Neoadjuvant checkpoint blockade for cancer immunotherapy

Suzanne L. Topalian*, Janis M. Taube, Drew M. Pardoll

BACKGROUND: Immunotherapies that target the interaction of programmed death 1 (PD-1) with its ligands, PD-L1 and PD-L2, have ushered in the modern oncology era. The PD-1 pathway is a key mediator of local immunosuppression in the tumor microenvironment (TME) but can also modulate T cell priming against tumor antigens in secondary lymphoid tissues. In advanced inoperable cancers refractory to other treatments, drugs that block the PD-1 receptor on lymphocytes or the PD-L1 ligand on tumor and/or immune cells [anti–PD-(L)1] can mediate tumor regression. Although anti–PD-(L)1 treatment is broadly active and is regarded as a “common denominator” for cancer therapy, many tumors demonstrate de novo or acquired resistance. Using anti–PD-(L)1 therapies in the neoadjuvant (presurgical) setting, when the tumor is potentially “resectable for cure,” presents a potential solution. There is ample oncologic precedent for this approach with presurgical chemotherapies in breast and lung cancer, associating pathologic response with improved long-term clinical outcomes. Our Review focuses on the development of neoadjuvant immunotherapies in the era of PD-1 pathway blockade, highlighting particular considerations for immunological mechanisms, clinical development, and pathologic response assessments.

ADVANCES: The immunologic effects of the PD-1 pathway on T cell priming, effector function, and exhaustion suggest distinct mechanisms underlying neoadjuvant immunotherapy versus chemotherapy. Whereas neoadjuvant chemotherapy can “bulk up” tumors preoperatively, neoadjuvant immunotherapy aims to enhance systemic immunity against tumor antigens, eliminating micrometastatic tumor deposits that would otherwise be the source of postsurgical relapse. Furthermore, neoadjuvant PD-(L)1 blockade while the primary tumor is in place, as opposed to adjuvant therapy directed only against micrometastatic disease after resection, leverages higher levels of endogenous tumor antigen present in the primary tumor to enhance T cell priming.

We discuss scientific evidence that supports two different but not mutually exclusive models by which neoadjuvant PD-(L)1 blockade may promote systemic antitumor immunity. First, anti–PD-(L)1 rejuvenates tumor-specific cytotoxic T cells that already reside in the TME, causing their activation, proliferation, and trafficking to micrometastatic deposits. Second, tumor-draining lymph nodes (TDLN) appear to be the focal point for anti–PD-(L)1 1 activity, where dendritic cell presentation of tumor antigens to T cells is enhanced; these tumor-specific T cells then enter the bloodstream and migrate to tumor sites. The destruction of micrometastases is central to the notion that neoadjuvant PD-1 blockade should result in enhanced relapse-free and overall survival in operable patients who would otherwise relapse after surgery alone.

There is a strong rationale for evaluating neoadjuvant immunotherapy across tumor types. Presurgical drug administration provides abundant on-therapy tissue for in-depth mechanistic and biomarker studies. We discuss data from recent clinical trials of neoadjuvant anti–PD-(L)1 that show that pathologic tumor regression can outpace radiographic regression and demonstrate the involvement of diverse cellular subsets in this process.

OUTLOOK: At present, more than 100 clinical trials of neoadjuvant anti–PD-(L)1 blockade, as monotherapy or combination therapy, are ongoing or planned. Combining anti–PD-1 with anti–CTLA-4 (cytotoxic T lymphocyte–associated protein 4), or with multidrug chemotherapies for triple-negative breast and lung cancer, has yielded substantial pathologic response rates that are encouraging but require longer follow-up. Next-generation trials may help assign patients to postsurgical observation or intervention depending on the degree of pathologic response, similar to the precedent established with non-immunologic neoadjuvant therapies in breast cancer. Tumors resected after neoadjuvant immunotherapy provide sufficient materials for in-depth scientific interrogations that are expected to further illuminate mechanisms of response and resistance, revealing pathways and molecules that can be cotargeted in new treatment combinations to increase the efficacy of anti–PD-(L)1 therapy.

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Neoadjuvant checkpoint blockade for cancer immunotherapy

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Cancer immunotherapies that target the programmed cell death 1 (PD-1)–programmed death-ligand 1 (PD-L1) immune checkpoint pathway have ushered in the modern oncology era. Drugs that block PD-1 or PD-L1 facilitate endogenous antitumor immunity and, because of their broad activity spectrum, have been regarded as a common denominator for cancer therapy. Nevertheless, many advanced tumors demonstrate de novo or acquired treatment resistance, and ongoing research efforts are focused on improving patient outcomes. Using anti–PD-1 or anti–PD-L1 treatment against earlier stages of cancer is hypothesized to be one such solution. This Review focuses on the development of neoadjuvant (presurgical) immunotherapy in the era of PD-1 pathway blockade, highlighting particular considerations for biological mechanisms, clinical trial design, and pathologic response assessments. Findings from neoadjuvant immunotherapy studies may reveal pathways, mechanisms, and molecules that can be cotargeted in new treatment combinations to increase anti–PD-1 and anti–PD-L1 efficacy.

