Clinical Development of Gene Therapies: The First Three Decades and Counting

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In the past three decades the field of gene therapy has made remarkable progress, surging from mere laboratory experiments to US Food and Drug Administration (FDA)-approved products that bring significant reduction in disease burden to patients who previously had no therapeutic options for their serious conditions. Herein, we review the evolution of the gene therapy clinical research landscape and describe the gene therapy product development programs evaluated by the FDA in Investigational New Drug applications received in 1988–2019. We also discuss the clinical development programs of the first six oncolytic and gene therapy products approved in the United States.

Introduction

More than 150 years of research and discovery have elapsed between Gregor Mendel’s pea-crossing experiments and the therapeutic use of gene therapies in clinical practice. The hope and promise of curing human diseases have continued to drive the many scientific and technological advances, along with the societal and policy considerations, that made the development of gene therapies possible.

Human gene therapy products include all products that mediate their effects by transcription or translation of transferred genetic material or by specifically altering human genetic sequences. Examples include nucleic acids (e.g., plasmids, in vitro transcribed ribonucleic acid), genetically modified microorganisms (e.g., viruses, bacteria, fungi), engineered site-specific nucleases used for human genome editing, and ex vivo genetically modified human cells.1 Gene therapy products intended for therapeutic purposes that are currently used in both clinical research and clinical practice exert their effects on somatic cells. Hence, the treatment results are limited to the treated individuals and not passed on to their offspring.

Gene therapy products intended to treat human diseases are regulated as biological products.1 In the United States, conducting human research with an investigational new drug or biological product requires submission of an Investigational New Drug (IND) application to the Food and Drug Administration (FDA). In addition to assuring safe and ethical use of investigational products, the IND application pathway permits FDA and IND sponsors to exchange pertinent information and facilitate product development. In 1974, the National Institutes of Health (NIH) established the Recombinant DNA Advisory Committee (RAC) to provide recommendations and be a public forum for discussion of the scientific and ethical issues related to research involving recombinant nucleic acid molecules. In the 1980s, the Human Gene Therapy Subcommittee of the RAC was created to review and discuss gene therapy clinical trials. Carefully embracing innovation, NIH through its RAC and FDA through its IND pathway independently reviewed clinical protocols for gene therapies proposed between 1988 and 2018. Once the field had advanced and the experience had grown, NIH and FDA collaboratively made a call for change. In 2018, while FDA maintained the oversight of gene therapy clinical trials, NIH refocused the RAC’s role to provide advice on issues associated with emerging biotechnologies and renamed the RAC the Novel and Exceptional Technology and Research Advisory Committee (NExTRAC).2,3

The submission of an IND application to FDA signifies the IND sponsor’s intent to begin clinical studies. During development, many factors can change the course of a product program, which may vary from expediting the development,4,5 to repurposing for another disease, to discontinuing the program. When safety or critical trial design issues arise, FDA may place an IND application on hold, which can be subsequently lifted following acceptable responses to the issues that led to the hold. If no study activity occurs for ≥ 2 years, the IND application becomes inactive. Either FDA or the sponsor can discontinue the IND application: FDA by terminating for various reasons, including safety and product quality concerns, and the sponsor by withdrawing for safety issues, lack of efficacy, manufacturing problems, or a business decision to discontinue the program.6 Sponsors intending to license their products must generate data that provide substantial evidence of effectiveness and safety to support the regulatory approval.

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Throughout its history, the field of gene therapy has experienced many failed products: some for absence of therapeutic effects, some for serious adverse events. One of the most tragic events in gene therapy clinical research was the death of Mr. Jesse Gelsinger, an 18-year-old participant in a trial investigating an adenoviral vector-based gene therapy carrying a normal ornithine transcarbamylase (OTC) gene for the treatment of X-linked OTC deficiency.7

In response to this event, FDA and other stakeholders working with gene therapies undertook a series of steps to ensure that all gene therapy IND sponsors strengthen the systems they had in place for product quality assurance and clinical trial oversight and monitoring. In March 2000, FDA issued the “Gene Therapy Letter” to sponsors of all gene therapy INDs, requesting to submit yearly reports summarizing various aspects of their product development, including product quality, manufacturing, animal safety, and clinical trial conduct. For the next 14 years, sponsors of gene therapy INDs submitted their product, preclinical, and clinical information to FDA for evaluation and feedback. Public advisory committee meetings were held at the time to discuss the information received and identify ways to address common issues experienced in the field.8

In addition, administration of some retroviral vector-based products caused leukemia and clonal cell proliferation in the early trials investigating therapies for X-linked severe combined immunodeficiency (SCID),9,10 Wiskott-Aldrich syndrome,11 and X-linked chronic granulomatous disease.12 These observations prompted the field13,14 and the regulators15,16 to make improvements in the risk-based approach to vector integration studies with evaluating vector replication potential and employing in vitro and in silico analytical methods for identification of potential off-target effects, along with the long-term follow-up (LTFU) of patients receiving gene therapies.17

During that time, FDA published a series of guidance documents relevant to gene therapy products, developed additional educational resources,18 and participated in numerous outreach activities broadly applicable to gene therapies. It took the gene therapy field more than a decade to recover from the consequences of the observed serious adverse events, reconsider many aspects of gene therapy product development, and continue the quest for cures for devastating genetic diseases.

Despite the setbacks, much progress has been made over the years, leading to greater realization of the therapeutic potential of gene therapies. In this article we describe the IND applications with gene therapy product development programs received by the Office of Tissues and Advanced Therapies (OTAT) and its predecessor offices in the Center for Biologics Evaluation and Research (CBER) at FDA in the years 1988–2019. We discuss the evolution of the gene therapy clinical research landscape and take a closer look at the programs of the approved marketed products. In summarizing more than 30 years of data for the field of gene therapy, we hope to share our experience and to highlight the unique aspects of this field of research, while supporting further development of these novel treatments.

