Immuno-Oncology: Identifying Next Generation Targets for Cancer Immunotherapy

T cell activation requires two signals: 1) recognition of the antigenic peptide/major histocompatibility complex (MHC) by the T cell receptor (TCR) and 2) antigen-independent co-stimulation induced by interactions between co-signaling molecules expressed on antigen-presenting cells (APCs) and their T cell-expressed receptors. In addition, T cell activation can be negatively regulated by co-inhibitory molecules present on APCs, which is crucial for maintaining self-tolerance and regulating the duration and magnitude of immune responses. Tumors frequently exploit these negative regulatory pathways, known as immune checkpoints, to evade the host’s immune system. As a result, researchers have focused on targeting these pathways for cancer immunotherapy using either agonists of co-stimulatory receptors or antagonists of inhibitory receptors to magnify the antigen-specific T cell response. Some of these studies have shown remarkable promise, but have also shed light on some significant obstacles that need to be addressed. For example, monoclonal antibodies directed against the T cell co-inhibitory receptors, CTLA-4 and PD-1 have been shown to have potent anti-tumor effects, but blocking CTLA-4 or PD-1/PD-L1 has proven to be effective in only a minority of patients. Furthermore, researchers have found that patients that do respond, can become resistant or relapse after an initial positive response due to an upregulation of other immune checkpoint pathways. As a result, additional immune checkpoint regulators that may serve as immunotherapeutic targets, either alone or in combination, are being sought. Many of these emerging targets also function as T cell co-inhibitory or co-stimulatory molecules, including members of the butyrophilin, CD2/SLAM, TIM, CD226, and TNF receptor families, along with additional members of the B7/CD28 families such as B7-H2/ICOS Ligand, B7-H3, B7-H4, and VISTA/B7-H5. Others, such as CD47/SIRP-α, Indoleamine-2,3-dioxygenase (IDO), LAG-3, leukocyte immunoglobulin-like receptors (LILR), CD94/NKG2A, and killer immunoglobulin receptors (KIR) are molecules commonly expressed by macrophages, dendritic cells, T cells, and/or natural killer (NK) cells that have also been found to be utilized by tumor cells to negatively regulate anti-tumor immune responses.

Members of the B7/CD28 Families of T Cell Co-Signaling Molecules have been Successfully Targeted for Cancer Immunotherapy

Co-stimulatory

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<td>B7-H1/PD-L1</td>
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<tr>
<td>2Ig B7-H3 (Mouse)</td>
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Co-inhibitory

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B7 and CD28 Family Interactions Regulate T Cell Activation. Interactions between B7 family proteins and T cell-expressed immune receptors belonging to the CD28 family (CD28, CTLA-4, PD-1, ICOS, and BTLA) can transduce both T cell co-stimulatory and co-inhibitory signals, as well as have bidirectional effects (indicated by the two-headed arrows). Tumor cells frequently overexpress B7 family proteins that negatively regulate T cell activation, allowing them to inhibit anti-tumor immune responses. As blocking antibodies against CTLA-4, PD-1, and PD-L1 have been shown to improve the immune response in multiple different cancer models, additional molecules belonging to the B7 and CD28 families are being investigated as targets for cancer immunotherapy.
Several Members of the Butyrophilin, CD2/SLAM, TIM, CD226, and TNF Receptor Families Regulate T Cell Co-Signaling and are being Investigated as Next Generation Targets for Cancer Immunotherapy. In addition to members of the B7/CD28 families, molecules belonging to several other families including members of the butyrophilin, CD2/SLAM, TIM, CD226, and TNF receptor families, have also been shown to regulate T cell activation. As a result, these molecules are being investigated as potential next generation immuno-oncology targets.

