L'inattivazione dei patogeni: tecnologie e prospettive

ISTITUTO SCIENTIFICO UNIVERSITARIO
SAN RAFFAELE

Silvano Rossini

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Servizio di Immunomatologia e Medicina Trasfusionale

Il percorso di oggi

I patogeni emergenti in Medicina Trasfusionale

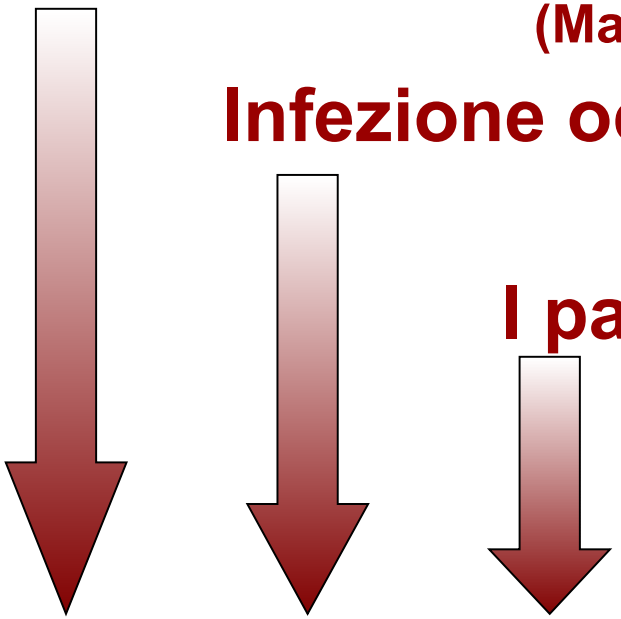
(Massimo Clementi

Infezione occulta da Virus dell'Epatite B

(A. Tagger)

I patogeni storici riemergenti

(G. Gesu)



Cosa possiamo fare ?

Transfusion safety

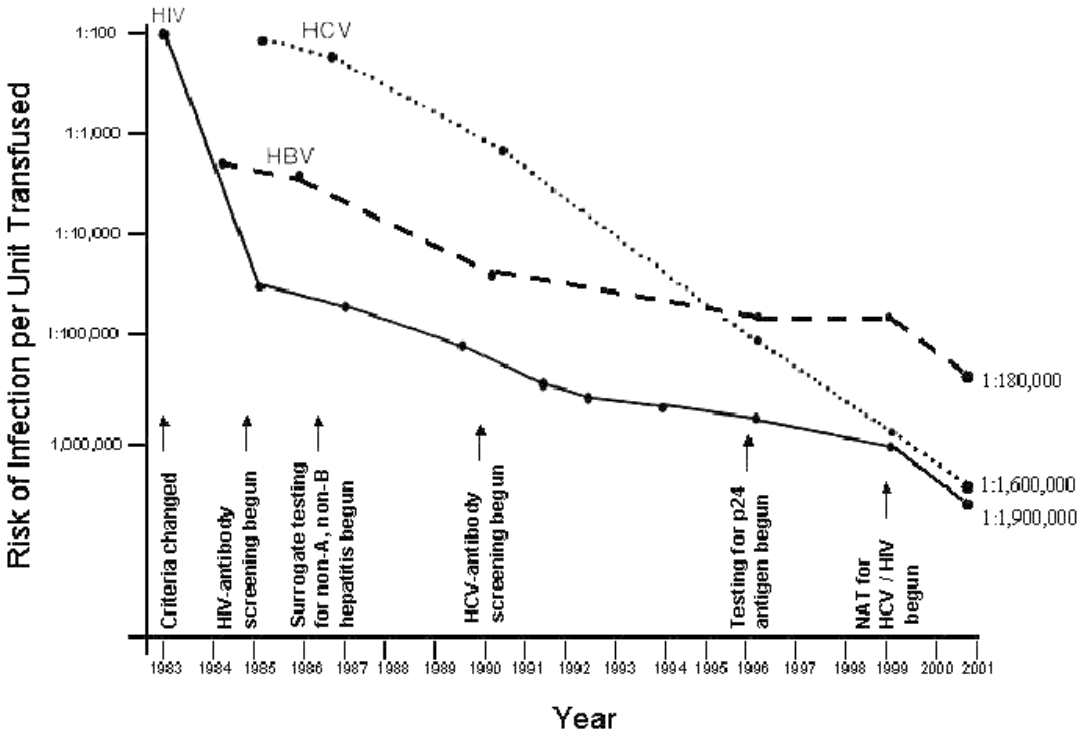
recruit

screen donor

collect & prepare

infection disea

luate



product

process

W. Dzik, 2003

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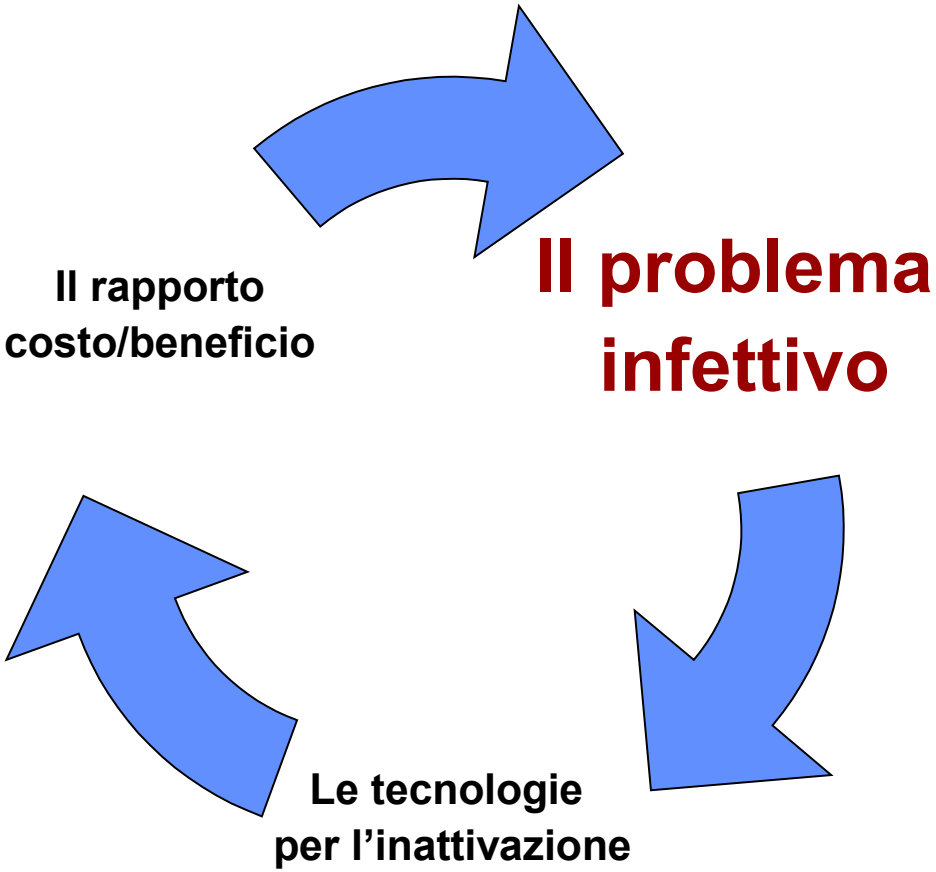

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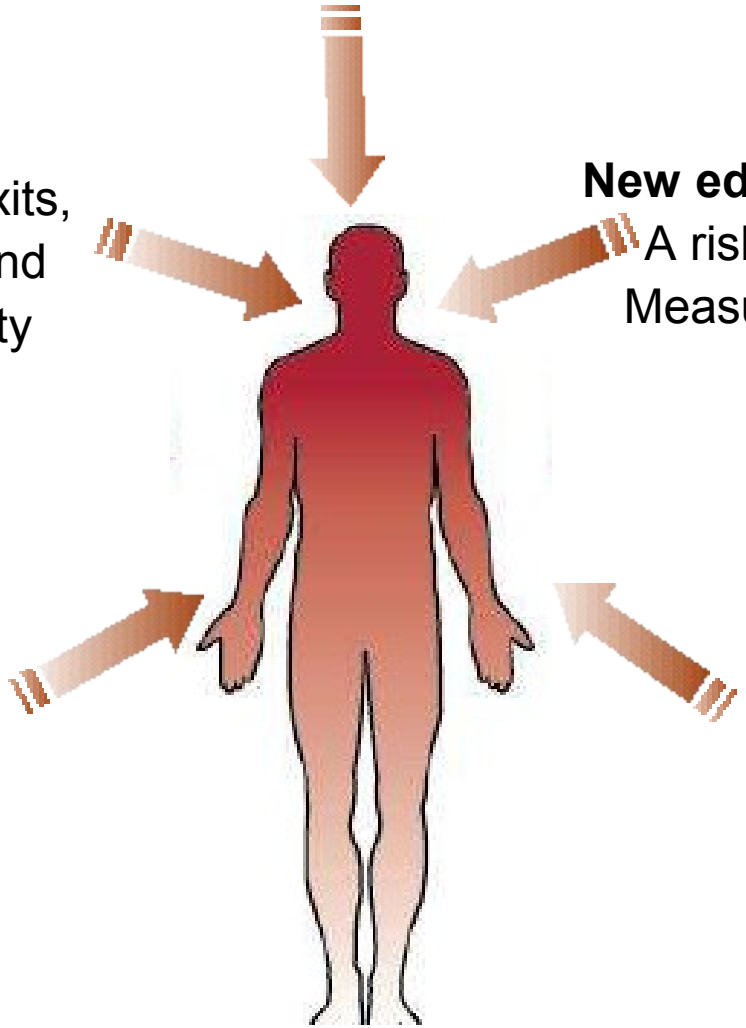
Screening limitations

