

DR. PEYTON S. METZEL

Received his PhD from the University of Illinois at Urbana-Champaign in 1979 in Cell Biology & Virology. Over the ensuing twelve years, Dr. Metzel assumed positions of increasing responsibilities in diagnostics development at Abbott, Ciba Corning and Amersham. In 1991, he joined Baxter's Fenwal Division as Director, Pathogen Inactivation. There he led a multidisciplinary team, evaluating various technologies for their use in pathogen inactivation of blood products.

In 1993, Baxter signed a development agreement with Cerus Corporation. Dr. Metzel successfully managed a multifaceted team, guiding the platelet and FFP pathogen inactivation programs through pre-clinical development, clinical trials, and regulatory submission. In 2001, he assumed the position of Director of Scientific Affairs, where he is responsible for presentations of INTERCEPT and scientific assessment of new technologies.

Demonstration of Inactivation of WNV and Other Flaviviruses

Pathogen Inactivation of Blood Components

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Outline

- » Baxter
- » Helinx Technology
- » Inactivation of Pathogens
- » Inactivation of Flaviviruses
 - Hepatitis C Virus (HCV)
 - Bovine Viral Diarrhea Virus (BVDV)
 - West Nile Virus (WNV)
- » Conclusions

Baxter International Inc.

- » Global medical products and services company
- » Focus on critical therapies for life-threatening conditions
- » Over 50% of sales from outside United States
 - Represented in more than 100 countries
- » Employs 48,000 people worldwide
- » Sales \$7.7 billion (2001, U.S.)
- » Invests over \$1 million per day in research and development
- » Corporate headquarters located in Deerfield, Illinois

Baxter Corporation

- » Canadian subsidiary of Baxter International Inc.
- » Canadian headquarters in Mississauga, Ontario
 - Manufacturing facilities in Alliston, Ontario; Sherbrooke, Quebec
- » Employs nearly 1,000 people
 - 550 in manufacturing facilities
- » Sales over \$375 million (2001)

Helinx Technology

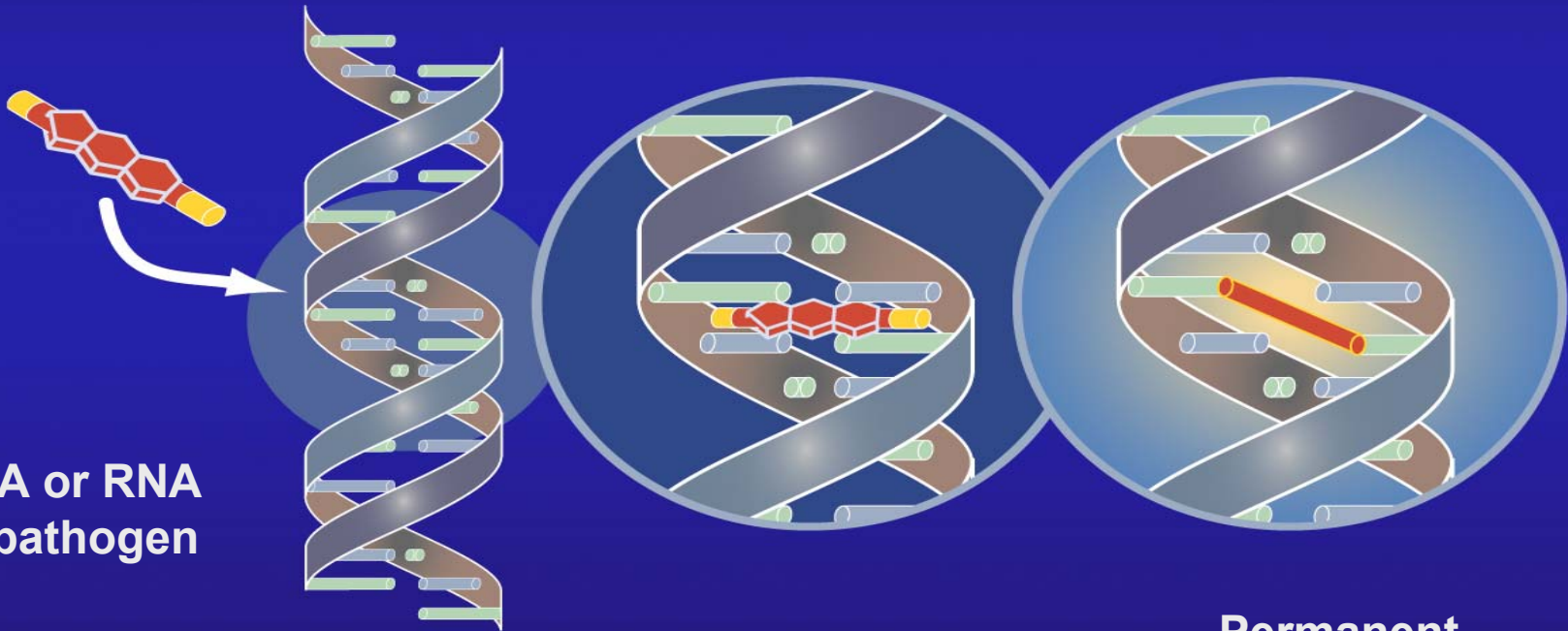
- » Pathogens and leukocytes require nucleic acids for replication
- » Blood components do not require nucleic acids for therapeutic function
- » **Helinx technology targets and modifies nucleic acids to prevent replication of viruses, bacteria, protozoa, and leukocytes**
- » Helinx technology for blood components
 - Platelet: Amotosalen HCL (S59)
 - Plasma: Amotosalen HCL (S59)
 - RBC: S-303

