CURADIGM

Overcome hepatic challenge for a better efficacy of IV administered therapeutics

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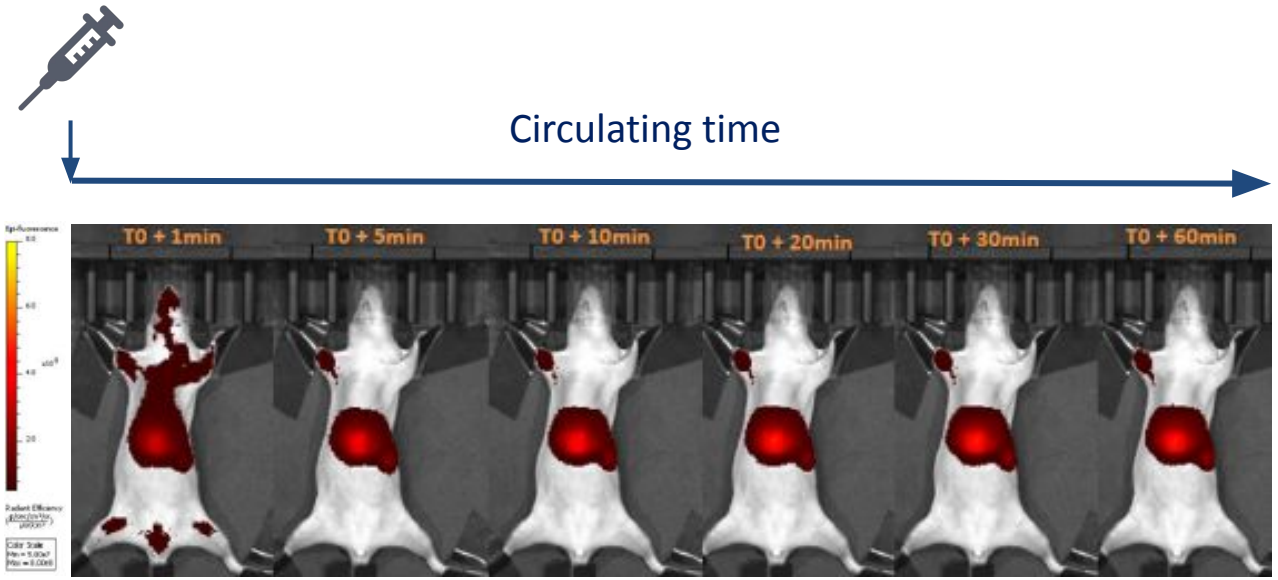
Advanced Delivery Science

Therapeutic bioavailability: an unmet need

Traditional therapeutic intravenous administration



Organ Trapping

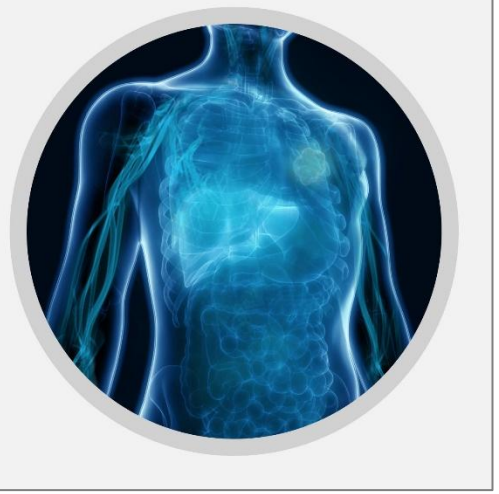
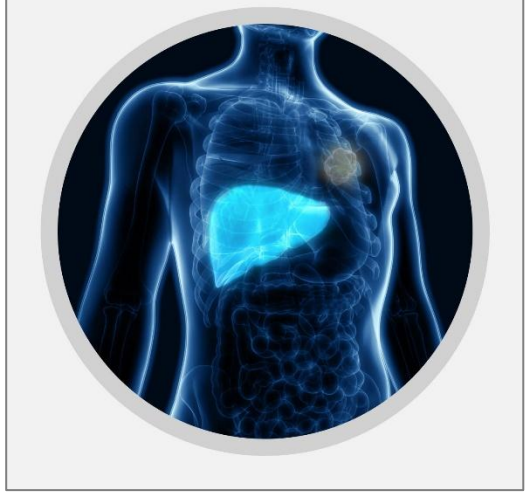
The therapeutic is rapidly trapped by the liver

Therapeutic bioavailability is still an issue with generally only few % of therapeutic dose eventually reaching the target tissue leading to decrease efficacy or safety issues

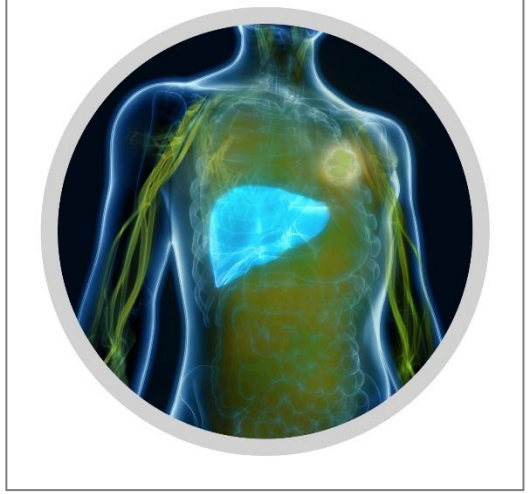
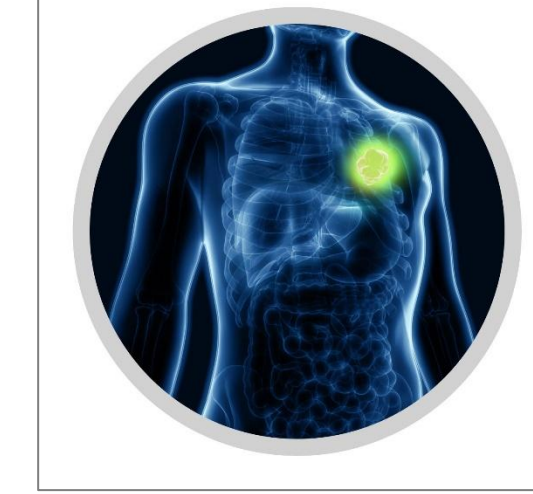
- Mice treated with one injection of nanoparticle (FluoSpheres carboxylate-modified, 200 nm, dark red (660/680); 2,6 g/l; 2,5ml/kg) alone or 10min after injection of the Nanoprimer (5ml/kg)
- Fluorescence by in vivo imaging system

