Those in the precision medicine field recognize that measuring a single-analyte is insufficient to capture the complexity of disease and accurately chart the best path of treatment. By way of example, the European Society for Medical Oncology (ESMO, 2017) highlighted the need for a multivariate approach to predictive diagnostics, citing that:

“PD-L1 IHC positivity [alone] is an imperfect biomarker of response and currently not suitable as a definite biomarker for selection for therapy with PD-1/PD-L1 inhibitors. It is likely that a more complex, multicomponent predictive biomarker system will be required to refine appropriate patient selection for PD-1/PD-L1 blockade.”

In 2019, this was reinforced in an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. The authors found that in the 45 FDA drug approvals across 15 tumor types from 2011 until April 2019

“PD-L1 was predictive in only 28.9% of cases, and was either not predictive (53.3%) or not tested
The need for multivariate, predictive biomarkers has led to new approaches that are enabling diagnostics to move from discrete, static measurements to holistic, multidimensional assays. But not only are new analyses available—these approaches allow clinicians to ask more comprehensive questions that test more than one hypothesis at a time.

Powered by the dynamic nature of RNA models and the utility of machine learning to identify useful signals and patterns, Predictive Immune Modeling untangles the inherent complexity of biology first in detecting immune response and then building biomarker assays for predictive diagnostics. With Predictive Immune Modeling, we are on track to develop our own diagnostic global position system (see inset on GPS model), and help clinicians better navigate a patient’s treatment path (see more details on the ImmunoPrism Assay at https://cofactorgenomics.com/immunoprism-assay-2/).

Case Study 1 – Calibrate Your Location

The first step in building any useful global positioning system is triangulating your location. How do we know we are where we think we are? In the diagnostic space, this reckoning is analogous to a technical or analytical validation.

In 2020, the first analytical validation of a Predictive Immune Modeling platform was published in the Journal of Molecular Diagnostics by the team at Cofactor Genomics.3 Here, the authors describe how to determine immune cell composition accurately in formalin-fixed, paraffin embedded (FFPE) tissue sections by building a database of immune Health Expression Models, or iHEMs (Figure 1). These iHEMs are made up of hundreds of RNA signals, which uniquely define an individual immune cell or state in the heterogeneous mixture of tumor tissue and the tumor microenvironment.

To illustrate the importance of multidimensional gene expression analysis of a complex tumor environment, consider the example of T-regulatory cells or T-regs, known to be important in tumor immune escape, and “often associated with a poor clinical outcome.”4 In most studies, the cellular marker FOXP3 is used to identify this cell population using flow cytometry or immunohistochemistry (IHC). Flow cytometry, on the one hand, is highly sensitive and specific at detecting populations of cells with multiple immune markers, but requires fresh tissue for analysis. Fresh tissue is difficult to acquire and store in a clinical setting, and FFPE archiving remains the recognized clinical standard. On the other hand, IHC, while suitable for FFPE tissue analysis in the clinic, only allows for the detection of single-analytes. Multiplex imaging technologies address this, but have yet to move from the research and translational setting into clinical practice.5

In this context, RNA-based approaches overcome the shortcomings of both flow cytometry and IHC assays; FFPE molecular protocols are now commonly used to quantify transcriptomic signals in a highly-multiplexed fashion. Notably, RNA-based diagnostics have already been shown to have utility in the clinic, with Oncotype DX6, Veracyte Affirma7, and Agenda MammaPrint8, specifically used in the oncology setting. While other software-based immune cell deconvolution methods have been reported on, they are powered primarily by public data.
sets – including microarray data – which is often very noisy. The team at Cofactor sought to build a new database and approach with a standardized molecular protocol to overcome historic challenges and build on the clinical use of RNA. New ways of leveraging RNA data to measure the complex immune system are necessary to address the needs of immune oncology; this publication marked the first example of moving beyond ranked gene lists, gene profiles, and gene signatures into machine learning-derived models.

From this need, immune Health Expression Models have emerged. In short, these RNA models were built using multiple examples of isolated immune cells from deep-sequenced, transcriptomic profiles of each immune cell type. Then, using machine learning technologies, multi-gene models of RNA expression levels that uniquely define each immune cell type are generated. In the previously cited Cofactor Genomics’ publication, Schillebeeckx et al use increasingly complex mixtures of known cells for an analytical validation of this approach using orthogonal technologies such as flow cytometry and IHC. They found that these models enabled the quantification of immune cells in heterogeneous tissue at the same detection level as flow cytometry, yet accessible for FFPE specimens. Bringing this back to our T-regulatory cell example, data (unpublished, internal Cofactor Genomics) comparing iHEMs to another platform that uses expression levels of only one gene to measure these immune cells (FOXP3) shows that the iHEMs are more accurate in quantification of known mixtures (Figure 2).

In itself, this is a significant step towards better characterizing the immune response at the site of the tumor. However, knowing our “location” with higher accuracy is only one step in building a clinical map or diagnostic.

