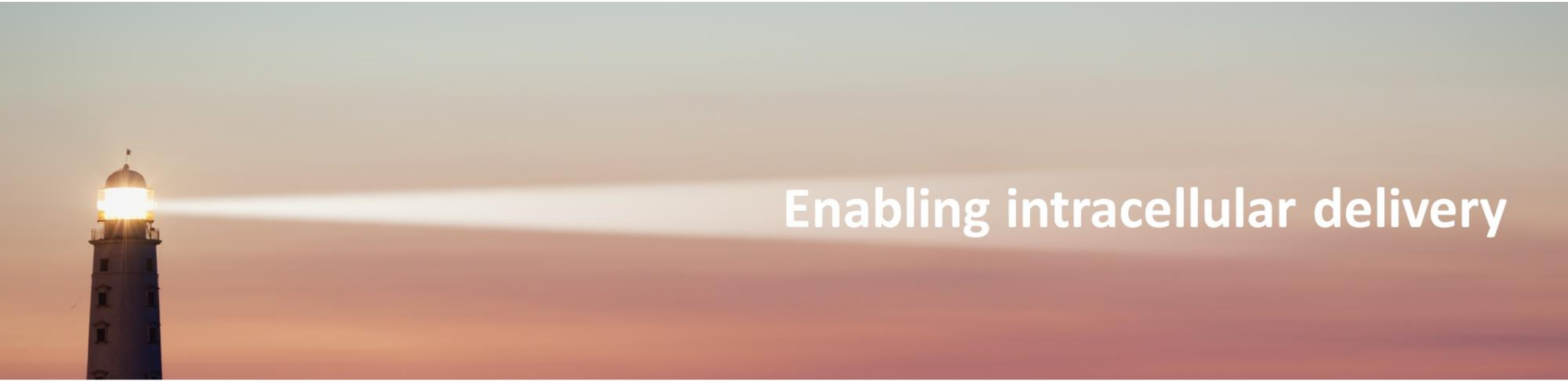
PCI Biotech



fima*NAc* – site-directed intracellular delivery of mRNA and oligonucleotides

SMi RNA Therapeutics 10-11 February 2021

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PCI Biotech

PCI Biotech – Enabling Intracellular Delivery

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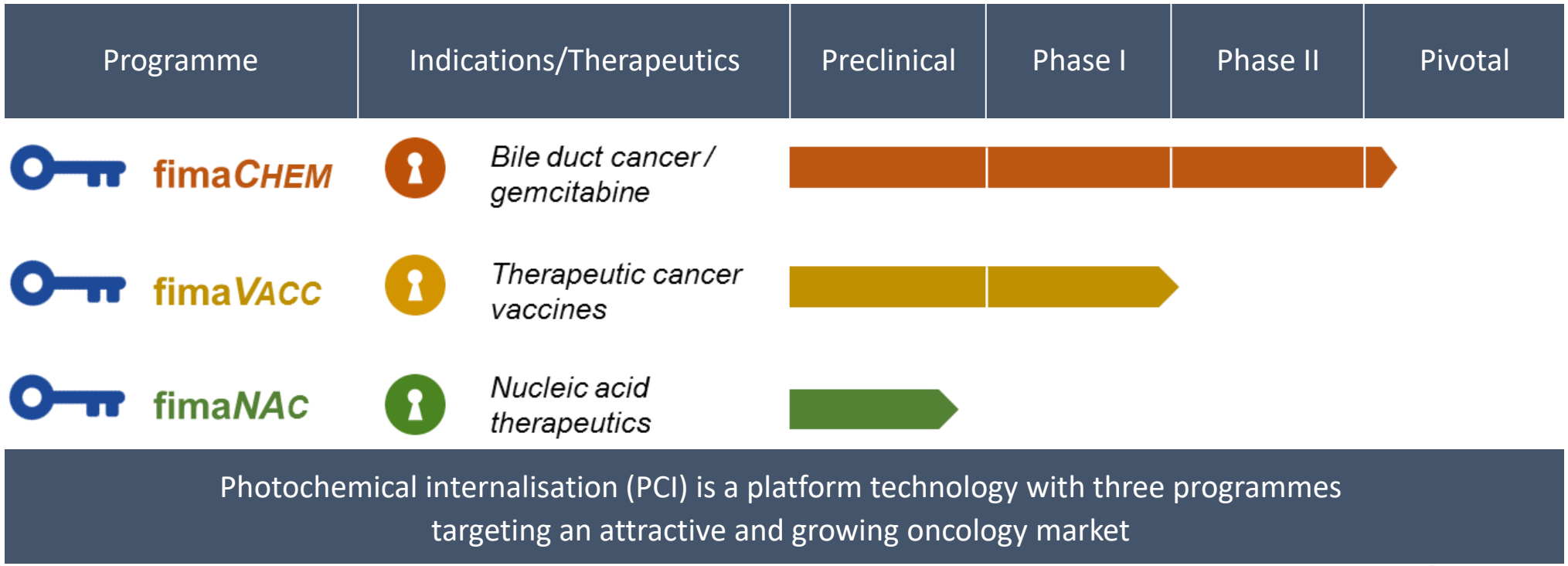
PCI Biotech – Enabling Intracellular Delivery

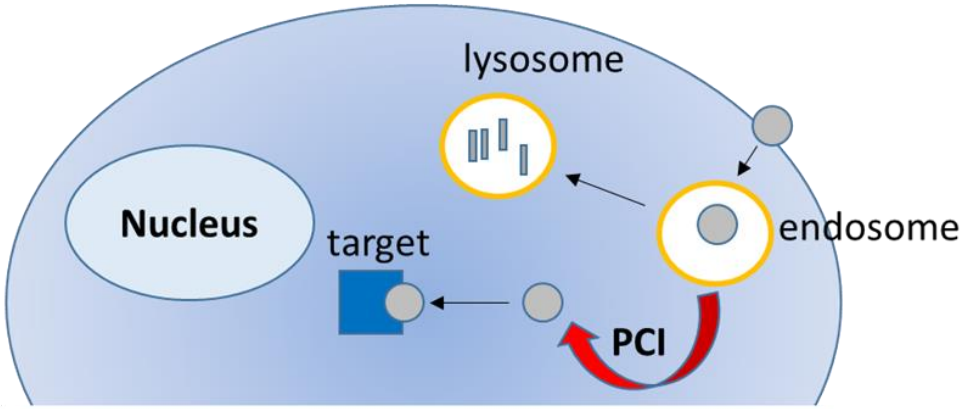
- A biotech company with an oncology focused pipeline
  - A listed (PCIB:NO) cancer-focused biotech company
  - Photochemical internalisation ("PCI") technology
  - One platform technology with three well differentiated assets



PCI TECHNOLOGY

- Using light to enable drugs to reach intracellular therapeutic targets

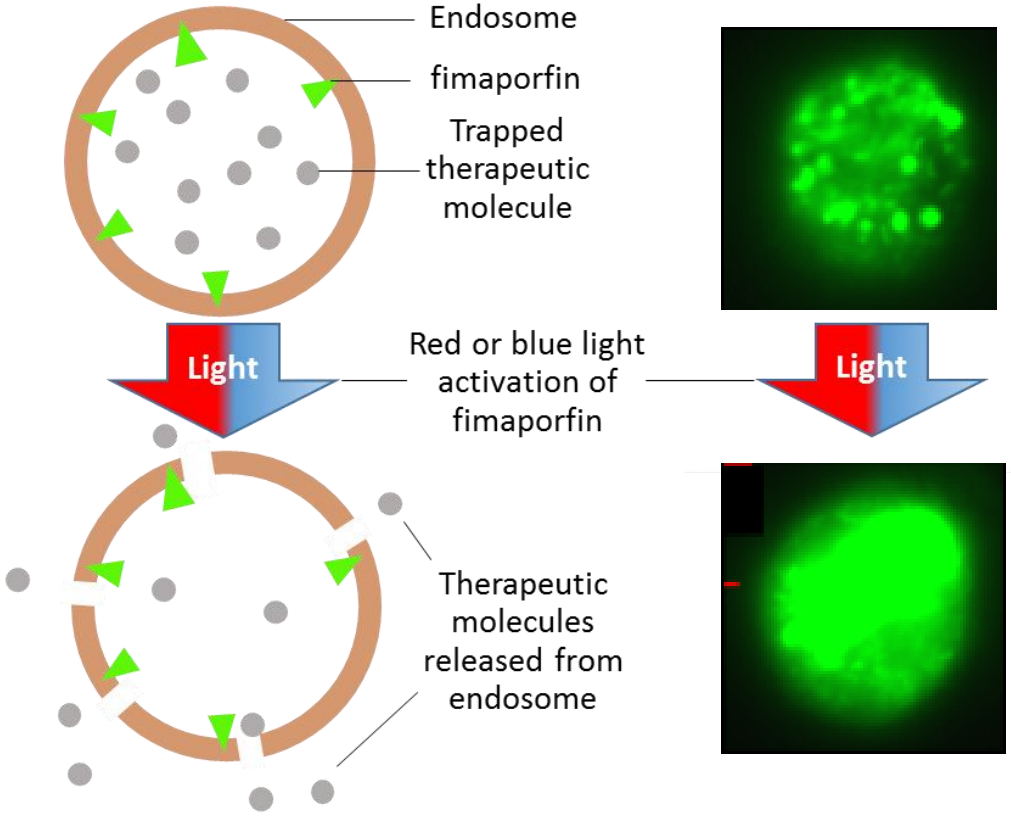
CELL SYSTEM



therapeutic molecule

- Small molecules (chemotherapeutics – fima*CHEM*)
- Antigens (peptides/proteins – fima*VACC*)
- Nucleic acids (mRNA, siRNA, plasmids - fima*NAC*)

