Microtubule-Depolymerizing Agents Used in Antibody–Drug Conjugates Induce Antitumor Immunity by Stimulation of Dendritic Cells

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Abstract

Antibody–drug conjugates (ADC) are emerging as powerful treatment strategies with outstanding target-specificity and high therapeutic activity in patients with cancer. Brentuximab vedotin represents a first-in-class ADC directed against CD30+ malignancies. We hypothesized that its sustained clinical responses could be related to the stimulation of an anticancer immune response. In this study, we demonstrate that the dolastatin family of microtubule inhibitors, from which the cytotoxic component of brentuximab vedotin is derived, comprises potent inducers of phenotypic and functional dendritic cell (DC) maturation. In addition to the direct cytotoxic effect on tumor cells, dolastatins efficiently promoted antigen uptake and migration of tumor-resident DCs to the tumor-draining lymph nodes. Exposure of murine and human DCs to dolastatins significantly increased their capacity to prime T cells. Underlining the requirement of an intact host immune system for the full therapeutic benefit of dolastatins, the antitumor effect was far less pronounced in immunocompromised mice. We observed substantial therapeutic synergies when combining dolastatins with tumor antigen–specific vaccination or blockade of the PD-1–PD-L1 and CTLA-4 coinhibitory pathways. Ultimately, treatment with ADCs using dolastatins induces DC homing and activates cellular antitumor immune responses in patients. Our data reveal a novel mechanism of action for dolastatins and provide a strong rationale for clinical treatment regimens combining dolastatin-based therapies, such as brentuximab vedotin, with immune-based therapies. Cancer Immunol Res; 2(8); 741–55. ©2014 AACR.

Introduction

Major scientific advances have refined our understanding of the protective immunity against tumors (1). Experiments with immunodeficient mice have clearly demonstrated a dual host-protective and tumor-promoting role for the immune system during cancer development. The plethora of pathways active in cancer patients to evade immune destruction is recognized as one of the emerging hallmarks of cancer (2). Immunotherapeutic approaches, in particular blockade of inhibitory receptors on T cells, such as CTLA-4, are now showing clinical efficacy and provide overall survival benefit in randomized phase III clinical trials in patients with metastatic cancer (3). More recently, blockade of additional immune checkpoints, such as the PD-1–PD-L1 interaction, has been shown to efficiently overcome immune resistance and produce durable clinical responses even in heavily pretreated patients with non–small cell lung cancer, melanoma, and renal cell cancer (4–6).

Besides immunotherapy, other approaches can also support antitumor immunity. It has recently been demonstrated that treatment with selected chemotherapeutics, such as anthracyclines and oxaliplatin, may induce an immunogenic type of tumor cell death (7), thereby enhancing antitumor immune responses. Therapeutic induction of tumor-cell apoptosis combined with triggering of dendritic cell (DC) maturation may represent an attractive alternative approach. Owing to their highly sophisticated antigen-presenting machinery, DCs are central to the initiation and regulation of anticancer immunity (8). Tumors may, however, hamper the maturation and antigen-processing capacity of tumor-residing DCs (9–11). In contrast to mature DCs that efficiently launch immune responses, immature or dysfunctional DCs are rather immunosuppressive.
Indeed, the latter property is exploited by tumors, allowing them to avoid antitumor immune responses (8, 12, 13). Therapeutic approaches that activate DCs, thereby promoting the priming of tumor antigen-specific T cells, may therefore induce durable immunity against cancer. Nevertheless, only a few studies have been reported that have investigated the capacity of antitumor therapeutics to improve DC function. Recent work has identified several cytotoxic agents, including the mitotic spindle inhibitor vinblastine, as potent activators of DC maturation (14–16). Although the underlying mechanism is still poorly defined, these data clearly highlight a previously unrecognized immunostimulatory activity for vinblastine.

Dolastatin 10, originally isolated from the Indian Ocean hare Dolabella auricularia (17), is a five-subunit penta-peptide, which potently inhibits microtubule assembly by interacting with tubulin at the "peptide sub-site" of tubulin’s "Vinca domain." The binding site is in close physical proximity to vinblastine, with dolastatins acting as noncompetitive inhibitors of vinblastine binding to tubulin (17). Of note, dolastatin 10 is a far more potent inhibitor of tumor growth in vitro and in vivo when compared with vinblastine (18). Subsequently, dolastatin 10 analogues, such as monomethylauristatin-E (MMAE) and dolastatin 15, characterized by different substituted or unsubstituted methyl groups from the N-terminal nitrogen has been synthesized, which share an identical chemical structure of the core region, and therefore, display the same molecular mode of action, but have been functionalyzed in the terminal domains (19). In particular, one of the methyl groups from the N-terminal nitrogen has been removed, thus converting the tertiary amine into a secondary amine, allowing for antibody conjugation.

Antibody–drug conjugates (ADC) allow the selective delivery of highly potent cytotoxic agents to tumor cells using a targeting mAb that specifically binds to a tumor cell–specific membrane receptor (20). Brentuximab vedotin represents an ADC, which contains MMAE conjugated via a protease cleavable linker to an anti-CD30 Ab, and has been shown to induce durable objective responses with tumor regression in most patients with relapsed or refractory CD30-positive lymphomas (21). Importantly, treatment with brentuximab vedotin was well tolerated. Although the exact mode of action is yet unknown, it is assumed that the complex is internalized following binding to CD30 expressed on tumor cells, leading to the intracellular release of MMAE, disruption of the microtubule network, cell-cycle arrest, and tumor-cell apoptosis (22).

The recent clinical success of brentuximab vedotin highlights the importance of cytotoxic drugs targeting the tubulin Vinca domain. Given the similarity to vinblastine in terms of tubulin binding and inhibition, we hypothesized that dolastatins (and subsequently ADCs containing dolastatin analogues, i.e., brentuximab vedotin) may achieve substantial therapeutic efficacy by augmenting host immunity, in particular through direct effects on DCs in addition to the killing of tumor cells. Our data demonstrate that upon treatment DCs are matured in vivo, which results in the activation of T cells in tumor models and in patients with relapsed Hodgkin lymphoma. We found that treatment was dependent on intact host immunity to be efficient, and synergized with immune-based therapies in the eradication of established tumors in mice. These findings support the rational clinical development of combination therapies based on ADCs using microtubule-depolymerizing agents as cytotoxic payload, such as brentuximab vedotin, and immunotherapies.

