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BHS Educational Course on Transfusion and Cell Therapy

Hematopoietic stem cell mobilization and collection



Daan Dierickx – UZ Leuven
BHS Educational Course – Seminar 5
Hof Ter Musschen, 12th February 2022

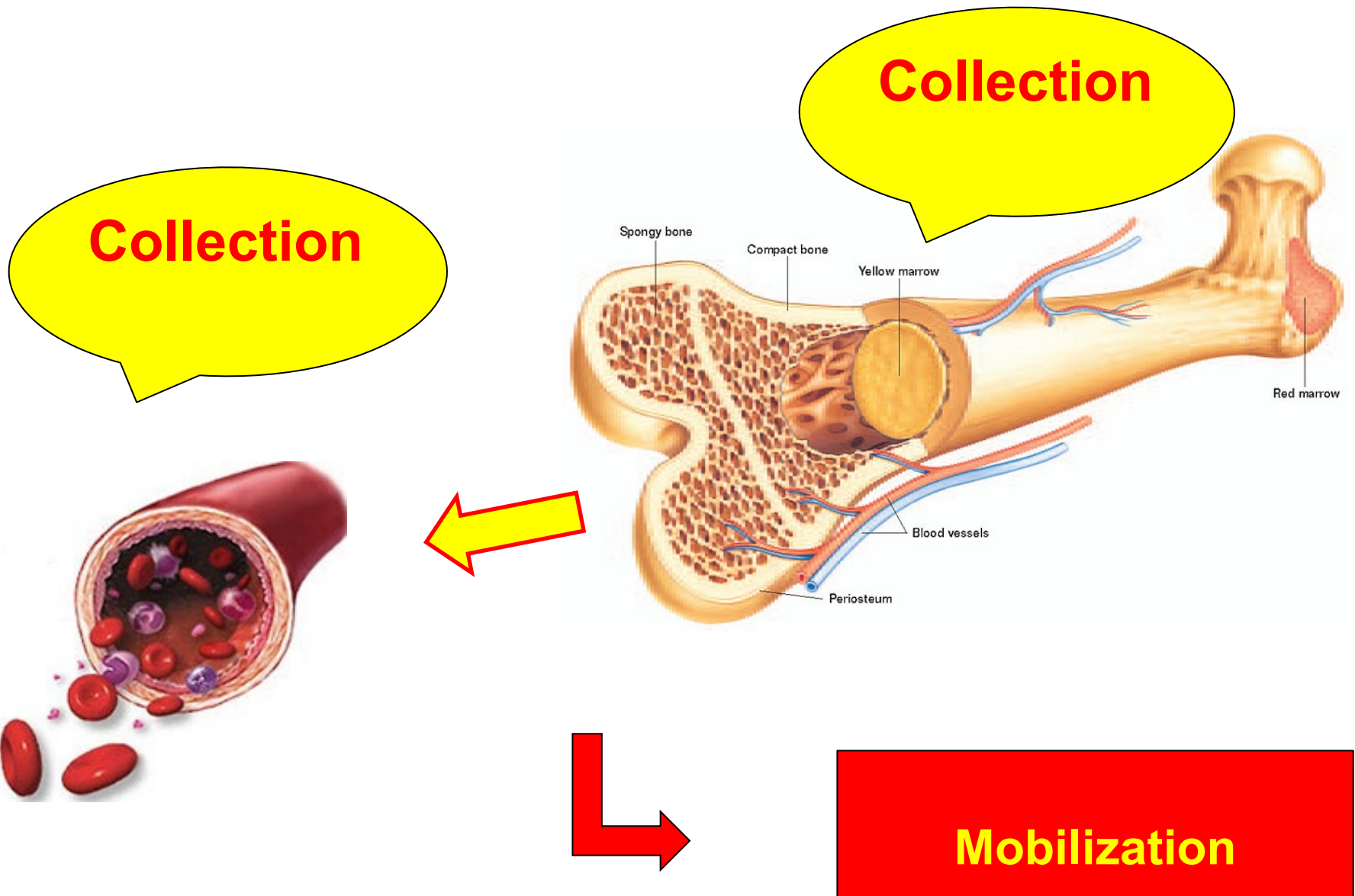
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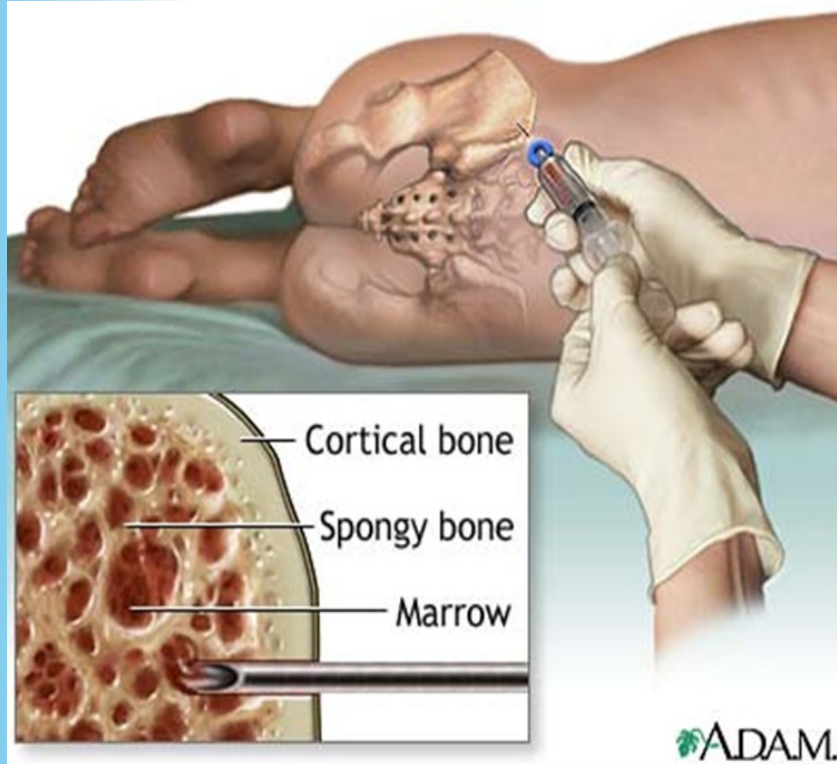
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Introduction

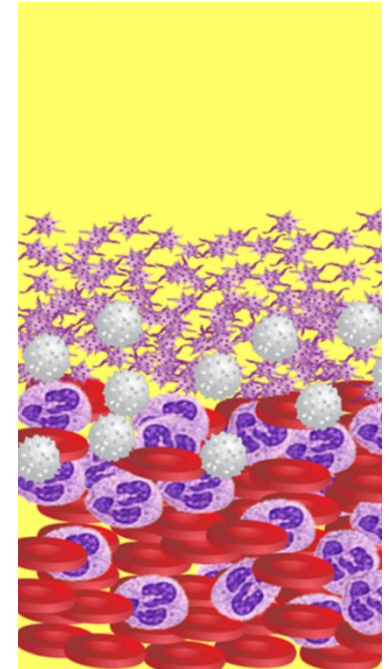


Introduction

Bone marrow stem cells (HPC-M)



Peripheral blood stem cells (HPC-A)



Introduction

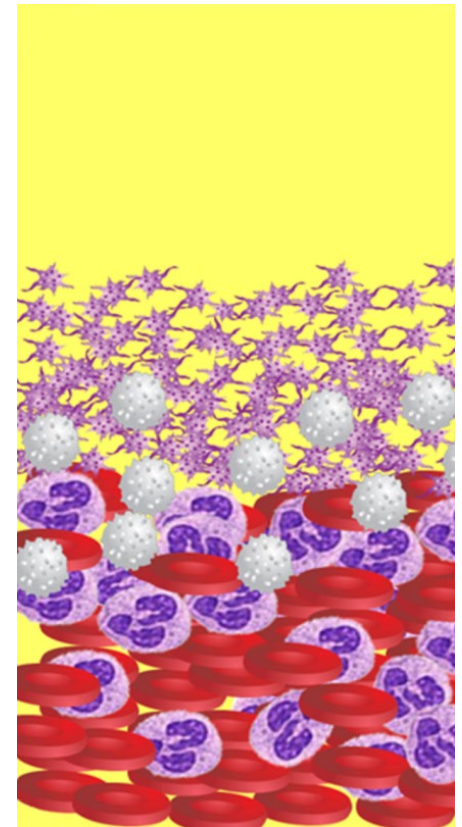
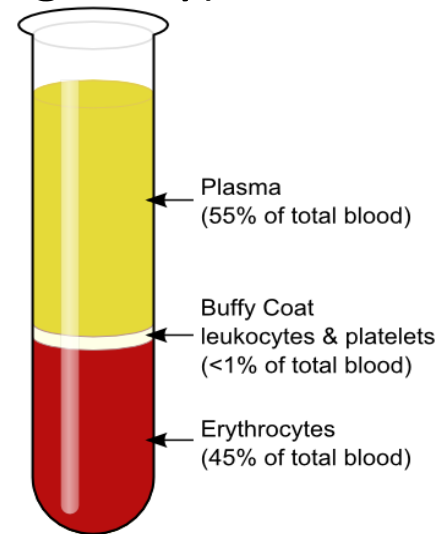
- Apheresis
- **Extracorporeal system:** whole blood from patient/donor → device: specific component separated → remaining components back to patient/donor
- **Aim:**
 - Removal of harmful component (removal, exchange, depletion)
 - Collection of desired component (collection)



Introduction

- Separation of blood components based on density
- Whole blood: different layers: (specific gravity)

– Plasma	1.025-1.029
– Platelets	1.040
– Lymphocytes	1.050-1.061
– Monocytes	1.065-1.070
– Granulocytes	1.087-1.092
– Erythrocytes	1.093-1.096




Introduction

Substance / Need for substitution	Plasma (plasmapheresis)	Cell (cytapheresis)
Yes	Plasma exchange	Cellular exchange
No	Plasma removal	Peripheral stem cell / T cell collection

Introduction

Flashback 1997: collection of hematopoietic progenitor cells by peripheral blood apheresis after stimulation with granulocyte-colony-stimulating factor

Willy A. Flegel , Karen M. Byrne, and Harvey G. Klein



Here's a video I mentioned to you about peripheral blood stem cell collection.

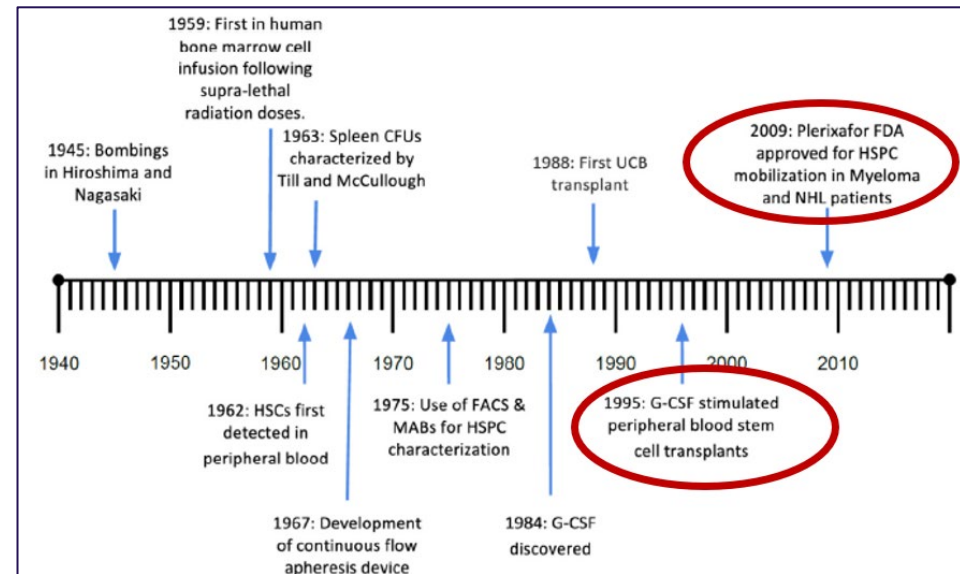
Mire, este es el video que yo le había comentado, que le explica el procedimiento de la recaudación



Efficacy



Safety



Introduction

HPC-A: replaced HPC-M for most indications (adults)

Mobilization necessary:

Chemotherapy
Chemotherapy + G(M)-CSF
G(M)-CSF steady state
Association of plerixafor (Mozobil®)

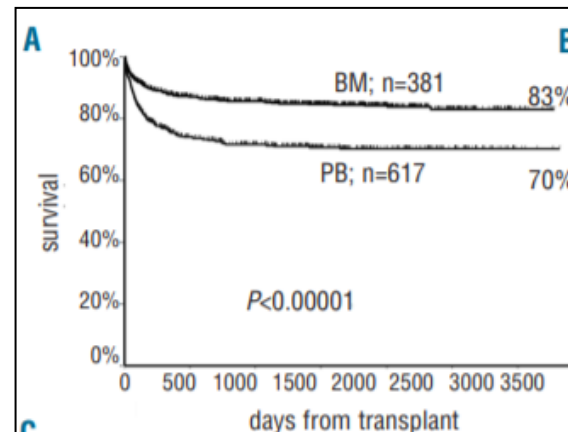
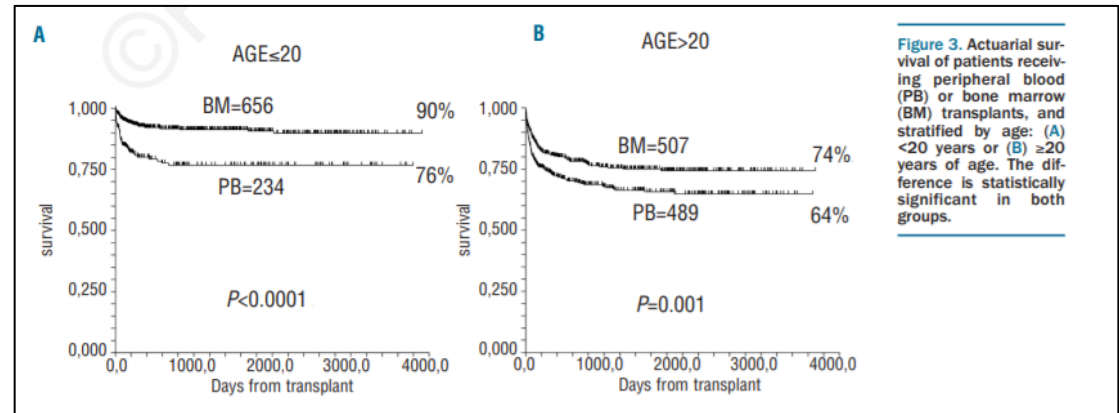
Advantages:

