Mechanisms of action of a killed, bacteria-based, multiple immune receptor agonist (Decoy platform) in development for pulsed anti-tumor immunotherapy

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CICON 2025 Abstract #203 (Friday Sept. 12 Session B 12:00 pm - 3:00 pm)

Background

Activation of immune receptors, such as Toll-like (TLR), NOD-like (NLR) and Stimulator of Interferon Genes (STING) is required for efficient innate and adaptive immunity. Gram-negative bacteria (G-NB) contain multiple TLR, NOD and STING agonists. Potential utility of G-NB for cancer immunotherapy is supported by observations of tumor regression in the setting of infection and Coley's Toxins. Coley reported that intravenous (i.v.) administration was likely most effective but produced toxicity. The discovery of TLRs and their agonists, particularly the potent TLR4 agonist lipopolysaccharide (LPS)-endotoxin, comprising ~75% of the outer membrane of G-NB, suggests that it may be both a critical active ingredient and responsible for dose-limiting toxicity of i.v. G-NB. LPS analogues are also clinically validated vaccine adjuvants.

We have produced killed, intact bacteria products from non-pathogenic G-NB with ~95% reduction of LPS activity. One product, Decoy10, contained TLR2,4,8,9, NOD2 and STING agonist activity and exhibited reduced i.v. toxicity in mice and rabbits relative to unprocessed cells. Decoy10 and closely related Decoy20 produced single agent activity or combination-mediated durable regressions, with immunological memory, in mice with syngeneic or human colorectal, hepatocellular, pancreatic carcinomas or non-Hodgkin's lymphoma. Regressions were observed in combination with chemotherapy, a non-steroidal anti-inflammatory drug, anti-PD-1, or rituximab, were associated with induction of 18-26 plasma cytokines/chemokines, activation of innate and adaptive immune pathways in tumors, and were dependent on NK, CD4+ and CD8+ T cells (1).

Preliminary clinical results demonstrating rapid clearance of Decoy20 with transient induction of >50 plasma cytokines and chemokines (2-4) are supportive of a "pulse-prime" mechanism, whereby Decoy bacteria produce transient, but broad innate and adaptive immune activation (NCT05651022).

Results Summary

We have analyzed immune activation by Decoy bacteria *in vitro* using human peripheral blood mononuclear cells (PBMCs). Decoy10 induced immature to mature M1 macrophage maturation, monocyte-derived dendritic cell maturation, Th1 CD4+ T cell polarization and inhibited Treg production. Decoy10 induced activation markers in Natural Killer (NK), Natural Killer T (NKT), NKT-Like, CD4+ T, CD8+ T and CD4+CD8+ double positive T cells, and stimulated production of gamma-delta (γδ) T cells. Some results were dependent on or enhanced by Decoy10 combination with IL-2. Finally, Decoy10 enhanced PBMC-mediated tumor cell killing. The results demonstrate that Decoy bacteria stimulate maturation, polarization or activation of cellular mediators of innate and adaptive anti-tumor immune responses, inhibit an immune-suppressive mechanism, and enhance immune cell killing of tumor cells.

Methods

Decoy10 was prepared as described (1) with final assay concentrations representing per mL values. Experiments were carried out at HD Biosciences (San Diego, CA) in triplicate or duplicate when PBMCs from three donors were tested (all 96-well format) using complete (GlutaMAX) RPMI medium (supplemented with L-glutamine, sodium pyruvate, non-essential amino acids, Hepes, penicillin/streptomycin, and β-2 ME) with fetal bovine serum (human serum for the macrophage assay). Frozen human PBMCs were obtained from StemExpress (Placerville, CA) or AllCells (Alameda, CA), or isolated from whole human blood purchased from the San Diego Blood Bank using LymphoPrep/SepMate from StemCell Technologies. Flow cytometry studies were carried out with an Attune Flow Cytometer from ThermoFisher.

CD16-CD14+ monocytes were isolated from PBMCs with the StemCell 19359 EasySep Kit. M1 macrophage maturation was assessed with immature M1 macrophages produced by incubating 5x10^4/well monocytes for 4 days with 50 ng/mL GM-CSF. After centrifugation-based washing the cells were incubated with Decoy10 or a positive control (50 ng/mL IFN-γ + IL-6, 20 ng/mL LPS) for 3 days followed by analysis of surface CD80 and intracellular iNOS.

