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| Study Title: | A Phase II Randomised, Double-Blind, Placebo-Controlled Study of the Efficacy, Safety and Tolerability of Oral NP202 in Adults who have paroxysmal atrial fibrillation and a cardiac device. |
| Protocol Number: | NP202-AF-001 |
| CTN Number: | AU/1/5B61312 |
| Test Drug: | NP202 |
| Indication: | Attenuation of paroxysmal atrial fibrillation in adults with an implanted cardiac device. |
| Version: | 3.0 |
| Date: | 24 Jan 2018 |
|  |

**PROTOCOL SYNOPSIS**

|  |  |
| --- | --- |
| Study Title: | A Phase II Randomised, Double-Blind, Placebo-Controlled Study of the Efficacy, Safety and Tolerability of Oral NP202 in Adults who have Paroxysmal Atrial Fibrillation and a Cardiac Device. |
| Study Period: | 2017 – 2020 |
| Objectives: | Primary objective:* To evaluate the efficacy of NP202 compared to placebo, when administered once daily for 1 month, in attenuating paroxysmal atrial fibrillation (AF).

The secondary objectives are:* To assess the safety and tolerability of NP202 compared to placebo, when administered once daily for 1 month.
 |
| Study Plan | This is a single-centre, randomised, double blind, placebo controlled study to assess the efficacy, safety and tolerability of NP202.Subjects will be screened during routine interrogation of their cardiac device. Eligible subjects will have a high burden of paroxysmal atrial fibrillation. They will be randomised and administered their first dose of investigational product (IP) on Study Day 1.Subjects will take their IP dose once a day for 1 month (30 days). During this treatment period they will return to the site for study visits at Week 2, and Months 1, 2, and 3. Month 3 is the end of the Treatment Period. Subject will return for follow up and the final study visits at Months 4 and 5.A Data Monitoring Committee (DMC) will review safety data at agreed recruitment and progression milestones to provide independent oversight of subject safety. |
| Number of participants: | It is planned that 20 participants will be randomised in a 1:1 ratio to one of two treatment arms:* 10 will receive NP202 1000 mg once daily for 1 month, followed by 1 month washout, and then placebo once daily for 1 month.
* 10 will receive placebo once daily for 1 month, followed by 1 month washout, and then NP202 1000 mg once daily for 1 month.
 |
| Key inclusion criteria: | Participants who:* Age between 18-80 years.
* Paroxysmal (not lasting more than 7 days) atrial fibrillation.
* An implanted device (loop recorder, pacemaker or defibrillator).
* An AF burden between 1-90% over the past 6 months.
 |
| Key exclusion criteria: | Participants with:* Pregnant or breastfeeding females.
* Estimated glomerular filtration rate (eGFR) <30ml/min.
* Liver function tests 3 x ULN due to non-cardiac disease.
* Have received any investigational research agent within 30 days or 5 half-lives (whichever is longer) prior to the first dose of IP.
 |
| Test product, dose, and mode of administration: | NP202 will be presented as capsules containing NP202 and microcellulose as required.NP202 will be encapsulated into gelatine capsules, to be administered as doses of 1000mg per day.Placebo will be presented in identical capsules and will contain microcellulose |
| Duration of study per subject: | Approximately 5 months: Up to 5 days screening, 3 months treatment and 1-2 months follow up. |
| Criteria for evaluation: | Primary efficacy endpoint* Change from baseline in total burden of atrial fibrillation per month, expressed as a percentage, as determined by device interrogation.

Secondary efficacy endpoints:* Change from baseline in frequency of atrial fibrillation events per month, expressed as a number, as determined by device interrogation.
* Change from baseline in duration of atrial fibrillation per month, measured in minutes, as determined by device interrogation.

Safety and Tolerability Endpoints* Major adverse cardiac and cerebrovascular events (MACCE) (non-fatal MI, non-fatal stroke, CV death, cardiac hospitalisation due to heart failure).
* All adverse event (AE) recording and assessments.
* Safety laboratory evaluations (biochemistry, haematology, prostate specific antigen (PSA), urinalysis).
* Vital signs.

Serum Biomarkers* Changes from baseline in serum biomarker levels at 1, 2 and 3 months.
* Absolute serum biomarker levels at 1, 2, 3 and 4 months.
 |
| Statistical methods and analyses: | Analysis Sets* Full analysis set (FAS): All subjects randomised into the study. Subjects will be analysed according to the treatment to which they were randomised. Efficacy analyses performed in the FAS are considered supportive of analyses performed in the mITT set.
* Safety set: All randomised subjects who received at least one dose of study medication. Subjects will be analysed according to the treatment received. All safety analyses will be conducted in the Safety set.
* Modified Intention-to-treat (mITT) set: All randomised subjects who received at least one dose of study medication. Subjects will be analysed according to the treatment to which they were randomised. The primary efficacy analysis will be performed in the mITT set.
* Per-protocol (PP) set: All subjects from the mITT population who completed the study in compliance with the protocol and who reported no major violation of the study protocol. Subjects will be analysed according to the treatment to which they were randomised. The final decision to exclude a subject from the PP set will be taken during a blinded data review meeting before database lock. Efficacy analyses performed in the PP set are considered supportive of analyses performed in the mITT set.

Sample SizeA sample size of 20-50 subjects provides 46-80% power to detect a treatment difference of at least 50% reduction in the primary efficacy endpoint. Power was calculated for a two-sided t-test with a 5% Type I error rate. Statistical AnalysesContinuous variables will be reported as mean ± standard deviation or as median and percentiles if appropriate. Normally distributed variables will be compared using the paired Student’s t-test. Otherwise comparisons between both the groups will be performed using the Mann–Whitney U test. Categorical variables will be stated as absolute and relative frequencies and compared using the χ2 test. All tests are two-tailed. A P-value of <0.05 will be considered as statistically significant. Sensitivity analyses of the primary endpoint will be performed in the FAS to assess the impact of missing data on the robustness of the primary analysis. The primary analysis will also be repeated in the PP set.The following secondary endpoints will be analysed and summarised using the methods described for the primary endpoint:* Change from baseline in frequency of atrial fibrillation events per month, expressed as a number, as determined by device interrogation.
* Change from baseline in duration of atrial fibrillation per month, measured in minutes, as determined by device interrogation.

