

PATENTS

Emerging patent landscape for non-viral vectors used for gene therapy

An analysis of the emerging patent landscape of gene therapies under development, focusing on non-viral vectors.

The possibility of persistently modifying mammalian cells represents an attractive field for molecular and cell biologists. A system that provides efficient, persistent and stable expression of transferred genes in mammalian cells may be a useful tool for a variety of applications, such as gene regulation, disease modeling, drug testing and gene supplementation for therapeutic correction. In 1990, researchers at the US National Institutes of Health conducted the first licensed gene therapy on an individual born with a rare genetic disease called severe combined immunodeficiency. This trial was a success and encouraged the expansion of the gene therapy field with the aim of treating other patients. However, in the following years, patients treated with gene therapy presented substantial adverse events. In 1999, the first severe adverse event was reported when an individual suffering from ornithine transcarbamylase deficiency had received a dose of an adenoviral vector carrying a corrected gene and developed a massive immune response, leading to multiple-organ failure and brain death¹. Owing to this and other clinical setbacks in 1999–2002, the field of gene therapy experienced a steep and persistent decline in investment in the years that followed. Meanwhile, many technical advances continued to be made that ensured safer introduction of foreign DNA into mammalian cells and organisms². For example, improvements in the design of gene delivery systems have led to safe delivery of cytokines, short interfering RNA and several other gene replacement strategies, and have been effective in vitro, in vivo and in ex vivo in preclinical and clinical applications.

The intense development of new gene therapies has been marked by the active use of intellectual property rights by companies, along with robust investments in research and development. Here we provide a scenario of the current trends under development, focusing on the emerging technology of non-viral vectors, which are considered a safer alternative to viral vectors and appear to be rekindling the interest of

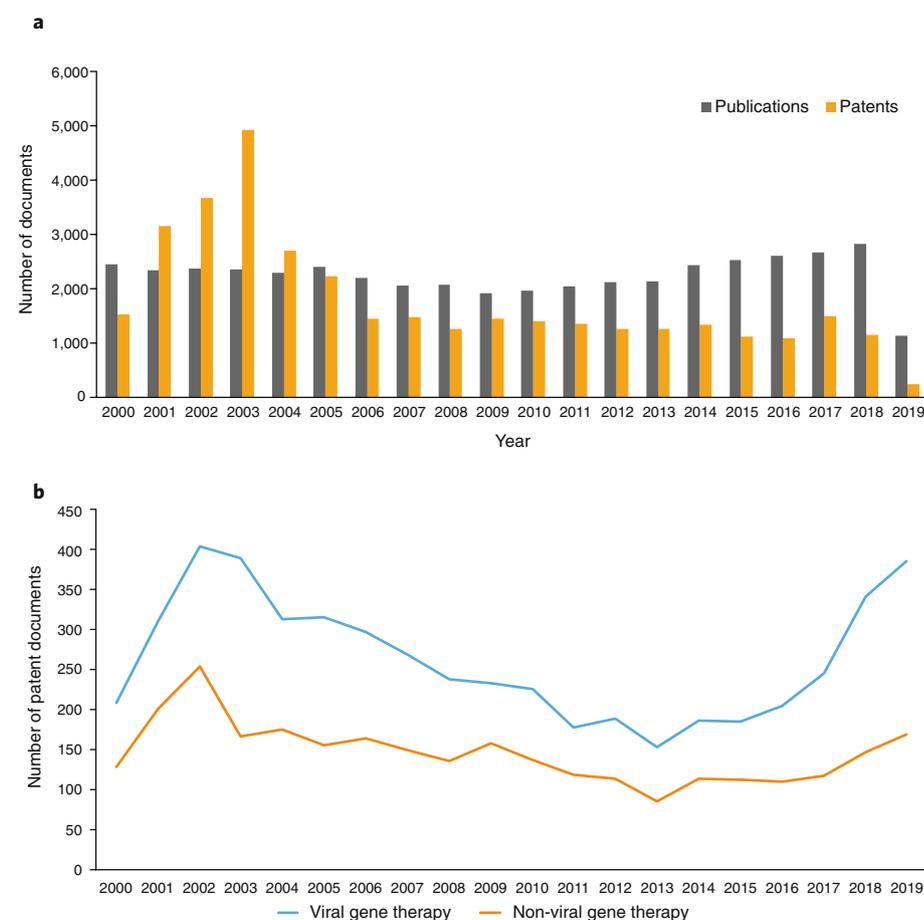


Fig. 1 | An overview of the publications and patents related to the term 'gene therapy'. a, Coverage of publications and patents related to the term 'gene therapy' since 1990. **b**, Current overview of patents and publications related to gene therapy.

clinicians and pharmaceutical industry in gene therapy.

