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Pioneering science delivers vital medicines™

Cardiovascular Toxicity Assessment in Oncology Trials Workshop

Organized by FDA, AACR, ACC, AHA & ASCO

Session 3: Nonclinical

Overview: What nonclinical studies are available to interrogate potential cardiovascular risk of an oncology product?

Hugo M. Vargas, PhD, DSP

Integrated Discovery & Safety Pharmacology

Comparative Biology and Safety Sciences

Amgen, Inc

General nonclinical study requirements

	ICH M3 (Small molecules)	ICH S9 (Small/large molecule)	ICH S6 (Large molecules)
Repeat-dose studies	≤1 mo, supports P1	1 mo, supports P1 and P2	≤1 mo, supports P1
Repeat-dose chronic studies	6 mo (rodent) and 9 mo (non-rodent) as needed for clinical dosing duration, supports filing	3 mo, supports P3/filing	6 mo (1 sp) as needed for clinical dosing duration, supports filing
Safety Pharm	Dedicated Functional Studies (CV, CNS, Respiratory)	**No dedicated studies unless warranted (SPiT approach)**	No dedicated studies unless warranted (SPiT approach)
Genotoxicity	Supports P1	For marketing	Not warranted
Repro Tox	Fertility and embryo-fetal, supports P3; Peri-postnatal, supports marketing	Embryo-fetal, supports marketing	Embryo-fetal, supports P3; Peri-postnatal, supports marketing
Carcinogenicity	2 species, supports marketing	Not warranted	Not generally warranted
Misc	Local tolerance: conduct Phototoxicity, Impurities: as indicated by data	Phototoxicity, Local tolerance, Impurities: assessment and justification with filing	Phototoxicity: not warranted Tolerance: not warranted Impurities: as indicated by data

Major differences in regulatory requirements

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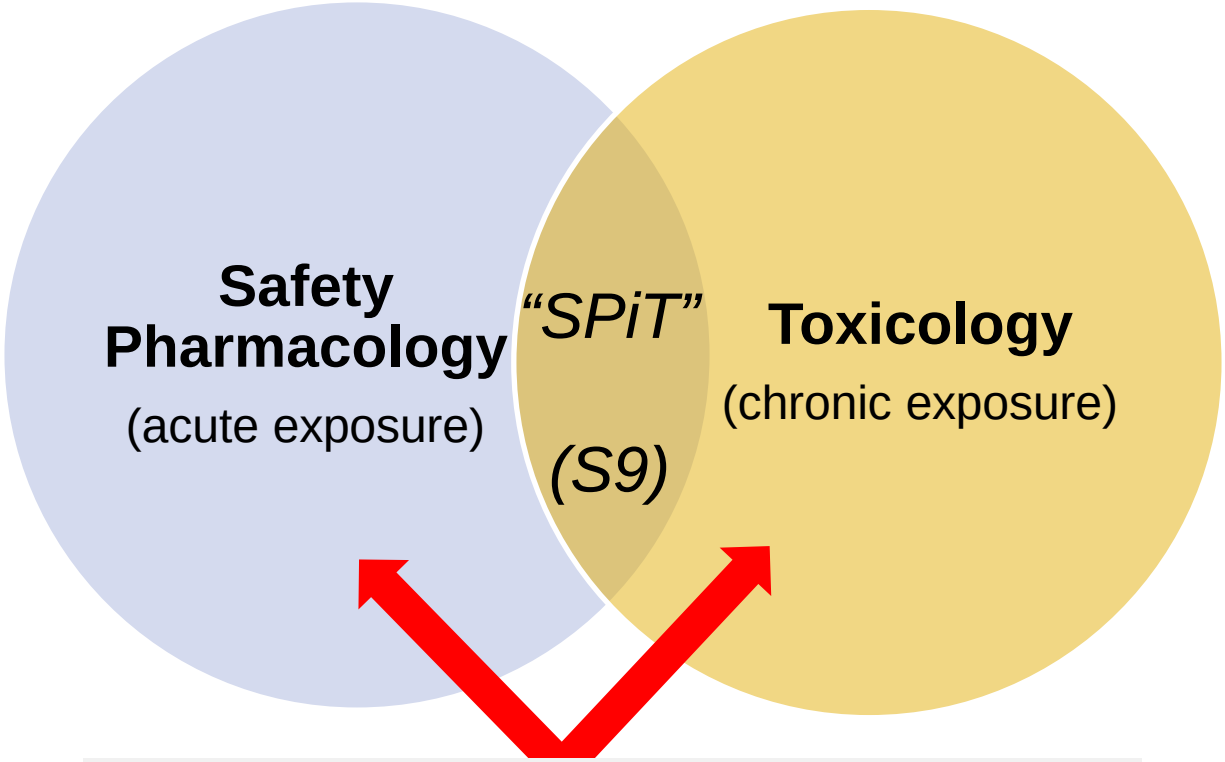
CV Safety Pharmacology Assessment:
Nonclinical Evaluation of Oncology Products



Acute *and* chronic functional effects to be considered

SPiT: Safety Pharm endpoints In Toxicology studies (ICH S9)

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Cardiovascular Risk Evaluation: "The Tool Box"

Safety Pharmacology Society Survey-2016 (85 respondents)

	Nonclinical Methods	Frequently Used (%)	Rarely Used (%)	Count (N)
In vitro - Ex vivo	**hERG & non-hERG** (Patch clamp)	**89**	11	66
	hERG trafficking	42	**58**	72
	Ion Channel Binding	**57**	43	68
	Human iPSC-cardiomyocytes	46	**54**	68
	Isolated heart (Langendorff)	38	**62**	63
	Cardiac wedge prep	18	**82**	51
	APD recordings (Purkinje fibers, etc)	**51**	49	61
In vivo	**Anesthetized animal models**	**53**	47	58
	Telemetry (implant; non-rodent)	**88**	12	67
	Telemetry (jacket; non-rodent)	**62**	38	63
	Pro-arrhythmia models	12	**88**	49
	Zebrafish models	4	**96**	47

>50% (majority)

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Assessing CV Liability for an Oncology Drug:
The oncology drug target co-exists in the myocardium

Drugs Stage	Safety Activity	Comments
Discovery	**Target liability review**	Review of literature; human data of relevance; genetic info; competitor info.
Screening	**hERG & non-hERG potency** **Isolated rabbit heart**	Pro: evaluate off-target and target issues Con: solubility limitations?
Lead Optimization	**Rat telemetry: BP & LVP**	Pro: small animal; single or multi-day dose Con: Is rat relevant, e.g. target expression?
	Non-rodent telemetry: BP & LVP	Pro: single and multi-day dosing Con: PK/PD relationship to CV toxicity
Exploratory toxicity	**Non-rodent: Toxicity: 7-14 day** -Jacket Telemetry (ECG/HR) -Echocardiography -CV biomarkers	Pro: repeat-dosing; dedicated telemetry is an option Con: small groups (N≤3); timing of CV evaluation relative to PK
IND-enabling Safety Studies (GLP)	**Rodent /Non-rodent Toxicity: 28 day** -Jacket Telemetry (ECG/HR) -Echocardiography -CV biomarkers	Pro: larger group sizes; multi-day dosing Con: will CV toxicity emerge in 4 weeks; CV evaluation confounded by other effects (e.g., GI toxicity; dehydration, etc)?
FIH and beyond	**BP, HR, ECG, vital signs** -Echocardiography? -CV biomarkers?	Pro: monitoring of functional CV effects Con: what is a safety signal of concern? unanticipated events?

