Focusing and sustaining the antitumor CTL effector killer response by agonist anti-CD137 mAb

Bettina Weigel^{a,1}, Elixabet Bolaños^{b,1}, Alvaro Teixeira^b, Ivan Martinez-Forero^b, Sara Labiano^b, Arantza Azpilikueta^b, Aizea Morales-Kastresana^b, José I. Quetglas^b, Esther Wagena^a, Alfonso Rodríguez Sánchez-Paulete^b, Lieping Chen^c, Peter Friedl^{a,2}, and Ignacio Melero^{b,2,3}

^aDepartment of Cell Biology, Radboud University Nijmegen Medical Center, 6525 GA Nijmegen, The Netherlands; ^bCentro de Investigación Médica Aplicada (CIMA) and Clínica Universidad de Navarra, University of Navarra, 31008 Pamplona, Spain; and ^cDepartment of Immunobiology, Yale University School of Medicine, New Haven, CT 06511

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Cancer immunotherapy is undergoing significant progress due to recent clinical successes by refined adoptive T-cell transfer and immunostimulatory monoclonal Ab (mAbs). B16F10-derived OVA-expressing mouse melanomas resist curative immunotherapy with either adoptive transfer of activated anti-OVA OT1 CTLs or agonist anti-CD137 (4-1BB) mAb. However, when acting in synergistic combination, these treatments consistently achieve tumor eradication. Tumor-infiltrating lymphocytes that accomplish tumor rejection exhibit enhanced effector functions in both transferred OT-1 and endogenous cytotoxic T lymphocytes (CTLs). This is consistent with higher levels of expression of eomesodermin in transferred and endogenous CTLs and with intravital live-cell two-photon microscopy evidence for more efficacious CTL-mediated tumor cell killing. Anti-CD137 mAb treatment resulted in prolonged intratumor persistence of the OT1 CTL-effector cells and improved function with focused and confined interaction kinetics of OT-1 CTL with target cells and increased apoptosis induction lasting up to six days post-adoptive transfer. The synergy of adoptive T-cell therapy and agonist anti-CD137 mAb thus results from in vivo enhancement and sustainment of effector functions.

CD137 | Cytotoxic T lymphocyte | adoptive T-cell therapy | immunotherapy | costimulation

Adoptive T-cell therapy is being developed following different approaches including infusion of expanded tumor-infiltrating lymphocytes to preconditioned lympho-depleted hosts (1) and adoptive transfer of T cells genetically engineered to express tumor-specific T-cell receptors or chimeric antigen receptors (CARs) (2). The dazzling clinical success of CARs against leukemias (3, 4) is related to the fact that these chimeric receptors intracellularly include both signaling elements of the CD3-TCR (CD3 ζ) and of costimulatory molecules (3). The intracellular costimulatory signaling domain with best reported effects so far is that of CD137 (4-1BB) (5).

CD137 (4-1BB) is a TNFR family costimulatory receptor (TNFRSF9) that is expressed on activated T (6) and NK cells (7) and mediates costimulation of both types of lymphocytes (8). On CD8⁺ T cells ex vivo, CD137 ligation with the agonist antibodies determines increased proliferation, survival, memory formation and stronger effector functions in terms of both cytotoxicity and cytokine production (9). In vivo, anti-CD137 mAb protects adoptively transferred CTLs from activation-induced cell death resulting in better antitumor efficacy in a mouse myeloma model (10). Significant therapeutic effects against transplanted tumor models (11) have provided the rationale for currently ongoing phase I and II clinical trials (NCT01471210; NCT01775631; NCT01307267) (8).

NK cells up-regulate CD137 and ligation by anti-CD137 mAb enhances NK-mediated antibody-dependent cellular cytotoxicity functions resulting in synergistic effects with anti-CD20 (12), anti-HER2 (13) and anti-EGFR (14) mAb. CD137 expression can also be induced on dendritic cells (15), tumor endothelial cells (16), B cells (17), and myeloid leukocytes (18) upon activation.

