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Review

Immune Response to Cancer Therapy: Mounting an Effective Antitumor Response and Mechanisms of Resistance

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Chemotherapy and radiotherapy have been extensively used to eradicate cancer based on their direct cytocidal effects on rapidly proliferating tumor cells. Accumulating evidence indicates that these therapies also dramatically affect resident and recruited immune cells that actively support tumor growth. We now appreciate that mobilization of effector CD8$^+$ T cells enhances the efficacy of chemotherapy and radiotherapy; remarkable clinical advances have been achieved by blocking regulatory programs limiting cytotoxic CD8$^+$ T cell activity. This review discusses immune-mediated mechanisms underlying the efficacy of chemotherapy and radiotherapy, and provides a perspective on how understanding tissue-based immune mechanisms can be used to guide therapeutic approaches combining immune and cytotoxic therapies to improve outcomes for a larger subset of patients than is currently achievable.

Trends

Conventional chemotherapy and radiation therapy alter immune cell function in addition to their direct cytocidal effects on rapidly proliferating tumor cells.

CD8$^+$ T cells are often found in immunosuppressive tumor microenvironments.

These immunosuppressive microenvironments are generally tumor subtype-specific and can be mediated by a multitude of immune cells, including macrophages.

Immune checkpoint inhibitors provide durable responses in a subset of patients for many cancer subtypes.

Overcoming additional tissue-based mechanisms of immunosuppression embedded within the tumor microenvironment may improve clinical response rates.

Antitumor Immunity: CD8$^+$ T cells and their Regulation

Surrounding a nest of neoplastic tumor cells is a microenvironment consisting of diverse mesenchymal support cells (fibroblasts, adipocytes, etc.), cells forming hematogenous and lymphatic vasculature, immune cells, and a dynamically regulated extracellular matrix (ECM), all of which influence neoplastic progression to the malignant and/or metastatic state [1,2]. Resident or recruited immune cells present within the tumor microenvironment (TME) represent a diverse assemblage of both lymphoid and myeloid cells, and, depending on their activation state and phenotype, these can either promote or inhibit various aspects of tumor development [3] as well as regulate response to anticancer therapy [4,5].

Antitumor immunity is largely imposed by antigen-specific CD8$^+$ T cells [6], although tumoricidal macrophages do play a role [7]. Antigens (Ags), typically foreign substances of environmental, viral, or bacterial origin, products of somatically altered proteins, or debris from dying (apoptotic) cells, are processed and presented by major histocompatibility complex (MHC) on Ag-presenting cells (APCs), including (but not limited to) dendritic cells, macrophages, and B cells. CD8$^+$ T cells utilize T cell receptors (TCRs) to recognize MHC-presented peptides and subsequently mount an antigen-specific cytolytic attack [8,9]. In particular, Ag–TCR engagement ultimately leads to the activation and proliferation of CD8$^+$ T cells that play a crucial role in autoimmunity, response to pathogens, and tumor suppression [10–13]. Genetic rearrangement of TCRs during T cell development enables the recognition of a broad spectrum of processed Ags in the adult.

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Individual T cells vary with respect to precise structure of TCRs, and this variation endows each T cell with a different specificity for a distinct foreign entity [8]. There is growing evidence that solid tumors contain T cells specific to tumor Ags, but have either been rendered tolerant or inhibited from killing tumor cells by expression of suppressive cytokines such as interleukin (IL)-10 [14] and arginase [15], expression of ligands for immune checkpoints molecules [16], downregulation of MHC class I on tumor cells [17], or other immunosuppressive factors expressed within the TME [18]. Therefore, a major goal of immune-based therapies is to quell regulatory programs limiting $CD8^+$ T cell responses to tumor Ags. For example, selective checkpoint inhibition can unleash endogenous antitumor programs; this approach has resulted in dramatic and durable clinical responses for several tumor types. In particular, antibodies blocking cytotoxic T lymphocyte-associated protein 4 (CTLA-4) [19–21] or the programmed death (PD)-1 pathway have resulted in durable responses for various malignancies [22–26]. Despite these clinical successes, the endogenous immune responses of many patients are insufficient to mediate antitumor programs when checkpoints are blocked, possibly because of high-level expression of suppressive molecules expressed by regulatory T or B cells, or myeloid cells present within the TME.

