Synapse-Functionalized Lipid Nanoparticles Loaded with Neoantigen mRNA Enhance Dendritic Cell Cross-Presentation and CD8$^+$ T Cell Memory

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ORIGINAL RESEARCH

Abstract

Neoantigen-based mRNA vaccines depend on efficient cross-presentation by dendritic cells (DCs) to prime tumor-specific CD8$^+$ T cell responses, yet endosomal entrapment and inefficient cytosolic escape remain major barriers to potent immunogenicity. Here we report a Synapse-functionalized ionizable lipid nanoparticle (Synapse-LNP) platform engineered to enhance cytosolic mRNA delivery and MHC class I cross-presentation in murine bone-marrow-derived dendritic cells (BMDCs). Synapse-LNPs encapsulating mRNA encoding three validated B16F10 melanoma neoantigens achieved 91.4% encapsulation efficiency, a mean hydrodynamic diameter of 84.2 nm, and a polydispersity index below 0.15. Compared with unmodified LNPs, Synapse-LNPs increased DC uptake by 2.3-fold and upregulated the maturation markers CD86, CD80, and surface MHC-I by 1.8–2.6-fold at 24 h post-treatment. In vivo, C57BL/6 mice immunized with Synapse-LNP vaccine exhibited a 24.6% frequency of interferon-γ^+ CD8$^+$ T cells among splenocytes, an 11.7-fold increase over free-peptide immunization and a 7.0-fold increase over unmodified LNPs. Vaccinated mice challenged with B16F10 tumor cells showed a 68% reduction in tumor volume at day 21 and a significant survival extension relative to controls (log-rank $p < 0.001$). These findings identify Synapse functionalization as an effective strategy for overcoming cytosolic delivery bottlenecks in mRNA neoantigen vaccines and support further preclinical development of the platform for personalized cancer immunotherapy.

Keywords: lipid nanoparticles, mRNA vaccine, cross-presentation, dendritic cells, CD8$^+$ T cells, tumor neoantigens, immunological memory, cancer immunotherapy

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1 Introduction

Therapeutic vaccination against tumor neoantigens has emerged as one of the most promising strategies in precision oncology, offering the possibility of eliciting durable, tumor-specific cytotoxic T lymphocyte responses while sparing healthy tissue (Castillo and Torres, 2024). Unlike shared tumor-associated antigens, neoantigens arise from somatic mutations unique to an individual tumor and are not subject to central immune tolerance, making them attractive targets for personalized immunotherapy (Reyes and Falk, 2023). Messenger RNA (mRNA) has become the delivery modality of choice for encoding these antigens, combining rapid, sequence-agnostic manufacturing with the ability to encode multiple epitopes in a single construct (Anand and Whitfield, 2025).

The clinical success of mRNA vaccines nevertheless hinges on efficient delivery to antigen-presenting cells, and in particular on cross-presentation: the process by which dendritic cells (DCs) route exogenously acquired antigen into the MHC class I pathway to prime naive CD8$^+$ T cells (Lindgren and Okafor, 2022). Conventional ionizable lipid nanoparticles (LNPs) achieve efficient endosomal uptake by DCs, but most internalized cargo remains trapped within maturing endosomes and is degraded before reaching the cytosol, where proteasomal processing and MHC-I loading must occur (Bianchi and Marlowe, 2021). Fewer than 2% of endocytosed LNP cargo is estimated to achieve productive cytosolic release under standard formulations, a major bottleneck for cross-presentation efficiency (Okonkwo and Petrov, 2023). Strategies explored to improve escape, including pH-responsive lipids and fusogenic peptide conjugates, often introduce cytotoxicity or rely on triggers difficult to translate to systemic administration (Alvarez and Dubois, 2024).

We reasoned that a surface-functionalization strategy targeting DC-specific uptake and trafficking, rather than a bulk lipid-chemistry change, could improve cytosolic delivery while preserving formulation simplicity, building on evidence that C-type lectin receptor engagement can route nanoparticle cargo through DC-selective uptake pathways (Falk and Reyes, 2020). Here we describe Synapse-LNP, an ionizable lipid nanoparticle platform surface-functionalized with a DC-targeting glycopeptide ligand (the "Synapse" moiety) designed to engage C-type lectin receptors on conventional type 1 DCs and promote early endosomal escape. Our objectives were to:

1. Formulate and physicochemically characterize Synapse-LNPs encapsulating mRNA encoding validated B16F10 melanoma neoantigens.

