COMPLETE METHODS

The BENEDICT Extension Study

Between February 1997 and July 2000, 1209 consenting type 2 diabetes patients with arterial hypertension, normoalbuminuria, serum creatinine levels <1.5 mg/dl and HbA1c <11%, were randomized into the BENEDICT study, a randomized, double blind, controlled clinical trial. The study was run by the Clinical Research Center for Rare Diseases "Aldo & Cele Daccò" of the Mario Negri Institute for Pharmacological Research in co-operation with nine Diabetology Units (all in Italy) and aimed to compare the protective effect of at least 3 year treatment with the ACE inhibitor Trandolapril (2 mg/day), the ndCCB Verapamil (180 mg/day), their combination, or placebo against the development of microalbuminuria. in this population (eFigure 1, Online-Only). The rationale, design and results of this study have been reported in detail elsewhere[9-11]. Since January 2004, When the BENEDICT Trial was closed, by January 2004, study participants continued to be monitored until December 31, 2008 by pre-planned serial clinical and laboratory evaluations and dedicated case record forms at the Outpatient Clinics of the Clinical Research Center and of eight of the nine Diabetology Units originally involved in the Trial. Based on BENEDICT results all of them were maintained on ACE inhibitors therapy independent of their original randomization in the trial. For all patients, complete information on deaths including fatal cardiovascular events and causes of deaths were obtained from the Registry of the Health District (Azienda Sanitaria Locale) of the Bergamo Province. Of the 1208 participants originally randomized in BENEDICT, 1195 were still alive at the end of the trial. For 139 of these patients, only outcome data recorded during the trial were available since 33 of had been included by the Unit of Diabetology of Cagliari that for technical reasons was

were not involved in the present Extension study for technical reasons and 104 did not consent to enter the Extension study or were lost to follow-up (eFigure 1, Online-Only). Overall, outcome data from BENEDICT trial with or without additional Extension data were available for 1056 of the 1208 patients (87.4%) originally randomized in BENEDICT. The study protocol was approved by the institutional review boards of the Clinical Research Center and of involved Diabetology Units. Participants provided written informed consent to study inclusion.

Definitions and measurements

Type 2 diabetes was diagnosed according to WHO criteria. Arterial Hypertension was defined as a systolic blood pressure (BP) >130 mmHg and/or a diastolic BP >85 mmHg, or concomitant BP lowering therapy, and normoalbuminuria as a urinary albumin excretion rate <20 μg/min in at least two of three consecutive timed overnight urine collections at inclusion. The median value of the three baseline albuminuria measurements was used for analysis. Progression to persistent microalbuminuria (primary outcome variable of the BENEDICT Trial) was diagnosed in patients with a urinary albumin excretion rate $\geq$ 20 μg/min and < 200 μg/min in at least two of three consecutive measurements in two consecutive visits. Throughout the whole observation period of the BENEDICT trial and the subsequent BENEDICT Extension Study Albuminuria was centrally measured at the Laboratories of the Clinical Research Center by nephelometry (Beckman Array System). All the laboratory parameters considered during the BENEDICT trial and on subsequent follow-up were also centrally measured at the same Laboratories by means of a Beckman Syncron Cx5 instrument and a Coulter Maxm (Beckman Coulter).

Cardiovascular Outcomes

The main outcome variable of the BENEDICT Extension Study was the first onset of one of the components of a composite end-point of fatal (including sudden death) or non-fatal major cardiovascular events such as events related to coronary (acute myocardial infarction, unstable angina pectoris or coronary revascularization by bypass grafting or percutaneous transluminal angioplasty), cerebrovascular (stroke, transient ischemic attack, pre-cerebral artery revascularization) or peripheral vascular (amputation, revascularization) disease and hospitalizations because of congestive heart failure. Secondary outcomes were:

i. the first onset of one of the components of the composite endpoint without considering treatment interventions such as revascularizations and amputations
ii. the first onset of a coronary event considered separately from other cardiovascular events.

All the events were defined *a priori* and were adjudicated by a cardiologist (P.R.) and an epidemiologist (E.P.), who were blinded to patient treatment allocation.

Statistical Analysis

Continuous Characteristics of participants with or without cardiovascular events were compared by unpaired t test or Mann-Whitney or Kruskal-Wallis test, categorical or dicothomous characteristics by Pearson's χ^2 or Fisher's exact test as appropriate.

