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Key considerations for successfully maintaining a rodent colony to support oncology drug development

June 13, 2016 - Helmut Ehall, Director of Veterinary Services EU

Company overview

Who We Are

We are a global contract research products and services company dedicated to helping our customers achieve the potential of their products which improve human life, advance animal welfare, and protect the environment and global food security.

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Our Brand Promise

Our company places the customer at the center of everything we do. We focus on what matters to you, our customer, and work closely with you to secure the potential of your research and products. We are dedicated to research that makes a difference in the lives of people all over the world.

Together, we make the world a safer and healthier place to live.

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Capacity and Reach

52 locations

Global availability of research models
and CRO services

5 continents

Extensive reach across the Americas, Europe, Asia, and the Middle East

3,800 employees

Serving over 65 countries

150 years

Combined industry experience

$500 million

Approximately $500m in annual revenues

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Research Models and Services (RMS) locations

Envigo production sites in North America, Europe, the Middle East and Asia

North America

Corporate HQ:
Indianapolis, IN

- Dublin, VA
- Frederick, MD
- Haslett, MI
- Indianapolis, IN
- Livermore, CA
- Madison, WI
- Mexico City, Mexico

Middle East

- Jerusalem, Israel
- Rehovot, Israel
- Yokneam, Israel

Europe

European Office:
Venray, Netherlands

- Blackthorn, UK
- Bresso, Italy
- Gannat, France
- Horst, Netherlands
- Loughborough, UK
- Udine, Italy
- Wyton, UK

India

- Hyderabad, India

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Contract Research Services (CRS) locations

Envigo laboratories are located in The United States of America, Europe and the Middle East

United States of America

+ Princeton, New Jersey

Europe

Corporate HQ: Huntingdon, England

+ Huntingdon, England
+ Eye, England
+ Ely, England
+ Shardlow, England
+ Rossdorf, Germany
+ Barcelona, Spain
+ Valencia, Spain

Middle East

+ Rehovot, Israel

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What are animal models of cancer?

Why use animals to model cancer?

- Cancer is an extremely complex range of diseases
- Research to understand its origin, progression, and treatment is continually evolving as better models become available
- Researchers use animals to study cancer for many reasons
- Rodents have…
  - …shorter lifespans, more rapid tumour generation and disease progression times than humans
- Rodent studies enable…
  - …laboratory control of multiple variables; the environment, diet, exposure to infectious agents, etc.
  - …actual investigation of living test systems, rather than just in vitro and in silico modelling that aid in better predicting and developing clinical solutions

What types of animal models are used for oncology?

- Many different approaches are taken to studying cancer in animal models, using a variety of different species and approaches
- Models in which cancer occurs spontaneously without any modification to the test system
- Animals which are genetically modified to that they more readily develop spontaneous tumours
- Animals that are exposed to environmental factors (chemicals or radiation) in order to induce spontaneous tumour formation
- Models where tumours or cell lines are engrafted

In vivo pharmacology

- Human tumour xenografts (rates and mice)
  - Immunocompromised rodent models are engrafted with cell lines derived from human tumours that have been cultured *in vitro* prior to implantation.
- Patient derived xenografts (PDX)
  - Immunocompromised rodent models are engrafted with primary or secondary tumours that have been cultured *in vivo* not in tissue culture
- Syngeneic models
  - Immunocompetent rodents are engrafted with murine tumour cell lines so that therapeutics can be evaluated in the presence of a functional immune system
- Orthotopic models
  - A variety of cell lines can be engrafted in particular tissues of interest in the test system, mimicking human disease
- Environmental/radiation exposure models
  - Many approaches may be taken to induce tumours in the test system

Non-clinical safety assessment

- Many different animal species are used to assess the safety of therapeutics intended for the treatment of cancer
- Rodents, dogs, non-human primates, pigs, mini-pigs, rabbits, guinea pigs and many other custom bred species are used
- The quality of the animals, the way they are bred, housed, trained, dosed, fed and maintained are all critical considerations in generating the data required to bring a drug to market
- Envigo is as passionate about working with our customers to build a healthier and safer world, whilst caring about each other, our animals and the environment

Do housing and husbandry matter?

Mandy Horn, MS
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Outline

- Why do housing and husbandry matter?
- What parameters should be considered?
- Management considerations
- Case-study review

Why do housing and husbandry matter?

The design of animal facilities combined with appropriate animal housing and management are ***essential contributors*** *to animal well-being, the quality of animal research and production, teaching or testing programs involving animals, and the health and safety of personnel.*

Microenvironmental conditions can directly affect physiologic processes and behavior and may alter disease susceptibility

Guide, 2011

What parameters should be considered?

- Bedding
- Diet
- Enrichment
- Handling
- Noise
- Vibration
- Olfactory stimuli
- Lighting
- Temperature
- Humidity
- Air flow
- Caging
- Cage density
- Experimental variables
- Inability/ability to socialize
- Pain/distress
- Restraint
- Transportation

Scenario review

Stress? What stress?

- Background
  - 12-wk study of Zucker rats to monitor blood glucose, body weight, and HbA1c
- Problem
  - Non-fasted glucose values were significantly greater during wk 7 compared to other weeks, atypical of model phenotype
- Investigation
  - Reviewed various parameters, including timing of glucose monitoring, light changes, personnel changes, confirmation of bedding/diet, cage cleaning schedule

Stress? What stress?

- Conclusion
  - Confirmed with site management that cages were cleaned prior to blood glucose measurement as animal technicians wanted to ensure best possible environment and cleanliness for experiment
- Resolution
  - Research technician discussed importance of not performing cage cleaning prior to measurement of blood glucose as this event can cause stress and impact results

I'm picking up *bad* vibrations…

- Background
  - Customer received timed-mated Sprague Dawley rats at early gestation for a number of years from the same facility
- Problem
  - Recent findings of non-pregnant rats with resorptions, increased cannibalization, death in dams and/or pups
- Investigation
  - Customer investigating their institution with veterinarian; discussing bedding, diet, cage type, animal technicians, overall vivarium, outside vendors, as well as external environmental changes, including new construction project nearby

I’m picking up *bad* vibrations…

- Conclusion
  - New construction project next to vivarium involved large amounts of noise and vibration; reported reproductive issues not only with timed-mated animals, but also internal colonies, leading to losses at the institution
- Resolution
  - Construction could not be stopped; however, increased awareness to discuss further with outside company

Am I missing something?

- Background
  - Immunodeficient animal model previously exhibited young pup death; a root cause was initiated and the addition of enrichment reduced pup death
- Problem
  - In this immunodeficient mouse model, the facility began to report an increased incidence of young pup death
- Investigation
  - Multiple factors investigated, including humidity, lighting, isolator location, diet, bedding, enrichment, animal technicians, and genetic results

Am I missing something?

- Conclusion
  - Investigation revealed new technician overseeing colony failed to utilize crinkle-paper enrichment in breeding cages over a two week period, resulting in an increase in pup death
- Resolution
  - Enrichment was added to breeding cages again, and training occurred in proper management of this model

Research summary: Garner and Gaskill

- Review of 7 years' of studies by Garner and Gaskill, published on March 30th, 2012
  - In general research, mice are kept too cold (without nesting material)
  - Effects on well being & study outcome (due to elevated metabolic rate)
  - With nesting material they can naturally regulate their temperature
  - At 20/24 degrees: less aggressiveness, more milk production, unhappy pups
  - At 18/20 degrees: immune function change and start of growth retardation
  - Nest is not only for warmth, also physical comfort, form of protection, decreasing anxiety and stress level
  - Nest provokes less food intake
  - Females prefer warmer temperature than males: plus 5 degrees

Summary

- Communication is key!
- Be knowledgeable about practices and procedures in your institution, and potential impacts on animal well-being or study outcome
- Animal technicians, veterinary staff, vivarium manager, and investigators should work closely, especially when any changes occur

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Genetic background as a research variable

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What genetic background does your model have?

Congenics, trangenics and knockouts… Oh my!

- Congenic
- Transgenic
- Targeted mutants
- Spontaneous mutants

Nomenclature

- Follow proper rules for nomenclature
- Don’t be tempted to abbreviate
- The correct nomenclature can tell you a lot about a model:
  - C57BL/6NHsd
  - RccHan®:WIST
  - B6.V-Lepob/OlaHsd
  - FVB/N-Tg(MMTVneu)202Mul/J
  - C57BL/6NTac-IL15tm1Imx N5
- Guidelines: http://www.informatics.jax.org/mgihome/nomen/gene.shtml

Now that you know what you have...

Genetic change is a research variable

- Genetic contamination
- Genetic drift

Genetic contamination

- Accidental mismating between unrelated models
- Can be identified through coat color changes
- Can be easily missed
  - Same coat color in same room
  - Poor cage labeling / record keeping
  - No routine genetic monitoring program
  - Difficult to assess in outbred stocks
- Inaccurate research data
- Lose years research time, data and money

Genetic contamination - prevention

- Know what you are starting with
- Separate coat colors
- Be sensitive to phenotypic changes
- Good record keeping
- Genetic testing program

Genetic drift

- Spontaneous mutations
  - Spontaneous mutations that occur randomly throughout the genome
  - Typically occurs in non-coding DNA
  - Can result in phenotypic changes
- Selection pressure
  - Can change allelic frequencies in outbred stocks
  - Can lead to the elimination of desired trait
  - Can lead to the introduction of non desirable trait
- Bottlenecks
  - Breeding closely related animals in an outbred colony which results in the loss of alleles and/or large changes in allelic frequencies

Minimizing genetic change

- Maintain the appropriate number of animals for the colony type
- Avoid selection pressure
- Follow a specific breeding protocol
- Excellent record keeping
- Monitor your colonies

Summary

- Know the genetic background of your model
- Minimize genetic change in your colony
  - Genetic contamination
  - Genetic drift
- Remember that genetic background can be a large research variable

Do housing and husbandry matter

Genetic background as a research variable

Feeding rodent models used in oncology studies

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Teklad Diets Europe

Introduction

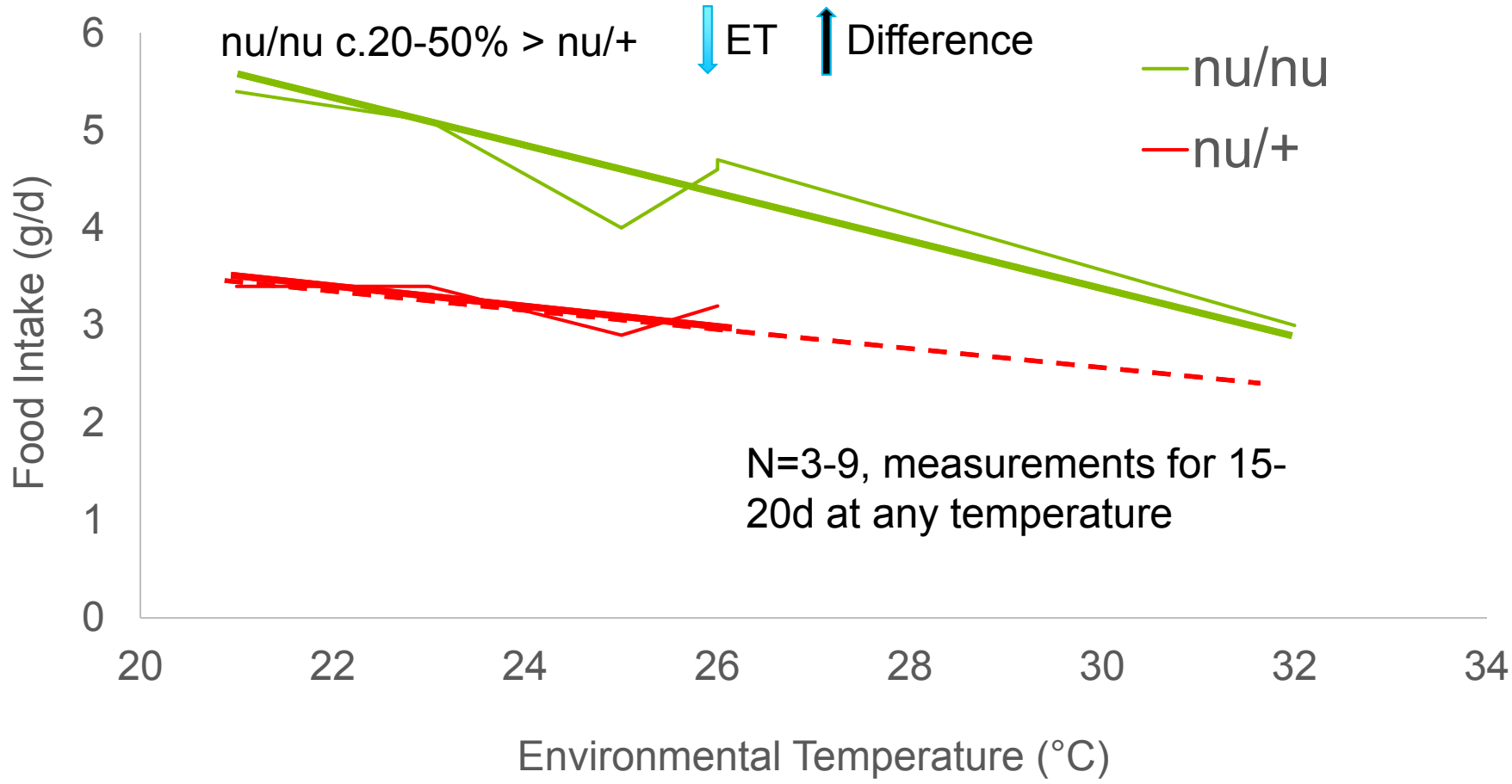
- Do immunocompromised mice have different nutrient requirements compared to immunocompetent animals?
  - Many immunocompromised mice are nude, with consequential effects on heat production for temperature regulation
  - Nutrient requirements for tumour growth
- What effect might different dietary ingredients have on tumour growth?
  - Bioactive substances in ingredients
  - Contaminants

