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Efficacy, Safety and Response Predictors of Adjuvant Astragalus for Diabetic Kidney Disease (READY) – Study Protocol of an Add-on, Assessor-blind, Parallel, Pragmatic Randomised Controlled Trial

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Running Title

Randomised controlled trial of astragalus for diabetic kidney disease

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Abstract

Introduction

Diabetic kidney disease (DKD) is a prevalent and costly complication of diabetes with limited therapeutic options, leading the cause of end-stage renal disease in most developed regions. Recent big data studies showed that add-on Chinese medicine (CM) led to reduced risk of end-stage renal disease and mortality among chronic kidney disease patients with diabetes. Astragalus, commonly known as huang-qi, is the most prescribed CM or used dietary herb in China for diabetes and DKD. *In vivo* and *in vitro* studies showed that astragalus could ameliorate podocyte apoptosis, foot process effacement, mesangial expansion, glomerulosclerosis and interstitial fibrosis. Nevertheless, the clinical effect of astragalus remained uncharacterised. This pragmatic clinical trial aims to evaluate the effectiveness of add-on astragalus on type 2 diabetic patients with stage 2 to 3 chronic kidney disease and macroalbuminuria and identify related response predictors.

Methods and analysis

This is an add-on, assessor-blind, parallel, pragmatic randomised controlled clinical trial.118 patients diagnosed with DKD will be recruited and randomised 1:1 to a 48-week add-on astragalus or standard medical care. Primary endpoints are the changes in estimated glomerular filtration rate and urine albumin-to-creatinine ratio between baseline and

treatment endpoint. Secondary endpoints include adverse events, fasting blood glucose, glycated haemoglobin, lipids and other biomarkers. Adverse events are monitored through self-complete questionnaire and clinical visits. Outcomes will be analysed by regression models. Subgroup and sensitivity analyses will be conducted for different epidemiological subgroups and statistical analyses. Enrollment started in July 2018.

Ethics and dissemination

This study was approved by the Institutional Review Board of the University of Hong Kong / Hospital Authority Hong Kong West/East/Kowloon Central clusters (UW 16-553/HKEC-2019-026/REC (KC/KE)-19-0049/ER-4). We will report the findings in medical journals and conferences. The dataset will be available upon reasonable request.

Trial registration

This study is prospectively registered on ClinicalTrials.gov (NCT03535935) on 24 May 2018.

STENGTHS AND LIMITATIONS

Strengths

1. Existing epidemiological data suggested that Chinese medicine was associated with retarded progression of renal function among diabetic kidney disease patients and it is timely to perform a clinical trial on astragalus, the most used herbs in diabetes and diabetic kidney disease with unclear clinical effectiveness.
2. The inclusion / exclusion criteria, primary outcome measurement and the corresponding analyses are designed according to conventionally used parameters to facilitate further meta-analysis with other clinical studies for wide range of audience.
3. A responder analysis is built into the trial as secondary analysis to identify possible factors (including biomarkers and symptom-based diagnosis) that could lead to more personalised the use of astragalus.
4. We conducted a focus group interview series to explore the expectations of patients and clinicians (both conventional and Chinese medicine) to refine the study design (drug form, dosage, administration route, frequency, health services delivery and outcome measurement) for better clinical translation.

Limitations

1. As the trial is open-label, subjective outcomes including quality of life could not be assessed.

Keywords

Diabetic kidney disease; clinical trial; astragalus, huangqi; effectiveness; renal medicine

Introduction

In 2019, it was estimated that 463 million (9.3%) people was living with diabetes worldwide and the figure was projected to reach 578 million by 2030, with the highest prevalence in North America at present. (1) In 2017, The healthcare expenditure on diabetes reached US$ 850 billion globally (11.6% of global health expenditure). (2, 3) Diabetic kidney disease (DKD) refers to the chronic kidney disease (CKD) caused by long-standing diabetes. DKD presents in more than one third of all diabetic patients and is the leading cause of end-stage renal disease in many developed regions which requires replacement therapy including dialysis and transplantation. (4, 5) In Hong Kong, the incidence of diabetes-related end-stage renal disease increased from 26.2% in 1996 to 49.6% in 2013 (6) and end-stage renal disease increased 5.23 times the annual direct medical cost to the local public health system. (7) Furthermore, DKD was accounted for 23.4% (31.1% vs 7.7%) absolute increase in 10-year mortality in US (8) and 16 years shorter life-expectancy in Taiwan (9) when compared to those without diabetes and kidney diseases.

The risk factors and pathogenesis of DKD are heterogeneous (5) involving metabolic, (10, 11) inflammatory, (12-14) hemodynamic, (15-18) and many other pathways. (5, 19) Conventional blockade on the renin–angiotensin–aldosterone system (RAAS) offers limited effect on clinical outcomes. (20-23) In a previous meta-analysis of 9797 patients with stage 3 to 5 CKD, RAAS blockade did not reduce all-cause mortality and only provided a mild risk

reduction in the composite endpoint of replacement therapy initiation or doubling of serum creatinine when compared to placebo or other antihypertensive agents. (21) RAAS blockade with combined angiotensin-converting enzyme inhibitor (ACEI) and angiotensin II receptor blocker (ARB) resulted in increased adverse events but not the expected synergistic effect. (22, 24) More therapies with different working mechanisms are needed.

Chinese Medicine (CM) has been extensively used among diabetes and DKD patients in Asia. (25, 26) Previous observational studies from Taiwan with 47,876 and 24,971 subjects showed that the use of add-on prescribed CM is associated with 40% reduction of mortality (26) and 59% risk reduction of end-stage renal disease, respectively. (25) *Astragalus membranaceus*, commonly known as huang-qi, is the most frequently used CM or dietary herb for DKD. (27) Systematic reviews showed that astragalus could enhance creatinine clearance, reduce albuminuria and reduce blood pressure among CKD and DKD patients. (28-30) Meta-analysis also showed that astragalus' effect in improving renal clearance and reducing albuminuria was better than routine care (without ACEI or ARB) and the efficacy was comparable to ACEI or ARB. (30) *In vivo* and *in vitro* evidence suggested that astragaloside IV, an active ingredient of astragalus, could ameliorate podocyte apoptosis, foot process effacement, mesangial expansion, glomerulosclerosis and interstitial fibrosis through regulating the NF-κB and TGF-β_1 signalling pathway, which partly explained the renoprotective effect. (31, 32) Nevertheless, the methodological reporting and quality of the

existing clinical trials were inadequate and further evaluation is needed. Based on our preliminary result of ongoing trials, CM formulations containing astragalus is likely to retard the progression of DKD. (33, 34) Considering the extensive currently use of astragalus, clinical study could be considered before preclinical investigation as suggested by the World Health Organisation. (35)

Methods/Design

Objective

This pragmatic clinical trial aims to evaluate the effectiveness of add-on astragalus on type 2 diabetic patients with stage 2 to 3 chronic kidney disease and macroalbuminuria, and to identify related response predictors for subsequent large-scale health services research.

Study Design

Add-on, assessor-blind, parallel, pragmatic randomised controlled trial.

Inclusion and exclusion criteria

Patients with 1) type 2 diabetes for at least 5 years; 2) estimated glomerular filtration rate (GFR) $\geq$ 30 and < 90 mL/min/1.73m^2 confirmed by repeated testing over three months calculated by the abbreviated MDRD study equation; (36, 37) 3) persistent macroalbuminuria with spot urine albumin-to-creatinine ratio (UACR) $\geq$ 300 mg/g confirmed by at least 2 consecutive first morning void urine samples; 4) age between 35 to 80 years old; 5) stable dose of anti-diabetic agent(s) including insulin for at least 12 weeks; and 6) stable dose of ACEI or ARB for at least 12 weeks will be recruited.

Patients will be excluded if with 1) UACR $\geq$ 5000 mg/g; 2) a known history of glomerulonephritis, polycystic kidney disease, systemic lupus erythematosus or any suggestive evidence of nondiabetic glomerulopathy; 3) known history of kidney transplant; 4) concurrent severe disorders of heart, brain, liver, and hematopoietic system, tumor, mental

disorder; 5) deranged liver function; 6) poorly controlled blood pressure; 7) known history of intolerance or malabsorption of oral medications; 8) uncontrollable urinary infection; 9) experiencing pregnancy; and 10) participating in other clinical trial(s) within 30 days.

Sample size calculation

Since the primary objective of this trial is to evaluate key clinical outcomes and to perform a preliminary analysis on potential response predictors, we calculated the sample size based on the control of inflation factor (IF) to the estimation of sample size for the subsequent large-scale studies (38, 39). 118 patients (around 60 per group) are needed.

$$IF = S_{ucl} / S_{obs} = \text{sqrt}\,[(n-1)/\chi^2_{1-\alpha,\ n-1}]$$

$$N_{adj}/N_{unadj} \approx IF^2 \approx n_{unadj} * IF^2$$

$$N \approx [2(\square_{1-\alpha/2}+\square_{1-\beta'})^2(IF*s)^2]/(\mu_1-\mu_2)^2] = [2(\square_{1-\alpha/2}+\square_{1-\beta})s^2]/(\mu_1-\mu_2)^2]$$

$$\square_{1-\beta'} = \square_{1-\alpha/2}(IF^{-1}-1) + \square_{1-\beta} * IF^{-1}$$

where IF = Inflation factor

S_{ucl} = Standard deviation of upper confidence interval

S_{obs} = Observed standard deviation in pilot study

α = Chosen confidence level

β = Nominal power set for main study

ß' = Actual power achieved for main study by using pilot standard deviation for sample size calculation

n = Sample size of pilot study

N = Sample size of main study

N_{unadj} = Sample size of main study with no adjustment on standard deviation

N_{adj} = Sample size of main study with adjustment on standard deviation

The standard deviation used for sample size calculation for large-scale main studies is often underestimated by small-scale pilot studies, therefore an IF is needed for adjustment in sample size calculation. (38, 39) IF is calculated based on the size of pilot study and the confidence level of achieving at least the desired power in subsequent main studies. Therefore, the actual achieved power of the main studies depends on the nominal power set for the main study and the IF.

In order to be 95% confident (two-sided) that the main study achieves a power of 70% with nominal power set at 80% (i.e., a 10% power forfeit), the IF should be controlled to less than 1.13. At IF = 1.13, a sample size of 100 is therefore needed to attain 95% one-sided confidence that the main studies will achieve the nominal power to test the hypothesis of add-on astragalus could be more effective in stabilising the GFR among DKD patients when compared to standard care. To allow a 15% attrition rate, a sample size of 118 patients is

therefore needed for this pilot study.

Currently, there is limited evidence on the symptom-based response predictors of astragalus. A general recommendation for power estimation is to have 10 events per variable (40). From the previous systematic review, we estimate that around 60% of patients will have stabilised GFR after receiving astragalus. (30) 118 subjects with 15% attrition will power up to 6 variables for the screening of predictors. A univariable screening on the 11 pre-specified potential symptom-based predictors will be conducted to reduce the number of predictors for the subsequent multivariable regression analysis, in order to maximise the power of the regression analysis.

Recruitment and randomisation

Patients will be recruited from general and specialist outpatient clinics of Queen Mary Hospital, Queen Elizabeth Hospital, Hospital Authority Hong Kong East Cluster through consultations, and the community via public health campaigns. The details of study will be explained by principal investigators (PIs) or co-investigators (Co-Is) before written consent is obtained from each participating patient. All patients will undergo a 2-week run-in period, during which the dosage of their medications will be stabilised. Blood and urine sample will be sent to an independent local laboratory for screening. Patients are considered eligible for the study if their liver functions are normal and fulfil the inclusion criteria. Recruitment started in July 2018 and the recruitment is on-going.

A random sequence was generated and encrypted with computer by an independent staff of the University of Hong Kong and kept in sealed opaque envelopes. The password of the sequence is kept in a sealed, duly signed opaque envelop locked by research assistants (RAs). The allocation sequence is concealed from PIs, Co-Is, CM physicians and all research staffs that are responsible for patient screening, randomisation or sample analysis. Eligible patients will be randomised to either receive active intervention along with standard care or standard care alone. The allocation is masked from the outcome assessor (laboratory technicians). The study subjects could not be masked due to the nature of treatment. Since the primary clinical outcomes under investigation are objectively assessed and the outcome assessor is blinded, placebo effect and outcome measurement bias should be minimised. The flow of study is presented in **Figure 1**. Under no circumstances the primary outcome assessors will be unblinded.

Intervention and control

The intervention under investigation is astragalus. Patients under intervention will receive astragalus daily on top of standard medical care for 48 weeks. The CM physicians will advise on the dose and possible adverse events of astragalus based on his/her professional knowledge. Existing literature supports a safe dosage of raw astragalus from 15 to 50 g/day. (41, 42) According to the China Pharmacopeia, the recommended therapeutic dosage of astragalus is below 30 g/day. To ensure the safety of patients, CM physicians are reminded

not to propose dosage exceeding 30 g/day. All patients will continue their standard medication and follow-up with the same consultation schedule with CM physicians.

