IMMUNOWATCH

EDITION N°4 - JANUARY 2022

GENE THERAPY

MABDESIGN
THE IMMUNOTHERAPY NETWORK

INTRODUCTION

MabDesign's Immunowatch is a one-of-a-kind information monitoring newsletter in the field of biologics. Its aim is to provide members of our association with the most recent and pertinent data gathered or generated through the key expertise of MabDesign and its collaborators in scientific research, business intelligence, market analysis and intellectual property.

Each edition will focus on trending type of biologics. Its general format includes market study research, financial and economic data, invited contributions from scientific teams working in the industry or in academia and a section dedicated to intellectual property. The content of each edition is decided by an editorial composed of two field experts. Decision concerning the theme and conception of each newsletter is done in-house by the permanent members of our editorial team.

Finally, we would like to acknowledge the support of the Ambition Recherche & Développement (ARD) Biomédicaments 2020 Phase II programme, funded by the Centre Val de Loire region during the initial phases of launching this newsletter.

BIOPHARMACEUTICALS
Innovation Dynamics in Health
IN REGION CENTRE-VAL DE LOIRE

MABDESIGN
THE IMMUNOTHERAPY NETWORK

Région Centre-Val de Loire

Table of content

EDITORIAL

Nicola Beltraminelli

Lysogene

The past years have seen an incredible expansion in the cell and gene therapies area, with the first products successfully reaching the market after decades of efforts. Spinraza, the three cell therapies Kymriah, Yescarta, Tescartus and the in vivo gene therapies Luxturna and Zolgensma paved the way for the treatment of incurable and life-threatening diseases and opens new perspectives to target a broader spectrum of pathologies. In this relatively novel arena of technologies and treatments, an increasing number of French actors are very actively participating in the generation of tools and therapies with innovative approaches. I am honored to introduce this edition specifically dedicated to cell and gene therapies. After a general introduction to the technologies and their applications, this number provides a global overview of the ongoing clinical trials and the market potential of the therapies, as well as case studies of product candidates in development by some French pioneers. Finally, an in-depth analysis on the challenging IP around CRISPR/CAS9 is provided. Enjoy the reading!

Home - Lysogene

Gerald Perret

Genethon

The gene therapy revolution is definitely underway. After the first successes observed with the ex vivo gene therapy including the CART-Cell technology, the clinical proof of concept has been achieved and confirmed for the in vivo gene therapy. For this approach, some additional challenges need to be addressed. This is particularly true for in vivo gene therapy delivered by systemic route. The amount of vectors required to treat a whole tissues or multiple organs is much larger. The tropism and the pre-existing as well as the reactive immunity are among of the safety issues. Innovations and advances observed in gene therapy in recent years have brought great hope for many serious unmet medical needs, especially for genetic disorders. It is a pleasure to present this edition, which provide an overview of the field, from market and IP analysis to recent scientific and medical developments.

https://www.genethon.com/

BioAgilytix

Biology. Agility. Analytics.

Small and large molecule bioanalysis to help your therapeutics reach patients faster.

Discuss your project with our scientists today.

European Headquarters
Lademannbogen 10, 22339
Hamburg, Germany

USA Headquarters
2300 Englert Drive,
Durham NC 27713

Boston Laboratory
1320 Soldiers Field Road,
Boston MA 02135

San Diego Laboratory
39050 Camino Santa Fe,
San Diego CA 92121

Melbourne Laboratory
85 Commercial Road Melbourne,
VIC, 3004 Australia

Brisbane Laboratory
37 Kent St, Woolloongabba,
Queensland, 4102 Australia

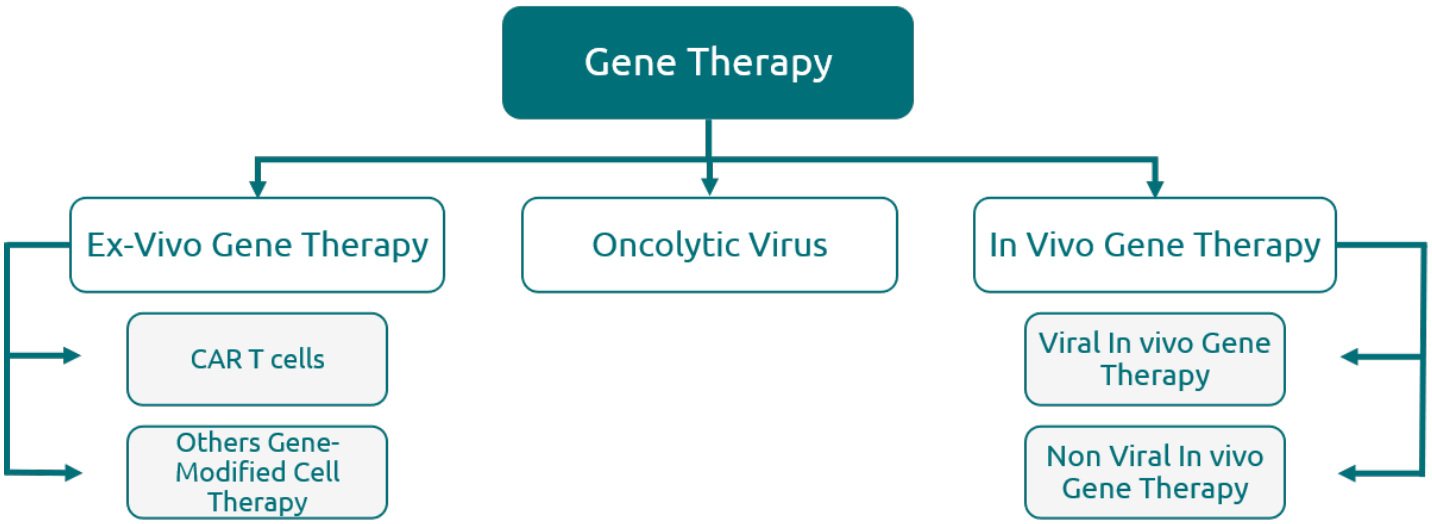
visit **BioAgilytix.com** to learn more
info@bioagilytix.com

GLOBAL GENE THERAPY market

Discover the available products, pipeline candidates, major deals and biopharmaceutical companies

Gene therapy: marketed products

Scope of gene therapy



Marketed gene therapies

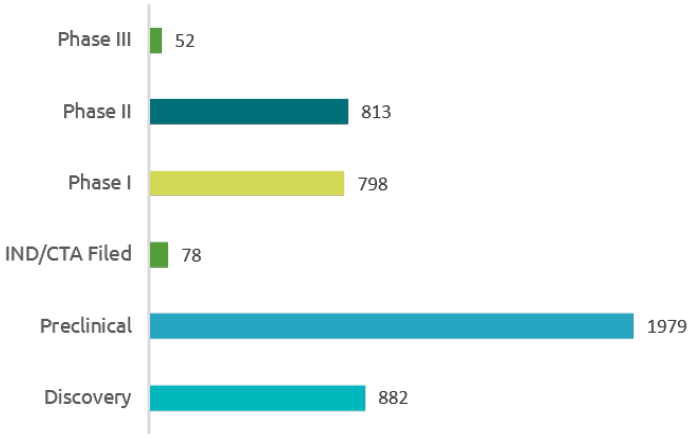
	Drug name	Brand name	Company	Vector
CAR T cells	axicabtagene ciloleucel	Yescarta	Gilead Sciences	Retrovirus
	tisagenlecleucel	Kymriah	Novartis	Lentivirus
	brexucabtagene autoleucel	Tecartus	Gilead Sciences	Retrovirus
	idecabtagene vicleucel	Abecma	Celgene	Lentivirus
	lisocabtagene maraleucel	Breyanzi	Juno Therapeutics	Lentivirus
	relmacabtagene autoleucel	Carteyva	JW Cayman Therapeutics Co	Lentivirus
Other Gene-Modified Cell Therapy	Strimvelis	Strimvelis	Orchard Therapeutics	Retrovirus
	atidarsagene autotemcel	Libmeldy	Orchard Therapeutics	Lentivirus
	betibeglogene autotemcel	Zynteglo	Bluebird Bio	Lentivirus
	elivaldogene autotemcel	Skysona	Bluebird Bio	Lentivirus
	nalotimagene carmaleucel	Zalmoxis	AGC Biologics SpA	Retrovirus
	tonogenchoncel-L	Invossa	Mitsubishi Tanabe Pharma Corp	Retrovirus
In vivo Gene Therapy	onasemnogene abeparvovec	Zolgensma	Novartis	Adeno Associated Virus (AAV)
	Gendicine	Gendicine	Shenzen SiBiono GeneTech Co Ltd	Adenovirus (AV)
	voretigene neparvovec	Luxturna	Novartis	Adeno Associated Virus (AAV)
	alipogene tiparvovec	Glybera	UniQure	Adeno Associated Virus (AAV)
	beperminogene perplasmid	Collategene	AnGes Inc	Plasmid
	Neovasculgen	Neovasculgen	Human Stem Cells Institute	Plasmid
Oncolytic Virus	human adenovirus type 5 (recombinant)	Oncorine	Shanghai Sunway Biotech Co Ltd	
	talimogene laherparepvec	Imlygic	Amgen Inc	
	teserpaturev	Delytact	Daiichi Sankyo Co Ltd	

** All data has been generated by MabDesign unless stated otherwise*
Source: Globaldata
Data from December 2021

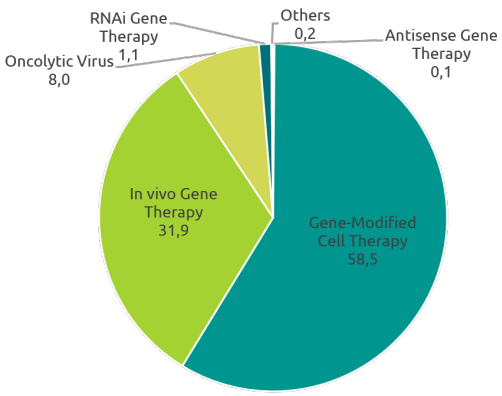
Gene therapy: products in development

Pipeline

Phase distribution



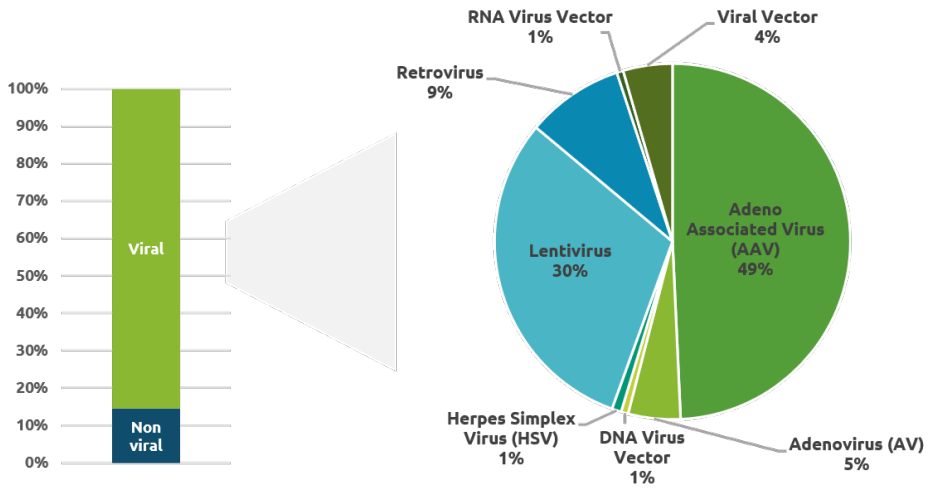
Type of gene therapy



Characteristics of the main viral vectors

Parameters	Retrovirus	Lentivirus	AAV	Adenovirus
Genetic Material	ssRNA	ssRNA	ssDNA	dsDNA
Genome Size	3-9 kb	3-9 kb	5 kb	36-38 kb
Coat	Enveloped	Enveloped	Non-Enveloped	Non-Enveloped
Packaging capacity	8 kb	8 kb	4,5 kb	7,5 kb
Tropism	Dividing Cells	Broad	Broad	Broad
Host Genome Interaction	Integrating	Integrating	Non Integrative	Non Integrative

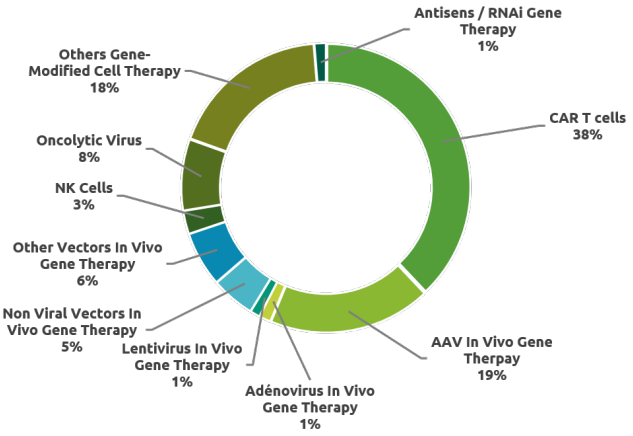
Type of vector



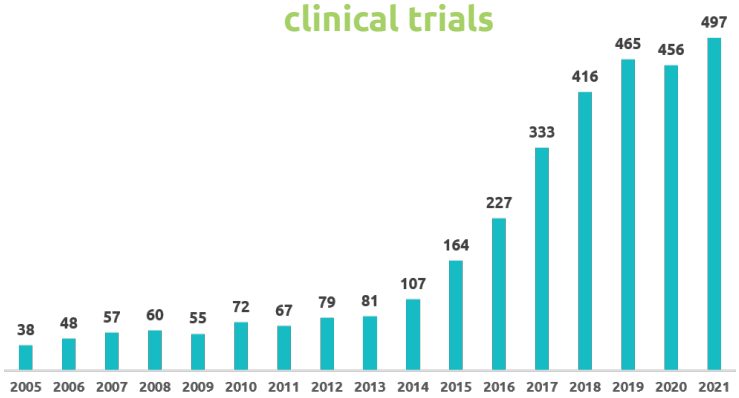
** All data has been generated by MabDesign unless stated otherwise*
Source: Globaldata
Data from December 2021

Gene therapy: products in development

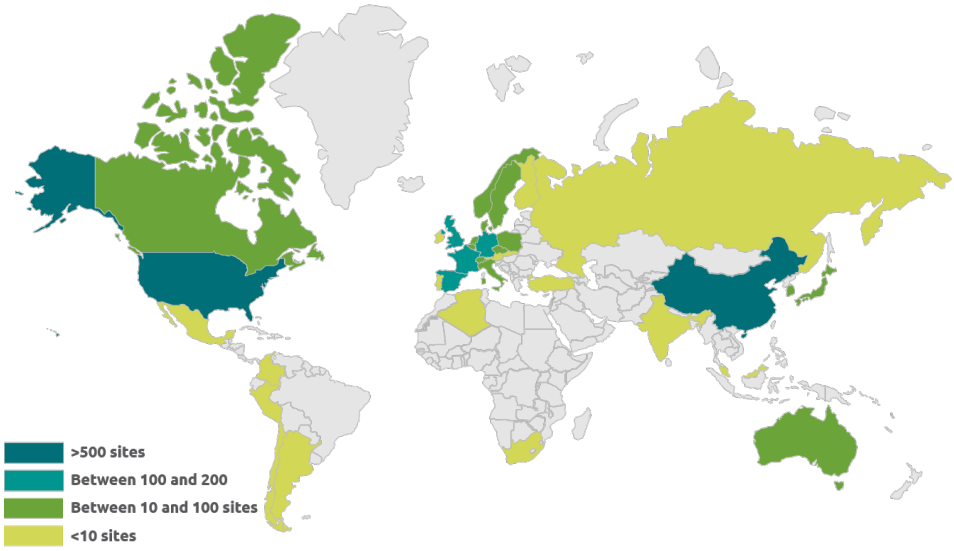
Gene therapy technologies



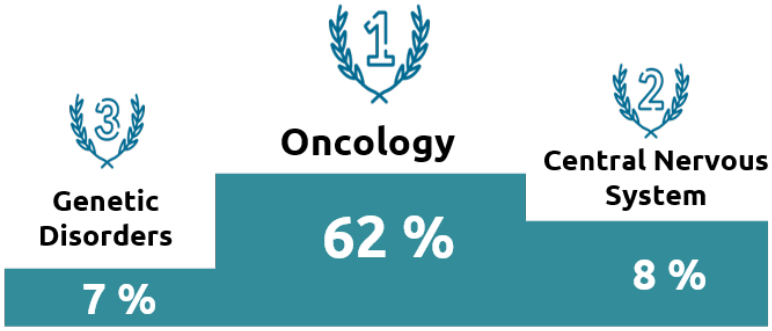
Evolution of the number of clinical trials