Effect of dietary protein level on spontaneous mammary tumours in female C3H mice

Group	No. mice	Mice with mammary tumours		Age of mice at appearance of tumours (weeks)	
		Number	Percent	Range	Mean
9% Casein	50	48	96	29-92	51.5 ± 2.2
18% Casein	50	47	94	28-86	47.0 ± 2.3
27% Casein	50	49	98	26-94	49.5 ± 2.4
36% Casein	50	46	92	23-103	50.6 ± 2.9
45% Casein	50	42	84	2-98	48.6 ± 2.6

After Tannebaum and Silverstone, 1949

Effect of environmental temperature on the food intake of 3-4m old NMRI nude mice



Based on data from Weihe, 1984

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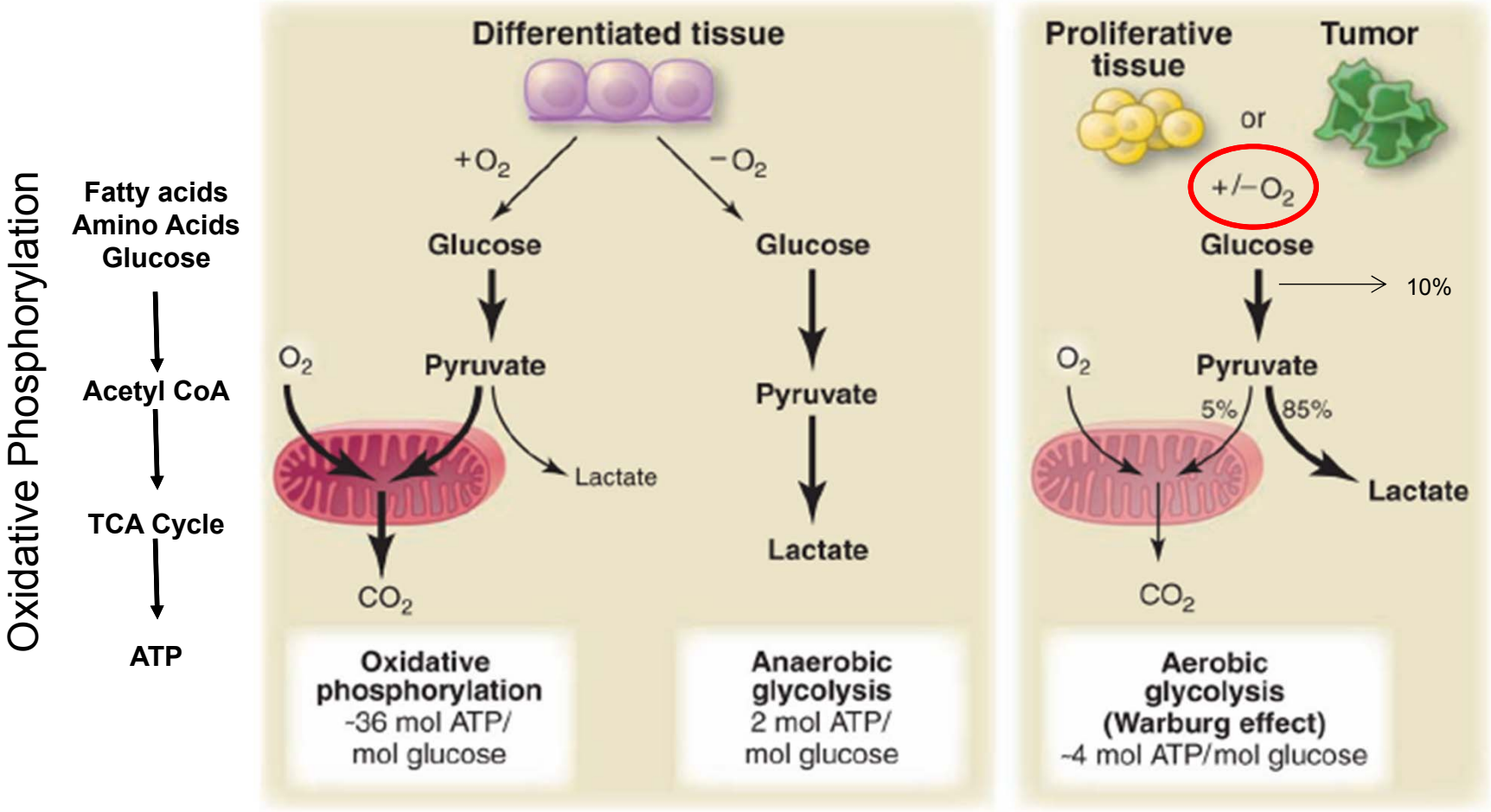
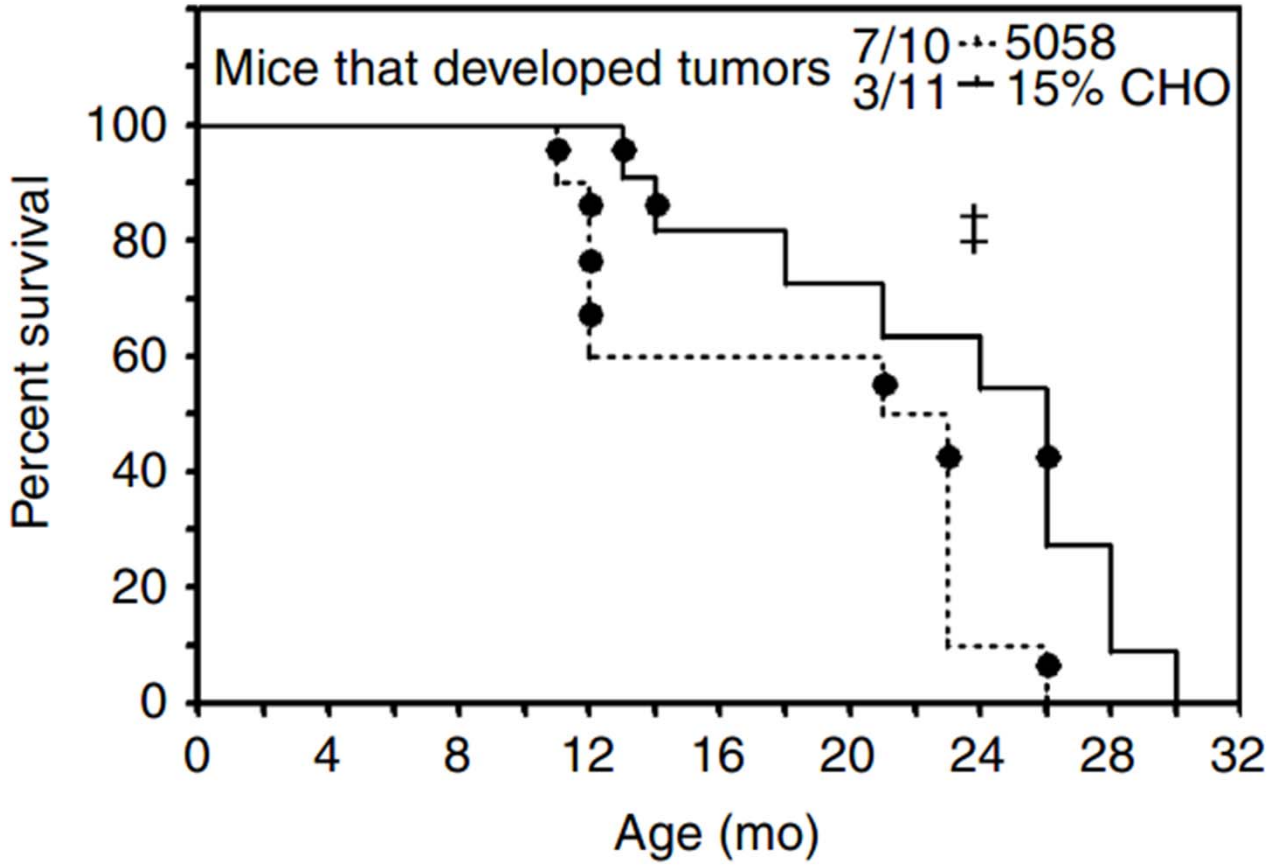
Warburg effect on energy provision in tumours



After van der Heiden *et al*., 2009

The 15% CHO diet reduces the incidence of tumors in a female NOP spontaneous mouse model of breast cancer

5058 – CHO 55%



After Ho *et al*., 2011

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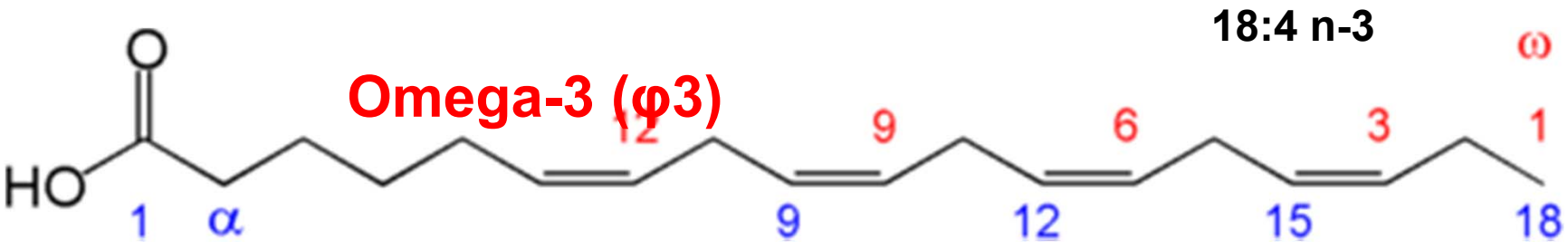
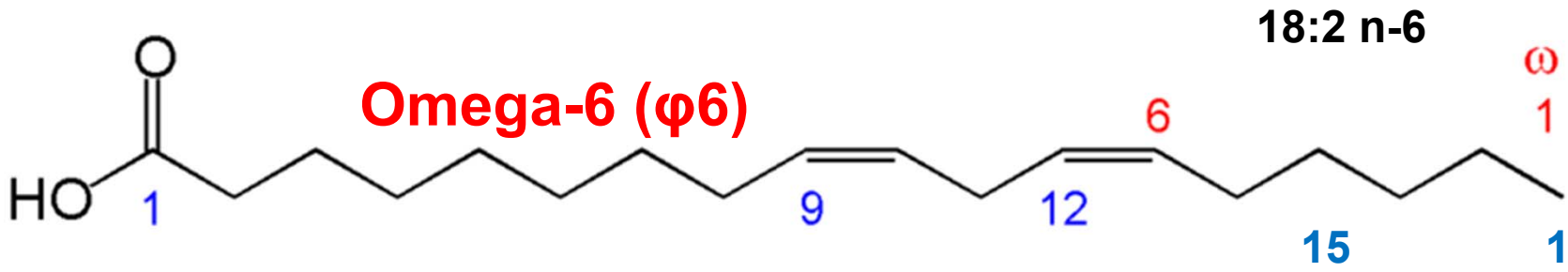
Types of fat

