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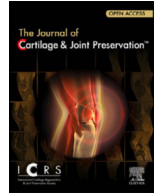


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Narrative Review

## Gene therapies for osteoarthritis: progress and prospects

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## ABSTRACT

**Introduction:** Osteoarthritis (OA), the most common form of joint disease, affects more than 500 million people worldwide. This painful and debilitating disease imposes a huge socioeconomic cost worldwide. Despite years of promising research, no etiological drug has been successfully introduced into daily clinical practice. In this context, gene therapy (GT) is emerging as a tool capable of meeting an increasingly specialized medical need. Five GT drugs for OA are currently under clinical evaluation, demonstrating the relevance of this tool. However, the widespread use of GT is still limited by considerations of safety, long-term efficacy, controlled and specific targeting, and the presence of neutralizing immune responses. Cartilage, a tissue of interest to target in OA, is a complex tissue to penetrate with the various GT vectors.

**Methods:** This manuscript reviews current clinical trials involving DNA-based GT for OA and suggests ways to improve recombinant adenoviral and adeno-associated viral vectors, including capsid engineering and transgene sequence optimization to achieve long-term expression of a given transgene exclusively in the target joint tissue, including cartilage.

**Results:** This review then highlights that the use of hybrid serotypes and/or chemical modifications of capsids are promising for improved tissue targeting. In addition, the choice of promoter and type of vectorized nucleic acid (single- or double-stranded DNA) appears to be critical for efficient transgene expression.

**Conclusion:** Finally, the combination of increasing knowledge about biocompatible materials and viral vectors should also be a way to improve transduction efficiency, increase the stability of transgene expression, and allow escape from neutralizing antibodies.

## Introduction

Joint disorders are a prevalent and often chronic source of pain and disability, resulting in significant socioeconomic costs worldwide. Osteoarthritis (OA) is the most common form, affecting over 500 million people in the world. Age and obesity are the 2 primary risk factors for OA (Fig. 1), contributing to its increasing prevalence in modern society. Despite long-standing intensive research, no disease-modifying OA drug (DMOAD) has yet been successfully translated into daily clinical practice. Therefore, finding

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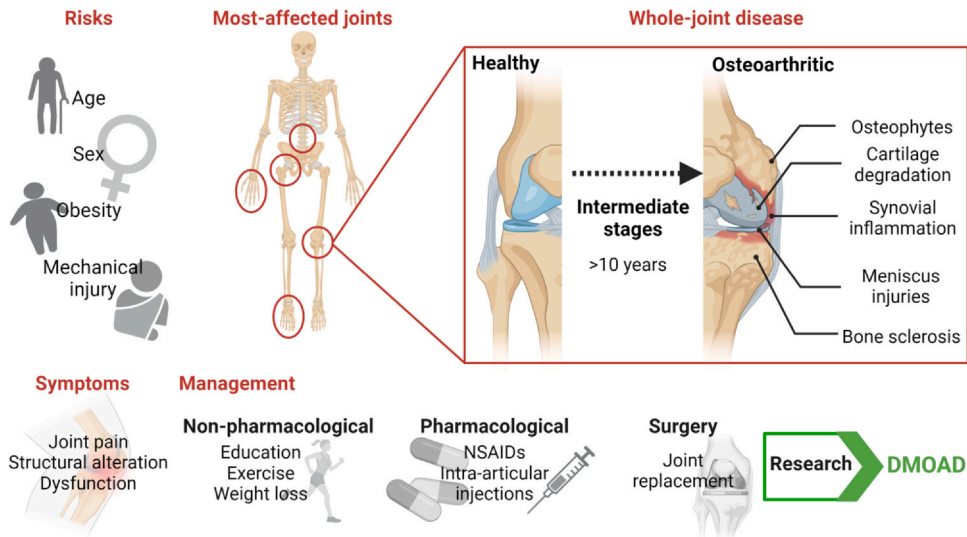
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**Fig. 1.** Osteoarthritis and its management at a glance. Major OA risk factors are age and obesity. Women and patients with a history of trauma are also more prone to OA. OA affects various joints, such as the knee, hip, hand, foot, and intervertebral disc, and all joint tissues. The main disabling symptom is pain. Currently, only management of painful symptoms is available. OA management consists of nonpharmacological options such as weight loss, pharmacological treatments including nonsteroidal anti-inflammatory drugs (NSAIDs) as well as intra-articular injections of glucocorticoids, and ultimately prosthetic surgery for replacement of the defective joint. Emergence of a DMOAD from this research area is still awaited. OA, osteoarthritis; DMOAD, disease-modifying OA drug. Created with [BioRender.com](https://www.biorender.com).

a cure for OA is needed.<sup>1</sup> Gene therapy (GT) could be considered a clinically useful approach for the safe and long-term expression of therapeutic factors in the absence of treatment. A promising therapy is the development of a GT vector platform that can transfer the appropriate nucleic acids targeting each pathological joint tissue. This manuscript reviews current clinical trials involving DNA-based GT for OA and suggests ways to improve recombinant adenoviral and adeno-associated viral (rAAVs) vectors, including capsid engineering and transgene sequence optimization. The search was performed on Pubmed, restricting it to articles (of all types) published between 2014 and the end of 2023, with a specific focus on GT with adeno-associated virus (AAV), adenovirus (Ad), or retrovirus in the field of OA, cartilage, and joint degeneration. For the improvement part, a special focus was placed on in vivo DNA-based GT (more specifically, AAV vector and Ad vector), which we consider the most relevant and advanced strategy to achieve clinical use.

