Detection of soluble immune checkpoint molecules with ProcartaPlex panels

Background

The human immune system has evolved to protect the body against malignant cells and external threats such as pathogenic organisms. To enable the balance between recognition of nonself and prevention of autoimmunity, the activity of immune cells needs to be strictly controlled.

Many new therapies aiming to engage the power of the immune system to combat cancer are under investigation. The goal of cancer immunotherapy is to initiate a self-sustaining cycle of cancer immunity, overcome negative feedback mechanisms, and avoid immunosuppressive molecules generated during cancerogenesis.

T cells fighting cancer: the cancer immunity cycle

For effective killing of cancer cells in an anticancer immune response, a series of events involving different immune cells needs to be initiated and allowed to proceed. The steps in the cancer immunity cycle (Figure 1) start with the release of tumor cell antigens, which are recognized and which lead to the killing of cancer cells by cytotoxic T cells. This immune response is modulated by a variety of stimulatory and inhibitory factors, which lead to either a self-propagating T cell response that fights cancer cells, or negative regulatory feedback mechanisms.

Immune checkpoint molecules play a crucial role in the regulation of T cells in the cancer immunity cycle. These checkpoint molecules lead to T cell exhaustion or stimulation, thereby modifying the antitumor immune response. Immune checkpoint molecules are recognized as intact transmembrane proteins; however, soluble isoforms or shed variants are described for the majority of these molecules.

Soluble checkpoint molecules can function as immune adjuvants or decoy receptors and thereby regulate the checkpoint pathways. Several therapeutic antibodies targeting immune checkpoint molecules are approved as immunotherapeutic drugs (e.g., ipilimumab targeting CTLA4, and nivolumab targeting PD-1). Clinical efficacy of these checkpoint modulator drugs may be influenced by soluble checkpoint molecules, as has been shown for soluble CTLA4, which enables the discrimination of responders and nonresponders to ipilimumab.

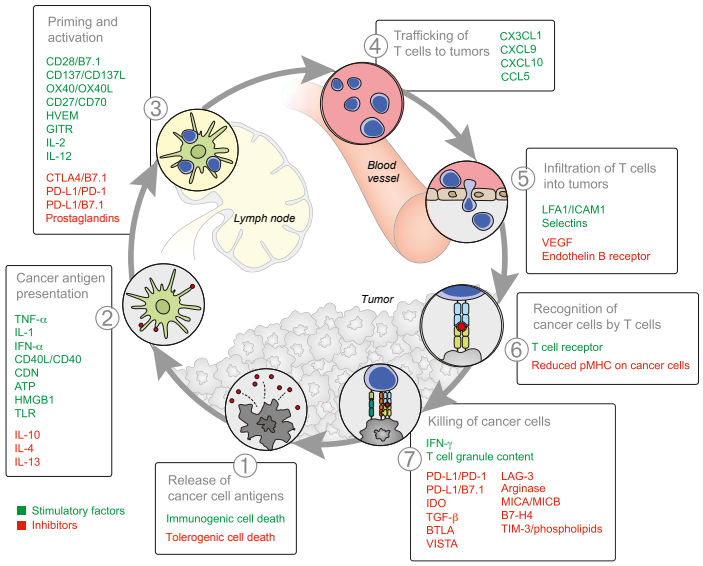

Figure 1. The cancer immunity cycle.

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ProcartaPlex Immuno-Oncology Checkpoint Panel 1

The Invitrogen™ ProcartaPlex™ Immuno-Oncology Checkpoint Panel 1 targets a set of selected molecules, including stimulatory factors that promote immunity and inhibitory factors that reduce immune activity and prevent autoimmunity (Table 1). The panel allows the simultaneous detection of multiple soluble immune checkpoint molecules and helps give a comprehensive picture of cancer immunity in a blood sample.

Table 1. Detection targets of ProcartaPlex Immuno-Oncology Checkpoint Panel 1.