Herbal safety

Soluble herbal granules prepared by PuraPharm (listed in US Pharmacopeia as dietary ingredient: VER-DI-PUR-09) are used. The production process is in strict compliance with standards of Good Manufacturing Practice (GMP). Fully registered CM physicians from the School of Chinese Medicine, The University of Hong Kong will be responsible for the clinical diagnosis and prescription. After 4 to 6 weeks of randomisation, all patients will undergo liver function tests and renal function tests to monitor acute changes of renal and liver function.

Outcome measurement

The primary outcome measures are the changes of estimated GFR and UACR from baseline (week 0) to treatment endpoint (week 48). Secondary outcome measures include adverse events, and changes in CKD stage, fasting blood glucose (FBG), haemoglobin A_{1c} (HbA_{1c}), lipids, urinary monocyte chemotactic protein 1 (MCP-1), urinary nephrin, urinary cystatin C and urinary TGF-β_1 from baseline to the midpoint (week 24) and the end of treatment.

Data collection

Patient demographics including age, gender, body mass index (BMI), duration of diabetes, other medical history and concurrent medications will be retrieved by the electronic clinical

management system of Hospital Authority by Co-Is and RAs. Estimated GFR, UACR, FBG, HbA_{1c}, lipids and liver function tests will be assessed by an independent laboratory (Chan & Hou Medical Laboratories Limited) which is accredited by College of American Pathologists, Royal College of Pathologists Australasia and Quality Control for Molecular Diagnostics UK. MCP-1, nephrin, cystatin C and TGF-β_1 will be assessed at lead-PI's research laboratory by an independent RA with commercially available kits. Blood pressure will be taken during consultation. Blood and urine samples will be taken at an overnight (>8 hours) fasting state.

Estimated GFR will be calculated using the MDRD equation with serum creatinine, age, ethnicity and gender. Clinical presentations and CM symptom-based diagnosis will be assessed in a structured consultation developed for this purpose. To ensure consistency and reliability of assessment and to minimise bias from investigators across the study, only 3 synchronised CM physicians will assess the patients.

A self-complete questionnaire will be distributed to the subjects to monitor adverse events, and they are advised to inform the PIs, Co-Is, CM physicians or RAs immediately if adverse events arise. All adverse events will be coded based on Common Terminology Criteria for Adverse Events (CTCAE) 5.0, following the recommendation of CONSORT (Consolidated Standards of Reporting Trials) Extension for Chinese Herbal Medicine Formulas. (43)

Follow-up consultations will be held for all patients bi-weekly in the first month and

monthly subsequently until the end of treatment for all patients. Minor adjustments are allowed based on clinical needs. Evaluation of outcomes will be performed at baseline, week 24 (treatment midpoint) and 48 (end of treatment). The follow up schedule is summarised below in **Table 1**.

Data management

A trial management committee (TMC) formed by lead-PI, Co-Is and RAs will centralise all trial data. Co-Is and RAs will collect, clean and send the data to TMC weekly. All data will be double entered, secured and cleaned before analysis to prevent data entry errors. TMC will have regular meetings monthly to discuss the progress and double check the data of the trial. Only PI, Co-Is and regulatory bodies will have access to the patient data to protect data privacy. An independent Data and Safety Monitoring Board (DSMB) (VCH Chung, W Wong, JWF Yeung) has been established with expert in methodology, biostatistics and clinical medicine to monitor the progress of the trial, including adverse events and change in protocol. DSMB will have meetings twice a year. No competing interests has been reported from DSMB. Trial result will be published in academic journal and trial subjects will be notified.

Handling of withdraw and dropout

In order to maximise subjects' compliance, we will provide a triple thorough consent process for all participants covering details of the study schedule, potential side effects of treatment, and the responsibilities of the subjects. An independent e-mail account and a direct telephone

line is available for this study to enable active communication with patients. Extra visits will be arranged for patients if necessary. To monitor the adherence of study medication, we will arrange irregular visits for patients and count the unfinished medication.

Termination criteria

The treatment will be terminated for a specific subject if he/she: 1) develops serious adverse event (SAE); 2) develops hypersensitivity towards astragalus; and 3) participates in other clinical trial. The whole study will be terminated under the following circumstances: 1) presence of clustered SAE(s) related to astragalus with supportive evidence; and 2) completion of all follow-up assessments.

SAE includes adverse events that result in death, require either hospitalisation or the prolongation of hospitalisation, are life-threatening, result in a persistent or significant disability/incapacity, result in a congenital anomaly/birth defect or events classified as Grade 3 or above in CTCAE 5.0. Other important medical events, based upon appropriate medical judgement, may also be considered SAEs if a patient's health is at risk and intervention is required to prevent an outcome mentioned.

Data analysis

Missing values will be imputed by multiple regression. The analysis will follow intention-to-treat principle that all randomised patients will be included in the analysis. STATA and PRISM will be used for the analysis.

Demographics will be presented as mean ± standard deviation or percentage. UACR will be log-transformed and reported as geometric means. Smoking history will be stratified into non-smoker, ex-smoker and current smoker. Rapid renal progression is pre-defined as a consecutive annual GFR drop of over 5 ml/min/1.73m^2 or a cumulative GFR drop of over 25 ml/min/1.73m^2 for 5 years. (44, 45) Differences in mean and proportion between groups will be tested by t-test and χ^2 test.

Mixed regression models will be used to compare the rate of change in estimated GFR and UACR. Analysis of covariance (ANCOVA) will be used to compare the adjusted mean of outcomes at week 48 between intervention group and control group with the corresponding baseline values as covariates. Data will be presented as the difference in adjusted means between the groups with 95% CI and the corresponding p-value.

The adverse events will be recorded according to CTCAE 5.0 and categorised into 5 grades (Grade 1: mild, asymptomatic or mild symptoms, clinical or diagnostic observations only, no intervention indicated; Grade 2: moderate, minimal, local or non-invasive intervention indicated, limiting age-appropriate instrumental activities of daily living; Grade 3: severe or medically significant but not immediately life-threatening, hospitalisation or prolongation of hospitalisation indicated, disabling, limiting self-care activities of daily living; Grade 4: life-threatening consequences, urgent intervention indicated; and Grade 5: death related to adverse events). The percentage of all adverse events with more than 1 case

will be compared between groups. SAE will be analysed case by case descriptively.

To minimise Type I error inflation, the analysis will follow a hierarchical approach in the order of 1) comparison of baseline to end of treatment on estimated GFR and UACR; 2) comparison of baseline to end of treatment on other outcome measurements; 3) comparison of baseline to treatment midpoints on estimated GFR and UACR and 4) comparison of baseline to treatment midpoints on other outcome measurements.

For the assessment of predictive factors as secondary analysis, the dependent variable will be the treatment response which is categorised into:

1. Improved or stabilised renal function, defined as estimated GFR after 48-week treatment being higher or equal to baseline.

2. Non-responder, defined as patients having estimated GFR decreased at a rate of less than 5 mL/min/1.73m^2 after 48-week treatment compared to baseline.

3. Rapid deteriorating renal function, defined as estimated GFR of more than 8 mL/min/1.73m^2 after 48-week treatment compared to baseline.

Potential prognostic variables (baseline values) will include:

1. Demographics: age, gender, BMI, systolic blood pressure, history and duration of smoking and alcohol consumption

2. Symptom-based diagnosis: presence of CM-based symptom-based subtype (e.g. *spleen and kidney qi deficiency*) based on the presentation of standardised and commonly documented signs and symptoms (46)

3. Biochemical profile: GFR, UACR, HbA_{1c}, lipids

All potential predictors will first be included into a multivariable stepwise regression analysis. Variables that are not significant at a 5% level will be excluded.

Subgroup analyses will be performed for 1) CKD stages stratified into stage 2, 3a and 3b; (47) 2) UACR levels stratified by 100 mg/mmol; (48) 3) gender and 4) age groups. Sensitivity analyses will be performed for 1) per-protocol cohort; 2) estimation of GFR by c-MDRD (49) and CKD-EPI (50) equations; 3) missing data imputed with last-observation-carried-forward and 4) different analytical approaches (change-score) and categorisations of primary outcomes.

Patient and public involvement

We conducted a focus group interview series to collate the experience and expectations of patients and clinicians (both conventional and Chinese medicine) on the study design (drug form, dosage, administration route, frequency, health services delivery and outcome measurement) for this trial. (51) The study results will be disseminated to diabetes patient groups and the participants via public workshops and talks.

Discussion

Diabetes and DKD are significant public health burdens and astragalus is the most used herbs among these patients with unclear clinical effectiveness. There is an urgent need to characterise the effect and response predictors of astragalus to prevent unnecessary consumption and to increase the cost-effectiveness of administration. Also, the assessment of response predictors of both biomarkers and symptom-based factors will facilitate the integration and clinical translation of generated evidence between conventional medicine and CM physicians. Based on our preliminary result of ongoing trials, CM formulations containing astragalus is likely to retard the progression of DKD. (33, 34) This trial aims to evaluate the effect of astragalus and identify related response predictors for more personalised application and further large-scale health services research.

To facilitate further meta-analysis with other clinical studies for wide range of audience, the inclusion / exclusion criteria, primary outcome measurement and the corresponding analyses are designed according to conventionally used parameters similar to other pharmaceutical studies. (52) A responder analysis is included as secondary analysis to identify possible factors (including biomarkers and symptom-based diagnosis) that could lead to more personalised the use of astragalus. Besides, we conducted a focus group interview series to explore the expectations of patients and clinicians (both conventional and Chinese medicine) to refine the study design for better clinical

translation. (51) The major limitation of this trial is the open-label nature. The study subjects could not be masked due to the nature of treatment. Since the clinical outcomes under investigation are objective and the outcome assessor is blinded, placebo effect and outcome measurement bias should be minimised. However, subjective outcomes including quality of life could not be assessed.

List of abbreviations

ACEI – angiotensin-converting enzyme inhibitors

ANCOVA – analysis of covariance

ARB – angiotensin II receptor blockers

BMI – body mass index

CI – confidence interval

CM – Chinese medicine

Co-I – co-investigator

CONSORT – Consolidated Standards of Reporting Trials

CTCAE – Common Terminology Criteria for Adverse Events

DKD – diabetic kidney disease

CKD – chronic kidney disease

FBG – fasting blood glucose

GMP – Good Manufacturing Practice

HbA_{1c} – haemoglobin A_{1c}

MCP-1 – monocyte chemotactic protein 1

NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells

PI – principal investigator

RA – research assistant

RAAS – renin–angiotensin–aldosterone system

SAE – serious adverse event

TGF-β_1 – transforming growth factor beta-1

UACR – urine albumin-to-creatinine ratio

DECLARATION

Ethics approval and consent to participate

This study was approved and monitored by the Institutional Review Board of the University of Hong Kong / Hospital Authority Hong Kong West/East/Kowloon Central clusters (Ref: UW 16-553/HKEC-2019-026/REC (KC/KE)-19-0049/ER-4). Written consent will be obtained from all subjects, including change of protocol. This protocol is prospectively registered on ClinicalTrials.gov (NCT03535935) on 24 May 2018 and reported according to SPIRIT-TCM Extension 2018. SPIRIT checklist and flow diagram are enclosed **(Supplementary File)**. We publish this protocol after the first DSMB meeting.

Consent for Publication

Not applicable. No personal information is included.

Availability of data and materials

The datasets used and/or analysed during the current study will be available from the corresponding author on reasonable request.

Competing interests

None declared.

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Authors' Contributions

KW Chan and SCW Tang designed the study. KW Chan, AKS Kwong, PN Tsui, SCY Cheung, GCW Chan, MMY Wong, KCB Tan and WF Choi recruited the patients and provided clinical consultation. WH Yiu provided expert opinion and support in biochemical analysis. L Lao, Z Zhang, Y Zhang provided expert opinion on the study design. KW Chan and SCW Tang drafted the manuscript. All authors involved in the manuscript revision.

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REFERENCES

1. Sinclair A, Saeedi P, Kaundal A, Karuranga S, Malanda B, Williams R. Diabetes and global ageing among 65-99-year-old adults: Findings from the International Diabetes Federation Diabetes Atlas, 9(th) edition. *Diabetes Res Clin Pract.* 2020:108078.

2. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract.* 2018;138:271-81.

3. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract*. 2017;128:40-50.

4. Thomas MC, Cooper ME, Zimmet P. Changing epidemiology of type 2 diabetes mellitus and associated chronic kidney disease. *Nature Reviews Nephrology*. 2016;12(2):73-81.

5. Tang SCW, Yiu WH. Innate immunity in diabetic kidney disease. *Nature Reviews Nephrology*. 2020;16(4):206-22.

6. Leung CB, Cheung WL, Li PK. Renal registry in Hong Kong-the first 20 years. *Kidney Int Suppl*. 2015;5(1):33-8.

7. Jiao F, Wong CKH, Tang SCW, Fung CSC, Tan KCB, McGhee S, et al. Annual direct medical costs associated with diabetes-related complications in the event year and in

subsequent years in Hong Kong. *Diabetic Medicine*. 2017;34(9):1276-83.

8. Afkarian M, Sachs MC, Kestenbaum B, Hirsch IB, Tuttle KR, Himmelfarb J, et al. Kidney disease and increased mortality risk in type 2 diabetes. *J Am Soc Nephrol.* 2013;24(2):302-8.

