PRIME-XV FreezIS DMSO-Free: Non-clinical Cryopreservation Toxicology Studies

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INTRODUCTION

Cryopreservation plays a vital role in cell and gene therapy production, enabling long-term storage and transport of highly precious cells. As a very effective cryoprotectant agent, DMSO continues to dominate cryopreservation protocols, despite the risks of cell stress and toxicity that ultimately affect cell viability. Because DMSO is harsh on cells, its use can become more limited, especially with more sensitive applications. The solution is to replace DMSO with an alternative that offers similar cryoprotective efficacy while minimizing cellular toxicity and maximizing cell viability.

FUJIFILM Irvine Scientific has developed PRIME-XV FreezIS DMSO-Free – a chemically defined cryopreservation solution that uses a nontoxic cryoprotective agent (CPA) as an alternative to the DMSO used in traditional cryopreservation methods. In order to confirm that PRIME-XV FreezIS DMSO-Free is nontoxic, toxicological data was obtained from two different testing laboratories where the product was injected into animal models.

The proprietary cryoprotectant (CPA) in this product is a nontoxic replacement for DMSO.

MATERIAL AND METHODS

Study 1: Investigated the effects of PRIME-XV FreezIS DMSO-Free via different administration routes

Experimental Design: Intermediate blood samples and body weight measurements were taken at day -2, day 0, day 7, day 14, and day 21 of the study. At the day of sacrifice (day 28), organs were processed for weight and general histopathology via hematoxylin and eosin staining as shown in Table 1 below.

Nr.	Test Compound	Route	Volume / 25 g (Body Weight)	Dosing at Day 0	N Mice	Sacrifice Timepoint
1	PRIME-XV FreezIS DMSO-Free medium	IP	1 mL	Single dose	3	Day 28
2	Negative control (PBS)					
3	PRIME-XV FreezIS DMSO-Free medium	IV	0.2 mL			
4	Negative control (PBS)					
5	PRIME-XV FreezIS DMSO-Free medium	SC	1 mL			
6	Negative control (PBS)					
7	PRIME-XV FreezIS DMSO-Free medium	PO	0.5 mL			
8	Negative control (PBS)					

Table 1 lists the different compounds tested, the route of administration (intraperitoneal “IP”, intravenous “IV”, subcutaneous “SC”, per os/oral “PO”), dosage, dosing period, sample size, and duration of study.

Study 2: Compared the effects of IV-injected stem cells in PRIME-XV FreezIS DMSO-Free to those in DMSO-containing cryomedium or PBS

Experimental Design: Animals were observed daily for any abnormal signs, conditions, or health concerns. Any animals exhibiting severe clinical signs or found moribund were euthanized. Animals were assessed for survival after 7 days.

Tab	Route/Dose	Test Material	Number per Group	Post-inoculation Observation Period
Guinea pigs	IV = 0.5 mL	AlloRX stem cells in DMSO-containing cryomedia	3	7 days
		AlloRX stem cells in PRIME-XV FreezIS DMSO-Free	3	
		AlloRX stem cells in PBS	3	
		PRIME-XV FreezIS DMSO-Free	3	
		PBS	2	
Mice	IV = 0.2 mL	AlloRX stem cells in DMSO-containing cryomedia	5	7 days
		AlloRX stem cells in PRIME-XV FreezIS DMSO-Free	5	
		AlloRX stem cells in PBS	5	
		PRIME-XV FreezIS DMSO-Free	5	
		PBS	2	

Table 2 lists animal models, administration route (intravenous “IV”) and dose, a description of the test material, which is either with or without AlloRX stem cells, sample size, and study duration.

RESULTS

Study 1: Normal growth following IV injection of PRIME-XV FreezIS DMSO-Free

Bodyweight

No abnormalities in growth progression were observed between vehicle control and compound treated mice for any of the dosing routes, indicating that the PRIME-XV FreezIS DMSO-Free medium, injected via different routes, does not affect growth progression in mice (e.g. IV injection, as shown in Figure 1).

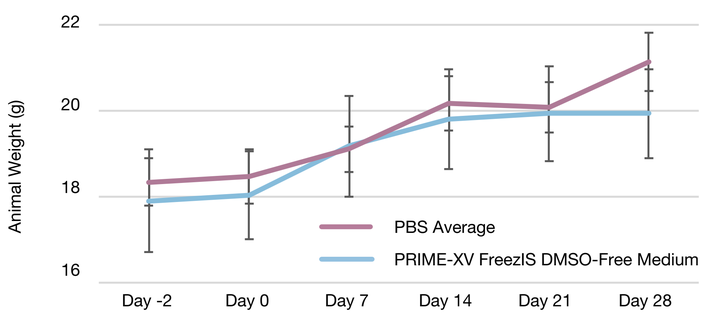

Figure 1 shows that intravenous (IV) injection of PRIME-XV FreezIS DMSO-Free does not affect growth, in terms of body weight, of mice over the 28-day period of the study. Error bars are SEM. Similarly, intraperitoneal (IP), subcutaneous (SC) and per os/oral (PO) administration did not impact body weight either (data not shown).

