Powerful synergistic effects of a STING agonist and an IL-2 superkine in eliciting NK and T cell responses against MHC I- and MHC I+ tumors

Natalie K. Wolf[1], Cristina Blaj[1], Lora Picton[2,3], Gail Synder[1], Lily Zhang[1], Christopher J. Nicolai[1], Chudi Ndubaku[4], Sarah McWhirter[4], K. Christopher Garcia[2,3] and David Raulet[1]

[1]Department of Molecular and Cell Biology, University of California Berkeley, [2] Department of Molecular and Cellular Physiology and Structural Biology, Stanford University, [3]Howard Hughes Medical Institution and [4]Aduro Biotech

Abstract

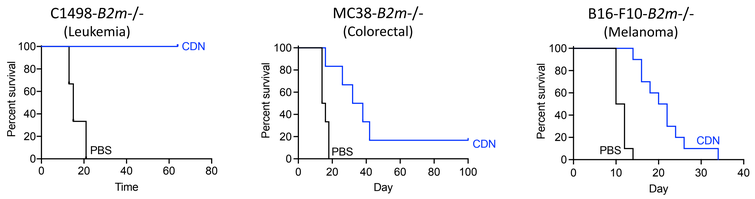
Most current cancer immunotherapies are based on mobilizing CD8 T cell responses. However, many types of tumors evade CD8 T cell recognition by displaying few or no antigens, or losing expression of MHC I. These considerations underlie the need for complementary therapies that mobilize other antitumor effector cells, such as NK cells, which preferentially kill MHC I-deficient cells. Cyclic dinucleotides (CDNs) activate the cGAS-STING pathway of the innate immune system and are candidates as immunotherapy agents. Intratumoral CDN injections induce type I IFNs and other mediators that amplify the CD8 T cell response and induce tumor regression (1). CDN therapy also induces long-term tumor regressions in some MHC I-deficient tumor models, mediated primarily by NK cells (2).

To extend the efficacy of CDN therapy, we have combined the IL-2 superkine, H9, or half-life extended H9, with CDNs to target and activate NK cells and T cells in the tumor microenvironment and prevent or delay the onset of NK cell desensitization (3,4). In these studies, we utilized B16-F10 and MC38 tumor cells lacking *B2m* to examine effects of the combination therapy on MHC I-deficient tumor growth as well as to examine the activation of NK cells by flow cytometry and cytotoxicity assays. We also utilized B16-F10 WT and the spontaneous tumor model, MCA, to assess the effect of the combination therapy on MHC I+ tumors.

Here we show that H9 synergized with CDN therapy to mobilize much more powerful antitumor responses against MHC I-deficient tumors than CDN alone. The responses were mediated by NK cells and in some cases CD4 T cells, and were accompanied by increased recruitment to and sustained activation of NK cells in the tumor. This combination therapy regimen activated NK cells systemically, as shown by antitumor effects distant from the site of CDN injection and enhanced cytolytic activity of splenic NK cells against tumor cell targets ex vivo. Finally, the same combination therapy regimen synergistically mobilized powerful CD8 T cell responses in the case of MHC I+ tumor cells, suggesting the generality of the approach. The approach was effective against primary sarcomas, as well, especially when combined with checkpoint therapy, leading to tumor regressions and long-term survival of many mice with MCA-induced sarcoma.

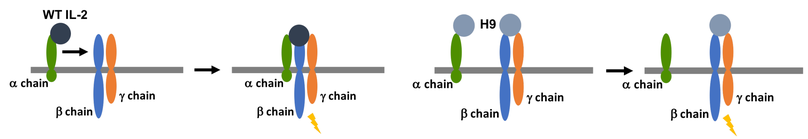
Overall, our work demonstrates the impact of a novel combination therapy in mobilizing powerful NK and T cell-mediated antitumor activity, providing important justification for evaluating this approach for treating cancers that are refractory to available treatment options.

Are CDNs viable as a therapy for MHC I-deficient tumors?



