In three of the studies which were performed in patients with CHF, Natrecor was administered as a bolus or as multiple boluses (Studies # 704.305, # 704.309, # 704.310). In two of these studies, Natrecor was administered as a continuous intravenous infusion (Study # 704.306, # 704.307). In the only study in patients with post-operative hypertension Natrecor was administered as a single and if necessary a second bolus (Study # 704.312).

The number of subjects per study and the doses studied are shown in the Table intr-1A total of 206 patients are included in this section of the primary review.

Table Intr-1: Descriptive Outline of The Six Studies Reviewed in This Document

Study Number	Number Enrolled	Bolus/Infusion	Patient Population	Doses	Number Treated with PBO[1]	Number treated with Natrecor[1]
#704.305	30	Bolus	CHF	0, 0.3, 1, 3, 10, 15 and 20 ug/kg	4	24
# 704.306	16	Infusion	CHF	0, 0.025 and 0.05 ug/kg/min	4	12
#704.307	20	Infusions	CHF	Incremental rates of infusions maximal dose =0.1 ug/kg/min; subsequently decreased to 0.03 ug/kg/min	19	20
#704.309	60	Intermittent Bolus	CHF	0 ug/kg Q 4 H 0 ug/kg Q 6 H 5 ug/Kg Q 4H 10 ug/kg Q 4 H 10 ug/kg Q 6 H for 24 hours	15	45
#704.310	60	Intermittent Bolus	CHF	0, 3.0, 5 and 10 ug/kg Q4 H x 6 doses	17	43
#704.312	20	Bolus	Post-CABG	5, 10, 15, 20 or 25 ug/kg	0	20

[1] when subjects were crossed over into the other regimen they were counted in both regimens.

When Natrecor was administered as a bolus, the highest single dose which was administered was 25 ug/kg (study #704.312- to post-operate CABG patients). In studies (# 704.309 and #704.310), Natrecor was administered as multiple intravenous boluses. In these studies Natrecor was administered for 24 hours with bolus frequency either Q4 or Q 6 Hours. The largest cumulative dose was 60 ug/kg (10 ug/kg Q 4 hour).

When Natrecor was administered as an infusion, the longest duration of infusion was 7.5 hours (later decreased to 6 hours; study # 704.307). Patients who received 6 hours of infusion had their dose escalated during the infusion. These subjects received the highest dose (0.1 ug/kg/min) only for 3 hours. The dose was subsequently discontinued because of one or more subjects who had severe as prolonged hypotension. In the other study Natrecor was infused as a dose of either 0.025 or 0.05 ug/kg/min. The largest cumulative dose in the infusion studies was therefore 21.8 ug/kg (or approximately 70 ug/kg/24 hours, study #704.307) or 12 ug/kg (or 72 ug/kg/24 hours, study # 704.309).

In addition to kinetics, invasive hemodynamics, vital signs and urine output were collected in most of these small studies.

Kinetics:

The kinetics of hBNP, the data was reasonably well fit in some subjects by a one-compartment model and for other by a two-compartment model. All data points reflected the measured plasma hBNP concentrations that were corrected by subtracting baseline endogenous plasma hBNP levels. Endogenous levels varied in the CHF population and ranged from < 0.1 ng/ml to > 3.4 ng/ml.

When fit to a two-compartment model the terminal phase of decay which accounts for approximately 70% of the AUC. The half-life of decay was approximately 20 minutes. The half-life for the less prominent alpha phase (when measured) was approximately 1 minute. The kinetic duration was usually not followed for greater than 1 ½-2 hours. If there was a longer terminal half-life i.e. if there were a tri-phasic or higher order decay process, the database could not detect these higher order decay processes. Furthermore, at later time point the concentrations of endogenous hBNP concentrations are nearly equivalent to those generated during the bolus, Consequently, it is unlikely that a more accurate or detailed kinetic model would be readily derivable.

When Natrecor was administered as a bolus, C_{max} was approximately 35 ng/ml (normalized to the 5 ug/kg bolus) (study #704.309 and # 704.310). Peak concentrations after a 10-ug/kg bolus (first dose) were approximately 60-90 ng/ml. After 4-6 boluses, among those who completed the study # 704.309, peak concentrations were also approximately 70 ng/ml.

After a 10 ug/kg dose, the corrected concentration (with endogenous [hBNP] subtracted) at 1 hour was approximately 2-5 ng/ml. For the 5-ug/kg dose the concentrations at 1 hour were approximately 0.4 –4 ng/ml. By 1-hour post dose, the post bolus, the concentrations of hBNP (after baseline subtraction) are likely to be equivalent endogenous concentrations.

When Natrecor was administered as a continuous infusion at sequentially higher doses the concentrations at steady state was approximately dose proportional. The concentration at 0.1 ug/kg/min was approximately 12 ng/ml. At a dose of 0.01 ug/kg the concentration at steady state is approximately 1.2 ng/ml (study # 704.307).

Blinding:

All studies should be interpreted with full knowledge that the data were not collected in an entirely blinded manner. The on-site pharmacist was aware as to treatment and dose. All doses were sent to the floor prior to inserting the Swan-Ganz catheter and prior to measuring baseline hemodynamics and, therefore, adequately defining the subject as eligible for enrollment. If a subject was subsequently found ineligible, the subject was discontinued and not considered as having been enrolled. The number of such subjects and whether these subjects were equally distributed between placebo and the individual doses of active treatment was never tabulated.

Hemodynamics:

Most studies did not declare a primary end-point. The statistical section generally describes the primary goal of the study as descriptive. Although several studies implied that the primary end point would to be changes in PCWP, the time point of interest was often left ambiguous or defined as the interdosing interval time point. At the inter-dosing interval none of the hemodynamic or blood pressure effects appeared to be consistently altered by treatment.

Among the studies reviewed in this section, the results of study #704.307 are the most suggestive evidence that Natrecor modifies cardiac hemodynamics. At each of several dose infusion rates, the effect of concentrations of Natrecor on hemodynamics were fit to an E_{max} model. Hemodynamics and concentrations were measured as > 60 minutes of infusion at each of the infusion doses. EC_{50} for Natrecor for PCWP, cardiac index and system vascular resistance were approximately 2.4-3.1 pg/ml (see table 3.5 of this review). The maximal effect (E_{max}) of PCWP was

approximately 16 mm Hg; the effect on SVR was –450 dynes sec cm-5, the effect on cardiac index was 0.68 L/min/M^2. There was substantial persistence of effect at 1 hour post-dose for PCWP, MRAP and SVR when (see Tables 3.4 and 3.4a) concentrations of Natrecor have substantially declined.

The relationship between hemodynamics and concentration were largely derived from single measurements that were made during the ascending phase of the dose titration scheme. Since only a single hemodynamic value was available for each infusion rate, there is little convincing evidence that dynamic steady- state was achieved. It is therefore, not possible to tease out whether the effects that were measured are only related to concentration or are partly related to the duration of infusion. The persistence of effect 1-hour post infusion is also suggestive of dissociation between hemodynamic effect and concentrations of h-BNP.

After single, or multiple individual intravenous boluses of Natrecor, the maximal hemodynamic effect peaks at approximately ½-1 hour after the bolus. The effect of this bolus persists for an additional 1-2 hours (see Figures 1.1, 2.2 and 4.3 for PCWP results; Figures 1.2 and 4.4 for Cardiac Index or cardiac output and Figures 1.3 and 3.5 for SVR). The data from acute bolus administration is also consistent with a dissociation or delay in hemodynamic response from acute changes plasma h-BNP concentrations.

In none of the studies were ANP concentrations measured, either at baseline or as a consequence of hBNP infusions. Since both ANP share the same receptors as well as the second messenger cascade and furthermore, since acute or subacute changes in cardiac dimensions could alter secretion of BNP and ABP it is not entirely surprising that there is a dissociation between hBNP concentration and hemodynamic effects.

