



Neoadjuvant Immunotherapy for High-Risk, Resectable Malignancies: Scientific Rationale and Clinical Challenges

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Abstract

Neoadjuvant immunotherapy involves administering immune checkpoint inhibitors before surgical resection in high-risk resectable disease. This strategy was shown to have a high pathological response rate and prolonged relapse-free survival in randomized trials in melanoma, glioblastoma, and colon cancer with small numbers of patients. In resectable cancers, immune checkpoint inhibitors such as anti-programmed cell death-1 (PD1) and anti-cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) can enhance antitumor immunity by activating antigen-specific T cells found in the primary tumor. These tumor-reactive T cells continue to exert antitumor effects on remaining neoplastic cells after the resection of the primary tumor, potentially preventing relapses from occurring. Based on the scientific rationale and early clinical observations with surrogate survival endpoints, neoadjuvant immunotherapy may provide an effective alternative to other therapeutic strategies such as adjuvant treatment. However, this can be determined only by conducting randomized controlled trials comparing neoadjuvant immunotherapy with the current standard of care for each tumor site. This review discusses the cellular mechanisms that occur during successful neoadjuvant immunotherapy and highlights the clinical data from the available human studies that support the preclinical mechanistic data. Here we also discuss strategies required for successful neoadjuvant immunotherapy, including combination treatment strategies and resistance mechanisms to neoadjuvant treatment.

Surgery remains the most effective treatment modality to cure early-stage cancers (1). However, removing the tumor in its entirety with no residual cells remains challenging, because micrometastases and isolated tumor cells are hard to detect by current imaging techniques and may reside in other locations throughout the body. Immunotherapy may be combined with surgery in a neoadjuvant setting to take advantage of the immune system's ability to eradicate micrometastases, thus lowering the probability of recurrence (2). Immune checkpoint inhibitors (ICIs) used as a single agent or in combination with other types of ICIs or chemotherapy has markedly improved patient outcomes in advanced and metastatic settings (3-7). These drugs are currently the backbone treatment in many advanced and metastatic cancers such as metastatic melanoma and metastatic non-small cell lung cancer (NSCLC) (8,9).

Neoadjuvant immunotherapy is an evolving strategy in oncology that consists of administering an ICI, either anti-programmed cell death-1 (PD1), its ligand (PD-L1), anti-cytotoxic

T lymphocyte-associated protein 4 (CTLA-4) monoclonal antibodies, or in combination before surgical resection (Figure 1) (2). PD1 is highly expressed on exhausted T cells that progressively lose their effector functions such as cytokine production and cytotoxicity on continuous antigen engagement (10). Anti-PD1 and anti-PD-L1 drugs reinvigorate exhausted cytotoxic T cells, subsequently enhancing antitumor immunity (11). Anti-CTLA-4 decreases the activation threshold of naïve T cells, increasing immune responses to weak antigens such as tumor antigens (12). Activated T cells can then induce tumor cell death, which effectively reduces microscopic tumor burden and improves the probability of achieving a complete resection with surgery (1).

Neoadjuvant immunotherapy also can attenuate the immunosuppressive effects of surgery, such as the systemic release of glucocorticoids, which suppress T-cell proliferation and induce apoptosis of naïve T cells (13-15). This surgery-mediated immunosuppression creates a supportive environment for tumors to recur postoperatively. The inevitable

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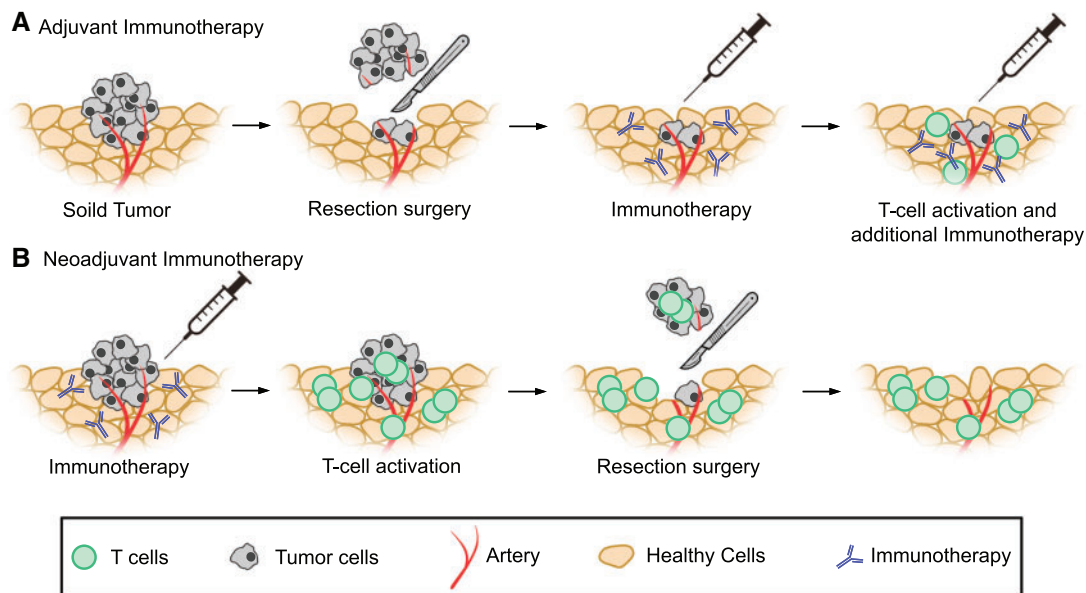


Figure 1. Comparison of adjuvant and neoadjuvant immunotherapy treatment regimens. **A)** Adjuvant immunotherapy is administered after resection surgery. Infusions may continue until complete response is seen or adverse effects become unmanageable. Immunotherapy increases the frequency of activated T cells that can eliminate residual cancer cells in the tumor bed after surgery. **B)** Neoadjuvant immunotherapy is administered before surgery. One or 2 infusions may be given depending on the tumor type. Immunotherapy can reduce the size of the primary tumor as well as eliminating residual cancers cells left after surgery.

immunosuppressive side effect of surgery is clinically significant because the immune system is a critical host mechanism for preventing relapse of cancer (16). The increase in activated T cells after neoadjuvant immunotherapy may reduce the severity of immunosuppression after surgery and lower the probability of disease relapse (17).

