【S-10】

Doxorubicin and Herceptin Combined Cardiac Dysfunction in Cynomolgus Monkeys: a Follow Up Study.

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Herceptin is a recombinant DNA monoclonal antibody that binds to human epidermal growth factor receptor protein (HER2). Herceptin has been shown to inhibit the proliferation of human tumor cells that express HER2. When used as an adjuvant therapy with chemotherapeutic agents, metastatic breast cancer has significantly increased disease free survival. Reduced cardiac function has been noted clinically when herceptin was used in conjunction with chemotherapeutic agents such as doxorubicin. This outcome was not anticipated by the preclinical studies. Thus, further studies were requested to evaluate the cardiac dysfunction. A previously developed doxorubicin model was employed to create a stable albeit significant level of heart failure in eight females Cynomolgus monkeys. Animals were periodically dosed with 2 mg/kg every week for 3 weeks followed by 1 mg/kg every other week until evaluation by echocardiography and telemetry showed evidence of heart failure. Doxorubicin reduced shortening fraction and velocity of circumferential shortening by nominally 35%. These animals were then either dosed with herceptin in 10 administrations of 25 mg/kg over four weeks or vehicle. Additional decrements in cardiac function were with herceptin as compared to vehicle by increased systolic and diastolic function and increased in E point septal separation. Thus, it appeared that herceptin exacerbated the decrease in cardiac function caused by doxorubicin. A subsequent study was performed in 20 monkeys. The dose groups were: vehicle, herceptin, doxorubicin, and herceptin + doxorubicin. Dose levels were as previously described. The doxorubicin groups exhibited increasing systolic and diastolic dimensions and increased E point septal separation. These parameters partially normalized with doxorubicin alone. Cardiac function continued to worsen with doxorubicin and herceptin. Cardiac function was not different from vehicle with herceptin alone. These data suggest that herceptin may exacerbate previously produced cardiac dysfunction. Several mechanistic hypotheses are being investigated.

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Doxorubicin (Adriamycin) and Herceptin (Trastuzum ab) Combined Cardiac Dysfunction in Cynomolgus Monkeys *A Follow-up Study*

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Doxorubicin (Adriamycin)

- Potent cytotoxic anthracycline antibiotic used for cancer chemotherapy.
- Effect on malignant cells presumably via intercalation with DNA.
- A known cardiotoxic at higher dose levels with the cardiotoxicity often delayed.
- Free radical formation has been implicated in Doxorubicin cardiotoxicity by means of Co (II) and Fe (III) reduction at the cellular level.

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Doxorubicin (Adriamycin) and Herceptin (Trastuzumab) Combined Cardiac Dysfunction

- Herceptin (Trastuzumab) is a recombinant DNA-derived humanized monoclonal antibody which binds to human epidermal growth factor receptor protein (HER2).
- Herceptin shown to inhibit proliferation of human tumor cells that express HER2.
- Herceptin as adjuvant therapy for metastatic breast cancer significantly increases disease-free survival.

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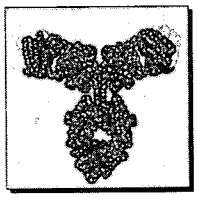
Trastuzumab (Herceptin®): Humanized Anti-HER2 Antibody



- Targets HER2 oncoprotein, present in 20% to 25% of patients with breast cancer
- High affinity (Kd = 5nM) and specificity
- 95% human, 5% murine
  - Decreased potential for immunogenicity
  - Increased potential for recruiting immune effector mechanisms

Carter et al, 1992; Park et al, 1993; Slamon et al, 1987; Genentech, data on file

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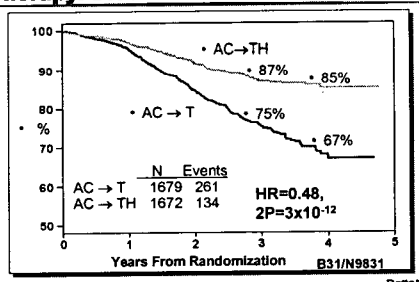
Herceptin (Trastuzumab) as Adjuvant Therapy: Disease-Free Survival



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Complications

- Reduced cardiac function was noted clinically when Herceptin (Trastuzumab) was used in conjunction with chemotherapeutic agents.
- This outcome was not anticipated by the preclinical trials. Thus, further studies were requested to evaluate combined cardiac dysfunction.

