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Polymeric Nanoparticle Platform Technologies for RNA & Gene Therapeutics

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Nanoparticle R&D in the Aigner lab

Polymer-based Nanoparticles for the delivery of nucleic acids *in vitro* & *in vivo*

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Nanoparticle
(PEI + nucleic acid)

PEI
- Linear
- Branched

Nucleic acid
- siRNA
- miRNA
- mRNA
- DNA

→ *Novel therapeutic drugs*

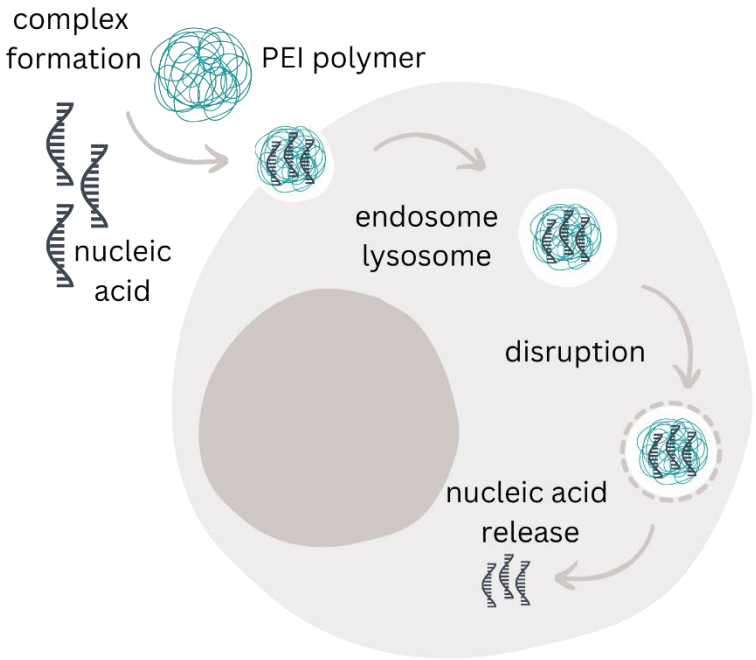
Chemical modification
- Tyrosine
- Lipopolyplexes
- PEGylation
- Disulfide bridges

→ *Enhanced safety & efficacy*

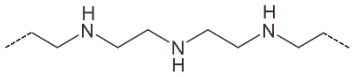
Ligand coupling
- Antibodies

→ *Targeted uptake*

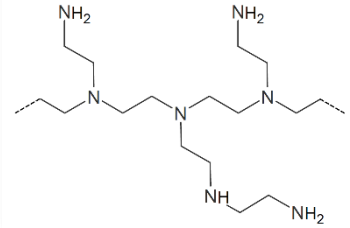
Cellular uptake of Nanoparticles



LINEAR PEI



BRANCHED PEI



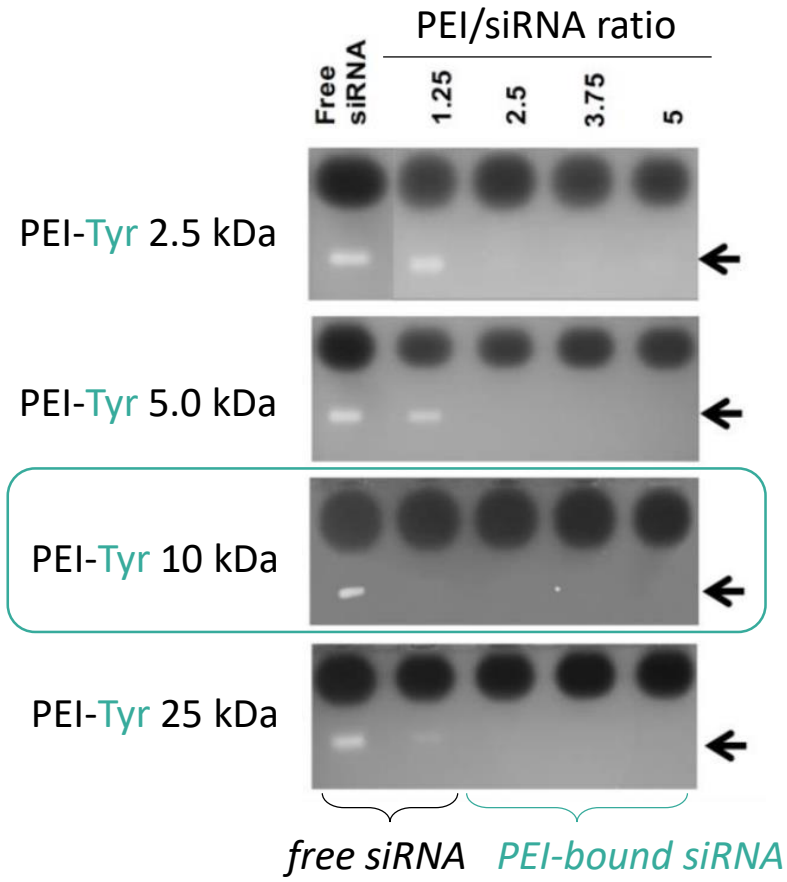
1. Nanoparticle formation - *Nucleic acids protected from degradation*
2. Cellular internalization - *Endocytosis*
3. Intracellular nanoparticle release - *Escape from the endo-/lysosome*
4. Release of nucleic acids - *at their site of action*

