

Cytotoxic T-Lymphocyte–Associated Antigen-4

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Abstract

Cancer immunotherapy relies on the ability of the immune system to target tumor-specific antigens to generate an immune response. This initial response requires both binding of the MHC/antigen peptide to T-cell receptor complex, along with a second costimulatory signal created by the binding of CD28 on the T cell, with B7 located on the antigen-presenting cell. Regulatory checkpoints, such as cytotoxic T-lymphocyte–associated antigen-4 (CTLA-4), serve to attenuate this signal, thereby preventing autoimmunity. Its key role in regulating the immune system has made CTLA-4 an attractive therapeutic target for cancer, with the development of fully human monoclonal antibodies that have successfully targeted CTLA-4 in clinical trials. Augmentation of the immune response via blockade of CTLA-4 represents a significant advance in the field of oncology and has shown an improvement in survival for patients with metastatic melanoma. An increased understanding of the components of this pathway and the identification of other methods to modulate the immune system hold great promise for future therapy. *Clin Cancer Res*; 17(14); 4622–8. ©2011 AACR.

Background

The generation of an immune response requires the careful coordination of a number of signals. An intricate system of checks and balances has evolved to ensure an appropriate response to antigenic stimuli. Dysregulation of these signals can lead to autoimmunity, thereby causing damage to otherwise normal tissue. In the field of oncology, the notion that tumors themselves may be antigenic and have the capability to induce an immune response has long been recognized. Attempts to exploit this phenomenon, in order to induce an immune-mediated antitumor response, have, until recently, been hindered by a lack of understanding of these regulatory mechanisms. Cytotoxic T-lymphocyte–associated antigen-4 (CTLA-4), a member of the immunoglobulin superfamily, is among the best characterized of these regulatory molecules. Monoclonal antibodies that target CTLA-4 have been in clinical development for the past decade, and promising results were seen in early-phase trials across a variety of cancer types. Recent data have now convincingly shown that CTLA-4 blockade has the potential to affect survival in patients with metastatic melanoma, and it is likely that the indications for CTLA-4–directed therapy will continue to expand.

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T-cell activation

Activation of naïve T cells in response to a specific antigen requires multiple concerted signals. Priming is initiated when tumor antigens are processed within an antigen-presenting cell (APC) and peptides are presented on the surface via the MHC molecules, for recognition and binding by the T-cell receptor (TCR; ref. 1). This initial signal, however, is insufficient to generate an immune response, and an additional costimulatory signal is required. B7–1 (CD80) and B7–2 (CD86), present on the APC surface, must bind with CD28 on the T-cell membrane (2). This second event, along with additional costimulatory signals, serves to initiate T-cell activation and expansion in response to a specific antigenic epitope (3, 4). Once bound to B7, activated CD28 stimulates the phosphoinositide 3-kinase (PI3K)/AKT pathway, upregulating the transcription factor NF- κ B, as well as other prosurvival signals, such as Bcl-2 and Bcl-X_L. Additionally, numerous adapter proteins mediate interactions with *c-jun* NH, kinase (JNK), Src family kinases, and interleukin 2 (IL-2) inducible kinase, leading to upregulated cytokine production and further expansion (Fig. 1A; ref. 5). Unchecked T-cell proliferation, however, can lead to deleterious effects and is, therefore, tightly regulated in order to prevent immune-mediated damage to normal tissues (6, 7). CTLA-4 is one such immunologic checkpoint, although others, including programmed death-1 (PD-1), also clearly play an important role in regulating peripheral tolerance (5, 8).

Although it is homologous to CD28, CTLA-4 is located in the intracellular compartment, and it is only minimally expressed on the surface of resting T cells (9). Activation of the TCR complex along with the costimulatory signal induces upregulation of CTLA-4, and cell surface expression is tightly regulated in a cyclical fashion (10). ADP ribosylation factor-1 (ARF-1) and phospholipase D (PLD)

