Statistical Analysis Plan

A Phase 1/2, Randomized, Double-blind, Placebo-controlled, Multiple-ascending-dose Study to Evaluate the Efficacy, Safety, and Pharmacokinetics of MEDI0382 in Overweight and Obese Subjects with a History of Type 2 Diabetes Mellitus

Protocol Number: D5670C00002

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List of Abbreviations

Abbreviation or Specialized Term	Definition
ADA	antidrug antibody
AE	adverse event
AESI	adverse event of special interest
AUC	area under the concentration-time curve
BP	blood pressure
C_{max}	maximum observed concentration
CRO	contract research organization
CSSRS	Columbia Suicide Severity Rating Scale
Red████	████████████████
CV	coefficient of variation
DEC	Dose Escalation Committee
ECG	electrocardiogram
eCRF	electronic case report form
Redact███	████████████
GFR	glomerular filtration rate
GIP	gastric inhibitory peptide
GLP-1	glucagon-like peptide-1
HbA1c	hemoglobin A1c (glycated hemoglobin)
HDL	high-density lipoprotein
Hg	mercury
HR	Heart rate
ITT	intent-to-treat
IV	intravenous(ly)
IXRS	interactive voice or web response system
LDL	low-density lipoprotein
MAD	multiple-ascending dose
MedDRA	Medical Dictionary for Regulatory Activities
MMT	mixed-meal test
NASH	non-alcoholic steatohepatitis
NOAEL	no-observed-adverse-effect level
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
Reda███	████████████
PR(PQ)	ECG interval measured from the onset of the P wave to the onset of the QRS complex
QRS	ECG interval measured from the onset of the QRS complex to the J point
QT	ECG interval measured from the onset of the QRS complex to the offset of the T wave

Abbreviation or Specialized Term	Definition
QTc	cardiac QT interval corrected for heart rate
QTcF	cardiac QTc interval corrected for heart rate by the formula of Fridericia
RR	The time between corresponding points on 2 consecutive R waves on the ECG
SAE	serious adverse event
SID	subject identification
SC	subcutaneous(ly)
T2DM	type 2 diabetes mellitus
TG	triglycerides
T_{max}	time to maximum observed concentration
ULN	upper limit of normal
w/v	weight per volume

1 INTRODUCTION

This document describes the statistical analysis for protocol D5670C00002, an investigation of MEDI0382 in overweight and obese subjects with a history of Type 2 Diabetes Mellitus. Redacted

This study is designed to assess the efficacy, safety/tolerability, and PK of MEDI0382 and pharmacodynamics (PD) effect on blood glucose, following SC administration of multi-ascending doses. This document details the statistical analyses that will be performed and describes the general conventions and definitions that will be used.

In addition, a set of table templates and specifications is planned to be created in a statistical programming plan (SPP) to complement this document.

2 STUDY OVERVIEW

2.1 Study Objectives

2.1.1 Primary Study Objective(s)

The primary objective of this study is to assess the effect of MEDI0382 on glucose control (as measured by the standardized MMT glucose data) and body weight from baseline to the end of a 4-week period at a stable dose.

2.1.2 Secondary Study Objectives

1. To assess the effect of MEDI0382 on glucose control as measured by the standardized MMT, HbA1c, and fructosamine data from baseline through end of treatment
2. To characterize the safety profile of MEDI0382 following SC administration of multiple-ascending doses
3. To characterize the PK and immunogenicity of MEDI0382
4. To characterize the PD effect of MEDI0382 on glucose metabolism following a MMT

2.1.3 Exploratory Study Objectives

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5. To explore the effect of MEDI0382 on the volume and fat/water content of the liver
6. To explore the effect of MEDI0382 on visceral and subcutaneous fat

2.2 Study Design

This is a randomized, double-blind, placebo-controlled study in 2 parts (A and B) with approximately 75 overweight or obese subjects with relatively well controlled T2DM (without significant late diabetic complications) planned for enrollment across up to 10 study sites.

Part A is designed to establish a dose titration regimen for MEDI0382 and a maximal effective dose after titration in T2DM subjects maintained on metformin therapy and Part B is designed to establish the efficacy of MEDI0382 on weight and glycemic control.

