RNAi therapeutics in rheumatoid arthritis

An improvement on current therapies?

Nicole Pruijssers, S3450279

16-08-2021

Rijksuniversiteit Groningen

Supervisor: Dr.J.Westra

Summary:

Rheumatoid arthritis is an auto-immune disease which attacks the tissue of the joints, which if left untreated, can lead to extensive damage to the cartilage and bones, and even to deformation and handicap. Nowadays, sufficient medical treatments exist to prevent the worst outcomes. However, there is much still to be gained in the area of rheumatoid arthritis medications.

RNAi, or RNA interference, is a recently discovered innate pathway proven capable of strongly suppressing the production of a protein. As the mechanisms of RA depend strongly on the functions of several immune molecules, RNAi could be the next step of development in the battle against rheumatoid arthritis through the suppression of the production of the involved molecules.

Many obstacles still exist in exploring and perfecting the possibilities of RNAi therapeutics, but they hold much promise for the future development of treatments and should thus be further researched in the near future.

Table of contents:

What is rheumatoid arthritis?

Rheumatoid arthritis is a disease characterised by inflammation of the joints and the thereof resulting pain (McWilliams. 2019). The cause is a dysfunction of the immune system, which leads it to attack primarily the lining of the joints, called the synovium (Hitchon). This inflammation, other than causing pain, can also eventually lead to erosion of the bones and deformity of the joints (McWilliams. 2019). In up to 40% of the cases, the disease can also spread to and damage other tissues, such as the lungs, skin, eyes, heart and cardiovascular system (Shaw).

Typically, this disease tends to first affect the smaller joints in the hand and feet, particularly those at the base of the toes and fingers. In later stages, the inflammation and resulting damage can spread to the wrists, ankles, knees, elbows, shoulders and hips (Mayoclinic). The affected joints become painful, swollen and difficult to move (NHS). The symptoms usually present themselves in flare-ups, in which symptoms worsen considerably (McWilliams. 2017). These flare-ups are difficult, if not impossible to predict (NHS). Treatment can reduce the amount of flare-ups, lengthen the time in between them and mitigate, or even prevent long-term tissue damage (NHS).

Symptoms that can accompany rheumatoid arthritis are fatigue, fever, loss of appetite and a predisposition for a variety of other conditions and diseases, but most characteristic for this disease is the morning stiffness that fades after exercise (Sierakowski).

Neither the exact mechanism through which the immune system attacks the synovium, nor the reason or cause why it does are yet clear. It is however known that the cause is not only genetic, but that a family history of the disease predisposes one to it, which suggests genetics can make an individual more susceptible to developing it (Kurkó). Other risk factors include; being female, being middle-aged (Zhang), smoking (Aho) and being overweight (Qin).

According to the current statistics, approximately 0,5% of the adult population worldwide suffer from rheumatoid arthritis, the estimations reaching from 0,2% to 1,2% (Carmona). The life expectancy of rheumatoid arthritis patients can drop 3 to 10 years, depending on the severity of the particular case and the availability of tools for diagnosis and treatment (Carmona). This drop in life expectancy can be attributed to, among others, the increased risk at cardiovascular diseases, opportunistic infection

due to compromised immune system and malignant cancers facilitated by the persistent inflammation (Carmona).

Essential to understanding any auto-immune disease, including rheumatoid arthritis, is to remember that it is in principle an immune reaction like any other, the issue being that it is misaimed and attacks the body's own cells.

The immune system is a complex structure with many different cells and molecules that work together to reach their final goal. Each of these components has their own function, characteristics and mechanisms. The main components observed to be active in the pathology of rheumatoid arthritis are the T-cells, the B-cells and the interaction of pro-inflammatory cytokines (Choy). Among the cytokines themselves, primarily TNF-alpha and IL-6 appear to play key roles. Additionally, IL-1 and IL-17 seem to play smaller, but still significant roles (Choy).

Looking at the pathology of rheumatoid arthritis as an immune reaction, the very first step would be the activation of the innate immune system (Choy). As the term implies, the immune cells become activated as a result and antigen-presenting cells, including dendritic cells, macrophages and B-cells will being presenting arthritis-associated antigens to the T-cells (Choy). Concurrently, CD4+ T-cells, secreting cytokines, infiltrate the synovium (Choy). Adding to this, B-cells also being to produce (auto)- antibodies and cytokins (Choy). Furthermore, T-cell and B-cell activation leads to cytokine activation, which in turn leads to more T-cell and B-cell activation, putting a feedback loop in effect (Choy).

In case the disease is left untreated, the damage it causes to tissues can progress rapidly and spread throughout the body, leading to permanently deformed joints and a wide array of other health issues (Hopkins arthritis center) related to the chronic inflammation (Rodriguez), and even a highly increased chance at early death (Kelly).

