Pathogen Inactivation

State of the Art

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Objectives

- Context
- History
- Technologies
- Clinical Studies
- Problems
- Benefits
- Costs

Current Strategy

- **Surveillance & testing strategy**
- **Costs money and donors**
- **1% donors lost through true and false positive screening**
- **Window periods – infectious but not detectable**
- **Geographic exclusion and faulty donor memories regarding travel**

Custer B Transfusion 2004;44:1417-26

Proactive Strategy

- **HIV – 12,000 patients before clinical case recognized**
- **Early days, 1% blood in SF from HIV+ donors[2]**
- **Today – babesia, dengue, chickungunya[3,4]**
- **74 infectious diseases with possible of transfusion transmission[5]**
- **5-6 newly discovered viruses per year[6]**
- **Malaria deferral, 1-3% 150,000 deferred + 580,000 self deferrals**

2.Busch MP Transfusion 1991;31:4-11
3. Lanter MC Transfusion 2012 52:1634-39
4. Teo D Transfus Med 2009;19:66-77
5. Stramer SL Transfusion 2009;49 suppl(2):1s-29s
6. Stramer SL ISBT Science Series 2014;9:1381-9

Developing World Issues

- Not enough donors
- Not enough money for testing
- High disease endemic population
- 13 million units not tested for HIV, HBV or HCV
- PI increases safety without cost of testing

Qualities Needed for PI Process

- High kill levels to prevent disease transmission
- Preserve structure, function and protein quality of blood components
- Non-toxic, non-immunogenic, non-mutagenic
- Easy to use

Pathogens in Components

- **Plasma – viruses**
- **Platelets – bacteria, viruses**
- **Risk of contamination due to low bacteria levels at sampling and false negatives[7]**
- **Bacterial detection sensitivity – 40%[8]**
- **Red Blood Cells – viruses, intra and extra-cellular parasites, bacteria**

7. Benjamin R Transfusion 2007;47:1381-9 8. Murphy WG VoxSang;95:13-9

Early PI

- Plasma derivatives – Pasteurization and heat
- Solvent detergent – 1% TNBP and 1% TritonX-100
- 1% TNBP – solvent to extract lipids
- 1% Triton X-100 – detergent to disrupt lipid bilayers
- SD plasma – Octaplas for patient transfusion
- Active against lipid enveloped viruses but not against non-enveloped viruses HAV or parvo
- No reported cases of HIV, HBV, HCV in plasma concentrates since 1987 [9]
- SD plasma standard of care – Norway, Belgium, Ireland, France