No anesthesia needed
Less painful
Faster engraftment

Introduction

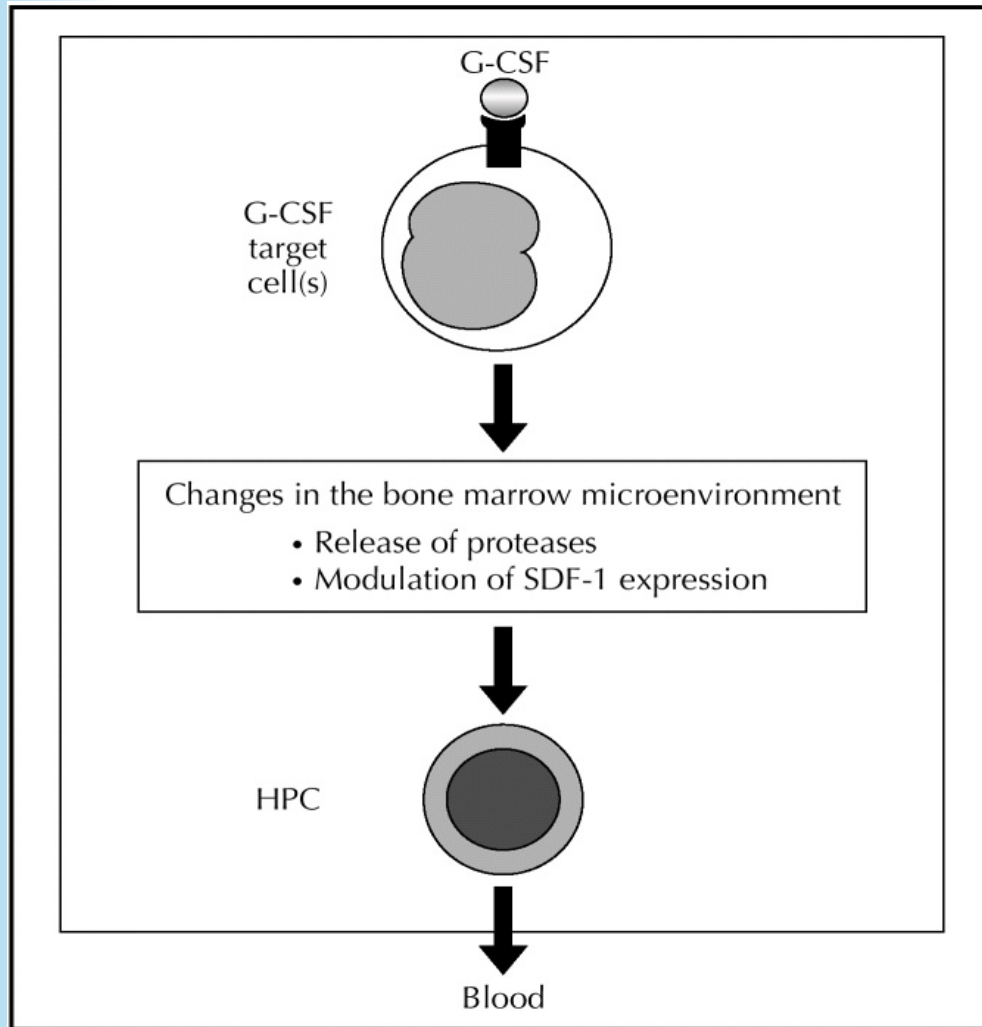
More CD34+ cells
Faster engraftment

More T-cells
More cGvHD

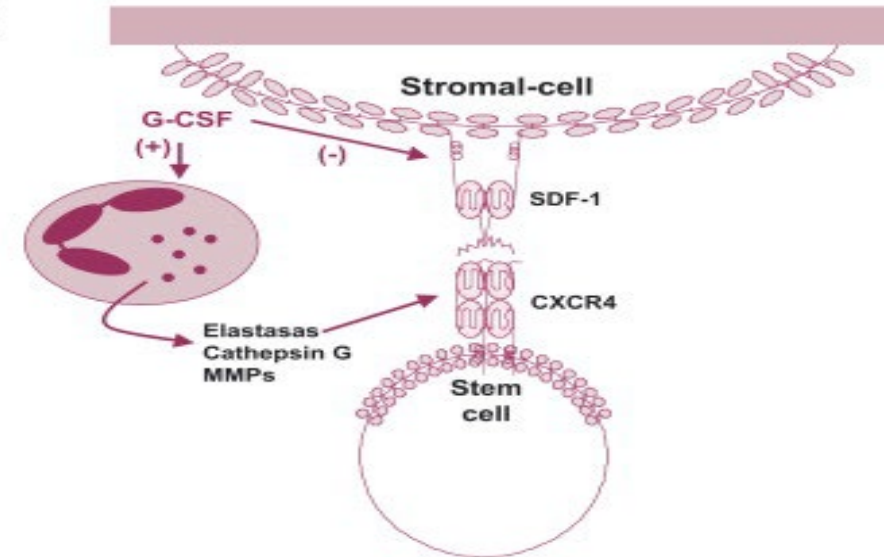


Aplastic anemia

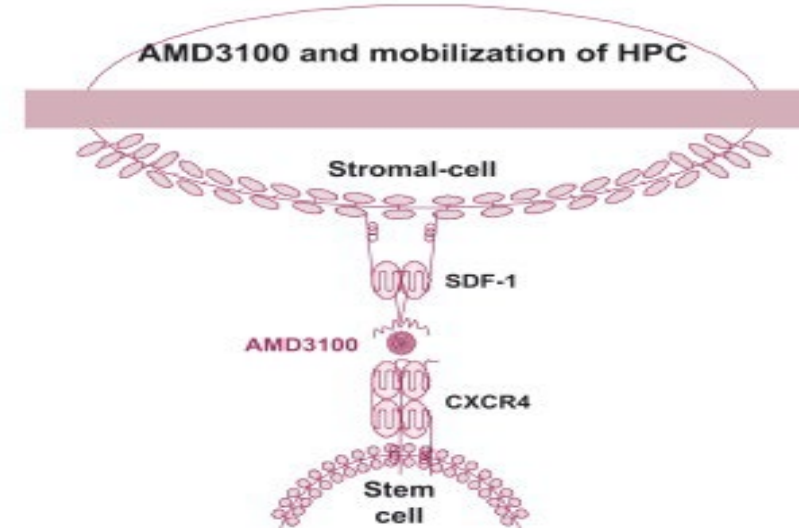
HPC-A



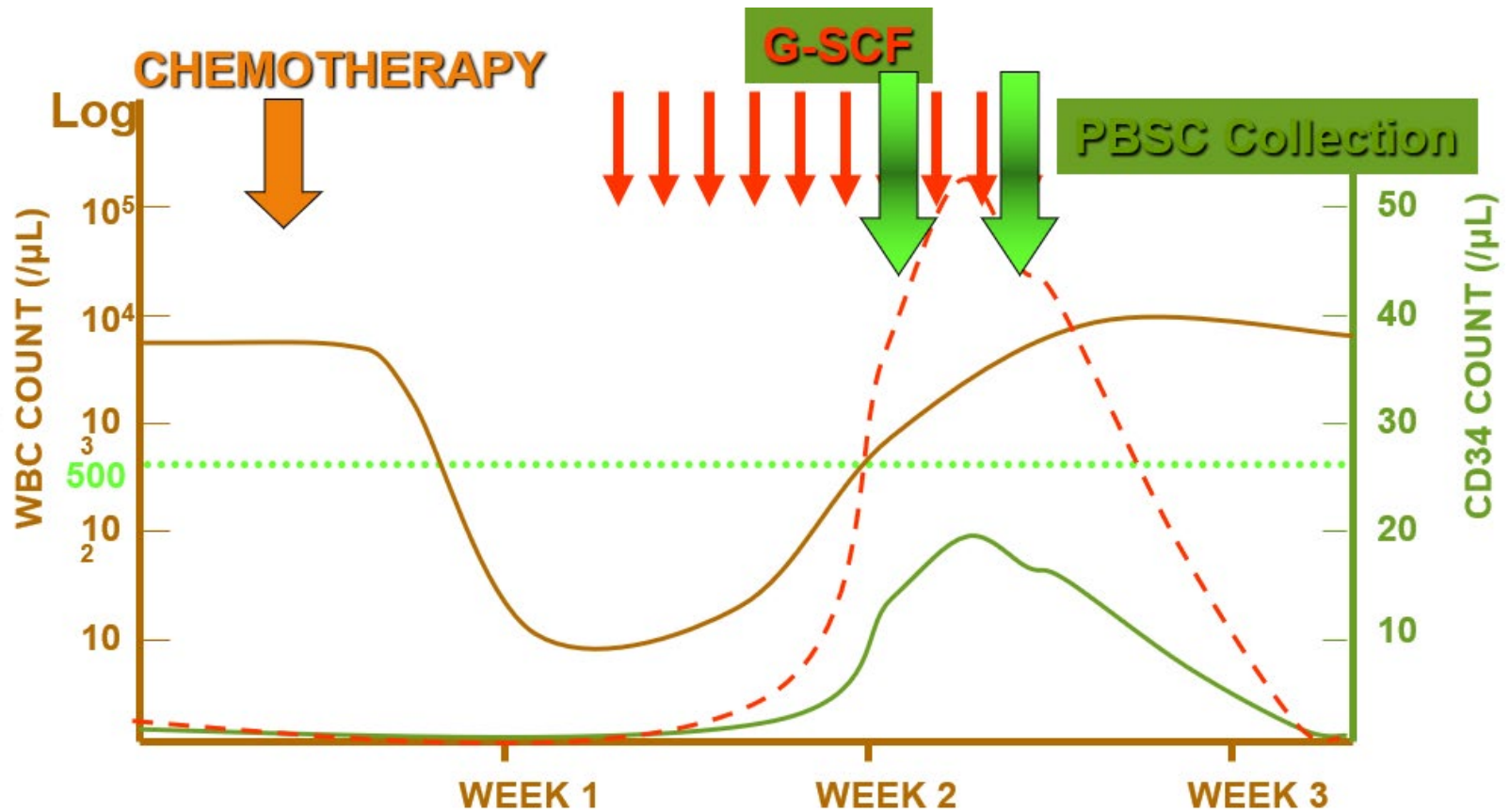
(a)



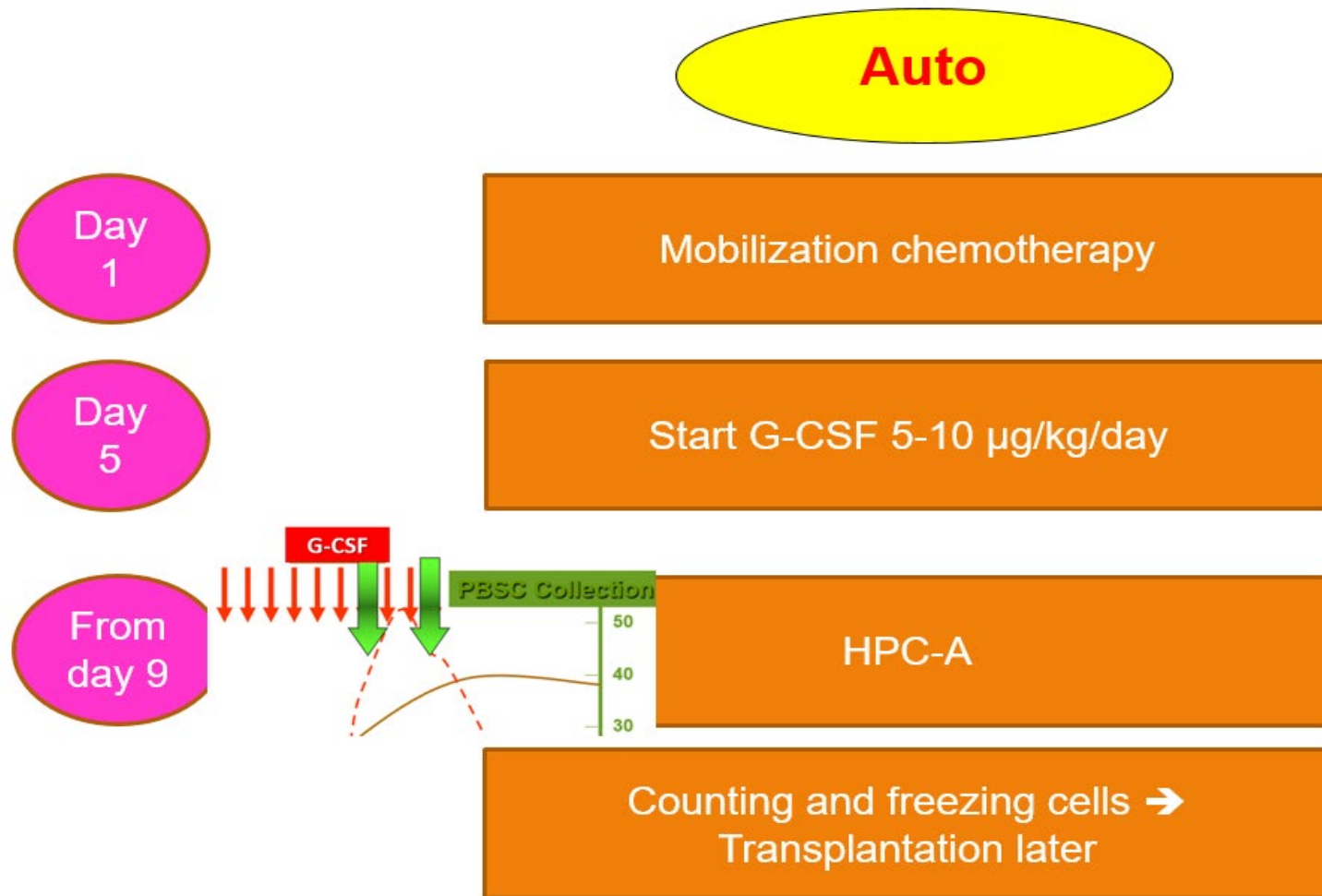
(b)