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Pivotal Clinical Trial Cardiac Dysfunction Summary Data

	H alone (n = 213)	H + AC (n = 143)	AC (n = 135)	H + P (n = 91)	P (n = 95)
Any cardiac dysfunction	7%	28%	7%	11%	1%
Class III-IV	5%	19%	3%	4%	1%

From Herceptin (Trastuzumab) *Product Insert*

H = Herceptin (Trastuzumab)
AC = Anthracyclin + Cyclophosphamide
P = Pacitaxel

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Model Development

- A primate Doxorubicin (Adriamycin) heart failure model was previously developed at Battelle in Rhesus monkeys (1981). The model was subsequently adapted for use in a combined Doxorubicin/Herceptin evaluation in Cynomolgus monkeys (2003).
- A primate model was essential since Herceptin (Trastuzumab) cross-reactivity is limited to the primate.

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Effects of Exercise Stress in Rhesus Monkeys ~ Compromised by Doxorubicin (Adriamycin) Toxicity

- Cardiomyopathies occur in some individuals treated with Doxorubicin (Adriamycin) one year or more after treatment (latent cardiotoxicity).
- Concern that an increased demand on the myocardium, such as repeated exercise, may contribute to or precipitate the cardiotoxicity.

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Doxorubicin (Adriamycin) Dosing Schedule

- 2 mg/kg per week for three consecutive weeks
- 1 mg/kg every other week
- Periodic cardiovascular evaluation via echocardiography until a "stable" and significant decrease in cardiac function was noted.
- Post-treatment one-half of the animals were exercised for 12 months

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Cardiovascular Evaluation via Echocardiography

- Echo scan of each animal's heart was performed periodically during the experiment.
- Ten baseline evaluations of each animal were the basis for determining adequate drug treatment for each individual animal.
- Parameters selected to provide basis treatment termination:
  - percent shortening fraction
  - velocity of circumferential fiber shortening
  - pre-ejection period/left ventricular ejection time.
- When any one of these three measures consistently exceeded ±2SD from baseline mean, the treatment with Adriamycin was terminated.

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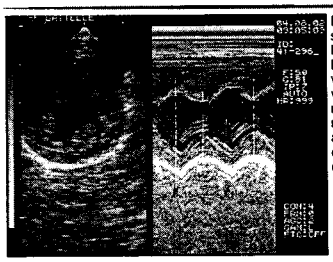

Right parasternal short-axis B-mode (left) and M-mode (right) view of the left ventricle of the monkey used to measure systolic and diastolic chamber dimensions

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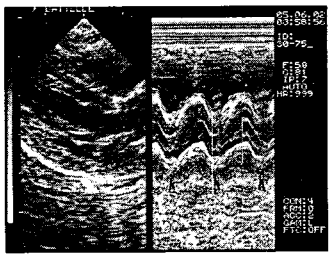

Left parasternal B-mode (left) and M-mode (right) view of the left ventricular outflow tract of the monkey. The M-mode tracing allows visualization of aortic valve action and measurement of left ventricular time intervals

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Study Design

Rhesus Monkeys

Both Sexes 3 to 5 kg

	Exercise	Non-Exercise
Vehicle	4	4
Adriamycin	20	20

Number of Animals on Study

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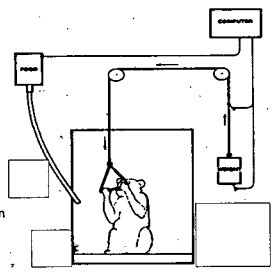
Exercise

- After treatment, 24 of the animals were trained to exercise.
- An automated exercising system for simultaneously exercising 24 Rhesus monkeys was designed.
- Each animal was behaviorally conditioned to lift half of his body weight 16 inches (40.6 cm), 25 times to receive a 1 gram food pellet.