9. CDC MMWR 2003;51:1152-54

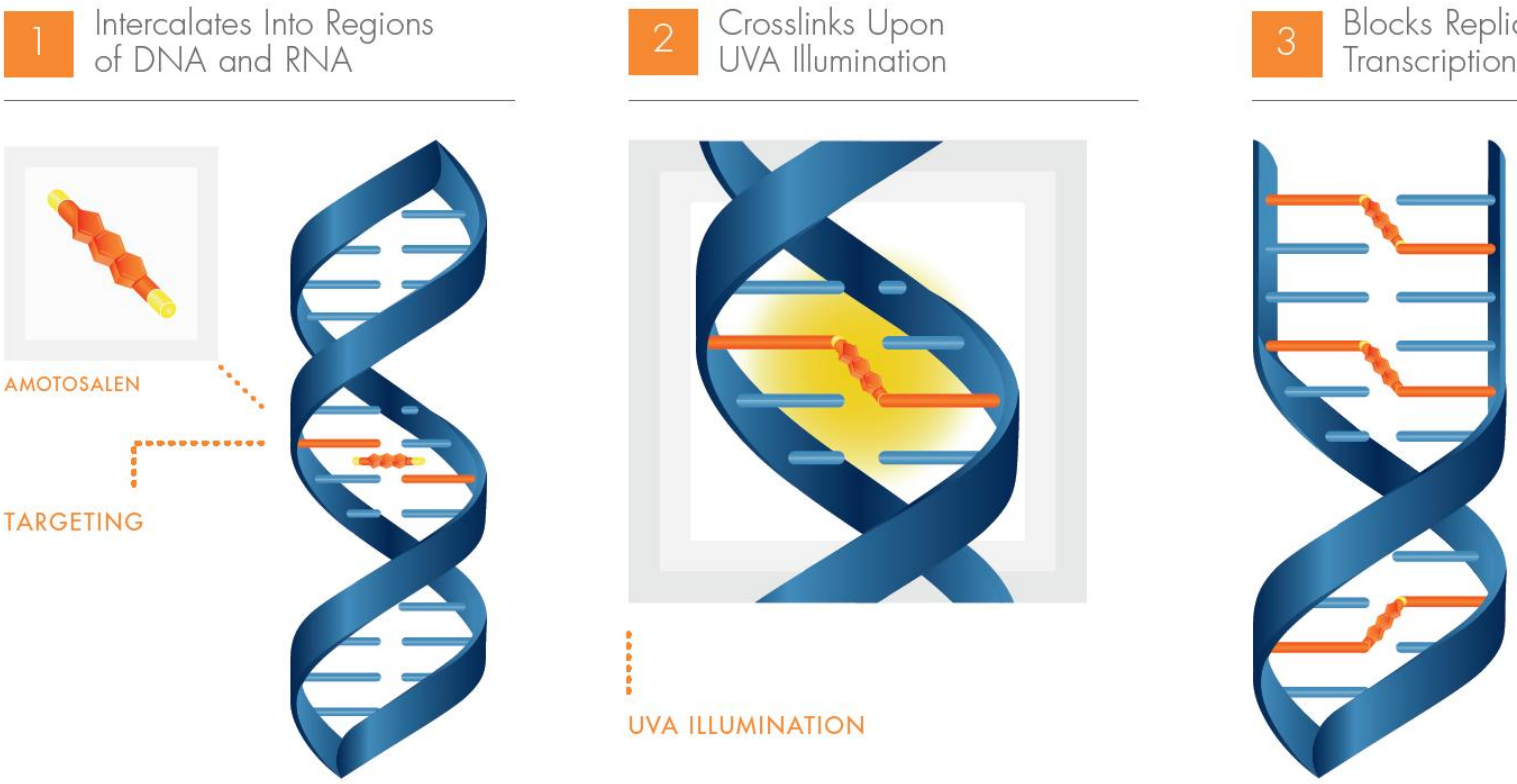
Methylene Blue

- Used in Europe for 20 years for plasma
- Photoactive +charged phenothiazine dye
- Binds guanine produces singlet oxygen on light exposure
- Filter out methylene blue
- Not for intracellular viruses but freezing and leukoreduction reduce and rupture wbcs
- Not licensed in US, used in Germany, Austria, Switzerland, Spain and UK

Current Technology

- **Intercept and Mirasol,CE approved for Europe for platelets and plasma**
- **Intercept, FDA approved for US for platelets and plasma**
- **Photochemical techniques – psoralen or riboflavin added and then exposed to UV light**
- **Genotoxicity and mutagenicity studies done across range of species and dosages**

