PFIZER GLOBAL RESEARCH & DEVELOPMENT

The challenges of pre-clinical models and endpoints in clinical translation in endometriosis

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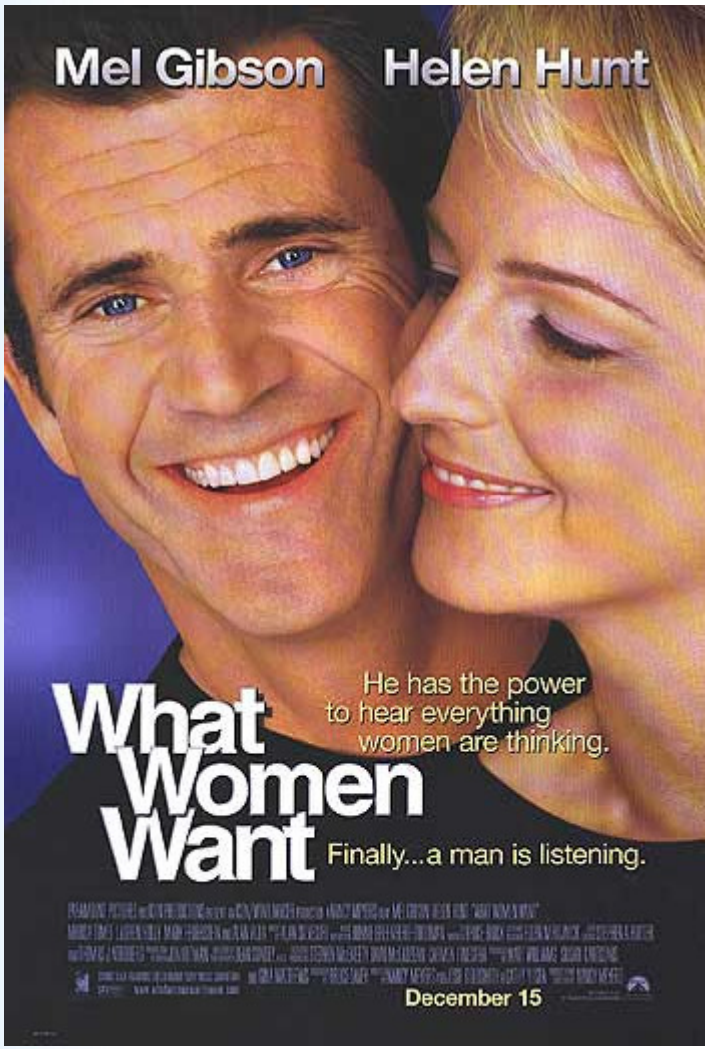
The clinical challenge



- **Disease presentation**
  - Diversity of 'phenotype'
  - No validated non-invasive diagnostic test
- **What does efficacy look like: Unmet medical need?**
  - Patient perspective
    - Symptom management: which symptoms are the most bothersome (menstrual/non-menstrual)?
    - Improved Quality of Life
    - Fertility outcome
  - Physician-Payor: improved disease management
    - Reduced frequency of surgical intervention
    - Decreased loss of work days
    - Decreased healthcare costs
- **How do you measure efficacy?**

rAFS as a direct measure of efficacy: 'disease burden'

- **Invasive measure**
- **Poor correlation between rAFS and endometriosis symptoms (Vercellini *et al.* 2007)**
- **High inter and intra-observer variability (Hornstein *et al.* 1993; Buchweitz *et al.* 2005)**
- **Ethical justification of repeat measure**
  - Consideration with respect to placebo controlled studies
- **Assumes**
  - Healthcare costs are proportional to rAFS
  - A reduction in rAFS is a desirable clinical outcome for Patient and Payor

Benefits of surgical intervention

- **Surgery reduces rAFS disease burden pain**
  - Significant placebo effect of laparoscopy
- **Relationship to improved fertility outcome not clear**
- **Mean return of symptoms ~6m**

TABLE 1

Operative findings at surgeries 1 and 2.

	Surgery 1			Surgery 2		
	DSG (n=19)	ISG (n=20)	DSG vs. ISG *P*	DSG (n=18)	ISG (n=16)	DSG vs. ISG *P*
No disease	—	—	—	0	9 (56)	$\chi^2(1) = 13.77$, $P<.0001$
rAFS Stage I, n (%)	0	1 (5)	1	2 (11)	3 (18)	1
rAFS Stage II, n (%)	8 (42)	9 (45)	0.85	4 (22)	1 (8)	.34
rAFS Stage III, n (%)	2 (10)	2 (10)	1	2 (11)	0	.49
rAFS Stage IV, n (%)	9 (48)	8 (40)	0.75	10 (66)	3 (18)	$\chi^2(1) = 4.85$, $P=.02$
Median rAFS score (range)	27 (6–142)	16 (3–142)	0.84	46 (3–142)	0 (0–142)	.0001

Note: rAFS = revised American Fertility Society; Stage I = minimal disease; Stage II = mild disease; Stage III = moderate disease; Stage IV = severe disease.

TABLE 2

Change in overall level of pain reported after surgery.

	DSG	ISG	DSG vs. ISG
Surgery 1	n = 19	n = 20	
Any improvement in pain, n (%)	6 (32)	16 (80)	
No change/worse pain, n (%)	13 (68)	4 (20)	$\chi^2 = 9.3$, $P=.002$
VAS[a] change in pain, score (range)	0 (0–100)	30 (0–95)	$Z = -2.5$, $P=.012$
Surgery 2[b]	n = 18	n = 15	
Any improvement in pain, n (%)	15 (83)	8 (53)	
No change/worse pain, n (%)	3 (17)	7 (47)	$\chi^2 = 3.88$, $P=.13$
VAS change in pain, score (range)	82.5 (0–100)	50 (0–100)	$Z = -1.22$, $P=.26$

Note: Patients were asked to report on their pain relief 6 mo after surgery. For surgery 1, this was immediately before surgery 2 and for surgery 2 this was 12 mo from surgery 1.

