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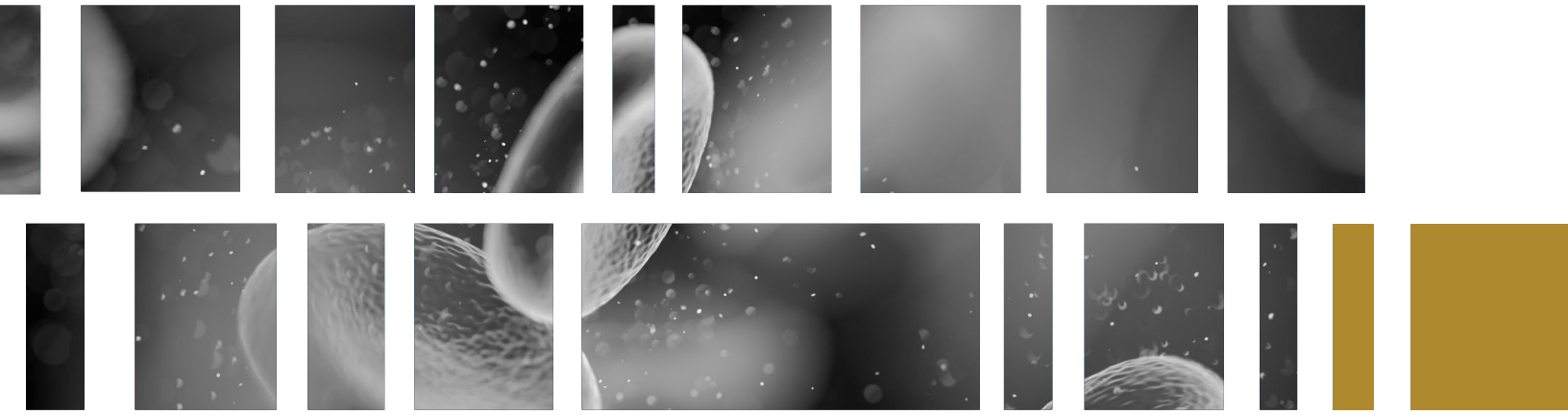

A WHITEPAPER FROM INSTITUTE@PRECISION

Exploring the Intricacies of the Immune System

A Practical Guide to Immune Monitoring Across Therapeutic Interventions

Darren Davis, PhD | *Senior Vice President*
Deborah Phippard, PhD | *Chief Scientific Officer*

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Introduction

Immune monitoring has become an essential strategy for understanding, predicting, and monitoring the response of the immune system to therapeutic interventions. Increasingly, researchers are integrating immune monitoring techniques into clinical trials to gain insight into mechanism of action, to select those patients who are most likely to respond or to have an adverse reaction to a therapeutic of interest, or to monitor treatment response or disease progression.

Key research immune-monitoring platforms—Cytokine Profiling, Cell-Based Immunophenotyping, Gene Expression, Sequencing, Circulating Tumor Markers, and Immunohistochemistry and Multiplex Immunofluorescence—are explored in this white paper to provide real-world insight into common questions researchers have about the practical application of these technologies.

Immune Monitoring in Immuno-Oncology

Breakthroughs in our understanding of cancer biology and the antitumor immune response have been the basis of paradigm shifts in oncology treatment over the past decade. However, there is still much to learn about how the immune system can be harnessed and unleashed to attack cancer without causing broad immunosuppression or triggering autoimmune disease.

To have the greatest impact on long-term survival, immuno-oncology therapeutics must be designed to address fundamental challenges in cancer treatment:

- There is a need for earlier diagnosis and approaches that implement biomarkers for distinguishing among potential responders, nonresponders, and those likely to experience serious adverse effects
- The development and expansion of resistance mutations limit the efficacy of chemotherapy and targeted therapies
- To date, immuno-oncology therapeutics have generally been effective with immunologically “hot” tumors that demonstrate homing of T cells and abundant T-cell infiltration. The ability to distinguish between hot and “cold” tumors—and ultimately the capability to turn cold tumors hot—is an area of active research
- The dynamic relationship between cancer and the immune system is complex, and tumor cells are adept at evading or suppressing the antitumor immune response. Therefore, immunooncology therapies are often given in combination with other moieties that help modulate the immune system, whether by expansion of the immune cell populations needed to make the immunomodulating treatment effective or by depletion/inactivation of regulatory cells

These challenges highlight the critical role of biomarkers at every stage of immuno-oncology therapeutic development. Given the trend toward use of immune system modulating therapies, immune monitoring is integral for shedding light on how the immune system responds to multiple treatment modalities and enabling a truly personalized approach to cancer treatment.

