

Background

Xenotropic murine leukemia-related virus (XMRV) is a newly identified retrovirus detected in humans. Results from several studies suggest an association of XMRV and/or MLV-related viruses with prostate cancer¹ and chronic fatigue syndrome²⁻⁷ (CFS). Some studies reported that up to 6.8% of healthy blood donors may be carriers of these viruses^{2,3}. XMRV and MLV-related viruses are infectious and blood-borne, thus have the potential to be transmitted by transfusion. Currently there are no regulatory approved blood screening assays for XMRV or MLV-related viruses. To mitigate the potential risk of transfusion transmission, blood services in the U.S., Canada, Australia, New Zealand and UK have decided to either discourage or defer donors with CFS from giving blood. Alternatively, pathogen inactivation (PI) technologies could be implemented to safeguard the blood supply from XMRV and MLV-related viruses, as is the case for plasma fractions (PPTA Press Release April 7, 2010). The INTERCEPT Blood System™ has robust inactivation capacity against a broad spectrum of viruses, bacteria, and parasites as well as leukocytes in platelet concentrates (PC), plasma, and red blood cell (RBC) components, including known human retroviruses, HIV-1, HTLV-I and HTLV-II (Table 1). This study evaluates the sensitivity of XMRV and MLV-related viruses to INTERCEPT treatment and determines the level of viral inactivation in PC and RBC.

- Schlager et al, PNAS 2009;106(38):16351-6
- Lombardi et al, Science 2009;326:585-589
- Lo et al, PNAS. 1006901107
- Hanson et al, Reviews in Antiviral Therapy & Infectious Diseases 2010;(8):10
- Mikovits et al, Reviews in Antiviral Therapy & Infectious Diseases 2010;(8):12
- Chen et al, Reviews in Antiviral Therapy & Infectious Diseases 2010;(8):32
- Pfost et al, Reviews in Antiviral Therapy & Infectious Diseases 2010;(8):35

Table 1: Inactivation of Retroviruses in Platelet, Plasma and RBC Components With INTERCEPT Treatment

Organisms	Extent of Inactivation (Log ₁₀ reduction)		
	Platelets ^a	Plasma ^b	RBC
HIV-1 (cell-associated)	>6.1	>6.1	>5.9 ^c
HIV-1 (cell-free)	>6.2	>6.8	NA
HTLV-I	4.7	≥4.5	>4.2 ^d
HTLV-II	5.1	>5.7	>5.1 ^d

- a. Lin et al. Transfusion 2005; 45: 580-90
b. Singh et al. Transfusion 2006; 46: 1168-77
c. Mufti et al. Biologicals. 2010; 38: 14-9
d. Data on file at Cerus; NA=not available

Aims

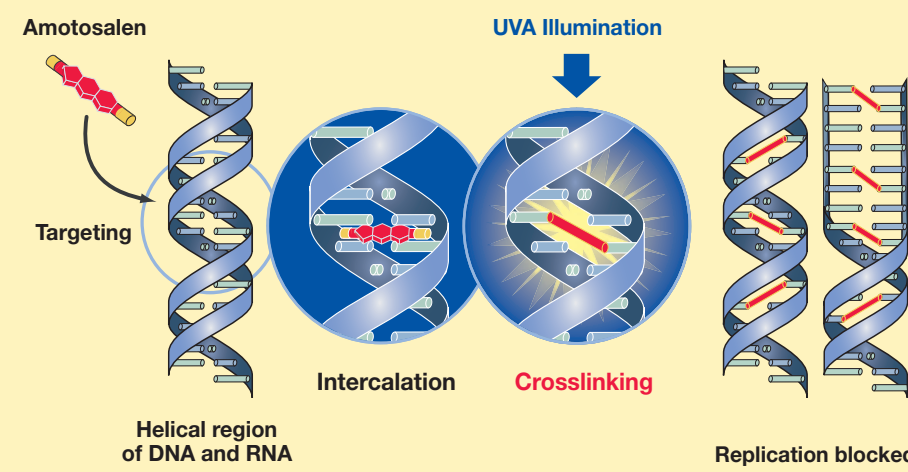
To evaluate the level of XMRV and MLV-related virus inactivation in platelet concentrates and red blood cell (RBC) concentrates prepared with the INTERCEPT Blood System

