Armed Oncolytic Virus Enhances Immune Functions of Chimeric Antigen Receptor–Modified T Cells in Solid Tumors

Nobuhiro Nishio1, Iulia Diaconu1, Hao Liu2, Vincenzo Cerullo3, Ignazio Caruana1, Valentina Hoyos1, Lisa Boucher-Hayes4, Barbara Savoldo1,4, and Gianpietro Dotti1,5,6

Abstract

The clinical efficacy of chimeric antigen receptor (CAR)-redirected T cells remains marginal in solid tumors compared with leukemias. Failures have been attributed to insufficient T-cell migration and to the highly immunosuppressive milieu of solid tumors. To overcome these obstacles, we have combined CAR-T cells with an oncolytic virus armed with the chemokine RANTES and the cytokine IL15, reasoning that the modified oncolytic virus will both have a direct lytic effect on infected malignant cells and facilitate migration and survival of CAR-T cells. Using neuroblastoma as a tumor model, we found that the adenovirus Ad5Δ24 exerted a potent, dose-dependent, cytotoxic effect on tumor cells, whereas CAR-T cells specific for the tumor antigen GD2 (GD2.CAR-T cells) were not damaged. When used in combination, Ad5Δ24 directly accelerated the caspase pathways in tumor cells exposed to CAR-T cells, whereas the intratumoral release of both RANTES and IL15 attracted CAR-T cells and promoted their local survival, respectively, increasing the overall survival of tumor-bearing mice. These preclinical data support the use of this innovative biologic platform of immunotherapy for solid tumors. Cancer Res; 74(18); 5195–205. ©2014 AACR.

Introduction

Adoptive transfer of T cells genetically modified to express a CD19-specific chimeric antigen receptor (CAR) coupled with costimulatory endodomains has shown significant clinical impact in lymphoid leukemias (1–3). In contrast, the clinical relevance of this approach has been more limited for solid tumors (4–6).

Insufficient migration of CAR-T cells to the tumor site and suboptimal persistence within the immunosuppressive tumor environment are all critical factors that limit the impact of T-cell immunotherapies in solid tumors. Insufficient migration of tumor-specific T cells may result from unfavorable chemokine gradients, as tumor-specific T cells may lack the appropriate chemokine receptors for chemokines secreted by tumor cells (7). Alternatively, tumor cells or stromal cells produce chemokines that preferentially attract T cells with regulatory function rather than T cells with antitumor activity (8, 9). Even if tumor-specific T cells reach the tumor environment, multiple mechanisms exploited by tumor cells themselves or by surrounding stromal cells can block an effective immune response. Among these mechanisms, downregulation of costimulatory molecules (CD28 and CD86) by tumor cells (10), abundant TGFβ production (11), and infiltration by regulatory T cells (Treg; ref. 9) significantly impair activation and proliferation of tumor-specific T cells, whereas overexpression of death receptor ligands by tumor cells, including FasL and PD-L1, may directly favor premature apoptosis of T lymphocytes (12, 13).

Engineering of CAR-T cells to include costimulatory endodomains can rescue T-cell activation through the autocrine production of IL2 (14–17), but this may still be insufficient to reverse the pro-anergic properties of the tumor environment (18, 19). CAR-T cells will also need to be further modified not only to migrate to the tumor site through co-expression of specific chemokine receptors matching relevant tumor-secreted chemokines such as Gro-α (20), CCL17 (21), and CCL2 (22) but also to specifically counter the local inhibitory milieu, for example, due to TGFβ (23) and CCL24 directly accelerated infiltration by regulatory T cells (Treg; ref. 9) significantly impair activation and proliferation of tumor-specific T cells, whereas overexpression of death receptor ligands by tumor cells, including FasL and PD-L1, may directly favor premature apoptosis of T lymphocytes (12, 13).

Engineering of CAR-T cells to include costimulatory endodomains can rescue T-cell activation through the autocrine production of IL2 (14–17), but this may still be insufficient to reverse the pro-anergic properties of the tumor environment (18, 19). CAR-T cells will also need to be further modified not only to migrate to the tumor site through co-expression of specific chemokine receptors matching relevant tumor-secreted chemokines such as Gro-α (20), CCL17 (21), and CCL2 (22) but also to specifically counter the local inhibitory milieu, for example, due to TGFβ (23) and CCL24 directly accelerated infiltration by regulatory T cells (Treg; ref. 9) significantly impair activation and proliferation of tumor-specific T cells, whereas overexpression of death receptor ligands by tumor cells, including FasL and PD-L1, may directly favor premature apoptosis of T lymphocytes (12, 13).

