UNIVERSIDADE DE LISBOA

Faculdade de Farmácia

ADVANCED THERAPY MEDICINAL PRODUCTS: NEW STRATEGIES FOR CLINICAL APPLICATIONS OF CELL AND GENE THERAPY

Marta Alexandra Bogalho Rodrigues de Carvalho

Orientadores: Prof. Doutora Ana Paula Mecheiro de Almeida Martins Silvestre Correia
Prof. Doutor Bruno Miguel Nogueira Sepodes

Tese especialmente elaborada para a obtenção do grau de Doutora em Farmácia, na especialidade de Farmacologia e Farmacoterapia

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DECLARATION

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The thesis was supervised by Professor Ana Paula Mecheiro de Almeida Martins Silvestre Correia, Assistant Professor at the Faculty of Pharmacy, University of Lisbon, Portugal, and Principal Investigator of the Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL) and Instituto de Saúde Baseada na Evidência (ISBE).

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Marta Carvalho participated in the design and conduct of all studies as well as in the analysis, interpretation of data, and preparation of all the manuscripts as part of the present dissertation. Full acknowledgements were made where the work of others has been cited or used.

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We used to think that our fate was in our stars,
but now we know that, in large measure,
our fate is in our genes.

James Watson, 1989

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Contents

PUBLISHED ARTICLES

Carvalho, M., Sepodes, B. and Martins, A., (2017). Regulatory and Scientific Advancements in Gene Therapy: State-of-the-Art of Clinical Applications and of the Supporting European Regulatory Framework. *Frontiers in Medicine*, 4. doi:10.3389/fmed.2017.00182

Carvalho, M., Martins, A. and Sepodes, B., (2019). Hurdles in gene therapy regulatory approval: a retrospective analysis of European Marketing Authorization Applications. *Drug Discovery Today*, 24(3), pp.823-828. doi:10.1016/j.drudis.2018.12.007

Carvalho, M., Sepodes, B. and Martins, A., (2020). Patient access to Gene Therapy Medicinal Products: a comprehensive review. *BMJ Innovation*, Epub ahead of print: [28Nov2020]. doi:10.1136/ bmjinnov-2020-000425

RESUMO

Os medicamentos de terapia avançada têm um enorme potencial para dar resposta a doenças onde existem necessidades médicas não satisfeitas. Especificamente, os medicamentos para terapia genética poderão representar a cura para diversas doenças genéticas. Apesar de muita investigação realizada nesta área, existe apenas um número modesto de produtos com Autorização de Introdução no Mercado (AIM). Esta tese foca-se nos medicamentos de terapia avançada e pretende identificar e compreender os obstáculos regulamentares e de acesso ao doente, no que diz respeito à utilização de terapia genética.

No **Capítulo 1**, é explorada a investigação realizada nesta área nas últimas décadas, bem como diferentes aplicações clínicas investigadas globalmente. Estes medicamentos experimentais baseiam-se em diversas estratégias que variam desde a substituição ou adição direta de genes até edição de genes específicos ou RNA *targeting*. Riscos de segurança importantes, eficácia limitada, obstáculos associados à produção destes medicamentos ou conflitos éticos podem representar desafios no sucesso de um potencial candidato a terapia genética. Durante o programa de desenvolvimento, é fundamental ter em consideração esses aspectos e estabelecer estratégias que permitam ultrapassar estas barreiras.

Em seguida, o atual quadro jurídico Europeu dos medicamentos de terapia avançada é revisto, dando uma visão geral do processo para pedidos de AIM em produtos de terapia genética. Na Europa, o regulamento dos medicamentos de terapia avançada foi totalmente implementado em 2009 e, nessa data, foi criado o Comité de Terapias Avançadas (CAT) como um grupo dedicado de especialistas para avaliar estes medicamentos que requerem conhecimentos específicos nessa área.

No **Capítulo 2,** foram identificadas as principais objeções, questões ou preocupações levantadas durante o pedido de AIM para terapias genéticas, entre 2009 e 2017. Durante os primeiros anos após o estabelecimento do CAT, os problemas de qualidade foram frequentemente identificados como deficiências importantes, enquanto questões no nível não clínico pareciam ser menos frequentes. Os aspectos clínicos de eficácia e segurança pareciam ter um papel muito

significativo nos pedidos de AIM com resultado negativo. A maioria das deficiências foi resolvida através de esclarecimentos prestados pelo requerente durante o pedido de AIM ou nos requisitos de pós-comercialização. O procedimento de obtenção de AIM para terapia genética é complexo e prevê-se que quanto maior for o número de novos produtos que obtenham AIM, maior será a experiência acumulada por parte do Regulador e dos Promotores, reduzindo assim a taxa de atrito para aprovação.

Apesar de obterem AIM, isso não significa necessariamente que estes produtos estejam a ser utilizados na prática clínica. No **Capítulo 3**, um conjunto abrangente de obstáculos que potencialmente impedem o acesso ao doente de terapias genéticas é identificado com base na literatura mais recentemente disponível. Foi realizada uma síntese da evidência mais atual disponível, através de uma abordagem sistemática, utilizando duas bases de dados, que incluiu publicações entre 2012 e 2018. Foram identificados sete tópicos principais como possíveis obstáculos de acesso ao doente, nomeadamente acessibilidade, avaliação de valor, desenvolvimento de terapia, fatores éticos / sociais, geração de evidência, implementação operacional e obstáculos regulamentares. Desses, vinte e cinco sub-temas adicionais foram identificados. O obstáculo mais frequentemente mencionado na literatura está relacionado com o aspecto da acessibilidade, principalmente no elevado custo da terapia (84%) e no seu financiamento sobretudo por via do co-pagamento de um terceiro pagador (51%). É importante salientar que a geração de evidência associada a resultados limitados dos ensaios clínicos (81%) parece ser um forte obstáculo no acesso dos doentes a essas terapias.

No **Capítulo 4**, é apresentada uma discussão global sobre os resultados obtidos nos capítulos 2 e 3. Estes são explorados no contexto do atual corpo de evidência, bem como no panorama atual de terapias genéticas aprovadas.

Espera-se que um número crescente de terapias genéticas seja desenvolvido e disponibilizado aos doentes e profissionais de saúde. Esta tese contribuiu para a compreensão de todos os obstáculos, de forma abrangente e integrada, para que estratégias sejam estabelecidas em tempo útil, garantindo que os benefícios da terapia genética alcancem os doentes e a sociedade.

Palavras-Chave: Medicamentos de Terapia Avançada, Terapia Genética, Pedido de Autorização de Introdução no Mercado, Acesso ao Doente, Acessibilidade

ABSTRACT

Advanced therapy medicinal products (ATMPs) have a massive potential to address existing unmet medical needs. Specifically, gene therapy medicinal products (GTMPs) may potentially provide cure for several genetic diseases. Despite much research conducted in this field, only a modest number of products are approved and available. This thesis intends to develop an end-to-end understanding of ATMPs, identifying regulatory and patient access hurdles on gene therapy use

In **Chapter 1**, broad research conducted in this field over the last few decades is explored as well as different clinical applications investigated worldwide. These are based on diverse strategies that range from direct gene replacement or addition to more complex pathways such as specific gene editing or RNA targeting. Important safety risks, limited efficacy, manufacturing hurdles, or ethical conflicts may represent challenges in the success of a candidate GTMP. During the development process, it is fundamental to take such aspects into account and establish overcoming strategies.

Then, the current European legal framework of ATMPs is reviewed and an overview of the clinical applications for approved and investigational GTMPs is provided. In Europe, the ATMP regulation was fully implemented in 2009 and, at this point, the Committee for Advanced Therapies was created as a dedicated group of specialists to evaluate medicinal products requiring specific expertise in this area.

In **Chapter 2**, major objections, issues, or concerns raised during the Marketing Authorization Application (MAA) for GTMPs between 2009 and 2017 were identified. During the first few years following CAT establishment, quality issues were often identified as major deficiencies, whereas issues at the nonclinical level appeared to be less frequent. Clinical efficacy and safety issues appeared to have a major role in unsuccessful MAA outcome for GTMPs. Most deficiencies were addressed through clarification during the MAA review or in post-marketing settings. The MAA procedure for GTMPs is complex and it is anticipated that continuous MAA submissions will further enhance the experience of both regulators and applicants, reducing the attrition rate for approval.

Despite having a positive Marketing Authorization, this does not mean that these products are being used in clinical practice. In **Chapter 3**, a full set of hurdles potentially preventing patient access to Gene Therapies is identified based on the most recently available literature. A review of the literature using a systematic approach in two distinct databases was performed by identifying relevant, peer-reviewed publications, between 2012 and 2018. Seven major topics were identified as potential patient access hurdles, namely affordability, assessment of value, development of therapy, ethical/social factors, evidence generation, operational implementation and regulatory hurdles. From these, twenty-five additional sub-themes were further identified. The most frequently mentioned obstacle in the literature is related to the affordability aspect especially focusing on high cost of therapy (84%) and therapy payment/reimbursement (51%). Importantly, the evidence generation focusing on limited trial outcomes (81%) seems to be a strong obstacle in patient access to these therapies.

In **Chapter 4**, a global discussion on the results obtained in chapter 2 and 3 is presented and summarized in the context of the current body of evidence, as well as the current GTMP landscape.

A growing number of Gene Therapies are expected to be developed and made available to patients and health care professionals. This thesis contributed to understanding all hurdles, in a complete and integrated fashion, so that strategies are timely established to ensure gene therapy's benefits are provided to patients and to the society.

Key words: Advanced Therapy Medicinal Products, Gene Therapy, Marketing Authorization Application, Patient Access, Affordability

ACRONYMS

AAV: Adeno-Associated Virus

ADA: Adenosine Deaminase

ADA-SCID: Adenosine Deaminase Severe Combined Immunodeficiency

ALL: Acute Lymphoblastic Lymphoma

ART: Anti-Retroviral Treatment

ATMP: Advanced Therapy Medicinal Product

B-ALL: B acute lymphoblastic leukemia

BLA: Biologics License Application

CARs: Chimeric Antigen Receptors

CAR-T: Chimeric Antigen Receptor T-Cells

CAT: Committee for Advanced Therapies

CBER: Center for Biologics Evaluation and Research

CCR5: Chemokine Receptor 5

CF: Cystic Fibrosis

CFTR: Cystic Fibrosis Transmembrane Conductance Regulator

CHMP: Committee for Medicinal Products for Human Use

CID: Combined Immunodeficiencies

CLG: Contusugene Ladenovec

CNS: Central Nervous System

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeat

CRS: Citokine Release Syndrome

CS: Clock stop

CTL: Cytotoxic T Lymphocyte

D120: Day 120

D180: Day 180

DA: Duration of MAA assessment

DD: Duration of MAA decision

DFS: Disease Free Survival

DLBCL: Diffuse Large B-cell Lymphoma

DNA: Deoxyribonucleic acid

EC: European Commission

EMA: European Medicines Agency

EPAR: European Public Assessment Report

EU: Europe

FDA: Food and Drug Administration

FIX: Human Clotting Factor IX

G-BA: Federal Joint Committee

GCP: Good Clinical Practices

GM-CSF: Granulocyte Macrophage Colony-Stimulating Factor

GMP: Good Manufacturing Practices

GT: Gene Therapy

GTMP: Gene Therapy Medicinal Product

GvHD: Graft versus Host Disease

HCM: Hypertrophic Cardiomyopathy

HE: Hospital Exemption

HF: Heart Failure

HIV: Human Immunodeficiency Virus

HLA: Human Leukocyte Antigen

HN: hemaglutinin-neuraminidase

HSC: Hematopoietic Stem Cells

HSCT: Hematopoietic Stem Cells Transplantation

HSV: Herpes Simplex Virus

HTA: Health Technology Assessment

ICER: incremental cost-effectiveness ratio

ICH: International Council for Harmonisation

IIT: Innovative Task Force

IM: Insertional Mutagenesis

IMP: Investigational Medicinal Product

IND: Investigational New Drug

INN: International Nonproprietary Name

iPSC: induced Pluripotent Stem Cells

IQWiG: Institut für Qualität und Wirtschaftlichkeit im Gesundheitswesen

IRB: Independent Review Board

LIM: Lin-11 Isl-1 Mec-3

LMO2: LIM domain only-2

LoOI: List of Outstanding Issues

LoQ: List of Questions

LPL: Lipoprotein Lipase

LPLD: Lipoprotein Lipase Deficiency

LTR: Long Terminal Repeat

MAA: Marketing Authorization Application

MACI: Matrix-applied characterised autologous cultured chondrocytes

MSC: Multipotent Stromal Cells

Nab: Neutralizing Antibodies

NICE: National Institute for Health and Care Excellence

NSPCs: Neural Stem/Progenitor Cells

OCTGT: Office of Cellular, Tissue and Gene Therapy

OE: Oral Explanation

OS: Overall Survival

OTC: Ornithine Transcarbamylase

OTCD: Ornithine transcarbamylase deficiency

PD: Pharmacodynamics

PDCD-1: Programmed Cell Death Protein 1

PEG: Polyethylene Glycol

pEP: primary endpoint

PFS: Progression Free Survival

PK: Pharmacokinetics

ppCM: post-prandial Chylomicron

PRIME: PRIority MEdicines scheme

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Q&A: Questions and Answers

QP: Qualified Person

RBA: Risk Based Approach

RCA: Replication Competent AdenoVirus

RMP: Risk Management Plan

RNA: Ribonucleic acid

RNAi: interference RNA

SA/PA: Scientific Advice / Protocol Assistance
SCID: Severe Combined Immunodeficiency
sCTMP: Somatic Cell Therapy Medicinal Product
SERCA2a: Sarcoplasmic Reticulum Ca-ATPase
shRNA: small hairpin RNA
siRNA: small interference RNA
SMC: Scottish Medicines Consortium
SME: Small and Medium Enterprise
TALEN: Transcription Activator-like Effector Nuclease
TCR: T-Cell Receptor
TDT: Transfusion-Dependent β-thalassaemia
TEP: Tissue Engineered Product
tracrRNA: trans activating crRNA
US: United States
WAS: Wiskott-Aldrich Syndrome
WASP: Wiskott-Aldrich Syndrome Protein
WPAR: Withdrawal Assessment Report
ZFN: Zinc Finger Nucleases

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Chapter 1

General introduction

1.1 Clinical Applications of Gene Therapy

Advanced Therapy Medicinal Products (ATMPs) represent a major class of innovative therapies that differ substantially from traditional therapeutic agents. ATMPs include gene therapy medicinal products (GTMPs), somatic cell therapy medicinal products (sCTMPs) and tissue-engineered products (TEPs). Both sCTMP and TEP are often referred to as cell-based medicinal products, as per **Figure 1**(1).

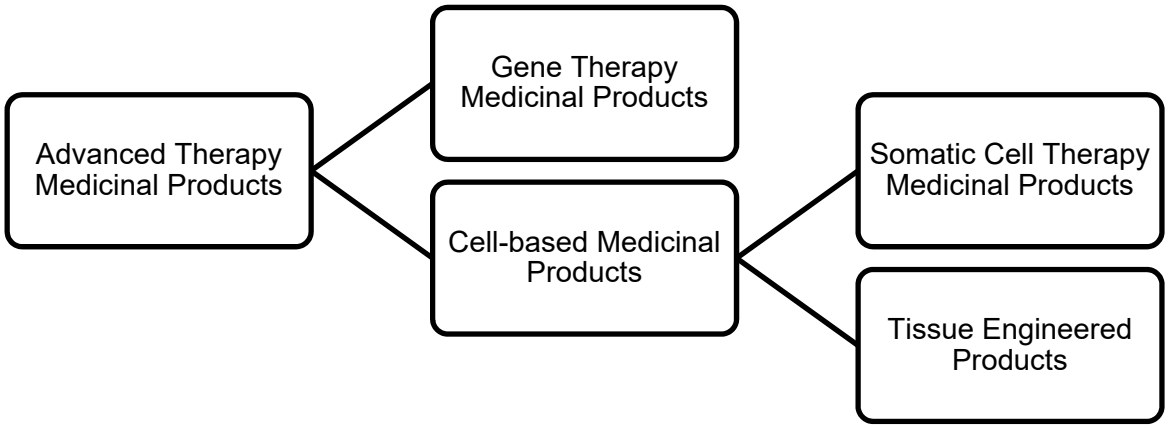


Figure 1 – ATMP types

Extensive research is being conducted to study ATMPs as they have the potential to address highly unmet medical needs. In a study by Hanna, *et al*., between 1999 and 2015, there were almost 1000 clinical trials worldwide investigating ATMPs, mainly in cancer and cardiovascular diseases. More than half of these trials studied sCTMPs, while the other half was equally split between GTMPs and TEPs (2). Data from a European survey is aligned, highlighting that between 2009 and 2015, around 500 new trials were submitted. Here, the proportion of clinical trials studying TEPs was higher (45%), followed by sCTMPs (30%) and GTMPs (25%)(3).

Therapeutic products based on the use of genes to prevent or treat diseases are not a new concept and were hypothesized as medicinal products since the discovery of recombinant DNA technology. A high number of diseases have underlying genetic causes, ranging from defects in a single gene (e.g. haemophilia) to more complex disorders affecting multiple genes (e.g. cancer)(4). Human gene

therapy is based on the simple principle that if a disease is caused by a defective gene, then curing such illness would be as simple as replacing the faulty genetic sequence with a functional copy. Gene therapy consists of using recombinant nucleic acids as the active pharmaceutical ingredient (API), where the effect is directly related to either the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence (5–8).

1.1.1 First steps in gene therapy

The first direct human gene therapy trial took place in 1974. In this study, the wild-type Shope papilloma virus was administered intravenously to two female patients suffering from hyperargininemia, a urea cycle disorder, with the intention of introducing the gene for arginase. It was believed that the Shope papilloma virus encoded the gene for arginase activity and that the gene could be transferred by administering the virus to the patients. Unfortunately, the trial was unsuccessful and there was neither a change in the arginine levels, nor in the clinical course of the hyperargininemias (9,10).

Michael Blaese was the first investigator to conduct a trial using a therapeutic gene (11). In 1990 the FDA approved, for the first time, a gene therapy trial with therapeutic attempt in humans. Two adenosine deaminase deficiency (ADA-SCID) paediatric patients were administered with autologous *ex vivo* modified white blood cells. ADA-SCID is a monogenetic disease leading to severe immunodeficiency where lymphocyte counts are virtually absent. The clinical manifestations of this disease go beyond the immune system, and may include deafness, behavioural problems, costochondral abnormalities and hepatotoxicity (12,13). The cells were modified to express the normal adenosine deaminase gene. Although the treatment was shown to be safe, its efficacy was not fully demonstrated as the patients still required maintenance treatment with enzyme replacement therapy using polyethylene glycol adenine deaminase (PEG-ADA), and the ADA transduced stem cells were unable to reconstitute the recipient's immune system. Later on, an ADA-SCID trial was also conducted in Europe (14) and further gene transfer trials were started for several diseases.

No major safety concerns were raised until the unfortunate death of a patient in a gene therapy trial, in 1999, for partial deficiency of ornithine transcarbamylase

(OTC). This event took place in the University of Pennsylvania, in Philadelphia. The patient was administered with a very high dose of an adenovirus carrying the missing gene. His immune system responded immediately and after just a few days the patient died as a result of multiorgan failure (15,16).

The first country to approve a gene therapy based product for clinical use was China, in 2003 (Gendicine™). This treatment was based on an adenoviral gene delivery system that was capable of inserting the p53 gene into tumor cells, thereby stimulating cell death. Gendicide™ was approved for the treatment of head- and neck squamous cell carcinoma (6). In Europe, Regulation (EC) No. 1394/2007, also known as the ”ATMP Regulation”, was put in place, but was only effective a couple of years later. In June 2009, ChondroCelect® was the first product with a positive opinion by the Committee for Advanced Therapies (CAT) in relation to an initial marketing authorization also supported by the Committee for Medicinal Products for Human Use (CHMP). This cell-based medicinal product was comprised of characterized viable autologous cartilage-forming cells expanded *in vivo*, expressing specific marker proteins, intended for the repair of single symptomatic cartilage defects of the femoral condyle of the knee, in adult patients (17).

In the meantime, in 2008, Cerepro®® became the first gene therapy to be assessed by the CAT/CHMP, in Europe. It was an adenoviral vector based therapy, which completed a phase III clinical trial (18). The treatment consisted in administering the herpes simplex virus gene for thymidine kinase (TK) encased in a non-replicating adenovirus vector, followed by administration of ganciclovir, in patients with operable, high-grade malignant glioma. Transduced cells express TK which phosphorylates ganciclovir that is further phosphorylated by several cellular kinases. The final product is ganciclovir triphosphate which is incorporated into DNA of dividing cells, as opposed to deoxyguanosine triphosphate, causing chain termination and apoptosis(19,20). A Marketing Authorization Application (MAA) was submitted but the CHMP adopted a negative opinion in December 2009, and the Sponsor requested re-examination. During this period, in early 2010, the applicant requested withdrawal of the application based on the inability to demonstrate the Committee that its main study provided clear evidence of a clinically meaningful benefit in relation to risk (21).

Finally, in July 2012, the EMA recommended for the first time a gene therapy product (Glybera®, alipogene tiparvovec) for approval in the European Union. Glybera® is based on an adeno-associated viral (AAV) vector and gained approval for the treatment of a genetically inherited metabolic disorder related to the gene encoding the lipoprotein lipase (LPL). Lipoprotein lipase is a key enzyme in the metabolism of lipoproteins following fat intake with diet. The lack of functional LPL results in severe hypertriglyceridemia, episodes of abdominal pain, acute pancreatitis and eruptive cutaneous xanthomatosis (22).

Glybera® paved the way for the approval of other gene therapy products in Europe. Since then, and until the end of 2019, six additional GTMPs have been granted marketing authorization, namely Imlygic® (Talimogene laherparepvec)(23), Strimvelis® (Autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence)(24), Kymriah® (Tisagenlecleucel)(25), Yescarta® (Axicabtagene ciloleucel)(25), Luxturna™ (Voretigene neparvovec)(26) and Zynteglo® (Betibeglogene autotemcel)(27).

1.1.2 Gene delivery vectors

Over the years, one of the most significant challenges of gene therapy has been the effective and safe delivery to its target. In light of the multiple extra and intracellular barriers gene delivery strategies came into picture, specifically through vehicles also known as vectors (4,28).

The ideal gene delivery system should have:

- high gene transfer efficiency,
- low toxicity to the cells,
- single cell specificity to the intended target and
- the ability to simultaneously treat heterogeneous systems with many different cells (8).

Generally, gene delivery methods are divided in two separate categories, based on whether they comprise of non-viral vectors or viral vector. Furthermore, current non-viral gene delivery methods may be grouped into two different types: physical or chemical. Physical gene delivery strategies use a wide variety of physical methods such as microinjection, needle injection, jet injection, gene gun / DNA injection /

DNA-coated particle bombardment, electroporation, sonoporation, hydrodynamic gene transfer and mechanical massage. On the other hand, examples of chemical gene delivery methods include calcium phosphate precipitation, cationic lipids (lipossomes), cationic polymers and lipopolyplexes (5,7,8).

When considering non-viral vectors, a number of advantages should be taken into consideration, such as easy scale-up production, ability to carry large molecular size genes and lack of viral component, i.e. low immunogenicity. On the other hand, the high vulnerability to intra- and extracellular degradation, with subsequent low cellular uptake is a major drawback as well as the low transgene expression, i.e. low efficacy(7).

Viral vectors are based on removing the pathogenicity of specific virus in order to use them as carriers of the therapeutic genetic content. Some of the most frequently used viral vector families include Adenovirus (AdV), Adeno-associated virus (AAV), Herpes simplex virus (HSV) and Retrovirus (such as gamaretrovirus and lentivirus). A summary of the main differences among viral vectors is presented in **Table 1** (29–32).

Table 1 – Viral vectors overview

Viral vector family	Immunogenicity	Genomic integration	Transgene expression	Packed genome size	ATMP examples (commercial name)
Adenovirus (AdV)	High	Non-integrating	Transient	Intermediate	Advexin®, Cerepro®
Adeno associated virus (AAV)	Low	Non-integrating	Potentially long lasting	Low	Glybera®, Luxturna™
Herpes simplex virus (HSV)	High	Non-integrating	Potentially long lasting	Intermediate	Imlygic®
Retrovirus (gama-retrovirus and lentivirus)	Low	Integrating	Long lasting	High	Strimvelis®, Kymriah®, Yescarta®, Zynteglo®

Advantages of viral vectors include the high cellular uptake, the high transduction efficacy and long-term gene expression. In contrast, safety concerns including immunogenicity are considered major drawbacks. Choosing a vector with low immunogenicity such as AAV as opposed to AdV reduces the risk of severe unwanted immunologic responses. On the other hand, integrating vectors such as

those based on lentivirus will pose a higher risk for oncogenicity, compared to, for instance, AAV. Additionally, poor target cell specificity may be a concern. For instance, recombinant AAV's tropism is largely dependent on the capsid. Capsids may be covered by signalling peptides or "shuffled" (pseudotyped) to generate new capsids (33). Finally, inability to transfer high molecular weight genes and high production costs represent significant disadvantages when considering these types of vectors to incorporate potential ATMPs (7).

1.1.2.1 Gene therapy strategies: from in vivo modification to ex vivo gene transfer

Essentially, gene therapy may be performed by one of two approaches. *In vivo* gene therapy consists of directly administering the vector carrying the therapeutic gene into the target tissue. It involves administration of the vector directly in the patient and genetic modification occurs in the host. Alternatively, *ex vivo* gene therapy is typically used in diseases where a specific type of cell is affected. It is possible to modify cells outside the body of a patient or donor to express specific genes. The first step is to isolate the target stem, progenitor or differentiated cell. Then, the cells are expanded with or without genetic modification. Lastly, the product is reinfused back to the patient (34).

When compared to *in vivo* gene therapies, *ex vivo* gene therapy comprises two important advantages. On the one hand, this method prevents direct human exposure to the vector which, in theory, decreases its immunogenicity, contributing to a stronger safety profile. On the other hand, it is possible to select the target cells of transduction, thus improving specificity and efficacy (34).

Ideally, easy to isolate and to manipulate *ex vivo* cells would be the perfect choice to apply this strategy. Hematopoietic stem cells (HSC) fit both criteria. Additionally, a long-term therapeutic effect is expected to be obtained as HSC originate several cell types, such as red blood cells and major immune cells (4). In the early 2000's, in Italy, 10 children with ADA-SCID were treated with HSC transduced with a retroviral vector, which successfully engrafted and differentiated into myeloid cells containing ADA gene (35). Another example, also from Italy, showed promising results after treating 3 children with Wiskott-Aldrich syndrome (WAS), an inherited immunodeficiency caused by mutations in the gene encoding a

regulating cytoskeleton protein (WASP). Hematopoietic stem/progenitor cells of the patients were genetically modified using a lentiviral vector encoding the functional WASP gene. The children were reinfused with the corrected cells after reduced-intensity conditioning regimen (36).

Other cell types used in ex vivo gene therapy include T cells. An established cell and gene therapy application is adoptive immunotherapy, where T cells are modified to better act against malignancies, infections and autoimmune diseases (34). Multiple studies were carried out by expanding and genetically modifying this cell type, particularly in the treatment of some lymphoproliferative diseases. In Acute Lymphoblastic Lymphoma (ALL), a specific type of B-cells accumulates in the body. Lymphadenopathy impairs immunity, allows opportunistic infections, and may compress adjacent body organ structures. In 30-50% of patients, the lymphoblasts infiltrate bone marrow, causing unsuccessful hematopoiesis. In ALL CD19+, the proportion of immature B cells expressing the CD19 marker is high. Chimeric Antigen Receptor (CAR) therapy represents a therapeutic alternative recently approved by the US FDA and EMA for a specific subset of patients, namely relapsed and refractory CD19 malignancies. Novartis' Kymriah®™ (tisagenlecleucel) consists of genetically modified autologous T cells expressing an Anti-CD19 CAR and it has shown great promise in several clinical trials, with complete remission (CR) rates ranging from 67% to 90%. Kite Pharma's Yescarta® (axicabtagene ciloleucel) is another CAR-T example, recently approved in Europe(25,37–41).

In 2006, Yamanaka and his team managed to reprogram differentiated cells into induced Pluripotent Stem Cells (iPSC), by transducing skin fibroblasts with viral vectors carrying specific gene transcription factors. These transcription factors were not randomly chosen but rather identified as key in the maintenance of pluripotency in both early embryos and embryonic stem cells. The development of iPSC technology was such an important milestone that Yamanaka was awarded with the Nobel Prize in Physiology/Medicine, in 2012 (42,43). Combining ex vivo gene transfer with iPSC may have high potential for the treatment of a number of genetic disorders. For example, transducing iPSC with a functional copy of β-globin gene showed promising results both in the treatment of β-thalassemia whether in in vitro (44) and in in vivo models (45). However, further studies are needed on this topic as it has been shown that iPSC may implicate some unacceptable safety risks in clinical

application. For example, the presence of reprogramming factors (such as c-Myc), could induce tumorigenesis (46,47).

Other types of cells that may be used for ex vivo gene transfer and yielded positive results in the potential treatment of several diseases include, but are not limited to, epidermal and limbal stem cells, neural stem/progenitor cells (NSPCs), cardiac stem cells and multipotent stromal cells (MSCs) (34).

In ex vivo gene therapy, the goal is to permanently modify the host genome, and then expand the cells prior to reinfusion (4). Retroviral vectors are the preferred choice for ex vivo gene therapy, since these require proviral integration into the host genome for transduction, and generally infect only dividing cells. The use of lentiviral vectors, mostly derived from Human Immunodeficiency Virus (HIV), which have a stronger safety profile and also transduce non-dividing cells may be preferred over gamaretroviral vectors (4,48).

An alternative option to viral vectors is applying targeted genome editing using clustered regularly interspaced short palindromic repeat (CRISPR)–associated systems (CRISPR–Cas). The potential for gene editing associated with the CRISPR/Cas-9 technology was developed in the US by Jennifer Doudna and Emmanuelle Charpentier. It generally consists of cutting genomic DNA in a sequence-specific fashion, allowing for disruption or repair of that region. The greatest advantage of this method over using viral vectors is related to the low risk of immunogenicity but also low probability of insertional mutagenesis (4,49). The most significant limitation of CRISPR/Cas-9 is related to off target mutations, which is discussed in further detail in section 1.1.3.4.3.

DNA transposition is a process by which discrete DNA portions, called DNA transposons, change their positions within the genome via a ‘cut and paste’ mechanism. The process is mediated by the transposase enzyme which is responsible for removing the element from its donor plasmid, followed by reintegration of the transposon into a specific chromosomal site. Transient transfection of a transposase, together with a donor plasmid containing the gene of interest can also be a strategy for ex vivo gene transfer (34,50).

1.1.3 Clinical applications

1.1.3.1 Monogenic diseases

Most of the investigation in gene therapy is focused on monogenic diseases, as these are perfectly characterized through a defective single gene, making gene replacement a straightforward strategy. Additionally, appropriate non-clinical animal models are relatively easy to be obtained and applied (28).

1.1.3.1.1 Lipoprotein lipase deficiency and Glybera®

Lipoprotein lipase (LPL) deficiency is a rare monogenic autossomal-recessive disease caused by a mutation in the gene encoding the LPL enzyme. LPL enzyme is involved in the fatty acids metabolism, by breaking them down into smaller molecules and allowing subsequent gastro-intestinal absorption. As a result, LPL deficient (LPLD) patients have an absence in the enzyme's activity and are restricted to a low-fat diet, suffering from recurrent life threatening pancreatitis. Therapeutic management of LPLD is mostly based on strict adherence to a low-fat diet. However, compliance with such a diet is variable and difficult (22).

Glybera®, the first GTMP approved by the EMA, in 2012, consists of a recombinant adeno-associated serotype 1 vector (rAAV) containing a functional copy of the LPL human gene. The drug administration is dependent on the patient's weight, and requires some level of anaesthesia, since it involves several intramuscular injections. The gene is transduced within myocytes and results in production of LPL to compensate the loss-of-function, as depicted in **Figure 2**, in such a way that the vector in unable to reproduce itself.

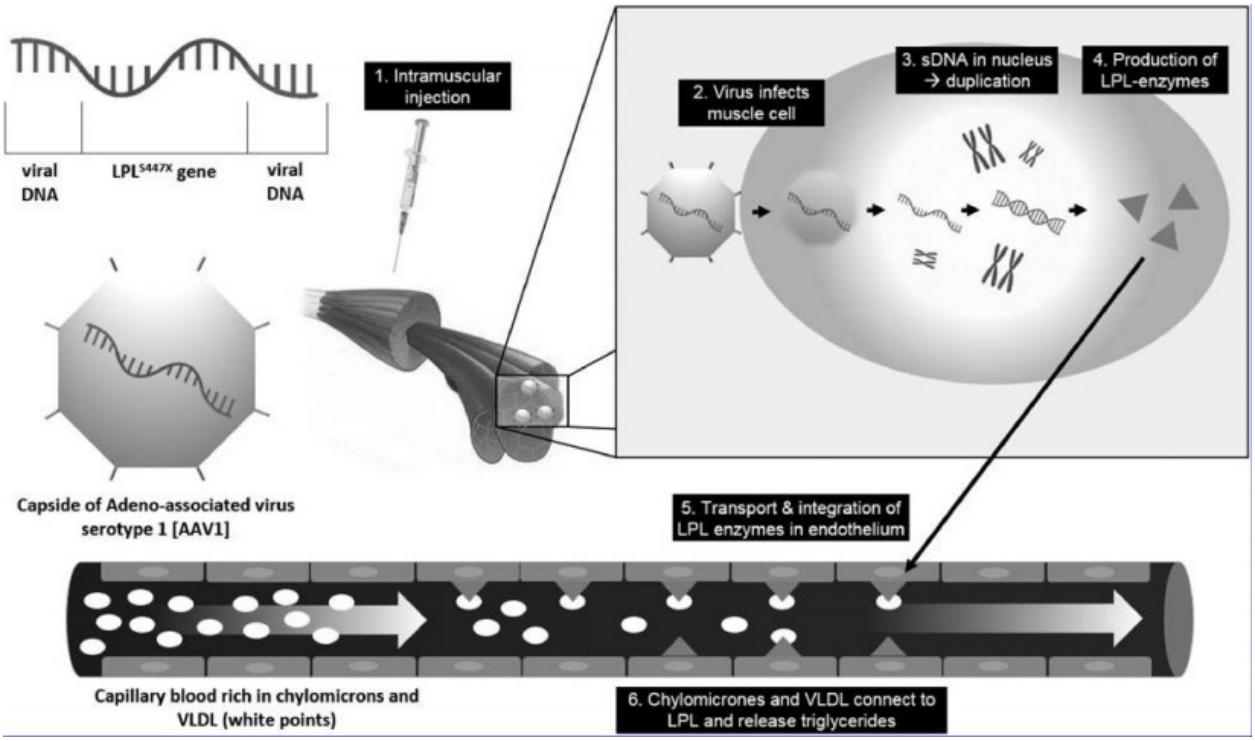


Figure 2 – Molecular mechanism of Glybera

Figure adapted from Kassner, et al., 2018 (51). Step 1: Gene therapy is administered via intramuscular injection. A capside of adeno-associated virus serotype 1 is used as a carrier vector. Step 2: Viral vector infects muscle cells. Step 3: sDNA is released in nucleus with consequent duplication. Step 4: cells initiates production of LPL enzymes. Step 5: LPL enzymes are transported in blood stream and integrated in endothelium. Step 6: Chylomicrones and VLDL connect to LDL consequently releasing tryglicerydes. DNA, deoxyribonucleic acid; LPL, lipoprotein lipase; s, serotype; VLDL, very low density lipoprotein.

As an orphan medicine, Glybera® was evaluated by the regulators having limited clinical data in a very small number of patients. The clinical development programme included three open label uncontrolled studies, which treated an overall number of 27 patients. The process underwent two re-evaluations before final approval. In terms of safety, most of adverse reactions are local and self-limiting within few days after the treatment. The risks associated with Glybera® include significant tissue swelling caused by multiple injections and subsequent thrombogenicity, and risks associated with 3-month course of immunosuppression (recommended after drug administration) (52).

The primary efficacy endpoint presented in the regulatory submission package consisted on the reduction of serum triglycerides. However, this was not consistently achieved and, when it was observed, it was not sustained. Further analysis concluded that serum triglycerides were simply too variable in these patients, requiring the applicant to propose a new primary endpoint. The measurement of postprandial serum chylomicrons before and after gene therapy made biological

sense. The data was compelling in the few subjects in which it was measured (52,53).

1.1.3.1.2 Severe Combined Immunodeficiency and Strimvelis®

One of the clinical applications of *ex vivo* gene therapy is to reconstitute dysfunctional cell lineages and this can be accomplished by genetic replacement, for example, in the treatment of Severe Combined Immunodeficiency using Hematopoietic Stem Cells that undergo *ex vivo* modification.

Combined Immunodeficiencies (CID) comprise a heterogeneous group of genetic disorders that result in impaired development, function, or both of T lymphocytes, associated with a defective antibody response. In the most severe forms of CID, also known as severe combined immunodeficiency (SCID), there are practically no functioning peripheral T cells (12,13,34).

Just about half of all SCID cases are due to a defective development of T cells and NK cells as a result of mutations in the gene encoding interleukin 2 receptor-γ (IL2RG). This is called X-linked SCID, as it is related to a mutation in the X-chromosome. It is also generally known as the “Bubble Boy Disease”, named after a case in the late 70’s of a young boy who lived over 10 years in a protective sterile plastic bubble, and then unfortunately died after an ineffective bone marrow transplant (54). Full activation of the IL2RG results in T-cell proliferation, antigen-induced cell-death and boosting of cytolytic activity of NK cells. This mechanism is significantly impaired in patients with X-linked SCID (12,55,56).

Another highly common type of SCID is ADA-SCID, where a deficiency in adenosine deaminase is found. The lack of ADA enzyme results in (de)adenosine compounds accumulation, which in turn induce cell death, particularly of lymphoid progenitors. Patients with ADA-SCID have nearly full absence of lymphocytes, either T, B or NK cells (12).

For both X-linked and ADA-SCID, Hematopoietic Stem Cells Transplantation (HSCT) represent life-saving standard of care therapy. The clinical prognosis in primary immunodeficiencies after HSCT is influenced by multiple factors, including molecular defect, disease status, donors, stem cell source and chemotherapy conditioning regimen. Conditioning aims at creating space in the recipient marrow enabling donor stem cells to engraft more easily (57). Risks include infection during

the transplant period, as patients undergo strong immunosuppressant regimen, as well as development of acute and/or chronic graft versus host disease (GvHD). GvHD occurs in allogenic transplants where newly transplanted cells attack the transplant recipient's body. Here, gene therapy represents a significant advantage as the patient's own cells are modified and reinfused back into the patient. This means that the donor receives his/her own cells (autologous transplant). GvHD is less likely to occur with Human Leukocyte Antigen (HLA) matching donor (58,59).

In the early 1990's, Michael Blaese and his team were first to conduct a trial using a therapeutic gene, by treating children with ADA-SCID (11). It was not until 2016 that a GTMP was authorized, in Europe, to treat ADA-SCID. Strimvelis® is comprised of patient's own CD34+ enriched cell fraction containing CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence.

Strimvelis®' intends to treat ADA-SCID patients who cannot undergo bone marrow transplant as they have no suitable donor. Prior to treatment administration, a conditioning regimen with busulfan is required, after bone marrow collection. The patients are then given transduced autologous cells via intravenous administration (60).

As far as manufacturing, Strimvelis® requires particular cell processing capabilities, in a short time frame, taking into account the cells viability. This process takes place in Italy (Molmed) which currently is the only approved manufacturing site. The patients are expected to travel to Italy in order to receive treatment (60).

As for Glybera®, Strimvelis® is proposed as a one-time administration to address an orphan disease. The pivotal study included a very limited number of patients (12 subjects). In terms of efficacy, and considering that ADA-SCID is a fatal disease where patients do not survive over the first year of life, the EMA considered that there was compelling evidence of benefit. Indeed, all patients were alive after a median follow-up of seven years (60,61).

Immune reconstitution appears to be much slower with gene therapy when compared to HSCT. Therefore, the risk related to infections was considered high by the EMA, especially during the first year after the treatment. Autoimmune serious adverse events were noted namely hemolytic anemia, aplastic anemia, hepatitis, thrombocytopenia and Guillain-Barré syndrome. However, considering the strong

efficacy data, the risk benefic balance was positive, as per the regulator's assessment (60).

1.1.3.1.3 RPE65 mutations and Luxturna™

Leber's congenital amaurosis (LCA) type 2 is an inherited autosomal recessive disease where the retinal pigment epithelium 65 kDa protein (*RPE65*) gene is mutated. This protein is fundamental in the visual perception biochemical process, specifically in the conversion of light energy to electrical signalling by retinal photoreceptors in the eye. The process involves consumption and regeneration of a derivative of vitamin A (i.e. 11-cis-retinal), through RPE65. Patients with LCA2 are unable to regenerate intra-ocular 11-cis-retinal leading to a profound impairment in the detection of light. Consequently, a severe vision loss and abnormal eye movements (nystagmus) is experienced, particularly in early infancy and childhood. Until recently, there was no treatment for LCA and usually it progresses to total blindness by the third or fourth decade of life(62).

In September 2018, in Europe, Luxturna™ (voretigene neparvovec) received positive opinion towards Marketing Authorization. This GTMP is an adeno-associated viral type 2 vector with a cytomegalovirus enhancer and chicken beta actin promoter driving expression of normal human *hRPE65* gene. It is intended for single use and to be administered by an experienced surgeon to the sub-retinal space of each eye. Luxturna™ is indicated for the treatment of adult and pediatric patients with vision loss due to inherited retinal dystrophy caused by confirmed biallelic *RPE65* mutations and who have sufficient viable retinal cells(26,63).

The pivotal clinical trial involved 31 patients with inherited retinal dystrophy due to *RPE65* mutations. The main effectiveness outcome was how well patients performed in a mobility test, under various light settings. After one year of treatment, patients treated with Luxturna™ improved their scores by 1.8 points, while patients who were not given Luxturna™ improved their scores by 0.2 points. Additionally, 13 of the 21 patients (62%) treated with Luxturna™ passed the mobility test at the lowest light level of 1 lux (similar to conditions of a poorly lit pavement at night), whilst none of the patients not given the medicine were able to do so. The improvement in patients' vision was sustained for at least three years(63).

1.1.3.1.4 β -Thalassemia and Zynteglo®

β-Thalassemias are characterized by a reduction or deficiency of β-globin chains and, consequently, an imbalance in globin chains of the haemoglobin molecule. This leads to impaired erythropoiesis. More than 200 mutations have been documented to affect the β-globin gene, for which patients may be either homozygous or heterozygous. Phenotypic effects, therefore, range widely from slight impairment to the complete inhibition of β-globin chain synthesis(64).

The clinical implications are, on the one hand, patients lack sufficient red blood cells and haemoglobin to effectively transport oxygen throughout the body, resulting in severe anaemia. On the other hand, an ineffective erythropoiesis can lead to morbidities such as splenomegaly, marrow expansion, concomitant bone deformities, and/or iron overload. Treatment strategies include blood transfusion, splenectomy, fetal hemoglobin induction and hematopoietic stem-cell transplantation. However, iron overload and associated morbidities remain a major challenge in the management of transfusion-dependent β-thalassaemia (TDT) and treatment-related complications are the primary source of mortality(64,65).

Zynteglo® (betibeglogene autotemcel) is a GTMP, approved in Europe since 2019(27). The product is indicated for the treatment of patients 12 years and older with TDT who do not have a β0/β0 genotype, for whom HSCT is appropriate but HLA-matched related HSC donor is not available.

Pivotal trials included two studies where the GTMP showed to reduce the need for blood transfusion in patients with TDT who required regular blood transfusions. In these studies, out of the 14 patients who did not completely lack beta-globin and were given Zynteglo®, 11 of them had sufficiently high levels of red blood cells so that they did not need blood transfusions for at least 1 year after treatment(65).

1.1.3.1.5 Hemophilia B and scAAV2/8-LP1-hFIXco

Hemophilia B is a severe inherited blood disorder caused by a deficiency in the gene encoding human clotting factor IX (FIX). As a result of this loss-of-function, patients with hemophilia have low levels of FIX, and a high risk of spontaneous bleeding while performing daily activities. A specific group of patients shows a severe bleeding phenotype which results in spontaneous musculoskeletal and soft tissue hemorrhages in the absence of appropriate treatment (66,67).