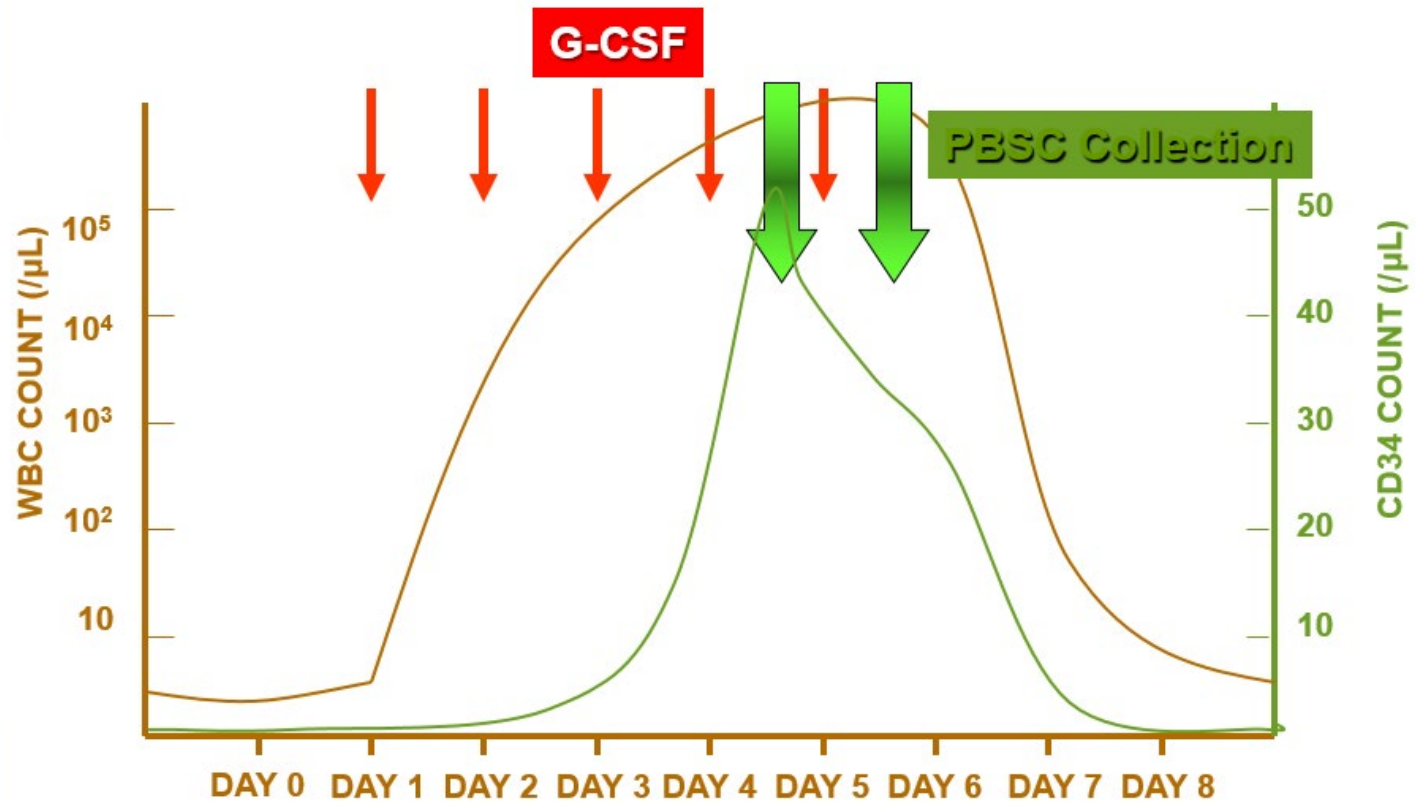
HPC-A: chemotherapy + G-CSF



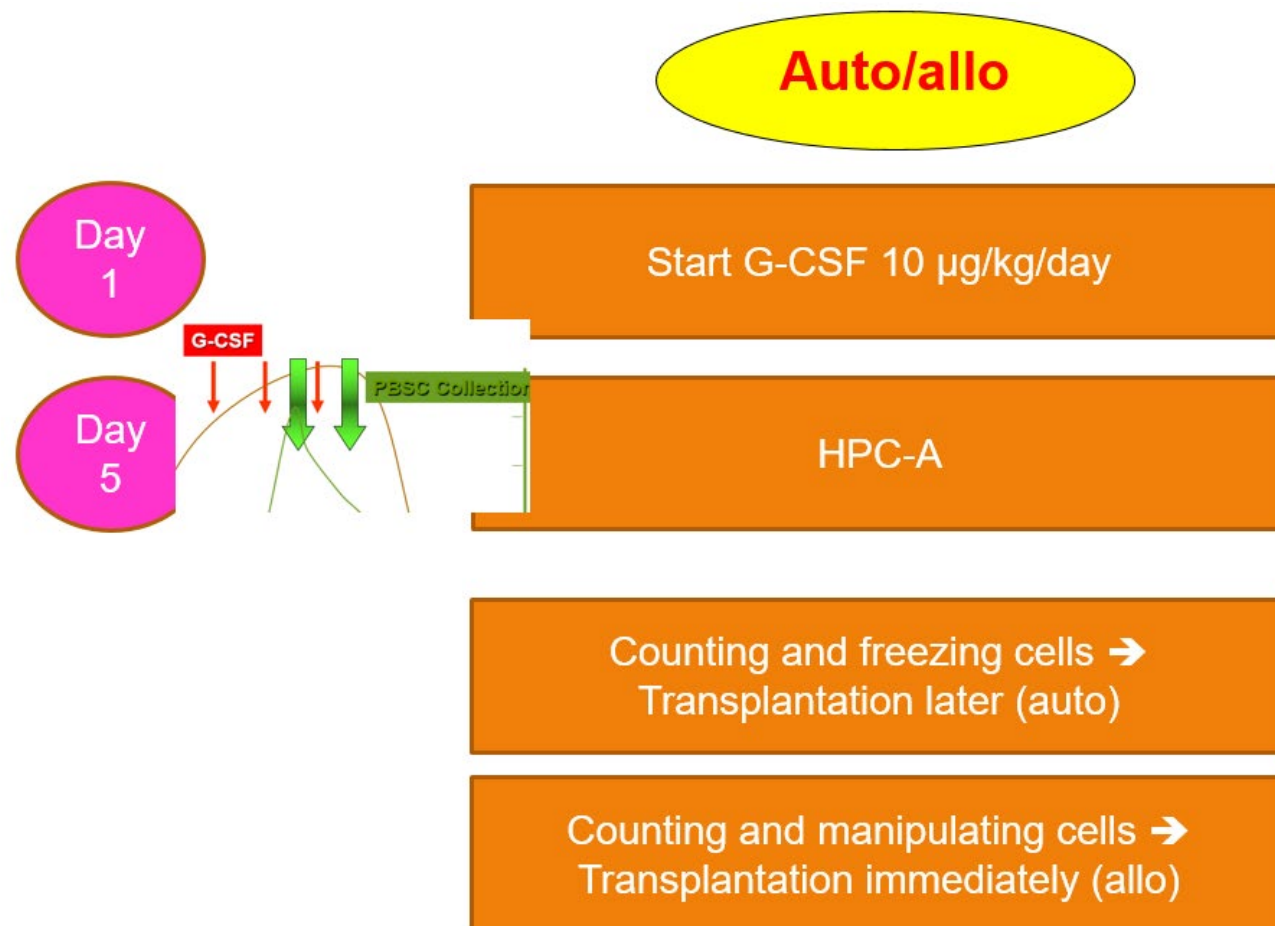
HPC-A: chemotherapy + G-CSF



HPC-A: G-CSF only



HPC-A: G-CSF only



HPC-A

- **Timing collection + estimation yield:** based on
 - WBC count
 - CD34+ cell count
 - ➔ CD34+: more predictive (Yu J, et al. *Transfusion* 1999;39:442-50; Tohoku J, et al. *J Exp Med* 2003;199:111-8)

- **Start collection** if CD34-count > 10/μL (literature: 5-20)

- **Prediction:**
 - **Yield:**
 - Yield (x 10 CD34+cells/kg) = (CD34-count pre-apheresis / 10) if 4x TBV
 - **Volume needed to process:**
 - Amount of blood to be processed =

$$\text{Target CD34+ cells} \times \frac{\text{weight patiënt (kg)}}{(\text{CD34+cells}/\mu\text{L} \times \text{collection efficiency})}$$

HPC-A

- **Yield:** dependent from
 - Number of progenitor cells in peripheral blood
 - Collection efficiency of device
 - Blood volume needed to be processed
 - Other factors
- **Blood volume to be processed:**
 - Conventional: 1-2 x TBV or procedure over 4 hours
 - Large volume leucapheresis (LVL): 3-5 x TBV
 - ➔ prospective trial: 1x 25L vs 2 x 15L ➔ similar yield

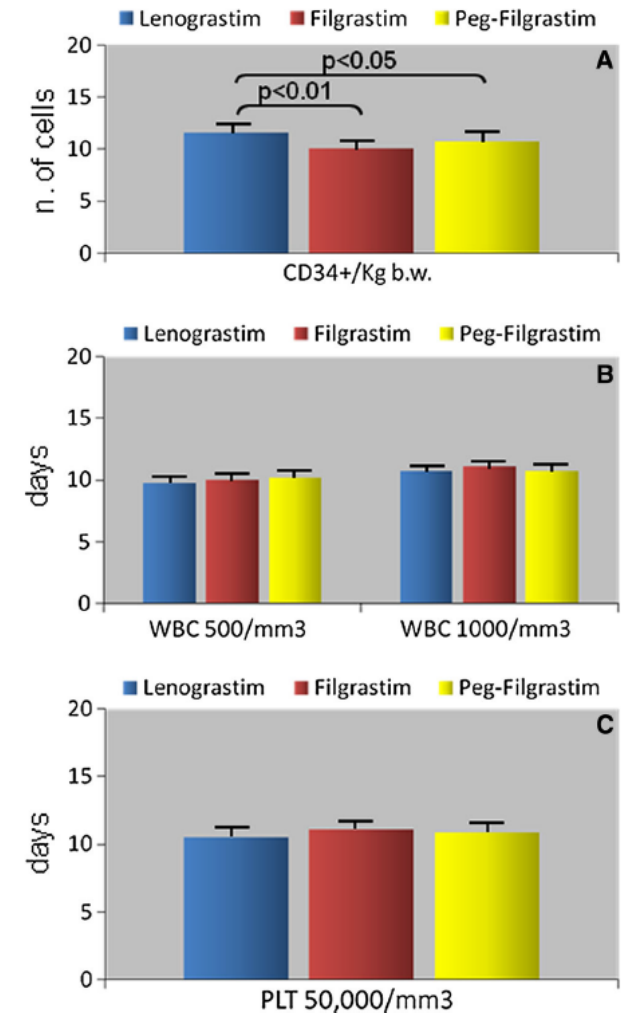
(Bolan CD, et al. Br J Haematol 2003;120:801-7)

HPC-A

- (First) apheresis:
 - Day 5 following start G-CSF monotherapy
 - Day 9-14 following last dose of chemotherapy if chemotherapy + G-CSF
- Target:
 - $> 2-3 \times 10^6$ CD34+ cells/kg if back up or 1 ASCT planned
 - $> 5-6 \times 10^6$ CD34+ cells/kg if tandem ASCT planned
 - $> 5-10 \times 10^6$ CD34+ cells/kg if alloTx (dependent from graft manipulation)
 - $> 10 \times 10^6$ CD34+ cells/kg if haplo-identical alloTx with T cell depletion
- CAVE loss of red blood cells and platelets (20-30%, LVL up to 50%)

HPC-A

- Differences between subtypes of G-CSF?
 - Glycosylated = lenograstim
 - Non-glycosylated = filgrastim
 - Pegylated = pegfilgrastim
- Lenograstim > filgrastim



