Study Protocol

The development of an *in vitro* human skin explant safety assay for the detection of immunogenicity and hypersensitivity reactions.

REC REFERENCE: 10/H0906/58
IRAS ID: 55396

This protocol has regard for the HRA guidance

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature: Date: 14/02/2024

.....

Name (please print):
Anne Dickinson

.....

Position:
CEO.....

Chief Investigator:

Signature: Date: 14/02/2024

.....

Name: (please print):
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List of Abbreviations

AuToDeCRA-2	Autologous TolerogenicDendritic Cells for Rheumatoid Arthritis (2nd phase trial)
AE	Adverse Event
ANOVA	Analysis of Variance
AR	Adverse Reaction
bDMARD	Biological Disease-modifying antirheumatic drug
CI	Chief Investigator
CRP	C-reactive protein
csDMARDs	Conventional synthetic disease modifying anti-rheumatic drugs
CTIMP	Clinical Trial of an Investigational Medicinal Product
DHRs	Drug Hypersensitivity Reactions
DMARD	Disease-modifying antirheumatic drug
ESR	Erythrocyte Sedimentation Rate
GCP	Good Clinical Practice
GVHD	Graft Versus Host Disease
HLA	Human Leukocyte Antigen
LMW	Low Molecular Weight
MHRA	Medicines and Healthcare products Regulatory Agency
NuTH	The Newcastle upon Tyne Hospitals NHS Foundation Trust
PICS	Patient Identification Centres
PI	Principle Investigator
PLNA	Popliteal Lymph Node Assay
RA	Rheumatoid Arthritis
REC	Research Ethics Committee
RGF	Research Governance Framework for Health and Social Care: Second Edition
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
Swollen Joint Count	SJC
Tender Joint Count	TJC
TGN 1412	Trial name for a type of monoclonal antibody therapy manufactured by German firm TeGenero
UAR	Unexpected Adverse Reaction

STUDY SUMMARY

Study Title	Development of an in vitro assay for drug safety and efficacy and skin sensitivity
Internal ref. no. (or short title)	Safety testing of novel compounds and drugs
Study Design	Laboratory
Study Participants	Healthy Volunteers / Rheumatoid Arthritis (RA) Patients /Surgical Patients *(Sub-Studies)*
Planned Size of Sample (if applicable)	<u>Healthy Volunteer</u> Recruit 4-6 participants per week 1 set of skin punch biopsy (2 biopsies per set) per healthy volunteer 60-70ml blood per healthy volunteer For patients who have opted in to consent to provide further samples a maximum of 3 sets of skin punch biopsies (2 biopsies per set) and 3 sets of 70 ml of blood. <u>Sub-Study : Rheumatoid Arthritis Patients</u> Recruit 40-50 per 12 months, 80-100 over 24 months 1 x 60-70ml blood and further maximum 120ml in serial (20ml x 6 blood samples) <u>Sub-study :Surgical Patients</u> 1x 70ml blood at pre-surgical clinic visit Excess skin sample taken at time of surgery (minimum 2x 4mm)
Follow up duration (if applicable)	NA
Planned Study Period	5 years
Research Question/Aim(s)	The development and use of in vitro skin assays to predict adverse responses to compounds including drugs and cellular therapies
Funder and financial and non-financial support given	Alcyomics Ltd - Payment of research staff, research nurses, funding study consumables, vouchers for participants. <u>Vouchers</u> <u>Healthy Volunteer:</u> • £50.00 voucher for 1 set of skin biopsies (2 biopsies per set) and 1 set of 70ml blood sample For patients who have opted in to consent to provide further samples: • £25.00 voucher per 70 ml blood sample and £25.00 voucher per 1 set of skin punch biopsy (2 biopsies per set)

	• A maximum of £150.00 for blood and skin punch biopsies samples Rheumatoid Arthritis Patients Sub Study 1 • £50.00 voucher for skin biopsies (x2) and 1x blood sample • £25.00 voucher per blood sample () • A maximum of £150.00 for a series of blood samples Rheumatoid Arthritis Patients Sub Study 2 • £25.00 voucher per blood sample • A maximum of £150.00 for a series of blood samples Surgery Patients Sub Study 3 • £25.00 voucher per blood sample
Role of study sponsor and funder	Alcyomics Ltd is responsible for the study design, conduct, data analysis and interpretation, manuscript writing, and dissemination of results and controls the final decision regarding any of these aspects of the study.
Key Words:	Safety and efficacy drug testing ; immunotoxicology; adverse immune reactions ; *in vitro* pre-clinical testing, novel diagnostics

1. BACKGROUND

The main aim of the study is to use an *in vitro* human skin based assay that will enable drug sensitivities and allergies to be detected at the early stage of drug development i.e. before animal or human trials.

Animal models provide only a partial guide to drug responses in man and drug companies are becoming increasingly reliant upon human tissue-based assays to improve drug development and bring drugs closer to market in a shorter period of time, thus ultimately reducing drug costs.

The drug trial conducted at the Northwick Park Hospital in 2006 made shocking headlines when a serious incident occurred in human volunteers. It was reported that TGN 1412 was tested on monkeys at doses 500 times greater than that given to the volunteers, but the human test subjects became critically ill within minutes of being given the drug.

An interim report issued by the Medicines and Healthcare products Regulatory Agency (MHRA) notes that the volunteers had a life-threatening incident of cytokine-release syndrome, which occurs when the substances released by the immune cells produce a type of systemic inflammatory response. This was an extreme example of what can go wrong when new drugs are introduced to man for the first time. This exemplifies the need for more effective human drug safety testing.

This is especially true when one considers the increasing number of biological drugs (e.g. monoclonal antibodies and cellular therapies) planned or in development. The problem is compounded by the lack

