

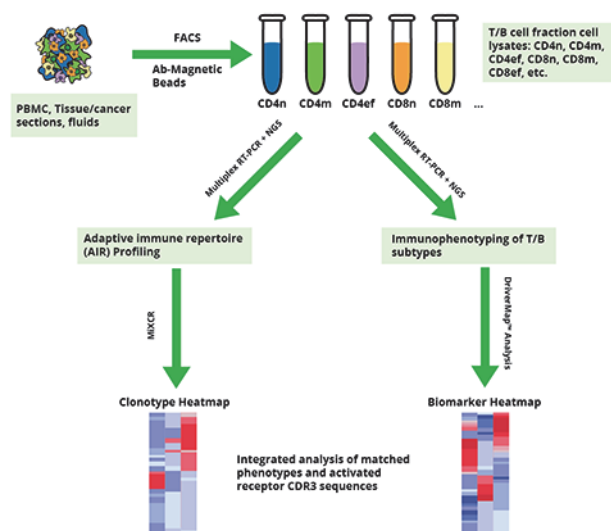
3 ADAPTIVE IMMUNE RECEPTOR REPERTOIRE PROFILING FOR BIOMARKER DISCOVERY

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Background Adaptive Immune Receptor (AIR) Repertoire profiling and characterization of antigen-activated immune cells is essential for the discovery of novel prognostic and predictive biomarkers and for studying immune response mechanisms in cancer, auto-immune and other diseases.

Methods To facilitate these studies, we developed a novel technology for combined, unbiased profiling of all human TCR and BCR variable regions; and phenotypic characterization of immune cells in bulk, sorted fraction and single immune cells (figure 1). This method involves multiplex RT-PCR amplification and sequencing of CDR3 regions of TCR and BCR genes along with immunophenotyping with a set 500 highly expressed T- and B-cell subtyping and activation marker genes. Bioinformatic analysis of next-generation sequencing (NGS) data allows comprehensive AIR repertoire profiling, identification of antigen-activated TCR and BCR clonotypes, and detailed phenotypic characterization of T and B cells induced by adaptive immune responses.

Results Preliminary phenotypic AIR profiling studies in metastatic tumor samples and Humira®-treated rheumatoid arthritis cases indicate that AIR immunophenotyping technology has unparalleled throughput and sensitivity for the discovery of immunity biomarkers.



Abstract 3 Figure 1 Integrated AIR repertoire profiling & immunophenotyping

Fig. 1 The workflow illustrates integrated adaptive immune receptor (AIR) repertoire profiling and immunophenotyping directly in sorted cells without RNA purification using multiplex RT-PCR. High-resolution immunophenotyping (matching) of top TCR/BCR clonotypes is based on the expression of 300 key cell typing and activation T and B markers. Candidates were selected from a set of 3000 candidate genes described in >100 public databases, commercial assays, and peer-reviewed publications

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