[a] Visual analogue scale, where 0 = no change in pain and 100 = complete relief of pain.

[b] Patients were asked to report on the improvement in overall level of pain after surgery 2, not compared with baseline.

Abbott. Excision of endometriosis for pain: RCT. Fertil Steril 2004.

Correlation with medical therapy ?

- **Majority of approved therapeutic treatments for endometriosis suppress ovarian function – [Mel, is this what women want?]**
- **Lead to improvements in rAFS and endometriosis disease symptoms**
  - Poorly tolerated by patients
  - No clear benefit on fertility outcome
  - Poorly effective on non-menstrual pain component
- **Is it possible to eliminate bias from RCTs studying the effects of ovulation suppression on endometriosis disease and symptoms?**

‘Patient Reported Outcome’ measures of efficacy

- **Most widely used symptom measure for assessing outcomes in endometriosis – Endometriosis Symptom Severity Scale (ESSS)**
  - Developed by Biberoglu & Behrman (1980), modified by Miller (2000)
  - Assesses dysmenorrhea, dyspareunia, pelvic pain, pelvic tenderness and induration
  - 4-week retrospective recall → recall bias → daily pain diary
- **Others**
  - Endometriosis Health Profile-30 (Jones *et al.* 2004)
  - VAS Pain
- **Can you mimic ‘disease symptoms’ pre-clinically?**

Simple hypothesis

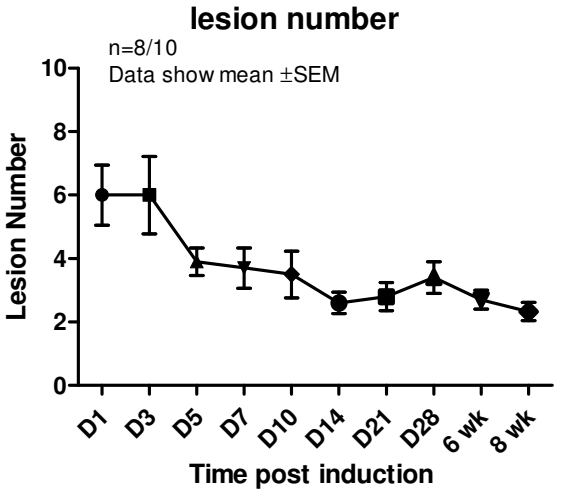
- **If reduction in disease burden [by surgery or medical therapy] leads to improvements in disease symptoms then Pharma should focus on mechanistic approaches which reduce disease burden**

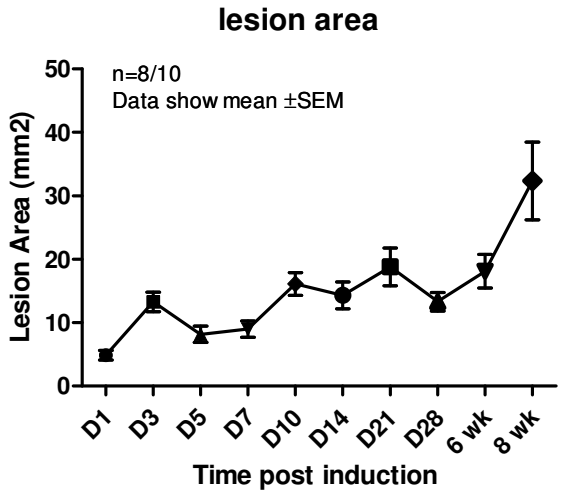
The Pharma challenge

- **To get to a clinical proof-of concept with a novel investigational new agent costs *ca* $50m**
- **The vast majority of approaches tested are either**
  - Poorly tolerated – Safety is the Issue
  - Poor PK/absorption – the Drug is the Issue
  - Don't work and we don't know why – Mechanism is the Issue
- **Validation of mechanistic or efficacy biomarkers is key to development of new medicines**
  - Needs partnership

Pre-clinical endometriosis models: Rodent

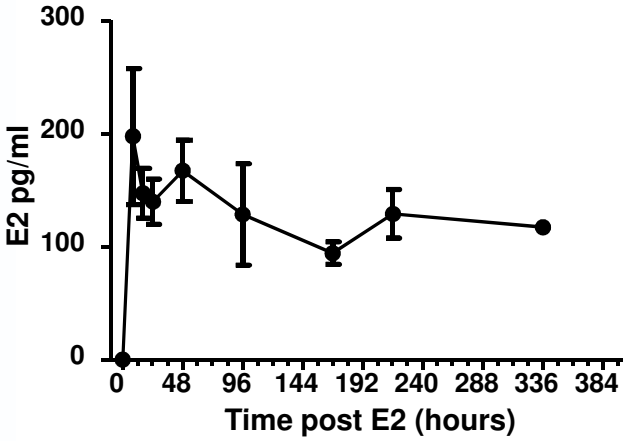
- **Isogenic mouse/Syngeneic rat**
  - Surgical: placement known
  - *i.p.* injection: placement unknown
  - Durable engraftment
  - Disease burden measured by microcalipers at end of study
- **Nude mouse- human xenograft**
  - Impaired immune function/lack T cells
  - Potentially allows for understanding of recipient-host interactions
- **Others**
  - *K-ras* mouse (Dinulescu *et al.* 2005)
  - *NOD/SCID/*γc^{null} xenograft (Masuda *et al.* 2007)
- **Lesions have similar histological appearance to human**
  - Markers of neovascularisation and neurogenesis
- **Non-menstruating species**
  - 4d cycle

