Cancer Immunotherapy with Immunomodulatory Anti-CD137 and Anti–PD-1 Monoclonal Antibodies Requires BATF3-Dependent Dendritic Cells

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ABSTRACT
Weak and ineffective antitumor cytotoxic T lymphocyte (CTL) responses can be rescued by immunomodulatory mAbs targeting PD-1 or CD137. Using Batf3−/− mice, which are defective for cross-presentation of cell-associated antigens, we show that BATF3-dependent dendritic cells (DC) are essential for the response to therapy with anti-CD137 or anti–PD-1 mAbs. Batf3−/− mice failed to prime an endogenous CTL-mediated immune response toward tumor-associated antigens, including neoantigens. As a result, the immunomodulatory mAbs could not amplify any therapeutically functional immune response in these mice. Moreover, administration of systemic sFLt3L and local poly-ICLC enhanced DC-mediated cross-priming and synergized with anti–CD137- and anti–PD-1–mediated immunostimulation in tumor therapy against B16-ovalbumin–derived melanomas, whereas this function was lost in Batf3−/− mice. These experiments show that cross-priming of tumor antigens by FLT3L- and BATF3-dependent DCs is crucial to the efficacy of immunostimulatory mAbs and represents a very attractive point of intervention to enhance their clinical antitumor effects.

SIGNIFICANCE: Immunotherapy with immunostimulatory mAbs is currently achieving durable clinical responses in different types of cancer. We show that cross-priming of tumor antigens by BATF3-dependent DCs is a key limiting factor that can be exploited to enhance the antitumor efficacy of anti–PD-1 and anti–CD137 immunostimulatory mAbs. Cancer Discov; 6(1); 71–9. ©2015 AACR.

INTRODUCTION
Tumor cells are antigenic as a result of abundant mutated sequences in their exomes (1). However, they are poorly immunogenic to prime cytotoxic T lymphocyte (CTL) responses because antigen presentation takes place in the absence of appropriate co-stimulation and in a strongly immunosuppressive environment (2). The immune response to cell-associated antigens requires the interplay of specialized and professional antigen-presenting cells called dendritic cells (DC). Among the variety of DC subsets, certain DCs excel at redirecting cell-associated phagocytosed proteins to the

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MHC class I antigen presentation pathway (3), a process termed cross-presentation, or cross-priming if it results in CD8+ T-cell activation. There is evidence that tumor antigens are efficiently cross-presented in vivo (4).

Two DC subsets have been identified in mice as the most efficient at cross-priming in vivo: lymphoid-tissue resident CD11c+CD8α+CD103−Clec9a+DNGR-1−XCR1+ DCs and migratory CD11c+CD103−Clec9a+DNGR-1+Clec9a+DCs (5). Differentiation of both DC subsets shows an absolute requirement for FLT3L, and is largely affected by the absence of BATF3 (6). Notably, the absence of BATF3 impairs not only numbers but also functional responses in the remaining CD11c+Clec9a+DNGR-1−Clec9a+DCs, such as cell-associated cross-presentation or IL12 production (7,8). Notably, Batf3−/− mice show impaired immunity against syngeneic immunogenic fibrosarcomas (6) and regulate T-cell infiltration in models of melanoma (9). However, other BATF3-independent DC subsets mediate the immune system–dependent antitumor activity of anthracyclines (10) and mediate tumor rejection under activating conditions in BATF3-deficient mice (11). Recent reports further support an important role for intratumoral BATF3-dependent DC103+ DCs in priming a CTL response through IL12 production (12,13). In humans, an equivalent BATF3-dependent DC subset characterized by expression of CD11c, CD141, Clec9a and XCR1 has been identified in peripheral blood and lymphoid organs (14).