Mechanism of Action

Amotosalen
or S-303

Activation

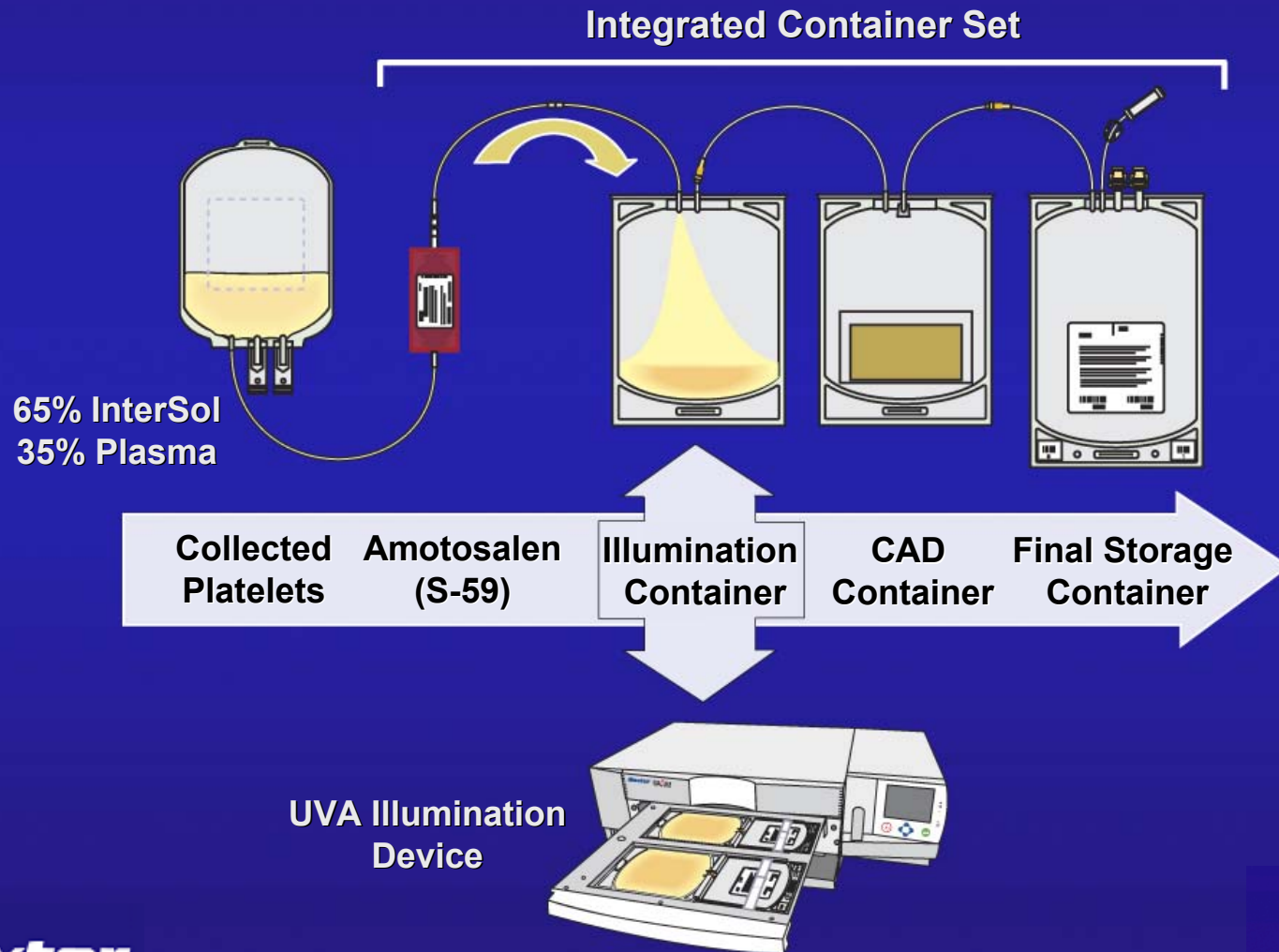
DNA or RNA
of pathogen



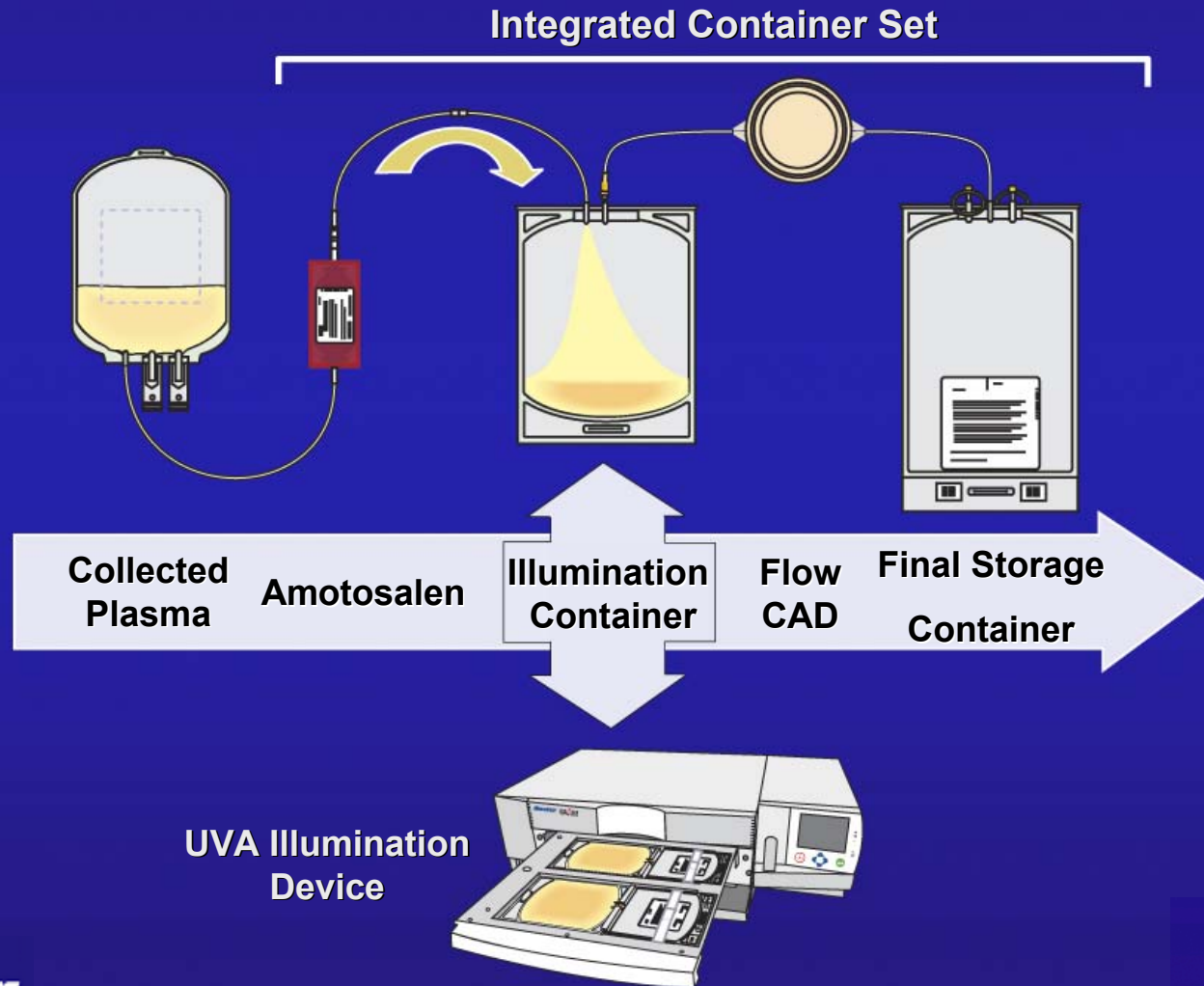
Intercalation

Permanent
Crosslinking

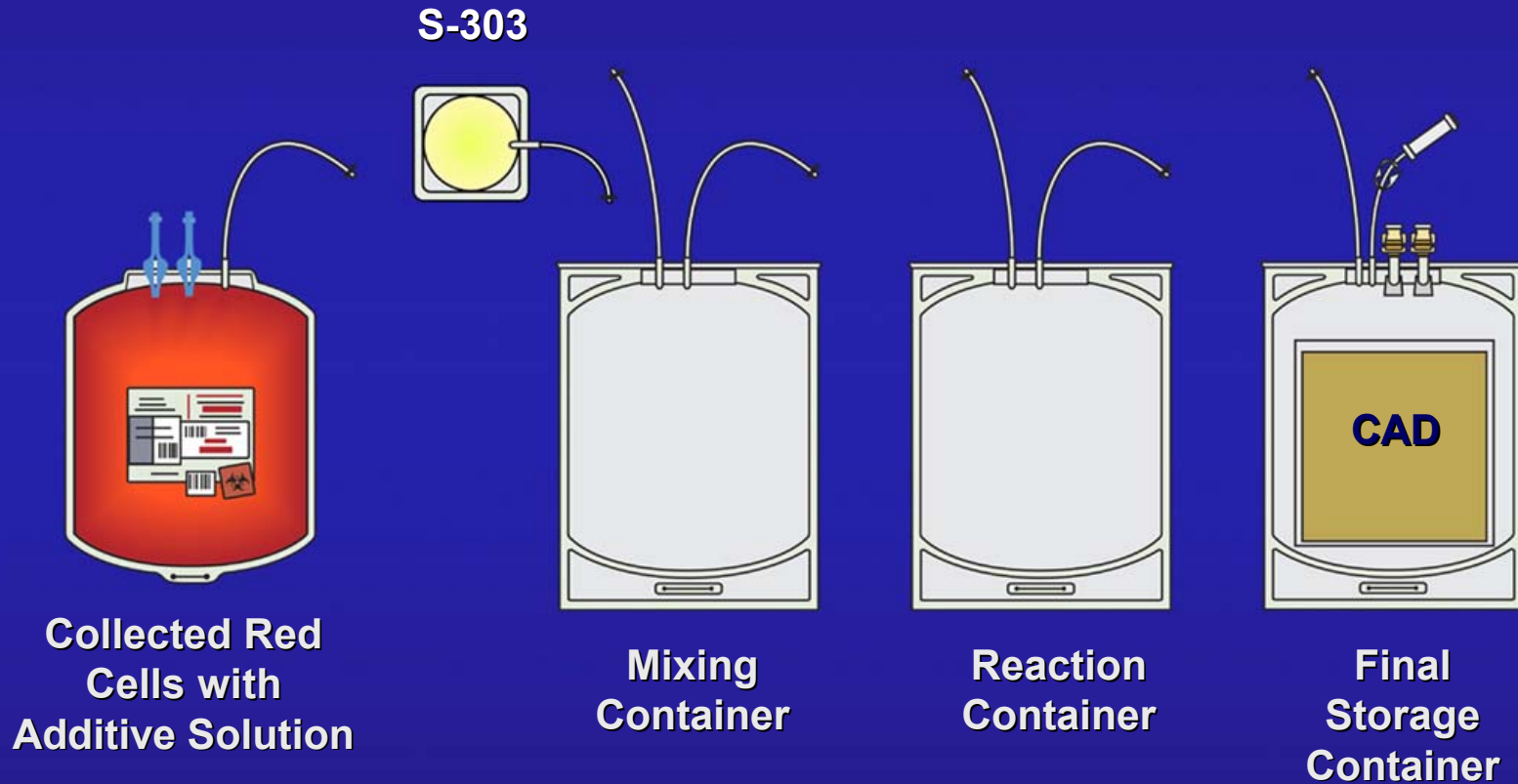
INTERCEPT Platelet System



INTERCEPT Plasma System



INTERCEPT RBC System



Study Design for the Validation of Pathogen Inactivation

- » Full-sized, therapeutic units of platelet concentrate, plasma, and RBC were used.
- » Each unit was spiked with $\sim 10^6$ infectious units/mL or with highest titered stocks available.
- » The contaminated units were treated with
 - Platelets/Plasma: 150 μM amotosalen and 3 J/cm^2 UVA
 - RBC: 200 μM S-303
- » Inactivation kinetics were measured.
- » The infectivity of each pathogen was assayed using culture methods or animal models.