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Exercise Results

- All animals successfully learned the positive reinforcement exercise paradigm
- Daily, the typical exercised animal performed an average 3375 repetitions and consumed an average 135 pellets of 1 gram each within 4 hours
- Non-exercise animals received 100 pellets

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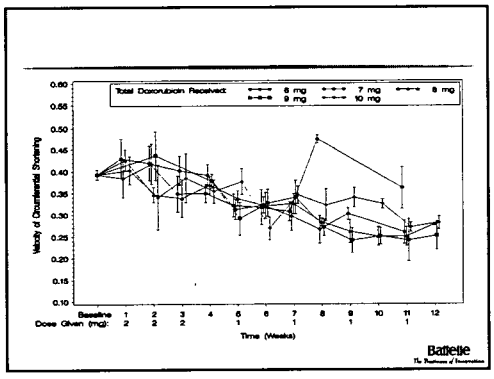

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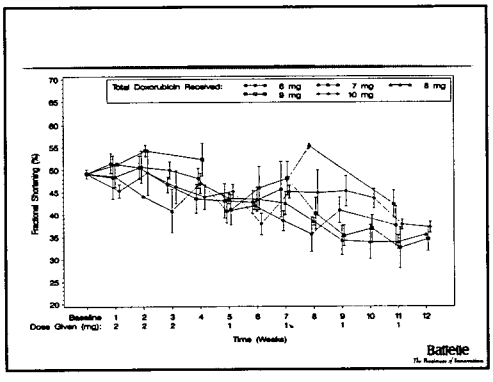

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Analysis

- PSF and VCF were reduced between baseline and post-treatment (over 110 days) for all the drug-treated control animals
- The reductions were substantially greater for the drug-treated animals than (α <.001) for the control animals
- Animals exercised (12 months) exhibited less reduction than non-exercised animals (NS) suggesting that exercise was a beneficial second-order effect

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Histology

- At total doses of 6 mg/kg or 7 mg/kg (72 mg/m^2 or 84 mg/m^2) little or no damage microscopically
- Extensive damage at 10 mg/kg or 11 mg/kg (120 mg/m^2 or 132 mg/m^2)
- Variable damage 8 mg/kg or 9 mg/kg (96 mg/m^2 or 108 mg/m^2)
- No correlation between cardinal echocardiographic parameters and histologic changes

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Conclusions

- Exercise did not further decrease myocardial function (possibly beneficial) in Rhesus monkeys that had been previously treated with Doxorubicin (Adriamycin).
- Echocardiographic parameters were useful in monitoring for and predicting Doxorubicin-induced cardiotoxicity in rhesus monkeys but they were not good indicators of histologic changes.
- Most monkeys in this program were administered an amount of Doxorubicin (Adriamycin) which induced a decrease in myocardial function as demonstrated by echocardiographic parameters and myocardial degeneration as demonstrated by histologic evaluation.

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Complications

- Reduced cardiac function was noted clinically when Herceptin (Trastuzumab) was used in conjunction with chemotherapeutic agents.
- This outcome was not anticipated by the preclinical trials. Thus, further studies were requested to evaluate combined cardiac dysfunction.

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Primate Model Development of Combined Doxorubicin and Herceptin Cardiac Dysfunction

Pilot Study 1.

The Cardiotoxic Potential of Doxorubicin (Adriamycin) Alone versus Doxorubicin (Adriamycin) Followed by Herceptin (Trastuzumab) in Female Cynomolgus Monkeys

- Eight female cynomolgus
- Telemetry and Echocardiography

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Sequential Dosing: Study Design

Groups	Doxorubicin (Adriamycin)	Herceptin (Trastuzumab)
1 Dox alone	2 mg/kg/wk x 3 1 mg/kg/eow	
2 group 1 followed by		25 mg/kg/4Xwk1; 2x wks 2,3,4
3 group 1 followed by		Herceptin vehicle

- Doxorubicin (Adriamycin) until objective evidence of heart failure
- Followed by 25 mg Herceptin (Trastuzumab)/kg or vehicle daily for four consecutive days followed by twice per week for three weeks (end of study)

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Doxorubicin (Adriamycin) Administered to Each Female Cynomolgus Monkey

Animal Number	Total Dox. Received (mg/kg)[a]	Treatment After Dox. Administration[b]
101	9	Herceptin
102	13	Herceptin
103[c]	8	Herceptin Vehicle
104	8	Herceptin
105	12	Herceptin
106	12	Herceptin Vehicle
107	13	Herceptin Vehicle
108	10	Herceptin Vehicle

a. The first three Doxorubicin administrations were performed on Days 1,8 and 15 at 2 mg/kg; 2 mg/mL; 1 mL/kg per dose. The remaining Doxorubicin administrations began on Day 29 at 1 mg/kg; 2 mg/mL; 0.5 mL/kg per dose.
b. The Herceptin was administered at 25 mg/kg; 21 mg/mL; 1.19 mL/kg per dose. The Herceptin vehicle was administered at 1.19 mL/kg per dose.
c. Animal 103 was euthanized on Day 63 following its 5th Herceptin vehicle dosing due to an exposed and damaged blood pressure catheter.