**Landscape of Product Development**

Over the three decades, there has been a gradual increase in the IND applications with gene therapy product development programs submitted to FDA, a trend reflective of the overall growth of this field (Figure 1). The first IND application involving genetic modification came to FDA in 1988 and investigated tumor-infiltrating lymphocytes (TILs) obtained from tumors of patients with advanced refractory melanoma. As an initial step in innovation, this first program tested only the possibility of gene transfer, cell survival, and trafficking, and not the therapeutic potential of gene correction. The investigation sought to examine the effects of TILs modified ex vivo
By transduction with a retroviral vector containing a gene encoding for neomycin resistance (NeoR). The purpose of modifying the autologous TIL genome with the NeoR gene was to mark these cells for both selection of TILs during product manufacturing and detection of TILs in blood and tumor samples of the treated patients.

That first trial achieved important goals. The ex vivo transduction of autologous cells, their growth in culture, and administration to patients in the clinical setting were all shown to be feasible. The infused genetically modified cells survived, circulated in the bloodstream, and homed to the target tumor tissue. Nonetheless, the product later failed to demonstrate efficacy, and the program was eventually discontinued. Yet, the execution of these first investigations blazed the trail for subsequent clinical trials that would employ human gene transfer for therapeutic purposes.

It was not until 2 years later, in 1990, when such proposals arrived: TILs transduced with a retroviral vector carrying a Tumor Necrosis Factor gene to enhance the tumor lysis for the treatment of metastatic melanoma, and autologous lymphocytes transduced with a retroviral vector carrying the gene encoding human adenine deaminase enzyme to treat, for the first time, a genetic disorder—adenosine deaminase-deficient severe combined immunodeficiency SCID-ADA.

In the ensuing years, new technologies of cellular transfection and nucleic acid delivery continued to develop, leading to more products entering the clinical phase (Figure 1). The influx of programs submitted to FDA in IND applications steadily increased between 1988 and 1999, followed by a visible decline, with the nadir in 2002. The decline occurred after the fatal event in the OTC deficiency study, for which the respective IND was placed on hold in 1999. The subsequent slowdown in clinical investigations was reflected by the relatively level numbers of IND applications submitted between 2003 and 2012. For many reasons, including safety concerns and the need for further research to reassess product characterization, manufacturing, tissue delivery, and clinical monitoring, it took more than a decade for the field to regain its momentum. Recent years, however, have shown remarkable growth: the number of product programs initiating clinical studies doubled between 2012 and 2015, then again between 2015 and 2018, and continued to trend upward.

However, despite promising results in animals, many products failed in clinical studies. Over the three decades, the higher rates of discontinued and inactive INDs were observed with the earlier product programs (Table 1). On average, 97% of INDs submitted in the first decade halted development following an average program duration of 8.6 years. Although most of these product programs have been abandoned, some products may have been modified and repurposed for future development. The rates of attrition remained high for IND applications submitted in the second decade (67%), with an average duration of 7.5 years. Although attrition appears lower in the last decade, it is expected to increase because insufficient time has elapsed for the recent programs to interpret their products’ effects or encounter issues with their development. Notwithstanding, the knowledge accumulated in the field, the technological advances in product manufacturing, and the growing experience with conducting clinical investigations with gene therapies will likely help more products to be developed successfully.

The distribution of the ongoing gene therapy programs by therapeutic area is shown in Figure 2. One half of the programs aim to treat solid cancers (50%), followed by hematological malignancies (20%); neurological (5%), eye (4%), and blood (4%) disorders; and infectious diseases (3%). All other therapeutic areas combined (cardiac, pulmonary, endocrine, dermatological, rheumatic, gastrointestinal, vascular, and other conditions) constitute the remaining 14% of the ongoing gene therapy programs submitted in IND applications. Among the ongoing programs, 59% include gene therapy products intended to treat rare diseases.

The scientific complexity of discovery and development of gene therapies is largely reflected in that many product programs are initiated in academic institutions, by small groups of researchers, or by academic spinoffs that become small biotechnology companies. For many years, more INDs submitted each year came from academic entities (Figure 3). The trend reversed in 2016, when more applications were submitted by commercial sponsors. Overall, these recent changes demonstrate that the field has matured to the point where the potential for commercialization of gene therapies is now being realized by the biotechnology and pharmaceutical sectors.

### Table 1. Rates of Attrition of IND Applications with Gene Therapy Product Programs by Year 2019

| Submitted | Rates of Attrition |
|-----------|--------------------|
| 1988–1998 | 97% 96% 98% |
| 1999–2008 | 67% 61% 71% |
| 2009–2019 | 13% 10% 15% |

*a11 years included: no INDs were submitted in 1989; year 2019 added to the third decade.

*bProgram duration, mean = 8.6 years, range [<1; 24].

*cProgram duration, mean = 7.5 years, range [<1; 19].

*dAverage program duration is too early to calculate.

As a science-based regulatory agency, FDA issues guidance documents intended to assist stakeholders, including industry and academic sponsors, in the development of new therapies. The issuance of guidelines is a public process. During this process, FDA typically publishes a draft guidance and requests public comments on the contents of the published draft. When the period of public comments ends, the Agency reviews the comments, incorporates any necessary revisions, and publishes the final guidance. Once published, final guidelines reflect FDA’s thinking on specific topics of product development. Final guidelines can be updated or replaced.
by newer recommendations to ensure that the regulatory advice is kept abreast of the scientific progress.

Prior to arrival of any IND application for a gene therapy, FDA had anticipated the emergence of the fields of cell and gene therapy and began developing a guidance document to assist sponsors of gene therapy products. After a few years of preparation, FDA published its first guidance in this area titled “Points to consider in human somatic cell and gene therapy, 1991.” It outlined the recommendations for characterization of cell populations, lot-to-lot manufacturing control and release testing, preclinical studies, and considerations for clinical trials. As the field accumulated experience, the first guidance was replaced by its next iteration in 1998.