Myeloid cells, including various subsets of monocytes, neutrophils, and macrophages, are implicated in T cell suppression [27]. Macrophages exist along a continuum of subtypes in tumors with classically activated M1-like macrophages at one end, and alternatively activated M2-like macrophages at the other [28]. In breast cancers, tumors vary with respect to relative proportions of macrophages (in general) and $CD8^+$ T cells. High macrophage ratios correlate with decreased overall survival (OS), progression-free survival (PFS), and pathologic complete response to therapy (pCR), with the most significant stratification of these parameters in women with $HER2^+$ (human epidermal growth factor receptor 2 positive) and basal/triple negative breast cancer [29,30]. Several have reported that macrophages present in several types of solid tumors are predominantly immunosuppressive (M2-like) and play a major role in suppressing the actions of $CD8^+$ T cells, as well as fostering malignancy by providing pro-growth, survival, and angiogenic molecules crucial for rapid tumor development [2,31–36]. A more extensive summary of the protumorigenic functions of macrophages has been recently reviewed elsewhere [4,37].

The functional significance of macrophage:T cell ratios has been examined in preclinical mouse models of cancer development. The data indicate that either reprogramming immunosuppressive macrophages towards an proinflammatory T helper 1 (T_H1) state or eliminating macrophages altogether, depending on the tumor context, limits tumor growth by fostering the infiltration of interferon (IFN)-γ or granzyme B-producing $CD8^+$ T cells [30,34]. These approaches yield significantly improved outcomes for tumor-bearing mice when delivered in combination with cytotoxic chemotherapy (CTX) or radiotherapy (RT), some targeted therapies, or other immunotherapeutic approaches [29,38–47]. Discussed below are implications of these findings with regards to how immunomodulatory agents interact with cytotoxic therapies and may drive potent and durable antitumor immune responses for improved clinical outcomes.

Immune Response to CTX and RT

Tumor-Intrinsic Effects

CTX and RT have tumoricidal properties that are directly linked to their ability to arrest cell cycle progression leading to cell death. Several classes of CTX (e.g., alkylating agents, nitrosureas, platinum agents, antimetabolites, antitumor antibiotics, anthracyclines, epipodophyllotoxins, vinca alkaloids, taxanes, and camptothecin analogs) have been developed, all of which kill proliferating cells by either cell cycle-specific or cell cycle-nonspecific mechanisms [48]. On the other hand, RT (e.g., ionizing radiation) leads to cell death by inducing single- and double-strand

breaks in DNA, and can be synergistic when used in combination with CTX. While initially thought to be immunologically silent, it is now clear that the massive cell death resulting from these modalities also leads to release of stress molecules and antigens into the TME, and these, in part, sculpt local (and likely systemic) immune responses to 'damage' and subsequently impact therapeutic response [49–51].

Tumor-Extrinsic Immune Effects

The fact that CTX and RT exert significant effects on tumor-infiltrating leukocytes was unappreciated in the 1950s and 60s when these modalities were being developed as mainstays for cancer therapy. Although both treatments can lead to transient depletion of resident leukocytes and/or myelosuppression via direct cytocidal activity, rebound effects following therapy do occur [52] and are known to impact response to therapy.

Immune Effects of RT

While early experimental studies revealed that RT induces tumor regression by $CD8^+$ T cell dependent mechanisms [53,54], later work specifically demonstrated that release of IFN-γ by $CD8^+$ T cells mediates these effects [55,56]. Following RT, pre-apoptotic exposure of calreticulin on tumor cell surfaces presages release of high-mobility group box 1 (HMGB1) during late apoptosis. HMGB1 subsequently binds to toll-like receptor (TLR)4 on dendritic cells (DCs), leading to antigen processing and presentation, as well as cytotoxic T cell activation [57–59]. We now appreciate that dying tumor cells release numerous stress factors in addition to HMGB1, including ATP, heat shock proteins (HSPs), and other danger-associated molecular patterns (DAMPs) that signal through TLRs on DCs, leading to DC maturation and cross-presentation of tumor antigens [60,61].

