Exploring clonal dynamics of adaptive immune cell infiltrates in solid tumors

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Abstract

Characterizing the antigen receptor repertoire of B and T cells in solid tumors is crucial for unraveling the dynamics of adaptive immune responses and tertiary lymphoid structures (TLS) across diverse cancer types. In this research project, we develop innovative experimental and bioinformatic approaches to map full-length immunoglobulin and T cell receptor sequences at spatial and single-cell level. We combine Nanopore long-read sequencing, high-accuracy basecalling, and Unique Molecular Identifiers (UMIs) to enhance the accuracy of our sequencing analyses.

Our methods form a powerful toolkit for robust assessment of antigen receptor repertoires and clonal lineage tracing of tumor-associated B and T cells.

The different bulk, single-cell and spatial methods will find application to study clonal and spatial dynamics of adaptive immune responses in different human immuno-oncological research projects.

Tertiary Lymphoid Structures in Solid Tumors

- Highly organized immune aggregates in solid tumors
- Hubs for anti-tumor immune responses in several cancer types[1,2]
- TLS present in 15 % of human glioma[3]

<u>Aim:</u> Establishment of innovative experimental and computational approaches to analyze the adaptive immune cell receptor repertoire in TLS-associated solid tumors

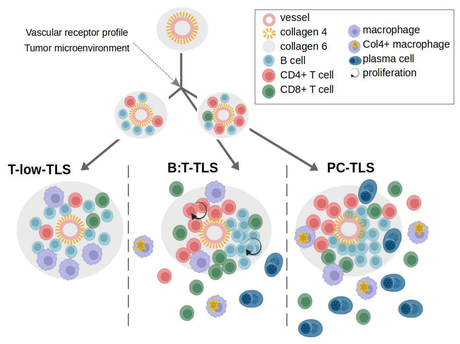


Fig. 1: Model of the differentiation of tertiary lymphoid structures (TLS) into the three subtypes [3].

Adaptive Immune Cell Diversity and Clonality

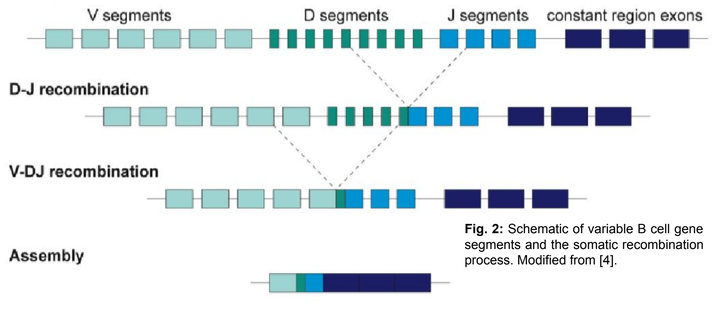


Fig. 2: Schematic of variable B cell gene segments and the somatic recombination process. Modified from [4].

Highly diverse and highly specific receptor repertoire (IG and TCR)

- Antigen specific receptors
- Somatic recombination, junctional diversity, somatic hypermutation **(IG)**
- **Analysis of receptor repertoire diversity and receptor evolution**

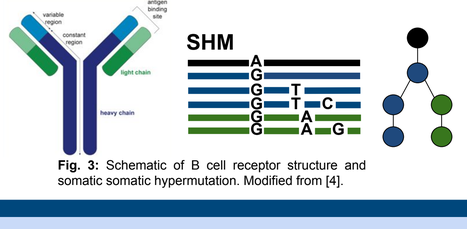
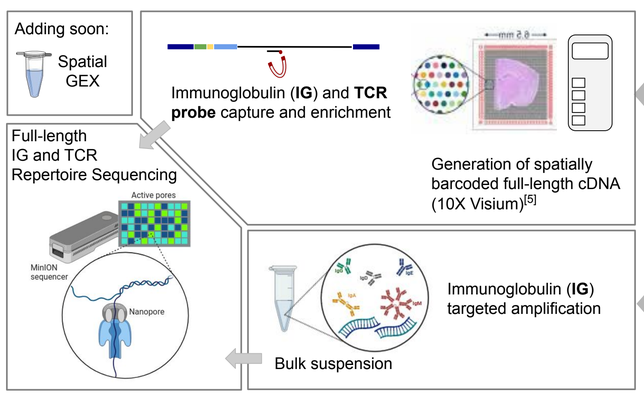


Fig. 3: Schematic of B cell receptor structure and somatic somatic hypermutation. Modified from [4].