The mechanistic and preclinical immunological data support a role for immunotherapy in earlier treatment settings when a patients' immune system is less negatively affected by common cancer treatments such as chemotherapy and radiation therapy. With neoadjuvant immunotherapy, high pathological responses were observed in melanoma, colon cancer, and urothelial carcinoma (18-20). Moreover, in a small randomized study (n=35), neoadjuvant treatment improved overall survival compared with adjuvant therapy in patients with glioblastoma (GBM) (21). Most available clinical data on neoadjuvant immunotherapy to date consist of small early-phase studies; therefore, there are currently no US Food and Drug Administration (FDA) approvals for this approach.

These early-phase trials with small numbers of patients mainly use surrogate endpoints that cannot prove long-term benefits to patients; however, such studies are instrumental in identifying the safest and most effective ways to use single-agent and combination immunotherapy drugs. These studies also provide a unique opportunity for studying the immune mechanisms involved in effective neoadjuvant treatment that may help identify biomarkers for efficacy and toxicity. The primary endpoint of these small trials in melanoma is the pathological response rate, and although relapse-free survival appears to correlate with response rate, trials with longer follow-up are required to show long-term relapse-free survival benefit. To change the standard of care, randomized controlled trials with large numbers of patients are necessary to prove the superiority of neoadjuvant ICI therapy over adjuvant therapy that is the current standard approach.

In this review, we discuss the cellular mechanisms and biomarkers that contribute to predict successful neoadjuvant immunotherapy. This includes a discussion on currently known resistance mechanisms as well as responses to neoadjuvant treatment in various tumor types. Responses to neoadjuvant immunotherapy can be optimized by drug choice, dose, and scheduling of treatment, with the option of combinatorial strategies. Finally, we compare the neoadjuvant setting with adjuvant immunotherapy and clinical challenges of neoadjuvant immunotherapy.

Immune Mechanisms of Neoadjuvant Anti-PD1 Therapy

A single injection of pembrolizumab, an anti-PD1 agent, in resectable stage III or IV melanoma patients resulted in the expansion of Ki67⁺ PD1⁺ CTLA-4⁺ CD8⁺ T-cells in the peripheral blood of patients 7 days postinjection (Table 1). This response declined thereafter; however, the Ki67⁺ population—an indicator of cell proliferation—retained similar expression of various immune markers (PD1, CTLA-4, CD45RA, CD27, and CD39) after 3 weeks in peripheral blood, indicating similar qualitative early and late responses to neoadjuvant anti-PD1 therapy (Figure 2; Table 1) (19). Notably, this Ki67⁺ CD8⁺ T-cell population was present in patients' peripheral blood before neoadjuvant treatment, highlighting the reinvigorating properties of anti-PD1 therapy on a preexisting immune response as established before (30). These are crucial observations because neoadjuvant anti-PD1 treatment might not benefit patients who lack preexisting antitumor immunity against their cancer.

Nevertheless, 1 study reported that preexisting T-cells have limited regeneration capacity, and after anti-PD1 treatment new clones of T-cells ultimately replace them (23). This study

Table 1. Summary of the immune and cellular markers and their main function^a

Immune marker	Ligand(s)	Function
CD27	CD70	A member of the TNF receptor superfamily. Expressed on CD4 ⁺ and CD8 ⁺ T cells, B cells, and NK cells and is involved in costimulation and generation of T-cell memory (22).
CD39 (ENTPD1)	ATP, ADP	CD39 is expressed by Tregs, B cells, and tumor-specific CD8 ⁺ T cells. CD39 facilitates the hydrolysis of ATP. Its expression on T cells is indicative of antigen-induced activation, and tumor-specific T-cells express CD39, bystander T-cells in tumors lack CD39 expression (23, 24).
CD45RA	—	Mainly expressed on naïve T-cells
CD137 (4-1BB)	4-1BBL	CD137 is a member of TNF receptor superfamily with T-cell costimulatory functions. Binding of this receptor promotes T-cell proliferation and survival and enhances cytolytic effector functions (25).
CTLA-4	CD80 (B7-1), CD86 (B7-2)	Expressed on Tregs and activated T-cells. CTLA-4 competes with CD28 for binding to CD80 and CD86 expressed on antigen presenting cells and attenuates TCR signaling (11).
Ki67	—	Expressed in several cell types and is a marker of cell proliferation also observed in activated T cells (26)
PD1	PD-L1, PD-L2	Expressed on activated T-cells, NK cells, NKT cells, B cells, and some myeloid cells. It downregulates T-cell activation when bound by PD-L1 or PD-L2 expressed on tumor cells or antigen presenting cells (11).
PD-L1	PD-1, CD80 (B7-1)	Can be expressed by immune cells or tumor cells and attenuates T-cell effector functions (27, 28)
TIM-3	Galectin-9, PtdSer, HMGB1, CEACAM-1	Negatively regulates Th1 responses (28, 29)
LAG-3	MHC-II, LSECtin	Negative regulation of T-cell expansion (28)
TIGIT	CD155, CD112	Negative regulator of T-cell activity (28)

^aCTLA-4 = cytotoxic T-lymphocyte-associated protein 4; ENTPD1 = Ectonucleoside triphosphate diphosphohydrolase-1; HMGB1 = High mobility group box 1; LAG-3 = Lymphocyte-activation gene 3; MHC-II = Major Histocompatibility Complex-II; NK = Natural Killer; NKT = Natural Killer T cells; PD-1 = Programmed Cell Death Protein 1; PD-L1 = Programmed Cell Death Protein Ligand 1; PD-L2 = Programmed Cell Death Protein Ligand 2; PtdSer = Phosphatidylserine; TIGIT = T-cell immunoreceptor with Ig and ITIM domains; TIM-3 = T-cell immunoglobulin and mucin-domain containing-3; TNF = Tumor Necrosis Factor.

