European Journal of Cancer 198 (2024) 113501

Contents lists available at ScienceDirect

European Journal of Cancer

journal homepage: www.ejcancer.com

Review

The future of targeting cytotoxic T-lymphocyte-associated protein-4: Is there a role?☆

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ARTICLE INFO

Keywords:
Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)
Programmed cell death protein 1 (PD1)
PD1 Ligand (PDL1)
Immunotherapy
Resistance

ABSTRACT

The 2022 yearly Think Tank Meeting in Siena, Tuscany (Italy), organized by the Italian Network for Tumor Biotherapy (NIBIT) Foundation, the Parker Institute for Cancer Immunotherapy and the World Immunotherapy Council, included a focus on the future of integrating and expanding the use of targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). The conference members exchanged their views on the lessons from targeting CTLA-4 and compared the effect to the impact of blocking Programmed cell death protein 1 (PD1) or its ligand (PDL1). The increasing experience with both therapeutic approaches and their combination suggests that targeting CTLA-4 may lead to more durable responses for a sizeable proportion of patients, though the specific mechanism is not entirely understood. Overcoming toxicity of blocking CTLA-4 is currently being addressed with different doses and dose regimens, especially when combined with PD1/PDL1 blocking antibodies. Novel therapeutics targeting CTLA-4 hold the promise to reduce toxicities and thus allow different combination strategies in the future. On the whole, the consent was that targeting CTLA-4 remains an important strategy to improve the efficacy of cancer immunotherapies.

1. Introduction

Blocking checkpoints of T-cell activation is a key step to overcoming resistance to current cancer therapies. The importance of this discovery was recognized by awarding the Nobel Prize in Physiology and Medicine to Professors James Allison and Tasuku Honjo [106]. Based on their discovery, recent research has targeted similar checkpoints to improve therapies containing monoclonal antibodies (mAb) against CTLA-4 and PD1 or its ligand PDL1 [53]. The Siena Think Tank 2022 meeting reviewed the role of targeting CTLA-4 as a template to devise new therapeutic approaches in oncology [29,63–65].

CTLA-4 was initially recognized as a negative regulator of T cell responses in animals, when pre-established tumors were rejected after treatment with anti-CTLA-4 antibodies [56]. This observation supported the clinical development of ipilimumab, a fully human, IgG1κ monoclonal anti-CTLA-4 antibody [40,46]. In patients with metastatic melanoma, median overall survival (OS) was 10.0 months among patients receiving ipilimumab plus gp100, as compared with 6.4 months among patients receiving gp100 alone (hazard ratio for death, 0.68; $P < 0.001$). This considerable improvement in OS (3.6-months) led subsequently to the approval of ipilimumab [40,45]. A couple of years later, a pooled analysis of 1861 patients enrolled in the initial Phase 2 and 3 studies, demonstrated a median OS of 11.4 months (95% CI: 10.7–12.1 months). However, among these patients there were 254 patients with at least 3

☆ This Article is dedicated to the memory of Professor Dr. Soldano Ferrone, who deeply influenced the Think Tank meetings in Siena with his scientific curiosity.

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https://doi.org/10.1016/j.ejca.2023.113501
Received 13 December 2023; Accepted 13 December 2023
Available online 21 December 2023

years of survival follow-up. Three-year survival rates were 22%, 26%, and 20% for all patients, treatment-naïve and previously treated patients, respectively [81]. Due to this unprecedented OS, ipilimumab has been studied in other malignancies and has received approvals in tumors such as renal cell carcinoma, non-small cell lung cancer (NSCLC), malignant pleural mesothelioma, colorectal cancer and esophageal squamous cell carcinoma [51]. Currently, there is only one other approved CTLA4-targeting mAb, tremelimumab [50,62]. Tremelimumab is a human IgG2 mAb and was approved in combination with the PDL1 mAb durvalumab for the treatment of adult patients with unresectable hepatocellular carcinoma [50]. .

The success of ipilimumab, with its long, durable responses in cutaneous melanoma, even after disease recurrence (Colucci, D'Alonzo et al., 2022), subsequently encouraged the clinical evaluation of similar checkpoint inhibitors, such as the humanized or fully human monoclonal anti-PD1 antibodies, pembrolizumab and nivolumab [21,66,93]. PDL1 targeting mAbs such as atezolizumab, avelumab, and durvalumab, followed soon after as additional treatment options [96]. Thousands of clinical trials are underway, testing anti-CTLA4 and anti-PD/PDL1 mAbs in combination with each other, and a growing body of evidence suggests that these immunotherapeutic agents can have additional anti-tumor activities when combined with chemotherapy, anti-angiogenic treatments or radiation [83,86].