- Saturated
- Unsaturated
  - Monounsaturated
  - Polyunsaturated (PUFA)





Adapted from GB HealthWatch

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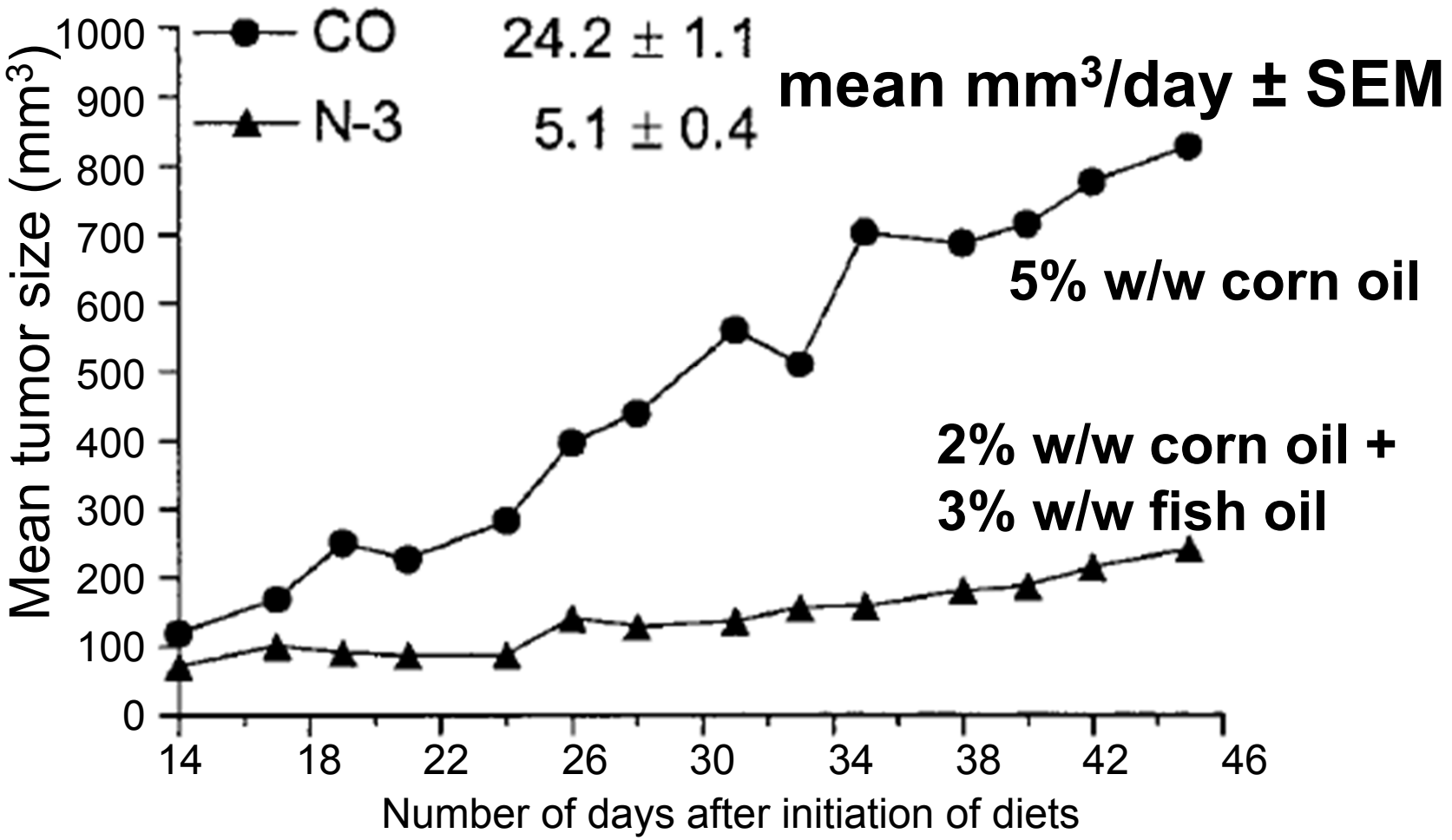
Sources of omega-3 and omega-6 fatty acids

	Omega-3 %	Omega-6 %	Ratio
Oil, corn	0.71	**59.16**	0.01
Margarine-butter blend	0.91	**19.08**	0.05
Oil, sunflower	0.20	**3.67**	0.05
Oil, olive	0.61	**8.07**	0.08
Shortening	1.05	**13.93**	0.08
Oil, wheat germ	7.04	**55.90**	0.13
Oil, soybean	6.94	**52.02**	0.13
Butter oil, anhydrous	1.49	**2.31**	0.64
Fish oil, sardine	**26.99**	3.75	7.19
Fish oil, menhaden	**30.72**	3.31	9.29
Fish oil, herring	**14.09**	1.43	9.88
Fish oil, cod liver	**20.57**	1.86	11.07
Fish oil, salmon	**37.92**	2.21	17.16

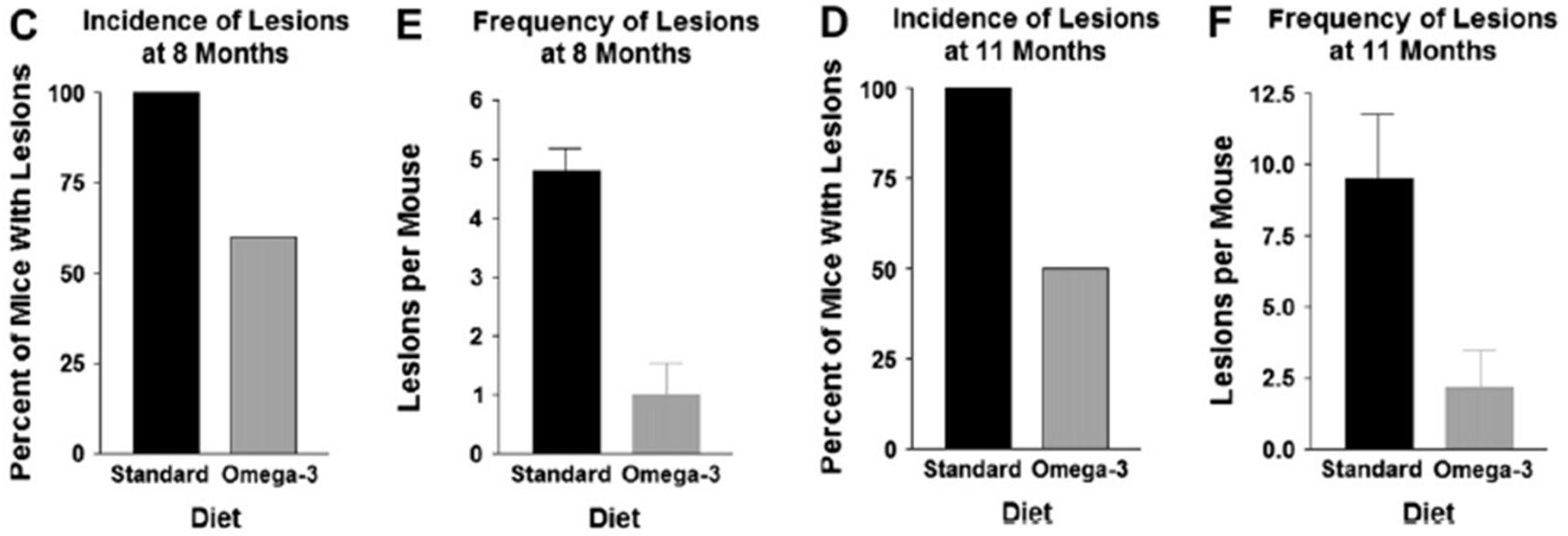
Values are % of the oil or fat

Mean growth rate of MDA-MB 231 xenografts in 3-month old female athymic nude mice



After Hardman *et al*., 2001

Effect of omega-3 fatty acids on pancreatic precancerous lesions in elastase-Kras mice



Omega-3 diet - Menhaden fish oil included at 23% w/w
No difference in body weight

After Strouch *et al*., 2011

Effect of nutrients on tumour growth

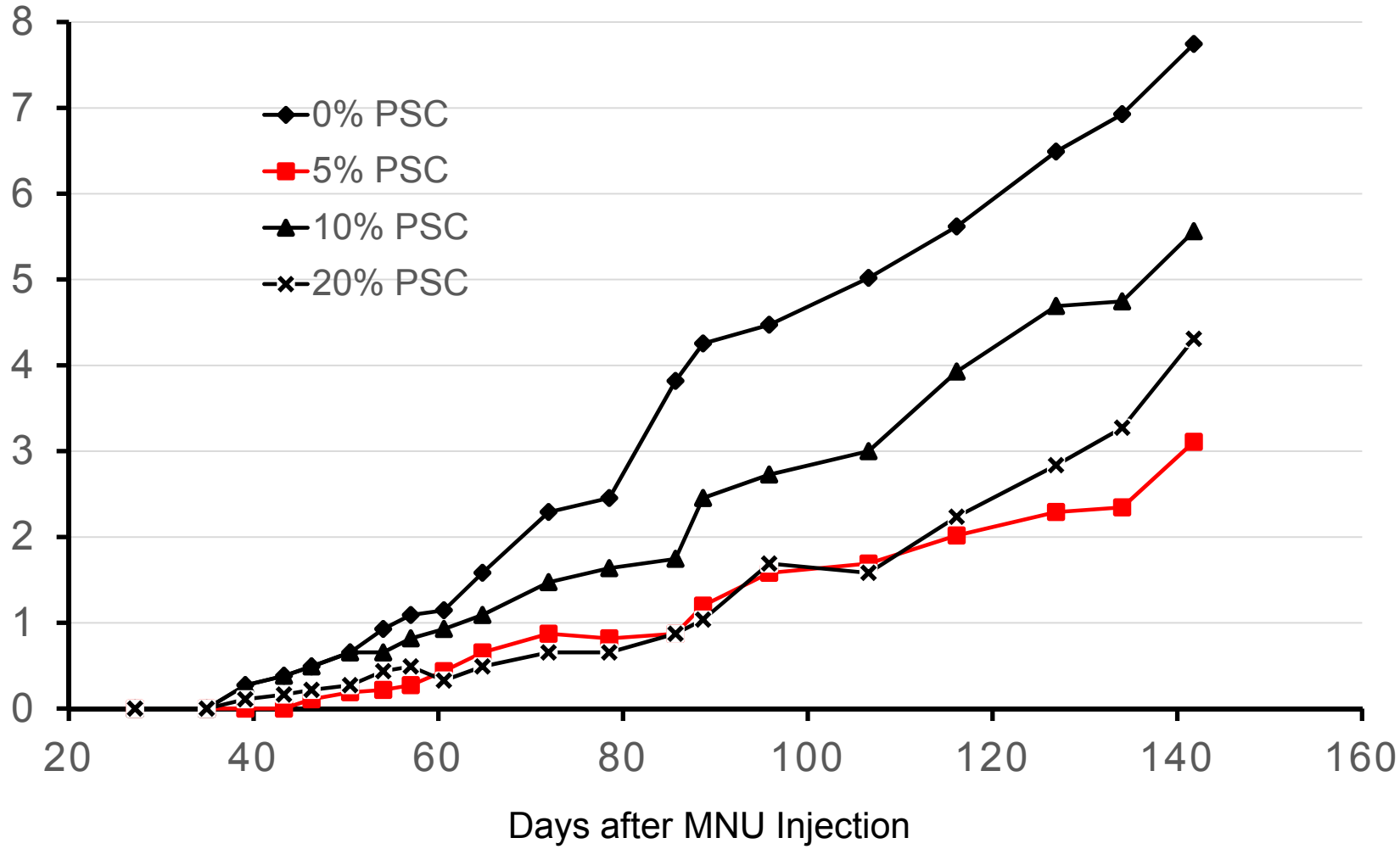
- ↓ Carbohydrate: Slows tumour growth and prevents cancer initiation
  - Warburg effect ? Cancer cells rely on glycolysis rather than oxidative phosphorylation [cancer cells hypoxic]
- Protein: no significant effect over a wide range, but very high inclusion may suppress tumour growth
- ↑ Fat: Tumour promoting, but inhibition with omega-3 fatty acids (especially fish oils)

