Molecular Pathways: Co-Expression of Immune Checkpoint Molecules: Signaling Pathways and Implications for Cancer Immunotherapy

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Abstract

The expression of immune checkpoint molecules on T cells represents an important mechanism that the immune system uses to regulate responses to self-proteins. Checkpoint molecules include CTLA-4 (Cytotoxic T Lymphocyte Antigen-4), PD-1 (Programmed Death-1), LAG-3 (Lymphocyte Activation Gene-3), TIM-3 (T cell Immunoglobulin and Mucin protein-3) and several others. Previous studies have identified individual roles for each of these molecules, but more recent data show that co-expression of checkpoint molecules occurs frequently on cancer-specific T cells, as well as on pathogen-specific T cells in chronic infections. While the signaling pathways associated with each checkpoint molecule have not been fully elucidated, blocking multiple checkpoints with specific monoclonal antibodies results in improved outcomes in several chronic viral infections as well as in a wide array of pre-clinical models of cancer. Recent clinical data suggest similar effects in patients with metastatic melanoma. These findings support the concept that individual immune checkpoint molecules may function through non-overlapping molecular mechanisms. Here we review current data regarding immune checkpoint molecule signaling and co-expression, both in cancer and infectious disease, as well as the results of preclinical and clinical manipulations of checkpoint proteins.

Background

One of the most important decisions made by the immune system involves modulating both the breadth and magnitude of an evolving response. As a whole, the immune system is capable of sterilizing immunity against a wide variety of pathogens, and maintains memory responses for future encounters. Therefore, an immune response is tightly regulated, and multiple mechanisms are in place to prevent autoimmune reactions to self-proteins. The
devastating and lifelong effects of many autoimmune diseases evidence the importance of these mechanisms. Over the past 20 years, a broad class of extracellular “checkpoint molecules” has been found to modulate T cell responses to self-proteins [1]. However, many of these molecules also have a role in regulating T cell responses to chronic infections and tumor antigens. Checkpoint molecules include CTLA-4, PD-1, LAG-3, and TIM-3 as well as several others[1, 2]. Recent clinical data on single-agent CTLA-4[3] and PD-1[4,5] blockades in cancer patients demonstrate that these pathways play a critical role in the maintenance of tumor tolerance in humans, since single-agent checkpoint blockade is associated with objective tumor responses and improved overall survival. Furthermore, very recent data combining PD-1 and CTLA-4 blockade in melanoma patients showed an increased rate of objective tumor responses as compared to blocking either checkpoint alone, supporting the notion that combinatorial checkpoint blockade may result in increased clinical benefit[6].

Signaling Through Immune Checkpoint Molecules

While the precise molecular pathways by which these checkpoint proteins signal are poorly understood, pre-clinical data from studies in which multiple checkpoints were blocked simultaneously suggest that the pathways utilized by different checkpoint proteins may be relatively unique and potentially non-redundant. This may provide a clinical rationale for blocking multiple checkpoints to enhance anti-tumor immunity. Among checkpoint molecules, CTLA-4 blockade was the first shown to augment anti-tumor immunity[7], and is the checkpoint molecule for which signaling is best understood. CTLA-4 is a homolog of CD28, and plays a significant role in the development of peripheral tolerance to self-proteins, as demonstrated by studies of CTLA-4 knockout mice[8,9]. These animals are moribund by 3-4 weeks of age, have significant up regulation of T cell activation markers, and exhibit severe pancreatitis, myocarditis, and T cell
infiltration of the liver, heart, lung, and pancreas. In terms of signaling, the major ligands for CTLA-4 are B7-1(CD80) and B7-2(CD86), which transmit an inhibitory signal to CTLA-4 expressing T cells. Initial data suggesting that the signaling pathway for CTLA-4 directly involves events downstream of T cell activation also came from studies using knockout mice, and showed that in the absence of CTLA-4 signaling, there was constitutive activation of the protein tyrosine kinases FYN, LCK, and ZAP-70[10]. To regulate the function of these kinases (and down-modulate T cell function), CTLA-4 recruits two phosphatases, SHP2[10] and PP2A[11]. As shown in Figure 1, the association of CTLA-4 with SHP2 results in dephosphorylation of the CD3ζ chain, reducing the signaling potential of the T cell receptor. Furthermore, CTLA-4 recruitment of PP2A results in decreased downstream AKT phosphorylation[12], further dampening the signaling cascade initiated by T cell receptor (TCR) engagement. Taken together, these data show that CTLA-4 signaling dampens T cell activation through both proximal and distal mechanisms.

