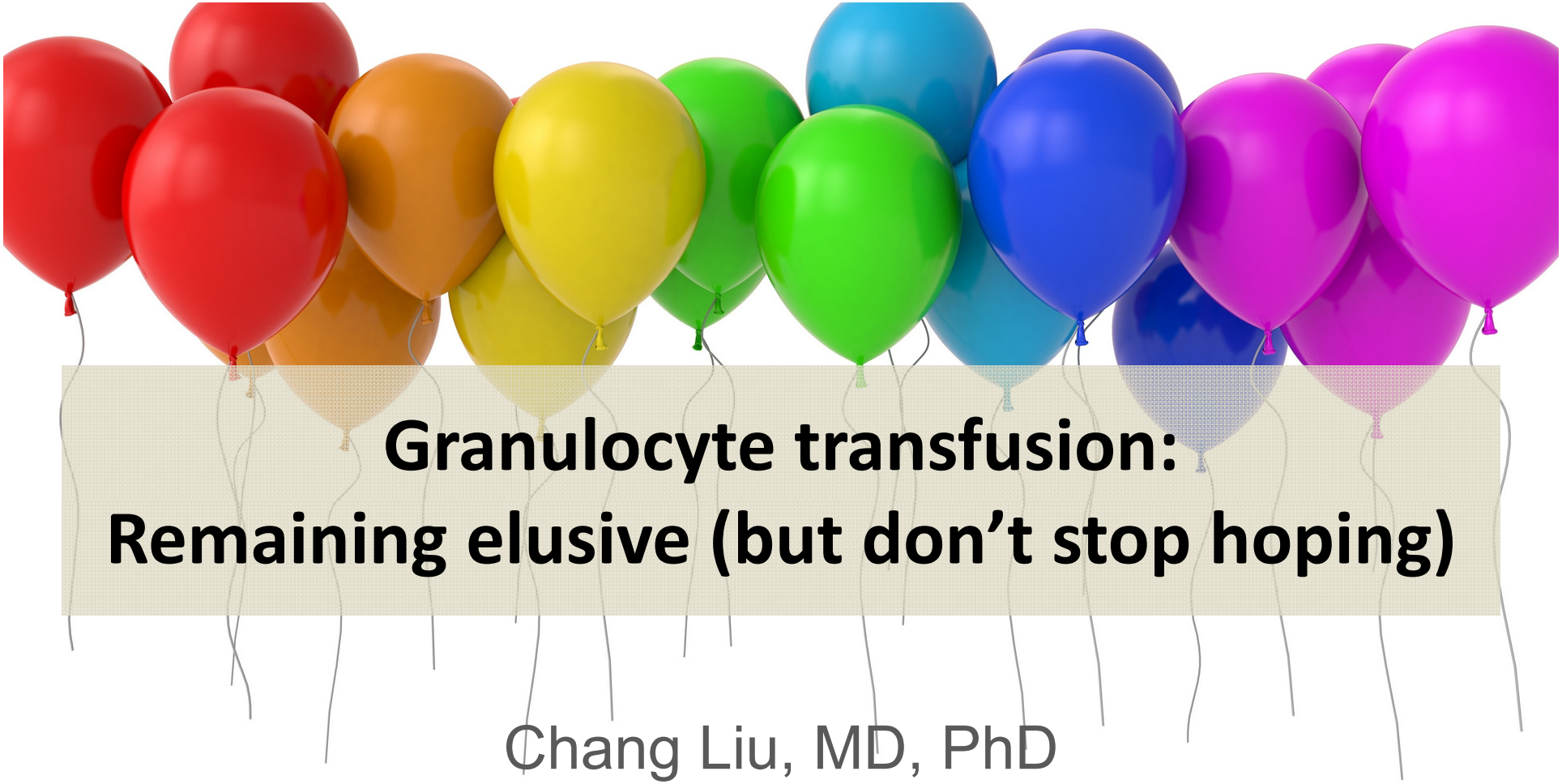

Granulocyte transfusion: Remaining elusive (but don't stop hoping)

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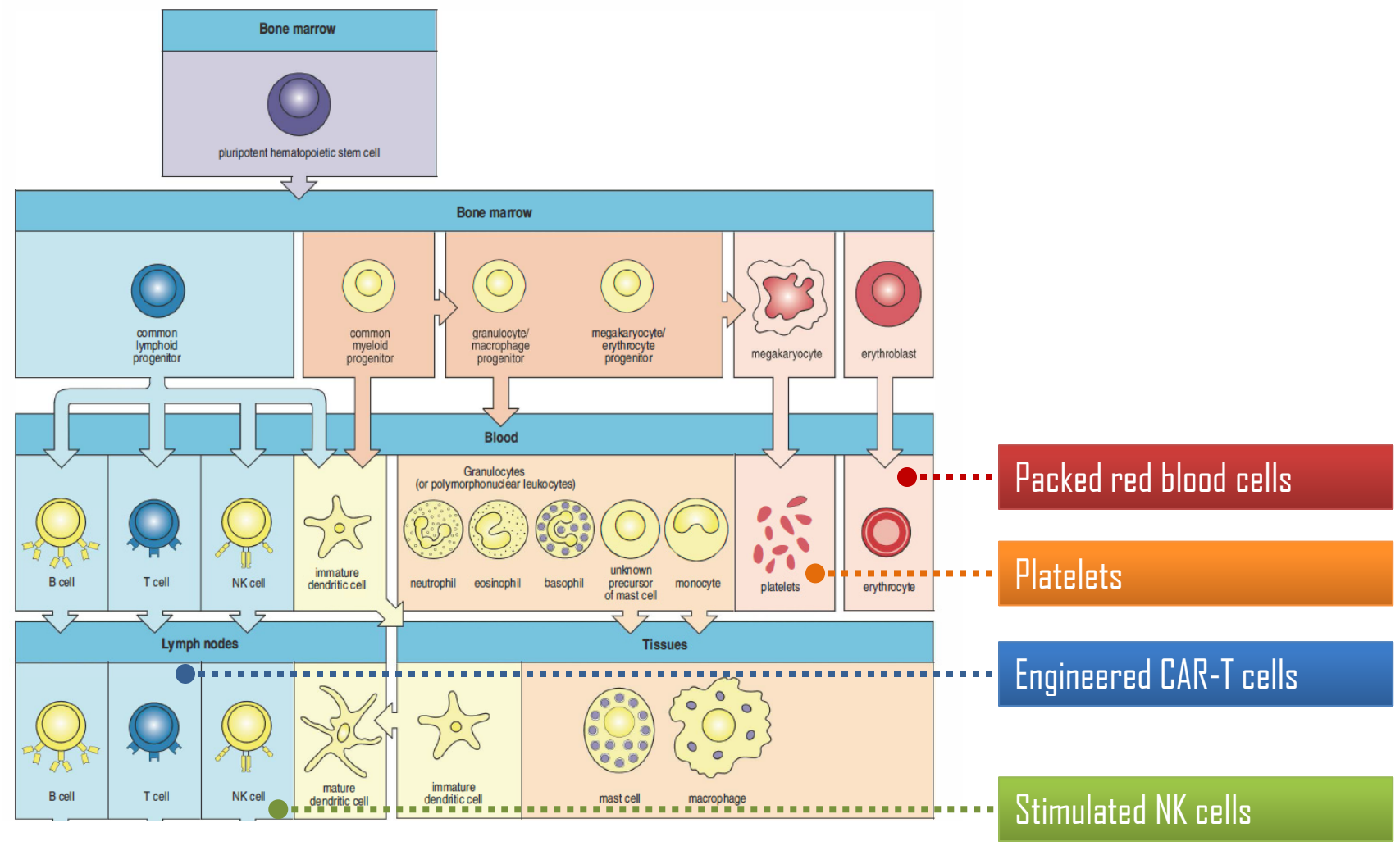
HAABB, St. Louis, MO | September 21, 2016

THIS SPEAKER HAS NOTHING TO DISCLOSE

Granulocyte transfusion

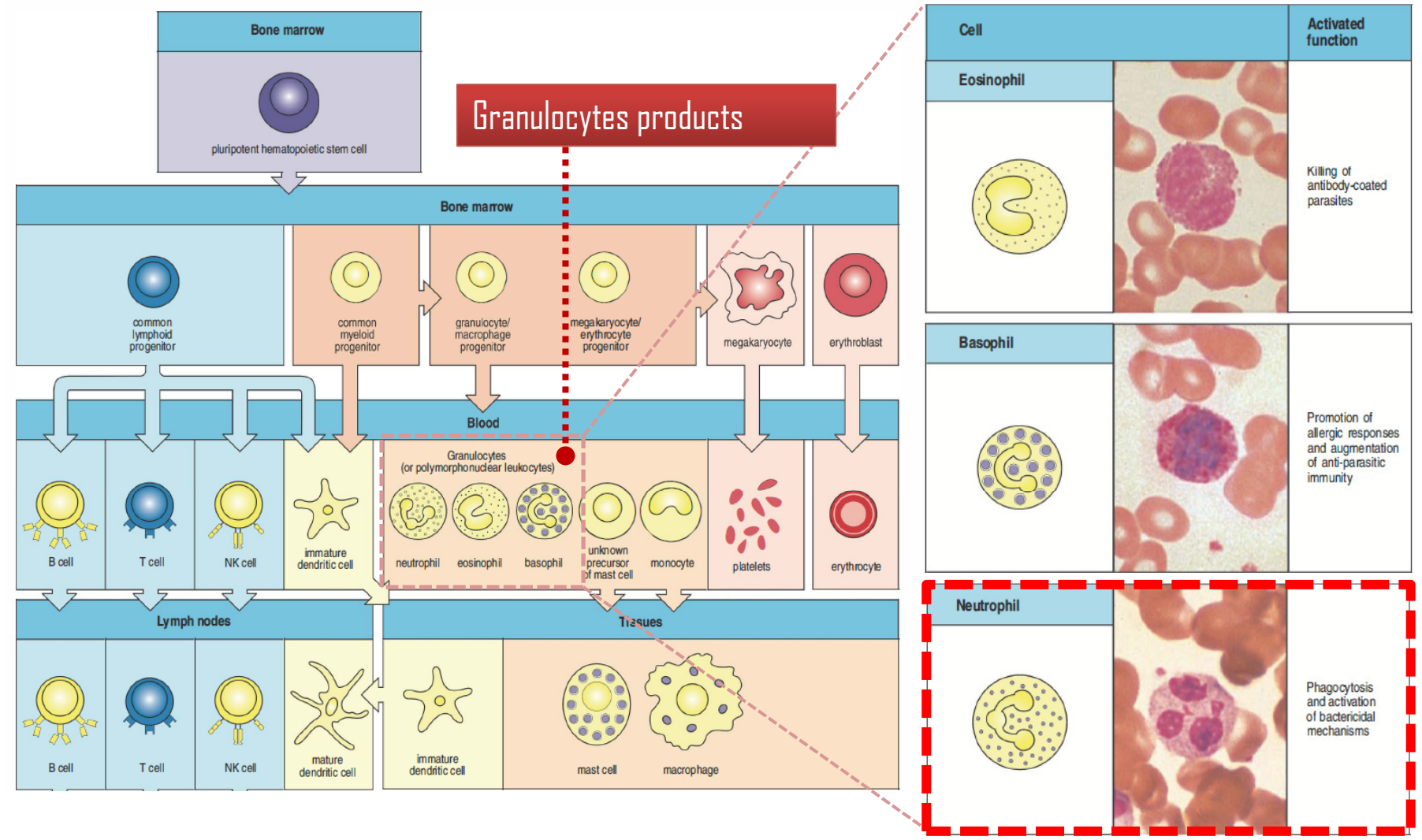
- Rationale and indications for granulocyte transfusion
- Evidence: RING trial and meta-analysis (*importance of dose*), and adverse reactions
- Collection and donor-related issues
- Future directions – *don't stop hoping*