Immune Monitoring in Autoimmune Diseases

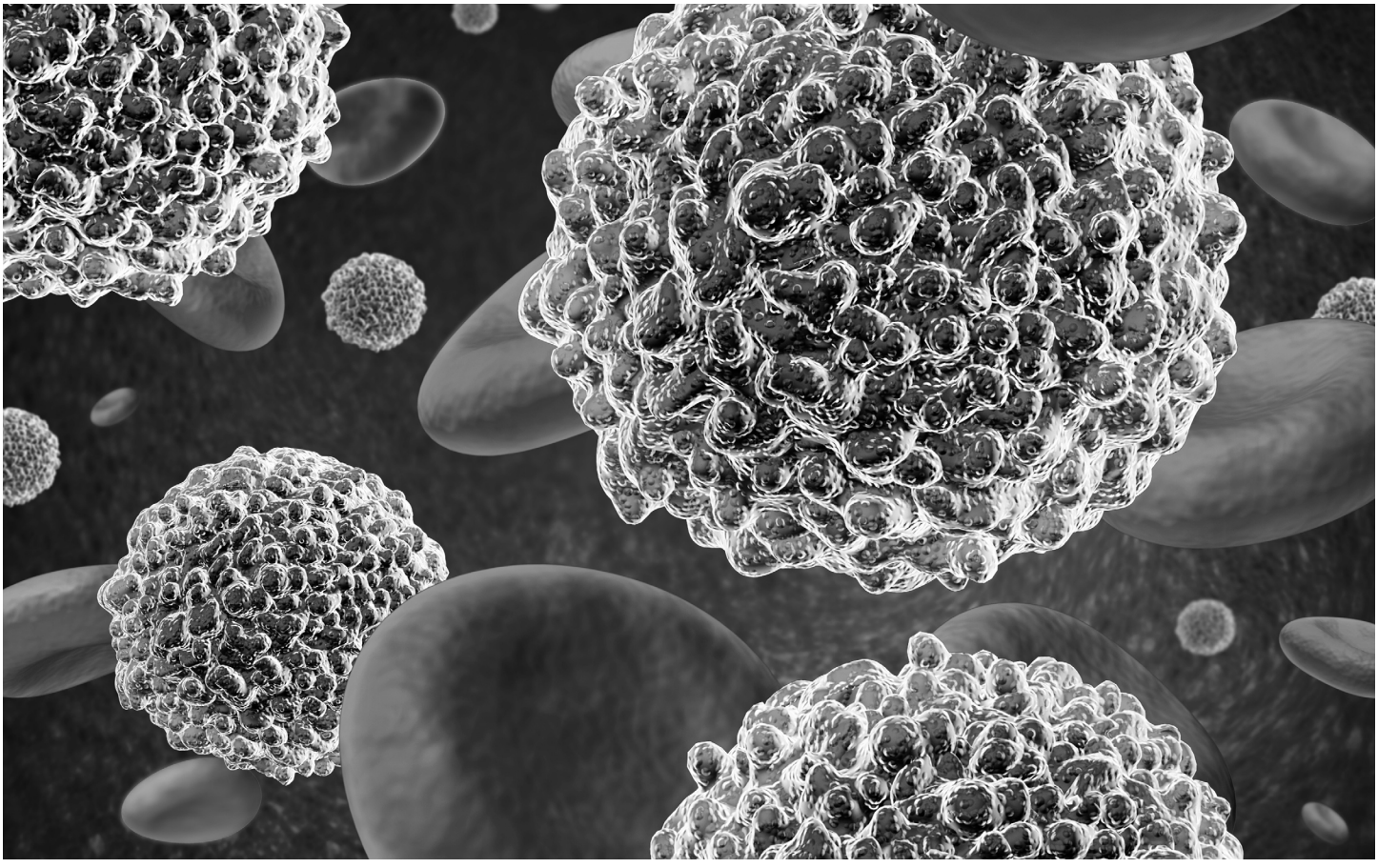
Failures of immune regulation are integral to the pathogenesis of autoimmune diseases, and immune monitoring can provide critical insights into disease etiology, progression, and treatment. There remains a substantial need to identify biomarkers specific to autoimmune disease, particularly ones that can be used clinically to monitor progression and therapeutic response.

In addition, there is a trend toward using the knowledge gained from testing immuno-oncology drugs to treat autoimmune diseases that target the same immune pathways. For example, chimeric antigen receptor (CAR) T cells can target autoantigens to suppress autoimmune responses, and immune checkpoints may be used to decrease the activity of the pathogenic T cells that drive autoimmunity.[1] Immune monitoring is essential for evaluating treatment response and assessing adverse events in patients treated with these therapies.

About This Guide

Advances in technology have led to an expansion of immune monitoring techniques, each with multiple applications. Utilizing the right technique on the appropriate sample type at the right time is critical for gaining biomarker insights needed to develop safe, effective immunotherapies.

In this guide, we explore key research platforms, including Cytokine Profiling, Cell-Based Immunophenotyping, Gene Expression, Sequencing, Circulating Tumor Markers, and Immunohistochemistry and Multiplex Immunofluorescence, and answer key questions researchers have regarding the utility of these technologies.