## Osteoarthritis

OA is a syndrome that affects various joints, including the knee, hip, shoulder, hand, and intervertebral disc.<sup>2</sup> OA is driven by the activation of a wide range of underlying pathways, resulting in joint destruction through mechanical, proinflammatory, and metabolic factors.<sup>3</sup> The disease is characterized by abnormal remodeling of the whole joint (Fig. 1).<sup>4,5</sup>

Although long underestimated in OA pathogenesis, tendons, menisci, and ligaments are also affected and appear essential to the stability of the joint.<sup>6</sup> Moreover, the synovium and the infrapatellar fat pad have emerged as a potential unit, physically and functionally linked, influencing synovitis and sustaining chronic low-grade inflammation.<sup>7</sup> The subchondral bone also plays a key role in the initiation and progression of OA with an uncoupled remodeling process and osteophyte formation at the joint margins that may participate in stabilizing the joint.<sup>8–11</sup> Articular cartilage, the tissue that initially received all the attention, exhibits a poor ability for spontaneous healing. In OA, articular cartilage undergoes striking changes in the extracellular matrix composition and structure, leading to damages ranging from surface fibrillation to progressive cartilage fragment delamination and exposure of calcified cartilage and underlying bone.<sup>12</sup>

With the importance of all these tissue players, the whole joint needs to be considered when looking for a DMOAD.

From a clinical perspective, pain is the primary OA symptom and the leading cause of health care use (Fig. 1). The presence and severity of pain are associated with specific OA features such as bone marrow lesions and synovitis.<sup>12</sup> Other symptoms that may aid in clinical diagnosis include transient morning stiffness, crepitus on joint motion, joint instability (buckling or giving way), swelling, muscle weakness, bony enlargement, and fatigue. However, in the early stages of the disease, symptoms are often intermittent and barely detectable but become more frequent and severe as the disease progresses.

General guidelines for managing OA focus on treating the core symptoms of pain and joint dysfunction from nonpharmacological methods to the ultimate step of joint replacement (Fig. 1).<sup>13</sup>

Efficient drugs that modify the underlying pathophysiological mechanisms and potentially prevent OA progression are currently lacking.<sup>14</sup> The completed clinical trials, despite the absence of patients' pain reduction, have highlighted the importance of better-stratifying patients to assess drug efficacy and the need for long-term follow-up.<sup>15–18</sup> Identifying DMOADs is an active area of

research, with no fewer than 2432 clinical studies related to OA currently underway (clinicaltrials.gov, November 3, 2023, Condition/disease: Osteoarthritis and Other terms: Gene therapy). Future therapies could enable long-term combined actions on various mechanisms and different target tissues. GT may make this possible, but further developments are still necessary. On the one hand, identifying the correct factor to vectorize is crucial, and on the other hand, making gene transfer technologies as efficient and safe as possible is essential. This review primarily focuses on GT vectors to highlight current advances and future challenges that will make them an essential tool for developing the next DMOADs.

## GT of OA

### GT: Presentation

The GT concept appeared in the early 1970s with the advent of molecular biology.<sup>19</sup> Initially, GT was designed to restore a gene responsible for a monogenic disease. However, naked DNA has a half-life of only 10 minutes after intravenous injection in mice and can enter cells with difficulty due to its negative charge.<sup>20</sup> The intrinsic properties of nucleic acids appear to be an issue for the GT concepts development, highlighting the need for a suitable delivery vehicle, that is, a vector, to efficiently transport and protect the DNA until it reaches and enters the cells of interest *in vivo*.

The concept of GT has now no longer been restricted to DNA delivery but extended to the delivery of all nucleic acids (micro RNA, short hairpin RNA, etc).<sup>21</sup> These tremendous possibilities have enlarged the spectrum of GT applications from monogenic diseases to more complex diseases such as neurological diseases, cardiovascular diseases, and cancer, leading to a 2-fold increase in GTs clinical trials since the 2000s. As of the first quarter of 2023, 28 GT DNA-based products have been approved by regulatory agencies (Table 1). Despite these increasing successes, GTs development still faces several issues, such as a limited specific targeting of cells of interest, potential limitations at the entry and processing steps in these cells leading to reduced efficiency of vectorized nucleic acids, and the immunogenicity of the vector used and/or the product of the transferred nucleic acids (transgene).<sup>22,23</sup> Two types of vectors can be used for GT, synthetic and viral ones. Nonviral or synthetic vectors are engineered from scratch, whereas viral vectors are based on hijacking the viral machinery.

Synthetic vectors consist of chemical-based particles able to encapsulate, protect, and deliver nucleic acids. All synthetic vectors share several advantages, they are preserved from pre-existing immunity due to their non-natural origin, they can package nucleic acids of almost unlimited size, and from an industrial point of view, easier large-scale manufacturing compared to viral vectors.<sup>24</sup> Despite these advantages, of the 29 DNA-based GTs approved to date (excluding RNA-based products), only 2, Neovasculgen (Russia, Ukraine) and Collategen (Japan) (Table 1), are nonviral vectors for the treatment of limb ischemia. These limited clinical successes are due to their insufficient stability to reach and deliver intact nucleic acids in their target cells. However, nonviral delivery technologies, boosted by their success in delivering mRNA vaccines (Moderna and Pfizer/BioNTech), are growing rapidly. In this way, nonviral delivery tools could be used in an OA context.

Viral vectors have an important advantage over synthetic vectors due to their natural ability to infect cells. Viruses, from which they are derived, are organized by families, each based on several characteristics, including nature (RNA or DNA, single or double-stranded), size of the genome, structure (presence or absence of capsid and/or envelope surrounding the viral genome), and shape. These wild-type characteristics can be modified to generate recombinant viruses, namely viral vectors. Although the wild-type capsid and/or envelope is not modified at first glance, the expression cassette (promoter, transgene, and poly-A sequences) replaces all or part of the viral genome. Among viruses, lentiviruses, Ads, and AAVs, are the most frequently used to deliver nucleic acids. Due to their specific characteristics (summarized in Table 2), each viral vector type can be tailored to specific needs. For example, Ad-derived vectors are highly immunogenic, which makes them of preferential use for vaccination strategies, as recently illustrated for severe acute respiratory syndrome coronavirus 2 (AstraZeneca, Vaxzevria), while retroviruses and lentiviruses are the vectors of choice in clinical trials for *ex vivo* GT, and AAVs are well established in clinical trials for *in vivo* GT (Table 1).