In addition to fostering DC maturation and cross-presentation to $CD8^+$ T cells, RT also promotes $CD8^+$ T cell recruitment into tumors via two mechanisms: (i) CXCR6 (CXC chemokine receptor 6)-expressing $CD8^+$ T cells are recruited to tissue in response to CXCL16 production induced by RT [62]; and (ii) RT upregulates expression of adhesion molecules E-selectin and ICAM-1 (intercellullar adhesion molecule 1) on surrounding vasculature, thereby enhancing leukocyte endothelial transmigration and entry into tissue [63]. The consequences of DC maturation and $CD8^+$ T cell mobilization are most clearly appreciated in the abscopal effect following RT where efficacy is observed in distant metastatic sites after irradiation of primary tumors [64]. Both cytotoxic $CD8^+$ T cells and Ag-presenting DCs mediate the abscopal effects, at least in animal models. In a syngeneic mammary carcinoma model, tumor growth outside the irradiation field was stunted following RT, but only in combination with FLT3 (FMS-like tyrosine kinase 3) ligand, a growth factor responsible for recruitment and maturation of DCs from bone marrow [65]. This effect was impaired in mice lacking functional T cells, indicating that cross-presentation of tumor antigens to $CD8^+$ T cells mediates this phenomenon [65]. However, it should be noted that these results are rarely observed in the clinic with RT monotherapy, likely due to an immunosuppressive TME.

Supporting this rationale are studies demonstrating that tumor-promoting monocytes and macrophages are recruited to TMEs following RT, likely mediated by upregulation of growth factors [30] or chemokines such as CXCL12 in RT-induced hypoxic regions [66,67]. Recruited macrophages in this scenario are generally immunosuppressive, support angiogenesis, and generate a protumor microenvironment. Efforts to prevent macrophage recruitment or deplete them once they have arrived following RT delays tumor regrowth when combined with RT in squamous [68], mammary [38], and prostate [39] cancers, as well as in glioblastoma [66]. Together, these studies indicate that while the ability to produce a potent and durable antitumor $CD8^+$ T cell response is induced by RT, there is often a macrophage-regulated immunosuppressive TME that thwarts those efforts. Thus, combining immunotherapies designed to

dampen macrophage-mediated immunosuppression and enhance CD8$^+$ T cell antitumor responses may prove to be more efficacious than the use of either approach alone.

Immune Effects of CTX

While the effects of the immune system on efficacy of CTX have been intensely examined and recently reviewed [4,5], the converse effects of CTX on the immune system are less studied. In many instances, treatment with CTX results in an enhanced cytotoxic CD8$^+$ T cell response. Dose-dense treatment of ovarian cancer with either cisplatin or paclitaxel yields enhanced IFN-γ production by CD8$^+$ T cells in both murine models and in patients [69]. Similarly, treatment of murine mammary carcinoma and fibrosarcoma tumors with doxorubicin results in IFN-γ-producing CD8$^+$ T cell proliferation and their recruitment to tumors [70]. As is similarly observed with RT, some types of CTX (e.g., anthracyclines, cyclophosphamide, and oxaliplatin) also activate immunogenic cell death pathways whereby cell surface expression of calreticulin is followed by release of ATP, HMGB1, and HSPs, thereby leading to DC-mediated cross-presentation of tumor antigens to CD8$^+$ T cells [71,72]. Corroborating in vitro evidence indicates that exposure of cancer cells to 5-fluoruracil or doxorubicin stimulates HSP release and promotes engulfment of cell debris by DCs, thereby promoting cross-presentation to CD8$^+$ T cells [73,74]. Similarly, when doxorubicin-treated cancer cells are injected into syngeneic mice, DCs phagocytose cell debris and generate a tumor-specific CD8$^+$ T cell antitumor immune response [75].

While CD8$^+$ T cells certainly elicit tumor cell death, natural killer (NK) cells also mediate tumor cell death and are affected by CTX, albeit with varying outcomes. NK cell effector functions have been found to be impaired in breast cancer patients treated with high-dose cyclophosphamide [76,77]. However, dosing may influence this effect because low-dose cyclophosphamide treatment was instead found to stimulate NK activity in late-stage cancer patients [78]. Moreover, CTX enhances NK recruitment and bioeffector functions in several cancer models [79–81], indicating that CTX can regulate NK cell recruitment and activity, but may be dependent on cancer type or dosing strategy.