The current scenario of gene therapy: publications and patents

From 2000 to 2003, the number of publications on gene therapy increased steadily, and a slight decrease was apparent in the years that followed (Fig. 1a). In 2011–2012, publication numbers again began to increase, which may be attributed to the emergence and rapid evolution of ex vivo gene therapy strategies. The patent trend for gene therapy is similar to that

of publications, with a much higher rate of patenting in the early 2000s, followed by a decline in subsequent years. This trend suggests that the intensity of gene therapy investments by both biotech and pharmaceutical companies declines after the 2000s. The negative outlook associated with early gene therapy treatments may have had an impact on the interest of biotech and pharma companies in persisting with their research investments. The increase in patents and publications indicates that gene therapy is promising and is being considered for the treatment of various

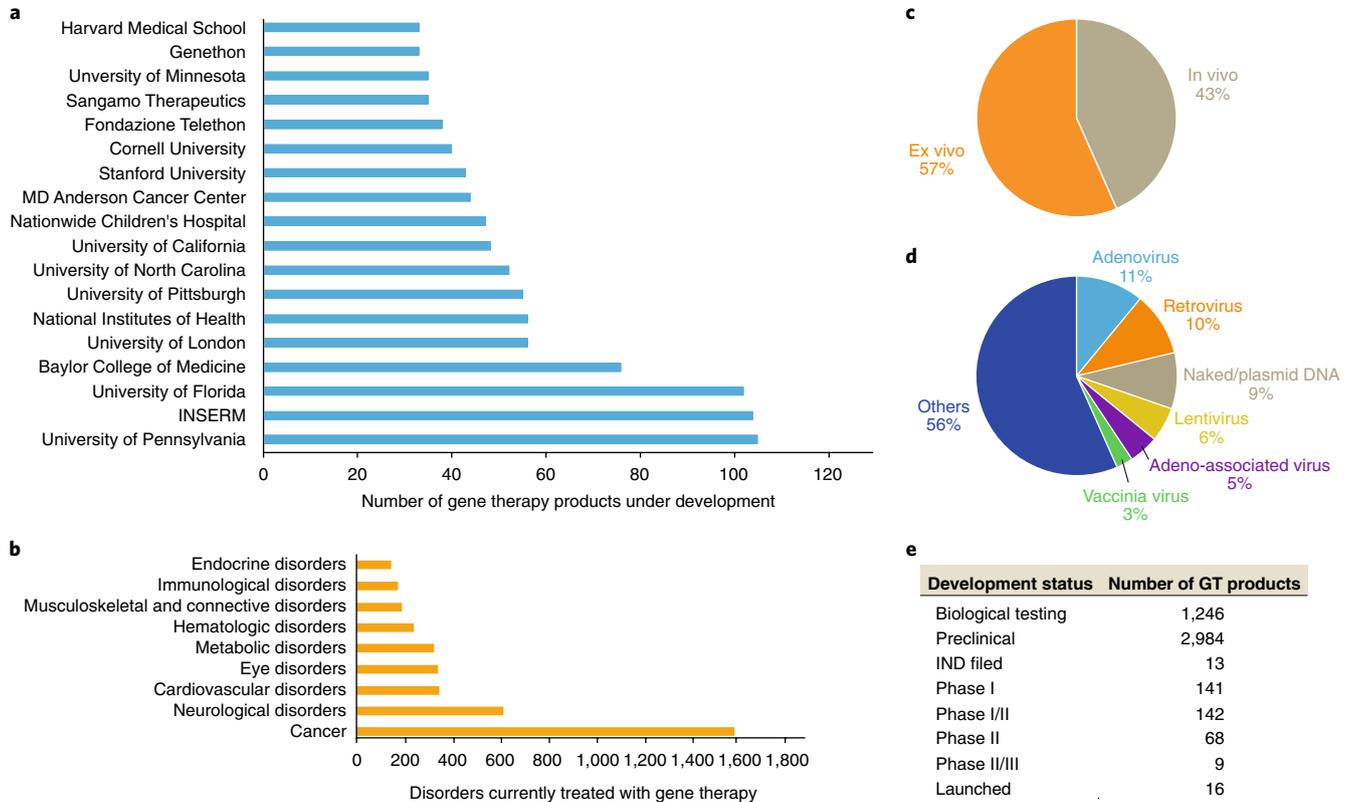


Fig. 2 | Current trends in the development of emerging gene therapy products. a, The top 18 organizations working on gene therapy. **b**, The top diseases currently treated with gene therapy. **c**, Gene delivery strategies. **d**, Type of vector. **e**, Current development status. GT, gene therapy.

diseases. The distribution of data suggests that although the vast majority of patents and publications are on viral vectors, the amount related to non-viral vectors may be experiencing a renewed increase since 2016 (Fig. 1b).