Mechanisms Underlying Anti-CTLA-4 and Anti-PD1/PDL1 Checkpoint Blockade – A Template for Future Drug Discovery?

Anti-CTLA4 and anti-PD1/PDL1 therapies differ in their induction of immune responses as assessed by $CD4^+$ and $CD8^+$ T cell responses, respectively (Table 1). In a murine tumor model, immune checkpoint blockade differentially modulated infiltrating T cells: with CTLA-4 blockade a reduction of Treg subsets was observed, while PD1 blockade had a lesser effect. Also, anti-CTLA-4 treatment led to a swift increased expression of inducible co-stimulator (ICOS) on Th1-like $CD4^+$ effector T cells that express the Tbet transcription factor, which represented a novel subset of T cells [13,58]. This effect was not observed with anti-PD1 treatment [24,99]. Dual blockade of CTLA-4 and PD1 led to the expansion of Th1-like $CD4^+$ effector T cells (identified as $PD1^+ICOS^+Tbet^+$) and to activated, terminally differentiated $CD8^+$ effector T cells (identified as $PD1^+LAG3^{int}$ $TIM3^{int}$). These observations showed a complementary anti-tumor effect when both CTLA-4 and PD1 were blocked [98]. ICOS, a T-cell-specific surface molecule that is structurally related to CD28 and CTLA-4, had been previously shown to play a role in anti-CTLA-4 mediated anti-tumor responses [58]. Thus, it became clear that ICOS is required for optimal anti-tumor responses during CTLA-4 blockade [35]. This was further confirmed in animal studies, where concomitant CTLA-4 blockade and ICOS engagement increased antitumor responses [32]; however, in spite of these intriguing results, combined targeting of CTLA-4 and ICOS has unfortunately not reached the clinic. Therefore, T cell differentiation plays a key role during immune checkpoint therapy: blockade of CTLA-4 can result in clonal diversity and differentiation (including differentiation of $ICOS^+CD4^+$ effector T cells), while PD1 blockade has less of an impact on the expansion of $CD8^+$ T cell phenotypes [100]. Finally, blocking CTLA-4 or PD1/PDL1 may have a differential effect on regulatory T cells (Tregs), because CTLA-4 is preferentially expressed on immune suppressive Tregs [101,72,73]. Although, the current anti-CTLA-4 antibodies (ipilimumab and tremelimumab) do not deplete FOXP3 + regulatory T cells in humans [84].

2. Rationale for the development of combination strategies

Compared to PD1/PDL1 inhibition, CTLA-4 blockade faces two main hurdles: lower rate of responses and higher toxicity [60]. For example, in a randomized study of patients with cutaneous melanoma receiving ipilimumab or nivolumab as adjuvant therapy, nivolumab showed better 12-month recurrence-free survival than ipilimumab (70% vs 61%). In addition, immune-related adverse events (irAE) were observed in 14% of nivolumab-treated patients and in 46% of ipilimumab-treated patients [97]. Despite this difference, combination of ipilimumab with anti-PD1 therapy has improved response and survival rates in multiple advanced melanoma trials [103,44,54,88]. This has resulted in sustained long-term OS at 6.5 years of 57% in the nivolumab-ipilimumab combination, compared to 43% in the nivolumab arm and to 25% in the ipilimumab arm ([55,104].). Initial signs of activity of ipilimumab combined with fotemustine have been reported in a subset of 20 metastatic melanoma patients with active, asymptomatic brain metastases, with a 3-year survival rate of 28% [23,25]. Two subsequent phase II studies reported the efficacy of ipilimumab combined with nivolumab in the same population of patients (Twabi, Forsyth et al., 2018, [61][27]). Consistently, the phase III NIBIT-M2 study showed a 41% 5-year overall survival (OS) of melanoma patients with asymptomatic brain metastases treated with ipilimumab plus nivolumab (Di Giacomo, Chiarion-Sileni et al., 2021). The benefit of combining anti-CTLA-4 with