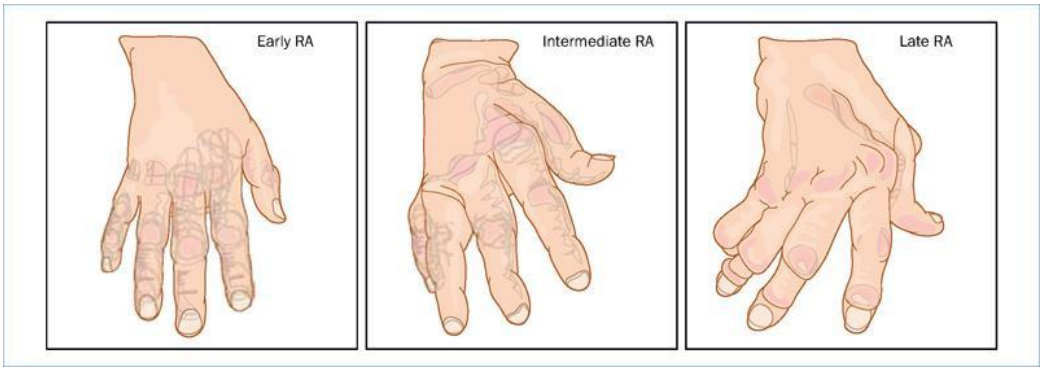

Figure 1. The progression of joint deformation in untreated rheumatoid arthritis. Rheumatoid arthritis and its effects on oral health - DentalNursing (dental-nursing.co.uk)

Fortunately, effective treatments to halt the progression of the disease have been developed and are in use today. This treatments focus primarily on early and aggressive intervention to reduce, and if possible, stop the inflammation, thus reaching a state of remission (Oh). Tight control of the disease and the treatment is required to keep the inflammation at the lowest level possible (Oh).

The first step in the treatment options are usually csMARD's, or conventional synthetic disease-modifying rheumatic drugs (Köhler). This is a collective name for many types of drugs that are used to suppress the immune system in a broad manner to treat rheumatoid arthritis (Iliades). Through the viewing and analysis of the patient's biological markers, as well as a process of trial-and-error, the best suited variant has to be selected (Köhler). However, the side-effects of these medications can be severe at times, including liver damage and lung infections (Mayoclinic).

The second option would be medications referred to as biological DMARD's, which include TNF-alpha inhibitors and IL-1 inhibitors. These newer type of drugs have a narrower effect as they block specific cytokines instead of the immune system in general (Iliades). However, these drugs come with an higher risk of side-effects, including opportunistic infections (Benjamin). Both these drugs require extensive health screening of the patient prior to administration come with the increased chance at infections and other complications (Köhler).

A recently developed third option would be a synthetic targeted DMARD, the Jak-inhibitor. The targeted Jak-stat pathway supports the function of many cytokines (Harrington). This treatment, too, comes with an increased risk at infections among other side-effects (Harrington).

In addition, glucocorticoids can be used to quickly subdue the symptoms, but are not recommended for anything other than short periods of use (Köhler). NSAID's, nonsteroidal anti-inflammatory drugs, can be administered to combat pain and other surface symptoms, but do neither stop nor slow down the progression of the disease (Crofford).

Nowadays, these medications can prevent extensive joint damage due to rheumatoid arthritis. However, this is often accompanied by immune suppression and a wide array of potential side-effects. As of such, there is still a long way to go in the field of anti-rheumatic treatments. The next step on it may lay in a recently discovered pathway: RNAi.

The RNAi pathway; what is it and what can it do?

The first recorded incident in which the effects of the RNAi pathway was observed happened in 1990 by Napoli and Jhopensen. The initial aim of their research was to investigate the molecular pathways in the coloration of petunias. When overexpressing the involved gene, expecting the petunias to develop a purple colouration, they found the opposite result. The gene they had overexpressed, appeared to have become inactive instead. In combination with reports of similar events, scientists reached the conclusion that the introduction of homologous RNA sequences led to the suppression of the endogenous gene (Sen).

In 1998, Fire and Mello proposed the possibility that the then-time widely used technique of silencing genes through hybridization with single-stranded RNA, which had been found effective up until that point, was not, in fact, effective. But rather, that the previous successes should be ascribed to contamination with double-stranded RNA, which was the real active agent. The subsequent success at proving their theory created the need to revise the entire existing framework regarding the mechanisms of RNA-induced gene silencing, or more specifically, how double-stranded DNA was capable of interfering with gene expression (Sen).

Soon after, the conclusion was reached that, to reach the silencing effect of double-stranded RNA, there had to be a silencing mediator, a stable molecule that put into effect the gene interference. It was initially assumed that the double-stranded RNA had to unwind in order to hybridise with the target sequence. However, no one succeeded at detecting the fully unwound sequence. What they did find were unwounded RNA segments of approximately 25 nucleotides in length, which appeared to be the required length for RNAi specificity. Further research indicated that the double-stranded RNA was converted into even slightly shorter fragments, 21-23 nucleotides long in specific, which were dubbed siRNA's, also small interfering RNA's. These siRNA's can bind to the target RNA and induce cleavage. Further refinement of the knowledge regarding what length and type of siRNA's were optimal for their function improved the resulting RNAi techniques to the point where their use could be expanded from flies, worms and plants to the use in mammalian cells (Sen).

Still, the question by which mechanisms exactly siRNA's had the potential to induce the cleavage of target mRNA's was left unanswered. The first step in elucidating this mystery was the discovery that the process of converting double-stranded RNA to siRNA and the process of cleaving the target

mRNA were carried out by two separate pathways. The pathway responsible for cleaving the target mRNA is currently known as the RNA-induced silencing complex, or RISC, and was referred to as the effector phase. The process of reverting double-stranded RNA to siRNA was called the initiation phase (Sen).