Curadigm's technology: priming the body to receive the treatment

PRIME with Nanoprimer

Nanoprimer Administration	Nanoprimer Accumulation
	

TREAT with the Therapeutic

Therapeutic Administration	Therapeutic Accumulation in Target Tissue
	

The Nanoprimer has key differentiating factors

Increase of Blood Bioavailability

- Transient blood bioavailability increase [2]
- Increased accumulation in targeted organ

Safety / Toxicity

- Nanoprimer is safe [1, 2]
- Biodegradable (lipid-based)
- Decreases potential hepatotoxicity of the therapeutics

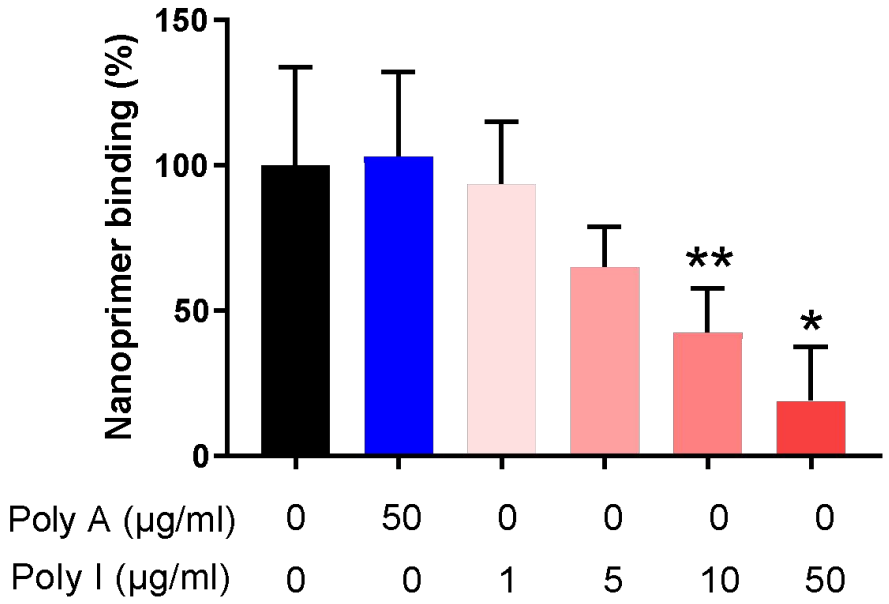
Specific MoA

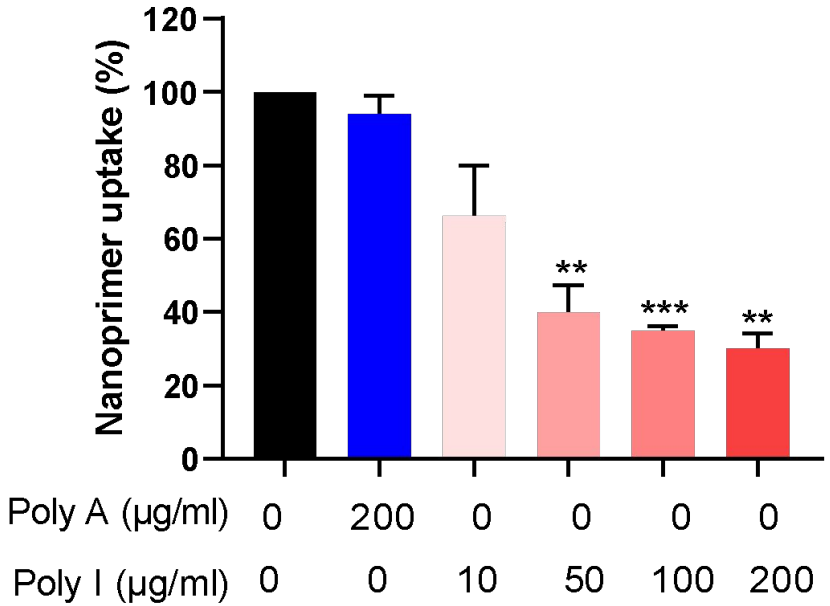
- Occupies clearance pathways in a highly specific way due to specific physico-chemical properties (no API)
- Does not modify therapeutic [2]
- No impact on cytochrom metabolization pathway

(1): A Nanoprimer to improve the systemic delivery of siRNA and mRNA. Saunders N. et. al. Nano Letters V 20, 6, 4264–4269 (2020)
(2): Priming the body to receive the therapeutic agent to redefine treatment benefit/risk profile. Germain M. et. al. Scientific Reports V 8, Article number: 4797 (2018)

Nanoprimer mode of action

The Nanoprimer binding and uptake can be competitively inhibited by poly I, a scavenger receptor type A ligand