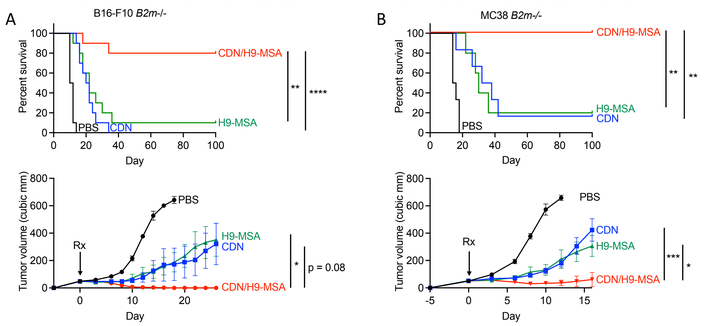
CDNs have therapeutic effects with CD8-T cell resistant MHC I-deficient tumors, generated by knocking out *B2m*. C1498-*B2m*-/-, (B) MC38-*B2m*-/- and (C) B16-F10-*B2m*-/- tumors were established in B6 mice to a size of 50 mm^3 before CDNs were injected intratumorally. Tumors were eliminated (left) or delayed (right) depending on the model.

Engineered IL-2 signals more effectively to NK cells



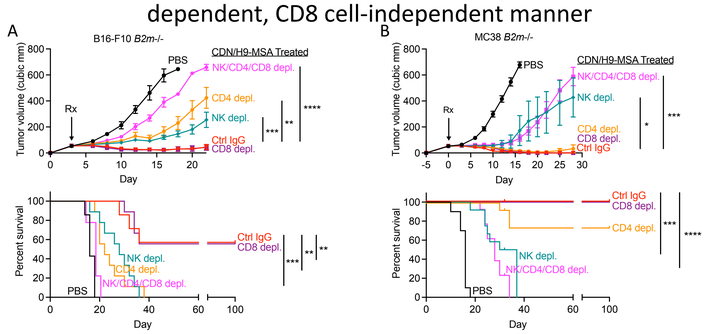
H9 "superkine" has been engineered to bind IL-2Rβ with high affinity and stimulates NK cells better than WT IL-2 (3). H9 also restores NK-mediated antitumor responses against MHC I-deficient tumors in vivo, in which NK cells become desensitized (4). We employed H9 fused to mouse serum albumin (H9-MSA) to extend the in vivo half life of the cytokine.

H9-MSA (i.p.), combined with CDNs (i.t., once), shows profound anti-tumor effects for MHC I-deficient tumors



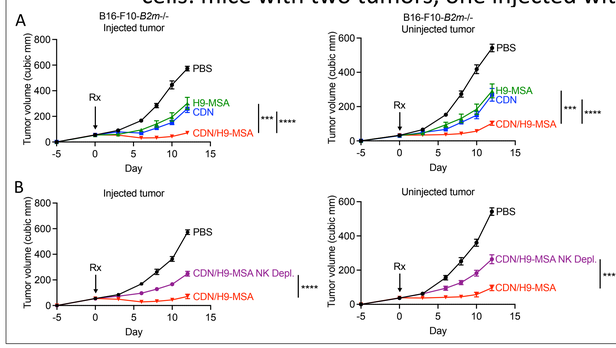
Tumors were established with 4 x 10^6 cells implanted s.c. in C57BL/6J mice and grown to approximately 50 mm^3 (day 0). Tumors were injected once i.t. with 50 ug of CDN or PBS. Some mice were also injected i.p. with 10 ug H9-MSA or PBS on day 0 and repeated every three days until euthanization or 1 week after complete tumor clearance. Tumor growth curves and survival of mice with B16-F10-*B2m*-/- (A) and MC38-*B2m*-/- (B) tumors. CDN/H9-MSA combination therapy led to dramatic tumor rejection in both hard-to-treat models

CDN+H9-MSA combination therapy delays tumor progression in an NK cell-dependent, CD8 cell-independent manner