PEI = Polyethylenimine

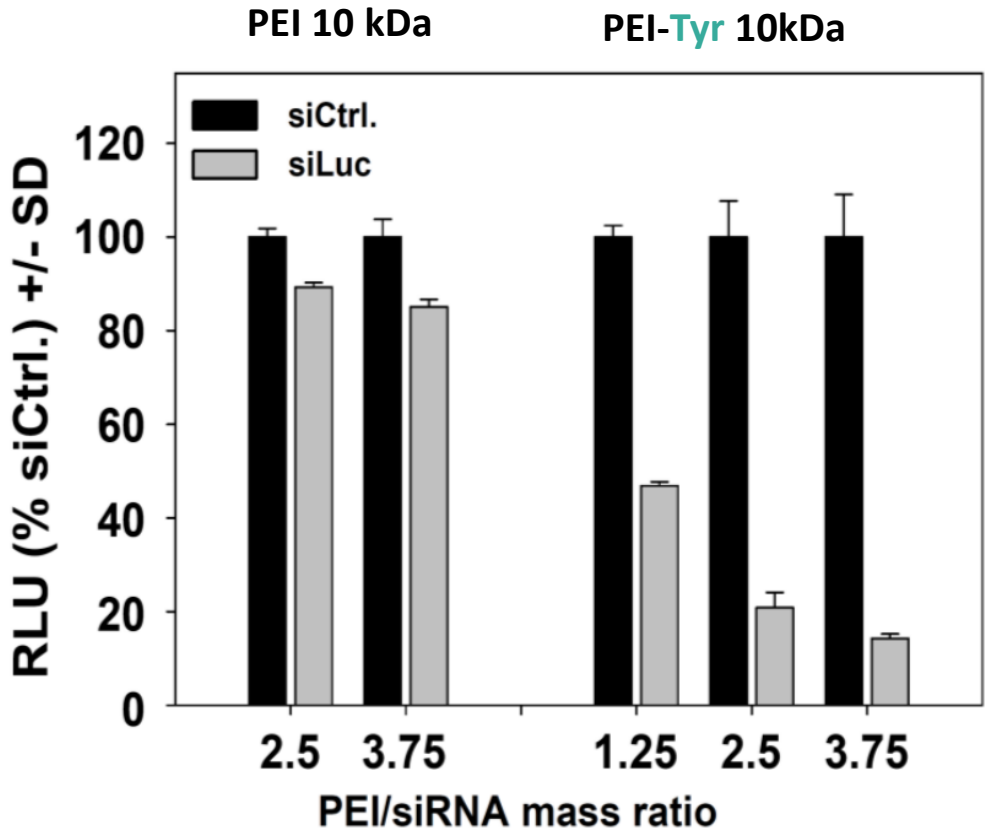
The polymer: PEI-Tyr + siRNA (*in vitro*)

High efficient complex formation by Tyrosine modification

Efficiency of siRNA complexation



Luciferase Reporter Gene Assay (PC3 prostate cancer cells)