Cardiac Contractility & Dysfunction
An Emerging Safety Concern for Oncology Drugs

- **Several anti-cancer drugs cause contractile dysfunction*:**
  - Oncology SM: Doxorubicin, epirubicin, mitoxantrone, cyclosphamide, 5-FU, capecitabine, sunitinib
  - Oncology LM: Trastuzumab
  - >30 kinases known to alter myocardial mechanics/function

- **Oncology patients may be susceptible to drug-induced cardiac dysfunction due to: age, co-morbidities, concomitant meds, prior chemotherapy**

- **Echocardiography: Essential tool for clinical LV function testing**

- **Nonclinical use in "SPiT" paradigm:**
  - Case-study with Doxorubicin in NHP

*: Slordal and Spigset, 2006; Force et al, 2006; Cheng & Force, 2010; Force and Kolaja, 2011; Eschenhagen et al (2011: Position paper from European Society of Cardiology)

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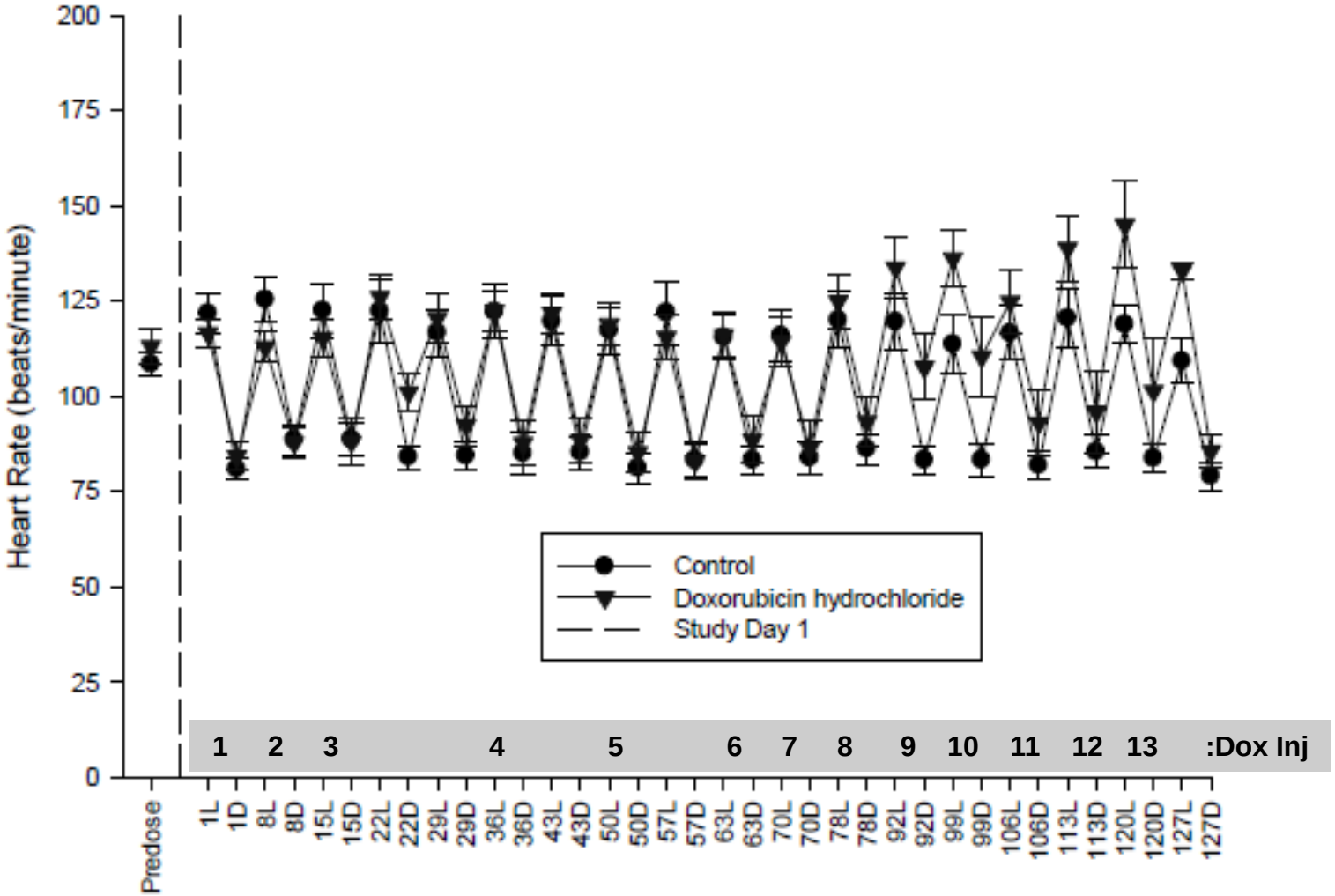
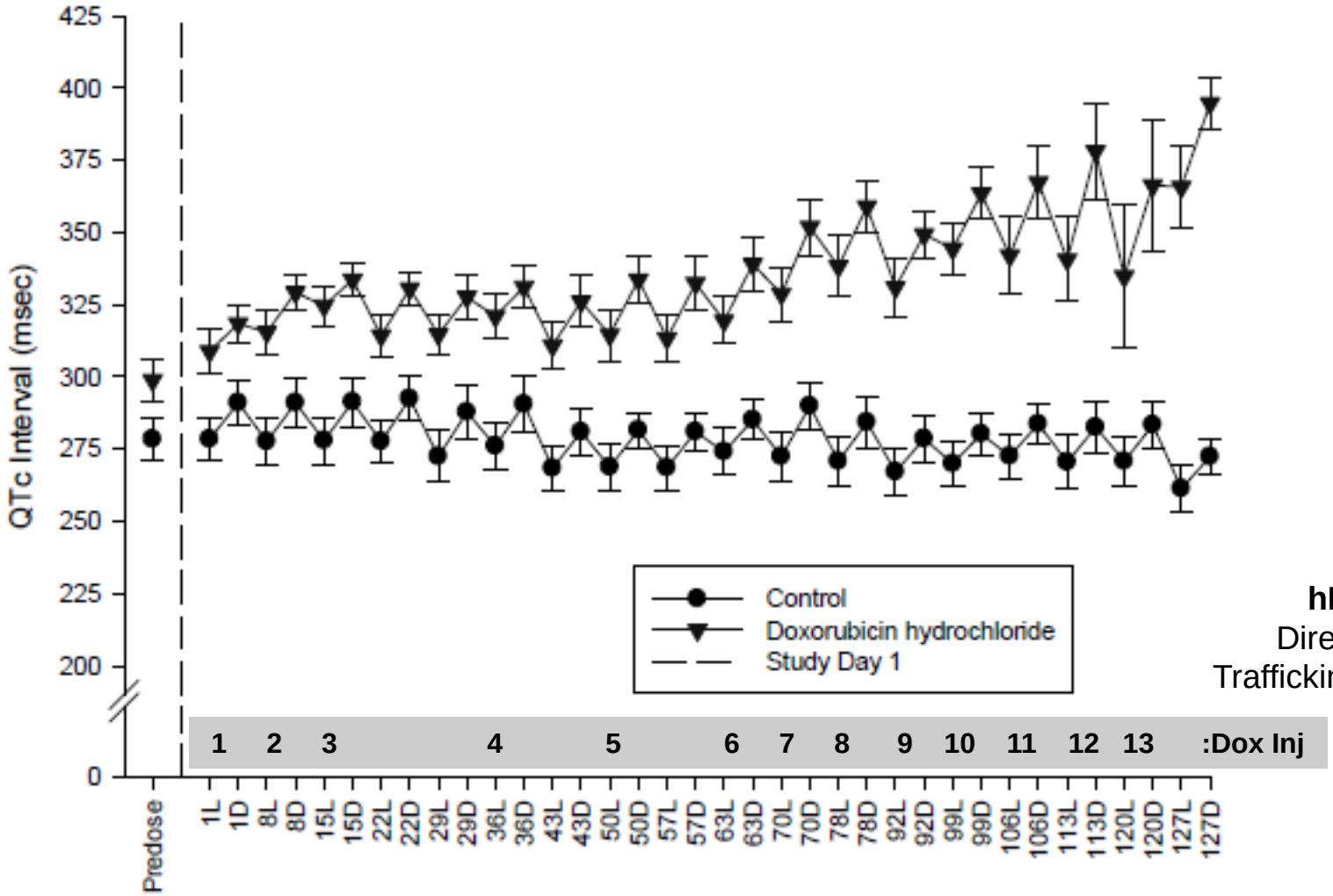
DOX-induced LV Dysfunction in NHP:
Study Design