Current trends in the development of emerging gene therapy products

Despite the adverse events mentioned earlier, several advances have been made in the field of gene therapy. Many technical advances have been achieved with the aim of introducing foreign DNA into mammalian cells, and also many efforts have been made in improving and refining several expression vector systems. To identify major trends in the discovery and development of novel 'gene therapy drugs', we analyzed data available in the Integrity database (Clarivate Analytics). At the time of our latest analyses, there were 4,692 drugs registered in the database that are related to gene therapy, and these were subdivided further into groups according to the main organizations participating in this technological field, the disease target or the therapeutic development status (Fig. 2).

When we examined the organizations with respect to the number of gene therapy products in development (Fig. 2a), the University of Pennsylvania appeared in first place with 106 drug candidates, focused on products for the treatment of Crigler-Najjar syndrome, spinal muscular atrophy, influenza, hemophilia A, ornithine transcarbamylase deficiency and CAR-T cells. The organization with the second greatest number of drug candidates related to gene therapy was INSERM (National Institute for Health and Medical Research, France), with 104 drugs, focused mainly on sickle cell anemia, microRNAs, and CAR-T cells. The organization with the third greatest number of drug candidates in gene therapy was the University of Florida, with 102 drugs, including the development of newer and safer agents for the delivery of therapeutic genes to patients with genetic diseases such as cystic fibrosis and alpha 1-antitrypsin deficiency.

Regarding the disease type, cancer was the most commonly treated disease with gene therapy. In fact, multiple gene therapy strategies have been developed to treat a wide variety of cancers, including gene therapy with suicide genes, oncolytic viruses,

anti-angiogenic genes and therapeutic gene vaccines. Neurological diseases appeared in second place, and recent progress in gene therapy has allowed for promising novel treatments for neurodegenerative diseases including Parkinson's disease and Huntington's disease (Fig. 2b). Among the two main gene delivery strategies used in clinical trials, approximately 56% of the clinical trials use ex vivo and 43% use in vivo approaches (Fig. 2c). Although viral vectors are the most widely used in clinical trials (Fig. 2d), these vectors are still associated with risks of integration into the host-cell genome and immune response against the viral vectors. Therefore, 9% of current clinical trials are using plasmid or naked DNA (non-viral) as an alternative to viral vectors. Evaluation of the number of gene therapy products currently in development revealed that the majority of studies are still in the biological testing or preclinical phases (Fig. 2e).

There currently are 16 gene therapy products on the market (Table 1), of which 13 are based on viral vectors and three are non-viral products. Neovascugen, the first non-viral gene therapy product, was launched by the Human Stem Cells

Table 1 | Gene therapy drugs launched in the market

Product	Vector type	Application	Highest phase	Gene	Organization
Gendicine	Adenovirus	Cancer, head and neck (squamous cell carcinoma)	Launched 2004	p53	SiBiono
Oncorine	Adenovirus	Cancer, rhinopharyngeal	Launched 2006	p53	Shanghai Sunway Biotech
DeltaRex-G	Retroviral	Cancer, solid tumor	Launched 2007	Dominant-negative mutant of cyclin G1	Epeius Biotechnologies
Neovasculogen	DNA plasmid	Peripheral arterial disease	Launched 2012	VEGF	Human Stem Cells Institute
Imlygic	Herpes simplex virus 1	Melanoma, metastatic	Launched 2015	GM-CSF	Amgen
Strimvelis	Retroviral	Adenosine deaminase deficiency	Launched 2016	Adenosine deaminase	Orchard Therapeutics
Invossa	Retroviral	Osteoarthritis, knee	Launched 2017	Transforming growth factor beta1 (TGF- β 1)	Kolon Life Science
Zalmoxis	Retroviral	Hematologic, blood cancer	Launched 2017	HSV-TK/Neo fusion suicide gene	MolMed
Kymriah	Lentiviral	Leukemia, acute lymphocytic	Launched 2017	CART-19	Novartis
		Lymphoma, diffuse large B-cell	Launched 2018	CART-19	
		Leukemia, B-cell acute lymphocytic	Launched 2019	CART-19	
Yescarta	Retroviral	Lymphoma, B-cell, diffuse large B-cell, primary mediastinal large B-cell	Launched 2017	CART-19	Kite Pharma
Spinraza	No vector	Spinal muscular atrophy	Launched 2017	SMN2-directed antisense oligonucleotide	Biogen
Glybera	Adeno-associated	Lipoprotein lipase deficiency	Withdrawn 2017	Lipoprotein lipase	Chiesi Farmaceutici
Luxturna	Adeno-associated	Retinal dystrophy	Launched 2018	Retinal pigment epithelium-specific (RPE65)	Spark Therapeutics
Zolgensma	Adeno-associated	Spinal muscular atrophy type 1	Launched 2019	Survival motor neuron 2 (SMN2)	AveXis
Collategene	DNA plasmid	Critical limb ischemia	Registered 2019	Hepatocyte growth factor	AnGes
Lentiglobin	Lentiviral	Thalassemia, beta (major)	Registered 2019	Hemoglobin subunit beta	bluebird bio

Institute (Russia). It consists of a plasmid-based vector encoding vascular endothelial growth factor (VEGF-165). The second product is Collategene, a DNA plasmid encoding the human hepatocyte growth factor (HGF) gene developed by AnGes (Japan). The last product in the market is Spinraza (nusinersen), an antisense oligonucleotide indicated for treatment of spinal muscular atrophy in children and adults.