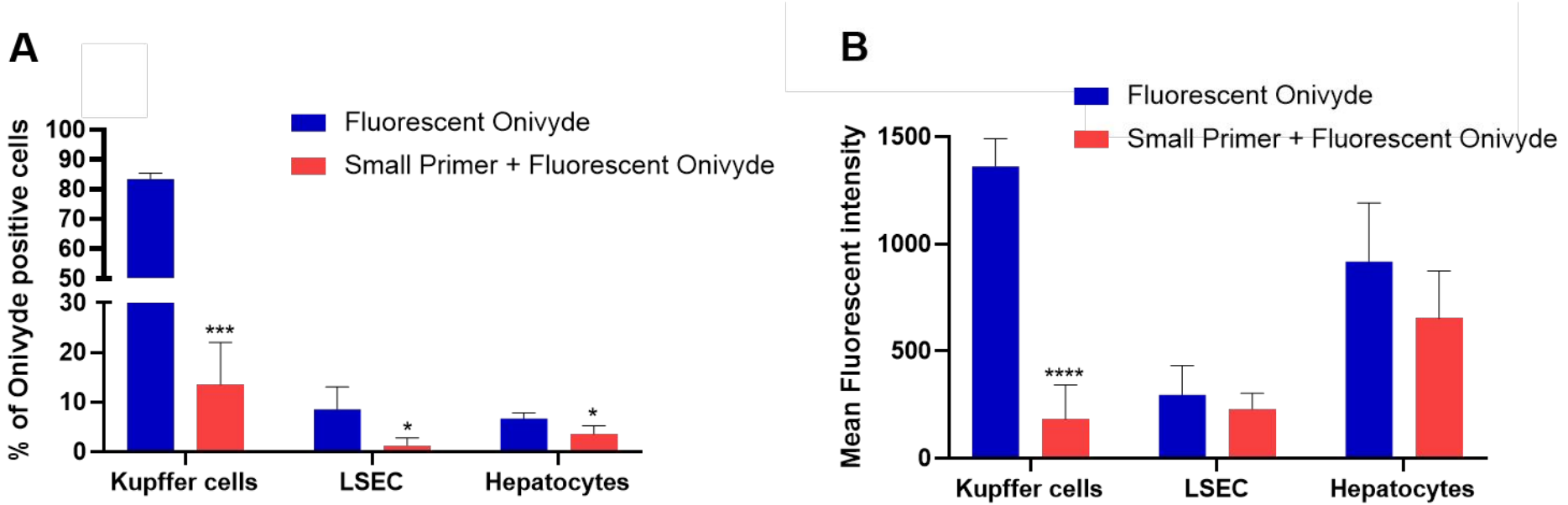


Nanoprimer uptake into Kupffer cells is mainly scavenger receptor-mediated

Immortalized rat Kupffer cells were incubated with polyadenylic acid (poly A = negative control) or increasing concantration of polyinosinic acid (poly I = ligand of scavenger receptor) before fluorescent Nanoprimer incubation at 4°C for binding assay or 37°C for Nanoprimer uptake for 30min. Cells were analyzed by flow cytometry

Decreased sequestration of liposomes by Kupffer cells, LSEC and hepatocytes *in vivo*

Uptake of fluorescent Onivyde-like liposomes by different liver cell populations in the presence of Nanoprimer

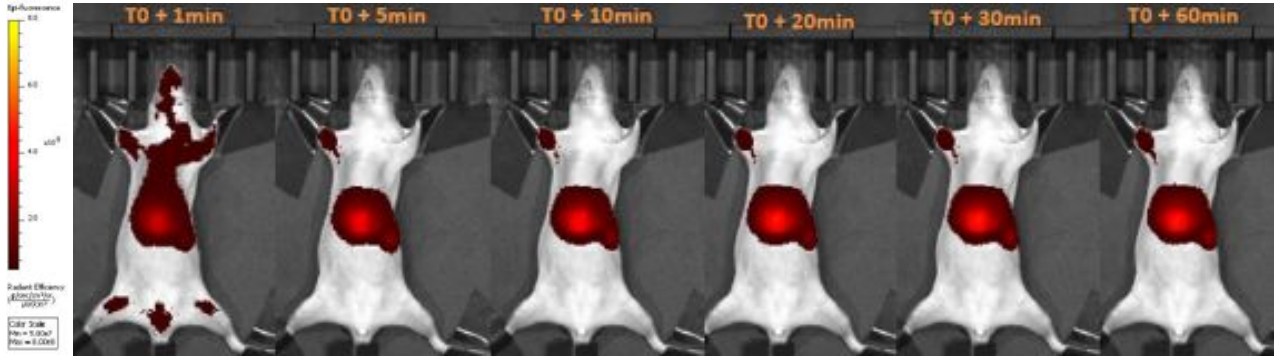


Nanoprimer injection decreases liposome uptake by Kupffer cells, LSEC and hepatocytes

- Mice treated with one injection of fluorescent liposomes (Onivyde-like pegylated liposome formulation containing 0.05% of the fluorescent lipid 18:0 Cy5.5; 93 nm; 30mM; 3,5ml/kg) alone or with Nanoprimer (5ml/kg)

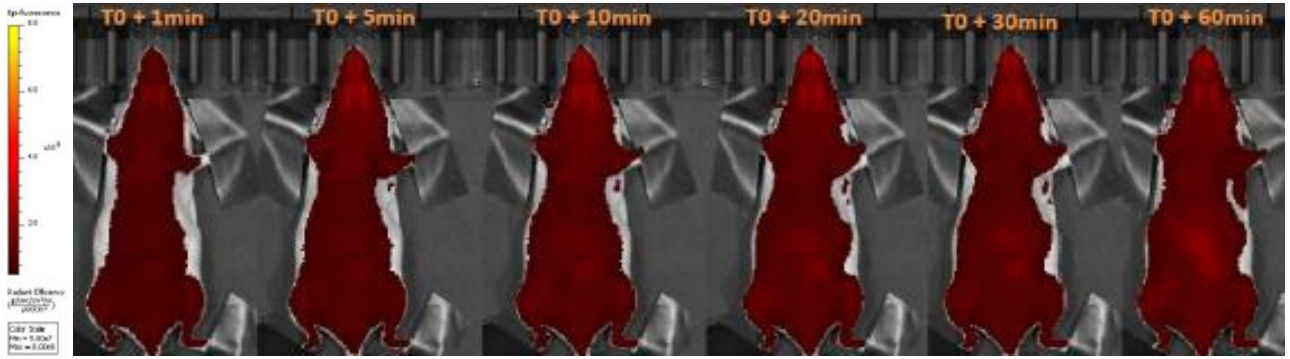
Curadigm's technology improves poor therapeutic bioavailability

