Research Protocol

Research project title (full):

A randomised double-blind placebo-controlled trial of topical sirolimus in chemoprevention of facial squamous cell carcinomas

Research project title (short): SiroSkin

Study investigators

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1. INTRODUCTION

Keratinising carcinomas are extremely common in the general population, especially in Australia. Solid organ transplant recipients (SOTRs) develop over 60-fold more skin cancers than the general population, therefore keratinocyte carcinomas are a major burden affecting mortality and morbidity in SOTRs.[1,2] Skin cancers also have a propensity to recur in the same location on the body after an individual's first skin cancer.[3,4] The gold standard of treatment is surgery and completely excised lesions enable complete remission and a cure in the majority of cases. Despite this, there is no effective way of preventing new cancers from developing in the same area, although sun protection can reduce the burden. Therefore, the high numbers of skin cancers result in SOTRs requiring recurrent skin checks and frequent skin cancer surgery.

Oral sirolimus is a selective immunosuppressant agent which has proven to reduce the burden of skin cancer however it is poorly tolerated due to side effects.[5,6,7] Topical sirolimus has proven effective in reducing the skin cancer burden in animal models and is safe on the face of patients with tuberous sclerosis.[8] A 12-week phase II clinical trial recently conducted by the research team suggested topical sirolimus to be safe and effective, as it reduced the early signs of skin cancer without any major side effects.

In this phase III randomized, double-blind, placebo-controlled study, we propose using 1% topical sirolimus to the face on a regular basis for 6 months. We plan to determine if this topical cream can fill a major gap in our current therapies by reducing the onset of new skin cancers and therefore reduce the burden of disease in terms of number of biopsies and surgeries, and potential hospitalisations and death.

2. BACKGROUND

Keratinocyte cancers, a major health burden in organ transplant recipients

Immunosuppressive therapies are important in SOTRs and recent advances in therapies have significantly improved short-term outcomes. However, this immunosuppression also results in a major increase in the risk of keratinocyte cancer (KC), specifically squamous cell carcinomas (SCC) and basal cell carcinomas (BCC). SOTRs have one to two orders of magnitude higher risk and incidence ratio for developing SCC and BCC.[9,10] This translates in SOTR incidence rates for SCC of 379/1000 patient-year for heart transplant recipients and prevalence of 11% for SCC at any given time for kidney transplant recipients.[11,12] Similar observations are seen with early skin cancers such as intra-epidermal carcinoma and actinic keratosis. There is also a slight increase in melanoma and Merkel cell carcinoma rates. In addition to higher rates occurring in SOTRs, the BCCs and SCCs have a more aggressive course. Aggressive cancers occur in 2% of heart and lung transplant recipients over 2 years, which is a 66 to 83-fold higher standardized mortality ratio regarding KCs compared to the general population.[1,2] Overall, keratinocyte carcinomas are indisputably a major burden in terms of morbidity, mortality and cost in solid organ transplant recipients.

Current therapy of keratinocyte cancers does not address the disease burden

Currently the most common treatment for KC is surgical excision with adequate margin. However, this treatment option results in significant disease burden for SOTRs, especially as it is well established there is an increased risk of additional skin cancers in a field of pre-cancerisation.[3,4] Multidisciplinary clinics have been established in recent years to enable

diagnosis and immediate treatment of skin cancers in SOTRs. One study reviewed 101 patients attending such a clinic and recorded over 300 excisions of suspected lesions over 3 months of follow up.[13] Overall, although surgery for individual KCs resulted in complete remission of individual lesions, it did not prevent the onset of additional cancers in the same field of exposure.

Aside from UV-related primary prevention such as sunscreen and barrier clothing, additional preventive or adjuvant measures have been suboptimal in reducing the onset of new KCs. Field therapy - usually photodynamic therapy or topical fluorouracil - of photodamaged skin or actinic keratosis is often recommended for SOTRs. Photodynamic therapy has shown only a clear preventative role in SCC development if performed in unrealistic 4-weekly cycles.[14,15,16] Topical fluorouracil (Efudix) has shown short term benefit with a 75% reduction in SCCs in immunocompetent patients at 1 year.[17] However, this effectiveness is not found at 2-5 years subsequently. In addition, topical fluorouracil appears not to prevent the formation of BCCs.[17,18] There is some deliberation as to whether imiquimod or other immunotherapies should be used in transplant patients. Regardless, their effectiveness is mainly for actinic keratosis and squamous cell carcinoma rather than basal cell carcinoma.

Oral chemopreventive regimens have been trialled specifically in SOTRs however significant side effects have prevented widespread use. Retinoid (acitretin) therapy is an additional adjuvant which prevents occurrence of new SCCs. Doses of 25 to 30mg per day (0.3mg/kg) reduce SCCs by 13% in treated groups compared to a 28% increase in placebo groups.[19] Real-world application of acitretin is limited in SOTRs due to high rates (52%) of side effects and withdrawal from trials.[20] Similarly, oral fluorouracil (capecitabine) reduces SCC and BCC incidence by three-fold compared to the pre-treatment period.[21,22] However, 70% of patients experienced significant side effects and dose adjustment with interrupted cycles of therapy. More recently, nicotinamide has been proposed as a chemopreventive measure for KC.[23] Although not formally proven effective in SOTRs due to its innocuous nature, it is widely prescribed.

Inhibition of mTOR pathway reduces skin cancer occurrence

Modulation of the immunosuppression was another strategy investigated to prevent skin cancer formation. Mammalian target of rapamycin inhibitors (mTORi), such as sirolimus (rapamycin) or everolimus, are immunosuppressive but do not increase in SCC or BCC incidence.[24] In patients with SCCs, changing calcineurin inhibitors to sirolimus resulted in a nearly two-fold reduction in SCC risk.[6,7,8] Unfortunately, these trials of oral sirolimus had poor tolerance with approximately 50% of patients withdrawing from the trial and 94% in the sirolimus group reporting at least one adverse event. The side effects associated with oral sirolimus are commonly difficult to manage and usually result in withdrawal of the drug. These include acne, albuminuria, mouth ulceration, oedema, rash and pneumonitis. Despite poor tolerance, the benefit oral sirolimus achieved in reducing skin cancer occurrence suggested sirolimus may have specific anticancer properties.