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Multiple Additional Molecules that Regulate the Activities of a Variety of Other Immune Cell Types are also being Investigated as Emerging Cancer Immunotherapy Targets. In addition to the molecules that regulate T cell activation, multiple molecules involved in regulating the activities of a variety of other immune cell types are also being investigated as potential immuno-oncology targets. These include members of the killer immunoglobulin-like receptor (KIR) family and the leukocyte immunoglobulin-like receptor A/B (LILRA/B) family, CD94-NKG2 heterodimeric receptors, CD47/SIRP-α, Indoleamine-2,3-dioxygenase (IDO), and LAG-3. The KIR family and CD94-NKG2 heterodimeric receptors are both primarily expressed by natural killer (NK) cells, while both the immune stimulatory and inhibitory LILRA/B receptors are expressed by a much wider range of hematopoietic cell types, including monocytes, macrophages, dendritic cells, T cells, NK cells, B cells, and granulocytes. SIRP-α and IDO are primarily expressed by macrophages and dendritic cells. LAG-3 is expressed on regulatory T cells (Tregs), along with CD8+ T cells. LAIR-1 is expressed by NK cells, B cells, T cells, and dendritic cells. The effects of each of these molecules on the different immune cell types is indicated in the figure. The domain key for the structures is located on the previous page.
## Products for Studying Immuno-Oncology Targets

### Recombinant Proteins

R&D Systems offers an unparalleled selection of recombinant proteins that can be used to characterize the effects of immuno-oncology targets. Our stringent production and purification standards ensure that R&D Systems® proteins will provide researchers with industry-leading bioactivity, low endotoxin levels, and lot-to-lot consistency. In addition to our standard recombinant proteins, we also offer Animal-Free™ and Animal Component-Free Process recombinant proteins, GMP-grade recombinant proteins, and custom protein development services.

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### Killer Immunoglobulin-like Receptors (KIRs)

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Learn more | rndsystems.com/proteins
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**Y-set and Immunoglobulin domain-containing (VSIGs) Family**

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**BTN1A1 Inhibits Anti-CD3-Induced IL-2 Production by Human T Cells.** Human T cells were incubated with an immobilized Mouse Anti-Human CD3ε Monoclonal Antibody (Catalog # MAB100; 1 µg/mL) and the indicated concentrations of Recombinant Human BTN1A1 (Catalog # 8467-BT). IL-2 secretion was measured in cell culture supernatants using the Human IL-2 Quantikine® ELISA Kit (Catalog # D2050). The ED_{50} for this effect is typically 0.5–2.5 µg/mL. Recombinant Human BTN1A1 (Catalog # 8467-BT; 1 µg/lane) was assessed by SDS-PAGE analysis under reducing (R) and non-reducing (NR) conditions and visualized by silver staining (inset).

**VSIG-3 Inhibits Anti-CD3-induced Human CD3ε T Cell Proliferation.** Human T cells were incubated with an immobilized Mouse Anti-Human CD3ε Monoclonal Antibody (Catalog # MAB100; 1 µg/mL) and the indicated concentrations of Recombinant Human VSIG-3 (Catalog # 4646-VS) for 72 hours. Cell proliferation was assessed by a fluorometric assay using the redox-sensitive dye, Resazurin (Catalog # AR002). IgG-Fc controls did not alter anti-CD3-induced CD3ε cell proliferation (data not shown).
R&D Systems offers multiple anti-mouse and anti-human antibodies for blocking/neutralization of first generation immuno-oncology targets and emerging targets. In addition, we offer a wide selection of unconjugated and fluorochrome-conjugated antibodies that are qualified for flow cytometry, immunofluorescence/immunocytochemistry, immunohistochemistry, and/or Western blot, along with antibodies that are CyTOF-ready or CyTOF-reported.