Blood products from different lineages



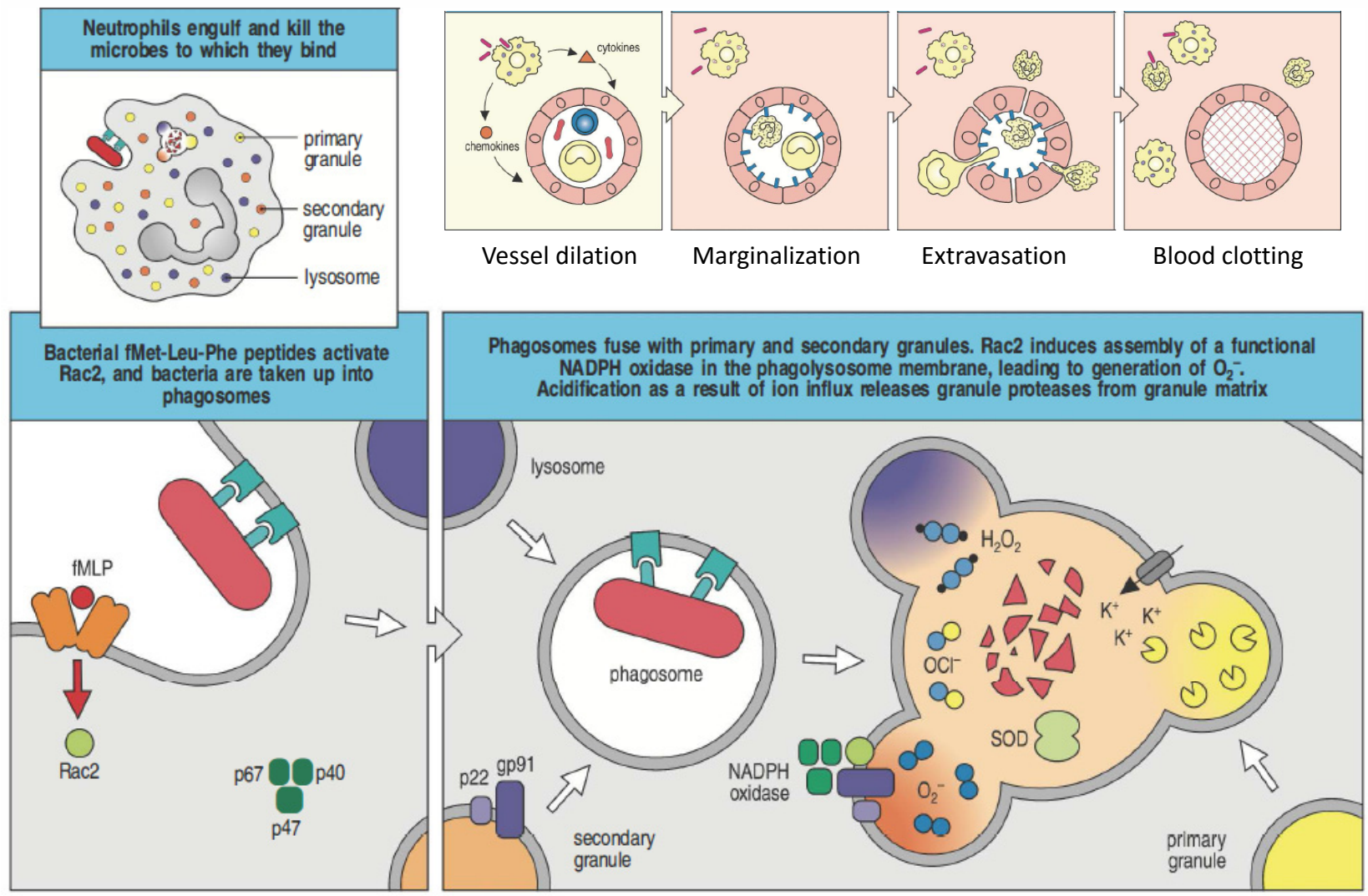
Adapted from Murphy 2012, Janeway's Immunobiology 8th edition

Granulocytes in the lineage tree



Adapted from Murphy 2012, Janeway's Immunobiology 8th edition

Uptake and killing of pathogens by granulocytes



Adapted from Murphy 2012, Janeway's Immunobiology 8th edition

Indications for granulocyte transfusion

- Severe neutropenia (absolute neutrophil count < $5x10^9$/L) or neutrophil dysfunction

➢ **Hereditary or congenital**
- *Chronic granulomatous diseases*
- *Leukocyte adhesion deficiency*
- *Severe congenital neutropenia*

➢ **Neonate sepsis and neutropenia**

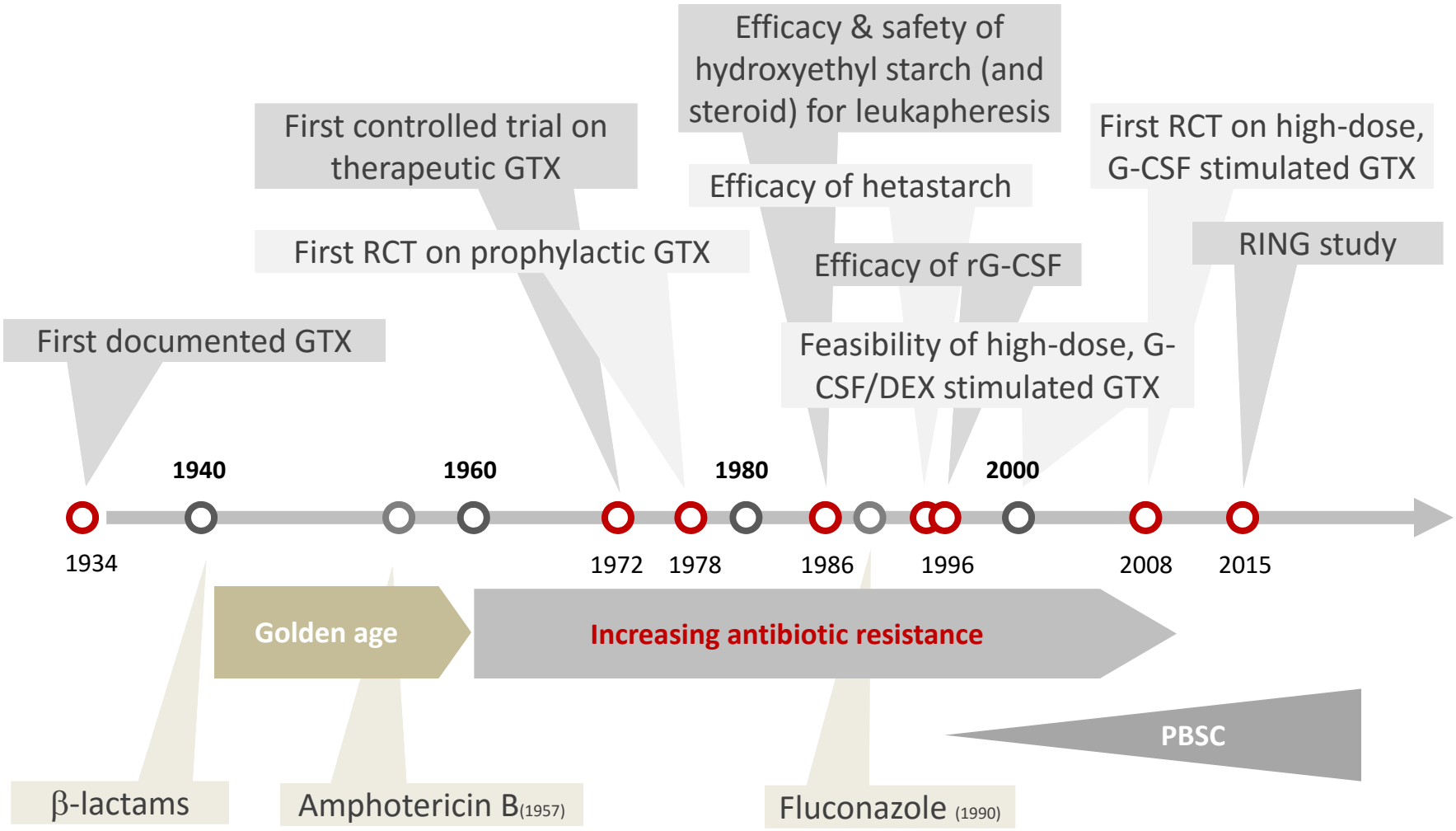
➢ **Acquired, reversible neutropenia (disease and treatment related)**
- *Hematological malignancy*
- *Chemotheray*
- *Stem cell transplantation*

➢ **To prevent infection (<u>prophylactic</u>)**

➢ **To treat severe infection not controlled by antimicrobial therapy (<u>therapeutic</u>)**

