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Biomedicine Law Coursework

Treatment for Rheumatoid Arthritis

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I. Introduction:

Rheumatoid arthritis, or RA, is an autoimmune and inflammatory disease that occurs when your immune system mistakenly attacks your own body's healthy tissues, causing inflammation in the affected parts of the body. RA can potentially affect, besides the joints, a wide variety of other body systems, including the skin, eyes, lungs, heart and blood vessels Unlike the wear-and-tear damage of osteoarthritis, rheumatoid arthritis affects the lining of the joints, causing a painful swelling that can eventually result in bone erosion and joint deformity. While new types of medications have improved treatment options dramatically, severe rheumatoid arthritis can still cause physical disabilities[1].

Early rheumatoid arthritis tends to affect smaller joints first — particularly the joints that attach the fingers to the hands and the toes to the feet. As the disease progresses, symptoms often spread to the wrists, knees, ankles, elbows, hips and shoulders. In most cases, symptoms occur in the same joints on both sides of the body. About 40% of the people who have rheumatoid arthritis also experience signs and symptoms that do not involve the joints. Rheumatoid arthritis can affect many non-joint structures, including the skin, the eyes, the heart, the kidneys etc. Rheumatoid arthritis signs and symptoms may vary in severity and may even occur occasionally. Periods of increased disease activity, called flares, alternate with periods of relative remission — when the swelling and pain fade or disappear. Over time, rheumatoid arthritis can cause joints to deform and shift out of place.

Rheumatoid arthritis occurs when an individual's immune system attacks the synovium -the lining of the membranes that surround the joints. The resulting inflammation thickens the synovium, which can eventually destroy the cartilage and bone within the joint. The tendons and ligaments that hold the joint together weaken and stretch. Gradually, the joint loses its shape and alignment. The exact causes are not yet known, although a genetic component appears likely. While a person's genes do not actually cause rheumatoid arthritis, they can increase the susceptibility to environmental factors — such as infection with certain viruses and bacteria — that may trigger the disease. Risk factors that may increase the probability of rheumatoid arthritis are, among others, the individual's sex -women are more likely than men to develop rheumatoid arthritis; age- even though rheumatoid arthritis can occur at any age, it most commonly begins in middle age; family history; smoking; environmental exposures; obesity[2].

Despite the improved understanding, in the last 20 years, of RA pathophysiology and the plethora of antirheumatic drugs, drug therapy is palliative, and the disease remains

incurable. Even though many treatments reduce inflammation and its associated symptoms, cartilage degradation remains refractory. The relative failure of conventional strategies led to the development of new therapies. Biologically based approaches appear to hold the greatest promise. The pathophysiology of RA is complex and involves immune dysfunction, the synovial infiltration and activation of various cell populations (macrophages, dendritic cells, CD4+ T cells, and B cells), and the release of many inflammatory mediators including cytokines (TNF, IL-1, and IL-6). These cytokines, released in the synovial microenvironment have autocrine (activating the same cell), paracrine (activating nearby cells), and endocrine (acting at distant sites) effects and accounting for many systemic manifestations of disease. There are many shared functions of TNF, IL-1, and IL-6, and these cytokines in turn upregulate the expression of the others.

Among the important effects of these cytokines are the induction of cytokine synthesis; the upregulation of adhesion molecules; the activation of osteoclasts; the induction of other inflammatory mediators including prostaglandins, nitric oxide, mtrix metalloproteinases; the induction of the acute phase response (e.g. C-reactive protein, increased ESR), while they also cause fatigue, fever and cachexia and they lead to the activation of B cells (IL-6), as mentioned above. More cytokines are also increasingly described in RA, among which IL-8 which is involved in cellular recruitment, GM-CSF involved in macrophage development, IL-15 involved in T cell proliferation, IL-17 which has pleiotropic effects on multiple cell types including osteoblast expression of RANK leading to osteoclast activation, and IL-23 involved in increasing TH17 cell differentiation[3].

Numerous biologic therapeutic strategies have been designed using mainly monoclonal antibodies (eg, anti-CD4 monoclonal antibody, anti-TNF), recombinant forms of natural inhibitors (eg, recombinant IL-1 receptor antagonist [rIL-1Ra], recombinant soluble TNF receptor [rTNFsR:Fc]), or anti- inflammatory cytokines (eg, IL-4, IL-10). Two such products, TNFsR:Fc (Enbrel, Boehringer Ingelheim, CT) and anti-TNF antibodies (Remicade, Centocor, Inc.) are approved by the US Food and Drug Administration (FDA) for the treatment of patients with RA[4].

