Statistical Analysis Plan: PICASSo Trial

Prophylactic InCisional Antiobiotics in Skin Surgery (PICASSo trial)

Section 1: Administrative information		
Trial and Trial registration	1a	**Statistical Analysis Plan: PICASSo Trial** Prophylactic InCisional Antiobiotics in Skin Surgery
	1b	Trial registration number: ACTRN12616000364471
SAP Version	2	SAP version 1.1 [October 15, 2021]
Protocol Version	3	Trial Protocol 1.1 [October 15, 2021]
SAP revisions	4a	SAP revision history 1.0 [September 17, 2021]: Initial version 1.1 [October 15, 2021]: Analysis changed from lesion assessment according to postoperative date to lesion assessment according to highest POWI score
	4b	Justification for each SAP revision 1.1: Some lesions were assessed multiple times, and it was realized that the highest POWI score exposed and captured all relevant SSIs
	4c	Timing of SAP revisions in relation to interim analyses 1.1: revision was instituted at study conclusion (there was no interim analysis)

Roles and responsibility	5	Bert Van der Werf. Senior Biostatistican. University of Auckland School of Population Health, Department of Epidemiology and Biostatistics. Jon A Mathy. Principal Investigator. Auckland Regional Plastic & Reconstructive Surgery Unit. University of Auckland School of Medicine.
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	6b	Senior statistician responsible Bert Van der Werf 
	6c	Chief investigator/clinical lead Jon A Mathy 

Section 2: Introduction		
Background and rationale	7	Skin cancer is by far the most common cancer worldwide. It also represents one of the most expensive cancers. Surgical site infections (SSIs) represent one of the most significant, potentially avoidable factors that negatively impact on skin cancer treatment costs, patient experience and outcomes. The Auckland Regional Skin Cancer Treatment Centre at Counties Manukau District Health Board (CMDHB) is one of the busiest skin cancer treatment centres in New Zealand, receiving >2000 patient referrals per year. The rate of SSI at this centre in a recent evaluation fell between 3.5% and 14.3%, and published rates of SSI after cutaneous surgery internationally have been reported to be 2.3-4.3%. While the routine adoption of evidence-based antibiotic prophylaxis has been associated with substantial decrease in the rate of SSIs in other surgical streams, there is a general lack of high-quality studies evaluating the role of prophylactic antibiotics in skin cancer surgery, and no evidence-based guidelines directly applicable to skin cancer surgery exist, either regionally or globally. Furthermore, there is an increasingly urgent need to develop antimicrobial prophylaxis approaches that incorporate good antibiotic stewardship and reduce the devastating potentiation of antibiotic resistance.
Objectives	8	The primary objective of the Prophylactic InCisional Antibiotics in Skin cancer Surgery (PICASSo) trial is to evaluate the efficacy and safety of a single dose of preoperative locally infiltrated incisional antibiotics for decreasing the incidence of SSIs in patients undergoing skin cancer surgery. The overarching goal is to lead the way in delivering high quality economical skin cancer treatment, while filling a void for evidence-based guidelines in skin cancer surgery that would be applicable worldwide
Section 3: Study Methods		
Trial design	9	This is a prospective, randomized, double-blind, placebo-controlled study All patients undergoing skin cancer surgery at the Auckland Regional Platic Surgery Unit