Materials and Methods

Reagents

Dolastatin 10 and 15 were kindly provided by the National Cancer Institute (Bethesda, MD); MMAE and anti-Thy1.1-MMAE ADC by Seattle Genetics. OVA protein was purchased endotoxin-free from Hyglos Biotechnology. OVA257–264 peptide was purchased from Abbiotech; OVA327–264 Peptide (T4) from PepGen & Elephants. The following human mAbs were used: α-CD3-APC, α-CD3-APC-H7, α-CD4-EC, α-CD4-PB, α-CD8-PE-Cy7, α-CD8-PerCP-Cy5.5, α-CD11c-APC, α-CD11b-PE-Cy7, α-CD11b-AF700, α-CD14-FITC, α-CD16-PB, α-CD19-AF780, α-CD19-AF700, α-CD20-PO, α-CD25-PE-Cy7, α-CD40-APC, α-CD45-Biotin, Streptavidin-BV605, α-CD62L-EC, α-CD69-APC-Cy7, α-CD80-FITC, α-CD83-Biotin, Streptavidin-PE-Cy7, α-CD86-PE-Cy7, α-CD86-PE, α-CD123-PE, α-CD154-FITC, α-CD161-PE, α-CTLA-4-PE-Cy7, α-FoxP3-APC-F488 (clone 259D), IgG1-isotype-AF488, α-ICOS (CD278)-PE, α-HLA-A2-PE, α-HLA-DR-PB, α-HLA-DR-PerCPCy5.5, and α-PD-1-APC (all Beckman Coulter or BD). Dead cells were stained with SytoxGreen (Invitrogen). The following murine mAbs were used: α-CD4-PE-Cy7, α-CD8-BV421, α-CD11b-FITC, α-CD11c-FITC, α-CD40-PE, α-CD80-PE, α-CD11c-PE-Cy7, α-CD45-PerCP, (all BioLegend), α-CD86-APC, α-I-A/E-Pacific Blue, α-CD8-Pe-Cy7, α-FoxP3-PE, α-IFNγ-APC (all eBioscience), and α-CD4- V450 (BD Horizon). The therapeutic Abs α-CTLA-4 (clone 9D9) and α-PD-1 (clone RMP1-14), and matched isotype controls used in vivo were produced by BioXCell. Dosing per injection was 250 μg for each of the Abs.

Cell lines

The immature mouse DC cell line SP37A3 (kindly provided by Merck KGaA) was cultured in Iscove’s Modified Dulbecco’s Medium (IMDM; Sigma) supplemented with 10% heat-inactivated and endotoxin-tested FBS (PAA), sodium pyruvate (Gibco), penicillin/streptomycin (Gibco), Eagle’s Minimum Essential Medium (MEM) nonessential amino acids (Sigma), Ciproxin (Bayer), and 0.05 mmol/L 2-mercaptoethanol (Gibco). IMDM complete medium was supplemented with 20 ng/ml recombinant mouse GM-CSF and 20 ng/ml recombinant mouse M-CSF (both Peprotech). The murine tumor cell lines EG7 and 3LL-OVA were obtained from ATCC or provided by Douglas T. Fearon (Cancer Research UK Cambridge Institute, Li Ka Shing Centre, University of Cambridge, Cambridge, UK), respectively. MC38 and RMA-Thy1.1 tumor cells were provided by Mark Smyth (Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia) and Angelo Corti (San Raffaele Scientific Institute, Milan, Italy), respectively. Murine tumor cells as well as human lymphoma cell lines Karpas-299, L-540, and Ramos (kindly provided by Jürg Schwaller, Department of Biomedicine, University of Basel, Basel, Switzerland) were cultured in DMEM with GlutaMAX (Gibco) supplemented with 10% heat-inactivated FBS, sodium pyruvate, penicillin/streptomycin, 1-glutamine mix, MEM
nonessential amino acids, Ciproxin (Bayer), and 0.05 mmol/L 2-mercaptoethanol. All cell lines were tested and validated to be Mycoplasma-free. Expression of OVA in EG7 and 3LL-OVA, and of Thy1.1 in RMAThy1.1, respectively, was confirmed; no genomic authentication was performed.

Mice
C57BL/6 mice were obtained from Charles River Laboratories, MyD88<sup>−/−</sup> and Rag2<sup>−/−</sup> mice on the C57BL/6 background were kindly provided by S. Rossi and E. Palmer, respectively (both at Department of Biomedicine, University Hospital Basel, Switzerland). OT-II TCR transgenic mice were obtained from J. Pieters (Biozentrum, University of Basel, Switzerland). CD11c-DTR mice, OT-I TCR transgenic mice, C57BL/6, C57BL/6-Ly5.1, and IFNyR<sup>−/−</sup> mice were bred in the animal facility of the Department of Biomedicine, University of Basel (Basel, Switzerland). All animals were housed under specific pathogen-free conditions and in accordance with Swiss federal regulations. Tumor cells (2.5–5 × 10<sup>5</sup>) were injected subcutaneously into the right flanks of the animals. Perpendicular tumor diameters were measured using a caliper, and tumor volume was calculated according to the formula, \( V = \frac{1}{2} \times d_1 \times d_2 \times d_3 \) where \( D_1 \) and \( d_2 \) are the longest and shortest diameter of the tumor in mm, respectively.

Assays in vitro
In mice, we used detection of cytokines and stimulation of OVA-specific OT-I and OT-II transgenic T cells as functional readouts.

Generation of bone marrow DCs
Bone marrow cells from C57BL/6 wild-type (WT) and MyD88<sup>−/−</sup> mice were activated on day 7 of culture. After 24-hour stimulation, the DC phenotype was assessed by flow cytometry and DCs were used in coculture assays.

Cytokine detection
IL1β, IL6, and IL12 in supernatants of murine DC cultures were detected by standard sandwich ELISA procedures using commercially available kits (eBioscience). Furthermore, cytokine secretion of SP37A3 DCs or bone marrow–derived DCs (BMDC) was characterized by flow cytometric analysis. For this purpose, cells were cultured in presence of dolastatin 10 (0.01 μmol/L) or lipopolysaccharide (LPS; 500 ng/mL) for 20 hours (IL12), 15 hours (IL6), or 6 hours (IL1β). Brefeldin A was added for the whole incubation time (IL1β) and IL6) or for the last 6 hours of culture (IL12). Cell surface staining of MHC-II and CD11c was performed following fixation, permeabilization, and intracellular cytokine staining.