Regarding the initiator molecule, it was discovered relatively easily through determining the specifications the molecule would need to fulfil its function. It was identified through a process of isolation and coined Dicer. As additional proof, it was verified that Dicer is present in all organisms which employ the RNAi pathway and depletion of this enzyme blocks the pathway (Sen).

In an attempt to identify the RISC-complex, the proteins argonaut 1 and 2 were isolated. It wasn't until 2004 that it was discovered that, even though all argonaut proteins could bind to the RISC complex, only argonaut 2 displayed slicer activity (Sen).

In 2005, it was established that, rather than being effected by helicases, it was the argonaut 2 protein that was responsible for separating the strands of the double-stranded RNA. In that same year, the entirety of the RISC complex was fully identified. It consists of three proteins, the already mentioned Dicer and Argonaut proteins, as well as what was already known as the HIV transactivating response RNA-binding protein, TRBP. This ternary complex proved to be sufficient for RNAi activity once it recruited a siRNA (Sen).

To summarise, RNAi is a pathway capable of supressing or shutting down gene expression through the RISC-complex, which utilises siRNA to identify the sequence of its target. Its use in mammalian cells is already a possibility. However, the knowledge surrounding this technique is relatively recent and likely still far from complete. Next, we will explore the possibility of use in human medicine.

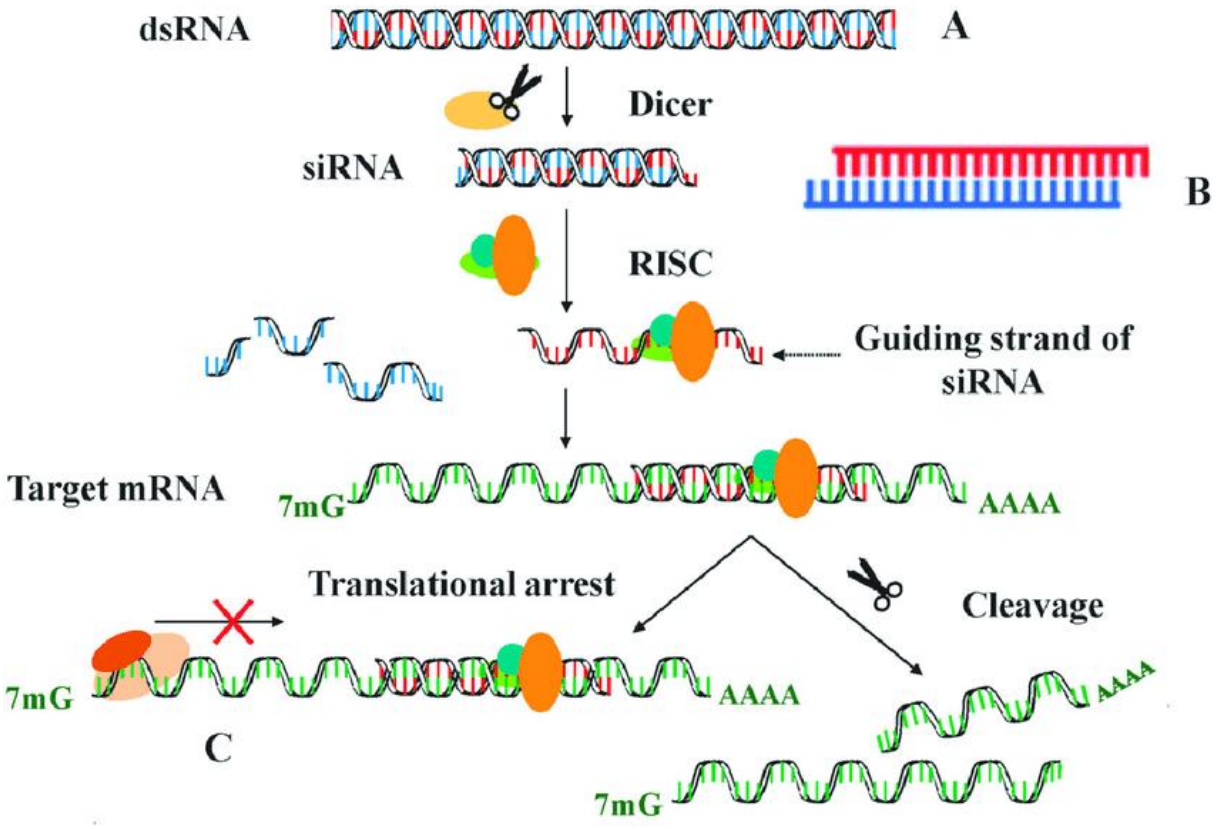

Figure 2. Steps of the RNAi pathway (Guo)

RNAi in modern medicine

Many of the characteristics of RNAi therapeutics lead it to have the potential to open a whole new world into treatment options in modern medicine. Among its strengths are its simplicity, reliability and the exceedingly high degree of target-specificness it can reach (Bumcrot).

In theory, as long as the disease depends on or is caused by the synthesis of a certain protein, be it wildtype or mutated, it should be possible to target its synthesis through the RNAi pathway as long as the sequence of the mRNA that translates to the protein in question is known (Bumcrot).

Once the sequence is known, siRNA's can be synthesized, which then only need to be delivered to the right location for it to activate the RNAi pathway, which will in turn suppress the production of the harmful protein (Bumcrot).

