The DIAMOND trial

A study to assess the Renoprotective Effects of the SGLT2 Inhibitor Dapagliflozin in Non-Diabetic Patients with Proteinuria: a Randomized Double Blind 6-Weeks Cross-Over Trial

STATISTICAL ANALYSIS PLAN

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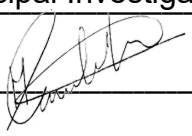
Signature: 

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1. Study synopsis

Despite optimal treatment with renin-angiotensin-aldosterone-system (RAAS) inhibitors, many patients with non-diabetic kidney disease show progressive kidney function loss, which is associated with high residual albuminuria. Novel treatment strategies are therefore required to further decrease albuminuria and to slow kidney function decline.
Dapagliflozin is a sodium-glucose transport (SGLT2) inhibitor and inhibits the reabsorption of glucose and sodium in the proximal tubule. The increased natriuresis following dapagliflozin administration normalizes tubuloglomerular feedback resulting in a reduction in intra-glomerular hypertension, which is in turn manifested by acute reductions in glomerular filtration rate and albuminuria. Since many etiologies of non-diabetic nephropathy are characterized by intraglomerular hypertension, we hypothesize that dapagliflozin acutely decreases GFR and albuminuria in patients without diabetes at risk of progressive kidney function loss via a glucose independent hemodynamic mechanism.
SGLT2 has a plausible therapeutic potential in individuals with non-diabetic nephropathy at risk of renal progression, and are likely to be well tolerated. Exploring the beneficial effects of these agents in non-diabetic proteinuric kidney disease may pave the way for a new indication for this class of drugs. In addition, trials in non-diabetic kidney disease may help to further characterize the non-glucose dependent effects, which may help to differentiate SGLT2i from other glucose lowering agents.

2. Study objectives

Our aim is to investigate two interventions that will be administered to the enrolled patients: Dapagliflozin 10 mg/day and matched placebo.

2.1. Primary Objective

- To assess the change baseline in 24-hr proteinuria with dapagliflozin for six weeks relative to placebo treatment in patients with non-diabetic kidney disease and proteinuria > 500 mg/day on stable ACEi or ARB treatment

2.2. Secondary Objectives

- To assess the effect of dapagliflozin 10 mg/d compared to placebo on Glomerular Filtration Rate (GFR) using iohexol clearance.
- To assess the effect of dapagliflozin 10 mg/d compared to placebo on systolic/diastolic blood pressure
- To assess the effect of dapagliflozin 10 mg/d compared to placebo on body weight
- To assess the effect of dapagliflozin 10 mg/d on selected neurohormones/biomarkers:
    - Hormones of the RAAS (plasma and urine)
    - Natriuretic peptides
    - Urinary adenosine
    - Co-peptin
    - Immunoglobulin G (plasma and urine)
- To characterize the safety of dapagliflozin vs. placebo by determining the number of hypoglycemic episodes between groups, and serious adverse events.

3. Study design

Randomized placebo controlled double blind cross-over trial. Eligible participants will be randomly assigned to one of the two treatment orders: placebo-dapagliflozin *or* dapagliflozin-placebo. Each treatment period lasts 6 weeks followed by a 6 week wash-out period to avoid cross-over effects. Patients will have their study visit in the morning in fasted condition.

3.1. General Design and Plan

A double blind randomized placebo controlled cross-over study will be conducted in subjects with Chronic Kidney Disease aged between 18 and 75 years, proteinuria levels between 500 and 3500 mg/24-hour, and an eGFR ≥ 25 ml/min/1.73m2 will be enrolled. Patients with type 1 or type 2 diabetes, polycystic kidney disease, glomerulonephritis with flares (lupus or anti-neutrophil cytoplasmic antibodies (ANCA) associated vasculitis) or ongoing active renal inflammation will be excluded. Since each subject is his/her own control, this design requires fewer patients as compared with a parallel study design. A wash-out period after each treatment period has been included in the design to avoid cross-over effects.
The study will consist of a screening visit, a 4-week run-in phase for those subjects not on stable ACEi/ARB treatment, 2 consecutive 6-week double blind treatment periods separated by 6-week wash-out period. Subjects will be randomly assigned to one of the treatment orders as depicted in figure 1.