- **Cynomolgus monkey (NHP): DOX (N=12) and Vehicle (N=12)**
  - Vehicle (IV): 0.9% NaCl for Injection (USP)
- **DOX: 2 mg/kg/wk x 3 wks, then by 1 mg/kg/wk every other week**
  - IV dose regimen selected to produce a gradual change in cardiac function and avoid overt bone marrow depression
    - Malik et. al., Proc Amer Assoc Cancer Res, Volume 45, 2004
  - Study Length: 4 mon
- **Cardiovascular Endpoints and Monitoring:**
  - Implant Telemetry: BP, LVP & ECG
  - Echocardiography
  - Cardiac Biomarkers: cTnI
- **Measurement Frequency:**
  - pre-study (baseline) & regularly (7-14 days) following first dose

Heart Rate


Heart Rate (beats/minute)
200
175
150
125
100
75
50
25
0
Control
Doxorubicin hydrochloride
Study Day 1
1
2
3
4
5
6
7
8
9
10
11
12
13
:Dox Inj
Predose
1L
1D
8L
8D
15L
15D
22L
22D
29L
29D
36L
36D
43L
43D
50L
50D
57L
57D
63L
63D
70L
70D
78L
78D
92L
92D
99L
99D
106L
106D
113L
113D
120L
120D
127L
127D

QTc



hERG-IC50:
Direct: >30 uM
Trafficking: >30 uM

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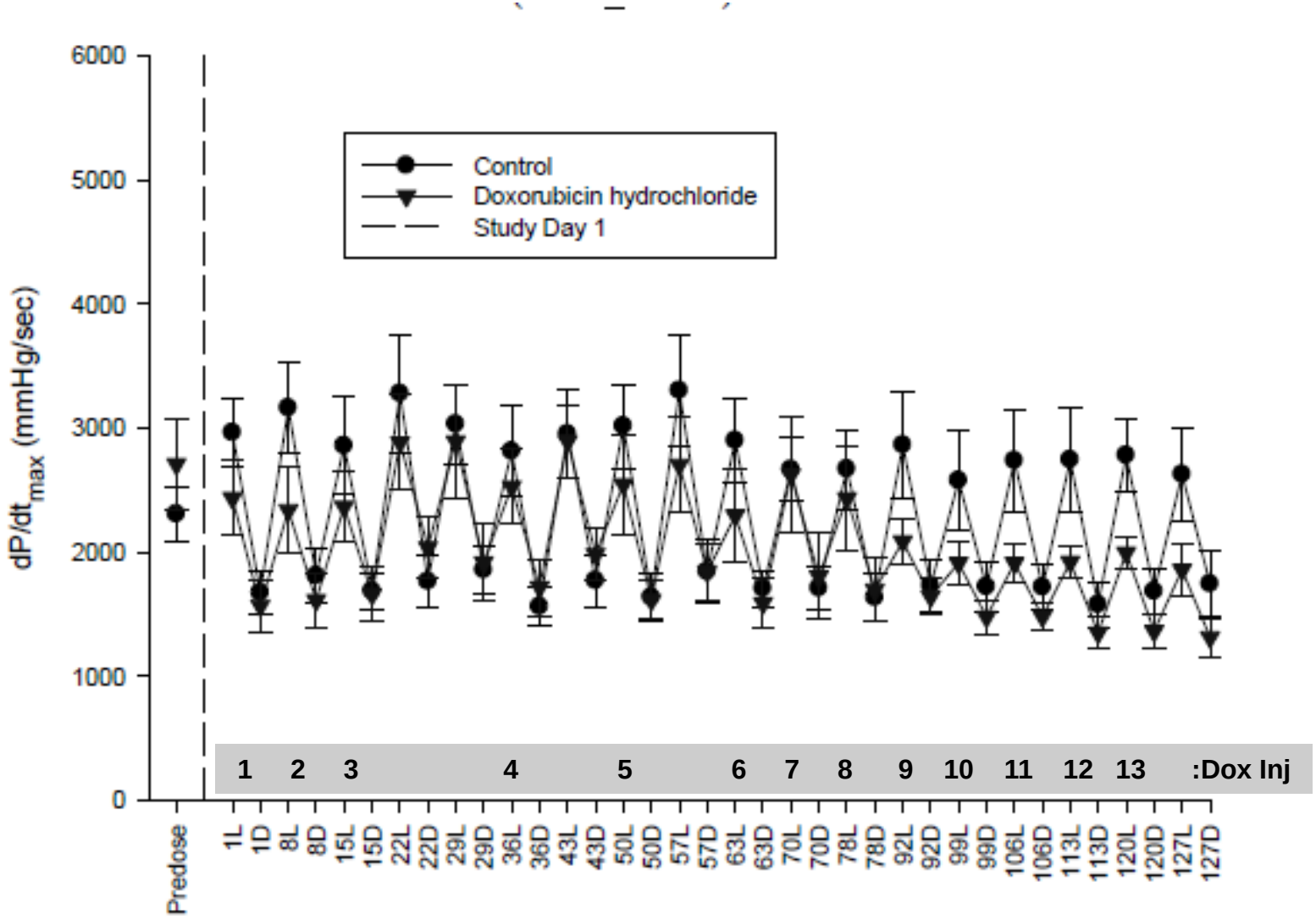
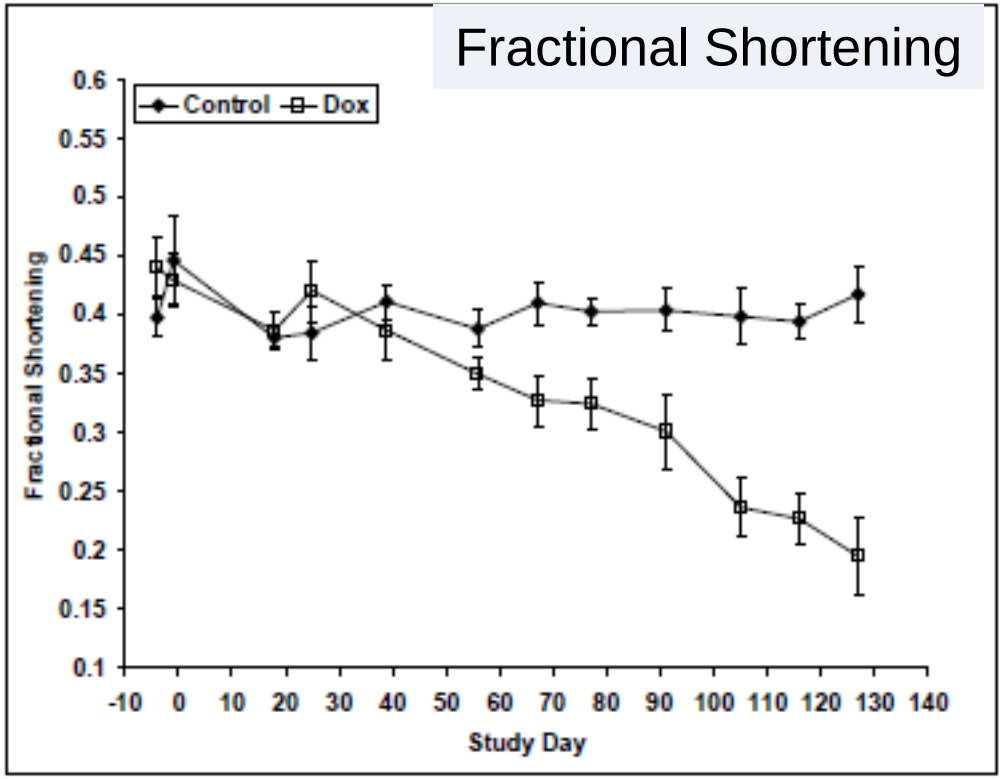
Index of LV Contractility: dP/dt max