Past studies support the importance of mTOR pathway activation in the keratinocyte proliferative response to UVB irradiation.[25] This activation as part of the AKT pathway is an important driver of epidermal proliferation and is speculated to have a cancer promotion effect on cells carrying oncogenic mutations.[26,27] Preclinical models of UV and chemically induced skin cancer showed that both systemic and topical rapamycin significantly reduced SCC formation and resulted in a significant increase in the time to first SCC. This supported anti-tumour effects of mTOR inhibitors. Importantly, this effect remains in animals

concomitantly receiving cyclosporine.[28] Overall, there is good preclinical and clinical evidence that mTOR pathway inhibition has a role in preventing skin cancer in SOTRs, however widespread use of systemic sirolimus is limited due to side effects.

Topical sirolimus is a safe way to inhibit mTOR pathway in the skin

To circumvent the side effects of systemic sirolimus therapy, topical sirolimus has recently been explored.[8] In addition to multiple case reports and case series, a phase II randomized controlled trial found that topical 0.2% sirolimus was effective in treating tuberous sclerosis-related angiofibromas, a genetic disease with a mutation upstream of the mTOR pathway resulting in its activation. The sirolimus preparation was applied twice daily for 12 weeks to the face to both children and adults. There were no serious adverse events with only mild skin dryness (65%) and mild irritation (50%) reported, compared to placebo. These side effects were manageable with emollients. In addition, systemic sirolimus levels were 0.25ng/mL, considerably lower than the toxic levels of 5-15ng/ml. Similarly, a 1% topical preparation applied daily to the face for up to 9 months in a paediatric population had no safety concerns and remained below 0.2ng/ml in blood concentrations.[29]

Preliminary findings

In 2018-19 we conducted a pilot randomized, double blind, placebo controlled, phase I-II, feasibility and safety study of the use of topical sirolimus in chemoprevention of skin cancer (ACTRN12618001961235). From 280 patients screened, 30 patients enrolled in the study and consented to receive topical sirolimus 1% in a gel versus the placebo gel, randomly assigned to the right or left arm for 12 weeks. The primary outcome measures were safety and feasibility indicated by recruitment and retention rates, and acceptability of the intervention. Treatment effectiveness was the secondary outcome, assessed by change in number of actinic keratoses after 12 weeks of active vs placebo intervention and the development of KC at 12 and 24 months.

Of the 280 patients screened, less than half were eligible due to concurrent sirolimus or everolimus therapy orally (7%), or recent field therapy with topical fluorouracil (57%), surgery required on field (5%), or did not meet the criteria of at least 5 actinic keratoses or 5 keratinocyte cancers (10%). Among those eligible, about half did not agree to participate and overall, 11% (30) of those considered consented to take part in the study. The predetermined number of 30 ended the recruitment period for the trial. Overall, the use of topical fluorouracil was the most common reason for ineligibility. 18 patients completed the treatment regimen and could be evaluated at 12 weeks. One patient was further excluded because of the initiation of oral everolimus during the trial. All 30 patients continued to be followed three monthly for skin checks and therefore 29 could be evaluated for skin cancer development.

Of the 30 participants, only 1 participant experienced an adverse event secondary to the trial intervention. This participant experienced irritant contact dermatitis on the forearm where topical sirolimus was applied. However, in total 5 participants developed serious adverse events that were mostly related to hospital admission for other causes (sepsis n=2, back pain, coronary disease, vitreous detachment). Of importance, many of the patients with adverse events (including 2/5 with SAE) continued therapy for up to 12 weeks. In the majority of cases, patients who did not complete the trial chose to stop the intervention for personal reasons, forgot the intervention or did not like applying the gel. In addition, 4 of the 29 patients had detectable serum sirolimus levels 2 weeks after initiating treatment. These levels were below 2.3ng/mL and never reached therapeutic or toxic levels. A repeat blood test at 12 weeks was always negative for these patients.

The number of keratotic lesions was reduced by 31+/-5% (in average from 9.7+/-1.1 to 7.3+/-1.0 keratotic lesions) in the treated group whereas it increased marginally in the control group (in average from 7.9+/-1.0 to 7.9+/-1.0) (p=0.0006). This translated into 8/18 in the sirolimus treated areas versus 1/18 in the vehicle treated area achieving a 30% or more reduction in keratotic lesions (p=0.0178). We then followed patients for an average of 25.5 months after initiating therapy in the trial. During this period patients developed over 150 KCs that required surgical excision and were verified by pathology. Of these, 32 histologically confirmed cancers occurred on the intervention areas and included 1 BCC, 15 SCCs and 16 IECs. When comparing the sirolimus treated and untreated sides for all 29 randomised patients, there was a significant decrease in IECs at 24 months (4 in treated areas versus 12 in vehicle areas, p=0.05). No difference was observed for IECs at 12 months.

Many of the SCCs and IECs were observed either during the 12-week intervention or in the immediate time following the intervention. Therefore, we speculated topical sirolimus is a preventive agent and rather than a direct anti-cancer therapy, sirolimus had no opportunity to prevent these early cancers. We therefore analysed the number of IECs and SCCs occurring after the 3 months following the intervention. If these early cancers are discounted, 9 SCCs and 7 IECs occurred in the 24-month follow-up period. No IECs were recorded on the treated side, whereas 2 and 7 IECs developed on the vehicle side at 12 and 24 months respectively (p=0.002). Similarly, time to IEC development was significantly different between the treated and untreated side (p=0.0025). Regarding SCCs, a strong trend towards increased SCCs remained on the sirolimus treated side, however this trend reversed when considering SCCs developing in the second year of follow-up. Importantly, in the second year, no SCCs or IECs were recorded on the treated side, as opposed to 3 SCCs (p=0.18) and 6 IECs (p=0.007) on the vehicle side.

Limitations of this study were related to use of the right versus left forearms randomly to compare treatment effectiveness. Unfortunately, in Australia, the right arm is much more likely to develop skin cancer and more right forearms received the intervention. Hence in this new study we plan to use the face as the main site of investigation. This is also because most cancers resulting ultimately in patient death emanate from the head (scalp/face). Moreover, given the limited efficacy in this pilot trial on SCC chemoprevention, we propose to increase the duration of therapy to 24 weeks. Indeed, many studies have used topical sirolimus for up to 12 months without side effects.