Figure 1: Study design

Screening	Run-in 4 weeks	Placebo 6 weeks	Wash-out 6 weeks	Dapa 10 mg 6 weeks	Wash-out 6 weeks
Screening	Run-in 4 weeks	Dapa 10 mg 6 weeks	Wash-out 6 weeks	Placebo 6 weeks	Wash-out 6 weeks

3.2. Sample Size

Sample size calculations estimated 50 patients completing the study would provide 80% power to detect a 25% reduction in albuminuria. Assuming that approximately 5% of patients will discontinue the study prematurely, we intend to enrol 53 subjects. This calculation assumed a conservative standard deviation of 0.7 in log transformed proteinuria (within-subject standard deviation of 0.475) in order to detect (with alpha at 0.05) at least the 25% reduction in mean albuminuria (i.e, delta=0.288 on the log-transformed proteinuria scale) in dapagliflozin compared to placebo.

4. Study population

Male and female subjects with Chronic Kidney Disease aged between 18 and 75 years, proteinuria levels between 500 and 3500 mg/24-hour, and an eGFR ≥ 25 ml/min/1.73m2 will be enrolled. Patients with type 1 or type 2 diabetes, polycystic kidney disease, glomerulonephritis with flares (lupus or anti-neutrophil cytoplasmic antibodies (ANCA) associated vasculitis) or ongoing active renal inflammation will be excluded. Patients have to be treated with a stable dose of an ACEi or ARB for at least 4 weeks prior to enrolment.

The study population will be selected from the outpatient clinical from hospitals based in the Netherlands (Groningen, Almelo, Amsterdam), Canada, (Toronto, Calgary, Vancouver) and Malaysia (Kuala Lumpur) with the following pre-specified inclusion and exclusion criteria.

Inclusion criteria

- Age ≥18 and ≤75 years
- Diagnosis of IgA nephropathy, focal segmental glomerulosclerosis, or membranous nephropathy. A biopsy proven diagnosis is not a prerequisite for participation in this study.
- Urinary protein excretion > 300 mg/g and ≤ 3500 mg/g in a 24-hr urine collection
- eGFR ≥ 25 mL/min/1.73m^2
- On a stable dose of an ACEi or ARB for at least 4 weeks prior to randomization
- Willing to sign informed consent
- Women of Child-Bearing Potential (WOCBP):
    - WOCBP must be using an acceptable method of contraception to avoid pregnancy throughout the study and for up to 4 weeks after the last dose of study drug in such a manner that the risk of pregnancy is minimized.
    - WOCBP must have a negative serum or urine pregnancy test result (minimum sensitivity 25 IU/L or equivalent units of HCG) within 0 to 72 hours before the first dose of study drug.
    - Women must not be breast-feeding.

WOCBP comprises women who have experienced menarche and who have not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or who are not post-menopausal.

Exclusion criteria

- Diagnosis of type 1 or type 2 diabetes mellitus
- Urinary protein excretion > 3500 mg/day
- Autosomal dominant polycystic kidney disease
- Indication for immunosuppressants as per the treating physician's judgment.
- Treatment with corticosteroids or immunosuppressant within the last 6 months
- Active malignancy aside from treated squamous cell or basal cell carcinoma of the skin.
- Any medication, surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of medications including, but not limited to any of the following:
    - History of active inflammatory bowel disease within the last six months;
    - Major gastrointestinal tract surgery such as gastrectomy, gastroenterostomy, or bowel resection;
    - Gastro-intestinal ulcers and/or gastrointestinal or rectal bleeding within last six months;
    - Pancreatic injury or pancreatitis within the last six months;
    - Evidence of hepatic disease as determined by any one of the following: ALT or AST values exceeding 3x ULN at inclusion visit, a history of hepatic encephalopathy, a history of esophageal varices, or a history of portocaval shunt;
    - Evidence of urinary obstruction of difficulty in voiding at screening
- History of severe hypersensitivity or contraindications to dapagliflozin
- Subject who, in the assessment of the investigator, may be at risk for dehydration or volume depletion that may affect the interpretation of efficacy or safety data
- Participation in any clinical investigation within 3 months prior to initial dosing.
- Donation or loss of 400 ml or more of blood within 8 weeks prior to initial dosing.
- History of drug or alcohol abuse within the 12 months prior to dosing, or evidence of such abuse as indicated by the laboratory assays conducted during the screening.
- History of noncompliance to medical regimens or unwillingness to comply with the study protocol.
- Any surgical or medical condition, which in the opinion of the investigator, may place the patient at higher risk from his/her participation in the study, or is likely to prevent the patient from complying with the requirements of the study or completing the study.
- Pregnancy or breastfeeding

- WOCBP who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and up to 4 weeks after the last dose of study drug.

