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Learning from clinical trials of neoadjuvant checkpoint blockade

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Neoadjuvant checkpoint inhibition, in which the therapy is administered before surgery, is a promising new approach to managing bulky but resectable melanoma, and is also being explored in other cancers. This strategy has a high pathologic response rate, which correlates with survival outcomes. The fact that biopsies are routinely available provides a unique opportunity for understanding the responses to therapy and carrying out reverse translation in which these data are used to select therapies in the clinic or in trials that are more likely to improve patient outcomes. In this Perspective, we discuss the rationale for neoadjuvant immunotherapy in resectable solid tumors based on preclinical and human translational data, summarize the results of recent clinical trials and ongoing research, and focus on future directions for enhancing reverse translation.

Immune checkpoint inhibition with CTLA-4 or PD-1 blockade, as monotherapy or in combination with other therapeutic modes, has revolutionized the management of patients with advanced malignancies, including melanoma[1–3], bladder cancer[4], non–small-cell lung cancer (NSCLC)[5,6] and renal cell carcinoma[7]. Additionally, the use of checkpoint inhibitors has evolved to become an important pillar in the multidisciplinary management of high-risk early-stage malignancies, including melanoma, in conjunction with surgery. Both adjuvant anti-PD-1 and anti-CTLA-4 monotherapy have improved the relapse-free survival (RFS) in resected stage III melanoma[8–10]; however, more than 30% of patients treated with these therapies relapse within the first two years[8–10], and a further 15–20% progress rapidly before adjuvant therapy can commence[11]. In many advanced malignancies, checkpoint inhibitors induce durable tumor control and improve overall survival[2,12,13], and, importantly, tumor burden has been associated with higher response rate and increased likelihood of a complete response[14–16]. Because melanoma is the poster child for cancer immunotherapy, with now over three years of follow-up in the neoadjuvant setting, this Perspective will focus mostly on developments with the neoadjuvant approach in melanoma.

Broad immune activation, characterized by the activation of many different T cell clones, depends on exposure to a broad range of antigens. This is because T cell activation is critically dependent on T cell receptor (TCR) signaling, which occurs in response to antigen recognition[17,18]. Thus, it is hypothesized that checkpoint inhibition in the early stages of melanoma and other cancers, such as breast, bladder, head and neck, lung and colon cancer, may be more effective when the drug is administered while the tumor is still present—in the approach known as neoadjuvant therapy—rather than after complete resection, known as adjuvant therapy. Neoadjuvant checkpoint inhibition studies, both in preclinical mouse models[19] and in humans[20], show that T cell expansion is indeed greater when checkpoint inhibition is given before complete surgical resection of the tumor compared with after surgery, and that this induces positive clinical results.

In addition, neoadjuvant therapies provide an unparalleled opportunity to explore the genetic signatures associated with responses to checkpoint blockade in homogenous patient cohorts, and more importantly, to explore the molecular mechanisms of resistance, because they yield abundant tissue samples, often including lymph node dissection material, collected after the neoadjuvant period. Harmonization of neoadjuvant trials, as proposed by the International Neoadjuvant Melanoma Consortium (INMC)[21,22], will allow intertrial comparison and accelerate the identification of biomarkers and therapeutic targets.

In this Perspective we will discuss the rationale for neoadjuvant immunotherapy in resectable solid tumors based on preclinical and human translational data, summarize the results from recent clinical trials and ongoing research, and focus on future directions to enhance reverse translation.

Rationale for neoadjuvant checkpoint inhibition in solid tumors

The reasoning underlying the strategy of neoadjuvant immunotherapy rests on its ability to induce T cell expansion, its greater utility at earlier stages of cancer when T cell function is less impaired, the routine feasibility of assessing the effects of treatment via routine biopsy of surgical specimens and the potential of immunotherapy to reduce tumor size before surgery, possibly improving surgical outcome.

T cell expansion. In a preclinical mouse model of breast cancer, neoadjuvant checkpoint inhibitor combination therapy with anti-PD-1 and anti-CD137 induces a stronger early expansion of tumor-specific $CD8^+$ T cells than the same combination applied in the adjuvant setting and is directly associated with long-term survival[19]. Furthermore, in a trial of 20 patients with macroscopic stage III melanoma, known as OpACIN (Study to Identify the Optimal Adjuvant Combination Scheme of Ipilimumab and Nivolumab in Melanoma Patients), patients treated with checkpoint inhibitors in the neoadjuvant setting showed expansion of a larger number of tumor-resident T cell clones in the peripheral blood than patients who received the same therapy in the adjuvant setting[20]. Interestingly, if a patient did not experience expansion of T cell clones that were undetectable at baseline, and thus was not capable of broadening his or her