of effective in vitro models and the continued reliance on animal testing. This is one of the reasons that 90% of compounds that drug-makers take into human clinical trials ultimately fail to become viable medicines, and occurrences like the one described above are a very real threat.
Alcyomics Ltd is looking to offer solutions to these problems, which will help pharma and biotech companies to be more innovative while at the same time decreasing their risk of failure. Current expertise will enable us to test drugs for potential allergic or hypersensitivity reactions, including individual responses to stem cells and cellular therapies.
In addition, a large number of low molecular weight (LMW) drugs fail in clinical development. Lack of efficacy along with drug hypersensitivity reactions (DHRs) are a major cause of failure or withdrawal of new LMW drugs (Hay et al., 2014). Despite a battery of clinical and pre-clinical tests used for evaluating the safety of drugs, DHRs are often undetected until drugs are tested in large clinical trials or marketed (Pourpak et al., 2008). This late failure of drugs due to DHRs clearly indicates the need for predictive tools during the early stages of pre-clinical drug development. DHRs can be divided into 2 main groups, allergic reactions caused by adaptive immune mechanisms and non-allergic reactions not involving adaptive immune responses. The latter is the more common type of a DHR response and is often predictable, dose-responsive, and can be observed pre-clinically. In contrast, allergic drug reactions are rarely observed during pre-clinical drug development. There are currently no in vitro pre-clinical tools available which have been validated to screen for potential allergic DHRs. However, there are some in vivo models such as the modified popliteal lymph node assay (PLNA) (Warbrick et al., 2001, Pieters, 2001), the lymph node proliferation assay (LNPA)(Weaver et al., 2005) and the mouse allergy model (Whritenour et al., 2014, Zhu et al., 2015), which show some potential for predicting DHRs. Drug hypersensitivity can be related to genetic predisposition (Pirmohamed, 2006) occurring in only a minority of the population and therefore can be difficult to predict, especially using animal models (Bala et al., 2005). Consequently, in vitro testing using human material may be an attractive alternative. Tests developed more specifically for determining sensitisation potential of topically administered chemicals/compounds such as the human cell line activation test (h-CLAT), direct peptide reactivity assay (DPRA) or KeratinoSens™, (Nukada et al., 2012, Gerberick et al., 2004, Emter et al., 2010) could be potentially further investigated as suitable tools for predicting DHRs for systemically administered compounds.
In order to further develop the skin assay Alcyomics is developing a human *in-vitro* 3D skin model using healthy volunteer skin cells (keratinocytes and fibroblasts) to form 3D skin model for testing in a 96 well format. The objective of our study is to use and evaluate a human in vitro skin explant assay for predicting DHRs to drugs, chemicals, cosmetics, biologics and peptides. The skin explant assay used in these studies was modified from the original predictive test, which has been extensively used to understand the underlying mechanism and immunobiology of graft versus host disease (GvHD) (Dickinson et al., 1999, Dickinson et al., 1988, Vogelsang et al., 1985), (Dickinson et al., 2002, Jarvis et al., 2002, Ruffin et al., 2011, Mavin et al., 2012, Ahmed et al., 2015). The modified assay (commercially known as Skimune®) has been used as a method for predicting skin sensitisation potential of chemical compounds (Ahmed et al., 2016). It has also recently been extended to test systemically administered LMW drugs that are associated (or not) with hypersensitivity reactions in the clinic. As previously described by Ahmed and colleagues, the assay is used in an autologous setting, when the different cell types and skin used in the assay are derived from the same donor. The assay involves isolation of peripheral blood mononuclear cells and separation of monocytes to derive dendritic cells which are activated with the compound (or peptide) of interest. The assay can then assess T cell proliferation, cytokine release, and histopathological damage to skin tissue due to any adverse immune reaction. The severity of the skin reaction is scored in severity from grade I-IV (Lerner et al., 1974) and a score of a grade II or above is used to predict a positive DHR.
In addition the assay can be used to predict any adverse immune reactions due to monoclonal antibody therapy – in this regard peripheral blood mononuclear cells are added directly onto autologous skin in the presence or absence of the test monoclonal.
We also aim to use the assay including the 3D skin assay for sensitivity testing other types of compounds e.g. cosmeceuticals which if the results is successful may reduce animal resting in the future and enable the design of safer drugs and compounds.

We will also use the assay to develop a model of atopic dermatitis and potentially other skin diseases such as scleroderma acute and chronic Graft versus Host Disease ,to understand disease development, which will include the infiltration of cells into the skin (neutrophils, T cells, eosinophils and other cells) which will enable us to correlate the results to maximise the testing and efficacy of novel drugs and compounds
In summary, the project will enable the use of an important faster assay, for different markets and novel patents associated with drug safety efficacy and sensitivity testing.
We have published using this assay:-
Ahmed, S.S, Wang, X.N, Fielding, M, Kerry, A, Dickinson, I, Munuswamy, R, Kimber, I, Dickinson, A. M. An in vitro human skin test for assessing sensitization potential. Applied Toxicology. 2015 DOI: 10.1002/jat.3197
Dickinson A, Nong Wang X, Ahmed S. An In Vitro Human Skin Test for Assessing Adverse Immune Reactions and Sensitization Potential. In: Alternatives for Dermal Toxicity Testing. Springer International Publishing, 2017, pp.437-448.
Ogese, M. O., Ahmed, S., Alferivic, A., Betts, C. J., Dickinson, A., Faulkner, L., French, N., Gibson, A., Hirschfield, G. M., Kammuller, M., Meng, X., Martin, S. F., Musette, P., Norris, A., Pirmohamed, M., Park, B. K., Purcell, A. W., Spraggs, C. F., Whritenour, J. & Naisbitt, D. J.New Approaches to Investigate Drug-Induced Hypersensitivity. Chem Res Toxicol, (2017)30, 239-259.
Dickinson, A, Nong Wang X, Ahmed S. An In Vitro Human Skin Test for Assessing Adverse Immune Reactions and Sensitization Potential. In: Alternatives for Dermal Toxicity Testing. Springer International Publishing, (2017), 437-448.

Sub Study- Rheumatoid Arthritis (RA) Patients

We also aim to extend the study to a small group of RA patients in order to:

1) Test peptides in the skin assay by activating autologous dendritic cells with the peptide of interest and testing on autologous skin samples. These peptides are of potential value in novel RA clinical trials and the assays will allow assessment of dose response as well as safety.

2) Develop assays to assess which biologic will work most efficiently for an individual patient and assess the effect post-therapy.

The background and rationale for this study is as follows:

1) The use of peptides in a local phase II clinical trial AuToDeCRA-2 (Principle Investigator Professor John Isaacs) will commence late 2019/early 2020. The peptides and dose response for instigation in the clinical trial will be tested in healthy volunteers and RA patients who have a particular HLA genotype for peptide recognition.

2) Due to the number of biologic therapies available to rheumatologists for the treatment of RA Patients, there is a critical need for better tools to inform RA disease management. The ability to identify patients, including to respond to first time biologics prior to their treatment would allow tailored therapies for an individual patients (Thomson, Lescarbeau et al. 2015, Smolen, Aletaha et al. 2016).

Alcyomics aims to develop *in-vitro* assays using only blood samples from RA Patients to assess their response to a number of biologics prior to therapy ultimately enabling therapy to be chosen by the clinician on an individual patient basis. This is especially due to some patients developing anti-drug antibodies which neutralise the therapy and therefore negate the treatment. We will therefore aim to develop an *in vitro* test for predicting the potential to develop anti-drug antibodies in individual RA patients (Moots , Xavier et al 2017) .