Even though there are many different approaches to the genetic modification of cells, the most efficient way to introduce genetic material into cells remains well represented by viral gene-delivery systems, as reflected in the number of launched products (Table 1). Viral vectors are efficient in generating persistently modified mammalian genomes. However, they also present several limitations and drawbacks. First, in the case of retroviral (including lentiviral) vectors, there is a risk of insertional mutagenesis. Various studies have shown that integration

target site selection is not random³⁻⁵ and the viral vector targets some regions preferentially, generating cis-activation of proto-oncogenes or suppression of tumor-suppressor genes, as well as trans-activation of endogenous genes due to their interaction with viral proteins. Second, with viral vectors, there is a risk of an immune response and enhanced inflammation. Several mechanisms may explain the immunogenicity of viral vectors. For example, viral DNA is more prone to be recognized by the cellular RNA-DNA sensing system⁶. Nuclear and cytosolic proteins can bind 'non-self' DNA or RNA and trigger the innate immunity of cells, which leads to the epigenetic silencing of the viral DNA, inducing cell death and the activation of adaptive immunity⁷. Third, viral DNA is prone to epigenetic silencing when it is introduced as foreign DNA in mammalian cells^{6,7}. Fourth, the substantially higher costs required for viral production⁸ hinder translation of additional viral vector systems into clinical applications.

Patents and trends related to non-viral vectors used for gene therapy

To provide a patent landscape and to identify technologies that use non-viral vectors, we performed search queries using Derwent Innovation and PatSnap to assess the patenting trend in the technology field for non-viral vectors (Fig. 3a). The patent applications follow the same pattern seen in our search query of scientific publications. The highest volume of applications was observed in the early 2000s, followed by a decrease in the years that followed. A geographic breakdown showed the distribution of the patents in the technology fields across different jurisdictions. The United States is the country with the most (24.04%) patents first deposited, followed by Europe and China (Fig. 3b).

To understand how the different applications of using non-viral vectors for gene therapy have changed over time in terms of the direction of technology and investment trends, we analyzed the annual International Patent Classification

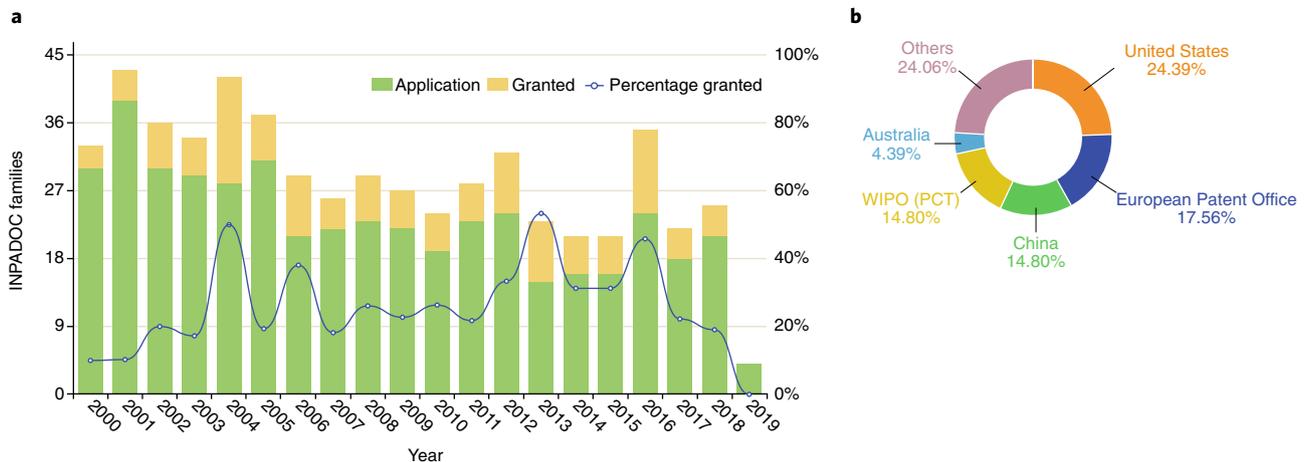


Fig. 3 | Patenting trends in the technology field of non-viral vectors. **a**, Annual patenting rate. The published patent applications are displayed in green, and the published granted patents displayed in yellow. **b**, Percentage of the patents across jurisdictions. WIPO, World Intellectual Property Organization; PCT, Patent Cooperation Treaty.

