CLINICAL TRIAL PROTOCOL

This protocol has regard for the HRA guidance.

SENTINEL

FULL TRIAL TITLE:

Efficacy and mechanism of sentinel skin flap reduction of solid organ (lung) transplant rejection: A randomised controlled trial

SHORT TRIAL TITLE: Sentinel skin flap in lung transplant RCT

Protocol version 2.0 15th November 2023

Trial website: <insert URL>

Contents

1 RESEARCH REFERENCE NUMBERS

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Conflict of Interest statement:	None of the protocol authors/contributors have declared a potential conflict of interest
Confidentiality Statement	In accordance with the NIHR Open Access policy, the protocol will be published and made freely and openly accessible to all.

2 KEY STUDY CONTACTS

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PROTOCOL SIGNATURE PAGE

This protocol has been approved by the Sponsor, Chief Investigator and Senior Statistician. Approval of the protocol is documented in accordance with OCTRU Standard Operating Procedures.

All parties confirm that findings of the trial will be made publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any important deviations and serious breaches of GCP from the trial as planned in this protocol will be explained.

3 TRIAL SYNOPSIS

Full Trial Title:	Efficacy and mechanism of sentinel skin flap reduction of solid organ (lung) transplant rejection: A randomised controlled trial	
Short Title:	Sentinel Skin flap in Lung Transplant RCT	
Trial Acronym:	SENTINEL	
Trial Design:	SENTINEL is a multicentre, parallel group, two-arm, superiority, open-label RCT.	
Trial Participants/Target Population:	Adults (aged 18 years or over) on the waiting list to receive a lung transplant (single or bilateral and including those in combination with other organs) where offered a simulatnaeous lung and skin transplant	
Eligibility criteria:	**Inclusion:** 1. Intended recipient of a lung transplant 2. Aged 18 years or over 3. Capable of giving informed consent **Exclusion:** 1. Any 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 comply with study procedures 2. Severe peripheral vascular disease with no vessels available for inset of the skin flap	
No. of trial arms	2	
Intervention	Lung transplant with sentinel skin flap transplant	
Comparator	Lung transplant	
Planned Sample Size:	152 lung transplant recipients (76 per trial arm)	
Planned Recruitment Period	36 months	
Target no. of centres:	5 NHS cardiothoracic transplant centres in the UK	
Follow-up duration:	Each randomised participant who receives a lung transplant will be followed-up for 12 months from the point of transplantation.	
	Objective	**Outcome Measure**

Primary objective and outcome measure	To assess the efficacy of the sentinel skin flap (SSF) at reducing rates of acute lung rejection by 12 months post-transplantation	Lung rejection events in the first 12 months, defined by clinical criteria diagnosis and/or biopsy diagnosis of rejection (ISHLT grade A≥1 for lung)
	Refer to the OBJECTIVES AND OUTCOME MEASURES section of the protocol for full study objectives and outcome measures.	

LAY SUMMARY/PLAIN ENGLISH SUMMARY

Around 4300 organs are transplanted in the UK every year; around 200 are lung transplants. Rejection of the transplanted organ occurs when the body's immune system recognises the transplanted organ as being foreign. Rejection is reduced by immunosuppression medication that interferes with the body's immune system and hinders rejection. Despite these medications, rejection can still occur. Rejection causes an inflammatory reaction leading to organ failure. The sooner that rejection is detected and treated, the less damage is sustained by the organ. As a result, there have been many previous studies, attempting to find methods to detect early rejection.

In lung transplant recipients, attempts are made to detect rejection by outpatient tests, such as spirometry; and by frequent hospital visits for chest x-rays, blood tests and biopsies of the transplanted lung. These tests are performed at regular intervals or if the patient has symptoms of lung disease such as coughing. These tests look for evidence of inflammation of the transplant, as this may indicate rejection. Unfortunately there is no specific indicator of rejection until the rejection is severe.

We have discovered a new technique that we believe has a twofold benefit to patients. Potentially, it may permit a more rapid detection of development of rejection than the current standard of care, thereby facilitating early treatment and preventing organ rejection injury. Secondly, it reduces the likelihood of developing rejection by changing how the immune system responds to the transplanted organs.

The technique is called a sentinel skin flap (SSF) transplant. This involves transplanting a small patch of skin from the same donor onto the arm of the patient at the same time they receive the organ transplant. Skin seems to reject earlier than other organs and is easily visible. If rejection develops, a rash appears on the skin. Medical professionals can then treat the rejection as soon as the rash appears, to try to prevent the organ also rejecting. The researchers conducting this studyhave tested this technique for some other solid organ transplants such as pancreas transplants for diabetes, and intestine transplants. We have found that this skin rash indicates rejection much more quickly than were we to rely solely on the intermittent standard tests. Additionally, rather than having invasive tests every week or so, patients can just look at the skin everyday to check for a rash. This study is to check if the flaps do or do not do the same things in individuals who have a lung transplant.

This study is being conducted to see if a small piece of skin attached at the same time as a lung transplant can act as a rejection monitor for lung transplants. This could then

potentially avoid the regular tests and hospital visits, reduce the immune suppression drug levels, and avoid rejection injury to the transplanted lung. We need 152 adults who have a lung transplant from the five NHS lung transplant hospitals in England to know if the skin attachment does or does not work as a rejection monitor.

Participants who agree to take part in this study, and who then are matched with a lung donor as part of the standard NHSBT allocation, if the donor/donor family has also consented to the giving of some skin and lungs, will be randomly allocated to receive either a lung transplant alone, or a lung transplant with a flap of skin transplanted from the same donor at the time of their offer of a transplant.
The skin transplant will be a small patch (eye-shaped and approximately 10x3 cm, which is about the size of two fingers together) of skin attached ideally to the under surface of the forearm. Participants will be taught to examine the skin for signs of a rejection rash. If such a rash is seen, a tiny biopsy from the skin will be taken to confirm the presence of rejection. Apart from the addition of the skin patch (flap) transplant, all other treatment, visits and care will be the same. Participants will come back to clinic for their usual follow-up visits.

4 ABBREVIATIONS

ACR	Acute cellular rejection
AE	Adverse Event
AR	Adverse Reaction
AWT	Abdominal Wall Transplant
AZT	Aziothioprine
BMI	Body Mass Index
CI	Chief Investigator
CIs	Confidence Intervals
CLAD	Chronic Lung Allograft Dysfunction
CRF	Case Report Form
DAS-24	Derriford appearance scale-24
DBD	Donation after Brain Death
DCD	Donation after Circulatory Death
DMSC	Data and Safety Monitoring Committee
DNA	Deoxyribonucleic acid
DSA	Donor Specific Antibodies
DSP	Digital Spatial Profiling
FBC	Full Blood Count
FEV1	Forced expiratory volume in one second
FFPE	Formalin fixation and paraffin embedding
GCP	Good Clinical Practice
GP	General Practitioner
GVHD	Graft versus Host Disease
HRA	Health Research Authority
HRQoL	Health-related Quality of Life
HTA	Human Tissue Authority

HOT	Human Organ Transplant panel
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ISHLT	International Standards for Heart and Lung Transplantation
ISRCTN	International Standard Randomised Controlled Trials Number
ITT	Intention to Treat
LFT	Liver Function Test
MedDRA	Medical Dictionary for Regulatory Activities
MMF	Mycophenolate mofetil
NHS	National Health Service
NHSBT	NHS Blood and Transplant
NORs	National Organ Retrieval Team
NPV	Negative predictive value
NRES	National Research Ethics Service
OCHRe	Oxford Centre for Histological Research
OCTRU	Oxford Clinical Trials Research Unit
OUH	Oxford University Hospitals NHS Foundation Trust
PI	Principal Investigator
PIC	Patient Identification Centre
PIS	Participant Information Sheet
PPV	Positive predictive value
PTLD	Post Transplant Lymphoproliferative Disorder
QA	Quality Assurance
QC	Quality Control
QoL	Quality of Life
REC	Research Ethics Committee
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard definition
SFQ	Site Feasibility Questionnaire
SNs	Specialist Nurses
SITU	Surgical Intervention Trials Unit
SOP	Standard Operating Procedure
SOT	Solid Organ Transplant
SSF	Sentinel Skin Flap
SUSAR	Suspected Unexpected Serious Adverse Drug Reaction
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
TRIG	Transplantation Research Immunology Group
U&E	Urea and electrolytes
VCA	Vascularised Composite Allograft

5 BACKGROUND INFORMATION AND RATIONALE

5.1 Lung transplantation in the United Kingdom

Approximately 4300 organs are transplanted per year in the UK; 200 of these are lung transplants(1). Immunological rejection of the transplanted organ is one of the biggest hurdles in transplantation. Patients require life-long immunosuppression to reduce the risk of rejection. Current immunosuppressive regimens are less than ideal. They are non-personalised, require lifelong use, can damage the transplanted tissues and significantly increase morbidity and mortality by increasing the risk of infections (viral -up to 50% of recipients, fungal-up to 59% of recipients, and often multi-drug resistance bacterial infections -up to 45% of patients). They also increase the risk of malignancies, including lymphoproliferative disorders (up to 20%), increasing the risk of cardiovascular disease, hypertension, kidney failure and diabetes, resulting in a significant reduction in life expectancy. Life expectancy for lung transplant patients has remained unchanged in the last 15 years. Survival is currently ~80% at 1-year and decreases to 35% at 10-years, mainly due to organ rejection and immuno-suppression associated morbidity(2).

Despite immunosuppression, acute cellular rejection (ACR) is prevalent in the first year, affecting ~30-80% of lung transplant recipients(3). ACR must be detected and treated to prevent loss of the organ. However, the symptoms are non-specific, consisting of inflammatory organ dysfunction and often occur late in the ACR process. Consequently, the early diagnosis of ACR is very difficult and usually made by excluding other causes of inflammation by biochemical, imaging and histological tests. Weekly to monthly organ biopsies are often performed to exclude asymptomatic milder forms of rejection during the first year.

Apart from the threat of immediate loss of the transplanted organ, the number, severity and duration of ACR events significantly increases the longer-term risk of organ fibrosis, chronic rejection, loss of function, organ failure and death. Therefore, early diagnosis and treatment of rejection is critical. This will prevent and minimise organ injury from the immune response, prolong organ function, and minimise immunosuppression morbidity.

Despite the importance of prevention by early detection and treatment of rejection, there are currently no predictive markers of impending rejection, nor any definitive markers of early rejection(4). The diagnosis of early rejection is difficult to determine and easily confused with infection(5). Current monitoring and diagnostic approaches lack the sensitivity and specificity needed to identify ACR early enough to avoid damage to the transplant. Lung monitoring is by spirometry, with some centres also doing surveillance biopsies. Histological early rejection changes are nonspecific, not sensitive and non-predictive(6). Alternative methods, such as gene expression profiling of immunoregulatory genes (trialled in kidney and heart transplants) showed that it could safely replace regular biopsies for the diagnosis of moderate -severe rejection. However, it did not detect early rejection and did not predict or prevent rejection(7).

Though some techniques such as cell-free DNA are useful in moderate to severe rejection, there are currently no validated biomarkers to predict or detect *early* rejection in Solid Organ Transplants (SOTs) (4). Therefore, irreversible damage has often occurred by the time

abnormal organ function or biopsies are identified. Better and more responsive biomarkers may permit personalised reduction of immunosuppression, reducing morbidity.

5.2 Innovation

Developing accurate techniques that predict or detect rejection before the transplant sustains injury whilst also recognising the absence of rejection is crucial. In response, we developed the use of a simultaneous skin transplant to monitor intestinal and pancreas/kidney transplants. Experience with successful hand and face transplants (8) encouraged us to transplant similar skin-bearing vascularised composite allografts (VCA) in the form of abdominal wall transplants, to facilitate abdominal closure after intestinal transplant. We observed that the skin of the abdominal wall transplant allowed early detection and diagnosis of rejection, as the skin showed a rash on rejection. This could be easily and painlessly biopsied to confirm and distinguish rejection from other inflammatory conditions such as infection or allergy. The practice of co-transplantation of a patch of skin with the intestine was extended to all intestinal transplant patients and to some pancreas/kidney transplant patients at the Oxford Transplant Centre. We reported that the transplant of an SSF is safe and adds value in diagnosing rejection, differentiating the causes of organ dysfunction. Unexpectedly, the skin flap patients had a significantly lower than expected rate of transplant organ rejection.

It is important, therefore, that use of SSFs be investigated in other transplant organ types (9). Lung transplantation is the ideal organ type to study, as it is very similar to intestinal transplantation in rejection rates and outcomes. It reflects the fact that both are epithelial organ transplants with direct communication with the external environment. They have susceptibility to direct contact /injury from inhaled or ingested objects and infection. Furthermore, lung transplants are performed much more frequently than intestinal transplants.

5.3 Skin flaps as transplant sentinels

Through the process of performing abdominal wall transplants with intestinal transplants, we discovered that the transplanted skin, in addition to showing a rash when rejection was occurring, also reduced the number of intestinal and total rejection events compared to intestinal transplants in which no skin was used (10–14).The skin also provided useful clinical information when there was intestinal dysfunction, to help discriminate between infection or rejection as the cause of dysfunction. The transplanted skin proved to be an extremely sensitive and responsive monitor of immune response. A few weeks after reducing the dose the patient noticed that the transplanted skin became red and swollen in the hour before the next dose was due. Once the dose was taken the redness and swelling returned to normal until the dose wore off just before the next dose was due. This indicated to us that the dose level was too low and after correcting this the redness and swelling no longer appeared
Such immediate feedback could help individualise immunosuppression dosing, reducing morbidity.

Following the success of the transplanted abdominal wall skin in providing monitoring of intestinal transplants, we went on to transplant a smaller patch of vascularised skin (VCA) onto the forearms of intestinal transplant recipients who did not require an abdominal wall

transplant. The patch of skin measured 10cm x 3-4 cm and was retrieved from the forearm of the donor, vascularised by the radial artery. It was placed into an incision on the under surface of the recipient's forearm and re-vascularised onto the ulnar artery and vein. This smaller patch also proved useful in the detection of rejection.

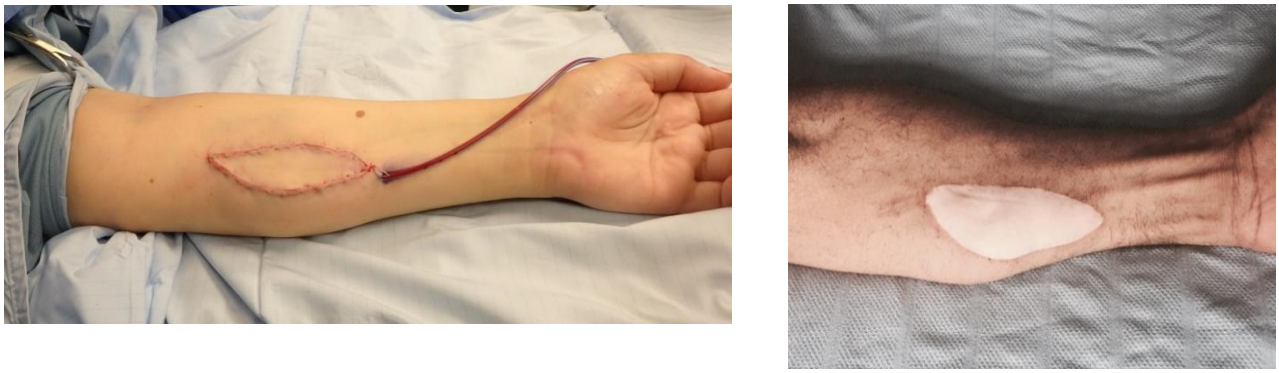


Figure 1. a) and b) Sentinel skin flap transplanted onto the under surface of the recipient's forearm.

Figure 2. The sentinel skin flap showing the characteristic rejection rash. The tape is over a punch biopsy taken to confirm the diagnosis of acute rejection and classify severity of rejection.
photographs (with consent).

A series of intestinal transplant patients co-transplanted with a skin flap (n=34; 24 AWT; 10 forearm SSF) were compared to a contemporary cohort of intestinal transplant patients without skin (n=19). The incidence of intestinal transplant rejection of 77% in the first year dropped to 22% when skin was co-transplanted ($p<0.01$). (Figure 3). Not only did the combined co-transplantation of skin flap and intestine have some immune-modulatory effect, but the sensitivity and visibility of the skin to rejection meant that when rejection was detected in the intestine, it was less severe than in the cohort without the SSF (unpublished data).