2. STUDY OBJECTIVES

2.1 Primary Objectives

- Use an *in vitro* human skin based assay that will enable drug sensitivities to be detected at the early stage of drug development i.e. before animal or human trials. To extend and improve our current skin explant assay technology by researching a new assay using 3D skin modelling and bio printing which will enable capacity building and standardisation for use to the pharmaceutical industry for drug safety testing
- Results enable assessment of target genes. This may lead to the identification of novel protein targets for therapeutic purposes.
- Further develop the assays to enable a reduction in animal testing as required by EU legislation (European Directive 86/609/EEC and the 7th Amendment to the Cosmetics Directive (Directive 76/768/EEC2.
- Enable the development of a novel assay for atopic dermatitis drug testing
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2.2 Secondary Objectives– Rheumatoid Arthritis (RA) Patients

- Sub-study 1: To assess the safety of peptides for potential use in clinical trial.
- Sub-study 2: To assess the responses of RA patients to biologics and monitor the response post therapy.

3. STUDY DESIGN

3.1 Healthy Volunteers

- Assays will be conducted in the laboratory to assess the effect of the novel drugs or compounds on healthy volunteer blood or tissue.
- A minimal of 6 volunteers will be tested per compound at various dilutions in order to assess potency and effect of the compound on the blood or tissue.
- Lymphocyte proliferation (known as T cell responses) will be measured as a surrogate to the potential proliferation of T cells and thus adverse effect of the drug on the healthy volunteer.
- The skin biopsy will be assessed for histopathological damage due to the effect of the T cells on the skin.
- Results from the assays (n=6 -10) will be analysed using non parametric statistical tests e.g. ANOVA and Mann –Whitney U tests using a PRISM statistical package

The analysis will be carried out by trained post-doctoral senior scientists and Prof Dickinson at Alcyomics
All data will be coded with regard to the samples from the healthy volunteers.

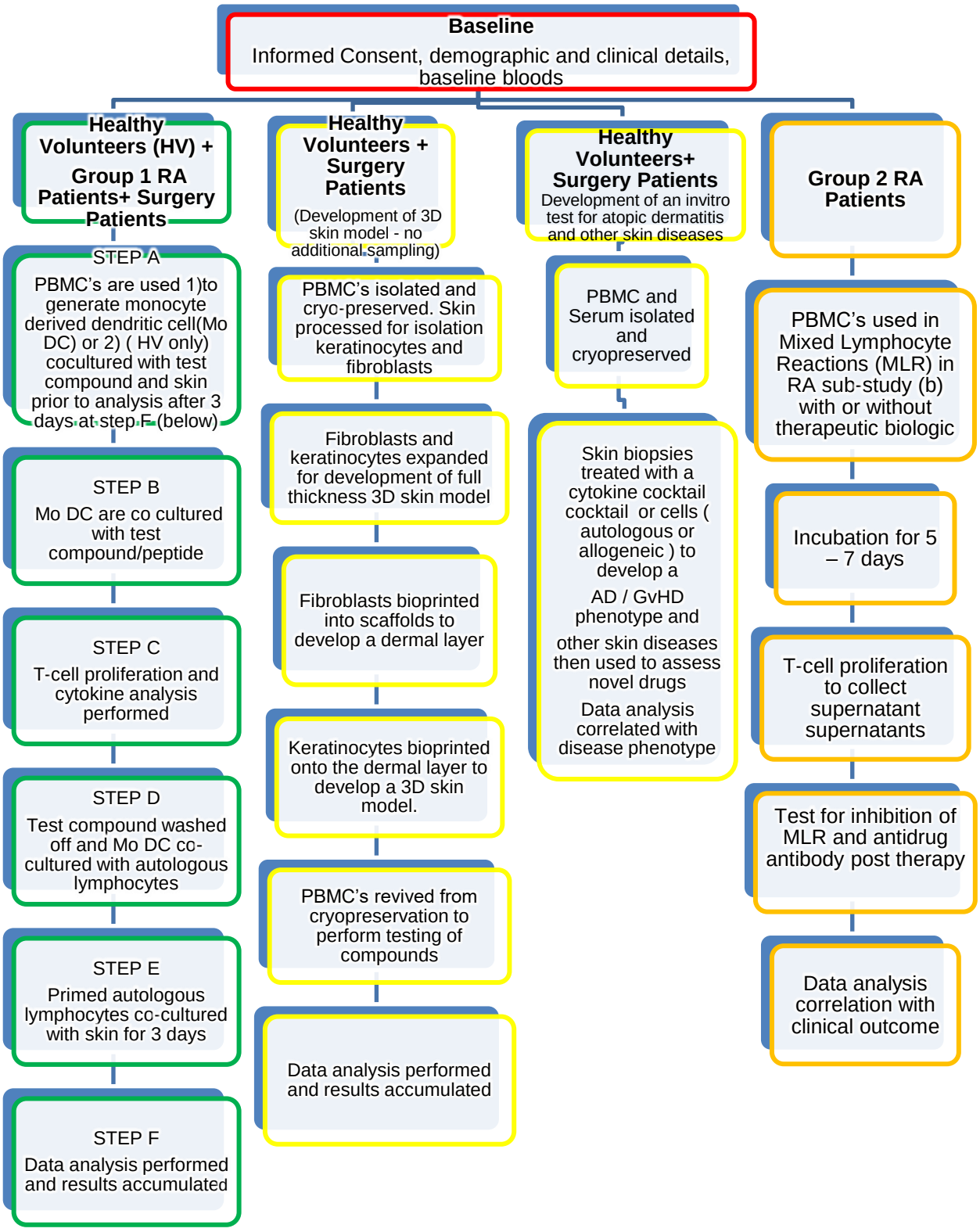
3.2 Sub-Study – Rheumatoid Arthritis (RA) Patients

Sub-study Group (1). PBMC and skin biopsies obtained at a single time-point from 6-10 RA patients will be used to assess the safety of peptides as described for healthy volunteers (above in 3.1).
Sub-study Group (2). A cohort of 40-50 RA patients will be assessed for anti-drug antibody responses before and after antibody therapy over two years. This will involve a 70ml peripheral blood sample being taken pre-treatment (baseline) and 20ml blood sample being obtained post-treatment (12, 24, 36 .48, 60, 72, weeks). Skin biopsies will not be required from volunteers in this group.

3.3 Sub-Study– Surgical Patients

A single blood sample of 70ml will be obtained at a pre-clinic surgery visit. Excess skin samples taken at the time of surgery, which would normal be thrown away, (eg for breast reduction or amputation) will be used to carry out the experiments as per the healthy volunteer study design.

3.4 Flow Chart of the Study



4. STUDY SETTING

Single centre study based in England with no other countries involved outside of the UK.
Recruiting volunteers will be a joint role for the NHS team and Alcyomics. Sampling, obtaining consent and clinical procedure will be held at an NHS site, Newcastle upon Tyne. Scientific Review and statistical significance will be held at the private offices/laboratories of the study sponsor, Alcyomics Ltd.

