Improved Quality Systems in Stem Cell Processing to avoid Graft Failure Incidents

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Declaration of interests:

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Commercial: None

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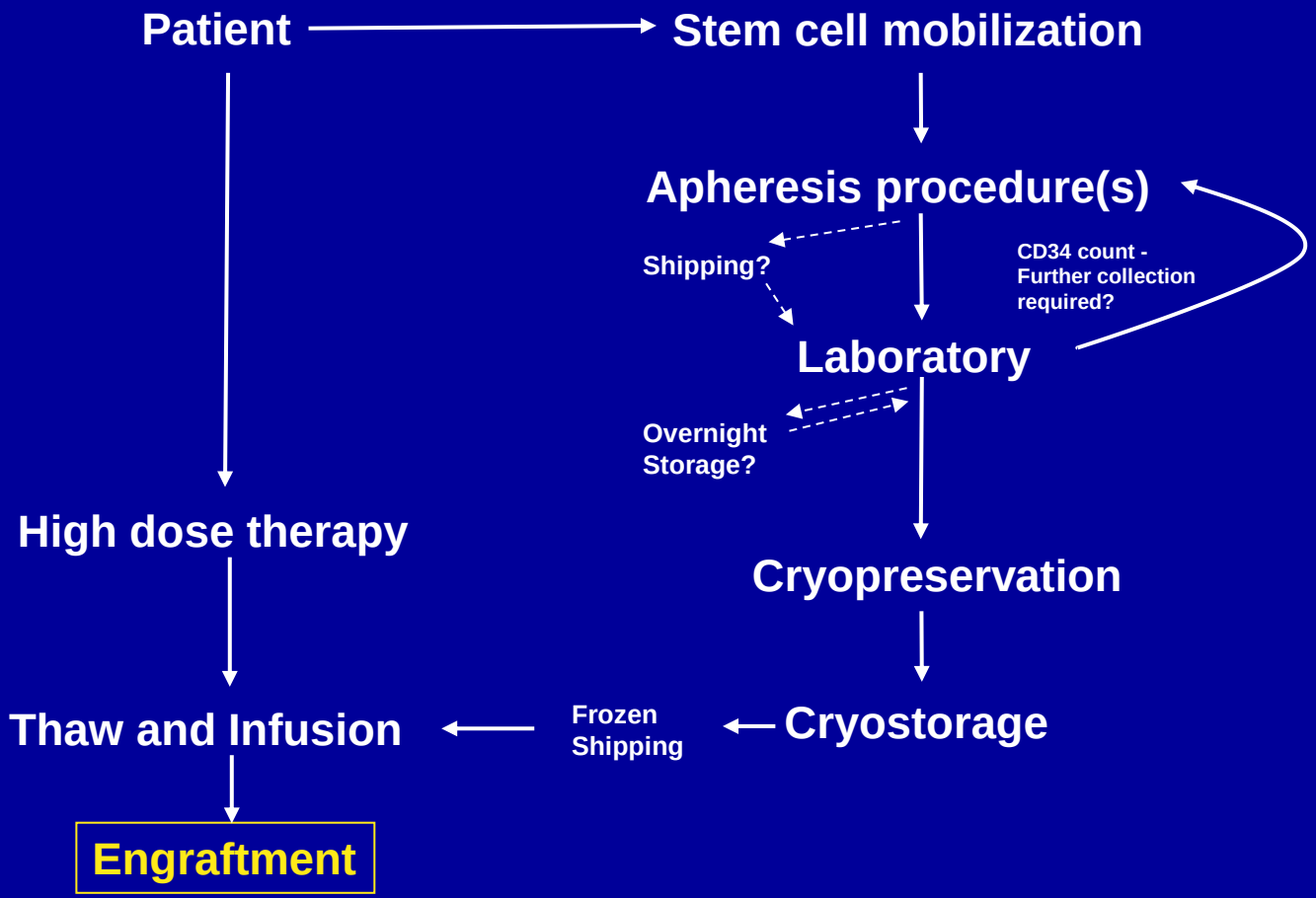
Autograft Stem Cell Pathway

