in vivo-jetPEI®, an alternative to lipid-based reagents or viral vectors for nucleic acid-mediated therapies

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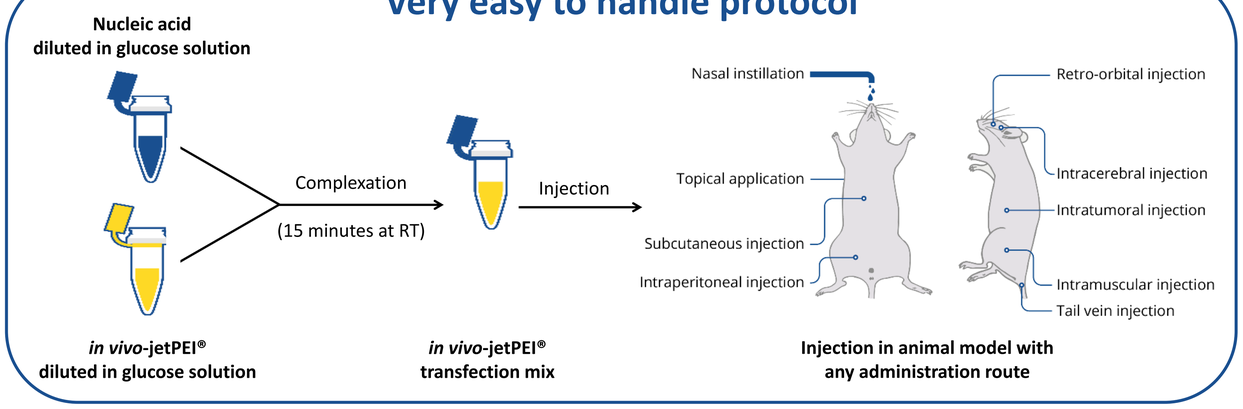
Abstract

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 safe delivery of these anionic macromolecules to their intended sites of action. Our cationic polymer-based reagent, *in vivo*-jetPEI®, was tested for plasmid DNA and siRNA *in vivo* delivery through intravenous and intraperitoneal injections in mice to evaluate its efficacy, safety and biodistribution.

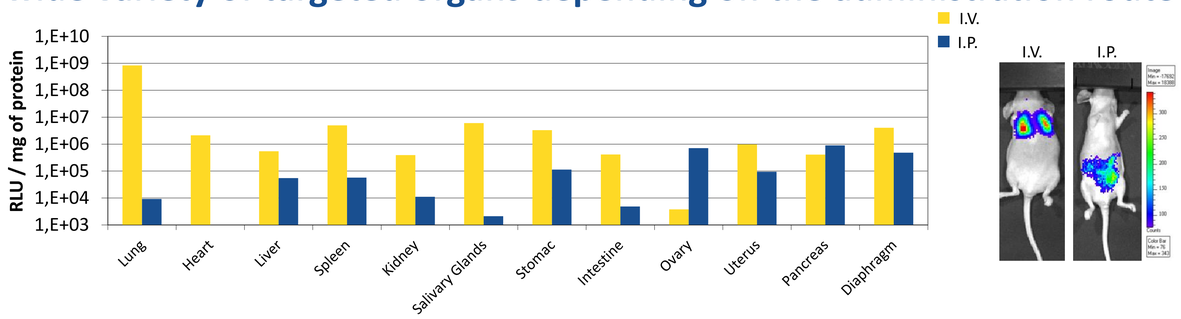
Here we demonstrate that *in vivo*-jetPEI® can efficiently and safely deliver various nucleic acids *in vivo* to target a wide range of tissues, through various routes of administrations.

Furthermore, *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 cancer therapies. To fulfill all the quality requirements associated to its use in Human, Polyplus® supplies a cGMP grade *in vivo*-jetPEI® reagent for a growing number of clinical trials.