The advent of immunotherapies that target the interaction of programmed cell death 1 (PD-1) with its major ligands, PD-L1 and PD-L2, has ushered in the modern oncology era (1). The PD-1 pathway is a key mediator of local immunosuppression in the tumor microenvironment (TME). In advanced inoperable cancers, blocking this pathway by inhibiting the PD-1 receptor on immune cells, or the PD-L1 ligand on tumor and/or immune cells, can mediate the rejection of established cancers that are refractory to other therapeutic modalities. To date, three monoclonal antibodies that block PD-1 (pembrolizumab, nivolumab, and cemiplimab) and three that block PD-L1 (atezolizumab, durvalumab, and avelumab) have been approved for use by the U.S. Food and Drug Administration (FDA) across 17 different types of advanced unresectable cancers, in the first- and/or later-line treatment settings. The antitumor effects of these drugs are remarkably consistent, with major response rates for individual tumor types running the gamut between ~15 and ~65%. A higher likelihood of response has been associated with various biological factors, including tumor mutational burden, PD-L1 protein expression, and oncogenic virus integration (2–5). Because of the broad activity spectrum of these drugs, PD-1 pathway blockade is regarded as a “common denominator” for cancer therapy. Nevertheless, in many patients, tumors demonstrate de novo or acquired resistance to anti–PD-1 or – PD-L1 (anti–PD-(L)1) treatment; furthermore, some cancer types are particularly resistant to this treatment approach (such as pancreatic and prostate cancer). Ongoing clinical and basic scientific research efforts aim to overcome these limitations. Using anti–PD-(L)1 therapies against earlier stages of cancer is hypothesized to be one solution.

The clinical efficacy of anti–PD-(L)1 drugs, coupled with a relatively modest rate of serious side effects (10 to 20% of patients), supports the testing of these drugs against earlier stages of cancer. Recent retrospective analyses showed that in the advanced metastatic treatment setting, patients with lower tumor burdens were more likely to experience long-term survival after anti–PD-1 therapy (6, 7). This suggests that postoperative (adjuvant) anti–PD-1 therapy directed against residual micrometastatic disease in cancer types known to be responsive to anti–PD-1 might prolong relapse-free survival (RFS) and overall survival (OS). In surgically resectable metastatic melanoma (stages IIIA to IV), randomized phase 3 clinical trials demonstrated the efficacy of adjuvant anti–PD-1 therapy, leading the FDA to approve nivolumab and pembrolizumab for this indication (8, 9). Because a proportion of these patients would have been cured by surgery alone, special attention was paid to risk/benefit considerations in clinical trial design. Protocol-eligible patients were at substantial risk for tumor relapse after potentially curative surgery, according to clinicopathologic staging (10).

The neoadjuvant (presurgical) application of immunotherapy occurs at an even earlier stage of cancer development, when cancer is considered potentially “resectable for cure.” There is ample precedent for this approach with the presurgical administration of chemotherapy in breast cancer and lung cancer, for which pathologic response is associated with improved long-term outcomes (RFS and OS) (11, 12). There is also some experience with neoadjuvant immunotherapies from the era before anti–PD-1 treatment, such as anti–CTLA-4 (cytotoxic T lymphocyte–associated protein 4) treatment for bladder cancer and melanoma (13, 14) and a cancer vaccine combined with chemoradiation for pancreatic cancer (15). This Review will focus on the development of neoadjuvant immunotherapies in the era of PD-1 pathway blockade, highlighting distinct considerations for biological mechanisms, clinical development, and immune-related pathologic response assessment.

Mechanistic rationale for neoadjuvant immunotherapies based on anti–PD-1

The known immunologic effects of the PD-1 pathway on T cell priming, effector functions, and exhaustion suggest a clinical utility for neoadjuvant checkpoint blockade based on mechanisms different than neoadjuvant chemotherapy. Although neoadjuvant chemotherapy is used to “debulk” tumors preoperatively, the hypothesis that launched the current wave of neoadjuvant immunotherapy trials posits that this form of treatment will enhance the systemic T cell response to tumor antigens (Fig. 1). This systemic response is predicted to result in enhanced detection and killing of micrometastatic tumor deposits disseminated beyond the resected tumor, which are ultimately the source of postsurgical relapse. A key corollary to this hypothesis is that neoadjuvant PD-1 blockade while the primary tumor is in place, as opposed to adjuvant therapy directed only against micrometastatic disease after resection, will leverage the higher levels of endogenous tumor antigen present in the primary tumor to enhance T cell priming. Essentially, the higher tumor antigen load present in the body in the context of neoadjuvant therapy relative to postsurgery adjuvant therapy will hypothetically result in presentation to and thus priming of more tumor-specific T cells circulating systemically.

This hypothesis was directly tested preclinically by Liu, Teng, and colleagues (16) using two spontaneously metastasizing transplantable mouse breast cancer models. When the 4T1.2 and E0771 breast cancer lines are established in mammary fat pad, the primary tumor seeds metastatic cells to distant sites. The metastases are not initially observable but ultimately grow and kill the mice within 30 days after implantation, regardless of whether the primary tumor is removed. Immunotherapy with anti–PD-1, anti–PD-1 plus an agonistic antibody directed against CD137 (a tumor necrosis factor receptor family costimulatory receptor), or regulatory T cell (Treg cell) depletion was given...
either before surgical removal of the primary tumor (neoadjuvant) or afterward (adjuvant). Survival was significantly better with neo-adjuvant versus adjuvant immunotherapy, even when treatment commenced at the same time after tumor implantation. Enhanced survival was associated with greater numbers of tumor-specific CD8+ T cells in the lungs and blood and enhanced cytokine production in response to an endogenous retroviral antigen expressed by tumor cells, indicating that systemic antitumor immunity had been generated. In follow-up studies, these authors showed that a specific interval between the initiation of neoadjuvant immunotherapy and surgery was critical for the enhanced systemic antitumor effect. Performing surgery too soon after immunotherapy initiation, or waiting too long, diminished the neoadjuvant effect (17). Although it is difficult to temporally compare mouse and human data, these studies highlight the importance of systematically exploring the optimal duration of neoadjuvant immunotherapy in clinical trials.

