

KIDNEY CANCER UPDATE

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The Science of Checkpoint Inhibition in Kidney Cancer

Based on a presentation by Gordon J. Freeman, PhD, at the ASCO Genitourinary Cancers Symposium

Immunotherapy has offered the hope of a cancer cure for 100 years, according to Gordon J. Freeman, PhD. Although a cure remains elusive, “the door is now open,” Dr Freeman said. Programmed death 1 (PD-1) inhibitors have provided “a good foundation to build upon” for the treatment of kidney cancer and other cancers.

Dr Freeman, who is a professor of medicine at Harvard Medical School in Boston, Massachusetts, made his remarks during the scientific half of the renal cancer keynote lecture at the 2016 Genitourinary Cancers Symposium of the American Society of Clinical Oncology (ASCO) in San Francisco, California. His talk addressed the role of the PD-1/programmed death ligand 1 (PD-L1) pathway, why only stimulating the immune response does not cure cancer, the role of predictive biomarkers, how the immune system attacks a tumor, and why the future of treatment is combination therapy.

The Role of the PD-1/PD-L1 Pathway

Checkpoint blockade is the strategy of blocking the pathways that tumors use to turn off the antitumor immune response, said Dr Freeman. The immune response depends upon T-cell activation, which requires 2 signals: a T-cell receptor (TCR) signal, which provides specificity to the immune response, and a costimulatory or checkpoint signal, which either activates the immune response or diminishes it.

Dr Freeman’s laboratory 15 years ago identified PD-L1 and PD-L2 and showed that they are ligands of the PD-1 molecule^{1,2} and therefore targets that drugs can block. Dr Freeman explained that PD-L1 and PD-L2 can engage PD-1, leading to phosphorylation of the cytoplasmic domain of PD-1. This in turn recruits the tyrosine phosphatase SHP-2, which dephosphorylates the proximal signaling kinases in the TCR signaling pathway.

“This has the effect of reducing the TCR signal, which leads to less cytokine production and less ability to lyse a target cell,” said Dr Freeman. The change also alters lymphocyte motility and impairs the ability of the T cell

to use the nutrients glucose and glutamine effectively in the tumor environment. “The effect is to turn the T cell off,” he said.

Dr Freeman explained that although PD-1 stands for programmed death 1, this is something of a misnomer because PD-1 does not kill cells directly, such as by activating caspases. Instead, PD-1 works by reducing the activity of the T cell. PD-1 also may have an indirect effect on T-cell death by reducing the activity of antiapoptotic Bcl-xL and increasing the activity of proapoptotic Bim.

PD-1 plays an important role in the body because it serves to diminish the immune response after the elimination of disease and prevent the immune response from becoming strong enough to damage tissues. “This suggests to me that you don’t want to block this pathway for a lifetime,” said Dr Freeman.

Dr Freeman said he was surprised to discover that PD-L1 is expressed in solid tumor cell lines,¹ which led his team to speculate that PD-L1 expression is a strategy that cancer uses to shield itself against the immune system.

Further studies, such as those by Dr Leiping Chen, Dr Sabina Signoretti, and their colleagues, found PD-L1 expression on the surface of solid tumor cells, including those of renal cell carcinoma.³⁻⁵ “In general, there’s expression of PD-L1 on the cell surface in about 30% of solid tumors and selected hematologic malignancies, and this expression inhibits antitumor immune responses.”

When the researchers created antibodies that blocked PD-L1 or PD-1 in the laboratory, the result was increased cytokine production and increased killing of target cells⁴ (Figure). This finding led to the development of nivolumab (Opdivo, Bristol-Myers Squibb) and pembrolizumab (Keytruda, Merck) by researchers at drug companies.

The other effective checkpoint blockade molecule is cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), which works primarily on the antigen-presenting cells in the lymph node, where its B7 ligands are located.