Site distribution



Top 3 therapeutic areas

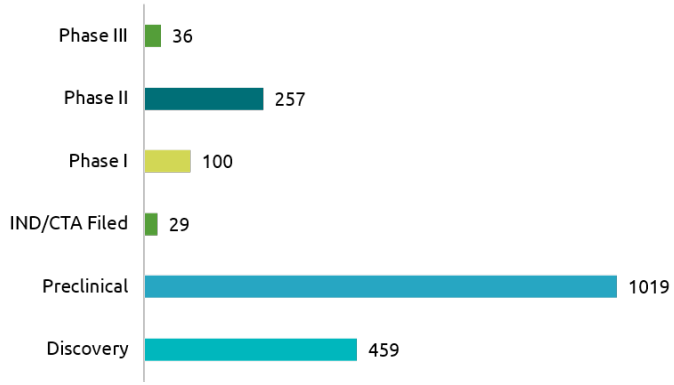


** All data has been generated by MabDesign unless stated otherwise*
Source: Globaldata
Data from December 2021

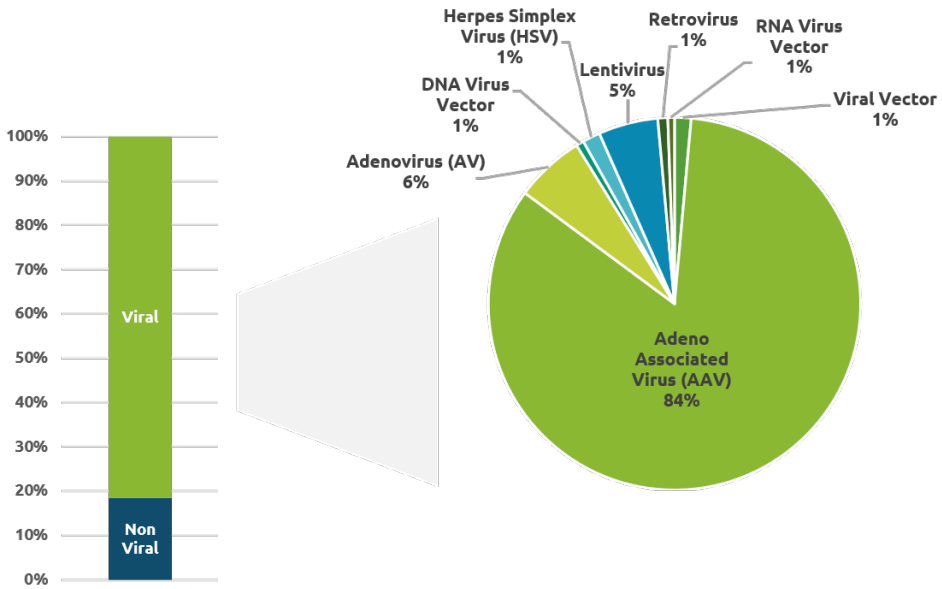
IN VIVO
Gene Therapy*

Pipeline

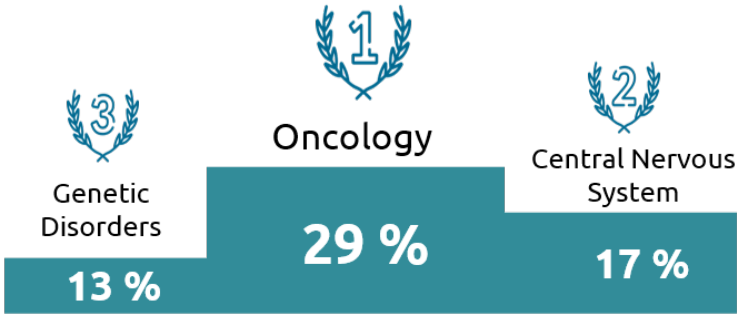
Phase distribution



Type of vector



Top 3 therapeutic areas



Top 5 companies

** Including oncolytic virus*

** * All data has been generated by MabDesign unless stated otherwise*

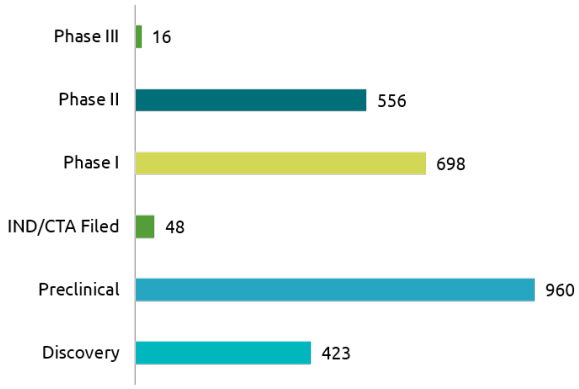
Source: Globaldata

Data from December 2021

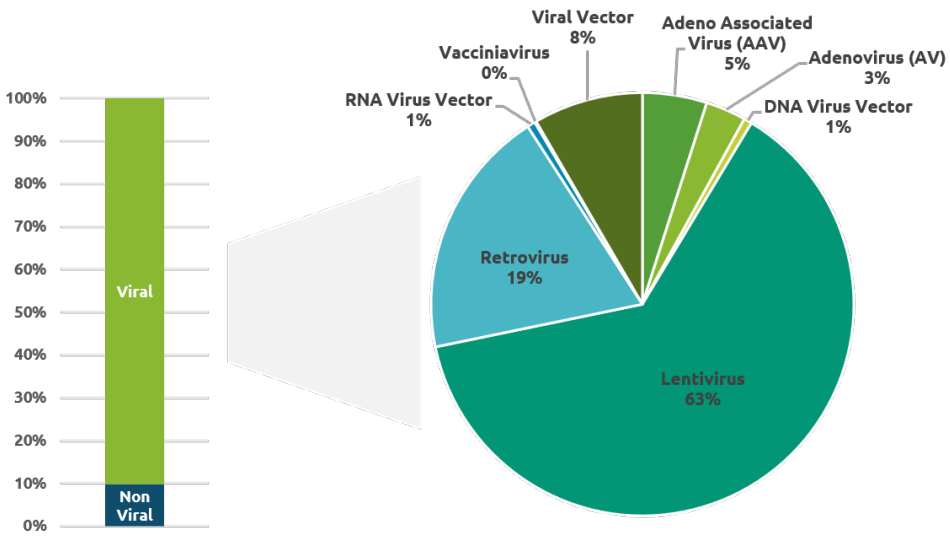
EX VIVO
Gene Therapy*

Pipeline

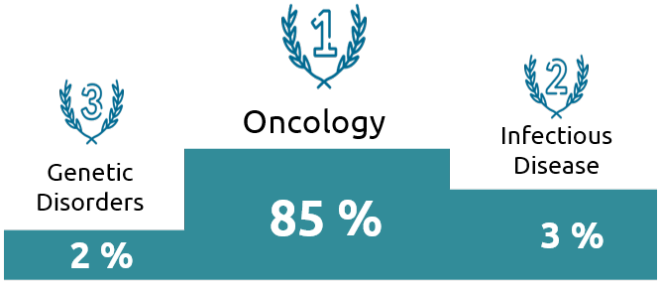
Phase distribution



Type of vector



Top 3 therapeutic areas



Top 5 companies

** Including oncolytic virus*

** * All data has been generated by MabDesign unless stated otherwise*

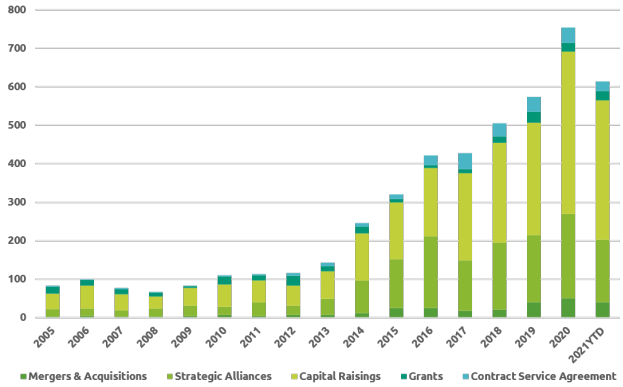
Source: Globaldata

Data from December 2021

Deals and companies

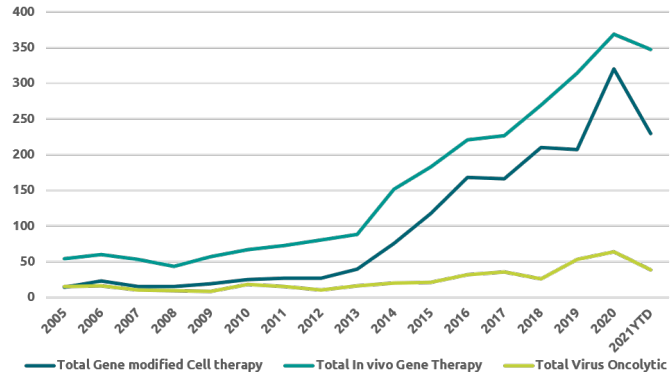
Deals concerning companies working in gene therapy

Total in number of deals



Total number of Deals registered according to the type of therapy

(Included Acquisitions, Strategic Alliances; Capital Raisings, Grants and Contract Service Agreement)



Top 3 deals

Acquisition		
Acquirers	Issuer	Deal value
Bristol-Myers Squibb	Celgene	89 (billions US$)
Gilead Sciences	Kite Pharma	12 (billions US$)
Celgene	Juno Therapeutic	9 (billions US$)

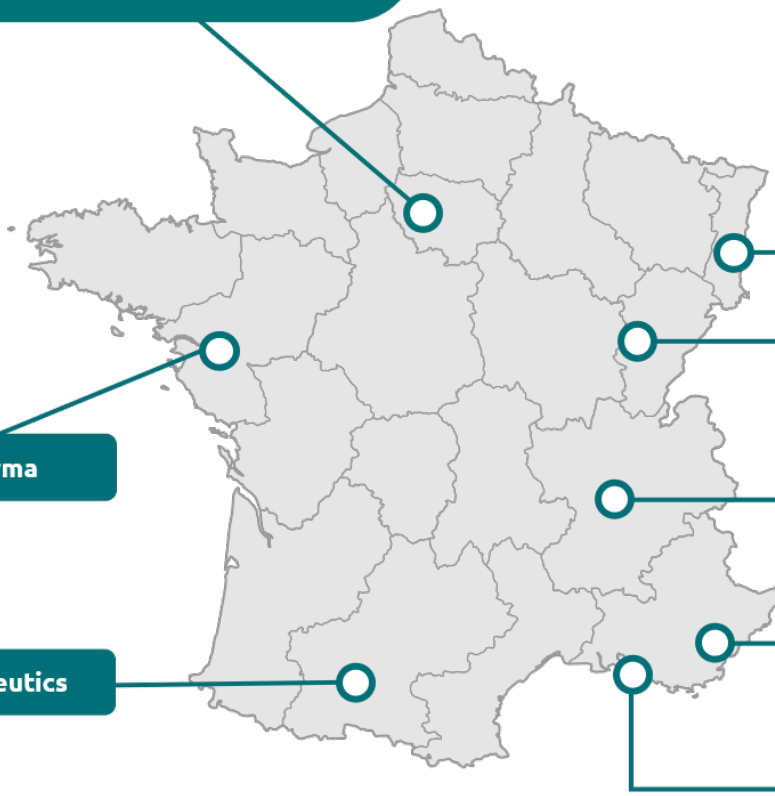
Capital Raising	
Acquirers	Deal value
BioMarin Pharma	911 (millions US$)
Beam Therapeutics	800 (millions US$)
Intellia Therapeutics	690 (millions US$)

Strategic Alliances			
Partners		Deal value	Deals description
Takeda Pharmaceutical	Poseida Therapeutics	3,6 (billions US$)	Takeda Pharma Enters into Licensing Agreement with Poseida Therapeutics
Genentech	Adaptimmune	3,25 (billions US$)	Genentech to Enter into Licensing Agreement with Adaptimmune
Fate Therapeutic	Janssen Biotech	3,1 (billions US$)	Fate Therapeutics Enters into Co-Development Agreement with Janssen Biotech

** All data has been generated by MabDesign unless stated otherwise*
Source: Globaldata
Data from December 2021

French companies developing gene therapy

Aratinga.bio
Atamyo Therapeutics
BrainVectis
Cellectis
Coave Therapeutics
Corlieve Therapeutics
EG 427
Eukarys
Eyevensys
Genethon
GenSight Biologics
Honing Biosciences
Invectys
Mnemo Therapeutics
Lysogène
Oncovita
Onxeo
Servier
Smart Immune
SparingVision
Theravectys
Vivet Therapeutics
Variant



Transgene

CanCell Therapeutics

OGD2 Pharma

Alma Biotherapeutics

Innoskel

Flash Therapeutics

Medesis Pharma

** All data has been generated by MabDesign unless stated otherwise*

scientific articles

Read the different inputs from the scientific community on various aspects of gene therapy

GENE THERAPY FOR GENETIC DISEASES IN 2021, A YEAR OF SUCCESS AND CHALLENGES

Gerald Perret[1]

1 Genethon, Programs Management Department, 1 rue de l'internationale 9100 Evry - France

INTRODUCTION

Gene therapy medicinal products (GTMPs) is generally defined as a vector or a delivery formulation/system containing a genetic construct engineered to express a specific transgene for the regulation, replacement, addition or deletion of a genetic sequence. The active substance is the nucleic acid sequence(s), or genetically modified microorganism(s), virus(es) or cells. The active substance may be composed of multiple elements. By using such gene therapy constructs, in vivo genetic regulation or genetic modification of somatic cells can be achieved. Vectors used in GTMPs can be engineered to target specific tissues or cells or to ensure the safety of the GTMP (deletion of genes associated with virulence, pathogenicity, immunotoxicity or replication-competence).

The GTMP could be grouped basically into 2 categories:

- Viral vectors;
- Non viral vectors: Plasmid DNA, Artificial vectors, Chromosome-based vectors, and Transposon vectors;

To date, the most common vector systems used for gene therapy have been viral vectors and plasmid DNA vectors. Viral vectors may be replication defective, replication competent or replication-conditional. Each type requiring specific consideration with regards to design and safety. Plasmid DNA vectors may be administered either in a simple salt solution or may be complexed with a carrier or in a delivery formulation. New artificial carrier with specific ligands

Historically, many gene therapy approaches have been based on expression of a transgene encoding a functional protein with related therapeutic effect. Recently tools are available that directly target nucleic acid sequences such as microRNA, RNAi via short hairpin RNAs (shRNA), molecular scissor and gene editing approaches such as CRISPR-Cas9.

Today, the great majority of available and under development GTMP are CAR-T cells. This technology is mainly developed for cancer indications. Only 2% of this approach target other indications including HIV/AIDs and autoimmune diseases. Since a previous Immunowatch has already been dedicated to the CAR-T cells, this article is focused on Gene therapies for the treatment of genetic diseases. This scope includes all strategies allowing the correction of genetic diseases by delivering a therapeutic gene in targeted cells/tissues or by direct genome editing. Gene therapy for genetic diseases is a subtype of the gene therapy field that belongs itself to the family of advanced therapy medicine. In addition to cancer and genetics diseases, the other currently approved and developed targets by GTMP are cardiovascular diseases including ischemia syndromes and common neurological diseases like Parkinson and Alzheimer diseases.

CURRENT WORLDWIDE PIPELINE

Among the 19 genes therapies currently approved worldwide, 6 are targeting genetic diseases. The table below provide an overview of theses product as known by end of 2021:

Product name	Generic name	Year first approved	Disease(s)	Locations approved	Originator company	Ex or In Vivo
Strimvelis	autologous CD34+ enriched cells	2016	Adenosine deaminase deficiency	EU, UK	Orchard Therapeutics	*Ex*
Luxturna	voretigeneneparvovec	2017	Leber's congenital amaurosis retinitis pigmentosa	US, EU, UK, Australia, Canada, South Korea	Spark Therapeutics (Roche)	*In*
Zolgensma	onasemnogene abepar-vovec	2019	Spinal muscular atrophy	US, EU, UK, Japan, Australia, Canada, Brazil, Israel, Taiwan, South Korea	Novartis	*In*
Zynteglo	lentiviral beta-globin gene transfer	2019	Transfusion-dependant beta thalassemia	EU, UK	Bluebird Bio	*Ex*
Libmeldy	OTL-200	2020	Metachromatic Leukodystrophy	EU, IK	Orchard Therapeutics	*Ex*
Skysona	elivaldogeneautotemcel	2021	Adrenoleukodystrophy	EU	Bluebird Bio	*Ex*

The great majority of the approved gene therapy is based on an ex-vivo gene transfer approach: relevant cells from patients (i.e., hematopoietic cells) are extracted, isolated and treated before being re-injected to achieve the therapeutic effect. The same approach is used for the CAR-T cells. Among the two therapies approved with an in-vivo delivery, only one is based on systemic administration: ZolgenSMA. This product is indicated to treat Spinal muscular atrophy (SMA) an autosomal recessive neuromuscular disease caused by deletion or mutation of the SMN1 gene. It is a serious disease characterized by a progressive loss of motor neurons resulting in muscle weakness. The disease, one of the most common monogenic diseases, affects 1 in 11,000 live births and before the era of SMA treatment, it was a leading genetic cause of mortality in infants. This product coming from the Genethon research and developed by AveXis (now Novartis Gene Therapy) have paved the way of the in vivo gene therapies requiring a systemic administration. Such administration is required for numerous genetic diseases including neuromuscular, metabolism and coagulation diseases. Several challenges are linked to a systemic administration of GTMP. Most of them are related to the quantity of vector to be administered, and to the biodistribution. To date the recombinant adeno-associated viral (rAAV) is by far the most common vector used for in vivo GTMP.