In comparing the two immune checkpoint pathways targeted by FDA-approved therapeutic antibodies—CTLA-4 and PD-1—CTLA-4 is viewed as a “master” checkpoint. It restricts the initial priming of T cells (naive or memory) in secondary lymphoid organs by antagonizing the master costimulatory signal, CD28, which shares the same ligands with CTLA-4 (CD80 and CD86) (18–22). CTLA-4 was originally thought to restrain early T cell receptor (TCR)/CD28–mediated activation of conventional CD4+ T cells (Tconv cells) (23–25), whereas PD-1 is thought to predominantly restrain CD8+ effector T cell (Teff cell) responses in peripheral tissues (26–28). For these reasons, the primary mechanism of action for PD-1 blockade in cancer immunotherapy is generally thought to be unleashing tumor-specific cytotoxic T cells that already reside in the TME before treatment (Fig. 1) (29). Part and parcel of this “unleashed activity” by both CTLA-4 and PD-1 pathway inhibition (collectively termed checkpoint inhibition [CPI]) is the proliferation of tumor-specific T cells in the TME. Nonetheless, true intratumoral proliferation of tumor-specific T cells upon PD-1 blockade has not yet been proven in humans, although it has been surmised from findings of increased clonality of TCRs in melanomas after anti-PD-1 therapy (30). Findings of an enhanced density of T cell infiltrates in resected tumors after neoadjuvant anti-PD-1 therapy are compatible with, but do not prove, intratumoral stimulation of T cells. Because these studies do not address the antigen-specificity of the expanded clones, it is not known whether they have any specificity for tumor antigens or whether they are “by-stander” cells with specificities irrelevant to the tumor.

A number of elegant mouse studies clearly demonstrate an intratumoral dendritic cell (DC)–T cell presentation axis (Fig. 1, left). Labeling and intravital imaging studies show that CD103+ DCs with DC2 markers ingest tumor antigen and can present antigenic peptides when isolated and cocultured with antigen-specific T cells (31). A recent study by Garris, Pettit, and colleagues using intravital imaging provided evidence that DCs could present tumor antigen to T cells within the tumor itself (32). This intratumoral presentation involved DC–T cell cross-talk in which T cell responses depended on interleukin-12 (IL-12) produced by DCs in response to interferon-γ (IFN-γ) production by T cells. In some models, cross-talk between intratumoral natural killer (NK) cells and DCs amplified the ultimate activation of antitumor T cell responses within the TME (33). Siddiqui et al. showed that Tcf1+ antigen–experienced T cells within the TME are the primary pool of tumor-specific T cells activated by CPI (34).

Beyond the evidence for direct priming of T cell responses by DCs within the primary tumor, there is mounting evidence that tumor-draining lymph nodes (TDLN) are also a key site for tumor antigen presentation to tumor-specific T cells and that this process is enhanced by PD-1 pathway blockade. Over a decade ago, Chen, Pardoll, and colleagues demonstrated PD-1 expression emerging on the surface of T cells within 12 hours of first contact with antigen, by the time of first cell division in the lymph node (35, 36). Induction of anergy in autoreactive T cells through self-antigen presentation in the lymph node was partially mitigated in mice in which PD-L1 or PD-1 was knocked out (KO mice), or through antibody blockade (35–37). Tumors are known to induce immune tolerance to their own antigens (38). One could imagine from these findings that increased antitumor immunity after neoadjuvant anti-PD-1 therapy is generated not only within the TME, which possesses multiple metabolic (such as glucose and glutamine deprivation, hypoxia, and indoleamine-pyrrole 2,3-dioxygenase) and cytokine-based (such as transforming growth factor–β) general inhibitory mechanisms, but also at the level of TDLN by reversing tolerogenic antigen presentation (Fig. 1). Direct evidence that tumor antigens are brought to TDLN has come recently through a number of experimental strategies, including fluorescence labeling of tumors, engineering of tumors to express model neoantigens recognized by cognate TCR-transgenic T cells, analysis of DC subsets in tumor and TDLN by means of flow cytometry and single-cell RNA-sequencing, and DC-specific KO mice. In mouse models, Krummel and colleagues demonstrated that CD103+ migratory DCs (CD141+ cells) or CD103+ migratory DCs (CD141+) in humans could carry tumor antigen to TDLN and cross-present it to CD8+ T cells. These DCs were CD8+ and resembled the classical DCs that efficiently cross-present antigens to CD8+ T cells. By contrast, there was no evidence for tumor antigen–carrying macrophages in lymph nodes (39).
The trafficking of DCs carrying tumor antigen to TDLN was also demonstrated by Salmon et al. (40). They provided experimental evidence for classical DC (cDC) presentation of tumor antigen in TDLN. The absence of cDCs resulted in failure of CD8+ T cells to enter into the tumor parenchyma after anti–PD-1 treatment, suggesting that intratumoral T cell expansion was due to trafficking of T cells first activated in TDLN back into the tumor rather than primary intratumoral expansion. Using similar DC-specific conditional KO mice as those of Salmon et al., Liu et al. (41) showed that DCs were necessary for successful neo- adjuvant immunotherapy. A caveat in the interpretation of these studies is that DCs are absent from birth in DC KO mice, and thus the failure of anti–PD-1 to generate antitumor effects could be due to tumor-specific T cells never being “primed” to a T eff1 state (memory or exhausted) from which PD-1 pathway blockade could effect “reinvigoration.”