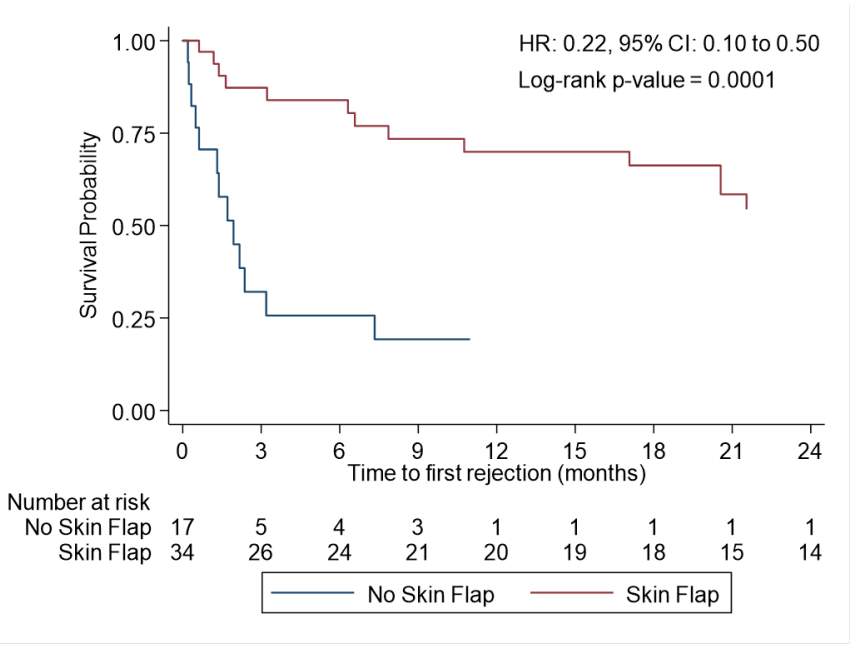


Figure 3. Kaplan Meier time to first rejection event (rejection free survival)

The ability of the SSF to diagnose rejection in the intestine transplant was found to have a sensitivity of 94%, specificity 89%, NPV 98%, PPV 44%, if assessed at time of test taken. We also saw that when the skin flap initially reported false positive (skin flap positive for rejection but the initial intestinal biopsy was negative for rejection) in seven out of nineteen events, the transplanted intestine biopsies became positive for rejection two to ten days later after repeated biopsy (despite treatment). This demonstrates that the skin changes indicating rejection were seen before the intestinal rejection changes occurred. This provides further evidence that the skin flap was useful for early detection of rejection before the intestine was affected. It is presumed that in the other twelve of nineteen events, treatment of the skin rejection prevented rejection appearing in the intestine. On the four occasions in which the intestinal biopsy signalled rejection before the skin biopsy, three had no skin biopsy taken at the time, and one had non-specific features.

We subsequently studied the use of an SSF transplant in patients having pancreas or pancreas-kidney transplants. The pancreas is the least successful organ to transplant due to difficulty in immune-monitoring (biopsy carries a significant risk to the graft) and the lack of symptoms until rejection is severe. We found a marked reduction in rejection events (0% vs 27%) and organ losses (0% vs 14%) in patients with the SSF. We have now transplanted 64 SSFs with encouraging outcomes. The potential benefits include 1) early diagnosis of rejection before SOT injury; 2) discrimination between infection and rejection as the cause of graft dysfunction/ inflammation; 3) reduced SOT rejection events; 4) no increased immunosuppression requirements; 5) no increase in donor specific antibody formation and 6) no deterioration in organ function with improved organ survival (unpublished data). SSFs have been used in hand and face transplants to provide an extra site to biopsy, and as an additional monitor for rejection, with reported concordant rejection between the SSF and face/hand transplant (15,16). The safety and utility of SSFs in SOT has been reviewed (9,14,17). However, experience outside our group is limited to fifteen kidney transplants (18,19) and fifteen abdominal wall transplants (20,21). In kidney transplants, parallel outcomes were reported, whereas the reported abdominal wall findings were inconclusive.

This was probably because some of the co-transplants had different donors, and the death rate exceeded 50%. In animal models, composite vascularised skin flaps, when transplanted in combination with visceral transplants, have been shown to reject prior to the visceral transplants when immunosuppression is withdrawn. Two different experimental rat studies on hind limb transplantation confirmed a small but significant delay of between one and seven days in rejection of the main graft compared with the skin VCA component. This was dependent on the rate of immunosuppression withdrawal (22)

5.4 Multi-organ transplants reduce rejection rates or may help induce tolerance

Multi-organ combination transplants from the same donor are linked to lower rejection rates and improved survival by unknown mechanisms. It is possible that immune modulation by the transplant of disparate tissue types; the presence of passenger donor leucocytes; the induction of suppressor T-cells; immune diversion or paralysis; immune exhaustion; and reduction of lymphocytotoxic antibodies(23–27) could all play a role. This feature has led to it being used in clinical use trials involving highly sensitised patients, to improve outcomes such as the combination of liver with kidney (28).

There is experimental evidence that the skin component in VCA transplants may confer some benefit due to chimerism (blood cells that are derived from both the individual and the donor's through transplanatation) and induction of tolerance (29). In combined heart and lung transplants though, the lung provides immune-protective effect to the heart. However, the heart does not seem to confer the same benefit to the lung (30). In our studies, the immune-protective effect seemed to work both ways. In addition to the reduction in SOT rejection events, there was a reduction in the expected rate of rejection in the skin flap VCA as well.

The reported rejection rates of skin in the first 12 months after hand or face transplant are 90%. However, when we combined SSF VCA transplant with a SOT, the observed skin flap VCA rejection rate reduced to less than 25%. The immune-modulatory effects of combination transplant with skin may be caused by large numbers of skin-derived recirculating T-effector memory cells (capable of recirculation between skin and blood but not equipped to enter lymph nodes). This would reduce the systemic response, as 10% of skin T-cells are T-regulatory cells. These are under the influence of Langerhans cells with an immuno-suppressive role both locally and systemically (31,32). Either the presence of skin dendritic cells (33) or the high antigenic load of skin and its microbiome may present a continual exposure of antigen, which is required for maintaining tolerance (34–36)

5.5 Safety issues of skin and solid organ transplantation

The high frequency of rejection in skin bearing a VCA raised concerns that adding a skin flap VCA transplant to the SOT would increase rejection events or require increased immunosuppression. We found that not only did this not occur, conversely, the overall rejection rate (skin +SOT) was less than expected compared to SOT alone or skin flap VCA alone. This suggests that combining the transplants confers some immune-protection(11). We were also concerned that the addition of skin flap VCA may increase the development of antibodies against the transplants, resulting in increased antibody mediated rejection, chronic rejection associated fibrosis and vascular occlusion. However, we found no such increase in the development of donor-specific antibodies or chronic rejection features (37).

The few reports of skin transplants and SOT showed that combination transplants of this nature were safe (17).

5.6 Health related quality of life

All our intestinal transplant patients were asked to complete SF-36 and EQ-5D health-related quality of life questionnaires (HRQoL) at pre-transplant, 3, 6, 12 months and then annually after transplantation. We observed that HRQoL outcomes were significantly better in our transplant patients who had SSFs compared to those who did not in both physical (p=0.018) and mental (p=0.027) components of the SF-36 questionnaire. Qualitative interviews suggested that this may be due to patients feeling they have improved control and involvement over their transplant and that the ability to self-assess immunological function reduced the anxiety and fear of rejection. In addition, we found that the skin flap reduced hospital visits and permitted remote monitoring of intestinal transplant patients (38).

Due to concerns about converting these patients from having no visible reminder of their transplant to having a visible transplant, we asked patients to complete a patient-reported outcome measure of aesthetic appearance and function (the DAS-24 Derriford appearance scale-24) (33). We also asked patients about concerns regarding appearance during qualitative interviews. Surprisingly, the results were better in the abdominal wall skin transplant cohort, perhaps reflecting improved abdominal scars in those whom received an AWT (39). Patients reported an increased awareness of the gift of transplantation they had received, most accepted the visibility of the skin flap as a beneficiary component to their transplant, and only 2 (out of 40) have asked for their skin flap VCA to be removed after the study period.

5.7 Why this research is needed now

A review of research priority setting in transplantation (40) identified the following priorities, which are targeted in this study:

1. Optimizing and individualising regimens to improve outcomes;
2. Developing ways to assess response to therapy including surrogate marker(s) for adequacy of immunosuppression;
3. Improving strategies for monitoring the level of immunosuppression; and
4. Reducing the need for immunosuppression.

Adopting SSFs into routine transplant practice would greatly reduce the demands of follow-up, which is currently weekly visits for 3 months, then blood and other tests every 6 weeks, and 3-monthly visits for life. In our previous studies, the skin flap led to significant changes in clinical practice after the first year, with patient self-monitoring, reducing the need for regular endoscopy and organ biopsy (38).

Patients may benefit from being able to self-monitor their immunological status, titrate and reduce their suppression dosing. This reduces the associated morbidity, reduces their hospital visits and the number of interventions and tests. It may result in better organ function and longer organ survival, with increased psychological well-being at being able to reassure themselves, better self-control and involvement in their health.

5.8 Co-enrolment

Co-enrolment defined as the enrolment of a participant in two or more clinical trials either concurrently or sequentially. Co-enrolment will be permitted with other studies/trials provided that the burden is acceptable to the participants and study office. Co-enrolment should not affect the study outcomes, for example if another study involved immunosupression.

6 OBJECTIVES AND OUTCOME MEASURES

6.1 Aims

The aims of the study are to further establish the impact and safety of SSF use in solid organ transplants; to determine immunological mechanisms of early rejection and combination transplantation, and to identify biomarkers of acute rejection to aid diagnosis of rejection and identify potential therapeutic targets.

6.2 Primary objective and outcome measure

Objective	Outcome measure	Time point(s) of evaluation of this outcome measure (if applicable)	Data required	Source data (including location)
To assess the efficacy of the sentinel skin flap (SSF) at reducing rates of acute lung rejection by 12 months post-transplantation	Lung rejection event in the first 12 months, diagnosed by clinical criteria and/or biopsy (ISHLT grade A≥1 for lung)[1]	Time to diagnosis of first rejection event in first 12 months post transplant	Date of transplant; date of first diagnosed lung rejection; date of death (if prior to diagnosis of lung rejection); date of withdrawal (if prior to diagnosis of lung rejection).	Participant's medical notes (clinical diagnosis); Histology report (biopsy diagnosis)

6.3 Secondary objectives and outcome measures

Objective	Outcome measure	Time point(s) of evaluation of this outcome measure (if applicable)	Data required	Source data (including location)
1. To compare the diagnostic accuracy of the sentinel skin flap to the current reference standard (organ biopsy and/or clinical diagnosis)[2]	Diagnostic accuracy (sensitivity, specificity, negative predictive value and positive predictive value) of the sentinel skin flap compared to current reference standard (biopsy and/or clinical diagnosis) [2]	Up to 12 months post transplant	Date of first diagnosed lung rejection; date and method of first diagnosed skin rejection.	Histology reports (skin and lung biopsies) Participant's medical notes
2. Safety of sentinel skin flap transplantation [2]	a. Number and severity of skin flap rejection episodes (defined by biopsy	Up to 12 months post transplant	Date of transplant date of first diagnosis of skin rejection; date of death (if prior to diagnosis of	Participant's medical notes and Histology reports (skin biopsies)

	BANFF grade ≥ 1) and time to first rejection diagnosis [2]		skin rejection); date of withdrawal (if prior to diagnosis of skin rejection); severity of rejection (BANFF grade).	
	b. Transplanted lung and patient survival[1]	Up to 12 months post transplant	Date of transplant; date of first loss of lung failure; date of death; date of withdrawal (if prior to lung loss or death)	Participant's medical notes/death record
	c. Transplant lung function [1]	12 months post transplant	Lung function tests (spirometry): Vital capacity (absolute values and % predicted); Forced expiratory volume (absolute values and % predicted)	Participant's medical notes
	d. Surgical complications and complications relating to the SSF (such as infection, skin loss, nerve injury) [1]	Up to 12 months post transplant	Surgical complications (flap failure, wound healing problems, infection, skin necrosis, nerve injury); Delayed complications (pain, hand symptoms including paraesthesia, numbness, weakness)	Participant's medical notes
	e. Development of de-novo donor specific antibodies [1]	Events up to 12 months post transplant	Presence of de-novo antibodies	Immunological laboratory report
	f. Development of graft versus host disease[1]	Events up to 12 months post transplant	Diagnosis of graft versus host disease	Participant's medical notes
	g. Evidence of chronic rejection in lung [1]	12 months post transplant	Chronic rejection	Histology report
3. To establish if the addition of a sentinel skin flap changes the immunosuppression requirements	Immunosuppression levels and requirements [1]	Events up to 12 months post transplant	Number and type of immunosuppressants given and target levels /doses (steroid, mycophenolate mofetil (MMF), tacrolimus, others);	Prescription for immunosuppressants
4. To establish if being able to personally check on immune response/rejection of transplant changes quality of life, or if rendering	HRQoL as measured by the following validated questionnaires: SF-36; EQ-5D-5L and DAS-24 [1]	Baseline, 3 and 12 months post transplant	SF-36; EQ-5D-5L; DAS-24	Participant-reported outcome measure

the transplant visible adversely effects QoL				

[1] Outcome assessed in both intervention and comparator;
[2] Outcome assessed in intervention arm only.

6.4 Exploratory/mechanistic objectives/outcomes

Objective	Outcome measure	Time point(s) of evaluation of this outcome measure (if applicable)	Sample type
1. To identify immune pathways in skin and solid organ transplant (SOT) rejection and compare these to known mechanisms	nCounter HOT (human organ transplant) panel	Up to 12 months post transplant	Lung and skin punch biopsies
2. To identify the mechanism of immune-modulation by simultaneous transplant of skin and SOT by exploring passenger leucocytes, suppressor T-cells, dendritic antigen presenting cells, immune diversion or paralysis, immune exhaustion, immune response termination sequences, and lymphocytotoxic antibodies	Gene expression	Up to 12 months post transplant	Lung and skin punch biopsies
3. To seek to demonstrate the relative proportions of T-cells of both donor and recipient origin including skin homing of T-reg cells, and the role of these cells in preventing rejection	Digital spatial profiling histology	Up to 12 months post transplant	Lung and skin punch biopsies
4. To investigate if serum cell- free DNA level correlates with detected rejection	Cell-free DNA Blood sample (cell free DNA)	Up to 12 months post transplant	Blood sample
5. To study the role of the cutaneous and lung microbiome on the immunological response	16sRNA analysis	Up to 12 months post transplant	Lung and skin punch biopsies and/or skin swabs, sputum/bronchial brushings/ bronchoalveolar lavage

6.5 Use of core outcome sets

There are no core outcome sets applicable for this study.

7 STUDY DESIGN AND SETTING

The SENTINEL study is a multi-centre, open-label, two-arm, parallel design, superiority randomised controlled clinical trial.

152 lung transplant recipients (76 in each of two trial arms) will be followed-up for 12 months from 5 sites in the UK. Participants will be randomised to either receive a lung

transplant with an accompanying sentinel skin flap (SSF) transplant (intervention arm) or a lung transplant only (control/comparator arm) if they are offered a suitable lung and skin organ.

A study flow chart is provided in Participant pathway graph at section 14.8 (Transplant pathway following consent).

7.1 Recruiting sites/site types

Participants will be recruited from the 5 specialist NHS cardiothoracic transplant centres who perform lung transplants in England.

7.2 Collection of outcome data and follow-up assessments

Participants will receive communications from the SENTINEL study team to collect outcome data and undergo research specific visits.
Refer to Section 17.3 for full details of outcome data collection and follow-up assessments.

For those who consent to take part (Stage 1) in the SENTINEL study. The following information will be recorded on a secure web-based form in the SENTINEL study database system (REDCap) by a member of the local study team to enable follow-up/contact:

- Participant details e.g. Name, Hospital number, address, NHS/CHI number, date of birth, telephone number, mobile number, email address, GP name and GP address.

The GP details are required to allow the central study team to send a letter to the participant's GP informing them of their SENTINEL participation after a transplantation procedure. The email address will enable a copy of the completed consent form to be sent to the participant or at their request a different individual for safekeeping. Depending upon participant preference the email /postal address and/or telephone may be utilised for follow up questionnaires, reminders/text messages and thank you letters. This information always be sent in an anonymised form without direct identifiers. Any confidential information that is sent will be marked confidential and will be encrypted and password protected.

After consent (Stage 1) baseline data and Health Related Quality of Life will be collected for the participants. Baseline HRQoL questionnaires will either be completed on paper or electronically on a tablet device/computer at the recruiting transplant centre. Participants who are randomised and receive a lung transplant will be sent HRQoL questionnaires again via e-mail or post (according to participant preference) at 3 months, and at 12 months post-transplant. Questionnaires may also be completed during clinic visits at these time points if the recipient has not already completed these when they come in for their visit.

On a suitable SENTINEL potential recipient / donor match that includes the offer of lungs and skin being found and flagged by NHSBT to the local SENTINEL Study Point of Contact, the potential recipient will be asked by the local team if they are still happy or not to participate in the study. A further consent is then taken and the participant is then randomised.

