

Title: *Vaccination against nerve growth factor is an effective pain treatment in murine osteoarthritis*

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Pain is the main symptom of osteoarthritis (OA), with nearly  $\frac{3}{4}$  of patients reporting constant pain (ARUK report 2017). Within the last decade, nerve growth factor (NGF) has emerged as a promising target for pain in OA. NGF mRNA is upregulated in whole knee joints of mice at the time of pain (McNamee 2010), and late OA pain can be blocked with the soluble NGF receptor (TrkA) (McNamee 2010). In humans, neutralising antibodies to NGF significantly suppress pain associated with late-stage OA (Lane 2010). Antibody therapy in OA is likely to be limited by cost. Vaccine technology potentially provides a mechanism to produce endogenous polyclonal anti-NGF antibodies with similar efficacy but at a lower cost.

We designed a vaccine targeting NGF using a novel technology in which murine NGF is presented on an immune-optimised virus-like particle (VLP) derived from cucumber mosaic virus. At 7 weeks of age 20 male C57BL6 mice were inoculated with the NGF-VLP construct by subcutaneous injection to the dorsal region, while 20 mice received the control-VLP mock vaccine. Mice were subsequently boosted two weeks later, thus one week before surgery, and again at 5 and 9 weeks after surgery. An additional sentinel cohort of 10 mice (n=5 each group) was used to assess blood titres of NGF antibodies across the duration of the study. Blood was sampled at different time points before and after surgery. The serum fractions were isolated and serial dilutions tested for anti-NGF immunoglobulin G (IgG) by enzyme-linked immunosorbent assay (ELISA), recording the fold dilution that yields the half-maximal absorbance (OD50). All mice (not including sentinels) underwent partial menisectomy at 10 weeks of age to induce OA. Painful behaviour was assessed daily for the first three days then weekly using the Linton incapacitance test, which measures weight distribution difference across hind limbs. All pain behaviour assessments were evaluated by a single investigator (IvL) who was blinded to the vaccination protocol. At the conclusion of the study, both contralateral and ipsilateral knees were imaged using microCT before being harvested for histology (n=24 mice), and RNA expression (n=16). Bilateral dorsal root ganglia (L3, L4) were collected from all animals for RNA expression. The sentinel cohort was used to obtain bone marrow, spleen and lymph nodes to investigate potential long-term immune responses.

Anti-NGF titres increased in response to NGF-VLP vaccination, with an OD50 of  $10^3$  compared with  $<10^{1.7}$  in the control group. Levels of anti-NGF increased with each boost and were maintained for around 3 weeks on each occasion. Mock-vaccinated animals started to display painful behaviour at week 10 post-surgery. NGF-VLP vaccinated animals were significantly protected from painful behaviour between weeks 11-13 which corresponded to high levels of serum anti-NGF IgG. By week 14 anti-NGF titres had dropped and the NGF-VLP vaccinated animals started to display painful behaviour and were indistinguishable from the VLP-

vaccinated control group. Molecular and histological analysis of the joints is in progress.

We have developed an effective vaccination against NGF that is analgesic in mice with OA-related pain. Vaccination to NGF provides an interesting alternative to blocking NGF using recombinant antibody and is potentially more cost-effective. Given current safety concerns using recombinant anti-NGF therapy, it is unlikely that this approach is ready for development in patients in the near future. However, as autoantigen VLP-vaccines tend not to induce a long-lived plasma cell population, the antibody responses generated are reversible in the absence of booster immunizations and are apparently not stimulated by endogenous NGF. Furthermore, such therapeutic immune responses can potentially be 'tailored,' to provide control over anti-NGF titres both immediately after and between vaccine boosts.