Intravenous administration of recombinant clotting factor concentrates represents the standard of care therapy. Due to its relatively short half-life, patients need to be administered rather frequently, around 2-3 times a week. PEGylated clotting factors may resolve this issue to a certain extent, by allowing treatment every 2 weeks. However, this is still not a curative approach and the risks of lifelong administration of PEGylated proteins are not completely known (66,67).

The first hemophilia gene therapy studies used AAV2 as a vector and different routs of administration. Intramuscular injection of AAV2-FIX in a group of 8 patients, showed no significant safety concerns though limited efficacy was observed, likely related to levels of FIX not rising above 1%. Conversely, improved efficacy was seen in a trial where 7 patients received FIX encapsulated in AAV2 vector administered directly in the hepatic artery. However, some safety issues related to immunogenicity towards the viral capsid were noted. Additionally, pre-existence of neutralizing antibodies could potentially impact successful gene transduction (68,69).

A group of London based investigators decided to use a different AAV serotype and a more straightforward route of administration. Early phase I dose escalation trial with 10 patients using a self complementary AAV serotype 8 vector expressing codon-optimized human FIX under the control of a liver specific promoter (scAAV2/8-LP1-hFIXco) have shown promising results, following a single systemic administration of the vector in severe hemophilia adult patients. AAV8 has an outstanding tropism for hepatic cells which is ideal as the synthesis of the defective clotting factor takes place in the liver. There was an evident analytic increase in plasma factor IX activity (from a baseline percentage of less than 1% to a percentage of 1-6% after treatment) and from a clinical perspective the average annual number of bleeding episodes was consistently lower after gene transfer, particularly in patients in the high-dose cohort. From a safety perspective, there were a number of cases of asymptomatic, transient elevation of serum liver enzymes, probably as a result of a cellular immune response to the AAV8 capsid, which rapidly disappeared after prednisolone treatment (70).

1.1.3.1.6 Cystic fibrosis and pGM169/GL67A

Cystic fibrosis (CF) is an autosomal recessive disorder which impacts the protein encoded by the Cystic Fibrosis Transmembrane Conductance Regulator

(CFTR) gene. The CFTR protein is present in epithelial membrane cells, widely distributed throughout the body, including in the pulmonary tract and gastrointestinal tract. Loss-of-function of the CFTR gene leads to intracellular accumulation of chloride, sodium and water which is of particular severity in the lungs, since it leads to formation of a thick mucus layer, impairing ciliary clearance pathway and being a perfect breeding media for microorganisms. Subsequent accumulation of inflammatory cells and other mediators may lead to bronchiectasis and gradually, overtime, airway remodelling takes place and the airway is destroyed (fibrosis). In late stages, CF leads to respiratory failure and chronic lung infection which is the main responsible for morbidity and mortality (71,72).

Therapeutic management of CF, especially displaying pulmonary exacerbations, is mainly based on administration of inhaled bronchodilators, mucolytic agents and use of oral antibiotics.

Epithelial respiratory cells are an attractive target which provide easy access when compared to other gene therapy strategies requiring more invasive forms of administration such as intramuscular or intravenous injection. Attempts to treat CF have been reported using both viral (73) and non-viral vectors (74) carrying the gene encoding the functional CFTR protein.

Repeated nebulisation of plasmid DNA encoding the CFTR gene complexed within a cationic liposome (pGM169/GL67A) was tested in CF patients. This phase 2-b trial enrolled 140 patients and showed proof-of-concept that non-viral gene therapy could beneficially impact lung function in CF patients. Treatment was well tolerated and a significant though modest effect was seen in the forced expiratory value in 1 second (FEV1) versus placebo after 12 months of treatment (74).

Dose increase or shortening of the administration interval were considered as an improvement strategy. On the other hand, more potent vectors like viral vectors were also tested in CF animal models. Lentiviral vectors have been investigated but since these vectors lack a natural tropism for lung tissue, pseudotyping with envelope proteins is required for the viral particles to reach their target. Promising results including a transduction of the gene in the respiratory epithelium of the murine nose *in vivo* at levels that may be relevant for clinical benefit in CF patients were reported by capsid pseudotyping with heamaglutinin-neuraminidase proteins from Sendai virus (73) (further details available in section 1.1.4.2).

1.1.3.2 Multifactorial diseases

As opposed to monogenic disorders, other more complex diseases may also be a suitable target for gene therapy. Here, gene replacement might not be the most suitable choice as for monogenic diseases. Conversely, gene addition in combination with other therapeutic agents has been studied in specific diseases and yielded interesting results.

1.1.3.2.1 Heart failure and AAV1/SERCA2a

Heart failure (HF) is a clinical syndrome where, generally, the heart fails to pump sufficient blood to meet the body's metabolic needs, as a result of a decrease in cardiac function. Underlying HF causes include post-acute myocardial infarction status. HF is characterized by shortness of breath, swollen ankles and fatigue and may be accompanied by signs such as elevated jugular venous pressure, pulmonary crackles and peripheral oedema (75).

Current therapeutic management in an outpatient basis consists of oral angiotensin-converting enzyme inhibitor (ACEI) or angiotensin-receptor blokers (ARB), beta-blockers and mineralocorticoid/aldosterone receptor antagonist. Recently, sacubitril/valsartan, belonging to a new class of therapeutic agents (angiotensin receptor-neprilysin inhibitor, ARNI) was added in the European Guidelines for HF management, as a replacement of first line ACEI/ARBs. However, HF has an overall prevalence that is increasing globally and, therefore, represents a major public health issue characterized by significant mortality, frequent hospitalization and poor quality of life(75).

Calcium is one of the most important ions involved in cardiac function and contractility. Deficient uptake of cytosolic calcium to the sarcopaslmatic reticulum has been identified in cardiac cells from failing human hearts. The enzyme involved in this process (the sarcoplasmic reticulum Ca-ATPase, also referred to as SERCA2a) was noted to have a reduced expression and activity in HF, not necessarily due to a defect in the corresponding genes (28,76).

The pilot dose-finding phase II CUPID study was the first human trial with gene transfer of SERCA2a. This was a small, placebo-controlled study in advanced HF patients which tested the percutaneous administration of a SERCA2a

gene encapsulated in an AAV serotype 1 vector on symptomatic, functional and structural efficacy endpoints. Thirty nine patients were on optimal medical treatment in addition to being administered with the vector directly in the coronary circulation and the results were very positive, without significant safety concerns (77,78).

However, a larger phase IIb trial with 250 patients (CUPID 2), which tested the same vector in a broader patient population, showed no evidence of improved outcomes, at the studied dose. Investigators provided several justifications including that the results of the pivotal trial were consequence of a chance finding and that the patients randomized to the placebo arm, in the CUPID trial, had a greater severity of illness. Another potential reason was related to the proportion of empty viral particles administered to the trial subjects that was higher in the CUPID trial when compared to the CUPID 2. These empty particles may improve transduction of the vector by binding to self-antibodies against the vector (79).

1.1.3.2.2 HIV infection and vectored immunoprophylaxis

Currently, HIV has no curative therapy though patients are able to live for many years while still infected if appropriate Anti-Retroviral Treatment (ART) is administered. ARTs suppress viral replication to low or undetectable levels, with a corresponding but variable increase in CD4 T-cell counts. Even though HIV infection has become a chronic but manageable disease, a significant decrease in survival is observed as a result of long-term complications in main organ systems such as accelerated cardiovascular disease, liver and renal failure and neurocognitive dysfunction. Additionally, resistance to certain ARTs suggest that further alternatives should be investigated (80).

A large number of attempts have been made at testing not only new treatment options but also preventative strategies, such as the development of vaccines. Here, the discovery of broadly neutralizing antibodies represents an important milestone. Natural infection induces the production of non-neutralizing or strain specific antibodies, especially during the early months after infection. Broadly neutralizing antibodies are antibodies against several strains of HIV-1 and can be found in approximately 20% of HIV-1 infected patients (81).

Intramuscular delivery of adeno-associated virus containing a gene encoding broadly neutralizing antibodies against Human or Simian immunodeficiency virus has

been tested in both rodent (82) and non-rodent animal models (83), with encouraging results. This strategy is also called vectored immunoprophylaxis (VIP), and efforts are currently underway for extending this strategy to humans, for the first time.

1.1.3.2.3 Chimeric Antigen Receptor T-Cells (CAR-T) therapies for Cancer: Kymriah® and Yescarta®

Cancer is a complex disorder where generally multiple genes are affected. Additionally, substantial differences can be found between tumour of different individuals and between tumours in the same patient. Gene addition as cancer treatment is not as straightforward as in monogenic diseases (84,85).

The goal of adoptive cancer immunotherapies is to induce the patient's own immune response against the tumour cells via specific tumour cell recognition and consequent induction of cytotoxicity. Specific tumor-associated antigens are involved in this process, generally recognized by genetically modified T-cell receptors or chimeric antigen receptors (CARs)(86). Such CAR-T cells recognize surface antigens regardless of MHC restriction. Available CAR-T treatments are based on *ex vivo* treatment of T cells with a vector containing the gene encoding the CAR, which after expansion is readministered to the patient, as per **Figure 3**. The progress in the development of CARs over the past three decades can be roughly grouped into five CAR generations based on the structure and composition of the endodomain (87,88).

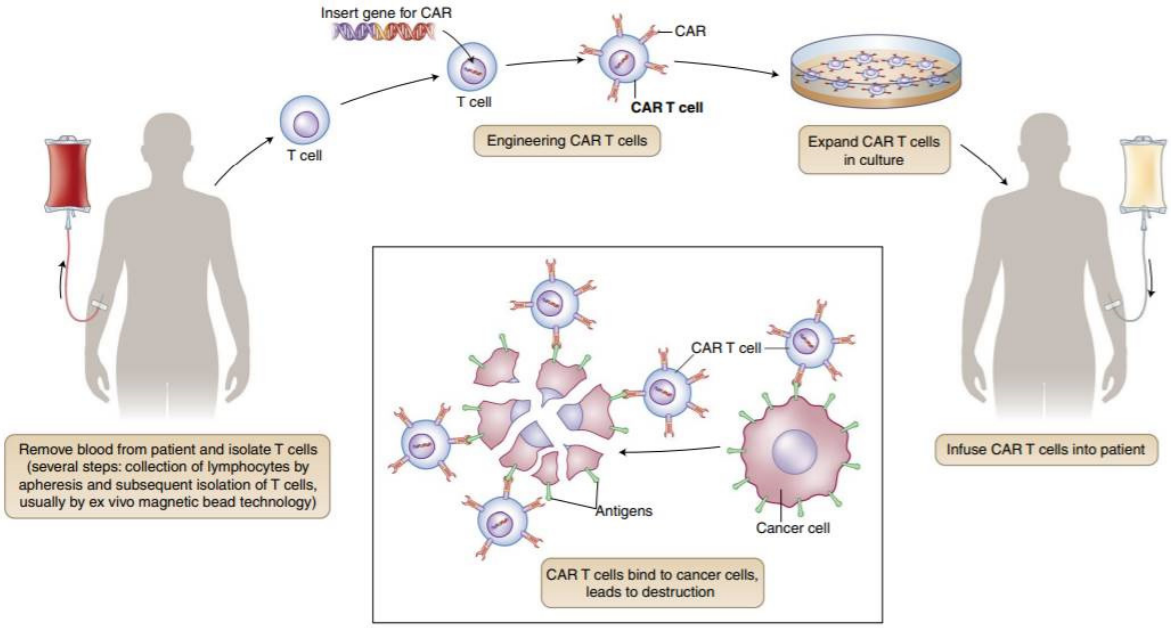


Figure 3 – CAR-T manufacture and administration

Figure adapted from Feigal, et al., 2019 (89). T cells are isolated from a patient's peripheral blood, then a viral or non-viral vector is used to insert the gene encoding the CAR into the genome of the T cells. The engineered T cells are expanded in cell culture and then infused back into the patient. The CAR expressed on the surface of the engineered T cells will recognize an antigen expressed on the surface of the tumor cells, activate the T cells, and target them for destruction. CAR: Chimeric Antigen Receptor.

In 2017, Kymriah® (tisagenlecleucel, an *ex vivo* genetically modified T-Cells to express the anti-CD19 Chimeric Antigen Receptor) was the first product based on gene therapy approved by the US FDA. Positive results were shown in relapsed and refractory Acute Lymphoblastic Lymphoma patients. Here, a lentiviral vector containing the gene encoding the Chimeric Antigen Receptor (CAR)-19 gene is transduced in patients own T-Cells and then reinfused back into the patient's circulation (37,38,41,85). Kymriah® was later approved in Europe in 2018(25).

In 2018, Yescarta®'s (axicabtagene ciloleucel) marketing authorization was granted in Europe, as the second CAR-T therapy available(25). This GTMP is an engineered autologous T-cell immunotherapy product where the patient's own T cells are harvested and genetically modified ex vivo by retroviral transduction to express a CAR comprising an anti-CD19 receptor. Since CD19 is expressed as a surface antigen in diffuse large B-cell lymphoma and other aggressive B-cell lymphomas, the transduced can recognize and eliminate CD19 expressing target cells. Yescarta® is indicated for the treatment of adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) and primary mediastinal large B-cell lymphoma, after two or more lines of systemic therapy(90).

1.1.3.2.4 Gene addition as Cancer treatment: Imlygic®

In 2016, Imlygic® (talimogene laherparepvec) was the 2nd gene therapy product approved in the EU, which takes advantage of a gene addition strategy for the treatment of advanced unresectable melanoma. Herpes Simplex Vector (HSV) is administered directly into the tumour. This vector was subjected to specific viral gene deletions, which result in replication inside tumour cells and consequent oncolysis. Furthermore, the vector contains a gene encoding the Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF), which triggers a systemic immune response, capable of fighting not only the injected tumour but also its metastasis. The main phase III trial which supported the MAA was based on a comparison between patients treated with subcutaneous GM-CSF versus Imlygic®. The study showed that the investigational treatment significantly improved the rate of responses lasting continuously for 6 or more months in patients with unresected stage IIIB to IV melanoma compared with subcutaneous GM-CSF. Imlygic®'s safety profile was considered acceptable, inducing minor adverse reactions mainly related to flu-like syndrome, following intralesional administration (91,92).

1.1.3.3 DNA down regulation through RNA targeting

RNA interference works by suppressing the expression of certain messenger RNAs, thereby preventing the accumulation of the corresponding toxic protein. Silencing a toxic gene may bring therapeutic benefit in specific genetic disorders.

1.1.3.3.1 HIV infection and shRNA against CCR5 gene

Virtually all HIV target cells are produced from hematopoietic stem cells, including T cells, macrophages, dendritic cells and brain microglia. Here, the virus is permanently incorporated forming 'reservoirs' of infected cells that are unable to be eliminated. The outstanding case of the 'Berlin Patient' raised great hope towards uncovering a cure for HIV. In 2007, an HIV infected patient was treated for relapsed acute myeloid leukemia with HSCT. This resulted in the first documented case of HIV cure, highlighting the importance of the chemokine receptor 5 (CCR5) in maintaining HIV infection. The transplanted cells had the CCR5 gene naturally silenced since the

donor was homozygous for a deletion in the CCR5 gene providing resistance against HIV-1 infection. From a molecular perspective, cellular infection with HIV-1 requires a CD4+ cell and a CCR5 receptor and by disabling the CCR gene the virus is unable to infect body cells. Up until today, the patient remained free of leukemia and also free of HIV rebound after discontinuing ART. However, this is not a feasible treatment option for the majority of HIV patients, since it would be very difficult to find an HLA-matching donor who would simultaneously be HIV-resistant by displaying the required CCR5 homozygous deletion, as per **Figure 4**(93).

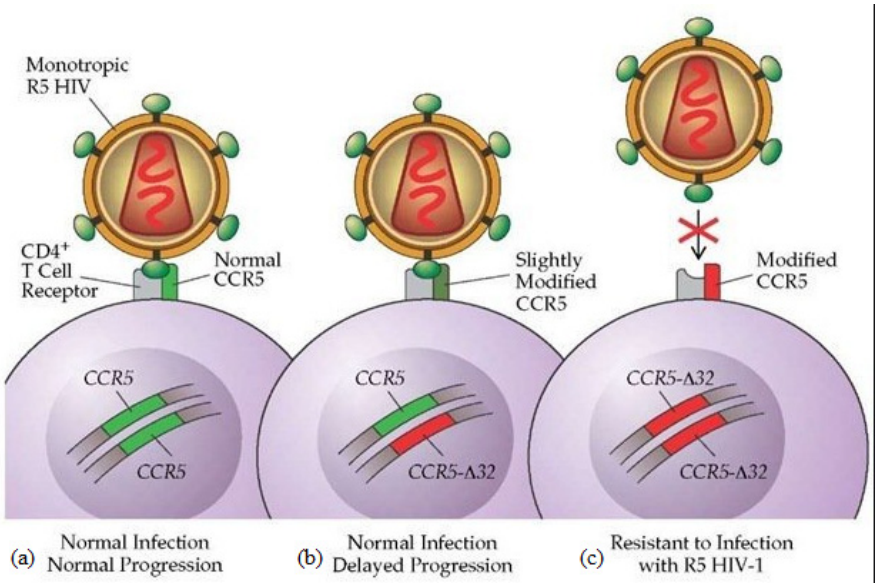


Figure 4 – HIV resistance and CCR5 mutations

Figure adapted from thehealthconnections.com (94). From left to right: (a) and (b) HIV viral particle may infect a cell with functional CCR5 on its surface. (c) Individuals with homozygous mutation in the CCR5 gene become resistant to HIV infection.

In contrast, the 'Berlin Patient' results were key for other gene therapy investigators to test administration of vectors containing anti-HIV genes. For example, in an attempt to knock down the CCR5 gene, several groups tested the administration of small hairpin RNA (shRNA) against CCR5 encapsulated within a lentiviral vector (95). shRNAs are vector-derived RNA interference structured, ultimately processed to produce siRNAs in the target cells (96).

The *in vitro* results showed that the cells gained HIV resistance. However, over expression of shRNA could induce cytotoxicity in human primary T lymphocytes. In an optimized animal model, no apparent adverse effects due to the shRNA were evident in transplanted primates for 3 years (95).

1.1.3.3.2 Paramyloidosis and Onpattro®

A similar strategy was used by a group of investigators, in the treatment of transthyretin amyloidosis. This is a dominant autosomal disease where hepatocyte-derived transthyretin amyloid deposits accumulate in several tissues and organs, namely peripheral nerves and in the gastrointestinal tract, heart and kidneys. The signs and symptoms include pain, paresthesia, muscular weakness and autonomic dysfunction.

Tafamidis, a small-molecule stabilizer of the transthyretin tetramer, is the only approved treatment, slowing the progression of neuropathy. Hepatic transplant eliminates the production of mutant transthyretin though there are obvious limitations regarding the broad application of this therapeutic option, such as HLA compatibility issues.

Onpattro® (patisiran) is an antitransthyretin small interfering RNA encapsulated in lipid nanoparticles that was tested in both rodents and humans. Clinical results showed that patisiran suppressed the production of both mutant and nonmutant forms of transthyretin, which may lead to an improvement of disease related symptoms. Besides infusion-related adverse reactions, the preliminary data on safety was satisfactory. A phase III study has established efficacy and safety of the investigational medicinal product (97,98).

In July 2018, the CHMP adopted a positive opinion, recommending the granting of a marketing authorisation for the medicinal product Onpattro®, intended for the treatment of hereditary transthyretin-mediated amyloidosis(99).

One potential challenge associated with RNA interference particularly impacts dominant genetic diseases, where there is one mutated allele and one normal allele. Here, RNAi inhibits the production of both the mutated and the normal protein, which can lead to a decline in the gene's normal function. A possible strategy to overcome this hurdle may include the administration of allele-specific RNAi towards the mutated allele, which has been tested by some investigators in some pathologies such as Huntington's disease (100).

1.1.3.4 Targeted gene editing

The greatest advantage of targeted gene editing when compared to gene replacement or addition is the highest control over the defective gene. Theoretically,

it corrects the problem directly in the source, rather than adding another genetic sequence. As simple as it may appear, targeting a single gene within a large genome may be challenging. This is probably the strategy that is being developed with the most caution due to potential important safety events, such as off target effects and also ethical implications about possible genetic changes in germline cells.

Three important strategies should be addressed including Zinc Finger Nucleases (ZFN), Transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeat (CRISPR)–associated systems (CRISPR–Cas) (28,101).

1.1.3.4.1 HIV treatment via CCR5 gene editing using ZFN

ZFN were the first genome editing nucleases to be described and are a type of gene-targeting reactants which combine both DNA recognition specificity of ZFN and the enzymatic activity of *Fok*I. The zinc finger domain comprises 30 amino acids and coordinates one zinc atom using two histidine and two cysteine residues. A specific DNA triplet is recognized by a α-helix in each domain. Multiple zinc finger domains are able to recognize long DNA sequences. *Fok*I is a nuclease responsible for the double-stranded break of DNA. The nucleases attached to ZFNs are required to function as dimmers, which mean that ZFNs can target any specific DNA sequence.

As per **Figure 5**, after this targeted cleavage, two DNA repair mechanisms can take place, including homologous recombination or nonhomologous end joining. Homologous recombination repairs the break while maintaining the original DNA sequence. This can be used for targeted gene replacement. Nonhomologous end joining can be used to edit a specific gene as it may result in deletion of a specific DNA sequence at the break site, causing permanent disruption of the primary DNA sequence (102).

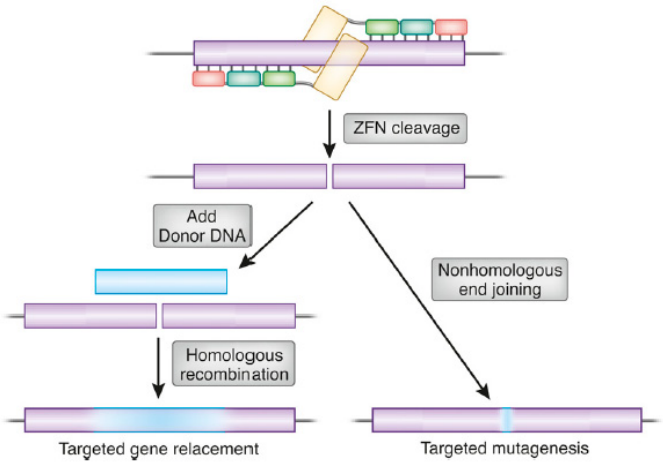


Figure 5 – Gene editing by Zinc Finger Nucleases

Figure adapted from Carroll, 2011 (102). Repair outcomes of a genomic double-strand break, illustrated for the case of ZFN cleavage. A pair of three-finger ZFNs is shown at the top in association with a target gene (open box). If a homologous donor DNA is provided (solid box, left), repair can proceed by homologous recombination using the donor as template. The amount of donor sequence ultimately incorporated will typically decline with distance from the original break, as illustrated by the shading. Alternatively, the break can be repaired by nonhomologous end joining, leading to mutations at the cleavage site. These may be deletions, insertions, and base substitutions, usually quite localized, but sometimes extending away from the break.

The first clinical trial using a nuclease for targeted gene editing (101) was conducted in 12 HIV patients where the CCR5 gene was silenced by treatment of patients' own CD4+ T cells with ZFN. In this phase I study the patients' own cells were treated *ex vivo* with ZFN in order to achieve CCR5 gene disruption and reinfused back into circulation. The study results included a significant increase in CD4+ T cells count after administration and long-term persistence of CCR5-modified CD4+ T cells in peripheral blood and other tissues. Overall, the results showed that artificial induction of HIV-resistance was a generally safe and feasible approach (103).

1.1.3.4.2 Leukemia and CAR-Ts developed with TALENs

TALENs have rapidly became an alternative genome editing tool to ZFN. The non-specific *Fok*I domain is used as the DNA cleavage element inducing double strand breaks. As depicted in **Figure 6**, the DNA binding domains comprise a series of tandem repeats, each including around 33 to 35 aminoacids capable of recognizing a single nucleotide. TALEN-DNA interactions are less complex when compared to ZFN. In addition, designing TALENs is generally simpler than ZFN. The bulky size of TALENs might be a limitation in clinical application (104,105).

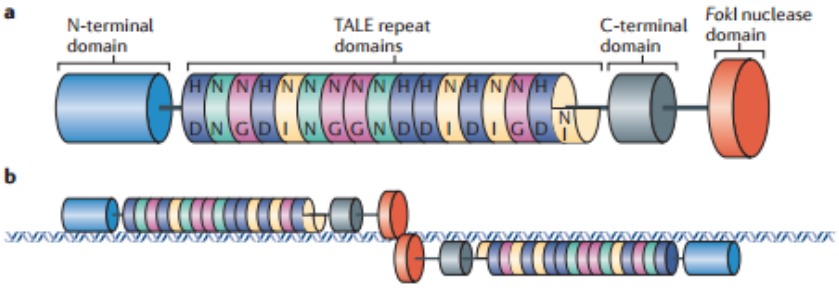


Figure 6 – Gene Editing by Transcription activator-like effector nucleases

Figure adapted from Joung and Sander, 2013 (104). (a)Schematic diagram of a transcription activator-like effector nuclease (TALEN). Transcription activator-like effector (TALE) repeats are shown as coloured discs with a final carboxy-terminal truncated half repeat. Letters inside each repeat represent the two hypervariable residues. TALE-derived amino-terminal and C-terminal domains that are required for DNA-binding are indicated. The nonspecific nuclease domain from the *Fok*I endonuclease is shown in red. (b) TALENs bind and cleave as dimers on a target DNA site. Note that the TALE-derived N-terminal and C-terminal domains flanking the repeats may also contact the DNA. Cleavage by the *Fok*I nuclease domains occurs in the 'spacer' sequence that lies between the two regions of the DNA bound by the two TALEN monomers.

The first published clinical application of TALEN refers to treatment of an 11-month old baby with B acute lymphoblastic leukemia (B-ALL). Phase I trials for this specific gene therapy medicinal product were underway, but the research group received a request for therapy on a compassionate basis for this infant with refractory relapsed B-ALL. Under UK special therapy regulations, this was the first patient treated with TALEN engineered Chimeric Antigen Receptor 19 T Cells. Analysis of the short follow up period, the intervention which included lymphodepletion and infusion of the manipulated CAR-T 19 T cells has induced molecular remission where previous conventional treatments had failed (106).

1.1.3.4.3 Immunosuppresion and CRISPR Cas-9

CRISPR technology allows gene editing with unprecedented accuracy and the potential to become a powerful gene editing tool was found by accident through a project on characterization of CRISPR associated protein 9 (Cas9 enzyme) by Jennifer Doudna and Emmanuelle Charpentier.

The term CRISPR refers to specific DNA sequences initially found in bacteria DNA as a series of short direct repeats interspaced with short sequences. The role of these sequences is related to protection from viral and plasmid infection. CRISPR DNA sequences within the host cell are specific for each virus. Transcription of this DNA to RNA is used to recognize a new virus attack. Together with a second small RNA, tracrRNA (trans activating crRNA), a Cas enzyme is able to recognize and neutralize viral DNA, preventing the infection.

Doudna envisioned that it would be possible for Cas9 to target a specific DNA sequence, by using a defined RNA template coupled to the enzyme so that it acts on the desired gene (49,107).

A group of Chinese investigators have generated genetically modified rodents and non-human primates by effectively disrupting specific genes, through the CRISPR-Cas9 technology in embryonic cells (108,109). This technology is on the verge of being tested for the first time in humans, by *ex vivo* removal of the Programmed Cell Death Protein 1 (PDCD-1) gene in T cells.

PDCD-1 is a key immune checkpoint receptor expressed by activated T cells and it is responsible for immunosuppression. Immunosuppressive PDCD-1 ligands are expressed by a number of tumour cells. Therefore, inhibition of this receptor may enhance T-cell response. Nivolumab is a monoclonal antibody, currently approved by the EMA, for the treatment of an array of cancer types such as melanoma, non-small cell lung cancer and renal cell carcinoma (110).

The same group of Chinese investigators are behind the first human trial involving the CRISPR-Cas9 technology in disrupting the PDCD-1 gene. To date, data from clinicaltrials.gov displays 4 planned first-in-human studies through the *ex vivo* modification of T cells so that the PDCD-1 gene is knocked out using CRISPR-Cas9. These cells are then reinfused back into patients' own circulation. The group has seen that the strategy is promising *in vitro*, by first applying it to human T cells from cancer patients (111,112).

In 2017, CRISPR-Cas9 made headlines again when a group of US investigators used the technique for the first time in viable human embryos to correct an inherited genetic mutation. Patients with an autosomal dominant genetic condition affecting the MYBPC3 gene may develop hypertrophic cardiomyopathy (HCM). This is a disease characterized by, among other clinical features, left ventricular hypertrophy. The tested embryos were not meant for implantation. Even though none of the embryos developed for more than a few days, the results were promising as not only the genetic mutation was corrected but two important safety issues seemed to be addressed. On the one hand, from the 58 tested embryos, only one showed signs of mosaicism. This is when in a single cell with different genetic sequence is found in the same embryo, which is unacceptable since it would make preimplantation genetic diagnosis challenging. Finally, there was no evidence of off-target mutations (113).

In November 2018, Chinese investigator He Jiankui and his team used the CRISPR gene-editing system to edit DNA in two human embryos to make them less susceptible to HIV. The edits were designed to disrupt a gene that codes for a protein that allows HIV to enter immune cell. This announcement has been highly controversial, considering that a Chinese court has sentenced He Jiankui, to three years in prison for “illegal medical practice”(114).

While the scientific community is excited about this technology and the expectation are high for first-in-human studies, some limitations have been reported for CRISPR technology. Off target mutations detected in higher proportions versus the intended gene edition are likely to occur, and are a major concern in clinical application. Several strategies, at the molecular level, to decrease the off-target mutations have been developed, as well as new approaches to detect them (115).

1.1.4 Challenges associated with gene therapy medicinal products’ development and use

When comparing to classic chemical or biologic therapies, ATMPs are substantially different in nature and, consequently, the evaluation of a MAA may not follow the same ‘standardized’ data submission package. In Europe, the EMA has developed a document outlining a risk-based approach for the evaluation of these specific medicinal products. The ‘risk-based approach’ is defined as “*a strategy aiming to determine the extent of quality, nonclinical and clinical data to be included in the MAA, in accordance with the scientific guidelines relating to the quality, safety and efficacy of medicinal products and to justify any deviation from the technical requirements as defined in Annex I, part IV of Directive 2001/83/EC*” (116). This is an optional approach that highlights some intrinsic risks as well as risk factors associated with candidate ATMPs. Interestingly, some of the risks and risk factors mentioned in this guideline are compatible with a number of pre-identified challenges in ATMP drug development. In this section, these and other challenges will be discussed as well as potential overcoming strategies.

1.1.4.1 Safety issues

1.1.4.1.1 Potential immunogenicity

Patients who suffer from Ornithine transcarbamylase deficiency (OCTD) have a rare X-linked genetic disorder characterized by complete or partial lack of the enzyme ornithine transcarbamylase (OTC). This is an enzyme involved in the urea cycle which prevents excessive accumulation of nitrogen, in the form of ammonia. Hyperammonemia may lead to neurotoxicity and, in extreme cases, result in coma and death.

In 1997, at the University of Pennsylvania, a group of investigators developed an adenovirus vector which contained a functional copy of the OTC gene. Eighteen patients with OTCD were enrolled in a phase I dose escalating study, which tested six different investigational product doses. The vector was administered through a femoral catheter into the right hepatic artery. In 1999, Jessie Gelsinger was enrolled and allocated to the highest dose cohort. Just 4 days after administration, a strong immune response against the vector was noted and the patient died due to multiorgan failure (16).

Following FDA inspection, the case unravelled major deficiencies in trial conduct, such as failure to report significant safety information to regulatory bodies, inadequate informed consent process, inclusion of ineligible patients and protocol amendment implementation prior to Independent Review Board (IRB) approval. Additionally, researchers' financial interest in positive trial results was pointed out as potential bias (117–119).

In return of such concerns, the US government agencies and academic institutions strengthened regulatory requirements on clinical research with special additional requirements for clinical gene therapy trials. For instance, at the time, it became mandatory for early phase studies to have Drug Safety and Monitoring Boards (118).

Initial Investigational New Drug (IND) included nonclinical data from the first-generation vector in mice and rhesus macaques. At the highest dose, syndrome of severe liver damage was noted in monkeys, which lead to death. However, in light of further scientific advancements between initial IND and trial approval, a third generation vector was used in clinical trials. Improved toxicity profile was seen in mice and baboons, compared to the first generation. Therefore, patients in the high

dose cohort were administered with vector dose that was 17-fold lower compared to the dose of first-generation vector that showed severe toxicity in primates. Researchers estimated that this would provide a 100- to 1000-fold margin of safety in terms of vector dose (117). Holistically, one can argue that the immunogenic profile of the vector was insufficiently characterized from a nonclinical standpoint, as well as that the researchers used potentially inadequate animal models. It is clear that the data at the time did not allow accurate prediction of the patient's massive immune response reaction.

Both the viral vector and the transgene product may exert these reactions. The unpredictability of innate and antigen-dependent immune responses in humans is a huge barrier. Additionally, suitable animal models to replicate these responses are difficult to be established (48).

Innate immunity is the first line human immune response, which is activated rather quickly after gene therapy administration. In a viral vector, capsid proteins as well as viral gene products may be recognized by the immune system as pathogens. When using non-viral vectors, naked DNA from plasmids may also exert innate and adaptive immune response. These have a higher proportion of unmethylated CpG motifs which have immunostimulatory effects (29).

Some vectors are more prone to induce unwanted immunogenic responses stressing the importance of choosing an appropriate vector type. During the manufacturing process, some vectors are more easily purified than others resulting in impurities in the finished product that may lead to immunogenic reactions. Additionally, the biodistribution to non-target sites which are more immunogenic may be a source of concern. Since antibodies have limited access to specific body areas, stronger neutralization may occur after intra-hepatic or respiratory administration (where antibodies can more easily access) when compared to more restricted body parts, such as intraocular (retina) or intracranial (brain) administration. Moreover, immunity varies with medical-procedure related factors (e.g. locally administered high dose may cause site inflammatory response due to immune reaction to a therapeutic protein), patient-related factors (e.g. genetic background) and type of transgene and transgene expression levels after administration (e.g. existence of DNA promoters within the therapeutic gene). The latter are of particular importance especially the cytokines present at the site of transgene expression. These may influence inhibition

or activation of promoters and, consequently, impact the expression of the gene of interest (29,116).

Administration of immunosuppressive agents prior or after gene therapy exposure may prevent immunogenicity. Modification of the vector structure at the capsid proteins level or by eliminating viral genes may also be an appealing option. The antigen of the vector may be changed and no longer display the immunogenic effect (29). The AdV vector is known to be highly immunogenic and the use of other types of less immunogenic vectors such as viral AAV or other non-viral vectors may be a strategy to overcome this issue (48). Additionally, *ex vivo* administration of gene therapy as opposed to *in vivo* delivery may also have the potential to exert less immunogenic responses (34).

1.1.4.1.2 Oncogenicity

Unwanted tumor formation may be a result of insertional mutagenesis (IM), which occurs when a gene vector integrates into the host genome, consequently activating/up regulating oncogenes or inactivating/down regulating tumor suppressing genes.

In 2002, a trial lead by Salima Hacein-Bey-Abina, in France, was the first to test *ex vivo* gene modification in patients with X-linked SCID. Five children underwent bone marrow harvesting and the CD34+ cells were then modified using a retroviral vector to express the gene encoding the common gamma chain (γc). Even though the transduction process had limited efficiency, the immune system of the 5 patients was partially repaired. At the time, these were very encouraging results and, additionally, no significant safety events were noted during the 30 month follow-up period (120). Later in 2004, the second X-linked SCID trial took place in the UK, enrolling four paediatric patients. Gene therapy strategy was very similar to the previously used by Hacein-Bey-Abina's team though the viral vector was pseudotyped. Patients were followed for 29 months displaying a substantial clinical and immunological benefit. On the other hand, no serious adverse events were noted, at that point (121).

Between late 2002 and beginning of 2003, reports that two of the French patients developed leukaemia alarmed the scientific community and the regulators.

As a result, French Health Authority immediately suspended SCID gene therapy trials (122,123).

The underlying cause was potentially related to the enhancer activity of the viral long terminal repeat (LTR) which activated an oncogene. The LMO2 (LIM domain only–2) is a cysteine rich *Lin*-11 *Isl*-1 *Mec*-3 (LIM) protein required for normal haematopoiesis. Retroviral integration in the proximity of the LMO2 proto-oncogene promoter resulted in abnormal transcription and expression of LMO2 triggered malignant cell proliferation. Since the two leukaemia patients were the youngest and those who received the highest cell dose, these were identified as putative contributing risk factor (12,124). It was not until June 2004 that the temporary halt was lifted. The HA required a protocol amendment in order to restrict the age of the patient population as well as to limit a maximum number of genetically modified cells to be administered (125).

Over the next few years, in total, reports of leukaemia were noted for 4 of the 9 patients. Unfortunately, in October 2004, one of the patients died. These events highlighted the importance of adequate assessment of IM risk in gene therapy and, currently, in Europe, when submitting a MAA, applicants are expected to have data on IM for those candidate GTMPs which have that potential. Minimization of the risk of IM could be at the level of appropriate genetic regulation. In the X-linked SCID case, a potentially safer vector could be engineered based on removing the LTR enhancer element and adding an internal promoter which would modulate the properties of the preintegration factor. Another potential strategy could be directing the integration into neutral region of the genome (‘safe harbour’) (12,101,126,127). Insertion profile as well as vector persistence should also be considered (116). Vectors that do not efficiently integrate into the host genome include AAVs, plasmids, or retroviral vectors modified to avoid integrations. Instead, the use of integrating vectors such as gamma-retroviruses, lentivirus, and transposons may increase the potential for oncogenesis (128). However, compared with gamma-retroviruses, lentiviruses such as HIV type 1 (HIV-1) are more likely to integrate within active transcription units not related to proliferation-associated genes or transcriptional start sites, which suggests a lower potential for triggering oncogenic adverse events (127).

Higher vector dose administration may have an increased potential for insertional mutagenesis, as the number of integrations/transduced cells is directly

proportional to the number of vectors present. Additionally, the mechanism of action of the transgene product may also influence potential mutations. For example, if this product is involved in cellular growth then accelerated occurrence of mutagenesis may be observed.

Finally, the target cell population/organ of the gene therapy medicinal product is highly likely to influence the oncogenic profile. Generally, the risk of oncogenic events appears to be inversely related to the maturity of cells/tissues. For instance, gamma-retroviral vectors can induce oncogenic events in HSC but not in mature lymphocytes, likely as a result of the different genetic program of the two cells types (128).

Several strategies were developed to evaluate the oncogenicity of gene therapy medicinal products. Non-clinical integration studies are required for drug candidates that are expected to have insertional mutagenesis potential. Moving on to the clinical studies, the oncogenic profile of a gene therapy product is difficult to predict considering the limited experience in humans with a low number of patients that have been treated with vector to date, the longer follow-up periods that are required and the possibility that the background disease could contribute to increase the risk (129).

Strategies to overcome potential oncogenicity include modification of vector design to prevent activation of oncogenic genes at the integration sites, utilization of non-integrating vectors or highly targeted genomic integration at the desired chromosomal loci (130).

Considering these challenges and the often irreversible effects of gene transfer, the CHMP Gene Therapy Working Party developed a range of scientific guidelines to minimize these risks (130). The safety follow-up requirements for patients administered with gene therapy medicinal products is one of the most important documents (131), detailing recommendations for clinical monitoring and safety follow-up in order to detect early or delayed signals of adverse reactions, prevent clinical consequences of such reactions, ensure timely treatment and gain insights on long-term safety and efficacy. The clinical follow-up activities described in this guideline should not be established in isolation but rather as an addition to the common pharmacovigilance requirements. Safety monitoring may be required within days, weeks or even years after gene therapy treatment administration. For example, an adverse reaction related to immunogenicity may be detected just a few hours after

treatment administration, as opposed to an oncogenic safety event which may take years to be noted. Most of the recommendations for the different GT products include follow-up at pre-treatment, 3, 6 and 12 months and then yearly thereafter for 5 years or longer. The decision on the extent and duration of clinical follow-up requires a case-by-case analysis since there are many different factors that should be taken into consideration (**Table 2**).

Table 2 – EMA guidance on safety follow-up after gene therapy administration

Factors that influence extent and duration of Gene Therapy clinical follow-up
1. *Potential for and extent of chromosomal integration of a vector/gene* 2. *Capacity of a vector/gene for latency/reactivation* 3. *Capacity of a vector for inadvertent replication after complementation by viruses causing escape from latency and reactivation and eventually leading to mobilisation* 4. *Persistence of expression of the gene/vector/gene product* 5. *Replication incompetence or competence of a vector* 6. *Potential for recombination or re-assortment* 7. *Altered expression of (a) host gene(s)* 8. *Biodistribution to target/non-target organ(s tissue(s)/cell(s)* 9. *Known interactions with concomitant treatments or known interactions associated with previous exposure to potent agents (chemotherapy, radiotherapy etc.).*

1.1.4.2 Efficacy issues

One of the biggest issues preventing candidate GTMPs from reaching further development phases is the low efficacy/treatment failure likely related to poor transduction rate (84,116).

Generally, viral vectors offer higher transduction efficiency and long-term gene expression, when compared to non-viral vectors (7). For instance, AAV2 was the first discovered adeno-associated virus serotype used in early neurodegenerative disorder studies, due to its high neurotropism. Direct injection in the brain parenchyma represents an advantage when compared to systemic administration since it overcomes the need of the vector to pass the blood-brain-barrier. Additionally, neurodegenerative disorders are often multifocal, affecting several Central Nervous System (CNS) structures. Widespread CNS distribution of the vector is essential for high treatment efficacy. However, after direct brain administration of the vector it was noted that AAV2 action was limited to the site of injection. Rather than having a strong transduction efficiency throughout the CNS, AAV2 was only able to transduce cells in a limited area. This seemed to be partially related to binding of extracellular matrix components which would prevent intracellular intake (30,33,101).

Viral tropism is the affinity to a specific cell or tissue. In recombinant vectors, the tropism is highly dependent on the capsid proteins. Improvement of transgene expression can be accomplished by using a vector with natural tropism for the target cell or engineering the vector's surface in order to change the original tropism to the desired target cell (pseudotyping). The latter consists of introducing viral genetic content into a different envelope or altering any capsid protein (48). A great example of viral pseudotyping is gene therapy development in cystic fibrosis. Direct airway drug delivery encounters a number of challenges such as low availability of relevant vector receptors, short contact time between vector and epithelium, and the barrier function of airway mucus (132). Lentiviral vectors are quite efficient in gene transduction. However, these do not have any natural lung tropism, as opposed to Sendai virus. Pseudotyping with the fusion (F) and hemaglutinin-neuraminidase (HN) protein from Sendai virus is a strategy to overcome lentivirus' natural tropism, depicted in **Figure 7** (72). A study showed that the F/HN-Pseudotyped Lentivirus had significantly greater *in vitro* transduction efficiency when compared to GL67A, the most efficient non-viral vector (133).

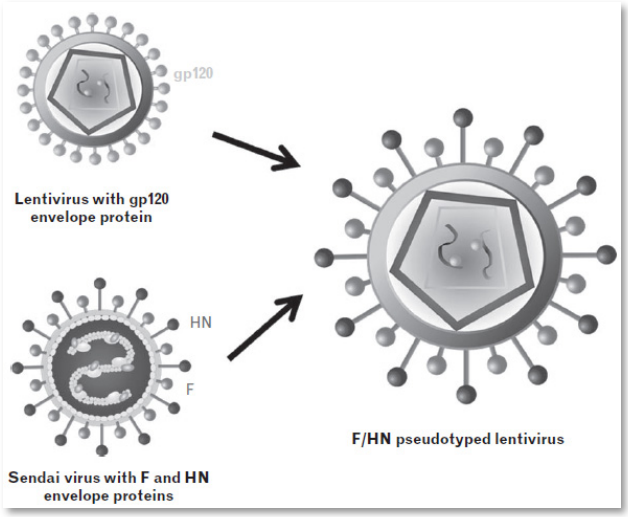


Figure 7 – Generation of F/HN-pseudotyped vector

Figure adapted from Griesenbach, et al., 2016 (72). In the development of F/HN-pseudotyped lentiviral vector, the gp120 protein on the lentivirus envelope glycoprotein was replaced with F and HN proteins from the Sendai virus. F, fusion; HN, hemagglutinin-neuraminidase

Another major hurdle for efficient gene transduction is the endogenous presence of neutralizing antibodies, either against the viral vector or the transgene product. Generally, these antibodies specifically recognize viral capsid proteins, preventing infection. This is of particular importance in therapeutic vectors since

these are produced from viruses and pre-existing humoral immunity may be an issue not only because it prevents transduction but also because it limits the gene therapy product administration more than once (29,48). On the other hand, antibodies against the transgene product may result in recruitment of immune cells to the therapeutic product production site with consequent inactivation of the protein (48).