9. Wen CP, Chang CH, Tsai MK, Lee JH, Lu PJ, Tsai SP, et al. Diabetes with early kidney involvement may shorten life expectancy by 16 years. *Kidney Int.* 2017;92(2):388-96.

10. Goh SY, Cooper ME. Clinical review: The role of advanced glycation end products in progression and complications of diabetes. *The Journal of Clinical Endocrinology and Metabolism.* 2008;93(4):1143-52.

11. Forbes JM, Cooper ME, Oldfield MD, Thomas MC. Role of advanced glycation end products in diabetic nephropathy. *J Am Soc Nephrol*. 2003;14(8 Suppl 3):S254-8.

12. Pichler R, Afkarian M, Dieter BP, Tuttle KR. Immunity and inflammation in diabetic kidney disease: translating mechanisms to biomarkers and treatment targets. *American Journal of Physiology Renal Physiology.* 2017;312(4):F716-f31.

13. Navarro-Gonzalez JF, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. *J Am Soc Nephrol*. 2008;19(3):433-42.

14. Navarro-Gonzalez JF, Mora-Fernandez C, Muros de Fuentes M, Garcia-Perez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. *Nature Reviews Nephrology*. 2011;7(6):327-40.

15. Alicic RZ, Rooney MT, Tuttle KR. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. *Clin J Am Soc Nephrol*. 2017;12(12):2032-45.

16. Premaratne E, Verma S, Ekinci EI, Theverkalam G, Jerums G, MacIsaac RJ. The impact of hyperfiltration on the diabetic kidney. *Diabetes & Metabolism*. 2015;41(1):5-17.

17. Tonneijck L, Muskiet MH, Smits MM, van Bommel EJ, Heerspink HJ, van Raalte DH, et al. Glomerular Hyperfiltration in Diabetes: Mechanisms, Clinical Significance, and Treatment. *J Am Soc Nephrol.* 2017;28(4):1023-39.

18. Palatini P. Glomerular hyperfiltration: a marker of early renal damage in pre-diabetes and pre-hypertension. *Nephrol Dial Transplant.* 2012;27(5):1708-14.

19. Yiu WH, Li RX, Wong DWL, Wu HJ, Chan KW, Chan LYY, et al. Complement C5a inhibition moderates lipid metabolism and reduces tubulointerstitial fibrosis in diabetic nephropathy. *Nephrol Dial Transplant.* 2018;33(8):1323-32.

20. Hsu TW, Liu JS, Hung SC, Kuo KL, Chang YK, Chen YC, et al. Renoprotective effect of renin-angiotensin-aldosterone system blockade in patients with predialysis advanced chronic kidney disease, hypertension, and anemia. *JAMA Internal Medicine.* 2014;174(3):347-54.

21. Nistor I, De Sutter J, Drechsler C, Goldsmith D, Soler MJ, Tomson C, et al. Effect of renin-angiotensin-aldosterone system blockade in adults with diabetes mellitus and advanced chronic kidney disease not on dialysis: a systematic review and meta-analysis. *Nephrol Dial*

Transplant. 2018;33(1):12-22.

22. Palmer SC, Mavridis D, Navarese E, Craig JC, Tonelli M, Salanti G, et al. Comparative efficacy and safety of blood pressure-lowering agents in adults with diabetes and kidney disease: a network meta-analysis. *The Lancet*. 2015;385(9982):2047-56.

23. Chan GC, Tang SC. Proteinuria reaffirmed as a risk modifier in diabetic chronic kidney disease. *Nephrol Dial Transplant.* 2018;33(11):1873-74.

24. Chan GC, Tang SC. Diabetic nephropathy: landmark clinical trials and tribulations. *Nephrol Dial Transplant*. 2016;31(3):359-68.

25. Lin MY, Chiu YW, Chang JS, Lin HL, Lee CT, Chiu GF, et al. Association of prescribed Chinese herbal medicine use with risk of end-stage renal disease in patients with chronic kidney disease. *Kidney Int*. 2015;5(10):226.

26. Hsieh CF, Huang SL, Chen CL, Chen WT, Chang HC, Yang CC. Non-aristolochic acid prescribed Chinese herbal medicines and the risk of mortality in patients with chronic kidney disease: results from a population-based follow-up study. *BMJ Open*. 2014;4(2):2013-004033.

27. Li Q, Zhang H, Fei Y, et al, Treatment of Diabetic Nephropathy by Integrative Medicine: A Multi-center Prospective Cohort Study. *Chinese Journal of Integrated Traditional and Western Medicine*. 2012;32(3):317-21.

28. Zhang HW, Lin ZX, Xu C, Leung C, Chan LS. Astragalus (a traditional Chinese

medicine) for treating chronic kidney disease. *The Cochrane Database of Systematic Reviews*. 2014;22(10).

29. Li M, Wang W, Xue J, Gu Y, Lin S. Meta-analysis of the clinical value of Astragalus membranaceus in diabetic nephropathy. *Journal of Ethnopharmacology*. 2011;133(2):412-9.

30. LIU Yi-hong, TIAN Hao-ming. Effect of Astragaulus Membranaceus on Diabetic Nephropathy:A Systematic Review. *Chinese Journal of Evidence-Based Medicine*. 2007;7(10):715-27.

31. Gui D, Guo Y, Wang F, Liu W, Chen J, Chen Y, et al. Astragaloside IV, a novel antioxidant, prevents glucose-induced podocyte apoptosis in vitro and in vivo. *PloS One*. 2012;7(6):22.

32. Sun L, Li W, Xiong L, Li G, Ma R. Astragaloside IV prevents damage to human mesangial cells through the inhibition of the NADPH oxidase/ROS/Akt/NFkappaB pathway under high glucose conditions. *Int J Mol Med*. 2014;34(1):167-76.

33. Chan KW, Chan CW, Leung CPS, Yiu WH, Lui SL, Ip TP, et al. Semi-individualised Chinese Medicine Treatment for Diabetic Kidney Disease – From users' perspectives to SCHEMATIC trial interim result and potential mechanisms. *Advances in Integrative Medicine*; Brisbane. Australia 2019. S12.

34. Chan KW, Ip TP, Kwong AS, Lui SL, Chan GC, Cowling BJ, et al. Semi-individualised Chinese medicine treatment as an adjuvant management for diabetic nephropathy: a pilot

add-on, randomised, controlled, multicentre, open-label pragmatic clinical trial. *BMJ Open*. 2016;6(8):e010741.

35. World Health Organisation. General guidelines for methodologies on research and evaluation of traditional medicine Geneva: World Health Organisation; 2000.

36. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med*. 1999;130(6):461-70.

37. Glassock RJ, Warnock DG, Delanaye P. The global burden of chronic kidney disease: estimates, variability and pitfalls. *Nature Reviews Nephrology*. 2017;13(2):104-14.

38. Sim J, Lewis M. The size of a pilot study for a clinical trial should be calculated in relation to considerations of precision and efficiency. *J Clin Epidemiol*. 2012;65(3):301-8.

39. Moore CG, Carter RE, Nietert PJ, Stewart PW. Recommendations for planning pilot studies in clinical and translational research. *Clinical and Translational Science*. 2011;4(5):332-7.

40. Pavlou M, Ambler G, Seaman SR, Guttmann O, Elliott P, King M, et al. How to develop a more accurate risk prediction model when there are few events. *BMJ*. 2015;11(351).

41. Li BC. The Combined Treatment of Astragalus and Vasartan for Early Stage Diabetic Kidney Disease. *China Foreign Medical Treatment*. 2011(23):98-100.

42. Huang H, Gao J. Astragalus Pharmacological Properties and Use of Research in

Diabetic Nephropathy. *Journal of Practical Traditional Chinese Internal Medicine*. 2011;25(10):80-2.

43. Cheng CW, Wu TX, Shang HC, Li YP, Altman DG, Moher D, et al. CONSORT Extension for Chinese Herbal Medicine Formulas 2017: Recommendations, Explanation, and Elaboration. *Ann Intern Med*. 2017;167(2):112-121.

44. Krolewski AS, Skupien J, Rossing P, Warram JH. Fast renal decline to end-stage renal disease: an unrecognized feature of nephropathy in diabetes. *Kidney Int.* 2017;91(6):1300-11.

45. Looker HC, Colombo M, Hess S, Brosnan MJ, Farran B, Dalton RN, et al. Biomarkers of rapid chronic kidney disease progression in type 2 diabetes. *Kidney Int.* 2015;88(4):888-96.

46. Zheng X. Guiding Principle of Clinical Research on New Drugs of Chinese Medicine (Trial). Beijing: China Medical Science Press; 2002.

47. Umanath K, Lewis JB. Update on Diabetic Nephropathy: Core Curriculum 2018. *American journal of kidney diseases*. 2018;71(6):884-95.

48. Chan KW. Integrative management for diabetic kidney disease – Patients' and clinicians' perspectives, clinical effectiveness and possible mechanisms [PhD thesis]. Hong Kong: The University of Hong Kong; 2018.

49. Ma YC, Zuo L, Chen JH, Luo Q, Yu XQ, Li Y, et al. Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. *J Am Soc Nephrol.*

2006;17(10):2937-44.

50. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150(9):604-12.

51. Chan KW, Lee PW, Leung CPS, Chan GCW, Yiu WH, Cheung HM, et al. Patients' and clinicians' expectations on integrative medicine services for diabetes: a focus group study. *BMC Complementary Medicine and Therapies*. 2020;20(1):205

52. Heerspink HJL, Parving HH, Andress DL, Bakris G, Correa-Rotter R, Hou FF, et al. Atrasentan and renal events in patients with type 2 diabetes and chronic kidney disease (SONAR): a double-blind, randomised, placebo-controlled trial. *Lancet*. 2019;393(10184):1937-47.

Figure legends

Figure 1 The Flow of Research

Table 1 Follow-up schedule

	STUDY PERIOD				
	Enrolment	Allocation	Post allocation – treatment period		
Timepoint	**Before treatment**	**Week 0, Day 1**	**Week 1-4 (+/- 3 days)**	**After 4-6 weeks (+/- 7 days)**	**After 24 and 48 weeks (+/- 7 days)**
ENROLMENT					
Eligibility screen	X				
Informed consent	X				
Medical history	X				
Allocation		X			
INTERVENTIONS					
Intervention (interventional group)		X	X	X	X
Routine care (all patients)		X	X	X	X
ASSESSMENTS					
Renal and liver function tests, other biomarkers (blood and urine tests)	X		X	X	X
Blood pressure, weight, hip-waist circumference		X	X	X	X
Demographics		X			
Clinical presentations		X	X	X	X
Adverse events		X	X	X	X

Appendix 1. World Health Organisation Trial Registration Dataset

Data Category	Information
Primary Registry and Trial Identifying Number	ClinicalTrials.gov (NCT03535935)
Date of Registration in Primary Registry	24 May 2018
Secondary Identifying Numbers	HMRF-14151731
Source(s) of Monetary or Material Support	Health and Medical Research Fund, Food and Health Bureau, Hong Kong
Primary Sponsor	The University of Hong Kong
Secondary Sponsor(s)	N/A
Contact for Public Queries	Prof TANG Chi-wai Sydney MD PhD Dr CHAN Kam-wa MSPH MD PhD Tel: +852 2255 3603 Email: scwtang@hku.hk / chriskwc@hku.hk
Public Title	Efficacy, Safety and Response Predictors of Adjuvant Astragalus Therapy for Diabetic Kidney Disease (READY)
Scientific Title	Efficacy, Safety and Response Predictors of Adjuvant Astragalus for Diabetic Kidney Disease (READY) – An Add-on, Assessor-blind, Parallel, Pragmatic Randomised Controlled Trial
Countries of Recruitment	Hong Kong SAR, China
Health Condition(s) or Problem(s) Studied	Diabetic kidney disease
Intervention (s)	Active comparator: Standard medical care with angiotensin converting enzyme inhibitor or angiotensin receptor blocker and oral hypoglycemic agents and/or insulin at stable dose Experimental arm: Semi-individualised dosage of astragalus on top of standard medical care
Key Inclusion and Exclusion Criteria	Ages eligible for study: between 35 and 80 years old Gender eligible for study: Both

	Healthy volunteers: Not accepted **Inclusion Criteria:** - diagnosed with type 2 diabetes for at least 5 years; - with an estimated glomerular filtration rate (GFR) ≥30 <90 mL/min/1.73m2 confirmed with repeat testing over three or more months calculated by the abbreviated MDRD study equation; - persistent macroalbuminuria with spot urine albumin-to-creatinine ratio (UACR) ≥ 300 mg/g confirmed by at least 2 out of 3 consecutive first morning void urine samples; - on stable dose of anti-diabetic drug including insulin for 12 weeks; - on stable dose of angiotensin-converting-enzyme inhibitor or angiotensin receptor blocker for 12 weeks; and - willing and able to give written informed consent **Exclusion Criteria:** - with known history of glomerulonephritis, polycystic kidney disease, systemic lupus erythematosus, any suggestive evidence of nondiabetic glomerulopathy; - with known history of kidney transplant; - with concurrent severe disorders of heart, brain, liver, and hematopoietic system, tumor and mental disorder; - with deranged liver function; - with poorly controlled blood pressure; - with known history of intolerance or malabsorption of oral medications; - with uncontrollable urinary infection; - experiencing pregnancy; or

	- participating in other clinical trial within 30 days
Study Type	Interventional Allocation: randomised Intervention model: parallel assignment (2 arms) Masking: Open label (Accessor of primary outcome measures blinded) Primary purpose: Treatment Phase: II/III Allocation concealment: Sealed opaque envelope prepared by an independent technical staff Sequence generation: computer generated random sequence
Date of First Enrollment	July 2018
Target Sample Size	118
Recruitment Status	Recruiting
Primary Outcome(s)	Changes in estimated glomerular filtration rate and spot urine to albumin ratio (time frame: 48 weeks)
Key Secondary Outcome(s)	Adverse events, changes in fasting blood glucose, glycated haemoglobin, lipids, blood pressure

Appendix 2. Sample Consent form

Patient/Subject Information Sheet

1. STUDY TITLE

Efficacy, safety and response predictors of adjuvant astragalus for diabetic kidney disease (READY) – An open-label randomised controlled trial with responder regression analysis

2. INVITATION PARAGRAPH

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your family doctor if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

3. WHAT IS THE PURPOSE OF THE STUDY?

Modern pharmacologic therapy using blockers of angiotensin II is unable to fully suppress the progression of chronic kidney disease (CKD). As a result, many patients progress to end-stage kidney disease and require either dialysis or transplantation. Recently, research data shows that astragalus has anti-fibrotic effect, slower the progression to kidney disease and have been using in addition to routine medical care in Hong Kong. However, the actual pharmacological and therapeutic effect of astragalus are unclear. The present study lasting 48 weeks aims to investigate whether astragalus consumption stabilises renal function and reduces albuminuria.