Gaps in current defenses exits, due to the window period and limited screening sensitivity



New ed emerging pathogens

A risk that current safety Measures cannot eliminate

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Screening limitations
Gaps in current defenses exits, due to the window period and limited screening sensitivity

New ed emerging pathogens
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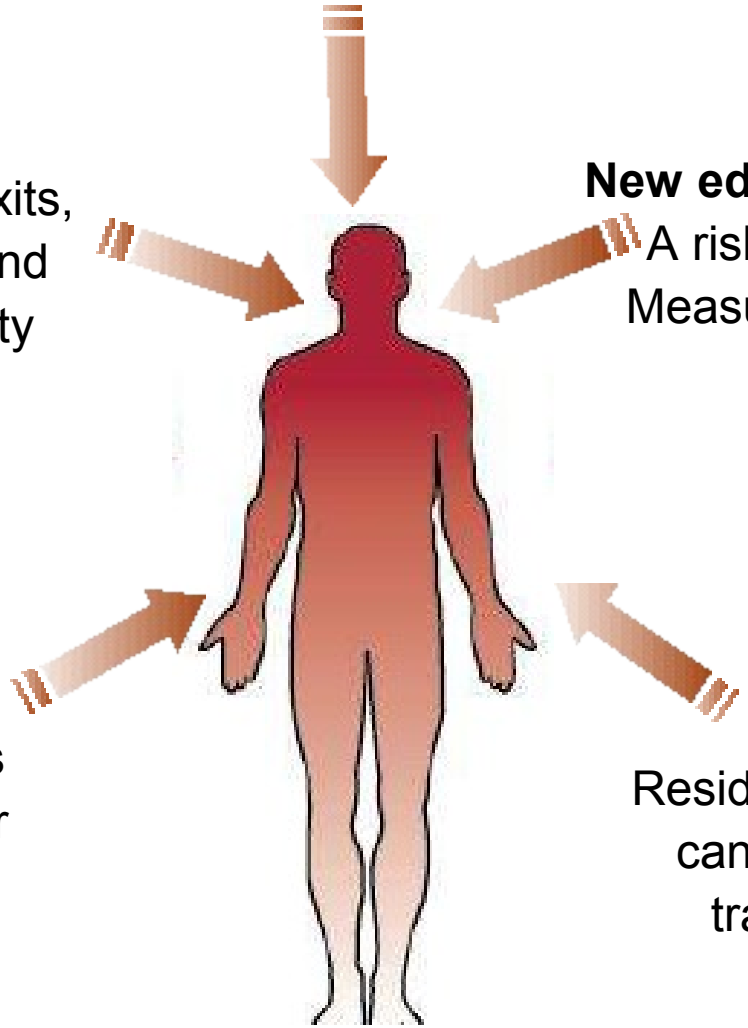

Know pathogens
Routine testing covers only a limited numeber

Leukocytes
Residual cells and cytokines can cause harmful post-transfusion reactions

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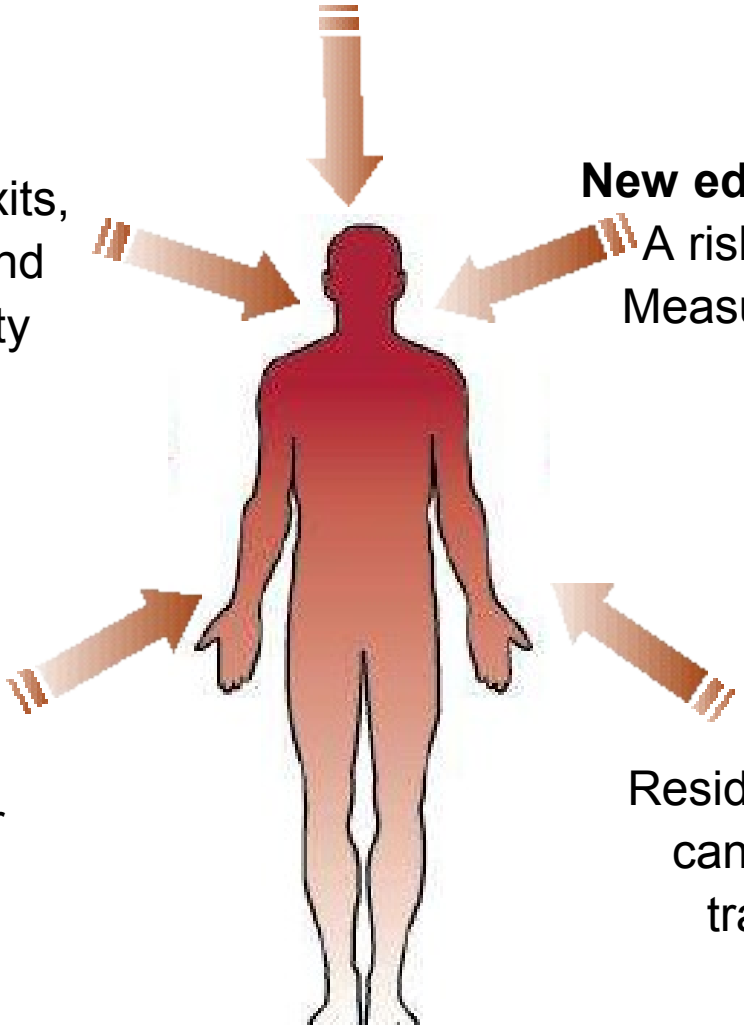
Bacteria
The most frequent transfusion-transmitted infection

Screening limitations
Gaps in current defenses exits, due to the window period and limited screening sensitivity

New ed emerging pathogens
A risk that current safety Measures cannot eliminate



Know pathogens
Routine testing covers only a limited numeber

Leukocytes
Residual cells and cytokines can cause harmful post-transfusion reactions

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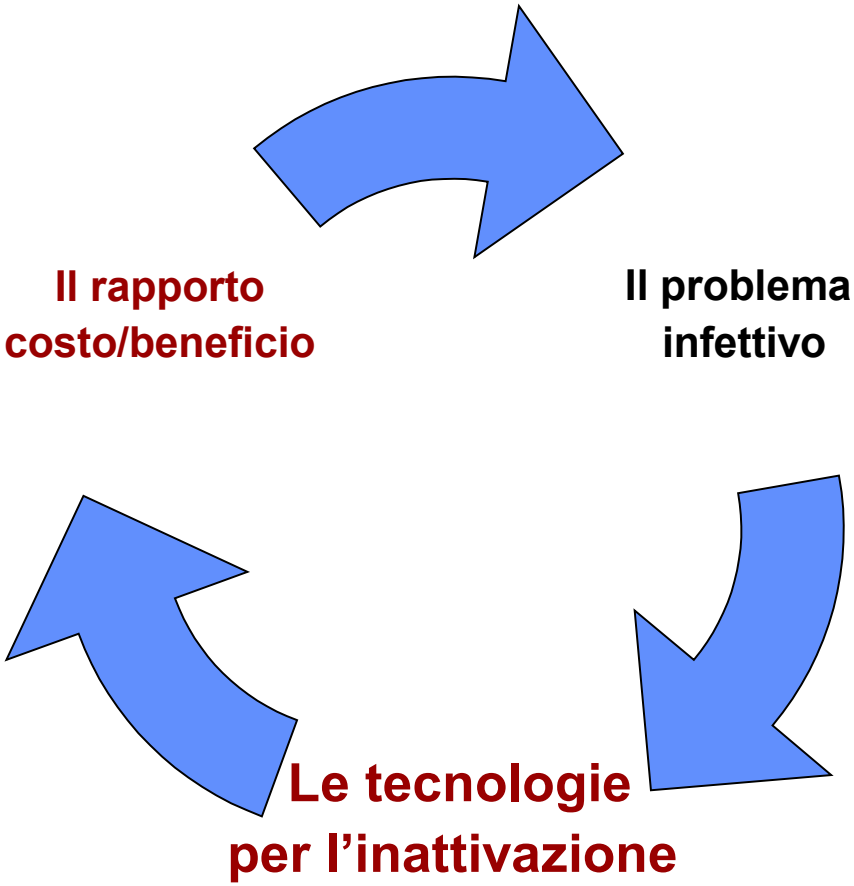
Il rischio sepsi