In Glybera®, limited efficacy was shown in pivotal studies, especially one year after administration, which is not compatible with the intended one-time treatment administration of the GTMP, as a sustained therapeutic effect was not obvious. Viability of retreatment with gene therapy may be achieved by using different serotype vectors, less likely to infect humans. A second administration may be possible if a vector derived from a different serotype is used (52).

Possible strategies to overcome humoral immunity in systemic gene transfer include:

- Select subjects with low-to-undetectable anti-vector neutralizing antibodies (Nab);
- Administer higher vector doses (although this may have an impact on safety events);
- Use empty capsids to adsorb anti-vector antibodies thus allowing transduction;
- Administer immune suppression to prevent or eradicate humoral immune responses;
- Switch vector serotype or engineer vector capsids that are less susceptible to Nab;
- Use repeated plasma exchange cycles to adsorb immunoglobulins and, therefore, reduce the anti-vector antibody titre (66).

However, in some cases, the low transduction rate is more than enough to have positive clinical results. In haemophilia B, gene therapy administration resulted in less than 10% of normal concentration of the missing clotting factor. This brought significant clinical benefit to a point where a proportion of the treated patients no longer needed artificial clotting factor replacement therapy (134).

1.1.4.3 Drug development issues (non-clinical and scale-up)

Because of its unique set of characteristics, the non-clinical development package of a gene therapy medicinal product is more complex than conventional medicinal products. Regulators soon recognized that ICH M3 (R2), the general guidance for non-clinical development requirements of new drugs, was inadequate in several aspects when discussing GTMPs. Therefore, in Europe, the EMA released in 2006 a scientific guideline which details the non-clinical studies required before first clinical use specifically targeted at GTMPs (135). One of the most important

differences is that the applicant is expected to have data on the vector particle/delivery system and on the therapeutic transgene(s) as included in the GTMP. The regulators are open to accept data obtained from other similar products. For example, if the same vector is used between two gene therapy candidates with a different transgene product, then the non-clinical studies on the vector can be used, although this may generally not be enough to support first clinical use.

This approach is currently being explored by a number of companies. For instance, Glybera®'s UniQure offers a modular AAV-based viral vector platform. Theoretically, the same viral vector could be used to treat different diseases, according to the disease-specific gene content. The greatest advantage would be to have a less extensive preclinical development package reducing time and cost when seeking regulatory approval (136).

Finding adequate animal models may also be an additional challenge and when these are not representative of the clinical situation, regulators encourage the use of homologous animal models (135). Several studies revealed that gene delivery in animal models does not always match clinical setting, from different immune responses to unmatched vector tropism (4).

In trials involving recombinant AAV, an immunological response in humans was observed, which was not seen in the corresponding animal models. This resulted in expression of transgene product levels lower than expected. For example, in a clinical trial for haemophilia patients where FIX was delivered to patients via AAV2 vector, two subjects developed an unexpected T Cell response to the vector's capsid 4 to 6 weeks after treatment administration (137). The FIX transgene expression declined to baseline values and around the same time there was an elevation in the hepatic transaminases, suggesting a destruction of transduced hepatocytes. This had not been seen in animal studies. The authors suggested this event was related to cytotoxic T lymphocyte (CTL) response to the vectors capsid, highlighting that humans are naturally infected by AAV, which is not the case for murine models (137–139).

In spite of the widespread use of rodent models, larger animal models such as nonhuman primates have proved to be more valuable when it comes to clinical translation, especially regarding toxicology and pharmacokinetics (4).

Manufacturing of gene therapy products is an additional complexity factor. From a regulatory standpoint, these products need to comply with additional guidelines. In Europe, the note for guidance which details the quality aspects of gene therapy medicinal products (140) was developed in 2001, several years before the implementation of the ATMP regulation or the Committee for Advanced Therapies (CAT), though a revision was made in 2015 (141).

In general, non-viral vectors are more straightforward to produce since they are synthetically developed as opposed to viral vectors (130). In a very simplistic approach, the manufacturing method of a viral vector includes upstream (i.e. the vector assembly) and downstream processes (i.e. vector purification) (142).

The manufacturing process should be GMP compliant, clearly described and performed in certified GMP facilities. For the starting materials, demonstrated evidence on source, quality and control is needed, for both chemical reactants and bacterial/cell/virus seed. On the other hand, the drug substance (i.e. genetic content) should have an extensive genotypic and phenotypic characterization. Its biologic activity should be tested through assessment of the level of transgene expression. Presence of contaminant substances to detect both product-related and process-related impurities (e.g. remaining solvent from purification process) should be carefully determined (141).

Whereas cost and the time are objective parameters in evaluating process efficiency, determining the quality of the production of a recombinant viral vector is not straightforward. Due to the limited experience and low number of approved gene therapy products, vector analytics are not standardized, and contaminants that are present could be completely different among different processes (e.g. residual helper virus vs. residual plasmid sequences, human cells vs. insect cells versus animal cells, etc.). Moreover, assays to test gene therapy products in respect to quality, safety, and efficacy must be developed and validated, which is an additional time consuming task (142,143). From a quality point of view, *ex vivo* modified cells represent an even higher complexity degree, whether allogenic or autologous cells are used.

Any changes in manufacturing methods may require an assessment of comparability to ensure that these changes have not affected the safety, identity, purity or efficacy of the product (144).

Due to its unique characteristics, gene therapy products require an environmental risk assessment/shedding studies, which intend to collect information about the likelihood of transmission to untreated individuals and measures to prevent such transmission. Shedding is the excretion/secretion of viral particles or bacteria that could be transmitted to other individuals than the patient (144).

Generally, vector manufacturing systems often provide relatively low yields, making clinical administration or non-clinical studies in large animal models quite difficult. Over the past few years, many research groups focused on improving manufacturing processes towards a better up scaling of the product (130). Joshua Grieger's group developed a strategy based on triple transfection for the production of AAV vectors (145). HEK293 packaging cell line unit is used as a basis where three different plasmids are added: a replication (Rep) and Capsid (Cap) plasmid, the desired recombinant vector genome plasmid, and a helper plasmid expressing adenoviral genes. AAV needs a helper virus, such as an adenovirus or a herpes simplex virus, for adequate replication. By using the third plasmid, addition of the helper virus is unnecessary and the biological hazard of the manufacturing process is reduced.

HEK293 cells are cultured in adherence using bovine serum-based growth media which means that an extensive area would be required to obtain good vector yields. However, Joshua Grieger's group addressed this challenge by developing a method where the cells grow in suspension in serum-free media, within 20 litters-bioreactors. The safety of the process was increased since the source of adventitious agents was removed, with reduced manufacturing costs. Conversely, larger scale up (to bioreactor with over 200 litters) has not yet been demonstrated.

When using HEK293 cells for rAAV production the very low yield is a major limitation. Recombinant baculvirus and insect cells may be an attractive alternative. In 2002, Masashi Urabe's team co-infected insect *Sf9* cells with 3 recombinant baculovirus with positive results. Comparing to vectors produced via HEK293 cells, the yield was several times higher and the resulting rAAVs were identical between the two processes (146). In the last few years, some research groups focused their work in fine tuning this process. Mario Mietzsch's group developed the OneBac in 2014, a system based on insect *Sf9* cell lines containing silent copies of AAV serotypes 1-12 rep and cap genes. Cell induction takes place upon infection with a

single baculovirus, carrying the rAAV genome. Besides being a scalable and high-titter production method, the greatest advantage of OneBac is to allow production of a broad spectrum of AAV serotypes (147). The downstream purification process many include centrifugation and chromatography to remove the empty capsids, which are critical in reducing immune responses due to capsid antigens. As expected, the centrifugation of large volumes is time consuming and a hurdle in up scaling (142,145).

1.1.4.4 Ethical considerations

The discussion on the bioethical hurdles of gene therapy is extensive and focuses on the controversial results that might come from using gene manipulation in both patients and healthy individuals.

Currently, at least in the Western countries, clinical use of gene therapy is limited to somatic cells for the treatment of a specific disease. In a consensus document from the Council of Europe's Convention on Human Rights and Biomedicine from 1997 it is defined that "*An intervention seeking to modify the human genome may only be undertaken for preventive, diagnostic, or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants"*. Therefore, the use of gene therapy in germline cells with corresponding genetic modification of human gametes or embryos, is not allowed (148).

The discovery of more advanced gene editing tools such as CRISPR/Cas-9 technology, transformed the otherwise academic and theoretical debate of germline genetic manipulation into an actual possibility. The CRISPR/Cas-9 technology was used in recent experiments where human germline cells were genetically manipulated, by a Chinese research group (149). Almost as a response to this paper, the members of the Organizing Committee for the International Summit on Human Gene Editing published a summit statement where it is highlighted that *in vitro* research including human germline manipulation is acceptable as long as the modified cells are not used to establish a pregnancy (148). To obtain strong and reliable safety and efficacy data, this would require the study of many generations. In 1985, French Anderson defined three conditions that should be met prior to any

attempt to undergo germline gene therapy in humans, which are still valid and up-to-date:

1. Considerable and well-built previous experience with somatic cell gene therapy in humans proving safety and efficacy of the approach;
2. Adequate animal research that set up the reproducibility, reliability, and safety of germline therapeutic interventions and
3. The informed public approval of the procedure, since this will impact generations to come and, therefore, the society as a whole (150).

Another important topic to address is the potential of using gene therapy for purposes other than disease treatment, such as enhancement of genetic engineering or eugenetics. Enhancement of genetic engineering refers to adding a single gene or making changes in a single gene in healthy individuals, while eugenetics can be defined as the attempt to change or improve complex human traits, related to a broader number of genes; for example, personality, intelligence, character. Consequences of such approaches are yet to be determined, in terms of safety or misuse. In this context, the widespread use of gene therapy may have the potential to make society less accepting of people who are different (148,150,151).

Patient access to gene therapy medicinal products raises an additional bioethical issue related to the affordability of these new innovative and potentially curing drugs. Economic difficulties, particularly with regard to unbalanced wealth distribution, may restrict the use of gene therapy products to those who are able to afford them. Glybera®, the first gene therapy to be commercially approved in Europe, set its market price at around a million euros (US$1.1 million) per treatment. For *ex vivo* gene therapies, where patients own cells are modified and then reinfused back into circulation, highly personalized and individualized manufacturing are required, potentially increasing even more the drug cost. Gene therapies have the potential to provide substantial, lifelong benefit to the patient on a single administration, which may compensate the cost of the standard treatment of the condition and its complications (34).

1.2 European Regulatory Background of Advanced Therapy Medicinal Products

1.2.1 From Directive 2003/63/EC to the ATMP Regulation

Legally, in Europe, the ATMP concept was first introduced in 2003 through Directive 2003/63/EC where ATMPs were defined as products "*based on manufacturing processes focused on various gene transfer produced bio-molecules, and/or biologically advanced therapeutic modified cells as active substances or part of active substances*" (152). Therefore, Tissue Engineered Products (TEPs) were excluded as medicinal products, leading to ambiguity across Europe. In order to address this gap, in 2007, Regulation (EC) number 1394/2007, also known as "ATMP Regulation", was created.

The ATMP regulation is considered a *lex speciallis* which intends to present a clear definition of ATMPs, outline the marketing authorization requirements and procedures and describe the post-authorization obligations, specifically focusing on efficacy, safety and risk management. ATMPs include:

- Gene Therapy Medicinal Products (GTMPs);
- Somatic Cell Therapy Medicinal Products (sCTMP);
- TEPs and
- Combined ATMPs (153).

The definition of GTMP can be found in Directive 2009/120/EC amending Directive 2001/83/EC, part IV of Annex I. GTMP are defined as biological medicinal products which include "*an active substance containing or consisting of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence. Its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence*". GTMP do not include vaccines against infectious diseases (154).

Generally, gene therapy can be divided into two categories: germ line gene therapy and somatic gene therapy. In somatic gene therapy, the genetic material is inserted within the target cells, though the change is not passed on to the next generation, whereas in germ line gene therapy the therapeutic or modified gene will be passed along to the next generation. This is a significant difference, since the current legislation only allows gene therapy on somatic cells (155). Detailed definition for sCTMP, TEPs and combined ATMPs can be found elsewhere (153,154).

1.2.2 The Committee for Advanced Therapies: a key player in Marketing Authorization Application for ATMPs

ATMPs' MAA should follow the centralized procedure on a compulsory basis. The benefits of centralized review include overcoming the scarcity of expertise in this area, ensuring a high level of scientific evaluation by a specialized committee. Since the outcome of the MAA process is applicable to all Member States, the centralized procedure aims at improving market access for these innovative therapies.

Generally, medicinal products for human use are assessed by the CHMP, the main scientific committee of the EMA. The scientific assessment of ATMPs is slightly different as the primary review is performed by the Committee for Advanced Therapies (CAT). This is an independent specialist committee which the main responsibility is to review MAAs for ATMPs and issue a draft opinion for the CHMP to make a recommendation to the European Commission (EC), which has the final authority to grant marketing authorization (**Figure 8**) (1,53).

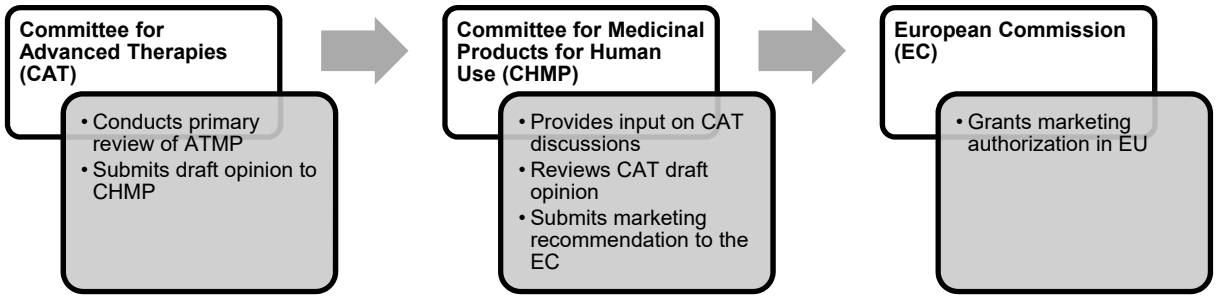


Figure 8 – European ATMP Marketing Authorization process overview

Figure adapted from Bryant, *et al.,* 2013. ATMP: Advanced Therapy Medicinal Product. CAT: Committee for Advanced Therapies. CHMP: Committee for Medicinal Products for Human Use. EC: European Comission. EU: Europe.

The assessment of a new application for an ATMP takes up to 210 'active' days, excluding the response time taken by the applicant. There are two independent review teams including a Rapporteur team and a Co-Rapporteur team. This latter considers the matter in parallel to, and independently from, the rapporteur. This active evaluation time is interrupted by at least one 'clock stop' (CS) at day 120 where the CAT adopts Day 120 (D12o) list of questions (LoQ). This list of questions includes major objections with regards to quality, non-clinical and clinical data. Another CS may occur at Day 180 (D180) where the CAT adopts a List of outstanding issues (LoOI) to be addressed by the applicant. Finally, an oral explanation (OE) of the applicant, if required, may be held in front of the CAT. The CHMP may express a divergent view than the CAT, although this should not be expected as a member of the CHMP is included in both the Rapporteur and the Co-rapporteur assessment teams ensuring alignment across both committees (**Figure 9**) (1).

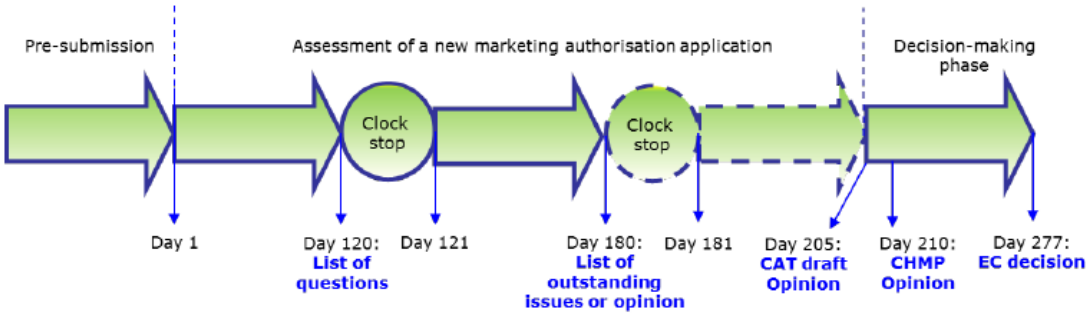


Figure 9 – Assessment of a new ATMP Marketing Authorization Application

The CAT is lead by an elected chair and includes members of the CHMP, representatives of each EU Member State, patients' organizations representatives and clinicians representatives nominated by the European Commission (1).

Besides reviewing applications for marketing authorization, another of the CAT's major tasks is to encourage the development of new ATMPs. Several regulatory strategies are currently in place to support ATMP development where the CAT plays a central role, such as i) the Innovative Task Force, ii) the ATMP Classification, iii) the ATMP Certification, iv) the Scientific Advice and v) the PRIority MEdicines (PRIME) scheme. Finally, the CAT should also scientifically assist in the elaboration of any documents related to the fulfilment of the objectives of the ATMP Regulation (153,156).

ATMPs without a centralized European MAA may anyhow be approved in individual member states. Due to the small scale and developmental nature of some intra-hospital ATMP applications, regulation 2001/83/EC includes the Hospital exemption (HE) concept for products not intended to be marketed. ATMPs approved via HE must be prepared on a non-routine basis, in a non-industrial manner and used as a custom made product for an individual patient. However, the definition of 'non-routine basis', 'industrial manner' and 'custom made' are not specified by the regulation. Therefore, different interpretations exist among European countries(157).

Obtaining Marketing Authorization Authorization of a gene and/or cell therapy product is a worldwide diverse process. Different steps and requirements may be needed depending on the evaluating regulatory body (158). For instance, in the US, gene and cell therapies are considered biologic therapies. Within the Food and Drug Administration (FDA), these products' primary oversight falls under the Office of Cellular, Tissue and Gene Therapy (OCTGT) which is a division of the Center for Biologics Evaluation and Research (CBER).

Initially, an IND application is needed for the investigational use of a biologic. It intends to support clinical use of the investigational product based on quality and non-clinical data. In order to market a biologic drug product, FDA requires Sponsors to hold an approved Biologics License Application (BLA). Timelines for evaluation range from 10 to 12 months from filing, depending on the pathway under which the BLA is reviewed. Like the EMA, the FDA has a number of initiatives in place in order to support the development of Gene and Cell therapies. These include i) Fast Track designation, ii) Breakthrough Therapy designation, iii) Accelerated Approval, and iv) Priority Review designation. As an example, in case the BLA is evaluated under Priority Review, a reduction to 6-month review time may be granted. Several web-based trainings hosted by OCTGT staff focusing on many regulatory topics can be easily found elsewhere(159) and additional supportive information is available in FDA's website(160).

1.3 Objectives and thesis outline

ATMPs have the potential to be preventive but also curative therapeutic approaches. Its possible application in diseases of high unmet medical need is promising. On the one hand, despite extensive research has been conducted and many clinical trials investigating ATMPs have been put in place, only a modest number of products have been approved, particularly in Europe(3). On the other hand, a significant impact in the health care systems is expected(2). For these reasons, broad understanding of ATMPs is fundamental to manage their availability and recommendation of use appropriately.

Much research has been conducted with the aim to analyze individual or a restricted group of obstacles preventing regulatory approval or post-marketing gene therapy availability. To the best of our knowledge, no research group has attempted to present a complete set of hurdles, towards gene therapy patient access. Therefore, the purpose of this research project is to develop an end-to-end understanding of ATMPs, from drug development to regulatory post-authorization use, by answering the following research questions:

1. Taking into consideration the particularities of ATMPs and the enormous potential to address unmet medical needs for serious conditions, what are the key factors influencing the regulatory approval process for obtaining a marketing authorization?
2. From those ATMPs already approved, what are the challenges preventing patient access to these innovative therapies?

This thesis is divided into four chapters. In **Chapter 1**, a description of strategic clinical applications is presented, particularly in gene therapy medicinal products, focusing on currently EU approved medicines as well as various promising investigational treatments. A number of pre-identified challenges in gene therapy development and post-authorization use are explored. Finally, a regulatory overview of the legal framework in Europe towards granting ATMP marketing authorization is provided.

Chapter 2 focuses on the regulatory analysis until the moment a Marketing Authorization is granted. Here, key aspects influencing regulatory GTMP approval, in

Europe, are identified. The analysis is fully based on publicly available data in the EMA's website, between March 2009 and December 2017. In chapter 2.1, MAAs for ATMPs submitted and assessed by the CAT are identified. Then, in chapter 2.2, the MAA regulatory process is fully characterized, especially focusing on the timelines, milestones and final outcome. Lastly, in chapter 2.3, a comparison of the major objections found in MAA assessment amongst GTMPs is presented and discussed.

The focal point of **Chapter 3** is the post-marketing setting. In this chapter, an analysis of the most recently available literature is provided, where the main objective is to identify a full set of hurdles potentially preventing patient access to Gene Therapies. This comprehensive review is based on a systematic approach, using data from two distinct databases and was conducted by identifying relevant, peer-reviewed publications, between the years of 2012 and 2018.

Chapter 4 comprises a general discussion of the research results obtained in chapters 2 and 3. The results are summarized and discussed, in light of similar studies, the current ATMP and gene therapy landscape, contextualized in the most up to date body of evidence. Study limitations are explored and future perspectives about the identified hurdles, both at the regulatory and post-marketing level, are presented.

1.4 References

1. Celis P. CAT--the new committee for advanced therapies at the European Medicines Agency. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz [Internet]. 2010 Jan;53(1):9–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20084354

2. Hanna E, Rémuzat C, Auquier P, Toumi M. Advanced therapy medicinal products: current and future perspectives. J Mark Access Heal Policy [Internet]. 2016 Jan 25;4(1):31036. Available from: http://www.jmahp.net/index.php/jmahp/article/view/31036

3. Boráň T, Menezes-Ferreira M, Reischl I, Celis P, Ferry N, Gänsbacher B, et al. Clinical Development and Commercialization of Advanced Therapy Medicinal Products in the European Union: How Are the Product Pipeline and Regulatory Framework Evolving? Hum Gene Ther Clin Dev [Internet]. 2017 Sep;28(3):126–35. Available from: http://online.liebertpub.com/doi/10.1089/humc.2016.193

4. Wang D, Gao G. State-of-the-art human gene therapy: part I. Gene delivery technologies. Discov Med [Internet]. 2015;18(97):67–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25091489

5. Medina-Kauwe LK, Xie J, Hamm-Alvarez S. Intracellular trafficking of nonviral vectors. Gene Ther [Internet]. 2005 Dec;12(24):1734–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16079885

6. Patil SD, Rhodes DG, Burgess DJ. DNA-based therapeutics and DNA delivery systems: a comprehensive review. AAPS J [Internet]. 2005 Apr 8;7(1):E61-77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16146351

7. Wang W, Li W, Ma N, Steinhoff G. Non-viral gene delivery methods. Curr Pharm Biotechnol [Internet]. 2013;14(1):46–60. Available from: http://benthamscience.com/journals/current-pharmaceutical-biotechnology/volume/14/issue/1/page/46/

8. Jin L, Zeng X, Liu M, Deng Y, He N. Current progress in gene delivery

technology based on chemical methods and nano-carriers. Theranostics [Internet]. 2014;4(3):240–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24505233

9. Rogers S, Lowenthal A, Terheggen HG, Columbo JP. Induction of arginase activity with the Shope papilloma virus in tissue culture cells from an argininemic patient. J Exp Med [Internet]. 1973 Apr 1;137(4):1091–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4348278

10. Terheggen HG, Lowenthal A, Lavinha F, Colombo JP, Rogers S. Unsuccessful trial of gene replacement in arginase deficiency. Z Kinderheilkd [Internet]. 1975 Dec;119(1):1–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25246403

11. Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M, et al. T lymphocyte-directed gene therapy for ADA- SCID: initial trial results after 4 years. Science (80-) [Internet]. 1995 Oct 20;270(5235):475–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7570001

12. Fischer A, Hacein-Bey-Abina S, Cavazzana-Calvo M. 20 years of gene therapy for SCID. Nat Immunol [Internet]. 2010 Jun;11(6):457–60. Available from: http://www.nature.com/doifinder/10.1038/ni0610-457%5Cnpapers3://publication/doi/10.1038/ni0610-457

13. Notarangelo LD. Primary immunodeficiencies. J Allergy Clin Immunol [Internet]. 2010 Feb;125(2 Suppl 2):S182-94. Available from: http://dx.doi.org/10.1016/j.jaci.2009.07.053

14. Bordignon C, Notarangelo LD, Nobili N, Ferrari G, Casorati G, Panina P, et al. Gene therapy in peripheral blood lymphocytes and bone marrow for ADA-immunodeficient patients. Science (80-) [Internet]. 1995 Oct 20;270(5235):470–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7570000

15. Stolberg SG. The Biotech Death of Jesse Gelsinger. The New York Times [Internet]. 1999 Nov 28; Available from: www.nytimes.com/1999/11/28/magazine/the-biotech-death-of-jesse-

gelsinger.html

16. Wilson RF. The death of Jesse Gelsinger: new evidence of the influence of money and prestige in human research. Am J Law Med [Internet]. 2010;36(2–3):295–325. Available from: http://heinonlinebackup.com/hol-cgi-bin/get_pdf.cgi?handle=hein.journals/amlmed36&section=14

17. European Medicines Agency. CAT Monthly Report June 2009 [EMEA/CAT/394179/2009] [Internet]. 2009. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Committee_meeting_report/2009/10/WC500005968.pdf

18. Wirth T, Samaranayake H, Pikkarainen J, Määttä A-M, Ylä-Herttuala S. Clinical trials for glioblastoma multiforme using adenoviral vectors. Curr Opin Mol Ther [Internet]. 2009 Oct;11(5):485–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19806496

19. Osborne R. Ark floats gene therapy's boat, for now. Nat Biotechnol [Internet]. 2008 Oct;26(10):1057–9. Available from: http://dx.doi.org/10.1038/nbt1008-1057

20. European Medicines Agency. Cerepro®'s Withdrawal Assessment Report [EMEA/203243/2008]. 2007; Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Application_withdrawal_assessment_report/2010/01/WC500068119.pdf

21. European Medicines Agency. Ark Therapeutics Ltd withdraws its marketing authorisation application for Cerepro® (sitimagene ceradenovec) [EMA/151854/2010] [Internet]. 2010. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2010/03/WC500075457.pdf

22. Burnett JR, Hooper AJ. Alipogene tiparvovec, an adeno-associated virus encoding the Ser(447)X variant of the human lipoprotein lipase gene for the treatment of patients with lipoprotein lipase deficiency. Curr Opin Mol Ther [Internet]. 2009 Dec;11(6):681–91. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/20072945

23. European Medicines Agency. CAT monthly report of application procedures, guidelines and related documents on advanced therapies - October 2015 [EMA/CAT/701141/2015] [Internet]. 2015 [cited 2020 Feb 26]. Available from: https://www.ema.europa.eu/en/documents/committee-report/cat-monthly-report-application-procedures-guidelines-related-documents-advanced-therapies-october_en-0.pdf

24. European Medicines Agency. CAT monthly report of application procedures, guidelines and related documents on advanced therapies - March 2016 [EMA/CAT/237075/2016] [Internet]. 2016 [cited 2020 Feb 26]. Available from: https://www.ema.europa.eu/en/documents/committee-report/cat-monthly-report-application-procedures-guidelines-related-documents-advanced-therapies-march-2016_en.pdf

25. European Medicines Agency. CAT monthly report of application procedures, guidelines and related documents on advanced therapies - June 2018 [EMA/CAT/435550/2018] [Internet]. 2018 [cited 2020 Feb 26]. Available from: https://www.ema.europa.eu/en/documents/committee-report/cat-monthly-report-application-procedures-guidelines-related-documents-advanced-therapies-june-2018_en.pdf

26. European Medicines Agency. CAT monthly report of application procedures, guidelines and related documents on advanced therapies - September 2018 [EMA/CAT/643189/2018] [Internet]. 2018 [cited 2020 Feb 26]. Available from: https://www.ema.europa.eu/en/documents/committee-report/cat-monthly-report-application-procedures-guidelines-related-documents-advanced-therapies-september_en-5.pdf

27. European Medicines Agency. CAT monthly report of application procedures, guidelines and related documents on advanced therapies - March 2019 [EMA/CAT/198680/2019] [Internet]. 2019 [cited 2020 Feb 26]. Available from: https://www.ema.europa.eu/en/documents/committee-report/cat-monthly-report-application-procedures-guidelines-related-documents-advanced-therapies-

march-2019_en.pdf

28. Wang D, Gao G. State-of-the-art human gene therapy: part II. Gene therapy strategies and clinical applications. Discov Med [Internet]. 2014 Sep;18(98):151–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25227756%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4440458

29. Bessis N, GarciaCozar FJ, Boissier M-C. Immune responses to gene therapy vectors: influence on vector function and effector mechanisms. Gene Ther [Internet]. 2004 Oct;11 Suppl 1(June):S10-7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15454952

30. Choudhury SR, Hudry E, Maguire CA, Sena-Esteves M, Breakefield XO, Grandi P. Viral vectors for therapy of neurologic diseases. Neuropharmacology [Internet]. 2016 Feb 21; Available from: http://www.ncbi.nlm.nih.gov/pubmed/26905292

31. Verlengia G, Miyagawa Y, Ingusci S, Cohen JB, Simonato M, Glorioso JC. Engineered HSV vector achieves safe long-term transgene expression in the central nervous system. Sci Rep [Internet]. 2017 Dec 4;7(1):1507. Available from: http://www.nature.com/articles/s41598-017-01635-1

32. J. Cann A. Principles of molecular virology - 5th edition [Internet]. Elsevier. 2005. 433 p. Available from: http://linkinghub.elsevier.com/retrieve/pii/0968000494900957

33. Li W, Asokan A, Wu Z, Van Dyke T, DiPrimio N, Johnson JS, et al. Engineering and selection of shuffled AAV genomes: a new strategy for producing targeted biological nanoparticles. Mol Ther [Internet]. 2008 Jul;16(7):1252–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18500254%5Cnhttp://www.nature.com/mt/journal/v16/n7/pdf/mt2008100a.pdf

34. Naldini L. Ex vivo gene transfer and correction for cell-based therapies. Nat Rev Genet [Internet]. 2011 May;12(5):301–15. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/21445084

35. Aiuti A, Cattaneo F, Galimberti S, Benninghoff U, Cassani B, Callegaro L, et al. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. N Engl J Med [Internet]. 2009 Jan 29;360(5):447–58. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19179314

36. Aiuti A, Biasco L, Scaramuzza S, Ferrua F, Cicalese MP, Baricordi C, et al. Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. Science (80-) [Internet]. 2013 Aug 23;341(6148):1233151. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23845947

37. Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med [Internet]. 2014 Feb 19;6(224):224ra25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24553386

38. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med [Internet]. 2014 Oct 16;371(16):1507–17. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa1407222%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/25317870

39. Frey N V., Porter DL. CAR T-cells merge into the fast lane of cancer care. Am J Hematol [Internet]. 2016 Jan;91(1):146–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26574400

40. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet [Internet]. 2015 Feb 7;385(9967):517–28. Available from: http://dx.doi.org/10.1016/S0140-6736(14)61403-3

41. Novartis. Novartis receives first ever FDA approval for a CAR-T cell therapy, Kymriah®(TM) (CTL019), for children and young adults with B-cell ALL that is refractory or has relapsed at least twice [Internet]. 2017 [cited 2017 Sep 9].

p. 1–13. Available from: https://www.novartis.com/news/media-releases/novartis-receives-first-ever-fda-approval-car-t-cell-therapy-Kymriah®tm-ctl019

42. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell [Internet]. 2006 Aug 25;126(4):663–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16904174

43. Takahashi K, Yamanaka S. Induced pluripotent stem cells in medicine and biology. Development [Internet]. 2013 Jun;140(12):2457–61. Available from: http://dev.biologists.org/cgi/doi/10.1242/dev.092551%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/23715538

44. Papapetrou EP, Lee G, Malani N, Setty M, Riviere I, Tirunagari LMS, et al. Genomic safe harbors permit high β-globin transgene expression in thalassemia induced pluripotent stem cells. Nat Biotechnol [Internet]. 2011 Jan;29(1):73–8. Available from: http://dx.doi.org/10.1038/nbt.1717

45. Yang G, Shi W, Hu X, Zhang J, Gong Z, Guo X, et al. Therapeutic effects of induced pluripotent stem cells in chimeric mice with β-thalassemia. Haematologica [Internet]. 2014 Aug;99(8):1304–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24816238

46. Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol [Internet]. 2008 Jan;26(1):101–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18059259

47. Ellis J, Baum C, Benvenisty N, Mostoslavsky G, Okano H, Stanford WL, et al. Benefits of utilizing gene-modified iPSCs for clinical applications. Cell Stem Cell [Internet]. 2010 Oct 8;7(4):429–30. Available from: http://dx.doi.org/10.1016/j.stem.2010.09.010

48. Kay MA. State-of-the-art gene-based therapies: the road ahead. Nat Rev Genet [Internet]. 2011 May;12(5):316–28. Available from: http://dx.doi.org/10.1038/nrg2971

49. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science (80-) [Internet]. 2014 Nov 28;346(6213):1258096. Available from: http://www.sciencemag.org/content/346/6213/1258096.long

50. Ivics Z, Izsvák Z. The expanding universe of transposon technologies for gene and cell engineering. Mob DNA [Internet]. 2010 Dec 7;1(1):25. Available from: http://www.mobilednajournal.com/content/1/1/25

51. Kassner U, Hollstein T, Grenkowitz T, Wühle-Demuth M, Salewsky B, Demuth I, et al. Gene Therapy in Lipoprotein Lipase Deficiency: Case Report on the First Patient Treated with Alipogene Tiparvovec Under Daily Practice Conditions. Hum Gene Ther [Internet]. 2018 Apr;29(4):520–7. Available from: http://www.liebertpub.com/doi/10.1089/hum.2018.007

52. European Medicines Agency. Glybera®'s European Public Assessment Report [EMA/882900/2011]. 2012; Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002145/WC500135476.pdf

53. Bryant LM, Christopher DM, Giles AR, Hinderer C, Rodriguez JL, Smith JB, et al. Lessons learned from the clinical development and market authorization of Glybera®. Hum Gene Ther Clin Dev [Internet]. 2013 Jun;24(2):55–64. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3992977&tool=pmcentrez&rendertype=abstract

54. Berg LJ. The "bubble boy" paradox: an answer that led to a question. J Immunol [Internet]. 2008 Nov 1;181(9):5815–6. Available from: http://www.jimmunol.org/content/181/9/5815%5Cnhttp://www.jimmunol.org/

55. Leonard WJ. Cytokines and immunodeficiency diseases. Nat Rev Immunol [Internet]. 2001 Dec;1(3):200–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11905829

56. Hacein-Bey-Abina S, Hauer J, Lim A, Picard C, Wang GP, Berry CC, et

al. Efficacy of gene therapy for X-linked severe combined immunodeficiency. N Engl J Med [Internet]. 2010 Jul 22;363(4):355–64. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2957288&tool=pmcentrez&rendertype=abstract

57. Gennery AR, Slatter MA, Grandin L, Taupin P, Cant AJ, Veys P, et al. Transplantation of hematopoietic stem cells and long-term survival for primary immunodeficiencies in Europe: entering a new century, do we do better? J Allergy Clin Immunol [Internet]. 2010 Sep;126(3):602-10.e1-11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20673987

58. Chinen J, Buckley RH. Transplantation immunology: solid organ and bone marrow. J Allergy Clin Immunol [Internet]. 2010 Feb;125(2 Suppl 2):S324-35. Available from: http://dx.doi.org/10.1016/j.jaci.2009.11.014

59. Slatter MA, Gennery AR. Stem Cell Transplantation for Primary Immunodeficiency. Immunodeficiency [Internet]. 2012;5(3):41–4. Available from: http://www.intechopen.com/books/immunodeficiency/stem-cell-transplantation-for-primary-immunodeficiency

60. European Medicines Agency. Strimvelis®' European Public Assessment Report [EMA/CHMP/323455/2016] [Internet]. 2016. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/003854/WC500208201.pdf

61. Touchot N, Flume M. Early Insights from Commercialization of Gene Therapies in Europe. Genes (Basel) [Internet]. 2017 Feb 17;8(2):78. Available from: http://www.mdpi.com/2073-4425/8/2/78

62. Maguire AM, High KA, Auricchio A, Wright JF, Pierce EA, Testa F, et al. Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. Lancet [Internet]. 2009 Nov 7;374(9701):1597–605. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19854499

63. European Medicines Agency. Luxturna™'s European Public Assessment Report [EMA/CHMP/700911/2018] [Internet]. 2018 [cited 2020 Mar

4]. Available from: https://www.ema.europa.eu/en/documents/assessment-report/Luxturna™-epar-public-assessment-report_en.pdf

64. Cappellini MD, Viprakasit V, Taher AT. An overview of current treatment strategies for β-thalassemia. Expert Opin Orphan Drugs [Internet]. 2014 Jul 15;2(7):665–79. Available from: http://www.tandfonline.com/doi/full/10.1517/21678707.2014.918503

65. European Medicines Agency. Zyntelgo's European Public Assessment Report [EMA/56140/2020/Corr] [Internet]. 2019 [cited 2020 Mar 4]. Available from: https://www.ema.europa.eu/en/documents/assessment-report/Zynteglo®-epar-public-assessment-report_en.pdf

66. High KH, Nathwani A, Spencer T, Lillicrap D. Current status of haemophilia gene therapy. Haemophilia [Internet]. 2014 May;20 Suppl 4(S4):43–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24762274

67. Lheriteau E, Davidoff AM, Nathwani AC. Haemophilia gene therapy: Progress and challenges. Blood Rev [Internet]. 2015 Sep;29(5):321–8. Available from: http://dx.doi.org/10.1016/j.blre.2015.03.002

68. High KA, Anguela XM. Adeno-associated viral vectors for the treatment of hemophilia. Hum Mol Genet [Internet]. 2016 Apr 15;25(R1):R36-41. Available from: https://academic.oup.com/hmg/article-lookup/doi/10.1093/hmg/ddv475

69. High KA. AAV-mediated gene transfer for hemophilia. Trans Am Clin Climatol Assoc [Internet]. 2003;114:337–51; discussion 351-2. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2194524&tool=pmcentrez&rendertype=abstract

70. Nathwani AC, Reiss UM, Tuddenham EGD, Rosales C, Chowdary P, McIntosh J, et al. Long-term safety and efficacy of factor IX gene therapy in hemophilia B. N Engl J Med [Internet]. 2014 Nov 20;371(21):1994–2004. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4278802&tool=pmcentrez&rendertype=abstract

71. Boucher RC. Airway surface dehydration in cystic fibrosis: pathogenesis and therapy. Annu Rev Med [Internet]. 2007 Feb;58(1):157–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17217330

72. Griesenbach U, Davies JC, Alton E. Cystic fibrosis gene therapy: a mutation-independent treatment. Curr Opin Pulm Med [Internet]. 2016 Nov;22(6):602–9. Available from: http://www.sciencedirect.com/science/article/pii/0959437X9490037X

73. Mitomo K, Griesenbach U, Inoue M, Somerton L, Meng C, Akiba E, et al. Toward gene therapy for cystic fibrosis using a lentivirus pseudotyped with Sendai virus envelopes. Mol Ther [Internet]. 2010 Jun;18(6):1173–82. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2889732&tool=pmcentrez&rendertype=abstract

74. Alton EWFW, Armstrong DK, Ashby D, Bayfield KJ, Bilton D, Bloomfield E V., et al. Repeated nebulisation of non-viral CFTR gene therapy in patients with cystic fibrosis: a randomised, double-blind, placebo-controlled, phase 2b trial. Lancet Respir Med [Internet]. 2015 Sep;3(9):684–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26149841

75. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J [Internet]. 2016 Jul 14;37(27):2129–200. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27894487

76. Hasenfuss G, Reinecke H, Studer R, Meyer M, Pieske B, Holtz J, et al. Relation between myocardial function and expression of sarcoplasmic reticulum Ca(2+)-ATPase in failing and nonfailing human myocardium. Circ Res [Internet]. 1994 Sep;75(3):434–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8062417

77. Giacca M, Baker AH. Heartening results: the CUPID gene therapy trial for heart failure. Mol Ther [Internet]. 2011 Jul;19(7):1181–2. Available from: http://dx.doi.org/10.1038/mt.2011.123

78. Jessup M, Greenberg B, Mancini D, Cappola T, Pauly DF, Jaski B, et al. Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID): a phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum Ca2+-ATPase in patients with advanced heart failure. Circulation [Internet]. 2011 Jul 19;124(3):304–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21709064

79. Greenberg B, Butler J, Felker GM, Ponikowski P, Voors AA, Desai AS, et al. Calcium upregulation by percutaneous administration of gene therapy in patients with cardiac disease (CUPID 2): a randomised, multinational, double-blind, placebo-controlled, phase 2b trial. Lancet [Internet]. 2016 Mar 19;387(10024):1178–86. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26803443

80. Hoxie JA, June CH. Novel cell and gene therapies for HIV. Cold Spring Harb Perspect Med [Internet]. 2012 Oct 1;2(10). Available from: http://www.ncbi.nlm.nih.gov/pubmed/23028130

81. Kwong PD, Mascola JR, Nabel GJ. Broadly neutralizing antibodies and the search for an HIV-1 vaccine: the end of the beginning. Nat Rev Immunol [Internet]. 2013;13(9):693–701. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23969737%5Cnhttp://www.nature.com/doifinder/10.1038/nri3516

82. Balazs AB, Ouyang Y, Hong CM, Chen J, Nguyen SM, Rao DS, et al. Vectored immunoprophylaxis protects humanized mice from mucosal HIV transmission. Nat Med [Internet]. 2014 Mar;20(3):296–300. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3990417&tool=pmcentrez&rendertype=abstract

83. Johnson PR, Schnepp BC, Zhang J, Connell MJ, Greene SM, Yuste E, et al. Vector-mediated gene transfer engenders long-lived neutralizing activity and protection against SIV infection in monkeys. Nat Med [Internet]. 2009 Aug;15(8):901–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2723177&tool=pmcentr

ez&rendertype=abstract

84. Brenner MK, Gottschalk S, Leen AM, Vera JF. Is cancer gene therapy an empty suit? Lancet Oncol [Internet]. 2013 Oct;14(11):e447-56. Available from: http://dx.doi.org/10.1016/S1470-2045(13)70173-6

85. Administration F and D. FDA approval brings first gene therapy to the United States [Internet]. 2017. p. 1–4. Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm

86. Salmikangas P, Kinsella N, Chamberlain P. Chimeric Antigen Receptor T-Cells (CAR T-Cells) for Cancer Immunotherapy – Moving Target for Industry? Pharm Res [Internet]. 2018 Aug 31;35(8):152. Available from: http://link.springer.com/10.1007/s11095-018-2436-z

87. Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells—challenges and opportunities in translating innovative treatment concepts. EMBO Mol Med [Internet]. 2017 Sep;9(9):1183–97. Available from: https://onlinelibrary.wiley.com/doi/abs/10.15252/emmm.201607485

88. Tokarew N, Ogonek J, Endres S, von Bergwelt-Baildon M, Kobold S. Teaching an old dog new tricks: next-generation CAR T cells. Br J Cancer [Internet]. 2019 Jan 9;120(1):26–37. Available from: http://dx.doi.org/10.1038/s41416-018-0325-1

89. Feigal EG, DeWitt ND, Cantilena C, Peck C, Stroncek D. At the end of the beginning: immunotherapies as living drugs. Nat Immunol [Internet]. 2019 Aug 15;20(8):955–62. Available from: http://www.nature.com/articles/s41590-019-0452-8

90. European Medicines Agency. European Public Assessment report: YESCARTA® [EMA/481168/2018] [Internet]. 2018 [cited 2020 Feb 26]. Available from: https://www.ema.europa.eu/en/documents/assessment-report/Yescarta®-epar-public-assessment-report_en.pdf

91. Andtbacka RHI, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, et al. Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma. J Clin Oncol [Internet]. 2015 Sep 1;33(25):2780–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26014293

92. European Medicines Agency. Imlygic®'s European Public Assessment Report [EMA/734400/2015] [Internet]. 2015. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002771/WC500201082.pdf

93. Hütter G, Nowak D, Mossner M, Ganepola S, Müssig A, Allers K, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. N Engl J Med [Internet]. 2009 Feb 12;360(7):692–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19213682

94. CCR5 and HIV infection [Internet]. [cited 2017 Jan 5]. Available from: http://thehealthconnections.com/2016/08/13/possible-cures-hivaids

95. Kitchen SG, Shimizu S, An DS. Stem cell-based anti-HIV gene therapy. Virology [Internet]. 2011 Mar 15;411(2):260–72. Available from: http://dx.doi.org/10.1016/j.virol.2010.12.039

96. Borel F, Kay MA, Mueller C. Recombinant AAV as a platform for translating the therapeutic potential of RNA interference. Mol Ther [Internet]. 2014 Apr;22(4):692–701. Available from: http://www.nature.com/doifinder/10.1038/mt.2013.285%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/24352214%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3982504

97. Coelho T, Adams D, Silva A, Lozeron P, Hawkins PN, Mant T, et al. Safety and efficacy of RNAi therapy for transthyretin amyloidosis. N Engl J Med [Internet]. 2013 Aug 29;369(9):819–29. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23984729

98. Suhr OB, Coelho T, Buades J, Pouget J, Conceicao I, Berk J, et al. Efficacy and safety of patisiran for familial amyloidotic polyneuropathy: a phase II

multi-dose study. Orphanet J Rare Dis [Internet]. 2015 Sep 4;10(1):109. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-84940759062&partnerID=tZOtx3y1

99. European Medicine Agency. Summary of opinion - Onpattro (patisiran) [EMA/CHMP/482444/2018] [Internet]. 2018 [cited 2020 Mar 2]. Available from: https://www.ema.europa.eu/en/documents/smop-initial/chmp-summary-positive-opinion-onpattro_en.pdf

100. Pfister EL, Zamore PD. Huntington's disease: silencing a brutal killer. Exp Neurol [Internet]. 2009 Dec;220(2):226–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19786020

101. Kumar SR, Markusic DM, Biswas M, High KA, Herzog RW. Clinical development of gene therapy: results and lessons from recent successes. Mol Ther Methods Clin Dev [Internet]. 2016;3(April):16034. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27257611%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4879992

102. Carroll D. Genome engineering with zinc-finger nucleases. Genetics [Internet]. 2011 Aug;188(4):773–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21828278

103. Tebas P, Stein D, Tang WW, Frank I, Wang SQ, Lee G, et al. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. N Engl J Med [Internet]. 2014 Mar 6;370(10):901–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24597865

104. Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol [Internet]. 2013 Jan;14(1):49–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23169466%5Cnhttp://www.nature.com/nrm/journal/v14/n1/pdf/nrm3486.pdf

105. Sun N, Zhao H. Transcription activator-like effector nucleases (TALENs): a highly efficient and versatile tool for genome editing. Biotechnol

Bioeng [Internet]. 2013 Jul;110(7):1811–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23508559

106. Qasim W, Amrolia PJ, Samarasinghe S, Ghorashian S, Zhan H, Stafford S, et al. First Clinical Application of Talen Engineered Universal CAR19 T Cells in B - ALL. Blood. 2015;126(23):2046.

