Historical Control Database of Spontaneous Tumors in Transgenic Tg.rasH2 Mice

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TgRasH2 Mouse Model

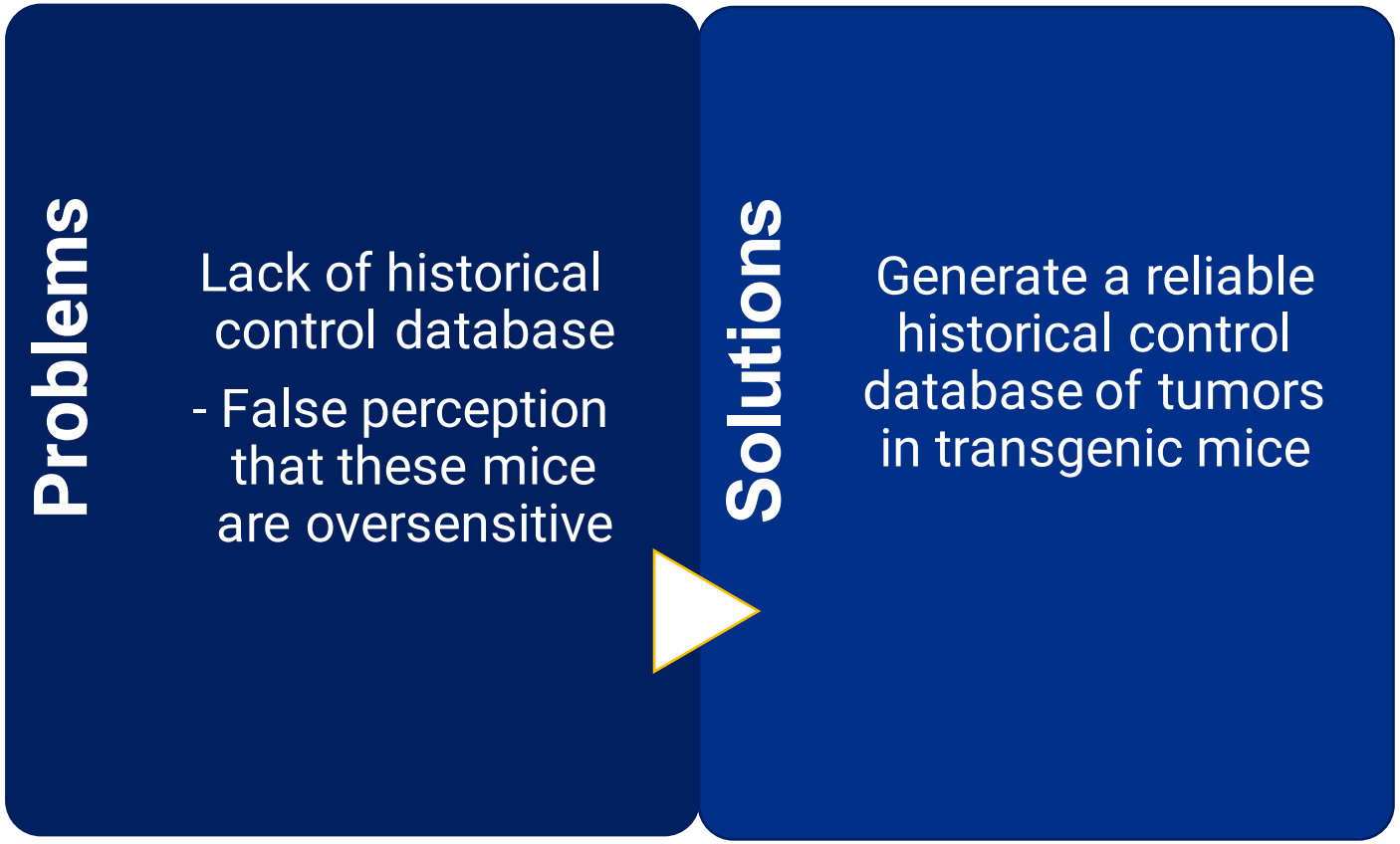
- CByB6F1-Tg(HRAS)2Jic (hemizygous c-Ha-ras) mice, obtained from Taconic Farms (Germantown, New York), were used in all the studies.
  - The knock-in Tg element (human prototype c-Ha-ras gene with its own promoter/enhancer) is injected into C57BL/6 x BALB/c F2 zygotes, which are crossed back to C57BL/6J forming C57BL/6JJic- Tg(HRAS)2Jic.
  - The CByB6F1-Tg(HRAS)2Jic (hemizy-gous c-Ha-ras) is the offspring from a cross of the C57BL/ 6JJic-Tg(HRAS)2Jic hemizygous male mice with the BALB/ cByJJic female mice.
- Each mouse was genotyped by Taconic to verify the presence of the transgene before being placed on study.
- There were a total of 52 studies conducted in male and 51 in female Tg.rasH2 Mice.
- 1615 male mice and 1560 female mice were examined in these studies.
- All studies were conducted at BioReliance.
- The opinions expressed in this talk are those expressed by speaker and co-authors.

Regulatory Clearance

- Tg.rasH2 mouse model was cleared by FDA for use in the 26-week carcinogenicity assays in 2002.
- BioReliance (BREL) did its first GLP study in Tg.rasH2 mouse model in 2003.
- However, the model was not accepted right away by the industry and its initial progress was painful.

(Morton, Daniel, *et al.*, 2002)

After the Tg.rasH2 Model was Available in 2003 It Took Nine–Ten Years for It to Become Acceptable



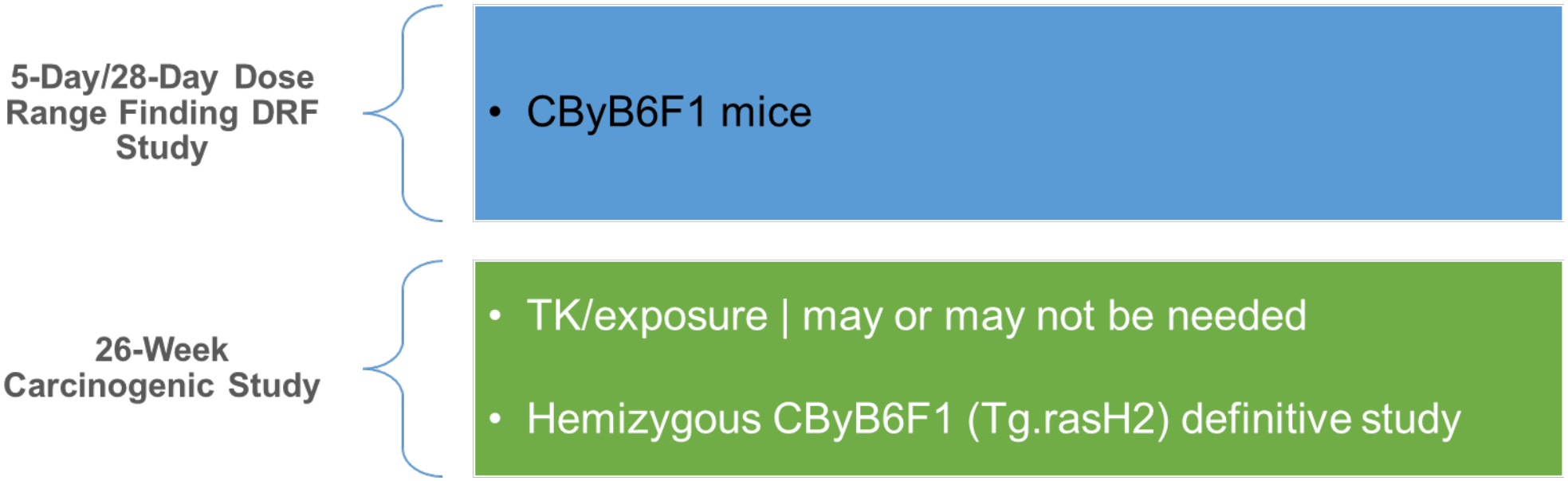
(Long, Gerald G., *et al.*, 2010)

(Storer, Richard D., *et al.*, 2010)

Publications that Gave a Boost to Tg.rasH2 Model Acceptance

- Nambiar, P. R., Turnquist, S. E., & Morton, D. (2012). Spontaneous tumor incidence in rasH2 mice: review of internal data and published literature. Toxicologic pathology, 40(4), 614–623. https://doi.org/10.1177/0192623311436181.
- Paranjpe, M. G., Elbekaei, R. H., Shah, S. A., Hickman, M., Wenk, M. L., & Zahalka, E. A. (2013). Historical control data of spontaneous tumors in transgenic CByB6F1-Tg(HRAS)2Jic (Tg.rasH2) mice. International journal of toxicology, 32(1), 48–57. https://doi.org/10.1177/1091581812471565.
- Paranjpe, Madhav G., Jessica L. Belich, Peter C. Mann, Marie E. McKeon, Reem H. Elbekai, Caren M. Brown, and Daniel J. Patrick. “A Comparison of Spontaneous Tumors in Tg.RasH2 Mice in 26-Week Carcinogenicity Studies Conducted at a Single Test Facility during 2004 to 2012 and 2013 to 2018.” Toxicologic Pathology 47, no. 1 (January 2019): 18–25. https://doi.org/10.1177/0192623318810202.

The 26-Week Carcinogenicity Study Process



Five-Day Study Design

- Conducted in wild-type CByB6F1 mice
- Generally, six dose groups including one vehicle and five test article treated groups
- 5 mice/sex/dose group
- The end points recorded include:
  - Initial and terminal body weights
  - Body weight
  - Clinical signs
  - Cage side observations
  - Unscheduled mortality
  - No necropsy or histopathology
- Used to define doses for the 28-day dose range finding studies

Dose Range Finder Study Design (28-Day) Main and TK Study Portions

- Conducted in wild type CByB6F1 mice.
- Main study: 10 mice/sex in each of the four groups (one vehicle and three test article treated groups)
- TK component: 5 mice/sex in vehicle dose groups and 20 mice/sex in test article treated groups, TK serum collection at protocol-defined time points
- For main study unscheduled terminations/deaths, full necropsy is performed.
- At study termination on Day 28, all surviving animals are necropsied (scheduled).
- Blood is collected for hematology and clinical pathology assessments.
- Organ weights are collected in the main study on protocol defined organs on scheduled deaths only.

Dose Range Finder Study Design Continued

- Entire animal is examined at necropsy and findings documented
- Protocol Required Tissues (PRT) are collected for each mouse and preserved in 10% Neutral Buffered Formalin (NBF)
- Microscopic examination of PRT from all terminal control and high dose animals and for all unscheduled deaths
- Potential target organs are read down in the lower dose groups
- At the end of the study, the Maximum Tolerated Dose (MTD) is determined for the 26-week study
- Parameters for MTD which are considered but not limited to are: mortality, BWG% differences, histopathology findings, clinical pathology findings, TK study findings, etc.

Tg.rasH2 Carcinogenicity Study Design (Six-Month)

- There are generally five groups:
  - One vehicle group
  - Three test article treated groups
  - One positive control group
- The positive control groups are treated with urethane or NMU: 10 mice/sex
- 25 mice/sex are used for the vehicle control and for each test article group
- Necropsies are performed on all unscheduled and scheduled deaths
- Organ weights are collected from PRT from all scheduled deaths
- PRT tissues are collected from all animals in 10% NBF
- PRT tissues are examined microscopically from all animals
- At the end of the study there is statistical analysis performed as defined in the protocol
- Generally, a peer review is performed on each study
- TK analysis may or may not be performed as defined in the protocol

Study Design: 26-Week

Group	Dose levels (mg/kg/day)	Number of Animals			
		Main Study (Tg mice)		TK Study (Wild-type)	
		Male	Female	Male	Female
Group 1 (Vehicle)	0	25	25	5	5
Group 2	LOW	25	25	20	20
Group 3	MID	25	25	20	20
Group 4	HIGH	25	25	20	20
Group 5 (Positive Control)	1000 (Urethane)	10	10	NA	NA
Total		110	110	65	65

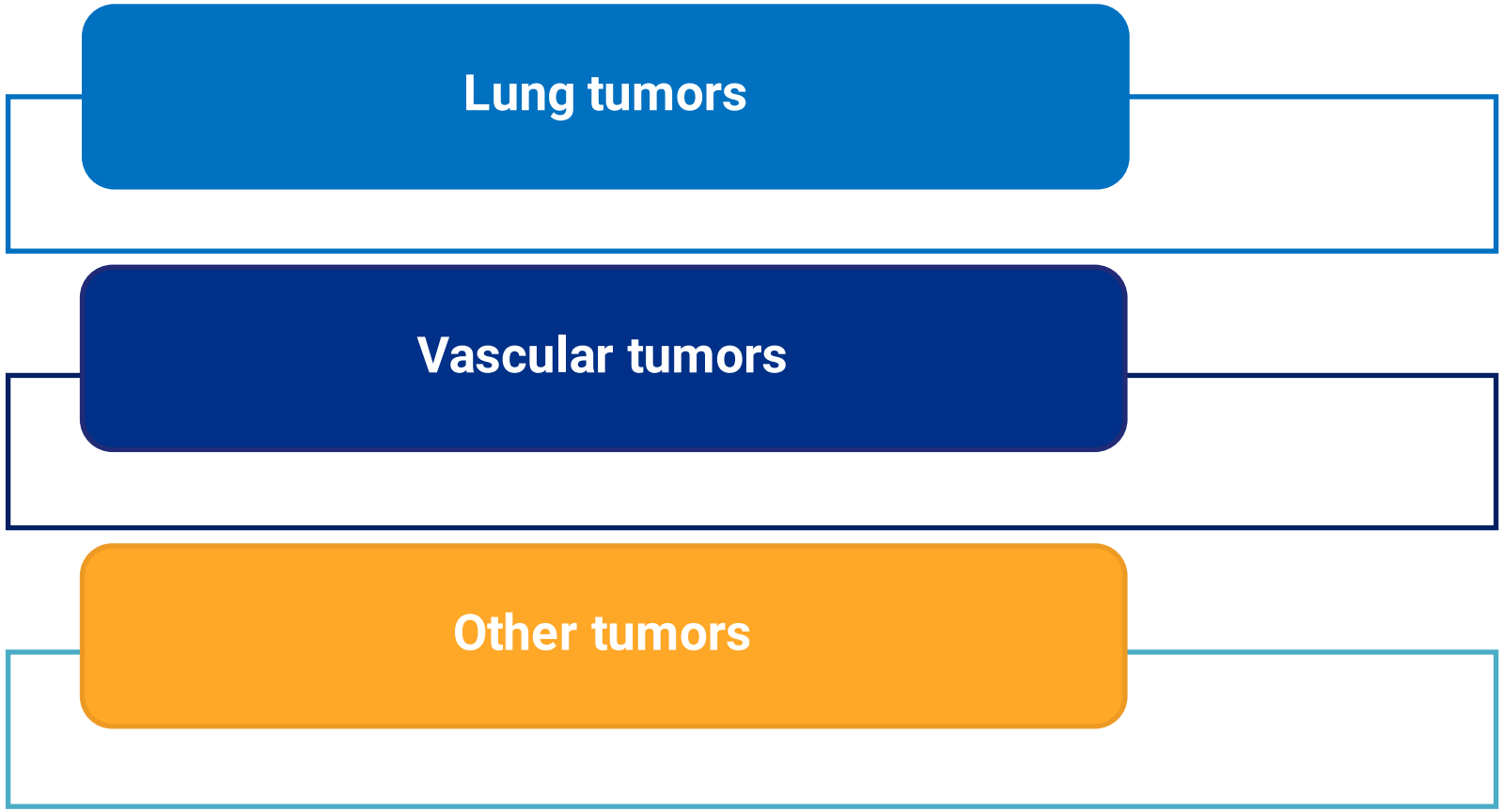
Negative control for unusual vehicles that have not previously been used in carcinogenicity studies.