4. WHY HAVE I BEEN CHOSEN?

You have CKD with unsatisfactory proteinuria control despite angiotensin blockade therapy, and are now being invited to participate in this study to investigate the potential beneficial effect of astragalus that is currently widely used in Hong Kong.

5. DO I HAVE TO TAKE PART?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

6. WHAT WILL HAPPEN TO ME IF I TAKE PART?

You will be randomised by computer after thorough assessment to either receive guidance on astragalus consumption in addition to your standard medications or continue standard medications. The observation period of this study is 48 weeks, and you will be followed up at the clinic in the usual manner, but with additional blood and urine tests as appropriate. You will need to attend 6 extra clinic visits for Chinese medicine consultation in addition to your usual visits over the next 48 weeks.

7. WHAT DO I HAVE TO DO?

There are no lifestyle restrictions by participating in this study, except for the need of practicing contraception. As you have CKD, you will be given dietary advice on salt and protein restriction which are necessary even if you are not participating in this study. You will take the astragalus on top of your therapy for your present condition.

8. WHAT IS THE DRUG OR PROCEDURE THAT IS BEING TESTED?

Astragalus has been widely consumed for years in Hong Kong although with limited clinical evidence. According to existing best available evidence, astragalus has anti-fibrotic effect and could slower the progression to kidney disease. Currently, no adverse events have been confirmed to associate with the use of astragalus.

9. WHAT ARE THE ALTERNATIVES FOR DIAGNOSIS OR TREATMENT?

An alternative treatment option of chronic kidney disease is standard medical care with angiotensin receptor blocker or angiotensin converting enzyme inhibitor alone.

10. WHAT ARE THE SIDE EFFECTS OF TAKING PART?

Astragalus is generally well tolerated. There are no known side effects in addition to those of conventional treatment when astragalus is being used within the reference range of Pharmacopeia of China. Nevertheless, astragalus may have unknown side effects. Full evaluation will be performed and adequate monitoring will be exercised once you start taking it. You will need to attend 6 extra clinic visits in addition to your usual visits over the next 48 weeks. Any claims on loss or injury attributable to the study will be arranged by the University of Hong Kong.

11. WHAT ARE THE DISADVANTAGES AND RISKS OF TAKING PART?

The safety of the astragalus to the human fetus is unclear, therefore women with child-bearing potential must practice contraception.

12. WHAT ARE THE BENEFITS OF TAKING PART?

We hope that astragalus will help you. However, this cannot be guaranteed. The information we get from this study may help us treat future patients with CKD better.

13. WHAT IF NEW INFORMATION BECOMES AVAILABLE?

Sometimes during the course of a research project, new information becomes available about astragalus that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

14. WHAT HAPPENS WHEN THE RESEARCH STUDY STOPS?

After the study stops, you will be advised whether or not to continue with astragalus according to clinical need. Astragalus will not be provided for free.

15. WHAT IF SOMETHING GOES WRONG?

Any claims on loss or injury attributable to the study will be arranged by the University of Hong Kong. If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal health service complaints mechanisms may be available to you.

16. WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

You have the rights of access to personal data and publicly available study results, if and when needed.

Under the laws of Hong Kong (in particular the Personal Data (Privacy) Ordinance, Cap 486), you enjoy or may enjoy rights for the protection of the confidentiality of your personal data, such as those regarding the collection, custody, retention, management, control, use (including analysis or comparison), transfer in or out of Hong Kong, non-disclosure, erasure and/or in any way dealing with or disposing of any of your personal data in or for this study. For any query, you should consult the Privacy Commissioner for Privacy Data or his office (Tel No. 2827 2827) as to the proper monitoring or supervision of your personal data protection so that your full awareness and understanding of the significance of compliance with the law governing privacy data is assured.

By consenting to participate in this study, you expressly authorize:

- the principal investigator and his research team and the ethics committee responsible for overseeing this study to get access to, to use, and to retain your personal data for the purposes and in the manner described in this informed consent process; and
- the relevant government agencies (e.g. the Hong Kong Department of Health) to get access to your personal data for the purposes of checking and verifying the integrity of study data and assessing compliance with the study protocol and relevant requirements.

17. WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?

The results of this study will be published in a medical journal. Your personal information will be kept confidential.

18. WHO IS ORGANISING AND FUNDING THE RESEARCH?

This study is supported by the Health and Medical Research Fund and you do not need to pay any extra cost. Your doctor will not be paid for including you in this study.

19. WHO HAS REVIEWED THE STUDY?

The Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Cluster has reviewed and approved this study. After recruitment, Each patient will receive HK$150 for each blood/urine investigation visit related to this study as travel support.

20. CONTACT FOR FURTHER INFORMATION

In case of enquiry, you may contact Mr Chris Chan or Prof Sydney Tang at 2255 3207. You will be given a copy of this information sheet and a signed consent form to keep. Thank you for taking part in this study!

From the Division of Nephrology
Department of Medicine
University of Hong Kong
Queen Mary Hospital

PATIENT/SUBJECT CONSENT FORM

Title of Project: Efficacy, safety and response predictors of adjuvant astragalus for diabetic kidney disease (READY) – An open-label randomised controlled trial with responder regression analysis

Name of Researcher: Prof Sydney C.W. Tang

Please initial box

1. I confirm that I have read and understood the information sheet dated __/__/__ for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of my medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I agree to take part in the above study. ☐

_____	_____	_____
Name of patient	*Date*	*Signature*
_____	_____	_____
Name of Witness (if applicable)	*Date*	*Signature*
_____	_____	_____
Name of person taking consent (if different from researcher)	*Date*	*Signature*
_____	_____	_____
Researcher	*Date*	*Signature*

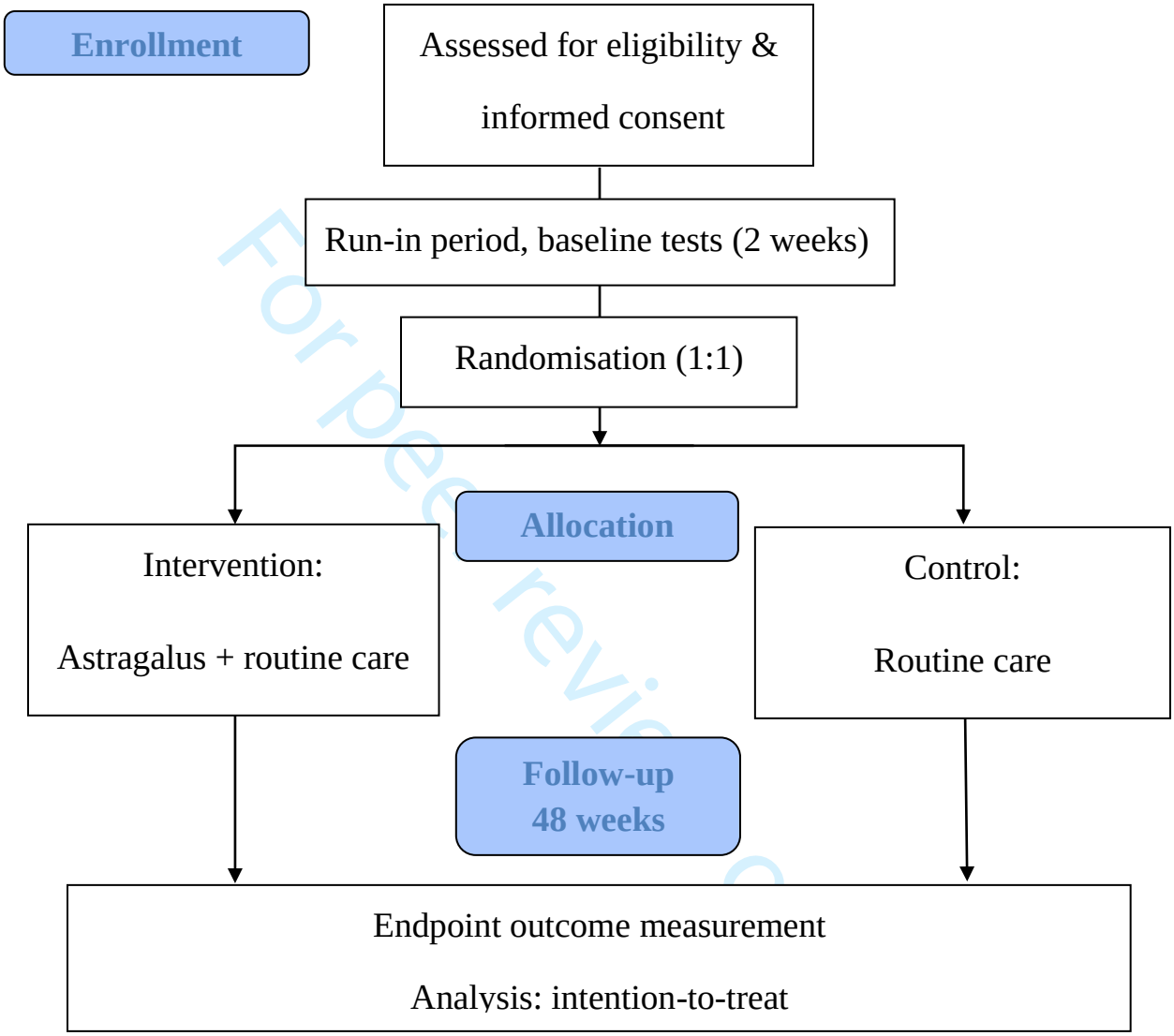
Copies to:

☐ Patient/Subject

☐ Researcher's File

☐ Hospital Record

Figure 1. Flow of study



SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_____1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_____4_____
	2b	All items from the World Health Organization Trial Registration Data Set	____Appendix__
Protocol version	3	Date and version identifier	____Appendix__
Funding	4	Sources and types of financial, material, and other support	____Appendix__
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	____Appendix__
	5b	Name and contact information for the trial sponsor	____Appendix__
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	____26, 27____
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	____17, 27____

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	8-10
	6b	Explanation for choice of comparators	10, 14
Objectives	7	Specific objectives or hypotheses	10
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	1, 10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	13
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10, 11, 15
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	14, 15
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	14, 17, 18
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	17
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	14
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	15
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1, Table 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11, 12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13, 17
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13, 14
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13, 14
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13, 14, 26
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	14
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	14
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	16, 17
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	17

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	17
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18-20
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	21
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	17, 26
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	18
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	16, 18
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	17
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	26
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	17, 26

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	17
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	26
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17, 26
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	27
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	26
Appendices			
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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Efficacy, Safety and Response Predictors of Adjuvant Astragalus for Diabetic Kidney Disease (READY) – Study Protocol of an Add-on, Assessor-blind, Parallel, Pragmatic Randomised Controlled Trial

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Efficacy, Safety and Response Predictors of Adjuvant Astragalus for Diabetic Kidney Disease (READY) – Study Protocol of an Add-on, Assessor-blind, Parallel, Pragmatic Randomised Controlled Trial

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Running Title

Randomised controlled trial of astragalus for diabetic kidney disease

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Abstract

Introduction

Diabetic kidney disease (DKD) is a prevalent and costly complication of diabetes with limited therapeutic options, leading the cause of end-stage renal disease in most developed regions. Recent big data studies showed that add-on Chinese medicine (CM) led to reduced risk of end-stage renal disease and mortality among chronic kidney disease patients with diabetes. Astragalus, commonly known as huang-qi, is the most prescribed CM or used dietary herb in China for diabetes and DKD. *In vivo* and *in vitro* studies showed that astragalus could ameliorate podocyte apoptosis, foot process effacement, mesangial expansion, glomerulosclerosis and interstitial fibrosis. Nevertheless, the clinical effect of astragalus remained uncharacterised. This pragmatic clinical trial aims to evaluate the effectiveness of add-on astragalus on type 2 diabetic patients with stage 2 to 3 chronic kidney disease and macroalbuminuria and identify related response predictors.