In addition to the effects on DCs and NK cells, macrophage phenotype can also be influenced by CTX. Generally speaking, the majority of macrophages infiltrating treatment-naïve tumors exhibit gene expression patterns corresponding to M2-like macrophages [28,82,83] that are immunosuppressive in nature towards NK and T cells [84]. Thus, targeting macrophages to relieve immunosuppression by reprogramming their activities towards M1-like states is an attractive therapeutic strategy that is being pursued clinically [4]. Context and tumor-specificity will likely underlie the therapeutic responses of these treatments. Preclinical mouse models have shown that, whereas low-dose cyclophosphamide reprograms M2-like macrophages towards an M1-like state in vivo [85], other tumor types may require additional immunomodulation for effective reprogramming [29,30,34,86,87] and the elaboration of functional antitumor CD8$^+$ T cell responses.

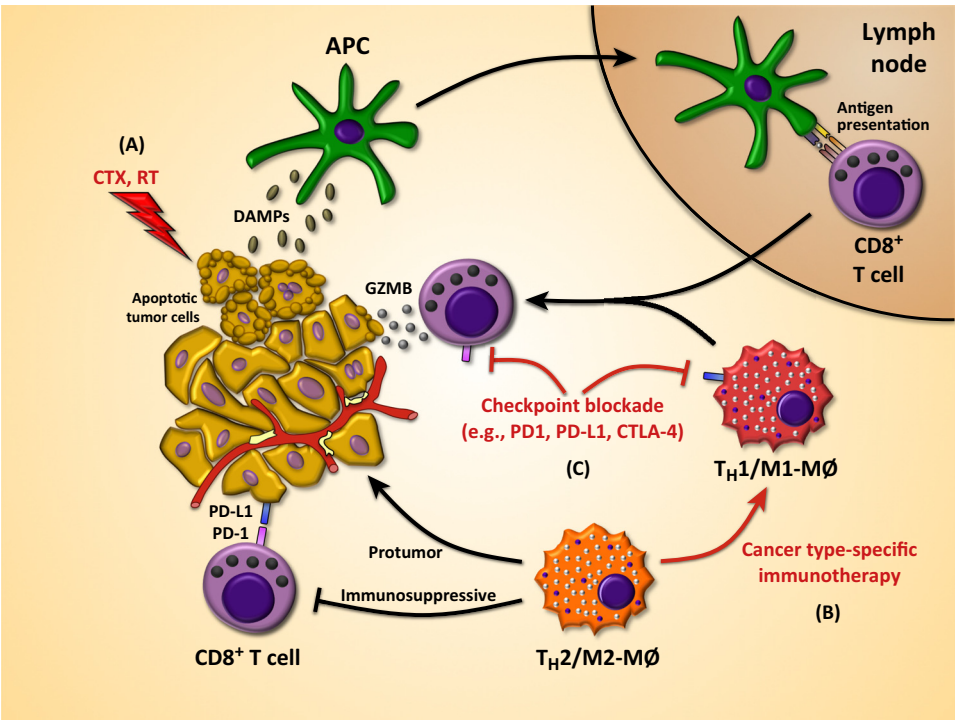
Mechanisms of Immune Resistance: Checkpoints

While the aforementioned mechanisms indicate that RT and CTX can produce robust tumor-specific immune responses through mobilization of CD8$^+$ T cells, there are often counter-regulatory mechanisms in place that restrict these responses. Most notably, immune checkpoint molecules function to limit activation of CD8$^+$ T cells following Ag stimulation by APCs [16]. While these checkpoints are necessary to dampen an active CD8$^+$ T cell immune response and to avoid autoimmune pathologies, these molecules are attractive targets for enhancing T cell mediated antitumor programs in the context of cancer. As such, therapies targeting CTLA-4 and PD-1 have been developed and evaluated clinically, and are showing tremendous efficacy in several tumor types [19–23,88–91].

