Alternatives to viral vectors for nucleic acid-mediated therapies

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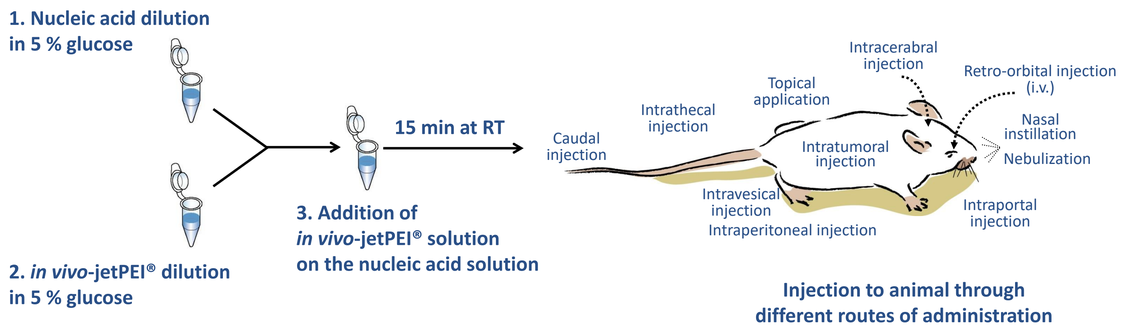
Abstract

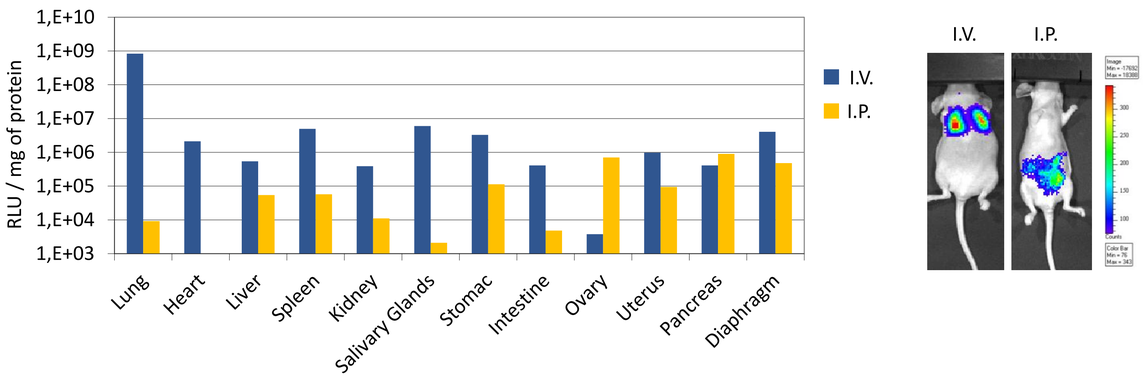
Nucleic acids have considerable potential as therapeutic agents in the treatment of pathologies including genetic diseases, viral infections, and cancer therapies. The major challenge for the use of nucleic acids in therapy lies in safely delivering these anionic macromolecules to their intended sites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



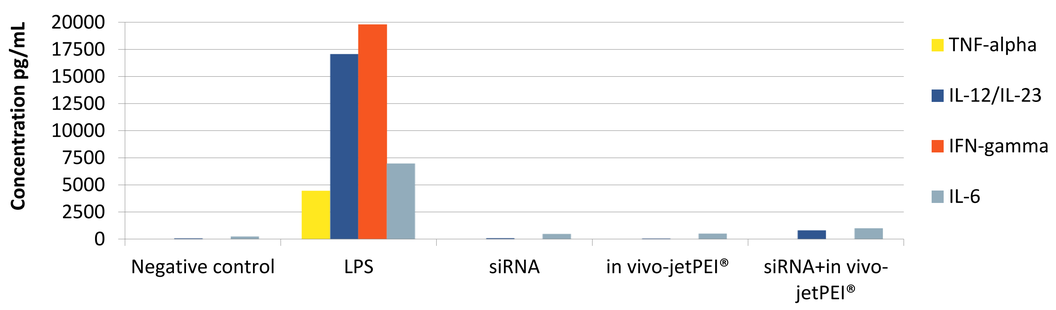
A wide variety of targeted organs depending on the administration route



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).

