



February 2021

ELLEGAARD WEBINAR SESSION

ANTICANCER DRUG DEVELOPMENT
COMPARISON OF TOXICITY IN MINIPIG AND
MOUSE

ELLEGAARD • •
GÖTTINGEN MINIPIGS

Drug Development

Crop Protection

Chemical Safety

great people, great work, real results

Increasingly Cancer Touches



All of Our Lives



Increased Survival Driven by Animal Research



Cancer Survival has Doubled in the Last 40 Years



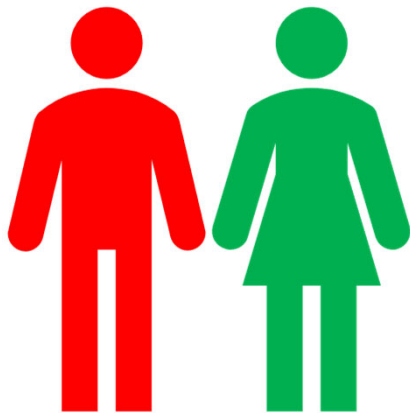
Animal Research Critical to this Progress



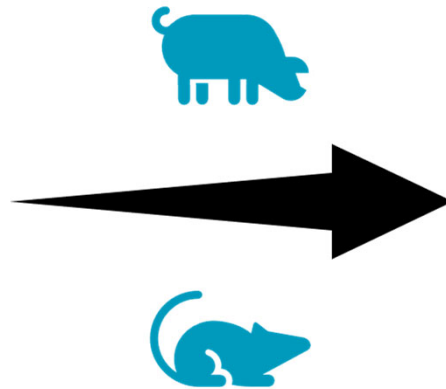
Continued Animal Work Vital to Save More Lives



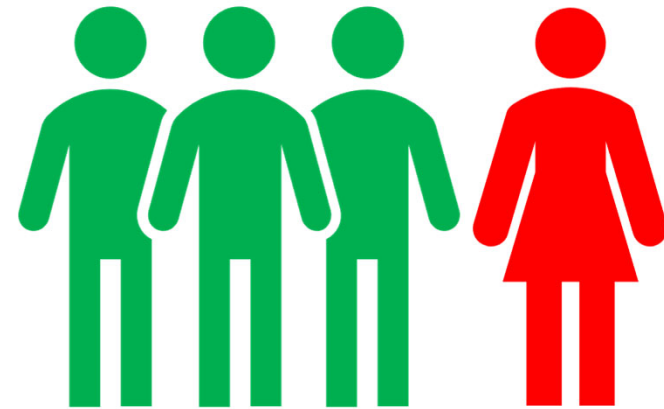
Forecast



Cancer will affect
1 in 2

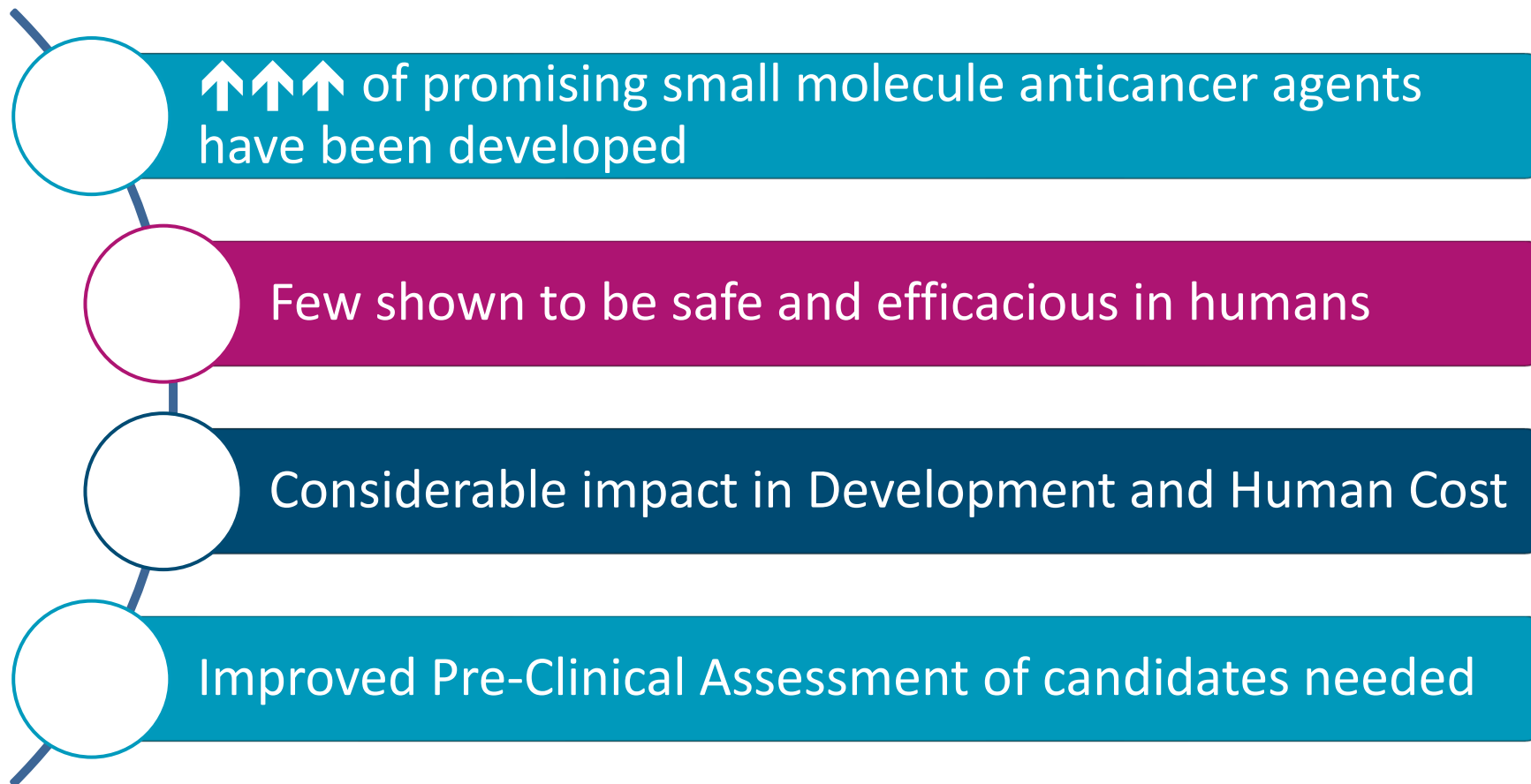


Aspiration

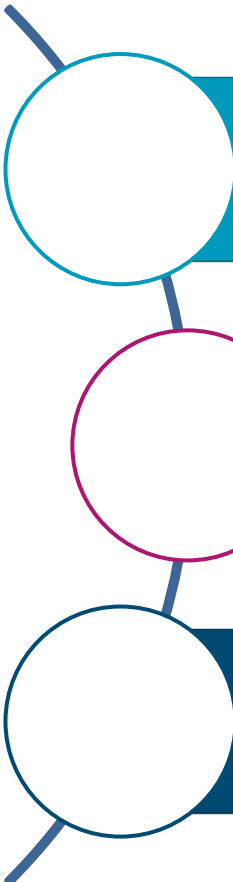


Aspiration
> 75% survival

Anticancer Drug Development



Clinical ethics drives minimising pre-clinical toxicology



Early stage clinical trials in cancer patients are often initiated with limited toxicology data

A clinical trial at a dose < efficacious is undesirable

A clinical trial producing unexpected severe toxicity is even worse



Most Commonly
Used Model

Historically the Only
Pre-Clinical Species

Similar to
Human
Genome



Variety of
Genetic
Models

Extensive
Background Data



Mouse



Not always reliable – drugs work well at preclinical stage but ineffective in clinical trials – e.g. 9-aminocamptothecin

Mouse bone marrow potentially less sensitive than human

Fundamental challenge for clinical cancer drug development



Other Species



NHP

- Likely similar bone marrow sensitivity to man
- Expensive
- Ethical concerns
- Disease status (immunosuppression)



Dog

- Possibly similar bone marrow sensitivity to man
- Prone to emesis
- Ethical concerns (charities)



Minipig

- Possibly similar bone marrow sensitivity to man
- Less prone to emesis
- High throughput – cost effective
- Reduced ethical concern