However, "biologics," initially used as single-agent therapies and now being tested in combination, revealed short-term effectiveness and some toxicity. The use of genes to deliver these therapeutic proteins, in contrast, has many theoretical advantages such as long-term expression with localized, endogenous production of high concentrations of the therapeutic gene product.

II. Gene Therapy for Rheumatoid Arthritis

As mentioned above, biologic therapy, currently used for the treatment of RA, presents significant limitations, including systemic side effects, a short half-life with requirement for frequent dosing, and a lack of curative response. Because of these limitations, the ideal therapy for RA is to be developed. Nowadays, progress in the field of gene therapy provides interesting and applicable methods to overcome many of these deficits. Below we will attempt to propose a vector, a therapeutic gene and a promoter for gene delivery in RA (under III), we will propose an animal model suitable for this purpose (under IV), while discussing the ethical issues arising from the choice of the suitable body part from which such a trial could begin (under V), the potential risks of the virus chosen shedding (under VI) and finally the necessary precautionary measures taken in order for this trial to be realized.

III. Proposed vector, promoter and therapeutic gene

a. Gene delivery vectors for Rheumatoid Arthritis:

When it comes to Rheumatoid arthritis both viral and non-viral vectors are available for gene transfer. Vectors are essentially the vehicles that transfer the genetic material into a wide variety of cells or tissues or even whole organs. The optimal vector and delivery system depends on the target cells and its characteristics, duration of expression and the size of the genetic material to be incorporated in the vector. The ideal vector should transfer a precise amount of genetic material into each target cell, thereby allowing for expression of the gene product without causing toxicity. Moreover, the ideal vector should deliver gene to a specific cell type, accommodate foreign genes of sufficient size, achieve the level and duration of transgenic expression sufficient to correct the defect and, most importantly, be non-immunogenic and safe[5].

The present vectors used for gene therapy are broadly classified as Viral vectors, Non-viral vectors and engineered vectors[6]. Non-viral vectors usually produce only low, transient expression of the transgene. Although this is inadequate for most purposes, non-viral systems can be proven valuable in certain contexts, such as vaccination and achieving immune deviation. Viral vectors, on the other hand, offer, in most circumstances, notably higher levels of gene expression for longer periods of time. Furthermore, with viral vectors, even lower dosages offer a higher level of transduction of cells since they infect cells more readily than

naked DNA delivery systems. In order to be used as vectors for gene therapy, viruses are genetically modified to reduce their pathogenicity and eliminate their ability to replicate, while, at the same time, retaining their infectivity.

-Viral vectors

i) Integrating Viruses

In human trials for rheumatoid arthritis, four types of viruses have been developed. Two of these, retrovirus and adeno-associated virus (AAV) are integrating while the other two, adenovirus and herpes simplex virus (HSV), are not. Integrating viruses were the starting point for the first useful gene therapy vectors, which have been used extensively in human clinical trials. Their ability to transduce only dividing cells has restricted their clinical use to ex vivo delivery. However, certain techniques can be used to permit in vivo delivery in particular experimental settings.

The ex vivo use of retroviruses in humans is a major safety advantage for clinical trials. By integrating into the host genome, retroviruses present advantages for achieving long-term gene expression. However, ex vivo procedures are laborious, involving the removal of cells from the body, their genetic modification, and then their re-implantation. Moreover, some viruses present theoretical risk of insertional mutagenesis. Other types of retroviruses derived from lentivirus, including Human immunodeficiency virus (HIV), have the advantage of being able to integrate their genome into those of nondividing cells. However, they require more development before they can be used for human gene therapy.

