Dr Christine Pham, a rheumatologist at the Washington University School of Medicine, is designing strategies to safely deliver nanomedicine in the treatment of inflammatory diseases, such as arthritis. Her strategies promise to target pathways associated with inflammation, while leaving other vital immune responses unaffected.

Rheumatoid arthritis (RA) is a chronic, incapacitating disease of the joints, characterised by painful swelling and progressive damage to the connective tissues affected. This is a major cause of disability and morbidity (the condition of being diseased), particularly in ageing populations. Although the underlying causes remain the subject of debate among researchers and clinicians, the symptoms are clear and well-documented.

Inflamed pain
An influx of immune cells from the bloodstream leads to swelling and inflammation of the synovial lining of the joints (the membrane that defines the joint space and retains the lubricating synovial fluid). This promotes the release of inflammatory molecules and degradative enzymes, resulting in damage to the connective tissue and the underlying bone of the joint in a vicious cycle that is painful and debilitating to the sufferer.

Although there are several treatment options for RA sufferers, the therapy is often associated with severe adverse effects and is ineffective in a large segment of the patient population. Dr Pham leads her team in investigating alternative treatment options, targeting the inflammation that underlies the pathogenesis of rheumatoid arthritis and other inflammatory conditions.

Interfering with RNA
RNA interference (RNAi) is a relatively new technique for preventing the DNA that encodes specific genes from being transcribed and translated into proteins. In the normal process, DNA is first transcribed into messenger RNA (mRNA), which can be translated into proteins by the cellular machinery. In mammalian cells, this is a carefully orchestrated process that controls a multitude of cellular activities and protects against hijacking of the cellular machinery by viruses. RNAi techniques exploit intrinsic cell mechanisms to ‘silence’ a gene by delivering small interfering RNA (siRNA) that binds to complementary strands of mRNA,
Rheumatoid arthritis and osteoarthritis are chronic, incapacitating diseases of the joints. Dr Pham is investigating novel treatment approaches to these conditions, as well as other pathologies of immune system dysfunction.

The Pham/Wickline team was able to show that intravenously injected peptide-siRNA nanocomplexes localised to the immune cells in the inflamed joints of an RA mouse model, while free siRNA, which has a half-life of minutes, was unable to do so. The peptide-siRNA complexes were not sequestered in “off-target” organs such as the liver or spleen, suggesting focused delivery of siRNA to the region of interest, the inflamed joint.

**THAT HITS THE SPOT**
Having shown they could target the right location, the team progressed to inserting the siRNA specific for the protein p65—a component of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway. The p65 protein is normally held in reserve in cells by the presence of inhibitors until activated during an immune response. When activated, it promotes the production of a wide range of pro-inflammatory molecules called cytokines—essential for the correct immune response to infection, but also extensively implicated in RA pathogenesis. Following injection of inflammatory arthritis in a mouse model, a three-day dosing regime of the peptide-p65 siRNA complexes “rapidly stabilised ankle swelling and significantly suppressed arthritis score”. Along with the significantly attenuated disease score there was a marked reduction in the recruitment of leucocytes (white blood cells, the primary players in an immune response), suppression of inflammatory cytokine production as well as a reduction in bone erosion and cartilage damage. These effects were not seen with intravenous administration of free siRNA, which the researchers posit is due to the inability of un-complexed material to enter the cell and block the target mRNA.

While the complex was effective at targeting only the area of interest it has no secondary effects elsewhere. This is particularly evident in the immune response, which was sufficiently robust despite the treatment regime, showing that this treatment has the potential to target just the elements of inflammation that have gone awry in this model of arthritis while sparing the immune system to respond to infection as needed.

**EXPANDING THE TREATMENT HORIZON**
While the researchers have taken advantage of the enhanced vascular permeability in RA (whereby certain molecules naturally accumulate more in inflamed than normal tissues through “leaky” blood vessels) to deliver siRNA intravenously, they also recognise the possibility of utilising the system to explore local delivery of the peptide-siRNA complex to avascular tissues that are otherwise inaccessible through systemic delivery.

Further processing of this basic concept could open the way for other disease processes to be targeted using the same technology, providing novel treatments for osteoarthritis, another extremely common form of arthritis with limited disease treatment options. Early work conducted in collaboration with Dr Sandell has shown that peptide-siRNA nanocomplexes targeting p65 may be effective in reducing cartilage cell death and damage—characteristics of osteoarthritis. The avascular cartilage is normally inaccessible even to locally delivered drugs due to the dense matrix that prevents their penetration, posing a challenge to osteoarthritis treatment. The peptide-siRNA complex can freely and deeply enter the cartilage matrix due to its size, thus overcoming a major drug delivery obstacle. As such, it presents a real option for osteoarthritis treatment.

Dr Pham and her collaborators are confident that the site-specific gene-silencing activity of this platform, coupled with the minimal collateral damage to other parts of the immune system may have, “real translational potential for the treatment of many inflammatory processes beyond arthritis.”