Optimum diet for tumour growth should have low/ moderate protein, high carbohydrate, and moderate (vegetable) fat

Ingredients are not just nutrient sources

- Many ingredients contain bioactive compounds
  - Soybeans *(Glycine max)*
    - Isoflavones (phytoestrogens)
    - Saponins
    - Lunasin (a soy peptide)
    - Glyceollins (soy phytoalexins)
    - Protease inhibitors
    - Conglycinin (7S Globulin)
- Some ingredients may contain contaminants
  - Fish meal
    - Organic mercury
    - Nitrosamines
    - PCBs, Dioxins, Brominated flame retardents, PAHs
      - But note geographical area
  - Maize
    - Zearalenone

Inhibition of mammary tumours induced by N-methyl-nitrosourea (MNU) in female SD rats at 50d age



Redrawn from Barnes et al., 1990

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Phytoestrogen characteristics

- Weak Estrogens
- High affinity for βER
- Selective Estrogen Receptor Modulators (SERM)
  - Agonist or antagonist effects
- Biphasic effects (U- or Bell-shaped)
- High concentrations in plasma of animals fed diets containing moderate levels of soya
- Conjugated forms (Glucuronides/Sulphate) largely biologically inactive

Effects of isoflavones on cancer

- Effects on cell signalling pathways
  - Controls cell proliferation, apoptosis, angiogenesis
- Cell-cycle arrest (at G0/G1 and G2/M transition steps)
- Topoisomerase inhibition
- Tyrosine protein kinase inhibition
- Inhibition of angiogenesis (blood supply)
- Inhibition of metastasis
- Suppression of inflammation
- Antioxidant effects
- Promotion of DNS demethylation
- Additional tumour-specific mechanisms
  - Inhibition of androgen-dependent carcinogenesis (e.g. Prostate-specific androgen)
  - Aromatase inhibition/upregulation
  - Competition for estrogen receptor sites

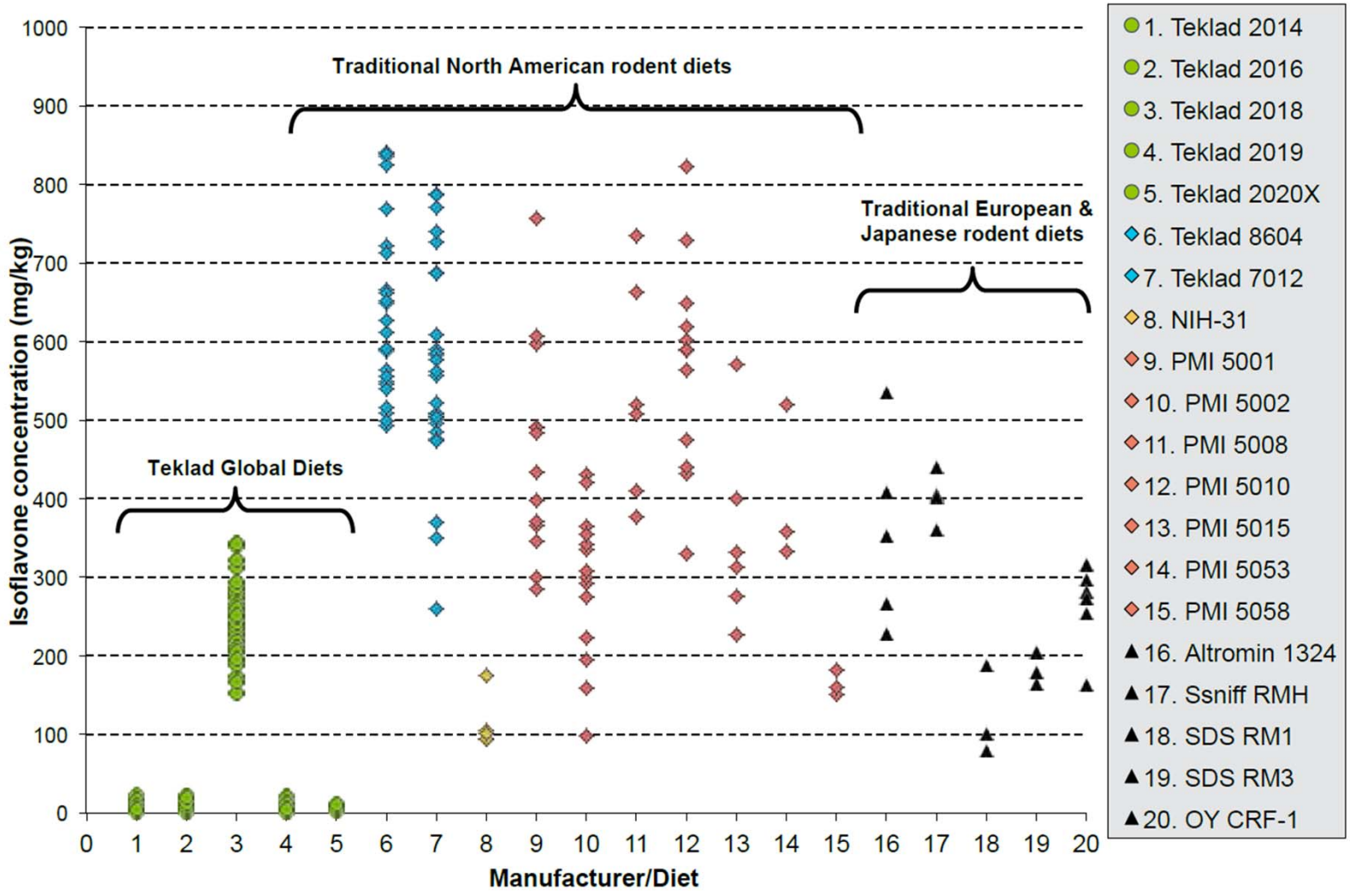
Soy and isoflavones affect tumours that originate by various routes

- Chemically-induced
- Xenograft
  - Estrogen-sensitive
  - Estrogen-independent
- Mutant Mice models
  - ApcMin mouse
- Genetically-modified animal models
  - MMTV-neu/ErbB-2 transgenic mice
  - TRAMP mice (**TRA**nsgenic **M**ouse model for **P**rostate cancer)
- Spontaneous

OECD guideline for the testing of chemicals: Carcinogenicity studies (2009)

- … the content of dietary contaminants, including but not limited to pesticide residues, persistent organic pollutants, **phytoestrogens**, heavy metals and mycotoxins, that might influence the outcome of the test, should be as low as possible

Variation in isoflavone levels in laboratory animal diets



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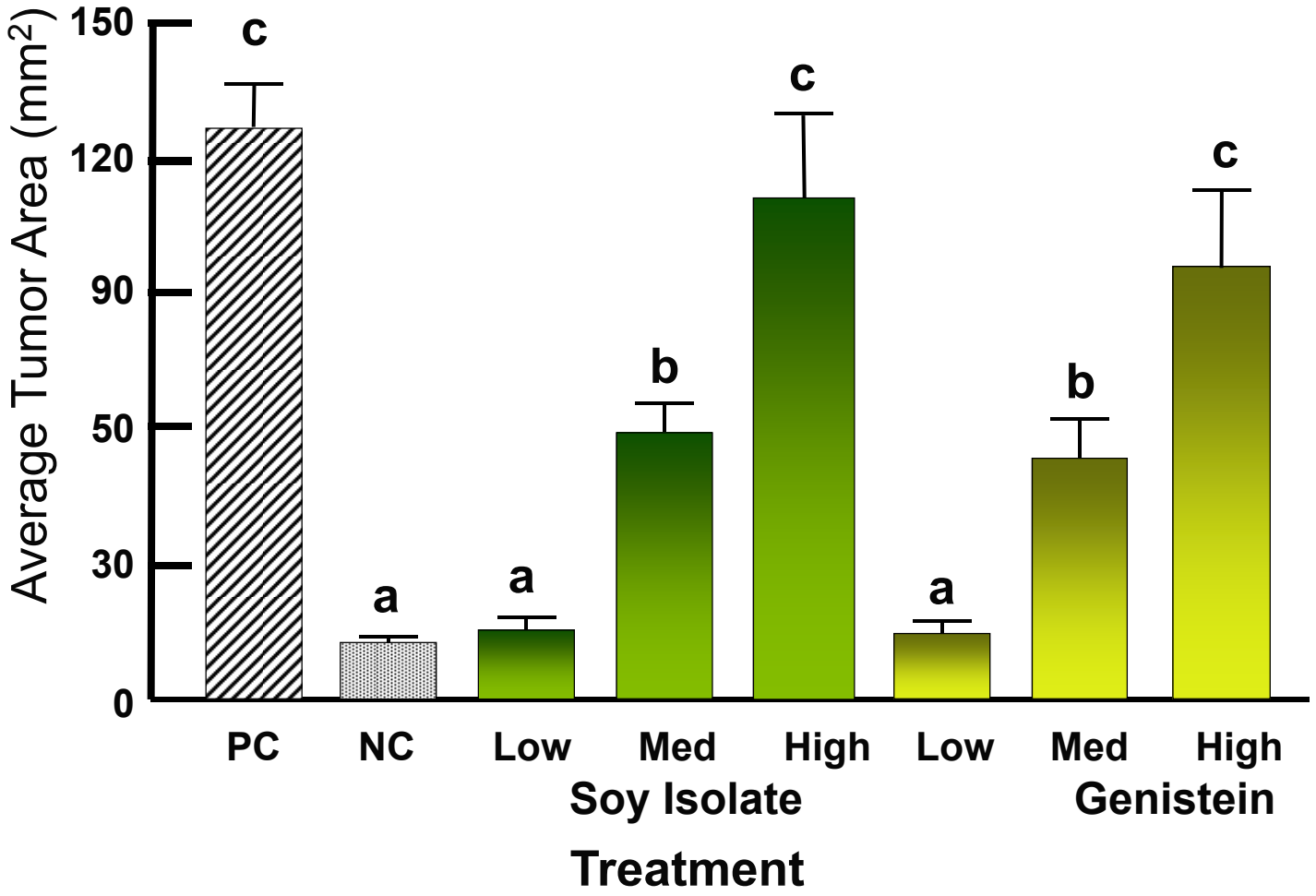
Growth of MCF-7 cells in ovariectomized athymic mice fed various levels of genistein (15, 150, 300 ug/g), either as soy isolate or genistein



