Identifying Putative Biomarkers for Rheumatoid Arthritis

Identification of biomarkers to diagnose disease, assess pathology, and select patients that will respond well to specific treatments has become an essential component of therapeutic drug development. In this application note, we present an effective and time-efficient approach using Cellecta's DriverMap Adaptive Immune Receptor Repertoire (AIR) and Targeted RNA-Seq Expression Profiling (EXP) assays to identify putative biomarkers associated with Rheumatoid Arthritis (RA) that correlate with treatment by a TNFα inhibitor.

Gene Expression and Immune Receptor Profiling

Comprehensive analysis of general changes in biological pathways that drive disease progression and therapeutic response provides a robust approach to identifying systemic variations linked to pathologies and therapeutic responses (Fig 1). DriverMap Profiling technology combines the sensitivity and specificity of RT-PCR with the power of next-generation sequencing (NGS) to comprehensively profile gene expression transcripts and immune receptors for each cell, tumor, whole blood, or PBMC sample.

RA is a chronic autoimmune and inflammatory disease that affects joints, causing pain, swelling, stiffness, and loss of joint function. In this study, we obtained 40 RA blood samples from Cureline, Inc. Samples were collected both before and after patients were treated with TNFα immunotherapy (Humira®). Based on the level of the adaptive immune response, as measured by the DriverMap-AIR Assay, the patients were classified into three groups — Non-responders, Early Responders, and Late Responders (Fig 2). We then ran the DriverMap AIR profiling assay and the DriverMap Targeted RNA-Seq Assay (Fig 3) on the blood samples with the goal of identifying putative biomarkers.

DriverMap AIR Assay Shows B-cell Role in Rheumatoid Arthritis

DriverMap Adaptive Immune Receptor (AIR) profiling showed significant differences in the B cells' IGH heavy chains repertoire in RA samples when compared to healthy control samples (Fig 4a).

No significant difference in the TCR repertoire was observed (Fig 4b). These findings align with published data that show differential antigenic reactivity in the B cell compartment between responders vs non-responders to TNFα immunotherapy[1] and suggest focusing on changes in the heavy-chain B-cell repertoire for immune response biomarkers to indicate treatment response to TNFα immunotherapy.

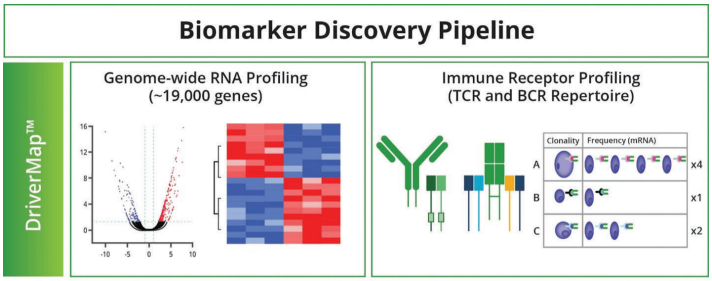

Figure 1. *DriverMap™ Technology Solution for biomarker discovery combines gene expression and immune receptor profiling to discover multifactorial disease biomarkers.*

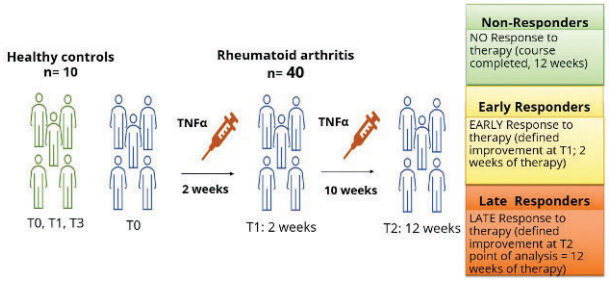

Figure 2. *Samples from 40 patients and 10 control samples were obtained from Cureline Inc. (Biobank). The patients are classified into three groups based on their response to TNFα immunotherapy (Nonresponders, Early Responders, and Late Responders).*

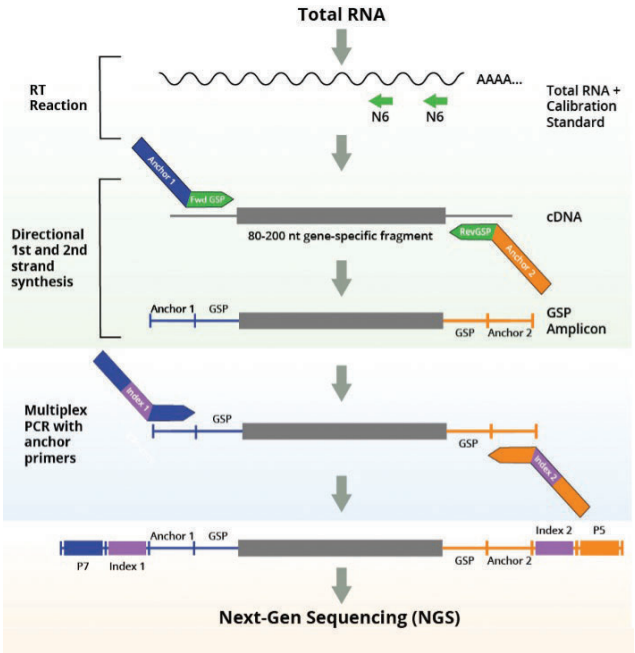

Figure 3: Outline of DriverMap Multiplex RT-PCR Technology. *The DriverMap workflow leverages multiplex RT-PCR to target and amplify a segment of the transcript from ~ 19,000 protein-coding genes in the human genome. The single-tube reaction makes use of 38,000 validated primers to target and amplify ~100-200 nt segments in each transcript expressed by each gene.*

Gene Expression Profiling Results from the DriverMap EXP Assay

We then employed the DriverMap Genome Wide Targeted RNA Expression Assay (EXP) to analyze transcript expression of all protein-coding genes in the RA samples. Distinct differences in gene expression were seen between the expression profiles of the 40 RA samples as compared with the 10 healthy controls, as seen in the Principal Component Analysis plot (Fig 5).