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The study design schematic is presented in Figure 2.2-1. More details on the study design can be found in section 3.1 in the protocol.

Part A:
Cohorts 1 through 3
Key endpoints:
Safety/tolerability and PK

Cohort 3: (6 MEDI0382 + 3 placebo)

Initial dose 4 days	Titration dose 1 4 days	Titration dose 2 7 days

Cohort 2: (6 MEDI0382 + 3 placebo)

Initial dose 4 days	Titration dose 1 7 days

Cohort 1: (6 MEDI0382 + 3 placebo)

Stable dose 7 days

Part B:
Cohort 4
Key endpoints:
Body weight, glycemic control, safety/tolerability

24 MEDI0382 + 24 placebo

Initial dose 4 days	Titration dose 1 4 days	Titration Dose 2 4 days	Titration dose 2 28 days at home	Titration dose 2 1 day (inpatient)

41 days dosing period per subject

Figure 2.2-1   Study Flow Diagram

2.3   Treatment Assignment and Blinding

Subjects will receive MEDI0382 or placebo. Subject assignment within a cohort is blinded. For Cohorts 1, 2, and 3, nine subjects in each cohort will be randomized in a 2:1 ratio to receive MEDI0382 or placebo. For Cohort 4, a total of 48 subjects will be randomized in a 1:1 ratio to receive MEDI0382 or placebo. The IXRS will assign a unique randomization code and treatment arm for the subject.

MEDI0382 and placebo are identically labeled and indistinguishable in appearance. As such, the pharmacist, the subject/legal representative, the investigator/ staff who are involved in the treatment or clinical evaluation of the subjects, and the CRO personnel responsible for managing and monitoring the study will NOT be aware of the treatment received. Medimmune will not be blinded to treatment allocation to allow for evaluation of data for

dose escalation decisions (including safety, and PK data) and for SAEs that are unexpected and are suspected to be causally related to an investigational product, and that potentially require expedited reporting to regulatory authorities.

2.4   Sample Size

The sample size of 6 subjects in the MEDI0382 group and 3 subjects in the placebo group for Cohorts 1, 2, and 3 was empirically determined to obtain adequate safety and tolerability evaluation. Under a two-sided 10% significance level, a sample size of 44 evaluable subjects (22 per arm) for Cohort 4 will provide 80% power to detect weight loss of 1.5 kg versus placebo (assuming a standard deviation of 1.9 kg), and 98% power to detect a 20% relative reduction in MMT glucose AUC (up to 240 minutes post-MMT) versus placebo at the end of treatment (assuming a coefficient of variation [CV] of 17%). (Internal data from another investigational agent for T2DM showed a CV of 17% for 24-hour glucose AUC, and the CV for glucose AUC [up to 240 minutes post-MMT] is expected to be similar.) The sample size for Cohort 4 is adjusted to 48 subjects (24 per arm) to account for a 10% drop-out rate. For Cohorts 5 and 6, under a one-sided 2.5% significance level the combination of 24 subjects in the MEDI0382 and 8 subjects in placebo from both cohorts will provide 80% power to rule out more than 20% of MEDI0382 subjects having > 5 mm Hg increase from baseline in diastolic BP on the seventh day of the 300 μg dose level (Day 22 visit of Cohort 5 and Day 17 visit of Cohort 6) relative to that of placebo subjects under the assumption that both placebo and MEDI0382 subjects have a true rate of 1% with > 5 mm Hg increase from baseline in diastolic BP. Moreover, under a one-sided 2.5% significance level, this sample size will provide 88% power to rule out > 20 bpm pulse rate increase from baseline on the seventh day of the 300 μg dose level in MEDI0382 subjects relative to that of placebo subjects with assumed standard deviation of 15 bpm.

3   STATISTICAL METHODS

3.1   General Considerations

Data will be provided in data listings sorted by treatment group and subject number. Tabular summaries will be presented by treatment group. Categorical data will be summarized by the number and percentage of subjects in each category. Continuous variables will be summarized by descriptive statistics, including mean, standard deviation, median, minimum, and maximum. Baseline values will be defined as the measurements at Day 1 pre-dose unless data are not available; in which case, baseline value will be the last assessment prior to the first administration of investigational product. Data analyses will be conducted using the SAS® System Version 9.3 or higher (SAS Institute Inc., Cary, NC).