Although positive effects of CD137 ligation for CD8⁺ T-cell memory generation are well explored (9, 19), its relevance for enhancing effector function in solid tumor lesions in vivo has not been established. In this study we show a synergy of adoptively transferred and endogenous CD8⁺ T cells against B16F10 melanoma that depends on the ability of both CTL populations to receive costimulation via CD137. Flow-cytometry of tumor-rejecting lymphocyte infiltrates and intravital microscopy of tumors provide evidence that anti-CD137 mAb therapy sustained the efficacy of more focused anti-tumor CTLs.

Results

B16F10-OVA Tumors Are Amenable to Eradication by Combined Adoptive Transfer of OT-1 CTLs and CD137 Costimulation. The B16F10-OVA cell line is a stable transfectant derived from B16F10 melanoma that expresses chicken ovalbumin (20). In our hands, it is a difficult-to-treat transplantable tumor model, at least by means of immunotherapy (21). Adoptive transfer of 2×10^6 cognate peptide-activated OT-1 cells to mice s.c. inoculated with 5×10^5 tumor cells and given no antibody or control antibody showed rapid exponential tumor growth (Fig. 1A). Treatment on day 3 with 100 μ g of the agonist anti-CD137 mAb 1D8 (22) showed no therapeutic impact in this model (Fig. 1B). Likewise, infusion of

Significance

Immunotherapy of cancer with immunomodulatory agents is achieving significant efficacy in an important fraction of patients. The stimulatory inducible receptor of T and NK lymphocytes known as CD137 or 4-1BB is being stimulated with agonist antibodies to enhance antitumor immunity in clinical trials. In addition, the intracellular signaling domain of CD137 is crucial as a component of successful anti-leukemia therapies with chimeric antigen receptors transduced into adoptively transferred T lymphocytes. In this study the marked synergistic effects of adoptive T cell and agonist anti-CD137 mAb therapies are studied, providing in vivo evidence for improved, more sustained and focused tumoricidal functions of antitumor cytotoxic T lymphocytes when under the influence of CD137-targeted pharmacological stimulation with immunostimulatory monoclonal antibodies.

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¹B.W. and E.B. contributed equally to this work.

²P.F. and I.M. contributed equally to this work.

³To whom correspondence should be addressed. Email: imelero@unav.es.

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Fig. 1. Synergistic combination of adoptive T-cell therapy and anti-CD137 mAb requires CD137 expression on both transferred OT-1 and endogenous CD8⁺ T lymphocytes. Mice were s.c. inoculated with 5×10^5 B16F10-OVA melanoma cells on day 0. Graphs represent individual follow-up of tumor size and the fraction of tumor-free mice at the end of the experiment. (A) Mice were left untreated or treated on day +3 with rat IgG or cognate peptide-activated OT-1 lymphocytes. (B) Mice were adoptively transferred with 2×10^6 polyclonally activated T cells from non-TCR-transgenic CD45.1 congenic mice and anti-CD137 mAb (1D8) on day +3. (C) Mice were adoptively transferred with activated OT-1 CD8⁺ T lymphocytes and 100 μ g of anti-CD137 mAb on day +3. (D) Mice were treated as in C but treatment was postponed to day +7 following tumor cell inoculation. (E) Mice were treated as in C but starting one day before treatment (day +2) mice received a depleting course of anti-CD4 mAb that was dosed every 5 d for up to four doses. (F) Mice were treated as in C, but OT-1 cells were harvested from OT-1 CD137-deficient mice. (G) B16F10-OVA-bearing CD137^{-/-} mice were treated as in C with CD137-sufficient OT-1 lymphocytes. (H) Tumor-bearing recipient mice were RAG1^{-/-} and were treated as in C. Experiments were repeated at least twice rendering comparable results with at least six animals per group.

polyclonal activated CD8⁺ T cells showed no efficacy, indicating that OVA-specificity was an absolute requirement (Fig. 1B). In striking contrast, combined treatment with activated OT1 CTL and anti-CD137 mAb accomplished complete regression of all tumors when treatment was instigated 3 days following tumor cell inoculation (Fig. 1C). However, if combination immunotherapy was delayed until day +7, no therapeutic effects were retained, and mice developed lethal tumors that progressed consistently, albeit with a slight delay (Fig. 1D) compared with controls (Fig. 1A). A single dose of 1D8 anti-CD137 did not alter hepatic function parameters but increased the content of liver CD8⁺ T, NKT and NK cells (Fig. S1).