Study Endpoints

Five- and 28-Day Studies in CByB6F1 Mice and 26-Week Studies in Tg.rasH2 Mice

Procedure	5-Day Study	28-Day Study		26-Week Study	
	Main Study	Main Study	TK Study	Main Study	TK Study
Body Weight	X	X	X	X	X
Moribundity and Mortality	X	X	X	X	X
Food Consumption	-	X	-	X	-
Daily Cage Side Observations	X	X	-	X	-
Detailed Clinical Observations	X	X	-	X	-
Clinical Chemistry (2) May add if used to est. MTD	-	X	-	-	-
Hematology (2) May add if used to est. MTD	-	X	-	-	-
Day 1 TK*	-	-	X	-	X
Term TK*	-	-	X	-	X
Full Necropsy and Gross Examination	-	X	-	X	-
Complete Histopathology (1) Read down if test article-related effects in high dose vs. control	-	X(1)	-	X-All	-

Tg.RasH2 |
Historical Control Database of Spontaneous Lesions

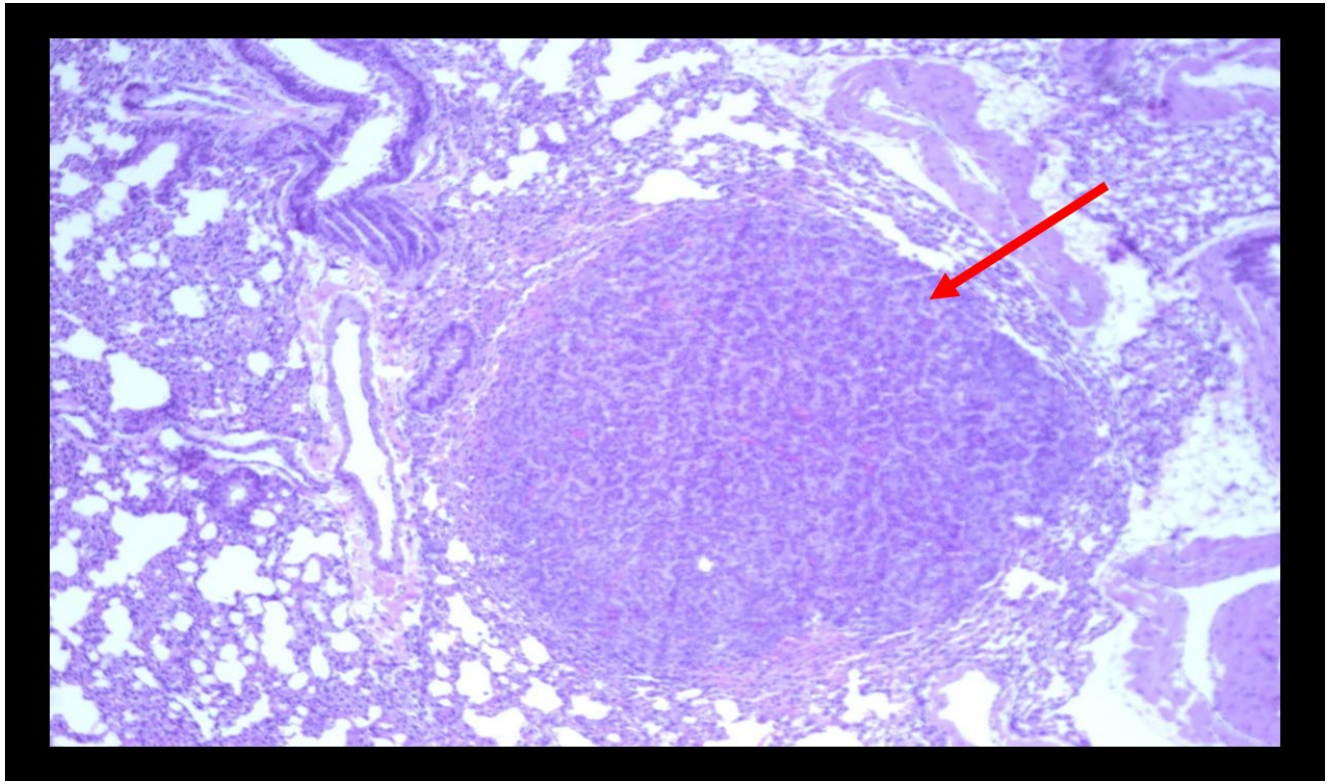


Lung Tumors

Lung nodules and a mass noted grossly:

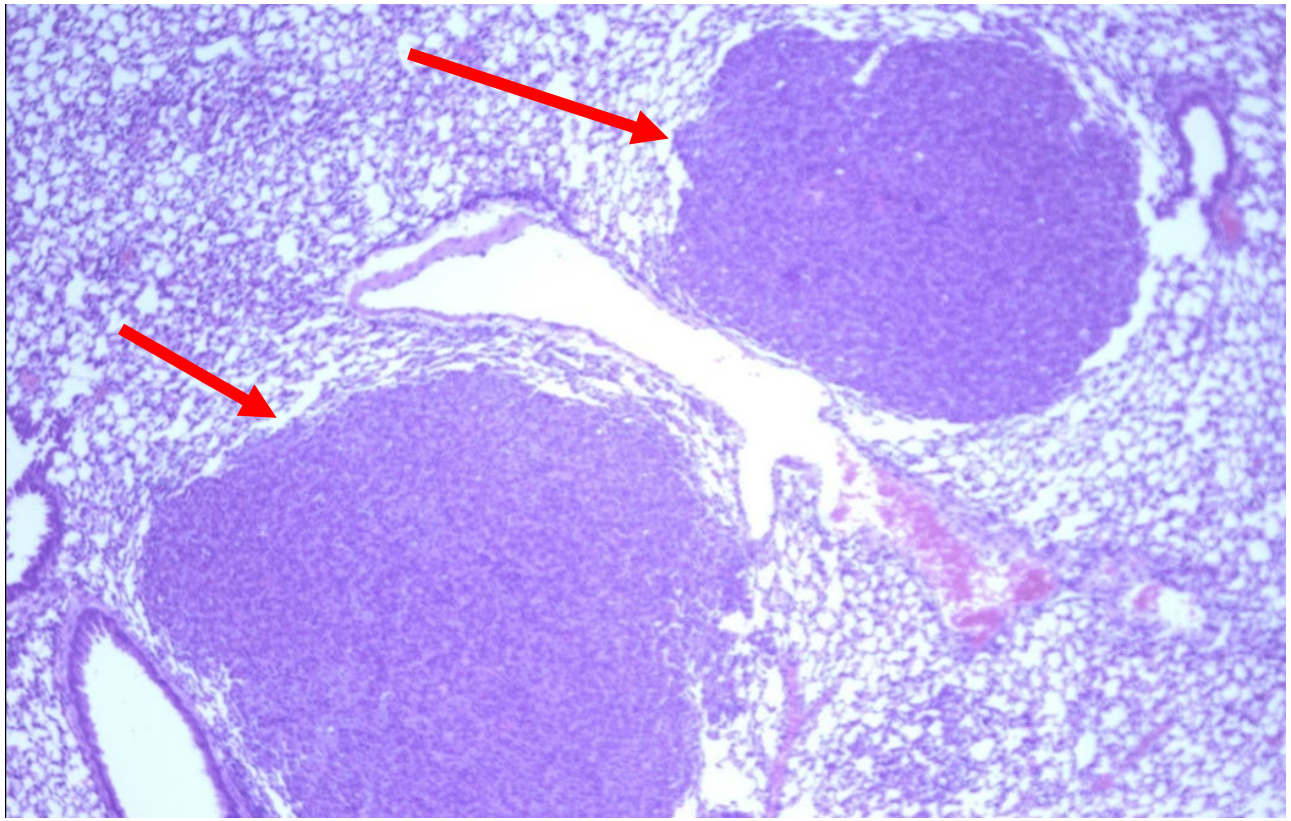
Lung

Single Bronchoalveolar Adenoma:



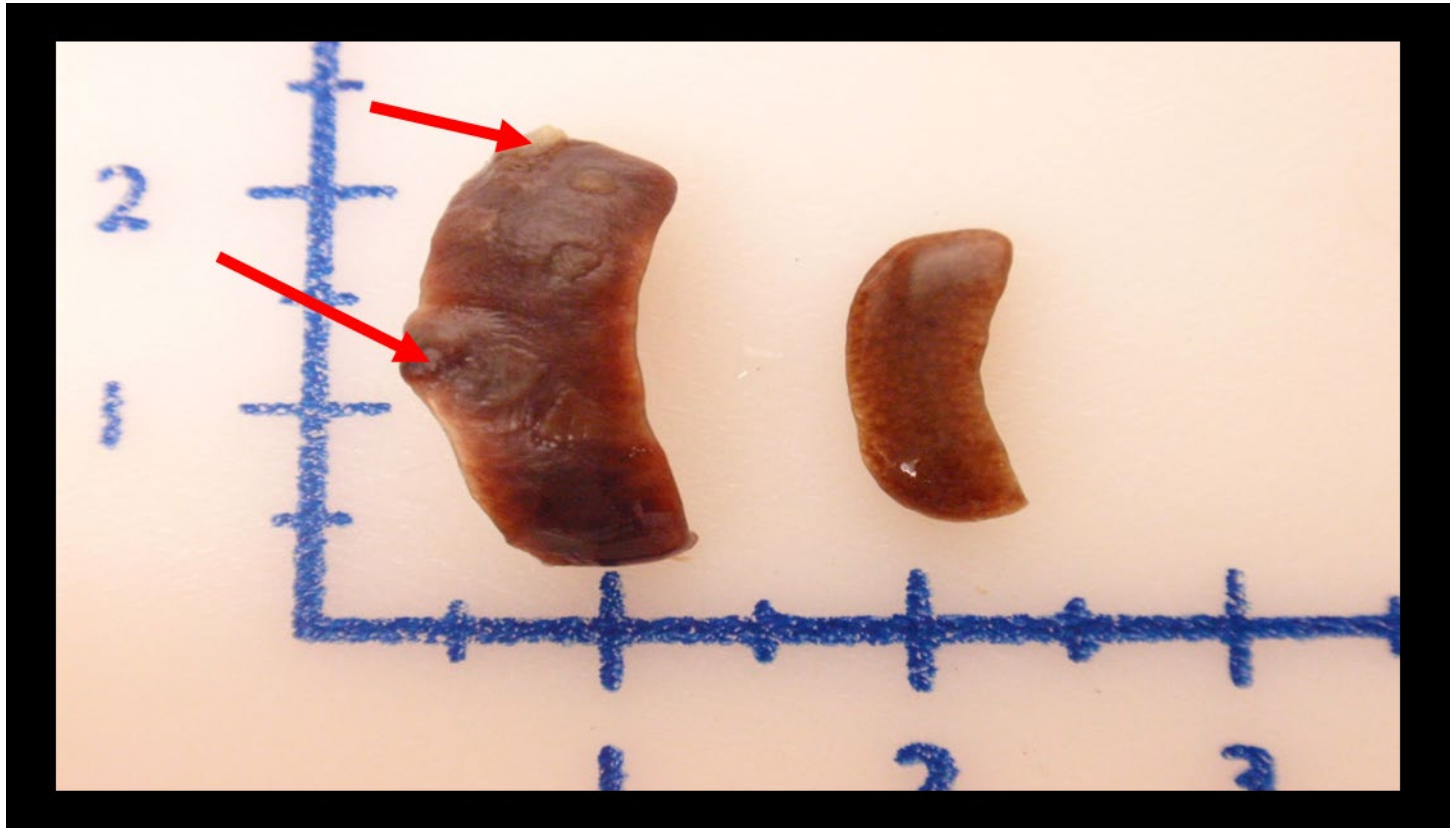
Lung

Multiple Broncho-Alveolar Adenomas:



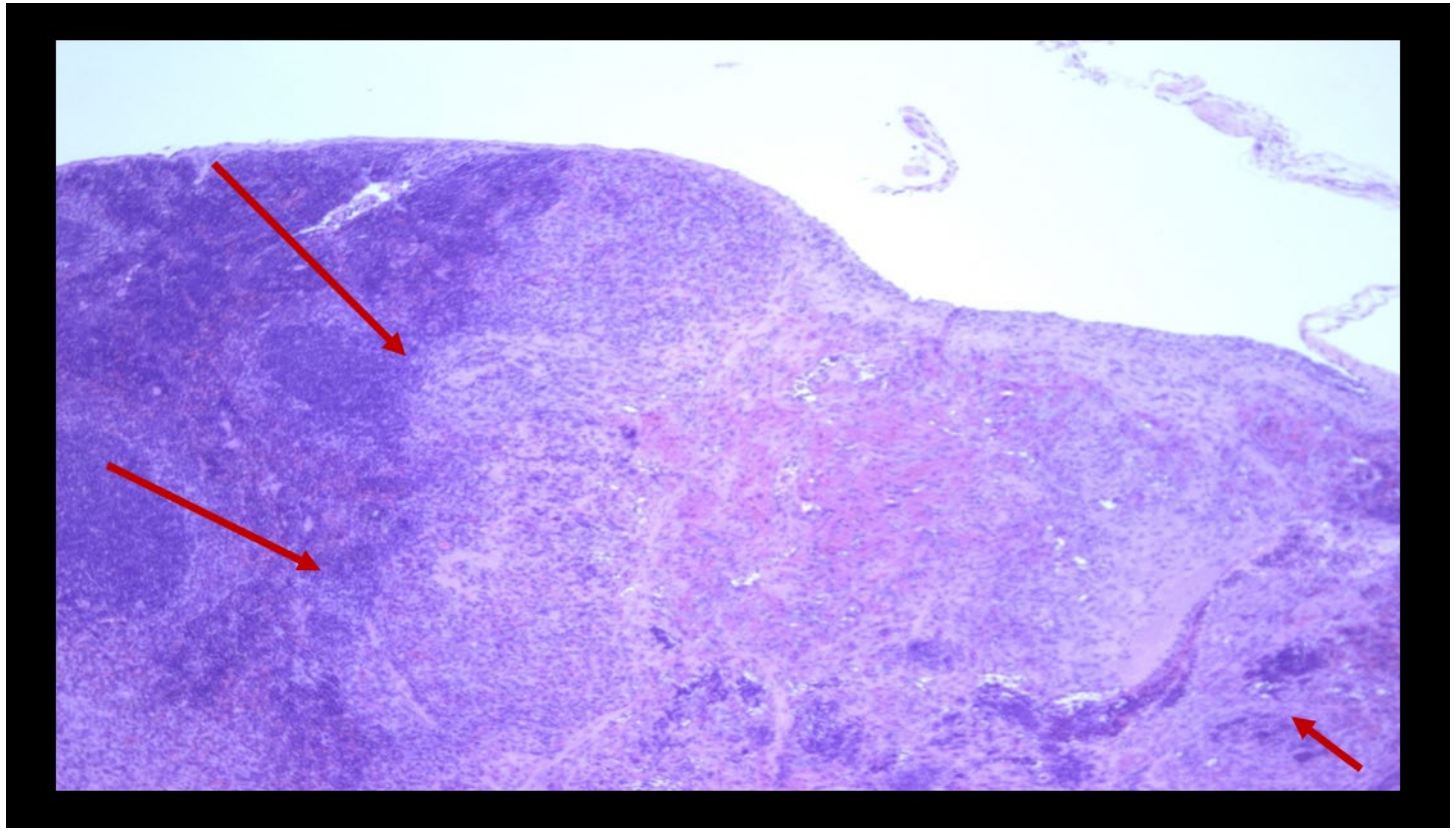
Spleen

Enlarged spleen with nodules and a mass:

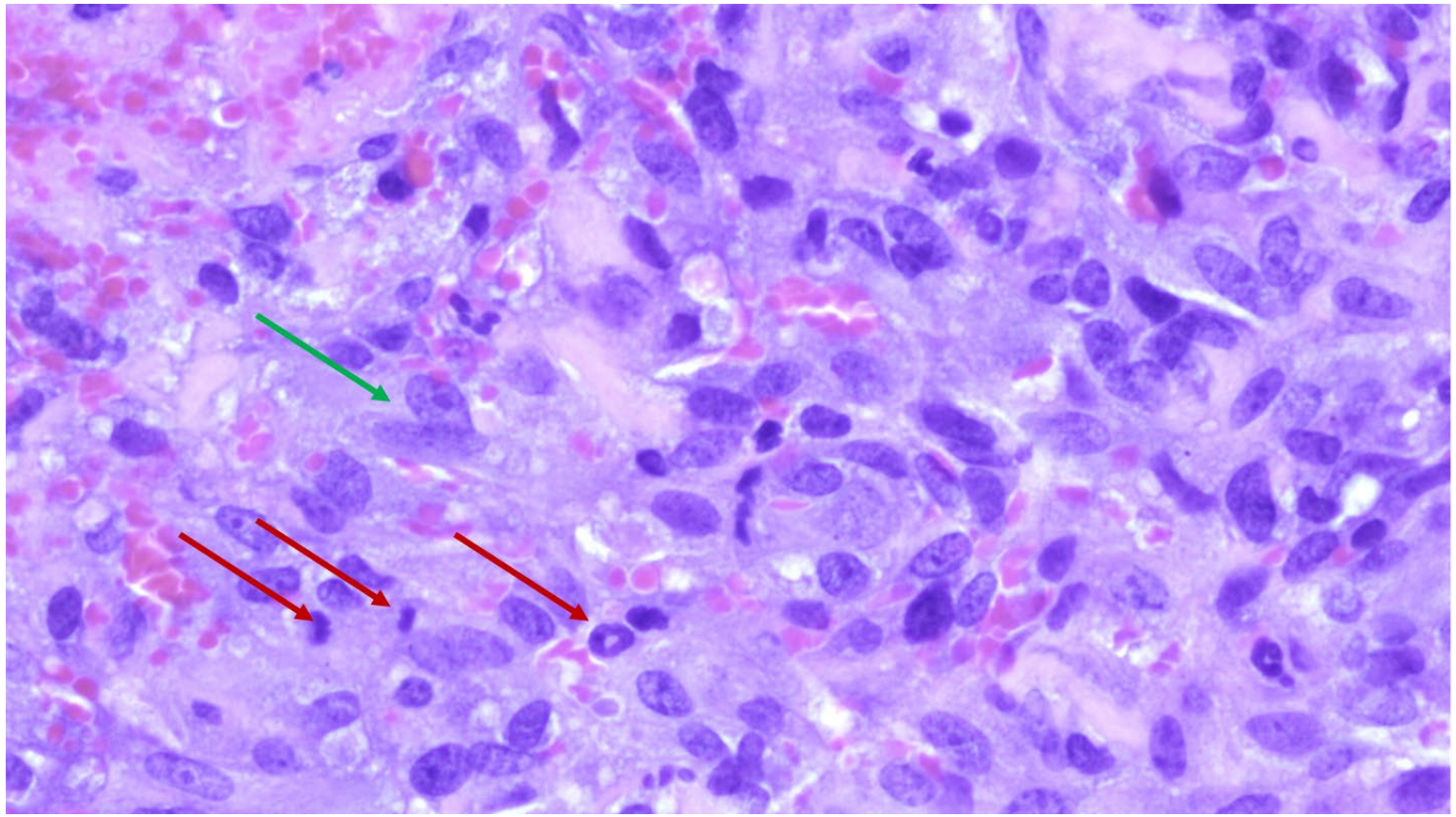


Spleen

Hemangiosarcoma



Hemangiosarcoma

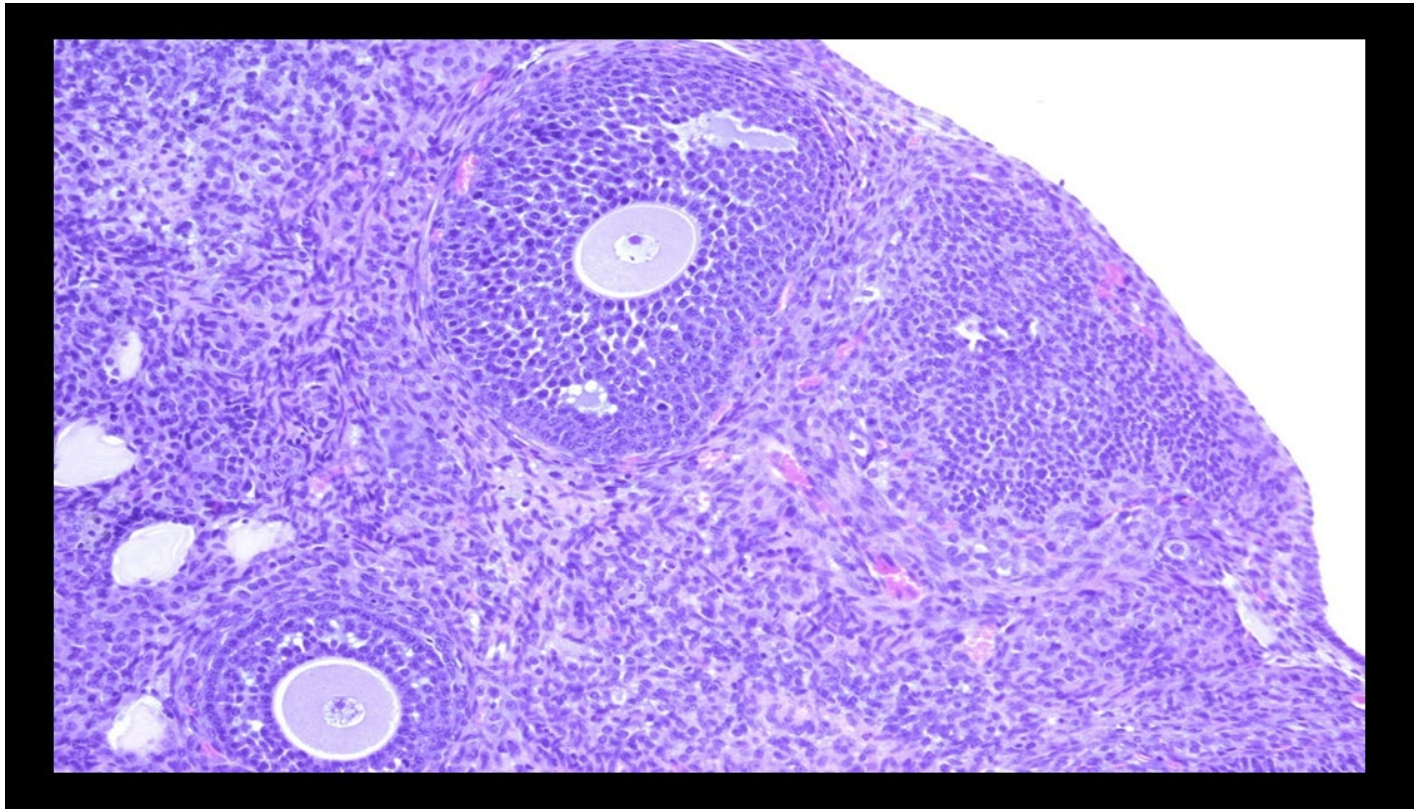


Ovary

Enlarged ovary with a dark mass:

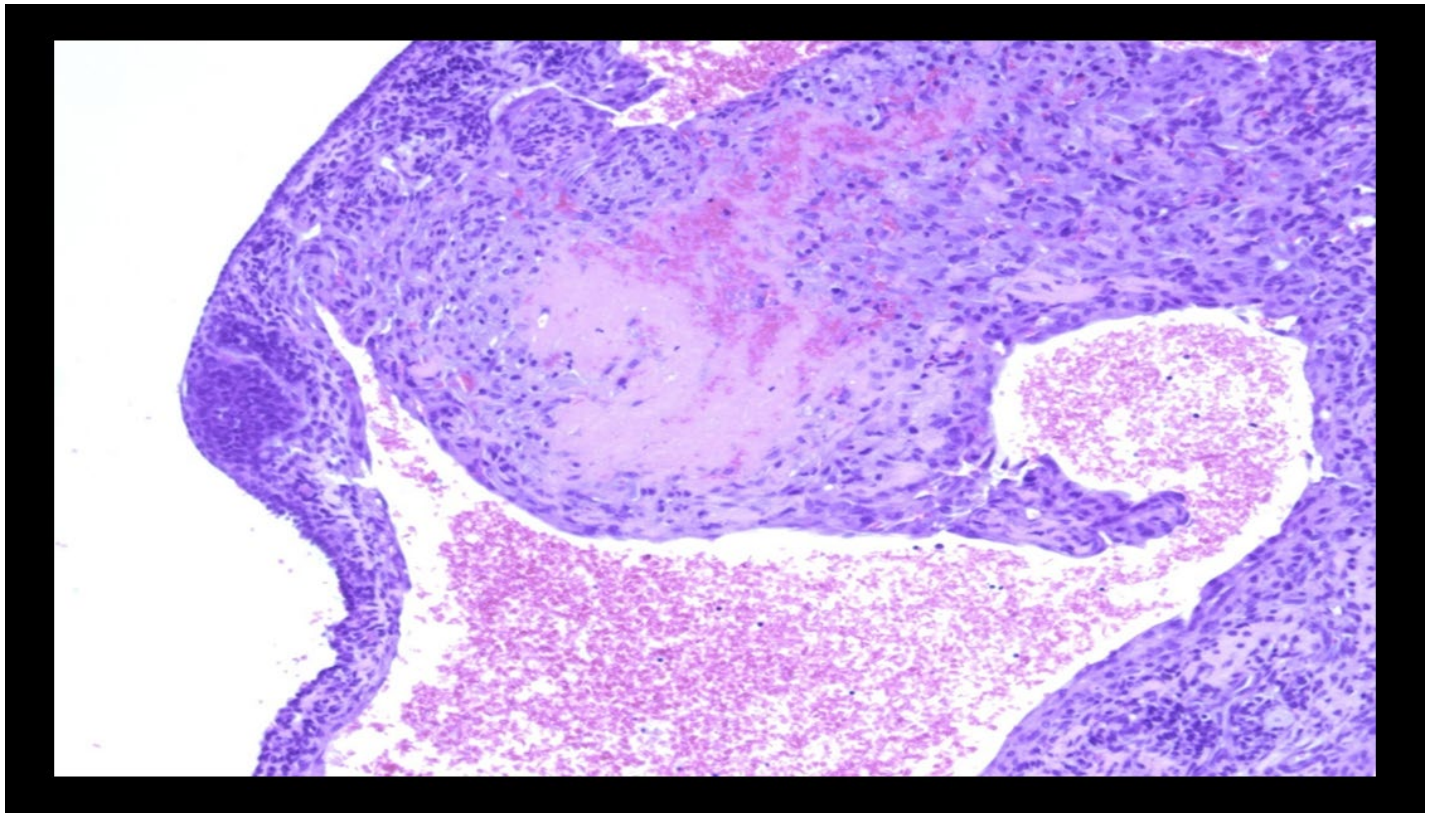
Ovary

Normal:



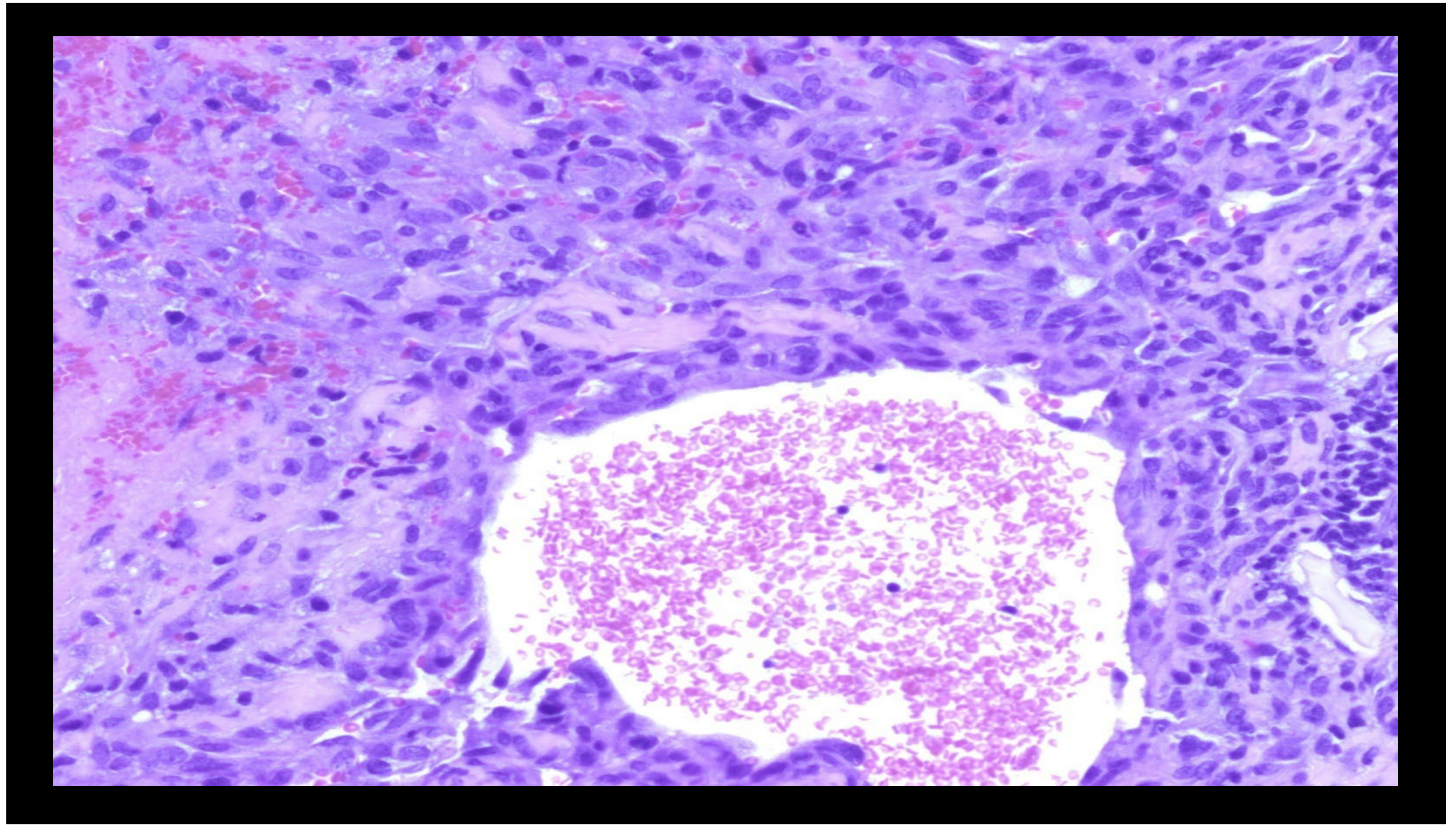
Ovary

Hemangiosarcoma:



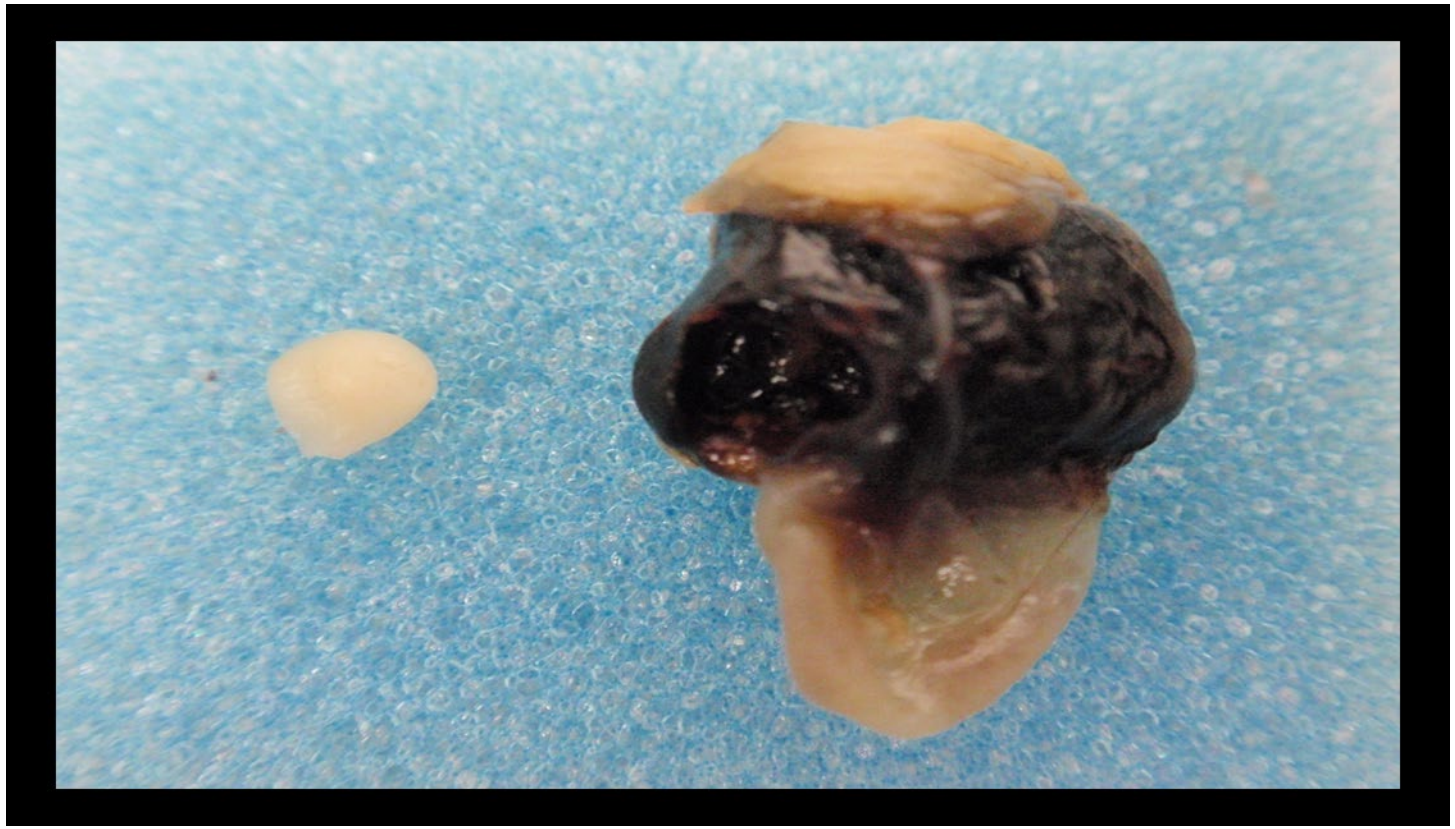
Ovary

Hemangiosarcoma:

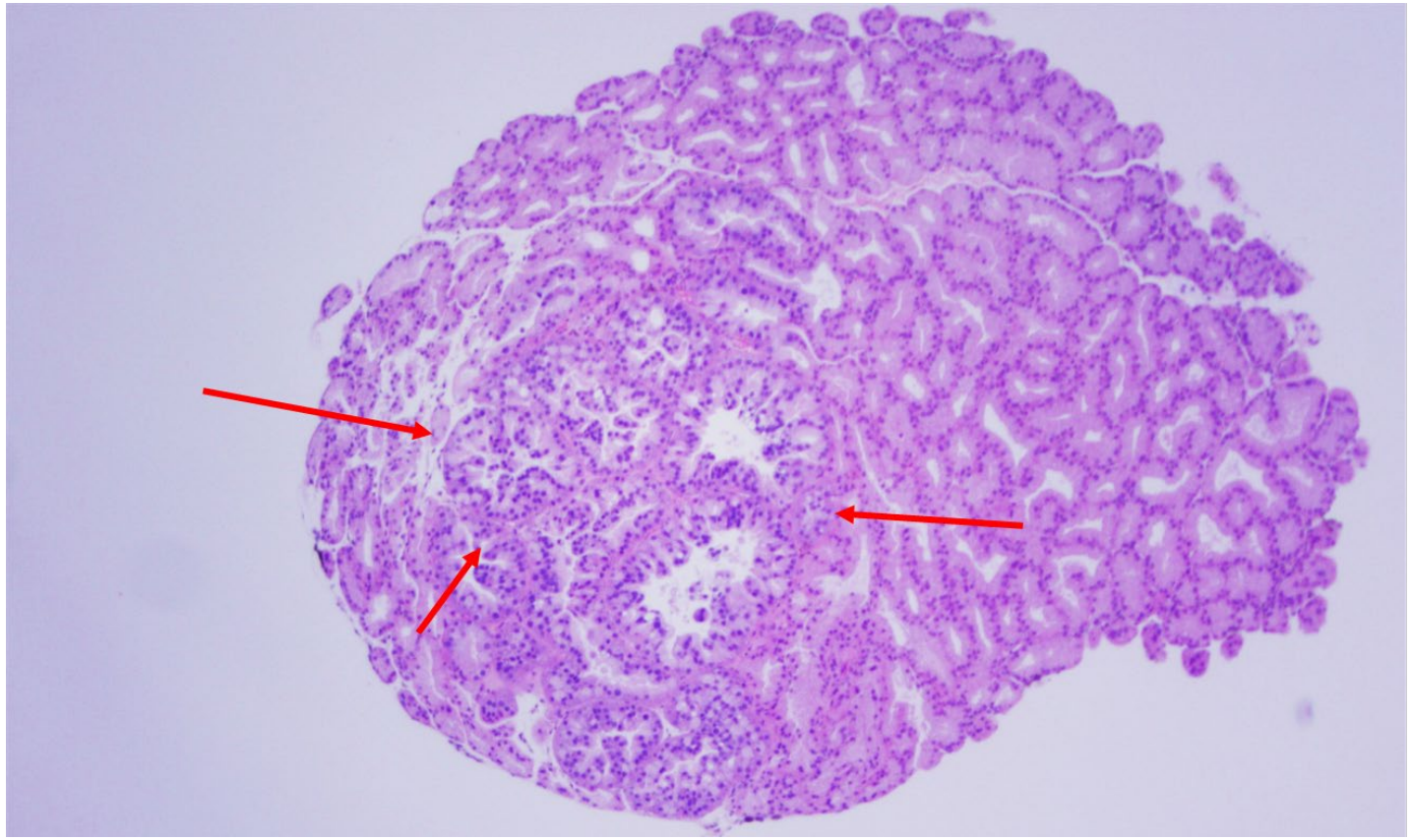


Urinary Bladder

Enlarged bladder with a dark mass:



Harderian Gland Adenoma



Vehicle Control Tumor Data Based on Histopathology Assessment

- 52 Male and 51 Female studies
- Number of animals: 1615 Males and 1560 Females
- Common tumors incidence >1%
  - Lung Tumors
  - Vascular Tumors
  - Harderian gland tumors
- All others were **uncommon**/rare tumors (incidence <1%)

Incidence of Lung Tumors

Lung tumors are the most common tumors in Tg.rasH2 mice with incidence >1%.

Sex	Male				Female			
Number of Studies	52				51			
Number of Animals	1615				1560			
	Total # of Animals	Incidence Range	Range% /study	Average %	Total # of Animals	Incidence Range	Range% /study	Average %
Number of Tumors: Combined Incidence	**184**	**0-6**	**0-24**	**11.39**	**126**	**0-6**	**0-24**	**8.08**

Vascular Tumors in Tg.rasH2 Mice

Splenic tumors are the second most common tumors in these mice exceeding the Incidence of 1%.