“Checkpoint blockade of CTLA-4 works primarily in the lymph node, whereas checkpoint blockade of PD-1 works more within the tumor microenvironment,” Dr Freeman said. PD-1 is expressed on T cells, and its ligand,

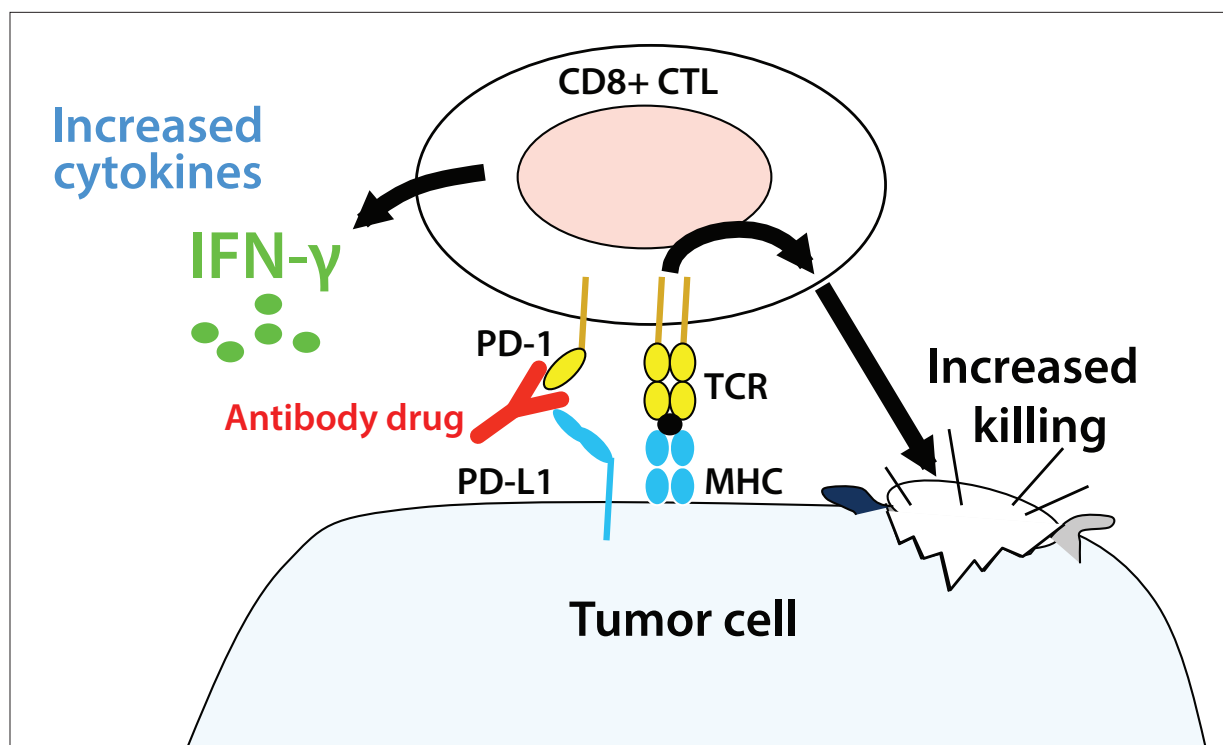


Figure. PD-1 or PD-L1 blockade stimulates antitumor immune response.

CTL, cytotoxic T lymphocyte; IFN- γ , interferon γ ; MHC, major histocompatibility complex; PD-1, programmed death 1; PD-L1, programmed death ligand 1; TCR, T-cell receptor

PD-L1, is expressed on the tumor and by cells in the tumor microenvironment, such as antigen-presenting cells, myeloid-derived suppressor cells, and stromal cells.

Why Only Stimulating the Immune Response Does Not Cure Cancer

Although researchers have attempted to harness the immune system to cure cancer for at least a century, most of their efforts have been aimed at directly stimulating the immune response. This does not work, however, in part because the tumor anticipates the immune response and expresses PD-L1. When researchers try to stimulate the immune response, T cells recognize the tumor and begin to produce interferon—but interferon increases PD-L1 expression and thus turns off the immune response. “So the harder you’re stepping on the gas, the harder you’re stepping on the brakes,” Dr Freeman said, a phenomenon that Janis Taube and colleagues have termed *adaptive resistance*.⁶ “The key is, we’ve learned to block PD-1 and stop the tumor from turning off the immune response. This enables all the stimulatory strategies that immunologists have developed.”

The Role of Predictive Biomarkers

A critical component of getting the right treatment to the right patients is the identification of predictive biomark-

ers. At least 5 good PD-L1 immunohistochemistry antibodies have recently been developed and made commercially available: 22C3, 28-8, SP142, E1L3N, and 9A11. “All of these can be used to do good immunohistochemistry on paraffin-embedded sections,” said Dr Freeman.