3.2   Analysis Populations

The analysis populations are defined in Table 3.2-1.

Table 3.2-1   Analysis Populations

Population	Description
Intent-to-treat (ITT) population	The intent-to-treat (ITT) population includes all subjects who are randomized and receive any investigational product analyzed according to the initial randomization.
As-treated population	The As-treated population includes all subjects who receive any investigational product analyzed according to the treatment they actually receive.
PD population	The PD Population includes all subjects who received at least 1 dose of investigational product and participated in at least 1 MMT (ie, gave at least 1 post-MMT PD blood sample).
PK population	The PK Population includes all subjects who received at least 1 dose of investigational product and had at least one PK sample taken that is above the lower limit of quantitation.

3.3   Study Subjects

3.3.1   Subject Disposition and Completion Status

The summary of subject status at the end of the study will include the number and percentage of subjects who completed the study and the number and percentage of subjects who did not complete the study due to reasons such as: lost to follow up, withdrawal of consent, death, or other. This summary will be presented by treatment group and for all subjects combined. The placebo subjects for the summary will be pooled from all Cohorts.

3.3.2   Demographics and Baseline Characteristics

Demographic information and baseline characteristics will be summarized by treatment group and for all subjects combined. The placebo subjects for the summary will be pooled from all Cohorts. Demographic information will include: gender, age (years), ethnicity, race, weight (kg), height (cm), and body mass index (BMI) (kg/m^2). A summary of baseline

characteristics may include but not limited to eGFR, baseline medication use, HbA1c, glucose, insulin, blood pressure, and pulse rate etc.

BMI= weight (kg) / [height (m)]^2

eGFR will be using the MDRD formula (Levy et al 2006).

eGFR = 175 × standardized Serum Creatinine $^{-1.154}$ × age$^{-0.203}$ × 1.212 [if black] ×

0.742 [if female]

All summaries of continuous characteristics will be based on non-missing observations. For categorical characteristics, percentage will be calculated, and the denominator for each percentage is the number of subjects with non-missing data for that particular demographic or baseline parameter.

No statistical test will be performed for comparison of any baseline measurement among treatment groups.

3.3.3   Study Drug Exposure

The number of study drug (investigational product) doses received will be summarized. In addition, the number and percentage of subjects who received all scheduled doses and reasons for full dose not received will be summarized by treatment group.

3.3.4   Concomitant Medications

Concomitant medications will be coded using current AstraZeneca Drug Dictionary (AZDD). Concomitant medications will be summarized using frequency count and percentage by the highest anatomical therapeutic chemical (ATC) class and preferred term. All concomitant medications will be presented in a data listing.

3.4   Efficacy Analyses

3.4.1   Primary Efficacy Endpoints and Analyses

3.4.1.1   Primary Efficacy Endpoints (Cohort 4 only)

1. Percent change from baseline in MMT glucose AUC (up to 240 minutes post-MMT) to end of treatment

2. Change from baseline in body weight in kg to end of treatment

3.4.1.2   Handling of Dropouts and Missing Data

Last observation carry forward (LOCF) will be applied for missing data for efficacy endpoints, that is, a missing measurement at the end of treatment will be replaced with the last available post-baseline measurement.

3.4.1.3   Primary Efficacy Analyses

The percent change in MMT glucose AUC (up to 240 minutes post-MMT) and change in weight from baseline (Day 1) to the end of treatment in Cohort 4 will be compared between MEDI0382 and placebo groups using an analysis of covariance by adjusting for baseline measurement and treatment group. The comparison will be conducted at a two-sided significance level of 0.1. The ITT Population in Cohort 4 will be used for the analyses.

3.4.2   Secondary Efficacy Endpoints and Analyses

3.4.2.1   Secondary Efficacy Endpoints

Change from baseline in HbA1c and fructosamine, and percent change from baseline (Day -1) in 24-hour glucose AUC post-MMT through end of treatment in Cohort 4, and percent change in MMT glucose AUC (up to 240 minutes post-MMT), and change in weight from baseline through end of treatment in all cohorts will be analyzed similarly to the primary efficacy endpoints.