Safety assessment will occur on all subjects who receive any study treatment. AEs will be coded to a standard set of terms using the MedDRA dictionary. Terminations/premature withdrawals, AEs, concomitant medications, and laboratory data will be tabulated.MACCE is defined as the occurrence of any one of the following individual events: non-fatal MI, non-fatal stroke, cardiac hospitalisation due to heart failure, and CV death. The number and percent of subjects with MACCE, overall and by each individual event will be tabulated by treatment arm. Ninety five (95%) confidence intervals for the MACCE event rate will be summarised by treatment arm. Survival analysis techniques will also be used to summarise and analyse time to the first occurrence of a MACCE. |

**Schedule of Procedures**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  VisitAssessment | Screening | Baseline | Treatment Period | End of Study |
| Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 |
|  | Day 1 | Day 14 ±2 | Day 30 ±2 | Day 60 ±2 | Day 90 +7 | Day 120-150 ±7 |
| Consent | X |  |  |  |  |  |  |
| Inclusion/exclusion criteria | X | X |  |  |  |  |  |
| Demographics | X |  |  |  |  |  |  |
| Medical history | X |  |  |  |  |  |  |
| (Abbreviated) PE | X | (X) | (X) | (X) | (X) | X | X |
| Vital signs | X | X | X | X | X | X | X |
| 12 lead ECG | X | X |  |  |  |  |  |
| Biochemistry | X | X | X | X | X | X | X |
| Haematology | X | X | X | X | X | X | X |
| Prostate specific antigen (male participants) |  | X |  |  |  | X |  |
| Coagulation studies | X | X | X | X | X | X | X |
| Urinalysis | X | X | X | X | X | X | X |
| Serum pregnancy test for WOCBP | X |  |  | X | X | X | X |
| Randomisation |  | X |  |  |  |  |  |
| Dispense IP |  | X |  |  | X |  |  |
| AE assessment |  | X | X | X | X | X | X |
| Concomitant medication assessment | X | X | X | X | X | X | X |
| IP accountability |  |  | X | X | X | X |  |

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**LIST OF ACRONYMS AND ABBREVIATIONS**

|  |  |
| --- | --- |
| **Abbreviation** | **Definition** |
| ACC | American College of Cardiology |
| ACE | Angiotensin Converting Enzyme |
| AE | Adverse Event |
| AHA | American Heart Association |
| ALP | alkaline phosphatase  |
| ALT | alanine aminotransferase |
| ANCOVA | Analysis of Covariance |
| ARB | Angiotensin II Receptor Blocker |
| AST | aspartate aminotransferase  |
| AUCinf | Area under (concentration-time) curve to infinity |
| AUClast | Area under (concentration-time) curve to last time-point |
| BNP | Brain natriuretic peptide |
| BP | Blood pressure |
| BUN | Blood urea nitrogen |
| C | Celsius |
| CEC | Clinical Endpoints Committee |
| CEO | Chief Executive Officer |
| Cave | Average plasma concentration over 24 hours |
| Cmax | Maximum observed plasma concentration |
| Cmin | Minimum observed plasma concentration |
| CNS | Central nervous system |
| COX-2 | cyclooxygenase-2  |
| CPK | creatine phosphokinase  |
| CRA | Clinical Research Associate |
| CRF | Case Report Form |
| DMC | Data Safety Monitoring Committee |
| ECG | Electrocardiogram |
| eGFR | estimated glomerular filtration rate |
| ESV | End systolic volume |
| F | Fahrenheit |
| FAS | Full Analysis Set |
| GCP | Good Clinical Practice |
| GGT | gamma glutamyl transferase  |
| GLP | Good Laboratory Practice |
| GRAS | Generally Regarded as Safe |
| HDPE | High density polyethylene |
| hERG | human ether-à-go-go-related gene |
| HIV | Human Immunodeficiency Virus |
| HPMC | Hydroxypropyl methylcellulose |
| hsCRP | high sensitivity C-reactive protein |
| IB | Investigator’s Brochure |
| ICH | International Conference on Harmonisation |
| ID | Identification |
| IEC | Independent Ethics Committee |
| IP | Investigational product |
| ITT | Intent-to-Treat |
| IUD | Intrauterine device |
| IV | Intravenous |
| IWRS | Interactive web response system |
| kg | Kilogram |
| LDH | lactate dehydrogenase |
| LS Mean | Least Squares Mean |
| LV | Left ventricular |
| LVEDVi | Left ventricular end diastolic volume (indexed) |
| LVEF | Left ventricular ejection fraction |
| LVESVi | Left ventricular end systolic volume (indexed) |
| MACCE | Major cardiac and cerebrovascular events |
| MAD | Multiple ascending dose |
| mg | milligram |
| MI | Myocardial infarction |
| min | Minute |
| mITT | Modified Intent to Treat |
| mL | Millilitre |
| MMRM | mixed model repeated measures  |
| MTD | maximum tolerated dose |
| nM | nanomols |
| NOAEL | No observed adverse effect level |
| NSAID | non-steroidal anti-inflammatory drug |
| NT-proBNP | Brain natriuretic peptide type B |
| NYHA | New York Heart Association |
| PAD | Pharmacologically active dose |
| PCEs | polychromatic erythrocytes  |
| PCI | Percutaneous coronary intervention |
| pg | picogram |
| PI | Principal Investigator |
| PICF | Subject Information and Consent Form |
| PK | Pharmacokinetic |
| PP | Per Protocol |
| PPP | PharmPackPro |
| PSA | Prostate specific antigen |
| PT | prothrombin time |
| PTT | partial thromboplastin time |
| QTc | Corrected QT interval |
| QTcB | Bazzet’s corrected QT interval |
| RBC | Red blood cell |
| SAD | single ascending dose |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SC | Steering Committee |
| SOP | Standard Operating Procedure |
| SRM | Study Reference Manual |
| STEMI | ST elevation myocardial infarction |
| T1/2 | Terminal elimination half-life |
| TK | Toxicokinetic |
| Tmax | Time to maximal concentration |
| ULN | Upper limit of normal |
| uM | micromol |
| WBC | White blood cell |
| WOCBP | Woman of child-bearing potential |

# Background and rationale

Investigators should be familiar with the current NP202 Investigator’s Brochure (IB).

## Ventricular arrhythmias and NP202

Armaron Bio Pty Ltd (previously NeuProtect Pty Ltd) has developed a novel compound, NP202, which has been shown reduce ventricular arrhythmias in sheep by inducing ischaemia and reperfusion. In this model, sheep were given a coronary ligation for 60 minutes. They were dosed with a single dose of study drug or placebo immediately prior to reperfusion. The study drug significantly reduced infarct size. In the placebo group, 9 of 26 sheep died from ventricular fibrillation. In the study group, none of the sheep died.

## Nonclinical Studies With NP202

A standard International Conference on Harmonisation (ICH)-recommended battery of nonclinical tests including *in vitro* and *in vivo* safety pharmacology, toxicokinetic and pharmacokinetic studies, acute, sub-chronic and chronic repeat-dose toxicity and genotoxicity studies have been conducted. More detailed discussion of these studies is provided in the current version of the IB.

### Pharmacology

NP202 is a synthetic flavonol. The mechanism of action is thought to be inhibition of pro-apoptotic kinases of the death-associated protein kinase family.

