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#3486-D294

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BACKGROUND & OBJECTIVE

Following ICH S6(R1) and ICH S5(R3) guidelines, transgenic mice are now a critical relevant species for the developmental and reproductive toxicity (DART) assessment of biotherapeutics, often as an alternative to non-human primates (NHPs). However, a major limitation is the paucity of practical data on their reproductive and developmental performance. This study aims to conduct a systematic review of the historical control data from DART studies accumulated at our facility involving various transgenic mouse models, and to evaluate the utility, inherent challenges, and critical design elements for DART assessment in these models.

METHODS

A retrospective analysis was conducted on 26 DART studies performed at our facility that utilized transgenic mouse models on C57BL/6N background for biopharmaceuticals. The dataset included 6 Fertility and Early Embryonic Development (FEED), 15 Embryo-Fetal Development (EFD), and 5 Pre- and Postnatal Development (PPND) studies. Models targeted key molecules such as IL-1β, IL-17A, CD22, and hIL6/hIL6R, among others. Reproductive performance parameters—including mating rate, pregnancy rate, early embryonic development, intrauterine embryo-fetal survival, and F1 generation litter size were evaluated in control animals to assess the robustness of these models for DART testing and to inform optimal study design.

RESULTS

Variable Reproductive Performance in Transgenic Mice

Analysis of mating and pregnancy data from 6 FEED and 15 EFD studies revealed that transgenic mice exhibited normal mating behavior at sexual maturity (8-11 weeks of age), with a high mean mating rate (98.02%) and most pairs cohabiting successfully within one estrous cycle (4-5 days). The mean pregnancy rate was 85.90% in FEED studies, which declined to a mean of 72.78% in the larger dataset of EFD studies, with considerable inter-study fluctuation (as low as 37.5%). Consequently, study designs must incorporate an increased number of initial mating pairs to ensure a sufficient number of pregnant animals for a robust evaluation.

Analysis of Early Embryonic and Embryo-Fetal Survival in FEED and EFD Studies.

FEED studies (GD18) identified post-implantation loss as the primary factor reducing live litter size, substantially exceeding pre-implantation loss rates. Data from EFD studies (GD 18) were consistent, showing post-implantation loss rates from 4.85% to 27.27%, primarily driven by early resorptions, with notable variability across studies. Although the incidence of late resorptions and dead fetuses was low, it was present in individual studies. Consequently, the mean number of live fetuses per litter in EFD studies ranged from 4.3 to 9. The data are summarized in Table 1.

Table 1. Data summary of early embryonic and embryo-fetal survival

DART Study		Parameters				
		number of live fetuses per litter	post-implantation loss (%)	pre-implantation loss (%)	incidence of early resorptions (%)	incidence of late resorptions and dead fetuses (%)
FEED	Mean±SD	-	12.09%±4.08	9.79	-	-
	Range	-	8.58%-18.14	-	-	-
	n	-	6	6	-	-
EFD	Mean±SD	7.37±1.24	13.75%±6.6	-	12.15%	1.11%
	Range	4.3-9	4.85%-27.27	37.5%-72.78	-	-
	n	15	15	15	15	15

PPND Studies are Challenged by Insufficient and Variable Offspring Numbers

Data from six PPND studies showed that the mean litter size for transgenic mice at birth (litter size) ranged from 5.9 to 8.5 pups (Mean ± SD: 7.6 ± 1.02). While individual litter sizes were highly variable (range: 1-12 pups), frequently accompanied by sex ratio bias.

RESULTS

This situation aligns with the challenges reported for the PPND study of the approved drug UPLIZNA (Inebilizumab-cdon, an anti-CD19 monoclonal antibody), where a CD19 transgenic mouse model used characterized by low pregnancy rate, low fecundity, and small litter size resulting in an insufficient number of F1 offspring and leading regulators to deem the neurobehavioral assessment inadequate.

This retrospective analysis underscores the critical need to:

1) Increase the number of initial mating pairs.

2) Implement litter standardization to a lower size (e.g., 6 pups/litter).

3) Carefully plan the allocation of animals to various postnatal subgroups and assessments.

CONCLUSION

The systematic collection of historical control DART data for transgenic mice is a cornerstone for their reliable use in DART assessment. Our data identify inherent limitations, including variable reproductive performance and limited offspring numbers, which must be proactively integrated into study design through optimized mating strategies, increased cohort sizes, and adjusted litter standardization practices. We recommend early regulatory engagement as a prudent strategy to align study designs with scientific and regulatory expectations for DART assessment using transgenic mice.

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