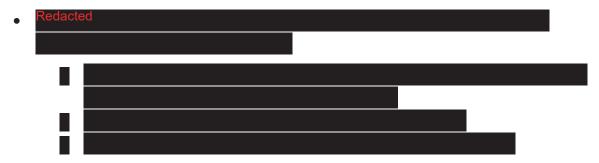### 3.4.2.2   Secondary Efficacy Analyses

Change from baseline (Day -2) in HbA1c and fructosamine, and percent change from baseline (Day -1) in 24-hour glucose AUC post-MMT will be analyzed similarly to the primary efficacy endpoints based on ITT population. The 24-hour glucose AUC analyses will include glucose measures from the pre-/post-MMT glucose metabolism panel as well as serum chemistry glucose levels and PD glucose samples, where those results are from unique time points.

3.4.3   Exploratory Efficacy Endpoints and Analyses

3.4.3.1   Exploratory Efficacy Endpoints

- Redacted

- Lipid endpoints: fasting high-density lipoprotein, low-density lipoprotein, triglycerides, non-esterified fatty acids, and beta-hydroxybutyrate

- Redacted

3.4.3.2 Exploratory Efficacy Analyses

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Lipid endpoints (fasting high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), non-esterified fatty acids, and beta-hydroxybutyrate)), and liver function endpoints as determined by MRI (Cohort 4 only) will be measured at the specified time points. They will be descriptively summarized by treatment group at specified time points. In addition, the change in those parameters from baseline to the end of treatment will be compared between MEDI0382 and placebo groups using an analysis of covariance by adjusting for baseline measurement and treatment group.

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ITT population will be applied for those analyses.

3.5 Pharmacodynamics Endpoint(s) and Analyses

3.5.1.1 Pharmacodynamics Endpoint(s)

PD endpoints (glucose metabolism panel):
   a. Glucose
   b. Beta cell health: insulin, pro-insulin, and c-peptide
   c. Incretins: GLP-1 (active and inactive), glucagon, gastric inhibitory peptide (GIP)

3.5.1.2 Analysis of Pharmacodynamics Endpoint(s)

PD endpoints will be descriptively summarized by treatment group at the specified time points. The summary will be provided for each cohort separately due to different MEDI0382 dose levels and/or different collection schedules across cohorts. Change in fasting measurement from baseline (Day -1) and percent change in 4-hour AUC from baseline of the PD endpoints to the end of treatment will be compared between MEDI0382 and placebo groups using an analysis of covariance by adjusting for baseline measurement and treatment group based on ITT population.

Beta cell function will be derived using the Homeostasis Model Assessment (HOMA) calculation from fasting glucose and insulin data, and insulin sensitivity will be derived from MMT data using the HOMA-2 IR algorithm and the modified Matsuda index calculation. Change from baseline (Day -1) to the end of treatment will be compared between MEDI0382 and placebo groups using an analysis of covariance by adjusting for baseline measurement and treatment group based on ITT population.

Change from baseline of glucose measured by glucometer on each measurement day will be descriptively summarized by daily average due to high variability nature of the glucose values. Change from baseline to the end of treatment will be compared between MEDI0382 and placebo groups using an analysis of covariance by adjusting for baseline measurement and treatment group based on ITT population.

3.6 Safety Analyses

The analyses will be based on the As-treated population. All safety summaries will be presented by treatment group for each cohort. Since the treatment schedule and follow up duration is different across cohorts, the placebo subjects will not be pooled across cohorts.

3.6.1 Adverse Events and Serious Adverse Events

Analysis of safety will include the type, incidence, severity and relationship to study investigational product summarized by MedDRA system organ class and preferred term by treatment group for each cohort. Specific AEs will be counted once for each subject for calculating percentages. In addition, if the same AE occurs multiple times within a particular subject, the highest severity and level of relationship observed will be reported. Non-treatment-emergent AEs/serious adverse events (SAEs) will be presented in the listings.