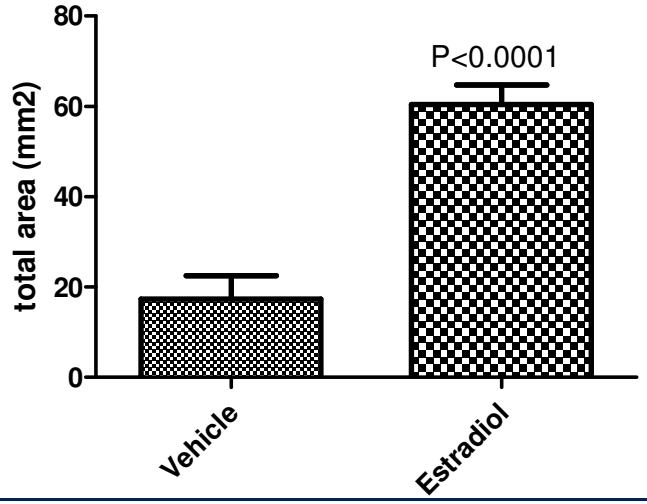




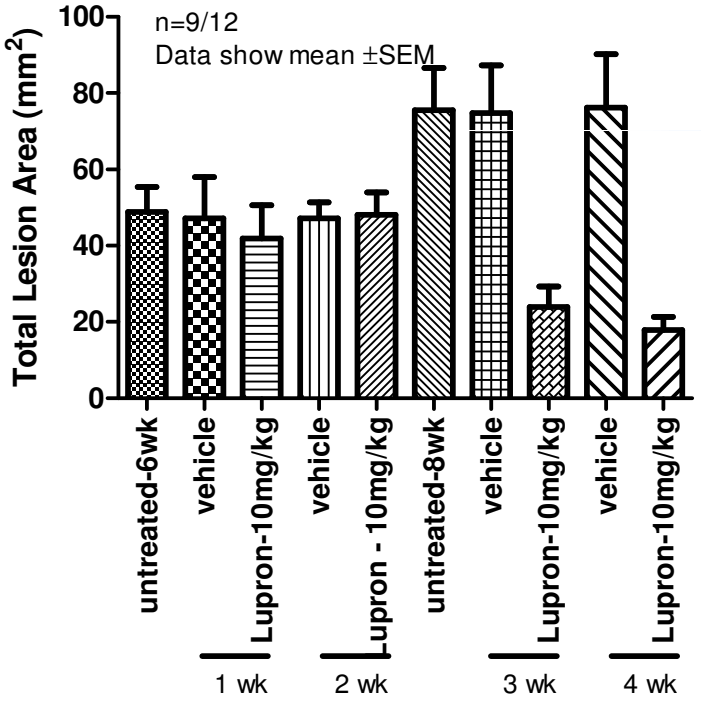
What constitutes model validation?

Sensitivity to estradiol and ovarian suppression





Lupron effect in regression: lesions allowed to establish for 5 weeks and then given weekly s.c injections of lupron out to 9 weeks

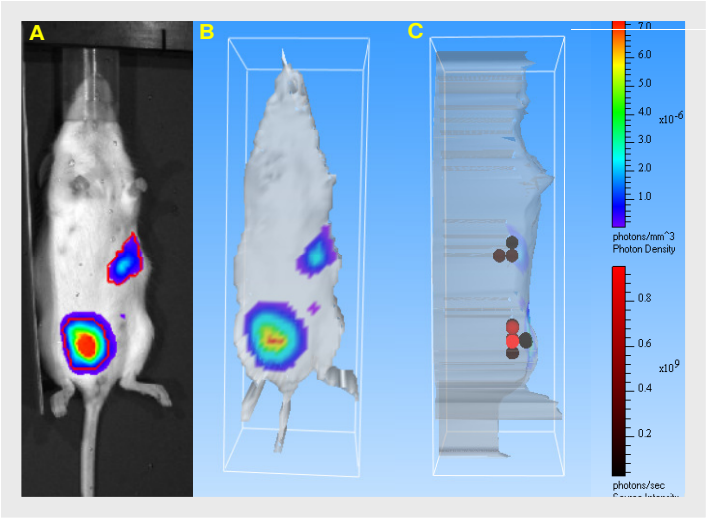


Assay sensitivity

- **If Lupron constitutes validation then ~60% reduction in disease burden in the isogenic mouse model is observed by ovarian suppression**
  - Blunt measure – not rAFS nor symptomatic
  - Large *n* to see effects on disease burden less than lupron
  - What % change would underwrite a clinical commitment?
- **Perhaps a longer time of treatment is needed to improve confidence in outcome.....**
- **Can we increase the sensitivity?**

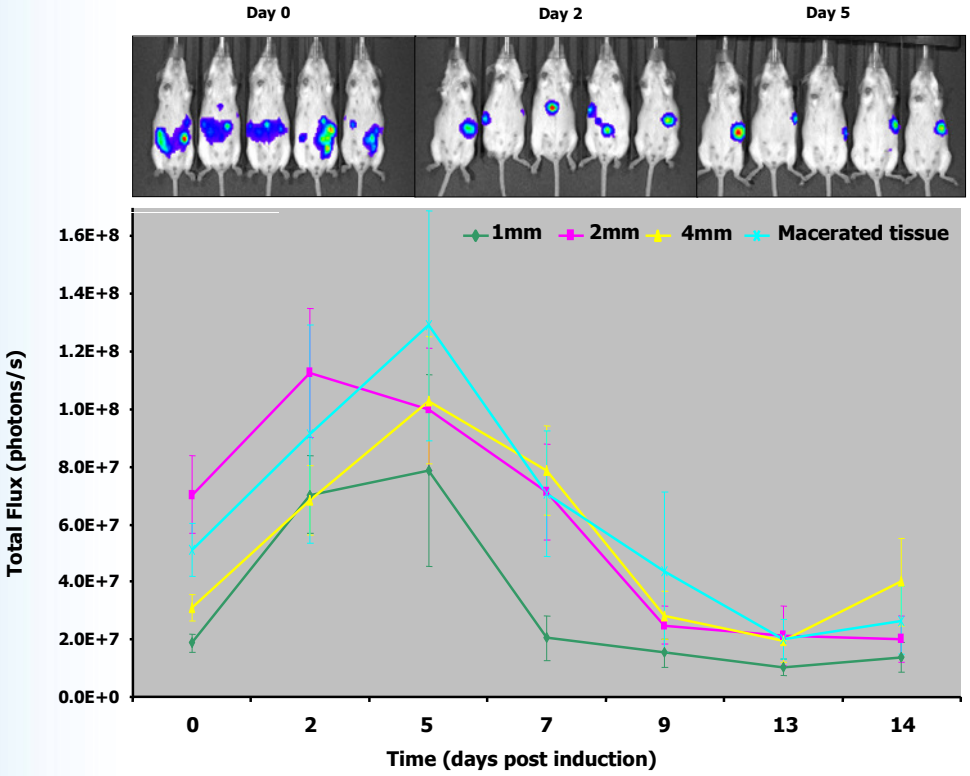
Increasing sensitivity and introducing temporal dynamics: IVIS Biophotonics