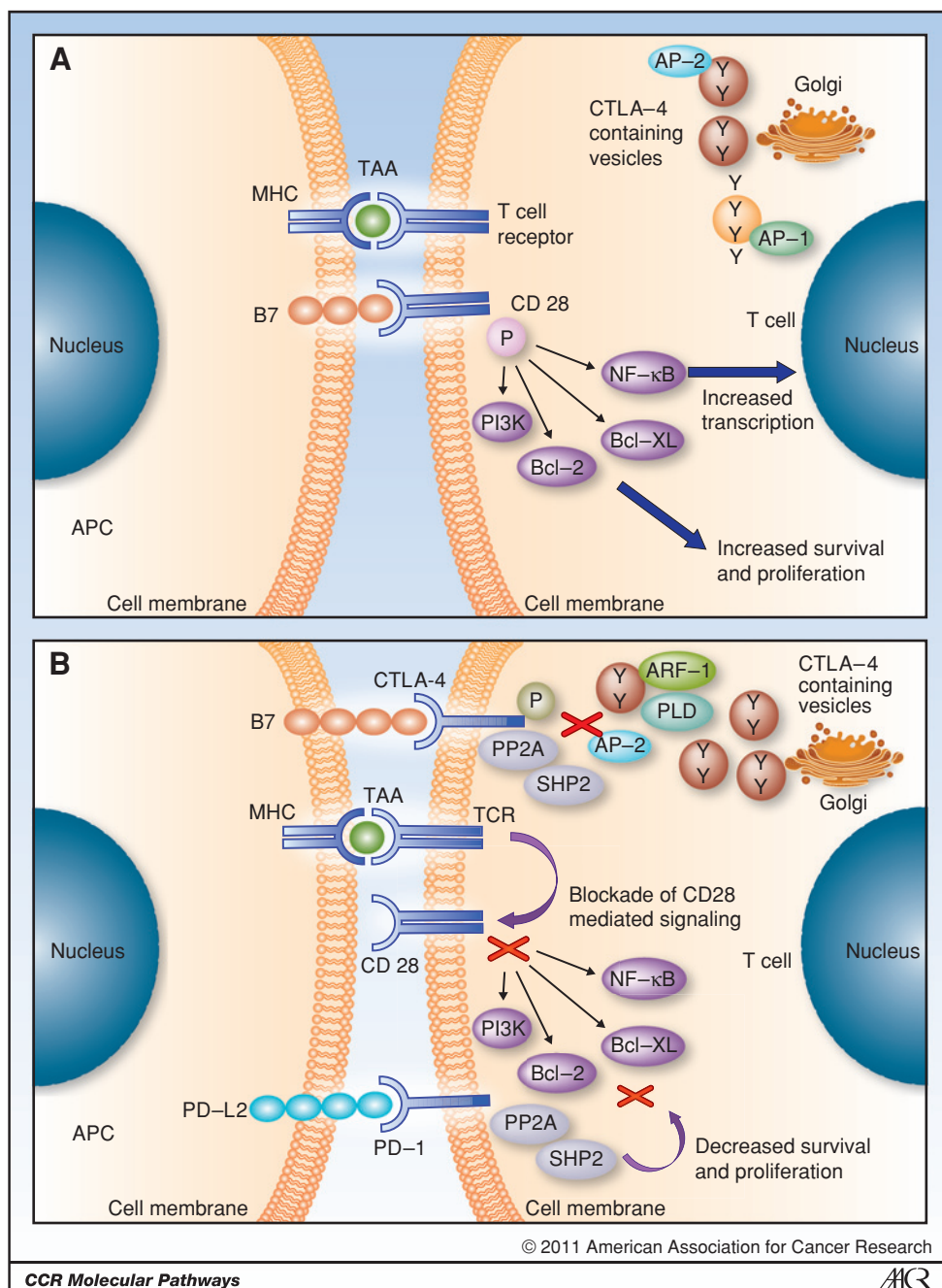


Figure 1. A, T-cell activation. T-cell priming in response to a specific antigenic epitope requires coordination of multiple signals. The initial signal is created when a full-length peptide is processed and presented on the surface of an APC. The resulting fragments, or tumor-associated antigens (TAA), are bound to MHC molecules present on the surface of the APC. This MHC/TAA complex then allows for detection and binding of the TAA by the TCR. A second costimulatory signal, however, is necessary to complete T-cell activation and expansion. The binding of CD28 on the T cell with B7 on the APC creates this second signal, which leads to activation of the PI3K/AKT pathway, upregulation of the antiapoptotic proteins Bcl-2 and Bcl-XL, and an increase in the nuclear transcription factor NF- κ B. This collectively leads to increased cellular proliferation, cytokine production, and prolonged survival. Initially, regulatory proteins like CTLA-4 are primarily inactive and remain complexed with AP-2 within the intracellular compartment. B, Upregulation of CTLA-4 and maintenance of immune tolerance. TCR activation induces upregulation of CTLA-4 via a number of mechanisms. ARF-1 and PLD bind to enhance the exocytosis of CTLA-4-containing vesicles as they exit the Golgi apparatus. Phosphorylation of the cytoplasmic tail of CTLA-4 prevents binding of AP-2, which normally functions to promote receptor internalization, resulting in an increase in CTLA-4 surface expression. CTLA-4 is then capable of directly competing with CD28 for binding of B7. CTLA-4 may also exert a direct negative effect on CD28 signaling, mediated by the binding of the phosphatases PP2A and SHP-2. Additional regulatory molecules, including PD-1, are also important in limiting T-cell activation and may also inhibit TCR-mediated signaling via blockade of specific downstream effectors. The resultant decrease in prosurvival signaling serves to limit T-cell activation and expansion.

promote the exocytosis of CTLA-4-containing vesicles as they exit the Golgi apparatus, whereas the adapter protein-1 (AP-1) targets CTLA-4 to the lysosomal compartment for degradation (11, 12). Phosphorylation of CTLA-4 by the tyrosine kinases Lck, Fyn, and resting lymphocyte kinase (RLK) prevents the binding of a second adapter protein-2 (AP-2) to the cytoplasmic domain of CTLA-4, a key mediator of receptor endocytosis, preventing internalization (2, 13). The net effect results in an overall increase in surface expression of CTLA-4, with both augmented transport to the cell surface and diminished endocytosis. Once CTLA-4 is dephosphorylated, AP-2 is then able to bind CTLA-4, causing the receptor to be endocytosed (14).