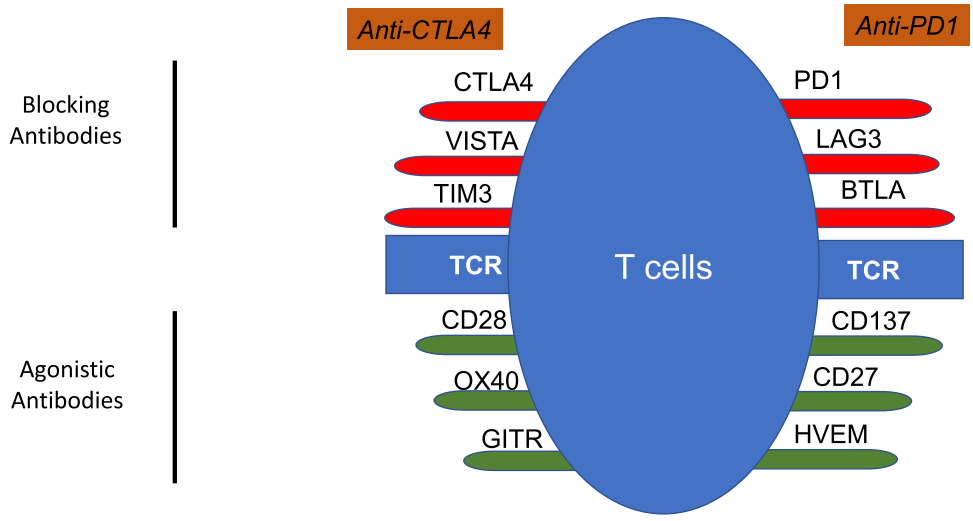

Fig. 1. Co-stimulatory and co-inhibitory interactions regulate T cell responses. Multiple co-stimulatory (e.g., CD28; CD137; CD27; OX40; GITR, glucocorticoid-induced tumor necrosis factor receptor-related protein; HVEM, herpesvirus entry mediator) and co-inhibitory molecules (e.g., CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; LAG3, lymphocyte activation gene 3; PD1, programmed cell death protein 1; VISTA, V-domain Ig suppressor of T-cell activation; TIM3, T cell membrane protein 3; BTLA, B and T lymphocyte attenuator) can regulate anti-tumor immune response and can be targeted by therapeutic monoclonal antibodies.

Table 1
Examples of Differences between CTLA-4 and PD1/PDL1 Blockade.

Anti-CTLA4	Reference	Anti-PD1/PDL1	Reference
Hard wired	[72][72]	Induces immune resistance	[72][72]
Targets CD28 pathway	Krummel et al 1995[52]	Targets TCR pathway	[34][34]
Involved during priming	[78] Rotte ($year$)[78]	Influences differentiated T cells	[99][99]
Expands clonal diversity	[100][100]	No expansion of clonal diversity	[100][100]
Response delayed	[102][102]	Response rapid (e.g., in combination with radiotherapy)	[17][17]
Primarily effects $CD4^+$ T cells	[99][99]	Primarily effects $CD8^+$ T cells	[99][99]
Can move T cells into "cold" tumors	Sharma et al 2015[85]	Limited/no impact on T cell recruitment into tumors	Sharma et al 2015[85]
Adverse events frequent	[72][72]	Adverse events less frequent	[72][72]
Tumor recurrence rare after CR/PR	[81][81]	Tumor recurrence occurs more frequently after CR/PR	Borcoman et 2018[7]
Induces $CD4^+ICOS^+Tbet^+$Th1-like Effector	[99][99]	Induces $CD8^+Tbet^+EOMES^+KLRG\text{-}1^+$ Effector	[99][99]
Induces $CD8^+Tbet^+$ $EOMES^+KLRG\text{-}1^+$ Effector	[99][99]	Induces exhausted CD8 + T cells (i.e., $CD8^+Tbet^+PD1^{++}LAG3^{++}TIM3^{++}$)	[99][99]

anti-PD1/PDL1 agents has also been reported in other cancer types, such as untreated advanced NSCLC, malignant pleural mesothelioma and unresectable sarcoma ([8–11,22,42,49], Calabrò, Morra et al., 2021, [82]).

Perhaps nowhere else has the combination of ipilimumab with an anti-PD1 shown a greater benefit than in the neoadjuvant setting. In a neoadjuvant study of patients with cutaneous melanoma, the most favorable benefit/risk ratio was observed in patients receiving the combination of 3 mg/kg nivolumab and 1 mg/kg ipilimumab, as this regimen had the lowest irAEs while maintaining a high pathological complete response (pCR) rate [67]. Similar impressive results were reported in the NICHE-2 neoadjuvant study of nivolumab combined with ipilimumab [16]. In this study, pathologic responses were observed after short-term neoadjuvant nivolumab plus ipilimumab treatment was given to colorectal cancer patients with deficient DNA mismatch repair (dMMR): 95% major pathological response (MPR) and 67% complete response, and no disease recurrence at 13 months follow-up were seen.