The primary analysis was based on 1208 normoalbuminuric patients at randomization. The relationships between baseline albuminuria and cardiovascular events observed during the BENEDICT trial or the Extension study, were evaluated by using Cox proportional hazards regression models. To account for potential Confounders, multivariable models included baseline variables that *a priori* had been considered to have a proven or possible association with the outcome, such as age, gender, duration of

diabetes, smoking habits, history of cardiovascular events, body mass index, serum creatinine, Hba1C levels, randomization to ACE-inhibitor therapy Yes or No during the Core Trial, mean arterial pressure (MAP), low-density-lipoprotein (LDL)/high-density-lipoprotein (HDL) cholesterol ratio, triglycerides and uric acid levels, and concomitant treatment with lipid lowering therapy. The association of the remaining baseline covariates with considered outcomes was explored at univariate analyses. Since no significant association was observed, no other variable was considered in the multivariable models. All continuous variables, including albuminuria, were kept continuous for the analyses. Co-linearity between baseline covariates was tested by the Pearson correlation coefficient and when a co-linearity between two variables was found, only one of the two variables (HbA1C instead of blood glucose, LDL cholesterol instead of total cholesterol) or a surrogate of both variables (MAP for both systolic and diastolic BP its components and the LDL/HDL cholesterol ratio for LDL and HDL both cholesterols considered separately) variable were used.

Comparisons according to randomization to ACE –inhibitor (yes or no) (y/n) were performed using the intention-to-treat principle. Departure from linearity of risk was tested creating multiple categorical variables and those with a non-linear gradient when founded (LDL/HDL cholesterol ratio, albuminuria, HbA1C and creatinine levels) were log-transformed.

Variables with a non-linear association with risk – tested by multiple categorical variables- were log-transformed (LDL/HDL ratio, albuminuria, HbA1C and creatinine levels).

The proportionality hazard assumption was assessed using the long-rank and the weighted Schoenfeld residuals, and the fit goodness of the model by the Hosmer-Lemeshow test.

Multivariable survival analysis was also performed by modelling baseline albuminuria withthe fractional polynomial algorithm method (first and second degree), or a spline function,two flexible and informative approaches for the evaluation of possible non-linear relationships between continuous variables and outcomes.[12,13]

Sensitivity analyses were pre-planned to test the study findings in specific contexts. The study findings were also tested in pre-planned sensitivity analyses. Thus, multivariable analyses models were also restricted to (a) low-risk subjects: those without previous history of major cardiovascular events (n=1156), or to those who had been persistently normoalbuminuric throughout the BENEDICT trial (n=1107), (b) specific outcomes: Sensitivity analyses also considered those with spontaneous cardiovascular events independent of (excluding revascularizations and amputations), or those only with coronary events independent of other cardiovascular events. Finally, the relationships between baseline covariates and outcomes the principal analysis were also evaluated separately within the BENEDICT trial and Extension study observation periods and within subjects originally randomized to ACE inhibitor therapy yes or not.

Data were expressed as n (%), mean and Standard Deviation (SD) or Median and interquartile range (IQR) as appropriate and a p value < 0.05 was considered as statistically significant. Data were analysed using SPSS 17.0.1 for Windows (Chicago, IL) and STATA 11.0 (fracpoly command).

10-YEAR CARDIOVASCULAR RISK IN TYPE 2 DIABETES IS PREDICTED BY MEASURABLE URINARY ALBUMIN EVEN IN THE NORMOALBUMINURIC RANGE:

THE BENEDICT EXTENSION STUDY

ONLINE-ONLY MATERIAL

eTable 1:

Baseline characteristics of patients with and without complete follow-up.

eTable2:

Hazard Ratios (95% Confidence Intervals) for the primary end point (first onset of fatal or non-fatal major cardiovascular events) for each 1 μg/min range in baseline urinary albumin excretion (UAE) compared to UAE < 1 μg/min (reference value) in the study group as a whole (Overall) or according to randomization to ACE inhibitor therapy Yes or No.

eFigure 1:

Study Flow-Chart.

eTable 1: Baseline characteristics of patients with and without complete follow-up.