patent trend. In the early 2000s, the two areas with the most patent applications were C12N15 for vector/plasmid design and A61K48 for medicinal preparation containing genetic material that is inserted into cells of the living body to treat genetic diseases (Fig. 4a). In recent years, the number of patents related to vector or plasmid design has grown again. The top ten cited patents are shown in Fig. 4b. The most cited patent in this field is related to a construction of a single gene encoding a signal-chain immunoglobulin-like molecule (US5892019), which facilitates ex vivo transfection of cells for gene therapy protocols. The subsequent two in the list of most cited patents (WO1993025673A1 and US5922687) are related to methods to improve gene delivery (for ex vivo or for in vivo gene therapy). The top patent inventors have included Thomas Weimer from CSL Behring, who is involved in 13 patents, followed by Stefan Schulte, who has been involved in 10 patents (Fig. 4c). When we queried the patent databases according to the main patent assignees, CSL Behring, Curevac and the Wisconsin Alumni Research Foundation (WARF) appeared in first place with 13 patents, followed by Genzyme with 12 patents (Fig. 4d).

To obtain a deeper understanding of the concepts relating to the main organizations in the field of non-viral vectors, we generated a cell diagram representation of the patent landscape, which provided a macroscopic glimpse of patenting trends and helped us to better visualize technology trends. To achieve this, we evaluated the keywords of the patents belonging to the main organizations. Relative coverage was represented by the number of cells

in each organization, with each cell representing the same number of patents (628 International Patent Documentation (INPADOC) families were used) (Fig. 5). The top five organizations currently focusing on viral vectors are CSL Behring, WARF, Genzyme, Curevac and Baylor College of Medicine.

CSL Behring is a multinational biopharma company with patents related to non-viral vectors applied in the production of coagulation recombinant factors. Through the acquisition of Calimmune, CSL is now focused on the development of ex vivo hematopoietic stem cell gene therapy for the treatment of sickle cell disease and β -thalassemia, which complements CSL Behring's current product portfolio and deep expertise in hematology. WARF is an independent nonprofit technology transfer organization serving the University of Wisconsin–Madison and Morgridge Institute for Research. WARF is involved in patents comprising plasmids to immortalize cells and is also investing in methods for delivering gene therapies to specific tissues. Genzyme has been developing patents to improve gene therapy delivery methods and also to develop CpG-reduced plasmid vectors with an ubiquitin promoter that is not inactivated over time in vivo. Curevac has been investing in mRNA as a drug that holds the potential to revolutionize vaccination, protein replacement therapies, and the treatment of genetic diseases. Curevac's patent portfolio encompasses the design of mRNA manufacturing and intracellular delivery methods. Baylor College of Medicine is investing in minicircle DNAs, composed of eukaryotic sequences only, which is an interesting

approach to increase the safety and efficiency of plasmid-based vectors for gene therapy.

Non-viral vectors as favorable emerging gene therapy products

We can envisage non-viral vectors as highly desirable since they are easier to produce on a large scale and to characterize chemically, enable a higher reproducibility of production on a large scale, harbor a greater transgene capacity and generate fewer concerns regarding biosafety. Non-viral systems are still relatively less efficient when compared to viral systems, as expression of the encoded transgenes remains transient. To overcome the challenges inherent to non-viral vectors, improvements are needed still to secure their stability and potency. This may include actions aiming to enhance transfection efficiency, vector establishment and vector maintenance in the host cell nucleus, as well as decreasing plasmid loss, cell toxicity and production costs. The combination of several of these improvements already under development could be transformative in making non-viral DNA vectors an increasingly favorable emerging gene therapy tool.

Over the past few years, several attempts have been made to generate more efficient non-viral vectors. Episomal vectors based on scaffold-matrix attached regions address the serious problem of plasmid loss and provide mitotic stability of established plasmids by attaching the DNA to the nuclear matrix during DNA segregation into progeny cells, as demonstrated by the prototype episomal vector known as pEPI⁹. Additionally, these plasmids achieve an increase in transgene expression by facilitating the access of