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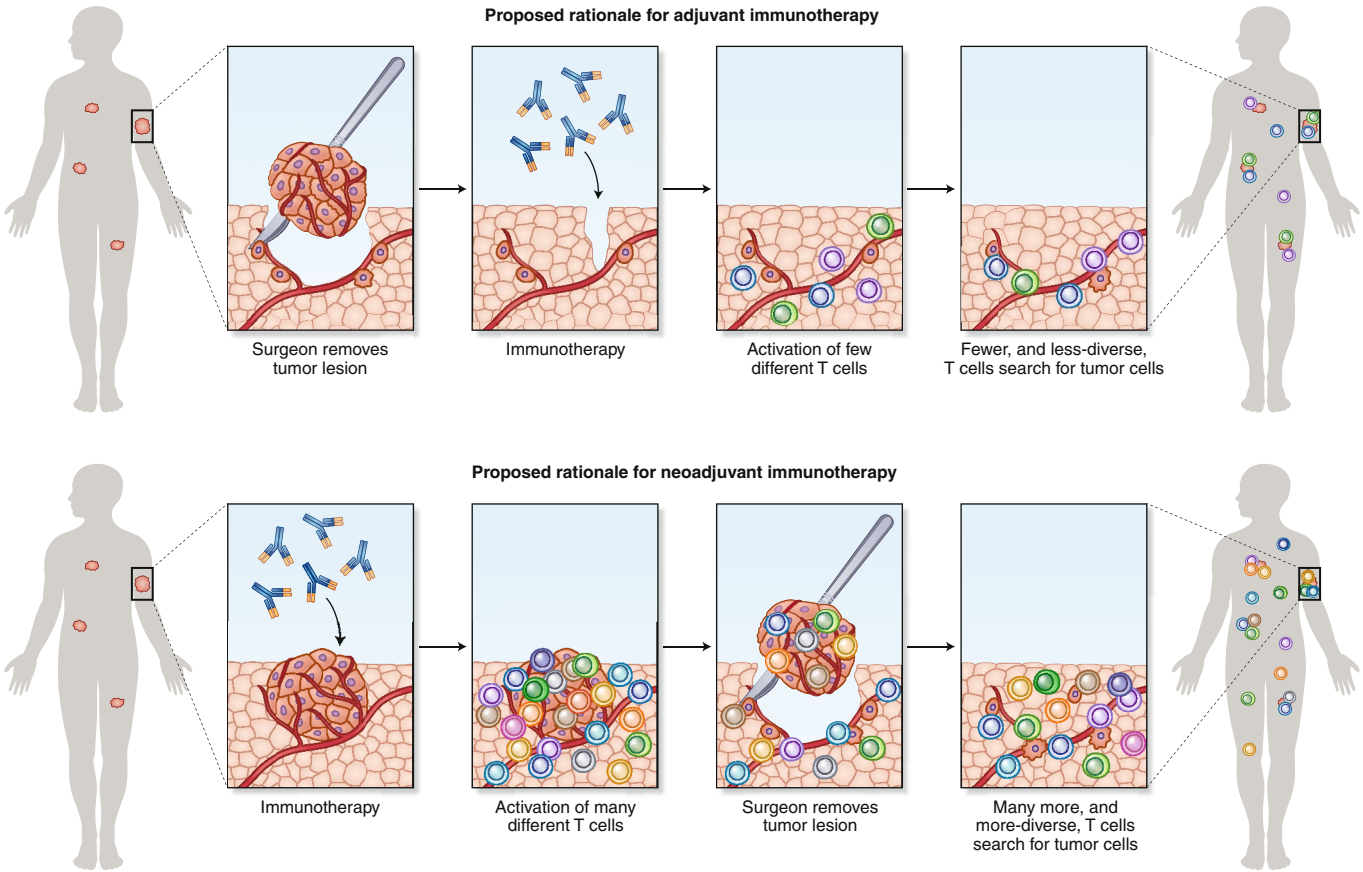

Fig. 1 | Neoadjuvant and adjuvant approaches to immunotherapy. In adjuvant approaches, shown above, immunotherapy (as indicated by the antibodies) is given after surgery, which results in the activation of T cells directed to different antigens, as indicated by the different colors. In neoadjuvant approaches, therapy is given before surgery, which results in the raising of a more diverse T cell response.

detectable T cell repertoire after neoadjuvant therapy, they relapsed. This greater T cell expansion, and greater likelihood of being free from relapses, observed in patients who received neoadjuvant therapy as compared with those treated in the adjuvant setting is consistent with the idea that the presence of the whole tumor during neoadjuvant checkpoint inhibitor therapy allows a triggering of a broader T cell response due to a larger repertoire of tumor antigen exposure (Fig. 1; it should be noted, however, that this trial was insufficiently powered for RFS comparison, and this should be confirmed in a larger cohort of patients). Similarly, three neoadjuvant checkpoint inhibitor trials demonstrated a strong expansion of tumor resident T cell clones in patients with melanoma and NSCLC[20,23,24]. Furthermore, a Batf3^{+} dendritic cell (DC) signature (indicating the presence of specialized antigen-presenting cells capable of cross-presentation within the tumor and of moving to the draining lymph node to present tumor antigen there) was associated with response and improved RFS in patients treated with neoadjuvant checkpoint inhibition in melanoma; this finding suggests a pivotal role for increased antigen presentation within the draining lymph node to explain the superiority of neoadjuvant compared with adjuvant checkpoint inhibition[20,25].

Given the induction of a stronger expansion of T cells in the neoadjuvant versus the adjuvant setting, there remains the question of what the optimal duration of neoadjuvant immunotherapy may be. This has not yet been addressed in randomized prospective human trials. The INMC has empirically selected six to eight weeks of neoadjuvant therapy as the preferred trial design, so that trial results may be pooled, tissue combined for analysis and patients kept safe without delaying potentially curative surgery too long[22].

Preclinical data suggests that timing of neoadjuvant checkpoint blockade may influence outcome[26]. In terms of human data, there have been only two trials of anti-PD1 as a neoadjuvant single agent, which used different schedules yet resulted in the same pathologic response rate of 30–33%: trial 1 used one cycle of pembrolizumab (i.e., three weeks of treatment)[27] and trial 2 used four cycles of two weekly nivolumab treatments (i.e., nine weeks of treatment)[23]. Thus, more research is needed for definitive definition of the optimal timing and convergence of the currently conflicting data from mice versus humans.

Impairment of T cell function. Incremental impairment of T cell function has been observed in early- versus late-stage (i.e., stage IV or advanced stage) melanoma patients[28], suggesting that higher tumor burden is associated with systemic immune suppression. In line with this idea, stage III patients treated with ipilimumab combined with nivolumab have higher rates of grade 3–4 immune-related toxicities than stage IV melanoma patients receiving the same dosing scheme[20,29]. The mechanism by which tumors exert systemic immunosuppression is poorly understood.

