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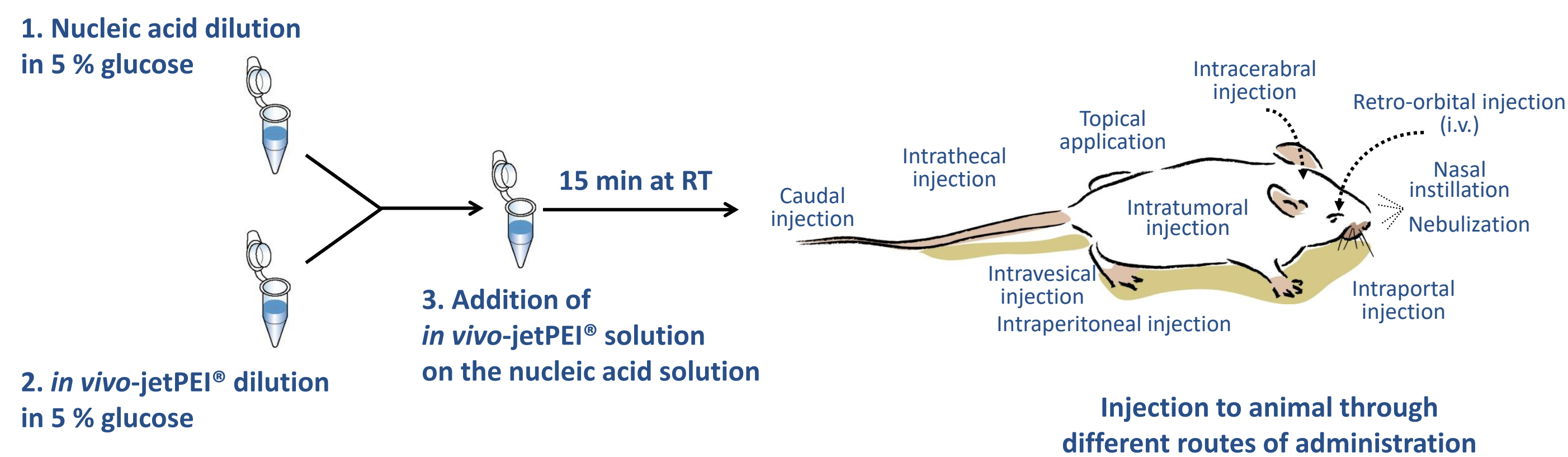
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Abstract

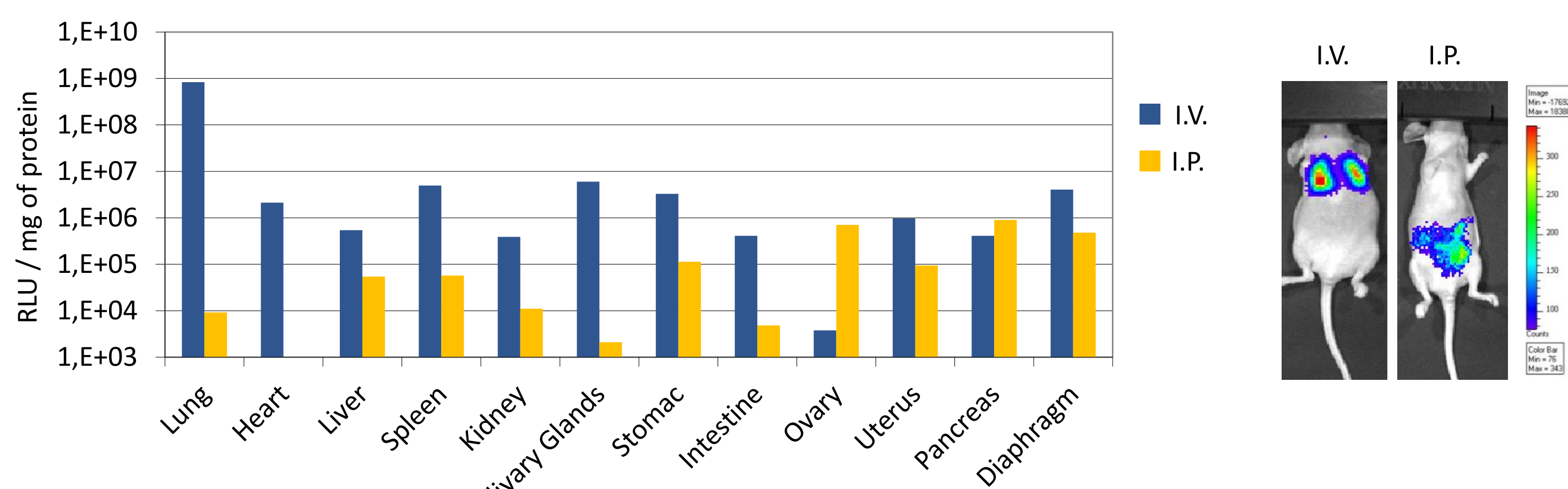
Nucleic acids have considerable potential as therapeutic agents in the treatment of pathologies including genetic diseases, viral infections, and cancer therapies. The major challenge for the use of nucleic acids in therapy lies in safely delivering these anionic macromolecules to their intended sites of action.