After Allred *et al*., 2001

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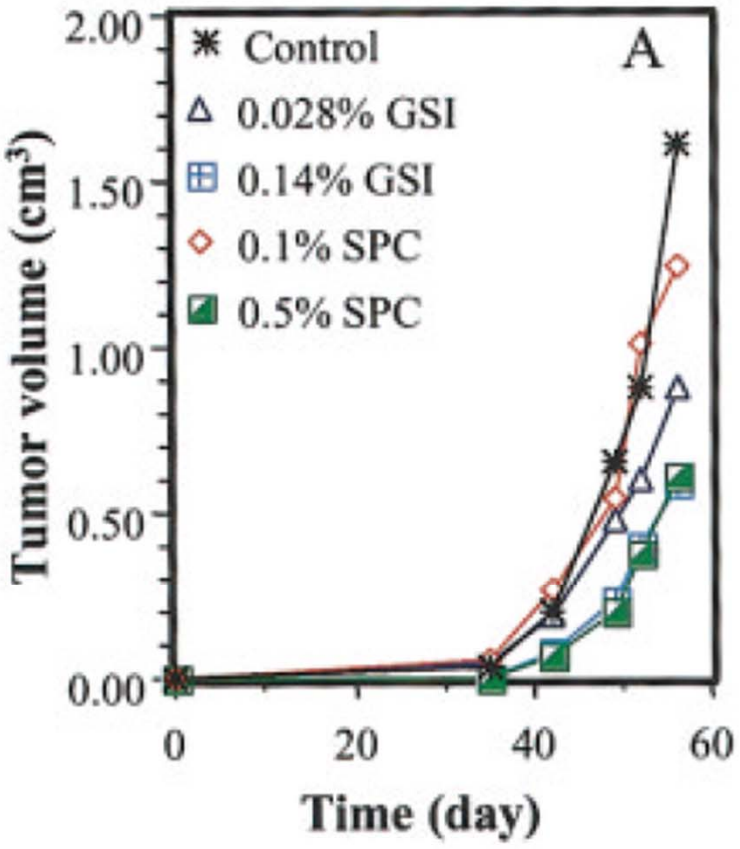
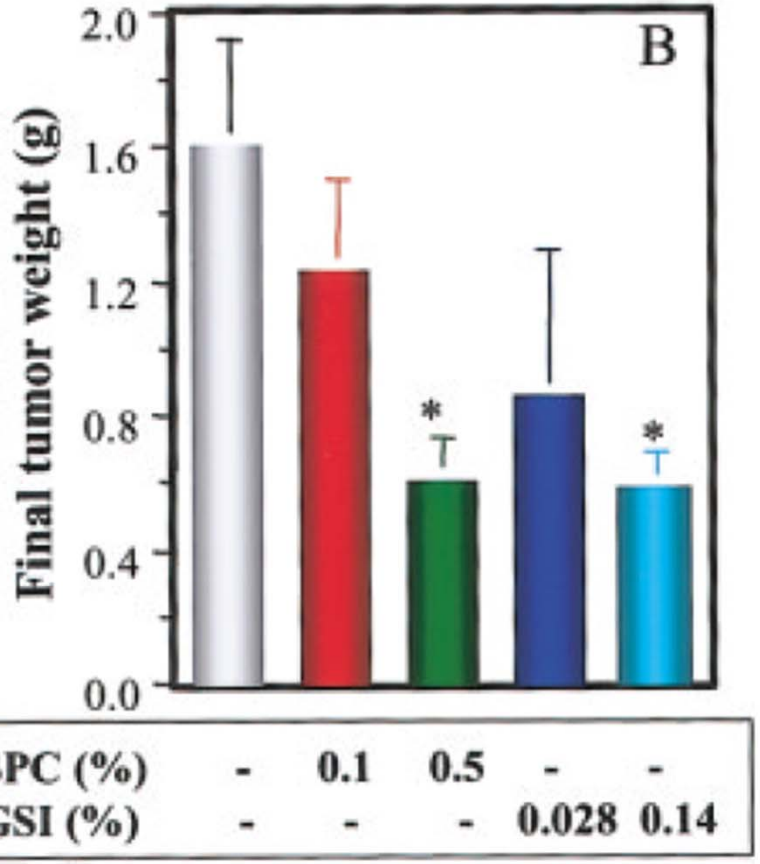
Effect of a dietary genistin-rich soy isoflavones mix and soya protein concentrate on MCF-7 tumour size in intact female scid mice predosed with estrogen and 8 weeks after injection of cells

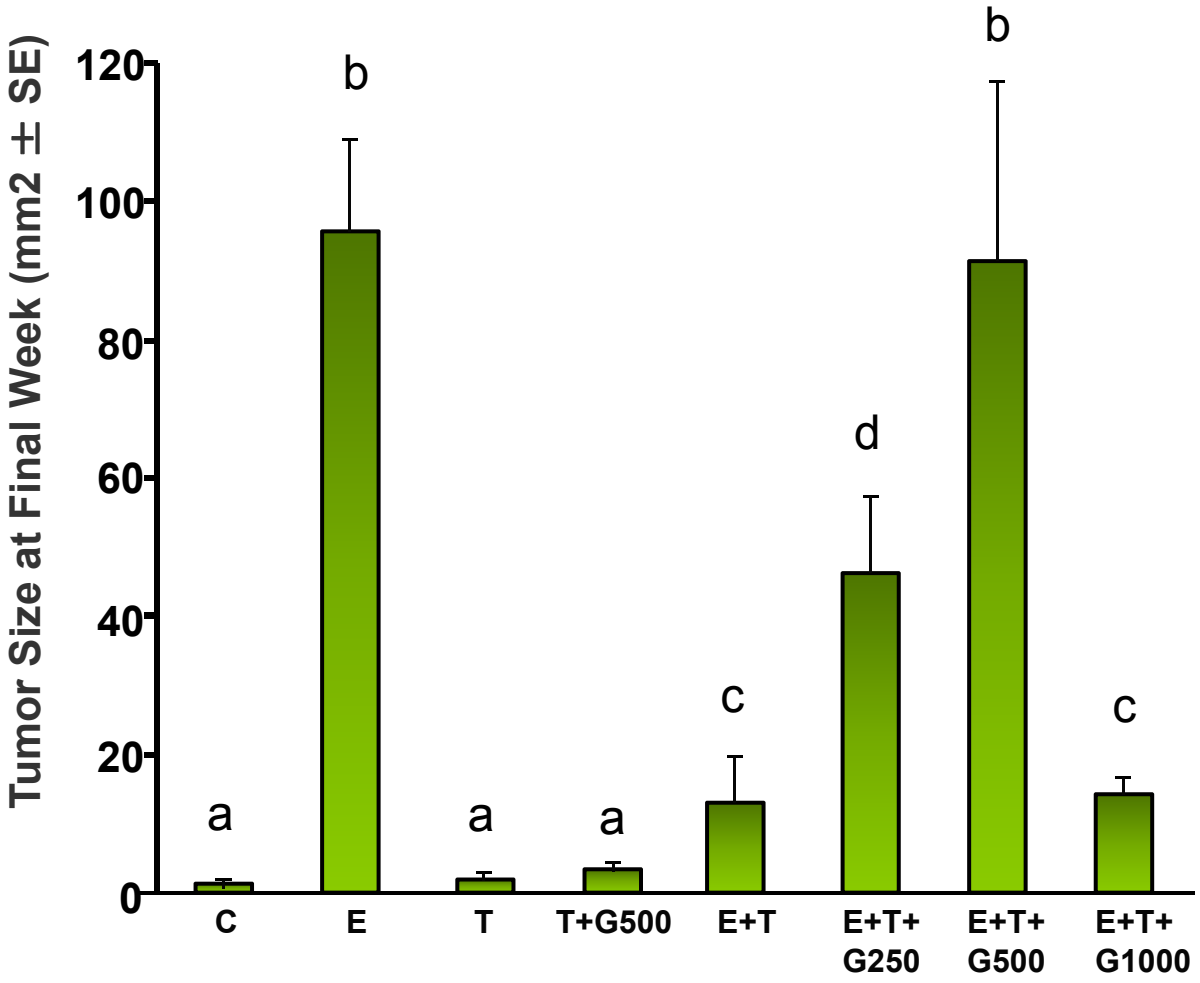




SPC – Soya Protein Concentrate
GSI – Genistin-rich Soy Isoflavone Mix

After Zhou *et al*., 2004

Interaction of Tamoxifen and genistein on MCF-7 tumours in ovariectomised athymic nude mice



Modified from Du *et al*., 2012

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Effect of soy and soy isoflavones on tumour proliferation, apoptosis and micro-vessel density in female scid mice injected with MCF-7 cells, and pre-dosed with estrogen

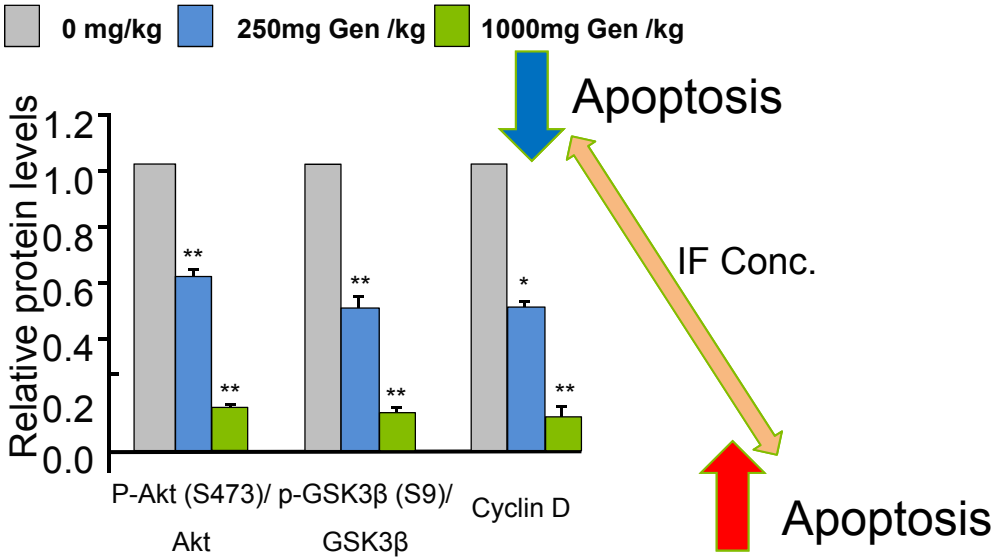
	Proliferation index (%)	Apoptotic index (%)	MVD (vessels/field)
Control	72.2 ± 4.3	3.32 ± 0.78	6.00 ± 2.65
SPC (0.1%)	48.7 ± 2.9[4]	4.98 ± 0.33	4.49 ± 1.29
SPC (0.5%)	46.3 ± 3.8[4]	4.39 ± 0.51	3.73 ± 0.81
GSI (0.028%)	47.1 ± 2.4[4]	4.34 ± 0.66	2.43 ± 0.72
GSI (0.14%)	45.3 ± 0.8[4]	4.94 ± 0.51	4.45 ± 1.61

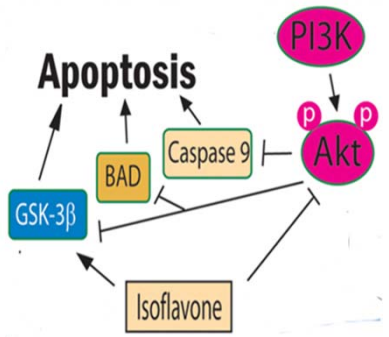
[1]Values are means ± SEM. Within the column, values with superscript are significantly different from the control value. MVD, microvessel density.–[2]$p < 0.05$.–[3]$p < 0.01$.–[4]$p < 0.005$.

SPC – Soya Protein Concentrate
GSI – Genistin-rich Soy Isoflavone Mix

After Zhou *et al*., 2004

Activation of the Akt pathway in the age-dependent prostate cancer progression in TRAMP/FVB and its inactivation by dietary genistein