Although these studies confirm that DC presentation of tumor antigens to tumor-specific T cells is important in the antitumor effects of CPI, the relative roles of intratumoral versus TDLN antigen presentation and priming of tumor-specific T cells has yet to be completely resolved. The demonstration that intratumoral DCs contain tumor antigen and the capacity to present it to antigen-specific T cells in vitro does not prove that presentation to T cells is occurring within the tumor, as opposed to within the TDLN, to which DCs traffic once they express CCR7. Defining the location of DC antigen presentation in vivo is in fact quite difficult. The primary experimental approach to determining the role of lymph node priming of tumor-specific T cells has used the sphingosine 1-phosphate (SIP) receptor inhibitor FYT720, which blocks the egress of T cells out of the lymph node through efferent lymphatics. Results with FYT720 have been contradictory in various animal models of cancer. Fransen et al. showed that FYT720 treatment abrogates the antitumor efficacy of anti–PD-1 treatment (42). By contrast, other groups have found that FYT720 treatment after tumor establishment does not inhibit accumulation of tumor-specific T cells in the tumor nor mitigate antitumor immune responses; this evidence favors intratumoral T cell priming as the predominant mechanism (33, 34). However, this pharmacologic technique for inhibition of T cell egress from the lymph node is not as clean as a genetic knockout. Early studies showed that although FYT720 rapidly diminishes numbers of circulating lymphocytes, depletion is not complete; circulating T cells are reduced to ~20% of control levels, and thus, lymph node–primed T cells can still leak out into the blood after the initiation of FYT720 treatment (43). Additionally, FYT720 effects were analyzed in the primary tumor implant, not in systemic micro-

metastases, the latter of which are most relevant for the ultimate clinical value of neoadjuvant CPI. The only experimentally defined way for T cells in tissues (including the primary tumor in situ) to migrate systemically is to first traffic to draining lymph nodes through afferent lymphatics and then enter the bloodstream through efferent lymphatics. Thus, T cells activated in the primary tumor through intratumoral DC presentation upon neoadjuvant CPI would ultimately need to pass through TDLN, where they might further encounter tumor antigen-presenting DCs before entering the blood by way of the effluent lymphatic vessels.

Mechanistically, an important outcome of enhanced priming of tumor-specific T cells by DCs is to change not only the activation state but ultimately the clonal dynamics of tumor-specific T cells. Studies of clonal dynamics in neoadjuvant CPI clinical trials are discussed below, and recent analyses in mouse models and in non-neoadjuvant clinical settings are beginning to shed light on this subject. Recently, Yost, Chang, and colleagues showed that after anti–PD-1 therapy of basal cell and cutaneous squamous cell carcinomas, T cell clonal expansion was due to new clones “appearing” in the TME rather than expansion of clones already in the tumor before initiation of anti–PD-1 therapy; these findings suggest that either clones not present initially in the tumor traffic into the tumor upon anti–PD-1 treatment (memory T cells from lymph nodes) or that subdominant T cell clones present intratumorally below the detection limit before treatment are selectively expanded (44). A caveat in this work is that the tumor specificity of the T cell clones is not known based on the TCR-sequencing data presented. However, in a neoadjuvant study of anti–PD-1 treatment in humans with non-small cell lung cancer (NSCLC), the frequency of validated neoantigen-specific T cell clones decreased substantially (roughly 10-fold) in the tumor 4 weeks after treatment initiation, whereas these same clones were increasing in the periphery (45). At the time of surgery, these clones were found at highest frequency in TDLN.

More direct evidence that anti–PD-1 treatment can expand “subdominant” T cell clones has also been reported. Kamphorst et al. reported that tumor killing by reinvigorated intratumoral tumor-specific T cells after PD-1 blockade may enable de novo T cell priming against new epitopes (46). Furthermore, it has been shown that anti–PD-1 treatment may allow subdominant T clones that could not be detected at baseline to be reinvigorated. Memarnejadian et al. showed in a mouse tumor model that anti–PD-1 treatment promotes epitope spreading in autologous CD8+ T cell reactivity by preventing the fratricidal death of subdominant clones to relieve immunodominance (47).

Continued elucidation of these processes is central to the notion that neoadjuvant PD-1 blockade will clinically result in enhanced relapse-free survival, a critical determinant of overall survival in operable patients who would otherwise relapse at distant sites after resection of the primary tumor alone.