7.3 Duration of participant involvement

Participants who are randomised and receive a lung transplant will be in the study for approximately 12 months from transplant to last protocol visit. Patient who take part in the study by consenting at stage 1 and/or stage 2 but do not receive a lung transplant do not participate beyond baseline data collection. They do not take part in the follow-up.

7.4 Post-trial treatment/care and follow-up

Following a participant's final protocol visit, they will receive standard care. It is expected that most skin flaps will be retained after the 12-month study period. The skin flaps can, however, be removed under local or general anaesthesia at the end of the study period if the participant wishes. Ongoing care for the flaps retained after the study period will be performed by the local transplant team with support from the local plastic surgery team.

Participants who receive a lung transplant will undergo routine clinical follow-up after transplantation, as part of standard care for all lung transplant recipients. The relevant participating transplant centres have standard protocols for the management of patients after lung transplantation. Typically, such patients are reviewed in an outpatient clinic very frequently in the first year: 10-15 times in the first 6 months and then 6 times in the second 6 months (this can be more frequent if problems arise). During these visits, standard clinical investigations performed by the relevant participating centres will be performed. These may include full blood count (FBC), renal function tests (U&Es), liver function tests (LFTs), C reactive protein, immunosuppressant medication levels and donor specific antibodies (DSA). There may also be organ-specific investigations such as spirometry, lung function tests, sputum, radiographs, bronchoscopies, bronchial brushings, bronchoalveolar lavage, and allograft biopsies.

Clinical outcomes of the lung transplant will be collected by local healthcare teams, as required by NHSBT and relevant data will be recorded in the case report forms.

Refer to the STUDY ASSESSMENTS/PROCEDURES section for full details of outcome data collection and follow-up assessments.

7.5 Training

Selection and appointments of plastic surgeons to perform the retrievals and transplantation of the skin flaps will ensure their competency in performing the surgery. The forearm flap design chosen for this study, is an established and well-known technique, in use since since 1980. All plastic surgeons should be competent and familiar with VCA transplantation. Further training regarding the specifics of organ retrieval and transplantation will be taught at the annual NHSBT retrieval masterclass course, designed and delivered by Chief Investigator of the study. Written material is available for guidance regarding the transplant procedure. Training of the specialist nurses for organ donation (SNs) involved in requesting and obtaining consent for organ donation will be undertaken by NHSBT. The material for this training and the standard operating protocol has been agreed with NHSBT. The skin biopsy procedure will be taught to the clinical researchers responsible for this procedure.

No pre-implantation blood samples or biopsies will be taken from the skin flap or lung for the purposes of the SENTINEL study.

All surgeons who are part of the study-specific plastic surgery retrieval and transplant team will receive study-specific training relevant to their role.

7.6 Central review procedures

Not applicable. There are no central review procedures for this study. Where site staff require a second expert opinion of the assessment of the skin rash this will be sought as described in section 16.8 Detecting, diagnosing and managing rejection.

7.7 Health Economics

There are no health economic analyses to be undertaken as part of the trial

7.8 Expected recruitment rate

During the 2019-2020 financial year, a total of 156 lung transplants were carried out across all centres in the UK. We estimate that approximately 75% of patients would meet the eligibility criteria for the trial, and that approximately 30-40% of eligible patients will consent to take part in the trial (which we consider is a conservative but realistic estimate). Therefore, once all sites are open, we expect recruitment of 3-5 patients per month (approximately 1 patient per month/per centre). We anticipate one centre per month, following the study opening to recruitment, with all 5 centres open by month 12 at the latest. Therefore, assuming staggered opening of sites, randomisation of 152 participants should be feasible within the 36-month recruitment period.

7.9 Participant Identification Centres

Participant Identification Centres (PICs) will not be used in this study. The identification of participants will be done by the recruiting centres only.

8 SUB-STUDIES/TRANSLATIONAL STUDIES/MECHANISTIC STUDIES

The following mechanistic studies will seek to:

1. Identify the immune pathways in skin and SOT rejection and compare these to known mechanisms;
2. Characterise the mechanisms by which simultaneous transplant of skin and SOT modulates the immune system. We will explore the role of passenger leucocytes, suppressor Tcells, immune diversion or paralysis, and lymphocytotoxic antibodies;
3. Examine the relative proportions of T-cells of both donor and recipient origin and the role of skin homing of T-reg cells in preventing rejection;
4. Establish whether serum cell-free DNA levels correlates with detected rejection;
5. Determine the role of cutaneous and lung microbiome on the immunological response.

Most studies have used samples collected when rejection and organ injury are already well-established. We have a unique collection of very early-phase rejection samples from intestinal and pancreas transplants. We will collect samples from lung transplants in this study to analyse and compare to non-rejection samples.

Within the Transplant Research Immunology Group, at the University of Oxford we will perform an un-biased high throughput assay to determine DNA, RNA and protein biomarkers related to the systemic and local alloresponse to the transplants, as well as targeted analysis of changes in known markers such as those involved in CXCL9, CXCL10, & IL-1.

Firstly, to characterise the local response in skin and lung transplant, NanoString nCounter gene expression analysis for mRNA quantification will be performed. We will use the human immunology panel v2, which measures 579 genes relating to the core pathways and processes of the immune system, with 15 internal reference genes included for normalization, and compare non rejection to rejection episodes.

Secondly, selected tissue biopsies will be further analysed using Digital Spatial Profiling. This will demonstrate exactly where within the tissue cells and genes of interest are being expressed.

Thirdly, SSF and SOT rejection biopsies will be analysed by expert transplant histopathologists. They will use quantitative multiplex immune-profiling to focus on those factors involved in rejection and tolerance, including $CD8^+$T cells, $CD4^+$T conventional cells ($CD4^+FoxP3^-$), Tregs ($CD4^+FoxP3^+$), CD68+ and PD-L1 using fluorescent antibodies against CD4, CD8, FoxP3 and nuclear stain to determine spatial distribution of immune infiltrates. Finally, the molecular profile of single cells within regions of interest will be determined using a panel of 40 immune-related barcoded antibodies or up to 800 RNA probes or by total single cell gene analysis. 16S ribosomal RNA gene sequencing will identify the microbiome changes over time and ACR.

Whenever a lung biopsy is taken as part of standard clinical care, an additional skin biopsy will also be taken at the same time for participants that have received a SSF transplant. In addition to routine follow-up and examination, surveillance biopsies of the SSF will be performed at routine appointments after transplantation at 3, 6 and 12 months.

9 ELIGIBILITY CRITERIA

9.1 Timing of eligibility assessment

Eligibility will be confirmed following Stage 1 consent, however once the patient has been matched with a suitable donor and confirmed their consent for study participation (Stage 2 consent) a final check of eligibility will be made prior to randomisation.

Patient eligibility will be confirmed by a suitably qualified and experienced individual who has been delegated to do so by the Principal Investigator. Eligibility will be assessed upon initial entry into the study and again as part of the Stage 2 consent process once the patient has been matched with a donor prior to randomisation.

9.2 Overall description of trial participants

The SENTINEL study will recruit adults aged 18 years and over awaiting lung transplantation, including single, bilateral or heart-lung transplantation.

Written informed consent must be obtained before any study specific procedures are performed. The Investigator will determine patient eligibility based on the following criteria. If a patient does not go on to receive the lung transplant their participation will end. Trial follow-up (including safety data) will be collected only for those who are randomised and go on to receive the lung transplant.

9.3 Inclusion Criteria for entry into the main trial

A patient will be eligible for inclusion in this study if all of the following criteria apply.

1. Intended recipient of a lung transplant
2. Aged 18 years or over
3. Willing and able to give informed consent

9.4 Exclusion Criteria for entry into the main trial

A patient will not be eligible for the trial if **ANY** of the following apply:

1. Any 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 trial, or the participant's ability to comply with trial procedures
2. Severe peripheral vascular disease with no vessels available for inset of the skin flap

9.5 Rationale for inclusion and exclusion criteria

Patients must already be on the lung transplant waiting list, having passed the rigorous process to establish the necessity for a lung transplant independently from the study.

Patients under the age of 18 are excluded, as they are perceived not to fully comprehend the risks and benefits of participating in the study.

Patients have to be able to give informed consent. Some patients may be intubated and ventilated and therefore unable to communicate with the trial staff and therefore will be deemed ineligible.

Patients with severe peripheral vascular disease in the recipient upper limb may be ruled ineligible as the risk of being unable to perform the skin flap transplant may be deemed too high. However, if an alternative healthier site for transplant can be agreed upon then they may still be eligible.

If, on donation the donor's relatives agree to lung donation only, then the recipient will be ineligible to be randomised in the study as the skin flap option is absent. In this case the recipient will proceed with lung transplantation alone outside of the study and no further data collection will occur.

9.6 Protocol waivers to entry criteria

Protocol adherence is a fundamental part of the conduct of a research study. There will be no waivers regarding eligibility i.e. each participant must satisfy all the eligibility criteria.

Changes to the approved inclusion and exclusion may only be made by a substantial amendment to the protocol.

Before enrolling a patient onto the study, the Principal Investigator or designee will confirm eligibility. If unsure whether the patient satisfies all the entry criteria and to clarify matters of clinical discretion, investigators must contact the Trial Office, who will contact the Chief Investigator or designated clinicians as necessary. If in any doubt, the Chief Investigator must be consulted before enrolling the patient. Details of the query and outcome of the decision must be documented in the TMF.

9.7 Clinical queries and protocol clarifications

Every care has been taken in drafting this protocol. Contact the SENTINEL Trial Office for clarification if any instructions seem ambiguous, contradictory or impractical. Clinical queries must also be directed to the Trial Office. All clinical queries and clarification requests will be logged, assessed and a written response provided. Minor administrative corrections or clarifications will be communicated to all trial investigators for information as necessary. For urgent safety measures or changes that require protocol amendment see Urgent safety measures section below.

10 SCREENING AND RECRUITMENT

10.1 Participant Identification

Participants will be recruited from all five lung transplant centres in England.
The following methods will be used to identify potentially eligible participants:

- Identification during routine clinic visits
- Searching of the lung transplant waiting list by the usual care team to identify individuals that may be eligible and are awaiting lung transplantation

Organs may be retrieved from donors at any hospital in England and Wales.

10.2 Identification of participants during routine clinic visits

Patients awaiting lung transplantation will undergo regular routine monitoring whilst on the transplant register. Potentially eligible patients identified during routine clinic visits will be provided with a Patient Information Sheet (PIS) by a member of their usual care team (who may also be a member of the research team) and asked to consider the study. Where their usual care clinician is not a member of the research team, potential participants will be asked for permission for the research team to contact them regarding the study. If permission is given, it should be recorded in their clinical notes. A member of the research team will make contact with them in a clinic visit or arrange a telephone or video call. Alternatively, patients may be given the participant information sheet and asked to call the number on it if they wish to find out more about the study.

10.3 Identification of participants via clinic records/hospital database

Potentially eligible patients may also be identified by searching clinic records/hospital databases by the local care team. Any patients who are thought to fulfil the inclusion/exclusion criteria may be sent a letter of invitation and patient information sheet by the local care team. A reply slip and postage paid envelope will be included, enabling potential participants to indicate how they would prefer to be contacted further about taking part in the study.

10.4 Use of screening logs

A screening log will be kept of all patients who are approached about the study. It will log the number of patients who decline further information about the study and their reasons. It will also log those who do not meet the inclusion criteria for the study, and record the reasons for exclusion. In addition, the number of eligible patients, and whether they consent to the study will be recorded. It will also provide information on participants who decline or later withdraw consent and their reasons for doing so.

10.5 Pre-study screening tests or investigations

There are no study-specific screening procedures.

11 INFORMED CONSENT

11.1 Consent Procedure

Prior to any study related procedures or data being collected the patient must personally sign and date the latest approved version of the Informed Consent Form (ICF).

Patients awaiting lung transplantation may remain on the lung transplant register for some time, therefore consent for study participation will be taken in two stages:

Stage 1 consent:	Initial consent for potential participation in the study (including baseline data collection) will be taken whilst the patient is on the lung transplant register and awaiting transplantation
Stage 2 consent:	Confirmation of final and full informed consent will be taken once the participant has been matched with a suitable donor (where a donation offer of both lungs and skin has been made)

Study participants awaiting a lung transplant will be matched with a suitable lung transplant donor as soon as they become available in accordance with NHSBT procedures. They may therefore be matched to a lung transplant donor where an offer of skin flap donation has not been made. In this situation the participant will not proceed to randomisation in the study and lung only transplantation will take place. In this situation, no-follow up will be collected on them, and their participation in the study ceases.

11.2 Timing of consent

Due to the emergency, unplanned nature of transplant procedures and the wish to have unhurried timely discussion with adequate period for digestion of the information, reflection and exchange of questions and answers; discussion about the study will be undertaken whilst patients are on the transplant waiting list. If patients agree to participate in the study consent will be taken and recorded whilst they are on the transplant waiting list (Stage 1 consent). In the event they are later matched with a donor and selected for transplantation, then consent will be confirmed prior to the procedure (Stage 2 consent).

11.3 Stage 1 consent (potential recipients)

A member of the responsible clinical team will briefly highlight the study to the patient and introduce a member of the local research team. If it is not possible for research staff to approach patients at the first point of contact, the patient can give their verbal permission

for their details to be passed to the local research team who will make contact with the patient by their preferred method and in accordance with the local site policy.
Potential participants will be given a current, approved version of the patient information sheet. They will also receive clear verbal information about the study from a member of the local research team. This will detail the nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be explained that they will be free to withdraw from the study at any time, for any reason, without prejudice to future care, and with no obligation to give a reason for withdrawal.

Due to the nature of the intervention, the timing of transplantation and the requirement for a dedicated transplant retrieval team, potential participants will also be advised that if they do join the study, it may not always be possible for them to receive an SSF or one may not always be available for transplantation. In this instance, the patient will not be able to take part in the study, but they will receive a lung transplant as planned and current NHS standard practice.

Patients will be given as much time as they wish to consider the information, and have the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. It will be clearly stated that the participant is free to withdraw from the study at any time, for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal.

The person obtaining consent must be suitably qualified, experienced and have been authorised to do so by the site's Principal Investigator. They are responsible for ensuring that the consent procedures comply with current applicable GCP Regulatory and ethical requirements. Informed consent discussions and outcomes must be well documented in the participants medical record.

11.4 Completion of the informed consent form (Stage 1)

The informed consent form (ICF) will usually be offered to participants in clinic as an electronic form on a tablet device (with the consent form being filled in directly on the study database, REDCap), however paper consent forms will also be made available for use in situations where electronic consent is not possible or suitable. Where it is not possible for a consent form to be completed in clinic, remote electronic consent may also be used.

A copy of the fully signed consent form will be given to the participant; where electronic consent is used and the participant has an email address they are willing to provide, an electronic version of the signed ICF will be automatically emailed to them. If the participant does not have/does not provide an email address the local team will be able to print a copy of the signed ICF and provide this to the participant. Consent forms will be e-mailed securely to the participant. The original signed consent form (paper consent only, for electronic consent this will be downloaded from the study database) should be placed in the Investigator Site File and a copy in the participant's medical record.
Remote eConsent (if required) will be obtained in accordance with OCTRU's standard operating procedures for obtaining consent. Where remote consent will be used, potential participants will be asked to provide an e-mail address for receiving consent documents, prior to obtaining written informed consent. Potential participants will receive a unique link via e-mail to an electronic consent form which may then be completed remotely. Once

completed, this form will be sent via e-mail to the participant as a PDF document. A member of the local site research team will be required to countersign all consent forms completed remotely, in the same way as for paper forms and verify the identity of the participant.

11.5 Stage 2 consent

If the intended recipient has given Stage 1 consent to be included in the study, they will be approached by the recipient point of contact at the recipient centre at the time of offer of organs. They will be asked to give confirmation of full informed consent (Stage 2) for the study in order to proceed. . This will be on or close to the day of transplantation. Due to the need to randomise participants in a timely manner to allow sufficient time for the dedicated skin flap retrieval team to travel to the donor site (for those participants randomised to this arm of the study), it may be necessary to discuss confirmation of consent over the telephone prior to the participants arrival at the transplant centre. This will permit randomisation and skin flap retrieval to proceed. Where telephone consent is taken, written consent will be sought as soon as it is practically possible.
Patients that give Stage 2 consent will be randomised to either proceed with lung and skin flap transplant or lung transplant alone when a set of lungs and skin flap are donated and matched by NHSBT.

11.6 Patients lacking capacity to consent

Patients that do not have capacity to consent to study participation will not be eligible to enter the study.