CD4⁺ T-cell depletion experiments with a monoclonal antibody ruled out a role for CD4⁺ T cells in this therapeutic setting, because tumors in mice depleted of CD4⁺ T cells were rejected as in nondepleted controls (Fig. 1E). NK1.1⁺ NK lymphocytes were also dispensable because complete depletion did not hinder the therapeutic outcome of treatment with 1D8 and 2A, two different anti-CD137 mAb (Fig. S2).

Mice cured with the combined therapy of anti-CD137 mAb 1D8 and adoptive OT1 transfer were able to reject an inoculum of B16F10-OVA given at day 90 posttreatment (Fig. S3, Left), showing effective memory state. OVA-negative B16F10 tumors progressed in all mice, irrespective of prior treatments, even though mice that had undergone CD4⁺ T-cell depletion experienced faster tumor progression (Fig. S3, Right).

Combined Adoptive T-Cell Therapy and Anti-CD137 mAb Require CD137 Expression on Transferred and Endogenous T Cells. To study which lymphocyte subsets needed to express CD137 for efficacy, we performed experiments using CD137^{-/-} syngenic mice as tumor recipients and CD8⁺ T cells from double transgenic OT-1 CD137^{-/-} mice. Mice treated with peptide-activated CD137^{-/-} OT-1 lymphocytes did not show activity upon combined treatment with anti-CD137 mAb, exhibiting only a transient delay in progression

(Fig. 1F). Surprisingly, if recipient mice were CD137^{-/-} and treated with CD137-sufficient OT-1 cells, tumors also progressed in every case after a brief period of transient tumor control (Fig. 1G).

To address whether endogenous T cells were indispensable, we performed similar experiments in RAG1^{-/-} deficient mice that lack T and B lymphocytes. Results in Fig. 1H indicate that dual-treatment with OT-1 cells and anti-CD137 mAb transiently controlled tumor growth even though all tumor lesions progressed after week three. Collectively, our results indicate that expression CD137 both on adoptively transferred T cells and on endogenous CD8⁺ T cells is mandatory to achieve complete tumor eradication upon combined immunotherapy.

Combined Therapy Results in Tumor Infiltrating CTLs with an Enhanced Effector Phenotype. To understand the mechanisms behind the therapeutic synergistic effects, we studied the CD8⁺ T lymphocytes present in the tumors on day 10 when the lesions start to shrink in size. Our first hypothesis was that a higher number of adoptively transferred T lymphocytes infiltrated the tumor lesion thus numerically explaining the synergistic effects. We performed quantitative experiments using WT or CD137^{-/-} mice as recipients and either CD137-sufficient or CD137^{-/-} OT1 cells. Adoptively transferred OT-1 T cells were CD45.1 in these experiments, which allowed their tracing and discrimination from the endogenous CD45.2 CD8⁺ T cells. Surprisingly, we observed that anti-CD137 mAb treatment did not increase the number of OT-1 T cells within the tumors in both wild-type and CD137^{-/-} recipient mice (Fig. 2A and B). However, when CD137^{-/-} OT-1 were used, virtually none of these cells were present in the infiltrate (Fig. 2A). Dot plots in Fig. 2C provide a reference at a glance of the relative abundance of transferred (CD45.1⁺) and endogenous (CD45.2⁺) CD8⁺ T lymphocytes in the different experimental groups. When treatment was given on day +7, absolute OT1 CTL numbers in the tumor increased but normalization by tumor weight was consistent with decreased OT-1 CTL density (Fig. S4A and B).