Immunotherapy of cancer is currently being revolutionized by the use of immunomodulatory mAbs. Interaction of Programmed Cell Death 1 (PD-1; CD279) with its ligands (PD-L1 or PD-L2, expressed on antigen-presenting cells and tumor cells) downregulates T-cell signaling (15,16). Interference with these interactions using mAbs to PD-1 or PD-L1 has proved effective in vitro (17) and in vivo, impairing cell-associated cross-presentation and the ability to produce IL12 in response to infectious challenge. The antitumor effects of immunostimulatory anti–PD-1 and anti–CD137 mAbs are contingent on an already-present baseline immune response, which is rescued and amplified by treatment (18). Based on the proposed role for BATF3-dependent DCs in immune surveillance (6), we hypothesized that the preexisting immune response rescued by the immunostimulatory mAbs might be mediated by BATF3-dependent cross-priming. Grafted MC38-derived tumors were lethal in C57BL/6 wild-type (WT) and BATF3-deficient mice, with slightly faster progression in Batf3−/− mice (Fig. 1A). In WT mice, tumor growth was delayed or curtailed by a course of treatment with anti–PD-1 or anti–CD137 mAbs, starting on day 4 after tumor cell inoculation. Combination treatment with both mAbs had a synergistic effect on their antitumor action (Fig. 1A and B), as previously reported in other tumor models (21). The antitumor efficacy of anti-CD137 and anti–PD-1 mAbs, used alone or in combination, was abolished in Batf3−/− mice (Fig. 1A and B), suggesting that BATF3-dependent DCs are responsible for the baseline immune response that is potentiated by immunostimulatory mAbs, as Batf3−/− mice only present some functional defects in CD8α+ resident DC or CD103+ migratory DC (6,7,12).

We explored whether the ability of BATF3-dependent DCs to specifically provide IL12 that boosts CTL function (8,13) could underlie the advantage of BATF3-dependent DCs to mediate basal antitumor response. We analyzed the ability of intratumorally injected IL12 to rescue the antitumor effect of systemic anti-CD137 mAb in the absence of BATF3. Repeat injections of recombinant IL12 in tumor lesions clearly potentiated the antitumor effects of systemic anti-CD137 mAb in WT mice, leading to rejection of most of the tumors (Fig. 1C). In stark contrast, no therapeutic effect was seen in identically treated Batf3−/− mice (Fig. 1C). Administration of IL12 is thus unable to compensate for the loss of a key function of BATF3-dependent DCs in the synergy with immunostimulatory anti-CD137 mAb.

**Impaired Ability of Batf3−/− DCs to Cross-Prime CTLs against Tumor Antigens**

To investigate the possible involvement of deficient cross-presentation in the nonresponsiveness of Batf3−/− mice to anti–PD-1 and anti–CD137 mAbs, we analyzed the ability of CD11c+ DCs to cross-present tumor-associated antigens to CD8+ T cells in vivo. For these experiments, we used MC38 cells transfected to express ovalbumin (OVA) as a surrogate tumor antigen (22). Two days after tumor-cell grafting, CD11c+ DCs from tumor-draining lymph nodes (LN) were magnetically sorted and cocultured at different ratios with OT-I OVA-specific CD8+ T cells. At all ratios tested, OT-I T cells cocultured with DCs from Batf3−/− mice produced markedly lower levels of intracellular and secreted IFNγ than cells cocultured with WT DCs (Fig. 2A and B), and also showed impaired proliferation (Fig. 2C), although there was some remaining cross-priming activity by Batf3−/− DCs.

To further investigate the DC subsets responsible for tumor cross-priming in WT and Batf3−/− mice, we FACSorted DC subsets from MC38-OVA tumor-draining LNs into resident CD11c+MHC-II+CD11b+ and CD11c+MHC-II+CD8α+ cells,
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Figure 1. Antitumor therapy with immunomodulatory mAbs is abrogated in Batf3−/− mice and is not rescued by IL12 administration. WT or Batf3−/− mice were s.c. inoculated with 5 × 10⁵ MC38 cells. A and B, mice were injected i.p. with 100 μg anti–PD-1 and anti-CD137 mAbs, alone or in combination (100 μg each), or with vehicle (untreated) on days 4, 7, and 10 after tumor cell inoculation. A, growth plots of individual tumors. B, overall survival charts show pooled results from 3 independent experiments with similar results. C, tumor-inoculated mice were injected i.p. with 100 μg anti-CD137 mAb on days 7, 10, and 13. The indicated groups of mice additionally received i.t. injections of recombinant mouse IL12 or saline on days 7, 9, and 11. IL12 was injected at 25 ng/dose into the tumor nodules. On the left, tumor area (mean ± SEM); on the right, overall survival. Fractions indicate the number of animals surviving at the end of the protocol. *, P < 0.05; **, P < 0.01; ***, P < 0.001.
Figure 2. Reduced ability of Batf3−/− DC to cross-prime CTLs against tumor antigens both in steady state and after treatment with anti-CD137 and anti-PD-1 mAbs. A–C, CD11c+ DCs from WT and Batf3−/− mice bearing MC38-OVA tumors were magnetically sorted from tumor-draining LNs and cocultured (see Methods) with purified naïve CD8+ OT-I TCR transgenic T cells over a range of DC:T cell ratios. A, left: representative flow cytometry dot plots of intracellular IFNγ staining in OT-I T cells cultured at a 1:4 DC:T cell ratio. Right: percentages of IFNγ-positive OT-I T cells at all ratios tested. B, IFNγ concentrations in the culture supernatants. C, percentages of proliferating OT-I cells by dilution of Cell Violet dye. D–F, WT and Batf3−/− mice grafted with MC38-OVA cells were treated with anti-CD137 (days 5 and 7) and tumor-draining LN analyzed on day 9 (see Methods). D, frequency of H-2Kb-OVA-tetramer+ cells among CD8+ T cells. E, intracellular IFNγ production induced by restimulation with OVA257–264 peptide in CD8+ T cells from tumor-draining LN. F, PD-1 surface staining on tumor-draining LN CD8+ T cells. G, frequency of PD-1+ lymphocytes among CD8+ TILs in mice treated as in D, H, WT and Batf3−/− mice grafted with MC38 cells were treated with anti-CD137 and anti-PD-1 mAbs on days 12 and 14, and tumor-infiltrating lymphocytes were analyzed on day 16 to detect CD8+ T lymphocytes specific for gp70 antigen (A–C) two-way and (D–H) one-way ANOVA with Bonferroni post-hoc test. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

