Background. Lipidic delivery systems relies on lipid components to condense nucleic acids into nanoparticles. They are usually constructed from several molecules for optimal performance. They could be liposomal with a liquid core bearing the nucleic acids, or solid with a closely packed structure of lipids/nucleic acids as a continuum. In such formulations, there is usually one lipid that ionically interacts with nucleic acids 1-to-1, while other components are fillers or have specialized functions. The latter include lipids for preventing fouling (PEG moieties) or targeting (e.g., to LDL receptor). Lipopolymers developed by RJH, on the other hand, are a single component system that contains a polycation with lipid domains (**Fig 1**). The polycations provide multivalent interactions with nucleic acids, that lead to more stable interactions and better condensation of the nucleic acids into nanoparticles. The cationic component is responsible for ionic interactions with nucleic acids, but lipids also contribute to stability by hydrophobic association. The nanoparticles are solid in nature, having an interpenetrating structure composed of polymers with nucleic acids. The cell specificity is dictated by the nature of the lipid component and the conjugation chemistry.

Efficiency. It is likely that both systems are functional to deliver different nucleic payloads to cells. RJH has specialized in developing lipopolymers for this purpose with >15 years of experience. Numerous publications have validated the lipopolymers for nucleic acid delivery. Lipopolymers have been compared with some lipidic systems used for transfection purposes (i.e., leading commercial reagents, which we assume to be most optimal for delivery of nucleic acids) and we have shown that lipopolymers are superior to most systems, if not all, available to us. Data is on file if requested.

Safety. Lipidic systems and LNPs have been approved for human use while lipopolymers from RJH are currently investigational. As such, lipidic systems probably provide sufficient (acceptable) safety for human use. Lipopolymers are made from endogenous lipids (unlike LNPs) so that we do not expect any safety issues with this component. The polymeric component in RJH reagents is cationic with low molecular weight, but high molecular weight cationic molecules are known to cause inflammatory reactions (as does synthetic lipids used in LNPs). Animal studies have provided no evidence of inflammatory reaction with lipopolymers sold by the RJH Biosciences.

Patentability. The patent landscape with lipidic nanoparticles is extensive due to their long history. Establishing a unique patent portfolio is difficult with purely lipidic systems. This requires synthesis of new, structurally distinct lipids, which is challenging given the long history with lipid chemistry. A 'use' based IP position might be feasible if certain unique formulations are found to be functional. This is unlike lipopolymer systems since they do not have such a long history of use and patent literature is expected to be sparse in this regard. RJH Biosciences owns IP on lipopolymers and continue to file additional patents.

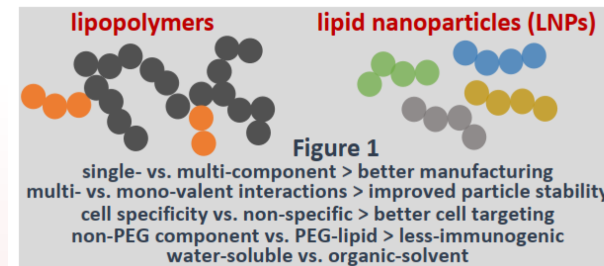

Figure 1
single- vs. multi-component > better manufacturing
multi- vs. mono-valent interactions > improved particle stability
cell specificity vs. non-specific > better cell targeting
non-PEG component vs. PEG-lipid > less-immunogenic
water-soluble vs. organic-solvent

Manufacturing. The multiple components (**Fig 1**) in lipidic systems poses manufacturing issues in reproducibility and scale-up. The lipopolymers from RJH are single component systems, hence easier to manufacture into a dosage form. Lipidic systems are soluble in organic solvents only, unlike water-soluble lipopolymers that allows flexible processing conditions. Finally, lipidic systems require specialized apparatus for thorough mixing in a reproducible way. These apparatuses could be expensive and may introduce other uncontrolled variables. The lipopolymers from RJH does not require any specialized system for use and can be readily implemented by simple mixing by the scientist or researcher.