To solve this complex task, we exploited oncolytic viruses that selectively infect, lyse, and replicate in malignant cells while leaving nonmalignant cells unaffected (29). Oncolytic viruses have sufficient cargo capacity to insert ectopic genes...
and have produced clinical responses in patients with solid tumors (30). We therefore armed an oncolytic adenovirus (Ad5Δ24) with the chemokine RANTES and the cytokine IL15 to enhance the subsequent trafficking and survival of T cells expressing a tumor-directed CAR.

Materials and Methods

Tumor cell lines

The neuroblastoma cell lines IMR-32, LAN-1, SKNLP, SK-N-SH, and SH-SY5Y and the lung carcinoma cell line A549 were obtained from the ATCC, whereas neuroblastoma cell lines LAN-5 and CHLA-255 were gifts from Dr. I.S. Metelitsa (Baylor College of Medicine, Houston, TX) and we verified that these lines retain the surface expression of the target antigen GD2. Cells were maintained in RPMI-1640 (IMR-32, LAN-1, LAN-5, SKNLP, SK-N-SH, and SH-SY5Y) or IMDM (CHLA-255 and A549). All media were supplemented with 10% FBS and 2 mmol/L L-glutamine (Invitrogen), with the exception of CHLA-255, which was supplemented with 20% FBS. Tumor cell lines were transduced with a gamma retroviral vector encoding enhanced GFP (eGFP) to obtain GFP-positive tumor cells.

Oncolytic virus

Oncolytic adenovirus Ad5Δ24 and nonreplicable control adenovirus Ad5-Luc1 were kindly provided by Dr. Akseli Hemminki (University of Helsinki, Helsinki, Finland). Ad5Δ24.RANTES, Ad5Δ24.II15, and Ad5Δ24.RANTES.II15 were generated and amplified according to standard procedures (31). For Ad5Δ24.RANTES.II15, the two genes were linked together using a 2A-like sequence (25).

Flow cytometry

The following monoclonal antibodies conjugated with fluorochromes were used: Coxackie-adenovirus receptor, GD2, CD95 (Fas), CD80, CD86, CD40L, OX40L, CD25, CD69, IFNγ, CD3, granzyme B (BD Biosciences); TRAIL, TRAIL-R1, and TRAIL-R2 (Biolegend). Expression of GD2.CAR by T cells was measured and propidium iodide (PI) was used as an internal control. The frequency of early apoptotic cells was determined as percentage of FITC-FAM Poly Caspases Assay Kit (Molecular Probes) according to manufacturer’s instructions. The level of late apoptotic cells was determined by the percentage of PI-positive cells. The frequency of late apoptotic cells was determined by the percentage of ICN Cookie-5 positive cells. The expression of GD2.CAR by T cells was determined as percentage of FAM-positive cells from the total cell population.

Retroviral production and CAR-T cell generation

The vectors encoding the GD2-specific CAR (GD2.CAR), incorporating the CD28 and OX40 costimulatory endodomain, the fusion protein eGFP-firefly luciferase (FFLuc), and the methodology for the production of retroviral supernatant and CAR-T cells have been described previously (15, 16, 33). For the T-cell proliferation assay in vitro, we used a first generation GD2.CAR that lacks both CD28 and OX40 signaling domains.

Coculture experiments

Tumor cells were seeded in 24-well plates (5 × 10⁴/well for cytotoxicity assay and 1 × 10⁶/well for T-cell proliferation assay), infected with Ad5Δ24 (50–100 vp/cell), and then cultured for 3 days. Control and GD2.CAR-T cells (3 × 10⁶/well for cytotoxicity assay and 5 × 10⁶/well for T-cell proliferation assay) were then added and cultured for additional 3 days. Residual GFP⁺ neuroblastoma cells and T cells were then counted on the basis of GFP and CD3 expression, respectively, using microbeads (CountBright Absolute Counting Beads, Invitrogen). Normalized residual tumor cells were calculated as 100 × tumor cell counts with treatment/tumor cell counts without treatment (%).