Adaptive Immune Cell Repertoire Analysis Workflow

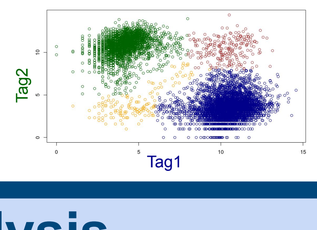
Full length IG/TCR Nanopore sequencing

- BCR / TCR targeted approach
  - 10X Visium Spatial
  - Bulk
- Full length IG / TCR transcripts
- Nanopore long read sequencing
- SUPer accurate (SUP) Basecalling
- UMI based error correction



Single-cell sequencing

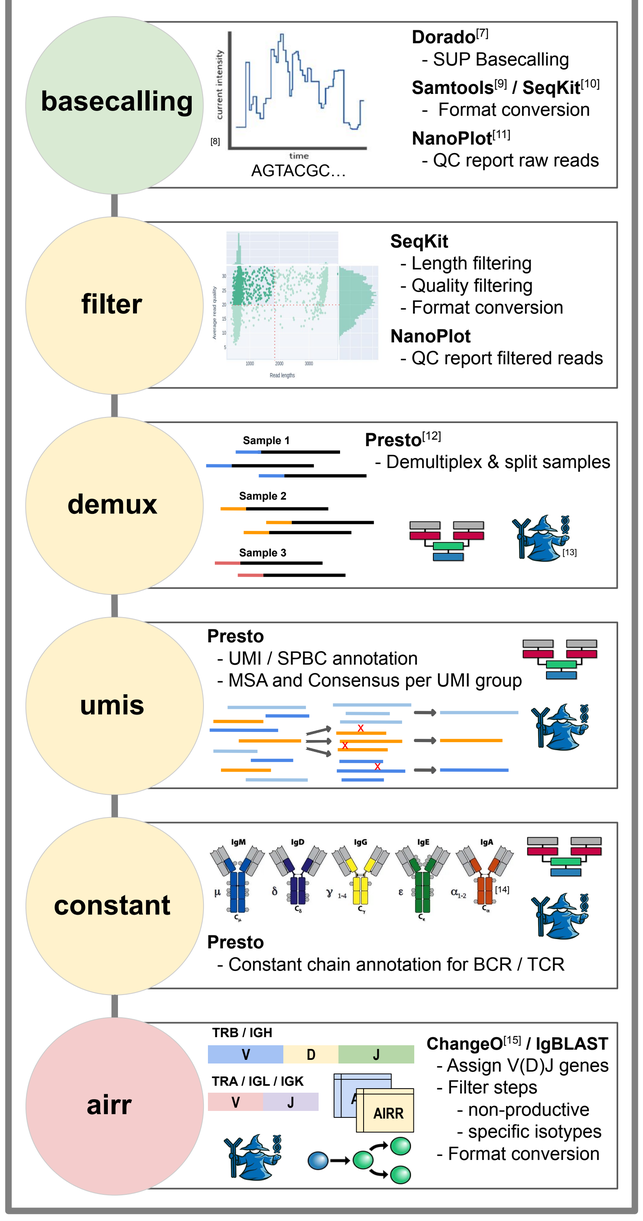
- **CD3+** and **CD19+** cell sorting of dissociated tumor samples
- 10X Chromium single-cell prep.
- Illumina short read sequencing
- Demultiplexing by feature barcode-Tags:

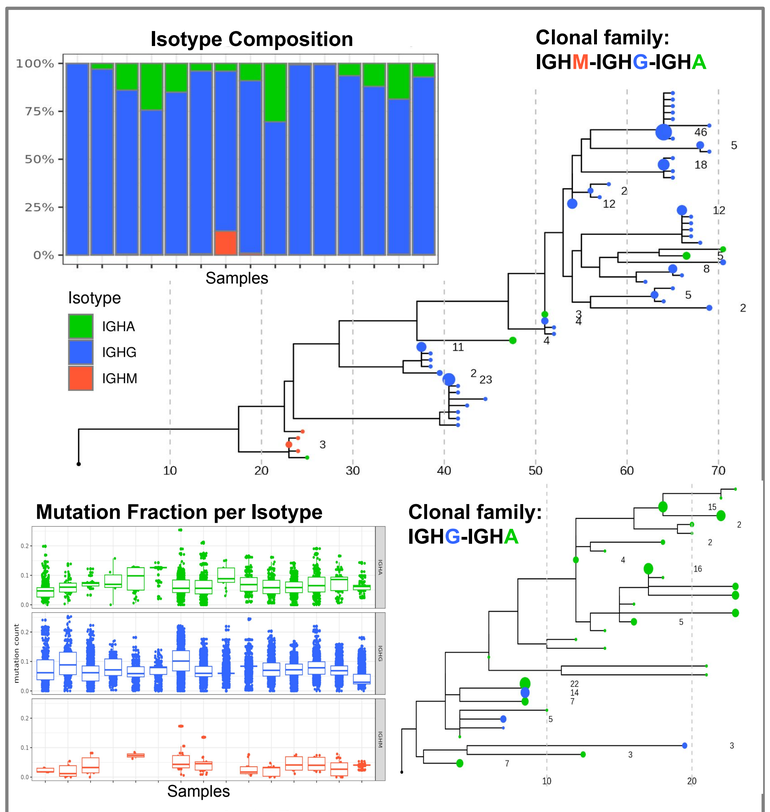


Spatial and Bulk Analysis

NanoAIRR

New bioinformatic method for the accurate preprocessing and annotation of **A**daptive **I**mmune **R**eceptors **R**epertoires using **Nano**pore sequencing:



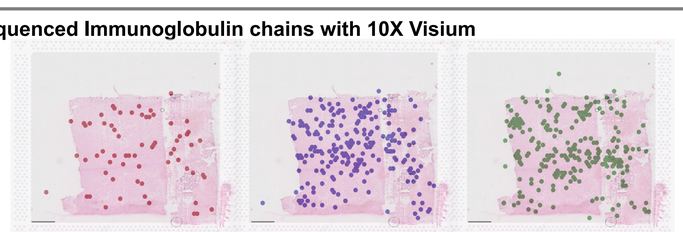


Repertoire downstream analysis

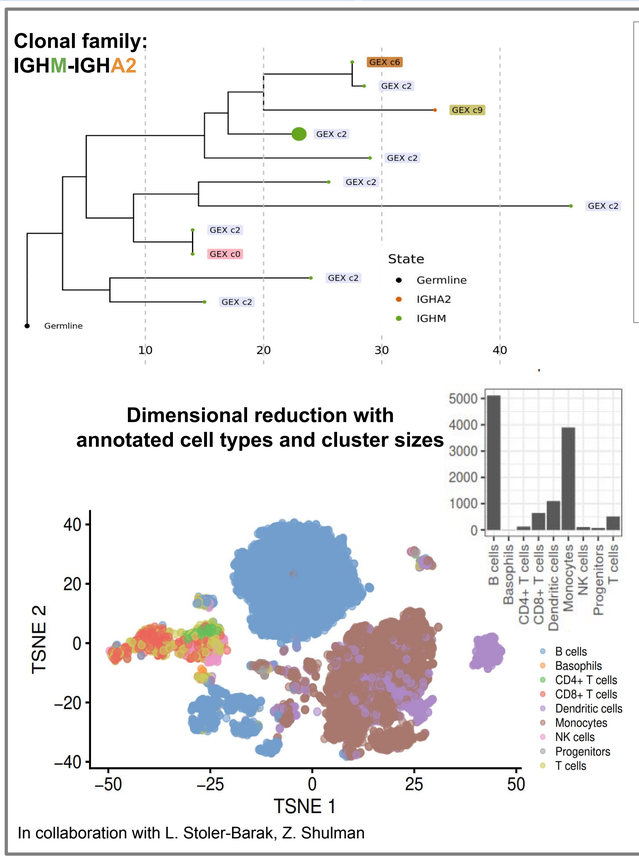
- Repertoire Isotype composition
- Clonal lineage tracing (**IG**)
- # of mutations in variable region
- Identification of dominant clones
- Mutational load between FWR and CDR region
- Repertoire diversity
- …

In collaboration with AG Plate, AG Reiss, FCI-Immunomonitoring

Sequenced Immunoglobulin chains with 10X Visium



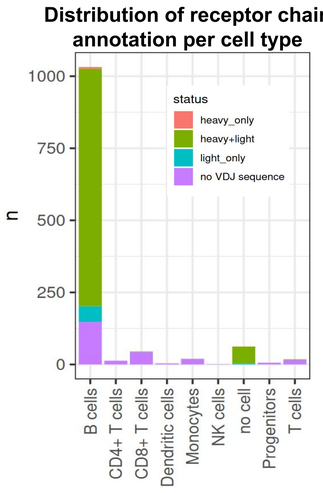
Single Cell Analysis



In collaboration with L. Stoler-Barak, Z. Shulman

Combined transcriptomics and receptor repertoire downstream analysis:

- Gene clustering and cell type annotation (subtype identification and annotation for T and B cells)
- Build clonal lineage trees with cluster annotation
- Repertoire diversity
- …



Conclusion

- Established experimental and bioinformatic approaches to map **full-length immunoglobulin and T cell receptor sequences** at **spatial, single-cell and bulk level**
- Combination of approaches enables the accurate identification and **characterization of tumor-associated adaptive immune cell repertoires**
- High-quality reads (>Q30) and UMI-based consensus enable **accurate error correction**, ensuring reliable downstream analysis
- Despite the highly immunosuppressive TME in glioma, this workflow accurately annotates IG / TCR reads, revealing a **high abundance of IgA and IgG across multiple samples**

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Online Version and References: