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Injections in the osteoarthritic knee: a review of current treatment options

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- Knee osteoarthritis is a degenerative condition characterized by progressive cartilage degradation, subchondral damage, and bone remodelling. Among the approaches implemented to achieve symptomatic and functional improvements, injection treatments have gained increasing attention due to the possibility of intra-articular delivery with reduced side effects compared to systemic therapies.
- In addition to well-established treatment options such as hyaluronic acid (HA), cortico-steroids (CS) and oxygen-ozone therapy, many other promising products have been employed in the last decades such as polydeoxyribonucleotide (PDRN) and biologic agents such as platelet-rich plasma (PRP) and mesenchymal stem cells (MSCs). Moreover, ultrasound-guided intra-meniscal injection and X-ray-guided subchondral injection techniques have been introduced into clinical practice.
- Even when not supported by high evidence consensus, intra-articular CS and HA injections have gained precise indications for symptomatic relief and clinical improvement in OA. Biological products are strongly supported by in vitro evidence but there is still a lack of robust clinical evidence. PRP and MSCs seem to relieve OA symptoms through a regulation of the joint homeostasis, even if their capability to restore articular cartilage is still to be proved in vivo.
- Due to increasing interest in the subchondral bone pathology, subchondral injections have been developed with promising results in delaying joint replacement. Nevertheless, due to their recent development and the heterogeneity of the injected products (biologic agents or calcium phosphate), this approach still lacks strong enough evidence to be fully endorsed.
- Combined biological treatments, nano-molecular approaches, monoclonal antibodies and 'personalized' target therapies are currently under development or under investigation with the aim of expanding our armamentarium against knee OA.

Keywords: injection; osteoarthritis; stem cells

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Introduction

Osteoarthritis (OA) is a degenerative disease with a tendency to worsen over time, characterized by articular cartilage degradation, subchondral damage, and bone remodelling, most commonly affecting weight-bearing joints such as the knee and hip. The aim of OA treatment is to control symptoms until the severity of the condition mandates surgical intervention; an early therapy may be a vital step for delaying the progression to end-stage disease. [1] Symptomatic control can be achieved through different therapeutic strategies such as lifestyle modifications, exercise therapy, pharmacological therapies and injections of various substances.[2] Pharmacological approaches based for example on non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol have been shown to be effective in controlling pain in OA,[3] but due to their systemic administration, they have also been associated with gastrointestinal, cardiovascular and renal adverse events, especially in patients already presenting with comorbidities.[4] Injections have therefore gained increasing attention thanks to their more direct effect on the target tissues and reduced side effects due to intra-articular delivery. Among the most frequently used products we include corticosteroids (CS), hyaluronic acid (HA), polynucleotides, oxygen-ozone therapy, platelet-rich plasma (PRP) and mesenchymal stem cells (MSCs). CS and HA are ranked first as the most frequently injected substances and their use is supported by a large amount of literature, although some controversial findings have emerged.[5] Conversely, there exists a more limited amount of evidence in support of the use of PRP and MSCs, due to

their more recent discovery and introduction as OA treatments. Autologous biologic products, according to their capability of modulating the joint environment by releasing a series of growth factors and immune-modulatory molecules, could play a beneficial role in reducing the local inflammation and promoting cartilage and synovial anabolism.[6] In addition, advances in research have not only brought about new products to be injected but also novel techniques of injection such as subchondroplasty and intra-meniscal application. The aim of the present narrative review is to summarize the different mechanisms of action of the various injectables for knee OA, presenting also their clinical results and potential areas of future development.

Off-the-shelf products

Corticosteroids (CS)

Intra-articular injection of CS is perhaps the most common conservative approach in the treatment of knee OA. The rationale behind its use relies on its immunosuppressive activity in the knee joint acting at different levels of the inflammatory cascade. In particular, it acts by blocking the synthesis of pro-inflammatory signalling molecules, such as interleukin 1 (IL-1), leukotrienes, prostaglandins and catabolic proteins such as metalloproteinases.[2] These combined actions may be accountable for the pain relief observed in patients treated with CS.

