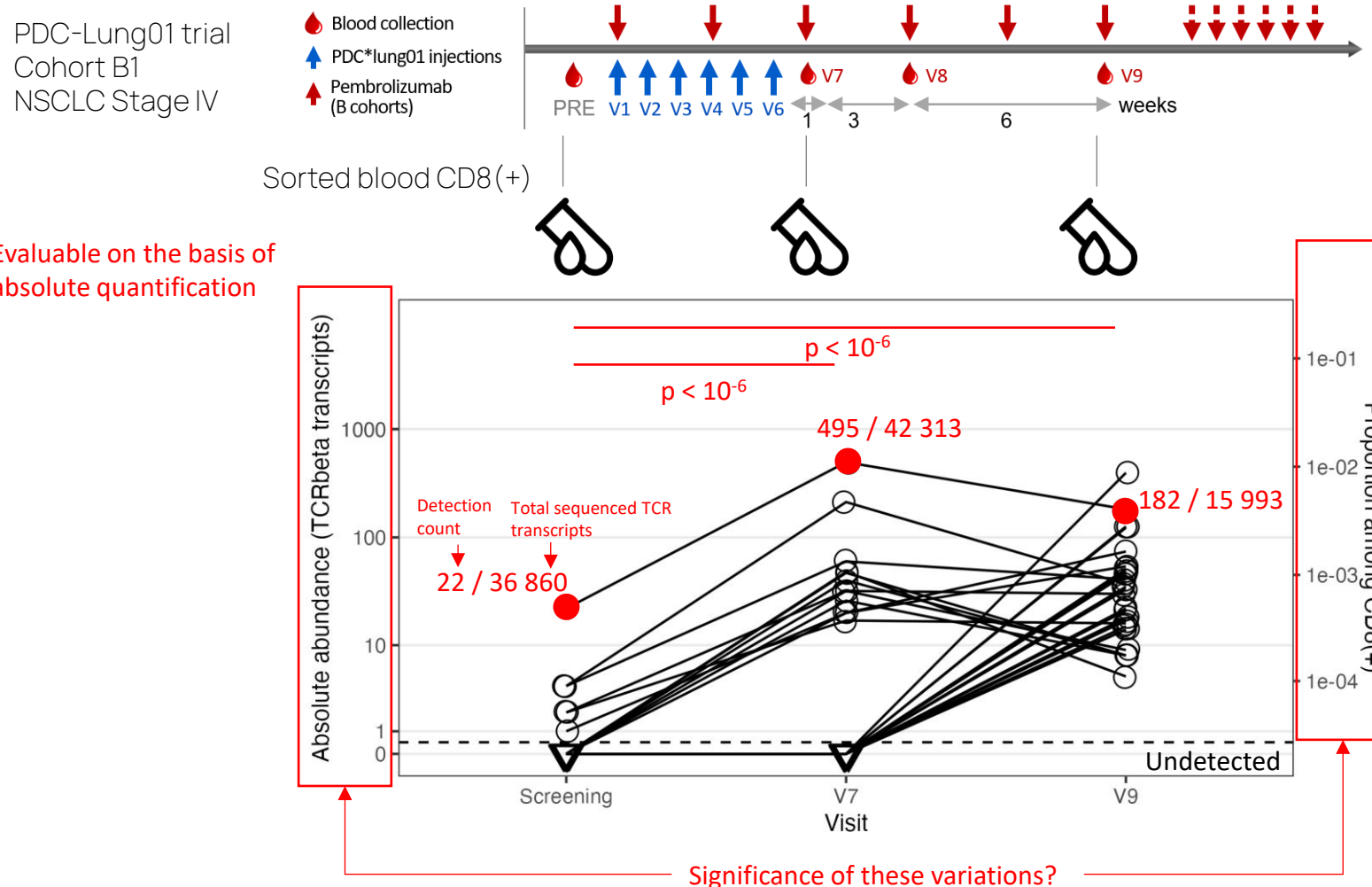
Case study: monitoring of targeted T cell clonotypes