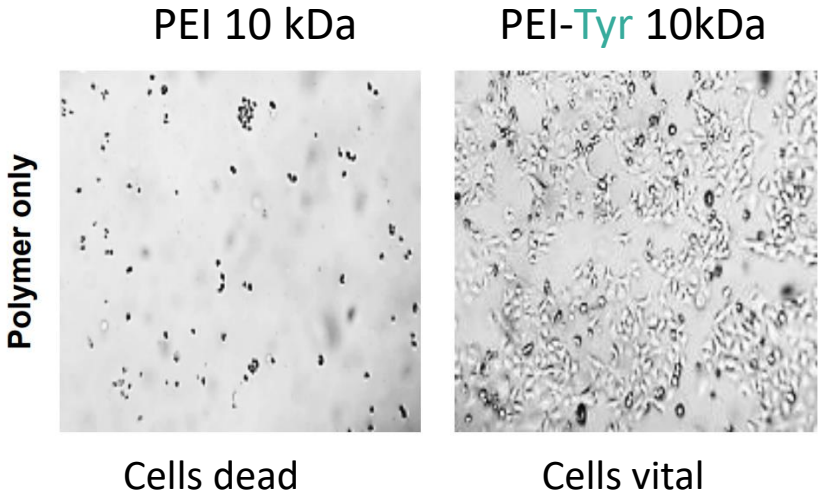


Tyrosine modification → high complexation efficiency & improved biological KD efficiency

The polymer: PEI-Tyr + siRNA (*in vitro*)

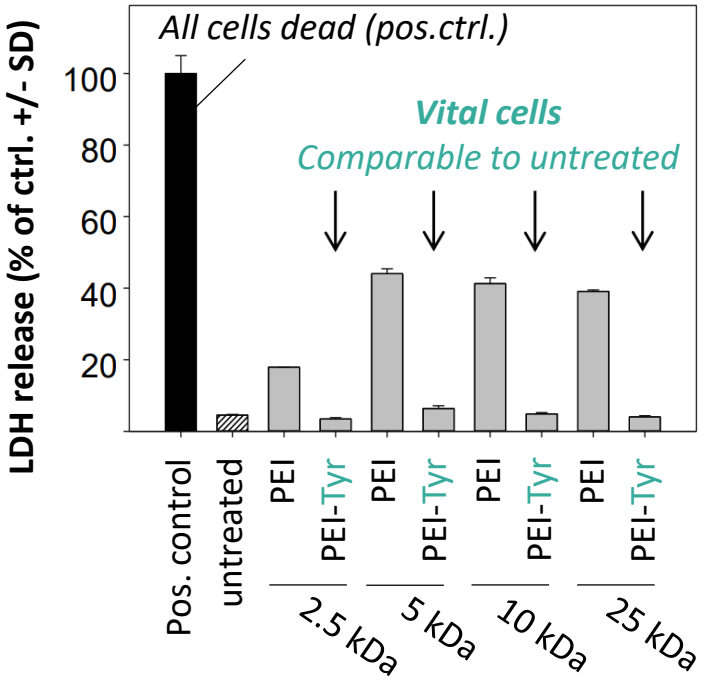
Increased biocompatibility by Tyrosine modification

Cell viability (PC3)



LDH Assay

Lactate dehydrogenase release
correlates with proportion of dead cells



Also no signs of

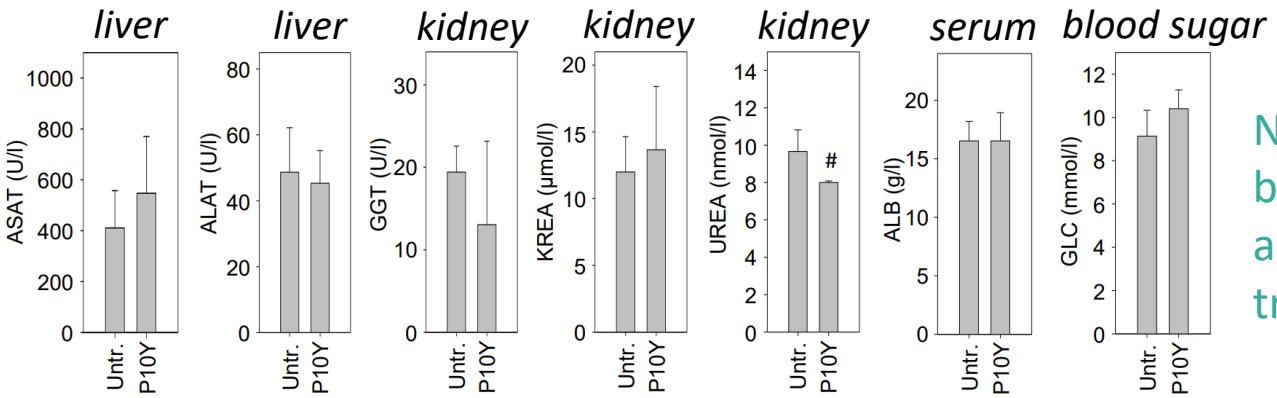
- DNA damage (COMET assay)
- Erythrocyte aggregation
- Hemolysis (Ery decay)

