Abstract 4198

Evaluation of a Cancer Immunotherapy With Engineered Arenavirus Vector and 4-1BB Agonists in a Preclinical Tumor Model

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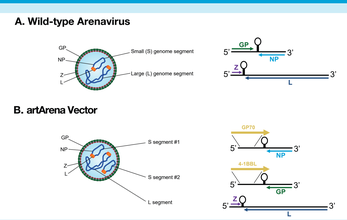
INTRODUCTION

- T cells play a central role in immune responses against cancer. However, within the tumor microenvironment, T cells are exposed to a plethora of negative regulators that can lead to varying degrees of dysfunction, exhaustion, and eventually tumor progression. Various costimulatory factors and cytokines can help prevent or delay the onset of exhaustion and instead augment effector functions and persistence of functional tumor-targeted T cells[1]
- Targeting 4-1BB (CD137), a member of the tumor necrosis factor receptor (TNFR) superfamily, has been shown to represent a promising strategy for inducing an activating signal in CD8+ T cells, resulting in increased proinflammatory cytokine secretion, cytotoxic function, and survival[2-4]
- Engineered arenavirus vectors based on lymphocytic choriomeningitis virus (LCMV) or Pichinde virus (PICV) have been shown previously to induce massive infiltration of tumor antigen-specific CD8+ T cells into the tumor in several preclinical cancer models[5-8]
- HOOKIPA is exploring whether enhanced co-stimulation of 4-1BB using 4-1BB agonists can further improve T cell responses and/or tumor control when administered in combination with replicating arenavirus-based vectors (artLCMV), including artLCMV-GP70. GP70, a product of the env gene of endogenous murine leukemia virus (MuLV), is expressed in multiple mouse tumor lines
- Here, we present preclinical data exploring whether immunotherapy with engineered arenavirus vectors combined with 4-1BB agonists can augment tumor associated antigen (TAA)-specific T cell responses within the tumor, leading to better tumor growth control and a higher rate of response

METHODS

- **artLCMV-GP70:** engineered arenavirus vector based on lymphocytic choriomeningitis virus (LCMV) and encoding the tumor-associated antigen GP70. artLCMV vectors are replication competent but stably attenuated by means of artificial genome organization
- **The 4-1BB agonists** used for experiments presented here were an **α4-1BB antibody** (clone LOB12.3, rIgG1) and the engineered arenavirus vector **artLCMV-GP70/4-1BBL,** which is based on LCMV and encoded GP70 on Segment 1 and 4-1BB ligand (4-1BBL) on segment 2. **Figure 1** depicts a schema of the arenavirus structure and how this vector was generated

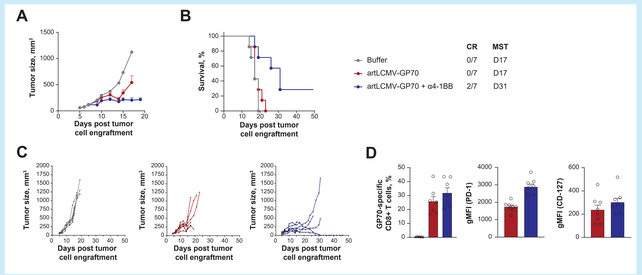
Figure 1. Arenavirus Vectors Are Engineered to Encode Target Antigens



Arenavirus particle composition and vector generation: (A) Schematic view of an arenavirus particle (left side) and genomic organization (right side). The ambisense RNA genome encodes for 4 viral proteins: GP (glycoprotein) and NP (nucleoprotein) on the S segment; L (RNA-directed RNA polymerase) and Z (RING finger protein Z) on the L segment. **(B)** To design artLCMV vectors, the LCMV genomes were modified by segregating the viral GP and NP onto artificially duplicated S segments. Each artLCMV vector thus contains 3 genome segments, including 1 L segment and 2 S segments in which the S segments encode either GP or NP but not both. The genetic modification of S segments absolutely prevents intersegmental recombination and reversion to a functional wild-type-like single S segment encoding both GP and NP. Because all 4 genes (L, Z, GP, and NP) are required for wild-type virus proliferation, all 3 genetic segments are required for artLCMV proliferation.