Increased expression of VCAM on tumor endothelial cells induced by 1D8 treatment of B16F10-OVA tumors growing in RAG^{-/-} T-cell-deficient mice indicated an inflammatory phenotype induced by direct effects on endothelial cells (16). However, combined treatment did not alter transcription of CTL-attracting chemokines in WT mice compared with mice treated with OT-1 and control antibody (Fig. S5).

Thus, rather than a mere numeric increase, these data implicate altered CTL function as the basis for improved therapeutic outcome.

CD107a (Lamp-1) is a cytotoxic granule protein that reaches the plasma membrane when CTLs degranulate on target cells. Surface CD107a was increased after treatment with OT-1 and anti-CD137, compared with treatment with OT-1 and control antibody (Fig. 3A) in both transferred OT-1 CTL and, importantly, endogenous CD8⁺ T cells of unknown antigen specificity. Levels of surface CD107a on transferred cells were reduced when the recipient mouse was CD137^{-/-} but undiminished on transferred CD137^{-/-} OT-1 lymphocytes in WT mice.

A similar picture emerged when surface KLRG1 was used as effector T-cell marker (Fig. 3B) and when immunostaining for intracellular IFN γ was carried out (Fig. 3C). Similarly, intracellular IFN γ expression in transferred OT1 and endogenous CTL tended to decrease when either transferred OT-1 cells or the recipient mouse were CD137 $^{-/-}$, suggesting cooperation between exogenous and endogenous lymphocyte subsets. When CTLs were restimulated in vitro with SIINKFEL IFN γ levels were increased in OT-1 TILs treated with 1D8 compared with control antibody, whereas endogenous TILs did not respond to the immunodominant OVA epitope (Fig. S6). Tumor draining lymph nodes showed similar but less obvious changes of CTL effector markers (Fig. S7). Moreover, transferred OT-1 CTLs expressed PD-1 and TIM-3 but anti-CD137 treatment did not modify their level of expression, whereas anti-CD137 enhanced the expression on endogenous CD8 $^{+}$ CTLs (Fig. S8). Of note,

tumors treated by the OT-1 + 1D8 combination tended to contain more CD4⁺ Foxp3⁺ Treg cells.

An interplay of transcription factors is involved in regulating the lymphocyte effector phenotype (23). Previous studies revealed a relation of eomesodermin and CD137 costimulation (24, 25). Eomes is involved in both favoring the expression of effector molecules (26) and favoring differentiation to memory T lymphocytes (27, 28).

We stained for intracellular expression of EOMES in tumor infiltrating CD8⁺ T cells. Combined treatment induced higher levels of intracellular EOMES (Fig. 4A). This was much more evident among endogenous TILs than transferred OT-1 CTLs (Fig. 4A). More importantly, the induction of EOMES critically required the ability to express CD137 in the recipient mouse but not in the transferred OT-1 cells. Thus, transcriptional regulation of eomesodermin satisfactorily explains a more robust effector phenotype. Further evidence for a more pronounced effector phenotype was attained by detecting multiple CTL effector markers by quantitative RT-PCR on whole tumor mRNA comparing the effect of OT-1 + 1D8 over OT-1 + control antibody treatments, including increased expression of EOMES, Granzyme B, perforin, FAS-L, BLIMP-1, and CXCR3 (Fig. S5). Conversely, the homologous and functionally interrelated T-bet

Fig. 4. EOMES is induced in transferred and endogenous intratumor CTLs by anti-CD137 mAb treatment. FACS analyses experiments as in Fig. 3 with intracellular staining for the transcription factors EOMES (A) and T-bet (B) in CD8⁺ TILs from the indicated experimental groups. In each graph, the adoptively transferred cells, the recipient mouse, and the antibody treatment are provided in the horizontal axis [WT: wild type; CD137KO: CD137^{-/-}]. Results in A are from two pooled experiments performed identically. Statistical differences were assessed with Mann-Whitney *u* test. n.s., not significant, *P ≤ 0.01.

transcription factor was not induced by anti-CD137 mAb treatment, although the CD137 sufficiency of the receptor mouse favored higher T-bet expression in adoptively transferred and endogenous CD8⁺ T cells (Fig. 4B and Fig. S5). Despite a similar induction of effector markers (including TIM-3 and PD-1), tumors surpassed immune control when treatment start was delayed until day +7 after tumor inoculation (Fig. S4B).