AAV (single-strand DNA viruses of the parvovirus family) are also integrating viruses and are able to transduce nondividing cells. Wild-type AAV integrates in a specific site on chromosome. No pathology seems to be associated with AAV infection, but they are difficult to produce and have only a small packaging capacity of approximately 4 kb.

ii)Non-integrating Viruses

In contrast to retroviruses, adenoviruses efficiently infect dividing and nondividing cells. Recombinant adenoviruses are easy to produce and are efficient vectors in many cases. They can engender high levels of transgene expression, notably when using strong viral promoters, such as the cytomegalovirus early promoter. However, transgenes are expressed

for a relatively short time, partly because adenoviruses rarely integrate the host genome. Transient gene expression can also be explained by immune responses triggered by adenoviruses. Cells infected with first generation adenoviral vectors express viral proteins on the surface of the transduced cells, leading to their immune recognition and elimination.

Vectors derived from HSV, another double-stranded DNA virus, also have the advantage of transducing non dividing cells with high efficiency, and their genomes may accept large inserts up to 40 kb or larger. Despite these advantages, they are difficult to produce, and, despite recent improvements, there is residual cytotoxicity[7].

What is important to take into consideration when it comes to viral vectors, it that vector particles containing viral proteins that are identical or similar to antigens that humans are exposed to, as a result of natural infection, may be neutralized by antibodies upon injection into in some humans because of pre-existing immunity. Recognition of viral structures (e.g., capsids or nucleic acids) by innate immune sensors may cause tissue infiltration by innate immune cells, may trigger the production of interferon (IFN)-α/β (type 1 IFN, hereafter abbreviated as T1 IFN), thereby inducing an antiviral state in the tissue and reducing transduction, and provides an activation signal for adaptive immune responses. Interestingly, pre-existing binding antibodies (that are not neutralizing) do not block gene transfer but may alter biodistribution of the vector[8].

Viral vector use in gene therapy has highlighted several safety concerns, including genotoxic events. Generally, vector-mediated genotoxicity results from upregulation of cellular proto- oncogenes via promoter insertion, promoter activation, or gene transcript truncation, with enhancer-mediated activation of nearby genes the primary mechanism reported in gene therapy trials. Vector-mediated genotoxicity can be influenced by virus type, integration target site, and target cell type; different vectors have distinct integration profiles which are cell-specific. Efforts have been made to develop viral vectors with less risk of insertional mutagenesis, including self-inactivating (SIN) vectors, enhancer-blocking insulators, and microRNA targeting of vectors, although insertional mutagenesis is not completely abrogated[9].

-Non-Viral vectors[10]

Non-viral vectors are Naked DNA, particle based and chemical based. They are administered by direct administration (plasmid DNA/Naked DNA)/ chemical /physical. Most of cardiovascular clinical trials use non-viral vectors as a mode of gene transfer[11]. When it comes to synthetic delivery vectors, they have the potential to address many of the limitations of viral vectors, particularly those related to safety. For systemic delivery of DNA, both lipid-based vectors and polymer-based vectors have been intensively investigated in experimental animals and in clinical trials.

The potential of mRNA for therapeutic protein expression in vivo has been investigated as an alternative to DNA-based gene therapy due to its unique advantages. Recent advances in chemical modifications of mRNA reduce stimulation of the immune system and improve stability when it is delivered in vivo. Small interfering RNA (siRNA) has great therapeutic potential, as it can silence nearly any targeted gene after introduction into cells. Lipid- and polymer-based siRNA nanoparticles and conjugate systems enable successful delivery of chemically modified siRNAs in humans. Levels of microRNA (miRNA) can be restored through the introduction of synthetic miRNAs or mimics as miRNA replacement therapy.

Delivery of genome editing systems — including zinc-finger proteins, transcription activator-like effectors and CRISPR–Cas (clustered regularly interspaced short palindromic repeat–CRISPR-associated) systems — facilitates gene editing at desired sites in the genome. Recent proof-of-concept studies in model organisms have shown that this approach may be used to cure genetic diseases, which is in contrast to the temporary expression or random insertion of a DNA fragment in conventional gene therapy.

b. Delivery methods

For the treatment of RA, one could consider two different gene delivery strategies. On the one hand, there is systemic delivery, which involves the transfer of genes to sites where the gene product, if secreted, has access to the systemic circulation. On the other hand, there is local delivery which implies gene delivery to selected, discrete sites, such as the joint, with reduced systemic exposure. For systemic therapy, genes may be delivered to sites such as skin, muscle, bone marrow, and liver. However, even though there have been some encouraging experimental results in animal models of RA[12], safety concerns prohibit

systemic delivery of genes to patients with RA. The intraarticular administration of genes, in contrast, raises fewer safety issues and is better suited to treat directly the arthritic joints.