107. Jiang F, Doudna JA. The structural biology of CRISPR-Cas systems. Curr Opin Struct Biol [Internet]. 2015 Feb;30:100–11. Available from: http://dx.doi.org/10.1016/j.sbi.2015.02.002

108. Shen B, Zhang J, Wu H, Wang J, Ma K, Li Z, et al. Generation of gene-modified mice via Cas9/RNA-mediated gene targeting. Cell Res [Internet]. 2013;23(5):720–3. Available from: http://dx.doi.org/10.1038/cr.2013.46

109. Niu Y, Shen B, Cui Y, Chen Y, Wang J, Wang L, et al. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell [Internet]. 2014 Feb 13;156(4):836–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24486104

110. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med [Internet]. 2012 Jun 28;366(26):2443–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22658127

111. Cyranoski D. Chinese scientists to pioneer first human CRISPR trial. Nature [Internet]. 2016;535(7613):476–7. Available from: http://www.nature.com/doifinder/10.1038/nature.2016.20302

112. Su S, Hu B, Shao J, Shen B, Du J, Du Y, et al. CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients. Sci Rep [Internet]. 2016 Jan 28;6:20070. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4730182&tool=pmcentrez&rendertype=abstract

113. Ma H, Marti-Gutierrez N, Park S-W, Wu J, Lee Y, Suzuki K, et al. Correction of a pathogenic gene mutation in human embryos. Nature [Internet].

2017 Aug 2;548(7668):413–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28783728

114. Cyranoski D. What CRISPR-baby prison sentences mean for research [Internet]. Nature. 2020 [cited 2020 Mar 2]. Available from: https://www.nature.com/articles/d41586-020-00001-y

115. Zhang X-H, Tee LY, Wang X-G, Huang Q-S, Yang S-H. Off-target Effects in CRISPR/Cas9-mediated Genome Engineering. Mol Ther Nucleic Acids [Internet]. 2015 Nov 17;4(11):e264. Available from: http://www.nature.com/doifinder/10.1038/mtna.2015.37%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/26575098

116. European Medicines Agency. Guideline on the risk-based approach according to Annex I , part IV of Directive 2001 / 83 / EC applied to Advanced Therapy Medicinal Products [EMA/CAT/CPWP/686637/2011] [Internet]. 2013. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/03/WC500139748.pdf

117. Wilson JM. Lessons learned from the gene therapy trial for ornithine transcarbamylase deficiency. Mol Genet Metab [Internet]. 2009 Apr;96(4):151–7. Available from: http://dx.doi.org/10.1016/j.ymgme.2008.12.016

118. Cornetta K, Smith FO. Regulatory issues for clinical gene therapy trials. Hum Gene Ther [Internet]. 2002 Jul 1;13(10):1143–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12133267

119. Deakin CT, Alexander IE, Kerridge I. Accepting risk in clinical research: is the gene therapy field becoming too risk-averse? Mol Ther [Internet]. 2009 Nov;17(11):1842–8. Available from: http://linkinghub.elsevier.com/retrieve/pii/S152500161630795X

120. Hacein-Bey-Abina S, Le Deist F, Carlier F, Bouneaud C, Hue C, De Villartay J-P, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. N Engl J Med [Internet]. 2002 Apr

18;346(16):1185–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11961146

121. Gaspar HB, Parsley KL, Howe S, King D, Gilmour KC, Sinclair J, et al. Gene therapy of X-linked severe combined immunodeficiency by une of a pseudotyped gammaretroviral vector. Lancet. 2004;364(9452):2181–7.

122. Check E. Regulators split on gene therapy as patient shows signs of cancer. Nature [Internet]. 2002 Oct 10;419(6907):545–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12374936

123. Check E. Second cancer case halts gene-therapy trials. Nature [Internet]. 2003 Jan 23;421(6921):305. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12540867

124. Hacein-Bey-Abina S. LMO2-Associated Clonal T Cell Proliferation in Two Patients after Gene Therapy for SCID-X1. Science (80-) [Internet]. 2003 Oct 17;302(5644):415–9. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.1088547

125. Check E. Gene therapists hopeful as trials resume with childhood disease. Nature [Internet]. 2004 Jun 10;429(6992):587. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15190314

126. Gonin P, Buchholz CJ, Pallardy M, Mezzina M. Gene therapy bio-safety: scientific and regulatory issues. Gene Ther [Internet]. 2005 Oct;12 Suppl 1:S146-52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16231047

127. Modlich U, Baum C. Preventing and exploiting the oncogenic potential of integrating gene vectors. J Clin Invest [Internet]. 2009 Apr;119(4):755–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19348042

128. Aiuti A, Cossu G, de Felipe P, Galli MC, Narayanan G, Renner M, et al. The committee for advanced therapies' of the European Medicines Agency reflection paper on management of clinical risks deriving from insertional mutagenesis. Hum Gene Ther Clin Dev [Internet]. 2013 Jun;24(2):47–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23862696

129. Naldini L. Gene therapy returns to centre stage. Nature [Internet]. 2015;526(7573):351–60. Available from: http://www.nature.com/doifinder/10.1038/nature15818

130. Schneider CK, Salmikangas P, Jilma B, Flamion B, Todorova LR, Paphitou A, et al. Challenges with advanced therapy medicinal products and how to meet them. Nat Rev Drug Discov [Internet]. 2010;9(3):195–201. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20190786

131. European Medicines Agency. Guideline on follow-up of patients administered with gene therapy medicinal products [EMEA/CHMP/GTWP/60436/2007] [Internet]. 2009. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/11/WC500013424.pdf

132. Yonemitsu Y, Kitson C, Ferrari S, Farley R, Griesenbach U, Judd D, et al. Efficient gene transfer to airway epithelium using recombinant Sendai virus. Nat Biotechnol [Internet]. 2000 Sep;18(9):970–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10973218

133. Griesenbach U, Inoue M, Meng C, Farley R, Chan M, Newman NK, et al. Assessment of F/HN-pseudotyped lentivirus as a clinically relevant vector for lung gene therapy. Am J Respir Crit Care Med [Internet]. 2012 Nov 1;186(9):846–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22955314

134. Nathwani AC, Tuddenham EGD, Rangarajan S, Rosales C, McIntosh J, Linch DC, et al. Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. N Engl J Med [Internet]. 2011 Dec 22;365(25):2357–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22149959

135. European Medicines Agency. Guideline on the Non-Clinical Studies Required before First Clinical Use of Gene Therapy Medicinal Products [EMEA/CHMP/GTWP/125459/2006] [Internet]. 2008. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003942.pdf

136. UniQure Gene Therapy Technology [Internet]. [cited 2017 Mar 12]. Available from: http://www.uniqure.com/gene-therapy/uniqure-technology.php

137. Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nat Med [Internet]. 2006 Mar;12(3):342–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16474400

138. Herzog RW. Immune responses to AAV capsid: are mice not humans after all? Mol Ther [Internet]. 2007 Apr;15(4):649–50. Available from: http://www.nature.com/mt/journal/v15/n4/full/6300123a.html%5Cnhttp://www.nature.com/mt/journal/v15/n4/pdf/6300123a.pdf

139. Mingozzi F, High KA. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. Blood [Internet]. 2013 Jul 4;122(1):23–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23596044

140. European Medicines Agency. Note for Guidance on the Quality, Preclinical and Clinical Aspects of Gene Transfer Medicinal Products [CPMP/BWP/3088/99] [Internet]. 2001. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500003987.pdf

141. European Medicines Agency. Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products [EMA/CAT/80183/2014] [Internet]. 2015. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/05/WC500187020.pdf

142. Ayuso E. Manufacturing of recombinant adeno-associated viral vectors: new technologies are welcome. Mol Ther Methods Clin Dev [Internet]. 2016;3(October 2015):15049. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26942207

143. Abou-El-Enein M, Bauer G, Reinke P, Renner M, Schneider CK. A roadmap toward clinical translation of genetically-modified stem cells for

treatment of HIV. Trends Mol Med [Internet]. 2014 Nov;20(11):632–42. Available from: http://dx.doi.org/10.1016/j.molmed.2014.08.004

144. Husain SR, Han J, Au P, Shannon K, Puri RK. Gene therapy for cancer: regulatory considerations for approval. Cancer Gene Ther [Internet]. 2015 Dec;22(12):554–63. Available from: http://dx.doi.org/10.1038/cgt.2015.58%5Cn10.1038/cgt.2015.58

145. Grieger JC, Soltys SM, Samulski RJ. Production of Recombinant Adeno-associated Virus Vectors Using Suspension HEK293 Cells and Continuous Harvest of Vector From the Culture Media for GMP FIX and FLT1 Clinical Vector. Mol Ther [Internet]. 2016 Feb;24(2):287–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26437810

146. Urabe M, Ding C, Kotin RM. Insect cells as a factory to produce adeno-associated virus type 2 vectors. Hum Gene Ther [Internet]. 2002 Nov 1;13(16):1935–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12427305

147. Mietzsch M, Grasse S, Zurawski C, Weger S, Bennett A, Agbandje-McKenna M, et al. OneBac: platform for scalable and high-titer production of adeno-associated virus serotype 1-12 vectors for gene therapy. Hum Gene Ther [Internet]. 2014;25(3):212–22. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3955967&tool=pmcentrez&rendertype=abstract

148. Hildt E. Human Germline Interventions-Think First. Front Genet [Internet]. 2016;7(MAY):81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27242891

149. Liang P, Xu Y, Zhang X, Ding C, Huang R, Zhang Z, et al. CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell [Internet]. 2015 May;6(5):363–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25894090

150. Anderson WF. Human gene therapy: scientific and ethical considerations. J Med Philos [Internet]. 1985 Aug;10(3):275–91. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/3900264

151. Freire JE da C, Medeiros SC de, Lopes Neto AV, Monteiro Júnior JE, Sousa AJS, Rocha AJ, et al. Bioethical conflicts of gene therapy: a brief critical review. Rev Assoc Med Bras [Internet]. 2014;60(6):520–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25650850

152. Commission Directive 2003/63/EC of 25 june 2003 amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use. Off J Eur Union. 2003;L159:46–94.

153. Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004. Vol. L324, Official Journal of the European Union. 2007. p. 121–37.

154. Commission Directive 2009/120/EC of 14 September 2009 amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use as regards advanced therapy medicinal products. Off J Eur Union. 2009;L242:3–12.

155. Wirth T, Parker N, Ylä-Herttuala S. History of gene therapy. Gene [Internet]. 2013 Aug 10;525(2):162–9. Available from: http://dx.doi.org/10.1016/j.gene.2013.03.137

156. European Medicines Agency. User guide for micro , small and medium-sized enterprises [Internet]. 2016. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/10/WC500004134.pdf

157. Eder C, Wild C. Technology forecast: advanced therapies in late clinical research, EMA approval or clinical application via hospital exemption. J Mark Access Heal Policy [Internet]. 2019 Jan 1;7(1):1600939. Available from: https://doi.org/10.1080/20016689.2019.1600939

158. Hayakawa T, Harris I, Joung J, Kanai N, Kawamata S, Kellathur S, et

al. Report of the International Regulatory Forum on Human Cell Therapy and Gene Therapy Products. Biologicals [Internet]. 2016 Sep;44(5):467–79. Available from: http://dx.doi.org/10.1016/j.biologicals.2016.06.002

159. Food and Drug Administration. Office of Cell, Tissue and Gene Therapy Learning website [Internet]. [cited 2017 Sep 28]. Available from: https://www.fda.gov/biologicsbloodvaccines/newsevents/ucm232821.htm

160. Food and Drug Administration. Cellular and Gene Therapy Guidances [Internet]. [cited 2017 Sep 28]. Available from: https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/default.htm

Chapter 2

Regulatory hurdles towards ATMP and Gene Therapy approval

Abstract

Background: In Europe, Regulation (EC) 1394/2007 ("ATMP Regulation") established a specific legal framework for Advanced Therapy Medicinal Products (ATMPs) but only a limited number of products were approved, despite the generalized growing number of clinical trials. Given the huge potential of ATMPs to address diseases of high unmet medical need, it is important to understand the reasons behind the low number of approved products, from a regulatory standpoint.

Objective: Identification of Marketing Authorization Application (MAAs) in the EU for ATMPs was conducted between implementation of the ATMP Regulation in 2009 up to December 2017 and the regulatory review process was analysed. In addition, for Gene Therapy Medicinal Products (GTMPs), the main major objections, issues or concerns noted in the MAA were identified.

Methods: ATMPs were identified thought the CAT's Monthly reports. European Public Assessment Reports (EPAR) were used to extract data on MAA process, in terms of regulatory milestones. For GTMPs, the EPARs were analysed to extract descriptive data on quality, non-clinical and clinical assessment. Products were classified as Successful or Unsuccessful MAA, if a positive MAA was obtained or not. Assessment timelines and regulatory milestones were analysed. Deficiencies in quality, non-clinical and clinical assessment were classified using a 4-level scale, based on whether any major objections, issues or concerns were found or not, as well as if these were considered satisfactorily addressed or not, by the regulator.

Results: 16 MAAs for ATMPs were identified and assessed. This represents a small and heterogeneous group of products, including 38% GTMPs, 38% TEPs and 25% sCTMP. The proportion of orphan and non-orphan products is identical across the study sample. An overall 63% success rate was noted in obtaining positive MAA outcome. The average number of requests for Scientific Advice/Protocol Assistance (SA/PA) was slightly higher in the successful group as opposed to the unsuccessful (2.6 vs. 2.0, respectively). A higher average number of Clock Stops (CS) was noted in the successful group comparing to the unsuccessful (3.1 vs. 2.0). For non-SME applicants, the success rate for obtaining MAA was higher (83%) comparing to SMEs (50%). Furthermore, a comparative analysis of GTMP MAA deficiencies is presented.

Clinical efficacy and safety issues were the most common, followed by quality deficiencies. Non-clinical data was the section with the least amount of objections.

Conclusion: Higher average number of requests for SA/PA, higher average number of CS and applicant status as non-SME were identified as positive predictors of ATMP MAA approval. For the assessment of GTMP MAAs specifically, in the early years of the CAT's activities, quality issues were often identified as major deficiencies. Issues at the non-clinical level appeared to be the less frequently noted. The analyzed data suggests that clinical efficacy and safety issues play a major role in unsuccessful MAA outcome for GTMPs. Most major objections, issues or concerns were addressed through clarification via oral explanation or written answer or submission of additional data (either during MAA review or post-marketing). In this context, RMP updates were noted in virtually all GTMPs.

Background

The development of ATMPs is a dynamic and fast-growing field, as these products have the potential to address highly unmet medical needs. On the other hand, a number of challenges may represent important hurdles in the development of such therapies, for instance safety concerns, efficacy issues or obstacles related to quality/scale-up (1). A recent research noted that the number of clinical trials using investigational ATMPs has almost doubled from 1999-2010 when compared to 2010-2015(2). It would be expected that a growing number of licensed ATMPs would follow this trend.

In Europe, Regulation (EC) 1394/2007 ("ATMP Regulation") which establishes a specific legal framework for ATMPs was implemented in 2009. Nevertheless, since then and until December 2017, only nine products have been granted MA, with the following distribution: three GTMPs, three sCTMP and four TEPs(3). The relevance of GTMPs is particularly significant, when considering orphan ATMPs. Farkas *et al.* analyzed all ATMPs that were granted orphan designation between 2001 and April 2016 and found that nearly 50% were GTMPs(4).

ATMPs are likely to be associated with high costs, due to its particular characteristic, in terms of technology development and manufacture. In addition, due to the fact that these may be curative rather than treatment approaches, a significant impact in health care systems should be anticipated. Therefore, a profound understanding of ATMPs is essential to manage their availability appropriately (1).

Therefore, it is relevant to identify key aspects influencing regulatory ATMP approval, in particular GTMPs, in Europe. Publicly available data in the EMA's website was analysed until December 2017. First, the MAAs for ATMPs are identified and reviewed in Europe. Then, the MAA regulatory process was characterized and reviewed. Finally, a comparison of the major objections found in MAA assessment amongst GTMPs is presented based on a 4-level scale, which takes into account if any major objections, issues or concerns were found, as well as if these were considered satisfactorily addressed, by the regulator.

2.1 Identification of ATMPs and products' baseline characteristics

2.1.1 Methodology and data collection

Several documents are publicly available in the EMA's website as part of the Agency's commitment to openness and transparency (5). The Committee for Advance Therapies' Monthly Reports include statistical data for the current year on the Committee's activities. In addition, a section on MAA is mentioned any time a draft opinion has been issued by the CAT (6), making these documents suitable to extract data for the first part of this research project.

CAT monthly reports were included in the analysis since March 2009 until December 2017, in a total of 97 reports reviewed. The final outcome on ATMP initial evaluation and the year of when the opinion was issued were collected. The final outcome for an initial evaluation was defined as:

- Successful MAA : positive CAT draft opinion
- Unsuccessful MAA : negative CAT draft opinion or MAA withdrawal

For those products which underwent re-examination procedure, the final outcome for initial evaluation was assessed based on the outcome of the last re-examination. Heparesc™ underwent one re-examination procedure resulting in final unsuccessful MAA outcome. Glybera® underwent two re-examination procedures resulting in a final successful MAA outcome. For Cerepro®, two MAA were assessed and both resulted in a negative opinion. As it is the same product, same indication and same final outcome this ATMP was counted as a single unsuccessful MAA.

Two MAAs were identified for contusugene ladenovec. The first with brand name Advexin® intended to be used for treatment of Li-Fraumeni cancer (an orphan disease) for which the MAA process was withdrawn by the applicant in 2008. A second MAA with brand name Contusugene Ladenovec Gendux (CLG) for treatment of adult patients with recurrent or refractory squamous cell carcinoma of the head and neck as monotherapy (non-orphan disease) for which the MAA process was also withdrawn by the applicant in 2009. For the purposes of this analysis, even though

this is the same active substance, both products were analysed separately, due to the different intended indications.

After identification of the products from the CAT's Monthly Reports, the product's Assessment Report was consulted for further data collection, including ATMP commercial name, INN, ATMP classification (on whether the product was gene therapy, somatic cell therapy or tissue engineered product), orphan designation, as well as the applicant's name. Applicants were additionally classified as Non-SMEs or SMEs, based on fulfilment of the legal definition outlined in Commission Recommendation 2003/361/EC(7). For the initial screening of the applicants, the EU's SME Register was used(8). If not found, then the applicant was further searched through EMA's report from SME, as detailed in the references section(9). Finally, if not found, then publicly available information on company's annual revenue and number of employees was used.

2.1.2 Results, data analysis and discussion

Sixteen products were identified and assessed for MAA by the CAT/CHMP. In four of these products the MAA was withdrawn before the CAT/CHMP issued an opinion, at either day 120 (Advexin®, CLG and Hyalograft® C autograft) or day 180 (OraNera) of the process. The main reason for withdrawal was the insufficiency of data submitted by the applicant to support a positive benefit-risk balance. In addition, some applicants justified the withdrawal through the inability to generate new data/analysis, as well as company's financial limitations.

On the other hand, twelve other products underwent full review process, where two of them were refused (Cerepro® and Heparesc™) and ten were issued a positive opinion (Alofisel®, ChondroCelect®, Glybera®, Holoclar®, Imlygic®, MACI®, Provenge®, Spherox, Strimvelis® and Zalmoxis®), as per **Figure 10**.

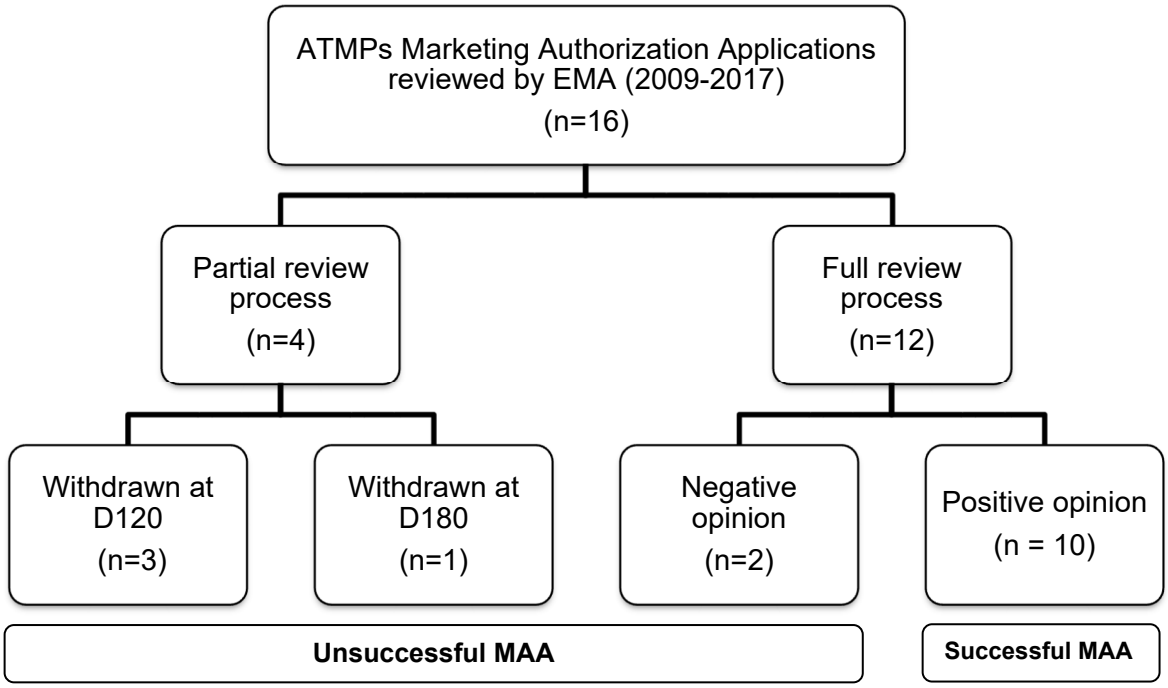


Figure 10 – MAAs analyzed. Full review process includes final CAT/CHMP opinion

ATMP: Advanced Therapy Medicinal Products. D120: Day 120. EMA: European Medicines Agency. MAA: Marketing Authorization Application.

Until 2012, only 2 ATMPs had successful MAA outcome while 3 other ATMPs resulted in unsuccessful MAA, highlighting a relatively slow start of the CAT's activities related to ATMP initial evaluation. The first TEP was approved in 2009 (ChondroCelect®), while the first GTMP was approved in 2012 (Glybera®) and the first sCTMP was approved in 2013 (Provenge®).

In 2014, for the first time, the number of successful MAA matched those with an unsuccessful MAA. Since 2016 the number of successful ATMPs surpassed the unsuccessful, a trend that has been sustained up until the end of 2017 (**Figure 11**).

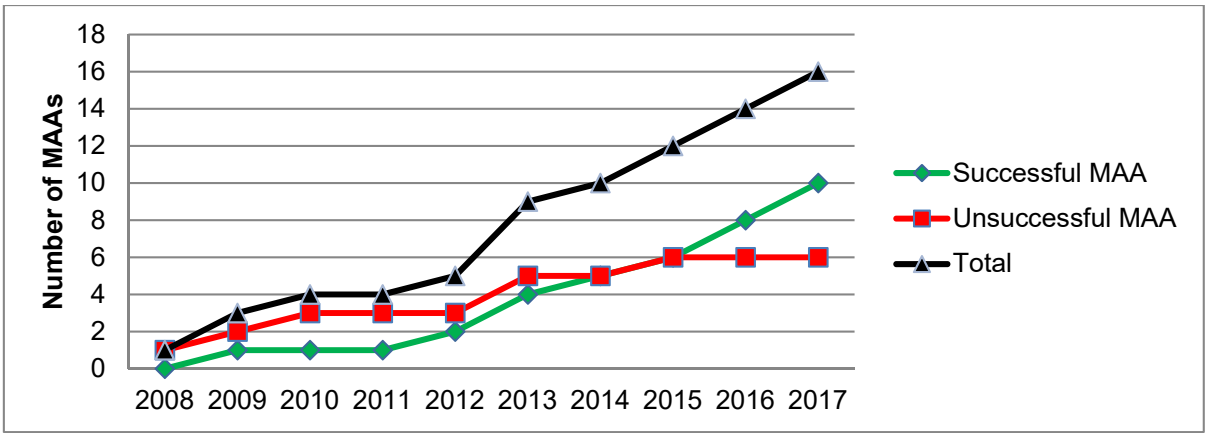


Figure 11 – Cumulative MAA outcome overtime for ATMPs

MAA: Marketing Authorization Application.

GTMPs and TEPs represent the categories of ATMPs with the highest number of applications for MA reviewed by the CAT (6 applications, each), followed by sCTMP (4 applications), according to **Table 3**.

By classification	**n=16**	**%**
Gene Therapy Medicinal Products	6	38%
Somatic Cell Therapy Medicinal Product	4	25%
Tissue Engineered Products	6	38%
By orphan designation	**n=16**	**%**
Non-orphan	8	50%
Orphan	8	50%
By MAA initial evaluation outcome	**n=16**	**%**
Successful	10	63%
Standard Approval	7	70%
Conditional Approval	2	20%
Approval Under Exceptional Circumstances	1	10%
Unsuccessful	6	38%
Negative Opinion	2	33%
Withdrawal at D120	3	50%
Withdrawal at D180	1	17%
Type of applicant	**n=16**	**%**
Non-SME	6	38%
SME	10	63%

Table 3 – Summary of ATMP MAA's characteristics

The success rate of obtaining MAA according to ATMP product type (**Table 4)** ranges from 50 to 75%, with GTMPs having less likelihood of obtaining MAA (50%),

followed by TEPs (67%) and by sCTMPs (75%). However, the number of MAAs included in this analysis is relatively low, thus these percentages might not be sufficiently informative, and interpretation of this data should be conducted with caution.

ATMP type	Unsuccessful MAA		Successful MAA		Total N (%)
	N (%)	ATMPs	N (%)	ATMPs	
GTMP	3 (50%)	Advexin®, CLG, Cerepro®	3 (50%)	Glybera®, Imlygic®, Strimvelis®	6 (100%)
sCTMP	1 (25%)	Heparesc™	3 (75%)	Provenge®, Zalmoxis®, Alofisel®	4 (100%)
TEP	2 (33%)	Hylograft Autograft, OraNera	4 (67%)	Spherox, Holoclar®, MACI®, ChondroCelect®	6 (100%)

Table 4 – Success rate of obtaining MAA according to ATMP product type

Half the number of assessed ATMPs were granted orphan designation (**Table 3**) which is aligned with the findings from Farkas and colleagues(4). This is likely related to the fact that ATMPs are being applied to diseases of high unmet medical need, particularly GTMPs, which hold the promise to become an important therapeutic option for a particular subset of rare diseases, namely those caused by a single gene defect. Interestingly, the success rate of obtaining MAA is the same, regardless of whether the ATMP is classified as orphan or not **(Table 5)**.

ATMP type	Unsuccessful MAA		Successful MAA		Total N (%)
	N (%)	ATMPs	N (%)	ATMPs	
Orphan	3 (37.5%)	Advexin®, Cerepro®, Heparesc™	5 (62.5%)	Glybera®, Holoclar®, Strimvelis®, Zalmoxis®, Alofisel®	8 (100%)
Non-Orphan	3 (37.5%)	CLG, Hylograft Autograft, OraNera	5 (62.5%)	ChondroCelect®, MACI®, Provenge®, Imlygic®, Spherox	8 (100%)

Table 5 – Success rate of obtaining MAA according to whether the ATMP is orphan or not

Over the analysed period, the global success rate of MAA was 63% (10 ATMPs with positive opinion out of 16 assessed), which means that more than one third of the candidate ATMP MAAs had unacceptable major objections affecting quality, efficacy and/or safety. For those products with a successful MAA outcome, 2 products (Holoclar® and Zalmoxis®) received conditional MA requiring further collection of clinical efficacy and safety data. One product (Glybera®) was only approved under exceptional circumstances, i.e. in a very restricted subset of patients. The remaining 70% were granted with a standard approval.

Importantly, almost two thirds of the applicants (63%) fulfil the SME definition, according to the legal EU framework, highlighting that ATMP development takes place in micro, small and medium enterprises, including as well academic institutions, as opposed to larger commercial companies. In some cases, for instance with Holoclar® and Strimvelis®, commercial agreements were established pre-MAA elevating the academic development to a partnership with larger pharmaceutical companies. This is in line with previous findings from other research groups and authors (13,14).

However, the success rate of ATMP MAA is higher for Non-SMEs (83% had successful MAA outcome) when compared to SME applicants (50%), as per **Table 6**. This aligned with other authors' work not only for advanced therapies(14) but also for other medicinal product types, such as biologics(15,16). It is hypothesized that this may potentially indicate that smaller structures with limited resources at the applicant's level could correlate with a lower probability of success in obtaining ATMP MA. SMEs are not as experienced as Non-SMEs in determining the required

regulatory procedures (i.e. limited regulatory expertise), as well they may not have enough resources to proceed with regulatory intelligence data-collection (i.e. restricted funding). In addition, these organizations have smaller product portfolio and thus less regulatory experience.

Full data used for this analysis may be consulted in **Appendix 2.1 – Characteristics of ATMPs assessed by the CAT**.

Applicant type	Unsuccessful MAA		Successful MAA		Total N (%)
	N (%)	ATMPs	N (%)	ATMPs	
Non-SME	1 (17%)	Hylograft Autograft	5 (83%)	MACI®, Provenge®, Holoclar®, Imlygic®, Strimvelis®	6 (100%)
SME	5 (50%)	Advexin®, CLG, Cerepro®, OraNera, Heparesc™	5 (50%)	ChondroCelect®, Glybera®, Zalmoxis®, Spherox, Alofisel®	10 (100%)

Table 6 – Success rate of obtaining MAA according to applicant type

2.2 Characterization of the MAA review process for ATMPs

2.2.1 Methodology and data collection

The ATMP Regulation establishes the Marketing Authorization requirements for ATMPs, in Europe. Generally speaking, the MAA should follow the centralized procedure on a compulsory basis (10). EMA publishes European Public Assessment Reports (EPARs) for all products for which a MAA underwent centralized procedure. These documents comprise steps taken for the assessment as well as the outcomes of the regulatory process, including a record of the scientific background on which a decision was made to approve or refuse a MAA. The publication of these reports is also mandatory for those products when applications for marketing authorisation were withdrawn or refused (5,11), making them ideal sources for data extraction.

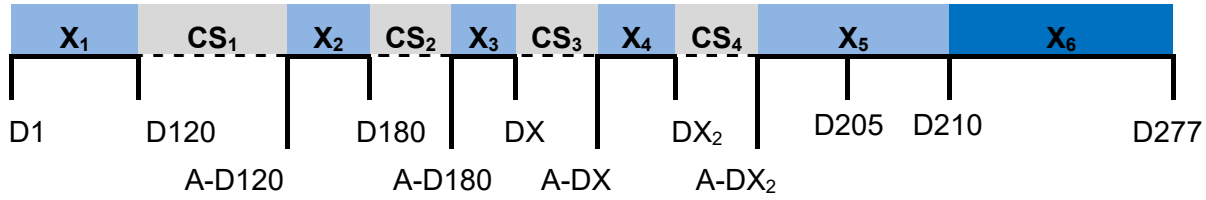
From those ATMPs identified, further analysis on the MAA review process was performed, based on the product's corresponding Public Assessment Report. Information on how the MAA was assessed by the EMA was reviewed, specifically from section "Steps taken for the assessment of the product". Milestone dates were collected for each individual ATMP, as per **Table 7**, in accordance with the process described in **Figure 12**. D277 was extracted from the corresponding EC Implementing Decision document.

Milestone	Definition
D1	Date when procedure started
D120	Date when consolidated list of questions (LoQ) was issued
A-D120	Date when Applicant submitted responses to D120 LoQ
D180	Date when list of outstanding issues (LoOI) was issued
A-D180	Date when Applicant submitted responses to D180 LoOI
DX, DX_2	Date when subsequent LoOI was issued
A-DX, A-DX_2	Date when Applicant submitted responses to subsequent LoOI
D205	Date when CAT issued draft opinion
D210	Date when CHMP issued draft opinion
OE	Date when Applicant provided any Oral Explanation
SA/PA	Date when Scientific Advice or Protocol Assistance was provided
D277	Date when European Commission (EC) issued MA

Table 7 – MAA milestone dates collected

After collection of the corresponding milestone dates, different variables were determined, with the support of Microsoft Excel software. The number and duration of

clock stops was calculated for each individual product. Clock stop (CS) was defined as the time allowed for the applicant to answer questions and issues raised during the assessment of new MAAs in the centralised procedure. Duration of MAA assessment (DA) was calculated as the number of active days taken since procedure start until CHMP opinion or MAA withdrawal, i.e. excluding the clock stops. Duration of MAA decision (DD) was calculated as the number of active days taken since Procedure start until EC decision, as per **Figure 12**, and as outlines in the corresponding EMA guideline(12). Number of oral explanations, as well as number of times each application requested Protocol Assistance or Scientific Advice, according to whether it was an orphan or non-orphan drug, respectively, was also collected.



$DA = X_1 + X_2 + X_3 + X_4 + X_5$

$DD = DA + X_6$

Figure 12 – MAA review process with detailed milestone dates

MAA review process is initiated at D1. Rectangles in light blue represent active time taken by the regulator to assess MAA. Rectangles in grey represent time taken by the applicant to respond to issues raised during the MAA review process (also known as clock stops). Rectangle in dark blue represents time taken by European Commission (EC) to issue final authorization. DA: duration of assessment. DD: duration of decision.

For the three MAA products which were withdrawn (CLG, Hyalograft® Autograft and OraNera) the milestone data were not collected from the Withdrawal Assessment Report, since the section “Steps taken for the assessment” is not available there. For these products, data was extracted from Q&A and Key Facts documents. The number of clock stops was collected, based on the day mentioned in the assessment report. In the case of withdrawn ATMPs, the number of CS was collected from the Q&A document. For Cerepro®, two assessment reports were available and each was analysed separately as 1st round of review and 2nd round of review, respectively. Analysis of Glybera®’s assessment report was the most complex as the same document included different MAA outcomes at specific time points. For Glybera®, the period between first procedure start on 20-Jan-2010 and final EC decision 25-Oct-2012 was considered. Any activities conducted between

January 2012 until 19-Apr-2012 were excluded from the analysis as the April 2012 CHMP opinion was considered void, after EMA legal scrutiny, because the CHMP adopted an opinion without having a formal CAT draft opinion.

Finally, average values, minimum and maximum for the following variables were calculated, for two groups of products identified in the first part (successful MAAs versus unsuccessful MAAs):

- Number of SA / PA requests
- Overall number of clock stops
- Duration of CS (in days)
- Number of oral explanations
- DA (in days)
- DD (in days)

2.2.1 Results, data analysis and discussion

2.2.1.1 Scientific Advice / Protocol Assistance

Overall results on milestone data in MAA review process for ATMPs are summarized in **Table 8**. All of the ATMPs included in the analysis requested at least once scientific advice / protocol assistance (SA/PA) (**Table 8**), hence to request or not SA/PA does not seem decisive in ATMP MAA success. This is aligned with other author's findings(15,17), where it is consistently shown that there are no differences in success rate for applicants who had prior scientific advice as opposed to those which had not.

Successful MAA for ATMPs have an average higher number of requests for SA/PA comparing to unsuccessful MAA (2.6 vs. 2.0, respectively) (**Table 8).** A higher number of SA/PA seems to suggest a higher probability of successful MAA outcome. This is a tendency that has been noted in other studies, where not only the number of requests of SA/PA by the applicant was identified as a key factor for obtaining MA but a more significant factor towards successful MAA was the compliance with the regulators recommendation described in SA/PA (15,16).

However, analysing the number of times an applicant requests SA/PA has its limitations. On the one hand, the initiative to request SA/PA comes primarily from the applicant, as well as the content of the advice that is sought. It seems fundamental to

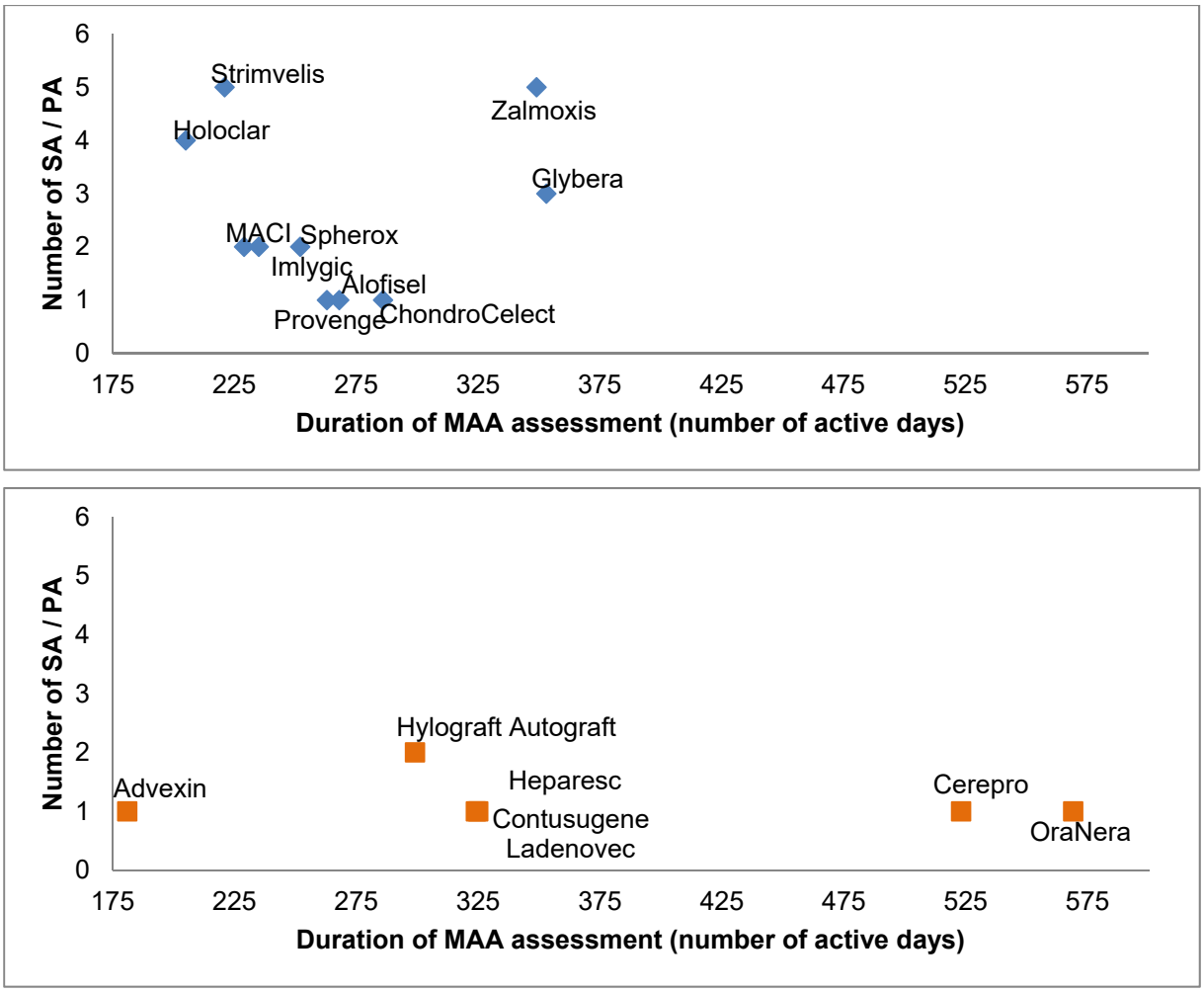
ask the right questions, on the right timing. One applicant may request advice only once and ask a high number of questions, while other applicant may decide to request multiple advices, at different time points, each containing a lower number of questions. In addition, and although it may be unexpected, non-compliance with the Regulator's advice can be accepted in some cases. For instance, for Imlygic®, advice was sought regarding the primary endpoint. While the EMA advised to use progression-free survival or overall survival, the applicant decided to use durable response rate, which was considered acceptable by the EMA, with proper justification(18).

Outcome	Unsuccessful MAA				Successful MAA			
	Average	Min	Max	N	Average	Min	Max	N
Number of SA/PA requests	2	1	2	6	2.6	1	5	10
Total number of CS	2	1	4	6	3.1	2	5	10
1st review process								
Duration of CS 1 (D120 - LoQ)	152	62	190	4	304.4	87	1330	10
Duration of CS 2 (D180 - LoOI)	61	59	63	2	80.8	1	239	10
Duration of CS 3 (DX - 2nd LoOI)	33	33	33	1	42.9	1	183	7
Duration of CS 4 (DX - 3rd LoOI)					12.0	5	19	2
Duration of CS 5 (DX - 4th LoOI)					1.0	1	1	1
2nd review process	95	95	95	1				
Duration of CS 1 (D120 - LoQ)								
Duration of CS 2 (D180 - LoOI)	38	38	38	1				
3rd review process								
Duration of CS					14.0	14	14	1
Number of oral explanations	1.17	0	4	6	1.3	0	6	10
DA (days)	370	181	569	6	266.1	205	353	10
DD (days)					337.4	266	451	10
Period between review processes 1-2	336	64	608.0	2	65.0	65	65	1
Period between review processes 2-3					217.0	217	217	1
Period between DA to DD					71.3	53	102	10

CS: Clock Stop; DA: Duration of Assessment; DD: Duration of Decision; LoOI: List of Outstanding Issues; LoQ: List of Questions; SA / PA: Scientific Advice / Protocol Assistance. For Glybera, the second review process did not include clock stops, only oral explanations. Hence, no data is shown for successful MAA 2nd review process.

