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EDITORIAL

The vicissitudes of gene therapy

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All fields of endeavour have their ups and downs, but gene therapy arguably suffers more than most. The predominant early concern was safety. In the broad sweep of events since the first approved gene transfer to a human in 1989,[1] there have been two major setbacks as a result of well-publicized patient deaths. The first, that of Jesse Gelsinger in 1999,[2] deflated what had until then been a decade of increasing optimism and achievement that saw the approval of approximately 485 gene therapy trials. After a hiatus, progress resumed only for momentum to be reversed once again, this time by the occurrence of leukaemia in children receiving gene therapy for severe combined immunodeficiency disease (SCID).[3] Nevertheless, at about this time, the Chinese authorities approved the world's first gene therapeutic, Gendicine (Shenzhen SiBiono GeneTech, Shenzhen, China), for the treatment of head and neck cancer (Table I).[4] Approval of a gene therapy by Western countries did not occur until 2012, when the European Medicines Association (EMA) authorized Glybera (UniQure, Amsterdam, The Netherlands) for lipoprotein lipase deficiency.[5].

Since then, in the absence of additional major safety issues and with technological advances in vector design[6] and manufacturing, 13 gene therapies have now gained full or conditional market approval in various parts of the world (Table I), although one of these has since been rescinded (Invossa; Kolon TissueGene, Rockville, Maryland) and another withdrawn for commercial reasons (Glybera). Another, conditionally approved product, Zalmoxis (MolMed, Milan, Italy), is on hold because the primary endpoint has not been met in a current Phase III trial. By the end of 2017, the last year for which complete data are available, approximately 2600 gene therapy clinical trials had been completed.[7]

Interest in orthopaedic applications of gene therapy began in the late 1980s and its development has been buffeted by many of the same issues affecting the field as a whole. Most progress has been made with the development of intra-articular gene therapies for treating arthritis,[8] where the introduction of cells expressing the interleukin-1 receptor antagonist (IL-1Ra) into rheumatoid joints was an early success.[9] Progress in the further development of this *ex vivo*, retroviral approach was prevented by a number of factors, including the risk from insertional mutagenesis of the type that caused the occurrence of leukaemia in the SCID trial mentioned above.

By then, adeno-associated virus (AAV) vector technology had improved considerably, and Phase I and II trials were conducted, which used this vector to deliver etanercept to joints with rheumatoid arthritis (RA).[10,11] Although these trials showed promise, a patient in the Phase II study died from a fungal infection.[12] After an investigation by the United States Food and Drug Administration (FDA), the clinical hold was lifted. However, there has been no further activity from this clinical programme. Elsewhere, recombinant AAV encoding interferon-beta under the transcriptional control of an inflammation-inducible promoter was developed for injection into joints with RA. This has shown promise in preclinical testing[13] and clinical trials are underway (NCT02727764, NCT03445715).

Meanwhile, a novel *ex vivo* protocol for the intra-articular treatment of osteoarthritis (OA) was introduced, using allogeneic chondrocytes transduced with retrovirus to express high levels of transforming growth factor-beta. This therapeutic, known as Invossa, was approved in South Korea in 2017 (Table I) and Phase III clinical trials in the United States began in 2018 (NCT03203330). Then everything stopped. Earlier this year, it came to light that the genetically modified cells being injected intra-articularly were not chondrocytes but HEK293 cells. The HEK293 line, established from human embryonic kidney, is often

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Table I. Gene therapy approvals worldwide

Indication	Vector (delivery method)	Gene product	Name	Jurisdiction (year approved)
Head and neck cancer	adenovirus (*in vivo*)	p53	Gendicine (Shenzhen SiBiono GeneTech, Shenzhen, China)	China (2003)
Solid tumours	Retrovirus (*in vivo*)	Mutant cyclin G1	Rexin-G (Epeius Biotechnologies, San Marino, California)	Philippines (2007)
Peripheral artery disease	Plasmid (*in vivo*)	Vascular endothelial growth factor	Neovasculgen (Human Stem Cell Institute, Moscow, Russia)	Russia (2011); Ukraine (2013)
Lipoprotein lipase deficiency	AAV (*in vivo*)	Lipoprotein lipase	Glybera (UniQure, Amsterdam, The Netherlands)	Europe (2012)
Melanoma	Herpes simplex virus (*in vivo*)	Granulocyte-macrophage colony stimulating factor	Imlygic (Amgen, Thousand Oaks, California)	United States (2015); Europe (2015); Australia (2018)
Adenosine deaminase deficiency	Retrovirus (*ex vivo*)	Adenosine deaminase	Strimvelis (Orchard Therapeutics, London, United Kingdom)	Europe (2016)
Restoration of host immune system after haematopoietic stem cell treatment	Retrovirus (*ex vivo*)	Human low-affinity nerve growth factor receptor; herpes thymidine kinase	Zalmoxis* (MolMed, Milan, Italy)	Europe (2016)
Osteoarthritis	Retrovirus (*ex vivo*)	Transforming growth factor-beta	Invossa† (Kolon TissueGene, Rockville, Maryland)	South Korea (2017)
Acute lymphoblastic leukemia	Lentivirus (*ex vivo*)	Chimeric antigen receptor	Kymriah (Novartis, Basel, Switzerland)	United States (2017); Europe (2018); Canada (2018); Switzerland (2018); Australia (2018)
Large B-cell lymphoma	Lentivirus (*ex vivo*)	Chimeric antigen receptor	Yescarta (Kite Pharma, Santa Monica, California)	United States (2017); Europe (2018); Switzerland (2018)
Biallelic RPE65 mutation-associated retinal dystrophy	AAV (*in vivo*)	Retinal pigment epithelium-specific 65 kDa protein	Luxturna (Spark Therapeutics, Philadelphia, Pennsylvania)	United States (2017); Europe (2018); Switzerland (2018)
Spinal muscular atrophy	AAV (*in vivo*)	Survival motor neuron-1	Zolgensma (Novartis, Basel, Switzerland)	United States (2019)
Beta-thalassaemia	Lentivirus (*ex vivo*)	Beta-globin	Zynteglo‡ (Bluebird Bio, Cambridge, Massachusetts)	Europe (2019)

