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Rabbit Models of Immune Response to Pathogen Inactivated Red Blood Cells

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**Presented at the 14th TIF International Conference
for Patients and Parents**

Antalya, Turkey • May 11 - 14th, 2011



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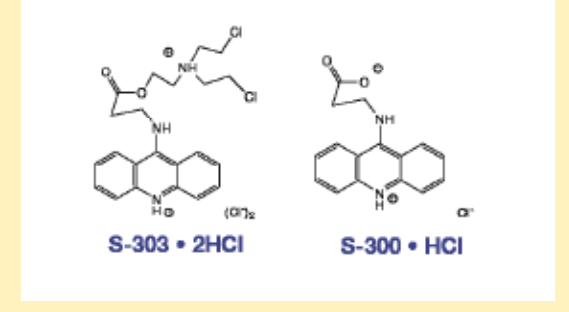
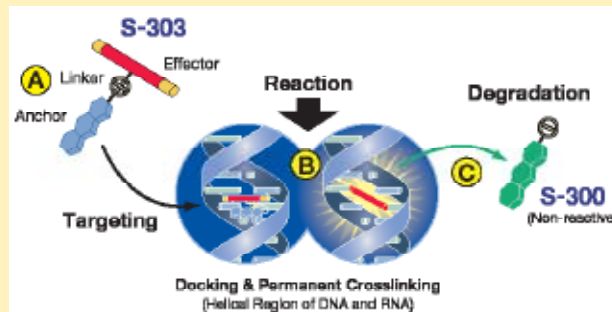
Background

Cerus has developed a pathogen inactivation (PI) system for red blood cells (RBC) based on S-303, an acridine compound that crosslinks nucleic acids of contaminating pathogens and leukocytes, preventing replication (Figure 1).

In a prior Phase 3 clinical trial evaluating transfusion support of patients with chronic anemia using RBC treated with the first generation process (HSRBC), low-titer antibodies to the acridine anchor of S-303 bound to RBC were detected in 2 of 17 patients although no physiologic activity or adverse outcomes were detected¹. To evaluate the consequence of repeat transfusion with

HSRBC, a model of chronic transfusion in New Zealand white rabbits hyperimmunized with S-303 conjugated to KLH (Keyhole Limpet Hemocyanin) was developed. With this model, we previously showed that HSRBC lifespan in rabbits pre exposed to S-303 treated RBC is normal whether or not hyperimmunized. However, in naïve animals, hyper-immunized against S-303, HSRBC were cleared faster than control RBC (CRBC)². Our efforts focused on reducing the amount of acridine on the surface of RBC through modifications of the treatment process while preserving the properties of RBC and retaining the ability to inactivate pathogens.

Figure 1: S-303 Treatment Process Mechanism of Action



- Anchor selectively targets nucleic acids
- Effector crosslinks nucleic acids
- Linker temporarily joins anchor and effector
- Cross-linking reaction is faster than linker degradation
- Degradation yields unreactive by-products

Aims

The objective of the study was to evaluate the immune response in rabbits with no prior S-303 treated RBC exposure using RBC with varying levels of surface bound acridine.

Methods

Chronic transfusion model

Rabbits, naïve to S-303, were transfused approximately biweekly for 29 weeks with allogeneic RBC (10mL/kg) with either untreated CRBC or with varying levels of RBC bound acridine molecules; Low, Intermediate and High (Table 1).

Antibody response to S-303 treated RBC was evaluated weekly in a heterologous system in which S-303 treated human RBC were incubated with rabbit serum and any bound anti-acridine antibody was measured with allophycocyanin (APC)-conjugated goat

anti-rabbit antibody and flow cytometry³. Median fluorescence intensity (MFI) was used to indicate the presence of antibody bound to cell targets. Potential positive samples (MFI > 2 fold above Control) were retested against treated and untreated RBC as confirmation of true positive. Pre-adsorption of sera with untreated human RBC was necessary to reduce non-specific fluorescence. Specificity of the response was determined using inhibition of IgG binding (measured as reduction in MFI) by competition with the S-303 related acridine compound, S-300.