Methods and analysis

This is an add-on, assessor-blind, parallel, pragmatic randomised controlled clinical trial.118 patients diagnosed with DKD will be recruited and randomised 1:1 to a 48-week add-on astragalus or standard medical care. Primary endpoints are the changes in estimated glomerular filtration rate and urine albumin-to-creatinine ratio between baseline and

treatment endpoint. Secondary endpoints include adverse events, fasting blood glucose, glycated haemoglobin, lipids and other biomarkers. Adverse events are monitored through self-complete questionnaire and clinical visits. Outcomes will be analysed by regression models. Subgroup and sensitivity analyses will be conducted for different epidemiological subgroups and statistical analyses. Enrollment started in July 2018.

Ethics and dissemination

This study was approved by the Institutional Review Board of the University of Hong Kong / Hospital Authority Hong Kong West/East/Kowloon Central clusters (UW 16-553/HKEC-2019-026/REC (KC/KE)-19-0049/ER-4). We will report the findings in medical journals and conferences. The dataset will be available upon reasonable request.

Trial registration

This study is prospectively registered on ClinicalTrials.gov (NCT03535935) on 24 May 2018.

STENGTHS AND LIMITATIONS

Strengths

1. Existing epidemiological data suggested that Chinese medicine was associated with retarded progression of renal function among diabetic kidney disease patients and it is timely to perform a clinical trial on astragalus, the most used herbs in diabetes and diabetic kidney disease with unclear clinical effectiveness.
2. The inclusion / exclusion criteria, primary outcome measurement and the corresponding analyses are designed according to conventionally used parameters to facilitate further meta-analysis with other clinical studies for wide range of audience.
3. A responder analysis is built into the trial as secondary analysis to identify possible factors (including biomarkers and symptom-based diagnosis) that could lead to more personalised the use of astragalus.
4. We conducted a focus group interview series to explore the expectations of patients and clinicians (both conventional and Chinese medicine) to refine the study design (drug form, dosage, administration route, frequency, health services delivery and outcome measurement) for better clinical translation.

Limitations

1. As the trial is open-label, subjective outcomes including quality of life could not be assessed.

Keywords

Diabetic kidney disease; clinical trial; astragalus, huangqi; effectiveness; renal medicine

Introduction

In 2019, it was estimated that 463 million (9.3%) people was living with diabetes worldwide and the figure was projected to reach 578 million by 2030, with the highest prevalence in North America at present. (1) In 2017, The healthcare expenditure on diabetes reached US$ 850 billion globally (11.6% of global health expenditure). (2, 3) Diabetic kidney disease (DKD) refers to the chronic kidney disease (CKD) caused by long-standing diabetes. DKD presents in more than one third of all diabetic patients and is the leading cause of end-stage renal disease in many developed regions which requires replacement therapy including dialysis and transplantation. (4, 5) In Hong Kong, the incidence of diabetes-related end-stage renal disease increased from 26.2% in 1996 to 49.6% in 2013 (6) and end-stage renal disease increased 5.23 times the annual direct medical cost to the local public health system. (7) Furthermore, DKD was accounted for 23.4% (31.1% vs 7.7%) absolute increase in 10-year mortality in US (8) and 16 years shorter life-expectancy in Taiwan (9) when compared to those without diabetes and kidney diseases.

The risk factors and pathogenesis of DKD are heterogeneous (5) involving metabolic, (10, 11) inflammatory, (12-14) hemodynamic, (15-18) and many other pathways. (5, 19) Thickening of glomerular basement membrane, mesangial expansion, effacement of foot process, formation of Kimmelstiel-Wilson nodules, glomerulosclerosis and interstitial fibrosis are classical histopathological features of DKD. (15, 20) Conventional blockade on the renin–

angiotensin–aldosterone system (RAAS) offers limited effect on clinical outcomes. (21-24) In a previous meta-analysis of 9797 patients with stage 3 to 5 CKD, RAAS blockade did not reduce all-cause mortality and only provided a mild risk reduction in the composite endpoint of replacement therapy initiation or doubling of serum creatinine when compared to placebo or other antihypertensive agents. (22) RAAS blockade with combined angiotensin-converting enzyme inhibitor (ACEI) and angiotensin II receptor blocker (ARB) resulted in increased adverse events but not the expected synergistic effect. (23, 25) More therapies with different working mechanisms are needed.

Chinese Medicine (CM) has been extensively used among diabetes and DKD patients in Asia. (26, 27) Previous observational studies from Taiwan with 47,876 and 24,971 subjects showed that the use of add-on prescribed CM is associated with 40% reduction of mortality (27) and 59% risk reduction of end-stage renal disease, respectively. (26) *Astragalus membranaceus*, commonly known as huang-qi, is the most frequently used CM or dietary herb for DKD. (28) Systematic reviews showed that astragalus could enhance creatinine clearance, reduce albuminuria and reduce blood pressure among CKD and DKD patients. (29-31) Meta-analysis also showed that astragalus' effect in improving renal clearance and reducing albuminuria was better than routine care (without ACEI or ARB) and the efficacy was comparable to ACEI or ARB. (31) *In vivo* and *in vitro* evidence suggested that astragaloside IV, an active ingredient of astragalus, could ameliorate podocyte apoptosis, foot process

effacement, mesangial expansion, glomerulosclerosis and interstitial fibrosis through regulating the NF-κB and TGF-β_1 signalling pathway, which partly explained the renoprotective effect. (32, 33) Nevertheless, the methodological reporting and quality of the existing clinical trials were inadequate and further evaluation is needed. Based on our preliminary result of ongoing trials, CM formulations containing astragalus is likely to retard the progression of DKD. (34, 35) Considering the extensive currently use of astragalus, clinical study could be considered before preclinical investigation as suggested by the World Health Organisation. (36)

Methods/Design

Objective

This pragmatic clinical trial aims to evaluate the effectiveness of add-on astragalus on type 2 diabetic patients with stage 2 to 3 chronic kidney disease and macroalbuminuria, and to identify related response predictors for subsequent large-scale health services research.

Study Design

Add-on, assessor-blind, parallel, pragmatic randomised controlled trial. The World Health Organisation Trial Registration Data Set (Appendix 1) and SPIRIT checklist (Supplementary File) are enclosed.

Inclusion and exclusion criteria

Patients with 1) type 2 diabetes for at least 5 years; 2) estimated glomerular filtration rate (GFR) ≥ 30 and < 90 mL/min/1.73m^2 confirmed by repeated testing over three months calculated by the abbreviated MDRD study equation; (37, 38) 3) persistent macroalbuminuria with spot urine albumin-to-creatinine ratio (UACR) ≥ 300 mg/g confirmed by at least 2 consecutive first morning void urine samples; 4) age between 35 to 80 years old; 5) stable dose of anti-diabetic agent(s) including insulin for at least 12 weeks; and 6) stable dose of ACEI or ARB for at least 12 weeks will be recruited.

Patients will be excluded if with 1) UACR ≥ 5000 mg/g; 2) a known history of glomerulonephritis, polycystic kidney disease, systemic lupus erythematosus or any

suggestive evidence of nondiabetic glomerulopathy; 3) known history of kidney transplant; 4) concurrent severe disorders of heart, brain, liver, and hematopoietic system, tumor, mental disorder; 5) deranged liver function; 6) poorly controlled blood pressure; 7) known history of intolerance or malabsorption of oral medications; 8) uncontrollable urinary infection; 9) experiencing pregnancy; and 10) participating in other clinical trial(s) within 30 days.

Sample size calculation

Since the primary objective of this trial is to evaluate key clinical outcomes and to perform a preliminary analysis on potential response predictors, we calculated the sample size based on the control of inflation factor (IF) to the estimation of sample size for the subsequent large-scale studies (39, 40). 118 patients (around 60 per group) are needed.

$$IF = S_{ucl} / S_{obs} = \text{sqrt}\,[(n-1)/\chi^2_{1-\alpha,\, n-1}]$$

$$N_{adj}/N_{unadj} \approx IF^2 \approx n_{unadj} * IF^2$$

$$N \approx [2(\square_{1-\alpha/2}+\square_{1-\beta'})^2(IF*s)^2]/(\mu_1-\mu_2)^2] = \quad [2(\square_{1-\alpha/2}+\square_{1-\beta})s^2]/(\mu_1-\mu_2)^2]$$

$$\square_{1-\beta'} = \square_{1-\alpha/2}(IF^{-1}-1) + \square_{1-\beta} * IF^{-1}$$

where IF = Inflation factor

S_{ucl} = Standard deviation of upper confidence interval

S_{obs} = Observed standard deviation in pilot study

α = Chosen confidence level

ß = Nominal power set for main study

ß' = Actual power achieved for main study by using pilot standard deviation for sample size calculation

n = Sample size of pilot study

N = Sample size of main study

N_{unadj} = Sample size of main study with no adjustment on standard deviation

N_{adj} = Sample size of main study with adjustment on standard deviation

The standard deviation used for sample size calculation for large-scale main studies is often underestimated by small-scale pilot studies, therefore an IF is needed for adjustment in sample size calculation. (39, 40) IF is calculated based on the size of pilot study and the confidence level of achieving at least the desired power in subsequent main studies. Therefore, the actual achieved power of the main studies depends on the nominal power set for the main study and the IF.

In order to be 95% confident (two-sided) that the main study achieves a power of 70% with nominal power set at 80% (i.e., a 10% power forfeit), the IF should be controlled to less than 1.13. At IF = 1.13, a sample size of 100 is therefore needed to attain 95% one-sided confidence that the main studies will achieve the nominal power to test the hypothesis of add-

on astragalus could be more effective in stabilising the GFR among DKD patients when compared to standard care. To allow a 15% attrition rate, a sample size of 118 patients is therefore needed for this pilot study.

Currently, there is limited evidence on the symptom-based response predictors of astragalus. A general recommendation for power estimation is to have 10 events per variable (41). From the previous systematic review, we estimate that around 60% of patients will have stabilised GFR after receiving astragalus. (31) 118 subjects with 15% attrition will power up to 6 variables for the screening of predictors. A univariable screening on the 11 pre-specified potential symptom-based predictors will be conducted to reduce the number of predictors for the subsequent multivariable regression analysis, in order to maximise the power of the regression analysis.

Recruitment and randomisation

Patients will be recruited from general and specialist outpatient clinics of Queen Mary Hospital, Queen Elizabeth Hospital, Hospital Authority Hong Kong East Cluster through consultations, and the community via public health campaigns. The details of study will be explained by principal investigators (PIs) or co-investigators (Co-Is) before written consent is obtained from each participating patient. All patients will undergo a 2-week run-in period, during which the dosage of their medications will be stabilised. Blood and urine sample will be sent to an independent local laboratory for screening. Patients are considered eligible for

the study if their liver functions are normal and fulfil the inclusion criteria. Recruitment started in July 2018 and the recruitment is on-going.

A random sequence was generated and encrypted with computer by an independent staff of the University of Hong Kong and kept in sealed opaque envelopes. The password of the sequence is kept in a sealed, duly signed opaque envelop locked by research assistants (RAs). The allocation sequence is concealed from PIs, Co-Is, CM physicians and all research staffs that are responsible for patient screening, randomisation or sample analysis. Eligible patients will be randomised 1:1 to either receive active intervention along with standard care or standard care alone. The allocation is masked from the outcome assessor (technicians from an independent laboratory). The study subjects could not be masked due to the nature of treatment. Since the primary clinical outcomes under investigation are objectively assessed and the outcome assessor is blinded, placebo effect and outcome measurement bias should be minimised. The flow of study is presented in **Figure 1**. Under no circumstances the primary outcome assessors will be unblinded.

Intervention and control

The intervention under investigation is astragalus. Patients under intervention will receive astragalus daily on top of standard medical care for 48 weeks. The CM physicians will advise on the dose and possible adverse events of astragalus based on his/her professional knowledge. Existing literature supports a safe dosage of raw astragalus from 15 to 50 g/day.

(42, 43) According to the China Pharmacopeia, the recommended therapeutic dosage of astragalus is below 30 g/day. To ensure the safety of patients, CM physicians are reminded not to propose dosage exceeding 30 g/day. All patients will continue their standard medication and follow-up with the same consultation schedule with CM physicians. Standard care is used as control to best reflect the real-world practice and the future application scenario of this trial. *(44)*

Herbal safety

Soluble herbal granules prepared by PuraPharm (listed in US Pharmacopeia as dietary ingredient: VER-DI-PUR-09) are used. The production process is in strict compliance with standards of Good Manufacturing Practice (GMP). Fully registered CM physicians from the School of Chinese Medicine, The University of Hong Kong will be responsible for the clinical diagnosis and prescription. After 4 to 6 weeks of randomisation, all patients will undergo liver function tests and renal function tests to monitor acute changes of renal and liver function.