Clinical trials assessing neoadjuvant immunotherapies based on anti–PD-(L)1

Basic laboratory research has predicted the potential for neoadjuvant PD-1 pathway blockade to potentiate antitumor immunity in a productive and enduring way. Through ongoing clinical research, we are now learning whether and how these observations might translate in patients with cancer. Special considerations for clinical translation are centered on risk:benefit expectations in patient populations among which a proportion would be cured by surgery alone, although biomarkers are currently lacking to identify individuals destined to benefit. In general, neoadjuvant cancer therapies offer several potential clinical advantages, including tumor reduction before surgery and the ability to assess pathologic response as an early surrogate marker for RFS and OS. They also provide adequate tissue availability on-therapy for in-depth scientific studies to explore drug mechanism-of-action and efficacy biomarkers. However, these advantages must be weighed against the potential disadvantages of tumor progression during the neoadjuvant treatment period and the occurrence of drug-related adverse events resulting in undue surgical delay, either of which could lead to loss of a surgical option.

To date, published reports of neoadjuvant anti–PD-1–based immunotherapies have centered on relatively small investigator-initiated clinical trials with a rich correliative scientific component. In 2018, Forde et al. presented the first literature report of neoadjuvant anti–PD–1 therapy, describing an investigator-initiated phase 2 trial of nivolumab in 21 patients with high-risk (stages I to IIIA) NSCLC (45). Although all subjects were deemed “resectable for cure” by a surgical expert, the risk of postsurgical relapse and death within 5 years was estimated to be 50 to 80%. In this study, previously untreated patients received nivolumab preoperatively for a brief 4-week period. Surgery was then carried out according to standard practice, resecting the primary tumor mass along with surrounding normal lung tissue and TDLN. The treatment regimen was found to be safe, with no surgical delays, no unanticipated toxicities, one treatment-related grade 3 adverse event, and one patient who was found to have an unresectable tumor intraoperatively. Particularly revealing was a comparison of tumor response assessments conducted radiographically, with computerized tomographic (CT) scans at baseline and just before surgery, or histologically by examining the surgical specimen for evidence of pathologic response. Although partial radiographic
responses (defined as ≥30% decrease in the sum of tumor diameters) were observed in only 2 of 21 (10%) patients, major pathologic responses at the primary tumor site (≤10% viable tumor cells remaining) were seen in 9 of 20 (45%) operable cases. Thus, conventional radiographic studies underestimated the extent and rapidity of pathologic responses, which were characterized by an influx of immune cells and proliferative fibrosis, which is consistent with an immunological mechanism. The degree of pathologic response significantly correlated with tumor mutational burden and with the computationally predicted neoantigen burden. Furthermore, an in-depth study of one patient revealed that neoantigen-specific T cell clones that were present intratumorally and in TDLN expanded in the peripheral blood during neoadjuvant nivolumab therapy; these clones persisted in the periphery for weeks after tumor resection. This example appears to recapitulate findings from mouse tumor models of neoadjuvant immunotherapy described above, supporting the notion that neoadjuvant immunotherapy can amplify systemic antitumor immunity and that these effects may persist even after surgical removal of the primary tumor and regional lymph nodes. A follow-up analysis of T cell clonal dynamics from this study showed that pathologic response was correlated with

Table 1. Anti–PD-1–based neoadjuvant therapy trials in melanoma. AJCC, American Joint Commission on Cancer (10). Stage III, regional lymph node metastases; stage IIIIB/C, macroscopic regional lymph node metastases; stage IV, disseminated metastases. ORR, objective response rate (complete + partial responses) based on radiographic and clinical assessments according to RECIST1.1 (83). pCR, pathologic complete response; MPR, major pathologic response, ≤10% viable tumor cells remaining; pPR, pathologic partial response, ≤50% viable tumor cells remaining. AE, treatment-related adverse event (toxicity).

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<td>Only 10% of patients completed therapy, due to toxicity.</td>
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<td>6 weeks nivolumab 3 mg/kg + ipilimumab 1 mg/kg identified as optimal regimen.</td>
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<td>Immunologic correlates of response</td>
<td>Radiographic response associated with higher tumor PD-L1 expression at baseline, and higher CD8 T cell density, lymphoid gene expression, and TCR clonality in tumor at 3 to 4 weeks.</td>
<td>Relapse-free survival associated with pathologic response; higher PD-L1, CD3, B2M, and IFNγ–related gene expression in baseline tumor; and greater number of newly detected tumor-resident T cell clones in the peripheral blood at week 6.</td>
<td>Relapse-free survival associated with pathologic response; higher expression of genes reflecting T cell activation and IFNγ signaling in baseline tumor; brisk TILs with increased exhausted T cells (EOMES[58], Tbet[26]) and decreased proliferating Treg cells in tumor at week 3; and gene expression reflecting intratumoral angiogenesis and B cells.</td>
<td>Relapse-free survival associated with pathologic response and higher IFNγ gene expression signature in baseline tumor.</td>
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*An extension cohort of approximately 100 patients who will receive the selected optimal neoadjuvant regimen provides for limited surgery in patients achieving a pCR or MPR on biopsy.
†Anti–PD-1, nivolumab in (49) and pembrolizumab in (51).
‡In specimens resected after neoadjuvant therapy.
§Anti–PD-1 (nivolumab) plus anti-CTLA-4 (ipilimumab).
posttreatment increases in the peripheral blood of clones that were highly represented in pretreatment tumor biopsies, further supporting the notion that peripheral mobilization of intratumoral T cells is correlated with intratumoral response (48). As mentioned above, these results do not distinguish the anatomic location of tumor-specific T cell activation through neoadjuvant PD-1 pathway blockade.

