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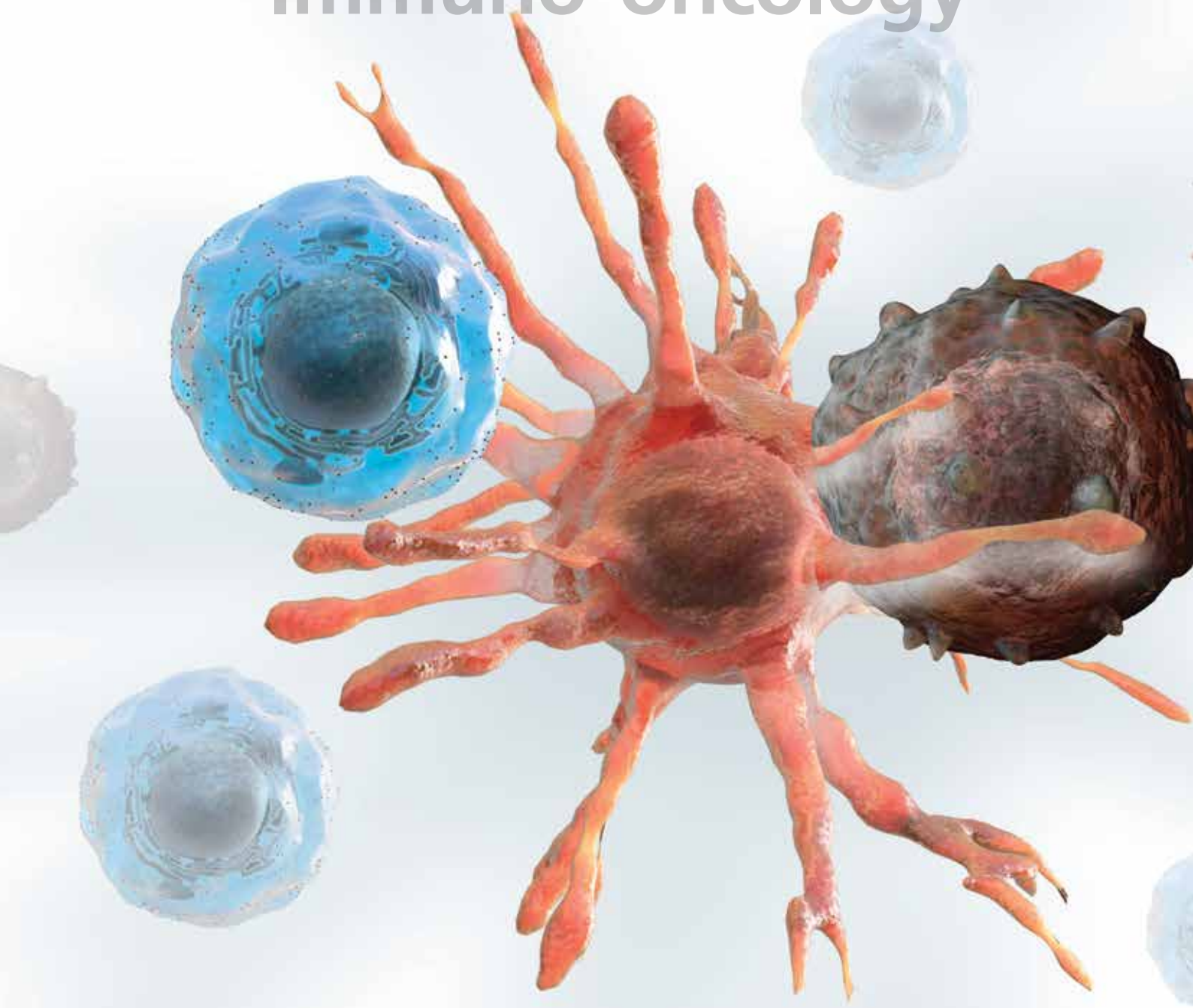
Biology for a better world

Knowledge wins

Take the 360° view

Immuno-oncology



eBioscience GeneChip USB

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Introduction

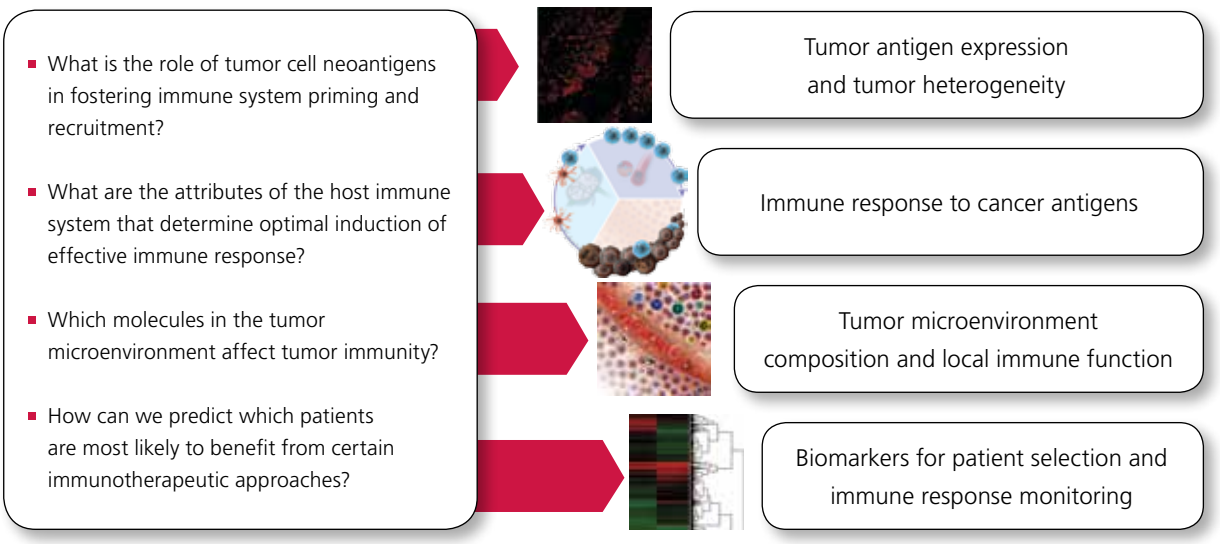
Immunotherapy of cancer: from vision to clinical reality

The immune system is an elegant and complex network that has evolved to protect the integrity of multicellular organisms against external threats such as viruses, bacteria, and parasites. Harnessing the power of the immune system to combat cancer has been a dream of visionary scientists and physicians for more than a century. Until recently, however, countless efforts in the laboratory as well the clinic have been unsuccessful, with very few exceptions.

In recent years, however, there has been dramatic progress in this field resulting from the advent of agents, primarily biologicals, able to direct and regulate T lymphocyte proliferation and function. Today, several antibodies targeting key regulators of T cell activation are approved as therapeutic agents by regulatory authorities around the globe, or are in late-stage clinical development, including compounds targeting CTLA-4 (ipilimumab), PD-1 (nivolumab, pembrolizumab), and PD-L1. These drugs have demonstrated significantly improved clinical outcomes for patients with metastatic malignant melanoma, non-small-cell lung cancer, and kidney cancer; encouraging results have also been reported from clinical trials in other cancer indications, including lymphomas. Importantly, checkpoint inhibitors are able to induce long-term durable responses and potential cures in a subset of patients.[1,2]

Nevertheless, not all patients treated with single agent checkpoint inhibitors experience clinical benefit. Efforts to further improve clinical outcomes for an expanded base of cancer patients include attempts to combine checkpoint inhibitors with established treatment modalities such as targeted kinase inhibitors, with other checkpoint inhibitors (e.g., ipilimumab plus nivolumab), or with cancer vaccines. These drug combinations appear promising, yet maintaining acceptable safety and tolerability still represents a major challenge.[1,2] In addition to these combination therapy approaches, novel T cell markers, such as TIM-1 and LAG-3, are also being explored as therapeutic targets. Significant additional clinical and translational research is required to discover new druggable targets and to elucidate markers on the tumor and in the tumor micro-environment which could ultimately serve as predictive biomarkers for treatment stratification.

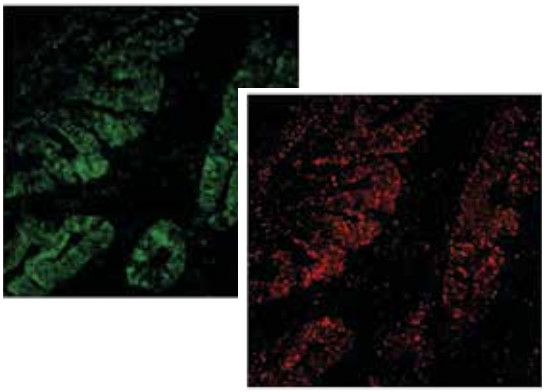
Key questions in immnuno-oncology[3]



Understanding how the immune system fights cancer and how cancer cells evade these attacks is the key to developing effective immunotherapies. A multi-omics approach providing a 360° view of tumor heterogeneity and the tumor microenvironment can yield a more comprehensive perspective contributing to clinical advances.