- **Prevalence of contaminated platelets:**
  - **1:2500 random platelet concentrates**
  - **1:5000 singol donor platelets (SDP)**
- **Risk of sepsis**
  - **1:50000 with SDP**

Castro E. et al., Trasfus Med 3005, Palavecino El et al., Transfusion 2006

Il "Challenge"

- **Agenti infettivi nuovi continuano ad emergere**
- **Agenti infettivi nuovi migrano rapidamente da ospiti non-umani all'uomo**
- **L'identificazione di patogeni emergenti, la comprensione dell'epidemiologia, lo sviluppo di test diagnostici e la protezione del sangue richiede tempo**



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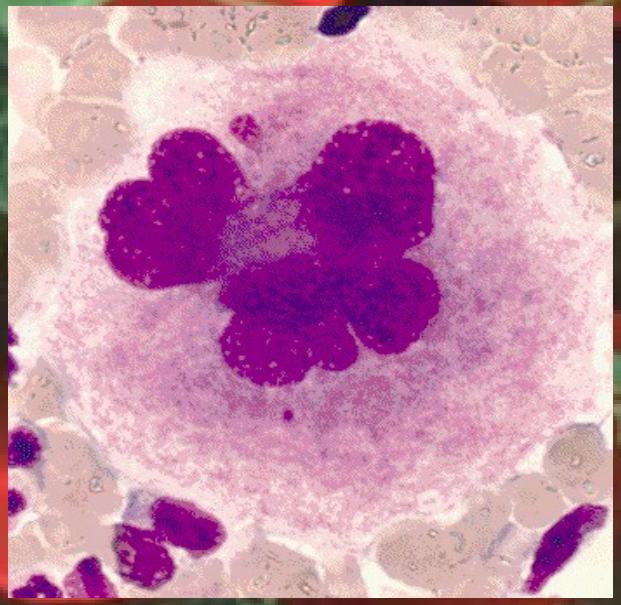
	Acellular Products	Cellular Products	
	Plasma and derivatives	Platelets	Red Cells
	Tecnique targeting nucleic acid		
	Tecnique targeting membrane		
Solvent-detergent	Yes	No	No
Methylene Blue	Yes	No	No



La (complessa) biologia piastrinica

L'idea

Gli acidi nucleici come bersaglio per bloccare la proliferazione dei patogeni

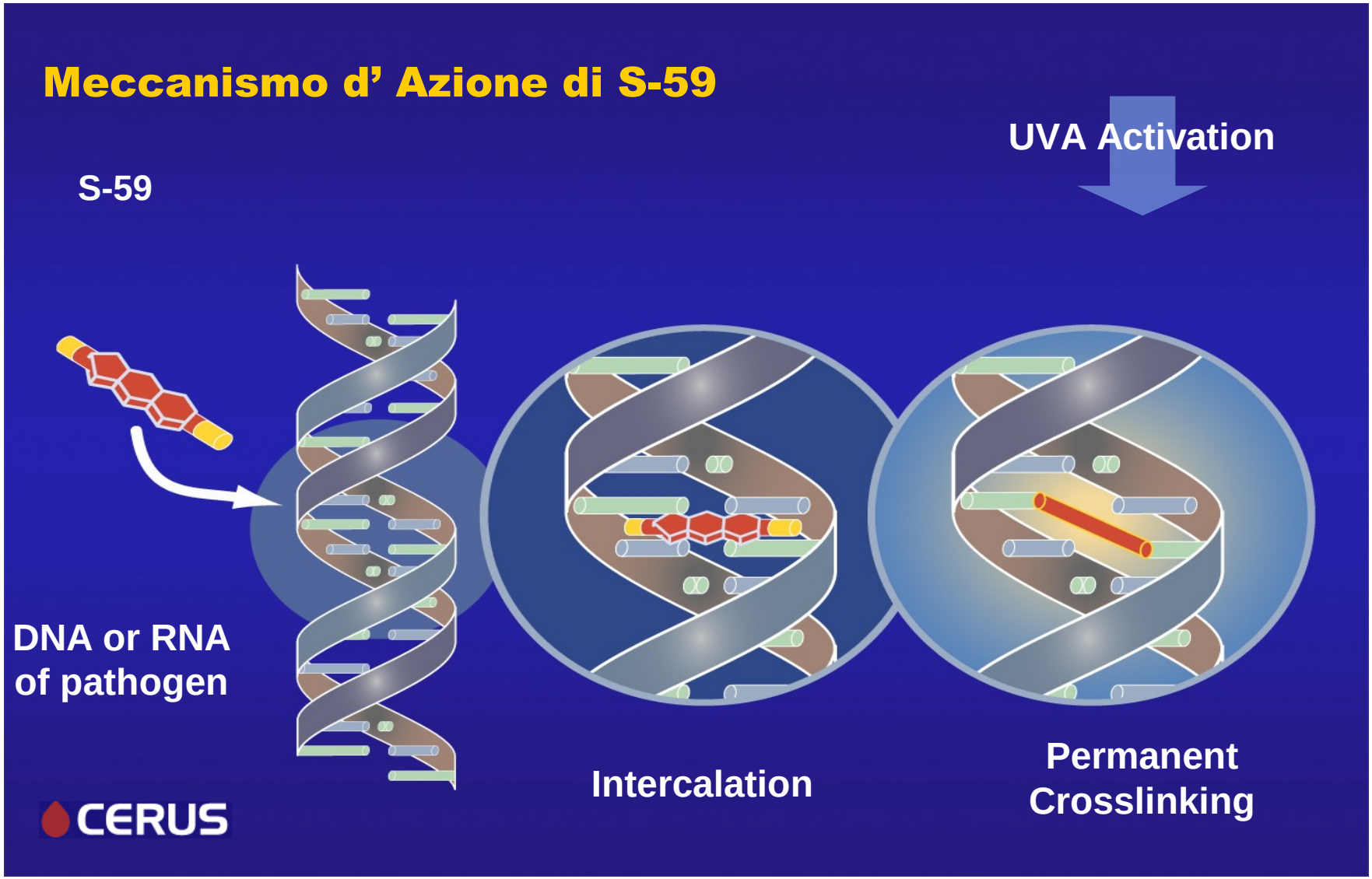
Le tecnologie proposte

Intercept – Cerus

Mirasol – Navigant

Macopharma – Theraflex

Intercept (I)


Meccanismo d' Azione di S-59
UVA Activation
S-59
DNA or RNA of pathogen
Intercalation
Permanent Crosslinking
CERUS

Intercept (II)