As noted earlier, in the 1990s and the early 2000s, clinical research with gene therapies stumbled upon concerns about the potential for replication-competent retrovirus (RCR) arising from retroviral vector-based gene therapy products. The development of lymphomas in rhesus monkeys administered hematopoietic stem cells transduced ex vivo with a gammaretroviral vector and the subsequent observations of clonal cell proliferations in human studies, along with many discussions in the field among the researchers and regulators, resulted in FDA’s publication of two guidance documents in 2006: one on testing for RCR in retroviral vector-based gene therapy products and during follow-up of patients in clinical trials, and the other on observing subjects receiving gene therapies for delayed adverse events. More than a decade later, much scientific experience has accrued with substantial data on safety of retroviral vectors supported by implementation of changes with different vector designs and the use of split plasmids and other methods, utilization of vector-producing cells, RCR detection assays, and patient monitoring. As the field continued to adopt more rigorous testing, safer vector designs, improved manufacturing, and long-term clinical follow-up, FDA yet again reevaluated its approach to ensure that the rigor of product evaluation is balanced by the release from any outdated recommendations. In January 2020, FDA issued three guidance documents; two of them replaced the previous guidelines from 2006 with more streamlined and less burdensome recommendations on RCR testing and LTFU, and one provided the most up-to-date recommendations on the information to be included in the Chemistry, Manufacturing, and Control module of IND applications for gene therapy products.

The experience gained from the early product failures and improvements that followed have paved the way to the more active research and development of novel gene therapies in the latest decade. Since 2010, FDA has continued issuing more guidances on different aspects of product evaluation, including design and analysis of vector shedding studies, development of microbial vectors, environmental assessment for gene therapies and other related recombinant products, preclinical evaluation of cell and gene therapies, and design of early-phase clinical studies for these products. More recently, additional work and successful experience with clinical research in some therapeutic areas catalyzed issuance of disease-specific guidances in blood and retinal disorders, recognizing a significant impact of gene therapies on the treatment of rare diseases, FDA also published guidances to assist stakeholders developing products for rare diseases.

### Product Categories

The product categories most frequently investigated over the first three decades of clinical research with gene therapies included genetically modified (GM) cells; plasmids; retroviral, adeno-associated viral (AAV), and microbial vectors; and, more recently, products incorporating genome-editing technologies (Figure 4). Other technologies, including herpes simplex virus (HSV), vaccinia, poxviridae, and other constructs, have also been used for gene delivery but individually contributed small percentages to the application pool.

A majority of gene therapy products that went into clinical development were ex vivo GM cells, including lymphocytes, bone marrow-derived cells, hepatocytes, fibroblasts, and autologous tumor cells. In fact, all programs initiated in 1988–1991 were with GM cells. Only later was in vivo administration of vectors proposed, due to concerns about the risks of unintended transfection of off-target cells. In the earlier years, GM cells transduced with retroviral, plasmid, and, later, adeno-viral vectors carrying genes of interest were the dominant design of most clinically researched gene therapy products.

Some of the early programs with plasmid transfection were proposed in 1992. Due to their low risk for genome integration, plasmids were considered safer than viral vectors. However, their short half-life, particularly in dividing cells, along with variable transfection efficiency and other factors, limited their use. The use of plasmids expanded from the late 1990s through early 2000s, but then shrank in the last decade, giving way to other product types. Nonetheless, plasmid-based gene delivery remains widely employed in both manufacturing and clinical gene therapy applications.

In 1993, clinical studies with in vivo administration of retroviral vectors were proposed. Retroviral vectors can integrate into the human...
genome, which enables their long-lasting effects. Early programs used primarily gammaretroviral vectors transducing dividing cells. Later, lentiviral vectors became more widely used for \textit{ex vivo} transduction, as they also transduce non-dividing cells. Despite the wide use of retroviral vectors in \textit{ex vivo} genetic modification of cells, their use for \textit{in vivo} gene delivery has been limited by concerns about vector replication\textsuperscript{16} and insertional mutagenesis.\textsuperscript{9–12} In the early 2000s, these concerns led to a shift toward vectors and vector designs with lower potential for these risks. As shown in Figure 4, following earlier modest use of \textit{in vivo}-administered retroviral vector-based products, only a few of these programs were in development after 2000 and mainly included lentiviral vectors, which underwent genetic modifications for replication incompetency and testing in integration studies for off-target effects. In the last two decades, the use of retroviral vector-based products has decreased, ranging from 6\% to 1\% of the respective IND pools in the years received.

The first programs with recombinant adenoviral vector-based products also appeared in 1993. Owing to their consistent efficiency of gene transfer and good tropism for pulmonary and other tissues, adenoviral vectors were one of the primary product types used for gene delivery in the late 1990s and early 2000s. Their use had gradually decreased from 28\% in 1998 to 6\% in 2019 for various reasons, among them the ability to trigger severe immunogenic and inflammatory responses.\textsuperscript{7} More recently, adenoviral vectors have continued to find their application in different therapeutic areas.

Development of recombinant AAV vectors in the late 1980s enabled their use in gene therapies, with first clinical proposals appearing in 1995. AAV, unlike other viral vectors, requires the presence of a “helper” virus\textsuperscript{33} as well as AAV genes (\textit{rep} and \textit{cap}) in \textit{trans} to enable AAV vector replication. Although AAV has a relatively simple genome, vector manufacturing had been complicated for a long time by the need for the second “helper” virus, low vector yield, and the difficulty in removing manufacturing impurities such as empty capsids, plasmid-, and host cell DNAs. Several recent manufacturing advances have both increased the yield and improved the quality of AAV vectors. Non-pathogenic during native infection, available in multiple serotypes, and exhibiting wide tissue tropism, AAV vectors are attractive for pseudotyping and capsid modification that can be optimized to target specific tissues, including neural and muscular. Because wild-type AAV is encountered in childhood, an adaptive immune response with production of neutralizing antibodies has been one of the issues hindering development of AAV-based gene therapies. Selection of serotypes, screening for antibody status, investigation of immunogenicity in preclinical studies, and utilization of various immunosuppressive regimens have considerably improved clinical use of AAV vectors. Their research penetration began slowly, with fewer programs initially reaching clinical trials, but increased over time, ranging from 14\% to 28\% and comprising the largest category of viral vector-based therapies in the past 6 years.