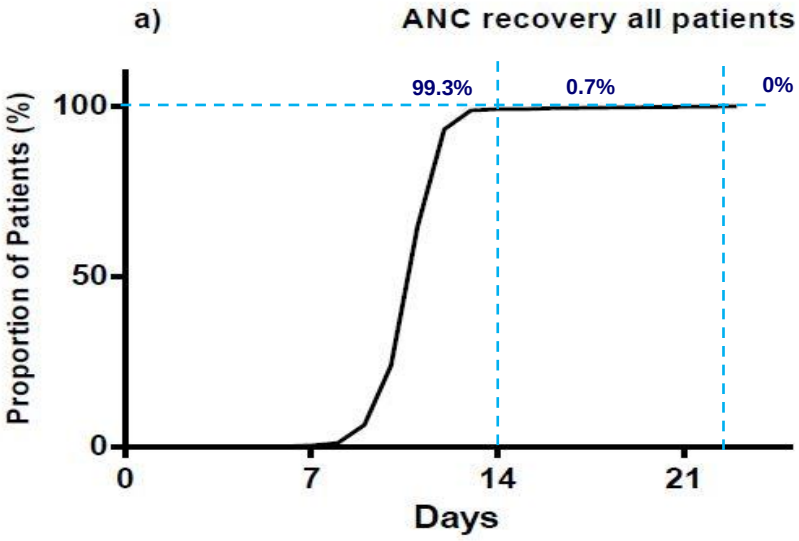
Watts et al 2016 (BJH review in proof)

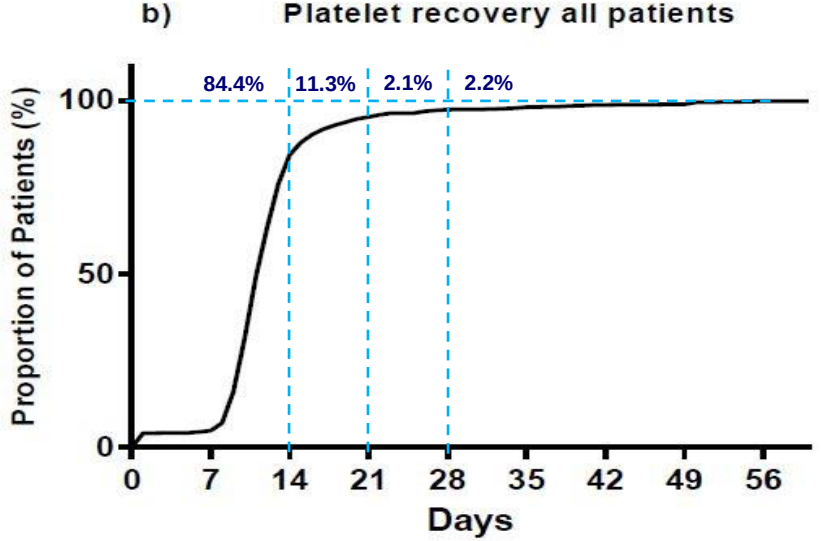


Autologous PBSCT Engraftment QA/QC

Watts and Linch 2016 BJH online (JACIE v6 standards C4.7.3: D 4.7.3: B4.7.3.1)

Pt no.	CD34 $x10^6$/kg	CD34 $x10^6$/kg	GM-CFC $x10^5$/kg	Days to ANC 0.5 $x10^9$/l	Days to PLT 20 $x10^9$/l	Release criteria UCLH
n=697	≥ 2	3.3	9.4	11 (6-23)	11 (0-67)	CD34 > 2 No GM-CFC required
n=107	< 2	1.6	4.7	12 (7-18)	13 (9-49)	CD34 1< 2 and GM-CFC ≥$2x10^5$/kg





ALERT : ANC >14 days in a single patient

ACTION: ANC >14 days in a second of the next 20 patients or
ANC in a single patient >28 days