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### B7/CD28 Families

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### Butyrophilins

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**Application Key:**
- B/N: Blocking/Neutralization
- CyTOF-ready or CyTOF-reported
- Mass Cytometry
- E: ELISA
- FA: Functional Assay
- FC: Flow Cytometry
- ICC/IF: Immunocytochemistry/Immunofluorescence
- IHC: Immunohistochemistry
- KO: Knockout
- SW: Simple Western
- WB: Western Blot

**Fluorochrome Key for FAB/IC Catalog Numbers Ending In:**
- A: Allophycocyanin
- C: PerCP
- F: Fluorescein
- G: Alexa Fluor® 488
- N: Alexa Fluor® 700
- R: Alexa Fluor® 647
- S: Alexa Fluor® 750
- T: Alexa Fluor® 594
- U: Alexa Fluor® 350
- V: Alexa Fluor® 405
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**Application Key:**
- B/N: Blocking/Neutralization
- CyTOF-ready or CyTOF-reported
- Mass Cytometry
- ELISA
- Functional Assay
- FC Flow Cytometry
- ICC/IF Immunocytochemistry/Immunofluorescence
- IHC Immunohistochemistry
- KO Knockout
- SW Simple Western
- WB Western Blot

**Fluorochrome Key for FAB/IC Catalog Numbers Ending In:**
- A: Allophycocyanin
- C: PerCP
- F: Fluorescein
- G: Alexa Fluor® 488
- N: Alexa Fluor® 700
- P: Phycoerythrin
- R: Alexa Fluor® 647
- S: Alexa Fluor® 750
- T: Alexa Fluor® 594
- U: Alexa Fluor® 350
- V: Alexa Fluor® 405

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**Kynurenine Pathway**

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**Killer Immunoglobulin-like Receptors (KIRs)**

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Detection of KIR2DL3 on Human Peripheral Blood Mononuclear Cells by Flow Cytometry.
Human peripheral blood mononuclear cells (PBMCs) were stained with a PE-conjugated Mouse Anti-Human KIR2DL3 Monoclonal Antibody (Catalog # FAB2014P) and an APC-conjugated Mouse Anti-Human NCAM-1/CD56 Monoclonal Antibody (Catalog # FAB2408A).

Detection of KIR3DL1 on Human Peripheral Blood Mononuclear Cells by Flow Cytometry.
Human peripheral blood mononuclear cells (PBMCs) were stained with an APC-conjugated Mouse Anti-Human KIR3DL1 Monoclonal Antibody (Catalog # FAB12251A) and a PE-conjugated Mouse Anti-Human NCAM-1/CD56 Monoclonal Antibody (Catalog # FAB2408P). Quadrant markers were set based on control antibody staining (Catalog # IC003A).
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Application Key: B/N Blocking/Neutralization  CyTOF-ready or CyTOF-reported  Mass Cytometry  E ELISA  FA Functional Assay  FC Flow Cytometry  ICC/IF Immunocytochemistry/Immunofluorescence  IHC Immunohistochemistry  KO Knockout  SW Simple Western  WB Western Blot

Fluorochrome Key for FAB/IC Catalog Numbers Ending In: A: Allophycocyanin; C: PerCP; F: Fluorescein; G: Alexa Fluor® 488; N: Alexa Fluor® 700; P: Phycoerythrin; R: Alexa Fluor® 647; S: Alexa Fluor® 750; T: Alexa Fluor® 594; U: Alexa Fluor® 350; V: Alexa Fluor® 405

Detection of TIGIT on Human Peripheral Blood Mononuclear Cells by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) gated on CD3 cells were stained with an APC-conjugated Mouse Anti-Human TIGIT Monoclonal Antibody (Catalog # FAB7898A) and a PE-conjugated Mouse Anti-Human NCAM-1/CD56 Monoclonal Antibody (Catalog # FAB2408P). Quadrant markers were set based on control antibody staining (Catalog # IC0041A).

Detection of DNAM-1/CD226 on Human Peripheral Blood Mononuclear Cells by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) gated on CD3 cells were stained with a Fluorescein-conjugated Mouse Anti-Human DNAM-1/CD226 Monoclonal Antibody (Catalog # FAB666F) and a PE-conjugated Mouse Anti-Human NCAM-1/CD56 Monoclonal Antibody (Catalog # FAB2408P). Quadrant markers were set based on control antibody staining (Catalog # IC002F).
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**Application Key:**  B/N Blocking/Neutralization  CyTOF-ready or CyTOF-reported  Mass Cytometry  E ELISA  FA Functional Assay  FC Flow Cytometry  ICC/IF Immunocytochemistry/Immunofluorescence  IHC Immunohistochemistry  KO Knockout  SW Simple Western  WB Western Blot

