A multi-centre, double blind, randomised, placebo-controlled, parallel group, phase II trial to determine the efficacy of intra-nodular injection of anti-TNF to control disease progression in early Dupuytren's disease, with a dose response

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Trial Title: A multi-centre, double blind, randomised, placebo-controlled, parallel group, phase II trial to determine the efficacy of intra-nodular injection of anti-TNF to control disease progression in early Dupuytren's disease, with a dose response.

Lay title: Repurposing anti-TNF for treating Dupuytren's disease.

Short title: RIDD

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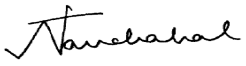

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, OCTRU, the Investigator Team, host organisations, the MHRA and the Research Ethics Committee, unless authorised to do so.

Conflict of interest

Professor Sir Marc Feldman and Professor Jagdeep Nanchahal have interests in the company funding the purchase of adalimumab, the investigational medicinal product to be used in this trial.

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1. KEY TRIAL CONTACTS

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2. SYNOPSIS

Trial Title	A multi-centre, double blind, randomised, placebo-controlled, parallel group, phase II trial to determine the efficacy of intra-nodular injection of anti-TNF to control disease progression in early Dupuytren's disease (DD), with a dose response.	
Lay title	Repurposing anti-TNF for treating Dupuytren's disease	
Short title	RIDD	
Clinical Phase	Phase II	
Trial Design	A multi-centre, double blinded, randomised, placebo-controlled, parallel group, phase II trial, with a dose response.	
Trial Participants	**Part 1**. Dose response: Patients with **established Dupuytren's disease** who are scheduled for surgery for this disease. **Part 2.** Randomised controlled trial: Patients with **early Dupuytren's disease.**	
Planned Sample Size	**Part 1**. Up to 40 participants **Part 2.** Up to 200 participants	
Treatment duration	**Part 1**. One-off treatment given approximately 2 weeks before surgery. **Part 2.** 4 treatments over 9 months, at baseline, 3, 6 & 9 months with 18 month follow-up.	
Follow up duration	**Part 1**. 12 weeks after surgery **Part 2**. 18 months	
Planned Trial Period	**Part 1**. 12 months **Part 2**. 45 months	
	Objectives	**Outcome Measures**
Part 1. **Primary Objective**	To establish an effective dose of adalimumab for downregulating the myofibroblast phenotype in participants with Dupuytren's disease.	Expression of mRNA for α-SMA from participants on each treatment.
Secondary Objectives	To determine the effectiveness of adalimumab for myofibroblast inhibition in participants with Dupuytren's disease and nodules. Monitor for adverse events	For participants on each treatment 1. Expression of mRNA for COL-1A1, COL-3A1 and cadherin 11 2. Levels of α-SMA and collagen proteins. 3. Hardness of selected nodule 4. Ultrasound imaging of nodule size. Adverse event assessment comparing active and placebo groups using visual inspection of injection site, surgery site and laboratory reports.

	To assess whether adalimumab affects the healing of the surgical incisions or subsequent scarring.	Visual comparison of surgical wounds using hand photographs of all participants on each treatment.
Tertiary Objectives	To determine circulating levels of adalimumab and antibodies to adalimumab in the blood. To assess if DD injection therapy would be acceptable to patients, and to evaluate the health economics of current clinical care.	Circulating levels of adalimumab and antibodies to adalimumab in the blood. Analysis of questionnaire data on the acceptability of the injection and their return to paid work. Analysis of EQ-5D-5L
Exploratory Objective	To investigate changes in other molecular markers relevant to the progression of Dupuytren's disease.	Change in level of the markers.

Part 2. **Primary Objective**	To determine if injection with adalimumab is superior to placebo injection of normal saline in controlling disease progression in participants with early Dupuytren's disease.	Change in hardness of selected nodule for participants on each treatment between baseline and 12 months after first treatment.
Secondary Objectives	To determine if injection with adalimumab is superior to placebo injection of normal saline in controlling disease progression in participants with early Dupuytren's disease.	Change in hardness of selected nodule for participants on each treatment at baseline, 3, 6, 9, 12 & 18 months after first treatment.
	To compare the development of Dupuytren's nodules and associated cord, flexion deformities of the fingers and impairment of hand function for participants on each treatment.	For participants on each treatment. 1. Ultrasound imaging of nodule size. 2. Range of motion of the affected digit. 3. Grip strength. 4. Participant Reported Outcomes: Michigan Hand Outcomes Questionnaire (MHQ) 5. Participant identified activity most restricted by DD scored on a scale of 1-10. 6. Progression to surgery of the digit being assessed
	To assess acceptability of injection to patients	Injection experience
	Monitor for adverse events	Adverse event monitoring comparing active and placebo

		groups using visual inspection of injection site.
Tertiary Objectives	To assess if early DD injection therapy represents good value for money compared to current clinical care. To determine circulating levels of adalimumab and antibodies to adalimumab in the blood.	Analysis of EQ-5D-5L data to estimate utilities using quality-adjusted life years (QALYs) from participants on each treatment. Analysis of resource use data to estimate the cost of each treatment. Monitor circulating levels of adalimumab and antibodies to adalimumab in the blood.
Exploratory Objective	To investigate changes in other molecular markers relevant to the progression of Dupuytren's disease	Change in level of the markers.
Investigational Medicinal Products	Adalimumab (anti-TNF) and Placebo	
Formulation, Dose, Route of Administration	Administration will be by subcutaneous injection into the DD nodule. Adalimumab is provided as a clear solution in two strengths – either 40mg in 0.8ml or 40mg in 0.4ml. The formulation will not be diluted. When mass and volume are stated together the corresponding formulation will be used. If the volume is not stated either formulation may be used and will be recorded. **Part 1**. Dose cohorts include: * 15mg adalimumab in 0.3ml carrier or for placebo assigned participants 0.3ml saline * 35mg adalimumab in 0.7ml carrier or for placebo assigned participants 0.7ml saline * 40mg adalimumab in 0.4ml carrier or for placebo assigned participants 0.4ml saline * Other doses, up to a maximum of 80mg and a maximum of 0.8ml carrier, may be used based on the results of interim analyses (see section 6). **Part 2.** 40mg adalimumab in 0.4 ml carrier, or for placebo assigned participants the equivalent volume of saline.	

3. ABBREVIATIONS

AE	Adverse Event
anti-TNF	anti-Tumour Necrosis Factor

AR	Adverse Reaction
CI	Chief Investigator
CRF	Case Report Form
CT	Clinical Trials
CTRG	Clinical Trials and Research Governance
CXR	Chest X-ray
DD	Dupuytren's disease
DSMC	Data and Safety Monitoring Committee
DSUR	Development Safety Update Report
EMA	European Medicines Agency
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GP	General Practitioner
IB	Investigators Brochure
ICF	Informed Consent Form
IgG	Immunoglobulin G
IMP	Investigational Medicinal Product
IRB	Independent Review Board
MHRA	Medicines and Healthcare products Regulatory Agency
MHQ	Michigan Hand Outcomes Questionnaire
NDORMS	Nuffield Department of Orthopaedics, Rheumatology and Musculosketal Science
NHS	National Health Service (UK)
NICE	National Institute for Health and Care Excellence
NRES	National Research Ethics Service
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
QALYs	Quality Adjusted Life Years
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SC	Safety Committee
SDV	Source Data Verification
SMA	Smooth muscle actin

SmPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
TSG	Oxford University Hospitals Trust / University of Oxford Trials Safety Group

4. BACKGROUND AND RATIONALE

Introduction

Dupuytren's disease (DD) is extremely common, affecting 4% of the general UK population (Hindocha, McGrouther & Bayat, 2009). The mean age of patients undergoing surgical treatment for the disease is 63 years (Chen, Shauver & Chung, 2011). It exhibits a strong hereditary basis (Hurst et al., 2009) and is much more prevalent in individuals of northern European ancestry, affecting 20% of people in Scotland over the age of 65 years (Ling, 1963). Factors such as extra-palmar disease, radial sided palmar and early onset disease have been shown to relate to the patients' Dupuytren's diathesis (Abe et al., 2004). The literature would suggest that the disease tends to be more aggressive in people with onset of DD before 50 years of age. Diagnosis is made by clinical examination of the palm. Between 35-50% of patients with early DD manifest as nodules on the palmar aspect of the hand, progress to develop finger contractures (Gudmundsson, Arngrimsson & Jonsson, 2001, Reilly, Stern & Goldfarb, 2005). The nodules are typically quiescent for a period and then become active, with progression to flexion deformities over a period of months (Bayat & McGrouther, 2006). The mainstay of treatment is surgical excision (fasciectomy) of the diseased tissue or cords (Davis, 2013), which is recommended when patients develop flexion deformities of the digits of 30 degrees or more of the finger joints and suffer impaired hand function (Legge & McFarlane; 1980, Rayan, 2007). Over 90% of the 12,900 patients who have surgery for DD per annum in the UK undergo fasciectomy (Gerber et al., 2011). 10-12% of patients develop recurrence within 3 years following surgery (Ullah, Dias & Bhowal, 2009; van Rijssen, ter Linden & Werker, 2012) and are treated with more extensive surgery that involves excision of the diseased tissue and overlying skin (dermofasciectomy). Post-operatively, patients require 3-6 months of hand therapy and splintage (Hughes et al., 2003; Larson & Jerosch-Herold, 2008). Complications occur in approximately 20% of surgical patients (Bulstrode, Jemec & Smith, 2005; Crean et al. 2011). Alternative, less invasive techniques have been developed to disrupt the cords of diseased tissue with either a needle (Beaudreuil et al., 2012) or collagenase digestion (Hurst et al., 2009). However, recurrence rates are high, affecting 70% of patients treated with percutaneous needle fasciotomy (van Rijssen et al., 2012) and 35% of those treated with collagenase (Peimer et al., 2013) at 3 years. The complication rate is 20% following needle fasciotomy (Crean et al., 2011) and over 70% after collagenase injection (Hurst et al., 2009). The flexion contractures are irreversible without treatment. There is currently no approved drug therapy for the treatment of early DD. Intralesional steroid injection has been proposed based on a retrospective review of 63 patients with early DD treated with steroid injection into the nodules at 6 week intervals (Ketchum & Donahue, 2000).

Following a mean of 3.2 injections, 60-80% subjective improvement was noted in 97% of patients and disease reactivation occurred in 50% of patients 1 to 3 years after the last injection. There was no control group and observers were not blinded. Given the paucity and quality of data, this treatment modality has found limited acceptance. Radiotherapy has also been used and, based on clinical assessment of nodule size and softness, regression or lack of disease progression was reported in approximately 30-50% of patients (Seegenschmeidt, Olschewski & Guntrum, 2001, Pohl et al., 2002, Betz et al., 2010). However, 20-30% of patients developed long term adverse effects, including dry skin, desquamation, skin atrophy, telangiectasia, erythema, and altered heat and pain sensation (Seegenschmeidt et al., 2001; Pohl et al., 2002; Betz et al., 2010). Based on the published data, NICE does not recommend radiotherapy (NICE 2010) or steroid injection for early DD.

Need for a trial

There is a need to develop an effective therapy to prevent progression of early DD which avoids the necessity for invasive procedures and also prevents the development of recurrent disease following surgery, needle fasciotomy or collagenase injection in patients with established finger contractures.

Investigational medicinal products (IMP): Adalimumab (active IMP) and saline (placebo)

Adalimumab is an anti-Tumour Necrosis Factor (anti-TNF) drug, approved for use by the US Food and Drug Administration (FDA) since 2002, and European Medicines Agency (EMA) since 2003. It is used to treat rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis with 40 mg administered by subcutaneous injection once every 2 weeks. No dose adjustment is required for those aged 65 years or older. It is also prescribed for Crohn's disease and ulcerative colitis with 160 mg initially administered by subcutaneous injection, followed by 80 mg 2 weeks later and then 40 mg every 2 weeks. The prescribing information gives the maximum dose to be given at one site as 40mg. In clinical trials, the most common adverse reactions were injection site reactions (erythema, and/or itching, haemorrhage, pain or swelling) but most were described as mild and did not necessitate drug discontinuation. Because of its effects on the immune system, adalimumab may increase susceptibility to infections and patients are monitored for tuberculosis (TB) and Hepatitis B. In common with all anti-TNF drugs, there may be a slightly increased risk of certain types of cancer, but this link is not proven and is currently being researched. However, in our study risks should be lower as anti-TNF will be given at relatively low doses, less frequently and for a limited duration. In this trial, adalimumab is being used off-label.

Comparator: normal saline: Normal saline (0.9% NaCl) will be used as the comparator (placebo).

Non IMP

Participants will be offered a local anaesthetic applied topically to the hand prior to injection to numb the area. If used, the anaesthetic will be either **lidocaine/prilocaine cream 5%** (2.5% lidocaine and 2.5% prilocaine) or **Ametop gel** (40mg/g tetracaine; Smith & Nephew Healthcare Ltd). Ametop gel has a faster onset of action than lidocaine/prilocaine cream, 30 minutes compared with one hour, and will be used preferentially. However, in some cases, such as the participant having epilepsy or a known sensitivity to tetracaine, lidocaine/prilocaine cream will be used.

Non-clinical study findings

Whilst the majority of cells in nodules of early DD are myofibroblasts, we also found significant numbers of macrophages (Fig 1A&B adapted from Fig 1, Verjee et al., 2013). We are the first group to study freshly

disaggregated cells from Dupuytren's nodules and found that *in vitro* they secrete TNF (mean±SD: 78±26pg/ml) and variable amounts of TGF-β1 (236±248pg/ml; range, 4–852pg/ml) (Fig 1C adapted from Fig 1, Verjee et al., 2013).

We tested the effects of recombinant cytokines on dermal fibroblasts from the palm of patients with DD (PF-D), non-palmar fibroblasts from Dupuytren's patients (NPF-D) and palmar fibroblasts from normal individuals (PF-N), and found TGF-β1 increased the contractility of all 3 types of fibroblasts at concentrations of 1–10ng/ml (Fig 2A adapted from Figs 2 and 3, Verjee et al., 2013), which is in excess of the range produced by freshly disaggregated tissue (236±248pg/ml). We found that cells cultured to passage 2 secreted very little TNF (4±4pg/ml) but produced nearly three times more TGF-β1 (654±158pg/ml) compared to fresh tissue (Verjee et al., 2013). TGF-β1 is known to drive the development of myofibroblasts, and cultured myofibroblasts upregulate its production in an autocrine manner, so as cells are cultured the concentration of TGF-β1 becomes artificially high. Previously, results from cells cultured up to passage 5 have implicated TGF-β1 as a therapeutic target. This may explain the lack of efficacy of TGF-β1 inhibition in all late phase clinical trials to date for fibrotic disorders (Varga & Pasche, 2009; Hawinkels & Ten Dijke, 2011). Furthermore, global inhibition of TGF-β1 is undesirable due to its role in a wide range of physiological processes (Varga & Pasche, 2009) and increased inflammation, tumour promotion, and cardiac toxicity seen in animal studies (Budd & Holmes, 2012).

