

Therapeutic use of anti-CTLA-4 antibodies

Christian U. Blank¹ and Alexander Enk²

¹Department of Medical Oncology and Division of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066CX Amsterdam, The Netherlands

²Department of Dermatology, The University of Heidelberg, Im Neunheimer Feld 440, 69115 Heidelberg, Germany

Correspondence to: C. U. Blank; E-mail: c.blank@nki.nl

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Abstract

Targeting CTLA-4 represents a new type of immunotherapeutic approach, namely immune checkpoint inhibition. Blockade of CTLA-4 by ipilimumab was the first strategy to achieve a significant clinical benefit for late-stage melanoma patients in two phase 3 trials. These results fueled the notion of immunotherapy being the breakthrough strategy for oncology in 2013. Subsequently, many trials have been set up to test various immune checkpoint modulators in malignancies, not only in melanoma. In this review, recent new ideas about the mechanism of action of CTLA-4 blockade, its current and future therapeutic use, and the intensive search for biomarkers for response will be discussed. Immune checkpoint blockade, targeting CTLA-4 and/or PD-1/PD-L1, is currently the most promising systemic therapeutic approach to achieve long-lasting responses or even cure in many types of cancer, not just in patients with melanoma.

Keywords: CTLA-4, immune checkpoint blockade, ipilimumab, PD-1, PD-L1, T cell, tremelimumab

Introduction

Cancer immunotherapy has been announced as the “breakthrough of the year” in oncology in 2013 (1). The euphoria is mainly based on the clinical successes of antibodies that modulate immune checkpoints by targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1). Immune checkpoints are inhibitory pathways that regulate the strength and duration of co-stimulatory signaling between T cells and antigen-presenting cells (APCs). Blocking CTLA-4 by ipilimumab is the first systemic treatment in 30 years of intensive clinical research to show improved overall survival (OS) in stage IV melanoma patients in phase 3 trials (2, 3). Updated OS data from phase 1/2 trials testing ipilimumab indicate that long-term benefit or even cure is possible in this patient population, previously facing a mean OS of about 6 months (4, 5). Phase 1/2 data testing antibodies targeting PD-1 or PD-1 ligand (PD-L1) as monotherapy or in combination with ipilimumab indicate even higher response rates and efficacy beyond melanoma (6–9).

In this review we will discuss recent understanding about the mechanisms of action of CTLA-4 blockade, its current and future therapeutic use, and the intensive search for biomarkers predicting response to CTLA-4 blockade.

CTLA-4 blockade—recent ideas about the mechanism of action

CTLA-4 (CD152) is member of a growing family of molecules that modify T cell activation. Among these, CTLA-4, CD28, PD-1 and inducible co-stimulator (ICOS) and their ligands

B7 (i.e. B7-1 or B7-2; CD80 or CD86), PD-L1 and ICOSL are members of the B7 family (within the immunoglobulin superfamily), whereas e.g. OX40 is a member of the TNF receptor (TNFR) superfamily (for a recent review, see (10)). These molecules have in common that they modulate, as the so-called second signal (co-stimulation or co-inhibition), the intensity of the first signal delivered to T cells from the interaction of the TCR with the (tumor-) antigen presented in the MHC.

CTLA-4 was the first molecule identified as a co-inhibitory molecule, and it is the counterpart of the co-stimulatory B7–CD28 axis (11, 12). Following activation, T cells up-regulate surface expression of CTLA-4 that binds B7 with a higher avidity, and thus outcompetes CD28's positive co-stimulatory signal. This dominance of negative signals results in reduced T cell proliferation and decreased IL-2 production (11, 13). Blocking CTLA-4, and thus freeing B7 for interaction with the co-stimulatory molecule CD28, resulted in the rejection of tumors and induced immunity to a secondary tumor challenge (14).