4.1. Patients recruitment, eligibility and measurements

The target population will be invited for screening. Subjects who meet all entry criteria and who are already on a stable dose (no changes in dose or type of drug) of ACEis or ARBs for at least 4 weeks proceed directly to randomization. Subjects who had their ACEi or ARB medication changed during the preceding 4 weeks of the screening visit proceed to a run-in phase during which the type and dose of these drugs are stabilized. Subjects will be randomly assigned to one of the two treatment orders as depicted in figure 1. Each treatment period lasts 6 weeks followed by a 6 week wash-out period to avoid cross-over effects. Subjects will have their study visit in the morning in fasted condition.
Subjects will be instructed to take the study medication once daily, in the morning, except on study days; on those days, the study drug will be taken after the patient visit.

Measurements

- During the double blind treatment period, and wash-out period patients will collect a 24-hour urine collection at each visit for measurement of 24-hour protein, albumin, glucose, sodium, potassium, creatinine, and urea excretion. Twenty-four hour urine collections will be performed at:
    - Screening visit
    - Day 1, week 3, and week 6 (last day on treatment) of each treatment period
- Office systolic and diastolic blood pressure measurements will be performed at day 1, week 3 and 6 (last day on treatment) of each treatment period
- Vital signs (heart rate and body weight) and blood will be sampled for measurement of HbA1c, glucose, complete blood count, plasma albumin, RAAS biomarkers, natriuretic peptides, co-peptin, neurohormones at day 1, week 3 and 6 of each treatment period.
- Blood and urine samples will be stored for future exploratory biomarker analyses to study the effect of dapagliflozin in this study population.

4.2. Randomization and blinding

Treatment sequence of dapagliflozin and placebo will be randomized. A computer generated randomized code generated by the sponsor will be used. The pharmacy of each participating centre will store the randomization code. Unblinding is not to be performed for any reason, other than an emergency where unblinding is required.

5. Statistical analysis

The flow of patients through the study (screened, fulfilled eligibility criteria, excluded, randomized and potential drop-outs) will be graphically displayed in a CONSORT diagram.
Baseline characteristics will be described by sequence group. All tests are to be two-sided with nominal level alpha set at 5%.
Descriptive statistics will be used to analyse the means and distribution between all the study variables. The means of the normal distributed variables will be compared with the student T-test and non-parametric tests (Mann-Whitney U-test for continuous and Chi-Square test for categorical variables) will be used for the variables that present a particularly skewed distribution.
The base-case analyses of the treatment effects will be unadjusted (with the exception of the period effect).

5.1. Analysis of primary outcome

The log-transformed 24-hour proteinuria values will be analyzed by a mixed-effects general linear regression model with treatment and period as fixed factor and patients (nested in sequence) as a random factor. This type of model, which accounts for repeated observations within individuals (measurement occasion at level one, individuals at level two) will allow us to estimate the treatment effect (estimated by restricted maximum likelihood). We will also explore the period-specific treatment effect adding in the model the interaction between the treatment and period covariates. If the baseline characteristics will be considerably different across the three regions included (Malaysia, North America, Europe) we shall consider to include region as a layer in the multilevel model fitted or as fixed effect covariate. We will also analyse the log-transformed 24-hour albuminuria values (a specific protein using the same model as described above).

The change in secondary outcomes, mGFR, eGFR, body weight and systolic blood pressure will be analyzed by the same model as showed for the proteinuria outcome.

In general, the models employed will be:

$$y_{it} = \beta_{0i} + \beta_1 Treat_{it} + \beta_2 Period_{it} + \beta_3 Interaction + \varepsilon_{it}$$

$$\beta_{0i} = \beta_0 + u_{0i}$$

$$\varepsilon_{it} \sim N(0, \sigma_\varepsilon^2)$$

Where y_{it} is the outcome for the individual *i* at time point t, β_{0i} is the intercept for individual *i* comprised of a sample-average fixed effect β_0 and an individual effect u_{0i} and ε_{it} is the residual error.