Traditional therapeutic administration



← Organ (liver) Trapping

Curadigm technology + Therapeutic



← Increased blood bioavailability

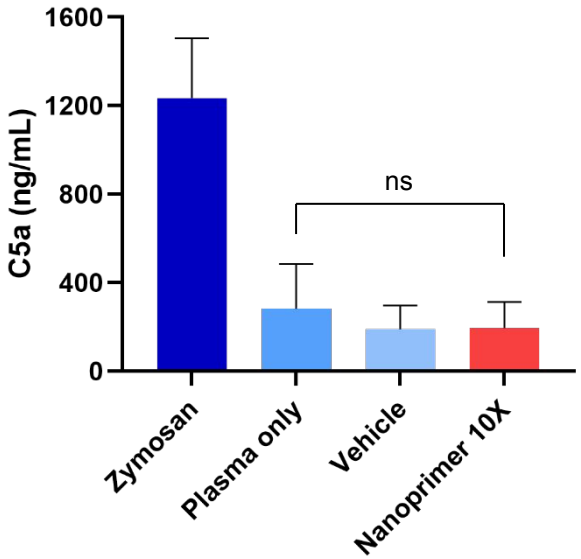
Curadigm's technology increases blood bioavailability, enabling increased accumulation in target tissues and reduced accumulation in liver

- Mice treated with one injection of nanoparticle (FluoSpheres carboxylate-modified, 200 nm, dark red (660/680); 2,6 g/l; 2,5ml/kg) alone or 10min after injection of the Nanoprimer (5ml/kg)
- Fluorescence by in vivo imaging system

In vitro safety of the Nanoprimer: complement activation

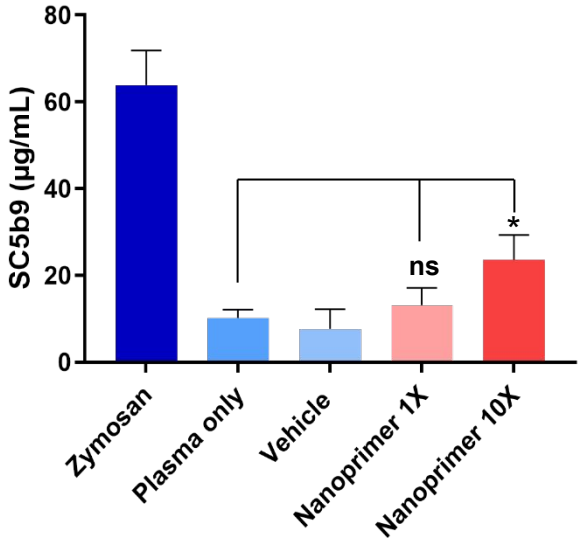
Complement Activation (C5a and SC5b9 concentrations)

C5a Concentration in Plasma



✔ No impact of the Nanoprimer (10X dose) on C5a level

SC5b9 Concentration in Plasma



✔ Moderate increase of SC5b9 level at 10X

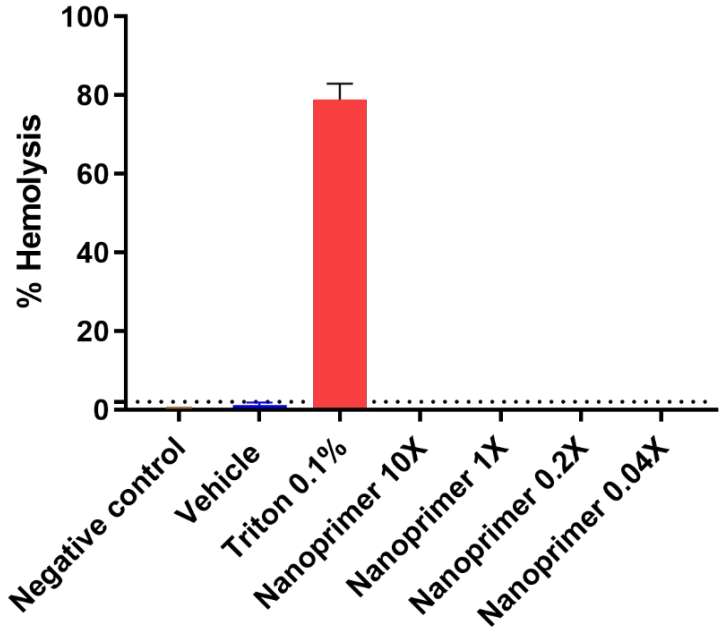
✔ No impact on SC5b9 at 1X

The Nanoprimer does not trigger any complement-mediated immune response

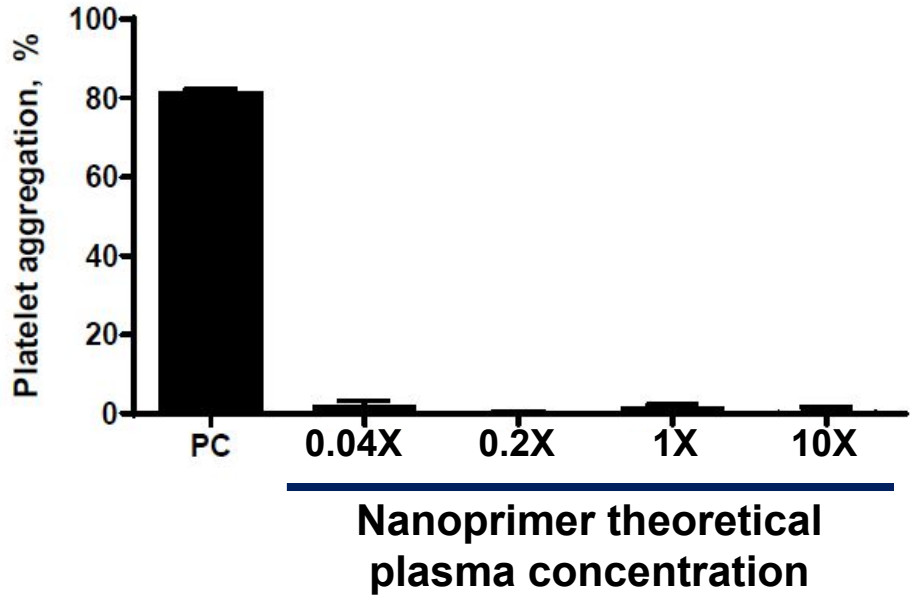
Human plasma was exposed to the Nanoprimer (1X = Theoretical plasma concentration = 0,37 mg/ml = 0,6 mM)