Tyrosine modification minimizes potentially toxic effects on cells

The polymer: PEI-Tyr + siRNA (*in vivo*)

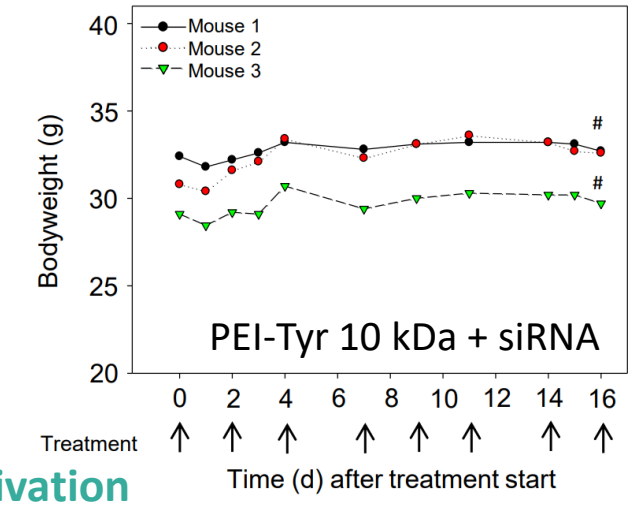
No toxic effects of Tyrosine-modified PEIs in mice