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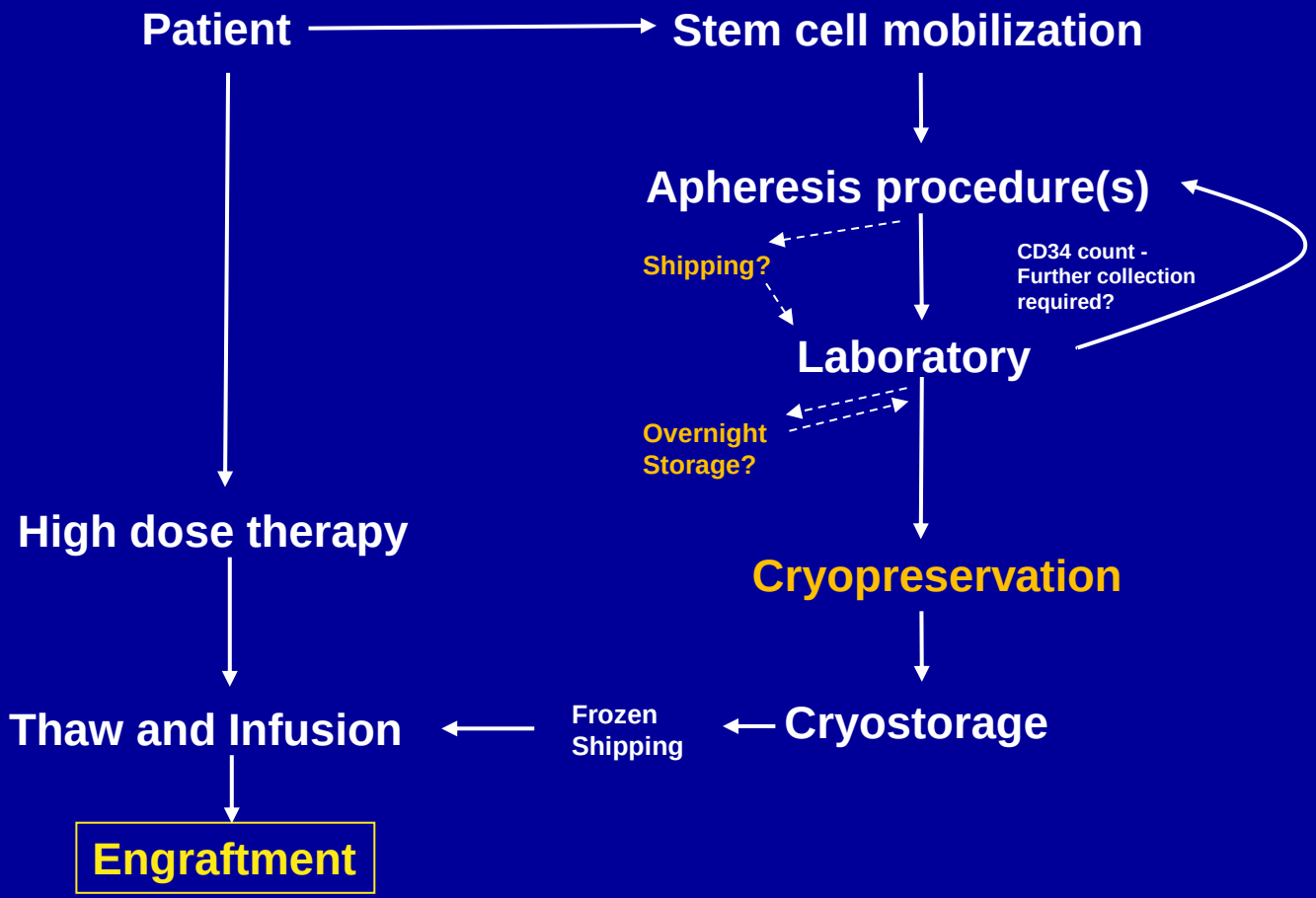