In this brochure, you will learn how Affymetrix can support your efforts in the field of immuno-oncology research with tools for cell-, protein-, and gene-level analysis. Together we can generate new insights.

Tumor heterogeneity



All malignant tumors are believed to be initially of monoclonal origin, harboring mutations in oncogenes and tumor suppressor genes.

As a tumor expands, it acquires additional genomic mutations and epigenetic aberrations. Occasionally, these mutations provide a survival advantage to individual cells and result in the outgrowth of more aggressive subclones. Genetic heterogeneity is therefore a characteristic feature of the majority of tumors at the time of diagnosis. Therapeutic intervention, whether by conventional chemotherapy, endocrine therapy, targeted agents, or immunotherapy, almost invariably results in the selective elimination of some cells and the resistance and continued proliferation of others. Understanding the plasticity of cancer genomes and of the tumor microenvironment represents the most important obstacle in the development of curative therapy.

Tumor microenvironment heterogeneity revealed by RNA flow cytometry

PrimeFlow® assay technology allows for the simultaneous detection of up to three RNA transcripts and protein in millions of single cells by flow cytometry. The assay employs the use of a proprietary fluorescent *in situ* hybridization (FISH) and branched DNA (bDNA) amplification technique. RNA detection may be combined with intracellular and cell surface antibody staining without laborious cell sorting, elevating your understanding of single-cell dynamics.

Flow cytometry is the method of choice for characterizing heterogeneous cell populations because it can simultaneously acquire and analyze millions of cells, adapts to multiplexing, and detects both intracellular and surface proteins. Nevertheless, it is constrained by the lack of antibodies to measure all analytes of interest. Non-coding RNA, messenger RNA, viral transcripts, unique model organisms, and troublesome targets for antibody development which remain elusive, have historically forced researchers to conduct numerous disconnected experiments to analyze these cellular systems.

A recent paper in Nature shows that the PrimeFlow® assay technology enabled the detection of a rare (0.04%-3%) B cell population in the microenvironment of castration-resistant prostate carcinomas in mice. Importantly, the presence of these IgA-expressing B plasmocytes was associated with resistance to oxaliplatin in three different models, and genetic or pharmacological depletion restored drug sensitivity. Of note, IgA-expressing B cells have similarly been observed in therapy-resistant and metastatic human prostate cancer.[4]

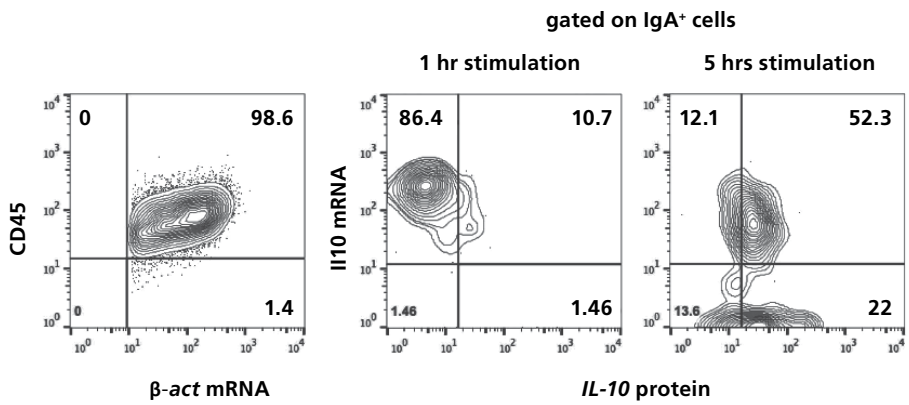


Figure 1: Flow cytometry analysis of Actb (β-actin) mRNA, IL10 protein and IL10 mRNA in tumor-infiltrating IgA⁺ cells using PrimeFlow® assay technology.

- Specific and rare (0.04–3% of total) B cell population in the tumor microenvironment correlates to an immuno-therapeutic response
- Mouse model of castration-resistant prostate cancer
- Compare kinetics of both RNA and protein in the same cell without laborious cell sorts

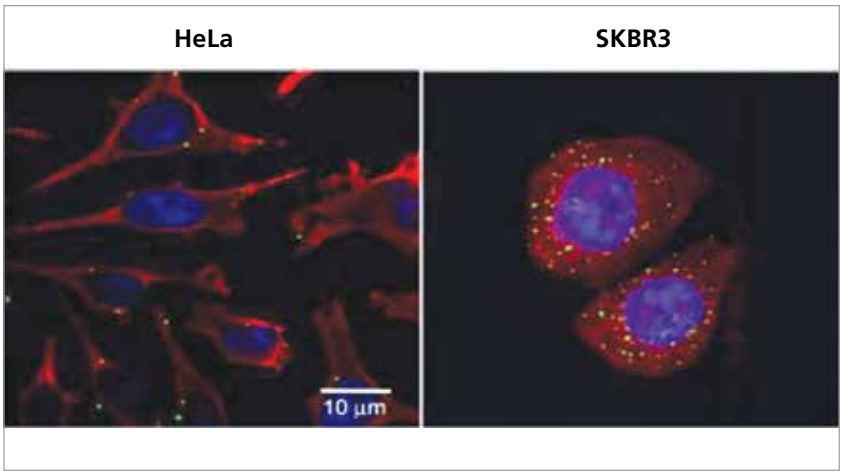
Reprinted by permission from Macmillan Publishers Ltd: ***NATURE***, Shalapour, *et al.*, Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy, **521**(7550):94–98 (2015).

Quantitation of expression heterogeneity with RNA FISH

Fluorescent *in situ* hybridization (FISH) is a powerful technique that allows specific visualization of RNA targets in fixed cells at single-cell resolution. Traditional FISH techniques that rely on oligonucleotides directly labeled with a few fluorophores are generally limited by high background and low detection sensitivity due to non-specific binding and insufficient signal-to-noise ratios.

ViewRNA® ISH Cell Assays incorporate a proprietary probe design and branched DNA (bDNA) signal amplification technology that results in excellent specificity, low background, and high signal-to-noise ratios, and makes ViewRNA ISH Cell Assay the most sensitive and specific RNA ISH method on the market. The assay enables simultaneous visualization of up to four RNA transcripts down to a single RNA molecule in single cells. Under equivalent imaging conditions, the ViewRNA ISH Cell Assay is 100 times brighter than traditional FISH, creating at minimum a 2–3 times higher signal-to-noise ratio.

A



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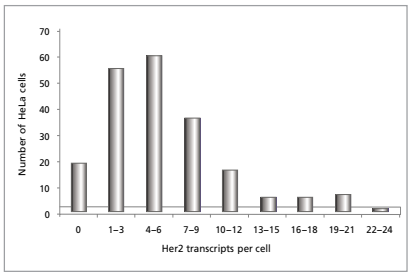


C

Assay	Ave. number of transcripts/cell
ViewRNA® Assay	5.4
QuantiGene® 2.0 Assay	6.4±2.3

Figure 2: Visualization and quantitation of transcript heterogeneity in cell lines

A: ViewRNA® Assay multiplex analysis of Her2 mRNA (green) and control 18S rRNA (red) in HeLa cells (left image) and SKBR3 cells (right image). Nuclei were stained with DAPI (blue) and visualized by fluorescence microscopy.

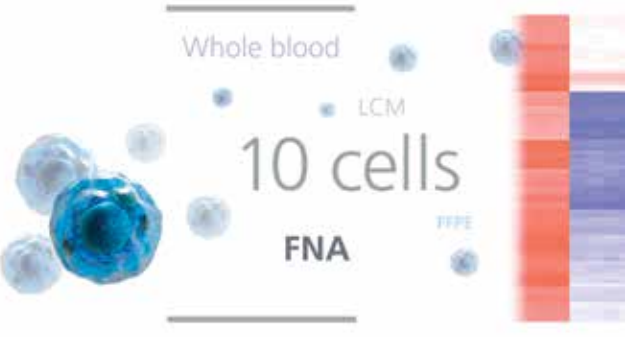
B: Histogram of Her2 expression per HeLa cell based on counting dots per cell for 200 cells.

C: Comparison of Her2 expression results using the ViewRNA® Assay for *in situ* transcript visualization vs. the QuantiGene® 2.0 Lysate Assay.

Whole-transcriptome profiling of very small samples

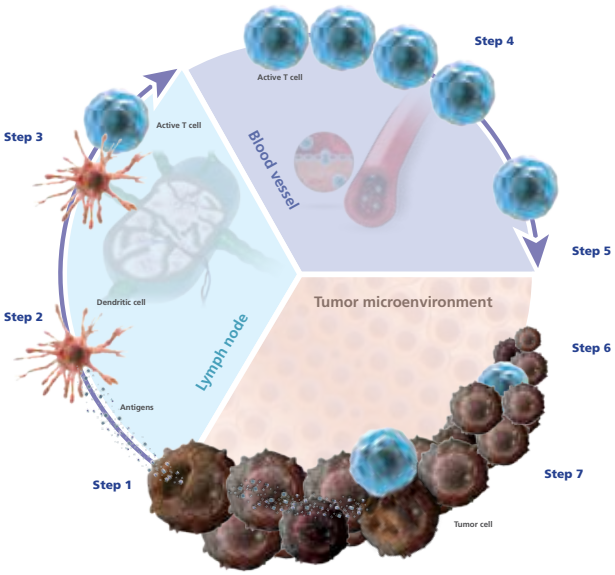
Traditional whole-transcriptome expression analysis depends on the examination of a large number of cells, which by definition will yield an average of expression signals masking potentially important differences between individual cells or subpopulations. To better understand subtle differences in gene expression and evaluate tumor heterogeneity, analysis of specific sub-populations is required. **GeneChip® WT Pico Kit** enables the measurement of a broad range of RNA expression changes from as few as 10 cells-worth of genomic material.