11.7 Consent Procedure – transplant (lung and skin flap) donor's families

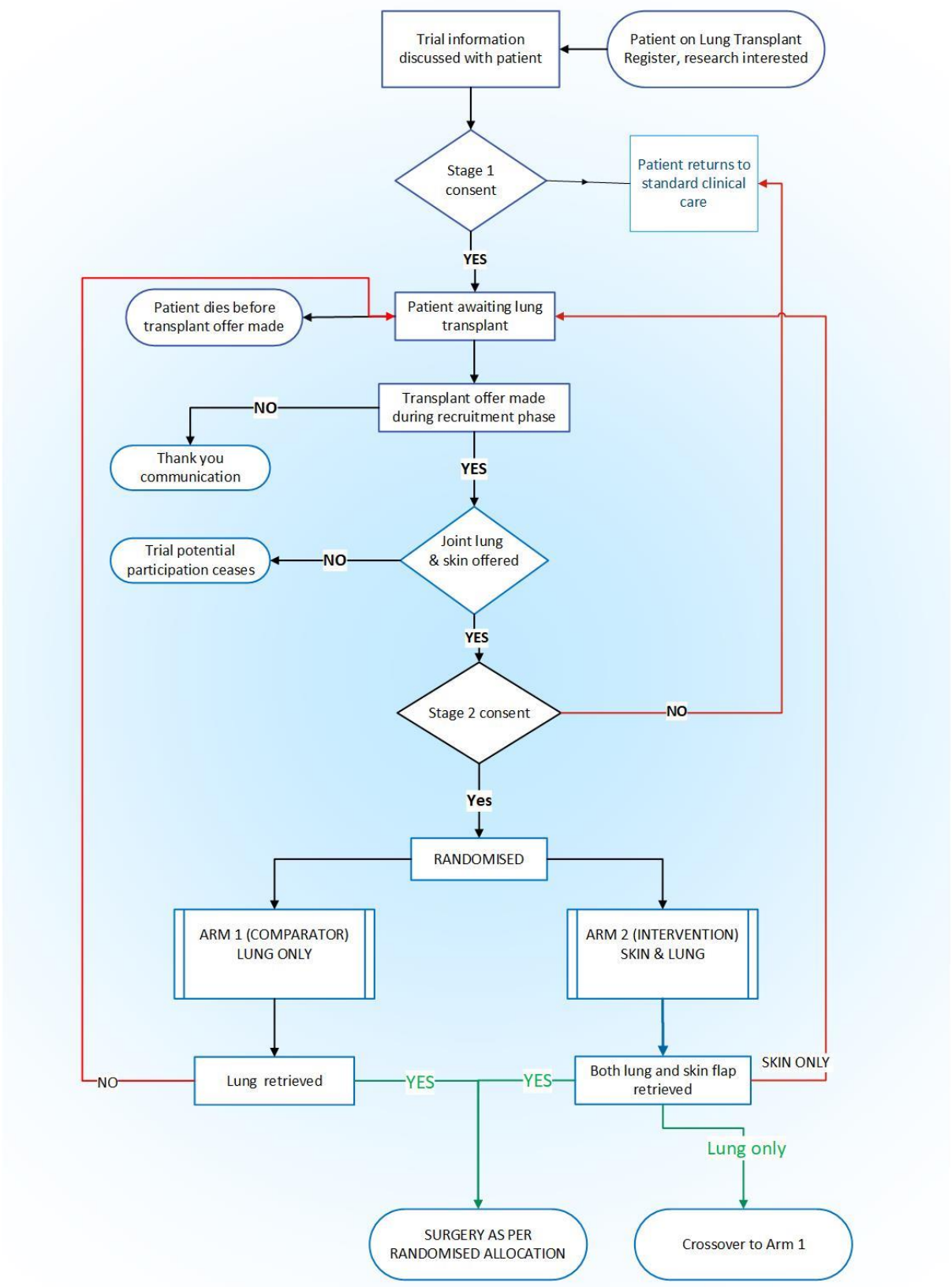
Note: This is as per standard UK practice – the process is not affected by the study nor details recorded by the study team.
Consent for donation of lung will be taken in accordance with usual NHS blood and transplant service (NHSBT) policies and guidelines. Current practice in the UK is for the NHSBT Specialist Nurses (SNs),as part of discussions if the offer of donation is made, to discuss organ donation with families and seek consent for donation using a standard NHSBT consent form. The Specialist Nurses will receive comprehensive study-specific training to an SOP which will be available for them to follow electronically when out on call. Training records will be recorded and kept by NHSBT. The SOP and training will include any information that needs to be given to the family and there will also be a step-by-step process for the SNs to follow to facilitate the skin flap donation process including giving the SENTINEL relative information sheet for the donors relatives to read. Donor families will be approached for consent for donation of the forearm SSF as a VCA which will be recorded as an organ for transplantation on the standard NHSBT consent form.

Note: All offers of donation will be recorded on NHSBT consent forms/paperwork – donor families will not complete any SENTINEL documentation.

11.8 Transplant pathway following consent

Patients awaiting lung transplantation may be on the waiting list for a transplant for some time. Following initial agreement to join the study (Stage 1 consent), numerous factors will determine whether a participant will proceed in the study. The flow chart overleaf provides an overview of the participant pathway following consent to the point of transplantation.

Study flowchart



11.9 GP notification

Permission from the participant will also be obtained to inform their GP of their inclusion in the study. An approved GP letter will be sent by the research team to the participant's GP informing them of their participation in the trial post-transplantation.

11.10 Re-consenting

Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the study, continuing consent will be obtained using an amended consent form which will be signed by the participant.

11.11 Participants who lose capacity during the study

Patients that initially consented to the study (Stage 1 consent) but have lost capacity and are unable to provide confirmation of consent (Stage 2 consent) immediately prior to transplantation will not be eligible to be randomised and will be excluded from being able to be randomised and their participation will cease.

If any participants permanently lose capacity during the course of the study after they have consented, randomised and been transplanted (this will be decided by their clinical care team, then we will continue to use data already collected and no further data will be collected.

12 RANDOMISATION AND BLINDING

12.1 Timing of randomisation

The NHSBT Hub Operations communicates any donor offers and the extent of any donor offers (i.e. does the offer include skin flaps) to the recipient centres.

If an organ becomes available for a consented participant but a skin flap is not offered then the participant will not be randomised. They will not continue to participate in the study and will receive the lung as part of their routine clinical care outside of the study.

If the offer of lung and skin is accepted by the recipient centre and matched to a patient who has consented to take part in the SENTINEL study, the recipient centre will reconfirm study consent (Stage 2 Consent) with the recipient. If consent is given, the study team will randomise the participant to receive either the lung transplant alone or a lung transplant and skin sentinel transplant.

The outcome of the randomisation will be communicated to the relevant parties involved in coordinating the retrieval and transplant (this may include recipient centre, SN, NHSBT Hub Operations, NORS team and recipient transplant teams by the local study team/ randomiser).

12.2 Randomisation procedure

Participants will be randomised via a centralised validated computer randomisation program through a secure (encrypted) web-based service,), provided by the Oxford Clinical Trials Research Unit (OCTRU); accessed via the REDCap study database.
Participants will be randomised to one of the following treatment arms:

Arm	Details
Lung transplant with sentinel skin flap (SSF) transplant (intervention)	Transplant of a skin flap of the same donor as the lung, transplanted at the same time as lung transplantation.
Lung transplant only (control arm)	Lung transplantation in accordance with usual procedure.

The randomisation outcome is communicated as per the 'PATHWAY FOR ORGAN DONATION AND RETRIEVAL' detailed in APPENDIX 1 – pathway for organ donation and RETRIEVAL

Randomisation outcome will be communicated directly back to the recruiting site by the randomiser immediately after randomisation (with an e-mail confirmation of randomisation sent to the site Principal Investigator, and the central study office and anyone else requested by the site). The SN's and NHSBT central Hub operations will also be informed whether or not skin retrieval is also required.

12.3 Randomisation methodology

Consenting participants will be allocated randomly (1:1) to either lung transplant with SSF transplant or lung transplant only.

Allocations will be generated using permuted block randomisation, with varying block sizes, stratifying for recruiting centre.

The randomisation schedule(s) will be designed by the OCTRU study statistician and full details will be detailed in a study randomisation and blinding plan.

12.4 Justification for stratification factors

The randomisation schedule is stratified for centre to balance for differences in routine practice, particularly in terms of assessing lung rejection (clinical or biopsy).

12.5 Re-randomisation following non-transplant

Randomised patients (i.e. those that have consented to the study and been offered and accepted an organ) who do not receive transplantation will return to the waiting list.. If they are offered lung and skin donation in the future they will be re-randomised. Patients will repeat stage 2 consent prior to re-randomisation and their HRQoL questionnaires will remain valid. If they are not offered lung and skin but offered lung only then they will not participate in the trial.

12.6 Back-up randomisation procedure

In case the web system fails, back-up emergency allocations will be prepared using opaque sealed envelopes. The emergency randomisation procedure is described in the study's randomisation and blinding plan along with study specific instructions for implementation with additional security measures. Emergency randomisation is undertaken by personnel not involved in recruitment.

12.7 Blinding

Table 1 provides an overview of the blinding status of all individuals involved on the conduct and management of the trial.

Table 1: Blinding status of those involved in trial conduct and management

Role in trial	Blinding status	Additional information
Participants	Not blinded	It is not possible to blind due to nature of the intervention. Participants will be told their treatment allocation immediately after randomisation.
Site research staff including Principal Investigator	Not blinded	It is not possible to blind due to the nature of the intervention. Following randomisation, an email will be sent to the PI and/or member of the site research team performing the randomisation (as delegated) confirming treatment allocation.
Chief Investigator	Not Blinded	It is not possible to blind the Chief investigator as they may be the primary clinician for those participants recruited at their site, however they will be blinded to allocations for participants at other sites. In instances where serious adverse events are reported, the CIs will become unblinded (if not already) to complete the full causality assessment.
Database programmer	Not blinded	The database programmer is responsible for the management of randomisation system and the REDCap database and will have access to all unblinded datasets within both systems.
SENTINEL Trial Management staff within the Surgical Intervention Trials Unit (SITU)	Not blinded	Trial Management staff within SITU will not be blinded to treatment allocations as site staff may require support for randomisation, or participants may contact the trial team directly. Serious Adverse Event reports will also be handled by the trial management team which will contain allocation information.
Data Management	Not blinded	Data management staff will have access to the unblinded datasets within the trial randomisation system and database to ensure data quality and undertake central monitoring activities.
Trial statistician and Senior Trial Statistician	Not blinded	The trial and senior trial statisticians will have access to treatment allocations or data needed for generating the Data Safety and Monitoring Committee (DMSC) closed reports and the final analysis.
Central laboratory staff	Blinded	Laboratory staff will only have access to the samples CRF – they will not be able to access trial allocations

12.8 Code break/ unblinding

Not applicable as there is no unblinding required. A pre-specified statistical analysis plan will be written in advance of un-blinding the data and any comparative analyses.

12.9 Summary: SENTINEL participants

All potential transplant recipients that have given consented are SENTINEL participants. However, due to the nature of the study and the timing of randomisation (which cannot be any other time), not all of them can contribute to the outcome. Participants cease to be part of SENTINEL if they revoke study consent at any stage or they get randomised without receiving a lung transplant (and are available for re-randomisation). Baseline data will be collected for all participants, but no follow-up data will be collected for those who never received a lung transplant, as they could never receive the intervention. Follow-up data will be collected for all randomised participants who received a lung transplant.

13 STUDY INTERVENTION AND COMPARATOR

13.1 Procedure for organ transplantation

NHSBT provide a blood and transplantation service to the NHS, looking after blood donation services in England and transplant services across the UK. This includes managing the donation, storage and transplantation of blood, organs, tissues, bone marrow and stem cells, and researching new treatments and processes (https://nhsbt.nhs.uk).

The National Organ Retrieval Team (NORs) will retrieve the lung in accordance with their usual care protocols and the skin flap will be retrieved by a dedicated SENTINEL study-specific skin flap retrieval and transplant team.

The SENTINEL study-specific plastic surgery retrieval and transplant team will generally be available 24 hours a day/7 days a week for timely travel to the donating hospital anywhere in the UK. They will be responsible for the retrieval of the donated skin flaps which will be performed at the same time as the lung retrieval at the donating hospital. The skin flap will be transported to the recipient hospital transplant unit, and then simultaneous surgical transplantation of the skin flap and lung will take place. Each completed episode will take on average 12-24 hours. The SSF retrieval will not be permitted to interfere nor delay retrieval or transport of the other organs.

13.2 Dedicated skin flap retrieval and transplant team

The dedicated study-specific skin flap retrieval and transplant team will consist of plastic surgeons and senior plastic surgery trainees familiar with performing radial forearm flaps. Radial forearm flaps are identical to the SSFs used in this study and are a common flap performed in plastic surgery. The dedicated skin flap retrieval and transplant team will be trained in the retrieval process and inset transplant process in part by attending the NHSBT masterclass in retrieval. This is a cadaver and classroom-based training course at which the Chief Investigator has taught for a number of years. The team will be deployed to donor hospital sites to retrieve SSFs from transplant donors for the study.

The skin flap retrieval and transplant team will be geographically dispersed so as to be able to cover all the hospitals in England and Wales at which retrievals may occur. The transplant team will be based at the transplanting hospitals, apart from Harefield which does not have an onsite plastic surgery unit and will be supplied by the plastic surgery team from Oxford. Backup support and supervision will be performed by the Chief Investigator, Prof. Henk Giele; as well as a core plastic surgery team all of whom will have an honorary contract at all 5 transplant hospitals.

The SSF will be taken to support the organ transplantation. In these circumstances, retrieval of such tissues that are essential to re-establishing functionality in the recipient is covered by the same licence as retrieval of organs for implantation. This is discussed in more detail within the HTA's framework document in paragraphs 54 – 57. Although retrieval of SSFs is covered by the same retrieval licence under which organ retrieval takes place, we would request each licence holder to advise the HTA so that their records can be updated.

A Flow Chart of the process from NHSBT receiving an offer of organ donation to the retrieval of the organs is provided in APPENDIX 1 – pathway for organ donation and RETRIEVAL.
The SENTINEL skin flap retrieval and transplant team will have a single contact number through which they will be contacted when an offer of skin and lung has been received, to ensure that a retrieval team are available. The team receiving the call will coordinate retrieval and transplant process as follows: The offer, once accepted by the recipient site will be checked to determine whether the proposed recipient has consented to participate in the study. If they have consented to participate and are cross-matched appropriately, consent will be confirmed, and then the patient will be randomised.

If a participant is randomised to receive skin as well as lung, the study-specific skin flap retrieval and transplant team will liaise with the lung NORS team and attend alongside them to retrieve the skin flap. Either the same plastic surgery team who removed the skin flap or the local recipient site plastic surgery team will attend the transplant procedure and inset the skin flap.

13.3 Management of skin retrieval

Not all lung donor families will agree to the donation of a skin flap as well. In order to avoid waste of retrieved, under-utilised skin flaps, the offer of both lung and skin donation must be confirmed prior to the randomisation. If the patient is randomised to lung only, the skin flap will not be retrieved. If the patient is randomised to lung and skin then both organs will be retrieved.

Around 50% of the time, the offer of the lung transplant is rejected by the transplant team as the lung is unfit for transplant. This decision could possibly be made as late as after randomisation has been performed and retrieval has already commenced. If skin flap retrieval has commenced prior to the decision not to proceed with lung retrieval and transplantation then the skin flap will be aesthetically sutured in place on the donor forearm and dressed. Obviously, the transplant and intervention cannot occur and the patient will return to the transplant waiting list. The patient will not retain their randomisation, as the next offer they receive may not include the offer of skin. They will need to be newly randomised next time, but only if their next offer has also agreed to skin flap donation.

If the decision is made at the recipient hospital, then both lung and skin flap will follow NHSBT policy and either be offered for research (if the donor family had consented for this) or discarded.

13.4 Lung transplantation procedure (usual care/comparator)

Participants randomised to receive lung transplant only will undergo transplantation by a transplant surgical team in line with routine clinical practice and will be followed up by current standard of care.

13.5 Lung transplantation with skin sentinel flap transplant (intervention)

Participants randomised to receive lung transplantation with an additional SSF transplant will receive a SSF from the same donor as the lung, at the same time as the lung transplant. The SSF (10cm x 3cm) will be anastamosed onto the ulnar artery of the recipient's non-dominant forearm at the same time as lung transplantation. The two procedures will be performed simultaneously to ensure that total operative and anaesthetic time is not prolonged. The arm will be abducted away from the body onto an arm table or anaesthetic arm board, to avoid interference with the lung transplant team. Inset of the forearm skin flap takes 1-2 hours. Lung transplantation takes an average of 4-12 hours, depending on the complexity of the procedure.