Evidence for More Effective CTL Activity in the Microenvironment of B16F10-OVA Tumors Upon Combined Immunotherapy. To address whether anti-CD137 mAb therapy enhances local antitumor CTL efficacy, frozen tumor sections were stained for CD8 and cleaved Caspase-3 to identify apoptotic cells. Tumors undergoing combined treatment revealed an increase of apoptotic tumor cells (Fig. S9) together with an increased total number and relative ratio of CTLs in direct contact with caspase-3-positive, dead, or dying tumor cells (Fig. S9A–C). Thus, combined treatment increases tumoricidal events between CTL and tumor cells. Both CTL-tumor cell conjugates and cytotoxic efficacy observed upon combined treatment were partially reduced when recipient mice were CD137^{-/-} deficient (Fig. S9C and D). Analysis of OT-1 CD137^{-/-} lymphocytes did not permit relevant observations due to the paucity of such T cells in the tumors.

Anti-CD137 mAb Therapy Prolongs in Vivo Efficacy of CTL Effector Function. To dissect how anti-CD137 therapy improves CTL effector function and tumor growth control or regression, the intratumor migration, interactions, and viability of adoptively transferred dsRed2, OT1 CTL were directly monitored by intravital multiphoton microscopy in B16F10-OVA tumors expressing histone-2B/mCherry as readout for mitosis, apoptosis, or necrosis (Fig. S10A). In control mice, adoptively transferred OT1 CTL induced a transient growth delay with exponential regrowth of the tumor thereafter, whereas combined therapy of OT1 transfer + anti-CD137 mAb resulted in a significantly increased suppression of tumor growth (Fig. S10A and B).

To directly address whether individual CTL show enhanced effector function, we quantified CTL effector dynamics by long-term 4D time-lapse microscopy. Migrating OT1 CTL efficiently infiltrated the tumor margin with decreasing CTL densities towards the tumor core and with reduced migration speed of $2 \pm 1.7 \mu\text{m}/\text{min}$ in OVA expressing tumors compared with B16F10 parental tumors ($4 \pm 2.8 \mu\text{m}/\text{min}$) and increased confinement of migration (Fig. 5A and B). This interaction pattern explains cognate antigen recognition and active effector function (29). When combined with anti-CD137 mAb, tumor infiltrating CTL showed an even more focused effector phenotype, defined by further reduced migration speed ($1.5 \pm 1.2 \mu\text{m}/\text{min}$) during interactions with tumor cells resulting in prolonged dwell time per focus (Fig. 5A–D and Movie S1). Quantification of OT1 CTL–tumor cell interactions and outcome showed that 75% of tumor cell apoptosis were directly preceded by an OT1 contact, indicating cell-contact dependent cytotoxicity as major mechanism of apoptosis induction (Fig. 6A and Movies S2 and S3). Combined treatment of OT1 transfer and anti-CD137 mAb resulted in mildly enhanced frequency but substantially prolonged effector window of apoptosis induction by OT1 CTL (Fig. 6B). Concurrently, tumor cell proliferation was impaired (Fig. 6B), but OT1 CTL proliferation increased and apoptosis rates decreased, consistent with transiently increased CTL densities in