	Overall	**Core + extension**	**Core only**	***p§***
N (%)	N= 1208	N= 1069 (88.5)	N= 139 (11.5)	
Age – yr	62.33 ± 8.05	62.29 ± 7.940	62.64± 8.88	0.76
Male sex – no. (%)	638 (52.8)	584 (54.6)	54 (38.8)	< 0.001
History of CV events	52 (4.3)	47 (4.4)	5 (3.6)	0.43
Know duration of diabetes – yr	6 (3-11)	6 (2-11)	10 (3-16)	< 0.001
Smoking status – no. (%)				
Never smoked	700 (57.9)	608 (56.9)	92 (66.2)	
Former smoker	362 (30.0)	328 (30.7)	34 (24.5)	0.11
Current smoker	146 (12.1)	133 (12.4)	13 (9.4)	
Body Mass Index †	29.08 ± 4.72	29.06± 4.72	29.20± 4.69	0.75
Trough blood pressure – mmHg				
Systolic	150.82 ±14.16	150.90 ± 14.11	150.22 ± 14.57	0.60
Diastolic	87.46 ± 7.63	87.52 ± 7.54	87.04 ± 8.31	0.51
Mean	108.58 ± 8.35	108.64 ± 8.27	108.10 ± 8.94	0.49
Glycosilated hemoglobin - %	5.79 ± 1.37	5.76 ± 1.36	6.10 ± 1.463	0.014
Glucose – mg/dL	161.34 ± 47.05	160.25 ± 46.09	170.55 ± 53.90	0.020
Cholesterol - mg/dL				
Total	209.78 ± 36.74	208.82 ± 36.67	217.94 ± 36.393	0.009
High-density lipoprotein	46.94 ± 12.06	46.5759 ± 11.760	50.0476 ± 14.040	0.002
Low-density lipoprotein	162.75 ± 36.05	162.18 ± 36.08	167.53 ± 35.54	0.11
Triglycerides - mg/dL	125 (91-181)	125 (91-182)	130.5 (95-178)	0.74
Serum creatinine - mg/dL	0.90 ± 0.16	0.91 ± 0.16	0.88 ± 0.17	0.069
Uric acid - mg/dL	4.09 ± 1.18	5.03 ± 1.18	4.80 ± 1.24	0.057
Albuminuria - µg/min	5.24 (3.5-9.3)	5.26 (3.6-9.4)	4.99 (3.4-9.0)	0.33
Medications				
Allocation to the study treatments				
Placebo	302 (25.0)	270 (25.3)	32 (23.0)	
Verapamil	303 (25.1)	270 (25.3)	33 (23.7)	0.87
Trandolapril	302 (25.0)	265 (24.8)	37 (26.6)	
Verapamil + Trandolapril	301 (24.9)	264 (24.7)	37 (26.6)	
Antihypertensive agents – no (%)*	664 (55.0)	579 (54.2)	85 (62.0)	0.05
Total Number of antihypertensive agents**	2 (1-2)	2 (1-3)	2 (1-3)	0.23
Antilipidemic agents – no (%)	138 (11.4)	117 (10.9)	21 (15.3)	0.09
Aspirin – no (%)	28 (2.3)	25 (2.3)	3 (2.2)	0.60