Dendritic cell maturation was assessed with immature DCs by incubating 1.25x10^5/well CD16-CD14+ monocytes for 2 days with 500 ng/mL GM-CSF + IL-4. After centrifugation-based washing, the immature DCs were incubated for 3 days in fresh medium containing GM-CSF + IL-4 and maturation cytokines (2,000 ng/mL PGE2, 10 ng/mL IL-6, IL-1β, TNF-α; positive control), or GM-CSF + IL-4 and Decoy10 followed by analysis of surface CD80, CD83 and CD86. Greater than 90% of double positive cells were also triple positive (not shown).

CD4 Th1 polarization was assessed after 6-day PBMC incubation (2.5x10^5/well) without or with 1 μg/mL anti-CD28, 1 μg/mL IL-4, 20 U/mL IL-2 and 20 ng/mL IL-12p70 (positive control), or with Decoy10 (± 20 U/mL IL-2 + 1 μg/mL anti-CD28), followed by analysis of surface CD4 and intracellular IFN-γ. The effect of Decoy10 on CD4 Treg polarization was assessed by incubating PBMCs as above with 1 μg/mL anti-CD28, 100 U/mL IL-2 and 5 ng/mL TGF-β ±Decoy10 followed by analysis of CD4, CD25, and FoxP3 expression.

Activation of NK (CD56+, CD4-, CD8-), NKT (CD56+, CD4+, CD8-), NKT-Like (CD56+, CD4-, CD8+), CD4+ T (CD56-, CD4+, CD8-), CD8+ T (CD56-, CD4-, CD8+) and CD4+/CD8+ double positive T (CD56-, CD4+, CD8+) cells was assessed by incubating PBMCs (2.5x10^5/well) for two days with 100 U/mL IL-2 (positive control for NK cells and all T cells), or with 2.5 μg/mL anti-CD28 in wells pre-coated overnight with 100 μL of 5 μg/mL OKT3 anti-CD3 antibody (additional positive control for all T cells) ± 1x10^7 Decoy10/mL, or with Decoy10 alone. Cell activation was assessed by analysis of surface CD69, CD226, and intracellular IFN-γ, Perforin, and Granzyme B. Immune activation studies were carried out with three independent PBMC donors (Red, Green, Blue) in duplicate. Results for one donor were not included for NKT-Like cells due to significant discrepancies between duplicates.

Induction of CD3+CD4-CD8- γδ2 T cells was carried out by incubating PBMCs at 3x10^5 per well for 6 days with 1-Hydroxy-2-methyl-2-butenyl 4-pyrophosphate (HMBPP, Sigma #95098) or Decoy10 followed by staining for CD3, CD4, CD8, Vγ9, and Vδ2.

Killing of human MDA-MB-231 breast carcinoma cells (ATCC) by Decoy10 + PBMCs was assessed with IncuCyte™ NucLight™ Red Lentivirus-transfected tumor cells (reagents from Essen Biosciences, Ann Arbor, Michigan). Tumor cells were plated at 2x10^4/well and incubated overnight in complete RPMI medium. PBMCs (1x10^4 per well) and/or Decoy10 (3x10^6 per mL) were added, and the incubation was continued for 7 days followed by image analysis using the IncuCyte™ system (Sartorius).

Statistical significance for flow cytometry experiments was analyzed with GraphPad Prism software using a standard, unpaired t-test with comparison to the appropriate no addition, vehicle, or single agent control, with results indicated by standard p≤0.05-0.0001 (*, **, ***, ****) notation.

Discussion

Induction of maturation, polarization or activation of multiple innate and adaptive immune cell types, and enhancement of PBMC-mediated tumor cell killing by Decoy10 is consistent with our pre-clinical *in vivo* data demonstrating that tumor eradication by Decoy10 combination therapy is dependent on NK, CD4 T and CD8 T cells, and is associated with innate and adaptive immune pathway activation (1).

The data are also consistent with our preliminary Phase 1 clinical trial results, where we have observed broad plasma cytokine and chemokine-associated immune activation (2-4).

The current data also suggest that Decoy bacteria may produce additive or synergistic effects with IL-2 in some settings.