Höglund M, et al. *Eur J Haematol* 1997;59:177-83

Watts MJ, et al. *Br J Haematol* 1997;98:474-9

Fisher JC, et al. *Br J Haematol* 2005;130:740-6

Ings SJ, et al. *Br J Haematol* 2006;134:217-25

Ria R, et al. *Clin Exp Med* 2015;15:145-50

HPC-A

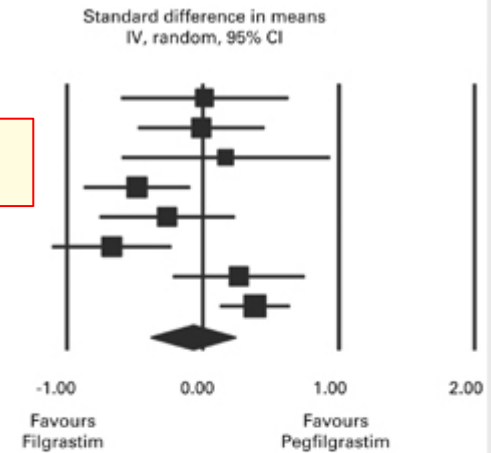
- Pegfilgrastim *versus* filgrastim

- Biosimilars

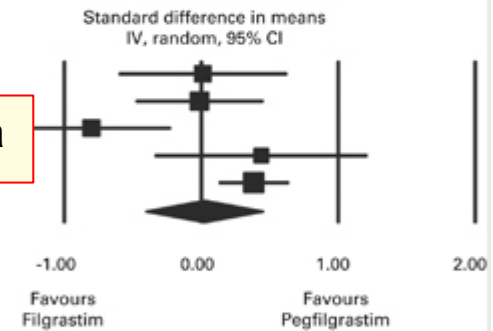
Brand name Europe	Marketing company	Manufacturing company
Ratiograstim	Ratiopharm GmbH, Germany	SICOR Biotech UAB, Vilnius, Lithuania
Tevagrastim	Teva GmbH, Germany	SICOR Biotech UAB, Vilnius, Lithuania
Zarzio	Sandoz GmbH, Austria	Sandoz GmbH, Kundl, Austria
Filgrastim Hexal	Hexal AG, Germany	Sandoz GmbH, Kundl, Austria
Nivestim	Hospira UK Ltd, United Kingdom	Hospira Zagreb d.o.o., Croatia
Grastofil	Apotex Europe BV, Netherlands	Intas Biopharmaceuticals Ltd, Ahmedabad, India
Accofil	Accord Healthcare Ltd, United Kingdom	Intas Biopharmaceuticals Ltd, Ahmedabad, India

the reference product Neupogen. Mobilisation of CD34 + cells as well as reported adverse events are also found to be comparable, although there is still a lack of long-term follow up for both Neupogen and filgrastim biosimilars. No evidence is found of a higher risk of filgrastim antibody formation using filgrastim biosimilars. Based on this increased experience, the WMDA therefore recommend that Stem Cell Donor Registries can use filgrastim biosimilars for the mobilisation of peripheral blood progenitor cells in healthy donors, provided that they are approved by national and/or regional agencies.

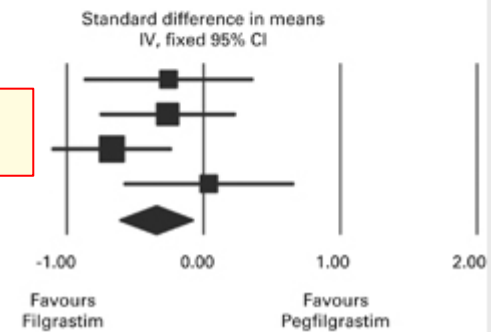
Total



Lymphoma



MM



HPC-A

- Once-dialy versus twice-daily G-CSF??

Table I. Collection and immunophenotyping data.

Variable	Group A (n = 40) 6 × 2/d	Group B (n = 41) 12 × 1/d	P-value
Donor TBV (l)	4.6 ± 1.3	4.9 ± 0.9	0.32
No. of TBVs processed	2.8 ± 0.2	2.8 ± 0.3	0.66
CD34 ⁺ (× 10 ⁶ cells/kg recipient)*	5.6 ± 3.3	5.6 ± 4.3	0.94
CD34 ⁺ (× 10 ⁶ cells/l blood processed)*	30 ± 17.2	30.4 ± 19.5	0.92

- Timing of collection

TABLE 4 Expected day of first PBSC collection depending on mobilizing chemotherapy regime. This Table was developed following audit of historical data from 3 large UK centers, and is now in routine use at several centers in the UK

Chemotherapy regime	Recommended weekday to start chemo	Anticipated first apheresis day	Anticipated weekday for first apheresis
CHOP	Friday	10	Monday
Cyclo 1.5 or 2 g/m ²	Monday	8–9	Tuesday or Wednesday
Cyclo 3 or 4 g/m ²	Friday	10–11	Monday or Tuesday
Cytarabine 4–6 g/m ² (Nordic protocol for mantle cell lymphoma)	Thursday	12–14	Tuesday to Thursday
DHAP	Wednesday	13	Tuesday
ESHAP	Wednesday	13	Tuesday
IVE	Wednesday	13	Tuesday
GDP	Tuesday or Wednesday	13	Monday or Tuesday
MATRIX	Tuesday	13	Monday
Etoposide 2.4 g/m ²	Tuesday or Wednesday	14	Tuesday or Wednesday
IVAC	Tuesday or Wednesday	14	Tuesday or Wednesday
TIP	Friday	11	Tuesday
VIDE	Tuesday	13–14	Monday or Tuesday

“Poor mobilizers”

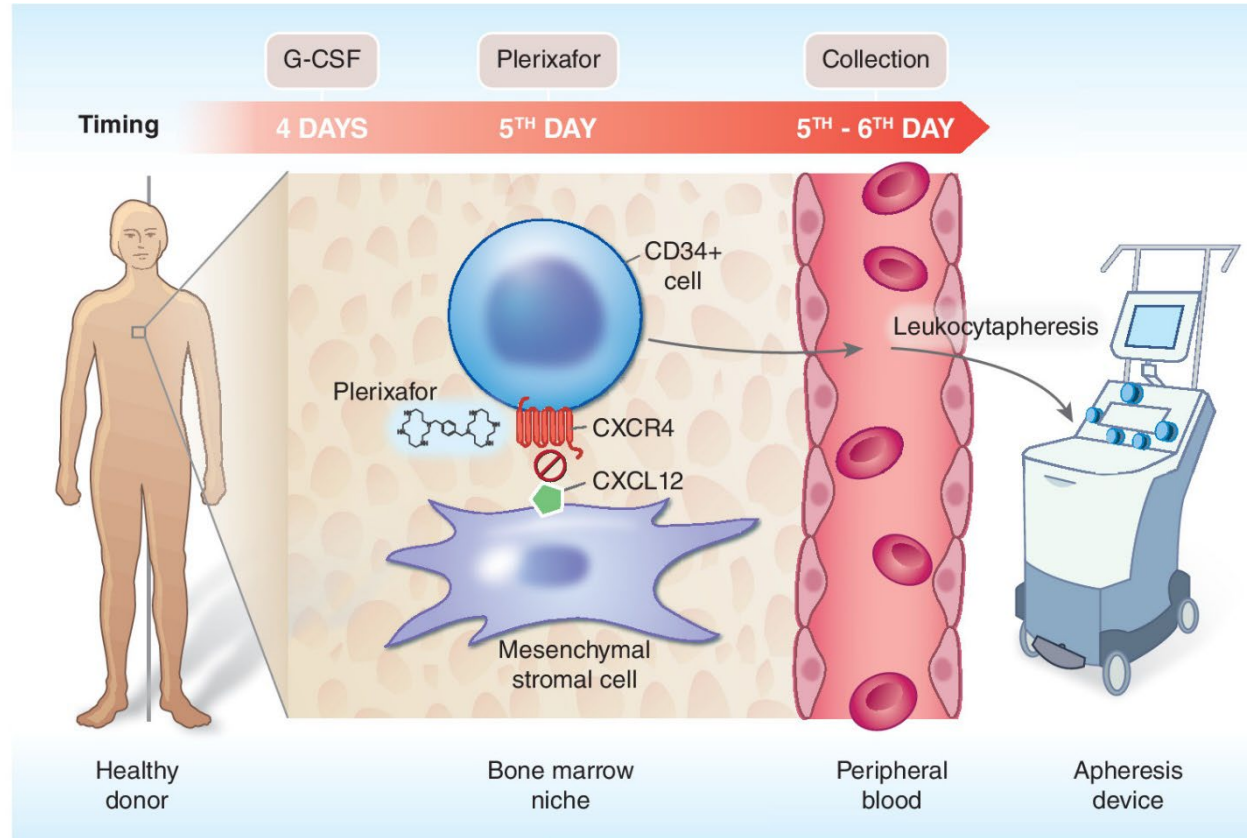
Risk Factors Associated with Poor Mobilization

Baseline	At Time of Mobilization
<p>Treatment-related</p> <ul style="list-style-type: none"> • Numerous cycles of previous chemotherapy • Previous exposure to melphalan, fludarabine, platinum-containing regimens, alkylating agents, or lenalidomide • Previous radiation therapy to the bone marrow <p>Patient-related</p> <ul style="list-style-type: none"> • Advanced age • Diagnosis of NHL • Diabetes <p>Bone marrow–related</p> <ul style="list-style-type: none"> • Bone marrow involvement • Thrombocytopenia 	<p>Low steady-state PB CD34⁺ cell count</p> <p>Steady-state thrombocytopenia</p> <p>Low preapheresis PB CD34⁺ cell count</p> <p>Low day 1 apheresis yield</p>

Daratumumab

(CASSIOPEIA: VTD vs D-VTD:
8% vs 22% plerixafor need)

“Poor mobilizers”



“Poor mobilizers”: chemotherapy + G-CSF

Auto

Day
1

Mobilization chemotherapy

Day
5

Start G-CSF 5-10 $\mu\text{g}/\text{kg}/\text{day}$

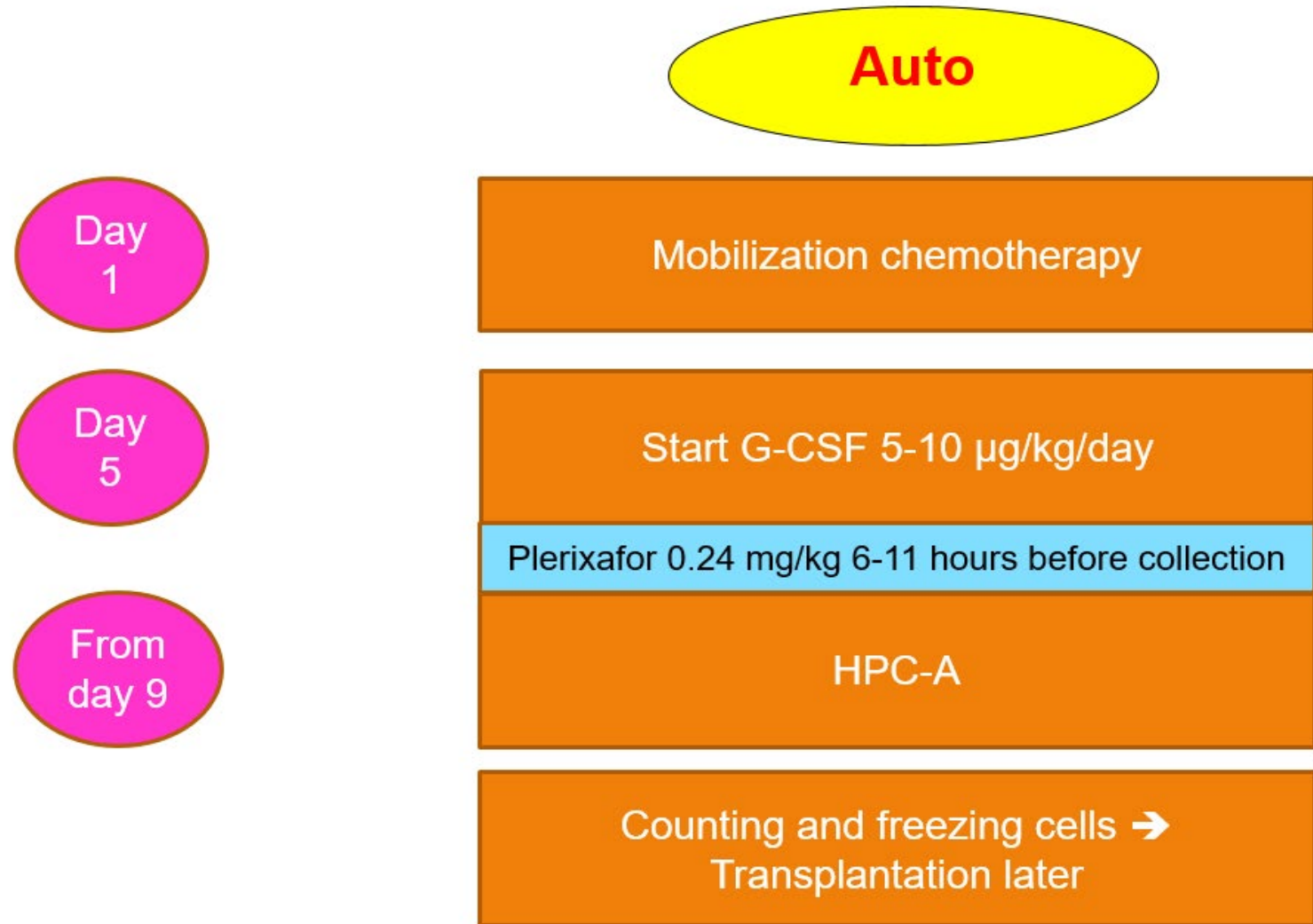
Plerixafor 0.24 mg/kg 6-11 hours before collection