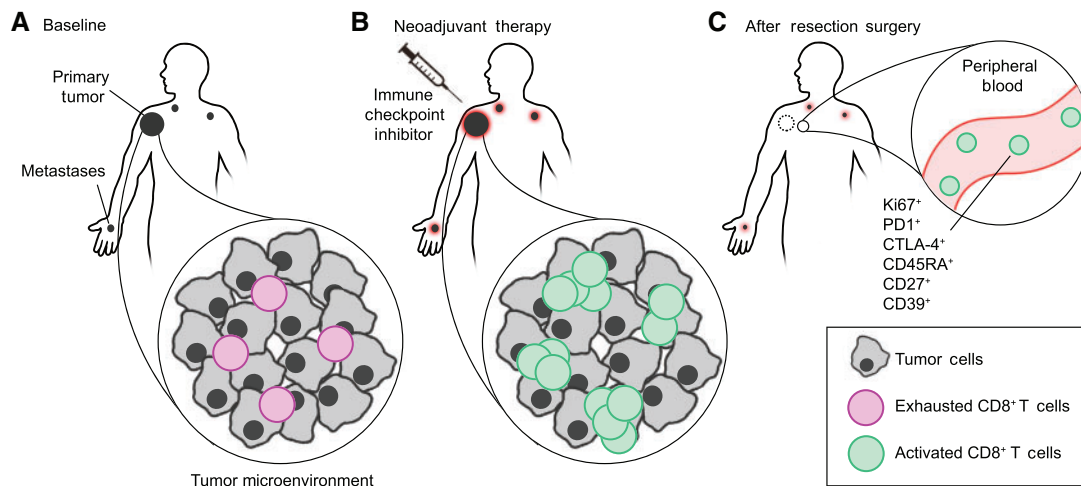


Figure 2. Expansion of CD8⁺ T cells during neoadjuvant therapy. **A)** Before neoadjuvant treatment, the primary tumor lacks activated tumor-infiltrating lymphocytes (TILs). **B)** Immune checkpoint inhibitors (ICIs) are administered before resection surgery with the intention of priming an antitumor T-cell response towards both primary tumor and any disseminated micrometastases. ICIs can induce increased infiltration TILs into the tumor and/or proliferation of T cells within the tumor. **C)** After resection surgery, activated T cells continue to circulate in the peripheral blood, eliminating micrometastases and alleviating the immunosuppressive effects of surgery.

proposes a new model for the effectiveness of anti-PD1 treatment with an emphasis on the importance of new T-cell recruitment to the tumor post-anti-PD1 treatment (23). These observations highlight the potential of neoadjuvant immunotherapy, because the presence of the primary tumor—the source of antigens—can improve activation of new T-cell clones

that may otherwise be lost in the absence of the source antigens after resection of the primary tumor. Recognition of a specific tumor antigen from the primary tumor often drives the expansion of tumor-reactive T-cells. This phenomenon known as T-cell clonality contributes to the presence of limited T-cell receptor repertoires among tumor-infiltrating lymphocytes (TILs)

and in peripheral blood. T-cell clonality can contribute to improving antitumor responses (31).

An increase in total CD8⁺, CD4⁺, and immunosuppressive regulatory T-cell (Tregs) populations in peripheral blood after anti-PD1 neoadjuvant treatment does not necessarily reflect the same phenomenon in the tumor microenvironment (TME) (19). Although CD8⁺ T-cells increase in proliferation in the tumor, CD4⁺ and Tregs often do not proliferate in the tumor despite the accumulation of Tregs in the tumor posttreatment (19). An increased number of CD8⁺ T-cells in tumors on anti-PD1 treatment may be the consequence of 1) increased proliferation of preexisting antitumor CD8⁺ T-cells within the tumor, 2) increased infiltration of proliferative CD8⁺ T-cells into the tumor, or 3) a combination of both phenomena. Current studies do not distinguish between those possibilities and attribute an increase in the number of CD8⁺ T-cells to higher infiltration of these cells into the tumor. This is a plausible explanation, given the increase in the same population of cells in peripheral blood following anti-PD1 therapy. However, this theory fails to explain why many patients who do not respond to anti-PD1 therapy still have increased proliferation of CD8⁺ T-cells despite not showing an increase in CD8⁺ T-cell numbers in their tumors.

Another critical observation in comparing peripheral CD8⁺ T-cells with TILs is the coexpression of various exhaustion markers by TILs that are absent on peripheral blood CD8⁺ T cells (19). The coexpression of exhaustion molecules indicates the presence of a highly immunosuppressive TME that does not exist in the peripheral blood. The immunosuppressive TME can explain the increase of PD1⁺ CTLA-4⁺ CD8⁺ exhausted T-cells in the tumor despite an increase in the frequency of Ki67⁺ PD1⁺ CTLA-4⁺ CD8⁺ proliferating T-cells in the blood 3 weeks after neoadjuvant anti-PD1 therapy. The lack of Ki67 expression on exhausted T-cells indicates that they are not proliferative in the TME. Nevertheless, an increase in total CD8⁺ T-cell populations in tumors is often associated with better clinical outcomes in many cancers, providing a rationale for neoadjuvant immunotherapy (32,33).