Recent work on PD-L1-carrying exosomes, extracellular vesicles containing PD-L1, derived from PD-L1-expressing tumors may provide an explanation for tumor-burden-mediated systemic immunosuppression[30]. Expression levels of PD-L1 on exosomes are significantly higher when the exosomes are derived from metastatic

melanoma cell lines than when they are derived from primary melanomas. Also, the PD-L1 levels in exosomes are higher in patients with metastatic melanoma than in healthy donors. Baseline levels of exosomal PD-L1 correlate with impaired outcome upon PD-1 blockade in stage IV melanoma[30]. Additionally, the levels of PD-L1 in exosomes in melanoma patients correlate with the levels of circulating interferon-γ (IFN-γ) and the tumor burdens, both of which are associated with poor prognosis[30]. In preclinical experiments in prostate cancer and colon cancer models, PD-L1 expression on tumor-derived exosomes, but not on the tumor cells themselves, led to the tumor escaping the immune system[31], indicating that PD-L1-mediated tumor escape might be occurring in the draining lymph node, and not only at the T cell–tumor interaction site.

Another mechanism hypothesized to mediate systemic immune suppression is cancer-associated inflammation, which is less prevalent in early-stage cancer. In preclinical experiments, a tumor with a COX-dependent inflammatory signature induced a shift toward T helper type 2 (Th2)-type immune responses[32]. This result is supported by clinical data showing that patients who responded to therapy with tumor-antigen-derived melanoma antigen gene-6 epitopes (MAGE-6), and were disease free, exhibited either a weak mixed Th1/Th2-type or a strongly polarized Th1-type response to the same epitopes[33]. COX inhibition combined with neoadjuvant checkpoint inhibition is currently being tested in a clinical trial in colorectal cancer[34]. Additionally, high C-reactive protein (CRP) levels, as a surrogate marker for cancer-associated inflammation, are associated with impaired outcome in several cancers[35]. Aside from being a surrogate marker for an inflammatory cancer response and for high interleukin-6 (IL-6) levels, CRP directly inhibits T effector cells[36]. Baseline high CRP and high IL-6 levels have been associated with impaired response to checkpoint inhibition, as well as with a shorter overall survival in metastatic melanoma[36–38].

Finally, cancer-cell intrinsic metabolic changes can also affect the metabolism, differentiation and function of tumor-infiltrating immune cells. Low glucose within the tumor environment, due to the high glucose consumption of the cancer cell, gives rise to metabolic competition between the cancer cells and activated immune cells, as both show the Warburg effect[39,40]. Aerobic glycolysis leads not only to nutrient deprivation, but also to high lactate production and subsequent acidification of the tumor environment, which is known to inhibit T cell functions[41,42].

All of the systemic inhibitory mechanisms discussed above are weaker or absent in early-stage disease[43,44].

Patient-centered factors. Neoadjuvant treatment has two practical advantages in the clinic: first, in contrast to adjuvant therapy, the individual patient's response to checkpoint inhibition is assessed by a pathologist upon surgical resection of the cancer (pathologic response)[21], and thus the de-escalation or escalation of additional systemic adjuvant therapies may be planned based on the pathologic response. Second, the tumor burden before surgery can be reduced, which may improve surgical resectability and thus reduce morbidity. In patients achieving major pathologic responses (MPR; <10% vital tumor) in an index lymph node (the largest lymph node metastasis marked before neoadjuvant therapy) after neoadjuvant immunotherapy, one might even omit extensive surgery. It has been shown that response in the index node is representative of the response in the whole lymph node bed[45], and omitting surgery in the case of MPR in the index node is an approach currently being examined in the PRADO trial[46].

Potential disadvantages. Patients who do not respond to neoadjuvant immunotherapy might deteriorate while awaiting their operation during the neoadjuvant period and miss potential curative surgery. Indeed, this has been observed in several melanoma patients receiving neoadjuvant anti-PD-1 monotherapy[23], although it may be argued that in many such cases, surgery in and of itself would be unlikely to save a patient from aggressive recurrent melanoma. Regardless, tumor progression during the neoadjuvant period was not observed in neoadjuvant trials testing combined ipilimumab and nivolumab in melanoma patients[20,47].

Another concern is that immune-related toxicity might delay timely surgery. In the OpACIN-neo trial, only one patient of 86 treated in the neoadjuvant setting did not undergo surgery due to toxicity (this patient, however, achieved an ongoing radiologic complete response)[47]. The high rate of toxicity associated with checkpoint blockade is a potential problem for patients with stage III melanoma, as observed in the OpACIN and other trials[20,23,48], as these patients are likely to have a more robust immune system compared to those with advanced melanoma. Alteration of the dosing schemes of combined ipilimumab and nivolumab in the OpACIN-neo trial substantially reduced toxicity, with only 20% of individuals developing grade 3–4 toxicities when ipilimumab was given at 1 mg/kg compared to 40% of individuals at a standard dosing of ipilimumab at 3 mg/kg, while the high pathologic response rate was preserved[47]. The high-grade toxicity of immune checkpoint blockade is largely driven by CTLA-4 blockade, but the possibility of omitting it completely was dismissed, as the cost in loss of efficacy is too high for the relative decrease in toxicity: the pathologic response rates drop from 74–78% for ipilimumab-containing schemes[20,47] to 30–33% in anti-PD-1 monotherapy schemes[23,27].

Reverse translation

Reliable access to high-volume blood and tumor biospecimens before and after treatment in standardized neoadjuvant immunotherapy trials[22] enables high-quality translational research in homogeneous populations focused on mechanisms of response and primary resistance, as well as biomarkers. The neoadjuvant platform affords a unique opportunity for reverse translation: there is a rapid and efficient method of gaining abundant, high-quality human tissue to examine primary resistance to immune checkpoint blockade therapies and identify signatures of responders. Using these data, novel therapies or therapeutic strategies can be designed. Reverse translation begins with real-time patients and clinical trial data, in contrast to the situation with bench-to-bedside research, which has a linear trajectory from laboratory research to clinical trials[49].

Examples of reverse translation. A prominent example of reverse translation as a result of further understanding derived from the neoadjuvant approach was the identification of the inducible T cell co-stimulator ICOS as being strongly associated with response in prostate cancer and bladder cancer in neoadjuvant trials of ipilimumab. The relevance of ICOS ligation to the response to therapy was confirmed in preclinical mouse models and finally returned to the clinic in the form of an agonistic anti-ICOS antibody, which is currently being tested in combination with CTLA-4 blockade in clinical trials with bladder cancer patients[50–52].