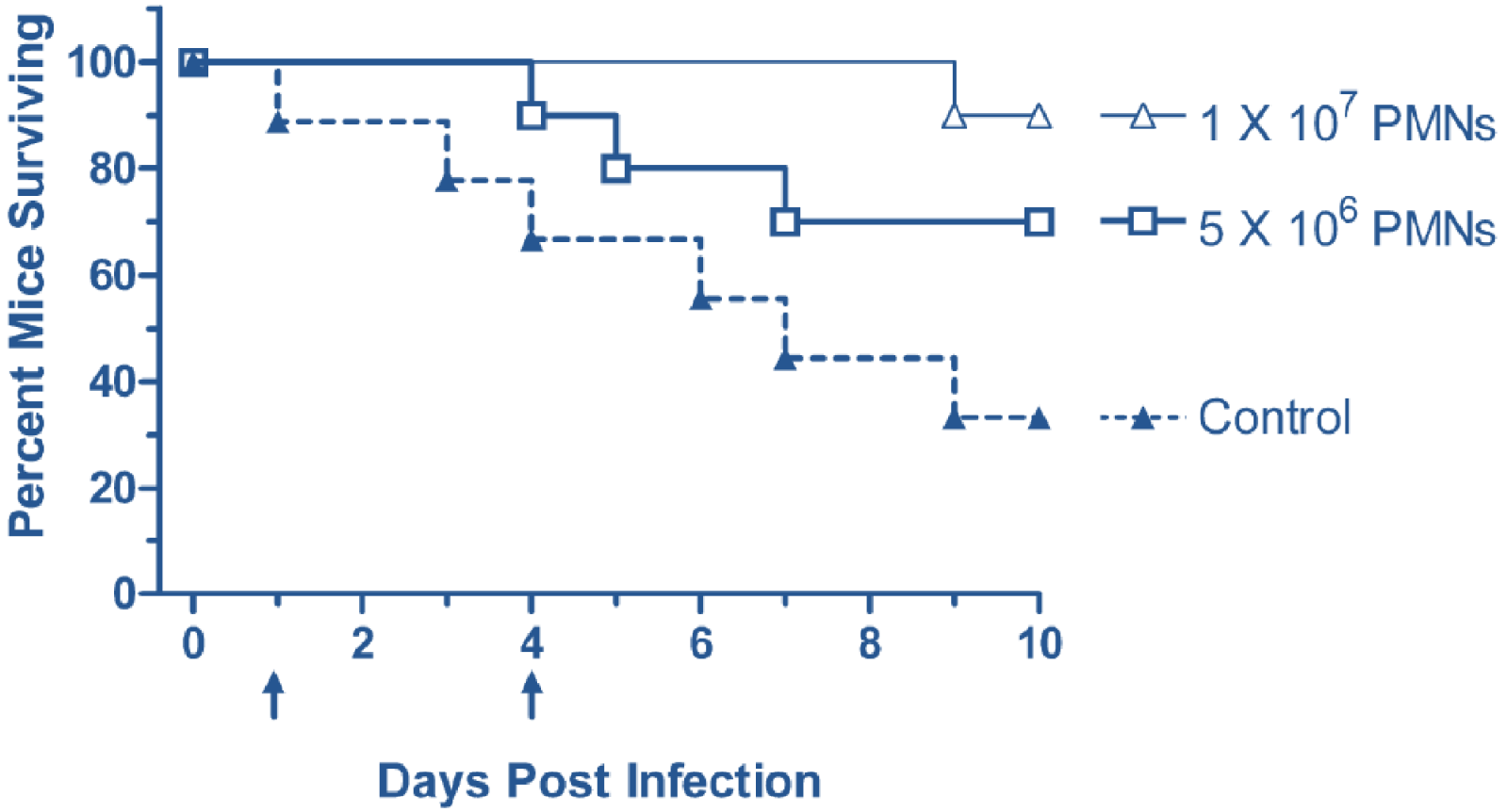
- *Bacterial infection (E. coli, Klebsiella, Pseudomonas, S. aureus, etc.)*
- *Fungal infection (Candida, Zygomycetes, Aspergillu, Fusarium etc.)*
- *Bloodstream or tissue infection (pulmonary, skin, soft tissue, sinus etc.)*

Historical perspective



Davies & Davies, Microbiology and Molecular Biology Reviews 2010;74:417-433. Strauss, Chapter 23 Rossi's Principles of Transfusion Medicine 5th edition. Cancelas, Blood 2015;126:2082-3. Graw et al., NEJM 1972;287:367-71. Clift et al., NEJM 1978;298:1052-7. Strauss et al., Transfusion 1986;26:258-64. Lee et al., Blood 1995;86:4662-6. Price et al., Blood 1996;88:335-40. Price et al., Blood 2000;95:3302-9. Anasetti et al., NEJM 2012;367:1487-96. Seidel et al., BMT 2008;42:679-84. Price et al., Blood 2015;126:2153-61.

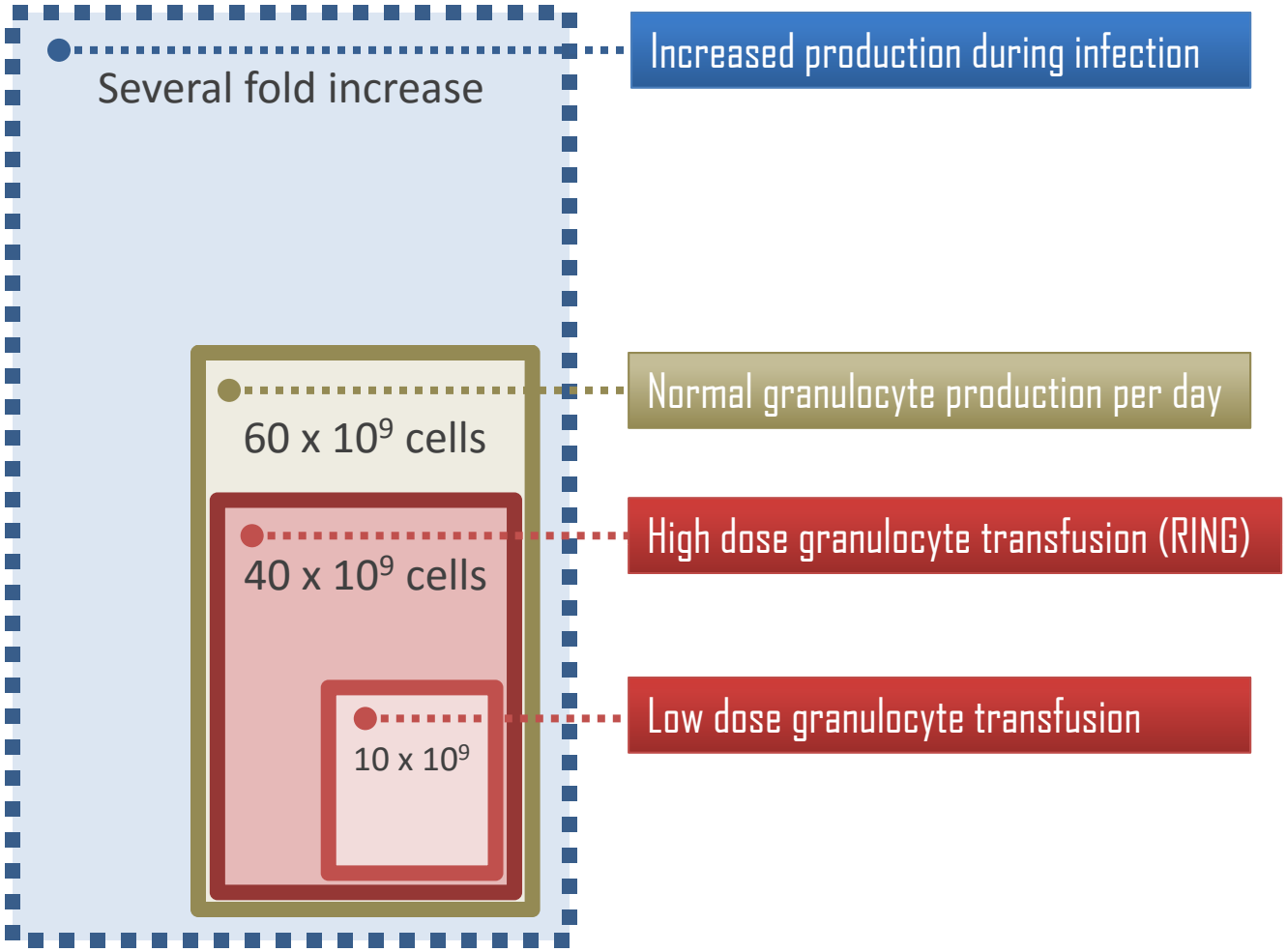
Dose matters: evidence from animal studies



Mouse aspergillosis model; receiving G-CSF stimulated mouse granulocytes

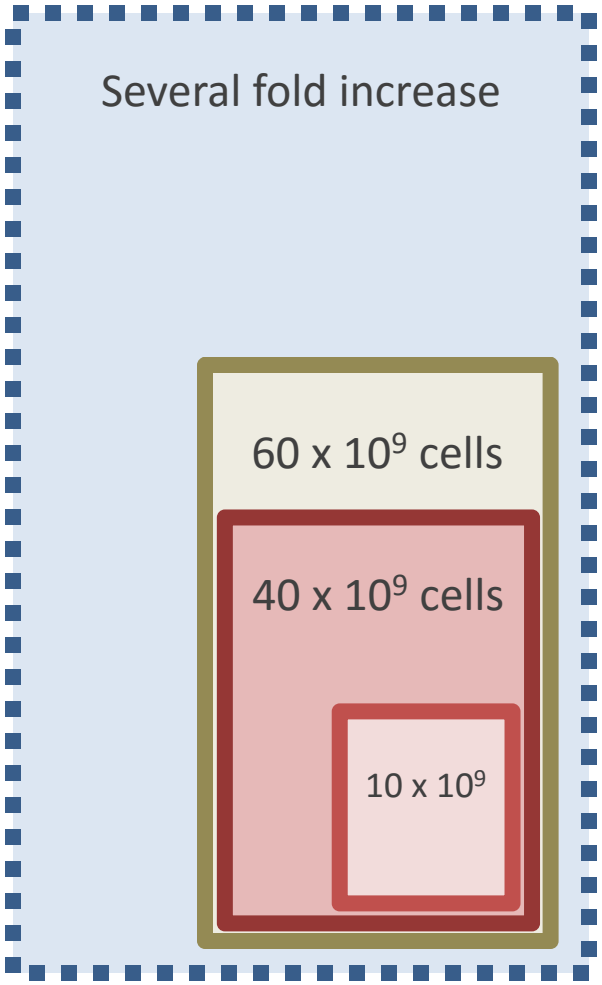
Martinez et al., Antimicrobial Agents and Chemotherapy. 2013; 57(4): 1882-7.