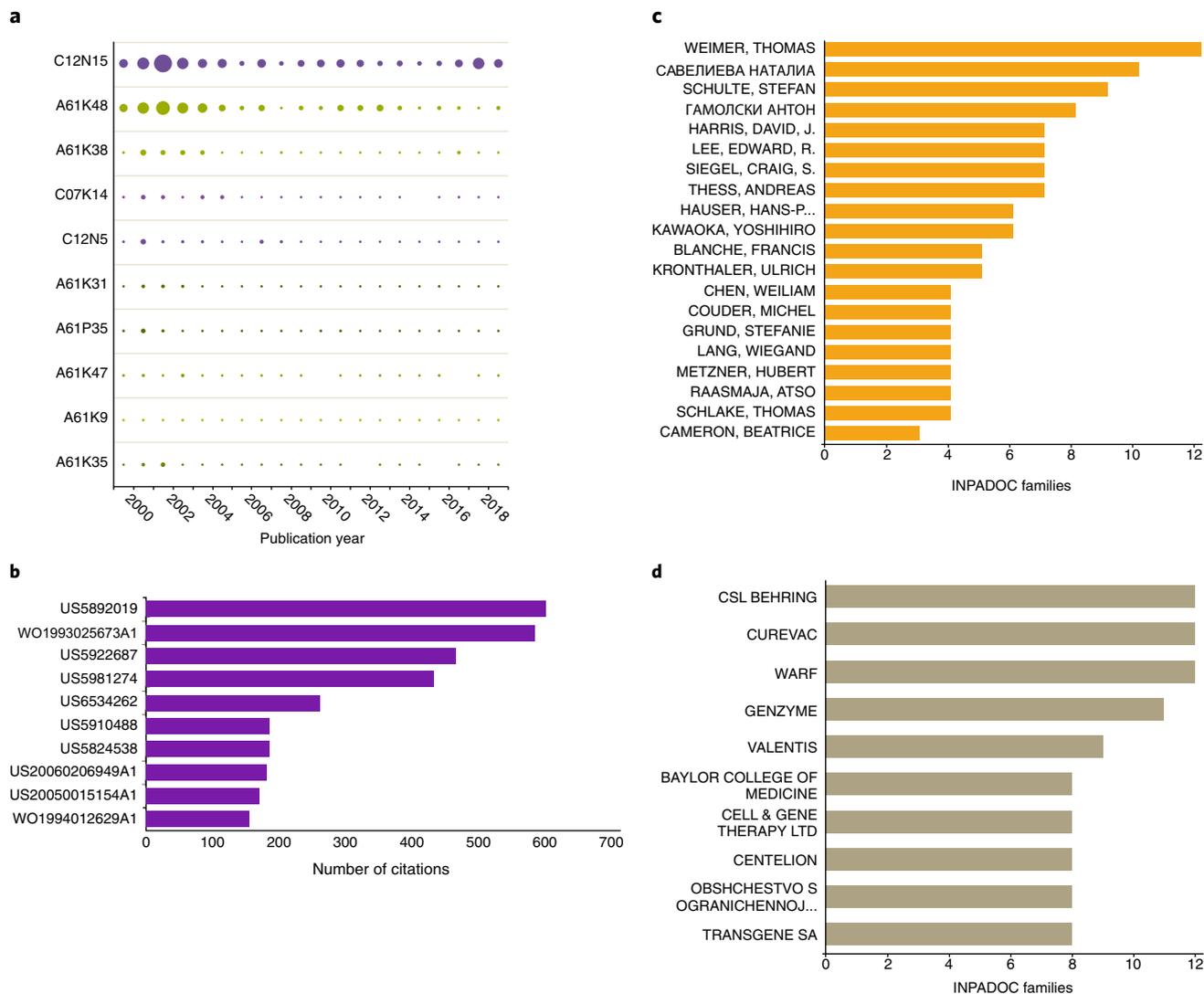


Fig. 4 | Direction of technology and investment trends analyses. **a**, Time evolution of patent family publication according to International Patent Classification (IPC) codes (for further information on IPC codes, visit <https://www.wipo.int/classifications/ipc/en/>), with circle sizes representing the number of patents registered in that particular year. C12N15: mutation or genetic engineering; DNA or RNA concerning genetic engineering, vectors, for example, plasmids, or their isolation, preparation or purification; use of hosts thereof. A61K48: medicinal preparations containing genetic material which is inserted into cells of the living body to treat genetic diseases; gene therapy. A61K38: medicinal preparations containing peptides. C07K14: peptides having more than 20 amino acids; gastrins; somatostatins; melanotropins; derivatives thereof. C12N5: undifferentiated human, animal or plant cells, for example, cell lines; tissues; cultivation or maintenance thereof; culture media thereof. A61K31: medicinal preparations containing organic active ingredients. A61P35: antineoplastic agents. A61K47: medicinal preparations characterized by the non-active ingredients used, for example, carriers or inert additives; targeting or modifying agents chemically bound to the active ingredient. A61K35: medicinal preparations containing materials or reaction products thereof with undetermined constitution. A61K39: medicinal preparations containing antigens or antibodies. **b**, Top ten cited patents related to non-integrating vectors. **c**, Top inventors. **d**, Top organizations working with non-integrating vectors.

transcription factors to gene regulatory regions to drive transcription. Modifications of pEPI include chromosomal elements, such as a replication-initiation region¹⁰, ubiquitous chromatin opening elements¹¹, and the *chs4* insulator from the chicken β -like globin gene cluster, which were successfully incorporated into pEPI vectors, contributing to the formation of autonomously replicating genetic episomes^{11,12}. Furthermore, the reduction of CpG content in the pEPI vector

backbone resulted in plasmids that achieved more efficient transgene expression *in vitro* and *in vivo*, generating the vector pEPITO¹³. All of these modifications have resulted in substantial improvements of cell stability and expression from non-integrating vectors for a long period of time (3 to 8 months *in vitro*), compared to non-integrating vectors free from viral sequences that still need to be optimized and refined to enter the pipeline for promising applications relating to gene

therapy and to ultimately show success in clinical trials.