Methods

Experiments were carried out by contaminating blood components with natural viral isolates from a CFS patient and then measuring the level of inactivation using an infectivity assay. The virus stock was prepared from a WPI cell line expressing both XMRV and MLV-related viruses. The cell-free virus stock in phosphate buffered saline has a reverse-transcription polymerase chain reaction (RT/PCR) titer of ~10⁷ copies/mL and an infectivity titer of ~10⁶ IU/mL. Two replicate experiments each were performed for PC and RBC. 30 mL of each PC containing 3x10¹⁰ platelets in 35% plasma/65% InterSol™ were inoculated with 1.5 mL of stock virus and treated with 150 μM amotosalen and 3 Joules/cm² UVA light (Figure 1). 20 mL of each RBC in SAG-M (50% hematocrit) were inoculated with 1 mL of stock virus and treated by incubation with 0.2 mM S-303 and 20 mM glutathione (GSH) for 3 hours at room temperature (Figure 2). For each experiment, samples were withdrawn before and after treatment and assayed for the level of viral infectivity.

Figure 1: INTERCEPT Blood System for Platelets (Mechanism of Action)

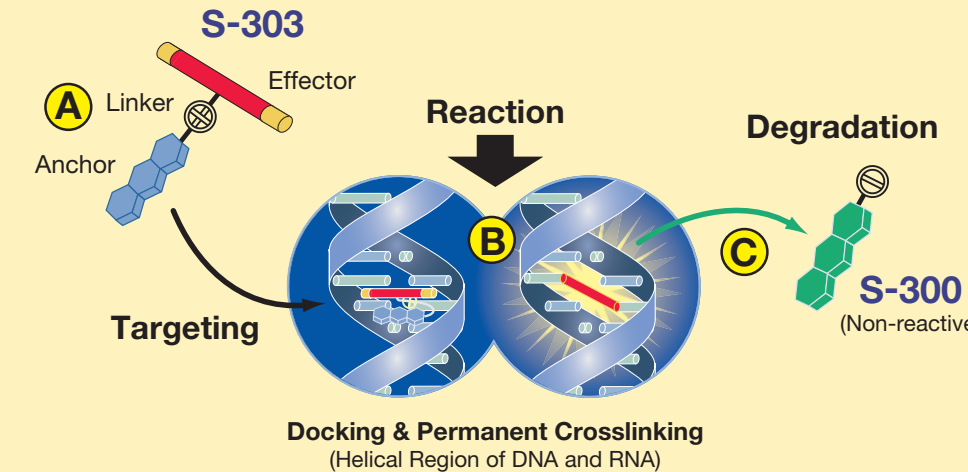
The INTERCEPT Blood System for platelets uses a combination of amotosalen HCl and long wavelength ultraviolet A (UVA) light. The amotosalen compound penetrates cellular and nuclear membranes and intercalates into the helical regions of DNA and RNA. Covalent crosslinks to the nucleic acid base pairs form upon exposure to UVA light, blocking DNA and RNA replication.



To quantify the level of virus, samples were titered on DERSE cells in six-well culture plates (Figure 3). 300 μL of serially diluted samples in RPMI media were overlaid onto each well containing sub-confluent DERSE cells. Plates were spun twice at 1,800 rpm for 10 minutes and incubated up to 2 hours at 37°C and 5% CO₂ to promote virus adsorption. Samples were aspirated, and cells were overlaid with 2 mL culture media (RPMI/10%FBS) and incubated at 37°C until confluent. After two passages, cells were harvested and analyzed by flow cytometry to detect viable XMRV/MLV-related viruses (Figure 4). The pre-treatment viral infectivity titer was estimated from the sample volume of the highest dilution in which virus was detected. The post-treatment viral infectivity titer was estimated from the sample volume of the lowest dilution in which virus was not detected. The inactivation was expressed as Log₁₀ reduction based on the ratio of the viral titer pre-treatment to post-treatment. Greater than symbols indicate no detectable residual virus in the volume of post-treatment sample assayed.

Figure 2: INTERCEPT Blood System for RBC (Mechanism of Action)

The INTERCEPT Blood System for RBC uses a combination of a FRALE compound S-303 and a quencher glutathione (GSH). S-303 rapidly passes through membranes (A). The anchor selectively targets nucleic acids. The effector crosslinks nucleic acids (B). The linker temporarily joins the anchor and the effector. Linker degradation yields the unreactive by-product S-300 (C). GSH minimizes non-specific reactions of S-303.