At Polyplus-transfection, we develop powerful non-viral vectors to safely deliver nucleic acids *in vivo* to target a wide range of tissues, through various routes of administrations. Of these reagents, *in vivo-jetPEI*[®] is widely acknowledged to deliver nucleic acids in animals; and coherently is selected as the delivery vector of choice in several drug development programs, notably for immunotherapies. To fulfill all the quality requirements associated to its use in Human, Polyplus-transfection[®] supplies preclinical grade and cGMP grade *in vivo-jetPEI*[®] reagents for a growing number of plasmid and oligonucleotide based-preclinical studies and clinical trials.

Very easy to handle protocol



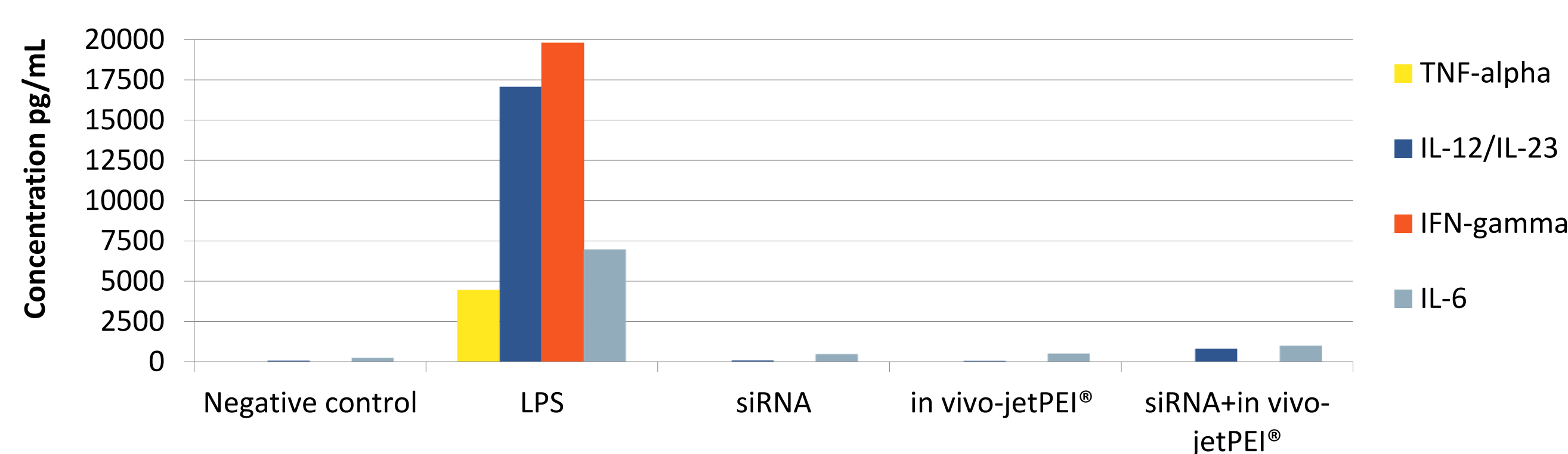
A wide variety of targeted organs depending on the administration route