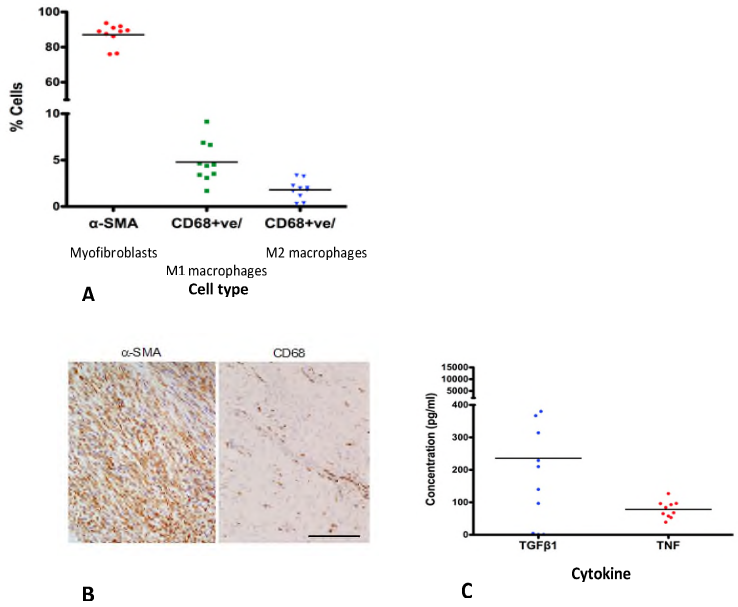

Fig 1. *(A) Flow cytometric analysis of cells isolated from freshly disaggregated Dupuytren's nodular tissue. Intracellular α-SMA–positive (myofibroblasts mean±SD: 87±6.1%), cell surface CD68-positive CD163-negative (classically activated M1 macrophages 4.8±2.2%), and CD68-positive CD163-positive (alternatively activated M2 macrophages 1.8±1.0%) cells were identified. (B) Serial histological sections of Dupuytren's nodular tissue stained for α-SMA+ (myofibroblasts) and CD68+ (monocytes) cells. (Scale bar 100 µm) (C) Cytokines released by freshly isolated nodular cells in monolayer culture using electrochemiluminescence. Adapted from Verjee et al., 2013.*

In contrast, TNF only converted cells from the palmar tissues of patients with DD into myofibroblasts, whereas non palmar tissue from Dupuytren's patients and palmar tissue from normal individuals became less contractile (Fig 2A). The optimal dose for conversion of palmar fibroblasts from Dupuytren's patients was 100pg/ml, similar to the amount found in fresh Dupuytren's tissue (78±26pg/ml). Therefore, anti-TNF therapy may specifically target the cells responsible for DD.

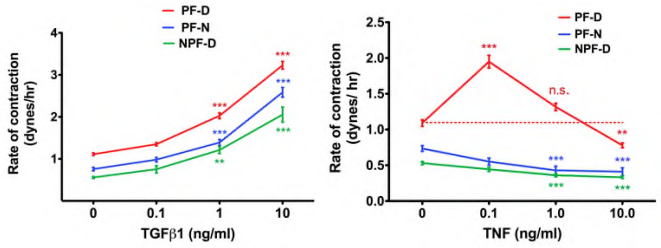

Fig 2. *(A) TGF-β1 led to increased contractility of all three types of fibroblasts. (B) Contraction of palmar fibroblasts from Dupuytren's patients (PF-D) peaked on addition of 0.1 ng/mL TNF. In contrast, TNF treatment palmar fibroblasts from normal individuals (PF-N) and non-palmar fibroblasts from Dupuytren's patients (NPF-D) led to a dose-dependent decrease in contractility. Adapted from Verjee et al., 2013.*

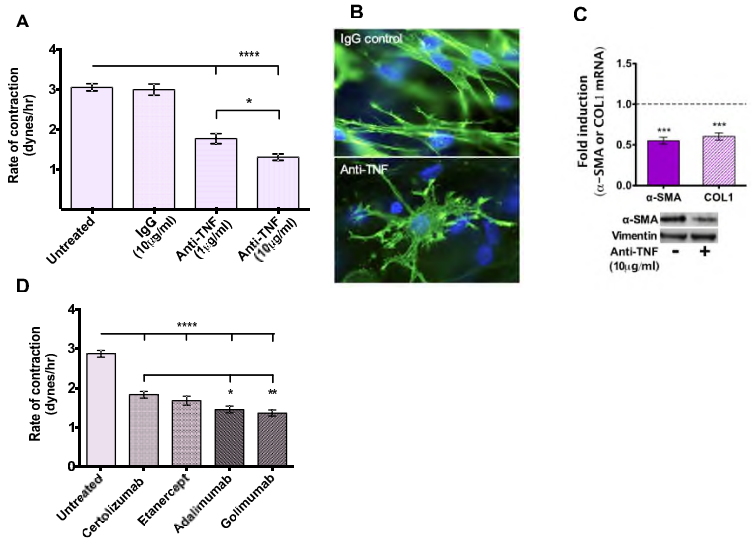

Fig 3. *(A) Anti-TNF led to a dose dependent inhibition of myofibroblasts contractility in vitro. (B) Immunofluorescence staining of Dupuytren's myofibroblasts seeded in 3D collagen matrices. Actin shown in green and nuclei blue. Anti-TNF led to disassembly of the α-SMA with no effect on cell viability (C) TNF inhibition led to reduction in mRNA expression of α-SMA and COL1 in Dupuytren's myofibroblasts with concomitant reduction in α-SMA protein. (D) Comparison of current anti-TNF preparations approved by FDA/EMA for subcutaneous administration on the contractility of Dupuytren's myofibroblasts. Doses calculated based on 25% of recommended dose in rheumatoid arthritis (certolizumab 50μg/ml (200 mg in 1 mL every 2 wk), etanercept 6.25μg/ml (50 mg in 1 mL every week), adalimumab 10μg/ml (40 mg in 0.8 mL every 2 wk), golimumab 12.5μg/ml (50 mg in 0.5 mL every 4 wk). All data shown are from n ≥ 3 patients (each in triplicate). Data expressed as mean ± SEM. *P < 0.05, **P < 0.01, ****P < 0.001, Adapted from Verjee et al., 2013.*

Anti-TNF led to dose-dependent inhibition of myofibroblast contractility (Fig 3A) with disassembly of their contractile apparatus (Fig 3B). TNF inhibition also reduced expression of the myofibroblast marker α-SMA and COL-1A1 for collagen (Fig 3C, Fig 3 adapted from Fig 3 Verjee et al., 2013). We found that of all the anti-TNF preparations approved by the FDA/EMA for subcutaneous administration, the two fully human IgG antibodies, adalimumab and golimumab, were the most effective in downregulating Dupuytren's myofibroblast phenotype (Fig 3D) (Verjee et al., 2013).

The reason for the selective effect of TNF on palmar fibroblasts from Dupuytren's patients may partly be due to their higher expression of TNF receptors (Fig 4, adapted from Fig 3 Verjee et al., 2013).

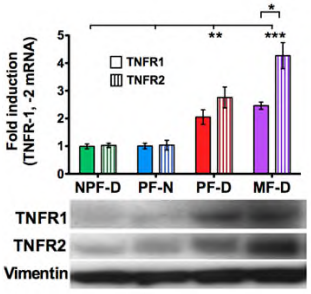

Fig 4. *Baseline gene expression of TNFR1 and TNFR2 was significantly higher in myofibroblasts and palmar fibroblasts from Dupuytren's patients (PF-D) compared with both palmar fibroblasts from normal individuals (PF-N) and non-palmar fibroblasts from Dupuytren's patients (NPF-D). In Dupuytren's myofibroblasts (MF-D), TNFR2 expression was significantly greater than TNFR1. Fold change was normalised to the baseline expression of NPF-D. Adapted from Verjee et al., 2013.*

We also found that TNF directly controls myofibroblast differentiation via the Wnt/β-catenin signaling pathway (Verjee et al., 2013). This is consistent with a genome-wide association study (GWAS) that demonstrated that Wnt signaling is involved in Dupuytren's disease (Dolmans et al., 2011). GWAS data from a much larger discovery cohort of 3,499 cases and 4,678 controls (D Furniss, unpublished data) confirmed the role of the Wnt pathway and also identified a novel locus for an enzyme known to be involved in TNF signaling.

Our published data (Verjee et al., 2013) demonstrate that anti-TNF downregulates the phenotype of existing myofibroblasts and also prevents the conversion of fibroblasts from the palm of patients with DD into myofibroblasts.

Therefore, TNF is a valid therapeutic target for downregulating the phenotype of existing myofibroblasts and preventing the development of new myofibroblasts.

IMP regime

The earliest manifestation of DD is palmar nodules, which we can reliably visualise and measure using an ultrasound scan (Fig 5), and are composed mainly of myofibroblasts. Even in the later stages with digital contractures, we found that the myofibroblasts remain aggregated in histological nodules (Verjee et al., 2009).

Our end user survey of 31 patients affected with DD (Table 1) clearly indicated that both early disease and established disease patients would accept injection therapy that reduced need for future surgery and the associated prolonged post-operative rehabilitation.

Extremely or very likely accept:	Early Dupuytren's disease (n=14)	Post-surgery for Dupuytren's disease (n=17)	Combined (n=31)
1 injection per year for lifetime	93%	94%	94%
3 injections per year for lifetime	57%	71%	65%

Table 1. *Summary of responses to questionnaire regarding acceptability of injection therapy that would prevent the progression of disease and hence avoid the necessity for future surgery (unpublished data).*

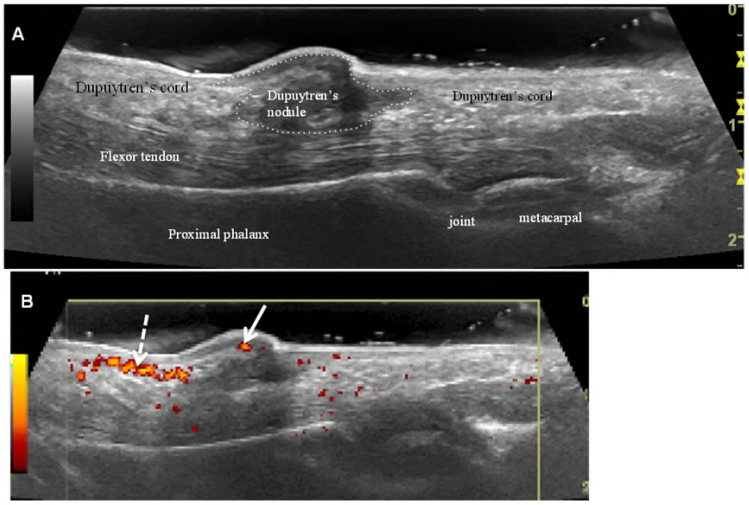

Fig 5. *(A) Gray scale ultrasound image of a patient with early stage Dupuytren's disease showing a well-defined hypoechoic nodule. (B) Power Doppler image of the same area. Arrow indicates hypervascular area in Dupuytren's nodule. Dotted arrow indicates digital artery. Overall, the nodules of Dupuytren's disease were less vascular than the subcutaneous tissues in the palm (unpublished data).*

Our proposal is to inject adalimumab directly into the nodule, thus directly targeting the affected area. Our recent data also demonstrate that Dupuytren's myofibroblasts function in a coordinated manner to contract the matrix (Verhoekx et al., 2013). Hence, in the event of incomplete penetration of adalimumab throughout the nodule, downregulation of the contractility of some cells will indirectly affect their neighbours. This effect may be magnified since the absence of tension causes myofibroblasts to disassemble their α-SMA stress fibres within hours (Hinz et al., 2001).

We have shown that DD is a localised inflammatory condition and macrophages that secrete the TNF responsible for the development and contractility of myofibroblasts are co-localised within the nodules (Fig 1A,B) (Verjee et al., 2013). The injected nodule and surrounding diseased tissue will be removed during scheduled surgery approximately two weeks after treatment. We aim to investigate whether adalimumab

down-regulates the myofibroblast phenotype in this excised nodule tissue. Based on our data we expect that adalimumab will reduce expression of both α-SMA as a marker for myofibroblasts and collagen as a marker for fibrosis (Verjee et al., 2013). There is no animal model for DD and based on our *in vitro* data we are ready to progress to clinical trials using a drug that has an excellent safety record and is approved for other diseases, including rheumatoid arthritis (Feldmann & Maini, 2003) and inflammatory bowel disease (Lawson, Thomas & Akobeng, 2006, Behm & Bickston, 2008).

The main aims of the study are to find an effective dose of adalimumab for downregulating the myofibroblast phenotype in the Dupuytren's nodule (Part 1: Dose response) and to demonstrate the efficacy of adalimumab to treat early DD (Part 2: Randomised controlled trial). The doses expected to be used in the dose response part of the trial have been selected to cover an effective range based on therapeutics for other indications and our *in vitro* data. The dose which will be used in the RCT part of the trial is based on therapeutics for other indications as the IMP has not previously been used for the treatment of DD. We have selected 40 mg adalimumab, which is comparable with the typical fortnightly dose of 40 mg used to treat conditions such as rheumatoid arthritis, psoriasis and Crohn's disease.

