Synthesis, Uptake and Biological Activity of Multimers of C-miR146a: a Conjugate of CpG Rich Deoxynucleotide D19 and Micro-RNA miR146a.

Veronica Go[1], Dongfang Wang[1], Angelina Roderer[2], Jonathan Miles Brown[3], Kristin Neuman[3], Piotr Swiderski[1], Philipp Hadwiger[2], and Marcin Kortylewski[1].

1) Beckman Research Institute, City of Hope National Medical Center, Duarte, CA, USA; 2) Axolabs GmbH, Kulmbach, Germany; 3) MPEG LA, LLC, Chevy Chase, MD, USA.

Summary

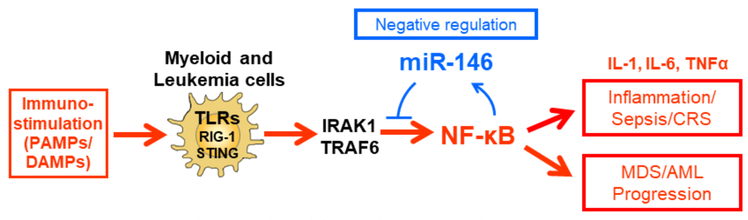
Single-stranded deoxyoligonucleotides containing unmethylated CpG motifs are internalized through scavenger receptor-dependent mechanism and activate intracellular Toll-like Receptor 9 (TLR 9) in myeloid immune cells and B cells. We previously used this SR/TLR9-dependent uptake and endosomal release mechanism for the delivery of C-miR146a, comprising a miR146 mimic oligonucleotide conjugated to the CpG containing deoxy-oligonucleotide D19, into myeloid cells both *in vitro* and *in vivo*. Unlike unconjugated miR146a, C-miR146a was rapidly internalized by these cells and reduced the expression of target IRAK1 and TRAF6, thereby blocking activation of NF-κB, a key transcriptional regulator of inflammation and tumorigenesis.

Separately, we also showed that siRNAs prepared in multimeric form exhibited increased serum half-lives with increasing molecular weight, and that hetero-multimers delivered biologically active siRNAs to hepatocytes in precise stoichiometries after conjugation to tri-antennary GalNAc ligand.

Accordingly, we have now prepared multimers of C-miR146a as a potential means of improving the serum half-life and hence the uptake and biological activity of C-miR146a via the SR/TLR9-mediated myeloid cell targeting. Here we report that the uptake of a hetero-tetramer of C-miR146a, prepared via a mono-DTME derivative and a split-strand annealing strategy, has been found to be significantly greater than that of the monomer in both *in vitro* cell culture and *in vivo* after i.v. administration, in some cases an increase approaching 2 orders of magnitude . Bioactivity studies on this and other multimers are under way.

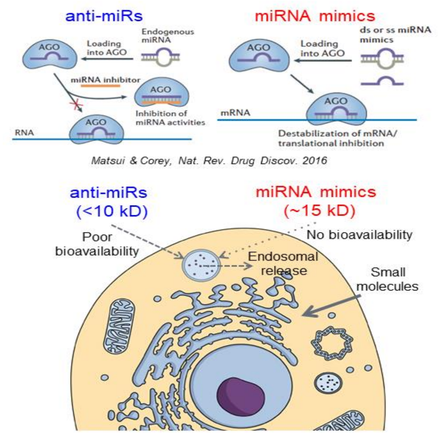
NF-κB

- **NF-κB is a key regulator of inflammation and cancer progression, with an important role in leukemogenesis.**
- **Despite therapeutic potential, targeting NF-κB proved challenging for pharmacologic inhibitors.**
- **miR-146a is a well-characterized negative feedback inhibitor of NF-κB with tumor suppressor activity.**