Microbial vectors have been in clinical research since the early 2000s. They include bacteria genetically modified to express human genes of interest in the target cells and tissues. One of the early products of this type was genetically modified \textit{Salmonella typhimurium} to treat advanced cancers.\textsuperscript{34}

Initial proposals for clinical trials employing genome-editing technologies date back to 2009.\textsuperscript{35} Most genome-editing technologies used in clinical studies are based on the ability to induce double-stranded DNA breaks in a nuclease-dependent or nuclease-independent manner in precise locations followed by repair of the broken DNA with endogenous processes through homology-directed repair (HDR) and non-homologous end-joining (NHEJ). The earlier genome-editing technologies used introduction of zinc finger-guided nucleases (ZFNs) or transcription activator-like effector-guided
nucleases (TALENs) into the cells of interest. A more recent advancement is the clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein (Cas) systems for which various types of intracellular delivery can be used, including viral vector delivery and electroporation. In June 2016, the RAC publicly discussed the first trial with CRISPR technology, generating both scientific and ethical debates in the field. Similar to the initial developmental stages of the gene therapy field, most current products employing genome-editing technologies are ex vivo GM cells.

**Overcoming Translational Challenges**

Initiation of clinical studies under an IND application is an important first step in the translation of scientific discovery and research from bench to clinical outcomes at bedside. In product development programs that successfully transition from the laboratory to the clinical stage, critical product development issues are recognized and addressed early on.

At the preclinical stage, it is important to have a good understanding of the disease manifestations and course of progression, the underlying genetic variations, and the pathogenetic mechanisms. Bypassing the critical knowledge of the disease and targeting only the pathway directly affected by a product may limit product development and negatively impact the design of subsequent clinical investigations. Reproducible and accurate demonstration of functional activity and potency of the investigational product are weighty milestones in product characterization and must be done well and sufficiently early to further enable successful product development. Control for impurities, particularly with viral vector production, and early identification of any potential off-target effects of gene therapies help shape the product toxicity profile at the preclinical stage and optimize the approach to safety monitoring during subsequent clinical investigations. Establishment and validation of adequate assays for product characterization and lot release along with delineation of the critical quality attributes are other important parts of successful transition into the clinical stage.

The translation of a product development program from bench to bedside also depends on the preclinical toxicology studies. Data generated in appropriately designed studies in biologically relevant animal species and disease models, as well as use of in vitro and in silico evaluations, serve to demonstrate proof of concept and describe product biodistribution and safety to justify proceeding to clinical studies. A well-conducted preclinical program will inform selection of a potentially safe starting clinical dose and dose-escalation regimen, support patient eligibility criteria, and help identify future elements of clinical monitoring.

Upon submission of an IND application, design of the first-in-human studies must not only address the anticipated safety concerns but also incorporate safeguards for recognition and management of any unexpected events. Other important features that support both transition into the clinical stage and efficiency of the overall product development program are adequate study design and selection of...
Table 2. Features of the Development Programs for Six Approved Oncolytic and Gene Therapy Products

|                              | Talimogene Laherparepvec | Tisagenlecleucel | Axicabtagene Ciloleucel | Voretigene Neparvovec | Onasemnogene Abeparvovec | Brexucabtagene Autoleucel |
|------------------------------|--------------------------|------------------|-------------------------|----------------------|-------------------------|--------------------------|
| **Indication** to address unmet medical needs | recurrent melanoma       | relapsed and refractory ALL | relapsed and refractory DLBCL | retinal dystrophy     | spinal muscular atrophy | relapsed and refractory MCL |
| **Serious disease**          | ✓                        | ✓                | ✓                       | ✓                    | ✓                       | ✓                         |
| **Rare disease**             | ✓                        | ✓                | ✓                       | ✓                    | ✓                       | ✓                         |
| **Product construct**        | oncolytic HSV with transgene for GM-CSF | T cells with CAR to CD19 transduced with LV vector | T cells with CAR to CD19 transduced with g RV vector | AAV2 vector with transgene for RPE65 | AAV9 vector with transgene for SMN1 | T cells with CAR to CD19 transduced with g RV vector |
| **Route of administration**  | intralesional            | intravenous      | intravenous             | subretinal           | intravenous             | intravenous             |
| **Significant modifications in product manufacturing during development** | ✓                        | ✓                | ✓                       | ✓                    | ✓                       | ✓                         |
| **Product comparability studies completed** | ✓                        | ✓                | ✓                       | ✓                    | ✓                       | ✓                         |
| **Non-clinical studies conducted** | in vitro studies with the human product and in vivo (TB and non-TB rodents) studies to assess AT activity, safety, and BD after IT and IV administration | in vitro and in vivo (TB and non-TB rodents) studies to assess specificity, AT activity, safety, and BD after IV administration | in vitro studies with the human product and in vivo (TB rodents) studies with an analogous murine CAR construct to assess specificity, AT activity, and safety after IV administration | in vivo studies in RPE65 mutant and normal-sighted dogs, and normal-sighted NHP to evaluate POC safety, immunogenicity, and safety, and BD after single and repeat IV administration | in vivo studies in a murine spinal muscular atrophy model, healthy mice, and NHP to evaluate POC safety, and BD after single IV administration | in vitro studies with the human product and in vivo (TB rodents) studies with an analogous murine CAR construct to assess specificity, AT activity, and safety after IV administration |
| **Clinical studies demonstrating the primary evidence of effectiveness [number of patients (n), study duration]** | one multicenter trial [n = 436, ~3.75 years] | one multicenter trial [n = 88, ~1.75 years] | one multicenter trial [n = 111, ~2 years] | one two-center trial with crossover of control to treatment at 1 year followed up to 2 years of observation [n = 31, ~4 years] | one multicenter ongoing trial with external control from natural history data [n = 21, ~1.5 years] | one multicenter ongoing trial [n = 74, ~3.25 years] |
| **Open label**               | ✓                        | ✓                | ✓                       | ✓                    | ✓                       | ✓                         |
| **Randomized, two arm, with concurrent control** | ✓                        | ✓                | ✓                       | ✓                    | ✓                       | ✓                         |
| **Single arm**               | ✓                        | ✓                | ✓                       | ✓                    | ✓                       | ✓                         |
| **Novel primary endpoint**   | ✓                        | ✓                | ✓                       | ✓                    | ✓                       | ✓                         |
| **Natural history data used** | ✓                        | ✓                | ✓                       | ✓                    | ✓                       | ✓                         |
| **First-in-human study in children** | ✓                        | ✓                | ✓                       | ✓                    | ✓                       | ✓                         |
| **Time from initial IND to approval** | 10 years                | 8 years          | 9 years                 | 10 years             | 6 years                 | 12 years                 |
| **Type of initial IND**       | commercial              | academic research | academic research       | academic research    | academic research       | academic research        |
| **Fast Track designation at ~ years before approval** | ✓                        | ✓                | ✓                       | ✓                    | ✓                       | ✓                         |
| **Breakthrough designation at ~ years before approval** | 4 years                 | 1.5 years        | <2 years                | 3 years              | 3 years                 | 2 years                  |