Fig. 5. Intravital microscopy shows focused effector dynamics of adoptively transferred OT1 CTL. (A) OT1 CTL migration dynamics and pattern. Heat map of CTL dwell time shows confined CTL migration tracks (arrow heads) in CD137 cotreated tumors. (B) OT1 CTL migration speed in parental, OVA expressing and anti-CD137 mAb treated tumors. (C) The mean square displacement relates to the area covered by the migrating cells at increasing time intervals. For each time interval Δt , the mean squared displacement is plotted for tracks of OT1 CTL with and without anti-CD137 treatment. Significance test, two-way ANOVA. (D) Cumulative OT1 CTL–tumor cell interactions. Quantifications are based on at least three independent experiments with at least 180 CTL tracks of 75 min length analyzed per condition. Black lines indicate the median. Statistical differences were assessed using the Mann-Whitney *u* test; ***P ≤ 0.0001. (Scale bars, 50 μm .)

Fig. 6. Intravital microscopy shows improved CTL viability and sustained effector function of adoptively transferred OT-1 T cells. (A) Percentage of tumor cell apoptosis events preceded by OT1 CTL contacts. Error bars, SD. (B) Image sequence of B16F10/OVA apoptosis and mitosis visualized by H2B-mCherry. Arrowheads point to nuclear fragmentation and mitotic nuclei, respectively. Dot plots: Quantification of tumor cell apoptosis and mitosis rates per hour. (C) OT1 CTL numbers in the tumor. Error bars, SD. (D) Image sequence of CTL mitosis and apoptosis in the tumor. Arrowheads indicate apoptotic fragmentation. Dot plots: Quantification of OT1 CTL apoptosis and mitosis rates per hour. Data were obtained from time lapse recordings of $350 \times 350 \times 100 \mu\text{m}$ stacks scanned at 2-min frame rate and recorded at ≥ 3 independent positions per tumor. $n = 3$ independent tumors with total observation times of ≥ 20 h per condition and time point. Statistical differences were assessed using the Mann-Whitney u test; n.s., not significant, $*P \leq 0.01$, $**P \leq 0.001$, $***P \leq 0.0001$.

the tumor under combined anti-CD137 mAb treatment (Fig. 6D). Thus, combining adoptive CD8⁺ T-cell therapy with anti-CD137 mAb induces a local effector phenotype with prolonged and more focused cytotoxic activity against tumor cells.

Discussion

In this study we observed effective synergism of CTL infusion combined with anti-CD137 mAb treatment, resulting in complete regression of s.c. and efficient growth control of intradermal B16F10-OVA melanoma tumors in a CD4 and NK cell independent fashion. However, when treatment is postponed to day +7, the therapy loses efficacy probably as a result of immunosuppressive mechanisms deployed by the larger tumor (30) and because the rapidly increasing tumor cell burden overwhelms the immunotherapeutic regimen despite transient increases in CTL tumor infiltration.

The synergistic effects between adoptive CTL transfer and antibody-induced enhanced CD137 signaling could operate and be exploited at multiple levels: (i) CD137 ligation is known to promote proliferation (6) and prevent activation induced cell death (31) in T cells. (ii) CD137 is expressed on the endothelial cells of tumor vessels which, when stimulated in this location, enhances T-cell trafficking into tumors (16). In our case this mechanism appears dispensable for recruiting adoptively transferred T cells into tumors, but we observe higher numbers of endogenous CD8⁺ T cells, that are lost if the recipient mouse is CD137^{-/-}, indicating an effect of anti-CD137 therapy on endothelial cells and/or on the tumor homing capability of endogenous CD8⁺ T lymphocytes. Of note, endogenous CD8⁺ T cells outnumbered by about 8- to 10-fold those adoptively transferred and their function is crucial to sustain the complete response over time, as found in experiments with recipient RAG1^{-/-} mice deficient in T cells.