Cost. The overall cost of reagents is significantly lower with a lipopolymeric delivery mode vs. lipid delivery mode. The starting materials for lipopolymers are significantly cheaper and processing conditions during the manufacturing is likely to keep the costs down. One needs to consider the final cost of performing transfection for both *in vitro* and *in vivo*. Our estimate in this regard suggest that an equivalent lipopolymeric nucleic acid formulation is 20% cheaper to produce compared to a lipidic system, ultimately reducing the transfection costs.

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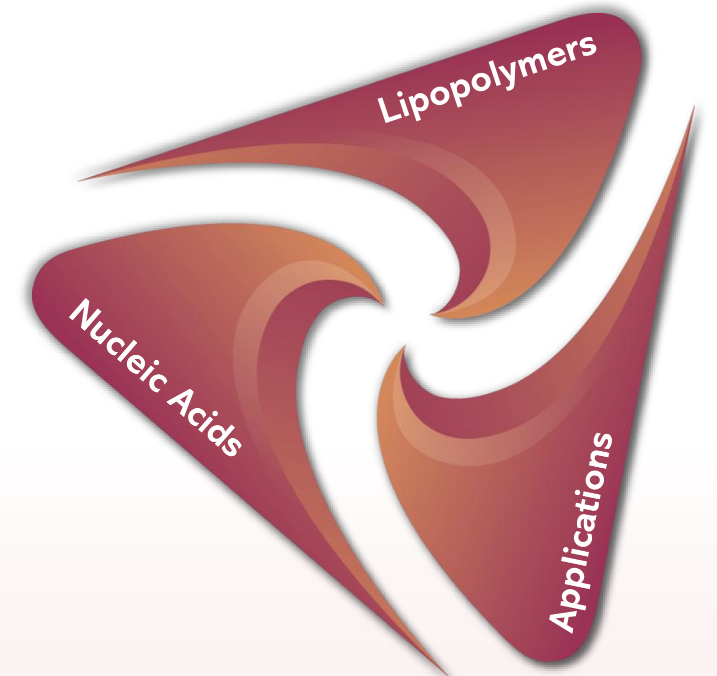
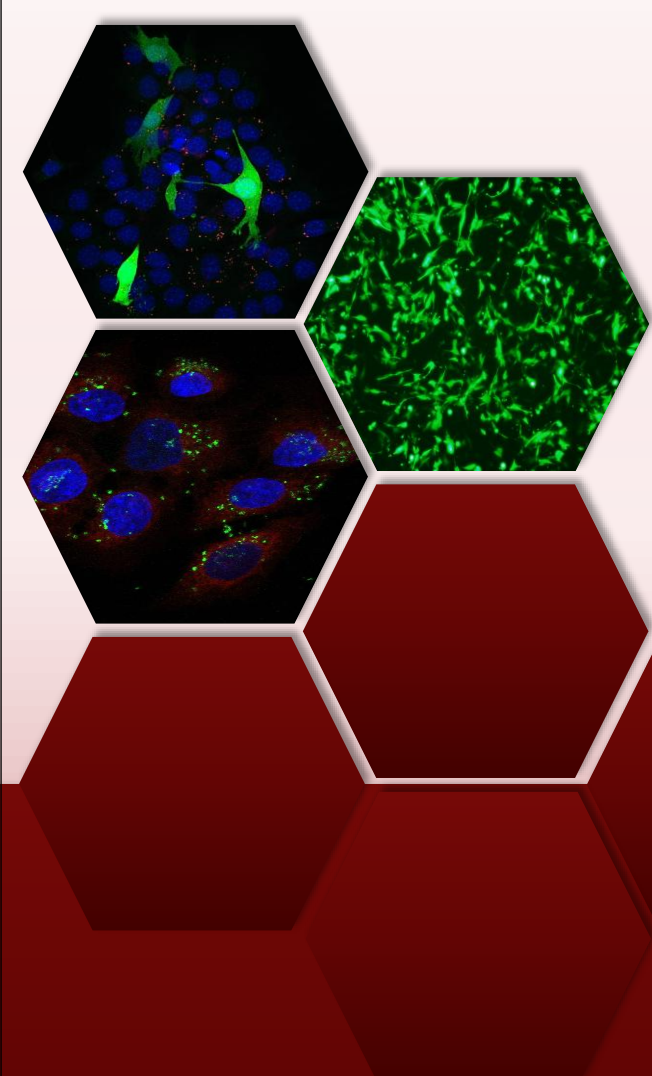
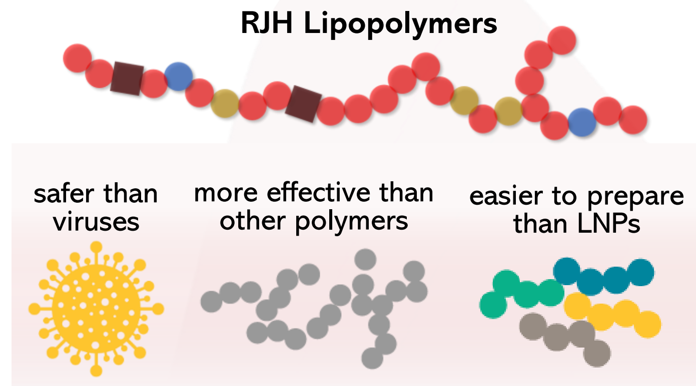