Inactivation of Currently Tested Pathogens

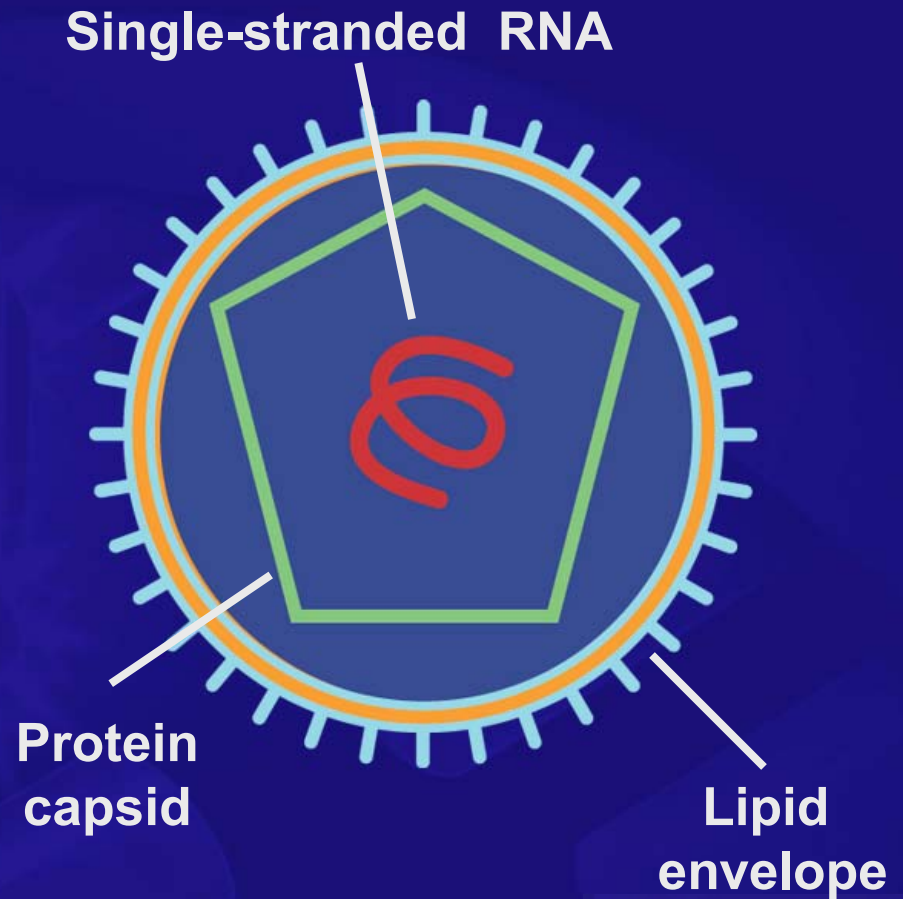
Log Reduction

	Platelets	Plasma	RBC
HIV-1, cell-free	> 6.2	> 5.9	> 6.5
HIV-1, cell-associated	> 6.1	6.4	> 6.5
HIV-1, clinical isolate	> 3.4		
HIV-2, clinical isolate	> 2.5		
HBV (Human MS 2)	> 5.5	> 4.5	
HCV (Human Hutchinson Strain)	> 4.5	> 4.5	
CMV	> 5.9		
HTLV-I	4.7		
HTLV-II	5.1		
<i>Treponema pallidum</i> * (Syphilis)	6.8 - 7.0		

* Tested in animal infection assays

Flavivirus

- » Yellow Fever
- » Japanese encephalitis
- » St. Louis encephalitis
- » Border Disease
- » Hog cholera
- » Dengue
- » Usutu
- » Hepatitis C
- » Bovine viral diarrhea
- » West Nile Virus