In vitro safety of the Nanoprimer: impact on hemolysis and platelet aggregation

hemolysis



platelet aggregation

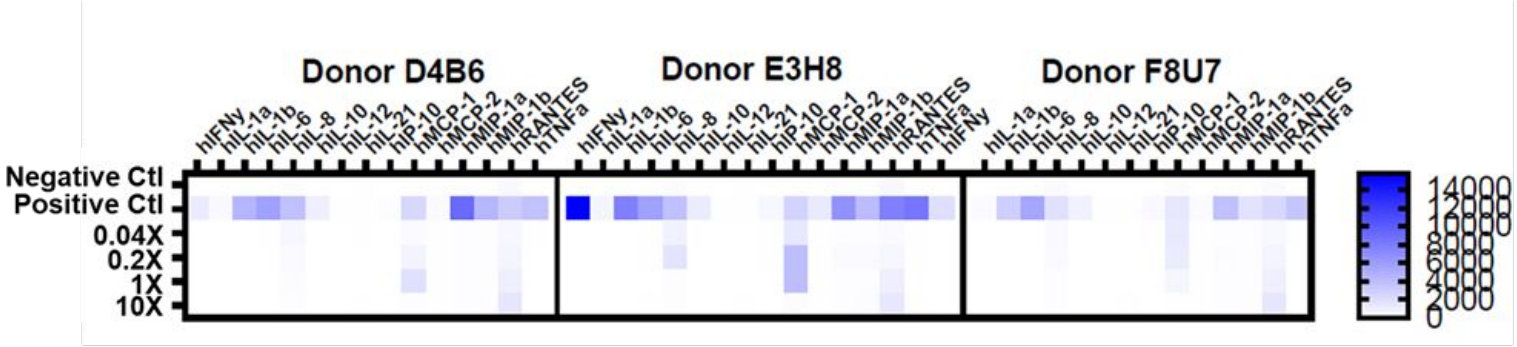


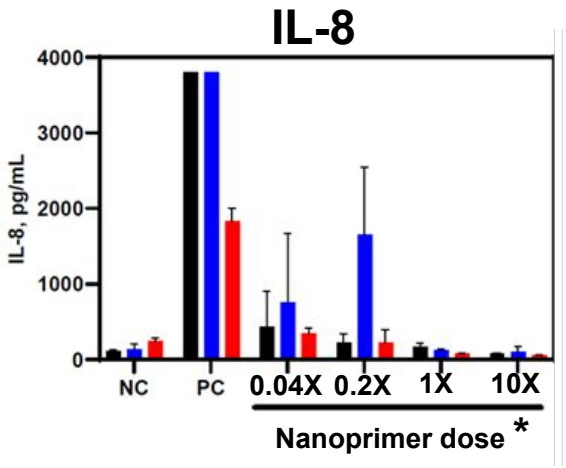
The Nanoprimer is not hemolytic and does not induce platelet aggregation

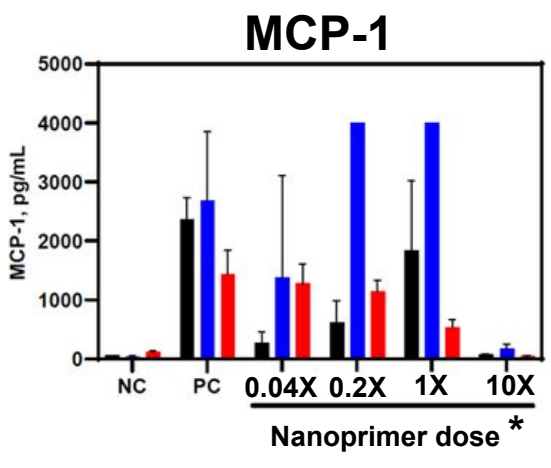
Rabbit blood was exposed to 4 concentrations of Nanoprimer (1X = Theoretical plasma concentration = 0,37 mg/ml = 0,6 mM)

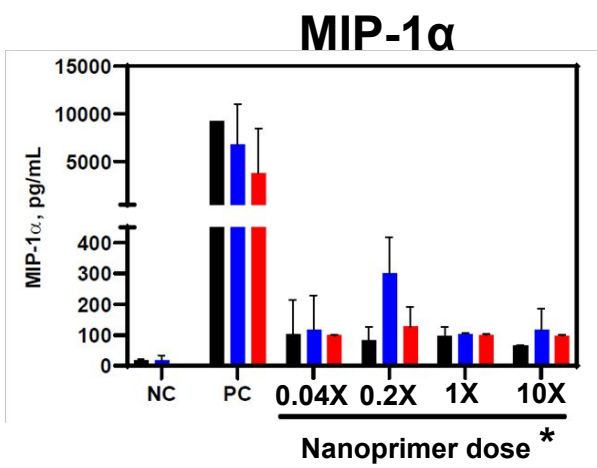
Nanoprimer Theoretical plasma concentration 1X = 0,36mg/ml
Nanoprimer Maximized plasma concentration 10X = 3,6mg/ml

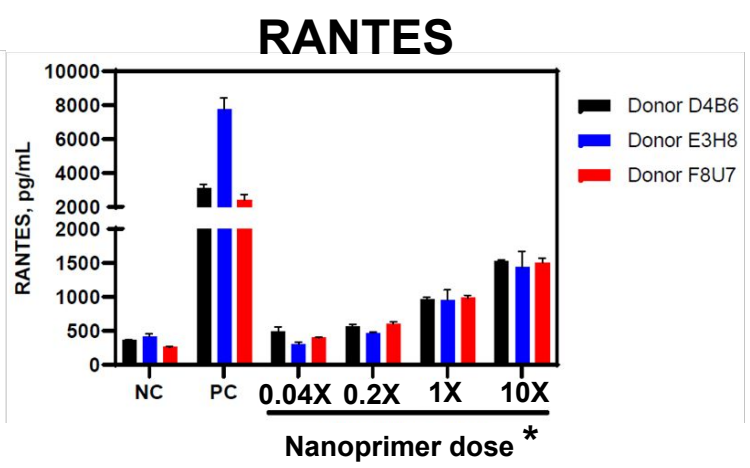
In vitro safety of the Nanoprimer: impact on cytokine/chemokine expression profile