Another example of reverse translation, although not through neoadjuvant treatment, is the discovery of the biology behind the finding that microsatellite instability (MSI) tumors are highly responsive to anti-PD1 therapy. It is postulated that tumors with high mutational burden present more neoantigens and thus are highly recognizable to the immune system[53]. Indeed, in MSI-high tumors patients responding to pembrolizumab (anti-PD-1), a rapid *in vivo* expansion of neoantigen-specific T cell clones was observed, and they were reactive to neoantigens found in the patient's tumor[54]. The US Food and Drug Administration approved pembrolizumab in 2017 for the treatment of adult and pediatric patients with unresectable or metastatic MSI-high or MMR-deficient solid tumors, regardless of tumor site or histology[55]. The approval was based on data from 149 MSI-high or MMR-deficient cancer patients enrolled in five small trials, who showed response rates of up to 40%, of which

89% lasted more than 6 months[55]. Interestingly, the concept that patients with early-stage cancers have a more functional immune system than those with advanced cancer was demonstrated when 100% of patients with MSI-high colorectal carcinoma had a pathologic response to the neoadjuvant ipilimumab plus nivolumab[34], which again seems to compare favorably to the response rate observed in advanced MSI-high colorectal cancer[56].

Pathologic response as a surrogate marker of relapse-free survival. Neoadjuvant administration of drug therapy allows efficacy to be determined by pathological examination of the fully resected tumor within the individual patient. Assessing the response to a therapy after six weeks of neoadjuvant therapy in melanoma patients by pathology is superior to radiologic assessment[47], and this response can be used as a surrogate marker for RFS and OS, as shown in a pooled analyses of four neoadjuvant trials in melanoma[57]. Correlation of pathologic complete response and improved overall survival and/or distant-metastasis-free survival has been shown in neoadjuvant trials for several cancer types[58–62], and this is a longstanding accepted surrogate endpoint in breast cancer[58,63].

Different histological patterns in tumors have been identified, classified and correlated with good pathologic response to neoadjuvant therapy in melanoma; preliminary data suggests that following checkpoint blockade, tumor beds are densely inflamed and show signs of cell death, tumor regression and tissue[64] whereas after neoadjuvant targeted therapy there is more necrosis and hyalinized fibrosis[21]. Interestingly, pathologic response seems to be a stronger surrogate marker of RFS in neoadjuvant checkpoint inhibition than with neoadjuvant targeted therapies[57,65] or chemotherapy[58,63,66,67]. This difference in the prognostic value of pathologic response might be due to different mechanisms by which checkpoint inhibition and targeted therapy mediate tumor killing. For neoadjuvant checkpoint inhibition, pathologic response has yet to be accepted as an endpoint by regulatory bodies, although the INMC is working to provide the evidence[22].

Histopathologic features of response after PD-1 blockade have been described in NSCLC[68] and are characterized by fibrosis, neovascularization, cholesterol clefts, copious tumor-infiltrating lymphocytes (TILs) and tertiary lymphoid structures (TLSs). Melanoma tumors responding to neoadjuvant treatment with immune checkpoint inhibition show a denser tumor immune infiltration, plasma cell aggregate, macrophages infiltration, proliferative fibrosis and neovascularization[21].

Given the emerging heterogeneity of resistance mechanisms to checkpoint blockade in advanced cancers, it is likely that more mechanisms beside the select set outlined are important for resistance to neoadjuvant checkpoint blockade.

Tertiary lymphoid structures. TLSs are thought to have an essential role in supporting both local and systemic T and B cell antitumor responses[69]. Lymphoid neogenesis occurs in non-lymphoid organs such as tumors upon local chronic inflammation, and the presence of TLSs in the tumor microenvironment is associated with a favorable clinical outcome for cancer patients[69]. Within a tumor, $CD4^+$ T cells are focused in TLSs, whereas $CD8^+$ T cells infiltrate all areas[69]. Therefore, new therapeutic combinations that can induce TLS formation in 'cold tumors'—i.e., tumors with little immune infiltration or evidence of inflammation—might be a promising approach for patients who are identified as unlikely to respond to neoadjuvant ipilimumab plus nivolumab. Consistent with this, there is a tendency toward lower B cell signatures in patients who relapse on neoadjuvant ipilimumab plus nivolumab than in those who do not relapse[70].

Intratumoral $CD8^+$ T cells. The presence of tumor-infiltrating T cells in the tumor microenvironment is also associated with prolonged survival in several malignancies[71,72]. The absence of such cells may reflect inefficient T cell priming (T cells that are not activated in the draining lymph node due to the absence of tumor antigen, activated antigen-presenting cells or dominance of inhibitory checkpoints) or lack of T cell attraction to the tumor[43]. Increased $CD8^+$ T cell densities at the invasive margin in pretreatment tumor samples have been associated with response to PD-1 blockade in metastatic melanoma[73]. Melanoma tumors serially biopsied during anti-PD-1 treatment show a parallel increase in T cells at both the invasive margin and the tumor center in responding patients, but not in progressing patients[73]. Reduced T cell tumor infiltration and a lower productive T cell clonality within the tumor in baseline samples are also found in melanoma patients who relapse, as compared to those who are free of relapse, after treatment with ipilimumab plus nivolumab, administered either neoadjuvantly or adjuvantly[20].

Gene-expression signatures. Certain T cell gene-expression signatures are associated with improved outcomes in neoadjuvant checkpoint inhibition in melanoma[27,70,74]. Another signature indicative of an intact T cell effector response, the IFN-γ signature, was associated with improved pathologic response and freedom from relapse after neoadjuvant checkpoint inhibition in multiple studies with melanoma patients[20,23,47]. In the OpACIN trial the IFN-γ signature, T cell inflammatory signature and $Batf3^+$ DC signature were associated with improved clinical outcome[25,74–76], with the IFN-γ signature being the most discriminative signature.