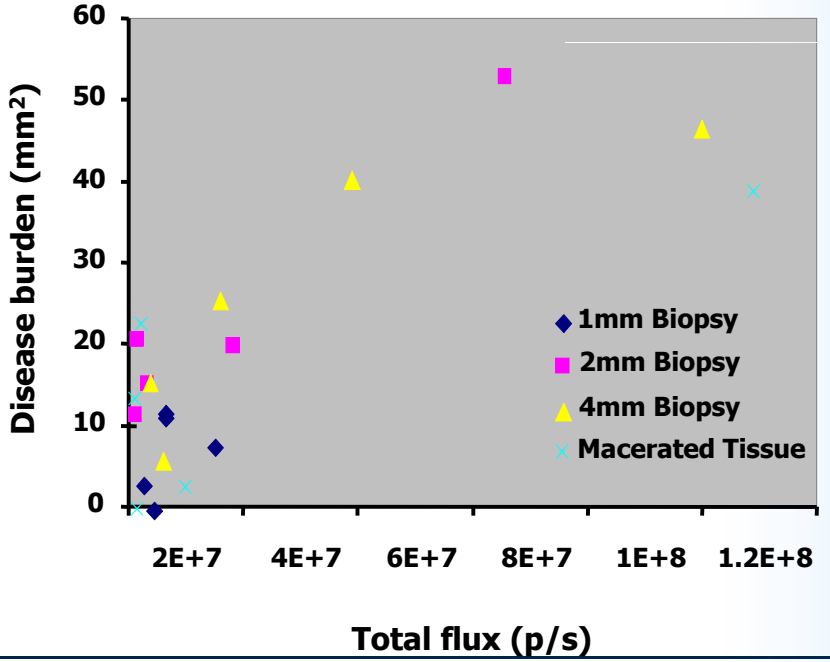
- **β-actin luciferase Tg mice replaces WT FVB donor**
  - Detection by biophotonics – measures of 'flux units' from transgene
- **Longitudinal studies possible with recovery anaesthesia**



Longitudinal profile

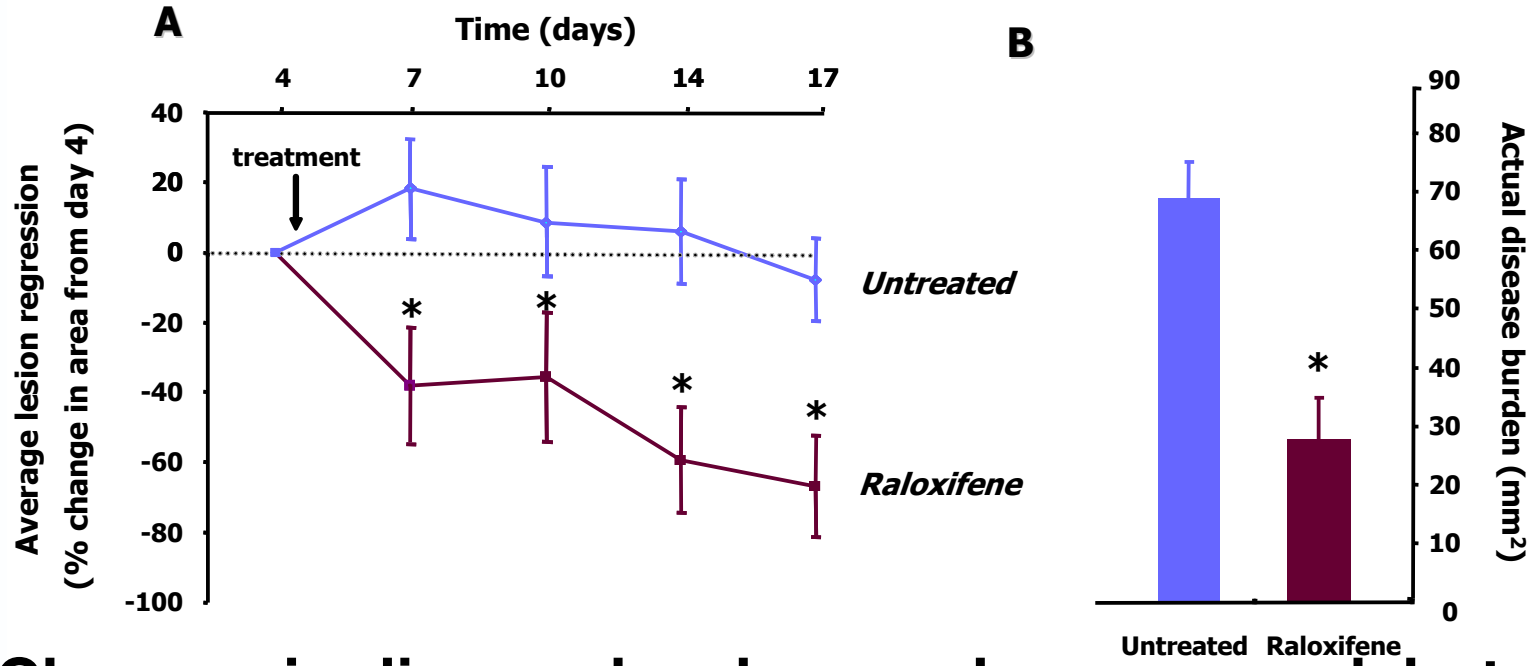
- **Initial ‘flare’ in response**
- **Reasonable correlation between lesion size and flux at early timepoints**