Programmed Death-1 (PD-1) is a 55 kDa transmembrane protein that, like CTLA-4, down-regulates T cell function[13,14]. Consistent with that role, PD-1 knockout mice show some evidence of autoimmunity; they have elevated serum levels of IgG2b as well as IgA, and develop mild lupus-like autoimmunity[15], as well as dilated cardiomyopathy[16], although this phenotype has not been universally observed. Additionally, these disease phenotypes are strain-specific, occur later in life, and are markedly less prominent than those observed in CTLA-4 knockout animals[2]. PD-1 signaling involves binding to several discrete ligands, including PD-L1 and PD-L2, as well as to the co-stimulatory molecule B7-1[17]. Under certain (inflamed) conditions, PD-L1 can be expressed on most cell types, including cancer cells, epithelial cells, lymphoid cells, myeloid cells, and professional antigen presenting cells. PD-L2, by contrast, is expressed primarily on professional antigen presenting cells, though recent data from several labs, including ours, suggest that PD-L2 may be expressed on several cancer cell
lines (CJN, unpublished data). Structurally, PD-1 has a cytoplasmic Immunoreceptor Tyrosine-based Inhibitory Motif (ITIM), as well as an Immunoreceptor Tyrosine-based Switch Motif (ITSM), and has been found to be capable of recruiting the phosphatases SHP-1 as well as SHP-2[18], although only SHP-2 recruitment has been confirmed in vivo (Figure 1). Furthermore, PD-1 signaling may result in dephosphorylation of the CD3ζ chain, mediating decreased TCR signaling[19]. Taken together these data support a model in which PD-1 and CTLA-4 both inhibit T cell function in part by inhibiting Akt activation, although PD-1 may operate primarily at a more membrane-proximal level[20]. Despite these similarities in the known signaling pathways of PD-1 and CTLA-4, early experiments by Blazar et al. demonstrated that these two inhibitory pathways do not serve fully redundant roles. In a murine model of graft vs. host disease where heavily irradiated hosts were given MHC mismatched bone marrow, blockade of either PD-1 or CTLA-4 exacerbated the disease by an IFNγ dependent mechanism[21]. However, combinatorial blockade had the greatest effect, demonstrating that these two pathways have distinct effects in maintaining self-tolerance.

A third immune checkpoint molecule which may be important in the immune response to cancer[22] is Lymphocyte Activation Gene-3 (LAG-3), a CD4 homolog with four extracellular Ig-like domains[23]. Like CD4, LAG-3 has been found to bind MHC Class II molecules[24]. However, unlike CTLA-4 or PD-1 knockout animals, LAG-3 knockout mice do not develop overt autoimmunity[25], suggesting that LAG-3 plays a more subtle role in modulating T cell function than either CTLA-4 or PD-1. Nevertheless, LAG-3 clearly restrains T cell function under several conditions [26]. This is particularly notable in the Non-Obese Diabetic (NOD) model of diabetes, where knocking out LAG-3 results in significantly accelerated disease, marked by an increased CD4+ and CD8+ T cell infiltration of the pancreas[27]. Furthermore, LAG-3 knock out CD4 and CD8 T cells show increased expansion in response to SEB activation, in vivo peptide stimulation, and to Sendai Virus[28], suggesting that LAG-3 may function by regulating T cell
expansion in immune reactions that have already been initiated. Other important data suggest a more prominent role for LAG-3 in regulatory T cells (Treg) function[29], in that enforced expression of LAG-3, but not a LAG-3 mutant, enhanced Treg suppressive capacity in vitro. The molecular pathways that mediate LAG-3 signaling are still largely unknown, although it is clear that the unique intracellular KIEELE domain is required for its function[28] (Figure 1). Based on the currently available data, it is not possible to determine whether PD-1 and LAG-3 signaling pathways overlap significantly, although recent data in several models (to be discussed below) would suggest that this is not the case.