In vitro stimulation of OVA-specific OT-I and OT-II T cells
SP37A3 or day 7 BMDCs were pulsed for 1 hour with OVA full-length protein (0.1 μg/mL) with activation with dolastatin 10 (0.1 μmol/L) or with OVA<sub>257</sub>−<sub>264</sub> peptide (T4)/OVA<sub>323</sub>−<sub>339</sub> peptide (500 ng/mL; after activation) and added at the indicated ratios to CD8<sup>+</sup>/CD4<sup>+</sup> T cells purified from OT-I/OT-II transgenic mice (2 × 10<sup>5</sup> total cells/well, 96-well round-bottomed plate). CD4<sup>+</sup> T cells were loaded with the proliferation dye eFluor670 (eBioscience) before coculture. Proliferation was assessed after 3 days using flow cytometry.

Phenotypic and functional analysis in vivo
We measured the maturation of skin Langerhans cells (LC), DC homing to tumor-draining lymph nodes (LN), and stimulation of antigen-specific CD8 and CD4 T cells. In mice, we assessed lymphocyte subsets upon treatment with brentuximab vedotin by flow cytometry and used immunohistochemistry for staining of tumor biopsies before and after brentuximab administration (see Supplementary Materials and Methods for preparation and analysis protocols for human studies).

In vivo activation of skin LCs
Cytotoxic compounds or PBS only were injected intradermally into the ears of C57BL/6 mice. Analysis was performed after 24 hours using flow cytometry and immunofluorescence. Epidermal sheets were digested with Accutase (PA), collagenase IV (Worthington), hyaluronidase (Sigma), and DNase type IV (Sigma). Single-cell suspensions were prepared and stained with anti-CD45, anti-CD11c, anti-MHC-II, and anti-CD86 antibodies. Dead cells were excluded using SytoxBlue (Invitrogen). For immunofluorescence, epidermal sheets were stained overnight with anti-MHC-II and anti-CD86 antibodies and analyzed using an Olympus BX61 fluorescence microscope.

Analysis of DC homing to tumor-draining LNs
For detection of DC homing upon injection of free dolastatin, mice bearing subcutaneous EG7 tumors were injected intratumorally with FITC-conjugated dextran (100 μg/mouse; Sigma) together with dolastatin 10 (10 μg/mouse) or PBS (mock control). For analysis of DC homing upon systemic administration of the anti-Thy1.1-MMAE ADC, mice bearing subcutaneous RMAThy1.1 tumors were injected intravenously with anti-Thy1.1-MMAE ADC (30 mg/kg) or PBS 24 hours before intratumoral injection of FITC-conjugated dextran (100 μg/mouse). Single-cell suspensions from tumor-draining and nondraining LNs were prepared 48 hours after injection of dolastatin or ADC and analyzed by flow cytometry.

In vivo stimulation of antigen-specific CD8 and CD4 T cells
LNs and spleen cells from naïve OT-I and OT-II transgenic mice (Ly5.2) were labeled with eFluor670 and adoptively transferred into C57BL/6-Ly5.1 mice. After 24 hours, mice were immunized via tail-base injection with OVA<sub>257</sub>−<sub>264</sub> peptide (T4: SIINFEKL; low-affinity variant of SIINFEKL) or OVA<sub>323</sub>−<sub>339</sub> peptide together with dolastatin 10 (1 μg) or LPS (25 μg). Proliferation of OT-I CD8<sup>+</sup> and OT-II CD4<sup>+</sup> T cells was assessed 4 days after adoptive transfer by flow cytometry.

Tumor challenge protocol
For tumor challenge, 7- to 10-week-old mice were injected subcutaneously with 2.5 to 5 × 10<sup>5</sup> tumor cells in 100 μL DMEM without phenol red (Gibco) into the right flank. For the dolastatin 10/vaccination treatment combination, a single dose of dolastatin 10 (0.4 mg/kg) was administered intravenously 15 days after tumor-cell injection. On days 17 and 24, mice were immunized intramuscularly with 5 × 10<sup>7</sup> plaque...
forming units of replication-deficient adenovirus type 5 encoding for chicken OVA (Ad-OVA). For the dolastatin 10/antibody treatment combination, two doses of dolastatin 10 (0.3 mg/kg) were administered intravenously on days 16 and 19 after tumor challenge. Treatment with four intraperitoneal doses (250 μg/mouse per dose) of anti-CTLA-4/PD-1 was initiated on day 16 (without dolastatin treatment) or on day 21 (in combination with dolastatin treatment). For T-cell depletion, mice were injected with anti-CD4 (clone: GK1.5) or anti-CD8 (clone: 53–6.72) antibodies (both were from BioXCell) at 10 mg/kg on days 14, 15, 19, 23, and 27 after tumor-cell implantation. Dolastatin 10 treatment was initiated on day 16. For IFNγ neutralization, mice were injected with anti-IFNγ (clone: XMG1.2; BioXCell) at 25 mg/kg on days 14, 15, 19, 23, and 27 after tumor-cell implantation. DCs were deleted in CD11c-DTR mice by a single injection of 4 ng/g diphtheria toxin on the day before dolastatin 10 injection. Tumor growth, determined as a function of tumor size over time, was measured every second day. Tumor volume was calculated according to the formula, $V = \frac{1}{2} \times d_1 \times d_2$, with $D$ and $d$ being the longest and shortest diameter of the tumor in mm, respectively. According to the animal regulations, mice were euthanized when tumors reached a size of 1,500 mm³.

**Analysis of tumor-infiltrating lymphocytes**

Mice that were 7 to 10 weeks old were injected subcutaneously with 5 × 10⁶ MC38 tumor cells in 100 μL DMEM without phenol red into the right flank. On days 16, 17, and 18 after tumor challenge, mice were treated with dolastatin 10 (0.3 mg/kg) intravenously, followed by three doses of anti-CTLA-4/PD-1 (250 μg each, i.p.) on days 20, 22, and 24. Mice receiving anti-CTLA-4/PD-1 only were treated on days 16, 18, and 20. On day 26, tumors were dissociated mechanically and digested using Accutase (PAA), collagenase IV (Worthington), hyaluronidase (Sigma), and DNAse type IV (Sigma). Single-cell suspensions were prepared, and stained for the indicated markers for flow cytometric analysis. For detection of IFNγ-producing cells, single-cell preparations were cultured overnight in the presence of anti-CD3/CD28 antibodies and monensin (BioLegend).

**Therapeutic protocols and human studies**

All protocols for therapeutic studies and analysis of human specimens are described in details in Supplementary Materials and Methods.

**Study approval**

Tumor biopsies and blood drawing from patients with cancer who received brentuximab were performed in accordance with the guidelines required by the local Institutional Review Board. Animals were maintained and treated in compliance with the guidelines of the Swiss Federal and the Cantonal Veterinary Office Basel-Stadt.

**Statistical analysis**

Statistical values were calculated using a two-tailed paired Student $t$ test for T-cell proliferation and DC activation marker expression. Kaplan–Meier survival plots were analyzed using a log-rank test (Mantel–Cox). $P < 0.05$ was considered statistically significant for all biologic tests.