Four reports of neoadjuvant immunotherapy regimens in resectable melanoma have illustrated opportunities to further deepen our mechanistic understanding of immune checkpoint blockade while underlining the practical challenges of balancing risk and benefit in clinical trial design (Table 1) (49–52). These melanoma reports represent the largest neoadjuvant anti–PD-1 immunotherapy literature in a single cancer type to date. In two studies by Amaria et al. and Blank et al., high rates of radiographic and pathologic response were achieved by combining nivolumab (anti–PD-1) with ipilimumab (anti–CTLA-4) for 8 to 9 weeks preoperatively (49, 50); this drug combination was already FDA-approved as a standard of care in advanced unresectable melanoma (53). However, efficacy in the neoadjuvant setting came at the cost of high rates of severe toxicities, which limited the conduct of these trials. Severe toxicity rates in the neoadjuvant treatment population appeared to exceed those previously documented in the advanced unresectable melanoma patient population; the investigators hypothesized that this might reflect a heightened impact of combined PD-1/CTLA-4 blockade in patients with earlier cancers and intact immune systems. By contrast, a study by Huang et al. using pembrolizumab (anti–PD-1) monotherapy for only 3 weeks preoperatively showed a lower rate of side effects but also a lower response rate (57). Subsequently, a trial reported by Rozeman et al., testing three randomized combination treatment arms, yielded a modified dosing regimen of nivolumab plus ipilimumab that had a substantial response rate and fewer severe toxicities (52). Preliminary evidence from these melanoma trials suggests that RFS may be prolonged in patients achieving a substantial pathologic response, compared with those with minimal or no pathologic response (51, 52). These results, requiring validation in larger trials, suggest that pathologic response after neoadjuvant immunotherapy may be an early surrogate marker for long-term clinical outcomes, similar to the general experience with neoadjuvant chemotherapies in breast and lung cancer (11, 12).

The anti–PD-1-based neoadjuvant trials in melanoma, although relatively small, also proffered preliminary evidence for baseline and on-treatment tumor biomarkers associated with radiographic response, pathologic response, and/or RFS (Table 1). Response markers, including increased densities of tumor-infiltrating T cells and IFN-γ–related gene expression signatures at baseline and on treatment, were subsequently also reported in a study of pembrolizumab in muscle-invasive bladder cancer (54). The melanoma trial by Blank et al. comparing neoadjuvant versus adjuvant regimens of anti–PD-1 plus anti–CTLA-4 found a greater expansion of tumor-resident T cell clones in the peripheral blood of patients enrolled on the neoadjuvant arm (50). These preliminary findings in small groups of patients mirrored preclinical studies in a mouse breast cancer models (described above) that showed superior tumor control and persistence of peripheral tumor-specific T cells with neoadjuvant compared with adjuvant immunotherapy, although they await validation in larger randomized trials (16). Similarly, a randomized trial of neoadjuvant versus adjuvant anti–PD-1 (pembrolizumab) in recurrent glioblastoma showed significantly improved RFS and OS in the neoadjuvant group, whose resection specimens were characterized by enhanced IFN-γ–related gene expression profiles and PD-L1 expression and whose on-treatment blood specimens showed expansion of T cell clones that were also found intratumorally (55). Another study in glioblastoma, which combined neoadjuvant plus adjuvant anti–PD-1 (nivolumab), found increased intratumoral chemokine gene expression, activated CD8+ T cells, and TCR diversity in on-treatment tumor specimens compared with tumors from patients who did not receive anti–PD-1 treatment (56). The systemic persistence of tumor-associated or tumor-reactive T cells suggests that a period of continued immunotherapy after surgery may further boost these immune responses and avert tumor relapse. In melanoma, for which two adjuvant anti–PD-1 therapies are already approved as standards of care, these regimens have been added to the design of some neoadjuvant immunotherapy trials (Table 1).

Surgeons are crucial clinical partners for medical oncologists and pathologists in the development of neoadjuvant immunotherapies, not only for determining the candidacy of individual patients, general guidelines for patient selection, and optimal preoperative treatment intervals but also for addressing new challenges that arise from early experience with these regimens. For example, tumors that regress rapidly or completely during the neoadjuvant treatment interval may be difficult to locate intraoperatively. This has led some investigators to suggest interventional placement of radiographic tumor markers before commencing neoadjuvant immunotherapy (57). Furthermore, if an aggressive or cosmetically disfiguring surgery would have been recommended in the absence of neoadjuvant therapy, would the same surgical approach still be required for tumors exhibiting a major response to neoadjuvant treatment? Some have suggested that surgery could be avoided entirely in the setting of a clinically and radiographically documented complete response (52), or that limited surgical interventions could be used in patients whose on-treatment tumor biopsies show a complete or major pathologic response (for example, as provided in an extension cohort of NCT02977052) (Table 1). These issues, some of which were not foreseen before the advent of active neoadjuvant immunotherapies, are the subject of ongoing and future investigations.

**Pathologic response assessment**

In oncology drug development, the gold-standard endpoint for assessing therapeutic benefit is OS, which is usually determined in large randomized trials conducted over several years. However, with the rapid development of neoadjuvant immunotherapy regimens, it would be advantageous for the field, and most importantly for patients, to identify an early indicator of long-term benefit. Pathologic response is a candidate early surrogate endpoint for OS and RFS, which might allow for expedient resulting of clinical trials, redirecting ongoing trials in real time, and making rational therapeutic decisions for individual patients.