We cross-referenced differentially expressed genes in the two sample populations with the KEGG Pathway database, and found marked differences in the Rheumatoid Arthritis pathway along with the MAPK, RAP1, and Ras signaling pathways, involved in producing inflammatory mediators such as TNFα, IL-1, IL-2, and IL-6 responsible for inflammation in RA disease.

More detailed pairwise comparison differences between responders at different time points, namely T0 (baseline) and T1/T2 (time points 1 and 2), identified a subset of genes associated with responders to TNFα immunotherapy (Fig 6). In particular, there were 46 unique genes found to be up- or down-regulated in the responder population and 141 up- down-regulated genes in the non-responder population (141 genes), (Fig 7).

We compared the data from the DriverMap EXP Assay to normal whole blood RNA-seq data from the GREIN dataset[2]. We found high concordance in high- and low-expressed genes in RA disease[3]. The top 20th percentile genes in the DriverMap EXP assay had a 70% overlap with the top 20th percentile of the GREIN dataset. This alignment demonstrates the robustness of our methodology and provides additional confidence in the identified genes.

Conclusion

The results from this study demonstrate that the DriverMap Adaptive Immune Receptor Profiling Assay together with the DriverMap Targeted RNA Sequencing Expression Profiling Assay offer a high-throughput and effective method for discovering potential biomarkers from starting material of total RNA derived from whole blood patient samples. Our platform identified 141 unique genes for non-responders and 46 unique genes for responders, which were related to RA, and the MAPK, RAP1, and Ras signaling pathways, known to be involved in inflammatory response in RA disease. While further research needs to be conducted on a larger sample size, this study provides a panel of ~200 putative biomarkers that are associated with response to TNFα immunotherapy and highlights the importance of studying the B-cell repertoire in RA biomarker discovery.

References

1. https://www.medrxiv.org/content/10.1101/2021.11.26. 21266347v1
2. https://www.nature.com/articles/s41598-019-43935-8
3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7027163/

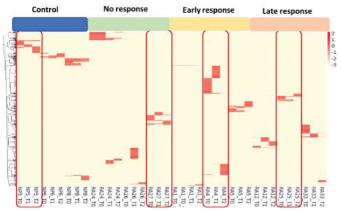

Figure 4a. *The DriverMap™ AIR BCR profiling assay shows significant patient-specific differences in BCR (IGH clonotype) repertoire in healthy control and RA blood samples before and after TNFα therapy.*

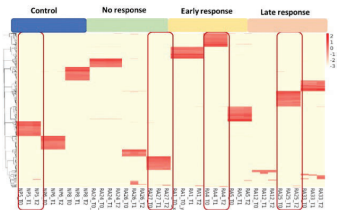

Figure 4b. *The DriverMap™ AIR TCR profiling shows no differences in TRB clonotype repertoire in normal control and RA blood samples before/after TNFα therapy.*

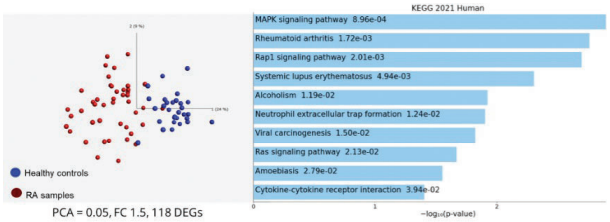

Figure 5. *Principal Component Analysis (PCA) of DriverMap EXP gene expression profiles shows significant differences in gene expression between patient and control samples (PCA= 0.5).*

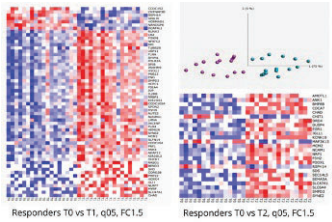

Figure 6. *Pairwise comparison for responders for different time points (T0 and T1/T2) helps identify a subset of genes associated with responders to TNFα immunotherapy.*

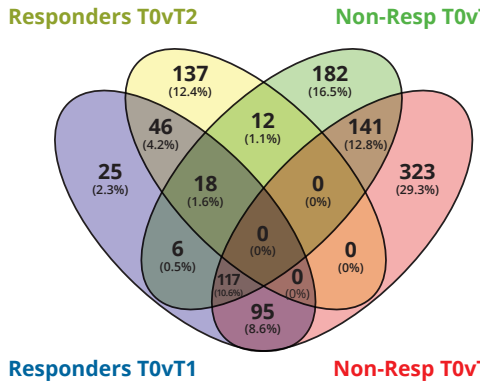

Figure 7. *141 genes were unique to nonresponders, and 46 genes were unique to responders.*

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