Viral and nonviral vectors are both the subject of promising studies in the field of OA, which are presented below.

### Nonviral vectors-based GT in OA

Because nonviral vectors have been thoroughly reviewed elsewhere,<sup>25,26</sup> we only discuss the promising XT-150 GT product here, currently undergoing evaluation in a phase II clinical trial in knee OA patients (NCT04124042) (Table 3). XT-150 is a plasmid DNA encoding a variant of the human anti-inflammatory cytokine interleukin-10 (pDNA-hIL-10v). Surprisingly, while naked DNA is the least effective in infecting target cells, using d-mannose as an adjuvant allowed increased uptake of the naked plasmid by phagocytic cells such as M2-type macrophages. This strategy thus drove long-lasting relief of chronic neuropathic pain.<sup>27</sup> While attractive, this strategy is specific to M2-type macrophage targeting. More efficient organic and inorganic vectors are still needed to carry other transgenes.<sup>26</sup>

### Retrovirus-based GT in OA

Among the retroviral RNA viruses subfamily, named as such for their unique ability to express retrotranscriptase, lentiviruses are widely used as vectors. Lentiviral vectors allow long-term transgene expression due to their ability to integrate the host genome (Table 2). However, these integrations can also lead to random mutagenesis, which, if occurring in a proto-oncogene or a tumor suppressor, can lead to tumor development. New generations of increasingly safer lentivirus vectors have been developed, leading to

**Table 1**  
Summary of authorized gene therapies on the market as of the first quarter of 2023.

	Trade name (Generic name)	Disease targeted	Year of market approval	Locations approved
<i>Nonviral vectors</i>				
In vivo GT	Neovasculgen (Cambiogenepiasmid) Collatogene (beperminogene perplasmid)	Peripheral vascular disease, limb ischemia Limb ischemia	2011 2019	Russia, Ukraine Japan
<i>Retroviruses</i>				
Ex vivo GT	Zalmoxis (onasemnogene abeparvovec-xioi) Strimvelis (autologous CD34+ enriched cells)	Adjunctive treatment in haploidentical hematopoietic stem cell transplantation with high-risk hematological malignancies Severe combined immunodeficiency due to adenosine deaminase deficiency	2016-2019 (Withdrawal after unfavorable post-approval results) 2016	EU EU, UK
	Inovassa (K/TissueGene-C Tonogenchoncet-L)	Knee osteoarthritis	2017-2019 (Withdrawal after false data discovered)	Korea
	Kymriah (tisagenlecleucel-t)	Acute lymphocytic leukemia, diffuse large B-cell lymphoma, follicular lymphoma	2017	US, EU, Japan, Australia, Canada, South Korea, Switzerland
	Yescarta (axicabtagene ciloleucel)	Diffuse large B-cell lymphoma, non-Hodgkin lymphoma, follicular lymphoma	2017	US, EU, UK, Japan, Canada, China
	Zynteglo (betibeglogene autotemcel) Tecartus (brexucabtagene autoleucel) Limbely (atidarsagene autotemcel) Relma-cel (relmacabtagene autoleucel) Abecma (idecabtagene vicleucel) Breyanzi (lisocabtagene maraleucel) Skysona (elivaldogene autotemcel) Carvykti (ciltacabtagene autoleucel) Lyfgenia (lovotibeglogene autotemcel) Rexin-G (mutant cyclin-G1 gene)	Transfusion-dependent beta thalassemia Mantle cell lymphoma, acute lymphoblastic leukemia Metachromatic leukodystrophy Diffuse large B-cell lymphoma, follicular lymphoma Multiple myeloma Diffuse large B-cell lymphoma, follicular lymphoma Early cerebral adreno-leukodystrophy Multiple myeloma Sickle cell disease Solid tumors	2019 2020 2020 2021 2021 2021 2021 2022 2023 2006	US, EU US, EU, UK EU, UK China US, Canada, EU, UK, Japan US, Japan, EU, Switzerland, UK, Canada US US, EU, UK, Japan US Philippines
In vivo GT	Imlygic (Talinogene laherparepvec) Delytact (Teseptaturev)	Melanoma Malignant glioma	2015 2021	US, EU, UK, Australia Japan
<i>Adenovirus</i>				
In vivo GT	Gendicine (recombinant p53 gene) Oncorine (E1B/E3 deficient adenovirus) Adstiladrin (nadofaragene firadenovec)	Head & neck squamous cell carcinoma Head and neck carcinoma, nasopharyngeal carcinoma Bladder cancer	2004 2005 2022	China China US
<i>AAV</i>				
In vivo GT	Glybera (alipogenic tiparvovec) Luxturna (voretigene neparvovec) Zolgensma (onasemnogene abeparvovec)	Familial lipoprotein lipase deficiency Leber's congenital amaurosis Spinal muscular atrophy (type 1) in children (< 2 years old)	2012-2017 (Withdrawal after insufficient cost-benefit balance) 2017 2019	EU US, EU, UK, Australia, Canada, South Korea Korea US, EU, UK, Japan, Australia, Canada, Brazil, Israel, Taiwan, South Korea EU, UK EU, UK US, EU, UK US
	Upstaza (eladocagene exuparvovec) Roctavian (valoctogene roxaparvovec) Hemgenix (etranacogene dezaparvovec) Elevidys (delandistrogene moxeparvovec-rokl)	Aromatic L-amino acid decarboxylase deficiency Hemophilia A Hemophilia B Duchenne myopathy	2022 2022 2022 2023	US, EU, UK

Abbreviations: AAV, adeno-associated virus; GT, gene therapy; HSV-1, Herpes simplex virus 1. Only DNA (and not RNA-approved)-gene therapies are listed.