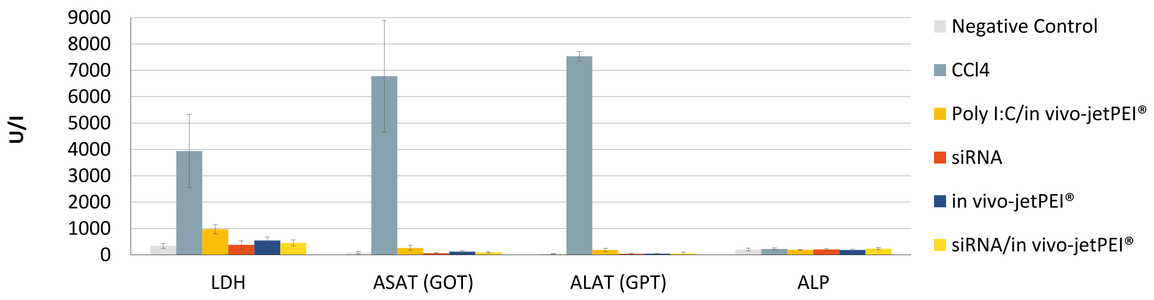
Safe method of delivery, with no major inflammatory response triggered

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

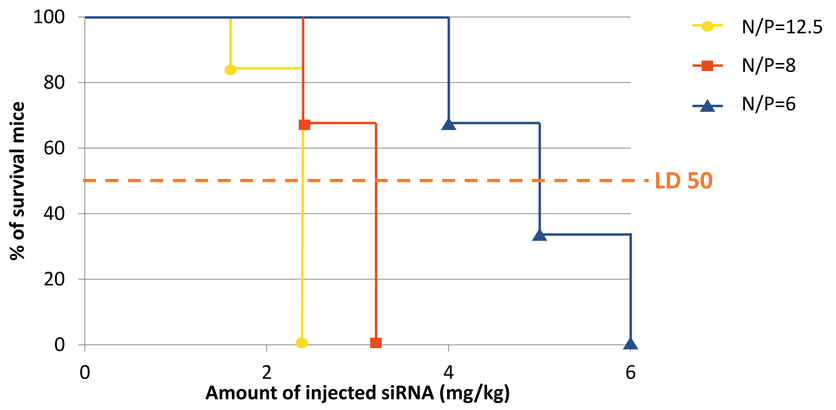


Complexes were formed in 200 µl of 5% glucose using 40 µg of luciferase expressing plasmid with in vivo-jetPEI® at an N/P ratio of 8, and injected through retro-orbital sinus. 24 hours after injection, blood was collected and the level of LDH, ASAT, ALAT and ALP was measured. Each value corresponds to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

Bonnet et al., 2008

Bonnet, M.E., P. Erbacher, and A.L. Bolcato-Bellemin. 2008. *Pharmaceutical research*. 25:2972-2982.
Bonnet, M.E., J.B. Gossart, E. Benoit, M. Messmer, O. Zounib, V. Moreau, J.P. Behr, N. Lenne-Samuel, V. Kedinger, A. Meulle, P. Erbacher, and A.L. Bolcato-Bellemin. 2013. *Journal of controlled release*. 170:183-190.
Buscail, L., B. Bournet, F. Vernejoul, G. Cambois, H. Lulka, N. Hanoun, M. Dufresne, A. Meulle, A. Vignolle-Vidoni, L. Ligat, N. Saint-Laurent, F. Pont, S. Dejean, M. Gayral, F. Martins, J. Torrisani, O. Barbey, F. Gross, R. Guimbaud, P. Otal, F. Lopez, G. Tiraby, and P. Cordelier. 2015 *Molecular therapy*. 23:779-789.
Matouk, I., E. Raveh, P. Ohana, R.A. Lail, E. Gershtain, M. Gilon, N. De Groot, A. Czerniak, and A. Hochberg. 2013. *International journal of molecular sciences*. 14:4298-4316.
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.