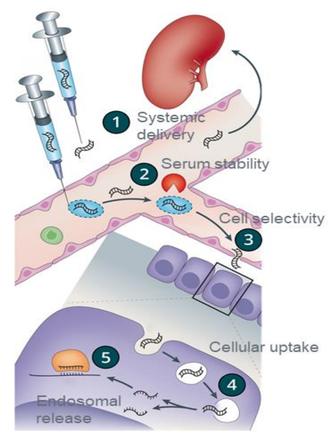


Based on Mehta and Baltimore, Nat. Rev. Immunol. 2016

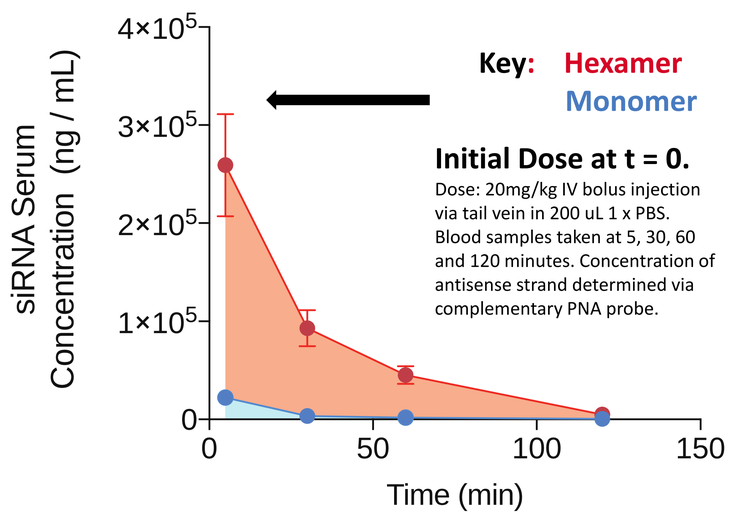
Challenges in the Development of miRNA Therapeutics



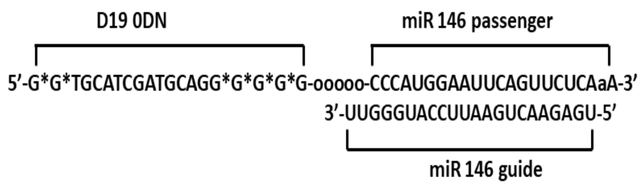
Matsui & Corey, Nat. Rev. Drug Discov. 2016

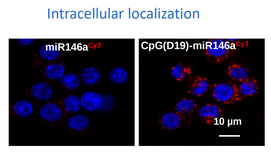


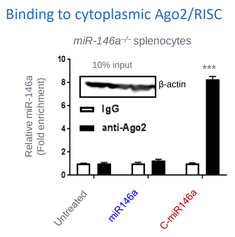
Multimers have Greater Serum Half-lives: FVII Hexamer vs monomer……

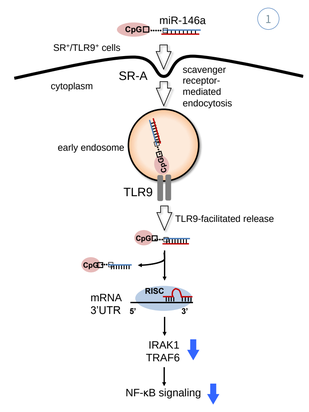


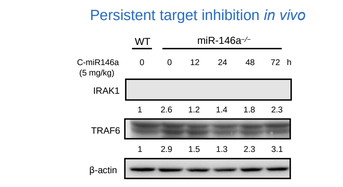
C-mir146 Mimic Suppressing Dangerous Inflammation and AML