***: 0.04X, 0.2X, 1X, 10X of theoretical plasma concentration**

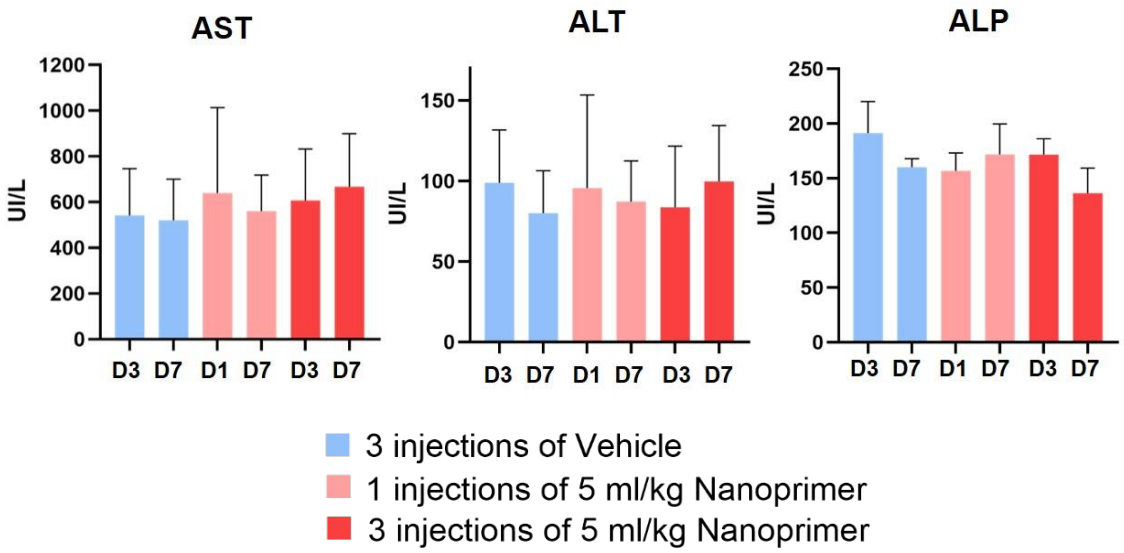
The Nanoprimer induces chemokines (IL-8, **MCP-1, MIP1α, MIP-1β**, **RANTES**; bold font highlights chemokines that are consistent between donors)

This finding is consistent with NCL's experience with other liposomal and lipid-based carriers

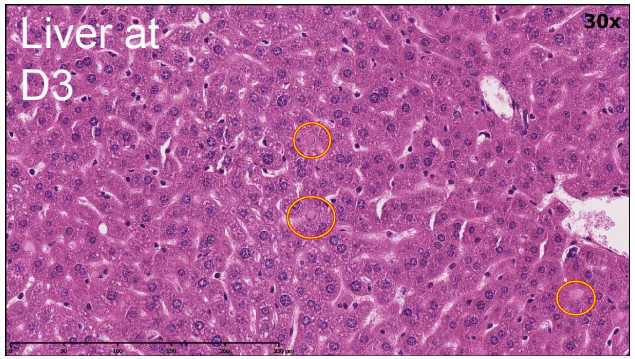
Human PBMC were exposed to 4 concentrations of Nanoprimer (0,36 mg/ml = Theoretical plasma concentration) or positive control (LPS for inflammatory cytokines, ODN2216 for type I interferons, PHA-M for Type II interferon)

In vivo safety of the Nanoprimer:

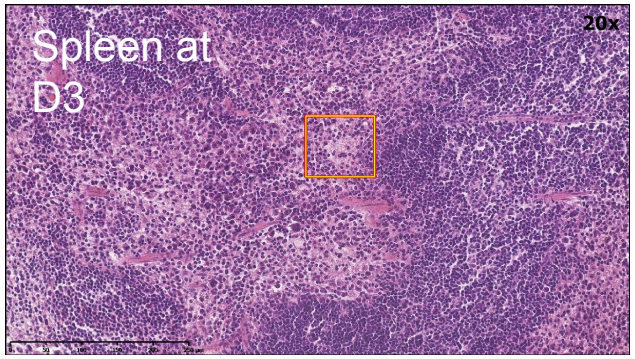
The Nanoprimer does not induce any changes to the hematological or biochemical parameters



The Nanoprimer does not induce any significant tissue structural or cellular damages.



Few and transient cytoplasmic vacuolar inclusions



Transient infiltration by large and active macrophages

The Nanoprimer has a favorable safety profile at the therapeutic dose

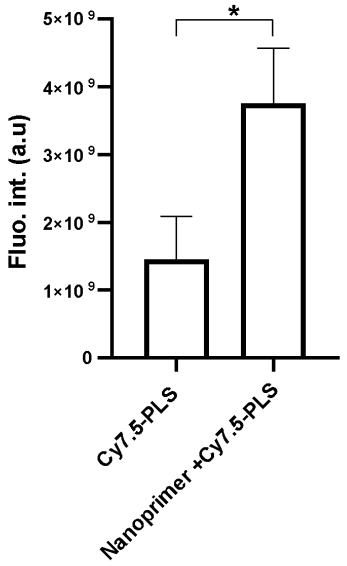
Mice received a single injection of Nanoprimer 3mL/kg or 10mL/kg and were sacrificed 24h or 7 days after injection (n=5)