Similarly, in a clinical trial with neoadjuvant tremelimumab plus durvalumab in cisplatin-ineligible patients who had localized bladder cancer, treatment led to pCR rate of 37.5%, and even in patients with large tumor burden (cT4a disease), there was an observed pCR of 42% [37].

The question of whether a CTLA-4 blockade is necessary or whether, in the neoadjuvant setting, the dose of CTLA-4 inhibition may be lower compared to more advanced conditions remains unresolved. For example, patients with dMMR rectal cancer who were treated with the PD1 inhibitor dostarlimab had 100% complete response rate [15]. Along this line, patients with advanced or recurrent dMMR endometrial cancer had a 42% objective response rate when treated with dostarlimab monotherapy (Oaknin, Thinker et al., [70] 70). Hence, it appears that CTLA-4 blockade may not be necessary in all cases, including dMMR tumors. In contrast, in a neoadjuvant study of patients with head and neck squamous cell cancer (HNSCC) who received the combination of ipilimumab and nivolumab, 35% experienced a major pathological response (MPR, 90–100% response), while patients receiving monotherapy nivolumab had an MPR rate of 17% [95].

2.1. Resistance in tumors – role of CTLA-4

With the approval of ipilimumab and subsequently of PD1/PDL1 inhibitors, the real-world percentage of responders is now 12.46% (95% CI: 12.37–12.54%) for all known malignancies [41]. Thus, there is still a large proportion of patients who do not benefit from immunotherapy. In metastatic melanoma patients who progressed following prior first-line anti-PD-1 immunotherapy and who were re-treated with either ipilimumab alone or combination of ipilimumab plus anti-PD1, OS lasted longer in the ipilimumab plus anti-PD1 group (median OS 20.4 months [95% CI: 12.7–34.8]) compared to those who received ipilimumab alone (8.8 months [6.1–11.3]; hazard ratio [HR] 0.50, 95% CI: 0.38–0.66; $P < 0.0001$) [74]. These initial results were confirmed by two subsequent clinical trials [71,92]. The observation that patients who are resistant to prior anti-PD1 treatment can still respond to immunotherapy that includes CTLA-4 indicates that such patients retain an ability to mount an immune response, most likely because their tumor contains activated T cells. Factors that contribute to resistance to immunotherapies include dysregulation of antigen-presenting machinery (e.g., MHC down-regulation), lack of IFN-γ pathway up-regulation, and reduced response of the innate immune system. For example, mutations in the interferon-receptor–associated Janus kinase 1 (*JAK1*) or Janus kinase 2 (*JAK2*) genes [36], and concurrent deletion of the wild-type allele can contribute to resistance [107]. Similarly, a truncating mutation of the gene for beta-2-microglobulin (*B2M*) can also lead to resistance [107].

2.2. Toxicity is a key limitation

Despite practice-changing results, ipilimumab-associated toxicities remain a concern; mainly because some of the irAEs (i.e., endocrine) are not reversible. Despite tremelimumab has also been approved, most of the observations on how to potentially overcome toxicities associated with a CTLA-4 inhibitor are based on ipilimumab. Currently, it is assumed that both CTLA-4 blocking agents are similar in their mode of action, with tremelimumab being dosed at a lower drug concentration compared to ipilimumab. Treatments against CTLA-4-associated toxicities are being investigated. Abatacept is a neutralizing CTLA-4-Ig Fc fusion agent, which can mitigate irAEs but that can reduce ipilimumab's therapeutic activity. Recently, CTLA-4 mutants that bind to B7–1 and B7–2, but not to clinical anti-CTLA-4 antibodies, including belatacept, were found to abrogate irAEs without affecting cancer immunotherapy efficacy. Thus, clinically used belatacept may emerge as a broadly applicable drug to abrogate irAEs while preserving the therapeutic efficacy of CTLA-4-targeting immune checkpoint inhibitors [59]. Stopping ipilimumab when irAEs appear, and restarting treatment after resolution, also seems to be associated with a reduced toxicity profile [1]. Finally, it remains mandatory that patients, treating physicians and general practicians should be educated on the possible immune-related toxicities deriving from anti-CTLA-4 containing regimens, though most centers today are well versed in dealing with irAEs. The most common complications are mainly observed in patients treated with combination therapies; it is therefore recommended that specialized centers should treat and follow such patients.