5. RECRUITMENT

5.1 Subject selection

Two broad subject groups will be selected as follows:

Healthy Volunteer Adults

We wish to recruit healthy adult volunteers to donate two 4mm skin punch biopsies and a 70 ml blood sample. The skin and blood sample will be used for drug safety testing by the development of an assay that can be used to identify adverse reactions i.e. skin sensitisation and immunogenicity to various products including new drugs such as monoclonal antibodies, small molecule drugs and cellular therapies. This is a commercial assay. To date no such reliable assay exists to predict responses to new drugs in the laboratory and without the use of animal testing. The same samples will also be used for the development of an assay for atopic dermatitis
The number of subjects will be maximum of 300 per annum. The maximum sample size is determined by the estimated availability of participants and the resources in the laboratory to process samples

Rheumatoid Arthritis (RA) Patients

Our target populations of RA patients will be enrolled according to distinct but overlapping criteria to account for two pre-defined sub-study groups as follows:
Sub-study Group (1). RA patients known to have one of the following tissue types: HLADRB0401 or 0404 positive, irrespective of their background DMARD therapy.
Sub-study Group (2). RA patients being started on one of four classes of biologic DMARD (bDMARD; namely anti-TNF, anti-IL-6R or anti-CTLA4 or anti-CD20) that they have not previously received. Such patients will have experienced an inadequate response to conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs), and may also have experienced an inadequate response to one or more treatment with a bDMARD (in one of the aforementioned classes) and/or targeted janus kinase inhibitor DMARD (tDMARD).
The number of RA subjects will be maximum of 40-50 per annum.

Surgery Patients

The surgery patients for example undergoing breast reduction or amputation surgery or patients where any surgery results in excess skin being thrown away, will be recruited at a pre-surgery clinic visit .

5.2 Enrolment criteria

Healthy Volunteers

Inclusion:

a. No underlying disease, such as cold or flu symptoms.
b. No serious illness such as cancer requiring medication.
c. Must speak and understand English

Exclusion:

a. Skin pathology at the biopsy site (lower abdomen or buttock)
b. Untreated/unresolved infective illness at the time of screening and/or baseline visit.
c. Use of immunosuppressive medicines
d. Evidence of allergy from questionnaire- see Appendix

Sub-Study – Rheumatoid Arthritis (RA) Patients
For RA Sub-study Group (1)
(To assess the safety of peptides for potential use in clinical trial).

Inclusion:
a. Clinical diagnosis of RA by a consultant rheumatologist with reference to accepted disease classification criteria.
b. Known to be HLA-DRB1*0401 or *0404 positive
c. Able and willing to provide written informed consent for study participation.
d. Stable or no corticosteroid therapy within 4 weeks prior to screening visit.
e. Must speak and understand English

Exclusion:
a. Receipt of intramuscular/intra-articular corticosteroid and/or change in oral dose corticosteroid within 4 weeks of baseline visit.
b. On >10mg daily dose prednisolone or equivalent corticosteroid therapy.
c. Untreated/unresolved infective illness at the time of screening and/or baseline visit.
d. Skin pathology at the biopsy site (lower abdomen or buttock)
e. Evidence of allergy from questionnaire (*see Appendix)*

For RA Sub-study Group (2)
(To assess the responses of RA patients to biologics and monitor the response post therapy).
Inclusion:
a. Clinical diagnosis of RA by a consultant rheumatologist with reference to accepted disease classification criteria.
b. Treated with conventional and/or biologic and/or targeted therapy for RA in accordance with NICE guidelines.
c. Clinical decision in accordance with current NICE guidelines to commence one of the following bDMARDs ((licensed biosimilar equivalents permitted): infliximab, adalimumab, etanercept, tocilizumab, abatacept or rituximab.
d. Baseline blood sample must obtained prior to first dose of bDMARD to be prescribed in (c).
e. Able and willing to provide written informed consent for study participation.
f. Stable or no corticosteroid therapy within 4 weeks prior to screening visit..
g. Enrolment of patients switching from one class of biologic therapy to another biologic (e.g. etanercept to adalimumab (both anti-TNF biologics) or an anti-IL6R) is permitted.
h. Must speak and understand English

Exclusion:
a. Receipt of intramuscular/intra-articular corticosteroid and/or change in oral dose corticosteroid within 4 weeks of baseline visit.
b. On >10mg daily dose prednisolone or equivalent corticosteroid therapy.
c. Contraindication to biologic therapy under NICE guidance and/or local practice.
d. Untreated/unresolved infective illness at the time of screening and/or baseline visit

Surgery Patients

Inclusion:
a. No acute underlying disease, such as cold or flu symptoms.
b. No serious illness such as cancer requiring medication.
c. Must speak and understand English

Exclusion:
a. Skin pathology at the biopsy site (lower abdomen or buttock)

b. Untreated/unresolved infective illness at the time of screening and/or baseline visit.
c. Use of immunosuppressive medicines
d. Evidence of allergy from questionnaire- see Appendix

5.3 Study Timeline

Healthy Volunteers
Months 1-59:
Recruitment of patients
Data Collection

Month 60: Completion of Study

RA Patients
Months 1-18:
Recruitment of patients
Data Collection
Disease activity calculations

Months 18-24:
Data Analysis Correlation with clinical outcomes

Month 24: Completion of study

Surgery Patients

Months 1-59:
Recruitment of patients
Data Collection

Month 60: Completion of Study

6. STUDY PROCEDURES

6.1 Subject Identification

Healthy Volunteers:

Healthy donor participants will be recruited publicly using two separate procedures:

Procedure 1 - Paper poster advertisements detailing contact information i.e. email and telephone number of the Dermatology Research Team will be placed around local buildings including NHS, University and local office sites. Dermatology Research Team will also use the NHS intranet site to advertise the study. When the volunteers contact the Dermatology Research Team they will be sent

the study information sheet, study consent form and screening questionnaires by email to complete and return. They will also be given the option to complete the details via the online link that will take them through procedure 2. Once returned to the Dermatology Research Team, volunteer will be screened for eligibility .Those eligible will be invited to attend a research clinic appointment.

Procedure 2 – Posters will be distributed in areas of high footfall i.e. Nexus Metro stations. The poster will give brief detail of the volunteers that we are looking to recruit and the contact details will be for Alcyomics Ltd. There will be a QR code for potential volunteers to access further information. The QR code will take the person to a webpage linked to the Alcyomics website. On this page they will find the full detail of the study including the information sheet and study consent form including a link to the Alcyomics privacy policy After consents are completed, they will be taken to the questionnaire to complete. The details received by Alcyomics will be sent to the Dermatology Research Team for them to book a time for the healthy volunteer toattend a research clinic appointment..

The following will then take place during their research clinic appointment:

The research nurse will confirm participant's identity and eligibility.
Confirm participant understands the study, and is willing to proceed with their appointment. The participants will have previously consented to the study however they will be asked if there have been any changes to the information given since their original consent and also asked to physically sign consent for the study on this visit. Take the samples by following the study protocol.
The study protocol and standard operating procedures will be used.