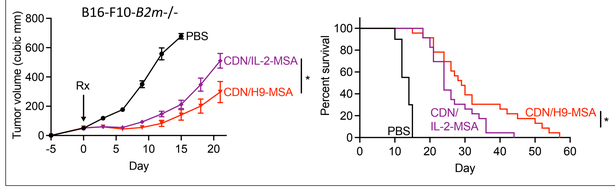
Tumors were established and treated as before, except groups of mice were injected i.p. on days -2, -1 and every 6 days thereafter with antibodies to deplete NK cells, CD8 T cells and/or CD4 T cells, or with control IgG. Tumor growth curves and survival of mice with B16-F10-*B2m*-/- (A) and MC38-*B2m*-/- (B) tumors, where depletion of NK cells and, in the case of B16-*B2m*-/- tumors, CD4 T cells, reduced efficacy.

CDNs and H9-MSA synergize in creating a systemic effect dependent on NK cells: mice with two tumors, one injected with CDN



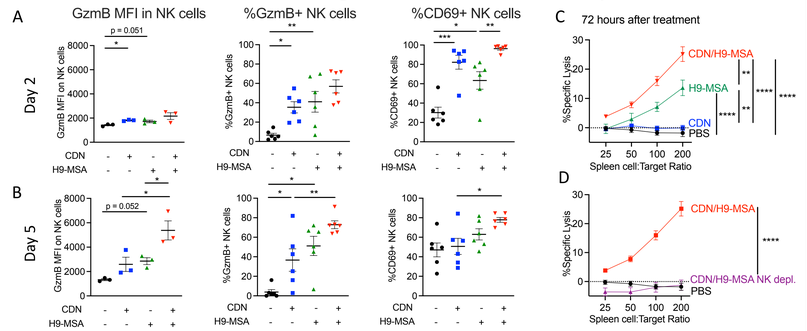
On day -5, B16-F10-*B2m*-/- tumor cells were injected s.c. on both flanks of C57BL/6J mice. Mice were depleted of CD4 cells and CD8 cells 2 days and 1 day before initiation of treatment. On day 0, the tumor on the right flank was injected once i.t. with 50 ug CDN or PBS. The mice received i.p. injections of PBS or 10 ug H9-MSA starting on day 0 and again every three days until mice were euthanized. (A) Growth of injected (left panel) vs contralateral uninjected tumors (right panel) receiving the indicated therapies. (B) Tumor growth following CDN/H9-MSA therapy in mice from which NK cells were depleted or not.

H9-MSA is more effective than WT IL-2-MSA in combination with CDNs



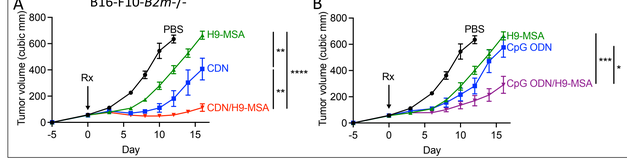
Comparison of CDN in combination with H9-MSA (CDN/H9-MSA) or IL-2-MSA (CDN/IL-2-MSA) using the schedule used before before (one dose of 50 ug CDN i.t. and repeated 10 ug i.p. doses of the cytokine fusion proteins i.p.). H9-MSA is more effective than WT IL-2-MSA. All mice were depleted of CD4/CD8 T cell to focus on the NK-mediated effects.

CDN+H9-MSA combination therapy mobilized more activated intratumoral NK cells with greater functional activity