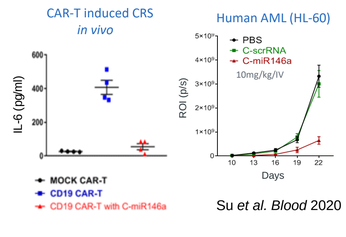












Su *et al. Blood* 2020

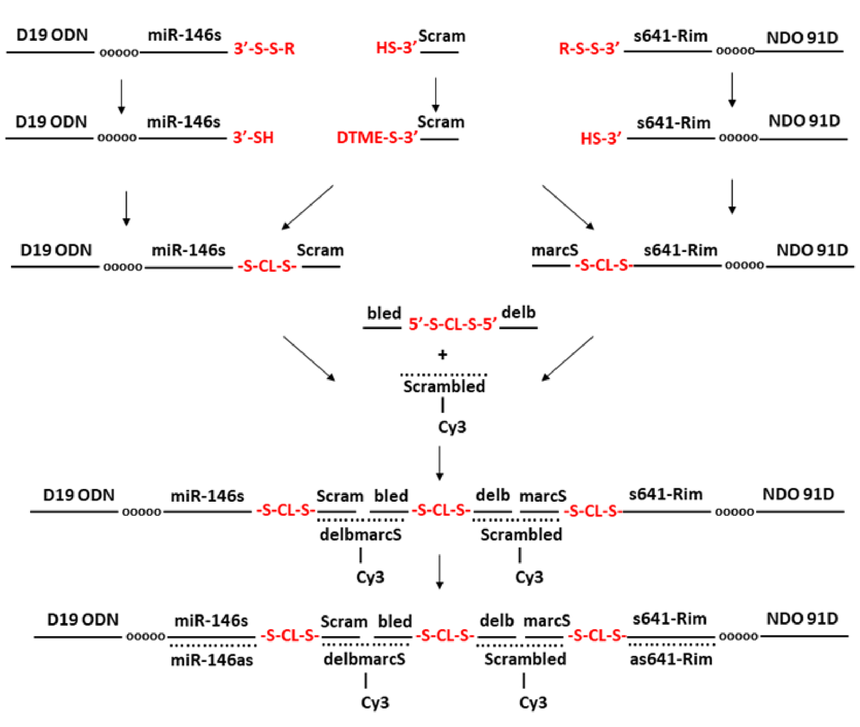
Hetero-tetramer of C-miR146

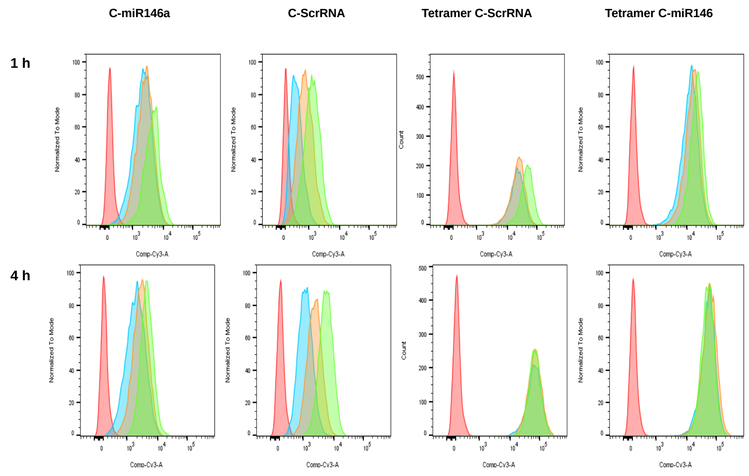
Synthesis

via Mono-DTME derivative + Split-Strand Annealing

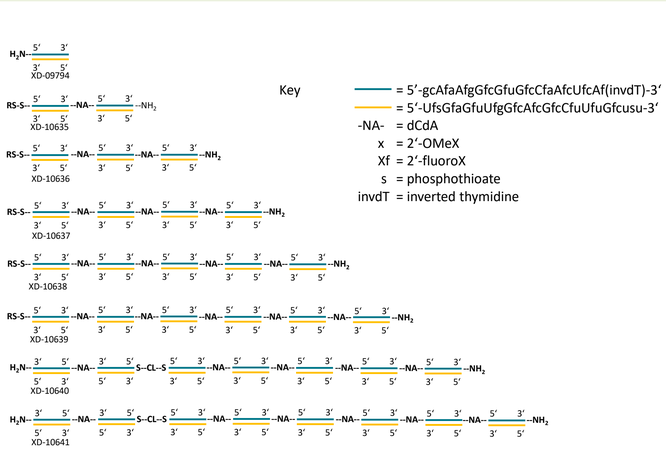
Uptake

by mouse macrophage *in vitro*

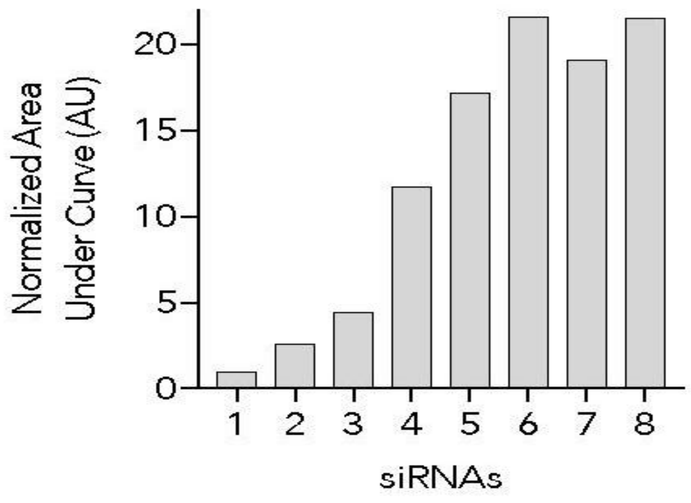




Comparison Serum Half-lives 1-8 mers…