This interference into the interaction of APCs and T cells within the tumor-draining lymph node has been a long-standing model for (tumor) immune enhancement by CTLA-4 blockade (Fig. 1A). The caveat of this model, that B7 is nearly always absent within the tumor environment, was regularly neglected. Recent data argue for a secondary mechanism of anti-CTLA-4 antibodies, which could occur within the tumor itself. CTLA-4 has been found to be expressed in tumors at higher levels on regulatory T cells (T_{reg} cells) as compared with intra-tumoral effector T cells (T_{eff} cells), resulting in the hypothesis of anti-CTLA-4 preferentially impacting the T_{reg} -cell

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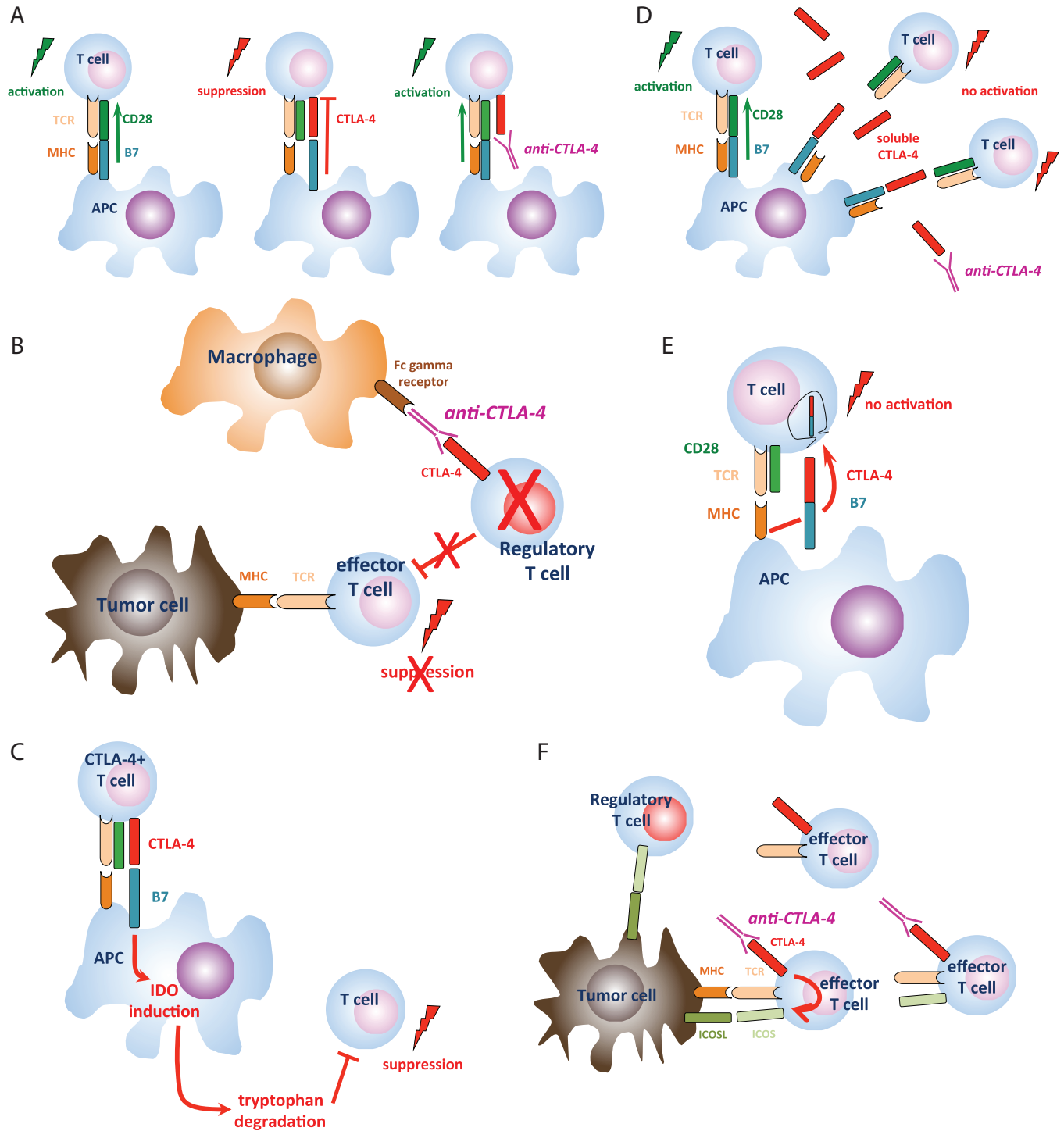