**Table 2**  
Features of viral vectors.

	Lentivirus	Adenovirus	AAV
Virus capsid	Enveloped capsid	Nonenveloped icosahedral capsid	Nonenveloped icosahedral capsid
Virus diameter	80-120 nm	60-90 nm	20-25 nm
Viral genome	ssRNA	dsDNA	ssDNA
Viral genome size	8 kb	36 kb	4.7 kb
Immunogenicity	Low	High	Very low
Target cells	Dividing or not	Dividing or not	Dividing or not
Transduction efficiency	Moderate	High	Moderate
Transgene process	Integrative	Nonintegrative (episomal)	Nonintegrative (episomal)
Advantage	Long transgene expression	Low to no risk of mutagenesis	No human disease associated with AAV
Disadvantage	Risk of mutagenesis	High immunogenicity	Low packaging capacity

Abbreviations: AAV, adeno-associated virus; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; ssRNA, single-stranded RNA.

their use in several authorized treatments (Table 1).<sup>28</sup> In addition, all approved treatments using retroviruses are ex vivo GT (or cell-mediated GT), that is, in vitro transduction of allogeneic cells of interest before reimplantation in patients, thus limiting the risk of off-target mutagenic integration.

Interestingly, among the approved retrovirus-based GTs, Invossa-K/TissueGene-C, which was the first GT approved for degenerative joint disease, used a Moloney-murine leukemia virus-based retrovirus. It was withdrawn from the market in 2019 by the Korean Ministry of Food and Drug Safety due to a lack of information regarding the product composition. Despite this point to be clarified, a phase III clinical trial evaluating TissueGene-C in patients with OA (Kellgren-Lawrence grade 2-3 and medial joint space narrowing grade 1-2) is ongoing (NCT03203330) and encouraging long-term results in a rat model of OA pain have been recently released.<sup>29</sup>

Finally, retrovirus-derived vectors are considered an interesting strategy when restricted to cell-mediated ex vivo GT and could thus be applied for the repair of osteochondral defects.

#### Ad-based GT in OA

Ads are composed of a linear double-stranded DNA genome packed into a nonenveloped icosahedral capsid of 60 to 90 nm in diameter (Table 2). Different serotypes of Ad, characterized by their capsid structure, exist. The Ad serotype 5, the most widely used serotype for gene transfer, has a 36 kb wild-type genome.

Gendicine and Oncorine were the two first Ad-based GT products authorized in the market in 2004 and 2005, respectively (Table 1). They are indicated in cancer patients and for vaccination strategies.<sup>30,31</sup> In the OA GT context, the immune response mediated by an Ad-based vector remains the most challenging issue<sup>32</sup> responsible for rapid clearance of infected cells, short-term expression of the transgene, as well as possible immune-driven cytotoxicity.<sup>33</sup> This has been addressed with the development of 3 different Ad vector generations, depending on the progressive viral gene deletion. The third generation, called helper-dependent adenoviruses (HD-Ads), is depleted of all its viral genome except a small part needed for genome replication and packaging.<sup>34</sup> In addition to being the less immunogenic Ad vectors, it allows the transport of 36 kb transgenes, opening GT to long sequences that may contain several genes.<sup>35,36</sup> For example, Stone et al,<sup>37</sup> used HD-Ad coding for Prg4 or interleukin-1 receptor antagonist (IL-1Ra) alone or in combination, and showed a greater efficiency in mice OA models of the combinatorial treatment compared to the single gene transfer approach (Table 4). Moreover, HD-Ads have permitted long-term transgene expression (> 1 year) in several animal models.<sup>36,38</sup> However, HD-Ad production requires a helper virus expressing adenoviral genes, which complicates the purification of HD-Ad and has hindered its use in clinics.<sup>39</sup> A variety of efficient production strategies are currently being developed, leading to HD-Ad evaluation in various clinical trials.<sup>40</sup> Among HD-Ad in clinical trials, Flexion Therapeutics Inc has developed an HD-Ad-based GT for patients with knee OA, FX201 (Table 3). FX201 coding for IL-1Ra is currently in phase I clinical trial (NCT04119687). Previous preclinical evaluations of HD-Ad-IL-1Ra in mice, rats, and equine models of OA exhibited no adverse events, good tolerability, improved joint function, and protection from severe OA-induced pain (Table 4).<sup>41,42</sup> If the phase I clinical trial of FX201 results in good tolerability and safety, it would open a promising avenue for developing synergistic HD-Ad-based GT strategies that will involve the packaging of 2 or more genes encoding therapeutic molecules.

However, 1 downside still exists. Despite HD-Ad being the least immunogenic of Ad-based vectors, humans naturally develop antibodies that neutralize these viral vectors. Thus, most humans have pre-existing antibodies directed against Ad, including Ad serotype 5, which can limit or even abolish gene transfer. In the final section of this review, we will discuss some of the most promising strategies for altering neutralization and improving transduction specificity.