*Zalmoxis was conditionally approved pending the outcome of a Phase III trial. This trial has been suspended because an interim analysis suggested the primary endpoint has not been met
†Invossa was withdrawn in 2019
‡Zynteglo, was conditionally approved pending additional clinical outcome data
AAV, adeno-associated virus

engineered to produce retrovirus vectors of the type used to prepare Invossa. The circumstances under which the HEK293 cells contaminated Invossa and other matters surrounding this case are under investigation. Meanwhile, the Korean licence for Invossa has been revoked and the Phase III United States trial has been suspended by the FDA.

In the latest arthritis gene therapy protocol to start clinical trials, knee joints of nine patients with OA will be injected with recombinant AAV that encodes IL-1Ra (NCT02790723); the first patient in this Phase I study was injected in June 2019. ClinicalTrials.gov also reports a Phase I study where plasmid DNA encoding a variant of human interleukin (IL)-10 will be injected into the knees of patients with OA (NCT03477487). In August 2019, its status was given as "active, not recruiting".

There is considerable interest in using gene transfer in the context of orthopaedic tissue regeneration.[14] The underlying strategy is to deliver regenerative gene products, especially morphogens and growth factors, in the sustained fashion necessary for robust healing. Traditional delivery methods, in contrast, implant these proteins in combination with a scaffold, which usually results in suboptimal, rapid burst release kinetics. Gene transfer holds additional promise when delivering products such as transcription factors and non-coding RNA, whose sites of action are intracellular.

Applications in bone healing, cartilage repair, and the regeneration of intervertebral disc, tendons, and ligaments largely remain at a preclinical stage of research, but show promise in rodent and rabbit models. It has proved difficult to replicate these successes in large animal models, although Bez et al[15] recently achieved impressive healing of critical size, tibial defects in pigs using bone morphogenetic protein-6 delivered via plasmid DNA in conjunction with sonication. Invossa has been implanted within a fibrin gel for the repair of human cartilage defects (NCT01825811) with encouraging, but unpublished, results.

There has been relatively little research into the application of gene therapy for treating genetic diseases of the skeletal system. These are quite rare and the most common, osteogenesis imperfecta, is a dominant negative mutation that not only requires transfer and expression of a wild-type cDNA, but also repression of the mutant gene. In such cases, gene editing using CRISPR-Cas technology may offer a more straightforward path forward.

Although cancer gene therapy is a thriving field, so far there has been little clinical application to malignancies of

orthopaedic interest beyond early trials using CAR-T cells (NCT01953900) and Rexin-G (Epeius Biotechnologies, San Marino, California) to target osteosarcoma (Table I).[16]

As safety concerns recede and the number of approved gene therapeutics increases, the field of gene therapy has gathered considerable recent momentum. Particularly encouraging is the rapidly expanding involvement of large pharmaceutical companies with the experience and resources to accelerate the clinical development of gene therapeutics.

However, a number of constraints continue to limit progress. In particular, the production of vectors under Good Manufacturing Practice (GMP) conditions remains inefficient and expensive. In many cases, contract manufacturers have long queues. These factors partly explain the very high cost of gene therapeutics. Glybera became the world's first million-dollar drug; it sold poorly and was withdrawn from the market in 2017. The latest gene therapy to be approved, Zolgensma (Novartis, Basel, Switzerland) for spinal muscular dystrophy, has been priced at $2.1 million per dose, another new record.

Genetic drugs for treating disorders of bones and joints should be much more affordable. Not only is the patient pool for diseases such as OA very large, but most applications envisage local treatment with a relatively small amount of vector. Under these conditions, orthopaedic conditions could become the domain where gene therapy becomes widely applied.[17]

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