Case Study 2 – Chart Your Route

In our precision medicine “GPS” paradigm, charting a path means enabling clinicians to use this molecular information to make more informed treatment decisions. Predictive Immune Modeling enables us to move from traditional single-analyte, ‘multigene’, or panel-based approaches, to true multidimensional biomarkers. In oncology, this means integrating multiple immune-related signals into a classifier. An example protocol for starting down this path of biomarker discovery to diagnostic development was published by Cofactor Genomics in the Journal of Visualized Experiments. Importantly, because the assay leverages RNA data, we are also able to measure and integrate known signals such as immune escape/co-inhibitory and costimulatory gene expression. In doing so, we build a more comprehensive and more accurate predictive model.

Step 1: Collect information from individual patients

First, we can use the iHEMs to quantify the immune composition of individual patients by analyzing RNA collected from patients’ solid tumor tissues. Other immune-related transcriptomic signals are also measured and reported for each patient (workflow summarized in Figure 3).

Step 2: Repeat for well-defined and well-annotated clinical cohort to identify and validate biomarker

Following recruitment of a well-defined and well-annotated cohort in a retrospective study, we can begin to build biomarkers or clinical decision tools with this data. For precision medicine in oncology, an example cohort would consist of responders and non-responders to immunotherapy. The signals in the individual patient profiles are considered alongside their clinical response data, and again using machine learning, a multidimensional biomarker is generated that marries together the most useful signals to uniquely define response (see process schematic Figure 4). The resulting biomarker may have predictive ability, reported as sensitivity, specificity, false positive, and false negative
Figure 4: The process of building a multidimensional biomarker relies on collecting immune profiling data from a well-defined patient cohort. This data is used in machine learning software to identify the combination of analytes that will result in the highest predictive value. The performance of the biomarker is evaluated, and then validated.

values (to be further validated with an additional patient cohort). At this stage, as we formalize the process in a framework such as that supported by CAP/CLIA, we begin moving from biomarker to laboratory-developed diagnostic (LDT) with documented procedures and reports.

Step 3: Deploy Diagnostic in Clinical Setting
Once the diagnostic test is validated, we are ready to deploy in the clinic, as shown in Figure 5. RNA from the tumor tissue of a newly diagnosed patient may be analyzed and the results compared to our validated multidimensional biomarker to provide guidance on whether the patient is likely to respond or not respond to a specific therapy, as determined in our retrospective studies.

Together, these steps take us from defining a map, to measuring a known location, to plotting the best course moving forward, akin to the process of programming a precision medicine GPS.

Case Study 3 – Providing Directions
Thus far, we have described the utility of a multidimensional predictive diagnostic in the immune oncology setting very generally. What is a specific example of where this technology might make a significant impact? When might a clinician look to predictive diagnostics to provide information to address a clinical question?

Consider the particular example of recurrent and metastatic squamous cell carcinoma of the head and neck (or RM-HNSCC). Immune checkpoint inhibitors (ICIs) are a class of immunotherapies that have shown promise for treating RM-HNSCC, as well as across multiple indications. The specific clinical question in this setting is whether to treat a patient with an ICI. The response to Pembrolizumab alone or with chemotherapy was evaluated in the KEYNOTE-048 study.12 Here, and for pembrolizumab therapy in general, the on-label diagnostic indication is once again PD-L1 IHC. In this study, the diagnostic is used to drive clinician confidence in prescribing pembrolizumab. If the patient’s tumor tissue shows high levels (≥20 combined positive score, or CPS) then the patient is considered to be a likely responder to pembrolizumab and therefore, this is the most promising clinical path. As we now know, this assay alone does not provide adequate characterization of a patient’s immune profile and has limited predictive ability. Many clinicians familiar with the study and the clinical data lack confidence that KEYNOTE-048 does not establish the measurement of a single analyte, PD-L1, as an adequate predictor of response.

As such, studies to integrate more immune markers into predictive diagnostics for this clinical decision have the potential to make an impact in patient outcomes and cost of healthcare. An unpublished economic study (contact author for detail) showed that in addition to greatly increasing confidence in predicting patient
The Future of Precision Medicine Relies on Predictive Diagnostics

As the future unfolds with benchtop sequencers and cloud computing growing in power and decreasing in cost, coupled with an expansion of virtual trials and study management, we anticipate that Predictive Immune Modeling studies will be launched for all immune-related diseases and therapy combinations. While the direct link to the immune system and the response to immunotherapies is obvious, there is growing evidence that shows other standard-of-care therapies including chemotherapy and radiation therapy induce and are influenced by immune responses that impact treatment outcome.15,16

At Cofactor, we believe in the power of Predictive Immune Modeling. We’re confident that the future of diagnostics requires the three facets of this new category including (a) predictive insights for confident treatment decisions, (b) a focus on immune response and markers, and (c) a holistic, multidimensional view enabled by machine learning. Using the approach outlined here, with RNA-based multidimensional biomarkers integrated into clinical diagnostics, the treatment decision-making paradigm can be streamlined while greatly expanding the information leveraged for each therapy decision. Predictive Immune Modeling is the key to closing the precision medicine gap that exists between powerful therapies, and the patients that will benefit most.17

References