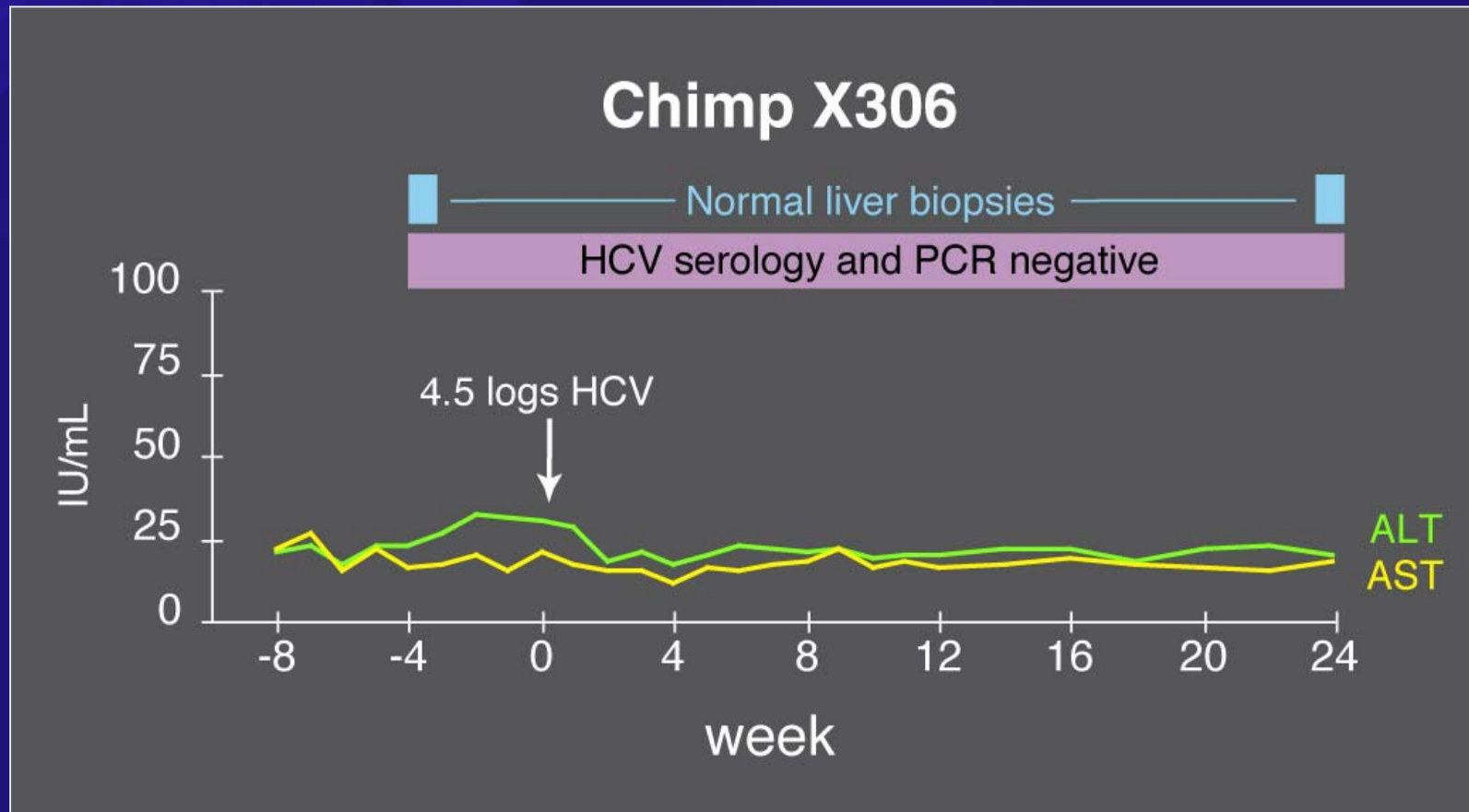


Inactivation of HCV in Platelets

Study Design

- » $10^{4.5}$ CID of the well-characterized Hutchinson strain of HCV were spiked into 3 full-sized apheresis platelet concentrates in 35% plasma/65% PASIII and treated with 150 μ M amotosalen and 3 J/cm² UVA.
- » Each unit (300 mL) was infused into a seronegative chimpanzee, which was followed for 6 months for development of hepatitis as well as molecular and biological markers of HCV infection.
- » Infection of Hutchinson strain of HCV in chimpanzees has been shown to be uniform and consistent.

Inactivation of HCV Infectivity in Platelets Results



Inactivation of BVDV

Study Design

- » Approximately 10^{5-6} pfu/mL of BVDV (strain NADL, ATCC) were spiked into full-sized platelet, plasma, or RBC units.
- » The contaminated units were treated with
 - Platelets/Plasma: 150 μ M amotosalen and 3 J/cm² UVA
 - RBC: 200 μ M S-303
- » The viral titer in a sample was measured using a plaque assay on bovine turbinate cells.