Concentrati piastrinici

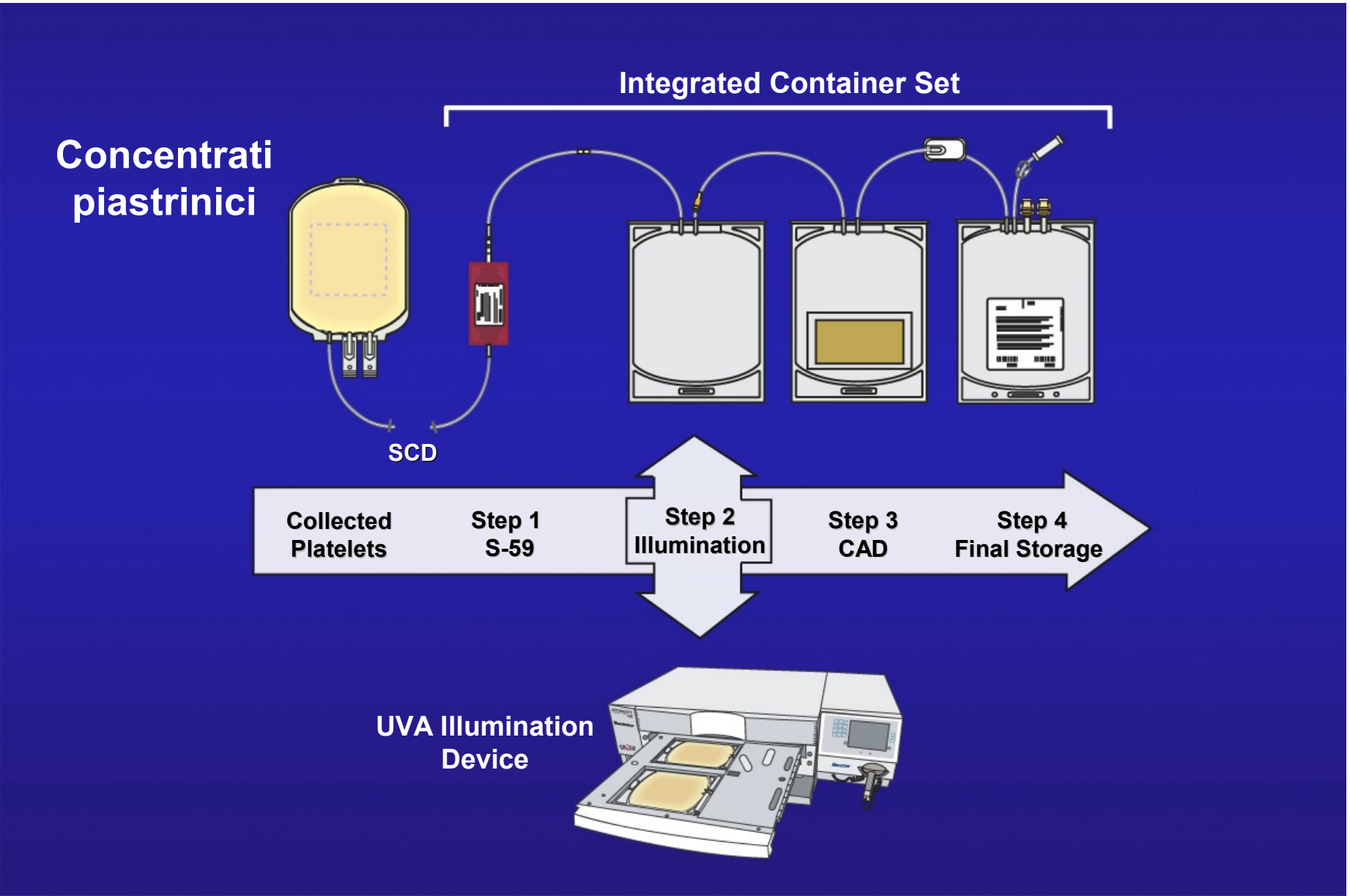


Table 2. Inactivation of Viruses and Microbial Pathogens by Treatment With Amotosalen and UV-A Light* [109–113]

Viral Pathogens	Genome	Enveloped	Infectivity Log Reduction Amotosalen/UV-A Platelets
Human immunodeficiency 1/2	ss-RNA	+	>6.2 cell free; >6.1 cell associated
Human T-cell lymphotropic I/II	ss-RNA	+	4.7/5.1 cell associated
Hepatitis B	ds-DNA	+	>5.5
Hepatitis C	ss-RNA	+	>4.5
West Nile	ss-RNA	+	>6.0
Human erythro B19	ss-DNA	–	4.0–4.9
Cytomegalo	ds-DNA	+	>5.9 ± 0.3 cell associated

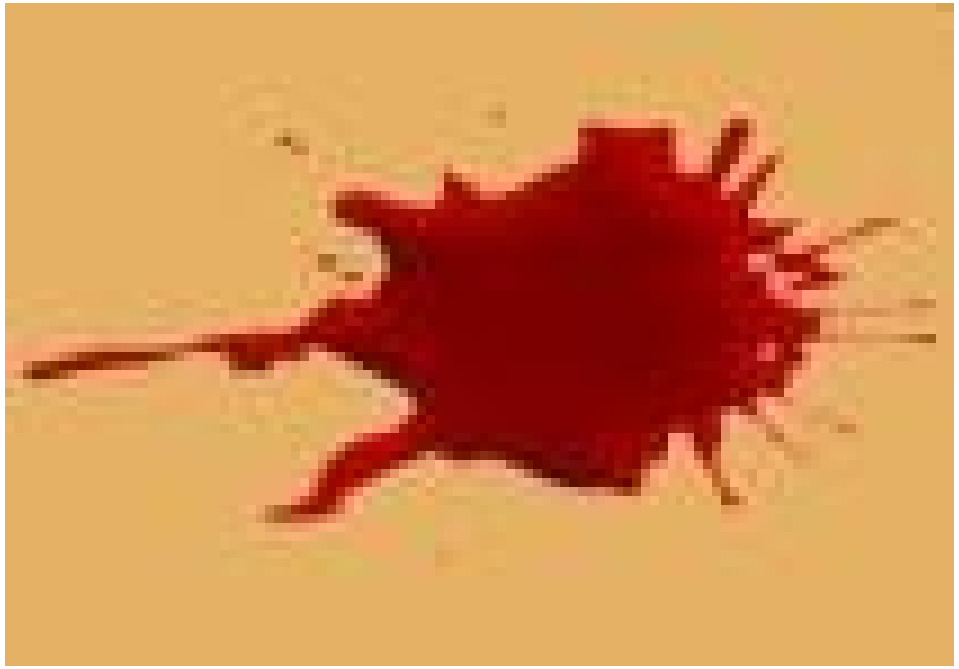
Microbial Pathogens	Gram	Aerobes Vs Anaerobes	Infectivity Log Reduction Amotosalen/UV-A Platelets
Escherichia coli	Neg	Aerobe	>6.4 ± 0.1
Serratia marcescens	Neg	Aerobe	>6.7 ± 0.1
Klebsiella pneumonia	Neg	Aerobe	>5.6 ± 0.2
Pseudomonas aeruginosa	Neg	Aerobe	4.5 ± 0.2
Salmonella choleraesuis	Neg	Aerobe	>6.2 ± 0.1
Yersinia enterocolitica	Neg	Aerobe	>5.9 ± 0.2
Enterobacter cloacae	Neg	Aerobe	5.9 ± 0.1
Staphylococcus aureus	Pos	Aerobe	6.6 ± 0.1
Staphylococcus epidermidis	Pos	Aerobe	>6.6 ± 0.1
Streptococcus pyogenes	Pos	Aerobe	>6.8 ± 0.1
Listeria monocytogenes	Pos	Aerobe	>6.3 ± 0.1
Corynebacterium minutissimum	Pos	Aerobe	>6.3 ± 0.1
Bacillus cereus	Pos	Aerobe	>5.5 ± 0.2
Lactobacillus species	Pos	Facultative anaerobe	>6.4 ± 0.1
Propionibacterium acnes	Pos	Facultative anaerobe	>6.2 ± 0.2
Clostridium perfringens	Pos	Anaerobe	>6.5 ± 0.2
Bifidobacterium adolescentis	Pos	Anaerobe	>6.0 ± 0.4

Microbial Pathogens	Class	Infectivity Log Reduction Amotosalen/UV-A Platelets
Treponema pallidum	Spirochete	6.8–7.0
Borrelia burgdorferi	Spirochete	>6.9 ± 0.1
Trypanosoma cruzi	Protozoa	>5.3
Plasmodium falciparum	Protozoa	>7.0
Leishmania mexicana	Protozoa	>5.0
Leishmania major	Protozoa	>4.5

Intercept: utilizzo clinico

- **Marchio CE**
- **3 trial clinici in Europa (euroSPRITE)**
  - **166 pazienti**
    - **Comparable post transfusion platelet count increment**
- **Trial SPRINT**
  - **645 pazienti**
    - **4719 trasfusioni**
    - **PCT platelet were hemostatically equivalent to the control platelets**

SARS : un patogeno emergente Implicazioni per la sicurezza del sangue



HSR: experimental evaluation

Amotosalen photochemical inactivation of severe acute respiratory syndrome coronavirus in human platelet concentrates
D. Pinna*, A. Sampson-Johannes†, M. Clementi‡§, G. Poli*§, S. Rossini¶, L. Lin† and E. Vicenzi* 1
Transfusion Medicine. 2005, 15. 269-276

L'epidemia di SARS é stata contenuta, tuttavia....