GeneChip® WT Pico Kit enables analysis of the whole-transcriptome from as few as 10 cells



Immune response to cancer antigens

First published in the journal *Immunity* in 2013, the Cancer-Immunity Cycle developed by Dan Chen and Ira Mellman is a model that describes a multistep series of immune events triggered by immunogenic cell death[5].



Step 1: Mutations in tumor cells result in the formation of tumor-derived antigens (TDA) that are released upon immunogenic cell death (ICD) and captured by dendritic cells.

Step 2: Dendritic cells (DCs) engulf and process the tumor antigens, mature and travel to the lymph node where they present TDA to T cells.

Step 3: DCs prime and activate T cells by presenting the TDA to naïve T cells, initiating the immune response against cancer cells; both effector and memory T cells are formed and enter the circulation.

Step 4: Primed and activated effector T cells expressing chemokine receptors are attracted to the tumor bed by a series of chemokines.

Step 5: Primed and activated effector T cells infiltrate the tumor tissue in order to attack the malignant cells.

Step 6: T cells recognize and bind to malignant cells; this interaction is mediated by T cell receptors and tumor antigen-derived peptides presented by MHC class I molecules.

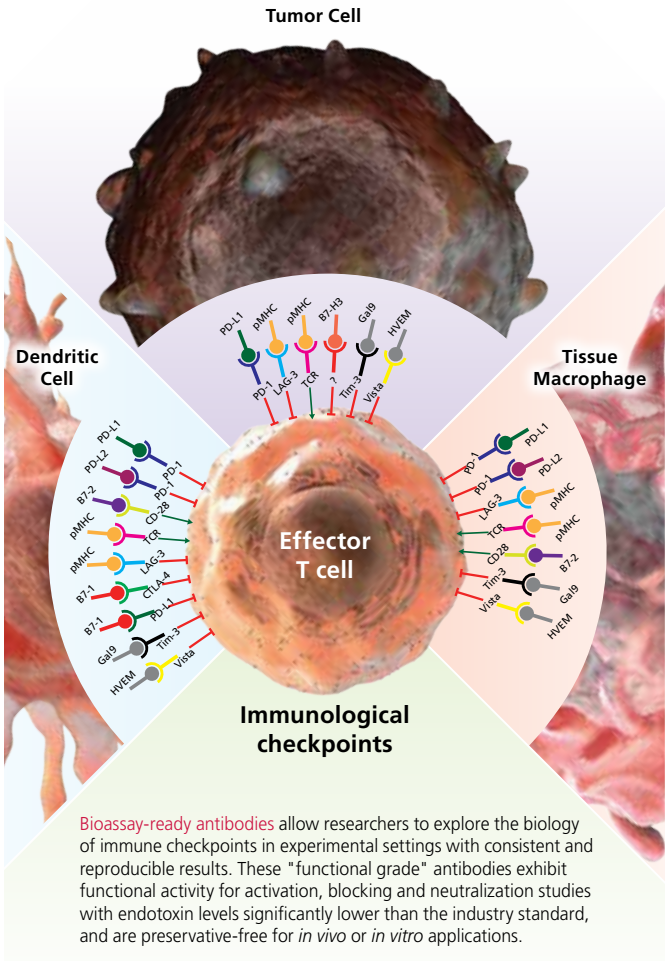
Step 7: Effector T cells destroy cancer cells and subsequently more tumor antigens are released. The cancer-immunity cycle begins again.

Adapted from Chen, D. S., *et al.* Oncology meets immunology: The cancer-immunity cycle. *Immunity* **39**(1):1–10 (2013).

Immunological checkpoints

Among the most promising approaches to activating therapeutic anti-tumor immunity is the blockade of negative regulators of the immune system, so-called immunologic checkpoints. Tumors exploit certain immune-checkpoint pathways as a major mechanism of immune resistance, particularly against T cells specific for tumor antigens. Because many of the immune checkpoints are initiated by interactions between membrane-bound ligands and receptors, they can be readily blocked by antibodies against these ligands or receptors.[6]

Antibodies against the checkpoint proteins CTLA-4 and PD1 have achieved regulatory approval for use in cancer therapy. Additional checkpoint regulators, including PD-L1, TIM-1, LAG-3, and BTLA have been discovered and the therapeutic utility of targeting these receptors and ligands is currently being explored. Clearly, many unresolved issues regarding the complex biology of T cell activation remain, and the full activity spectrum of checkpoint-blocking drugs, used alone or in combination, remains to be determined.[6]



Bioassay-ready antibodies allow researchers to explore the biology of immune checkpoints in experimental settings with consistent and reproducible results. These "functional grade" antibodies exhibit functional activity for activation, blocking and neutralization studies with endotoxin levels significantly lower than the industry standard, and are preservative-free for *in vivo* or *in vitro* applications.

Immune response monitoring

When evaluating immunotherapies, particularly in experimental settings, it is essential to monitor the immune response elicited or modified by the specific treatment. Immuno-monitoring delivers evidence of immunogenicity, guides the choice and dosage of antigens, assesses the effects of immune modulators and therapy combinations, and has the potential to reveal biomarkers of clinical efficacy.

Tumor antigen-specific $CD4^+$ and $CD8^+$ T cells, as well as regulatory T cells and myeloid-derived suppressor cells, are of particular interest. In addition, the role of cytokines activating or suppressing the immune response to a tumor is increasingly recognized.

Multicolor flow cytometry allows for simultaneous analysis of multiple cellular markers to assess phenotype, functional properties and quantification of cell subsets that are typically found at low frequencies in the peripheral blood or in the tumor microenvironment.[7]

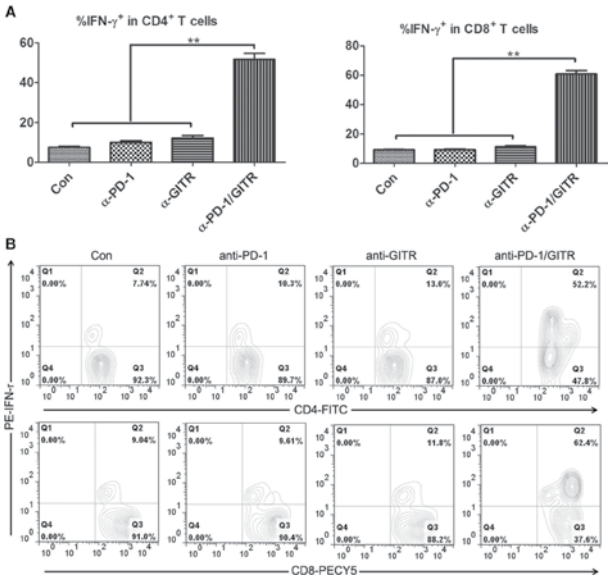


Figure 3: Immune response after combined PD-1 blockade and GITR triggering in a murine ovarian cancer model.
Functional analysis showed that significantly elevated frequencies of IFN gamma-producing cells were seen in tumor-associated $CD4^+$ and $CD8^+$ T cells from combined mAb treated mice (Figure 3A). Representative dot plots are shown in Figure 3B.[8]

Reprinted from Lu, L., *et al.* Combined PD-1 blockade and GITR triggering induce a potent antitumor immunity in murine cancer models and synergizes with chemotherapeutic drugs. *Journal of Translational Medicine* **12**:36 (2014).

Visualization of immune response

In situ hybridization allows for the visualization and quantitation of transcripts for any gene of interest. With this technique, it is possible to monitor cytokine induction at the single-cell level and analyze heterogeneity of cell populations using a one-day assay based on fluorescence rather than radioactivity.