Vital Signs: When Natrecor was administered as a constant infusion (study # 704.307), there appeared to be dose related decreases in both systolic and diastolic blood pressures as well as increases in heart rate, which persisted through the 1-hour post infusion time point (Tables 3.4 and 3.4a).

When Natrecor was administered as chronic intermittent boluses (study #704.309), mean systemic blood pressures decreased but the effect was not consistent across doses. With the 10-ug/kg Q 6-hour regimen the effect was on systolic pressures, with a 10-ug/kg Q 4-hour regimen the effect was on diastolic blood pressures. The effect on heart rate was variable.

Among patients who were treated with Natrecor post-Cardiac surgery there appeared to be a drop in Blood pressure and a concurrent increase in heart rate. In the post-CABG population (given the small sample size) no dose relationship was obvious.

Safety:

No deaths occurred during the infusion period. There were three deaths (two-placebo and one-5 ug/kg q 6h bolus) that occurred between 6-30 days after the start of the infusion.

There were more patients who were discontinued for worsening CHF or whose CHF was considered as severe or serious (6/60 (10%) versus 4/144 (3%)) among those treated with placebo than those treated with Natrecor among those with CHF. Most of the discontinuations occurred during study 704.309 and among those who discontinued most were recruited from a single study

center (Center # 315). This center enrolled 13% of the subjects but accounted for 67% of the dropouts.

Hypotension was only noted in the CHF cohort who were treated with Natrecor. There were 11 subjects who either discontinued due to hypotension or whose episode of hypotension was considered serious or severe.

There was one patient (#359-002, study # 704.309) who developed a decrease in urine output and worsening of renal function beginning on day 4 (3 days post boluses of 10 ug/kg q 6 hours). She eventually required ultrafiltration and dialysis.

APPEARS THIS WAY
ON ORIGINAL

1. Study 704.305: vol. 37 98 to 39 307

Title of Study: A Phase I/II Double-Blind, Randomized, Placebo-Controlled, Ascending Dose Study of the Hemodynamic and Renal Effects of Single Intravenous Bolus of NATRECOR® hBNP in Subjects with Congestive Heart Failure.

The protocol, as well as two amendments, was included within this submission. There were, however, a total of five amendments. The remaining three amendments were only described. Line listings were included. CRFs were available for all deaths, dropouts and discontinuations on CD-ROM.

Investigator and Sites: (Table 1.1)

Robert E. Hobbs, MD The Cleveland Clinic Foundation Cleveland, OH	Leslie Miller, MD St. Louis Univ. Medical center St. Louis, Mo

Formulation: The formulation was produced by the synthetic peptide methodology. Lot # E0013A1.

Study Summary:

This was a two-center, single-dose intravenous bolus study. Natrecor was administered as a rapid (< 30 seconds) intravenous bolus. There were five subjects per dose (initially the protocol had seven subjects/group but the number/group was amended to five/group at the same time the number of groups were expanded to seven). Of those enrolled into each group, all but one received Natrecor. This one patient received placebo. Natrecor was administered at doses of doses 0.3, 1.0, 3.0, 10, 15, and 20 ug/kg[1]. The cohort of subjects were first enrolled into the lowest dose and only when safety was assured for this dose were subjects enrolled into the next higher dose. There was to be at least one-week interval between cohorts.

Subjects were eligible to enroll if they were > 18 years old and were NYHA CHF classification II-IV, with left ventricular systolic dysfunction (EF < 25%) as determined by ECHO or radionuclide ventriculography (within the three previous months). Patients were excluded if they had a recent MI (within 3 months); unstable angina (within 2 weeks); valvular heart disease; hypertrophic, restrictive or obstructive cardiomyopathy; recent VT or Vfib (within 2 weeks), 2nd (Mobitz II) or third degree block (unless pacemaker was inserted); recent stroke (within 3 months); renal or liver impairment or any acute medical condition or laboratory abnormality values such as sodium <120 or > 160 meq/L; potassium < 3.0 or > 5.5. meq/L.

The following table lists the following procedures during screening and the day of the bolus:

[1] The initial protocol stipulated the following doses: 0.3, 1.0, 3.0 and 10 ug/kg. The following additional doses were then added: 15. 20 or 25 ug/kg. A subsequent amendment discontinued the highest dose.

Table 1.2 Listing of Procedures Study 704.305

Procedure	Screen	Pre-dose(min)			After Bolus (min)																	
			-15	-1	0	2	5	10	15	30	45	60	75	90	105	120	150	180	210	240	24 Hr
Informed Consent, Medical History, Height, Weight, ECG, Pregnancy Test	x																				
Physical Exam, Vital Signs	x																•				
Blood Chemistry, Hematological Tests	x																			x	
Urinalysis	x																			x	
Swan-Ganz Placement		x																			
Drug Administration					x																
BP (systolic/diastolic)			x	x				x	x	x	x	x	x	x	x	x	x	x	x	x	
PAP, PCWP, mean RAP, Cardiac Output, Heart Rate*			x	x					x	x	x	x	x	x	x	x	x	x	x	x	
Urine Collection	Q4 hour aliquots starting 4 hours prior to the study																				
Blood for Drug levels			x		x	x	x	x	x	x		x		x							
Anti BNP antibodies																					

*hemodynamic measurements should also be captured during any significant adverse event.

Subjects were subsequently seen on day 2 for a follow-up physical exam, vital signs, blood chemistries and hematology. They were seen again after two weeks at which time any adverse events that occurred during the two interval were noted. Subjects also returned somewhere between 3 and 6 weeks after the bolus for the determination of anti-hBNP antibodies.

Some Definitions: Three sequential measurements of hemodynamics were to be performed at each of the time points. If these measurements did not differ by more than 15% (of the greater or lesser?), these values were then averaged. If these measurements differed by more than 15%, two additional measurements were taken and the highest and lowest discarded before the remaining three measurements averaged. Urine was collected through the four-hour period after the bolus. Sodium and potassium excretion was determined.

Statistical Issues: The study was described as descriptive, no hypothesis was tested. Patients who were not successfully instrumented were not considered as having been randomized. One subject who was randomized to receive 10 ug/kg Natrecor, actually received only approximately 5% of the randomized dose. This subject was not included in calculating the hemodynamic response. He was included in the 0.3 ug/kg dose for safety.

Blinding: The pharmacist on-site was unblinded both to treatment and dose. For those who were to receive placebo, only D5W was administered. For Natrecor patients, the pharmacist formulated the infusion based on the randomized dose and weight of subject, so that the bolus was generally less than 10 ml (and usually greater than 1 ml).

Results: Since there are concentrations of hBNP even in the absence of treatment, the measured endogenous pre-bolus values of hBNP were subtracted from all measurements (this correction assumes that there was no major diurnal variation or time course to hBNP levels). Among those who received placebo, the background concentrations ranged from approximately 200-1700 pg/ml (0.2-1.7 ng/ml). These values were on the same order of magnitude as those of bolus doses of 1 ug/kg or less. The hBNP concentrations of those who received $\geq$ 3 ug/kg were generally an order of magnitude higher than these placebo baseline measurements.

The kinetics of hBNP were fit both to one- or two- compartment models, with the model that fit best by the Akaike criteria accepted as the most appropriate way to handle each individual's data. For some subjects, the individual's kinetics was best fit to a two-compartment model. Among those whose data were best fit by the two-compartment model, the initial $t_{1/2}$ was on the order of 1-2 minutes. The area under the curve for this α-portion of the curve was approximately 30% of the total AUC. 70% of the AUC was accounted by the β-portion of the curve. The β-half-life (or the terminal half-life where no rapid decline in concentrations were noted) was generally between 16-20 minutes.

Effects: Though not stipulated by protocol, the placebo patients were all combined and analyzed as a single group. The PCWP and CO results are shown in Figures 1.1 and 1.2, respectively. The placebo response is quite small. The effect of Natrecor appears dose-related, with the 3 higher dose groups demonstrating a drop in wedge pressures as well as a modest increase in cardiac output.