Pathologic response criteria for neoadjuvant cancer therapy were first developed in the context of chemotherapy as a parameter portending clinical outcomes. Pathologic complete response (pCR), the most stringent criterion, is defined as the absence of any viable tumor in the definitive surgical resection specimen. It has been variably defined to refer only to the primary tumor site or to include assessment of TDLN (11). pCR has been correlated with OS in patients who received chemotherapy for muscle-invasive bladder cancer, gastric or gastroesophageal junction cancers, breast cancer, and NSCLC, with average pCR rates of 28.6, 7, 21, and 8%, respectively. (58–61) Although informative for those patients achieving a pCR, this readout misses a potential opportunity to prognosticate and make treatment decisions for the vast majority of patients. To that end, “major pathologic response” (MPR), describing a treatment effect resulting in ≤10% residual viable tumor (RVT), was proposed as an alternative endpoint (62). However, retrospective studies have suggested that OS correlates with a much larger spectrum of RVT, implying that if assessments beyond pCR and MPR could be performed, prognostication could potentially be available for all patients (12, 63).

To date, pCR and MPR are the most commonly used metrics for assessing response to neoadjuvant immunotherapy, although differences exist both within and across tumor types as to how these pathologic criteria have been assessed. For example, some studies of neoadjuvant immunotherapy in melanoma grouped patients as having a pCR, “near pCR” (MPR, ≤10% RVT), “partial pathologic response” (pPR,
to be determined as data from ongoing neoadjuvant immunotherapy trials mature. Ultimately, informative pathologic response thresholds may vary by tumor type (65). It will be necessary to collect and report data regarding %RVT in a uniform and reproducible fashion to allow for valid cross-study comparisons.

Recently, immune-related pathologic response criteria (irPRC) have been developed with the aim of assessing the full spectrum of response to immunotherapy in the complete resection specimen—that is, scoring 0 to 100% RVT at 10% intervals (66). This approach, first described in the context of neoadjuvant anti–PD-1 monotherapy in NSCLC (45), has been extended to include other tumor types, tumors from multiple anatomic locations, and combination treatment regimens based on anti–PD-1 (67). The ability to assess regional lymph nodes as well as the primary tumor site provides additional information regarding treatment response; excluding regional lymph nodes from pathologic response assessments may alter long-term outcome correlations (11, 45). Some neoadjuvant immunotherapies are now being tested in cancer types in which the primary tumor is often surgically absent at the time that immunotherapy commences, such as melanoma and Merkel cell carcinoma (57, 68). In these cases, neoadjuvant immunotherapy is directed against resectable metastases in lymph nodes and/or distant organ sites. This has necessitated the development of pathologic response scoring systems that evaluate response in metastatic sites with or without an accompanying primary tumor (64, 66, 67). New scoring systems that assess the full spectrum of potential pathologic response will require educating pathologists in academic centers as well as in the community. This will be particularly important as neoadjuvant immunotherapies become standard of care.

In addition to emphasizing pathologic assessment of the entire surgical specimen, irPRC recognize the distinct histologic characteristics of anti–PD-1–based treatment response (Fig. 2). Accurate identification of the regression bed has been shown to lead to more reproducible assessments of %RVT than historical scoring systems that do not detail this component (66, 67). The features of this zone may also provide important insights into the mechanism of action of immune checkpoint inhibitors. They include activation of diverse immune cell types such as lymphocytes, macrophages, and plasma cells associated with tertiary lymphoid structures (TLS); the stigmata of organized tumor cell death; and features of tissue repair, such as proliferative fibrosis and neovascularization (66). It is recognized that TLS support the organization of antitumor B cell and T cell responses, and an appreciation of their role in this setting could potentially be leveraged therapeutically, such as by using

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**Fig. 2. Immune-mediated tumor regression.** (A) Representative photomicrograph from a definitive surgical resection specimen from a patient with NSCLC responding to neoadjuvant immunotherapy. Hematoxylin and eosin staining was used, with 100× original magnification. HEV, high endothelial venule. (B) Schematic of the tumor bed immunoarchitecture, displaying features consistent with both T cell– and B cell–mediated local antitumor immune responses. The regression bed—the area where the tumor used to be—is characterized by hallmarks of tissue repair and wound healing, such as neovascularization and proliferative fibrosis. (C) Percent RVT using irPRC is calculated by the surface area of the RVT/surface area of the tumor bed. The tumor bed surface area includes RVT + tumor-associated stroma + necrosis + regression bed. Schematics show examples of 100, 50, 10 (MPR), and 0% (pCR) RVT.
chemokines or even synthetic scaffolds to induce TLS formation in otherwise "noninflamed" tumors (69). High expression of genes associated with angiogenesis and B cell receptor pathways was associated with prolonged RFS after neoadjuvant anti–PD-1 therapy in a melanoma study (Table 1) (51), supporting the histologic description of a tumor regression bed that extends beyond a simple T cell signature. The potential role of B-lineage cells in tumor rejection after anti–PD-1 therapy has been relatively understudied. The expansion of B cells after anti–PD-1 therapy is consistent with the original basic functional description of the PD-1 pathway (70); however, their increased detection in the TME does not necessarily equate with a requirement for tumor rejection. Additional research will be needed to define the potential role of B cells in tumor regression and to determine whether their contributions may be tumor type or context dependent (56, 71, 72). The above-described features of robust inflammation and fibrosis in responding tumors have been shown to account for apparent discrepancies between radiographic and pathologic response assessments after neoadjuvant immunotherapy (45, 52, 66, 73). Such discrepancies may depend on the kinetics of anti–PD-1 response in certain tumor types, in the context of the neoadjuvant treatment interval before radiographic restaging and surgery.