Mechanism of T-cell inactivation

The exact mechanism by which CTLA-4 regulates TCR/CD28 signaling remains to be fully elucidated. CTLA-4 has a much higher affinity for binding of B7 and is thought to directly compete with CD28 to prevent initiation of the costimulatory signal, leading to decreased T-cell activation (2). Some evidence also suggests that CTLA-4 may inhibit TCR signaling at the immune synapse, either by affecting T-cell motility, directly inhibiting CD28 and its downstream effectors, or by regulating the availability of specific cofactors necessary for TCR signaling (10, 15). Although the cytoplasmic tail of CTLA-4 itself lacks intrinsic enzymatic activity, it has been shown to bind a number of signaling molecules, including the phosphatases PPA-2 and SHP-2, which may be important for mediating its effects (Fig. 1B; refs. 5, 16).

The ability of CTLA-4 to modulate T-cell activation is complemented by another regulatory molecule, PD-1. PD-1 is expressed on the surface of both activated B and T cells. Its ligands, PD-L1 and PD-L2, exhibit differential expression, with PD-L1 being found on leukocytes and nonhematopoietic cells, whereas PD-L2 is restricted to dendritic cells and monocytes. Evidence suggests that although CTLA-4 may be involved in the initiation of immune tolerance, PD-1 may act at a later point to maintain immune homeostasis (5). Similar to CTLA-4, PD-1 has the capacity to bind phosphatases that inhibit TCR complex-induced signaling, albeit via distinct signaling pathways. The intricate balances between CTLA-4 effects and those involving numerous members of the family of immune regulatory targets are actively being pursued both preclinically and in clinical development.

Effects on regulatory and memory T cells

Although CTLA-4 has a clear role in limiting T-cell activation, additional evidence also suggests that CTLA-4 is an important component in the functioning of both regulatory T cells and CD8⁺ memory T cells. CTLA-4 directs proper functioning of FoxP3⁺ regulatory T cells, critical in maintaining peripheral tolerance and preventing autoimmunity (17). Preclinical and pathologic evidence, in post-treatment biopsies, also suggests that whereas CTLA-4 blockade may increase effector and regulatory cell populations, the intratumoral ratio of CD8⁺ T cells to FoxP3⁺

T cells determines the extent of tumor cell death and potential for efficacy (18). Additionally, *in vivo* data suggest that CTLA-4 blockade may enhance memory CD8⁺ T-cell expansion (19). The strong preclinical data suggest an important role for CTLA-4 blockade as a regulatory checkpoint for therapeutic development.