$Batf3^+$ DCs that express CLEC9 and XCR1 have the special capacity to take up tumor antigen, transport it to the draining lymph node and present the antigen there[25]. Additionally, they are important in the recruitment of $CD8^+$ effector T cells. In β-catenin-expressing tumors, activated $Batf3^+$ DCs enabled the migration of T cells into the tumor[25]. Expression of $Batf3^+$ DC–associated genes, such as CLEC9, is positively associated with survival, as seen in the $Batf3^+$ DC signature[25,77].

Knowledge is lacking regarding what effect the presence of other myeloid cells has on the outcome of patients treated with neoadjuvant immune checkpoint blockade. In the OpACIN trial, myeloid cells were not associated with relapse after neoadjuvant checkpoint blockade, and in addition, the myeloid subsets of macrophages and DCs had no association with pathologic response[70].

Given these findings regarding the role of $CLEC9^+$ $Batf3^+$ DCs, up-front resistance to neoadjuvant immunotherapy may be more likely to result from insufficient T cell activation within the draining lymph node, rather than from a defect in T cell homing or effector functions within the tumor itself. This makes the targeting of mechanisms that mediate systemic immune suppression (for example, PD-L1-expressing exosomes) an attractive approach for neoadjuvant combination checkpoint inhibition.

Tumor mutational burden. In late-stage or advanced cancer, higher tumor mutational burden (TMB) is associated with response to immunotherapy in a broad range of malignancies[78–80] because of the higher number of potential neoantigens that can be recognized in these tumors[53,81]. In several neoadjuvant trials in melanoma, high TMB was associated with a higher rate of pathologic response[23,82]; furthermore, combining the IFN-γ signature and TMB showed that patients with a high baseline IFN-γ signature and high TMB achieved an unparalleled pathologic response rate of 100% after six weeks of ipilimumab plus nivolumab in the neoadjuvant setting[82]. Interestingly, only 37% of patients with a low baseline IFN-γ signature and low TMB had a pathologic response, and those scoring high on only one of the two markers had pathologic response rates of 80% and 90%[82]. This observation not only allows the up-front identification of patients who are less likely to respond, but creates an opportunity to examine the mechanisms of resistance within the resected tumors, and design novel drug therapies that restore the IFN-γ signature in this unfavorable patient group.

Table 1 | Completed neoadjuvant immunotherapy trials in melanoma

Trial	Population	Neoadjuvant regimen	No. patients	Timing surgical resection	MPR rate	pPR rate	Median follow-up
Blank et al.[20] NCT02437279	Clinical stage III	2× ipi 3 mg/kg + nivo 1 mg/kg q3w	10[a]	6 w	67% (6/9[b])	79% (7/9[b])	25.6 mo
Rozeman et al.[47] NCT02977052	Clinical stage III	Arm A: 2× ipi 3 mg/kg + nivo 1 mg/kg q3w Arm B: 2× ipi 1 mg/kg + nivo 3 mg/kg q3w Arm C: 2× ipi 3 mg/kg + 2× nivo 3 mg/kg	86	6 w	Arm A: 70% (21/30) Arm B: 63% (19/30) Arm C: 46% (12/26)	Arm A: 80% (24/30) Arm B: 77% (23/30) Arm C: 65% (17/26)	7.7 mo
Amaria et al.[23] NCT02519322	Clinical stage III, resectable stage IV	Arm A: 4× nivo 3 mg/kg q2w Arm B: 3× ipi 3 mg/kg + nivo 1 mg/kg q3w	23	8–9 w	Arm A: 25% (3/12) Arm B: 54% (6/11)[c]	Not reported	15.6 mo
Huang et al.[27] NCT02434354	Clinical stage III, resectable stage IV	1× pembro 200 mg	29	3 w	30% (8/27[b])	Not reported	25 mo
Tarhini et al.[98] NCT01608594	Clinical stage III	Arm A: ipi 3 mg/kg q3w + IFN 20 MU/m^2 5 d/w 4 w + 10 MU/m^2 q.a.d. 2 w Arm B: ipi 10 mg/kg q3w + IFN 20 MU/m^2 5 d/w 4w + 10 MU/m^2 q.a.d. 2w	30	6–8 w	Arm A: 36% (pCR; 5/14) Arm B: 29% (pCR; 4/14)	Not reported	32 mo

[a]Only neoadjuvant patients taken into consideration. [b]Not all patients were evaluable for pathologic response. [c]Personal communication. ipi, ipilimumab; nivo, nivolumab; pembro, pembrolizumab; IFN, high-dose interferon-α2b; q2w/q3w, once every two/three weeks; q.a.d., every other day; MU, million units; d, days; w, weeks; mo, months; MPR, major pathologic response (defined as a complete or near-complete response, thus ≤10% viable tumor cells present)—when not reported, pCR (pathologic complete response: 0% viable tumor cells present) is reported; pPR, pathologic partial response (defined as <50% viable tumor cells present).

Applying the knowledge from reverse translation

The knowledge obtained from these studies can be used for reverse translation in which these data are used to select therapies in the clinic or in trials that are more likely to improve patient outcomes.

Developing baseline markers predicting non-response. Moving checkpoint inhibition into the neoadjuvant setting, and personalizing the drug therapy combination based on the patient's individual tumor signature, will assist in eventually achieving the pathologic response rates of 90–100% that are already a reality in melanoma[82], and there are promising results in other malignancies. For example, neoadjuvant ipilimumab plus nivolumab in MSI-high colorectal carcinoma, NSCLC and bladder cancer resulted in a 100% pathologic response rate[34], a 44% MPR rate[83] and a 46% pathologic complete response (pCR) rate[84], respectively. Thus, the challenge in moving forward is now to define the patients who are unlikely to respond up-front, and either develop alternative treatment combinations or escalate to tolerable multidrug therapy regimens in these patients. Below, we discuss promising approaches to achieve this.