Table 8 – Characterization of milestone data in MAA review process for ATMPs

The duration of assessment ranged from 205 and 353 days in the successful MAAs, as opposed to the unsuccessful MAAs, where the range was wider, between 181 and 569 days (as per **Figure 13)**. This data combined with the fact that successful MAAs requested more often SA / PA compared to the unsuccessful MAAs may support that a higher number of SA / PA could result in a shorter duration of MAA assessment, as per **Figure 13**. It appears that if an applicant is more often counselled by the regulators, at an early stage of product development, then this is an applicant with better preparation for the any requests from regulators during the MAA process review, which is a conclusion also in line with other authors (15,16). In addition, it can even be hypothesized that the potential questions raised by regulators during the MAA review process could be addressed by means of this early form of dialogue with the regulator.



MAA: Marketing Authorization Application; SA / PA: Scientific Advice / Protocol Assistance. Successful ATMPs are marked in blue and Unsuccessful ATMPs are marked in orange.

Figure 13 – Number of requests for Scientific Advice / Protocol Assistance versus Duration of MAA review

2.2.1.2 Clock stops

The average total number of clock stops seems to be slightly higher for the Successful MAAs when compared to Unsuccessful MAAs (3.1 vs. 2.0, respectively). This is related to the fact that the unsuccessful MAA group includes 6 ATMPs, where 4 of them were withdrawn during MAA review, therefore having only one or two clock stops during the process. Here, it is clear that the farthest an ATMP goes in review process the more likely it is having a higher number of clock stops.

All products, regardless of successful or unsuccessful MAA, have at least one clock stop with an observed maximum number of five clock stops amongst all analysed MAAs. The first clock stop, at D120, is consistently the longest and the

average duration almost twice as much in the successful MAA versus the unsuccessful (304.4 vs. 152 days, respectively) (**Table 8**). Nevertheless, this has limited significance since Alofisel® is a clear outlier in the successful MAA group, with a first clock stop duration of 1330 days. If we exclude this value, then the average duration of this first clock stop between the unsuccessful and successful group is more similar (152 vs. 190.4 days). Nevertheless, the successful MAA group continues to have longer average first clock stop duration. Unfortunately, the content of the D120 List of Questions or D180 List of Outstanding Issues is not publicly available, due to the confidential nature of the issues raised. Therefore, as only publicly available data was used in this analysis, we did not reflect on the nature of the initial regulatory deficiencies noted which appear to take a substantial period of time for the applicant to address such questions.

It is also noted that the duration of CSs has a clear tendency to decrease throughout the MAA assessment process and the average number of CS days between both groups appears to be quite similar. This is also noted regardless of review cycle. Therefore, it does not seem to be a tendency for the duration of clock stops to be related with success of ATMP MAA.

2.2.1.3 Oral explanations

The purpose of an oral explanation is to give the Applicant a final opportunity to substantiate the products' MAA, generally after D180 and prior to CAT issuing a draft opinion(12). Unsuccessful MAA ATMPs have an average number of 1.17 oral explanations comparing to 1.3 in the successful MAA ATMPs group (**Table 8**). At a first glance, it does not appear that the number of oral explanations seem to be a predictor of MAA success nor failure. However, a closer analysis of the data indicates the opposite. All ATMPs included in the Successful MAA group presented between zero and two oral explanations, except for Glybera®, a clear outlier, which was the product with the highest number of OEs among all ATMPs (six, in total). In the unsuccessful group, all 4 withdrawn ATMPs had zero OEs simply because these did not reach the MAA review process far enough for that. The other two MAAs had 3 (Cerepro®) and 4 (Heparesc™) OEs, resulting in a negative opinion. Therefore, a higher number of oral explanations may be a predictor of MAA failure. It is hypothesized that this due to the higher number of issues raised by the regulatory agency.

2.2.1.4 Duration of Assessment

For unsuccessful ATMPs, the average DA was generally higher when compared to the successful ATMP group (370 days vs. 266.1 days, **Table 8**). As per the EMA guidelines, the duration of assessment takes up to 210 active days until CHMP draft opinion is issued(12). If we exclude the products that were withdrawn during the assessment, in 92% of the MAA processes included in this analysis, a draft opinion was issued by CHMP after day 210. Only one product (Holoclar®) was able to obtain CHMP draft opinion prior to day 210, highlighting that the MAA review process for these products is complex and often needs more assessment time than standard medicinal products. Cerepro®, included in the unsuccessful MAA group, is the product with highest DA, reaching to 523 days. Conversely, Glybera® and Zalmoxis® presented the longest DA in the successful MAA group, with 353 days and 349 days, respectively. There seems to be a tendency that longer durations of assessment could result in a lower probability of ATMP approval.

2.2.1.5 Duration of Decision

Sixty-seven days after CHMP draft opinion, the EC issues a formal decision on Marketing Authorization. This final decision-making process includes the linguistic development of product and labelling information in all official EU languages(12). According to our analysis, the process took on average 71.3 days, for successful ATMPs (**Table 8**). For 60% of the Successful MAAs the EC issues a decision in less than 67 days, while the maximum time noted between DA and DD was 102 days. Four ATMPs (Alofisel®, ChondroCelect®, Glybera® and Provenge®) exceed the 67-day mark.

Results of the data extraction in detail are available as **Appendix 2.2 – Characterization of milestone data in the MAA review process for ATMPs.**

2.3 Major objections found in MAA assessment for GTMPs

2.3.1 Methodology and data collection

Identified GTMPs were further analysed, based on the information available in the European Publicly Assessment Report or in the Withdrawal Assessment Report. Even though the overall structure of both EPAR and WPAR is, to some extent, different and has evolved during the analysed time period, the core structure remains generally kept. Each MAA was compared as per the EPAR/WPAR headings, whenever possible. This allowed comparison of GTMPs with regards to quality, non-clinical and clinical assessment of MAA.

For the purpose of this analysis, Advexin® and CLG were assessed separately as well as Cerepro®'s MAA of 2007 and 2010. This allowed uncovering major objections, issues or concerns during the entire MAA process, as opposed to performing the analysis focusing on only one (i.e. the most recent) assessment report.

Each section of the corresponding assessment report was reviewed in detail. The information was categorized based on whether it was considered satisfactorily assessed or not, by the regulator. Sections including data to support an immediate satisfactory assessment by the regulator were classified as A (green). Any sections with data mentioning major objections, issues or concerns were classified as B (yellow) or C (orange), depending on whether these items were resolved or not at the time of CAT/CHMP opinion. In some cases, no data was mentioned in the report and this was categorized as F (grey) (refer to **Table 9**). Data for each GTMP was entered in three-level matrix (quality, non-clinical and clinical) where the successful MAA products were separated from unsuccessful MAA.

Description	Category
Immediate satisfactory assessment	A
Satisfactory assessment after resolution of objection, issue or concern	B
Objection, issue or concern were found resulting in unsatisfactory assessment	C
Not mentioned in EPAR / withdrawal report	F

Table 9 – Categorization of data extracted from assessment reports

2.3.2 Results, data analysis and discussion

2.3.2.1 Quality Data Assessment

Deficiencies related to quality aspects were found in all products except one (Imlygic®), regardless of whether the products were in the successful MAA group or the unsuccessful MAA group (**Table 10**). Importantly, during the review of quality data, the most frequently discussed objections were related to drug product and substance manufacture/specification at the level of 1) issues with production process 2) issues with drug specification 3) issues regarding release assay data. Each of these three topics are described in detail below.

Changes in the production process of the drug substance were common, raising comparability issues. This was observed in 2 of 4 unsuccessful MAA (both Cerepro® MAAs) and 2 of 3 successful MAAs (Glybera® and Strimvelis®). Regulators are aware that these changes occur not only during development process but also in the post-authorization setting. It is hypothesized that since GTMP drug development is often initiated at the academic level, where the resources are often limited, optimization of the manufacturing process prior to clinical drug test is not a priority. Ideally, any changes in the manufacturing process, should take place as early as possible in product development, to reduce the impact of potential comparability issues during regulatory approval(19). Positive comparability data should indicate that, regardless of manufacturing process, the resulting drug product or drug substance are equivalent for clinical use in terms of product safety, identity, purity and potency(20). However, this might not always be possible for applicants, especially for GTMPs, where more knowledge of the product features is obtained during development.

Issues in specification of drug substance and/or drug product were often encountered. Per EMA's Guidance, the applicant should provide adequate criteria for acceptance or rejection of a production batch. The specifications should cover, among others, identity, purity, content and activity(21). As ATMPs are generally considered more complex entities comparing to small molecules or other biologic agents, variability between batches is acknowledged(22).

Inadequate release assay validation (Advexin®, CLG and Glybera®) or insufficient/unacceptable release criteria (Cerepro® and Glybera®) were the most common issues found regarding specification. There are no validated assays with

associated reference standards for many of these parameters. Additionally, regulators have not established a complete set of release criteria(23), although a non-exhaustive list is available as guidance(21). An additional guideline regarding validation of analytical methods is available, as well(24). Therefore, at the time of MAA, each applicant is compelled to define a suitable validated assay with a cut-off value for release criteria. It became clear that this was a massive challenge, given the scarce experience with these innovative products. Importantly, variability in the product manufacturing process makes this task even more difficult(25). Issues with release criteria were noted at the drug substance level (e.g. unacceptable specification of potency) and drug product level (e.g. unacceptable process-related impurities and Replication Competent AdenoVirus (RCA)).

	Unsuccessful MAA				Successful MAA		
Commercial name	Advexin®	CLG	Cerepro®	Cerepro®	Glybera®	Imlygic®	Strimvelis®
Year of opinion	2008	2009	2007	2010	2012	2015	2016
QUALITY							
Drug Substance							
Manufacture	C	C	B	A	B	A	B
Characterization	C	C	A	A	B	A	A
Specification	C	C	B	B	B	A	B
Stability	F	C	A	A	A	A	A
Drug Product							
Pharmaceutical Development	A	A	A	A	A	A	A
Manufacture	C	C	B	A	A	A	A
Specification	C	C	C	A	B	A	A
Stability	C	C	C	A	A	A	A
Adventitious Agents	C	A	A	A	B	A	A
NON-CLINICAL							
Pharmacology							
Primary pharmacodynamics	A	A	A	A	A	A	A
Secondary pharmacodynamics	C	C	A	A	A	A	A
Safety pharmacology programme	F	F	A	A	F	A	A
Pharmacodynamic drug interactions	A	A	A	A	A	A	A
Pharmacokinetics							
Biodistribution, persistence, clearance	C	C	A	A	A	A	A
Germline transmission	C	C	A	A	B	A	A
Shedding	F	F	F	F	F	A	A
Toxicology							
Single dose toxicity	A	A	A	A	A	A	B
Repeat dose toxicity with toxicokinetics	C	C	A	A	A	A	A
Genotoxicity	F	F	A	A	B	A	A
Carcinogenicity	F	F	A	A	B	A	A
Reproduction Toxicity	F	F	A	A	B	A	A
Local tolerance	F	F	A	A	A	A	A
Other toxicity studies - immunogenicity/toxicity	F	F	A	A	A	A	F

	Unsuccessful MAA				Successful MAA		
Commercial name	Advexin®	CLG	Cerepro®	Cerepro®	Glybera®	Imlygic®	Strimvelis®
Year of opinion	2008	2009	2007	2010	2012	2015	2016
CLINICAL							
GCP							
GCP	F	C	C	B	A	A	B
Clinical Pharmacology							
Pharmacokinetics	C	C	C	B	B	B	A
Pharmacodynamics	C	C	C	C	B	A	A
Clinical Efficacy							
Dose selection and schedule	C	C	A	A	B	A	A
Clinical efficacy data	C	C	C	C	B	B	B
Clinical Safety							
Clinical safety data	C	C	C	C	B	B	B
Pharmacovigilance system	C	C	C	A	A	A	A
Risk Management Plan	C	C	C	C	B	B	B
Environmental Risk assessment	C	C	A	A	A	A	A

Table 10 – Major objections, issues of concerns noted in the assessment of GTMPs at the level of quality, non-clinical and clinical data

Legend: A, Immediate satisfactory assessment. B, Satisfactory assessment after resolution of objection, issue or concern. C, Objection, issue or concern were found resulting in unsatisfactory assessment. F, Not mentioned in EPAR / withdrawal report. CGG: Contusugene Ladenovec Gendux. MAA: Marketing Authorization Application.

The EMA Quality data certification is part of a set of initiatives promoted by the CAT to foster the development of ATMPs. Quality data certification procedure involves the scientific evaluation of this data and intends to identify any potential issues early on, so that these can be addressed prior to the submission of a MAA. This is a well-recognized incentive which could be instrumental in the development of GTMPs and considered a powerful tool for the early phase GTMP developers. This procedure is viewed as leverage regarding future partnerships with commercial stakeholders. However, quality data certification is available exclusively to applicants holding SME status according to the European SME Regulation(10). This is a limitation since non-profit organizations (i.e. academia, hospitals and charities) are, in general, the majority of ATMP sponsors(26). Many of these may not hold the SME status and, consequently, would not benefit from the certification procedure(4).

Quality data assessment via scientific advice or protocol assistance (for orphan drugs) is also an important instrument, where quality deficiencies could be identified prior to request for MAA(13).

In general, over the years, there is a clear trend regarding quality data acceptability by CAT/CHMP. This could either be a result of regulators' and applicants' increased experience with GTMP assessment or the submission of more robust quality data by the applicants. This could be verified, for instance, considering the overall difference between the assessments of Cerepro® in 2007 comparing to 2010. Here there is a clear improvement, to the point where there are no deficiencies precluding GTMP approval, in Cerepro®'s 2010 MAA submission, as far as quality data is concerned.

2.3.2.2 Non-clinical Data Assessment

At least one deficiency related to non-clinical aspects was found in all GTMP assessments except Cerepro® and Imlygic® **(Table 10)**. Below are described the main deficiencies noted at the level of pharmacodynamics, pharmacokinetics and toxicology.

Regarding pharmacodynamics, finding the adequate animal models to demonstrate the mode of action is a recurrent issue in GTMP non-clinical development, reported in the literature (22,25,27). Our analysis shows that there

were no deficiencies noted in this group of GTMPs regarding animal model suitability. Conversely, issues were raised on secondary pharmacodynamics. Two of four unsuccessful MAAs (Advexin® and CLG) were reported to exhibit unresolved objections related to the unclear role of RCA. RCA presence in adenoviral batches to be used in human patients is undesirable, as these may replicate in an uncontrolled manner in the patient, resulting in potential safety risks(28).

With regards to the assessment of pharmacokinetics, 2 of 4 unsuccessful MAAs (Advexin® and CLG) presented major objections. Methodological deficiencies were noted especially regarding the use of unqualified and not validated assays, as well as lack of GLP compliance, as described elsewhere(22). For 2 of 4 unsuccessful MAAs (Advexin® and CLG), as well as for 1 of 3 successful MAAs (Glybera®) objections were raised regarding pharmacokinetics of germline transmission, where the data submitted was considered insufficient. The possibility of vertical germline transmission of expression/transfer vector DNA raises ethical and safety concerns(21,29). For Advexin® and CLG, these concerns were unresolved at the time of opinion. For Glybera®, submission of an additional breeding study in mice resolved this concern, indicating that there was no paternal germ line transmission of drug. This study was also able to resolve the issue on reproduction toxicity.

For the assessment of toxicology, deficiencies were noted regarding repeat dose toxicity studies in 2 of 4 unsuccessful MAAs (Advexin® and CLG). Safety data limitations as well as study design not adequately reflecting the intended clinical use resulted in an unsatisfactory regulatory opinion. Assessment of insertional mutagenesis risk was not applicable to products using non-integrating vector, such as Advexin®, CLG and Cerepro® (adenoviral vector) as well as Imlygic® (herpes simplex vector). The risk is higher in products using integrating vectors such as Glybera® (AAV vector) and Strimvelis® (retroviral vector). The tumorigenic risk of Glybera® is associated with two elements: 1) potential for insertional mutagenesis and 2) inclusion of woodchuck post transcriptional element. On the one hand, the applicant highlighted that there were no further practical methods to assess the risk of tumourigenicity and the available evidence suggested that the risk was very low. Theoretically, the product could integrate and cause a tumour. However, the CAT/CHMP agreed with the applicant that no further animal testing or experiments could usefully address these concerns. For Strimvelis®, even though it theoretically

exhibited a higher insertional mutagenic potential considering all GTMPs included in this analysis, due to the nature of the vector used, carcinogenicity studies have not been conducted as no adequate animal model was available to evaluate the tumourigenic potential. The main reason was the inability to achieve long-term engraftment of transduced cells in mice.

Similarly, to quality assessment, applicants are able to use the certification procedure to have a regulatory and scientific evaluation of non-clinical data already collected, prior to MAA, along with a request for scientific advice or protocol assistance. The ATMP non-clinical data certification can only be used by SMEs(10). Although not legally binding, these allow the identification of concerns from a non-clinical perspective prior to request for MAA(13).

Non-clinical data seems generally satisfactorily accepted considering the low number of deficiencies identified. Because of its unique nature, the non-clinical development of GTMPs may be supported by a risk-based approach (RBA), a strategy to determine the extent of data to be included in the MAA.

2.3.2.3 Clinical Efficacy Data Assessment

Regarding GCP aspects, major objections, issues or concerns were found in 3 of 4 unsuccessful MAAs (CLG and both Cerepro® submissions) and 1 of 3 successful MAAs (Strimvelis®) **(Table 10),** especially during the academic phase of the trials. This supports that prior experience and Applicant's resources pose as key factors in regulatory approval. Importantly, the GCP findings noted for Cerepro®'s assessment of 2007 appeared to have an impact on the overall regulatory assessment, considering not only the nature of the findings but also the fact that this was a pivotal single site trial(30).

Concerning the analysis of clinical Pharmacokinetics and Pharmacodynamics, three main issues were identified during MAA, namely regarding data collection methods, data analysis and study design. Submission of additional data generally addressed these concerns.

In terms of clinical efficacy, dose identification does not seem to be a recurrent objection. Instead, the administration frequency, treatment duration and concomitant therapeutic regimens were highlighted as concerns in 2 of 4 unsuccessful MAAs (Advexin® and CLG) and 1 of 3 successful MAAs (Glybera®).

The most frequent objections in clinical efficacy assessment were related to:

- Primary demonstration of efficacy in 3 of 4 unsuccessful MAAs (CLG and both Cerepro®'s assessments) and 2 of 3 successful MAAs (Glybera® and Imlygic®)
- Change or use of a non-validated primary endpoint (pEP) in 2 of 4 unsuccessful MAAs (both Cerepro®'s assessments) and 3 of 3 successful MAAs (Glybera®, Imlygic®, Strimvelis®)
- Efficacy claims based on post-hoc and sub-group analysis in 2 of 4 unsuccessful MAAs (Advexin® and 2010's Cerepro® assessment) and 1 of 3 successful MAAs (Imlygic®)

Efficacy demonstration has been persistently identified as a key challenge in gene therapy development(13,31,32). We found this to be one of the most frequent objections in MAA assessment. One GTMP was found to be more harmful than the comparator (CLG had a more negative effect on survival versus standard treatment). For Cerepro®'s both MAA, no statistically significant difference was seen compared to standard of care. For Glybera®, the long-term beneficial effects were not clear. Analysis of pancreatitis events as surrogate markers of efficacy was proposed to support the product's positive efficacy profile, but methodological issues hampered the conclusions. Independent adjudication of pancreatitis events by an expert panel according to defined criteria was reviewed and accepted by the CAT/CHMP, in a restricted patient population. For Imlygic®, concerns were noted over the potential delay in next line treatment for non-responders. Additional studies submission as part of Risk Management Plan (RMP) addressed this objection.

The change or use of novel and non-validated pEP was reported as one of the most common objections found in GTMP assessment (23,25,31). For gene therapy, and particularly concerning rare diseases, the use of standard validated endpoints may not be as informative as for traditional medicinal products. Application of more innovative endpoints may be an option though demonstration of validity might ultimately play an important role in the assessment. From the analysed GTMP, the majority (5 of 7 MAAs) were intended to be used as anticancer treatment. The selection of the primary endpoint in clinical trials in oncology has been typically the subject of strong discussion. In this context, the EMA guideline on evaluation of anticancer medicinal products recommends cure rate, Overall Survival (OS) and

Progression Free Survival (PFS) or Disease Free Survival (DFS) as acceptable primary endpoints(33,34).

For Cerepro®'s both MAAs, the survival pEP was updated from patient's lifetime after surgery to time to death or time to reoperation, which was considered by the CAT/CHMP a significant methodological deficiency. Even though the updated pEP was assessed by an independent re-intervention committee, this did not compensate the potential bias due to the open label nature of the study. For Strimvelis®, the survival endpoint was initially defined as time to death related to disease to all-cause mortality, upon CAT/CHMP recommendation. It is well acknowledged that the accuracy of disease specific mortality depends on correctly identifying the cause of death(35), and the updated endpoint was considered acceptable. For Glybera®'s, the change in pEP was based on the evolution of knowledge around disease. Initially, triglycerides reduction was used as pEP, which was later updated to post-prandial Chylomicron (ppCM) reduction. Considering the rarity of the disease, the CAT/CHMP recognized that the pEP update is common. An additional problem with this change was that ppCM was a novel and non-validated endpoint. To address this concern, the applicant proposed to conduct a post-authorization study to assess ppCM metabolism in patients previously treated with Glybera®. Imlygic®'s applicant applied the Durable Response Rate (DRR) as primary endpoint. The CAT/CHMP acknowledged that DRR captured a relevant clinical effect of the treatment, so this issue was considered resolved.

Efficacy claims based on not pre-specified post-hoc analysis were reported for 2 of 4 unsuccessful GTMPs (Advexin® and Cerepro® 2010) as well as 1 of 3 successful GTMP (Imlygic®). These analyses are useful especially if the trial population is heterogeneous. However, interpretation should be carefully conducted as there are a number of commonly known disadvantages(36,37). Methodological issues resulted in data being regarded as hypothesis generating rather than confirmatory. The intended patient population for treatment with Imlygic® was based on a post-hoc analysis. Here, even though the CAT expressed concerns over the post-hoc nature, the regulator acknowledged that these were conducted in compliance with the appropriate EMA Guideline(38).

From the 7 GTMP MAAs analysed, 5 were intended to be used as orphan drugs. Challenges in generating efficacy and safety data are known. Often the trials

are limited by low patient numbers due to recruitment difficulties, inadequate follow-up and trial design issues (i.e. open label nature)(39–41). Expedited regulatory approval pathways such as conditional approval or approval under exceptional circumstances may be useful tools to bring orphan drugs to the market. Glybera® was intended to be used in an ultra-rare indication and the data and the follow-up period presented at submission were incredibly limited, which resulted in an approval only in a small sub-set of patients (i.e. approval under exceptional circumstances). On the other hand, for Strimvelis®, despite the recruitment issues as a pediatric study in an orphan disease, the data as a whole was more compelling and the follow-up period was more extensive, which resulted in a standard approval.

2.3.2.4 Clinical Safety Data Assessment

All MAAs reported at least one deficiency regarding safety assessment and unsurprisingly similar results were obtained for RMP, taking into account that most safety concerns were addressed through this tool **(Table 10).** The most common observations were limited or incomplete safety database (3 of 4 unsuccessful GTMPs and in 2 of 3 successful GTMPs), as well as specific safety concerns over immunogenicity (2 of 4 unsuccessful GTMPs and 3 of 3 successful GTMPs). The risk of immunogenicity has been previously reported as an important hurdle in the GTMP development(32).

Immunogenicity safety concerns regarding Advexin® and CLG were noted as the local immune response risk described in the literature was not adequately assessed by the applicant. For Glybera®, delayed humoral and cellular immunogenicity were identified across all studies. The 3-month immunosuppressive regimen intended to address this risk, though data showed no reduction of unwanted humoral and cellular immunogenicity. The CAT/CHMP raised the concern on the need of the immunosuppressive regimen, though after extensive discussions the regulator concluded that removing the immunosuppressant treatment would represent a major change in therapeutic protocol, potentially affecting patient outcome. Additionally, considering the short-term regimen, the immunogenicity concerns were addressed. For Strimvelis®, the CAT/CHMP reported that the applicant assumed a low immunogenicity risk so the evaluation of anti-adenosine deaminase (ADA) antibodies was not conducted. This issue was addressed as the applicant agreed to assess ADA-antibodies in a post-marketing setting.

Deficiencies regarding RMP were identified in all MAAs. For Advexin® and Cerepro®'s 2007 assessment, the risk minimization measures were not enough to assess important safety risks. The CAT/CHMP required a more robust RMP to be proposed prior to positive Marketing Authorization could be granted. On the other hand, for CLG and Cerepro®'s assessment of 2010, the RMP was not sufficient based on the inability to establish efficacy and safety of the products'. For the successful MAA products, the applicants accepted updates to the RMP requested by the CAT/CHMP. Some measures included, for instance, collection of additional long-term safety data via patient registry or implementation of educational programs for health care professionals (e.g. Glybera®).

Concerning Environmental Risk Assessment, deficiencies were noted for Advexin® and CLG. For all other MAAs, the data presented was considered satisfactory to support that the risk for human health (other than patients) and for the environment was negligible. The main issue concerned immunocompromised individuals who were at high risk, due to presence of RCA in the medicinal product (which may be transferred if immunocompromised individuals came into contact with treated patients, in a potentially sustained fashion), shedding of Ad5-p53 vector and possible horizontal transmission. Risk management strategies were considered insufficient to address this concern in both MAAs.

2.4 Conclusion

Sixteen ATMPs were identified to be assessed by the CAT/CHMP for MAA in the analysed time period, up to December 2017. The CAT's operations seem to have initiated with a slow start and 2016 was identified as a turning point, where the number of successful ATMP MAAs finally surpassed the unsuccessful, a trend that has been sustained until presently.

This is a relatively small and heterogeneous group of products; hence any interpretation of data should be done with great caution. The majority of the MAAs are GTMPs and TEPs (6 of each, i.e. 38%), and half have been granted orphan designation. The overall MAA success rate is 63% and from the successful MAAs there are 70% granted a standard approval, while the other 30% are subject to further data or more limited indications. Almost two thirds of applications come from SMEs highlighting that the development of such innovative products takes place in academic and smaller business companies. Further analysis showed that GTMPs have overall less likelihood of obtaining MAA when compared to TEPs or sCTMPs, but due to the low number of analysed products this finding may have limited value. Having an orphan designation does not seem to be related with higher MAA success compared to non-orphan products. On the other hand, a positive trend for obtaining successful MAA outcome was noted for Non-SME applicants (83%), comparing to SMEs (50%). Here, it is hypothesized that applicants with less complex structures and limited funding may have a lower probability of MAA success. Limited regulatory expertise and restricted experience through smaller product pipeline may also be contributing factors.

Requesting SA/PA or not does not seem to be decisive in terms of successfully obtaining MAA. However, our analysis showed that successful MAAs present an average higher number of requests for SA/PA, comparing to the unsuccessful MAAs (2.6 vs. 2.0, respectively). Though it should be noted that the number of SA/PA requests provides only limited information, comparing to analysis of the content and further compliance with the SA/PA. All MAAs had at least one clock stop where the D120 CS is consistently the longest, in both groups. The CS duration tends to decrease throughout the MAA review process. Successful MAAs present a higher average number of clock stops, when comparing to the unsuccessful group (3.1 vs.

2.0), probably because the latter includes 6 products where 4 were withdrawn prior to opinion, i.e. these MAAs did not go as far in the review process. Generally, a higher number of requests for SA/PA, lower number of oral explanations and shorter duration of assessment were associated with better chance of obtaining a successful MAA.

GTMPs are complex in nature and each individual component may have an impact on the efficacy and safety profile of the product, including the vector, the inserted sequence(s), the target cells modified by the vector or the protein encoded by the vector(13). For GTMPs, major objections, issues or concerns in terms of quality, non-clinical and clinical data were reviewed. Though in the beginning of the CAT's work the Quality data was significantly noted as a deficiency, over the years there have been substantial improvements in this area. Manufacture changes (raising comparability issues) and deficiencies regarding specification of drug product and/or drug substance are highlighted as common objections. Often drug development is initiated in academic setting, where the resources are limited, and optimization of the manufacturing process prior to non-clinical and clinical drug testing is not a priority. With regards to Advexin® and CLG, a necessary consequence of being the first to be assessed for MAA is that there was none or limited past experience as to how the product should be evaluated. It is hypothesized that the submission dossiers were either quite deficient or the regulatory assessment was incredibly strict.

Non-clinical data seems to be the section with the least frequent number of major objections. Non-clinical PK/PD data as well as toxicology are the most frequent concerns, though the importance of the using a risk-based approach (RBA) for the assessment of non-clinical data is highlighted. The RBA is defined as an optional strategy to determine the extent of quality, non-clinical and clinical data to be included in the MAA dossier. Since ATMPs are very diverse in nature the applicant may use a flexible approach to address and evaluate potential risks associated with the clinical use(42).

Clinical assessment is, without a doubt, the section where consistently the CAT/CHMP tends to encounter issues. Particularly, the demonstration of clinical efficacy and safety are the most important points. In terms of clinical efficacy, the data shows that primary demonstration of efficacy, change or use of a non-validated primary endpoint and efficacy claims based on post-hoc and sub-group analysis

constitute the most predominant objections. On the other hand, in terms of safety, the limited database and inadequately addressing immunogenicity concerns are highlighted as the most frequently raised objections.

In the case of successful GTMPs, the majority of the major objections, issues or concerns were addressed through the clarification of the concern via oral explanation or written answer or submission of additional data (either during MAA review or post-marketing). In this context, RMP updates were noted in practically all GTMPs.

Although quantitative data on the request or use of the EMA's initiatives to support ATMPs' development (e.g. ATMP certification, classification, IIT, PRIME) was not analysed, this is acknowledged to be an advantage.

This analysis provided valuable insights, particularly, for future ATMP applications for Marketing Authorization. Clearly, the benefit–risk assessment of ATMPs with subsequent issuing of a successful MAA is a complex and multi-factorial exercise. Experience in the assessment of these products has been accumulating over the years, since the implementation of the CAT. The expectation is that these products will continue to be at the forefront of innovation and become important treatment strategies.

2.5 References

1. Carvalho M, Sepodes B, Martins AP. Regulatory and Scientific Advancements in Gene Therapy: State-of-the-Art of Clinical Applications and of the Supporting European Regulatory Framework. Front Med [Internet]. 2017 Oct 26;4(October). Available from: http://journal.frontiersin.org/article/10.3389/fmed.2017.00182/full
2. Hanna E, Rémuzat C, Auquier P, Toumi M. Advanced therapy medicinal products: current and future perspectives. J Mark Access Heal Policy [Internet]. 2016 Jan 25;4(1):31036. Available from: http://www.jmahp.net/index.php/jmahp/article/view/31036
3. European Medicines Agency. CAT monthly report of application procedures, guidelines and related documents on advanced therapies - December 2017 [EMA/CAT/830803/2017] [Internet]. 2017 [cited 2018 Apr 15]. Available from: https://www.ema.europa.eu/en/documents/committee-report/cat-monthly-report-application-procedures-guidelines-related-documents-advanced-therapies-december_en-2.pdf
4. Farkas AM, Mariz S, Stoyanova-Beninska V, Celis P, Vamvakas S, Larsson K, et al. Advanced Therapy Medicinal Products for Rare Diseases: State of Play of Incentives Supporting Development in Europe. Front Med [Internet]. 2017;4(May):1–6. Available from: http://journal.frontiersin.org/article/10.3389/fmed.2017.00053/full
5. Papathanasiou P, Brassart L, Blake P, Hart A, Whitbread L, Pembrey R, et al. Transparency in drug regulation: public assessment reports in Europe and Australia. Drug Discov Today [Internet]. 2016 Nov;21(11):1806–13. Available from: http://dx.doi.org/10.1016/j.drudis.2016.06.025
6. European Medicines Agency. Committee for Advanced Therapies Monthly Report [EMEA/CAT/169206/2009]. 2009. p. 27–9.
7. Commission Recommendation of 6 May 2003 concerning the definition of micro, small and medium-sized enterprises (2003/361/EC). Official Journal of the European Union. 2003. p. 36–41.
8. European Medicines Agency. SME Registry [Internet]. Available from: https://fmapps.emea.europa.eu/SME/index.php

9. European Medicines Agency. Report from SME Office on its 3rd Year of Operation [EMEA/617936/2008]. 2008.
10. Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004. Vol. L324, Official Journal of the European Union. 2007. p. 121–37.
11. Regulation (EC) No 726/2004 Of The European Parliament And Of The Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agen [Internet]. Vol. L 269, Official Journal of the European Communities. 2000. p. 1–15. Available from: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/reg_2004_726/reg_2004_726_en.pdf
12. European Medicines Agency. Procedural advice on the evaluation of combined advanced therapy medicinal products in accordance with Article 8 of Regulation (EC) No . 1394 / 2007 [EMA/630043/2008]. 2018. p. 1–18.
13. Schneider CK, Salmikangas P, Jilma B, Flamion B, Todorova LR, Paphitou A, et al. Challenges with advanced therapy medicinal products and how to meet them. Nat Rev Drug Discov [Internet]. 2010;9(3):195–201. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20190786
14. de Wilde S, Guchelaar HJ, Herberts C, Lowdell M, Hildebrandt M, Zandvliet M, et al. Development of cell therapy medicinal products by academic institutes. Drug Discov Today [Internet]. 2016;21(8):1206–12. Available from: http://dx.doi.org/10.1016/j.drudis.2016.04.016
15. Regnstrom J, Koenig F, Aronsson B, Reimer T, Svendsen K, Tsigkos S, et al. Factors associated with success of market authorisation applications for pharmaceutical drugs submitted to the European Medicines Agency. Eur J Clin Pharmacol [Internet]. 2010 Jan 20;66(1):39–48. Available from: http://link.springer.com/10.1007/s00228-009-0756-y
16. Ziogas C. A regulator’s instights for SMEs in the biologics and advanced therapies sectors. TOPRA Regul Rapp. 2016;13(2):23–5.
17. Hofer MP, Hedman H, Mavris M, Koenig F, Vetter T, Posch M, et al. Marketing authorisation of orphan medicines in Europe from 2000 to 2013. Drug Discov

Today [Internet]. 2018 Feb;23(2):424–33. Available from: http://dx.doi.org/10.1016/j.drudis.2017.10.012

18. Bravery CA, Ball O, Robinson S. EU market authorisation strategy: lessons from the first 22 ATMP submitted to the EMA. Cell Gene Ther Insights [Internet]. 2019 Jun 24;5(6):759–91. Available from: https://insights.bio/cell-and-gene-therapy-insights/journal/articles/eu-market-authorisation-strategy-lessons-from-the-first-22-atmp-submitted-to-the-ema/
19. European Medicines Agency. Reflection paper on design modifications of gene therapy medicinal products during development [EMA/CAT/GTWP/44236/2009]. 2011.
20. European Medicines Agency. Note for Guidance on the Quality, Preclinical and Clinical Aspects of Gene Transfer Medicinal Products [CPMP/BWP/3088/99] [Internet]. 2001. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500003987.pdf
21. European Medicines Agency. Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products [EMA/CAT/80183/2014] [Internet]. 2015. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/05/WC500187020.pdf
22. Rousseau CF, Mačiulaitis R, Śladowski D, Narayanan G. Cell and Gene Therapies: European View on Challenges in Translation and How to Address Them. Front Med [Internet]. 2018 May 28;5(May):158. Available from: www.frontiersin.org
23. Bryant LM, Christopher DM, Giles AR, Hinderer C, Rodriguez JL, Smith JB, et al. Lessons learned from the clinical development and market authorization of Glybera®. Hum Gene Ther Clin Dev [Internet]. 2013 Jun;24(2):55–64. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3992977&tool=pmcentrez&rendertype=abstract
24. European Medicines Agency. Guideline on bioanalytical method validation [EMEA/CHMP/EWP/192217/2009] [Internet]. 2011. Available from: http://www.falk-bretschneider.eu/biblio/biblio-index.htm

25. Husain SR, Han J, Au P, Shannon K, Puri RK. Gene therapy for cancer: regulatory considerations for approval. Cancer Gene Ther [Internet]. 2015 Dec;22(12):554–63. Available from: http://dx.doi.org/10.1038/cgt.2015.58%5Cn10.1038/cgt.2015.58
26. Boráň T, Menezes-Ferreira M, Reischl I, Celis P, Ferry N, Gänsbacher B, et al. Clinical Development and Commercialization of Advanced Therapy Medicinal Products in the European Union: How Are the Product Pipeline and Regulatory Framework Evolving? Hum Gene Ther Clin Dev [Internet]. 2017 Sep;28(3):126–35. Available from: http://online.liebertpub.com/doi/10.1089/humc.2016.193
27. Gonin P, Buchholz CJ, Pallardy M, Mezzina M. Gene therapy bio-safety: scientific and regulatory issues. Gene Ther [Internet]. 2005 Oct;12 Suppl 1:S146-52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16231047
28. Fallaux FJ, van der Eb AJ, Hoeben RC. Who's afraid of replication-competent adenoviruses? Gene Ther [Internet]. 1999 May 14;6(5):709–12. Available from: http://www.nature.com/articles/3300902
29. European Medicines Agency. Guideline on Non-Clinical Testing for Inadvertent Germline Transmission of Gene Transfer Vectors [EMEA/273974/20059]. 2006. p. 4–6.
30. European Medicines Agency. Points to consider on GCP inspection findings and the benefit-risk balance [EMA/868942/2011] [Internet]. Vol. 44. 2012. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Other/2013/01/WC500137945.pdf
31. de Wilde S, Coppens DGM, Hoekman J, de Bruin ML, Leufkens HGM, Guchelaar H-J, et al. EU decision-making for marketing authorization of advanced therapy medicinal products: a case study [Internet]. Vol. 23, Drug Discovery Today. Elsevier Ltd; 2018. p. 1328–33. Available from: https://doi.org/10.1016/j.drudis.2018.03.008
32. Kumar SR, Markusic DM, Biswas M, High KA, Herzog RW. Clinical development of gene therapy: results and lessons from recent successes. Mol Ther Methods Clin Dev [Internet]. 2016;3(April):16034. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27257611%5Cnhttp://www.pubmedcentral

.nih.gov/articlerender.fcgi?artid=PMC4879992

33. Dabisch I, Dethling J, Dintsios C-M, Drechsler M, Kalanovic D, Kaskel P, et al. Patient relevant endpoints in oncology: current issues in the context of early benefit assessment in Germany. [EMA/CHMP/205/95 Rev.5] [Internet]. Vol. 4, Health economics review. 2014. p. 2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24460706
34. European Medicines Agency. Guideline on the evaluation of anticancer medicinal products in man [EMA/CHMP/205/95 Rev.5]. 2017;44(September 2017). Available from: www.ema.europa.eu/contact
35. Penston J. Should we use total mortality rather than cancer specific mortality to judge cancer screening programmes? Yes. BMJ [Internet]. 2011 Oct 13;343:d6395. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16375258
36. Lagakos SW. The challenge of subgroup analyses-reporting without distorting. N Engl J Med [Internet]. 2006 Apr 20;354(16):1667–9. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMp068070
37. Hemmings R. An overview of statistical and regulatory issues in the planning, analysis, and interpretation of subgroup analyses in confirmatory clinical trials. J Biopharm Stat [Internet]. 2014;24(1):4–18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24392975
38. European Medicines Agency. Guideline on the investigation of subgroups in confirmatory clinical trials [EMA/CHMP/539146/2013]. 2014;44(January):1–20.
39. Picavet E, Cassiman D, Hollak CE, Maertens J a, Simoens S. Clinical evidence for orphan medicinal products-a cause for concern? Orphanet J Rare Dis [Internet]. 2013 Oct 16;8(1):164. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3852769&tool=pmcentrez&rendertype=abstract
40. Winstone J, Chadda S, Ralston S, Sajosi P. Review and comparison of clinical evidence submitted to support European Medicines Agency market authorization of orphan-designated oncological treatments. Orphanet J Rare Dis [Internet]. 2015 Oct 28;10:139. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4625856&tool=pmcentrez&rendertype=abstract
41. Giannuzzi V, Landi A, Bosone E, Giannuzzi F, Nicotri S, Torrent-Farnell J, et

al. Failures to further developing orphan medicinal products after designation granted in Europe: an analysis of marketing authorisation failures and abandoned drugs. BMJ Open [Internet]. 2017 Sep 11;7(9):e017358. Available from: http://bmjopen.bmj.com/lookup/doi/10.1136/bmjopen-2017-017358

42. European Medicines Agency. Guideline on the risk-based approach according to Annex I , part IV of Directive 2001 / 83 / EC applied to Advanced Therapy Medicinal Products [EMA/CAT/CPWP/686637/2011] [Internet]. 2013. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/03/WC500139748.pdf

Appendix 2.1 – Characteristics of ATMPs assessed by the CAT

Year of opinion	ATMP brand name	ATMP INN	ATMP classification	MAA assessment outcome	Orphan designation	Type of applicant
2008	Advexin®	Contusugene ladenovec	Gene Therapy Medicinal Product	Unsuccessful Withdrawn at D120	Yes	SME
2009	ChondroCelect®	Characterised viable autologous cartilage cells expanded *ex vivo* expressing specific marker proteins	Tissue Engineered Product	Successful standard approval	No	SME
2009	CLG	Contusugene ladenovec	Gene Therapy Medicinal Product	Unsuccessful Withdrawn at D120	No	SME
2010	Cerepro®	Sitimagene ceradenovec	Gene Therapy Medicinal Product	Unsuccessful Negative opinion	Yes	SME
2012	Glybera®	Alipogene Tiparvovec	Gene Therapy Medicinal Product	Successful approval under exceptional circumstances	Yes	SME
2013	MACI®	Matrix-applied characterised autologous cultured chondrocytes	Tissue Engineered Product	Successful standard approval	No	Non-SME
2013	Hyalograft ® C autograft	Characterised viable autologous chondrocytes expanded in vitro, seeded and cultured on a hyaluronan-based scaffold	Tissue Engineered Product	Unsuccessful Withdrawn at D120	No	Non-SME
2013	OraNera	Multilayered cell-sheet of autologous oral mucosal epithelial cells	Tissue Engineered Product	Unsuccessful Withdrawn at D180	No	SME
2013	Provenge®	Autologous peripheral-blood mononuclear cells activated with prostatic acid phosphatase granulocyte-macrophage colony-stimulating factor (Sipuleucel-T)	Somatic Cell Therapy Medicinal Product	Successful standard approval	No	Non-SME
2014	Holoclar®	*Ex vivo* expanded autologous human corneal epithelial cells containing stem cells	Tissue Engineered Product	Successful Conditional approval	Yes	Non-SME

Year of opinion	ATMP brand name	ATMP INN	ATMP classification	MAA assessment outcome	Orphan designation	Type of applicant
2015	Heparesc™	Human heterologous liver cells	Somatic Cell Therapy Medicinal Product	Unsuccessful Negative opinion	Yes	SME
2015	Imlygic®	Talimogene laherparepvec	Gene Therapy Medicinal Product	Successful standard approval	No	Non-SME
2016	Strimvelis®	Autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence	Gene Therapy Medicinal Product	Successful standard approval	Yes	Non-SME
2016	Zalmoxis®	Allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2)	Somatic Cell Therapy Medicinal Product	Successful Conditional approval	Yes	SME
2017	Spherox	Spheroids of human autologous matrix-associated chondrocytes	Tissue Engineered Product	Successful standard approval	No	SME
2017	Alofisel®	Darvadstrocel	Somatic Cell Therapy Medicinal Product	Successful standard approval	Yes	SME

ATMP: Advanced Therapy Medicinal Product. CLG: Contusugene Ladenovec. INN: International Nonproprietary Name. MAA: Marketing Authorization Application. MACI: Matrix-applied characterised autologous cultured chondrocytes . Non-SME: Non-Small Medium Enterprize. SME: Small Medium Enterprize.