Clinical Outcome Measures

Nodule hardness (**Part 1** and **Part 2**): Previous authors have relied on subjective assessment of ease of injection during subsequent steroid injections (Ketchum & Donahue, 2000), clinical impression following radiotherapy (Seegenschmidt et al., 2001) or tonometry following surgery (Thurston, 1987). Tonometry has also been used to measure change in skin elasticity in lymphoedema (Chen et al., 1988, Liu & Olszewski, 1992, Pallotta et al., 2011), and scar pliability and hardness (Magliaro & Romanelli, 2003, Corica et al., 2006, Lye et al., 2006). In our ultrasound trial, (REC Ref. 11/SC/0447) 'Ultrasonographic (grey scale) characteristics of DD and their relationship to the clinical features and functional severity of the disease: a pilot study', we tested our outcome measures, informing the measurements for use in our trial (unpublished). Our preliminary data (Table 2) from 25 patients with early DD, demonstrate that the palmar tissues of patient's with untreated early DD are significantly firmer (53±8) than the corresponding areas of age and sex matched controls (32±3). We will assess nodule hardness using a portable durometer, with all participating centres using the same model.

	Early Dupuytren's (mean±SD) n=25	Normal (mean±SD) n=12	P value
Tonometry	53±8	32±3	<0.0001

Table 2. *Unpublished data showing the palmar tissue of patients with early DD is firmer than for controls.*

Grip strength (**Part 2** only)**:** Grip strength will be measured using a Jamar meter (CE marked) to monitor for change following injection. Previous studies of grip strength with patients with DD pre- and post-operatively have reported no significant change (Ball, Pratt & Nanchahal, 2013). Therefore it is anticipated that there will be no change in grip strength.

Range of motion of the digits (Part 1 and Part 2)**:** Range of movement is the most frequently reported measure used to monitor DD progression and has demonstrated reliability, validity and sensitivity (Ball et al., 2013). Individual range of movement of finger joints will be measured using a goniometer.

Clinical assessment of the hand (Part 1 and Part 2): Prior to treatment, information collected will include participant reported disease duration, age at onset, occupation, family history of DD, the digits involved, configuration of the Dupuytren's cords, joint involvement and whether the flexion deformities are fixed or can be passively corrected, presence of Garrod's knuckle pads and Ledderhose's disease of the feet. Clinical assessments after treatment has started will record the injected nodule and adjacent cord.

Ultrasound (Part 1 and Part 2): The size of the nodule will be determined from ultrasound images to monitor for change following injection(s). In our previous trial (REC Ref. 11/SC/0447) 'Ultrasonographic (grey scale) characteristics of DD and their relationship to the clinical features and functional severity of the disease: a pilot study', we found that nodules could be clearly visualised and that there was a trend for larger nodule areas to be associated with greater extensor deficit (r_s (23) = 0.267, p = 0.07). In **Part 2,** to ensure that the maximum size of the nodule is measured a short video of the ultrasound will be taken in the coronal plane. These will be reviewed by a member of the research team and the maximum nodule size, including area, perimeter, depth and maximum ferret, recorded.

Participant Reported Outcome Measures

Michigan Hand Outcomes Questionnaire (MHQ) (Part 2 only)**:** The MHQ is a hand-specific measure that, unlike some other instruments, allows the user to separately score each hand. It takes approximately 15 minutes to complete and comprises six distinct scales that assess overall hand function, activities of daily living, pain, work performance, aesthetics, and satisfaction with hand function. The MHQ shows high test-retest reliability (correlation coefficient 0.81-0.97) (Chung et al., 1998, Shauver & Chung, 2013) and internal consistency (Cronbach alpha 0.81-0.98 depending on the domain assessed) (Shauver & Chung, 2013). The MHQ has good construct validity depending on the condition assessed and good responsiveness (Shauver & Chung, 2013). It is a validated outcomes measure and has shown to be sensitive to change when used to assess improvement in hand function following surgery for established DD (Johnston et al., 2008, Herweijer et al., 2007). The MHQ was recommended following our systematic review of outcome measures for DD (Ball et al., 2013).

Preliminary data from our ultrasound trial, REC Ref. 11/SC/0447 'Ultrasonographic (grey scale) characteristics of DD and their relationship to the clinical features and functional severity of the disease: a pilot study' show that the Michigan Hand Questionnaire (MHQ) score was significantly lower in patients with early DD (mean ± SD, 80±17) compared to age and sex matched controls (93±8) (p=0.0058). In this study we will not collect the MHQ information beyond the six scales, i.e. data on the person's gender, ethnicity, education, income, health insurance and changes in jobs.

Participant identified activity most restricted by DD scored on a scale of 1-10 (Part 2 only)**:** Using a person centred approach for outcome measurement, participants will initially be asked to identify the activity most restricted by DD and to rate the restriction on a scale of 1-10. At later points in the trial, participants will be asked to again rate the restriction of the same activity. When applied to DD, significant improvement in median scores have been reported following fasciectomy (Engstrand, Boren and Liedberg, 2009).

Health utilities using EQ-5D-5L (Part 1 and Part 2): The EQ-5D-5L questionnaire is a preference-based instrument widely used in economic evaluation of health care technologies to assess quality of life and is the instrument preferred by the National Institute for Health and Care Excellence (NICE) to estimate utilities for the calculation of quality-adjusted life years (QALYs, National Institute for Health and Care Excellence, 2013). The responsiveness of the EQ-5D-5L instrument in early DD has not been formally evaluated, but the instrument was successfully used in a recent clinical trial of strengthening and stretching of the hand for people with rheumatoid arthritis (Heine et al., 2013). Moreover, recent qualitative research has shown that the main impact of DD on quality of life is on the performance of daily activities (Wilburn et al., 2013). The EQ-5D-5L includes domains on self-care and usual activities. Therefore, in Part 2, it is likely this instrument will be able to capture improvements or deteriorations in quality of life in this group of patients. In Part 1, the EQ-5D-5L will inform the health economics modelling when comparing the cost efficacy of adalimumab with the current standard of care.

Permission has been granted for the use of the MHQ and EQ-5D-5L from the relevant agencies.

Injection experience questionnaire (Part 1 and Part 2): Participants will report on the experience of each research injection using an injection questionnaire which incorporates a numeric rating scale. In Part 1, participants will reflect back on the injection experience and its acceptability as a therapy at 12 weeks after surgery.

Adverse Event monitoring

Injection site assessment: The most common adverse reaction with adalimumab is injection site reactions. The injection site will be monitored for adverse events using an 'Injection Site Response Form'. The injection responses are based on injection site reactions in the prescribing literature and in clinical trials. In placebo-controlled trials in patients with rheumatoid arthritis, 14% of patients treated with adalimumab developed injection site reactions (erythema and/or itching, hemorrhage, pain or swelling), compared to 8% of patients receiving placebo (SmPC for Humira (adalimumab), updated 26 March 2015). Most injection site reactions were described as mild.

Surgery site assessment (Part 1 only): The surgery site will be monitored using wound assessment criteria from the Centers for Disease Control and Prevention (CDC; www.cdc.gov/nhsn).

Progression to surgery of the digit being assessed (Part 2 only): Progression to surgery of the digit being assessed, if applicable, will be recorded during the 18 month follow-up.

Further measures

Monitor blood circulating levels of adalimumab and antibodies to adalimumab: 12.5ml of blood will be collected pre- and post-injection to measure circulating levels of adalimumab and antibodies to adalimumab. Local delivery of anti-TNF by intra-articular injection for arthritis has resulted in improvement of symptoms for more than 4 months (Fisher & Keat 2006). Monitoring circulating levels of adalimumab will facilitate our understanding of the kinetics of drug absorption following intranodular injections and may provide further information regarding the optimal frequency of intranodular injection.

Exploratory Objectives – Part 1 & Part 2:

In addition to those markers specifically listed above, others including newly identified indices of myofibroblasts and myofibroblast activity pertinent to Dupuytren's disease, will be investigated. In the case of limited tissue/blood, priority will be given to completing the outcome measures for the primary, secondary and teriary endpoints.

5. OBJECTIVES AND OUTCOME MEASURES

See Appendix C for schedule of procedures and visit windows

Research hypothesis: To determine whether experimental treatment by injection with adalimumab will control the progression of early Dupuytren's disease more than control placebo treatment by injection with normal saline.

Part 1. Objectives	**Outcome Measures**	**Timepoint(s) of evaluation of this outcome measure (if applicable)**
Primary Objective To establish an effective dose of adalimumab for down regulating the myofibroblast phenotype in participants with Dupuytren's disease.	Expression of mRNA for α-SMA from participants on each treatment.	Analysis of tissue removed during surgery at 12-18 days post-treatment.
Secondary Objectives To determine the effectiveness of adalimumab for myofibroblast inhibition in participants with Dupuytren's disease and nodules.	For participants on each treatment 1. Expression of mRNA for COL-1A1, COL-3A1 and cadherin 11 2. Levels of α-SMA and collagen proteins. 3. Hardness of selected nodule 4. Ultrasound imaging of nodule size.	1 and 2. Analysis of tissue removed during surgery at 12-18 days post-treatment. Measures 3 and 4 measured before treatment and at 2 weeks post-treatment.
Monitor for adverse events	Adverse event assessment comparing active and placebo groups using visual inspection of injection site, surgery site and laboratory reports.	Observation at each research visit. Injection site assessment immediately after injection and 2

		weeks later. Health check phone call 1 week post treatment. Surgery site assessment at 2 weeks post-surgery.
To assess whether adalimumab affects the healing of the surgical incisions or subsequent scarring.	Visual assessment of surgical wounds using hand photographs of all participants on each treatment.	2 and 4 weeks post surgery.
Tertiary Objectives To determine circulating levels of adalimumab and antibodies to adalimumab in the blood.	Circulating levels of adalimumab and antibodies to adalimumab in the blood.	Analysis of blood samples taken pre-injection and at 2 weeks post-treatment.
To assess if DD injection therapy would be acceptable to patients, and to evaluate the health economics of current clinical care.	Analysis of questionnaire data on the acceptability of the injection, injection experience and their return to paid work. Analysis of EQ-5D-5L	Injection experience at baseline. Health economic data at 12 weeks post-surgery.
Exploratory Objective To investigate changes in other molecular markers relevant to the progression of Dupuytren's disease.	Change in level of the markers.	Analysis of tissue removed during surgery at 12-18 days post-treatment. Analysis of blood samples taken pre-injection and at 2 weeks post-treatment.

Part 2. Objectives	**Outcome Measures**	**Time point(s) of evaluation of this outcome measure (if applicable)**
Primary Objective		

To determine if injection with adalimumab is superior to placebo injection of normal saline in controlling disease progression in participants with early Dupuytren's disease.	Change in hardness of selected nodule for participants on each treatment baseline and 12 months after first treatment..	12 months after first treatment.
Secondary Objectives To determine if injection with adalimumab is superior to placebo injection of normal saline in controlling disease progression in participants with early Dupuytren's disease.	For participants on each treatment. Change in hardness of selected nodule for participants on each treatment at baseline, 3, 6, 9, 12 & 18 months after first treatment.	At 3, 6, 9 and 18 months after first treatment
To compare the development of Dupuytren's nodules and associated cord, flexion deformities of the fingers and impairment of hand function for participants on each treatment.	1. Ultrasound imaging of nodule size. 2. Range of motion of the affected digit. 3. Grip strength. 4. Participant Reported Outcomes: Michigan Hand Outcomes Questionnaire (MHQ) 5. Participant identified activity most restricted by DD scored on a scale of 1-10. 6. Progression to surgery of the digit being assessed	Outcome measures 1, 2, 3 and 4 recorded before, and at 3, 6, 9, 12 and 18 months after first treatment. 5. During the 18 month post first treatment. 6. 18 months post first treatment.
To assess acceptability of injections to patients	Injection experience	After each injection at baseline, 3, 6 and 9 months.
Monitor for adverse events	Adverse event monitoring comparing active and placebo groups using visual inspection of injection site.	Observation at week 0 and 3, 6, 9 and 12 months.
Tertiary Objectives To assess if early DD injection therapy represents good value for money compared to current clinical care.	Analysis of EQ-5D-5L data to estimate utilities using quality-adjusted life years (QALYs) from participants on each treatment. Analysis of resource use data to estimate the cost of each treatment.	EuroQol EQ-5D-5L before, and at 3, 6, 9, 12 and 18 months after first treatment.
To determine circulating levels of adalimumab and antibodies to adalimumab in the blood.	Monitor circulating levels of adalimumab and antibodies to adalimumab in the blood.	Analysis of blood samples pre-injection and 3 and 12 months post first treatment.
Exploratory Objective To investigate changes in other molecular markers relevant to the progression of Dupuytren's disease.	Change in level of the markers.	Analysis of blood samples taken pre-injection and at 3

		and 12 months post first treatment.

6. TRIAL DESIGN

The trial is a multi-centre, double blind, randomised, placebo-controlled, parallel group, phase II trial to determine the efficacy of intra-nodular injection of anti-TNF in delaying disease progression in early DD, with a dose response.

Part 1. Dose response

For the dose response, study participants will be recruited with **established DD** who are scheduled for surgery for excision of the diseased tissue to correct flexion deformities of the fingers, to assess the efficacy of adalimumab in down regulating the phenotype of myofibroblasts. A maximum of 40 participants will be randomised to participate in the study if they meet the inclusion criteria and provide written consent. All participants will be screened for TB, Hepatitis B and C and HIV. If results are positive for any test, the participant will not be eligible to enter the trial and will be informed and counseled about the result of their test. Consented participants will enter the trial and receive the injection(s) two weeks prior to the scheduled surgery date. Intra-nodular injections will be delivered into the most prominent nodule due to be excised by surgery. If participants do not proceed to surgery at 12-18 days following injection, the reason will be recorded and they will continue with normal care. These participants may be replaced if still during the recruitment phase of the dose response study.

The diseased tissue excised during surgery, which would normally be discarded as waste, will be collected and analysed centrally for α-SMA, COL-1A1, COL-3A1 and cadherin 11 mRNA expression, and α-SMA and, where sufficient material exists, collagen protein levels. As other markers and assays of myofibroblasts become available these may also be used. The primary outcome measure will be mRNA for α-SMA measured using RT-PCR for doses of adalimumab compared to equivalent volumes of saline.

Study participants will be seen at 4 time points after screening: week 0, week 2, week 2 post-surgery and week 12 post-surgery according to the summary chart (Appendix A). The schedule, duration of each visit and visit window is detailed in the Schedule of procedures (Appendix C: Schedule of procedures Part 1).

The safety profile of adalimumab is well known; with the most common adverse reactions being mild injection site reactions (see Section 4 Background). Participant safety will be monitored for three months after surgery, and if more than one participant in a dose cohort has an unplanned admission to hospital or unplanned surgery related to the surgery for Dupuytren's disease of the affected digit, or if any participant experiences a SUSAR, we will refer for advice to the Safety Committee.