Modulating tumor T cell infiltration. The neoadjuvant setting is a good platform with which to explore novel combinations of therapies, and this is being done in many tumor types, particularly melanoma (Tables 1 and 2). Although most neoadjuvant trials focus on pCR as an end point, the MPR and the pathologic partial response are also good predictors of prolonged RFS, as observed in the OpACIN and OpACIN-neo trials[82,85].

Continuous application of chemotherapy with checkpoint inhibition may hamper the induction of a broad T cell response, owing to the killing of proliferating T cells by the chemotherapy. A broad T cell response, however, has been shown to be critical for the induction of a durable anticancer immune response[20]. The approach taken in the TONIC trial for patients with stage IV triple-negative breast cancer[86], in which chemotherapy is used only in a short induction phase, is a more rational approach to the use of chemotherapy in neoadjuvant immunotherapy combination trials. The aim of the TONIC trial was to induce tumor T cell infiltration before resection, which may be effective in patients with stage III melanoma with low IFN-γ and T cell signatures. Therefore, testing compounds that increase T cell infiltration or induce IFN signatures, including targeted therapies, in small neoadjuvant trials could be a straightforward approach[65,87].

Another strategy to increasing T cell infiltration is the use of oncolytic viruses. Talimogene laherparepvec (T-VEC) has been shown to increase immune cell infiltration in a small cohort of melanoma patients[88]. Neoadjuvant T-VEC demonstrated an improved two-year RFS of 50.5%, versus 30.2% for up-front surgery alone, in resectable and injectable stage III or IV melanoma patients, and will be trialed in combination with neoadjuvant nivolumab in melanoma (J.B.A.G. Haanen, personal communication).

Targeted therapies can also increase T cell infiltration. In the Neo-trio trial[89,90], short-term BRAF plus MEK inhibition is used to increase tumor T cell infiltration and is combined with PD-1 blockade in resectable stage III melanoma with the aim to increase the pathologic response rate[91,92]. In the earlier mentioned NICHE trial, COX inhibition, which targets cancer-associated inflammation, is combined with neoadjuvant checkpoint inhibition in MMR colorectal cancer, with the aim of reversing COX-mediated Th2 type responses toward Th1-like responses[34].

The chemokine CXCL9/CXCL10/CXCL11-CXCR3 axis mainly contributes to immune cell migration, differentiation and activation[93]. The three CXCR3 ligands are, among others, expressed on effector CD8$^+$ T cells and are strongly related to a Th1 immune response, which can evoke an antitumor response[93]. The chemokines are induced by IFN-γ, and CXCL11 has the highest affinity for the receptor CXCR3[93]. AMG487, an antagonist of CXCR3, inhibited lung metastasis in an *in vivo* model of osteosarcoma cells[94]. So far,

Table 2 | Overview of recruiting neoadjuvant immunotherapy combination trials or combined immunotherapy and targeted therapy[a]

clinicaltrials.gov identifier	Tumor type	Trial name	Compounds	Phase of trial	Estimated enrollment
NCT03768531	Biliary tract cancer	Safety and Tolerability Study of Nivolumab and Cabiralizumab for Resectable Biliary Tract Cancer	Nivolumab (anti-PD-1) Cabiralizumab (anti-CSF1R)	Phase II	16
NCT03532451	Bladder cancer	Phase Ib Feasibility Trial of Neoadjuvant Nivolumab/Lirilumab in Cisplatin-Ineligible Muscle-Invasive Bladder Cancer	Nivolumab (anti-PD-1) Lirilumab (anti-KIR2DL1/2L3)	Phase I	43
NCT03520491	Bladder cancer	A Study to Test the Safety of Immunotherapy with Nivolumab Alone or With Ipilimumab Before Surgery for Bladder Cancer Patients Who Are Not Suitable for Chemotherapy	Ipilimumab (anti-CTLA-4) Nivolumab (anti-PD-1)	Phase II	45
NCT03661320	Bladder cancer	A Study of Chemo Only Versus Chemo Plus Nivo with or without BMS-986205, Followed by Post-Surgery Therapy with Nivo or Nivo and BMS-986205 in Patients With MIBC	Nivolumab (anti-PD-1) BMS-986205 (IDO-1 inhibitor)	Phase III	1,200
NCT03234153	Bladder cancer	Neoadjuvant Immunotherapy with Durvalumab and Tremelimumab for Bladder Cancer Patients Ineligible for Cisplatin (NITIMIB)	Durvalumab (anti-PD-L1) Tremelimumab (anti-CTLA-4)	Phase II	68
NCT03472274	Bladder cancer	DUrvalumab (MEDI4736) and TREmelimumab in NEOadjuvant Bladder Cancer Patients (DUTRENEO)	Durvalumab (anti-PD-L1) Tremelimumab (anti-CTLA-4)	Phase II	99
NCT03773666	Bladder cancer	A Feasibility Study of Durvalumab +/− Oleclumab as Neoadjuvant Therapy for Muscle-invasive Bladder Cancer (BLASST-2)	Durvalumab (anti-PD-L1) Oleclumab (anti-CD73)	Phase I	24
NCT03534492	Bladder cancer	Durvalumab Plus Olaparib Administered Prior to Surgery of Resectable Urothelial Bladder Cancer (NEODURVARIB)	Durvalumab (anti-PD-L1) Olaparib (PARP inhibitor)	Phase II	29
NCT03546686	Breast cancer	Peri-Operative Ipilimumab+Nivolumab and Cryoablation Versus Standard Care in Women with Triple-negative Breast Cancer	Ipilimumab (anti-CTLA-4) Nivolumab (anti-PD-1)	Phase II	150
NCT03594396	Breast cancer	Window of Opportunity Trial of Neoadjuvant Olaparib and Durvalumab for Triple Negative or Low ER+ Breast Cancer	Olaparib (PARP inhibitor) Durvalumab (anti-PD-L1)	Phase I-II	25
NCT03802604	Breast cancer	Combination of Talimogene Laherparepvec With Atezolizumab in Early Breast Cancer (PROMETEO)	Talimogene laherparepvec (cancer vaccine) Atezolizumab (anti-PD-L1)	Phase I	30
NCT03026140	Colon carcinoma	Nivolumab, Ipilimumab and COX2-inhibition in Early Stage Colon Cancer: An Unbiased Approach for Signals of Sensitivity (NICHE)	Ipilimumab (anti-CTLA-4) Nivolumab (anti-PD-1) Celecoxib (NSAID)	Phase II	60
NCT02754856	Colorectal cancer	Tremelimumab and Durvalumab in Treating Patients with Colorectal Cancer With Liver Metastases That Can Be Removed by Surgery	Durvalumab (anti-PD-L1) Tremelimumab (anti-CTLA-4)	Phase I	35
NCT03003637	Head and neck carcinoma	ImmunoModulation by the Combination of Ipilimumab and Nivolumab Neoadjuvant to Surgery in Advanced or Recurrent Head and Neck Carcinoma (IMCISION)	Ipilimumab (anti-CTLA-4) Nivolumab (anti-PD-1)	Phase I-II	32
NCT03299946	Hepatocellular carcinoma	Feasibility and Efficacy of Neoadjuvant Cabozantinib Plus Nivolumab (CaboNivo) Followed by Definitive Resection for Patients with Locally Advanced Hepatocellular Carcinoma	Cabozantinib (multitargeted kinase inhibitor) Nivolumab (anti-PD-1)	Phase I	15
NCT02519322	Melanoma	Neoadjuvant and Adjuvant Checkpoint Blockade in Patients with Clinical Stage III or Oligometastatic Stage IV Melanoma	Ipilimumab (anti-CTLA-4) Nivolumab (anti-PD-1) Relatlimab (anti-LAG-3)	Phase II	53
NCT02858921	Melanoma	Neoadjuvant Dabrafenib, Trametinib and/or Pembrolizumab in BRAF Mutant Resectable Stage III Melanoma (NeoTrio)	Dabrafenib (BRAF inhibitor) Trametinib (MEK inhibitor) Pembrolizumab (anti-PD-1)	Phase II	60
NCT02977052	Melanoma	Optimal Neo-adjuvant Combination Scheme of Ipilimumab and Nivolumab (OpACIN-neo) - PRADO extension cohort	Ipilimumab (anti-CTLA-4) Nivolumab (anti-PD-1)	Phase II	110