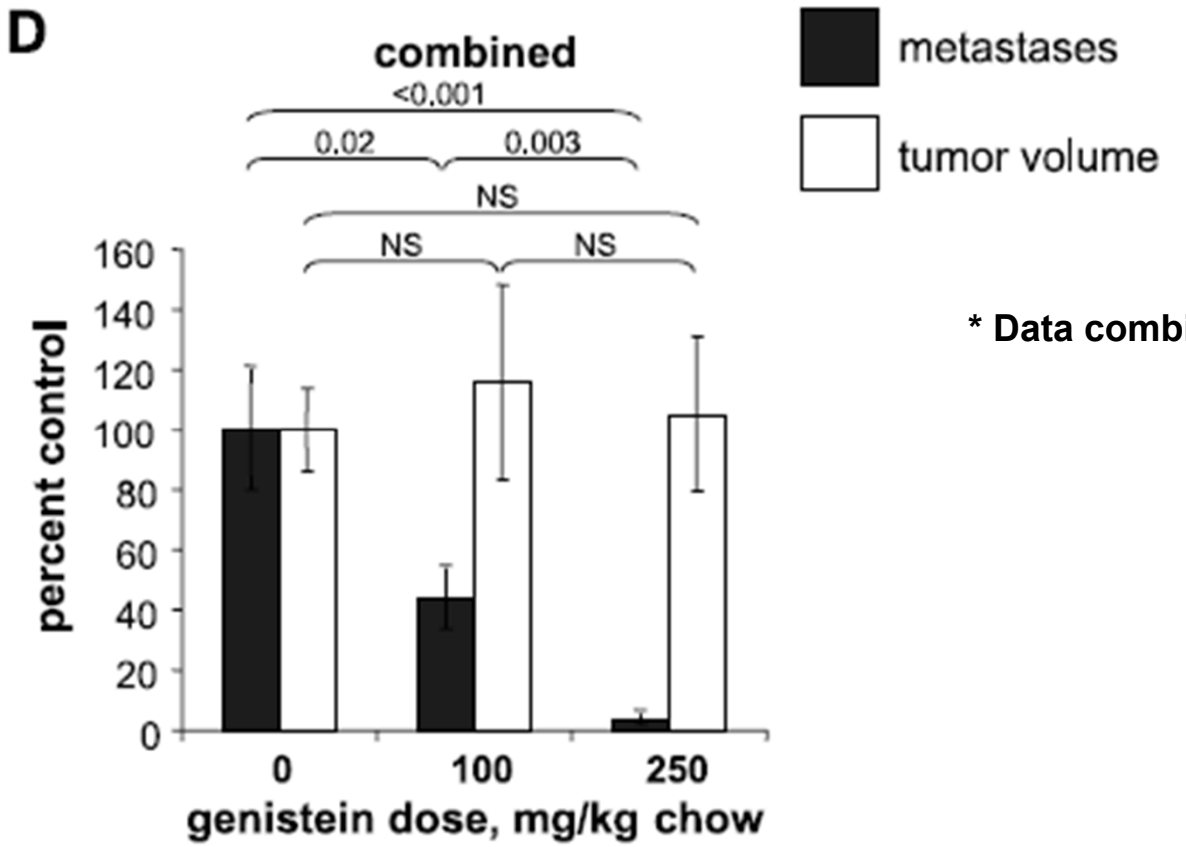




1. IF inhibit phosphorylation of Akt protein
2. Decreased phosphorylation of Akt protein lifts the inhibition of the GSK-3β system, BAD and Caspase thus increasing apoptosis and inhibiting tumour formation

Modified from Touny and Banerjee, 2007

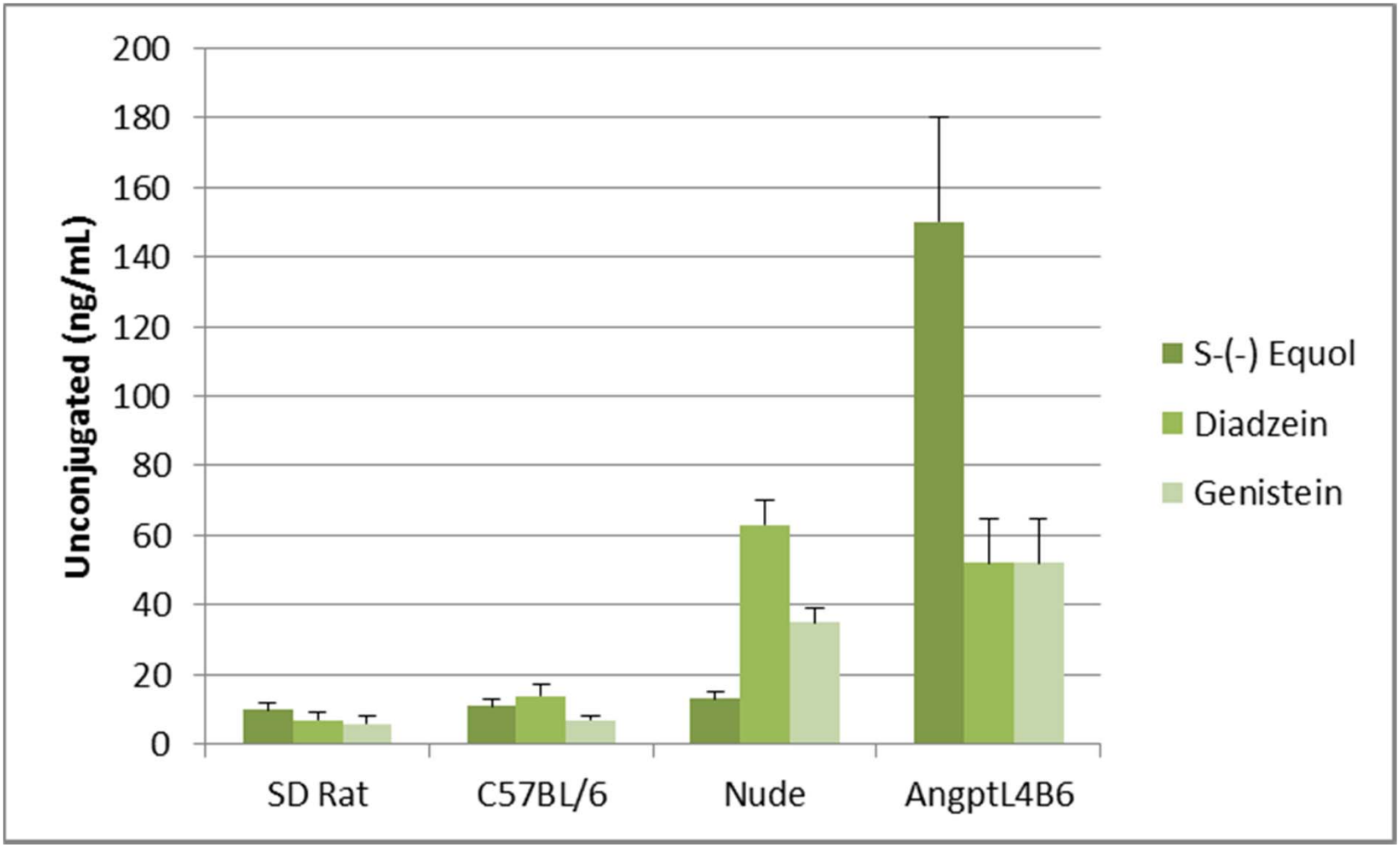
Genistein inhibition of prostate cancer metastasis in male athymic Balb/c mice



*** Data combined from two studies**

After Lakshman *et al*., 2008

Comparison of the steady state concentrations of unconjugated plasma isoflavones in adult Sprague-Dawley rats and in 3 different strains of mice that were fed commercial soy-containing diets



Adapted from Setchell *et al*., 2011

ENVIGO

Summary: The ideal diet for oncology

- Nude immunocompromised mice have a greater requirement for energy to compensate for a higher heat loss, and this amounts to about 20-50% over typical ambient temperatures
- This can probably be met through normal regulation of energy balance and the control of food intake, providing that food is easily available and eatable
- It is unlikely that the amount of dietary protein within reasonable limits (10-25% w/w) has a significant effect on growth of the animal or of tumours

Summary: The ideal diet for oncology

- Optimal tumour growth is likely to take place with moderate levels of carbohydrate and unsaturated fats, especially if the fat contains a high proportion of omega-6 fatty acids and low levels of omega-3 fatty acids
- Ideally fishmeal and fish oils should be avoided because of the relatively high levels of omega-3 fatty acids and, although a low risk, potentially carcinogenic levels of nitrosamines, dioxins, and other persistent organic pollutants
- Soybean meal (and alfalfa) should be excluded from the diet, and in regulatory studies is an OECD objective

Feeding rodent models used in oncology studies

Health monitoring and new oncology research models

Stephen Hillen, DVM
Director, Veterinary Science, Europe

Importance of health monitoring programs

FELASA guidelines

- Agreement/consensus in between users and breeders in Europe
- List of micro-organisms that can (but do not have to) influence research results should be mentioned in published articles
- FELASA makes a distinction in between immunocompetent and immunodeficient animals
- FELASA recommends 10 animals per quarter in an ‘open cage’ environment
- This will only detect with a certainty close to 100% infections with a prevalence of more than 30%

FELASA health monitoring recommendations vs Envigo barrier reporting (serology)

VIRUSES		TEST	Mouse (EU)		Rat (EU)		Hamster (US)		Guinea pig (EU)		Rabbit (EU)	
		METHOD (*)	A(1)	B(2)	A(1)	B(2)	A(1)	B(2)	A(1)	B(2)	A(1)	B(2)
Ectromelia (mouse pox) virus		Bead	1	12								
Guinea Pig Adenovirus	GpAd	ELISA							4	12		
Guinea Pig Cytomegalovirus	GpCMV	IFA							1	---		
Guinea Pig Parainfluenzavirus	GpPI-3	ELISA							4	---		
Hantaan Virus (3)	HTN	Bead / ELISA	---	12	1	12						
Kilham Rat Virus	KRV	ELISA			4	12						
Lactic Dehydrogenase-Elevating Virus (3)	LDEV	ELISA	---	1								
Lymphocytic Choriomeningitis Virus	LCM	Bead / ELISA	1	12	---	12	4	12	---	4		
Minute Virus of Mice	MVM	Bead	4	12								
Mouse Adenovirus type 1 (FL)	MAd	Bead / ELISA	1	12	1	12						
Mouse Adenovirus type 2 (K87)	MAd	Bead / ELISA	1	12	1	12						
Mouse Cytomegalovirus (3)	MCMV	Bead	---	12								
Mouse Hepatitis Virus	MHV	Bead	4	12								
Mouse K-virus (3)	K	ELISA	---	1								
Mouse Parvo Virus	MPV (NS-1)	Bead	4	12								
Mouse Polyoma virus (3,5)	POL	ELISA	---	1								
Mouse Rotavirus	EDIM	Bead	4	12								
Mouse Thymic Virus (3)	MTV	IFA	---	1								
Murine Noro Virus	MNV	Bead	4	12								
Pneumonia Virus of Mice (5)	PVM	Bead / ELISA	1	12	4	12	---	12				
Rabbit Haemorrhagic Disease Virus	RHDV	ELISA									4	12
Rabbit Pox Virus (Myxomatosis) (6)	Myxo	ELISA									---	12
Rabbit Rotavirus	Rota	ELISA									4	12
Rat Minute Virus	RMV	ELISA			4	12						
Rat Parvo Virus	RPV	ELISA			4	12						
Rat Respiratory Virus (see Pneumocystis)	RRV	PCR			1	12	---	4				
Rat Theilovirus	RTV	ELISA			4	12						
Reovirus Type 3	Reo 3	Bead / ELISA	1	12	1	12	---	12				
Sendai	Sendai	Bead / ELISA	1	12	1	12	4	12	4	12		
Simian virus 5	Simian	ELISA					---	12				
Sialodacryoadenitis / (Rat Coronavirus)	SDA/(RCV)	ELISA			4	12						
Theiler's Murine Encephalomyelitis Virus	TMEV (GD VII)	Bead	4	12								
Toolan H1	H1	ELISA			4	12						
Total tests			31	184	34	168	8	64	13	28	8	36

ENVIGO

FELASA Health monitoring recommendations vs Envigo barrier reporting (bacteriology)

Bacteria and fungi	TEST	Mouse (EU)		Rat (EU)		Hamster (US)		Guinea pig (EU)		Rabbit (EU)	
	METHOD	A(1)	B(2)	A(1)	B(2)	A(1)	B(2)	A(1)	B(2)	A(1)	B(2)
Bordetella bronchiseptica (4)	Culture			---	12	---	4	4	12	4	12
CAR bacillus (3,5)	ELISA	---	1	1	12					1	---
Campylobacter jejuni	PCR					---	4				
Citrobacter rodentium	Culture	1	12								
Citrobacter bovis (HAC)	PCR	Immune deficient animals + SOPF									
Chlamydia psittaci	IFA							---	12		
Clostridium piliforme	Bead / ELISA	1	12	4	12	1	12	1	4	4	12
Corynebacterium kutscheri (4)	Culture	1	12	---	12	1	4	4	12		
Dermatophytes (5,7)	Culture							---	12		12
Helicobacter spp. (all subspecies)	PCR	4	12	4	12	1	4				
Klebsiella pneumoniae (3,4)	Culture	Immune deficient animals + SOPF				---	4				
Klebsiella oxytoca (3,4)	Culture	Immune deficient animals + SOPF				---	4				
Lawsonia intracellularis (6)	PCR					---	4				
Mycoplasma pulmonis	Bead/ELISA/PCR	1	12	4	12	---	4				
Pasteurellaceae (3,4,5,6,7)	Culture										
Pasteurella spp.	Culture	---	12	---	12			---	12	---	12
Pasteurella multocida	Culture	---	12	---	12			---	12	4	12
Pasteurella pneumotropica	Culture	4	12	4	12	4	4	---	12	---	12
Pneumocystis spp (4)	PCR			---	12	---	4				
Pneumocystis carinii (RRV) / murina (3)	PCR	Imm.Def. + SOPF	1	1	12						
Pseudomonas aeruginosa (3,4,5)	Culture	Immune deficient animals + SOPF				---	4				
Proteus spp.	Culture	Immune deficient animals + SOPF									
Salmonella spp.	Culture	1	12	1	12	1	12	1	12	1	12
Staphylococcus aureus (3,4,5,7)	Culture	Immune deficient animals + SOPF				---	4				
Streptobacillus moniliformis	Culture	1	12	1	12			1	12		
Streptococci Beta-haemolytic (not Group D)	Culture	4	---	4	---			4	12		
Streptococci Beta-haemolytic (A and/or G Group)	Culture	---	12	---	12						
Streptococci Beta-haemolytic (Group B)						---	4				
Streptococcus pneumoniae	Culture	4	12	4	12	---	4	4	12		
Yersinia pseudotuberculosis (5)	Culture							---	12		
Treponema cuniculi (7)	IFA									---	12
Total tests		22	145	28	168	8	80				