While the CTLA-4 and the PD-1 pathways both have the capacity to dampen antitumor responses, their mechanisms of action are distinct. T cell expression of CTLA-4 functions to dampen T cell function by outcompeting for binding and costimulation of the TCR by CD28 [92]. Instead of functioning within the TME as is the case with PD-1, CTLA-4 functions to dampen T cell function in secondary lymphoid organs where cross-presentation occurs. It should be noted that CTLA-4 is primarily expressed on $CD4^+$ T cells and that efficacy of αCTLA-4 therapy likely relies on T helper function, although direct action of CTLA-4 on $CD8^+$ T cells as has been reported [93]. On the other hand, PD-1 is primarily expressed on $CD8^+$ T cells and has two ligands that are differentially expressed, PD-L1 and PD-L2. While PD-L2 is primarily expressed on APCs, PD-L1 is more broadly expressed and is found on neoplastic cells, immune cells, and endothelial cells [16,94,95]. Engagement of PD-1 with PD-L1 or PD-L2 within the TME dampens

Key Figure

Strategies to Bolster Antitumor Immunity



Trends in Cancer

Figure 1. (A) Apoptotic tumor cells release high-mobility group box 1 (HMGB1), ATP, heat shock proteins (HSPs), and other danger-associated molecular patterns (DAMPs) into the stroma following chemotherapy and/or radiation therapy. DAMPs signal through TLRs on DCs and lead to maturation, trafficking to lymph nodes, and cross-presentation of tumor antigens to cytotoxic T cells. (B) $CD8^+$ T cells present within the tumor microenvironment (TME) are often rendered tolerant via immunosuppressive factors released by M2 macrophages or a T helper 2 (T_H2) microenvironment, as well as expression of immune checkpoint inhibitors by various cells within the microenvironment. By utilizing cancer type-specific immunotherapy, macrophages can be repolarized such that $CD8^+$ T cells can be recruited and activated within the TME. (C) Expression of checkpoint inhibitors, such as PD-1 (programmed death 1), PD-L1, and CTLA-4 (cytotoxic T lymphocyte-associated protein 4) limits the activity of $CD8^+$ T cells. By blocking checkpoint molecules, $CD8^+$ T cells are able to mount a cytoloytic attack against tumor antigens. By targeting several or all of the pathways outlined above, the immunosuppressive TME can become immune-stimulatory and is likely to offer potent and durable antitumor immune responses in the clinic. Abbreviations: APC, antigen-presenting cell; GZMB, granzyme B; MΦ, macrophage.

the effector functions after TCR stimulation [96]. Moreover, PD-1 engagement can lead to increased migration of $CD8^+$ T cells, thereby reducing the time available for TCR stimulation and effectively rendering tumor cells invisible [97] (see [98] for a more thorough and recent review on immune checkpoints).

Several studies have evaluated patients treated with αCTLA-4 and found that T cell responses to therapy can be monitored in peripheral blood [99]. These data reveal the possibility of identifying circulating biomarkers that may be indicative of tumor response to therapy. By sequencing TCRβ chains to define diversity and frequency of T cell clones in patients with prostate cancer treated with αCTLA-4 therapy, CTLA-4 blockade was found to induce global remodeling of the T cell repertoire, a response that could be monitored in blood [99]. Although CTLA-4 blockade induced both gains and losses in the frequency of specific TCR clones, a gain in clonotype frequency predominated in patients on therapy, thereby revealing increased T cell diversity [99]. Similarly, TCR pattern changes were observed in melanoma patients treated with various immune checkpoint inhibitors within 4 weeks of treatment [100]. Of particular interest, patients with the most favorable outcomes were those whose most frequent TCRs were able to maintain an undiminished frequency during therapy, indicating that some patients have in place a set of T cells primed and ready to attack, needing only immunotherapy to unleash them, and that TCR measurements conducted at two timepoints can identify patients likely to benefit.

The success of CTLA-4 blockade led to rapid clinical evaluation of monoclonal antibodies targeting the PD-1 pathway. Antibodies against PD-1, like those targeting CTLA-4, have demonstrated significant clinical efficacy in non-small-cell lung carcinoma, melanoma, renal cell carcinoma [22], and Hodgkin's lymphoma [101]. Significantly, αPD-1 antibodies have been found to be efficacious in CTLA4-refractory disease [102]. Similarly, αPD-L1 antibodies have demonstrated efficacy in non-small-cell lung carcinoma, melanoma, renal cell carcinoma [23], and bladder cancer [103]. Because the mechanisms regulating CTLA-4 and PD-1 function are distinct with regards to regulating T cell function [104,98], combination approaches have also been investigated, and two recent clinical trials in advanced melanoma demonstrated greater efficacy using αCTLA-4 combined with αPD-1 [25,105].