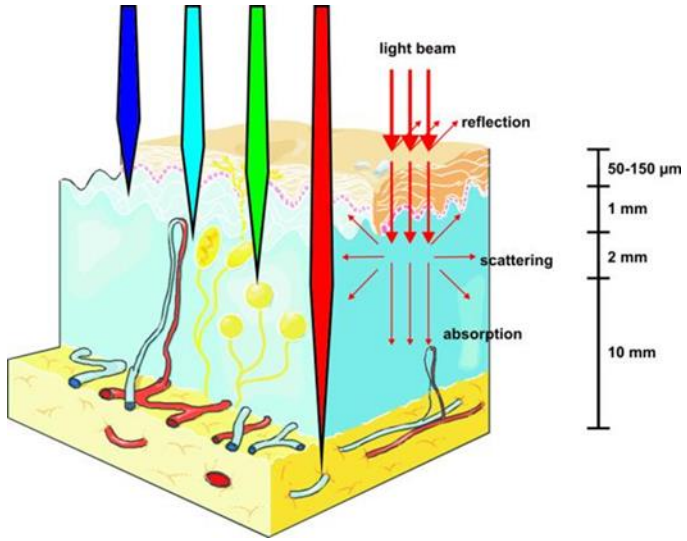
TRIGGERED ENDOSOMAL RELEASE

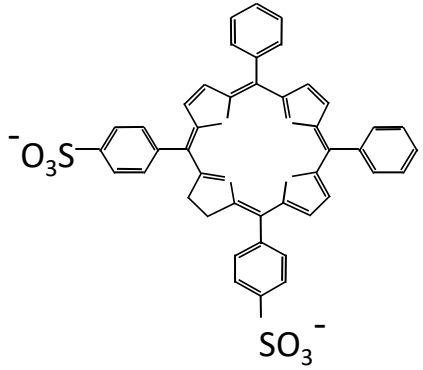


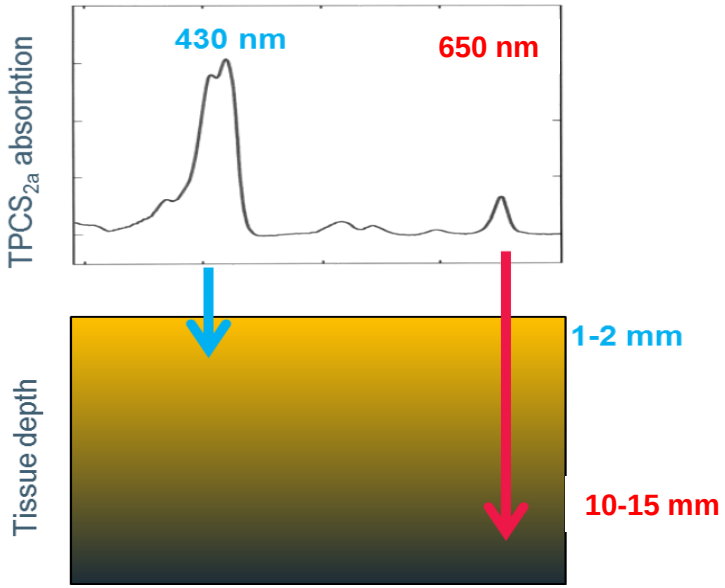
PCI TECHNOLOGY

- Effect dependent on interaction between photons and photosensitiser molecule

- Different wavelengths have different tissue penetration





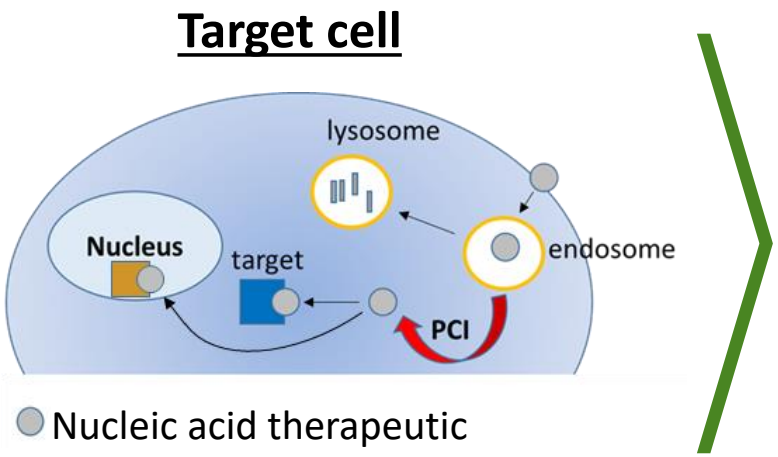


Fimaporfin

- Activated by blue or red light
- Easily synthesized
- Low toxicity
- GMP material in stock
- Very stable, can be autoclaved
- Can be mixed with nucleic acids in aqueous solution
- Also compatible with various delivery vehicles

PCI TECHNOLOGY

- **fimaNAc** – local intracellular delivery of nucleic acid therapeutics



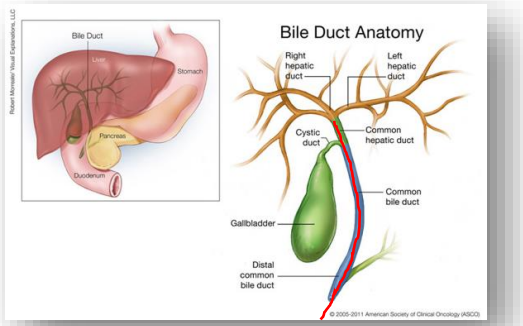
Nucleic acids successfully delivered by fimaNAc – functional assays

Type of nucleic acid	Delivery vehicle
Plasmids	PEI, cationic peptides, cationic lipids, polylysine, ++ Targeting to EGF-R, transferrin-R
siRNA	PEI, cationic peptides, dendrimers, lipofectamine, DOTAP, nanogels, chitosan ++
PNA (peptide nucleic acids)	None, cationic amino acids attached
mRNA	PEI, protamine, naked, lipofectamine, chitosan
Adenoviral vectors	None, cationic polymers
AAV vector	None

Light-Induced Delivery of Nucleic Acid Therapeutics

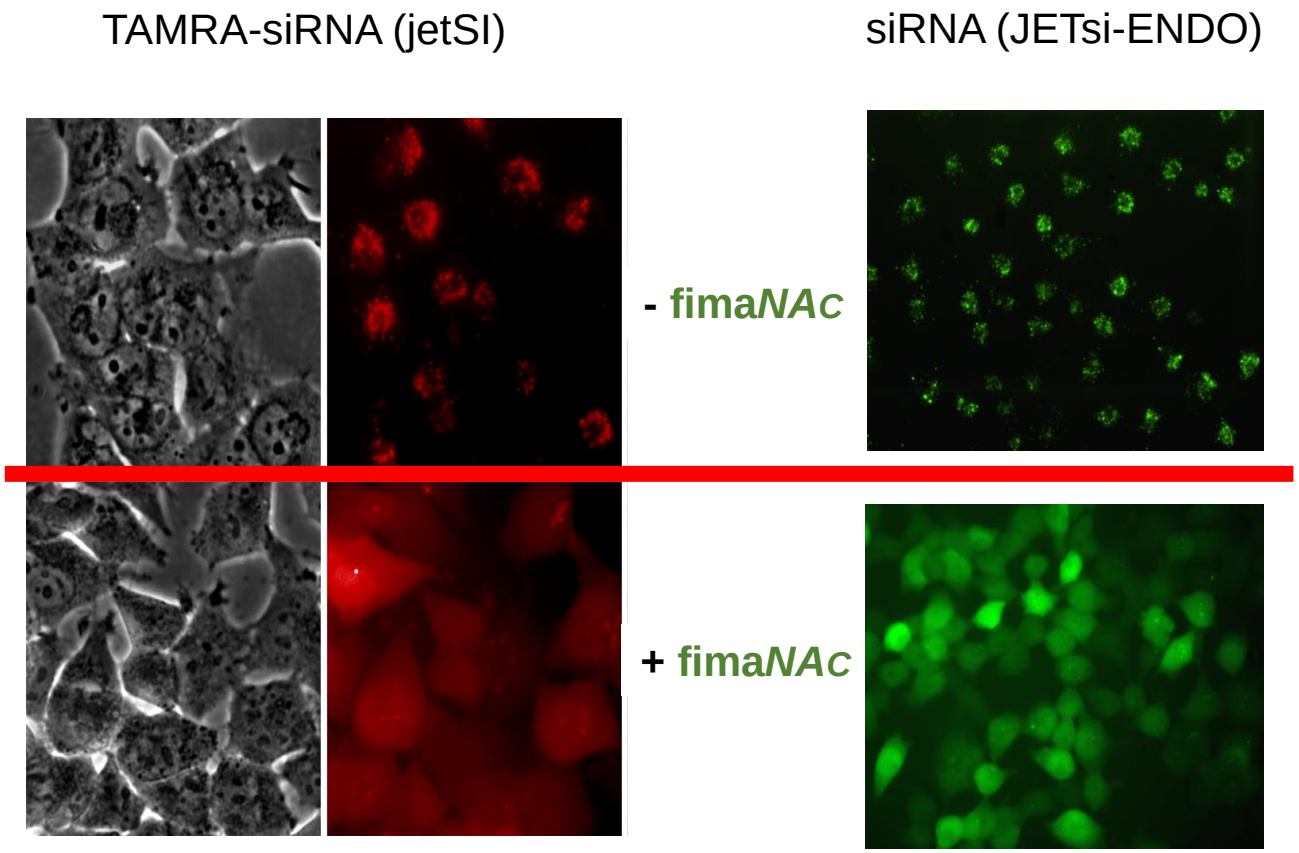
Possibilities and fields of use

- Enhance and site-direct delivery of therapeutic RNAs
  - Possible to strictly target effect to illuminated areas, strongly diminishing off-target effects
  - Most relevant with local administration, but can in principle also be used to enhance the effect of systemically administered RNA in defined target tissues/areas
- Target tissue must be accessible for illumination
  - Surface of the body
  - Internal cavities (airways, gastrointestinal tract, urogenital tract etc.) via optical fibre in endoscope
  - Eye
  - Intratumoural, e.g. via a catheter
  - In connection with surgical procedures
- Normal visible light is used, for surface illumination simple ordinary light tubes or LED-based light sources are sufficient