Sex	Males				Females			
No. of Studies	**52**				**51**			
No. of Animals Examined	**1615**				**1560**			
Tissue/Organ	**Total Number of Animals**	**Incidence Range**	**Range (%/Study)**	**Average (%)**	**Total Number of Animals**	**Incidence Range**	**Range (%/Study)**	**Average (%)**
Spleen	**61**	**0-4**	**0-16**	**3.78**	**58**	**0-4**	**0-16**	**3.72**
Testes	**7**	**0-1**	**0-4**	**0.43**	**NA**	**NA**	**NA**	**NA**
Seminal Vesicles	**2**	**0-1**	**0-4**	**0.12**	**NA**	**NA**	**NA**	**NA**
Penis	**1**	**0-1**	**0-4**	**0.06**	**NA**	**NA**	**NA**	**NA**
Epididymis	**1**	**0-1**	**0-4**	**0.06**	**NA**	**NA**	**NA**	**NA**
Liver	2	0-2	0-8	0.50	0	0-0	0-0	0.00
Lungs	4	0-1	0-4	0.25	2	0-1	0-4	0.13
Nasal cavity	2	0-1	0-4	0.12	1	0-1	0-4	0.06
Skin/Subcutis/Muscles	3	0-1	0-4	0.19	8	0-1	0-4	0.51
Bone	1	0-1	0-4	0.06	2	0-1	0-4	0.13
Multicentric	5	0-1	0-4	0.31	7	0-1	0-4	0.45

Vascular Tumors: *Continued*

Sex	Males				Females			
No. of Studies	52				51			
No. of Animals Examined	1615				1560			
Tissue/Organ	Total Number of Animals	Incidence Range	Range (%/Study)	Average (%)	Total Number of Animals	Incidence Range	Range (%/Study)	Average (%)
Ovary	NA	NA	NA	NA	7	0-1	0-4	0.38
Vagina	NA	NA	NA	NA	1	0-1	0-4	0.06
Uterus	NA	NA	NA	NA	11	0-2	0-8	0.71
Kidney	1	0-1	0-4	0.06	2	0-1	0-4	0.13
Urinary Bladder	2	0-1	0-4	0.12	1	0-1	0-4	0.06
Bone Marrow	1	0-1	0-4	0.06	2	0-1	0-4	0.13
Spinal Cord, Lumbar	2	0-1	0-4	0.12	0	0-0	0-0	0.00
Lymph Node	3	0-2	0-8	0.50	0	0-0	0-0	0.00
Prostate Gland	1	0-1	0-4	0.06	NA	NA	NA	NA
Mesentery	1	0-1	0-4	0.06	0	0-0	0-0	0.00
Preputial Glands	1	0-1	0-4	0.06	0	0-0	0-0	0.00
Stomach, glandular, serosa	0	0-0	0-0	0.00	1	0-1	0-4	0.06
Mammary Gland	NA	NA	NA	NA	1	0-1	0-4	0.06
Vascular Tumors Combined Incidence	101	NA	NA	NA	104	NA	NA	NA

Other Tumors: Nonvascular, Nonpulmonary

	Males				Females			
Tissue/Organs	Total # of Animals	Incidence Range	Range %/Study	Average %	Total # of Animals	Incidence Range	Range %/Study	Average %
Lymphoid Tissue Lymphoma/Leukemia	4	0-1	0-4	0.25	14	0-2	0-8	0.90
Lymphangioma, Various Tissues	1	0-1	0-4	0.06	3	0-1	0-4	0.19
Sarcoma, Various Tissues	5	0-1	0-4	0.31	2	0-1	0-4	0.13
Mesothelioma	6	0-1	0-4	0.37	12	0-2	0-8	0.77
Squamous Cell Carcinoma (oral and skin)	1	0-1	0-4	0.06	4	0-1	0-4	0.26
Skin, Papilloma	8	0-2	0-8	0.50	2	0-1	0-4	0.13
Harderian Gland, Adenoma	**22**	**0-2**	**0-8**	**1.36**	**31**	**0-4**	**0-16**	**1.99**
Harderian Gland, Carcinoma	**14**	**0-3**	**0-12**	**0.87**	**20**	**0-2**	**0-8**	**1.28**
Stomach, Non-Glandular Papilloma	2	0-1	0-4	0.12	5	0-1	0-4	0.32
Stomach, Non-Glandular Squamous Cell Carcinoma	5	0-1	0-4	0.31	2	0-1	0-4	0.13
Stomach, Glandular Adenocarcinoma	0	0-0	0-0	0.00	1	0-1	0-4	0.06

Other Tumors: *Continued*

	Males				Females			
Tissue/organs	Total # of Animals	Incidence Range	Range %/Study	Average %	Total # of Animals	Incidence Range	Range %/Study	Average %
Prostate, Carcinoma	2	0-1	0-4	0.12	NA	NA	NA	NA
Liver, Adenoma	7	0-1	0-4	0.43	4	0-2	0-8	0.26
Thyroid, Follicular Adenoma	5	0-1	0-4	0.31	0	0-0	0-0	0.00
Thyroid, C-cell Adenoma	1	0-1	0-4	0.06	0	0-0	0-0	0.00
Thymus, Thymoma	1	0-1	0-4	0.06	13	0-3	0-12	0.83
Nasal Cavity, Adenoma	3	0-2	0-8	0.19	0	0-0	0-0	0.00
Nasal Cavity, Carcinoma	2	0-1	0-4	0.12	6	0-1	0-4	0.38
Nasal Cavity, Osteosarcoma	1	0-1	0-4	0.06	0	0-0	0-0	0.00
Mammary Gland, Adenocarcinoma	NA	NA	NA	NA	1	0-1	0-4	0.06
Lung, Osteosarcoma	0	0-0	0-0	0.00	1	0-1	0-4	0.06
Ovary, Teratoma	NA	NA	NA	NA	1	0-1	0-4	0.06
Ovary, Leiomyosarcoma	NA	NA	NA	NA	1	0-1	0-4	0.06
Kidney, Adenoma	4	0-2	0-8	0.25	0	0-0	0-0	0.00
Uterus, Endometrial Stromal Polyp	NA	NA	NA	NA	1	0-1	0-4	0.06
Other Tumors Combined Incidence	26	NA	NA	NA	28	NA	NA	NA

Common and Uncommon Tumors in Tg.rasH2 Mice

- Lung tumors are the most common tumors.
- Splenic vascular tumors are the second most common tumors.
- Harderian gland tumors are the thirs most common tumors.
- They all exceed the incidence of 1%
- All other tumors have an average incidence of <1% and are therefore considered rare.

Advantage of Tg.rasH2 Study Over Conventional Two-Year Mouse Study

- Low incidence of spontaneous tumors
- Shorter duration of the studies
- Fewer number of animals
- Less test article required

Thank You to My Colleagues Who Contributed to This Project:

- Jessica Belich
- Melissa Denton
- Peter Mann
- Marie McKeon
- Reem Elbekai
- Karen Brown
- Dan Patrick
- Sudhir Shah
- Marty Wenk
- Eias Zahalka
- Michelle Hickman

A Proposal for New Strategies in Dose Selection Process for 26-Week Tg.Rash2 Carcinogenicity Studies

Definition of MTD

What is MTD?

- It is an estimated MTD (EMTD)
- The dose of a test article estimated from dose range-finding studies, which is then applied as the high dose for the carcinogenicity study
- When that dose is given for the duration of the carcinogenicity study, it is expected to elicit minimal signs of toxicity
- It should not cause >10% decrease in the body weights compared to concurrent controls
- It should not shorten the animal's normal longevity or unduly compromise normal well being of the animal, except for the effects of carcinogenicity.

(ICH 2008; Sontag, *et al.*, 1976; Haseman, 1985; Haseman and Seilkop, 1992; ILSI 1984; Paranjpe, Madhav G., et al, 2015)

Maximum Tolerated Dose (MTD)

- Derivation of EMTD/MTD for the 26-week carcinogenicity study are based on findings from dose range-findings (DRF) studies.
- Once determined, these doses are applied to the carcinogenicity study.
- In general, if the MTD is chosen for the high dose groups, then the medium and low doses were set at 40% and 20% of the MTD, respectively.

What Happened in the Real World

In Two-Year Rodent (Rat and Mouse) Carcinogenicity Studies:

- Retrospective Analyses Have Shown that the MTD chosen for carcinogenicity studies is often overestimated resulting in some level of toxicity at the high dose.
- Decreased body weights (>10%)
- Increased mortality
- A decreased incidence of tumors at this dose
- This increase in toxicity may defeat the very purpose of the carcinogenicity assays.

Retrospective Analysis of Tg.RasH2 Studies

Such retrospective analysis of the dose selection process was not performed in 26-week Tg.rasH2 Studies.

What Stimulated this Investigation for Tg.rasH2 Mice?

- For this assessment, data from 29 individual 26-week carcinogenicity studies in Tg.rasH2 mice were reviewed.
- Statistically significant dose-related increases in incidence of tumors were not observed in any of these studies in either sex.
- Additionally, there were no statistically significant flags or increased number of tumors in high dose groups of either sex.
- A statistically significant flag occurred uncommonly for low or medium dose group in a rare individual study.

Why Were NO Individual Tg.rasH2 Studies Statistically Positive?

- Our review indicated that toxicity in the high dose group was excessive when the EMTD was derived from DRF studies.
- The DRF studies were conducted in CByB6F1 mice:
  - These mice weigh 10-15% more than Tg.rasH2 mice.
  - We proposed Tg.ras H2 mice may not tolerate significant reductions in body weight exceeding 10%.
- Range finding studies in CByB6F1 mice are only 28 days in duration, whereas test article is given to Tg.rasH2 mice for an extended period of 26 weeks.
- We proposed that the EMTD derived from CByB6F1 mice is not well tolerated in Tg.rasH2 mice due to reduced body weights.

Analysis of Parameters in Tg.rasH2 Carcinogenicity Studies

We conducted a retrospective analysis of 29 Tg.RasH2 26-week carcinogenicity studies where the following parameters were available for each mouse:

1) Initial body weights (IBW)
2) Food Consumption (FC)
3) Terminal body weights (TBW, not fasted)
4) Mortality and its cause
5) Tumor numbers and incidence
6) Tumor data in these studies was statistically analyzed:
    1) Pair wise (Fisher's exact and Poly 3 pair wise test)
    2) Trend tests (Cochran-Armitage overall trend, Peto overall Trend Test, Poly 3 Overall Trend test)

Statistical Analysis of 29 Studies

- The data from these 29 individual studies was pooled into a single data set for vehicle and test article treated groups at low, mid, and high dose.
- Statistical analysis was performed for the combined data set by using nonparametric Dunn's test (Hollander and Wolfe, 1973) for the IBW, FC, % BWG, and % tumor incidence.
- Fisher's exact test was applied for analysis of % mortality.
- Linear treatment–related trend analysis was performed for % BWG, % mortality, and % tumor incidence.
- The COD (cause of death) was analyzed by chi square test for independence.
- The statistical analysis for the Fisher's exact, trend test, and chi square test were performed by SAS1 Proprietary Software, Version 9.2 (SAS1 2008).
- For each sex, the comparison for each parameter was made between the control and the test article-treated groups (low, mid, and high).

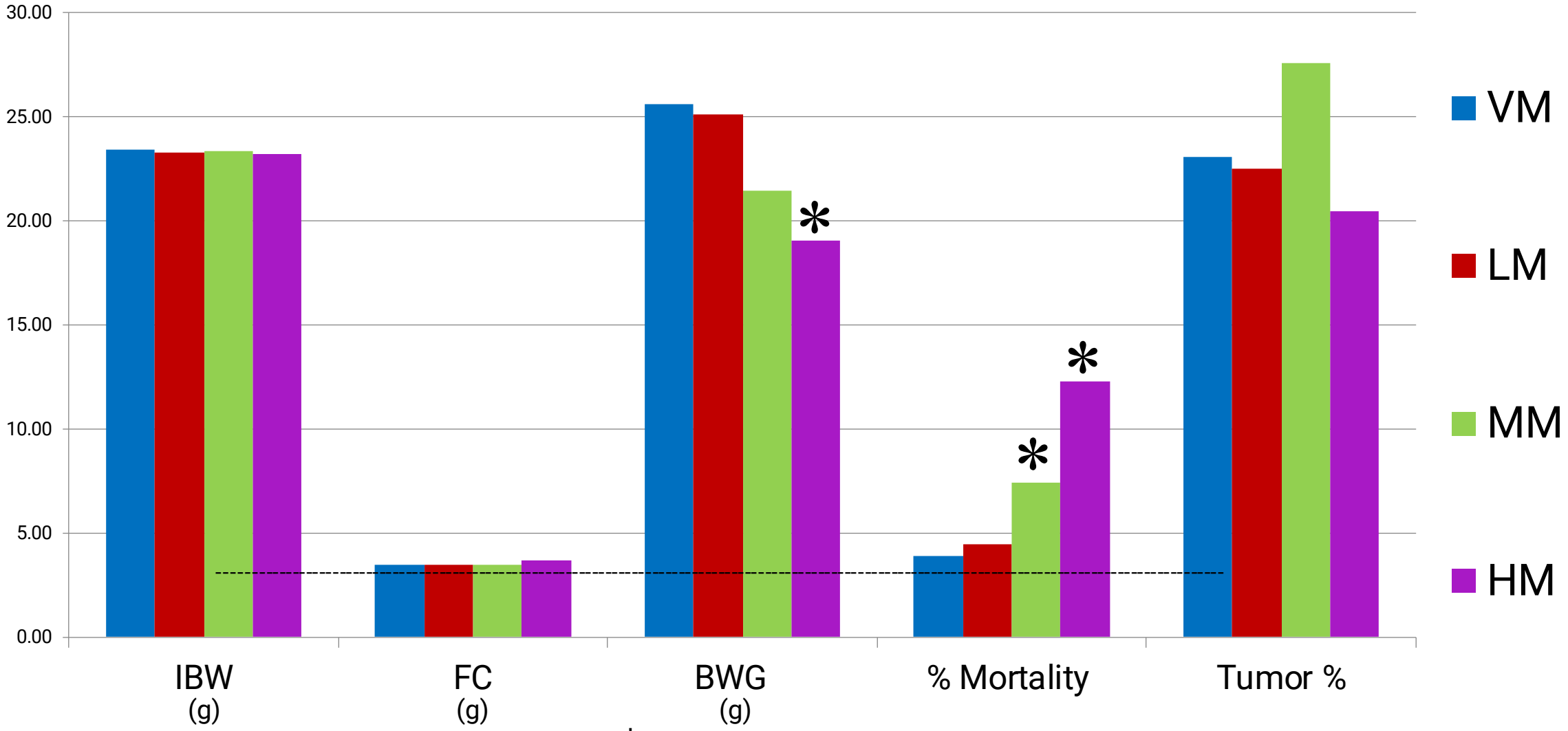
Combined 26-Week Tg.rasH2 Studies (29): Males