Graft Failure due to Poor HPC Storage and/or Cryopreservation

1. Storage ‘cell stress’ and loss of CFC activity

2. Cryopreservation of ‘stressed cells’ and major CFC loss

3. Sub-optimal cryopreservation method and major CFC loss

Graft Failure due to Poor HPC Storage and/or Cryopreservation

Graft Failure Incident	Quality System Failure
Lazarus 2009 BBMT, 15:589	Delayed platelet recovery HPC, BM (allo) : transit >20 hours at ambient temperature
Jansen 2009 BMT43: 499	Delayed platelet recovery HPC,A (allo) : transit >48 hours (WBC 245 ±76 x10^9/l) poor temperature control
Watts 2003 Blood 102(11): 40	Pre cryopreservation storage stress: HPC,A (auto): 4 hour ambient shipping plus 4^0C overnight storage, WBC 261±153 x10^9/l : Slow/delayed ANC with 'offsite' frozen cells. 101 harvests affected: 65 adequate HPC collections lost (thaw GM-CFC <1x10^5/kg) 23 re-harvested
Lioznov 2008 BMT 42:121-8	Pre cryopreservation storage stress: HPC,A (auto): 48 hrs transit with poor temperature control, WBC 220± 50 x10^9/l : Graft failure in 9/33 patients
Morgenstern 2016 BJH (online)	'Fast freeze' CFC damage ANC >30 days HPC,A (allo) x 2, HPC,A (auto) x 6 : Four deaths engraftment failure primary contribution in one case
Bavley & Karash : Oct 2008 pg1 Kansas City Star	'Fast freeze' CFC damage HPC,A (auto): 40 patients received 'fast freeze HPC' Delayed engraftment increased morbidity, mortality, 8 died within 100 days, 20 dead in 2 years
Abrams 1980 Lancet Aug 23rd p385	'Fast freeze' CFC damage : HPC, BM (auto) rescue for Ewings therapy, 13 'rapid' ANC recovery but 3 children receiving 'fast freeze' cells ANC >40 days and comparable with two patients where no autologous bone marrow cells were available
Gorin 1983 Eur J Cancer Clin Oncol 19:485	'Fast freeze' CFC damage: HPC, BM (auto): 8/35 deaths with engraftment failure – three deaths directly attributable, 5 contributory

HPC Quality Assessment: Viability and Potency

HPC QUALITY	TEST	PROS	CONS
POTENCY	**Engraftment**	• Ultimate potency measure	• Retrospective
VIABILITY BY DEAD CELL EXCLUSION	**All Cells** e.g. Trypan Blue, 7-AAD	• Rapid	• Functionally uninformative as CFC <1% of bulk harvest and heterogeneous cell populations
	CD34+7AAD	• Highly predictive of potency potential of fresh harvest • Rapid, standardised	• May give overly optimistic measure of CFC survival • Thaw viable CD34 not standardised
VIABILITY BY FUNCTION	**Colony Assays**	• Potency potential • Proven 14 day CFC survival • 'bad freeze' investigation	• Poorly standardised • Two week incubation

Storage Factors Affecting HPC Potency

1. Temperature
2. White cell concentration
3. Time

Loss of PBSC Viability and Potency During Storage

Jansen *et al* Cytotherapy 2009:11:79 (& coolbox validation 2010:12:919)

Storage Conditions	Storage Temperature	Progenitor survival (%) after 48 hours in storage	
		GM-CFC	Viable CD34 (7AAD-)
Temperature (WBC 200x10^9/l)	22^0C	6	18
	17^0C	18	67
	13^0C	50	80
	4^0C	86	96
Cell Concentration WBC (x10^9/l)			
200	22^0C	6	19
100		9	43
50		25	55
25		51	81
200	4^0C	86	95
100		93	98
50		98	98
25		91	95

Supporting evidence: Pettengell *et al* 1994, Jestice *et al* 1994, Sugrue *et al* 1998, Petzer et al 1999, Kao et al 2011, Fry et al 2013