Rather than increasing the peak activity in the lesion, here we show that exposure to the CD137 agonist results in prolonged CTL-mediated cytotoxicity as the basis for higher efficacy at killing tumor cells. These phenotypic changes were dependent on

the ability of CD137 to be expressed both on endogenous cells of the recipient mouse and on adoptively transferred OT1 cells. This finding is consistent with the *in vivo* treatment efficacy that required the expression of CD137 on both endogenous and adoptively transferred cells. Our results are compatible with those on targeting hematologic malignancy using bone marrow chimeras (32) which showed that endogenous T cells were required for the efficacy of transferred memory-like CD8⁺ T cells and anti-CD137 mAb combined treatment against experimental immunogenic EG7 lymphomas. In this case of high CTL efficacy, the ability to express CD137 on endogenous or adoptively transferred cells was mutually dispensable, being required only in one of the subsets. The apparent discrepancy with our results indicates the different degree of immunogenicity of EG7-OVA lymphomas compared with the B16F10-OVA melanomas and, possibly, different efficacy of reactivated memory-like OT-1 lymphocytes (32) versus the recently activated OT-1 used here.

Our observations using the very aggressive B16F10-OVA melanoma which shows poor immunogenicity toward endogenous effector cells strongly advocate for translational research of this immunotherapy combination. Indeed, we have recently reported synergy against spontaneous liver cancer expressing transgenic OVA of a combination of anti-OVA OT-1 + OT-2 T cells in conjunction with a combination of immunostimulatory mAb (33).

Our work is also consistent with previous reports on the critical role of EOMES as a transcription factor in the effects of CD137 costimulation (24, 25). EOMES up-regulates transcription of molecules that CTLs use for killing (26), and likely supports the overall enhanced performance of transferred and endogenous CTLs. However, the transcriptional and epigenetic control networks that would mediate the effects of CD137 costimulation are likely more complex (34) involving an interplay of transcription factors regulating CTL differentiation.

Live-cell imaging of tumors undergoing rejection clearly showed that CTLs were associated with more tumor cell death that was correlated with more frequent and prolonged interaction of CTLs

with target cells. The perforin-granzyme machinery represents the predominant tumor-killer effector mechanism upon anti-CD137 mAb treatment (35), which is consistent with more focused behavior of the OT-1 lymphocytes inside the tumor and strongly suggests enhanced stringency of CTL engagement with target cells, degranulation, and apoptosis induction.

Previous intravital imaging studies indicated that long-lasting CTL:tumor cell interactions support tumor cell killing (36) and tumor immunotherapy models show that CTLs initiate relevant tumor regression few days after adoptive transfer. Besides initial activation, CTL are required to maintain a sustained effector phenotype within the tumor microenvironment (37) for prolonged time periods to efficiently eradicate tumors. Our data indicate that CD137 mAb therapy, besides directly stimulating cytotoxic function, prolongs the CTL effector phase and viability in the local tumor microenvironment, which sustains local cytotoxicity.

The combination of adoptive T-cell therapy and anti-CD137 mAb is likely to involve various application possibilities. First, CD137 expression can be used for immunomagnetic selection of tumor reactive TILs (38), and subsequent culture of T cells in the presence of CD137 renders more efficacious phenotypic features (39, 40). Second, the effector performance (11), survival (10), and memory differentiation (41) can be enhanced by coadministration of the agonist antibody in vivo. Lastly, as shown here, anti-CD137

infusion may enhance adoptive immunotherapy by CTL by a dual mechanism, enhancing endogenous effector functions as well as focusing and prolonging the efficacy of therapeutically transferred lymphocytes.

Materials and Methods

See SI Materials and Methods. In brief, experimental designs were based on adoptive transfer of OT-1 TCR transgenic T cells. As needed, OT-1 donor mice were crossed to become CD137^{-/-} or double transgenic for green or red fluorescent proteins. Adoptively T-cell transferred mice were dosed anti-CD137 agonist antibodies (1D8 or 2A) or control antibodies to subsequently follow up tumor size, retrieve intratumoral lymphocytes for flow cytometry evaluation or to perform imaging experiments by intravital microscopy of tumors implanted in dorsal skin-fold chambers, using a customized multiphoton microscope (TriMScope-II, LaVision BioTec).

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