Very easy to handle protocol



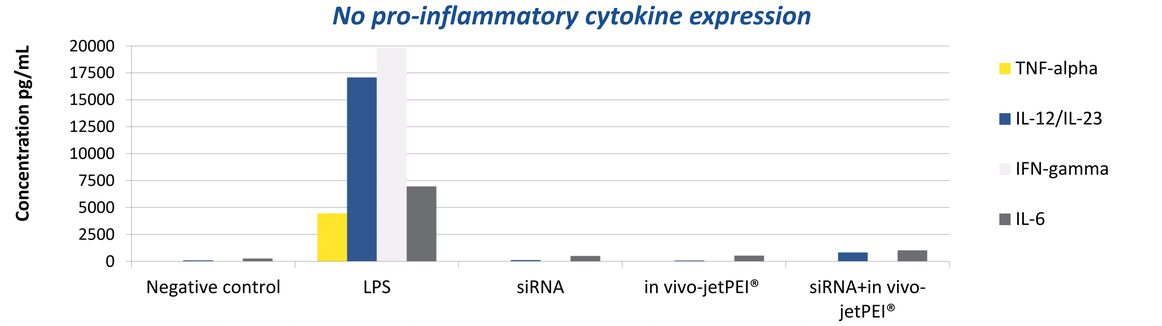
A wide variety of targeted organs depending on the administration route



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).

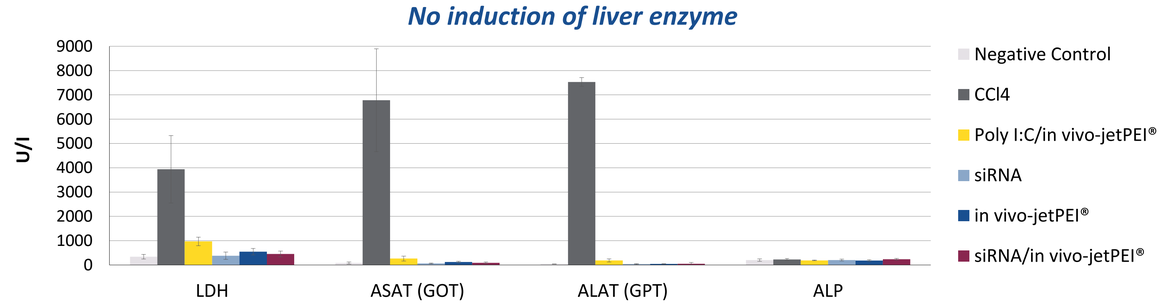
Safe method of delivery, with no major inflammatory response triggered

No pro-inflammatory cytokine expression



Complexes were formed in 200 µl of 5% glucose using 40 µg of 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

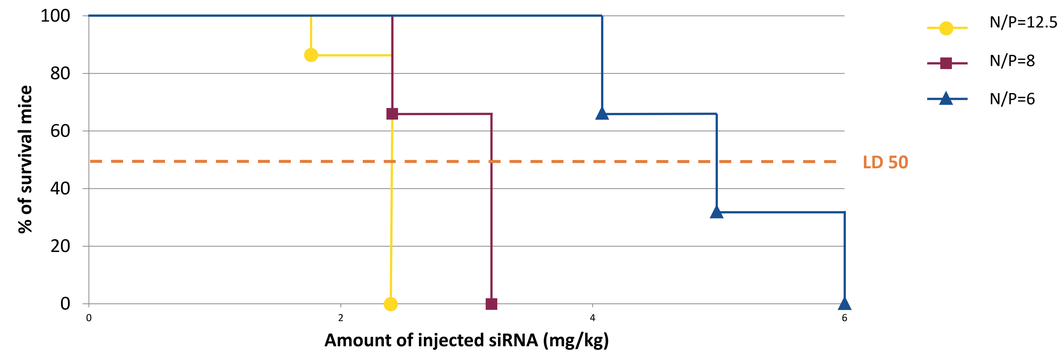


Complexes were formed in 200 µl of 5% glucose using 40 µg siRNA with in vivo-jetPEI® at an N/P ratio of 8 and injected through retro-orbital sinus. 24 hours after injection, blood was collected and the level of LDH, ASAT, ALAT and ALP was measured. Each value corresponds to the mean ± SD (n=8). As a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