ENVIGO

FELASA Health monitoring recommendations vs Envigo barrier reporting (parasitology)

Parasitology	TEST	Mouse (EU)		Rat (EU)		Hamster (US)		Guinea pig (EU)		Rabbit (EU)	
	METHOD	A(1)	B(2)	A(1)	B(2)	A(1)	B(2)	A(1)	B(2)	A(1)	B(2)
Ectoparasites (5)	Micr.	4	12	4	12	4	12	4	12	4	12
Endoparasites (6)	Micr.	4	12	4	12	4	12	4	12	4	12
Encephalitozoon cuniculi (3,4)	ELISA	---	1			---	4	1	12	4	12
Total tests		8	25	8	24	8	28	9	36	12	36

(1,2) Frequency per year A(1) = FELASA, B(2) = ENVIGO (3) = FELASA Optional for rats (4) = FELASA Optional for hamsters

(5) Ectoparasite screening includes:	(6) Endoparasite screening includes:
Glericola porcelli	Aspicularis tetraptera
Myocoptes musculinus	Balantidum sp.
Myobia musculi	Chilomastix sp.
Octodectes cyanotis	Cryptosporidia
Radfordia ensifera	Dentostomella translucida
Sarcoptes scabiei	Eimeria sp.
	Entamoebe sp.
	Giardia sp.
	Hymenolepis nana
	Spironucleus sp.
	Syphacia muris
	Syphacia oblevata
	Trichomonas sp.
	Tritrichomonas sp.

Health monitoring program: What are we looking for?

- Serology - serum (ELISA - HI - IFA - MFI/Bead)/indirect
- Bacteriology - culture (direct)/serum/PCR
- Parasitology - micro/macroscopic/serum
- Pathology - micro/macroscopic

Health monitoring program

- Frequency and profile types
- Age (sensitivity)
- Strain (sensitivity)
- Test method (sensitivity)
- A ‘good’ health monitoring program requests a statistically significant sample size and an adequate frequency
- Sample size (at NL 12 mice - 10 rats per barrier/month + bedding Sentinels)
- FELASA Recommendations (Nicklas *et al*., 2002; Maehler *et al*., 2014)
- Additional organisms related to study type (on request)
- Build up historic data as prevalence differ

Sample size - Detection limits

Confidence Limits for Detecting Infection using Different Sample Sizes and Assumed Rates of Infection[a]

	Assumed Infection Rate (%)											
Sample size (N)[b]	1	2	3	4	5	10	15	20	25	30	40	50
5	0.05	0.10	0.14	0.18	0.23	0.41	0.56	0.67	0.76	0.83	0.92	0.97
10	0.10	0.18	0.26	0.34	0.40	0.65	0.80	0.89	0.94	0.97	0.99	
15	0.14	0.26	0.37	0.46	0.54	0.79	0.91	0.95	0.99			
20	0.18	0.33	0.46	0.56	0.64	0.88	0.95	0.99				
25	0.22	0.40	0.53	0.64	0.72	0.93	0.98					
30	0.25	0.45	0.60	0.71	0.79	0.96	0.99					
35	0.30	0.51	0.66	0.76	0.83	0.97						
40	0.33	0.55	0.70	0.80	0.87	0.99						
45	0.36	0.69	0.75	0.84	0.90	0.99						
50	0.39	0.64	0.78	0.87	0.92	0.99						
60	0.45	0.70	0.84	0.91	0.95							
70	0.51	0.76	0.88	0.94	0.97							
80	0.55	0.80	0.91	0.96	0.98							
90	0.60	0.84	0.94	0.97	0.99							
100	0.63	0.87	0.95	0.98	0.99							
120	0.70	0.91	0.97	0.99								
140	0.76	0.94	0.99									
160	0.80	0.96	0.99									
180	0.84	0.97										
200	0.87	0.98										

[a] ILAR, 1976

[b] $N = \frac{\log(1 - \text{probability of detecting infection})}{\log(1 - \text{assumed infection rate})}$

Dr. Colin White and Department of Epidemiology and Public Health at Yale University and the year 1976

Overview Envigo health monitoring profiles mice

Health Monitoring profiles for Envigo immunocompetent mouse stocks and strains bred in barriers. **Category - FELASA M-I**

M-I	M-II(J)	M-IA
Ectromelia virus	Ectromelia virus	Ectromelia virus
Hantavirus	Hantavirus	Hantavirus
Lymphocytic choriomeningitis virus	Lymphocytic choriomeningitis virus	Lactic Dehydrogenase-Elevating Virus
Minute virus of mice	Minute virus of mice	Lymphocytic choriomeningitis virus
Mouse adenovirus type 1 (Mad FL)	Mouse adenovirus type 1 (Mad FL)	Minute virus of mice
Mouse adenovirus type 2 (Mad K87)	Mouse adenovirus type 2 (Mad K87)	Mouse adenovirus type 1 (Mad FL)
Mouse cytomegalovirus	Mouse cytomegalovirus	Mouse adenovirus type 2 (Mad K87)
Mouse hepatitis virus	Mouse hepatitis virus	Mouse cytomegalovirus
Mouse norovirus	Mouse norovirus	Mouse hepatitis virus
Mouse parvovirus	Mouse parvovirus	Mouse K virus
Mouse rotavirus (EDIM)	Mouse rotavirus (EDIM)	Mouse norovirus
Pneumonia virus of mice	Pneumonia virus of mice	Mouse parvovirus
Reovirus type 3	Reovirus type 3	Mouse rotavirus (EDIM)
Sendai virus	Sendai virus	Mouse thymic virus (MTV)
Theiler's murine encephalomyelitis virus	Theiler's murine encephalomyelitis virus	Pneumonia virus of mice
		Polyoma virus
		Reovirus type 3
		Sendai virus
		Theiler's murine encephalomyelitis virus
Bordetella bronchiseptica	*Bordetella bronchiseptica*	
Citrobacter rodentium	*Citrobacter rodentium*	
Clostridium piliforme	*Clostridium piliforme*	
Corynebacterium kutscheri	*Corynebacterium kutscheri*	
Escherichia coli (haemolytic)	*Escherichia coli* (haemolytic)	
Helicobacter spp	*Helicobacter* spp	
Corynebacterium bovis (HAC)	*Corynebacterium bovis* (HAC)	
Klebsiella oxytoca	*Klebsiella oxytoca*	
Klebsiella pneumonia	*Klebsiella pneumonia*	
Mycoplasma pulmonis	*Mycoplasma pulmonis*	
Pasteurella spp	*Pasteurella* spp	
Pneumocystis spp	*Pneumocystis* spp	
Proteus spp	*Proteus* spp	
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	
Salmonella spp	*Salmonella* spp	
Staphylococcus aureus	*Staphylococcus aureus*	
Streptobacillus moniliformis	*Streptobacillus moniliformis*	
Streptococci Beta-haemolytic (group A and/or G)	Streptococci Beta-haemolytic (group A and/or G)	
Streptococcus pneumoniae	*Streptococcus pneumoniae*	
Yersinia pseudotuberculosis	*Yersinia pseudotuberculosis*	
		CAR Bacillus
		Citrobacter rodentium
		Clostridium piliforme
		Corynebacterium kutscheri
		Helicobacter spp
		Mycoplasma pulmonis
		Pasteurella spp
		Salmonella spp
		Streptobacillus moniliformis
		Streptococci Beta-haemolytic (group A and/or G)
		Streptococcus pneumoniae
Ectoparasites	Ectoparasites	Ectoparasites
Endoparasites	Endoparasites	*Encephalitozoon cuniculi*
		Endoparasites
Performed 11 times a year on barrier animals	Performed 12 times a year on barrier juvenile animals	Performed once a year on barrier animals

Health Monitoring profiles for Envigo immunodeficient nude mouse stocks and strains bred in isolators. **Category – FELASA M X**

M NUDE	M SENTINEL FULL	M SENTINEL SHORT
Ectromelia virus	Ectromelia virus	Ectromelia virus
Hantavirus	Hantavirus	Hantavirus
Lymphocytic choriomeningitis virus	Lymphocytic choriomeningitis virus	Lymphocytic choriomeningitis virus
Minute virus of mice	Minute virus of mice	Minute virus of mice
Mouse adenovirus type 1 (Mad FL)	Mouse adenovirus type 1 (Mad FL)	Mouse adenovirus type 1 (Mad FL)
Mouse adenovirus type 2 (Mad K87)	Mouse adenovirus type 2 (Mad K87)	Mouse adenovirus type 2 (Mad K87)
Mouse cytomegalovirus	Mouse cytomegalovirus	Mouse cytomegalovirus
Mouse hepatitis virus	Mouse hepatitis virus	Mouse hepatitis virus
Mouse norovirus	Mouse norovirus	Mouse norovirus
Mouse parvovirus	Mouse parvovirus	Mouse parvovirus
Mouse rotavirus (EDIM)	Mouse rotavirus (EDIM)	Mouse rotavirus (EDIM)
Pneumonia virus of mice	Pneumonia virus of mice	Pneumonia virus of mice
Reovirus type 3	Reovirus type 3	Reovirus type 3
Sendai virus	Sendai virus	Sendai virus
Theiler's murine encephalomyelitis virus	Theiler's murine encephalomyelitis virus	Theiler's murine encephalomyelitis virus
Bordetella bronchiseptica	*Bordetella bronchiseptica*	*Bordetella bronchiseptica*
Citrobacter rodentium	*Citrobacter rodentium*	*Citrobacter rodentium*
Clostridium piliforme	*Clostridium piliforme*	*Clostridium piliforme*
Corynebacterium kutscheri	*Corynebacterium kutscheri*	*Corynebacterium kutscheri*
Escherichia coli (haemolytic)	*Escherichia coli* (haemolytic)	*Escherichia coli* (haemolytic)
Helicobacter spp	*Helicobacter* spp	*Helicobacter* spp
Corynebacterium bovis (HAC)	*Corynebacterium bovis* (HAC)	*Corynebacterium bovis* (HAC)
Klebsiella oxytoca	*Klebsiella oxytoca*	*Klebsiella oxytoca*
Klebsiella pneumonia	*Klebsiella pneumonia*	*Klebsiella pneumonia*
Mycoplasma pulmonis	*Mycoplasma pulmonis*	*Mycoplasma pulmonis*
Pasteurella spp	*Pasteurella* spp	*Pasteurella* spp
Pneumocystis spp	*Pneumocystis* spp	*Pneumocystis* spp
Proteus spp	*Proteus* spp	*Proteus* spp
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
Salmonella spp	*Salmonella* spp	*Salmonella* spp
Staphylococcus aureus	*Staphylococcus aureus*	*Staphylococcus aureus*
Streptobacillus moniliformis	*Streptobacillus moniliformis*	*Streptobacillus moniliformis*
Streptococci Beta-haemolytic (group A and/or G)	Streptococci Beta-haemolytic (group A and/or G)	Streptococci Beta-haemolytic (group A and/or G)
Streptococcus pneumoniae	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*
Yersinia pseudotuberculosis	*Yersinia pseudotuberculosis*	*Yersinia pseudotuberculosis*
Ectoparasites	Ectoparasites	Ectoparasites
Endoparasites	Endoparasites	Endoparasites
Performed 6 times a year on nude juveniles	Performed 2 times a year on haired animals	Performed 3 times a year on haired animals

