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Neoadjuvant immune checkpoint inhibitors in cancer, current state of the art

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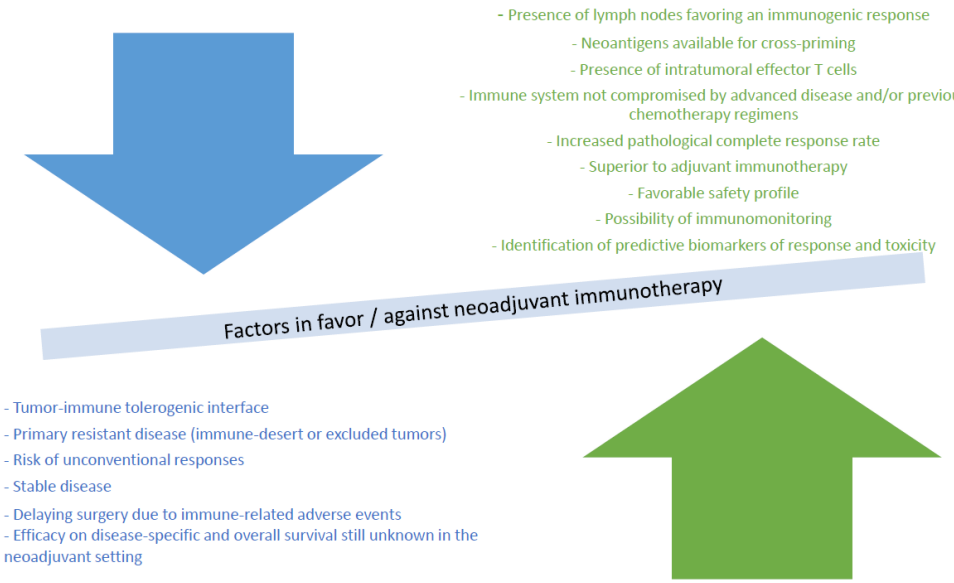
Graphical abstract



HIGHLIGHTS

1. Preclinical data support the use of immunotherapy (IO) in the neoadjuvant setting.
2. Two clinical trials reported higher efficacy of neoadjuvant IO versus adjuvant IO.
3. Neoadjuvant trials are a unique opportunity to identify predictive biomarkers.
4. Neoadjuvant IO present limits: unconventional responses, toxicity and resistance.

Abstract: Immunotherapy has been a revolution in cancer management in the metastatic setting. This has led to a prompt evaluation of such therapies in earlier stages. This article discusses the still limited amount of data finding the rationale to assess such therapy in this setting and reviews preclinical and clinical data available. Overall, neoadjuvant immunotherapy is a promising approach for the treatment of cancers and the rationale supporting its use is strong. Neoadjuvant immunotherapy resulted, in the majority of clinical trials, in improved pathologic complete response rates with a favorable toxicity profile and no delay in surgery. Various regimens were effective: inhibitory immune check-point blockers (IICPB) alone, combination of PD-1 and CTLA-4 inhibitors, combination of chemotherapy (CT) and IICPB, phased CT and IICPB (either IICPB before CT or IICPB after CT). Yet the question whether neoadjuvant immunotherapy will benefit to patients in terms of disease-free and, ultimately, overall survival remains unknown.

Keywords: immunotherapy; cancer; neoadjuvant; preclinical data

Introduction

Neoadjuvant therapy refers to a systemic treatment administered prior to local treatment (surgery or radiotherapy) for any type of cancer. Neoadjuvant therapy usually takes the form of chemotherapy, but endocrine therapy or targeted therapies have also been described as alternatives in some cancers such as breast cancer[1,2]. Neoadjuvant therapy has the purpose of downstaging tumor size allowing less extensive surgery or resectability in order to achieve local control, identifying sensitivity to systemic treatment, improve the pathological response rate and preventing early metastatic risk[1,3,4].

Immunotherapy and especially inhibitory immune check-point blockers (IICPB) have drastically transformed the landscape of cancer. IICPB restore anti-tumor functions of T cells via blockade of negative regulatory signals such as PD1/PD-L1 and CTLA-4. Conceptually, CTLA4 blockade primarily acts at sites of priming in which CD28-positive costimulation is involved (e.g., tumor draining lymph nodes) whereas PD-1/PD-L1 blockade primarily acts in inflamed peripheral tissues (e.g., tumor)[5]. Checkpoint inhibition was shown to be associated with significantly prolonged survival and long-lasting disease control for metastatic cancers. Consequently, IICPB emerged as front-line options for various cancers such as metastatic melanoma, lung cancer, renal clear cell carcinoma, bladder or urothelial cancers, Hodgkin's lymphoma and head and neck cancers. Currently, many trials are ongoing in the neoadjuvant setting due to the major impact of immunotherapy in the metastatic setting. Yet, few data are available in the literature on the rationale for using immunotherapy in the neoadjuvant setting and only preliminary efficacy results were published with no FDA approval yet.

The present review highlights available data on immunotherapy in the neoadjuvant setting, the rationale supporting the use of neoadjuvant immunotherapeutic approaches, the potential advantages and limits of this approach, available clinical data, and implications for future management of cancers.

Material and methods

**Trial selection*

In June 2019, all reports on immune checkpoint inhibitors in the neoadjuvant setting were identified. The research was performed using Text Words "Avelumab", "Pembrolizumab", "Ipilimumab", "Tremelimumab", "Atezolizumab", "Durvalumab", "Nivolumab", "immunotherapy", "CTLA-4 inhibitor", "PD-1 inhibitor", "PD-L1 inhibitor", "neoadjuvant" and "preoperative". Resources used for this research included the electronic database Pubmed, relevant articles retrieved from references, personal data and abstracts presented at the American Society of Clinical Oncology (ASCO), the American Association for Cancer Research (AACR), the European Society for Medical Oncology (ESMO) congresses and the San Antonio Breast Cancer Symposium. Included reports were published in English.

1. Rationale supporting the use of immunotherapeutic approaches in the early stages of cancer

1.1. Mechanistic arguments

From a mechanistic point of view, the availability of neoantigens for cross priming, the possibility of lymphatic migration of antigen-presenting cells (APC), the interaction between APC and naïve T cells in lymph nodes, the lymphatic recirculation of effector cells and the presence of tumor-infiltrating lymphocytes (TILs) are necessary to initiate an immune response. In case of lymphadenectomy or tumor resection, this immune response might be jeopardized[6]. On the other hand, tumor cells can release immunosuppressive factors modulating immune cells to become tolerogenic. Tolerogenic APC and regulatory cells use lymphatics and blood flow respectively to migrate into the tumor and lymph nodes can act as a tumor- immune tolerogenic interface. These arguments are in favor of the use of IICPB in the neoadjuvant setting.

Moreover, until recently, IICPB were mostly evaluated in heavily pretreated patients or in patients with advanced disease, e.g. with an immune system already compromised by tumor progression[7] and/or previous regimens of chemotherapy[8]. It was shown in melanoma and renal cell carcinomas that the host immune response strikingly differ between earlier stages with micro metastatic disease and more advanced stages with measurable disease[9]. For instance, healthy donors or patients free of disease following therapy tended to present tumor antigen specific mixed Th1/Th2 type or Th1-type polarized immune response while patients with advanced renal cell carcinoma or melanoma displayed increased tumor antigen-specific Th2-type polarization[7,10,11].

Therefore, it may be more efficient to prescribe this type of anticancer drug earlier in the history of the disease when an anti-tumor immune response (Th1) can still be amplified/restored.

1.2. Impact of surgery on the immune system

Surgery may have an impact on the immune system. Indeed, in breast cancer patients, Péguillet and colleagues reported that the number of effector CD4+ blood T cells decreases ($P \leq 0.017$) after primary surgery. In addition, the total number of CD4+ blood T cells was not modified by adjuvant therapy but significantly increased during neoadjuvant chemotherapy[12]. IICPB treatment aims to restore the functions of tumor effector T cells. Tumor removal will induce a decrease of intratumoral antigen-experienced effector T cells that can circulate between blood, tissue and secondary lymphoid organs. Therefore, prescribing IICPB after surgery may not be relevant.