**Fluorochrome Key for FAB/IC Catalog Numbers Ending In:** A: Allophycocyanin; C: PerCP; F: Fluorescein; G: Alexa Fluor® 488; N: Alexa Fluor® 700; P: Phycoerythrin; R: Alexa Fluor® 647; S: Alexa Fluor® 750; T: Alexa Fluor® 594; U: Alexa Fluor® 350; V: Alexa Fluor® 405
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Detection of TIM-3 in Th1-stimulated Human Peripheral Blood Mononuclear Cells by Flow Cytometry. Unstimulated and Th1-stimulated human peripheral blood mononuclear cells (PBMCs) were stained with an Alexa Fluor® 647-conjugated Rat Anti-Human TIM-3 Monoclonal Antibody (Catalog # FAB2365R) and a PE-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (Catalog # FAB3791P). Quadrant markers were set based on control antibody staining (Catalog # IC006R).
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Application Key:  
B/N Blocking/Neutralization  
CyTOF-ready or CyTOF-reported  
Mass Cytometry  
E ELISA  
FA Functional Assay  
FC Flow Cytometry  
ICC/IF Immunocytochemistry/Immunofluorescence  
IHC Immunohistochemistry  
KO Knockout  
SW Simple Western  
WB Western Blot

Fluorochrome Key for FAB/IC Catalog Numbers Ending in:  
A: Allophycocyanin;  
P: PerCP;  
G: Alexa Fluor® 488;  
N: Alexa Fluor® 700;  
P: Phycoerythrin;  
R: Alexa Fluor® 647;  
S: Alexa Fluor® 750;  
T: Alexa Fluor® 594;  
U: Alexa Fluor® 350;  
V: Alexa Fluor® 405
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ELISA Kits for Quantifying Immuno-Oncology Targets

R&D Systems offers complete, ready-to-run Quantikine® ELISA Kits and the more flexible DuoSet® ELISA Development Systems for detecting soluble immuno-oncology targets.

Quantikine® ELISA Kit Features

- Complete, ready-to-use kits
- Exhaustively tested for superior quality and reproducibility
- Detailed protocol booklets
- Colorimetric detection

DuoSet® ELISA Development System Features

- Contains all of the essential components required to develop an immunoassay for a specific target, but requires the user to set up the assay
- Contains carefully selected and validated antibodies, reducing development time
- Provides sufficient reagents for five or fifteen 96-well plates
- Includes mass-calibrated recombinant standard, reducing assay variability
- Can be adapted for use across multiple platforms

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Fc Receptors

| CD23/Fcc RII     | Human            | DCD230                         | DY123                             |
|                 | Mouse            |                                | DY6900                            |
| FCyRIIB/C (CD32b/c) | Human           |                                | DY1875                            |

Kynurenine Pathway

| IDO             | Human            |                               | DY6030                            |

LILR Family Receptors & ANGPT/ANGPTL Ligands

<p>| Angiopoietin-1  | Human            | DANG10                         | DY923                             |
| Angiopoietin-2  | Human            | DANG20                         | DY623                             |
|                 | Mouse/Rat        | MANG20                         |                                   |
| Angiopoietin-like Protein 3 | Human | DANL30                         | DY3829                            |
|                 | Mouse            | MANL30                         | DY136                             |
| Angiopoietin-like Protein 4 | Human |                               | DY3485                            |
| Angiopoietin-like Protein 6 | Human |                               | DY7846                            |</p>
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<td>VSIG8</td>
<td>Human</td>
<td>DY9200</td>
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<td>Other Related Ligands, Receptors, and Enzymes</td>
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<td>CD47</td>
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<td>DPP11/CD26</td>
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<td>DC260B</td>
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<td>Mouse</td>
<td>DY954</td>
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<td>LAG-3</td>
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<td>DY2319B</td>
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<td>TSLP R</td>
<td>Mouse</td>
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Monitor Immune Responses with Multiplex Assays

Luminex® Assays and Instruments

Luminex® Assays offer flexibility, require only a small sample volume (<50 µL), and are cost-effective. Select one of our pre-defined High Performance Assays or choose from over 340 analytes and build your own panel of up to 100 analytes.