In cases in which definitive surgical resection after neoadjuvant immunotherapy would result in serious functional or cosmetic consequences—for example, locally advanced gynecologic or genitourinary carcinomas, or scalp or facial tumors—it is possible that on-treatment biopsies could be used to assess therapeutic response by using the above-described iPRC. These criteria, originally developed for assessing response to neoadjuvant anti–PD-1-based immunotherapies, have recently been applied to core needle tumor biopsies taken from patients with advanced unresectable cancers while receiving the same therapies. In a melanoma study, such assessments have already been shown to correlate with 5-year OS (73), suggesting the potential for innovations in pathologic evaluation to allow for prognostication, therapeutic decision making, and organ sparing. Association of iPRC with OS in this latter scenario supports the concept that iPRC may ultimately be correlated with long-term patient outcomes in the neoadjuvant setting. Over time, more advanced imaging tools may also become available to capitalize on the observed features of the regression bed or otherwise more accurately reflect the residual tumor burden.

Future development

More than 100 clinical trials of neoadjuvant anti–PD-(L)1 therapy are now ongoing in diverse tumor types, in which anti–PD-(L)1 is administered as monotherapy or in combination with other immunotherapies, radiation therapy, chemotherapy, kinase inhibitors, tumor-targeted antibodies, or endocrine or metabolic modulators [reviewed in (74, 75)]. Treatment combinations designed to recruit more immune cells into the tumor—such as intratumoral therapeutics (oncolytic viruses or interferon pathway agonists), cancer vaccines, and kinase inhibitors—hold promise. Furthermore, treatment combinations with increased antimelanoma efficacy might accelerate response kinetics, thus shortening the optimal presurgical treatment interval. The first wave of neoadjuvant trials has emphasized cancer types in which anti–PD-(L)1 mono- or combination therapies have already shown some efficacy in the advanced metastatic disease setting, hypothesizing that applying these treatments earlier in the course of cancer evolution will be beneficial. Early reports of safety and substantial pCR rates from neoadjuvant combinations of anti–PD-1 with anti–CTLA-4 (52), or with multidrug chemotherapies for triple-negative breast cancer (76, 77) or NSCLC (78), are encouraging but require longer follow-up for assessment of clinical efficacy endpoints. Next-generation trials may assign patients to postsurgical observation or intervention depending on the degree of pathologic response, similar to the precedent established with nonimmununologic neoadjuvant therapies in breast cancer (79).

Although conventional computerized tomographic imaging at early time points on neoadjuvant therapy often underestimates the extent of pathologic changes occurring in tumor tissues, advanced methods for CT image analysis and interpretation are currently under evaluation and may provide previously unidentified on-treatment markers of response to guide clinical decision-making (80, 81). Moreover, nuclear imaging with positron emission tomography (PET) may operationalize markers specific for immune cells, checkpoint molecules, or metabolic processes associated with neoadjuvant treatment response or resistance (82).

Tumor specimens obtained after neoadjuvant immunotherapy provide a rich source of materials for in-depth scientific interrogations that are expected to further illuminate mechanism of action for anti–PD-1 drugs. A surprisingly high B cell component observed in responding tumors has drawn attention to the potential role of this cell type in cooperating to mediate anti–PD-1 responses. The quantity of tissue obtained at surgery will allow much more extensive single-cell analyses of on-therapy immune responses within the TME, as well as analyses of residual viable tumor cells, which have been overlooked in many studies. It will be of great interest in the future to better understand the particular characteristics of these two compartments with regard to immunoreactivity, cytokine signatures, and cellular functional states, including tumor cell signaling and immune evasion mechanisms in cases with residual tumor. Findings from such studies may reveal pathways, mechanisms, and molecules that can be targeted in new treatment combinations to increase the efficacy of anti–PD(L)1 drugs.

REFERENCES AND NOTES


4. B7-h1 expression in human melanocytic lesions supports an
T cell immunity. requires T cell-dendritic cell crosstalk involving the cytokines


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Neoadjuvant checkpoint blockade for cancer immunotherapy

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Presurgical immune checkpoint blockade

Checkpoint blockade immunotherapy using antibodies that inhibit the programmed cell death 1 (PD-1) or cytotoxic T lymphocyte–associated protein 4 (CTLA-4) pathways has resulted in unprecedented clinical outcomes for certain cancers such as melanoma. Topalian et al. review advances in neoadjuvant (presurgical) immunotherapy as an important next step for enhancing the response of early-stage tumors to immune checkpoint blockade. They highlight the mechanistic rationale for neoadjuvant immunotherapy and recent neoadjuvant clinical trials based on anti–PD-1 or anti–PD-1 ligand 1 (anti–PD-L1) therapy. Pathological assessment criteria that may provide early on-treatment biomarkers to predict patient response are also discussed.

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