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Successful *in-vivo* oral delivery of biologically active and therapeutic anti-inflammatory mRNA-targeted oligonucleotides with a lipid nanocrystal delivery platform

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Abstract

Background:

There has been little progress in the oral delivery of nucleic acid therapeutics beyond the liver. Matinas BioPharma's lipid nanocrystal (LNC) platform has successfully delivered oral amphotericin-B in patients with cryptococcal meningitis; other ex-vivo work has shown avid LNC uptake by innate immune cells. Prior studies have shown the *in vitro* efficacy of two mRNA-targeted oligos – one knocking down *IL-17A*, the other knocking down *TNFα*. LNC formulations of both have shown greater cytokine knock-down than "naked" oligos *in vitro*. The present work evaluated the *in vivo* efficacy of oral LNC formulations of these oligos in two different inflammatory disease models.

Methods:

Psoriasis in BALB/C mice was induced with 31.25 mg of 5% Imiquimod (IMQ) applied daily for 6 days. There were 5 treatment groups (n=10 per group): untreated controls, IMQ alone, IMQ plus one of two different LNC-oligo formulations administered daily by oral gavage, and IMQ plus anti-IL17A antibodies. Skin erythema and scaling was scored daily. The study was terminated at day 7; cytokine mRNA levels in the psoriatic skin lesions were determined by qRT-PCR.

Colitis in C57BL/6 mice was induced with 3.5% DSS in drinking water for 5 days. There were 6 treatment groups: untreated controls, DSS alone, DSS plus one of two different LNC-oligo formulations, DSS plus LNC-formulated scrambled oligos, and DSS plus a TNFα neutralizing antibody. Daily disease activity scores were measured; animals were sacrificed at day 14 and serum TNFα and tissue (colon) *TNFα* mRNA (qRT-PCR) were measured.

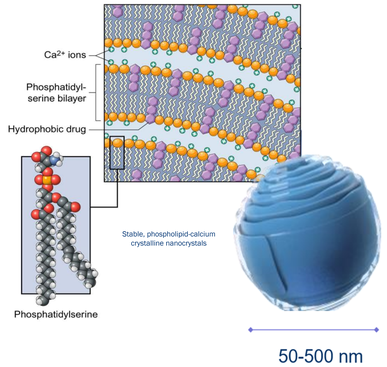
Results:

In the imiquimod psoriasis model (**Figure 1**) daily oral administration of an LNC formulation of the *IL-17A*-targeted oligos resulted in both knock-down of skin IL-17A mRNA and significant improvement in clinical parameters of redness and scaling.

Similarly, in the DSS colitis model (**Figure 2**), daily oral administration of an oral LNC formulation of the *TNFα*-targeted oligo resulted in reductions of colon *TNFα* mRNA and significant reductions in serum TNFα levels, as well as significant improvements in disease activity scores. Thus, we have shown successful oral delivery of two RNAi oligos targeting different inflammatory cytokines in two different disease models, with documented biological/molecular activity as well as therapeutic efficacy.

Conclusions:

The LNC delivery platform can successfully orally deliver biologically active (and potentially therapeutic) oligonucleotides targeting key cytokines in inflammatory disease models. While these initial results are promising, there is still some individual heterogeneity of response; future work will be focused on optimizing the LNC formulations to improve delivery efficiency, increase their potency, and extend the application of oral cytokine-targeting oligo therapeutics to other inflammatory disease models.



LNC oligo formulations maintained full cytokine knockdown capabilities *in vitro* even after gastric fluid exposure

Delivery of small molecules and small oligonucleotides

- Successful oral delivery of therapeutics in infectious disease, inflammation, and oncology

Extra-hepatic targeting

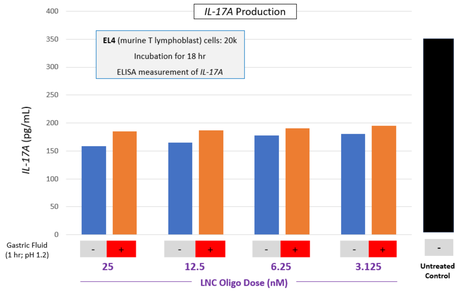
- Selective delivery to targeted tissues facilitated by phosphatidylserine
- Validated Blood-Brain-Barrier penetration with MAT2203 in cryptococcal meningitis