This obviously leads to the question; can this system be used to suppress the production of the proteins involved in rheumatoid arthritis pathology? What proteins would these be and what benefits would this have compared to the already existing medications?

But first, we will touch upon the progress already made in the field of RNAi therapeutics in human medicine. Human trials for RNAi-based treatments have already been conducted during the past ten years (Davis) and the very first of such medication has already been approved to be introduced onto the market, Patirisan (Hu). This drug targets and reduces the synthesis of the protein transthyretin, combating the deposits of this protein that occur in hereditary transthyretin amyloidosis (Adams). At the moment, thirty more candidate drugs are at various stages in the long and careful process of human trials (Hu). Based on these facts, we can conclude that, with the usual scientific reserve, that RNAi therapies are safe and effective in the battle against various diseases.

Furthermore, there exist roughly two ways to approach the use of the RNAi pathway in medicine, dependent on the type of disease it is supposed to combat. If the disease in question is caused by a mutation or otherwise deviant mRNA, it is possible to target this mutation product specifically by utilising the difference in sequence (Alnylam). The wildtype allele should not be affected due to the high specificity of the RNAi pathway (Uprichard). In case the disease is not caused by a mutation, but is still dependent on one or more proteins or mRNA's, it should be possible to suppress the synthesis of the wildtype proteins as well (Alnylam). The latter would have a much broader effect, which means it is accordingly effective in broader types of diseases, such as deviant levels of cholesterol and blood pressure (Alnylam). This leads to the next questions, which type of drugs would be best used in treating rheumatoid arthritis?

Potential targets

Rheumatoid arthritis is a disease with complex mechanisms which depend heavily on a wide array of immune cells and molecules (Glocker). And as a general rule, none of the involved cells carry mutations within their genetic material (Stanislavsky). However, the cell surface receptors and antibodies might have specific sequences that could be targeted.

It is certainly a good possibility to target the creation of new molecules and cell components. This will, however, have a rather broad effect on the overall immune system. Therefore, it is important to select the targets that come with the lowest possible among of collateral damage once suppressed.

As for the question whether it is possible to target specific receptors or antibodies, the following facts need to be considered.

Rheumatoid arthritis is mediated by many different types of antibodies (Bas). Most known are the rheumatoid factor, the first RA-involved antibody discovered, ACPA, which attacks citrullination process, and anti-CarP antibodies, which attack the carbamylation process (van Delft). In some cases, these antibodies can be detected years before the onset (van Delft), but not every individual with these antibodies develop the disease (hhs.edu). However, if it does develop, there is usually already a wide variety of these antibodies and their isotypes present within the immune system (van Delft). Certain natural processes that normally enhance the effectiveness of the immune system, including isotype switching and avidity maturity, inevitably contribute to the effectivity of the RA pathology as well (van Delft). It is important to note that this profile of antibodies differs from patient to patient (van Delft).

Second, past research has experimented with the possibilities of specifically targeting disease-specific T-cell receptors while leaving the healthy T-cell untouched. While this was successful in simple models with a previously known T-cell receptor sequence, there has been no success so far with replicating these results in more complex models of spontaneously induced auto-immune diseases, in which the variety of T-cells is much larger and not known beforehand (Kotzin). As this is generally the case in rheumatoid arthritis, targeting specific T-cell receptors is not a viable option according to current knowledge.

Instead, it would be in the patients' best interest to focus on treatments that can be realised within a reasonable amount of time. As already mentioned, another, and more reliable possibility would be to target the immune cells and cytokines that play a role in the immune reaction that eventually attacks the synovial tissues. The cytokines IL-1, IL-6, IL-17 and TNF-alpha have already been established as being pivotal in the disease pathways. Immune cells such as the T-cells and B-cells should also be considered as potential targets. However, since it is not yet a viable option to target specific receptors, it would mean that the production or function of all the T-cells or B-cells within the body would have to be targeted. As the most significant strength of RNAi therapies would be their specificity, it would remain the question whether that course of action will have any benefit in regards to the already existing treatments.

It is important to note that the already existing therapies do not shut down the T-cells themselves or in their entirety, but rather partially block the costimulatory signals that activate the T-cells in order to render these T-cells inactive (Rosenblum). This process seems to have a certain specificity for auto-reactive cells (Rosenblum). Targeting such supporting pathways would make for a far more specific treatments than any one that interferes with T-cells on a broad level, since T-cells are vital to the functioning of the immune system.

B-cells are also essential in the disease mechanisms, as they produce the antibodies and cytokines that play a role in the attack on the synovial tissues (Silverman). As of such, it is logical to conclude that lowering the effectivity or production of B-cells will lead to a decrease in the auto-immune activity. B-cell depletion in auto-immune diseases is already practiced through a medication called Rituximab (Clark). While effective, it targets entire subsets of B-cells and can thus be argued to be a medication with a broad effect and it still has potential points of improvement (Clark). Alternatively, it is still a possibility to ascertain which of the B-cells' products contribute to the RA pathology and target those. Concluding, targeting B-cells falls within the possibilities to be considered, however, it might not necessarily be an as promising target compared to the more specific alternatives.

That brings us to the next option; targeting specific cytokines. To give a clearer picture of these molecules, here follows a short description of each of the aforementioned cytokines.