2.3. Reduced dosing

In Checkmate 511, two different doses of ipilimumab and nivolumab were investigated [57]. This study demonstrated that reducing ipilimumab dose from 3 mg/kg to 1 mg/kg lowers the incidence of treatment-related grade 3–5 irAEs without having an impact on efficacy. Given that there are different dose and dose schedule recommendations for various malignancies, it is possible that the recommend dose of ipilimumab may depend on the type of malignancy. It appears that in some tumors, such as mesothelioma or HNSCC, at least 3 mg/kg ipilimumab are needed, while in cutaneous melanoma this may not be required.

Nevertheless, in patients with advanced melanoma ipilimumab 10 mg/kg resulted in significantly longer overall survival than did ipilimumab 3 mg/kg, but with increased treatment-related adverse events [4]. In patients with brain metastases (e.g., from cutaneous melanoma) 3 mg/kg of ipilimumab is generally a recommended dose. 0.3 mg/kg or any dose below 1 mg/kg appears to be inefficacious and this because no clinical efficacy data have been published or no activated T cells have been detected. Dose levels above 10 mg/kg of ipilimumab are instead associated with auto-immune reactions, some of which appear to induce self-reactive T cells.

2.4. Reduced dose regimen

Alternative dose schedules may also help mitigate toxicity – for instance, ipilimumab dosing every 6 weeks instead of every 3 weeks has been explored in NSCLC [43]. The half-life of ipilimumab, while maintaining anti-tumor effect, may further justify more spaced-out dosing [14]. More importantly, it appears that limited dosing might be sufficient to achieve anti-tumor responses compared to long-term continuous dosing; two doses of CTLA-4, for example, appear to be sufficient. Furthermore, dose regimens may differ between different tumor types or even clinical settings. For example, in the neoadjuvant setting a reduced and limited dose of ipilimumab may be sufficient because the overall anti-tumor response may require a lower stimulus compared to advanced malignancies, or those where the baseline immune response is particularly suppressed.

2.5. Novel CTLA-4 mAb – new approaches

Recent efforts have focused on finding new CTLA-4 inhibitors with a reduced irAEs profile. Given that patients with CTLA-4 deficiency have a phenotype similar to those treated with anti-CTLA-4 mAb, which includes a reduction in Treg and B-cell immune deficiencies, as well as autoimmune symptoms [47,68], strategies for developing novel CTLA-4 mAb have mainly focused on reducing undesirable immune co-activation (e.g., by optimizing the immunoglobulin frame). Among those are, designing the release of CTLA-4 as close as possible to the tumor microenvironment (e.g., pH-dependent release of mAb) or improving the binding affinity of novel anti-CTLA-4 mAb. In several animal models, optimization of the Fc domain appeared to maintain the anti-tumor response while reducing irAEs [3,48,80]. Novel anti-CTLA-4 agents engineered for either higher binding affinities or for enhanced Treg depletion, include quavonlimab, zalifrelimab, GIGA-, CBT-509, AGEN1181, HCAb, 4003–2, pH sensitive Abs (Table 2). Also, bispecific anti- CTLA-4/PD1 antibodies are being developed and are designed to reduce irAEs.

2.6. Utility of Anti-CTLA-4 in the neoadjuvant setting

Anti-CTLA-4 seems to have a particularly potent role in the neoadjuvant setting by expanding diversity and amplitude of T cell clones [6]. The first neoadjuvant clinical trial with anti-CTLA-4 was conducted in 2006 in a small cohort of patients with localized bladder cancer prior to any FDA-approvals of immune checkpoint therapy, which clearly established the safety and feasibility of this approach and reported pCR rate of 25%, providing the first data to demonstrate efficacy of immune checkpoint therapy in bladder cancer [13,58]. In a pooled analysis of the NIMC (Neoadjuvant Immunotherapy Melanoma Consortium), a 26% major pathologic response (MPR) rate of 26% was observed for neoadjuvant anti-PD1 monotherapy, whereas a 61% MPR rate was observed for the combination of anti-CTLA-4 plus anti-PD1 ([67], Amaria, [2,67]). Moreover, in the macroscopic melanoma stage IIIC-D setting it has been demonstrated that the combination of ipilimumab 1 mg/kg plus nivolumab 3 mg/kg is as effective in terms of MPR and recurrence-free survival (RFS) and OS as the much more toxic combination of ipilimumab 3 mg/kg plus nivolumab 1 mg/kg ([79], Versluijs, Menzies et al., 2023). Again, in the PRADO trial a MPR rate of 61% was observed which resulted in 60% of patients not needing a lymph node dissection or further adjuvant therapy after neoadjuvant ipilimumab 1 mg/kg plus nivolumab 3 mg/kg [76,108]. The neoadjuvant trials in melanoma clearly indicated that a IFN-γ signature of the tumor identified patients more likely to obtain an MPR (Reijers, [94]).