From
day 9

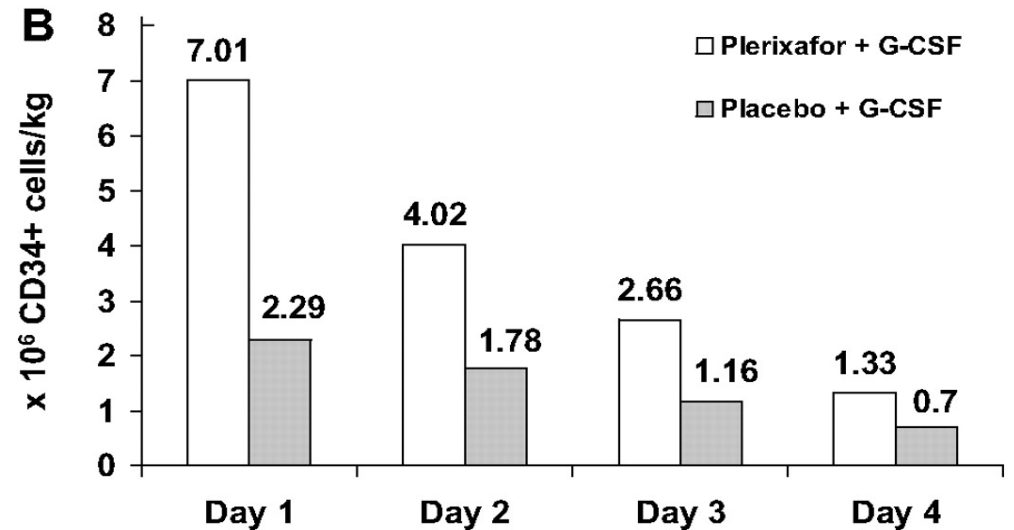
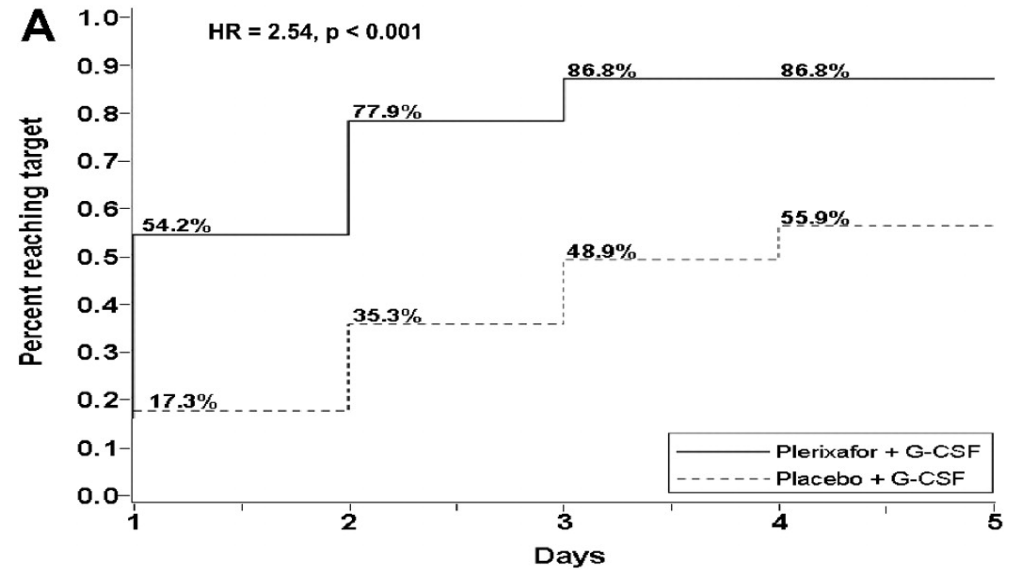
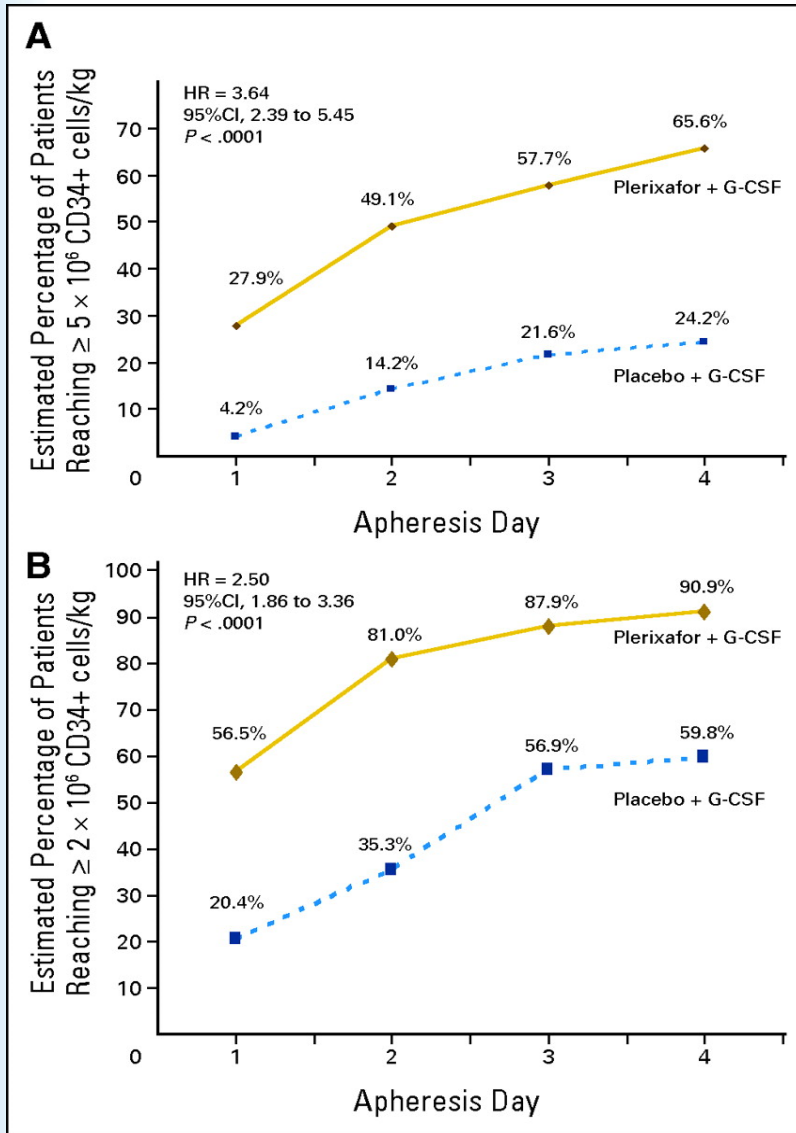
HPC-A

Counting and freezing cells →
Transplantation later

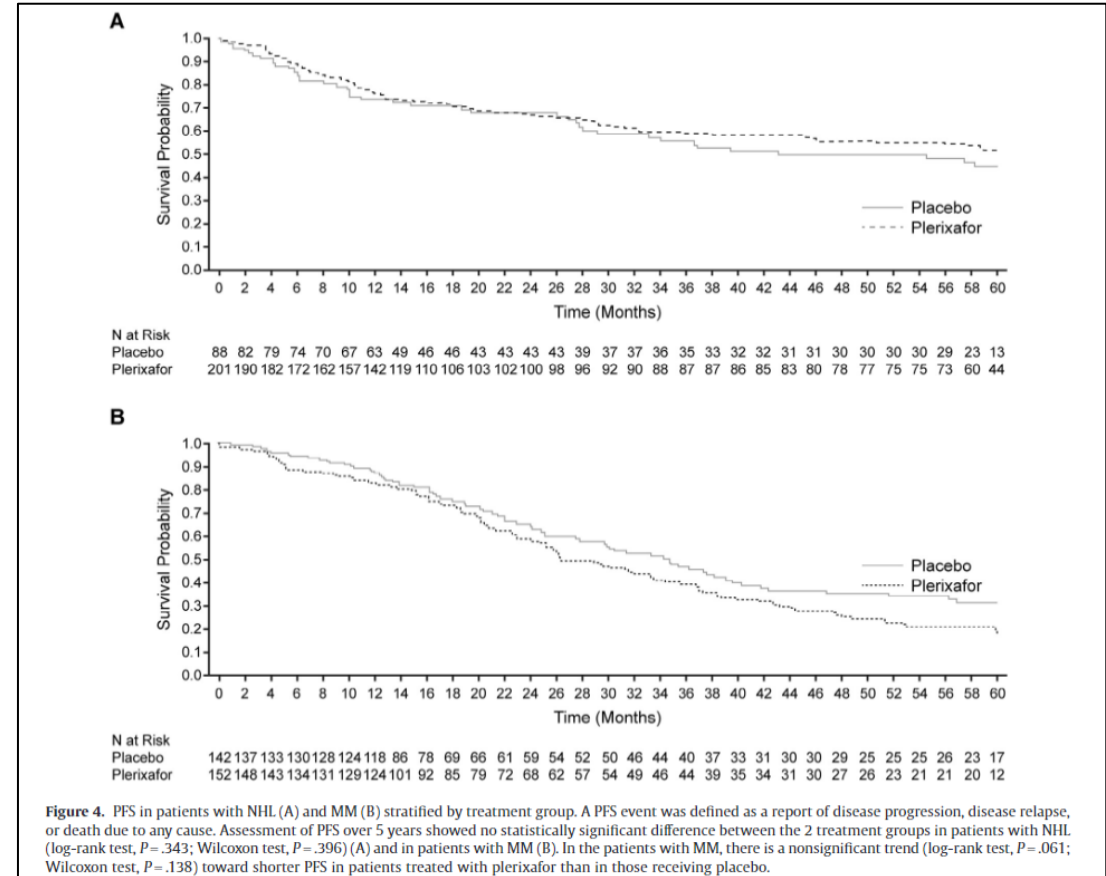
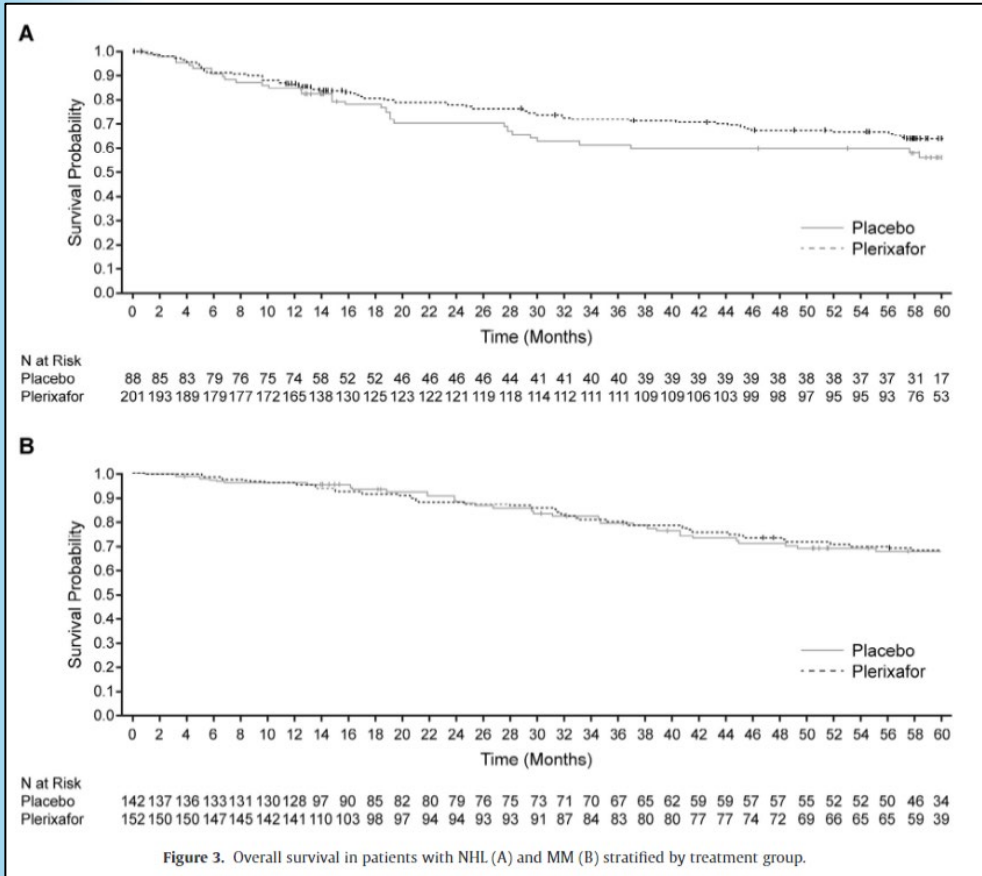
“Poor mobilizers”: G-CSF only



“Poor mobilizers”



“Poor mobilizers”



“Poor mobilizers”

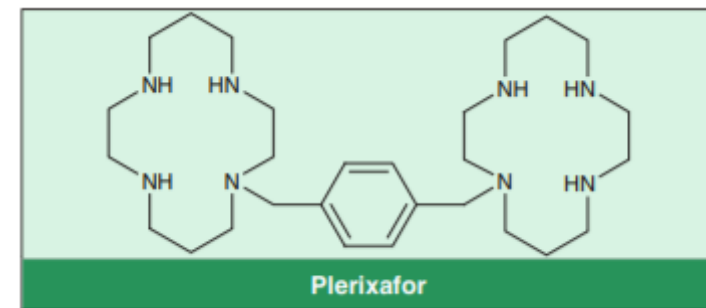
Table 2. Summary of mobilization strategies and outcomes

<i>Author, year</i>	<i>Strategy</i>			<i>>2 × 10⁶ CD34+ cells/kg, %</i>	<i>HSCT, %</i>
	<i>Upfront, %</i>	<i>Preemptive, %</i>	<i>Salvage, %</i>		
Arcaini, 2011 ²⁵	0	P+S	P+S	37	17
Attolico, 2012 ⁴⁰	0	32	68	73	65
Basak, 2011 ²³	0	30	66	78	65
Basak, 2011 ⁴¹	0	84	16	66	56
Calandra, 2008 ³¹	0	P+S	P+S	66	76
D’Addio, 2011 ²⁶	0	100	0	100	39
Douglas, 2012 ²⁴	0	19	81	95	71
Duarte, 2011 ⁴²	0	4	96	75	63
Hubel, 2011 ²⁸	0	0	100	75	67
Hubel, 2012 ²⁹	0	0	100	74	NA
Selleslag, 2011 ³⁰	0	0	100	64	59
Shaughnessy, 2013 ³⁹	98	0	0	92	87
Worel, 2011 ³²	0	P+S	P+S	63	48

