Novel Ionizable lipids for delivery of nucleic acids (mRNA, DNA, siRNA) (Ramot)

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Background

Chemotherapy treatment is generally accompanied by adverse effects, which can be significantly reduced using site specific, targeted delivery systems, such as antibody-coated LNPs (immuno-LNPs). However due to inter-and intra-tumor heterogeneity targeting with a single targeting moiety is not sufficient in many cases and in others may induce cancer relapse due to the survival of side cancer cell population also known as cancer stem cells. Constructing an arsenal of carriers, coating with different targeting moieties will enable the optimal treatment by choosing the most suitable targeting moieties for each individual patient.

The ability to target any cell subsets: Tel Aviv researchers have shown the ability to target subsets of immune cells and deliver various payloads including genome editing approaches, siRNAs, pDNA and mmRNA. In addition, they have shown the ability to deliver very large payloads (> 10 Kb) in a safe and highly efficient manner.

Challenges

There are multiple challenges facing NA delivery. For example, carrying large payloads is not possible with virus vectors (e.g. AAV – cannot carry more than 5Kb), Our novel ionizable lipids are designed to entrap large mmRNA or pDNA payloads (> 10Kb) forming ~ 100nm in diameter payloads that can deliver in a safe manner these large payloads that currently cannot be delivered.

In addition, constructing immuno-LNPs is a process that consumes massive resources. Immuno-LNPs composed of antibody chemically conjugated to a drug carrier. This demand access amounts of antibodies. In some cases, the conjugation reduces antibody affinity due to possible conjugation in its variable domain. Moreover, chemical conjugation may place antibody Fc in a position that will be recognized by Fc receptors on immune cells and elevate immune response and clearance. Most importantly, chemical conjugation requires calibration to each different antibody due to the different amounts and position of functional groups. All of the above motivates companies to pinpoint one targeting moiety with the highest potential and to invest only in it.

Technology Overview

Researchers developed a novel set of ionizable lipids (60 new families) that enable the entrapment of different types of nucleic acids (siRNAs, miRNAs, lncRNA, mmRNA, pDNA) including co-entrapment of sgRNAs and mRNA encoding CAS9 (via CRISPR/cas strategy) and very large payloads (over 10Kb). They have shown that these lipids in the form of lipid nanoparticles (LNPs) are safer compared to Alnylam's DLin-MC3-DMA in mice, rats and non-human primates from liver toxicity (Liver enzyme release, pathology) standpoint and from an immune standpoint (lymphocytes activation, interferon response, cytokine induction and complement activation).

In addition to the new ionizable lipids, the researchers are able to direct these LNPs to different subsets of cells using a novel strategy of a universal linker that enables selection of specific targeting moiety, allowing adjustment to target virtually any cell type including in vivo examples of inflammatory cells and cancer cells. The universal targeting vehicle is based on a unique mediator (linker) protein, which allows self-assembly of the vehicle that is made from novel ionizable lipids with a variety of selected primary antibodies (Figure 1).

Benefits

The current strategy and the use of the novel lipids can be assembled once and then every isotype mAb could be easily attached to these nanoparticles via the linker. This will allow efficient drug development process with multiple options for potential therapeutic payloads in a personal manner.

In addition, the use of novel, safe ionizable lipids open the door for novel applications when there is a need to carry large payloads for genome editing, gene manipulation or replacement therapy in different diseases (including rare diseases, autoimmunity, cancer and neurodegenerative diseases) . This strategy serves as an example to target cells beyond the liver even for massive payloads (> 10Kb).

Applications

- **Proof-of-concept in several mouse models and safety profile in non-human primates**
  Utilizing monoclonal antibodies (mAbs) for targeted delivery of small interfering (si)RNAs has been recently shown to facilitate specific gene silencing in a desired cell population. However, clinical translation of targeted siRNAs has not occurred, in part because of high development and production costs. It would be ideal to have an arsenal of carriers harboring a wide range of mAbs for treating immune-related diseases and cancer. However, considering the high costs, this is not feasible with current technologies. Here we present a modular platform to target specific cell types that enables the development of a theoretically unlimited repertoire of targeted delivery carriers. siRNA-loaded lipid nanoparticles (LNP) are coated with oriented, targeting antibodies noncovalently bound to a membrane-anchored lipoprotein that recognizes their Fc domain. Unlike chemically conjugated antibodies, these oriented antibodies maintain their high affinity and the LNPs avoid scavenging by Fc receptors on macrophages. Notably, each mAb can be utilized for targeting with almost no need for calibration or optimization. As proof-of-concept, we show that simply switching 8 different targeting antibodies (against CD44, CD34, Ly6C, CD3, CD4, CD25, CD29 and Itgb7) redirects the LNP for exquisitely specific uptake in diverse leukocyte subsets in vivo and specific knockdown even in difficult-to-transfect CD4+ cells. Intravenously injected anti-Ly6C-coated LNP encapsulating TNF siRNAs were taken up selectively by Ly6C+ monocytes and activated tissue macrophages, suppressed TNF-a expression in the colon and ameliorated inflammatory bowel disease symptoms in a DSS-induced colitis mouse model. Anti-CD29-coated LNPs entrapping Polo-like kinase 1 (PLK1) siRNAs prolonged survival of mice bearing Mantle Cell Lymphoma xenografts. These data demonstrate the platform’s potential utility for studying gene function in vivo and for targeted therapeutic applications. In addition, carrying large payloads (such as > 10Kb) was demonstrated in a rare disease indication and in a safe manner without inducing cytokine storm, interferon response and lymphocytes activation.
- **Translation to human**
  To translate the universal platform from mouse to human therapy we are constructing a scFv that is design to mediate LNPs coating with human IgG primary antibodies.

Patents

- WO2014041544 titled “Immunoparticles and methods of generating and using same”
- WO 2018/087753 A1 application titled “CATIONIC LIPIDS FOR NUCLEIC ACID DELIVERY AND PREPARATION THEREOF”
- US20150246135A1 application titled “Immunoparticles and methods of generating and using same”
  WO2018015881A1 application titled “Modular platform for targeted therapeutics”

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