The special value of anti-CTLA-4 of increasing MPR rates was also demonstrated in head and neck tumors by the studies performed at NKI-Amsterdam (Vos, Elber et al., 2021).

The neoadjuvant immunotherapy revolution is currently unfolding across tumor types and anti-CTLA-4 will be an important component of this revolution ([30,65,91], Garbe, Drummer et al., [38]).

2.7. Recommendation and Considerations for the Future

It is somehow fortuitous that ipilimumab has enabled the development of PD1/PDL1 inhibitors and, in particular, that it has provided an ideal combination partner for many malignancies. The following considerations were discussed during the Siena NIBIT Foundation Think Tank 2022 meeting:

2.8. Clinical trials with novel agents

Rather than conducting large clinical trials, smaller and biomarker-focused trials should help in finding novel drug combinations. For example, trials consisting of approximately 10 patients where pre- and on-treatment biopsies are obtained, may guide the investigation of novel agents. Neoadjuvant design, in particular, may offer the possibility to study changes occurring in the tumor tissue. Ideally, one should compare three cohorts, each with 10 patients, treated with ipilimumab plus a novel agent, PD1/PDL1 inhibitors plus a novel agent, or ipilimumab plus PD1/PDL1 plus a novel agent. The rationale to keep ipilimumab in the mix clearly comes from the observation that CTLA-4 blockade appears to be consistently active even in patients with PD1/PDL1 resistance.

2.9. The use of ipilimumab is necessary

In certain malignancies, such as prostate cancer, immune responses are barely observed and only regimens containing ipilimumab have shown evidence of an activated T cell responses (Gao et al., 2017; [8,87]). In mesothelioma, CTLA-4 blockade monotherapy was initially used with signs of activity [9,10]. However, the latter was substantially improved when PD1/PDL1 blockade was added ([11], Calabrò, Morra et al., 2021, [12,33]), leading to the approval of ipilimumab combined with nivolumab in first line mesothelioma patients (Baas, Sherpereel et al.[5], 2021). Amongst the participants of the Siena Think Tank meeting, there was the consensus that CTLA-4 inhibition will become the backbone in the treatment of several malignancies, with additional agents aimed at targets that will likely depend on the type of malignancy, the presence of activated immune cells (e.g., activated NK cells, dendritic cells, B cells), specific cancer-associated mutations (e.g., EGFR) and the ability to release the “break” of the activated immune cells.

2.10. New combination options

Among the various checkpoint inhibitors identified to date, their benefits as novel treatment options have not been fully determined. For example, targeting of ICOS in monotherapy has not yet resulted in clinical efficacy, perhaps because most ICOS agonists are not sufficiently selective [105]. Such a pleiotropic profile with a residual antagonistic activity may blunt the immune system. In contrast, combinations with the Lymphocyte-Activation Gene 3 (*LAG3)* inhibitor relatlimab and nivolumab in previously treated patients with metastatic cutaneous melanoma showed a benefit [89]. Following these recent encouraging

Table 2
List of CTLA-4 Targeting Agents* .

CTLA- 4 agents

Novel IgG1 mAb Fc-enhanced Bispecific CTLA-4 x PD-1 BsAb CTLA-4 x LAG3 BsAb OX40 and others