A fourth immune checkpoint molecule with potential relevance to cancer immunology is T cell Immunoglobulin Mucin-3 (TIM-3), a glycoprotein that has both immunoglobulin and mucin domains on its extracellular portion. Like LAG-3 knockout mice, TIM-3 knockout animals do not develop overt autoimmunity[30], suggesting that TIM-3 and LAG-3 have similarly subtle effects in controlling T cell function. In concordance with this hypothesis, TIM-3 blockade also accelerates the disease phenotype in models prone to the development of autoimmunity, including NOD mice[30], as well as experimental autoimmune encephalitis[31]. Functionally, TIM-3 binds to galectin-9 (as well as several other ligands), as supported by data showing that administration of galectin-9 in vitro causes cell death of TH1 cells in a TIM-3 dependent manner[32]. Furthermore, galectin-9 treatment in vivo suppresses TH1 mediated EAE by inducing the death of IFN-γ producing CD4 T cells. TIM-3 signaling is dependent on Y265 phosphorylation by inducible T cell kinase[33], and recent data in autoimmune models suggest that the cytoplasmic protein Bat3, important in modulating cellular proliferation, serves as an important adaptor protein[34]. In this model, Bat3 is bound to TIM-3 at rest, and protects the T cell from TIM-3 signaling. However, when TIM-3 binds to Galectin-9, Bat3 dissociates from TIM-3, and TIM-3 can now down-modulate production of IFN-γ and T cell proliferation.
Immunologically, chronic infections are in some ways quite similar to tumors, in that lymphocytes are persistently exposed to their cognate antigens, resulting in non-functionality, or tolerance. In models of chronic viral infection, checkpoint molecules have been individually found to play a role in down-modulating a pathogen-specific immune response. However, recent studies have begun to home in on the expression of a checkpoint signature, wherein multiple checkpoint molecules are co-expressed on the same T cell. Many of these studies have focused on a murine model of chronic infection (Lymphohoriomeningitis Virus or LCMV), in which CD8 T cells specific for viral epitopes persist, but lose their lytic function as well as the capacity to secrete cytokines over time[35]. Using the LCMV model, a seminal study by Wherry et. al. showed that non-functional antigen-specific CD8 T cells co-express multiple checkpoint molecules, including PD-1, LAG-3, 2B4, and CD160[36]. Expression of multiple checkpoint molecules correlated with decreased cytokine production, in which virus-specific CD8 T cells first lost lytic ability, then their ability to secrete IL-2, TNF-α, and IFN-γ in that order. In this model, certain combinations of immune checkpoint molecules were more commonly co-expressed; in particular PD-1 was commonly expressed along with LAG-3, 2B4 and/or CD160. Of potential clinical relevance, it was noted that combination PD-1 / LAG-3 blockade was superior in terms of restoring IFNγ secretion and viral clearance than blocking either checkpoint alone[36]. A related study in the LCMV model also showed co-expression of PD-1 and TIM-3[37] correlated with decreased production of IFNγ, TNFα, and IL-2. In both studies, there was a clear hierarchy of checkpoint expression: in addition to dual expressing cells (cells expressing PD-1 and either LAG-3 or TIM-3), PD-1 single positive cells could be found, but LAG-3 or TIM-3 single positive cells were relatively rare. We found similar results for PD-1 and LAG-3 in a model of self-antigen tolerance in vivo [38]. While those results focused mostly on CD8 T cells, in a model of chronic parasitic infection (Plasmodium yoelii), CD4 T cells were also found to co-
express PD-1 and LAG-3, and similar to the LCMV model, blocking both checkpoint molecules was superior in restoring production of IFNγ and TNFα, leading to increased clearance of the parasite[39]. Taken together, these data support the notion that immune checkpoint molecules are often co-expressed in response to persistent antigens from infectious agents, and that blocking multiple checkpoints may significantly improve T cell immune responses.