- Non ci sono farmaci antivirali
- Non c'è un vaccino
- Non è stato identificato l'ospite naturale
- Nessuno può garantire che non possa riemergere

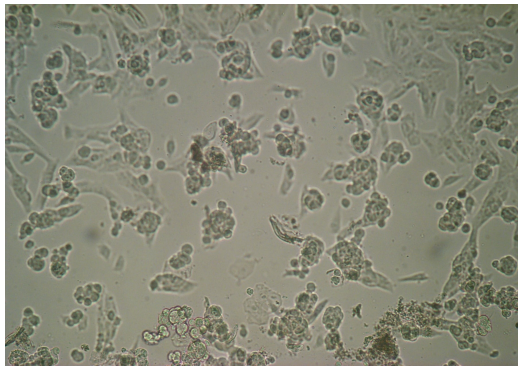
SARS Coronavirus è presente nel sangue degli individui infettati

Inattivazione di SARS-CoV HSR1 dopo Trattamento con 150 µM S-59 e 3 J/cm² UVA in Concentrati Piastrinici

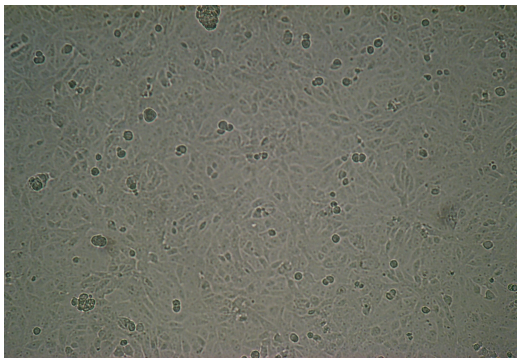
SARS-CoV	Pre-trattamento (pfu/mL)	Volume testato (mL)	Riduzione (log)
Concentrati piastrinici N = 5	$10^{4.7}$	100 mL (no virus)	> 7.4

Inattivazione del SARS-Coronavirus da INTERCEPT

Prima



Dopo



HSR: Clinical evaluation

	N. Plt X 10^3/ml average /range	Dose X 10^{11} average /range	CI in 1°h X 10^3/ml average /range	CCI 1°h Effectiveness	CI 24°h X 10^3/ml average /range	CCI 24°h Effectiveness	Temperature Haemorrhage Viral therapy antibiotic therapy
Platelets with treatment	3862 5000-17000	3,16 2,3-3,54	7058 11400-3000	14/29	4018 6000-2000	23/29	13/29 2/29 9/29 22/29
Platelets without treatment	10000 4000-16000	2,72 1,95-3,5	9837 15000-4000	18/24	6560 8500-2500	19/24	1/24 1/24 2/24 17/24

$$CCI = \frac{\text{(Posttransfusion count- Pretransfusion count) X Body surface area } (m^2)}{\text{Platelet dose } (\times 10^{-11})}$$

Effectiveness is proved whenever:

- CCI 1° h>7,5 X 10^9/L
- CCI 24° h>2,5 X 10^9/L

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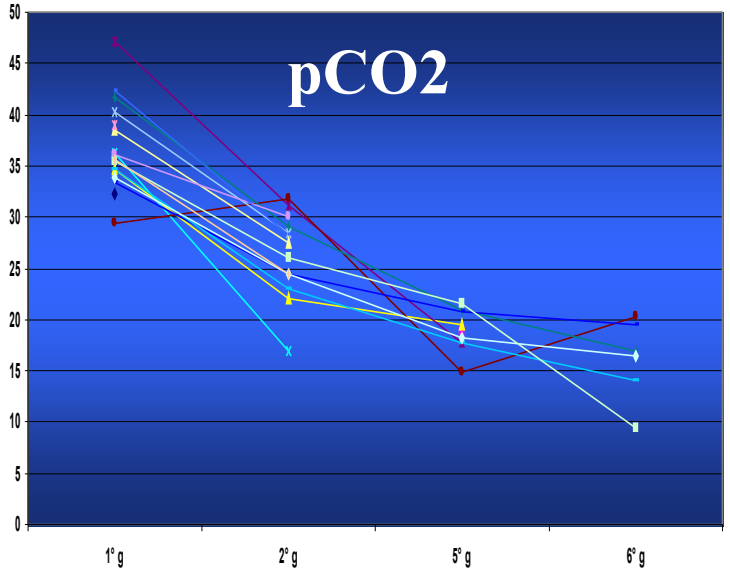