	IBW (g)	FC (g)	TBW (g)	% Mortality	Tumor %
Vehicle	23.41	3.47	25.62	3.94	23.09
Low Dose	23.33	3.52	25.13	4.51	22.54
Mid Dose	23.35	3.49	21.45	7.44*	27.6
High Dose	23.26	3.68	19.1*	12.27*	20.45

*** Indicates statistical significance.**

Red indicates no statistically significant difference but BWG% decreased by >10%

Combined 26-Week Tg.rasH2 Studies (29): Males



*** Indicates statistical significance.**

VM=Vehicle male, LM=Low dose male, MM=Mid dose male, HM=High dose male

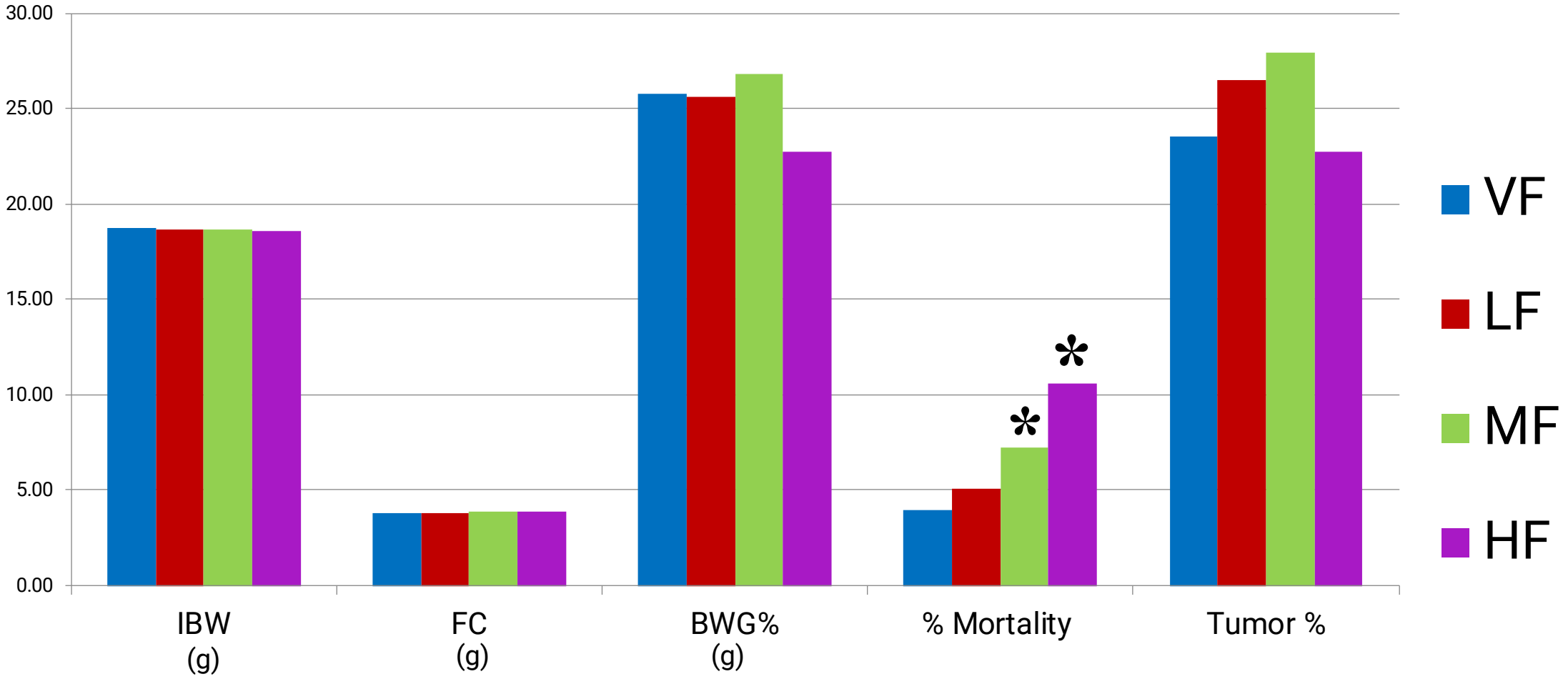
Combined 26-week Tg.rasH2 Studies (29): Females

	IBW (g)	FC (g)	TBW (g)	% Mortality	Tumor %
Vehicle	18.74	3.79	25.79	3.94	23.52
Low Dose	18.63	3.81	25.61	5.07	26.48
Mid Dose	18.62	3.86	26.8	7.27*	27.93
High Dose	18.55	3.85	22.74	10.61*	22.73

*** Indicates statistical significance.**

Red indicates no statistically significant difference but BWG% decreased by >10%

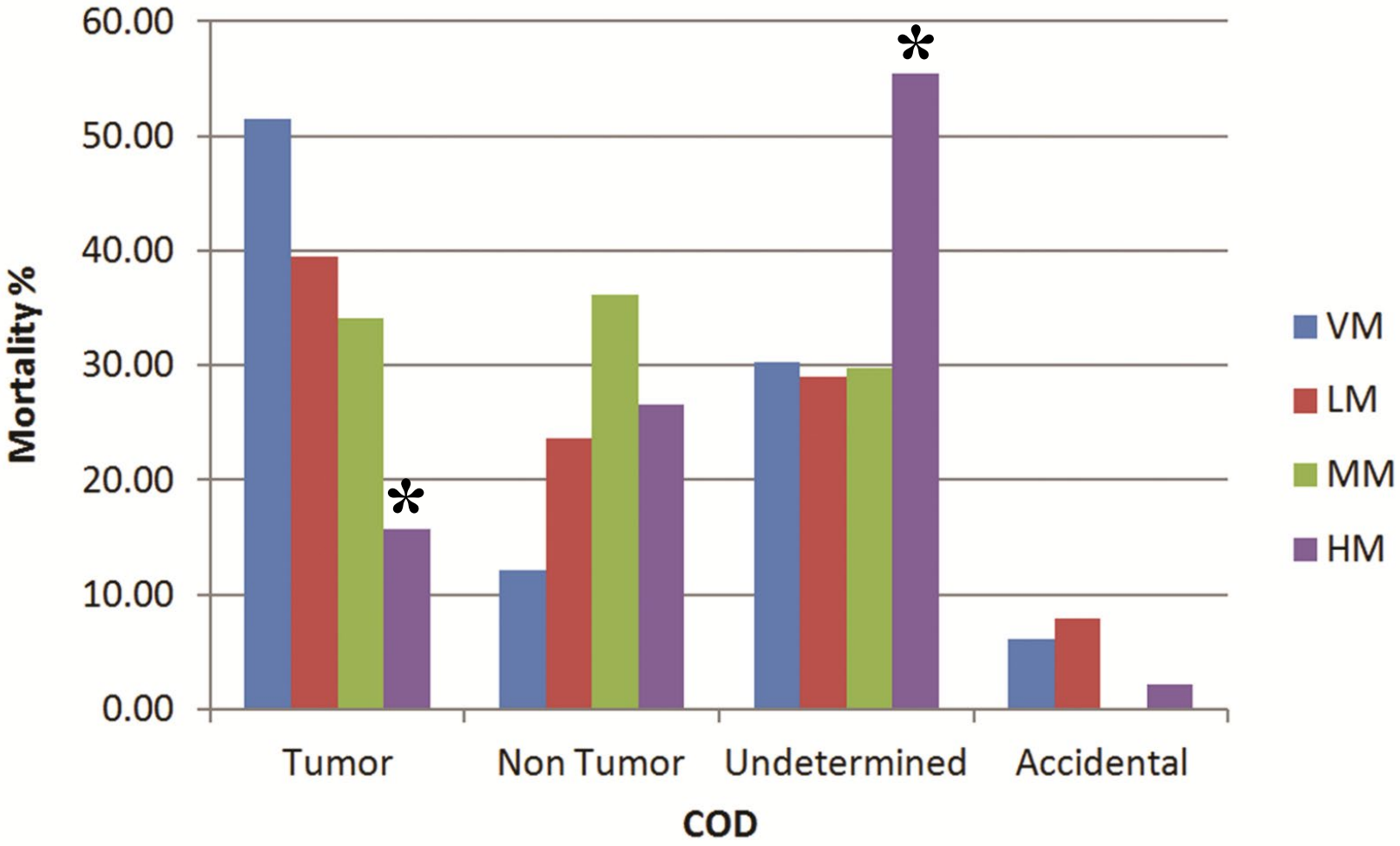
Combined 26-Week Tg.rasH2 Studies (29): Females



***Indicates statistical significance**

VF=Vehicle female, LF=Low dose female, MF=Mid dose female, HF= High dose female

Cause of Death in Males

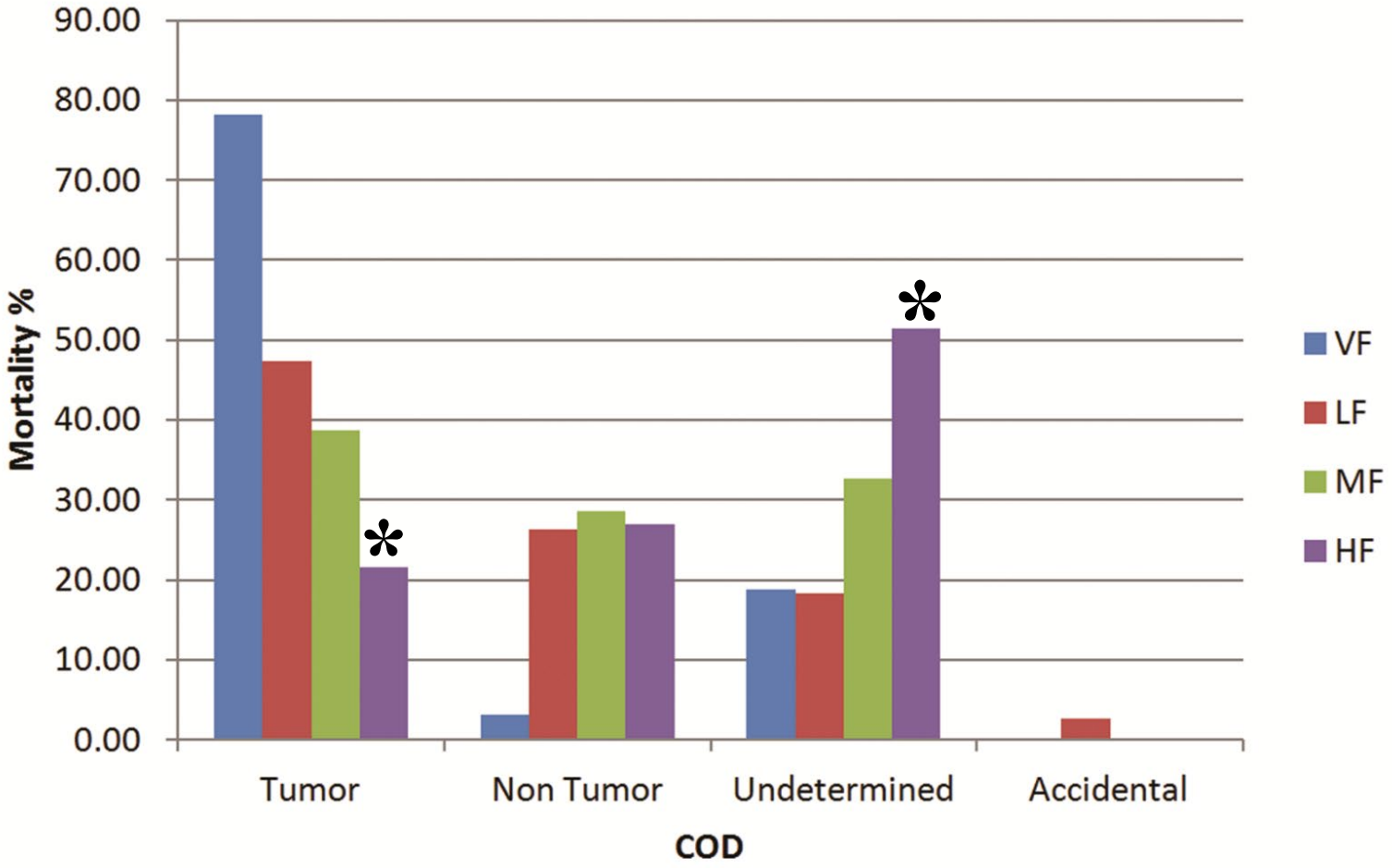


Undetermined: When a COD was not determined by gross and microscopic examination.

*** Indicates statistical significance.**

VM=Vehicle male, LM=Low dose male, MM=Mid dose male, HM=High dose male

Cause of Death in Females



Undetermined: When a COD was not determined based on gross and microscopic examination.

*** Indicates statistical significance.**

VF=Vehicle female, LF=Low dose female, MF=Mid dose female, HF=High dose female

What Does It All Mean?

- Analysis of 29 studies show that in the High Dose groups (when compared to controls):

Males	**Females**
Body weights decreased by 25.45%	Body weights decreased by 11.83%
Mortality increased by 211.42%	Mortality increased by 169.29%
Incidence of tumors decreased by 11.43%	Incidence of tumors decreased by 3.36%

- A Dose Responsive Relationship was not maintained between low, mid, and high dose groups for tumors.
- A Dose Responsive Relationship was somewhat maintained between the low and mid dose group tumors, if high dose group was eliminated.
- The high dose group did not behave in an expected manner, but the low and mid dose groups behaved as they were expected to behave.

What is the Bottom Line?