Assay Type	Utility
Cytokine Profiling	Assessment of inflammatory responses
Flow Cytometry	Immunoprofiling
EpiontisID **Epigenetic immune cell quantification**	Immunoprofiling
qPCR	Mutation detection Gene expression
ddPCR	Mutation detection Gene expression
NanoString	Gene expression MicroRNA analysis Copy number variation detection Spatial profiling of RNA and protein targets Immune profiling to determine hot/cold tumor status
RNA-Seq	Gene expression
NGS (DNA) Whole Exome Sequencing (WES) or Targeted Panels	Biomarker discovery Mutation detection
NGS (RNA) Whole Transcriptome Sequencing (WTS) or Targeted Panels	Gene expression Mutation detection Transcriptome profiling
CTC Analysis	Surrogate for tumor biopsy Use in downstream assays, such as multiplex immunofluorescence for protein detection or molecular assays for DNA/RNA detection
Immunohistochemistry + Pathologist Review	Protein expression Pharmacodynamics Tumor characterization Tissue assessment Diagnosis Patient stratification
Multiplex Immunofluorescence **Using Pre-developed or Custom Panels**	Protein expression Pharmacodynamics Tumor characterization Tissue assessment Diagnosis Patient stratification Assessment of tumor microenvironment Immune cell interactions Spacial profiling Tumor cell marker expression

Figure 1. Utility of Key Biomarker Research Tools

Cytokine Profiling

Cytokine profiling provides insight into immunological processes, such as the inflammatory response. It can also be used to measure disease progression or the immune response to therapy. There are several cytokine profiling platforms that can be used for measurement of cytokine release, diagnosis classification among disease stages, and therapeutic surveillance. At Precision for Medicine, we are experts in recommending an appropriate platform and format on the basis of sample volume, sample matrix, required dynamic range and sensitivity, and multiplexing needs.

Common Questions

Question 1: How does one select the most appropriate method for measuring cytokines in a clinical study?

This will depend largely on the number of analytes to be examined and whether a validated assay is required. If the data are to be used for exploratory end points, a validated assay is probably not necessary. However, if the data will be reported to the FDA (eg, in the investigation of a therapy associated with the risk of cytokine elevation or cytokine storm), a validated assay is required.

If the researcher is looking at one or two analytes, enzyme-linked immunoassay (ELISA) might be a good choice. ELISA is an economical option with good performance and allows for assay validation if needed.

If a multiplex solution is required, Meso Scale Discovery (MSD), Luminex, and Quanterix™ all offer multiplexing capabilities (Figure 2). MSD assays work on the same principle as do ELISA assays but use an electrochemiluminescent signal as the detection method. MSD is a good choice when a validated assay is required for clinical studies, but this platform can multiplex up to only 10 analytes. The Luminex platform uses fluorescently labeled beads bound to capture antibodies for quantitative detection of cytokines or other analytes of interest. Luminex can multiplex up to 100 analytes, and a single 96-wellplate run can be completed in approximately 45 minutes. Because of its high multiplexing capability, Luminex may be preferred in the exploratory stages of development when the target analytes have not yet been defined. The technology's manufacturer does not offer validated assays, but the assays can be validated if needed. Quanterix Simoa® is a specialized platform that uses digital bead counting to increase sensitivity. For researchers looking for lowlevel markers, such as markers of neuroinflammation using peripheral blood rather than cerebrospinal fluid, Quanterix would be the platform of choice.

Precision for Medicine offers all three platforms, and our experts are available to assist with selection of the appropriate technology for your clinical study.

	ELISA	MSD	LUMINEX®	SIMOA®
Sensitivity	ng/mL - pg/mL	pg/mL	pg/mL	fg/mL
Dynamic Range	2 logs	>4 logs	>4 logs	>4 logs
Multiplex Capabilities	1	10	50	6
Sample Volume	50 - 100 µL	25 µL	50 µL	1 - 100 µL
Use	All stages	All stages	Early stages	All stages

Figure 2. Comparison of Cytokine Profiling Capabilities

Question 2: Are there regulatory considerations that could impact one's choice of cytokine platform?

Yes. If the intent is to report data to the FDA (eg, primary or secondary end point assays), this will impact the technology selected for cytokine analysis. In this scenario, Luminex might not be an ideal choice, given that there can be significant lot-to-lot variation. This can be mitigated by, for example, using one lot of reagents for the entire study or asking Luminex to validate the assay and to provide lot-to-lot data. The latter would, of course, come with a budgetary effect. In addition, there is a high likelihood that the assay would also need to be validated by the laboratory performing the test.

For FDA reporting purposes, one would want to be able to validate the assay to GxP, the relevant good-practice quality guideline. ELISA and MSD are generally easier to validate, though it is possible to validate Luminex assays. The complexity of the validation protocol will increase with the number of analytes to be measured simultaneously. It is also worth considering that multiplexing comes with trade-offs—for example, if one is measuring many analytes, it is likely that the universal buffer chosen will not be optimal for all analytes, resulting in decreased sensitivity. In addition, before selecting a platform, one should check whether the platform provides GxP-validated software if one plans to use it for FDA reporting.

Question 3: How much sample volume needs to be collected for a cytokine assay, and does it need to be in a separate collection tube?

