A polymeric phosphoantigen prodrug nanoparticle delivery vehicle to program antitumor γδ T cells

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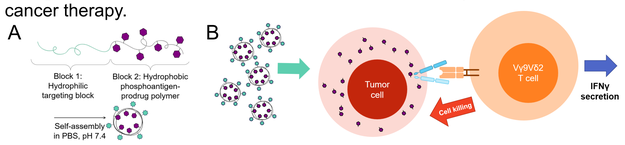
Abstract

Vγ9Vδ2 T cells, a subset of unconventional T cells, can efficiently eradicate tumors upon activation. Adoptive transfer therapies of *ex vivo*-activated Vγ9Vδ2 T cells have been tested in the clinic, but systemic administration of cells and Vγ9Vδ2-activating small molecule phosphoantigens (pAgs) has been met with limited efficacy, likely due to rapid clearance of pAgs and lack of Vγ9Vδ2 tumor localization. Furthermore, adoptive cell therapies are available only in select locations and come with a high price tag. We hypothesize that targeted delivery of large payloads of pAgs directly to the tumor site can facilitate localized Vγ9Vδ2 activation and subsequent tumor cell killing. We have developed a novel **polymeric phosphoantigen prodrug nanoparticle** (**P^{3}NP**) which has the potential to activate Vγ9Vδ2 T cells *in situ*, sidestepping limitations associated with adoptive cell transfer and systemic delivery of activation agents. We synthesized multiple novel polymers with drug loading over 40% and self-assemble into nanoparticles which demonstrate preferential tumor cell uptake over untargeted controls as well as Vγ9Vδ2-mediated tumor killing *in vitro*. We will next assess P^{3}NPs *in vivo* in xenograft tumor models. If successful, P^{3}NPs could be a powerful, cell-free technology for accessible cancer therapy.



Scheme 1. (a) Visual representation of block copolymer self-assembly into pAg-loaded nanoparticles. (b) Proposed mechanism of action for nanoparticle internalization and subsequent γδ T cell activation.

Introduction

- Vγ9Vδ2 T cells are a subset of γδ T cells that can eliminate tumor cells and recruit pro-inflammatory immune cells upon activation.[1] These unconventional T cells have advantages over other immune cell types in antigen recognition and cell activation (**Table 1**).[2,3]

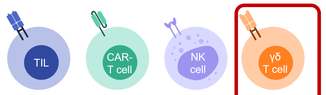
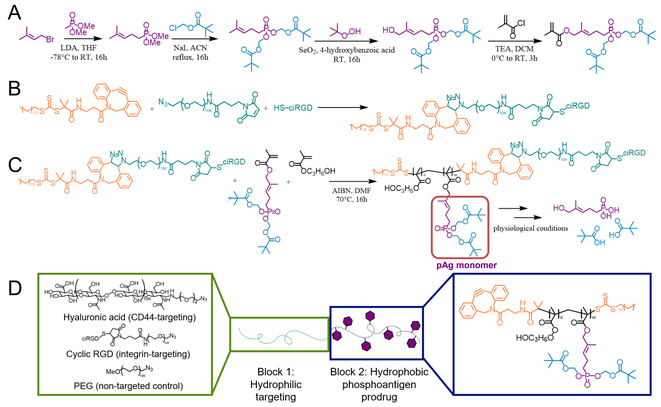


Table 1. Considerations for adoptive transfer with various immune cell types

	TIL	CAR-T cell	NK cell	γδ T cell
Does not require MHC presentation or antigen processing	X	✓	✓	✓
Not susceptible to treatment resistance due to antigen escape	X	X	✓	✓
Not susceptible to MHC-I, "self" markers	✓	✓	X	✓
Long-lived responses due to T cell expansion	✓	✓	X	✓

- Although therapies composed of *ex vivo*-activated Vγ9Vδ2 T cells with bolus injections of pAgs have been tested in the clinic, several roadblocks have stymied the clinical efficacy of these approaches:[4,5]
  1. Adoptive cell therapies tend to be expensive and inaccessible due to limited number of centers for sterile cell expansion.
  2. Free pAgs are small, hydrophilic molecules and thus have fast clearance kinetics in systemic circulation. Systemic exposure to pAgs may also result in global Vγ9Vδ2 anergy.
  3. Lack of pAg tumor localization may also result in limited Vγ9Vδ2 tumor killing.
- Vγ9Vδ2 T cells are activated by internalization of small molecule phosphoantigens (pAgs) by malignant cells.[6] pAgs are small and hydrophilic, making them difficult to encapsulate in traditional NP vehicles.[7]
- To overcome issues associated with current experimental Vγ9Vδ2 therapies, we developed a drug delivery platform engineered to maintain activation of Vγ9Vδ2 cells by **providing durable, localized delivery of therapeutic quantities of pAg to tumor cells**.
- We synthesized RAFT polymers that **incorporate pAg directly into the biomaterial backbone** and **target/retain nanoparticles to tumors**.
- We utilized click chemistry to combine hydrophobic and hydrophilic polymer blocks to allow for **self-assembly into nanoparticles.**

Click-functionalized prodrug RAFT polymers demonstrate high pAg loading capacity



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Polymer Structure	Polymer Name	Mw	PDI	pAg wt%
	HA-poly(HPMA-pAg)	150,000	1.036	50%
	RGD-poly(HPMA-pAg)	38,000	1.365	46%
	PEG-poly(HPMA-pAg)	93,000	1.224	53%

Fig 1. Synthetic schemes for (A) pAg prodrug methacrylate monomer, (B) RGD RAFT agent and (C) RGD-poly(HPMA-pAg) polymer. Biodegradation of covalent ester bonds on pAg monomer under physiological conditions. (D) general structure schematic of pAg prodrug polymers. (E) structure, name, weight-average molecular weight (Mw), polydispersity index (PDI) and pAg loading weight percentage (wt%) of P^{3}NP component polymers as determined by gel permeation chromatography and nuclear magnetic resonance.