Effect of Raloxifene (0.4 mg/kg s.c) in Biophotonics mouse

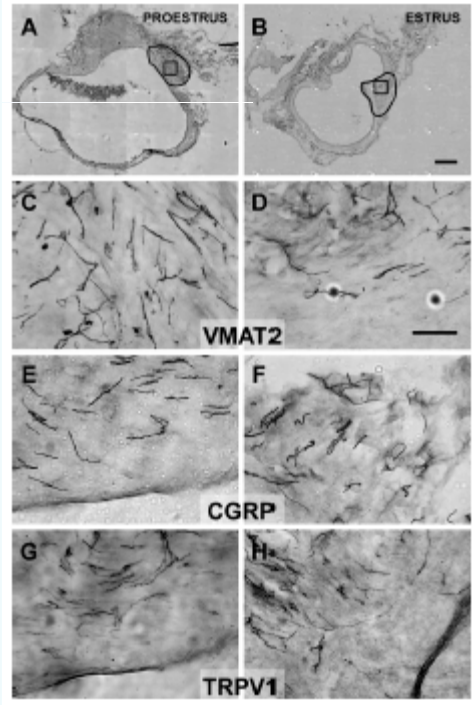
- **Significant reduction in animal use needed to generate regression data, compared with endpoint alone generated data**



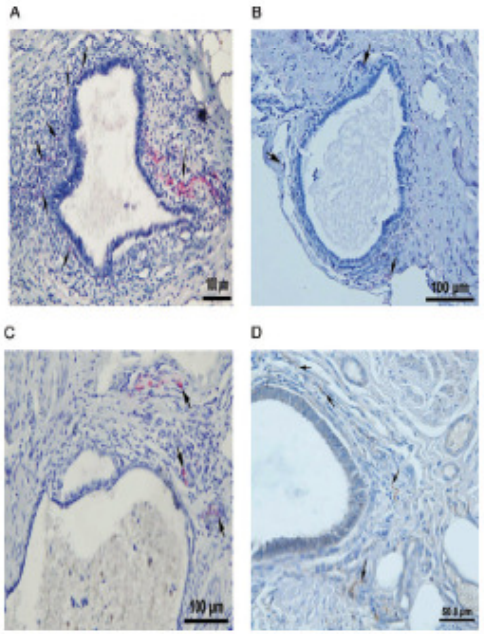
- **Changes in disease burden can be measured, but does this translate to symptomatic improvement?**

What about disease associated pain?

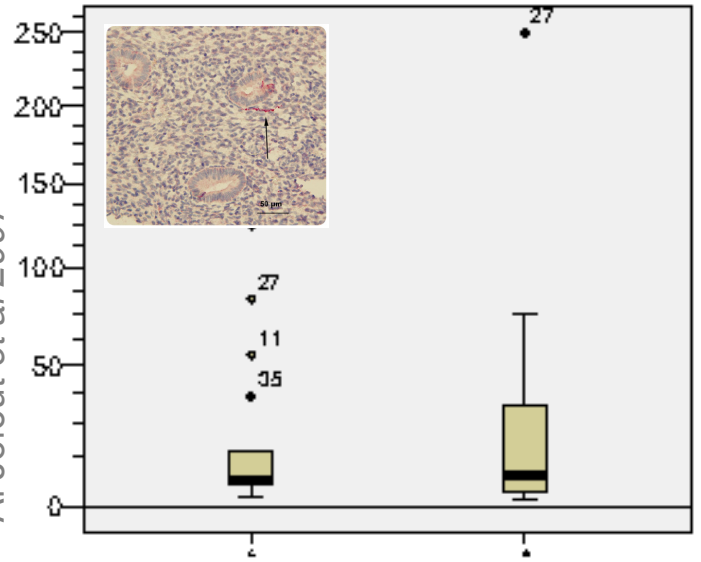
- **Ectopic endometriotic lesions in syngeneic rat appear to be innervated with sensory afferents (sympathetic, C & Aδ fibre)**
  - Elevation in CGRP, NGF
  - Neurogenesis appears to accompany neovascularisation process
- **Relevance to Patients: Sensory fibres have been observed in ectopic lesions as well as eutopic endometrium**



Zhang *et al* 2007



Tokushige *et al* 2006

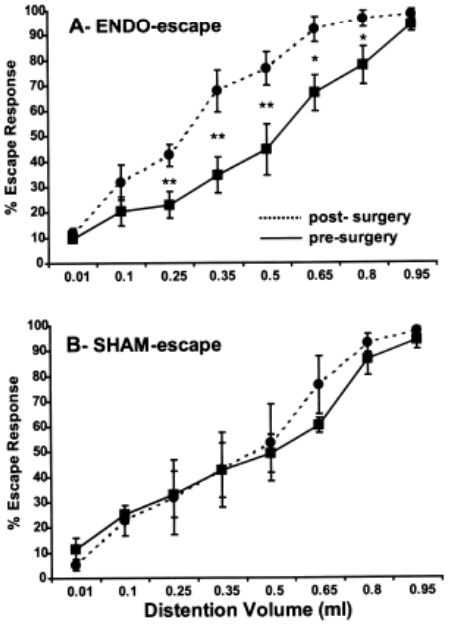


Al Jefout *et al* 2007

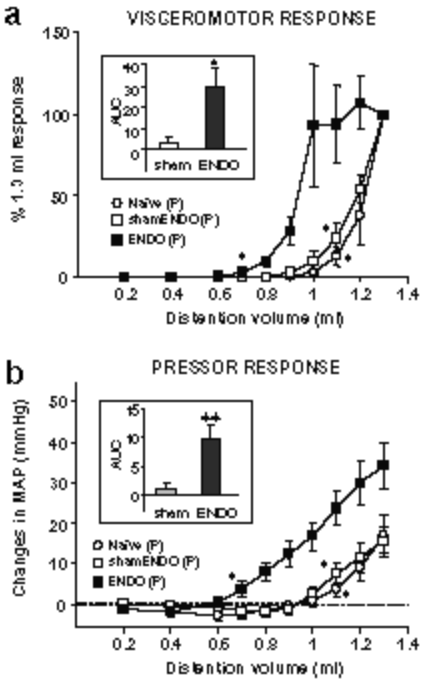
Endometriosis Surrogate Pain models: Rat