Fig. 1. Postulated mechanisms of action of CTLA-4. CTLA-4 is up-regulated upon T cell activation and has a higher affinity for CD80 and CD86 (B7.1 and B7.2, respectively) than the positive co-stimulatory receptor CD28 does, thereby preferentially binding to B7 and preventing ligation of CD28. In addition it has been postulated that CTLA-4 delivers an inhibitory signal to the T cells upon ligation with B7. Blocking CTLA-4 frees B7 for stimulation of CD28 (A). CTLA-4 is expressed within tumors to a higher extent on T_{reg} cells than on T_{eff} cells. Binding of anti-CTLA-4 allows Fc γ R-dependent depletion of T_{reg} cells (B). CTLA-4 induces, by way of retrograde signaling via B7, IDO induction within APCs and subsequent tryptophan degradation, resulting in local suppression of T_{eff} cells (C). Soluble CTLA-4 can be bound by anti-CTLA-4 mAb, preventing occupation of B7 by soluble CTLA-4 (D). CTLA-4 bound to B7 can mediate internalization of B7 (transendocytosis) and subsequent absence of B7 for co-stimulation via CD28 (E). ICOS is expressed to higher levels on T_{reg} cells as compared with T_{eff} cells, shifting the T_{eff}/T_{reg} ratio towards T_{reg} cells. CTLA-4 blockade on T_{reg} cells (and not on T_{reg} cells) increases ICOS expression on T_{eff} cells, improving T_{eff} cell proliferation, and subsequently shifting the T_{eff}/T_{reg} ratio in favor of T_{eff} cells (F). The figure is based in part on data and figures published elsewhere as follows: (A) Hoos *et al.* (21), (B) Blank (10), (C–E) Walker and Sansom (22) and (E) Fan *et al.* (23).

pool within the tumor-infiltrating lymphocyte (TIL) pool. This could happen either via depletion of T_{reg} cells or by altering their suppressive activity (15, 16). Indeed, it has been shown that anti-CTLA-4 needs to bind to T_{reg} cells and to T_{eff} cells, to induce full tumor protection (17). Furthermore, anti-CTLA-4-mediated tumor destruction was regularly associated with an increased ratio of intra-tumoral $CD4^+ T_{eff}/T_{reg}$ cells and an increased ratio of intra-tumoral $CD8^+ T_{eff}/T_{reg}$ cells (18–20).

Finally, three groups independently showed that CTLA-4 antibodies mediate an $Fc\gamma$ receptor ($Fc\gamma R$)-dependent intra-tumor T_{reg} -cell depletion, which holds also true for anti-GITR (glucocorticoid-induced TNFR-related protein) or anti-OX40, both molecules that are also highly expressed on T_{reg} cells (24–27) (Fig. 1B). While being an attractive model to explain local effects within the tumor environment, this idea has not yet been confirmed in human tumors, e.g. by correlating the pretreatment intra-tumor content of T_{reg} cells and/or $Fc\gamma R^+$ macrophages to responses upon CTLA-4 blockade.

Additional cell-extrinsic functions of CTLA-4 have been postulated, which potentially can be targeted by CTLA-4-blocking antibodies. Reverse signaling triggered by CTLA-4 via B7 has been shown to induce indoleamine 2,3-dioxygenase (IDO) in APCs (28, 29). IDO catabolizes the amino acid tryptophan, resulting in a local tryptophan depletion and subsequent inhibition of T cell proliferation (30) (Fig. 1C).

The identification of alternatively spliced mRNA encoding a soluble CTLA-4 molecule that lacks its transmembrane domain supported the idea of T cells secreting soluble CTLA-4 molecules (31, 32). These soluble CTLA-4 molecules could impair the availability of B7.1/B7.2 for co-stimulation of other T cells (Fig. 1D).

Similarly, CTLA-4 expressed on T_{reg} cells could reduce B7 expression on murine and human APCs, which could be blocked by anti-CTLA-4 antibodies (33, 34). This reduced expression of B7 molecules can result both from reduced up-regulation during APC maturation and from down-regulation of B7 on mature dendritic cells (DCs) (35–37). Such sequestration of B7 molecules from the APC surface can be mediated by the recently observed CTLA-4-dependent transendocytosis of B7-1 and B7-2 (38) (Fig. 1E).

While conflicting data concerning the significance of cell-extrinsic CTLA-4 effects exist, as discussed by Walker and Sansom (22), the existence of these cell-extrinsic mechanisms has been recently corroborated by coculture experiments using CTLA-4 deficient T cells (39, 40).