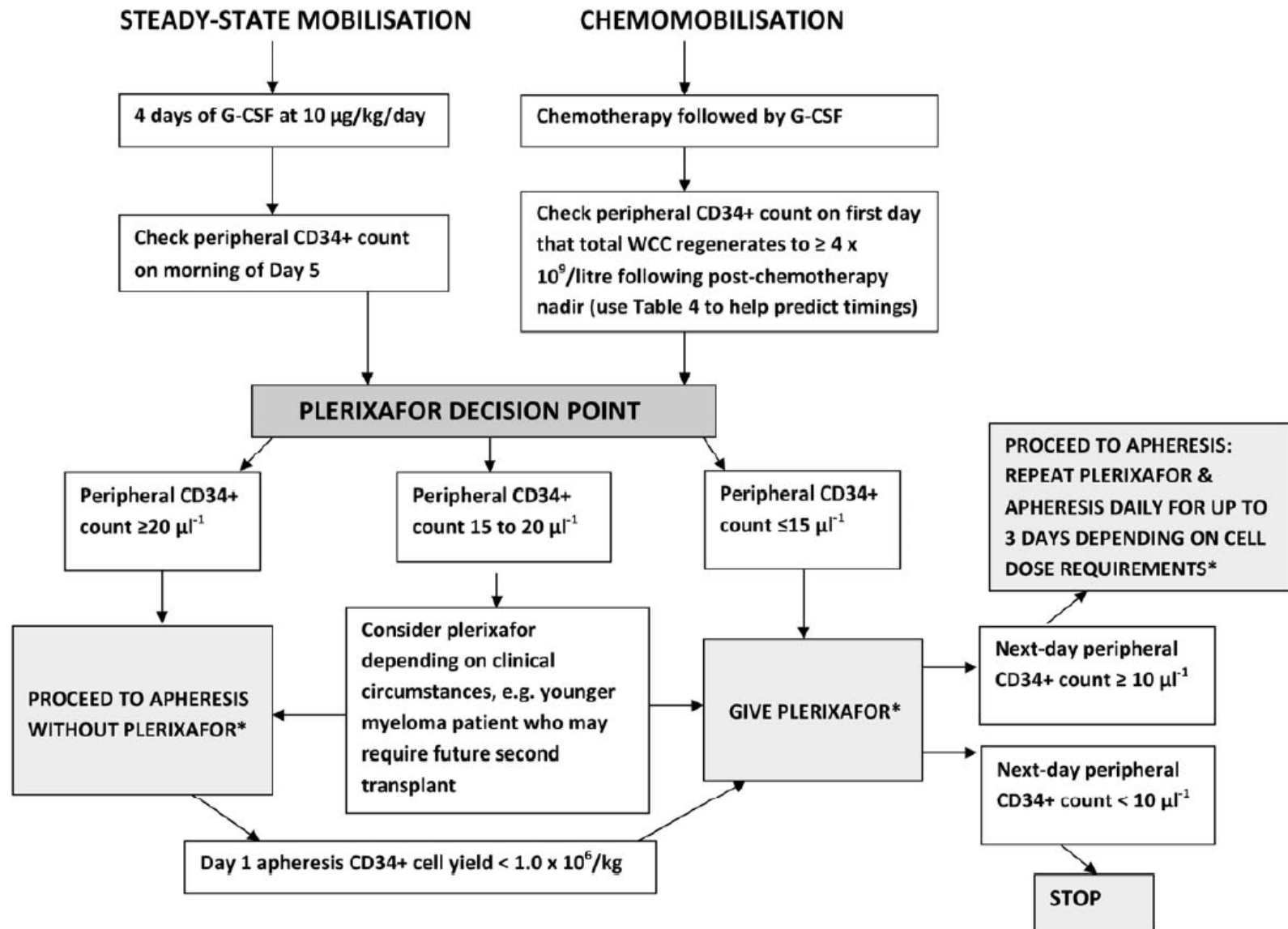
Abbreviations: HSCT = hematopoietic SCT; NA = not available; P+S = preemptive and salvage strategies used although proportions not stated.

Plerixafor

- **Dose:** 0.24 mg/kg/day (renal insufficiency: 0.16 mg/kg/day) 6-11 hours before collection (real life: one vial 20 mg)
- **Reimbursement:** only for MM and lymphoma!
 - < 10-15 CD34+ cells/ μ L blood after mobilization
 - OR**
 - < 2×10^6 CD34+ cells/kg following collection
- **Side effects:**
 - Local injection reaction
 - Headache
 - Gastrointestinal complaints



Plerixafor



* G-CSF should also be continued daily.

Plerixafor

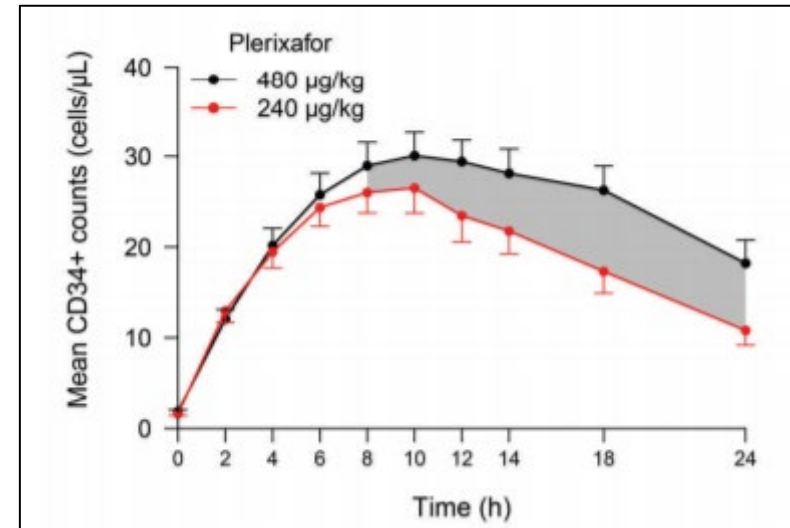
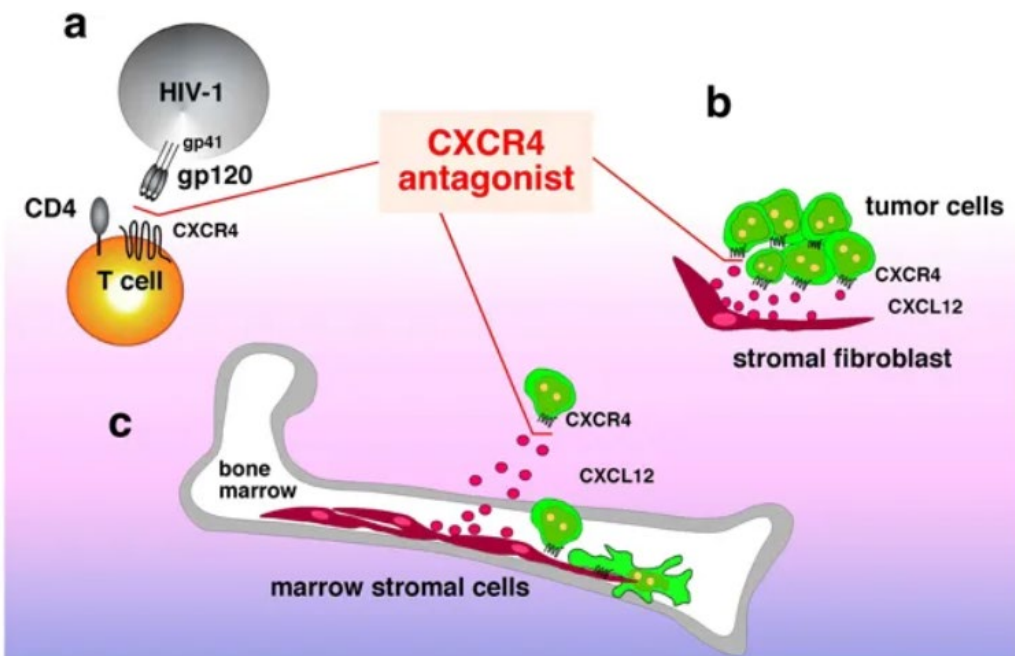
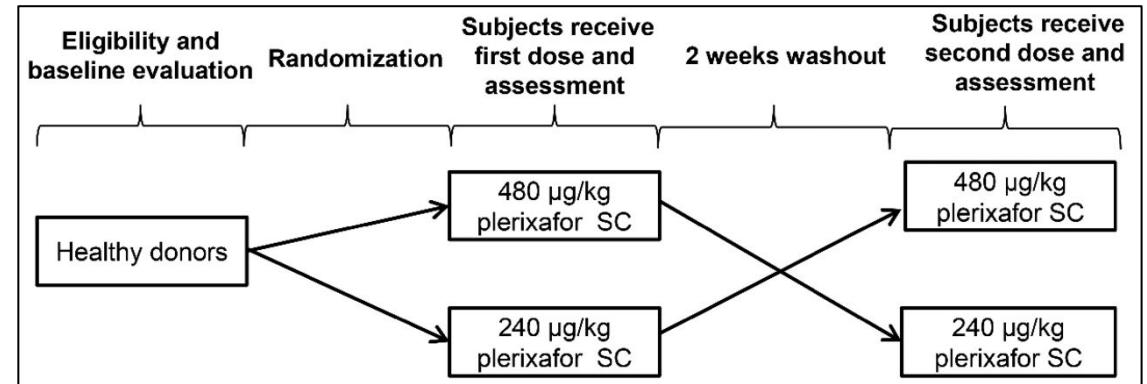
First author [ref]	Healthy related donors		Mobilization strategy		CD34+ cells in peripheral blood				CD34+ cells collected ($\times 10^6$ /kg recipient)			
	n	Gender (M/F)	Age (years)	G-CSF (μ g/kg/day)	Plerixafor (mg/kg)	After G-CSF (μ l)	After plerixafor (μ l)	Increase	Timing ^a (h)	Without plerixafor	With plerixafor	Total
Devine [22]	25	20 M/25 F	24-60	No	0.24	No	16 (4-54)	No	4	No	2.9 (1.2-6.3)	2.9 (1.2-6.3)
Hauge [23]	6	All F	44 (35-55)	10-16 (3-4 days)	0.24	19 (10-41)	96 (19-157)	3.2 (1.6-12.8)	10	1.4 (0.4-3.4)	7.0 (0.9-13.3)	10.2 (2.3-13.3)
Gattillo [24]	10	5 M/5 F	57 (37-73)	10 (4 days)	0.35 (0.24-0.47)	27 (11-34)	41 (17-147)	2.8 (1.1-4.3)	9-11	1.2 (1.1-3.9)	4.2 (1.3-8.5)	5.9 (1.8-9.5)
Schroeder [25]	50	NR ^b	NR ^b (18-70)	No	0.08-0.48 (SC and IV)	No	23 (4-157)	No	4	No	2.9 (2.0-9.7)	2.9 (2.0-9.7)
Teipel [26]	35	NR ^b	NR ^b	7.5-10 (4 days)	0.24	NR ^b	NR ^b	NR ^b	10	1.0 (0.5-1.9) ^c	2.2 (0.9-3.8) ^f	NR ^b
De Greef [27]	23	16 M/7F	47 (24-60)	No	0.32	No	26 (9-71)	No	10 (8-11)	No	3.3 (1.9-6.5)	3.9 (1.9-6.5)
Chen [28]	64	41 M/23F	56 (range: 18-65)	No	0.24	No	19 (range: 1.7-52)	No	4	No	2.8 (0.3-9.6)	4.7 (0.9-9.6)
Zubicaray [29]	9	NR ^b	3 (range: 1-17)	10 (4 days)	0.24	NR ^b	208	Yes	6-11	NR ^b	11.42 (5-17.85)	NR ^b
Hölig [30]	37	17 M/20F	34 (IQR: 26-48)	15 (4 days)	0.24	15 (IQR: 12-18)	44 (IQR: 38-61)	Yes	12	1.31 (IQR: 0.8-1.65)	2.8 (IQR: 2.26-4.69)	5.16 (IQR: 3.06-6.10)
Kharya ^d [31]	25	10 M/15F	35 (9-51)	10 (5 days)	0.24	NR ^b	NR ^b	NR ^b	10-12	NR ^b	NR ^b	10
Cid [32]	30	9 M/21F	51 (IQR: 34-59)	10-16 (IQR: 4-6 days)	0.24	17 (IQR: 11-27)	56 (IQR: 39-81)	3.3 (IQR: 2.8-4.8)	11 (IQR: 10-12)	1.6 (IQR: 0.9-2.5)	5.0 (IQR: 3.5-5.8)	6.1 (IQR: 4.8-7.3)



No significant differences:

- Time to engraftment
- aGvHD/cGvHD
- Overall survival

“Poor mobilizers”