1.3. Prognostic impact of PD-L1 expression

A meta-analysis of 61 studies showed that PD-L1 overexpression can predict a poor overall survival (OS) (HR=1.58, 95% CI=1.38-1.81, P<.000) and disease- and progression-free survival (DFS/PFS) (HR=1.72, 95%

CI=1.26-2.33, P=.001) in various solid tumors[13]. PD-L1 expression was also shown to be associated with a higher risk of recurrence in surgically resected non-small cell lung cancer[14], in human urothelial cancers[15] and in hepatocellular carcinoma[16]. Furthermore, multivariate analysis indicated that tumor-associated PD-L1 was a more significant prognostic factor than WHO grade for postoperative recurrence in human urothelial cancers[15].

As most tumors expressing immune co-inhibitory molecules such as PD-L1 exhibit a poor prognosis and a higher risk of relapse following surgery, neoadjuvant immunotherapeutic strategies could be beneficial in combination with surgery in order to decrease relapse risk.

1.4. Presumed efficacy

For metastatic disease, ORR is higher with immunotherapy compared to chemotherapy in various immunogenic cancers such as melanoma or lung cancer, which are the two cancers in which immunotherapy has first proven its efficacy. In lung cancer, in a phase III trial comparing pembrolizumab to platinum-based chemotherapy in front-line, ORRs for pembrolizumab and platinum-doublet chemotherapy were 45% and 28%, respectively and the time to response was equal in both arms and estimated at 2.2 months[17]. In combination with standard first-line therapy (carboplatin and pemetrexed), pembrolizumab showed an ORR of 55% vs 29% with chemotherapy alone. In melanoma, dacarbazine (standard first-line chemotherapy until recently) was compared to nivolumab[18] and to dacarbazine + ipilimumab[19]. In the first trial, nivolumab achieved an ORR of 40% versus 13.9 % with dacarbazine alone[18]. Complete response rate (CRR) was 7.6 % in the experimental arm versus 1.0% in the comparative standard arm[18]. In the second trial, ipilimumab + dacarbazine resulted in an ORR of 15.2% versus 10.3% and a CRR of 1.6 % vs 0.8%[19]. In the phase III KEYNOTE-006 trial which compared pembrolizumab (anti-PD-1) to ipilimumab (anti-CTLA-4), ORR was 37% vs 13% respectively and CRR was 12% vs 5% respectively[20]. In the CheckMate 067 trial, nivolumab + ipilimumab followed by nivolumab was compared to nivolumab alone and to ipilimumab alone. ORR were 58, 44, and 19%, respectively. The CRR were 19, 16, and 5%, respectively[21].

In these pivotal trials, immunotherapy resulted in an improvement of the ORR and the CRR suggesting an increased depth of response that could be interesting in the neoadjuvant setting for resectability and preservation of function. Yet, whether these results in the advanced setting can be extrapolated to the neoadjuvant setting is unknown.

1.5. Safety profile

Another potential advantage of IICPB in the neoadjuvant setting is its intrinsic safety profile. Neoadjuvant therapy-related adverse events may affect post-operative morbidity and/or mortality and/or delay surgery, thus

jeopardizing surgery efficacy. As IICPB are generally well-tolerated, they are particularly interesting in the neoadjuvant setting. IICPB are associated with peculiar adverse events (AEs) commonly defined as immune-related AEs (irAEs). IrAEs are usually mild to moderate in severity and reversible when they are rapidly detected and when immunosuppressive and/or immunomodulatory strategies are promptly initiated[22]. A meta-analysis of twenty randomized trials with 10794 patients reported that treatment discontinuations and grade 3-5 (G3-5) AEs were less frequent for programmed-death-1 (PD-1) or PD-ligand-1 (PD-L1) inhibitors and cytotoxic-T-lymphocyte-associated-antigen-4 (CTLA-4) inhibitors than for standard cytotoxic chemotherapy[23].

1.6. Preclinical data

In two mouse models of spontaneous metastatic mammary tumors (orthotopic 4T1.2 and E0771 tumors)[24], Liu et al. demonstrated that the proportion of long-term mice survivors was more important in mice receiving neoadjuvant immunotherapy compared to adjuvant immunotherapy. Four different immunotherapies were assessed: complete depletion of regulatory T cells with an anti-CD25, anti–PD-1 alone and anti-PD-1 in combination with anti-CD137. Neoadjuvant immunotherapy-treated mice displayed significantly longer survival compared with those that received adjuvant immunotherapy. For instance, in the neoadjuvant T regulatory-depleted group (Treg), almost all mice (19/20) displayed long-term survival compared with the adjuvant Treg-depleted mice group in which only 5 out of 20 mice displayed long-term survival. In both 4T1.2 and E0771 tumor models, the efficacy of neoadjuvant therapy was dependent on interferon gamma (IFNγ) as there were no long-term survivors when it was neutralized. All three immune cell types, CD8, CD4, and NK cells, were also required. After neoadjuvant immunotherapy, an increase in tumor specific CD8+ T cells was detected. They displayed an effector/memory phenotype (CD44+ CD62L−), were proliferative and produced IFNγ. It is noteworthy that neoadjuvant immunotherapy given only 2 days before surgery still had a beneficial impact in this preclinical study.

Similarly, in the murine B16F10 melanoma model, neoadjuvant vaccination provided superior protection against tumor relapse following surgery compared with adjuvant vaccination[25].

2. First Clinical data

Efficacy results, tolerance and impact on surgery of the main clinical trials evaluating immune checkpoint inhibitors in the neoadjuvant setting are reported in table 1.

In conclusion, neoadjuvant immunotherapy resulted, in the majority of clinical trials, in improved pathologic complete response rates with a favorable toxicity profile and no delay in surgery. Various regimens were effective: IICPB alone, combination of PD-1 and CTLA-4 inhibitors, combination of chemotherapy (CT) and IICPB, phased CT and IICPB (either IICPB before CT or IICPB after CT). The majority of novel trials evaluating neoadjuvant immunotherapy in solid cancers use pathological response as a surrogate endpoint for survival which is interesting because gives access to earlier results. Yet the question whether neoadjuvant immunotherapy will benefit to patients in terms of disease-free and, ultimately, overall survival remains unknown and we lack sufficiently validated association with OS in the era of immunotherapy. Concerns were raised from one study evaluating nivolumab monotherapy which was stopped prematurely due to rapid progression. Finally, two trials, in glioblastoma and melanoma, compared neoadjuvant IICPB *versus* neo and adjuvant IICPB and reported similar results suggesting that neoadjuvant may be more efficient than adjuvant immunotherapy which confirms preclinical data.