The blood volume available for biomarker testing is often limited, and the sample volume required will vary from assay to assay. It is also important to clarify the sample type (eg, serum or plasma) for which the assay one is using has been validated. Keep in mind that an assay that has been validated by the manufacturer for serum can usually be validated for plasma, but the laboratory will require time to perform that validation.

If one has an integrated translational approach to biomarker testing, one may be able to perform multiple assays from a single tube. For instance, peripheral blood mononuclear cells (PBMCs) and plasma can be isolated from the same tube. However, some commercial assays have been validated on serum, not plasma. Thus, it is important to understand what the downstream assays will be and what level of validation will be required on the basis of how the resulting data will be used and where they will be reported.

Cell-Based Immunophenotyping

Immune cell profiling has become a central part of immuno-oncology drug development. Insights gained from cell-based immunophenotyping can be used to optimize therapeutic design and treatment strategies and to monitor immune status, disease progression, and treatment response. While flow cytometry is the most-studied method of quantifying and typing immune cell populations, epigenetic immune monitoring has emerged during the past decade as a complementary approach.

Common Questions

Question 1: What is the optimal sample type for a flow cytometry assay?

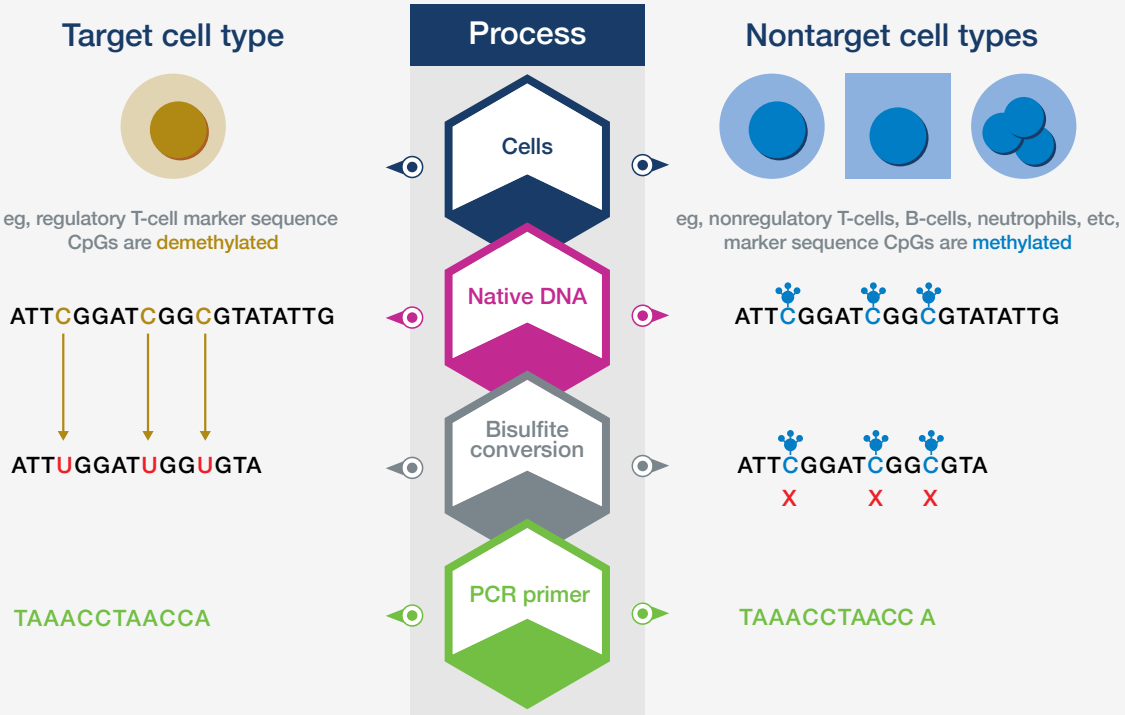
This generally depends on the analytes to be measured and the assay to be performed. Some cell types or markers (eg, CD62L) may be affected by the freeze/thaw process. Activated cells are often sensitive to delays in processing, and these are often the cells of most interest. If the researcher is looking for absolute counts per milliliter (mL) of blood (eg, in the case of a treatment that depletes one or more cell types), flow cytometry using whole blood and counting beads would give cell counts per mL. If the researcher is looking for subtle changes in populations over time, and markers are stable in cryopreserved cells, flow cytometry of banked cryopreserved samples will probably yield the most robust, accurate data. For measuring receptor occupancy, whole blood would be optimal given that the procedures for isolating PBMCs may reduce sensitivity as cells are washed and cryopreserved.

Question 2: Stabilization buffers are intended to preserve immunofluorescence staining of cells intended for downstream flow cytometric analysis. Do stabilization buffers actually work?

At Precision for Medicine, we have successfully run many whole blood assays by using blood that has been stabilized or partially fixed. We also maintain a library of markers that have been shown to be unaffected or minimally affected by stabilization buffers. Whether the stabilization buffer works— and the duration of that stability—would need to be tested for each marker of interest. Stabilization buffer technology for flow cytometry has come a long way, and newer products such as SMART tubes allow whole blood to be frozen prior to use in flow cytometry. In fact, at Precision for Medicine, we frequently use the SMART technology to support better stabilization and have successfully used SMART tubes for receptor occupancy assays.