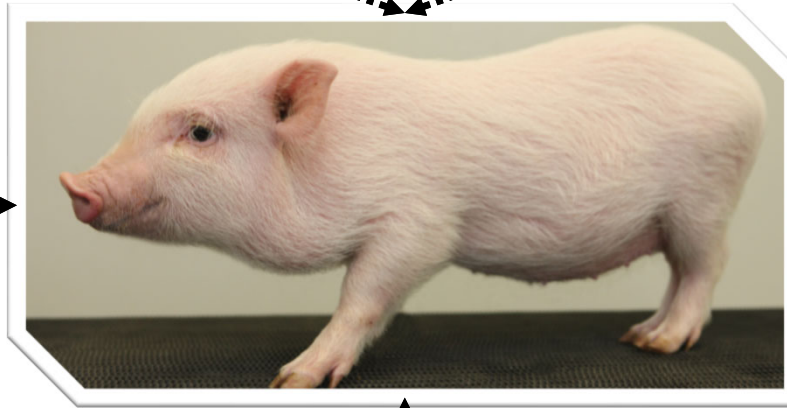


Alternative species

Growing use – well
accepted non-
rodent species

Regulatory pressure
to use two species

Similar to
Human
Genome



Increasing #
Genetic
Models

Extensive
Background Data



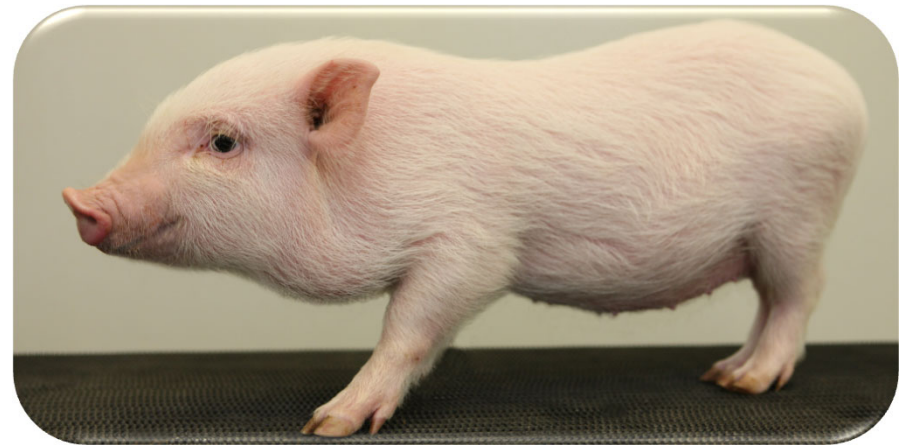


sequani
NON CLINICAL

great people, great work, real results

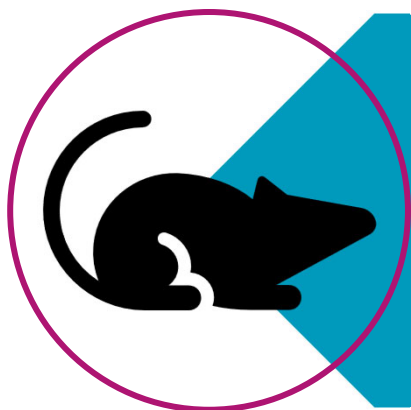
STUDY DATA COMPARISON

Mouse versus Minipig

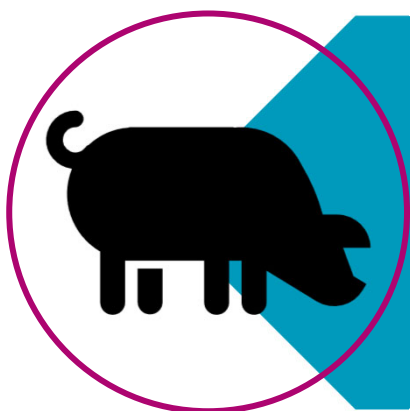


Non-Clinical Studies conducted

Test Item : Novel Oral Anti-cancer drug (non-solid tumours)



Preliminary and 14 Day Study
in the Mouse



- MTD and Range Finder in the Minipig
- 28 Day Minipig with a 28 Day Treatment-Free Period



Study Design

Group	Dose level (mg/kg bid)	Number of animals		Duration of dosing
		Males	Females	
Preliminary phase				
5	150	2	2	7 days
6	225	2	2	up to 7 days
7	100	2	2	7 days
8	125	2	2	7 days
Dose range finding phase				
1	75	12	12	14 days
2	125	12	12	12 days
3	75	3	3	14 days
4	125	3	3	13 days



Study Design - Phase 1

Group	Animal	Dose level (mg/kg bid) on						
		Days						
		1 - 4	5 - 11	12 - 25	26 - 32	33	34 - 37	
1	Male 95	0	6	ND	9	ND	12	Necropsy (Day 34)
	Female 98	0	6	ND	9	ND	12	Necropsy (Day 37)

Study Design – Phase 2

Group	Animal Numbers		Dose (mg/kg bid)
	Males	Females	
2	97	99	6
3	101	100	9





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NON CLINICAL

great people, great work, real results

Minipig – 28 Day Study with 28 Day Treatment- Free Period

Study Design

Group	Number of animals		Animal ID numbers		Dose level (mg/kg bid)	Dose concentration (mg/mL bid)
	Males	Females	Males	Females		
1	5	5	33 - 37	51 - 53, 57, 58	Control	0
2	3	3	38 - 40	46 - 48	3	0.6
3	5	5	41 - 45	49, 50, 54 - 56	6	1.2



Dose Level Comparison

	Mouse	Minipig
Dose Level	mg/kg BID	
Low	75	3
High	125	6

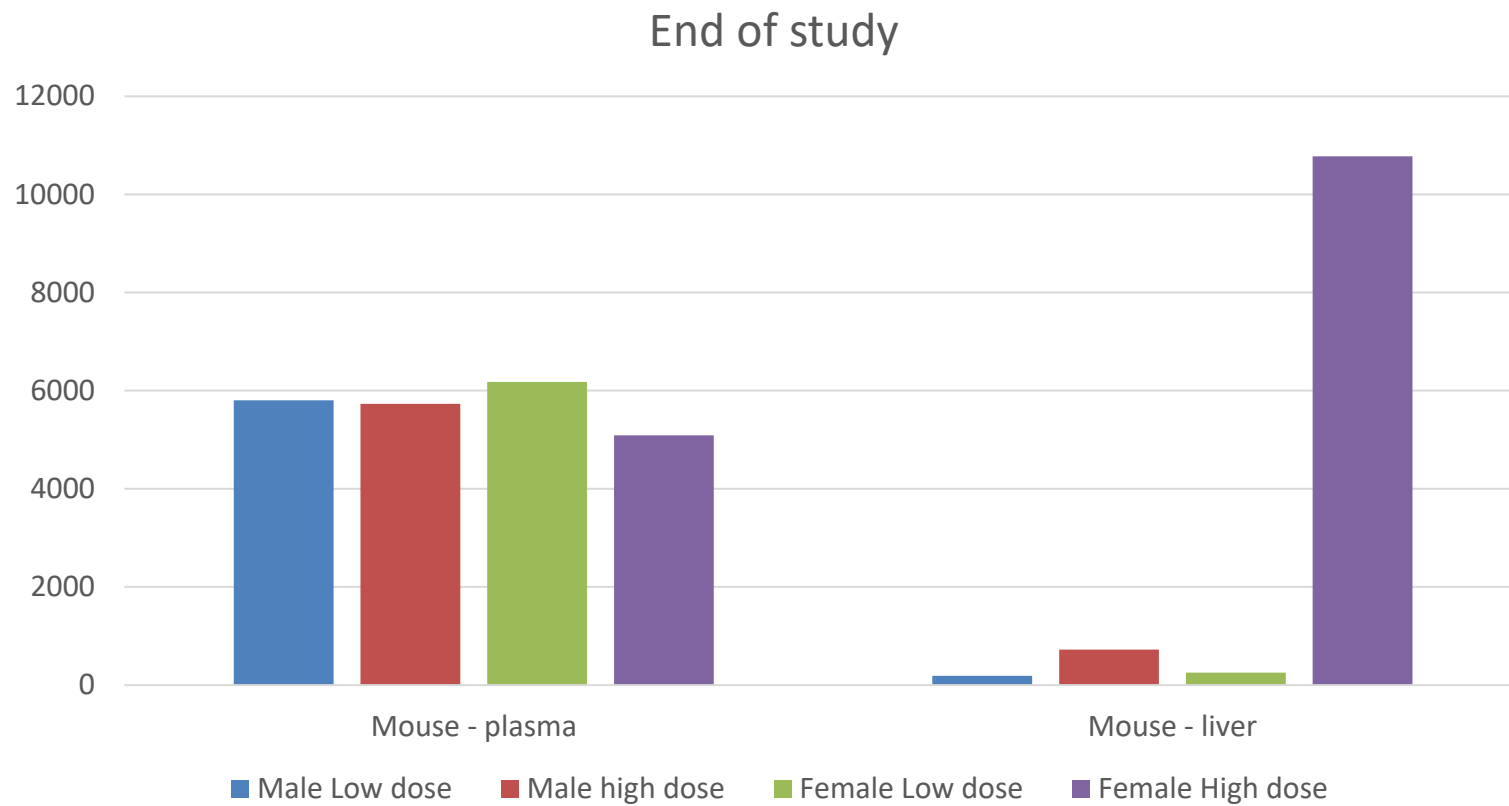
Minipig dose levels more in line with human dose levels

Measured Study Endpoints

	Mouse	Minipig
Clinical Observations	post-dose and daily	post-dose and daily
Body weights	twice weekly, daily	weekly
Food consumption	twice weekly	
Ophthalmoscopy		acclimatisation and end of study
Electrocardiograms		acclimatisation and end of study
Haematology	end of study	acclimatisation and end of study (additional 0.1 mL taken twice weekly)
Blood Chemistry	end of study	acclimatisation and end of study
Urinalysis		at necropsy, by cystocentesis
Proof of Absorption/TK	end of study	Day 1 and Day 28
Organ weights		
Pathology		