- **Enhanced escape & pressor responses to vaginal distension**
  - maximal responses @ E2 peak
  - Vaginal hyperalgesia as a surrogate for dyspareunia
- **Limitations**
  - Formal validation has not been published
  - Potential for observer bias

Berkley *et al* 2001

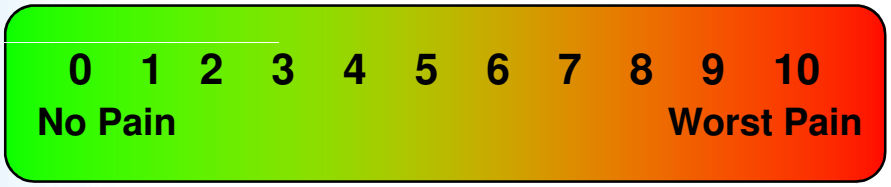


Nagabukuro & Berkley 2007



A Cautionary Tale: Importance of observer bias

Humans can report the level of pain that they are feeling



In the rat an observer measures pain by evoking paw withdrawal to an applied stimulus

Static allodynia

Dynamic allodynia

Potential for bias unless the observer is blinded

Eliminating observer bias

Trt.\Rat	1	2	3	4	5	6
1	B	B	B	B	B	B
2	E	E	E	E	E	E
3	D	D	D	D	D	D
4	A	A	A	A	A	A
5	F	F	F	F	F	F
6	C	C	C	C	C	C

single-blind

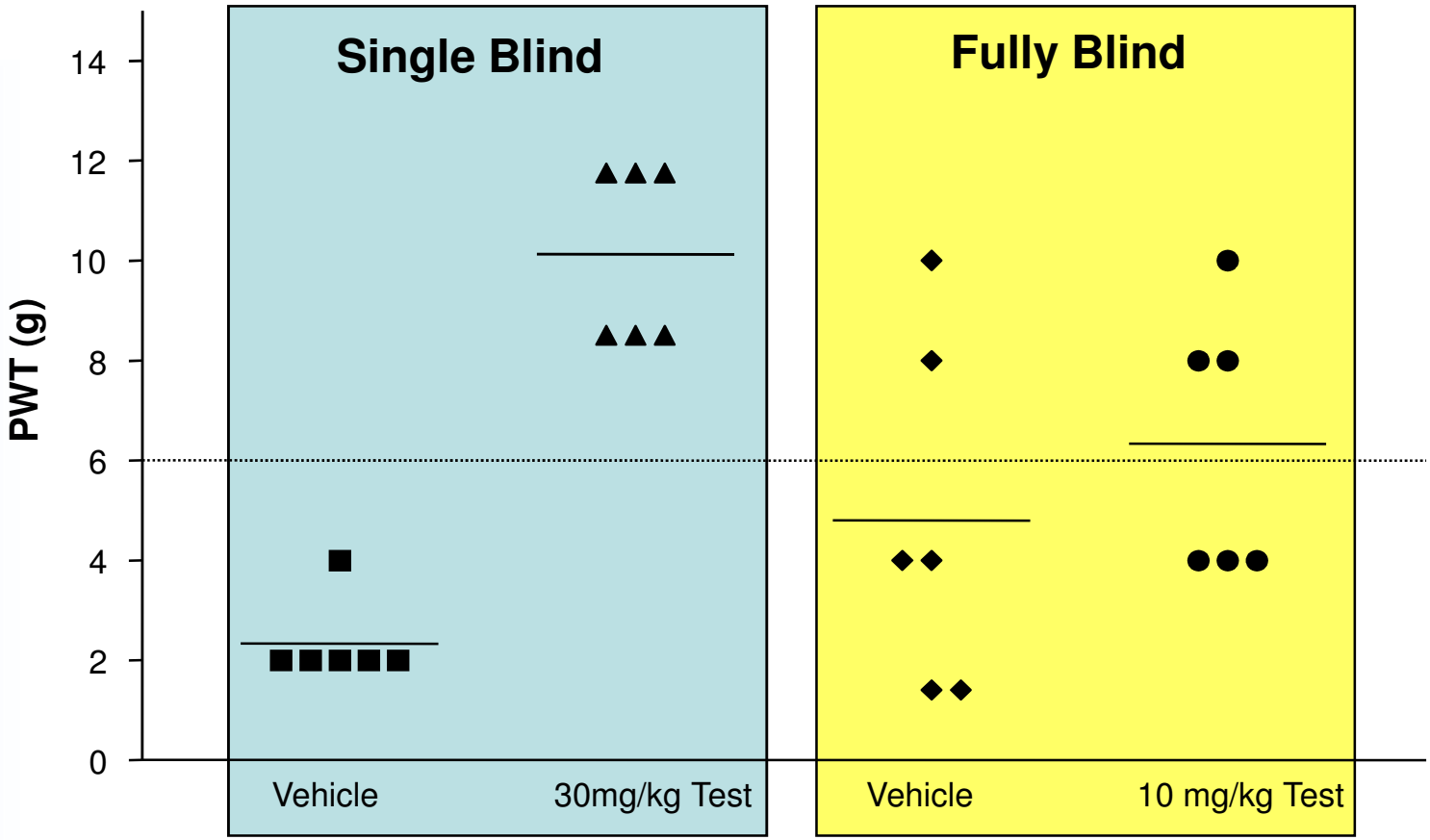
Single blind: Rats not randomised within groups. Observer dosed their own rats but were blinded to treatment.