We further analyzed the response against gp70, a well-described endogenous antigen in MC38 colon cancer cells (23). Notably, CD8+ TILs specific for gp70 were increased in a Batf3-dependent fashion upon anti-CD137 and anti-PD-1 mAb treatment, as detected by pentamer staining (Fig. 2H). A similar analysis of the response to the ADPGK-mutated neoantigen (24) showed some positive responses in WT but not Batf3-deficient mice (Supplementary Fig. S2A and S2B).

Priming of CD137+ PD-1− Antigen-Specific TILs by Activated Batf3-Dependent DCs

We hypothesized that expansion and activation of Batf3-dependent DCs with sFLT3L and the TL3 adjuvant poly-ICLC would synergize with immunostimulatory mAbs to enhance priming of tumor-specific CD8+ T cells. To extend our results to an alternative tumor model, we used B16-OVA melanoma cells grafted subcutaneously. Hydrodynamic injection of a plasmid expressing sFLT3L markedly promoted the expansion of cross-presenting DCs (Supplementary Fig. S3A). Intratumoral administration of poly-ICLC increased some activation markers including CD40 and PD-L1 in DCs from the spleen, tumor, and tumor-draining LNs, particularly in the TL3R-expressing CD103+ DCs (Supplementary Fig. S3B–S3D). Immunity to B16-OVA was estimated from the number of tumor-infiltrating lymphocytes among CD8+ T cells (Fig. 2E). Notably, priming of CD8+ T cells resulted in upregulation of surface PD-1 in CD8+ T cells at the tumor-draining LNs in WT mice, and this was impaired in Batf3−/− mice (Fig. 2F). Tumor-infiltrating lymphocytes (TILs) were basally activated and expressed high PD-1 levels that were not further increased by anti-CD137 treatment (Fig. 2G). However, TILs expressed much lower levels of PD-1 in Batf3−/− mice (Fig. 2G), which correlates with their reduced potential to respond to immunomodulatory mAb therapy. These results show that BATF3-dependent DCs are crucial for the priming and concomitant induction of targets for immunostimulatory mAbs by tumor-specific CD8+ T cells.
were paralleled by an increased frequency of CD137+CD8+ T cells in WT mice treated with sFLT3L and poly-ICLC and the impairment of this effect in Batf3−/− mice (Fig. 3B). Notably, antigen-specific TILs showed higher surface expression of PD-1 and CD137 compared with the bulk of CD8+ infiltrating T cells (Fig. 3C). These results show that expansion and activation of BATF3-dependent DCs increase the frequency of primed CD8+ T cells that upregulate markers of activation and exhaustion and are sensitive to immunostimulatory mAb treatment because of the expression of the targets for such agents.