**Results**

**Dolastatins trigger maturation of DCs**

To test the impact of dolastatins on the maturational state of DCs, we used a previously established primary murine DC line (SP37A3), which resembles immature DCs (23). Although the cancer cell growth-inhibitory properties of dolastatins are several-fold greater than those of vinblastine, we included the latter agent into our experimental set-up based on previously published data showing its capacity to mature DCs (15, 16). Following 24-hour culture of SP37A3 cells in the presence of dolastatin 10, dolastatin 15, vinblastine, or LPS, we determined the surface expression of the DC maturation markers CD80, CD86, CD40, and MHC-II (Fig. 1A and Supplementary Fig. S1). We observed a dose-dependent upregulation of these molecules during treatment with all compounds; the most pronounced effects being induced by dolastatin 10. These results were confirmed using mouse BMDCs (Supplementary Fig. S2A). DC viability did not change significantly or differ between the three drugs at any of the drug concentrations tested, as determined using SytoxGreen staining (Supplementary Fig. S1).

Supernatants from the DC cultures were analyzed for proinflammatory cytokines that have been demonstrated to play critical roles in regulating T-cell function and antitumor immune responses (24). Dolastatin 10 and, to a lesser extent, vinblastine triggered the production of IL1β, IL6, and IL12 (Fig. 1B). Intracellular cytokine staining using SP37A3 and BMDCs confirmed the pattern of cytokine expression, whereas cytokine production in BMDCs was generally less efficient (Fig. 1B and Supplementary Fig. S2). To assess the capacity of drug-treated DCs to activate naïve T cells, SP37A3 cells and BMDCs, exposed to dolastatin 10 and LPS as positive control, were loaded with either OVA267–274 or OVA323–339 peptide. Notably, upon coculture with naïve eFluor670-labeled CD8+ and CD4+ T cells isolated from OVA-specific T-cell receptor transgenic OT-I and OT-II mice, respectively, we noticed a robust T-cell proliferation when incubated with dolastatin 10 (Fig. 1C and D and Supplementary Fig. S2C and S2D). In the next step, DCs were loaded with recombinant OVA full-length protein. Both SP37A3 and BMDCs were able to induce significant proliferation of both OT-I and OT-II transgenic T cells. Thus, dolastatin treatment of DCs allows efficient antigen uptake and processing for both MHC class II and I antigen-presentation, the latter pathway being commonly referred to as cross-presentation (Fig. 1C and D; Supplementary Fig. S2C and S2D; ref. 25). Of note, DCs exposed to dolastatin 10 exhibited cytokine release and supported T-cell proliferation to levels comparable with that of LPS-stimulated DCs.

We next investigated the capacity of dolastatins to activate skin-resident LCs by injecting dolastatin 10 or 15 into the ears of C57BL/6 mice. Consistent with our in vitro observations, dolastatin 10 and, to a lesser extent, dolastatin 15 induced the expression of CD86 and MHC-II on LCs. Compatible with in situ maturation of LCs (Fig. 2A and B), immunofluorescence staining revealed an enlarged cell size, profound morphologic changes, such as dendrite hyper-elongation, a strong upregulation of the costimulatory molecule CD86, and a marked
Figure 1. Dolastatins induce phenotypic and functional DC maturation in vitro. A, representative histograms (n = 3) for the expression of MHC-II and costimulatory molecules CD80, CD86, and CD40 by SP37A3 murine DCs exposed to dolastatin 15 (0.1 μmol/L), dolastatin 10 (0.1 μmol/L), vinblastine (0.1 μmol/L), or LPS (500 ng/mL). Mean fluorescence intensity (MFI) was assessed by flow cytometry. B, secretion of IL1β, IL6, and IL12 by dolastatin 10 (0.01 μmol/L) or LPS (500 ng/mL)-pretreated DCs was assessed by intracellular staining and flow cytometric analysis (right). Controls indicate mock-treated DCs. Three experiments in triplicates were performed with similar results. C and D, DCs were treated with dolastatin 10 (0.01 μmol/L) or LPS (500 ng/mL) for 12 hours before or after loading with specific protein or peptide, respectively. Controls indicate mock-treated, antigen-pulsed DCs. DCs were pulsed with OVA267–293 or OVA263–299 peptide (left) or loaded with OVA protein (right) and treated with the indicated agents before irradiation and addition to microwells of transgenic CD8+ OT-I (C) or CD4+ OT-II T cells (D). DC:T cell ratios are indicated. Proliferation of OT-I/OT-II T cells was assessed by flow cytometry. Data show representative histograms from one of at least three independent studies.

Dolastatins activate antigen-specific T cells in vivo

We adoptively transferred naive CD8+ and CD4+ T cells from OT-I and OT-II transgenic mice (Ly5.2) into congenic C57Bl/6 (Ly5.1) recipient mice. Subsequently, we measured T-cell responses following immunization with a weak agonist peptide derived from the original OVA263–293 peptide SIINFEKL (26) and the OVA267–293 peptide, respectively. A much stronger proliferation of both OT-I and OT-II T cells was observed in mice receiving peptide plus dolastatin 10 compared with dolastatin 10 or peptide alone (Fig. 2D). Therefore, under in vivo conditions, dolastatin 10 is capable of inducing efficient antigen presentation, thereby augmenting antigen-specific T-cell responses comparable with those achieved with LPS. Most decrease in LC density, which may reflect the migration of LCs to the draining LN (Fig. 2C).

The exact mechanism by which dolastatins induce the maturation of DCs is unknown. To determine whether activation of DCs requires MyD88 during exposure to dolastatin 10, we analyzed its effect on the maturation of BMDCs from WT and MyD88−/− mice and injected dolastatin 10 into the ears of WT and MyD88−/− mice as described in Supplementary Fig. S3. The TLR9 ligand CpG1668 was used as a positive control. Unlike CpG, which is not able to upregulate CD86, CD40, and MHC-II in MyD88−/− mice, the activation of DCs during exposure to dolastatin 10 occurred independently of MyD88 signaling, both in vitro and in vivo.
importantly, we observed the same degree of T-cell proliferation in tumor-draining as well as non-draining LNs.

To further describe the migratory behavior of dolastatin-activated DCs, we used FITC-dextran, a carbohydrate with a high molecular mass, which is readily taken up by DCs during early activation (27). In mice treated with vehicle alone, DCs from tumor-draining LNs showed almost no increased FITC signal. In stark contrast, FITC-dextran–bearing DCs could be robustly detected and correlated with high CD86 expression in the tumor-draining LNs of mice treated with dolastatin 10 (Fig. 2E and F). FITC-dextran–bearing DCs could not be detected in non–tumor-draining LN in both cases, providing further evidence for the local, tumor-selective effects of dolastatin treatment (Supplementary Fig. S4).