Mechanism of Action: Nucleic Acid Targeting

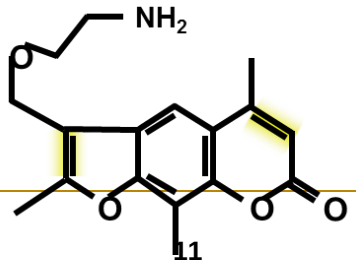


Helical region of single- or double-stranded DNA or RNA

Intercalation

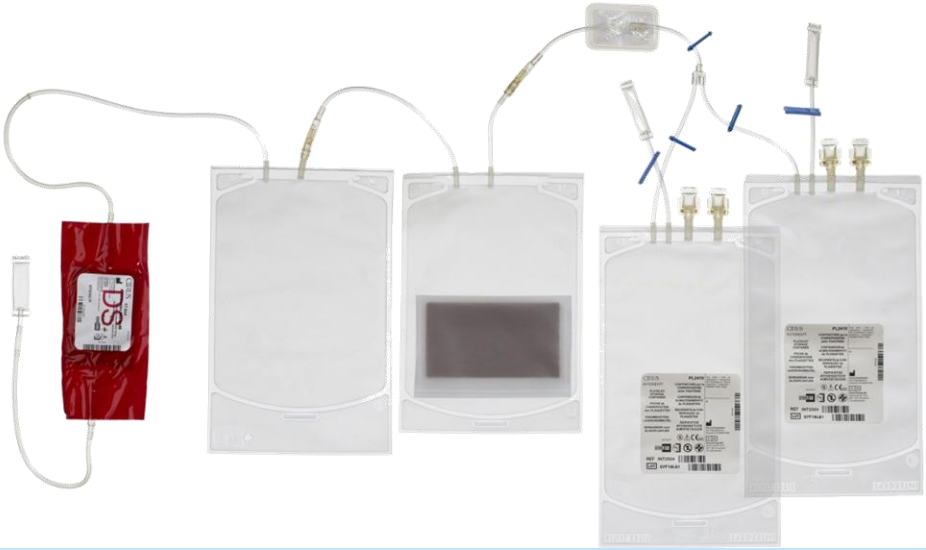
Crosslinking

Amotosalen (S-59)



The INTERCEPT Blood System

INTERCEPT Platelet Processing Set
(DS shown; SV, LV also available)



INTERCEPT Illuminator

Step 1 Amotosalen | **Step 2** Illumination | **Step 3** CAD | **Process Complete** Storage

INTERCEPT Plasma Processing Set

New York Blood Center

Cerus Intercept

- **Intercept is the only licensed PI in the US**
- **Loss of 7% of platelets due to retention in bag set**
- **Patient receives around 1 ug of amotosalen**
- **LD50 in rates 1 g/kg**
- **Safety margin 10^5 fold [10]**

10. Irsch J Transfus Med Hemother 2011;38:19-31

Intercept Efficacy

- **Effective against wide range of gram+ and gram- bacteria[11]**
- **Weakness – Bacillus cereus spores and high levels of Pseudomonas and Enterobacter**
- **Phase 1 – Slight but significant reduction in recovery 15% and lifespan of platelets 20%[12]**
- **Phase 2 – Thrombocytopenic patients – Statistical decrease in 1 hour CI and CCI**
- **No difference in improvement of bleeding times between PI and non-PI platelets[13]**

11. Lin L Transfusion 2004;44:1496-154
12. Snyder E Transfusion 2004;44:1732-40
13. Slichter SJ Transfusion 2006;46:731-40

Eurosprite[14]

- 103 patients RCT, PI vs. non-PI platelets
- CIs lower in PI but dosage was lower 3.9 vs. 4.6x 10^{11} platelets
- CCIs at 1 hour were similar
- More transfusions in PI platelet cohort 7.5 vs. 5.6
- Time to transfusion for PI shorter 3 vs. 3.4 days
- Total platelet dosage – no statistical difference
- No statistical difference in hemorrhagic events or adverse transfusion events though lower in PI 1.6% vs. 5% in non-PI

14. Van Rhenen D Blood 2003;101:2426-33

Sprint[15]

- 645 patients RCT PI vs. non-PI platelets
- Primary endpoint bleeding – WHO scale 1-4
- No statistical difference – Grade 2 or higher bleeding, time to onset Gr 2 bleeding, overall Gr 3 and 4 bleeding
- More transfusion in PI group 8.4 vs. 6.2
- Smaller dosage of platelets in PI 3.7 vs. $4x10^{11}$
- CCI lower in PI even after adjusting for dosage
- PI as effective in preventing or treating bleeding as non-PI platelets[16]

15. McCullough Blood 2004;104:1534-41

16. Murphy S Transfusion 2006:46:24-33

Sprint Adverse Events

- **No statistical difference in number or grade of adverse events – almost all patients had adverse events**
- **Using MedDRA – would appear PI patients had more petechiae, occult fecal blood, skin rash**
- **Concern about ALI in PI group proved to have no statistical difference between the groups[17,18]**
- **In-vitro and animal studies of Mirasol show no increase in priming or generation of TRALI[19]**