**BATF3-Dependent DC Activation Enhances Antitumor Ability of Immunomodulatory mAbs**

We next sought to establish how FLT3L- and poly-ICLC-enhanced priming of CD8+ T cells affects the antitumor efficacy of anti-CD137 and anti-PD-1 mAbs. For this analysis, we used the B16-OVA model, which in our hands responds weakly or not at all to anti-PD-1 or anti-CD137 mAb treatment (Fig. 4A and B). Hydrodynamic injection of sFLT3L was concomitant with tumor inoculation, and intratumoral injection of poly-ICLC at day 7 was administered with or without anti-PD-1 or anti-CD137 mAbs at days 4, 7, and 10 after tumor inoculation. The triple combinations retarded tumor progression and significantly extended overall survival in WT mice (Fig. 4A and B) but had no significant effect in Batf3−/− mice (Fig. 4C and D). Furthermore, we found that quadruple combination immunotherapy encompassing sFLT3L + poly-ICLC + anti-CD137 + anti-PD-1 mAbs exerted marked antitumor effects against parental B16F10-derived melanomas (Supplementary Fig. S4A), while completely eradicating B16-OVA-derived tumors (Supplementary Fig. S4B). Functional enhancement of BATF3-dependent DCs thus cooperates synergistically with anti-CD137 and anti-PD-1 mAbs, indicating that baseline BATF3-dependent cross-priming is a key limiting factor that can be targeted to enhance antitumor immunity.

**DISCUSSION**

This study shows the immunodynamic interactions between professional cross-priming DCs and immunostimulatory mAbs that target CD137 and PD-1. The observations are fully consistent with an essential presentation of tumor antigens to CD8+ T cells by BATF3-dependent DCs. Both migratory CD103+ DCs and LN-resident CD8α+ DCs are functionally or ontogenically impaired in Batf3−/− mice (6, 7, 12), as they are also in Irf8−/− mice (12). Our results support a model in which at least one of these DC subsets is crucial for the basal antitumor response that is amplified by immunostimulatory mAbs.

BATF3-dependent DC subsets have been identified in the tumor environment, where they are functional and even have positive prognostic significance (12). These DCs are effective at taking up antigen from tumor cell debris for MHC class I cross-presentation. We find that these DCs mediate CTL priming at the malignant tissue or migrate via lymphatic afferent vessels to reach the draining LNs and meet naïve or...
central memory CD8+ T cells. These primed CTLs upregulate surface CD137 and PD-1, making them suitable targets for immunostimulatory mAbs. Our results show that expansion and activation of BATF3-dependent DCs result in increased antitumor priming and more effective tumor rejection in response to immunostimulatory mAbs. The dependency of anti-CD137 mAb treatment on DCs was suggested by the decreased efficacy of treatment upon depletion of CD11c cells (25). In the case of anti-PD-1 mAb, treatment synergizes with vaccines consisting of tumor cells transfected with GM-CSF or FLT3L, whose activity depends on attraction and differentiation of DC subsets (26).

Our data are consistent with the recent results from Gajewski and colleagues, elegantly showing that BATF3-dependent CD103+ DCs play an important role in regulating the infiltration of T cells in the tumor. Notably, intratumoral injection of cultured FLT3L-derived DCs rescues the response to anti-CTLA-4 and anti-PD-L1 immunomodulatory mAbs in terms of inducing antitumor CTLs and exerting antitumor activity (9). Previous studies from the same group had indicated a role for CD8α+ DCs in the baseline CTL response to a transplantable melanoma model (27).

CD103+ DCs were recently shown to be responsible not only for priming in the draining LNs, but also for IL12-dependent
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promotion of a productive CD8+ T-cell response locally in the tumor (12, 13), suggesting that expansion and activation of BATF3-dependent DCs might favor the generation of antitumor responses at several levels. Although professional cross-priming DCs have been characterized as key IL12 producers in infections and also in the tumor environment (8, 12, 13), we find that treatment of tumor-bearing mice with exogenous IL12 is unable to rescue a key BATF3-dependent function needed for synergy with immunostimulatory mAbs. Therefore, although IL12 production might be involved in the action of BATF3-dependent DCs, other functions of cross-priming DCs are absolutely needed. It is becoming apparent that effective anti–CTLA-4 or anti–PD-1 mAb therapy requires the presence of a measurable preexistent CTL response to the tumor mutatome epitopes in both humans and mice (28). It is now crucial to identify whether such responses are caused by direct presentation of antigens by tumor cells or by cross-priming of tumor cell-associated antigens in the tumor or in the tumor-draining LNs. Our data suggest that basal antitumor responses that are amplified by immunostimulatory mAbs have a critical requirement for professional cross-priming by DCs.