#### AAVs-based GT in OA

AAVs consist of a 20 to 25 nm diameter nonenveloped icosahedral capsid, which contains a single-stranded DNA genome of ~4.7 kb (Table 2) composed of 3 open-reading frames, *rep*, *cap*, and *AAP*, respectively required for replication, capsid protein production, and capsid assembly. These 3 open-reading frames are flanked by inverted terminal repeats, which are essential for replication and genome packaging into the capsid. rAAV only contains the capsid and the inverted terminal repeat sequences inside

**Table 3**  
Summary of clinical trials related to gene therapy for OA.

Drug name	GT vector	Transgene	Clinical trial phase	Route of administration	Enrolled patients	Primary outcomes evaluated	ClinicalTrials.gov identifier
XT-150 TissueGene-C	Naked plasmid Lentivirus	CAG- <i>hIL-10v</i> <i>TGF-β1</i>	II III	Knee injection Knee injection	Painful knee OA (KL 2-3, WOMAC ≥8) Knee OA (KL 2-3, Osteoarthritis Research Society International Medial JSN 1-2)	KOOS and WOMAC WOMAC, knee pain assessed by VAS	NCT04124042 NCT03203330
ART-102	AAV2/5	NF-κB prom- <i>IFNβ</i>	I/II	Hand joints injection	Inflammatory hand arthritis (RA or OA diagnosis)	Adverse events	NCT02727764
ICM-203	AAV2.5	<i>Nkx-3.2</i> (also called <i>Bqpx1</i> )	I/IIa	Knee injection	Knee OA (KL 2-3)	Adverse events, knee pain and function, articular cartilage grade, JSW	NCT05454566
sc-rAAV2.5IL-1Ra	AAV2.5	<i>IL-1Ra</i>	I	Knee injection	Knee OA (moderate)	Adverse events	NCT02790723
GNSC-001	AAV	<i>IL-1Ra</i>	Ib	Knee injection	Knee OA (KL 2-3, WOMAC > 20)	Adverse events	NCT05835895
FX201	HD-Adenovirus	<i>IL-1Ra</i>	I	Knee injection	Knee OA (KL 2-4)	Adverse events	NCT04119687

Abbreviations: AAV, adeno-associated virus; CAG, cytomegalovirus early enhancer/chicken β-actin; GT, gene therapy; HD, helper-dependent; IL-1Ra, interleukin-1 receptor antagonist; JSN, joint space narrowing; JSW, joint space width; KL, Kellgren-Lawrence; KOOS, Knee injury and osteoarthritis outcome Score; NF-κB, nuclear factor-kappa B; OA, osteoarthritis; RA, rheumatoid arthritis; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Arthritis Index.

**Table 4**  
Preclinical studies evaluating HD-Ad efficiency in OA animal models.

Animal (OA induction)	Transgene	Promoter	Injected particles	Age at the time of surgery	Injection time (relative to surgery)	End time point of outcome measurements	Outcomes
Mice (CLT)	<i>Prg4</i>	?	1E8 vp or 1E9 vp	8 wk	2 d before (preventive) or 2 wk after surgery (curative)	4 wk postsurgery (preventive) or 8 wk postsurgery (curative)	Preventive treatment: no dose-effect, decrease in OARSI score, curative treatment: dose-effect, decrease in OARSI score with the higher dose
Mice (CLT)	murine <i>IL-1Ra</i>	NF-κB endothelial leukocyte adhesion molecule	1E8 vp	8 or 12 wk	2 d after surgery	6 wk postsurgery	OARSI score improvement, cartilage volume improvement, no synovitis score change, protection against thermal hyperalgesia
Rats (ACLT)	rat <i>IL-1Ra</i>	NF-κB endothelial leukocyte adhesion molecule	3.6E7 gc or 3.1E8 gc	8-9 wk	7 d after surgery	12 wk postsurgery	Dose-dependent mitigation of OA-like changes of cartilage, bone, and synovial membrane
Rats (ACLT)	rat <i>IL-1Ra</i> or human <i>IL-1Ra</i> (FX201)	NF-κB endothelial leukocyte adhesion molecule	Rat: 3.2E8 gc or 3.1E9 gc or 4.3E10 gc/human: 4.1E10 gc	8-9 wk	28 d after surgery	36, 57, and 120 d postsurgery	Biodistribution: FX201 remains locally, low copy number detected in spleen, liver, lung, lymph nodes, and well tolerability throughout the study
Horses (osteocondral fragmentation)	equine <i>IL-1Ra</i>	NF-κB endothelial leukocyte adhesion molecule	2E11 vp (low dose) or 2E12 vp (high dose)	3-6 y	5 d after surgery	72 d	No local adverse events after injection, good tolerability, joint mobility improvement, better cartilage status
Mice (DMM and CLT)	<i>Prg4</i> and <i>IL-1Ra</i>	Elongation factor-1 (driving <i>Prg4</i> ) and inflammation-sensitive NF-κB promoter (driving <i>IL-1Ra</i> )	1E8 vp of each HD-Ad	8 wk	2 wk after surgery	2.5 or 3.5 mo after surgery	Combinatorial injection of HD-Ad-IL-1Ra and HD-Ad- <i>Prg4</i> provides higher benefit on pain and cartilage degradation

Abbreviations: ACLT, anterior cruciate ligament transection; CLT, cruciate ligament transection; DMM, destabilization of the medial meniscus; gc, genome copies; HD-Ad, helper-dependent adenovirus; NF-κB, nuclear factor-kappa B; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International; Vp, viral particles.



**Table 5**  
AAV serotypes, their preferential tropism, and their described receptor to be processed.

AAV serotypes	Preferentially targeted cells	Known receptor interactions	References
AAV 1	Skeletal muscle, central nervous system (CNS)	$\alpha$ -2,3- and $\alpha$ -2,6-N-linked sialic acids	Wu et al <sup>43</sup> and Zhang et al <sup>44</sup>
AAV 2	Kidney	Heparan sulfate proteoglycan, hepatocyte growth factor, fibroblast growth factor receptor 1, integrin $\alpha$ V $\beta$ 5/ $\alpha$ 5 $\beta$ 1	Summerford and Samulski <sup>45</sup>
AAV 3	Liver	Heparan sulfate proteoglycan, hepatocyte growth factor receptor	Ling et al <sup>46</sup>
AAV 4	CNS	$\alpha$ -2,3-O-linked sialic acid	Kaludov et al <sup>47</sup>
AAV 5	CNS	$\alpha$ -2,3-N-linked sialic acid	Kaludov et al <sup>47</sup>
AAV 6	Skeletal muscle	$\alpha$ -2,3- and $\alpha$ -2,6-N-linked sialic acids	Wu et al <sup>43</sup>
AAV 7	Skeletal muscle	-	
AAV 8	Liver, skeletal muscle, pancreas, heart	-	
AAV 9	Liver, skeletal muscle, lung	Galactose	Shen et al <sup>48</sup>

Abbreviation: AAV, adeno-associated virus.

which the expression cassette is placed. The different AAV serotypes, defined by the *Cap* sequence, differ in their cellular tropism and the prevalence of pre-existing immunity to each of them in the human population (Table 5). Thus, choosing the appropriate rAAV serotype is essential for OA GT. Nevertheless, rAAVs are a popular gene transfer tool for GT developments in OA, leading to 4 ongoing or recently completed clinical trials (Table 3).