Outcome measurement

The primary outcome measures are the changes of estimated GFR (45) and UACR from baseline (week 0) to treatment endpoint (week 48). As the progression of kidney disease is slow, we believe reporting 1-year (48-week) change in GFR is necessary to avoid extrapolation while extended observation may lead to substantial attrition and is limited by

resources. Secondary outcome measures include adverse events, and changes in CKD stage, haemoglobin A_{1c} (HbA_{1c}), lipids, urinary monocyte chemotactic protein 1 (MCP-1) and urinary cystatin C from baseline to the midpoint (week 24) and the end of treatment.

Data collection

Patient demographics including age, gender, body mass index (BMI), duration of diabetes, other medical history and concurrent medications will be retrieved by the electronic clinical management system of Hospital Authority by Co-Is and RAs. Estimated GFR, UACR, HbA_{1c}, lipids and liver function tests will be assessed by an independent laboratory (Chan & Hou Medical Laboratories Limited) which is accredited by College of American Pathologists, Royal College of Pathologists Australasia and Quality Control for Molecular Diagnostics UK. MCP-1 and cystatin C will be assessed at lead-PI's research laboratory by an independent RA with commercially available kits. Blood pressure will be taken during consultation. Blood and urine samples will be taken at an overnight (>8 hours) fasting state.

Estimated GFR will be calculated using the MDRD equation with serum creatinine, age, ethnicity and gender. Clinical presentations and CM symptom-based diagnosis will be assessed in a structured consultation developed for this purpose. To ensure consistency and reliability of assessment and to minimise bias from investigators across the study, only 3 synchronised CM physicians will assess the patients.

A self-complete questionnaire will be distributed to the subjects to monitor adverse

events, and they are advised to inform the PIs, Co-Is, CM physicians or RAs immediately if adverse events arise. All adverse events will be coded based on Common Terminology Criteria for Adverse Events (CTCAE) 5.0, following the recommendation of CONSORT (Consolidated Standards of Reporting Trials) Extension for Chinese Herbal Medicine Formulas. (46)

Follow-up consultations will be held for all patients bi-weekly in the first month and monthly subsequently until the end of treatment for all patients. Minor adjustments are allowed based on clinical needs. Evaluation of outcomes will be performed at baseline, week 24 (treatment midpoint) and 48 (end of treatment). The follow up schedule is summarised below in **Table 1**.

Data management

A trial management committee (TMC) formed by lead-PI, Co-Is and RAs will centralise all trial data. Co-Is and RAs will collect, clean and send the data to TMC weekly. All data will be double entered, secured and cleaned before analysis to prevent data entry errors. TMC will have regular meetings monthly to discuss the progress and double check the data of the trial. Only PI, Co-Is and regulatory bodies will have access to the patient data to protect data privacy. An independent Data and Safety Monitoring Board (DSMB) (VCH Chung, W Wong, JWF Yeung) has been established with expert in methodology, biostatistics and clinical medicine to monitor the progress of the trial, including adverse events and change in protocol. DSMB will

have meetings twice a year. No competing interests has been reported from DSMB. Trial result will be published in academic journal and trial subjects will be notified.

Handling of withdraw and dropout

In order to maximise subjects' compliance, we will provide a triple thorough consent process for all participants covering details of the study schedule, potential side effects of treatment, and the responsibilities of the subjects. An independent e-mail account and a direct telephone line is available for this study to enable active communication with patients. Extra visits will be arranged for patients if necessary. To monitor the adherence of study medication, we will arrange irregular visits for patients and count the unfinished medication.

Termination criteria

The treatment will be terminated for a specific subject if he/she: 1) develops serious adverse event (SAE); 2) develops hypersensitivity towards astragalus; and 3) participates in other clinical trial. The whole study will be terminated under the following circumstances: 1) presence of clustered SAE(s) related to astragalus with supportive evidence; and 2) completion of all follow-up assessments.

SAE includes adverse events that result in death, require either hospitalisation or the prolongation of hospitalisation, are life-threatening, result in a persistent or significant disability/incapacity, result in a congenital anomaly/birth defect or events classified as Grade 3 or above in CTCAE 5.0. Other important medical events, based upon appropriate medical

judgement, may also be considered SAEs if a patient's health is at risk and intervention is required to prevent an outcome mentioned.

Data analysis

Missing values will be imputed by multiple regression. The analysis will follow intention-to-treat principle that all randomised patients will be included in the analysis. STATA and PRISM will be used for the analysis.

Demographics will be presented as mean ± standard deviation or percentage. UACR will be log-transformed and reported as geometric means. Smoking history will be stratified into non-smoker, ex-smoker and current smoker. Rapid renal progression is pre-defined as a consecutive annual GFR drop of over 5 ml/min/1.73m^2 or a cumulative GFR drop of over 25 ml/min/1.73m^2 for 5 years. (47, 48) Differences in mean and proportion between groups will be tested by t-test and χ^2 test.

Mixed regression models will be used to compare the rate of change in estimated GFR and UACR. Analysis of covariance (ANCOVA) will be used to compare the adjusted mean of outcomes at week 48 between intervention group and control group with the corresponding baseline values as covariates. Data will be presented as the difference in adjusted means between the groups with 95% CI and the corresponding p-value.

The adverse events will be recorded according to CTCAE 5.0 and categorised into 5 grades (Grade 1: mild, asymptomatic or mild symptoms, clinical or diagnostic observations

only, no intervention indicated; Grade 2: moderate, minimal, local or non-invasive intervention indicated, limiting age-appropriate instrumental activities of daily living; Grade 3: severe or medically significant but not immediately life-threatening, hospitalisation or prolongation of hospitalisation indicated, disabling, limiting self-care activities of daily living; Grade 4: life-threatening consequences, urgent intervention indicated; and Grade 5: death related to adverse events). The percentage of all adverse events with more than 1 case will be compared between groups. SAE will be analysed case by case descriptively.

To minimise Type I error inflation, the analysis will follow a hierarchical approach in the order of 1) comparison of baseline to end of treatment on estimated GFR and UACR; 2) comparison of baseline to end of treatment on other outcome measurements; 3) comparison of baseline to treatment midpoints on estimated GFR and UACR and 4) comparison of baseline to treatment midpoints on other outcome measurements.

For the assessment of predictive factors as secondary analysis, the dependent variable will be the treatment response which is categorised into:

1. Improved or stabilised renal function, defined as estimated GFR after 48-week treatment being higher or equal to baseline.

2. Non-responder, defined as patients having estimated GFR decreased at a rate of less than 5 mL/min/1.73m^2 after 48-week treatment compared to baseline.

3. Rapid deteriorating renal function, defined as estimated GFR of more than 8 mL/min/1.73m^2 after 48-week treatment compared to baseline.

Potential prognostic variables (baseline values) will include:

1. Demographics: age, gender, BMI, systolic blood pressure, history and duration of smoking and alcohol consumption
2. Symptom-based diagnosis: presence of CM-based symptom-based subtype (e.g. *spleen and kidney qi deficiency*) based on the presentation of standardised and commonly documented signs and symptoms (49)
3. Biochemical profile: GFR, UACR, HbA_{1c}, lipids

All potential predictors will first be included into univariable regression models followed by multivariable stepwise regression analysis. Variables that are not significant at a 5% level will be excluded.

Subgroup analyses will be performed for 1) CKD stages stratified into stage 2, 3a and 3b; (50) 2) UACR levels stratified by 100 mg/mmol; (51) 3) gender and 4) age groups. Sensitivity analyses will be performed for 1) per-protocol cohort; 2) estimation of GFR by c-MDRD (52) and CKD-EPI (53) equations; 3) missing data imputed with last-observation-carried-forward and 4) different analytical approaches (change-score) and categorisations of primary outcomes.

Patient and public involvement

We conducted a focus group interview series to collate the experience and expectations of patients and clinicians (both conventional and Chinese medicine) on the study design (drug form, dosage, administration route, frequency, health services delivery and outcome measurement) for this trial. (54) The study results will be disseminated to diabetes patient groups and the participants via public workshops and talks.

Ethics and dissemination

This study was approved and monitored by the Institutional Review Board of the University of Hong Kong / Hospital Authority Hong Kong West/East/Kowloon Central clusters (Ref: UW 16-553/HKEC-2019-026/REC (KC/KE)-19-0049/ER-4). The patient information sheet and consent form are enclosed in Appendix 2. Result will be disseminated as conference presentations and journal publications upon completion.

Discussion

Diabetes and DKD are significant public health burdens and astragalus is the most used herbs among these patients with unclear clinical effectiveness. There is an urgent need to characterise the effect and response predictors of astragalus to prevent unnecessary consumption and to increase the cost-effectiveness of administration. Also, the assessment of response predictors of both biomarkers and symptom-based factors will facilitate the integration and clinical translation of generated evidence between conventional medicine and CM physicians. Based on our preliminary result of ongoing trials, CM formulations containing astragalus is likely to retard the progression of DKD. (34, 35) This trial aims to evaluate the effect of astragalus and identify related response predictors for more personalised application and further large-scale health services research.

To facilitate further meta-analysis with other clinical studies for wide range of audience, the inclusion / exclusion criteria, primary outcome measurement and the corresponding analyses are designed according to conventionally used parameters similar to other pharmaceutical studies. (55) A responder analysis is included as secondary analysis to identify possible factors (including biomarkers and symptom-based diagnosis) that could lead to more personalised the use of astragalus. Besides, we conducted a focus group interview series to explore the expectations of patients and clinicians (both conventional and Chinese medicine) to refine the study design for better clinical

translation. (54) The major limitation of this trial is the open-label nature. The study subjects could not be masked due to the nature of treatment. Since the clinical outcomes under investigation are objective and the outcome assessor is blinded, placebo effect and outcome measurement bias should be minimised. However, subjective outcomes including quality of life could not be assessed.

List of abbreviations

ACEI – angiotensin-converting enzyme inhibitors

ANCOVA – analysis of covariance

ARB – angiotensin II receptor blockers

BMI – body mass index

CI – confidence interval

CM – Chinese medicine

Co-I – co-investigator

CONSORT – Consolidated Standards of Reporting Trials

CTCAE – Common Terminology Criteria for Adverse Events

DKD – diabetic kidney disease

CKD – chronic kidney disease

GMP – Good Manufacturing Practice

HbA_{1c} – haemoglobin A_{1c}

MCP-1 – monocyte chemotactic protein 1

NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells

PI – principal investigator

RA – research assistant

RAAS – renin–angiotensin–aldosterone system

SAE – serious adverse event

TGF-β_1 – transforming growth factor beta-1

UACR – urine albumin-to-creatinine ratio

DECLARATION

Ethics approval and consent to participate

This study was approved and monitored by the Institutional Review Board of the University of Hong Kong / Hospital Authority Hong Kong West/East/Kowloon Central clusters (Ref: UW 16-553/HKEC-2019-026/REC (KC/KE)-19-0049/ER-4). Written consent will be obtained from all subjects, including change of protocol. This protocol is prospectively registered on ClinicalTrials.gov (NCT03535935) on 24 May 2018. We publish this protocol after the first DSMB meeting.

Consent for Publication

Not applicable. No personal information is included.

Availability of data and materials

The datasets used and/or analysed during the current study will be available from the corresponding author on reasonable request.

Competing interests

None declared.

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study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Authors' Contributions

KW Chan and SCW Tang designed the study. KW Chan, AKS Kwong, PN Tsui, SCY Cheung, GCW Chan, MMY Wong, KCB Tan and WF Choi recruited the patients and provided clinical consultation. WH Yiu provided expert opinion and support in biochemical analysis. L Lao, Z Zhang, Y Zhang provided expert opinion on the study design. KW Chan and SCW Tang drafted the manuscript. All authors involved in the manuscript revision.

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REFERENCES

1. Sinclair A, Saeedi P, Kaundal A, Karuranga S, Malanda B, Williams R. Diabetes and global ageing among 65-99-year-old adults: Findings from the International Diabetes Federation Diabetes Atlas, 9(th) edition. *Diabetes Res Clin Pract.* 2020:108078.

2. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract.* 2018;138:271-81.

3. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract*. 2017;128:40-50.

4. Thomas MC, Cooper ME, Zimmet P. Changing epidemiology of type 2 diabetes mellitus and associated chronic kidney disease. *Nature Reviews Nephrology*. 2016;12(2):73-81.

5. Tang SCW, Yiu WH. Innate immunity in diabetic kidney disease. *Nature Reviews Nephrology*. 2020;16(4):206-22.

6. Leung CB, Cheung WL, Li PK. Renal registry in Hong Kong-the first 20 years. *Kidney Int Suppl*. 2015;5(1):33-8.

7. Jiao F, Wong CKH, Tang SCW, Fung CSC, Tan KCB, McGhee S, et al. Annual direct medical costs associated with diabetes-related complications in the event year and in

subsequent years in Hong Kong. *Diabetic Medicine*. 2017;34(9):1276-83.

