Efficacy of injectable antigen presenting nanoparticles (AIM INJ) in solid tumor models

Haiyun Liu, Duong Nguyen, Durgadas Cherukaraveedu, Adam Parks, Bryan Hahn, Daniel Dembrow, Sojung Kim, Jack Ragheb, Aniket Wadajkar, Mathias Oelke
NexImmune Inc., Gaithersburg, MD

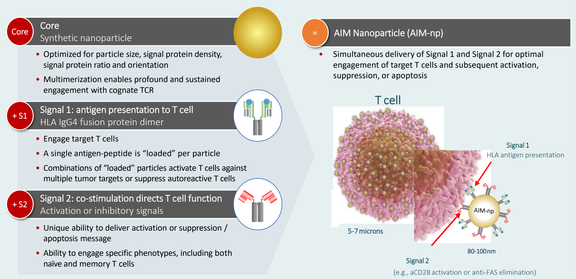
INTRODUCTION

NexImmune's Artificial Immune Modulation (AIM) nanoparticle (NP) platform (AIM INJ) is an injectable multi-antigen specific off-the-shelf immunotherapy designed to directly modulate T cell responses in vivo. It consists of PLA-PEG NPs conjugated with two proteins, a dimeric IgG MHC-class I fusion protein loaded with a tumor peptide and an anti-CD28 antibody for co-stimulation. Multiple batches of NPs, loaded with a single peptide, are mixed to create a multi-antigen specific NP product. AIM INJ NP expanded T cells are antigen-specific, polyfunctional and consist of memory phenotypes associated with anti-tumor activity and immunologic memory.

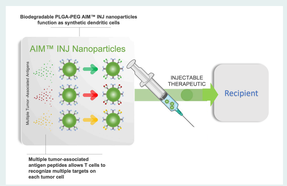
The ability of AIM INJ NPs to activate and expand antigen-specific T cells in vivo has been demonstrated in various mouse models. In vivo efficacy data in B16F10-OVA and B16F10 tumor model are presented.

AIM PLATFORM

AIM nanoparticles designed to act as synthetic dendritic cells to deliver precise instructions to targeted T cells



AIM INJ – Direct Injection Therapy

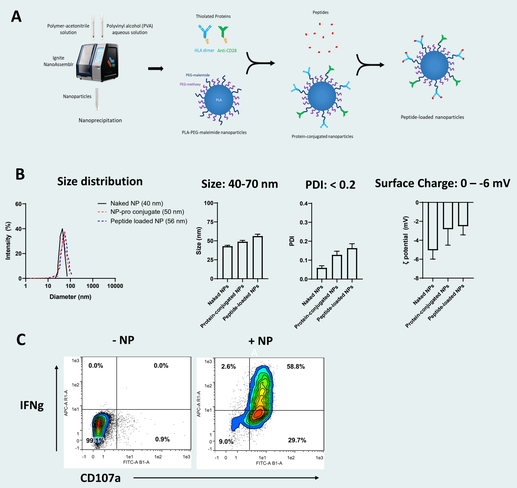


Batches of AIM nanoparticle can be loaded with individual antigen and later combined to create a desired antigen cocktail for specific disease.

Key platform and modality benefits

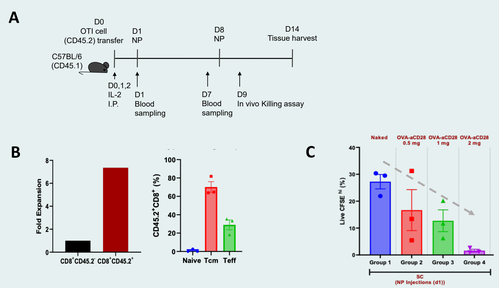
- **Disruptive potential** as a highly targeted multi- antigen specific SOC or as novel backbone therapy
- **Direct *in vivo* engagement** with cognate CD8+ T cell receptors
- **"Off-the-shelf"** products increases and simplifies access, speed to HCP administration
- Ability to target **multiple antigens** in a single product
- Can deliver **up-regulatory** or **down-regulatory** signals directly to targeted T cells (or T cell populations) without impairing healthy tissue or immune function
  - Break tolerance: Activation signals for oncology, infectious disease
  - Establish tolerance: Suppression or apoptotic signals to treat autoimmune diseases

AIM INJ Nanoparticle Characterization



(A) AIM INJ nanoparticles are synthesized using nanoprecipitation on the Ignite NanoAssemblrTM microfluidic system. AIM INJ nanoparticles are formulated by conjugating Signal 1 and 2 proteins, followed by loading antigenic peptides. (B) Physicochemical characterization demonstrates homogenous and monodispersed PLA-PEG Nanoparticles with near-neutral surface charge. Size, PDI, and surface charge of nanoparticles were measured by DLS. (C) shows one representative example of functional quality control. AIM INJ nanoparticle conjugated with H-2D^b and murine anti-CD28 and loaded with gp100 peptide stimulates and induces effector function such as IFNg production and CD107a upregulation in Pmel transgenic CD8 T cells in vitro.