Participants will not be recruited through Patient Identification Centres (PICs)
Participants will be recruited by publicity, displaying posters and by advertisement on the NHS intranet website

Alcyomics' contact with the volunteer will be to discuss the project to see if they are suitable, and review the screening questionnaire. The sample information received by Alcyomics will be anonymised and there will be no link to trace the identity of the sample donors.

Sub-Study – Rheumatoid Arthritis (RA) Patients:
The process of patient identification and recruitment will differ according to the target group (*RA Sub-study Group [1]* or *RA Sub-study Group [2]* as described).
RA Sub-study Group (1)
(To assess the safety of peptides for potential use in clinical trial).
To facilitate identification of RA patients known to be of HLADRB0401 or 0404 tissue types due to participation in previous observational research, a departmental database of such individuals who have consented to be approached for future studies may be utilised. Such individuals will be approached during planned hospital visits by a member of their usual care team or the rheumatology research team, and provided with a written information sheet. To facilitate identification of RA patients known, due to participation in previous observational research, to be of HLADRB0401 or 0404 tissue types, *or* to be autoantibody seropositive (and hence most likely to be HLADRB0401 or 0404 on screening), a departmental database, of those who have consented to be approached for future studies, may be utilised. Such individuals will be approached during planned hospital visits by a member of their usual care team or the rheumatology research team, and provided with a written information sheet. Alternatively, information sheets may be posted to such individuals by a member of the rheumatology or dermatology research team, together with contact information for the research nurse to discuss further. If the patient wishes to join the study, they can inform the research team who will arrange for a screening appointment.

RA Sub-study Group (2)
(To assess the responses of RA patients to biologics and monitor the response post therapy).

RA patients will be identified the Rheumatology outpatient department. Potentially eligible individuals will be approached by a member of their usual clinical care team and, if interested in participation will be provided with a written information sheet by the same individual or a member of the rheumatology research team. Potentially eligible patients who are retrospectively identified by their clinical team can also be recruited to the study. In this instance, a member of their clinical team may arrange for a participant information sheet to be posted to the patient together with contact information for the rheumatology research team to discuss further. If the patient wishes to join the study, then they can inform the rheumatology research team who will arrange for a screening appointment. The study will also be advertised on posters displayed in rheumatology out-patient departments. Patients who contact the trial team via the poster campaign will be discussed with their primary consultant before being sent trial specific information as above. Finally, patients may be identified by consulting a departmental database; such patients will also be discussed with their primary consultant before being sent trial specific information as above.

Surgery Patients
Participants will be recruited at a pre-surgery clinic visit. The surgery patients will be given an Information sheet about the study. Participants will be screened by the completion of a Surgery Patient Consent form and Questionnaire.
The surgical team identify the participants and take the blood samples at a predetermined clinic visit and the excess skin samples at the time of surgery by following the study protocol.
The study protocol and standard operating procedures will be used.
Participants will not be recruited through Patient Identification Centres (PICs)

6.2 Screening Visit and Consent

Before any study procedures can take place all participants must have provided full written informed consent to participate in the study.

The Chief Investigator is responsible for ensuring that informed consent for study participation is given by each subject. An appropriately trained Doctor or Research Nurse may take consent. If no consent is given a subject cannot be entered into the study.

Healthy Volunteers:
The consent process for healthy volunteers is as follows:

The potential volunteer will make contact with either the research nurse (procedure 1 above) or Alcyomics via the website link (procedure 2 above) Either method, they will be provided with a study information sheet, study consent form and screening questionnaire. The volunteer reads the information sheet and has at least 24 hours to decide if they want to participate. The participant will complete the screening questionnaire and consent forms and send back to the dermatology team OR they will complete these electronically and they will be sent on to the Dermatology Team.
At the research clinic appointment a research nurse will discuss the completed forms, ensuring that the detail is still correct and making any changes if applicable. The study, the nature of the research and what is involved is discussed with the participant and they have the opportunity to ask any questions. The research nurse ensures the potential volunteer understands the benefits (or lack of benefits), risks and burdens before gaining a second consent The participant is able to make a free choice if they wish to proceed and must be capable of signing the study consent form for themselves.. The consent form will then be countersigned by the research person taking consent The study consent form will be retained in the study investigator site file, a copy will be entered into the patient notes electronic medical record and a copy will be given to the participant.

Healthy volunteers have the option to agree to consent to provide further study samples at the sponsor's request. This is entirely voluntary as stated in the Patient Information Sheet. A maximum of

3 sets of skin punch biopsies (2 biopsies per set) and 3 further 70ml blood samples may be taken after a minimum of 7 days from the previous sample collection date.

Sub-Study – Rheumatoid Arthritis (RA) Patients:
The consent process for RA patients is as follows:

For RA Sub-study Group (1)
(To assess the safety of peptides for potential use in clinical trial).
All potential participants will have at least 24 hours to consider their willingness to participate in the study, and the opportunity to discuss the study further with a study investigator before signing the consent form. It will be made clear to patients that they are free to withdraw their consent to participate in the study at any time, and that withdrawal from the study will not impact their routine clinical care.
RA Sub-study Group (2)
(To assess the responses of RA patients to biologics and monitor the response post therapy).
All potential participants will have at least 24 hours to consider their willingness to participate in the study, and the opportunity to discuss the study further with a study investigator before signing the consent form. At each study visit, the patient will be asked to confirm their willingness to continue participation in the study. It will be made clear to patients that they are free to withdraw their consent to participate in the study at any time, and that withdrawal from the study will not impact their routine clinical care.

Surgery Patients

The consent process for surgery patients is as follows:
The research team makes contact with the potential surgery patient volunteer via email or telephone and provides information about the study both verbally and written in the form of the surgery patient information sheet. The surgery patient reads the information sheet and has at least 24 hours to decide if they want to participate. The research team will arrange to go through the surgery patient consent form (REC approved), explaining the study and what is involved in order to understand the purpose and nature of the research. The research team ensures the potential surgery patient understands what the research involves, its benefits (or lack of benefits), risks and burdens, is able to make a free choice and capable of consenting for themselves. The potential surgery patient is given the opportunity to ask question to clarify any aspects of the study. The subject will be asked to sign the consent form which will then be countersigned by the person taking consent and will be retained in the study site file, with a copy placed in the patient notes.

Surgery patients may be asked to return to the study to provide further blood samples. Patients will be given the option to consent to this element of the study using the consent form. This is entirely voluntary as stated in the Surgery Patient Information Sheet.

6.3 Withdrawal of Consent
All patients are able to withdraw consent at any time in the process of the study. In doing this they will no longer be actively participating, but all data collected up to this point may be included in the study analysis unless the patient also withdraws the consent to use the data.