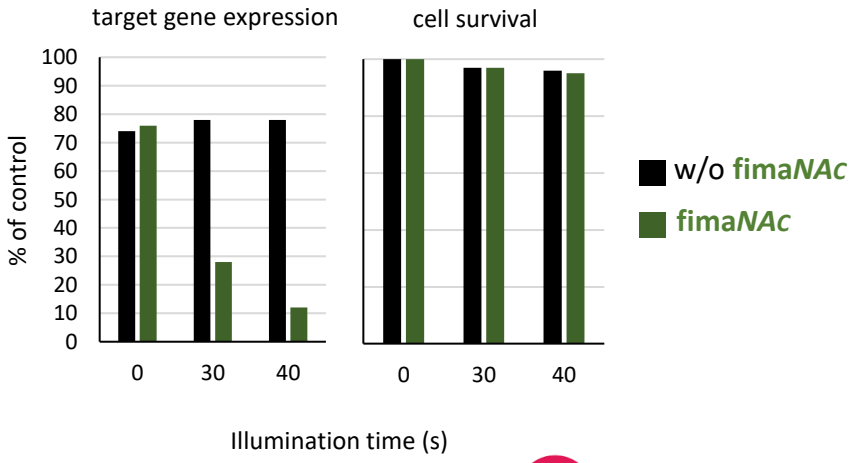


fimaNAc RELEASES OLIGONUCLEOTIDES FROM ENDOSOMES

- Effect with many types of oligonucleotides

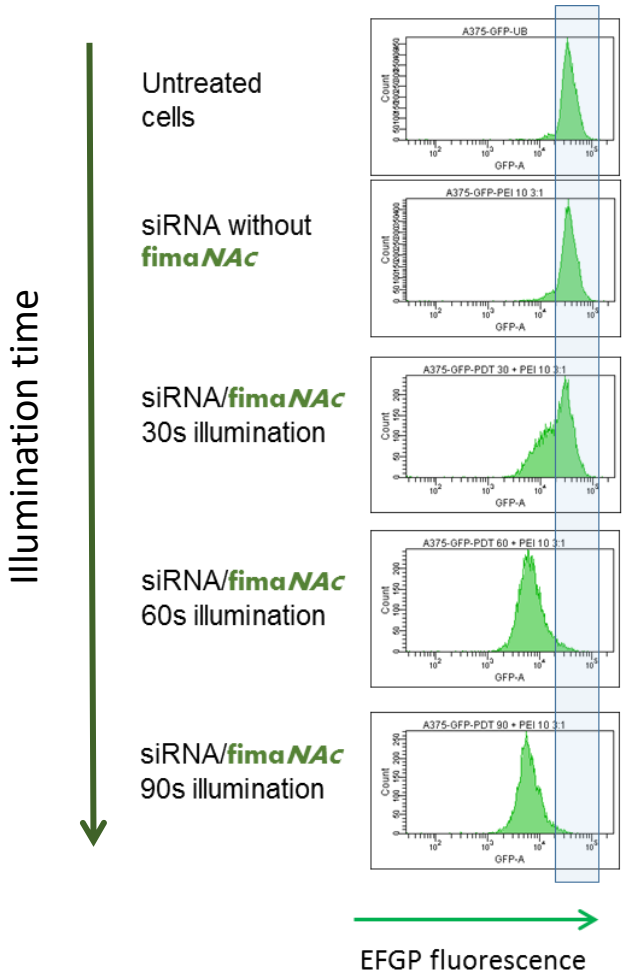


- Effect with all types of oligonucleotides tested (siRNA, DNA oligos, PNA)
- Works well both with naked oligos and with oligos delivered by delivery vehicles
- **fimaNAc** enhances siRNA (JETsi-Endo) biological activity without affecting cell viability

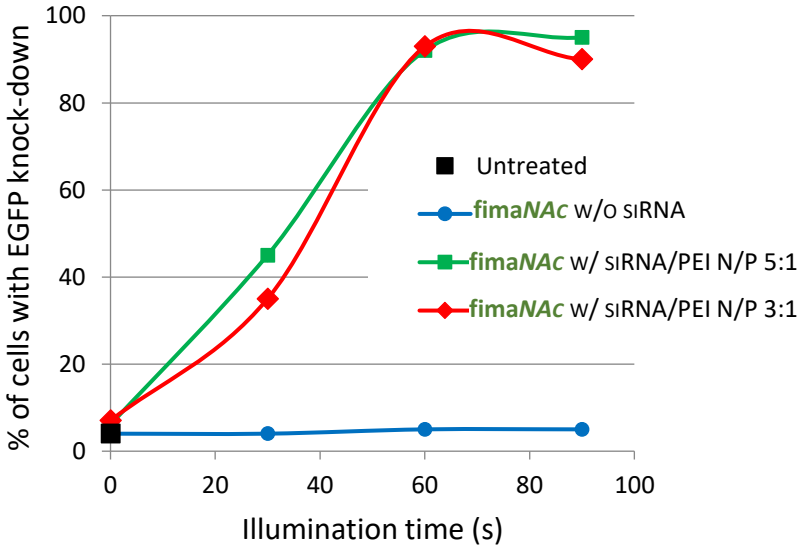


fimaNAc CAN STRONGLY ENHANCE *IN VITRO* siRNA DELIVERY

- Strongly enhanced siRNA (PEI complex) activity in A375-EGFP cells



Knock-down is dependent on illumination

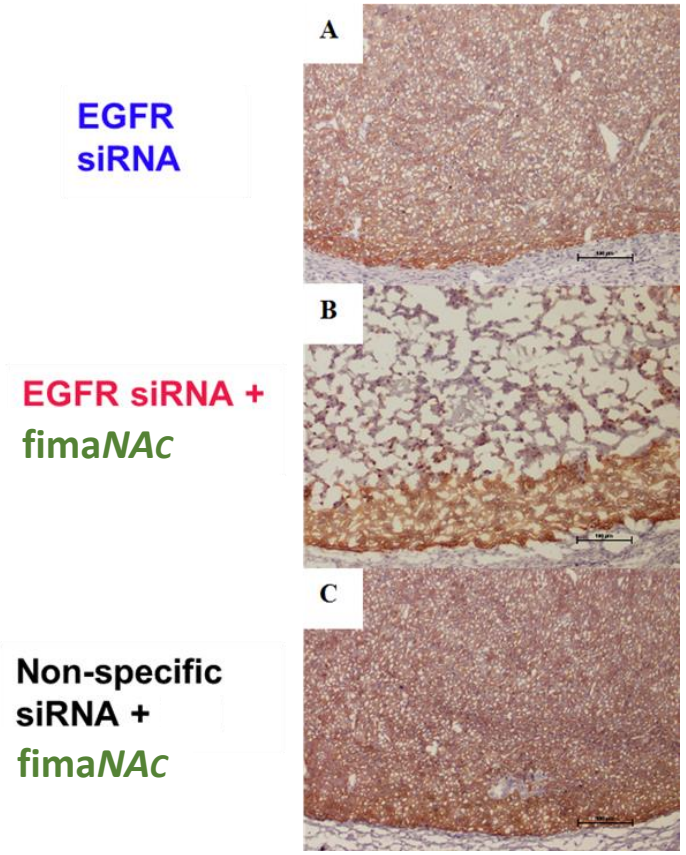


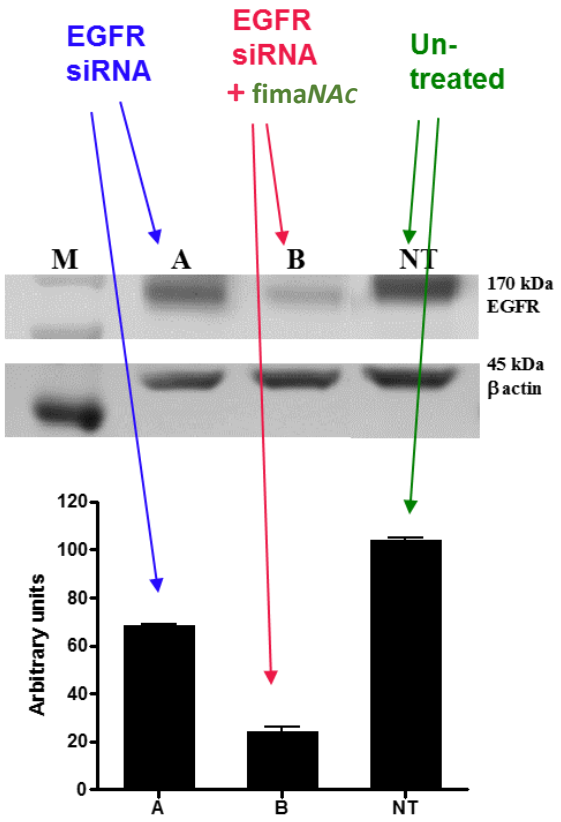
PEI 10 000 (branched)