The need for cross-priming in the antitumor immune response also indicates possible relationships with mechanisms of immunogenic tumor cell death (10). Recent results show a crucial role for BATF3-dependent CD103+ DCs in priming a CTL response through IL12 production in the context of tumor cell death induced with paclitaxel (12, 13). However, doxorubicin-mediated immunogenicity against F244 sarcoma cells is BATF3-independent (10), and BATF3-deficient mice are able to reject tumors under conditions with exogenously provided IL12 (11). Therefore, the precise role of BATF3-dependent CD103+ DCs may depend on the context of the ongoing baseline immune response in the tumor, which will be eventually modulated by the treatment with immunostimulatory mAbs.

Each addition to our knowledge in this area of tumor antigen cross-priming has the potential to provide predictive biomarkers for the efficacy of immunostimulatory mAbs, because cross-priming against tumor neoantigens seems to be a key determinant of the variable efficacy of these treatments in mice and humans (1, 12, 28). Moreover, more effective vaccines could be prepared by immune sorting or targeting these cross-priming DC populations or their differentiation in culture from precursors (29).

Overall, our results raise important pointers for improving therapy with immunostimulatory mAbs. The cross-priming function of DCs is essential for the therapeutic effect of immunostimulatory mAbs, but the baseline CTL-priming function is suboptimal. These observations suggest the potential to devise exogenous or in situ tumor vaccination therapies to enhance cross-priming of tumor antigens and thereby increase the efficacy of immunostimulatory mAbs.

METHODS

Mice

Mice were bred at the Centro Nacional de Investigaciones Cardiovasculares Carlos III (CINIC) and the Center for Applied Medical Research (CIMA), University of Navarra, in specific pathogen-free conditions. Batf3+/− on C57BL/6 background (kindly provided by Dr. Kenneth M. Murphy, Washington University, St. Louis, MO) were further back-crossed with C57BL/6 mice at the CNIC to establish WT and Batf3−/− cousin colonies from the heterozygotes. Animal studies (protocol approval 150/12) were approved by the local ethics committee. All animal procedures conformed to EU Directive 2010/63/EU and Recommendation 2007/526/EC regarding the protection of animals used for experimental and other scientific purposes, enforced in Spanish law under Real Decreto 1201/2005.

Cell Lines, Culture Conditions, and Tissue Processing

MC38, MC38-OVA, B16F10, and B16-OVA cells were cultured in RPMI medium (Gibco) supplemented with 10% decomplemented and filtered PBS (Sigma Aldrich) containing 50 μmol/L β-2-mercaptoethanol, 100 U/mL streptomycin (all from Gibco). MC38 cells were provided by Dr. Karl E. Hellström (University of Washington, Seattle, WA) in September 1998. B16F10 cells were purchased from the ATCC in June 2006. B16-OVA cells were a kind gift from Dr. Lieping Chen (Yale University, New Haven, CT) in November 2001. These cell lines were authenticated by Idexx Radil (Case 6592-2012) in February 2012. MC38-OVA–transfected cells were kindly provided by Dr. Cornelis Melief (Leiden University Medical Center, the Netherlands) in November 2013 and were further verified. All cell lines were cultured at 37°C with 5% CO2. Isolated LNs were incubated in collagenase/DNase for 15 minutes at 37°C, followed by mechanical disaggregation using frosted slides. Single-cell suspensions were then stained for flow cytometry.

Flow Cytometry

Acquisition was performed using a FACS Canto II flow cytometer (BD Biosciences). The antibodies used included FITC-conjugated αPD-1 (29F.1A12), and εC404 (3/23); PE-conjugated εCD11b (M1/70), εCD137 (17B), and ofFlt3 (XMG1.2); PrPCy5.5-conjugated εCD103 (E7) and εCD11c (N418); APC-conjugated εCD11b (M1/70), εPD-L1 (1F7,9G2), εCD8 (53-6.7), and ofεK1 (ZE7); BV6 (εCD89) and εCD4 (5 μg/mL); and ofε45 (εCD4) (RM4-5). For identification of epitope-specific T cells, phycoerythrin– or Alexa Fluor 647–conjugated H-2Kb–OVA 257–264 tetramer (MBL and NIH Tetramer Facility), H-2Dd–ASMTNMELM dextramer (Immudex), or H-2 Dk–ASMTNMELM dextramer (ADPGK, Immudex) were used. For intracellular staining, cells were fixed and permeabilized using Cytofix/Cytoperm buffer and then incubated with fluorochrome-conjugated antibodies in PermWash buffer (BD Biosciences).