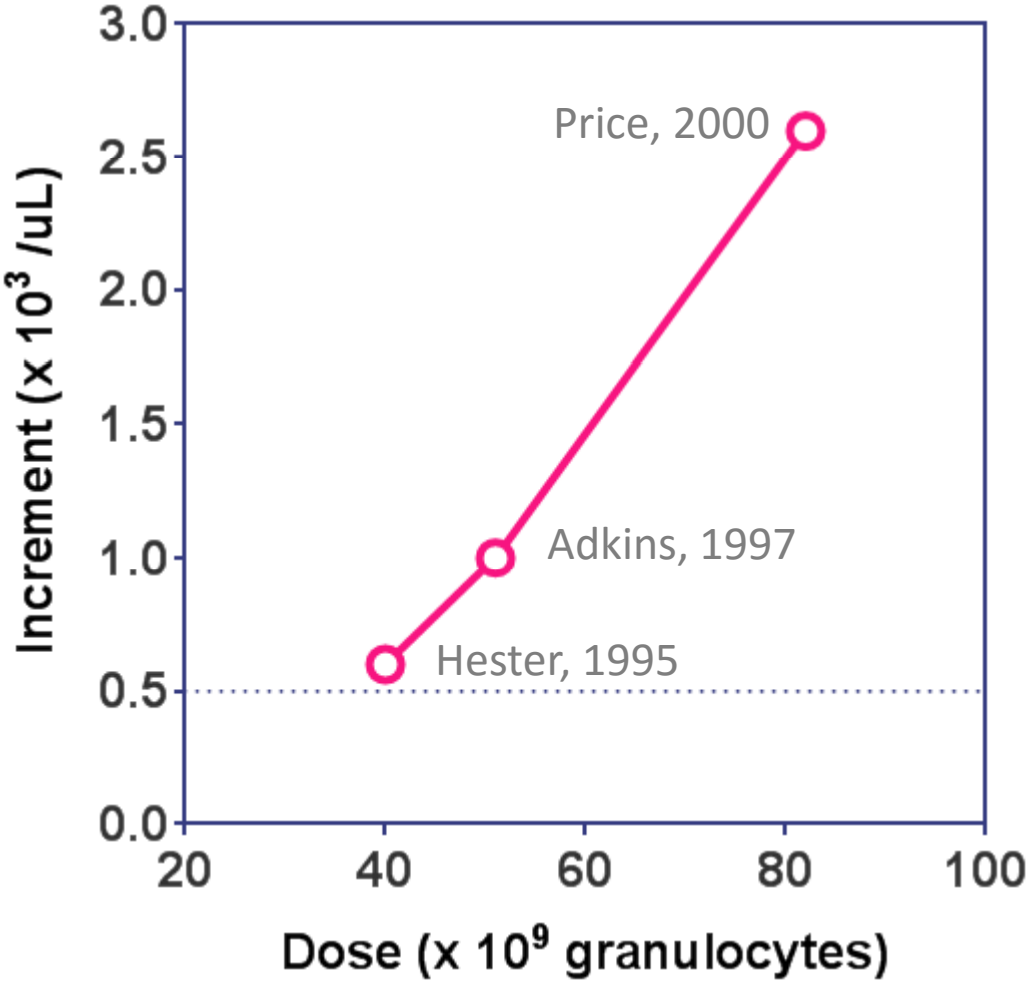
Dose matters: a *numerical* perspective



Price, J Clin Apher 2006;21:65-71. Dancey et al., JCI 1976;58:705-15.

Dose matters: a *numerical* perspective





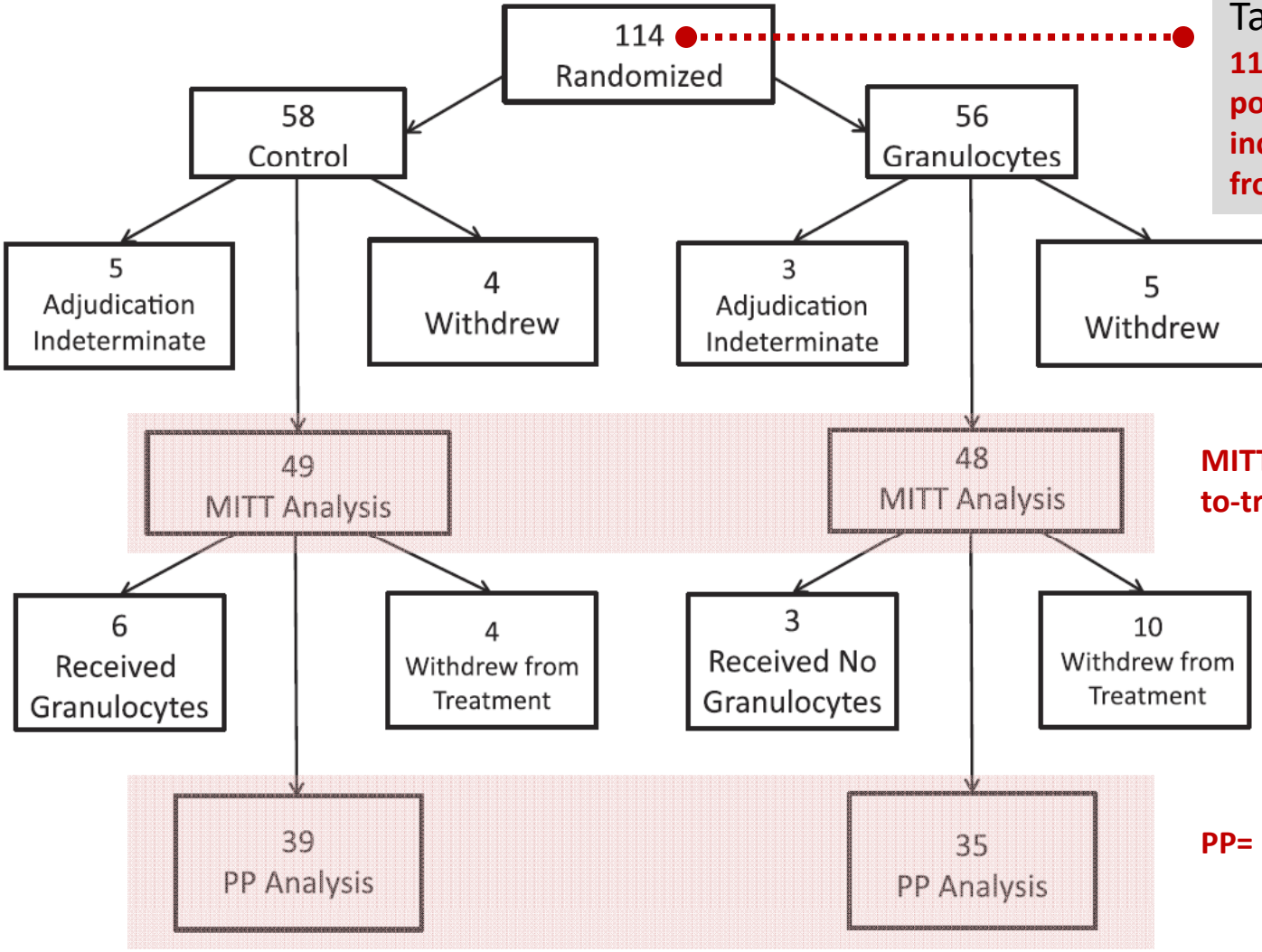
Price, J Clin Apheresis 2006;21:65-71. Hester et al., J Clin Apheresis 1995;10:188-193. Adkins et al., Transfusion 1997;37:737-48. Price et al., Blood 2000;95:3302-9.

RING study

- RING: *Resolving Infection in Neutropenia with Granulocytes*
- Aim: To determine the **efficacy of high-dose granulocyte transfusion (GTX, G-CSF/Dexamethasome stimulated)** as an adjunct treatment to standard antimicrobial therapy in adult and pediatric patients with (1) several **neutropenia (ANC < 500/μL)** secondary to chemotherapy or HSCT conditioning regimen and (2) proven or probable **bacterial/fungal infection**.
- Study design: two-arm RCT, open-label, multi-center (14)
- Primary endpoint: a composite of survival plus microbial response at 42 days.

Price et al., Blood. 2015;126(18):2153-61.

RING: flow diagram



Target sample size:
118 per arm for an 80% power to detect an increase in success rate from 50% to 70%

MITT= Modified intention-to-treat analysis

PP= per-protocol analysis

Price et al., Blood. 2015;126(18):2153-61.

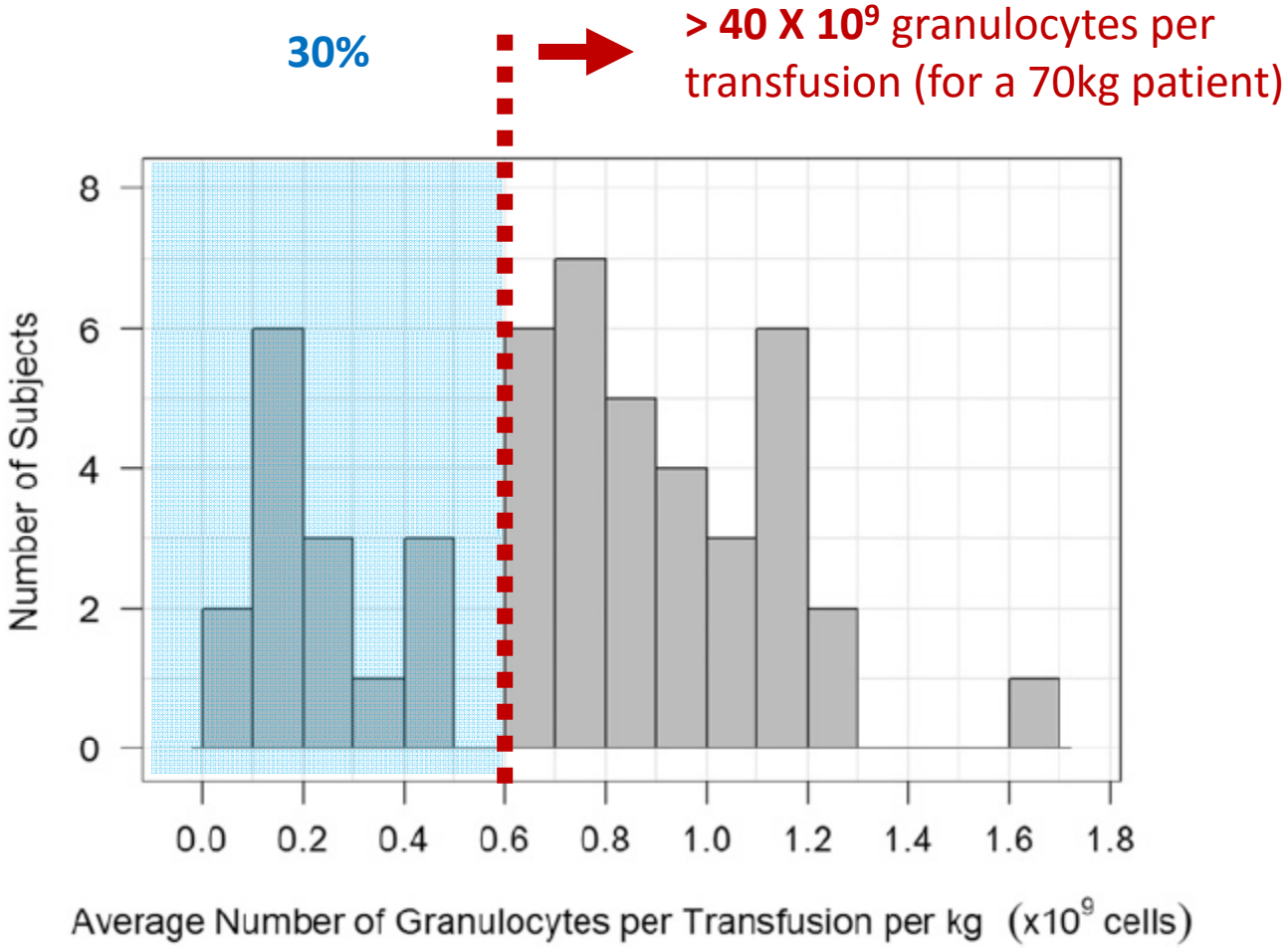
RING: baseline characteristics (MITT, N=97)