In addition to the approved products, 3 GTMPs for genetic diseases were in pre-registration step end of 2021:

- Valoctocogene roxaparvovec: AAV5 vector, Hemophilia A, (Biomarin)
    - o In EU and the UK
- Lenadogene nolparvovec: AAV2 vector, Leber hereditary optic neuropathy, (Genethon, GenSightBiologics)
    - o In EU and the UK, compassionate use (ATU) available in France
- Eladocagene exuparvovec: AAV2 vector, Aromatic L-amino acid decarboxylase (AADC) deficiency, (PTC Therapeutics)
    - o In EU and the UK

There are 2 CART-cell GTMPs at a similar stage. This recent pipeline evolution illustrates a trend

towards a significant increase in the proportion of in-vivo GTMPs for genetic diseases currently in an advanced development stage. However, the majority of gene therapies in preclinical development through pre-registration (about 1,900 candidates by end of 2021) remains ex-vivo approaches with 75% of the candidates. Among them the CAR T-cell therapies continue to dominate pipeline by representing more than 50% of the ex-vivo GTMPs currently in development.

The main genetic diseases for which GTMPs are being developed at clinical stage are:

- Retinitis pigmentosa (10 candidates)
- Hemophilia A (8 candidates)
- Sickle cell anemia (7 candidates)
- Duchenne's muscular dystrophy, Hemophilia B, Thalassemia (5 candidates, each)
- Achromatopsia, Fabry's disease, Leber's congenital amaurosis, Mucopolysaccharidosis IIIA (4 candidates, each)

MAIN 2021 EVENTS: SUCCESS AND CHALLENGES

The main issues and success observed in 2021 reflect quite well the identified challenges for development of GTMPs. They can be classified in 3 categories: (I) Stabilized efficacy / (II) Safety and immune response / (III) Biomanufacturing. The improvement of the regulatory paths and the identification of strategies to accelerate the development of GTMP especially for rare diseases are as well identified challenges.

The long-term efficacy is clearly an objective for GTMPs aiming to treat genetic diseases. The concept had already been demonstrated more than 20 year ago in the first age of gene therapy with the treatment of SCID-X1, a serious genetic immunodeficiency () based on first-generation gammaretroviral vectors. The clinical results demonstrated a good long-term immune reconstitution in most treated patients despite the occurrence of vector-related leukemia in a few of them. This long term effect conducted regulatory agencies to recently release recommendations regarding a 15-years clinical follow-up after ex-vivo integrative (i.e. using gammaretroviral or lentiviral vector) gene therapy. However, a similar demonstration for an in-vivo approach, particularly with a systemic administration, is still pending. The past year brought very encouraging data with notably the results of George LA et al. The team reported clinical long term results with the Spark Therapeutic product, an investigational AAV-LK03 (SPK-8011) to treat hemophilia A (lack of factor VIII). For most of patients treated in the cohort (16/18), factor VIII expression was maintained for more than 2 years after the infusion, and a one-stage factor VIII assay showed no apparent decrease in factor VIII activity over time. These results confirm the potential of GTMP to treat hemophilia, as reported the year before by Biomarin (K John Pasi et al) with the AAV5-hFVIII-SQ vector. In this study, three years after infusion seven participants treated with the high dose displayed a significant factor VIII expression. From a clinical point of view, the median number of annualized treated bleeding events was 0, and the median use of exogenous factor VIII was reduced from 138.5 infusions to 0 infusions per year. In the neuromuscular field, the long-term efficacy of ZolgemSMA was recently confirmed in an oral presentation by Jerry R. Mendell at the 2021 MDA Virtual Clinical and Scientific Conference. The gene therapy benefits were sustained more than five years after dosing.

Immune response is associated with several serious adverse events observed with recent GTMPs developed for genetic diseases, especially for the in-vivo rAAV-based approaches. The current purification processes are able to provide highly purified vectors with low process and product-related impurities generally known to contribute to immunogenicity in classical biotechnological

therapeutic products (i.e recombinant proteins). However, the rAAV vector is composed of a viral capside with viral proteins, and a genetic expression cassette containing the transgene with potential CpG motifs. These two components are naturally immunogenic. In addition, the protein generated by the transgene is expected to have immunogenic potential. This is particularly true for patients with genetic mutation leading to a total lack of expression for this protein. rAAV vector capsids, genetic cassette and their transgene products can provide targets for humoral and cellular immunity and for both, the innate and specific immunity could be involved.

The parameters below, can be involved in an immune clinical related effects.

- The route of administration (0% of SAE with intrathecal ad. Vs 88% with IV administered rAAV)
- A pre-existing immunity against the vector or the transgen protein: humoral (neutralising or total antibodies) or cellular
- The individual setting in innate immunity (i.e. plasmatic level of complement modulators)
- The CPG content of the transgen (non clinical proof)
- The dose * proportion of empty capside * weight of the patient = total rAAV infused
- The residual process and product related impurities (main product related impurities represented by the empty or partially full capsids)
- The age of the patient (maturation of the immune system from born to teenage)
- Treatment(s) of the patient (i.e use of corticoids before the infusion)
- The underlying physiopathological condition (i.e inflammation context related to the pathology)
- The level of transgen protein expression and the tropism of the vector
- The pre-existence of the protein in similar conformation, total or truncated

Dysregulation of the complement pathway, a major player of the innate immunity, could lead to severe effects including kidney injury (Atypical hemolytic uremic syndrome = aHUS), thrombocytopenia or thrombotic microangiopathy (TMA). A complement dysregulation has been involved in different serious events observed in in-vivo rAAV GTMP clinical trials leading to clinical holds or pauses. It was the case for the Pfizer and Solid trials using AAV9 vector to treat the Duchenne's muscular dystrophy. Dreepa et al described the TMA in 3 infants observed following ZolgenSMA infusion. An increased activation of the complement system was evident in two children. A review of different cases of complement activations investigated in non human primate presented by Juliette Hordeaux at the 2021 ASCGT congress suggest the involvement of the alternative complement pathway relatively independently of an antibody response that rather trigger the classical pathway. A better understanding of the underlying causes is still required. To date, the availability of Eculizimab, an anti-Complement 5 protein (C5), provides a potential solution for clinicians to manage complement upper-regulation.

The hepatoxicity is one other major serious adverse effect observed at clinical level with in vivo GTMP. ASPIRO is a gene therapy trial sponsored by Astellas Pharma using an AAV8 to treat the X-Linked Myotubular Myopathy (XLMTM) coming from Genethon research. The XLMTM is a severe muscular disease caused by mutations in the MTM1 gene that affects 1 in 50,000 live male births. Patients present generalized muscle hypotonia and respiratory failure at birth, and most of them die during early infancy. The first results of clinical efficacy were released early 2018 by Audentes

(now part of Astellas) showed significant improvements in neuromuscular and respiratory functions which were further confirmed in the second cohort of patients (a total of 23 patients treated), as presented in January 2021 by Dr. Perry Shieh at the ESGCT 2021 Congress. However, the trial has been put on hold two times after fatal events were observed in 2020 (3 deaths) and 2021 (1 death). These tragic events are all associated with serious liver dysfunctions. The underlying cause is still unknown and there is no evidence to date that an immune response is implicated in these events. The main other hepatotoxicities observed through rAAV-based GT trials (hemophilia, SMA) are associated with early transient elevation in liver enzymes that could range from asymptomatic event to liver injury with jaundice.

An improvement in the tropism of the vector by capsid elements and/or the control of expression by regulatory genetic element (including mir / microRNA approach) are significant leverages to increase the efficacy/risk balance. Sarah E Sinnet et al reported an significant improvement of safety without compromising efficacy by using a miR based auto-regulatory element into the miniMECP2 gene expression cassette. The AAV9/miniMECP2 vector is developed to treat the Rett syndrome, an X-linked neurodevelopmental disorder, and the uncontrolled expression of the MECP2 transgen conducted to a global toxicity.

In September 2021, the FDA have organized a global meeting dedicated to the toxicity risks of rAAV vectors for Gene Therapy. The meeting exchanges and minutes provide an exhaustive overview on this topic.

The immune system is also a significant challenge to treat patient with pre-existing anti-vector antibodies or to consider a retreatment if required. Pre-exiting anti-vector antibodies is a major non-inclusion criterion for clinical trials or a contraindication for marketed in vivo GTMPs. Last year, Christian Leborgne et al. and Zachary C Elmore et al. reported encouraging results with an IgG-degrading enzyme rapidly and transiently degrading anti-vector antibodies before administration of the GTMP. This approach is expected to be more transient and tolerated than the full clearance of lymphocyte B by using anti-CD20 antibodies. IgG-degrading enzyme represents a promising approach for the treatment of currently non-eligible patients. However, it is not clear today that such approach would open a path for the retreatment. The immune response observed after rAAV infusion and the related anti-drug antibodies (ADA) is usually much stronger than the natural immunisation related to wild circulating AAV (generating the pre-existing anti-vector antibodies). The approach developed by Selecta called ImmTOR and based on encapsulated rapamycin in nanoparticle, is intended to generate an immuno-tolerance when co-administered with rAVV. ImmTOR nanoparticles can be added to new or existing biologics without the need to modify or reformulate the biologic drug. The ability of ImmTOR to mitigate the formation of ADAs has been demonstrated at non clinical level in mice and in non-human primates for several biologics as well as for rAAV in a model of repeat dosing. In 2021, Selecta provided update on several partnerships in the gene therapy field including Sarepta and Askbio. The company announced the launch of a clinical trial in collaboration with Akbio to demonstrate the tolerogenic effect of ImmTOR with an empty rAAV8. The top line results were recently reported. At day 30, in subjects administered with a single dose of ImmTOR, Selecta reported a median anti-AAV8 neutralizing antibody titer 250-fold lower level than that observed in subjects dosed with AAV8 capsid alone. In collaboration also with Askbio, a clinical trial has been launch with this time a candidate GTMP. However, in November 2021, the U.S. Food and Drug Administration (FDA) has placed a clinical hold on this Phase 1/2 clinical trial of SEL-302 aiming to treat the Methylmalonic Acidemia-U.S (which consists of gene therapy candidate MMA-101 plus ImmTOR) due to CMC related questions for the MMA-101 part of this combination product.

The development of new generation of vectors with lower immunogenic properties is another promising way to overcome the immune challenge. Ying Kai Chan at al reported a new strategy based on rAAV vectors intrinsically less immunogenic by incorporating short DNA oligonucleotides that antagonize TLR9 (pathogens receptor of the innate immunity) activation directly into the vector genome. For such approach, that may request high throughput testing, the artificial intelligence (AI) and the machine learning could constitute a critical support.

The biomanufacturing remains to date a challenge for GTMP field. For the in-vivo GTMP, the main issues of biomanufacturing are at least at two levels: production costs and empty particles removal.

The production costs are mainly driven by the yield performance and the manufacturing scale. The yield could be improved at both cell culture (Upstream, USP) and purification (Downstream DSP) levels. Several process development works were inspired from methods and technologies originally designed for recombinant proteins products including monoclonal antibodies (MAbs). However, chromatography resins optimized for MAbs and recombinant proteins are not as well suited to the nature and the size of viral vectors such as rAAVs and lentiviruses (LVs). Emerging technologies must be purpose-built for viral vector manufacturing and purification. As analysed by Daniella Stell in BioProcess International, one other challenge is to have an homogeneous and parallel improvement between upstream and downstream technologies. This in order to avoid what have been observed for Mabs, with upstream titers that were not compatible with purification capacities. The economy of scale is an obvious leverage to improve the manufacturing costs. However, large bioreactors are rare among the GTMP CDMO. Early in 2021, Regenxbio announced the launch of an AAV program for the treatment of DMD (RGX-202). In the same press-release, the company specified that commercial-scale cGMP material has already been produced at 1000L capacity and will be used in the clinical development of RGX-202. Latter this year, StrideBio, a gene therapy company with preclinical pipeline for rare genetic diseases with a proprietary structure rAAV vector engineering platform (STRIVE™), announced to have completed construction of GMP manufacturing facility with 1000L production capacity (1000L single use bioreactor).

Removal of empty capsids from manufacturing lots is a major issue in the DSP of rAAV clinical-grade batches. Most of the SAE observed during the first weeks following the infusion of clinical AAV seems to be associated with the total viral load. A part of this load is related to the proportion of empty capsids. Because of similar physico-chemical characteristics, the rAAV capsid populations totally lacking or containing partial viral DNA are difficult to separate from the desired vector capsid populations. The ultracentrifugation (UC) remains the most effective separation method and has been extensively used at small scale but the scale-up appears very challenging. In 2020, Solid was able to lift the second FDA hold on the IGNIT DMD clinical trial by providing notably data regarding a new manufacturing process able to remove most of empty rAAV. The process strategy to achieve this result was not published. In 2021, Joshi et al reported robust, scalable and versatile anion-exchange chromatography (AEX) method for removing empty capsids and subsequent enrichment of vectors of AAV serotypes 5, 6, 8, and 9. In order to support the development of such innovative purification approaches, the improvement of analytical methods is critical to better understand the different population of vectors that compose the full and empty capsids. The work reported by Bia Separation (Pete Gagnon et al) described new HPLC method for the analysis of empty capsids and capsids encapsidating genetic material in a purified rAAV. This approaches could be an interesting complementary methods to the analytical ultracentrifugation (AUC).

The 2021 year brought numerous success in the field of GTMP for rare diseases. In consistency with the observed progress, several clinical trial have been launched and promising partnerships

were settled. However, many challenges remain to be managed. In addition to those mentioned here, numerous other challenges could be discussed. For instance, the adaptation of the regulatory guidelines (EMEA, FDA and ICH) to the specificity of GTMP is still in progress but something more specific could be expected for rare and ultra-rare diseases. Another example is the definition relevant clinical endpoints for rare and not well-known genetic diseases that is always challenging. We can wish that 2022 with, hopefully the end of the Covid-19 sanitary situation, will be a year of even greater success.

REFERENCES

Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products 22 March 2018 EMA/CAT/80183/2014 Committee for Advanced Therapies (CAT)

ASGCT-Pharma-Intelligence-Quarterly-Reports-2021

Cavazzana M, Six E, Lagresle-Peyrou C, André-Schmutz I, Hacein-Bey-Abina S. Gene Therapy for X-Linked Severe Combined Immunodeficiency: Where Do We Stand?. Hum Gene Ther. 2016;27(2):108-116. doi:10.1089/hum.2015.137

Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, Gross F, Yvon E, Nusbaum P, Selz F, Hue C, Certain S, Casanova JL, Bousso P, Deist FL, Fischer A. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science. 2000 Apr 28;288(5466):669-72. doi: 10.1126/science.288.5466.669. PMID: 10784449.

George LA, Monahan PE, Eyster ME, Sullivan SK, Ragni MV, Croteau SE, Rasko JEJ, Recht M, Samelson-Jones BJ, MacDougall A, Jaworski K, Noble R, Curran M, Kuranda K, Mingozzi F, Chang T, Reape KZ, Anguela XM, High KA. Multiyear Factor VIII Expression after AAV Gene Transfer for Hemophilia A. N Engl J Med. 2021 Nov 18;385(21):1961-1973. doi: 10.1056/NEJMoa2104205. PMID: 34788507; PMCID: PMC8672712.

Strauss K, Muntoni F, Farrar MA, et al. Onasemnogene abeparvovec gene therapy in presymptomatic spinal muscular atrophy (SMA): SPR1NT study update in children with 2 copies of SMN2. Presented at 2021 MDA Virtual Clinical & Scientific Conference. March15-March 18, 2021; Poster 67.

Strauss K, Muntoni F, Farrar MA, et al. Onasemnogene abeparvovec gene therapy in presymptomatic spinal muscular atrophy (SMA): SPR1NT study update in children with 3 copies of SMN2. Presented at 2021 MDA Virtual Clinical & Scientific Conference. March15-March 18, 2021; Poster 68.

Mendell JR, Finkel R, Mercuri E, et al. Long-term follow-up (LTFU) of onasemnogene abeparvovec gene therapy in spinal muscular atrophy (SMA). Presented at 2021 MDA Virtual Clinical & Scientific Conference. March15-March 18, 2021; Poster 63.

Hamilton BA, Wright JF. Challenges Posed by Immune Responses to AAV Vectors: Addressing Root Causes. Front Immunol. 2021 May 17;12:675897. doi: 10.3389/fimmu.2021.675897. PMID: 34084173; PMCID: PMC8168460

Sinnett SE, Boyle E, Lyons C, Gray SJ. Engineered microRNA-based regulatory element permits safe high-dose miniMECP2 gene therapy in Rett mice. Brain. 2021 Nov 29;144(10):3005-3019. doi: 10.1093/brain/awab182. PMID: 33950254.

Völkner M, Pavlou M, Büning H, Michalakis S, Karl MO. Optimized Adeno-Associated Virus Vectors for Efficient Transduction of Human Retinal Organoids. Hum Gene Ther. 2021 Jul;32(13-14):694-706. doi: 10.1089/hum.2020.321. Epub 2021 Jun 29. PMID: 33752467.

BRIEFING DOCUMENT- Food and Drug Administration (FDA), Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) Meeting #70: Toxicity Risks of Adeno-associated Virus (AAV) Vectors for Gene Therapy (GT) September 2-3, 2021

Cataland SR, Wu HM. Diagnosis and management of complement mediated thrombotic microangiopathies. Blood Rev. 2014 Mar;28(2):67-74. doi: 10.1016/j.blre.2014.01.003. Epub 2014 Jan 31. PMID: 24534133.

Gomez-Ganda L, Benitez-Carabante MI, Fernandez-Polo A, Muñoz-Lopez M, Renedo-Miro B, Ariceta G, Diaz De Heredia C. Use of Eculizumab in Pediatric Patients With Transplant Associated Thrombotic Microangiopathy. Front Pediatr. 2021 Nov 11;9:761726. doi: 10.3389/fped.2021.761726. PMID: 34858907; PMCID: PMC8632356.