Participants may withdraw from the study at any time and will continue normal NHS clinical care. Information collected up to the point of withdrawal may still be used. To monitor safety, if the participant withdraws after they have received the medication their medical notes may be reviewed for 3 months after the baseline visit. Samples donated by participants for research are difficult to destroy on request once samples are processed for storage. Participants will be informed that they will not be allowed to request their samples to be destroyed at a later date.

The first participants will be recruited to 15 mg (in 0.3 ml carrier) and 35 mg (in 0.7 ml carrier) intra-nodular adalimumab cohorts. Each of these cohorts will initially recruit eight participants, of which two participants in each cohort will be assigned to placebo. A further eight participants will be recruited to a 40 mg (in 0.4 ml carrier) intra-nodular adalimumab cohort, with two participants in this cohort assigned to placebo.

Interim analyses of the excised disease tissue may be used to guide further decisions on cohort size and dose selection. However, the frequency of interim analyses is limited by the necessity for samples to be compared within each analysis run. The Trial Management Group (TMG) will monitor recruitment and will recommend the points at which any interim analyses will be performed.

Blinded data from interim analyses, together with safety data, will be presented to the Safety Committee for a decision on how best to proceed with the study. All decisions on cohort size and dose selection will be documented in the TMF. The Safety Committee may recommend to stop the dose response study at this point, to increase the participant numbers (either for all or specific cohorts), or to collect data at (an)other dose(s). Decisions on dose cohorts will adhere to the following rules:

- A maximum of 40 participants will be recruited
- The dose administered into the nodule will not exceed 80 mg adalimumab or 0.8 ml in volume.

Part 2. Randomised controlled trial

For the randomised controlled part of the trial, participants will be recruited with **early DD** to assess the efficacy of injections of adalimumab in controlling disease progression including nodule hardness.

A maximum of 200 participants will be randomised to participate in the study if they meet the inclusion/exclusion criteria and provide written consent. We will select participants with early disease nodules who also give a clear history of activation and progression of the disease in the previous 3-6 months. Only participants with flexion deformities of ≤30° at the metacarpophalangeal and/or at the proximal interphalangeal joint of their fingers will be recruited, with a maximum total flexion deformity of 60°. All participants will be screened for TB, Hepatitis B and C and HIV. If results are positive for any test, the participant will not be eligible to enter the trial and will be informed and counseled about the result of their test. The participants will be randomised (1:1) to receive injections of either adalimumab or saline (placebo) into the active nodule. The dose will be an injection into the nodule of 40 mg adalimumab, or the equivalent volume of saline. The adalimumab formulation of 40 mg in 0.4 ml formulation will be used. The adalimumab or placebo will be administered at 3 monthly intervals for the first year i.e. each participant will be injected on 4 occasions over a 9 month period (baseline, 3, 6 and 9 months) and followed-up for a further 9 months. If, on a returning visit, the nodule has reduced in size to such an extent that the clinician is not satisfied they can accurately deliver a repeat injection, then the clinician may decide to not inject on that visit. In this event, the participant will be invited to all further visits as originally planned, and future planned injections may be given if the nodule recurs.

Study participants will be seen at 6 time points after screening: week 0, 3 months, 6 months, 9 months, 12 months and 18 months according to the flow chart (Appendix B: Trial Flow Chart Part 2). The schedule, duration of each visit and visit window is detailed in the Schedule of procedures (Appendix C: Schedule of procedures Part 2). Study participants will be contacted by telephone by a member of the research team at 1 week post baseline to monitor for Adverse Events.

Participants may withdraw from the study and will continue normal NHS care. If they withdraw, they may keep in contact with the study organisers to let us know their progress. Information collected up to the point of withdrawal may still be used. To monitor safety, if the participant withdraws after they have received medication their medical notes may be reviewed for 18 months after the baseline visit. Samples donated by participants for research are difficult to destroy on request once they are processed. Participants will be informed that they will not be allowed to request their samples to be destroyed at a later date.

7. PARTICIPANT IDENTIFICATION

7.1. Trial Participants

Part 1. Dose response

Participants with **established DD** leading to contractures ≥30° at either the metacarpophalangeal joint or the proximal interphalangeal joint and awaiting scheduled surgery of the hand will be invited to join the study. Flexion deformities of ≥30° are associated with impairment of function and form the criteria used by the clinical commissioning groups for surgical referral. The diagnosis of DD is made on the basis of the history and clinical examination. Only a single nodule will be injected in each participant and the most prominent nodule due to be excised will be selected.

Part 2. Randomised controlled trial

Participants with **early DD** nodules who also have shown or report progression of the disease in the previous 6 months will be invited to join the study. Only participants with flexion deformities of ≤30° at the metacarpophalangeal and/or at the proximal interphalangeal joint of their fingers will be recruited, so total flexion deformity could be up to 60°. The participants will be randomised (1:1) to receive injections of either adalimumab or saline into the active nodule.

7.2. Inclusion Criteria

The participant may enter the trial if:

- Participant is willing and able to give informed consent for participation in the study.
- Male or Female, aged 18 years or above.
- **Part 1**: Diagnosed with DD affecting the fingers resulting in flexion deformities of ≥30° at the metacarpophalangeal joint and or the proximal interphalangeal joint with impaired hand function and awaiting surgery.

  or

  Part 2: Participants with early disease nodules who have shown or reported progression of the disease in the previous 6 months with flexion deformities of their fingers of ≤30° at the metacarpophalangeal and/or at the proximal interphalangeal joint, i.e. total flexion deformity of up to 60°.

- The DD nodule to be treated must be distinct and identifiable.
- Female participants of child bearing potential, and male participants whose partner is of child bearing potential, must be willing to ensure that they or their partner use effective contraception throughout the treatment period and for 5 months following the last research injection. Acceptable methods of contraception include: a combination of male condom with either cap, diaphragm or sponge with spermicide (double barrier methods), injectables, the combined oral contraceptive pill (at a stable dose for at least 3 months before entering the study), an intrauterine device, vasectomised partner, or true sexual abstinence (when this is in line with the preferred and usual lifestyle of the participant).
- Participant results from safety screening tests within normal ranges within 12 weeks of enrolment, with the exception that an earlier clear CXR result may be used where this is in accordance with the time frames of local standard procedures for anti-TNF screening.
- Able (in the Investigators opinion) and willing to comply with all study requirements.
- Willing to allow his or her general practitioner to be notified of participation in the study.
- Sufficient language fluency to ensure informed consent is obtained and to complete the questionnaires pertaining to hand function.

7.3. Exclusion Criteria

The participant may not enter the trial if ANY of the following apply:

- **Part 1**: Participant has previously had fasciectomy, dermofasciectomy, needle fasciotomy, collagenase injection, steroid injection or radiotherapy to treat Dupuytren's disease in the **digit** concerned.

or

Part 2: Participant has previously had fasciectomy, dermofasciectomy, needle fasciotomy, collagenase injection, steroid injection to the **digit** to be treated or radiotherapy to treat Dupuytren's disease in the **hand** concerned.

- Female participant who is pregnant, lactating or planning pregnancy during the course of the study and for 5 months following last injection.
- Male participant who is planning a pregnancy during the course of the study and for 5 months following last injection.
- Significant renal or hepatic impairment.
- **Part 1.** Scheduled elective surgery or other procedures requiring general anaesthesia during the study other than the scheduled Dupuytren's surgery

- Participant who has ever been diagnosed with cancer, is terminally ill or is inappropriate for placebo medication
- Systemic inflammatory disorder such as RA or inflammatory bowel disease.
- Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate in the study.
- Participated in another research study involving an investigational medicinal product in the past 12 weeks.
- Known allergy to any anti-TNF agent.
- Have HIV or hepatitis B or C.
- Known to have an infection or history of repeated infections.
- History of Tuberculosis (TB).
- Have Multiple Sclerosis (MS) or other demyelinating disease.
- History of local injection site reactions.
- Needle phobia.
- Have moderate or severe heart failure.
- **Part 1**: Being treated with coumarin anticoagulants, such as warfarin.
- Have known lung fibrosis (thickening of lung tissue).
- Being treated with concomitant biologic DMARDS.
- Have received a live vaccine within the previous 4 weeks. Participants may receive concurrent vaccinations but must avoid the use of live vaccines for 12 weeks after their last injection.
- **Part 1**: Have received parenteral steroid within the previous 6 weeks.
- **Part 2.** Participants at risk of Hepatitis B infection.

Note: Participants with epilepsy or a known allergy to tetracaine may take part in the study but will not receive Ametop gel as a local anaesthetic. Participants with a known allergy to lidocaine or prilocaine will not receive lidocaine/prilocaine cream as a local anaesthetic.

8. TRIAL PROCEDURES

See Appendix C: Schedule of procedures **Part 1** and **Part 2**

8.1. Recruitment

Participants will be recruited through the following routes:

Part 1: Dose response

Patients with established DD and who are due to be scheduled for surgery for this disease, will be identified and approached by hand specialists who will be assessing patients in an out-patient clinic. Potential participants will be given the relevant Participant Information Leaflet (PIL) which contains a telephone number and an e-mail address to request further information. Screening tests for the study are combined with the pre-operative assessment for the surgery, and the standard clinical care pathway varies between the two sites: for one site, after the need for surgery has been confirmed by hand specialists, the patient is sent home to wait for a pre-operative assessment appointment; but at the other site the pre-operative assessment for the surgery is done immediately after the need for surgery has been confirmed. At this second site consent must therefore be taken during the same out-patient clinic in which the potential participant was identified. Although this reduces the time the participant has to consider the information it should be noted that the IMP is not administered on this visit, it is the screening tests which are performed. In all cases the potential participant will be given enough time to read the PIL carefully and the opportunity to ask questions about the study.

Permission will be requested from the potential participant that they may be contacted by a member of the trial team by telephone, post or e-mail to invite them to attend study visits (such as screening or baseline). If the recruiting site requires the trial team in Oxford to co-ordinate the participant's visits, then permission will be requested from the potential participant that their contact details will be sent securely to the Trial centre in Oxford. All participant information will be stored securely as described in section 15.5.

Part 2. Randomised controlled trial

Participants with early disease will be recruited through the following routes:

(i) Patients will be approached by hand specialists consulting patients in out-patient clinics at centres participating in the trial.

(ii) Patients diagnosed with early DD who took part in our preliminary study of outcome measures (data shown in Table 2) and also those who completed the end user questionnaire (Table 1) will be contacted. We have also identified patients with early disease who in the past have said that they would like to be informed of any new clinical trials into DD.

(iii) GPs primarily but not exclusively within 20 miles of participating centres will be informed about the study providing details of our clinical trial, inviting them to display a poster and advise relevant patients of the opportunity to participate in this research. The patient records of some GP sites may also be searched for patients with Dupuytren's disease, potentially suitable patients will be sent the PIL for the study. The same poster will also be displayed at participating sites on noticeboards including in out-patient clinics.

(iv) Information about the trial, including contact information for potential participants to self-refer, will be placed

a) through web-based arenas such as:

The Oxford Clinical Trials Research Unit website http://www.octru.ox.ac.uk/

The British Dupuytren's Society http://dupuytrens-society.org.uk/

As well as a trial specific website (www.ridd.octru.ox.ac.uk), Facebook page and Twitter account.

The trial will also be added to the trial registries (which are searchable by the general public) including:

NIHR UK Clinical Trials Gateway http://www.ukctg.nihr.ac.uk/

ISRCTN registry website http://www.isrctn.com/

NIHR CRN portfolio website http://public.ukcrn.org.uk/search/

https://clinicaltrials.gov/

b) In publications and correspondance such as newletters including to those previously diagnosed with Dupuytren's disease.
c) In the public domain via text, visual and audio adverts about the trial.

Potential participants from (iii) and (iv) will contact the RIDD trial office in Oxford. Potential participants from (i) to (iv) will be contacted by a member of the trial team or site staff to discuss the research. Potential participants will be given/sent the relevant Participant Information Leaflet (PIL) which contains a contact details to request further information or to make an appointment for consent and screening to participate in the study.

When potential participants contact the Trial Office via telephone, post or e-mail, or has been approached at clinic at a participating site, and has been provided with the PIL, if no further response is received the Trial Office/site may contact the potential participant a maximum of 2 further times to determine if they wish to participate in the trial. If during either of these subsequent contacts the potential participants agrees to or requires the staff member to ring them again (e.g. medical input is needed to respond to a question from the potential participants, or the potential participants requests that the staff member rings them back at a later date), then further contact with the potential participant is permitted. Potential participants who do not respond to the 2 follow up contacts will not be contacted by the Trial Office/site team again, unless the potential participants contacts the Trial Office/site to advise they do wish to join the trial.

Self-referring potential participants wishing to join the trial will speak with a member of the RIDD Trial office or site staff to discuss the research and to determine if based on the potential participants reported medical history they are suitable to proceed to screening (e.g. their DD is of the appropriate stage; no prior treatment for DD; no excluded concurrent medical conditions). If appropriate the trial office and/or site staff will arrange an appointment for consent and screening at a participating site when a member of the trial team will be present.

Diagnosis of early DD will be confirmed by clinical examination. To ensure the nodule is active and the disease is progressing, the patient may be invited back for another meeting up to 6 months later, in other cases the person's self-reported history or their clinical notes may be sufficient.

All participant information will be stored securely as described in section 15.5.

8.2. Informed Consent

The participant must personally sign and date the current approved version of the Informed Consent Form before any trial specific procedures are performed.

Written versions of the PIL and Informed Consent form will be presented to the participants detailing the exact nature of the trial, what it will involve for the participant, the implications and constraints of the protocol and the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as they wish to consider the information, and the opportunity to question the Investigator, their GP/family doctor or other independent parties to decide whether they will participate in the trial. Written informed consent will then be obtained by means of a participant dated signature and the dated signature of the person who presented and obtained the informed consent. The person who obtained the consent will be suitably qualified and experienced, and authorised to do so by the Principal Investigator, at each site. A copy of the signed Informed Consent Form will be given to the participant. The original signed form will be retained at the trial site.

8.3. Screening and Eligibility Assessment

Part 1 and **Part 2**

Visit 1 (screening) 65-75 minutes

Informed consent for participation in the study will be obtained and the participant will be screened for eligibility to enter the study.

Medical history and demographics

Relevant medical history and medications will be recorded as well as demographic data including age, sex, smoking habits and alcohol consumption.