		under local anesthetic over the 6 month recruitment period will be eligible. Exclusion criteria are allergies to both penicillin and clindamycin, preoperative systemic intake of any antibiotic within 7 days of skin surgery, or inability to return for face-to-face postoperative wound assessment. Patients can be enrolled and randomized more than once if they underwent additional procedures within the study period, assuming all eligibility criteria were still met. Participants will be randomized to receive incision site injection of buffered local anesthetic alone (1% lidocaine plus adrenaline 1:100,000 standard solution buffered 1:10 with 8.4% sodium bicarbonate; "control"), buffered local anesthetic + micro-dosed flucloxacillin (500 µg/cc), or buffered local anesthetic + micro-dosed clindamycin (500 µg/cc). Surgical excision, reconstruction, and perioperative management will proceed as per the usual standard of care, and all interventions and assessments will be performed by investigators unaware of treatment allocation. Lesions will be infiltrated using a pre-filled infiltration syringe based on randomization number and corresponding to the allocated treatment group that had been prepared by a trial pharmacist (Baxter Pharmaceuticals) in 10cc aliquots. To promote blinding integrity, syringes will be labelled in a random order within each batch. Syringes will be stored at 4ºC, protected from light, and replaced with a fresh batch every 48 hours. Patients and all members of the surgical and follow-up teams will remain blinded to allocation until study conclusion. If more than one syringe is required (e.g. large infiltration area and/or multiple sites), the original allocation arm will be maintained for that patient-presentation. Participants enrolled more than once will be independently randomized at each presentation. Any deviations to treatment received compared with protocol will be recorded. Surgical excision, wound closure/reconstruction, and postoperative management will be performed according to standard of care. Patients will be monitored for adverse reactions perioperatively and questioned about sensitivity reactions elicited at each follow-up visit. Safety will be monitored by an independent data monitoring committee throughout the trial. All assessments will be performed by clinical staff blinded to treatment group allocation. All participants will undergo standardized postoperative assessment by a consistent trial nursing team between 7 and 21 days postoperatively. Additional assessments will also

		be also performed opportunistically during any further encounters (for example in case of wound healing concerns and/or surgical site infections requiring further management) as required. Each assessment will include a patient questionnaire eliciting any side effects experienced, adverse events, and/or antibiotics prescribed for any reason since the time of operation and/or last assessment. A standardized wound assessment will be performed for each operative site using a previously validated Post-Operative Wound Infection (POWI) scoring system [scale, 0-7, with 0 reflecting no signs or symptoms of infection, and 7 reflecting obvious signs and symptoms of severe infection]. When more than one post-operative assessment is made, the highest POWI score recorded for each lesion will be used for analysis. Additional data recorded based on surgical type will includ length of closure and any dehiscence (for wounds closed directly), percent skin graft take (grafted wounds), and area of local tissue rearrangement (skin flaps). Patient-specific factors will be recorded for each participant including: immunosuppression; glucose intolerance; history of prior Surgical Site Infection (SSI); smoking status; and, for extremity wound sites, peripheral edema score, presence of venous insufficiency and peripheral pulse palpability. In any case of overt infection, culture swabs will be obtained for microbiological analysis and to guide tailored antibiotic treatment.
Randomization	10	Randomizations will be performed among arms 1:1:1 using a a blinded schedule generating a randomization number, with random block sizes. Patients randomized to the flucloxacillin arm with a penicillin allergy will be automatically re-allocated to the clindamycin arm (second line antibiotic, also blinded, assuming no clindamycin allergy), and patients randomized to clindamycin who with a clindamycin allergy will be automatically re-allocated to control arm.
Sample size	11	A pre-recruitment power analysis calculation was performed based on a retrospective analysis of Surgical Site Infections encountered in skin surgery undertaken at the Auckland Regional Plastic Surgery Unit (same unit as current study) over a 6-month period at the same as follows: In a retrospective analysis, ICD10 codes were used to identify all outpatient skin excisions performed at the unit over 6 months in 2015. Medical records were individually reviewed, and data obtained on patient demographics, comorbidities, surgical type, and utilisation of perioperative antibiotics. A total of 2,088 lesions were excised from 938 patients in 1053 encounters over the 6-

month evaluation period. Seven patients (0.7%) were readmitted for wound infection within 28 days of excision, clinic letters noted infection in 56 patients (5.2%), while 143 (13.4%) were prescribed antibiotics in the community within 21 days after surgery. It was concluded that the actual rate of SSI was between 3.5% and 14.3% based on these direct versus direct + indirect indicators, respectively. These rates were averaged to estimate the expected SSI rate of 7%.