Juliette Hordeaux, Adeno-Associated Virus-related toxicities in non-human primates. Presented at 2021 ASCGT

Chand DH, Zaidman C, Arya K, Millner R, Farrar MA, Mackie FE, Goedeker NL, Dharnidharka VR, Dandamudi R, Reyna SP. Thrombotic Microangiopathy Following Onasemnogene Abeparvovec for Spinal Muscular Atrophy: A Case Series. J Pediatr. 2021 Apr;231:265-268. doi: 10.1016/j.jpeds.2020.11.054. Epub 2020 Nov 28. PMID: 33259859.

Philippidis A. Fourth Boy Dies in Clinical Trial of Astellas' AT132. Hum Gene Ther. 2021 Oct;32(19-20):1008-1010. doi: 10.1089/hum.2021.29182.bfs. PMID: 34662231

Louis Jeune V, Joergensen JA, Hajjar RJ, Weber T. Pre-existing anti-adeno-associated virus antibodies as a challenge in AAV gene therapy. Hum Gene Ther Methods. 2013 Apr;24(2):59-67. doi: 10.1089/hgtb.2012.243. Epub 2013 Apr 3. PMID: 23442094; PMCID: PMC3732124

Leborgne C, Barbon E, Alexander JM, Hanby H, Delignat S, Cohen DM, Collaud F, Muraleetharan S, Lupo D, Silverberg J, Huang K, van Wittengerghe L, Marolleau B, Miranda A, Fabiano A, Daventure V, Beck H, Anguela XM, Ronzitti G, Armour SM, Lacroix-Desmazes S, Mingozzi F. IgG-cleaving endopeptidase enables in vivo gene therapy in the presence of anti-AAV neutralizing antibodies. Nat Med. 2020 Jul;26(7):1096-1101. doi: 10.1038/s41591-020-0911-7. Epub 2020 Jun 1. PMID: 32483358.

Elmore ZC, Oh DK, Simon KE, Fanous MM, Asokan A. Rescuing AAV gene transfer from neutralizing antibodies with an IgG-degrading enzyme. JCI Insight. 2020 Sep 17;5(19):e139881. doi: 10.1172/jci.insight.139881. PMID: 32941184; PMCID: PMC7566709.

Kishimoto TK. Development of ImmTOR Tolerogenic Nanoparticles for the Mitigation of Anti-drug Antibodies. Front Immunol. 2020 May 20;11:969. doi: 10.3389/fimmu.2020.00969. PMID: 32508839; PMCID: PMC7251066.

Chan YK, Wang SK, Chu CJ, Copland DA, Letizia AJ, Costa Verdera H, Chiang JJ, Sethi M, Wang MK, Neidermyer WJ Jr, Chan Y, Lim ET, Graveline AR, Sanchez M, Boyd RF, Vihtelic TS, Inciong RGCO, Slain JM, Alphonse PJ, Xue Y, Robinson-McCarthy LR, Tam JM, Jabbar MH, Sahu B, Adeniran JF, Muhuri M, Tai PWL, Xie J, Krause TB, Vernet A, Pezone M, Xiao R, Liu T, Wang W, Kaplan HJ, Gao G, Dick AD, Mingozzi F, McCall MA, Cepko CL, Church GM. Engineering adeno-associated viral vectors to evade innate immune and inflammatory responses. Sci Transl Med. 2021 Feb 10;13(580):eabd3438. doi: 10.1126/scitranslmed.abd3438. PMID: 33568518; PMCID: PMC8409505.

Wec AZ, Lin KS, Kwasnieski JC, Sinai S, Gerold J, Kelsic ED. Overcoming Immunological Challenges Limiting Capsid-Mediated Gene Therapy With Machine Learning. Front Immunol. 2021 Apr 27;12:674021. doi: 10.3389/fimmu.2021.674021. PMID: 33986759; PMCID: PMC8112259.

Gagnon P, Goricar B, Mencin N, Zvanut T, Peljhan S, Leskovec M, Strancar A. Multiple-Monitor HPLC Assays for Rapid Process Development, In-Process Monitoring, and Validation of AAV Production and Purification. Pharmaceutics. 2021 Jan 17;13(1):113. doi: 10.3390/pharmaceutics13010113. PMID: 33477351; PMCID: PMC7830902.

Rieser R, Koch J, Faccioli G, Richter K, Menzen T, Biel M, Winter G, Michalakis S. Comparison of Different Liquid Chromatography-Based Purification Strategies for Adeno-Associated Virus Vectors. Pharmaceutics. 2021 May 18;13(5):748. doi: 10.3390/pharmaceutics13050748. PMID: 34070226; PMCID: PMC8158740.

Joshi PRH, Bernier A, Moço PD, Schrag J, Chahal PS, Kamen A. Development of a scalable and robust AEX method for enriched rAAV preparations in genome-containing VCs of serotypes 5, 6, 8, and 9. Mol Ther Methods Clin Dev. 2021 Mar 23;21:341-356. doi: 10.1016/j.omtm.2021.03.016. PMID: 33898632; PMCID: PMC8056178.

Daniella Steel, Reducing Cell and Gene Therapy Development Time and Cost with New Purification Strategies, December 8, 2021, BioProcess International

Beattie S; Alliance for Regenerative Medicine (ARM), European Federation of Pharmaceutical Industries and Associations (EFPIA), European Association for Bioindustries (EuropaBio). Call for More Effective Regulation of Clinical Trials with Advanced Therapy Medicinal Products Consisting of or Containing Genetically Modified Organisms in the European Union. Hum Gene Ther. 2021 Oct;32(19-20):997-1003. doi: 10.1089/hum.2021.058. Epub 2021 May 24. PMID: 33843251.

PALL CORPORATION

Poster Content as Presented at ASGCT and ISCT 2021 Virtual Conferences, May 2021

Scale Up of a Lentiviral Production Process from the iCELLis® Nano Bioreactor to the iCELLis 500+ Bioreactor

Isabelle Pelletier[1], Volga Pasupuleti[1], Pragati Agnihotri[1], Young Do[1], Ziv Sandalon[1], Kaitlynn Bayne[2], Odette Becheau[2]

[1]Advanced BioScience Laboratories, Inc., 9800 Medical Center Drive, Bldg D, Rockville, MD 20850, USA

[2]Pall Corporation, 20 Walkup Drive, Westborough, MA 01581, USA

INTRODUCTION

Lentivirus is an increasingly important viral vector for the production of CAR-T and gene therapies. Traditionally, viral vectors are produced in various multi-layered flatware vessels for adherent cell processes. Biotechnology companies are interested in scaling up from flatware to commercial scale as quickly as possible, which necessitates using adherent cell culture methods. The gold-standard bioreactor in the industry for adherent cell culture for gene therapy is the iCELLis bioreactor.

The iCELLis bioreactor system is a closed, automated, single-use, fixed-bed bioreactor that provides excellent cell growth conditions for adherent cells. The iCELLis bioreactor fixed bed is comprised of carriers made of non-woven medical-grade polyethylene terephthalate (PET) fibers that allow for simplified and predictable process scale up from bench to manufacturing scale.

Pall's iCELLis 500+ bioreactor

Key features of the iCELLis bioreactor include:

- Fully integrated, single-use iCELLis 500+ bioreactor with disposable, pre-installed calibrated probes
- Unique waterfall system for shear-free oxygenation and CO_2 stripping
- Compact high cell density, fixed bed bioreactor providing a significant increase in volumetric productivity vs. traditional stirred tank bioreactors
- Keeping factors such as media volume per surface area, perfusion rate, fixed bed height, and fixed bed compaction constant, processes can be easily scaled from the bench-scale iCELLis Nano bioreactor to the large-scale iCELLis 500+ bioreactor

Advanced BioScience Laboratories, Inc. (ABL) is a global contract development and manufacturing organization (CDMO) providing clinical supply solutions for vaccines, immunotherapies, oncolytic and genetic therapy agents, and other large molecule products. They have invested in the iCELLis bioreactor technology and are now demonstrating their use of the technology for lentivirus production at the typical production scale for CAR-T applications. ABL's goal is to help customers reach the market quickly and efficiently with the iCELLis bioreactor.

Experimental Goals

- Scale up to the iCELLis 500+ bioreactor quickly with very little development work at the bench scale
- Produce lentiviral titers in the iCELLis 500+ bioreactor greater than or equal to the iCELLis Nano bioreactor
- Use nutrient and metabolite analysis to determine if cell growth is similar in the iCELLis Nano and the iCELLis 500+ bioreactors
- Utilize perfusion after transfection to collect product

MATERIALS AND METHODS

Cell Expansion

HEK293LTV cells from Cell Biolabs were cultured in DMEM+6 mM L-glutamine + 10% fetal bovine serum (FBS) in CellSTACK® 10s culture chambers and inoculated into the iCELLis Nano and iCELLis 500+ bioreactors at 8,000 cells/cm².

Cell Density and Metabolite Analysis Monitoring

Carrier strips from the iCELLis Nano bioreactor were removed from the fixed bed daily to determine cell density using lysis buffer and nuclei counts on the NucleoCounter® NC-200® cell counter. Metabolite concentrations were measured from both the iCELLis Nano and the iCELLis 500+ bioreactors daily using the Nova Biomedical BioProfile FLEX® automated cell culture analyzer. These samples were obtained by removing medium from the aseptic sampling port on the iCELLis Nano and iCELLis 500+ bioreactors.

Transfection

Cells were transfected on day 4 using the parameters used in Table 1. At this time, the cell density was 252,000 cells/cm² in the iCELLis Nano bioreactor. Because direct cell counts cannot be obtained from the iCELLis 500+ bioreactor, it was estimated that cells were at a similar cell density at time of transfection due to the similarities in nutrient and metabolite profiles. A complex volume of 10% of the working volume was used. 10% of the bioreactor working volume was removed from the bioreactors which was then replaced by the transfection complex via hand-pump (iCELLis Nano bioreactor) or gravity (iCELLis 500+ bioreactor).

Production

Perfusion was started 4 hours post-transfection. A constant flow rate was used for both bioreactors. A bottle or tote with fresh medium was connected to the 'media in' pump of each bioreactor and an empty bottle or tote was connected to the 'media out' pump of each bioreactor. Slowly, new medium was pumped in while virus-containing medium was pumped out.

Analysis

Samples were collected and stored at -80 °C until ready to be analyzed. Samples were then thawed and clarified by centrifugation before RNA extraction for RT-PCR.

Pall's iCELLis Nano bioreactor

Table 1

Parameters used in iCELLis Nano and iCELLis 500+ bioreactors. The iCELLis Nano bioreactor scales up to the iCELLis 500+ bioreactor based on fixed bed size and compaction. The 0.53 m² iCELLis Nano bioreactor and the 66 m² iCELLis 500+ bioreactor have a bed height of 2 cm and a compaction of 96 g/L.

Process Parameter	iCELLis Nano Bioreactor	iCELLis 500+ Bioreactor
Surface area (m²)	0.53	66
Culture duration (days)	7	7
Seeding density (cells/cm²)	8,000	8,000
Volume per surface area during cell growth (mL/cm²)	0.13	0.13
Media change prior to transfection (day)	4	4
Day of transfection (day)	4	4
Cell density at time of transfection (cells/cm²)	252,000	*
DNA concentration (µg/cm²)	0.2	0.2
µg DNA/million cells	0.8	*
µg DNA : µg PEI ratio	1:2	1:2
Transfection complex volume	10% of working volume	10% of working volume
Perfusion rate post-transfection (mL/cm²/day)	0.067	0.067
pH setpoint	7.2 ±0.1	7.2 ±0.1
DO setpoint	40%	40%
Linear speed during attachment (cm/s)	2	1.3
Linear speed during cell growth (cm/s)	1.2	0.7
Linear speed during transfection (cm/s)	2	1.3
Linear speed during production phase (cm/s)	1.2	0.5

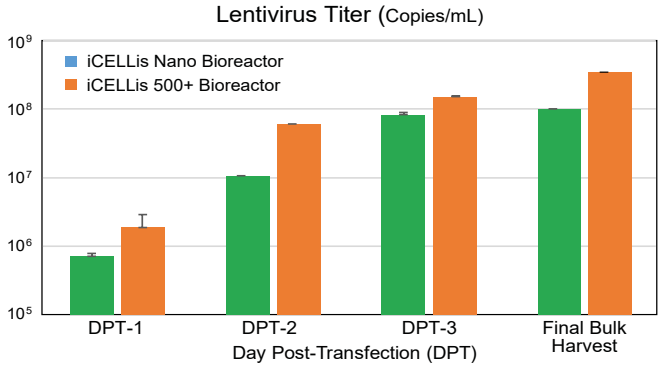
** Cell counts for iCELLis 500+ bioreactor estimated based on counts from the iCELLis Nano bioreactor*

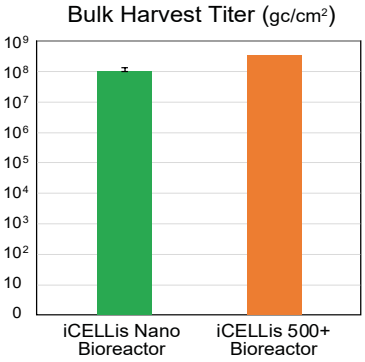
RESULTS

Viral Titer

Figure 1

Left: Titer obtained via qRT-PCR for the iCELLis Nano and the iCELLis 500+ bioreactors. Samples were collected from the bioreactor vessels 1, 2, and 3 days post-transfection (DPT). Final bulk harvest was collected from the perfusion collection tote at the end of the run when the contents of the vessel were drained into it. Right: Total titer yield from each bioreactor, normalized to surface area



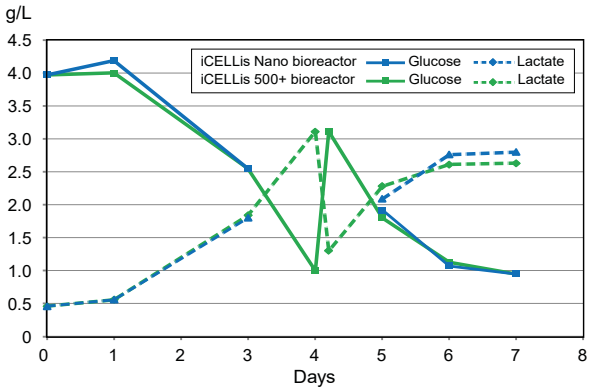


- The final bulk harvest had a similar titer to DPT-3, suggesting the concentration of virus in the perfusion out drum was the same as inside the iCELLis bioreactor vessel.
- The titer (gc/cm²) was 1.02 x 10^8 gc/cm² in the iCELLis Nano bioreactor and 3.47 x 10^8 gc/cm² in the iCELLis 500+ bioreactor.

Metabolites

Figure 2

Nutrient and metabolite concentrations in the iCELLis Nano and iCELLis 500+ bioreactors in g/L



- Concentrations of both glucose and lactate were similar between the iCELLis Nano and the iCELLis 500+ bioreactors, suggesting similar cell growth and cell density.

CONCLUSION

- **A robust process was scaled up quickly from the iCELLis Nano bioreactor to the iCELLis 500+ bioreactor with an N=1 for each bioreactor.**
- **The iCELLis 500+ bioreactor produced 3.47 x 10^8 gc/cm² while the iCELLis Nano bioreactor produced 1.02 x 10^8 gc/cm².**
- **The concentration of nutrients and metabolites were similar between the iCELLis Nano and the iCELLis 500+ bioreactors throughout the entire run, suggesting similar cell growth between the two scales.**

PALL PALL CORPORATION

ABL inc. ADVANCED BIOSCIENCE LABORATORIES

GN 21.10406, 6/21

GENE THERAPY FOR NEUROLOGICAL DISEASES: AN OVERVIEW OF LYSOGENE'S PIPELINE

Ralph Laufer[1], Nicola Beltraminelli[1], Michaël Hocquemiller[1], Marie Deneux[1], Stéphane Durant des Aulnois[1] and Karen Aiach[1]

[1]Lysogene, 8 Rue Jacques Dulud, 92200 Neuilly-sur-Seine, France

1. DESCRIPTION OF THE COMPANY

LYSOGENE is a leading gene therapy platform company focused on the research and development of AAV gene therapy solutions for neurological diseases, including neurodegenerative and neurodevelopmental disorders.

Since its inception, LYSOGENE has focused on the development of novel gene therapy candidates for rare genetic diseases of the central nervous system (CNS), for which no treatment is available. The company initially focused its efforts on the development of drug candidates for lysosomal storage disorders («LSDs») that affect the CNS (Platt et al. 2018). The company's most advanced drug candidates are LYS-SAF302 for the treatment of Sanfilippo A disease (MPS IIIA) and LYS-GM101 for the treatment of GM1 gangliosidosis. These two fatal neurodegenerative pediatric diseases currently have no treatment.

In addition to these two drug candidates, the company is exploring other gene therapy opportunities for CNS diseases, notably Fragile X syndrome, neuronopathic Gaucher disease and Parkinson disease.

LYSOGENE is also developing next-generation capsids with improved properties, which will constitute valuable tools for future program developments.