Safety Screening Tests

Laboratory Tests

Participants will have a maximum of 30ml peripheral blood taken to screen for suitability for anti-TNF therapy. The following tests are required:

- screening for Hepatitis B and C and HIV,
- testing for latent TB,
- full blood count, liver function tests (LFTs), U&Es and CRP.

All laboratory results will be reviewed by the investigator who will record in the CRF whether the participant is suitable for anti-TNF therapy.

X-Ray

A CXR will be taken to screen for TB. This is a routine requirement prior to commencing anti-TNF treatment. X-rays expose people to radiation. However, for a CXR this is equivalent to a few days' background radiation and is associated with a less than 1 in a million chance of causing cancer (http://www.nhs.uk/Conditions/X-ray accessed 17 Jul 2015). If the potential participant already has a clear CXR result in their medical record then this may be accepted in place of a new scan where this is in accordance with local standard procedures for anti-TNF screening.

8.4. Randomisation, blinding and code-breaking

For Part 1 and Part 2, randomisation will be undertaken prior to or at the baseline visit prior to receiving the trial injection. The randomisation procedure and allocation ratios for the two parts of the trial will differ:

Part 1: participants will be randomised 1:3 to placebo or adalimumab within each dose cohort. Each participant will be assigned a Trial ID as they enter the study. Pharmacy will refer to the allocation log, which is securely stored in pharmacy, to ensure they dispense the IMP corresponding to the Trial ID. To enable unblinding, the randomisation will retrospectively be added to the RRAMP system (see below) by an unblinded member of the trial team.

Part 2: participants will be randomised 1:1 to placebo or adalimumab with stratification by age and centre. Participants will be randomised through a secure online service provided by the Oxford Clinical Trials Research Unit (OCTRU) called RRAMP. RRAMP is used to randomise participants and this will generate a Trial ID and prompt an email to Pharmacy which informs them of which IMP (drug or placebo) to dispense.

The randomisation procedures will be fully documented, reviewed, tested and approved prior to the start of the study.

The methods for blinding will be influenced by the packaging of the adalimumab being used. Where possible the adalimumab will be supplied in a single use glass vial. However, a pre-filled syringe may be the only option if using the 40 mg in 0.4 ml formulation (see section 9.1).

Procedure for single use glass vials:

Participants, treating physicians and healthcare professionals involved with administering the IMP or administering any trial procedure from the injection onwards will be blinded to treatment.

The IMP (which will be stored in pharmacy) will be dispensed with accountability to a member of the research team who will take the IMP to a clinic room separate from the participant. In this separate room, a non-blinded member of the research team, who is not involved in administering the IMP or assessing the participant, will prepare and draw up the adalimumab or normal saline in a syringe according to the randomisation, and label the syringes with the participant's ID. The label will not reveal the identity of the IMP. Both the IMP and placebo have a similar viscosity and appearance so that the two treatments, adalimumab or saline, will be indistinguishable. The syringe, without any identifying packaging, will be taken to the trial healthcare professional (blinded to treatment allocation) to inject the participant. Once adalimumab has been drawn up, it tends to lose potency and this precludes preparation of the syringes before the participant presents for treatment. There is no stipulated time limit. For this trial, no more than 1 hour will elapse before the injection is given.

Procedure for pre-filled syringes:

Due to the distinctive appearance of the syringe, it is not possible to blind the healthcare professional administering the injection to treatment allocation. To protect the quality of the data, any person injecting with a pre-filled syringe will NOT be involved in administering any further trial procedures.

The IMP (which will be stored in pharmacy) will be dispensed with accountability to a member of the research team who will take the IMP to a clinic room separate from the participant. A non-blinded member of the research team will prepare the saline injection for the placebo treatment allocation. All syringes (adalimumab or saline) will be labelled with the participant's ID. Care will be taken to ensure any healthcare professionals blinded to treatment allocation are not present for the injection, and the syringe will be hidden from the participant's view. A pre-filled syringe of adalimumab can be stored at up to 25°C for up to 14 days, therefore the one hour time limit described for glass vials is not applicable with pre-filled syringes.

Any healthcare professionals involved with administering any trial procedure, including outcome assessments, after the injection of IMP will be blinded to treatment.

Participants will be blinded to treatment.

Based on the well-known safety profile of the IMP, it is not anticipated that there will be any serious unexpected adverse reactions. However in the unlikely event of an emergency and the need to unblind for safety reasons, the participants will be provided with a laminated card to present to the treating physician with the contact details of whom to contact at the Trial Office. The appropriate and authorised person at the Trial Office will unblind the participant through RRAMP and inform the treating physician of the allocation. The RRAMP system will automatically and immediately notify the Trial Manager and the Chief Investigator that a participant has been unblinded.

During part 1 of the trial this unblinding facility will be available 24 hours a day, 7 days a week.

During part 2 of the trial, this unblinding facility will be available Monday to Friday 9 am to 4 pm (UK time), excluding UK Bank Holidays. This is deemed adequate by the Trial team as emergency treatment would not be affected by the treatment allocation and participants are not due to have any planned surgery. This facility will be available until 1 month after the last participant has received their final treatment injection. This is deemed adequate by the Trial team due to the half life of adalimumab. After this time unblinding requests can still be made via the Trial Office but the Trial Office may not always be staffed during the times specified.

In summary, participants and healthcare professionals involved in participant follow-up and outcome assessment will always be blinded to treatment allocation. Laboratory staff will be blinded to treatment allocation. Where possible, health care professionals delivering the injection will also be blinded to treatment allocation.

8.5. Baseline Assessments

Part 1 and **Part 2**

Visit 2 week 0 (Baseline including injection) (112-132 minutes)

The maximum amount of time between screening and baseline is 12 weeks. There is no minimum time between screening and baseline. The trial team will verbally check the participant is happy to continue to give consent for the trial. The results of the screening investigations performed on the previous visit will have been checked by a clinician to confirm that all the parameters are within normal range prior to this appointment. Any change in health or medication will be recorded and checked to ensure the participant is still eligible.

- Participants will have an ultrasound imaging examination to assess Dupuytren's nodule size (greyscale).
- Participants will undergo clinical examination of the hand to establish baseline disease status. Information collected will include participant reported, age at onset, occupation, family history of DD, the digits involved, configuration of the Dupuytren's cords, joint involvement and whether the flexion deformities are fixed or can be passively corrected, presence of Garrod's knuckle pads and Ledderhose's disease of the feet.
- The hardness of the palmar tissue overlying the Dupuytren's nodule will be measured using tonometry.
- A digital photograph of the palm showing the selected nodule and indicating the nodule and associated affected finger will be recorded.
- Participants will have their finger range of movement measured using a goniometer.
- Participants will have a maximum of 12.5ml peripheral blood taken. This blood will be transported to the central laboratory for assessment of pre-treatment levels of adalimumab and antibodies to adalimumab.
- Ametop gel or lidocaine/prilocaine cream may be applied 30 minutes to 1 hour prior to injection.

Note: Participants with epilepsy or a known allergy to tetracaine may take part in the study but will not receive Ametop gel as a local anaesthetic. Participants with a known allergy to lidocaine or prilocaine will not receive lidocaine/prilocaine cream as a local anaesthetic.

- Participants will complete a Health Quality of Life Questionnaire, the EQ-5D-5L.
- **Part 1** only: The **injection will be administered** into a single Dupuytren's nodule scheduled for surgical removal.
- **Part 2** only: The **injection will be administered** into a single Dupuytren's nodule identified by the Investigator and recorded on the CRFs.
- Participants will then answer 2 questions about the experience of the injection.
- The injection site will be assessed by a trial healthcare professional blinded to treatment allocation to monitor for local adverse effects using an injection site response form.

Part 2 only

- Participants will complete the MHQ hand function questionnaire and will be asked to identify the activity most restricted by DD and score it on a scale of 1-10.
- Grip strength will be measured using a JAMAR Dynamometer.

Injections may be administered by any clinician who has been trained and is on the site delegation log. In Part 2, it is not essential that the same clinician gives all injections to a participant.

8.6. Subsequent Visits

(See Appendix C for schedule of procedures)
At each visit we will verbally check the participant continues to freely give consent for the trial. At all visits, any change in health or medication will be recorded and checked to ensure the participant is still eligible. All procedures will be done by a trial healthcare professional blinded to treatment allocation.

Part 1 Dose response

One week after treatment participants will be phoned by a hand therapist, Investigator or other member of site staff to monitor for adverse events. Any concerns raised by this call will be brought to the urgent attention of the site PI or other Investigator on the site delegation log promptly.

Visit 3 week 2, prior to surgery (37 minutes)

- Review of possible adverse events.
- Participants will have a maximum of 12.5ml peripheral blood taken. This blood will be transported to the central laboratory for assessment of levels of adalimumab and antibodies to adalimumab.
- The injection site will be assessed to monitor for local adverse effects using an injection site response form.
- Participants will have ultrasound scan to assess the size of the injected nodule.
- A digital photograph of the palm showing the selected nodule will be recorded.
- The hardness of the palmar tissue overlying the Dupuytren's nodule will be measured using tonometry.

Following the **standard care pathway**, participants will have surgery under general anaesthetic or regional block and the excised Dupuytren's tissue, which is normally discarded, will be collected and sent to the central laboratory for analysis (See 8.7 Sample handling). The samples will be collected and transported as per the Sampling handling protocol.

Participants will continue to follow the standard care pathway following surgery for DD.

Visit 4 (2 weeks after surgery) (12 minutes) + standard consultation time

Following the standard care pathway following surgery for DD, participants will be reviewed in the hand clinic on an outpatient basis. If non-dissolvable sutures have been used, they will be removed at this stage.

- Review of possible adverse events.
- The participant's wound will be assessed.
- A digital photograph of the palm will be recorded.

Visit 5 (12 weeks after surgery) (15 minutes) + standard consultation time

Following the standard care pathway following surgery for DD, participants will be reviewed in the outpatient hand clinic.

- Review of possible adverse events.
- Participants will complete a Health Quality of Life Questionnaire, the EQ-5D-5L.

Part 2 Randomised Controlled Trial

One week after the first treatment, participants will be phoned by a member of site staff to monitor for adverse events. If any of the information provided by the participant indicates that there may be a

problem with the hand that was injected this is to be brought to the urgent attention of the local PI, or other clinician on the site delegation log.

Visit 3, 3 months (post baseline visit) (1 hour 47 minutes)

- Review of possible adverse events.
- Participants will have a maximum of 12.5ml peripheral blood taken. This blood will be transported to the central laboratory for assessment of levels of adalimumab and antibodies to adalimumab.
- Participants will have an ultrasound scan to assess the size (grey scale) of the previously injected nodule.
- The injected nodule will be clinically assessed for suitability for injection.
- The hardness of the palmar tissue overlying the Dupuytren's nodule will be measured using tonometry.
- A digital photograph of the palm showing the selected nodule and indicating the nodule and associated affected finger will be recorded.
- Participants will have their finger range of movement measured using a goniometer.
- Grip strength will be measured using a JAMAR Dynamometer.
- Ametop gel or lidocaine/prilocaine cream may be applied 30 minutes to 1 hour prior to injection.
- Participants will complete the MHQ hand function questionnaire, and a Health Quality of Life Questionnaire, the EQ-5D-5L, and the Resource Use Questionnaire. Participants will consider the activity most restricted by DD identified at baseline and score it on a scale of 1-10.
- The injection will be administered into the previously injected Dupuytren's nodule (unless the clinician is not satisfied they can accurately deliver a repeat injection).
- Participants will then answer 2 questions about the experience of the injection.
- The injection site will be assessed by a trial healthcare professional blinded to treatment allocation to monitor for local adverse effects using an injection site response form.

Visit 4, 6 months and
Visit 5, 9 months (1 hour 42 minutes)

Both visits 4 and 5 are the same as visit 3 except that **no** blood will be drawn at these visits.

Visit 6, 12 months (57 minutes)

- Review of possible adverse events.
- Participants will have an ultrasound scan to assess the size (grey scale) of the injected nodule.
- The injected nodule will be clinically assessed.
- Participants will have a maximum of 12.5ml peripheral blood taken. This blood will be transported to the central laboratory for assessment of levels of adalimumab and antibodies to adalimumab.
- The hardness of the palmar tissue overlying the Dupuytren's nodule will be measured using tonometry.
- A digital photograph of the palm showing the selected nodule and indicating the nodule and associated affected finger will be recorded.
- Participants will have their affected finger range of movement measured using a goniometer.
- Grip strength will be measured using a JAMAR Dynamometer.
- Participants will complete the MHQ hand function questionnaire, and a Health Quality of Life Questionnaire, the EQ-5D-5L and the Resource Use Questionnaire. Participants will consider the activity most restricted by DD identified at baseline and score it on a scale of 1-10.

Visit 7, 18 months (52 minutes)

- Review of possible adverse events.
- Participants will have an ultrasound scan to assess the size (grey scale) of the injected nodule.
- The injected nodule will be clinically assessed.
- The hardness of the palmar tissue overlying the Dupuytren's nodule will be measured using tonometry.
- A digital photograph of the palm showing the selected nodule will be recorded.
- Participants will have their finger range of movement measured using a goniometer.
- Grip strength will be measured using a JAMAR Dynamometer.
- Participants will complete the MHQ hand function questionnaire, and a Health Quality of Life Questionnaire, the EQ-5D-5L and the Resource Use Questionnaire. Participants will consider the activity most restricted by DD identified at baseline and score it on a scale of 1-10.
- Any progression to surgery of the digit being assessed will be recorded.

If a participant is unable or unwilling to attend a follow up appointment but happy to complete the questionnaires, the questionnaires may be provided to the participant by email or post for completion. Other follow up data such as progression to surgery can also be collected by telephone and/or from the medical notes. The site must also check with the participant for any AE or SAE that require to be reported (if applicable) and submit the relevant CRF(s) as required.

8.7. Sample Handling

Part 1 only.

Tissue: All tissue (removed during surgery) will be analysed in laboratories within the Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences (NDORMS), University of Oxford, in Oxford, UK. Following surgery, the anonymised tissue samples will be transported as per instructions specified in a Sample Handling Manual to NDORMS laboratories. A scientist not involved with the clinical assessment will dissect the nodule and extract the mRNA and protein. PCR will be carried out as previously described (Verjee et al., 2013). Protein levels will be measured using electrochemiluminescence (Meso Scale Diagnostics, Maryland, USA) or other validated assays in accordance with manufacturer's instructions or published protocols. The scientist will be blinded to treatment allocation.