3.6.2 Adverse Events of Special Interest

The following AESIs have been identified specifically for this protocol and are to be reported as described in the Sections of Recording of Adverse Events and Reporting of Serious Adverse Events in the protocol:

1. Any arrhythmia deemed by the investigator to be clinically significant.
2. Any vomiting (vomiting is known to be associated with GLP-1 receptor agonist use)

Hepatic function abnormality meeting the definition of Hy's law is considered an AESI. See the Section of Hepatic Function Abnormality in the protocol for the definition and reporting of AESIs of hepatic function abnormality.

The AESIs will be summarized overall, as well as categorized by MedDRA system organ class and preferred term by treatment group for each cohort.

3.6.3 Deaths and Treatment Discontinuations due to Adverse Events

Death and AEs resulting in discontinuation from the study drug permanently will be summarized. The summary includes overall, categorized by MedDRA system organ class, and preferred term.

3.6.4 Clinical Laboratory Evaluation

Hematology, serum chemistry, and urinalysis laboratory evaluations will be conducted during the study. The hematology and serum chemistry parameters as well as their changes from baseline will be summarized with descriptive statistics (number of subjects, mean, and standard deviation, median, minimum and maximum) by treatment group at each of the time points specified in Treatment and Follow-up Period Study Procedures in the protocol. The

hematology and serum chemistry results will also be classified into low, normal, and high. The urinalysis results will be classified into normal and abnormal. The shift from baseline hematology, serum chemistry, and urinalysis results will be summarized by treatment group at each of specified time points. The criteria for each parameter will be provided in statistical programming plan (SPP).

3.6.5 Other Safety Evaluation

3.6.5.1 Vital Signs

Vital signs including pulse rate (bpm), blood pressure (mm Hg), temperature (ºC), weight (kg), and respiratory rate (/min), as well as the change from baseline for each of those parameters will be descriptively summarized by treatment group at each of the specified time points in the protocol.

Pulse and BP during the at-home self-administration period for Cohorts 4, 5, and 6, as measured by the ABPM, will be summarized and listed separately from vital signs data. Pulse and diastolic BP in Cohorts 5 and 6 will be compared between MEDI0382 and placebo groups using an analysis of covariance by adjusting for baseline measurement. In addition, a one-sided 2.5% upper confidence interval will be provided for the difference between MEDI5884 and placebo groups in percentages of subjects with > 5 mmHg increase from baseline in diastolic BP on seventh day of the 300 mcg dose level for Cohorts 5 and 6 combined.

3.6.5.2 Electrocardiogram

Electrocardiogram parameters will be assessed using telemetry and standard 12-lead electrocardiography. The following ECG parameters as well as the change from baseline for each of those parameters will be reported and descriptively summarized by treatment group at each of the specified time points for Part A and Part B in the protocol: Heart rate, RR, PR, QRS and QT intervals as well as derived parameters QT corrected interval QTcF (Fridericia's formula $\frac{QT}{\sqrt[3]{RR}}$).

3.6.5.3 Columbia-Suicide Severity Rating Scale (C-SSRS) score

Due to small sample size and short follow up duration, suicide-related events are expected to be rare. A listing and a table (if data is warranted) will be provided as following. No formal statistical hypothesis testing will be performed. As-treated population will be used for the analyses.

3.7 Immunogenicity

Immunogenicity of MEDI0382 will be assessed on the time points specified in the tables of Schedule of Treatment Period Procedures and early discontinuation. The number and percentage of subjects in each treatment group showing an immunological response to MEDI0382 will be summarized by study visit, for As-Treated population and by cohort. In the summary, all post-dose immunogenicity results will also be summarized under "Any Visit" category. A subject will be counted as having detectable antibodies at "Any Visit", if the subject has detectable antibodies at any post-dose visit. The subject will be counted as not having detectable antibodies at "Any Visit" if all post-dose immunogenicity assessments have no MEDI0382 antibody. The subject will be counted as having a missing result at "Any Visit" if all post-dose results for the given test for the subject are missing or if the subject has at least one missing post-dose result for the given test and the remaining results are normal.

For those with a positive post-baseline assessment the percentage who was persistent positive and transient positive will also be presented.

Persistent positive is defined as positive at ≥2 post-baseline assessments or positive at last post-baseline assessment.

Transient positive is defined as negative at last post-baseline assessment and positive at only one post-baseline assessment.

