charles river

SAFETY ASSESSMENT

Carcinogenicity Testing Services

Research Models, Services and Safety Assessment

Regulatory guidelines necessitate carcinogenicity testing of human pharmaceuticals intended for long-term therapeutic use (e.g., six months or more of continual or intermittent administration) or chemicals expected to have prolonged human exposure. Of note, compounds with a positive for carcinogenic potential during genotoxicity testing may also require long-term testing. Carcinogenicity studies expose animals (mouse or rat in most cases) to the test compound for a major portion of their lifespan and assess for the development of non-neoplastic and neoplastic lesions in multiple organs and organ systems. Carcinogenicity studies performed to support pharmaceutical candidates are normally completed before submission of an application for marketing approval. Unless there is a particular cause for concern, or specific patient populations involved, carcinogenicity studies are not generally needed in advance of late-phase (Phase II or III) clinical trials.

Given the cost of carcinogenicity testing, study duration, and the number of animals involved, it is imperative that sponsors work with a contract research organization (CRO) that can deliver with flawless execution and that has the experience to appropriately assess the tumorigenic potential of a compound and relevance of risk to humans. Charles River has built a worldwide reputation for excellence in the conduct of conventional design, transgenic, and diet-optimized rodent carcinogenicity programs. Our global scientific network offers experience, knowledge, and services that are second to none in the industry.

Services:

- Study design considerations:
  - Selection of a model
  - Route of administration
  - Dose level selection
  - Housing and feeding regimens
  - Endpoints and evaluations
- The rasH2 alternative transgenic model
- Comprehensive background data and experience
- Pathology
- Non-animal alternatives

Carcinogenicity testing is offered at five Charles River locations throughout North America and Europe. Our facilities offer full-service laboratory support and knowledgeable staff to enable a sponsor to perform all aspects of a carcinogenicity study within a single GLP-compliant facility. Our experts have been conducting carcinogenicity studies for over 40 years, and collectively, perform 20 to 30 of these studies per year. Our team can advise sponsors on specific study design options, considerations for the requirements for pharmaceuticals, agrochemicals or food additives. Most importantly, we have extensive background tumor and survival databases to facilitate the interpretation of carcinogenicity data for regulatory review and approval.

Study Design Considerations

Selection of a Model

Carcinogenicity studies are generally conducted using either the rat or mouse. Hamsters may be used as an alternative species. These species are selected due to their susceptibility to tumor induction and the extensive database of information available on both physiology and pathology. Strain selection should be made at the start of the development program so that data from the previous general toxicology and range-finding studies can be used later in the program for the selection of dose levels for the carcinogenicity study. The strain of the selected rat or mouse can impact survivability and, consequently, the group size, statistical analysis and data interpretation. Charles River typically selects the Sprague-Dawley rat, Han Wistar rat, or CD-1 mouse, a decision guided by our comprehensive historical control databases that include survivability, tumor types, and tumor incidence/prevalence in these strains. An extensive historical control data set is essential for the interpretation of neoplastic histopathological findings in relation to normal background pathology. The studies are conducted over the normal lifespan of these species, with a typical design lasting up to 104 weeks for the pharmaceutical industry or 78 to 80 weeks for the chemical sector.

Charles River also offers the rasH2 transgenic mouse model as an alternative to the traditional “two-year” mouse study. We have participated in the International Life Sciences Institute (ILSI) Alternatives to Carcinogenicity Testing program that began in 1997 to validate alternative mouse models. Before embarking on the use of a transgenic mouse model as a replacement for a conventional mouse study, regulatory agency agreement of the plan should be obtained because most regulatory agencies approve alternative models on a case-by-case basis.

Route of Administration

The selected route of administration in animals should mimic as closely as possible the intended or expected route of human exposure. The most common route of administration is oral, whether by gavage or via a mixture with diet and/or drinking water. As pharmaceutical development involves diverse routes of administration, Charles River offers common and specialized routes of administration including gavage, dietary, weekly subcutaneous injections, intranasal, dermal, and inhalation (nose only).