Table 1: Levels of acridine on RBC for each rabbit group relative to the first generation process. Number of Responders indicates the number of animals that gave a positive anti-acridine response during chronic transfusion of SRBC

Group	Relative level of acridine compared to first generation process	Number of responders
Control (CRBC)	NA	0/6
Low (LSRBC)	25%	0/8
Intermediate; (ISRBC)	50%	2/8
High (HSRBC)	100%	4/8

Results

A novel rabbit model for allo-immunization to RBC antigens was used to evaluate the potential for immune response to S-303 treated RBC. The acridine levels on the transfused RBC represented those of the first generation process (HSRBC), the second generation process (LSRBC) and an intermediate level (ISRBC) (Table 1). Potential positive sera (displaying a two-fold or higher signal over CRBC) were retested against RBC units that were either untreated (CRBC) or treated with S-303 (SRBC). The comparison of immune response pattern identified the specificity of the antibody and eliminated other non-specific reactions (Figure 2A-D).

Using this methodology, 4 positive responders were identified in HSRBC group (Figure 3B), 2 in the ISRBC group (Figure 3A) and no positive response in the LSRBC group (summarized in Table 1). The specificity of the response was determined by a competition assay in which MFI could be reduced by preincubation of serum with S-300 (Figure 4).

Figure 2 (A-D): Confirmation of a true positive from a false positive response

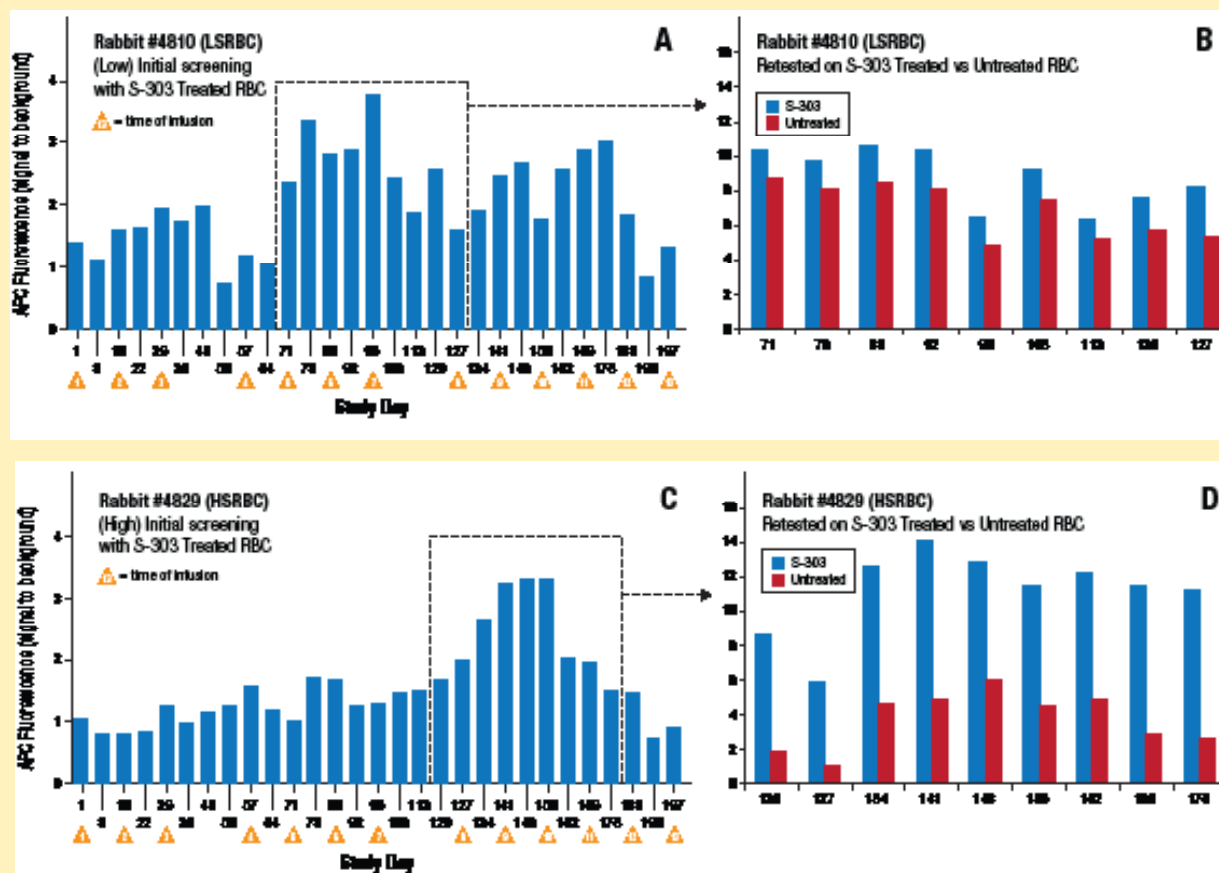


Figure 4: Competition Assay inhibition to confirm acridine specificity of response