In a cohort of patients with resectable stage III or IV melanoma, some patients had an increase in the frequency of Tregs and PD-L1 expression in their tumors after neoadjuvant treatment with pembrolizumab (19). Within the tumor, an increase in Tregs and PD-L1-expressing cells post neoadjuvant therapy indicates an immunoregulatory feedback mechanism following the increase of CD8⁺ T-cells. This can contribute to limiting the efficacy of anti-PD1 therapy, hence inducing adaptive resistance. In fact, following neoadjuvant treatment, an increase in Ki67⁺ Tregs proliferation was associated with recurrence and reduced disease-free survival in patients (19). It appears that baseline proliferative CD8⁺ T-cell frequency within the tumor—Ki67⁺ CD8⁺ T-cells—is the primary driver of response and may inhibit Tregs proliferation.

The most compelling determinant of response to neoadjuvant anti-PD1 therapy in melanoma patients so far appears to be the pretreatment existence of a T-cell-inflamed phenotype in the tumor (30). Such a phenotype in tumors is often defined by an increase in the infiltration of activated T-cells, a type I interferon (IFN) gene signature, and the presence of immunostimulatory chemokines and antigen-presenting cells (30,34). The improved response of tumors with a T-cell-inflamed phenotype to neoadjuvant ICI treatment is similar to unresectable and metastatic melanoma settings where ICIs have been the standard treatment for several years. More randomized clinical trials are required to establish the role of a preexisting T-cell-

inflamed phenotype in conferring clinical benefit to patients treated with neoadjuvant immunotherapy.

A T-cell-inflamed phenotype in a treatment-naïve primary tumor can be determined by assessing the T-cell-inflamed gene expression profile known as the gene expression profile 18 score. This method examines the expression of 18 genes associated with a T-cell-inflamed signature from a tumor biopsy specimen (35). Perhaps such testing can be done in tertiary hospitals in the future. However, for current clinical practice, this T-cell-inflamed phenotype needs to be characterized by 1 or 2 key markers, such as the presence of PD1⁺ CD8⁺ T-cells and PD-L1 expression in the tumor. Hospital-based pathology laboratories should be equipped to determine the extent of T-cell infiltration by staining tumor biopsies for these markers. This may inform oncologists' decisions regarding which patients are better candidates for neoadjuvant immunotherapy. Moreover, randomized controlled trials are required to determine whether neoadjuvant immunotherapy can transform a non-T-cell-inflamed primary tumor to an inflamed phenotype. These trials should examine different treatment schedules and combinations of ICIs to increase T-cell infiltration into the primary tumor without inducing unacceptable toxicity. Conducting comprehensive preclinical studies in conjunction with small-scale translational phase I trials may also shed light on appropriate treatment schedules and drug combinations for more extensive randomized neoadjuvant studies.

Antigen-presenting cells are also crucial for neoadjuvant immunotherapy efficacy. A randomized phase Ib study of neoadjuvant vs adjuvant ipilimumab (anti-CTLA-4) and nivolumab (anti-PD1) combination in stage III melanoma patients revealed that patients who relapsed had a low expression of Batf3⁺ dendritic cell-associated genes in their tumors before treatment (36). Batf3⁺ dendritic cells are responsible for presenting tumor antigens to CD8⁺ T-cells—a phenomenon known as cross-priming—and a reduction of these cells can reduce T-cell activation.

Immune Cells Required for Effective Neoadjuvant Immunotherapy

Murine models are necessary to determine cellular mechanisms that can be further tested in clinical trials as potential biomarkers. In preclinical models of neoadjuvant immunotherapy with anti-PD1 and anti-CD137 antibodies, the efficacy of therapy mainly relied on CD8⁺ T-cells and NK cells, and partially on CD4⁺ T cells (37). An increase in the IFN- γ -producing tumor-specific CD8⁺ T-cells in the periphery and within the primary tumor of treated animals was also critical to the efficacy of treatment (37). Neoadjuvant treatment increased the frequency of tumor-specific CD8⁺ T-cells in various organs of treated animals, including the liver, spleen, and lungs. These CD8⁺ T-cells displayed an effector memory phenotype (CD44⁺ CD62L⁻), were more proliferative (Ki67⁺), and produced more effector cytokines (IFN- γ and TNF). This observation highlights the biological importance of CD8⁺ T-cells in neoadjuvant immunotherapy and explains the long-term protective effect of neoadjuvant immunotherapy against highly metastatic cancer cell lines (37).

Mechanisms of Resistance to Neoadjuvant Immunotherapy

Although establishing predictive biomarkers of response to neoadjuvant immunotherapy is crucial for patient selection, determining the mechanisms of resistance to such treatment is of