The first clinical trial concerns ART-I02, encoding interferon-beta under the control of an element of the nuclear factor-kappa B promoter in a rAAV5 vector. The rAAV5 was chosen for its preferential tropism for synovial cells compared to other serotypes.<sup>49</sup> Although preclinical studies in rats and rhesus monkeys have shown that intra-articular injection of ART-I02 was well tolerated and exhibited no adverse events, the phase I/II clinical trial was stopped due to insufficient safety and tolerability.<sup>49–51</sup>

The second clinical trial involves ICM-203, an Nkx-3.2 coding carried by a rAAV2.5 vector. Serotype 2.5 is a hybrid serotype designed to address various GT issues encountered with the native AAV2 capsid, such as pre-existing neutralizing antibodies limiting transgene expression and a suboptimal articular tropism.<sup>52</sup> In this context, AAV2.5 was used to enhance cartilage targeting for expressing Nkx-3.2, which was shown to slow the development of OA in murine preclinical models.<sup>53,54</sup>

Finally, sc-rAAV2.5IL-1Ra (NCT02790723) and GNSC-001 (NCT05835895) aim to deliver *IL-1Ra*. Interestingly, sc-rAAV2.5IL-1Ra is based on a self-complementary rAAV (scAAV), which differs from the classical single-stranded rAAV in its already double-stranded genome, a strategic option discussed later in this review. These 4 clinical trials pave the way for new developments in GT therapies for OA.

### What improvements can be made to viral vector-based GTs of OA?

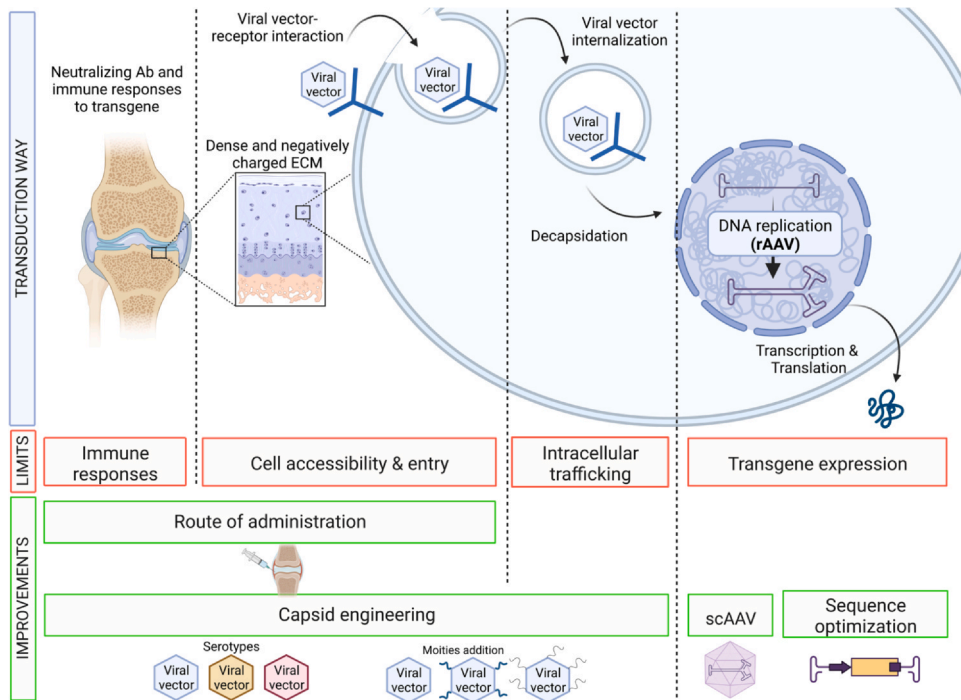
Despite promising GT product developments, viral vectors, among which the most promising in OA are Ad- and rAAV-based vectors, still suffer from 2 main limitations: the host immune response and the efficacy and specificity of transgene expression in the joint tissue (Fig. 2). To address these issues, modification of the viral vectors and optimization of the carried nucleic acid sequence have been contemplated with growing interest (Fig. 2).

#### Capsid modifications

Capsid, which defines serotypes, is responsible for the natural ability of viruses to preferentially target one cell type over another (Table 5). Indeed, capsid proteins are the first element in contact with the targeted tissues/cells and the key to efficient intracellular trafficking. Unfortunately, surface capsid proteins of Ads or AAVs can also be recognized by neutralizing antibodies, present not only in the circulation but also in the synovial fluid.<sup>55–57</sup> Thus, to design a GT for use in the largest number of patients, it will be interesting to develop vectors for which there are no pre-existing neutralizing antibodies. In this context, capsids naturally appear as the targets of choice to improve viral vector-based GT. In addition to the capsid modification strategy extensively described hereafter, a very promising strategy is to encapsulate viral vectors in biocompatible materials that can be finely tuned.<sup>58–61</sup> Such biomaterial-mediated GT takes advantage of the considerable knowledge of biocompatible materials already widely used for cartilage regeneration. This combinatorial strategy should improve transduction efficiency, increase the stability of transgene expression, and allow escape from neutralizing antibodies (for a review see Balakrishnan and David<sup>62</sup>). Moreover, in a regenerative context, a combination of vectors with scaffolds or gene-activated matrices, could be of interest to specifically modified cells within the defect.<sup>63</sup>

#### AAV capsids

Considering rAAV, some variable regions encoding surface capsid proteins are responsible for specific interaction with targeted cells through membrane receptors (Table 5). These capsid proteins, required for rAAV internalization and intracellular processing, are essential for subsequent transgene expression.<sup>64,65</sup> These variable regions are the targets of rAAV capsid modifications in which directed evolution and rational design are the primary technological strategies used.<sup>66,67</sup> Briefly, directed evolution allows the creation of new proteins with improved functions through repeated cycles of mutation-selection from a functional capsid protein.