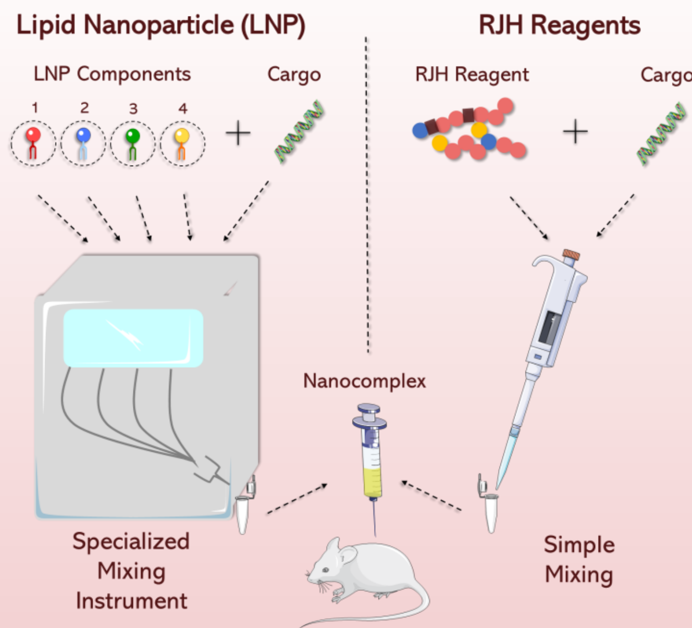
Next-Level Delivery
The RJH Advantage



RJH Biosciences is a biotechnology company based in Edmonton, Alberta, Canada. We develop novel **transfection reagents** and **delivery systems** that transport different types of **nucleic acids** to a range of human cells and cell lines. Our reagents are designed for biomedical R&D enterprise and for preclinical and clinical applications. **Our platform** is based on **lipopolymers** for effective delivery of **nucleic acids:** lipopolymers that are optimized for size, composition, stability and architecture. They display superior performance as compared to lipofection reagents and lipid nanoparticles (LNPs)