With respect to the time course of effect, Natrecor's effect appears prolonged and disproportionate to the concentrations of hBNP. After the single intravenous bolus, peak response both for wedge pressure and CO generally occurred at 30-60 minutes after the bolus. The time course of decline in hemodynamic effect appears prolonged for a drug with an approximately 16-20 minute half-life.

The time course of the effect of Natrecor on SVR is similar (Figure 1.3) to that of wedge pressure and cardiac output, though there appears to be some deviation for a Dose-Response relationship, the 20 ug/kg dose having less of an effect than the 10 and 15 ug/kg doses.

Figure 1.1

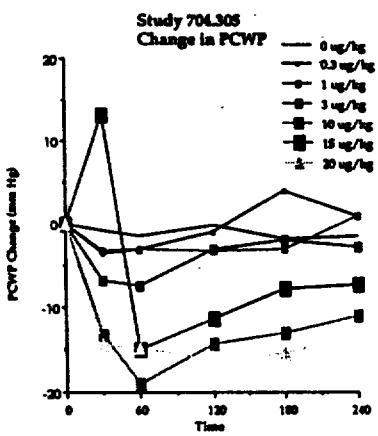
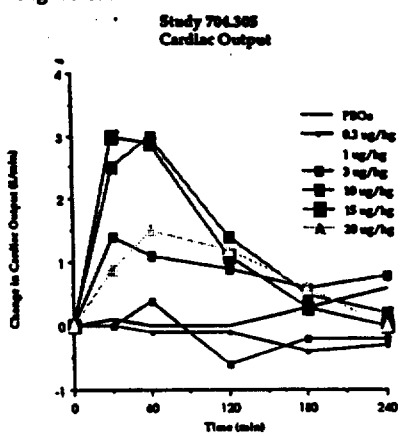
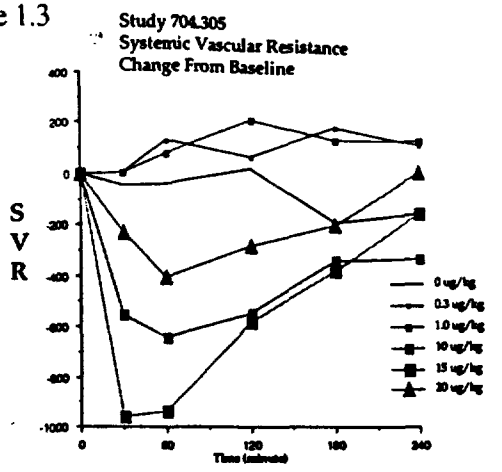


Figure 1.2



Systolic blood pressure (data not shown) decreased for the 10 and 15 ug/kg dose cohorts. The 20-ug/kg dose cohort did not display a systolic blood pressure effect different than placebo.

Figure 1.3



Safety: No deaths or discontinuations were reported.

Conclusion: This was a very small, single-intravenous dose study that allows for some tentative conclusions. The β-phase half-life of hBNP was approximately 20 minutes. This estimate of terminal half-life was nearly the same for those whose had concentration that were an order of magnitude greater than their endogenous hBNP levels and, therefore, this estimate of half-life is based on credible data.

For PCWP and CO there was some relationship between dose and effect. The time course, however, showed that the maximum effect occurs at 30-60 minutes following the bolus. The persistence of effect seems to be much longer than the kinetic half-life. At four hours (equivalent to 12 half-lives) the effect at the higher doses is still measurable.

2. Study: Study 704.306 vol. 40-42.

Title of Study: A Phase I/II Randomized, Double-Blind, Placebo-Controlled, Ascending Dose Response Study of the Hemodynamic, Renal and Neurohormonal Effects of a Continuous Infusion of NATRECOR hBNP in Subjects with Chronic Congestive Heart Failure.

Protocol, Amendments, Line Listings and CRFs: The protocol as well as two amendments was included (there were a total of five amendments). Line listings were included. CRFs were available for all deaths, dropouts and discontinuations as representations on CD-ROM. The first of the amendments (dated 25 April 1994) modified the infusion to be given though a peripheral intravenous line, discontinued urinary bicarbonate measurements and included serum insulin as part of the routine labs.

The second amendment (dated 29 August 1994) changed the doses to be infused. The original doses to be infused were 0.025, 0.1 and 0.2 ug/kg/min. The high dose group was dropped and a 0.05 ug/kg/min infusion rate included.

Investigator and Sites: William T. Abraham
University of Colorado Health Science
Denver, CO

Formulation: Lot no E0013A1. No placebo was supplied.

Study Summary:
Protocol:

This was a single center study. Originally, the study proposed to enroll three cohorts of eight patients. These patients were to be treated with one of the following doses: 0.025, 0.1 and 0.2 ug/kg/min for four hours. The protocol was subsequently amended. The high infusion rate was dropped and an intermediate dose 0.05 ug/kg/min was added. Patients, however, were only enrolled into the two lowest infusion doses. Of the eight subjects in each cohort, six received the active drug and the other two received placebo. Patients were enrolled into the next dose infusion only after those enrolled in the lowest dose group successfully completed the infusion.

Patients eligible for enrollment were of either gender, $\geq$ 18 years old, with symptomatic heart failure (NYHA Classification II-IV) and evidence of left systolic dysfunction (i.e. left ventricular ejection fraction of < 30%) as determined either by radionuclide ventriculography or echocardiography, within 6 months of enrollment. Those enrolled must demonstrate a CI < 2.5 l/min/m^2 and a PCWP of $\geq$ 15 mm Hg.

Patients were excluded if they had a recent MI (within 3 months); unstable angina (within 2 weeks); valvular heart disease; hypertrophic, restrictive or obstructive cardiomyopathy, recent VT or Vfib (within 2 weeks); 2nd (Mobitz II) or 3rd degree block (unless pacemaker was inserted); recent stroke (within 3 months); recent cardiac surgery (<3 months), or any anesthesia (within 2 weeks), SBP < 85 mm Hg, abnormal serum [Na+] (<120 or > 160 meq/L); or [K+](< 3.0 or > 5.5 meq/L); or renal or liver impairment or any acute medical condition or laboratory abnormality that might increase the risk to Natrecor infusion.

Blinding: The pharmacist on-site was unblinded, as to treatment and dose. Only D5W was supplied for placebo patients (not a true placebo i.e. a vial of excipients without the active drug). For Natrecor

patients, the pharmacist formulated the infusion, based on dose and weight of the subject, so that the infusion rate, independent of dose was to be 25 ml/h.

The duration of infusion was 4 hours. A Swan-Ganz catheter was placed the day prior to the infusion. A listing of the procedures is shown below.

Table 2.1: List of Procedures.

	Screen	Baseline	Day 1 Infusion	Day 1 Post infusion	Day 2	Day 7-10	Day 21 and 42
Informed Consent, Medical History, Height, Weight, Vital Signs, Urine Analysis, Serum Digoxin Levels, ECG, Chest x-ray, Pregnancy Test(if appropriate), Medications discontinued	x				•		
Physical Examination	x				x	x	
Blood Chemistry, Hematology[1]	x	x	x	x	x	x	
Urine Collection[2]							
Hemodynamic Measurments[3]							
Neurohormonal Levels							
Vital Signs							
Infusion of Inulin and PAH							
hBNP Levels[4]		x	x	x			x
anti-hBNP Levels		x					
Lithium Carbonate[5]							

1. At -4 and O hours prior to the infusion, at the end of infusion, at 4 hours post infusion, on day 2 and day 7-10
2. A 24 -hour urine collection , to be completed prior to the study. Four-hour urine collections beginning 4 hours prior to the start of the infusion till 4 hours post-infusion.
3. Measured at baseline and –4, -3, -2, -1 and 0 hours prior to the infusion and 15, 30, 45. 60. 90, 120, 180 and 240 minutes during the infusion and 15, 30, 45, 60, 90, 120 180 and 240 minutes post-infusion.
4. Measured at baseline and at 0, 10, 30, 90, 180 and 240 minutes during the infusion and at 5, 10, 30, 60, 120 and 180 minutes post- infusion.
5. Lithium carbonate at a dose of 600 mg was administered at 10 p.m. prior to the day of the infusion.