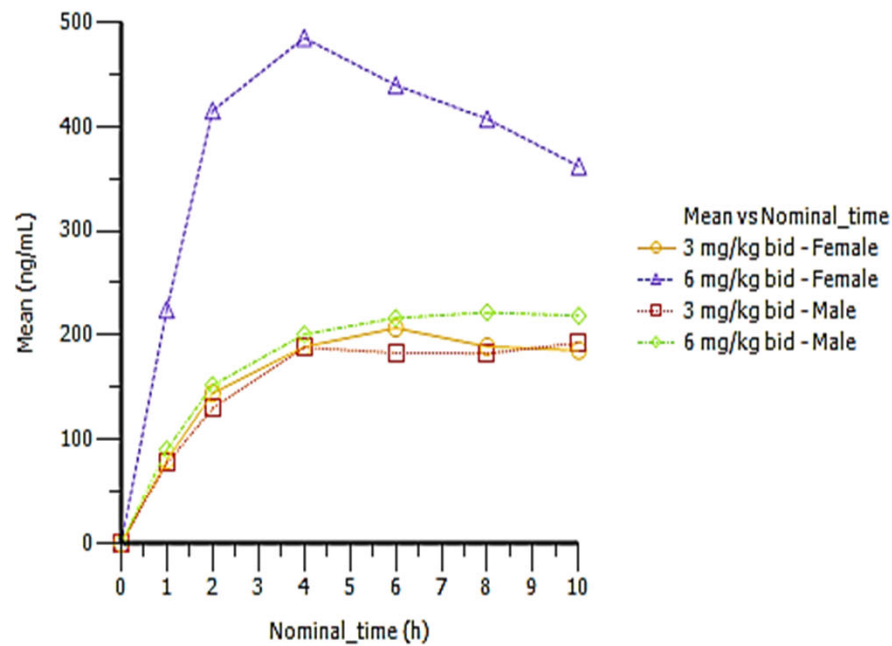


Plasma and Liver concentrations

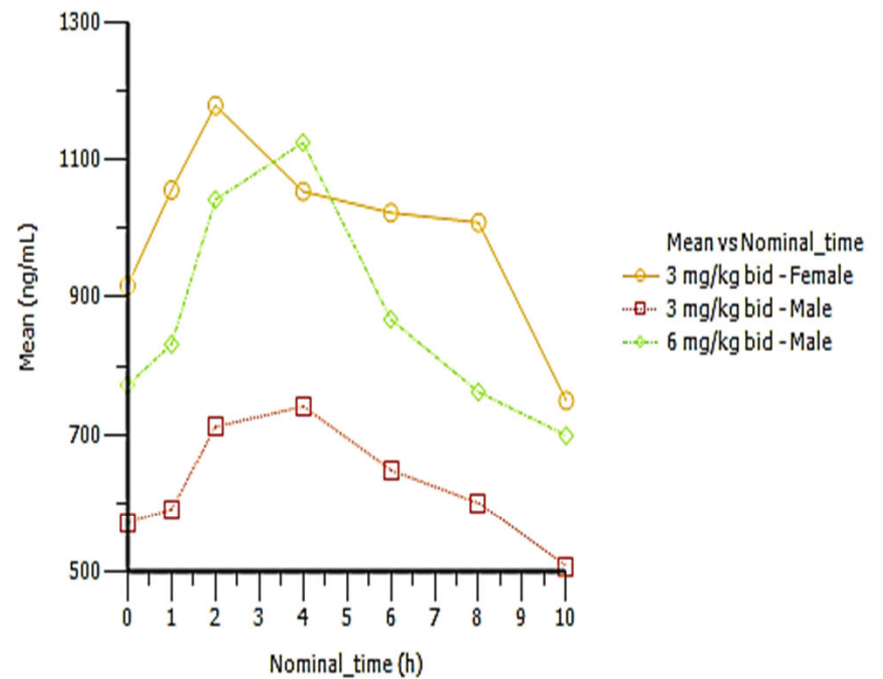


Mean Plasma Profiles

Day 1



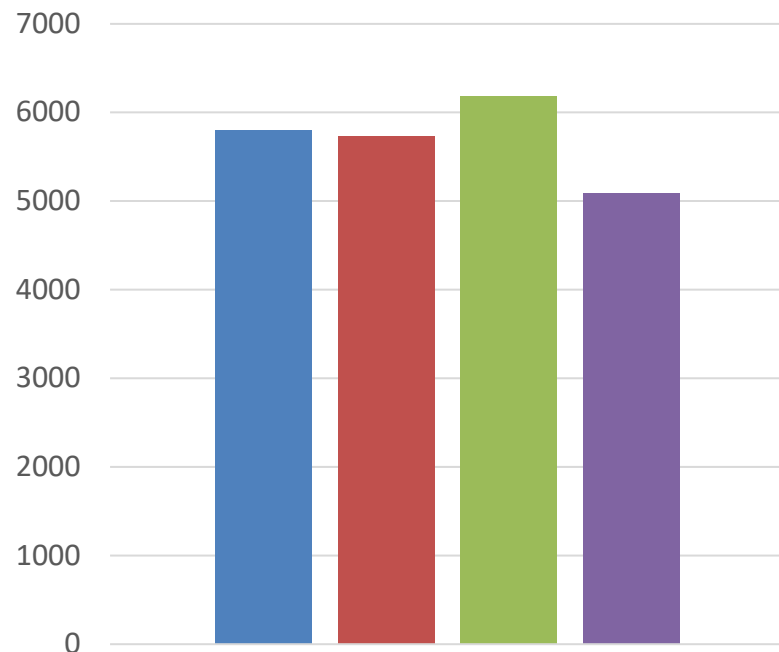
Day 28



Proof of Absorption comparison

Plasma concentrations – end of study (1 hour)

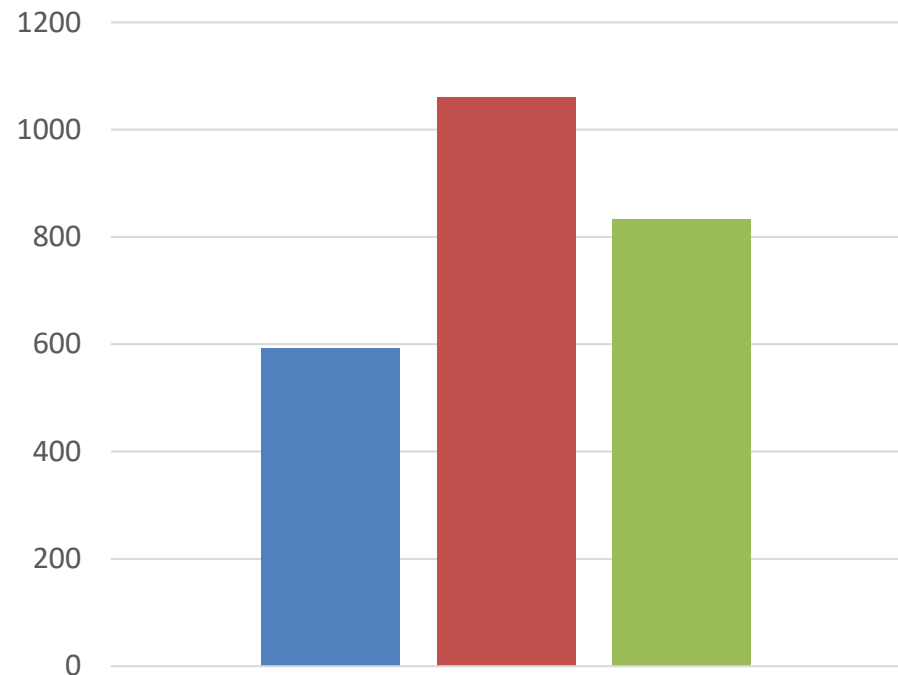
Mouse



Mouse - plasma

■ Male Low dose ■ Male high dose
■ Female Low dose ■ Female High dose

Minipig



Minipig plasma (Day 28)