Indeed, the latest 2019 Osteoarthritis Research Society International (OARSI) guidelines have assigned to intra-articular CS injections a recommendation level of 1B ('high consensus'), the same level as for HA. In particular, their use is suggested for short-term pain relief compared to hyaluronic acid, which instead requires a longer time to provide its more beneficial effects in terms of pain control (2–4 weeks).[7]

Concerning the indications for CS injections, synovitis has been seen as a single predictor of treatment response: therefore, patients with an inflammatory phenotype of OA, characterized by stiffness, joint swelling and effusion, are more likely to respond to CS compared to HA.[8]

The latest Cochrane meta-analysis of 27 randomized controlled trials (RCTs) found CS to be more beneficial in pain reduction (lower visual analogue scale (VAS)) and function improvement (measured with the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score) than control interventions, but this difference was gradually lost after up to 13 weeks of follow-up, at which point no statistical difference was detected. Nevertheless, the reliability of these results is poor due to the low methodological quality of the included RCTs, hence the quality of evidence in support of CS injections for knee OA was graded as 'low'.[9] Most recent studies found CS injections to be superior to oral NSAIDs, even if the author suggests a possible bias represented by the intra-articular (IA) placebo effect.[10]

Despite its beneficial effects, CS knee injections are associated with some known side effects. The latest meta-analysis on the safety of this procedure reported rare and mild side effects such as temporary joint pain, erythema and itching, but no cases of joint infection.[11]

Hyaluronic acid (HA)

Hyaluronic acid (HA) is a glycosaminoglycan that provides joint lubrication and shock absorbency and acts as the backbone for the proteoglycans of the extracellular matrix. In normal adult knees, HA concentration ranges from 2.5 to 4.0 mg/ml, whereas in OA it decreases by 33–50%.[12] Besides their different origins and biological characteristics, viscosupplements currently available are classified based on the molecular weight (MW), method of preparation, and dosage. Currently, no clinical trials indicate a clear advantage of one product over another, even though a higher MW allows optimal binding on cell surfaces. In addition, there is no evidence to support that HA viscosupplementation affects OA progression. HA effects may last up to 26 weeks.[12] The benefits in terms of pain relief and function improvement associated with HA have been recently rediscussed.[13] Osteoarthritis Research Society International (OARSI) guidelines assigned a level of recommendation 1B/2 to the use of HA in the treatment of knee OA.

Intra-articular HA is particularly recommended for long-term treatment, as it is associated with symptom improvement beyond 12 weeks (i.e. more durable effects compared to CS), and has demonstrated a more favourable safety profile than repeated corticosteroids injections.[7] On the other hand, the American Academy of Orthopaedic Surgeons (AAOS), in light of inconclusive evidence, has neither endorsed nor discouraged HA use. However, the real benefits of HA in the early stages of joint degeneration need to be confirmed by specifically designed studies.

Polynucleotides

Polydeoxyribonucleotide (PDRN) is composed by polymers of various chain lengths capable of binding large amounts of water molecules, hence capable of reorganizing the cartilage surface.[14] Moreover, PDRN in vitro experiments have shown enhancement of chondrocyte survivorship if compared to HA exposure, with a reduced proteoglycans degradation.[15]

PDRN effects seem to be related to viscoelastic properties, cell grown induction, collagen and cell migration and anti-inflammatory capabilities. Moreover, in animal models, not only symptomatic improvement has been reported, but also a decrease of proinflammatory factors,

such as IL-6, tumor necrosis factor-alpha (TNF-α) and high mobility group box 1 (HMGB-1). Despite the need for further studies, PDRN has been shown to reduce cartilage oligomeric matrix protein (COMP) levels, which themselves have recently been investigated as a biomarker of arthritis.[16]