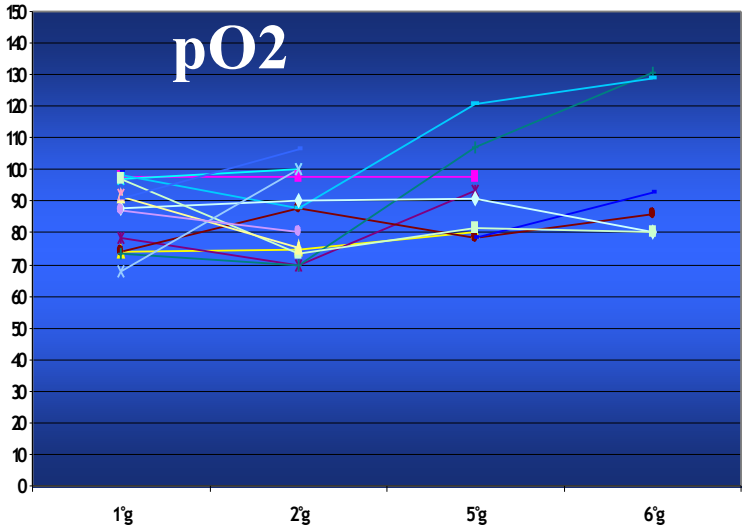

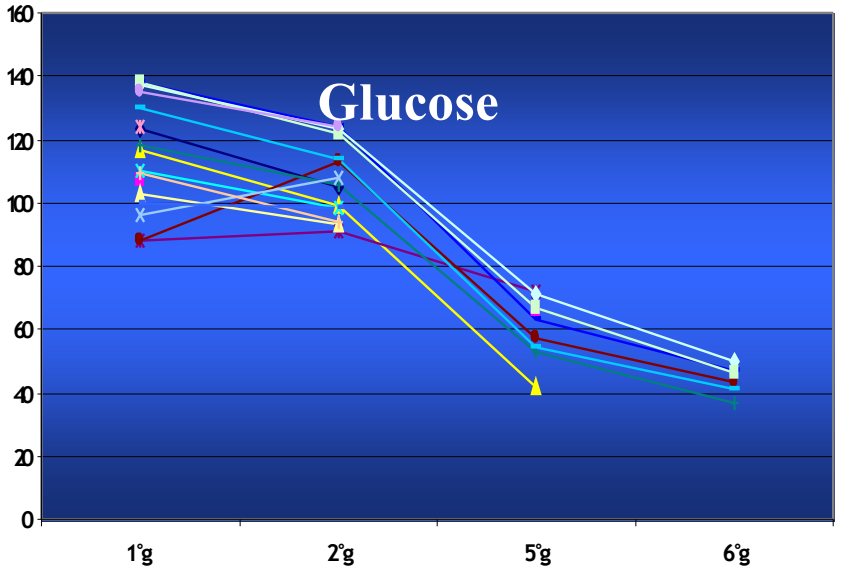

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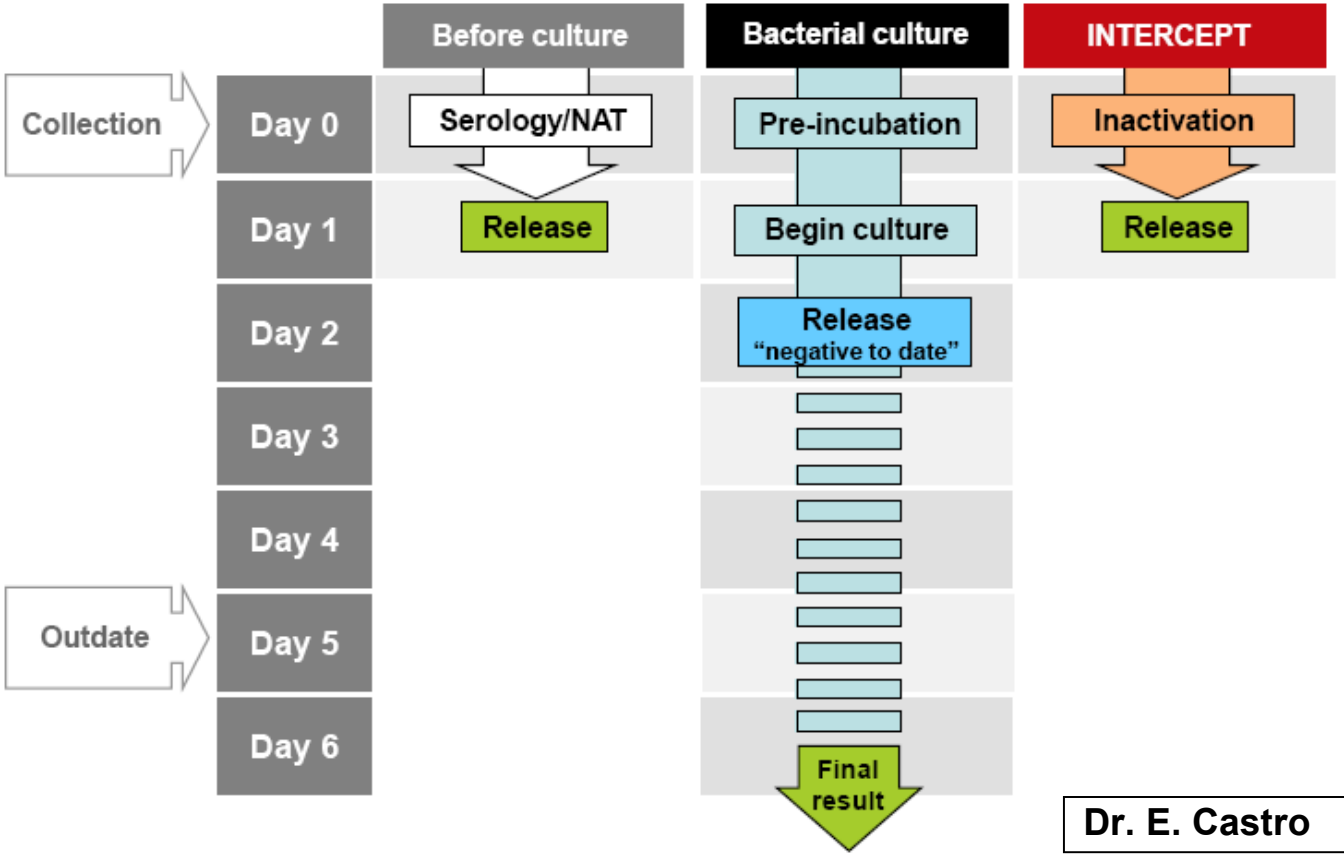
Five years of experience with INTERRCEPT blood system in routine use.

Pathogen inactivation allows timely release of platelet units to maximize shelf-life



Dr. E. Castro

Le tecnologie proposte

Intercept – Cerus

Mirasol – Navigant

Macopharma – Theraflex

Mirasol

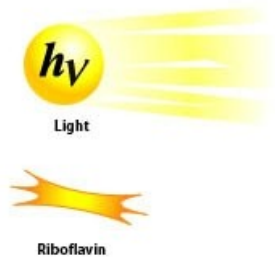
- **RIBOFLAVIN Vitamin B2**
- Essential nutrient. RDA ~1.3-1.7mg/d
- Intake 1>10-50mg/d in food
- I/V therapy ~ 30-50mg/d
- Up to 1000mg/d 'safe'.
- Plasma level *m* 24, *range* 9-79nM [USA]†

- LUMICHROME formed naturally in riboflavin solutions
- Dose in IV preparations. 5-25mg/d
- Normal plasma level *m* 11; range 0-75nM [USA] †

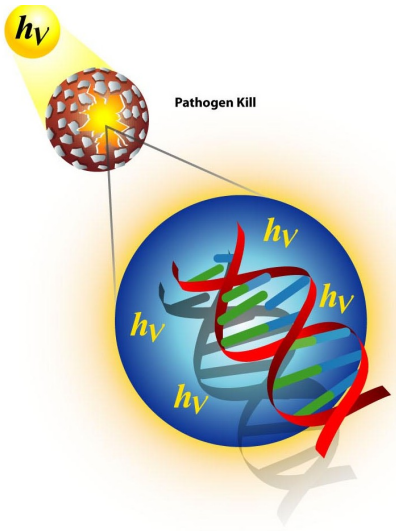
- Phototherapy of Neonatal Jaundice.
- ~2-3d @ 420-470nm; 4μW/cm2.
- Lumichrome etc formed *in vivo*.
- Riboflavin supplements given; 300-400 μg/kg/d IV or PO, 4+d

- No harmful effects. No excess neoplasia
- [Olsen et al 1996, Cancer Causes & Control, 7, 411-414; 55120 children followed 16 years]

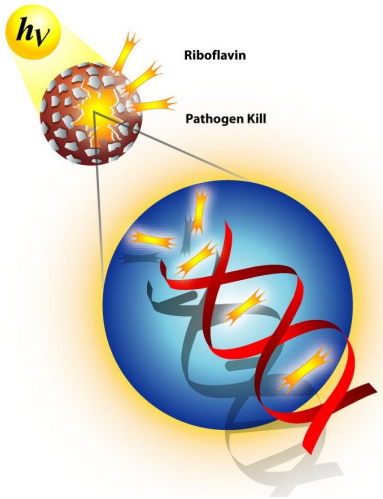
MIRASOL PRT system – Modes of action



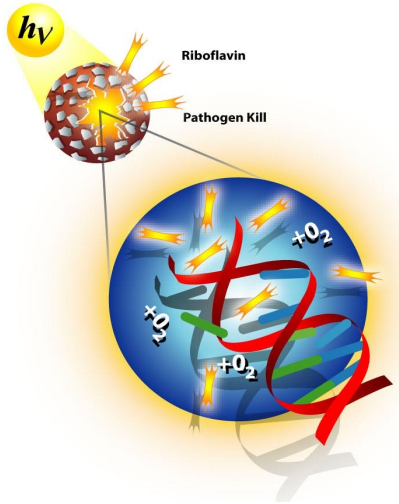
The Mirasol PRT system applies light* and riboflavin to reduce the pathogen load in labile blood products by several mechanisms including the three distinct modes of action shown below.



In blood products, viral, bacterial, and parasitic nucleic acids absorb low wavelength photons ($h\nu$) resulting in nucleic acid damage (direct UV light effect).



Riboflavin interacts with nucleic acids, causing additional irreversible nucleic acid damage through electron transfer chemistry, primarily between riboflavin and guanine.



In all blood products, "Reactive Oxygen Species" (ROS) are generated by riboflavin and light, resulting in irreversible nucleic acid damage through various oxidative reactions.

*The Mirasol PRT system uses low wavelength light.

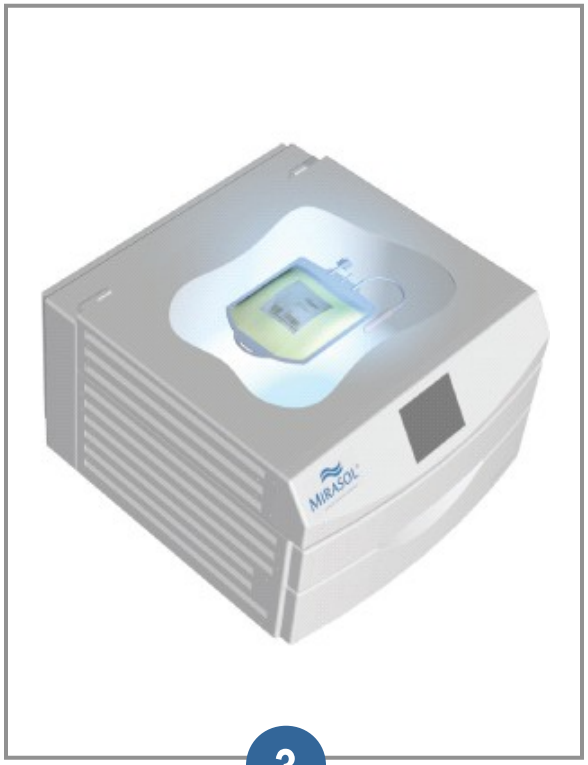
MIRASOL PRT system

1

Transfer PLT product to Mirasol Illumination bag

2

Add Riboflavin Solution
(2nd sterile dock)



3

Illuminate for ~ 10 min.