Therapeutic synergy of dolastatins and immune-based therapies

We next assessed the requirement of T cells and/or IFNγ for the efficacy of dolastatin 10–based treatments using MC38 adenocarcinoma tumors (Fig. 3A). In immunocompetent syngeneic WT mice, systemic treatment with dolastatin 10 was sufficient to suppress the growth of MC38 tumors. Depletion of CD8+ cells or neutralization of IFNγ with mAbs administered before dolastatin 10 treatment severely abrogated the antitumor effect of the drug in an established tumor setting with significant loss of tumor growth suppression (Fig. 3A). Abrogation of dolastatin 10 efficacy was not observed in mice depleted of CD4+ cells. To investigate the specific role of CD11c+ DCs in dolastatin 10–mediated tumor rejection,
Figure 3. Dolastatin 10 treatment requires intact host immunity for full therapeutic efficacy and synergizes with antibody-mediated blockade of T-cell inhibitory receptors PD-1 and CTLA-4. A and B, tumor growth during treatment with dolastatin 10 in MC38 tumor-bearing C57BL/6 WT control mice and upon depletion of CD4 or CD8 T cells or neutralization of IFNγ with mAbs (A) and in CD11c-DTR mice (depleted of CD11c DCs) bearing 16-day established subcutaneous MC38 tumors; control: tumor-bearing, mock-treated mice (B). C and D, tumor growth in C57BL/6 WT mice bearing 16-day established subcutaneous MC38 tumors during treatment with dolastatin 10, anti-PD-1/CTLA-4, or the combination. Control, tumor-bearing mice received matched isotype control Abs. All data are expressed as mean ± SEM (n = 11–12). Two independent experiments were performed, and the pooled data are shown. Results are depicted as individual tumor-growth curves (A), cumulative tumor volume over time (D), and as a Kaplan–Meier survival plot (E); the x-axis depicts post-tumor implantation (days).

Before dolastatin 10 treatment, CD11c-DTR transgenic mice (28) with established MC38 tumors were treated with diphtheria toxin, which results in the depletion of CD11c-expressing DCs. Depletion of CD11c+ cells was sufficient to abrogate the antitumor effect of dolastatin 10 (Fig. 3B). In addition, we have used RAG2−/− and IFNγR1−/− mice to investigate tumor growth kinetics in EG7 and 3LL-OVA tumors upon treatment with dolastatin 10. In immunocompetent syngeneic WT mice, treatment with dolastatin 10 was sufficient to induce persistent regression of transplanted tumors. In both RAG2−/− and IFNγR1−/− mice, we noticed a significant reduction of its therapeutic effect during treatment with dolastatin 10 (Supplementary Fig. S3A and S5B). These data underline the importance of CD11c+ DCs and, subsequently, T cells and IFNγ as critical determinants of the antitumor effects of dolastatins.

Given its capacity to augment antitumor immunity, we hypothesized that dolastatin may synergize with and enhance the efficacy of immunomodulatory agents. In the first experiment, mice with established ovalbumin-expressing 3LL tumors were treated with systemic administration of dolastatin 10 and subsequent vaccination using a recombinant adenovirus expressing ovalbumin (Adeno-OVA). The overall survival of mice treated with Adeno-OVA plus dolastatin 10 was significantly longer than that of mice given dolastatin 10 or vaccination alone (Supplementary Fig. S6A–S6C). Thus, dolastatins improve the efficacy of antigen-specific vaccination in a setting with established tumors.

To delineate further therapeutic synergies between dolastatins and immune checkpoint inhibition, we treated mice with established MC38 tumors using a combination of anti-CTLA-4/PD-1 antibodies (29) and systemically administered...
dolastatin 10. Slower tumor outgrowth was observed with monotherapy using either dolastatin 10 or anti-CTLA-4/PD-1 compared with the control group. In the dolastatin 10 group, 1 of 12 mice experienced complete tumor regression compared with 3 of 12 mice in the anti-CTLA-4/PD-1 group. In contrast, concomitant treatment with dolastatin 10 and anti-CTLA-4/PD-1 achieved complete tumor rejection in 7 of 12 mice and significantly delayed outgrowth in the rest of the mice (Fig. 3C–E). Our results demonstrate therapeutic synergy between dolastatins and immunomodulatory treatment approaches, leading to potent induction of antitumor immunity and finally tumor rejection.

Combination therapy of dolastatin 10 and CTLA-4/PD-1 blockade increases the intratumoral effector to regulatory T-cell ratio
To define the immunologic mechanism of action of a treatment approach combining dolastatins and anti-CTLA-4/PD-1 antibodies, we analyzed tumor-infiltrating lymphocytes (TIL) 10 days after treatment onset. To this end, we determined the impact of the indicated treatments on the frequency of regulatory T cells (Treg) and IFNγ-producing intratumoral CD8 effector T cells (Teff). We observed a significantly lower frequency of intratumoral Tregs in dolastatin- or anti-CTLA-4/PD-1–treated tumors, which was even more pronounced in tumors exposed to the combination treatment (Fig. 4A and C). Similarly, we observed a relative increase in the number of IFNγ-producing CD8 T cells in dolastatin-treated tumors, in particular in combination with anti-CTLA-4/PD-1–blocking antibodies (Fig. 4B and C). Previous work has demonstrated a correlation between the therapeutic efficacy of immunotherapies and a shift in the intratumoral Teff-to-Treg ratio (30). Accordingly, we documented a significant increase in the CD3+IFNγ+ and CD8+IFNγ+ Teff-to-Treg ratio in the combination group compared with nontreated, dolastatin only-, or anti-CTLA-4/PD-1 only–treated tumors (Fig. 4C).

Absolute cell numbers of the respective cell populations are provided in Supplementary Fig. S7. Overall, the therapeutic efficacy of the combined treatment with dolastatin and CTLA-4/PD-1 blockade correlates with a shift in the intratumoral balance between Teffs and Tregs toward a more antitumorigenic profile.