RGD-P^{3}NPs display preferential tumor cell uptake

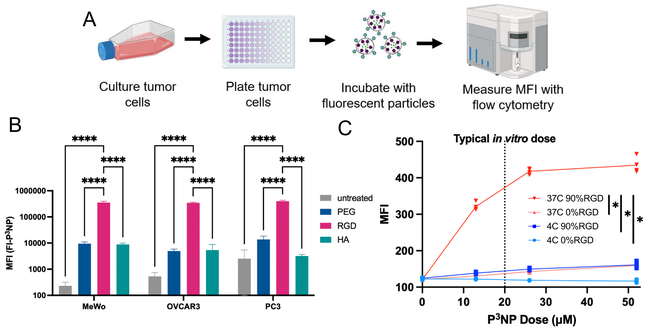

Fig 2. (A) Methods for Fl-P^{3}NP uptake (B) Fl-P^{3}NP uptake in multiple cancer cell types. n = 4; Two-way ANOVA with Tukey's multiple comparisons. **** = p < 0.0001. All other comparisons ns. Comparisons between treatments within each cell type. (E) Fl-P^{3}NP uptake in PC3 tumor cells at 4C and 37C. n = 4; One-way ANOVA with Tukey's multiple comparisons. * = p < 0.05. All other comparisons ns.

RGD-P^{3}NPs facilitate γδ-mediated tumor cell killing across cancer cell types

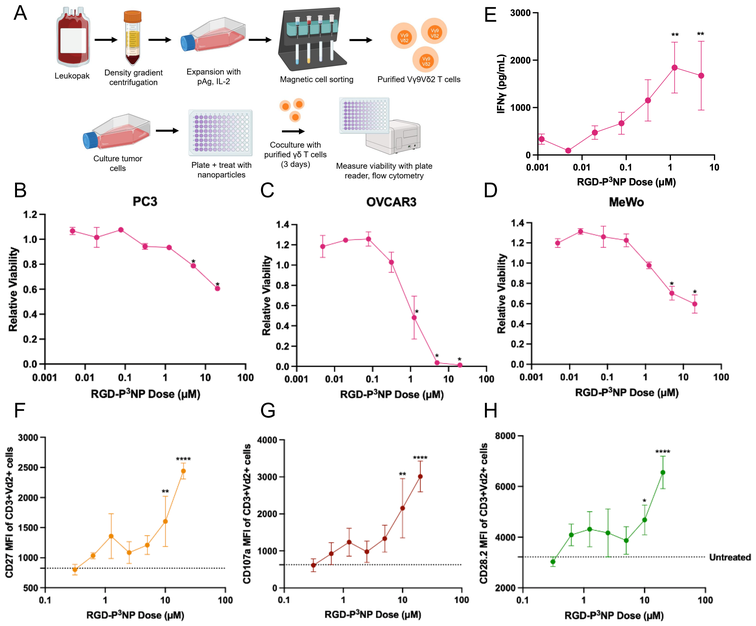

Fig 3. (A) Methods for isolation of PBMC-derived Vγ9Vδ2 T cells and coculture with tumor cells. (B,C,D) Relative viability of tumor cells after 4h treatment with RGD-P^{3}NPs and subsequent 72-hour culture for three tumor cell types. Viability of co-cultured tumor cells normalized to equivalently treated cells in monoculture. Ordinary one-way ANOVA with Dunnett's multiple comparisons test, comparisons to untreated control, * = p<0.0001. All other comparisons ns. (E) Levels of IFNγ in the supernatant media of cultures in (B) as measured by ELISA. Statistical comparisons to lowest dose. ** = p<0.01. All other comparisons ns. (F,G,H) Surface expression of CD27, CD107a, and CD28.2 of Vγ9Vδ2 cells treated with RGD-P^{3}NPs as determined via flow cytometry. Statistical comparisons to untreated control, ** = p<0.01; **** = p < 0.0001, all other comparisons ns.

Conclusions

- Prodrug polymers successfully incorporate pAg at levels three-fold higher than traditional NP encapsulation.[8]
- RGD-P^{3}NPs outperform other P^{3}NP formulations and display active uptake *in vitro*
- P^{3}NPs demonstrate γδ T cell-mediated cytotoxicity and pro-inflammatory activation, indicating potential translatability to an *in vivo* tumor model.
- We are currently exploring a murine xenograft tumor model to assess the efficacy of P^{3}NPs *in vivo.*

Acknowledgements

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Figures contain assets from BioRender.

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