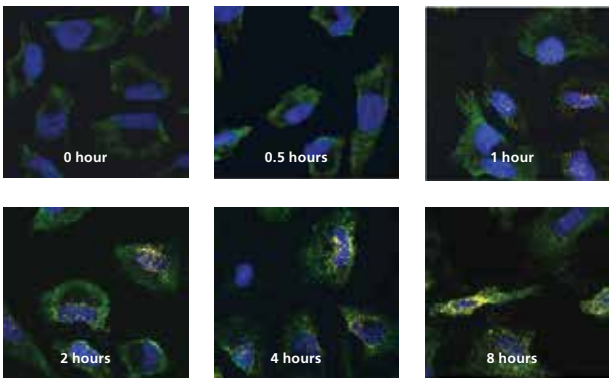
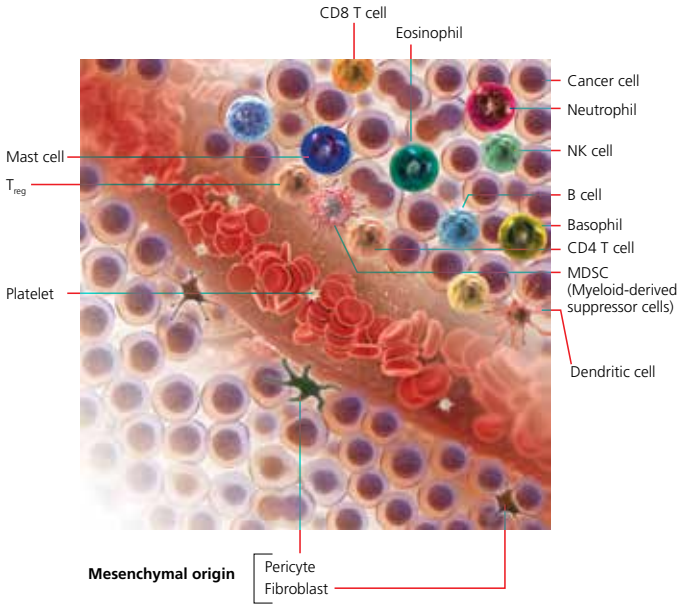


Figure 4: HeLa cells were treated with PMA for 0–8 hours. Cells were fixed and analyzed using **ViewRNA® ISH Cell Assay** for the induction of **IL-6** (red), **IL-8** (yellow), and **ACTB** (green) expression. Nuclei (blue) were stained with DAPI. The results show post-induction peaks for IL-6 at 0.5–2 hours and 8 hours for IL-8.

The tumor microenvironment

A solid tumor is a complex ecosystem composed of multiple cells performing a variety of functions. In addition to malignant cells, this ecosystem includes stromal fibroblasts, extracellular matrix proteins, multiple types of infiltrating leukocytes, as well as endothelial and perivascular cells from the blood and lymphatic systems. These players interact with each other by direct cell-to-cell contacts (such as co-stimulatory receptors on T lymphocytes and their ligands expressed by malignant or dendritic cells) as well as by secreted proteins and low-molecular weight compounds. This ecosystem, referred to as the tumor microenvironment, plays a crucial role in cancer progression, supporting tumor invasion, growth and the formation of metastatic lesions. In addition, the tumor microenvironment protects the tumor from host immunity and promotes resistance to therapeutic intervention.

Tumor microenvironment



Identification of immune cells by multiplex immunohistochemistry

Infiltration of tumors by leukocytes is an important step in the host response to cancer. Identification, characterization, and quantification of these populations of infiltrating immune cells has been limited by the small number of proteins that can be examined simultaneously within a single tissue section using standard colorimetric immunohistochemistry (IHC). The combination of multiple fluorophore-labeled antibodies overcomes this limitation and allows for simultaneous visualization of both classic cancer markers as well as a variety of immune cell antigens.

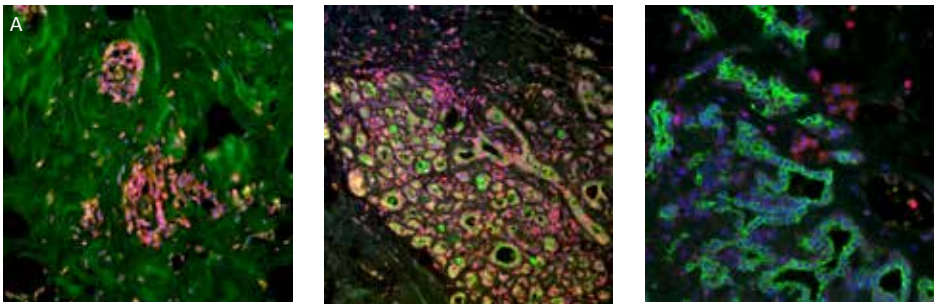


Figure 5: Identification of T and B cell populations in the tumor microenvironment.
Formalin-fixed paraffin embedded (FFPE) human breast cancer tissue (infiltrating ductile carcinoma (IDC) or ductile carcinoma *in situ* (DCIS)) was stained with 3 different eBioscience antibodies.

Numerous CD45RB-positive cells (NK, myeloid, T, and B cells) localize in clusters adjacent to duct regions. CD45RB-positive cells (red) are interspersed among pan-cytokeratin-positive (green) and HER-4 (ErbB4)-positive cells (orange). Nuclei are blue.

Gene expression analysis in the tumor microenvironment

Analysis of gene expression signatures enables characterization of the immune status within the tumor microenvironment and is conducive to longitudinal studies exploring the impact of immunotherapy on leukocyte subtypes, leukocyte activation, cytokine/chemokine expression, as well as the identification and evolution of other microenvironmental markers and tumor-associated antigens. Bead-based, multiplex assays for use on the Luminex® instrument platform enable sensitive PCR-free direct quantitation of RNA or DNA copy numbers from even the most troublesome samples.

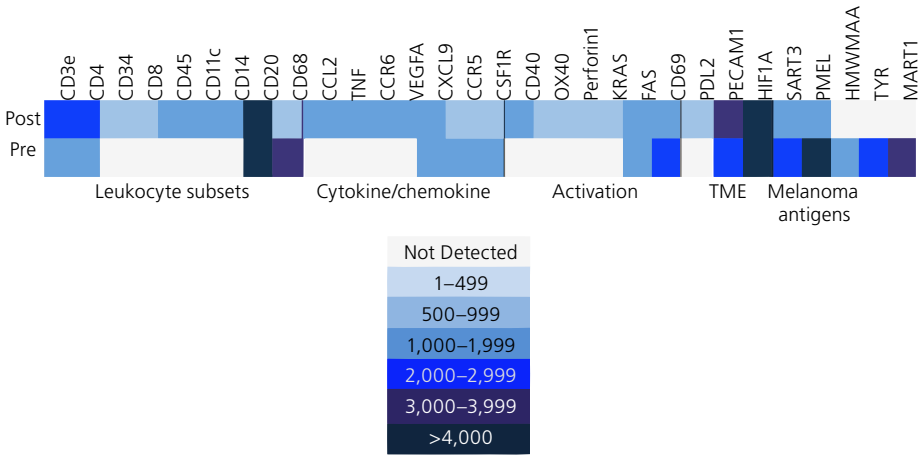


Figure 6: Relative gene expression in the tumor of a patient with melanoma before and after treatment with CD40 antibody CP-870.893.[9] Multiplex gene expression analysis was performed on FFPE-resected tumor samples collected before and after treatment. RNA quantification was performed directly from FFPE lysates without enzymatic amplification using branched DNA signal amplification via the **QuantiGene®** platform.

Reprinted from *Cancer Immunology Research*, 2014, **2**(11): 1051–1058, David L. Bajor, *et al.*, Immune activation and a 9-Year ongoing complete remission following CD40 antibody therapy and metastasectomy in a patient with metastatic melanoma, with permission from AACR.

Visualization of tumor-relevant markers using RNA *in situ* hybridization (ISH)

RNA transcripts of any gene can be directly localized at the cellular level using **ViewRNA® Assay** technology for RNA *in situ* hybridization. These assays, in contrast to conventional IHC, do not involve any protein-protein interactions. ViewRNA® Assays offer an alternative to IHC for detection of expression of protein-encoding genes and the unique option to detect targets for which antibodies suitable for IHC are not available, as well as transcripts of non-coding RNAs. In addition, these RNA ISH assays allow a precision view of cells expressing cytokines, chemokines, and other secreted proteins which is particularly useful since localization of secreted proteins by IHC is notoriously challenging due to diffusion of the target protein throughout the tissue.

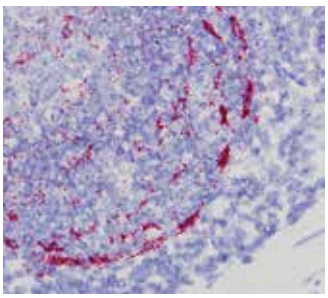


Figure 7: CXCL13 expression.
Reactive lymph node showing CXCL13 expression in parafollicular T cells.

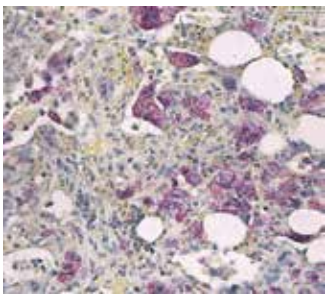


Figure 8: TGF-beta 1 expression.
Bone marrow biopsy showing TGF-beta 1 expression in megakaryocytes.