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Representative ECHO Changes Consistent with Development of Cardiomyopathy Following DOX Treatment

Parameter (following DOX and as compared to baseline)	Group 2 Herceptin[a]	Group 3 Herceptin Vehicle[a]
Fractional shortening (FS)	31%	33%
Velocity of Circumferential Shortening (vcf)	36%	29%

[a] At conclusion of Doxorubicin (Adriamycin) dosing and prior to receipt of Herceptin (Trastuzumab) or vehicle. Thus, the animals before Herceptin (Trastuzumab) vehicle dosing had a similar decreases in Doxorubicin (Adriamycin) induced cardiomyopathy.

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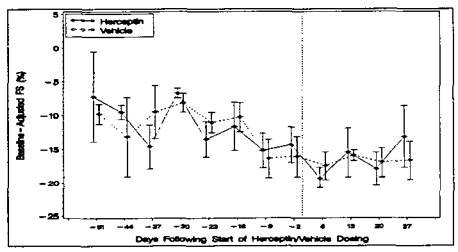
Fractional Shortening of Baseline-Adjusted Measurements by Study Day



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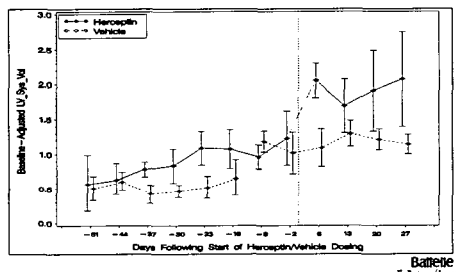
Left Vent. Volume Dose Group Means of Baseline-Adjusted Measurements



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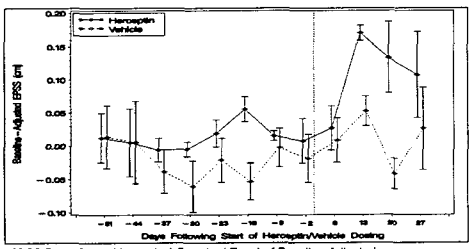
EPSS Dose Group Means of Baseline-Adjusted Measurements by Day



EPSS Dose Group Means (± 1 Standard Error) of Baseline-Adjusted Measurements by Study Day, Relative to Start of Herceptin (Trastuzumab)/Vehicle Dosing

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Cardiovascular Safety Pharmacology – Telemetry Data

- No alterations attributable to Doxorubicin (Adriamycin) or Herceptin (Trastuzumab):
  - Blood pressure
  - Heart rate
  - Body temperature
- Arrhythmia analyses –
  - Ventricular ectopic beats present to some degree in all animals following Doxorubicin (Adriamycin), continued into Herceptin (Trastuzumab)/vehicle phase of study

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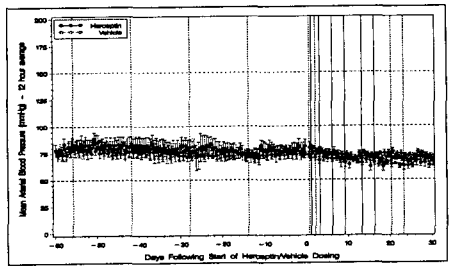
Mean Arterial Blood Pressure



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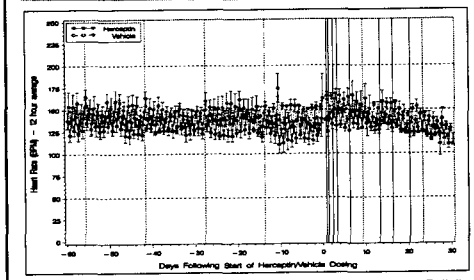
Heart Rate



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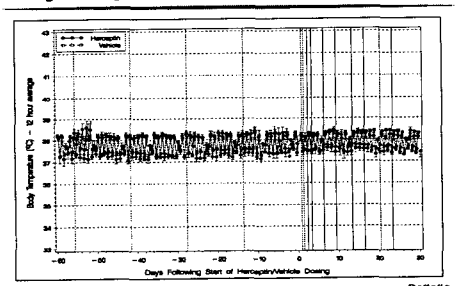
Body Temperature



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Selected Clinical and Anatomic Pathology

- Cardiac chemistry
  - No alterations in total CPK, CK-MB or CK-MM
  - Troponin T & I - inconsistent and solitary minimal elevations noted rarely and randomly
- Histopathology
  - Minimal or mild cardiac degeneration
  - No difference between Herceptin (Trastuzumab) vs. vehicle following Doxorubicin (Adriamycin) administration

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Pilot study 2.