IL-1 is a family of cytokines that is strongly associated with the innate immune system. While it increases both the nonspecific resistance to infection and the response to foreign antigens, it is also seems to be correlated with inflammation-induced damage more so than any other known cytokine (Dinarello). In line with its association with the innate immune system, the effects of this cytokine are aimed for non-specific targets rather than a specific type of invader or threat (Dinarello). Research so far has implicated high levels of IL-1 with the induction of rheumatoid arthritis and low levels of IL-1 with lower amounts of joint damage, thus proving it plays a large role in the pathology of rheumatoid arthritis (Kay). Given that its reduction has been proven to be correlated with reduced joint damage, it holds strong potential as a target for RNAi therapies.

IL-6 is acutely produced and transiently present in case of tissue injury or infection (Tanaka). It has pleiotropic functions and induces the production of many immune system-associated proteins (Tanaka). It has previously been established that suppression of this cytokine though synthesized antibodies is an effective treatment against rheumatoid arthritis (Tanaka). For this cytokine, the main question would be whether reverting the treatment into RNAi therapies would have beneficial effects compared to the treatment with antibodies.

IL-17 is a cytokine capable of inducing powerful immune reactions, including but not limited to the mobilisation of neutrophils (Zenobia). Recent research has also associated it with Th17-cells (Gaffen). Although there is some disagreement between various studies, there is a strong indication this cytokine plays a large role in RA pathogenesis (Gaffen). Given its potent immune effects and its association with rheumatoid arthritis, this cytokine is a promising candidate for becoming a target in RNAi therapies.

TNF-alpha has a wide array of effects on the immune system (Vasanthi). Drugs targeting this cytokine are already in use in patients that do not respond favourable to DMARD's and have been shown to improve these patients' conditions (Jin). As such, this cytokine should certainly be included as a potential target for RNAi therapies.

Future research might be able to find even more cytokines connected to RA pathology, providing even more targets and expanding the field of RA treatments even further.

Benefits, potential and downsides of RNAi therapeutics

Now that both the mechanisms of RNAi and several potential targets in the battle against rheumatoid arthritis are clear, it's time to move to both the benefits and downsides of these potential new therapies in comparison to the already existing ones.

In essence, the RNAi pathway is a cellular mechanism that serves to regulate gene expression post-transcriptionally (Pauley), rather than targeting the resulting protein products. Often, this mechanism bring about more effective down-regulation of targeted gene products than techniques such as ribozymes and DNA oligonucleotides (Aagaard). Due to this high efficiency, or potency, these drugs should be able to carry out their function at a much lower concentration, which is an important consideration in the creation and administration of drugs (Aagaard).

Besides its potency, another strength of the RNAi pathway is its specificity. As stated before, it should even be possible to target a mutated, disease-causing allele, differing only one or a few nucleotides from the wildtype, while leaving the healthy allele untouched (Uprichard). This possibility has already

been demonstrated in previous research (Uprichard). It is important to note that despite its specificity, it has been shown that suppression of off-target genes can still occur if there's sufficient homology to the target gene (Aagaard). Three questions remain about these off-target effect; can it be resolved with further refinement of the technique, whether the minimally achievable off-target effects are tolerable in regards to the disease it treats, and whether different mechanisms of the off-target effects, in contrast with the mechanisms of protein-targeted drugs, hold new possibilities to be explored.

Additionally, the RNAi pathway has the potential to target molecules that have previously been impossible to target through the already established techniques utilising antibodies or other small molecules, since it RNAi targets the production pathway rather than the effecting pathway of the disease-causing protein itself (Hu).

Furthermore, the delivery methods of RNAi therapeutics merit some further explanation to fully appreciate its potential. These methods can be roughly divided into two types. One consists of delivering the siRNA into the cells (Aagaard). Whereas this method is simple and still potent, it would require constant administration to maintain its effects (Aagaard). It is the second method that could be an answer to this almost universal problem in treating chronic disease.