Determining whether a tumor is positive for PD-L1 is less straightforward than it may seem, however. In a study that was published in *Cancer Immunology Research*,⁵ Callea and colleagues examined both primary and metastatic sites in 53 patients with metastatic clear cell renal cell carcinoma (RCC). In 33 patients, the tumors were negative in both primary and metastatic sites and so were concordant. In 20 patients, either the primary tumor or the metastatic lesion tested positive for PD-L1. Of these 20 patients, 3 had PD-L1 expression in the metastatic lesion but not the primary tumor, and 8 had PD-L1 expression in the primary tumor but not the metastatic lesion—so just 9 of the 20 patients had PD-L1 expression in both the primary and the metastatic sites, for a total discordancy rate of 21%. This result highlights the possibility of sampling error and the need to analyze both metastatic lesions and primary tumors. PD-L1 positivity was heterogeneous within the tumors and detected almost exclusively in areas with a high nuclear grade. Pathologists should therefore select tumor areas with a high nuclear grade for PD-L1 immunohistochemistry analysis to avoid false-negative results.

A study by Dr Toni Choueiri and colleagues⁷ found PD-L1 positivity in 11 of 101 patients with non-clear cell RCC (11%); 2 of 36 patients with chromophobe RCC (6%), 5 of 50 patients with papillary RCC (10%), 3 of 10 patients with Xp11.2 translocated RCC (30%), and 1 of 5 patients with collecting duct carcinoma (20%).

Although PD-1 is a predictive marker for response to PD-1 therapy in lung cancer, bladder cancer, and melanoma, the phase 3 study of nivolumab vs everolimus (Afinitor, Novartis) by Motzer and colleagues⁸ found that PD-L1 was not associated with a PD-1 treatment benefit in RCC. “We do not completely understand the reasons for this.”

Dr Freeman proposed that other information, such as The Cancer Genome Atlas (TCGA) messenger RNA expression data, might be used to guide the further development of immunotherapy in kidney cancer. For example, TCGA data show that kidney cancer is particularly inflamed, as reflected by high levels of CD8 (cytolytic T cells) and CD14 (myeloid cells). Other checkpoint targets of the B7 family that are highly expressed in clear cell RCC include PD-L2, B7-H3, V-domain immunoglobulin suppressor of T-cell activation (VISTA), and HERV-H LTR-associating 2 (HHLA2), along with a surprising decrease in B7-H4. Dr Freeman said that RCC is “extraordinary” because “the T cells are in the tumor and there are a lot more checkpoint blockade targets in RCC that can be exploited” than in other types of cancer, such as prostate cancer. “Kidney cancer is really a good target for immunotherapy.”

How the Immune System Attacks a Tumor

When the immune system tries to attack a tumor, it recognizes changes in the protein-coding sequences—called tumor neoantigens—that are caused by mutations. There are 2 evolutionary processes occurring in cancer: the well-known accumulation of DNA mutations that lead to oncogenesis and the more recently recognized development of immune evasion.

DNA mutation occasionally results in a kinase driver mutation that supports tumor growth. A driver mutation may be an excellent target for a kinase inhibitor but is rarely a good neoantigen. DNA mutation also generates many passenger mutations, some of which contribute to tumor development but most of which are neutral. Some of these passenger mutations occur in protein-coding regions and constitute neoantigens that can be processed into peptide antigens, which are recognized by the immune system and are targets for the immune response.

Regarding immune evasion, the immunologist Dr Robert Schreiber and his colleagues have shown that many early tumors are eliminated by an antitumor immune response before they become medical problems.⁹ This means that a tumor that has grown large enough to

become a medical problem has learned to evade the immune response. Immune evasion mechanisms include expression of PD-L1, indoleamine 2,3-dioxygenase (IDO), transforming growth factor beta (TGF- β), and interleukin 10 (IL-10), along with loss of major histocompatibility complex (MHC) class I proteins.

Because a tumor evolves continually and is very heterogeneous, a system that attacks it successfully must also evolve rapidly. “Indeed, the immune system is a rapidly evolving, very diverse system with millions of T-cell receptors, millions of antibody specificities, and hundreds of pattern-recognition molecules, which can attack the changes in the tumor and sense the genomic instability and stress of a tumor cell.”