Name/ molecule	Company	Status	Structure	Indications/ potential Tas	Combination therapy	MoA/ description
Abatacept	Bristol-Myers Squibb	FDA-approved 2005	IgG1 fused to extracellular domain of CTLA-4	Rheumatoid arthritis, psoriatic arthritis	–	Selective T-cell costimulation blocker; inhibits full activation of T cells
Belatacept	Bristol-Myers Squibb	FDA-approved 2011	IgG1 linked to extracellular domain of CTLA-4	Organ rejection in kidney transplant patients	Transient calcineurin inhibitor	Selective T-cell costimulation blocker; immunosuppressant
Ipilimumab	Bristol-Myers Squibb	FDA/EMA-approved	Fully human IgG1 mAb	Melanomas, renal cell carcinoma, NSCLC, mesothelioma.	Nivolumab (PD-1 inhibitor)	Human IgG1 binds CTLA-4, preventing T-cell inhibition; half-life 12-14 days
Tremelimumab	AstraZeneca	FDA-approved (EMA 2023)	Fully human IgG2 mAb	Hepatocellular carcinoma	Durvalumab (PD-L1 inhibitor)	IgG2 isotype form of a CTLA4-blocking antibody; half-life 22.1 days
AK104 (Cadonilimab)	Akeso	Phase 2 in US; China approval 2022	Bispecific CTLA-4 x PD-1 antibody	Relapsed or metastatic cervical cancer, carcinomas	–-	Tetravalent PD-1/CTLA-4 bispecific antibody with crystallizable fragment (Fc)-null design, leading to lower irAEs
GIGA-564	GigaGen	Phase 1 (expected to launch in 2023) Preclinical	Depletes intratumoral Tregs via enhanced Fc receptor activity instead of blocking CTLA-4–B7 ligand interaction	Advanced solid tumors	–	3rd-gen novel agent: binds to a CTLA-4 epitope very close to that of ipilimumab, resulting in enhanced anti-tumor activity & reduced irAEs
Quavonlimab	Merck. Sznol	Phase 3 Phase 1&2	Humanized IgG1 anti–CTLA-4 mAb	Renal cell carcinoma, advanced solid tumors, NSCLC	Pembrolizumab (PD-1 inhibitor), Favezelimab (anti-LAG3)	Novel IgG1 mAb with ostensibly higher CTLA-4 binding affinity than ipilimumab; combined with a LAG-3 inhibitor
Zalifrelimab (AGEN1884)	Agenus	Phase 1&2	Fully human IgG1 mAb	Cervical cancers, solid tumors	Balstilimab (AGEN2034; PD-1 Inhibitor)	Impressive Phase 2 response rates in cervical cancer
Botensilimab (AGEN1181)	Agenus	Phase 3 (expected to launch in 2023) Phase 1&2	Next-gen Fc-enhanced anti-CTLA-4 antibody	non-MSI-H colorectal cancer, advanced melanoma, metastatic pancreatic cancer, advanced or metastatic soft tissue sarcoma, metatastic clear cell renal cell carcinoma	Balstilimab (AGEN2034; PD-1 Inhibitor), chemotherapy	Promotes intratumoral regulatory T cell depletion and reduces complement fixation
BMS-986218	Bristol-Myers Squibb	Phase 1&2	Version of ipilimumab that is nonfucosylated in Fc region	Prostate cancer, other advanced cancers	Nivolumab; degarelix (GnRH antagonist)	Increases binding affinity to activating FcγR, CD16, thus increasing intratumoral Treg depletion

(continued on next page)

Table 2 (*continued*)

Lorigerlimab	MacroGenics	Phase 1 & 2	Bispecific CTLA4 x PD-1 BsAB	Microsatellite-stable colorectal cancer, NSCLC, mCRPC, melanoma	Chemotherapy	DART® Protein Binding; maintains maximal PD-1 blockade on PD-1-expressing cells
Volrustomig (MEDI5752)	AstraZeneca/ MedImmune	Phase 3 Phase 1&2	Monovalent bispecific antibody targeting PD-1 and CTLA-4	Locally advanced cervical cancer, NSCLC, advanced renal cell carcinoma, gastric cancer, mature tertiary lymphoid structures solid tumors	Monotherapy biologic + chemotherapy	Novel bispecific antibody that preferentially targets CTLA-4 on PD-1 expressing T-cells
Vudalimab	Xencor	Phase 2	Bispecific CTLA-4 x PD-1 BsAb	Metastatic castration-resistant prostate cancer, metastatic anaplastic thyroid or hurthle cell thyroid cancer, advanced gynecologic and genitourinary malignancies, advanced rare cancers, advanced biliary tract cancers, high-risk patients with colorectal cancer	Regorafenib (multi-kinase inhibitor)	Engineered to eliminate Fc gamma receptor (FcγR) binding to prevent activation and/or depletion of T cells via FcγR-expressing cell engagement
Bavunalimab (XmAb22841, formerly pavunalimab)	Xencor	Phase 1&2	Bispecific CTLA-4 x LAG3 BsAb	Advanced/ metastatic melanoma	XmAb104 (investigational bispecific antibody targeting PD-1 and immune co-stimulatory receptor ICOS)	Targets two T cell membrane proteins responsible for regulation of T cell activity, offering potential immunologic and safety advantages over other therapies
ATOR-1015	Alligator Bioscience	Phase 1	Bispecific CTLA-4 x OX40 BsAb	Oncology	–	Next-gen CTLA-4 x OX40 human bsAB generated by linking an optimized version of Ig-like V-type domain of human CD86, a natural CTLA-4 ligand, to an agonistic OX40 antibody
MEDI0562	MedImmune	Phase 1 & 2	Humanized IgG1κ OX40 mAb	Advanced solid tumors, ovarian cancer	Tremelimumab, durvalumab	An agonistic humanized $IgG1_k$ mAb that specifically binds to the costimulatory molecule OX40
PRS-344/S095012	Pieris Pharmaceuticals	Phase ½	PD-L1x4-1BB bispecific antibody-anticalin fusion protein (4-1BB is a co-stimulatory receptor belonging to the TNFR superfamily)	Solid tumors	–	T-cell stimulation mediated by 4-1BB agonism via bispecific molecule that blocks PD-1/PD-L1 axis and localizes 4-1BB costimulation to a PD-L1+ tumor microenvironment (TME) so as to address resistant/ refractory tumors