PDRN offers mechanical protection towards the damaged cartilage, replacing the synovial fluid and restoring an ideal microenvironment for matrix production. The PDRN preparation appears colourless, transparent, viscoelastic and it is provided in pre-filled glass sterile disposable syringes containing a solution of 2 ml (the concentration of polynucleotides is 20 mg/ml). PDRN intra-articular treatment has been shown to produce faster improvement of activities of daily living compared to HA.[17] According to a meta-analysis, intra-articular PDRN injections provided more significant pain reduction for up to two months after the procedure and equal functional improvements if compared to HA.[18] Therefore, the clinical advantages of PDRN, unlike HA, could be better exploited by extending the interval between the different injections, thus reducing the frequency of injections. However, there are still no large-scale RCTs determining the effect of PDRN injections on knee OA.

Oxygen-ozone therapy

The rationale behind the use of intra-articular ozone (O_3) therapy arose from the assumption that chronic oxidative stress plays an important role in OA. Ozone is a molecule discovered in the mid-19th century consisting of three atoms of oxygen in a dynamically unstable structure. Clinical experiences and research have considered O_3 as a powerful anti-inflammatory, immune-modulatory substance. Due to its high reactivity, it may be able to reduce oxidative stress, stimulate fibroblastic joint repair and may promote new cartilage growth. It can be safely administered intra-articularly as an O_3-O_2 gas mixture (Fig. 1). When dissolved into the synovial fluid, it can generate reactive oxygen species (ROS) and lipid oxidation products that may inhibit proteolytic enzymes. Indeed, O_3 therapy leads to a localized increase in oxygen delivery by promoting vasodilation and angiogenesis.[19]

Despite controversial results, O_3 therapy has shown better results in terms of pain relief, joint function and quality of life compared to placebo or corticosteroids injections. The kinematics of O_3 effects seem to be slow but lasting. In some trials HA treatment was clinically superior to O_3 therapy[20] and therefore oxygen-ozone therapy still lacks a large consensus. In terms of safety profile, O_3 proved to be a safe procedure with almost zero adverse events: O_3 is bacteriostatic, fungicidal, and viricidal, therefore the infection risk is minimal.[21]

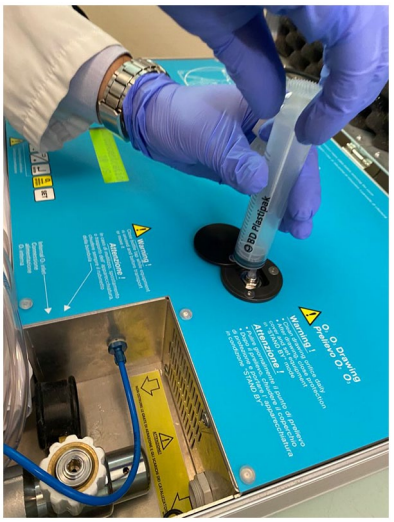

Fig. 1 Oxygen-ozone preparation before intra-articular injection.

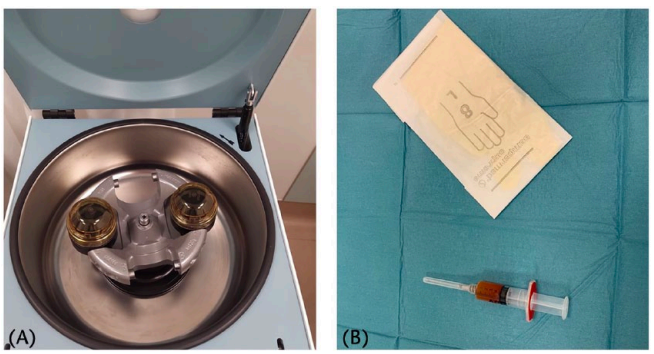

Fig. 2 PRP preparation process. (A) Centrifugation after blood sample collection; (B) final output consisting in this case of leukocyte-poor PRP.

Note. PRP, platelet-rich plasma.