Mutation frequency varies in different tumor types. Melanoma, lung cancer, and bladder cancer have the most mutations, a feature consistent with their high rate of response to checkpoint blockade. Treating the tumor relies on preventing it from turning off the antitumor immune response. The strength of the tumor—its high mutation rate—then becomes a weakness that can be attacked by the immune response. Dr Freeman said it is surprising that kidney cancer, which is in the middle of the mutagenesis profile, also responds well to checkpoint blockade. Because different tumor types have different immune environments, additional organ-specific mechanisms of immune evasion should be identified in resistant tumors.

Understanding the immunology and genetics of tumors has helped researchers identify tumors that respond well to PD-1/PD-L1 therapy. These include tumors with very high mutation rates due to defects in DNA repair, such as colorectal tumors with microsatellite instability, which have a 62% response rate. For example, the 87% response rate of Hodgkin lymphoma to PD-1/PD-L1 inhibitors is logical given that PD-1 and PD-L2 are genetically amplified and overexpressed in this disease.⁹ Tumors that express foreign antigens, such as viral antigens from human papillomavirus and Merkel cell polyomavirus, also are good candidates for immunotherapy. Further work should identify other especially responsive tumor types.

We now realize the antitumor immune response goes on for years, and if a tumor grows, the T cells have tried to attack the tumor, failed, and become “exhausted.” Dr Rafi Ahmed first described “exhausted” T cells in his studies of chronic viral infection. As Dan Barber, John Wherry, and colleagues have shown in their laboratory, PD-1 blockade can revive exhausted T cells.¹⁰ “We have shown that tumor-infiltrating T cells behave like these ‘exhausted’ T cells, and now we think of the antitumor immune response as a chronic immune response with exhausted T cells.”

Exhausted T cells express not only PD-1 but also other inhibitory receptors, such as T-cell immunoglobulin-

lin and mucin domain-containing protein 3 (TIM-3), lymphocyte activation gene 3 (LAG3), CD244, T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), and other molecules—all of which are druggable targets of tumor immunotherapy that are under development.

Why the Future of Treatment Is Combination Therapy

PD-1 inhibitors work well only in approximately 20% to 30% of patients; although this is encouraging, improvement is greatly needed. The fact that many other druggable targets exist, and that some have been shown to improve the rate of response to PD-1 inhibitors in preclinical models and clinical trials, illustrates that “the future is clearly combination therapy.” For example, Dr Jedd Wolchok’s group has shown that response rates in melanoma are higher with the combination of PD-1 blockade plus CTLA-4 blockade than with either of these alone.¹¹ Early data also suggest that the combination works better than either one alone in renal cancer.

PD-1 inhibitors also may be used in combination with blockade of other immunoinhibitory molecules, such as TIM-3, LAG3, TIGIT, CD244, and CD160. Another possible combination is PD-1 blockade plus immunostimulation, such as with OX40, CD137, inducible costimulator (ICOS), interleukin 2 (IL-2), or Toll-like receptor (TLR) ligands. PD-1 blockade can be used in combination with kinase inhibitors, angiogenesis inhibitors, radiation, histone deacetylase inhibitors, cancer vaccines, oncolytic viruses, or chimeric antigen receptor (CAR) T cells.

PD-1 antibody does not block all the immunoinhibitory possibilities of PD-L1 and PD-L2, however. PD-L1 has B7-1 as an alternate receptor, and PD-L2 has repulsive guidance molecule b (RGMB) as an alternate receptor. As Dr Freeman and colleagues showed in recently published work,¹² RGMB and PD-L2 interact to deliver an immunoinhibitory signal. In a mouse model of colorectal cancer, RGMB blockade alone does not work and PD-1 alone works moderately well, but a combination of the two works even better. Therefore, there is a good rationale for combining PD-1 and RGMB.

Questions remain. How can oncologists identify patients who will respond to PD-1 blockade, what are the mechanisms of primary failure to respond, and what are the mechanisms of secondary failure to respond? The

answers to these questions will allow us to develop therapies for the 70% to 80% of patients who do not respond well to PD-1 blockade.

Conclusion

Dr Freeman concluded by saying that this is “a wonderful time to be an oncologist or researcher and a better time to be a patient.” PD-1 blockade works on a wide range of tumors and has a good safety profile, although only a moderate percentage of tumors respond. He said he was impressed by the “enormous amount of human creativity that has been unleashed” now that we have an effective strategy. “We’re learning to do better, and we think we can win.”

Disclosures

Dr Freeman is a consultant for Novartis, Bristol-Myers Squibb, Roche, Lilly, and Seattle Genetics.

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