(Continued on next page)
of clinically meaningful, reliable endpoints even for the preliminary evaluation of product efficacy. To this end, early partnership with patient communities to determine the clinical impact of the disease and support study recruitment can become an asset to any new product development program. Finally, successful transition into the clinical stage requires knowledge of the regulatory processes for opening an IND application. Because many initial studies with gene therapies historically come from academic institutions, the investigators conducting the research usually assemble IND packages and become IND sponsors. In order to reduce the burden on IND sponsors, FDA issues guidance documents and provides educational and other resources available to stakeholders seeking to open an IND application.

To foster development of new therapies, FDA has put in place different procedures enabling sponsors to meet with the Agency and ask questions before IND submission. Sponsors may request a pre-IND meeting to receive regulatory advice and expert recommendations from different review disciplines for any concerns specific to their products. In addition, based on the increasing numbers of novel gene therapies developed for various clinical indications, reviewers from OTAT and its predecessor office (Office of Cellular, Tissue, and Gene Therapies: OCTGT) recognized the importance of an earlier interaction with sponsors on issues of product manufacturing and design of preclinical studies. Thus, a communication initiative called “pre-pre-IND interaction” was started approximately 15 years ago and subsequently evolved into the INitial Targeted Engagement for Regulatory Advice on CBER productTs (INTERACT) meeting program. INTERACT meeting is an informal non-binding communication and advice intended for innovative investigational products at an early stage of development on issues that are not yet at the pre-IND meeting phase. Acknowledging the rapid development of novel manufacturing technologies, CBER established another process enabling stakeholders to request meetings with CBER Advanced Technologies Team (CATT) in order to promote dialog, education, and input between CBER and prospective innovators and developers of advanced manufacturing technologies to discuss issues related to the implementation of these technologies in the development of novel products.

### Approved Oncolytic and Gene Therapies

To date, six products with genetic modifications have been approved by FDA: an oncolytic viral therapy, three autologous CAR T cell therapies, and two AAV vector-based therapies.

Talimogene laherparepvec is a genetically modified replication-competent HSV, which acts by infecting tumor cells and producing viral-induced cell lysis. Although it was approved as oncolytic viral therapy, the product’s construct contains genetic modifications; therefore, we include the description of its development program in this section.

### Table 2. Continued

|                         | Talimogene Laherparepvec | Tisagenlecleucel | Axicabtagene Calolecucel | Voretigene Neparvovec | Onasemnogene Abeparvovec | Brexucabtagene Autoleucel |
|-------------------------|--------------------------|------------------|--------------------------|----------------------|--------------------------|--------------------------|
| Orphan Product designation at ~years before approval | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Rare pediatric disease voucher | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Accelerated approval | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Review cycle duration | 15 months | 7 months | 6.5 months | 7 months | 8 months | 7.5 months |
| Approved in 1st review cycle | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Post-marketing LTFU | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| PMR safety study | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Risk evaluation and mitigation strategy | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

ALL, acute lymphoblastic leukemia; DLBCL, diffuse large B cell lymphoma; MCL, mantle cell lymphoma; GM-CSF, granulocyte-macrophage colony-stimulating factor; RPE65, retinal pigment epithelium protein; SMN1, survival motor neuron 1 protein; BD, biodistribution; IV, intravenous; IT, intratumoral; SR, subretinal; AT, antitumor; HSV, herpes simplex virus; CAR, chimeric antigen receptor; LV, lentiviral; RV, retroviral; AAV, adeno-associated viral; POC, proof-of-concept; TB, tumor-bearing; NHP, non-human primates; LTFU, long-term follow-up; PMR, post-marketing requirement.

*Only first approved indications are included.

+Autologous.

+Cell targeting, vision, and behavior.

+Cell targeting, survival, and motor function.

+Study duration represents an approximate time from enrollment of the first subject to the data cutoff accepted for evaluation in the marketing application.
unmet medical needs. Consistent with the trend in the field, these programs are examples of early development taking place in academic centers, with five initiated as academic research INDs and one as a commercial IND started by an innovator biotechnology company. For each of these programs, the 3- to 4-year time before approval marked the IND transfer to a new commercial sponsor; two had another change of sponsor < 1 year before approval.

The time from the IND submission to approval ranged from 6 to 12 years. This time, however, does not account for the gargantuan work that goes into engineering a product, establishing a controlled manufacturing process, and conducting preclinical studies. While the years from IND initiation to approval represent a visible part of the proverbial iceberg, a much less recognized aspect of making a new product is the availability of other technological, scientific, and clinical knowledge that plays a catalytic role in the development of a novel treatment. For example, the prototype construct for talimogene laherparepvec was described in 2003, but some experimental work that supported this construct dated back to the early 1990s. Similarly, some of the ground work for the chicken β-actin promoter used in the approved AAV-based products was conducted more than two decades before the respective clinical programs were initiated. In CAR T cell development, the concept of using genetically modified lymphocytes to treat hematological malignancies was supported by the observations of immunocompetent donor T cells mediating antileukemic effects, which were made almost 40 years before approval of CAR T products.