■ Male Low dose ■ Male high dose
■ Female Low dose





Clinical Observations - Similarities to Human

Mouse

Piloerection

Pale Extremities

Decreased Activity

Hunched Posture

Minipig

Tremors

Vomiting

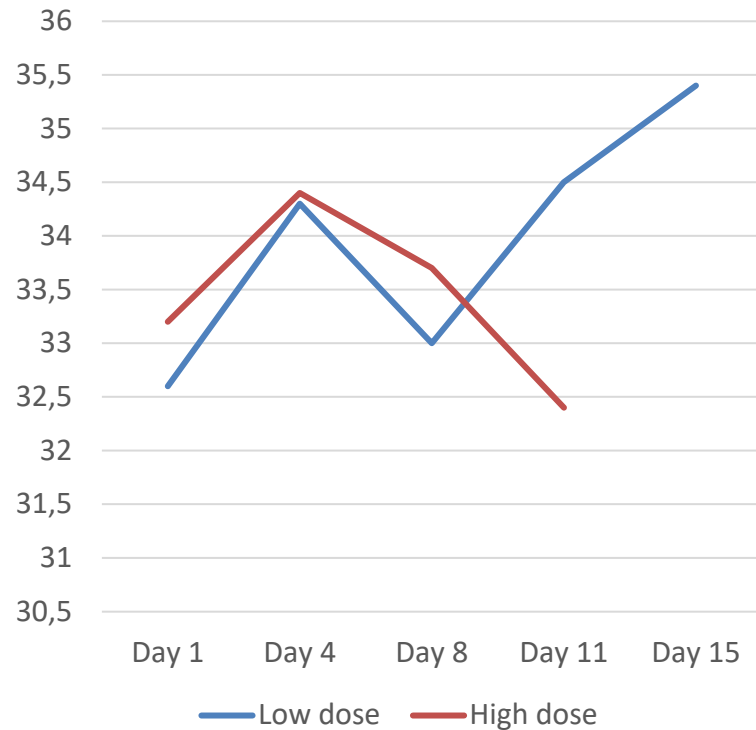
Subdued Behaviour



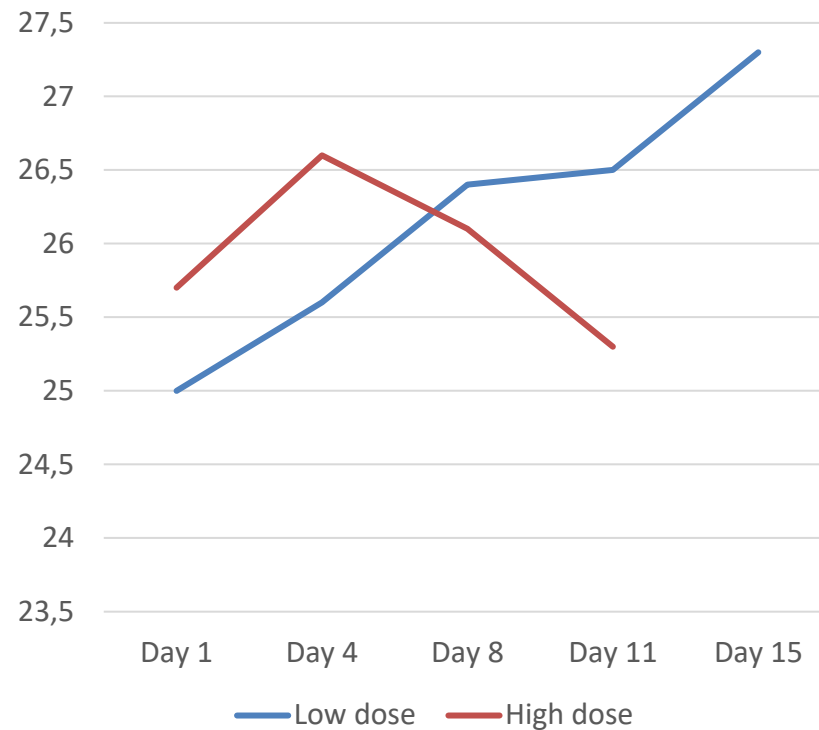
In-Life Findings – Body weights

Mouse

Males