equal importance, because many patients will recur despite neoadjuvant treatment. Comparison of recurrent tumor samples with resected samples in stage III or IV melanoma patients treated with a single neoadjuvant anti-PD1 treatment did not show a statistically significant difference in the number of predicted neoantigens. Two patients displayed fewer infiltrating Ki67⁺ CD8⁺ and PD1⁺ CD8⁺ T-cells and a measurable increase of immunosuppressive CD163⁺ myeloid cells (19). These myeloid cells prevent T-cell infiltration into tumors, and depletion of these cells in preclinical models promotes tumor regression (38). The observation of a deleterious single-nucleotide variant at the TP53 gene can somewhat explain this change in the balance of immune populations in the recurrent samples. TP53 codes the tumor suppressor p53 protein that promotes apoptosis in cells on irreparable DNA damage and was absent in resected samples. The loss of p53 in the tumor can further suppress antitumor immunity by promoting myeloid-derived suppressor cell differentiation and Tregs (39). One patient with recurrent disease who showed statistically significant T-cell infiltration of the tumor with high proliferation capacity (Ki67⁺ CD8⁺ T-cells) had a loss of heterozygosity in Beta-2-microglobulin (B2M) on recurrence (19). The lack of B2M will render tumors resistant to CD8⁺ T-cell-mediated cytotoxicity and may explain the recurrence of the disease despite high infiltration into the tumor (40). More scientific analysis of clinical samples from recurrent tumors in patients treated with neoadjuvant immunotherapy may help us understand the underlying mechanisms of resistance from such a treatment strategy. Understanding what factors lead to resistance to neoadjuvant immunotherapy may provide valuable information to oncologists in determining the most logical subsequent lines of therapy. As mentioned above, a B2M mutation renders a tumor completely resistant to CD8⁺ T-cell-mediated killing because of the lack of Major Histocompatibility Complex (MHC) class I molecules on the surface of the tumor cells. Therefore, a next line of treatment involving anti-PD1 therapy may not be beneficial to the patient, and a different treatment strategy should be considered.

Biomarkers of Response to Neoadjuvant Immunotherapy

Immune Markers Associated With a Positive Response

It is desirable to determine if there are biomarkers associated with response in the neoadjuvant setting due to variable responses with immunotherapy. In a clinical trial testing of neoadjuvant atezolizumab (anti-PD-L1) in patients with urothelial carcinoma, the prominent biomarker of response was high pretreatment levels of activated tumor-infiltrating T-cells. This was determined by measuring baseline levels of tGE8, a transcriptional signature of 8 genes (*IFNG*, *CXCL9*, *CD8A*, *GZMA*, *GZMB*, *CXCL10*, *PRF1*, and *TBX21*) that involve IFN signaling, which indicates a T-cell-inflamed phenotype. Responders in this study were more likely to have T-cell-inflamed tumors before treatment (18).

In a phase Ib study of resectable stage III or IV melanoma, 29 patients were treated with a single dose of neoadjuvant pembrolizumab. Those with a major pathologic response (MPR), defined as a complete or near-complete response, 3 weeks after treatment demonstrated an accumulation of TILs. The majority of those T-cells exhibited an exhausted CD8⁺ T-cell phenotype coexpressing various checkpoint molecules, including PD1,

TIM-3, LAG-3, CTLA-4, and TIGIT (19). Notably, these CD8⁺ T-cells also expressed CD39, a marker indicating tumor reactivity; many of those T-cells were also bound with pembrolizumab 3 weeks posttreatment (41). Two patients who recurred showed a low percentage of exhausted T-cells in both resection and recurring tumors, indicating the clinical importance of immune infiltration into the tumors post neoadjuvant treatment. The increase in CD8⁺ T-cells in the tumor posttreatment correlated with Tregs proliferation. The increase of Tregs may indicate a tumor response to increased CD8⁺ T-cell accumulation in the tumor post neoadjuvant treatment. A T-cell-inflamed TME and increased TIL density were more common among GBM patients treated with neoadjuvant immunotherapy with higher overall survival than patients treated with adjuvant immunotherapy (21). These results in melanoma and GBM demonstrate the statistical significance of immune infiltration into tumors for clinical benefit post neoadjuvant treatment regardless of the vast differences between the 2 types of cancer. Nevertheless, these exploratory trials included only a small number of patients, and well-powered randomized controlled trials are required for proper assessment of clinical benefit with neoadjuvant treatment. Larger trials are also required to prospectively validate these immune biomarkers in the neoadjuvant setting.

Major Pathological Response

MPR is generally defined as $\leq 10\%$ residual viable tumor cells in the surgical specimen, while pathologic complete response (pCR) is the absence of any viable tumor cells at the time of resection (42,43). Both of these surrogate endpoints serve as predictors of relapse-free survival. In breast cancer, where neoadjuvant chemotherapy is routinely used, pCR was associated with improved disease-free survival (42). Also, pCR is being used as a primary endpoint in neoadjuvant immunotherapy studies (44,45). Melanoma patients with a complete or MPR after treatment with 1 dose of neoadjuvant pembrolizumab remained disease free after a follow-up of 25 months (19). At this time, pCR is being used as a surrogate endpoint for relapse-free survival in trials of neoadjuvant immunotherapy and will require further exploration in a large randomized controlled trial with extended follow-up. Different assessment criteria might be necessary to define pathological response for neoadjuvant immunotherapy because of different mechanisms of action between chemotherapy and immunotherapy. Therefore, an immune-related pathologic response criterion—the percentage of viable tumor in posttherapy pathology specimens—has been proposed and should be considered for neoadjuvant immunotherapy studies (45). We should note that the use of pCR and disease-free survival in the neoadjuvant immunotherapy setting should be validated in a larger randomized controlled trial.

Tumor Mutation Burden (TMB) and Mismatch Repair Deficiency

TMB refers to the number of mutations within the genome of the tumor and can be a potential predictor of a positive response to immunotherapy in some cancers (46). As the tumor acquires more mutations, resulting mutated peptides may be presented on MHC class I molecules. T cells will recognize a small proportion of these neoantigens and initiate an immune response (47). Therefore, the more mutations in a tumor genome, the higher probability of stimulating tumor-specific

T cells. In fact, responsiveness to anti-CTLA-4 and anti-PD1 was found to correlate with TMB in metastatic melanoma and advanced NSCLC (48,49). High TMB may be caused by dysfunctional DNA repair mechanisms, thus accelerating the number of spontaneously acquired mutations within the genome (50). Since TMB is an emerging biomarker for ICIs in advanced settings, more clinical studies are required to examine its predictive role in the neoadjuvant setting as well.