Subsequently, the first notable reports on what would become CAR T cells appeared in the late 1980s. The first-generation CARs, although able to recognize antigens on the target tumor cells, failed to work in the absence of costimulatory signaling. Over the years, the design of CARs had to undergo modifications to first include and then optimize the costimulatory and cytoplasmic signaling domains before the desired antitumor effect of the approved CAR T products was achieved.

As shown in Table 2, all six products demonstrated clinical benefit, with large quantitative or previously unseen qualitative therapeutic effects. Each product program included one pivotal study and supportive confirmatory evidence, overall demonstrating the substantial evidence of product effectiveness that formed the basis for regulatory approval. Notwithstanding, all product programs had other clinical studies conducted during development, some with the final product and some with its earlier versions. When significant manufacturing changes were made during product development, comparability studies had to be conducted. Natural history (NH) data were used in two programs: for RPE65-associated retinal dystrophy, NH data helped understand the progression of blindness and supported the development of a novel trial endpoint; for spinal muscular atrophy, NH data provided a valid comparison with outcomes of the progressive disease.

Various regulatory incentives were used for these programs to expedite their development: fast track, breakthrough, and orphan disease designations; three out of six were granted the rare pediatric disease voucher, and one product received accelerated approval. Consistent with the recommendations for LTFU, patients treated with gene therapies continue to be followed clinically after product approval. LTFU is separate from the requirement to conduct post-marketing studies evaluating risks of infections with talimogene laherparepvec and secondary malignancies with CAR T products. Sponsors of the CAR T products have implemented risk evaluation and mitigation strategies to manage cytokine release syndrome and neurotoxicity associated with these treatments.

Conclusions and Perspectives
In this article we presented the evolution of gene therapy clinical product development as witnessed by FDA since the beginning of clinical research with gene therapies. In addition to the extraordinary scientific advances and the great clinical advantages offered by this field, its story is remarkable for the ability to overcome challenges and realize successes. More than three decades after the first clinical study with gene transfer into humans, six approved products are available to benefit patients with serious diseases. Despite the many failures in the early decades, the field continues to grow, with increasing numbers of products tested in clinical trials. Not all of them will reach the market with proven safety and effectiveness, but those that become approved and continue showing beneficial treatment effects and safety after approval will be welcome additions to the therapeutic options for patients with serious diseases. The scientific progress made over the years will continue furthering the field’s interdependent components. More systems will be created around storage and manufacturing of quality cell banks and viral banks used for production of genetically modified cells and vectors. As the gene therapy field is actively looking for improvements in the capabilities of cell and vector production to reduce costs and increase outputs, an eventual rebalancing of the economic value of product manufacturing will decrease the barriers to entry into the field and attract more researchers and companies to use the available technologies for targeting new treatments. At the same time, the backbone of the gene therapy research in academic institutions and biotechnology companies will continue refining vector designs to improve target delivery of gene therapies to the intended cells and tissues and to enable evasion of the immunological responses, thus improving efficacy and safety of new products at the stage of design. Newer technologies of genome editing, which already made their rapid entry into the field, will continue being rigorously researched to better understand their safety and long-term effects. In silico computational methods employed for identification of off-target effects and various types of modeling will further penetrate the different domains of product development. On the clinical side, assurance of safety and sufficiently large treatment effect of gene therapies will continue influencing study designs, allowing FDA to exercise the flexible and feasible approaches to support efficient product development and expedite availability of new treatments to patients. To monitor the increasing numbers of patients treated with gene therapies, disease- or product-based registries will be created or consolidated from the existing venues, with likely transition of the long-term patient care from researchers to regular healthcare practitioners. New challenges will undoubtedly appear along the way and yet again will require multifaceted and collective problem solving.
Consistent with FDA’s mission of protecting and promoting public health, OTAT will continue implementing science-based and data-driven policies and undertaking measures to facilitate safe and ethical development, timely availability, and safe use of novel gene therapies. In addition to issuing guidance documents and providing advice to sponsors at all stages of product life cycle, we collaborate with stakeholders in the field and various national and international organizations to address challenging areas for gene therapies, including standards development, vector manufacturing,34 immunogenicity,35 and development of individualized therapeutics,35 among many others.

As the field of gene therapies continues to grow, improvements in the economies of scale and scope for vector production and product manufacturing, fine-tuning of genome-editing technologies, and ascertaining the dominant designs of transgene delivery systems will likely become the next catalytic steps critical for this industry. Moving forward, as the first-approved gene therapies are replaced by next-generation constructs with improved safety profiles and enhanced effectiveness, their clinical use will be optimized further. Patients with serious conditions, including rare genetic disorders that were once considered incurable, will have the greatest potential to benefit from the next frontiers in the development of gene therapies.

AUTHOR CONTRIBUTIONS
L.L. analyzed IND data, designed the figures and tables, conceptualized, wrote, and revised the manuscript; T.P.S. reviewed IND data and wrote and revised the manuscript; M.S. designed table content, revised the manuscript, and advised on historical aspects; R.K.P. reviewed IND data, wrote and revised the manuscript, and advised on historical aspects of the gene therapy field development.

CONFLICT OF INTEREST
The authors declare no competing interests.