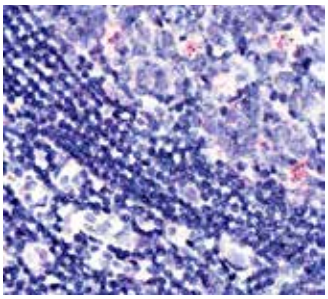


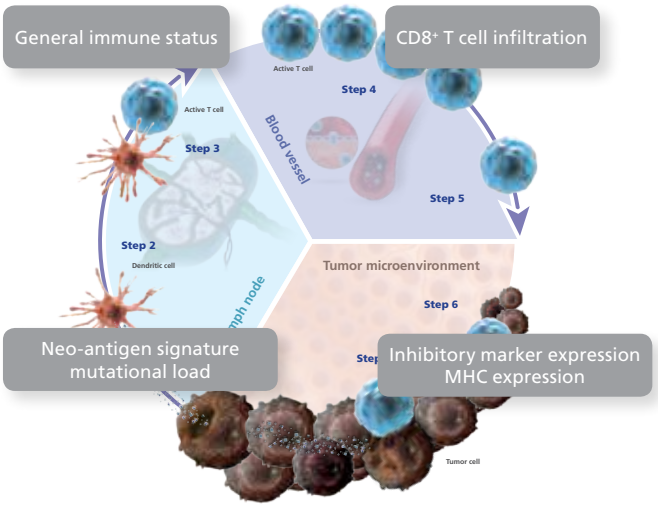
Figure 9: PD-L1 expression.
Reactive lymph node showing PD-L1 expression in antigen-presenting cells such as macrophages and dendritic cells.

Biomarkers in immuno-oncology

Although immunotherapy is likely to become a key part of the clinical management of cancer across multiple indications, biomarkers that can aid in patient stratification are still missing.

Biomarker targets across the cancer immunity cycle have recently been reviewed by Schumacher *et al.*[10] including

- Tumor-antigens recognized by T cells
- Markers measuring the immune status of the patient
- Quantification of cytotoxic T lymphocytes within the tumor microenvironment
- Expression of checkpoint molecules by malignant cells and tumor-infiltrating lymphocytes



Adapted from Chen, D. S., *et al.* Oncology meets immunology: The cancer-immunity cycle. *Immunity* **39**(1):1–10 (2013), according to Schumacher, T. N., *et al.* Biomarkers in Cancer Immunotherapy. *Cancer Cell* **27**(1):12–14 (2015).

Whole-transcriptome analysis and biomarker validation

Discovery of tumor antigens requires whole-transcriptome analysis of gene- and exon-level expression of both coding and long non-coding RNA as well as alternative splicing events. Sample size and quality of the available RNA samples, often partially degraded or derived from archival FFPE samples, may present technical challenges. Affymetrix human transcriptome array technology allows for global expression profiling from limited and often degraded nucleic acids derived from FFPE samples.

Das *et al.* (2015)[11] illustrated the power of **GeneChip® Transcriptome Array 2.0** to monitor transcriptional changes subsequent to therapy with antibodies to CTLA-4 and PD-1. Therapy-induced changes in gene expression, including alternative splicing, was observed in purified peripheral blood T lymphocytes and also in affected non-coding RNAs. Pathway analysis indicated that blockade of CTLA-4 mainly resulted in changes in cell proliferation in a subset of T cells, whereas PD-1 inhibition affected genes of relevance for cytolytic effector functions; interestingly, combination therapy resulted in changes in a much larger number of genes, including genes not affected by single-agent therapy.

As biomarkers that can predict immune response to treatment are identified, robust assays are needed to validate these biomarkers across a large set of samples in order to optimize clinical management. Expression signatures of immune response genes, for example, can be validated directly in FFPE tissue homogenates with **QuantiGene® Plex Assay**.[12]

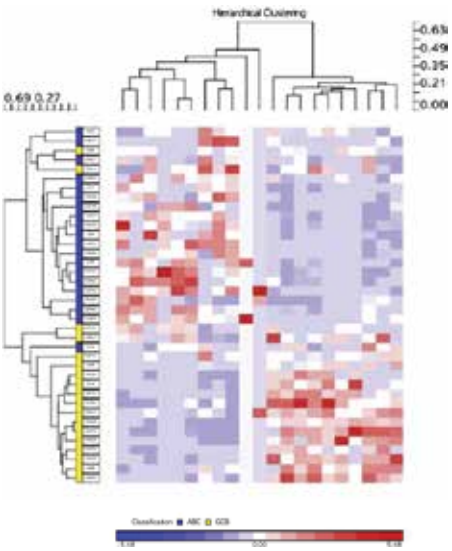


Figure 10: Cluster dendrogram and heatmap stratifying diffuse large B cell lymphoma samples.

Reprinted from: Hall, J.S., *et al.* QuantiGene® Plex represents a promising diagnostic tool for cell-of-origin subtyping of diffuse large B-cell lymphoma. *Journal of Molecular Diagnostics* **17**(4): 402-11 (2015).

Protein biomarker analysis using ELISA

Less than 25% of stage IV metastatic melanoma patients treated with the anti-CTLA-4 antibody ipilimumab benefit from this therapy, while the potential for serious side effects is a major concern and treatment is costly. Biomarkers to predict which patients may have the highest probability of benefiting from ipilimumab treatment are thus urgently required.

Speed, sensitivity, and specificity, as well as compatibility with standard clinical laboratory equipment, make immunoassays, and in particular ELISAs, the method of choice for biomarker evaluation. For example, quantification of soluble CTLA-4 (sCTLA-4) concentrations by ELISA could be a promising method to enrich for ipilimumab responders given that elevated levels correlate with clinical benefit (Figure 11, left), and patients with elevated sCTLA-4 show significant survival benefit compared to those with low sCTLA-4 levels (Figure 12, right).

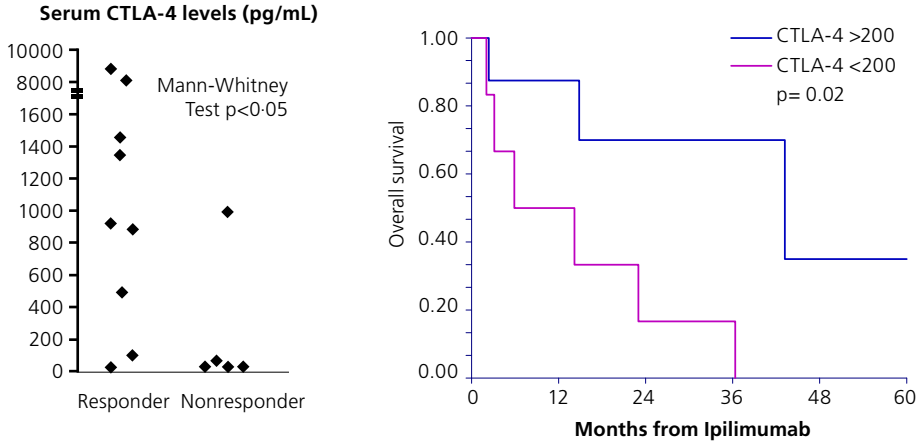


Figure 11 (left): sCTLA-4 levels were measured by ELISA and individual values plotted according to clinical responses.

Figure 12 (right): Overall survival (5 years) of patients treated with ipilimumab comparing those with greater than 200 pg/mL serum sCTLA-4 to those with less than or equal to 200 pg/mL.[13]

Reprinted from: Leung A.M., *et al.* Clinical benefit from ipilimumab therapy in melanoma patients may be associated with serum CTLA-4 levels. *Frontiers in Oncology* **4**:110 (2014).

Protein multiplexing for biomarker investigation

In addition to CTLA-4, PD-1, and PD-L1, several additional T cell activation checkpoint regulators have been identified, including TIM-3, LAG-3, and BTLA; a new wave of agents targeting these proteins is expected to enter clinical development in the near future. Soluble isoforms or shedded variants, detectable in serum, have been described for the majority of these checkpoint molecules, presumably functioning as immune adjuvants or decoy receptors and thus regulating the checkpoint pathways and potentially influencing the clinical efficacy of the respective drug candidates.

Broad analysis and quantitation of these soluble biomarkers may shed light on the biology of the respective pathways and may point to a characteristic responder profile of patients likely to benefit from treatment with individual drugs or drug combinations. In addition, multiplexed checkpoint protein analysis with assays like **ProcartaPlex® Immuno-oncology Checkpoint Markers Panel**, may be a useful tool for monitoring the immune status of patients during treatment with agonistic or antagonistic antibodies.

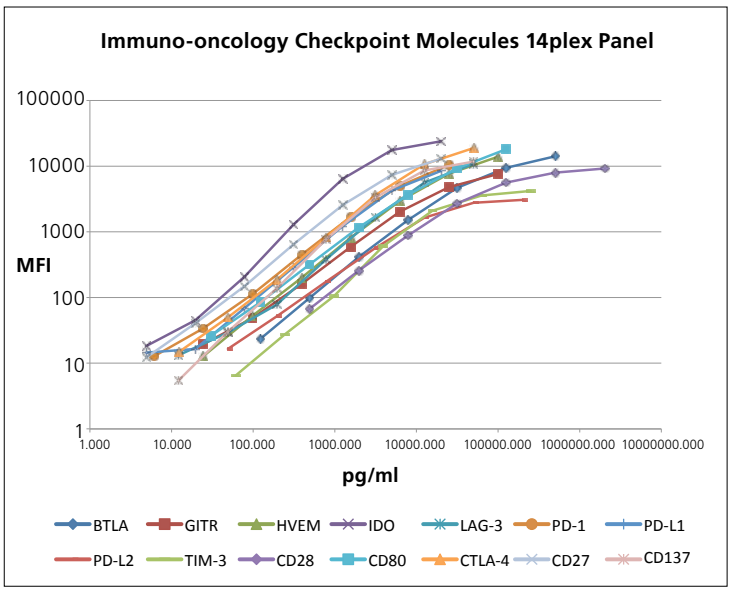


Figure 13: Standard curves for the human ProcartaPlex® immuno-oncology checkpoint 14plex panel.