**Combined Checkpoint Blockade in Cancer: Pre-Clinical Models**

Since tumors represent a fairly obvious example of persistent antigen expression, one might reason that tumor-specific lymphocytes should express multiple immune checkpoints, and that combination checkpoint blockade might mediate increased therapeutic benefit. Indeed, early data showed that combinatorial blockade of PD-1 and CTLA-4 resulted in significantly increased anti-tumor immunity when compared to blocking either single checkpoint alone [40]. Data supporting this hypothesis were generated in a murine melanoma model, in which PD-1 and CTLA-4 blockade was combined with vaccination [41]. In these studies, vaccination with irradiated tumor cells expressing Flt3 ligand was important, most likely in order to initiate an anti-tumor response to a poorly immunogenic tumor. The combination of vaccination plus dual PD-1 / CTLA-4 blockade resulted in increased survival of mice bearing B16 melanoma flank tumors in comparison to vaccination alone or to vaccination combined with single agent blockade of either CTLA-4 or PD-1. In terms of immunological mechanism, the combination of vaccination along with dual CTLA-4 / PD-1 blockade significantly increased the ratios of both CD4 and CD8 effector T cells to regulatory T cells (Treg). Further studies in the MB49 bladder cancer model showed that combined blockade of PD-1 and CTLA-4 increased survival and decreased tumor growth in both small and large established flank tumors without additional vaccination [42]. However, more recent studies blocking PD-1 and CTLA-4 in a model of ovarian
cancer also required vaccination for optimal pre-clinical benefit\cite{43}. Taken together, these studies are important as they confirm the potential of blocking multiple immune checkpoint molecules in cancer models; however, they also raise the issue of whether specific vaccination might be required for maximal clinical benefit.

In other recent studies, the role of the immune checkpoint molecule TIM-3 was studied in several murine cancer models\cite{44}, including CT26 colon carcinoma, 4T1 mammary carcinoma, and B16 melanoma. Interestingly, TIM-3 was nearly universally co-expressed with PD-1, and TIM-3 / PD-1 double positive cells represented the majority of infiltrating T cells. Co-expression of both checkpoint molecules corresponded to a more exhausted phenotype, defined as a T cell’s ability to proliferate and secrete IFN$_\gamma$, IL-2 and TNF$_\alpha$. Combined blockade was more effective in controlling tumor growth than blocking either checkpoint alone, confirming the notion that combined immune checkpoint blockade could be a potential treatment strategy to a wide variety of cancers, and that, besides CTLA-4, other checkpoints might synergize with PD-1 to down-modulate T cell responses to tumors.

In related work, we examined the relationship between the immune checkpoints LAG-3 and PD-1. In prior studies, we found that LAG-3 is relatively over-expressed on non-functional CD8 T cells in models of both self-tolerance and tumor tolerance\cite{26}. In those studies, blocking LAG-3 alone resulted in a significant, but incomplete, recovery of function, with evidence for a cell intrinsic effect on CD8 T cells. Based on emerging data underscoring the importance of the immune checkpoint PD-1, we crossed LAG-3 knockout mice to PD-1 knockout animals. Unlike either single knockout animal, loss of both LAG-3 and PD-1 resulted in multi-organ lymphocytic infiltration, and in death between 6 and 8 weeks of age\cite{45}. Nearly identical results were obtained earlier by a group studying autoimmunity\cite{46}, reinforcing the notion that LAG-3 and PD-1 are potentially synergistic in regulating T cell function. Mechanistically, adoptive transfer of CD4 and CD8 T cells from double knockout mice into mice lacking B and T cells (Rag -/-)
resulted in a similar, fatal autoimmune phenotype, confirming that the primary drivers of this autoimmunity are CD4 and CD8 T cells. Interestingly, in three separate tumor models (Sa1N, MC38, B16), we found significant expression of PD-1 and/or LAG-3 on both CD4 and CD8 expressing TILs. Tumors implanted onto PD-1/LAG-3 double knockout mice were mostly rejected, while PD-1 single knockout mice showed delayed tumor growth. In that regard, LAG-3 knockout mice were not significantly different from wild type mice in terms of tumor growth, underscoring the more subtle nature of the LAG-3 checkpoint. In preclinical studies, we treated established Sa1N and MC38 tumors by blocking either LAG-3, PD-1 or both. Similar to studies with TIM-3, anti-PD-1 monotherapy showed some efficacy (including a small percentage of “cured” animals), anti-LAG-3 monotherapy delayed tumor growth, and quite strikingly, combined blockade resulted in the majority of tumors being rejected, without any evidence of autoimmune side effects. These results were mathematically synergistic, and seemed to be mediated by increased secretion of effector cytokines such as IFN$\gamma$ and TNF$\alpha$ by tumor-infiltrating lymphocytes.