Question 3: How does epigenetic immune cell quantification work?

Epigenetic immune monitoring uses highly cell type–specific methylation imprints at individual gene loci to distinguish immune cell types. Epigenetic modification of DNA by methylation is one of the mechanisms that control gene expression. Epigenetic immune cell quantification exploits these (de)-methylation phenomena for the quantitative assessment of immune cell populations by using quantitative polymerase chain reaction (qPCR)-based assays that provide highly reproducible data. A significant advantage of epigenetic immune cell quantification is that it is DNA based and can be applied to fresh, frozen, or paper-spotted dried blood and other bodily fluids or tissues. This eliminates the need for special care during sample storage and transport, which can streamline logistics and reduce overall cost. At Precision for Medicine, we offer a range of more than 30 prevalidated assays on our patented Epiontis ID epigenetic phenotyping platform and are continually adding new validated assays.



Legend. Epiontis ID identifies cell types through their epigenetic signature. With bisulfite, which converts unmethylated cytosines to uracils while leaving methylated cytosines intact, Epiontis ID uses specifically designed primers and qPCR to count target cells. qPCR results are largely operator independent, and assays can be completed rapidly, so Epiontis ID delivers fast, consistent results even across large, multisite trials.

Figure 3. Epiontis ID Identifies and Quantifies Cell Types by Detecting Epigenetic Changes in Cell Type–Specific Genes

Gene Expression

Genomic profiling provides opportunities for therapeutic target identification, optimized patient selection, refinement of immuno-modulatory therapies, and improved insight into immunotherapy response. Research has shown that gene expression profiling can be used to detect changes in the global transcriptome to provide insight into the activity or toxicity of immunotherapies. In addition, gene expression analysis of immune cells can reveal distinct therapy-induced pharmacodynamic changes.[2]

A meta-analysis of phase 2 clinical trials in diverse cancers revealed that personalized arms using genomic biomarkers demonstrated increased overall survival or relapse-free survival compared with protein overexpression assays.[3] In recent years, technologies such as NanoString and RNA-Seq have become powerful tools for both translational research and clinical trials. With NanoString, researchers have the capability to quickly analyze several hundred distinct gene or protein targets simultaneously. In addition, NanoString assays are compatible with low abundance samples like cell-free DNA isolated from liquid biopsies and low quality samples such as formalin-fixed paraffinembedded (FFPE) specimens.

Common Questions

Question 1: How should changes in gene expression be measured?

Measuring such changes generally depends on the number of genes of interest. If there are only a few genes of interest, qPCR or Droplet Digital™ PCR (ddPPCR) would be a good choice (Figure 4). If the expression level of a greater number of genes is required, NanoString would be recommended for up to 800 genes. NanoString is compatible with various sample types, including FFPE specimens, and does not require amplification, complementary DNA (cDNA) conversion, or library preparation steps.

NanoString offers a variety of panels, including oncology, immunology, and neuroscience, and these panels can be customized if needed. NanoString's PanCancer IO 360™ gene expression panel is a 770-gene CodeSet designed for profiling tumor biopsies and characterizing gene expression patterns associated with the tumor, the immune response, and the tumor microenvironment. Among the gene signatures contained in the panel is the Tumor Inflammation Signature which measures pre-existing peripherally suppressed adaptive immune responses in tumors.[4]

	Advantages	Disadvantages
qPCR	• Wide dynamic range • High throughput	• Lower precision when low-fold changes (<2-fold) need to be detected • Relies on the standard curve for absolute quantification
ddPCR	• Ability to detect small-fold changes (10%) • Can be used for direct quantification without a standard curve	Depending on the application and level of sensitivity required: • More costly • High-throughput limitations • Limited reagents
NanoString	• Multiplexing capability up to 800 distinct genes • Ability to directly detect RNA for accurate quantification • Compatible with low-abundance or low-quality samples • Availability of predesigned panels of 770 genes that can be customized with an additional 30 genes	• Not as sensitive as other PCR-based methods • Need to perform assay optimization for each tissue or species of interest • Less cost-effective for lower numbers of samples

Figure 4. Comparison of Technologies for Measuring Gene Expression

NanoString also offers the nCounter Vantage 3D™ RNA: Protein Immune Cell Profiling Assay, a highly multiplexed assay designed to quantitate mRNA and proteins using low levels of sample input. This assay can be used for identifying and quantifying immune cells, assessing immunological function and response to immuno-modulation, identifying and quantifying tumor-specific antigens, and performing longitudinal monitoring of response to immunemodulating cytokine treatments.[5]

If NanoString panels cannot cover all the genes of interest, targeted or whole transcriptome RNASeq can be used. RNA-Seq is a technology-based technique that uses next-generation sequencing (NGS) to isolate, sequence, and quantify the complement of RNAs in a given sample. RNA-Seq can detect subtle changes in expression (10%), identify novel transcripts, and produce absolute expression values.