Results

PC (1:3 dilution) and RBC (1:5 dilution) demonstrated no cytotoxicity to the DERSE indicator cells and had no effect on the infectivity of XMRV or MLV-related viruses. The average titer of virus in PCs and RBC was calculated as the mean of two experiments after at least two passages of DERSE cells.

Before INTERCEPT treatment, XMRV and MLV-related viruses were detected in 60 μL of all PC dilution samples ranging from 1:3 to 1:15,625 in both experiments, suggesting a mean infectious titer of 5.2 x 10⁴ IU/mL. After treatment, no viable viruses were detected in 200 μL of each PC at

Table 2: Inactivation of XMRV and MLV-related Viruses in Platelet Concentrates With INTERCEPT Treatment

Experiment	Infectivity titer (IU/mL)		Log ₁₀ reduction
	Pre-treatment	Post-treatment	
1	5.2 x 10 ⁴	<5	>4.0
2	5.2 x 10 ⁴	<5	
mean	5.2 x 10 ⁴	<5	

Log reduction is calculated as Log (mean input titer/mean post treatment titer)

the dilution of 1:3, suggesting a residual titer of < 1 IU/200 μL or < 5 IU/mL. Therefore, the log reduction in PC was >4.0 (Table 2).

Similarly, before INTERCEPT treatment, XMRV and MLV-related viruses were detected in 60 μL of all RBC dilution samples ranging from 1:5 to 1:78,125 (exp#1) or 1:15,625 (exp#2), suggesting an average titer of 1.6 x 10⁵ IU/mL. After treatment, no viable viruses were detected in 60 μL of each RBC at the 1:5 dilution, suggesting a residual titer of < 1 IU/60 μL or < 17 IU/mL. Therefore, the viral log reduction in RBC was >4.0 (Table 3).

Table 3: Inactivation of XMRV and MLV-related viruses in RBC With INTERCEPT Treatment

Experiment	Infectivity titer (IU/mL)		Log ₁₀ reduction
	Pre-treatment	Post-treatment	
1	2.6 x 10 ⁵	<17	>4.0
2	5.2 x 10 ⁴	<17	
mean	1.6 x 10 ⁵	<17	

Log reduction is calculated as Log (mean input titer/mean post treatment titer)