- In the high dose groups of both sexes, the MTD was overestimated resulting in overt toxicity, which caused:
  - >10% decrease in body weight gains of both sexes
  - More so in males than in females
  - Females show more resistance to changes reflecting lower toxicity
- Our analysis was based on the fact that if the high doses were set at 100%, then the mid and low doses were set at 40% and 20%, respectively or just above the clinical dose. Based on the knowledge of these doses and in order to bring weight decreases in males and females below 10%, we proposed that the MTD in males be set at ½ of the EMTD and that MTD in females be set at 2/3 of EMTD derived from DRF studies.

Additional Investigations: Why the MTD was Overestimated in the 26-Week Tg.rasH2 Studies

- The 28-day studies conducted prior to 26-week Tg.rasH2 carcinogenicity studies were selected
- The criteria used for selection of studies:
  - Both the 26-week carcinogenicity studies and earlier 28-day DRF studies must have been conducted at one facility,
  - Both type of studies must have been conducted with same vehicle, same test article and same doses
- Using the above criteria, 24 studies qualified for the analysis.
- We analyzed initial body weights, terminal body weights, food consumption, body weight gains/drops, mortality in each of the 24, 28-day DRF studies in CByB6F1 mice and compared them with first 4 weeks of the associated carcinogenicity studies conducted in Tg.rasH2 mice.

Statistical Analysis for Selected 24 Studies

- The parameters compared included IBW, TBW, BWG, FC, and mortality collected for 28-day DRF studies (CByB6F1 mice) and data collected for the first 28 days of the 26-week carcinogenicity studies (Tg.rasH2 mice).
- Comparisons were made for each sex and dose level, testing for differences between the strains using the summary data for each study.
- Statistical Analysis was done using the Statistical Analysis Software (SAS) system 2009, version 9.2.

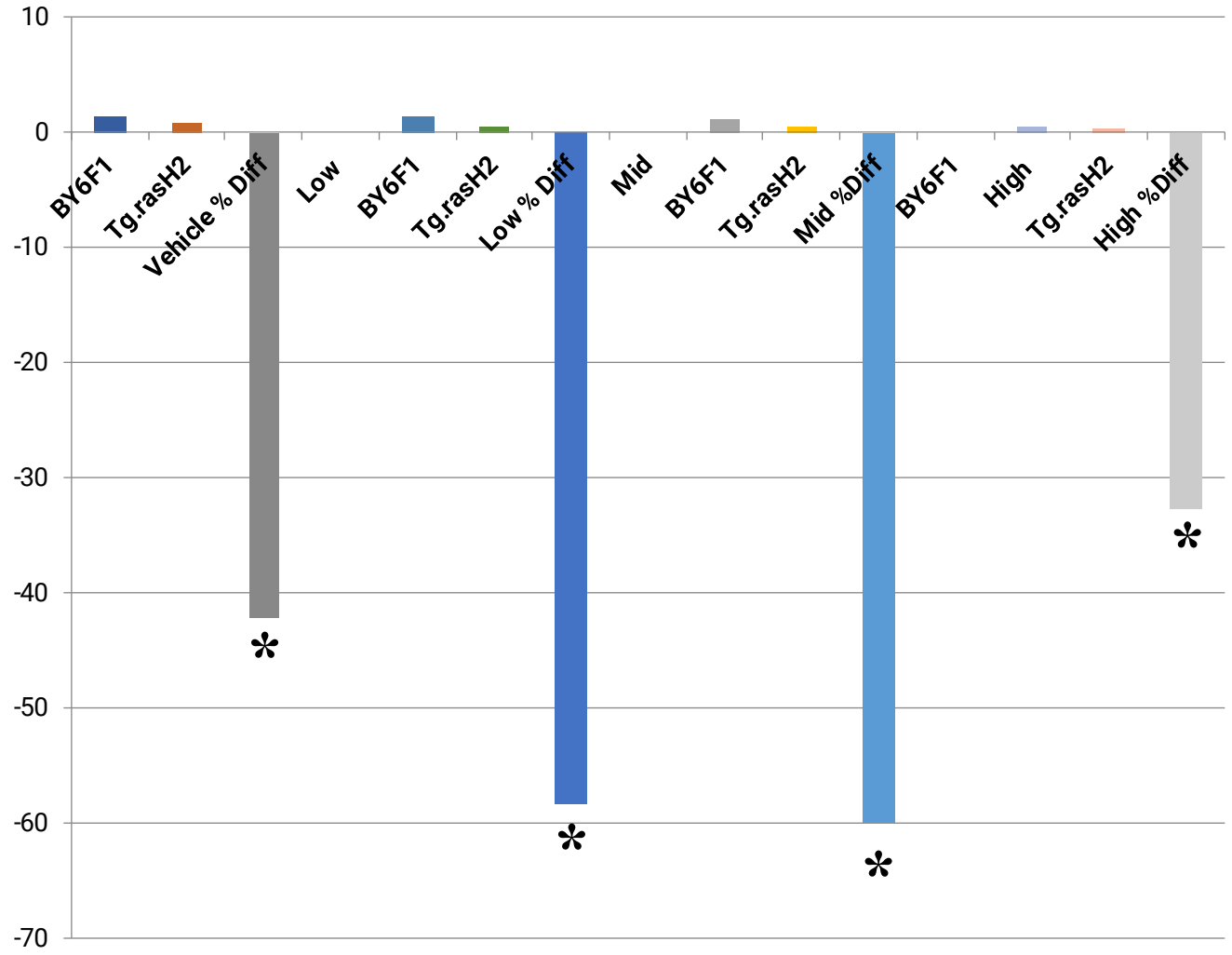
Breakdown of the Selected Studies

Doses	Males	Females
Vehicle	24	24
Low	22	21
Medium	22	21
High	8	8

- 24 selected male and 24 selected female studies had same vehicle
- 22 studies in males and 21 in females were given the same low and mid TA dose
- 8 studies in high dose groups of each sex received the same high dose.
- Remaining 16 (24-8) studies with different high dose levels in the CByB6F1 mice for 4-week DRF studies exhibited overt toxicity either because of excess drop in body weights or mortality and were not included in further evaluation.

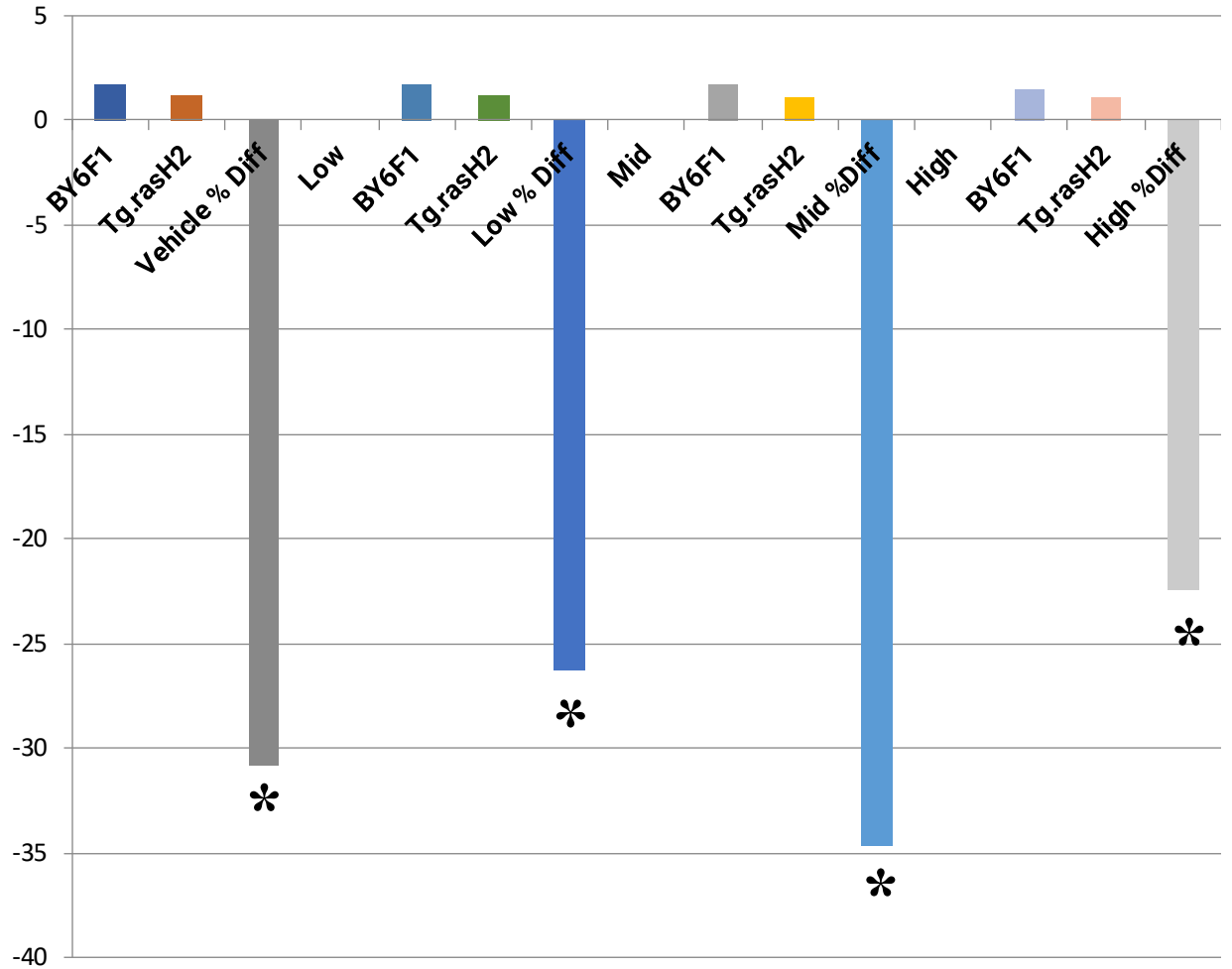
BWG Differences (%) in Tg.rasH2 vs. CByB6F1 (WT) Mice at Day 28: Males

Dose	CByB6F1 BWG (g)	Tg.rasH2 BWG (g)	% Difference in BWG
Vehicle	1.33	0.77	-42.1053*
Low	1.27	0.53	-58.2677*
Mid	1.12	0.45	-59.8214*
High	0.52	0.35	-32.6923*



BWG Differences (%) in Tg.rasH2 vs. CByB6F1 (WT) Mice at Day 28: Females

Dose	CByB6F1 BWG (g)	Tg.rasH2 BWG (g)	% Difference in BWG
Vehicle	1.72	1.19	-30.8146*
Low	1.67	1.23	-26.3473*
Mid	1.73	1.13	-34.6821*
High	1.47	1.14	-22.449*



Results of Body Weight Gain Analysis: 24 Selected Studies

- The comparison of % of body weight gain shows that there is a statistically significant decrease in the body weights of Tg.rasH2 in all four dose groups of both sexes compared with CByB6F1 mice at Day 28.
- Because of both smaller size and extended duration of doses in Tg.rasH2 mice, doses derived from 28-day DRF studies in CByB6F1 mice that are 10-15% more in weight are inappropriate and potentially toxic when applied in Tg.rasH2 mice
- This results in decreased terminal body weights and increased mortality which are not due to tumors in high dose groups of Tg.rasH2 mice compared to the control Tg.rasH2 mice.
- Based on this analysis, we propose that the Tg.rasH2 strain and not the CByB6F1 strain should be used in future DRF studies.

Additional Research

- 26 additional carcinogenicity studies using Tg.rasH2 mice for 26 weeks between 2015 and 2020 were reviewed.
- The same parameters were evaluated in these new studies as in the previous set of 29 studies.
- This research is conducted to confirm or refute the findings of the first 29 studies.
- All studies were conducted in the same facility, under GLP regulations, were read by a single pathologist, each study was peer reviewed, and each study had undergone tumor data statistics prior to finalization.

26 New Studies

- Each of the individual 26-week studies went through the same statistics as explained before for the individual studies.
- Similarly, the combined data from 26 new studies was analyzed the exact same way as the first 29 combined studies.