Confocal microscopic video imaging

GFP-labeled CHLA-255 cells were seeded into 8-well chamber slide (Lab-TekII, Thermo Scientific; 10⁴ cells/well), infected with Ad5Δ24 (100 vp/cell), and cultured for 3 days. Control and GD2.CAR-T cells were then added to the well (10⁵ cells/well). GFP⁺ neuroblastoma cells stained with Annexin V (Invitrogen) were imaged using a spinning disk confocal microscope for 16 hours. Imaging data were acquired and analyzed using Zen software (Zeiss).

Migration assay

Migration assays were conducted as previously described (21) with minor modifications using 5-μm pore 24-well Transwell plates (Corning Life Science). The percentage of migrating cells was calculated as follows: 100 × (cell count of experimental sample – cell count of negative control)/cell count of positive control – cell count of negative control.

ELISA and Milliplex assay

To measure the in vitro production of chemokines and cytokines, tumor cells were plated at 5 × 10⁵ cells/mL in 24-well plates and infected with viruses (50–100 vp/cell). Supernatants were collected 72 hours later and analyzed for the production of RANTES, MIP-1α, MIP-1β, MCP-1, IP-10, and IL15. To measure the in vivo production of RANTES and II15, tumor and blood samples were collected 14 to 18 days after virus inoculation. Tumor homogenates and serum were
separated and finally assayed using specific ELISA kits (R&D Systems). Human IL17F, GM-CSF, IFNγ, IL10, CCL-20, IL12p70, IL13, IL17α, IL22, IL9, IL1β, IL33, IL2, IL21, IL4, IL23, IL5, IL6, IL25, IL27, IL31, TNFα, TNFβ, and IL28B and mouse G-CSF, GM-CSF, IFNγ, IL1α, IL1β, IL2, IL4, IL5, IL6, IL7, IL9, IL10, IL12p40, IL12p70, IL13, IL15, IL17, and TNFα in the serum were measured using Milliplex assay kits (Millipore) following manufacturer's protocols.

Neuroblastoma xenograft animal model

To assess antitumor effects and persistence of GD2.CAR-T cells, we used NOD.Cg-Neuroblastoma xenograft animal model measured using Milliplex assay kits (Millipore) following IL12p40, IL12p70, IL13, IL17, GM-CSF, IFNγ, IL2, IL4, IL5, IL6, IL7, IL9, IL10, IL12p40, IL12p70, IL13, IL15, IL17, and TNFα in the serum were measured using Milliplex assay kits (Millipore) following manufacturer's protocols.

Combining Armed Oncolytic Virus with CAR-T Cells

To assess antitumor effects and persistence of GD2.CAR-T cells, we used NOD.Cg-Neuroblastoma xenograft animal model measured using Milliplex assay kits (Millipore) following IL12p40, IL12p70, IL13, IL17, GM-CSF, IFNγ, IL2, IL4, IL5, IL6, IL7, IL9, IL10, IL12p40, IL12p70, IL13, IL15, IL17, and TNFα in the serum were measured using Milliplex assay kits (Millipore) following manufacturer's protocols.

Combined therapy of GD2.CAR-T cells with Ad5Δ24 enhances apoptosis of neuroblastoma cells in vitro