Biologic products

Platelet-rich plasma (PRP)

PRP is an autologous blood derivate characterized by a higher platelet concentration than peripheral blood (Fig. 2). The rationale behind the employment of platelets relies on the ability of the latter to release biologically active proteins that are able to promote tissue healing; a property that becomes even more relevant when the target tissue has low healing potential as cartilage.[22] In particular, this beneficial effect of PRP on knee homeostasis is exerted by means of a number of growth factors released by platelets as insulin-like growth factor (IGF), tissue growth factor (TGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF), vascular endothelium growth factor (VEGF) and fibroblast growth

factor (FGF).[23] In particular, PRP effects can be ascribed to its effect on the Wnt/β-catenin pathway, which is implicated in OA development.[24] The Wnt family of proteins plays a central role in inflammation signalling cascades stimulating the release of catabolic molecules such as metalloproteinases, which are responsible for cartilage degradation and progressive degeneration of all the articular tissues. Moreover, the Wnt pathway is significantly involved in Type II collagen degradation and chondrocyte apoptosis.[25] The clinical application of PRP injections for knee OA has been investigated by an increasing number of clinical studies, including many RCTs, that have demonstrated its safety and overall clinical benefits. Looking at high-quality studies, the majority have shown that PRP is superior to hyaluronic acid (HA), especially in the case of low-grade articular degeneration,[26,27] whereas in severe OA less satisfactory outcomes have been documented, with results quite similar in comparison to viscosupplementation.[26,28] A potential explanation for these contradictory results might be the high variability of PRP products. Indeed, PRP formulations may be prepared by different methods resulting in different compositions, thus acting as a source of bias in the analysis of clinical outcomes. This limitation in the ability of clinical studies to investigate the real efficacy of PRP may be overcome by future studies employing a novel coding system as proposed by Kon et al.[29] An example of PRP's formulation variability is the amount of leukocytes present.[29] In fact, both leukocyte-rich PRP and leukocyte-poor PRP products are available. Nevertheless, comparison between these two formulations has been performed showing no inter-product differences; similarly, no clear evidence exists on the ideal number of injections and their timing to maximize the clinical results.[30]

Mesenchymal stem cells (MSCs)

MSC treatment consists of intra-articular injections of stem cells associated to a pool of immune-modulatory and anti-inflammatory stromal molecules. Many adult tissues are populated by MSCs (adipose tissue, muscles, dermis, periosteum, synovial membrane, synovial fluid, etc.),[31] but, in clinical practice, they are usually harvested from either bone marrow or adipose tissue. Even if many issues are still to be verified, MSCs, given their capacity to differentiate in mesenchymal derived tissue such as osteoblasts, chondrocytes and adipocytes, may have not only an anti-inflammatory, pro-angiogenetic and anti-apoptotic function, but also a reparative or regenerative role.[32,33] Their effects are consequences of direct cell–cell interaction and secretion of factors.[34] Similar to PRP, MSCs affect Wnt/β-catenin expression, thus controlling OA progression.[25,35] However, differentiation potential is dependent on several factors such as architectural extracellular and inter-cellular segmental characterization, environmental factors, growth factors, and adequate pool of MSCs.[36] In addition, having low expression of antigen-presenting molecules, MSCs are non-immunogenic;[37] moreover, cartilage tissue, due to lack of vascular and lymphatic system, is particularly immune-privileged. That is why the use of allogenic MSCs may be feasible, and preliminary phase I and II studies are demonstrating their long-term efficacy and safety.

In clinical practice, MSCs can be used as a cell suspension, after expansion in culture or enzymatic digestion, or they can be concentrated directly in the operating room (OR), which is currently the favoured approach due to the stringent regulations and higher costs affecting the procedures undergoing extensive manipulation in the lab. MSC products differ markedly in composition, and the most suitable strategy is far from clear, with biological and practical considerations currently guiding the development of treatment strategies. Expanded MSCs allow a more reproducible treatment but a two-step procedure is needed with an increase in costs, manipulation-related infection risks and invasiveness. Cell expansion transforms MSCs in advanced-therapy medicinal products (ATMPs), subjected to more rigorous regulatory requirements, similar to those of conventional drugs. This is why in both the USA and Europe 'minimally manipulated' MSCs, such as bone marrow aspirate concentrate (BMAC) and adipose-derived stromal vascular fraction (SVF) are the most exploited strategies for clinical application.