Overall, this early trial despite significant drop-out rates demonstrated the safety of topical sirolimus and offers a positive viewpoint on its efficacy long term. The benefit observed in intraepidermal carcinomas, an earlier form of SCC, combined with the speculation of topical sirolimus being a chemoprevention agent is encouraging the pursuit of a larger and longer-term study. In this proposed study, we improved the design to increase recruitment eligibility and increase participant retention to the completion of the trial. This includes stratifying participants to include topical fluorouracil use within the last 12 months and altering the topical formulation from a gel to a cream to make it more tolerable to patients.

3. AIM(S) OF RESEARCH

This randomised control trial (RCT) will investigate the routine use of topical 1% sirolimus by SOTRs in reducing the occurrence and number of facial keratinocyte carcinomas. The research team has a specific interest in the number of invasive SCCs at the completion of 6 months of application, and then subsequently at 6, 12 and 24 months post completing application. We also aim to investigate if this regime is safe, feasible and tolerated by SOTRs. In addition, the cost-effectiveness from a health economics perspective will be assessed.

4. OBJECTIVES

5a. Primary Objectives

To determine if 1% sirolimus applied topically to the face on a daily basis for six months is effective at reducing the occurrence and number of invasive SCC in the treated area at 6, 12 and 24 months.

5b. Secondary Objectives

1. To determine if 1% sirolimus applied topically to the face on a daily basis for 6 months is effective at reducing the occurrence and number of intraepidermal carcinomas (IECs), BCCs and subtypes of SCCs or BCCs at 6, 12 and 24 months.

2. To determine if 1% sirolimus applied topically to the face on a daily basis for 6 months can reduce the number of actinic keratoses at 6 months compared to baseline.

3. To determine if 1% sirolimus applied topically to the face for a period of 6 months is feasible and safe in patients.

4. To evaluate the participants' experience by monitoring side effects, issuing participant surveys, determining the number of patients who completed the 24-week application course and the number of doses applied during this time.

5. To determine the cost-effectiveness of using topical sirolimus therapy in comparison to the current standard of care, being surgical intervention, in the management of KCs.

5. HYPOTHESIS

5a. Primary Hypothesis

In this study, we hypothesize that applying topical 1% sirolimus daily to the face for 6 months is safe and effective in reducing the occurrence and number of invasive SCCs at the completion of the study and at 6,12 and 24 months post intervention. We propose to conduct a phase 3 randomized, double-blind, placebo-controlled study to evaluate the use of topical sirolimus in this situation.

5b. Secondary Hypotheses

1. We hypothesize topical 1% sirolimus applied on a regular basis to the face for 6 months will reduce the occurrence and number of intraepidermal carcinomas (IECs), BCCs and subtypes of SCCs or BCCs at each of the aforementioned time-points.

2. We hypothesize topical 1% sirolimus applied on a regular basis to the face for 6 months will reduce the number of actinic keratoses of each patient at the completion of 6 months topical therapy compared to initiation day on photographic images and counts.

3. We hypothesize topical 1% sirolimus applied on a regular basis to the face for 6 months is feasible and safe.

4. We hypothesize that compared to the current standard of care, applying topical 1% sirolimus on a regular basis to the face for 6 months will be more cost-effective.

6. RESEARCH DESIGN

We propose to conduct a phase 3 randomized, double-blind, placebo-controlled trial to evaluate the use of topical 1% sirolimus versus placebo applied every night for 6 months in solid organ transplant recipients.

The Phase I-II earlier trial assessing topical sirolimus on the arms supported the efficacy of topical sirolimus. However, although the previous trial was tolerated on the arms, it is important to assess the acceptability by participants of applying a cream every night to the face. Therefore, an initial 3-month pilot study has been incorporated to be conducted with the first 20 participants recruited to ensure the acceptability of applying topical sirolimus every night to the face. This research design will be the same process and format as the phase 3 trial described below. However, an additional safety and feasibility questionnaire (see Appendix 1) will be conducted at the completion of three months of applying the topical cream to assess the acceptability and safety of applying topical sirolimus. The phase 3 randomized, double-blind, placebo-controlled trial will be continuous after the first 20 patients have been recruited, and the continuation of the trial will be evaluated at the completion of the pilot study. The pilot study participants will also continue their treatment to complete the full 6-month topical application to the face and be included in the evaluation of the phase 3 trial.

Solid organ transplant recipients will be recruited at the Transplant Skin Clinics at both the Princess Alexandra Hospital and The Prince Charles Hospital during the patient's routine clinic appointment. At this appointment, the patient will be reviewed and assessed by either a dermatologist, dermatology registrar or dermatology senior house officer (SHO) who will

contact research staff. The patient will be provided with a Patient Information Sheet and Consent Form (PICF) if eligible and interested. The potential participant will take the PICF home to read and consider involvement. They will be encouraged to contact the study team with any questions. They will be able to inform the medical staff of their decision on the day or over the phone in the following week. Written consent will be obtained from the participant by the research personnel. After consent has been obtained, baseline photos of the participant face will be taken and the EuroQol 5-dimensional (EQ-5D-5L) and Basal and Squamous Cell Carcinoma QoL (BaSQoL) will be completed.[30,31] This questionnaire will be utilised to evaluate the cost-effectiveness of topical sirolimus compared to the current standard of care.

Participants will be randomised into an intervention and placebo group. The intervention topical cream consists of sirolimus 1% in a vehicle while the placebo topical cream consists of the vehicle only. Both topical creams will be prepared by a compounding pharmacy and will be dispensed to both PAH and TPCH pharmacies to provide 2 consecutive 3-month supplies of topical cream to each participant. The second 3-month supply will be collected at the participant's 3-month review. The randomization will be performed by the research team but the participant allocation will be undertaken by each pharmacy. Packaging of the creams will be identical and will not reveal ingredients.

The topical cream received by participants will be applied once every night to the entire face. A recording sheet will be used to document each successful application and any side effects experienced. Participants will also be contacted monthly either via email, phone call or text message to gauge their use over the past month, to remind participants to apply the cream and ask about any possible side effects. They will also have a 3-month and 6-month in-person review. These 3-monthly reviews may be an extra appointment or may coincide with the routine 3-monthly appointment in the Transplant Skin Clinic. It is endeavoured to align the research reviews on the same day as the participants regular 3-monthly review, however additional reviews may be required to be scheduled. During this review the participants will also complete a further 5Q-5D-5L and BaSQoL questionnaire.