Dolastatin 10 and its synthetic analogue MMAE enhance the T-cell stimulatory capacity of human DCs
To determine whether human DCs react similarly as their murine counterparts, we analyzed the maturation stage of monocyte-derived DCs (moDC) from healthy blood donors during exposure to dolastatin 10. Furthermore, we included the dolastatin analogue MMAE, the cytotoxic component of brentuximab vedotin, in all tests performed on human cells. In agreement with the data from the murine SP37A3 DC line, we observed a dose-dependent upregulation of the surface expression of CD86, CD83, CD40, and MHC-II (HLA-DR) by human moDCs after dolastatin 10 exposure (Fig. 5A and B). In addition, we confirmed the potential of the analogue MMAE to induce upregulation of costimulatory molecules on mouse SP37A3 DCs (Supplementary Fig. S8A) and human moDCs (Supplementary Fig. S8C). SP37A3 DCs readily produced the proinflammatory cytokines IL1β, IL6, and IL12 upon stimulation with MMAE (Supplementary Fig. S8B). DC viability did not significantly change as determined by SytoxGreen staining using dolastatin 10 or MMAE at the indicated concentrations (data not shown). To further address the functional activation of human DCs during treatment with dolastatin 10 or MMAE, we analyzed the capability of pretreated moDCs to induce proliferation of allogeneic CD8 T cells in mixed-lymphocyte cultures. We consistently observed a >2-fold increase in T-cell proliferation following pretreatment of DCs with dolastatin 10 (P < 0.0001) or MMAE (P = 0.0002). Similar results were obtained when human DCs were stimulated using LPS (P < 0.0001; Fig. 5C). To directly demonstrate maturation of tumor-resident DCs, we incubated matched pieces of human tumor resections with dolastatin 10, MMAE, or LPS as positive control and, after 24 hours, analyzed the expression of CD86 and HLA-DR as indicators of DC maturation. Single-cell suspensions of treated and control tumor pieces were analyzed by flow cytometry. We observed a >2-fold upregulation of CD86 by tumor-infiltrating DCs (CD45+CD11c+CD11b+) treated with dolastatin 10 (P < 0.0001) or MMAE (P < 0.003; Fig. 5D; Supplementary Table S1). Interestingly, LPS treatment was not able to reverse the immature state of tumor-resident DCs in terms of CD86 expression (P = 0.062, not statistically significant) to the same degree as dolastatins. In contrast, expression of HLA-DR was induced to the same extent by all three agents, namely dolastatin 10 (P < 0.05), MMAE (P < 0.03), and LPS (P < 0.02). Overall, we demonstrate that dolastatin 10 and its analogue MMAE are able to phenotypically and functionally mature human moDCs and, most importantly, both agents are capable of reversing the immature, hence rather tolerogenic (31), state of tumor-resident DCs in human tumor explants.

MMAE-coupled ADCs induce DC homing in vivo, mature human moDCs in lymphoma cell–DC cocultures, and activate adaptive immunity in patients with Hodgkin lymphoma
To determine whether systemically administered, tumor-targeted MMAE-coupled ADCs allow sufficient release of free MMAE in the tumor vicinity to induce maturation of tumor-resident DCs, we synthesized an ADC, which binds specifically to a mouse tumor in syngeneic, fully immunocompetent mice. To this end, MMAE was conjugated to an antibody specific to the model antigen Thy1.1 (anti-Thy1.1-MMAE ADC) and has been tested in tumor-bearing animals using Thy1.1-transfected RMA lymphoma cells. To directly show DC activation, which is reflected by early antigen uptake and migration of tumor-resident DCs to the tumor-draining LNs, fully immunocompetent mice bearing subcutaneous RMA-Thy1.1 tumors were injected intratumorally with FITC-conjugated dextran together with systemic administration of anti-Thy1.1-MMAE ADC or PBS/carrier. Single-cell suspensions from tumor-draining and nondraining LNs were prepared 48 hours after injection and analyzed by flow cytometry. In mice treated with vehicle alone, DCs from tumor-draining LNs showed almost no increased FITC signal. In stark contrast, FITC-dextran–bearing DCs
could be robustly detected and correlated with high CD86 expression in the tumor-draining LNs of mice systemically treated with anti-Thy1.1-MMAE ADC (Fig. 6A and B). FITC-dextran–bearing DCs could not be detected in non–tumor-draining LN in both cases, providing further evidence for the local, tumor-selective effects of the treatment with anti-Thy1.1-MMAE ADC (Supplementary Fig. S9). To further substantiate our findings with clinically relevant ADCs, we explored whether brentuximab vedotin elicits maturation of human moDCs in coculture with human lymphoma cell lines. Brentuximab vedotin was highly potent and selective against the CD30+ tumor cell lines L-540 (HL) and Karpas-299 (ALCL) with an IC₅₀ of 10 and 30 ng/mL, respectively, but more than 1,000-fold less active on the CD30− tumor cell lines Raji and Ramos (both Burkitt non-Hodgkin lymphoma; data not shown). In contrast to coculture of human moDCs with brentuximab vedotin–treated CD30− lymphoma cells (Ramos) or brentuximab vedotin only, we observed a substantial upregulation of the surface expression of CD86 by human moDCs after coculture with brentuximab vedotin–treated CD30+ L-540 and Karpas-299 lymphoma cells (Fig. 6C and D), comparable with the maturation capacity of free MMAE.

We next assessed the induction of antitumor immune responses upon systemic treatment with brentuximab vedotin. To this end, we collected peripheral blood mononuclear cells (PBMC) from 6 patients with relapsed Hodgkin and CD30+ B cells.
A

Fold increase MFI CD86

Concentration (μmol/L)

Fold increase MFI CD83

Concentration (μmol/L)

Fold increase MFI CD40

Concentration (μmol/L)

Fold increase MFI MHC-II

Concentration (μmol/L)

B

CD86

CD83

CD40

MHC-II

C

% Dividing CD8 T cells

Control

Dolastatin 10

LPS

Control

MMAE

LPS

eFluor 670

D

Fold change: CD86 (intratumoral DCs)

Dolastatin 10

MMAE

LPS

Fold change: MHC-II (intratumoral DCs)

Dolastatin 10

MMAE

LPS

Paired, two-tailed t test (CD86):

Dola P value: <0.0001 ****

MMAE P value: 0.0023 ***

LPS P value: 0.062 n.s.

