Biomarkers associated with anti-PD-1/PDL-1 therapies:

Farcast has curated a universe of 549 biomarkers including differential gene/protein in the tumor microenvironment, mutations, epigenetic modifications, immune cell sub-populations, signaling pathway signatures and peripheral plasma factors. Of these, 379 are associated with response while 170 are associated with resistance to PD-1/PD-L1 therapy. This curated set of biomarkers is linked with 21 tumor histologies with melanoma being the most studied followed by lung cancer, breast cancer, renal cell carcinoma (RCC) and several others.

Response Biomarkers

Response biomarkers are classified into three major categories

1. Gene or protein level regulation: Gene mutations, epigenetic changes, transcriptional regulations, and post translational modifications
2. Immune cell sub-populations: T cell, B cell, Dendritic cell and macrophage
3. Tumor micro-environment response pathways : TCR clonality, gut microbiota, HPV, EBV positivity, chromatin alterations, secreted factors in peripheral blood etc

Response markers	# of unique biomarkers
Gene/protein	267
Immune sub-populations	44 predictive, 24 On-treatment response
Pathways	36

Farcast has identified 267 unique genes that drive response across multiple cancer types and the table summarizes the top 5 upregulated genes for both predictive and on-treatment response and the source of sample to be measured in :

Gene	Measured in
CD274	Tumor tissue
IFNG	Tumor tissue, serum, PBMCs, cell lines,
CD8A	Tumor tissue, BALF
CXCL9	Tumor tissue, BALF
GZMB	Tumor tissue, BALF, PBMCs

PBMC- Peripheral blood mononuclear cells| BALF- Broncho-Alveolar Lavage Fluid

To receive a comprehensive table of all 267 response associated genes, write to us at biopharma@farcastbio.com

Spatial contexture of anti-PD-1/PD-L1 response markers and their tumor indication specific prevalence are as important as the form (gene, RNA or protein) in which they are measured. Some examples are listed in the table below:

Gene regulation	Measured as	Spatial context	Tumor type	Treatment
4.1BB, CD274 (PD-L1), CD4, CD27, CD45RO, CD25, CD44, VISTA, GZMB, CD127, TIM3, CD8	Protein	High expression of T cell activation markers within TLS compared to outside of TLS was predictive	Melanoma, Renal cell carcinoma (RCC)	Nivolumab Nivolumab + Ipilimumab or Nivolumab + Bevacizumab
IDO1, CD3, CD8A, CD11c, HLA-DR, TIM3, CD20, B2M	RNA	High expression of these markers in tumor (S100+HMB45 melanocyte) compartment was predictive	Melanoma	PD-1/PD-L1 inhibitor
CD274 (PD-L1), CD8A, CD3, B2M, TIM3	RNA	High expression in macrophage (CD68+) compartment was predictive for response	Melanoma	PD-1 inhibitor/PD-L1 inhibitor

To receive a comprehensive table along with literature references, write to us at **biopharma@farcastbio.com**

Biomarkers conferring resistance to anti-PD-1/PD-L1 therapies.

Resistance biomarkers can be classified into three major categories;

1. Gene or protein level regulation: Somatic and germline gene mutations, transcriptional regulations and post translational modifications
2. Immune cell sub-populations: Lymphoid cell and myeloid cells
3. Tumor micro-environment response pathways: serum kynurenine/tryptophan ratio etc

Biomarker category for resistance markers	Number of unique biomarkers
Gene/protein	166
Immune cell sub-populations	26
Pathways	30

Farcast has curated 166 unique genes that cause resistance in different cancer types. Spatial contexture of markers conferring resistance to anti-PD-1/PD-L1 and their tumor indication specific prevalence are as important as the form (gene, RNA or protein) in which they are measured. Top five markers implicated in primary and acquired resistance are listed in the table below:

Genes	Regulation	Resistance Type	Measured as	Measured in	Tumor type
IL-8	Up	Primary/acquired resistance	RNA/protein	Tumor tissue, PBMC, plasma	Pan-cancer
HLA-A	Down	Primary/acquired resistance	RNA	Tumor tissue	Melanoma, Merkel cell carcinoma
TGF-β1	Up	Primary resistance	RNA	Tumor tissue, cell line	HNSCC, Urothelial / Bladder cancer
Beta-2 microglobulin	Down	Primary/acquired resistance	RNA	Tumor tissue	Melanoma, Lung cancer
HLA-B	Down	Primary/acquired resistance	RNA	Tumor tissue	Melanoma, Lung cancer, Merkel cell carcinoma

PBMC- Peripheral blood mononuclear cells

To receive a comprehensive list of all 166 resistance associated genes write to us at **biopharma@farcastbio.com**

Summary:
There are more than 250 biomarkers that are reported to predict response and over 150 that are associated with resistance to treatment with anti-PD-1/PD-L1 therapies. Spatial contexture of these markers vis-à-vis the cell/tissue compartment where it is expressed, the form (gene, RNA or protein) they are to be measured and the temporal order of expression in response to treatment are critical to evaluate response to treatment. The complexity caused by interplay between different signaling pathways, molecular targets, gene expression patterns, and stromal/immune spatial orientation makes it nearly impossible to associate response or resistance with any single biomarker on a single dimensional assay platform. Most of the existing preclinical models have a low predictive power precisely due to the failure to capture this complex response that is crucial in anticipating success of failure of any therapy.

TruTumor is a functional human Tumor Microdynamics platform that pools multiple signals from living near native human tumor microenvironments. Orthogonal datasets are powered by intelligent algorithms that combine gene signatures, protein expression, cytokine secretion as well as spatial orientation of tumor, immune and stromal compartments to predict response more accurately. Biosignatures developed in the translational phase, power not only better designed clinical trials, but also lead to superior personalized precision therapies that have the potential for far higher response rates in clinic.
Contact us at biopharma@farcastbio.com for a free no-obligation session to dive deep into Farcast exclusive datasets