The safety pharmacology profile for NP202 has been evaluated in a comprehensive program of Good Laboratory Practices (GLP)-compliant studies. The results of these studies indicate that NP202 is without effect on the cardiovascular system as evidenced by a lack of an *in vitro* effect on hERG (human ether-à-go-go-related gene) channel current and a lack of effect on blood pressure and electrocardiogram (ECG) parameters following oral administration in conscious telemetered dogs. In addition, no effects of oral NP202 were observed on the central nervous system (CNS) or the respiratory system of rats.

### Pharmacokinetics and Metabolism

The pharmacokinetics (PK)/toxicokinetics (TK) of NP202 have been evaluated in a number of studies in rats and dogs. These include 5 studies in rats in which the oral (4 studies) and/or IV (2 studies) routes were evaluated and 4 studies in dogs in which the oral (3 studies) or IV (1 study) routes were evaluated.

NP202 is rapidly converted (hydrolysed) to NP201 *in vivo* by non-specific plasma esterases. The cumulative data from nonclinical PK/TK and metabolism studies suggest that NP202 is absorbed in a dose-dependent manner following oral administration, undergoes metabolism (via hydrolysis) to form NP201 and subsequently various Phase II‑mediated conjugated forms of NP202 and NP201 (e.g. glucuronidated, O‑methylated, and/or sulfated metabolites) that contribute to overall plasma exposure, and is excreted along with its metabolites in the urine. The metabolic pathways for NP202 and NP201 appear to be similar in rats, dogs, and humans with slight differences noted in the rate and extent of metabolite generation.

Studies have not been conducted to evaluate the distribution of NP202.

### Toxicology

The results from a comprehensive program of toxicology studies support a favourable safety profile for NP202 in rats and dogs. Single-dose oral studies indicate that NP202 is well-tolerated in rats and dogs at dose levels up to 2,000 mg/kg body weight and 1,000 mg/kg body weight, respectively (the highest dose levels evaluated in each species). Results from pivotal, GLP-compliant, repeat-dose studies indicate that exposure to NP202 is dose‑dependent in rats and dogs administered oral doses of 100, 300, and 1,000 mg/kg body weight/day. The available data indicate that NP202 appears to be well-tolerated and without adverse effects when administered for up to 90 days at oral dose levels of up to 1,000 mg/kg body weight/day in rats (the highest dose level evaluated) and 300 mg/kg body weight/day in dogs (the mid-dose level).

Positive data were reported in a single bacterial strain in a bacterial reverse mutation assay conducted with NP202 and NP201, but NP202 was negative in an *in vitro* chromosome aberration assay in mammalian cells and did not cause an increase in micronuclei formation in bone marrow polychromatic erythrocytes (PCEs) of rats following administration of a single oral dose of up to 1,500 mg/kg body weight (the maximum tolerated dose (MTD) in this study as determined in a preliminary test). It is considered that the positive bacterial reverse mutation assay data for NP202 and NP201 represent a minimal concern with regard to oral administration of NP202 in humans. This is based on a review of the available information which includes the negative findings for NP202 in a mammalian chromosome aberration assay and an *in vivo* micronucleus assay in rats, the negative carcinogenicity profile (even with a positive bacterial reverse mutation profile) for the model flavonol quercetin [which is structurally similar to NP202 and has Generally Recognised As Safe (GRAS) status for use as a food ingredient], and the demonstrated metabolism of NP202 (and NP201) in the intact mammalian system that is not simulated under the conditions of the bacterial reverse mutation assay.

## Clinical Studies With NP202

NP202 has been administered in single doses up to 1600mg, and multiple doses up to 1000mg in a single study. A phase 1 single ascending dose (SAD) and multiple ascending dose (MAD) study was conducted in healthy volunteers. A placebo-controlled Phase 2 trial is currently in progress using 1000 mg once daily dosing for the efficacy, safety and tolerability of NP202 in in adults who have left ventricular systolic dysfunction following myocardial infarction.

### Safety

In the SAD cohorts 8 males per cohort received a single dose of 50, 200, 600, 1000 or 1600mg of NP202, or placebo, in a 3:1 ratio. NP202 was very well tolerated, with no serious adverse events (SAE) and few adverse events (AEs). There were no AEs associated with vital signs, ECG, haematology or biochemistry.

In the MAD cohorts 8 males per cohort received a dose of 400 or 1000mg of NP202, or placebo, once a day for 14 days, in a 3:1 ratio. NP202 was very well tolerated, with no SAEs and few AEs. The most common AE was semen discolouration, reported in most subjects (10 of 12) taking NP202. Semen was noted to be a green or yellow colour. Samples were taken to test sperm count, motility and morphology – no clinically significant changes were seen, and no trends observed. There were no AEs associated with vital signs, ECGs, haematology or biochemistry.

### Pharmacokinetics

Intense PK samples were taken in each SAD and MAD cohort. Plasma concentrations of NP202 and NP201 were determined using a validated assay. However, in all samples only very low levels of NP202 were observed, generally less than 60nM, and no NP201 was observed.

This is consistent with other compounds in the class and is due to the high level of gut and/or first-pass hepatic metabolism, which results in little or no parent compound observable in the plasma.

SAD: PK samples were taken from the single ascending dose cohorts at 6 and 24 hours for analysis post sulfatase hydrolysis. These data show a dose dependent increase in NP202 and NP201 plasma levels at 6 hours and 24 hours post dosing, with the concentration of NP201 significantly higher than NP202 at both time points, for each dose level.

The plasma concentration of NP202 at 6 hours was reduced by 24 hours post dose. In contrast the plasma levels of NP201 at 24 hours were increased over those observed at 6 hours post dose. Significant levels of NP201 were observed in the plasma 24 hours after a single oral dose of NP202.

MAD: Samples were taken at 24 hours post dose to give trough levels, and analysed using the sulfatase hydrolysis procedure. Not all NP202 related compounds are cleared by 24 hours, and therefore some accumulation is observed. The data suggests that steady state plasma concentrations are achieved after approximately 3-4 days. Mean plasma trough PK from 400mg dose was 3.5uM, and from 1,000mg was 6uM.

The cumulative data suggest that NP202 is absorbed in a dose-dependent manner following oral administration, undergoes metabolism to form NP201 and various conjugated forms of NP202 and NP201 that contribute to overall plasma exposure, and is excreted along with its metabolites in the urine.

Based on the available data, the metabolic fate of NP202 appears to be comparable to that of other flavanols such as quercetin and fisetin where the amount of “free” parent substance present in the plasma after oral or intra venous (IV) administration is considerably less than the amount of conjugated metabolites[[1]](#endnote-2), [[2]](#endnote-3). Thus, the observed prolonged presence of conjugated NP202-derived metabolites in plasma may represent a source of pharmacologic activity similar to what has been demonstrated in studies with other conjugated flavonols[[3]](#endnote-4), [[4]](#endnote-5).