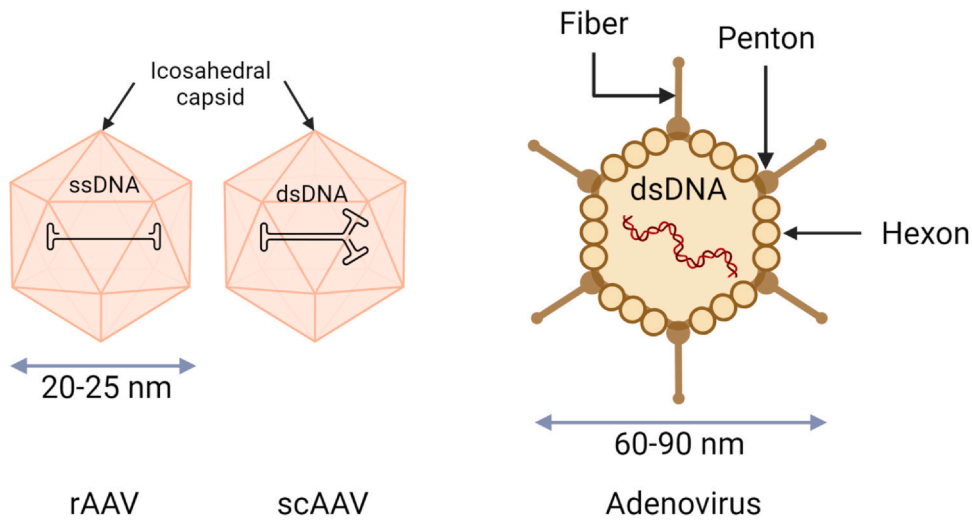


**Fig. 2.** Limitations of intra-articular gene transfer and improvements to enhance viral vector transduction. There are multiple impediments to efficient expression of transgenes in target cells. The viral vector harboring the transgene must evade neutralizing antibodies, find the cells even when they are embedded in a dense extracellular matrix, penetrate them until it decapsids, and finally releases the transgene. To finish, the cell must process the transgene from DNA replication (for the particular example of rAAV) to translation. All the steps in this pathway can limit viral vector transduction. To improve transduction, intra-articular injection of the vector with the appropriate capsid and transgene should be considered. Ab, antibody; ECM, extracellular matrix; rAAV, recombinant adenoviral and adeno-associated viral; sCAAV, self-complementary rAAV. Created with BioRender.com.

Using this technology, several improved pseudoserotypes or hybrid serotypes have emerged such as rAAV-DJ, which has already been successfully used in a preclinical rat model of OA.<sup>68</sup> The rAAV-DJ mainly comprises some rAAV2, rAAV8, and rAAV9 capsid sequences,<sup>64</sup> and was selected for its improved transduction efficiency in different cell types *in vitro*.<sup>69</sup> Grimm et al showed that this improvement may be due to enhanced intracellular trafficking, which could explain its high transduction efficiency in many cell types. Another hybrid serotype of interest in OA is rAAV2.5, used in ICM-203 formulation. rAAV2.5 is a chimera of rAAV1 and rAAV2 in which 5 rAAV1 amino acids substitute 5 others from rAAV2 to exhibit rAAV2 affinity for heparan sulfate and evade the pre-existing immune response against rAAV2.

The variable regions of the rAAV capsid can also be the target of genetic modifications.<sup>70</sup> As proposed by Müller et al,<sup>71</sup> inserting a suitable peptide sequence selected from a random peptide library in an AAV sequence can improve cell-type specific targeting. Although, to our knowledge, this design approach has not yet been evaluated in OA, Eichhoff et al<sup>72</sup> designed a rAAV preferentially targeting cells overexpressing the P2X<sub>7</sub> purinergic receptor, as described in OA chondrocytes compared to healthy chondrocytes.<sup>73</sup> This strategy could also be applied to several receptors overexpressed by OA joint cells, such as the cannabinoid receptors<sup>74</sup> or receptor tyrosine kinase like orphan receptor 2 in chondrocytes.<sup>75</sup> To target OA joint cells, other genetic modification strategies of interest include adding stimulus-dependent motifs that can activate or deactivate the ability of rAAV to interact with the target cell. For example, given the significant secretion of matrix metalloproteases during OA, adding matrix metalloprotease cleavable moieties as tetra-aspartic peptides to the rAAV capsid could allow better control of the infection timing and specificity of target cells.<sup>76</sup>

Finally, rAAV capsid can also be chemically modified to avoid tricky AAV genome manipulations. Due to their high chemical reactivity, lysines and tyrosines that are highly exposed at the surface of the capsid (> 300 moieties) are a relevant target for the chemical engineering of rAAV capsids.<sup>77</sup> This allows a wide range of chemical compounds to be coupled to the capsids, from the chemotherapy drug paclitaxel to ligands such as N-acetylgalactosamine or mannose.<sup>77-79</sup> In addition to more specific targeting, this strategy can also prevent rAAV recognition by neutralizing antibodies. Similarly, as previously suggested with genetic modifications, chemically added peptides may target proteins overexpressed in OA joint cells. Peptides, such as chondrocyte-affinity peptide discovered by phage display,<sup>80</sup> could enhance chondrocytes targeting by rAAVs.<sup>81</sup>



**Fig. 3.** Schematic representation of the current most promising viral vectors for OA GT. Capsid and genome representations of rAAVs and Ads highlighting differences between the single-stranded DNA (ssDNA) genome of rAAV and the double-stranded DNA (dsDNA) genome of self-complementary rAAV (sCAAV). Ads, adenoviruses; GT, gene therapy; OA, osteoarthritis; rAAV, recombinant adenoviral and adeno-associated viral. Created with [BioRender.com](https://www.biorender.com).