Impact of the Nanoprimer on tumor accumulation of polymeric drug delivery system

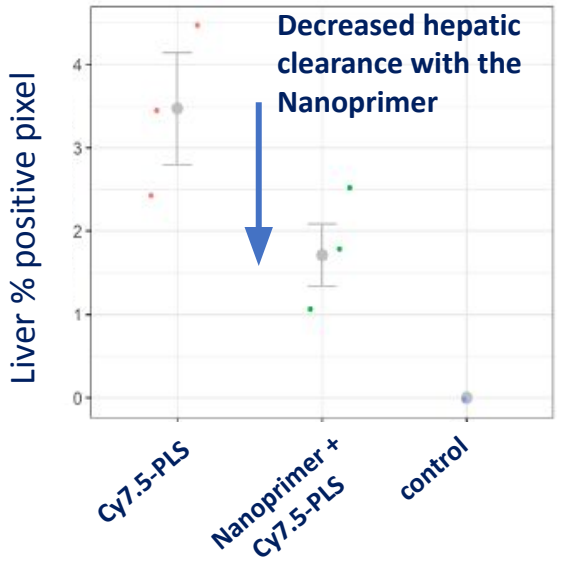
NCL Nanotechnology Characterization Laboratory

tumor associated macrophages accumulation of the fluorescent poly(L-lysine succinylated) (Cy7.5-PLS) Polymer in the 4T1 mouse model

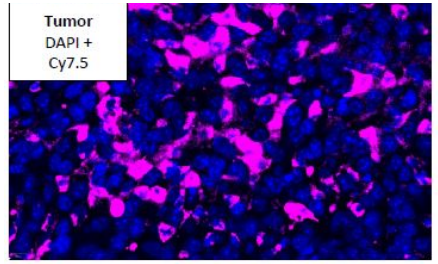
Tumor accumulation of Cy7.5 PLS polymer



Histological observation of Cy7.5 PLS Positive Kupffer cells in liver



Histological observation of CY7.5 accumulation in tumor associated macrophages



Cy7.5 signal localized in cells with smaller nuclear profiles than tumor cells,

positive cells have nuclei that are often indented, consistent with TAMs

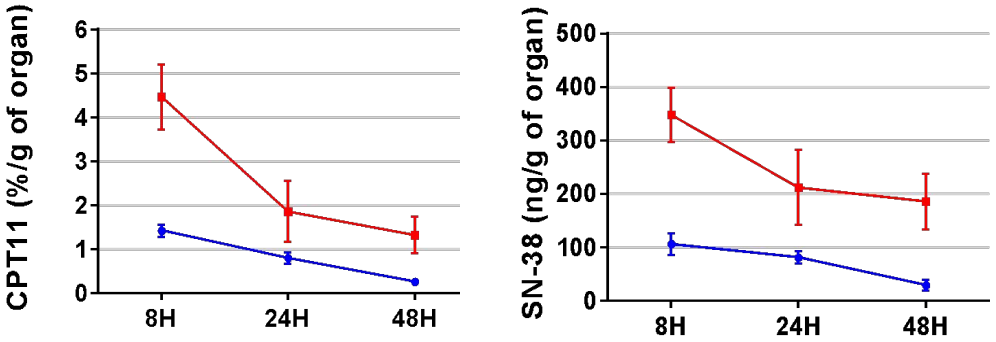
Nanoprimer is able to increase accumulation of polymer-based drug delivery system in tumor-associated macrophages by 3-fold

- 200mm^3 4T1 breast adenocarcinoma on BALB/c mice (n=5)
- Nanoprimer is injected at 120mM, 10 min before the Cy7.5 PLS, both by tail vein injection
- Animal are sacrificed 6h after injection

Impact of the nanoprimer on biodistribution and efficacy of small molecule loaded liposomes