We next explored whether combining Ad5Δ24 and GD2.CAR-T cells enhanced the elimination of neuroblastoma cells in vitro in coculture experiments. When CHLA-255 cells were incubated with 75 vp/cell of Ad5Δ24 and a low ratio of GD2.CAR-T cells (effectortarget = 3:5), tumor cells were effectively eliminated by day 7 of culture compared with tumor cells incubated with each agent alone. Residual tumor cells were significantly decreased to less than 5% in the presence of Ad5Δ24 together with GD2.CAR-T cells, compared with 30% ± 7% and 33% ± 7% in the presence of GD2.CAR-T cells (P = 0.01) and Ad5Δ24 combined with control T cells (P = 0.01; Fig. 2A). Comparable results were obtained when 2 other neuroblastoma cell lines IMR-32 and SKNLP were used (Fig. 2A). To clarify the mechanism behind the superior antitumor effects of the combined treatment, we explored whether Ad5Δ24 potentiated the function of T cells by upregulating infected tumor cells the GD2 antigen, death receptors (Fas and TRAIL R1/R2), or costimulatory molecules and ligands (CD80, CD86, OX40L, and CD40L). However, no upregulation of any of these molecules by neuroblastoma cells was detected upon infection with Ad5Δ24 (Supplementary Fig. S1). We also investigated whether Ad5Δ24-infected neuroblastoma cells could indirectly increase the activation or effector function of CAR-T cells. However, neither CD25, CD69, IFNγ, granzyme B nor TRAIL was significantly upregulated by GD2.CAR-T cells exposed to Ad5Δ24-infected neuroblastoma cells (Supplementary Fig. S2). In contrast, we found that Ad5Δ24-infected neuroblastoma cells underwent more rapid apoptosis when exposed to GD2.CAR-T cells. As illustrated in Fig. 2B and Supplementary Movie S1, we conducted sequential confocal microscope imaging of GFP+ CHLA-255 cells cocultured with GD2.CAR-T cells. We found that neuroblastoma cells preincubated for 72 hours with Ad5Δ24 became Annexin-V+ within 4 hours after exposure to GD2.CAR-T cells, whereas noninfected CHLA-255 cells exposed to GD2.CAR-T cells required more than 16 hours to bind detectable levels of Annexin-V. We quantified the active caspases in neuroblastoma cells and found in response to GD2.CAR-T cells a consistent increase in the percentage of early apoptotic cells when neuroblastoma cells were preincubated with Ad5Δ24 compared with neuroblastoma cells that had not been exposed to Ad5Δ24 (FAM+/PI-) cells were 20% ± 2% vs.15% ± 1% at 2 hours, P < 0.05; 24% ± 2% vs.18% ± 2% at 4 hours, P < 0.05; Fig. 2C and D). These results indicate that the caspase activity and apoptosis in neuroblastoma cells are more pronounced after incubation with CAR-T cells if neuroblastoma cells are infected with Ad5Δ24.
Combined GD2.CAR-T cells and Ad5Δ24 have more robust antitumor activity in vivo

We examined whether the combination of GD2.CAR-T cells and Ad5Δ24 was also effective in a neuroblastoma xenograft mouse model. Mice engrafted subcutaneously with the neuroblastoma cell line CHLA-255 were inoculated intratumorally with either PBS or Ad5Δ24 and then infused intravenously with either control or GD2.CAR-T cells (10^7 cells). Control mice, inoculated with PBS and control T cells, had rapid tumor progression and were euthanized within 20 days (Supplementary Fig. S3). When mice were inoculated with a high dose of Ad5Δ24 (10^7 vp), tumor growth was controlled irrespective of T-cell transfer (Fig. 3A). However, when mice were inoculated with lower doses of Ad5Δ24, the addition of CAR-T cells improved tumor control compared with Ad5Δ24 plus control T cells (P = 0.04 in 10^6 vp; Fig. 3A).

We also found that GD2.CAR-T cells showed enhanced, although still transient, persistence in vivo at the tumor site when tumors were inoculated with Ad5Δ24. As shown in Fig. 3B and C, FFluc-labeled GD2.CAR-T cells localized and persisted up to day 6 in Ad5Δ24-inoculated tumors compared with PBS-inoculated tumors (1.8 × 10^5 ± 0.63 × 10^5 p/s/cm^2/sr vs. 1.5 × 10^5 ± 0.2 × 10^5 p/s/cm^2/sr, P < 0.01) and then became undetectable. These findings raise the possibility that Ad5Δ24-infected neuroblastoma cells may release T-cell chemoattractant factors. However, measurement of the chemokines RANTES, MIP-1α, MIP-1β, MCP-1, and IP-10 in the culture neuroblastoma cells infected with Ad5Δ24 showed no significant production of these cytokines before and after infection, with the exception of the SK-N-SH neuroblastoma cell line that spontaneously produced a high level of MCP-1 (Supplementary Table S1). In vitro migration assays confirmed that supernatants collected from neuroblastoma cells infected with Ad5Δ24 did not promote T-cell migration, excluding the possibility that other unknown factors that favor T-cell migration are released by Ad5Δ24-infected neuroblastoma cells (Fig. 3D). Thus, the transient beneficial effects of the combined Ad5Δ24 and GD2.CAR-T cells support the need for the engineering of Ad5Δ24 to increase the antitumor benefits.