Complete case analyses, that is analysing records that present the whole set of information (outcome and covariates) for every visit of the patients will be performed. Patterns of missing data (for the outcomes and the covariates) will be reported. If the proportion of missing records is higher than 5% and/or we will suspect of particular missing mechanisms we will impute the missing values by multiple imputation using the full conditional specification.
Further, if there will be baseline measurements that are missing we will use the values measured at the screening visit: we will document the frequency of this imputation.
As a complementary analysis we will also include week 3 (mid-period 1 visit) and week 15 (mid-period 2 visit) data points so to explore potential "immediate" treatment effects: this will be done for the primary outcome and the secondary outcomes for which the data is available (for instance, no eGFR is collected at week 3 and 15) .
A fully-adjusted model will be also considered for the primary outcome including – among the others – the following time-varying variables: mGFR, systolic blood pressure, body weight and Hba1c.

5.2. Subgroup and sensitivity analyses

The primary and secondary outcomes will be analyzed in the following subgroups:

1. Age (≥ or < 60 years or median)
2. Gender (Male / Female)
3. Region (Asia, Europe, North Am)
4. Proteinuria (≥ or < 1000 mg/24hr)
5. mGFR (≥ or < 45 ml/min/1.73m^2 or median)
6. Systolic blood pressure (≥ or < 130 mmHg or median)
7. Body mass index (≥ or < 25 kg/m^2 or median)
8. Kidney Diagnosis
9. Diuretic use (Yes/No)

We will flag patients who have started or changed the dose of any medication that my influence the primary outcome (e.g. NSAID, corticosteroids, diuretic treatment, mineralocorticoid receptor antagonists, and Vitamin D during the double blind treatment periods and perform a sensitivity analysis on the primary outcome excluding them.

Another sensitivity analysis will be performed on the primary outcome where we will use the average of the screening and randomization 24-hr proteinuria value.

All the analyses described in the SAP are based on the intention to treat principle (that is, patients analysed according to the group they were originally assigned). We will also perform the analyses on the primary outcome(s) considering "on treatment" patients defined as those with adherence to study medication ≥80% (as determined by pill count).

5.3. Supplementary analyses

We will assess the change in primary and secondary outcomes during the dapagliflozin treatment periods with the change in proteinuria/albuminuria after stopping dapagliflozin;

This will be assessed by a longitudinal model as described above assessing the appropriate contrasts.

We will also assess the effect of dapagliflozin using 24-hr protein:creatinine ratio (and albumin:creatinine ratio as dependent variables. The model used for the primary outcome analysis will be used for this analysis.

Finally, the proportion of patients who achieve a proteinuria level <500 mg/g during follow-up will be summarized between dapagliflozin and placebo periods. Additionally, the proportion of patients with 30%, 40%, 50% reduction in proteinuria during follow-up will also be summarized between dapagliflozin and placebo group. McNemar and/or Mainland-Gart tests will be conducted depending on the nature of proportions and on the importance of the sequence order

Adherence on treatment, defined as pill count at least 80% during follow-up, will be summarized by treatment arm and study visits.

6. Evaluation of safety parameters

Counts and percentage per treatment arm will generally summarize all categorical indicators. A breakdown of SAEs stratified by fatal/non fatal status will be presented.

7. Proposed tables and figures

Table 1: Baseline characteristics for all randomised subjects by treatment group and in overall

Baseline Characteristics	Dapa (N=xxx)	Placebo (N=xxx)	Overall (N=xxx)
Categorical variables: n/N (%)			
XXX	xxx xxx	xxx xxx	xxx xxx
XXX	xxx xxx	xxx xxx	xxx xxx
XXX	xxx xxx	xxx xxx	xxx xxx
XXX	xxx xxx	xxx xxx	xxx xxx
Continuous variables:			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Q1 Q2 Q3	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
min max	xxx xxx	xxx xxx	xxx xxx

Table 2: Baseline characteristics for all randomised subjects by group and by period

Baseline Characteristics	Group 1 Period 1 (N=xxx)	Group 1 Period 2 (N=xxx)	Group 2 Period 1 (N=xxx)	Group 2 Period 2 (N=xxx)
Categorical variables: n/N (%)				
XXX	xxx xxx	xxx xxx	xxx xxx	xxx xxx
XXX	xxx xxx	xxx xxx	xxx xxx	xxx xxx
XXX	xxx xxx	xxx xxx	xxx xxx	xxx xxx
XXX	xxx xxx	xxx xxx	xxx xxx	xxx xxx
Continuous variables:				
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Q1 Q2 Q3	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
min max	xxx xxx	xxx xxx	xxx xxx	xxx xxx