c. Candidate therapeutic genes:

There are a few genes which are good candidates for use in the gene therapy of RA, and one should consider multiple parameters before choosing the suitable one, always bearing in mind the vector that will be selected as well. Numerous experimental gene therapies, mainly based on cytokine inhibition, have been performed successfully in several animal models of RA. Other strategies, involving the inhibition of intracellular signaling pathways, are also being investigated, and, even if less studied, they are encouraging[13]. Generally, RA is considered as a Th1 disease, and therefore, the injection of a viral vector expressing Type 2 cytokine genes could improve RA in various RA animal models. In this regard, according to the anti-inflammatory criteria, interferon β and IL-10 have been considered as appropriate targets for gene therapy as satisfactory results have been reported about them in the treatment of multiple sclerosis and RA[14]. The antiarthritic activities of anti-inflammatory cytokines could be because of their independent anti-inflammatory properties, for example, IL-4, as well as IL-13, are strongly able to protect from bone and cartilage destruction[15].

When the production of two traditionally opposing cytokines, IL-4 and interferon γ (IFN-γ), by colonic mononuclear cells is compared, IFN-γ levels dominate in early disease, yet the relative concentration of these two cytokines normalizes to control amounts in late disease. Given these findings, it is not surprising that biologic treatments which disrupt IFN-γ biased effector mechanisms are excellent therapies during early disease and useless in late disease[16]. However, there are some side effects, the most common being “flu-like,” such as fever, headache, chills, myalgia, or fatigue. Other common side effects include rash, injection site erythema or tenderness, diarrhea and nausea, and leukopenia[17]. As preclinical work has demonstrated that IL-13, IL-4 and their receptors are involved in the expulsion of parasites[18], the immune response to malignant cells[19] and cardiac repair[20], modulation of these pathways could potentially increase susceptibility to certain infections, malignancy or cardiovascular events.

Finally, transforming growth factor β (TGF-βt), with both immunosuppressive effects and preventing from chondrogenesis appears to be beneficial for treating RA, but unexpected

results (massive fibrosis induction, osteoporosis, and cartilage destruction) were observed[21]. Therefore, it seems that TGF-β gene therapy may not be suitable in some RA models.

d. Regulation of gene expression- Promoters:

The regulation of the level of the therapeutic drug is of high importance to any successful therapeutic regimen. When it comes to gene therapy, this is related to the activation or turning on of selected genes during the active period of the disease, while being able to turn off the expression at disease remission. Drug-inducible promoters provide a targeted method to control transgene expression. One method developed for this purpose is an antibiotic-inducible system. An alternative approach to drug-inducible systems are inflammation-responsive promoters. In this approach, proinflammatory cytokines or transcription factor regulatory elements are used to control gene expression.

Both tumor necrosis factor-alpha (TNFα) and Interleukin-1 (IL-1) are cytokines required for activating the innate immune response[22], mediating the recruitment, activation, and adherence of circulating phagocytic cells (macrophages and neutrophils), and terminating the innate immune response[23]. The transcription factor NF-κB regulates multiple aspects of innate and adaptive immune functions and serves as a pivotal mediator of inflammatory responses. NF-κB induces the expression of various pro-inflammatory genes, including those encoding cytokines and chemokines, and also participates in inflammasome regulation. In addition, NF-κB plays a critical role in regulating the survival, activation and differentiation of innate immune cells and inflammatory T cells. Consequently, deregulated NF-κB activation contributes to the pathogenic processes of various inflammatory diseases[24].

Another method of gene regulation comes in the form of small molecules capable of regulating gene expression once the transgene has been delivered. Proteasome inhibitor (PI) administration has been shown both in vitro and in vivo to upregulate transgene expression. Interestingly, although the effect is transient, repeated PI administration was able to reinduce gene expression.[25]

e. Our choice

Taking into consideration the above, we think that the AAV is the vector of choice in our situation. In *in vivo* gene delivery to the joint by direct intra-articular injection, although a number of different vectors can successfully transduce cells in intra-articular tissues,

following the injection to the joint, many are unsuitable for clinical translation because they are inflammatory, immunogenic, unsafe or provide only short- term transgene expression. Adeno- associated virus (AAV) is preferred because it is safe, effective, and less immunogenic than other vectors. Moreover, AAV provides extended periods of intra-articular transgene expression[26].