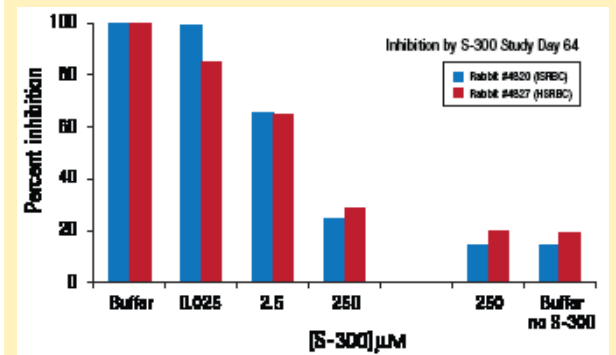
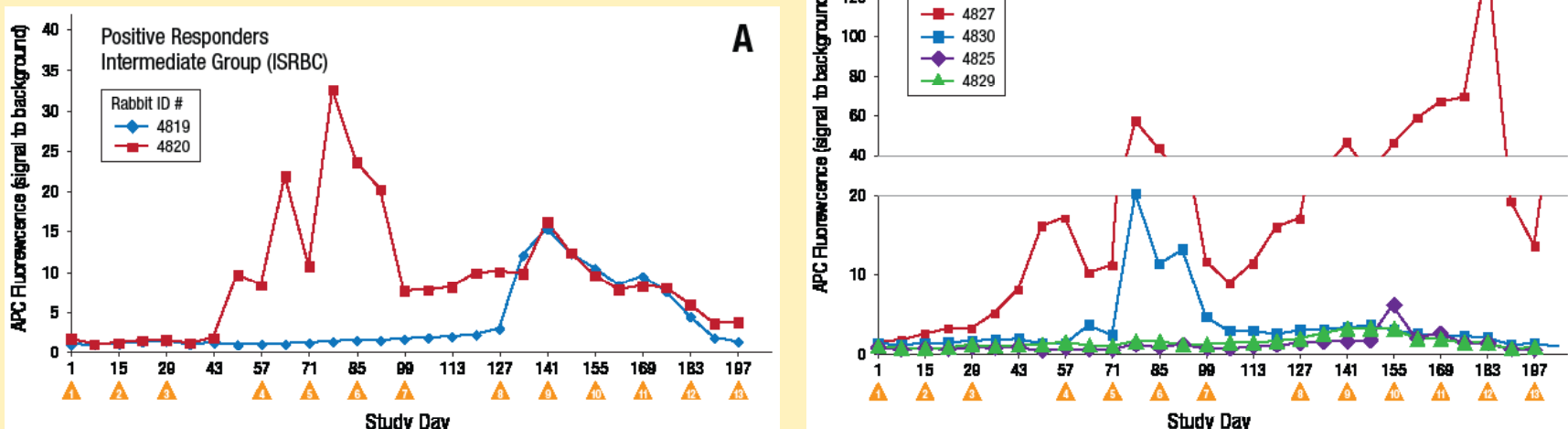


Figure 3 (A & B): Positive responders from the intermediate acridine level group (ISRBC) and the high acridine level group (HSRBC). The break in the Y-axis on Figure 3B allows scale expansion to include rabbit #4827 which had a much higher response than the other animals



Conclusions

- A chronic transfusion rabbit model was successfully developed and reproduced the immune response observed in a prior Phase 3 clinical trial with HSRBC
- The immune response is specific to the acridine anchor of the S-303 molecule
- S-303 RBC prepared using the second generation process (LSRBC) did not elicit immune responses after repeated exposures

References

- MG Conlan, E Vichinsky, E Snyder, et al. *Vox Sang* 2005;89(s1):121.
- MA Schott, GM Castro and A Stassinopoulos *Vox Sang* 2005;89 (s1):138
- V Lee, A Erickson, K Bahjat, E Lemmens and A North *Vox Sang* 2007;93(s1):168