17. Snyder E Transfusion 2005;45:1864-75
18. Corash L Blood 2011;117:1014-20
19. Silliman CC VoxSang 2010;98:525-530

Intercept and Additive Solution

- **43 patient RCT, apheresis platelets, PI stored in additive solution, non-PI in 100% plasma**
- **CCI decreased in PI patients but not significant 11,600 vs. 15,100**
- **No difference in number of transfusions, occurrence of bleeding or adverse events[20]**

20. Janetzko K Transfusion 2005;45:1443-52

Hovon Trial[21]

- Three arms, platelets in plasma, platelets in additive solution, PI platelets in additive solutions
- Primary endpoint 1 hour CCI
- Secondary endpoints: 24 hour CCI, bleeding, transfusion requirements, platelet transfusion interval, adverse reactions
- PI platelets significantly lower 1 hour CCI and increase in bleeding events
- No difference in bleeding site, rbc transfusions, platelet dose or transfusion reactions
- Flaws: lack of blinding, absence of bleeding assessment by independent trained observers, use of a bleeding scale other than WHO, underpowered to assess bleeding

21. Kerkoffs JL Br J Haematol 2010;150:209-217

Hemovigilance Programs

- **Intercept used in over 700,000 plasma and platelet transfusions**
- **Hemovigilance studies Switzerland, France, Belgium [22-26] show similar results:**
  - **Fewer overall adverse transfusion reactions**
  - **Fewer severe adverse transfusion reactions**
  - **No increase in platelet transfusions or dosage**
  - **No increase in rbc transfusion**
  - **No neoantigen/antibody formation[27]**

22. Infanti L Trasfus Apher Sci 2011;45:175-81 23. Cazenave JP Transfusion 2010;50:1210-19
24. Cazenave JP Transfusion 2011;51:622-9 25. Osselaer JC Transfusion 2009;49:1412-22
26. Osselaer JC VoxSang 2008;94:315-23 27. Lin L Transfusion 2005;45:1610-20

Intercept Plasma

- **Reduction in fibrinogen and factors ranging from 11-28% [28-31]**
- **RCT in TTP patients undergoing plasma exchange – no difference in efficacy between PI or non-PI plasma [32]**
- **RCT in acquired coagulopathies due to liver disease – no difference in clinical hemostasis and correction of PT between PI or non-PI plasma [33]**

28 Osselaer JC Transfusion 2008;48:108-17
29. deAlarcon P Transfusion 2005;45:1362-72
30. Singh Y Transfusion 2006;49:2167-72
31. Cid J Transfus Apher Sci 2008;39:114-21
32. Mintz L Transfusion 2006;46:1693-704
33. Mintz L Blood 2006;107:3753-60

Mirasol

- **Riboflavin (vitamin B2) and UV A&B light**
- **Associates at guanine base, light generate reactive oxygen = disruption of nucleic acids**
- **Riboflavin**
  - **water soluble**
  - **excreted in urine, 50% within 12 hours[34]**
  - **no toxicity[35]**
- **Patient receives about 5 mg after platelet transfusion[36]**

34. Reddy Transfus Med Rev 2008;22:133-53 35. Unna J Pharmacol Exp Ther 1942;76:75-80
36. Marschner S Transfus Med Hemother 2011;38:8-18

Mirasol Efficacy

- **Efficacy against wide range of gram+ and gram- bacteria[37-38]**
- **Reduced action against Staph aureus and Acinetobacter baumannii**
- **Phase 1 – significant reduction in post transfusion recovery and lifespan of treated platelets 27%[39]**

37. Marschner S et al. Transfus Med Hemother 2011;38:8-18
38.Goodrich RP Transfusion 2009;49:1205-16
39. AuBuchon JP et al. Transfusion 2005;45:1335-41

MIRACLE Trial[40]

- 110 patients RCT
- Primary endpoint 1 hour CCI, non-inferiority limit 30%
- Analysis – 1 hour CCI significantly lower, 11,725 PI vs. 16,939 non-PI, non-inferiority was not allowed
- No statistical difference in:
  - Mean days between transfusion
  - Mean dose of transfusion
  - Mean number of transfusions per patient
  - Grade 2 or higher bleeding
- Second study had similar efficacy and safety results[41]
- No neoantigen or antibodies detected[42]