8. Afkarian M, Sachs MC, Kestenbaum B, Hirsch IB, Tuttle KR, Himmelfarb J, et al. Kidney disease and increased mortality risk in type 2 diabetes. *J Am Soc Nephrol.* 2013;24(2):302-8.

9. Wen CP, Chang CH, Tsai MK, Lee JH, Lu PJ, Tsai SP, et al. Diabetes with early kidney involvement may shorten life expectancy by 16 years. *Kidney Int.* 2017;92(2):388-96.

10. Goh SY, Cooper ME. Clinical review: The role of advanced glycation end products in progression and complications of diabetes. *The Journal of Clinical Endocrinology and Metabolism.* 2008;93(4):1143-52.

11. Forbes JM, Cooper ME, Oldfield MD, Thomas MC. Role of advanced glycation end products in diabetic nephropathy. *J Am Soc Nephrol*. 2003;14(8 Suppl 3):S254-8.

12. Pichler R, Afkarian M, Dieter BP, Tuttle KR. Immunity and inflammation in diabetic kidney disease: translating mechanisms to biomarkers and treatment targets. *American Journal of Physiology Renal Physiology.* 2017;312(4):F716-f31.

13. Navarro-Gonzalez JF, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. *J Am Soc Nephrol*. 2008;19(3):433-42.

14. Navarro-Gonzalez JF, Mora-Fernandez C, Muros de Fuentes M, Garcia-Perez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. *Nature Reviews Nephrology*. 2011;7(6):327-40.

15. Alicic RZ, Rooney MT, Tuttle KR. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. *Clin J Am Soc Nephrol*. 2017;12(12):2032-45.

16. Premaratne E, Verma S, Ekinci EI, Theverkalam G, Jerums G, MacIsaac RJ. The impact of hyperfiltration on the diabetic kidney. *Diabetes & Metabolism*. 2015;41(1):5-17.

17. Tonneijck L, Muskiet MH, Smits MM, van Bommel EJ, Heerspink HJ, van Raalte DH, et al. Glomerular Hyperfiltration in Diabetes: Mechanisms, Clinical Significance, and Treatment. *J Am Soc Nephrol.* 2017;28(4):1023-39.

18. Palatini P. Glomerular hyperfiltration: a marker of early renal damage in pre-diabetes and pre-hypertension. *Nephrol Dial Transplant.* 2012;27(5):1708-14.

19. Yiu WH, Li RX, Wong DWL, Wu HJ, Chan KW, Chan LYY, et al. Complement C5a inhibition moderates lipid metabolism and reduces tubulointerstitial fibrosis in diabetic nephropathy. *Nephrol Dial Transplant.* 2018;33(8):1323-32.

20. Alsaad KO, Herzenberg AM. Distinguishing diabetic nephropathy from other causes of glomerulosclerosis: an update. *Journal of Clinical Pathology*. 2007;60(1):18-26.

21. Hsu TW, Liu JS, Hung SC, Kuo KL, Chang YK, Chen YC, et al. Renoprotective effect of renin-angiotensin-aldosterone system blockade in patients with predialysis advanced chronic kidney disease, hypertension, and anemia. *JAMA Internal Medicine.* 2014;174(3):347-54.

22. Nistor I, De Sutter J, Drechsler C, Goldsmith D, Soler MJ, Tomson C, et al. Effect of

renin-angiotensin-aldosterone system blockade in adults with diabetes mellitus and advanced chronic kidney disease not on dialysis: a systematic review and meta-analysis. *Nephrol Dial Transplant*. 2018;33(1):12-22.

23. Palmer SC, Mavridis D, Navarese E, Craig JC, Tonelli M, Salanti G, et al. Comparative efficacy and safety of blood pressure-lowering agents in adults with diabetes and kidney disease: a network meta-analysis. *The Lancet*. 2015;385(9982):2047-56.

24. Chan GC, Tang SC. Proteinuria reaffirmed as a risk modifier in diabetic chronic kidney disease. *Nephrol Dial Transplant.* 2018;33(11):1873-74.

25. Chan GC, Tang SC. Diabetic nephropathy: landmark clinical trials and tribulations. *Nephrol Dial Transplant*. 2016;31(3):359-68.

26. Lin MY, Chiu YW, Chang JS, Lin HL, Lee CT, Chiu GF, et al. Association of prescribed Chinese herbal medicine use with risk of end-stage renal disease in patients with chronic kidney disease. *Kidney Int*. 2015;5(10):226.

27. Hsieh CF, Huang SL, Chen CL, Chen WT, Chang HC, Yang CC. Non-aristolochic acid prescribed Chinese herbal medicines and the risk of mortality in patients with chronic kidney disease: results from a population-based follow-up study. *BMJ Open*. 2014;4(2):2013-004033.

28. Li Q, Zhang H, Fei Y, et al, Treatment of Diabetic Nephropathy by Integrative Medicine: A Multi-center Prospective Cohort Study. *Chinese Journal of Integrated*

Traditional and Western Medicine. 2012;32(3):317-21.

29. Zhang HW, Lin ZX, Xu C, Leung C, Chan LS. Astragalus (a traditional Chinese medicine) for treating chronic kidney disease. *The Cochrane Database of Systematic Reviews*. 2014;22(10).

30. Li M, Wang W, Xue J, Gu Y, Lin S. Meta-analysis of the clinical value of Astragalus membranaceus in diabetic nephropathy. *Journal of Ethnopharmacology*. 2011;133(2):412-9.

31. LIU Yi-hong, TIAN Hao-ming. Effect of Astragaulus Membranaceus on Diabetic Nephropathy:A Systematic Review. *Chinese Journal of Evidence-Based Medicine*. 2007;7(10):715-27.

32. Gui D, Guo Y, Wang F, Liu W, Chen J, Chen Y, et al. Astragaloside IV, a novel antioxidant, prevents glucose-induced podocyte apoptosis in vitro and in vivo. *PloS One*. 2012;7(6):22.

33. Sun L, Li W, Xiong L, Li G, Ma R. Astragaloside IV prevents damage to human mesangial cells through the inhibition of the NADPH oxidase/ROS/Akt/NFkappaB pathway under high glucose conditions. *Int J Mol Med*. 2014;34(1):167-76.

34. Chan KW, Chan CW, Leung CPS, Yiu WH, Lui SL, Ip TP, et al. Semi-individualised Chinese Medicine Treatment for Diabetic Kidney Disease – From users' perspectives to SCHEMATIC trial interim result and potential mechanisms. *Advances in Integrative Medicine*; Brisbane. Australia 2019. S12.

35. Chan KW, Ip TP, Kwong AS, Lui SL, Chan GC, Cowling BJ, et al. Semi-individualised Chinese medicine treatment as an adjuvant management for diabetic nephropathy: a pilot add-on, randomised, controlled, multicentre, open-label pragmatic clinical trial. *BMJ Open*. 2016;6(8):e010741.

36. World Health Organisation. General guidelines for methodologies on research and evaluation of traditional medicine Geneva: World Health Organisation; 2000.

37. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med*. 1999;130(6):461-70.

38. Glassock RJ, Warnock DG, Delanaye P. The global burden of chronic kidney disease: estimates, variability and pitfalls. *Nature Reviews Nephrology*. 2017;13(2):104-14.

39. Sim J, Lewis M. The size of a pilot study for a clinical trial should be calculated in relation to considerations of precision and efficiency. *J Clin Epidemiol*. 2012;65(3):301-8.

40. Moore CG, Carter RE, Nietert PJ, Stewart PW. Recommendations for planning pilot studies in clinical and translational research. *Clinical and Translational Science*. 2011;4(5):332-7.

41. Pavlou M, Ambler G, Seaman SR, Guttmann O, Elliott P, King M, et al. How to develop a more accurate risk prediction model when there are few events. *BMJ*. 2015;11(351).

42. Li BC. The Combined Treatment of Astragalus and Vasartan for Early Stage Diabetic

Kidney Disease. *China Foreign Medical Treatment*. 2011(23):98-100.

43. Huang H, Gao J. Astragalus Pharmacological Properties and Use of Research in Diabetic Nephropathy. *Journal of Practical Traditional Chinese Internal Medicine*. 2011;25(10):80-2.

44. Ford I, Norrie J. Pragmatic Trials. *N Engl Med*. 2016;375(5):454-63.

45. Grams ME, Sang Y, Ballew SH, Matsushita K, Astor BC, Carrero JJ, et al. Evaluating Glomerular Filtration Rate Slope as a Surrogate End Point for ESKD in Clinical Trials: An Individual Participant Meta-Analysis of Observational Data. *J Am Soc Nephrol.* 2019;30(9):1746-55.

46. Cheng CW, Wu TX, Shang HC, Li YP, Altman DG, Moher D, et al. CONSORT Extension for Chinese Herbal Medicine Formulas 2017: Recommendations, Explanation, and Elaboration. *Ann Intern Med*. 2017;167(2):112-121.

47. Krolewski AS, Skupien J, Rossing P, Warram JH. Fast renal decline to end-stage renal disease: an unrecognized feature of nephropathy in diabetes. *Kidney Int.* 2017;91(6):1300-11.

48. Looker HC, Colombo M, Hess S, Brosnan MJ, Farran B, Dalton RN, et al. Biomarkers of rapid chronic kidney disease progression in type 2 diabetes. *Kidney Int.* 2015;88(4):888-96.

49. Zheng X. Guiding Principle of Clinical Research on New Drugs of Chinese Medicine (Trial). Beijing: China Medical Science Press; 2002.

50. Umanath K, Lewis JB. Update on Diabetic Nephropathy: Core Curriculum 2018. *American Journal of Kidney Diseases*. 2018;71(6):884-95.

51. Chan KW. Integrative management for diabetic kidney disease – Patients' and clinicians' perspectives, clinical effectiveness and possible mechanisms [PhD thesis]. Hong Kong: The University of Hong Kong; 2018.

52. Ma YC, Zuo L, Chen JH, Luo Q, Yu XQ, Li Y, et al. Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. *J Am Soc Nephrol.* 2006;17(10):2937-44.

53. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150(9):604-12.

54. Chan KW, Lee PW, Leung CPS, Chan GCW, Yiu WH, Cheung HM, et al. Patients' and clinicians' expectations on integrative medicine services for diabetes: a focus group study. *BMC Complementary Medicine and Therapies*. 2020;20(1):205

55. Heerspink HJL, Parving HH, Andress DL, Bakris G, Correa-Rotter R, Hou FF, et al. Atrasentan and renal events in patients with type 2 diabetes and chronic kidney disease (SONAR): a double-blind, randomised, placebo-controlled trial. *Lancet*. 2019;393(10184):1937-47.