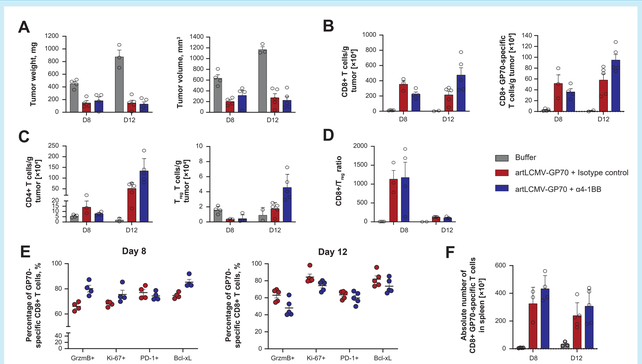
RESULTS

Figure 2. Treatment With artLCMV-GP70 in Combination With an Agonistic α4-1BB Antibody Improves Tumor Control and Survival in B16.F10 Tumor-Bearing Animals



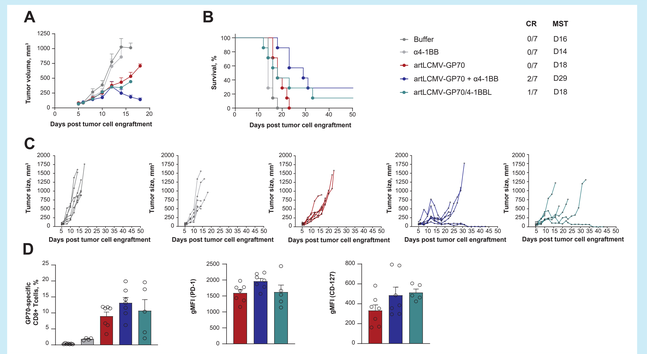
The anti-tumor effect of artLCMV-GP70 vector administration in the B16.F10 model was further enhanced by a single injection of agonistic α4-1BB antibodies. C57BL/6 mice were subcutaneously injected with 2×10^5 B16.F10 cells. Animals were immunized intravenously with 1×10^5 RCV FFU of artLCMV-GP70 vectors when tumors reached approximately 100 mm^3 (day 7). On the day of vector application (day 7), animals were treated intraperitoneally with α4-1BB (100 µg). Tumor growth **(A and C)** and survival **(B)** were monitored. On day 14, the frequency of tumor antigen-specific CD8+ T cells as well as their PD-1 and CD127 expression (gMFI – gated on GP70-specific CD8+ T cells), was analyzed in the blood. **(D)** Data shown are mean ± SEM, n=7 per group. CR, complete responder; gMFI, geometric mean fluorescence intensity; FFU, focus-forming unit; MST, mean survival time; PD-1, programmed death 1; RCV, replication-competent virus; SEM, standard error of the mean.

Figure 3. α4-1BB Has a Moderate Effect on artLCMV-GP70-Induced Tumor Infiltration of T cells but Affects Cytotoxicity, Proliferation, and Survival of GP70-specific CD8+ T Cells



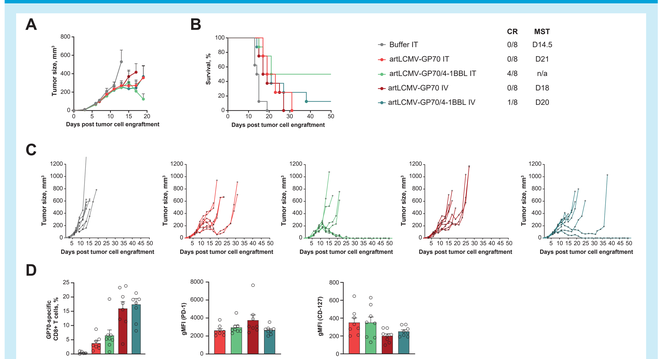
artLCMV-GP70 treatment induced tumor infiltration of immune effector cells, which was further enhanced in combination with α4-1BB. C57BL/6 mice were subcutaneously injected with 2×10^5 B16.F10 cells. Animals were immunized intravenously with 1×10^5 RCV FFU of artLCMV-GP70 vectors when tumors reached approximately 100 mm^3 (day 8). Agonistic α4-1BB (100 µg) was administered intraperitoneally on the day of vector administration (day 8). On day 8 and day 12 post vector application (D16 and D20 post tumor cell engraftment), tumors and spleens were isolated and lymphocytes were quantified. **(A)** Tumor weight and volume on the day of isolation **(B)** Number of CD8+ and GP70-specific CD8+ T cells/g tumor. **(C)** Number of CD4+ and regulatory T cells (CD4+FoxP3+CD25+)/g tumor. **(D)** Ratio of CD8+ to regulatory T cells. **(E)** Percentages of granzyme B-, Ki-67-, PD-1- and Bcl-xL- expressing cells among antigen-specific GP70-specific CD8+ T cells in the tumor. **(F)** Absolute numbers of antigen-specific CD8+ T cells in the spleen on day 8 and day 12 post vector administration. Data shown are mean ± SEM, n=5 per group. Bcl-xL, B-cell lymphoma-extra large; GrzmB, granzyme B.