Fully blinded to treatment and group. Treatments randomised within groups and dosed by separate colleague.

Trt.\Rat	1	2	3	4	5	6
1	B	D	A	E	C	F
2	E	B	A	F	D	C
3	D	F	B	C	E	A
4	A	E	C	A	F	B
5	F	A	E	B	C	D
6	C	B	F	D	A	E

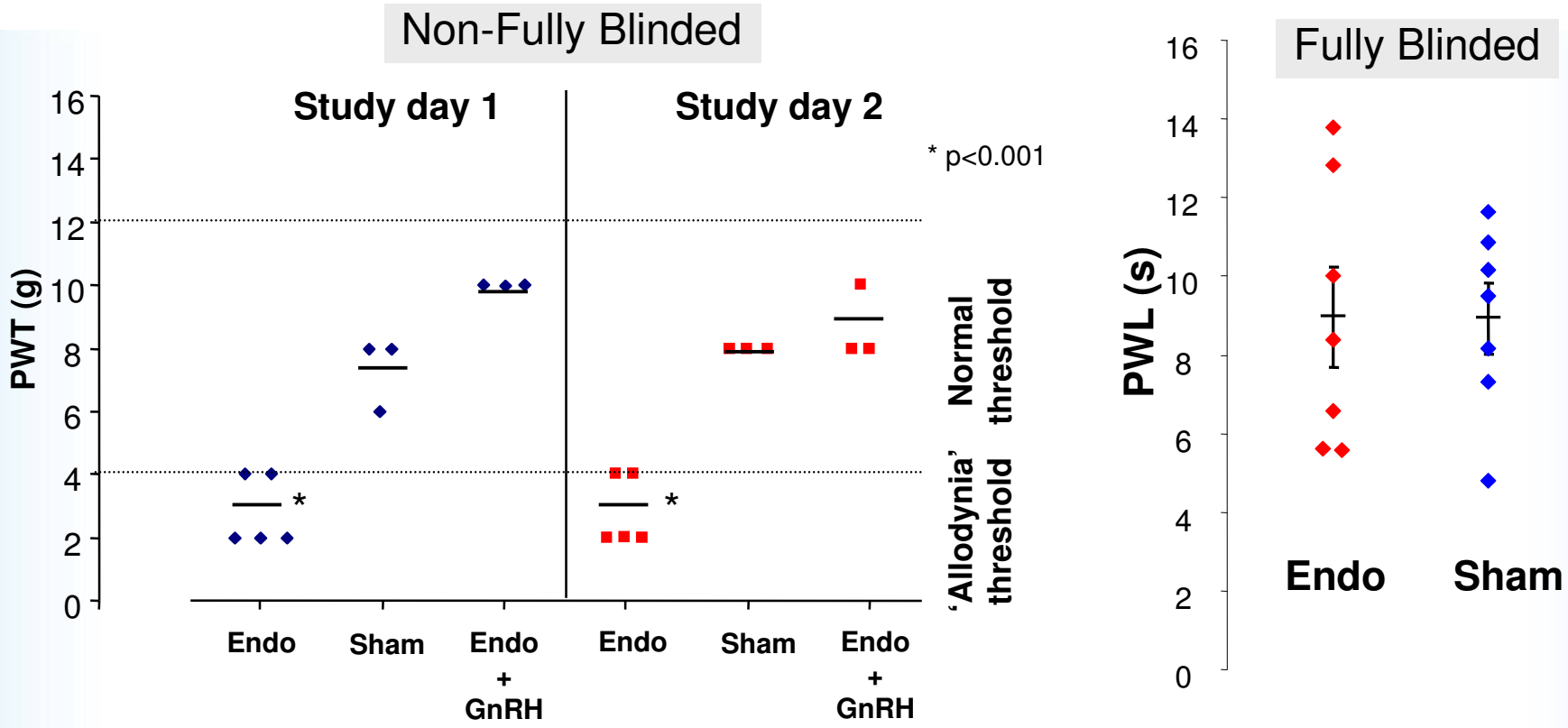
fully-blind

Effect of observer bias in blinding on pre-clinical data



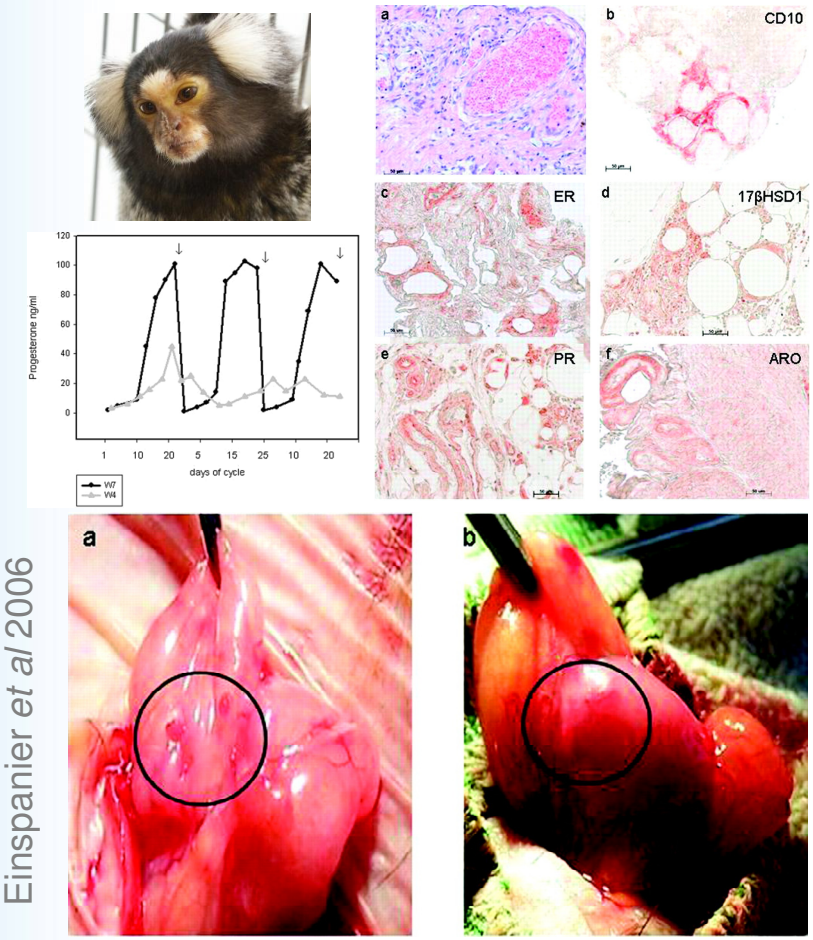
- **Rat Chronic Constriction Injury model of Neuropathic Pain**
  - Behavioural effects of 30 mg/kg treatment group → 10 mg/kg dose
- **Fully blinding reveals the true variability of vehicle effect**
- **Impact of blinding = Larger sample sizes to realise statistical significance**