### Efficacy

Efficacy was not assessed in the phase 1 study.

## Study And Dose Rationale

NP202 has been shown to reduce rhythm disturbances in heart muscle in the ventricles, and it is compelling to discover if it similarly reduces rhythm disturbances in heart muscle in the atria. As AF is the most common rhythm disturbance in the atria, it is the most suitable target to investigate a benefit.

Potential risks to subjects are summarised in the IB.

In determining the dose level to be evaluated in this Phase 2 study, consideration was given to the available clinical and nonclinical data for NP202.

Preliminary data from the Phase 1 clinical trial support the safety and tolerability of NP202 when administered at oral dose levels of up to 1000mg/day for 14 days. Preliminary PK data from the MAD cohorts show that steady state plasma concentrations of NP202 and NP201 conjugates appear to have been achieved after approximately 3 to 4 days of dosing. Mean plasma trough concentrations of NP202-related species were approximately 3.5µM in the 400mg/day cohort and approximately 6µM in the 1000mg/day cohort.

The nonclinical data from 90‑day repeat-dose toxicity studies support the safety of NP202 when administered at oral dose levels of up to 1000mg/kg body weight/day in rats and 300mg/kg body weight/day in dogs (the NOAELs in the respective studies). These dose levels are equivalent to 161 and 167mg/kg body weight/day in humans, respectively (U.S. FDA, 2005). Notably, the PK profile and metabolic pathways for NP202 appear to be similar in rats, dogs, and humans, with NP202 being absorbed in a dose-dependent manner following oral administration and undergoing metabolism to form NP201 and various conjugated forms of NP202 and NP201 that contribute to overall plasma exposure.

Based on the following considerations, a dose level of 1000mg/day (equivalent to 16.7mg/kg body weight/day for a 60kg individual) can be supported for evaluation in this proof-of-concept Phase 2 study in post-MI participants:

* Safety
	+ 1000mg/day is the highest dose level evaluated in Phase 1 MAD cohorts.
	+ 1000mg/day is approximately 10‑fold less than the HED values of 161 and 167mg/kg body weight/day that were calculated from the NOAEL values from 90‑day repeat-dose toxicity studies in rats and dogs, respectively.
* Efficacy
	+ 1000mg/day is anticipated to demonstrate positive signs of activity based on the observation of efficacy at a dose level with a lower HED of 3.2mg/kg body weight/day (equivalent to 192mg/day for a 60kg individual) in a rat model of cardiac remodelling post-infarction.
	+ 1000mg/day is anticipated to provide similar Cmax and Cave, and considerably higher trough levels, as the efficacious dose in a rat model of cardiac remodelling post-infarction.

# Study Objectives

The objectives of this study are to assess the efficacy, and safety of oral NP202.

## Primary Objective

To evaluate the efficacy of NP202 compared to placebo, when administered once daily for 1 month, in attenuating the burden of atrial fibrillation in adults with an implanted cardiac device.

## Secondary Objectives

To evaluate the safety and tolerability of NP202 compared to placebo, when administered once daily for 1 month, in treating atrial fibrillation in adults with an implanted cardiac device.Study Plan

## Overall Trial Design

This is a single-centre, randomised, double blind, placebo controlled study to assess the efficacy, safety and tolerability of NP202.

Subjects will be screened during routine interrogation of their cardiac device. Eligible subjects will have a high burden of paroxysmal atrial fibrillation. They will be randomised and administered their first dose of investigational product (IP) on Study Day 1.

Subjects will take their IP dose once a day for 1 month (30 days). During this treatment period they will return to the site for study visits at Week 2, and Months 1, 2, and 3. Month 3 is the end of the Treatment Period. Subject will return for follow up and the final study visit at Month 4.

A Data Monitoring Committee (DMC) will review safety data at agreed recruitment and progression milestones to provide independent oversight of subject safety.

## Number Of Subjects

It is planned that approximately 20-50 adult participants aged 18 to 80 years (inclusive) will participate in the study.

## Study Period/Duration Of Subject Participation

The duration of the study for each subject includes a Screening Period, followed by a 1 month (30 day) Treatment Period, 1 month Washout Period, 1 month Crossover Treatment Period, and a 1 month Follow Up Period. The screening period will allow sufficient time to review blood tests results prior to treatment beginning. Total study duration for each participant is therefore approximately 5 months.

It is anticipated that recruitment will take approximately 24-36 months. The study period is expected to be 2017 – 2020.

## Subject Selection And Withdrawal

### Trial selection record

Investigators must keep a record of subjects who were considered for the study but were not enrolled.

### General considerations

Only subjects who meet all of the inclusion and none of the exclusion criteria will be eligible to participate in the study.

### Inclusion criteria

Subjects who:

1. Age between 18-80 years.
2. Paroxysmal (not lasting more than 7 days) atrial fibrillation.
3. An implanted device (loop recorder, pacemaker or defibrillator).
4. An AF burden between 1-90% over the past 6 months.
5. Agree to use contraception according to section 2.6.5 for the duration of the study, or are of non-child bearing potential.
6. Are able to provide written informed consent.

### Exclusion criteria

1. Pregnant or breastfeeding females.
2. Estimated glomerular filtration rate (eGFR) <30ml/min.
3. Liver function tests 3 x ULN due to non-cardiac disease
4. Have received any investigational research agent within 30 days or 5 half-lives (whichever is longer) prior to the first dose of IP.
5. History of severe or life-threatening drug allergy and/or known drug hypersensitivity.

### Contraception requirements

The reproductive effects of NP202 have not been tested. Women of child-bearing potential (WOCBP) must agree to use two methods of contraception – at least one being highly effective as listed below - for the duration of the study and until at least 3 months after the last intake of study drug.

Highly effective methods of contraception are those with a failure rate of less than 1% per year and include;

* Implants
* Injectables
* Combined oral contraceptive pill
* Progesterone or copper eluting intrauterine device (IUD)
* True abstinence in line with the preferred and usual lifestyle of the subject
* Sexual intercourse with a vasectomised partner.

A condom is acceptable as a second form of contraception.

Male subjects must agree not to father a child for the duration of the study and until 3 months after the last intake of study drug. Approved methods of contraception for males include the following;

* Vasectomy at least six months prior to screening, with documentation of no sperm in ejaculate.
* Female partner is postmenopausal (at least 12 months since last menstruation), has undergone surgical sterilisation (e.g. tubal occlusion, hysterectomy, bilateral salpingectomy, or bilateral salpingo-oophrectomy) or uses a highly effective method of contraception as listed above, plus condom.

If a study participant or female partner of a male participant does become pregnant during the trial period, they will be asked to consent to the follow up of the pregnancy outcome by the Sponsor.

### Concomitant Medications

Any medications taken other than the IP must be documented in the subject notes and the Case report Form (CRF). This record should include the drug name, the dose and frequency, route of administration, the start and stop date of administration, and the indication.