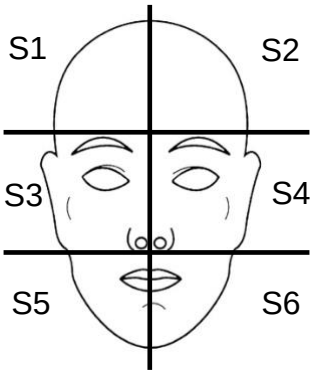

Figure 1: Face sextants

If a participant is required to have a biopsy, skin excision or spot treatment of a lesion on the face during the trial, interruptions of cream application will be allowed. The face will be divided into 6 sextants (see Figure 1) and the cream application will be paused in the corresponding sextant(s) to enable successful treatment and healing. Once healed, sirolimus application to the entire face will resume. A break from application to the whole face will not impact the duration of treatment, and sextant(s) not receiving topical application will be documented on the participant's nightly application recording sheet (as seen in Appendix 2).

For all biopsy or skin excisions undertaken during the 6 months of topical cream application, an extra pathology form will be included to request extra tissue blocks or sections to be prepared by the pathologist. This extra tissue block will be prepared after the pathologist has made the diagnosis. The extra tissue block or sections will be transferred to the University of Queensland PC2 certified facility located at the Translational Research Institute (TRI) in Woolloongabba, Brisbane QLD.

At the completion of six months of topical cream application, photos of the participant's face will be taken to compare the numbers of AKs at the commencement and completion of the topical sirolimus. Participants will complete their third EQ-5D-5L and BaSQoL questionnaires. In addition, a blood test will be taken by either the PAH, TPCH or an external laboratory to check systemic sirolimus levels. All attempts will be made to coordinate this blood test with the participant's routine blood tests to avoid any additional procedures.

Patients will be followed up at 6, 12 and 24 months post-completion of the intervention. Final EQ-5D-5L and BaSQoL questionnaires will be mailed to patients and responses collected by phone, digitally or by mail. The clinical follow up will be passive for the participant as it will be undertaken by monitoring the routine appointments attended by the SOTRs as part of their post-transplant management.

7. RESEARCH PROJECT SETTING/LOCATION

This will be a multi-centre research trial conducted in the Transplant Skin Clinics at both the Princess Alexandra Hospital and The Prince Charles Hospital.

8. RESEARCH POPULATION

This research trial will be conducted on SOTRs reviewed at the Transplant Skin Clinics at the Princess Alexandra Hospital and The Prince Charles Hospital. Therefore, the population will consist of heart, lung, kidney and renal transplant patients. Patients attending these clinics will be considered if they received their transplant over 12 months ago, have experienced at least 1 SCC/BCC in the past 5 years and have at least 5 keratotic lesions on the face at inclusion. If there are any lesions that require treatment at recruitment or throughout the trial, appropriate treatment will be provided, and participants will withhold applying the trial cream provided in the sextant the lesion is within (see Figure 1). Keratotic lesions include Actinic Keratosis which do not require immediate treatment.

The recruitment target is 146 participants to be randomized to receive either topical 1% sirolimus or placebo with an anticipated 73 people in each arm.

9. ELIGIBILITY CRITERIA

SOTRs reviewed at the Transplant Skin Clinic of the Princess Alexandra Hospital and The Prince Charles Hospital will be considered if they meet the inclusion criteria without having any specified exclusions. In addition, the study will not exclude nicotinamide.

9a. Inclusion criteria

To be eligible, individuals must:

- Be aged 18 years or older
- Have received an organ transplant 12 months ago or earlier
- Have had at least 1 SCC/BCC in the past 5 years
- Have at least 5 keratotic lesions on their face at inclusion

9b. Exclusion criteria

An individual will be excluded from participation if they:

- Are unable to provide informed consent
- Are currently receiving sirolimus or everolimus orally
- Have a skin cancer on their face requiring treatment
- Have an open wound on their face requiring treatment
- Are pregnant or planning to become pregnant in the next 6 months
- Are medically unstable
- Have difficulty understanding and signing the PICF document (are non-English speaking or intellectually impaired)

10. RESEARCH OUTCOMES

10a. Primary outcome

The primary outcome measure is the occurrence of invasive SCC on the treated area at the completion of 6 months of topical 1% sirolimus then at 6, 12 and 24 months follow up.

10b. Secondary outcome(s)

1. The number of biopsy-proven invasive SCC at the completion of 6 months of topical 1% sirolimus, then at 6, 12 and 24 months follow up.
2. The occurrence and number of intraepidermal carcinomas (IECs), BCCs and subtypes of SCCs or BCCs at each of the aforementioned time-points.
3. The number of actinic keratoses of each patient at recruitment compared to the end of the study on photographic images and counts[32]
4. The safety and feasibility of applying topical sirolimus every night to the face for 6 months
5. The cost-effectiveness of utilising topical sirolimus therapy on SOTRs
6. The occurrence and type of intervention-related side effects
7. The number of doses applied during the 24 weeks
8. The number of patients completing the 24-week course
9. Participant satisfaction on applying regular topical sirolimus

11. RESEARCH PROCEDURES

11a. Recruitment of patients/participants

A dermatologist, dermatology registrar or dermatology SHO in the Transplant Skin Clinics at the PAH or TPCH will meet a potential participant during the patient's regular scheduled appointment, assess the patient for their eligibility and subsequently ask research personnel to offer a Patient Information Sheet and Consent Form (PICF) if appropriate. If interested, the potential participant will take the PICF home to read and consider involvement. They will be encouraged to phone the study team if they have any questions. Written consent will be obtained from the participant by the research staff.

Approximately 12 and 16 patients are reviewed in the TPCH Transplant Skin Clinic and PAH Transplant Skin Clinic per week respectively. We estimate that half will be interested and eligible to participate. We expect it will take three weeks to recruit the required number of patients for the pilot study and ten months to recruit the required number of participants for the RCT.

11b. Randomisation

This is a randomised double-blinded placebo-controlled trial comparing an intervention being topical 1% sirolimus with a topical vehicle-only placebo. This will be randomised at patient level, with a 1:1 ratio between intervention arms. The trial will be stratified, as seen in Figure 2 below, initially by hospital and then into higher risk (>10 SCCs in the past) and lower risk (<10 SCCs in the past). Further stratification will include whether the patient has or has not used 5-fluorouracil (5-FU/Efudix) in the past 12 months or is on acitretin for the duration of the trial. There will be an equal division of patients receiving intervention and control.