6.4 Additional screening procedures
All consenting individuals will undergo the following procedures at screening:

- Eligibility check. This is to ensure potential participants fulfil the eligibility criteria for one of the target populations to be enrolled in the study (see *Section 5.2*).
- Recording of demographic data including age, gender and race.

- Recording of previous DMARD usage.
- Recording of medical history and concomitant medications.

If not known, blood may be drawn to determine HLA tissue type of RA patients being considered for the RA Sub-study Group (1).

Note that the baseline visit may occur simultaneously with the screening visit providing all eligibility criteria are confirmed as being fulfilled at that time. If Screening and Baseline visits are not combined the Baseline visit should take place a maximum of 28 days after the Screening visit.

7. STUDY ASSESSMENTS

7.1 Biological Samples

Healthy volunteers or RA Patients Sub-Study 1 and Surgery Patients will be asked to provide blood samples at baseline and RA Sub Group 2 only to provide blood samples during therapy as indicated in the study flow chart and detailed below.
For Healthy volunteers and RA Patients Sub-Study 2x 4mm skin biopsies will be taken either at the time of the baseline blood sample or + 7 to 14 days after the baseline blood samples depending on the testing protocol. The HV and RA Patients (sub-group 1) will be asked to return to the clinic for removal of the skin biopsy suture one week later. All samples will be collected using standard NHS protocols. Samples and data will be held until such time as they become unusable for study, or until a participant asks for them to be withdrawn from further study, at which point they will be disposed of according to regulations in force at the time.

Skin samples from Surgery Patients will only be obtained as excess skin samples at the time of their surgery.

7.2 Baseline Visit.

Study procedures to be carried out during the baseline visit will differ according to the study population into which the volunteer has been enrolled, as outlined below.

7.2.1 Healthy Volunteers:

These procedures will be carried out by the Dermatology Research Team in the Dermatology Clinic, RVI.

- 70ml blood sample will be taken at the first clinic visit which will include screening for common viruses and HLA (Human Leucocyte Antigen) genotypes. Blood is used to isolate peripheral blood mononuclear cells (PBMC's) which are used in the assays or further processed to generate monocyte derived dendritic cells (Mo DC). 5mLs of the blood is used for serum collection which is used in the skin assay
- 2 x 4mm Skin biopsy (see Section 7.3). The skin biopsies are dissected and used for the skin assay.

7.2.2 Sub-Study – Rheumatoid Arthritis (RA) Patients:

<u>For RA Sub-study Group (1)</u>
(To assess the safety of peptides for potential use in clinical trial).

- Blood draw for research (60ml). Blood is used to isolate peripheral blood mononuclear cells (PBMC's) which are used in the assays or further processed to generate monocyte derived dendritic cells (Mo DC). 5mLs of the blood is used for serum collection which is used in the skin assay.

<u>For RA Sub-study Group (2)</u>

(To assess the responses of RA patients to biologics and monitor the response post therapy). These procedures will be carried out by the Rheumatology Research Team at the Musculoskeletal Unit, Freeman Hospital.

- Patient Global Health questionnaire (visual analogue scale).
- Physician-reported Global Assessment questionnaire (visual analogue scale).
- 28-joint swollen and tender joint count (SJC, TJC).
- Blood test for CRP and ESR (NHS laboratories).
- Blood draw for research (70ml). Blood is used to isolate peripheral blood mononuclear cells (PBMC's) which are used in mixed lymphocyte inhibitor assays using therapeutic antibodies.
- The DAS28-CRP, DAS28-ESR and CDAI scores will be calculated (awaiting results of contemporaneous blood results where needed).

7.2.3 Surgery Patients

These procedures will be carried out by the Surgical team at the RVI or Freeman Road Hospital.

- A 70ml blood sample will be taken at a pre-surgery clinic visit.
- Blood draw for research (70ml). Blood is used to isolate peripheral blood mononuclear cells (PBMC's) which are used in the assays or further processed to generate monocyte derived dendritic cells (Mo DC). 5mLs of the blood is used for serum collection which is used in the skin assay

The collected excess skin samples are dissected and used for the skin assay

7.3 Skin Biopsy

Skin Biopsies will be performed in line with NuTH routine procedures. Two 4mm punch biopsies will be obtained from either the lower back, buttock or lower abdominal areas depending on volunteer or RA patient preference. The biopsy will be dissected into 10 pieces for the Healthy Volunteer and Group 1 RA study. Healthy volunteer biopsies may also be dissected and skin cells, fibroblasts and keratinocytes isolated and a 3D skin model developed in line with a high throughput assay development.

7.4 Follow-up Samples

7.4.1 Healthy Volunteer / RA Patient Sub-Study Group 1

- Samples (taking of 2x 4mm biospies or extra 70ml blood sample) will be taken either at the same time as the baseline visit or + 7 to14 days after baseline as directed according to laboratory requirements and patient availability:

  2 x 4mm Skin biopsy (defined as 1 x skin biopsy). The skin biopsies are dissected and used for the skin assay.

Patients enrolled in this group will only require further follow up for the removal of sutures, as indicated in the study flow chart, one week after the biopsy (see *Schedule of Events, 7.5.1, Table 1*).

7.4.2 RA Sub-study Group 2

These patients will be followed prospectively in order (i) to determine treatment response and (ii) to monitor for the development of anti-drug antibodies by the developed *in vitro* test and confirmatory serum anti-drug antibody assays and drug levels. Follow-up visits will take place at weeks 12(+/- 2 weeks), 24

(+/- 2 weeks), 36 (+/- 2 weeks), 48 (+/- 2 weeks), 60 (+/- 2 weeks), and 72 (+/- 2 weeks),. At each of these visits the following procedures will be undertaken (see also *Section 7.5*, *Table 2*):

- Patient Global Health questionnaire (visual analogue scale).
- Physician-reported Global Assessment questionnaire (visual analogue scale).
- 28-joint swollen and tender joint count (SJC, TJC).
- Blood test for CRP and ESR (NHS laboratories).
- Blood draw for research (20ml). 15ml blood is used to develop the anti-drug antibody assay and 5ml as serum to screen for anti-drug antibodies.
- The DAS28-CRP, DAS28-ESR and CDAI scores will be calculated (awaiting results of contemporaneous blood results where needed)

7.5 Schedule of Events

Procedures	**Screening Day -90 - Day 0.**	**Baseline Day 0**	**Follow-up[5] Day 7 to14 days after baseline**	**Follow-up +7 days**
Informed consent	x			
Eligibility check	x			
Demographic data	x			
Medical History	x			
Concomitant medication	x			
NHS blood[1]		X[1]		
Research blood[2]	X[3]	X[4]		
Skin Biopsy[5]		x	Or x	
Removal of biopsy Suture			x	Or x

7.5.1 Healthy Volunteers + Sub-study Group [1] - To assess the safety of peptides for potential use in clinical trial and use of HV skin and blood sample for development of 3D skin model.