An intermediate mechanism of CTLA-4 involves the induction of ICOS expression on TILs upon CTLA-4 blockade (23). ICOSL is expressed at high levels in human melanoma cells and promotes expansion of T_{reg} cells that constitutively express higher levels of ICOS, as compared with T_{eff} cells (41). The blockade of CTLA-4, and subsequent up-regulation of ICOS on T_{eff} cells, shifts the intra-tumor ratio of $CD8^+ T_{eff}/T_{reg}$ cells and of $CD4^+ T_{eff}/T_{reg}$ cells in favor of the effector cells, which improves tumor control (20, 23) (Fig. 1F).

Clinical development of CTLA-4 blockade

On the basis of several pre-clinical murine models showing improved tumor control after CTLA-4 blockade (14, 42, 43), human monoclonal antibodies that block CTLA-4 were developed. Two CTLA-4-targeting antibodies have been developed

and tested in phase 3 clinical studies: ipilimumab (IgG1k, also known as MDX-010, MDX-101 and BMS-734016); and tremelimumab (IgG2, previously known as ticilimumab or CP-675,206). Phase 1/2 studies showed that both antibodies were safe and showed some activity (preferable in melanoma) as monotherapy or in combination with IL-2, gp100 vaccination, or chemotherapy (44–48).

A series of phase 2 studies evaluated ipilimumab in patients with late-stage melanoma (49–52) observing a new kind of adverse events, namely immune-related adverse events (irAE), and unique kinetics of response, namely response after initial progression and objective response sometimes as late as 6–12 months after treatment initiation (53). Similarly, median time to response after tremelimumab treatment was 21 weeks and the same, regularly observed irAEs, like colitis, rash, pruritus or fatigue, were reported (44, 54). Most responses were durable, for both ipilimumab and tremelimumab. Thus, it was recognized that the OS was a better endpoint to capture the activity of CTLA-4 blockade. Therefore the primary endpoint in the phase 3 studies was OS for tremelimumab, and was changed to OS for both ipilimumab phase 3 trials (2, 3, 55).

Tremelimumab failed to significantly improve the OS of late-stage melanoma patients as compared with chemotherapy (55). There has been much speculation about the potential reasons for this clinical result, because both phase 3 clinical trials testing ipilimumab succeeded in showing improved OS. First, the immunoglobulin classes are different between ipilimumab and tremelimumab. Murine anti-CTLA-4 of the IgG2a subclass (which correlates with human IgG1, the ipilimumab subclass) has been shown to be most potent in mediating $Fc\gamma$ -dependent T_{reg} -cell depletion (26). Considering the fact that human IgG1 binds with a higher affinity to $Fc\gamma R$ s than human IgG2 (the subclass of tremelimumab) does (56) one might speculate that tremelimumab mediates the CTLA-4 antibody mediated T_{reg} -cell depletion to a lesser extent, a mechanism observed in animal models, and discussed above (24, 25).

A second, and more profane, possibility could have been that patients in the chemotherapy control arm switched to ipilimumab in the expanded access programs (EAPs; i.e. given outside the clinical-trial group). Indeed the forest blot of the patients treated in the United States favors the chemotherapy arm (where many centers had the ipilimumab EAP already open), whereas the rest of world patients favor tremelimumab (where fewer centers had access to the ipilimumab EAP at that time) (55).

Tremelimumab is currently being tested as monotherapy in mesothelioma and in combination with PD-L1 blockade (MEDI4736) or the CD40-agonistic antibody CP-870,893 in non-small-cell lung cancer (NSCLC) and metastatic melanoma (Table 1).

As mentioned above, both ipilimumab phase 3 trials—MDX010-20 (ipilimumab versus ipilimumab + gp100 vaccination versus gp100 vaccination only) and CA184-024 [ipilimumab + dacarbazine (DTIC, dimethyl-triazeno imidazole carboxamide) versus dacarbazine + placebo]—showed significant improvement of OS. The results of study MDX010-20 were the basis for approval by the regulatory authorities of ipilimumab (3 mg kg^{-1} body weight) for previously treated metastatic melanoma, and in some countries (including the United States), for treatment-naive metastatic melanoma (53). In 2013,

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and based on data from the early phase 2 trials and EAPs, ipilimumab was also approved as first-line therapy for melanoma in the EU. Meanwhile, ipilimumab has become one of the standard treatments in melanoma and is currently being tested in various combinations with chemotherapy, targeted agents, vascular endothelial growth factor receptor (VEGFR)-targeting agents,

cytokines, irradiation and radiofrequency ablation (RFA) (www.clinicaltrials.gov; www.trialregister.nl). A selection of interesting combination trials, which cannot be comprehensive due to the vast numbers of trials, is shown in Table 2.