Static allodynia : rat endometriosis



- **Loss of static allodynia signal between sham and endometriosis rats when observer is blinded to group**

Would non-human primate be better?

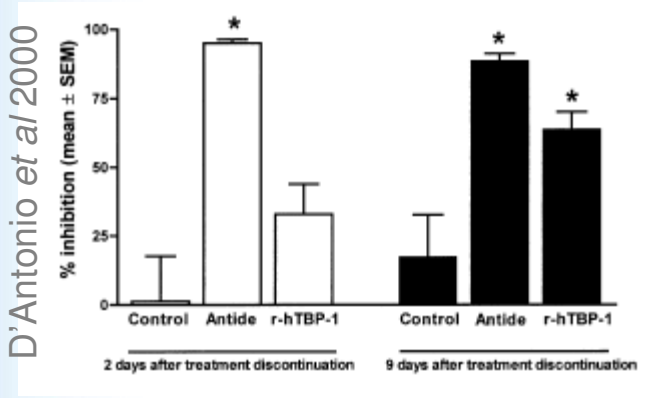


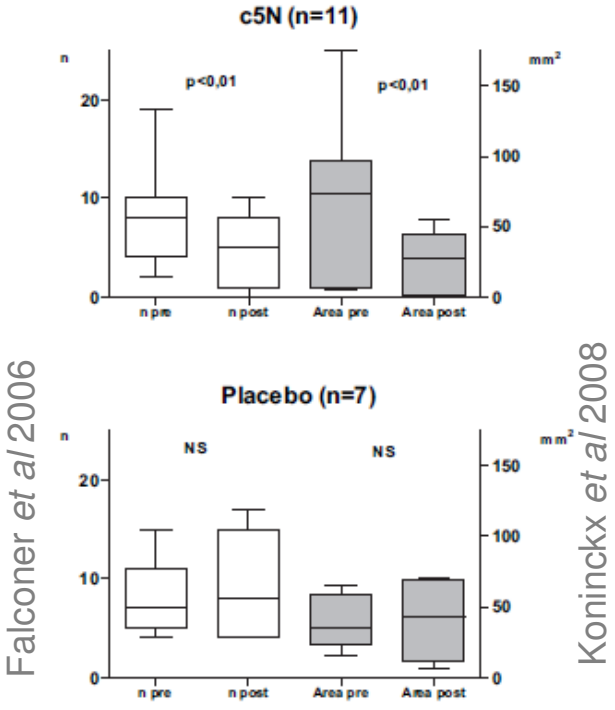
Einspanier *et al* 2006

- **Surgical models established in marmoset, macaque and baboon**
  - 'Sampson' methodology of retrograde uterine washings or by surgical engraftment
  - Ovarian E2/P4 cycle faithful to disease
- **Limitations**
  - High study cost
  - Specialism
  - Similar uncertainty on endpoints needed to underwrite outcome
  - Validated subjective pain endpoints?

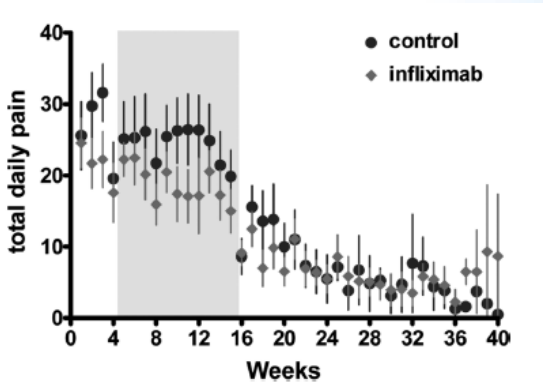
Do we have translational models? Not yet

- **Levels of TNFα elevated in patients with endometriosis**
  - Reduction in disease burden observed in multiple pre-clinical disease models
- **No apparent translation clinically**
  - Small study (n=7 placebo, n=13 infliximab), powered to detect a B&B decrease of 3 points with 90% confidence





No apparent change in TVU from baseline



Rat: Disease burden

Baboon: Disease Burden

Patient: Daily Pain

Summary

- **No pre-clinical model of endometriosis disease exists which predicts clinical outcome**
  - Clinical outcome measures are blunt markers – more sophisticated pre-clinical endpoints are also needed
- **Blinding and randomisation of behavioural studies suggests that observer bias can mask inherent variability and result in 'false positive' findings.**
  - We need to apply the same rigor of clinical trial randomisation and blinding to our pre-clinical studies
  - Accepted consensus across the industry that all studies should be fully blinded