Measurements: The measurements, which were collected, included:

- vital signs (systolic, diastolic, mean arterial pressures and heart rate);
- renal function: GFR and RBF as well as urine output, urine electrolytes (sodium potassium, chloride), urine excretion of urea nitrogen, creatinine, urine osmolarity, urine cGMP levels and lithium clearance.

Statistical: The study did not pose a primary hypothesis. Any conclusions of the study were descriptive with respect to the effects of hBNP on hemodynamics, renal function and neurohormone changes.

Results: Only 16 subjects were enrolled. These subjects were allocated to the two low dose cohorts (0.025 and 0.05 ug/kg/min). According to the sponsor, the higher dose was never implemented because patients who received this dose in a different protocol developed substantial hypotension.

The effect of Natrecor at doses of either 0.025 or 0.05 ug/kg/min on cardiac output or pulmonary capillary wedge pressure are shown in Figures 2.1 and 2.2. The effect of Natrecor on cardiac output was highly variable and did not differ between the two active doses and placebo.

Pharmacokinetics:
Endogenous baseline hBNP ranged from concentrations of 140-3415 pg/ml. Concentrations of hBNP during the infusion at steady state ranged from approximately factors of 2-3 above baseline to those an order of magnitude greater than baseline. All kinetic data was the concentration minus the baseline endogenous concentration values. The kinetics were fit to a one-compartment model. The

terminal half-life was approximately 20 minutes, but there was large variability in the estimate of the terminal half-life (Range: 4-46 minutes).

Hemodynamics:

With respect to PCWP, both Natrecor doses differed from placebo shortly after the start of the infusion: The two active doses did not substantially differ from each other. The differences between placebo and active doses, however, appeared to widen upon continuation of the infusion. Upon cessation of the infusion, PCWP remained below that of placebo for several hours.

Figure 2.1

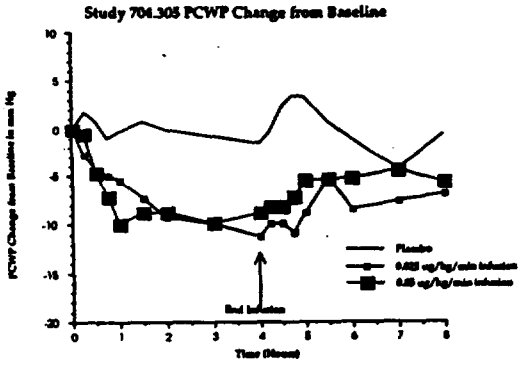
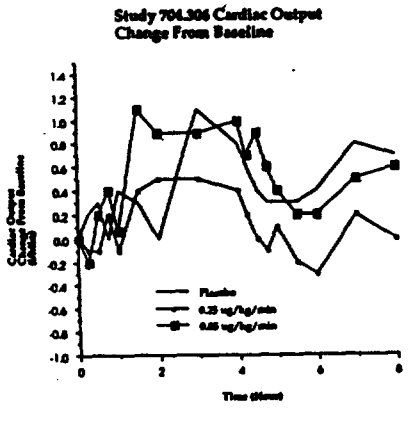


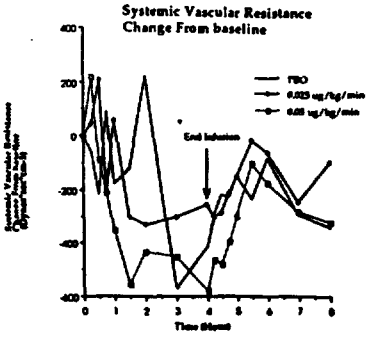
Figure 2.2



Cardiac output (Figure 2.2) was more variable, with virtually no differences among the two active doses and placebo for the first hour of the infusion (Steady state kinetics should have been approached by this time).

Systemic vascular resistance (Figure 2.3) was highly variable and I could not discern either a dose or time dependent effect.

Figure 2.3



Renal Function:

The sponsor claims that there were no baseline changes in renal function. Urine output, however, was numerically less on Natrecor. Urine output the day prior to the infusion, as well as the four-hour period immediately prior to the infusion, was higher for the placebo than the two active infusion groups. The sponsor claims, there were no significant differences in urinary sodium excretion from baseline (comment: it is unclear what defined baseline).

Safety:

Deaths: One placebo patient (**pt #324-010**) died 15 days into the study (well past the infusion day). This was a 57-year old white male with NYHA Class III and an underlying ischemic cardiomyopathy, mitral and tricuspid valve regurgitation and pulmonary hypertension. The subject was dobutamine dependent at baseline. At the time of enrollment the patient was hospitalized and was awaiting cardiac transplantation. The patient died post transplantation. He could not be weaned from the bypass.

Dropouts and Discontinuations: One subject allocated to the 0.05 ug/kg/min infusion group discontinued from the study (summarized from sponsor's capsular summary). **Patient #425-009** was a 33 year old white male with a history of NYHA Class III and an ejection fraction of 11%. The underlying cause of the CHF was alcoholic cardiomyopathy, amphetamine abuse and alcoholic cardiomyopathy. After three hours of infusion the patient developed symptomatic hypotension (68/48). Wedge pressure at the time of the hypotension was 10 mm Hg (at baseline it was 29 mm Hg). The hypotension apparently responded to drug discontinuation and fluid replacement (I could not find a description of the time course of blood pressure recovery).

Serious Adverse Events: The sponsor reports two adverse events (the one discontinuation described above and this event). **Patient #324-011** was a 66 year old white male with a history of NYHA Class III CHF due to ischemic cardiomyopathy. He also had atrial fibrillation, a history of renal failure and hypertension. At the end of the infusion the subject developed a high fever (40.3°) associated with shaking chills. The subject was treated with inotropes and antibiotics (it is unclear why the patient was treated with inotropes) with the presumptive diagnosis as staphylococcus bacteremia.

Three severe events were listed: **pt # 324-010** is listed as having severe CHF. This subject is listed above under deaths. **pt # 324-011** listed above with shaking chills and hyperthermia. **Pt # 324013** treated with 0.05 ug/kg infusion had a severe headache.

Adverse Events: Overall Adverse Events Through Day 14 are shown in Table 2.2

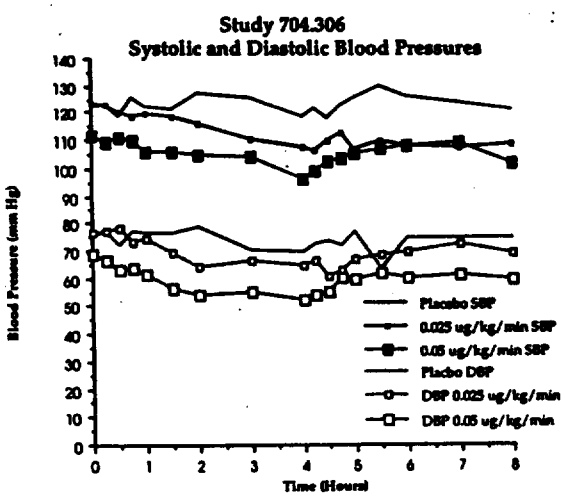
Table 2.2 Adverse Events Through Day 14.

System	PBO (n=4)	Dose of hBNP ug/kg/min 0.025 (n=6)	0.05 (n=6)		System	PBO (n=4)	Dose of hBNP ug/kg/min 0.025 (n=6)	0.05 (n=6)
Body as A Whole	2	2	4		Respiratory	1	2	1
Cardiovascular	1	3	3		Skin and Appendages	1	1	1
Metabolic and Nutritional Disorder	2	3	0		Digestive	1	1	0
Nervous	1	1	2		Musculoskeletal	1	0	1
					Urogenital	0	1	0

There were too few patients to associate the adverse events with a particular dose.