Target	Function
Stimulatory factors	
CD28	Costimulatory in T cell activation
CD80 (B7-1)	Ligand of stimulatory CD28
CD137	Costimulatory in T cell activation
CD27	Costimulatory immune checkpoint molecule
HVEM	Costimulatory in T cell activation
GITR	Costimulatory immune checkpoint molecule
Inhibitory factors	
CD80 (B7-1)	Ligand of inhibitory CTLA4
HVEM	Coinhibitory in T cell activation
CTLA4	Coinhibitory in T cell activation
PD-1	Coinhibitory in T cell activation and cancer killing
PD-L1	PD-1 ligand
PD-L2	PD-1 ligand
IDO	Immunomodulatory enzyme
BTLA	HVEM ligand
LAG-3	Negatively regulates proliferation, activation, and homeostasis of T cells
TIM-3	Immune checkpoint, regulates macrophage activation

Natural killer (NK) cells as targets for immunotherapy

In addition to the important role of adaptive immunity shown in the cancer immunity cycle, innate immune cells, especially NK cells, also contribute to anticancer immune responses. The major role of NK cells is to recognize virus-infected cells that show alterations on their cell surface. NK cells also recognize and attack transformed malignant cells lacking major histocompatibility complex (MHC) class I. In addition to MHC class I deficiency, NK cell activity is mediated by multiple activating and inhibitory receptors that determine if the NK cell becomes cytotoxic.

Activated NK cells act either directly via perforin, which disrupts the target cell membrane, or indirectly by secreting proinflammatory cytokines. These cytokines stimulate the adaptive immune response and T cell response as shown in the cancer immunity cycle discussed previously. However, during tumor progression, cancer cells develop immunosuppressive mechanisms that circumvent NK cell–mediated killing. Tumor cells shed soluble ligands for NK cell–activating receptors, as a mechanism to escape immunosurveillance. Therefore, as for T cells, immunotherapeutic agents that stimulate NK cells' lytic capacity and support their ability to recognize and eradicate tumors are being investigated.

The major NK cell receptors that are potential immunotherapeutic targets are shown in Figure 2. Activating receptors of NK cells described so far are NKG2D, NCR, and DNAM-1, which can act synergistically. Ligands of NKG2D, like MICA, MICB, and the UL16-binding proteins (ULBPs), are upregulated in cellular stress response and expressed by many tumor cells.

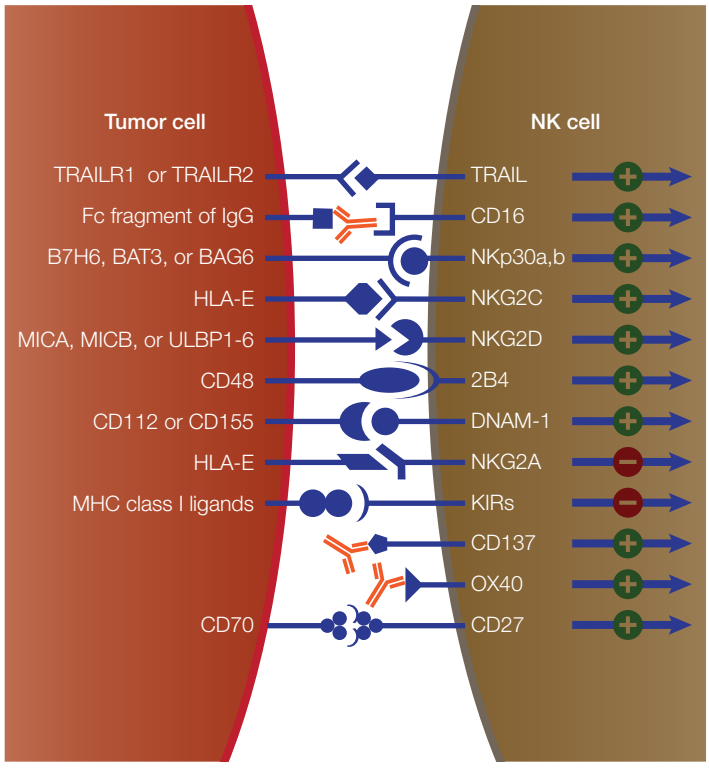

Figure 2. NK cell immunomodulation.

ProcartaPlex Immuno-Oncology Checkpoint Panel 2

Soluble forms of receptors and ligands are described for NK cells. These can be detected using the Invitrogen™ ProcartaPlex™ Immuno-Oncology Checkpoint Panel 2 (Table 2). Both ProcartaPlex Immuno-Oncology Checkpoint Panel 1 and Panel 2 can be further combined with cytokines, chemokines, or adhesion molecules that play important roles in shaping local and systemic immune responses. Various cytokines have been demonstrated to be associated with chronic inflammation, which is cancer-promoting, or with acute inflammation, which facilitates cancer rejection.

Table 2. Detection targets of ProcartaPlex Immuno-Oncology Checkpoint Panel 2.