Clinical Translation Advances: Co-Expression of Immune Checkpoints on Human T Cells

Recent studies of virus-specific T cells in humans corroborate the results discussed above involving murine models of chronic infection. Specifically, in patients with chronic hepatitis C (HCV), CD8 T cells specific for HCV co-expressed combinations of PD-1, 2B4, and CD160[47]. Furthermore, cells co-expressing multiple checkpoint proteins expressed low levels of CD127, indicating that these cells were actively responding to the virus. As in the murine models, co-expression of multiple checkpoint molecules correlated with decreased proliferative capacity in vitro. TIM-3 has also been found on HCV specific CD8 T cells. Surprisingly, in patients transitioning from acute to chronic HCV infection there was a significant increase in the expression of TIM-3 on HCV specific CD8 T cells in the peripheral blood, as well as significant co-expression of PD-1 and TIM-3[48]. Furthermore, the majority of intrahepatic CD8 T cells
expressed PD-1 and TIM-3, followed by a population expressing PD-1 alone, mirroring the data in tumor-infiltrating lymphocytes. Blocking TIM-3 and PD-1 during in vitro re-stimulation also restored proliferative function of T cells to HCV peptides, suggesting that combinatorial blockade could also be of clinical utility in chronic infections.

Another chronic infection in which checkpoint proteins have been implicated is HIV. A recent report examining the role of checkpoint proteins on HIV specific CD8 T cells found increases in PD-1, CD160, and 2B4 expression[49]. Curiously, there was no significant increase in LAG-3 expression on these CD8 T cells, suggesting once again that while checkpoint molecules act in concert, their signaling and expression is likely not redundant. Expression of PD-1 and CD160 decreased following HAART therapy in these patients, and, as in the pre-clinical models, there were distinct patterns of combinatorial expression. Also similar to the murine models, the most prevalent subpopulations expressed PD-1 and a combination of other markers, in this case CD160. Furthermore, the number of checkpoint proteins expressed correlated with an inability to produce IFN-γ upon restimulation in vitro. Together these data mirror the preclinical murine data, and suggest a potential clinical strategy involving combinatorial checkpoint blockade to treat chronic infectious diseases in patients.

In cancer, recent studies have begun to investigate co-expression of immune checkpoint molecules on either tumor infiltrating or tumor-specific T cells. Some of the earliest studies involved isolation of peripheral blood lymphocytes (PBL) and TIL from women with ovarian cancer [50]. Cells specific for the cancer-testis antigen NY-ESO-1 were found to co-express LAG-3 and PD-1, with the double positive cells being most impaired in terms of IFN-γ secretion. Of clinical relevance, blocking both immune checkpoint molecules during in vitro T cell priming augmented both proliferation as well as cytokine secretion, again suggesting combined checkpoint blockade as a potential therapeutic intervention. Similar results have been reported for the combination of TIM-3 and PD-1 in melanoma patients[51]. Perhaps the most
comprehensive analysis of immune checkpoint co-expression was recently reported by the Speiser group, who examined the expression of CTLA-4, PD-1, LAG-3, and TIM-3, in addition to CD160, 2B4 and BTLA [52]. These data are fascinating, suggesting that naïve T cells are controlled primarily by TIM-3 and BTLA, while effector T cells that infiltrate tumors co-expressed a wide variety of combinations of checkpoint molecules, depending to some degree on anatomical location. The conclusion of those studies was that further work is necessary to define the relative role of different checkpoint molecules in patients.

Clinically, a variety of checkpoint blocking agents are being developed to block PD-1 and CTLA-4 signaling. These include a wide variety of monoclonal antibodies blocking CTLA-4, PD-1, or PD-L1 as well as PD-2 and LAG-3 fusion proteins (Figure 1). Currently, several early stage, ongoing clinical trials are exploring combined monoclonal antibody based immune checkpoint blockade in cancer patients, and a Phase III trial in melanoma has been announced (Table 1). These studies all involve the combination of anti-CTLA-4 (Ipilimumab, BMS), which is FDA-approved for treating patients with melanoma, and anti-PD-1 (Nivolumab, BMS) which is currently in Phase III trials in several tumor types. Recently, a study investigating stage III or IV, unresectable melanoma (NCT01024231) was published with quite striking results [53]. Across all dose levels, concurrent delivery of anti-PD-1(Nivolumab) and anti-CTLA-4 (Ipilimumab) resulted in objective responses in 40% of patients. When given at the maximum tolerated dose, 53% of patients had objective responses. Furthermore, these responses were rapid: all responding patients had a tumor reduction of 80% or more by their first scheduled assessment. Studies in kidney cancer (NCT01472081) and non-small cell lung cancer (NCT01454102) recently opened combined anti-CTLA-4 / anti-PD-1 arms, and it will be interesting to see if the melanoma results extend to other disease histologies. It also remains to be seen whether this combination will prove tolerable, or whether further dose and schedule optimization is necessary.
Conclusions