Graft Failure due to Poor Storage and/or Cryopreservation

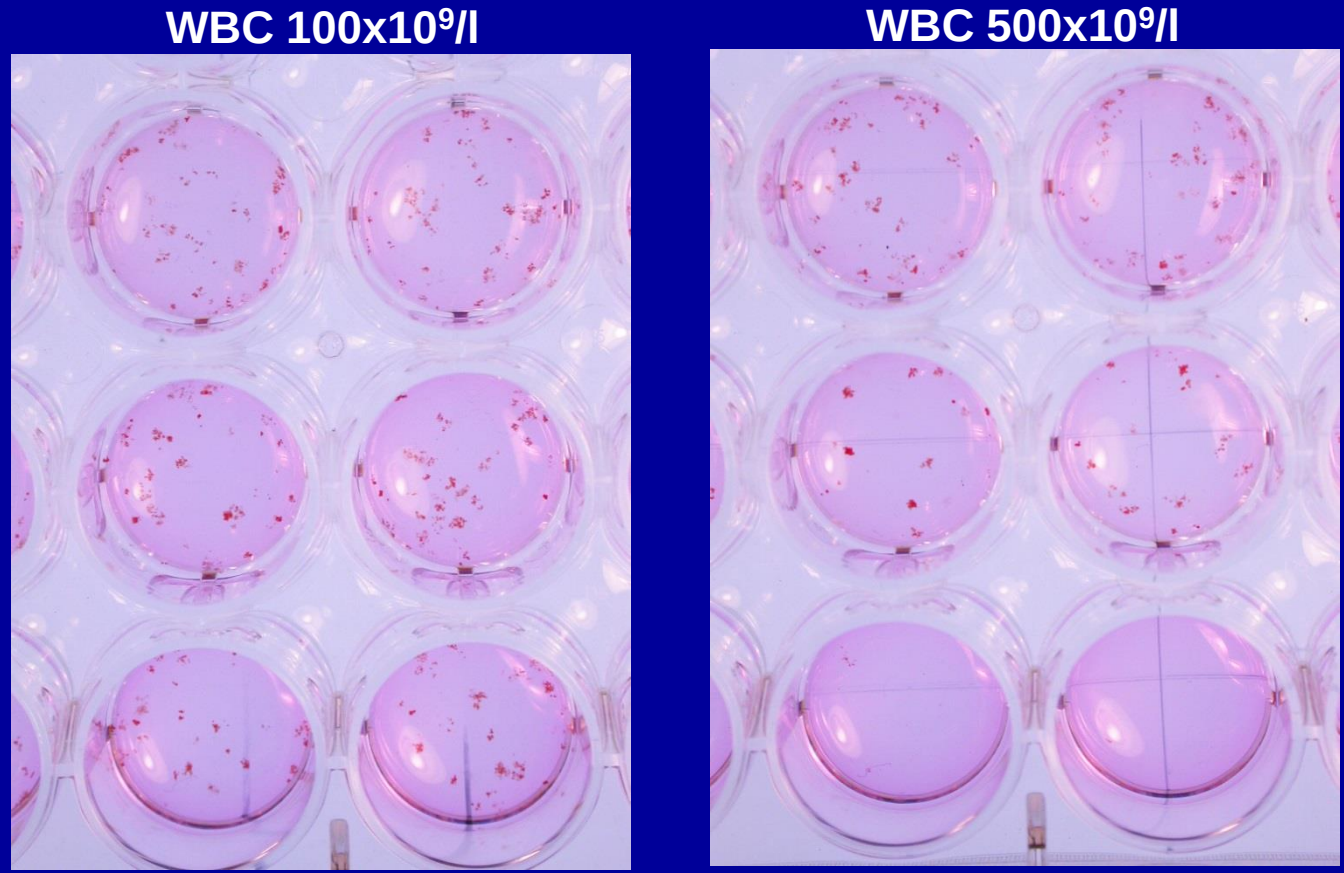
1. Storage ‘cell stress’ and loss of CFC activity

2. Pre-freeze stored ‘stressed cells’ and major CFC loss

3. Sub-optimal cryopreservation method and major CFC loss

Thaw colonies of PBSC samples adjusted to low and high WBC and stored prior to cryopreservation

Watts *et al* 2003 Blood 102(11): p.40a.



Certified Transit for HPC (2-10^{0}C)

International Safe Transit Association www.ista.org

JACIE v6 standards
Assess storage risks and control D2.3 & D.2.3.1

Credo Cube™ Pelican Bio Thermal

- Frozen ‘tic plate’ enclosure
- Certified up to 96 hour transit time
- Requires -20^{0}C freezer for plates
- Smaller box - hand delivery suitable

Transmed range, Sarstedt Ltd

- End plates and frames chilled at 4^{0}C
- Certified for 48 hour transit
- No freezer required
- Larger package - vehicle delivery

Graft Failure due to Poor Storage Conditions and/or Cryopreservation

1. Storage ‘cell stress’ and loss of CFC activity

2. Pre-freeze stored ‘stressed cells’ and major CFC losses

3. Sub-optimal cryopreservation method and major CFC loss

Patients Affected – ANC recovery >30 days viable CD34+ cell dose infused

Morgenstern *et al* 2016 BJH

Subject	Fresh CD34 $x10^6$/kg	Thaw viable CD34 $x10^6$/kg (CD34+7AAD-)
Allo 1	8.56	8.59
Allo 2	2.92	3.00
Auto 1	6.60	6.12
Auto 2	5.61	4.84
Auto 3	3.88	2.94
Auto 4	4.00	3.39
Auto 5	17.99	14.63
Auto 6	5.22	4.01