In Vivo Tumor Experiments

Cultured tumor cells were trypsinized before reaching confluence and suspended in PBS. Unless specified otherwise, 5 × 106 cells in 50 μL PBS were used for inoculation. Cells were injected s.c. using 29G syringes into the shaved right flank of 8- to 12-week-old C57BL/6 Batf3−/− and WT mice. Tumor size was measured twice weekly and calculated as the product of orthogonal diameters. Anti–CD137 (1D8) antibody was produced as described (19). Anti–PD-1 (RMP1-14) antibody was purchased from BioXcell. Antibodies (100 μg) were administered i.p. in PBS on days 4, 7, and 10 after tumor inoculation. Reombinant mouse IL12 (25 ng/dose; Milenyi) was administered intratumorally (i.t.) on days 7, 9, and 11. In experiments involving injection of IL12, anti–CD137 was administered on days 7, 10, and 13. For in vivo DC expansion, 10 μg of sFLTL3–coding plasmid (pUMVC3–mFLex, Aldevron) or a control empty plasmid were injected i.v. to achieve hydrodynamic liver gene transfer. For in vivo stimulation of DCs, 100 μg poly-ICLC (Hiltonol; Oncovir) were injected i.t. on day 7 or when tumors reached 25 to 50 mm3. PBS was injected as control.

Ex Vivo Cross-Presentation of Surrogate Tumor Antigen

To test the ex vivo cross-presentation capacity of LN DCs, sFLTL3 plasmid-injected mice were bilaterally inoculated s.c. with 2 × 106
MC38-OVA cells. LNs were extracted 48 hours later. CD11c+ cells were magnetically sorted with CD11c microbeads in an AutoMACS Pro Separator (Miltenyi) and further FACSorted where indicated. OT-I CD8+ T lymphocytes were magnetically sorted from the spleens of C57BL/6 mice using CD8 microbeads (Miltenyi). Cell Violet-labeled (Thermo Fisher) OT-I lymphocytes were cocultured with OVA-reactive T cells were restimulated ex vivo with 1 μg/ml SIINFEKL peptide-pulsed DCs served as positive controls. After 72 hours, culture supernatants were collected, and OVA-reactive T cells were restimulated ex vivo with 1 μg/ml SIINFEKL peptide for 5 hours, with Brefeldin A (10 μg/ml; Sigma-Aldrich) added for the last 4 hours. Cells were then stained for membrane markers before being fixed and permeabilized for staining of intracellular IFNγ. Secreted IFNγ was measured in culture supernatants with the BD Biosciences OptEIA Mouse IFNγ ELISA Kit.

**Analysis of T-cell Priming by Tumor Antigens**

WT and Batf3−/− mice were inoculated s.c. with 2 × 10^6 MC38-OVA cells. Mice were injected i.p. with 100 μg anti-CD137 or an isotype control at days 5 and 7 after tumor inoculation. LNs and tumors were extracted at day 7. LNs were incubated at 37°C in Liberase TL (Roche; 20 minutes) and tumors in Liberase TL/DNase I (30 minutes). Then both LN and tumors were dissociated with a 70-μm cell strainer (Fisher Scientific). Single-cell suspensions were stained and analyzed by flow cytometry.

**Statistical Analysis**

Tumor growth data were analyzed with Prism software (GraphPad Software, Inc.). Mean diameters of tumors over time were fitted using the formula γ = A x e^δt/(1 + e^δ(t-t0)), where t represents time, A the maximum size reached by the tumor, and B its growth rate. Treatments were compared using the extra sum-of-squares F test. Tumor survival was compared with log-rank (Mantel–Cox) tests. All other analyses among groups were performed as described in figure legends.

**Disclosure of Potential Conflicts of Interest**

M. Jure-Kunkel has ownership interest (including patents) in Bristol-Myers Squibb. I. Melero reports receiving commercial research grants from Bristol-Myers Squibb and Pfizer and is a consultant/advisory board member for AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, and Roche-Genentech. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.R. Sánchez-Paulete, F.J. Cueto, M.E. Rodriguez-Ruiz, M. Jure-Kunkel

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**References**

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