All valid assay results from subjects who receive any investigational product will be included in immunogenicity summaries. Study discontinuation blood samples will be summarized at the closest nominal time point that does not already have a value. All assay results along with their titer information and cross reactivity to GLP-1 and glucagon will be listed will be shown in a by-subject listing.

If warranted by the data, the association of ADA positivity with observed PK data and safety may be explored.

3.8 Pharmacokinetics

Actual time of sampling, rather than nominal (planned) sampling time, will be used to derive PK parameters. Nominal sampling time will be used for the summary of PK concentrations and will be utilized in the descriptive summaries in mean and median plots. Missing PK parameters will not be imputed.

For subjects in each active treatment group the following pharmacokinetic parameters will be determined from the plasma concentration-time data for MEDI0382 if data allow. The pharmacokinetic parameters will be calculated by standard non-compartmental analysis according to current working practices and using Phoenix WinNonlin 6.3. All calculations of non-compartmental parameters will be based on actual sampling times.

The following PK parameters of MEDI0382 will be determined if data allow for each subject from the serum concentration–time data:

- (C_{max}): The first occurrence of the maximum observed plasma concentration determined directly from the raw concentration-time data

- (t_{max}): The time at which C_{max} is observed will be determined directly from the raw concentration-time data.

- (C_{min}): Minimum observed concentration

- $C\tau$: trough concentrations from the plasma concentration-time data at the end of the dosing interval (i.e. 24 hours).

- t half: The apparent terminal elimination half-life ($t_{1/2}$) obtained as the ratio of $\ln2/\lambda_z$, where λ_z is the terminal phase rate constant estimated by linear regression analysis of the log transformed concentration-time data

- $AUC_{(0-last)}$: The area under the plasma concentration-time curve to the last quantifiable concentration determined using the linear trapezoidal rule for increasing concentrations and the logarithmic trapezoidal rule for decreasing concentrations.

- $AUC_{(0-\infty)}$: The AUC extrapolated to infinity will be calculated, where data permit, as the sum of $AUC_{((0-t)}$ and $C_{t/z}$, where C_t is the observed plasma concentration obtained from the log-linear regression analysis of the last quantifiable time-point and z is the terminal phase rate constant.

- %$AUC_{\infty extrap}$: The percentage of $AUC_{(0-\infty)}$ obtained by extrapolation will be calculated as the ratio of [$AUC_{(0-\infty)}$ minus $AUC_{(0-t)}$] to $AUC_{(0-\infty)}$.

- CL/F : The apparent clearance will be calculated as CL/F=Dose/AUC(0-∞).

- AUC(0-τ): area under the plasma concentration-time curve from time zero to the time at the end of the dosing interval (i.e. 24hours) hours post-dose.

- Ro: observed accumulation ratio will be calculated for each cohort if data permit according to the dose titration scheme as follows: Ro = AUC(0-tau)day i/AUC(0-tau)day 1

All the derived parameters described above will be listed. For each of these parameters, except t_{max} and $\%AUC_{\infty extrap}$, the following summary statistics will be calculated for each active treatment group: median, maximum, minimum, arithmetic mean, 95% confidence interval for the arithmetic mean, standard deviation, coefficient of variation, geometric mean, 95% confidence interval for the geometric mean and standard deviation of logarithmically transformed data. For t_{max} and $\%AUC_{\infty extrap}$, median, maximum, minimum, arithmetic mean, 95% confidence interval for the arithmetic mean, and standard deviation will be calculated. The first point, last point and number of points used in the determination of λ_z will be included on the listing of the derived parameters.

With respect to metformin PK, changes in metformin levels over time will be evaluated, as will any association between baseline metformin dose and change in metformin levels over time in the clinical study. In the event that a reduction in metformin levels is seen in Cohorts 1-3, an exploratory analysis will be performed to examine the relationship between changes in metformin level and glucose control over time.

4 INTERIM ANALYSIS

Interim analyses of the safety and PD data will be conducted after the last subject of Cohort 4 and Cohorts 5/6 has completed dosing. PK data will be analyzed if available at the time of analysis.

5 REFERENCES

Levy AS, Coresh J, Greene T, Stevens, LA, Zhang YL, Hendriksen S, et al. Chronic Kidney Disease Epidemiology Collaboration. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med 2006;145(4):247-54