NAME OF THE TRIAL OR FIRST	PHASE	PRIMITIVE TUMOR TYPE	NUMBER OF PATIENTS	TYPE OF THERAPY EVALUATED	EFFICACY	TOXICITY	SURGICAL OUTCOMES
	y randomized phase II	negative breast cancer		b + chemotherapy	TNBC (pCR 60% vs 20%) HR+/HER2- (34% vs 13%)	insufficiencies (n=6/69)	
KEYNOTE-173[27]	Phase Ib	TNBC	20	Pembrolizumab + chemotherapy	ypT0 ypN0 pCR rate: 50% to 80%	1 grade 2/3 alanine aminotransferase elevation	NA
KEYNOTE-522[28]	Phase III	TNBC	602	Pembrolizumab + chemotherapy	pCR rate 64.8% with the pembrolizumab/chemotherapy regimen compared to 51.2% (standard arm) Benefit more important in lymph node positive TNBC compared to lymph node negative TNBC	grade 3 or higher treatment-related adverse event (AE) rates: 78.0% - experimental arm- compared to 73.0% - standard arm. Incidence of death: 0.4% versus 0.3%.	NA
GEPARNUEVO[29]	Randomized phase II	TNBC	31	Durvalumab + chemotherapy (CT)	Patients who started durvalumab before chemotherapy+durvalumab presented more pCR (OR=2.22 [95%CI 1.06-4.64]. This phased administration of IICPB called "window of opportunity" resulted in a higher pCR rate 61.0% compared to 41.4% of the patients in the control arm (p=0.052)	Treatment delay in 54.8% of patients receiving the combination vs 40.0 to 67.7% patients treated with chemotherapy alone	NA
NEOTRIPAPDL1[30]	Phase III	TNBC	280	Atezolizumab + CT	pCR rate 43.5% vs. 40.8% not statistically significant	Immune-mediated adverse events of any grade: 8% >G3 infusion reactions: 1.4%	NA
MCARTHUR HL[31]	Pilot study	Breast cancer	19	Single dose ipilimumab +/- cryoablation	NA	Grade III unrelated rash in one patient	No delay in surgery
TARHINI AA[32]	NA	Melanoma	35	Ipilimumab	ORR was 9% (2 complete responses and 1 partial response)	Grade 3 diarrhea/colitis (5; 14%), hepatitis (2; 6%), rash (1; 3%), elevated lipase (3; 9%)	NA
OPACIN[33]	Two-arm phase Ib feasibility trial	Melanoma	20 (10 in the neoadjuvant arm)	Ipilimumab + Nivolumab	ORR 80% pCR 30% Estimated 30 months relapse-free survival rates were 80% for the neoadjuvant arm and 60% for the adjuvant arm and 30 months OS rates were 90% and 67%, respectively	High frequency of grade ¾ toxicity (n=18). G3 elevated lipase (n=8), G3 diarrhea and colitis (n=6)	No delay in surgery

3. Neoadjuvant immunotherapy as a platform for identification of efficacy and resistance biomarkers and for drug development

IICPB are not effective in all cancers nor on all patients, even with immunogenic tumors[48]. Predictive biomarkers of efficacy are urgently needed. Neoadjuvant immunotherapy offers the possibility of immune cells

						No surgery related AEs attributed to immunotherapy	
AMARIA RN[34]	Phase II	Melanoma	23	Ipilimumab + Nivolumab	ORR 73% pCR 45%	G3 irAEs 73%	Rapid disease progression for 17% of patients in the nivolumab monotherapy arm
TARHINI AA[35]	Randomized trial	Melanoma	30	Ipilimumab (3 or 10mg/kg) + high-dose interferon	pCR 35% (95CI[17-56])	One toxicity-related drug withdrawal. Grade ≥3 elevated transaminases (n=6, 20.0%). Grade ≥3 rash (n=7, 23.0%) Toxicities more frequent at 10mg/kg	NA
TOP1201 IPI[36]	Phase II	Non-small cell lung cancer (NSCLC)	24	Ipilimumab + chemotherapy	ORR (n=14, 58%)	Ipilimumab related AEs: grade 2 pneumonitis (n=1, 4%), grade 3 adrenal insufficiency (n=4, 17%), diarrhea/colitis 8 (grade 1 or 2: n=6, 25%; grade 3: n=3, 13%).	Prolonged air leak (n = 2, 15%) Urinary tract infection (n = 2, 15%) No apparent increased occurrence of adverse surgical outcomes compared to historical cohort.
NCT02259621[37]	Phase II	NSCLC	22	Nivolumab (2 doses, 4 weeks and 2 weeks prior to surgery)	ORR 10% (n=2) pCR 43.0% 95IC[24-63] Discrepancy between ORR and pCR suggests that some patients may derive benefit from immunotherapy that is not captured radiographically	Pneumonia grade≥3 (n=1,5%)	No delay in surgery
LCMC3[38]	Phase II	NSCLC	21	Atezolizumab	Major pathologic response: 21% (95% CI 6-46)	Gr 1 pyrexia, Gr 2 dyspnea for 2 patients	No major delay in surgery
NCT02296684[39]	Phase II	HPV negative HNSCC	25	Pembrolizumab	pCR 42.0% (n=10)	No serious drug-related AEs	No unexpected surgery delays or complications
NCT02641093[40]	Phase II	HNSCC	28	One dose Pembrolizumab 200mg	Major pathologic response (>70%): 32%	NA	NA
NCT01194271[41]	Phase II	Prostate	19	Ipilimumab + androgen deprivation therapy (ADT)	No pCR	NA	NA

CARTHON BC[42]	Controlled clinical trial	Bladder	12 (6 patients at 3mg/kg/dose with results on immune monitoring and 6 at 10 mg/kg/dose)	Ipilimumab	pCR 33.3 % (n=4/12)	Treatment completion rate (n=11/12) Reason for not receiving second dose = diarrhea grade 3.	No significant surgical delays due to irAEs in the 3 mg/kg/dose cohort Surgical delays (n=3, grade 2/3 diarrhea)
PURE-01[43]	Phase II	Muscle invasive urothelial bladder cancer	50	Pembrolizumab	Downstaging to pT0 (42%; 95% CI, 28.2% to 56.8%).	One discontinuation of pembrolizumab (grade 3 transaminase increase - 2%)	All patients underwent radical cystectomy
ABACUS[44]	Phase II	Muscle invasive urothelial bladder cancer	95	Atezolizumab	pCR rate 31% (95% CI: 21–41%)	No new safety signals	Grade I to II surgical complications (Clavien Dindo classification): 39 of 87 (45%)
GROOTSCHOLTEN C[45]	Phase II	Colon cancer	14 (mismatch repair proficient (pMMR) (n = 8) or mismatch repair deficient (dMMR) (n = 7)	Ipilimumab + Nivolumab	Major pathological responses (<5% viable tumor cells) 100% (n=7/7) CR 57% (n=4/7)	Well tolerated	No delays in surgery
NCT02210117[46]	Phase I	Kidney cancer	105	Nivolumab (Nivo) Nivo+Bevacizumab (Bev) Nivo+Ipilimumab (Ipi)	ORR: 55% nivo, 44% nivo + bev, 43% nivo + ipi	> Grade 3: 38% for nivo, 42% for nivo + bev (including 18% hypertension), and 47% for nivo + ipi	NA
NEO-NIVO[47]	Phase II	Glioblastoma	35 (16 in the neoadjuvant group and 19 in the adjuvant group)	Nivolumab	Benefit for the neoadjuvant group compared to the adjuvant group: • Increased OS: HR 0.39 (95%CI 0.17–0.94; P = 0.04). • Increased PFS: (HR 0.43; 95% CI 0.20–0.90; P = 0.03)	One grade 3 pneumonitis. One grade 4 elevation in alanine aminotransferase.	No delays in surgery

monitoring and identification of underlying response or resistance mechanisms given the access to blood, to tumor and even to fecal samples prior and post therapy. Understanding resistance mechanisms will help us

design next generation trials. Characteristics of the immune system evaluated in neoadjuvant trials testing IICPB and predictive factors of efficacy and toxicity are reported in table 2.

4. Limits

4.1. Atypical and unconventional responses

In most studies, tumor response is evaluated at week 8 or 12, yet pseudo progression on immunotherapy can occur at this point[49]. Activation/restimulation of T cell mediated immunity may initially delay tumor regression and appearance of new lesions along with progression of existent lesions may precede objective tumor response. Pseudo progression can occur early (≤12weeks) but can also be delayed (> 12weeks)[50]. This might have an impact on the time of surgery as one of the objectives of neoadjuvant therapy is to render operable an otherwise inoperable disease. Hyper progression disease, defined as a rapid progression after initiation of anti-PD1 and anti-PD-L1, was also reported[51,52]. Atypical responses are not so rare and approximately 15% of patients experience such responses[50]. Moreover, the expression of antigens by tumor cells is not homogeneous and immunologic heterogeneity within the tumor was described[53]. Intratumor heterogeneity influenced immune evasion[54]. This principle was first shown in patients presenting responding lesions and progressive lesions at the same time under high-dose IL-2 therapy[55]. Responding lesions showed brisk CD8+ T cells infiltration while progressive lesions showed poor lymphocyte infiltration. Unconventional dissociated responses were reported in patients treated with anti- PD1, anti-PDL1 and anti-CTLA-4. In a French retrospective analysis of all consecutive patients treated with anti-PD-1 or anti-PD-L1 Abs for advanced NSCLC, dissociated responses occurred in 8% of cases[56]. Atypical and unconventional responses are limiting factors in the prescription of immunotherapy in the neoadjuvant setting.