Target	Function
Stimulatory factors	
MICA	Ligand of activating NKG2D receptor
MICB	Ligand of activating NKG2D receptor
ULBP1	Ligand of activating NKG2D receptor
ULBP3	Ligand of activating NKG2D receptor
ULBP4	Ligand of activating NKG2D receptor
CD96 (tactile)	Stimulatory receptor
CD155 (PVR)	Costimulatory to DNAM-1
CD112 (nectin-2)	Costimulatory to DNAM-1
CD73 (NT5E)	Costimulatory to DNAM-1
Inhibitory factors	
CD155 (PVR)	Coinhibitory to TIGIT
CD112 (nectin-2)	Coinhibitory to TIGIT
CD73 (NT5E)	Coinhibitory to TIGIT
Arginase-1	Immunomodulatory enzyme, amino acid depletion
Siglec-7	Inactivating receptor, binds to sialylated glycans on tumor cells
Siglec-9	Inactivating receptor, binds to sialylated glycans on tumor cells
Other factors	
Perforin	Induces cell lysis; deficiency increases cancer risk
E-cadherin	Involved in epithelial–mesenchymal transition

Results of beta testing using ProcartaPlex Immuno-Oncology Checkpoint Panel 1 and Panel 2 show that the expression patterns of the soluble markers are dependent on disease state and cancer treatment (Figures 3–5) [1]. The systematic, simultaneous analysis of these soluble biomarkers should help to shed light on the biology of checkpoint pathways and provide a tool for monitoring response to therapeutic treatment. Such characteristic responder profiles could help to preselect patients who may benefit from treatment with a defined drug.

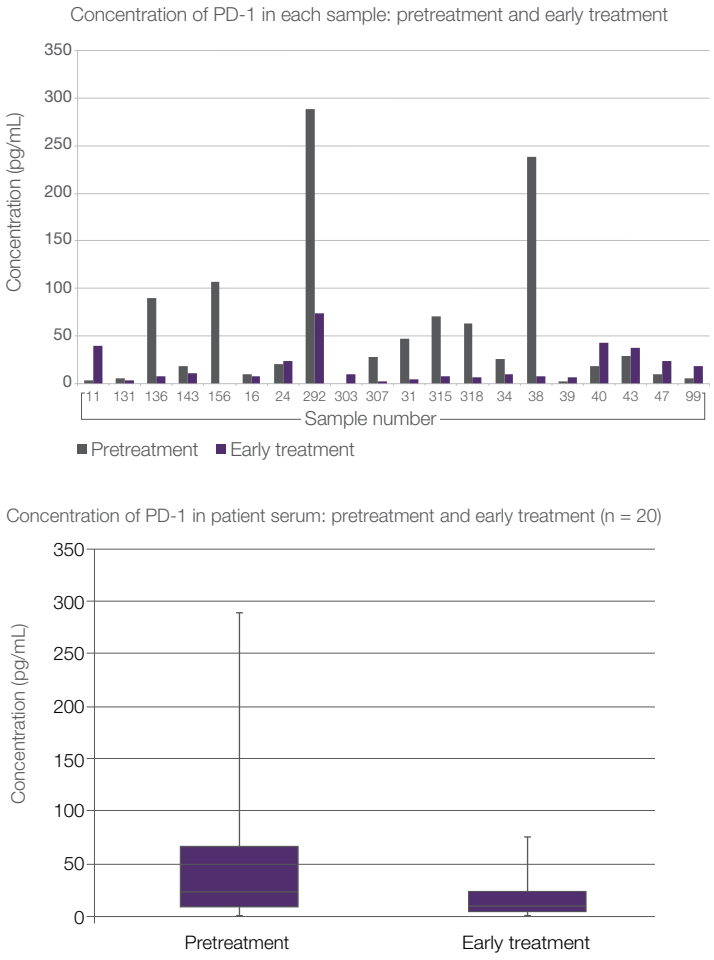

Figure 3. Serum levels of PD-1 in melanoma samples before and after treatment (data from one beta tester). The ProcartaPlex Immuno-Oncology Checkpoint Panel 1 was used. A reduction of PD-1 levels during treatment (early treatment compared to pretreatment) was detected. Correlation studies of checkpoint molecule levels and response to therapy are ongoing.