40. Miracle Study Group, Transfusion 2010;50:2362-2375
41. Schlenke P et al. Ann.Hematol;2011:1457-65
42. Ambruso DR et al. Transfusion 2009;49:2631-6

Mirasol Plasma

- **Treated plasma shows 20-40% reduction in fibrinogen and Factors V, VII, VIII, IX, X, XII[43-44]**
- **Good storage characteristics for two years[45]**
- **Can be used to make cryoprecipitate meeting US and European standards, 93 IU Factor8 and 262 mg/dl fibrinogen[46]**

43. Hornsey VS et al. Transfusion 2009;49:2167-72
44. Bihm DJ et al. Vox Sang 2010;98:108-15
45. Ettinger A et al. Transfus Apher Sci 2011;44:25-31
46. Ettinger A et al. Transfus Apher Sci 2012;46:153-8

Theraflex

- **Shortwave UVC light interacting with pyrimidine bases,no photochemicals**
- **Transfer to UVC permeable bag, illuminate for 1 minute, transfer to final storage bag**
- **Phase 1 – similar reduction in platelet recovery and lifespan as other PI technologies[47]**
- **Not validated for plasma yet**
- **Most bacteria 4 log reduction but reduced efficacy in high concentrations of Bacillus cereus, Klebsiella pneumoniae, and Proprionobacter acnes[48-49]**
- **Only 1 log reduction in HIV[48]**

47. Bashir S Transfus Med 2010:20(suppl 1):8
48. Mohr H et al. Transfusion 2009;49:2612-24
49. Seltsam A et al. Transfus Med Hemother 2011;38:43-54

PI Efficacy Intercept & Mirasol

Table 1
Degree of reduction of pathogens in log (adapted from [24] with Permission).

	Amotosalen/UVA	Riboflavin/UV	UVC
Enveloped virus			
HBV	>5.5	2.3	na*
HCV	>4.5	3.2	na
HIV (cell free)	>6.2	>5.9	1.4
HIV (cell-associated)	>6.1	>4.5	na
HTLV-I	4.7	na	na
CMV (cell-associated)	>5.9	na	na
West Nile virus	>6.0	>5.1	5.4
Chikungunya	>6.4	2.1	na
Influenza A virus	>5.9	>5	na
Nonenveloped virus			
HAV	0	1.8	na
Parvovirus B19	3.5 to 5.0	>5	5.46
Bacteria			
S. aureus	≥6.6	≥4	>4.8
S. epidermidis	≥6.6	4.2	4.8
P. aeruginosa	4.5	4.6	4.9
E. coli	≥6.4	4.4	>4
Spirochaete bacteria			
T. pallidum	>6.8	na	na
B. burgdorferi	>6.8	na	na
Parasite			
T. cruzi	>5.3	6	na
P. falciparum	>6	>3.2	na

* Information not available.

50. Prowse CV Vox Sang 2013;104:183-99

PI Efficacy[50]

- 2-6 log removal for viruses – higher log removal for enveloped than non-enveloped viruses
- 3-6 log removal for parasites
- 2-6 log removal for gram+ and gram- bacteria
- Intercept appears more effective than other PI[51]
- How much removal do we need?
- 6-8 copies of virus necessary for infection in window period, HIV, HBV, HCV[52]
- No claims for efficacy, safety, or sterility

50. Prowse CV Vox Sang 2013;104:183-99
51. Kaiser-Guignard J et al. Blood Reviews 28(2014) 235-241
52. Kleinman SH et al. Transfusion 2009;49:2454-89

PI Effects on Platelets

- **Weak impact on overall proteome[53]**
- **Fewer changes on day 1 vs. irradiated platelets**
- **Similar changes on day 5 but in different proteins**
- **Decreased response to agonists, more storage lesion changes[54]**
- **Slight activation – higher p-selectin expression[55]**
- **Increased metabolism, decrease in pH, increased lactate, increased glucose consumption[56]**
- **PI platelets showed shorter coagulation time[57]**
- **Low shear rates – same sheer adhesion[58]**
- **High shear rates – Surface deposition equal with decline in controls and Intercept but no decline in Mirasol platelets[59]**

