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Summary
The 6-month rasH2 transgenic mouse carcinogenicity study is increasingly used by biopharmaceutical companies as an alternative to the 2-year mouse carcinogenicity bioassay.

SAFETY ASSESSMENT

rasH2 Transgenic Mouse Carcinogenicity Study

Tumorigenesis in rasH2

The rasH2 mouse contains multiple copies of the human c-Ha-ras proto-oncogene as well as its native murine Ha-ras gene expressed in a large number of tissues. Transgenic mice with an activated/overexpressed oncogene are much more susceptible to carcinogens than normal mice, resulting in a more rapid induction of tumorigenesis, saving valuable time and resources.

Regulatory History and Acceptance

The International Conference on Harmonization (ICH) Guidance S1B, issued in 1997, specifies assessment of carcinogenicity in one long-term study conducted in a rodent species (the rat in most circumstances) plus one short-term or medium-term *in vivo* rodent study or another long-term carcinogenicity study in a second rodent species. ICH S1B accepts transgenic mouse models as a short- or medium-term rodent test system.

The rasH2, p53+/- and Tg.AC models are three primary transgenic mouse models that were initially accepted by regulatory agencies as the result of a multinational effort by The International Life Sciences Institute/Health and Environmental Sciences Institute Alternatives to Carcinogenicity Testing Project, in conjunction with the directives of ICH S1B, from 1996 to 2001. Since then, the rasH2 model has become the model of choice most often used as a replacement for a traditional 2-year mouse carcinogenicity study.

Advantages

Advantages of the rasH2 model as a replacement for a traditional 2-year mouse carcinogenicity study:

- Low incidence of spontaneous tumors
- Response to both genotoxic and non-genotoxic carcinogens, and acceptance by the FDA, the MHLW and the CPMP as appropriate for testing of compounds in either category
- Considered neither insensitive nor prone to false-positive results

As a transgenic model system, the rasH2 model also conforms to the 3Rs as a reduction since the experimental group size is typically 25/sex/group in comparison to ≥60/sex/group in a 2-year bioassay. In addition, as scheduled euthanasia in a 6-month transgenic mouse study occurs long before the senescence-related morbidity at the end of 2-year studies, the use of a transgenic mouse alternative, arguably, constitutes refinement.

Expertise

Charles River's experience and robust historical control data help distinguish background from treatment-related findings. Our spontaneous tumor incidences in negative control rasH2 mice and induced tumor incidences in positive control rasH2 mice are largely within the tumor incidence ranges of published data. (Tables 1 & 2)

For untreated or vehicle (negative) control mice, the most common spontaneous neoplasms in males and females are lung bronchioloalveolar adenomas (8.2% in males; 5.4% in females), followed by splenic hemangiosarcomas (3.7% in males; 4.1% in females), lung bronchioloalveolar carcinomas (1.8% in males; 2.3% in females), Harderian gland adenomas (1.8% in males and females), and Harderian gland adenocarcinomas (0.9% in males and females).

For rasH2 mice administered N-nitrosomethylurea as a positive control, the most common neoplasms are squamous cell papillomas in the stomach (78.1% in males; 67.6% in females) and malignant lymphomas (67.3% in males; 74.0% in females), followed by squamous cell papillomas in the skin (15.3% in males; 23.7% in females), squamous cell carcinomas in the stomach (17.8% in males; 11.7% in females), lung bronchioloalveolar adenomas (14.4% in males; 13.6% in females), adenomas in the small intestine (10.1% in males; 13.1% in females), and adenocarcinomas in the small intestine (10.0% in males; 8.8% in females).

Table 1: Comparison of Spontaneous Neoplasms in Vehicle/Untreated Control rasH2 Mice