Continued

Table 2 | Overview of recruiting neoadjuvant immunotherapy combination trials or combined immunotherapy and targeted therapy[a] (continued)

clinicaltrials.gov identifier	Tumor type	Trial name	Compounds	Phase of trial	Estimated enrollment
NCT03618641	Melanoma	Neoadjuvant Phase II Study of TLR9 Agonist CMP-001 in Combination with Nivolumab in Stage IIIB/C/D Melanoma Patients With Clinically Apparent Lymph Node Disease	Nivolumab (anti-PD-1) CMP-001 (TLR-9 agonist)	Phase II	32
NCT03639948	Melanoma	Neoadjuvant Combination Targeted and Immunotherapy for Patients with High-Risk Stage III Melanoma (NeoACTIVATE)	Atezoluzimab (anti-PD-L1) Vemurafenib (BRAF inhibitor) Cobimetinib (MEK inhibitor)	Phase II	30
NCT03918252	Mesothelioma	Neoadjuvant Immune Checkpoint Blockade in Resectable Malignant Pleural Mesothelioma	Ipilimumab (anti-CTLA-4) Nivolumab (anti-PD-1)	Phase II-III	30
NCT02259621	NSCLC	Neoadjuvant Nivolumab, or Nivolumab in Combination with Ipilimumab, in Resectable NSCLC	Ipilimumab (anti-CTLA-4) Nivolumab (anti-PD-1)	Phase II	30
NCT03794544	NSCLC	Neoadjuvant Durvalumab Alone or in Combination with Novel Agents in Resectable Non-Small Cell Lung Cancer (NeoCOAST)	Durvalumab (anti-PD-L1) Oleclumab (anti-CD73) Monalizumab (anti-NKG2A) Danvatirsen (anti-STAT3)	Phase II	160
NCT04006262	Oesogastric adenocarcinoma	Peri-operative Association of Immunotherapy (Pre-operative Association of Nivolumab and Ipilimumab, Post-operative Nivolumab Alone) in Localized Microsatellite Instability (MSI) and/or Deficient Mismatch Repair (dMMR) Oeso-gastric Adenocarcinoma (NEONIPIGA)	Ipilimumab (anti-CTLA-4) Nivolumab (anti-PD-1)	Phase II	32
NCT03153410	Pancreatic cancer	Pilot Study With CY, Pembrolizumab, GVAX, and IMC-CS4 (LY3022855) in Patients with Borderline Resectable Adenocarcinoma of the Pancreas	Cyclophosphamide (alkylating agent) GVAX (cancer vaccine) Pembrolizumab (anti-PD-1) IMC-CS4 (anti-CSF-1R)	Phase I	12
NCT03075423	Renal cell carcinoma	Randomized Phase-II Study of Nivolumab Plus Ipilimumab vs. Standard of Care in Untreated and Advanced Non-clear Cell RCC (SUNIFORECAST)	Ipilimumab (anti-CTLA-4) Nivolumab (anti-PD-1)	Phase II	306
NCT02762006	Renal cell carcinoma	Neoadjuvant MEDI 4736 +/− Tremelimumab in Locally Advanced Renal Cell Carcinoma	Durvalumab (anti-PD-L1) Tremelimumab (anti-CTLA-4)	Phase I	45
NCT04028245	Renal cell carcinoma	A Study of Combination Spartalizumab and Canakinumab in Patients with Localized Clear Cell Renal Cell Carcinoma (SPARC-1)	Spartalizumab (anti-PD-1) Canakinumab (anti-IL-1)	Phase I	14
NCT03680521	Renal cell carcinoma	Neoadjuvant Sitravatinib in Combination with Nivolumab in Patients with Clear Cell Renal Cell Carcinoma	Sitravatinib (multitargeted kinase inhibitor) Nivolumab (anti-PD-1)	Phase II	25
NCT02845323	Urothelial carcinoma	Neoadjuvant Nivolumab With and Without Urelumab in Patients With Cisplatin-Ineligible Muscle-Invasive Urothelial Carcinoma of the Bladder	Nivolumab (anti-PD-1) Urelumab (anti-4-1BB)	Phase II	44
NCT02812420	Urothelial carcinoma	Durvalumab and Tremelimumab in Treating Patients with Muscle-Invasive, High-Risk Urothelial Cancer That Cannot Be Treated with Cisplatin-Based Therapy Before Surgery	Durvalumab (anti-PD-L1) Tremelimumab (anti-CTLA-4)	Phase I	45