BMAC

BMAC is commonly obtained from the iliac crest using needle aspiration and concentration through centrifugation (one or multiple) occurring directly in the OR to obtain a product for immediate use (Fig. 3). Furthermore, recent technological developments have provided a concentration system without the need for centrifugation. With regard to clinical outcomes, even if BMAC has been shown to have a positive effect on function and pain, its regenerative effects and superiority compared to viscosupplementation and corticosteroids are still to be proved.[38,39]

SVF

SVF contains 300-fold more MSCs when compared to BMAC, and is composed of a heterogenous cell population: preadipocytes, vascular endothelial cells, smooth muscle cells and pericytes (ASCs), leucocytes, and erythrocytes.[40] Biologically, the maintenance of the stromal cell niche architecture seems to be a great advantage.[41] Indeed, this beneficial aspect is thought to arise from the cross talk between progenitors, resident cells and immune cells, possibly displaying a wound-healing phenotype (i.e. M2 alternatively activated macrophages). SVF has proved

Fig. 3 BMAC preparation in the OR. (A) Harvesting of bone marrow aspirate; (B) centrifugation; (C) final output rich in MSCs.
Note. BMAC, bone marrow aspirate concentrate; OR, operating room; MSCs, mesenchymal stem cells.

to be a safe treatment with positive clinical consequences and a radiological and histological improvement compared to BMAC.[42,43,44]

SVF products differ in terms of preparation methods: originally SVF was obtained by enzymatic digestion with collagenase and trypsin, but, following safety and regulatory concerns, minimally manipulative methods were developed,[45] exploiting mechanical forces such as centrifugation or microfragmentation in order to obtain a readily injectable product. Although we still lack clearly defined protocols to optimize the outcomes, such an approach may pave the way for a simpler application of adipose-derived MSCs in clinical practice by reducing operative times and costs.[46]

Potential contraindications

The aforementioned biologic products are reaching a wider number of clinics, who must be aware of some potential contraindications for their use. Although no absolute contraindication exists, there are some conditions warranting caution and that should be considered for correct patient counselling and management. In the case of PRP, low basal platelet count (< 150,000/mm^3), the presence of haematological diseases, coagulopathies (both congenital or acquired) or chronic anti-aggregant or steroidal therapy may impair or reduce the biologic efficacy of PRP.[47] PRP and MSCs should be also avoided in cases of recent diagnosis of malignancies, in particular hematologic ones: in fact, stem cells have theoretically an increased transformation and tumourigenic potential that suggests avoiding their use in oncological patients. More debated is the administration of biologic products in patients affected by concurrent rheumatic diseases: although in these cases a systemic therapy may be necessary to control the progression of the disease, intra-articular injections of PRP or MSCs may still contribute to reduce local symptoms and improve the joint functional status.

Combined therapies

Another strategy that has been attempted in the conservative treatment of knee OA is a combination therapy based on a single intra-articular administration of multiple substances. Different combinations have been investigated, among PRP, MSCs, HA and corticosteroids. A recent meta-analysis showed that combined IA injections of CS and HA were superior to HA alone in the reduction of pain both in the short and long-term outcomes.[48] Similar results were found in a study comparing PRP and HA against HA and PRP alone, where the combined therapy achieved better clinical outcomes.[49,50] Preliminary results on a PRP + MSCs combination showed significant reduction of symptoms up to 12 months after the procedure.[51] However, besides the preliminary encouraging results, combination therapy should be proposed with caution, especially when adopting biologic products, and further data should be collected before endorsing its routine application.