*As reported on clinical trials.gov as of 6 November 2023.

ABBREVIATIONS

CTLA-4: cytotoxic T-lymphocyte-associated antigen 4

mAb: monoclonal antibody
NSCLC: non-small cell lung cancer
irAEs: immune-related adverse events
BsAb: bispecific antibody
PD-1: programmed cell death protein 1
GnRH: gonadotropin hormone-releasing hormone
mCRPC: metastatic castration-resistant prostate cancer
ICOS: inducible costimulator

results, clinical investigation continues to target additional immune checkpoints, such as LAG-3, TIM-3, TIGIT, VISTA, LILRB4, OX40, TNFRSF, TNFRSF9. Combinations with the MEK inhibitor selumetinib and ipilimumab have been associated with re-programming of the immune microenvironment [75]. More recently, clinical studies targeting the PI3Kinase delta pathway have been shown to down-modulate the Treg cells while maintaining the effector lymphocyte population [28, 31].

2.11. Combinations of ipilimumab with agents other than PD1/PDL1

Including ipilimumab in triplet combination therapies leads to increased risk of toxicities. Therefore, novel combination strategies are considering alternative agents, other than PD1/PDL1 inhibitors, to add to the ipilimumab backbone. For example, agents targeting macrophage populations may enhance the activity of ipilimumab by blocking chemokines/cytokines (e.g., CCL2, CCL22) that are generally associated with inducing Treg cells (Cheng, Bai et al. [17]., 2021). Combinations with vaccines can likewise have a complementary effect [20]. For example, a study of ipilimumab plus talimogene laherparepvec suggested that ipilimumab could be a key modulator for the priming and expansion of the immune response following application of the therapeutic vaccine [19]. Combination of ipilimumab with epigenetic modifiers may represent a third approach for a novel treatment regimen that does not contain PD1/PDL1 inhibitors (Maio, Covre et al., 2015, [39, 90]). The combination of the DNA hypomethylating agent (DHA) guadacitabine and ipilimumab in the phase I, NIBIT-M4 study, was shown to result in the up-regulation of HLA class I on melanoma cells and an in an increase in $CD8^+$, $PD1^+$ T cells and $CD20^+$ B cells, with a promising clinical activity [26]. Furthermore, a five-year follow-up of the NIBIT-M4 study demonstrated a 5-year OS of 29%, while an integrated multiomic analysis showed that a genetic immunoediting index with an adaptive immunity signature stratifies patients into four distinct subsets and discriminates 5-year OS and progression free survival [69]. These preliminary results support the notion that DHA may represent the ideal "partner drug" to improve the therapeutic efficacy of immune-checkpoint blockade and of CTLA-4 containing regimens, including their foreseeable role in reverting PD1/PDL1 resistance, a hypothesis currently being tested in the NIBIT-ML1 study (NCT04250246) sponsored by the NIBIT Foundation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Acknowledgements and Funding

The meeting was organized with scientific support of the NIBIT Foundation. The contents and topics of the panel discussion were not influenced by the sponsors. Development of this manuscript was funded by the NIBIT Foundation. The authors thank Michael Smith and Chiara Beilin for providing scientific writing support.

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