Stored Harvests Thaw Tested for CD34 viability

Morgenstern *et al* 2016 BJH

Date Frozen	Fresh CD34 $x10^6$/kg (7AAD >98%)	Thaw CD34+ 7AAD- (%)	Thaw CD34+ 7AAD- $x10^6$/kg
19/04/2013	2.0	99	1.3
13/03/2013	4.8	99	3.6
30/07/2013	6.5	91	6.1
30/09/2010	3.4	94	2.8
28/09/2011	30.8	88	26.8

Stored Harvests Thaw Tested for GM-CFC

Morgenstern *et al* 2016 BJH

Date Frozen	Fresh CD34 $x10^6$/kg (7AAD >98%)	Thaw CD34+ 7AAD- (%)	Thaw CD34+ 7AAD- $x10^6$/kg	Thaw GM-CFC $x10^5$/kg*	Thaw GM-CFC expected (CD34:GM ratio 0.11) (Min UCLH $\geq$1 $x10^5$/kg)
19/04/2013	2.0	99	1.3	0.0	2.2
13/03/2013	4.8	99	3.6	0.58	5.3
30/07/2013	6.5	91	6.1	0.13	7.2
30/09/2010	3.4	94	2.8	0.16	3.7
28/09/2011	30.8	88	26.8	0.29	33.9

CD34+ cells ‘viable’ but poorly clonogenic

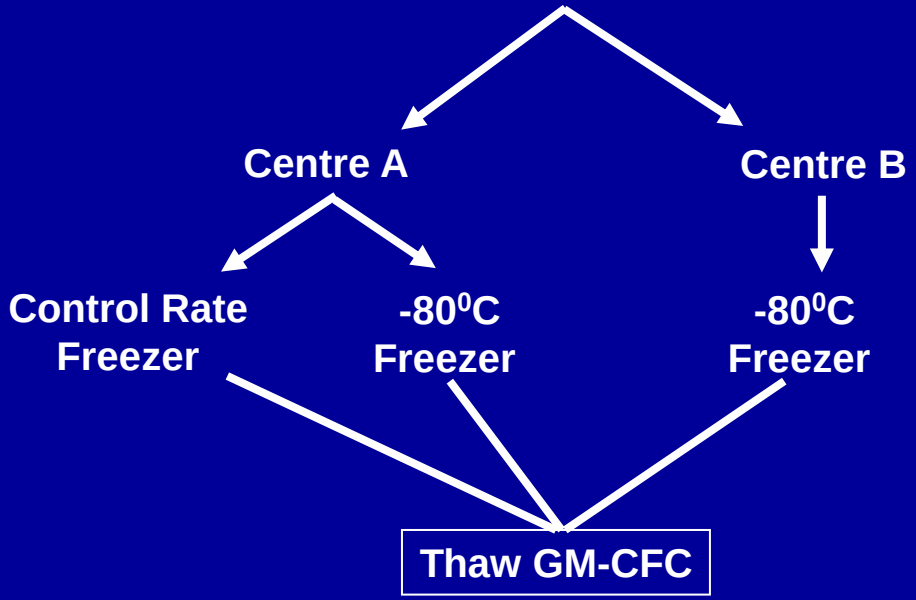
Split Harvest HPC,A Experiments to Investigate the Freeze Step