Neuroblastoma cells infected with Ad5Δ24 engineered to release RANTES and IL15 produce functional amounts of both proteins

We armed Ad5Δ24 with RANTES and IL15 (Ad5Δ24. RANTES, IL15) to induce migration and prolong persistence of GD2.CAR-T cells at the tumor site (Fig. 4A) and demonstrated that neuroblastoma cells infected with this virus release RANTES (709–2,247 pg/ml at 72 hours) and IL15 (864–2,131 pg/ml at 72 hours) in vitro (Fig. 4B). Both factors...
were functional because the supernatant of neuroblastoma cells infected with Ad5Δ24.RANTES.II15 improved the migration (19% ± 2% in Ad5Δ24.RANTES.II15 vs. 9% ± 2% in mock; P = 0.002, and vs. 13% ± 3% in Ad5Δ24; P = 0.01) and expansion (1.6 ± 0.1-fold in Ad5Δ24.RANTES.II15 vs. 0.71 ± 0.07 fold in mock; P = 0.01, and vs. 0.55 ± 0.04-fold in Ad5Δ24; P = 0.005) of GD2.CAR-T cells in vitro (Fig. 4C and D) without impairing the oncolytic property of the virus (Fig. 4E). Thus, neuroblastoma cells infected with Ad5Δ24.RANTES.II15 produce functional levels of both proteins.

**Ad5Δ24.RANTES.II15 enhances the persistence of GD2.CAR-T cells in vivo**

Mice engrafted with CHLA-255 cells were inoculated intratumorally with either Ad5Δ24 or Ad5Δ24.RANTES.II15 (10^7–10^8 vp) followed by one single intravenous infusion of FFluc-labeled GD2.CAR-T cells (10^7 cells). As shown in Fig. 5A and B, bioluminescence signals of GD2.CAR-T cells were higher at the site of tumors inoculated with Ad5Δ24.RANTES.II15 than in tumors inoculated with unmodified Ad5Δ24 (2.1 × 10^7 ± 1.0 × 10^7 p/s/cm²/sr vs. 5.6 × 10^8 ± 4.1 × 10^7 p/s/cm²/sr at day 12, P < 0.001). RANTES and IL15 secretion by Ad5Δ24.RANTES.II15-infected tumor did not promote accumulation of control T cells lacking CAR expression, indicating that antigen specificity of T cells is also necessary for T-cell persistence at the tumor site (Fig. 5C). The increase in photon intensity corresponded to an increased numeric infiltration of human T cells within the tumor as demonstrated by both flow cytometry and immunohistochemistry (Fig. 5D and E). Quantification of RANTES and IL15 in the sera and tumor biopsies confirmed that these two factors were produced only in mice inoculated with Ad5Δ24.RANTES.II15 (Fig. 5F). Moreover, both RANTES and IL15 were predominantly detected at the tumor site (5,890 ± 1,055 and 1,762 ± 360 pg/mL, respectively) rather than in serum (111 ± 46 and 357 ± 162 pg/mL, respectively;
RANTES; $P < 0.001$ and IL15; $P < 0.01$; Fig. 5F). Quantification of other human and mouse cytokines in serum by a Milliplex assays as indicated in Materials and Methods demonstrated no significant modifications except for an equal elevation of mouse granulocyte colony-stimulating factor in both Ad5Δ24 and Ad5Δ24.RANTES.II.15-treated mouse. Combination therapy with GD2.CAR-T cells and Ad5Δ24.RANTES.II.15 significantly enhanced the survival of mice compared with GD2.CAR-T cells and Ad5Δ24 (73% vs. 44% at day 45, $P = 0.03$; Fig. 5G). Finally, we found that the intratumoral inoculation of the Ad5Δ24.RANTES.II.15 is essential to sustain the persistence of CAR-T cells. When mice were indeed engrafted with tumor cells in both flanks, CAR-T cells significantly persisted and promoted antitumor activity only at the virus-inoculated tumor site (Supplementary Fig. S4).