Organ	Neoplasm	Vehicle/Untreated Control					
		rasH2 Male Mice			rasH2 Female Mice		
		BioReliance*	Pfizer**	Charles River Ohio	BioReliance*	Pfizer**	Charles River Ohio
		% Incidence (Range)	% Incidence (Range)	% Incidence	% Incidence (Range)	% Incidence (Range)	% Incidence
Harderian gland	**Adenoma**	1.4 (0–8)	1.2 (0–4)	1.8	2.8 (0–16)	0.8 (0–4)	1.8
	Adenocarcinoma	0.1 (0–4)	0.6 (0–4)	0.9	0.7 (0–8)	0.8 (0–4)	0.9
Hemolymphoreticular	**Lymphoma**	0.1 (0–4)	0	0	1.1 (0–8)	0.3 (0–1)	1.4
Liver	**Hepatocellular adenoma**	0.3 (0–4)	1.8 (0–9)	0.9	0	0	0
Lung	**Bronchioloalveolar adenoma**	11.7 (0–24)	9.9 (0–18)	8.2	6.5 (0–24)	3.9 (0–16)	5.4
	Bronchioloalveolar carcinoma	0.6 (0–8)	2.4 (0–5)	1.8	1.1 (0–4)	1.1 (0–4)	2.3
Skin	**Squamous cell papilloma**	0.6 (0–8)	1.2 (0–4)	0	0.1 (0–4)	1.1 (0–4)	0.5
	Squamous cell carcinoma	0	0	0	0	0.3 (0–0.1)	0.2
Small intestine	**Adenoma**		0	0		0.3 (0–4)	0
	Adenocarcinoma		0	0		0	0
Spleen	**Hemangiosarcoma**	3.7 (0–16)	3.0 (0–8)	3.7	3.7 (0–16)	3.9 (0–17)	4.1
Stomach	**Squamous cell papilloma**	0.3 (0–4)	0.3 (0–4)	0.9	0.7 (0–4)	0.3 (0–4)	0.5
	Squamous cell carcinoma	0.6 (0–4)	0.3 (0–4)	0	0.1 (0–4)	0	0

**Paranjpe et al. Historical control data of spontaneous tumors in transgenic CByB6F1-Tg(HRAS)2Jic (Tg.rasH2) mice. Int. J. Toxicol. 32(1):48-57, 2013.*

***Nambiar et al. Spontaneous tumor incidence in rasH2 mice: review of internal data and published literature. Toxicol Pathol. 40(4):614-623, 2012.*

Table 2: Comparison of Spontaneous Neoplasms in Positive Control rasH2 Mice

Organ	Neoplasm	Positive Control (NMU)			
		rasH2 Male Mice		rasH2 Female Mice	
		Pfizer*	Charles River Ohio	Pfizer*	Charles River Ohio
		% Incidence (Range)	% Incidence	% Incidence (Range)	% Incidence
Harderian gland	Adenoma	3.4 (0–7)	8.4	6.9 (0–13)	6.1
	Adenocarcinoma	0	1.5	4.6 (0–13)	0
Hemolymphoreticular	Lymphoma	65.5 (47–87)	67.3	66.7 (47–80)	74.0
Liver	Hepatocellular adenoma	0	0	0	0.7
Lung	Bronchioloalveolar adenoma	8.0 (0–20)	14.2	18.4 (0–27)	13.6
	Bronchioloalveolar carcinoma	2.3 (0–7)	6.1	2.3 (0–7)	5.3
Skin	Squamous cell papilloma	29.9 (7–60)	15.3	46.0 (20–53)	23.7
	Squamous cell carcinoma	6.9 (0–33)	0.8	10.3 (0–27)	12.2
Small intestine	Adenoma	4.6 (0–13)	10.1	2.3 (0–7)	13.1
	Adenocarcinoma	8.0 (0–27)	10.0	11.5 (0–25)	8.8
Spleen	Hemangiosarcoma	3.4 (0–20)	6.8	4.6 (0–13)	5.4
Stomach	Squamous cell papilloma	71.3 (47–83)	78.1	63.2 (33–87)	67.6
	Squamous cell carcinoma	34.5 (7–60)	17.8	29.9 (20–40)	11.7

**Nambiar et al. Spontaneous tumor incidence in rasH2 mice: review of internal data and published literature. Toxicol Pathol. 40(4):614-623, 2012.*

Summary

- A 6-month carcinogenicity study in rasH2 (CByB6F1-Tg(HRAS)2Jic) mice is accepted by regulatory agencies under ICH S1B as an alternative to a 2-year carcinogenicity study in mice.
- The rasH2 model provides a shorter testing duration (6 months), a more timely generation of the final report, fewer animals, lower background tumor incidence in control groups and substantially lower cost than a standard 2-year mouse bioassay.
- Since regulatory agencies such as the FDA tend to approve transgenic mouse alternatives on a case-by-case basis, the sponsor should first secure regulatory agency approval of the plan to use rasH2 mice. A 28-day range-finding study with full histopathology should be performed using CByB6F1 hybrid (non-Tg) mice prior to the actual carcinogenicity study.
- For studies intended for FDA submission, the results of the 28-day study and the proposed protocol for the 6-month transgenic mouse study should be submitted to the FDA Carcinogenicity Assessment Committee for prior review.