2. MPS IIIA GENE THERAPY

Mucopolysaccharidosis type IIIA (MPS IIIA, OMIM #252900) is a LSD caused by mutations in the SGSH gene that result in deficiency of the N-sulfoglucosamine sulfohydrolase (sulfamidase, EC 3.10.1.1) and subsequent accumulation of heparan sulfate (HS)-derived oligosaccharides (Valstar et al. 2010). Patients have relatively mild somatic symptoms, however the CNS is the primary site of pathology characterized by accumulation of HS and gangliosides leading to neuroinflammation and severe neurodegeneration. As a result, patients experience a wide range of CNS-based symptoms, including delayed neurocognitive development, mental regression, rapid loss of social skills and learning ability, disturbed sleep, aggression and hyperactivity with death usually occurring during the second decade (Heron et al. 2011). Therefore, the focus of new therapies is to treat the neurological manifestations associated with the disease (de Ruijter, Valstar, and Wijburg 2011).

Gene therapy using adeno-associated virus (AAV) vectors with neuronal tropism holds promise for delivering SGSH to the brain, which is the organ most susceptible to toxicity caused by SGSH deficiency. Even though some AAV capsid serotypes or vectors with engineered capsid variants have been reported to cross the blood-brain barrier (BBB) in mice(Deverman et al. 2016), intravascular administration of AAV vectors in primates is much less efficient (Gray et al. 2011). . In nonhuman primates, the most efficient route of delivery of an AAVrh.10 vector carrying the lysosomal enzyme arylsulfatase A was demonstrated to be direct injection into the subcortical white matter fiber tracts. This delivery route provided high enzyme expression and broad distribution throughout the primate brain, unlike administration by the intraventricular and intraarterial routes, which failed to demonstrate measurable enzyme levels above controls at the same dose level (Rosenberg et al. 2014).

Direct intraparenchymal delivery of AAV vectors has been used in several clinical trials for neurological diseases, including LSDs, as well as in preclinical disease models (Hocquemiller et al. 2016). LYSOGENE obtained proof of concept for this approach in a MPS IIIA mouse model, where unilateral intracranial injection of an AAVrh.10 vector carrying SGSH and the sulfatase cofactor SUMF1, referred to as LYS-SAF301, resulted in ipsilateral restoration of SGSH and reduction in HS storage, the number of activated microglia and at later stages reduced GM3 gangliosides and ubiquitin-positive lesions (Winner et al. 2016). LYS-SAF301 was used in a Phase I/II clinical trial for MPS IIIA (Tardieu et al. 2014), in which 4 patients received 7.2E+11 viral genomes simultaneously via six injection sites at two depths in 60 µL deposits bilaterally to the white matter anterior, medial and posterior to the basal ganglia. Safety data collected from inclusion, during the neurosurgery period and over the year of follow-up, showed good tolerance and absence of adverse events related to the injected product. Neuropsychological evaluations suggested a possible although moderate improvement in behavior, attention, and sleep in 3 out of 4 patients, with the youngest patient most likely to display a neurocognitive benefit.

To prepare for an efficacy trial of its investigational gene therapy, LYSOGENE conducted a 2-year natural history study in MPS IIIA patients. The results of this study, pooled with those of another natural history study carried out by Shapiro et al (Shapiro et al. 2016), will be used as an external control for the pivotal efficacy trial.

In parallel, LYSOGENE designed a second generation, improved gene therapy vector, referred to as LYS-SAF302. LYS-SAF302 is an AAVrh.10 vector containing a stronger gene promoter (CAG vs. murine phosphoglycerate kinase (mPGK) in LYS-SAF301), and carries SGSH as a single transgene. In short term (4 week) studies, LYS-SAF302 was shown to be about 3-fold more potent in directing brain expression of SGSH following intrastriatal administration in MPS IIIA mice (Gray et al. 2019). The ability of LYS-SAF302 to correct disease pathology was evaluated in a mouse model for MPS IIIA (Hocquemiller et al. 2020). LYS-SAF302 was administered to 5-week-old MPS IIIA mice at three different doses (8.6E+08, 4.1E+10, and 9.0E+10 vg/animal) injected into the caudate putamen/striatum and thalamus. LYS-SAF302 was able to dose-dependently correct or significantly reduce HS storage, secondary accumulation of GM2 and GM3 gangliosides, ubiquitin-reactive axonal spheroid lesions, lysosomal expansion and neuroinflammation, at 12-weeks and 25-weeks post-dosing. To study SGSH distribution in the brain of large animals, LYS-SAF302 was injected into the subcortical white matter of dogs (1.0 or 2.0E+12 vg/animal) and cynomolgus monkeys (7.2E+11 vg/animal) (Hocquemiller et al. 2020). Increases of SGSH enzyme activity of at least 20% above endogenous levels were detected in 78% (dogs 4 weeks after injection) and 97% (monkeys 6 weeks after injection) of the total brain volume. Taken together, these data validated intraparenchymal AAV administration as a promising method to achieve widespread enzyme distribution and correction of disease pathology in MPS IIIA and supported the initiation of a pivotal Phase II/III clinical study with LYS-SAF302 for the treatment of MPS IIIA.

The pivotal study of LYS-SAF302 (AAVance trial, NCT03612869) began in late 2018 with the activation of four centers in the United States and four centers in Europe. LYS-SAF302 was administered as a single injection of 7.2 E+12 vg into 6 different sites in the brain subcortical white matter. The first enrolled patient was treated in February 2019 and 19 patients had been treated by the end of March 2020. In parallel with the AAVance trial, LYSOGENE is carrying out a Video study of the patients (called PROVide) that captures real life behaviors in their home environment. At the end of 2020, the Company published initial positive biomarker data with LYS-SAF302 related to changes in cerebrospinal fluid (CSF) heparan sulfate (HS) concentration. In 9 patients analyzed, highly significant reductions in CSF HS concentration were observed at 6- and 12-months post-treatment compared to pre-treatment values. In addition, there was a reduction in the secondary storage products GM2

ganglioside and GM3 ganglioside in the CSF of treated patients relative to baseline values. These secondary storage products are thought to contribute to pathogenesis in MPS IIIA and other LSDs (Walkley 2004). Thus, these preliminary results indicate that LYS-SAF302 is biologically active and has the potential to provide therapeutic benefit in this patient population. The outcomes of the AAVance trial will be determined when all patients of the main cohort will have reached 2 years of treatment. The primary endpoint is the change in cognitive performance relative to the natural history data control group. Secondary endpoints include behavior, quality of life, MRI and fluid biomarkers. Localized MRI brain abnormalities have been observed at the injection site in patients treated in the clinical study. These are being monitored per protocol, and no directly attributable clinical sequelae have been reported.

In October 2018, LYSOGENE entered into a partnership with SAREPTA, a leading U.S. genetic precision medicine company, in the form of a collaboration and exclusive license agreement relating to the development, manufacturing and commercialization of the drug candidate LYS-SAF302. Under the terms of the agreement, SAREPTA has acquired exclusive commercial rights to LYS-SAF302 in the United States and markets outside Europe, while the Company retains commercial exclusivity for LYS-SAF302 in Europe. SAREPTA is responsible for the global manufacturing of LYS-SAF302 and will supply LYSOGENE in its markets.

LYS-SAF302 has been granted orphan designation in the EU and the US, and rare pediatric disease designation and fast track designation in the US.

3. GM1 GANGLIOSIDOSIS GENE THERAPY

GM1 gangliosidosis is a rare autosomal-recessive LSD that is always fatal in children and has no approved disease-modifying treatment (Arash-Kaps et al. 2019). It is due to mutations in the GLB1 gene, which encodes the lysosomal enzyme beta galactosidase (Beta-Gal), which metabolizes GM1 ganglioside. Deficiency of this enzyme leads to accumulation of GM1 ganglioside, which is particularly deleterious for neurons and therefore the major symptoms of the disease are neurological, including progressive psycho-motor decline and reduced lifespan. There are several subtypes of GM1 gangliosidosis, type I which is the rapidly progressing infantile form, type II with slower progression and more clinical variability, which is subdivided into late-infantile and juvenile forms, and type III which is the rare adult form. Interestingly, both the severity and the age of onset are related to the amount of residual Beta-Gal activity, from less than 1% in the infantile form to about 10% in the adult form. Asymptomatic heterozygote carriers of the disease have residual Beta-Gal activity that can be as low as 15 - 20% of normal activity.

LYSOGENE is developing an investigational gene therapy, designated LYS-GM101, consisting of an AAVrh10 vector that contains the functional GLB1 cDNA under a CAG promotor. LYS-GM101 is administered into the cerebrospinal fluid (CSF) via the cisterna magna (ICM), a space at the base of the skull that is filled with CSF and can be accessed from outside the skull without surgery through insertion of a needle under imaging guidance. It at been shown by several groups, including LYSOGENE, that injection of AAV vectors into the CSF achieves efficient transgene expression and broad distribution in the brain, spinal cord, and also peripheral organs. In contrast, when AAV vectors are injected into the bloodstream, e.g. via intravenous administration, only a low percentage of vector can pass through the blood-brain barrier (BBB) and reach the CNS, with most of the dose transducing the liver and other peripheral organs (Ballon et al. 2020).

Preclinical proof of concept was obtained using the GM1 knockout mouse model (GM1 mice), which does not express Beta-Gal (Hahn et al. 1997). A murine version of LYS-GM101 was administered to the CSF of 6-8 weeks old GM1 mice via intracerebroventricular (ICV) injection (because ICM

injection is technically challenging in mice of this age). This led to normalization of Beta-Gal activity in the cerebrum, cerebellum and spinal cord, and a concomitant correction of the GM1 ganglioside accumulation in these CNS regions.

In addition, experiments in GM1 cats were carried out. The GM1 cat expresses a naturally occurring mutant form of Beta-Gal with reduced enzymatic activity, causing a disease similar to juvenile GM1 gangliosidosis (Baker and Lindsey 1974). The feline version of LYS-GM101 was administered into the CSF using different routes, ICM, ICV or lumbar intrathecal (IT). Following euthanasia of the animals, Beta-Gal activity in different parts of the CNS was then visualized by staining. ICM dosing was found to yield the highest levels of expression in both brain and spinal cord. This increase in Beta-Gal activity following ICM injection was associated with a pronounced reduction of GM1 accumulation, as determined by filippin white staining in the gray matter of the brain and spinal cord.

IND-enabling GLP toxicology and biodistribution study was performed in nonhuman primates (NHP). LYS-GM101 was administered ICM at two doses and analyses were performed after 12 weeks and 6 months. Monkeys are more relevant to predict distribution in humans than mice or cats because their brain anatomy is more similar, and the size of the monkey brain is comparable (about 10-fold smaller) to that of a child. LYS-GM101 was found to be homogenously distributed in the CNS and also expressed in peripheral organs. Increases of 20% and 60% of Beta-Gal activity compared to vehicle treated animals were observed in NHP brain at 12 weeks after ICM injection of LYS-GM101 at the low and high dose, respectively. This magnitude of Beta-Gal activity suggests that at similar doses in humans, LYS-GM101 should be able to elicit clinical improvements in patients with GM1 gangliosidosis.

In early 2021, LYSOGENE obtained approvals from the MHRA in the United Kingdom, the FDA in the United States and the ANSM in France to start a clinical trial with LYS-GM101 in GM1 gangliosidosis patients (NCT04273269). This trial is an open-label, 2 stage adaptive clinical trial with natural history data as external control, conducted at 4 clinical sites (2 in US, one in France and one in UK). In parallel, a video outcome and parent interview study is being conducted, whose data will be complementary to the clinical endpoints. The trial will include 16 patients with early and late infantile GM1 gangliosidosis, 4 of which be part of an initial safety cohort, treated with ICM injection of LYS-GM101 at 8E12 vg/kg. The first two patients were treated in June and August 2021. In addition, LYSOGENE and Casimir Trials launched in early 2020 a collection of natural history data using interview and video assessments captured at home by parents/caregivers of their child with GM1 gangliosidosis (NCT04310163).

LYS-GM101 has been granted orphan designation in the EU and the US, and rare pediatric disease designation and fast track designation in the US.

4. OTHER PIPELINE PROJECTS

In June 2021, LYSOGENE entered into an exclusive, worldwide license agreement with SATT Conectus for the development and commercialization of a gene therapy candidate for the treatment of Fragile X syndrome (Hagerman et al. 2017). Fragile X syndrome (FXS) is the first cause of inherited intellectual disability (ID), affecting about 1/5000 males and 1/8000 females. FXS is associated with variable behavioral symptoms that include autism spectrum disorder, anxiety, hyperactivity, hypersensitivity, stereotypies, memory deficits and sleeping problems. FXS is a currently uncured condition that severely impacts the familial sphere and represents a considerable societal burden requiring life-long medico-social care. The disease is caused by silencing of the FMR1 gene due to CGG triplet expansions in its promoter region, leading to absence of the RNA-binding protein

FMRP. In a collaborative study between LYSOGENE and the lab of Dr. Hervé Moine at the IGBMC (Strasbourg), it was shown that intracerebral administration of an AAVrh10 vector expressing diacylglycerol kinase kappa (DGKk), the major downstream target of FMRP in neurons, rescued the core deficits of the Fmr1-KO mouse. LYSOGENE is now developing a gene therapy candidate for FXS based on these findings.

In July 2020, LYSOGENE entered into a research partnership with Yeda Research and Development Co Ltd, the commercial arm of the Weizmann Institute of Science. The objective is to develop innovative AAV gene therapy approaches for Gaucher disease, Parkinson's disease and other diseases associated with mutations in the GBA1 gene (Neumann et al. 2009). LYSOGENE provides expertise in AAV vector design and production, while Prof. Futerman's laboratory at the Weizmann Institute provides variants of the glucocerebrosidase enzyme with improved biological properties and conducts biological proof of concept studies.

5. TECHNOLOGY DEVELOPMENT

LYSOGENE is also conducting studies in the field of next-generation AAV capsid development and AAV producer cell lines, which will constitute valuable tools for internal development of LYSOGENE pipeline assets.

6. LYSOGENE'S BIOPRODUCTION CHALLENGES

The gene therapy field is among the most rapidly evolving ones over the past ten years. Several biomanufacturing players decided to be the pioneers in this field by developing CMC capabilities for the manufacture of AAV based therapies, and thanks to their contribution, research and GMP grade material was successfully generated for preclinical and clinical trials. Historically, AAV was mostly obtained by transient transfections on adherent cells and major industrial brands developed cell factories enabling to generate GMP grade material for clinical trials. This production system however has major limitations to scale up, thus impacting the use of this technique for diseases with larger patient populations. Furthermore, as the domain is expanding, the bioproduction demands are also massively increasing. In the past few years, several industry actors progressively implemented AAV productions based on cells cultured in suspension, and new facilities are currently under construction. Despite the significant increase in AAV-based gene therapy production capabilities, the demands are such that it remains a challenge to rapidly generate GMP grade material for large clinical trials and for commercial use.

7. PERSPECTIVES

LYSOGENE' objective is to reinforce its position as a global gene therapy platform for the development of gene therapies for CNS diseases. The company's business model is to continue expanding the pipeline through its internal discovery research, as well as by in-licensing early-stage programs from academic and industrial partners. LYSOGENE will develop the selected programs in a timely and cost-effective manner along all the steps required to progress these new assets into clinical trials. The company relies on its extensive experience acquired in the past ten years by advancing several programs from early research into pivotal clinical trials, more specifically in the pre-clinical, clinical, manufacturing, and regulatory domains, as well as its close connections with key opinion leaders and patient groups in the US and Europe.

REFERENCES

Arash-Kaps, L., K. Komlosi, M. Seegraber, S. Diederich, E. Paschke, Y. Amraoui, S. Beblo, A. Dieckmann, M. Smitka, and J. B. Hennermann. 2019. 'The Clinical and Molecular Spectrum of GM1 Gangliosidosis', J Pediatr, 215: 152-57 e3.

Baker, H. J., and J. R. Lindsey. 1974. 'Animal model: feline GM1 gangliosidosis', Am J Pathol, 74: 649-52.

Ballon, D. J., J. B. Rosenberg, E. K. Fung, A. Nikolopoulou, P. Kothari, B. P. De, B. He, A. Chen, L. A. Heier, D. Sondhi, S. M. Kaminsky, P. D. Mozley, J. W. Babich, and R. G. Crystal. 2020. 'Quantitative Whole-Body Imaging of I-124-Labeled Adeno-Associated Viral Vector Biodistribution in Nonhuman Primates', Hum Gene Ther, 31: 1237-59.

de Ruijter, J., M. J. Valstar, and F. A. Wijburg. 2011. 'Mucopolysaccharidosis type III (Sanfilippo Syndrome): emerging treatment strategies', Curr Pharm Biotechnol, 12: 923-30.

Deverman, B. E., P. L. Pravdo, B. P. Simpson, S. R. Kumar, K. Y. Chan, A. Banerjee, W. L. Wu, B. Yang, N. Huber, S. P. Pasca, and V. Gradinaru. 2016. 'Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain', Nat Biotechnol, 34: 204-9.

Gray, A. L., C. O'Leary, A. Liao, L. Agundez, A. S. Youshani, H. F. Gleitz, H. Parker, J. T. Taylor, O. Danos, M. Hocquemiller, N. Palomar, M. R. Linden, E. Henckaerts, R. J. Holley, and B. Bigger. 2019. 'An improved AAV vector for neurological correction of the mouse model of Mucopolysaccharidosis IIIA', Hum Gene Ther, 30: 1052-66.

Gray, S. J., V. Matagne, L. Bachaboina, S. Yadav, S. R. Ojeda, and R. J. Samulski. 2011. 'Preclinical differences of intravascular AAV9 delivery to neurons and glia: a comparative study of adult mice and nonhuman primates', Mol Ther, 19: 1058-69.