Online randomization software will be used to generate two separate allocation sequences to be distributed to both the PAH and TPCH pharmacies. Although the allocation sequence is undertaken by the research team, the assigning of participants to their respective arm will be performed by the pharmacies. Hence, only these pharmacists will know whether participants are receiving the intervention or control. The pharmacies do not have any input into the assessment of the participants or the data collected.

In order to conceal arm allocation, the topical cream provided by the pharmacy to the patient will not identify ingredients. Only the pharmacy will have access to the randomisation allocation sequence during the trial.

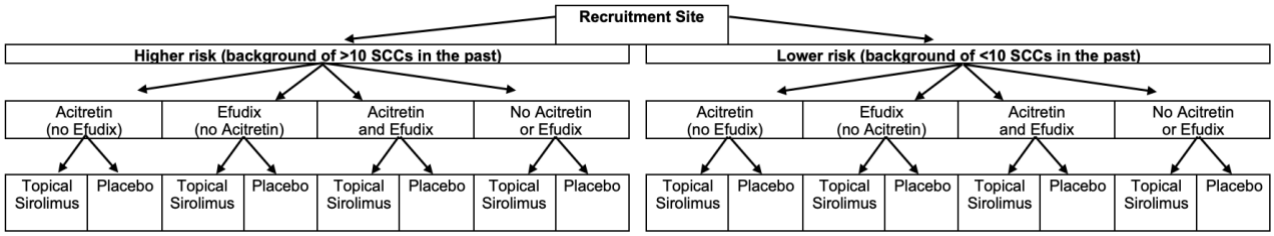

Figure 2: SiroSkin RCT stratification

11c. Research project process

Eligible SOTRs will be recruited during their usual clinic appointment in the Transplant Skin Clinics within the PAH and TPCH.

Figure 3a is a timeline of an individual's participation in the pilot trial:

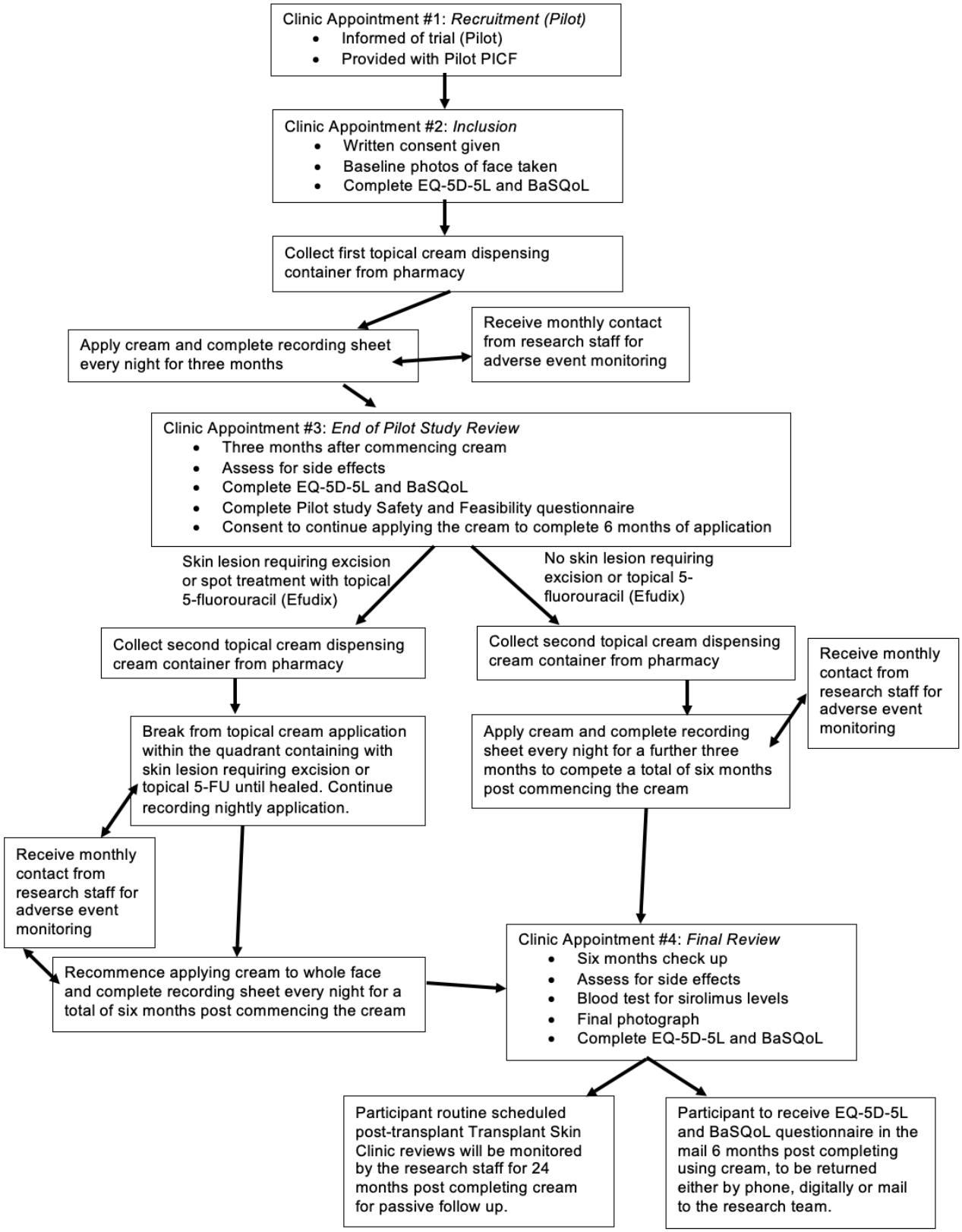

Figure 3a: Flow diagram of a patient participation in SiroSkin Pilot Study

Figure 3b is a timeline of an individual's participation in the RCT:

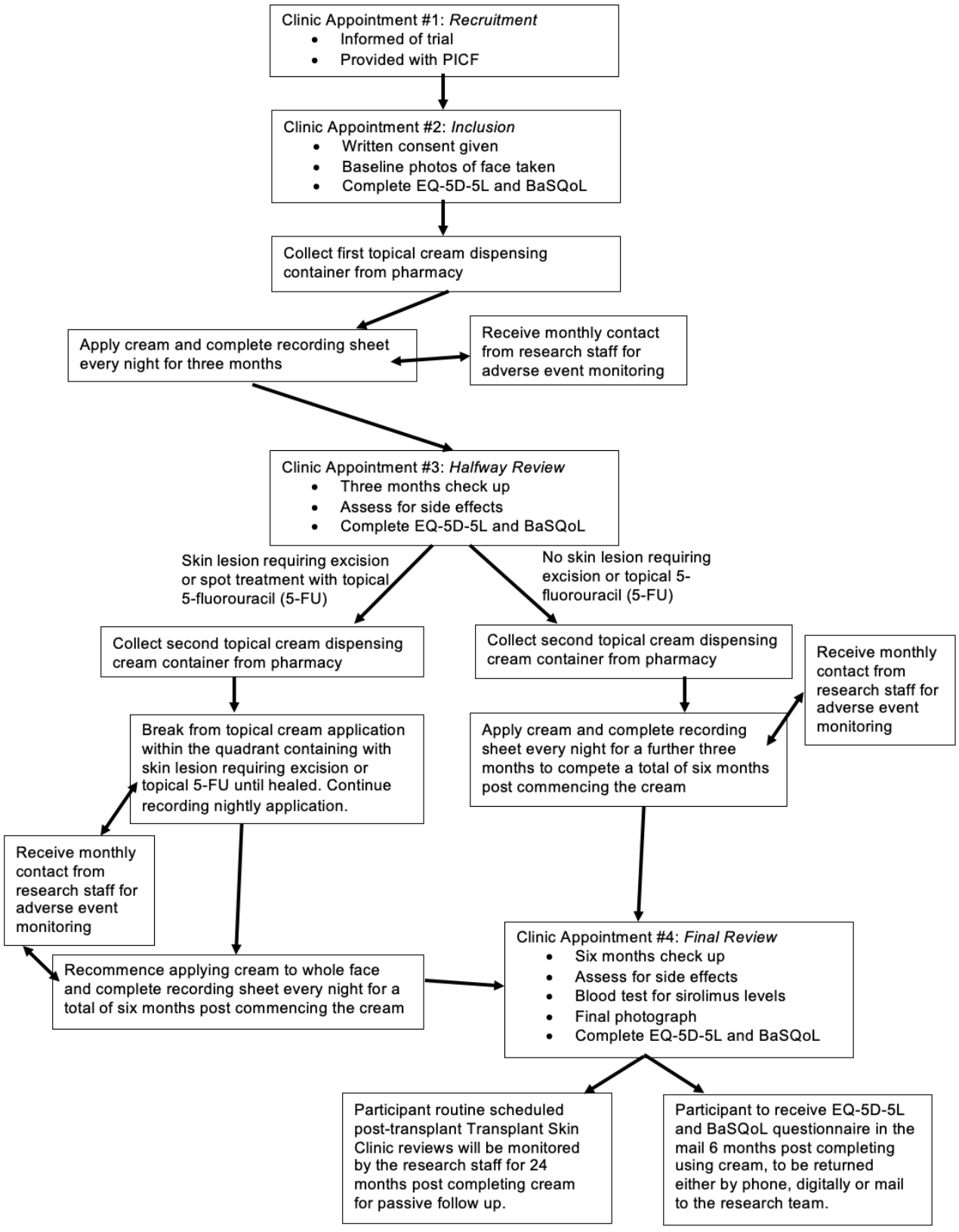

Figure 3b: Flow diagram of a patient participation in SiroSkin RCT

11d. Measurement tools used

The main measurement tool utilised will be counting histologically proven skin cancer lesions to assess the occurrence and number of SCCs, BCCs and IECs. Using only histological-proven lesions will ensure accuracy of data collection. The number of AKs will be compared at baseline to treatment completion. This will be done by a trained medical professional, such as a dermatology consultant, registrar or SHO either from clinical examination or digital photography and will be recorded at each clinic review.

Participants will also document throughout the 6 months all successful applications of the topical cream on a recording sheet to show the number and duration of applications used. This sheet will also include areas for the participants to document any side effects they have experienced or any sextant(s) breaks from treatment.

A blood test to measure serum sirolimus levels will be undertaken at the completion of 6 months of topical therapy. This sample will be collected by a trained phlebotomist

The cost-effectiveness of topical sirolimus will also be evaluated through utilising the EuroQol five-dimensional (EQ-5D-5L) which is a self-assessed, health-related and quality of life (QoL) questionnaire widely used to measure health-related QoL for the estimation of quality-adjusted life years.[30] It has five questions plus a visual analogue scale and takes less than a minute to complete. The EQ-5D-5L takes less than a couple minutes to complete and measures QOL on a 5-part scale including mobility, self-care, usual activities, pain/discomfort and anxiety/depression. The EQ-5D-5L is the most common measure worldwide for use in economic evaluations. In addition, the Basal and Squamous Cell Carcinoma QoL (BaSQoL) questionnaire will be utilised. This questionnaire specifically captures the issues experienced by individuals with SCCs and BCCs.[31] Both these questionnaires will be undertaken at the start, halfway point, completion of the application period and 6 months after completion of the application.

11e. Safety considerations including patient/participant safety

We consider that there are no significant risks and burdens associated with this research trial. Therefore, we consider the risk of harm and discomfort to be low. Safety considerations include:

- Sirolimus side-effects are usually minor and include skin dryness, irritation and folliculitis
- The possibility of an allergic or hypersensitive response to the sirolimus cream e.g. dermatitis, asthma, allergic rhinitis
- The chance that the treatment may fail
- Blood sampling may cause discomfort and bruising

11f. Data monitoring

A Data and Safety Monitoring Committee will be formed with a minimum of three specialist transplant consultants. This Committee will meet every six months, unless organised earlier, to meet and discuss all adverse events which have been sent to the committee, to determine the impact and if it relates to the intervention. All adverse events deemed related to the intervention will be declared to the Human Research and Ethics Committee (HREC).