There is always the problem with pre-existing antibodies to the AAV virus to be tackled, but, theoretically, repeat administration of the AAV vector is possible by switching the capsid sequence. However, such a strategy is complicated by the tendency of humans to produce cross-reactive antibodies and by the need to develop at least two products. An alternative approach is to apply immunosuppression. One protocol uses antibody-mediated B cell depletion combined with rapamycin, while rapamycin-containing nanoparticles have been tested in animal models[27].

Regarding the promoter, as mentioned above, the need for a pro-inflammatory gene is essential in order to induce the expression of the therapeutic gene locally and specifically when there is RA-related inflammation. For this purpose, TNFa, IL-1a, Cox2 or NF-kB would all be suitable to carry the therapeutic gene, as they are upregulated during inflammation.

Finally, the therapeutic gene needs to be an anti-inflammatory agent that will alleviate the phenomenon of inflammation in the joints. There are numerous choices here as well. We conclude that IL-4 and hIFN-β are among the best candidates due to their anti-inflammatory actions.

IV. Animal Model

After creating the construct of the pro-inflammatory gene as a promoter guiding the expression of the anti-inflammatory gene, we could test it to see if it practically works. As a proof of principle, we could use a synovial organoid[28],[29] to test if we can switch on the promoter, if the inflammation is blunted due to the expression of the therapeutic gene and finally, how long this effect lasts. To induce the inflammation in the first place, we could administer a pro-inflammatory stimulus such as LPS or IFNγ.

Once we have established that the construct works, we can move on with the preclinical stage, specifically injecting to a live animal. Unfortunately animals need to be used in order to test the safety and efficacy of the therapy before proceeding to a human patient; however the 3Rs Principle will guide all necessary experiments. In this case, where

we cannot completely replace the animals, we would try to reduce their number to the absolute minimum in order to gain clear and hopefully statistically significant results, while also refining our experiments so that the lab animals can live a comfortable life and not experience distress or pain at any moment.

Regarding the issue of which animal model would be the best choice for our study, we should firstly state that there are a few species which we could examine, the most common being the mice, rats, sheep and pigs. Although the mice are easy to handle and economical to house, they are very small animals and this would not give us a clear image of the process taking place inside their wrist. Pigs and sheep are larger animals whose joints would surely resemble more those of humans', however the ethical issues raised are more serious and it would also increase the cost of the study dramatically since their purchasing and housing is more expensive. Therefore, for the sake of this study, we would choose to proceed with the rats. For this species, Collagen-Induced Arhritis (CIA) is one of the most commonly used models of RA. In CIA, several different cartilage-derived proteins, including type II collagen (CII)[30], type XI collagen (CXI)[31] and cartilage oligomeric matrix protein (COMP)[32]. Nevertheless, almost all gene-mapping studies have been performed using CII-induced arthritis, so it would also be safe for us to proceed with this animal model[33]. Finally, if this study turns out to have good results in our chosen animal model, we could also confirm our findings using a small amount of larger animals before testing it on humans, preferrably pigs[34].

V. Injection in the Wrist

The wrist is a rather complex joint, made up of multiple small joints. When healthy, the bones glide easily over each other during movement, protected by smooth cartilage that coats the joint surfaces. Rheumatoid arthritis damages this cartilage and, as the disease progresses, there is a gradual loss of cartilage. Without a smooth joint surface, the bones rub against each other, causing wearing and rupture of the tendons that straighten the fingers. This furthermore leads to joint damage and loss of function in the hand that cannot be repaired[35].

Scientists in our scenario have chosen to start the injection from the wrist due to it being large and easy to X-ray and monitor. Several studies have identified complications into the wrist joint arising as results of arthrocentesis and corticosteroid injections[36]. Bearing these

into consideration we would advise to avoid the injection to be done into the wrist. The most significant issue that has been highlighted is the risk of infection and because of that, precaution must always be taken to use sterile techniques. Moreover, there is the risk of septic arthritis following aspiration or corticosteroid injection, which has been estimated to be between 1 in 2000 and 1 in 15,000 procedures[37].