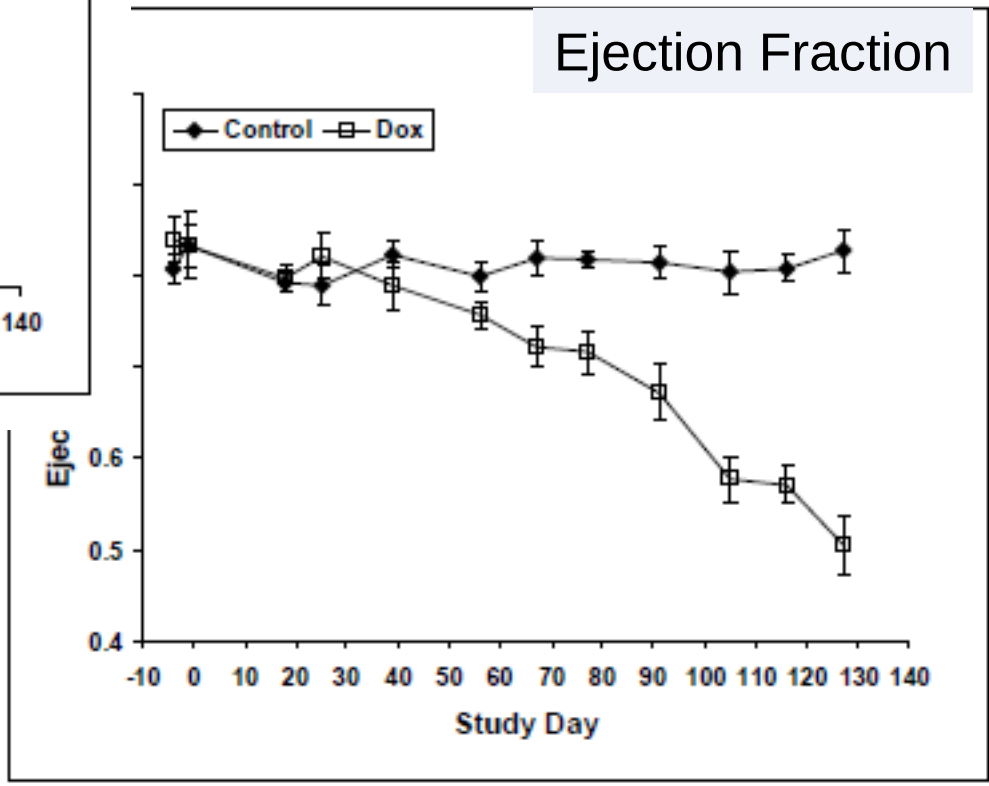
Doxorubicin-Induced Heart Failure in NHP:
Functional and Structural Changes over 4 months

Fractional Shortening



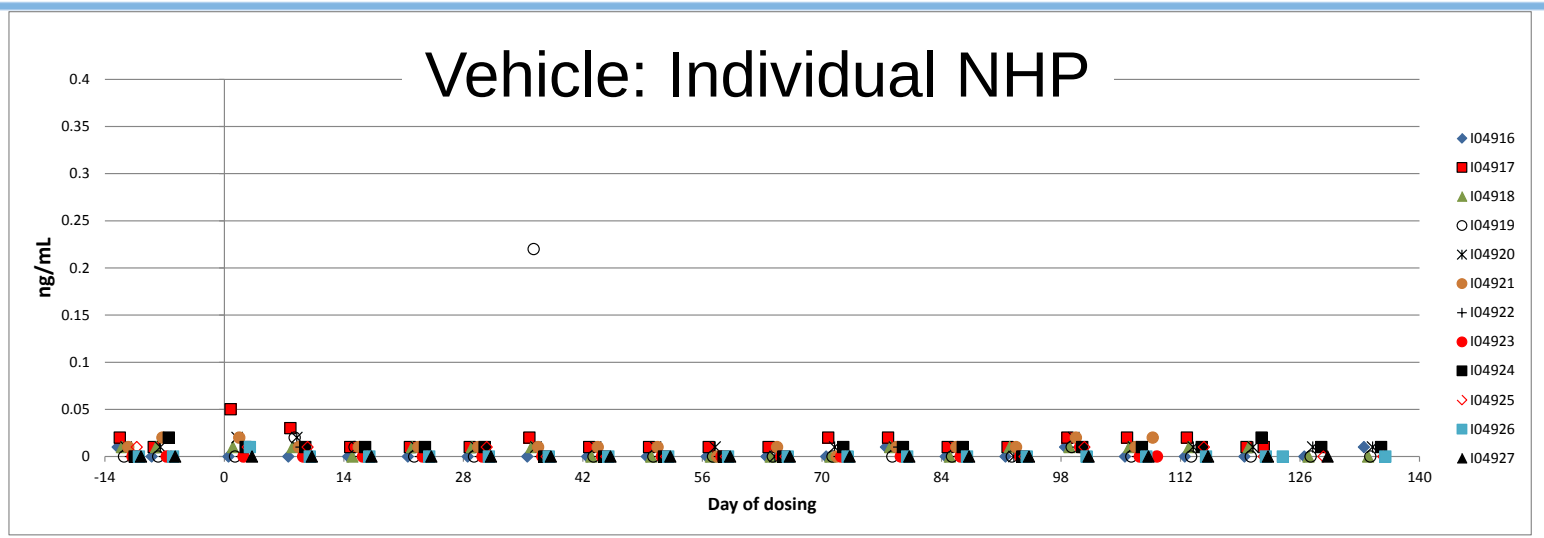
Sonographer: Dr. Meg Sleeper
Echo collection under Ketamine anesthesia

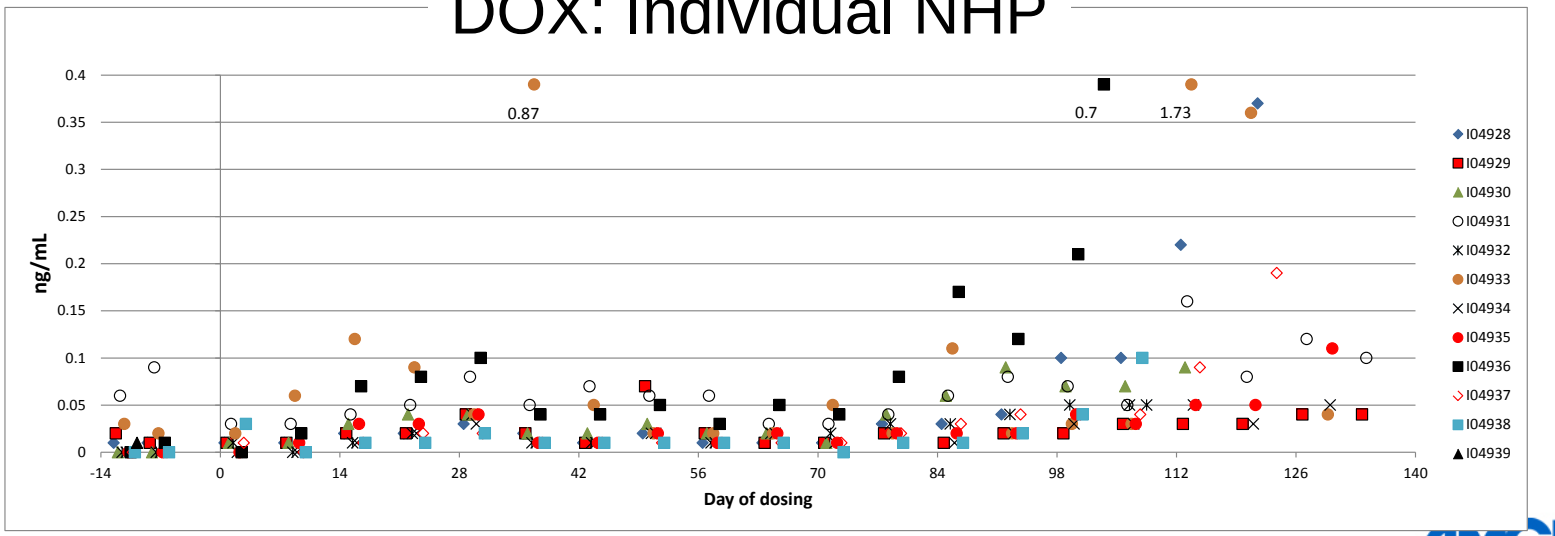
Ejection Fraction



DOX: 2 or 1 mg/kg (weekly)