Blood levels

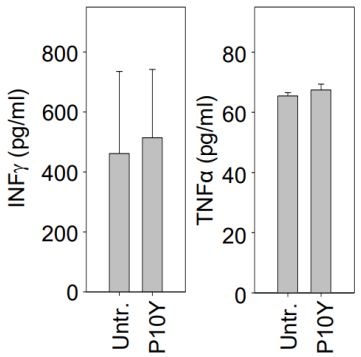


No differences between untreated and PEI-Tyr/siRNA treated mice

Stable body weight after PEI-siRNA treatment



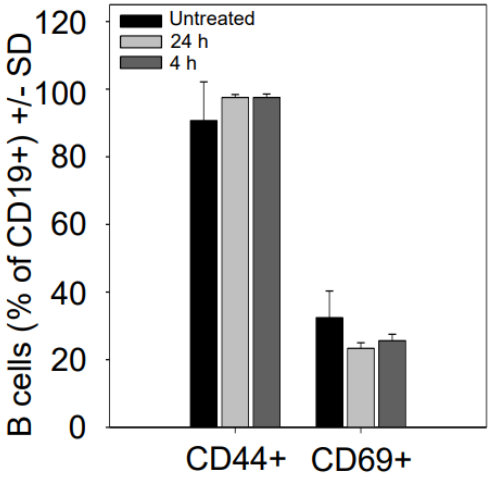
IFNγ TNFα



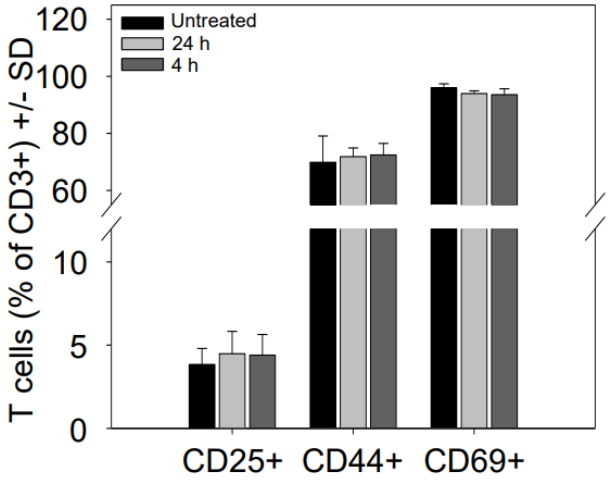
(anti viral) *(inflammation)*

No activation of innate immune response by PEI-siRNA

B cell activation



T cell activation

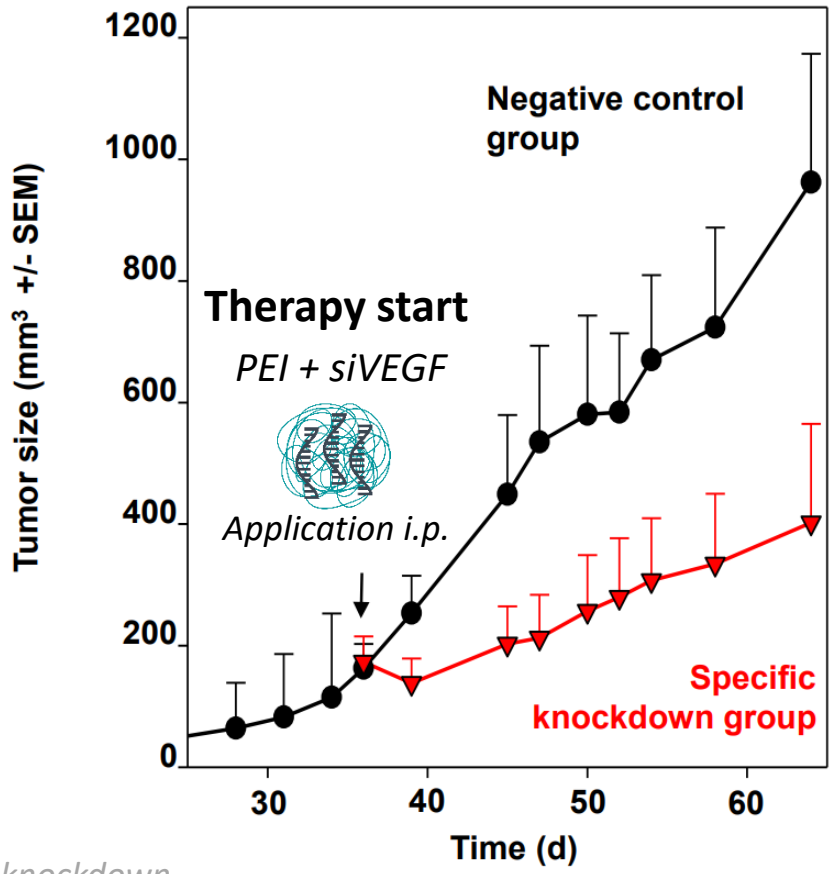


Activation markers

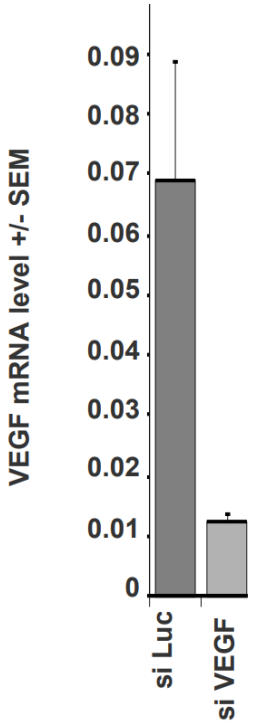
No activation of adaptive immune response by PEI-siRNA

