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

Application note

PRODUCT INFORMATION

PRIME-XV FreezIS DMSO-Free (Catalog #91140) cryopreservation medium from FUJIFILM Irvine Scientific, is a DMSO-free, chemically defined cryogenic preservation solution for human mesenchymal stromal cells (MSCs), human hematopoietic and progenitor cells (HSPCs), and somatic, mature, effector T lymphocytes. PRIME-XV FreezIS DMSO-Free is a high-quality, high-performing, nontoxic product manufactured with raw materials of the highest grade available. All incoming raw materials are tested prior to use, and each lot of the finished cryomedium is tested for sterility, pH, osmolality, endotoxins, mycoplasma, and performance.

The proprietary cryoprotectant agent (CPA) in this product is a nontoxic replacement for DMSO. Reassurance of the CPA raw material can be taken from the biological and clinical evaluations performed on other FUJIFILM Irvine Scientific media products that contain the same raw material; specifically, those used with Assisted Reproductive Technology (ART) medical devices. The ART media have contact with gametes and embryos during *in vitro* fertilization and culture, and incidental contact with the uterus. These media have been approved by regulatory agencies for over a decade, including the FDA and the EU MDR. To receive and maintain regulatory approval, substantial safety data is required, including biocompatibility studies for cytotoxicity, irritation, and sensitization. Furthermore, post-market clinical surveillance data is collected to confirm that there are no long-term safety concerns.

The characteristics and qualities of this component, which has been thoroughly tested in the ART field, appeals to those working with cells, such as progenitor cells, and T cells in the development of a broad spectrum of autologous and allogenic therapies within the regenerative and cell and gene therapy market.

TOXICOLOGY TESTING

In addition to the toxicity data obtained from ART media products containing the CPA in PRIME-XV FreezIS DMSO-Free, further toxicological testing of the cryomedia has been performed in animal models. Testing was performed at two independent, certified testing laboratories. Study 1 was in depth and diagnostically neutral, with the aim of investigating the effects of PRIME-XV FreezIS DMSO-Free via different administration routes. Study 2 was an applied study that compared the effects of intravenous injection of cells in PRIME-XV FreezIS DMSO-Free to those in commercially-available DMSO-containing cryomedium or PBS.

The results of these studies are presented below and demonstrate that it is nontoxic under these experimental conditions.

STUDY 1

STUDY DESIGN

Table 1. Study Design

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 the study.

Treatment Regimen

C57BL/J6 mice were administered with one injection of the test medium, as indicated in Table 1, on day 0 of the study.

Testing Regimen

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.

RESULTS

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, introduced via different entry routes, does not affect growth progression in mice (example for intravenous injection shown in Figure 1).

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, indicating that the DMSO-free cryopreservation media is not affecting organ weight progression after introduction via different entry routes (p-values noted in Table 2).

Figure 1. Normal growth following IV injection of PRIME-XV FreezIS DMSO-Free

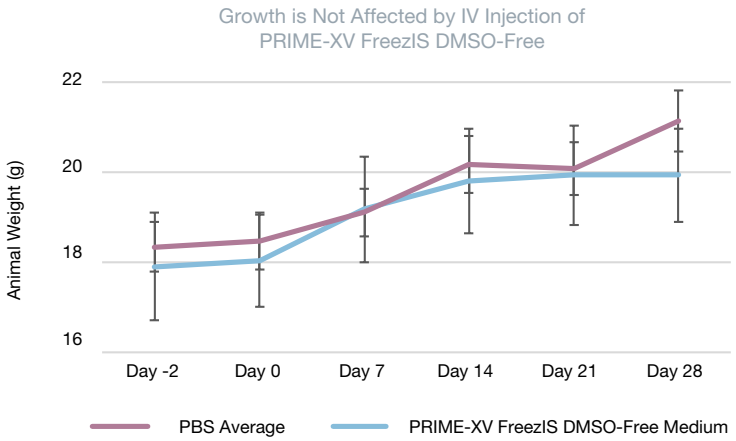

Figure 1 shows that intravenous (IV) injection of PRIME-XV FreezIS DMSO-Free does not affect growth, in terms of body weight, of mice over the period of 28 days for the study. Error bars are SEM. Similarly, intraperitoneal (IP), subcutaneous (SC), and per os/oral (PO) administration did not impact body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

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). 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.

Table 2. PRIME-XV FreezIS DMSO-Free does not affect organ weight

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 2 shows that, for all of the administration routes (intraperitoneal "IP", intravenous "IV", subcutaneous "SC", per os/oral "PO"), the p-value indicates that there is no statistical difference in organ weights measured 28 days post-administration and are not affected by PRIME-XV FreezIS DMSO-Free.

CONCLUSION

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.

STUDY 2

STUDY DESIGN

Table 3. Study Design

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 3 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.

Treatment Regimen

Hartley guinea pigs and CD-1 mice were administered with one injection of the test medium, as indicated in Table 3, on day 0 of the study.

Testing Regimen

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.

RESULTS

Viability

Acceptance criteria were that ≥ 80% of the animals would survive and have no abnormal observation present during the observation period. Survival rates are presented in Table 4. Percent survival is set as passing at 80% to allow for the fact that an animal might die for reasons not linked to the injection. No abnormal observations were present.

Table 4. Intravenous administration of PRIME-XV FreezIS DMSO-Free, either alone or combined with stem cells, does not affect survival

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.

CONCLUSION

In this applied study, 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 findings during the observational period. 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.

SUMMARY

Toxicological data obtained from two different testing laboratories demonstrate that PRIME-XV FreezIS DMSO-Free is nontoxic when injected in animal models. This data, along with the safety data obtained for regulated ART medical device media products that contain the CPA component, should provide reassurance to customers when using PRIME-XV FreezIS DMSO-Free for their cell and gene therapy applications.

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