Sample Mean: MFI

Dolastatin 10 239

MMAE 232

LPS 91.5

Control 79.5

Paired, two-tailed t test (MHC-II):

Dola P value: 0.0426 *

MMAE P value: 0.0020 *

LPS P value: 0.015 *

Sample Mean: MFI

Dolastatin 10 10522

MMAE 305

LPS 332

Control 134

(Legend on the following page)
T-cell lymphoma before and after brentuximab vedotin administration. All patients exhibited marked clinical and metabolic responses using PET-CT scans (data not shown). Surprisingly, a significant decrease in the number of CD4+CD25+FoxP3+ Tregs was observed when comparing PBMCs before and after brentuximab vedotin administration (Fig. 7A), while the relative numbers of CD4 and CD8 T cells remained unchanged (data not shown). We were able to document a significant increase of both CD4 and CD8 T-cell activation, determined by the expression of CD25 (Fig. 7B). To determine the activation of peripheral DCs and B cells, the latter being increasingly recognized as potent antigen-presenting cells and key players in antitumor response, we analyzed the expression of CD86 and MHC-II (HLA-DR) on DCs after exposure to dolastatin 10 and MMAE, respectively, and subsequently used in a mixed lymphocyte reaction (MLR) with allogeneic CD8+ T cells. T-cell proliferation was measured after 4 days. The experiments were repeated with PBMCs from different donors to exclude DC death. D, CD86 mean fluorescence intensity (MFI) fold-change as compared with untreated moDCs; graphs show data from 11 independent experiments.

Figure 6. MMAE-coupled ADCs induce DC homing in vivo and mature human moDCs in tumor cell cocultures. A and B, C57BL/6 mice bearing subcutaneous RMA-Thy1.1 tumors of approximately 100 mm³ in size were treated as outlined. Briefly, anti-Thy1,1-MMAE ADC was administered systemically 24 hours before intratumoral (i.t.) injection of FITC-dextran. On day 2 after ADC treatment, tumor-draining LNs were examined by flow cytometry for the presence of FITC-CD86⁺ DCs. A, representative plots depict FITC⁺ DCs detected in tumor-draining LNs after PBS/DMSO or ADC injection. B, pooled data from two independent experiments. C and D, CD30⁺ Karpas-299 and L-540 as well as CD30⁺ Ramos cells were incubated with brentuximab vedotin for 3 days. On day 3, immature moDCs were added and cocultured for 24 hours. C, CD86 expression by DCs after coculture with brentuximab vedotin-treated L-540 cells (orange). Control DCs were left untreated (black), incubated with L-540 cells alone (blue), or incubated with MMAE at 0.1 μmol/L for 24 hours (red; left). DCs were cultured in the presence of brentuximab vedotin without tumor cells at the indicated concentrations (middle) and with L-540 cells treated with three different chemotherapeutics at 100 μmol/L (right) to exclude DC maturation induced by tumor cell death. D, CD86 mean fluorescence intensity (MFI) fold-change as compared with untreated moDCs; graphs show data from 11 independent experiments.

Figure 5. Human DCs exposed to dolastatin 10 or MMAE upregulate costimulatory molecules and display enhanced T-cell-stimulatory capacity. A, expression of CD86, CD83, CD40, and MHC-II (HLA-DR) by dolastatin-treated human moDCs was assessed by flow cytometry. Graphs show mean fluorescence intensity (MFI) change compared with mock-treated cells, which were set as 1. All data are representative of at least three independent experiments. Data are depicted as mean ± SD. B, representative histograms for CD83, CD86, CD40, and HLA-DR expression after exposure to dolastatin 10 (0.1 μmol/L), LPS (500 ng/mL), or carrier (control). C, monocyte-derived DCs were exposed to dolastatin 10 and MMAE, respectively, and subsequently used in a mixed lymphocyte reaction (MLR) with allogeneic CD8⁺ T cells. T-cell proliferation was measured after 4 days. The experiments were repeated with PBMCs from 9 healthy blood donors yielding similar results. Representative flow cytometry plots of one experiment are shown. D, fresh human tumor biopsies were incubated with dolastatin 10 (0.1 μmol/L), LPS (500 ng/mL), or carrier (control) for 24 to 36 hours. Expression of CD86 and MHC-II by tumor-resident DCs (CD45⁺CD11c⁺CD11b⁻) in treated and untreated (mock control) tumor pieces was assessed by flow cytometry. Graphs summarize all performed experiments and show fold-change of MFI compared with mock-treated specimens, which were set as 1. Histograms depict analysis of one representative tumor explant.
immunity, we assessed the expression of CD86 on lin−/CD11c+CD11blow DCs and CD20+ B cells before and after brentuximab vedotin (BV) treatment using flow cytometry. As shown in Fig. 7C, expression of this marker substantially increased after brentuximab vedotin administration. Taken together, our results demonstrate that brentuximab vedotin leads to the induction of cellular immunity (fewer Tregs and increased activation of T and B cells) in patients with relapsed Hodgkin lymphoma, reflecting the activation of cellular immunity.

**Brentuximab vedotin induces marked T-cell infiltration into human lymphomas**

To analyze the changes in the degree and type of TILs in response to treatment with brentuximab vedotin, skin biopsies were performed on a patient with relapsed CD30+ cutaneous T-cell lymphoma before (pre) and after (post) brentuximab vedotin treatment. In addition to H&E staining, immunohistochemical reactions were carried out using specific antibodies for CD30 (lymphoma) and CD4 (Th) as well as CD8 (cytotoxic) T cells. Compatible with the clinical response upon treatment with brentuximab vedotin, we observed a decrease in the number and density of CD30+ lymphoma cells. Notably, we detected a substantial increase in total lymphocytes from pre- to post-tumor specimens. Immunohistochemistry revealed a pronounced increase in both CD4 and CD8 lymphocytic infiltrates in the post-skin biopsy (Fig. 7D). We have performed a similar analysis in a patient with relapsed CD30+ Hodgkin lymphoma. Comparably, we have observed an increase in total lymphocytes.
lymphocyte numbers with a preferential accumulation of T cells (data not shown).

**Discussion**

Dolastatins, potent inhibitors of tubulin polymerization and cytotoxic components of brentuximab vedotin, have been shown to bind to tubulin via the Vinca domain, that is, close to the binding site of vinblastine and other Vinca alkaloids (32). Because of the resemblance in binding properties and the capacity of vinblastine to induce DC maturation (14–16), we hypothesized that dolastatins, and ultimately ADCs using dolastatins, may exhibit similar effects and promote antitumor immunity in addition to their direct cytotoxic effects on tumor cells. In this study, we showed first that dolastatins trigger a program of phenotypic and functional activation of tumor-resident DCs. Second, upon antigen uptake and maturation, these DCs migrate to the tumor-draining LNs, where they encounter and activate tumor antigen-specific T cells. Third, the adaptive immune system is essential for the full therapeutic activity of dolastatins. Fourth, when combined with tumor antigen-specific vaccination or checkpoint inhibitor-blocking antibodies, dolastatins show synergistic antitumor activity and promote tumor destruction. Fifth, ADCs coupled to the dolastatin analogue MMAE, such as brentuximab vedotin, induce DC homing in murine mouse models, and the maturation of human DCs in lymphoma cell–DC cocultures as well as the activation of T and B cells in patients, reflecting augmentation of tumor-specific immunity.