The choice of delivery technologies is crucial for the success of gene delivery. *In vivo* gene delivery has been gaining a lot of attention, as it avoids *ex vivo* manipulation of cells, making the procedure simpler and cheaper. Non-viral vectors are considered safer, especially for *in vivo* administration. However, the efficiency of transfecting host cells is relatively low in

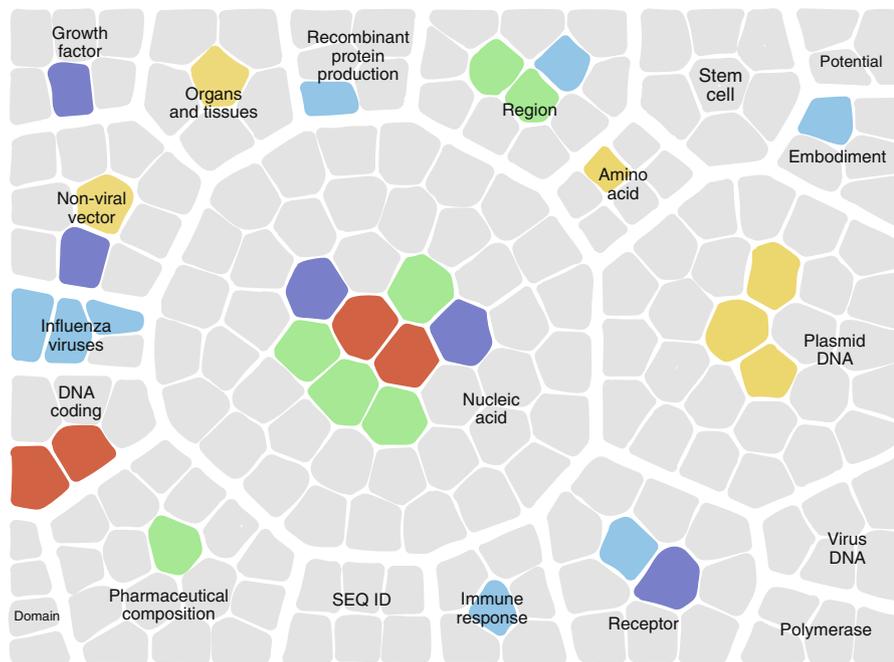


Fig. 5 | Cell diagram representing the most prevalent keywords from the patents belonging to the top organizations in the technology field. Red, CSL Behring; blue, Wisconsin Alumni Foundation; yellow, Genzyme; green, Curevac; purple, Baylor College of Medicine; gray, other.

non-viral systems compared to viral systems, and recently different synthetic materials for enhancing gene delivery have been developed to maximize therapeutic benefits and minimize toxicity. Many materials have augmented the non-viral transfection efficiency for enhancing delivery. Some examples, including the use of polymers, nanoparticles and liposomes, show enhanced delivery of non-viral vectors and hence have augmented non-viral transfection efficiency. Innovations in the field of gene delivery could substantially impact the more efficient and rapid translation of non-viral vectors toward emerging product candidates in the next few years.

Conclusions

Genetic therapies are considered the most complex therapeutic system ever developed. Many of these therapies have already demonstrated therapeutic efficacy in preclinical and clinical studies conducted in the academic setting and are now undergoing commercial development. This type of therapy is in transition from the academic model to an industrial drug development model, with partnerships with biotech companies whose expertise will be essential for a broad clinical application of gene therapy. Despite many advances, the vast majority of clinical trials still use viral

vectors and several challenges remain, including addressing genotoxicity from integrating vectors, improving gene transfer, achieving ideal expression levels and avoiding immune responses to viral vector sequences. The possibility of using non-viral and also potentially episomal vectors for gene therapy represents one of the most interesting and intriguing fields of gene therapy research. New innovations will certainly continue to arise to generate systems using non-viral vectors, with the potential to be scaled up and become suitable for clinical use.

Methods

Patent search. This study has some limitations due to the search methodology and the availability of information in a dynamic and rapidly evolving technological field. Our analyses are based on a set of keyword-selected and patent families; therefore, irrelevant documents may be included, and some documents relevant to the topic may have been missed from the analyses owing to the scope of the search queries. We believe that the limitations are minimized because of our deep understanding of the technical field, as well as professional patent research skills that optimize search queries to maximize the integrity of the analyses presented in this study.