ENVIGO

Isolator report
Immunodef. mice

- Compared to barrier reared
  - Klebsiella (oxytoca/pneumonia)
  - Proteus spp
  - Pseudomonas aeruginosa
  - Staphylococcus aureus
  - Hyperkeratinosis Associated
  - Corynebacterium spp (HAC)

Facility	Isolator			Species		
Envigo RMS S.r.l.	Isolator 26			Mouse		
Viruses	**Test Frequency**	**Latest Test Date**	**Latest Results**	**Testing Laboratory**	**Test Method**	**Historical Results***
Ectromelia virus	6 months	22.03.16	0/3	Italy	Bead	0/9
Hantavirus	6 months	22.03.16	0/3	Italy	Bead	0/9
Lactate dehydrogenase elevating virus	12 months	19.01.16	0/3	Italy	ELISA	0/6
Lymphocytic choriomeningitis virus	6 months	22.03.16	0/3	Italy	Bead	0/9
Minute virus of mice	2 months	17.05.16	0/3	Italy	Bead	0/30
Mouse adenovirus type 1 (MAd FL)	6 months	22.03.16	0/3	Italy	Bead	0/9
Mouse adenovirus type 2 (MAd K87)	6 months	22.03.16	0/3	Italy	Bead	0/9
Mouse cytomegalovirus	6 months	22.03.16	0/3	Italy	Bead	0/9
Mouse hepatitis virus	2 months	17.05.16	0/3	Italy	Bead	0/30
Mouse K-virus	12 months	19.01.16	0/3	Italy	ELISA	0/6
Mouse Norovirus	6 months	22.03.16	0/3	Italy	Bead	0/9
Mouse parvovirus	2 months	17.05.16	0/3	Italy	Bead	0/30
Mouse polyoma virus	12 months	19.01.16	0/3	Italy	ELISA	0/6
Mouse rotavirus (EDIM)	2 months	17.05.16	0/3	Italy	Bead	0/30
Mouse thymic virus	12 months	19.01.16	0/3	Italy	IFA	0/6
Pneumonia virus of mice	2 months	17.05.16	0/3	Italy	Bead	0/30
Reovirus type 3 (Reo 3)	6 months	22.03.16	0/3	Italy	Bead	0/9
Sendai virus	2 months	17.05.16	0/3	Italy	Bead	0/30
Theiler's murine encephalomyelitis virus	2 months	17.05.16	0/3	Italy	Bead	0/30
Bacteria, Mycoplasma and Fungi						
CAR Bacillus	12 months	19.01.16	0/3	Italy	ELISA	0/6
Citrobacter rodentium	2 months	17.05.16	0/6	Italy	Culture	0/60
Clostridium piliforme	2 months	17.05.16	0/3	Italy	Bead	0/30
Corynebacterium kutscheri	2 months	17.05.16	0/6	Italy	Culture	0/60
Helicobacter spp	2 months	17.05.16	0/3	Italy	PCR	0/30
Hyperkeratinosis Associated *Corynebacterium* spp	2 months	17.05.16	0/3	Italy	Culture/PCR	0/30
Klebsiella oxytoca	2 months	17.05.16	0/6	Italy	Culture	0/60
Klebsiella pneumoniae	2 months	17.05.16	0/6	Italy	Culture	0/60
Mycoplasma pulmonis	2 months	17.05.16	0/3	Italy	Bead	0/30
Pasteurella spp	2 months	17.05.16	0/6	Italy	Culture	0/60
Pneumocystis spp	2 months	17.05.16	0/3	Italy	PCR	0/30
Proteus sp	2 months	17.05.16	0/6	Italy	Culture	0/60
Pseudomonas aeruginosa	2 months	17.05.16	0/6	Italy	Culture	0/60
Salmonella spp	2 months	17.05.16	0/6	Italy	Culture	0/60
Staphylococcus aureus	2 months	17.05.16	0/6	Italy	Culture	0/60
Streptobacillus moniliformis	2 months	17.05.16	0/6	Italy	Culture	0/60
Streptococci Beta-haemolytic (group A and/or G)	2 months	17.05.16	0/6	Italy	Culture	0/60
Streptococcus pneumoniae	2 months	17.05.16	0/6	Italy	Culture	0/60
Parasites						
Ectoparasites	2 months	17.05.16	0/6	Italy	Microscopy	0/60
Encephalitozoon cuniculi	12 months	19.01.16	0/3	Italy	ELISA	0/6
Endoparasites	2 months	17.05.16	0/6	Italy	Microscopy	0/60
Pathological Lesions						
External	2 months	17.05.16	0/6	Italy	Pathology	0/60
Necropsy	2 months	17.05.16	0/6	Italy	Pathology	0/60

ENVIGO

Health monitoring SCID mice

- Meeting the FELASA 2014 recommendations
- Screening frequency: every 2 months
- Fixed number of mice
- Fixed screening profiles
    - Scid and sentinel full/short

In general immunodeficient mice must be negative for opportunistic bacteria, to include: *Klebsiella* spp., *Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus* spp.

Health monitoring complication - SCIDs

- Problems with serology
- Sentinel programme using Hsd:NIHBS
- SCID isolators have sentinel animals in the same isolator
  So all data on the same report

Mouse Strains Held In Isolator
Mutant Mice
C.B-17/IcrHan®Hsd-*Prkdc*^{scid}
Sentinel (Outbred) Mice
Hsd:NIHBS

Summary of Findings

No significant findings.

Serological testing for antibodies to pathogenic agents in *scid* mice is not appropriate because of a lack of a fully functioning immune system in these mice. Consequently, the serology results for the scid mice held in this Isolator are obtained using sentinel animals.

Report Reference: 15-2521, Report Printed: December 21, 2015, Isolator Populated: March 25, 2008
Data are expressed as number positive/number tested. *Cumulative data up to 18 months.
All testing is performed by Envigo using standard operating procedures.
Results are controlled through a secure LIMS and released on the approval of the Laboratory Services Manager.

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Health monitoring nude mice

- Meeting the FELASA 2014 Recommendations
- Screening frequency: every 2 months
- Fixed number of mice
- Fixed screening profiles
  - Nude and sentinel full/short
  - In this case the sentinel is the haired heterozygous mouse

In general immunodeficient nude mice must be negative for opportunistic bacteria, to include: *Klebsiella* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus* spp. and *Corynebacterium* bovis (HAC)

Isolator husbandry

- Isolators and equipment sterilized at set-up
- 3 months consecutive health testing prior to release for sale
- Sterilized equipment and irradiated consumables
- Weekly cage change
- Automatic water
  - 0.2 micron filtered
  - Chlorinated to 8-10 ppm
  - Acidified to pH of 5.8-6.0
- Irradiated Teklad Diet and bedding (Global Diet 2918 or 2919)

Model selection considerations

What are researchers looking for in a model?

- Will it **grow** a tumor?
- What will **take rate** of tumor be?
- Will the model become **leaky**?
- Docile, easy to handle
- Acceptable health status
- Economical
- **Robust**, vigorous
- Availability

Considerations in choosing optimal model

Nudes

- Inexpensive (outbred)
- Robust model (outbred)
- Mild temper/docile
- Hairlessness - easier to observe/measure tumor growth
- High throughput, economical for drug screening

SCIDs

- Multiple mutations/defects = €
- Increased sensitivity to irradiation & cytotoxic agents
- Some stocks/strains more aggressive (NOD.SCID, e.g.)
- Haired (except SHrN) - not desirable for imaging
- Models with combined mutations are useful for study of immunological role in tumor growth & compound efficacy

New oncology models

SHrNTM model: Background

- S Hr N = “SCID” “Hairless” “NOD” mouse
- Developed by Harlan (renamed Envigo)
- Created by backcrossing the hairless (Hrhr) mutation on to a NOD.SCID mouse
- Hair loss commences at approximately 10 days of age
  - Complete hair loss by 5-6 weeks of age
- Nomenclature
  - NOD.Cg-Prkdcscid Hrhr/NCrHsd

SHrNTM model: Characteristics

- SHrNTM ("Shorn") Hairless NOD.SCID mouse
  - Triple-immunodeficient model with distinct benefits, well-suited for tumor xenografts
    - Deficient in T and B cells
    - NK cell functional deficit
    - No circulating complement
    - Decreased macrophage function
    - Decreased granulocyte function
    - Decreased dendritic cells

www.envigo.com/shrn

SHrN™ model: Benefits

- Severely Immunocompromised
  - More immunocompromised than other hairless SCID models
  - Preliminary phenotyping results show promising advantages in cell uptake and tumor growth
  - Decreased leakiness compared to other SCID models
  - Enhanced acceptance and growth of cells for humanization
- Inbred
  - Less variability in research
- Hairless
  - Eliminates need to shave model, saving time and labor
  - Improved tumor imaging clarity and observation of tumor growth

Flow cytometry: Summary data

Models (Females) 6-7 Weeks Old	Average Percentages								
Model Name	% B Cells	% NK Cells	% Dendritic Cells	% NKT Cells	% T Helper	% T Cells	% Granulocytes	% Macrophages	% Delta Gamma T
NOD SCID (n=5)	0.29	16.35	2.56	0.14	0.01	0.04	15.09	9.80	0.23
SCID Beige (n=10)	0.29	6.46	0.81	0.01	0.07	0.01	10.35	12.36	0.01
SHrN Female (n=10)	0.32	10.14	1.25	0.19	0.03	0.02	25.19	9.12	0.53
NOG Female (n=6)	0.04	0.02	3.40	0.07	0.00	0.01	8.58	4.43	0.02

Models (Males) 6-7 Weeks Old	Average Percentages								
Model Name	% B Cells	% NK Cells	% Dendritic Cells	% NKT Cells	% T Helper	% T Cells	% Granulocytes	% Macrophages	% Delta Gamma T
SHrN Male (n=10)	0.41	7.82	0.78	Error	0.29	0.57	22.17	7.08	0.11
NOG Male (n=5)	0.09	0.03	1.38	Error	0.10	0.46	6.71	0.05	0.03

Key Point: NK Cells & Dendritic Cells are the main differences between the SHrN & NOG*

**Flow cytometry experiment conducted by UMass*

SHrN™ model: Cell line references

- Our scientists summarized the publication data on the SHrN into a simple chart
- Research details can be located on our cell line chart tool or the SHrN model web page

Referenced Envigo model

Human Tissue	Cell Lines*	Mice
Breast	MCF-7 [354]	SHrN™
	MDA-MB-231 [354]	SHrN™
Lung	NCI-H69 [354]	SHrN™
	NCI-H841 [354]	SHrN™
Skin	(HPV16)BC-1-Ep/SL-LF [314]	SHrN™

*Visit www.envigo.com/onco for links to publications

Peer-reviewed publications show proven performance in multiple lung and breast cancer cell lines

R2G2 (Rag2 DKO) model: Background

- **R2G2** = "**R**ag**2**" "IL**2**R**G**" **double** knockout mouse
- Model licensed from an institution in the United States
- Created by backcrossing the IL2RG (common gamma chain) mutation on to a Rag2 mouse
- Background is a mixture of C57 and 129
- Nomenclature:
  - B6;129-*Rag2*^{tm1Fwa}*Il2rg*^{tm1Rsky}/DwlHsd

R2G2 (Rag2 DKO) model: Characteristics

- Ultra (triple) immunodeficient mouse model
  - Deficient in T and B cells
  - Deficient in NK cells
  - No circulating complement
  - Decreased macrophage function
- Reduced leakiness
  - Reduced leakiness as compared to other SCID mice (varies with background)
- Less radiosensitivity
  - Less sensitive to radiation as compared to SCID mice (including NOG/NSG)
- Flow cytometry data
  - Overall results similar to NOG mouse*
  - NK cell counts at level similar

**Must repeat flow cytometry data with larger sample sizes*

Health monitoring and new oncology research models

Thank You

ENVIGO

Come and visit us at our booth

F001/003 and E002/004