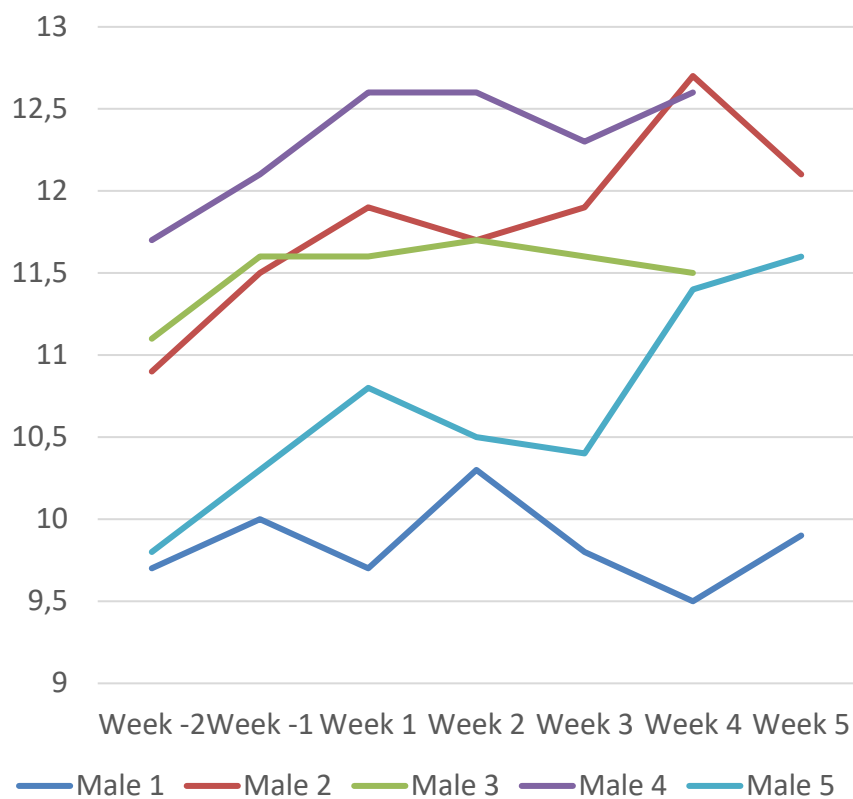
Females



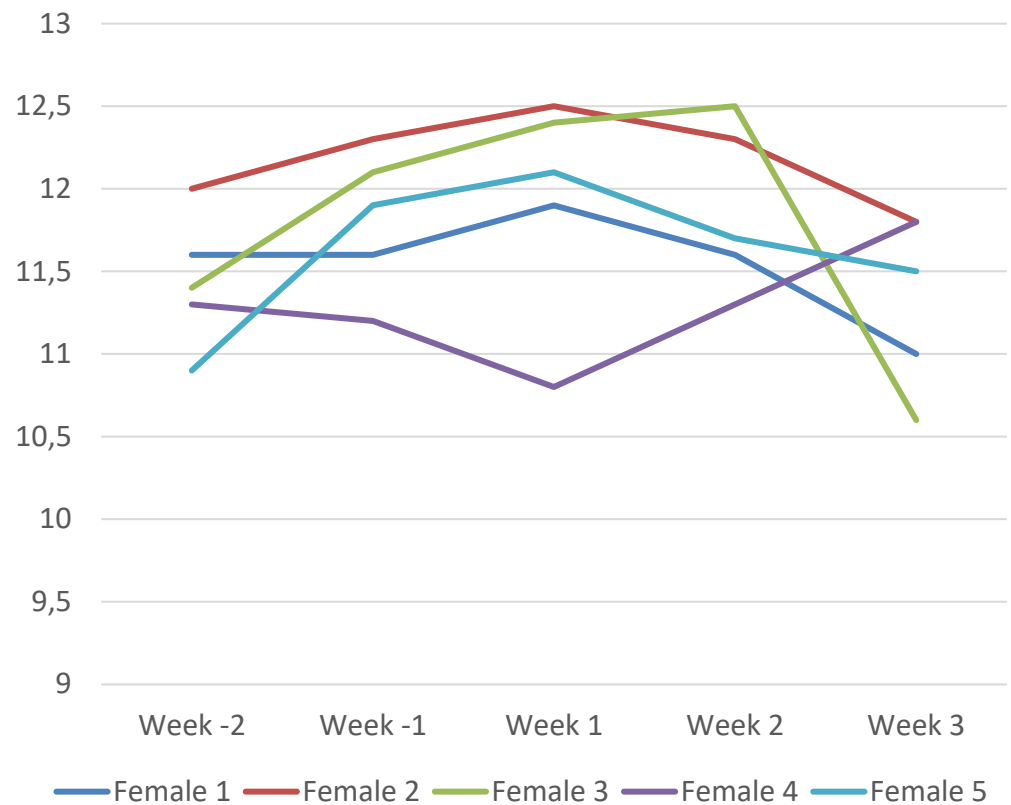
In-Life Findings – Body weights

Minipig

Males – High dose

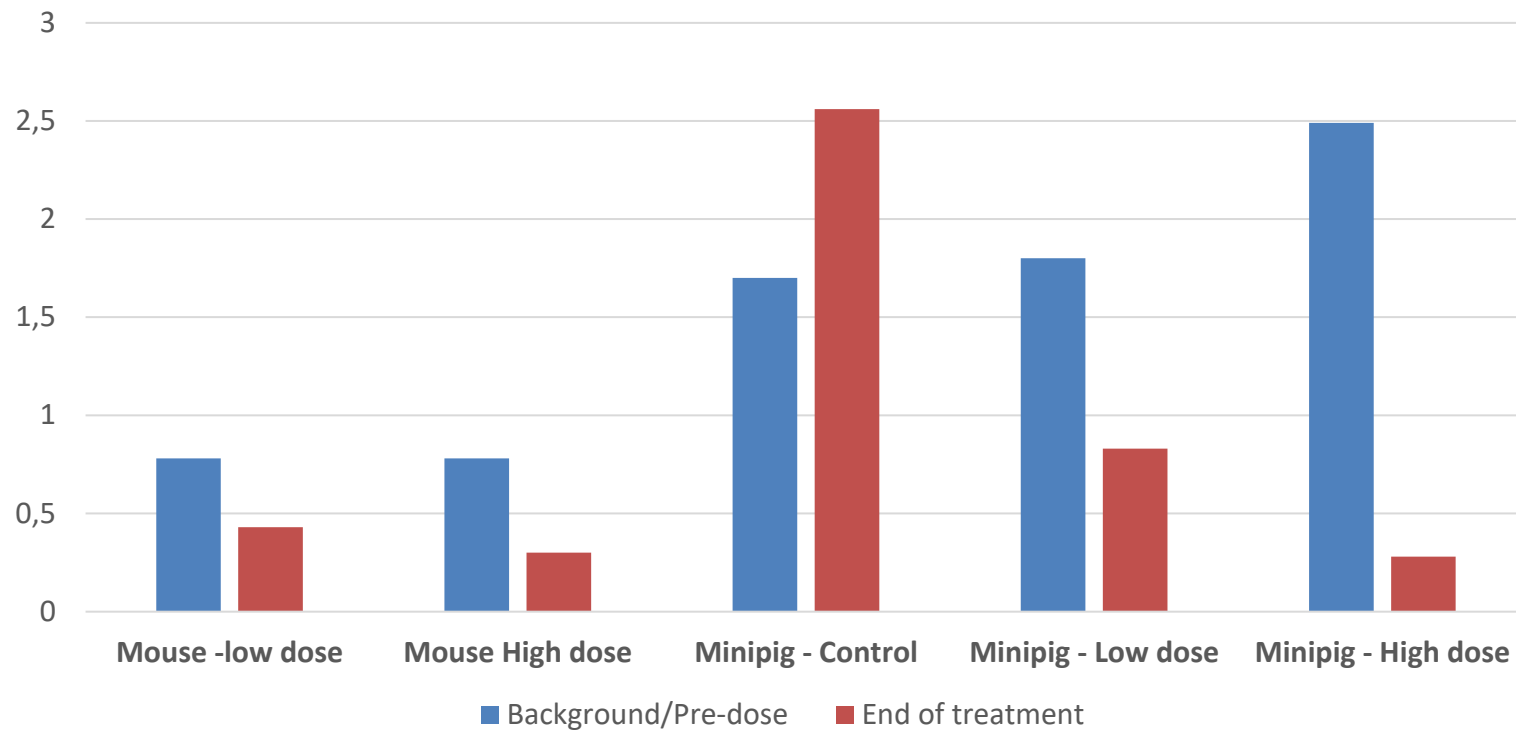


Females – High dose



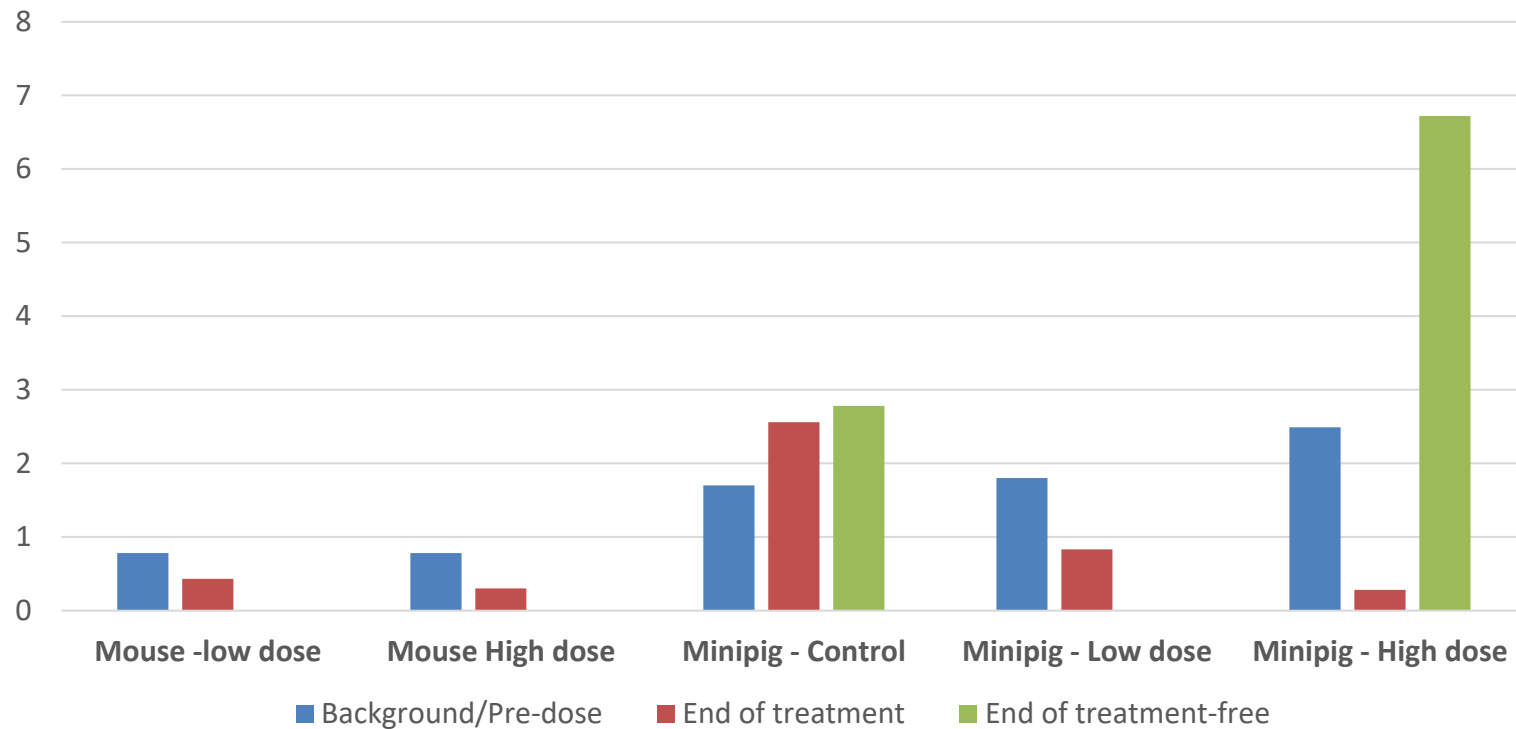
Clinical Pathology Results - Males

Neutrophil counts 10^3uL

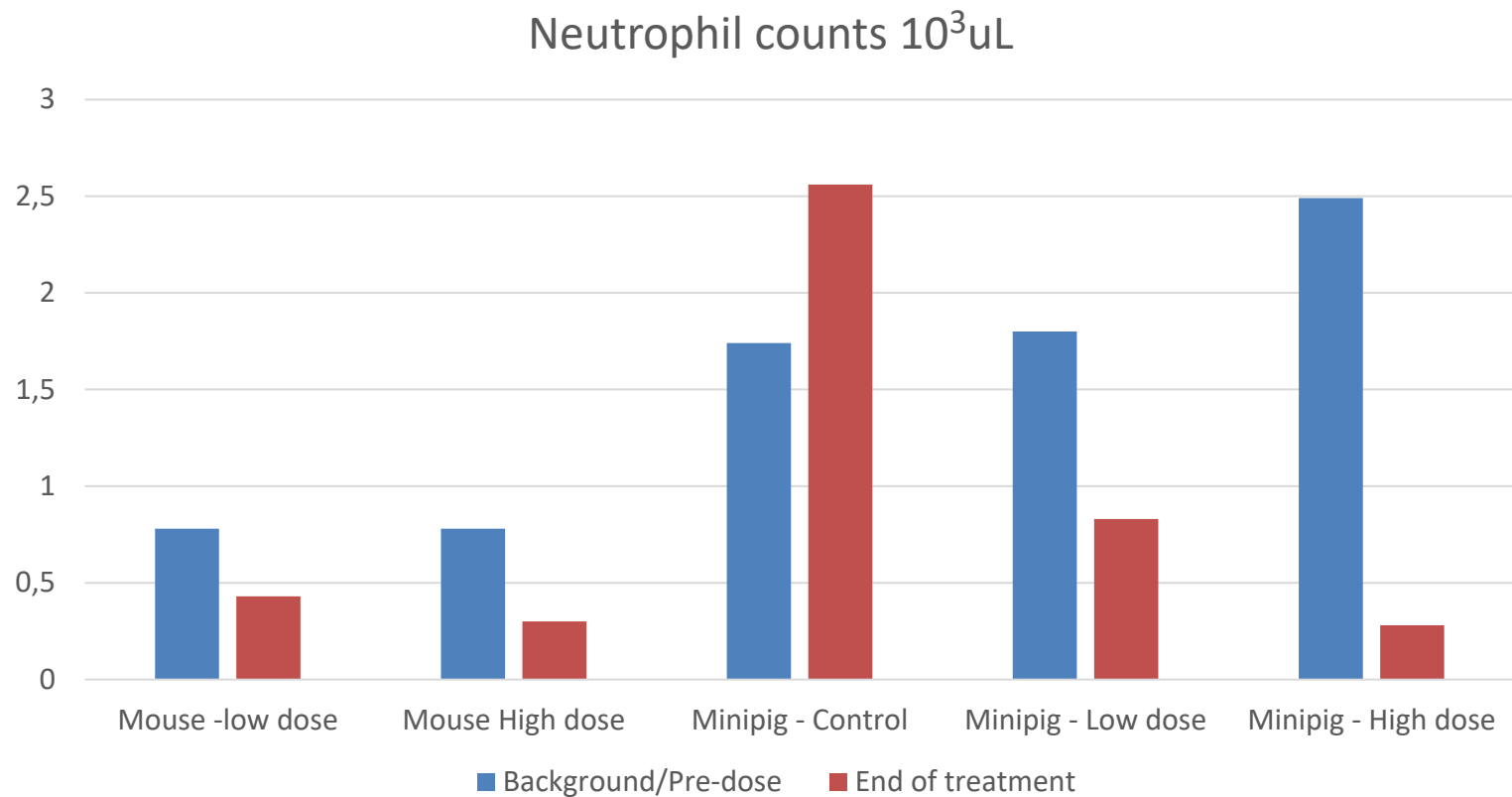