4.2. Stable disease is a marker of IICPB efficacy

Through its direct cytotoxic effect, chemotherapy yields measurable effects within a few weeks. This phenomenon is often expressed as partial responses defined as a significant decrease in the size of the tumor. In contrast, stable disease is often transient and not reflective of the true benefit. On the contrary, for immunotherapy, stable disease was described as a surrogate endpoint for improved survival outcome[57,58]. Some IICPB were shown to improve OS without improving PFS[59]. Durable stable disease is even one of the four immune-related response patterns[49]. Consequently, stable disease is associated with a true clinical benefit for patients, however, this clinical benefit may not be sufficient when the objective is to render an inoperable tumor operable.

4.3. Primary resistant disease

Interesting results in terms of duration of responses and response rates with IICPB were reported in inflamed tumors. Yet, some patients still present primary resistance to IICPB. In this context, combination with chemotherapy might be interesting as it has been shown to further increase the ORR in lung cancer[60].

Furthermore, immune-desert and immune-excluded tumors exhibit primary resistant to currently available immune checkpoint inhibitors[61,62]. This indicates that selection of patients is mandatory in the neoadjuvant setting in order to give our patients the best opportunity of treatment. Microsatellite instability, TMB or PD-L1 expression could be used.

4.4. Adverse events

Another important consideration to take into account is the potential risk of serious immune-related adverse events in the first few cycles of neoadjuvant immunotherapy that could prevent or delay surgery. For this type of therapy, severe and even life-threatening AEs were described with treatment-related deaths occurring in up to 2% of patients[63]. Yet, consensus recommendations on how to detect and treat irAEs have recently been published by the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group and will most likely reduce the rate of serious AEs[22]. Furthermore, the impact on the tumor microenvironment and the hemostatic effects after immunotherapy are unknown. The normal wound-healing process involves an inflammation phase with neutrophils and lymphocytes infiltration and recruitment of monocytes that will differentiate into macrophage[64]. Immunotherapy might jeopardize wound-healing and therefore delay adjuvant therapy (if needed) impacting negatively the prognostic of the patient. Finally, there is also a threat of prolonged toxicity, particularly affecting the lung with interstitial inflammation and resulting difficulties for subsequent surgery in the neoadjuvant setting with immunotherapy which could preclude curative resection for lung cancer. This is different from the ,-although more frequent-, but often relatively transient toxicity of neoadjuvant chemotherapy.

4.5. Window of opportunity studies versus neoadjuvant trials

Window of opportunity studies differ from neoadjuvant trials in that no therapeutic effect is expected. Window of opportunities are trials in which patients receive the investigational compound between their cancer diagnosis and standard of care treatment[65]. Tumor biopsies before and after the investigational treatment are collected for translational research. These trials have the potential to reveal pharmacodynamics effect of a therapeutic compound and to assess predictive biomarkers of efficacy or resistance. These trials could therefore be more efficient than neoadjuvant studies in selecting patients who might benefit from a given therapy.

4.6. Adjuvant versus neoadjuvant trials

Currently, immunotherapy is being tested in various settings in cancer and sometimes without a global strategy. For example, in melanoma, neoadjuvant IICPB versus neo and adjuvant IICPB reported similar results suggesting that neoadjuvant may be more efficient than adjuvant immunotherapy which confirms preclinical data[33]. However, at present, the study of neoadjuvant immunotherapy for melanoma is in the exploratory stage with no mature research result. On the other hand, adjuvant immunotherapy has already been associated with

improved results for nivolumab[66] and pembrolizumab[67] and are approved by the US Food and Drug Administration (FDA) for this indication. The positive results of these large phase III trials with adjuvant immunotherapy might be an obstacle for many ongoing neoadjuvant immnotherapy trials, particularly in earlier stages, as has happened with neoadjuvant chemotherapy after adjuvant treatment became standard of care.

4.7. Uncertainty about timing of neoadjuvant immunotherapy

Using orthotopic 4T1.2 and E0771 mouse models of spontaneously metastatic mammary cancer, Liu et al. demonstrated that a short duration (4-5 days) between first administration of neoadjuvant immunotherapy and resection of the primary tumor was necessary for optimal efficacy, while extending this duration (10 days) abrogated immunotherapy efficacy. However, efficacy was also lost if neoadjuvant immunotherapy was given too close to surgery (2 days). Interestingly, an additional 4 adjuvant doses of treatment following a standard 2 doses of neoadjuvant immunotherapy, did not significantly improve overall tumor-free survival regardless of the combination treatment (anti-PD-1+anti-CD137 or anti-CTLA4+anti-PD-1). Furthermore, biochemical immune-related adverse events (irAEs) increased in tumor-bearing mice that received the additional adjuvant immunotherapy suggesting that shorter doses of neoadjuvant immunotherapy scheduled close to the time of surgery may optimize effective anti-tumor immunity and reduce severe irAEs[68].

Usually, immunotherapy is either repeatedly given alone or in combination with chemotherapy. However, contradictory results were reported in human studies. In breast cancer, interesting results were reported when immunotherapy (durvalumab) was started before chemotherapy[29]. In non-small-cell lung cancer, Lynch and colleagues found that phased ipilimumab plus paclitaxel and carboplatin improved immune-related PFS and PFS but not concurrent ipilimumab plus paclitaxel and carboplatin in a phase II study[69], but the addition of phased ipilimumab to first-line chemotherapy did not prolong OS compared with chemotherapy alone in patients with advanced squamous NSCLC in the phase III study[70].. In head and neck squamous cell carcinoma (HNSCC), neoadjuvant pembrolizumab monotherapy resulted in a high pCR in two phase II trials [35,36] even with only one dose of 200mg[40]. Taken together, these data suggest that the optimal timing administration (before, concomitant or after chemotherapy) is not completely understood. Moreover, the efficacy of immunotherapy could be prolonged and only one exposure could be sufficient.

5. Conclusion and implications for future management of cancers

Immunotherapy has been a revolution in cancer management with long-term survivors in the metastatic setting in diseases such as melanomas, for which prognosis was poor a few years ago. This has led to a prompt evaluation

of such therapies in earlier stages of cancer with the objective to cure patients. This article discussed the still limited amount of data finding the rationale to assess such therapy in this setting and reviewed the preclinical and clinical data available.

Overall, neoadjuvant immunotherapy is a promising approach for the treatment of cancers and the rationale supporting its use is strong. However, durable responses were reported only in a minority of patients. In order to understand why immune therapies work or fail, and how they can be improved to reach their hoped-for potential as a broadly transformative treatment for cancer, the best way is to assess those drugs in the neoadjuvant setting where we have access to samples pre-and post-treatment. T-cell-Inflamed tumors usually respond well to IICPB. In this regard, inflamed tumors are an appropriate model for IICPB monotherapy in the neoadjuvant setting in order to increase OS[61]. For immune desert or excluded tumors, the neoadjuvant setting is a good option to test new combinations[71] (with chemotherapy, with radiotherapy, with targeted therapies,…) or novel immuno-oncology drugs[61]. In addition to anti-PD-1, anti-PD-L1 and anti-CTLA-4, novel immunologic therapeutic approaches being developed in the neoadjuvant setting that could be of interest in immune desert and excluded tumors are represented by toll-like receptor 8 agonist (ClinicalTrials.gov Identifier: NCT02124850), and oncolytic viruses for example (ClinicalTrials.gov Identifier: NCT03259425). Moreover, bispecific antibodies, cancer vaccines and adoptive T-cell therapy are emerging therapies in immune-oncology that could be relevant in the neoadjuvant setting. In this context, cancer vaccine sipuleucel-T was evaluated in prostate cancer. Forty-two patients with untreated localized prostate cancer were treated on an open-label phase II study prior to planned radical prostatectomy (RP)[72]. However, downstaging was not observed at the time of RP relative to baseline. The authors described a greater than three-fold increase in infiltrating CD3(+), CD4(+) FOXP3(-), and CD8(+) T cells in the radical prostatectomy tissues compared with the pretreatment biopsy ($P < .001$). This level of T cell infiltration was observed at the tumor interface. The majority of infiltrating T cells were PD-1(+) and Ki-67(+) in favor of activated T cells.