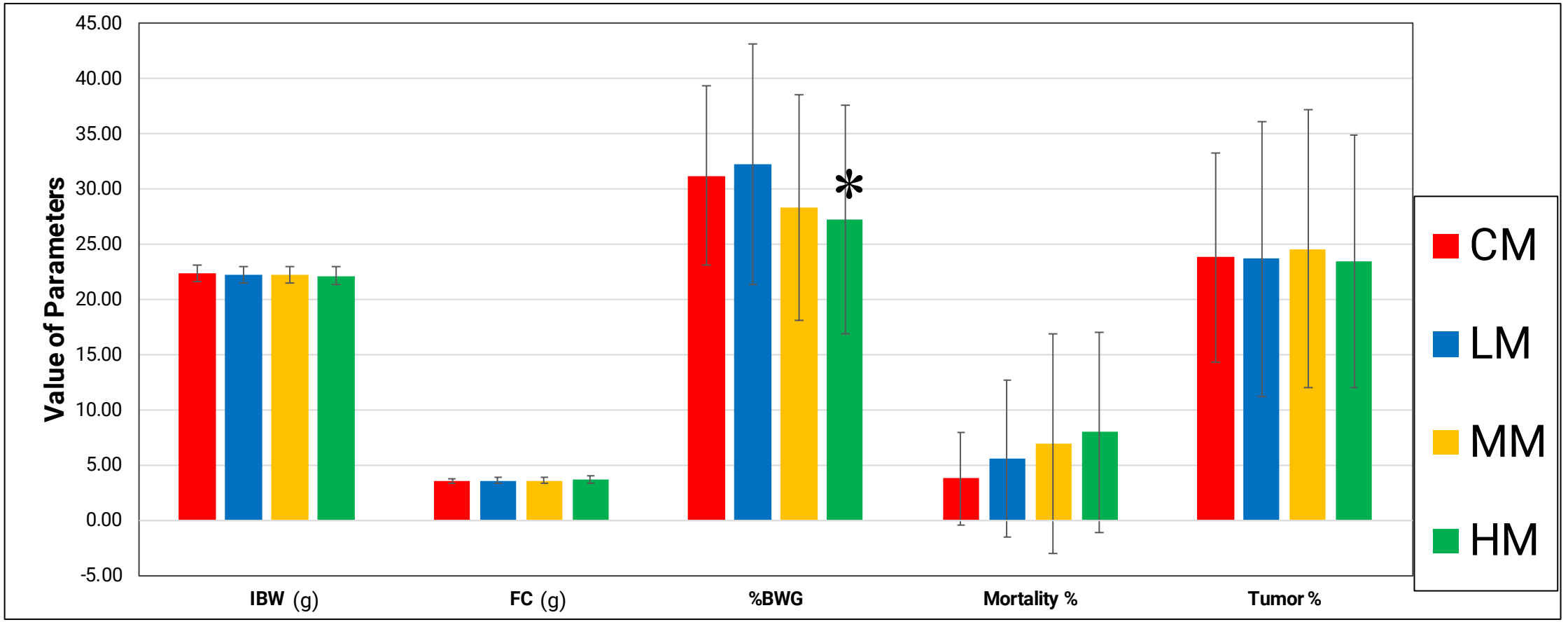
Combined 26-Week Tg.rasH2 Studies (26): Males

	IBW (g)	FC (g)	TBW (g)	Mortality %	Tumor %
Control	23.22	3.50	26.19	3.82	23.82
Low	23.19	3.58	26.73	5.56	23.70
Mid	23.21	3.52	**22.30**	6.92*	24.60
High	23.16	3.68	**26.14***	8.00*	23.45

*** Indicates statistical significance.**

Red indicates no statistically significant difference but BWG% decreased by >10%

Combined 26-week Tg.rasH2 Studies (26): Males



IBW: initial body weights, FC: food consumption, %BWG: body weight gain % difference

*** Indicates statistical significance**

- **CM=vehicle male. LM=Low dose male. MM=Mid dose male. HM=high dose male.**

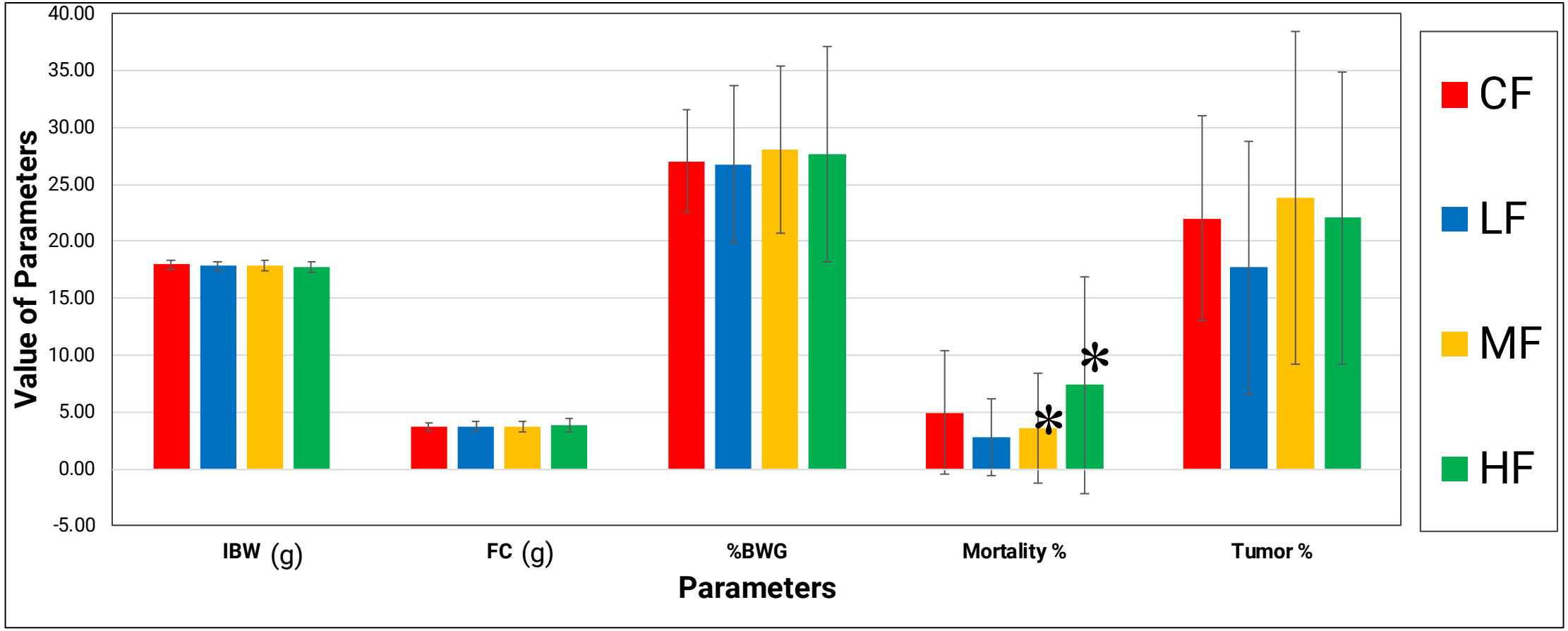
Combined 26-week Tg.rasH2 Studies (26): Females

	IBW	FC	TBW	% Mortality	% Tumor
Control	18.6	3.82	26.2	4.10	23.6
Low	18.6	3.87	26.8	4.53	25.6
Mid	18.6	3.94	27.2	**6.86***	28.6
High	18.5	3.95	**23.2**	**9.72***	23.9

*** Indicates statistical significance.**

Red indicates no statistically significant difference but BWG% decreased by >10%

Combined 26-week Tg.rasH2 Studies (26): Females

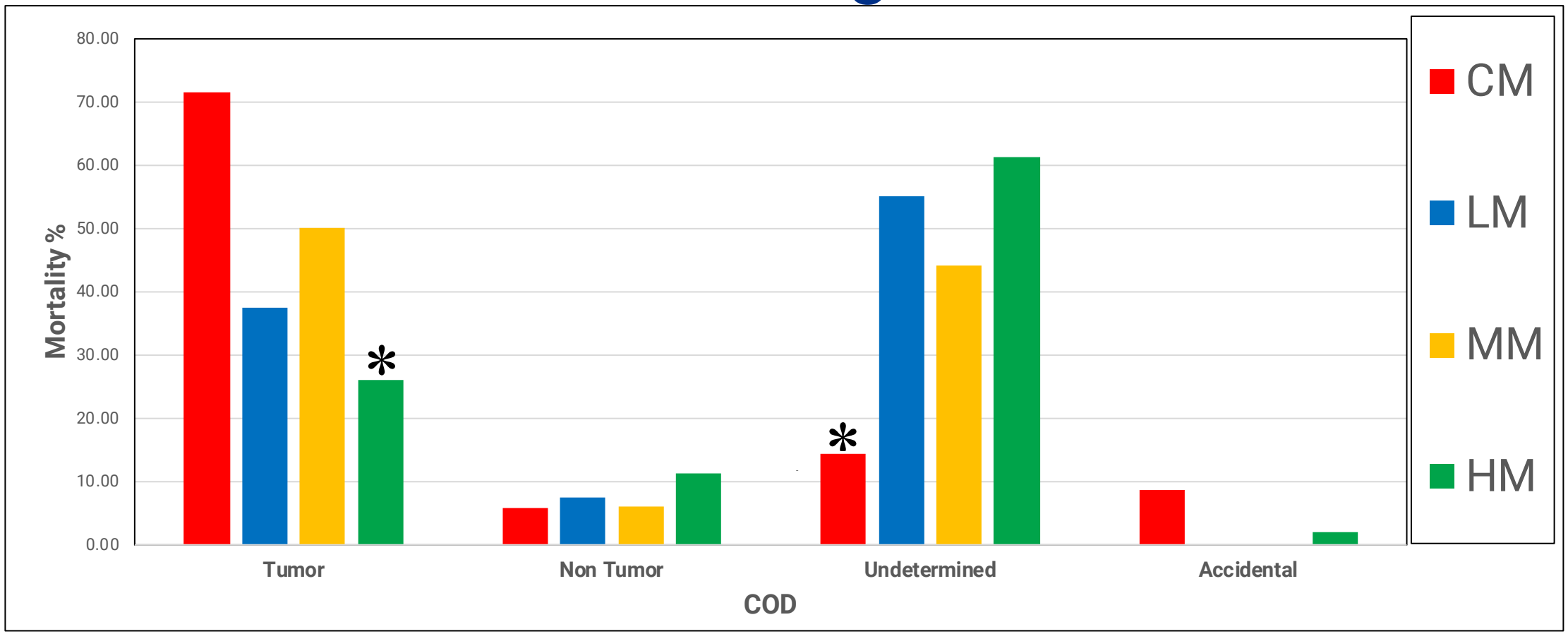


IBW: initial body weights, FC: food consumption, %BWG: body weight gain % difference

*** Indicates statistical significance.**

- **CF=Vehicle female, LF=Low dose female, MF=Mid dose female, HF=High dose female**

Cause of Death in Tg.rasH2 Males

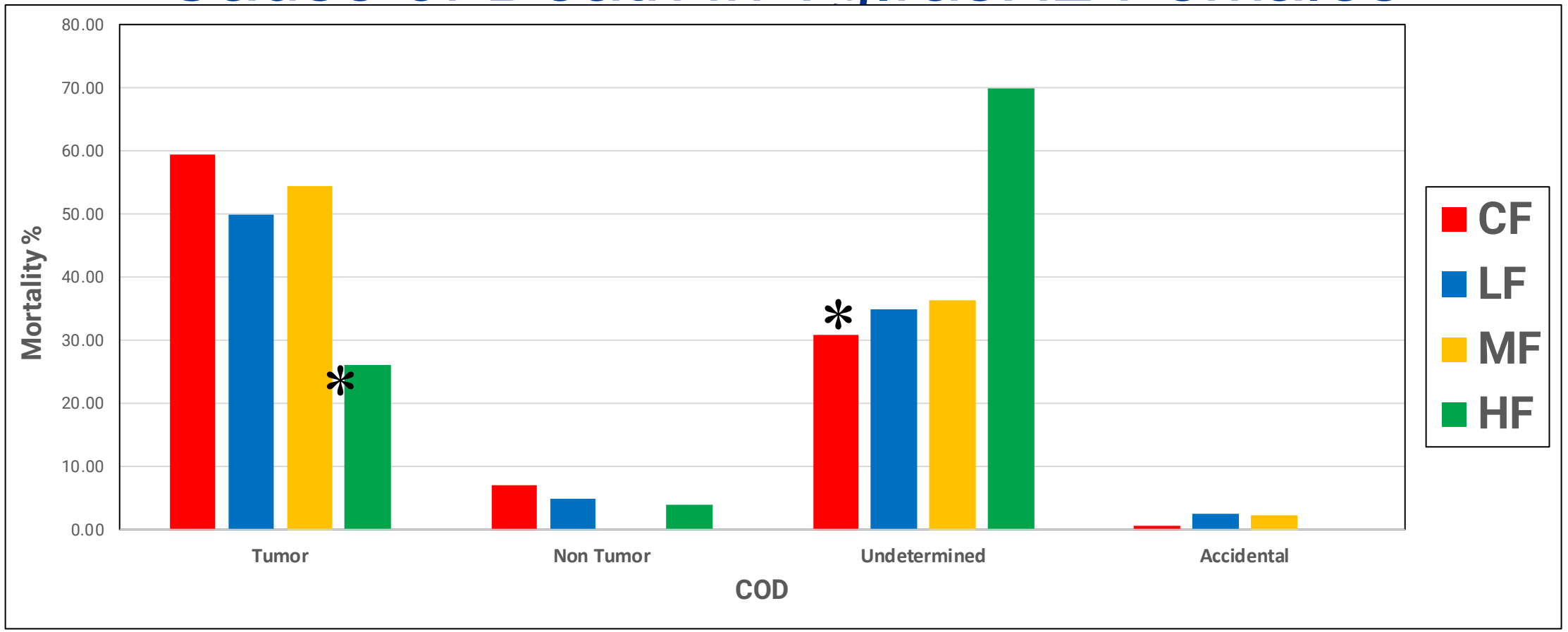


Undetermined: When a COD was not determined by gross and microscopic examination.

*** Indicates statistical significance.**

CM=Vehicle male, LM=Low dose male, MM=Mid dose male, HM= High dose male

Cause of Death in Tg.rasH2 Females



Undetermined: When a COD was not determined based on gross and microscopic examination.

*** Indicates statistical significance.**

CF=Vehicle female, LF=Low dose female, MF=Mid dose female, HF= High dose female

Overall Results

- The results of these newer (26) 26-week studies are very similar to earlier (29) 26-week studies.
- The decrease in body weights, decrease in tumors and mortality is smaller in both sexes of Tg.rasH2 studies in the second study of group of 26 studies compared to the group of first 29 studies.

Overall Results

- The main problem here is that body weights of CbyB6F1 mice are 10-15% higher than the TG.rasH2 mice.
- Thus, the EMTD derived from 28-day CByF6B1 mice is too high and is toxic to the smaller Tg.rasH2 mice.
- The Tg.rasH2 mice are getting doses at MTD derived from CBYB6F1 mice for 26-week duration which is for much longer duration than that is used for 28 days in CByB6F1 mice.

Overall Results

- For 26-week studies, Tg.rasH 2 mice had decreased terminal body weight, increased mortality and decreased incidence of tumor.
- Tumors as the COD in control mice of both sexes was higher than high dose groups.
- The “undetermined” as COD in control mice is much lower than the high dose mice of both sexes.
- The increased undetermined COD in high dose mice is likely due to overt toxicity.
- The lower incidence of tumors in high dose mice may defeat the purpose of this assay.

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- Jessica Belich
- Marie McKeon
- Caren Brown
- Peter C. Mann
- John Martinek

Thank you as well to Drs. Nelson and Reindel for spending enormous amount of time with me for shaping this presentation!

Thank you!

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