REFERENCES
1. U.S. Food and Drug Administration (2020). Chemistry, Manufacturing, and Control Information for Human Gene Therapy Investigational New Drug Applications: Guidance for Industry. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/chemistry-manufacturing-and-control-cmc-information-human-gene-therapy-investigational-new-drug.
2. National Institutes of Health. Novel and Exceptional Technology and Research Advisory Committee https://osp.od.nih.gov/biotechnology/main-nextrac/.
3. Collins, F.S., and Gottlieb, S. (2018). The Next Phase of Human Gene-Therapy Oversight. N. Engl. J. Med. 379, 1393–1395.
4. U.S. Food and Drug Administration (2014). Guidance Document. Expedited Programs for Serious Conditions – Drugs and Biologics, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/expedited-programs-serious-conditions-drugs-and-biologics.
5. U.S. Food and Drug Administration (2019). Expedited Programs for Regenerative Medicine Therapies for Serious Conditions. Guidance for Industry, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/expedited-programs-regenerative-medicine-therapies-serious-conditions.
6. U.S. Food and Drug Administration (2019). Code of Federal Regulations Title 21. 21CFR 312.38-312.45. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=312.
7. Wilson, J.M. (2009). Lessons learned from the gene therapy trial for ornithine transcarbamylase deficiency. Mol. Metab. 86, 151–157.
8. U.S. Food and Drug Administration (2014). 2014 Meeting Materials. Cellular, Tissue, and Gene Therapy: Advisory Committee Meeting. http://wayback.archive-it.org/7993/2017011127931/http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/CellularTissueandGeneTherapiesAdvisoryCommittee/ucm380847.htm.
9. Hacein-Bey-Abina, S., von Kalle, C., Schmidt, M., Le Deist, F., Walffraat, N., McIntyre, E., Radford, I., Villeval, J.L., Fraser, C.C., Cavazzana-Calvo, M., and Fischer, A. (2003). A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. N. Engl. J. Med. 348, 255–256.
10. Marwick, C. (2003). FDA halts gene therapy trials after leukemia case in France. BMJ 326, 181.
11. Braun, C.J., Boztug, K., Paruzynski, A., Witzel, M., Schwarzer, A., Rehle, M., Modlich, U., Beier, R., Göhring, G., Steinemann, D., et al. (2014). Gene therapy for Wiskott-Aldrich syndrome–long-term efficacy and genotoxicity. Sci. Transl. Med. 6, 227ra33.
12. Stein, S., Ott, M.G., Schulzke-Strasser, S., Jauch, A., Burwinkel, B., Kinner, A., Schmidt, M., Krämer, A., Schwibble, J., Glimm, H., et al. (2010). Genomic instability and myelodysplasia with monosomy 7 consequent to EV11 activation after gene therapy for chronic granulomatous disease. Nat. Med. 16, 198–204.
13. Nyberg, K., Carter, B.J., Chen, T., Dunbar, C., Flotte, T.R., Rose, S., Rosenblum, D., Simek, S.L., and Wilson, C. (2004). Workshop on long-term follow-up of participants in human gene transfer research. Mol. Ther. 10, 976–980.
14. Vannucci, L., Lai, M., Chiuppi, F., Ceccherini-Nelli, L., and Pistello, M. (2013). Viral vectors: a look back and ahead on gene transfer technology. New Microbiol. 36, 1–22.
15. Wilson, C.A., Ng, T.H., and Miller, A.E. (1997). Evaluation of recommendations for blood safety and gene therapy advisory committee meeting. http://wayback.archive-it.org/7993/2017011127931/http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/CellularTissueandGeneTherapiesAdvisoryCommittee/ucm380847.htm.
16. U.S. Food and Drug Administration (2020). Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up. Guidance for Industry, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/testing-retroviral-vector-based-human-gene-therapy-products-replication-competent-retrovirus-during.
17. U.S. Food and Drug Administration (2020). Long-Term Follow-up After Administration of Human Gene Therapy Products. Guidance for Industry, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/long-term-follow-after-administration-human-gene-therapy-products.
18. U.S. Food and Drug Administration. OTAT (formerly OCTG) Learn Webinars. https://www.fda.gov/vaccines-blood-biologics/news-events-biologics/otat-learn.
19. Rosenberg, S.A., Arbersold, P., Cornetta, K., Kasid, A., Morgan, R.A., Moen, R., Karson, E.M., Lotze, M.T., Yang, J.C., Topalian, S.L., et al. (1990). Gene transfer into humans—immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N. Engl. J. Med. 323, 570–578.
20. Rosenberg, S.A., Anderson, W.F., Blaese, M., Hwu, P., Yannelli, J.R., Yang, J.C., Topalian, S.L., Schwartzzentruber, D.J., Weber, J.S., Ettinghausen, S.E., et al. (1993). The development of gene therapy for the treatment of cancer. Ann. Surg. 218, 455–463, discussion 463–464.

21. Candotti, F., and Blaese, R.M. (1998). Gene therapy of primary immunodeficiencies. Semin. Immunopathol. 19, 493–508.

22. U.S. Food and Drug Administration (1998). Guidance for Human Somatic Cell Therapy and Gene Therapy. Guidance for Industry, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-human-somatic-cell-therapy-and-gene-therapy.

23. Donahue, R.E., Kessler, S.W., Bodine, D., McDonagh, K., Dunbar, C., Goodman, S., Agricola, B, Byrne, E., Rafeld, M., Moen, R., et al. (1992). Helper virus induced T-cell lymphoma in nonhuman primates after retroviral mediated gene transfer. J. Exp. Med. 176, 1125–1135.

24. U.S. Food and Drug Administration (2015). Design and Analysis of Shedding Studies for Virus or Bacteria-Based Gene Therapy and Oncolytic Products. Guidance for Industry, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/design-and-analysis-shedding-studies-virus-or-bacteria-based-gene-therapy-and-oncolytic-products.

25. U.S. Food and Drug Administration (2016). Recommendations for Microbial Vectors Used for Gene Therapy. Guidance for Industry, September 2016, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/recommendations-microbial-vectors-used-gene-therapy.

26. U.S. Food and Drug Administration (2015). Determining the Need for and Content of Environmental Assessments for Gene Therapies, Vacceted Vaccines, and Related Recombinant Viral or Microbial Products. Guidance for Industry, https://www.fda.gov/media/91425/download.

27. U.S. Food and Drug Administration (2013). Preclinical Assessment of Investigational Cellular and Gene Therapy Products. Guidance for Industry, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/preclinical-assessment-investigational-cellular-and-gene-therapy-products.