We also constructed Ad5Δ24 encoding either RANTES alone (Ad5Δ24.RANTES) or IL15 alone (Ad5Δ24.II.15) to determine the specific role of each single component (Fig. 6A). Both viruses were functional, as infected neuroblastoma cells produced either RANTES or IL15 in vitro (Fig. 6B), and both viruses retained oncolytic activity (Fig. 6C). When tested in vivo, GD2.CAR-T cell persistence was superior in mice that received Ad5Δ24.RANTES.II.15 ($1.9 \times 10^7 \pm 0.9 \times 10^7$ p/s/cm$^2$/sr) compared with those that received either Ad5Δ24.RANTES ($1.0 \times 10^6 \pm 0.2 \times 10^6$ p/s/cm$^2$/sr, $P < 0.01$) or Ad5Δ24.II.15 ($3.9 \times 10^6 \pm 2.8 \times 10^5$ p/s/cm$^2$/sr, $P < 0.05$; Fig. 6D and E). Thus, the combination of intratumoral injection of Ad5Δ24.RANTES.II.15 and intravenous infusion of GD2.CAR-T cells produces significant improvements in the control of tumor growth.

**Discussion**

We have demonstrated that the Ad5Δ24 armed with RANTES and IL15 increases the number of T cells infiltrating a solid tumor, likely through the enhanced trafficking and prolongation of T-cell survival that these agents respectively induce. These effects were achieved without compromising the intrinsic lytic activity of the oncolytic virus or CAR-T cells, and the combination of both agents resulted in better control of the tumor growth and prolonged survival of tumor-bearing animals.

The rationale for combining oncolytic viruses and CAR-T cells for the treatment of solid tumors stems from two main experimental evidences. First, Ad5Δ24 engineered to accommodate genes supporting T-cell function remain toxic to tumor cells without damaging or compromising CAR-T cell activities, even when the viruses were used at high concentrations. Second, Ad5Δ24-infected neuroblastoma cells become more susceptible to the lytic effects of CAR-T cells. It was previously described that oncolytic adenoviruses induce...
activation of caspase-3 in infected tumor cells (34). Here we support this observation and demonstrated that Ad5Δ24 further accelerates and amplifies the occurrence of caspase-induced cell death of neuroblastoma cells mediated by CAR-T cells. In turn, the faster lysis of tumor cells promoted by CAR-T cells may facilitate the spread of the virus, which in solid tumors is usually limited to the surrounding area of the virus inoculation (35). As a consequence, when unmodified Ad5Δ24 and CAR-T cells are combined, better control of the neuroblastoma tumor growth is observed in animals.

Despite the superior cytolytic activity observed with the combined treatment described above, we found that the overall infiltration and persistence of CAR-T cells at the tumor site was not robustly improved, as Ad5Δ24-infected neuroblastoma cells did not release factors that promote T-cell migration and survival. Even though the GD2-specific CAR we used is engineered to co-express 2 costimulatory molecules such as CD28 and OX40 (16, 22), this combination is evidently insufficient to sustain the persistence of the few CAR-T cells that reach the tumor microenvironment. These limitations strongly support our strategy to engineer oncolytic virus to produce not only a T-cell chemoattractant factor but also a T-cell growth factor.

For optimized T-cell trafficking and survival, we selected the chemokine RANTES and the cytokine IL15. While we and others have previously focused on identifying chemokines specifically produced by tumor cells and on engineering CAR-T cells with the cognate receptor (20–22), here we selected RANTES as a broadly applicable chemokine because its receptors CCR1, CCR3, and CCR5 are retained by ex vivo expanded T cells (22, 36). This assures that upon adoptive transfer, T cells will migrate to multiple types of tumors if they are forced to release RANTES. We selected IL15 as a T-cell growth factor because in addition to its...
multiple beneficial effects on T cells (19, 37), this cytokine is not produced by CAR-T cells upon activation and thus the oncolytic virus provides a growth factor that is generally absent in the tumor environment (25). In addition, we previously demonstrated that IL15 is preferentially used by effector T cells rather than Tregs (19) in the unfortunate event that RANTES also attracts this cell subset (38). We found that only Ad5×24 co-expressing RANTES and IL15 produced substantive increases in CAR-T cell numbers at tumor sites, which lead to improved tumor control in vivo.

Of note, the detection of RANTES and IL15 in our model was mostly confined to the tumor site, indicating a preferential local expression of both factors and thereby circumventing the toxicities associated with systemic administration of cytokines (39). To further potentiate the safety of this strategy for the clinical application, antiviral agents, such as cidofovir, or the inclusion of the thymidine kinase gene of the human herpes virus type I can be used to abort any potential toxicity of oncolytic viruses (40, 41).