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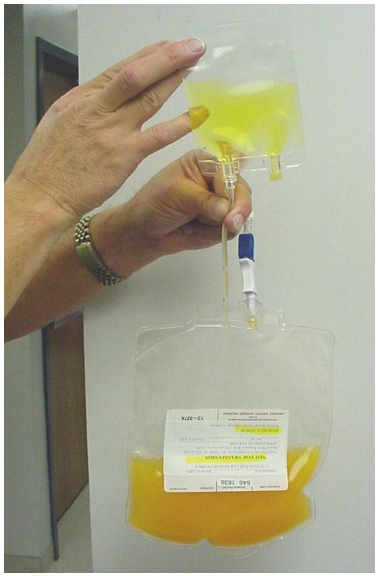


- Safe – Use of a natural compound with known safety profile
- Effective against broad range of viruses, bacteria, parasites, as well as white cells
- Simple – Process does not require removal of compounds

Table 3. Inactivation of Viruses and Microbial Pathogens by Treatment With Riboflavin and UV-A Light* [81–84]

Viral Pathogens	Genome	Enveloped	Infectivity Log Reduction Riboflavin/UV-A Platelets
Human immunodeficiency 1/2	ss-RNA	+	5.93 ± 0.20 cell associated and cell free; 4.46 ± 0.39 intracellular
West Nile	ss-RNA	+	5.19 ± 0.50
PPV (surrogate for human erythro B19)	ss-DNA	−	≥5.03

Microbial Pathogens	Gram	Aerobes Vs Anaerobes	Infectivity Log Reduction Riboflavin/UV-A Platelets
Escherichia coli	Neg	Aerobe	≥4.38
Staphylococcus epidermidis	Pos	Aerobe	≥4.5

Microbial Pathogen	Class	Infectivity Log Reduction Riboflavin/UV-A Platelets
Leishmania donovani infantum	Protozoa	≥5.0

* Apheresis platelets were treated with 50μM riboflavin and 6.2 J/mL UV-A light. PPV indicates porcine parvovirus; ss, single stranded; ds, double stranded; +, presence of an envelope; −, no envelope; Neg, gram negative; and Pos, gram positive.

Le tecnologie proposte

Intercept – Cerus

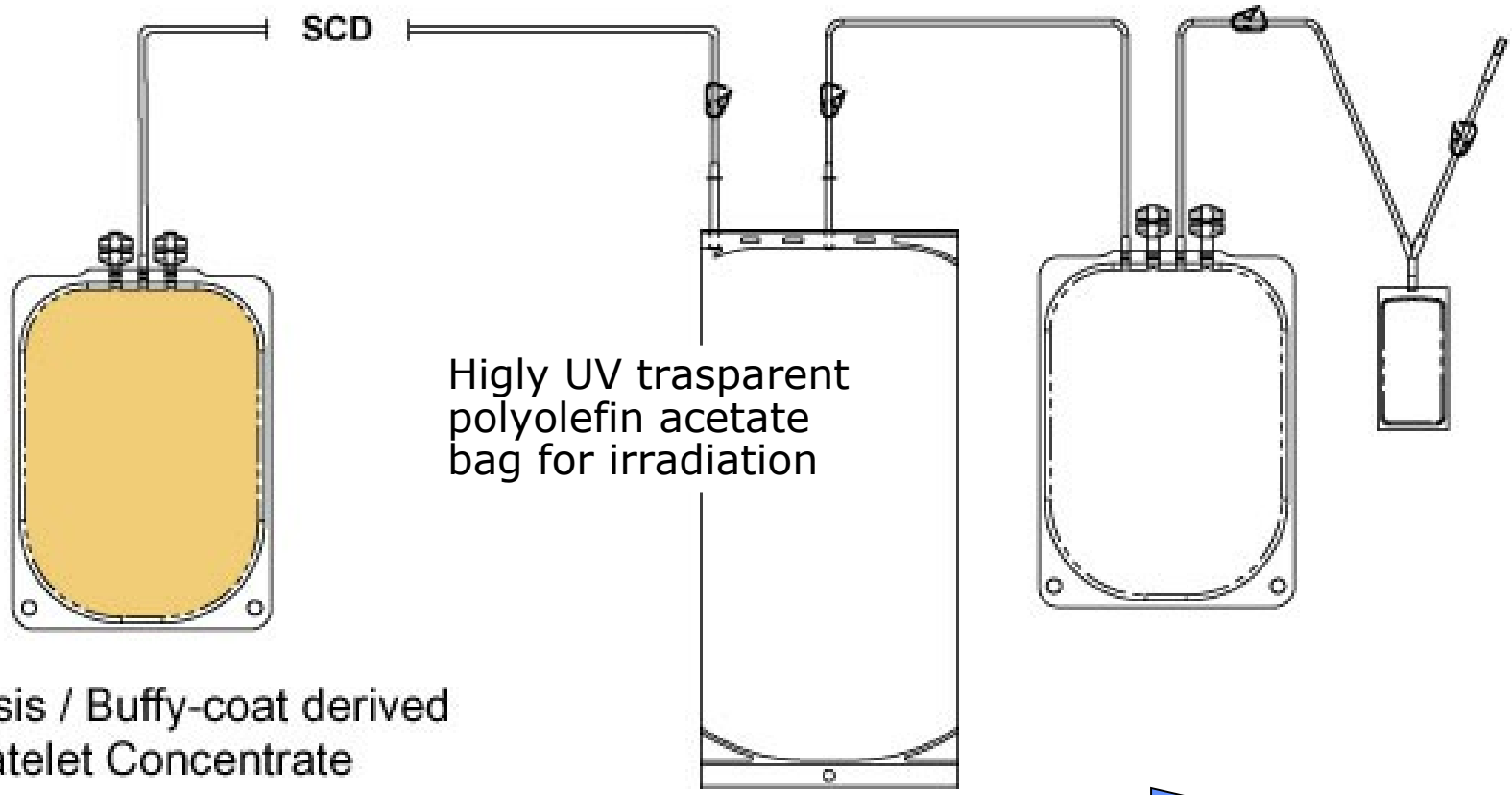
Mirasol – Navigant

Macopharma – Theraflex

Theraflex

- Short wave UVC (254 nm)

Theraflex Kit



Transfer of plasma-reduced platelet concentrates in SSP+

UVC Illumination (< 60 sec)

Storage/ Transfusion

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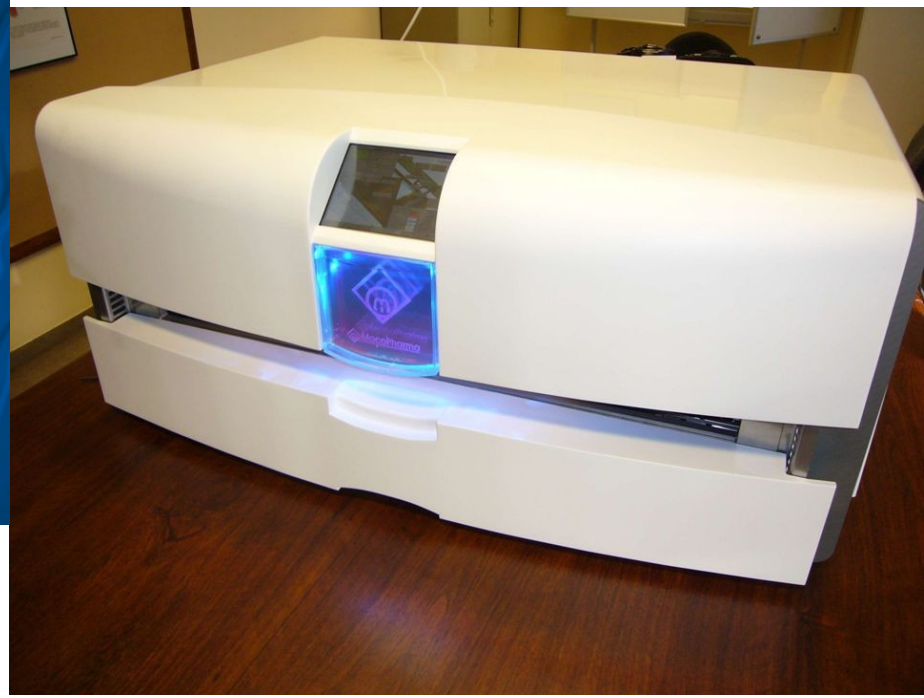
THERAFLEX UV System for Platelets

THERAFLEX Kit (CE certified)



MACOTRONIC UV Illuminator (CE pending)

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Status / Planning – THERAFLEX UV Platelets:

Preclinical phase A
- efficiency (bacteria, viruses, leucocytes)
- platelet quality