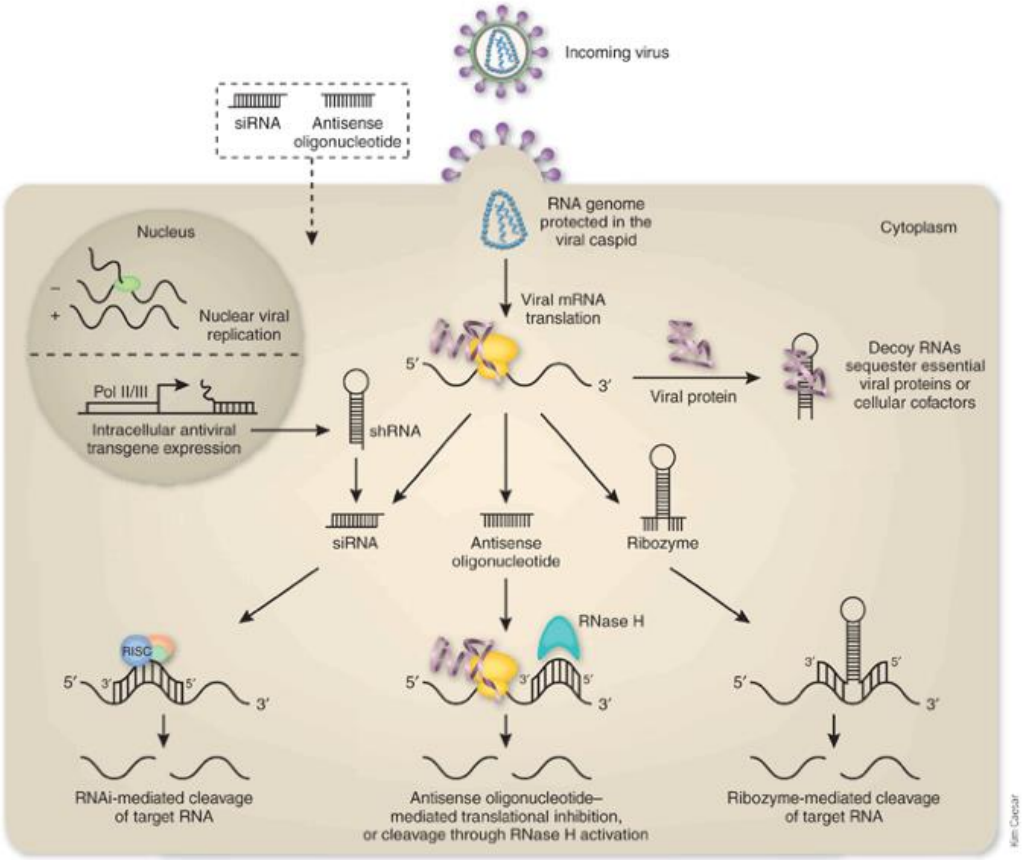

Figure 3. mechanisms of viral RNAi delivery (Haasnoot)

This second method is based on the delivery of genes meant to start producing short hairpin RNA through a viral vector into the nucleus (Aagaard). The resulting shRNA will be transported to the cytoplasm where it will be converted into siRNA, which will then trigger the RNAi pathway to target the mRNA's of interest (Aagaard). This method, even though much more complicated and more risky, would come with the potential that only a few or even a single treatment would be enough to maintain the effects on long-term, since it reprogrammes cells into producing the needed molecule themselves (Aagaard). This could not only considerably bring down the cost of treatment but also prevent life-long dependency on medication.

However, this DNA-based, virally delivered therapy is still firmly within the developing phases, as there are still many obstacles to overcome and safety concerns to be addressed (Pauley). Still much is to be discovered in the areas of how to deliver the vectors to the right cells, finding and effecting the optimal features of the molecules and the prevention of immune responses to the viral molecules (Pauley). Altogether, this method of delivery, while holding promise, is still needs much research and refinement before it is ready for practical and public use against the variety of diseases it is theoretically capable of combating.

In summarization, RNAi therapies hold both much promise as it holds many yet-to-be overcome obstacles. However, it has made rapid progress in the recent past and might continue to do so. Time will have to tell.

Will RNAi therapeutics contribute in the battle against rheumatoid arthritis?

This brings us to the final and ultimate question about RNAi therapeutics in rheumatoid arthritis; will it contribute something new and useful to the field that conventional medicine cannot yet? The answer is simple; likely so, but it is still too early to tell. This essay has posed many questions and possibilities, but not yet enough answers and certainties.

Theoretically, RNAi therapeutics have a higher potency and target-specificity than already existing RA treatments targeting the same molecules. However, it remains to be seen if theory carries on in practice. In the same manner, it is theoretically possible to integrate the medication into the

patients' genetic code to prevent life-long medication dependency, bringing this into practice is yet considered to be too hazardous.

What matters in the end is that these possibilities become realities that can be used to improve the lives of thousands of patients. Until then, speculation remains speculation. So perhaps a better question would be; is the potential worth the time and effort that will go into overcoming these obstacles?

It might still be decades until RNAi therapeutics becomes widespread and effective in modern medicine, and even longer before it reaches optimal efficiency. However, given its promise and the strides already made, the answer to the question would be yes.

After all, no treatment has been created, investigated and approved within a day. Much of the knowledge we take for granted today has taken decades if not centuries to unravel. Researchers should keep looking in the direction of RNAi in their work against rheumatoid arthritis, if not for the smaller benefits on the short-term, then for the distant possibility that some day in the far future, we'll find a way to incorporate medication into the DNA that produces siRNA's specific against the each patient's unique array of auto-immune T-cell receptors.

Literature list

Aagaard, L. and Rossi, J., 2007. RNAi therapeutics: Principles, prospects and challenges. *Advanced Drug Delivery Reviews*, 59(2-3), pp.75-86.

Adams, D., Gonzalez-Duarte, A., O'Riordan, W., Yang, C., Ueda, M., Kristen, A., Tournev, I., Schmidt, H., Coelho, T., Berk, J., Lin, K., Vita, G., Attarian, S., Planté-Bordeneuve, V., Mezei, M., Campistol, J., Buades, J., Brannagan, T., Kim, B., Oh, J., Parman, Y., Sekijima, Y., Hawkins, P., Solomon, S., Polydefkis, M., Dyck, P., Gandhi, P., Goyal, S., Chen, J., Strahs, A., Nochur, S., Sweetser, M., Garg, P., Vaishnaw, A., Gollob, J. and Suhr, O., 2018. Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. *New England Journal of Medicine*, 379(1), pp.11-21.

Aho, K. and Heliövaara, M., 2004. Risk factors for rheumatoid arthritis. *Annals of Medicine*, 36(4), pp.242-251.