The most promising combination seems to us to be ipilimumab plus nivolumab, a PD-1-blocking antibody, which is

Table 1. Selected current trials testing combinations with tremelimumab

Trial number	Phase	Combination	Rationale
NCT01975831	1	tremelimumab + MEDI4736 (anti-PD-L1 mAb) in solid tumors	Synergistic effects of CTLA-4 and PD-1/PD-L1 blockade have been shown in mouse and human melanoma (9, 20)
NCT01103635	1	tremelimumab + CP-870,893 (CD40 agonist mAb) in late-stage melanoma	CD40 ligation on DCs in combination with anti-PD-L1 improves T cell proliferation (57)
NCT01853618	1	tremelimumab + TACE or RFA in patients with advanced HCC	Both TACE and RFA have been shown to induce peripheral immune responses in HCC (58, 59)
NCT02141542	1	tremelimumab + MEDI3617 (angiopoietin-2-blocking mAb) in late-stage melanoma	Synergistic effects between CTLA-4 blockade and anti-angiogenesis have been shown in melanoma (60)
NCT02040064	1	tremelimumab + gefitinib in EGFRi-resistant NSCLC	Correlation between EGFR pathway activation and CTLA-4 expression (61)

EGFRi, epidermal growth factor receptor inhibitor; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer; RFA, radiofrequency ablation; TACE, trans-arterial catheter chemoembolization.

Table 2. Selected current trials testing combinations with ipilimumab

Trial number	Phase	Combination	Rationale
NCT01844505	3	ipilimumab versus ipilimumab + nivolumab versus nivolumab in previously untreated advanced melanoma	synergistic effects of CTLA-4 and PD-1/PD-L1 blockade have been shown in mouse and human melanoma (9, 20)
NCT02089685	1/2	ipilimumab + MK3475 in advanced melanoma or RCC	synergistic effects of CTLA-4 and PD-1/PD-L1 blockade (see above)
NCT01988077	2	ipilimumab + TIL ACT in stage IV melanoma	CTLA-4 is expressed on PD-1 ⁺ TIL (62)
NCT01708941	2	ipilimumab + high-dose IFN- α 2b versus ipilimumab in surgical non-removable stage III–IV metastatic melanoma	ipilimumab and interferon have both shown some activity in adjuvant trials (63, 64)
NCT01750580	1	ipilimumab + BMS-986015 (anti-KIR mAb) in solid tumors	possible synergistic improvement of T cell and NK cell activity
NTR3488 (trialregister.nl)	1/2	ipilimumab + RFA in hepatic metastasized uveal melanoma	RFA + anti-CTLA-4-induced anti-tumor immunity in a murine model (65)
NCT01832870	1	ipilimumab + sipuleucel-T in advanced prostate cancer	possible synergy between both agents showing activity in prostate cancer (66, 67)
NCT01740297	1b/2	ipilimumab + talimogene laherparepvec (T-VEC) versus ipilimumab in metastatic melanoma	induction of immunogenic cell death as a possible adjuvant for ipilimumab (68)
NCT01767454	1	ipilimumab + dabrafenib \pm trametinib in V600E/Kmut melanoma	ipilimumab and dabrafenib \pm trametinib have been shown to have activity in V600 melanoma, dabrafenib + trametinib is less toxic, and thus might succeed in contrast to ipilimumab + vemurafenib (2, 69, 70)
NCT01604889	1b/2	ipilimumab + INCB024360 (oral IDO inhibitor) or placebo in advanced melanoma	possible dual targeting of IDO production (see Fig. 1C) and synergism observed in a murine model (71)
NCT01689974	2	ipilimumab versus ipilimumab + radiotherapy in metastatic melanoma	abscopal effects have been observed in combination of RT and ipilimumab (72, 73)
NCT01970527	1/2	ipilimumab + SBRT in advanced melanoma	synergistic effects between RT and ipilimumab have been postulated while synergistic effects between IL-2 and RT have so far only been seen when using high-dose SBRT (74)
NCT01323517	2	ipilimumab + ILI with melphalan and dactinomycin in advanced unresectable melanoma of the extremity	ILI mediated induction of tumor-specific T and NK cells that might synergize with CTLA-4 blockade (75, 76)