	Treatment arm		
	Control, n = 49	Granulocytes, n = 48	P*
Age, y	46.9 + 20.2	54.9 + 17.1	.04
<18, n (%)	6 (12)	4 (8)	.24
18-64, n (%)	33 (67)	27 (56)	
>65, n (%)	10 (20)	17 (35)	
Male sex, n (%)	27 (55)	28 (58)	.84
Race, n (%)			.18
White	31(63)	37 (77)	
Asian	4 (8)	1 (2)	
Black/African American	1 (2)	3 (6)	
Other/unknown	13 (27)	7 (15)	
ANC at randomization, cells/μL	43 ± 88	59 ± 100	.38
Cause of neutropenia, n (%)			.83
HSC transplantation	8 (16)	8 (17)	
Chemotherapy	36 (73)	37 (77)	
Other	5 (10)	3 (6)	
Underlying disorder, n (%)			.73
Acute lymphocytic leukemia	6 (12)	6 (13)	
Acute nonlymphocytic leukemia	31 (63)	32 (67)	
Chronic myelogenous leukemia	2 (4)	0 (0)	
Chronic lymphocytic leukemia	1 (2)	0 (0)	
Non-Hodgkin lymphoma	3 (6)	4 (8)	
Myelodysplasia	1 (2)	2 (4)	
Myeloma	1 (2)	0 (0)	
Aplastic anemia	2 (4)	0 (0)	
Other malignancy	0 (0)	1 (2)	
Other	2 (4)	3 (6)	

	Treatment arm		
	Control, n = 49	Granulocytes, n = 48	P*
Infection at enrollment, n (%)			.48†
Proven fungal	15 (31)	11 (23)	
Bloodstream			
Candida	6 (12)	3 (6)	
Zygomycetes	1 (2)	0 (0)	
Mold not specified	1 (2)	0 (0)	
Pulmonary			
Aspergillus	1 (2)	2 (4)	
Zygomycetes	1 (2)	0 (0)	
Facial/sinus			
Aspergillus	0 (0)	2 (4)	
Zygomycetes	0 (0)	1 (2)	
Skin/soft tissue			
Aspergillus	1 (2)	1 (2)	
Fusarium	0 (0)	1 (2)	
Disseminated			
Candida	1 (2)	2 (4)	
Fusarium	1 (2)	0 (0)	
Other			
Aspergillus	1 (2)	0 (0)	
Mold not specified	1 (2)	0 (0)	
Probable/presumptive pulmonary fungal	9 (18)	11 (23)	
Typhlitis	2 (4)	5 (10)	
Invasive bacterial	13 (27)	8 (17)	
Bacteremia alone	10 (20)	13 (27)	

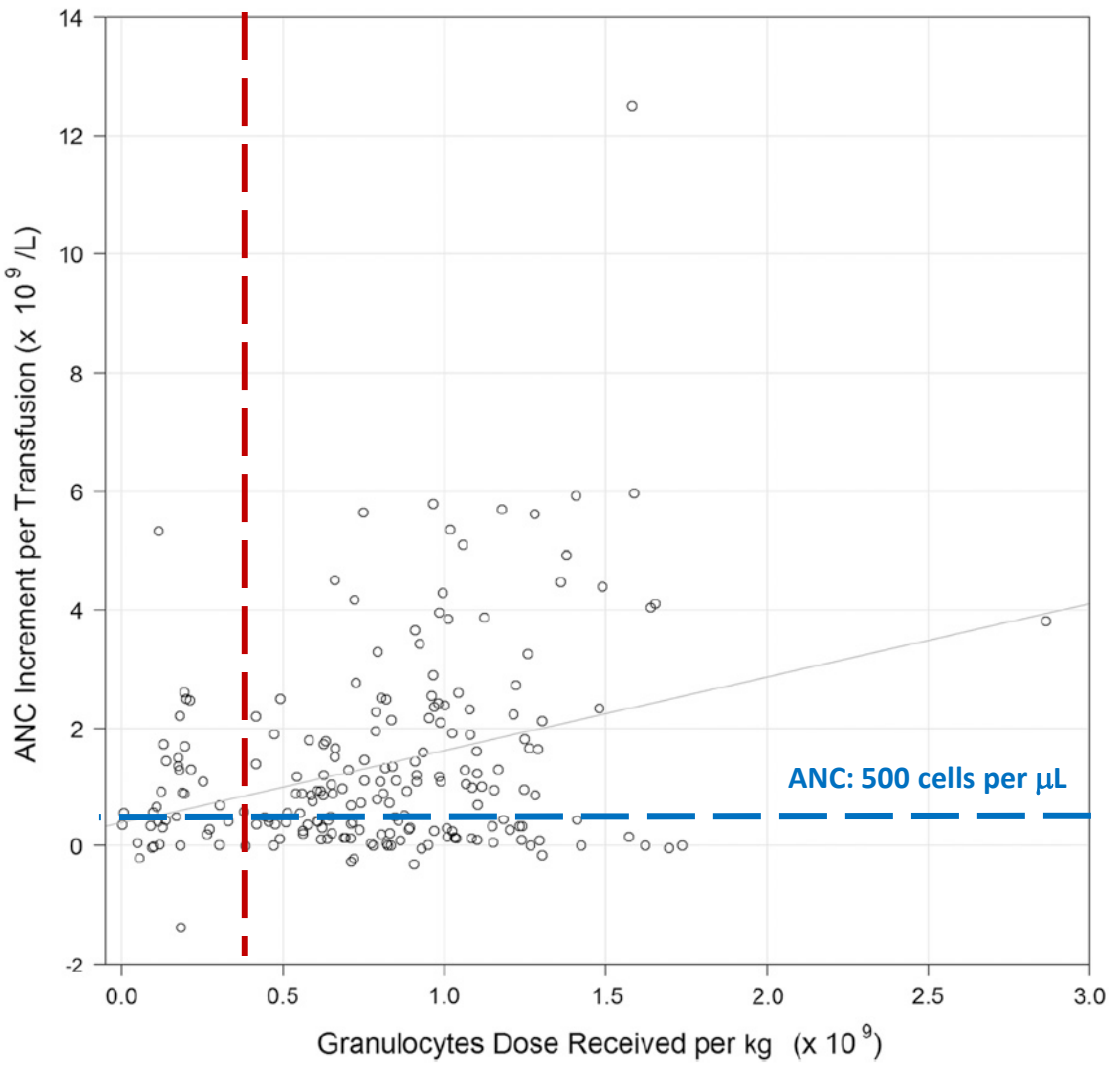
Price et al., Blood. 2015;126(18):2153-61.

RING: was high-dose GTX achieved?



Price et al., Blood. 2015;126(18):2153-61.

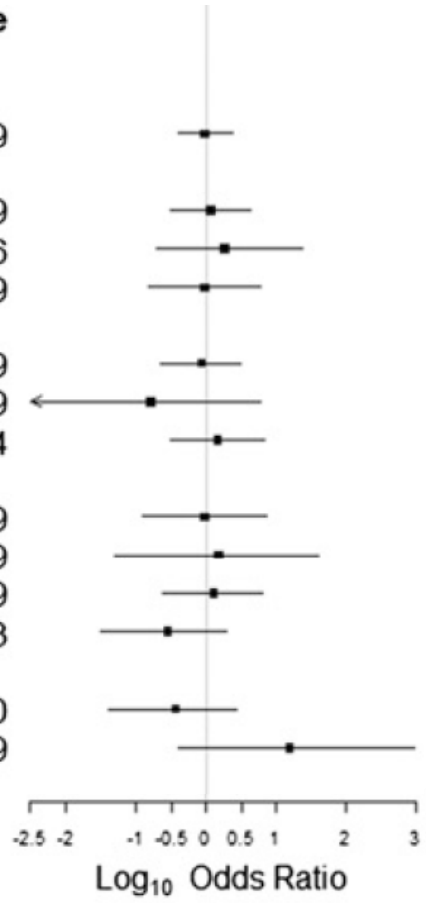
RING: hematologic response to GTX



Price et al., Blood. 2015;126(18):2153-61.