Evaluation of the Cardiotoxic Potential of Doxorubicin (Adriamycin) or Herceptin (Trastuzumab) Alone and in Combination in Cynomolgus Monkeys

- Twenty male and female cynomolgus
- Echocardiography

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Concomitant Dosing: Study Design

Groups	Animal N; gender	Doxorubicin	Herceptin
1 Vehicle	2 M/F	0	0
2 Herceptin	2 M/F	0	25 mg/kg/4Xwk1; 2x wks 2-12
3 *Doxorubicin*	2 M/F	2 mg/kg 1X wk 1-3; 1 mg/kg 1X Wk 5, 7, 9	0
4 Herceptin + Doxorubicin	4 M/F	2 mg/kg 1X wk 1-3; 1 mg/kg 1X Wk 5, 7, 9	25 mg/kg/4X wk1; 2x wks 2-12

Objectives:

- Evaluate effect of fixed dose of 9 mg/kg Doxorubicin (Adriamycin) on cardiac pathology in the cynomolgus monkeys
- *Evaluate Herceptin (Trastuzumab) alone with intensive cardiac monitoring*

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Decreased Systolic Function in Both Doxorubicin (Adriamycin) Treated Groups

Increasing diastolic and systolic LV internal diameters and EPSS

- Doxorubicin (Adriamycin) group - partially normalized toward the end of the study (following cessation of dox)
- Doxorubicin (Adriamycin) + Herceptin (Trastuzumab) group – consistent worsening of systolic function with none appearing to improve toward end of study (following cessation of dox)
- The Herceptin (Trastuzumab) group was no different from vehicle

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Relevance of Preclinical Safety Assessment to Humans

- Risk of cardiac failure was not recognized pre-clinically.
- Mechanistic work continues, progress but mechanism not yet elucidated.
- Significant patient benefit despite recognized risk.
  - Manage risk
    - Appropriate patient selection (targeted therapy)
    - Screen and monitor cardiovascular function

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Hypotheses for Mechanisms of Activity Herceptin (Trastuzumab) Associated Cardiotoxicity

- Direct effect of Herceptin (Trastuzumab) on cardiomyocytes.
- Interference with cardiomyocyte survival or repair signals
  - Herceptin (Trastuzumab) may block or alter cell survival signaling
  - Herceptin (Trastuzumab) may down regulate erbB2 and thereby prevent cell survival signaling.
- There may be a feedback loop involving neuregulin and erbB2 (as a co-receptor) as part of a cell survival mechanism.
- Cardiac physiological stress or damage can be exacerbated by Herceptin (Trastuzumab).
- Observational artifact (extensive surveillance in the face of imperfect tests).

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Thank You!

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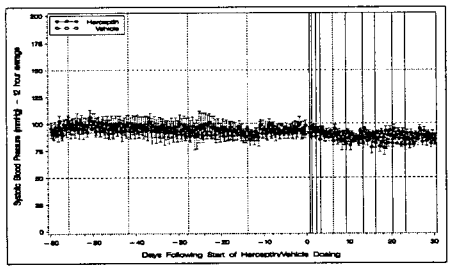
Systolic Blood Pressure



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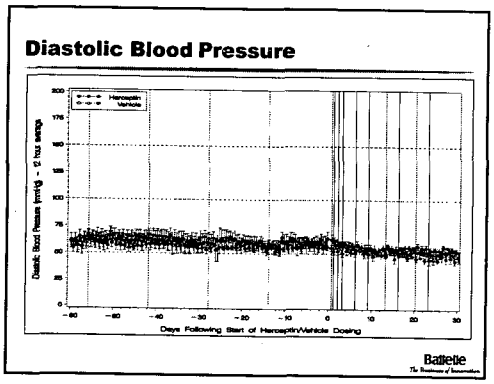
Diastolic Blood Pressure
Herceptin
Vehicle
Diastolic Blood Pressure (mmHg) - 6 hour average
Days Following Start of Herceptin/Vehicle Dosing
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