Other complications can arise from misplaced injections. The best-described complication is tendon rupture following corticosteroid injections for tendinitis. The risk of this complication can be minimized by avoiding injection into the tendon itself. No therapeutic agent should be injected against any unexpected resistance. Occasionally, nerve damage can also result from a misplaced injection (eg, median nerve atrophy following attempted injections for carpal tunnel syndrome).

Following the aforementioned information, it is clear that there are some considerable ethical issues, given the fact that if anything goes wrong with a potential injection to the wrist, it would result in severe effects. In the worst case scenario, a failure of the treatment could incapacitate the whole hand leading to a serious deterioration of the individual's quality of life. Therefore, we believe that it would be safer and more ethically correct to choose another joint for the beginning of this study. We would advise starting with a joint in a finger being affected by RA, specifically on the hand that is not the one mainly used for the everyday life activities, and we could also argue that in the scenario in which something goes wrong, a finger could be more easily replaced than a wrist.

Finally, as far as a mitigation procedure is concerned, synovectomy could be considered in our case. Synovectomy is a surgical procedure used to treat synovitis and some other conditions that affect the synovium, a thin membrane that lines the inside of certain joints such as a knee, shoulder or elbow. In a synovectomy procedure, much of the synovium is removed. A normal synovium, which is usually one or two cell layers thick, produces synovial fluid that helps lubricate the joint. When the synovium grows too bulky, it produces too much synovial fluid, which contains an enzyme that, in large quantities, 'eats away' at the articular cartilage on the joint surface. In patients with inflammatory arthritis, excessive growth of synovium is part of an abnormal immune response in which the body recognizes cartilage as a foreign substance that must be attacked. Loss of cartilage eventually leads to damage to the joint surface as well as the stiffness and pain characteristic of all types of arthritis. (Osteoarthritis, the more common form of arthritis, does not involve this type of inflammatory response. Other causes, including injury, wear-and-tear, and heredity are

thought to contribute to the degeneration of cartilage in osteoarthritis.) Moreover, the vitamin D hormone may mitigate cytokine-induced upregulation of MMP and PGE2 by synovial fibroblasts in RA[38]

VI. Viral Shedding

Information regarding shedding duration, excretion routes, biodistribution, and other components vital to appropriate risk assessment of vectors is available from basic research studies and human gene therapy trials. Shedding is defined as the dissemination of the AAV vector through secretions and/or excreta from the animal model or patient, whereas biodistribution relates to the spreading of the AAV vector within the animal model and the patient's body from the site of administration[39]. In general, AAV is considered to be safe and is not known to cause disease and causes a very mild immune response, while the synovial membrane is considered to not be very permeable. The fibroblast-like synoviocytes manufacture a long-chain sugar polymer called hyaluronan, together with a molecule called lubricin, which lubricates the joint surfaces. The water of synovial fluid is not secreted as such but is effectively trapped in the joint space by the hyaluronan[40].

Wild type AAV virus is dependent for replication on the presence of adenovirus or herpesvirus and will, in the absence of helper virus, stably integrate into the host cell genome at a specific site on the human chromosome 19 and remain latent. Potentially at a later time when a helper virus is present, AAV can be reactivated and produce infection. Therefore, AAV may not be as safe as previously thought and in order to be fully covered, we would advise the patients to get blood tests every once in a while to ensure that the AAV is not found in the circulation.

VII. Precautionary Measures

In assessing risk regarding viral vector use in animal research, one must consider the hazards inherent to the viral vector itself, the animal model, the inserted gene construct, and the proposed research manipulations. A plethora of information is known regarding the original virus from which the vectors are genetically engineered, including replicative ability,

oncogenic potential, environmental stability, tissue and cellular tropism and pathogenic characteristics—all of which helps to guide risk assessment[41].

In our case we have chosen as a vector the Adeno-associated virus (AAV). Adeno-associated virus (AAV) and recombinant adeno-associated virus (rAAV) are commonly used for gene expression with notably fewer associated biosafety concerns, especially when compared to viral vectors which are persistent and able to integrate into the genome[42].

Adeno-associated viral vectors (AAV) are generally classified under RG1. Exceptions include synthetic or recombinant AAV constructs produced in the presence of a helper virus and those that contain a potentially harmful transgene[43]. In our case, we should take into consideration the potentially detrimental effects that the gene that is expressed by the vector could likely have, and since the therapeutic gene of choice is a cytokine, BSL2 should be used as a minimum. In this biosafety level, there are laboratories that work with agents associated with human diseases (i.e. pathogenic or infections organisms) that pose a moderate health hazard.