Part 1 and **Part 2**

Blood: The participants in the trial will have:

Part 1: A maximum of 12.5ml of blood collected at baseline and 2 weeks post-injection will be sent to the NDORMS laboratories in Oxford.

Part 2: A maximum of 12.5ml of blood collected at baseline, 3 months, and 12 months post first injection, will be sent to the NDORMS laboratories, in Oxford.

The transportation procedure is described in the Sample Handling Manual. On arrival the serum will be stored at -80 degrees Celsius. Serum concentration of adalimumab and antibodies to adalimumab will be measured using a commercial ELISA kit.

Participants will have provided informed consent approving collection, long-term storage, and future use of tissue and blood. As new mechanisms of action, safety and efficacy may evolve during or following the trial, which are unknown at present, biospecimens will be stored for a maximum of 15 years from the end of trial, after which time they will be destroyed. All samples will be identified by a trial identification number and stored securely.

8.8. Discontinuation/Withdrawal of Participants from Trial or Trial Treatment

Each participant has the right to withdraw from the study treatment at any time. In addition, the investigator may discontinue a participant from the study treatment at any time if the investigator considers it necessary for any reason including:

- Pregnancy
- Significant protocol deviation
- Significant non-compliance with treatment regimen or study requirements
- An adverse event which requires discontinuation of the study medication or results in inability to continue to comply with study procedures
- Hand surgery to remove the nodule being treated.

The reason for withdrawal will be recorded in the CRF. Following withdrawal from study treatment, participants will be encouraged to continue to attend for follow up appointments.

In the event that a participant were to undergo intervention (e.g. surgical excision, PNF, collagenase) to the digit/ray with the treated nodule during the course of the trial, the participant should be encouraged to attend the next follow up appointment. However, physical measures, including tonometry, range of motion and ultrasound assessments are not required (note: hand photograph is required). They should also be encouraged to attend the 12 month follow up appointment so that a blood sample can be obtained. Should the participant have further follow up appointments due (after attending the subsequent and 12 month appointments), or be unwilling to attend for a follow up appointment but agree to complete the questionnaires, the questionnaires may be provided to the participant by email or post for completion. The site must also ensure that any AE or SAE for which reporting is required are identified and submit the relevant CRF(s) as required.

Note: In the event that the participant undergoes surgery to a digit other that the one being treated in the trial, trial treatment and follow up should continue as per protocol.

Part 1

Participants may withdraw consent from the study at any time and will continue on the standard care pathway of hand surgery and aftercare. Participants who do not proceed to surgery may be replaced if this occurs during the recruitment phase of **Part 1**.

Part 2

Participants may withdraw consent from the study at any time and will continue on the standard care.

Part 1 and **Part 2**

Information collected up to the point of withdrawal may still be used. To monitor safety, if the participant withdraws after they have received medication their medical notes may be reviewed for the usual follow-

up period to capture protocol required safety data (i.e. grade 3 or above AE) . Intention-to-treat analyses will be carried out requiring admission of data to analysis for participants that are withdrawn.

8.9. Definition of End of Trial

The end of trial for each part of the study is the date of the last visit of the last participant. The overall end of the trial is the last visit of the last participant in part 2, or if the last participant is unable/unwilling to attend, the date of receipt of their completed questionnaires.

9. INVESTIGATIONAL MEDICINAL PRODUCTS (IMP)

9.1. IMP Description

Adalimumab is a human monoclonal antibody with Marketing Authorisation, but will be used off-label for this study. Adalimumab is available as both a 40 mg in 0.8 ml formulation, and (as of 2016), a 40 mg in 0.4 ml formulation. These formulations differ in the excipients they contain, with the 40 mg in 0.4 ml containing only polysorbate 80 and mannitol, and the 40 mg in 0.8 ml contains several other excipients, including citrate.. The 40 mg/0.8 ml formulation will be supplied in a single use glass vial and this can be used to provide variable doses lower than 40 mg. At the time of writing, the 40 mg/0.4 ml formulation is not available as a single use glass vial and so will be supplied in a pre-filled syringe. If during the trial, it becomes possible to obtain single use glass vials for this formulation then these may be used instead. Normal saline will be used as placebo. The cost of the drug will be paid for by 180 Therapeutics LP.

9.2. Storage and labelling of IMP

The adalimumab IMP will be purchased from AbbVie Ltd by the sites participating in the study. Pharmacy will receive the trial supply and label it 'For clinical trial use only' with other details such as the name of the investigator, sponsor and EudraCT number. No special packaging/blinding will be required as the person collecting and preparing the drug will not be blinded as described in section 8.4. It will be stored according to the manufacture's recommendations in a refrigerator at 2ºC to 8ºC in the original container, protected from light, until it is used.

The saline IMP will be purchased by the site and similarly labelled and stored in a refrigerator at 2ºC to 8ºC.

The IMP will be issued on prescription.

9.3. Preparation of IMP

The pre-filled syringe is fitted with a non-removable fine gauge needle and the research team have found that extremely high pressures are required to inject directly into the fibrotic nodule of Dupuytren's disease. Participants experience a lot of pain as a result and it is possible that the injection through a small calibre needle at high pressures may denature the adalimumab.

To prevent these problems, the IMP will be ejected into the barrel of an empty 1ml syringe the plunger of which has been withdrawn sufficently to accommodate the volume of adalimumab [0.4 ml]. This

procedure will be performed by an experienced medically qualified practitioner, who will ensure sterility throughout by following a no-touch technique for the contents of the barrel of the syringe. An appropriate gauge needle (typically 25 gauge) will be fitted, air bubbles evacuated and nodule injected without any delay. Care will be taken to ensure that none of the IMP is lost during the transfer. Transfer of the IMP will be done immediately prior to use.

Also see section 8.4.

9.4. Compliance with Trial Treatment

In **Part 1** compliance will not be applicable as treatment is completed in a single visit and administered by a trial healthcare professional. We will record administration of the IMP in the CRF with details of the dose, date and time, and ease of injection. Ease of injection will be recorded as 1 (easy), 2 (moderately easy) or 3 (difficult).

In **Part 2** compliance will be defined as attending not less than 75% of injection visits i.e. 3 visits. All injections will be administered by a trial healthcare professional. We will record administration of the IMP in the CRF with details of the dose, date, and ease of injection. Ease of injection will be recorded as 1 (easy), 2 (moderately easy) or 3 (difficult).

9.5. Accountability of the Trial Treatment

Pharmacy at each site will account for the drug and placebo. Any unused, partly used or used IMP including packaging will be stored separately from IMP awaiting allocation for accountability. Accurate records will be kept for all trial products by pharmacy at each site.

9.6. Concomitant Medication

Concomitant administration with biologic DMARDS is contraindicated. For Part 1 only, coumarin anticoagulants are contraindicated. Participants may receive concurrent vaccinations but must avoid the use of live vaccines for 12 weeks after their last injection.

Throughout the study Investigators may prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care. Any medication, other than the study medication taken during the study will be recorded in the CRF.

9.7. Post-trial Treatment

Part 1: Participants transfer to normal care for surgery and remain in normal care. There will be no provision of the IMP beyond the trial period.

Part 2: Participants will transfer to normal care at the finish of the trial. There will be no provision of the IMP beyond the trial period.

10. SAFETY REPORTING

10.1. Definitions

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor representative as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the summary of product characteristics (SmPC) for the IMP.

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific

event, which may be of relatively minor medical significance. “Seriousness” is the regulatory definition supplied above.

Any pregnancy occurring during the clinical trial and the outcome of the pregnancy will be recorded and followed up for congenital abnormality or birth defect, at which point it would fall within the definition of “serious”.

10.2. Causality

The relationship of each adverse event to the trial medication will be determined by a medically qualified individual according to the following definitions:

Related: The adverse event follows a reasonable temporal sequence from trial medication administration. It cannot reasonably be attributed to any other cause.

Not Related: The adverse event is probably produced by the participant’s clinical state or by other modes of therapy administered to the participant.

10.3. Procedures for Recording Adverse Events

The safety profile of Adalimumab is well known, with the most common adverse reactions being mild injection site reactions (see section 4). Common Terminology Criteria for Adverse Events (CTCAE) v4.0 (U.S. Department of Health and Human Services) will be used to guide recording Adverse Events including grading of the event.

Part 1. All AEs graded 3 and above occurring during the trial and until 12 weeks **after surgery** that are observed by the Investigator or reported by the participant will be recorded on the CRF, whether or not attributed to trial medication.

Part 2. All AEs graded 3 and above occurring during the trial until 28 days after the last injection of trial medication that are observed by the Investigator or reported by the participant and are attributed to trial medication or the injection of the trial medication will be recorded on the CRF. The capture all of AEs in the trial CRFs is deemed not necessary due to the period of time for which the IMP has had a marketing authorisation and it’s well established safety profile.

The following information will be recorded: description, date of onset and end date, severity, reason for seriousness, assessment of relatedness to trial medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

AEs considered related to the trial medication as judged by a medically qualified investigator will be followed either until resolution, or the event is considered stable.

It will be left to the Investigator’s clinical judgment to decide whether or not an AE is of sufficient severity to require the participant’s removal from treatment. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must be given appropriate care under medical supervision until symptoms cease, or the condition becomes stable.

10.4. Reporting Procedures for Serious Adverse Events

SAEs occurring after the first injection of trial treatment and up to 28 days after the last injection of trial treatment must be reported to the RIDD Trial Office. Only SAEs that are related to trial medication or the injection of the trial medication require to be reported. The reporting of SAEs not related to the IMP/injection is deemed not necessary due to the period of time for which the IMP has had a marketing authorisation and it's well established safety profile.

SAEs must be recorded on the trial specific SAE form and reported within 24 hours of the Site Study Team becoming aware of the event. Causality must be assessed by a medically qualified doctor. The SAE form must be sent to the Trial Office. Details are as follows:

Trial Office contact: RIDD@kennedy.ox.ac.uk Tel: +44 (0)1865 612610

The Trial Office will review the information on the SAE and raise immediate queries for any key data which is missing. In the event where the reporting site does not provide an assessment of causality the Trial Office will assume that the event is related to the IMP.

The SAE form will also be sent to an independent clinician as soon as all the relevant information is available but no later than 3 calendar days from when the Trial Office is made aware of the event as per OCTRU SOPs for safety reporting. The independent clinician will assess the SAE for causality and expectedness and provide a response as per OCTRU safety reporting. In the event where the independent clinician disagrees with the assessment of the site PI, a discussion will be encouraged between the two to re-assess the event. If either the PI or the independent clinician continue to assess the event as related, both assessments will be recorded. If the event is also related and unexpected this will be reported as a SUSAR by the Trial Office or delegate to the relevant Competent Authorities, UK Ethics, Sponsor and other bodies in with the nation and local regulations of participating sites, as per below.

All SAEs will be followed till resolved or no further information is expected. SAEs will only be considered closed when signed off by the local PI.

SAEs will be reviewed at the next Safety Committee meeting (Part 1) or Data and Safety Monitoring Committee meeting (Part 2).

10.5. Expectedness

Expectedness will be determined by the independent clinician according to the reference safety information (RSI) section of the Summary of Product Characteristics of for the 40 mg solution for injection in pre-filled syringe.

10.6. SUSAR Reporting

All SUSARs will be reported by the Trial Office or delegate to the relevant Competent Authorities, UK Ethics, Sponsor and other bodies in line with the nation and local regulations of participating sites, as applicable. For fatal and life-threatening SUSARs, this will be done no later than 7 calendar days after the Sponsor is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

Treatment codes will be un-blinded for specific participants.

10.7. Development Safety Update Reports

The Development Safety Update Reports will be prepared by the CI and submitted to the relevant Competent Authorities, UK Ethics, Sponsor and other bodies in line with the nation and local regulations of participating sites, and to the Sponsor within the timelines as per the relevant OCTRU SOP.

11. STATISTICS

Full details of the statistical analysis will be detailed in a statistical analysis plan (SAP) which will be finalised prior to any data analysis.

Stata (StataCorp LP) or other appropriate validated statistical software will be used for analysis.

11.1. Inclusion in Analysis

The intention-to-treat population is all randomised participants, grouped by the treatment allocated as opposed to the treatment received.

The per-protocol population includes those participants who complied with the protocol in terms of eligibility, treatment and availability of outcome measurements. Full details of the exclusions from this population will be detailed in the SAP.

The safety population is made up of all participants who started treatment, that is, who received at least one injection.

11.2. Description of Statistical Methods

During the dose response (**Part 1**), analysis will be carried out using data from all participants randomised to Part 1. In the RCT (**Part 2**), all analyses will be performed on participants randomised to Part 2 only, on an intention-to-treat basis with sensitivity analyses run on the per protocol population.

Part 1

Descriptive statistics will be used to describe the demographics between the intervention groups for each dose. This difference in the mean between cases and controls will be reported with 95% confidence intervals for all continuous outcome variables. P-values will be reported to 3 decimal places.

The primary outcome measure for **Part 1** is expression of mRNA for α-SMA. Expression will be quantified with PCR using the standard curve method using GAPDH, B2M and PGK1 as housekeeping genes to normalise the samples.

The secondary outcome measures to be analysed include expression of mRNA for COL-1A1, COL-3A1, adherin 11 and levels of α-SMA and collagen proteins, nodule size and nodule hardness.

For continuous variables, the difference in the means and the corresponding 95% confidence interval will be reported for each dose group and overall. For categorical variables, the number (and percentage) of participants in each category will be reported for each dose group and overall.

Part 2

The primary outcome measure is change in nodule hardness from baseline to 12 months. There will only be one dose level used in **Part 2**, which will be 40 mg of adalimumab. It is expected that all nodules will be large enough to accommodate the injection, but if not the volume of residual IMP will be recorded. The difference in the mean change of nodule hardness between the two groups will be reported with 95% confidence intervals. The comparison of change in nodule hardness between interventions will be analysed primarily using Analysis of Covariance (ANCOVA) adjusting for baseline nodule hardness, if it is normally distributed. As a secondary confirmatory analysis, ANCOVA adjusting for baseline nodule hardness as well as the stratification factors which are centre (Oxford, Edinburgh or Groningen) and age group (18-49 or ≥ 50 years) will be conducted.