Clinical Pathology Results - Males

Neutrophil counts 10^3uL

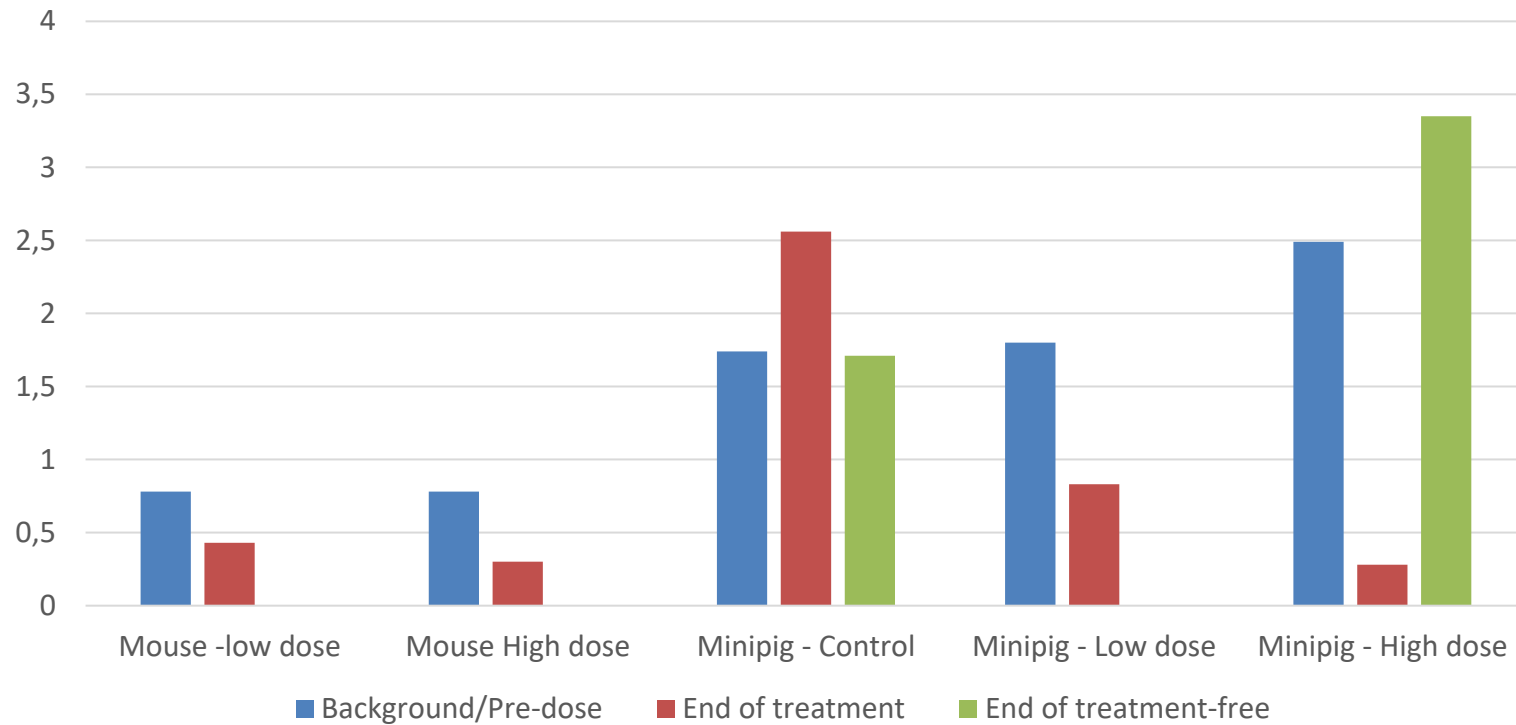


Clinical Pathology Results - Females



Clinical Pathology Results - Females

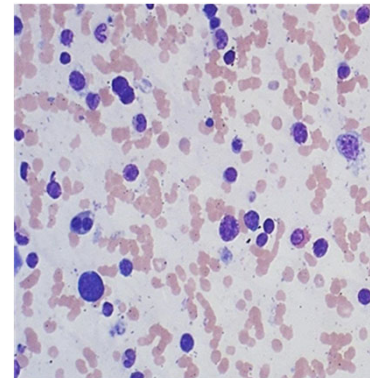
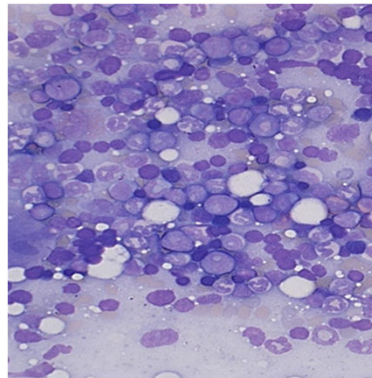
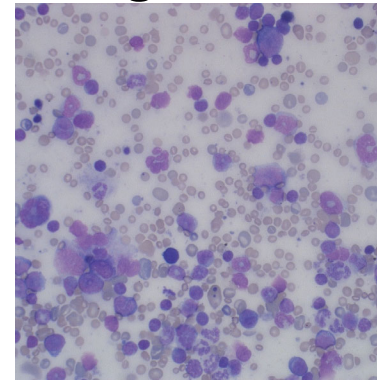
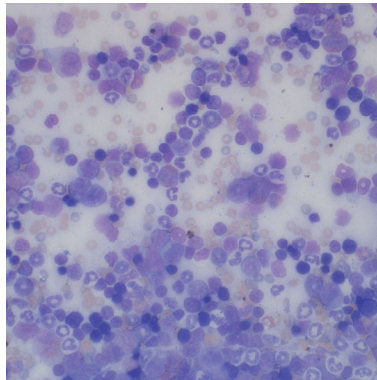
Neutrophil counts 10^3uL