In the unlikely event that there is an anatomical or personal reason that a SSF cannot be anastomosed onto the non-dominant forearm, then it can be implanted onto their dominant forearm. Alternatively, another site, such as the groin or axilla can be chosen, or the participant can be withdrawn from the study. The options will be described within the patient information sheet and discussed and confirmed with the patient pre-operatively.

13.6 Retrieval of the lung

Retrieval of the lung will be performed according to current practice as directed by NHSBT by the National Organ Retrieval Teams. Organ donation is coordinated by the NHSBT. The specialist nurses (SNs) discuss organ donation with the families and seek consent for donation. Some families refuse any donation. Some refuse donation of a skin flap but permit lung donation. Despite consent being obtained for organ donation, not all donated organs will be utilised. This commonly in the case of donation after circulatory death (DCD) as compared to donation after brainstem death (DBD) where the logistics are more predictable.

13.7 Sentinel skin flap retrieval

If a suitable donor, with consent obtained for both lung and skin donation is matched with a participant, their local participating site will be informed (in accordance with NHSBT procedures). The dedicated retrieval team will be contacted by the participating site to ensure availability for skin retrieval, as there may be situations where transplantation procedures are already underway elsewhere and retrieval of the skin flap is not possible. If the patient confirms their consent to take part in the study, they will be randomised. If they are randomised to receive a SSF, the dedicated retrieval team will be placed on standby to travel to the donor hospital to retrieve the SSF alongside the usual lung retrieval team.

If skin flap retrieval is not possible or viable then lung retrieval and transplantation will proceed as planned without skin flap transplantation.

Where a skin flap is retrieved and not transplanted, the NHSBT will be informed and the skin flap will be discarded or used in other research approved by NHSBT provided the family has consented for such use. The study database will record that the skin flap was retrieved and not used. Skin flaps not used for the purpose of this study will be tracked and documented within NHSBT systems to ensure that they are handled and managed appropriately and in accordance with relevant approvals.

In the unlikely event that two donations are approved at the same time, and the skin flap retrieval team are not available to retrieve both SSFs, then the donation logistically/geographically nearest to the retrieval team would take priority; and only that recipient randomised. If that participant is randomised to lung only then the second recipient may be randomised.

Rarely, a skin flap may be required for both a lung transplant, as part of this study, and a intestinal transplant, as part of another study. In this instance, the SSF will be split in two for each recipient or prioritised for the intestinal transplant.

13.8 Detecting, diagnosing and managing rejection

If any events occur that suggest lung transplant rejection, such as a clinically significant drop in forced expiratory volume in 1 second (FEV1) of >10% or new symptoms of lung failure, then the usual sequence of clinical investigations will be performed and may include a tissue biopsy. The decision to perform a biopsy will be made in accordance with local site practice.

If a lung biopsy is performed, a skin biopsy will also be taken by the local health team for any participants with a SSF. The biopsy samples taken (lung and/or skin) will be examined by the local pathology team. Remaining samples embedded in paraffin blocks will be sent to the University of Oxford for the mechanistic studies at the completion of the 12 month follow up period;

If signs of skin rejection occur, the participant will either be examined in person by a study clinician (e.g. during a routine or extra visit) or they will be asked to send a photo of their skin flap to the research team/local healthcare transplant teams. The sending of any photographs from a participant to the research team or between local healthcare team and the research team will be done in accordance with local site practice for remote consultations; photographs will be stored within the participant's medical notes.

Consensus determination of rejection change in the skin will trigger an outpatient visit. Clinical features of the rash, investigations such as blood tests, sputum or bronchial brushings/, bronchoalveolar lavage if available, and lung function assessment and skin and/or lung biopsies (if taken) will be analysed for rejection.

All investigations for rejection will be performed at the discretion of the participating site/clinician and considered part of standard of care. This does not include the protocol skin biopsies taken at 3, 6 and 12 months which are required for the purpose of the study only.

A summary of the biopsy requirements for suspected rejection is provided in Table 2.

Table 2: Biopsy requirements/investigations for suspected rejection events

	Lung biopsy	**Skin biopsy required (sentinel skin flap recipients only)**
Signs/symptoms of lung rejection WITHOUT visible skin changes/rash only	In accordance with local policy*	Yes
Signs/symptoms of lung rejection WITH visible skin changes/rash only	In accordance with local policy*	Yes
Visible skin changes/rash	In accordance with local policy if signs of lung rejection present	Yes

*Where it is local policy to biopsy the lung, the date and result of the biopsy will be recorded on the case report form, and some of the biopsy sample will be stored for later mechanistic analysis

13.9 Monitoring the sentinel skin flap

For participants randomised to the intervention and go on to receive the intervention, both participants and healthcare staff will be taught by the research team how to monitor the skin for signs of rejection. The skin will be monitored daily by the participant for visible signs of rejection, such as erythema or rash.
Participants that experience a skin rash may be asked to take a photograph of their skin rash to send to their local research team prior to being asked to return to the site for any investigations.

Any photos sent to the local research team will be done so in accordance with the local Trust's policies on remote consultations and any approved NHS systems. The images will not be stored within the study database.

Whilst site staff will receive full training on the study, many will have limited or no experience in SSF transplantation. They can seek further assurance regarding the assessment of the skin for signs of rejection from the Chief Investigator or designated point of contact. Where this is required, the local study team may send a photograph of the skin flap to the Chief Investigator for review using secure nhs.net e-mail. Images will not identify the participant and will be transferred in accordance with applicable site policies. A consensus will be made between the Chief Investigator and local site clinician.

13.10 Treatment of rejection

Acute rejection of the lung will be detected and treated according to established practice at the participating transplant centre. If rejection in either skin or lung is confirmed by clinical findings or pathology (BANFF grade>1 skin and ISHLT grade A>1 for lung) this will be recorded. Decision on instituting treatment will be at the discretion of the local care team. Treatment given, treatment date and outcome of treatment for rejection will be recorded.

Normal local transplant follow-up protocols will continue with the addition that the skin flap will also be biopsied whenever the lung is biopsied.

The usual treatment of biopsy proven acute rejection of the SSF for Banff Grade 1 rejection will be a single 500mg dose of intravenous methylprednisolone with correction or escalation of daily immunosuppression if required, and for Banff Grade 2-4 rejection, is 3 daily doses of 500mg intravenous methylprednisolone with escalation of daily immunosuppression if required. However, any such treatment is at the determination of the clinical relevance by the local transplant team.

13.11 Immunosuppression following transplantation

Immunosuppression will be managed by the healthcare team according to standard practice at the participating transplant centre. Maintenance immunosuppression will be changed at the discretion of the clinical team according to factors such as serum levels, rejection episodes, renal function, neutrophil count, viral infections and side effects. Details of the immunosuppression and changes to the immune-suppression regimen that the participant receives will be collected on the case report form.

13.12 Removal of the sentinel skin flap

The SSF may be removed from the recipient at any time due to either flap complications or participant request. Flap complications are rare but include ischemia due to vessel thrombosis and persistent rejection that is resistant to treatment. Removal of the skin flap involves a minor procedure under local, regional or general anaesthetic, according to patient choice. During this procedure all donor tissue is removed and the skin closed to leave a longitudinal scar. This procedure will be undertaken at the patients' local centre by the Oxford or local research team and will take approximately 45 minutes.

14 STUDY ASSESSMENTS/PROCEDURES

14.1 Overview

Table 3 shows scheduled assessments including sampling for the study.

Participants who receive a lung transplant will undergo routine clinical follow-up after transplantation as part of standard care for all lung transplant recipients. The relevant participating transplant centres have standard protocols for the management of patients after lung transplantation. In addition, participants who receive a SSF will be given a Skin Flap Information Leaflet. This provides important information about what to look out for on their skin flap after transplantation and what to do if they notice a skin rash developing. During the first year following transplantation, typically, such patients are reviewed in an outpatient clinic at least 10 times in the first 6 months and then 6 times in the second 6 months (this can be more frequent if problems arise). During these visits the usual monitoring and clinical investigations will be performed by the relevant participating centres. These may include full blood count (FBC), renal function tests (U&Es), liver function tests (LFTs), C reactive protein, immunosuppressant levels and donor specific antibodies (DSA). In addition, there may be organ specific investigations such as respiratory function tests, sputum, radiographs, bronchoscopies, bronchoalveolar lavage, bronchial brushings and allograft biopsies.

Trial-specific follow-up assessments/subsequent visits will coincide with usual care follow-up clinic visits.

Table 3: Summary of study assessments.

PROCEDURE	**Pre-baseline**	**Baseline**		**3 months +/-4WKS**	**6 months +/- 4 WKS**	**12 months +/- 4WKS**	**Rejection Event**
	On transplant waiting list	Upon donor matching	Day of transplant				
Screening/eligibility	X						
Stage 1 consent	X						
Stage 2 consent		X					
Final eligibility check		X					
Baseline CRF data collection	X	X					
Transplant (+/- skin flap)			X				
HRQoL questionnaires (SF-36, EQ-5D-5L, DAS-24)	X			X		X	
Clinical outcome data collection (CRF)			X	X	X	X	X
Study Samples							
Recipient lung microbiome biopsy or bronchial brushing*			X				
Transplanted lung biopsy (if and when routinely performed, centre specific)**			X[1]	X	X	X	X
Transplanted lung bronchial brushing or broncho-alveolar lavage or sputum (if and when routinely performed, centre specific)*			X	X	X	X	X
Skin swab of flap (those randomised to the lung and sentinel skin flap group).			X[1]	X	X	X	X

PROCEDURE	Pre-baseline	Baseline		3 months +/-4WKS	6 months +/- 4 WKS	12 months +/- 4WKS	Rejection Event
Skin flap biopsy (those randomised to the lung and sentinel skin flap group)*			X[1]	X	X	X	X
Recipient native skin swab microbiome sample (both groups)			X[1]	X	X	X	X
Blood sample (both groups)			X[1]	X	X	X	X

*When taken, these samples will be divided into two and for immediate analysis and to Oxford for later analysis.
X[1] after transplant re-vascularisation.

Samples

Refer to SAMPLES FOR LABORATORY ANALYSIS section for full details on study samples, including taking, processing and shipping, where applicable.

14.2 Data Collection

Baseline data

Once Stage 1 consent has been obtained, baseline data collection can commence, including the baseline HRQoL questionnaires (SF-36, EQ-5D-5L and DAS-24). Where possible, baseline questionnaires will be completed electronically by the participant. Paper questionnaires may also be used where use of electronic means is not possible or suitable. Where paper-based questionnaires are used, data will be entered into the study database by the local site research team.

Completed at hospital by local study team member from medical notes or with participant prior to transplant

Sourced/collected by local study team	Direct patient report

• Date of baseline visit
• Participant demographics which will include:
  ○ Date of birth
  ○ Sex
  ○ Height and weight (to calculate BMI)
  ○ Ethnicity
  ○ Reason for requiring lung transplant
  ○ Co-morbidities
• Contact details of participant
• Participant's NHS/CHI number
	• Health-related quality of life*
	  ○ SF-36 (unmodified)
	  ○ EQ-5D-5L (unmodified)
	  ○ DAS-24 (unmodified)

Collected after transplant:
• Transplant outcome
• Date of transplant
• Skin flap transplant details to include:
  ○ Location of transplanted flap
  ○ Operating time/duration
• Reason for not receiving a transplant or not receiving the sentinel skin flap (if randomised to skin flap arm)
• Transplant type
• Organs transplanted
• Details of donor matching^
• Donor age, sex and weight^
• Cold ischaemia time (lung)
• Operating time/duration
• Details of induction and regular immunosuppression
• Surgical complications
• Biopsy results	

^ information regarding the donor matching accompanies the transplanted organ(s). Further information regarding the donor (age, sex and weight) is collected routinely by NHSBT and will be accessed by the research team from the medical notes.

Completed by dedicated skin flap retrieval and transplant team after transplant

Sourced/collected by local study team

• Details of skin flap retrieval which will include:
  ○ Skin flap retrieval outcome
  ○ Skin flap transplanted
  ○ Reason for non-transplant
• Presence of an arterial line prior to skin flap retrieval.

Follow-up data

3 months post-transplantation

Sourced/collected by local study team	**Direct patient report**

• Surgical complications relating to the SSF
• Biopsy-proven or clinical rejection
• Transplant organ function
• Antibodies
• Immunosuppression
• Check that lung and/or skin biopsy samples have been collected and sent for clinical analysis and storage at the participating sites pathology labs until the end of the trial when they will be transferred to Oxford for the mechanistic work.
	• Health-related quality of life*
	  ○ SF-36 (unmodified)
	  ○ EQ-5D-5L (unmodified)
	  ○ DAS-24 (unmodified)

• Check that blood and microbiological swabs and half of the skin biopsy have been collected and sent to Oxford for storage for later analysis. • Graft and patient survival	

6 months post-transplantation

Sourced/collected by local study team	Direct patient report
• Surgical complications relating to the SSF • Biopsy-proven or clinical rejection • Transplant organ function • Immunosuppression levels and requirements • Check that lung and/or skin biopsy samples have been collected and sent for clinical analysis and storage at the participating sites pathology labs until the end of the trial when they will be transferred to Oxford for the mechanistic work. • Check that blood and microbiological swabs and half of the skin biopsy have been collected and sent to Oxford for storage for later analysis. • Graft and patient survival	N/A

12 months post-transplantation

Sourced/collected by local study team	Direct patient report
• Surgical complications relating to the SSF • Biopsy-proven or clinical rejection • Transplant organ function • Immunosuppression levels and requirements • Check that lung and/or skin biopsy samples have been collected and sent for clinical analysis and storage at the participating sites pathology labs until the end of the trial when they will be transferred to Oxford for the mechanistic work. • Check that blood and microbiological swabs and half of the skin biopsy have been collected and sent to Oxford for storage for later analysis. • Graft and patient survival	• Health-related quality of life* ○ SF-36 (unmodified) ○ EQ-5D-5L (unmodified) ○ DAS-24 (unmodified)

*Patients will be sent HRQoL questionnaires via e-mail or post (depending on participant preference) at baseline, 3 months, and 12 months post-randomisation, with an option to be contacted by telephone by the Surgical Intervention Trials Unit (SITU).

Upon detection of a rejection event:

Sourced/collected by local study team	Direct patient report
• Method of diagnosis (clinical/biopsy)	N/A

• Reason for suspecting rejection • Investigations for rejection • Biopsies taken and results • Check that lung and/or skin biopsy samples have been collected and sent for clinical analysis and storage at the participating sites pathology labs until the end of the trial when they will be transferred to Oxford for the mechanistic work. • Check that blood and microbiological swabs and half of the skin biopsy have been collected and sent to Oxford for storage for later analysis. • Treatment of rejection event • Resolution date	

14.3 Assessments for investigating signs of rejection

Certain events that indicate possible rejection will trigger an additional visit and procedures. These events are:

- Skin rash/ oedema on sentinel skin flap confirmed as rejection
- Clinically significant drop in lung function on spirometry
- Clinically significant change in chest x-rays
- New symptoms suggestive of rejection such as cough, breathlessness, fever

14.4 Qualitative assessments

No qualitative research will be performed as part of the trial.

14.5 Withdrawal of Participants

Withdrawal of consent means that a participant has expressed a wish to withdraw from the study altogether or from different aspects of the study.

Data and samples collected up to the point of withdrawal will be used in the study analysis. For complete withdrawal from the study: Under these circumstances, the site needs to document all relevant discussions in the participant's medical notes. No subsequent data (including routine care data) should be collected. The site should notify the SENTINEL study office, which will allow them to mark all future CRFs as not applicable. However, under these conditions, investigators are still responsible for following up any SAEs, and should continue to report the SAE to resolution in the CRF and to the study centre.

For withdrawal from different aspects of the study participants can decide which aspects of the study they wish to stop participating in.
The type of withdrawal will be collected on the CRF.
The following types of withdrawal will be captured within the CRF with appropriate action taken by the study to ensure compliance with the participant's wishes:

- No longer willing to complete study questionnaires
- No longer willing to provide study samples
- No longer willing to receive study-related communications
- No longer willing to attend study visits
- No longer willing to be contacted by the research team to obtain outcome data

- No longer willing to have intervention (Skin-flap) (Request for participant to have skin flap removed)

14.6 Withdrawal of participant by the clinician

Withdrawal from the study or parts of the study may come from clinicians if they believe the participant needs to be withdrawn. Under these circumstances, the site needs to document the reason for the clinician's withdrawal of the individual in the participant's medical notes. The site should notify the SENTINEL study office, which will allow them to mark all future CRFs as not applicable. However, under these conditions, investigators are still responsible to follow up any SAEs, and continue to report the SAE to resolution in the CRF and to the study centre.

The type of withdrawal will be collected on the CRF.
Data and samples collected up to the point of withdrawal will be used in the study analysis.