RING: success rate (MITT analysis)

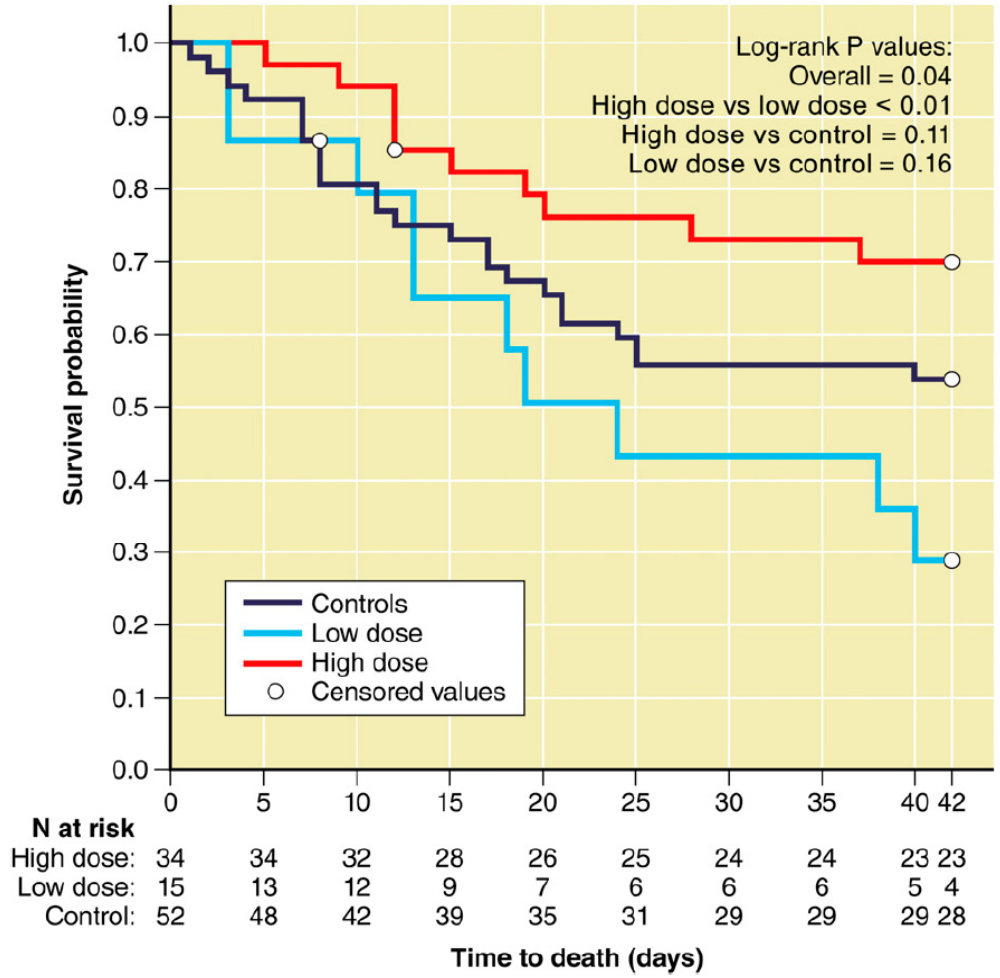
	Total N (% Success)	Granulocytes N (% Success)	Control N (% Success)	P-Value
MITT				
Overall	97 (42%)	48 (42%)	49 (43%)	>0.99
Bacterial Infection	51 (33%)	26 (35%)	25 (32%)	>0.99
Bacteremia Only	25 (24%)	14 (29%)	11 (18%)	0.66
Tissue Bacterial Infection	26 (42%)	12 (42%)	14 (43%)	>0.99
Fungal Infection	46 (52%)	22 (50%)	24 (54%)	>0.99
Fungemia Only	11 (64%)	3 (33%)	8 (75%)	0.49
Tissue Fungal Infection	35 (49%)	19 (53%)	16 (44%)	0.74
High Risk with Invasive Mold Infection	22 (55%)	13 (54%)	9 (56%)	>0.99
Low Risk with Invasive Mold Infection	9 (44%)	4 (50%)	5 (40%)	>0.99
High Risk with other Infection	41 (27%)	17 (29%)	24 (25%)	>0.99
Low Risk with other Infection	25 (56%)	14 (43%)	11 (73%)	0.23
Mold Pulmonary/Chest	23 (57%)	13 (46%)	10 (70%)	0.40
Mold ExtraPulmonary	10 (40%)	4 (75%)	6 (17%)	0.19



- No significant difference by per-protocol analysis either
- No significant difference in day 42 survival status

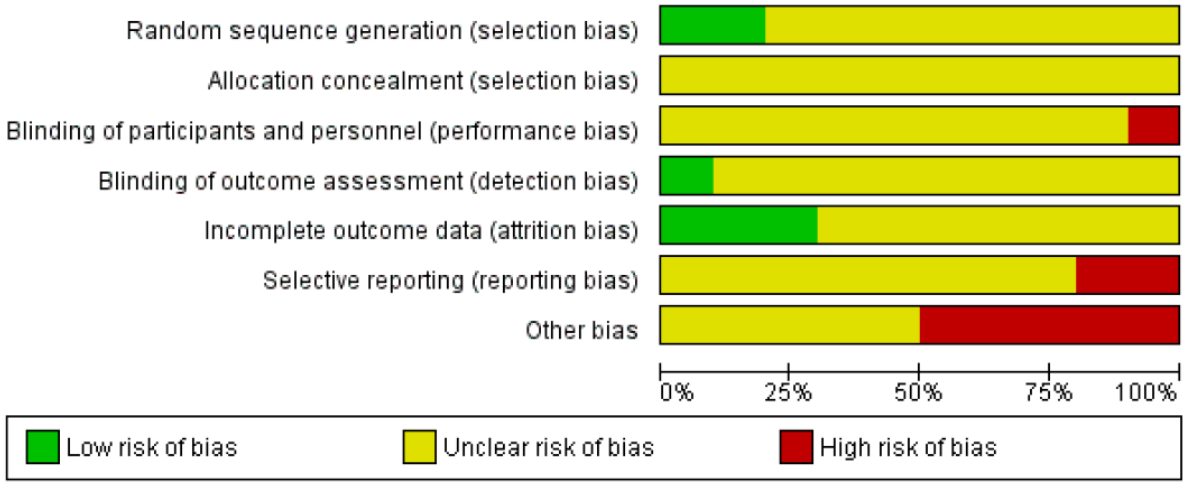
Price et al., Blood. 2015;126(18):2153-61.

RING: post hoc analysis



Price et al., Blood. 2015;126(18):2153-61. Cancelas, Blood. 2015; 126(18): 2082-3.

Meta-analysis: therapeutic GTX



Comparison 1. Therapeutic granulocyte transfusions versus no therapeutic granulocyte transfusionsMortality

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Overall mortality (up to 30 days) fixed effects	6	321	Risk Ratio (M-H, Fixed, 95% CI)	0.75 [0.54, 1.04]
1.1 Mortality at 20 to 22 days	5	226	Risk Ratio (M-H, Fixed, 95% CI)	0.73 [0.52, 1.02]
1.2 Mortality at 5 days	1	95	Risk Ratio (M-H, Fixed, 95% CI)	0.98 [0.30, 3.16]
2 Clinical reversal of concurrent infection	5	286	Risk Ratio (M-H, Fixed, 95% CI)	0.98 [0.81, 1.19]
3 Pulmonary complications	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only

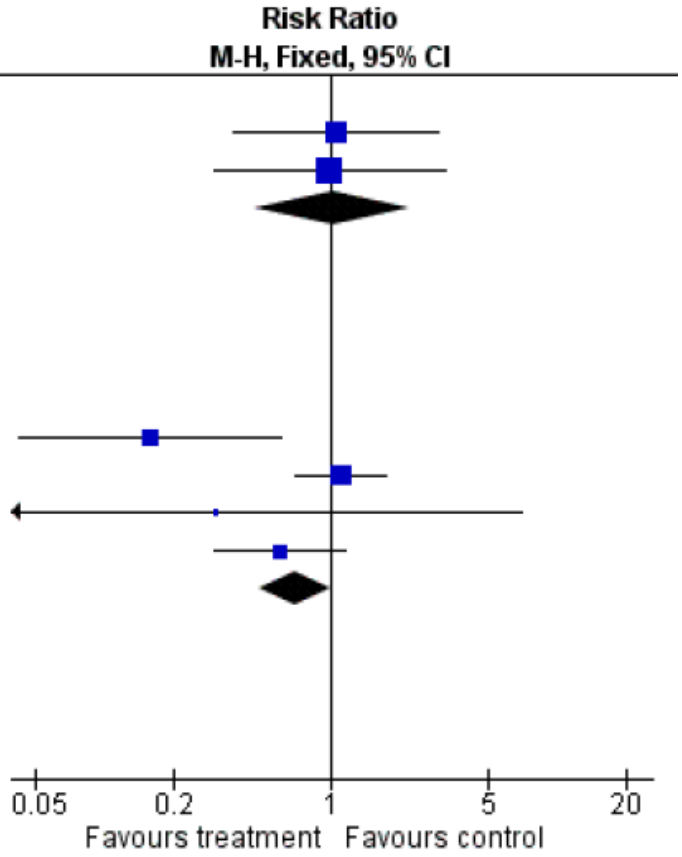
Estcourt et al., Cochrane Database Syst Rev 2016;**4**: Cd005339.

Meta-analysis: therapeutic GTX

Comparison 2. Sub group analyses for studies transfusing < and ≥ 1 x 10^{10} granulocytes per transfusion