An adverse event (AE) is any untoward medical occurrence in a study participant which is temporally associated with the use of an investigational product, whether or not it is

considered related to the investigational product. A serious adverse event (SAE) is any untoward medical occurrence that results in death, is life-threatening, results in disability or requires hospitalisation. Although not expected, all participants will be informed and provided with contact information for the research team to report any events and it will be recommended to either call Emergency Services on 000, visit a General Practitioner or report to the clinic. All AE/SAE will be resolved according to usual medical practices and procedures. All SAEs will be investigated to determine whether it is safe for participants to continue on the trial. All AEs/SAEs will be followed up until the event stabilises or resolves, and the clinic will be informed of the progression and outcome of the event.

An independent auditor will be used at the beginning and end of the trial to audit research conduct. In addition, an Annual Progress Report will be submitted to the approving Human Research Ethics Committee (HREC).

Participants will be provided with the contact details of the Principal Investigator or delegate to allow any questions or concerns to be addressed in a timely fashion. These will be on the PICF provided and on the recording sheet completed nightly by participants. Participants will also be advised to cease the topical cream and seek medical attention immediately if they experience any symptoms. 3-month clinic reviews will provide an opportunity to review any previously undisclosed AEs. Long term, routine follow up in the same dermatology clinic provides extra assurance that close monitoring will continue.

The trial may be prematurely ceased if a similar study with great significance has been published, or a study published a SAE related to the intervention or a study published an unpredicted SAE.

12. STATISTICAL CONSIDERATIONS AND DATA ANALYSIS

12a. Statistical power

Assuming that the IECs detected at 6 months in our preliminary study will become invasive at a later stage, we used the average number of IECs per patient to inform the sample size calculation of this trial. Based on that pilot data, in which 12 IECs were diagnosed in 29 placebo arms in 2 years and 4 IECs were detected in 29 sirolimus arms in the same time period, we assume that the underlying diagnosis rates in the placebo and sirolimus arms are 0.414 and 0.138 SCC/patient respectively over 2 years. To achieve power of 80% with a two-sided Type I error rate of 0.05, 47 patients are required to be tested in each arm with a 1:1 enrolment ratio between arms. Factoring in the expected high level of trial dropouts (36%), we will aim to recruit a minimum of 73 patients per arm totalling 146 patients.

12b. Statistical methods

The data will be analysed using a Poisson regression model with number of KCs per patient as the dependent variable and treatment arm (sirolimus vs. placebo) as the independent (predictor) variable. A 2-sided p-value of less than 0.05 will be deemed significant.

The cost-effectiveness of topical sirolimus will be evaluated by quantifying in both arms: (a) the costs of the intervention and all healthcare interactions (including cost of sirolimus gel, diagnosis and treatments of skin lesions and managing adverse events) and (b) the benefits of the intervention as quality-adjusted life years (QALYs) and skin-cancer related quality of life using the Basal and Squamous Cell Carcinoma Quality of Life (BaSQoL) questionnaire and (c) skin cancers avoided with topical sirolimus. The EQ-5D-5L measures QOL on a 5-part scale including mobility, self-care, usual activities, pain/discomfort and anxiety/depression and is the most common measure worldwide for use in economic evaluations. The two questionnaires will be undertaken at the start, halfway point, and completion of the application period and at 6, 12 and 24 months aligning with skin cancer counts. Incremental cost-per-effect ratios will be generated which represent the additional cost and health benefits (QALYs, cancers avoided, improved QOL) for the intervention, compared with the current standard of care (biopsies, surgeries, hospitalization). Extensive sensitivity analyses will be undertaken.

A statistician will be involved throughout the statistical analysis.

13. ETHICAL CONSIDERATIONS

All ethical considerations have been contemplated for this research trial. The RCT will be conducted in full conformance with principles of the “Declaration of Helsinki”, Good Clinical Practice (GCP) and within the laws and regulations of Australia. The protocol and all relevant documents will be submitted for approval to the Metro South Health HREC.

In addition, a consumer from the targeted study population was contacted to gauge their response to the participants involvement in the trial. There was strong support expressed for any intervention that would be well tolerated and could reduce the need for surgery on the face.

The recruitment strategy takes account of all ethical considerations. The recruitment process is considered fair in that potential participants will be approached on a chronological basis and only those who fit the eligibility criteria will be targeted. There are large numbers of potential participants being seen over two large public hospitals, therefore this reduces the risk of unnecessary coercion and exploitation. All participants are required to give informed consent hence the exclusion of non- English-speaking persons and those who are considered cognitively impaired. In addition, specific racial groups are considered much less likely to be at high risk of skin cancers due to skin characteristics (e.g. indigenous Australians and Pacific Islanders).

Before enrolment in the study, all prospective participants will be provided with a detailed full explanation of the purpose and process of the study and provided with a copy of the PICF to review before consent. All participants will provide informed written consent before being enrolled. The completed original consent form will be retained by the Investigator and a copy will be provided to the participant.

Potential risks will be managed as well as possible and we will endeavour to ensure that the participants clearly understand the risks they are assuming. Of paramount importance is that the participants communicate any concerns to medical staff (GP, dermatologist) and seek advice as soon as possible. A contact phone number for the research team will be provided in the participants PICF to enable timely assistance.

The main risks to participants associated with this research include a reaction to the sirolimus cream (which is likely to be minor) and the discomfort of blood tests (which will coincide with their routine blood tests when possible). These risks of harm and discomfort are justified by the potential to decrease the very high risk of developing keratinocyte carcinoma in the treated areas. The pilot study assessing topical 1% sirolimus on the arms had minimal side effects, however to ensure it is tolerated by participants on the face a 3 month pilot study has been incorporated into the initial part of this RCT.

Although participants will not be financially reimbursed, it is not envisaged participants will be 'out of pocket' as they will be recruited in their usual and regular clinic surveillance appointments which they would have been attending with or without the trial occurring. In addition, every endeavour will be made to align subsequent 3-monthly reviews into the participant's regular review to avoid participants attending extra clinic visits for the purpose of the trial. However, an extra appointment may be required if this is unable to be arranged.