Alnylam. 2021. *RNAi Therapeutics | How RNA Interference Works | Alnylam® Pharmaceuticals*. [online] Available at: <https://www.alnylam.com/our-science/the-science-of-rnai/> [Accessed 8 August 2021].

Arthritis.org. 2021. [online] Available at: <https://www.arthritis.org/diseases/rheumatoid-arthritis> [Accessed 2 August 2021].

Bas, S., 2002. Diagnostic tests for rheumatoid arthritis: comparison of anti-cyclic citrullinated peptide antibodies, anti-keratin antibodies and IgM rheumatoid factors. *Rheumatology*, 41(7), pp.809-814.

Benjamin, O., Bansal, P., Goyal, A. and Lappin, S., 2021. *Disease Modifying Anti-Rheumatic Drugs (DMARD)*. [online] Ncbi.nlm.nih.gov. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK507863/> [Accessed 11 August 2021].

Bumcrot, D., Manoharan, M., Koteliansky, V. and Sah, D., 2006. RNAi therapeutics: a potential new class of pharmaceutical drugs. *Nature Chemical Biology*, 2(12), pp.711-719.

Carmona, L., Cross, M., Williams, B., Lassere, M. and March, L., 2010. Rheumatoid arthritis. *Best Practice & Research Clinical Rheumatology*, 24(6), pp.733-745.

Choy, E., Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis, *Rheumatology*, Volume 51, Issue suppl_5, July 2012, Pages v3–v11, https://doi.org/10.1093/rheu matology/kes113

Clark, E., 2021. *How does B cell depletion therapy work, and how can it be improved?*.

Crofford, L., 2013. Use of NSAIDs in treating patients with arthritis. *Arthritis Research & Therapy*, 15(S3).

Davis, M., Zuckerman, J., Choi, C., Seligson, D., Tolcher, A., Alabi, C., Yen, Y., Heidel, J. and Ribas, A., 2010. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature*, 464(7291), pp.1067-1070.

Dinarello, C., 2017. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunological Reviews*, 281(1), pp.8-27.

Gaffen, S., 2009. The role of interleukin-17 in the pathogenesis of rheumatoid arthritis. *Current Rheumatology Reports*, 11(5), pp.365-370.

Glocker, M., Guthke, R., Kekow, J. and Thiesen, H., 2005. Rheumatoid arthritis, a complex multifactorial disease: On the way toward individualized medicine. *Medicinal Research Reviews*, 26(1), pp.63-87.

Guo, J., Fisher, K., Darcy, R., Cryan, J. and O'Driscoll, C., 2010. Therapeutic targeting in the silent era: advances in non-viral siRNA delivery. *Molecular BioSystems*,.

Haasnoot, J., Westerhout, E. and Berkhout, B., 2007. RNA interference against viruses: strike and counterstrike. *Nature Biotechnology*, 25(12), pp.1435-1443.

Harrington, R., Al Nokhatha, S. and Conway, R., 2020. JAK Inhibitors in Rheumatoid Arthritis: An Evidence-Based Review on the Emerging Clinical Data . *Journal of Inflammation Research*, Volume 13, pp.519-531.

A. Hitchon, C., 2011. The Synovium in Rheumatoid Arthritis. *The Open Rheumatology Journal*, 5(1), pp.107-114.

Hu, B., Weng, Y., Xia, X., Liang, X. and Huang, Y., 2019. Clinical advances of siRNA therapeutics. *The Journal of Gene Medicine*, 21(7). *Everyday Health*. [online] EverydayHealth.com. Available at: <https://www.everydayhealth.com/hs/rheumatoid-arthritis-treatment-management/dmards-biologics/> [Accessed 11 August 2021].

Jin, J., Chang, Y. and Wei, W., 2010. Clinical application and evaluation of anti-TNF-alpha agents for the treatment of rheumatoid arthritis. *Acta Pharmacologica Sinica*, 31(9), pp.1133-1140.

Johns Hopkins Arthritis Center. 2021. *Rheumatoid Arthritis : Symptoms, Diagnosis and Treatment*. [online] Available at: <https://www.hopkinsarthritis.org/arthritis-info/rheumatoid-arthritis/> [Accessed 2 August 2021].

Kay, J., 2004. The role of interleukin-1 in the pathogenesis of rheumatoid arthritis. *Rheumatology*, 43(suppl_3), pp.iii2-iii9.

Kelly, C. and Hamilton, J., 2006. What kills patients with rheumatoid arthritis?. *Rheumatology*, 46(2), pp.183-184.

Köhler, Günther, Kaudewitz and Lorenz, 2019. Current Therapeutic Options in the Treatment of Rheumatoid Arthritis. *Journal of Clinical Medicine*, 8(7), p.938.

Kotzin, B. and Kappler, J., 1998. Targeting the T cell receptor in rheumatoid arthritis. *Arthritis & Rheumatism*, 41(11), pp.1906-1910.

Kristen, A., Ajroud-Driss, S., Conceição, I., Gorevic, P., Kyriakides, T. and Obici, L., 2019. Patisiran, an RNAi therapeutic for the treatment of hereditary transthyretin-mediated amyloidosis. *Neurodegenerative Disease Management*, 9(1), pp.5-23.