14.7 Participants that do not proceed to lung transplant

Patients who consent (Stage 1) to be included in the study may not proceed to transplantation during the study period as:

1) they may not receive a suitable offer of a lung and skin organ;
2) They may received a lung offer but not accepted by the clinical team and/or patient
3) They receive a suitable offer but the lung is not retrieved
4) may become too unwell or die
5) may recover and no longer need transplantation.
6) ;

14.8 Participants that are offered a transplant where skin has not been agreed to be also donated

Participants who consent to be included in the study may proceed to transplantation but not be randomised. If an organ is only offered for donation without skin – a matched individual will be offered the organ and if they take the transplant offer up – and it proceeds this will end their participation in the study. A thank you letter will be sent to the individual at this point.

14.9 Participants that are offered a transplant where skin is available and to which they are randomised to an SSF with their transplant but they do not receive the SSF but they still receive the lungs

Participants who consent and are randomised may not receive their allocation because the either the skin or SSF transplant does not proceed.

If the lung is not transplanted these participants will return to the lung transplant waiting list and their participation ceases. They will, however, have a further opportunity to participate should they get a future offer of lung and skin. They will be re-randomised in this event.
If the lung is transplanted but the skin flap is not transplanted, these patients will cross over to the lung only arm of the trial.

14.10 Communication with study participants by the central trial team

Participants who have requested to receive their study questionnaires by e-mail will be sent e-mails from the central study team at the relevant time points with links to complete their study questionnaire. A reminder e-mail will be sent if this is not returned within 14 days and a further remind a week later.

Participants who have not completed their study questionnaire after the second reminder will be contacted by telephone by the central study team.

15 SAMPLES FOR LABORATORY ANALYSIS

15.1 Overview of study samples

Table 4 provides a summary of all samples required by this trial protocol

Table 4: Sampling requirements

Sample Type	Time point	Standard of care or trial-specific?	Analysis by local Trust lab or other
Skin flap biopsy (those randomised to the lung and sentinel skin flap group)	Baseline 3 months post-transplant 6 months post-transplant 12 months post-transplant and Upon suspicion of rejection	Study-specific	Half of the biopsies sample sent to local laboratory and half sent to central laboratory (Oxford) for mechanistic studies
Skin swab of flap (those randomised to the lung and sentinel skin flap group)	Baseline 3 months post-transplant 6 months post-transplant 12 months post-transplant and Upon suspicion of rejection	Study-specific	Sent to central lab (Oxford) for mechanistic studies
Recipient native skin swab sample (both groups)	Baseline 3 months post-transplant 6 months post-transplant 12 months post-transplant and Upon suspicion of rejection	Study-specific	Sent to central lab (Oxford) for mechanistic studies
Transplanted Lung biopsy	Upon suspicion of rejection (only where local site practice is to perform lung biopsies to confirm rejection)	Where Standard of care*	Biopsies sent to local laboratory for analysis; and at end of follow up period to be sent to Central laboratory (Oxford) for mechanistic studies.

Sample Type	Time point	Standard of care or trial-specific?	Analysis by local Trust lab or other
Transplanted Lung biopsy (only where local site practice is to perform lung biopsies for monitoring)	Baseline 3 months post-transplant 6 months post-transplant 12 months post-transplant	Where standard of care*	Biopsies sent to local laboratory for analysis; and at end of follow up period to be sent to Central laboratory (Oxford) for mechanistic studies.
Transplanted Lung bronchial brushing or bronchoalveolar lavage or sputum (only where local site practice)	Baseline, 3 months post-transplant 6 months post-transplant 12 months post-transplant and On suspicion of rejection	Where standard of care*	Some of sample sent to local laboratory and some sent to Central laboratory (Oxford) for mechanistic studies
10 ml blood sample (both groups)	Baseline, 3 months post-transplant 6 months post-transplant 12 months post-transplant and On suspicion of rejection	Study-specific	Sent to central lab (Oxford) for mechanistic studies

*sites will take lung biopsies in accordance with local site practice; clinical diagnosis of lung rejection may be used in place of biopsy-confirmed rejection where this is also local site practice.

15.2 Sample handling for standard of care samples and trial-specific samples to be analysed in local Trust's laboratories

Standard of care samples and trial-specific samples to be analysed in local Trust laboratories will be taken and processed by laboratories in accordance with local site practice. This includes labelling of samples with standard patient identifiers. Results will be reported back in the usual way according to local practice and accessed by the site research team, and entered into the study database.

Sample Type	Time point	Test(s) to be undertaken on the sample
Lung biopsy	Upon suspicion of rejection, and when taken for monitoring as standard of care	Biopsy will be analysed by local pathology laboratory for signs of rejection. Results will be reported back on histopathology report.
Skin biopsy	Upon suspicion of rejection, and at baseline, 3, 6 and 12 months.	Biopsy will be analysed by local pathology laboratory for signs of rejection. Results will be reported back on histopathology report.

Other samples required for routine clinical follow-up following transplantation will be taken and processed by local Trust laboratories as required e.g. blood samples, broncho-alveolar samples, sputum samples.

15.3 Sample handling for trial-specific samples analysed in a central laboratory

Sample handling (including any local processing) and storage will be managed according to separate written instructions/sample handling manual. Table 5 summarises the arrangements for collection, timings, and analytical laboratories responsible

Table 5: Study-specific samples analysed by a central laboratory

Sample Type	Time point	Tests to be undertaken on the sample
Skin flap biopsy (those randomised to the lung and sentinel skin flap group)	Baseline 3 months post-transplant 6 months post-transplant 12 months post-transplant Upon suspicion of rejection	Immunological studies including HOT panel, DSP, flow cytology, immunohistochemistry
Skin swab of flap(those randomised to the lung and sentinel skin flap group)	Baseline 3 months post-transplant 6 months post-transplant 12 months post-transplant Upon suspicion of rejection	Microbiome analysis
Swab of recipient native skin (both groups)	Baseline 3 months post-transplant 6 months post-transplant 12 months post-transplant Upon suspicion of rejection	Microbiome analysis
Transplanted Lung biopsy (if taken)	Baseline 3 months post-transplant 6 months post-transplant 12 months post-transplant Upon suspicion of rejection	Immunological studies including HOT panel, DSP, flow cytology, immunohistochemistry
Transplanted Lung Bronchial brushing or brochoalveolar lavage or sputum (if taken)	Baseline 3 months post-transplant 6 months post-transplant 12 months post-transplant Upon suspicion of rejection	Microbiome analysis
10 ml blood (both groups)	Baseline 3 months post-transplant 6 months post-transplant 12 months post-transplant Upon suspicion of rejection	Cell free DNA analysis RNA and gene expression

Provision of sample collection packs

Sites will be provided with biological transport packs for the transport of blood, skin swab and skin flap samples to Oxford where they will be documented and stored with the trial

code, trial patient identifier, and date. Sites will also be provided with 4 and 6mm skin punch biopsy instruments and sample collection tubes, microbiological swabs and transport tubes for the skin swabbing. Blood is collected and stored in Streck Cell-Free DNA blood collection tube and Tempus tubes.

At each sample event either a single 6mm punch biopsy is cut in half, or two x 4mm punch biopsies will be taken. Both biopsies will be stored in formalin with one biopsy to be sent to Oxford for later analysis and one sent to the local histology laboratory for paraffin embedding and clinical reporting. These blocks may be useful for the mechanistic investigation later in the study.

The lung samples will be sent for fixation in formalin and paraffin embedding (FFPE) and clinical analysis at the local laboratory. At the end of follow up these samples will be sent to Oxford for storage for later analysis for the mechanistic studies.

Samples may be stored for up to 5 years by Oxford after conclusion of the clinical component of the trial, during which they will be analysed for the mechanistic component of the study. We can only analyse the samples for the mechanistic elements once the clinical outcomes are known as correlation to clinical progress is needed to select the samples for analysis and comparison.

15.4 Labelling and confidentiality of trial-specific samples

Study-specific samples that are not requested and processed in the same manner as standard of care clinical samples will be labelled as study samples in accordance with the separate sample handling manual.

All samples sent to analytical laboratories will be labelled with the study code, study patient number, initials and date taken. Should a laboratory receive any samples carrying unique patient identifiers the recipient must immediately obliterate this information and re-label and report a data breach to the Trial Office.

15.5 Use of tissue banks

No tissue samples will be retrieved from or sent to any tissue banks in the trial.

15.6 Samples for Biobanking

Not applicable for this study

15.7 Clinical reporting of exploratory research assay results

The results of the mechanistic investigations are exploratory and are not intended to influence the individual participant's medical care. Findings will not be reported routinely to the responsible clinician.

15.8 Retention of samples at the end of the trial

The Chief Investigator has overall responsibility for custodianship of the trial samples. Laboratories are instructed to retain any surplus samples pending instruction from the Chief Investigator on use, storage or destruction. It is possible that new or alternative assays may be of future scientific interest. At the end of the research study any surplus samples may be retained (if consented by patient) for use in other projects that have received ethical approval. Hence, any surplus study samples may be transferred to a licensed tissue bank where they will be managed in accordance with applicable host institution policies and the Human Tissue Act (HTA) requirements.

15.9 Withdrawal of consent for sample collection and/or retention

A participant may withdraw consent to provide samples for research at any time without giving a reason. The Investigator must ensure that their wishes are recorded in their medical notes and will inform the SENTINEL study office accordingly. The investigator should discuss with participants the valuable use of samples that have already been provided and under circumstances where these samples have already been processed and anonymised, it would not be possible to destroy such samples.

16 SAFETY REPORTING

16.1 Safety reporting period

Safety reporting for each participant who is randomised and receives a lung transplant will begin from the date of transplant and will end when the participant has reached their final main follow-up time point, at 1 year post-transplant surgery. All complications will be recorded in both groups.

16.2 Definition of Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical study participant. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporarily associated with study procedures, whether or not considered related to the procedures.

A serious adverse event (SAE) is any untoward medical occurrence that:

- Results in death
- Is life-threatening[1]
- Requires inpatient hospitalisation or prolongation of existing hospitalisation[2]
- Results in persistent or significant disability/incapacity
- Consists of a congenital anomaly or birth defect
- Is otherwise considered medically significant by the Investigator[3]

Significant medical events are medical events that may jeopardise the participant and may require an intervention to prevent one of the outcomes listed above.

[1] 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

[2] In general, hospitalisation signifies that the participant has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting.

[3] Medical events that may jeopardise the participant or may require an intervention to prevent one of the above characteristics/consequences. Such events should also be considered as 'serious' in accordance with the definition.

An AE does include a / an:

1. exacerbation of a pre-existing illness;
2. increase in frequency or intensity of a pre-existing episodic event or condition;

3. condition detected or diagnosed after the start of the study even though it may have been present prior to the start of the study;
4. continuous persistent disease or symptoms present at baseline that worsen followings the start of the study

An AE does NOT include a / an:

1. medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); but the condition that led to the procedure is an AE;
2. pre-existing disease or conditions present or detected at the start of the study that did not worsen;
3. situations where an untoward medical occurrence has not occurred (e.g., hospitalisations for cosmetic elective surgery, social and / or convenience admissions);
4. disease or disorder being studied, or sign or symptom associated with the disease or disorder unless more severe than expected for the participant's condition;
5. overdose of concurrent medication without any signs or symptoms

Seriousness vs severity

A distinction is drawn between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined using the criteria above. Hence, a severe AE need not necessarily be serious.

16.3 Reporting of Serious Adverse Events (SAEs) from sites to the central study team

Only serious adverse events considered by to be related (possibly, probably or definitely) to any of the study procedures will be reported to the central study team. Such events must be reported within 24 hours to the central study team using the SAE form within the REDCap study database. On notification/receipt of an SAE the central study team will perform an initial check of the report and request any additional information from the site team. The SAE will be assessed by the CI for relatedness (see 19.5). The SAE will also be reviewed by the Nominated Person for the trial, who will perform the assessment of expectedness, according to section 19.4.

The **Surgical complications** listed below should not be reported on a SAE form unless the complication is (in the opinion of the CI or designee) considered more severe in nature than might be expected, or unexpected, for the particular intervention received.

16.4 Surgical complications

The following treatment-related complications will be collected on the peri- and post-operative complications case report forms (CRFs) and should not be reported separately on a SAE form unless the complication is (in the opinion of the site investigator) considered **more severe in nature than might be expected, or unexpected, for the particular intervention** received by the participant.

Peri-operative complications (events occurring intraoperatively, during the immediate post-operative period, or in the first 14 days post-procedure):

Skin Flap:

- Bleeding/Haematoma
- Wound dehiscence

- Infection (spreading wound inflammation, purulent discharge requiring additional antibiotics)
- Arterial supply problem to the flap
- Venous supply problem to the flap
- Vascular supply deficiency
- Nerve supply to hand (numbness, paraesthesia, weakness, paralysis, palsy, stiffness, carpal tunnel syndrome)
- Delayed wound healing
- Flap necrosis: partial %/total

Lung Transplant:
- Pneumothorax
- Haemothorax
- Bleeding/Haematoma
- Wound dehiscence
- Wound Infection (spreading wound inflammation, purulent discharge requiring additional antibiotics)
- Empyema
- Vascular supply problem to the lung transplant
- Delayed wound healing
- Air leaks
- Bronchial anastamotic leak
- Bronchial stenosis
- Bronchial malacia
- Infection- cmv/other respiratory virus/aspergillus/mycobacterial/other
- Cryptogenic organising pneumonia
- Pulmonary embolism
- Pulmonary infarction

Post-Operative: Events occurring at any time during follow-up period likely related to a study intervention: (3 – 12 months) *from 2 weeks.

Skin Flap:
- Nerve supply to hand (numbness, paraesthesia, weakness, paralysis, palsy, stiffness, Carpal tunnel syndrome)*
- Infection (spreading wound inflammation, purulent discharge requiring additional antibiotics)*
- Arterial supply problem to the flap*
- Venous supply problem to the flap*
- Flap necrosis*
- Vascular supply deficiency
- Pain
- Tendon dysfunction

Lung Transplant:
- Bronchial dehiscence*

- Bronchial stenosis
- Bronchial malacia
- Infection- cmv/other respiratory virus/aspergillus/mycobacterial/other
- Cryptogenic organising pneumonia
- Pulmonary embolism
- Pulmonary infarction
- PTLD (post transplant lymphoproliferative disorder)
- GVHD (graft versus host disease)
- Chronic rejection
- Upper lobe fibrosis
- Post trans-bronchial biopsy related complications
- Bronchogenic carcinoma
- Lung failure (reasons: vascular failure, acute rejection, chronic rejection, pneumonia, other organ failure, other)
- Lung explanted

16.5 Assessment of SAEs

Relatedness/causality assessment

The assessment of "relatedness" to the study intervention is the responsibility of the CI, or medically qualified designee, who has signed the delegation log.

All AEs judged as having a reasonable suspected causal relationship to the study intervention/procedure(s) are considered to be adverse events. The assessment of relatedness is made using the following:

Relationship to intervention	Attribution (Causality)	Description
Unrelated	Unrelated	The AE is clearly NOT related to the intervention
	Unlikely	The AE is doubtfully related to the intervention
Related	Possible	The AE may be related to the intervention
	Probable	The AE is likely related to the intervention
	Definite	The AE is clearly related to the intervention

16.6 Expectedness

The Nominated person (NP) will be appropriately trained to carry out this assessment. They will use the listed complications in section 19.4 to determine whether an event is considered expected, taking into consideration the study intervention received by the participant and the timing of the event in relation to the study intervention. Any event that might not ordinarily be expected in a participant undergoing the study intervention (sentinel skin flap transplant) received should be considered unexpected. In addition, any events that might be expected but are more severe in nature than might be expected should also be considered unexpected, and these should include any event deemed related to the intervention which presents as a life-threatening event.

17 REPORTING OF UNEXPECTED SAES TO THE RESEARCH ETHICS COMMITTEE (REC)

All SAEs that are considered to be both related (i.e. resulted from administration of any of the research procedures) and unexpected (that is, the type of event is not expected for the

study intervention) will be submitted to the REC within 15 days of the CI or CTU becoming aware of the event, using the HRA report of serious adverse event form.

Expected events will be collected during the follow-up visits, and these will be recorded on the peri- and post-operative complications CRFs.

Transplant rejection is a study outcome and will be recorded in the peri- and post-operative complications CRFs and should not be reported as a SAE.

18 FOLLOW-UP OF SERIOUS ADVERSE EVENTS

A follow-up report must be completed when the SAE resolves, is unlikely to change, or when additional information becomes available. Follow-up information must also be provided as requested by the study office.

19 ADVERSE EVENT CODING

All serious adverse event terms will be coded by the central study team using MedDRA version 25.1.

20 PREGNANCY

Pregnancy is a contra-indication to receiving a transplant. If an individual does become pregnant in this study, it does not need to be reported due to the nature of the intervention of this study.