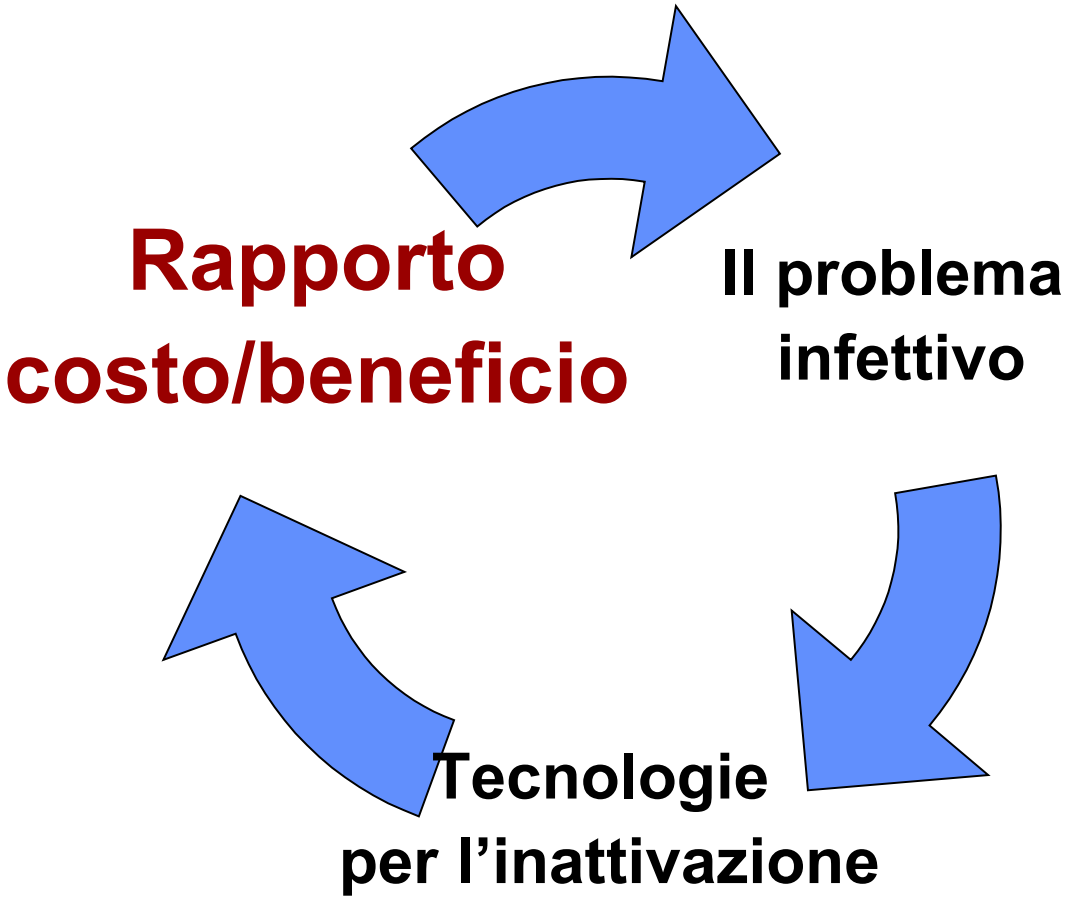
- Preclinical Phase B
  - neoantigenes,
  - cytokines, parasites

- Clin. Phase I study (recov. & survival)
- Clin. Phase I, full unit, Volunteer, Tolerance Study
- Phase III Clinical Study (Thrombocytop. Patients)

- CE registration

	Acellular Products	Cellular Products	
	Plasma and derivatives	Platelets	Red Cells
	Tecnique targeting nucleic acid		
	Tecnique targeting membrane		
Solvent-detergent	Yes	No	No
Methylene Blue	Yes	No	No
Psoralen (S59) Amotosalen	Yes	Yes	No
Riboflavin	Yes	Yes	?
UV-C		Yes	?

	Acellular Products	Cellular Products	
	Plasma and derivatives	Platelets	Red Cells
	Tecnique targeting nucleic acid		
	Tecnique targeting membrane		
Solvent-detergent	Yes	No	No
Methylene Blue	Yes	No	No
Psoralen (S59) Amotosalen	Yes	Yes	No
Riboflavin	Yes	Yes	?
UV-C		?	?
FRALE (S503)			Yes
Inactine			Yes


Rapporto costo/beneficio
Il problema infettivo
Tecnologie per l'inattivazione

Pathogen reduction: risks versus *benefits*

- **Risks**
  - **Damage to transfusion product**
  - **Toxicity to processing personnel**
  - **Toxicity to recipient**
- **Benefits**
  - **Reduction of known viruses**
  - **Reduction of bacteria**
  - **Reduction of parasites**
  - **Potential reduction of unknown pathogens**
  - **Eventually elimination of lymphocytes**

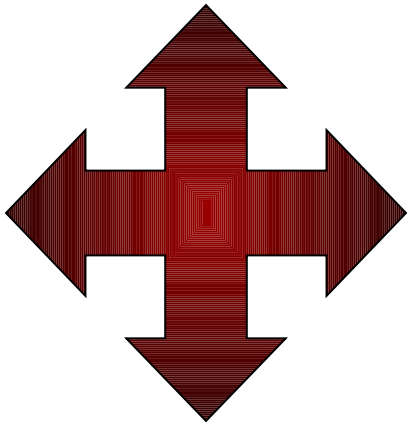
Inattivazione: si o no ?

opzione scientifica

(vincolo etico)

risorse disponibili

(vincolo economico)



legge

(vincolo legale)

paziente

Blood Bank: a new perception

- **Organization providing a community service to patients, hospital and donors**

HIV

- Drug manufacturing firms that produce and distribute a variety of injectable products.

Legge 21 ottobre 2005, n. 219

" Nuova disciplina delle attività trasfusionali e della produzione nazionale degli emoderivati "

ORGANIZZAZIONE DEL SISTEMA TRASFUSIONALE

Art. 5.

(Livelli essenziali di assistenza sanitaria in materia di attività trasfusionale)

, *i servizi e le prestazioni erogati dalle strutture del Servizio sanitario nazionale in rapporto alle specifiche competenze disciplinari, con esenzione dalla partecipazione alla spesa, in materia di attività trasfusionali comprendono:*

4) esecuzione delle indagini di laboratorio e ***delle procedure di inattivazione dei patogeni*** *finalizzate alla certificazione dei requisiti di qualità e sicurezza previsti dalla legislazione vigente per le unità di sangue e gli emocomponenti, con particolare riferimento alla prevenzione delle malattie trasmissibili con la trasfusione;*

Inattivazione: un problema di economia sanitaria ?

- **Assessment of the economic value of the Intercept blood system in Belgium.**
  - **K. Moeremans, Transfusion Medicine, 2006; 16, 17-30**
    - **3459200 € - 195364 € Quality Adjusted Life Years (QALY)**
    - **NAT: 2-3 million per lifeyear**

- **Cost-effectiveness of pathogen inactivation for platelet transfusions in the Netherland.**
  - **M.J. Postma, Transfusion Medicine 2005; 15, 379-387**
  - **554000 € net cost per life year gained (LYG)**

La decisione sulle nuove tecnologie

- **Il ruolo delle Società Scientifiche**
- **Consensus Conference – Canada (2007)**
  - **Is the current risk of transfusion-transmitted disease acceptable in relation to other risk of transfusion** ?
  - **What mininum acceptable safety and efficacy criteria should be put into place for the pre-approval assessment of pathogen-inactivated products ?**
  - **For PI technologies that have been approvated by the regulatory autorithies, what implications should be considered prior to their widespread adoption ?**

Large adequately powered **randomized clinical trials** should be performed to evaluate and/or to confirm the effectiveness of **any new PI technology**. Post-licensure phase IV studies should be integrated with haemovigilance systems to enhance the ability to detect adverse events**.**

The Canadian Consensus Conference on Pathogen Inactivation in March 2007

- During the Consensus Conference on Pathogen Inactivation the consulting panel of experts recognized that based on the relatively low rates of post-tranfusion infections with the most well-known agents (e.g. HIV or HBV), pathogen reduction **cannot be recommended.**

- However, the same panel acknowledged that **emerging pathogens** have been detected in blood donors at an increasing rate since the HIV epidemic.

- The strategy of surveillance, identification and development of test, allows a new agent spread widely even before the disease can be recognized. Such situations undermine confidence in the safety of blood supply when they reach public opinion

- A proactive approach in accordance to the precautionary principle would lead to the reduction of the theoretical risk, and help to sustain public confidence in the blood supply.

- The precautionary principle is a different way of making decsion to manage great risks when there is significant scientific uncertainity, to meet society expectations that the risk is covered.

- The panel believes that pathogen inactivation should be implemented, when feasible technologies were available and were capable of inactivating a broad spectrum of infectiuos agents

L'inattivazione dei patogeni: una nuova sfida per la nostra mission

Quality in the clinical use of blood products implies administring the *right quantity* of the *right blood product* in the *right way* at the *right time* to the *right patient*, and *appropriate documentation of process and the outcome*

Blood safety in the European Community:

An initiative for optimal Use 20-22 May 1999, Germany

S. Rossini

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