#### Ad capsids

Ad capsid is composed of a total of 252 proteins divided into 3 protein types: hexons, pentons, and fibers (Fig. 3).<sup>82</sup> Like rAAV capsid variable regions, both pentons and fibers contribute to Ad interaction with cellular receptors and Ad internalization for further transgene processing, whereas the outer surface of hexons is the primary target of neutralizing antibodies. For more than 20 years, PEGylation strategies (addition of polyethyleneglycol [PEG] moieties) of Ad capsid have been developed. Different PEG sizes and covalent attachment strategies have been tested in various contexts, allowing limitation of off-target effects as well as escape from neutralizing antibodies.<sup>83–87</sup> Other polymers such as poly-*N*-(2-hydroxypropyl) methacrylamide (HPMA) have also been widely used to overcome the undesirable properties of Ad.<sup>88</sup> To our knowledge, these Ad modifications have not been evaluated in the context of OA or joint diseases, but it would be interesting, to effectively escape from neutralizing antibodies present in the synovial fluid and compare the transduction efficiency of modified or unmodified HD-Ad after intra-articular injections. Besides chemically adding fragments, genetic manipulation of capsid sequences is also currently used. The most promising genetic manipulation is to generate chimeric Ads by replacing the fiber domains with their homologs from other serotypes.<sup>89</sup> As for rAAV pseudotyping, this chimeric Ad has acquired a new tropism and can escape neutralizing antibodies directed against the parental Ad serotype. The peptide can also be genetically included in the fiber end to enhance gene transfer into the targeted cells.<sup>90</sup> These strategies have not yet been evaluated in OA joint cells.

#### Viral vector genome optimization

To improve the final steps of the transduction process for producing a therapeutic protein, transgene engineering should be considered (Fig. 2). Depending on the desired effect, inducible or ubiquitous promoters can be used. As illustrated in OA, an inflammation-sensitive promoter like the nuclear factor-kappa B promoter used in the ART-I02 clinical trial could be considered when the transgene of interest is an anti-inflammatory molecule. The design of complementary DNA sequences for optimal transduction and translation should be considered, along with the addition of regulatory sequences such as the Kozak sequence. The termination site can also be tailored to the viral packaging capacity. Finally, preliminary studies are crucial when selecting a ubiquitous promoter, as transgene expression can vary between cell types, even among different ubiquitous promoters. For example, IL-1Ra with a chicken  $\beta$ -actin hybrid promoter compared to cytomegalovirus promoter can induce similar IL-1Ra concentrations in chondrocytes, but approximately 10,000 pg/mL for chicken  $\beta$ -actin hybrid against 24,000 pg/mL for cytomegalovirus in synoviocytes.<sup>91</sup>

Among the final steps of the transduction process, rAAVs have a disadvantage compared to Ads. rAAVs have a single-stranded genome that requires replication before transgene expression can occur (Fig. 2). The use of sCAAV, which is composed of a double-stranded DNA genome, allows this step to be bypassed (Fig. 3).<sup>92</sup> ScAAVs have been widely used to transduce chondrocytes, which have a very low proliferation rate and may not have very active DNA replication machinery.<sup>93,94</sup> However, sCAAVs have a limited capacity to package nucleic acid sequences, with a maximum size of 2.2 kb, thus limiting their use to short complementary DNA sequences, small interfering RNA, or micro RNA. To deliver larger genes, dual AAV vectors can be considered, employing strategies such as homologous recombination or trans-splicing, as recently done in the retina.<sup>95</sup> Thus, in addition to the viral vector used, particular attention must also be paid to the sequence to be transferred.

## Conclusion

This review outlines the many possibilities that exist around GT for OA. Among viral vectors, Ad and rAAV appear to be the most promising. However, as illustrated by the growing number of clinical trials aimed at developing therapeutics based on rAAV vectors rather than Ad, rAAV has more recently emerged as the vector of choice in a setting where inflammation may be deleterious and long-term expression of the transgene is required after a single injection. Nevertheless, the significant progress made with Ad-based vectors is leading the scientific community to reconsider their use, particularly in cases where the transgene to be delivered is long and cannot be carried by an rAAV-based vector. To further improve Ad-based vectors for OA GT in the future, it will be very interesting to apply the knowledge gained from rAAV modifications for OA to Ads. However, improvements in both Ad and rAAV vectors are still needed, as illustrated by the recent disappointing tolerability results of ART-I02.<sup>50</sup>

Finally, progress is also needed in the production and manufacture of rAAVs to make them more accessible at a controlled price to the many patients suffering from OA.<sup>96</sup> As well-reviewed by Escandell et al,<sup>97</sup> several challenges need to be addressed to meet the growing demand for clinical-grade rAAVs.

In conclusion, the range of possibilities for improvement of already promising GT tools in OA suggests great future clinical successes.

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## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships, which may be considered as potential competing interests: Claire Vinatier reports no competing interests or personal relationships that could have appeared to influence the work reported in this paper. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Ethics approval

The authors declare that informed patient consent was not provided for the following reason: It is a review article with no data coming from patients.

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