Besides TMB, cancers that are mismatch repair deficient (dMMR) or microsatellite instability-high (MSI-H) often show improved response to ICIs regardless of the type of cancer. Therefore, the FDA has approved pembrolizumab for the treatment of all dMMR or MSI-H solid tumors regardless of histology (30). The ICI sensitivity of dMMR and MSI-H tumors is mainly attributed to the presence of more neoantigens and numerous insertions and deletions that accumulate within the genome (30,51). Chalabi et al. (52) compared the antitumor response in dMMR and mismatch repair proficient advanced colon cancers when treated with neoadjuvant ipilimumab and nivolumab (NCT03026140). While pathological response was present in all dMMR patients, MPR were observed in 19 of 20 dMMR patients. Twelve of these patients (60%) had a pCR. However, only 4 of 15 (27%) mismatch repair proficient patients showed pathological responses. This indicates that when choosing candidates for neoadjuvant immunotherapy, dMMR and MSI-H status of tumors should be accounted for, because those with tumors with higher neoantigen levels such as many dMMR colon cancers are more likely to benefit.

Neoadjuvant vs Adjuvant Immunotherapy

Whereas neoadjuvant therapy is administered before surgery, adjuvant therapy is administered afterward (Figure 1). In a small randomized trial, 35 patients with recurrent and resectable GBM were treated with pembrolizumab either before or after surgery. The median overall survival was almost twice as long with neoadjuvant therapy compared with adjuvant therapy. A substantial proportion of patients in the neoadjuvant arm had activated T-cell gene signatures in their tumors compared with those who received adjuvant therapy. Moreover, patients who received neoadjuvant pembrolizumab showed a downregulation of cell cycle-associated genes—an indication of aggressive tumor growth—in their tumors compared with patients in the adjuvant arm (21). This suggests that neoadjuvant therapy may be more effective because it increases the number of activated T cells.

In the clinical trial setting, neoadjuvant therapy usually involves fewer infusions of immunotherapy than adjuvant therapy (19-21). From a biological standpoint, our knowledge about the long-term effect of continuous anti-PD1 treatment on the available pool of T-cell clones in the body is not well understood. Adjuvant treatment provides an evolutionary pressure, at least in theory, to select resistant clones that would not respond to available immunotherapeutics in relapsed patients. Tumors from relapsed metastatic melanoma patients commonly show loss-of-function mutations in IFN-receptor-associated Janus kinase 1 (JAK1), Janus kinase 2 (JAK2), or B2M (53). Both JAK1 and JAK2 are required for sensitivity to IFN γ , which has antiproliferative effects (54,55). B2M is also essential for peptide presentation, and thus, T-cells cannot be activated in its absence. Whole-exome sequencing revealed that both baseline and posttreatment biopsies shared multiple mutations. However, JAK1 and JAK2 mutations were present only in

posttreatment biopsies in melanoma patients who had a relapse following an initial response to anti-PD1 treatment (53). This suggests that mutations were acquired and expanded due to the continuous selection pressure of immunotherapy. The same mechanism of resistance potentially takes place in the adjuvant setting where the standard duration of treatment is 1 year. Therefore, a purely neoadjuvant treatment that provides reduced exposure to immunotherapy may avoid the development of potentially immunotherapy-refractory clones in patients who relapse.

Neoadjuvant Immunotherapy in Immune-Hot and -Cold Tumors

Immunologically hot tumors are those with a T-cell-inflamed phenotype that demonstrate high T-cell infiltration and an IFN signature (30). These tumors often respond better to ICIs than tumors with an immunologically cold phenotype (lack of T-cell infiltration and IFN signature) and low TMB such as GBM. Neoadjuvant immunotherapy has the potential to prime antitumor immunity in the presence of the primary tumor. Priming not only enhances the antitumor immunity in patients with hot tumors but also benefits patients with cold tumors that might not experience meaningful immune priming before surgery. Furthermore, the initial priming of antitumor immunity in a neoadjuvant setting can, in theory, improve patients' response to adjuvant treatment with immunotherapy in melanoma or chemotherapy in GBM, breast, and pancreatic cancer. Neoadjuvant combination of ipilimumab (3 mg kg⁻¹) and nivolumab (1 mg kg⁻¹) given as 2 courses before and 2 after surgery in stage III melanoma patients (n = 10) was feasible in a randomized phase Ib trial, and all patients underwent surgery on schedule despite clinically significant toxicity. Pathological responses were observed in 7 of 9 (78%) of the patients treated with neoadjuvant combination immunotherapy with no relapses at a median follow-up of 25.6 months (56). These findings suggest that neoadjuvant therapy provides an additional opportunity to further prime the antitumor immune response against an immune-hot tumor such as melanoma.

Another trial of neoadjuvant therapy combined ipilimumab and nivolumab and compared it with neoadjuvant nivolumab alone in high-risk stage III melanoma patients (n = 23) showing a high objective response rate in the combination arm (73%) vs a modest objective response rate in the nivolumab arm (25%). pCR was also much higher in the combination arm (45%) compared with nivolumab alone (25%). However, treatment-related toxicities were also much higher in the combination arm with 73% grade 3 immune-related adverse events (ir-AEs) compared with only 8% in the nivolumab arm (20). Nevertheless, larger randomized studies are required to confirm these findings.

One dose of nivolumab administered to patients with GBM, an immune-cold tumor, before surgery increased the expression of chemoattractant cytokines compared with patients undergoing standard radiation and chemotherapy treatment after resection. Nivolumab treatment increased T-cell clonality even after surgery (57). In fact, those who had higher T-cell receptor clonality had more prolonged overall survival. However, patients receiving nivolumab did not have a statistically significant change in the percentage of T cells within the tumor, and this change was comparable with the standard treatment. The

mutated intracranial immune response may be due to the corticosteroids administered during the study (57).