Any use of concomitant medications taken in the 7 days prior to Day 1 will be recorded on the prior medication CRF. Any use of medications for the treatment of pre-existing conditions or AEs will be recorded on the concomitant medication CRF.

### Withdrawal of Subjects from Study

Subjects can terminate their study participation at any time and without giving a reason, without prejudice to further treatment. Subjects who discontinue from the trial should always be asked about the reason(s) for their discontinuation and about the presence of any AEs. If possible, they should be seen and assessed by an investigator and have an Early Termination Visit. AEs should be followed up until resolved or stable and determined to be chronic.

The Investigator or the Medical Monitor can exclude a subject from continuing in the trial.

Possible reasons for discontinuing a subject may include:

* Subject withdrawal of consent
* Any unacceptable AEs, in the judgement of the Investigator
* Subject’s non-compliance with the protocol

### Stopping Rules / Discontinuation Criteria

There are no specific study stopping rules, however, the DMC will review safety data at time points as specified in the charter, and will be notified of any SAE in the time period defined in the charter.

## Study Treatments

### Description of the Investigational Product

NP202 is a light yellow crystalline powder. It is a synthetic compound produced in four steps from commercially available starting materials, with purity typically greater than 98%. NP202 will be presented as capsules containing NP202 and microcellulose, as required. NP202 will be encapsulated into gelatine capsules.

Identical placebo capsules will contain microcellulose.

### Packaging and labelling

The study formulations will be supplied by Armaron Bio Pty Ltd. The NP202 drug substance was manufactured by Piramal Healthcare, 110 Industrial Parkway North, Aurora, Ontario L4G 3H4, Canada.

The drug product is manufactured by PharmPackPro (PPP), Port Melbourne, Australia. Labels will be in accordance with all applicable regulatory requirements. Product will be labelled by PPP.

IP will be supplied as oral gelatine capsules containing 500mg of NP202, or placebo, and packaged in high density polyethylene (HDPE) bottles. IP will be supplied to the study sites after receipt of required documents in accordance with all applicable regulatory requirements. Each bottle will contain capsules for 30 days plus sufficient overage.

### IP storage and handling

All IP supplies must be stored at or below 25°C (77°F) (not frozen) and protected from light,

Only subjects enrolled in the study may receive IP. Prior to dispensing, IP supplies will be stored securely under the appropriate conditions at the clinical trial site in a secure area with access limited to authorised staff, and according to relevant laws

The Principal Investigator (PI) or authorised designee will ensure that the IP at site is safely stored in compliance with the storage requirements. The PI is responsible for ensuring that the IP is dispensed in accordance with the protocol and only to subjects enrolled in the study. Authorised study personnel will dispense the IP according to the randomisation schedule.

### Accountability of study supplies

All material supplied is for use only in this clinical study and should not be used for any other purpose.

The PI or designee is responsible for IP accountability, reconciliation and record maintenance. The PI or designated site staff must maintain IP accountability records throughout the course of the study including records of the amount of IP received, the identification of the subject for whom it was dispensed, and the date(s) and quantity of the IP dispensed.

IP supplies, including unused, partially used or empty bottles, will either be returned by the site to Armaron Bio Pty Ltd or designee, or destroyed on site if facilities and procedures are available. Records shall be maintained by the PI of any disposition of the IP. These records must show the identification and quantity of IP disposed of, the method of destruction, and the person who disposed of the IP.

### Doses and treatment regimens

Approximately 20-50 subjects, who fulfil all the entry criteria, will be randomised in the study.

Subjects will be randomly assigned to one of the following treatment arms:

* 10 will receive NP202 1000 mg once daily for 1 month, followed by 1 month washout, and then placebo once daily for 1 month.
* 10 will receive placebo once daily for 1 month, followed by 1 month washout, and then NP202 1000 mg once daily for 1 month.

Subjects will be instructed to take two capsules once per day during the treatment period.

### Method of assigning subjects to treatment group

Subject eligibility will be established before randomisation. Eligible subjects will be assigned to either NP202 or placebo in a 1:1 ratio according to the randomisation schedule.

At each site, a subject identification (ID) number will be allocated to each subject who provides informed consent, so that subjects can be identified without making assumptions about their subsequent eligibility for the main trial. Subjects will be allocated to sequential, ascending 3-digit ID numbers (001, 002, 003 *etc.*), which will provide a unique identifier. The subject will retain the same ID number for the duration of the study.

If a subject fails screening and is not randomised, or discontinues from the trial, the subject’s ID number will not be reused.

### Blinding and procedures for breaking the blind

At each study drug dispensing visit, subjects will be allocated a bottle number by an interactive web or voice response system (IW/VRS). The subject, site, sponsor and monitoring personnel will be blind to whether the subject is receiving NP202 or placebo.

Due to the colour change that NP202 causes in semen, it is possible that subjects or study staff may be unblinded. However, as the study efficacy endpoints are objective and will be assessed by blinded reviewers, this does not compromise the study objectives.

The IW/VRS will provide the option for an investigator to break the blind. Sites will be instructed to break the blind only in situations in which the investigator determines that adequate medical care cannot be provided without knowing the treatment assignments. If a code break must occur, the investigator or designee must contact the study Medical Monitor before unblinding. Details for contacting the Medical Monitor will be provided in the Study Reference Manual (SRM).

If the blind has been broken, the investigator must document the date and the reason the blind was broken in the subject’s notes and CRF.

### Treatment compliance

Subject compliance will be assessed at each study visit by capsule count. Subjects will be considered compliant if they take ±10% of scheduled doses. Subjects who are non-compliant should be reminded of the dosing requirements and advised that they may be withdrawn from the study if compliance does not improve.

# study procedures

## Subject Information

The Investigator must provide adequate information regarding the study conduct and obtain written informed consent from the subject before any tests or investigations outlined in the study protocol are carried out.

The subject will be given time to read and understand the Participant Information and Consent Form (PICF) and have any questions answered. They may wish to take the PICF and consider it further, or to discuss it with their family before signing.

The PICF must be personally signed and dated by both the Investigator obtaining consent and the subject.

## Screening Visit

The purpose of the Screening visit is to confirm subject eligibility against inclusion and exclusion criteria.

Once written informed consent has been obtained, the following visit assessments and procedures will be performed;

* Review eligibility criteria.
* Record subject demographic data.
* Review and record medical history.
* MI symptoms, treatment and intervention history
* Physical examination including height and weight (see Section 5.2).
* Vital signs (see Section 5.2).
* 12-lead ECG (see Section 5.2).
* Safety laboratory tests including haematology, biochemistry, coagulation studies and urinalysis (see Section 5.2). Laboratory tests performed any time post PCI may be used as Screening results, if relevant (i.e. it is not required to repeat tests specifically for study screening if the tests have already been done and meet entry criteria)
* Serum pregnancy test for WOCBP.
* Concomitant medication review.