Hagerman, R. J., E. Berry-Kravis, H. C. Hazlett, D. B. Bailey, Jr., H. Moine, R. F. Kooy, F. Tassone, I. Gantois, N. Sonenberg, J. L. Mandel, and P. J. Hagerman. 2017. 'Fragile X syndrome', Nat Rev Dis Primers, 3: 17065.

Hahn, C. N., M. del Pilar Martin, M. Schroder, M. T. Vanier, Y. Hara, K. Suzuki, and A. d'Azzo. 1997. 'Generalized CNS disease and massive GM1-ganglioside accumulation in mice defective in lysosomal acid beta-galactosidase', Hum Mol Genet, 6: 205-11.

Heron, B., Y. Mikaeloff, R. Froissart, G. Caridade, I. Maire, C. Caillaud, T. Levade, B. Chabrol, F. Feillet, H. Ogier, V. Valayannopoulos, H. Michelakakis, D. Zafeiriou, L. Lavery, E. Wraith, O. Danos, J. M. Heard, and M. Tardieu. 2011. 'Incidence and natural history of mucopolysaccharidosis type III in France and comparison with United Kingdom and Greece', Am J Med Genet A, 155: 58-68.

Hocquemiller, M., L. Giersch, M. Audrain, S. Parker, and N. Cartier. 2016. 'AAV based gene therapy for CNS diseases', Hum Gene Ther.

Hocquemiller, M., K. M. Hemsley, M. L. Douglass, S. J. Tamang, D. Neumann, B. M. King, H. Beard, P. J. Trim, L. K. Winner, A. A. Lau, M. F. Snel, C. Gomila, J. Ausseil, X. Mei, L. Giersch, M. Plavsic, and R. Laufer. 2020. 'AAVrh10 Vector Corrects Disease Pathology in MPS IIIA Mice and Achieves Widespread Distribution of SGSH in Large Animal Brains', Mol Ther Methods Clin Dev, 17: 174-87.

Neumann, J., J. Bras, E. Deas, S. S. O'Sullivan, L. Parkkinen, R. H. Lachmann, A. Li, J. Holton, R. Guerreiro, R. Paudel, B. Segarane, A. Singleton, A. Lees, J. Hardy, H. Houlden, T. Revesz, and N. W. Wood. 2009. 'Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease', Brain, 132: 1783-94.

Platt, F. M., A. d'Azzo, B. L. Davidson, E. F. Neufeld, and C. J. Tifft. 2018. 'Lysosomal storage diseases', Nat Rev Dis Primers, 4: 27.

Rosenberg, J. B., D. Sondhi, D. G. Rubin, S. Monette, A. Chen, S. Cram, B. P. De, S. M. Kaminsky, C. Sevin, P. Aubourg, and R. G. Crystal. 2014. 'Comparative Efficacy and Safety of Multiple Routes of Direct CNS Administration of Adeno-Associated Virus Gene Transfer Vector Serotype rh.10 Expressing the Human Arylsulfatase A cDNA to Nonhuman Primates', Hum Gene Ther Clin Dev, 25: 164-77.

Shapiro, E. G., I. Nestrasil, K. A. Delaney, K. Rudser, V. Kovac, N. Nair, C. W. Richard, 3rd, P. Haslett, and C. B. Whitley. 2016. 'A Prospective Natural History Study of Mucopolysaccharidosis Type IIIA', J Pediatr, 170: 278-87 e1-4.

Tardieu, M., M. Zerah, B. Husson, S. de Bournonville, K. Deiva, C. Adamsbaum, F. Vincent, M. Hocquemiller, C. Broissand, V. Furlan, A. Ballabio, A. Fraldi, R. Crystal, T. Baugnon, T. Roujeau, J. M. Heard, and O. Danos. 2014. 'Intracerebral administration of AAV rh.10 carrying human SGSH and SUMF1 cDNAs in children with MPSIIIA disease: results of a phase I/II trial', Hum Gene Ther.

Valstar, M. J., S. Neijs, H. T. Bruggenwirth, R. Olmer, G. J. Ruijter, R. A. Wevers, O. P. van Diggelen, B. J. Poorthuis, D. J. Halley, and F. A. Wijburg. 2010. 'Mucopolysaccharidosis type IIIA: Clinical spectrum and genotype-phenotype correlations', Ann Neurol.

Walkley, S. U. 2004. 'Secondary accumulation of gangliosides in lysosomal storage disorders', Semin Cell Dev Biol, 15: 433-44.

Winner, L. K., H. Beard, S. Hassiotis, A. A. Lau, A. J. Luck, J. J. Hopwood, and K. M. Hemsley. 2016. 'A Preclinical Study Evaluating AAVrh10-Based Gene Therapy for Sanfilippo Syndrome', Hum Gene Ther, 27: 363-75.

THERAPEUTIC GENE CORRECTION WITH PRECISION GENOME EDITING

Pierre Billon[1,2,3]

1 The University of Calgary, Cumming School of Medicine, Department of Biochemistry and Molecular Biology, 3330 Hospital Drive N. W., Calgary, Alberta T2N 4N1, Canada
2 Robson DNA Science Centre
3 Arnie Charbonneau Cancer Institute

THE RISE AND FALL OF GENE THERAPY

Millions of individuals are living with an inherited genetic disorder worldwide. Alterations in the sequence that compose our genome can cause debilitating conditions that result in suffering, early aging, and eventually death. Gene therapy is a process in which an exogenous DNA fragment encoding a normal copy of a defective gene is randomly introduced inside patient's genome to offer a therapeutic value (Friedmann and Roblin, 1972). Despite initial encouraging clinical results, gene therapy suffered from dramatic side effects due to the random genomic insertion of DNA fragments (Branca, 2005; Hacein-Bey-Abina et al., 2003a; Hacein-Bey-Abina et al., 2003b; Teichler Zallen, 2000). While the field of gene therapy was still recovering, several technological breakthroughs that enable direct modifications of nucleic acid sequences have accelerated the promises of correcting pathogenic mutations in humans.

In 1994, Jasin revealed that introducing a site-specific double-strand break in the genome of living cells induces permanent changes in the targeted nucleic acid sequence (Rouet et al., 1994). This finding leverages cellular DNA repair mechanisms to introduce genetic alterations at the targeted genomic sequences (Yeh et al., 2019). Since this seminal discovery, highly specific and accurate innovative technologies that stimulate different DNA repair mechanisms have been developed for precision genome editing with high promises for therapeutic applications (Anzalone et al., 2020).

CRISPR, FROM AN OBSCURE BACTERIAL ADAPTIVE IMMUNE SYSTEM TO A POWERFUL GENOME EDITING TECHNOLOGY

Between 2007 and 2011, an obscure prokaryotic immune system that defends bacteria against predation was identified for its ability to introduce double-strand breaks in the genome of bacteriophages (Barrangou et al., 2007; Deltcheva et al., 2011; Garneau et al., 2010). The adaptive immune system was named CRISPR for Clustered Regularly Interspaced Short Palindromic Repeats to describe the presence of repeated sequences encoded in the bacterial genome, which are interspaced by variable DNA fragments. Fundamental research on the mechanism of action demonstrated that these variable sequences are excised from the genome of bacteriophages as fingerprints of previous infections. When a bacteria is infected a second time, a small non-coding RNA called guide RNA (gRNA) is produced to "guide" the Cas9 nuclease to the invaders' genome. The gRNA-Cas9 complex introduces a double-strand break at a location directed by the gRNA inducing the degradation of the phage genome, thereby resulting in premature abortion of the infection (Nussenzweig and Marraffini, 2020).

The ease of programming a small non-coding RNAs to direct the introduction of a double-strand break at desired genomic sequences has democratized the use of genome editing technologies for fundamental research and has facilitated its applications in biology and medicine (Pickar-Oliver and Gersbach, 2019). The programmable nature of the CRISPR-Cas9 system was revealed in 2012 by

Doudna and Charpentier. For this discovery, they were awarded the Nobel prize of Chemistry in 2020.

DNA repair machineries are dedicated to repairing DNA double-strand breaks as they represent the most dangerous type of DNA lesions (Ciccia and Elledge, 2010). Two major cellular repair machineries are involved in repairing DNA double-strand breaks in human cells: homologous recombination, which enables the introduction of desired new sequences; and non-homologous end-joining, which removes or adds one or several nucleotides at the break site. The ability to introduce mutations into virtually any cellular and animal system has revolutionized fundamental research for the generation of gene knock-out by non-homologous end-joining or for the insertion of desired genomic changes by homologous recombination. The CRISPR-Cas9 system allows the unprecedented engineering of genomes and is progressing towards clinical trials to correct mutations underlying genetic diseases (Doudna, 2020). For example, CRISPR was tested in a phase I clinical trial to engineer T cells to improve antitumor immunity against refractory cancers (Stadtmauer et al., 2020). The encouraging results demonstrated minimal immunogenicity or toxicity, prompting the continuation of clinical trials. More recently, a single injection of the gRNA/Cas9 complex reduced disease manifestations in patients suffering from beta-thalassemia and sickle cell disease (Frangoul et al., 2021), the most common human inherited disorders.

LIMITATIONS OF THE CRISPR-CAS9 SYSTEM FOR THERAPEUTIC GENOME EDITING

However, despite promising results, many challenges might potentially prevent CRISPR-Cas9 from wide adoption for therapeutic genome editing. Controlling precise correction of genomic sequence relies on repairing of the double-strand break by homologous recombination, which is non-functional in somatic cells. Moreover, double-strand breaks can generate mutagenesis at on- and off-target loci, induce catastrophic chromosome rearrangements, cause the generation of chromosome fragments into micronuclei, activate innate immunity and the p53 checkpoint that triggers cell cycle checkpoint and cellular apoptosis (Haapaniemi et al., 2018; Ihry et al., 2018; Leibowitz et al., 2021).

To limit the damages associated with the introduction of double-strand breaks, new classes of more precise genome editing technologies that edit genomic sequences without introducing double-strand breaks have recently been developed (Anzalone et al., 2020).

NEXT-GENERATION PRECISION TOOLS FOR HIGH-EFFICIENCY GENOME EDITING

One of the important lessons gained from sequencing human genomes is that most pathogenic variants are single nucleotide changes. Transition mutations, in which C:G base pairs are mutated into T:A, and T:A base pairs are mutated into C:G represent ~50% of genetic variants. Therefore, the development of technologies that can precisely correct transition mutations would be highly desirable for therapeutic genome editing.

In 2016, Liu developed a highly innovative technology, named base editing, that utilizes the CRISPR system to localize corrupted genomic sequences and a deaminase that reorganizes the atoms of the targeted nucleotides without altering the structure of the DNA (Gaudelli et al., 2017; Komor et al., 2016). They developed two types of base editing systems: A cytosine base editor which converts C:G base pair into T:A base pair and the adenine base editor which modifies A:T base pair into G:C base pair. These breakthrough technologies provide unprecedented single-nucleotide resolution for interrogating the genome (Billon et al., 2017; Cuella-Martin et al., 2021; Hanna et al., 2021). Moreover, base editing utilizes DNA repair machineries present in all cell types, including somatic cells, providing high potential for therapeutic genome editing. In only a few years, multiple animal

models of human genetic diseases have been corrected with high precision with base editing, and clinical trials have started (Porto et al., 2020).

In 2019, the same group developed another revolutionary CRISPR-based genome editing technology named prime editing (Anzalone et al., 2019). Unlike base editing, which is limited to transition mutations, prime editing can rewrite segments of the genome. Prime editing is a technology that utilizes an engineered gRNA that can localize a targeted genomic sequence and that primes a reverse transcriptase to replace the targeted genomic sequence with the desired changes. Prime editing is an exciting emerging technology and has the immense potential to correct all types of genetic mutations, including transversion mutations, small insertions and deletions.

Recent advances in prime editing have further advanced genome editing to directions, previously inaccessible with other genome editing technologies. Prime editing can insert gene-size fragments at desired locations in the genome, without introducing double-strand breaks, with high sensitivity, specificity, and accuracy (Anzalone A., 2021; Choi et al., 2021; Ioannidi, 2021; Lin et al., 2021). The ability to introduce corrective genes in cells was the driving force of gene therapy, but its inability to insert fragments safely caused the complications.

The advent of genome editing technologies has transformed our abilities to manipulate genomes for basic research, agriculture, and medicine. Although precision genome editing technologies are in their infancy, the rapid pace of development and their potential for safe, accurate, and high-efficiency gene correction hold exciting promises for personalized treatment and potentially offer cures for people living with genetic disorders (Rees et al., 2021).

REFERENCES

Anzalone A., G.X., Podracky C., Nelson A., Koblan L., Raguram A., Levy J., Mercer J., Liu D. (2021). Programmable large DNA deletion, replacement, integration, and inversion with twin prime editing and site-specific recombinases. BioRxiv.

Anzalone, A.V., Koblan, L.W., and Liu, D.R. (2020). Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. Nat Biotechnol 38, 824-844.

Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M., Chen, P.J., Wilson, C., Newby, G.A., Raguram, A., et al. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576, 149-157.

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D.A., and Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. Science 315, 1709-1712.

Billon, P., Bryant, E.E., Joseph, S.A., Nambiar, T.S., Hayward, S.B., Rothstein, R., and Ciccia, A. (2017). CRISPR-Mediated Base Editing Enables Efficient Disruption of Eukaryotic Genes through Induction of STOP Codons. Mol Cell 67, 1068-1079 e1064.

Branca, M.A. (2005). Gene therapy: cursed or inching towards credibility? Nat Biotechnol 23, 519-521.

Choi, J., Chen, W., Suiter, C.C., Lee, C., Chardon, F.M., Yang, W., Leith, A., Daza, R.M., Martin, B., and Shendure, J. (2021). Precise genomic deletions using paired prime editing. Nat Biotechnol.

Ciccia, A., and Elledge, S.J. (2010). The DNA damage response: making it safe to play with knives. Mol Cell 40, 179-204.

Cuella-Martin, R., Hayward, S.B., Fan, X., Chen, X., Huang, J.W., Taglialatela, A., Leuzzi, G., Zhao, J., Rabadan, R., Lu, C., et al. (2021). Functional interrogation of DNA damage response variants with base editing screens. Cell 184, 1081-1097 e1019.

Deltcheva, E., Chylinski, K., Sharma, C.M., Gonzales, K., Chao, Y., Pirzada, Z.A., Eckert, M.R., Vogel, J., and Charpentier, E. (2011). CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature 471, 602-607.

Doudna, J.A. (2020). The promise and challenge of therapeutic genome editing. Nature 578, 229-236.

Frangoul, H., Altshuler, D., Cappellini, M.D., Chen, Y.S., Domm, J., Eustace, B.K., Foell, J., de la Fuente, J., Grupp, S., Handgretinger, R., et al. (2021). CRISPR-Cas9 Gene Editing for Sickle Cell Disease and beta-Thalassemia. N Engl J Med 384, 252-260.

Friedmann, T., and Roblin, R. (1972). Gene therapy for human genetic disease? Science 175, 949-955.

Garneau, J.E., Dupuis, M.E., Villion, M., Romero, D.A., Barrangou, R., Boyaval, P., Fremaux, C., Horvath, P., Magadan, A.H., and Moineau, S. (2010). The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 468, 67-71.

Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I., and Liu, D.R. (2017). Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. Nature 551, 464-471.

Haapaniemi, E., Botla, S., Persson, J., Schmierer, B., and Taipale, J. (2018). CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. Nat Med 24, 927-930.

Hacein-Bey-Abina, S., von Kalle, C., Schmidt, M., Le Deist, F., Wulffraat, N., McIntyre, E., Radford, I., Villeval, J.L., Fraser, C.C., Cavazzana-Calvo, M., et al. (2003a). A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 348, 255-256.

Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M., McCormack, M.P., Wulffraat, N., Leboulch, P., Lim, A., Osborne, C.S., Pawliuk, R., Morillon, E., et al. (2003b). LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 302, 415-419.

Hanna, R.E., Hegde, M., Fagre, C.R., DeWeirdt, P.C., Sangree, A.K., Szegletes, Z., Griffith, A., Feeley, M.N., Sanson, K.R., Baidi, Y., et al. (2021). Massively parallel assessment of human variants with base editor screens. Cell 184, 1064-1080 e1020.

Ihry, R.J., Worringer, K.A., Salick, M.R., Frias, E., Ho, D., Theriault, K., Kommineni, S., Chen, J., Sondey, M., Ye, C., et al. (2018). p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. Nat Med 24, 939-946.

Ioannidi, E., Yarnall, M., Schmitt-Ulms C., Krajeski R., Lim J., Villiger L., Zhou W., Jiang K., Roberts N., Zhang L., Vakulskas C., Walker J., Kadina A., Zepeda A., Holden K., Gootenberg J., Abudayyeh O. (2021). Drag-and-drop genome insertion without DNA cleavage with CRISPR-directed integrases. BioRxiv.

Komor, A.C., Kim, Y.B., Packer, M.S., Zuris, J.A., and Liu, D.R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 533, 420-424.

Leibowitz, M.L., Papathanasiou, S., Doerfler, P.A., Blaine, L.J., Sun, L., Yao, Y., Zhang, C.Z., Weiss, M.J., and Pellman, D. (2021). Chromothripsis as an on-target consequence of CRISPR-Cas9 genome editing. Nat Genet 53, 895-905.