Choice of Therapeutic Agents for Neoadjuvant Immunotherapy

Several FDA-approved monoclonal antibodies are available for PD1 blockade (pembrolizumab and nivolumab), PDL-1 blockade (atezolizumab, durvalumab, and avelumab), and CTLA-4 blockade (ipilimumab and tremelimumab). These agents are approved for several types of advanced unresectable cancers (58). Some of these agents are approved for overlapping indications, yet little is known about which drug has the best efficacy with the lowest risk of serious adverse events. In the randomized phase III CheckMate 238 trial that compared ipilimumab with nivolumab in the adjuvant setting for patients with resectable stage III or IV melanoma, nivolumab was associated with a superior relapse-free survival with a hazard ratio for recurrence or death of 0.65 ($P < .001$) (5). Patients in the ipilimumab group were more likely to discontinue treatment because of grade 3 or 4 ir-AEs (5). These results show that nivolumab is superior in efficacy and toxicity when used as adjuvant treatment in resected stage III or IV melanoma. In BRAF wild-type melanoma, adjuvant anti-PD1 therapy is the standard of care, whereas ipilimumab is no longer recommended as standard adjuvant treatment in the National Comprehensive Cancer Network guidelines. Furthermore, regulatory approval for ipilimumab was not sought from Health Canada or the European Medicines Agency. Early-phase studies in the neoadjuvant settings are ongoing. Of 10 stage III melanoma patients treated with the standard ipilimumab and nivolumab doses before and after surgery, 9 patients experienced grade 3 or 4 ir-AEs. Only 1 patient was well enough to receive all 4 treatments (56). This suggests that more clinical studies are required to find effective doses of this neoadjuvant combination treatment and the optimum number of treatments to minimize severe toxicities that may be life-threatening or potentially delaying curative surgery.

Timing and Treatment Schedule of Neoadjuvant Immunotherapy

The time interval between initial immunotherapy administration and resection can determine the response to neoadjuvant immunotherapy. However, the safety of delivering an effective dose should be considered, because high toxicity can potentially delay curative surgery in patients with resectable disease. The standard ipilimumab plus nivolumab dosing schedule in melanoma patients was more effective compared with monotherapy in inducing pathological response in most patients, yet the rate of severe toxicity prevented its use in larger clinical trials (20,56). The observation led to the OpACIN-neo trial that compared various dosing schedules of ipilimumab plus nivolumab in stage III melanoma patients with the goal of identifying a less toxic but similarly effective dosing schedule (59). Investigators compared 3 different dosing schedules of ipilimumab and nivolumab before surgery in stage III melanoma patients and found that 2 cycles of ipilimumab (1 mg/kg) plus nivolumab (3 mg/kg) before surgery was tolerable for most patients, with a high proportion of patients having a pathological response in their tumors (59). The OpACIN-Neo trial has an ongoing expansion cohort (PRADO trial) that is showing both tolerability and efficacy of this dosing schedule and showing promise for future phase III studies (60). It is important to note that similar studies

are required for other malignancies to determine a safe yet effective neoadjuvant dosing schedule.

A preclinical study showed that overall survival was improved when immunotherapy was given 4-5 days before surgery compared with 10 days before surgery. Notably, efficacy was also lost if neoadjuvant treatment was administered within 2 days of surgery (61). The optimal window to conduct surgery after neoadjuvant immunotherapy could be determined by the time of maximal T-cell activation. Huang et al. (19) have shown that the percentage of Ki67⁺ CD8⁺ T cells peaked 7 days after anti-PD1 administration in melanoma patients with resectable disease. There is also evidence that CD39⁺ tumor-reactive T cells continue to expand until 9 days after anti-PD1 (41). These observations suggest that the optimal time to conduct surgery might be approximately 1 week after anti-PD1 administration. However, achieving the optimal timing may pose a logistical challenge for smaller hospitals, where access to operation rooms may not always be available on a specific day. Nevertheless, more clinical studies are required to determine the optimal timing of neoadjuvant immunotherapy before surgery.

Combination Strategies in the Neoadjuvant Immunotherapy Setting

Single-agent anti-PD1 neoadjuvant therapy did not effectively induce long-term survival in a mouse model of triple-negative breast cancer. However, the neoadjuvant combination treatment of anti-PD1 and anti-CD137 resulted in 50% of the treated animals' long-term survival, providing a preclinical rationale for combination neoadjuvant studies in humans (37). The combination of different ICIs in the neoadjuvant setting is currently under investigation (17). Here, we discuss the rationale for combining immunotherapy with more traditional cancer treatment strategies, such as radiation therapy and chemotherapy, in the neoadjuvant setting.

Combination of Neoadjuvant Immunotherapy and Neoadjuvant Radiation Therapy

Radiation therapy was first used to treat ulcerated breast cancer and has continued to be effective for local disease control by inducing lethal damage to the tumor DNA (62). In addition to reducing tumor burden, radiation can also induce an immune response. The dying tumor cells expose tumor-specific antigens that dendritic cells uptake and use to prime T cells (63). Therefore, combining immunotherapy with radiation in the neoadjuvant setting could further amplify T-cell responses. Thus, such a combination may have synergistic effects as neoadjuvant therapy, particularly in immune-cold tumors.