- **fimaNAc** induces target gene knock-down in almost 100% of the cells, while siRNA-PEI alone has almost no effect
- With PEI as delivery vehicle **fimaNAc** can literally turn siRNA activity on

fimaNAc Enhances *in vivo* Local Delivery of Lipofectamine-Complexed siRNA

- Intratumoral delivery of EGF receptor (EGFR) siRNA





- **fimaNAc** induces target gene knock-down in a large fraction of the tumour cells
- siRNA-lipofectamine alone has almost no effect

Oliveira, S. et al. (2008). *Curr. Pharm. Design* 14, 3686-97

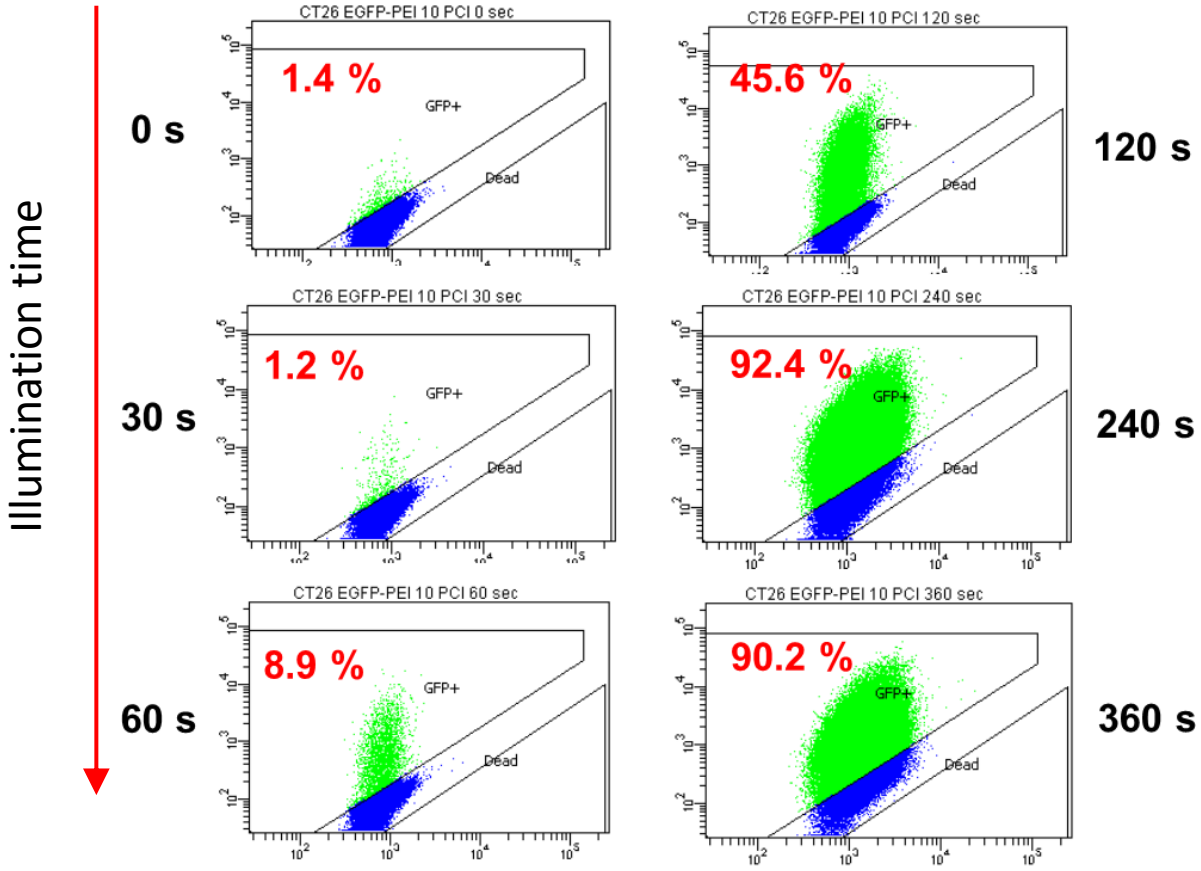
fimaNAc WITH OLIGONUCLEOTIDES - SUMMARY

- fimaNAc enhances oligonucleotide delivery mediated by many different types of delivery vehicles
- Relative effect often better when low amounts of vehicles are used
  - Vehicle-induced endosomal release can be dependent on amount of vehicle inside the endosome
  - *In vivo*, using low amounts of vehicles advantageous – toxicity
- Effect of fimaNAc varies between cell types
  - Differences in endocytosis and intracellular trafficking process?
- Good effects observed with several types of naked oligonucleotides
- *In vivo*, fimaNAc -mediated local enhancement of siRNA effect has been demonstrated in tumours and in skin
- fimaNAc is well suited for local oligonucleotide delivery to a variety of tissues

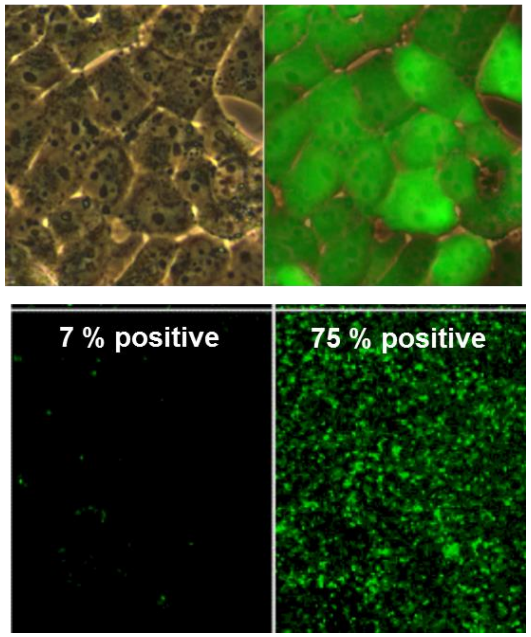
fimaNAc FOR ENHANCEMENT OF mRNA DELIVERY

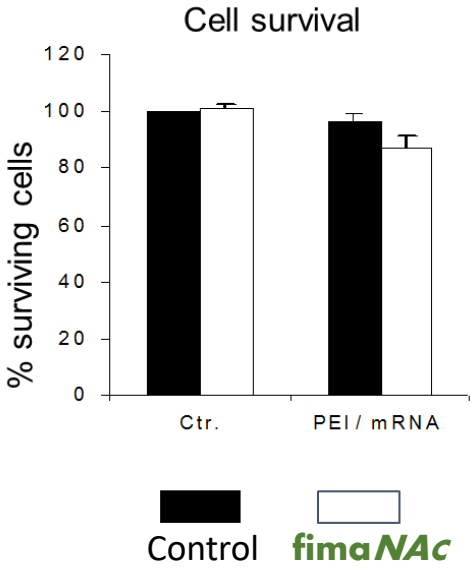
- Illumination strongly enhances *in vitro* mRNA delivery with PEI vehicle (> 60 times improvement)
- Excellent cell survival

fimaNAc with polyethylenimine (PEI) vehicle

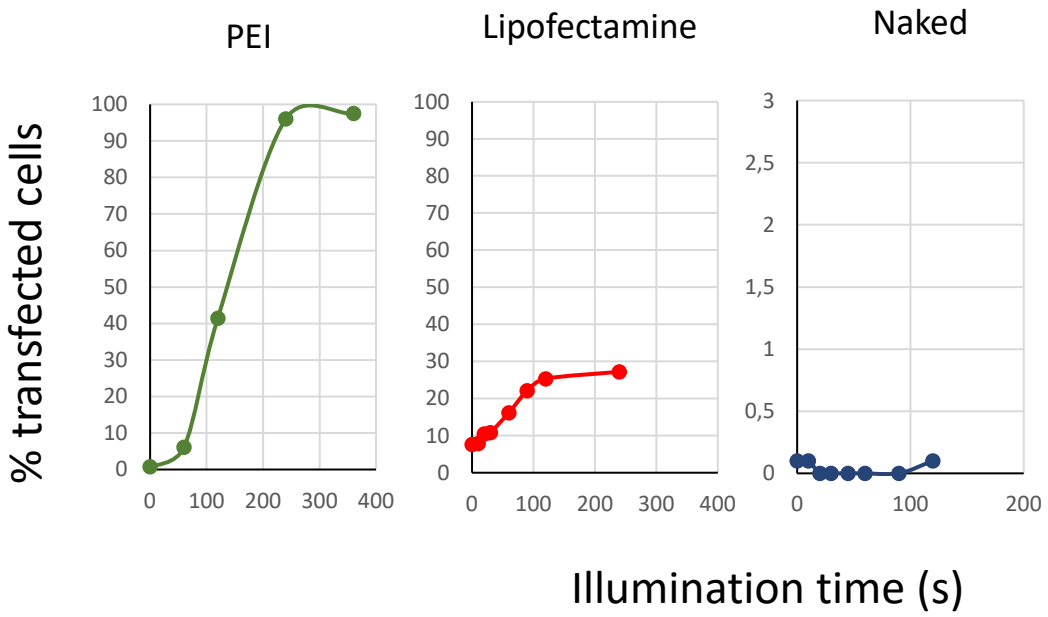


Control:





IN VITRO, fimaNAc ENHANCES mRNA DELIVERY WITH DIFFERENT TYPES OF DELIVERY VEHICLES



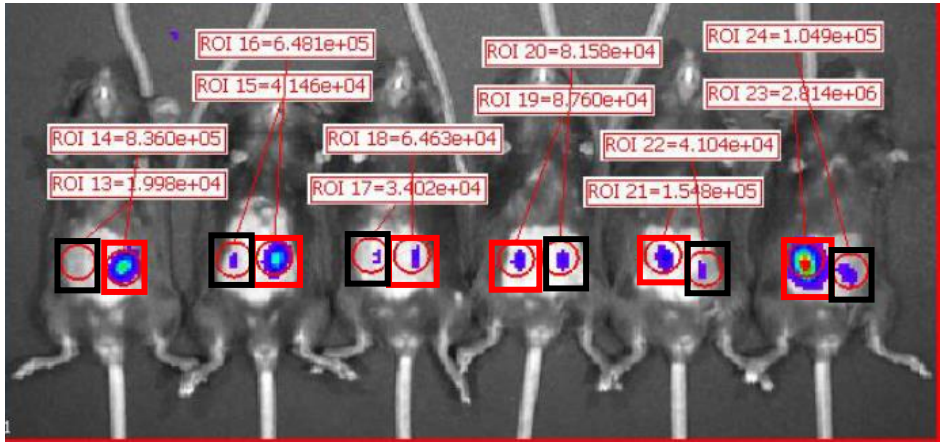
- **fimaNAc** strongly enhances mRNA delivery with different types of delivery vehicles *in vitro*
- Polyplex vehicles in general performs best, but also significant enhancement with lipid-based vehicles
- Good conditions for using **fimaNAc** for delivery of naked mRNA *in vitro* has yet to be established
- Effect with different vehicles strongly depends on cell line

fimaNAc FOR *IN VIVO* mRNA DELIVERY

- **fimaNAc** has been explored for local delivery to skin, skeletal muscle and tumours
- With lipofectamine and PEI enhancement in delivery to skin of 4-6 times has been observed as compared to delivery with vehicle alone
- Lack of correspondence between *in vitro* and *in vivo* results
  - Vehicle:mRNA ratios optimal *in vitro* did not work well *in vivo*
  - **fimaNAc** strongly enhances *in vivo* delivery of naked mRNA, but this has not yet been possible to establish *in vitro*

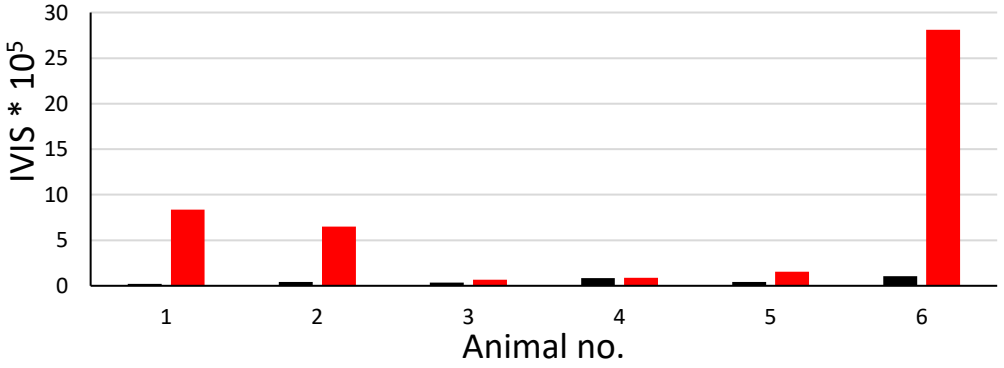
Strongly Improved Naked mRNA Delivery to Skin

- Control and **fimaNAc** site in the same animal – both sites illuminated (IVIS imaging results)



Control **fimaNAc**

fimaNAc with corresponding control in the same animal.



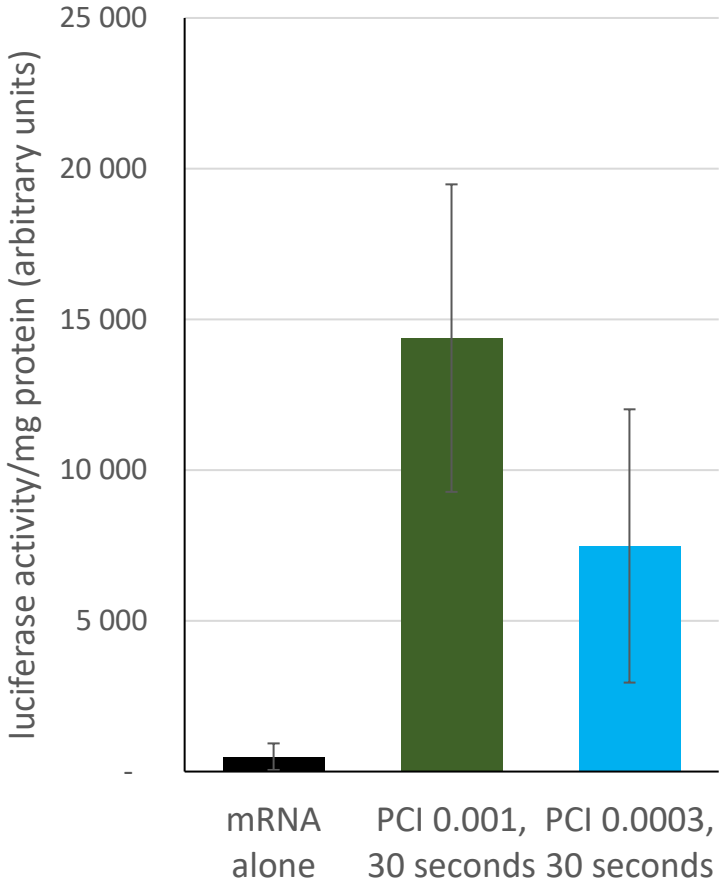
2 µg mRNA alone

2 µg mRNA + fimaporfin

- 2 µg naked luciferase mRNA mixed with fimaporfin, or mRNA alone (control,) injected intradermally at two different sites in the same mouse
- Both sites illuminated
- 14-fold increase by **fimaNAc**

fimaNAc Strongly Improves Naked mRNA Delivery to Skin

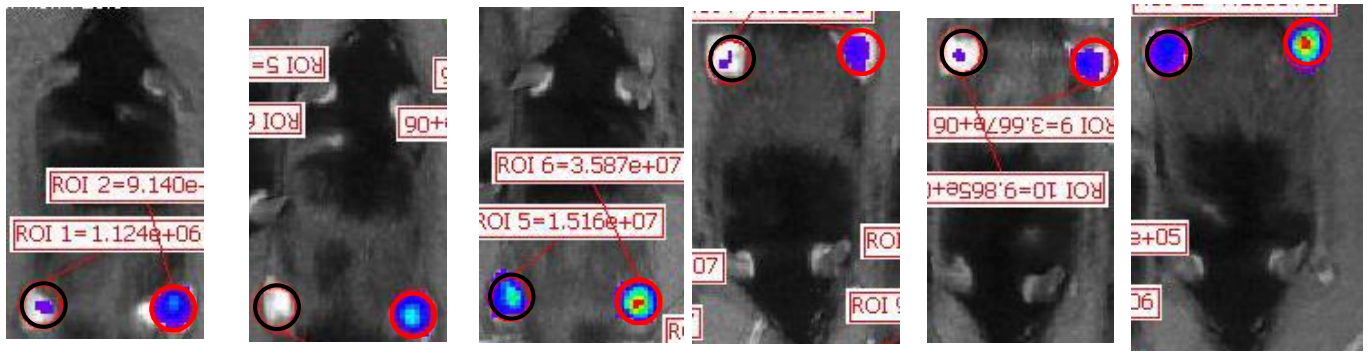
- A 29-fold increase is observed (luciferase assay) with **fimaNAc**