cTnI (Troponin) Levels





DOX Exposure-Response: NHP vs Human

Dose Injection	Dose (mg/m^2)	Cardiovascular Findings: Cynomolgus	Cardiovascular Findings: Human	Reference
1	24	↑QTc	Ventricular arrhythmia	A
2	48	↑QTc	Ventricular arrhythmia	A
3	72	↑QTc		
4	84	↑QTc		
5	96	↑QTc; ↓EF/FS		
6	108	↑QTc; ↓EF/FS		
7	120	↑QTc; ↓EF/FS		
8	132	↑QTc; ↓EF/FS		
9	144	Above+ ↑HR; ↓dP/dtmax (min)		
10	156	Above+ ↑HR; ↓dP/dtmax (min)		
11	168	Above+ ↑HR; ↓dP/dtmax (min)		
12	180*	Above+ ↑HR; ↓dP/dtmax (min)		
13	192*	Above+ ↑HR; ↓dP/dtmax (min)		
14	204*	Above+ ↑HR; ↓dP/dtmax (min)	↓EF (<55%)	B
-	300	-	↓EF (<55%)	B
-	400	-	↓EF (CHF: ≤5%)	C
-	500	-	↓EF (CHF: 7-26%); ↑QTc	C, D
-	>700	-	↓EF (CHF: 18-48%)	C

*: not tolerated

Conclusions

- **CV functional assessment is needed → repeat-dose paradigms**
  - Oncology Products → balance regulatory & safety needs
  - Augment CV safety assessment in chronic toxicity studies
    - 3Rs: Minimize animal use, especially non-rodents

- **Methods are available for high quality functional data capture**
  - Non-invasive methods: ideal for CV toxicity application
    - Jacket telemetry & Minimally-invasive BP implants
    - Echocardiography: clinical translation
  - Invasive telemetry: best practice for chronic CV evaluations

- **"SPiT": Opportunity for CV Hazard Identification & Risk Assmt.**
  - Pro: Evaluation multiple endpoints over long time periods
  - Con: Risk of false signals; timeframe for CV toxicity unknown; right species used; confidence in translation to human?

Acknowledgements

Amgen

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- Covance
  - Safety Pharmacology Group (Madison, WI)

Recent Publications on "SPiT":
Assess CV Safety after Repeated Dosing

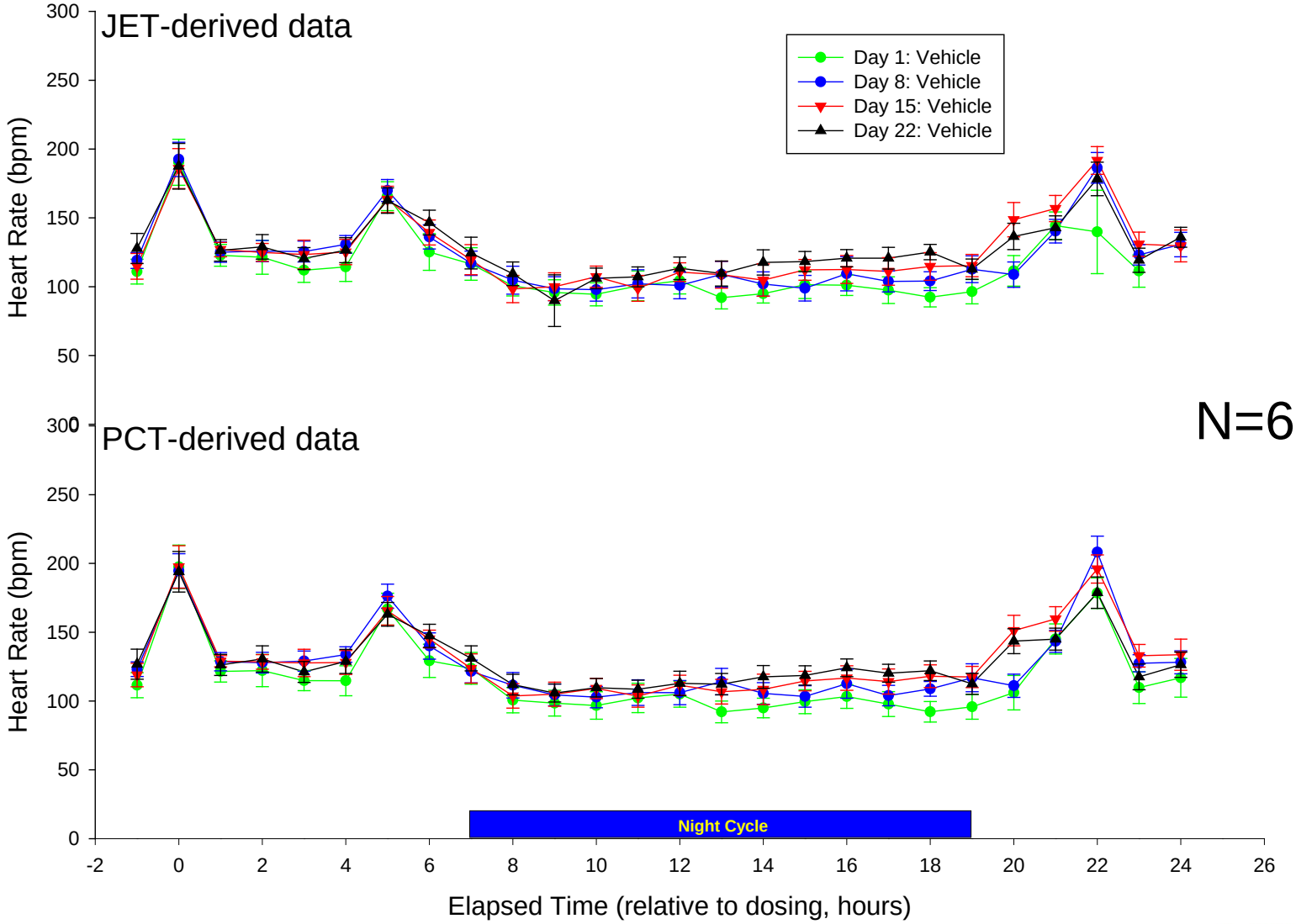
- **Authier et al. (2013): Safety pharmacology investigations in toxicology studies: an industry survey.**
  J. Pharmacol. Toxicol. Methods 68:44-51
  - Industry experience growing; regulatory acceptance of jacket telemetry

- **Redfern et al. (2013): Functional measurements in repeat-dose toxicity studies: the art of the possible.**
  Toxicology Research DOI: 10.1039/c3tx20093k
  - Pros/cons of SPiT; how to do it right

- **Derakhchan et al. (2014): Detection of QTc interval prolongation using jacket telemetry in conscious non-human primates: comparison with implanted telemetry.**
  British J. Pharmacol. 171:509-22
  - Amgen validation experience with a 1 month study design
  - Builds on prior jacket-based ECG collection in dogs (Chui et al., 2009)