Appendix 2.2 – Characterization of milestone data in the MAA review process for ATMPs

	Negative		Withdrawn				Positive									
Parameter	**Cerepro®**	**Heparesc™**	**Advexin®**	**Contusugene Ladenovec**	**Hyalograft® C Autograft**	**OraNera**	**Glybera®**	**Imlygic®**	**Strimvelis®**	**ChondroCelect®**	**MACI®**	**Provenge®**	**Holoclar®**	**Zalmoxis®**	**Spherox**	**Alofisel®**
Scientific Advice / Protocol Assistance	1	1	1	1	2	1	3	2	5	1	2	1	4	5	2	1
Overall number of clock stops	4	3	1	1	1	2	3	3	2	4	3	3	2	5	3	3
1st review process Duration of clock stop 1 (D120 - LoQ)	190	180	176			62	187	97	87	193	270	144	398	187	1330	151
Duration of clock stop 2 (D180 - LoOI)	59	63					75	60	1	69	45	70	28	239	12	209
Duration of clock stop 3 (DX - 2nd LoOI)		33						1		183	38	42		25	9	2
Duration of clock stop 4 (DX - 3rd LoOI)										5				19		
Duration of clock stop 5 (DX - 4th LoOI)														1		
2nd review process Duration of clock stop 1 (D120 - LoQ)	95															
Duration of clock stop 2 (D180 - LoOI)	38															
3rd review process Duration of clock stop (CS)							14									
Number of oral explanations (OE)	3	4	0	0	0	0	6	1	0	2	0	1	0	1	1	1
DA_Duration for MAA assessment (Number of active days taken)	523	325	181	324	299	569	353	235	221	286	229	263	205	349	252	268

	Negative		Withdrawn				Positive									
Parameter	Cerepro®	Heparesc™	Advexin®	Contusugene Ladenovec	Hyalograft® C Autograft	OraNera	Glybera®	Imlygic®	Strimvelis®	ChondroCelect®	MACI®	Provenge®	Holoclar®	Zalmoxis®	Spherox	Alofisel®
DD_Duration for MAA decision (Number of active days taken)							451	290	276	388	292	334	266	405	305	367
Period between review processes 1-2	608	64					65									
Period between review processes 2-3							217									
Period between DA to DD							98	55	55	102	63	71	61	56	53	99

Blank cells represent data that is “Not applicable” since the MAA process did not reach that specific milestone. DA: Duration for MAA assessment. DD: Duration for MAA decision. D120: Date when consolidated list of questions (LoQ) was issued. D180: Date when list of outstanding issues (LoOI) was issued. DX: Date when subsequent LoOI was issued. MAA: Marketing Authorization Application. OE: Oral Explanation. SA/PA: Scientific Advice or Protocol Assistance.

Chapter 3

Patient access hurdles towards Gene Therapy use

Abstract

Background: Gene therapies have the potential to be a curative approach to a large number of genetic diseases. However, a positive Marketing Authorization does not equal patient access to therapy.

Objectives: The purpose of this paper is to identify a full set of hurdles potentially preventing patient access to Gene Therapies based on the available literature.

Methods: A review of the literature using systematic approach in two distinct databases was performed by identifying relevant, peer-reviewed publications, between 2012 and 2018.

Results: Seven major topics were identified as potential patient access hurdles, namely affordability, assessment of value, development of therapy, ethical/social factors, evidence generation, operational implementation and regulatory hurdles. From these, twenty five additional sub-themes were further identified. The most frequently mentioned obstacle in the literature is related to the affordability aspect especially focusing on high cost of therapy (84%) and therapy payment/reimbursement (51%). Importantly, the lack of sufficient evidence generation focusing on limited trial outcomes (81%) seems as a strong obstacle in patient access to these therapies.

Conclusions: A growing number of gene therapies are expected to be developed and made available to patients and health care professionals. Improvement of patient access to gene therapies can only be achieved by understanding all hurdles, in a complete and integrated fashion, so that strategies are timely established to ensure gene therapy's benefits are provided to patients and to the society.

Background

Gene Therapy Medicinal Products (GTMP) are exceptionally promising treatment strategies, with the potential to cure a wide array of genetic diseases(1). Extensive research has been conducted in the field. According to Hanna *et. al*, the number of clinical trials using GTMP as medicinal product has increased steadily over the past few years(2).

This trend is aligned with the growing number of commercialized GTMPs worldwide. In Europe, the first GTMP approved was Glybera®, in 2012. Since then, and until end of 2019, six additional products reached a positive Marketing Authorization outcome (Imlygic®, Strimvelis®, Kymriah®, Yescarta®, Luxturna™ and Zynteglo®). Conversely, in the United States (US), the first GTMP reached the market in 2017, while in the same time period a total of five approved products (Imlygic®, Kymriah®, Yescarta®, Luxturna™ and Zolgensma®) GTMPs are available(3,4)

Development of GTMPs is a challenging process. In chapter 2, our research suggested that the main driver for negative Marketing Authorization outcome in Europe is insufficient clinical efficacy evidence as well as safety issues, while issues at quality or non-clinical level play a secondary role in the MAA outcome. Regulators are aware that Advanced Therapy Medicinal Products (ATMPs) aim at diseases of high unmet medical need. Therefore, in Europe, several strategies have been implemented to expedite the MAA process, such as the implementation of the Innovative Task Force (ITF), the ATMP Classification, the ATMP Certification, the PRIority MEdicines (PRIME) scheme and Scientific Advice(1,5).

However, a positive MAA outcome should not be considered an immediate synonym of therapy availability to patients. In Europe, after regulatory approval, health technologies are assessed by many countries, at national level, for their value with subsequent (or parallel) pricing and reimbursement negotiations(6). In the case of gene therapy, this aspect is of particular importance considering the significant budget impact in Healthcare Systems that GTMPs may elicit. Although high cost and budget impact are undoubtedly critical aspects in patient access to innovative therapies, additional factors should be taken into account.

This research intends to provide a comprehensive review of patient access to gene therapy by indentifying a full set of hurdles. A review of the literature using systematic approach in two distinct databases was performed to identify relevant, peer-reviewed publications, between the years of 2012 and 2018. Data extraction was performed and qualitative synthesis is here presented.

3.1 Methodology

3.1.1 Search strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for conducting systematic reviews were followed. This study included publications available in Medine (accessed via Pubmed) and Embase (accessed via Ovid) published between 1974 and 20 Jan 2019. The search strategy was purposefully designed to be broad, in order to ensure all relevant material was included.

Our search included both mapped and un-mapped terms. Within the conducted search “Boolean Operator” rules were utilized. The terms used were searched using ‘AND’ to combine the keywords listed and using ‘OR’ to remove search duplication where possible. Full search strategy is available as **Appendix 3.1 – Search strategy** Embase and **Appendix 3.2 – Search strategy Medline**.

The process of identification, screening and inclusion of papers for this review is detailed in

Figure 14. Records were extracted to EndNote X8. The software de-duplication functionality was used to identify duplicate references. Additionally, manual de-duplication was performed. Following full text review, references were further excluded based on eligibility criteria described in **Table 11.**

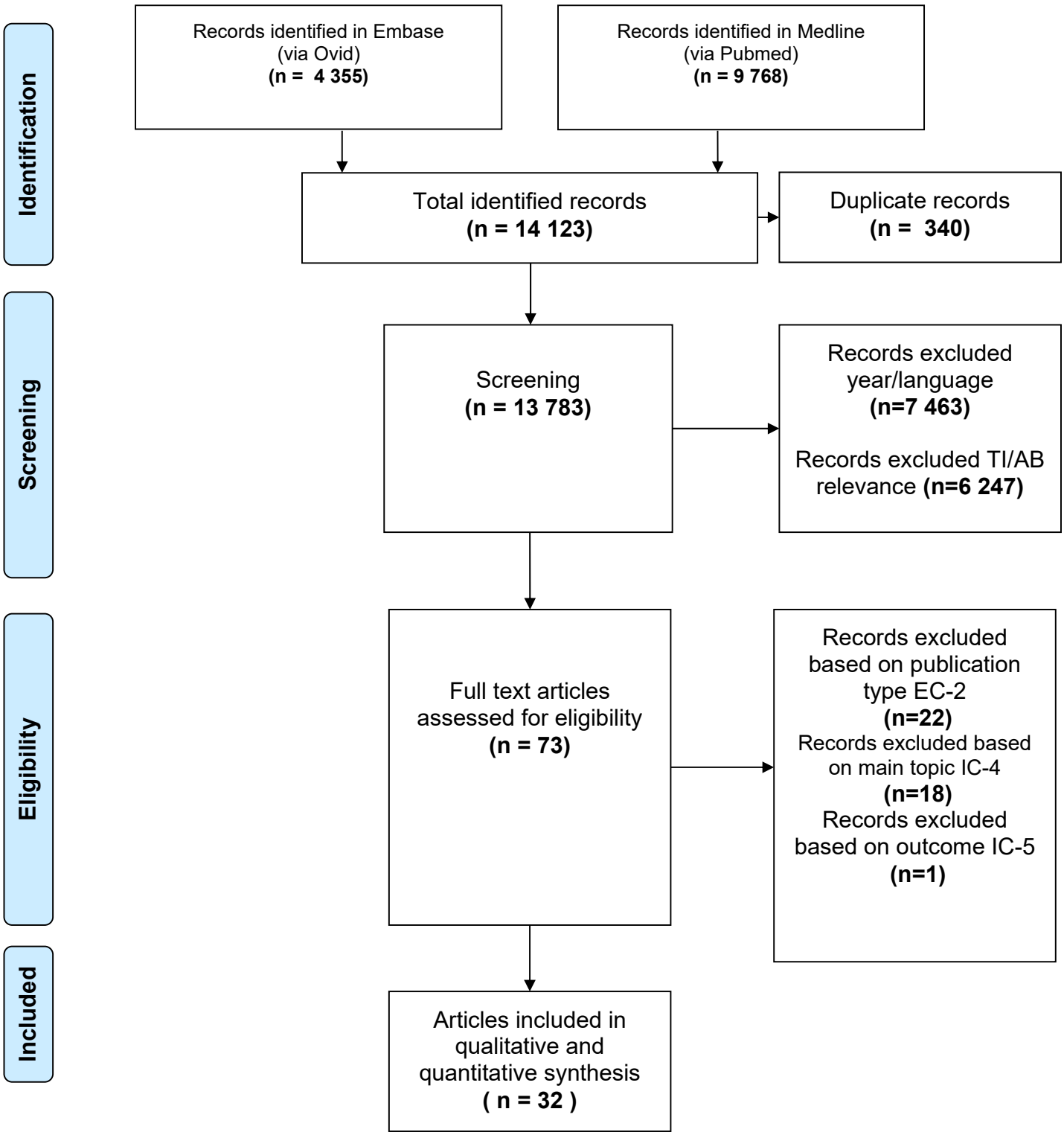


Figure 14 – Process of identification, screening, eligibility and inclusion in this comprehensive review

EC: Exclusion criteria. IC: Inclusion criteria. TI/AB: Title or abstract

Inclusion Criteria		
Number	**Category**	**Description**
IC-1	Publication year	2012-2018
IC-2	Publication type	Full-text articles in peer review journals
IC-3	Publication quality	Full text article should include clear objective, methodology, analysis/discussion, conclusion and a defined set of references
IC-4	Type of medicine	Including but not limited to gene therapy
IC-5	Outcomes	Full text article should include at least one challenge related to patient access
Exclusion Criteria		
Number	**Category**	**Description**
EC-1	Language	Articles not in English
EC-2	Publication type	Book, Book chapters, News articles/press-release, Congress abstracts/posters

Table 11 – Study inclusion and exclusion criteria

EC: Exclusion criteria. IC: Inclusion criteria.

3.1.2 Publication selection

Eligibility criteria were developed in order to reflect the research aim. Firstly, papers were included if they were published between 2012 and 2018. The year of 2012 was selected as lower cut-off date as it was the year that the first GTMP was approved in Europe. Only full-text articles published in peer review journals were included.

Articles which referred to the topic including but not limited to GTMP were included as well. This means that if a paper discussed cell and gene therapy simultaneously, this publication was included in the analysis. Additionally, at least one challenge related to patient access had to be extracted from the full text review, in order to include the publication in the analysis.

Publications were excluded if not written in English. Other publication types such as books, book chapters, news articles/press-release, and congress abstracts/posters were excluded. Eligibility criteria are fully detailed in **Table 11**.

3.1.3 Data extraction and analysis

Publication characteristics were extracted from all relevant articles and were recorded in an extraction table. One researcher (MC) compared and extracted data and discussed any discrepancies with other researchers (BS, APM), when required. An overview of the identification process is documented in the PRISMA diagram, in **Scheme 1**.

Hurdles towards patient access were extracted from the full-text review of the articles. Major themes and sub-themes were pulled from the data, until no more major topics and sub-topics could be identified. Narrative synthesis of the articles was performed. A qualitative and quantitative analysis of the extracted hurdles is here presented.

3.2 Results

The search in both databases identified 14 123 publications. After removing 340 duplicates, a total of 7 463 references were excluded based on year and language. Then, all remaining titles and abstracts were reviewed by MC for relevance, in alignment with the main objective of this research project, where 6 247 were excluded. A total of 73 full text articles were reviewed. Studies were excluded if they did not meet the eligibility criteria specified in the study. Twenty two records were excluded based on exclusion criteria 2 (publication type), 18 records were excluded based on inclusion criteria 4 (main topic) and 1 record was excluded based on inclusion criteria 5 (outcome).

In this analysis, 32 publications were included in qualitative and quantitative synthesis. These publications generated 7 major themes with 25 sub-themes, which are described in the **Table 12.**

A frequency graph was generated which presents the number of publications out of the 32 which mention a specific hurdle, in each sub-theme (**Figure 15**).

The six most common hurdles found in this comprehensive review belong to the themes/sub-themes described below:

- Issues related to therapy cost / price were reported in 27 publications (84%);
- Issues related to therapy payment / reimbursement were reported in 18 publications (51%);
- Issues related to operational implementation (infrastructures) were reported in 14 publications (44%);
- Issues related to payer's budget were reported in 11 publications (34%);
- Issues related to patient related health benefits (assessment of value) were reported in 11 publications (34%).

Each theme and sub-themes are described in more detail in the discussion section.

Major Themes	Sub-Themes
Affordability	Payer's Budget
	Therapy Cost / Price
	Therapy Payment / Reimbursement
Assessment of value	Criteria
	Non-Patient related health benefit
	Patient related health benefits
Development of therapy	Intellectual property
	Manufacturing
	Non-Clinical
	Positioning
	Resources
Ethical / Social factors	Patient's convictions
	Patient's perception
	Socio-economical
Evidence generation	Trial Design
	Trial Conduct
	Trial Outcomes
	Post-Authorization
Operational implementation	Infrastructures
	Patient burden
	Health Care Professionals
Regulatory hurdles	Marketing Authorization Application Process
	Quality standards
	Pricing Regulations
	Parallel Access

Table 12 – Major themes and sub-themes extracted from the included literature

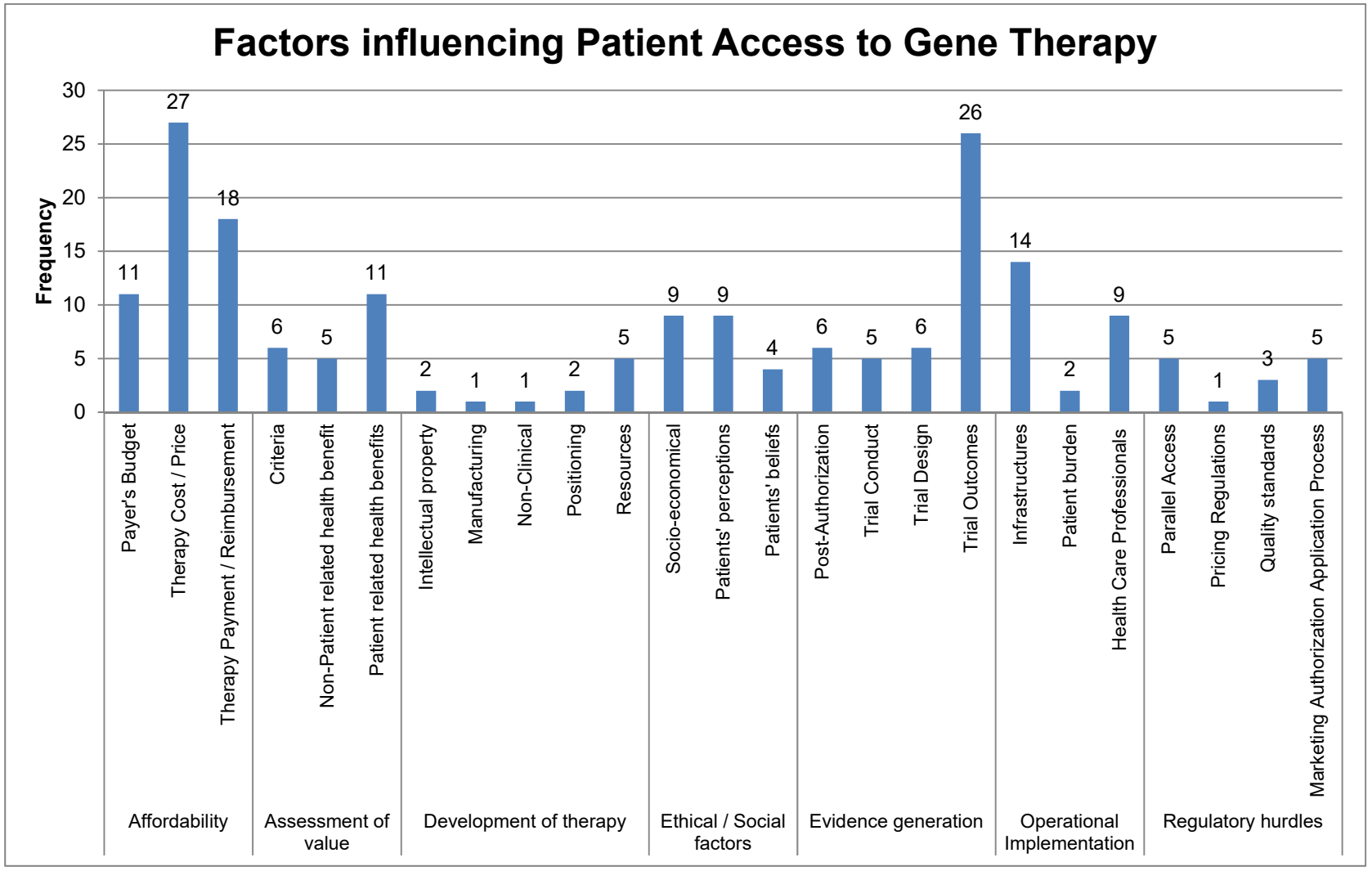


Figure 15 – Frequency of factors influencing Patient Access to Gene Therapy

3.3 Discussion

3.3.1 Affordability

3.3.1.1 Therapy cost / price

Excessive high cost of these therapies is reported in several publications (2,7–25), though the therapy cost/price hurdle does not exclusively related to this. The price level of these types of therapy is mainly justified by the high cost of development and manufacture (23). Some authors also mention that the increased medical follow-up associated with detecting late toxicities contributes to added costs (12,26). Furthermore, higher therapy cost is a possibility in special scenarios. For instance, gene therapy for haemophilia in patients who develop some level of immunogenicity requires multiple administrations, therefore increasing the therapy cost, due to high levels of anti-drug antibodies. In these cases, retreatment may be required in order to achieve a certain level of efficacy) (14).

On the other hand, many gene therapy products were developed using government/public funds. However, when setting up the final price, this is not taken into account (20,26). Estimating annual and lifetime costs is challenging due to variability in disease presentation, type, frequency of treatments required, access to follow-up care and payer source (13).

3.3.1.2 Payer's budget

Such an elevated price clearly has a significant impact on the healthcare budget impact (2,8,9,12,17,20,23,25,27). For example, in the US it is estimated that 25–30 million Americans have a rare condition related to a genetic defect. Considering the initial pricing experience with gene therapy in Europe, if more gene therapy products are made available at US$1–2 million price, the cumulative budget impact would be substantial and perhaps unsustainable. Assuming that gene therapies are developed to treat only one in ten patients with a genetic condition, the cumulative budget impact at that price could rise to US$3 trillion, which is as much as the current spent in a year on all health care in the US(28).

Hurdles at the level of payer's budget are even more marked considering the high pressure to control healthcare budget(12,23). This is especially true considering, for instance, in Europe, the period of recovery after a financial crisis and flattening of gross domestic product growth(2). In March 2017, in addition to the cost effectiveness recommendation, a budget impact test was introduced in England, which assesses whether a new therapy's aggregate additional cost to the healthcare budget exceeds the threshold value of £20 million per year. If the additional cost associated with the new therapy is expected to exceed this threshold in any of the first three years after launch, then additional commercial negotiations and potential restrictions may apply(9).

3.3.1.3 Therapy Payment/Reimbursement

Some authors mentioned the heterogeneity in reimbursement/payment strategies across different countries that geographically lead to different levels of access(20,27). It was clear from our research that standard reimbursement strategies may not be adequate to cope with super high cost treatments (13,20,23,24). Moreover, the reimbursement decision takes place after positive Marketing Authorization, and this process may be lengthy in some cases (20). Even after the full assessment, health insurers or healthcare payers may refuse to reimburse therapy. Some publications mentioned a lack of willingness to pay from governments and payers leading to no reimbursement after approval (11,14,26).

An issue occurring in countries with a competitive private insurance healthcare system (e.g. US or Switzerland) is related to the uncertainty on how to manage patients switching health plans. The first insurance company may be stuck with full upfront payment and no downstream benefit in case the patient decides to switch insurance company(13,20,22).

In the context of cost-effectiveness model, and with regards to discount rates, it is not clear how to reach the appropriate discounting rate. Gene therapies are likely to involve high intervention costs occurring years before all health effects have emerged(25). Often, clinical trials are limited in time, not allowing a full characterization of long-term outcomes. There is, therefore, high uncertainty that needs to be incorporated in the decision making, not only in terms of costs but also in terms of benefits.

Finally, in case a payment based on performance model is implemented, there is uncertainty on which outcomes to monitor. Hard endpoints are preferred, though this may not be possible in all cases(20).

3.3.2 Assessment of value

3.3.2.1 Criteria

Heterogeneity in economic and clinical value assessment systems and budgets across different countries, results in different coverage recommendations based on how Health Technology Assessment (HTA) agencies perceive evidence and uncertainties. These different recommendations clearly lead to different levels of patient access to therapy. Additionally, several publications reported that gene therapy products may be assessed by HTA agencies using the same criteria and scrutiny than other classic therapies, which may be inadequate, considering the specificities of gene therapy. HTA systems appear to not be prepared to assess curative therapies(8,13,15,25,28,29).

3.3.2.2 Non-patient related health benefits

Some publications included in this analysis also highlighted that HTA models may not account for all relevant elements for assessment of value for the health system and society. For instance the ability of patients to go back to work, the work productivity or the impact on caregiver burden, are often not considered for treatment reimbursement or HTA assessment (13,14,25,27,28).

3.3.2.3 Patient related health benefits

A number of publications included in this analysis highlighted that endpoints that matter to patients (e.g. quality of life) are often not aligned with efficacy outcomes for reimbursement (e.g. disease survival). There seems to be a strong mismatch between payers' and regulators' needs(8,13,15,20,24–26). This divergence may lead to a drug being approved by the Health Authorities for commercialization but not reimbursed and, therefore, with a low level of market uptake.

Similarly to the issues noted at the level of non-patient related health benefits, some publications reported that HTA models may not account for all relevant elements for assessment of value for patients(13,14,25,28). For instance, most gene therapies have the potential to be curative therapies. These may be valued more highly by society as opposed to non-curative therapies. Cure of a disease at a young age could help produce significant gains in many aspects such as omitting the cost of avoidable co-morbidities, lifelong management of complications but also reducing the economic impact over individual patients and their caregivers/families (i.e. work productivity) compared with treatments that bring marginal gains over many years. There is little evidence that such balance is currently being included in gene therapy HTA assessments (14,28).

3.3.3 Development of therapy

3.3.3.1 Intellectual property

It is known that the academia plays a strong role in the development of gene therapy products. A survey conducted in 2016 reviewed ATMPs clinical trials in the EU and reported that the majority of the sponsors (62%) were non-profit organisations, representing academia, hospitals and charities(30). GTMP development is often initiated at the academic level and then leveraged by larger pharmaceutical companies after commercial agreements are established.

The Academia/Industry partnerships influence remains uncertain, as the merger of academic intellectual freedom with big business focus on value may create conflict(29,31). If these divergences remain unresolved or take too long to reach a solution, this may impact patient access to GTMPs.

3.3.3.2 Manufacturing

Another issue, particularly regarding gene therapy products based on genetic cell modification (e.g. Chimeric Antigen Receptor (CAR) or T-Cell Receptor(TCR)), is related to the composition of the cell product. Uncertainties remain regarding the content of drug product/drug substance. Upon drug development, investigators question which sub-types of cells should be included. The selection of specific cell subtypes may increase even more the manufacturing costs, impacting therapy price and, ultimately, patient access. The choice of cells is key for therapy success.

Additionally, how to measure such success may pose challenges. Currently, high uncertainty exists regarding therapeutic success biomarkers. Without measurable efficacy biomarkers, the therapy will never reach patients(31).

3.3.3.3 Non-Clinical

During gene therapy development process, animal testing is key for advancing to clinical trials. This is a challenging process and lack of appropriate non-clinical testing data may have a therapy fail before even reaching to first-in-human studies. Understanding cross-species variability, particularly regarding viral vectors tropism and transduction efficiency, is critical for predicting clinical outcomes. Appropriate development of validated preclinical assays is key to clinical experience(29).

3.3.3.4 Positioning

External competitive landscape may have a significant impact in GTMP development with consequences to patient access. For instance, in the case of haemophilia, if the companies that bring GTMPs to market already have traditional haemophilia products within their portfolio, their incentive to offer gene therapy for a low price may be lacking because the new technology would disrupt their existing market (14).

Another example is the ongoing innovation on regular monoclonal antibody therapy that can directly compete with antibody gene therapy. The classic therapy is a less costly alternative, with less administration burden and may potentially offer higher efficacy(32).

3.3.3.5 Resources

Developer resources levels may also be a key factor influencing patient access. As previously mentioned, development of gene therapy often starts in non-profit organizations. In our research, some authors noted the lack of manufacturers experience(2,33) as well as lack of preparedness from market access strategy and launch sequence(2), as key aspects impacting patient access. Additionally, limited resources for translational research from academia and early clinical trials(29) and

lack of reimbursement after approval may lead to disincentive for small business manufacturers to develop breakthrough therapies(9).

3.3.4 Ethical / Social factors

3.3.4.1 Patients' beliefs

Core individual values and beliefs may influence whether patients' choose to have GTMP treatment or not. In two publications, it was reported that some patients may be unwilling to receive GTMPs due to religious beliefs(34,35). Other patients may be intrinsically against germline genome manipulation, thereby refusing treatment(19). Finally, a study showed that one of the biggest fears about receiving gene therapy was that patients would not receive all the relevant information from their health care professionals prior to receiving treatment. This apprehension is directly related to the assumption that gene therapy may alter features such as identity and personality(35).

3.3.4.2 Patients' perception

Patients' perceptions on gene therapy may play a powerful role in the level of access to therapy. Several publications highlight a general lack of genetic literacy, not only from patients but also from caregivers(16,17,36,37), which in turn contributes to an inaccurate perception of gene therapy. In one publication, the potential irresponsible use of novel technologies and unrealistic expectation of cure (e.g. in the case o HIV) was also noted as a barrier related to patients' perception(18). Furthermore, the fear that genetic therapy will be utilized by those with means to improve intellect, physical abilities and longevity, thereby enhancing social inequality, was also noted as a potential access barrier(21,35).

Additionally, patients may be unwilling to receive genetic therapy due to psychological challenges (e.g. receiving news about testing positive for a genetic marker of disease)(34).

Finally, a study showed that the degree of gene therapy acceptance by the public is directly related with the seriousness of the condition. If the disease is very serious, then patients will be more willing to accept gene therapy (35).

3.3.4.3 Socio-economical factors

Socio-economical, cultural and geographical factors may potentially restrict access to gene therapy. Different price setup according to geographic regions will result in different GTMP availability. Consequently, others may become "treatment tourisms". Finally, according to geographic region, there may be differences in standard of care therapy cost. These differences lead to different comparisons and recommendations on gene therapy reimbursement, which may cause discrepancies in patient access(14,16–18,20,21,27,34,38).

3.3.5 Evidence generation

3.3.5.1 Trial Design

A US publication by Hampson and colleagues reported the implementation of fully blinded, placebo controlled studies with specific GTMPs, in specific indications, would require unethical sham procedures(23) (e.g. those GTMPs that require invasive methods of administration like Glybera®, where the patient is administered with multiple intramuscular injections).

Furthermore, challenges at the level of comparator identification have been reported. Here, those therapies developed for diseases where there is no treatment are the most affected (13,23,25). In many cases, there is no other choice but to assess data resulting from single-arm, open label or even observational studies, which are known to be less robust for benefit-risk evidence generation.

Finally, finding easily measured patient-centered outcomes to assess efficacy was reported as an important hurdle related to clinical trial design. Trials evaluating gene therapy may rely on surrogate outcomes, as opposed to clinical outcomes. For instance, in oncology setting, the use of data from progression free survival as a surrogate endpoint rather than data from overall survival as a clinical endpoint allows implementation of shorter duration trials, contributing to a more expedited regulatory assessment of a Marketing Authorization. On the other hand, other less known surrogate endpoints may be used and, in that case, these need to be developed and validated, with limited data and limited time.

Weighing up the benefits and risks of any medicine is a complex process, as it involves the evaluation of a large amount and diverse type of data. The actual

benefits and risks of any medicine are determined based on the information that is available at a given point in time, which often involves a fair level of uncertainty.

In case surrogate endpoints are used, frequently there is considerable uncertainty because these may not allow capturing the combined benefit–risk profile of a technology and a surrogate may not translate to benefits for a clinical endpoint. (23,25).

3.3.5.2 Trial Conduct

With regards to clinical trial conduct, four publications reported that getting patients diagnosed and recruited into clinical trials, as well as promoting adherence to medical follow-up is an important hurdle. Patients seems to be inherently reluctant to share their data and participate in clinical translation(17,23,25,26). This may potentially be related to patient's limited knowledge of GTMPs. A study on patient's perspectives regarding gene therapy for Sickle Cell Disease reported lack of knowledge of gene therapy from patients (e.g. patients had fear of getting HIV if the vector was based on inactivated HIV virus) and a perception that treatment with gene therapy would be unacceptably unsafe (e.g. negative effects of concomitant chemotherapy in sickle cell gene therapy, potential new onset of cancer due to gene therapy, potential infertility problems)(39).

3.3.5.3 Trial Outcomes

Upon reviewing the data generated through pivotal clinical trials, some hurdles have been identified which could potentially be an obstacle to patient access. Firstly, a strong uncertainty related to safety data, whether short, medium or long term has been reported(13,15,23,25,29,31,32). For instance, for CAR-T gene therapy product Yescarta®, a number of patients experienced Citokine Release Syndrome (CRS) and unexplained neurotoxicity. CRS symptoms ranged from fever and myalgias to life-threatening unstable hypotension and respiratory failure. While treatable for most cases, fatalities have been reported. On the other hand, the use of integrating vectors has an inherent potential genotoxicity risk, which is of particular importance following past reports that primary immunodeficiency children treated with retroviral vectors developed cancer.

Additionally, uncertain long term efficacy of gene therapy products has been reported as a hurdle in patient access by several authors(8–10,17,23,27,29,32). On the one hand, most of the clinical trials for candidate new GTMPs are conducted in a limited patient population (i.e. rare diseases) where the main clinical efficacy endpoint is fairly new to the regulators and scientific community. These endpoints may not be the best choice, but this only becomes clear after some time, based on the evolution of knowledge around the disease. A great example of this was the European regulatory assessment of Glybera®, where the initially assessed primary endpoint was triglyceride reduction but later it was noted that this surrogate endpoint was too variable from patient to patient and postprandial chylomicron reduction was used instead. Upon regulatory approval, long term efficacy is extrapolated from pivotal clinical trials and when such uncertainty is raised at the pivotal trial level, it is even more difficult to predict effectiveness.

Moreover, the durability of clinical effect remains questionable. On the one hand, this may be due to the unpredictability of transgene expression. Immunogenicity may limit a prolonged expression, which could potentially be related to a decreased clinical effect. On the other hand, tissue targeting refinement may be needed to improving transduction efficiency(32).

Based on the way trials are designed and conducted (limited patient population, limited follow-up time, limited experience in primary clinical efficacy/safety endpoint analysis) it becomes clear that both efficacy and safety evidence at launch may be extremely immature. This may have an impact on limiting therapy access to patients(2,9,12,13,15,16,20,23–25)

Overall, there is the need for improved understanding of the role of specific disease factors in gene therapy outcomes. As time goes by, more knowledge is built, leading to a better selection of patient population, biomarkers and endpoints(29).

3.3.5.4 Post-Authorization

After regulatory approval, post-authorization data is a mandatory requirement not only for safety but also effectiveness monitoring. Securing drug reimbursement is also often based on obtaining real world evidence. This is particularly important for GTMPs, where approval/reimbursement may be obtained with incredibly limited

number of patients and open-label, uncontrolled clinical trials which are generally very limited in time (8,9,25,27).

In some instances, there is the need of implementing a patient registry. Several challenges related to this method of collecting post-authorization data have been reported(20,40), including but not limited to:

- Low number of patients (considering rare diseases landscape);
- Long term follow-up which may lead to low retention rate;
- High administrative burden (e.g. establishing site contract, local ethics committee approval, site staff training, etc.);
- High associated costs (e.g. registry oversight, costs associated with multiple sites, database setup, etc.);
- Limited data quality (e.g. who is contributing to the registry, i.e. only physicians? Patients? Family/caregivers?);
- Limited resources (e.g. regulatory agencies often approve gene therapy conditional to the implementation of a disease registry. From an Industry perspective, Sponsors prefer a registry based on drug-use, while Regulatory agencies favour a broader disease-based registry);
- Data privacy issues (e.g. in US, if reimbursement is based on implementation of a patient registry, the legislation would need to change due to issues with privacy legislation).

3.3.6 Operational Implementation

3.3.6.1 Infrastructures

Gene therapy manufacturing and quality control process is lengthy and complex (e.g. difficulty in large-scale production of clinical-grade vectors). Besides not being readily available, certain GTMPs have generally short shelf-life, which may be particularly challenging in cases of urgent need of therapy (e.g. acute diseases)(7,25,29).

Access to therapy may also be influenced by the need for adequate healthcare infrastructures regarding gene therapy manufacturing, administration and pre/post-administration medical monitoring(8,12,15,16,18–20,23,38). One publication reported that major health system changes are required before gene therapy can be

fully implemented, highlighting the current limitations in information technology systems and limited support tools for clinical use of the information(37).

Finally, many therapies in precision medicine, and especially gene therapy, need to have an appropriate validated companion diagnostic test approved by regulatory agencies, which the availability may differ from country to country(17).

3.3.6.2 Patient burden

Generally, gene therapy administration involves a heavy patient burden(7,8). Patients need to be hospitalized to receive therapy. The hospitalizations may be for a variable period of time, since it may also include either pre-administration preparation and/or post-administration medical monitoring. The majority of traditional drugs are self-administered by the patient, or even administered by a close caregiver, in the comfort of their home environment. For the case of gene therapy, due to its unique characteristics, administration in the hospital setting is likely to be the rule. Here, one should take the patient's perspective where an additional itineration from patient's home to a specific healthcare facility (in this case, hospital setting) could potentially be a hurdle for patient access, in many aspects, such as additional time spent or additional resources. Strimvelis® is an example of gene therapy administered only at one reference site, in Italy, meaning that patients have to travel to that specific clinical setting to receive treatment.

3.3.6.3 Health Care Professionals

As a unique and very distinctive therapeutic strategy, compared to classic treatments, gene therapy requires formal health care professional training (e.g. with regards to safety and rescue therapy should any life-threatening toxicity occur)(7,8,13,18,20,25,37). Also, physicians should be adequately trained to clearly explain patients and caregivers the benefits and risks(17) of gene therapy.

Finally, a higher than usual administrative burden is expected for gene therapy(12), related to electronic patient medical records completion by health care professionals as well as other administrative documents (e.g. health insurance forms). Overall, the specific training and higher administrative burden will likely result on an increase in human resource workload.

3.3.7 Regulatory hurdles

3.3.7.1 Marketing Authorization Application Process

Hurdles related to Marketing Authorization Application (MAA) process have been identified to contribute to different levels of patient access to gene therapies. Firstly, there is a lack of regulatory harmonization regarding ATMPs definition(33). In Europe, the definition of ATMP, is included in Regulation 1394/2007/EC. However, when a Sponsor requests a classification from the European Medicines Agency (EMA), this it is not legally binding, and each member state may classify the same product differently.

Additionally, there is a lack of regulatory harmonization towards MAA approval resulting in a different number of approved ATMPs across different geographic regions. For instance, up to 2017, in Europe there were 9 cell and gene therapy products approved through centralized procedure, while in the US there were 17 products(41). From a patient access perspective, this may generate differences in accessing treatment according to geographic region. Legislative flexibility exists in different jurisdictions, specifically created to facilitate access to therapy for products not yet centrally authorized, although this means additional time and resources spent.

One publication specifically focusing on academic developers also reported the lack or limited interaction with regulators which decreases chances of a positive MAA(33).In Europe, several regulatory strategies are currently in place to support new ATMPs early in the development process, such as requesting for Scientific Advice / Protocol Assistance (SA/PA). A recent study conducted by Bravery and colleagues(42) analyzed the first 22 ATMP MAA submissions to the EMA suggests that requesting SA/PA does not seem to be decisive in terms of successfully obtaining MAA, since all Sponsors requested it. Large pharmaceutical companies requested more SA/PA compared to Small-Medium Enterprises (SME), where academic developers are included. On the one hand, the initiative to request SA/PA comes primarily from the applicant, as well as the content of the advice that is sought. It seems fundamental to ask the right questions, on the right timing. In addition, and although it may be unexpected, non-compliance with the Regulator's advice can be accepted in some cases. For instance, for Imlygic®, advice was sought regarding the primary endpoint. While the EMA advised to use progression-

free survival or overall survival, the applicant decided to use durable response rate, which was considered acceptable by the EMA, with proper justification.

Finally, two publications highlighted the lack of reimbursement after approval which may cause withdrawal of MAA(9,41). For instance, in Europe, the Sponsor of Provenge® (Sipoleucel-T) requested withdrawal of MAA in May 2015. The MAA of Glybera® (Alipogene tiparvovec) expired in October 2017 and the Sponsor chose not to review it due to commercial reasons.

3.3.7.2 Quality standards

Academic centers are important contributors to GTMP development. A study by Pearce *et. al* (33) has shown several interesting barriers at the level of quality standards that may have an impact on patient access. Firstly, even though it is considered an essential process, GMP manufacture adds significant costs and complexity to the production process. Secondly, there are unrealistic expectations of product qualification by some national health authorities in terms of manufacturing process. Lastly, in the EU, there is the statutory requirement for a qualified person (QP) for the release of investigational medicinal products. QP release of each batch when a single batch treats a single patient is prohibitively expensive and may even be logistically impossible in some cases.

3.3.7.3 Pricing Regulations

While there are heterogeneous pricing regulations across different geographical areas, it is clear that GTMPs with elevated price will increase financial pressure on healthcare budget. Payers are less and less willing to pay for therapy with immature evidence, given the continuously increasing healthcare spent. However, in diseases of high unmet medical need, society is likely to exert pressure on politicians to enable access to therapies. In this context, pricing policies and regulations need to be reconsidered, taking into account the growing number of high-cost gene therapy products approaching the market(2).

3.3.7.4 Parallel Access

In Europe, Hospital Exemption (HE) is an alternative pathway to centralized Marketing Authorization. HE is a permission that can be granted by EU member states for unauthorized ATMPs to be used on a named-patient basis in a hospital setting, within the same member state, only and under the exclusive responsibility of the treating physician. While theoretically this should promote patient access to GTMPs, the less stringent requirements in HE may put public health at risk(26,33,41). For instance, HE has been criticized because its implementation varies between Member States, which has been said to put patients at risk (e.g., due to non-routine processing in small batches). In addition, a successful pharmaceutical industry's lobbying resulted in attaining such a level playing field (i.e. comparable competition environment/setting), in which the conditions for applying HE are kept as narrow as possible. Consequently, hospitals have more difficulty in competing with commercial actors manufacturing ATMPs. This has resulted in some valuable established therapies risking to become unavailable for patients in need of them(41,43). Additionally, the abusive use of parallel access pathways may result in withdrawal of centralized Marketing Authorization(33,41).

3.4 Conclusion

A limited number of GTMPs have successfully been granted successful Marketing Authorization, in Europe. In chapter 2, a retrospective study focusing on hurdles that GTMPs face during the MAA process was reported. Clinical efficacy and safety issues appeared to have a major impact resulting in unsuccessful MAA outcome for GTMPs.

However, a positive MAA does not necessarily mean that the therapy is actually being used by patients and health care professionals. This research used a systematic approach to provide a broad overview of items that may potentially impact patient access to gene therapy, based on available literature between 2012 and 2018, from two separate databases.

From this comprehensive review, seven major themes were identified as potential patient access hurdles and twenty five sub-themes were further identified. The major themes are outlined below:

1. Affordability
2. Assessment of value
3. Development of therapy
4. Ethical / Social factors
5. Evidence generation
6. Operational Implementation
7. Regulatory hurdles

Affordability issues especially related to therapy cost/price (84%) but also to therapy payment/reimbursement (51%) are those most mentioned throughout the publications included in this analysis. There is no question that providing a potentially curative therapy comes at a certain price, most of the times unprecedentedly high. Throughout the years, this has not been the case for traditional medicines, as often the new products are intended to treat rather than cure diseases. Overall pressure to control healthcare budget is elevated. The assessment of value provides a link between therapy benefits for the patient and for the health care system and the willingness to pay. The payment/reimbursement decision-making process is based on the generated evidence which is often fairly limited, not only in patient numbers but also in the follow-up time, as most gene therapy products target rare diseases.

This uncertainty contributes to different levels of access to gene therapy, since with the same data it has been noted that one product is reimbursed in one country but not in another, due to different criteria. Our research results seem aligned with other author(44,45) in the sense that evidence generation (trial outcomes) and affordability (reimbursement issues) present two of the most relevant hurdles in GTMP patient access. Additionally, value appreciation is noted as an important hurdle for patient access impacting reimbursement. HTAs and Payers are heterogeneous group of decision makers across jurisdictions and diverse assessment methodologies have an impact on the decision process.

The lack of relevant information (comparative data versus potential comparators, robust QoL data, collection of relevant outcomes, short trial durations) raises high uncertainty regarding the long-term efficacy and safety for most gene therapies. HTA bodies use different methodologies to minimize this uncertainty whilst accepting high cost GTMPs. Despite this, to date, most gene therapies have successfully been granted reimbursement, with more or less delay in terms of assessment timelines, as described elsewhere(46).

In less extent, ethical and social aspects related to the use of genetic therapy also seem to impact patient access. It became clear that the more serious a medical condition is, the more likely the patient is willing to use gene therapy. Operational implementation of gene therapy also rises as an important access aspect, especially related to the need of having specific infrastructures for administration of therapy and medical follow-up, as well as trained health care professionals. Some hurdles (e.g. patient perception, beliefs, etc.) are applicable to all patients, regardless of geography. Upon identification of country-specific hurdles, we attempted to identify at all times its geographic origin, whilst integrating them in the overall context of the patient access hurdles.

Society and healthcare systems must adjust to this new reality. It is expected in the near future that more and more GTMPs are developed and made available to patients and health care professionals. Improvement of patient access and GTMP availability can only be achieved by understanding all hurdles, in a complete and integrated fashion. It is important to have these hurdles present so that clear strategies are set to overcome them since the significant benefits of gene therapy will not be realised unless patients have access to it.