BSL-2 laboratories maintain the same standard microbial practices as BSL-1 labs, but also include enhanced measures due to the potential risk of the more hazardous microbes. Personnel working in BSL-2 labs must take precautions to prevent injuries such as cuts and other breaches of the skin, as well as ingestion and mucous membrane exposures. In addition to BSL-1 expectation, the following practices are required in a BSL 2 lab setting: Appropriate personal protective equipment (PPE) must be worn, including the BSL 2 lab coats and double gloves. The procedure must take place inside a BSC class II, and even though the glass existing can be used as an appropriate shield, we still propose the use of eye protection and face shields. When it comes to proper disposals, an autoclave or an alternative method of decontamination must be available. In this kind of laboratory there are self-closing, lockable doors as well as a sink and an eyewash station, readily available. There should exist biohazard warning signs and in general, access to a BSL-2 lab must be far more restrictive than a BSL-1 lab. Outside personnel, or those with an increased risk of contamination, are often restricted from entering when work is being conducted[44].

When it comes to the extent of infection control precautions, they should be determined for this clinical study by the infection control personnel collaborating with members of their local Institutional Review Board (IRB) and Institutional Biosafety Committee (IBC). Educating the lab's or hospital's employees within these areas is pivotal in order to promote safe patient and product handling and to minimize employee exposure.

When it comes to gene therapy patients admitted to the hospital, they must be tracked and the personnel should provide them with information about necessary infection control precautions. Patients should be treated only in areas approved by the institutional biosafety committee.

Vector preparations shipped from a commercial vendor should be brought into the healthcare facility with a procedure which can ensure safe receipt, preparation, dispensing, and storage of gene therapy products and maintain records of their use. The preparation of vectors should be done by experts who have training in biosafety and an understanding of infection control requirements. Work should not be performed on an open bench, but as already mentioned above, in an appropriate biosafety cabinet (BSL-2). Considering the safe transport of the product to the patient's room/to the place of administration of the therapy, protocols should be established; protocols should also be put in place for the preparation of the administration of the gene therapy agent at the bedside. These should include techniques to clear air from syringes or intravenous line tubing to prevent aerosols.

When it comes to delivering the viral construct into the patient's joints with echo-guidance, ultrasound is generally considered to be safe with very low risks. However, the risks may increase with unnecessary prolonged exposure to ultrasound energy, or when untrained users operate the device. It is based on non-ionizing radiation, so it does not have the same risks as X-rays or other types of imaging systems that use ionizing radiation. Although ultrasound imaging is generally considered safe when used by appropriately trained health care providers, ultrasound energy has the potential to produce biological effects on the body. Ultrasound waves can heat the tissues slightly. In some cases, it can also produce small pockets of gas in body fluids or tissues (cavitation). The long-term consequences of these effects are still unknown. Because of the particular concern for effects on fetuses, organizations such as the American Institute of Ultrasound in Medicine External Link Disclaimer have advocated prudent use of ultrasound imaging in pregnancy. Therefore, expecting mothers working as part of the medical staff should take extra precautions and have adequate training. Ultrasound imaging does introduce energy into the body, and laboratory studies have shown that diagnostic levels of ultrasound can produce physical effects in tissue, such as pressure oscillations with subsequent mechanical effects and rise in temperature. Therefore, FDA recommends that health care providers consider ways to minimize exposure while maintaining diagnostic quality when using ultrasound. As with all other imaging

modalities, the principles of As Low As Reasonably Achievable (ALARA) should be practiced by health care providers[45].

When it comes to the disposal of gene therapy waste (i.e. glass, vials, sharps, syringes, etc.), it should be placed in a puncture-resistant container with an easily recognized biohazard label. Personal protective equipment, gauze, or other soft waste should be disposed of in red biohazard bags and handled as other regulated waste. Special attention should be given to emergency spill procedures and the management of spills must depend on the amount of material spilled. Small spills (<10mL) should be wiped up, and the surface disinfected with an appropriate germicidal agent. Kits should be prepared in advance to manage these spills and be readily available in areas where the vector will be prepared or administered. For larger spills, personnel should evacuate the area, notify the appropriate authorities, warn others, control traffic, dispose of contaminated clothing and materials, clean contaminated skin with soap and water, and clean up the spill using an appropriate disinfectant.