Figure 3: DERSE Indicator Cell Clones to Detect XMRV Replication

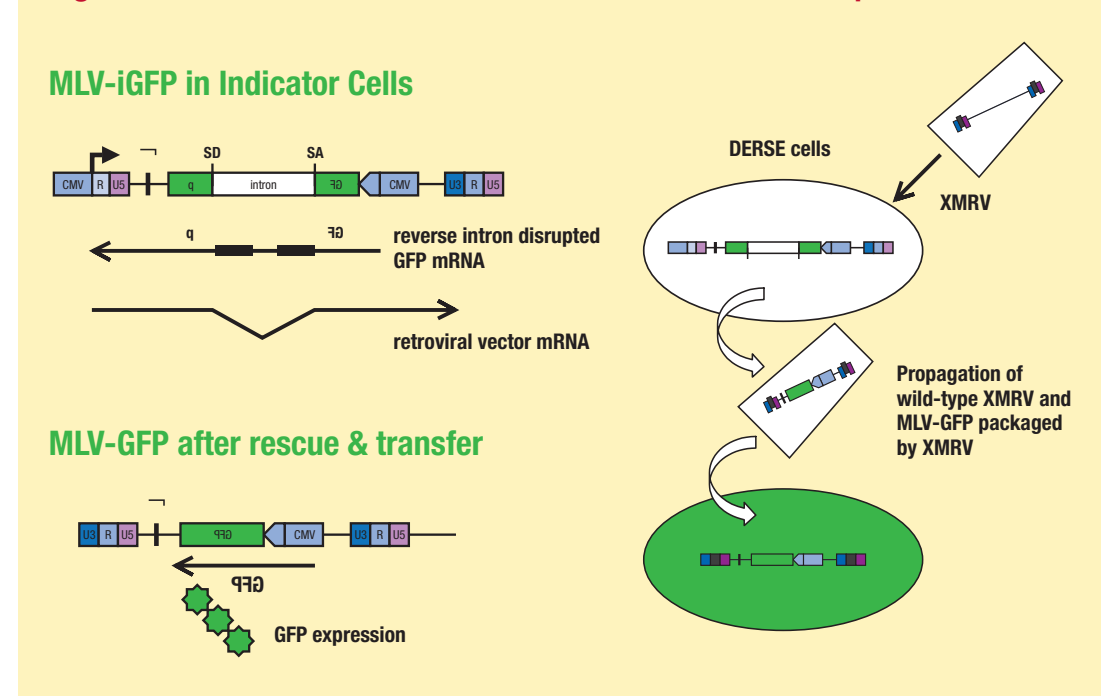
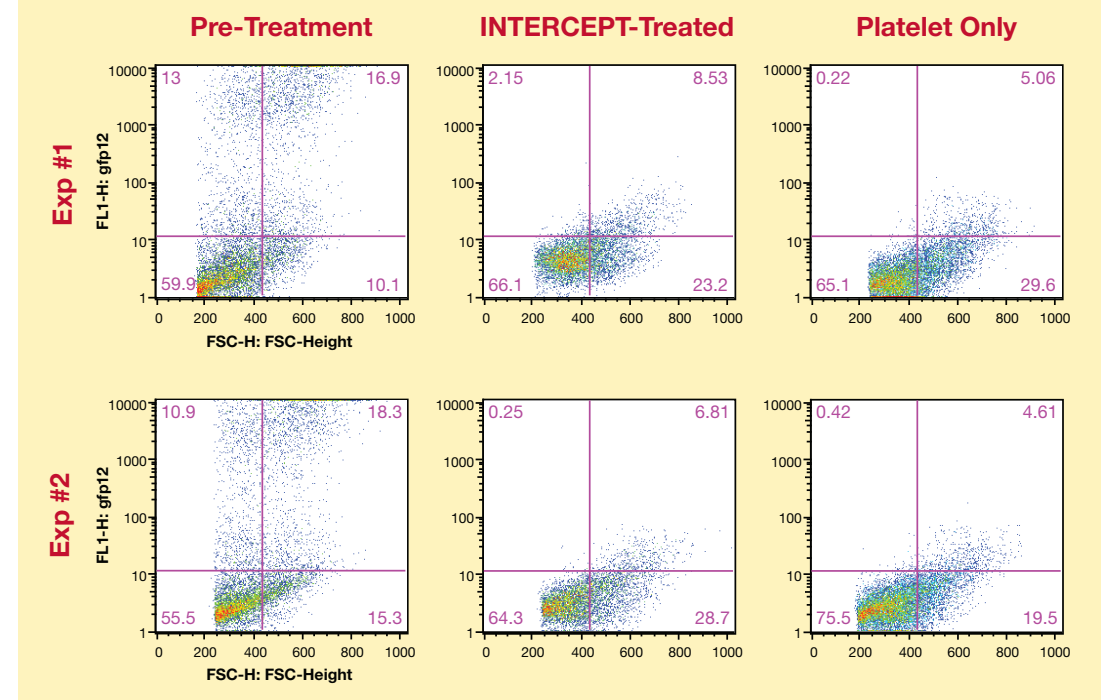


Figure 4: Representative Samples of Flow Cytometry Analysis of DERSE Cells Inoculated With Untreated or INTERCEPT Treated Platelets

300 μL of each sample were overlaid onto DERSE cells, after two passages, cells were harvested and analyzed by flow cytometry. Viable XMRV was detected in pre-treatment samples (left), no XMRV was detected after INTERCEPT treatment (middle). Platelet only samples (right) are the control platelets without virus inoculum and INTERCEPT treatment. All PC samples shown are at a 1:3 dilution. Positive cells are presented in the upper left and right quadrants above the FL1 intensity of 100.



Conclusions

- The results of this study demonstrated that high levels of XMRV and MLV-related viruses are inactivated in both PC and RBC components by treatment with the INTERCEPT Blood System.
- Pathogen inactivation with INTERCEPT has the potential to prevent transfusion-transmitted XMRV and MLV-related viral infection.
- Further studies will be performed to confirm XMRV and MLV-related virus inactivation in plasma with INTERCEPT treatment.