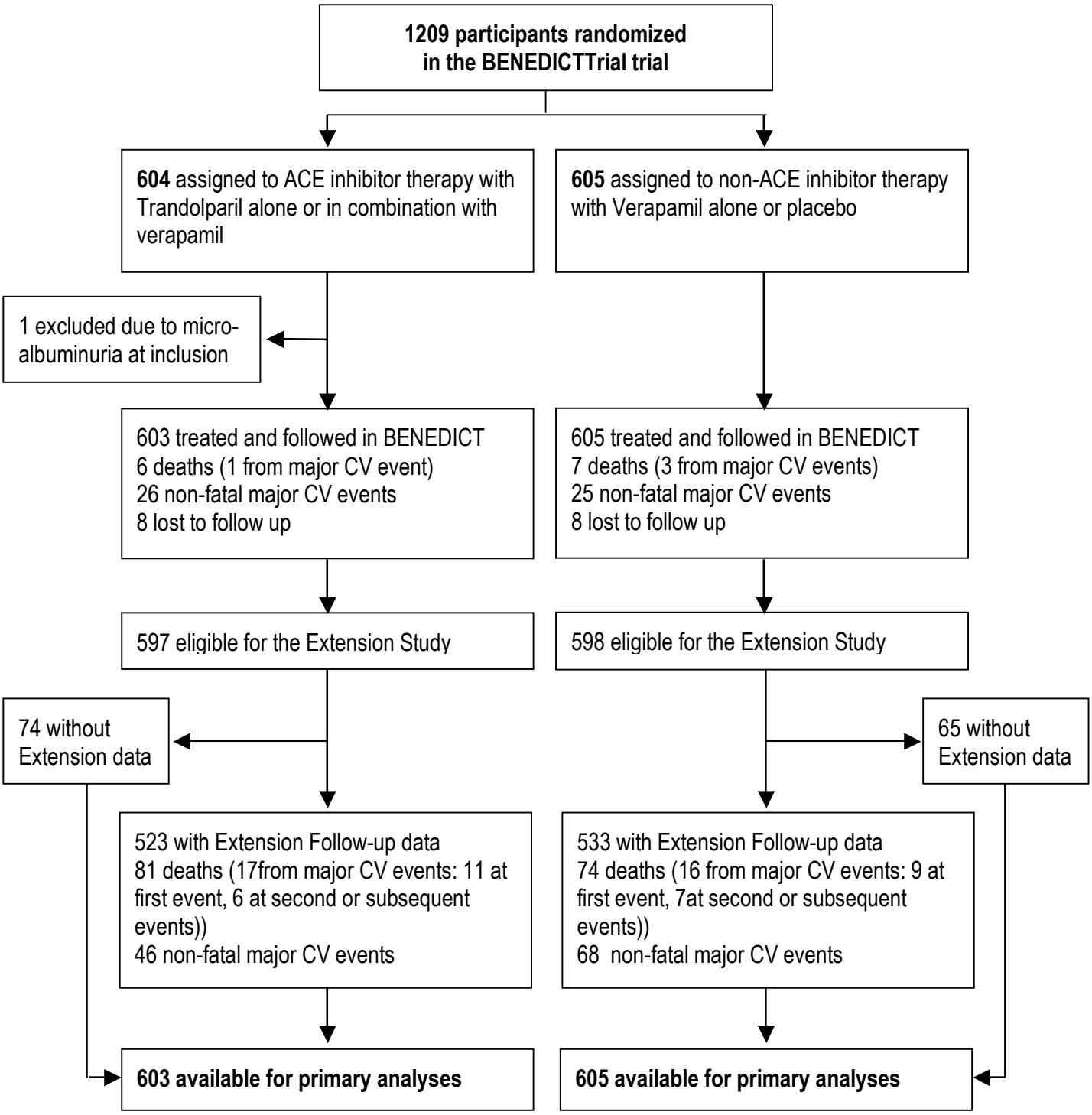
* Different from the study treatment. **Includes study treatment and other antihypertensive drugs.

§: comparisons between patients with and without complete follow-up.

eTable2:

Hazard Ratios (95% Confidence Intervals) for the primary end point (first onset of fatal or non-fatal major cardiovascular events) for each 1 μg/min range in baseline urinary albumin excretion (UAE) compared to UAE < 1 μg/min (reference value) in the study group as a whole (Overall) or according to randomization to ACE inhibitor therapy Yes or No. In the study group as a whole and in patients not randomized to ACEi therapy, each 1 μg/min UAE range is associated with a significant excess cardiovascular risk compared to the reference value. In patients originally randomized to ACEi therapy, the risk of events associated with each UAE range is never significantly different from that associated with the reference value. Albuminuria is modeled with the second degree fractional polynomial transformation. Multivariable Cox proportional hazards regression analyses adjusted for the same variable shown in Table 3.

