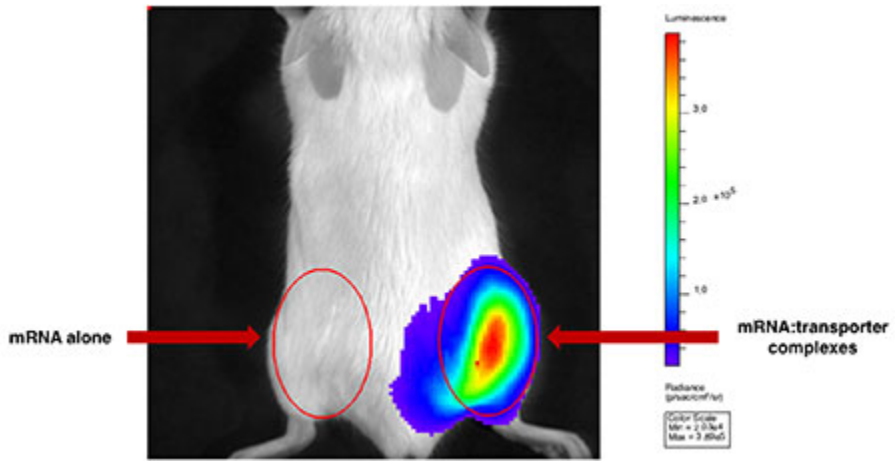
Docket #: S16-157

CART- a transfection delivery system for efficient intracellular mRNA delivery

Stanford researchers have developed a new class of materials that enable new strategies for the efficient delivery of messenger RNA (mRNA) into cells and animals. The delivery materials are easily prepared (2 steps), stable and readily tuned. Upon simple mixing, they form non-covalent complexes with mRNA that protect, deliver and release mRNAs of varied lengths. While mRNA therapeutics have the potential to transform disease treatment, their large size, polyanionic nature and susceptibility to degradation by nucleases make delivering mRNA across tissues and into cells a formidable challenge. Clinical utility is hampered by the lack of safe and effective delivery methods. Thus new delivery systems are needed to allow the intracellular delivery of mRNA *in vitro* and *in vivo*. To address this need the inventors developed a new, tunable, effective class of synthetic biodegradable materials called charge-altering releasable transporters (CARTs). CARTs operate through an unprecedented mechanism, serving initially to non-covalently complex, protect and deliver the mRNA and then change their physical properties through a degradative charge-neutralizing intramolecular rearrangement to release the mRNA in cells. This technology provides a tool for effective mRNA delivery that results in functional protein expression both in cells and animals. It can also be extended to other RNAs and has shown efficacy with plasmid DNA with up to 10,000 negative charges.



Bioluminescence image shows CART provided increased mRNA delivery and local luciferase expression (right- mRNA:transporter complexes).

Stage of research

CART transfection efficiency was tested *in vitro* using multiple cell lines, including hard to transfect J774 cells. CARTs showed significantly better transfection efficiencies than commercial reagents; achieving greater than 98% transfection. Animal (mouse) studies showed mRNA expression is highly effective *in vivo* via multiple (IM, IV, SC) routes of administration.

Applications

- mRNA delivery for:
  - Vaccination
  - Cancer immunotherapy
  - Gene editing
  - Protein replacement/augmentation therapy
  - Stem cell differentiation
  - Research

Advantages

- Ease of synthesis (2 steps)
- Metal free, scalable
- Readily tuned length, lipid and charge composition
- Material is:

    - Stable on storage
    - Biodegradable
        - pH responsive degradation
        - Degrades to know metabolites
    - Customizable:
        - Lipid and charge composition can be varied to fit needs
        - Rate of mRNA release can be varied
        - Amenable to targeting
    - Biocompatible
- Readily formulated:
    - Simply mix with mRNA and incubate (cells) or inject (animals)
    - Can be used with various lengths of mRNA
    - Protects mRNA from degradation
- Effective and general:
    - Achieved greater than 98% transfection efficiency
    - Works in multiple cell lines
    - Higher transfection efficiency *in vitro* than Lipofectamine
- Well tolerated in animal studies performed including IM, IV and SC administration
- Can be used *in vitro* or *in vivo*
- Avoid harsh delivery agents and clinically unacceptable permeablizing conditions

Publications

- Ole A. W. Haabeth, Timothy R. Blake, Colin J. McKinlay, Robert M. Waymouth, Paul A. Wender, and Ronald Levy, "mRNA vaccination with charge-altering releasable transporters elicits human T cell responses and cures established tumors in mice," PNAS September 25, 2018 115 (39) E9153-E9161.
- Colin J. McKinlay, Jessica R. Vargas, Timothy R. Blake, Jonathan W. Hardy, Masamitsu Kanada, Christopher H. Contag, Paul A. Wender, and Robert M. Waymouth, "Charge-altering releasable transporters (CARTs) for the delivery and release of mRNA in living animals," PNAS January 24, 2017 114 (4) E448-E456.
- Colin J. McKinlay, Nancy L. Benner, Ole A. Haabeth, Robert M. Waymouth, and Paul A. Wender, "Enhanced mRNA delivery into lymphocytes enabled by lipid-varied libraries of charge-altering releasable transporters," PNAS June 26, 2018

115 (26) E5859-E5866.
- Nancy L. Benner, Katherine E. Near, Michael H. Bachmann, Christopher H. Contag, Robert M. Waymouth, and Paul A. Wender, "Functional DNA Delivery Enabled by Lipid-Modified Charge-Altering Releasable Transporters (CARTs)," Biomacromolecules, 2018, 19 (7), pp 2812–2824.

Patents

- Published Application: WO2018022930
- Published Application: 20180028688
- Published Application: 20240277870
- Published Application: 20260207774
- Issued: 12,527,882 (USA)
- Issued: 12,622,981 (USA)

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