Complexes were formed using 40 µg or 100 µg of luciferase expressing plasmid and *in vivo-jetPEI*[®] at an N/P ratio of 8, in 200 µl or 1 ml of 5% glucose and injected either through retro-orbital sinus (IV) or intraperitoneally (IP), respectively. 24 hours after injection, different organs were extracted and luciferase expression was measured or live imaging was performed using IVIS system (Perkin Elmer).

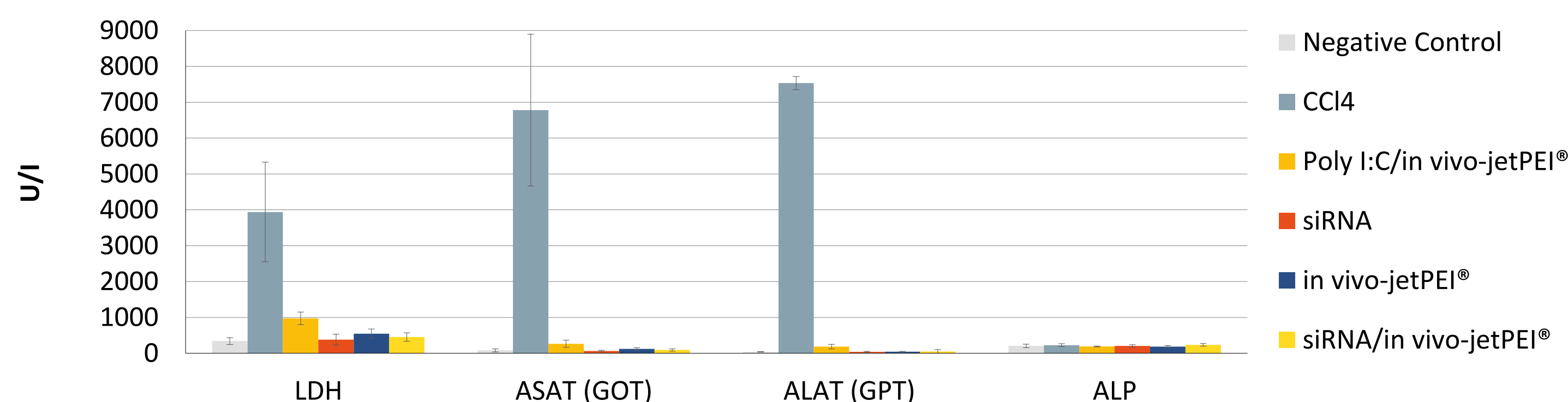
Safe method of delivery, with no major inflammatory response triggered

No pro-inflammatory cytokine expression



Complexes were formed in 200 µl of 5% glucose using 40 µg of luciferase siRNA with *in vivo-jetPEI*[®] at an N/P ratio of 8, and injected through retro-orbital sinus. 1 to 6 hours after injection, blood was collected and the level of TNF, IFN and IL-6 was measured by ELISA (n=8). As a positive control, LPS was injected intraperitoneally.