Protein and RNA profiling from a single sample

A large number of cytokines and chemokines are involved in shaping local and systemic immune responses. Therefore, the potential for analyzing polarization in the cytokine repertoire or differences in patterns of their production by immune or tumor cells and relating them to a specific clinical response has tremendous appeal. Combining protein- and RNA-multiplexing technologies allows for simultaneous analysis of numerous proteins and RNA transcripts from a single sample and thus enables comprehensive characterization of the cytokine milieu in the sample of interest. Most importantly, the composition of the multiplex assays can be customized by selecting from our portfolio of more than 150 human protein targets (plus additional mouse, rat, canine, porcine, and non-human primate proteins) and any RNA transcript.

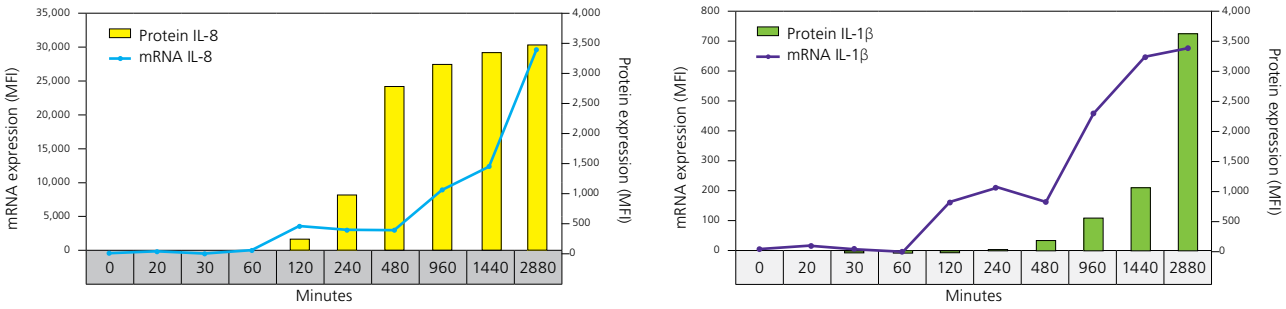
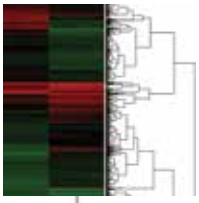


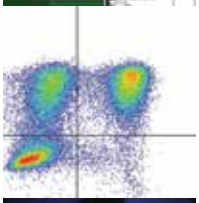
Figure 14: Analysis of 20 different cytokines; IL-8 and IL-1β are shown as examples.
Cell line: Human histiocytic lymphoma cells; U-937 treated with LPS
Samples: Cell culture supernatant samples and corresponding lysed cells
Assays: **ProcartaPlex® Multiplex Immunoassay Kit** and **QuantiGene® Plex reagent system**

Solutions for single-cell analysis

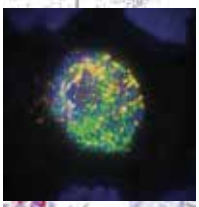
Expression



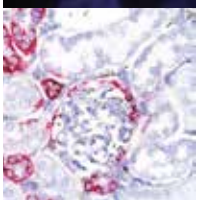
GeneChip® transcriptome-view pico assays
The simplest, most flexible, rapid, and cost-effective method to deliver the broadest measurement of transcriptome-wide expression changes **using as few as 10 cells**



PrimeFlow® RNA Assay
Simultaneous detection of RNA and protein expression in millions of single cells by flow cytometry

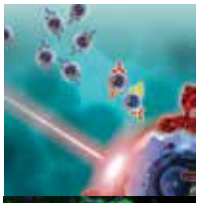


ViewRNA® ISH Cell Assay
RNA visualization with single-copy sensitivity and single-cell resolution

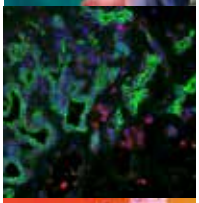


ViewRNA® Assays
Transcript visualization and quantification within the tissue microenvironment using manual or automated assays

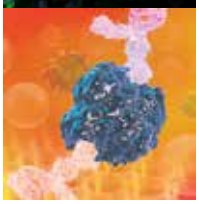
Protein



Flow cytometry antibodies
The broadest portfolio of fluorochrome-conjugated antibodies, supported by exceptional technical and panel-design support



Antibodies validated for IHC
Performance-validated antibodies for microscopy applications on cells, as well as frozen and paraffin-embedded tissues



Ready-Set-Go!® ELISPOT Sets
Evaluate cytokine production of individual cells

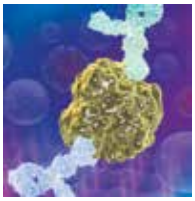
Solutions for multiplexing and ELISA

Protein and RNA multiplexing

ProcartaPlex® Multiplex Immunoassays
Choose from more than 300 assays for quantification of your proteins of interest

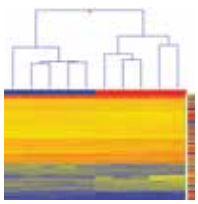
QuantiGene® Plex Assay
The highest throughput solution for quantitative multiplexed gene expression analysis, with no RNA purification needed

ELISAs



ELISAs
Multiple platforms for analyte assessment and biomarker profiling delivered by stringent development and quality-validation processes

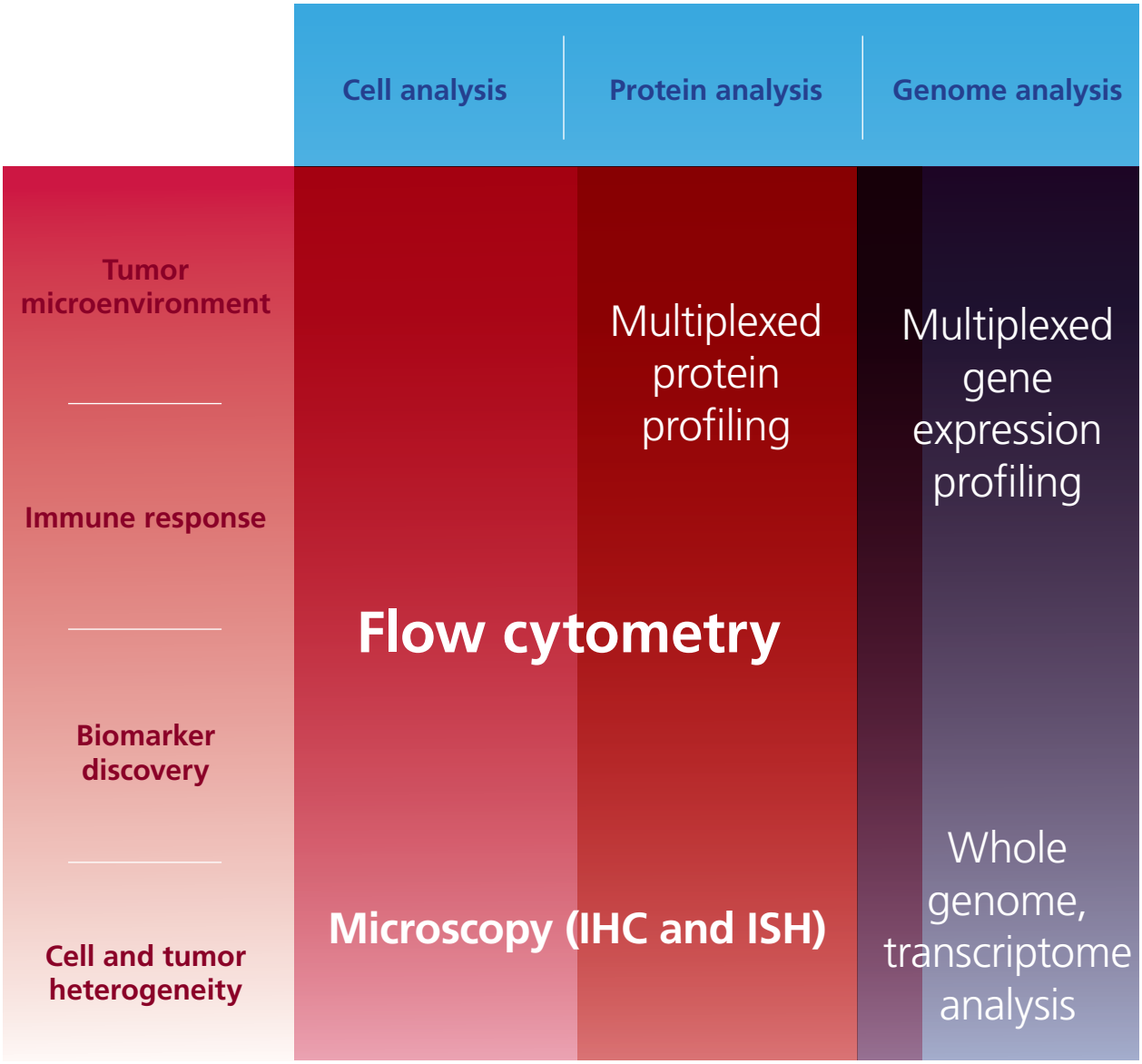
Genome-wide analysis



GeneChip® transcriptome-view assays
Delivering the most comprehensive view of the transcriptome