impact of the Nanoprimer on SN-38 liposomes

Tumor accumulation of SN-38 liposomes

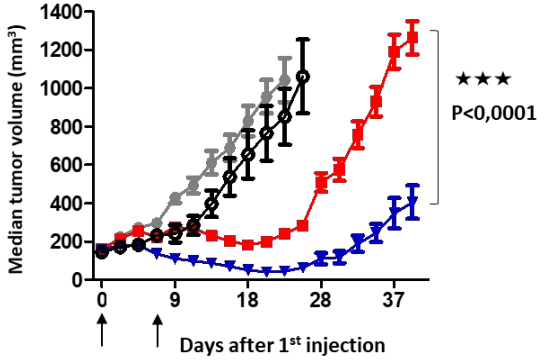


CPT 11 SN-38

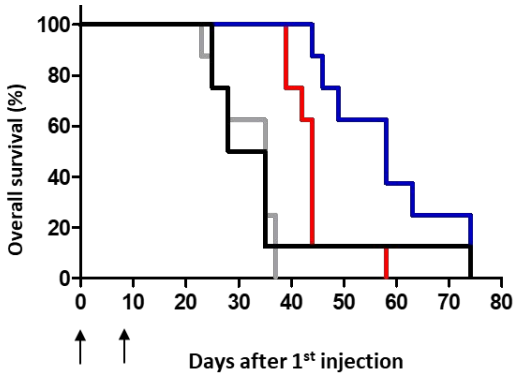
SN-38 liposome alone

Nanoprimer + SN-38 liposomes

Anti tumor efficacy of SN-38 liposomes



tumor growth delay



Increased Overall survival

SN-38 liposomes alone

Nanoprimer + SN-38 liposomes

Vehicle

Nanoprimer alone

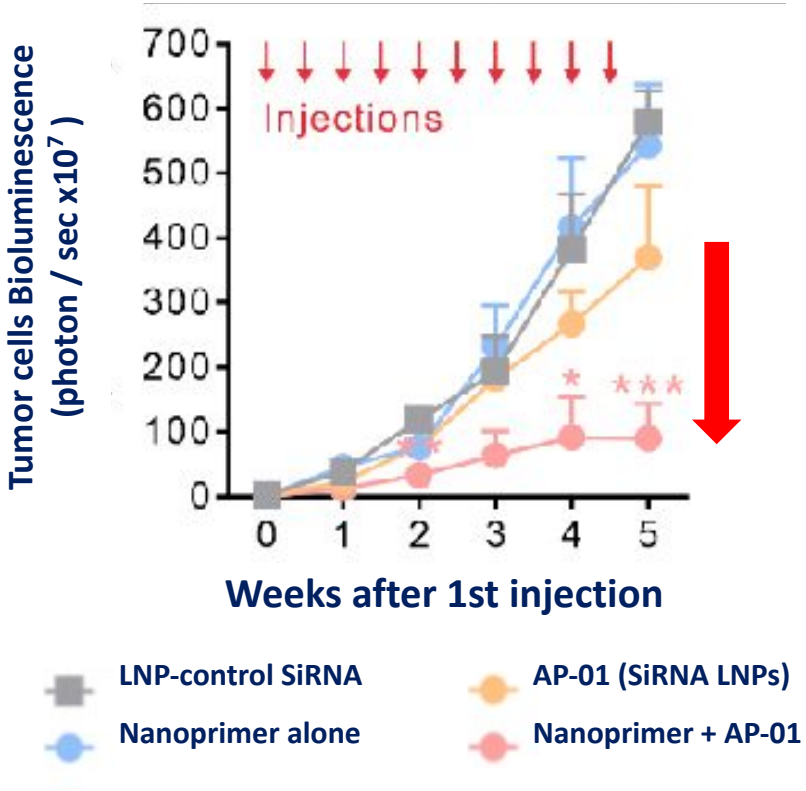
Clear correlation between impact of the Nanoprimer on biodistribution and efficacy increase

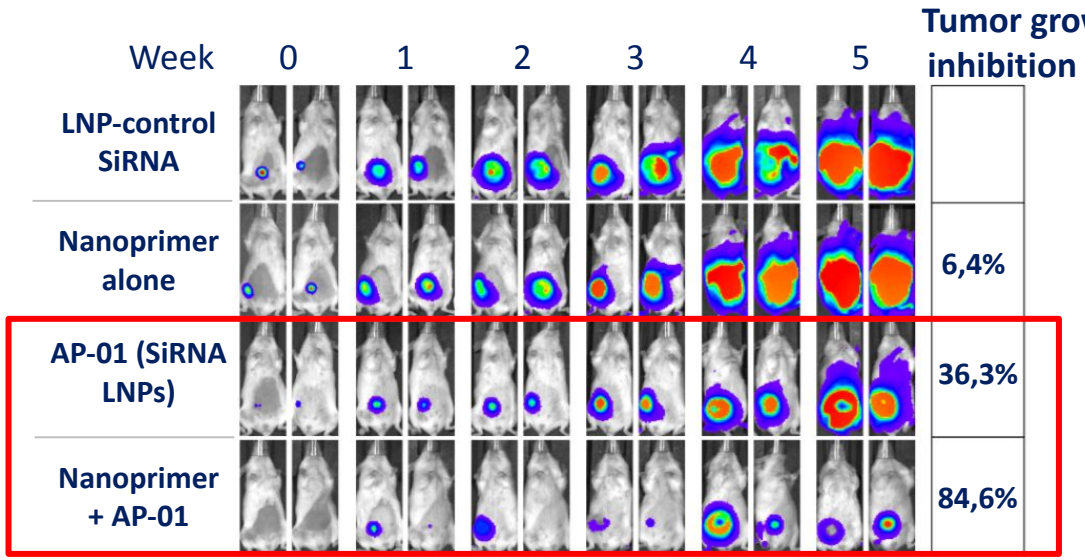
- HT-29 human colorectal adenocarcinoma cancer cell line on NMRI nude mice (n=5)
- Nanoprimer 5mL/kg; IV injected 10min before CPT-11 liposomes 15mg/kg; IV injected

- HT-29 human colorectal adenocarcinoma cancer cell line
- NMRI nude mice (n=8)
- CPT-11 liposomes 30mg/kg; IV injected on days 0, 7 (dark arrows)
- Nanoprimer 5mL/kg; IV injected 10min before each onivyde injection

Curadigm platform validation by partner with Si-RNA based therapeutic

Anti tumor efficacy of siRNA based therapeutic





Tumor bioluminescence

Constitutive expression of luminescence by living tumor cells

The Nanoprimer improves significantly efficacy of SiRNA-based therapeutic on orthotopic TNBC tumor model

Orthotopic model of TNBC: FF-Luc-expressing MDA-MB-436 cells
NOD/SCID mice (n=5)
Si-RNA LNPs were administered biweekly during 5 weeks
Nanoprimer was injected at 5mL/kg; 10 min before each injection of Si-RNA LNPs.

Take-home messages

- First preclinical safety data are very encouraging

- The Nanoprimer could bring significant benefit for several nature of therapeutic agents
  - Lipid-based nanomedicines loaded with nucleic acid or small molecules
  - Polymer based nanomedicines
  - …

- Positive impact on various therapeutic areas
  - Oncology
  - Rare diseases
  - CNS delivery

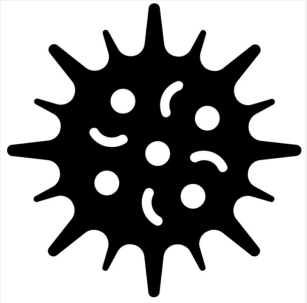
The Nanoprimer technology could also be leveraged to secure partnerships in other areas of interest

The Nanoprimer:

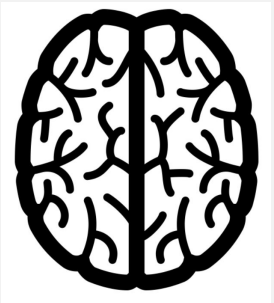
A platform to leverage the technology and improve efficacy of various nanomedicine-based therapeutics in different therapeutic areas

Nanomedicines

- **Oncology**



- **CNS**



- **Rare Diseases**

Potential synergies with your technology???

Acknowledgements

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antapodia THE NEXT FRONTIER

Kelvin TSAI

NANOBIOTIX EXPANDING LIFE