References

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2. Newman M. et al., J. Immunother. Cancer 2023; 11 782-E
3. Waltzman R. et al., J. Clin. Oncol. 2024, 42 (16) Suppl 2583
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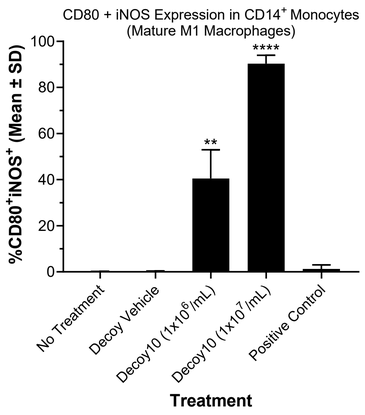
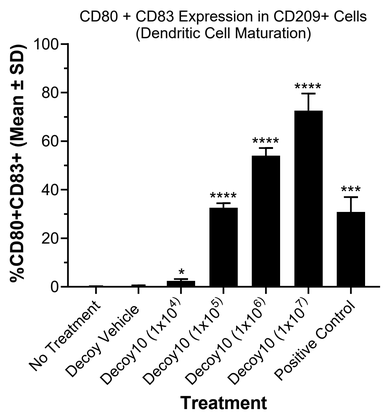
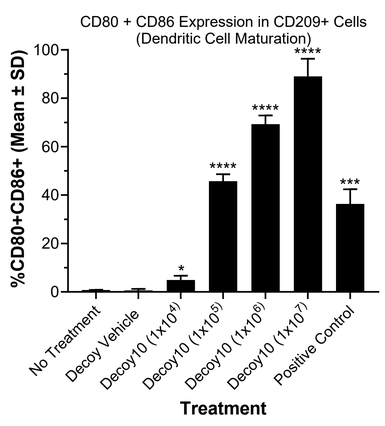
Decoy10 Induced M1 Macrophage Maturation, DC Maturation, CD4+ Th1 Polarization, Treg Inhibition, Activation Markers in NK, NKT, CD4+ and CD8+ T Cells, Production of Gamma-Delta T Cells and Enhanced PBMC-Mediated Killing of Tumor Cells

Decoy10 Induces M1 Macrophage Maturation

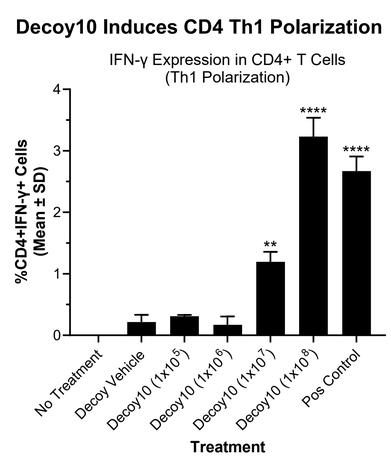


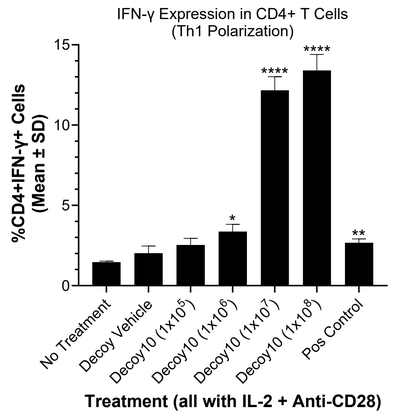
Decoy10 Induces Dendritic Cell Maturation