OncoScan® FFPE Assay Kit
Genome-wide copy-number analysis of solid tumors

Tools for a 360° view of immuno-oncology



Selected products

Antibodies for flow cytometry, immunohistochemistry (IHC), and bioassays

Selected antibodies for flow cytometry, immunohistochemistry (IHC), and bioassays																						Validated for IHC				
Antigen	Cat. No.	Purified	Functional Grade	Biotin	eFluor® 450	eVolve® 605	eVolve® 655	FITC	Alexa Fluor® 488	PerCP-Cyanine5.5	PerCP-eFluor® 710	PE	PE-eFluor® 610	PE-Cyanine5	PE-Cyanine5.5	PE-Cyanine7	Alexa Fluor® 532	APC	eFluor® 660	Alexa Fluor® 700	eFluor® 780	Purified	Functional Grade	Biotin	FITC	eFluor® 660
Anti-Canine CD28	0283		■																							
Anti-Canine CD28	0282																	■								
Anti-CD27	0271	■	■	■				■			■	■				■		■			■					
Anti-Human B7-H4	5949	■	■	■								■						■								
Anti-Human CD27	0279	■		■	■			■			■	■	■	■		■		■		■	■					
Anti-Human CD28	0288		■																							
Anti-Human CD28	0289	■	■	■	■			■		■		■	■			■		■			■	■				
Anti-Human CD40	0409	■	■		■			■			■	■				■		■				■				
Anti-Human CD80 (B7-1)	0809	■	■	■				■			■	■		■												
Anti-Human CD86 (B7-2)	0869	■	■	■					■		■	■		■		■										
Anti-Human CD112	1128																	■								
Anti-Human CD134 (OX40)	1347	■						■				■						■				■			■	
Anti-Human CD137 (4-1BB)	1379							■			■	■														
Anti-Human CD150	1509	■		■				■				■										■				
Anti-Human CD152 (CTLA-4)	1528											■														
Anti-Human CD152 (CTLA-4)	1529	■	■	■							■	■				■			■							
Anti-Human CD154 (CD40 Ligand)	1548	■	■		■			■			■	■	■			■		■			■					
Anti-Human CD155	1550										■	■														
Anti-Human CD160	1609								■			■							■							
Anti-Human CD223 (LAG-3)	2239				■			■			■	■	■			■		■								
Anti-Human CD244	2449	■	■																							
Anti-Human CD244	5838	■	■	■								■														
Anti-Human CD244	5837							■										■								
Anti-Human CD258 (LIGHT)	2589										■	■						■								
Anti-Human CD270 (HVEM)	5969	■		■								■														
Anti-Human CD272 (BTLA)	5979	■	■	■																						
Anti-Human CD273 (B7-DC)	5888	■	■	■							■	■						■								
Anti-Human CD274 (PD-L1, B7-H1)	5983	■	■	■	■						■	■				■		■				■				
Anti-Human CD275 (B7-H2)	5889		■	■								■						■					■	■		
Anti-Human CD276 (B7-H3)	2769																	■								
Anti-Human CD277	2779	■																								
Anti-Human CD278 (ICOS)	9948	■	■	■	■			■			■	■	■			■		■			■	■				
Anti-Human CD279 (PD-1)	2799	■		■							■	■	■			■		■			■	■				
Anti-Human CD279 (PD-1)	9989	■	■																			■				
Anti-Human CD279 (PD-1)	9969	■						■			■	■						■				■				
Anti-Human CD357 (AITR/GITR)	5875	■			■				■		■	■	■			■		■								
Anti-Human CD366 (TIM3)	3109		■		■			■			■	■				■		■								
Anti-Human DR3	6603	■		■								■														
Anti-Human IDO	9477										■	■							■							
Anti-Human IDO	9750	■																	■			■				■
Anti-Human TIGIT	9500		■								■	■				■		■								
Anti-Human TSLP Receptor	5499	■									■	■						■								
Anti-Human/Mouse CD27	0272	■	■																			■	■			

More than 5000 additional antibodies with detailed product descriptions can be found at **www.ebioscience.com**

Selected antibodies for flow cytometry, immunohistochemistry (IHC), and bioassays (Continued)																						Validated for IHC				
Antigen	Cat. No.	Purified	Functional Grade	Biotin	eFluor® 450	eVolve® 605	eVolve® 655	FITC	Alexa Fluor® 488	PerCP-Cyanine5.5	PerCP-eFluor® 710	PE	PE-eFluor® 610	PE-Cyanine5	PE-Cyanine5.5	PE-Cyanine7	Alexa Fluor® 532	APC	eFluor® 660	Alexa Fluor® 700	eFluor® 780	Purified	Functional Grade	Biotin	FITC	eFluor® 660
Anti-Mouse B7-H4	5970	■										■														
Anti-Mouse B7-H4	5972	■	■									■														
Anti-Mouse CD28	0281	■	■	■						■		■		■		■		■				■				
Anti-Mouse CD40	0401	■	■	■							■	■		■				■				■				
Anti-Mouse CD80 (B7-1)	0801	■	■	■	■			■			■	■		■		■		■				■				
Anti-Mouse CD86 (B7-2)	0861	■	■									■														
Anti-Mouse CD86 (B7-2)	0862	■	■	■	■			■				■		■		■		■				■				
Anti-Mouse CD134 (OX40)	1341	■	■								■	■				■		■				■				
Anti-Mouse CD137 (4-1BB)	1371	■	■	■	■						■	■				■		■								
Anti-Mouse CD137 Ligand (4-1BB Ligand)	5901			■							■	■														
Anti-Mouse CD150	1501	■	■	■				■				■						■								
Anti-Mouse CD150	1502				■			■			■	■				■		■								
Anti-Mouse CD152 (CTLA-4)	1521		■																							
Anti-Mouse CD152 (CTLA-4)	1522	■		■								■	■					■								
Anti-Mouse CD154 (CD40 Ligand)	1541	■	■	■	■						■	■				■		■								
Anti-Mouse CD155	1551																	■								
Anti-Mouse CD160	1601	■	■	■								■							■							
Anti-Mouse CD223 (Lag-3)	2231	■	■		■			■			■	■				■		■								
Anti-Mouse CD244.1	2440										■							■								
Anti-Mouse CD244.2 (2B4)	2441	■						■				■				■		■								
Anti-Mouse CD252 (OX40 Ligand)	5905	■	■									■						■				■				
Anti-Mouse CD270 (HVEM)	5962	■										■						■								
Anti-Mouse CD272 (BTLA)	5950	■	■	■							■	■														
Anti-Mouse CD272 (BTLA)	5955	■																								
Anti-Mouse CD272 (BTLA)	5956			■								■						■								
Anti-Mouse CD273 (B7-DC)	5986	■	■	■								■										■				
Anti-Mouse CD273 (B7-DC)	9972	■						■			■	■														
Anti-Mouse CD274 (PD-L1, B7-H1)	5982	■	■	■							■	■				■						■				
Anti-Mouse CD274 (PD-L1, B7-H1)	9971	■		■																						
Anti-Mouse CD275 (B7-H2)	5985	■	■	■								■							■							
Anti-Mouse CD276 (B7-H3)	5973	■	■	■								■														
Anti-Mouse CD278 (ICOS)	9940										■															
Anti-Mouse CD278 (ICOS)	9942	■	■	■				■				■		■		■										
Anti-Mouse CD279 (PD-1)	9982	■	■																							
Anti-Mouse CD279 (PD-1)	9985	■	■	■	■			■			■	■	■			■		■			■	■				
Anti-Mouse CD279 (PD-1)	9981	■		■	■			■			■	■						■								
Anti-Mouse CD357 (GITR)	5874		■	■	■			■			■	■				■		■								
Anti-Mouse CD365 (TIM1)	5861	■		■																						
Anti-Mouse IDO	9473										■								■							
Anti-Mouse PD-1H	5919		■								■															
Anti-Mouse TIGIT	9501										■	■							■							
Anti-Mouse TIM3	5870	■										■				■										
Anti-Mouse TIM3	5871	■	■	■	■							■						■								
Anti-Mouse TIM4	5866										■	■														
Anti-Mouse TSLP	5491		■																							
Anti-Mouse/Rat CD40	0402	■	■	■	■			■										■				■				
Anti-Mouse/Rat CD278 (ICOS)	9949	■	■	■				■				■		■		■		■				■				

More than 5000 additional antibodies with detailed product descriptions can be found at **www.ebioscience.com**