Figure 4. artLCMV Vector Encoded GP70 and 4-1BBL Slightly Improves Antitumor Efficacy After Intravenous Dosing



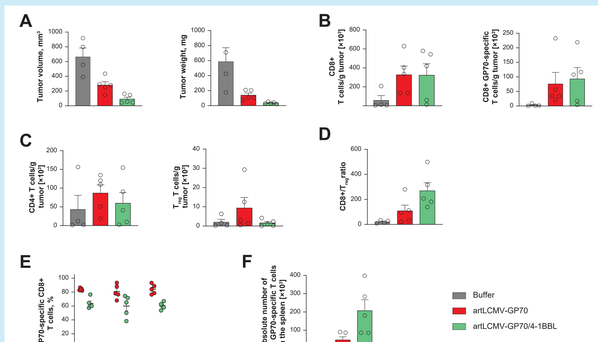
Intravenous treatment with vectors encoding GP70 and 4-1BBL slightly enhanced antitumor efficacy compared with vectors encoding GP70 alone. C57BL/6 mice were subcutaneously injected with 2×10^5 B16.F10 cells. Animals were immunized intravenously with 1×10^5 RCV FFU of artLCMV-GP70 or artLCMV-GP70/4-1BBL vectors when tumors reached approximately 100 mm^3 (day 6). Agonistic α4-1BB antibodies (100 µg) were administered intravenously on the day of vector administration. Tumor growth **(A and C)** and survival **(B)** were monitored. On day 13, the frequency of tumor antigen-specific CD8+ T cells, as well as their PD-1 and CD127 expression (gMFI – gated on GP70-specific CD8+ T cells), was analyzed in the blood. **(D)** Data shown are mean ± SEM, n=7 per group.

Figure 5. artLCMV-GP70/4-1BBL Shows Strongest Antitumor Effect When Applied Intratumorally



IT treatment with artLCMV-GP70/4-1BBL vectors had stronger antitumoral effects compared with IV treatment and led to an increase in CRs. C57BL/6 mice were subcutaneously injected with 2×10^5 B16.F10 cells. Animals were immunized intravenously or intratumorally with 1×10^5 RCV FFU of artLCMV-GP70 or artLCMV-GP70/4-1BBL vectors when tumors reached around 100 mm^3 (day 7). Tumor growth **(A and C)** and survival **(B)** were monitored. On day 14, the frequency of tumor antigen-specific CD8+ T cells as well as their PD-1 and CD127 expression (gMFI – gated on GP70-specific CD8+ T cells) was analyzed in the blood **(D)** Data shown are mean ± SEM, n=8 per group. IT, intratumorally; IV, intravenously.

Figure 6. Intratumoral Treatment With artLCMV-GP70/4-1BBL Increases the CD8+ T Cell to T_{reg} Ratio in the Tumor and Leads to Higher Peripheral Tumor Antigen-Specific CD8+ T Cell Responses



IT treatment with artLCMVGP70/4-1BBL vectors increased the CD8+ to T_{reg} cell ratio in the tumor and enhanced tumor antigen-specific immune responses in the periphery. C57BL/6 mice were subcutaneously injected with 2×10^5 B16.F10 cells. Animals were immunized intratumorally with 1×10^5 RCV FFU of artLCMV-GP70 or artLCMV-GP70/4-1BBL vectors when tumors reached appoximately 100 mm^3 (day 7). On day 7 post vector application (D14 post tumor cell engraftment), tumors and spleens were isolated and lymphocytes were quantified. **(A)** Tumor weight and volume on the day of tumor isolation. **(B)** Number of CD8+ and GP70-specific CD8+ T cells per gram tumor. **(C)** Number of CD4+ and regulatory T cells (CD4+FoxP3+CD25+) per gram tumor. **(D)** Ratio of CD8+ to regulatory T cells. **(E)** Percentages of granzyme B-, PD-1- and Bcl-xL- expressing cells among GP70-specific CD8+ T cells in the tumor. **(F)** Absolute number of GP70-specific CD8+ T cells in the spleen. Data shown are mean ± SEM, n=4-5 per group. T_{reg}, regulatory T cells.

CONCLUSIONS

- The engineered arenavirus platform induced strong tumor antigen-specific CD8+ T cell responses in the periphery and tumor microenvironment, leading to transient control of tumor growth in a stringent mouse model
- Combination with 4-1BB agonists, either in the form of antibodies or encoded within the vector genome, lead to better tumor growth control and a higher rate of complete response
- Vector-encoded 4-1BBL had its strongest effect when the vector was applied intratumorally
- These data continue to support the potential of replicating, engineered, arenavirus-based vectors as novel anticancer treatments with broad applicability across various tumor types and in combination with other treatment modalities, such as 4-1BB agonists

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