Dose Level Selection

Appropriate dose level selection is critical for the successful outcome of the study. In general, studies should be conducted using three dose levels. The highest dose should produce a minimal toxic effect that does not physiologically compromise the animal or alter the normal life span, other than as a result of the effects of tumors. The lowest dose should be two to three times the maximum human therapeutic dose or the dose that produces a pharmacological effect in animals and for agrochemicals not lower than 10 percent of the highest dose. Typically, study designs include one concurrent untreated or vehicle-control group, but a second pair-fed control group that is feed-restricted may be needed in dietary studies where reduced palatability or dietary intake of approximately 20% or more is expected. Sometimes the control group is doubled to two concurrent control groups to produce a large histopathological database.

Dose level selection should also take into consideration the results of earlier toxicity studies, including a dose range-finding study of at least three months (13-week) in duration. It is also important to ensure that the definitive carcinogenicity studies include the same elements of study design incorporated into the 13-week study, including multiple dose groups, toxicokinetic satellite animals, and complete histopathology on the control and high dose groups, target organs, and any gross lesions.

Housing and Feeding Regimens

Recent changes in social housing requirements, in part per EU guidance, have resulted in changes to the housing environment; as such, more studies are being performed using solid-bottom bins with bedding material. Charles River has extensive experience with different housing and feeding regimens and can advise and accommodate most study designs, including group housing and restricted feeding in rats. In general, rats are socially housed by sex; however, due to natural aggression observed in group-housed male mice, only female mice are housed together if/when requested for mouse studies. Restricted feeding has been known to limit overall weight gain in some strains and, consequently, influence survivability and tumor latency and incidence.

Endpoints and Evaluations

A traditional carcinogenicity study is composed of four (or more) dose groups of rats or mice, with 50-75 animals/sex/group and additional satellite animals for toxicokinetic assessments. Sentinel animals housed in the same room are critical to monitor the general health of the study animals during the study.

Carcinogenicity studies should commence as soon as possible after weaning (i.e., as soon as the animals are accustomed to their diet and surroundings), which is typically six weeks of age. Body weights, food consumption, overt signs of toxicity, palpable masses, and ophthalmoscopy are routinely monitored. In addition, the monitoring of hematological parameters should be considered during the study, depending on applicable regulatory requirements and previous findings in shorter-term studies. A complete histopathologic examination of all study groups, followed by a peer review, is performed for all carcinogenicity studies. At Charles River, mortality and tumor data are statistically analyzed by experienced statisticians familiar with using the Kaplan Meier methodology, as approved by the US FDA and other relevant industry/regulatory authorities.

Incorporation of an evaluation milestone at the mid-point (e.g., 52-weeks) may be appropriate in some cases, but more often for agrochemicals or food additives. In addition, in some instances, an *in utero* exposure phase may also be necessary for food additives. Depending on the mechanism of action and/or intended therapeutic use for the pharmaceutical or test compound, additional measurements may also be needed. We offer a comprehensive range of standard and specialized endpoints such as clinical biochemical parameters, urinalysis, bone densitometry, neurotoxicity, immunotoxicology, immunohistochemistry and stereology for inclusion in carcinogenicity studies.

Toxicokinetics of the test compound is typically assessed at the start of dosing and again at appropriate intervals. Satellite animals are generally used to provide blood samples for exposure analysis and to allow for serial blood sampling without compromising the main study population. A typical toxicokinetic phase duration is 6 to 12 months. We provide full laboratory support, including bioanalysis and modeling of data as well as toxicokinetic data reporting and interpretation.

At study finalization, a submission-ready electronic version (a linked, searchable PDF) of the complete audited report is provided to the client. Electronic files containing body weight, food consumption and tumor data can be provided in FDA format following issuance of the final version of the report, including standard data format/terminology to meet SEND requirements. Data analysis and the reporting process are monitored to ensure that reports are submitted according to scheduled timelines.

The rasH2 Alternative Transgenic Model

Charles River offers the rasH2 mouse model as an alternative to the traditional two-year mouse bioassay. We have performed over 60 studies using the rasH2 mouse model in the last several years, including both 28-day range-finding studies and 6-month carcinogenicity studies. The use of the rasH2 transgenic mouse model for carcinogenicity testing offers a shorter testing duration, potential mechanistic data, 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. Regulatory agencies such as the FDA have routinely approved the substitution of a 6-month rasH2 carcinogenicity study for a 2-year mouse carcinogenicity study. A 28-day range-finding study with full histopathology should be performed using CByB6F1 wild-type littermates prior to a 6-month rasH2 carcinogenicity study.