Bone Marrow Smear

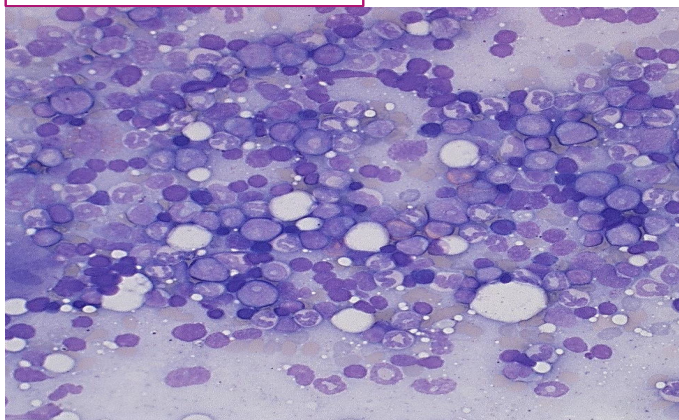
Control

High Dose

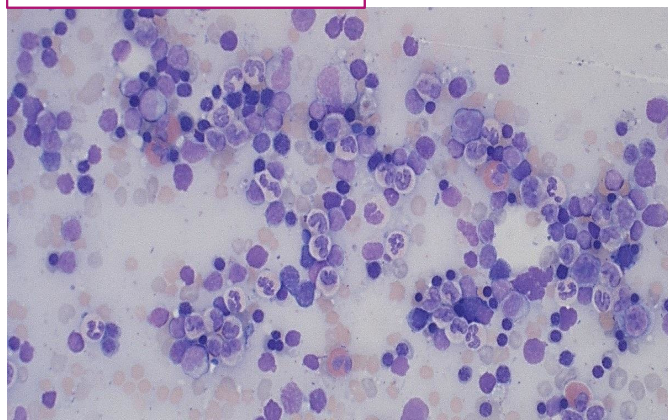


Bone Marrow Smear Depletion

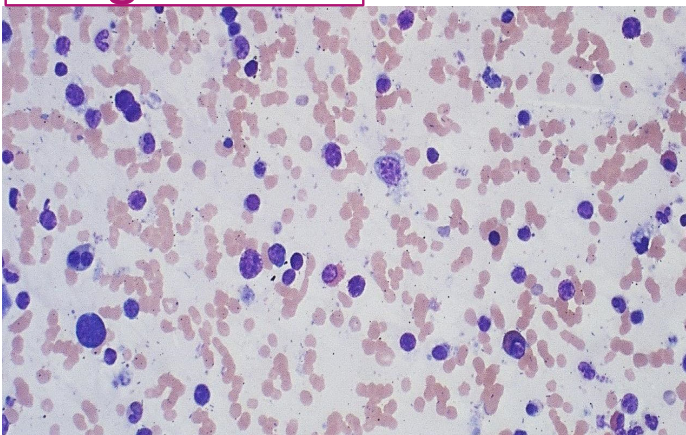
Control



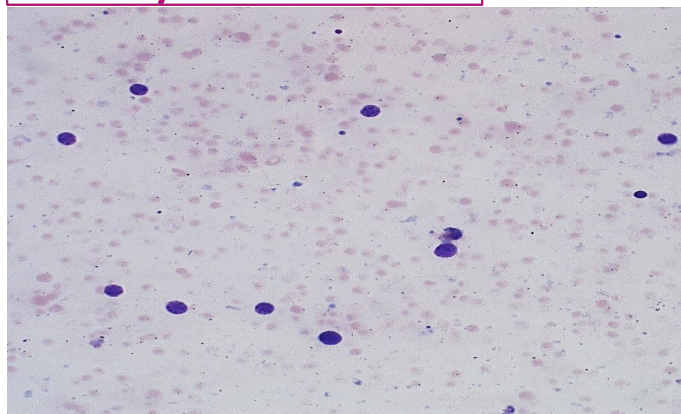
Low Dose



High Dose

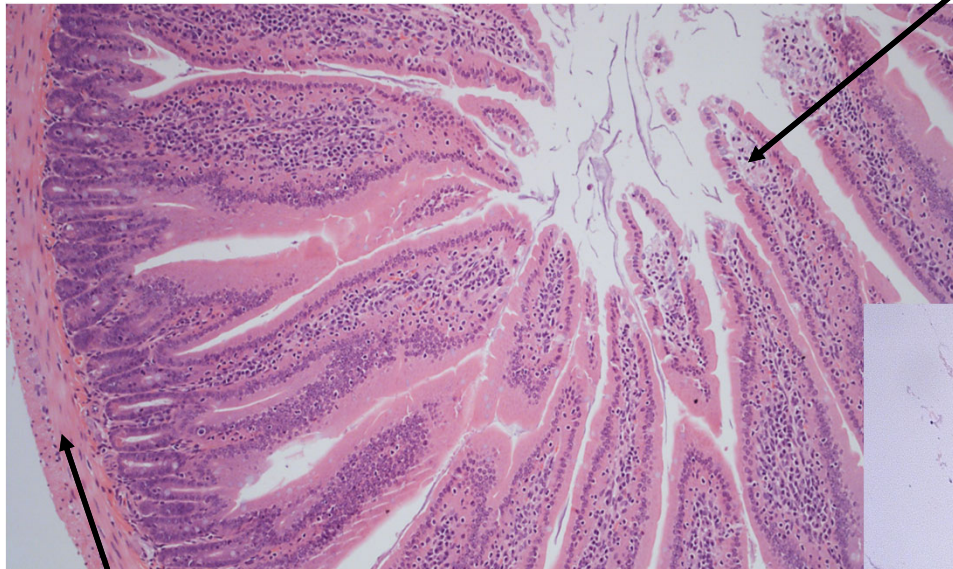


Early decedent



Mouse Pathology

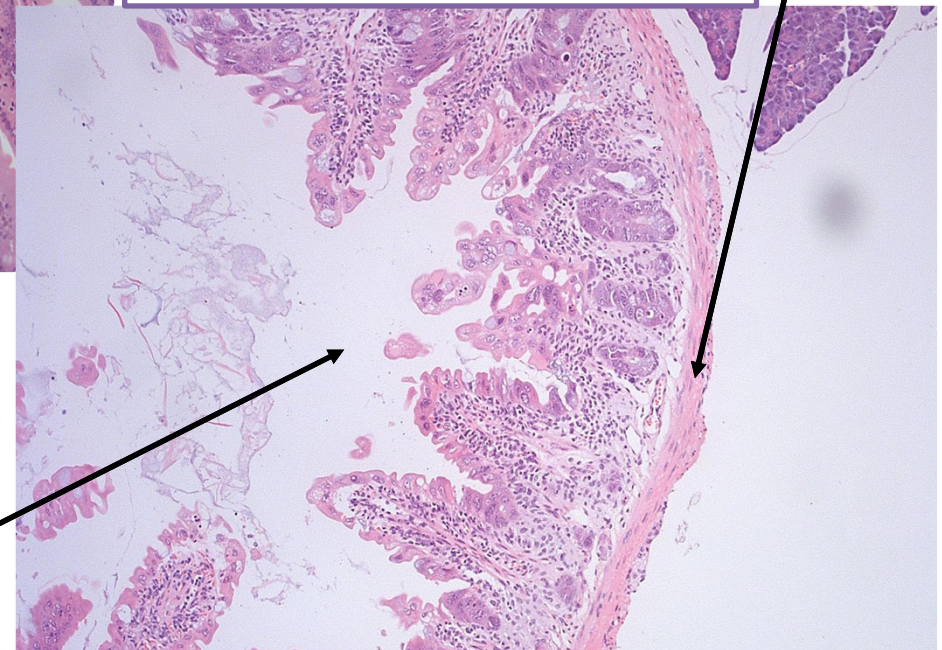
Control mouse- duodenum



Crypt cells, nicely basophilic

Villi protruding into centre region

Treated mouse - duodenum



Very little basophilia

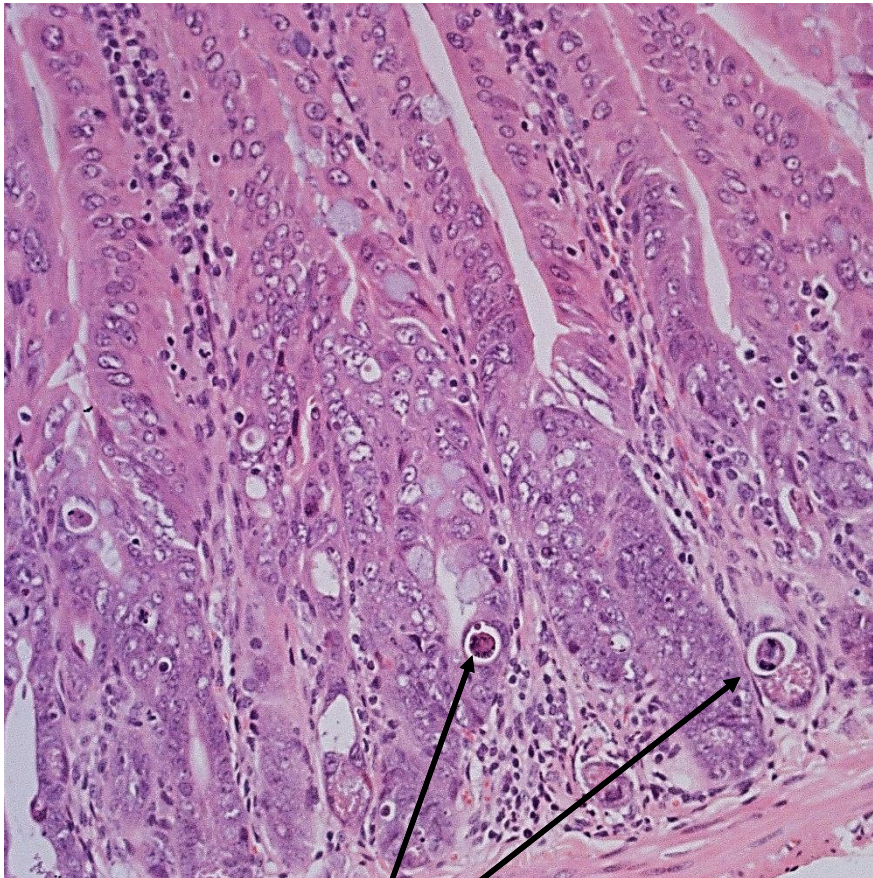
No/limited replenishment of cells



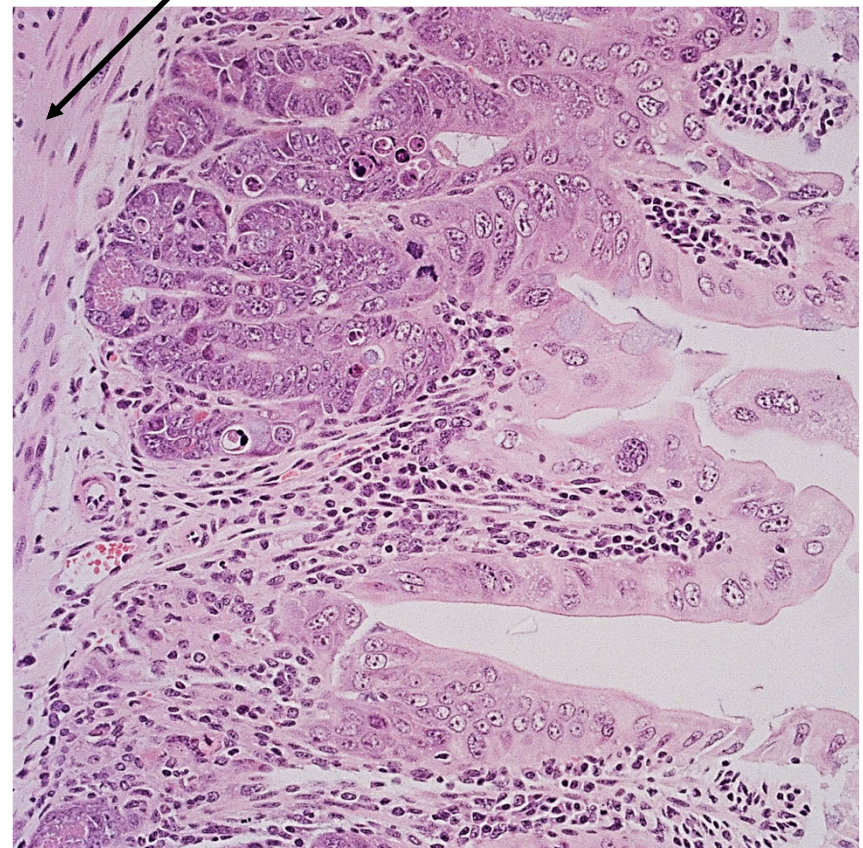
Pathology

Treated mouse – duodenum

Crypt region knocked out

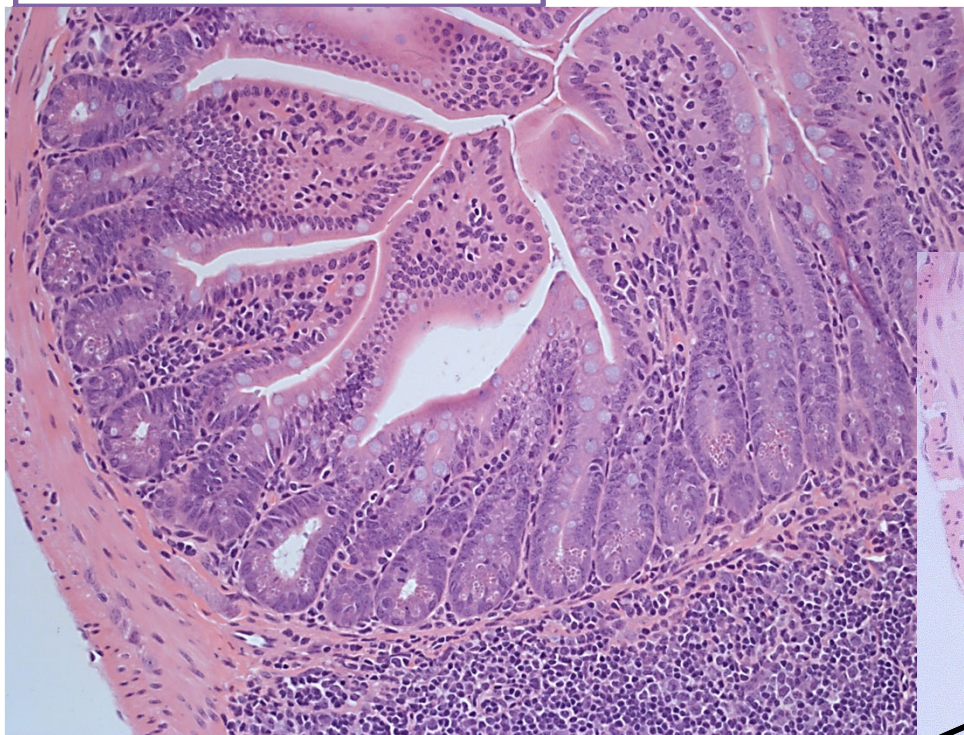


Apoptotic bodies

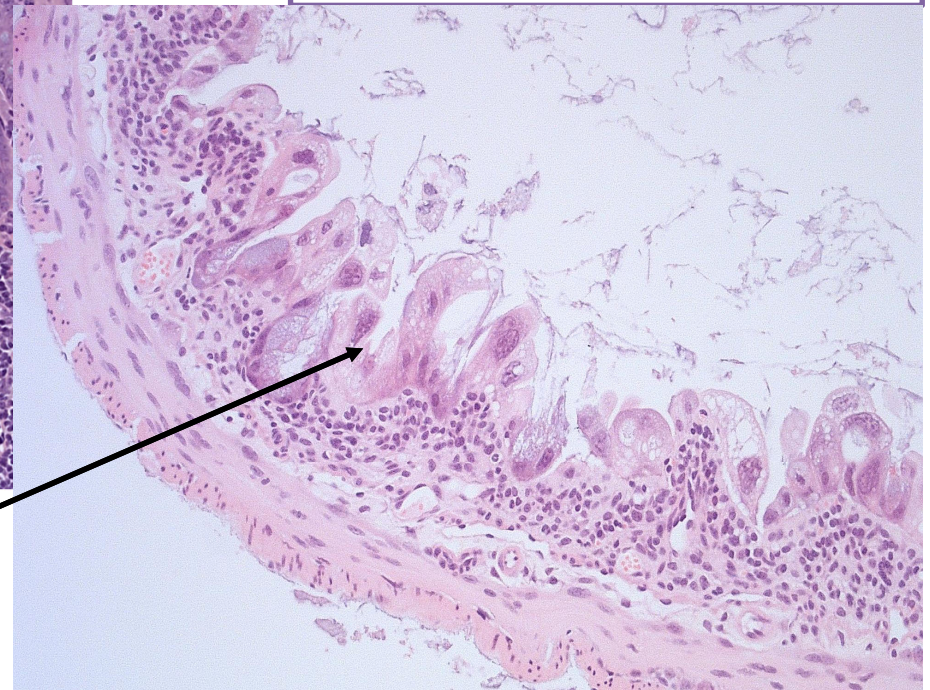


Mouse Pathology

Control mouse- ileum



Treated mouse – ileum



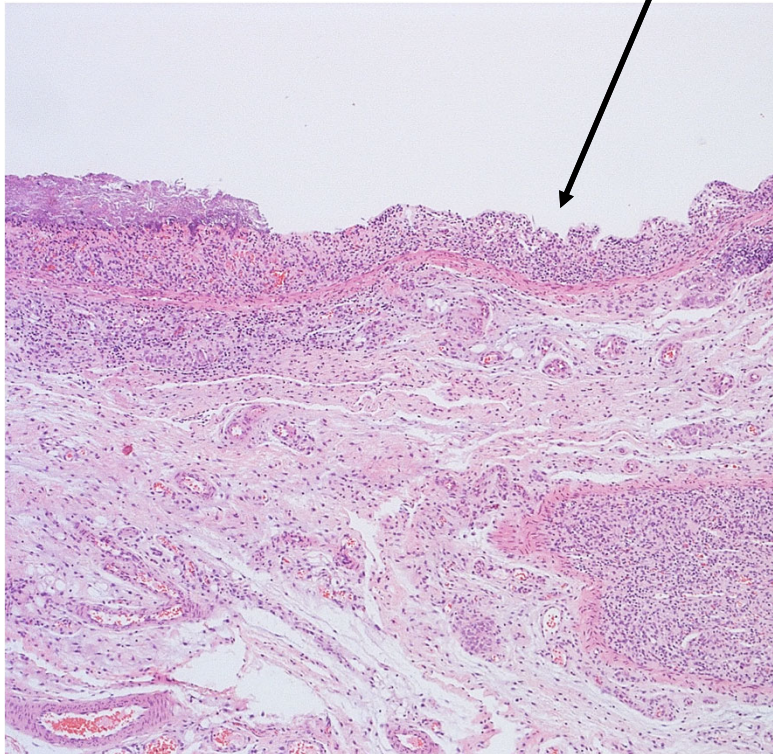
Abnormally shaped nuclei and
abnormal cell turnover



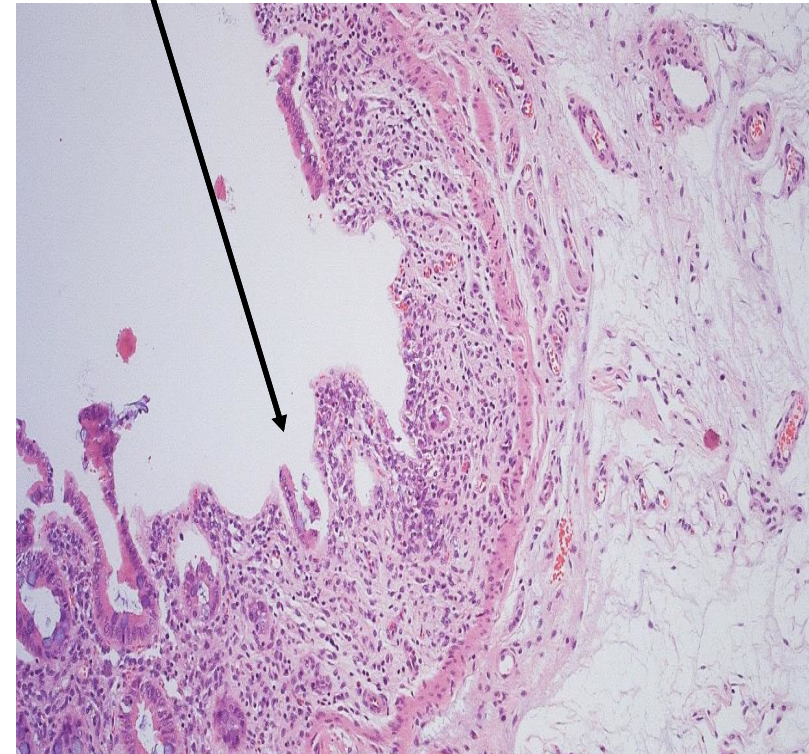
Minipig Pathology

Surface focal erosions

Caecum



Colon



Longer duration repeat dose toxicity study

Animals closely monitored (haematology) and taken off dose when necessary

Clinical signs and pathology similar to man

Haematology: reduction in total white cell count (neutropenia, lymphocytopenia)