RESULTS (CONT.)

Organ Weight

The PRIME-XV FreezIS DMSO-Free treated group does not show alterations (Mann-Whitney U test) in organ weight compared to the vehicle control group, thereby indicating that the DMSO-free cryopreservation media is not affecting organ weight progression after injection via different entry routes as shown in Table 3.

PRIME-XV FreezIS DMSO-Free Versus PBS (p-value)				
	IP	**IV**	**SC**	**PO**
Total Kidney	0.1	> 0.9	0.4	0.2
Liver	0.8	0.4	0.4	0.1
Heart	0.7	> 0.9	> 0.9	0.4
Lungs	> 0.9	> 0.9	0.1	0.1
Spleen	0.7	0.4	0.4	0.1
Brain	0.5	> 0.9	0.4	> 0.9
Thymus	0.1	0.2	0.4	0.7
Lymph Nodes	0.4	0.7	> 0.9	0.2
Stomach	N/A	N/A	N/A	0.2

Table 3 lists the different compounds tested, the route of administration (intraperitoneal “IP”, intravenous “IV”, subcutaneous “SC”, per os/oral “PO”), dosage, dosing period, sample size, and duration of study.

Serum Chemistry

The results obtained from day 2 to day 21 showed comparable AST/ALT, creatinine, and urea concentrations in the test and control samples when evaluated for each route of administration. Based on these serum chemistry parameters, there is no evidence for an effect of DMSO-free cryopreservation medium on liver function, kidney function and general metabolic homeostasis in these mice.

Histopathology

At sacrifice, the following organs were carefully dissected and processed for histopathology and hematoxylin and eosin staining: heart, liver, kidneys, brain, lymph nodes, spleen, lungs, thymus, and injection site (stomach, skin, peritoneum, and tail). The histopathological analysis did not show adverse toxicity effects on the organs after injection with the PRIME-XV FreezIS DMSO-Free media as organs appeared normal compared to the vehicle control group.

Study 2: PRIME-XV FreezIS DMSO-Free cryomedium is considered nontoxic

Viability

Acceptance criteria included a survival rate of ≥ 80% and an absence of abnormal clinical observations during the experiment. Survival rates are presented in Table 4. A passing percent survival was set at 80%, due to the fact that an animal might die for reasons not linked to the injection. No abnormal clinical observations occurred.

Phase	Test Material	Number of Hosts Inoculated	Number of Hosts to Survive 24 Hours	Number of Hosts to Survive Observation Period	% Survival
Guinea pigs	AlloRX stem cells in DMSO-containing cryomedia	3	1	1	33
	AlloRX stem cells in PRIME-XV FreezIS DMSO-Free	3	3	3	100
	AlloRX stem cells in PBS	3	3	3	100
	PRIME-XV FreezIS DMSO-Free	3	3	3	100
	PBS	2	2	2	100
Mice	AlloRX stem cells in DMSO-containing cryomedia	5	4	4	80
	AlloRX stem cells in PRIME-XV FreezIS DMSO-Free	5	4	4	80
	AlloRX stem cells in PBS	5	5	5	100
	PRIME-XV FreezIS DMSO-Free	5	5	5	100
	PBS	2	2	2	100

Table 4 shows that PRIME-XV FreezIS DMSO-Free did not affect survival of guinea pig or mice hosts post-intravenous injection when compared to the PBS control. This was true both for PRIME-XV FreezIS DMSO-Free intravenous injection alone or with AlloRX stem cells.

SUMMARY AND CONCLUSION

In **Study 1**, PRIME-XV FreezIS DMSO-Free solution did not show toxic effects after administration through different entry routes. No clear pathological changes in blood biochemistry or organ histopathology were observed throughout the study in comparison to the PBS control and animals did not show any clinical signs, with growth curves and organ weights being as expected.

In **Study 2**, IV administration of cells in DMSO-containing and PRIME-XV FreezIS DMSO-Free cryomedia was compared to PBS. The survival rates for the inoculated hosts of AlloRX cells in PRIME-XV FreezIS DMSO-Free cryomedium, and PRIME-XV FreezIS DMSO-Free cryomedium were 80% or higher, and the hosts presented with no abnormal observations. However, survival of cells in DMSO-containing cryomedium in guinea pigs was only 33%, which was below the acceptance criteria for the study. Therefore, it is determined that PRIME-XV FreezIS DMSO-Free cryomedium is considered nontoxic via intravenous administration into guinea pigs and post-weaning mice.

The toxicological data obtained from this study demonstrates that PRIME-XV FreezIS DMSO-Free is nontoxic when injected in animal models. Therefore, this study should provide reassurance to cell and gene therapy customers when using PRIME-XV FreezIS DMSO-Free for their cell and gene therapy applications.*

*PRIME-XV FreezIS DMSO-Free is indicated for research or further manufacturing use.

ACKNOWLEDGEMENT

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