Question 2: Is ddPCR more sensitive than qPCR?

Yes, ddPCR improves upon the sensitivity of qPCR by partitioning each sample into thousands of individual reactions or "droplets." After amplification, each droplet is analyzed for presence or absence of the target sequence and the absolute number of molecules present in the sample is calculated using Poisson statistical analysis. In addition to not requiring a standard curve for quantification, ddPCR provides the required sensitivity to quantify rare targets in complex backgrounds.

Question 3: How can distribution and persistence of a viral vector transgene/cell therapy be measured?

Generally, we would recommend ddPCR for this purpose as it demonstrates better performance than qPCR in terms of robustness and assay variance. One thing to keep in mind is the importance of sample preparation prior to PCR analysis.[6]

Sequencing

Sequencing enables the study of immunotherapy response factors, identification of biomarkers, and ability to personalize immuno-oncology treatments. It also contributes to the discovery and understanding of the processes involved in cancer cell proliferation, immune evasion, invasion, and metastasis. Targeted sequencing techniques such as NGS and RNA-Seq enable rapid identification of both common and rare genetic variations in specific genomic and transcriptomic regions of interest.

Common Questions

Question 1: What is the best way to look for mutations in a gene of interest?

The choice of technology will typically depend on the number of mutations or single nucleotide polymorphisms (SNPs) to be detected. In most cases, researchers are interested in a high throughput method for confirming known mutations and detecting novel variants through interrogation of hundreds or thousands of genes or gene regions. NGS, also known as massive parallel sequencing, can sequence millions of fragments simultaneously per run and has a high sensitivity for detecting low-frequency variants. One of the most important applications of NGS is to identify mutations by mapping NGS reads to a reference genome and detecting variations—including single nucleotide variants (SNVs), small indels, copy number variation (CNV), and structural variation—from the mapped data.

RNA-Seq leverages the advantages of NGS to detect and quantify known and novel variants, such as alternative splice sites, novel isoforms, and post-transcriptional modifications. This technology can be applied to a range of scientific questions, including gene expression profiling and identification of disease-associated SNPs and gene fusions.

Question 2: Should whole exome sequencing or targeted sequencing be used?

If the researcher is unsure of the genes of interest, whole exome sequencing (WES) may be preferred because it can help provide a comprehensive view of the genetic landscape by sequencing thousands of genes. If the researcher has a specific group of genes they want to study, targeted sequencing would be preferred because it lowers the cost and can achieve much deeper coverage by focusing on a small genomic region. This enables the identification of low-frequency variants and makes the resulting data sets more manageable.

	Key Features	Applications
NGS	• Sequences key genes or regions of interest to high depth • Identifies variants at low allele frequencies • Provides cost-effective findings and manageable data sets • Uses predesigned or custom panels that include genomic regions of interest	• Mutation detection • Disease diagnosis • Identification of mutation targets for prognosis or patient selection • Testing for tumor mutation burden (TMB)
RNA-Seq	• Offers ability to detect novel transcripts as it does not require species- or transcript-specific probes • Quantifies expression across a large dynamic range • Provides high specificity and sensitivity, especially for genes with low expression	• Variant detection • Identification of coding and non-coding transcriptional activities • Measurement of gene expression levels

Figure 5. Key Features and Applications of NGS and RNA-Seq

Question 3: Does Precision for Medicine have validated panels?

Yes, Precision for Medicine routinely runs panels that have been validated internally and by the manufacturer. For example, TruSight Oncology 500 (TSO500) performs comprehensive genomic profiling of tumor samples, which supports identification of relevant DNA and RNA variants implicated in various tumor types. In addition, TSO500 accurately measures key immunotherapy biomarkers, tumor mutation burden (TMB), and microsatellite instability (MSI).

This assay has been validated to:

- Detect mutations at 5% variant allele frequency (VAF)
- Accurately distinguish between somatic and germline mutations
- Determine TMB in FFPE tissue samples

Another example of a validated panel is the TruSeq RNA Exome assay for RNA-Seq, which features a highly optimized probe set that delivers comprehensive coverage of coding RNA sequences. TruSeq RNA Exome is compatible with RNA isolated from a variety of sources, including FFPE specimens and other low-quality samples. Data generated by this assay can be analyzed to provide detailed gene expression of the captured targets and identification of SNPs and transcript variants.

Liquid Biopsy Markers

Liquid biopsy-based biomarkers have generated considerable interest as they are minimally invasive, cost-effective and can be repeated longitudinally throughout follow-up.[7] There are a variety of liquid biopsy–based approaches, including soluble proteins, circulating tumor cells (CTCs), cell-free DNA (cfDNA), cell-free RNA (cfRNA), and exosomes or other vesicles transmitting proteins or nucleic acids.

Common Questions

Question 1: Why would one want to capture CTCs?