53. Prudent M et al. Transfus Med Rev 2014;28:72-83
54. Thiele T Blood Transfus 2012;10:s63-70
55. Middelburg RA et al. Transfusion 2013;53:1780-7
56. Picker SM et al. Transfusion 2004;44:320-9
57. Tynngard N et al. Transfus Aph Sci 2008;38:85-8
58. Lozano M et al. Transfusion 2007;47:666-71
59. Picker SM et al. Transfusion 2009;49:1224-32

Meta Analysis - 3

- 1st – Including all PI papers[60]
  - Significant reduction in 1 and 24 hour CCIs,
  - Mean difference 3260 and 3315 respectively
  - Increased risk of overall bleeding OR 1.58
  - Clinically significant bleeding OR 1.54
- 2nd – same author restricted to Intercept only with expanded Sprint safety analysis[61]
  - Increased risk of overall bleeding OR1.52
  - No increase in severe bleeding[62]
- 3rd – different author, Intercept only but use of double blind studies only – Hovon study dropped[63]
  - No increased risk of bleeding

60. Vamvakas EC Transfusion 2011;51:1058-1071
61. Snyder E et al. Transfusion 2005;45:1864-75
62. Vamvakas EC Vox Sang 2012;102:302-16
63. Cid J et al. VoxSang 2012;103:322-30

Cochrane Analysis[64]

- 10 trials of treated vs. untreated platelets
- Primary outcomes: mortality, any bleeding, clinically significant bleeding, severe bleeding
- No increase in odds ratios for any of the four primary outcomes
- No difference in acute transfusion reactions, adverse events, or rbc transfusion requirements
- No bacterial contamination or TRALI seen
- Increase in refractoriness in PI treated group
- Required 7% more transfusions with half day shorter interval to transfusion
- Lower 1 hour and 24 hour CCIs in treated group

90 Butler C et al. Cochrane Database Syst Rev 2013:28;3:1-90

Can We Get to 7 day Platelets?

- **Five day limit related to risk of bacterial contamination**
- **FDA guidance requires secondary testing and 7 day storage bag**
- **TESSI trial – RCT, non-inferiority trial for transfusion of 6-7 day treated and untreated platelets[65-66]**
- **Primary endpoint – 1 hour CCI, inferiority set at 30%**
- **Non-inferiority of PI platelets, no significant difference in bleeding, use of rbcs and median time to next platelet transfusion**

65. Lozano M et al. Br. J. Haemotol 2011;153:393-401
66. Lozano M et al. Vox Sang 2010;99:13

Whole Blood and Red Blood Cells - Cerus Intercept

- **Problem – UV light absorbed by hemoglobin**
- **Original Cerus Intercept – use of S-303 acridine derivative**
- **Alkylating agent binds to nucleic acid activated at neutral pH**
- **Glutathione as quencher**
- **Incubation period 20 hours – 6 for inactivation, 14 for breakdown,**
- **Removed by centrifugation[67]**

67. Henschler et al. Transfus med Hemother 2011;38:33-42

Problems and Solutions

- **Clinical studies were stopped due to neoantigen formation with antibody formation[68]**
- **Process modified to include more glutathione and changing pH of solution**
- **Modest decrease in rbc lifespan though lower levels of extracellular potassium, higher glucose levels and lower lactate levels in PI rbcs**
- **Pathogen reduction 4-6 log removal[69]**
- **Phase 1 – 24 hour recovery, 88% at day 35 equivalent to untreated rbcs[70-71]**
- **Median lifespan shorter for PI rbcs, 32 days vs. 39 days**
- **Cerus trial on treated rbcs in cardiovascular surgery**

68. Benjamin RJ et al. Transfusion 2005;45:1739-49
69. Mufti NA et al. Biologicals 2010;38:14-19
70. Winter KM et al. Transfusion 2014;54:1798-1807
71. Cancelas JA Transfusion 2011;51:2367-76

Whole Blood and Red Blood Cells - Mirasol

- Working with military
- In theater, over 10,000 fresh whole blood transfusions, no testing
- Phase 1 – riboflavin and increasing light intensity
- Variability in recovery and lifespan dependent on light intensity – akin to irradiated rbc
- Hemolysis 1 - 1.5% dependent on light dosage[72]
- Normal subject study showed FDA acceptable data for survival and hemolysis
- Phase 3 trial to begin
- Reward is different dependent on population, benefit for military not equal to shorter rbc lifespan for thalassemic