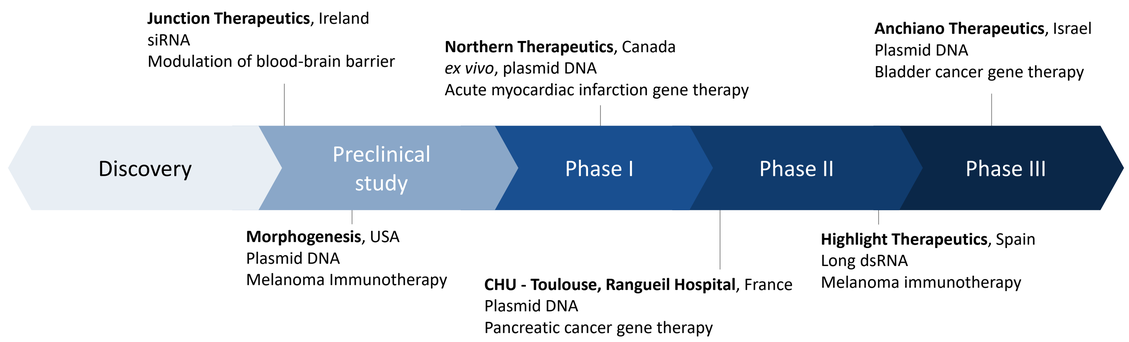
Mice were treated via intravenous injection with increasing amounts of siRNA delivered with in vivo-jetPEI® at an N/P ratio of 6, 8 and 12.5 (n=6 per group). The N/P ratio is a measure of the ionic balance within the complexes and is defined as the number of nitrogen residues of in vivo-jetPEI® per nucleic 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).

in vivo-jetPEI® enables a seamless transition from Drug Discovery to Human clinical trials

Basic Research/Discovery	Drug development		Commercialization
Proof of Concept study Screening and validation of nucleic acid therapy approach: • Target gene • Nucleic acid (DNA, siRNA, mRNA) • Vector delivery (Administration method, dose, route, etc.)	**Preclinical study** Tests on animals to evaluate: • Properties & effects • Pharmacology • Pharmacokinetics • Biodistribution • Toxicity	**Human Clinical trials** **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.	**Patient therapy** Regulatory Approval and Commercialization of the drug for nucleic acid-mediated therapy.
***in vivo*-jetPEI®**		***in vivo*-jetPEI® GMP**	

in vivo-jetPEI® is available for applications ranging from in vivo Fundamental research or Proof-of-Concept experiments to Preclinical studies in animals (pharmacodynamics, biodistribution, toxicology studies…). Then, to meet quality and regulatory requirements for Human use in clinical trials and on the market, a cGMP-compliant grade is available: in vivo-jetPEI® GMP. In addition, Polyplus® also offers Regulatory support with:
• a Drug Master File (DMF) submitted to the US FDA that can be cross-referenced for IND applications & BLA,
• a Documentation describing the Chemistry, Manufacturing and Control (CMC) section for IMPD submission in Europe,
• a Quality agreement

Clinical trials with *in vivo*-jetPEI® GMP



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

- **GMP compliant**: Manufactured in compliance with US and EU cGMP guidelines since 2007
- **Successful and proven**: trusted excipient used in several Human clinical trials worldwide (Buscail et al., 2015; Sidi et al., 2008; Matouk et al., 2013) for different applications such as in cancer therapy, immunization, modulation of blood-brain barrier, etc.
- **Ready-to-use**: 2-step protocol requiring no equipment or formulation expertise
- **Polyvalent**: Used for *in vivo* delivery of any nucleic acid, to target any organ, in any animal model. Plus, different administration routes can be used, including systemic delivery
- **Fully supported**: Expert Regulatory and Scientific Support teams