21 STATISTICAL CONSIDERATIONS

21.1 Statistical Analysis Plan (SAP)

The statistical aspects of the study are summarised here with details fully described in a statistical analysis plan that will be drafted early in the study and finalised prior to the start of the data analysis. The SAP will be written by the Trial Statistician in accordance with the current OCTRU SOPs. The TSC and DSMC will review and, if necessary, provide input on the SAP.

A summary of the planned statistical analysis is included within this section.

21.2 Sample Size/Power calculations

The anticipated 12-month rejection rates for lungs in the control arm by 12 months is 80%. Published rates vary between 33-100% (43–46) depending on definition and grade of rejection and method of analysis. However, a comprehensive study of regular transbronchial biopsies in 299 lung transplant patients showed a cumulative incidence of rejection (≥grade A1) of 91.7% in the first 12 months and (≥grade A2) of 77.9% (46).

In the SSF intestinal study, we observed a 72% reduction in biopsy-proven acute rejections from the 77% rejection rate in the control arm reducing to 22% in the with skin arm. However, as a more conservative estimate for this study, we consider a relative reduction of a third from 80% to 53.33% (corresponding to an absolute difference of 26.67%) to be clinically meaningful. 128 participants who have had a transplant are required for the analysis to achieve 90% power at a two-sided significance level of 5%. To allow for a 15% loss to follow-up or death by 12 months (76 per trial arm) 152 participants need to have been randomised and transplanted. In total, we expect approximately 254 randomisations

will be required to achieve the required sample sizeand account for participants not proceeding with transplant.

Assessment of the diagnostic accuracy of the SSF compared to the current reference standard (organ biopsy) is a secondary objective of this study, and is undertaken in participants who received an SSF. Assuming approximately 80% specificity, and 90% specificity, based on previous studies, 95% confidence intervals (Wilson method) around the sensitivity, specificity, positive and negative predicted values will have a width of approximately 20-30%, depending on the observed sensitivity, specificity and event rates. A sample size of 64 participants receiving SSF and providing one-year follow-up data, an expected rejection rate of 53%, and anticipated values of 80% and 90% for sensitivity and specificity, respectively, will provide approximately 80% power (at a one-sided 5% significance level) to show that the observed sensitivity and specificity in the study are statistically significantly higher than 60% and 70%, respectively.
Since the proposed mechanistic sub-studies are intended to be hypothesis generating, no formal sample size calculations have been performed.

21.3 Choice of primary outcome

The primary outcome is lung rejection events in the first 12 months post transplant. This is the common reporting period and the period in which the majority (90%) of first rejection events occur. After this period, the transplant is immunologically more stable. Although there is a correlation between rejection episodes and transplant organ loss and mortality, in order to demonstrate that reduced rejection events do translate into improved patient and organ survival, longer-term patient and transplanted organ survival and functional data will be analysed for 5 years, as part of a separate longer term study subject to funding. AE and SAE data will also be collected over this period. The data is already routinely collected by the transplant units and reported to NHSBT annually.

21.4 Description of Statistical Methods

Results will be reported in line with the CONSORT statement and relevant extensions (41)(42).

Available baseline data will be summarised for all participants: those who provided stage 1 consent only; those who also provided stage 2 consent; those who consented and randomised and those who also randomised and received a lung transplant. All outcomes will be summarised using descriptive statistics overall and, when collected for both arms, split by treatment groups. Binary and categorical data will be summarised by frequencies and percentages. Continuous data will be summarised by means and standard deviations (SDs), or median and inter-quartile range if data are skewed. Corresponding 95% confidence intervals will be presented where possible. Visual representation of outcomes will be considered and, where it will support interpretation, presented.

Analysis will be conducted using a complete case approach, no imputation methods for missing data are planned. Analysis of the primary and secondary outcomes will be done once the 12 month follow up has been reached by the last patient. Analyses of these outcomes will be pre-specified in a statistical analysis plan finalised prior to un-blinding of the data. Analysis of mechanistic outcomes, using sample data, will be done following completion of the primary and secondary outcomes.

An independent Data and Safety Monitoring Committee (DSMC) will meet early in the trial to agree its terms of references. They will review confidential interim analyses of accumulating data, including the interim analysis of the stop-go criteria and diagnostic accuracy.
It is anticipated that all statistical analysis will be undertaken using Stata (StataCorp LP, www.stata.com) or other well validated statistical software.

24.5 Primary outcome

The primary outcome of the study is absolute difference in rejection rates (clinical or biopsy) at 12 months post transplant. The absolute risk difference, and corresponding 95% CIs, will be obtained from a mixed-effects Cox proportional hazards models, including treatment and adjusted for centre as a random effect, analysing time to first lung rejection (43). If the proportional hazards assumption is not met, alternative parametric methods will be considered. The treatment groups will also be compared using Kaplan-Meier curves and log rank tests. Events, by severity, will be summarised descriptively. Sensitivity analyses for the primary outcome will include alternative definitions of the primary endpoint, including lung rejections of ISHLT grade 2 or higher, and ISHLT grade 3 or higher. The primary analysis will be repeated for the per-protocol population, excluding participants with major deviations from the study protocol. Details on the definition of analyses populations will be described fully in the study's Statistical Analysis Plan, along with consideration of cross-overs.

24.6 Secondary outcomes

For diagnostic accuracy of the first test taken, proportions of observed true positives, true negatives, false positives and false negatives will be presented. Estimates of sensitivity, specificity, positive and negative predictive values will be generated, as well as the positive and negative likelihood ratios together with corresponding 95% confidence intervals, using the Wilson method. Diagnostic accuracy will be calculated for rejection events diagnosed by both methods (biopsy and clinical) for the entire trial period. A sensitivity analysis will include data for the first three months of patient follow up (to which point all suspected rejections are diagnosed using biopsy). Skin flap rejection events, by severity, will be summarised descriptively. Time to first skin flap rejection, transplanted lung and patient survival will be analysed following the methods used for the primary outcome. Competing risks will be considered if the number of deaths not related to the transplanted organ is sufficiently high to warrant such analyses. Transplant lung function (vital capacity, forced expiratory volume) will be analysed using mixed-effects linear regression models, including treatment, and adjusted for centre as a random effect. Development of de-novo specific antibodies, development of graft versus host disease, and evidence of chronic rejection, will be analysed using a mixed-effects logistic regression model, including treatment, and adjusted for centre as a random effect. Safety events (AEs, SAEs, complications), immunosuppressive levels (and requirements) and immunological markers will be summarised descriptively. HRQoL outcomes will be analysed using mixed-effects linear regression model, including treatment and adjusted centre and patient as random effects. Supplementary analyses of HRQoL will also use the area under the curve statistics.

21.7 Mechanistic outcomes

Mechanistic outcomes will be summarised using descriptive statistics overall and, when collected for both arms, split by treatment groups. Binary and categorical data will be summarised by frequencies and percentages. Continuous data will be summarised by means and standard deviations (SDs), or median and inter-quartile range if data are skewed. Corresponding 95% confidence intervals (CIs) to support simple group comparisons will be presented where possible. Visual representation of outcomes will be considered and, where it will support interpretation, presented.

21.8 Additional analyses

A supplementary analysis, using the approaches for the primary outcome, will be run using a composite outcome of rejection free survival, whereby lung rejection (ISHLT A grade 1 or higher), graft failures (biopsy BANFF grade ≥ 1) and death are counted as events. As participants can have recurrent lung or skin rejection events, an exploratory analysis of the ordered multiple events will be undertaken using the Prentice, Williams and Peterson total time models (44). Visual representation of recurring events will also be presented.

21.9 Inclusion in analysis

The principle of intention-to treat (ITT), as far as practically possible will be the main strategy for the primary outcome and all secondary outcomes (except for Secondary Outcome 2d). ITT will analyse all randomised and transplanted patients, in the group to which they were allocated and for whom the outcomes of interest have been observed/measured. To check the robustness of analysis under ITT, a secondary sensitivity per-protocol analysis, which will mirror the ITT population but exclude participates defined with a major protocol deviation, will be conducted. Secondary outcome 2d (safety events) will be analysed according to the safety population, which will include all participants who receive a transplant, where groups are defined according to whether or not they received a skin transplant.

21.10 Subgroup analysis

Heterogeneity of the primary outcome, split by centre, will be explored using a forest plot.

21.11 Interim analyses

The main outcomes will be analysed as stated in the analysis plan and will not be analysed as an interim analysis.

21.12 Stopping Rules

No formal interim analyses with stopping guidelines are planned. An independent Data Safety and Monitoring Committee (DSMC) will review the accumulating data at regular intervals and may recommend pausing or stopping the trial in the event of safety concerns.

21.13 Level of Statistical Significance

All principal analyses will be performed at the 2-sided 5% significance level.

21.14 Procedure for accounting for missing, unused and spurious data

The procedure for handling spurious or missing data will be described in the SAP. The trial will attempt to collect data as completely as possible.

The main analysis will include participants for whom endpoint data are available, with other participants being censored after their last available relevant outcome measure. It is anticipated that there will be minimal participant attrition. The sample size calculation has been adjusted to account for loss to follow-up or patient death.

21.15 Procedures for reporting any deviation(s) from the original statistical analysis plan

Any deviation(s) from the original SAP will be described in the final report.

21.16 Health economics analysis

There are no health economic analyses to be undertaken as part of the trial.

21.17 Internal pilot/Decision Points

An internal pilot phase to assess the feasibility of recruitment will be conducted. This timepoint was chosen to ensure that five centres are open to recruitment and 20 participants are randomised. Recruitment is expected to last for 36 months, however there will be a formal review after an internal pilot phase of 9 months of recruitment. Stop-go criteria for this pilot phase are given in table 6 together with the progression guidance.

Table 6: Stop-go criteria for internal pilot phase

Progression guidance	Participants randomised
Continue with study – no action required	>20 participants
Continue with study – action required: • Review recruitment strategies • Report to TSC	13-20 participants
Stop	<13 participants

The Trial Management Group (TMG) will closely monitor the progression criteria during the internal pilot, and together with the Trial Steering Committee (TSC) and Data and Safety Monitoring Committee (DSMC) will perform a full review towards the end of the internal pilot. The TSC and funder will make the final decision to terminate the study.

The internal pilot will mirror the procedures and logistics undertaken in the main definitive study. Data from the internal pilot will contribute to the final analysis.
Should a decision be made to stop the study, all randomised participants who go on to receive a lung transplant will be followed up per protocol. It is intended that the study will progress seamlessly from the internal pilot phase to the main recruitment phase.

22 DATA MANAGEMENT

The data management aspects of the study are summarised here with details fully described in the Data Management Plan. See section on patient confidentiality for information on management of personal data.

22.1 Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. For this study, source data will include the following:

- Hospital medical records (from which data will be summarised into the CRF)
- Biopsy reports
- Patient-reported outcome measures that are submitted directly to the study office

22.2 Location of source data

The location of source data in the study is listed with the tables within the section OBJECTIVES AND OUTCOME MEASURES.

22.3 Case report forms (CRFs)

The Investigator and study site staff will ensure that data collected on each participant is recorded in the CRF as accurately and completely as possible. Details of all protocol evaluations and investigations must be recorded in the participant's medical record for extraction onto the CRF.
All appropriate laboratory data, summary reports and Investigator observations will be transcribed into the CRFs from the relevant source data held in the site medical record(s).

All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code, not by name.

22.4 Non-CRF data

All trial data will be recorded on the CRF. No additional data will be held outside of the CRF.

Translational study data from analysis of samples will be kept on the secure server identifiable only by study ID, and accessible only by appropriate members of the research team.

22.5 Access to Data

To ensure compliance with regulations, direct access will be granted to authorised representatives from the Sponsor and host institution to permit trial-related monitoring, audits and inspections. The data submitted by study participants directly via the study database (i.e. electronic patient reported outcomes) will also be made available to the participating site.

22.6 Data Recording and Record Keeping

The case report forms will be designed by members of the trial management team which will include the Chief Investigator, study statisticians and study trial manager.

Data will, wherever possible, be collected in electronic format with direct entry onto the study database by site staff or participants. Electronic data collection has the major advantage of building "data logic" into forms, minimising missing data, data input errors and ensuring the completeness of consent and assent forms. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

All data entered will be encrypted in transit between the client and server. All electronic patient-identifiable information, including electronic consent forms, will be held on a server located in an access-controlled server room at the University of Oxford. The data will be entered into a GCP compliant data collection system and stored in a database on the secure server, accessible only to members of the research team based on their role within the

study. The database and server are backed up to a secure location on a regular basis. Details of the data collected, where it is stored and who has access to it along with a fair processing statement will be available for the public to see on the study website. Paper forms with patient identifiable information will be held in secure, locked filing cabinets within a restricted area. The identifiable data will be kept separately from the outcome data obtained from/about the patients (both paper and electronic). Patients will be identified by a study ID only.

Direct access to source data/documents will be required for study-related monitoring and/or audit by the Sponsor, NHS Trust or regulatory authorities as required.

Contact details will be retained until the website link publishing the results of the study can be sent out, or for longer where consent is in place for future research. The data management and sharing plan will list explicitly when sensitive personal information will be destroyed.

Data captured during phone calls to participants or from paper-based study questionnaires returned to the SENTINEL study office will be entered into the study database by suitably trained central office staff. Full details will be recorded in the Data Management Plan. The participants will be identified by a unique study specific number in any data extract. Identifiable data will only be accessible by members of the study team with a demonstrated need (managed via access controls within the application) and only used to communicate with the participant (e.g. for study sending follow-up reminders for online form completion or telephone follow-up).

23 QUALITY ASSURANCE PROCEDURES

A rigorous programme of quality control will be implemented. The study management group will be responsible for ensuring adherence to the study protocols at the study sites. Quality assurance (QA) checks will be undertaken by OCTRU to ensure integrity of randomisation, study entry procedures and data collection. The OCTRU has a QA team who will monitor this study by conducting audits (at least once in the lifetime of the study, more if deemed necessary) of the Trial Master File and compliance with requirements in OCTRU SOPs. The study will undergo a formal check of the documentation as part of OCTRU giving the Green Light to open the study. Furthermore, the processes of obtaining consent, randomisation, registration, provision of information and provision of treatment will be monitored by the trials unit staff. Written reports will be produced for any oversight committees as applicable, informing them if any corrective action is required. Additionally, the study may be monitored, or audited by sponsor or host sites in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. A study-specific data management and monitoring plan will be in place prior to the start of the study.

23.1 Audit and regulatory inspection

All aspects of the trial conduct may be subject to internal or external quality assurance audit to ensure compliance with the protocol, GCP requirements and other applicable regulation or standards. It may also be subject to a regulatory inspection. Such audits or inspections may occur at any time during or after the completion of the study. Investigators and their

host Institution(s) should understand that it is necessary to allow auditors/inspectors direct access to all relevant documents, study facilities and to allocate their time and the time of their staff to facilitate the audit or inspection visit. Anyone receiving notification of a Regulatory Inspection that will (or is likely to) involve this trial must inform the Trial Office without delay.

23.2 Risk Assessment

This protocol is designed to deliver a risk-adapted approach to conducting the research. A risk assessment has been conducted and a monitoring plan will be prepared before the trial opens. The known and potential risks and benefits to participants have been assessed in comparison to those of standard of care. A risk management strategy is in place and will be reviewed and updated as necessary throughout the trial or in response to outcomes from monitoring activities. Monitoring plans will be amended as appropriate.

23.3 Trial monitoring

Monitoring will be performed according to a study-specific monitoring plan. Data will be evaluated for compliance with the protocol, completeness and accuracy. The investigator and institutions involved in the study will permit study-related monitoring and provide direct on-site access to all study records and facilities if required. They will provide adequate time and space for the completion of monitoring activities.

Study sites will be monitored centrally by checking incoming data for compliance with the protocol, consistency, completeness and timing. The case report data will be validated using appropriate set criteria, range and verification checks. The study site must resolve all data queries in a timely manner. All queries relating to key outcome and safety data and any requiring further clarification will be referred back to the study site for resolution.
***Note**: 'in a timely manner' means within no more than 7 working days of the data query and within 28 days of receipt of a data query unless otherwise specified.*
The Trial Office will decide the maximum CRF lag time based on protocol requirements and feasibility. In general, a 2 week turn around is an acceptable default for all non-urgent data.

Study sites will also be monitored remotely and/or by site visit, as necessary, to ensure their proper conduct of the trial. Trial Office staff will be in regular contact with site personnel to check on progress and deal with any queries that they may have. Where on-site monitoring is conducted monitoring reports will be sent to the site in a timely fashion. The Investigator is expected to action any points highlighted through monitoring and must ensure that corrective and preventative measures are put into place as necessary to achieve satisfactory compliance, within 28 days as a minimum, or sooner if the monitoring report requests.