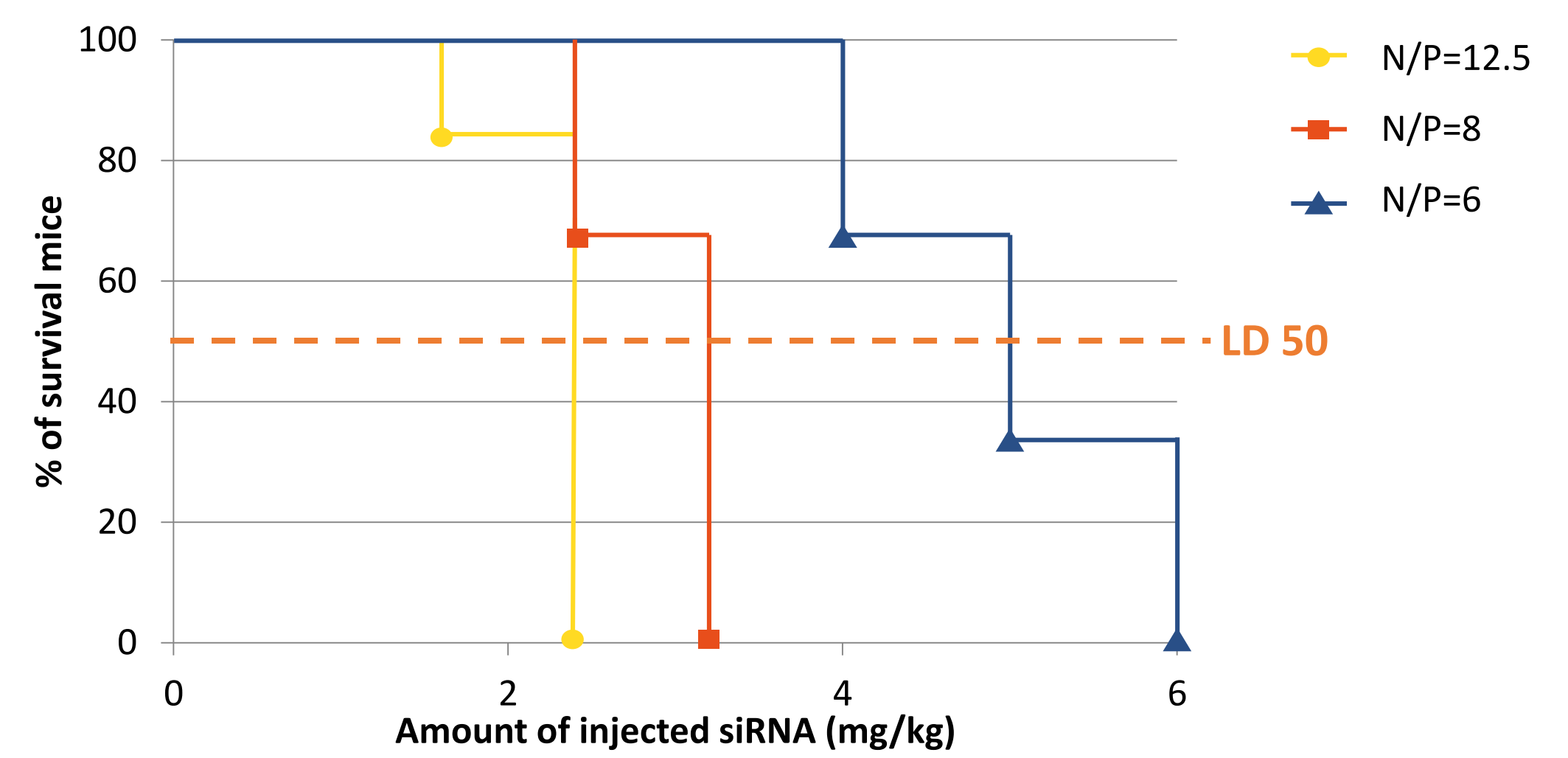
No induction of liver enzyme



Complexes were formed in 200 µl of 5% glucose using 40 µg of luciferase expressing plasmid with *in vivo-jetPEI*[®] at an N/P ratio of 8, and injected through retro-orbital sinus. 24 hours after injection, blood was collected and the level of LDH, ASAT, ALAT and ALP was measured. Each value corresponds to the mean ± SD (n=8). As a positive control, CCl4 was subcutaneously administered.