ACT, adoptive cell therapy; IDO, indoleamine 2,3-dioxygenase; HNSCC, head and neck squamous cell carcinoma; ILI, isolated limb infusion; KIR, killer cell immunoglobulin-like receptor; RCC, renal cell carcinoma; RFA, radiofrequency ablation; SBRT, stereotactic body radiation therapy; TILs, tumor-infiltrating lymphocytes.

currently being tested in a phase 3 trial for first-line treatment of unresectable metastasized melanoma. Data from the phase 1 trial indicate that at the maximum doses that were associated with an acceptable level of adverse events (nivolumab at a dose of 1 mg kg⁻¹ body weight and ipilimumab at a dose of 3 mg kg⁻¹), 53% (9 out of 17 patients) had an objective response, all with tumor reduction of 80% or more. In all concurrent regimen cohorts, a confirmed objective response according to modified World Health Organization (WHO) criteria was observed in 21 out of 52 patients (40%) (9). The 1-year OS of these patients was 82% (77). If this clinical efficacy can be confirmed by the phase 3 trial, we envision this anti-CTLA-4 combination therapy will become a standard therapy in late-stage melanoma, and most likely in other malignancies, too.

Clinical parameters associated with response upon CTLA-4 blockade

Despite the clinically significant improvement in OS from ipilimumab therapy, only about 20% of patients benefit long term (5). This raises the question of how to improve the treatment outcome upon CTLA-4 blockade. As discussed above, combinations with ipilimumab or tremelimumab are currently being tested with that intention. Meanwhile, the identification of patients who benefit from ipilimumab long term in a daily-life setting should be one main goal. In contrast to targeted therapy against e.g. BRAFV600 (which includes selective BRAF inhibitors), where the patient population benefitting from the therapy can be clearly defined, no such definitive biomarkers have been identified so far for CTLA-4 blockade. Several

Table 3. Retrospective studies on biomarkers predicting improved outcome after ipilimumab therapy

Identified biomarker (bold marks independent variables)	Single- or multi-center analysis/ name of center	Number of patients/ ipilimumab dose	Result/type of analysis	Reference
ALC	Single/MSKCC, EAP	51 patients/10mg/kg, q3wk, 4x	ALC > 1000 μl^{-1} at weeks 4 and 7 correlates with improved OS/Cox proportional hazards model	Ku <i>et al.</i> (83).
ALC ALC slope LDH	Single/University of Siena, EAP	27 patients/10mg/kg, q3wk, 4x, maintenance	ALC > 1500 μl^{-1} at week 7, positive ALC slope between baseline and week 4, and normal LDH correlate with OS/multivariate analysis	Di Giacomo <i>et al.</i> (84)
CRP ALC ALC slope LDH WHO-PS	Single/UZ Brussel	50 patients/3mg/kg, q3wk, 4x	WHO-PS < 2, LDH > ULN, ALC week 6 > 8007 μl^{-1} , pos ALC slope week 6 – baseline, and CRP < ULN, correlate in univariate with improved OS, CRP $\leq 5 \times$ ULN is the only marker in multivariate analysis	Wilgenhof <i>et al.</i> (85)
Number of courses ≥ 4 ALC ALC delta Eosinophil delta LDH	Single/Gustave Roussy	73 patients/3mg/kg, q3wk, 4x	ALC > 1000 μl^{-1} at week 4, delta ALC (or eosinophils) week 4 – week 7 > 200 μl^{-1} (> 100 μl^{-1}), LDH < ULN versus > ULN – < 2 \times ULN versus > 2 \times ULN/multivariate analysis	Delyon <i>et al.</i> (86)
WHO-PS M-stage ALC ALC slope S100 LDH ESR	Multiple/WIN-O Netherlands, Royal Marsden, Christie	230 patients (166 from NL, 64 from UK)/3mg/kg, q3wk, 4x	WHO-PS 0, M1a/b, ALC baseline and ALC week 6 > 1 \times ULN, ALC slope > 1.35, S100 < 1 \times ULN, LDH < 1 \times ULN, ESR > 1 \times ULN correlate in univariate with improved OS, LDH and ESR are the only independent markers	Kelderman <i>et al.</i> (87)
ALC slope LDH slope CRP slope T _{reg} slope	Multiple/National Cancer Institutes Italy	95 patients, 3mg/kg, q3wk, 4x	Between baseline and week 12 increase in ALC, and stable or decreased LDH, CRP, FoxP3 ⁺ T _{reg} -cell numbers were associated with improved OS/no multivariate analysis	Simeone <i>et al.</i> (88)