Therapeutic siRNA delivery *in vivo*

Pancreatic cancer xenografts
Antitumor effects

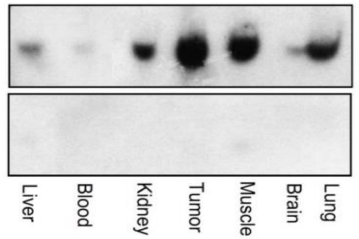


Target gene KD in tumor tissue



Tissue accessibility & biodistribution

PEI-siRNA 30min after i.p. injection



PEI + siRNA

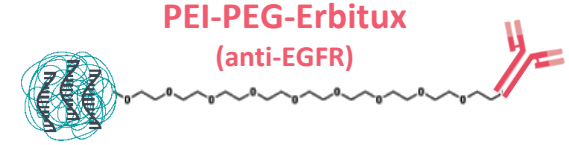
siRNA

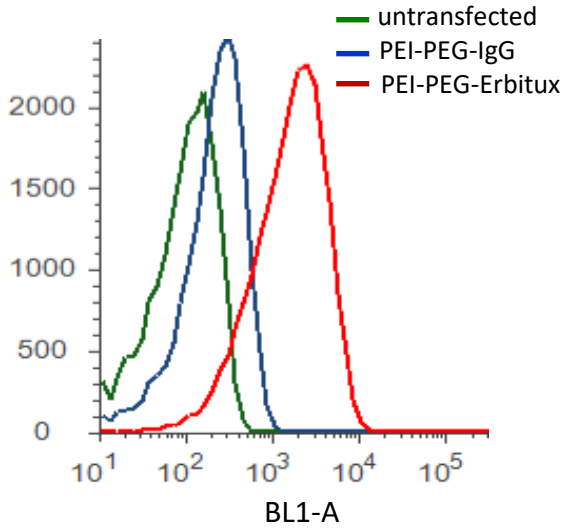
radioactively labeled siRNA

Targeted delivery & distribution options

- Other injection sites (i.v.)
- Nebulization and inhalation
- Change in surface charge (PEGylation)
- Formation of Lipoplexes
- Antibody-coupled PEIs

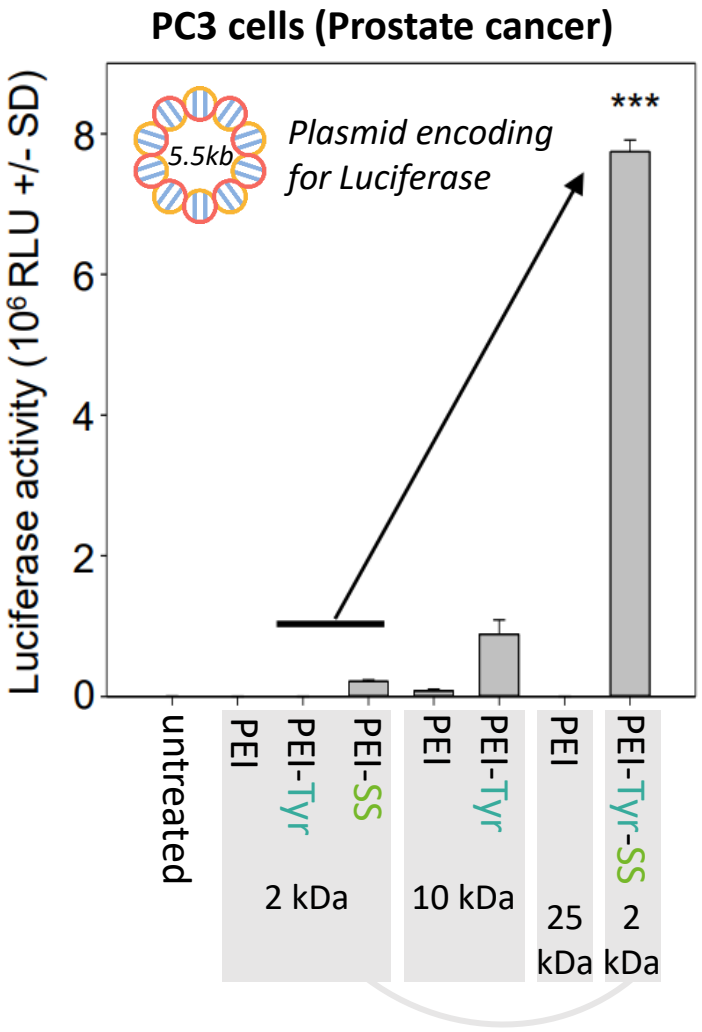
PEI-PEG-Erbitux
(anti-EGFR)