Inactivation of BVDV Infectivity Results

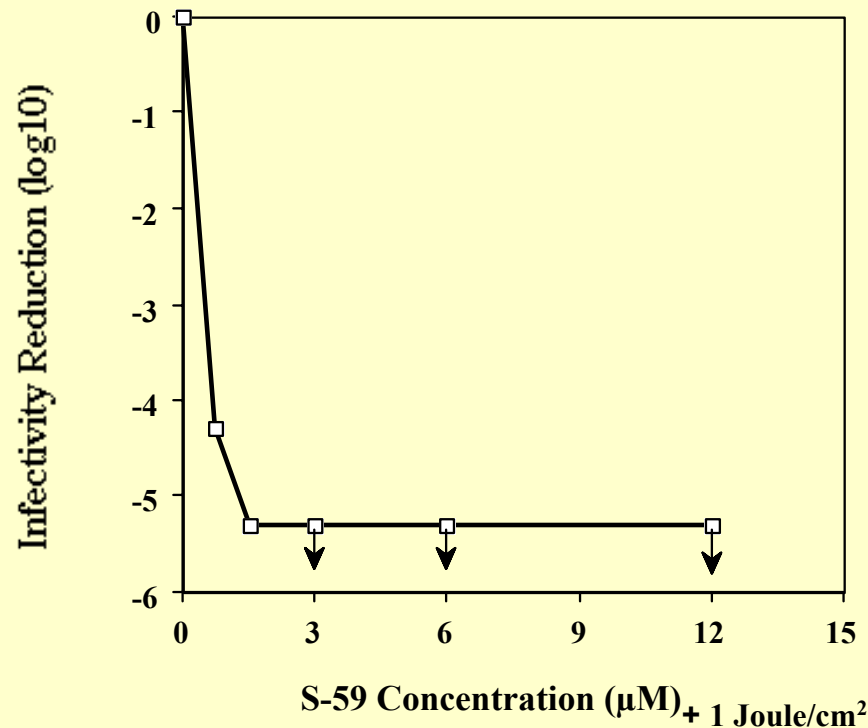
Log Reduction

	Platelets (N=4)	Plasma (N=4)	RBC (N=4)
BVDV	> 6.0	> 6.0	> 7.3

Kinetics of BVDV Inactivation

UVA Dose (J/cm ²)	BVDV Titer (log pfu/mL)	Log-Reduction (N=4)	Volume Assayed
0	4.8	0	
0.5	< -1.5	> 6.3	30 mL (No virus 4/4)
3	< -1.7	> 6.5	49 mL (No virus 4/4)

Amotosalen Dose Response of BVDV Infectivity



Inactivation of WNV

Viral Inoculum

- » This study was conducted in collaboration with Dr. Kristen Bernard of the New York State Department of Health.
- » The viral inoculum was prepared from BHK-21 cells infected with a full-length infectious clone of WNV.
- » The parental strain of WNV (lineage I) was isolated from the epicenter of New York City during the 2000 outbreak.
- » The infectivity and virulence of the cloned virus and the parental virus are similar.
- » The plaque morphology of the cloned WNV is indistinguishable from the parental virus.
- » Working viral stock: 1×10^8 pfu/mL

(Shi et al, J Virol. 76:5847-5856, 2002)

Inactivation of WNV

Study Design

- » Approximately 10^6 pfu/mL of the cloned WNV were spiked into full-sized units of platelet concentrate or RBC and treated with:
 - Platelets: 150 μ M amotosalen and 3 J/cm² UVA
 - RBC: 200 μ M S-303

- » The titer of WNV was measured using a plaque assay on Vero cells.