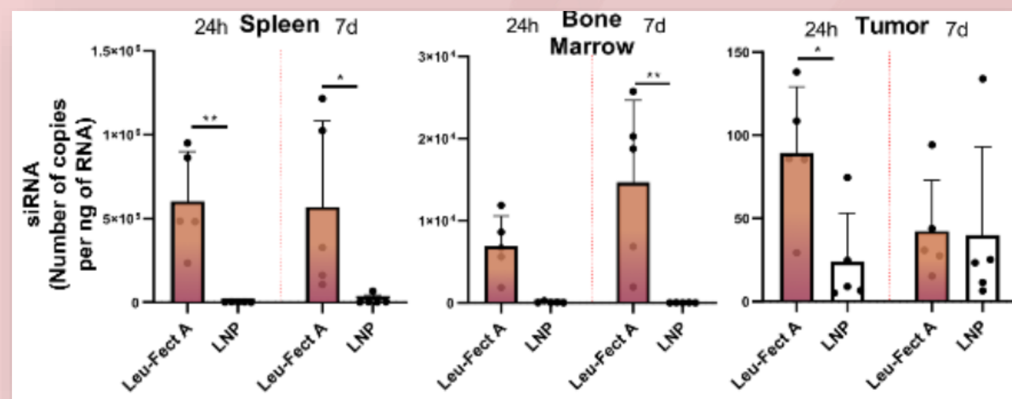
Nanocomplex Preparation



Simple Procedure for Nanocomplex Preparation

Preparing nanocomplexes using RJH reagents is simple. It does not require any costly and cumbersome mixing instrument for encapsulation and nanoparticle generation. It involves mixing the cargo (nucleic acid) of choice with the RJH reagent of choice using a pipette in a tube in aqueous medium, and the nanocomplexes will be ready to use after 30 minutes of incubation at room temperature.

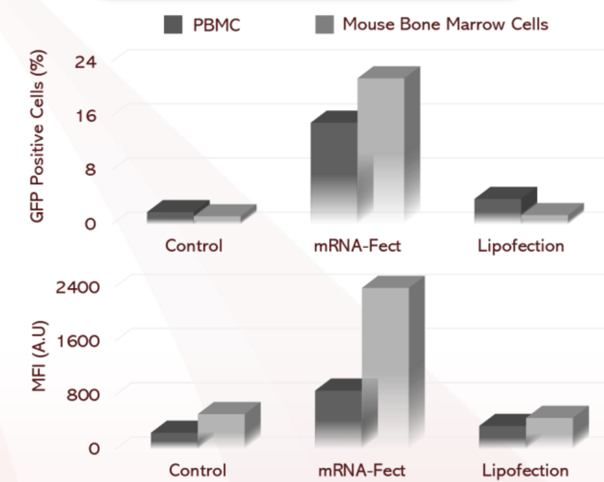
In Vivo Biodistribution



In vivo siRNA delivery efficiency with an RJH Reagent (Leu-Fect A) as compared to LNPs. Biodistribution of Bcr-Abl specific siRNA was determined in spleen, bone marrow and subcutaneous tumors in NCG mice after 24 hr and 7d of IV injection. The levels of siRNA were determined by a digital PCR using Bcr-Abl siRNA specific primers. LNP was formulated using a clinical protocol and D-Lin MC3 DMA as the ionizable lipid.

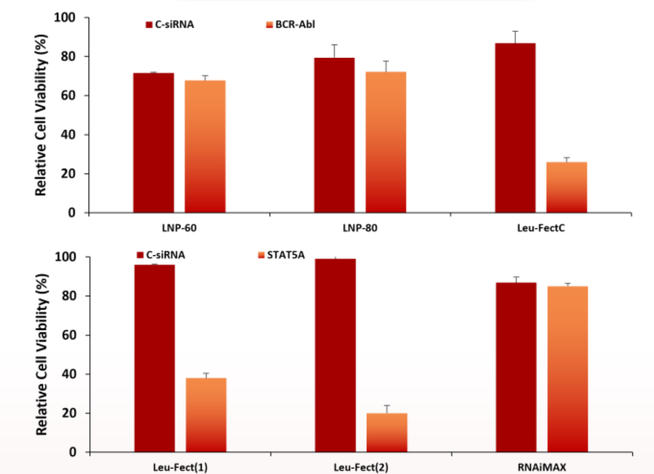
In vitro Lipofection and LNP Comparison

Transfection in Primary Cells



GFP mRNA delivery using mRNA-Fect in human PBMCs and mouse bone marrow cells. Lipofection was performed with Lipofectamine™ 2000.