Vital Signs: Systolic and diastolic blood pressures during the infusion and after cessation of the infusion are shown below:

Figure 2.4



Blood pressures differed at baseline among the three treatments. Those patients allocated to the high dose infusion had lower systolic and diastolic blood pressures. There appears to be some effect of infusion on blood pressures. The time course, however, is not easily described. If anything, the largest blood pressure drops occurred after several hours of infusion. The drop is SBP did not recover after the infusion was discontinued.

With respect to heart rates (data not shown) there did not appear any consistent pattern. Heart rate for the low dose group increased, for the high dose group there was substantial decreases. For the 4 placebo patients heart rate increased substantially.

Laboratory: The sponsor noted no "clinically relevant" laboratory changes. None of the subjects (10 of the 12 subjects enrolled) developed anti-hBNP antibodies.

Study # 704.307 vols. 43-45

Title of Study: A Phase II Randomized, Double-Blind, Placebo-Controlled, Crossover Study of the Hemodynamic Effects of an Intravenous Incremental Dose Infusion of NATRECOR hBNP in Subjects with Congestive Heart Failure.

Investigator and Site: Milton Packer, MD
Columbia Presbyterian Hospital
New York, NY

Formulation: Natrecor was produced by the synthetic peptide methodology (Lot no. E0013A1).

Protocols, Line Listings and CRFs: The protocol and 5 amendments were included. The amendments are summarized below:

Table 3.1. Amendments

Amendment and Date	Summary
1. 28 Dec 1993	This amendment modified the blood processing for hBNP levels.
2. 2 Feb 1994	This amendment modified the pre-study lower limits of hematocrit and sodium.
3. 6 May 1994	This amendment established criteria to limit titration of drug.
4. 16 Aug 1994	This amendment changed the requirement for stable baseline medication from 5 days to 48 hours.
5. 22 Dec 1994	This amendment changed the duration of infusion from 7.5 to 6 hours. An initial high dose of 0.1 ug/kg/min was deleted and duration of the 0.03 ug/kg/min was increased from 1.5 to 3 hours. One patient had a profound episode of hypotension that lasted several hours at the 0.1 ug/kg/min dose and required fluids and pressors before BP recovered.

Summary (The summary includes the amendments) :

The study is labeled as placebo-controlled, double blind, ascending dose crossover study. A total of approximately 20 subjects were randomly enrolled into one of two groups. The first group (n=10) received placebo on day 1 and hBNP on day 2. Group 2 (n=10) received the alternate sequence of treatments.

Eligible subjects were patients > 18 years old with stable CHF (defined by dyspnea or fatigue at rest or on exertion, corresponding to NYHA classification II-IV, for two months). Patients must have evidence of a decreased left ventricular systolic dysfunction as measured by an EF of < 35% (either by 2-D echocardiography or radionuclide ventriculography, within 3 months of enrollment). Patients with corrected valvular disease were eligible for enrollment. The subject required a PCWP (or pulmonary artery diastolic pressure) $\geq$ 15 mm Hg or a CI $\leq$ 2.5 L/min/m2.

Excluded, were patient with heart failure due to restrictive, hypertrophic or obstructive cardiomyopathy, constrictive pericarditis, primary pulmonary hypertension, or active myocarditis. Patients with recent MI (< 3 months), unstable angina (2 weeks) or stroke (<3 months) were not eligible for enrollment. Patients with sustained VT (hemodynamically significant) or VFib (if on drugs other than amiodarone) as well as patients with second degree A-V block (Mobitz type II) or third degree block were not eligible. Patients with abnormal [Na+] ($\leq$ 125 or $\geq$ 160 meq/l), [K+] ($\leq$ 3.5 or $\geq$ 5.5 meq/l), serum creatinine > 5 mg/dl, or Hct< 30% similarly were excluded. Similarly, patients with any acute disease process; or patients who are receiving beta-blockers, calcium channel blockers, hydralazine or long acting nitrates (within 5 days) were not eligible to enroll.

The infusion rate was to be 0.003 ug/kg/min for 1.5 hours, 0.01 ug/kg/min for the next 1.5 hours and 0.03 ug/kg/min the last 3 hours. The total infusion time was six hours. Those patients who develop asymptomatic hypotension (SBP < 75 mm Hg) did not have their doses escalated. Those who develop symptomatic hypotension were back titrated to a lower dose. Those who required fluid support or pressors, were discontinued.

Patients had right sided catheterization one day prior to the infusion. All medications for heart failure (except for anti-arrhythmic drugs) were withheld until after completion of the catheterization. Any medications for heart failure were given 12 hours prior to the initial measurements and 12 hours prior to the second day's infusion.

The list of the procedures are shown Table 3.2

Table 3.2 Listing of Procedures

		Day								
Procedure	Screen	0	1	2	3	7-10	15	21	42	
Infusion			x	x						
Medications D/C[1]	x									
Informed Consent, Medical History, Height and Weight	x				x	x				
Phys Ex, Vital Signs	x									
Blood Chemistries, Hematology [2]			x	x	x	x				
Urinalysis [3]			x	x						
ECG, Pregnancy Test (when necessary)										
Blood Pressure[4], PAP[5], PCWP[5], mean RAP[5], Cardiac Output[5]			x	x						
Heart Rate [6]										
Plasma hBNP levels[7]			x	x						
Urine Output			x	x						
Anti hBNP antibodies	x							x	x	

[1] Medications to be held before catheterization and during infusion. Medications will be given at night 12-hours prior to the hemodynamic studies
[2] Also measured after each day's infusion, day 3 if any abnormality on day 1 or 2
[3] Also measured after each day's infusion.
[4] Every 15 minutes during and for 1 hour following study drug infusion (times are noted as pre, 1, 1.5, 2.5, 3, 4, 4.5 5.5, 6 hours during infusion and within 1 hour post infusion on both days.
[5] At pre dose, 1, 1.5, 2.5, 3, 4, 4.5, 5.5, and 6 hours and post on each day
[6] Measured every 15 minutes during the infusion and for one- hour post infusion.
[7] Before infusion and at 1.5, 3, 4.5 , 6 and within 1 hour post infusion.

Blinding: The pharmacist on-site was unblinded to treatment. Each patient was randomized to a given treatment regimen prior to the successful placement of the catheter. If the catheter placement was unsuccessful or the measured hemodynamic parameters did not meet entry criteria, the subject was discontinued and replaced. Although randomized, the ineligible patient was no longer followed and considered as not having enrolled. The number of such patients is not stated.

Statistical Issues: The intent of this study was to describe the effect of hBNP on hemodynamic parameters. Among the parameters of interest was PCWP. The primary analysis consisted of the value of PCWP after the largest completed infusion of the day. An infusion was considered complete if, the duration of exposure to a given dose was at least 75 minutes. The protocol leaves the question of interim looks and the number of such looks ambiguous. The claim is that the stopping rule of Haybittle (1971, British Journal of Radiology) will be used, the study will be declared positive only if the p value was < 0.0027. No penalty was taken for the final analysis.

Results:

Demographics:

A total of 20 subjects were enrolled (17 males, 2 Females). The mean age ± SD was 57 ± 9 years. Twelve of those enrolled were Caucasians, 5 were black, 2 were Hispanics and one was Asian. The degree of CHF was as follows 2 (10%) were class II; 16 (80%) were Class III and 2 (10%0 were Class IV.