The anticipated SSI rate in the treatment group was then estimated to be 2% based on the expectation that intervention will render at least equal - and likely greater- benefit than that detected in a pilot trial previously performed by our group when measured to a threshold POWI >=5 (OR 0.367-0.563, POWI >=4). These results were presented at the 86th Annual Scientific Congress of the Royal Australasian College of Surgeons, May 2017 (Award for Best Scientific Paper), and the full statistical data tables from this trial are available on request. All eligible consenting patients presenting to the Auckland Plastic Surgery Unit over a 3-month period in 2016 were enrolled. Patients were randomly allocated to one of three treatment groups: buffered local anaesthetic (LA); buffered LA with micro-dosed clindamycin; or buffered LA with micro-dosed flucloxacillin. Solutions were infiltrated to the excision sites (and donor sites if applicable) in double-blinded fashion. Wounds were assessed using the same standardised POWI scoring system employed in the current study at 1 week and 1 month after surgery. The primary outcome was the overall rate of SSIs. Secondary outcomes included adverse events, SSI-associated co-morbidities, culture swabs and antibiotic use. A total of 332 patients were enrolled (519 lesions excised). Treatment groups were comparable at baseline.

The sample size calculations in the table below are based on achievement of 80% power at a 5% level of significance (to which a False Discovery Rate correction has been applied to allow comparison between each of the active treatments and placebo). These infection rate differences (7% vs 2%) and associated sample size are used to guide patient recruitment so that study power is maximised.

Group	Infection Rate - POWI ≥5 (%)	Required Group Size (# Lesions)	Required Sample Size
Placebo	7	299	987
Flucloxac	2	344	

		illin			
		Clindamycin	2	344	
		In summary, to achieve 80% power with a 5% level of significance (to which a False Discovery Rate correction was applied to allow comparison between each active treatment and control), it was calculated that 987 lesions would need to be recruited.
Framework	12	Superiority analysis of interventions versus standard of care (control)
Statistical interim analysis and stopping guidance	13a/b	n/a – no statistical interim analysis performed. No planned adjustment of significance level.
	13c	A safety monitoring committee will be active throughout recruitment. Recruitment will be paused or stopped in case of any adverse reactions are thought or found to arise as a result of trial participation.
Timing of final analysis	14	The final analysis will be performed collectively at completion of data collection (at least 3 weeks following final lesion recruitment).
Timing of outcome assessments	15	All participants will undergo standardized postoperative assessment by a consistent trial nursing team between 7 and 21 days postoperatively. Additional assessments will also be also performed opportunistically during any further encounters (for example in case of wound healing concerns and/or surgical site infections requiring further management) as required.
Section 4: Statistical Principals		
Confidence intervals and P values	16	The level of statistical significance will be at least 0.05, two sided tests.
	17	There will be no adjustments for multiplicity. Not all pairwise comparisons will be made, only the ones defined a-priori. Those comparisons are with the control value, and will only be done after the general test indicates a significant effect.

	18	Confidence intervals to be reported are the 95% confidence intervals
Adherence and Protocol deviations	19a	Adherence to intervention is defined as following study protocol. This is assessed by comparing intended treatment according to randomization (respecting patient allergies) and actual treatment administered.
	19b	The adherence to the intended intervention (and any deviations) will be presented via a CONSORT table
	19c	The definition of protocol deviations for the trial is defined as any deviation from intended treatment as randomized according to the study protocol, and as detailed in the SAP "study methods" sections above (section 9-10)
	19d	Any differences between the intended treatment and actual treatment will be prospectively collected and summarily reported at study conclusion.
Analysis populations	20	Definition of Intention To Treat (ITT) and Actual Treatment (AT) analysis sets are defined for analysis as follows: 1. Intention To Treat (ITT): Participants who were randomized and contributed to at least one measure of an outcome post-randomization will be deemed to belong to the ITT analysis set, with allocation corresponding to the randomization result, respecting patient-reported allergies as detailed in SAP study methods "randomization" section above(Item 10) 2. Actual Treatment (AT): Participants who were randomized and contributed to at least one measure of an outcome post-randomization, with allocation corresponding to the actual treatment received. This group will include any allocation errors (eg intended treatment post randomization is flucloxacillin arm but patient actually receives control injection).
Section 5: Trial Population		
Screening data	21	All patients screened (ie, assessed for eligibility) will be recorded irrespective of enrollment and randomization in order to help describe representativeness. Data collected at screening will include name, medical record number, demographic data including age, gender, and ethnicity. If a screened patient is not enrolled/randomized,