Overcoming Resistance

Despite the clinical success of checkpoint inhibition, the majority of patients still fail to respond to therapy, likely due either to a lack of ligand expression or due to compensatory mechanisms limiting productive T cell infiltration [94]. Given that CTX and RT both elicit immunogenic cell death, resulting in cross-presentation of tumor antigens to $CD8^+$ T cells, several studies have combined CTX or RT with checkpoint inhibitors to investigate synergy. Indeed, RT enhances TCR diversity and, when combined with αPD-1 or αCTLA-4 antibodies, reverses T cell exhaustion and promotes T cell expansion, respectively [106]. Moreover, in mouse models of pancreatic cancer, where macrophage antagonists combined with CTX foster $CD8^+$ T cell infiltration of tumors, tumor regression is restricted by simultaneous upregulation of PD-L1 and CTLA4 [47]. Importantly, PD-1 and/or CTLA4 blockade in this context potently elicited tumor regression, even in larger established tumors [47]. Collectively, these data indicate that a combinatorial therapy for myeloid cell reprogramming should also be considered when aiming for a long-term durable antitumor response to checkpoint inhibition.

Concluding Remarks

As mainstays of tumor therapy, CTX and RT have profound effects not only on rapidly-dividing tumor cells that are the intended targets but also on cellular components of the TME that in turn regulate overall response to therapy. Because both CTX and RT elicit immunogenic cell death in tumor cells, these can also serve as an endogenous vaccine to provide tumor antigens against

which CD8$^+$ T cells can be primed [107]. Unfortunately, the protumorigenic TME limits productive antitumor immune response and thereby restricts efficacy. Consequently, combating immunosuppression and T cell exhaustion are primary targets for immunotherapy (Figure 1, Key Figure). While many are hailing the emergence of checkpoint inhibitors as a panacea for anticancer therapy, because CD8$^+$ T cells have the capacity to recognize a virtually unlimited number of tumor Ags, there are still many issues to overcome. While checkpoint blockade is undoubtedly promising, in many cases less than 20% of patients have durable responses to therapy. This raises several questions (see Outstanding Questions). Regarding whether checkpoint expression changes in response to therapy, recent studies indicate that one mechanism of resistance to αCTLA-4 treatment in melanoma is mediated by upregulation of PD-L1 by tumor cells [106]. While this is one mechanism by which resistance can occur, there are likely other mechanisms of resistance or immune escape in response to monotherapy, and future directions in both clinical and basic research should aim to understand these pathways. An additional and significant question that remains to be addressed is whether, by overcoming counter-regulatory mechanisms by which CD8$^+$ T cells exert antitumor responses, therapies will lead to subsequent autoimmune pathology. A meta-analysis of clinical trial data from stage III–IV melanoma using various immunotherapies revealed that 3.4% of all patients subsequently develop autoimmune vitiligo, while patients treated with checkpoint inhibitors fared better, with a 2.0% incidence of vitiligo [108]. Importantly, appearance of vitiligo after immunotherapy was associated with response to therapy, where an increase progression-free survival and overall survival was observed [108]. With accumulating clinical data emerging, additional adverse events in several cancer types associated with checkpoint blockade are being identified [109]. For the most part, adverse events can likely be managed with corticosteroids, and, interestingly, immunosuppression with corticosteroids does not appear to alter the clinical benefits of checkpoint blockade [110]. Efforts are currently underway both in preclinical models and in the clinic to identify efficacious combinations of immunotherapies for a majority of patients. Whether these combined therapies will have broad applicability or will require personalization based on predictive biomarkers remains to be determined. Regardless of the outcome, there is optimism for these rational combinational immunotherapeutic strategies and the hope that they will provide potent and durable antitumor immune responses in a greater number of patients than is currently achievable.

Outstanding Questions

Is there significant patient variability in the expression of checkpoint inhibitors, or is expression cancer type-specific?

Is there similar intratumoral heterogeneity for checkpoint inhibitors, as has been observed for somatic mutations and gene expression?

Does checkpoint inhibitor expression vary as a function of neoplastic progression?

Does checkpoint inhibitor expression change in response to therapy?

Will checkpoint therapies lead to subsequent autoimmune pathology?

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