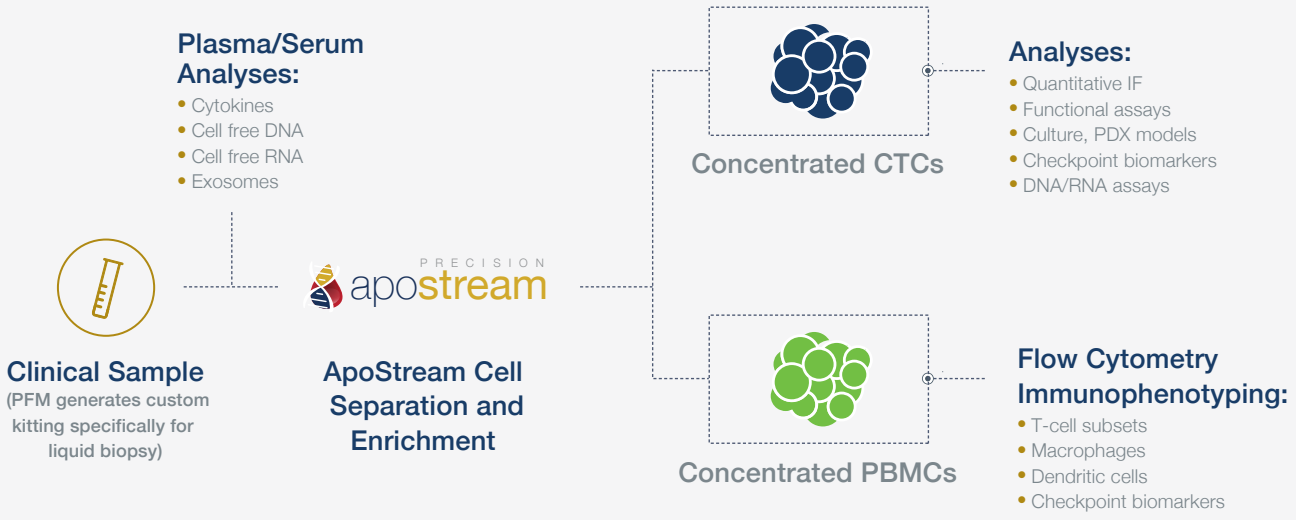
Due to the critical role they play in tumor dissemination and metastases, CTCs are an important clinical biomarker for cancer diagnosis and prognosis.8 As a surrogate for tumor tissue, CTCs can be used to evaluate protein expression, pharmacodynamic markers, mechanistic biomarkers, and drivers of tumor progression. CTCs can also be used to detect biomarkers, such as antigen expression, for patient stratification.

Question 2: Should circulating RNA or DNA or both be considered?

This generally depends on the strategy for the specific end point and the stage of the trial. cfDNA can be used to profile mutations or to align targeted therapies. cfRNA can be used upfront for profiling and over time for evaluating signatures of response using RNA-Seq. DNA and RNA can be extracted from viable CTCs and compared to cfDNA and/or cfRNA to determine changes in mutations over time and correlation with clinical outcome.

Question 3: What are the applications of circulating tumor markers in a phase 1 trial?

In phase 1 trials, circulating tumor markers can be used for selecting patients or evaluating pharmacodynamic markers or markers of resistance.



Legend. CTCs circulate at an extremely low frequency of approximately 1 in 1 billion cells. ApoStream isolates and enriches CTCs, facilitating any type of downstream analysis. ApoStream can also be used to isolate other rare cell types such as stem cells, progenitor and differentiated immune cells including CAR T cells, and other difficult-to-identify immune cell populations.

Figure 6. CTC Isolation and Enrichment Allows for Multiple Assays From a Single Tube

Immunohistochemistry and Multiplex Immunofluorescence

Immunohistochemistry (IHC) and immunofluorescence (IF) both utilize antibodies to provide visualization into protein abundance, distribution, and localization. Newer multiplexing approaches using these technologies can be used to help quantify immune cell subsets, their functional state, and their spatial arrangement and location within the tumor microenvironment.[9]

Common Questions

Question 1: Should one be using immunohistochemistry or immunofluorescence?

This depends on the scientific question being asked. IHC is useful when there is a single target or when using an existing or approved diagnostic such as PD-L1 in a clinical trial. One advantage of IHC over IF is that IHC staining is permanent so

IHC slides can be archived. Tissue architecture and morphology may also be more apparent in IHC stained samples. However, pathological analysis of IHC samples requires a board-certified pathologist for scoring.

If multiplexing is the priority, IF may be a better option. Multiplexing chromogenic IHC is often used for highlighting different, non-overlapping cell populations. However, IF is the method of choice for multiplexing, especially when co-localization is needed or more than 2 different antibodies are required.

Question 2: What are the advantages of multiplex immunofluorescence?

Multiplex IF allows for simultaneous detection of multiple target proteins in the same FFPE tissue section or even the same cell, while preserving the sample. Tissue multiplexing IF capabilities allow for the detection of up to 8 markers, providing information on biomarker expression levels and increasing the number of biomarkers that can be visualized simultaneously.

Question 3: What technology should be used to distinguish hot and cold tumors?

The ability to distinguish between "hot" and "cold" tumors—and ultimately the capability to turn cold tumors hot—is an area of active research.