Moreover, the timing of administration of immunotherapy is not well understood. Whether, IICPB present persistent immunomodulatory effects or prolonged exposure is necessary is unknown. Unlike vaccines, IICPBs are passively administered antibodies with uncertainty and variability in their ability to engage the adaptive immune system. It is unknown whether ongoing therapy is truly superior to limited treatment of a defined duration or to the use of a maintenance regimen with less frequent administration. Finally whether we need to administer neoadjuvant immunotherapy or adjuvant immunotherapy or both and how to combine

immunotherapy with other therapies either concurrently or sequentially are not known.. To this day, we do not know the best way to combine IICPB, a challenge for future clinical trials, indeed.

Conflict of interest

O Le Saux has received fees from Novartis, Lilly, MSD and Astrazeneca; and grants from Novartis, Fondation Hospira-Pfizer and Astellas outside of the submitted work.

I Ray-Coquard has received honoraria from AstraZeneca, Roche, Clovis, Tesaro, Genmab, MSD, Pfizer and PharmaMar; IRC has received research funding from MSD; IRC has received travel expenses from AstraZeneca, Roche, MSD, Tesaro and AstraZeneca.

The other authors declare no conflict of interest.

1. Cortazar, P. *et al.* Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet* **384**, 164–172 (2014).
2. Spring, L. M. *et al.* Neoadjuvant Endocrine Therapy for Estrogen Receptor-Positive Breast Cancer: A Systematic Review and Meta-analysis. *JAMA Oncol* **2**, 1477–1486 (2016).
3. Li, Z. *et al.* Correlation of pathological complete response with survival after neoadjuvant chemotherapy in gastric or gastroesophageal junction cancer treated with radical surgery: A meta-analysis. *PLoS ONE* **13**, e0189294 (2018).
4. Petrelli, F. *et al.* Correlation of pathologic complete response with survival after neoadjuvant chemotherapy in bladder cancer treated with cystectomy: a meta-analysis. *Eur. Urol.* **65**, 350–357 (2014).
5. Wei, S. C., Duffy, C. R. & Allison, J. P. Fundamental Mechanisms of Immune Checkpoint Blockade Therapy. *Cancer Discov* **8**, 1069–1086 (2018).
6. Melero, I., Berraondo, P., Rodríguez-Ruiz, M. E. & Pérez-Gracia, J. L. Making the Most of Cancer Surgery with Neoadjuvant Immunotherapy. *Cancer Discov* **6**, 1312–1314 (2016).
7. Bindea, G. *et al.* Spatiotemporal Dynamics of Intratumoral Immune Cells Reveal the Immune Landscape in Human Cancer. *Immunity* **39**, 782–795 (2013).
8. Verma, R. *et al.* Lymphocyte depletion and repopulation after chemotherapy for primary breast cancer. *Breast Cancer Res* **18**, 10 (2016).
9. Tarhini, A. A. & Iqbal, F. CTLA-4 blockade: therapeutic potential in cancer treatments. *Onco Targets Ther* **3**, 15–25 (2010).
10. Tatsumi, T. *et al.* Disease-associated Bias in T Helper Type 1 (Th1)/Th2 CD4+ T Cell Responses Against MAGE-6 in HLA-DRB10401+ Patients With Renal Cell Carcinoma or Melanoma. *J Exp Med* **196**, 619–628 (2002).
11. Tatsumi, T. *et al.* Disease Stage Variation in CD4+ and CD8+ T-Cell Reactivity to the Receptor Tyrosine Kinase EphA2 in Patients with Renal Cell Carcinoma. *Cancer Res* **63**, 4481–4489 (2003).

12. Péguillet, I. *et al.* High numbers of differentiated effector CD4 T cells are found in patients with cancer and correlate with clinical response after neoadjuvant therapy of breast cancer. *Cancer Res.* **74**, 2204–2216 (2014).
13. Wang, Q., Liu, F. & Liu, L. Prognostic significance of PD-L1 in solid tumor. *Medicine (Baltimore)* **96**, (2017).
14. Owonikoko, T. K. *et al.* PD-L1, PD-1, and CTLA-4 as prognostic biomarkers in resected non-small cell lung cancer. *JCO* **33**, 7551–7551 (2015).
15. Nakanishi, J. *et al.* Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. *Cancer Immunol. Immunother.* **56**, 1173–1182 (2007).
16. Gao, Q. *et al.* Overexpression of PD-L1 Significantly Associates with Tumor Aggressiveness and Postoperative Recurrence in Human Hepatocellular Carcinoma. *Clin Cancer Res* **15**, 971–979 (2009).
17. Reck, M. *et al.* Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N. Engl. J. Med.* **375**, 1823–1833 (2016).
18. Robert, C. *et al.* Nivolumab in previously untreated melanoma without BRAF mutation. *N. Engl. J. Med.* **372**, 320–330 (2015).
19. Robert, C. *et al.* Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N. Engl. J. Med.* **364**, 2517–2526 (2011).
20. Robert, C. *et al.* Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N. Engl. J. Med.* **372**, 2521–2532 (2015).
21. Larkin, J. *et al.* Combined Nivolumab and Ipilimumab or Monotherapy in Previously Untreated Melanoma. *N Engl J Med* **373**, 23–34 (2015).
22. Puzanov, I. *et al.* Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. *J Immunother Cancer* **5**, 95 (2017).

23. Man, J., Ritchie, G., Links, M., Lord, S. & Lee, C. K. Treatment-related toxicities of immune checkpoint inhibitors in advanced cancers: A meta-analysis. *Asia Pac J Clin Oncol* **14**, 141–152 (2018).
24. Liu, J. *et al.* Improved Efficacy of Neoadjuvant Compared to Adjuvant Immunotherapy to Eradicate Metastatic Disease. *Cancer Discov* **6**, 1382–1399 (2016).
25. Grinshtein, N., Bridle, B., Wan, Y. & Bramson, J. L. Neoadjuvant Vaccination Provides Superior Protection against Tumor Relapse following Surgery Compared with Adjuvant Vaccination. *Cancer Res* **69**, 3979–3985 (2009).
26. Nanda, R. *et al.* Pembrolizumab plus standard neoadjuvant therapy for high-risk breast cancer (BC): Results from I-SPY 2. *JCO* **35**, 506–506 (2017).
27. Schmid, P. *et al.* Abstract PD5-01: KEYNOTE-173: Phase 1b multicohort study of pembrolizumab (Pembro) in combination with chemotherapy as neoadjuvant treatment for triple-negative breast cancer (TNBC). *Cancer Res* **79**, PD5-PD5-01 (2019).
28. Schmid, P. *et al.* KEYNOTE-522: Phase III study of pembrolizumab (pembro) + chemotherapy (chemo) vs placebo + chemo as neoadjuvant therapy followed by pembro vs placebo as adjuvant therapy for triple-negative breast cancer (TNBC). *JCO* **36**, TPS602–TPS602 (2018).
29. Loibl, S. *et al.* A randomised phase II study investigating durvalumab in addition to an anthracycline taxane-based neoadjuvant therapy in early triple-negative breast cancer: clinical results and biomarker analysis of GeparNuevo study. *Ann. Oncol.* **30**, 1279–1288 (2019).
30. Antonio, S. Combining Atezolizumab with Neoadjuvant Chemotherapy Does Not Improve Pathologic Complete Response Rates for Patients with Triple- Negative Breast Cancer. 3.
31. McArthur, H. L. *et al.* A Pilot Study of Preoperative Single-Dose Ipilimumab and/or Cryoablation in Women with Early-Stage Breast Cancer with Comprehensive Immune Profiling. *Clin. Cancer Res.* **22**, 5729–5737 (2016).