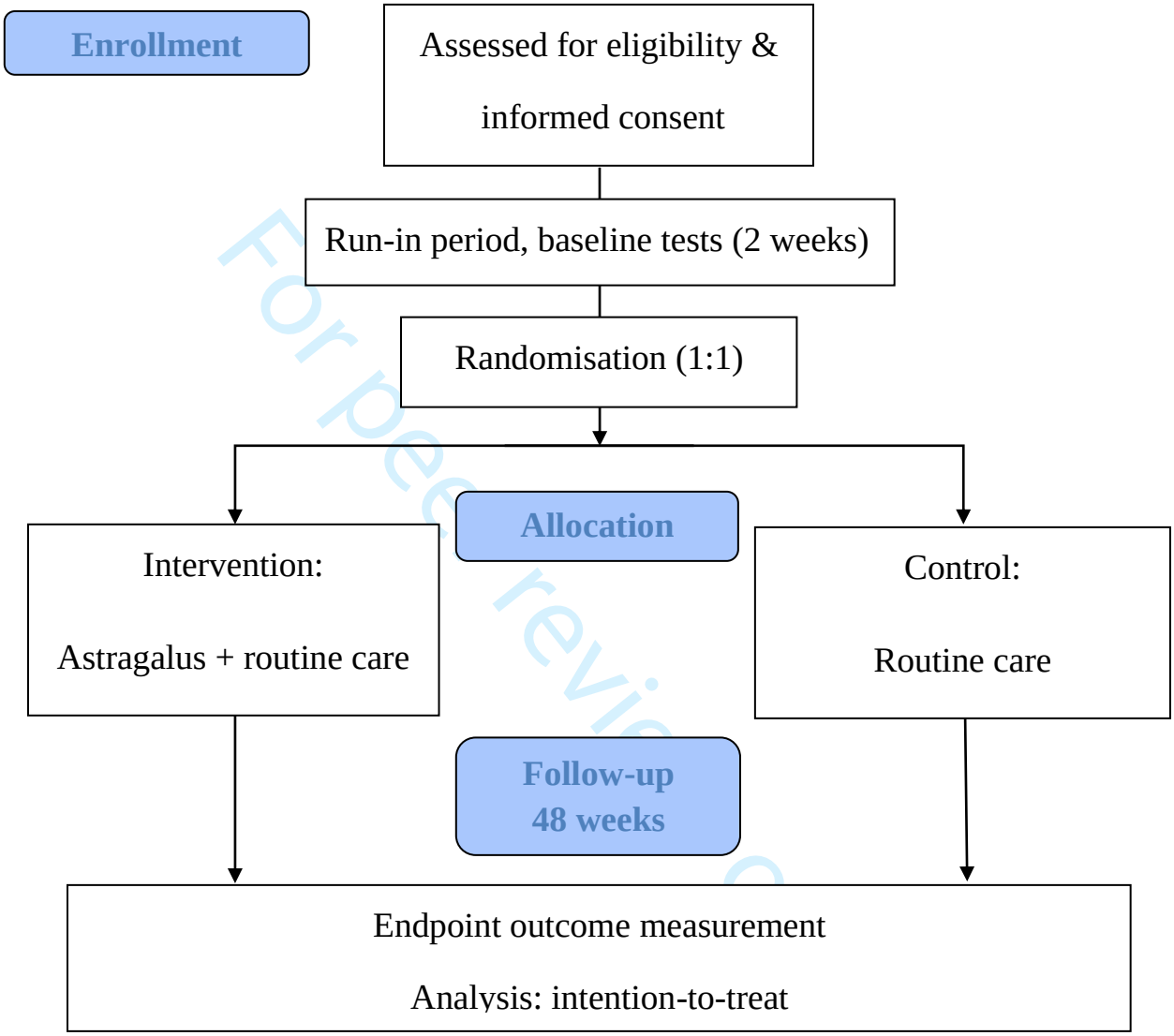
Figure legends

Figure 1 The Flow of Research

Table 1 Follow-up schedule

	STUDY PERIOD				
	Enrolment	Allocation	Post allocation – treatment period		
Timepoint	**Before treatment**	**Week 0, Day 1**	**Week 1-4 (+/- 3 days)**	**After 4-6 weeks (+/- 7 days)**	**After 24 and 48 weeks (+/- 7 days)**
ENROLMENT					
Eligibility screen	X				
Informed consent	X				
Medical history	X				
Allocation		X			
INTERVENTIONS					
Intervention (interventional group)		X	X	X	X
Routine care (all patients)		X	X	X	X
ASSESSMENTS					
Renal and liver function tests, other biomarkers (blood and urine tests)	X			X	X
Blood pressure, weight, hip-waist circumference		X	X	X	X
Demographics		X			
Clinical presentations		X	X	X	X
Adverse events		X	X	X	X

Figure 1. Flow of study



Appendix 1. World Health Organisation Trial Registration Dataset

Data Category	Information
Primary Registry and Trial Identifying Number	ClinicalTrials.gov (NCT03535935)
Date of Registration in Primary Registry	24 May 2018
Secondary Identifying Numbers	HMRF-14151731
Source(s) of Monetary or Material Support	Health and Medical Research Fund, Food and Health Bureau, Hong Kong
Primary Sponsor	The University of Hong Kong
Secondary Sponsor(s)	N/A
Contact for Public Queries	Prof TANG Chi-wai Sydney MD PhD Dr CHAN Kam-wa MSPH MD PhD Tel: +852 2255 3603 Email: scwtang@hku.hk / chriskwc@hku.hk
Public Title	Efficacy, Safety and Response Predictors of Adjuvant Astragalus Therapy for Diabetic Kidney Disease (READY)
Scientific Title	Efficacy, Safety and Response Predictors of Adjuvant Astragalus for Diabetic Kidney Disease (READY) – An Add-on, Assessor-blind, Parallel, Pragmatic Randomised Controlled Trial
Countries of Recruitment	Hong Kong SAR, China
Health Condition(s) or Problem(s) Studied	Diabetic kidney disease
Intervention (s)	Active comparator: Standard medical care with angiotensin converting enzyme inhibitor or angiotensin receptor blocker and oral hypoglycemic agents and/or insulin at stable dose Experimental arm: Semi-individualised dosage of astragalus on top of standard medical care
Key Inclusion and Exclusion Criteria	Ages eligible for study: between 35 and 80 years old Gender eligible for study: Both

	Healthy volunteers: Not accepted **Inclusion Criteria:** - diagnosed with type 2 diabetes for at least 5 years; - with an estimated glomerular filtration rate (GFR) ≥30 <90 mL/min/1.73m2 confirmed with repeat testing over three or more months calculated by the abbreviated MDRD study equation; - persistent macroalbuminuria with spot urine albumin-to-creatinine ratio (UACR) ≥ 300 mg/g confirmed by at least 2 out of 3 consecutive first morning void urine samples; - on stable dose of anti-diabetic drug including insulin for 12 weeks; - on stable dose of angiotensin-converting-enzyme inhibitor or angiotensin receptor blocker for 12 weeks; and - willing and able to give written informed consent **Exclusion Criteria:** - with known history of glomerulonephritis, polycystic kidney disease, systemic lupus erythematosus, any suggestive evidence of nondiabetic glomerulopathy; - with known history of kidney transplant; - with concurrent severe disorders of heart, brain, liver, and hematopoietic system, tumor and mental disorder; - with deranged liver function; - with poorly controlled blood pressure; - with known history of intolerance or malabsorption of oral medications; - with uncontrollable urinary infection; - experiencing pregnancy; or

	- participating in other clinical trial within 30 days
Study Type	Interventional Allocation: randomised Intervention model: parallel assignment (2 arms) Masking: Open label (Accessor of primary outcome measures blinded) Primary purpose: Treatment Phase: II/III Allocation concealment: Sealed opaque envelope prepared by an independent technical staff Sequence generation: computer generated random sequence
Date of First Enrollment	July 2018
Target Sample Size	118
Recruitment Status	Recruiting
Primary Outcome(s)	Changes in estimated glomerular filtration rate and spot urine to albumin ratio (time frame: 48 weeks)
Key Secondary Outcome(s)	Adverse events, changes in, glycated haemoglobin, lipids, blood pressure and other biomarkers

Appendix 2. Sample Consent form

Patient/Subject Information Sheet

1. STUDY TITLE

Efficacy, safety and response predictors of adjuvant astragalus for diabetic kidney disease (READY) – An open-label randomised controlled trial with responder regression analysis

2. INVITATION PARAGRAPH

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your family doctor if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

3. WHAT IS THE PURPOSE OF THE STUDY?

Modern pharmacologic therapy using blockers of angiotensin II is unable to fully suppress the progression of chronic kidney disease (CKD). As a result, many patients progress to end-stage kidney disease and require either dialysis or transplantation. Recently, research data shows that astragalus has anti-fibrotic effect, slower the progression to kidney disease and have been using in addition to routine medical care in Hong Kong. However, the actual pharmacological and therapeutic effect of astragalus are unclear. The present study lasting 48 weeks aims to investigate whether astragalus consumption stabilises renal function and reduces albuminuria.

4. WHY HAVE I BEEN CHOSEN?

You have CKD with unsatisfactory proteinuria control despite angiotensin blockade therapy, and are now being invited to participate in this study to investigate the potential beneficial effect of astragalus that is currently widely used in Hong Kong.

5. DO I HAVE TO TAKE PART?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

6. WHAT WILL HAPPEN TO ME IF I TAKE PART?

You will be randomised by computer after thorough assessment to either receive guidance on astragalus consumption in addition to your standard medications or continue standard medications. The observation period of this study is 48 weeks, and you will be followed up at the clinic in the usual manner, but with additional blood and urine tests as appropriate. You will need to attend 6 extra clinic visits for Chinese medicine consultation in addition to your usual visits over the next 48 weeks.

7. WHAT DO I HAVE TO DO?

There are no lifestyle restrictions by participating in this study, except for the need of practicing contraception. As you have CKD, you will be given dietary advice on salt and protein restriction which are necessary even if you are not participating in this study. You will take the astragalus on top of your therapy for your present condition.

8. WHAT IS THE DRUG OR PROCEDURE THAT IS BEING TESTED?

Astragalus has been widely consumed for years in Hong Kong although with limited clinical evidence. According to existing best available evidence, astragalus has anti-fibrotic effect and could slower the progression to kidney disease. Currently, no adverse events have been confirmed to associate with the use of astragalus.

9. WHAT ARE THE ALTERNATIVES FOR DIAGNOSIS OR TREATMENT?

An alternative treatment option of chronic kidney disease is standard medical care with angiotensin receptor blocker or angiotensin converting enzyme inhibitor alone.

10. WHAT ARE THE SIDE EFFECTS OF TAKING PART?

Astragalus is generally well tolerated. There are no known side effects in addition to those of conventional treatment when astragalus is being used within the reference range of Pharmacopeia of China. Nevertheless, astragalus may have unknown side effects. Full evaluation will be performed and adequate monitoring will be exercised once you start taking it. You will need to attend 6 extra clinic visits in addition to your usual visits over the next 48 weeks. Any claims on loss or injury attributable to the study will be arranged by the University of Hong Kong.

11. WHAT ARE THE DISADVANTAGES AND RISKS OF TAKING PART?

The safety of the astragalus to the human fetus is unclear, therefore women with child-bearing potential must practice contraception.

12. WHAT ARE THE BENEFITS OF TAKING PART?

We hope that astragalus will help you. However, this cannot be guaranteed. The information we get from this study may help us treat future patients with CKD better.

13. WHAT IF NEW INFORMATION BECOMES AVAILABLE?

Sometimes during the course of a research project, new information becomes available about astragalus that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

14. WHAT HAPPENS WHEN THE RESEARCH STUDY STOPS?

After the study stops, you will be advised whether or not to continue with astragalus according to clinical need. Astragalus will not be provided for free.

15. WHAT IF SOMETHING GOES WRONG?

Any claims on loss or injury attributable to the study will be arranged by the University of Hong Kong. If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal health service complaints mechanisms may be available to you.

16. WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

You have the rights of access to personal data and publicly available study results, if and when needed.

Under the laws of Hong Kong (in particular the Personal Data (Privacy) Ordinance, Cap 486), you enjoy or may enjoy rights for the protection of the confidentiality of your personal data, such as those regarding the collection, custody, retention, management, control, use (including analysis or comparison), transfer in or out of Hong Kong, non-disclosure, erasure and/or in any way dealing with or disposing of any of your personal data in or for this study. For any query, you should consult the Privacy Commissioner for Privacy Data or his office (Tel No. 2827 2827) as to the proper monitoring or supervision of your personal data protection so that your full awareness and understanding of the significance of compliance with the law governing privacy data is assured.

By consenting to participate in this study, you expressly authorize:

- the principal investigator and his research team and the ethics committee responsible for overseeing this study to get access to, to use, and to retain your personal data for the purposes and in the manner described in this informed consent process; and
- the relevant government agencies (e.g. the Hong Kong Department of Health) to get access to your personal data for the purposes of checking and verifying the integrity of study data and assessing compliance with the study protocol and relevant requirements.

17. WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?

The results of this study will be published in a medical journal. Your personal information will be kept confidential.

18. WHO IS ORGANISING AND FUNDING THE RESEARCH?

This study is supported by the Health and Medical Research Fund and you do not need to pay any extra cost. Your doctor will not be paid for including you in this study.

19. WHO HAS REVIEWED THE STUDY?

The Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Cluster has reviewed and approved this study. After recruitment, Each patient will receive HK$150 for each blood/urine investigation visit related to this study as travel support.

20. CONTACT FOR FURTHER INFORMATION

In case of enquiry, you may contact Mr Chris Chan or Prof Sydney Tang at 2255 3207. You will be given a copy of this information sheet and a signed consent form to keep. Thank you for taking part in this study!

From the Division of Nephrology
Department of Medicine
University of Hong Kong
Queen Mary Hospital

PATIENT/SUBJECT CONSENT FORM

Title of Project: Efficacy, safety and response predictors of adjuvant astragalus for diabetic kidney disease (READY) – An open-label randomised controlled trial with responder regression analysis

Name of Researcher: Prof Sydney C.W. Tang

Please initial box

1. I confirm that I have read and understood the information sheet dated __/__/__ for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of my medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I agree to take part in the above study. ☐

_____	_____	_____
Name of patient	*Date*	*Signature*
_____	_____	_____
Name of Witness (if applicable)	*Date*	*Signature*
_____	_____	_____
Name of person taking consent (if different from researcher)	*Date*	*Signature*
_____	_____	_____
Researcher	*Date*	*Signature*

Copies to:

☐ Patient/Subject

☐ Researcher's File

☐ Hospital Record

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
	2b	All items from the World Health Organization Trial Registration Data Set	Appendix 1
Protocol version	3	Date and version identifier	Appendix 1
Funding	4	Sources and types of financial, material, and other support	Appendix 1
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Appendix 1
	5b	Name and contact information for the trial sponsor	Appendix 1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	27, 28
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	17, 28

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7-9
	6b	Explanation for choice of comparators	8, 9, 14, 15
Objectives	7	Specific objectives or hypotheses	10
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	1, 10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	13
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10, 11, 14
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	14, 15
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	17, 18
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	18
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10, 14
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	15
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1, Table 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11, 12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13, 18
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13, 14
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13, 14
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13, 14, 28
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	14
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	14
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	15-17
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	18

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	19-21
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	21
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	19
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	17, 28
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	18
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	16, 18
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	17
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	25
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	17, 22

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	17
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	27
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17, 27
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	22
	31b	Authorship eligibility guidelines and any intended use of professional writers	28
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	27
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 2
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.