### Re-Screens

One repeat test of each individual screening assessment (e.g., laboratory tests, ECG) is allowed, within the screening period. Subjects who subsequently fail screening may not be re-screened.

## Baseline (Day 1)

The Baseline visit may be done on the same day as Screening, if all Screening results are available and confirmed. If done on the same day as Screening safety bloods, these may be used as baseline safety bloods.

At baseline, the following will be performed:

* Confirm subject continues to meet eligibility criteria.
* Document any changes in physical examination (abbreviated physical examination).
* Vital signs.
* 12-lead ECG.
* Safety laboratory tests including haematology, biochemistry, PSA (males), coagulation studies and urinalysis.
* Upon confirmation of eligibility and suitability to proceed, subjects will be randomised and receive their first dose of IP.
* AE and concomitant medication assessments.

## Days 14, 30 And 60

There is a ±2 day window on the each visit. Subjects will return to the site for the following assessments;

* Abbreviated physical examination.
* Vital signs.
* Safety laboratory tests including haematology, biochemistry, coagulation studies and urinalysis.
* Serum pregnancy test for WOCBP (Day 30 and Day 60 only).
* AE and concomitant medication assessments.
* Review of compliance and IP accountability.

## Day 90 (+7) / Early Termination

Subjects will return to the site for the following assessments;

* Physical examination.
* Vital signs.
* Safety laboratory tests including haematology, biochemistry, PSA (males), coagulation studies and urinalysis.
* Serum pregnancy test for WOCBP.
* AE and concomitant medication assessments.
* Review of compliance and IP accountability.

This is the end of the treatment period.

## Month 4/End Of Study

Subjects will return to the site for the following assessments;

* Physical examination.
* Vital signs.
* Safety laboratory tests including haematology, biochemistry, coagulation studies and urinalysis.
* Serum pregnancy test for WOCBP.
* AE and concomitant medication assessments.

## Early Termination Visit

Subjects who withdraw from the study prior to completion of the treatment period should be asked to return to the site for a final follow up visit, and have the Day 90 visit performed.

# Trial Endpoints

## Efficacy Endpoints

### Primary

* Change from baseline in total burden of atrial fibrillation per month, expressed as a percentage, as determined by device interrogation.

### Secondary

* Change from baseline in frequency of atrial fibrillation events per month, expressed as a number, as determined by device interrogation.
* Change from baseline in duration of atrial fibrillation per month, measured in minutes, as determined by device interrogation.

## Safety And Tolerability Endpoints

* Physical examination parameters assessed at visits throughout the study
* Incidence and severity of treatment emergent AEs throughout study
* Occurrence of MACCE throughout the study, including:
	+ Cardiovascular death
	+ Non-fatal myocardial infarction
	+ Non-fatal stroke
	+ Cardiac hospitalisation due to heart failure
* Safety laboratory evaluations (biochemistry, haematology, PSA (males), coagulation studies, urinalysis) throughout study
* Vital sign values assessed at visits throughout the study

# Trial Measurements

## Efficacy

The burden of AF will be assessed using a Cardiac Device Programmer. The AF burden will comprise of counting the episodes and duration of AF, and the total percentage of AF. Prior to commencing randomisation, if there is more than one month of cardiac device data available, the amount of AF will be averaged to represent a monthly burden of AF. After commencing the study, no further data will be averaged and only monthly burden of AF will be used.

## Safety And Tolerability

Safety will be assessed by recording of AEs, MACCE, vital signs, laboratory parameters, and physical examinations.

* Physical examinations during the study will include height (at Screening only) and weight, and assessment of skin, head, neck, lymphatic, eyes, ears, nose and throat, abdomen, and respiratory, cardiovascular/peripheral vascular, endocrine, central nervous, genitourinary and musculoskeletal systems as appropriate to determine general condition. New or worsening clinically significant abnormalities will be reported as an AE. An abbreviated PE will be conducted at some visits based on prior findings and symptoms.
* Subjects will be questioned and monitored throughout the study with regard to any AEs they may have experienced. See Section 7 for further details on recording AEs.
* Blood and urine samples will be taken throughout the course of the study for safety assessments. The following specific tests will be performed at the site’s local pathology laboratory:

Haematology: Haemoglobin, haematocrit, red blood cell (RBC) count, white blood cell (WBC) count with differential, and platelet count.

Serum Chemistry: Sodium, potassium, blood urea nitrogen (BUN), serum albumin, total protein, gamma glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, cholesterol, glucose, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), and creatinine (calculated creatinine clearance using the Cockcroft and Gault formula).

Prostate Specific Antigen: all male participants

Coagulation: Partial thromboplastin time (PTT), prothrombin time (PT), thrombin time

Urinalysis: Dipstick (specific gravity, pH, glucose, protein, blood, ketones, urobilinogen, nitrite, leukocyte esterase). If protein, nitrite AND leukocyte esterase are positive, microscopic examination of urine sediment will be performed (RBC, WBC, epithelial cells, crystals, casts, bacteria).

* Vital signs will be measured after the subject has been supine for 5 minutes and will include blood pressure (BP) on the same arm (if possible) throughout study, pulse rate, respiratory rate and temperature.

# STUDY OVERSIGHT

## Data Monitoring Committee

An independent DMC will be established prior to recruitment start, with appropriate charter that defines its roles and responsibilities. The DMC will monitor accruing trial safety results at intervals throughout the study. The main purpose of the DMC will be to protect the interests of the subjects included in the trial.

The charter will specify the intervals for formal DMC meetings.

The DMC will convey to the Investigators their recommendations as to whether the trial may continue as planned or if the trial should be modified or stopped. The final decision on whether the study should be modified or stopped will be the responsibility of Investigators, in consultation with the IEC.

# Adverse events

The definitions of AEs and SAEs are given below. It is extremely important that all staff involved in the trial are familiar with the content of this section. The PI is responsible for ensuring this.

## Adverse Event Definitions

An AE is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not related to the medicinal (investigational) product.

Laboratory reference ranges are defined by upper or lower limits of parameters of the laboratory. The Investigator should ensure that each parameter out of the normal range is assessed for clinical significance and potential for being an AE. It is at the discretion of the Investigator to document any change in laboratory result as an AE if he considers the change to be clinically significant, even if the absolute value is within the alert limit or reference range.

The subject must be instructed to inform the Investigator about all AEs and these must be documented in the subject records and Case Report Form (CRF) together with their intensity;

* Severe are those AEs which make normal daily routine impossible.
* Moderate AEs impact the normal daily routine
* Mild AEs do not impact normal daily routine.