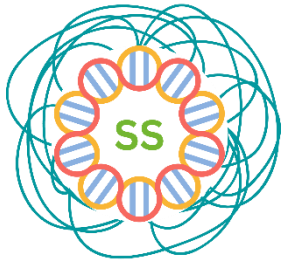




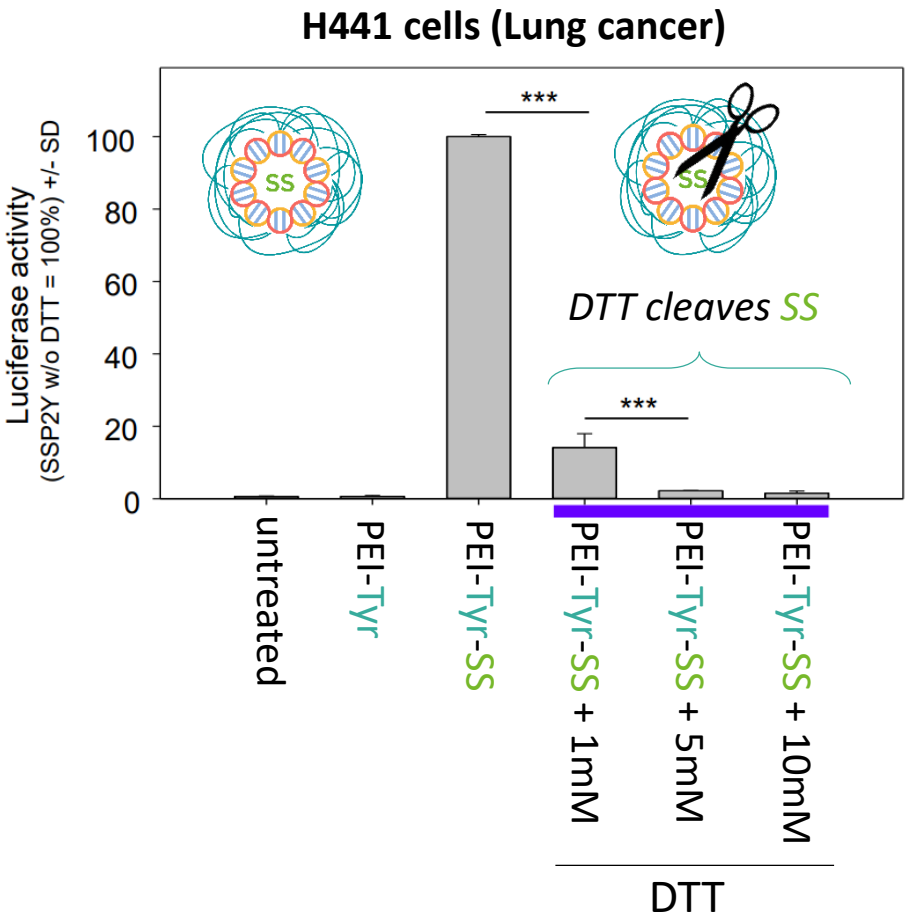
KD = knockdown

Delivery of DNA in vitro





- Delivery of DNA challenging
- Further modification of PEI-Tyr required → disulfide-bridges (SS)

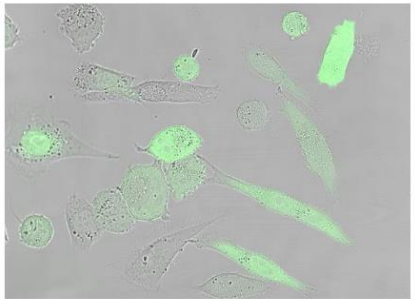


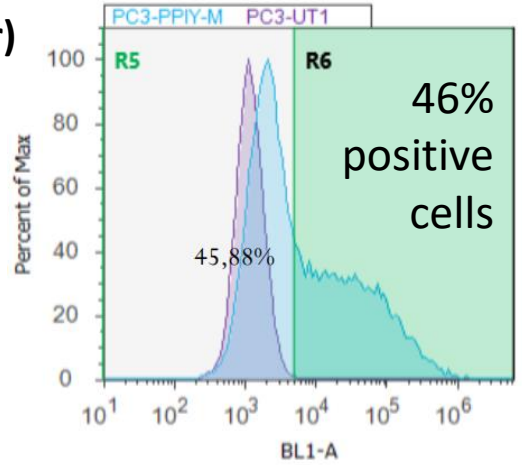
Delivery of mRNA in vitro

Recent and unpublished work

eGFP-mRNA

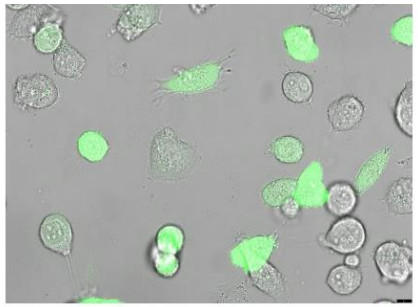
PC3 cells (Prostate cancer)