32. Tarhini, A. A. *et al.* Immune monitoring of the circulation and the tumor microenvironment in patients with regionally advanced melanoma receiving neoadjuvant ipilimumab. *PLoS ONE* **9**, e87705 (2014).
33. Rozeman, E. A. *et al.* Neoadjuvant ipilimumab + nivolumab (IPI+NIVO) in palpable stage III melanoma: Updated data from the OpACIN trial and first immunological analyses. *JCO* **35**, 9586–9586 (2017).
34. Amaria, R. N. *et al.* Neoadjuvant immune checkpoint blockade in high-risk resectable melanoma. *Nat. Med.* **24**, 1649–1654 (2018).
35. Tarhini, A. *et al.* Neoadjuvant ipilimumab (3 mg/kg or 10 mg/kg) and high dose IFN-α2b in locally/regionally advanced melanoma: safety, efficacy and impact on T-cell repertoire. *J Immunother Cancer* **6**, (2018).
36. Yang, C.-F. J. *et al.* Surgical Outcomes After Neoadjuvant Chemotherapy and Ipilimumab for Non-Small Cell Lung Cancer. *Ann. Thorac. Surg.* **105**, 924–929 (2018).
37. Forde, P. M. *et al.* Neoadjuvant PD-1 Blockade in Resectable Lung Cancer. *N. Engl. J. Med.* **378**, 1976–1986 (2018).
38. Rusch, V. W. *et al.* Neoadjuvant atezolizumab in resectable non-small cell lung cancer (NSCLC): Initial results from a multicenter study (LCMC3). *JCO* **36**, 8541–8541 (2018).
39. Uppaluri, R. *et al.* Neoadjuvant pembrolizumab in surgically resectable, locally advanced HPV negative head and neck squamous cell carcinoma (HNSCC). *JCO* **35**, 6012–6012 (2017).
40. Wise-Draper, T. M. *et al.* Phase II multi-site investigation of neoadjuvant pembrolizumab and adjuvant concurrent radiation and pembrolizumab with or without cisplatin in resected head and neck squamous cell carcinoma. *JCO* **36**, 6017–6017 (2018).
41. Gao, J. *et al.* VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer. *Nat. Med.* **23**, 551–555 (2017).
42. Carthon, B. C. *et al.* Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial. *Clin. Cancer Res.* **16**, 2861–2871 (2010).

43. Necchi, A. *et al.* Pembrolizumab as Neoadjuvant Therapy Before Radical Cystectomy in Patients With Muscle-Invasive Urothelial Bladder Carcinoma (PURE-01): An Open-Label, Single-Arm, Phase II Study. *J. Clin. Oncol.* JCO1801148 (2018) doi:10.1200/JCO.18.01148.
44. Powles, T. *et al.* Clinical efficacy and biomarker analysis of neoadjuvant atezolizumab in operable urothelial carcinoma in the ABACUS trial. *Nat. Med.* **25**, 1706–1714 (2019).
45. Grootscholten C, Voest EE, Chalabi M et al. LBA37_PRNeo adjuvant ipilimumab plus nivolumab in early stage colon cancer. Annals of Oncology 2018; 29.
46. Gao, J. *et al.* A pilot randomized study evaluating nivolumab (nivo) or nivo + bevacizumab (bev) or nivo + ipilimumab (ipi) in patients with metastatic renal cell carcinoma (MRCC) eligible for cytoreductive nephrectomy (CN), metastasectomy (MS) or post-treatment biopsy (Bx). *JCO* **36**, 4520–4520 (2018).
47. Cloughesy, T. F. *et al.* Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. *Nat. Med.* **25**, 477–486 (2019).
48. Topalian, S. L., Taube, J. M., Anders, R. A. & Pardoll, D. M. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat. Rev. Cancer* **16**, 275–287 (2016).
49. Wolchok, J. D. *et al.* Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin. Cancer Res.* **15**, 7412–7420 (2009).
50. Hodi, F. S. *et al.* Evaluation of Immune-Related Response Criteria and RECIST v1.1 in Patients With Advanced Melanoma Treated With Pembrolizumab. *J Clin Oncol* **34**, 1510–1517 (2016).
51. Champiat, S. *et al.* Hyperprogressive Disease Is a New Pattern of Progression in Cancer Patients Treated by Anti-PD-1/PD-L1. *Clin. Cancer Res.* **23**, 1920–1928 (2017).
52. Saâda-Bouzid, E. *et al.* Hyperprogression during anti-PD-1/PD-L1 therapy in patients with recurrent and/or metastatic head and neck squamous cell carcinoma. *Ann. Oncol.* **28**, 1605–1611 (2017).
53. Miller, F. R. Intratumor immunologic heterogeneity. *Cancer Metastasis Rev.* **1**, 319–334 (1982).

54. Caswell, D. R. & Swanton, C. The role of tumour heterogeneity and clonal cooperativity in metastasis, immune evasion and clinical outcome. *BMC Med* **15**, 133 (2017).
55. Topalian SL. Immunologic heterogeneity of cancer: Determinants of response and resistance to immunotherapy. [abstract]. In: Proceedings of the AACR Precision Medicine Series: Drug Sensitivity and Resistance: Improving Cancer Therapy; Jun 18-21, 2014; Orlando, FL. Philadelphia (PA): AACR; Clin Cancer Res 2015;21(4 Suppl): Abstract nr IA01.
56. Tazdait, M. *et al.* Patterns of responses in metastatic NSCLC during PD-1 or PDL-1 inhibitor therapy: Comparison of RECIST 1.1, irRECIST and iRECIST criteria. *Eur. J. Cancer* **88**, 38–47 (2018).
57. Hughes, T. *et al.* The prognostic significance of stable disease following high-dose interleukin-2 (IL-2) treatment in patients with metastatic melanoma and renal cell carcinoma. *Cancer Immunol. Immunother.* **64**, 459–465 (2015).
58. Tsujino, K. *et al.* Response rate is associated with prolonged survival in patients with advanced non-small cell lung cancer treated with gefitinib or erlotinib. *J Thorac Oncol* **4**, 994–1001 (2009).
59. Hodi, F. S. *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* **363**, 711–723 (2010).
60. Zhou, Y. *et al.* First-line treatment for patients with advanced non-small cell lung carcinoma and high PD-L1 expression: pembrolizumab or pembrolizumab plus chemotherapy. *J Immunother Cancer* **7**, (2019).
61. Chen, D. S. & Mellman, I. Elements of cancer immunity and the cancer-immune set point. *Nature* **541**, 321–330 (2017).
62. Sharma, P., Hu-Lieskovan, S., Wargo, J. A. & Ribas, A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell* **168**, 707–723 (2017).
63. Topalian, S. L. *et al.* Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* **366**, 2443–2454 (2012).
64. Guo, S. & Dipietro, L. A. Factors affecting wound healing. *J. Dent. Res.* **89**, 219–229 (2010).
65. K, K. & Dl, H. Cracking open window of opportunity trials. *J Clin Oncol* **30**, 2573–2575 (2012).

66. Weber, J. *et al.* Adjuvant Nivolumab versus Ipilimumab in Resected Stage III or IV Melanoma. *N Engl J Med* **377**, 1824–1835 (2017).

67. Eggermont, A. M. M. *et al.* Adjuvant Pembrolizumab versus Placebo in Resected Stage III Melanoma. *N Engl J Med* **378**, 1789–1801 (2018).