Jacket (JET) vs. Implant (PCT) Telemetry: NHP

Heart Rate following Vehicle (oral) over 4 weeks



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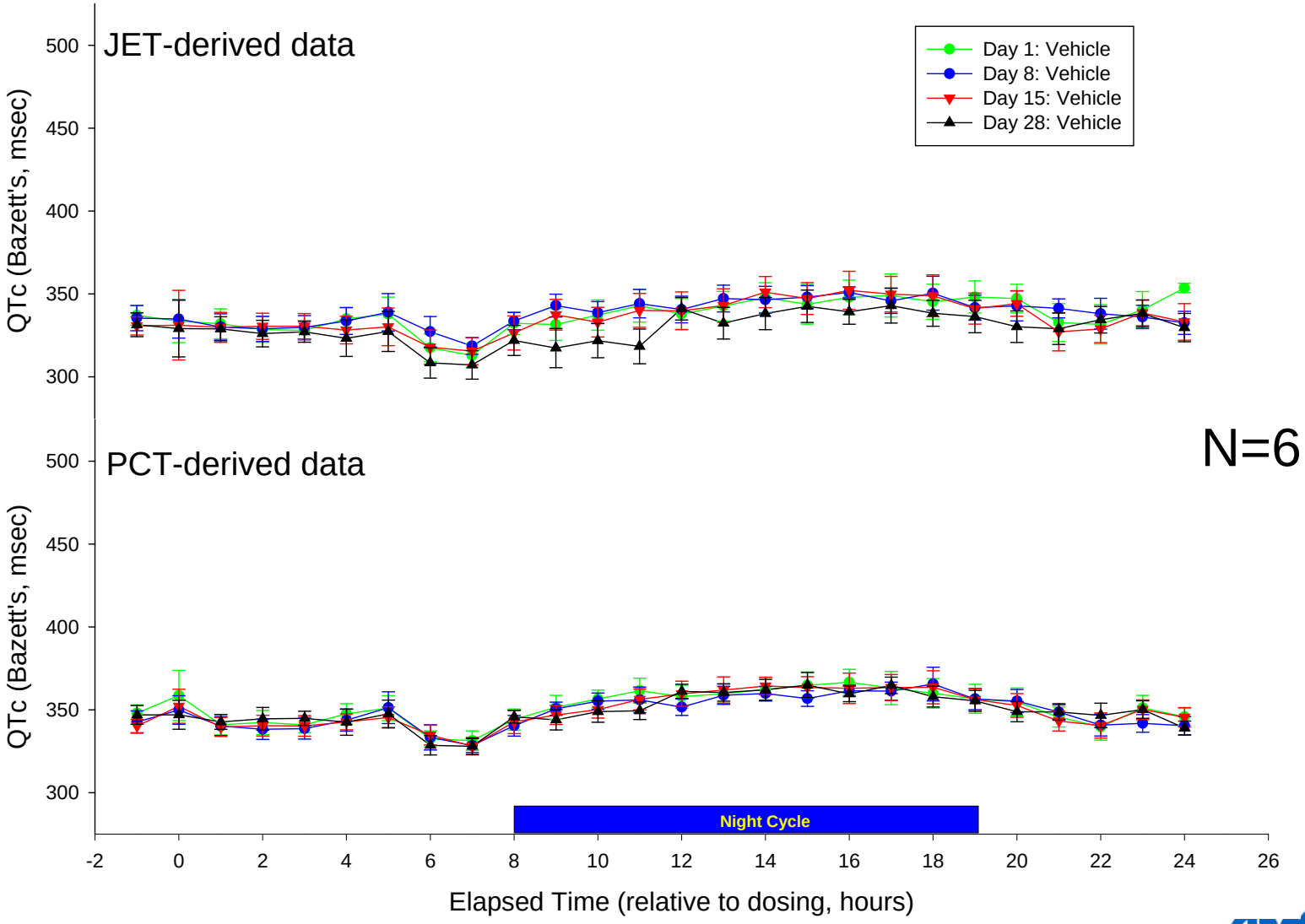
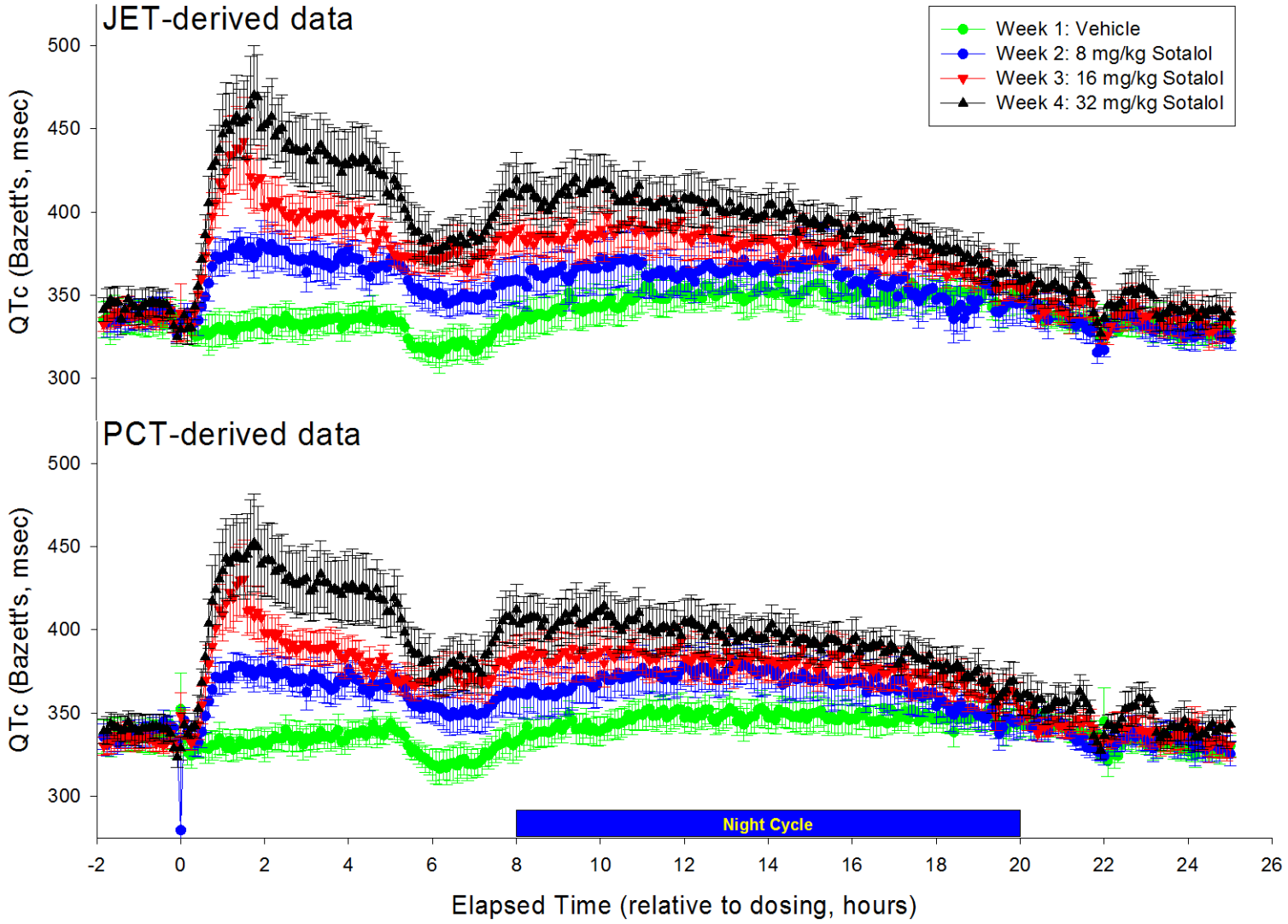