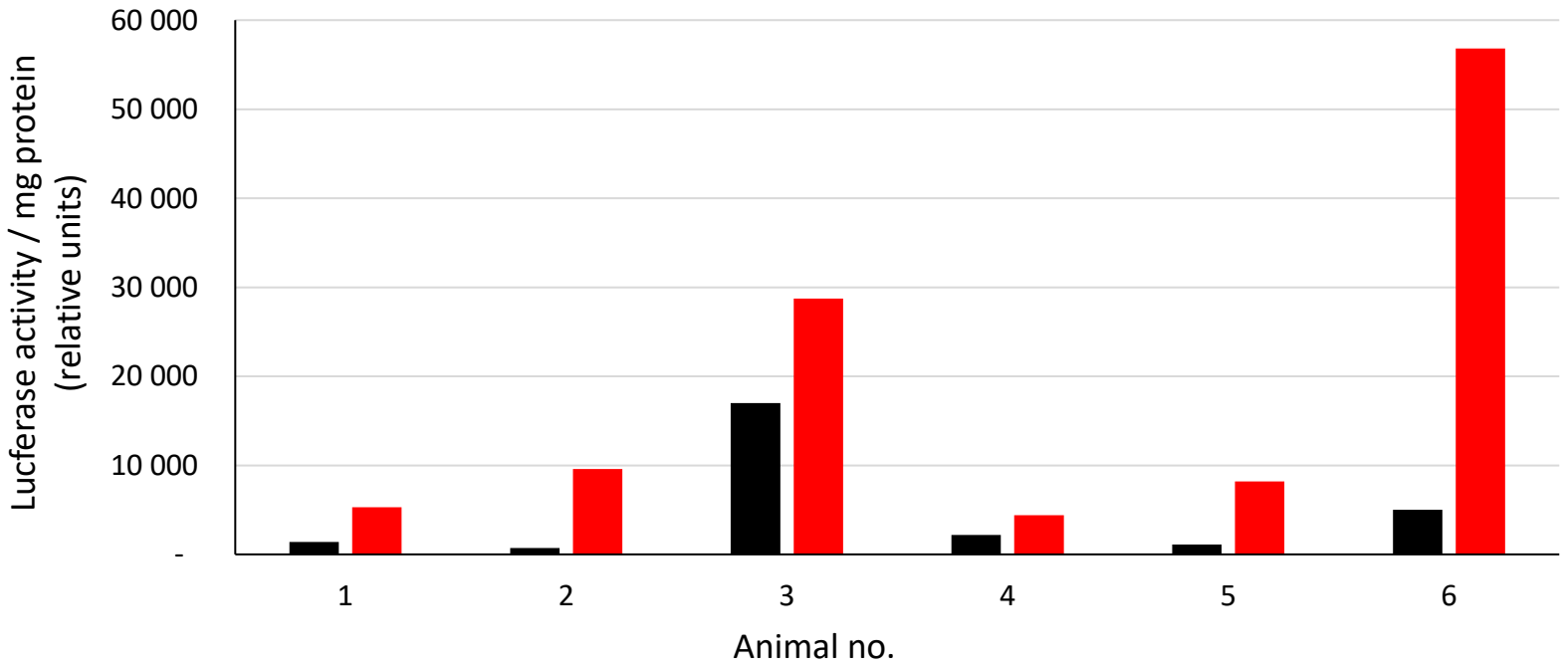


- 2 µg naked mRNA mixed with fimaporfin injected intradermally
- Illumination 60 s later
- With a fimaporfin dose of 0.001 µg, a 29-fold increase by **fimaNAc** is observed

fimaNAc Strongly Enhances Intramuscular Delivery of Naked mRNA

- Delivery to thigh muscle





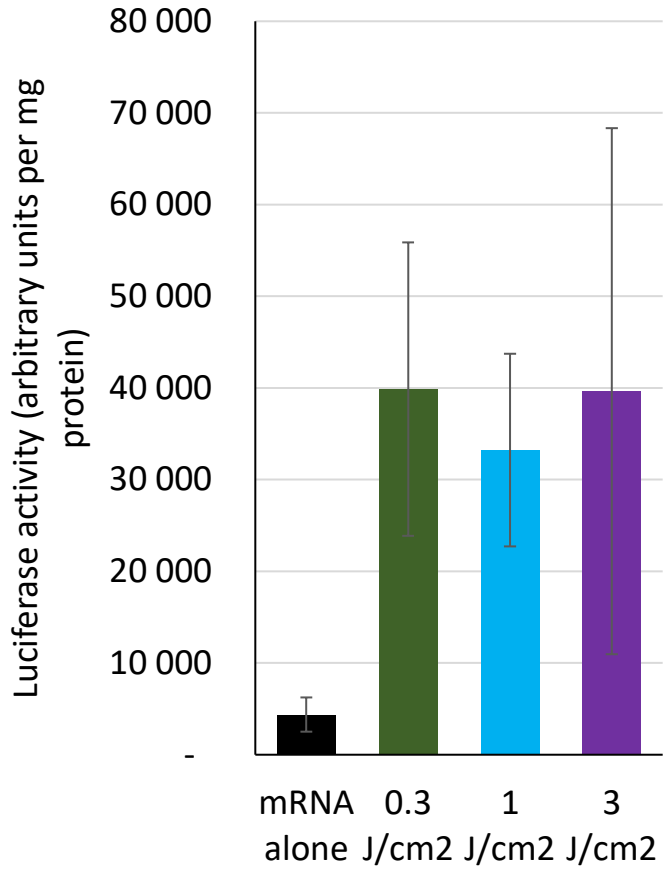
2 µg mRNA alone

2 µg mRNA + fimaporfin

- 2 µg naked mRNA mixed with 0.003 µg fimaporfin or mRNA alone (control) injected intramuscularly in each thigh in the same BL/6 mouse
- Both sites illuminated 5 min after injection
- Geometrical mean of fold increase **fimaNAc** /control in same animal = 4.9

fimaNAc Strongly Increases mRNA Delivery to Thigh Muscle

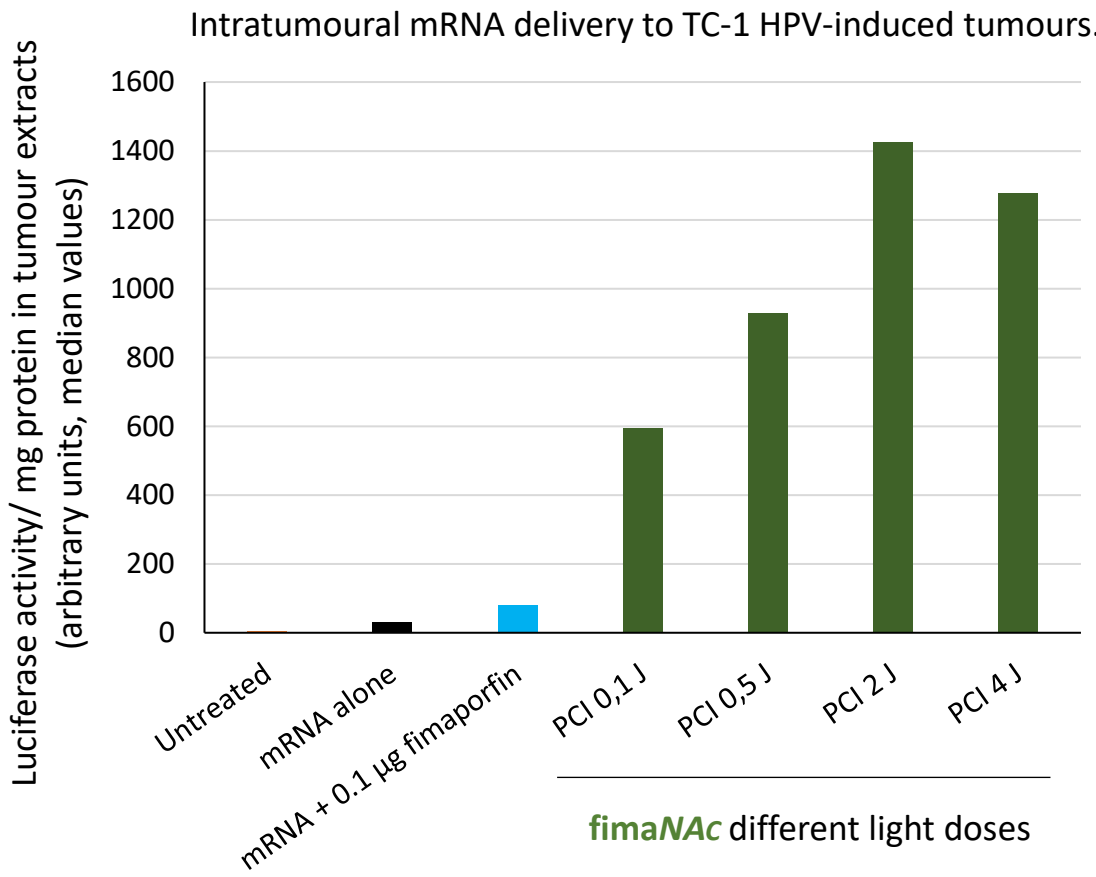
- Effect with short injection – illumination time intervals



- 8-9 fold increase by **fimaNAc** is observed over a light dose range of 0.3 – 3 J/cm^2
- Red light illumination 5 min after injection
- Good effects seen with injection – illumination intervals of 30 s to 10 min
- Injection and illumination can be performed in the same operation
- Photochemical internalisation via a direct permeabilization effect on the plasma membrane?

fimaNAc Strongly Enhances Intratumoural Delivery of Naked mRNA

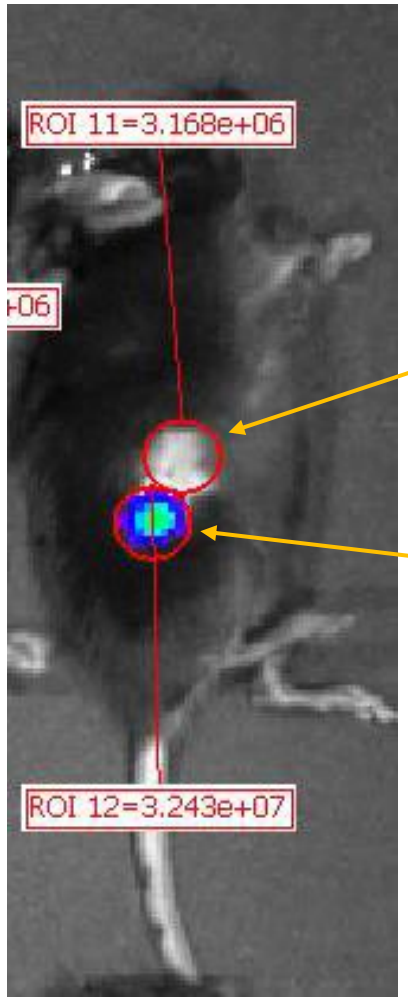
- TC-1 mouse tumour model



- fimaporfin was mixed with naked mRNA, the mixture was injected into the tumour, and the tumour was illuminated 60 min later (6 min illumination)
- **fimaNAc** gives light dose dependent enhancement of naked mRNA delivery
- At the best light dose (2 J/cm^2) nearly 50 x enhancement as compared to naked mRNA alone