Multiplex IF and image analysis can provide information on the spatial distribution and activation state of different immune cell populations within a sample. Using software to track each cell and its associated data, it is possible to explore the architectural context of the tumor microenvironment. This can help to distinguish hot and cold tumors and stratify patients for immunotherapy. In fact, there is increasing evidence that cell-to-cell topography and the resulting probability of cell-to-cell interactions can be correlated to clinical and prognostic parameters.[10]

Conclusion

In this guide, we have explored common questions about Cytokine Profiling, Cell-Based Immunophenotyping, Gene Expression, Sequencing, Circulating Tumor Markers, and Immunohistochemistry and Multiplex Immunofluorescence and provided insight into their utility in clinical trials. However, the application of these technologies will vary depending on the scientific question(s) being asked, phase of development, and the nuances of a particular study (Figure 7).

At Precision for Medicine, our philosophy is to offer multiple technology options for addressing a scientific question, thus allowing the investigator's requirements to drive selection of the optimal solution. Our goal is to maximize data generation with minimum sample requirements. The depth and breadth of expertise Precision offers goes beyond technical prowess—our scientists engage with innovative developers as a true scientific partner, committed to the best possible solutions for advancing medical research.

Phase of Development	Preclinical	Phase 1	Phase 2	Phase 3
• Primary objective(s of immune monitoring assays	• Research and development	• Safety • Dose • Mechanism of action • Exploratory end points • Patient enrollment	• Efficacy • Mechanism of action • Patient enrollment	• Correlation of biomarker with clinical response

Figure 7. Use of Biomarkers Throughout Therapeutic Development

Authors

Darren Davis, PhD

Darren Davis, PhD is a visionary leader with more than 25 years of distinguished biotechnology and clinical translational research experience. He founded ApoCell in 2004 and later was instrumental in developing and commercializing the ApoStream® rare-cell liquid biopsy technology. He is a globally recognized cancer researcher and the author of more than 100 peer-reviewed publications, dedicated and committed to improving the lives of patients with debilitating diseases.

Deborah Phippard, PhD

Deborah Phippard, PhD is a pharma industry veteran and expert at biomarker-driven clinical trial design and execution. She is a leader of biomarker and drug development programs for pharmaceutical and diagnostics companies, as well as the National Institutes of Health. Deborah has spearheaded the discovery of pharmacodynamic biomarkers and novel targets for inflammatory disease therapy.

At Precision for Medicine, we integrate clinical trial execution with deep scientific knowledge, technological and laboratory expertise, and advanced data sciences. This convergence of science, technology, and clinical trials— coupled with our regulatory and market access capabilities—enables us to engage as a partner throughout immunotherapy development, from translation to therapeutic approval.

References

1. Immunotherapies for autoimmune diseases [editorial]. *Nat Biomed Eng*. 2019;3(4):247. doi:10.1038/s41551-019-0394-3
2. Das R, Verma R, Sznol M, et al. Combination therapy with anti-CTLA-4 and anti-PD-1 leads to distinct immunologic changes in vivo. *J Immunol*. 2015;194(3);950-959. doi:10.4049/jimmunol.1401686
3. Schwaederle M, Zhao M, Lee JJ, et al. Impact of precision medicine in diverse cancers: a meta-analysis of phase II clinical trials. *J Clin Oncol*. 2015;33(32):3817-3825. doi:10.1200/JCO.2015.61.5997
4. Ayers M, Lunceford J, Nebozhyn M, et al. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. J Clin Invest. 2017;127(8):2930-2940. doi:10.1172/JCI91190
5. NanoString. Uncovering the tumor-immune system interaction using the nCounter Vantage 3D™ RNA: Protein Immune Cell Profiling Assay. Published September 2019. Accessed October 2, 2020. https://www.nanostring.com/download_file/view/820/3778
6. Dobnik D, Kogovšek P, Jakomin T, et al. Accurate quantification and characterization of adeno-associated viral vectors. *Front Microbiol*. 2019;10:1570. doi:10.3389/fmicb.2019.01570
7. Pantel K, Alix-Panabières C. Liquid biopsy and minimal residual disease—latest advances and implications for cure. *Nat Rev Clin Oncol*. 2019;16(7):409-424. doi:10.1038/s41571-019-0187-3
8. Agarwal A, Balic M, El-Ashry D, Cote RJ. Circulating tumor cells: strategies for capture, analyses, and propagation. *Cancer J*. 2018;24(2):70-77. doi:10.1097/PPO.0000000000000310
9. Taube JM, Akturk G, Angelo M, et al. The Society for Immunotherapy of Cancer statement on best practices for multiplex immunohistochemistry (IHC) and immunofluorescence (IF) staining and validation. *J Immunother Cancer*. 2020;8(1):e000155. doi:10.1136/jitc-2019-000155
10. Feng Z, Bethmann D, Kappler M, et al. Multiparametric immune profiling in HPV-oral squamous cell cancer. *JCI Insight*. 2017;2(14):e93652. doi:10.1172/jci.insight.93652

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