Table 3.3. Baseline Hemodynamics are shown below (Mean + SD) :

PCWP	24.6 ± 5.5		SVR	1481 ± 392
MRAP	10.8 ± 5.8		CI	2.1 ± 0.4
SBP	107 ± 13			

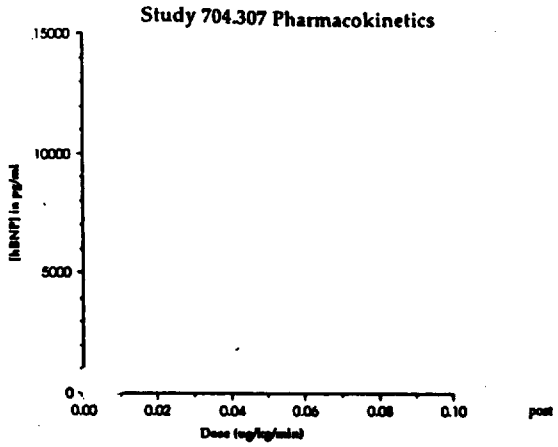
Table 3.3 combines those treated as Group 1 (Placebo on day 1, Natrecor on day 2) and Group 2 (Natrecor o on Day 1, Placebo on Day 2). The patients were clearly sick based on the high PCWP and low CI.

Kinetics: (This section summarizes the sponsor's analysis. I have not attempted to reproduce the sponsor's analysis. The kinetic analysis was performed under the direction of Nancy C. Sambol, PharmD, Department of Biopharmaceutical Sciences, UCSF.) The concentrations of hBNP were determined by the ELISA methodology (see Dr. Sadreih's review for more specifics).

Data was available for all subjects. One kinetic data point for patient #307-005 was excluded because it clearly was an outlier and substantially deviated from any near data point (I agree). One subject had data on Natrecor but discontinued prior to the placebo infusion. Only 9 patients (out of 20) had Natrecor infusions for the stipulated time at the highest dose (0.1 ug/kg/min).

The concentration versus dose effect is shown in Figure 3.1:

Figure 3.1



Only 9 patients received the 0.1 ug/kg/min infusion rate for an adequate duration of time.

Pharmacodynamics:

Two representations of the hemodynamic effects are shown below. Table 3.4 is the change from baseline. Table 3.4a is the placebo subtracted data. To obtain the double delta the baseline differences should also be subtracted.

Natrecor apparently decreases wedge pressures, mean right atrial pressures as well as systolic and diastolic blood pressures. Cardiac Index as well as heart rate increases. The effect at 1-hour post appears to persist despite a substantial drop in the plasma concentrations of Natrecor.

Table 3.4 Hemodynamics Mean ± SD –Change from Baseline Natrecor Infusion

Parameter	Baseline Value	Natrecor Infusion Rates in ug/kg/min				
		0.003	0.01	0.03	0.1	1-Hour Post infusion
PCWP (mm Hg)	24.8 ± 5.0	-3.2 ± 3.5	-6.0 ± 3.5	-10.2 ± 5.2	-14.6 ± 4.6	-10.9 ± 4.8
MRAP (mm Hg)	11.0 ± 5.1	-2.5 ± 1.9	-3.5 ± 1.7	-5.5 ± 2.4	-6.2 ± 2.7	-5.7 ± 3.5
SVR (dynes*sec*cm-5)	1514 ± 385	7 ± 236	-121 ± 348	-261 ± 348	-449 ± 361	-206 ± 349
CI (l/min/M2)	2.1 ± 0.4	-0.0 ± 0.3	0.2 ± 0.4	0.4 ± 0.6	0.6 ± 0.4	0.2 ± 0.46
DBP (mm (Hg)	74.1 ± 7	-2.1 ± 4.9	-3.3 ± 6.4	-7.4 ± 6.5	-9.6 ± 6.6	-8.5 ± 7.3
SBP (mm (Hg)	108 ± 14	-5 ± 4	-5 ± 5	-10 ± 8	-11 ± 8	-10.9 ± 4.8
Heart Rate (BPM)	74.8 ± 15	-0.5 ± 2.6	-0.5 ± 4.4	2.9 ± 7.9	11.5 ± 9.2	10.9 ± 10.7

[2] These were my calculations from data in Appendix 1a. With the exception of one subject, the data from the pre-infusion dose was taken as the baseline measurement, which was subtracted, from the measurement 1-hour post-infusion. For this last subject, I could not find a baseline value and I used the value obtained during the placebo infusion.

Table 3.4a Hemodynamic Effect –Placebo Subtracted

Parameter	Baseline Value	Natrecor Infusion Rates in ug/kg/min				
		0.003	0.01	0.03	0.1	1-hour Post infusion
PCWP (mm Hg)	1.7	-3.7	-6.8	-10.3	-15.5	-12.2
MRAP (mm Hg)	0.5	-2.1	-2.6	-4.7	-5.4	-5.0
SVR (dynes*sec*cm-5)	-117	10	-92	-224	-392	-151
CI (l/min/M2)	-0.1	-0.1	+0.1	+0.3	+0.6	+0.1
DBP (mm (Hg)	1.5	-4.2	-4.1	-7.9	-8.6	-9.8
SBP (mm Hg)	1.8	-4.7	-5.0	-9.0	-10.4	-12.7
Heart Rate (BPM)	-1.5	-2.6	-3.9	0.4	7.1	4.3

Pharmacokinetic/dynamic interaction:

The sponsor modeled the kinetics to an Emax model (see Equation 2)[3].

$$E = E_{base} + \frac{Emax \times Css^{\gamma}_{ss(k)}}{Css^{\gamma}_{ss(k)} + Css^{\gamma}_{50}} \qquad \text{(Equation 2)}$$

i= a particular individual; k= infusion dose and where Css is the measured hBNP – endogenous hBNPconcentrations.

(Dr. Ray Miller of the Agency is analyzing this study. Please refer to the biopharm review for additional details). The analyzed data did not include the one offset hemodynamic data point.

Table 3.5 Dynamic Parameters of Study 704.307 (95% Confidence Interval)

Parameter	Baseline	Emax	EC50 pg/ml	Comments
PCWP (mm Hg)	24.6 (22.4-26.7)	16.2 (13.6-18.8)	2400 (1500-3300)	Data reflects class II and III patients the two class IV patients had lower responses and were not included in this data
CI (L/min/M^2)	2.02 (1.90-2.15)	0.68 (0.27-1.08)	3100 (700-5500)	There appeared to be a significant negative relationship between weight and CI.
SVR (dynes.sec.cm^{-5}	1500 (1.347-1.653)	-450 (-18 to -750)	2400 (500-4300)	There appeared to be a significant negative effect of

The EC_{50} for the three measured parameters correspond to infusion rates of between 0.01 to 0.03 ug/kg/min. (The sigmoidicity factor γ=2.03).

Hysteresis? The sponsor does not address the question of hysteresis. Only a single point, approximately 1-hour post treatment, was available. There is no information as to whether this data point was included in the model.

Renal Effects: There was no statistical difference in urine output (placebo = 94 ± 55 versus Natrecor 112 ± 55).

Safety:

Deaths Dropouts/Discontinuations:

There were no deaths reported in this study. One subject discontinued the study.

[3] The sponsor developed a model for baseline measurements. These baseline measurements were tested for stability (i.e. lack of a placebo effect and for the potential of diurnal variation. Parameters were also tested for stability based on disease severity, age and weight. The values were the estimates of baseline value that was subsequently used in equation 2.

Patient 307-014 was a 64-year old black male with NYHA Class III CHF. His SBP at baseline was 93 mm Hg. Ten minutes after completing the 0.1 ug/kg/min infusion his SBP dropped to 64 mm Hg. Chest pain, nausea, headache and diaphoresis accompanied the drop of blood pressure. The subject's blood pressure remained labile for the next 5.5 hours. This subject was treated with intravenous fluids, dopamine and atropine (a heart rate of 51 was noted during this event). No acute ECG changes were reported. It is unclear if cardiac enzymes were sent.