→ changes fully reversible

Main pathology: bone marrow and intestines

There were non-responders on the study!



Clinical use in humans

Expected dose levels similar to those selected for minipigs, mice > 10x higher

Main pathology in humans:
Haematology and bone marrow

Responders and non-responders (man and minipig)



Pros and Cons

	Pros		Cons	
	Minipig	Mouse	Minipig	Mouse
Pre-clinical cost		√	√	
Additional Haematology monitoring	√			√
Similarity to humans :-				
Clinical signs	√			√
Haematology effects	√			√
Bone marrow effects	√			√

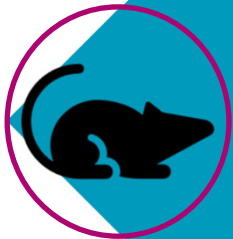


most frequently used model for anticancer drugs.

frequent disappointments when moving into clinical trials.

high cost in both financial and human terms of clinical failures.

better preclinical model is called for.

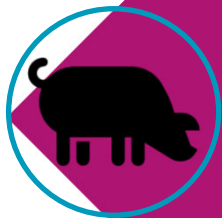


offers a viable non-rodent species or alternative to commonly used rodent models.


monitor parameters throughout the study.

although the initial cost is higher

outweighed by improved prediction of clinical efficacy.



Acknowledgements



**Client for
allowing me to
share the data**



Ellegaard



**Sequani
Personnel**





sequani
NON CLINICAL

great people, great work, real results

Thank you for your Attention