…normalized to monomer



ODN D19:

5′-G*G*TGCATCGATGCAGG*G*G*G*G-3′

Scram passenger:
5′-AfaUfaCfaCfgCfcAf*a-3′

X = Unmodified nucleoside.

• = phosphorothioate.

miR146a passenger:

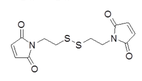
5′-CCCAUGGAAUUCAGUUCUCAaA-3′

bled passenger:
5′-Af*uUfuAfgCfcUfu-3′

x = 2′-MeO-X.

ooooo = $-[(CH_2)_3-PO_2]_5$

DTME =



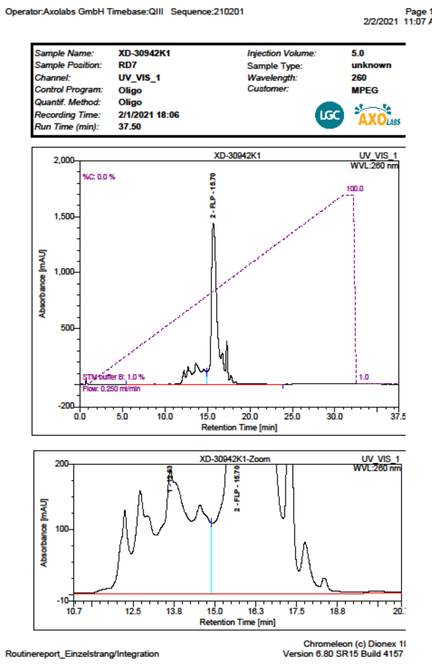
miR146a guide:

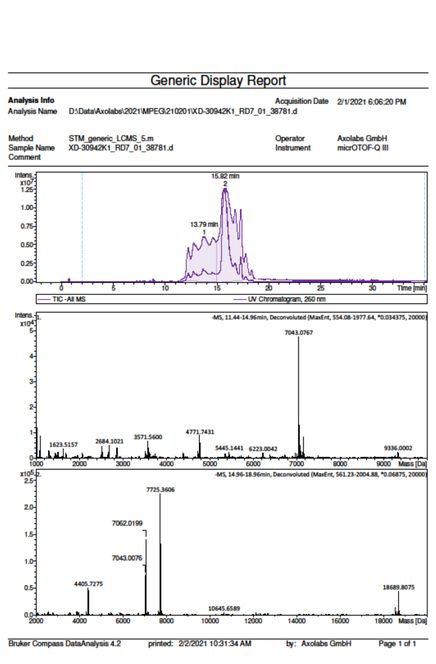
5′-UGAGAACUGAAUUCCAUGGGUU-3′

Scrambled guide:
5′-Gf*gCfgUfgUfaUfuAfaGfgCfuAfaAfuCf*u-3′

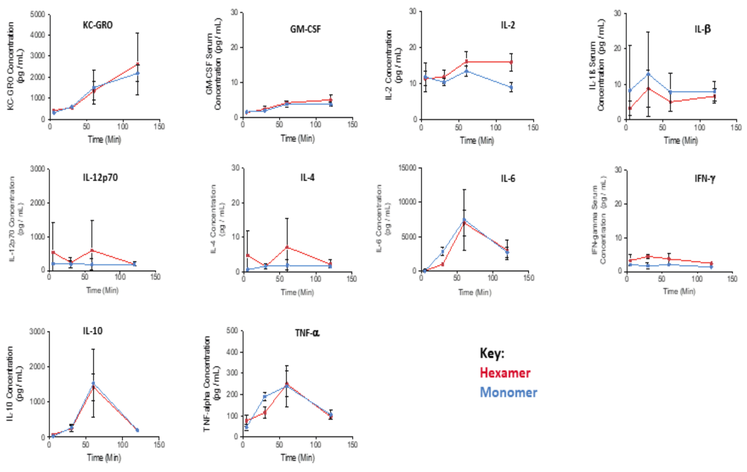
Xf= 2′deoxy-2′-fluoro-X.

-S-CL-S- = alkyl-thio-DTME-thioalkyl





….with minimal increase in toxicity



Determination of Levels of 10 Cytokines in blood samples taken at t= 5, 30, 60, and 120 minutes using MSD U-Plex platform.
Key: Hexamer Monomer

C-mir146a active
Yield: 1.2 µmol
HPLC-purity of duplex: 99.0 %

C-miR146a dimer
Yield: 490 nmol
HPLC-purity of duplex: 85.2 %

Tetramer active
Yield: 1.2 µmol
HPLC-purity of duplex: 78.4 %

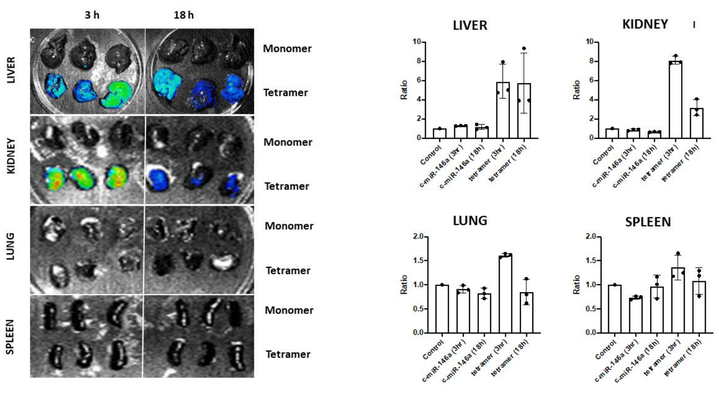
Modified scrambled miR
amount: 1.2 µmol
HPLC-purity of duplex: 96.0 %

C-mir146a control
Yield: 1.2 µmol
HPLC-purity of duplex: 98.7 %

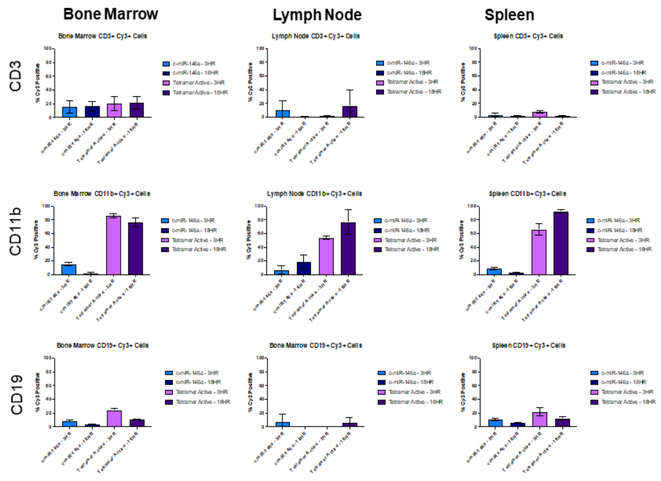
C-miR146a dimer control
Yield: 995 nmol
HPLC-purity of duplex: 90.6 %

Tetramer control
Yield: 1.2 µmol
HPLC-purity of duplex: 82.3 %

In vivo Tissue Distribution in Mice



In vivo Cellular Distribution in Mice



Conclusions

A hetero-tetramer of C-miR146a has significantly greater uptake by target cells wrt to monomer both *in vivo* and *in vitro*. Multimeric oligonucleotides have great potential for targeting cell/tissue/organs other than liver.