The rasH2 mouse was developed by Nomura and colleagues at Central Institute for Experimental Animals (CIEA) in Kawasaki, Japan. The human c-Ha-ras gene was microinjected into C57BL/6 x BALB/c F2 zygotes that were backcrossed to C57BL/6J mice. The mice are maintained at Taconic by crossing hemizygous C57BL/6JJic-Tg(HRAS)2Jic males with Balb/cByJJic females to produce CByB6F1-Tg(HRAS)2Jic mice. The rasH2 mouse has multiple copies of the human Ha-ras gene as well as its native murine gene, and Ha-ras expression is up to 3 times higher than normal. As a result of the overexpression of Ha-ras, the rasH2 mouse develops a higher tumor incidence with a more rapid onset when administered carcinogens compared to wild-type controls. Reports show that the rasH2 mouse has a lower spontaneous tumor incidence at six months and higher survival than that observed in two-year mouse studies, enabling the use of fewer animals per sex per group (e.g., 25/sex/group). Regulatory agencies including the US FDA, PMDA, and EMA consider this model to be appropriate for testing of either genotoxic or non-genotoxic compounds, and is appropriate across diverse routes of administration.

Comprehensive Background Data and Experience

The assessment of a compound to induce tumor growth in animals is widely accepted for predicting relative risk in humans. To this end, an understanding of the spontaneous background incidence of tumor formation and survival in the animal model used for carcinogenicity testing is critical for assessing tumorigenic potential and relevance of risk to humans. Charles River has one of the largest comprehensive historical control databases for carcinogenicity studies, including background data on tumor incidence, survival, histopathology, organ weights, clinical pathology, body weight and food consumption for each species and strain tested. Table 1 illustrates the total number of carcinogenicity studies we've performed at each of our sites between 2006 and 2017. Collectively, our sites have conducted well over 100 carcinogenicity studies in the last 5 years since 2011.

Pathology

The key assessment for carcinogenicity testing resides in the full pathological evaluation of tissues and organs, and a comprehensive assessment of tumorigenicity is critical in the evaluation of carcinogenicity studies. Histopathological assessments are supported by more than 25 veterinary pathologists who are board-certified by the American or European College of Veterinary Pathologists (ACVP and ECVP, respectively), or who hold membership with the Royal College of Pathologists (RCP). Their work is further supported by a group of experienced veterinary clinical pathologists. Our dedicated pathology team can also facilitate peer review, and has access to data sharing technology such as the Aperio virtual pathology system.

Non-Animal Alternatives

Non-animal alternatives to carcinogenicity studies include cell-based assays and computational prediction models. These approaches are of significant interest to sponsors, as well as scientists within Charles River; however, current alternatives/methods are not considered sufficient to serve as replacements for animal studies.

Table 1: Experience. Knowledge. Worldwide Reputation.

Charles River Site	Species/Strain	Number of Carcinogenicity Studies			
		Last 10 Years*	Last 5 Years	In Progress	Total
Montreal-Sherbrook Canada	Rat/CD®IGS	35	22	1	36
	Rat/Wistar Han	16	11	4	20
	Rat /Fisher F344	3	2	-	3
	Mouse/CD-1	25	18	1	26
	Mouse/rasH2	5	3	2	7
Spencerville, Ohio USA	Rat/CD®IGS	13	8	1	14
	Rat/Wistar Han	-	-	4	4
	Mouse/CD-1	10	5	1	11
	Mouse/rasH2	13	12	1	14
Edinburgh, UK	Rat/CD®IGS	4	4	-	4
	Rat/Wistar Han	14	7	2	16
	Mouse/CD-1	17	6	2	19
	Mouse/rasH2	-	-	-	-
Ashland, Ohio USA	Rat/CD®IGS	27	11	-	27
	Rat/Wistar Han	-	-	-	-
	Mouse/CD-1	17	6	-	17
	Mouse/rasH2	-	-	-	-
Lyon, France	Rat/CD®IGS	1	-	-	1
	Rat/Wistar Han	4	2	1	5
	Mouse/CD-1	5	2	-	5
	Mouse/rasH2	-	-	2	2
* Years represented are 2006-2017.				10-Year Total (all CRL sites)	231