Bonnet et al., 2008

Dose response survival study of siRNA/*in vivo-jetPEI*[®] complexes



Mice were treated via intravenous injection with increasing amounts of siRNA delivered with *in vivo-jetPEI*[®] at an N/P ratio of 6, 8 and 12.5 (n=6 per group). The N/P ratio is a measure of the ionic balance within the complexes and is defined as the number of nitrogen residues of *in vivo-jetPEI*[®] per nucleic acid phosphate. Percentage of survival is represented depending on the amount of siRNA. Optimal efficiency is obtained with the delivery of 1 to 1.5 mg/kg nucleic acid and a N/P ratio of 6 to 8 (Bonnet et al., 2013).

A range of different quality grade reagents for each step of nucleic acid-mediated therapy development

Basic Research/Discovery	Drug development	Commercialization
Proof of concept study Animal Screening and validation of nucleic acid therapy approach: • Target gene • Nucleic acid (DNA, siRNA, mRNA) • Vector delivery (Administration method)	Preclinical study Animal Test on small and larger animals to evaluate: • Properties & effects • Pharmacology • Biodistribution • Toxicity	Clinical trials Human Phase I: Study of tolerance and define dose/frequency of administration: 10-40 volunteers. Phase II: Confirmation of the drug activity at the defined dose: 40-80 volunteers. Phase III: Compare and/or combine the drug to a current treatment: 3000+ patients.
<i>in vivo-jetPEI</i>[®]	Preclinical grade <i>in vivo-jetPEI</i>[®]	cGMP <i>in vivo-jetPEI</i>[®]

in vivo-jetPEI[®] is available as a regular R&D grade for first proof of concept experiments. When moving into therapeutic development program, Polyplus-transfection can supply preclinical grade *in vivo-jetPEI*[®] to perform GLP preclinical studies in animal (pharmacodynamics, biodistribution, toxicology studies...). For further clinical studies, Polyplus-transfection is able to supply GMP grade *in vivo-jetPEI*[®].

Clinical trials with *in vivo-jetPEI*[®]

Discovery	Preclinical study	Phase I	Phase II	Phase III
Plasmid DNA Bladder instillation Bladder cancer gene therapy				
Plasmid DNA Local delivery in tumor Pancreatic cancer gene therapy				
Plasmid DNA Topical application HIV Vaccination				
Plasmid DNA Ex vivo cell therapy, Acute myocardial infarction gene therapy				
Long dsRNA Local delivery in tumor Melanoma immunotherapy				
Plasmid DNA Blood infusion Imaging and cancer therapy				
Plasmid DNA Local delivery in tumor Melanoma immunotherapy				
siRNA Blood infusion Modulation of blood-brain barrier				

in vivo-jetPEI[®] has been selected as a nucleic acid delivery vector for the development of a growing number of nucleic acid-mediated therapies. Type of nucleic acid delivered, administration route and therapeutic application are very diverse.

Conclusion

- Manufacturing of *in vivo-jetPEI*[®] in compliance with US and EU GMP guidelines since 2007.
- Several clinical trials worldwide using *in vivo-jetPEI*[®] for the delivery in human of **different types of nucleic acids** (DNA, siRNA, oligonucleotides...) (Buscaill et al., 2015; Sidi et al., 2008; Matouk et al., 2013).
- Different administration routes can be used, including **systemic delivery**
- Used in different applications such as in cancer therapy, immunization, modulation of blood-brain barrier.
- *in vivo-jetPEI*[®] mediated delivery of nucleic acids can be used as a treatment in **combination with chemotherapy**.
- One project entering **Phase III** in 2018.

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 Sidi, A.A., P. Ohana, S. Benjamin, M. Shalev, J.H. Ransom, D. Lamm, A. Hochberg, and I. Leibovitch. 2008. *The Journal of urology*. 180:2379-2383.