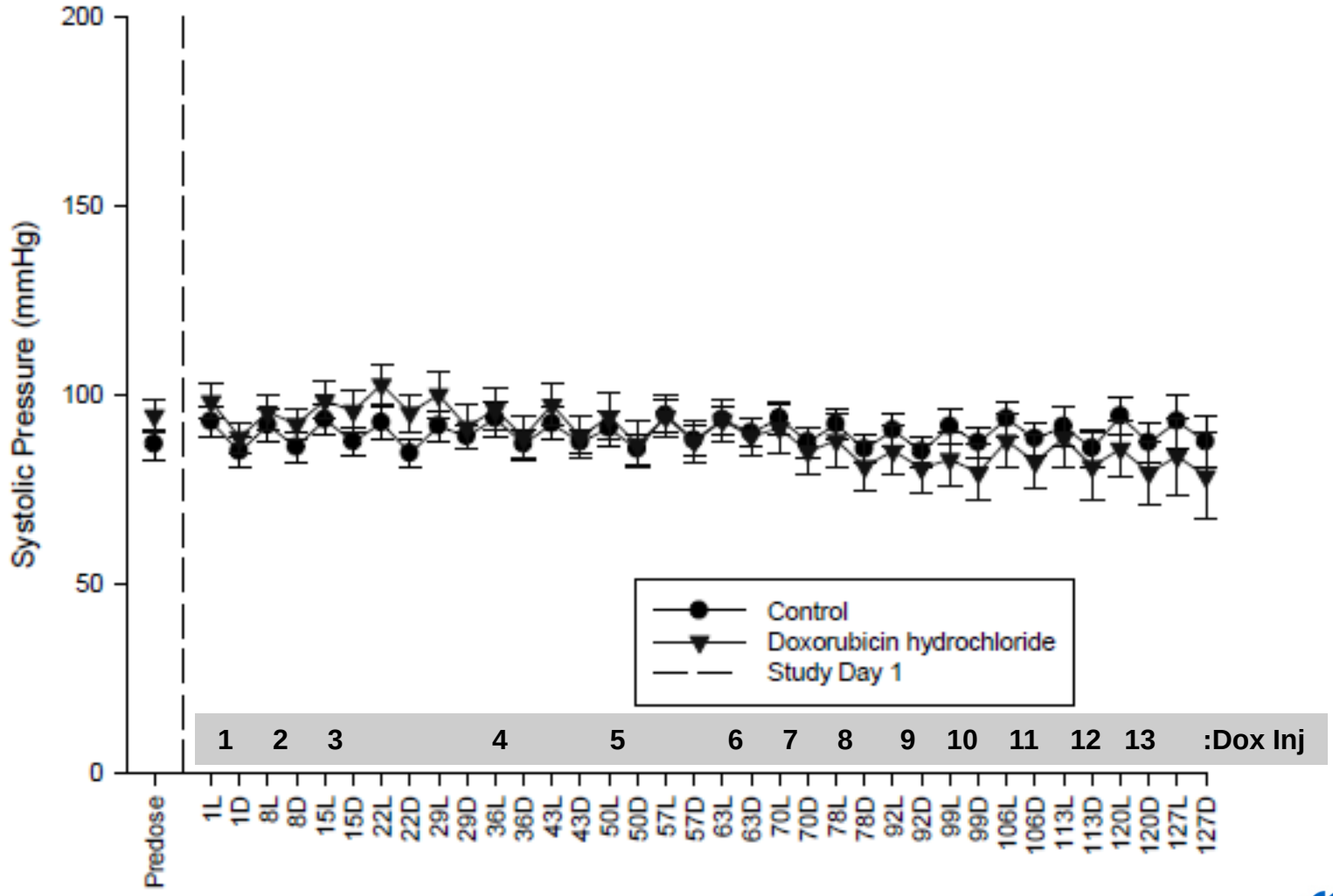
Jacket (JET) vs. Implant (PCT) Telemetry: NHP