If the data are not normally distributed, the data will be log-transformed in order to gain normality and geometric means with 95% confidence intervals will be reported. If data are not normally distributed after log-transformation, the non-parametric Mann Whitney test will be used with no adjustment for baseline, and the median change with 95% confidence intervals will be reported.

In order to analyse change in nodule hardness over the full time period, linear mixed effects regression will be used to compare nodule hardness between interventions utilising all time-points up to 18 months and adjusting for stratification and other important prognostic factors.

The secondary outcome measures for **Part 2** include those for **Part 1** and also grip strength, range of motion and participant reported outcomes.

For nodule size, grip strength, range of motion of the affected digit, Michigan Hand Outcomes Questionnaire, patient identified activity most restricted by DD, progression to surgery, and injection experience linear mixed effects regression models will be used. This procedure will allow for data from all follow-up time-points to be accounted for, and for adjustment for baseline measures to be made. The time (days) between baseline injection and each follow-up time-point will be included in the models as a random effect to account for patients not all having their follow-up assessments at the same time.

As per the primary outcome, initial analyses for all secondary outcomes will be unadjusted and additional adjusted analyses will be carried out further accounting for centre (Oxford, Edinburgh or Groningen) and age group (18-49 or ≥ 50 years). For all models, assessments for interaction between time since baseline treatment and baseline measurement will be carried out to assess if any effect of baseline measurement changes over time.

For all secondary outcome analyses, mean difference and associated 95% confidence intervals will be reported. If severe departures from normality are identified in secondary outcome measurements, the data will be log-transformed in order to gain normality and geometric means with 95% confidence intervals will be reported. If the data are not normally distributed – or near normally distributed – after log transformation, the non-parametric Mann-Whitney test will be used with no adjustment for baseline, and the median change with 95% confidence intervals will be reported.

11.3. The Number of Participants

Based on our *in vitro* findings for the primary outcome measure (α-SMA expression 1.03±0.18 in controls compared to 0.55±0.11 on treatment with anti-TNF), we estimate that, for **Part 1**, we will need 6 participants at each dose. As the *in-vivo* response is completely unknown a minimum of 8 participants will

be recruited at each dose randomised in a 3:1 ratio to adalimumab or placebo. Consenting participants who do not proceed to surgery will ideally be replaced. A maximum of 40 participants will be randomised.

For **Part 2**, the sample size is based on figures shown in Table 2. In our ultrasound trial (REC Ref. 11/SC/0447) there were 25 participants with mean 53±8 for cases and 32±3 for corresponding controls matched by age and sex. A minimum of 138 participants (69 per arm) are required to detect a moderate effect size of 0.62, based on a 5 point change in nodule hardness at 12 months and assuming a standard deviation of 8, 5% significance (2-sided) and 90% power, allowing for a 20% loss to follow up). These assumptions will be reviewed at the end of Part 1.

11.4. The Level of Statistical Significance

The level of significance to be used is $p \leq 0.05$.

11.5. Criteria for the Termination of the Trial

Part 1: Participant safety will be monitored for three months after surgery, and if any participant has an unplanned admission to hospital or unplanned surgery related to the surgery for Dupuytren's disease of the affected digit, or if any participant experiences a SUSAR, we will refer for advice to the Safety Committee (SC).

Part 2: If any participant experiences a SUSAR, we will refer to the advice of the Data and Safety Monitoring Committee (DSMC).

In the light of the interim data and other evidence from relevant studies, the SC (**Part 1**) or DSMC (Part 2) will inform the Trial Steering Committee (TSC) if there is proof beyond reasonable doubt that the data indicate the trial should be terminated. A decision to inform the TSC of such a finding will in part be based on statistical considerations (appropriate proof beyond reasonable doubt cannot be specified precisely).

Both the DSMC and TSC will follow appropriate charters drawn up prior to the start of the trial.

11.6. Procedure for Accounting for Missing, Unused, and Spurious Data.

Missing data will be described. The number and percentage of individuals in the missing category will be presented. All data collected on data collection forms will be used, since only essential data items will be collected. No data will be considered spurious in the analysis since all data will be checked and cleaned before analysis.

11.7. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

A detailed statistical analysis plan will be drawn up prior to participant recruitment or early in the trial with review and appropriate sign-off following OCTRU SOPs. Any changes to the statistical analysis plan during the trial will be subject to the same review and sign-off procedure with details of changes being included in the new version. Where appropriate these changes will be included in a protocol amendment. Any deviations from the statistical analysis plan will be reported and justified in the statistical section of the final report.

12. DATA MANAGEMENT

12.1. Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

We will record in the CRF:

Inclusion and exclusion criteria tick box list information from hospital records and confirmed by the participant

Informed consent confirmed

Demographic data (age, sex, height, weight, smoking habits, alcohol consumption) from hospital records and confirmed by the participant

Medical history (relevant medical history in other systems, medications taken) from hospital records and confirmed by the participant

Physical examination (vital signs: heart rate and blood pressure)

Printouts of laboratory analysis of blood and CXR results will be used to complete the relevant sections of the CRF.

Dupuytren's disease assessment

Participant reported outcomes measures (MHQ, EQ-5D-5L, activity limitation questionnaire, resource use)

Physical measures (finger range of movement, tonometry and grip strength)

Participant reported injection questionnaire

Injection site assessment

Wound assessment

We will record electronically:

Ultrasound images: Grey scale images or videos will be saved electronically with the relevant participant identifier and date on the portable ultrasound machine. Raw data from the ultrasound machine will be transferred to an appropriate media for transfer to the trial office where it will be stored securely and following the end of the trial archived with the trial data. Analysis will be carried out at the RIDD trial office using image assessment software such as ImageJ.

Digital photographic images: these will be saved with the relevant participant identifier and date and stored on a secure university server.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all trial-specific documents that leave the participating site, other than the signed consent form (where permitted by national regulations), the participant will be referred to by the trial participant number/code, not by name.

Sample analysis

All participant samples will be labelled with the participant trial number at site and then sent to the lab who will code further and thereafter only identified using unique codes. Data from the sample analysis (ELISA, PCR) will be stored on a secure university server. Printouts will be kept in the dedicated study laboratory book in a secure location or an electronic laboratory book on a secure university server. The data will be archived in line with the archiving of the trial.

12.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor including OCTRU, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

12.3. Data Recording and Record Keeping

All **Part 1** and **Part 2** trial data will be entered into the trial clinical database at the RIDD trial office. The participants will be identified by a unique trial specific number and/or code in the clinical database and their name will NOT be included.

Personal information including name, contact details, GP/family doctors details and national healthcare system/hospital identification number (e.g. NHS/CHI/MRN number) collected during the recruitment and screening process will be stored at the RIDD Trial Office in a secure manner and separately from the trial clinical database.

Participant files will be stored in numerical order and stored in a secure and accessible manner in the RIDD trial office. Participant files will be kept in storage onsite with the possibility of archiving offsite if the need arises. Trial documentation including data will be archived for a minimum of 15 years.

Sample derivatives will be stored in the NDORMS in secure -80^{0}C storage. Raw data will be analysed and stored in the NDORMS.

13. QUALITY ASSURANCE PROCEDURES

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. A risk assessment will be performed prior to the start of the trial and a monitoring plan, including central monitoring will be drawn up for the trial with the level and intensity of monitoring proportionate to the risks identified.

Trial committees

1. Trial Management Group (TMG)

The TMG will be responsible for day-to-day management of the research for **Part 1** and **Part 2**. Members will include members of the research team, the chief investigator, trial manager and statistician.

2. Safety Committee (SC) Part 1

The Safety Committee will include members of the Trial Management Group (TMG) and an independent clinician.

The aims of this committee include:

- To pick up any trends, such as increases in un/expected events, and take appropriate action
- To evaluate the risk of the trial continuing and make recommendations to the PI.

3. Data and safety monitoring Committee (DSMC) Part 2

The Data and Safety Monitoring Committee (DSMC) will review recruitment, study conduct and participant safety in **Part 2** and will consist of at least two independent clinicians and a statistician. This committee will review the accumulating data some of which will be analysed separately by treatment arm. They will make recommendations to the TSC. The terms of reference will be according to a DSMC charter.

4. The Trial Steering Committee (TSC)

The Trial Steering Committee (TSC) will be an independent committee who are ultimately responsible for making decisions about the continuation or otherwise of the trial. The TSC will oversee the whole trial and will have the overall responsibility on decisions to continue or stop early.

14. SERIOUS BREACHES

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.

A serious breach is defined as “A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) The safety or physical or mental integrity of the subjects of the trial; or

(b) The scientific value of the trial”.

In the event that a serious breach is suspected the trial team will follow OCTRU’s approved SOP on the management of serious breaches. In collaboration with the chief investigator the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee and Regulatory Authority within the legal timeframe for reporting.

15. ETHICAL AND REGULATORY CONSIDERATIONS

15.1. Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

15.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice. All investigators will have current GCP certification or equivalent.

15.3. Approvals

The protocol and all relevant supporting documentation such as the informed consent form, participant information sheet, any proposed advertising material, clinical trial label, and SmPC, will be submitted as required by country-specific national and local regulations, which in the UK will be to an appropriate Research Ethics Committee (REC), the UK Competent Authority (MHRA), and host institution(s) for written approval. Submissions for non-UK countries will be in line with country-specific national and local regulations.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

15.4. Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the UK REC and Sponsor. A Development Safety Update Report (DSUR) will be submitted annually to all relevant Competent Authories, in the UK to the REC, in non-UK countries in line with country-specific national and local regulations and to the Sponsor office. In addition, an End of Trial notification and final report will be submitted to the relevant Competent Authories, in the UK to the REC, host organisation and Sponsor and in non-UK countries in line with country-specific national and local regulations. .

15.5. Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials (where permitted by national regulation) and a participants ID number on the CRFs and any other documents that leave the study site, except the signed consent form (where permitted by national regulation), and in the trial clinical database. All documents and files, including those containing personal details collected during the UK recruitment process (which may include name and contact information, GP details, NHS/CHI/MRN number), will be stored securely and will be only accessible by trial staff and authorised personnel. Where necessary participant personal information will be communicated between the RIDD Trial Office and participating UK sites by secure methods (e.g. nhs.net email) as described in the PIL. The trial will comply with the General Data Protection Regulation (GDPR) and the Data Protection Act 2018, which requires data to be anonymised as soon as it is practical to do so.

15.6. Expenses and Benefits

Part 1 and **Part 2**. Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate.

15.7. Other Ethical Considerations

Some participants will be randomised to placebo injection of saline. In placebo controlled trials of adalimumab in patients with rheumatoid arthritis, 8% of patients receiving saline developed injection site reactions, mostly described as mild (SmPC for Humira (adalimumab), updated 26 March 2015). Very rarely a test used at the screening visit (physical examination or blood test) may lead to the detection of an unsuspected medical condition. The local PI at site will arrange for appropriate support and treatment if required.

16. FINANCE AND INSURANCE

16.1. Funding

We have successfully gained a Health Innovation Challenge Fund (HICF) for the research. This is a parallel funding partnership between the Wellcome Trust and the Department of Health.

Costs for the IMP are covered by 180 Therapeutics LP.

16.2. Insurance

UK:

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London, policy numbered: WD1200463). Standard NHS indemnity operates in respect of the clinical treatment which is provided.

Netherlands:

The University will arrange a site- and trial- specific insurance to provide for any harm which may be suffered by research participants, and which meets the requirements for the conduct of such research in the Netherlands. In respect of any `standard' [non- protocol specified] clinical treatment provided to participants, the usual indemnity arrangements operated by the organisation providing that treatment will apply.

17. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authorship will be determined in accordance with the International Committee of Medical Journal Editors (ICMJE) guidelines and other contributors will be acknowledged. The ICMJE Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals 2013) recommend that authorship be based on the following 4 criteria:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- Drafting the work or revising it critically for important intellectual content; AND

- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

The author will be able to identify which co-authors are responsible for specific other parts of the work.

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19. APPENDIX A: TRIAL SUMMARY

Part 1: Dose response

	What will happen?	Is this visit combined with usual hospital treatment?
Medical check-up *Approx. 1-2 hours.*	Eligibility assessment including chest X-ray and blood tests.	**Yes**, combined with pre-operation assessment
Injection visit *Approx. 2 hours.*	• Dupuytren's assessment • Blood test • Ultrasound scan • Hand photo • EQ-5D-5L Questionnaire • Nodule hardness • Injection and then assessment of injection site	**No**
Surgery day *Approx. 1 hour.*	• Assessment of injection site • Blood test • Ultrasound scan • Hand photo • Nodule hardness	**Yes**, while waiting for surgery
2 weeks after surgery *Approx. 20 mins.*	• Surgery site assessment. • Hand photo	**Yes**, combined with surgery aftercare
12 weeks after surgery *Approx. 15 mins.*	• EQ-5D-5L Questionnaire • Review of therapy record	**Yes**, combined with surgery aftercare

20. APPENDIX B: TRIAL FLOW CHART

Part 2: Randomised Controlled Trial

	What will happen?
Medical check-up *Approx. 1-2 hours.*	Eligibility assessment including chest X-ray and blood tests.
Injection visit 1 *Up to. 2 hours.**	• Dupuytren's assessment • Blood test • Ultrasound scan • Hand photo • EQ-5D-5L and hand function questionnaires • Nodule hardness, finger movement and grip strength • Injection and then assessment of injection site
+ 1 WEEK **Phone call**	• Researcher calls participant, no visit needed
Injection visit 2 **3 months later** *Up to. 2 hours.**	• Assessment of nodules and cords in hand • Blood test • Ultrasound scan • Hand photo • EQ-5D-5L and hand function questionnaires • Nodule hardness, finger movement and grip strength • Injection and then assessment of injection site • Resource Use Questionnaire
Injection visit 3 and 4 **6 and 9 months later** *Up to. 1 hour 45 mins.**	• As at 3 months but without blood test
12 months later *Approx. 1 hour.*	• As at 3 months but without injection
18 months later *Approx. 1 hour.*	• As at 3 months but without injection or blood test

* Appointment length is dependant on whether local anaesthetic cream is used as these need to be applied 30/60 minutes (depending on type) prior to the injection.