In a case study of 4 patients with unresectable stage III melanoma, patients received either pembrolizumab or nivolumab concurrently with fractionated doses of radiation. In all cases, lesions reduced in size, making surgery feasible, and 2 patients remained recurrence free 5 months after surgery (64). Several parameters should be explored, including the timing of radiation with immunotherapy administration. Studies have shown that this is therapy dependant. For example, a combination of ipilimumab and radiation is optimal if ipilimumab is administered before the radiation, but anti-OX40 was more effective when given the day after radiation (65). Another important variable in such combinations is the specific type of radiation administered. Stereotactic ablative radiotherapy (SABR) that precisely delivers high doses of radiation (usually 50-60 Gy) to

small targets has been proposed to have immunomodulatory effects that may increase the efficacy of immunotherapy in the neoadjuvant setting (66). However, regional and distant recurrence rates in NSCLC patients treated with SABR before surgery were not better than historically expected values. This finding suggests that SABR alone does not induce clinically meaningful immunological effects when delivered without immunotherapy (67). Platform trials that compare different methods of radiation therapy in combination with an immunotherapy agent before surgery can be informative in choosing the type of radiation therapy for more extensive randomized neoadjuvant trials.

Combination of Neoadjuvant Immunotherapy and Neoadjuvant Chemotherapy

Neoadjuvant chemotherapy is commonly used to reduce tumor size or downstage before surgery in several tumor types. Immunotherapy does not directly target tumor cells; thus, it does not have a direct cytotoxic effect like conventional chemotherapy (30). Therefore, neoadjuvant immunotherapy cannot replace neoadjuvant chemotherapy when the goal of neoadjuvant treatment is reducing tumor volume before surgery. Neoadjuvant immunotherapy can, in theory, prime antitumor immunity in the presence of the primary tumor. The goal of this treatment is to reinvigorate tumor-reactive T cells so that activated T cells can continue identifying and eliminating neoplastic cells after surgery.

While neoadjuvant combination immunotherapy generated long-term survivors in a preclinical model of breast cancer, neoadjuvant chemotherapy failed to produce comparable results (37). However, given the different mechanisms of action of neoadjuvant chemotherapy and immunotherapy, such combinations may work synergistically. Some chemotherapy agents, such as cyclophosphamide and oxaliplatin, among others, can induce immunogenic cell death (68). Immunogenic cell death releases tumor antigens to the environment; much like using a combination of radiation and immunotherapy as neoadjuvant therapies, the combination of chemotherapy and immunotherapy can also boost T-cell responses. For example, anthocyanins such as doxorubicin increase the expression of the “eat-me-signal” calreticulin on the surface of tumor cells. This increases phagocytosis by dendritic cells and subsequent activation of T cells (69). Tumor cells also release type I IFN on exposure to anthocyanins, which aids CD8⁺ T-cell proliferation (70). In a phase III trial in triple-negative breast cancer, pembrolizumab was combined with multiple conventional chemotherapeutic agents before surgery (paclitaxel, carboplatin, and doxorubicin–cyclophosphamide or epirubicin–cyclophosphamide). The percentage of patients who had pCR was 64.8% in the pembrolizumab-chemotherapy group and 51.2% in the placebo-chemotherapy group. Notably, there was a higher percentage of grade 3 or higher adverse events in the pembrolizumab-chemotherapy group than in the placebo-chemotherapy group (71). These findings suggest that immunotherapy and chemotherapy may be more effective when combined. However, more studies are required to identify an effective and safe dose for such combinations that might differ from the current standard doses.

Clinical Challenges and Risks of Neoadjuvant Immunotherapy

One of the main concerns with using neoadjuvant immunotherapy is the risk of delaying curative surgery to remove the primary tumor. Delays in surgery may take place because of

1) severe toxicities that are associated with immunotherapy, 2) misdiagnosing progression in the presence of pseudoprogression of the primary tumor because of induced immune activation and associated inflammation, and 3) actual progression on treatment rendering the tumor unresectable. Specific combination immunotherapies such as ipilimumab plus nivolumab are more likely to cause ir-AEs that could delay surgery. A combination of ipilimumab and nivolumab has shown a high toxicity rate in a small cohort of melanoma patients in the neoadjuvant setting when used at doses commonly used in advanced disease (56). Although this neoadjuvant combination did not delay surgery, it resulted in such frequent discontinuation of treatment that combination therapy at the current dosing would potentially limit broad application in a neoadjuvant setting.

Pseudoprogression is delayed tumor shrinkage following an increase in primary lesion size or appearance of new lesions in response to immunotherapy that is not due to actual progression or increased number of tumor cells. It is related to the inflammatory response associated with immunotherapy (72). Pseudoprogression can be mistaken for progressive disease, which can be determined only if the tumor decreases in size afterward. The main concern with pseudoprogression in neoadjuvant immunotherapy is the potential for delay in surgery because of the increased size of the primary tumor. We should note that despite the potential for pseudoprogression in the neoadjuvant setting, none of the reported studies of neoadjuvant immunotherapy have reported issues with delayed surgery so far, albeit small sample sizes.

Preclinical and clinical studies have both shown that neoadjuvant immunotherapy holds promise in improving long-term outcomes such as relapse-free survival. ICIs administered before surgery induce a robust immune response with antitumor effects even after primary tumor resection. Such immune responses may prevent metastases and prolong the period of relapse-free survival in patients. This clinical effect is likely due to T cells having broader access to antigens for activation before surgery. Therefore, understanding the underlying immune mechanisms of successful neoadjuvant immunotherapy is key in designing prospective clinical trials testing such strategy in different tumor settings.

Nevertheless, optimal dosing, schedules, and combination therapies are still under evaluation across tumor types. The clinical outcomes of such trials should be correlated to immune cell abundance and marker expression, where such findings can advance our understanding of biomarkers that may predict patient response and allow for more personalized treatment in the future. Finally, larger randomized controlled trials are required to prove survival benefit to patients who receive neoadjuvant immunotherapy, and the predictive role of surrogate endpoints such as pCR and immune-related pathologic response criterion should be further investigated.

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