23.4 Study committees

27.4.3 Trial Management Group (TMG)

A Trial Management Group (TMG) will be established for the study and operate in accordance with a study-specific TMG charter. The TMG will manage the trial, including the clinical and practical aspects and will meet approximately monthly to assess progress. Other specialities/ individuals will be invited as required for specific items/issues.

27.4.4 Data and Safety Monitoring Committee (DSMC)

An independent Data & Safety Monitoring Committee (DSMC) will be established for this study made up of independent experts external to the study who will assess the progress, conduct and critical outcomes of the study. The DSMC will adopt a DAMOCLES based charter, which defines its terms of reference and operation in relation to the oversight of the study. The DSMC will meet regularly throughout the study at time-points agreed by the Chair of the Committee and the Chief Investigator. At a minimum this will be on an annual basis. The DSMC will review study progress, accruing interim data and all safety aspects of the study and make recommendations as to whether any changes to the study should be undertaken, including stopping early for safety reasons. Full details of responsibilities are included in the DSMC Charter. Recommendations of the DSMC will be discussed between the CI, TSC, and the Sponsor.

27.4.5 Trial Steering Committee (TSC)

The TSC, which includes independent members, provides overall supervision of the study on behalf of the funder. The TSC will act in accordance with a TSC charter which will outline its roles and responsibilities. Full details including names will be included in the TSC charter. Meetings of the TSC will take place at least once a year during the recruitment period. An outline of the remit of the TSC is to:

- monitor and supervise the progress of the trial towards its interim and overall objectives
- review at regular intervals relevant information from other sources
- consider the recommendations of the DSMC
- inform the funding body on the progress of the study

The TSC will consider, and act, as appropriate, upon the recommendations of the DMC.

24 IDENTIFICATION AND MANAGEMENT OF PARTICIPATING SITES

24.1 Identification of recruitment sites

The five sites were selected as they are the only sites that conduct lung transplants in England.

24.2 Study site responsibilities

The Principal Investigator (the PI or lead clinician for the study site) has overall responsibility for conduct of the study, but may delegate responsibility where appropriate to suitably experienced and trained members of the study site team. All members of the study site team must complete delegation log provided by the central study team prior to undertaking any study duties. The PI must counter sign and date each entry in a timely manner, authorising staff to take on the delegated responsibilities.

24.3 Study site set up and activation

The Principal Investigator leading the investigational study site is responsible for providing all required core documentation. Mandatory Site Training which is organised by the study office (usually carried out as a telephone conference call or personal visit) must be completed before the site can be activated. The Study Office will check to confirm that the site has all the required study information/documentation and is ready to recruit. The site will then be notified once they are activated on the study database and are able to begin recruiting patients.

24.4 Training

Training in the study processes will be administered at site initiation visits (delivered face to face or online) by the Central Study team.
Further details are provided in STUDY DESIGN AND SETTING.

24.5 Study documentation

The study office will provide an Investigator File to each investigational site containing the documents needed to initiate and conduct the study. The study office must review and approve any local changes made to any study documentation including patient information and consent forms prior to use. Additional documentation generated during the course of the study, including relevant communications must be retained in the site files as necessary to reconstruct the conduct of the study.

25 ARRANGEMENTS FOR SITES OUTSIDE THE UK

It is not anticipated that this study will open in non-UK sites.

26 ETHICAL AND REGULATORY CONSIDERATIONS

26.1 Declaration of Helsinki

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

26.2 Guidelines for Good Clinical Practice

The Investigator will ensure that the study is conducted in accordance with relevant regulations and with Good Clinical Practice.

26.3 Ethical conduct of the study and ethical approvals

The protocol, patient information sheet, informed consent form and any other information that will be presented to potential trial participants (e.g. advertisements or information that supports or supplements the informed consent process) will be reviewed and approved by an appropriately constituted, independent Research Ethics Committee (REC), HRA, NHSBT and host institution.

26.4 NHS Research Governance

Once HRA & HCRW approval is in place for the trial, sites will confirm capability and capacity to participate in the study.

26.5 NHSBT Governance

The protocol and study have been approved by the NHSBT Research Innovation and Novel Technologies Advisory Group (RINTAG).

26.6 Protocol amendments

All amendments will be generated and managed according to the study office standard operating procedures to ensure compliance with applicable regulation and other requirements. Written confirmation of all applicable REC, regulatory and local approvals must be in place prior to implementation by Investigators. The only exceptions are for changes necessary to eliminate an immediate hazard to study patients (see below).

It is the Investigator's responsibility to update patients (or their authorised representatives, if applicable) whenever new information (in nature or severity) becomes available that might affect the patient's willingness to continue in the trial. The Investigator must ensure this is documented in the patient's medical notes and the patient is re-consented if appropriate.

26.7 Protocol Compliance and Deviations

Protocol compliance is fundamental to GCP. Prospective, planned deviations or waivers to the protocol are not allowed. Changes to the approved protocol need prior approval unless for urgent safety reasons.

A study related deviation is a departure from the ethically approved trial protocol or other trial document or process (e.g. consent process or IMP administration) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Deviations from the protocol will be captured within the study database either using a protocol deviation form or via suitably designed fields within the CRF which will be extracted from the trial database and reviewed regularly by the Trial Management Group (TMG). Deviations will be handled and reviewed in a timely manner in accordance with a study-specific Data Management and Monitoring Plan.

The investigator must promptly report any important deviation from Good Clinical Practice or protocol to the study office. Examples of important deviations are those that might impact on patient safety, primary/ secondary endpoint data integrity, or be a possible serious breach of GCP .

26.8 Urgent safety measures

The sponsor or Investigator may take appropriate urgent safety measures to protect study participants from any immediate hazard to their health or safety. Urgent safety measures may be taken without prior authorisation. The study may continue with the urgent safety measures in place. **The Investigator must inform the study office IMMEDIATELY if the study site initiates an urgent safety measure:**

The notification must include:

- Date of the urgent safety measure;
- Who took the decision; and
- Why the action was taken.

The Investigator will provide any other information that may be required to enable the study office to report and manage the urgent safety measure in accordance with the current regulatory and ethical requirements for expedited reporting and close out. The study office will follow written procedures to implement the changes accordingly.

26.9 Temporary halt

The sponsor and Investigators reserve the right to place recruitment to this protocol on hold for short periods for administrative reasons **or** to declare a temporary halt. A temporary halt is defined as a formal decision to:

- interrupt the treatment of participants already in the trial for safety reasons;
- stop recruitment on safety grounds; or

- stop recruitment for any other reason(s) considered to meet the substantial amendment criteria, including possible impact on the feasibility of completing the trial in a timely manner.

The study office will report the temporary halt via an expedited substantial amendment procedure. The study may not restart after a temporary halt until a further substantial amendment to re-open is in place. If it is decided not to restart the study this will be reported as an early termination.

26.10 Serious Breaches

A "serious breach" is a breach of the protocol or of the conditions or principles of Good Clinical Practice which is likely to affect to a significant degree –
(a) the safety or physical or mental integrity of the trial subjects; or
(b) the scientific value of the research.
In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the C.I., the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the approving REC committee and the relevant NHS host organisation.

26.11 Trial Reports

This protocol will comply with all current applicable Research Ethics Committee and Sponsor reporting requirements.

26.12 Transparency in Research

Prior to the recruitment of the first participant, the study will have been registered on a publicly accessible database (ISRCTN), which will be kept up to date during the study, and results will be uploaded to the registry within 12 months of the end of the study declaration. A Final Report will be submitted to the REC containing a lay summary of the study results which will be published on the HRA website.

The results of the study will be published and disseminated in accordance with the PUBLICATION AND DISSEMINATION section.

26.13 Participant Confidentiality

The study will comply with UK General Data Protection Regulation (UK GDPR) and Data Protection Act 2018, which will require data to be de-identified as soon as it is practical to do so. Personal data on all documents will be regarded as confidential. The processing of the personal data of participants will be minimised by making use of a unique participant study number on study documents and any electronic databases.

Study questionnaires sent via the post to participants will be labelled with the participant's study ID number and initials.

All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participant's personal data. The study staff will safeguard the privacy of participants' personal data. See section 22 DATA MANAGEMENT for more details.

The following personal identifiable data will be collected in the study:

- Participant contact details (e-mail address, postal address and telephone number). These will be used for the purpose of sending study questionnaires and contacting

participants regarding study follow-up and for sending a summary of the study results

NHS/CHI number for the purpose of this study. Study questionnaires sent directly to study participants will either be sent via post or e-mail with a unique URL link to the participant's study questionnaire; this will be unique to the participant's record, visit and case report form.

Site staff at participating sites will ensure that contact details for study participants are up to date when participants attend for study visits.

. The Investigator site must maintain the patient's confidentiality in all communications and reports related to the research. The Investigator site team must keep a separate log of enrolled patients' personal identification details as necessary to enable them to be tracked. These documents must be retained securely, in strict confidence. They form part of the Investigator Site File and are not to be released externally. Data Breaches will be highlighted to the relevant site staff and reported as required by the GDPR and Data Protection Act 2018. This will also be deemed a protocol deviation.

26.14 End of study

The end of study is the point at which all the clinical data has been entered and queries resolved in the study REDCap database, and all laboratory outcomes have been analysed and any queries also resolved.

The Sponsor and the Chief Investigator reserve the right to terminate the study earlier at any time. In terminating the study, they must ensure that adequate consideration is given to the protection of the participants' best interests.

27 PUBLIC AND PATIENT INVOLVEMENT

27.1 Impact of PPI on study design and protocol development

Consultation with transplant charities and patient groups, as well as national members of the Oxford Transplant Foundation Patient and Public Involvement Group and the NHSBT Cardiothoracic Patient Advisory Group has taken place prior to, and during the planning and designing of the study. Patients will continue to be involved throughout the running of the study. Feedback from the lay members of the ethic committee was also incorporated. The findings will be presented at national and international multi-disciplinary transplant and surgery meetings and will be published in suitable high-impact scientific journals. We anticipate that results of the study may have significant practice-changing impact for patients undergoing a solid organ transplant.

Participants taking part in the study will be informed of the findings via the study website.

The trial has strong support from NHSBT, the Oxford Transplant Foundation Patient and Public Involvement Group and the NHSBT Cardiothoracic Patient Advisory Group. Extensive PPI input has been sought regarding the effect of viewing donated skin and the location of the skin flap throughout the full development of the study. Although the majority of patients have indicated that they would accept a skin flap located on the under surface of the forearm, some have indicated that they would prefer that it is placed somewhere more discrete (such as in the groin crease, axilla, or upper arm). In our intestinal transplant studies, some patients preferred their skin flap to be sited on their abdomen and

others preferred the forearm. Patients will be asked before surgery their preference for where the skin flap will be located. The skin flap does, however, need to be located somewhere that is accessible for the patient to check for a skin rash. To date, only 2 of 64 patients have requested removal of their SSF after the study period.

28 EXPENSES/PAYMENTS TO PARTICIPANTS

There is no study funding to reimburse patient expenses incurred for attending additional research visits in excess of standard of care.

29 SPONSORSHIP, FINANCE AND INSURANCE

29.1 Sponsorship

The Sponsor has provided written confirmation of Sponsorship.

29.2 Funding and support in kind

Funder(s)	Financial and non-financial support given
National Institute for Health Research - Efficacy and Mechanism Evaluation Programme (EME)	Reference Number: NIHR130899

29.3 Insurance

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). NHS indemnity operates in respect of the clinical treatment that is provided.

30 CONTRACTUAL ARRANGEMENTS

Appropriate contractual arrangements will be put in place with all third parties.

This study is subject to the Sponsor's policy requiring that written contracts/agreements are agreed formally by the participating bodies as appropriate.

The Sponsor will also set up written agreements with any other external third parties involved in the conduct of the study as appropriate.

31 PUBLICATION AND DISSEMINATION

The Sponsor will retain ownership of all data arising from the trial.

Publication and dissemination of trial results and associated trial publications (e.g. the trial protocol, statistical analysis plan (SAP) and any secondary analyses) will be in accordance with the OCTRU Standard Operating Procedure and irrespective of study findings.

The study protocol will be published in an open-access peer-reviewed journal in accordance with the Standard Protocol Items: Recommendations for Interventional Trials statement (SPIRIT, www.spirit-statement.org/). The study results will be published in an open-access journal, in accordance with the NIHR's policy on open-access research. The study will be reported following the Consolidated Standards of Reporting Trials guideline (CONSORT)

including any applicable extensions to this. The Template for Intervention Description and Replication (TIDieR) statement will be used for reporting the intervention.

31.1 Study results

All data will be presented such that no individual participants can be identified. Dissemination of results will include the following methods:

Conference

The results of this study will be disseminated to the clinical community via presentations at national and international meetings. Traditional conference dissemination will focus on presentations to include the key professional stakeholders. It is expected that findings from this study will be presented at national (British Transplantation Society, British Association of Plastic, Reconstructive and Aesthetic Surgeons) and international (European Society for Organ Transplantation, American Transplant Congress) conferences.

Publications

Results will be published in peer-reviewed journals. Where possible, plain English summaries will be published alongside the full paper, along with links to other digital media on the trial website to explain the trial result in an accessible format – i.e. an explainer video and infographic.

Public Dissemination

To ensure a broad campaign we will target a range of social media outlets (this may include an explainer video and infographic). We will seek to engage the NHS Dissemination centre and seek to publish 'digital story' as part of the 'NIHR Signal'.

All participants will be asked at the time of recruitment if they would like to receive a copy of the study results. This document will be written collaboratively with clinicians and patient representatives and distributed accordingly. Newsletters, Facebook, Twitter etc. will be used to ensure the results of SENTINEL are communicated to the wider community once they are available.

The wider public will be alerted via links with relevant organisations/charities, and the Research Media Offices. Engagement with the NIHR Dissemination Centre will also be sought, to ensure global awareness of study findings. Moreover, the University of Oxford and Oxford University Hospitals NHS Trust have professional communication officers. It is anticipated that together these individuals, NHSBT and NIHR equivalents will agree upon effective communication strategies including co-ordinated press releases, interviews etc.

32 AUTHORSHIP

Authorship of any publications arising from the study will be determined in accordance with the ICMJE guidelines and any contributors acknowledged accordingly.

All publications arising from this study must acknowledge the funder, OCTRU, SITU, NHSBT and the Sponsor.

33 DEVELOPMENT OF A NEW PRODUCT/PROCESS OR THE GENERATION OF INTELLECTIAL PROPERTY (IP)

Ownership of IP generated by employees of the University vests in the University. The University will ensure appropriate arrangements are in place as regards any new IP arising from the trial.

34 ARCHIVING

During the study and after study closure the Investigator must maintain adequate and accurate records to enable the conduct of a clinical trial and the quality of the research data to be evaluated and verified. All essential documents must be stored in such a way that ensures that they are readily available, upon request for the minimum period required by national legislation or for longer if needed.

Retention and storage of laboratory records for clinical study samples must also follow these guidelines.

It is the University of Oxford's policy to store data for a minimum of 5 years following publication. Investigators may not archive or destroy study essential documents or samples without written instruction from the study office.

Study data and associated metadata will be retained electronically in a suitable format in a secure server area maintained and backed up to the required standard. Access will be restricted to the responsible Archivist and will be controlled by a formal access request. On completion of the mandatory archiving period the TMF and associated archived data sets will be destroyed or transferred as appropriate, according to any data sharing requirements.

34.1 Sponsor Trial Master File

All paper and electronic data including the Trial Master File and trial database will be archived in accordance with the OCTRU standard operating procedures and retained for at least 3 years after completion of the study.

34.2 Investigator Site File and participant medical records.

Archiving and eventual destruction of the Investigator Site File (ISF) is the responsibility of the Principal Investigate/site. The medical files of study participants must be retained for at least 3 years and in accordance with the maximum period of time permitted by the participating site. As part of the close-out procedure for each participating site, the Study Office will notify each participating site when the ISF may be destroyed. No documents will be destroyed prior to this.

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36 VERSION HISTORY

Previous versions of this protocol and a summary of the changes made are provided in the table below:

Protocol version no.	Protocol date	Summary of key changes from previous version
1.0	17April 2023	1st version of the protocol.
2.0	15Nov2022	(1) Removing reference to RRAMP (We will use REDCap to randomise participants (protocol pages 40 & 42), editing text to make it clear that only randomised and transplanted patients are participants for the analysis. (2) Changing Trial Steering Committee (TSC) Chair to Prof Joerg-Matthias Pollock, Lead Statistician to Dr Daphne Kounali, Co-Investigator Dr Jessica Scaife (also added to IRAS Q66). Protocol contributors Daphne Kounali and Begum Zeybek (pages 8-9) (3) Corrected the error field in funder details (protocol page3), added full address for Surgical Intervention Trial Unit. (4)

APPENDIX 1 – PATHWAY FOR ORGAN DONATION AND RETRIEVAL