		then the stated reason for non-participation will be recorded. After de-identification, number and reason for non-participation will be reported in the study CONSORT diagram, with additional data available upon request.
Eligibility	22	Screened patients are deemed eligible for randomization if the following criteria are met, as detailed in the SAP study method "trial design" above (section 9) All patients undergoing skin cancer surgery at the Auckland Regional Platic Surgery Unit under local anesthetic over the 6 month recruitment period will be eligible. Exclusion criteria are allergies to both penicillin and clindamycin, preoperative systemic intake of any antibiotic within 7 days of skin surgery, or inability to return for face-to-face postoperative wound assessment. Patients can be enrolled and randomized more than once if they underwent additional procedures within the study period, assuming all eligibility criteria were still met.
Recruitment	23	The following information will be collected on all recruited patients and reported in the study CONSORT flow diagram: 1. Number of patients (and independent lesions) recruited 2. Number of patients (and independent lesions) randomized to each trial arm as intended according to randomization protocol [ITT] by arm, including number (and independent lesions) affected by patient-reported allergies. 3. Number of patients (and independent lesions) according to actual treatment received [AT] by arm. Further detail regarding numbers of patients (and independent lesions) discrepant between ITT and AT groups will also be separately reported. 4. Number of patients (and independent lesions) completing follow up, by actual treatment arm 5. Number of patients (and independent lesions) included in analysis, in total and by actual treatment arm
Withdrawal/ Follow-up	24a	All patients randomized but not receiving intervention for any reason will be considered "withdrawn" and will be recorded by arm. These data will be disclosed in CONSORT table. Patients are given written and verbal instructions regarding follow up at time of intervention. Patients not appearing for scheduled follow up are called and invited for further follow up at least once.

		All patients randomized and receiving intervention but no returning for any follow up assessment will be considered “lost to follow up.” And will be recorded by arm. Both number of patients (and independent lesions) completing at least one follow-up and “lost to follow-up” will be disclosed in the CONSORT table.
	24b	Patients deemed “lost to follow up” if they refuse follow up or do not attend at least two personal invitations for scheduled follow up appointments.
	24c	Numbers and percentage of patients who are lost to follow up will be reported by arm.
Baseline patient characteristics	25a/b	The following baseline patient characteristics will be summarized (with details of how the characteristics will be descriptively summarized): 1. Gender (number and percentage) 2. Age (mean with standard deviation, and median) a. Age group, by decile (number and percentage) 3. Ethnicity (number and percentage) 4. Current smoking status (number and percentage) 5. Immunosuppression status (number and percentage) 6. Glucose intolerance status (number and percentage) 7. History of prior surgical site infection status (number and percentage) 8. Self-reported allergy to flucloxacillin and/or clindamycin (number and percentage) 9. Number of presentations per patient (number and percentage) 10. Number of lesions treated per patient (number and percentage) Additionally, the following study-specific baseline procedure- and lesion- characteristics will be summarized: 11. Lesion ulceration status (number and percentage, reported by arm; including p-value versus control) 12. Skin surgery type (number and percentage, reported by arm; including p-value versus control) 13. Skin surgery location (number and percentage, reported by arm; including p-value versus control) 14. Whether postoperative antibiotics were prescribed (number and percentage, reported by arm; including p-value versus control)
Section 6: Analysis		

<table>
<tr><td>Outcome definitions</td><td>26a</td><td>Primary outcome measure is Surgical Site Infection (SSI) as defined by maximal standardized Post-Operative Wound Infection (POWI) score recorded at any follow up visit, per lesion, with a value greater than or equal to 5 [scale, 0-7], by arm.