2. Quantify DC uptake, maturation, and cross-presentation efficiency in vitro relative to unmodified LNPs and free peptide.

3. Evaluate neoantigen-specific $CD8^+$ T cell responses, tumor growth control, and survival in an immunocompetent murine melanoma model.

Together, these experiments test whether targeted endosomal-escape engineering translates into a measurable gain in vaccine-induced antitumor immunity.

2 Materials and Methods

2.1 Nanoparticle Formulation and Characterization

Lipid nanoparticles were formulated by microfluidic mixing of an ethanolic lipid phase with an aqueous mRNA phase at a 3:1 flow rate ratio using a Meridian NanoAssemblr benchtop system. The lipid phase consisted of an ionizable amino-lipid, cholesterol, DSPC, and a PEG_{2000}-lipid at a molar ratio of 50:38.5:10:1.5. For the Synapse-LNP formulation, 1.5 mol% of the PEG-lipid was substituted with a Synapse glycopeptide–PEG-DSPE conjugate synthesized via maleimide-thiol coupling. All formulations encapsulated a 1:1:1 mixture of mRNAs encoding three validated B16F10 neoantigens ($Kif18b^{driver}$, $Cpne1^{mut}$, $Rpl18^{mut}$), each fused to an N-terminal ubiquitin-degron sequence to enhance proteasomal turnover. Hydrodynamic diameter and polydispersity index were measured by dynamic light scattering (Nexa Zetasizer Pro); zeta potential was determined by laser Doppler electrophoresis in 10 mM HEPES (pH 7.4); and mRNA encapsulation efficiency was quantified by a modified Ribogreen assay before and after LNP disruption with 1% Triton X-100. All measurements were performed in triplicate across three independent batches.

2.2 Dendritic Cell Uptake and Maturation Assays

Bone-marrow-derived dendritic cells (BMDCs) were differentiated from C57BL/6 bone marrow progenitors over 7 days in GM-CSF-supplemented medium. For uptake assays, BMDCs were incubated with Cy5-labeled LNPs at 2 μg/mL mRNA-equivalent dose for 2 h at 37°C, followed by flow cytometric quantification of $Cy5^+$ frequency and mean fluorescence intensity. Maturation was assessed 24 h post-treatment by staining for CD86, CD80, CD40, and surface MHC-I ($H\text{-}2K^b$) on a Vertex Aurora spectral cytometer with FlowJo v10.

2.3 In Vivo Immunization and Tumor Challenge

All procedures were approved by the Vertex University IACUC (protocol VU-IACUC-2025-0142). Female C57BL/6 mice (8–10 weeks, $n = 10$ per group) received two intramuscular immunizations, 14 days apart, of PBS, empty LNP, free neoantigen peptide pool (10 μg per peptide), unmodified LNP vaccine, or Synapse-LNP vaccine (each at 5 μg total mRNA). Splenocytes were harvested 7 days after the boost for intracellular cytokine staining following 6 h ex vivo restimulation with peptide pool in the presence of brefeldin A, and interferon-γ^+ $CD8^+$ T cell frequency was quantified by flow cytometry. For tumor challenge, a separate cohort was immunized identically and inoculated subcutaneously with 2×10^5 B16F10 melanoma cells 7 days after the boost, using a syngeneic model previously validated for neoantigen vaccine evaluation (Whitfield and Bianchi, 2022). Tumor volume was measured every 3 days ($V = \frac{1}{2} \times \text{length} \times \text{width}^2$), and animals were monitored to a humane endpoint of 1500 mm^3.

2.4 Statistical Analysis

Data are presented as mean $\pm$ SEM. Comparisons across groups used one-way ANOVA with Tukey's post-hoc correction; tumor growth curves were compared by two-way

repeated-measures ANOVA; survival was analyzed by the log-rank test. $p < 0.05$ was considered significant. Analyses were performed in GraphPad Prism 10.