Preclinical models of chronic infection, self-tolerance, and tumor tolerance have illuminated a role for combinations of checkpoint molecules in regulating the immune response. Remarkably, despite the differences in these models, several broad conclusions have emerged. First, in many pre-clinical models of T cell tolerance and exhaustion, as well as in human disease, multiple immune checkpoint molecules are co-expressed on CD4 and CD8 T cells. Second, certain combinations of checkpoint molecules are expressed more frequently than other combinations, in many cases involving co-expression of PD-1 with other molecules. A potentially central role for PD-1 in tumor tolerance is supported by data showing expression on TIL in many tumor types[1], both in mice as well as humans, as well as by data showing that PD-1 is up-regulated at the first division in a tolerogenic environment[54]. While patterns of checkpoint co-expression have only begun to be analyzed in cancer patients, those accumulating data could be quite valuable in designing combination regimens; in fact it could very well turn out that combination checkpoint blockade requires a personalized approach to achieve maximal efficacy. Lastly, and perhaps most important, are recent clinical data in melanoma patients; a great deal of additional clinical work is required to understand the potential for combined checkpoint blockade to induce long-term clinical responses in cancer patients.
Figure Legends;

**Figure 1**: Known Signaling Pathways of Selected Checkpoint Molecules and Current Therapeutics.

Upon binding B7-1 or B7-2, CTLA-4 recruits the phosphatases SHP2 and PP2A via the YVKM motif in its cytoplasmic domain. SHP2 recruitment results in attenuation of TCR signaling by dephosphorylating the CD3\(\zeta\) chain. PP2A recruitment results in downstream dephosphorylation of AKT, further dampening the T cell activation pathway. PD-1 ligation by PD-L1 or PD-L2 also recruits SHP2 to the ITIM domain, resulting in membrane proximal decreases in TCR signaling. LAG-3 signaling is dependent on interaction with its ligand, MHC II, as well as its intracellular KIEELE domain. TIM-3 binds to Galectin-9, as well as other ligands. In the absence of ligand binding, TIM-3 is associated with Bat3, protecting the cell from TIM-3 mediated inhibition and allowing for greater activation. However, once TIM-3 binds to Galectin-9, Y265 is phosphorylated and the interaction with Bat3 is disrupted, allowing TIM-3 to deliver inhibitory signals to the T cell. BTLA and CD160 bind to Herpes Virus Entry Mediator (HVEM). BTLA contains an intracellular ITIM domain that may be important in signaling. 2B4 binds to CD48, but further signaling mechanisms are poorly understood. Ig domains are depicted in orange, mucin domains in green, cysteine rich domains in brown, and GPI anchors are depicted as bolded black lines.

Current therapeutics to block checkpoint signaling molecules include both monoclonal antibodies and Ig fusion proteins. 1) Anti-CTLA-4: Ipilimumab (BMS-734016), Tremelimumab (CP-675,206) 2) Anti-PD-1: Nivolumab (BMS-936558, MDX1106), Lambrolizumab (MK-3475), CT-011 3) Anti PD-L1: BMS-936559 (MDX1105), MEDI4736 4) PD-L2 Ig: AMP224 5) LAG-3 Ig: IMP321

Table 1: Combined Immune Checkpoint Blockade: Current Clinical Trials
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Relevant Conflicts of Interest:

CJN – none. CGD has served as a paid consultant to Bristol Myers Squibb (BMS), CoStim Inc, and Pfizer. CGD has also received sponsored research funding from BMS. These relationships are managed through the Johns Hopkins Conflict of Interest Committee.
References


Decreased proliferation
Decreased cytokine production
Decreased cytolytic function

NF-κB activation
IL-2 production
mTOR activation
Bcl-xL activation

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Table 1: Combined Immune Checkpoint Blockade: Current Clinical Trials

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