FOR INTRATUMOURAL DELIVERY fimaNAc WITH NAKED MRNA WORKED BETTER THAN LNPS

- Intratumoural delivery to MC38 tumours



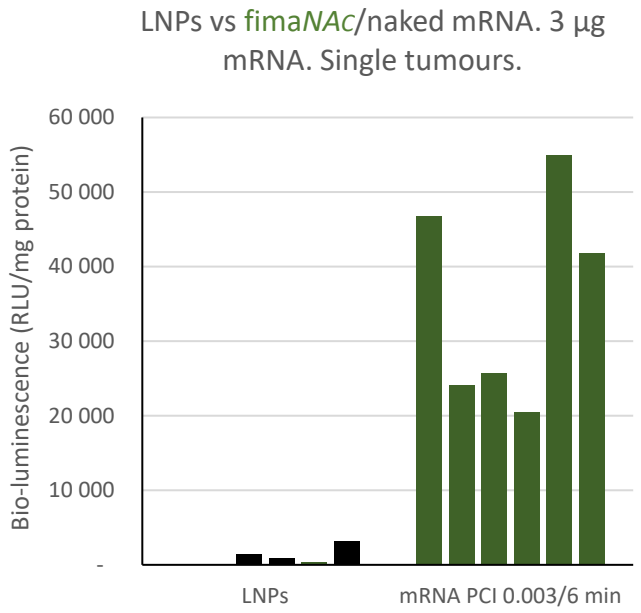
LNPs, 12 µg mRNA

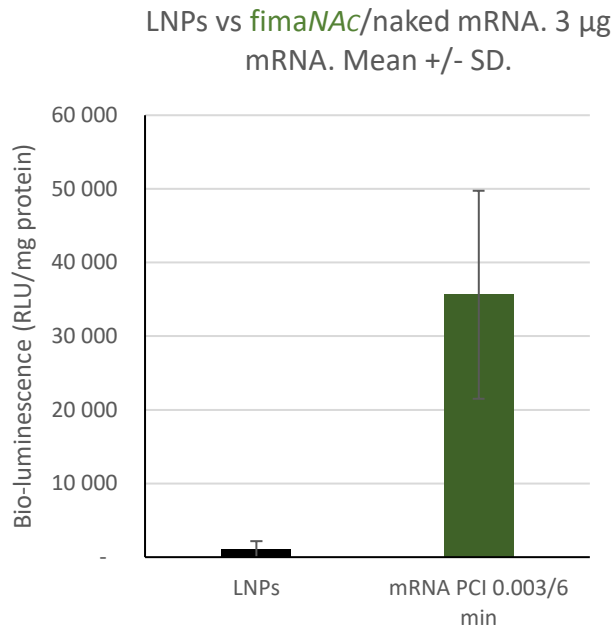
fimaNAc with 12 µg naked mRNA and 0.003 µg fimaporfin

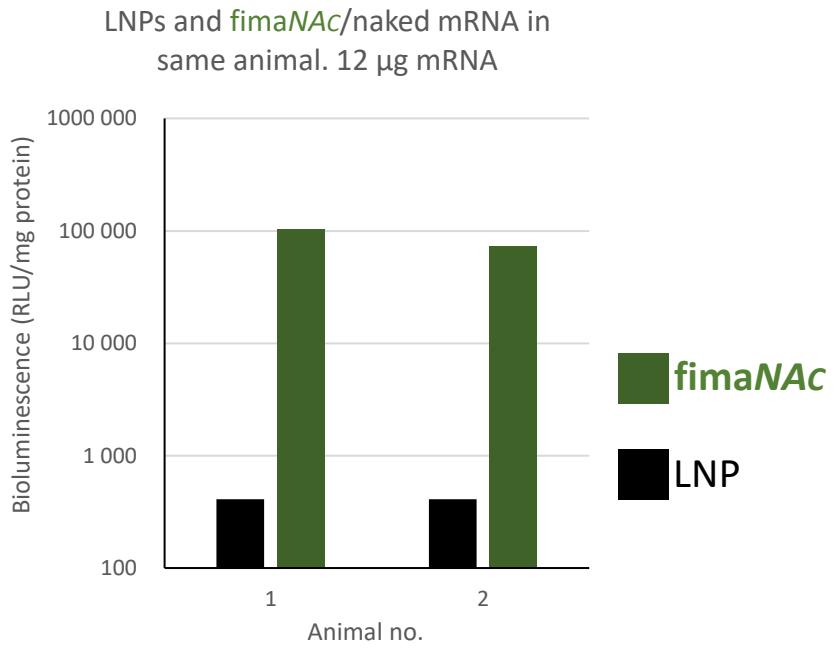
- LNPs:
  - MC3:DSPC:Chol:DMG-PEG at
  - mol% composition of 50:38.5:10:1.5;
  - 20:1 lipid to mRNA wt/wt ratio
- Two tumours per animal
- One injected with fimaNAc (naked luciferase mRNA + fimaporfin) and one with LNPs
- Both tumours illuminated
- *In vivo* chemiluminescence imaging of luciferase activity
- Enzymatic assay for luciferase activity in tumour extracts

INTRATUMOURAL DELIVERY WITH fimaNAc IS CONVINCINGLY SUPERIOR TO LNPS

- Consistently improves delivery to MC38 tumours compared to what is achieved with LNPs





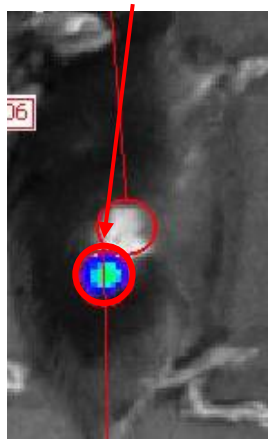


- **fimaNAc** with 3 µg mRNA increased luciferase activity about 35 times as compared to the LNPs
- In animals where one tumour was treated with **fimaNAc** and one with LNPs (12 µg mRNA) the observed fold increase was about 200 times (right panel)

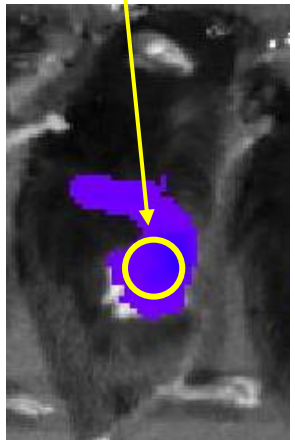
Preventing Undesirable Off-Target Delivery

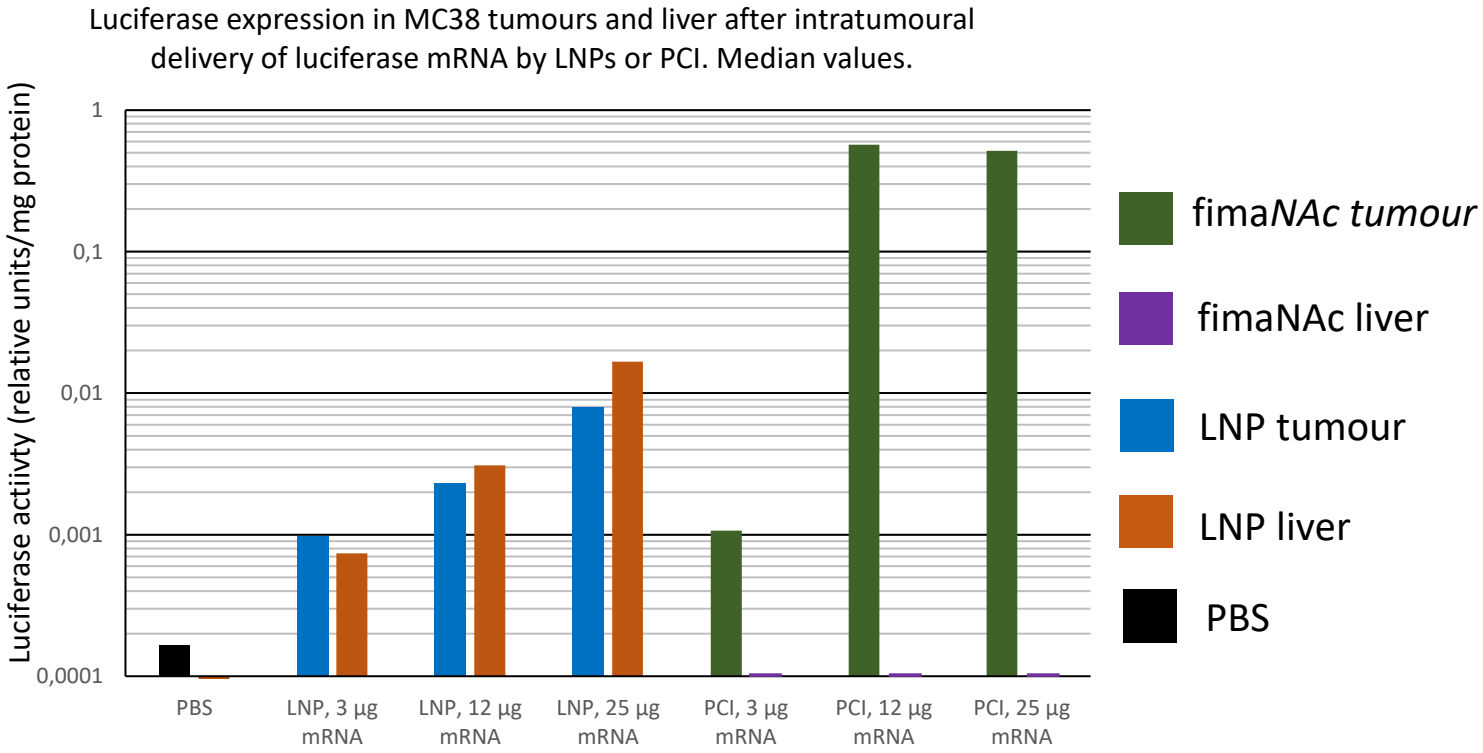
- With **fimaNAc**, mRNA expression is confined to tumour tissue