Side effects

Short term risks

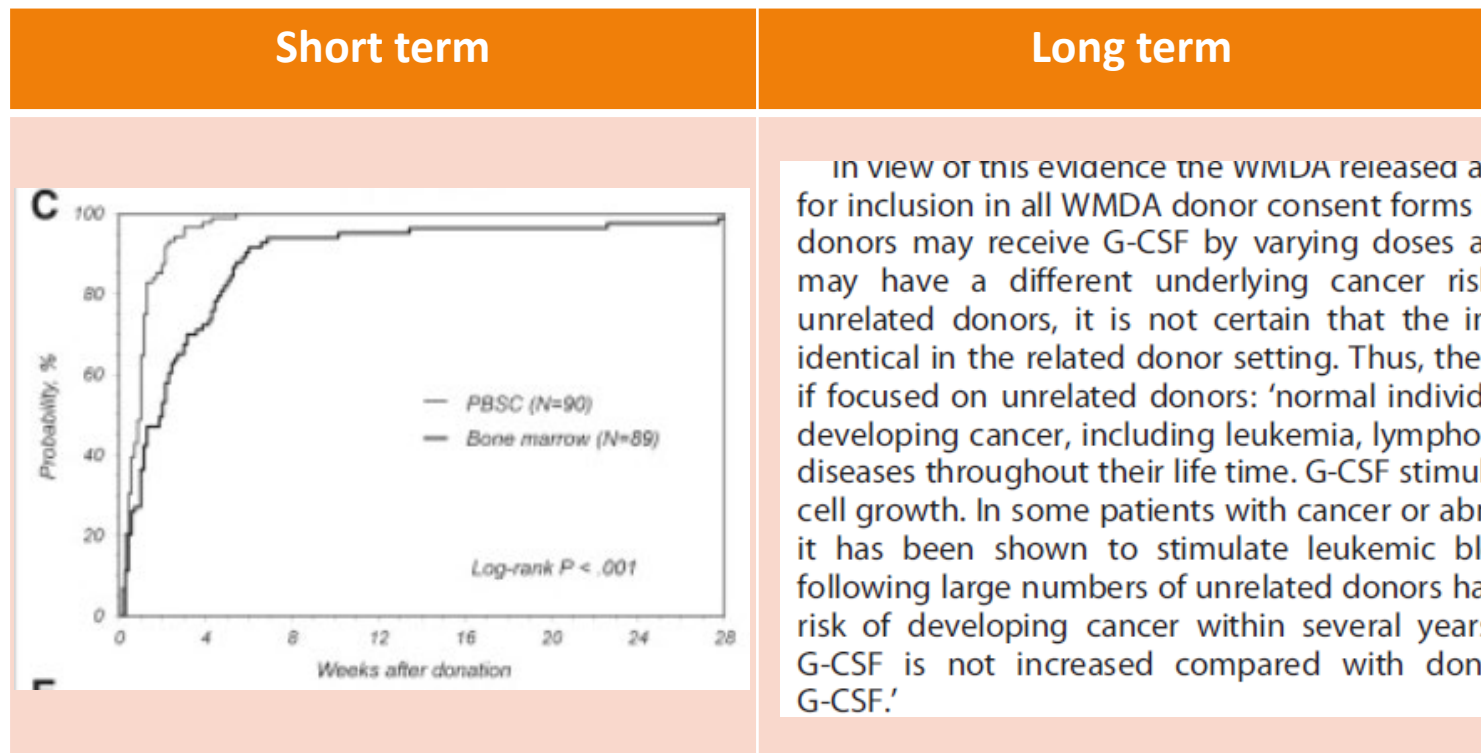
Mobilization-related	Procedure-related
Chemotherapy-related	Vasovagal reaction
G-CSF-related	Catheter-related
<i>Headache</i>	<i>Infection</i>
<i>Skeletal pain</i>	<i>Bleeding</i>
<i>Nausea</i>	<i>Thrombosis</i>
<i>Splenomegaly</i>	Citrate-induced hypocalcemia
<i>Fever</i>	
<i>Injection site reaction</i>	
Plerixafor-related	
<i>Headache</i>	
<i>Nausea & diarrhea</i>	
<i>Arthralgia</i>	
<i>Injection site reaction</i>	

Long term risks in healthy donors

Table 4. Publications reporting haematological malignancies in normal donors

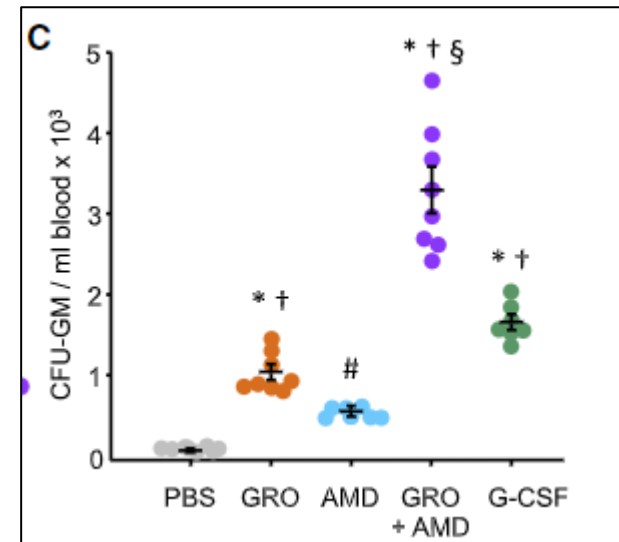
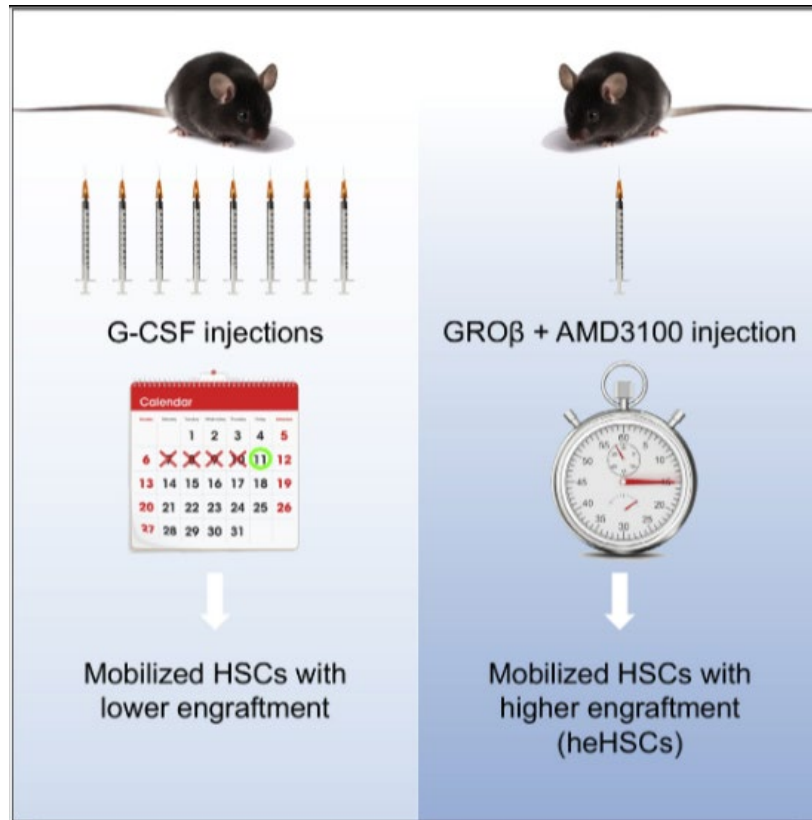
Reference	Study design	Type and number of donors	Years of donation	Median follow-up	% Survey compliance	Haematologic cancer	Comparison between stem cell source and to general population
Pulsipher <i>et al.</i> ³⁵	Prospective registration study	Unrelated, PBSC 6768 BM 2726	2004–2009	PBSC 36 m (range 1–89 m) 20570 donor years BM 36 m (1–86 m) 8642 donor years	Prospective	AML (1: PBSC) myeloma (1: PBSC) lymphoma (1: BM)	PBSC vs BM: NS
Kodera <i>et al.</i> ⁴¹	Prospective national registration study BM donor survey	Related PBSC 3114 BM 5291	2000–2010; 1991–2003		67%	AML (1)	Similar to sibling BM donors
Schmidt <i>et al.</i> ³⁷	Donor centre survey	Unrelated PBSC 8730 BM 3556 Both 273		30777 23037 1414 (Observation years)	81.3%	HD (2: PBSC)* plasmacytoma (1: PBSC) AML (1: BM) NHL (1:BM) CLL (1: both)	PBSC vs BM: NS No difference compared with normal population
Halter <i>et al.</i> ¹⁷	EBMT survey	Unrelated/ Related PBSC 23254 BM 27770	1993–2005	99 875 200 786 (Observation years)	65–78%	AML (2:BM, 1:PBSC) ALL (2: BM, 1:PBSC) NHL (4 BM, 3:PBSC) HD (1:PBSC) CLL (1:PBSC) plasmacytoma (1: PBSC) MPN (1:PBSC)	No difference compared with normal population
Hollig <i>et al.</i> ¹⁸	Single centre retrospective study	Unrelated PBSC 3928	1996–2008	Not given	Not given	AML (1) CLL (1) HD (2)*	AML/CLL NS HD significantly higher than normal population
Hollig <i>et al.</i> ³⁶		Unrelated PBSC 8290	1996–2012 (Overlaps with above study population)		67% at 4 weeks, 38% at 5 years	AML (2) CLL (1) HD (3) CML (1) ALL (1) (overlaps with above study population)	AML and HD significantly different from the natural incidence in the German population; others NS
Pulsipher 2008	Prospective registration study	Unrelated PBSC 2408	1999–2004	49 months	Prospective	CLL (1 case)	
De la Rubia <i>et al.</i> ¹⁹	National registry retrospective study	Unrelated PBSC 736	1998–2006	320 had been followed for 2 years or more	320 followed for 2 years or more	No cases	
Jeger <i>et al.</i> ⁴²	Single centre retrospective cohort study	Related BM 212 PBSC 119	1974–2001	13.8 years	92%	ALL (1:BM) CLL (1:BM)	No difference compared with normal population
Anderlini 2002	Single centre survey	Related PBSC 281	1994–1998	median time post donation 39 months	82%	No cases	
Cavallaro <i>et al.</i> ²²		Related PBSC 95	1993–1995	median time post donation 43.13 months	95%	No cases	
Wiersum-Osselton <i>et al.</i> ³⁸	Single centre survey	Related PBSC 268	1996–2006	Median follow-up 4.5 years	60%	HD (1)	No difference compared with normal population
Mueller <i>et al.</i> ⁴⁰	Prospective long-term follow-up study	Unrelated PBSC 198	2001–2006	367 donor years	30% at 5 years	No cases	

Long term risks in healthy donors

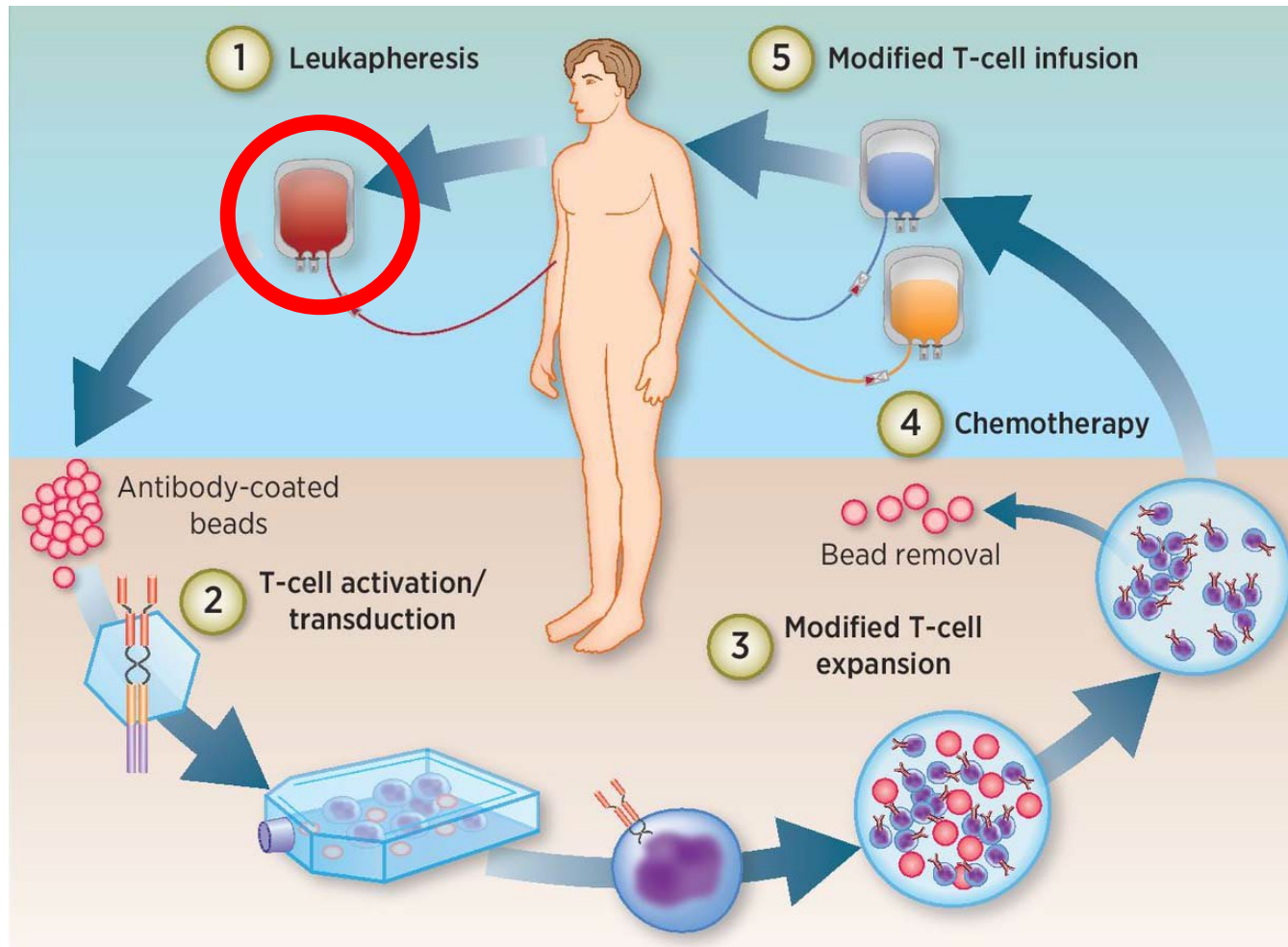


“Poor mobilizers”