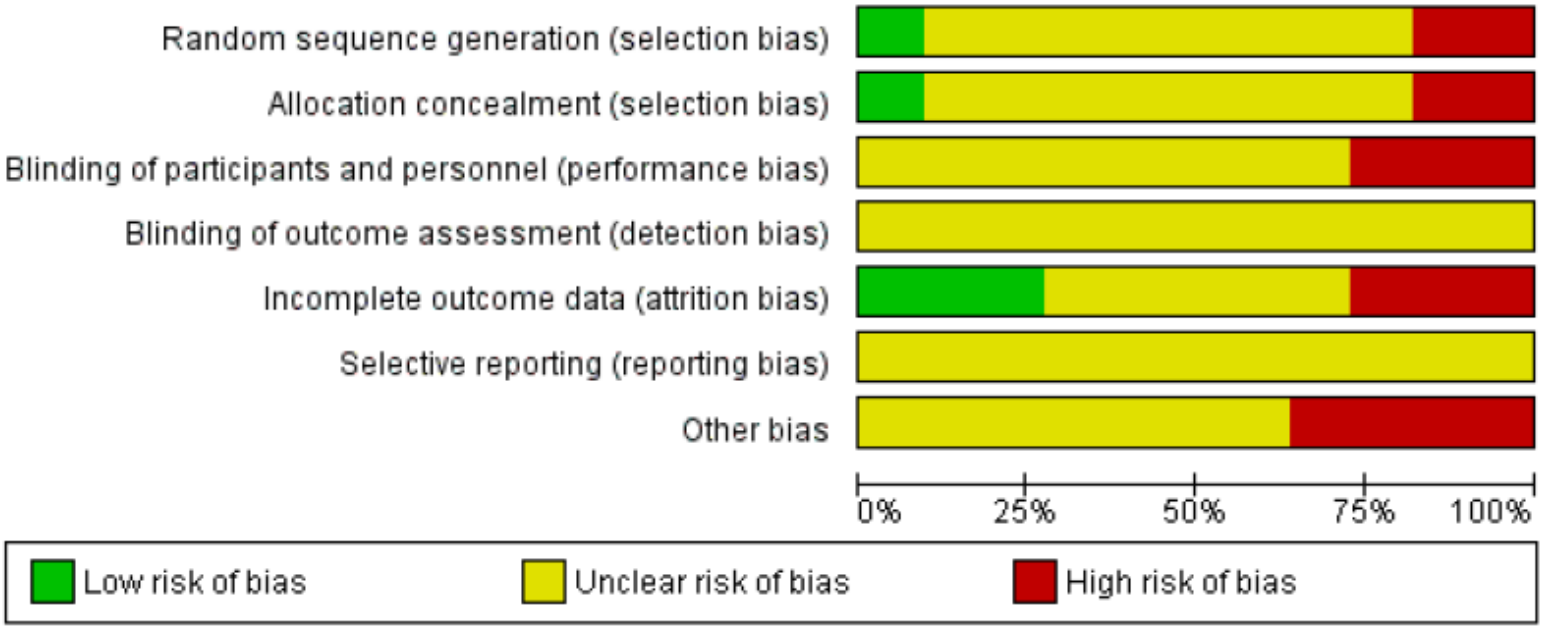
Study or Subgroup	Treatment Events	Treatment Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
2.1.1 granulocytes < 1 x 10^{10}						
Bow 1984	5	13	4	11	46.2%	1.06 [0.37, 3.00]
Winston 1982a	5	48	5	47	53.8%	0.98 [0.30, 3.16]
Subtotal (95% CI)		**61**		**58**	**100.0%**	**1.02 [0.46, 2.24]**
Total events	10		9			
Heterogeneity: Chi² = 0.01, df = 1 (P = 0.92); I² = 0%						
Test for overall effect: Z = 0.04 (P = 0.97)						
2.1.2 granulocytes ≥1 x 10^{10}						
Higby 1975	2	17	14	19	28.5%	0.16 [0.04, 0.60]
Price 2015	23	54	22	57	46.1%	1.10 [0.70, 1.73]
Scali 1978	0	13	1	12	3.4%	0.31 [0.01, 6.94]
Vogler 1977	7	17	9	13	22.0%	0.59 [0.30, 1.17]
Subtotal (95% CI)		**101**		**101**	**100.0%**	**0.70 [0.49, 0.99]**
Total events	32		46			
Heterogeneity: Chi² = 9.20, df = 3 (P = 0.03); I² = 67%						
Test for overall effect: Z = 2.01 (P = 0.04)						

Test for subgroup differences: Chi² = 0.73, df = 1 (P = 0.39), I² = 0%



Estcourt et al., Cochrane Database Syst Rev 2016;**4**: Cd005339.

Meta-analysis: prophylactic GTX, quality



- A total of **11 trials**, conducted between 1978 and 2006, were eligible involving **653 participants**.
- Overall, the quality of the evidence was **very low to low**; many of the studies were **at high risk of bias**; many of the outcome estimates being **imprecise**.

Estcourt et al., Cochrane Database Syst Rev 2015;**6**: Cd005341.

Meta-analysis: prophylactic GTX, outcomes

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 All-cause mortality up to 30 days	7	437	Risk Ratio (M-H, Fixed, 95% CI)	0.92 [0.63, 1.36]
1.1 Low-dose granulocytes	3	144	Risk Ratio (M-H, Fixed, 95% CI)	1.32 [0.64, 2.72]
1.2 Intermediate-dose granulocytes	4	293	Risk Ratio (M-H, Fixed, 95% CI)	0.74 [0.47, 1.16]
2 All-cause mortality up to 100 days	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
2.1 Granulocyte dose unknown	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3 All-cause mortality over 100 days	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
3.1 Low-dose granulocytes	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
4 Mortality due to infection up to 30 days	6	286	Risk Ratio (M-H, Fixed, 95% CI)	0.69 [0.33, 1.44]
4.1 Low-dose granulocytes	3	144	Risk Ratio (M-H, Fixed, 95% CI)	1.14 [0.46, 2.86]
4.2 Intermediate-dose granulocytes	3	142	Risk Ratio (M-H, Fixed, 95% CI)	0.25 [0.06, 1.10]
5 Mortality due to infection up to 100 days	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
5.1 Granulocyte dose unknown	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]

Estcourt et al., Cochrane Database Syst Rev 2015;**6**: Cd005341.

Meta-analysis: prophylactic GTX, outcomes

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
6 People with localised or systemic bacterial or fungal infections	9		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
6.1 Low-dose granulocyte transfusions	4	204	Risk Ratio (M-H, Fixed, 95% CI)	0.84 [0.58, 1.20]
6.2 Intermediate-dose granulocyte transfusions	4	293	Risk Ratio (M-H, Fixed, 95% CI)	0.40 [0.26, 0.63]
6.3 Unknown dose of granulocyte transfusion	1	112	Risk Ratio (M-H, Fixed, 95% CI)	0.97 [0.61, 1.55]
7 People with bacteraemia or fungaemia	9	609	Risk Ratio (M-H, Fixed, 95% CI)	0.45 [0.30, 0.65]
7.1 Low-dose granulocyte transfusions	4	204	Risk Ratio (M-H, Fixed, 95% CI)	0.51 [0.27, 0.96]
7.2 Intermediate-dose granulocyte transfusions	4	293	Risk Ratio (M-H, Fixed, 95% CI)	0.28 [0.14, 0.55]
7.3 Unknown dose of granulocyte transfusions	1	112	Risk Ratio (M-H, Fixed, 95% CI)	0.79 [0.39, 1.61]

Estcourt et al., Cochrane Database Syst Rev 2015;**6**: Cd005341.

Meta-analysis: prophylactic GTX, outcomes

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
8 People with bacteraemia or fungaemia (excluding study that compared prophylactic granulocyte transfusions versus prophylactic broad-spectrum antibiotics)	8	497	Risk Ratio (M-H, Fixed, 95% CI)	0.37 [0.23, 0.59]
8.1 Low-dose granulocyte transfusions	4	204	Risk Ratio (M-H, Fixed, 95% CI)	0.51 [0.27, 0.96]
8.2 Intermediate-dose granulocyte transfusions	4	293	Risk Ratio (M-H, Fixed, 95% CI)	0.28 [0.14, 0.55]
9 People with localised bacterial or fungal infection	6	296	Risk Ratio (M-H, Fixed, 95% CI)	0.75 [0.50, 1.14]
9.1 Low-dose granulocyte transfusions	2	42	Risk Ratio (M-H, Fixed, 95% CI)	0.46 [0.19, 1.11]
9.2 Intermediate-dose granulocyte transfusions	3	142	Risk Ratio (M-H, Fixed, 95% CI)	0.71 [0.38, 1.31]
9.3 Unknown dose of granulocyte transfusions	1	112	Risk Ratio (M-H, Fixed, 95% CI)	1.12 [0.54, 2.33]

Estcourt et al., Cochrane Database Syst Rev 2015;**6**: Cd005341.

Adverse effect of GTX (RING)

Grade	Clinical presentation	Frequency	
		By subject	By transfusion
1-2 (mild to moderate)	**Fever** **Chills** Modest change in BP	41%	28%
3-4 (severe to life-threatening)	**Hypoxia (n=7)** Tachycardia (n=1) Hypotension (n=1)	20%	<5%
4	**Hypoxia** requiring temporary ventilatory support (n=1)	2%	<1%
Death	N=0	0	0

- No significant association between granulocyte dose (cells per kilogram weight) and presence of a transfusion reaction (OR=1.07, 95CI: 0.58-1.95)
- The study did not select donors on the basis of HLA or granulocyte compatibility.

Price et al., Blood. 2015;126(18):2153-61. Price et al., Blood 2000;95:3302-9.

Pulmonary and other reactions with GTX

- Pulmonary reactions: ~ 5% of transfusions (Hester, 1995), characterized by varying degrees of **dyspnea, hypoxia and radiographic changes**
  - Alloimmunization to HLA, reverse TRALI
  - Not always reproduced in different studies
- Other reactions:
  - Volume overload
  - CMV transmission
  - Hemolysis and alloimmunization

Hester et al., J Clin Apheresis 1995;10:188-93. Stroncek et al., Transfusion 1996;36:1009-15. Narvios et al., Blood 2002;99:390-1.

Preparation of apheresis granulocytes (1)

4 different centers in the United States

- UT, MD Anderson (UT)
- National Institute of Health Clinical Center (NIH)
- Puget Sound Blood Center/U Washington (UW)
- U Iowa (UI)

- **Donor selection** for granulocytes collection?
  - Healthy volunteer, apheresis platelet donors [3] > patients relatives and social network [1]
  - CMV status: not considered [2] versus considered [2]
- **Mobilization regimen?**
  - G-CSF (480 μg or 600 μg single dose) SC (*off-label*) + dexamethasone (8 mg) PO 8-16 hours before collection [4]

Strauss et al., Vox Sanguinis. 2011, 100:426-433

Preparation of apheresis granulocytes (2)

