INTENT™ LNPs Induce Effective Anti-Tumor T cell Responses

Matthew J. Gold[1], Douglas G. Millar[2], Kirsten Olsen[1], Yu Wu[1], Yury Karpov[1], Haritha Menon[1], Aarushi Jain[1], Alisha Elford[2], Rajesh Krishnan[1], Jun Liu[1], Pamela S. Ohashi[2], Natalia Martin-Orozco[1]

[1]Providence Therapeutics Holdings, Inc., 8832 Blackfoot Trail SE Calgary, AB, T2J 0T2, [2]Princess Margaret Cancer Centre, 610 University Ave. Toronto, ON, M5G 2M9

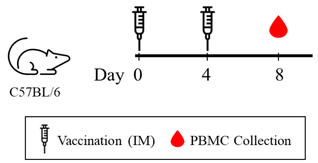
INTRODUCTION

Recently, lipid nanoparticle (LNP)-based mRNA vaccines have gained widespread attention and offer an attractive modality for personalized cancer therapies. However, inducing an effective anti-tumor response often requires the induction of a T cell response breaking self-tolerance mechanisms, a challenge for current therapies. Providence Therapeutics Holdings Inc. has developed a portfolio of ionizable lipids for the INTENT LNP platform. These LNP formulations were carefully screened for their desired end use application. While multiple INTENT LNP formulations are capable of inducing expansion of antigen specific T cells, the adjuvant activity of each INTENT LNP formulations are distinct. Utilizing an *in vivo* screening model identifying INTENT LNP formulations with adjuvant activity capable of breaking self-tolerance allows the selection of candidates with superior anti-tumor efficacy. In the well characterized mouse syngeneic colorectal cancer (CRC) model expressing model glycoprotein antigens from lymphocytic choriomeningitis virus (LCMV, MC38gp), intramuscular administration of this new INTENT LNP formulation as a monotherapy in a therapeutic setting significantly delayed tumor growth and cleared tumors in 50% of treated mice. The data suggests the applicability of our INTENT LNP formulations for the development of effective therapeutic mRNA cancer vaccines for multiple solid tumors.

METHODOLOGY

1. *In vivo* Immunogenicity – ψ-modified mRNA encoding 3 immunodominant epitopes to LCMV (3Gp: gp33, gp61 and gp276) was formulated with the novel ionizable lipids INTENT-1 or INTENT-2 into LNPs INTENT-1.1 and INTENT-2.1, respectively. LNPs were delivered to C57BL/6 animals intramuscularly (i.m.) to evaluate induction of antigen specific CD8+ T cells and CTL activity.
2. Breaking self-tolerance – INTENT LNP candidates were tested in the RIPgp model of autoimmune diabetes. These transgenic mice express LCMVgp under control of the Rat Insulin Promoter (RIPgp) resulting in gp-expression on pancreatic β islet cells. Induction of a T cell response against this "self" tissue can be monitored via peripheral blood glucose levels. This provides a robust assay for screening LNPs capable of inducing an immune response that can break self-tolerance against a target tissue.
3. Tumor control – INTENT LNPs displaying a positive result in RIPgp transgenic mice were further tested in a therapeutic setting as a monotherapy for the colon adenocarcinoma model (MC38gp) to assay potential for cancer therapeutics.

Immunization with INTENT LNPs induce antigen-specific CD8+ T cell responses *in vivo*



- Both INTENT-1.1 and INTENT-2.1 LNPs induce a robust expansion of antigen-specific CD8+ T cells.
- Antigen specific lysis of target cells (gp-peptide pulsed naïve splenocytes) was comparable between animals administered INTENT-1.1 or INTENT-2.1.

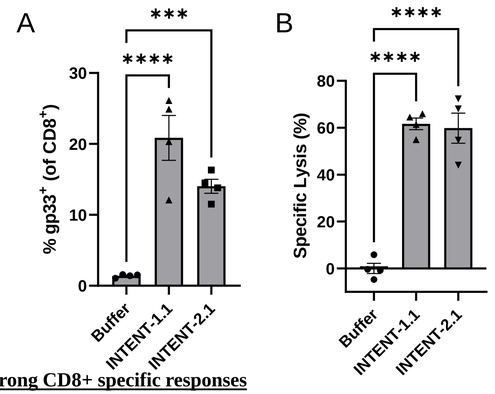
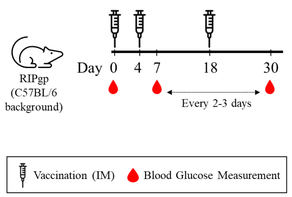


Figure 1. Both INTENT LNP formulations induce strong CD8+ specific responses
Female C57BL/6 mice (n=12) were immunized with 50µL/12.5µg of INTENT LNPs in the biceps-femoris (i.m.) on day 0 and 4 and (A) peripheral blood was collected on day 8 for staining with tetramers to gp33/H-2D^b or (B) labelled target cells were injected i.v. into immunized animals on day 8 and splenocytes collected 4hrs later for an *in vivo* CTL assay. *** p<0.001, *** p<0.0001.

Immunization with INTENT-2.1 LNPs can break immune tolerance to self-antigen in RIPgp mice



- Despite induction of antigen-specific T cells by INTENT-1.1 LNPs (Fig 1), the T cells induced lack the fitness or ability to effectively break self-tolerance.
- INTENT-2.1 LNPs provide necessary adjuvant stimulation to induce T cell infiltration and destruction of the pancreas tissue resulting in uncontrolled blood glucose and diabetes.
- INTENT-2.1 LNP mediated responses are dependent on both CD4+ and CD8+ T cells, as depletion of either significantly reduces diabetes incidence.