3 Results

3.1 Synapse Functionalization Yields Stable, Monodisperse Nanoparticles

Both unmodified and Synapse-functionalized LNPs formed stable, monodisperse particles suitable for parenteral administration (Table 1). Synapse functionalization did not substantially alter particle size or encapsulation efficiency, but modestly shifted the zeta potential toward neutrality, consistent with successful surface conjugation of the glycopeptide ligand.

Table 1: Physicochemical characterization of unmodified and Synapse-functionalized LNP formulations encapsulating B16F10 neoantigen mRNA. Values are mean ± SD across three independent batches.

Parameter	Description	Unmodified LNP	Synapse-LNP
Hydrodynamic diameter	Z-average, DLS	79.6 ± 3.1 nm	84.2 ± 2.8 nm
Polydispersity index	DLS PDI	0.128 ± 0.02	0.142 ± 0.02
Zeta potential	10 mM HEPES, pH 7.4	-4.8 ± 1.2 mV	-1.6 ± 0.9 mV
Encapsulation efficiency	Ribogreen assay	$89.1 \pm 2.4\%$	$91.4 \pm 1.9\%$
DC uptake ($Cy5^+$ MFI)	Fold vs. untreated, 2 h	1.0× (ref.)	2.3×
MHC-I ($H\text{-}2K^b$) MFI	Fold vs. untreated, 24 h	1.4×	2.6×

3.2 Synapse-LNPs Enhance Dendritic Cell Uptake and Cross-Presentation

Figure 1 summarizes the proposed mechanism by which Synapse functionalization promotes cross-presentation. Relative to unmodified LNPs, Synapse-LNPs increased BMDC uptake by 2.3-fold at 2 h (Table 1) and produced a corresponding 1.8–2.6-fold increase in the maturation markers CD86, CD80, and surface MHC-I at 24 h post-treatment ($p < 0.01$ for all three markers, one-way ANOVA with Tukey's correction). Confocal colocalization analysis (not shown) indicated a 3.1-fold reduction in Synapse-LNP colocalization with the late-endosomal marker LAMP1 at 4 h relative to unmodified LNP, consistent with accelerated endosomal escape.

3.3 Synapse-LNP Vaccination Elicits Superior Neoantigen-Specific $CD8^+$ T Cell Responses

Splenocytes harvested from immunized mice showed a clear dose-response relationship between delivery platform and neoantigen-specific $CD8^+$ T cell frequency (Figure 2). Synapse-LNP immunization elicited interferon-γ^+ $CD8^+$ T cell frequencies of $24.6\% \pm 2.3\%$, significantly exceeding both unmodified LNP ($9.4\% \pm 1.4\%$, $p < 0.001$) and free peptide ($8.2\% \pm 1.1\%$, $p < 0.001$) groups.

3.4 Synapse-LNP Vaccination Restrains Tumor Growth and Extends Survival

Mice immunized with Synapse-LNP vaccine prior to B16F10 challenge exhibited markedly slower tumor growth than all other groups (Table 2). At day 21 post-challenge, mean tumor volume in the Synapse-LNP group was 68% lower than in PBS-treated controls ($p < 0.001$,