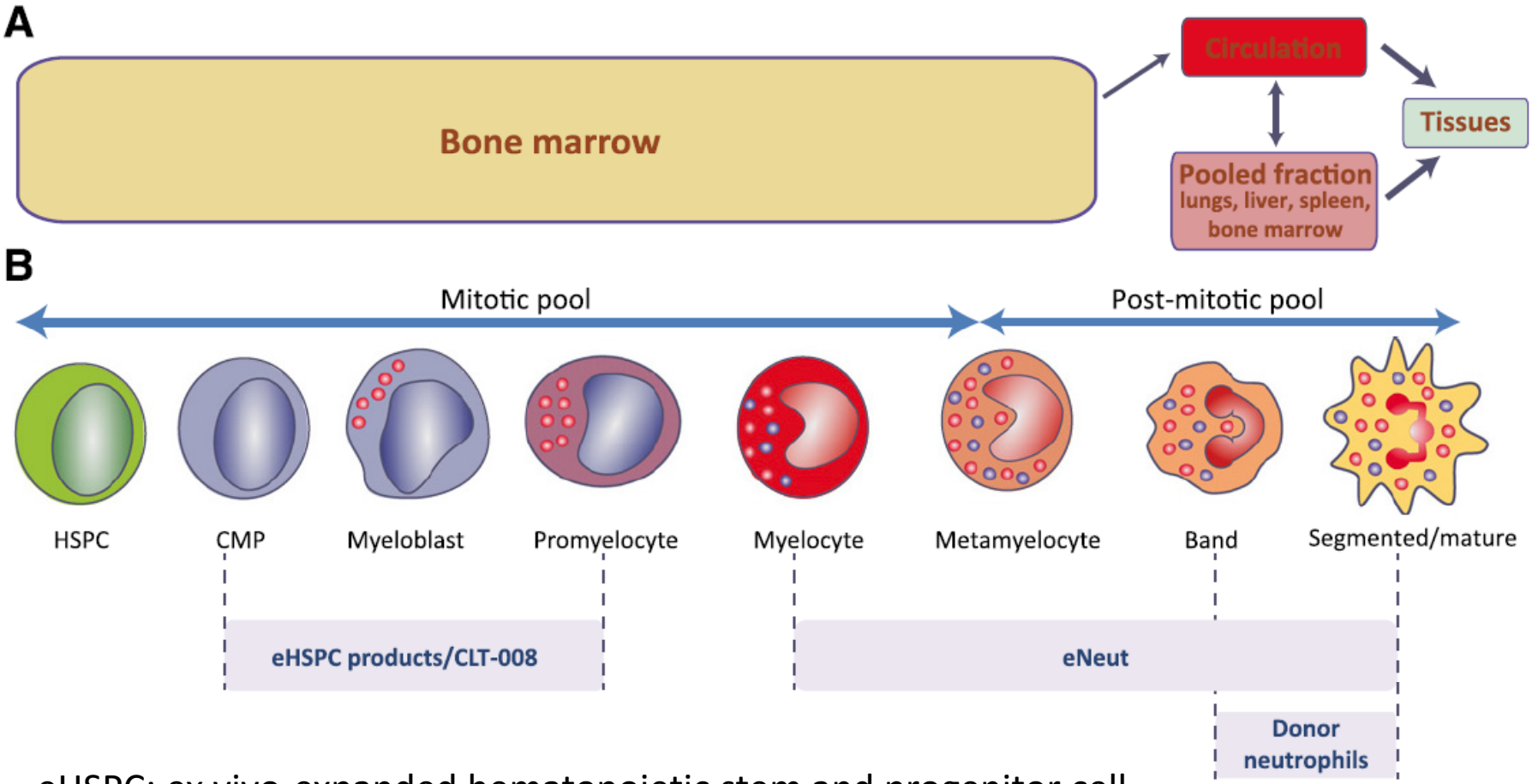
- **Frequency of donation?**
  - Every other day, weekly, every other week [1]; up to once a month [1]; up to once every 72 hrs, 8 donations per year [2]
- Sedimentation agents, **Hetastarch or pentastarch?**
  - 6% hetastarch [4] for much higher yield than pentastarch
- Compatibility tests, **ABO/Rh compatibility required?**
  - ABO compatibility: respected [3], ignored [1]
  - Rh compatibility: respected [3], ignored [1]
    - Mitigate the risk of incompatible GTX (if given due to medical necessity) by sedimentation and IV RhIG
- **Screening for leukocyte antibodies**? Not really [4]

Strauss et al., Vox Sanguinis. 2011;100:426-433

Donor reactions & risks

- G-CSF:
  - Bone pain, headache, fatigue, insomnia and flushing (ibuprofen or acetaminophen, q 4-6hrs)
- Dexamethasone:
  - Posterior subcapsular cataracts if stimulated repeatedly (eye exam every 2 years suggested)
- Hetastarch:
  - Fluid retention (monitor weight)
  - Allergic reaction
- Collection:
  - Processing 7-10 L of blood

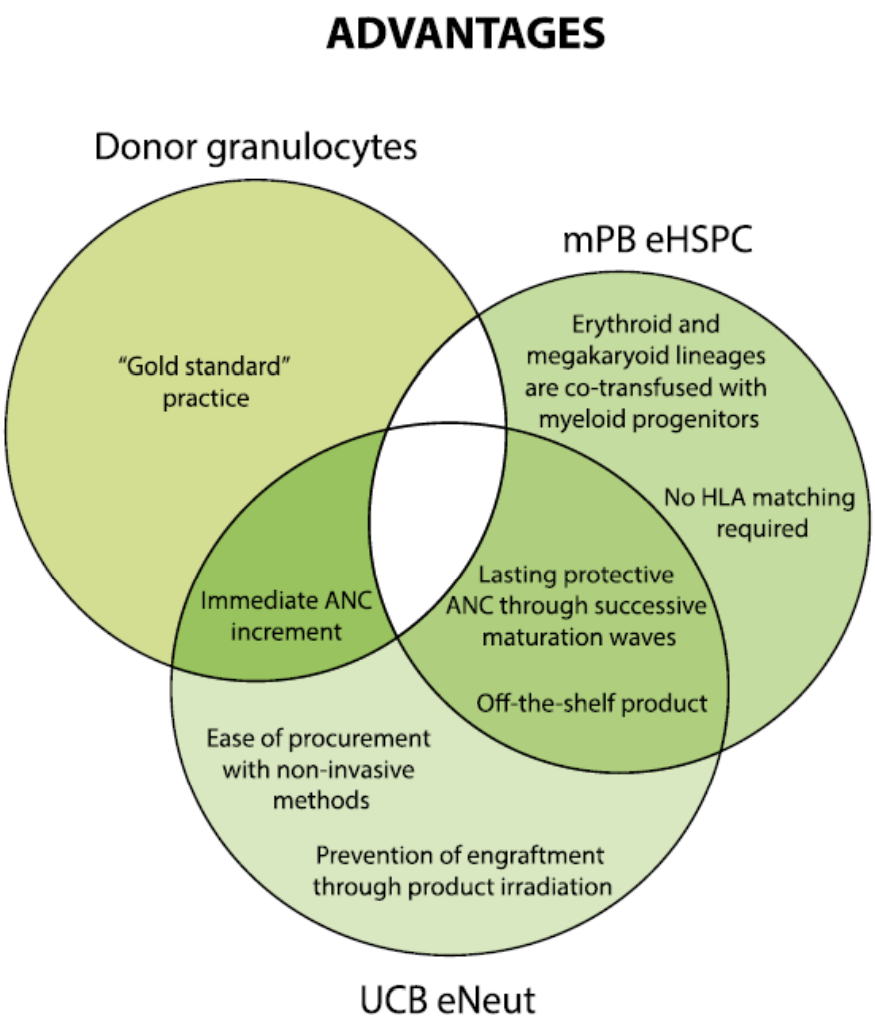
‘Next-generation’ granulocyte transfusion

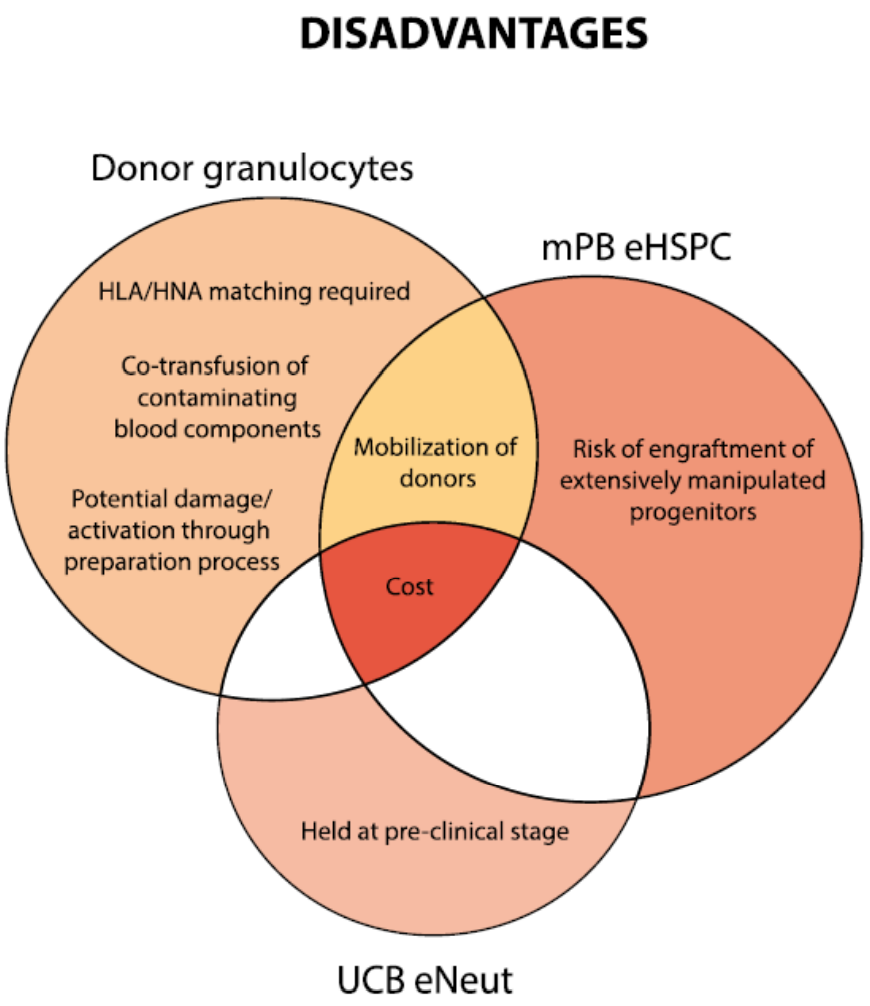


eHSPC: ex vivo-expanded hematopoietic stem and progenitor cell
eNeut: ex vivo-manufactured neutrophils

Brunck & Nielsen. Stem Cells Transl Med 2014;3:541-8

‘Next-generation’ granulocyte transfusion





Brunck & Nielsen. Stem Cells Transl Med 2014;3:541-8

Summary

- Granulocyte transfusion is indicated for patients with reversible neutropenia complicated by severe bacterial or fungal infections that are refractory to anti-microbial therapy
  - Especially when high-dose granulocyte transfusion can be achieved (> 10-40 x 10^9 cells per transfusion)

- Collection of granulocyte products:
  - Should try whatever works to improve the yield and assure adequate dose for transfusion
  - Donors should be informed about possible risks