Lin, Q., Jin, S., Zong, Y., Yu, H., Zhu, Z., Liu, G., Kou, L., Wang, Y., Qiu, J.L., Li, J., et al. (2021). High-efficiency prime editing with optimized, paired pegRNAs in plants. Nat Biotechnol.

Nussenzweig, P.M., and Marraffini, L.A. (2020). Molecular Mechanisms of CRISPR-Cas Immunity in Bacteria. Annu Rev Genet 54, 93-120.

Pickar-Oliver, A., and Gersbach, C.A. (2019). The next generation of CRISPR-Cas technologies and applications. Nat Rev Mol Cell Biol 20, 490-507.

Porto, E.M., Komor, A.C., Slaymaker, I.M., and Yeo, G.W. (2020). Base editing: advances and therapeutic opportunities. Nat Rev Drug Discov 19, 839-859.

Rees, H.A., Minella, A.C., Burnett, C.A., Komor, A.C., and Gaudelli, N.M. (2021). CRISPR-derived genome editing therapies: Progress from bench to bedside. Mol Ther 29, 3125-3139.

Rouet, P., Smih, F., and Jasin, M. (1994). Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. Mol Cell Biol 14, 8096-8106.

Stadtmauer, E.A., Fraietta, J.A., Davis, M.M., Cohen, A.D., Weber, K.L., Lancaster, E., Mangan, P.A., Kulikovskaya, I., Gupta, M., Chen, F., et al. (2020). CRISPR-engineered T cells in patients with refractory cancer. Science 367.

Teichler Zallen, D. (2000). US gene therapy in crisis. Trends Genet 16, 272-275.

Yeh, C.D., Richardson, C.D., and Corn, J.E. (2019). Advances in genome editing through control of DNA repair pathways. Nat Cell Biol 21, 1468-1478.

ImmunXperts
a Nexelis company

Gene Therapy Immunogenicity assessment and monitoring

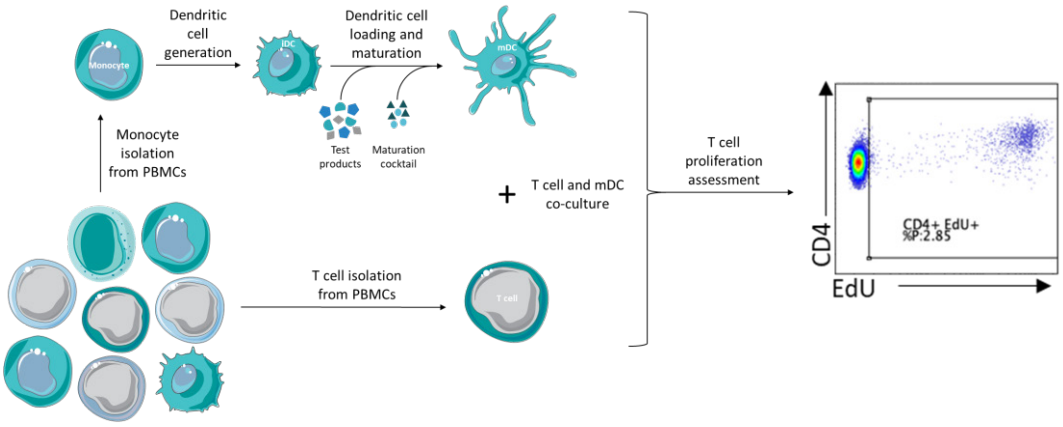
New modalities, new issues

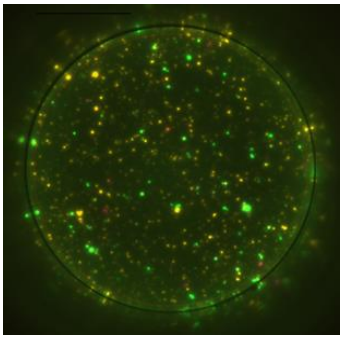
Introducing the gene therapy product into human cells requires a vector that will deliver the gene into the cells and incorporate those genes into the gene expression mechanism of those cells. One of the issues is the induction of an unwanted immune response that can have an influence on the efficacy and potency of the treatment. Additionally, pre-existing immunity towards AAV and CRISPR can also neutralize the therapeutic effect.

Comprehensive assessment of human immune responses to gene therapy candidates includes characterization of humoral and cellular immunogenicity, specific for both the viral vector and the expressed transgene (protein) product before and after dose administration.

Immunogenicity assessment

In vitro assays such as PBMC and DC:T cell proliferation assays can be used to assess this unwanted immunogenicity in an early phase. Additionally, innate assays such as dendritic cell activation assays can be applied for the evaluation of potential impurities and innate response inducing contaminants.





Immunogenicity monitoring

Sensitive Fluorospot assays can be carried out to monitor patient' specific immunogenicity both at the T and B cell level. The advantage of the Fluorospot technology is the sensitivity to detect responses on a single cell level and measure up to 4 analytes simultaneously.

Representative Fluorospot well showing the IFN-γ, IL-2 and TNF-α production of human PBMCs upon stimulation with peptides representing the vector of interest.

IMXP is an experienced partner for all your immunology projects, having performed over 90 immunogenicity screenings over the last 5 years. As a member of the EIP-NCIRA (Non-Clinical Immunogenicity Risk Assessment) and AAPS Immunogenicity Risk Assessment and Mitigation expert working groups, IMXP is in constant dialogue with regulatory authorities and up-to-date on all new, state-of-the-art developments in the field.

www.immunxperts.com - info@immunxperts.com

Intellectual property

Learn about the challenges of patenting gene therapy

CRISPR/Cas9: a nebula of patents

Lucile VERNOUX[ǂ] and Nicolas BOUQUIN [ǂ]

[ǂ] Regimbeau, Paris

CRISPR-Cas9 technology ("Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Associated Protein 9 (Cas9)") is already one of the most important scientific developments of this century. Its applications are wide and varied, and touch almost every aspect of biology. It has the ability to transform such important fields as e.g., agriculture and medicine. This technology is in particular poised to revolutionise medicine, with the potential to cure a range of genetic diseases, including neurodegenerative disease, blood disorders, cancer, and ocular disorders.

CRISPR-Cas9 is the most potent gene-editing tools to date. Sections of nucleic acids are edited in cells by insertion, deletion, or replacement at a specific target sequence. It is precise, fast, easy to implement, cheap, and uses components readily accessible.

The original CRISPR is a bacterial defence mechanism against phages. The CRISPR-Cas9 technology, as developed in the labs of Jennifer Doudna and Emmanuelle Charpentier, is a simple two-component system wherein an endonuclease (Cas9) is guided by a single guide RNA to the target sequence. This technology was then "used" as a programmable tool to cleave any nucleic acid sequence. It has made numerous achievements in the field of correcting pathogenic mutations, searching for essential genes for cancer immunotherapy, and solving key problems in organ xenotransplantation.

Improvements of the technology have been numerous and varied, including dead-Cas9, other endonucleases such as Cpf1, base editing systems, Cas9 variants, RNA editing, prime editing, etc., making CRISPR-Cas9 a sort of Swiss army knife for biologists. Indeed, its applications are seemingly limitless. In particular, this technology is widely used for the amelioration of plants and crops, whilst the recent announcement of successful treatment of transthyretin amyloidosis in clinical trials suggests that CRISPR–Cas9 gene editing can be deployed directly into the body to treat disease.

It is important for any party wishing to commercialise a technology to identify the relevant patent rights in order to assess their freedom to operate. For CRISPR-Cas9, this is complicated by the sheer number of patent applications filed. If the original CRISPR-Cas9 system was already the subject of

About Regimbeau

REGIMBEAU
Creative IP

REGIMBEAU, a French IP law firm, has been assisting companies and private and public project developers to protect, enhance, and defend their innovations and creations (patents, trademarks, designs) for more than 85 years. Fifteen partners head a team of more than 200 people whose skills are put into practice in every strategic aspect of Intellectual Property – business intelligence and information search, license agreements, IP portfolio audits, partnership negotiations, acquisition of industrial property rights, litigation. A dedicated team of technical and legal experts, with hands-on experience in tackling issues and challenges of innovation in immunology, can assist you in protecting your inventions with your best interest in mind. More info on our specific **webpage**.

of half a dozen competing applications, this number has exploded with the development of the applications of the technology as well as its various improvements. Finding their way in this nebula of patents is thus crucial for all interested parties.

The present article will not examine the intellectual property issues associated with every aspect of every development of CRISPR-Cas9. Rather, the present article aims at exposing the situation and the issues associated with the patent protection of the basic CRISPR-Cas9 technology. As described below, the situation is murky, as several parties hold competing rights over the technology. These parties, including notable academic institutions, are engaged in a string of judicial disputes. Moreover, each of these parties has used distinct licensing strategies.

All of this results in growing incertitude for anyone willing to develop commercial applications of the CRISPR-Cas9 technology.

1. CHRONOLOGY OF PATENTS FILING

As is the case most often than not, breakthrough discoveries do not happen in a vacuum. They are usually preceded and/or accompanied by a string of incremental improvements of earlier technologies. In addition, several groups may arrive at the same crucial results within moments of each other.

Two teams have received the most attention: the University of California (UC) team led by Jennifer Doudna at Berkeley and her colleague Emmanuelle Charpentier; and the team led by Feng Zhang at the Broad Institute (Broad). The Doudna and Charpentier labs showed that CRISPR and Cas9 could be programmed to cut a specific DNA molecule[1]. A few months later, the use of the technology in eucaryotic cells was described by the Zhang lab[2]. Both teams have filed various patent applications covering the very basics of the CRISPR-Cas9 technology.

Although the groups at UC and Broad have received the most attention, other actors should not be ignored. Notably, the lab of Virginijus Šikšnys at the University of Vilnius, demonstrated that the CRISPR-Cas9 system can be programmed to cut DNA at specific sites[3]. Scientists at Toolgen, a South Korean company, and Harvard University showed that the system could be used in human cells[4,5]. Researchers at Sigma-Aldrich, later acquired by the pharmaceutical company Merck KGaA, also deployed CRISPR on human cells.

These "secondary" actors have all filed various patent applications covering several aspects of the core CRISPR-Cas9 technology, thereby creating an interlacing of potential patent rights and clouding even more the situation for other parties.

Patents are granted for inventions which are new, inventive (i.e., non obvious), susceptible of industrial applicability (i.e., useful), and sufficiently disclosed (i.e., enabled). Hence prior disclosures will have serious impact on the patentability of each player's invention.

Figure 1 shows the dates of filing of priority applications, dates of filing of international applications (framed) and publication numbers of said international applications, of the six "earliest" parties in the game. Relevant scientific publications disclosing the technology are also indicated.

As illustrated in Fig. 1, each of the teams respected the rules - each patent application was filed before the publication of the corresponding article. Fig. 1 also illustrates the importance of validly claiming priority when the invention is disclosed (e.g., through the publication of an article in a scientific journal) between the priority date and filing date of the application: if the priority of the international application is found to be not valid, the scientific publication becomes part of the prior art and can destroy the novelty and inventive step of claims. Examples of such issues are described below in relation to the patent wars between all these parties

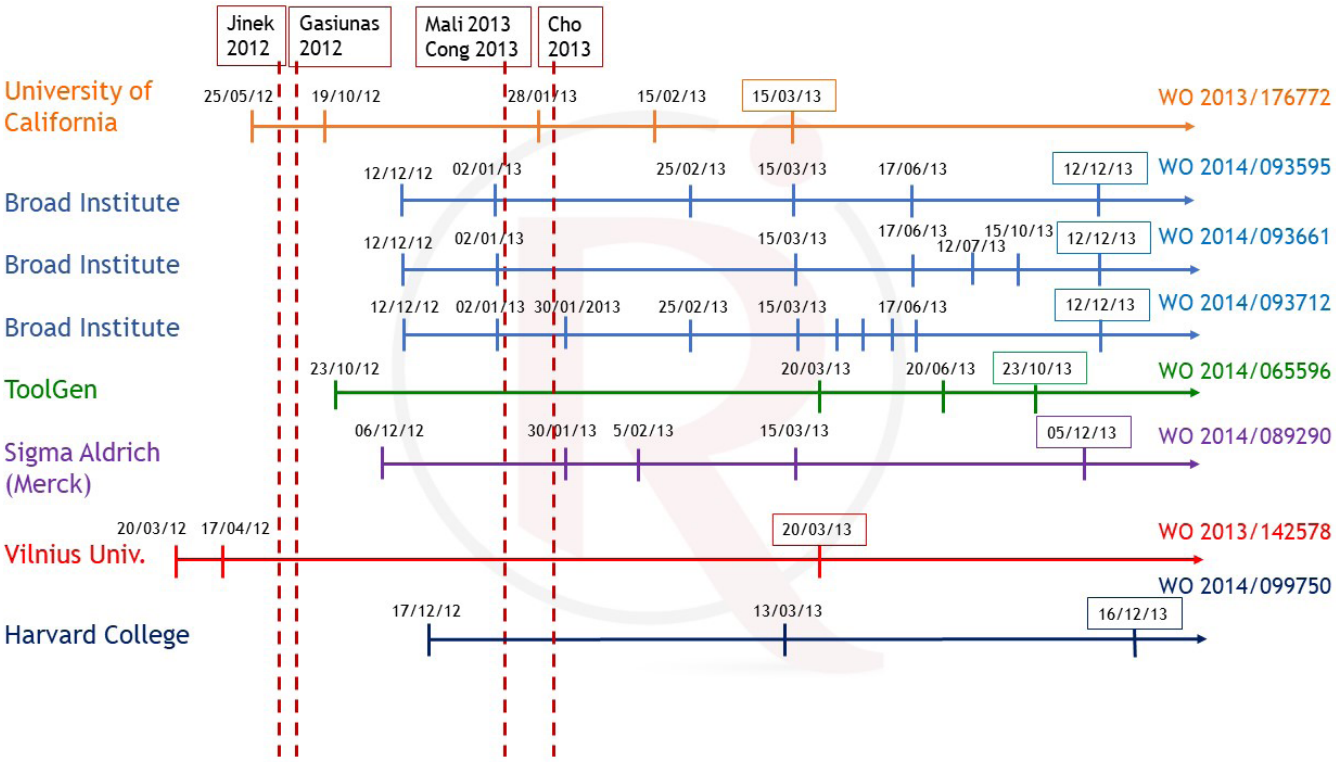


Figure 1: Timeline of patent applications filing and scientific publications

2. THE MAJOR PLAYERS

2.1 University of California (UC) with the University of Vienna and Dr Charpentier

The first player is UC, home of the Doudna lab. UC holds with the University of Vienna, where Emmanuelle Charpentier was based, a number of patents and applications relating to the CRISPR-Cas9 gene modification system in general. Claims relate to a synthetic DNA-targeting RNA and uses thereof to modify genomic DNA. Notably, the claims are not restricted to a specific cell type (i.e., procaryotic vs. eucaryotic). Indeed, the examples presented in the application concern both procaryotic and eucaryotic cells. However, these examples do not explicitly demonstrate that the technology is functional in eucaryotic cells.

Importantly, the first priority application had to be filed before the publication of the Jinek article in order to maintain novelty. UC could therefore only rely on the results obtained up to May 2012 in this priority application. Hence this priority application only contains examples relating to the use of CRISPR-Cas9 in procaryotes. Whether this priority application nonetheless taught how to use CRISPR-Cas9 in all types of cells, eucaryotes included, has become a crucial question in the patent battles which ensued. Different answers were given in the U.S. and in Europe.

2.2 Broad Institute (Broad) with the Massachusetts Institute of Technology (MIT), Harvard College and Rockefeller University

Shortly after the UC's earliest priority application was filed, Broad, the MIT, Harvard College, and Rockefeller University filed a patent application, directed specifically to eucaryotic applications of CRISPR-Cas9.

This application was based on data obtained in the Zhang lab at the Broad Institute (Broad). The principal improvement of Zhang's methods over his predecessors was the use of a nuclear localisation signal and, separately, codon optimisation to natively express Cas9. This first priority application was the basis for numerous applications, notably in the U.S. and in Europe,

all of which were directed to uses of the CRISPR-Cas9 technology in eucaryotic cells.

Whereas UC's applications had gone through prosecution without any particular haste, Broad's attorneys had sought to accelerate proceedings as much as possible, both in the U.S. and in Europe. As a result, they were issued patents whilst UC's applications were still being examined. This is important as there is a presumption of validity of granted patents.

However, the filing of the earliest priority applications has become crucial in the later disputes. Indeed, if the priority of these applications is not validly claimed, then the Cong paper becomes prior art and destroys the novelty of the claims. Once again different answers were given in the U.S. and in Europe.

2.3 ToolGen

Scientists at the South Korean company Toolgen published in March 2013 an article in Nature Biotechnology demonstrating the use of CRISPR-Cas9 technology in eucaryotes[4]. Before the publication of this paper, they had filed one priority application. However, data about the use of CRISPR in eucaryotic cells was not present in this earliest application.

Patents have been granted both in the U.S. and in Europe. They are directed to uses of the CRISPR-Cas9 system to effect site-specific modifications in eucaryotic cells, in particular human cells.

2.4 Sigma Aldrich, merged into Merck KGaA since 2014

Another major player – though often overlooked – is Sigma Aldrich. This company holds a significant patent portfolio relating to the applications of CRSPR-Cas9 in eucaryotic cells. Once again, claims relate to the use of the CRISPR technology in eucaryotic cells. Several priority applications were filed by Sigma Aldrich. The earliest was filed six days before Broad's earliest priority application. However, it is not before the latest priority applications that data supporting this use of CRISPR in eucaryotic cells was provided.