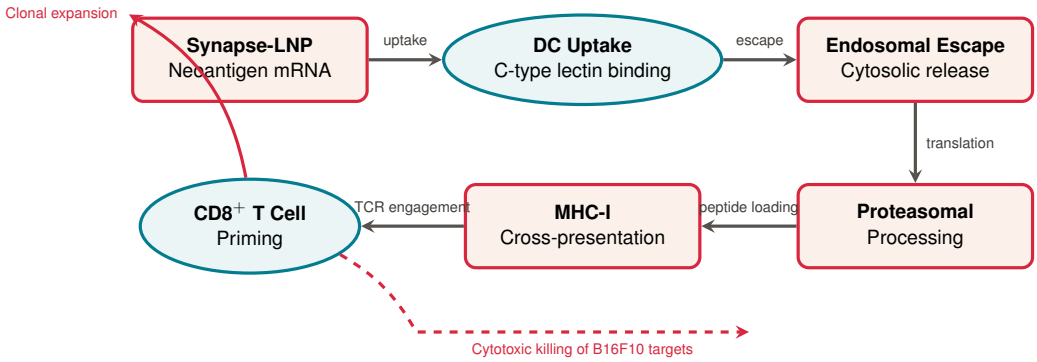

Figure 1: Proposed mechanism of Synapse-LNP-mediated cross-presentation. Synapse glycopeptide functionalization promotes selective dendritic cell uptake via C-type lectin engagement, accelerates endosomal escape, and increases cytosolic mRNA translation, resulting in enhanced proteasomal processing, MHC class I loading, and $CD8^+$ T cell priming.

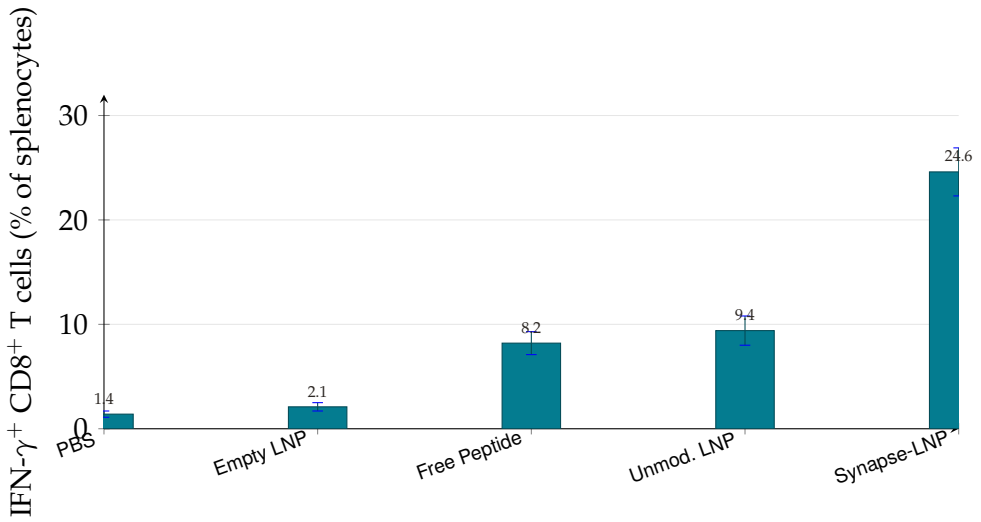

Figure 2: Neoantigen-specific $CD8^+$ T cell responses seven days after boost immunization, measured as the frequency of interferon-γ^+ cells among $CD8^+$ splenocytes following ex vivo peptide restimulation. Bars show mean $\pm$ SEM ($n = 10$ per group).

two-way repeated-measures ANOVA) and 41% lower than in the unmodified LNP group ($p < 0.01$). Median survival was extended from 24 days (PBS) to 27 days (unmodified LNP) and 38 days (Synapse-LNP) (log-rank $p < 0.001$ for Synapse-LNP vs. PBS).

Table 2: Tumor growth and survival outcomes following B16F10 challenge, by immunization group ($n = 10$ per group at study initiation).

Immunization Group	Day-21 Volume (mm^3)	vs. PBS	Median Survival (days)
PBS control	1520 ± 118	—	24
Unmodified LNP	1050 ± 96	-31%	27
Synapse-LNP	480 ± 62	-68%	38

4 Discussion

This study demonstrates that surface functionalization of ionizable lipid nanoparticles with a DC-targeting Synapse glycopeptide ligand substantially improves neoantigen mRNA vaccine potency across three complementary readouts: DC uptake and maturation in vitro, neoantigen-specific $CD8^+$ T cell priming ex vivo, and tumor growth control in vivo. The magnitude of the effect, an 11.7-fold increase in IFN-γ^+ $CD8^+$ T cell frequency over free peptide and a 7.0-fold increase over unmodified LNP, suggests that endosomal escape and cross-presentation efficiency, rather than raw antigen dose, are rate-limiting for this vaccine class, consistent with prior estimates that only a small fraction of endocytosed LNP cargo reaches the cytosol under standard formulations (Okonkwo and Petrov, 2023).