[a]Trials with either monotherapy and immunotherapy combined with either chemotherapy, radiotherapy or hormone therapy are excluded from this overview.

none of these interesting additional approaches have been analyzed in the neoadjuvant setting, but translational studies are underway.

Targeting additional T cell inhibitory mechanisms and improving antigen presentation and T cell help. Another approach to identify compounds that might overcome primary resistance to neoadjuvant anti-PD-1, with or without anti-CTLA-4, may be the addition of compounds that have been shown to induce responses in stage IV patients resistant to PD-1 blockade. The most promising compounds in this field are LAG-3 inhibitors and PEGylated cytokines such as IL-2 (NKTR-214) or IL-10[95–97]. Whereas LAG-3 blockade is thought to reverse T cell exhaustion[95], cytokines are hypothesized to mimic $CD4^+$ T cell help and to improve T effector cell maturation[96,97].

In early-stage NSCLC, the NeoCOAST trial tests the addition of several different drugs to durvalumab (anti-PD-L1) to overcome resistance to PD-1 blockade alone, including anti-CD73 (inhibiting the conversion of ATP to adenosine, the latter shown to inhibit $CD4^+$ helper and $CD8^+$ effector T cell functions), anti-NKG2A (induction of NK cell help) and anti-STAT3A (inhibiting expansion of myeloid-derived suppressor cells and regulator T cells).

Future directions. With the many neoadjuvant trials of PD-(L)1 plus CTLA-4 blockade (often investigator initiated as opposed to pharmaceutical company driven) underway or completed, there should soon be a plethora of response-associated genetic signatures for a range of different malignancies publicly available. We predict that many of the signatures may overlap across a range of malignancies, particularly if these signatures indicate the neoantigen load (for example, TMB), the reaction of the immune system toward the tumor or antigen load (for example, IFN-γ signature) and any defect in the response initiation (for example, $Batf3^+$ DC signature). With this structured and biological approach, we will be better able to rationally combine novel drug therapies for rapid clinical testing and study in the neoadjuvant trial setting, with a focus on patients with unfavorable signatures for response.

Already, such an individualized neoadjuvant phase 1b trial is underway (the DONIMI trial, NCT04133948) testing the combination of a histone deacetylase inhibitor (domatinostat) with ipilimumab and nivolumab in IFN-γ-signature-low melanoma patients.

With the unique availability of large amounts of tumor tissue collected from those patients without a pathologic response in the neoadjuvant setting, there are new opportunities to explore creative and sophisticated methods of assessing potentially active novel combinations in these patients. The patient-derived tumor fragment platform is one such example of an innovative method to obtain a readout of potentially effective novel drug combinations (D.S. Thommen, personal communication); for example, ex vivo exposure of patient-derived tumor fragments to checkpoint inhibition correlated with outcome to PD-1 blockade.

Conclusions

Neoadjuvant therapy induces a broader immune response than adjuvant therapy in humans and leads to superior survival in preclinical models. Large prospective randomized phase 3 trials are being planned to determine whether the high pathologic response rates, associated with a remarkable lack of recurrence, in the neoadjuvant setting translate into a prolonged RFS compared with adjuvant therapy. The high pathologic responses observed in phase 2 studies of neoadjuvant immune checkpoint inhibitors is likely to be due to a broad antigen exposure when the drug is administered in the presence of measurable tumor, along with the weaker systemic immune suppression in early-stage cancer patients. Supportive evidence for the latter mechanism comes from the observed higher rates of toxicity in early-stage high-risk melanoma (patients with resectable stage III melanoma in neoadjuvant and adjuvant studies) compared with stage IV disease, thus requiring dose adjustments from the standard stage IV dosing for the neoadjuvant setting.

Neoadjuvant immunotherapy allows evaluation of individual response in early-stage high-risk cancer patients, and neoadjuvant trials have the added advantage of a homogenous population. Genomic signatures of response and, even more importantly, of nonresponse are more easily developed in this trial setting and provide a unique opportunity for efficient reverse translation using sophisticated preclinical testing to develop novel neoadjuvant combinations to improve patient outcomes. In such a cycle of translational research, we expect highly efficacious personalized neoadjuvant therapies to be possible for the majority of cancer patients within the next decade.

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Author contributions

J.M.V. wrote the first draft of the review and processed writing and suggestions by the coauthors. This was done under supervision of, and final inspection by, G.V.L. and C.U.B.

Competing interests

J.M.V. declares no conflict of interests. Both G.V.L. and C.U.B. declare no direct conflicts with this work. For unrelated conflicts, G.V.L. is a consultant advisor to Aduro, Amgen, BMS, Mass-Array, Pierre-Fabre, Novartis, MERCK MSD and Roche. C.U.B. has received research funding from BMS, Novartis and NanoString; has an advisory role for BMS, MSD, Roche, Novartis, GSK, AZ, Pfizer, Lilly, GenMab, Pierre Fabre and Third Rock Ventures; and is a stock owner of Uniti Cars, Neon Therapeutics and Forty Seven.

Additional information

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