Health employees who will work with gene therapy patients, products, or waste must be adequately trained and thoroughly informed regarding the relative risks and hazards. Protocols for the management of exposed healthcare workers must be established before any patient is enrolled or treated. Details about the vector and transgene must be part of these protocols as well as a description of known or potential risks, recommended screening tests, treatment and follow-up, the timing of follow-up, and ways to contact investigators for consultation at all times when patients are being treated in the hospital or clinics. Finally, immunization against the AAV should be considered as a precautionary measure for the doctors and medical staff performing the injections, who should at the time of the injection wear gloves and who should be excluded from performing the injections if they meet any of the criteria mentioned below as exclusion criteria.

Since AAV does not cause any known disease, propagation of the vector in a gene therapy volunteer or their contacts is unlikely because it requires both superinfection by wild type AAV (to supply the normal AAV genes) and co-infection with a helper virus such as the adenovirus. AAV's relatively small size limits the amount of DNA that can be transduced (up to 5kb). When it comes to the survival of this virus on surfaces, in the case of potential spills, sodium hypochlorite or quaternary ammonium compound could be used to disinfect the area, since they are the recommended disinfectants against AAV.[46] Specifically concerning the animals used in this study and the potential risk caused by AAV, we should mention that in some animal models, the integration of recombinant AAV has been associated with an

increased incidence of tumor formation. However, this association has not been noted to occur in humans[47].

VIII. Exclusion Criteria from this trial

a) Patients under 18 years of age

b) Pre-existing immunogenicity either from wild-type AAVs or from previous AAV-based gene therapies. A significant portion of patient populations are environmentally exposed to wild-type AAVs, and many have developed antibodies and cell-mediated immune responses to those naturally occurring viruses. Moreover, patients who have been previously administered other AAV-based gene therapies can also have pre-existing immunogenicity. In these cases, pre-existing antibodies to the virus can impair the successful transduction of the vector and limit subsequent efforts to re-dose, hampering durability of the treatment's benefit.

c) Known hypersensitivity to Il4 or hINF-β or presence of neutralizing antibody (Nab) titers against Il4 or hINF-β

d) Contra-indication for intra-articular treatment.

e) Active infectious disease of any nature, including clinical active viral infections, or clinically relevant illness within two weeks of enrollment including fever > 38.2o C, vomiting more than once in 24 hours, seizure, or other symptom deemed contraindicative to new therapy

f) Pregnant or nursing mothers

g) Positive for human immunodeficiency virus (HIV) infection, hepatitis C antibodies or hepatitis B surface antigen.

h) Serious medical disease, such as severe liver or kidney disease, uncompensated congestive heart failure, myocardial infarction within six months, unstable angina, uncontrolled hypertension, severe pulmonary disease or active asthma, demyelinating neurological disease, depression or a history of depression, history of seizures or epilepsy, uncontrolled epilepsy, or history of cancer (other than cutaneous basal and squamous cell carcinoma or cervical intraepithelial neoplasia) with less than five years documentation of a disease-free state, recurrent opportunistic infections or other concurrent medical condition that, in the opinion of the investigator, would make the patient unsuitable for the study.

i) In addition, prevention of coinfection with adenovirus, herpesvirus, or vaccinia during administration may be of value.

IX. Precautionary measures after treating the RA patient

After treating the patients, we must consider which precautionary measures will have to be taken. When it comes to gene therapy where AAV is used as a vector and delivery of the transgene happens intravascularly, some pre-clinical studies have identified hepatotoxicity and genotoxicity as a theoretical long-term safety concern, with no published long-term clinical trial safety data extending beyond 3 years post-intravascular vector delivery, up to date[48]. However, the hepatotoxicity risk, which according to some studies is related to AAV delivery, has been identified as dose dependent, which means that on the one hand we should focus on administrating the correct dose and on the other, under the follow-up safety context, that maybe tests should be run in order to provide information regarding the patients' livers' functions.

Another theoretical long-term risk that could be caused from AAV is tumorigenesis, only when we have systemic AAV use. In our case this seems rather unlikely, since we aim for the therapy to be an one-time injection, still we propose some relating tests to be run in order to certify that no carcinoma has been caused.

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