68. Liu, J. *et al.* Timing of neoadjuvant immunotherapy in relation to surgery is crucial for outcome. *Oncoimmunology* **8**, (2019).

69. Lynch, T. J. *et al.* Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. *J. Clin. Oncol.* **30**, 2046–2054 (2012).

70. Govindan, R. *et al.* Phase III Trial of Ipilimumab Combined With Paclitaxel and Carboplatin in Advanced Squamous Non–Small-Cell Lung Cancer. *JCO* **35**, 3449–3457 (2017).

71. Chen, D. S. & Mellman, I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* **39**, 1–10 (2013).

72. Fong, L. *et al.* Activated lymphocyte recruitment into the tumor microenvironment following preoperative sipuleucel-T for localized prostate cancer. *J. Natl. Cancer Inst.* **106**, (2014).

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NAME OF THE TRIAL OR FIRST	PHASE	PRIMITIVE TUMOR TYPE	NUMBER OF PATIENTS	TYPE OF THERAPY EVALUATED	EFFICACY	TOXICITY	SURGICAL OUTCOMES
	y randomized phase II	negative breast cancer		b + chemotherapy	TNBC (pCR 60% vs 20%) HR+/HER2- (34% vs 13%)	insufficiencies (n=6/69)	
KEYNOTE-173[27]	Phase Ib	TNBC	20	Pembrolizumab + chemotherapy	ypT0 ypN0 pCR rate: 50% to 80%	1 grade 2/3 alanine aminotransferase elevation	NA
KEYNOTE-522[28]	Phase III	TNBC	602	Pembrolizumab + chemotherapy	pCR rate 64.8% with the pembrolizumab/chemotherapy regimen compared to 51.2% (standard arm) Benefit more important in lymph node positive TNBC compared to lymph node negative TNBC	grade 3 or higher treatment-related adverse event (AE) rates: 78.0% - experimental arm- compared to 73.0% - standard arm. Incidence of death: 0.4% versus 0.3%.	NA
GEPARNUEVO[29]	Randomized phase II	TNBC	31	Durvalumab + chemotherapy (CT)	Patients who started durvalumab before chemotherapy+durvalumab presented more pCR (OR=2.22 [95%CI 1.06-4.64]. This phased administration of IICPB called "window of opportunity" resulted in a higher pCR rate 61.0% compared to 41.4% of the patients in the control arm (p=0.052)	Treatment delay in 54.8% of patients receiving the combination vs 40.0 to 67.7% patients treated with chemotherapy alone	NA
NEOTRIPAPDL1[30]	Phase III	TNBC	280	Atezolizumab + CT	pCR rate 43.5% vs. 40.8% not statistically significant	Immune-mediated adverse events of any grade: 8% >G3 infusion reactions: 1.4%	NA
MCARTHUR HL[31]	Pilot study	Breast cancer	19	Single dose ipilimumab +/- cryoablation	NA	Grade III unrelated rash in one patient	No delay in surgery
TARHINI AA[32]	NA	Melanoma	35	Ipilimumab	ORR was 9% (2 complete responses and 1 partial response)	Grade 3 diarrhea/colitis (5; 14%), hepatitis (2; 6%), rash (1; 3%), elevated lipase (3; 9%)	NA
OPACIN[33]	Two-arm phase Ib feasibility trial	Melanoma	20 (10 in the neoadjuvant arm)	Ipilimumab + Nivolumab	ORR 80% pCR 30% Estimated 30 months relapse-free survival rates were 80% for the neoadjuvant arm and 60% for the adjuvant arm and 30 months OS rates were 90% and 67%, respectively	High frequency of grade ¾ toxicity (n=18). G3 elevated lipase (n=8), G3 diarrhea and colitis (n=6)	No delay in surgery

Table.1

						No surgery related AEs attributed to immunotherapy	
AMARIA RN[34]	Phase II	Melanoma	23	Ipilimumab + Nivolumab	ORR 73% pCR 45%	G3 irAEs 73%	Rapid disease progression for 17% of patients in the nivolumab monotherapy arm
TARHINI AA[35]	Randomized trial	Melanoma	30	Ipilimumab (3 or 10mg/kg) + high-dose interferon	pCR 35% (95CI[17-56])	One toxicity-related drug withdrawal. Grade ≥3 elevated transaminases (n=6, 20.0%). Grade ≥3 rash (n=7, 23.0%) Toxicities more frequent at 10mg/kg	NA
TOP1201 IPI[36]	Phase II	Non-small cell lung cancer (NSCLC)	24	Ipilimumab + chemotherapy	ORR (n=14, 58%)	Ipilimumab related AEs: grade 2 pneumonitis (n=1, 4%), grade 3 adrenal insufficiency (n=4, 17%), diarrhea/colitis 8 (grade 1 or 2: n=6, 25%; grade 3: n=3, 13%).	Prolonged air leak (n = 2, 15%) Urinary tract infection (n = 2, 15%) No apparent increased occurrence of adverse surgical outcomes compared to historical cohort.
NCT02259621[37]	Phase II	NSCLC	22	Nivolumab (2 doses, 4 weeks and 2 weeks prior to surgery)	ORR 10% (n=2) pCR 43.0% 95IC[24-63] Discrepancy between ORR and pCR suggests that some patients may derive benefit from immunotherapy that is not captured radiographically	Pneumonia grade≥3 (n=1,5%)	No delay in surgery
LCMC3[38]	Phase II	NSCLC	21	Atezolizumab	Major pathologic response: 21% (95% CI 6-46)	Gr 1 pyrexia, Gr 2 dyspnea for 2 patients	No major delay in surgery
NCT02296684[39]	Phase II	HPV negative HNSCC	25	Pembrolizumab	pCR 42.0% (n=10)	No serious drug-related AEs	No unexpected surgery delays or complications
NCT02641093[40]	Phase II	HNSCC	28	One dose Pembrolizumab 200mg	Major pathologic response (>70%): 32%	NA	NA
NCT01194271[41]	Phase II	Prostate	19	Ipilimumab + androgen deprivation therapy (ADT)	No pCR	NA	NA

CARTHON BC[42]	Controlled clinical trial	Bladder	12 (6 patients at 3mg/kg/dose with results on immune monitoring and 6 at 10 mg/kg/dose)	Ipilimumab	pCR 33.3 % (n=4/12)	Treatment completion rate (n=11/12) Reason for not receiving second dose = diarrhea grade 3.	No significant surgical delays due to irAEs in the 3 mg/kg/dose cohort Surgical delays (n=3, grade 2/3 diarrhea)
PURE-01[43]	Phase II	Muscle invasive urothelial bladder cancer	50	Pembrolizumab	Downstaging to pT0 (42%; 95% CI, 28.2% to 56.8%).	One discontinuation of pembrolizumab (grade 3 transaminase increase - 2%)	All patients underwent radical cystectomy
ABACUS[44]	Phase II	Muscle invasive urothelial bladder cancer	95	Atezolizumab	pCR rate 31% (95% CI: 21–41%)	No new safety signals	Grade I to II surgical complications (Clavien Dindo classification): 39 of 87 (45%)
GROOTSCHOLTEN C[45]	Phase II	Colon cancer	14 (mismatch repair proficient (pMMR) (n = 8) or mismatch repair deficient (dMMR) (n = 7)	Ipilimumab + Nivolumab	Major pathological responses (<5% viable tumor cells) 100% (n=7/7) CR 57% (n=4/7)	Well tolerated	No delays in surgery
NCT02210117[46]	Phase I	Kidney cancer	105	Nivolumab (Nivo) Nivo+Bevacizumab (Bev) Nivo+Ipilimumab (Ipi)	ORR: 55% nivo, 44% nivo + bev, 43% nivo + ipi	> Grade 3: 38% for nivo, 42% for nivo + bev (including 18% hypertension), and 47% for nivo + ipi	NA
NEO-NIVO[47]	Phase II	Glioblastoma	35 (16 in the neoadjuvant group and 19 in the adjuvant group)	Nivolumab	Benefit for the neoadjuvant group compared to the adjuvant group: • Increased OS: HR 0.39 (95%CI 0.17–0.94; P = 0.04). • Increased PFS: (HR 0.43; 95% CI 0.20–0.90; P = 0.03)	One grade 3 pneumonitis. One grade 4 elevation in alanine aminotransferase.	No delays in surgery