Although the immunodeficient mouse model we have used has limits in assessing the effect of the inhibitory components of the immune system such as Tregs, tumor-associated macrophages, and myeloid-derived suppressor cells, it is nevertheless relevant for a clinical translation. It demonstrates indeed that the infection of tumor cells with RANTES- and IL15-engineered oncolytic virus is sufficient to enhance the overall antitumor activity of CAR-T cells generated following the manufacturing procedures currently applied for the production of CAR-T cells for clinical use (33). There is also additional evidence that tumors infected with oncolytic virus can trigger danger signals by dendritic cells and promote cross-antigen presentation, ultimately leading to the elicitation of innate and adaptive immune responses (42). Thus, the positive effects of armed oncolytic viruses observed in our own and other models (36, 43) may be further amplified in patients due to the recruitment of other components of the immune system.

Figure 5. Ad5×24.RANTES.IL15 improves persistence of GD2.CAR-T cells. A and B, NSG mice engrafted subcutaneously with CHLA-255 cells were inoculated intratumorally with oncolytic viruses (10^5–10^6 vp) by days 10 to 14. Four days later, mice were infused intravenously with FFluc labeled GD2.CAR-T cells. T-cell bioluminescence was then measured. Data represent mean ± SEM. †, P < 0.05; ††, P < 0.01; †††, P < 0.001 by Student t test. C, NSG mice engrafted subcutaneously with CHLA-255 cells were inoculated intratumorally with oncolytic viruses (10^6 vp) by day 10. Four days later, mice were infused intravenously with FFluc labeled control T cells or FFluc-labeled GD2.CAR-T cells diluted with control T cells at 6:10 or 2:10 ratios. T-cell bioluminescence was then measured. Data represent mean ± SEM in 8 mice for each virus.
In our model, we selected to administer the oncolytic virus intratumorally, as this is the preferential route of infusion of oncolytic adenoviruses in clinical trials. We found that the local presence of the virus is essential in supporting the persistence of CAR-T cells. Thus, to be effective for metastatic tumors, our proposed combined approach will likely require the use of CAR-T cells. Therefore, to be effective for metastatic tumors, our proposed combined approach will likely require the use of oncolytic viruses that can be administered either intratumorally, as this is the preferential route of infusion of the infused T cells. Our data support the further exploration of this platform of biologic agents for therapy of solid tumors.

Disclosure of Potential Conflicts of Interest

G. Dotti and B. Savoldo have ownership interest (including patents) in the field of T cell and gene-modified T-cell therapy for cancer. The Center for Cell and Gene Therapy has a collaborative research agreement with Celgene and Bluebird bio.

Authors’ Contributions

Conception and design: N. Nishio, I. Diaconu, V. Cerullo, G. Dotti
Development of methodology: N. Nishio, I. Diaconu, I. Caruana, V. Hoyos, B. Savoldo, G. Dotti
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N. Nishio, V. Hoyos, L. Boucher-Hayes, B. Savoldo, G. Dotti
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N. Nishio, I. Diaconu, H. Liu, I. Caruana, V. Hoyos, B. Savoldo, G. Dotti
Writing, review, and/or revision of the manuscript: N. Nishio, I. Diaconu, B. Savoldo, G. Dotti
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): N. Nishio, I. Diaconu, G. Dotti
Study supervision: N. Nishio, I. Diaconu, G. Dotti

Acknowledgments

The authors thank Dr. Malcolm K. Brenner for the critical revision of the article.

Grant support

This work was supported in part by R01 CA142636 NIH-NCl W81XWH-10-10925 Department of Defense and Technology/Therapeutic Development Award, and NIH/NCl P01 CA094237. N. Nishio was supported by Strategic Young Researcher Overseas Visits Program for Accelerating Brain Circulation, Japan Society for the Promotion of Science.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 6, 2014; revised June 12, 2014; accepted June 17, 2014; published OnlineFirst July 24, 2014.
References


Modified T Cells in Solid Tumors

Armed Oncolytic Virus Enhances Immune Functions of Chimeric Antigen Receptor–Modified T Cells in Solid Tumors

Nobuhiro Nishio, Iulia Diaconu, Hao Liu, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-14-0697

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2014/07/25/0008-5472.CAN-14-0697.DC1

Cited articles
This article cites 44 articles, 14 of which you can access for free at:
http://cancerres.aacrjournals.org/content/74/18/5195.full.html#ref-list-1

Citing articles
This article has been cited by 6 HighWire-hosted articles. Access the articles at:
/content/74/18/5195.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.