fimaNAc with naked mRNA



LNPs

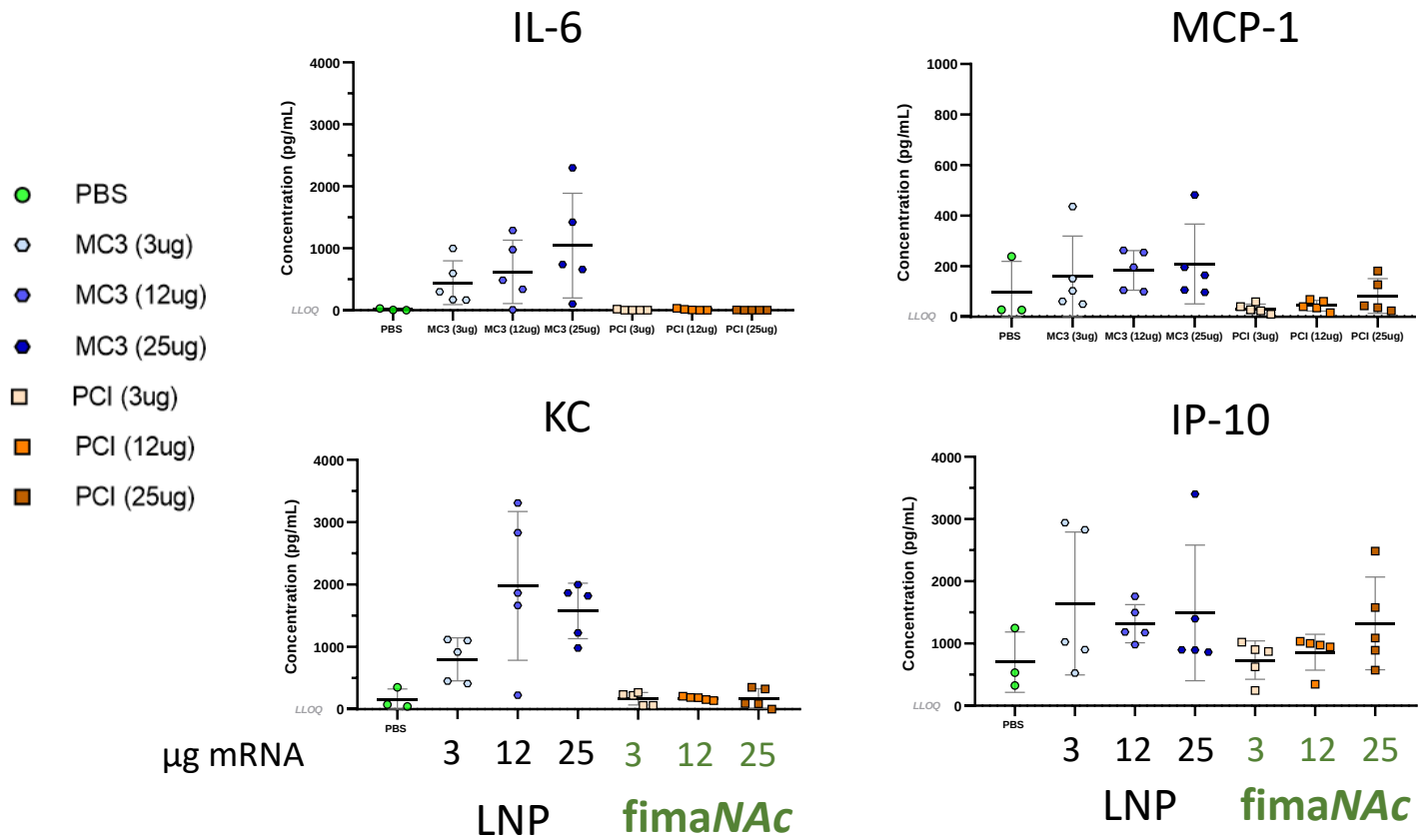




- With **fimaNAc** -mediated delivery of naked mRNA, expression is confined to the tumour
- LNPs seem to leak out of the tumour leading to unwanted expression in the liver, with similar expression levels as in the tumour

fimaNAc Does not Induce Undesirable Cytokine production

- Intratumoural mRNA delivery with **fimaNAc** does not increase cytokine levels in blood

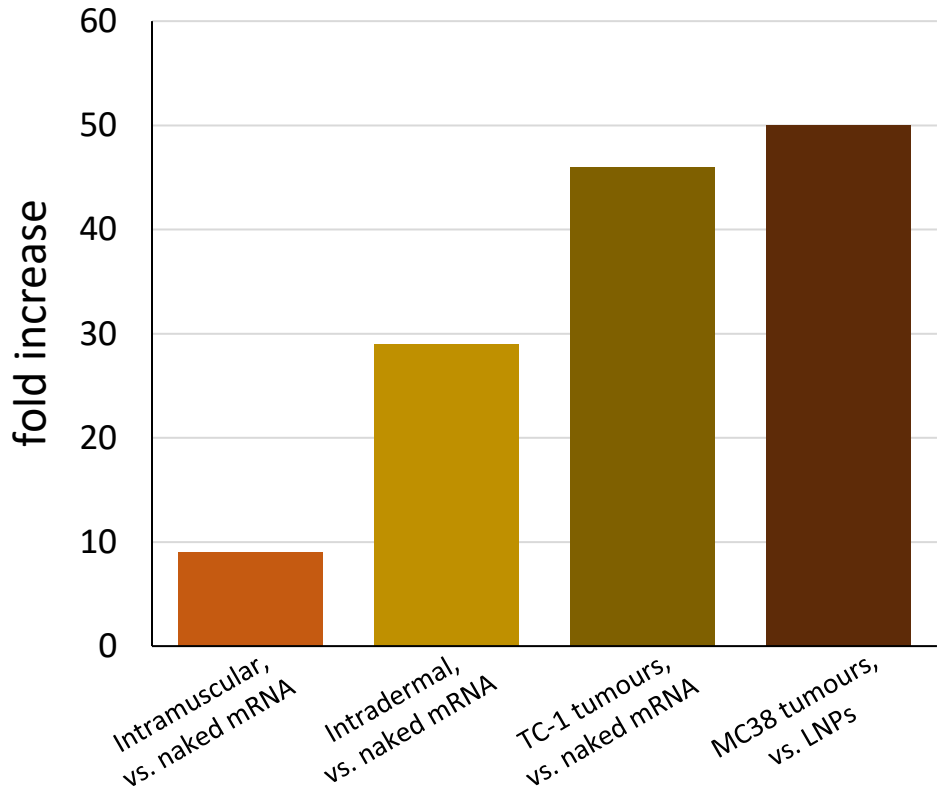


- **fimaNAc** delivery gives a lower inflammatory response (IL-6, KC and MCP-1) compared to delivery with LNP
- IP-10 read-out was less clear
- IL-1β was not detected for any of the treatments

NAKED MRNA DELIVERY WITH fimaNAc – DIFFERENT APPLICATIONS

- Best effect seen for intratumoural delivery

Fold increase of mRNA expression with fimaNAc



- Intratumoural immunotherapy
  - Systemic therapeutic effects can also be achieved
  - mRNA encoding antigens and immuno-stimulating factors
  - To avoid side effects of potent effector molecules it may be very important to confine mRNA expression to tumour
    - fimaNAc substantially better than LNPs
  - The photochemical treatment can also have an immunological adjuvant effect
    - Modulation of tumour microenvironment

NAKED MRNA DELIVERY WITH fimaNAc – SUMMARY

- Local delivery technology
  - mRNAs and fimaporfin can be mixed in aqueous solution and administered as one injection without local or systemic side effects
  - mRNA administration and illumination can be done in the same procedure
  - mRNA expression spatially restricted to illuminated area
- Clinically proven platform technology
  - The clinical fimaVACC and fimaCHEM programmes are using the same platform technology
  - Ample safety data in humans both for systemic and local administration of fimaporfin
- Applications where a local effect is desired
  - Skin, muscles, tumours, eye, joints, lymph nodes
- Substantially enhanced delivery to tumour, muscle and skin
  - Clearly improved characteristics compared to LNPs demonstrated in tumour

COLLABORATORS AND ACKNOWLEGDEMENTS

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Thank you