Inactivation of WNV Infectivity

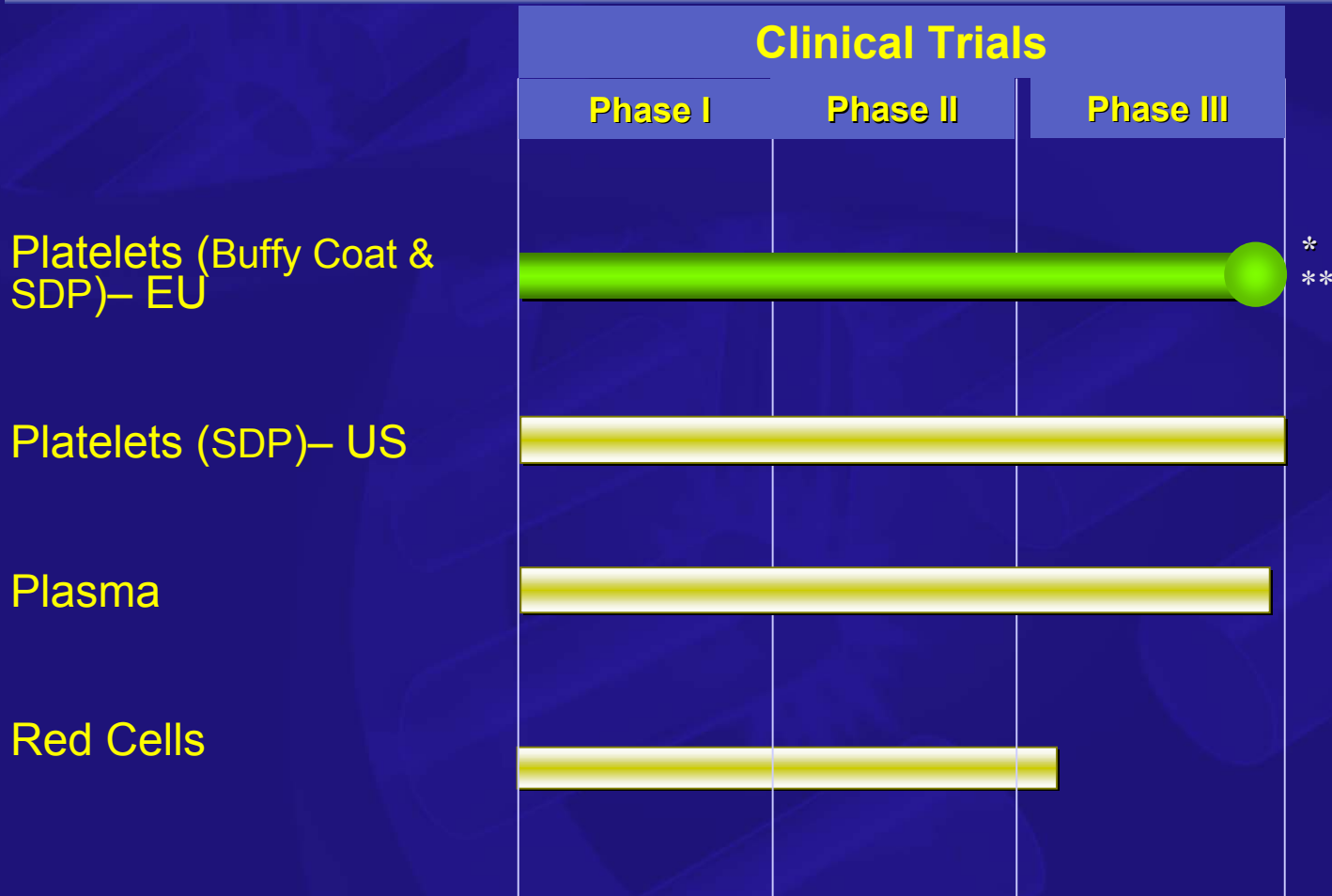
Preliminary Results

	Volume treated (mL)	Initial WNV Titer (pfu/mL)	Post-treatment WNV Titer (pfu/mL)	Log-Reduction (N=2)	Comments
Platelets	300	5.4×10^5	< 1	> 5.7	No recoverable virus in 1 mL in 2 of 2 experiments
RBC	300	9.1×10^5	< 1	> 6.0	No recoverable virus in 1 mL in 2 of 2 experiments

Advantages of Pathogen Inactivation

- » Proactive vs. reactive strategy (testing)
- » Independent of donor testing
- » May alleviate need for new tests
- » Minimizes impact of new infectious agents

INTERCEPT Program Status



* EU CE Mark approval October 14, 2002

** Canadian Medical Device License (Buffy Coat)– October, 2002

Conclusions

- » Helinx technology inactivates a broad spectrum of viruses, bacteria, protozoa, and leukocytes in platelets, plasma and RBC.
- » Preliminary results demonstrated inactivation of high levels of WNV in platelet concentrate and RBC components.
- » Both amotosalen and S-303 are effective against WNV.

Bacterial Inactivation in Platelets

<u>Gram-Negative</u>	<u>Log Inactivation</u>
<i>Escherichia coli</i>	> 6.4
<i>Serratia marcescens</i>	> 6.7
<i>Klebsiella pneumoniae</i>	> 5.6
<i>Pseudomonas aeruginosa</i>	4.5
<i>Salmonella choleraesuis</i>	> 6.2
<i>Yersinia enterocolitica</i>	> 5.9
<i>Enterobacter cloacae</i>	5.9

Parasite Inactivation in Platelets

<u>Parasite</u>	<u>Disease</u>	<u>Log Inactivation</u>
<i>Treponema pallidum</i>	Syphilis	>4.6
<i>Trypanosoma cruzi</i>	Chagas	>6.0
<i>Borellia burgdorferi</i>	Lyme	Planned
<i>Plasmodium falciparum</i>	Malaria	Planned
<i>Babesia microti</i>	Babesiosis	Planned

Leukocyte Inactivation

- » Inactivates > 5.4 logs of T-Lymphocytes (LDA assay)
- » Inhibits cytokine synthesis during platelet storage
- » Prevents Transfusion-Associated Graft-Versus-Host Disease in a murine transfusion model

INTERCEPT Red Cell System

Bacterial Inactivation Data

<u>Gram Positive</u>	<u>Log Inactivation</u>
<i>Listeria monocytogenes</i>	> 7.0
<i>Staphylococcus aureus</i>	> 5.2
<i>Staphylococcus epidermidis</i>	> 6.9
<i>Deinococcus radiodurans</i>	> 6.0

Toxicology Studies for INTERCEPT Systems Under Development

	Platelets	FFP	RBC
Acute Toxicology	✓	✓	✓
Repeated Dose – 1 month	✓	✓	✓
Repeated Dose – 3 months	✓	✓	✓
General Pharmacology	✓	✓	✓
Reproductive Toxicology	✓	✓	✓
Genotoxicity	✓	✓	✓
Carcinogenicity	✓	✓	✓
Phototoxicity	✓	✓	n/a
ADME	✓	✓	✓

✓ Complete

✓ In Progress

✓ Planned

n/a: Not applicable

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