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



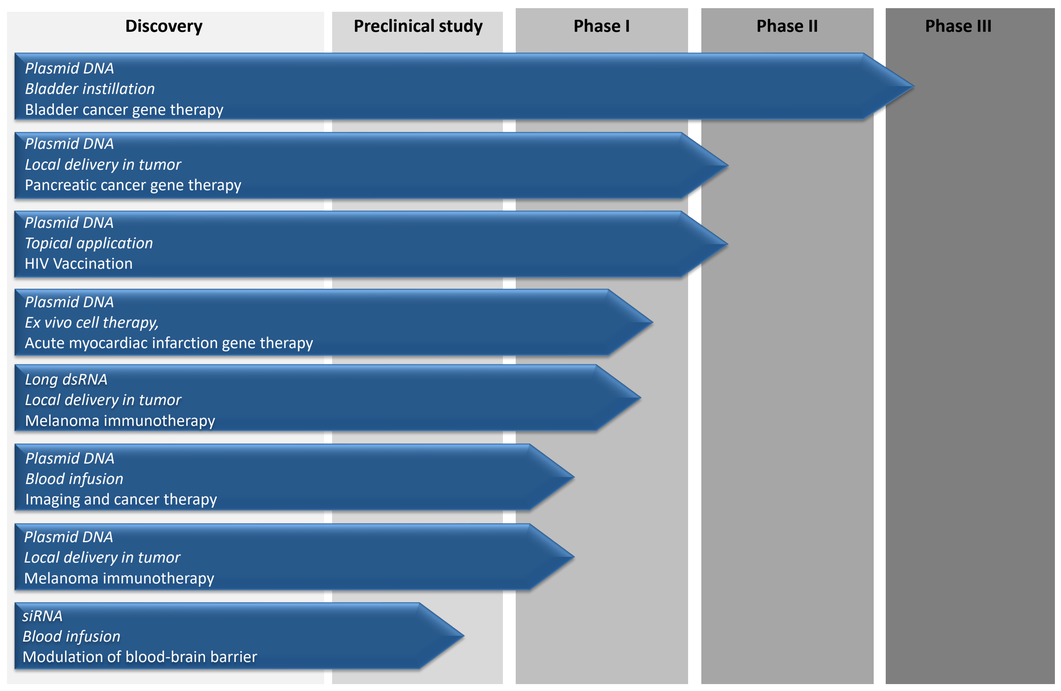
Mice were treated via intravenous injection with increasing amounts of siRNA delivered with in vivo-jetPEI® at an N/P ratio of 6, 8 and 12.5 (n=6 per group). The N/P ratio is a measure of the ionic balance within the complexes and is defined as the number of nitrogen residues of in vivo-jetPEI® per nucleic 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	**Preclinical study Animal**	**Clinical trials Human**	**Patient therapy**
Screening and validation of nucleic acid therapy approach: • Target gene • Nucleic acid (DNA, siRNA, mRNA) • Vector delivery (Administration method)	Test on small and larger animals to evaluate: • Properties & effects • Pharmacology • Biodistribution • Toxicity	**Phase I**: Study of tolerance and define dose/frequency of administration: 10-40 volonteers. **Phase II**: Confirmation of the drug activity at the defined dose: 40-80 volonteers. **Phase III**: Compare and/or combine the drug to a current treatment: 3000+ patients.	Commercialization of the drug for nucleic acid-mediated therapy.
***in vivo*-jetPEI®**	**Preclinical grade *in vivo*-jetPEI®**	**cGMP *in vivo*-jetPEI®**	

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®



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…) (Buscail 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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