21. APPENDIX C: SCHEDULE OF PROCEDURES

Part 1 Summary of schedule for dose response:

Procedure	Time in mins	Screening Visit 1	Baseline Visit 2 Week 0	1 week telephone call	Visit 3 Week 2		Visit 4 Week 2 post-surgery	Visit 5 Week 12 post-surgery
Visit window					±3 days		±1 week	±4 weeks
Informed consent	20	X						
Demographics	5	X						
Medical history	5	X						
Concomitant medications	5	X	X		X		X	X
Physical examination	5		X					
Chest X-Ray	20	X						
Blood for screening	5	X				HANDSURGERY		
Blood for research	5		X		X			
Dupuytren's assessment, inc. range of motion	15		X					
Eligibility assessment	5	X	X					
Health questionnaire	5		X					X
Nodule hardness (tonometry)	5		X		X			
Ultrasound imaging	15		X		X			
Digital photograph of palm	2		X		X		X	
Randomisation			X					
Topical anaesthetic and Injection of study drugs or placebo	40		X					
Injection questionnaire	5		X					
Adverse event assessment Injection site assessment	5		X		X			
Adverse event assessment Surgery site assessment	5						X	X
Telephone call to monitor for adverse events	10			X				
Total in minutes		**65**	**112**		**37**		**12**	**15**

Part 2 Summary of schedule for RCT

Procedures		Visits							
	Time in minutes	Screening Visit 1	Baseline Visit 2 week 0	1 week telephone call	Visit 3 3 month	Visit 4 6 month	Visit 5 9 month	Visit 6 12 month	Visit 7 18 month
Visit window			≤12 weeks of screening	± 4 days	±4 weeks	±4 weeks	±4 weeks	±4 weeks	±4 weeks
Informed consent	20	X							
Demographics	5	X							
Medical history	5	X							
Concomitant medications	5	X	X		X	X	X	X	X
Chest X-Ray	20	X							
Blood for screening	5	X							
Blood for research	5		X		X			X	
Dupuytren's assessment	10		X						
Eligibility assessment	5	X	X						
Health & Resource Use questionnaire	5		X[1]		X	X	X	X	X
Hand function questionnaires	10		X		X	X	X	X	X
Physical measures: grip, range of motion, tonometry	15		X		X	X	X	X	X
Ultrasound imaging	15		X		X	X	X	X	X
Digital photograph of palm	2		X		X	X	X	X	X
Randomisation			X[2]						
Topical anaesthetic and Injection of study drugs or placebo	≤40		X		X	X	X		
Injection questionnaire	5		X		X	X	X		
Adverse event assessment Injection site assessment	5		X		X	X	X	X	
Telephone call to monitor for adverse events	10			X					
Total in minutes		**75**	**132**		**107**	**102**	**102**	**57**	**52**

[1] Health questionnaire only.

[2] May be done in advance of baseline visit.

22. APPENDIX D: AMENDMENT HISTORY

	Details of changes made
Amendment No.: response to initial MHRA application **Protocol Version No.:** 2.0 **Date issued:**19 May 2015 **Author(s) of changes:** J Swettenham	Pg 27. Instructions on contraception use changed from 12 weeks after injection to 5 months, in line with SmPC.
Amendment No.: 1 **Protocol Version No.:** 3.2 **Date issued:**08 Jul 2015 **Author(s) of changes:** J Swettenham	**Screening procedure:** modified to those used in standard NHS practice to ensure suitability for anti-TNF therapy: only blood tests directly relevant to anti-TNF suitability will be performed, an ECG is not required. **Extra blood sample:** A research blood sample will be collected before the first injection to get baseline levels. **Exclusion criteria:** **(new)** Participant has previously had fasciectomy, dermofasciectomy, needle fasciotomy, collagenase injection or steroid injection to treat Dupuytren's disease in the digit concerned. **(modified)** Exclude participants who have ever been diagnosed with cancer instead of currently diagnosed with cancer. **(modified)** Exclude participants with any demyelinating disease, not just multiple sclerosis. **(modified)** Exclude participants with moderate or severe heart failure instead of existing or previous serious heart condition. **(removed)** Participants will not be excluded if they have donated blood in the previous 12 weeks. **(new)** Participant has received a live vaccine within 4 weeks, and they must avoid live vaccines for 12 weeks after the last injection. **(new)** Participants with epilepsy or a known allergy to tetracaine may take part in the study but will not receive Ametop gel as a local anaesthetic. If these patients are also allergic to either lidocaine or prilocaine they will be excluded. **(new)** Part 2 only: Participant at risk of Hepatitis B infection. **Part 1, progression to the next dose level.** Changed from clinician led judgement of delayed healing at 4 weeks post-surgery to "unplanned admission to hospital or unplanned surgery related to the surgery for Dupuytren's disease of the affected digit" at 2 weeks post-surgery. **IMP.** The doses of adalimumab to be administered have not been altered, but for some doses the associated volume of carrier had been miscalculated. Blood to be tested for antibodies to adalimumab, as well as adalimumab levels. The volume of blood taken for the research question will be a maximum of 12.5 ml and not 10 ml. Removal of the DASH questionnaire. Alteration to Michigan Hand Questionnaire: we will not ask questions on ethnicity, education, income and health insurance. Hand therapy treatment and adherence will not be collected. NIMP. Ametop gel will be used as an optional alternative local anaesthetic to EMLA cream. Ametop gel will be used in preference unless the participant is allergic to tetracaine or has epilepsy.

	Investigator addition: Assoc. Professor Oliver Rivero-Arias has been added as a health economist. Acceptable visit time windows have been broadened. **Inclusion criteria:** **(modified)** The nodule to be injected does not need to be seen on ultrasound for eligibility judgement. Contraception requirements altered. Unblinding will be done via trial centre not the site. The volume of the carrier associated with the IMP has been changed RRAMP will not be used for randomisation in Part 1. The cord associated with the nodule will also be assessed in Part 2. We may use mail-outs from GP surgeries to recruit to Part 2. In Part 1, the wound will be assessed using criteria from the CDC until it has healed enough to use the POSAS.
Amendment No.: 2 **Protocol Version No.:** 4.0 **Date issued:**13 Aug 2015 **Author(s) of changes:** J Swettenham	**7.2 Contraception:** True abstinence to be in line with preferred and usual lifestyle of the participant. Change made in response to MHRA request. **8.4 Unblinding:** unblinding will be possible 24 hours a day with a paper backup available at the Trial Office.
Amendment No.: 3 **Protocol Version No.:** 5.0 **Date issued:**15 Dec 2015 **Author(s) of changes:** J Swettenham	**Part 1 - secondary objectives:** Expression of COL-3A1 in excised nodule tissue has been added. Grip strength, range of motion, the Michigan Hand Outcomes Questionnaire (MHQ) and activity most restricted have been removed. **Part 2 - addition of blood sample 12 months post first treatment.** **Part 1 – fewer study visits:** The study visit one week after injection will be replaced with a phone call. The study visits one and four weeks after surgery have been removed. **Part 1 – selection of the dose to be used in Part 2** will be decided by the TSC based on all measured changes in the nodule, not just the primary outcome. **Inclusion criteria** – the nodule to be treated won't be removed in Part 2, this was never intended but was an error. **Exclusion criteria** – previous radiotherapy added. **Part 2 – recruitment:** As well as being sent the PIL potential participants may be sent a questionnaire about their Dupuytren's medical history. The websites details were updated. **Part 1 –** Western blotting and histology are not part of the trial procedure.
Amendment No.: 4 **Protocol Version No.:** 6.0 **Date issued:**09 Feb 2016 **Author(s) of changes:** J Swettenham	The choice of dose cohorts in Part 1 will be decided by the Safety Committee following analysis. This is to ensure the best doses are chosen to cover the response range. Doses will not exceed the maximum already stated.
Amendment No.: 5 **Protocol Version No.:** 7.0 **Date issued:** 24 Mar 2016 **Author(s) of changes:** J Swettenham	The dose to be used in the RCT will be 35mg into the nodule. In the RCT the visit at +1 week will be replaced with a phone call to check for adverse events. Modifications to inclusion/exclusion criteria: participants do not need to be on stable medication for 4 weeks, participants will be excluded if they have received parenteral steroid within 6 weeks, and anticoagulants have been clarified as coumarin type.

Amendment No.: 7* **Protocol Version No.:** 8.0 **Date issued:** 21 Sep 2016 **Author(s) of changes:** J Swettenham *Amendment 6 was addition of site and did not affect protocol.	**Objectives:** Ultrasound will not measure vascularity or cord extent and more information on ultrasound has been provided. Levels of α-SMA protein in the nodules (Part 1) will be measured. **Doses:** A new formulation of adalimumab has been released (40mg in 0.4ml). It has been made clear in the protocol which formulation will be used at which points. In Part 1 the maximum dose that may be used has been reduced to 80 mg or 0.8 ml and no cohorts will use abdominal injections. **Non-IMP:** Lidocaine/prilocaine cream of any brand may be used as a local anaesthetic. Participants may choose to not have local anaesthetic. **Part 1:** Up to a maximum of 40 people may participate in Part 1. At a minimum dose cohorts of 15 mg in 0.3 ml, 35 mg in 0.7 ml and 40 mg in 0.4 ml will be collected. **Nodules:** Ultrasound will not be used as a screening tool. The decision process for whether a repeat injection in Part 2 will be given has been clarified. **CXR:** If a participant has a suitable clear CXR result in their medical record this may be used if local procedures for anti-TNF screening allow. **Consent:** For one site in Part 1, consent may be taken on the same visit as the potential participant is identified. **Randomisation:** For Part 1, Pharmacy will consult their allocation log to dispense the IMP corresponding to the Trial ID. **Blinding: T**he 40mg in 0.4ml formulation is currently not available in glass vials and this has implications for blinding. The way blinding will be controlled has been described for both vials and pre-filled syringes. Participants and researchers involved in assessments from the injection onwards will remain blinded. **Heart rate and blood pressure:** These values will not be measured as part of the study but will be available from medical notes if required. **Patient outcome measures:** In Part 1, at 12 weeks after surgery, participants will be asked about the acceptability of the injection therapy and their return to paid work. **General corrections:** 'Patient' replaced with 'participant' when appropriate. One off treatment is given 2 weeks before surgery. Part 1 will not be used to optimise the dose for Part 2.
Amendment No.: 12 **Protocol Version No.:** 9.0 **Date issued:** 08Dec2017 **Author(s) of changes:** Anne Francis	**Objectives - Part 1:** addition of further markers to outcome measures for the 1st secondary objective; addition of secondary objective to monitor for adverse events and to assess healing; correction that injection experience is an outcome measure and additional of analysis of EQ-5D-5L to the 2nd tertiary endpoint; addition of a new exploratory objective. **Objectives - Part 2:** Changes to outcome measure and time points of evaluation of primary objective to clarify this is at 12 months. Addition of a secondary objective to evaluate changes in nodule hardness at other follow up time points (previously included in primary endpoint; removal of clinical assessment of the hand as an outcome measure for the 2nd secondary objective; addition of new secondary objective to assess acceptability of injections and to monitor for adverse events; removal of laboratory reports as an outcome measure; addition of analysis of resource use data as outcome measure for the 1st tertiary objective; addition of a new exploratory objective. **Inclusion/exclusion criteria - Part 2** – Change to define that Dupuytren's must affect fingers and not thumb; change that only treatment to the digit

	to be treatment, except radiotherapy, is an exclusion criterion; increase in permitted time between screening and baseline from 8 to 12 weeks. **Recruitment – Part 2:** Removal of distance of GP surgeries; addition of other methods of recruiting/advertising; clarification of process; addition of suitability discussion for self-referring potential participants prior to screening visit. **Unblinding:** Removal of option for unblinding to be via backup copy held by Trial Office as part 2 this is not possible; Removal of 24/7 unblinding for part 2. **3 month visit – Part 2:** Increase in window for this visit to +/- 4 weeks. **Part 2 – Subsequent visits**: Addition of requirement to complete resource use questionnaire. **IMP:** Addition of section about preparation of IMP when using pre-filled syringe. **Safety reporting:** Change to reporting requirements of AEs (including SAEs) to 28 days after last treatment and only if related. **Statistics section**: Correction to the time point of data being used for the primary outcome analysis. **Throughout protocol:** Update to make applicable and inclusive of recruiting centres outside of the UK, specifically in The Netherlands.
Amendment No.: 14 **Protocol Version No.:** 10.0 **Date issued: 18Jun2018** **Author(s) of changes:** Anne Francis	**Amendment to the following sections:** Update to address of the Sponsor **7.3 Exclusion criteria** - Removal of exclusion criteria 'Have received parenteral steroid within the previous 6 weeks' from part 2 of the trial. **8.3 Screening and Eligibility Assessment, Part 2** – Change from 'in line with local procedures' to protocol specified tests. Increase to maximum volume of blood to be taken to enable all sites to undertaken the protocol specified tests. **9.3 Preparation of IMP** – change to the technique of transferring the IMP from the PFS to the 1 ml syringe. **8.1 Recruitment, Part 2. Randomised controlled trial** – change to implement within the protocol the REC requirement as per their favourable ethical opinon letter for Amendment 12 to limit the number of follow up contacts post provision of the PIL to a potential participant.
Amendment No.: 15 **Protocol Version No.:** 11.0 **Date issued: 12Sep2018** **Author(s) of changes:** Anne Francis	**Amendment to the following sections:** Change to target recruitment from 138 to maximum of 200. **7.3 Exclusion criteria** - Removal of exclusion criteria: Part 2. Scheduled elective surgery or other procedures requiring general anaesthesia during the study Section 15.5 has been updated in line with GDPR.
Amendment No.: 17 **Protocol Version No.:** 12.0 **Date issued: 29Aug2019** **Author(s) of changes:** Anne Francis	**Amendment to the following sections:** **Synopsis** – increase in planned duration to 45 months. **8.4** – Addition of fixed duration for unblinding facility. **8.6 –** Additional of options for data collection if participants are unable or unwilling to attend an appointment. **8.8** – Addition of information regarding withdrawal from treatment due to surgery to the hand being treated and the follow up required. 8.9 – Change to reflect changes in section 8.6 **10.5** – Change to the definition of the Reference Safety Information.

	10.6 – Change to allow SUSAR reporting to be completed by a delegate of the Sponsor. **11.2** – Changes to the statistical methods for part 2. 12.3 – Increase in minimum archiving period to 15 years. **The following correction hase also been made:** **Appendix C: Part 2** – correction to remove physical measures, that has previously been removed from section 8.5.
Amendment No.: 18 **Protocol Version No.:** 13.0 **Date issued:** **Author(s) of changes:** Anne Francis	**Amendment to the following Section:** **8.8 Clarifiation on eroding for withdrawal.**