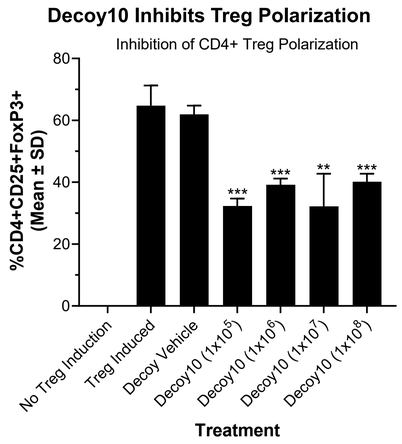


Decoy10 Induces CD4 Th1 Polarization

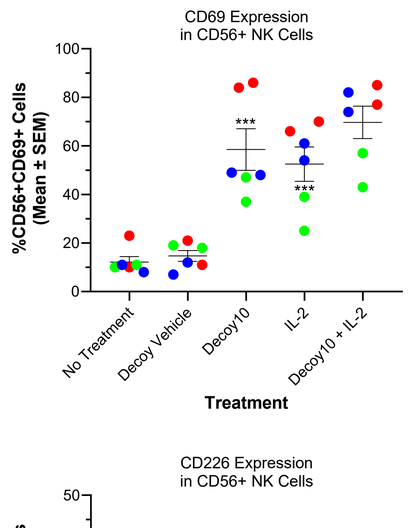


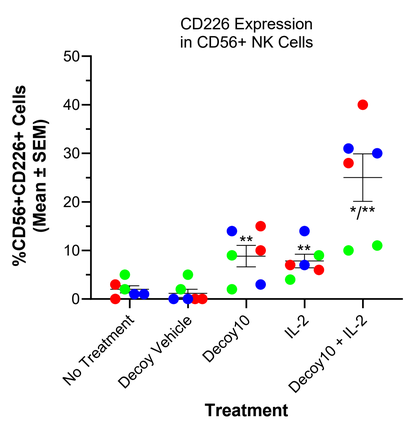


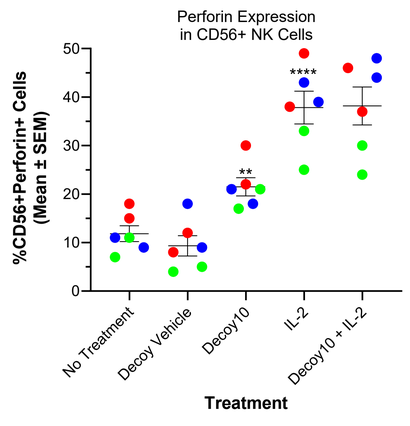
Decoy10 Inhibits Treg Polarization



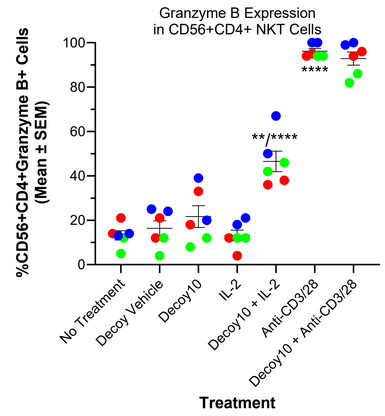
Decoy10 Induces Activation Markers in NK Cells (3 PBMC donors [red, green, blue])