We do not envisage any ethical dilemmas in relation to storage, access and destruction of data and samples. Any information that can identify the participant (i.e. the consent form) will remain confidential and will only be used for the purpose of the RCT. All PICFs will be scanned into IeMR before transfer to TRI. Consent forms will be stored in a locked compartment within the TRI facility. Study records may be viewed for the purposes of auditing by members of the HREC. All information will be transferred to computer files that will be password protected and the entrance to the TRI facility will require key code access. This will ensure that only study personnel will be able to access study information. As per QLD Government retention policy, all RCT documentation will be stored for 25 years and will then be destroyed by incineration.

The skin samples from the skin biopsies and excisions are an essential component of the research. These skin samples will be prepared by the laboratory only after the diagnosis process has been complete to ensure there is no compromising of the diagnosis process. The skin samples obtained for the purpose of this RCT will be transferred to University of Queensland PC2 certified facility located at the Translational Research Institute in Woolloongabba, Brisbane QLD. Storage within a secure laboratory can be assured as access is limited to authorised personnel only. All samples will be given a unique code (study number) and only the research team will be able to re-identify them (i.e. trace them back to the participant). Samples will be stored for a minimum of 7 years after the study and they will then be destroyed by incineration. Samples will not be used for commercial purposes. The research team will not be performing any genetic testing on skin or blood samples therefore there are no implications for the participant or their family.

The blood sample collected 6 months post-completion of the trial cream is associated with minimal risk (e.g. some participants may experience arm discomfort or minor bruising) and will be collected by a trained phlebotomist. To mitigate any additional risk and inconvenience, this blood test will be combined with other routine blood collections, where possible. If this is not possible, the participant can visit a blood collection centre of their

choice, and at no cost to them. All samples will be analysed by an external laboratory i.e. they will not be tested or stored by the research team.

It is anticipated that the results of this RCT will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that participants cannot be identified. Information about their participation in this RCT may be recorded in their health records. In accordance with relevant Australian and QLD privacy and other relevant laws, the participant has the right to request access to their information collected and stored by the research team. They also have the right to request that any information with which they disagree be corrected.

This study is funded by the Metro South Study, Education and Research Trust Account (SERTA) through the 2021 Metro South Heath Research Support Scheme (MSH RSS) Project Grant. However, the funders will not be involved in the designing or conducting of the study, interpreting the results or determining the reporting of the study results.

Participants will be advised they may voluntarily withdraw from the study at any time without affecting their future treatment or care. This withdrawal will be complete with no further participation or follow up unless the participant provides consent to allow ongoing passive follow up. A withdrawal form will be provided to participants to complete. Any data already collected will be retained. An investigator may terminate a participant's participation in the study if an exclusion criterion becomes apparent or a participant experiences an adverse event or other medical condition meaning their continued participation in the study would not be in their best interest.

14. OUTCOMES AND SIGNIFICANCE

The SiroSkin RCT addresses a high priority identified by SOTRs. This RCT has come out of the team's regular engagement with organ transplant recipients, including recipients that are most severely affected by KCs (through the multi-disciplinary skin clinics and Cancer Council QLD KC consumer groups). SiroSkin proposes to reduce the extreme burden that skin cancers and pre-cancers place on SOTRs through generating high-quality evidence of the efficacy of topical sirolimus as a chemopreventive therapy for SCCs in this population. This RCT has received strong support, with consumers keen to see well tolerated interventions that can reduce the need for surgery, especially on the face.

We expect that upon continuous regular therapy with topical sirolimus, a cream with minimal side effects, we will observe a major reduction in invasive SCCs, BCCs and IECs as well as a significant reduction in the number of actinic keratosis as compared to placebo. From the individual patient's perspective, a reduction in the risk of both sun damage to the skin, and skin cancer, will improve their quality of life and possibly their life expectancy. Notably there will be fewer clinic visits and medical procedures required and less anxiety and inconvenience for patients and family members. In addition to reducing the morbidity and mortality associated with keratinocyte cancers in this population, success of our research will translate into economic benefits for society at large.

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16. APPENDIX 1

THE UNIVERSITY OF QUEENSLAND AUSTRALIA

Queensland Government

SiroSkin Pilot Questionnaire

Date:
Patient number_____ Hospital: PAH / TPCH

Safety

Did you have any side effects from the cream? Yes / No
If yes, what were these? _____

_____

_____

While applying the cream, did you have any medical issues not due to the cream? Yes / No
If yes, what were these? _____

_____

_____

While applying the cream, did you have any medication changes not due to the cream? Yes / No
If yes, what were these? _____

_____

_____

Feasibility

Did you have any issues with the feel of the cream? Yes / No
If yes, what were these? _____

_____

Did you have any issues applying the cream every night? Yes / No
If yes, what were these? _____

_____

Did you miss any applications of the cream? Yes / No
If yes, how many?_____

If yes, why?_____

_____

Did you bring with you the nightly recording sheet to review today? Yes / No
Did you have any issues completing the nightly recording sheet? Yes / No
If yes, what were these? _____

_____

If effective, would you use this cream every night long-term? Yes / No
If no, why not? _____

_____

Did you complete the EQ-5D-5L Questionnaire?
At start of trial: Yes / No
At today's 3-month review: Yes / No

Did you have any issues completing the EQ-5D-5L? Yes / No
If yes, what were these? _____

_____

Did you complete the BaSQoL Questionnaire?
At start of trial: Yes / No
At today's 3-month review: Yes / No

Did you have any issues completing the BaSQoL? Yes / No
If yes, what were these? _____

_____

Will you continue using the cream for next three months as part of the trial? Yes / No
If no, why not? _____

_____

Sheet completed by: _____

17. APPENDIX 2

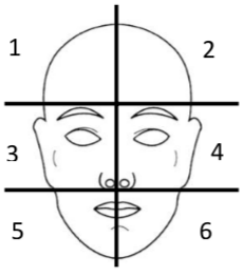
First month shown out of six months, all months are recorded the same.

The University Of Queensland AUSTRALIA Queensland Government

Daily Recording Sheet for SiroSkin Trial: Month 1

Name:_____

If any concerns or issues, please contact Dr Charlotte Cox 0486 030 731.
Please bring this sheet to all your Transplant Skin Clinic appointments.
Please complete this sheet every night after applying the cream to your face.



Face Areas

Date	Application Done	Any break from the cream?	If so, which area/s on the face? (see picture)	Used sunscreen during the day?	Any concerns/ issues?	Please share your concerns/issues:
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	
	Yes / No	Yes / No	1 2 3 4 5 6	Yes / No	Yes / No	