Table.2

NAME OF THE TRIAL OR FIRST AUTHOR	IMMUNE MONITORING	BIOMARKERS OF RESPONSE	BIOMARKERS OF TOXICITY
I-SPY2[26]	NA	NA	NA

KEYNOTE-173[27]	NA	NA	NA
KEYNOTE-522[28]	NA	No effect of PD-L1 PD-L1+: pCR 68.9% vs 54.9% PD-L1-: pCR 45.3% vs 30.3%	NA
GEPARNUEVO[29]	NA	NA	NA
NEOTRIPAPDL1[30]	NA	PD-L1–positive status according to immunohistochemistry (P < .0001)	NA
MCARTHUR HL[31]	Sustained peripheral elevations in: -Th1-type cytokines -activated (ICOS+) and proliferating (Ki67+) CD4+ and CD8+ T cells And high ratio of Ki67+ effector T cells/regulatory T cells within tumor	NA	
TARHINI AA[32]	Linear combination of 2 regulatory cytokines at baseline [TGF- β1 (ρ = 0.19) and IL-10 (ρ = -0.34)] significantly associated with PFS (HR 2.66; p = 0.035).	• Significant increase in circulating regulatory T cells • Significant increase in $CD8^+$ T cell • Increased tumor infiltration by fully activated ($CD69^+$) $CD3^+/CD4^+$ and $CD3^+/CD8^+$ T cells with evidence of induction/potentiation of memory T cells ($CD45RO^+$) • Significant decrease in circulating MDSC Lin1-/HLA-DR-/$CD33^+/CD11b^+$ • a 22 immune active and proinflammatory tumor microenvironment gene signature • Cytokine expression profile (IL-1β, VEGF, G-CSF, HGF, IL-13, IL-17, GM-CSF, MCP-1, IL-5, IL-7, IL-4, IL-10, IFN-γ, IL-8 and IL-2) from patients PBMCs (peripheral blood mononuclear cells) in response to NY-ESO-1	IL-17 at baseline (p = 0.02) correlated with the incidence of grade 3 diarrhea/colitis
OPACIN[33]	NA	Baseline PD-L1 and β2 microglobulin (absolute protein counts) B cells within Tertiary Lymphoid Structures (TLS)[49]	NA
AMARIA RN[34]	NA	Higher TMB (trend) Higher CD8+ T-cell infiltrate, tumor cell PD-L1 expression, and expression of lymphoid markers (Granzyme B, CD4, FoxP3, CD20, and PD-1) Expression of CD45RO, β2-microglobulin, T cell markers (CD3, CD8), B cell markers (CD19, CD20), cell proliferation (assessed by Ki67) within CD45+ cell Higher clonality of TCR B cells within TLSs[49]	NA
TARHINI AA[35]	NA	NA	NA
TOP1201 IPI[36]	• Significantly increased frequencies of highly activated T cells in the peripheral circulation • Significantly increase of CD4+ and CD8+ cells expressing ICOS, HLA-DR, CTLA-4 and PD-1 • Higher frequencies of activated TILs in resected tumors compared to PBMCs	NA	NA
NCT02259621[37]	NA	• Higher mutational and neoantigen burden • Mutation associated neoantigen specific TCR increase in peripheral blood • Responses in PD-L1-negative tumors	NA
LCMC3[38]	NA	NA	NA
NCT02296684[39]	NA	• PDL1, CD8, CD8/PD1 and CD4 in baseline biopsies • Serum secreted cytokines • Tumor mutational burden	NA
NCT02641093[40]	NA	Immune cell infiltration Increased PD-L1 and PD-L2	NA

NCT01194271[41]	• Increase in CD4+ and CD8+ T cells, including PD-1+ and ICOS+ subsets, increase in CD45RO+, granzyme-B (GrB)+, and CD68+ cells • Significantly greater immune cell infiltration in prostate tumors compared to ADT alone group • Significantly higher PD-L1 expression on CD4+ T cells, CD8+ T cells, and CD68+ macrophages after treatment • Increase in CD4+ PD-L1+ T cells from 0.2 to 0.7%), CD8 PDL1+ (4.4 to 21.3%) and CD68 PD-L1+ (from 2.5 to 25%) • Significantly higher expression of V-domain Ig-containing Suppressor of T-cell Activation (VISTA) on CD4 (0.0% to 4%), CD8 (0.0 to 7.0%) and CD68 macrophages (7 to 31%) • Significant changes in the expression of a total of 690 genes (increased PD-L1 and VISTA expression) • Significantly greater proportion of CD68+ macrophages with PD-L1 and VISTA expression in post-treatment prostate tumors in comparison to historical melanomas • increase in the frequency of PD-L1+ and VISTA+ macrophages with expression of CD163 and ARG1, suggesting an M2-like phenotype and function	NA	NA
CARTHON BC[42]	• ICOS expression in peripheral blood and tumor CD4 T cells is increased • IFNγ-Producing CD4+ICOShi Effector T Cells That Recognize the Tumor Antigen NY-ESO-1 in the Peripheral Blood of Anti-CTLA-4 Treated Patients • FOXP3 Expression Is Lower in CD4 T Cells in Tumor Tissues • IFNγ Expression Is Increased in Tumor Tissues • The Ratio of CD4+ICOShi Effector to CD4+FOXP3+ Regulatory T Cells Is Increased • Higher frequency of CD4+ICOShi T cells and IFN-γ mRNA levels in nonmalignant prostate tissues and incidental prostate tumor tissues removed at the time of radical cystoprostatectomy	Increased frequency of CD4+ICOShi T cells, sustained over a period of 12 weeks of therapy	NA
PURE-01[43]	NA	• PD-L1 CPS ≥ 10%: pT0 after RC in 54.3% of patients with PD-L1 CPS ≥ 10% (n = 35), vs 13.3% in those with CPS < 10% (n = 15). • Tumor mutation burden (TMB) - cutoff at 15 mutations/Mb.	NA
ABACUS[44]	• 5 patients changed from an excluded to an inflamed phenotype and 4 patients changed from an inflamed to an excluded phenotype.	• High presence of intraepithelial CD8+ cells (pCR rate of 40% (95% CI: 26–57%) compared to a rate of 20% (95% CI:9–35%) with absence of CD8 (P<0.05)	NA

		• A predefined eight-gene cytotoxic T cell transcriptional signature (tGE8) (P<0.01) • Expression of dually stained cells for CD8 and GZMB (14 out of 16 patients, 87% in responding patients versus 3 out of 10 in non responding patients, 30%; P<0.05) • Low FAP (Fibroblast Activation Protein) expression (P<0.01)	
GROOTSCHOLTEN C[45]	• T-cell infiltration, particularly CD8+ T-cells	Post-treatment IFNγ gene signatures Deficiency in the mismatch repair system	NA
NCT02210117[46]	• NA	• Tumor infiltrating CD8 T cells correlate with clinical responses to nivo or nivo + bev, but not to nivo + ipi • Tumor IFN pathway gene expression • B cells within TLSs[49] • PD-L1 status, tumor mutation or mutation burden, neoantigens did not correlate with response	NA
NEO-NIVO[47]	• Upregulation of T cell– and interferon-γ-related gene expression • Downregulation of cell-cycle-related gene expression within the tumor • Focal induction of programmed death-ligand 1 in the tumor microenvironment • Enhanced clonal expansion of T cells • Decreased PD-1 expression on peripheral blood T cells • Decreasing monocytic population	• Standardized baseline peripheral T cell receptor clonality (hazard ratio of 1.48 for each standard deviation increase of 1, P = 0.12). • Cell-cycle-related gene set variation analysis enrichment score (R2 = 0.57).	NA