It has become evident that some chemotherapeutics are capable of augmenting host immunity (33). Mizumoto and colleagues identified microtubule-depolymerizing drugs as inducers of in situ maturation and migration of tissue-resident DCs (14). Similarly, vinblastine was found to promote DC-mediated antigen presentation and tumor antigen–specific T-cell responses in vitro and in vivo (15, 16). In contrast, other cytotoxic drugs may block DC maturation, thereby antagonizing antitumor immunity (34, 35). Yet, the mechanism proposed here seems to be distinct from direct effects on DCs that have been described using low noncytotoxic concentrations of other classes of cytotoxic drugs (36). We have observed effects on DC maturation and T-cell activation over a wide range of drug doses with the highest doses inducing tumor-cell apoptosis in vivo and in vitro (data not shown). Although the mechanisms of cell death in DCs following cytotoxic treatment have not been elucidated in detail, non-/less-dividing cells, such as endothelial cells or DCs, may exhibit distinct sensitivity in apoptosis induction toward microtubule-targeting agents (37, 38), which may be reflected by the presence of specific targets/signaling pathways in these cells (39). Further investigations are necessary to characterize the microtubule dynamics in DCs upon dolastatin exposure.

It is unclear how dolastatins activate DCs. Previous studies have demonstrated a critical role for Toll-like receptor (TLR) signaling in DC differentiation (40). Among cytosolic adapter proteins, MyD88 is shared by most TLRs, except TLR3 and TLR4 (40). We used MyD88-deficient mice to test whether MyD88-dependent TLR signaling was required for dolastatin-induced DC maturation. In the absence of MyD88, both in vitro and in vivo DC maturation induced by TLR9 ligation (CpG-ODN) was abolished. In contrast, dolastatin-induced upregulation of CD40, CD86, and MHC-II occurred independently of MyD88, although we cannot rule out that TLR3, TLR4, or non-TLR pattern-recognition receptors may be involved in the molecular events induced by dolastatins for DC activation. In addition, the molecular mechanisms may be located downstream of TLR domain-containing cytosolic adapters. In fact, microtubule-depolymerizing agents can activate the NF-κB and MAPK signaling pathways efficiently (41, 42), although no specific receptors have been identified to date. Further experiments are necessary to address this issue and to define the molecular signaling cascades induced by dolastatins. Of note, we have observed only marginal effects on DC activation using agents with microtubule-polymerizing/stabilizing activity such as taxanes (data not shown). In addition to immunostimulatory effect on DCs, tumor cell death triggered by cytotoxic agents could lead to the release of cellular cues, which may elicit an antitumor immune response (7, 43). Although in our experiments tumor cell death induced by cytotoxic agents, such as cisplatin, mafosfamide, and etoposide, was not sufficient to activate DCs, and, more importantly, potent DC activation by dolastatin 10 has been observed in the absence of tumor cell death, we cannot exclude the possibility that dolastatin-exposed dying tumor cells could emit a series of danger signals that may elicit the recruitment and activation of antigen-presenting cells.

Brentuximab vedotin has been shown to induce sustained clinical responses in heavily pretreated patients (21). In addition to direct tumor cytotoxicity, it has been reported that brentuximab vedotin interferes with the immunosuppressive environment by decreasing the release of some cytokines (44). Moreover, we have reported a significant, lymphoma-specific increase of CD161 T cells in patients with CD30+ lymphomas that have been treated with brentuximab vedotin (45, 46). These data suggest that induction of tumor-specific immunity could play a more substantial role for the therapeutic efficacy of brentuximab than has been appreciated. Importantly, our data reveal a mechanism by which dolastatins, both as free compounds and as ADCs, such as brentuximab vedotin, induce DC maturation and subsequent T-cell activation. In humans, we have confirmed that free dolastatins, such as MMAE, can induce the maturation of peripheral, moDCs as well as intratumoral DCs obtained from primary resections of patients with cancer. The latter experiments suggest that dolastatins may convert, at least in part, the immature status of tumor-resident DCs, which is a hallmark of DCs residing in close proximity to tumor cells (47, 48), into an antitumorigenic one. Accordingly, brentuximab vedotin elicits maturation of human moDCs in coculture with CD30-expressing lymphoma cells, underlining that MMAE can diffuse from tumor cells into the vicinity, resulting in DC activation. In patients with CD30+ lymphomas, we were able to demonstrate that brentuximab vedotin applied as systemic treatment activates cellular immune responses. In the peripheral blood, we observed a consistent upregulation of costimulatory markers in both
T and B cells after brentuximab vedotin administration. The frequency of circulating Tregs substantially decreased, which may further support the induced/enhanced antilymphoma immune response. Of particular note, we found an increase in CD8 and CD4 TILs early after commencement of brentuximab vedotin treatment. Our data thus suggest that brentuximab vedotin favorably alters the balance between tumor-mediated immune suppression and antitumor immunity, which may contribute to therapeutic efficacy. Additional studies are required to delineate the specific immune response, in particular the generation of an immunologic memory upon treatment with brentuximab vedotin, and to correlate these findings with clinical outcome.

To our knowledge, this is the first study to show that dolastatins can be used to improve the therapeutic efficacy of immune-based therapies in advanced tumors in which these therapies are commonly less efficacious. As compared with monotherapy, we have demonstrated improved long-term survival in mice treated with dolastatins combined with both antigen-specific vaccination and immune-checkpoint inhibition. The combination therapy induced an increase in the number of effecter CDS T cells producing IFNγ and a substantial increase in the intratumoral ratio of Teffs (defined as IFNγ+ T cells) to Tregs, which is considered an indicator of a favorable immunologic antitumor response (30, 49). Mechanistically, we envision that the antitumor immune response, which is activated upon dolastatin administration, subsequently is dampened by the engagement of inhibitory receptors, such as CTLA-4 and PD-1 on tumor-infiltrating T cells. The concomitant application of antagonistic antibodies blocking these inhibitory receptors efficiently unleashes and sustains the T-cell response against the tumor.

In summary, we have shown that dolastatin-based therapies induce the full spectrum of maturational changes in DCs, which activates cellular immunity both in patients and in animal tumor models. Our data support the pivotal contribution of adaptive immunity for the therapeutic efficacy of dolastatin-based therapies and provide a strong rationale to explore combination therapies of drugs such as brentuximab vedotin and novel immune-based therapies.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Antibody–Drug Conjugates Potentiate Tumor-Specific Immune Responses


Microtubule-Depolymerizing Agents Used in Antibody–Drug Conjugates Induce Antitumor Immunity by Stimulation of Dendritic Cells

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