Tat-Fit: Tasteful tamoxifen for inducible transgenesis

Overall aim

This Challenge aims to develop a method that allows tamoxifen to be added to rodent chow without changing its palatability or consumption by the animals. The method must not alter the nutritional value of the food and the final product must be affordable and permit the addition of tamoxifen to the food by researchers and animal care staff.

Duration

Up to one year

Budget

Up to £100k

Sponsor

Mary Lyon Centre

Background and 3Rs benefits

Conditional inducible transgenesis is widely used in mice to temporally and spatially express or delete genes using CRE recombinase (CRE-ER) (Kim *et al*., 2018). Induced by the application of the drug tamoxifen, CRE-ER proteins translocate into the nucleus of cells expressing them, leading to recombination at specific Lox P sites flanking the fragment of DNA to be excised.

The CRE system has been widely used to study the function of genes either at specific life stages or in specific tissue types at controlled time points. Although this targeted and inducible transgenesis can help to avoid the lethality caused by some global knockouts where the gene of interest is deleted in every cell in the mouse, the use of tamoxifen can be problematic.

Rodent diet containing tamoxifen is commercially available. The bitter taste of tamoxifen however means that it is not readily consumed by the mice and this can lead to weight loss. Unpalatability can also cause variability in the experimental cohort and confounds findings with the animal ingesting varying amounts of the food and hence tamoxifen. As a result, experiments usually require direct administration of tamoxifen by oral gavage which requires repeated handling and scruffing of the mice and insertion of a gavage cannula, with dosing typically for seven days. This can be highly time consuming for staff. Some facilities administer the compound either intraperitoneally or subcutaneously and this can lead to inflammation at the injection site as tamoxifen is hydrophobic and

must be prepared in oil, typically corn oil. For studies involving pregnant mice, dosing particularly by the intraperitoneal route can result in spontaneous abortions.

Tamoxifen preparations can be variable depending on the source and approximately 10% of batches (data from the Mary Lyon Centre) have an adverse effect that results in the animals being removed from the study. The number of animals used is influenced by the challenges associated with effectively administering tamoxifen and the genotype required for the experiment. Some experiments use 192 mice with around 500 mice used in the associated breeding. Improved dosing could minimise the overall number of mice used.

This Challenge aims to deliver a palatable form of tamoxifen that can be prepared within a facility, using the required feed for the experiment. Providing food which contains tamoxifen but is otherwise indistinguishable for the animal will ensure that normal intake is maintained, reducing potential welfare concerns from lack of food and reducing dose variability. This will lead to more consistent recombinase activity, decreasing the variability of the phenotyping data yielded from such experiments, and resulting in a reduction in the number of animals used.

Other benefits

Many drugs are unpalatable, hence the widespread use of tablets in human medicine. It is not possible to dose small rodents with capsules or tablets, therefore any developed method which could potentially be applied in any facility and to any drug would be a significant advantage to the fields of preclinical medicine as well as discovery science (Turner *et al*., 2011).

Key deliverables

- A method of applying or incorporating the powdered form of tamoxifen into any food stuff by researchers or technical staff within a facility.
- The vehicle or approach must not alter the nutritional value of the food ingested.
- The product must be affordable [around £250 for 10kg] to ensure broad uptake.
- The product must be amenable to dosing appropriate quantities of food, as a guide, each mouse consumes between two to five grams of chow a day. Each cage would need approximately 250 grams per week.
- The product must be compatible with pelleted food eaten from a hopper.
- Animals on this food must not lose weight or eat less.
- Evidence that this method delivers tamoxifen effectively and at sufficient doses for good bioavailability (as measured by its efficacy at inducing recombination of conditional transgenes). The Sponsors will provide support with these tests.

It is important to note that the CRACK IT Challenges competition is designed to support the development of new 3Rs technologies and approaches, which will improve business processes and/or lead to new marketable products. The application must include a plan to commercialise the results into a product or service. This should be taken into consideration when completing your application.

Sponsor in-kind contributions

The Mary Lyon Centre will provide:

- Technical, animal care and logistics expertise.
- Trial of any method developed for mixing food with tamoxifen.
- A comparison study of this method of dosing and other conventional methods.
- As part of on-going efforts in assaying expression levels and patterns of mouse lines carrying CRE-ERT2, incorporate trials of dosed food and measure the biological efficacy (reporter gene assays).
- Disseminate any data at meetings frequently attended by Mary Lyon Centre staff.

References

Kim H *et al.* (2018). Mouse Cre-LoxP system: general principles to determine tissue-specific roles of target genes. *Laboratory Animal Research* 34(4):147-159.

Turner V *et al*. (2011) Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider. *Journal of the American Association for Laboratory Animal Science* 50(5): 600–613.