To search specifically for non-integrative vector patents, the PatSnap database was used. A search query was developed to retrieve patents related to gene therapy inventions that were mentioned in the title, abstract and claims (TAC) of patents. The following search query was used:

TAC: ((TAC:(“non viral vector” OR “non-viral vector” OR “non integrat* vector” OR “non-integrat* vector” OR (episom*) OR (plasmid*) OR (minivector) OR (minicircle) OR (miniplasmid)) AND TA:(“gene therapy” OR “Cell therapy”)) NOT TAC:(“adeno associated virus” OR (AAV) OR (Adenovir*) OR “oncolytic viral vector” OR (retrovir*) OR (lentivir*))).

The search was performed on 22 May 2019 and retrieved 628 INPADOCs families (2,362 documents). INPADOCs stands for International Patent Documentation and represents a set of patent documents of a single invention that were extended for many countries. The total records account for all patent documents that were not grouped into INPADOCs families.

Search for gene therapies under development.

To search for gene therapies under development and leading compounds, we used the Integrity Database from Clarivate Analytics. The search was performed in May 2019 using the “Drugs and Biologics” section following a search for ‘gene therapy’ in the ‘product type’ field. A total of 4,692 drugs and biologics were registered in the database. Using the information from the drug’s milestones, we analyzed the current trends in the development of new ‘gene therapy’ drugs, including the top 18 assignees of patents, the top diseases currently treated with Gene Therapy and current development status. In this study, we applied the following definitions of development status used by the Integrity Database. (i) Biological testing: products from patents are entered into this phase; synthesis and preliminary pharmacology (in vitro testing) data may be available. (ii) Preclinical: in vivo testing; testing on animals has started. (iii) Investigational new drug application (IND) filed: application has been filed with the competent authority requesting permission to test the drug on humans (IND filed in the United States). (iv) Phase I: initial studies have started to determine the metabolism and pharmacological actions of drugs in humans, the side effects associated with increasing doses, and to gain early evidence of effectiveness; usually conducted on healthy volunteers. (v) Phase I/II: studies that combine certain aspects of phase I trials and phase II trials (for example, a safety study in patients). (vi) Phase II: controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks. (vii) Phase II/III: studies that combine certain aspects of phase II trials and phase III trials. Phase III: expanded controlled and uncontrolled trials initiated after preliminary evidence suggests effectiveness of the drug has been obtained. These are intended to gather additional information to evaluate the overall relationship of the drug and provide an adequate basis for physician labeling benefit-risk. (viii) Launched: the drug is being marketed.

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Published online: 7 February 2020

<https://doi.org/10.1038/s41587-019-0402-x>

References

- Raper, S. E. et al. *Mol. Genet. Metab.* **80**, 148–158 (2003).
- Kim, T. K. & Eberwine, J. H. *Anal. Bioanal. Chem.* **397**, 3821–3826 (2010).
- Garçon, L. et al. *Blood* **122**, 912–921 (2013).
- de Sousa Russo-Carbolante, E. M. et al. *Biotechnol. Lett.* **33**, 23–31 (2010).
- Michieletto, D., Lusic, M., Marenduzzo, D. & Orlandini, E. *Nat. Commun.* **10**, 575 (2019).
- Atianand, M. K. & Fitzgerald, K. A. *J. Immunol.* **190**, 1911–1918 (2013).
- Tao, J., Zhou, X. & Jiang, Z. *IUBMB Life* **68**, 858–870 (2016).
- Gándara, C., Affleck, V. & Stoll, E. A. *Hum. Gene Ther. Methods* <https://doi.org/10.1089/hgtb.2017.098> (published online 1 February 2018).
- Jenke, A. C. W. et al. *Proc. Natl. Acad. Sci. USA* **101**, 11322–11327 (2004).
- Stavrou, E. F. et al. *Sci. Rep.* **7**, 40673 (2017).
- Hagedorn, C., Antoniou, M. N. & Lipps, H. J. *Mol. Ther. Nucleic Acids* **3**, e118 (2013).
- Saunders, F., Sweeney, B., Antoniou, M. N., Stephens, P. & Cain, K. *PLoS One* **10**, (e0120096 (2015).
- Haase, R. et al. *BMC Biotechnol.* **10**, 20 (2010).

Acknowledgements

We acknowledge support from the Basic Medical Sciences Department at the Purdue University School of Veterinary Medicine, Purdue Center for Cancer Research, and the Purdue Institute for Drug Discovery. This work was supported in part by NIH grants R01CA196947 and R01AR069079 and FAPESP grants 2017/25364-6, 2013/08135-2 and 2014/22500-8.

Author contributions

V.P.C., C.G.P., G.S.P. and M.L.F. designed the experiments, analyzed the data and wrote the manuscript; A.A. and D.C. discussed the data and wrote the manuscript.

Competing interests

The authors declare no competing interests.