ALC, absolute lymphocyte count; CRP, C-reactive protein; EAP, expanded access program; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; MSKCC, Memorial Sloan-Kettering Cancer Center; q3wk, every 3 weeks; ULN, upper limit normal; UZ, Universitair Ziekenhuis (University Hospital); WHO-PS, World Health Organization performance status; WIN-O, De Werkgroep Immunotherapie Nederland voor Oncologie (The Dutch working Group for Immunotherapy in Oncology).

preliminary biomarkers have been investigated, but none of these has been validated in confirmatory studies (51, 78–82).

Retrospective analyses from EAPs have repeatedly identified the same factors to be associated with improved outcome upon ipilimumab (Table 3). Interestingly, most of these correlations have not (or to a lesser extent) been found to be predictive in the clinical trials, emphasizing the need for meta-analysis of all currently available patient data.

As one of the first markers to be associated with improved outcome, the absolute lymphocyte count (ALC) ($>1000 \mu\text{l}^{-1}$), has been suggested, as the number at baseline, at week 4, or at week 7 (83). These data were extended by another single-center publication, suggesting that the ALC slope between baseline and week 6 predicted best the outcome upon ipilimumab (84).

In line with these data, a third single-center analysis of clinical and laboratory baseline variables associated with improved OS identified, in univariate analyses, WHO performance status (WHO PS) of <2 , lactate-dehydrogenase (LDH) below the laboratory upper limit normal (ULN), and C-reactive protein (CRP) below the ULN as significant parameters, whereas in the multivariate analysis CRP was identified as the strongest and single independent baseline variable (85). An ALC $>1000 \mu\text{l}^{-1}$ at the start of the second course and an increase in the eosinophil count $>100 \mu\text{l}^{-1}$ between the first and second infusions were correlated with an improved OS upon ipilimumab in the fourth single-center analysis (86).

In an initial attempt, the Dutch Working for Immunotherapy [De Werkgroep Immunotherapie Nederland voor Oncologie (WIN-O)] examined the proposed markers in 166 patients treated in The Netherlands with second-line ipilimumab (87). When analyzing the clinical and laboratory parameters (the eosinophil counts were not included at that time, but at a later analysis also identified this as a predictive marker, C.U. Blank, unpublished data), the previously suggested markers were reproduced, and a new marker, namely baseline S100, identified. However, multivariate analysis revealed only LDH, and to a lesser extent the absence of inflammation [in this case the erythrocyte sedimentation rate [ESR]] to be associated with long-term benefit from ipilimumab (87). When choosing an arbitrary cut-off of LDH $>2 \times \text{ULN}$, none of the patients had long-term benefit beyond 15 months, which was confirmed by an independent cohort of 64 patients treated in the UK with ipilimumab.

Similarly, another large analysis of 95 patients treated at the Italian National Cancer Institute, identified once more LDH and CRP (in this case their decrease between baseline and week 12) to be the strongest markers associated with response to ipilimumab (88).

Thus, currently ALC, LDH and CRP/ESR are the most frequently identified markers correlating with improved long-term outcome upon treatment with ipilimumab. As these markers are also known as prognostic markers, their predictive value remains unclear so far.

Conclusion

CTLA-4 blockade by ipilimumab has become the gold-standard of all T cell checkpoint molecules currently tested in melanoma and beyond. Combination of ipilimumab and

nivolumab seems to be the next step in inducing strong responses and so increasing the percentage of patients benefitting long term from combination therapy with CTLA-4 blockade. So far no predictive markers for long-term benefit from CTLA-4 blockade have been identified in a prospective manner. Retrospective analyses from patients treated with ipilimumab in EAPs indicate ALC, LDH and CRP/ESR to be prognosticators for long-term benefit from ipilimumab.

The success of CTLA-4 blockade has fostered the idea of a new therapeutic approach for cancer (aside from surgery, chemotherapy and radiotherapy), namely immunotherapy of cancer, which has been crowned as the breakthrough of the year last year.

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