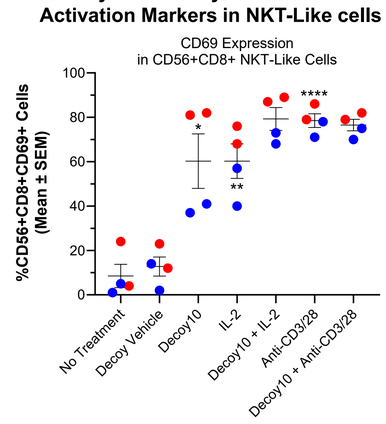


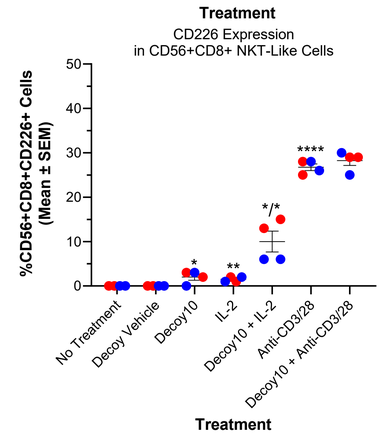


Decoy10 or Decoy10 + IL-2 Induce Activation Markers in NKT cells

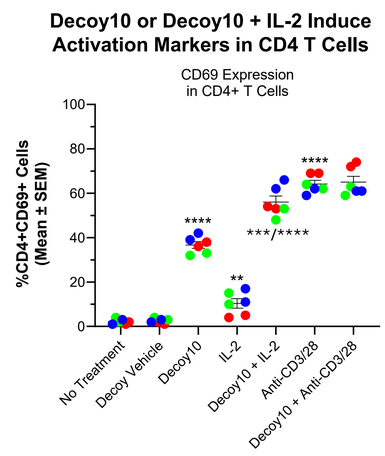


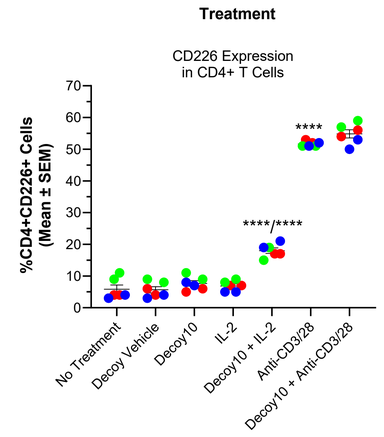
Decoy10 or Decoy10 + IL-2 Induce Activation Markers in NKT-Like cells

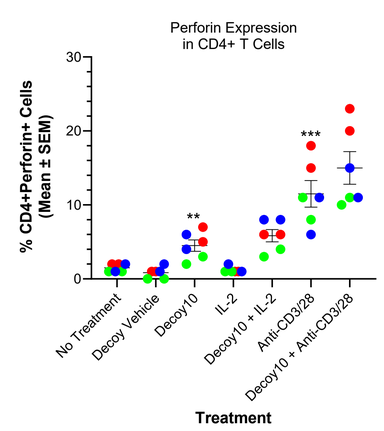




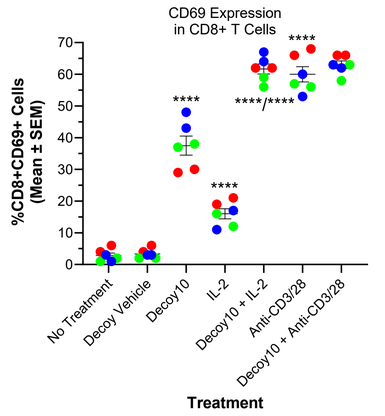
Decoy10 or Decoy10 + IL-2 Induce Activation Markers in CD4 T Cells

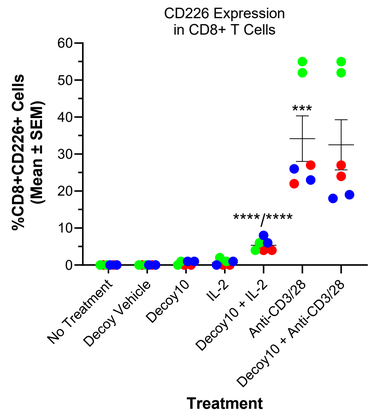




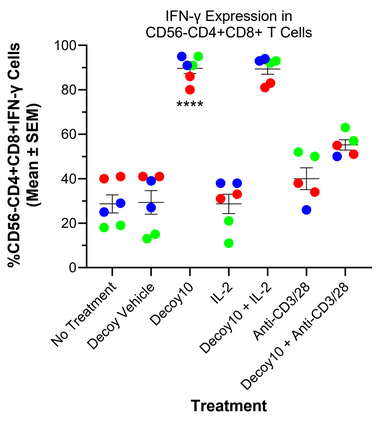


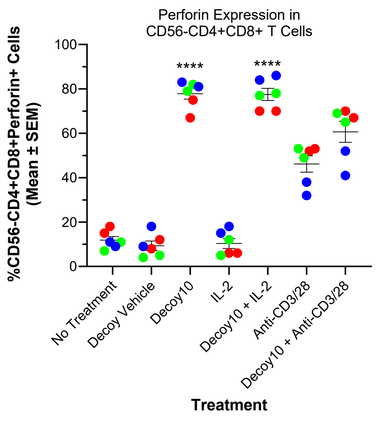
Decoy10 Induces Activation Markers in CD8 T Cells



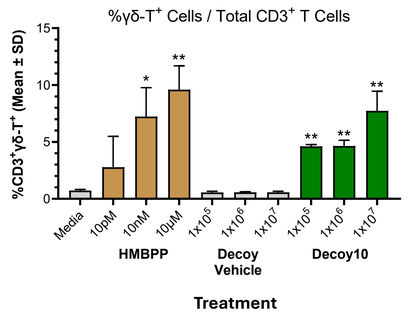


Decoy10 Induces Activation Markers in CD4+CD8+ Double Positive T Cells





Decoy10 induces γ9δ2-T Cells



Decoy10 Enhances Killing of Human Breast Carcinoma Cells by PBMCs