Two subjects discontinued drug infusions prematurely:

Patient 307-002 is a 61-year old white male with NYHA Class II, CAD and pulmonary hypertension. He was initially treated with the placebo infusion. When he was started on the Natrecor infusion, he tolerated the lowest dose infusion. When started on the 0.01 ug/kg/min infusion his SBP decreased to 76 mm Hg (the SBP had been between 92-110 mm Hg). His SBP stabilized and he was advanced to the 0.03 ug/kg/min infusion. After 2 hours of the infusion he again developed hypotension (BP =78/48 mm Hg, accompanied by lightheadedness.) Study drug was discontinued and blood pressure increased (heart rate = 72 BPM).

Patient 307-008 was a 47-year old white male, NYHA Class III and CAD. After 59 minutes of the high dose infusion of 0.1 ug/kg/min the subject became hypotensive (BP 74/50 mm Hg) accompanied by dizziness. The study drug was discontinued and the patient recovered. The adverse event form suggests that the hypotensive event lasted a little more than an hour. After discontinuation, the subject subsequently developed junctional bradycardia (HR= mid 40s). Digoxin was discontinued and the subject eventually recovered. A digoxin level was sent but the results were not reported.

Adverse Events:

Adverse Event Profiles, which occurred during the infusions, are shown below.

Table 3.6 Adverse Events During Infusions (Derived from Table 32A (vol. 43 pp 164-166) ≥ 2 events/group.

Event	BNP	Placebo		Event	BNP	Placebo
Headache	4	1		Pain	2	1
Hypotension	7	0		Angina	1	2
Dizziness	3	2		Nausea	2	0
Vomiting	2	0		Sweating	0	

Hypotension and headache was substantially more common in the hBNP group.

Vital Signs: Table 3.4 demonstrates a dose-related drop in systolic blood pressure.

EKGs: I could find no analysis of the ECG information for this study.

Laboratory: Thrombocytopenia was reported for two subjects. **Subject # 307-014** had a drop from 179 x 10^3/mm^3 at baseline to 106 x 10^3/mm^3 on day one, but recovered to 251 x 10^3/mm^3 on day 9.

Subject 307-016 had an initial platelet count of 183 x 10^3/mm^3 which decreased after the placebo infusion to 128 x 10^3/mm^3. His platelet count after the hBNP infusion was 125 X 10^3/mm^3. On day 9 the platelet count was 190 x10^3/mm^3.

Study # 4. Study 704.309 Vol. 46-49.

Title of Study: A Phase II, Randomized, Double-Blind, Placebo-Controlled, Dose Response Study of the Effects of a 24-Hour Course of NATRECOR hBNP Administered as an Intermittent Intravenous Bolus in Subjects With Congestive Heart Failure.

Investigator, Sites and Number of Subjects Enrolled.

Table 4.1 Investigators, Sites and Number Enrolled (Total = 60 Patients).

Site 352 Robert C. Bourge, MD U. of Alabama, Birmingham Birmingham, AL	4		Site 348 Robert J Cody, MD Ohio State U. Med Center Colombus, OH	17		Site 353 Wilson S. Colucci, MD Boston U. Med Center Boston, MA	4
Site 354 E. Michael Gilbert, MD U. of Utah Health Science Center Salt Lake City, UT	1		Site 357 Joshua Hare, MD The Johns Hopkins Hospital Baltimore, MD	2		Site 355 Ray Hershberger, MD Oregon Health Sciences Center Portland, OR	2
Site 306 Robert E. Hobbs, MD Cleveland Clinic Foundation Cleveland OH	3		Site 356 Walter Kao, MD Rush-Presbyterian-St-Lukes Med Center Chicago, IL	3		Site 359 Kenneth B. Margulies, MD Temple U Health Science Center Philadelphia, PA	3
Site 374 Jonathan Plehn, MD Dartmouth-Hitchcock Lebanon, NH and Site 381: White River Junction, VT	7		Site 315 Leslie Miller, MD St, Louis U. Med Center St Louis, MO	8		Site 360: P. Jacob Varghese, MD George Washington U Med Center Washington, DC	6

Protocol, Line Listings and CRFs. The protocol was dated 18 July 1994. CRFs for discontinuations and dropouts were supplied on CD-ROM.

Formulations: Active drug was either lot #E0013A1 or G0003A1- Placebo was merely intravenous fluids.

Blinding: The on-site pharmacist formulated the doses for the subjects from a bottle of Natrecor or sent D5W for the placebo-randomized subjects. The pharmacist was, therefore, fully aware as to the dosing of any patient. The dose was sent to the floor prior to the insertion of the Swan Ganz catheter. If the subject could not successfully be instrumented, or if the subject was found ineligible because of the baseline measurements, the patient was discontinued and was not considered as ever enrolled. It appears that these patients were censored.

Doses: Placebo: Group 1- (N=10) Placebo Q 4 hours for 6 doses (0 ug total dose)
Group 2: (N=5) Placebo Q 6 hour for 4 doses (0 ug total dose)
hBNP: Group 3: (N=15). hBNP 5 ug/kg Q 4hours for 6 doses (30 ug total dose)
Group 4: (N=15) hBNP 10 ug/kg Q 6 Hours for 4 doses (40 ug total dose)
Group 5: (N=15) hBNP 10 ug/kg Q4 hours for 6 doses (60 ug total dose).

Statistical issues: The was a descriptive study. PCWP was the hemodynamic endpoint of primary interest. Secondary hemodynamic parameters of interest include: cardiac index, systemic vascular resistance, mean right atrial pressures and heart rate. The time-point of primary interest is either the 4-hour time point (for the Q 4-hour dosing group) or 6 hours (for the Q 6-hour dosing group) after the last bolus (approximately 24 hours after the initiation of hBNP/placebo).

The data was analyzed using an one-way ANOVA model. The two placebo regimens were pooled. Investigators were, however, not included as a term in the model. The differences among treatment arms are to be tested by an omnibus F-test. The effect of dose was tested by linear contrasts using equally spaced scores for the total daily dose. Each of the measured values (at the different time points) was analyzed in the same way.

The protocol leaves the option of interim analyses. The stopping rule would require a $p < 0.0027$, the final analysis will be conducted at the 0.05 level

Renal endpoints of interest are the 24-hour urine output, urine sodium and urine potassium.

Protocol: Patients were eligible for enrollment if they were at least 18 years old with chronic CHF (NYHA II-IV) and demonstrated an ejection fraction ≤ 0.30 (as determined either by 2-D echocardiography or radionuclide ventriculography). Specifically excluded were patients with recent MI (2 months) or unstable angina (within 2 weeks). Patients with valvular stenosis, hypertrophic, restrictive or obstructive cardiomyopathy or active myocarditis were also excluded. Patients with rhythm or conduction abnormalities such as ventricular tachycardia or ventricular fibrillation, Mobitz type II block or third degree heart block (except if patient has permanent pacemaker) were excluded. Patients with a recent stroke (within 3 months) or patients with significant renal disease (SCr > 3.0); SBP < 85; hyponatremia or hypernatremia (<125 or > 160 meq/l); or those unable to tolerate cessation of cardiac medications (with the exception of antiarrhythmics) were excluded. Patients with other acute or chronic medical conditions or laboratory abnormalities, which could increase the risk of such patients to Natrecor infusion, will not be enrolled.

Screening: Subjects were screened for eligibility within one week of the initiation of the pretreatment procedures. The screen included a history (including NYHA class, cause of CHF), physical exam, vital signs, laboratories, ECG and chest X-ray.

Pretreatment: Within 12 hours of starting the infusion, the subject was admitted and placed on continuous telemetry monitoring. All cardiovascular medications except digoxin, diuretic and antiarrhythmic drugs were withheld. A Swan-Ganz catheter was placed. Only those patients whose PCWP was > 15 mm Hg and also had a CI of < 2.5 L/min/m^2 were enrolled into the study.