Sotalol-induced QTc Prolongation

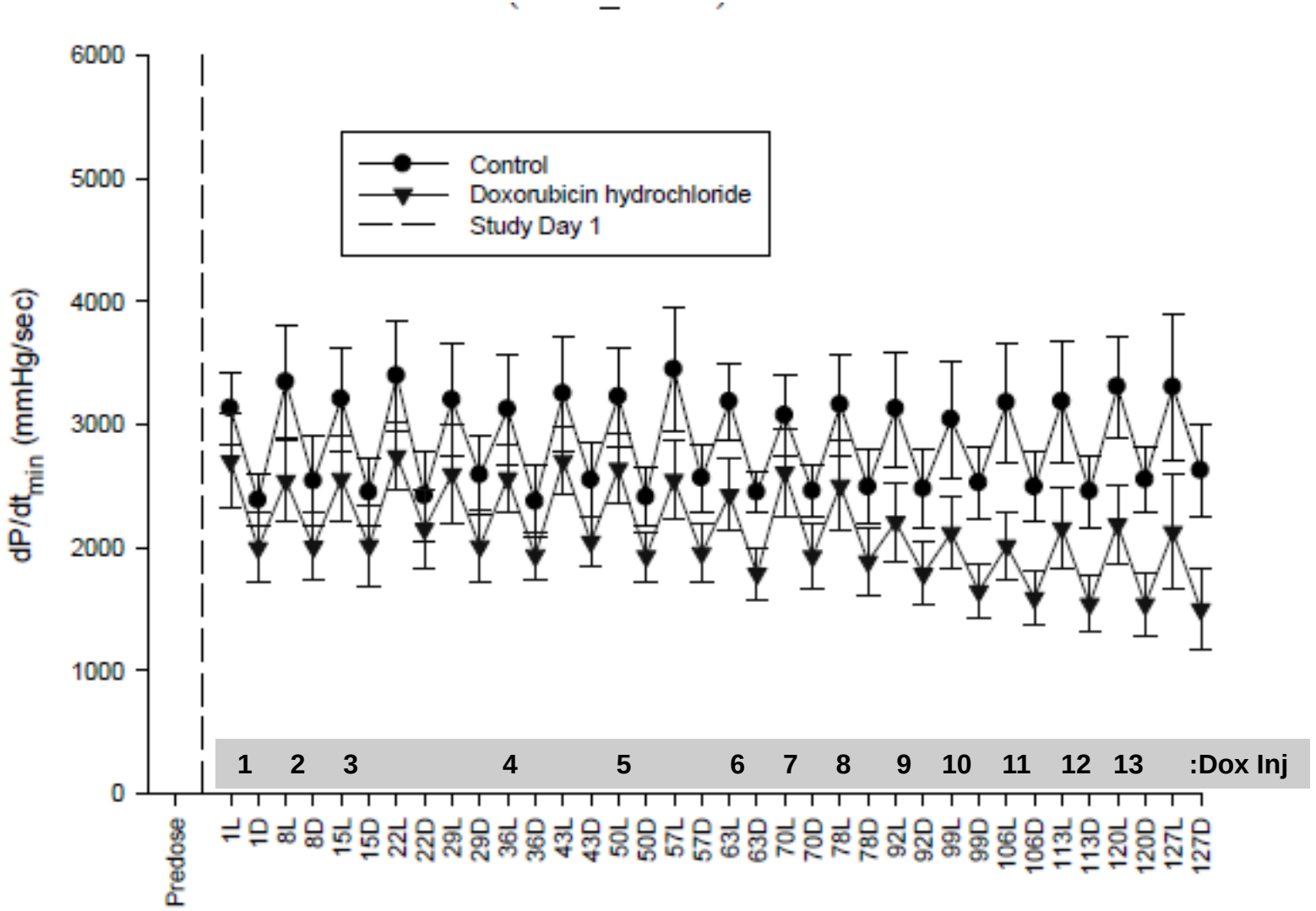


Derakhchan et al. (2014): *British J. Pharmacol*. 171:509-22

Systolic Arterial Pressure



Index of LV Relaxation: dP/dt min



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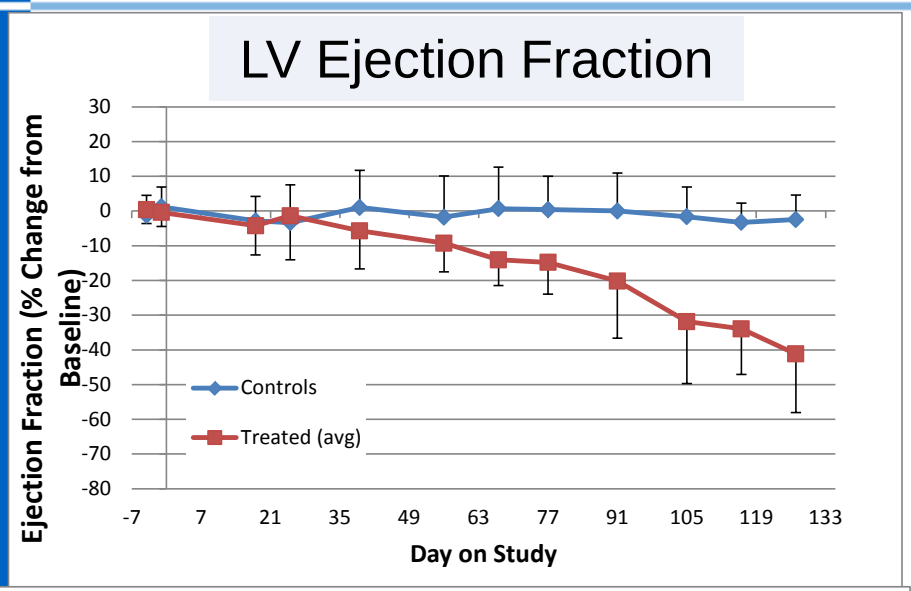
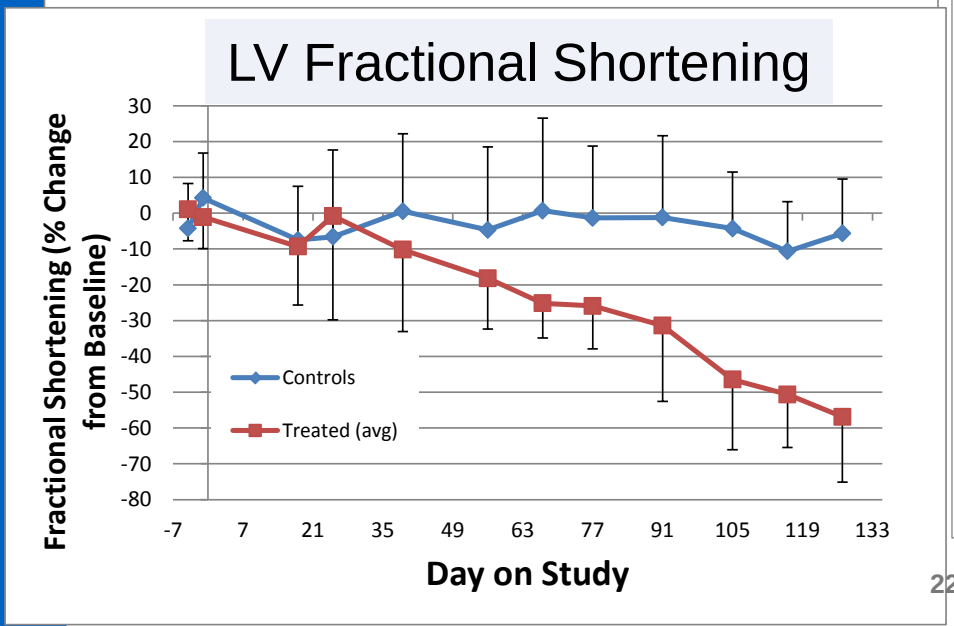
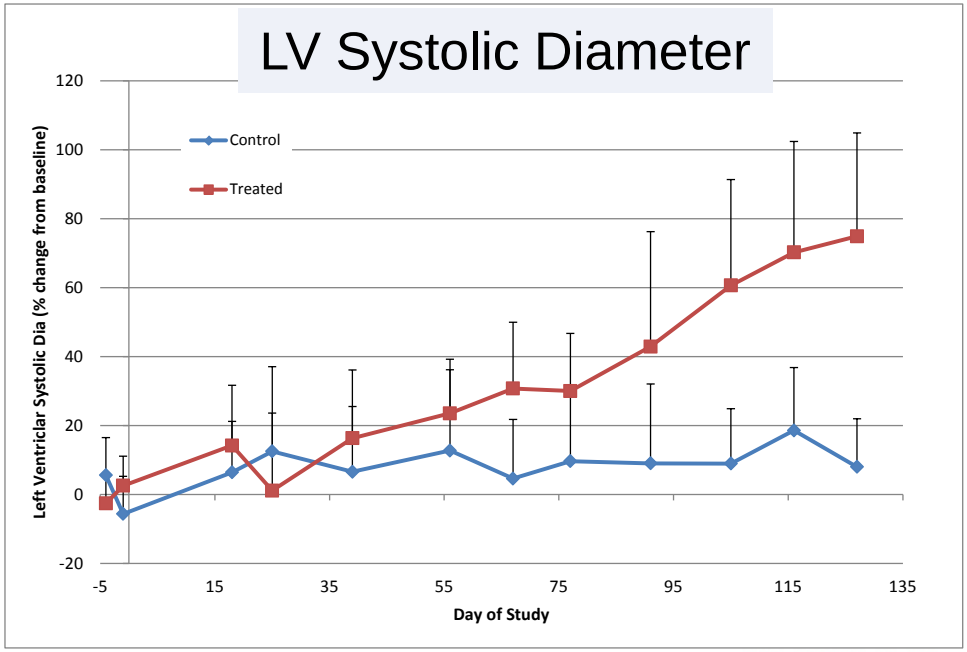


Sonographer: Dr. Meg Sleeper
Echo collection under Ketamine anesthesia

DOX: 2 or 1 mg/kg (weekly)





Histopathology

- **Minimal to mild degeneration of cardiomyocytes:**
  - **DOX: 7 of 12 animals**
  - **VEH: 0 of 12 animals**
- **Reduced immunoreactivity for cTnI and SOD2 in cardiomyocytes undergoing degeneration.**
- **Isolated instances of mild or moderate degeneration or necrosis in liver and mild or minimal fibrosis in the kidney**
  - **considered secondary to hemodynamic changes**

DOX CV TOX: Human References

A. J.S. Steinberg et al. Cancer 60:1213-1218 (1987)
   - Single IV dosing (34 ± 12 mg/m^2; 10 min) was associated with increased ventricular premature beats, ventricular couplets and supra-ventricular tachycardia over a 24 hr period (ambulatory ECG monitoring).

B. M.E. Caram et al. Breast Cancer Res Treat 152: 163-72 (2015)
   - 11.5% (19/166) of dox-treated patients (240-359 mg/m^2) had LVEF <55%

C. S.M. Swain et al. Cancer 97:2869-79 (2003)
   - 26% of dox-treated patients experienced congestive heart failure (CHF) at 500 mg/m^2
   - Age (>65 yrs) was a risk factor for dox-induced CHF.
   - >50% of dox-treated patients that developed CHF had LVEF <30% on study

D. T. Nousiainen et al. J. Internal Med. 245:359-363 (1999)
   - QTc increased at 500 mg/m^2; non-significant QTc prolongation at 200 and 400 mg/m^2

CV Testing Strategy for Oncology Drug:
Amgen Examples

- **Small Molecules: exploratory evaluation during lead optimization**
  - hERG blockade:
    - assess SAR and off-target potency
  - Isolated rabbit heart:
    - assess direct effects on electrophysiology & contractility
  - Rat Telemetry: profile drug for BP/HR & contractility effects
    - Single ascending doses or repeat (e.g., 4-day)
  - QTc/ECG evaluation integrated into repeat dose toxicology (non-rodent)
    - 14 day (exploratory; non-GLP) and/or IND-enabling studies (GLP)
  - Other approaches: case-by-case
    - target-based liability
    - non-rodent telemetry
    - echocardiography

- **Biologicals: case-by-case approach**
  - CV risk: low for direct hERG-mediated effects and off-target CV liabilities
    - QTc/ECG evaluation integrated into repeat dose toxicology (non-rodent)
  - LV function assessment: echocardiography
    - Assess: case by case based on target-based biology

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