Table 1. Schedule of Events - HV + RA Sub study Group 1

[1]Routine NHS bloods (RA patients only); additional blood may be taken for routine purposes.
[2]Total blood draw during the course of the study, including blood for NHS and Research, will not exceed 100mls (and blood draw for clinically indicated NHS testing will be prioritised within this volume if needed).
[3]10mls will be used for HLA typing at screening, collected in an EDTA tube. (Screening – 7 -28 days from baseline for HLA typing if not known to be of eligible HLA tissue type at screening).
[4]70mls blood for research at baseline visit will be drawn into the following tubes: 7x10ml Heparin tubes; screening and baseline visits may be combined IF already known to be of an eligible HLA tissue type at screening.
[5]Two 4mm skin punch biopsies from either the abdomen or buttocks will either be taken at baseline or + 7 to14 days after baselinefollowing the 70mls blood draw- depending on the assay configuration at the time of the patient recruitment and by arrangement with the patient (hence the "Follow-up" visit may be combined with the Baseline visit). This is done with local anaesthetic and will require a small stitch and will leave a small scar after the skin biopsy. The patient will be requested to return to the clinic one week after the biopsy has been taken in order to have the stitch removed.

Procedures	**Screening** Day -28 - Day 0.	**Baseline** Day 0	**Week 12** +/-14 days	**Week 24** +/-14 days	**Week 36** +/-14 days	**Week 48** +/-14 days	**Week 60** +/-14 days	**Week 72** +/-14 days
Informed consent	x							
Eligibility check	x							
Demographic data	x							
Medical History	x							
Concomitant medication	x							
Previous DMARD usage	x							
Patient Global Health questionnaire		x	x	x	x	x	x	x
Physician Global Assessment		x	x	x	x	x	x	x
SJC/TJC [28]		x	x	x	x	x	x	x
NHS blood[1]		x[3]	x[4]	x[4]	x[4]	x[4]	x[4]	x[4]
Research blood[2]		x	x	x	x	x	x	x
Disease activity calculations		x	x	x	x	x	x	x

7.5.2 RA Sub-study Group [2] - To assess the responses of RA patients to biologics and monitor the response post therapy

Table 2. Schedule of Events RA Sub-Study Group 2
[1]NHS bloods will include CRP and ESR; additional blood may be taken for routine purposes.
[2]Total blood draw, including blood for NHS and Research, will not exceed 70mls at baseline + 6x20ml (120mls) during therapy (and blood draw for clinically indicated NHS testing will be prioritised within this volume if needed).
[3]70mls blood for research at baseline visit will be drawn into the following tubes: 10ml Heparin tubes x 7; 1x 5ml serum tube
[4]15ml blood for research will be drawn into Heparin tubes and 5 ml into serum tube .

Procedures	Baseline Day 0 pre surgery clinic visit	Time of surgery
Informed consent	x	
Eligibility check	x	
Demographic data	x	
Medical History	x	
Concomitant medication	x	
Research blood[1]	X[1]	
Skin Sample		Excess skin only

7.5.3 Surgical Patients
70mls of blood will be drawn at baseline and put in the following tubes: 10ml Heparin tubes x 6; 1x 5ml serum tube

Table 3 Schedule of Events Surgical Patients

[1]70mls blood for research at baseline visit and will be drawn into the following tubes: 6x10ml Heparin tubes; 1x 5ml serum tube .

7.6 Recall of Healthy Volunteers and potentially Surgery Patients

In the case where healthy volunteers (or surgery patients) may be recalled for further testing, this recall is entirely voluntary as stated in the Patient / Surgery Patient Information Sheet. The healthy volunteer /surgery patient will sign a separate line on the Consent Form indicating their agreement. If it is necessary to contact the healthy volunteer/ surgery patient in the future a member of the study team will contact the healthy volunteer/ surgery patient and ask if they are willing to provide further biological samples. To a maximum of 3 skin biopsies (1 Skin biopsy = 2 x 4mm punch biopsies as defined in 7.4.1) and 3 further 70ml blood samples.

8. ETHICAL AND REGULATORY CONSIDERATIONS

8.1 Possible Adverse Effects

The possible adverse effects for the subjects involved in the study are related to the procedures. When taking blood samples, discomfort, hematoma and infection may occur. The taking of skin biopsies carries the risk of bleeding, discomfort, infection and scarring, similar to as if you would cut yourself accidentally. However, based on our last lasting experience with the described routine procedures we consider the risks of these side effects as low.

8.2 Management of risk/Safety Reporting

This non-CTIMP study does not impact patient treatment and has a low risk of causing adverse events. Where an adverse event occurs due to the collection of samples, such as infection or injury caused by blood extraction or biopsy, the Chief Investigator or Principal Investigator at participating sites will report to the main Research Ethics Committee any unexpected and SAEs in line with the National Research Ethics Service standard operating procedure on reporting of SAEs in non-CTIMP studies. The collection of samples will follow an study SOP.

8.3 End of Study

The study will terminate when the required number of patients have been enrolled and have completed follow-up.
However, the study will end prematurely if

- The Ethics Committee mandate so
- The sponsor mandates so

The approving REC will be notified in writing if the study has been concluded or terminated early.

8.4 Research governance and regulatory approvals

The study will comply with the principles, requirements and standards set out in the Research Governance Framework for Health and Social Care: Second Edition (RGF). Approval from a Research Ethics Committee is needed before the start of the study. Each site must give NHS management permission and provide evidence of such to the funding sponsor before commencement.

8.4.1 Ethics

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Following detailed discussion of the study, written, informed consent will be obtained from the participant. All investigators involved in this study will be Good Clinical Practice (GCP) compliant. This study will be in compliance with the applicable laws in England.

8.4.2 Research Ethics Committee (REC) and other Regulatory review & reports

The study was granted favourable opinion from the UK Health Departments Research Ethics Service NHS REC (NRES North East North Tyneside).
For NHS REC reviewed research:
Substantial amendments that require review by NHS REC will not be implemented until that review is in place and other mechanisms are in place to implement at site.
All correspondence with the REC will be retained.
It is the Chief Investigator's responsibility to produce the annual reports as required.
The Chief Investigator will notify the REC of the end of the study.
An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the study is declared ended.
If the study is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination.
Within one year after the end of the study, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the REC.

Regulatory Review & Compliance
Before any site can enrol patients into the study, the Chief Investigator/Principal Investigator or designee will ensure that appropriate approvals from participating organisations are in place. Specific arrangements on how to gain approval from participating organisations are in place and comply with the relevant guidance. Different arrangements for NHS and non NHS sites are described as relevant.
For any amendment to the study, the Chief Investigator or designee, in agreement with the sponsor will submit information to the appropriate body in order for them to issue approval for the amendment.
The Chief Investigator or designee will work with sites (R&D departments at NHS sites as well as the

study delivery team) so they can put the necessary arrangements in place to implement the amendment to confirm their support for the study as amended.