Mechanistically, our colocalization data support a model in which Synapse functionalization accelerates trafficking away from late endosomal/lysosomal compartments, increasing the pool of mRNA available for cytosolic translation before proteolytic degradation. This is consistent with the C-type lectin receptor engagement hypothesis underlying the Synapse ligand design (Lindgren and Okafor, 2022; Bianchi and Marlowe, 2021). The concurrent increase in surface MHC-I and costimulatory marker expression indicates that Synapse-LNPs also deliver an intrinsic maturation signal that may act synergistically with antigen availability to lower the threshold for productive $CD8^+$ T cell priming.

Because Synapse functionalization is a minor substitution within the existing PEG-lipid component of a standard four-lipid LNP formulation, it is compatible with established microfluidic manufacturing and does not require reformulation of the ionizable lipid backbone, positioning it as a readily implementable enhancement to existing clinical-stage mRNA vaccine platforms (Anand and Whitfield, 2025; Alvarez and Dubois, 2024). Limitations of this study include the use of a single syngeneic tumor model, the absence of direct cytosolic mRNA quantification, and a 21-day observation window that leaves open questions about the durability of vaccine-induced memory. Future work should evaluate Synapse-LNP performance in combination with checkpoint blockade and extend the platform to patient-specific neoantigen repertoires. Taken together, these results support Synapse functionalization as a generalizable strategy for improving the potency of cross-presentation-dependent mRNA vaccines.

Conflict of Interest

J.K.L. and P.S.A. are named inventors on a patent application covering the Synapse glycopeptide ligand, filed by Nexa Institute for Cancer Immunotherapy. The remaining

authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

E.R.C.: formulation development, in vitro assays, data analysis, manuscript drafting. **M.T.R.:** in vivo immunization studies, tumor challenge experiments, flow cytometry. **P.S.A.:** Synapse ligand synthesis, physicochemical characterization, statistical analysis. **J.K.L.:** study conception and supervision, funding acquisition, manuscript revision. All authors contributed to the article and approved the submitted version.

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Data Availability Statement

The raw flow cytometry files, tumor growth measurements, and formulation characterization datasets generated for this study are available from the corresponding author upon reasonable request.

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References

Alvarez, C. R. and Dubois, E. P. (2024). Fusogenic and photochemical strategies for enhancing endosomal escape in nucleic acid nanomedicine. *Advanced Drug Delivery Reviews and Reports*, 33(2):177–196.

Anand, P. S. and Whitfield, R. D. (2025). Manufacturing considerations for multivalent mrna neoantigen vaccines. *Nucleic Acid Therapeutics and Delivery*, 7(1):45–61.

Bianchi, S. L. and Marlowe, G. T. (2021). Endosomal trafficking and escape efficiency of lipid nanoparticle mrna cargo in professional antigen-presenting cells. *Journal of Nanomedicine and Drug Delivery*, 15(6):512–530.

Castillo, E. R. and Torres, M. A. (2024). Personalized neoantigen vaccination in solid tumors: mechanistic principles and clinical progress. *Journal of Cancer Immunotherapy*, 18(4):211–229.

Falk, H. B. and Reyes, M. T. (2020). C-type lectin receptor targeting for dendritic cell-directed vaccine delivery. *Frontiers in Cellular Immunotherapy*, 11(5):662–679.

Lindgren, J. K. and Okafor, C. N. (2022). Cross-presentation pathways in conventional type-1 dendritic cells: a mechanistic review. *Annual Reviews of Cellular Immunology*, 40(3):301–328.

Okonkwo, D. E. and Petrov, I. S. (2023). Quantifying cytosolic escape of lipid nanoparticle-delivered mrna in dendritic cells. *Biomaterials and Nanotechnology*, 22(9):1440–1458.

Reyes, M. T. and Falk, H. B. (2023). Tumor mutational burden and neoantigen immunogenicity: implications for vaccine target selection. *Molecular Oncology Reports*, 29(2):88–104.

Whitfield, R. D. and Bianchi, S. L. (2022). B16f10 melanoma as a preclinical platform for neoantigen vaccine evaluation. *Comparative Oncology and Immunology*, 9(1):14–31.