Subchondral injections

Subchondral injections refer to minimally invasive intra-osseous delivery under fluoroscopic guidance of products such as PRP, MSCs or as calcium phosphate (CaP) (Fig. 4); when the latter is employed the procedure takes the name of subchondroplasty.[52] The rationale behind this procedure stems from an increasing interest in the role of the subchondral bone in OA. The latter is defined as the bony component lying distal to the calcified cartilage and it is directly linked to it by means of channels, which transport a high number of tiny branches of vessels and nerves.[53]

Indeed, bone marrow lesions (BML), an alteration of the bone marrow visible on T2-weighted magnetic resonance imaging (MRI), is commonly identified in osteoarthritic knees.[54] In addition, subchondral sclerosis is generally recognized as a hallmark of OA.

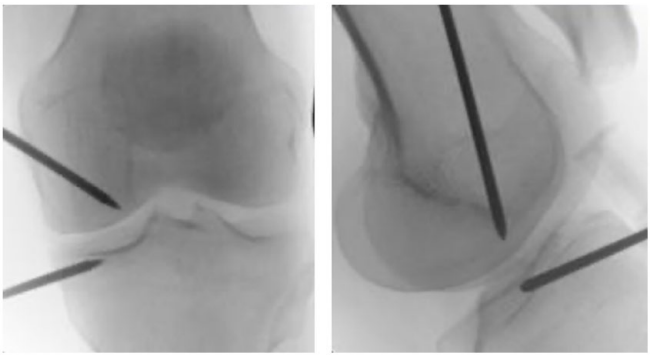

Fig. 4 Intra-osseous placement of the trocars, under fluoroscopic guidance. BMAC was injected into the bone marrow lesions of the medial femoral condyle and tibial plateau.
Note. BMAC, bone marrow aspirate concentrate.

There is an etiopathogenetic hypothesis suggesting that OA may start as subchondral bone pathology, leading to destruction of the overlying articular cartilage. Hence, subchondral injections exploit the possibility to fill the trabecular space within the BML with substances capable of ameliorating the bone integrity. Two recent systematic reviews on the topic[55,56] showed that, independently from the employed substance, the subchondral injection is a safe procedure able to relieve OA-related symptoms. In particular, subchondroplasty was able to reduce the rate of conversion to arthroplasty therefore delaying this procedure in patients with BML. This is particularly relevant if we consider that patients with BMLs are nine times more likely to progress to TKA compared to patients without BML within three years.[57]

Nevertheless, the quality of evidence in support of the efficacy of this procedure is poor. In addition, in spite of CaP being the most employed substance in subchondral injections, there is still no comparative analysis demonstrating its superiority over PRP and MSCs. Moreover, there is still no evidence regarding the quantity of substance that should be injected into the subchondral bone. One limitation of this technique may be represented by the fluoroscopic guidance for the intraosseous insertion of the cannula: in fact, this imaging technique, as opposed to MRI, is not able to perfectly localize the exact position of the bone marrow lesions. On the other hand, a potential strength of subchondroplasty is represented by the ability of this procedure to address osteoarthritis pathophysiology at its core: the affected area of the subchondral bone.

Intra-meniscal injections

Meniscal degenerative tear and/or meniscal degeneration are often an early sign of knee OA and could elicit significant pain even in the absence of relevant chondral damage.[58] Arthroscopic meniscectomy has shown conflicting results related to both the augmented risk of OA progression and reduced efficacy when compared to placebo surgery.[59] The first-line management of degenerative meniscal lesions without 'mechanical' symptoms is conservative.[60] The conservative approach appears challenging and consists of physiotherapy, NSAIDs, HA or corticosteroid intra-articular injections, and also the aforementioned biological products. In recent years there have been attempts to target selectively the meniscal tissue with intra-meniscal injections. Among them, PRP, due to its anti-inflammatory and regenerative effects, has recently been spreading in clinical practice. Guenoun et al performed an ultrasound-guided intra-meniscal, meniscal wall and intra-articular injection procedure, demonstrating that intra-meniscal PRP treatment is feasible, safe and efficient. A prolonged clinical and functional improvement was observed. Despite promising in vitro results, no MRI healing signs were noted.[61] Randomized controlled studies with a higher number of patients are needed to confirm these encouraging results.