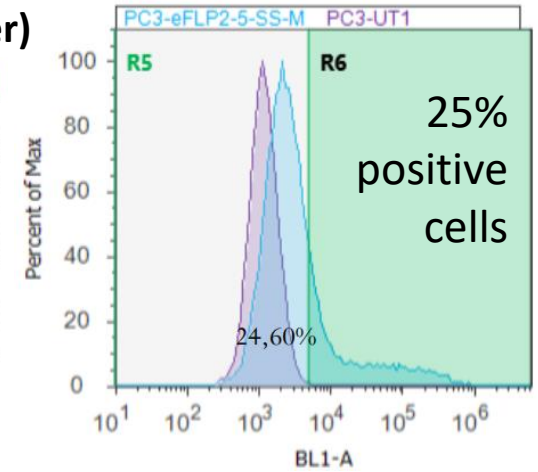




Polymer 1
Ratio 3.75
2µg mRNA
24h

PC3 cells (Prostate cancer)



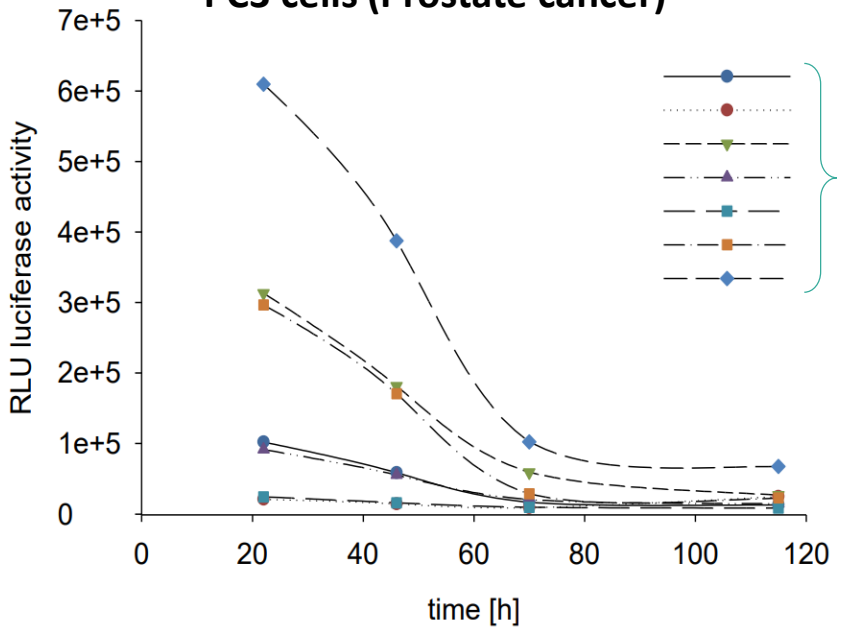


Polymer 2
Ratio 3.75
2µg mRNA
48h

Ongoing work on preventing time-critical mRNA expression

Luciferase-mRNA

PC3 cells (Prostate cancer)



Nanoparticle platform technology

Involvement of Aigner Lab technology around the globe

- Multiple national & international scientific collaborations & consortia
  - Martin-Luther-University Halle-Wittenberg (GER)
  - Ludwig-Maximilians-University Munich (GER)
  - University L'Aquila (ITA)
  - Friedrich-Alexander University Erlangen (GER)
  - Paracelsus University Salzburg / Medical University of Vienna (AUT)
  - Philipps-University Marburg (GER)
  - University of Kansas Medical Center (USA)
  - Northwestern University Chicago (USA)

- Delivery technology for US-based spin-off company (siRNA-based cancer therapeutics), Aigner-IP involved, patent filed

- Delivery technology for siRNA-based treatment of a bone disease (preclinical data: pharmacology & tox *in vivo*)

- Publications

Mirna et al., Cardiovasc Res (2022)
Müller et al., NAR Cancer (2020)
Helmschrodt et al., Mol Ther Nucleic Acids (2017)
Hermanns et al., Oncogene (2017)
Hampl et al., EMBO Mol Med (2013)

- Member of SaxoCell (Clusters4Future, BMBF) in project CAR-NK-AID for gene transfer of CAR-mRNA into NK cells

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Thank you for your attention!

Special Thanks to

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