Clinical-Translational Advances

Although tumor-associated antigens are capable of eliciting an immunogenic signal, the ability of cancer to evade detection and regulate the immune response has stifled the efficacy and enthusiasm of immunotherapy as a treatment for cancer. With an increased understanding of the mechanisms that govern immune regulation, the capability now exists to capitalize on this with therapeutic intent.

Preclinical studies

The role of CTLA-4 as a key immune regulator is evident by the development of a lethal lymphoproliferative phenotype at a young age in CTLA-4 knockout mice (20, 21). Distinct immunosuppression also results from a soluble CTLA-4 fusion protein capable of binding to B7 (22). Anti-CTLA-4-directed therapy has been shown to induce tumor regression in a number of mouse transplantable models, including prostate, colon, and renal carcinoma (4). The degree of response seemed to be directly related to the degree of immunogenicity of the tumor. For example, in the B16 melanoma model, a relatively nonimmunogenic transplantable tumor, minimal activity was seen. However, combination therapy with a whole tumor cell vaccine, engineered to secrete granulocyte macrophage colony stimulating factor (GM-CSF), was synergistic and enhanced efficacy (23, 24). Interestingly, the responses seen with this combination therapy resulted in loss of fur pigmentation in approximately half of the animals, suggesting loss of tolerance to normal differentiation antigens (25).

Clinical trials

Two fully human monoclonal CTLA-4 antibodies, ipilimumab and tremelimumab, are currently in clinical development. The majority of the evidence supporting the biologic activity of both agents results from trials in metastatic melanoma, although there is evidence of efficacy in other tumor types. Consistent with on-target effects of anti-CTLA-4-directed therapy, the side effect profile of these drugs is unique and is often manifested as inflammatory events (26). Furthermore, unique patterns of tumor responses have been identified with this class of agents, in that tumor sizes may increase in tumor volume initially or even new sites of disease develop, with subsequent stabilization or improvement over several weeks (27, 28). Initial phase I studies tested doses ranging up to 3 to 20 mg/kg, with some evidence to suggest increased benefit with higher doses, albeit at the risk of increased toxicity (29).

Tremelimumab in melanoma

Initial phase I testing of tremelimumab included 29 patients with measurable melanoma and resulted in 2 patients who experienced a complete response and an additional 2 patients who had evidence of a partial response. Importantly, these responses proved to be durable, lasting for several years in each case (30). Subsequent studies established a dose of 15 mg/kg every 3 months as the preferred regimen, which was compared in a phase III randomized controlled trial to dacarbazine or temozolomide in patients with melanoma. A total of 655 patients were enrolled. At the second planned interim analysis, the trial was closed for futility as the tremelimumab arm did not show a survival advantage. It was estimated that at least 10% of patients in the chemotherapy arm went on to receive ipilimumab on a different trial, offering one potential confounding variable (31). At the time of closure, patients in the tremelimumab arm had a median overall survival of 11.8 months, compared with 10.7 months in the control arm ($P =$ not significant). A follow-up analysis suggested that patients with low baseline C-reactive protein (CRP) benefited from tremelimumab, although the final analysis of this trial is pending (32).

Ipilimumab in melanoma

Initial clinical investigation of ipilimumab in metastatic melanoma patients revealed partial responses in 2 of 17 patients (33). Two phase II studies investigated ipilimumab at 10 mg/kg, with 1 single-arm study showing a median overall survival of 10.2 months (34). Additionally, a randomized trial of ipilimumab, 10 mg/kg, with or without the addition of budesonide in 115 patients, was significant for an approximately 40% 2-year survival rate in each arm (35). In another dose-ranging study of 217 patients, patients were randomized to receive ipilimumab at 0.3 mg/kg, 3 mg/kg, or 10 mg/kg. Response rate correlated with dose, with a best overall response rate of 11.1% in patients who received the highest dose. No grade 3 or 4 adverse events were seen at the lowest dose level, but side effects were manageable in the other 2 cohorts (36).