An AE that is assessed as severe should not be confused with a SAE. Severity is a category utilised for rating the intensity of an event, and both AEs and SAEs can be assessed as severe. An event is defined as ‘serious’ when it meets one of the pre-defined outcomes as described in Section 7.2.

The Investigator must assign causality to eachadverse event in relation to NP202 based on the following scale:

* **Not related:** AE for which there is evidence of another explanation, e.g. the adverse event is obviously explained by the subject’s disease(s), is in accordance with the known effect of a concomitant medication, or has occurred prior to first administration of NP202.
* **Unlikely related:** AE with a time to NP202 administration that makes a relationship improbable (but not impossible), and disease or other drugs provide plausible explanations
* **Possibly related:** AE with a reasonable time relationship to NP202 administration, but which could also be explained by disease or other drugs. Information on NP202 withdrawal may be lacking or unclear
* **Probably related:** AE with reasonable time relationship to NP202 administration that is unlikely to be attributed to disease or other drugs. Response to NP202 withdrawal is clinically reasonable. Rechallenge is not required
* **Definitely related:** AE with plausible time relationship to NP202 administration which cannot be explained by disease or other drugs. Response to NP202 withdrawal is plausible (pharmacologically, pathologically), and event is definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognised pharmacological phenomenon). Rechallenge, if performed/necessary, is satisfactory.

All AEs must be documented by the Investigator, regardless of causality.

Expected AEs are defined as all AEs stated in the IB. If an AE has not been previously reported (including type, degree, or frequency) in the IB, it is an unexpected adverse event.

If an AE leads to premature discontinuation of the study, the appropriate pages of the CRF must be completed.

## Serious Adverse Events (SAEs)

An AE shall be classified as serious if it:

* Results in death.
* Is life-threatening.

Life threatening in the definition of serious refers to an event in which the subject 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.

* Requires in-patient hospitalisation or prolongation of existing hospitalisation.

Hospitalisation is defined as in-patient admission or care regardless of duration.

Out-patient treatment in an emergency room is not in itself an SAE, although the reasons for it may be (e.g. bronchospasm, laryngeal oedema). Elective surgery, hospitalisation for social reasons (with no causal AE), or hospital admissions and/or surgical operations planned before or during this study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

* Results in persistent or significant disability/incapacity.
* Is a congenital anomaly/birth defect.
* Is an important medical event.

This includes events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed above.

## Recording Of Adverse Events

AEs will be captured from the time of informed consent until the final study visit. Subjects will be asked at each visit whether they have experienced any AEs. SAEs possibly related to NP202 occurring to a study subject after the AE reporting period will be reported to the sponsor if the Investigator becomes aware of them.

It is preferable that AEs are reported as diagnoses if one is able to be made, rather than individual signs and symptoms. The AE description, start and stop dates, intensity, causality and outcome must be recorded, as well as any actions taken.

Unless a diagnosis is made, or signs and symptoms are present, laboratory values or vital signs abnormalities should only be reported as AEs if they cause the subject to discontinue from the trial, the investigator feels it is clinically significant, or they meet a criterion for a SAE.

## Reporting Of Serious Adverse Events

All SAEs will be recorded in the subject records and the CRF. Study investigators areresponsible for informing the regulatory authorities of the SAE as appropriate.

The investigator must also notify their Independent Ethics Committee (IEC) of any SAEs occurring at their site, within the time period specified by the IEC.

## Follow-Up Of Adverse Events And Serious Adverse Events

All AEs and all SAEs must be followed by the Investigator until resolution, until the AE stabilises or is recognised as a permanent condition by the Investigator, or until the subject is lost to follow up, whichever comes first. Follow-up investigations may be necessary according to the Investigator’s medical judgement.

# DATA management

Data collection and entry into the CRF will be completed by authorised study site personnel designated by the Investigator. Appropriate training and security measures will be completed with the Investigator and all authorised study site personnel prior to the study being initiated and any data being entered into the system for any study subjects.

All data must be entered in English. The CRFs should always reflect the latest observations on the subjects participating in the trial; therefore, the CRFs are to be completed as soon as possible after the subject’s visit. The Investigator must verify that all data entries in the CRFs are accurate and correct. If some assessments are not done, or if certain information is not available or not applicable or unknown, this should be indicated in the CRF. The Investigator will be required to sign off on the clinical data.

# Statistical Analysis

The statistical analysis principles described below will be supplemented by a comprehensive statistical analysis plan (SAP) which will be finalised before the database is locked. Any changes to the statistical plans will be described and justified in the final report.

## Provisional Stastical Analysis Plan

Continuous variables will be reported as mean ± standard deviation or as median and percentiles if appropriate. Normally distributed variables will be compared using the paired Student’s t-test. Otherwise comparisons between both the groups will be performed using the Mann–Whitney U test. Categorical variables will be stated as absolute and relative frequencies and compared using the χ2 test. All tests are two-tailed. A P-value of <0.05 will be considered as statistically significant.

## Analysis Sets

The following sets will be used for the statistical analyses:

**Full analysis set (FAS):** All subjects randomised into the study.  Subjects will be analysed according to treatment to which they were randomised. Efficacy analyses performed in the FAS are considered supportive of analyses performed in the mITT set.

**Safety set:** All randomised subjects who received at least one dose of study medication. Subjects will be analysed according to the treatment received.

**Modified Intention-to-treat (mITT) set:** All randomised subjects who received at least one dose of study medication. Subjects will be analysed according to the treatment to which they were randomised. The primary efficacy analysis will be performed in the mITT set.

**Per-protocol (PP) set:** All subjects from the mITT population who completed the study in compliance with the protocol and who reported no major violation of the study protocol. Subjects will be analysed according to the treatment to which they were randomised. The final decision to exclude a subject from the PP set will be taken during a blinded data review meeting before database lock. Efficacy analyses performed in the PP set are considered supportive of analyses performed in the mITT set.

## Data Analysis Considerations

All efficacy and safety data will be listed and summarised using descriptive statistics by treatment group and nominal time. The descriptive summary for the categorical variables will include counts and percentages. The descriptive summary for the continuous variables will include means, medians, standard deviations and minimum and maximum values. Where possible, data from subjects who withdraw prematurely from the study will be included in any analysis. Further details on the handling of withdrawals and/or missing data will be specified in the SAP.

All statements of statistical significance will be based on a two-sided test at the 5% level of significance, unless stated otherwise. Further details will be specified in the SAP.

The baseline value for each clinic assessment will be the last pre-dose value obtained.

Full details of the statistical analyses will be presented in the SAP. Any deviations from the planned analyses detailed in the protocol will be documented in the SAP and final study report. If the study is prematurely discontinued, all available data will be listed and a review will be carried out to determine which statistical analyses are considered appropriate.

## Safety Data

### Extent of exposure

The duration of exposure and number of subjects exposed to study treatment will be summarised by treatment received.