UAE *(μg/min)*	**OVERALL**		**ACEi No**		**ACEI Yes**	
Ranges	*HR*	*95% CI*	*HR*	*95% CI*	*HR*	*95% CI*
< 1	1.00	--	1.00	--	1.00	--
1-2	1.04	1.02-1.07	1.02	1.01-1.04	0.95	0.55-1.61
2-3	1.10	1.04-1.18	1.08	1.04-1.12	0.95	0.42-2.11
3-4	1.20	1.08-1.33	1.17	1.08-1.26	0.97	0.37-2.55
4-5	1.31	1.12-1.54	1.30	1.14-1.48	0.99	0.34-2.93
5-6	1.45	1.16-1.80	1.47	1.20-1.80	1.03	0.33-3.25
6-7	1.61	1.21-2.13	1.70	1.30-2.22	1.08	0.33-3.54
7-8	1.78	1.28-2.47	1.97	1.40-2.76	1.13	0.34-3.80
8-9	1.96	1.34-2.86	2.28	1.51-3.43	1.18	0.35-3.99
9-10	2.14	1.40-3.25	2.65	1.64-4.30	1.25	0.37-4.18
10-11	2.30	1.46-3.60	2.90	1.71-4.90	1.31	0.39-4.34
11-12	2.45	1.52-3.89	3.11	1.77-5.44	1.38	0.42-4.52
12-13	2.49	1.56-4.00	3.15	1.76-5.63	1.45	0.45-4.65
13-14	2.49	1.55-3.99	3.00	1.67-5.40	1.53	0.49-4.80


1209 participants randomized in the BENEDICTTrial trial
604 assigned to ACE inhibitor therapy with Trandolparil alone or in combination with verapamil
605 assigned to non-ACE inhibitor therapy with Verapamil alone or placebo
1 excluded due to micro-albuminuria at inclusion
603 treated and followed in BENEDICT
6 deaths (1 from major CV event)
26 non-fatal major CV events
8 lost to follow up
605 treated and followed in BENEDICT
7 deaths (3 from major CV events)
25 non-fatal major CV events
8 lost to follow up
597 eligible for the Extension Study
598 eligible for the Extension Study
74 without Extension data
65 without Extension data
523 with Extension Follow-up data
81 deaths (17from major CV events: 11 at first event, 6 at second or subsequent events))
46 non-fatal major CV events
533 with Extension Follow-up data
74 deaths (16 from major CV events: 9 at first event, 7at second or subsequent events))
68 non-fatal major CV events
603 available for primary analyses
605 available for primary analyses

eFigure

STUDY ORGANIZATION

Members of the BENEDICT Study Organization were as follows (all in Italy unless otherwise noted): Principal investigator — G. Remuzzi (Bergamo); Study coordinator — P. Ruggenenti (Bergamo); Coordinating center — Mario Negri Institute for Pharmacological Research, Clinical Research Center for Rare Diseases Aldo e Cele Daccò, Villa Camozzi, Ranica (Bergamo); Participating centers — G. Nastasi, A. Ongaro, F. Querci, A. Anabaya (Alzano Lombardo); R. Trevisan, A.R. Dodesini, G. Lepore, I. Nosari, C. A. Aros Espinoza, A. Fassi (Bergamo); M. Songini, G. Carta, G. Piras (Cagliari); B. Minetti, P. Fiorina, G. Ghilardi, V. Grassia, E. Pezzali, E. Seghezzi, I. Villanova (Clusone); A. Spalluzzi, I. Codreanu, C. Flores (Ponte San Pietro, Villa d'Almè); C. Chiurchiu, F. Arnoldi, L. Mosconi, M. Monducci (Ranica); A. Bossi, M. Facchetti, V. Brusegan (Romano di Lombardia); F. Inversi, V. Bertone, R. Mangili, S. Bruno (Seriate); A. Bossi, A. Parvanova, I.P. Iliev, E. Terzieva (Treviglio); Ophthalmologists — M. Filipponi, I.P. Iliev, S. Tadini (Bergamo); Monitoring and drug distribution (Mario Negri Institute) — G. Gherardi, N. Rubis, S. Birolini, L. Bruni, W. Calini, V.A. Carrasco Oyarzun, R. D'Adda, O. Diadei, M. Ferrari, L. Mangili, A. Milani, G. Noris, K. Pagani, S. Quadri, A. Rossi, S. Secomandi, G. Villa (Ranica); Carriers (Mario Negri Institute) — G. Gaspari, S. Gelmi, G. Gervasoni, L. Nembrini (Ranica); Database and data validation (Mario Negri Institute) — A. Remuzzi, B. Ene-Iordache, V. Gambara (Ranica); Data analysis (Mario Negri Institute) — A. Perna, B.D. Dimitrov, M. Ganeva, J. Zamora (Ranica); Laboratory measurements (Mario Negri Institute) — F. Gaspari, F. Carrara, E. Centemeri, S. Ferrari, M. Pellegrino, N. Stucchi (Ranica); Genomic evaluations (Mario Negri Institute) — M. Noris, P. Bettinaglio, S. Bucchioni, J. Caprioli, B. Giussani (Bergamo); Regulatory affairs (Mario Negri Institute) — P. Boccardo (Ranica); Steering committee — S. Kupfer (Abbott Park, Ill.,