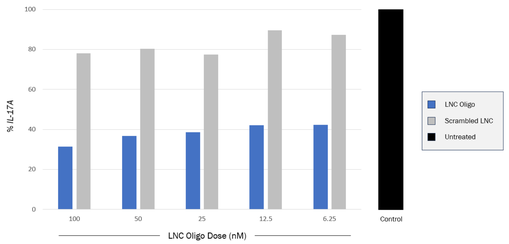
Oral delivery

- Unique structure protects cargo in GI tract
- Particle size obviates first-pass hepatic metabolism

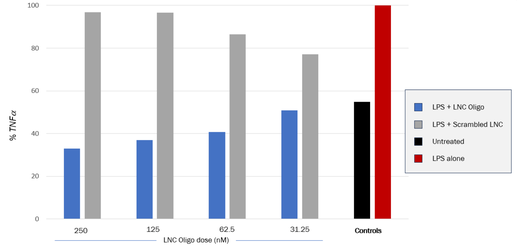
Safe & stable

- Deliver high-target tissue concentrations of drug with low plasma levels and greatly reduced uptake in non-target tissues
- No evidence of immunogenicity or cytotoxicity



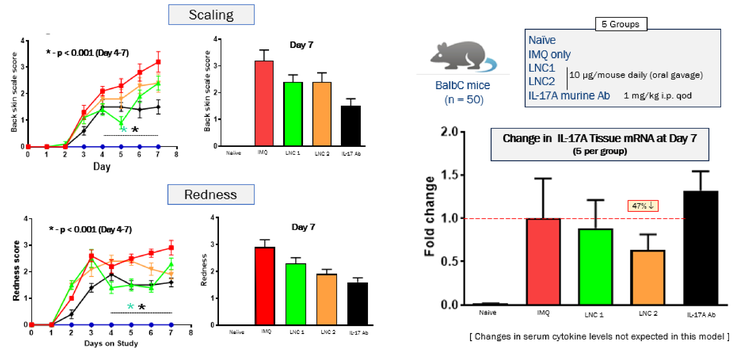


Substantial knock-down (up to 70%) of *IL-17A* with active LNCs
Comparable results in stimulated (IL-2) and unstimulated cells



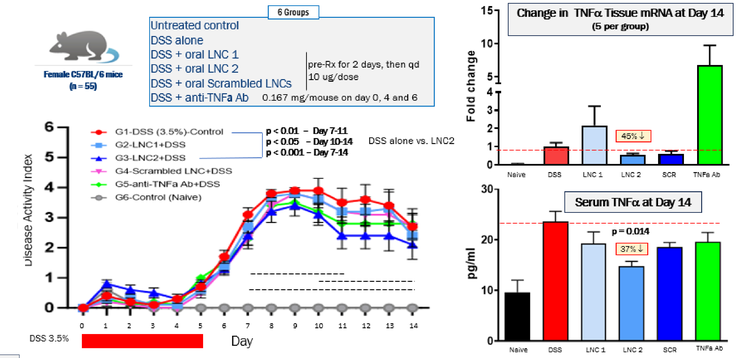
Substantial knock-down (up to 65%) of *TNFα* with active LNCs
No additional TNFα produced after LPS stimulation

Figure 1 Effect of Oral LNC *IL-17A* RNAi in a Murine Imiquimod (IMQ) Psoriasis Model



[Changes in serum cytokine levels not expected in this model]

Figure 2 Effect of Oral LNC-TNFα RNAi in a Murine DSS Acute Colitis Model



A lipid nanocrystal formulation was used to orally deliver small mRNA-targeted oligonucleotides in two different animal inflammatory disease models; each of the two oligos tested showed both biological activity and potential therapeutic effects.

Small therapeutic anti-inflammatory oligonucleotides can be orally delivered outside the liver.