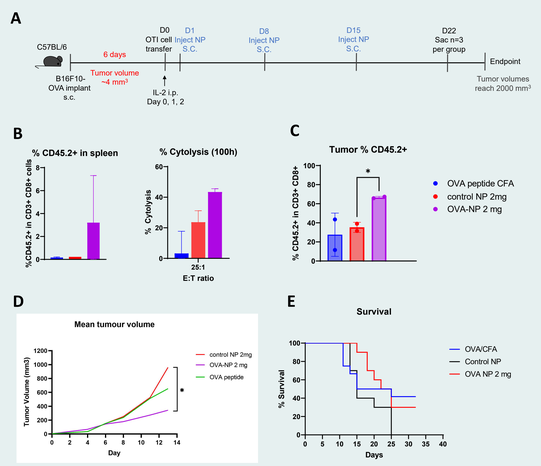
Antigen specific T cell expansion In vivo



(A) Functional ability of AIM INJ NPs to expand target antigen-specific T cells with desired memory phenotypes was demonstrated in vivo by adoptive transfer of naïve OVA-specific T cells (OT-1) into wild type mice and subsequently injecting them with OVA peptide-loaded H-2K^b/aCD28 NP. (B) Fold expansion and phenotype on day 7 were shown.

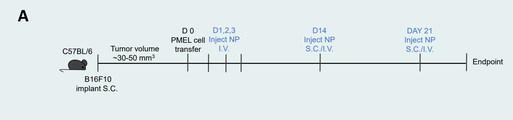
(C) To demonstrate functionality of expanded T cells, in vivo killing assay was performed on day 9. CSFE labeled, peptide pulsed targets were administered i.v. on day 9 and killing of target cells was measured by flow in splenocytes on day 10. NP dose-dependent target cell killing was demonstrated.

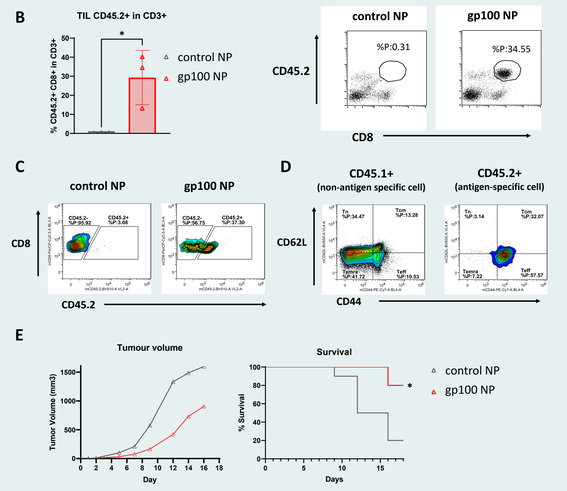
Efficacy of AIM INJ in B16F10-OVA tumor model



An *in vivo* efficacy study was performed to evaluate the anti-tumor effects of AIM INJ NPs in a syngeneic mouse B16F10-OVA solid tumor model. (A) A treatment scheme is shown. (B) On day 22, spleens were collected and analyzed for frequency and cytolytic activity of expanded OVA-specific T cells. OVA NP treated group had 15-fold more OT-1 cells than control group. T cells from the OVA NP treated group showed higher killing activity against OVA-expressing tumor targets than other treatment groups. (C) The OVA NP treated group generated significantly more OVA specific CD8+ tumor infiltrating lymphocytes (TIL, 67% of CD8+ T cells in tumor), compared to the control peptide unloaded NP treated (35% TIL) and OVA peptide/CFA treated (27% TIL) groups. (D) Treatment of mice with the OVA AIM INJ NPs significantly delayed B16F10-OVA tumor growth and (E) showed increased survival as compared to mice treated with peptide unloaded NPs.

Efficacy of AIM INJ in B16F10 tumor model targeting endogenous tumor antigen





AIM INJ NPs targeting endogenous tumor antigen gp100 are efficacious in the B16F10 tumor model. (A) A treatment scheme is shown. (B) On day 13, tumors were collected from 3 mice/group and analyzed for TIL. T cells from the gp100 NP treated group showed significantly more gp100 specific CD8+ TIL, compared to the control unloaded NP treated group. A representative flow plot from each group is shown. (C) gp100-specific TILs were still present in mice at terminal harvest. (D) Interestingly, while gp100-specific T cells (CD45.2+ CD8+) consisted of memory and effector T cells, non-antigen specific T cells (CD45.1+ CD8+) had significant amounts of terminal effector T cells. (E) Treatment of mice with the gp100 AIM INJ NPs delayed B16F10 tumor growth and increased survival as compared to mice treated with peptide unloaded NPs using a study endpoint when tumor size reaches 1500mm^3 in all mice in the control group.

CONCLUSION

AIM INJ NPs can activate and expand antigen-specific CD8$^+$ T cells that elicit anti-tumor activity in vivo. More than 80% of antigen specific T cells were memory phenotypes associated with durability, which persisted and maintained anti-tumor killing potential. The ability to load batches with individual antigen allows for multi-antigen targeted therapies to address the heterogeneity of each tumor type. These studies along with other in vivo pre-clinical studies, including further evaluation of a dose, regimen, and route of administration, will be used to support our INDs for using AIM INJ in Phase 1 studies for solid tumors. AIM INJ potentially addresses gaps in current immunotherapies by directly expanding multiple tumor antigen-specific T cells that traffic to the tumor, establishing T cell memory for persistence and durability. Off-the-shelf modality of AIM INJ supports easy access, scalability, and enables rapid multi-antigen product development within months (an "IND engine").

Abstract No. 1139

Presented at the Society for Immunotherapy of Cancer (SITC) 38th Annual Meeting, November 1–5, 2023, San Diego, CA