Within 1 hour prior to the infusion, several baseline measurements were made. These measurements included vital signs (1 hour and 15 minutes prior to the infusion) and hemodynamic measurements (2 sets within 30 minutes of starting the infusion). If the measurements were not within $\pm$ 15% of each other, an additional set of measurements were taken, with the last two values serving as baseline. Blood for chemistry and hematology screens (within 15 minutes of the infusion) and baseline hBNP levels (within 15 minutes of the infusion) and a blood sample to serve as a reference (reference to what?) were also drawn.

Treatment period: After each subject ate a light breakfast, they received the first of the planned boluses. The following measurements and times with respect to the first bolus when these

measurements were made are shown below. During any serious adverse event, both vital signs and hemodynamics will again be measured.

Vital signs was measured at 15, 30 and 45 minutes and 1, 1.5, 3, and 4 hours after the first and last bolus as well as immediately prior to all boluses.

Hemodynamics (with the exception of cardiac output) was measured at 30 minutes, 1 and 2 hour after the first bolus and immediately before each subsequent bolus, as well as 30 min 1, 2, and 4 or 6 hours after the last bolus. The last measurement for those who receive Q 4 hour boluses was at 4 hours and for those who receive Q 6 hour boluses it was at 6 hours.

Cardiac Output was measured 2 hours after the first bolus and before each additional bolus. After the last bolus, cardiac output was measured at 2 and 4 or 6 hours.

hBNP Concentrations: Blood was collected at 2, 5, 15 60 and 90 minutes after both the first and last bolus for hBNP measurements.

24-hour Urine: Began at the first bolus, with measurements of volume and urinary sodium and potassium.

Inputs: Total fluid intake (intravenous and oral).

Dosing Guidelines: Subsequent bolus doses were to be halved if the patient developed symptomatic hypotension, which did not require fluid or pressor support. If the hypotension requires fluid or pressor intervention, the patient was discontinued from further boluses. A patient whose status deteriorates was discontinued from the study and received appropriate treatment (i.e. .diuretics or inotropes) and was declared a treatment failure.

Post treatment. A follow-up visit was scheduled at 7 and 15 days via telephone and an clinic visit was scheduled on day 20 to 30, at which time a physical exam was performed and blood was drawn for chemistries/hematology., Blood was also drawn to determine anti-BNP antibodies.

Results:

Demographics: A total of 60 subjects were enrolled into the study. Table 4.1(above) contains the number of subject enrolled/site. The largest number of patients were enrolled into site # 348 (17/60 subjects). Site 315 enrolled 8 subjects. The demographics of those enrolled are shown in Table 4.2. Those enrolled were fairly young for CHF patients (median age 53). The majority was Caucasian (65%) with the rest black. The population was largely male (73%).

Table 4.2 Demographics of Those Enrolled into Study 704.309

	Placebo	5 ug/kg Q 4h	10 ug/kg Q6	10 ug/kg Q4	p value
Age (years)	52 ± 9.4	52 ± 13.5	56 ± 12.6	53 ± 8.3	0.77
Ethnicity (Black/Caucasian)	4/12	7/8	5/9	5/10	0.67
Gender (Male/Female)	11/5	9/6	11/3	132	0.39
Height (cm)	175 ± 9	173 ± 10	176 ± 13	173 ± 7	0.78
Weight (Kg)	81 ± 24	85 ± 26	87 ± 22	94 ± 17	0.54

Discontinuations. Nine subjects discontinued, per sponsor, for reason of worsening CHF. Five of those who discontinued were placebo patients, two were assigned to the 5 ug/kg Q4 hr group, one from the 10 ug/kg O6h. and one to the 10 ug/kg O 4h. Six of those who discontinued were from one

study center (# 315). Since only eight patients were enrolled from this site, a disproportionate fraction of those who discontinued were from this site..

Table 4.3 List of Dropouts for Worsening CHF.

315-001 (PBO)	315-009 (PBO)	348-017 (PBO)	353-001 (PBO)	315-006 (PBO	315-003 5 ug/kg Q4	355-001 5 ug/kg Q4	315-002 10 ug/kg Q6	315-004 10 ug/kg Q4

(Capsular summaries are listed under Safety).

Concomitant Medications. (This Data was derived from Listing 23 and 24). Concomitant medications that might alter either cardiac hemodynamics or renal function were fairly common. All but 16 patients (nearly equally divided among the four treatments) received a loop diuretic during the 24 hours in which hemodynamics and urine output were measured. A total of 8 patients received some form of inotropic support (iv dopamine or dobutamine), four of these were placebo patients. Seven of the those who received inotropic support were enrolled in a single study site (# 315).

Kinetics:

Kinetics profiles were measured both after the first and last drug/placebo bolus. Additional blood samples were to be drawn at trough, prior to each subsequent bolus. These trough samples, however, were never drawn. Plasma samples were measured by the antigen displacement methodology (described in Dr. Sadrieh's review). The kinetics for both the first bolus and the second bolus (either at 18 or 20 hours after the first bolus are shown in Figures 4.1 and 4.2)

Figure 4.1

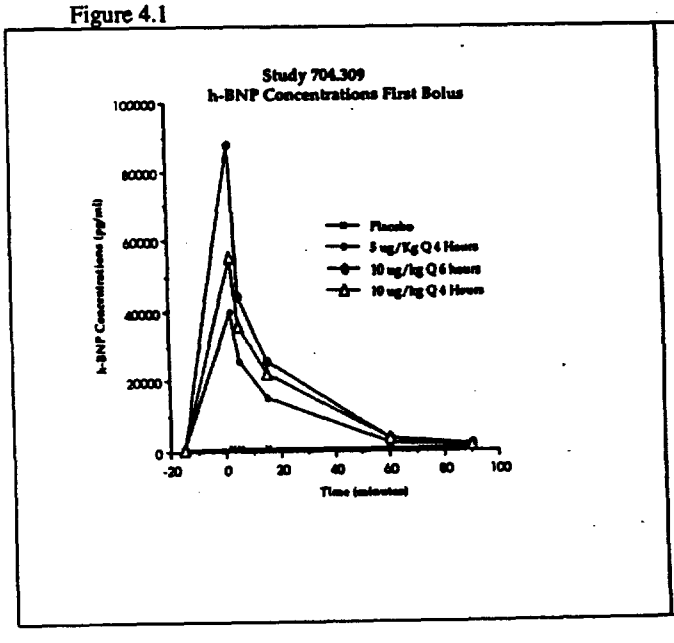
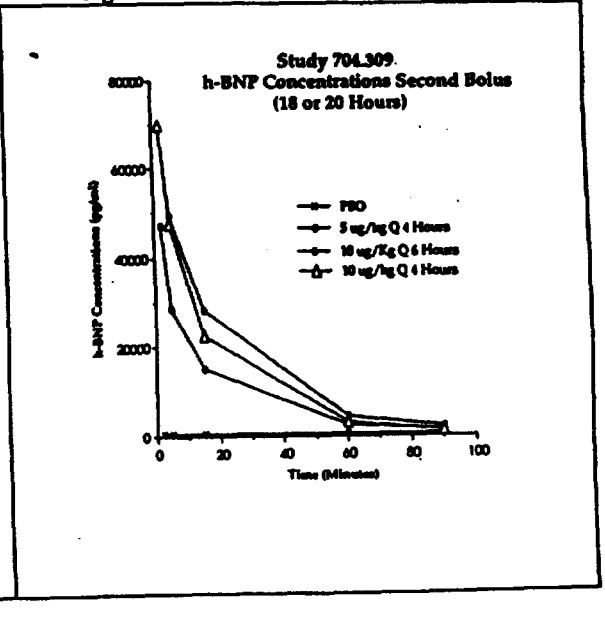


Figure 4.2



A substantial portion of data for the initial bolus of 2 patients (# 353-003 and #359-003), were excluded from the analysis. The data for the terminal bolus data was also excluded for this last patient (#359-003 as well as patient # 352-003). Data points for the 60 and 90 minutes for two subjects (#360-003 and #360-005), during the terminal bolus were also excluded. Data for those who prematurely discontinued clearly were not included in the measurement of the final bolus.