2.5 Vilnius University

Contrary to popular belief, the very first filed patent application regarding a method of site-specific modification of a target DNA molecule with the CRISPR-Cas9 technology is the US provisional application 61/613,373 filed on March 20, 2012 by Vilnius University (Lithuania).

In the U.S., a patent was granted as U.S. Patent No. 9,637,739 with claims directed to CRISPR-Cas9 complexes assembled in vitro and used for site-specific modification of target DNA sequences, in particular ex vivo. In Europe, a patent EP 2828386B1 has been granted with claims regarding in vitro methods only. A divisional patent application EP 3594341A1 is still pending, also related only to in vitro methods.

2.6 Harvard College

Harvard is one the co-applicants of Broad in three patent applications. On the other hand, it is also the sole proprietor of several patents and applications. All of them relate to the work of the lab of George Church, whose team demonstrated the use of CRISPR-Cas9 in human cells[5].

The first priority application in the portfolio was filed five days after the first priority dates of the Broad's applications.

As-filed claims relate to methods of modulating target gene expression comprising using guide RNAs and a nuclease-null Cas9 bearing effector domains, to multiplex activate or repress genes in vivo. Claims also relate to a method of altering a eucaryotic cell, as well as a method for altering human cells.

3. JUDICIAL DISPUTES

3.1 The "war" between the Broad Institute and the University of California

UC and Broad are the key players in the discovery and first uses of CRISPR-Cas9. The two are battling each other to determine which has the right to the claimed invention. The main issue is whether the initial UC's patent discloses the

use of this technology in eucaryotic cells, which would ensure that the University is entitled to this invention.

The main patent battlegrounds have been in the United States and in Europe, with slightly different questions asked in each jurisdiction, but receiving significantly different answers.

Because these patents claim priority earlier than 16 March 2013, the "old" system of first-to-invent applies in the US (it has now been replaced with a "first-to-file" system more akin to the rest of the world). Under this system, a patent applicant could use an interference proceeding to challenge whether another applicant should be granted a patent covering the same subject matter. For there to be no interference, it is only necessary to show that one party's claim would be considered novel and non-obvious (i.e., inventive) over the other party's claim. If this condition is not met, the proceedings would continue to determine which party was first to invent.

The present case pits 10 patent applications of UC against Broad's 13 patents and one patent application.

A first interference between the parties ended in 2018 after an appeal, which concluded that there was no interference. The Federal Circuit (i.e., the U.S. Federal Court of Appeal specialising in patents) found that Broad's invention, directed to CRISPR-Cas9 in eucaryotic cells, would not have been obvious in light of the University of California's invention, which claims the CRISPR-Cas9 generically.

Shortly after, UC filed new claims directed to CRISPR-Cas9 in eucaryotic cells. The scope of these new claims was tailored to be exactly identical to those of Broad which survived the first interference. Clearly UC was not satisfied with the outcome of the first interference. The USPTO examiner had no choice but to declare a new interference.

On 10 September 2020, the Patent and Trial Appeal Board (PTAB) decided key motions in this second interference. This decision addressed several important points for the rest of the proceedings. However, it is only an intermediary decision and the final word in this second interference will not be given until later in 2022 at least.

In their decision of September 2020, the PTAB decided notably that UC was only entitled to its third priority date of 28 January 2013 for this invention (CRISPR-Cas9 with a single guide RNA in eucaryotic cells), after Broad's priority date of 12 December 2012. However, the PTAB also decided that the dispute was only directed to a eucaryotic cell comprising CRISPR-Cas9 with a single guide RNA. This may be important when the Board decides on the interference because Broad's earliest proofs of invention are directed to the use of dual guide RNA.

This mix of outcomes – with Broad receiving an advantage on priority but with the University of California prevailing on the terms of priority contest – leaves both parties with considerable uncertainty. It cannot be excluded that the parties now feel an increasing pressure to settle. However, this looks unlikely, as they had plenty of opportunities to negotiate in all these years

Meanwhile, things have been going differently in Europe.

All of Broad's and UC' patents were individually opposed. Opposition is a mechanism which allows anyone to challenge a European patent in front of the European Patent Office (EPO) within of grant. Whereas in the US, interference aims at determining which Party has the right to the invention, opposition in Europe rather addresses the question of whether an invention is patentable at all. Hence an opposition ends either in the maintenance of the patent, as granted or as amended during the opposition, or in its revocation. A decision of an opposition division can be appealed in front of the EPO's Board of Appeals.

Since the Broad patents were the first granted, they were the first opposed. As is common for CRISPR patent challenges at the EPO, multiple opponents sought revocation of the patents on multiple grounds. In a landmark case[6], Broad's European patent No. EP2771468B1 was revoked for lack of novelty. In fact, the board of appeal concurred with the finding of the opposition division that the Broad patent did not validly

claim the earliest priorities because some of the applicants on these priority applications did not assign their rights to the invention to the Broad Institute and its co-applicants. As a result, the Cong paper - published on 3 January 2013 - became prior art and destroyed the novelty of the claims.

Far from being a technicality as Broad contends, the assignment of the priority applications from the original applicants to the applicants of the PCT application is an essential formal requirement of the European Patent Convention relating to priority. Since most, if not all, of the present Broad patents claim the same priorities, there is a strong chance that all these patents might be revoked for exactly the same reason.

Interestingly, following this earlier revocation of Broad's patent based on a successful priority challenge, UC's European patent No. EP 3241902 was revoked in opposition based on an invalid claim of priority. In this case, the claims were directed to a CRISPR-Cas9 system wherein the Cas9 protein has reduced nuclease activity. The opposition division considered that the earliest priority date of 25 May 2012 was not valid because it did not disclose credibly this invention. It followed that Jinek was prior art and that the claims were not new.

On the other hand, the parent patent EP 2800811, also held by UC, was found to be entitled to its earliest priority date of 25 May 2012. The opposition division considered that the claimed invention in that case (a CRISPR-Cas9 system in a procaryotic or a eucaryotic cell) was credibly enabled by the first priority application. Hence the opposition division of the EPO and the PTAB of the USPTO reached conclusions exactly opposite with regard to the teaching of the priority application of 25 May 2012, thus adding another layer of complexity to the case.

Needless to say, both decisions were appealed. The boards of appeal are not expected to decide on these cases before 2022.

3.2 The remaining parties

The conflict between Broad and UC has featured prominently in the media as the *ur*-CRISPR-patent battle, since it involves several universities and two Nobel prize winners, and has now dragged on for several years. However, this is an oversimplification, as new characters now enter the judicial scene.

3.2.1 Toolgen

For example, the South Korean company Toolgen is now facing two interference challenges of its application in the U.S., one against 14 patents and two patent applications of Broad, the other against 14 patent applications of UC. In Europe, Toolgen's corresponding patent has been opposed by multiple parties (as seems to be the case in all CRISPR-Cas9-related oppositions), resulting in revocation of the patent. Appeal is under way.

3.2.2 Sigma-Aldrich

Sigma-Aldrich petitioned the USPTO for having an interference declared between three of their U.S. applications and the same 10 UC's U.S. patent applications which were already involved in the interferences with Broad. Once again, what is at stake here is to determine the Party which was the first to invent the CRISPR-Cas9 technology. However, the PTAB has refused to consider Sigma-Aldrich's petition, dismissing it as "premature". This does not mean, though, that no interference will be filed later when Sigma-Aldrich patents issue.

In Europe, all six granted patents have been opposed by multiple parties. Two of them, EP 3138910B1 and EP 3138911B1, were revoked for lack of inventive step. It appears that the priority claims of these patents were considered valid, a welcome change from the earlier CRISPR-Cas9 decisions. This was not the case for EP 3138912B1 whose first auxiliary request was deemed not enjoy a valid priority and which was thus revoked. Note that the main request had been rejected on a very formal but very important basis, i.e., added subject-matter.

The decision against EP 3138910B1 was appealed by the proprietor. The two other decisions were rendered on 9 and 12 November

2021. An appeal can be formed against each of them within two months of the issuance of the written decision. In other words, Sigma-Aldrich has at least until 9 and 12 January 2022 to decide on this matter. However, it can reasonably be predicted that the other two decisions will also be appealed.

The remaining three patents are currently facing multiple oppositions.

3.2.3 Vilnius University

Surprisingly in view of all the CRISPR-related activity at the PTAB, the Vilnius US patent has not been involved in any interference. This may due to the fact that the claims are limited to CRISPR-Cas9 complexes assembled in vitro whereas the other relevant applications and patents claim CRISPR-Cas9 complexes for in vivo uses.

On the other hand, the European patent EP 2828386B1 was opposed by three opponents in April 2020. The proceedings are under way.

3.2.4 Harvard College

Applications and patents held by Harvard do not seem, to the best of our knowledge, to be involved in any interference proceedings. This may be explained by the fact that these patents appear to be directed to specific embodiments of CRISPR-Cas9 rather than to the most general technology.

In contrast, Harvard's European patent has been opposed by 4 opponents. Oral proceedings will be held on 22 & 23 March 2022.

4. THE CRISPR LICENSING LANDSCAPE

The grant of large number of CRISR-Cas9 patents with overlapping scopes has created a landscape that is difficult to navigate for would-be licensees.

For researchers and interested parties, the situation detailed above creates thorny issues around where to obtain the rights to use the CRISPR-Cas9 technique. In order to commercialise new CRISPR-Cas9 technologies and applications, interested parties will need to obtain commercial licences to the basic CRISPR-Cas9 patents. Notably, users of CRISPR technology need to obtain patent licences from UC, Broad, and others as the price of admission for operating in the space.

However, the continuing conflict between UC and Broad affects the evaluation any interested user must do. As of today, UC seems to have won the first round in Europe. On the other hand, in the U.S., the situation is a lot murkier. UC has claims to the use of CRISPR-Cas9 without further specification, whereas Broad has claims covering CRISPR technology in eukaryotes. It is therefore unclear whose patents a license is needed. For example, CRISPR-Cas9 users must decide whether they want to obtain a licence for patents which may later be declared invalid in one or more of the most important commercial markets. On the other hand, waiting before making a decision may expose them to a steep price hike if the patent is maintained in the US or in Europe by the competent legal authorities.

One way to facilitate easier access to technology created by multiple groups is to create a patent pool from which multi-party licenses can be obtained. A patent pool forms when multiple patentees combine their patents and use a single entity to license all the combined patents to third-parties as a single, non-exclusive licensing package.

In 2017, MPEG LA attempted to create a patent pool for a worldwide CRISPR licensing standard. Such patent pool would create a one-stop shop for commercial users to license CRISPR patents, without needing to navigate a complex patent and licensing landscape. The Broad Institute expressed interest in working with MPEG LA and other CRISPR patent holders to streamline non-exclusive access to the genome editing technology (except for human therapeutics applications). More recently, in July of 2019, Broad announced a joint CRISPR licensing framework with MilliporeSigma to "encourage innovation." With the intention of streamlining access for scientists, this licensing agreement includes patent rights from multiple key parties including: Broad, Millipore Sigma

(under the Sigma-Aldrich portfolio), Harvard University, MIT, New York Genome Center, The Rockefeller Center, and more. It is unclear how this new licensing venture will affect Broad's participation in MPEG LA.

On the other hand, up to now, University of California has not given any sign that they would be up to join any of the initiatives. Furthermore, these patent pools specifically exclude the possibility to request IP rights for human therapeutic and diagnosis applications and for agricultural uses.

The task is complicated by the fact that licences must be obtained from different sources. The owners of the core patent applications have granted their rights exclusively to marketing companies, with the mandate to grant exclusive or non-exclusive licences to private companies willing to invest in developing applications using CRISPR-Cas9. For example, for the development of human therapies, rights must be obtained from CRISPR Therapeutics, Intellia Therapeutics and Editas Medicine. CRISPR Therapeutics obtained its exclusive rights from Emmanuelle Charpentier, Intellia Therapeutics from UC and the University of Vienna, and Editas Medicine from the Broad Institute. For all other areas, the companies holding the relevant rights are ERS Genomics, Caribou Biosciences and the Broad Institute. ERS Genomics obtained its exclusive rights from Emmanuelle Charpentier, Caribou Biosciences from UC and the University of Vienna, while the Broad Institute licenses CRISPR IP non-exclusively for commercial research or to companies wishing to sell tools and reagents for genome editing.

To this day, no entity has been granted licenses for all CRISPR-Cas9 IP rights, whether held by one research group or the other. While the academics doing fundamental research with CRISPR Cas9 might pass over these IP questions, since they are usually exempt from the patent infringement regime under national laws, any commercial entity willing to obtain rights for using the technology will have to wait the end of legal controversies, or the creation of a patent pool.

5. CONCLUSION

The uncertainty about the CRISPR-Cas9 patent landscape presents a barrier to innovation.

One notable aspect of the CRISPR patent dispute is that it involves several large academic institutions. It has been rare for universities to sue one another over patents. This may dampen any spirit of scientific collaboration or even interaction between these institutions. As long as the legal battle is on, interested parties will thus not know for sure which patent owner they should contact for obtaining IP rights, neither how many licenses they would need. Unfortunately, the battle shows no sign of abating, suggesting that it is about something more than money[7].

REFERENCES

[1] Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012 Aug 17;337(6096):816-821

[2] Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013 Feb 15;339(6121):819-823.

[3] Gasiunas G, Barrangou R, Horvath P, Siksnys V. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci U S A. 2012 Sep 25;109(39):E2579-2586.

[4] Cho SW, Kim S, Kim JM, Kim JS. Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. Nat Biotechnol. 2013 Mar;31(3):230-232.

[5] Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM. RNA-guided human genome engineering via Cas9. Science. 2013 Feb 15;339(6121):823-826.

[6] T 844/18

[7] Jacob S Sherkow, Patent protection for CRISPR: an ELSI review, Journal of Law and the Biosciences, Volume 4, Issue 3, December 2017, Pages 565–576

About the authors

Lucile Vernoux

Lucile VERNOUX is a French and European Patent Attorney. She has a Ph.D. in Endocrinology and Cellular Interactions (University of Paris XI) and is a Graduate from the CEIPI Patents General Course. She started her Industrial Property career in 2001. After an initial experience in a biopharmaceutical start-up, she spent two years in the United States working in the Technology Transfer Office at Duke University. Lucile spent eight years working at REGIMBEAU, followed by 3.5 years in another renowned Industrial Property Law firm, before rejoining **REGIMBEAU** in 2017.

Lucile VERNOUX (vernoux@regimbeau.eu)

Nicolas Bouquin

Nicolas BOUQUIN is a French and European Patent Attorney. He has a Ph.D. in Genetics and Physiology of Microorganisms (University of Paris XI) and is a Graduate from both the CEIPI Patents General Course and the CEIPI Industrial Property Law LLM. He has also completed the course on Patent Litigation in Europe organized by the CEIPI. Nicolas has worked for several years as a scientist, both in France and abroad, in the academics and for a pharmaceutical company, before moving to IP law. After a few years as an in-house patent attorney in a big pharma, Nicolas joined **REGIMBEAU** in 2009. He assists his clients in protecting and defending their innovations in all aspects of biotechnologies, with a special focus on pharmaceuticals and antibodies.

Nicolas BOUQUIN (bouquin@regimbeau.eu)

REGIMBEAU
Creative IP

www.regimbeau.eu
info@regimbeau.eu
Twitter : @REGIMBEAU_IP
LinkedIN : REGIMBEAU

UPCOMING MABDESIGN EVENTS

Join us at our next scientific and networking events in 2022

10TH AIS 2022 Antibody Industrial Symposium

JUNE 28-29, 2022
MONTPELLIER, FRANCE

MAbimprove LabEx Tours Montpellier

MABDESIGN THE IMMUNOTHERAPY NETWORK

https://aiscongress.com/

About MabDesign

MabDesign, the French biotherapy industry association, aims to structure the biopharmaceutical industry in France from its R&D phases to biomanufacturing and marketing. Its objective is also to promote the creation of innovative start-ups resulting from academic research, to increase the visibility of the biopharmaceutical industry, to promote exchanges, to support the development and competitiveness of companies, and to stimulate innovation. Created in 2014 MABDESIGN is administered by ABL Europe, Biomérieux, DBV Technologies, Institut Pasteur, Lyonbiopôle, Pierre Fabre, Sanofi, Thermo Fischer, TreeFrog therapeutics and 3 independent field experts.

MABDESIGN OFFERS

STRUCTURE THE INDUSTRIAL SECTOR ...

DIRECTORY | COMMUNICATION | COSSF | EVENT

... to increase its **readability** and international **visibility**.

SUPPORTING THE INDUSTRIAL SECTOR...

CONTINUOUS TRAINING | INNOVATION FINANCING | MARKET STUDY | TECHNOLOGY SURVEY & IP | BUSINESS DEVELOPMENT

... to enhance its **value offer** and **its competitiveness**.

Operational since September 2015, MabDesign currently has more than 210 member companies, including pharmaceutical and biotech companies, service providers, training organizations, high-tech equipment suppliers and specialized consultants.

Next on Immunowatch

- **6th Update of COVID-19 Special Edition**
- **Cell Therapy (1st Semester)**

MABDESIGN
THE IMMUNOTHERAPY NETWORK

Follow us on
@Mabdesign_fr
MabDesign

Bât. L'Initial
17 , rue Crépet
69007 Lyon
Tél. 04 78 02 39 88
contact@mabdesign.fr
www.mabdesign.fr