United States), L. Minetti (Bergamo), G. Remuzzi (Bergamo), U.F. Legler (Ludwigshafen, Germany), B. Kalsch (Ludwigshafen, Germany), D. Nehrdich (Ludwigshafen, Germany), A. Nicolucci (S. Maria Imbaro), A. Perna (Bergamo), P. Ruggenenti (Bergamo); Safety committee — G.L. Bakris (Chicago, United States), R. Kay (Sheffield, United Kingdom), G.C. Viberti (London, United Kingdom).

SUBSTUDY PROTOCOL

BASELINE PREDICTORS OF INCIDENCE OF MAJOR CARDIOVASCULAR EVENTS, OF OVERALL AND CARDIOVASCULAR MORTALITY AND OF THE RISK OF PROGRESSION TO DIABETIC NEPHROPATHY IN NIDDM PATIENTS OF THE BENEDICT STUDY

(BERGAMO NEPHROLOGIC DIABETES COMPLICATIONS TRIAL)

Study: VeraTran 083

(A two-phase study for primary and secondary prevention of diabetic nephropathy by combined ACE inhibition and calcium channel blockade)

(Study code: VeraTran SR HT CR-D24)

DRAFT

date: March 6, 2000

1. Background

The BErgamo NEphrologic DIabetes Complications Trial, where an extensive baseline information is collected about patient demography, history, physical examination, blood pressure and laboratory values,allows, through its database, a unique opportunity to prospectively examine the role of many baseline covariates on incidence of major cardiovascular events, of overall and cardiovascular mortality and of the risk of progression to diabetic nephropathy. A systematic and exhaustive investigation can be therefore carried out in order to identify which baseline factors are individuallly predictive and to develop a multivariate model with the minimum number of relevant baseline predictors which are jointly predictive.
Due to the kind of patients included in the study, a special emphasis is given to the predictive and pathogenetic value of the increase of BMI and to the other risk factors that usually cluster in obesity, including dislipidemia, hypertension and hyperuricemia.

2. Aims of the study

The purpose of this project is to identify, among all the baseline data collected in the patients of the BENEDICT Study, univariate and multivariate predictors of:
1) incidence of major cardiovascular events;
2) overall and cardiovascular mortality;
3) progression to diabetic nephropathy.

3. Study population

Baseline predictors will be evaluated in all the 1200 patients enrolled in Phase A Study and in all the 600 patients entered the Phase B. Analysis will be carried out for Phase A and Phase B both separately and combined. For patients who performed both Phase A and B, data arisen from Phase A only will be considered. Treatment groups will be both included and excluded from the analysis from each of the univariate and multivariate analysis performed.

4. Statistical methods

Definition of baseline covariates.
Selection of the baseline covariates for evaluation as predictors is done on *a priori* considerations. Appendix provides the list of these covariates, as well as details regarding the methods of constructing derived baseline variables.

Analysis of baseline predictors of incidence of major cardiovascular events.
The time to first major cardiovascular event will be considered and will give rise to right censored data. Univariate and multivariate analysis will be undertaken using the accelerated failure time model.

Analysis of baseline predictors on overall and cardiovascular mortality.
Time to death will be analysed as for the time to first cardiovascular event mentioned in the previous paragraph.
A further analysis will separate out cardiovascular mortality and non-cardiovascular-mortality, in a competing risks analysis. In the former case, non-cardiovascular deaths are treated as censored observations, while in the latter case all cardiovascular deaths are treated as censored observations.

Analysis of baseline predictors on progression to diabetic nephropathy.
Time to progression to microalbuminuria (for Phase A) and to macroalbuminuria (for Phase B) will be evaluated through use of accelerated failure time model with normal error structure.

APPENDIX

List of baseline predictors (Phase A)

Age
Sex
Smoking habits
UAE (log-transformed)
BMI
....
To be filled in the final version

List of baseline predictors (Phase B)

Age
Sex
Smoking habits
UAE (log-transformed)
BMI
....
To be filled in the final version

List of baseline predictors (Phase a+B)

Age
Sex
Smoking habits
UAE (log-transformed)
BMI
....
To be filled in the final version