Amendments

Amendments will be notified to the national coordinating function of the UK country where the lead NHS R&D office is based.

Process for making amendments:

STEP 1. If the sponsor wishes to make a substantial amendment to the REC application or the supporting documents, the sponsor must submit a valid notice of amendment to the REC for consideration electronically via the IRAS system (https://www.myresearchproject.org.uk/). The PI has a personal log on for the IRAS site.

STEP 2. The REC will provide a response regarding the amendment within 35 days of receipt of the notice. It is the sponsor's responsibility to decide whether an amendment is substantial or non-substantial for the purposes of submission to the REC. NOTE: Non- substantial amendments e.g. changes to funding arrangements must also be notified to NHS R&D and a copy of the response kept on file

STEP 3. All substantial amendments will be automatically assessed by the HRA to confirm that the amendment meets the expected criteria and standards. An Assessor from the HRA will contact the PI via email *(*from hra.amendments@nhs.net) with a separate notification informing the PI that the HRA Assessment is complete. The amendment should NOT be implemented at participating NHS organisations in England until the outcome of the HRA assessment is confirmed, and the conditions detailed in the categorisation section have been met.

STEP 4. The outcome of the amendment should be communicated to the following participating organisations to assess whether the amendment affects the NHS permission for that site and to request their agreement that the changes can be implemented on site:-

Newcastle NHS R&D office and local research team

STEP 5. The outcome of the amendment i.e. REC Approval. HRA Approval and NHS R&D approval documents, should be sent to the local team for storage in the Investigator Site File (ISF) along with all modified study documentation clearly marked with the current versions. The Substantial Amendment summary sheet should be completed and counter signed/dated by the PI.

Other Key Points for consideration in the event of an Amendment:

The PI has overall responsibility for the decision to amend the protocol and for deciding whether an amendment is substantial or non-substantial*. The PI can be supported in this role by key protocol* contributors *(see 'Key Study Contacts' above)* Substantive changes will be communicated to relevant stakeholders (e.g., REC, R&D, regulatory agencies) via email.
The amendment history will be tracked to identify the most recent protocol version using a local 'Amendment History' tracking sheet counter signed by the PI.

8.5 Peer review

This is a commercial study and as such did not go through an initial peer review – however parts of the study i.e. the initial protocol, the development of a 3 D skin equivalent model and bio printing have been funded by grants i.e. Innovate UK ; EPSRC and have therefore been subject to a rigorous independent and expert peer review process.

8.6 Patient & Public Involvement

Healthy volunteers ,Surgery Patients and RA patients will be able to follow the project progress by accessing the Alcyomics website

8.7 Study Monitoring

Alcyomics will regularly review study compliance by including it on the monthly Alcyomics Board agenda and implementing a 6 monthly internal audit.
Accidental protocol deviations can happen at any time. They must be adequately documented on the AE relevant forms and reported to the Chief Investigator and Sponsor immediately. Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach.

8.8 Data protection and patient confidentiality

All investigators and study site staff will comply with the requirements of the Data Protection Act 1998 with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. The healthy volunteer and RA patient information is coded, depersonalised and replaced by an unrelated sequence of characters which is carried out by the research nurse. The coded data is kept only by the research nurse in an encrypted digital files within password protected folders and storage media. The coded data is only available to the research nurse. The confidentiality of data will be preserved since it is not required by sponsors and co-investigators. The data will be stored in conformance with the applicable regulatory requirements. The data custodian is the NHS Trust.

8.9 Indemnity

The Sponsor indemnifies and holds harmless the Participating Organisation and its employees and agents against all claims and proceedings (to include any settlements or ex gratia payments made with the consent of the Parties hereto and reasonable legal and expert costs and expenses) made or brought (whether successfully or otherwise):

by or on behalf of Study Subjects and (or their dependants) against the Participating Organisation or any of its employees or Agents for personal injury (including death) to Study Subjects arising out of the study required by the Protocol to which the Study Subjects would not have been exposed but for their participation in the Study;

by the Participating Organisation, its employees or Agents or by or on behalf of a Study Subject for a declaration concerning the treatment of a Study Subject who has suffered such personal injury.

The Sponsor will take out appropriate insurance cover or will provide an indemnity satisfactory to the Participating Organisation in respect of its potential liability.

The Participating Organisation and the Sponsor will each give to the other such help as may reasonably be required for the efficient conduct and prompt handling of any claim or proceeding by or on behalf of Study Subjects (or their dependents).

8.10 Access to the final study dataset

This is a commercial study (not a clinical trial) therefore the only individuals who will have access to the final dataset are the CEO /sponsor and scientific staff within Alcyomics Ltd.

9. PUBLICATION AND DISSEMINATION

9.1 Dissemination policy

Alcyomics Ltd owns the data arising from the study. The research will not be registered on a public database because it is not a clinical trial and there are potential commercial interests. The use of the assays will be reported and disseminated via a number of sources including peer reviewed scientific journals, conference presentations, publications on the company website, submission to regulatory

authorities and potential patent applications. Participants will be informed of the results of the use of the assay in general terms, via the company website www.alcyomics.com

9.2 Authorship eligibility guidelines

Alcyomics scientific staff only will have authorship on the final study report as is commercially sensitive.

10. REFERENCES

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5. Smolen, J. S., et al. (2016). "Rheumatoid arthritis." The Lancet 388(10055): 2023-2038.
6. Thomson, T. M., et al. (2015). "Blood-based identification of non-responders to anti-TNF therapy in rheumatoid arthritis." BMC medical genomics 8: 26-26.
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11. APPENDICIES

11.1 Appendix 1- Required documentation

List here all the local documentation you require prior to initiating a participating site (e.g. CVs of the research team, Patient Information Sheet (PIS) on headed paper etc.).

CVs of the research team; Dickinson. Ahmed and Pratt
Healthy Volunteer Information Sheet on headed paper
Healthy Volunteer Consent Form on headed paper
Healthy Volunteers Questionnaire
RA Patient Group 1 Information Sheet (PIS) on headed paper
RA Patient Group 1 Consent Form on headed paper
RA Patient Group 2 Information Sheet (PIS) on headed paper
RA Patient Group 2 Consent Form on headed paper
Surgery Patient Information Sheet on headed paper
Surgery Patent Consent Form on headed paper
Surgery Patient Questionnaire

11.2 Appendix 2 – Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
No.18	8	24.08.2023	AMD	Inclusion of surgical skin
No. 17 24.07.2023	7	24.07.2023	AMD	Part 1 Background- Addition of description of a lab test for atopic dermatitis which includes assessing the infiltration of cells into the skin. Part Part 3.3. Flowchart for HV