These and other trials raised important issues about the unique side effect profile of this class of agents. As might be expected with the blockade of a key immunoregulatory checkpoint, many adverse events are inflammatory in nature; these include dermatologic events, manifested by pruritis, rash, or vitiligo, and gastrointestinal events, with diarrhea, that can potentially lead to a fulminant colitis (26). Rare deaths have been associated with bowel perforation. Endocrinopathies, including thyroiditis and hypophysitis, as well as hepatitis have also consistently been reported in clinical trials but are significantly lower in incidence. Although less severe adverse events may be managed symptomatically, patients presenting with grade 3 or 4 events are less frequent but require close medical management and prompt initiation of high-dose systemic corticosteroids, as outlined in a number of prespecified algorithms (6, 37, 38). Patients who do not respond to steroids may require administration of an anti-TNF agent.

These measures can control adverse events well in the vast majority of cases.

Ipilimumab has also been studied in combination with other agents, including IL-2, vaccines, or cytotoxic chemotherapy, such as dacarbazine (29). Recently, results from a pivotal phase III trial, in which ipilimumab was compared with a peptide vaccine, have shown that ipilimumab improved overall survival in patients with metastatic melanoma (39). In this 3-arm trial, 676 patients were randomized in a 3:1:1 fashion to receive ipilimumab, 3 mg/kg, in conjunction with a gp100 peptide vaccine, ipilimumab alone, or the vaccine alone. Patients were required to be human leukocyte antigen (HLA)-A2 positive and have had a prior therapy. Previously treated central nervous system metastases were permitted. Patients who received ipilimumab (either alone or with the vaccine) showed a 4-month improvement in survival. Notably, a significant proportion of patients on the ipilimumab arms survived at least 1 to 2 years, suggesting that for those patients who received a clinical benefit to therapy, this may persist for months or years. In addition, clinical benefit was obtained in this patient population who historically has had relatively poor prognoses. This was the first randomized trial to show a survival benefit for patients with metastatic melanoma and provides the foundation for a novel class of oncology therapeutics. We await maturity of additional trials for metastatic melanoma patients, including a randomized combination trial with chemotherapy and an ongoing investigation of ipilimumab in the adjuvant setting (40).

Anti-CTLA-4 therapy in other cancers

Ipilimumab has also been examined in malignancies other than melanoma. In an early study, patients with metastatic castrate resistant prostate cancer (mCRPC) received 1 dose of ipilimumab at 3 mg/kg. Two patients experienced a $\geq 50\%$ decline in prostate-specific antigen (PSA) levels (41). Additional trials have explored the possibility of combination therapies. In a phase I trial of 24 patients with mCRPC, patients were treated with escalating doses of ipilimumab (0.5 mg/kg to 3 mg/kg) in conjunction with fixed-dose GM-CSF. In the cohort of 6 patients at the highest dose level, 3 patients experienced PSA declines of $>50\%$, including a partial response in a patient with visceral metastases (42). The therapy seemed to be well tolerated, although patients did experience a number of immune-related adverse events, including hypophysitis, temporal arteritis, and diarrhea. This trial also showed that, at higher doses of ipilimumab, an increase in activated CD8⁺ T cells was detected in the peripheral blood of patients. Additional trials have investigated the combination of ipilimumab with chemotherapy and hormonal therapy in prostate cancer, and trials in the neoadjuvant setting are ongoing (43, 44).

Promising activity for anti-CTLA-4-based therapy has also been seen in the setting of metastatic renal cell carcinoma. In a phase II study of 61 patients treated with either 1 mg/kg or 3 mg/kg of ipilimumab, 5 of 40 patients

receiving the higher dose had a partial response. In this study, approximately one third of patients experienced inflammatory adverse events (45). The combination of tremelimumab with sunitinib has also been investigated, although rapid-onset renal failure was seen in 4 of 20 patients who were treated with tremelimumab at 10 or 15 mg/kg, and this combination has not been further investigated (46).