Table 3: Descriptive results of follow-up measurements by visits

Variables during follow-up	Group 1 (N=xxx)	Group 2 (N=xxx)	Overall (N=xxx)
Vital signs, BP, Biomarkers:			
Week0			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week3 (if applicable)			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week6			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week12			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week15 (if applicable)			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week18			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week24			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx

Table 4: Descriptive results for primary outcome by visits

Variables during follow-up	Group 1 (N=xxx)	Group 2 (N=xxx)	Overall (N=xxx)
Primary outcome:			
Week0			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week3 (if applicable)			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week6			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week12			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week15 (if applicable)			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week18			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week24			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx

Table 5: Descriptive results for secondary outcomes by visits

Variables during follow-up	Group 1 (N=xxx)	Group 2 (N=xxx)	Overall (N=xxx)
Secondary outcome:			
Week0			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week3 (if applicable)			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week6			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week12			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week15 (if applicable)			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week18			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx
Week24			
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx

Table 6: Treatment effect for primary outcome

Primary outcome	Dapa (N=xxx)	Placebo (N=xxx)	Difference (Dapa – Placebo) (N=xxx)	p-value
Primary outcome:				
Change from baseline in overall				
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx
Change from baseline in period 1				
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx
Change from baseline in period 2				
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx

Table 7: Treatment effect for secondary outcomes

Secondary outcomes	Dapa (N=xxx)	Placebo (N=xxx)	Difference (Dapa – Placebo) (N=xxx)	p-value
Secondary outcomes:				
Change from baseline in overall				
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx
Change from baseline in period 1				
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx
Change from baseline in period 2				
N Mean (SD)	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx

Table 8: Treatment effect for primary outcome by pre-specified subgroups

Primary outcome	Dapa (N=xxx)	Placebo (N=xxx)	Difference (Dapa – Placebo) (N=xxx)	p-value
Change from baseline in overall	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx
Subgroup1				
Yes	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx
No	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx
Subgroup2				
Yes	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx
No	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx
Subgroup3				
Yes	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx
No	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx
Subgroup4				

Yes	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx
No	xxx xxx xxx	xxx xxx xxx	xxx xxx xxx	xxx
Subgroup XXX				

Figure 1: Patients flow chart based on CONSORT

Figure 2: Primary outcome over time by treatment groups and periods. (

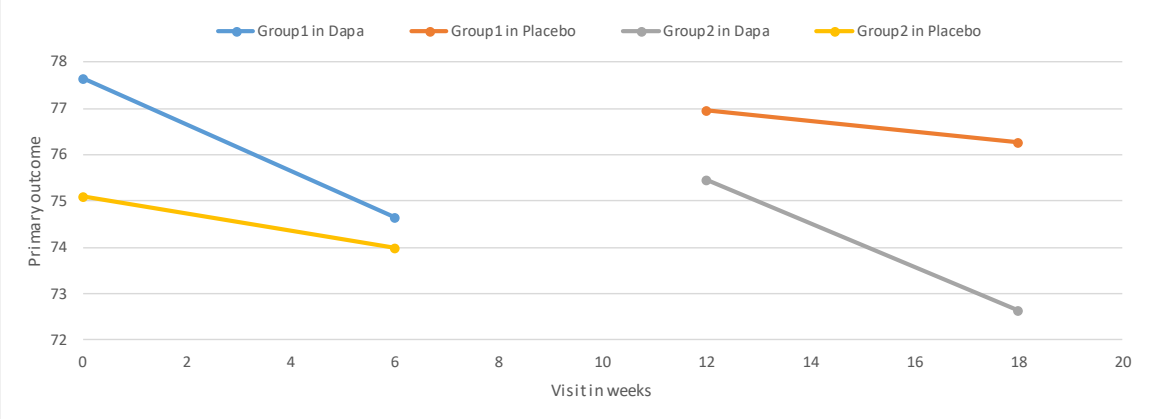

Figure 3: Bar charts with changes from baseline to week 6 for primary/secondary outcomes

Figure 4: Process measurements over time

Similar figure as above, but could add more time points in week 3 and week 15.

Figure 5: Forest plot for subgroup analysis for primary outcome