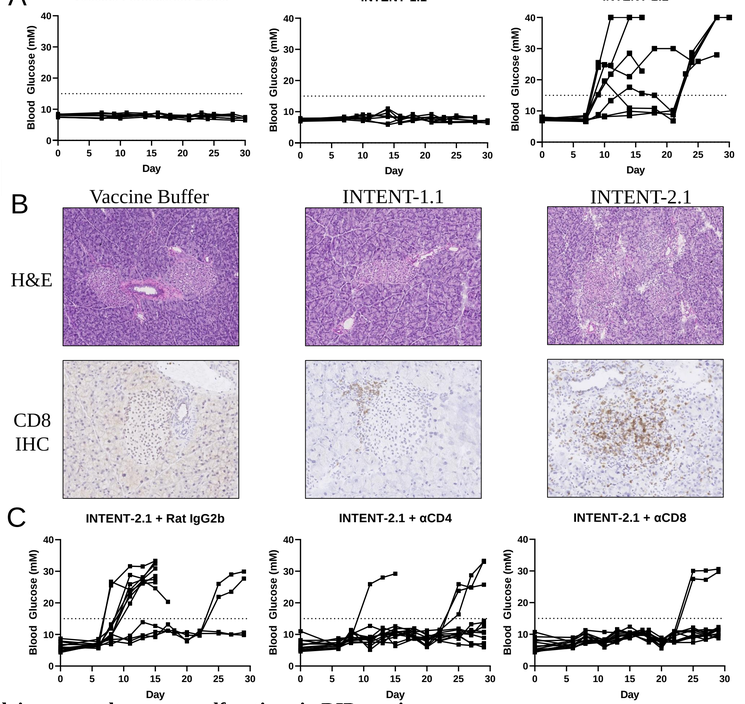
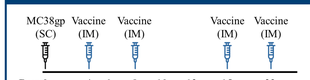


Figure 2. INTENT-2.1 LNPs break immune tolerance to self-antigen in RIPgp mice
(A) RIPgp transgenic mice were immunized with 50µL of INTENT™ LNPs in the biceps-femoris on days 0, 4 and 18 and peripheral blood glucose was monitored daily for signs of hyperglycemia (> 15mM). (B) Pancreas tissue was collected on day 7 and sectioned for H&E and CD8 IHC staining. (C) CD4 or CD8 T cells were depleted via i.p. administration of monoclonal antibodies or Rat IgG2b isotype control in RIPgp animals prior to INTENT-2.1 LNP treatment as performed in Fig 2A.

Immunization with INTENT-2.1 LNPs inhibits tumor growth and improves survival in MC38gp tumor model



- INTENT-2.1, showing success in the RIPgp model, was able to clear tumor burden in 50% of animals as a monotherapy.
- Depletion of CD8+ T cells abrogates INTENT-2.1 mediated therapeutic effect.

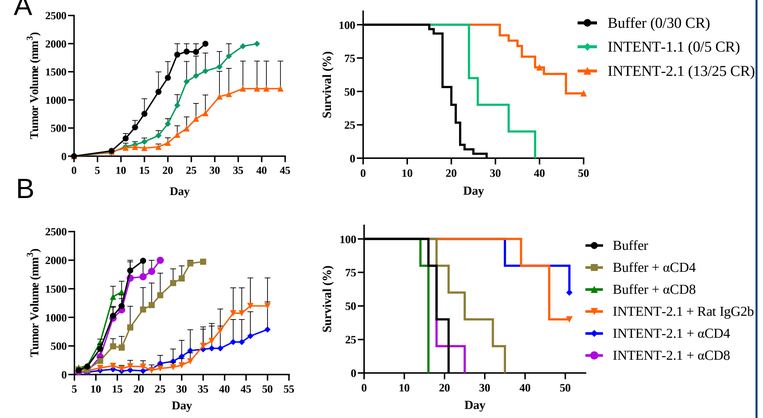


Figure 3. INTENT-2.1 LNPs induce potent anti-tumor immunity and promotes survival in MC38gp model dependent on CD8+ T cells
(A) Female C57BL/6 mice were inoculated s.c. with 1x10^6 MC38gp tumor cells on day 0 and treated with INTENT LNP candidates on days 4, 8, 15 & 22 (12.5µg/50µl/dose). Tumor volume was measured using digital calipers and calculated as Width^{2}xLength/2. (B) Tumor bearing animals as in (A) were treated with isotype (Rat IgG2b), CD4 or CD8 depletion antibodies (i.p.) prior to and during INTENT-2.1 LNP treatment.

CONCLUSIONS

- Multiple INTENT LNP formulations are effective at generating antigen-specific T cells, however the functional ability of these cells to break self-tolerance and kill target tissue is highly dependent on the adjuvant activity of the ionizable lipid component of the INTENT LNP formulation.
- The RIPgp transgenic model offers a useful screening tool to assess LNPs capable of breaking self-tolerance and likely to be efficacious when utilized as an anti-tumor mRNA/LNP based therapeutic treatment.
- Through enhanced adjuvant activity, INTENT-2.1 LNPs are capable of breaking self-tolerance and inducing autoimmune diabetes in the RIPgp model as well as offering long-term tumor control when utilized as a therapeutic monotherapy for established MC38gp tumors.

CONTACT

Business Development: bd@providencetherapeutics.com
Investor Relations: investor@providencetherapeutics.com