72. Cancelas JA Transfusion 2011;51:1460-8

PI vs. Irradiation

- **Licensed and used in Europe in lieu of irradiation**
- **Benefits over irradiation:**
  - **Quality of rbc – less extracellular potassium**
  - **Availability and inventory management[73]**
  - **NRC concern over cesium irradiators**
  - **Cost of irradiation at $50-60, 10% products irradiated, 2.3 million in 2006**

73. Mintz et al. Bone Marrow Transplant 2009;44:205-11

PI and GVHD

- **FDA has not licensed either PI for this indication though Intercept can claim 4 log reduction in viable T-cells in package insert**
- **Animal and in-vitro studies :**
  - **5-6 log reduction of viable T-cells**
  - **Elimination of cytokine synthesis**
  - **Prevention of murine GVHD[74]**
- **Comparable to irradiation, process may be more robust[75]**
- **Intercept trials 100's to 17,000 – no TA-GVHD[76-78]**
- **France and Belgium no longer irradiate platelets**
- **Mirasol – as effective as irradiation for inactivation of wbc in rbc and platelets[79-81]**
- **No TA-GVHD in Miracle trial[40]**

74. Corash L et al. Bone Marrow Transplant 2004;33:1-7
75. Schlenke P et al. Transfus Med Hemother 2005;32:45-46
76. Cazenave JP et al. Transfusion 2008;48(S2):36A
77. Osselaer JC et al. Transfusion 2008;48:1061-1071
78. Osselaer JC et al. Vox Sang 2008;94:315-23
79. Fast LD et al. Transfusion 2011;51:1397-1404
80. Marschner S et al. Transfusion 2010;50:2489-98
81. Fast LD et al. Transfusion 2013;53:373-81
40. Miracle Study Group, Transfusion 2010;50:2362-2375

Cost Models

- Canadian model 1.4 million per QALY all ages
- Under 39, $426,000 per QALY[82]
- NAT testing – 1.5 million per QALY[83-84]
- Areas of potential cost savings
  - Bacterial culture, irradiation,
  - CMV, HTLV, WNV, Chagas testing
- Abstract shows gross savings of $187 before cost of PI
  - Bacterial culture, POC testing, irradiation, reduced outdating with 7 day platelet, no false +,
  - CMV, WNV, Chagas, Syphilis testing and no new test for babesia and dengue[85]
- Chickungunya, dengue, babesia, malaria, chagas all sensitive to PI – no test or geographic deferral needed[86-94]

82. Custer B et al. Transfusion 2010;50:2461-2473
83. Jackson BR et al. Transfusion 2003;43:721-729
84. Marshall DA et al. VoxSang 2004;86:28-40
85. McCullough J et al. Transfusion 2014;54(2s):57a
86. Rasongles P et al. Transfusion 2009;49:1083-91
87. Vanlandingham DL et al. Transfusion 2013;53:284-90
88. Grellier P et al. Transfusion 2008;48:1676-84
89. Tonnetti L et al. Transfusion 2010;50:1019-27
90. Keil SD et al. Transfusion 2013;53:2278-86
91. El Chaar M et al. Transfusion 2013;53:3174-83
92. Owusu-Ofori S et al. Shock 2014
93. Castro E et al. Transfusion 2007;47:434-41
94. Tonnetti L et al. Transfusion 2012;52:409-16

Overcoming the Paradigm

- Conceptually people favor proactive stance[95]
- Resistance to paradigm change:
  - Cost
  - Timing
  - Dilution of safety factor without rbc PI
  - Dual inventory
- The risk is not what we know but what we don't know and what we don't know we don't know
- WNV testing 1 year delay, 4480 estimated WNV+ transfusions[95], only 23 recognized[96]
- How many infections of Babesia, Dengue, Chickungunya before testing starts OR PI begins?

95. Alter HJ Transfuse Med Rev 2008;22:97-102

96. Pealer LN et al. NEJM 2003;349:1236-45