28. U.S. Food and Drug Administration (2015). Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products. Guidance for Industry, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/considerations-design-early-phase-clinical-trials-cellular-and-gene-therapy-products.

29. U.S. Food and Drug Administration (2020). Human Gene Therapy for Hemophilia. Guidance for Industry, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-gene-therapy-hemophilia.

30. U.S. Food and Drug Administration (2020). Human Gene Therapy for Retinal Disorders Guidance for Industry, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-gene-therapy-retinal-disorders.

31. U.S. Food and Drug Administration (2020). Human Gene Therapy for Rare Diseases Guidance for Industry, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-gene-therapy-rare-diseases.

32. U.S. Food and Drug Administration (2020). Interpreting Sameness of Gene Therapy Products Under the Orphan Drug Regulations. Draft Guidance for Industry, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/interpreting-sameness-gene-therapy-products-under-orphan-drug-regulations.

33. Grimm, D., and Buning, H. (2017). Small but increasingly mighty: latest advances in AAV Vector research, design, and evolution. Hum. Gene Ther. 28, 1075–1086.

34. Lee, K. (2002). Comparison of additional regulatory requirements for conducting clinical trials on organisms and recombinant DNA in Europe, the United States, and Canada. Drug Inf. J. 36, 379–386.

35. Maier, D.A., Brentan, A.L., Jiang, S., Binder-Scholl, G.K., Lee, G., Plesa, G., Zheng Z., Cotte, J., Carpenito, C., Wood, T., et al. (2013). Efficient clinical scale gene modification via zinc finger nuclease-targeted disruption of the HIV co-receptor CCR5. Hum. Gene Ther. 24, 245–258.

36. 144th Meeting of the Recombinant DNA Advisory Committee, June 21-22 (2016). Minutes of Meeting (OSP), https://osp.od.nih.gov/wp-content/uploads/2017/02/Minutes_RAC_Jun2016_508_0.pdf.

37. U.S. Food and Drug Administration. INTERACT Meetings (Initial Targeted Engagement for Regulatory Advice on CBER Products). https://www.fda.gov/vaccines-blood-biologics/industry-biologics/interact-meetings.

38. U.S. Food and Drug Administration. CBER Advanced Technology Team (CATT) https://www.fda.gov/vaccines-blood-biologics/industry-biologics/cber-advanced-technologies-team-catt.

39. U.S. Food and Drug Administration (2019). IMLYGIC: Summary Basis for Regulatory Action, https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/imlygic-talmogene-laherparepvec.

40. U.S. Food and Drug Administration (2017). KYMRIAH: Summary Basis for Regulatory Action, https://www.fda.gov/media/107962/download.

41. U.S. Food and Drug Administration (2017). YESCARTA: Summary Basis for Regulatory Action, https://www.fda.gov/media/108788/download.

42. U.S. Food and Drug Administration (2020). TECARTUS: Summary Basis for Regulatory Action, https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/tecarts-breuxcabtagene-autoleucle.

43. U.S. Food and Drug Administration (2017). LUXTURNA: Summary Basis for Regulatory Action, https://www.fda.gov/media/110411/download.

44. U.S. Food and Drug Administration (2019). ZOLGENSMA: Summary Basis for Regulatory Action, https://www.fda.gov/media/127961/download.

45. Liu, B.L., Robinson, M., Han, Z.Q., Branston, R.H., English, C., Reay, P., McGrath, Y., Thomas, S.K., Thornton, M., Bullock, P., et al. (2003). ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. Gene Ther. 10, 292–303.

46. Niwa, H., Yamamura, K., and Miyazaki, J. (1991). Efficient selection for high-expression transfectants with a novel eukaryotic vector. Gene 108, 193–199.

47. Crowther, M.D., Svane, I.M., and Met, O. (2020). T-Cell Gene Therapy in Cancer Immunotherapy: Why It Is No Longer Just CARs on The Road. Cells 9, 1588.

48. Weiden, P.L., Flourny, N., Thomas, E.D., Prentice, R., Feyer, A., Buckner, C.D., and Storb, R. (1979). Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. N. Engl. J. Med. 300, 1068–1073.

49. Kuwana, Y., Asakura, Y., Utsunomiya, N., Nakashim, M., Arata, Y., Itoh, S., Nagase, F., and Kuroasa, Y. (1987). Expression of chimeric receptor composed of immunoglobulin-derived V regions and T-cell receptor-derived C regions. Biochem. Biophys. Res. Commun. 149, 960–966.

50. Gross, G., Waks, T., and Eshhar, Z. (1989). Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc. Natl. Acad. Sci. USA 86, 10024–10028.

51. Chung, D.C., McCague, S., Yu, Z.F., Thill, S., DiStefano-Pappas, J., Bennett, J., Cross, D., Marshall, K., Wellman, J., and High, K.A. (2018). Novel mobility test to assess functional vision in patients with inherited retinal dystrophies. Clin. Exp. Ophthalmol. 46, 247–259.

52. Al-Zaydi, S.A., Kolb, S.J., Lowes, L., Alfano, L.N., Shell, R., Church, K.R., Nagendran, S., Srouple, D.M., Feltner, D.E., Wells, C., et al. (2019). AVXS-101 (Onasemnogene Abeparvovec) for SMA1: Comparative Study with a Prospective Natural History Cohort. J. Neuromuscul. Dis. 6, 307–317.

53. National Center for Advancing Translational Sciences (2020). Workshop on expanding AAV manufacturing capacity for rare disease gene therapies, January, https://events-support.com/events/NCATS_Gene_Therapies_January_2020.

54. National Center for Advancing Translational Sciences (2020). Workshop on systemic immunogenicity considerations for AAV-mediated gene therapy, November, https://ncats.nih.gov/events/.

55. U.S. Food and Drug Administration (2020). Workshop: Facilitating end-to-end development of individualized therapeutics, March, https://www.fda.gov/vaccines-blood-biologics/workshops-meetings-conferences-biologics/facilitating-end-to-end-development-individualized-therapeutics-03032020-03032020.