Kurkó, J., Besenyei, T., Laki, J., Glant, T., Mikecz, K. and Szekanecz, Z., 2013. Genetics of Rheumatoid Arthritis — A Comprehensive Review. *Clinical Reviews in Allergy & Immunology*, 45(2), pp.170-179.

Mayo Clinic. 2021. *Rheumatoid arthritis - Symptoms and causes*. [online] Available at: <https://www.mayoclinic.org/diseases-conditions/rheumatoid-arthritis/symptoms-causes/syc-20353648> [Accessed 2 August 2021].

Mayoclinic.org. 2021. *Rheumatoid arthritis - Diagnosis and treatment - Mayo Clinic*. [online] Available at: <https://www.mayoclinic.org/diseases-conditions/rheumatoid-arthritis/diagnosis-treatment/drc-20353653> [Accessed 2 August 2021].

McWilliams, D., Rahman, S., James, R., Ferguson, E., Kiely, P., Young, A. and Walsh, D., 2019. Disease activity flares and pain flares in an early rheumatoid arthritis inception cohort; characteristics, antecedents and sequelae. *BMC Rheumatology*, 3(1). [Accessed 22 August 2021].

McWilliams DF, Walsh DA. Pain mechanisms in rheumatoid arthritis. Clin Exp Rheumatol. 2017 Sep-Oct;35 Suppl 107(5):94-101. Epub 2017 Sep 29. PMID: 28967354./> [Accessed 22 August 2021].

nhs.uk. 2021. *Rheumatoid arthritis*. [online] Available at: <https://www.nhs.uk/conditions/rheumatoid-arthritis/> [Accessed 2 August 2021].

Oh, Y. and Moon, K., 2020. Predictors of Flares in Patients with Rheumatoid Arthritis Who Exhibit Low Disease Activity: A Nationwide Cohort Study. *Journal of Clinical Medicine*, 9(10), p.3219.

Pauley, K. and Cha, S., 2013. RNAi Therapeutics in Autoimmune Disease. *Pharmaceuticals*, 6(3), pp.287-294.

Qin, B., Yang, M., Fu, H., Ma, N., Wei, T., Tang, Q., Hu, Z., Liang, Y., Yang, Z. and Zhong, R., 2015. Body mass index and the risk of rheumatoid arthritis: a systematic review and dose-response meta-analysis. *Arthritis Research & Therapy*, 17(1).

Results, U., 2021. *Understanding Rheumatoid Arthritis Lab Tests and Results*. [online] Hospital for Special Surgery. Available at: <https://www.hss.edu/conditions_understanding-rheumatoid-arthritis-lab-tests-results.asp> [Accessed 6 August 2021].

Rodriguez, D. and Alexa Meara, M., 2020. *The Risks of Untreated Rheumatoid Arthritis | Everyday Health*. [online] EverydayHealth.com. Available at: <https://www.everydayhealth.com/hs/rheumatoid-arthritis-treatment-management/untreated-ra-risks/> [Accessed 2 August 2021].

Rosenblum, M., Gratz, I., Paw, J. and Abbas, A., 2012. Treating Human Autoimmunity: Current Practice and Future Prospects. *Science Translational Medicine*, 4(125), pp.125sr1-125sr1.

Sen, G. and Blau, H., 2006. A brief history of RNAi: the silence of the genes. *The FASEB Journal*, 20(9), pp.1293-1299.

Shaw, M., Collins, B., Ho, L. and Raghu, G., 2015. Rheumatoid arthritis-associated lung disease. *European Respiratory Review*, 24(135), pp.1-16.

Sierakowski, S. and Cutolo, M., 2011. Morning symptoms in rheumatoid arthritis: a defining characteristic and marker of active disease. *Scandinavian Journal of Rheumatology*, 40(sup125), pp.1-5.

Silverman, G. and Carson, D., 2003. *Arthritis Research & Therapy*, 5(Suppl 4), p.S1.

Stanislavsky, A., 2021. *Rheumatoid arthritis | Radiology Reference Article | Radiopaedia.org*. [online] Radiopaedia.org. Available at: <https://radiopaedia.org/articles/rheumatoid-arthritis> [Accessed 15 August 2021].

Tanaka, T., Narazaki, M. and Kishimoto, T., 2014. IL-6 in Inflammation, Immunity, and Disease. *Cold Spring Harbor Perspectives in Biology*, 6(10), pp.a016295-a016295.

Uprichard, S., 2005. The therapeutic potential of RNA interference. *FEBS Letters*, 579(26), pp.5996-6007.

van Delft, M. and Huizinga, T., 2020. An overview of autoantibodies in rheumatoid arthritis. *Journal of Autoimmunity*, 110, p.102392.

VASANTHI, P., NALINI, G. and RAJASEKHAR, G., 2007. Role of tumor necrosis factor-alpha in rheumatoid arthritis: a review. *APLAR Journal of Rheumatology*, 10(4), pp.270-274.

Zenobia, C. and Hajishengallis, G., 2015. Basic biology and role of interleukin-17 in immunity and inflammation. *Periodontology 2000*, 69(1), pp.142-159.

Zhang, Y., Bo, L., Zhang, H., Zhuang, C. and Liu, R., 2014. E26 Transformation-Specific-1 (ETS1) and WDFY Family Member 4 (WDFY4) Polymorphisms in Chinese Patients with Rheumatoid Arthritis. *International Journal of Molecular Sciences*, 15(2), pp.2712-2721.