Selected antibodies for flow cytometry, immunohistochemistry (IHC), and bioassays (Continued)																					Validated for IHC				
Antigen	Cat. No.	Purified	Functional Grade	Biotin	eFluor® 450	eVolve® 605	eVolve® 655	FITC	Alexa Fluor® 488	PerCP-Cyanine5.5	PerCP-eFluor® 710	PE	PE-eFluor® 610	PE-Cyanine5	PE-Cyanine5.5	PE-Cyanine7	Alexa Fluor® 532	APC	eFluor® 660	Alexa Fluor® 700	eFluor® 780	Purified	Functional Grade	Biotin	FITC	eFluor® 660
Anti-Rat CD28	0280	■	■	■				■				■														
Anti-Rat CD80 (B7-1)	0800			■								■														
Anti-Rat CD86 (B7-2)	0860							■				■														
Anti-Rat CD134 (OX40)	1342																	■								
Anti-Rat CD152 (CTLA-4)	1520											■														

More than 5000 additional antibodies with detailed product descriptions can be found at **www.ebioscience.com**

Additional antibodies for IHC

Human immune cell antibodies											
Antigen	Clone	Cell Type*	FFPE Tissue	Frozen Tissue	Cat. No.	Purified	Biotin	Alexa Fluor® 488	eFluor® 570	eFluor® 615	eFluor® 660
Arginase-I	sl6arg	M/M	■		9779	■		■			■
CD3	UCHT1	T		■	0038	■					
CD4	N1UG0	T	■		2444	■	■		■		
CD4	RPA-T4	T		■	0049	■					
CD8a	AMC908	T	■		0008	■	■	■			■
CD8a	C8/144B	T	■		0085	■					
CD8a	HIT8a	T		■	0089	■					
CD11b (Mac-1α)	ICRF44	My		■	0118	■					
CD11b (Mac-1α)	LM2/1	My		■	BMS104	■					
CD11c	118/A5	My	■	■	9761	■	■		■		
CD11c	CBR-p150/4G1	My		■	BMS112	■					
CD11c	3.9	My		■	0116	■					
CD15	HI98	G	■	■	0159	■	■			■	
CD19	HIB19	B	■		0199	■					
CD20	L26	B	■	■	0202	■		■		■	■
CD25	GLLZDMY	B, T	■		0256	■					
CD45	CD45-2B11		■		9457	■	■				
CD45	HI30		■		0459	■					
CD45R (B220)	RA3-6B2	B	■	■	0452	■		■	■	■	■
CD45RB	PD7/26		■		9458	■		■			■
CD56 (NCAM)	5tukon56	NK, NKT	■		0565	■	■				■
CD56 (NCAM)	CMSSB	NK, NKT	■		0567	■	■				
CD57	TBO1	NK	■		0577	■					
CD68	815CU17	My	■		0687	■	■		■		
CD68	eBioY1/82A	My		■	0689	■					
CD68	KP1	My	■	■	0688	■					
CD74	VIC-Y1	M/M	■		0747	■					
FDC	CNA.42	F	■	■	9968	■	■				
Foxp3	236A/E7	Treg	■	■	4777	■	■	■	■		
Granzyme B	496B	NK, T	■		8889	■				■	
IDO	V1NC3IDO	My	■		9750	■					■
Macrophage	HAM56	M/M	■		6548	■					

*Cell type key: B = B cell; DC = Dendritic cell; F = Follicular dendritic cell; G = Granulocyte; M/M = Macrophage and Monocyte; My = Myeloid cell; NK = Natural killer cell; NKT = Natural killer T cell; T = T cell; Treg = Regulatory T cell.

Mouse immune cell antibodies												
Antigen	Clone	Cell Type*	FFPE Tissue	Frozen Tissue	Cat. No.	Purified	Biotin	FITC	Alexa Fluor® 488	eFluor® 570	eFluor® 615	eFluor® 660
CD3	17A2	T		■	0032	■		■				■
CD4	GK1.5	T		■	0041	■	■					
CD4	4SM95	T	■		9766	■	■			■		■
CD4	RM4-5	T		■	0042	■				■	■	
CD8a	53-6.7	T		■	0081	■	■				■	■
CD8a	4SM15	T	■		0808	■	■					
CD11b (Mac-1α)	M1/70	My		■	0112	■	■		■			■
CD11c	N418	DC		■	0114	■			■		■	
CD19	eBio1D3	B		■	0193	■						
CD45	30-F11			■	0451	■						
CD45R (B220)	RA3-6B2	B	■	■	0452	■			■	■	■	■
CD45RB	C363.16A			■	0455	■						
CD68	FA-11	My		■	0681	■						■
CD86 (B7-2)	GL1			■	0862	■						
CD170 (Siglec F)	1RNM44N	M/M		■	1702	■						
CD301b (MGL2)	11A10-B7	M/M		■	3011	■						
F4/80	BM8	M/M	■	■	4801	■				■		
Foxp3	FJK-16s	Treg	■	■	5773	■	■	■		■	■	■
Granzyme B	16G6	NK, T	■		8822		■					
Ly-6G (Gr-1)	RB6-8C5	M/M	■	■	5931	■						

*Cell type key: B = B cell; DC = Dendritic cell; F = Follicular dendritic cell; G = Granulocyte; M/M = Macrophage and Monocyte; My = Myeloid cell; NK = Natural killer cell; NKT = Natural killer T cell; T = T cell; Treg = Regulatory T cell.

Bioassay-ready antibodies and recombinant proteins

An extensive list of antibodies and recombinant proteins designed for optimal results when used in activation, neutralization, or blocking studies can be found at **www.ebioscience.com**

Immunoassays

ELISAs					
Antigen	Cat. No.	Ready Set Go!	Platinum	High Sensitivity	Instant
Human 4-1BB (CD137)	BMS289		■		
Human CD27	88-50370; BMS286INST	■			■
Human CD28	BMS290		■		
Human CD40	BMS265		■		
Human CD80 (B7-1)	BMS291INST				■
Human CD154 (CD40 Ligand)	BMS239; BMS293; BMS239INST		■	■	■
Human CD258 (LIGHT)	88-7258	■			

ELISAs (Continued)					
Antigen	Cat. No.	Ready Set Go!	Platinum	High Sensitivity	Instant
Human CD276 (B7-H3)	88-50370	■			
Human CTLA-4 (CD152)	BMS276		■		
Human OX40 (CD134)	BMS296		■		
Human PD-1*	BMS2214		■		
Human PD-L1*	BMS2212		■		
Human PD-L2*	BMS2215		■		
Human TSLP	88-7497	■			
Human TWEAK	BMS2006INST				■
Mouse TSLP	88-7490	■			

*Available soon

A complete listing of ELISAs can be found at **www.ebioscience.com**

ProcartaPlex® Multiplex Immunoassays		
Antigen/Description	Cat. No.	ProcartaPlex
Human 4-1BB (CD137)	EPX010-10289-901	Simplex
Human BTLA (CD272)	EPX010-12217-901	Simplex
Human CD27	EPX010-10286-901	Simplex
Human CD28	EPX010-10290-901	Simplex
Human CD80 (B7-1)	EPX010-10291-901	Simplex
Human CTLA-4 (CD152)	EPX010-10276-901	Simplex
Human GITR (CD357)	EPX010-12210-901	Simplex
Human HVEM (CD270)	EPX010-12218-901	Simplex
Human IDO	EPX010-12213-901	Simplex
Human LAG-3 (CD223)	EPX010-12211-901	Simplex
Human PD-1 (CD279)	EPX010-12214-901	Simplex
Human PD-L1 (CD274)	EPX010-12212-901	Simplex
Human PD-L2	EPX010-12215-901	Simplex
Human TIM-3 (CD366)	EPX010-12219-901	Simplex
ProcartaPlex® Human Immuno-Oncology Checkpoint Markers Panel	EPX140-15803-901	Multiplex

A complete listing of multiplex immunoassays can be found at **www.ebioscience.com**

Branched DNA gene expression products

All products are based on branched DNA technology. PrimeFlow® RNA Assay, ViewRNA® ISH Cell Assay and ViewRNA® Assay, as well as QuantiGene® Plex Assay are custom designed with your genes of interest.

To learn more about branched DNA technology, visit www.ebioscience.com

Whole-transcriptome analysis

GeneChip® transcriptome-view assays (arrays and reagents)		
Part No.	Description	Unit Size
902662	GeneChip® Human Transcriptome Pico Assay 2.0	Sufficient for 30 samples
902661	GeneChip® Human Transcriptome Pico Assay 2.0	Sufficient for 12 samples
902310	GeneChip® Human Transcriptome Assay 2.0	Sufficient for 30 samples
902309	GeneChip® Human Transcriptome Assay 2.0	Sufficient for 10 samples
900720	GeneChip® Hybridization, Wash, and Stain Kit	Sufficient for 30 samples

Genome-wide copy number analysis of solid tumors

OncoScan® FFPE Assay Kit provides a genome-wide view of DNA copy number variation that can provide insight into immunotherapy response pathways.

Genome-wide copy number analysis of solid tumors		
Part No.	Description	Unit Size
902293	OncoScan® FFPE Assay Kit	Sufficient for 24 samples
902305	OncoScan® Training Kit	Sufficient for 18 samples
902695	OncoScan® CNV FFPE Assay Kit	Sufficient for 24 samples
902693	OncoScan® CNV Training Kit	Sufficient for 18 samples

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