Additionally, recent data suggest a role for CTLA-4-directed therapy in non-small cell lung cancer (NSCLC). A phase II trial compared the addition of 10 mg/kg of ipilimumab to carboplatin and paclitaxel (CP) chemotherapy alone, in patients with stage IV NSCLC (47). Patients were randomized to receive ipilimumab either concurrently with chemotherapy, or in a phased schedule after receiving the first 2 cycles of CP. Patients who received either schedule of ipilimumab had an improvement in immune-related progression-free survival, and there was a trend for improved overall survival in patients who received ipilimumab in the phased schedule. This trial also highlights the unique response patterns that may be seen in patients treated with anti-CTLA-4 therapy, designated immune-related response criteria. In contrast to response to classic cytotoxic agents, patients treated with ipilimumab may initially have an increase in tumor size, or may continue to show continued tumor shrinkage well after the last exposure to therapy. These novel response criteria take into account these immune-mediated events and incorporate serial assessments over time to more accurately gauge clinical efficacy (27, 48). Although evaluated retrospectively, novel response criteria such as these need prospective validation to confirm their utility to predict clinical benefit.

Potential for future therapies

The realization of the ability to potentiate the immune response with clinical benefit has established a new era in cancer therapeutics. Although an increased understanding of immune regulatory mechanisms allowed for this advance, details about the pathways and costimulatory signals are constantly evolving. Multiple strategies are needed to continue building upon this platform, including combination with other agents and the identification of biomarkers to better select patients for therapy. Given that preclinical evidence suggests that CTLA-4 therapy may be most effective in immunogenic tumors, the combination of CTLA-4-targeted agents with vaccines could further increase their efficacy. In patients who had previously been vaccinated with irradiated, autologous tumor cells engineered to secrete GM-CSF (GVAX), the administration of ipilimumab showed clinical activity in the majority of melanoma patients, without evidence of significant side effects, possibly providing a therapeutic index for antitumor benefit (49). Application of this combination in larger groups of patients is difficult to pursue because of practical circumstances with autologous vaccine production. Currently, a randomized clinical trial of ipilimumab with or without the administration of systemic GM-CSF has been

initiated in the Eastern Cooperative Group and offers the opportunity to answer this combination question with a practical therapeutic regimen. The recent approval of a dendritic cell vaccine in prostate cancer showing a survival advantage again provides an intriguing platform for combinatorial approaches.

With the success of CTLA-4 blockade in the clinic, other immune modulators with distinct roles in immune regulation have the additional potential to affect oncology care. PD-1 has been shown to be an important modulator of tumor immune responses in preclinical models. One native ligand, PDL-1, is highly expressed in a number of tumors, including melanoma and renal cell carcinoma. This biology has direct relevance to immune regulation occurring within the tumor microenvironment. Besides single modality activity, the combination of CTLA-4 and PD-1 blockade seems to be synergistic in a B16 murine melanoma model, resulting in a 65% tumor rejection rate compared with 10% and 25%, respectively, when each agent was given alone (50). A phase I trial testing this combination is currently being conducted. Although therapy directed at PD-1 is earlier in clinical development, encouraging activity has nonetheless been seen (51). The possibility also exists that targeting PD-1 may be an effective way to overcome resistance after treatment with an anti-CTLA-4 agent.

Within the field of melanoma, great advances have also been made in the molecular basis of the disease, with the identification of specific mutations in *BRAF* and *KIT* as drivers of oncogenic signaling (52, 53). Targeted agents directed at tumors that harbor these mutations have induced dramatic response rates in early-phase testing, yet a large proportion of patients ultimately relapse (54–56). *In vitro* data suggest that selective inhibition of *BRAF*^{V600E} may increase T-cell recognition of melanoma antigens (57). One strategy to improve efficacy, for example, could involve the combination of a BRAF-targeted agent that provides significant tumor cell death with antigen presentation to the immune system, followed by anti-CTLA-4 that potentiates these immune responses. Such a principle may not only apply to melanoma, but also, importantly, to potential combinations of tyrosine kinase inhibitors relevant to other cancers and CTLA-4 blockade.

Modulation of the immune system via blockade of CTLA-4 represents a significant advance in the field of cancer therapy. The future remains brilliant for additional T-cell-modulating agents to be developed and, importantly, for combination testing. Improvement in our understanding of the regulation of tumor immunity should continue to provide a path for better outcomes for cancer patients.

Disclosure of Potential Conflicts of Interest

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