Analysis will also be performed by maximal standardized POWI score greater than or equal to 4 by arm</td></tr>
<tr><td></td><td>26b</td><td>Standardized POWI score is defined as follows:

<table><tr><th>Score</th><th>Condition</th></tr><tr><td>0</td><td>Normal healing</td></tr><tr><td>1</td><td>Normal healing but with one of the following signs of infection: erythema, edema, or increased pain</td></tr><tr><td>2</td><td>Normal healing but with two of the following signs of infection: erythema, edema, or increased pain</td></tr><tr><td>3</td><td>Normal healing but with three of the following signs of infection: erythema, edema, and increased pain</td></tr><tr><td>4</td><td>Hemoserous discharge combined with two of the following: erythema, edema, or increased pain</td></tr><tr><td>5</td><td>Pus combined with one of the following: erythema, edema, or increased pain; or hemoserous discharge combined with all three of the following: erythema, edema, and increased pain</td></tr><tr><td>6</td><td>Pus combined with two of the following: erythema, edema, or increased pain</td></tr><tr><td>7</td><td>Pus combined with all three of the following: erythema, discharge and increased pain</td></tr></table>.</td></tr>
<tr><td></td><td>26c</td><td>The main outcome of the trial is POWI classified in 7 groups. Depending on the sizes of the group it is possible that adjacent groups will be combined. This will done by checking the variances of the estimates. When the group size is small, the variances will be large compared to other groups. On medical grounds combining groups 5, 6 and 7 to create a POWI with 5 classes makes sense a priori.</td></tr>
<tr><td>Analysis methods</td><td>27a</td><td>Analysis method used and how treatment effects will be presented: the Logistic regression for multinomial observations will be used as described by Dobson and</td></tr>
</table>

		Barnett. Estimated POWI proportions for each treatment arm will be presented in histograms and comparisons between treatments and arms will be calculated on basis of estimated parameters and covariances from the model
	27b	Any adjustment for covariates: Analysis with and without covariate as interaction term. Only baseline characteristics are used as covariate.
	27c	The assumptions will be checked by using the methods given in the DHARMa package in "R."
	27d	Details of alternative methods to be used if distributional assumptions do not hold, e.g., normality, proportional hazards: if the assumptions do not hold, then this can maybe solved by adding covariates, assuming different distribution, maybe probit analysis or if all fails using sandwich estimators for the covariances.
	27e	Any planned sensitivity analyses for each outcome where applicable: No sensitivity analyses are planned
	27f	Any planned subgroup analyses for each outcome including how subgroups are defined: Subgroup analyses are defined as adding an interaction term with a covariate (e.g. Age group, surgical location, gender, ethnicity) assuming adequate number of observations in each subgroup.
Missing data	28	Reporting and assumptions/statistical methods to handle missing data: It is not expected that there will be many missing values. If there are a few missing values and it looks randomly distributed over the treatment arms then they will be ignored. If there are a mediate number of missing values and or biased distribution of those, then we will impute them with the help of the baseline characteristics.
Additional analyses	29	Additional pairwise comparisons with control are made using the estimated parameters and their covariances.

Harms	30	Adverse events are coded according to a combination of direct observation by medical staff at all patient encounters (eg, point of intervention and each subsequent assessment), and elicited patient reported outcomes at all patient encounters. These includes signs or symptoms of allergic/hypersensitivity reaction (ie, localized pruritis, erythema, induration, pain), and/or anaphylaxis (ie tachypnea/shortness of breath, wheeze/stridor, tachycardia, anxiety/confusion, loss of consciousness).
Statistical software	31	R version 4.03 will be used with the main packages lme4, lmerTest and Dharma. The package ggplot2 will be used to create graphics.
References	32a	How to analyze multinomial observations with logistic regression is not commonly known, but it is described in Dobson, A (1990), An introduction to generalized linear models, Chapman & Hall, New York.
	32b	Data Management Plan: Protected Health Information will be guarded at all times according to standard of care. All data will be returned to Principal Investigator at conclusion of analysis. Data will be accessible from PI upon reasonable request.
	32c	The Trial Master File and Statistical Master File are included in the Data Management Plan above.
	32d	Other documents adhered to in this SAP include the “PICASSo standard operating procedure (SOP)”