### Adverse events

AE data will be listed individually and incidence of AEs summarised by system organ class and preferred terms within a system organ class for each treatment group. When calculating the incidence of AEs, each AE, based on preferred terminology defined by Medical Dictionary for Regulatory Activities (MedDRA; Version 13.1, or later), will be counted only once for a given subject. A summary of the number and percent of subjects with the following treatment emergent AEs will be displayed by treatment groups:

* All AEs
* Drug-related AEs
* Severe AEs
* SAEs
* AEs leading to permanent discontinuation of NP202.

### MACCE analysis

MACCE is defined as the occurrence of any one of the following individual events: non-fatal MI, non-fatal stroke, cardiac hospitalisation due to heart failure, and CV death. The number and percent of subjects with MACCE, overall and by each individual event will be tabulated by treatment arm. Ninety five (95%) confidence intervals for the MACCE event rate will be summarised by treatment arm. Survival analysis techniques will also be used to summarise and analyse time to the first occurrence of a MACCE. Right censoring will be applied to non-CV deaths, early terminations, and study completion. Kaplan Meier estimates of MACCE will be tabulated over time by treatment arm. The treatment difference for time to MACCE will be assessed using the log-rank test.

### Clinical laboratory evaluations

Summary statistics will be presented by treatment group for each laboratory value and change from baseline in each laboratory value at every assessment.

Each laboratory value will be flagged to show whether it is a value within, below, or above the normal range.

### Other safety measures

Continuous variables will be summarised along with the change from baseline at each time point by treatment group. Other variables will be summarised as appropriate to the data.

# TRIAL management

## Quality Control and Quality Assurance

### Monitoring

Study monitoring will be performed in accordance with applicable regulations, ICH Good Clinical Practice (GCP), and study site\ Standard Operating Procedures (SOPs).

Before the start of the trial, the PI will ensure facilities are adequate and discuss responsibilities with the site staff with regards to following the protocol and regulatory and ethical requirements.

During the trial, the PI will regularly monitor and confirm protocol, regulatory and ethical adherence, confirm data accuracy and provide information and support as needed.

The PI agrees to allow the CRA direct access to all relevant documents, including electronic medical records, and to allocate his time and the time of his staff to the CRA to discuss findings and any relevant issues.

Site staff will be provided with CRA and back up contact details in the event they have queries or require assistance.

## Training Of Staff

Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective tasks.

The PI will maintain records of all individuals involved in the trial at their site. The PI will ensure that appropriate training relevant to the trial is given to all these staff, and that they will receive any new information relevant to the performance of this trial in a timely manner.

## Changes To The Protocol

If it is necessary for the trial protocol to be amended, the amended protocol must be approved by the IEC, unless the immediate safety of subjects is involved.

If a protocol amendment requires a change to the PICF, approval of the revised PICF by the IEC is required before the revised form can be used.

## Trial Agreements

The PI must comply with all the terms, conditions and obligations of the trial agreement for this trial. In the event of any inconsistency between this protocol and the trial agreement, the trial agreement shall prevail.

## Trial Timetable And Termination

The planned start date for this trial is February 2018. The proposed completion date is in 2020.

## Ethics Review

The protocol and the PICF will be submitted for approval to the IEC, and must be approved or given a favourable opinion in writing as appropriate.

Any amendment to the protocol will be sent to the IEC. No deviations from or changes to the protocol will be implemented without documented approval/favourable opinion from the IEC of an amendment, except where necessary to eliminate an immediate hazard to a trial subject, or when the changes involve only logistical or administrative aspects of the trial.

The deviations from or changes to the protocol which were implemented to eliminate an immediate hazard to a trial subject and the proposed amendment, if appropriate, should be submitted to the IEC for review and approval as soon as possible.

The PI must submit progress reports to the IEC according to local regulations and guidelines. The PI must also provide the IEC with any reports of SAEs from the trial site in accordance with the IEC requirements and timelines.

## Ethical Conduct Of The Study

The trial will be performed in accordance with the ethical principles in the Guidelines of the World Medical Association's Declaration of Helsinki in its revised edition (Fortaleza, Brazil, October 2013), ICH GCP, the approved study protocol, and applicable regulatory requirements.

## Insurance And Liability

Insurance and liability will be covered by Treasury Managed Funds, in accordance with NSW Health investigator-initiated trials.

## Subject Information And Informed Consent

The PI at each centrewill ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and potential benefits of the trial. Subjects must also be notified that they are free to discontinue from the trial at any time. The subject should be given the opportunity to ask questions and should be allowed time to consider the information provided.

The subject’s signed and dated informed consent must be obtained before conducting any procedure specifically for the trial. The site investigator must store the original, signed PICF. A copy of the signed and dated PICF must be given to the subject.

## Data Protection

The PICF will explain that trial data will be stored in a computer database, maintaining confidentiality in accordance with national data legislation. Subjects in this database will be identified by subject ID number only. The PICF will also explain that for data verification purposes, regulatory authorities, IECs or sites may require direct access to parts of the hospital or site records relevant to the trial, including personal subject information.

## Archiving

The PI is responsible for the archiving of the trial records for their site. Trial records include the subject files as well as the source data, the Investigator Site File, pharmacy records, and other study documents. Trial records must be archived for at least 15 years (or at least 2 years since the formal discontinuation of clinical development of NP202).

If the PI leaves the investigational site for whatever reason, the responsibility for all study related records must be transferred to another person at site.

## Publication Policy

The data is owned by the investigators. An investigator may publish any data related to this study (poster, abstract, paper, slide presentation, etc.) without having consulted with or received approval from Armaron Bio Pty Ltd in advance.

# References

1. Shia CS *et al*. Metabolism and Pharmacokinetics of 3,3′,4′,7-Tetrahydroxyflavone (Fisetin), 5-Hydroxyflavone, and 7-Hydroxyflavone and Antihemolysis Effects of Fisetin and Its Serum Metabolites. J. Agric. Food Chem. 2009, 57, 83–89 [↑](#endnote-ref-2)
2. Galindo P, Rodriguez-Go´mez I, Gonza´lez-Manzano S, Duen˜ as M, Jime´nez R, et al. (2012). Glucuronidated Quercetin Lowers Blood Pressure in Spontaneously Hypertensive Rats via Deconjugation. PLoS ONE 7(3): e32673. doi:10.1371/journal.pone.0032673 [↑](#endnote-ref-3)
3. Lodi F, *et al*. Glucuronidated and sulfated metabolites of the flavonoid quercetin prevent endothelial dysfunction but lack direct vasorelaxant effects in rat aorta. Atherosclerosis 204 (2009) 34–39 [↑](#endnote-ref-4)
4. Menendez C, *et al*. Vascular deconjugation of quercetin glucuronide: The flavonoid paradox revealed? Mol. Nutr. Food Res. 2011, 55, 1–11 [↑](#endnote-ref-5)