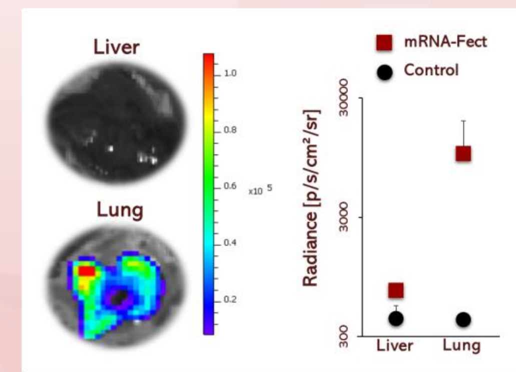
siRNA Treatment Effects



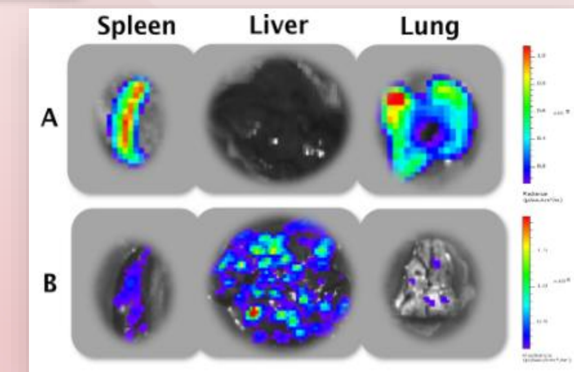
Top. Comparison of reagent Leu-Fect to LNPs for inhibition of K562 cell growth with Bcr-Abl siRNA. **Bottom**. Comparison of Leu-Fect to Lipofectamine™ RNAiMAX for inhibition of SUB-B15 cell growth with STAT-5A siRNA.

In vivo Delivery Systems

mRNA Delivery



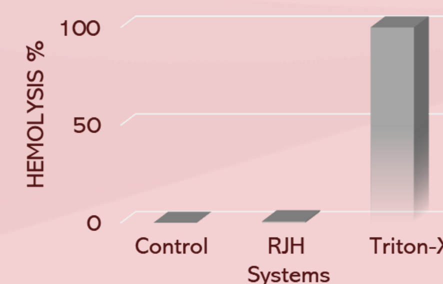
In vivo induced luciferase activity after delivery of mRNA-Fect/Luciferase mRNA complexes in BALB/c mice measured 24 hr post IV-injection. Note the strong luciferase expression in lungs, unlike the liver tissue that is the target of LNPs.



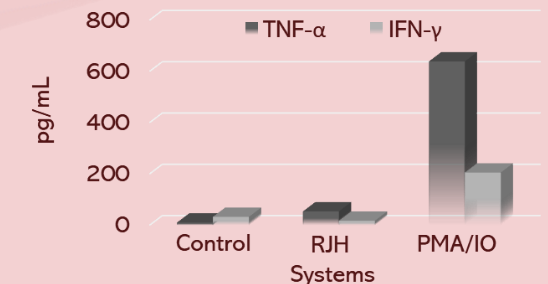
In vivo mRNA delivery with different RJH formulations. Expression of IV-injected Luciferase-mRNA in select organs after 24 hr in BALB/c mice. The mRNA was formulated with mRNA-Fect (A) and mRNA-Fect Kit (B) before injection.

Biocompatible Systems

Hemolysis



Cytokine Secretion



Left. Extent of hemolysis percentage in human Red Blood Cells (RBCs). **Right.** Pro-inflammatory cytokine secretion in human Peripheral Blood Mononuclear Cells (PBMC) after exposure to the RJH reagents.