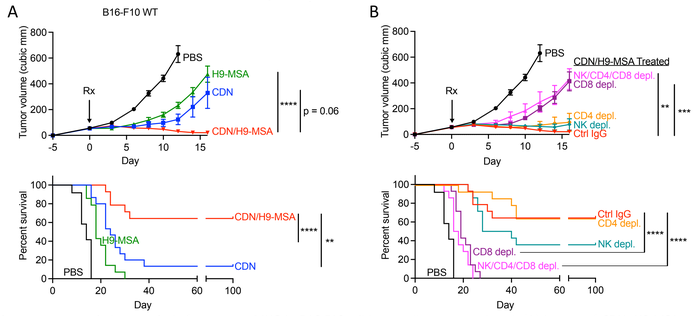
B16-F10-*B2m*-/- tumors were established and treated as described above. On d2 (A) and especially on d5 (B) after treatment initiation, flow cytometry analysis of tumors revealed increased GzmB MFI, %GzmB+ NK cells and %CD69+ NK cells in mice treated with CDN/H9-MSA compared to monotherapy. (C) Spleen cells from tumor bearing mice 72 hours after treatment as above with CDN/H9-MSA showed enhanced cytotoxicity of B16-F10-*B2m*-/- target cells. (D) Cytotoxicity by splenocytes was mediated by NK cells, as shown by depleting mice of NK cells prior to treatment.

A distinct innate activator, CpG oligonucleotide, also synergized with H9-MSA in providing antitumor efficacy



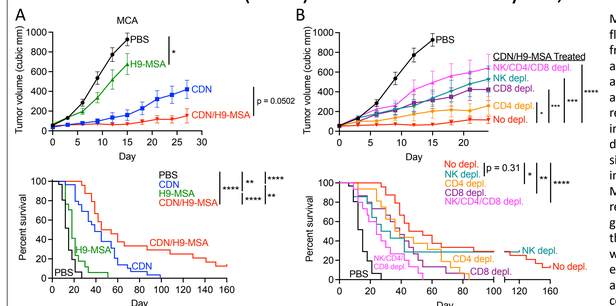
Tumors were established and treated as before. Comparison of the effects of CDN or CpG ODN (each at 50 ug dose, i.t.) alone or in combination with H9-MSA (10 ug doses) shows that H9-MSA amplified the efficacy of both innate activators. All mice were depleted of CD4/CD8 T cell to focus on the NK-mediated effects.

The combination of H9-MSA and CDNs is also highly effective for MHC I+ tumors, dependent on CD8 T cells



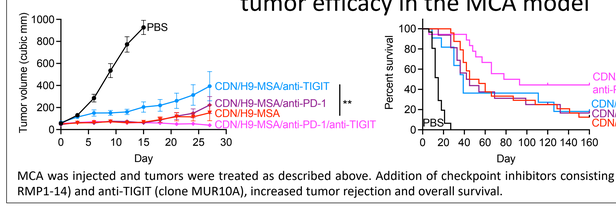
Experiments were carried out as in before except MHC I+ B16-F10 wildtype cells were employed. (A) Impact of CDN, H9-MSA or combination therapy. (B) Antitumor effects were reversed by depleting CD8 T cells (p<0.0001), with no significant impacts of depleting NK cells or CD4 T cells.

CDN+H9-MSA improves anti-tumor efficacy in the methylcholanthrene induced sarcoma (MCA) model mediated by NK, CD4 and CD8 T cells



MCA was injected s.c. on the right flanks and mice were monitored frequently for palpable tumors, which arose 8-10 weeks after carcinogen administration in 80% of the treated animals. When MCA-induced tumors reached ~50 mm^3 in size, tumors were injected i.t. with PBS or 50 ug CDN on days 0, 3, and 6 and repeated every 6 six days thereafter. Mice were also injected i.p. with PBS or 10 ug H9-MSA starting on day 0, which was repeated every three days. (A) Tumor growth and survival curves. (B) Mice that received combination therapy were injected i.p. on days -2, -1 and every 6 days thereafter with antibodies to deplete NK cells, CD8 T cells, CD4 T cells, or with control IgG. Tumor growth curves and survival of mice are shown.

Combining CDN+H9-MSA with anti-PD-1/anti-TIGIT therapy improves anti-tumor efficacy in the MCA model



MCA was injected and tumors were treated as described above. Addition of checkpoint inhibitors consisting of i.p. injections of anti-PD-1 (clone RMP1-14) and anti-TIGIT (clone MUR10A), increased tumor rejection and overall survival.

References and Acknowledgements

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