Micro-fragmented adipose tissue (MFAT) rich in SVF, once processed, may also be used for intra-meniscal injections; it acts as trophic mediator by secreting a variety of cytokines and growth factors, inhibiting fibrosis and apoptosis, enhancing angiogenesis and stimulating the differentiation of tissue-intrinsic reparative or stem cells. Mild mechanical forces may preserve the microarchitecture of the stromal vascular niche, where pericytes are located. MFAT may not only work as biological vehicle but also as an adipose tissue filler for meniscus regeneration. Malanga et al demonstrated that MFAT is a safe and potentially efficacious treatment option. A clinical improvement in pain, function, and quality of life measures, with no side effects, was achieved.[62]

Future perspectives

A strong limitation of the injective techniques is still the limited longevity of a sufficient drug dose inside the joint in order to exert a durable therapeutic effect. To overcome this limit, numerous new nano-technological approaches have been developed and are currently under testing.[63] Indeed, the possibility of employing nano-carriers to keep the injected substances inside the knee environment and slowly release their contents over time, may achieve what most injective strategies have failed so far to obtain: long-lasting symptomatic relief. Another new experimental approach is represented by gene delivery. The concept is to deliver intra-articularly viral vectors with cDNAs coding for therapeutic proteins such as TGF-β1, IL-1Ra, interferon-beta (IFN-β), aiming at a sustained, endogenous drug delivery system.[64]

In addition, new injectable substances have been developed in order to slow down, halt or even to reverse the

destruction of the joint tissues either by promoting cartilage anabolism or inhibiting its catabolism. In the first category we include the recombinant human fibroblast growth factor 18 (rhFGF-18, Sprifermin): it has been investigated both in vitro and in rat models showing its ability to expand hyaline cartilage-producing chondrocytes leading to an overall increase in cartilage volume.[65] However, despite these encouraging pre-clinical findings, the first RCT assessing its efficacy in humans found no difference between rhFGF-18 and a placebo, thus again revealing the challenges of addressing the complex in vivo human joint system.[66]

Among the cartilage catabolism inhibitors, we should mention IL-1 receptor antagonist (IL-1Ra): its use as an intra-articular injectable was found to be safe in humans and effective in reducing cartilage degeneration and synovial inflammation in animal models.[67] Moreover, monoclonal antibodies directed against nerve growth factor (NGF) (tanezumab, fulranumab, and fasinumab) have also been suggested as potentially therapeutic in OA. Indeed, anti-NGF injections for knee OA were found to be superior in pain reduction compared to a placebo [68]. There are also molecules able to simultaneously promote cartilage synthesis and taper inflammatory processes responsible for cartilage degradation, such as kartogenin. This small molecule has been reported to promote cartilage regeneration and inhibit joint inflammation in small animal models, but its efficacy in humans still needs to be investigated in clinical trials.[69]

Last but not least, all the therapeutic strategies mentioned in the present review should be viewed in the perspective of the 'personalized medicine'. Indeed, not only the development of new drugs is relevant, but also the correct indication to the right patient plays a key role. Radiologic and biochemical strategies for early diagnosis, a better understanding of the pathogenesis and prognostic factors of OA, which is a multi-faceted disease with different genotypes and phenotypes, and the patient's individual features should drive the choice of the 'right' injectables, modelling a more personalized therapeutic plan for each patient.[70]

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ICMJE CONFLICT OF INTEREST STATEMENT

BDM reports consultancy for Cartiheal Ltd, outside the submitted work.
EK reports consultancy for Cartiheal Ltd, Fidia Farmaceutici Spa and Greenbone Ortho Srl, payment for lectures including service on speakers' bureaus from Zimmer Biomet, and stock/stock options in Cartiheal Ltd, all outside the submitted work.
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