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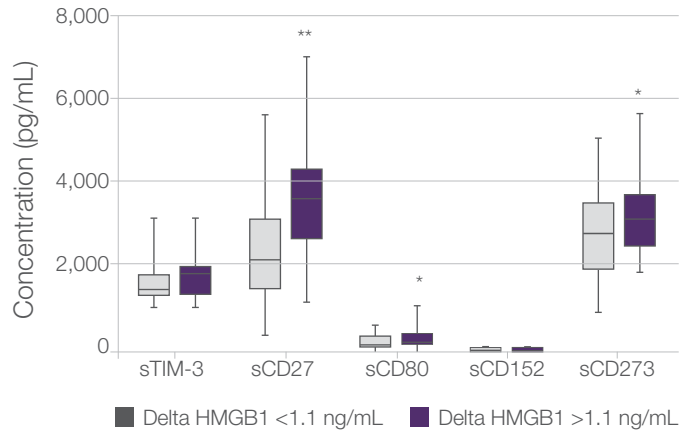

Figure 4. Plasma levels of checkpoint markers in human breast cancer samples after chemotherapy. Six immune checkpoint molecules (CD27, CD28, CD80/B7-1, CD152/CTLA4, CD273/PD-L2, and TIM-3) were detected. Interestingly, CD27, CD80/B7-1, and CD273/PD-L2 were higher in samples from subjects who had a better response to epirubicin/docetaxel combination chemotherapy and better 5-year survival (delta HMGB1 ≥1.1 ng/mL group). Statistically significant differences are indicated by asterisks (* $P < 0.05$; ** $P < 0.002$). Used with permission from Exner et al. [1].

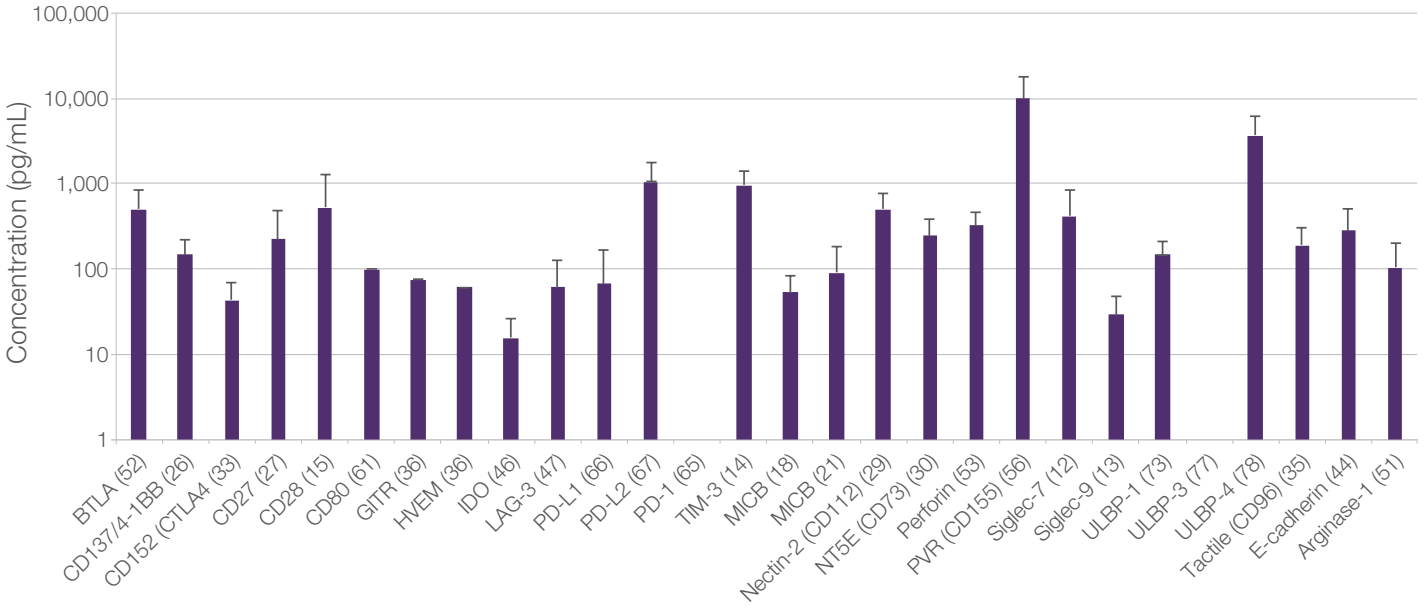

Figure 5. Serum levels of checkpoint markers in melanoma samples. Results as the mean of ungrouped human samples are shown for all targets of both panels.

Reference

1. Exner R, Sachet M, Arnold T et al. (2016) Prognostic value of HMGB1 in early breast cancer patients under neoadjuvant chemotherapy. *Cancer Med* 5(9):2350-8.

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