CXCR2 agonist + CXCR4 antagonist



CAR T-cells



CAR T-cells

Washout period	Agent
> 6 months	Alemtuzumab, ATG
> 12 weeks	Fludarabine, bendamustine
12 weeks	Allogeneic HSCT
8 weeks	Clofarabine
4 weeks	DLI, PegAsparaginase
2 weeks	Systemic chemotherapy, GVHD therapy, long acting growth factors, imatinib, dasatinib, ponatinib, blinatumomab
1 week	IT MTX, high dose steroids (dexa), lenalidomide
5 days	short acting growth factors, nilotinib
3 days	Short acting cytotoxic/antiproliferative medication (hydroxyurea, low dose steroids)

CAR T-cells

CAR-T yescarta: #MNC = #WBC * (lymfo+mono%) via LAG → in te geven in tool

Gewenste opbrengst: tussen 5-10 x10⁹ MNC (min. 12l en max 15l bloed bewerken)

CAR-T kymriah: CD3+/μl vóór (via Tx labo) → in te geven in de tool

Gewenste opbrengst: 2-4 x10⁹ CD3+ // ≥ 2 x 10⁹ TNC // CD3+: ≥ 3% van TNC

CAR-T Cartitude: #MNC = #WBC * (lymfo+mono%) via LAG → in te geven in tool

Gewenste opbrengst: ≥6x10⁹ MNC

CAR-T Karma 3: #WBC vóór en lymfo % via LAG → in te geven in tool om ALC te berekenen

ALC > 500/μL → 2 x TBV bewerken

ALC < 500/μL → 3 x TBV bewerken

CAR-T Zuma: #MNC = #WBC * (lymfo+mono%) via LAG → in te geven in tool

Gewenste opbrengst: 5-10x10⁹ MNC

CAR-T Shrink: #MNC = #WBC * (lymfo+mono%) via LAG → in te geven in tool

Gewenste opbrengst: ≥4 x10⁹ MNC

CAR-T Sangamo Nefro: #MNC = #WBC * (lymfo+mono%) via LAG → in te geven in tool

Gewenste opbrengst: ≥12.75 x10⁹ MNC

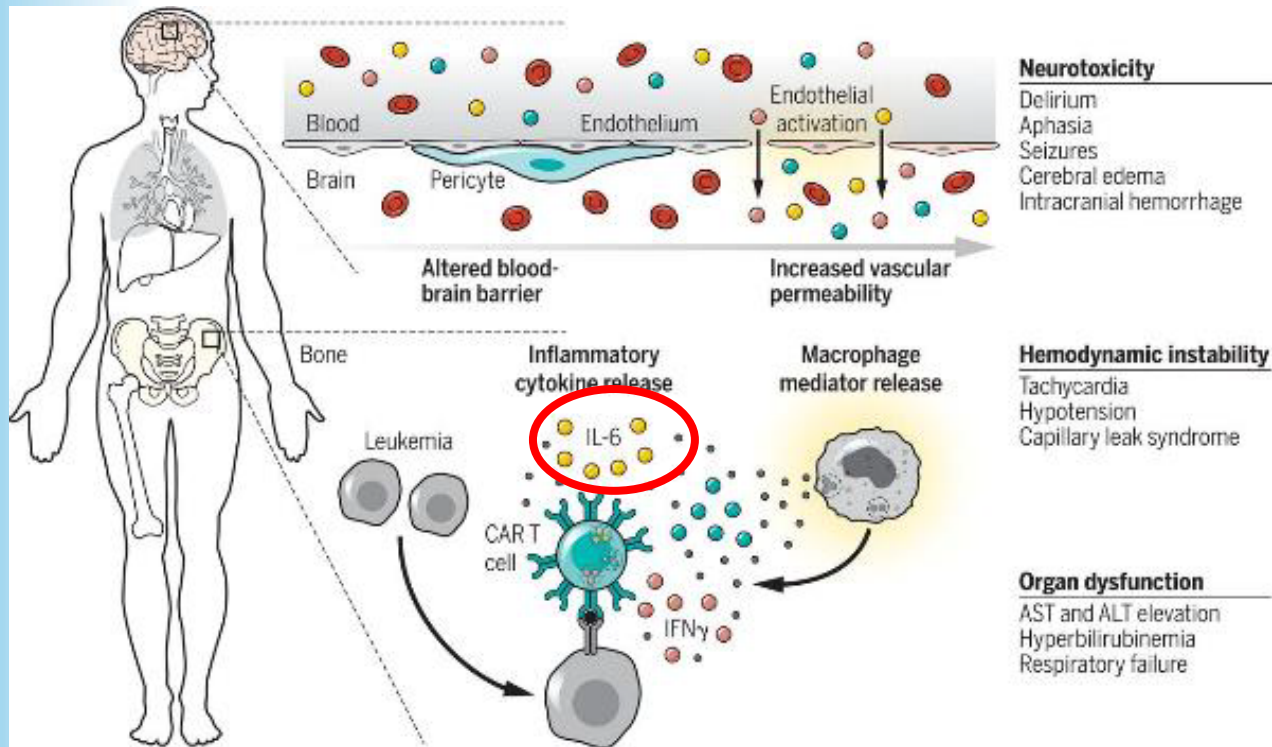
CAR-T Nefro TX200-KT02: #MNC = #WBC * (lymfo+mono%) via LAG → in te geven in tool

Gewenste opbrengst: ≥10-20 x10⁹ MNC

CAR-T Belinda/Novartis: CD3+/μl vóór (via Tx labo) → in te geven in de tool

Gewenste opbrengst: 2-4 x10⁹ CD3+ // ≥ 2 x 10⁹ TNC // CD3+: ≥ 3% van TNC

CAR T-cells

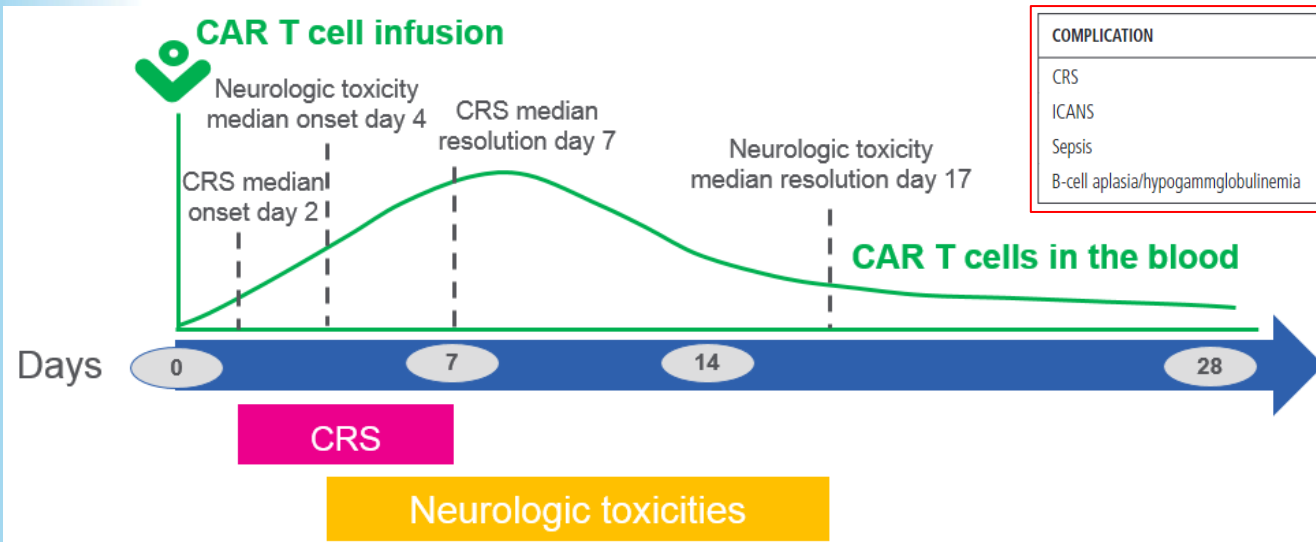


ICANS = immune effector cell-associated neurotoxicity syndrome

CRS = cytokine release syndrome











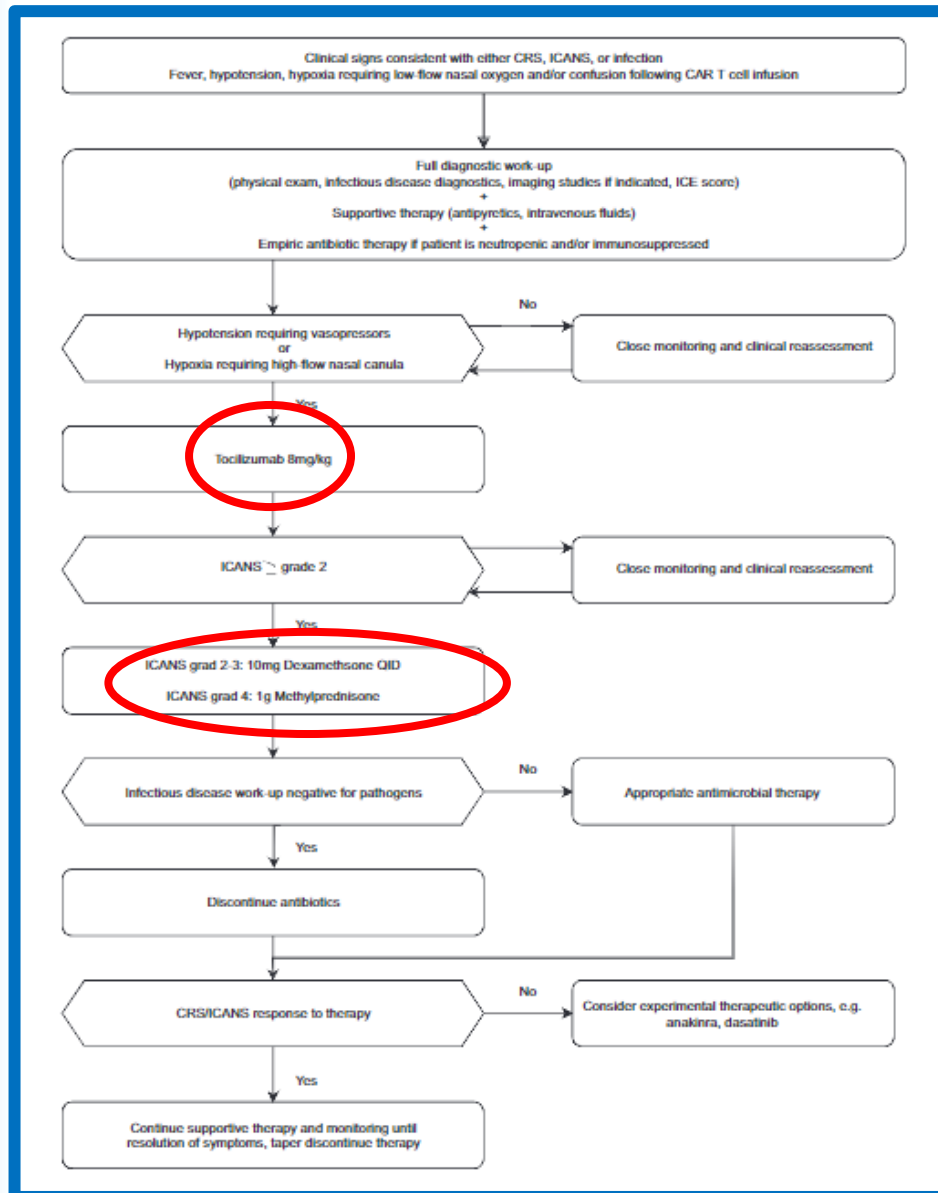
MAS = macrophage activation syndrome



COMPLICATION	FREQUENCY	TREATMENT OPTIONS
CRS	37% - 93%	Tocilizumab if no response to tocilizumab glucocorticoids
ICANS	23% - 67%	Glucocorticoids
Sepsis	8% - 16%	Empiric antibiotic therapy
B-cell aplasia/hypogammaglobulinemia	56% - 88%	Intravenous immunoglobulins

CAR T-cells

CRS				ICANS				
Hypotension	Hypoxia	Fever	Grade	ICE score	Consciousness	Weakness	Seizures	Edema
No	No	Yes	1	7-9	depressed level of consciousness but awakens spontaneously	No motor weakness	No seizures	No raised ICP or cerebral edema
Hypotension not requiring vasopressors	Hypoxia requiring low-flow nasal cannula	Yes	2	3-6	depressed level of consciousness but awakens to voice	No motor weakness	No seizures	No raised ICP or cerebral edema
Hypotension requiring one vasopressor with or without vasopressin	Hypoxia requiring HFNC, facemask, non-rebreather/venturi mask	Yes	3	0-2	depressed level of consciousness but awakens to tactile stimulus	No motor weakness	Any focal/generalized/nonconvulsive seizures that rapidly resolve	Focal/local edema on neuroimaging
Hypotension requiring multiple vasopressors (excluding vasopressin)	Hypoxia requiring positive pressure (CPAP, BiPAP, MV)	Yes	4	0 and unarousable	requires vigorous or repetitive tactile stimuli to arouse or stupor or coma	Deep focal motor weakness (hemiparesis, paraparesis)	Repetitive or life-threatening prolonged seizure (>5 min)	Clinical signs or imaging findings consistent with diffuse cerebral edema
								





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