<table>
<thead>
<tr>
<th>High Performance Assay</th>
<th>Analytes</th>
<th>Bead Format</th>
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<tbody>
<tr>
<td>Human XL Cytokine Discovery Panel</td>
<td>• CD40 Ligand • EGF • Eotaxin/CCL11 • FGF basic/FGF-2 • Flt-3 Ligand • Fractalkine/CX3CL1 • G-CSF • GM-CSF • Granulocyte Colony Stimulating Factor (G-CSF) • IFN-α • IFN-β • IFN-γ • IL-1α • IL-1β • IL-2 • IL-3 • IL-4 • IL-5 • IL-6 • IL-7 • IL-8/CXCL8 • IL-10 • IL-12 p70 • IL-13 • IL-15 • IL-17A • IL-17E/IL-25 • IL-33 • IP-10/CXCL10 • MCP-1/CCL2 • MIP-1α/CCL3 • MIP-1β/CCL4 • MIP-3α/CCL20 • MIP-3β/CCL21 • PD-1/B7H1 • PDGF-αA • PDGF-BB • RANTES/CCL5 • TGF-α • TNF-α • TRAIL • VEGF</td>
<td>Magnetic</td>
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<td>Human Cytokine Panel A</td>
<td>• CCL2/MCP-1 • CCL3/MIP-1α • CCL4/MIP-1β • CCL5/RANTES • CCL5/ENA-78 • CCL8/IL-8 • FGF basic/FGF-2 • GM-CSF • IFN-γ • IL-1α/IL-1F1 • IL-1β/IL-1F2 • IL-1ra/IL-1F3 • IL-2 • IL-4 • IL-5 • IL-6 • IL-10 • IL-17 • TNF-α • Thrombopoietin/Tpo • VEGF</td>
<td>Magnetic &amp; Polystyrene</td>
</tr>
<tr>
<td>Human High Sensitivity Cytokine Panel A</td>
<td>• CXCL8/IL-8 • GM-CSF • IFN-γ • IL-1α/IL-1F1 • IL-1β/IL-1F2 • IL-2 • IL-4 • IL-5 • IL-6 • IL-10 • IL-12 p70 • TNF-α • VEGF</td>
<td>Magnetic &amp; Polystyrene</td>
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</tbody>
</table>

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Our antibody arrays allow you to analyze the expression levels of up to 111 cytokines simultaneously, using only standard Western blotting equipment.

<table>
<thead>
<tr>
<th>Proteome Profiler™ Antibody Array</th>
<th>Measures</th>
<th>Catalog #</th>
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<tbody>
<tr>
<td>Human XL Cytokine Array</td>
<td>105 cytokines</td>
<td>ARY022B</td>
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<tr>
<td>Mouse XL Cytokine Array</td>
<td>111 cytokines</td>
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<td>Human Cytokine Array</td>
<td>36 cytokines</td>
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<td>Mouse Cytokine Array</td>
<td>40 cytokines</td>
<td>ARY006</td>
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<tr>
<td>Rat Cytokine Array</td>
<td>29 cytokines</td>
<td>ARY008</td>
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Simultaneous Detection of Multiple Analytes in IFN-γ, LPS-treated Human Acute Monocytic Leukemia Cell Culture Supernatants using the Proteome Profiler™ Human XL Cytokine Array Kit. The THP-1 human acute monocytic leukemia cell line was untreated or treated with 1 µg/mL Recombinant Human IFN-γ (Catalog # 285-IF) for 16 hours followed by 1 µg/mL lipopolysaccharide (LPS) for 8 hours. Cytokine levels in cell culture supernatants were analyzed using the Proteome Profiler™ Human XL Cytokine Array Kit (Catalog # ARY022B).

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