Monitoring T cell repertoire in anti-tumoral vaccines trial



S. Michel et al _ presentation CIMT 2024

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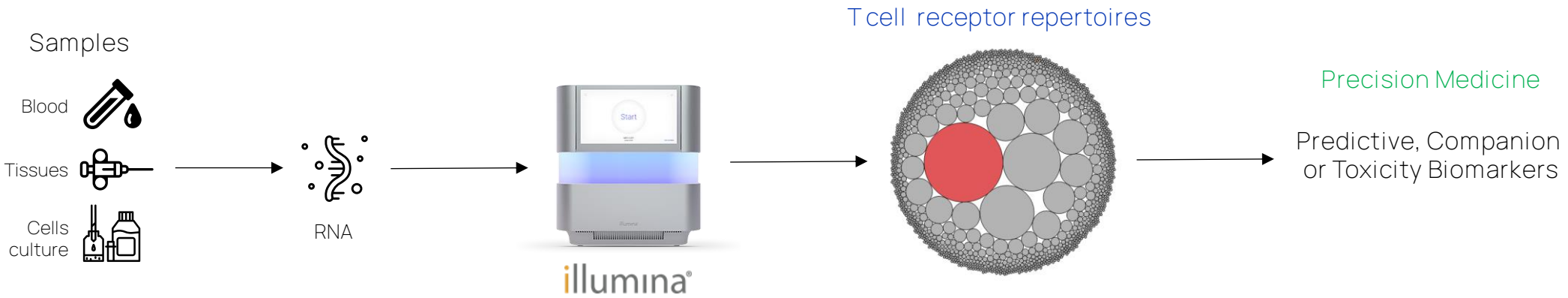
Applications and Perspectives

- Now
  - Better understanding of the adaptive immune response to immuno-therapies & vaccines (cancer and infectious diseases) at cellular & molecular level (T cells expansion)
  - Immuno-monitoring of patients in clinical trials
- Short term (3 to 5 years)
  - Development of biomarkers (predictive, companion and toxicity) for Precision Medicine
  - Applications for other pathologies: auto-immune diseases, transplantation/organ rejections, …



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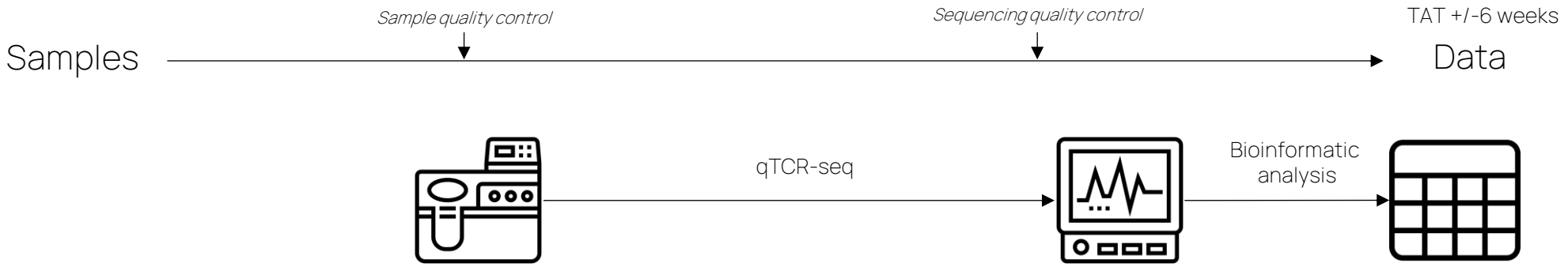
qTCR-seq workflow – RNA



Sample requirements

- Well preserved RNA (DV200≥ 60%; quality control done previous to start)
- RNA amount: 50 - 500 ng

Possible type of samples

- Blood sample or isolated peripheral blood mononuclear cell (PBMC)
- Frozen or later stored tissues (trucut biopsies, fine needle aspiration, surgical samples…)
- Isolated cells (sorted or cultured cells)

Deliverables:

1. Run basic report

2. Data files

Sequencing .fq data

Generated tabular data includes

- Variable genes used for each immune receptor
- CDR3 sequence for each rearrangement
- Functionality of the rearrangement
- Number of detected molecules per rearrangement

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Thank you for your attention

Questions?