3.5 References

1. Carvalho M, Sepodes B, Martins AP. Regulatory and Scientific Advancements in Gene Therapy: State-of-the-Art of Clinical Applications and of the Supporting European Regulatory Framework. Front Med [Internet]. 2017 Oct 26;4(October). Available from: http://journal.frontiersin.org/article/10.3389/fmed.2017.00182/full
2. Hanna E, Rémuzat C, Auquier P, Toumi M. Advanced therapy medicinal products: current and future perspectives. J Mark Access Heal Policy [Internet]. 2016 Jan 25;4(1):31036. Available from: http://www.jmahp.net/index.php/jmahp/article/view/31036
3. Food and Drug Administration. Approved Cellular and Gene Therapy Products [Internet]. 2019 [cited 2020 Jun 3]. Available from: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products
4. Vaggelas A, Seimetz D. Expediting Drug Development: FDA's New Regenerative Medicine Advanced Therapy Designation. Ther Innov Regul Sci [Internet]. 2019 May 12;53(3):364–73. Available from: http://journals.sagepub.com/doi/10.1177/2168479018779373
5. Carvalho M, Martins AP, Sepodes B. Hurdles in gene therapy regulatory approval: a retrospective analysis of European Marketing Authorization Applications. Drug Discov Today [Internet]. 2019 Mar;24(3):823–8. Available from: https://doi.org/10.1016/j.drudis.2018.12.007
6. N. T. Early insights from commercialization of gene therapies in Europe. Genes (Basel) [Internet]. 2017;8(2):78. Available from: http://www.mdpi.com/2073-4425/8/2/78/pdf
7. Abou-El-Enein M, Bauer G, Reinke P. The business case for cell and gene therapies. Nat Biotechnol [Internet]. 2014 Dec 9;32(12):1192–3. Available from: http://dx.doi.org/10.1038/nbt.3084
8. Jørgensen J, Kefalas P. Reimbursement of licensed cell and gene therapies across the major European healthcare markets. J Mark Access Heal Policy [Internet]. 2015 Jan 30;3(1):29321. Available from:

https://www.tandfonline.com/doi/full/10.3402/jmahp.v3.29321

9. Jørgensen J, Kefalas P. Annuity payments can increase patient access to innovative cell and gene therapies under England's net budget impact test. J Mark Access Heal Policy [Internet]. 2017 Jan 31;5(1):1355203. Available from: https://doi.org/10.1080/20016689.2017.1355203
10. Malik NN. Pay-for-performance pricing for a breakthrough heart drug: learnings for cell and gene therapies. Regen Med [Internet]. 2016 Apr;11(3):225–7. Available from: http://www.futuremedicine.com/loi/rme
11. Touchot N, Flume M. The payers' perspective on gene therapies. Nat Biotechnol [Internet]. 2015 Sep 8;33(9):902–4. Available from: http://www.nature.com/articles/nbt.3332
12. Kefalas P, Ali O, Jørgensen J, Merryfield N, Richardson T, Meads A, et al. Establishing the cost of implementing a performance-based, managed entry agreement for a hypothetical CAR T-cell therapy. J Mark Access Heal Policy [Internet]. 2018 Jan 20;6(1):1511679. Available from: https://doi.org/10.1080/20016689.2018.1511679
13. R. S, F. C, T. H, H.L. M, P. R, B. F-C. Addressing the Value of Gene Therapy and Enhancing Patient Access to Transformative Treatments. Mol Ther [Internet]. 2018;26(12):2717–26. Available from: https://www.journals.elsevier.com/molecular-therapy
14. Skinner MW. Gene Therapy for Hemophilia: Addressing the Coming Challenges of Affordability and Accessibility. Mol Ther [Internet]. 2013 Jan;21(1):1–2. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1525001616305524
15. Touchot N, Flume M. Early Insights from Commercialization of Gene Therapies in Europe. Genes (Basel) [Internet]. 2017 Feb 17;8(2):78. Available from: http://www.mdpi.com/2073-4425/8/2/78
16. Tuckson R V., Newcomer L, De Sa JM. Accessing Genomic Medicine. JAMA [Internet]. 2013 Apr 10;309(14):1469. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2013.1468
17. Salgado R, Moore H, Martens JWM, Lively T, Malik S, McDermott U, et al. Societal challenges of precision medicine: Bringing order to chaos. Eur J Cancer [Internet]. 2017 Oct;84:325–34. Available from:

https://linkinghub.elsevier.com/retrieve/pii/S0959804917311486

18. M. A-E-E, G. B. Gene therapy: A possible future standard for HIV care. Trends Biotechnol [Internet]. 2015;33(7):374–6. Available from: http://www.elsevier.com/locate/tibtech
19. Bauer G, Abou-El-Enein M, Kent A, Poole B, Forte M. The path to successful commercialization of cell and gene therapies: empowering patient advocates. Cytotherapy [Internet]. 2017 Feb;19(2):293–8. Available from: http://dx.doi.org/10.1016/j.jcyt.2016.10.017
20. Carr DR, Bradshaw SE. Gene therapies: the challenge of super-high-cost treatments and how to pay for them. Regen Med [Internet]. 2016 Jun;11(4):381–93. Available from: https://www.futuremedicine.com/doi/10.2217/rme-2016-0010
21. K. C, K. P, C.M. W. Equitable Access to Gene Therapy: A Call to Action for the American Society of Gene and Cell Therapy. Mol Ther [Internet]. 2018;26(12):2715–6. Available from: https://www.journals.elsevier.com/molecular-therapy
22. D. C, M. C, G. L, N. K. Insurance switching and mismatch between the costs and benefits of new technologies. Am J Manag Care [Internet]. 2017;23(12):750–7. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emexb&NEWS=N&AN=623516072
23. G. H, A. T, S.D. P, W.B. D. Gene therapy: Evidence, value and affordability in the US health care system. J Comp Eff Res [Internet]. 2018;7(1):15–28. Available from: http://www.futuremedicine.com/loi/cer
24. Hanna E, Rémuzat C, Auquier P, Toumi M. Gene therapies development: slow progress and promising prospect. J Mark Access Heal Policy [Internet]. 2017 Jan 3;5(1):1265293. Available from: http://dx.doi.org/10.1080/20016689.2017.1265293
25. Jönsson B, Hampson G, Michaels J, Towse A, von der Schulenburg J-MG, Wong O. Advanced therapy medicinal products and health technology assessment principles and practices for value-based and sustainable healthcare. Eur J Heal Econ [Internet]. 2018; Available from: http://link.springer.de/link/service/journals/10198/index.htm

26. G. B, M. A-E-E, A. K, B. P. The path to successful commercialization of cell and gene therapies: empowering patient advocates. Cytotherapy [Internet]. 2017;19(2):293–8. Available from: http://www.journals.elsevier.com/cytotherapy
27. Jørgensen J, Servos S, Kefalas P. The potential price and access implications of the cost-utility and budget impact methodologies applied by NICE in England and ICER in the US for a novel gene therapy in Parkinson's disease. J Mark Access Heal Policy [Internet]. 2018 Jan 6;6(1):1500419. Available from: https://doi.org/10.1080/20016689.2018.1500419
28. Hampson G, Towse A, Pearson SD, Dreitlein WB, Henshall C. Gene therapy: evidence, value and affordability in the US health care system. J Comp Eff Res [Internet]. 2018 Jan;7(1):15–28. Available from: https://www.futuremedicine.com/doi/10.2217/cer-2017-0068
29. O'Reilly M, Federoff HJ, Fong Y, Kohn DB, Patterson AP, Ahmed N, et al. Gene Therapy: Charting a Future Course—Summary of a National Institutes of Health Workshop, April 12, 2013. Hum Gene Ther [Internet]. 2014 Jun;25(6):488–97. Available from: http://www.liebertpub.com/doi/10.1089/hum.2014.045
30. Boráň T, Menezes-Ferreira M, Reischl I, Celis P, Ferry N, Gänsbacher B, et al. Clinical Development and Commercialization of Advanced Therapy Medicinal Products in the European Union: How Are the Product Pipeline and Regulatory Framework Evolving? Hum Gene Ther Clin Dev [Internet]. 2017 Sep;28(3):126–35. Available from: http://online.liebertpub.com/doi/10.1089/humc.2016.193
31. Barrett DM, Grupp SA, June CH. Chimeric Antigen Receptor– and TCR-Modified T Cells Enter Main Street and Wall Street. J Immunol [Internet]. 2015 Aug 1;195(3):755–61. Available from: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.1500751
32. Hollevoet K, Declerck PJ. State of play and clinical prospects of antibody gene transfer. J Transl Med [Internet]. 2017 Dec 7;15(1):131. Available from: http://translational-medicine.biomedcentral.com/articles/10.1186/s12967-017-1234-4
33. Pearce KF, Hildebrandt M, Greinix H, Scheding S, Koehl U, Worel N, et al.

Regulation of advanced therapy medicinal products in Europe and the role of academia. Cytotherapy [Internet]. 2014 Mar;16(3):289–97. Available from: http://dx.doi.org/10.1016/j.jcyt.2013.08.003

34. Cornetta K, Brown CG. Balancing Personalized Medicine and Personalized Care. Acad Med [Internet]. 2013 Mar;88(3):309–13. Available from: https://insights.ovid.com/crossref?an=00001888-201303000-00014
35. Robillard JM, Roskams-Edris D, Kuzeljevic B, Illes J. Prevailing Public Perceptions of the Ethics of Gene Therapy. Hum Gene Ther [Internet]. 2014 Aug;25(8):740–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24773182
36. H. S, M.J. M, A. G-L, L. S, P. M. Erratum to: Patient Perspectives on Gene Transfer Therapy for Sickle Cell Disease (Advances in Therapy, (2017), 34, 8, (2007-2021), 10.1007/s12325-017-0587-7). Adv Ther [Internet]. 2017;34(9):2177. Available from: http://www.springer.com/springer+healthcare/journal/12325
37. Vorderstrasse AA, Hammer MJ, Dungan JR. Nursing Implications of Personalized and Precision Medicine. Semin Oncol Nurs [Internet]. 2014 May;30(2):130–6. Available from: http://dx.doi.org/10.1016/j.soncn.2014.03.007
38. F.J. E-R, A. C-N, J.L. F. Into Action: Improving Access to Optimum Care for all Primary Immunodeficiency Patients. J Clin Immunol [Internet]. 2016;36(5):415–7. Available from: http://www.wkap.nl/journalhome.htm/0271-9142
39. H. S, M.J. M, A. G-L, L. S, P. M. Patient Perspectives on Gene Transfer Therapy for Sickle Cell Disease. Adv Ther [Internet]. 2017;34(8):2007–21. Available from: http://www.springer.com/springer+healthcare/journal/12325
40. Stirnadel-Farrant H, Kudari M, Garman N, Imrie J, Chopra B, Giannelli S, et al. Gene therapy in rare diseases: the benefits and challenges of developing a patient-centric registry for Strimvelis in ADA-SCID. Orphanet J Rare Dis [Internet]. 2018 Dec 6;13(1):49. Available from: https://ojrd.biomedcentral.com/articles/10.1186/s13023-018-0791-9
41. D.G.M. C, M.L. DB, H.G.M. L. Global Regulatory Differences for Gene- and Cell-Based Therapies: Consequences and Implications for Patient Access and

Therapeutic Innovation. Clin Pharmacol Ther [Internet]. 2018;103(1):120–7. Available from: http://www.nature.com/clpt/index.html

42. Bravery CA, Ball O, Robinson S. EU market authorisation strategy: lessons from the first 22 ATMP submitted to the EMA. Cell Gene Ther Insights [Internet]. 2019 Jun 24;5(6):759–91. Available from: https://insights.bio/cell-and-gene-therapy-insights/journal/articles/eu-market-authorisation-strategy-lessons-from-the-first-22-atmp-submitted-to-the-ema/
43. Mansnérus J. Encountering Challenges with the eu Regulation on Advance Therapy Medical Products. Eur J Health Law [Internet]. 2015 Oct 28;22(5):426–61. Available from: https://brill.com/view/journals/ejhl/22/5/article-p426_2.xml
44. Hanna E, Marre C, Toumi M. PBI59 The Reimbursement Status of Advanced Therapy Medicinal Products in Europe. Value Heal [Internet]. 2019 Nov 1;22:S428. Available from: https://doi.org/10.1016/j.jval.2019.09.163
45. R. M, Y. Q, M. Z, A. M, K. S. Long-Term Safety Follow-Up of Subjects Previously Treated with Non-Replicating Retroviral Vector-Based Gene Therapies. Mol Diagnosis Ther [Internet]. 2016;20(6):591–602. Available from: http://rd.springer.com/journal/40291
46. Qiu T, Hanna E, Dabbous M, Borislav B, Toumi M. Health Technology Assessment of Gene Therapies in Europe and the USA: Analysis and Future Considerations. Cell Gene Ther Insights [Internet]. 2019 Sep 4;5(8):1043–59. Available from: https://insights.bio/cell-and-gene-therapy-insights/journal/articles/health-technology-assessment-of-gene-therapies-in-europe-and-the-usa-analysis-and-future-considerations/

Appendix 3.1 – Search strategy

Embase

Database: Embase <1974 to 2019 January 18>

Search Strategy:

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1 exp health care access/ or exp health care delivery/ (2913739)

2 exp gene therapy/ (78311)

3 gene therapy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (91990)

4 genetic therapy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (575)

5 viral therapy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (1718)

6 recombinant nucleic acid.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (19)

7 DNA therapy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (47)

8 recombinant DNA.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (23441)

9 nucleic acid therapy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (46)

10 RNA therapy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (107)

11 Gene Transfer Techniques.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (466)

12 DNA Viruses.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (3475)

13 RNA Viruses.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (7197)

14 Genetic Vector.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (55)

15 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 (131367)

16 Market.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (89054)

17 patient.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (5159673)

18 healthcare.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (328210)

19 medicines.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (71975)

20 drugs.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (938723)

21 pharmaceuticals.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (47428)

22 16 or 17 or 18 or 19 or 20 or 21 (6180165)

23 Access.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (428847)

24 availability.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (245933)

25 accessibility.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (45388)

26 23 or 24 or 25 (686811)

27 22 and 26 (253478)

28 1 or 27 (3067123)

29 15 and 28 (4355)

Appendix 3.2 – Search strategy

Medline

("health services accessibility"[MeSH Terms] OR ((((((Market[All Fields] OR ("patients"[MeSH Terms] OR "patients"[All Fields] OR "patient"[All Fields])) OR ("delivery of health care"[MeSH Terms] OR ("delivery"[All Fields] AND "health"[All Fields] AND "care"[All Fields]) OR "delivery of health care"[All Fields] OR "healthcare"[All Fields])) OR ("Medicines (Basel)"[Journal] OR "medicines"[All Fields])) OR ("pharmaceutical preparations"[MeSH Terms] OR ("pharmaceutical"[All Fields] AND "preparations"[All Fields]) OR "pharmaceutical preparations"[All Fields] OR "drugs"[All Fields])) OR ("pharmaceutical preparations"[MeSH Terms] OR ("pharmaceutical"[All Fields] AND "preparations"[All Fields]) OR "pharmaceutical preparations"[All Fields] OR "pharmaceuticals"[All Fields])) AND ((Access[All Fields] OR availability[All Fields]) OR accessibility[All Fields]))) AND ((((((((((("genetic therapy"[MeSH Terms] OR ("genetic therapy"[MeSH Terms] OR ("genetic"[All Fields] AND "therapy"[All Fields]) OR "genetic therapy"[All Fields] OR ("gene"[All Fields] AND "therapy"[All Fields]) OR "gene therapy"[All Fields])) OR ("genetic therapy"[MeSH Terms] OR ("genetic"[All Fields] AND "therapy"[All Fields]) OR "genetic therapy"[All Fields])) OR ("oncolytic virotherapy"[MeSH Terms] OR ("oncolytic"[All Fields] AND "virotherapy"[All Fields]) OR "oncolytic virotherapy"[All Fields] OR ("viral"[All Fields] AND "therapy"[All Fields]) OR "viral therapy"[All Fields])) OR (recombinant[All Fields] AND ("nucleic acids"[MeSH Terms] OR ("nucleic"[All Fields] AND "acids"[All Fields]) OR "nucleic acids"[All Fields] OR ("nucleic"[All Fields] AND "acid"[All Fields]) OR "nucleic acid"[All Fields]))) OR ("genetic therapy"[MeSH Terms] OR ("genetic"[All Fields] AND "therapy"[All Fields]) OR "genetic therapy"[All Fields] OR ("dna"[All Fields] AND "therapy"[All Fields]) OR "dna therapy"[All Fields])) OR ("dna, recombinant"[MeSH Terms] OR ("dna"[All Fields] AND "recombinant"[All Fields]) OR "recombinant dna"[All Fields] OR ("recombinant"[All Fields] AND "dna"[All Fields]))) OR (("nucleic acids"[MeSH Terms] OR ("nucleic"[All Fields] AND "acids"[All Fields]) OR "nucleic acids"[All Fields] OR ("nucleic"[All Fields] AND "acid"[All Fields]) OR "nucleic acid"[All Fields]) AND ("therapy"[Subheading] OR "therapy"[All Fields] OR "therapeutics"[MeSH Terms] OR

"therapeutics"[All Fields]))) OR (("rna"[MeSH Terms] OR "rna"[All Fields]) AND ("therapy"[Subheading] OR "therapy"[All Fields] OR "therapeutics"[MeSH Terms] OR "therapeutics"[All Fields]))) OR ("gene transfer techniques"[MeSH Terms] OR ("gene"[All Fields] AND "transfer"[All Fields] AND "techniques"[All Fields]) OR "gene transfer techniques"[All Fields])) OR ("dna viruses"[MeSH Terms] OR ("dna"[All Fields] AND "viruses"[All Fields]) OR "dna viruses"[All Fields])) OR ("rna viruses"[MeSH Terms] OR ("rna"[All Fields] AND "viruses"[All Fields]) OR "rna viruses"[All Fields])) OR ("genetic vectors"[MeSH Terms] OR ("genetic"[All Fields] AND "vectors"[All Fields]) OR "genetic vectors"[All Fields] OR ("genetic"[All Fields] AND "vector"[All Fields]) OR "genetic vector"[All Fields]))

Chapter 4

Overall discussion

4.1 Thesis relevancy considering the overall and current health context

4.1.1 Pharmaceutical innovation

Pharmaceutical innovation aims at providing society with a therapeutic arsenal, which can safely and effectively address an unmet healthcare need. As further knowledge in disease mechanisms is built, a large number of new medicines become available, every year. Since its foundation in 1995 until 2018, the EMA has recommended authorisation of over 1 200 medicines for use in humans(1), meaning that, on average, around 50 new drugs are annually approved, in Europe. In the United States, from 1950 to 2008, the FDA approved a similar number of new molecular entities, including new biologics(2).

A review in the number of new active substances approved in Europe (**Table 13**) gives us a sense that, in recent years, the number of newly approved drugs has been gradually slowing down, away from the 50 new drugs per year, potentially highlighting that innovation in the pharmaceutical industry is progressively more difficult.

Year	New active substances approved in Europe
2015	39 (3)
2016	27 (4)
2017	35 (5)
2018	42 (6)
2019	30 (7)

Table 13 – New active substances approved in Europe (2015-2019)

The innovation triad in the pharmaceutical industry intends to improve health and wellbeing of patients, enhance health management for healthcare professionals, with budget savings for payers(8).

4.1.2 From Drug Development to Clinical Application

Drug development remains an expensive, long and high-risk industry with a high associated attrition rate (9,10). Generally, between 5 000 and 10 000 compounds are screened before one fortunate drug candidate successfully passes all the needed testing and a Marketing Authorization is granted. This process takes a variable amount of time, ranging from 10 to 15 years, from drug discovery to clinical use. Before proceeding with administration to humans, a wide number of *in vitro* and *in vivo* test procedures are conducted as well as non-clinical studies to assess the pharmacology and biochemistry of the drug. Afterwards, clinical phases of drug development include phase I in healthy volunteers to assess primarily pharmacokinetics, safety and tolerability, followed by phase II in patients with the target disease to establish efficacy and dose-response relationship. Large-scale phase III studies are subsequently conducted to confirm safety and efficacy. Once data from one or more successful pivotal trials is obtained, an overall benefit/risk balance is discussed and, if considered positive, a Marketing Authorization may be granted. However, the assessment of new medicinal product's safety continues beyond the initial drug approval through post-marketing monitoring of adverse event (10,11). In Europe, once a medicine has received Marketing Authorisation and before commercialization, the decisions about pricing and reimbursement take place at national and/or regional level (12), through a formal Health Technology Assessment process, which is followed by (or includes) pricing negotiations (13)**(Figure 16)**.

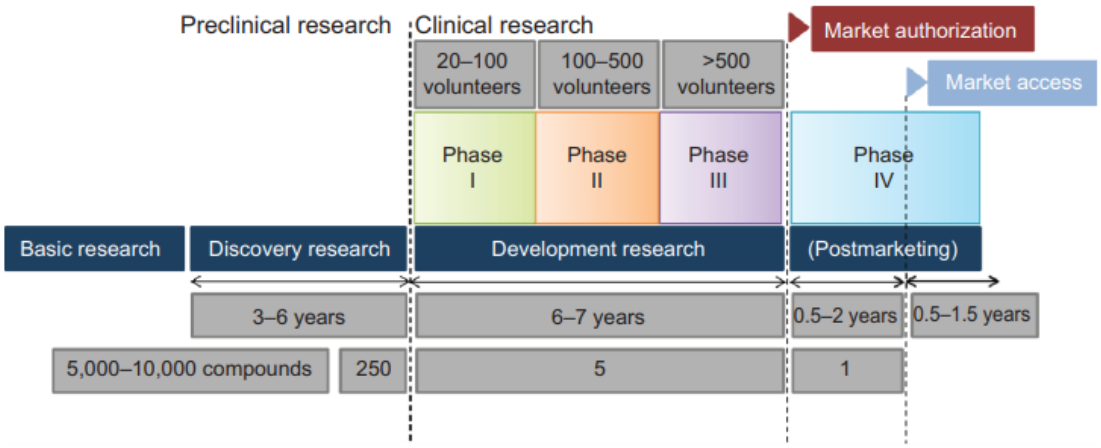

Figure 16 – Schematic representation of the drug development process

Figure adapted from Cianni and Jommi, 2014 (11). Process with timeline, attrition rate, and sample sizes of clinical studies is represented. Timing of different stages and sample sizes vary according to different countries, manufacturers, and indications

Generally, the drivers of drug development process comprise three main dimensions(10)(**Figure 17**). First, a **medical need** should be clearly defined. From a business standpoint, developing a new health technology for a disease of high unmet medical need is appealing. Different interpretations of the concept of unmet medical need are available and have been identified elsewhere(14). In Europe, the definition is included in European Regulation (EC) No. 507/2006 on conditional marketing authorization. Here, "*unmet medical needs means a condition for which there exists no satisfactory method of diagnosis, prevention or treatment in the Union or, even if such a method exists, in relation to which the medicinal product concerned will be of major therapeutic advantage to those affected*"(15).

Secondly, it is important to take into account the **disease prevalence**(10). Data from ClinicalTrials.Gov shows that, between 2005 and 2007, the majority of ongoing clinical studies focused on therapeutic areas such as oncology, infectious diseases, endocrinology and central nervous system disorders(16). Unsurprisingly, these are diseases of high prevalence and, therefore, developing a new drug with added benefit for any of these diseases is extremely attractive.

Lastly, the **likelihood of success** should be considered. This is a more heterogeneous dimension, for which factors related to the drug candidate itself should be considered, such as having promising early data on the candidate new molecule or in similar molecules. On the other hand, external factors should also be taken into consideration, for instance the competitive pharmaceutical landscape.

Figure 17 – Drug development drivers

4.1.3 Drug development in the era of precision medicine

Precision medicine is a promising approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person. In contrast to a “one-size-fits-all” approach, precision medicine allows a more accurate prediction of which treatments and/or prevention strategies will be effective in different patient groups (17,18).

Precision medicine has changed a number of aspects in the traditional drug development process. Development of the medicine is intended for those patient populations who are most likely to benefit, thus new treatments are created for smaller patient groups. In addition, precision medicine therapies are likely to require the co-development of diagnostic tools to identify the optimal treatment for individual patients(17). In terms of clinical development, the implementation of new trial designs(18), such as basket trials or umbrella trials, may be needed. For instance, in the development of personalized cancer therapies, basket trials are innovative trial designs which evaluate the effectiveness of a drug based on its underlying mode of action rather than strictly on the specific form of cancer it was intended to treat. Alternatively, in umbrella trials, genomically guided targeted treatments are provided to groups of patients with the same cancer type, and outcomes are compared to controls receiving only standard therapy(17).

Ultimately, precision medicine should ensure that patients get the right treatment, at the right dose at the right time, with maximum efficacy and safety(18). It stratifies clinical populations into mechanistic subgroups allowing a molecular classification of disease. This will potentially, in turn, result in a higher success rate within those molecularly defined subpopulations, thereby benefiting patients, health care professionals, drug developers, regulators and payers (17).

4.1.4 Gene therapy as a therapeutic innovation approach: the answer to diseases of high unmet medical need

Considering the precision medicine framework, it is clear that ATMPs, and specifically gene therapy, is a valuable and very relevant tool. Gene therapy offers groundbreaking new opportunities in the treatment of genetic diseases. These products present a more specific and targeted treatment in many rare diseases for which the specific underlying cause is known, e.g., a gene defect (19).

Gene therapy becomes highly relevant considering the specific framework of diseases of high unmet medical need, such as rare medical conditions. Although rare diseases affect small numbers of patients, an estimated total of 350 million patients globally are affected, corresponding to more than double the number of AIDS and cancer patients combined(20). In 2018, an estimated 27 to 36 million European citizens suffered from an orphan disease. There are more than 6000 rare diseases, from which 80% of rare diseases are of genetic origin, often chronic and life-threatening(21).

A study conducted by Farkas and colleagues, including all medicinal products that were granted orphan designation (OD) between 2001 and April 2016, highlighted that GTMPs represent the largest group among the requests for OD, with 49%(19).

More than 10 years after the implementation of the ATMP regulation, it is time to reflect on the current ATMP panorama, and specifically focusing on gene therapy, in an integrated and complete fashion. A number of authors and research groups have dedicated their time to analyze individual or a specific set of obstacles preventing regulatory approval or post-marketing gene therapy availability. However, to the best of our knowledge, none of them attempted to present a full set of hurdles, towards gene therapy patient access. This thesis intends to develop an end-to-end

understanding of ATMPs, particularly focusing on GTMPs, from drug development to regulatory post-authorization use.

4.2 Summary of findings in each chapter

The key findings in each chapter are summarized below, which will be further discussed in this chapter.

Regulatory hurdles - Chapter 2.1 and 2.2

- A relatively small and heterogeneous group of products (16 ATMPs) was identified to be assessed by the CAT/CHMP for MAA in the analyzed time period, up to December 2017.
- **Baseline ATMP MAA characteristics in Europe:**
    - The majority of the MAAs correspond to GTMPs and TEPs (6 of each, i.e. 38%)
    - Orphan designation was granted to 50% of ATMPs
    - The overall ATMP MAA success rate is 63%
    - SMEs were responsible for 63% of MAAs
    - From the successful MAAs there are 70% granted a standard approval, while the other 30% are subject to further data requirements or more limited indications
- **ATMP MAA hurdles in Europe:**
    - **ATMP type**: GTMPs have overall less likelihood of obtaining MAA (50%) when compared to TEPs (75%) or sCTMPs (67%)
    - **Orphan designation**: the same proportion of orphan ATMPs obtained successful MAA, comparing to non-orphan products (62,5%). As such, having an orphan designation does not seem to be related with higher MAA success compared to non-orphan products
    - **Applicant type**: a positive trend for obtaining successful MAA outcome was noted for Non-SME applicants (83%), comparing to SMEs (50%)
    - **Scientific Advice/Protocol Assistance (SA/PA):**
        - All ATMPs included in the analysis, regardless of successful or unsuccessful MAA outcome, requested SA/PA, at least once, SA/PA. Hence, requesting SA/PA or not does not seem to be decisive in terms of successfully obtaining MAA

        - Successful MAAs present an average higher number of requests for SA/PA, comparing to the unsuccessful MAAs (2.6 vs. 2.0, respectively)
    - **Clock stops:**
        - Successful MAAs report a higher average number of clock stops, when comparing to the unsuccessful group (3.1 vs. 2.0)
        - A higher average duration of D120 clock stop was noted for successful products (190.4 days) compared to the unsuccessful (152 days), when excluding Alofisel® as an outlier
    - **Oral explanations**: higher number of OE for successful ATMPs (median = 1) was noted comparing to the unsuccessful group (median = 0)

Regulatory hurdles - Chapter 2.3

- **GTMP MAA hurdles in Europe:**
    - 75% of unsuccessful GTMP MAAs presented unacceptable major objections, issues or concerns related to quality data.
        - Substantial improvement in quality data was noted as more MAAs were assessed
        - Manufacture changes (raising comparability issues) and deficiencies regarding specification of drug product and/or drug substance are highlighted as common objections.
    - 50% of unsuccessful GTMP MAAs presented unacceptable major objections, issues or concerns related to non-clinical data
        - Non-clinical PK/PD data as well as toxicology are the most frequent concerns
    - 100% of unsuccessful GTMP MAAs presented unacceptable major objections, issues or concerns related to clinical efficacy and safety data
        - Clinical efficacy: the most frequent objections noted during MAA assessment were related to primary demonstration of efficacy (3 of 4 unsuccessful MAAs and 2 of 3 successful MAAs), followed by the change or use of a non-validated primary endpoint (2 of 4

unsuccessful MAAs and 3 of 3 successful MAAs). Lastly, efficacy claims based on post-hoc and sub-group analysis were noted as objections in 2 of 4 unsuccessful MAAs compared to 1 of 3 successful MAAs.

        - Clinical safety: the limited database and inadequately addressing immunogenicity concerns are highlighted as the most frequently raised objections.

- **Resolving or preventing major objections, issues or concerns in Europe:**
    - In the case of successful GTMPs, most issues were addressed through the clarification via oral explanation or written answer or submission of additional data (either during MAA review or post-marketing). In this context, RMP updates were noted in practically all GTMPs.
    - Although quantitative data on the request or use of the EMA's initiatives to support ATMPs' development (e.g. ATMP certification, classification, IIT, PRIME) was not analysed, this is acknowledged to be an advantage and may prevent the regulator from raising objections during MAA assessment.

Patient access hurdles - Chapter 3

- Seven major themes (underlined in the following text) and 25 sub-themes were identified as worldwide hurdles for gene therapy patient access
    - The most commonly mentioned hurdle was related to **affordability** issues, especially regarding therapy cost/price (84% of the publications), followed by **evidence generation**, namely in trial outcomes (81%). Then, therapy payment/reimbursement issues (51%) were the third most common issue identified
    - **Operational implementation** hurdles (i.e. having specific infrastructures for administration of therapy and medical follow-up) were reported in 44% of the publications, as well as the need of having adequately trained health care professionals (28% of the publications)
    - **Ethical and social aspects** related to the use of genetic therapy also seem to impact patient access, as reported in 28% of the publications

- In less extent, the heterogeneity of criteria used on **value assessment** in different geographic locations was reported in 19% of the publications, followed by hurdles at the level of **development of therapy** and **regulatory** (16%)

4.3 Thesis results considering the existing body of evidence

In **Chapter 1**, a description of clinical applications was presented, particularly in gene therapy medicinal products, focusing on currently EU approved medicines as well as various promising investigational treatments. A number of pre-identified challenges in gene therapy development and post-authorization use were explored and were considered as a starting point for the research subsequently conducted. Issues such as safety signals, limited efficacy, drug development hurdles and ethical aspects were discussed. Importantly, a regulatory overview of the legal framework in Europe towards granting ATMP marketing authorization was provided, which was especially important to contextualize chapter 2.

4.3.1 Regulatory hurdles

More than a decade has now passed since the implementation of the European ATMP regulation and the approval of the first ATMP, through the centralized procedure. In **chapter 2**, our research showed that the CAT's operations were initiated with a slow start. The year of 2016 was identified as a turning point, where the number of ATMP successfully obtaining Marketing Authorization finally surpassed the unsuccessful group. For GTMPs, this turning point occurred two years later, in 2018, as per **Figure 18**. These trends have been sustained until the present days. It is widely acknowledged that the rate of new MAA for ATMPs is low, considering not only other types of medicinal products(22), but the growing number of new and ongoing clinical trials where the Investigational Medicinal Product is an ATMP(23).

In **chapter 2.1**, sixteen ATMPs were identified to be assessed in Europe by the CAT/CHMP for MAA, in the analyzed time period, up to December 2017. From these, 38% were GTMPs. Since the completion of the research described in chapter 2, we have seen an incredible progress in the GTMP setting, as depicted in **Figure 18.** In the last years, five additional ATMPs have been granted a successful MAA in Europe, all of them are GTMPs. From these 5 products, Kymriah®(24), Yescarta®(24) and Zynteglo®(25) are considered *ex vivo* gene therapies while Luxturna™(26) and Zolgensma®(27) are meant for *in vivo* gene therapy treatment.

One additional GTMP (Raligize) was assessed by the CAT, but the application was withdrawn before an opinion could be issued, due to concerns over the data from the main study not being sufficient to support the approval of the medicine(28). In just under three years, the number of assessed GTMPs by the European CAT has doubled, highlighting that the gene therapy landscape is a fast-growing highly innovative field. Our research included a relatively small and heterogeneous group of products, which may be considered as the first set of GTMP MAAs to be assessed from a regulatory standpoint, in Europe. Combined with the incredible growth recently seen means that any interpretation of data should be done with great caution.

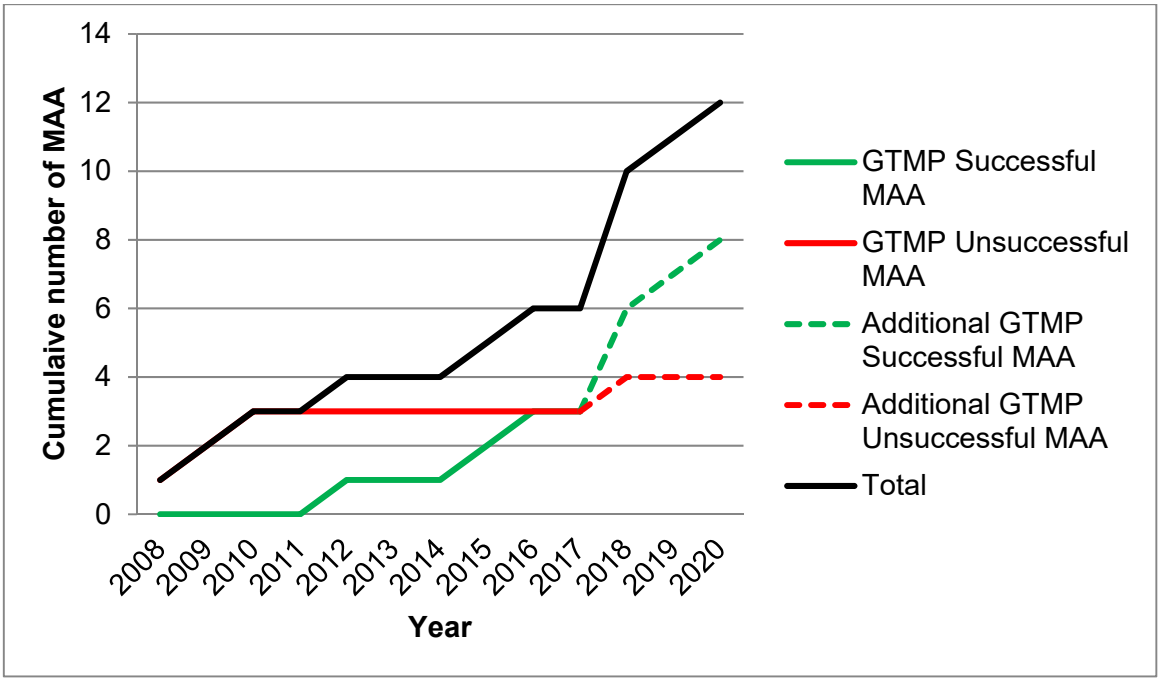

Figure 18 – Cumulative number of MAA for GTMPs (2008-June 2020)

From the 16 MAAs analyzed, the majority were either GTMPs or TEPs (6 products of each). As mentioned in the above paragraph, this proportion has changed dramatically in the last 3 years, with the assessment of 6 additional ATMP MAAs, all of them GTMPs. From the currently assessed 22 ATMP MAAs, twelve (55%) correspond to GTMPs, highlighting the importance of such products in the Advanced Therapies setting. With regards to ATMP MAA success factors, our research suggests that, in Europe, GTMPs have overall less likelihood of obtaining MAA (50%) when compared to TEPs (75%) or sCTMPs (67%), but due to the low number of analyzed products, this finding has limited value. In fact, looking at the

current landscape of 22 ATMPs(29), eight out of 12 GTMPs (66%) were considered successful MAAs. Therefore, considering the enormous growth of assessed GTMPs in the past recent years, the type of ATMP does not seem to affect the MAA outcome.

Half of these have been granted orphan designation, a proportion that is aligned with other authors' publications. The same percentage of orphan ATMPs was found in a study conducted by De Wilde and colleagues, in 2018, which analyzed the first 14 MAAs in EU for ATMPs(30). A more recent study conducted by Bravery and colleagues in 2019, included the first 22 MAAs for ATMPs submitted to the EMA and found that 60% had orphan designations(29). The higher proportion of orphan products found in the latter study is likely influenced by the addition of GTMPs approved in the past recent years. Moreover, we attempted to understand whether having an orphan designation affects MAA success. Our study suggests that obtaining orphan designation status does not seem to impact MAA outcome, since the same proportion of orphan ATMPs obtained successful MAA, comparing to non-orphan products (62.5%). However, Bravery and colleagues study(29) suggests that a tendency was observed for orphan ATMP to have higher approval rate (67%) compared to non-orphan products (50%). The higher proportion of orphan drugs in the ATMP group may explain this. Additionally, some studies expressed concerns on whether regulators have similar scientific and regulatory standards when reviewing and assessing the benefits and risks of orphan drugs comparing to non-orphan medicinal products. Some studies suggest that orphan drugs were authorized to the market with a less rigorous study design, less hard endpoints and more serious safety concerns than non-orphan drugs(31), while other studies suggest the contrary(32,33). The reasons behind such trend need to be further explored, and will certainly become clearer as more ATMPs are assessed for Marketing Authorization. Comparing to all medicines assessed between 2000 and 2013, no difference in orphan ATMP MAA success was noted, both having approval rates of around two thirds(34).

The overall MAA success rate for ATMPs calculated in the present research was 63%. This is in line with the current body of evidence where De Wilde's study found a success rate of 57%(30) while Bravery's study presents a rate of 59%(29). Unsurprisingly, the MAA success rate for ATMPs is lower comparing to all medicines

applications in Europe. Here, based on published data from all EMA submissions between 2000 and 2013, for all medicines applications, the MAA success rate is 76%(34).

From the successful MAAs, 70% were granted a standard approval, while the remaining 30% were approved via an expedited pathway (i.e. either through conditional approval or approved under exceptional circumstances). Interestingly, in De Wilde and colleagues study, where 8 successful ATMP MAAs were included, 62% of the MAAs is noted as having standard approval(30). On the other hand, Bravery's study highlights that from the 13 successful ATMPs, 75% had sufficient data for a full MA(29), concluding that, over the years, the proportion of ATMPs granted a standard approval has been increasing. This suggests that the data packages included in the MAA package has been more robust as more MAAs are assessed.

Almost two thirds of applications come from SMEs (63%). In Bravery's and colleagues study, this proportion is slightly higher, reaching 73%(29). This reinforces that the development of the majority of ATMPs takes place in academic and smaller business environment. In fact, data from an European survey on ATMPs in clinical trials between 2009-2015 shows that 62% of Sponsors were non-profit organizations, including academia, hospitals and charities(23). A positive trend for obtaining successful MAA outcome was noted for Non-SME applicants (83%), comparing to SMEs (50%), which is true for ATMPs but also for other medicinal products, such those of biological origin(35,36). Unsurprisingly, these numbers are the same in Bravery and colleagues study(29). We hypothesized that applicants with less complex structures and limited funding may have a lower probability of MA success. Limited regulatory expertise and restricted experience through smaller product pipeline may also be contributing factors, as previously mentioned by other authors(37).

In **chapter 2.2**, we focused on the characterization of the MAA process for ATMPs. The analysis on milestone data allowed us to draw conclusions on different regulatory aspects towards successfully obtaining a MA.

Requesting SA/PA or not does not seem to be decisive for ATMPs to successfully obtaining MAA, since all ATMPs included in the analysis requested SA/PA, at least once. Comparing to all EMA submissions (from all medicines,

regardless whether they are ATMPs or not) between 2000 and 2013, only 42% of applicants sought EMA for advice, before submission(34). This disproportion may be justified by the complex nature of ATMPs compared with other medicinal products, leading to Sponsors seeking regulatory advice more often than for traditional medicinal products.

Additionally, our analysis suggests that successful MAAs present an average higher number of requests for SA/PA, comparing to the unsuccessful MAAs (2.6 vs. 2.0, respectively). Data from the Bravery and colleagues study highlights a larger gap between groups (3.1 average number of advices for successful group and 1.2 average number of advices for unsuccessful group)(29), but a similar trend remains.

However, the number of SA/PA requests provides only limited information, and just gives us a sense on whether advice as requested to the Regulators or not. Other outcomes would have been important to analyze, such as the content and further compliance with the SA/PA, as well as the timing. In general, compliance with SA/PA recommendations on clinical trial design have previously shown to correlate with MAA success for medicines(36,38). Nevertheless, exceptions may be accepted, if adequately justified. For instance, in the case of Imlygic®, the applicant decided to use durable response rate as primary endpoint for the main study, contrary to advice from the EMA(29). Also, timing of SA/PA could have an impact on MA outcome. Bravery and colleagues data shows that 65% of requests for SA/PA for the first 22 ATMP, MAAs occurred after the main study was submitted for approval, which is undesirable, since the Regulator may have relevant feedback on major clinical trial elements, such as design or primary endpoint, which are difficult to update once the trial is ongoing.

All MAAs had at least one clock stop where the D120 CS is consistently the longest, between both groups. It is assumed that the most relevant major objections are raised at this point, taking the longest for Applicants to solve. The CS duration tends to decrease throughout the MAA review process. Successful MAAs present a higher average number of clock stops, when comparing to the unsuccessful group (3.1 vs. 2.0), probably since the latter includes 6 products where 4 were withdrawn prior to opinion, i.e. these MAAs did not go as far in the review process. With regards to the duration of the first clock stop, a higher average duration was noted for successful products (190.4 days) compared to the unsuccessful (152 days).

Our study also suggests that higher number of oral explanations (OE) may be a predictor of MAA failure. Here, the average number of OEs is not as informative, comparing to analysis of the individual data points. All ATMPs included in the successful MAA group reported between zero and two oral explanations, except for Glybera®, a clear outlier, which was the product with the highest number of OEs among all ATMPs (six, in total). In the unsuccessful group, all 4 withdrawn ATMPs had zero OEs simply because these did not reach the MAA review process far enough for that. The other two MAAs had 3 (Cerepro®) and 4 (Heparesc™) OEs, resulting in a negative opinion. We hypothesize that OEs were almost a regulatory "rescue" strategy in obtaining marketing authorization, for those products with some satisfactory level of efficacy and safety.

Limited data exists on the analysis of number and duration of clock stops and oral explanations, during MAA in Europe. As reported by other authors, D120 clock stop provides us an indication on the amount and complexity of major objections raised by the regulator, while subsequent clock stops, such as D180, may reflect differences of opinion between the applicant and the regulator(29). In addition, analysing such outcome has particular relevancy considering the overall time to approval, compared to other jurisdictions. It has been reported in the past that, in EU, medicines take longer to approve from a regulatory standpoint due to clock stops combined with the final decision making process where the EU Commission is involved(39,40). Our study found that for 92% of the MAA processes included in this analysis, a draft opinion was issued by CHMP after day 210 and in 40% of the successful MAAs the EC issues a decision after the 67 days mark. Further research is required to understand whether there is a difference in timings between ATMPs and other medicinal products. Also, it would be relevant to understand the reasons behind such tendency, although higher complexity of ATMPs may justify these numbers and additional factors may contribute, such as the Sponsor's experience, reflected in applicant type (i.e. SME or non-SME). Nevertheless, our study shows clearly that EMA offers an array of opportunities for applicants to resolve major objections (i.e. clock stops or oral explanations). Even when these opportunities seem exhausted, an expedited MA pathway may be offered, such as the case of Glybera®, where after 3 clock stops, 6 oral explanations and overall 353 days of assessment later, a MA under exceptional circumstances was granted.

In **chapter 2.3**, we focused on analysing assessment reports for seven GTMPs reviewed in Europe. We attempted to find MAA success factors for these products as outlined below. To our knowledge, this is the first that specifically reviews GTMPs, as opposed to other authors who chose to analyse ATMPs as a group(29,30,41,42). A summary on the research developed is depicted below in **Table 14** and further discussed.

	Unsuccessful MAA				Successful MAA		
GTMP	**Advexin®**	**CLG**	**Cerepro® (2007)**	**Cerepro® (2010)**	**Glybera®**	**Imlygic®**	**Strimvelis®**
Quality assessment	🔴	🔴	🔴	🟢	🟢	🟢	🟢
Non-clinical assessment	🔴	🔴	🟢	🟢	🟢	🟢	🟢
Clinical assessment	🔴	🔴	🔴	🔴	🟢	🟢	🟢

Table 14 – Summary of regulatory acceptability on MAA assessment

Red dots represent unacceptable major objections Green dots represent acceptability of the data, regardless whether major objections were found or not.

Overall, 75% of unsuccessful GTMP MAAs presented unacceptable major objections, issues or concerns related to quality data. Though in the beginning of the CAT's work the quality data was significantly noted as a deficiency, our research suggests that over the years there have been substantial improvement. This could either be a result of the increased regulators experience with GTMP assessment or the submission of more robust quality data by the applicants. For instance, comparing the assessments of Cerepro® in 2007 with 2010, there were no deficiencies precluding GTMP approval in the 2010 MAA as far as quality data. However, clinical deficiencies contributed to a negative benefit-risk assessment for this product.

Manufacture changes (raising comparability issues) and deficiencies regarding specification of drug product and/or drug substance are highlighted as common objections, for both successful and unsuccessful GTMPs. Often drug development is initiated in academic setting, where the resources are limited, and optimization of the

manufacturing process prior to non-clinical and clinical drug testing is not a priority. These findings are in line with other authors studies (29,30,41).

Quality and manufacturing issues are extremely important as being interlinked with clinical outcomes, as highlighted by Boráň and colleagues(43). Barkholt and colleagues note this is especially important for cell based products. Quality and manufacturing process is tightly linked to their functionality, since the cells depend on signals coming from their environment(41). In fact, Bravery and colleagues study mention that, overall, gene therapy products raise less quality major objections compared to cell based products(29).

With regards to Advexin® and CLG, a necessary consequence of being the first to be assessed for MAA is that there was none or limited past experience as to how the product should be evaluated. It is hypothesized that the submission dossiers were either quite deficient or the regulatory assessment was incredibly strict.

Overall, 50% of unsuccessful GTMP MAAs presented unacceptable major objections, issues or concerns related to non-clinical data, as depicted in **Table 14**, mainly related to toxicology data. De Wilde and colleagues study highlights that major objections at toxicology level were found in 4 out of 6 non-approved ATMPs(30). Other studies also found issues with biodistribution and toxicology more often in the non-clinical category(29,41), which is aligned with our results.

On the other hand, Bravery and colleagues research indicated that only 36% of ATMPs had major objections at non-clinical level, and were more likely for GTMPs (50%) comparing to cell-based products (23%). We hypothesized that such discrepancy is related to some non-clinical tests being more relevant to GTMPs, as opposed to cell-based products. For instance, with regards to biodistribution, the applicant should identify any off-target accumulation and present data on possible shedding of the viral vector into body fluids. Additionally, in the toxicology section, possible tumourigenicity risk may be higher for GTMPs compared to cell-based products, especially for retroviral vectors. Overall, immunogenicity of GTMPs can be studied in animals while for cell-based products such studies may not provide meaningful results, due to species differences.

Overall, deficiencies in non-clinical data very rarely result in major objections, but add uncertainty to clinical evaluation of efficacy and safety, similarly to previous

studies(41). Using a risk-based approach for the assessment of non-clinical data is highlighted as an important tool towards successful MAA(44).

Clinical assessment is, without a doubt, the section where consistently the CAT/CHMP tends to encounter issues, for unsuccessful GTMPs. In our research we found that 100% of unsuccessful GTMPs fail due to unacceptable objections at clinical efficacy and safety level, as per the summary presented in **Table 14**.

In terms of clinical efficacy, the three most frequent objections noted during MAA assessment were related to primary demonstration of efficacy (3 of 4 unsuccessful MAAs and 2 of 3 successful MAAs), followed by the change or use of a non-validated primary endpoint (2 of 4 unsuccessful MAAs and 3 of 3 successful MAAs). Lastly, efficacy claims based on post-hoc and sub-group analysis were noted as objections in 2 of 4 unsuccessful MAAs compared to 1 of 3 successful MAAs. Similarly to other authors research, we found that for the approved products, these major issues were considered resolved while the Applicants for the unsuccessful MAAs were unable to resolve upon final decision making(30). In the case of successful GTMPs, the majority of the major objections, issues or concerns were addressed during MAA assessment through the clarification of the concern via oral explanation or written answer or submission of additional data (either during MAA review or post-marketing). In this context, RMP updates were noted in practically all GTMPs.

These results are fully aligned with similar studies on ATMP major objections. The change in endpoint is reported as an important objection in De Wilde and colleagues study, as well as in the Coppens and colleagues publication(30,42). Deficiencies in the clinical data package (i.e. lack of randomization, issues with study design, conduct of clinical study and/or choice of control group) and issues related to indication (i.e. intended use not supported by the primary efficacy results) were noted in the Bravery and colleagues and Barkholt and colleagues study as the most frequent issues raised during MAA for ATMPs(29,41).

With regards to the assessment of clinical safety, the most common observations were limited or incomplete safety database (3 of 4 unsuccessful GTMPs and in 2 of 3 successful GTMPs), as well as specific safety concerns over immunogenicity (2 of 4 unsuccessful GTMPs and 3 of 3 successful GTMPs). De Wilde and colleagues study noted that 5 out of 6 unapproved products presented

major objections related to safety profile(30). Barkholt and colleagues study, as well as Bravery and colleagues publication, found that limited safety and efficacy follow-up and risk management were the 4th most frequently reported major objection for ATMPs(41)(29). These studies are aligned with our research, highlighting that the major objections reported for GTMPs in the clinical and efficacy assessment are no different than those noted for ATMPs overall.

Our research shows that, in Europe, 50% of ATMPs are orphan drugs and this proportion is even higher for GTMPs (4 out of 6 GTMP MAAs correspond to orphan products). The interpretation of our study results should, therefore, be in the context of orphan drugs. Lack of available treatment options and small patient populations may be the reason for the willingness of European regulators to accept high levels of uncertainty and non-confirmatory evidence for orphan gene therapy products approval. An early indication of clinical benefit, even if very modest, together with considerations on unmet medical need seem to prevail over efficacy and safety uncertainties, under conditions of substantial post-marketing requirements. The use of expedited authorization pathways, such as approval under exceptional circumstances or conditional approval may apply.

4.3.2 Patient access hurdles

In chapter 2, the European landscape regarding regulatory hurdles on gene therapy approval was analyzed and taken as a starting point for the subsequent chapter. In **chapter 3**, we attempted to provide a broad overview of items that could potentially impact patient access to gene therapy. Initially, when this study was designed, we considered to add in the inclusion/exclusion criteria an item to restrict the data on a geographical level, in an effort to align with the research conducted in chapter 2. However, we soon realized that this would not be adequate since some hurdles (e.g. patient perception, beliefs, etc.) are applicable worldwide. Therefore, upon identification of country-specific hurdles, we attempted to identify at all times its geographic origin, whilst integrating them in the overall context of the patient access hurdles.

A systematic approach was applied based on available literature between 2012 (date when first gene therapy was approved in Europe) and 2018, from two separate databases. From this comprehensive review, seven major themes were identified as

potential patient access hurdles and twenty five sub-themes were further identified. The major themes are outlined below:

- Affordability
- Assessment of value
- Development of therapy
- Ethical / Social factors
- Evidence generation
- Operational Implementation
- Regulatory hurdles

Unsurprisingly, in 84% of the publications included in this analysis, affordability issues especially related to sub-theme therapy cost/price were reported. It is true that gene therapy is associated with high price tags, as presented in **Table 15.**

GTMP commercial name	List price of full treatment (€) (45–47)	EU country*
Glybera®**	900 000	Germany
Imlygic®	73 480	UK
Strimvelis®	594 000	Italy
Kymriah®***	282 000	UK
Yescarta®***	300 000	UK
Luxturna™***	613 410	UK
Zynteglo®	1.58 million	Proposed price
Zolgensma®	1.95 million	Germany

Table 15 – Price of approved gene therapies in EU countries

*Prices are the list prices of gene therapy in the first EU country that gene therapy was marketed in

**Marketing authorization withdrawn in 2017

***Confidential commercial agreement in place between National Health Service (NHS) England and the manufacturer

Gene therapies are frequently meant to be one-time administration, and curative medicinal products, as opposed to traditional treatments. Very small patient pools and a complex manufacturing and research process contribute to the high price, comparing to conventional therapies(48).

The second most frequently found hurdle was related to evidence generation, namely trial outcomes (81%), followed by therapy payment/reimbursement issues (51%). These two items are intimately linked, since the reimbursement of therapy is directly dependent on the clinical efficacy and safety results obtained from clinical trials.

Considering most gene therapy products target rare diseases, the decision-making process on payment/reimbursement is often based on fairly limited evidence. This uncertainty contributes to different levels of access to gene therapy, since with the same data is has been noted that one product is reimbursed in one country but not in another, due to different criteria. Other authors have attempted to review GTMP reimbursement status and HTA decisions in major European countries and US, allowing us to reflect on gene therapy patient access in major European countries and in US(49,50).

In England, two GTMPs were reimbursed (Imlygic®, Strimvelis®) with patient access schemes. In addition, two CAR-Ts (Yescarta® and Kymriah®) were funded through the Cancer Drugs Fund (49,50). In Scotland, Kymriah® was accepted for B-cell acute lymphoblastic leukaemia treatment with a patient access scheme, while Yescarta® and Kymriah® for diffuse large B-cell lymphoma were rejected due to unjustified cost–effectiveness estimates (49). In Germany, three GTMPs had “non-quantifiable added benefit” due to insufficient data (Glybera®, Yescarta® and Kymriah®) and Imlygic® had “no-added benefit” due to inappropriate comparator use. However, this did not limit its reimbursement (49,50). Three GTMPs were reimbursed in France (Yescarta®, Kymriah® and Luxturna™), whilst Glybera® was not recommended, as it was considered to have ‘insufficient’ benefit due to its unsustainable and heterogeneous treatment effects (49,50). In Italy, one GTMP was reimbursed for hospital use with managed entry agreement (Strimvelis®)(50). In Spain, Kymriah® was recommended for use in specialized centers (50). In the USA, Kymriah®, Yescarta®, Luxturna™, and Zolgensma® were evaluated as having substantial net health benefits. However, a high certainty of conclusion for the assessment of Zolgensma® was established (49). No data on Zynteglo® was available for any of the EU5 countries either because the assessment is in progress or not assessed at all (50).

Overall, discrepancies among HTA bodies’ perception of GTMPs’ value were noticed. Hanna and colleagues highlight that uncertainty due to lack of robust and long-term evidence was the main limitation in securing reimbursement(50). On the other hand, conditional reimbursement is increasingly considered a useful strategy to mitigate uncertainty as it allows collection of long-term data whist minimizing the impact on patient access. Qiu and colleagues(49) refer that although the limitations

in pivotal studies resulted in substantial uncertainties regarding long-term treatment benefit, there was still a possibility for gene therapies to gain acceptance from HTA bodies. Most importantly, further evidence collection becomes the critical key, not only to reduce the uncertainty in reimbursement decisions, but also to increase the public's confidence in the use of gene therapies.

Neither HTAs nor Payers are a homogeneous group of decision makers. Different methodologies and factors have an impact on the decision process depending on these methodological differences, thereby influencing patient access to medicines. Characteristics of different payer types are available in **Table 16**. Additionally, HTA agencies may be grouped in three key archetypes(51). Agencies such as the German IQWIG/G-BA, base their decision on the clinical benefit assessment, while cost-effectiveness analysis is only conducted in case of disagreements during pricing negotiation. Others including National Institute for Health and Care Excellence (NICE) (England) and Scottish Medicines Consortium (SMC) (Scotland), mainly base their decisions on cost-effectiveness analysis using the incremental cost-effectiveness ratio (ICER). Some 'mixed' HTA frameworks combine both clinical benefit assessment and health economic assessment when making their decisions (cost-effectiveness and budget impact analysis). Here, decisions are mainly driven by budget impact analysis rather than cost-effectiveness analysis. For instance, in Italy and Spain, cost-effectiveness analysis and budget impact analysis are not mandatory for national pricing and reimbursement application, but they can be submitted by the manufacturers(52).

In Portugal, the key stakeholder for medicines pricing and reimbursement is the National Authority of Medicines and Health Products (Infarmed), operating under the Ministry of Health (MoH). The economic evaluation to decide on funding a medicine through the National Health Service (SNS) is conducted by Infarmed. The reimbursement regimens for inpatient and outpatient medicines define that public financing should be granted to new medicines that demonstrate that they are at least as efficacious/effective as therapeutic alternatives as well as less expensive or more cost effective. Mechanisms to control reimbursement are also in place, including re-evaluation and the potential decision to stop financing. Additionally, the possibility of entering into agreements, which in the case of hospital-only medicines is mandatory, ensures a more effective use of resources. In 2014, the National System of Health

Technology Assessment (SiNATS) was created with the mission of helping with financing decisions, notably including the feasibility of risk-sharing agreements(53).

Payer type	Description	Country
Private Insurance Markets	Free market environment with competing private insurance	US, Switzerland
Therapeutic Reference Markets	Relative therapeutic effectiveness index (demonstrated meaningful benefits over comparator)	Germany, France
Cost-effectiveness Markets	Rigid modelling and value thresholds	England
Budget Impact Markets	Cost to system to adopt new therapy	Italy, Spain

Table 16 – Key payer types

The current body of evidence(49,50) seems aligned with our research with special focus on the fact that evidence generation (trial outcomes) and affordability (reimbursement issues) are two of the most relevant hurdles in GTMP patient access. Qiu and colleagues also highlight that value appreciation constitutes an important factor for patient access impacting reimbursement, since different countries showed different perspectives on the weights allocated to each attribute. In our study, hurdles related to patient access related to criteria used in value assessment were found in 6 out of 32 publications (19%).

In less extent, operational implementation of gene therapy rises as an important access aspect, especially related to the need of having specific infrastructures for manufacturing, administration of therapy and medical follow-up (44% of the publications), as well as trained health care professionals (28% of the publications).

Ethical and social aspects related to the use of genetic therapy also seem to impact patient access. Here, a maximum of 28% of publications reported this major topic as a hurdle. It became clear that the more serious a medical condition is, the more likely the patient is willing to use gene therapy.

Development of therapy and regulatory hurdles were the major topics found less often in the literature. A maximum of 16% of publications mentioning resources as a hurdle in the development of therapy major theme was found. This is unsurprising since the majority of gene therapy development initiates or occurs in

small and medium sized companies where the resources are limited comparing to larger companies. This finding has been already discussed in chapter 2.

Additionally, the same percentage of publications (16%) reported hurdles at the level of parallel access and marketing authorization application process. While the latter has been extensively discussed in chapter 2, it is worth exploring the issue of parallel access. Current knowledge indicated that parallel access hurdle seems more relevant in the context of cell-based therapies as opposed to gene therapies. Limited data on the use of Hospital Exemption (HE) existed until recently. In fact, some authors have already suggested that creating a registry with product and facility information for all ATMPs manufactured under HE in the EU could facilitate coordination between public facilities and inform business opportunities and market access planning for industry(54). A study published in 2020 by Coppens and colleagues analyzed ATMPs manufacturing under the HE and other exemption pathways (such as compassionate use and named patient supply) in seven EU countries. This study found that manufactured ATMPs under HE were mainly somatic cell therapy medicinal products (n = 11/12), plus one combination ATMP (n = 1/ 12). No gene therapy medicinal products or genetically modified cell based products were manufactured under HE(55), reinforcing that this hurdle seems more important in the context of cell based therapies.

4.4 Research limitations

To the best of our knowledge, this thesis presents a complete array of hurdles towards gene therapy regulatory approval and patient access. Nevertheless, some limitations should be highlighted and discussed in the context of the reported findings.

In chapter 2, we analyzed a sample size composed of 16 ATMPs, of which 6 were GTMPs. Clear limitations regarding sample size were noted, especially taking into account that, overall, this was a very heterogeneous group of medicinal products. One resulting limitation of the small sample size is the inability to conduct inferential statistical analysis, particularly between the unsuccessful and the successful group of MAA, concerning regulatory milestone data. In this context, we chose to report tendencies on which factors most impacted MAA outcome.

In addition, we found that the ATMP landscape is rapidly evolving. Since this research was completed, particularly chapter 2, six additional ATMPs were assessed in EU, and all of them are GTMPs (i.e. Kymriah®, Yescarta®, Raliglize, Luxturna™, Zynteglo® and Raliglize). All products were approved except Raliglize which was withdrawn due to initial concerns expressed by the CAT that the data from the main study would not be sufficient to support the approval of the medicine. In addition, our analysis only includes one *ex vivo* gene therapy (Strimvelis®), whilst currently there are 3 additional *ex vivo* gene therapies approved in EU (i.e. Kymriah®, Yescarta® and Zynteglo®). These items may well impact the conclusions we have suggested.

With regards to the data source used for the analysis conducted in chapter 2, we chose to use publically available data from the EMA website only. This means that some confidential data was not included in the study. Additionally, the reporting style used on the public assessment reports is dependent, to some extent, on the responsible *rapporteur*. Theoretically, these limitations may potentially exclude some hurdles or relevant details on major objections mentioned in the public assessment reports.

Moreover, we used a 4-level scale to qualitatively classify the major objections, issues or concerns in chapter 2.3, which were found in the assessment report. While some authors previously used a similar scale(29,41), classifying the hurdles extracted remains fairly dependent on individual interpretation of the authors.

However, comparing our findings to studies where the confidential information, including official EMA major objection reports were analyzed(41), our results seem quite aligned, which leads us to conclude that this limitation had reduced impact on our findings.

Importantly, although quantitative data on the request or use of the EMA's initiatives to support ATMPs' development (e.g. ATMP certification, classification, IIT, PRIME) was not analysed, this is acknowledged to be an advantage.

With regards to chapter 3, the use of two databases may be perceived as a limitation. Generally, investigators searching for relevant references for a systematic review are advised to search multiple databases and to use additional methods to be able to adequately identify all literature related to the topic of interest. The Cochrane Handbook, for example, recommends the use of at least MEDLINE and Cochrane Central and, when available, EMBASE for identifying reports of randomized controlled trials. Disadvantages of using multiple databases include high burden related to translating a search strategy into multiple interfaces and search syntaxes, as well as being more time-consuming for reviewers who have to screen more, and likely irrelevant, titles and abstracts. In addition, access issues may apply, as not all publications are readily accessible. In the present study, and considering the limited timeframe when the study was designed, implemented and reported, we chose to include publications available in two of the most complete, contemporary and relevant scientific databases, i.e. MEDLINE (accessed via Pubmed) and EMBASE (accessed via Ovid). In addition, the search strategy was purposefully designed to be broad, in order to ensure all relevant material was included and all hurdles were reported. One consequence of this was that some small level of overlap was noted in the hurdles found between chapter 2 and 3, particularly regarding regulatory hurdles.

4.5 Conclusions, implications to practice and opportunities to research

This thesis undoubtedly contributed to the development of an end-to-end understanding of ATMPs, and particularly gene therapy, from drug development to regulatory post-authorization use. Through a combination of analysis of EMA publically available data and review of the latest literature using a systematic approach, this research was able to provide a complete and integrated set of hurdles, towards gene therapy regulatory approval and patient access. From the studies which were conducted, the most relevant findings are summarized in **Figure 19**.

In the near future, ATMPs and gene therapy in particular, are likely to have a strong impact in the public health landscape, not only due to its curative potential for diseases of high unmet medical need but for the anticipated high price and budget impact that these therapies are expected to have. More and more new clinical trials are in place where the investigational medicinal product corresponds to an ATMP, which is reflected in a modest growth of number of approved products over the last decade, particularly GTMPs in Europe.

A comprehensive understanding of regulatory hurdles in ATMP MAAs is critical and will certainly contribute to the design of more robust development programs of upcoming new ATMPs. The current analysis reflects EU regulatory hurdles for a small sample of first-generation ATMPs. Caution was taken when drawing conclusions for the future. Learning from past MAAs is essential for applications to come, both from the Regulator and from the Applicants perspective. Considering the orphan drug context and that the majority of ATMPs target diseases of high unmet medical need, our research suggests that EMA is prepared to accept efficacy and safety uncertainties, while putting in place adaptive approval strategies and/or substantial post-marketing obligations.

The benefits of ATMPs will not be realized unless patients have access to it. Commercial success is vital for patient access through the implementation of a

viable business. Despite the evidence generated by gene therapy developers often not matching the standard requirements of health technology assessment agencies, to date, most gene therapies have successfully secured reimbursement. To improve the efficiency of collecting relevant data for both regulatory and HTA, requesting parallel advice between EMA/HTA seems critical.

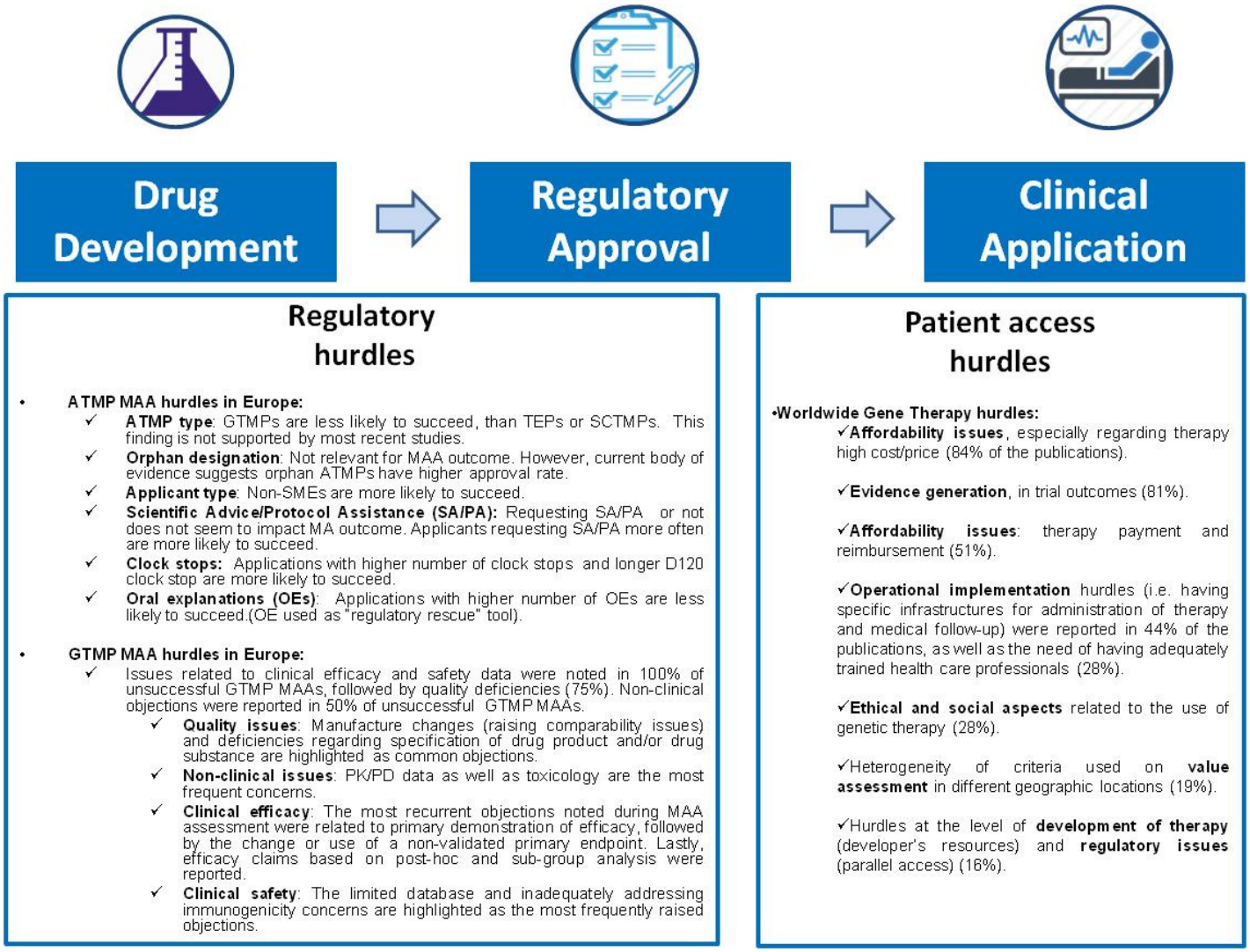

Figure 19 – Thesis key findings

4.6 Future perspectives

This thesis represents a robust starting point for discussing and proposing strategies to overcome ATMP regulatory and patient access hurdles.

In 2014, based on the experience accumulated since the implementation of the ATMP Regulation in Europe, the European Commission proposed strategies to support the translation of research into ATMPs(56). Details of such strategies are available in **Table 17**. It would be interesting to analyze to which extent these actions were implemented and its impact on ATMP regulatory approval and patient access.

- Clarification of the scope of the ATMP Regulation by fine-tuning the current definitions of ATMPs and by reflecting on the appropriate regulatory framework for new innovative products that many not be captured by existing provisions; - Considering measures to avoid disparities in the classification of ATMPs in the EU; - Clarification of the conditions for the application of the hospital exemption, as well as the role of data obtained there from in the context of marketing authorisation procedures; - Revising the requirements for the authorisation of ATMPs with a view to ensure that applicable requirements are proportionate and well-adapted to the specific characteristics thereof, having specific consideration to autologous products; - Streamlining the marketing authorisation procedures; - Extending the certification procedure and clarification of the link between the certification and the marketing authorisation procedure; - Creating a more favourable environment for ATMP developers working in an academic or non-for-profit setting, including by promoting early contacts with the authorities through the application of the fee reduction for scientific advice and by extending the certification scheme to these developers; - Considering possible fee incentives to reduce the financial impact of post-marketing obligations.

Table 17 – European Commission proposed strategies to support the translation of research into ATMPs

Our research identified affordability issues as the most relevant hurdle related to patient access. Exploring optimal business model and reimbursement strategies would be relevant. Several authors have attempted to present potential innovative pricing agreements(57–61). In **Table 18**, some examples of such strategies are presented(61). Analyzing which of these strategies is the most adequate for ATMPs towards improving patient access would be quite relevant. Of course that such analysis would certainly need to take into account specific factors related to the

particular indication/disease itself, as well as contextualizing in the specific geographic setting.

Innovative pricing agreement	Detail
Performance based risk sharing agreements	Under this style of agreement, the price paid for a therapy would depend upon the extent of the effectiveness of a therapy. This type of agreement could also be adapted for Managed Entry Agreements, where reimbursement is reduced until uncertain outcomes can be full assessed in post-market studies
Annuity payments	Annuity payment agreements may reduce upfront costs to payer bodies, many of which are not set up to provide large upfront costs for a one-off treatment. Annuity style payments could also be linked to long-term outcomes on a performance-based payment mechanism.
Leasing schemes	Leasing schemes could be used similarly to annuity schemes, particularly for end-of-life interventions. Therapies could be 'leased' using monthly payments for as long as progression-free patient survival occurs.

Table 18 – Examples of innovative pricing agreements for ATMPs

Table content adapted from Jenkins, *et. al.,* 2017 **(61)**.

4.7 References

1. European Medicines Agency. European Medicines Agency's 2018 Annual Report [Internet]. 2019 [cited 2020 May 9]. Available from: https://www.ema.europa.eu/en/documents/annual-report/2018-annual-report-european-medicines-agency_en.pdf
2. Munos B. Lessons from 60 years of pharmaceutical innovation. Nat Rev Drug Discov. 2009;8(December):959–68.
3. European Medicines Agency. Human medicines highlights 2015 [Internet]. 2015 [cited 2020 May 9]. p. 1–5. Available from: https://www.ema.europa.eu/en/documents/leaflet/human-medicines-highlights-2015_en.pdf
4. European Medicines Agency. Human medicines highlights 2016 [Internet]. 2016 [cited 2020 May 9]. Available from: https://www.ema.europa.eu/en/documents/leaflet/human-medicines-highlights-2016_en.pdf
5. European Medicines Agency. Human medicines highlights 2017 [Internet]. 2017 [cited 2020 May 9]. Available from: https://www.ema.europa.eu/en/documents/report/human-medicines-highlights-2017_en.pdf
6. European Medicines Agency. Human medicines highlights 2018 [Internet]. 2018 [cited 2020 May 9]. Available from: https://www.ema.europa.eu/en/documents/report/human-medicines-highlights-2018_en.pdf
7. European Medicines Agency. Human medicines highlights 2019 [Internet]. 2019 [cited 2020 May 9]. Available from: https://www.ema.europa.eu/en/documents/report/human-medicines-highlights-2019_en.pdf
8. IQVIA Consulting Services. Assessing person-centered therapeutic innovations [Internet]. 2019 [cited 2020 Apr 21]. Available from: https://www.iqvia.com/library/white-papers/assessing-person-centered-therapeutic-innovations
9. Katzung BG. Basic & Clinical Pharmacology. In: Basic & Clinical

Pharmacology. 14th ed. McGraw-Hill Companies, Inc.; 2018. p. Section 1.
10. Tamimi NAM, Ellis P. Drug Development: From Concept to Marketing! Nephron Clin Pract [Internet]. 2009;113(3):c125–31. Available from: https://www.karger.com/Article/FullText/232592
11. Ciani O, Jommi C. The role of health technology assessment bodies in shaping drug development. Drug Des Devel Ther [Internet]. 2014 Nov;8:2273. Available from: http://www.dovepress.com/the-role-of-health-technology-assessment-bodies-in-shaping-drug-develo-peer-reviewed-article-DDDT
12. European Medicines Agency. From laboratory to patient: the journey of a centrally authorised medicine [Internet]. 2018 [cited 2020 Apr 21]. Available from: https://www.ema.europa.eu/en/documents/other/laboratory-patient-journey-centrally-authorised-medicine_en.pdf
13. Touchot N, Flume M. Early Insights from Commercialization of Gene Therapies in Europe. Genes (Basel) [Internet]. 2017 Feb 17;8(2):78. Available from: http://www.mdpi.com/2073-4425/8/2/78
14. Vreman RA, Heikkinen I, Schuurman A, Sapede C, Garcia JL, Hedberg N, et al. Unmet Medical Need: An Introduction to Definitions and Stakeholder Perceptions. Value Heal [Internet]. 2019 Nov;22(11):1275–82. Available from: https://doi.org/10.1016/j.jval.2019.07.007
15. Regulation (EC) No 507/2006 of 29 March 2006 on the conditional marketing authorisation for medicinal products for human use falling within the scope of Regulation (EC) No 726/2004 of the European Parliament and of the Council. Official Journal of the European Union. 2006. p. 6–9.
16. Karlberg JPE. Trends in disease focus of drug development. Nat Rev Drug Discov [Internet]. 2008 Aug;7(8):639–40. Available from: http://www.nature.com/articles/nrd2618
17. Dugger SA, Platt A, Goldstein DB. Drug development in the era of precision medicine. Nat Rev Drug Discov [Internet]. 2018 Mar 8;17(3):183–96. Available from: http://dx.doi.org/10.1038/nrd.2017.226
18. Mirnezami R, Nicholson J, Darzi A. Preparing for Precision Medicine. N Engl J Med [Internet]. 2012 Feb 9;366(6):489–91. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMp1114866
19. Farkas AM, Mariz S, Stoyanova-Beninska V, Celis P, Vamvakas S, Larsson K,

et al. Advanced Therapy Medicinal Products for Rare Diseases: State of Play of Incentives Supporting Development in Europe. Front Med [Internet]. 2017;4(May):1–6. Available from: http://journal.frontiersin.org/article/10.3389/fmed.2017.00053/full

20. Tambuyzer E, Vandendriessche B, Austin CP, Brooks PJ, Larsson K, Miller Needleman KI, et al. Therapies for rare diseases: therapeutic modalities, progress and challenges ahead. Nat Rev Drug Discov [Internet]. 2020 Feb 13;19(2):93–111. Available from: http://dx.doi.org/10.1038/s41573-019-0049-9
21. European Comission. Rare diseases: A major unmmet medical need [Internet]. Publications Office of the EU. 2018 [cited 2020 May 14]. Available from: https://ec.europa.eu/research/health/pdf/rarediseases-leaflet-2018.pdf
22. Detela G, Lodge A. EU Regulatory Pathways for ATMPs: Standard, Accelerated and Adaptive Pathways to Marketing Authorisation. Mol Ther - Methods Clin Dev [Internet]. 2019 Jun;13(June):205–32. Available from: https://doi.org/10.1016/j.omtm.2019.01.010
23. Boráň T, Menezes-Ferreira M, Reischl I, Celis P, Ferry N, Gänsbacher B, et al. Clinical Development and Commercialization of Advanced Therapy Medicinal Products in the European Union: How Are the Product Pipeline and Regulatory Framework Evolving? Hum Gene Ther Clin Dev [Internet]. 2017 Sep;28(3):126–35. Available from: http://online.liebertpub.com/doi/10.1089/humc.2016.193
24. European Medicines Agency. CAT monthly report of application procedures, guidelines and related documents on advanced therapies - June 2018 [EMA/CAT/435550/2018] [Internet]. 2018 [cited 2020 Feb 26]. Available from: https://www.ema.europa.eu/en/documents/committee-report/cat-monthly-report-application-procedures-guidelines-related-documents-advanced-therapies-june-2018_en.pdf
25. European Medicines Agency. CAT monthly report of application procedures, guidelines and related documents on advanced therapies - March 2019 [EMA/CAT/198680/2019] [Internet]. 2019 [cited 2020 Feb 26]. Available from: https://www.ema.europa.eu/en/documents/committee-report/cat-monthly-report-application-procedures-guidelines-related-documents-advanced-therapies-march-2019_en.pdf

26. European Medicines Agency. CAT monthly report of application procedures, guidelines and related documents on advanced therapies - September 2018 [EMA/CAT/643189/2018] [Internet]. 2018 [cited 2020 Feb 26]. Available from: https://www.ema.europa.eu/en/documents/committee-report/cat-monthly-report-application-procedures-guidelines-related-documents-advanced-therapies-september_en-5.pdf
27. European Medicines Agency. CAT monthly report of application procedures, guidelines and related documents on advanced therapies - March 2020 [EMA/CAT/159950/2020] [Internet]. 2020 [cited 2020 Jun 29]. Available from: https://www.ema.europa.eu/en/documents/committee-report/cat-monthly-report-application-procedures-guidelines-related-documents-advanced-therapies-march-2020_en.pdf
28. European Medicines Agency. CAT monthly report of application procedures, guidelines and related documents on advanced therapies - July 2018 [EMA/CAT/514869/2018] [Internet]. Vol. EMA. 2018 [cited 2020 Jul 1]. Available from: https://www.ema.europa.eu/en/documents/committee-report/cat-monthly-report-application-procedures-guidelines-related-documents-advanced-therapies-july-2018_en.pdf
29. Bravery CA, Ball O, Robinson S. EU market authorisation strategy: lessons from the first 22 ATMP submitted to the EMA. Cell Gene Ther Insights [Internet]. 2019 Jun 24;5(6):759–91. Available from: https://insights.bio/cell-and-gene-therapy-insights/journal/articles/eu-market-authorisation-strategy-lessons-from-the-first-22-atmp-submitted-to-the-ema/
30. de Wilde S, Coppens DGM, Hoekman J, de Bruin ML, Leufkens HGM, Guchelaar H-J, et al. EU decision-making for marketing authorization of advanced therapy medicinal products: a case study [Internet]. Vol. 23, Drug Discovery Today. Elsevier Ltd; 2018. p. 1328–33. Available from: https://doi.org/10.1016/j.drudis.2018.03.008
31. Kesselheim AS. Characteristics of Clinical Trials to Support Approval of Orphan vs Nonorphan Drugs for Cancer. JAMA [Internet]. 2011 Jun 8;305(22):2320. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2011.769
32. Putzeist M, Mantel-Teeuwisse AK, Llinares J, Gispen-De Wied CC, Hoes AW,

Leufkens HGM. EU marketing authorization review of orphan and non-orphan drugs does not differ. Drug Discov Today [Internet]. 2013;18(19–20):1001–6. Available from: http://dx.doi.org/10.1016/j.drudis.2013.06.012

33. Putzeist M, Heemstra HE, Garcia JL, Mantel-Teeuwisse AK, Gispen-De Wied CC, Hoes AW, et al. Determinants for successful marketing authorisation of orphan medicinal products in the EU. Drug Discov Today [Internet]. 2012 Apr;17(7–8):352–8. Available from: http://dx.doi.org/10.1016/j.drudis.2011.10.027
34. Hofer MP, Hedman H, Mavris M, Koenig F, Vetter T, Posch M, et al. Marketing authorisation of orphan medicines in Europe from 2000 to 2013. Drug Discov Today [Internet]. 2018 Feb;23(2):424–33. Available from: http://dx.doi.org/10.1016/j.drudis.2017.10.012
35. Ziogas C. A regulator's instights for SMEs in the biologics and advanced therapies sectors. TOPRA Regul Rapp. 2016;13(2):23–5.
36. Regnstrom J, Koenig F, Aronsson B, Reimer T, Svendsen K, Tsigkos S, et al. Factors associated with success of market authorisation applications for pharmaceutical drugs submitted to the European Medicines Agency. Eur J Clin Pharmacol [Internet]. 2010 Jan 20;66(1):39–48. Available from: http://link.springer.com/10.1007/s00228-009-0756-y
37. de Wilde S, Guchelaar HJ, Herberts C, Lowdell M, Hildebrandt M, Zandvliet M, et al. Development of cell therapy medicinal products by academic institutes. Drug Discov Today [Internet]. 2016;21(8):1206–12. Available from: http://dx.doi.org/10.1016/j.drudis.2016.04.016
38. Hofer MP, Jakobsson C, Zafiropoulos N, Vamvakas S, Vetter T, Regnstrom J, et al. Impact of scientific advice from the European Medicines Agency. Nat Rev Drug Discov [Internet]. 2015 May 17;14(5):302–3. Available from: http://www.nature.com/articles/nrd4621
39. Hartmann M, Mayer-nicolai C, Pfaff O. Approval probabilities and regulatory review patterns for anticancer drugs in the European Union. Crit Rev Oncol / Hematol [Internet]. 2012;87(2):112–21. Available from: http://dx.doi.org/10.1016/j.critrevonc.2013.01.004
40. Shah RR, Roberts SA, Shah DR. A fresh perspective on comparing the FDA and the CHMP / EMA : approval of antineoplastic tyrosine kinase inhibitors. Br

J Clin Pharmacol. 2013;76(3):396–411.

41. Barkholt L, Voltz-Girolt C, Raine J, Salmonson T, Schüssler-Lenz M. European regulatory experience with advanced therapy medicinal products. Nat Rev Drug Discov [Internet]. 2019 Jan 30;18(1):8–9. Available from: http://www.nature.com/articles/nrd.2018.200
42. Coppens DGM, de Wilde S, Guchelaar HJ, De Bruin ML, Leufkens HGM, Meij P, et al. A decade of marketing approval of gene and cell-based therapies in the United States, European Union and Japan: An evaluation of regulatory decision-making. Cytotherapy [Internet]. 2018 Jun;20(6):769–78. Available from: https://doi.org/10.1016/j.jcyt.2018.03.038
43. Boráň T, Menezes-Ferreira M, Reischl I, Celis P, Ferry N, Gänsbacher B, et al. Clinical Development and Commercialization of Advanced Therapy Medicinal Products in the European Union: How Are the Product Pipeline and Regulatory Framework Evolving? Hum Gene Ther Clin Dev [Internet]. 2017 Sep;28(3):126–35. Available from: http://www.liebertpub.com/doi/10.1089/humc.2016.193
44. European Medicines Agency. Guideline on the risk-based approach according to Annex I , part IV of Directive 2001 / 83 / EC applied to Advanced Therapy Medicinal Products [EMA/CAT/CPWP/686637/2011] [Internet]. 2013. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/03/WC500139748.pdf
45. Thalassemia International Federation. Bluebird puts €1.58m price on gene therapy Zynteglo® [Internet]. 2020 [cited 2020 Jul 10]. Available from: https://thalassaemia.org.cy/news/bluebird-puts-e1-57m-price-on-gene-therapy-zynteglo/
46. PharmaPhorum. Germany first in EU to get Novartis' SMA gene therapy, costing almost 2m euros [Internet]. 2020 [cited 2020 Jul 10]. Available from: https://pharmaphorum.com/news/novartis-eyes-smart-deal-for-sma-gene-therapy-in-uk/
47. Covance. The challenges associated with evaluating the cost benefit of gene therapies and enabling patient access [Internet]. 2020 [cited 2020 Jul 10]. Available from:

https://www.covance.com/content/dam/covance/assetLibrary/whitepapers/Gene-Therapy-WPCMA017.pdf

48. Group DR. Cell and Gene Therapies: Five Key Access and Reimbursement Strategies [Internet]. 2019 [cited 2020 Jul 10]. Available from: https://decisionresourcesgroup.com/blog/cell-gene-therapies-five-key-access-reimbursement-strategies/
49. Qiu T, Hanna E, Dabbous M, Borislav B, Toumi M. Health Technology Assessment of Gene Therapies in Europe and the USA: Analysis and Future Considerations. Cell Gene Ther Insights [Internet]. 2019 Sep 4;5(8):1043–59. Available from: https://insights.bio/cell-and-gene-therapy-insights/journal/articles/health-technology-assessment-of-gene-therapies-in-europe-and-the-usa-analysis-and-future-considerations/
50. Hanna E, Marre C, Toumi M. PBI59 The Reimbursement Status of Advanced Therapy Medicinal Products in Europe. Value Heal [Internet]. 2019 Nov 1;22:S428. Available from: https://doi.org/10.1016/j.jval.2019.09.163
51. Angelis A, Lange A, Kanavos P. Using health technology assessment to assess the value of new medicines: results of a systematic review and expert consultation across eight European countries. Eur J Heal Econ [Internet]. 2018 Jan 16;19(1):123–52. Available from: http://link.springer.com/10.1007/s10198-017-0871-0
52. Universitet AM. Value Added Medicines - Time to Adjust the HTA Decision Frameworks [Internet]. 2017 [cited 2020 Jun 19]. Available from: https://www.medicinesforeurope.com/wp-content/uploads/2017/09/White-paper_HTAonValueaddedmedicines_Final.pdf
53. Markit I. Portugal Country Profile Report. 2018.
54. Driscoll D, Farnia S, Kefalas P, Maziarz RT. Concise Review: The High Cost of High Tech Medicine: Planning Ahead for Market Access. Stem Cells Transl Med [Internet]. 2017 Aug 27;6(8):1723–9. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/sctm.16-0487
55. Coppens DGM, Hoekman J, De Bruin ML, Slaper-Cortenbach ICM, Leufkens HGM, Meij P, et al. Advanced therapy medicinal product manufacturing under the hospital exemption and other exemption pathways in seven European Union countries. Cytotherapy [Internet]. 2020 Jun;000:1–9. Available from:

https://doi.org/10.1016/j.jcyt.2020.04.092

56. European Comission. Report from the Commission to the European Parliament and Council in accordance with Article 25 of Regulation (EC) No 1394/2007 of the European Parliament and of the Council on advanced therapy medicinal products and amending Directive 2001/83/EC and Regu [Internet]. 2014 [cited 2020 Jul 11]. Available from: https://op.europa.eu/en/publication-detail/-/publication/2dc18b82-b6c8-11e3-86f9-01aa75ed71a1
57. Kefalas P, Ali O, Jørgensen J, Merryfield N, Richardson T, Meads A, et al. Establishing the cost of implementing a performance-based, managed entry agreement for a hypothetical CAR T-cell therapy. J Mark Access Heal Policy [Internet]. 2018 Jan 20;6(1):1511679. Available from: https://doi.org/10.1080/20016689.2018.1511679
58. Hampson G, Towse A, Pearson SD, Dreitlein WB, Henshall C. Gene therapy: evidence, value and affordability in the US health care system. J Comp Eff Res [Internet]. 2018 Jan;7(1):15–28. Available from: https://www.futuremedicine.com/doi/10.2217/cer-2017-0068
59. Jönsson B, Hampson G, Michaels J, Towse A, von der Schulenburg J-MG, Wong O. Advanced therapy medicinal products and health technology assessment principles and practices for value-based and sustainable healthcare. Eur J Heal Econ [Internet]. 2018; Available from: http://link.springer.de/link/service/journals/10198/index.htm
60. Jørgensen J, Kefalas P. Annuity payments can increase patient access to innovative cell and gene therapies under England's net budget impact test. J Mark Access Heal Policy [Internet]. 2017 Jan 31;5(1):1355203. Available from: https://doi.org/10.1080/20016689.2017.1355203
61. M.J. J, P. B. Pricing pressures and market access outcomes of advanced therapy medicinal products (ATMPS) in the EU5. Value Heal [Internet]. 2017;20(9):A663. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed18&NEWS=N&AN=619024295