Morgenstern *et al* 2016 BJH

Cryoprotectant method, regents, disposables same on both sites

1. Cryoprotectant added to HPC,A (n=4) at clinical scale as for patient use

2. Split between Controlled Rate Freezer or -80^{0}C mechanical freezer



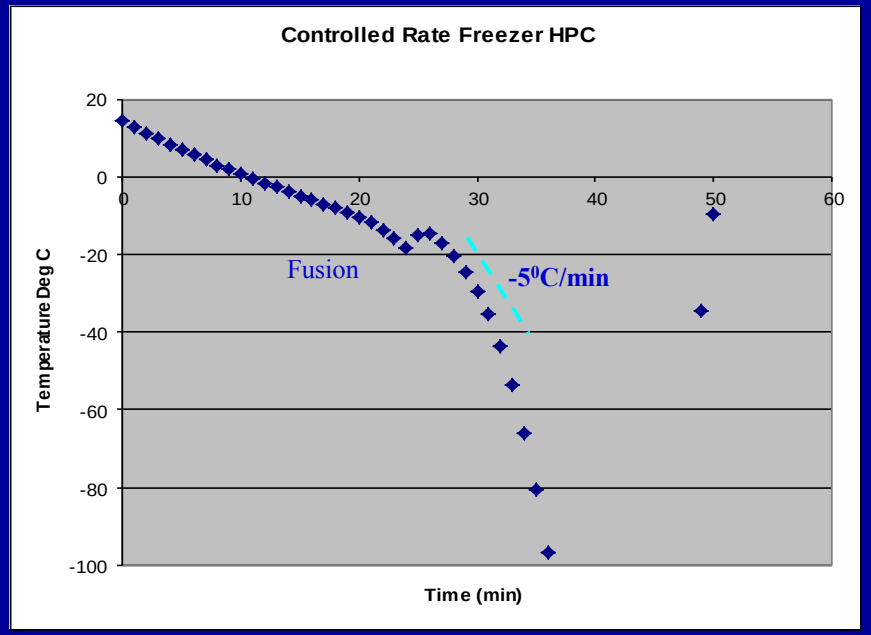
Split Harvest Thaw Colonies: CRF versus -80^{0}C Freezer

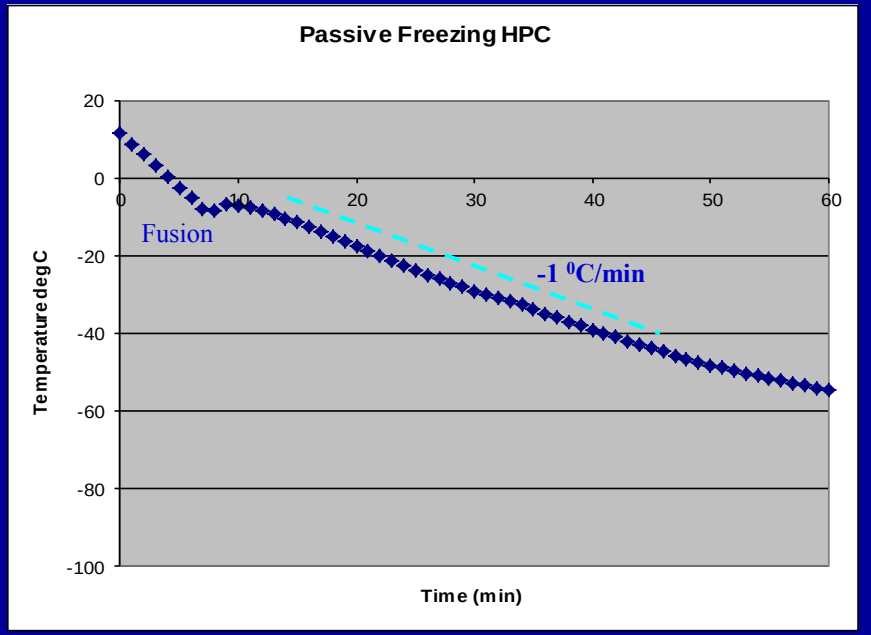
HPC,A	BFUE /well	GM-CFC /well	GM-CFC x10^{4}ml (yield%)
HPC 1 PRE	36	33	38 (100%)
Centre B -80^{0}C	19	17	23 (59%)
Centre A -80^{0}C	21	18	25 (64%)
Centre A CRF	0.5	0	0 (0%)
HPC 2 PRE*	15	6	9 (100%)
UCLH -80^{0}C	8	5	10 (106%)
Centre A -80^{0}C	9	5	10 (112%)
Centre A CRF	0	0	0 (0%)
HPC 3 PRE*	15	3	6 (100%)
UCLH -80^{0}C	7	4	7 (118%)
Centre A -80^{0}C	6	2	4 (47%)
Centre A CRF	0.3	0	0 (0%)
HPC 4 PRE	21	47	33 (100%)
Centre A -80^{0}C	14	29	23 (70%)
Centre A CRF	2	0	0 (0%)

* CD34 selection ‘flow through waste’ HPC

Freeze profile of split PBSC harvest in Controlled Rate Freezer versus Passive in a Mechanical Freezer (-80^{0}C)

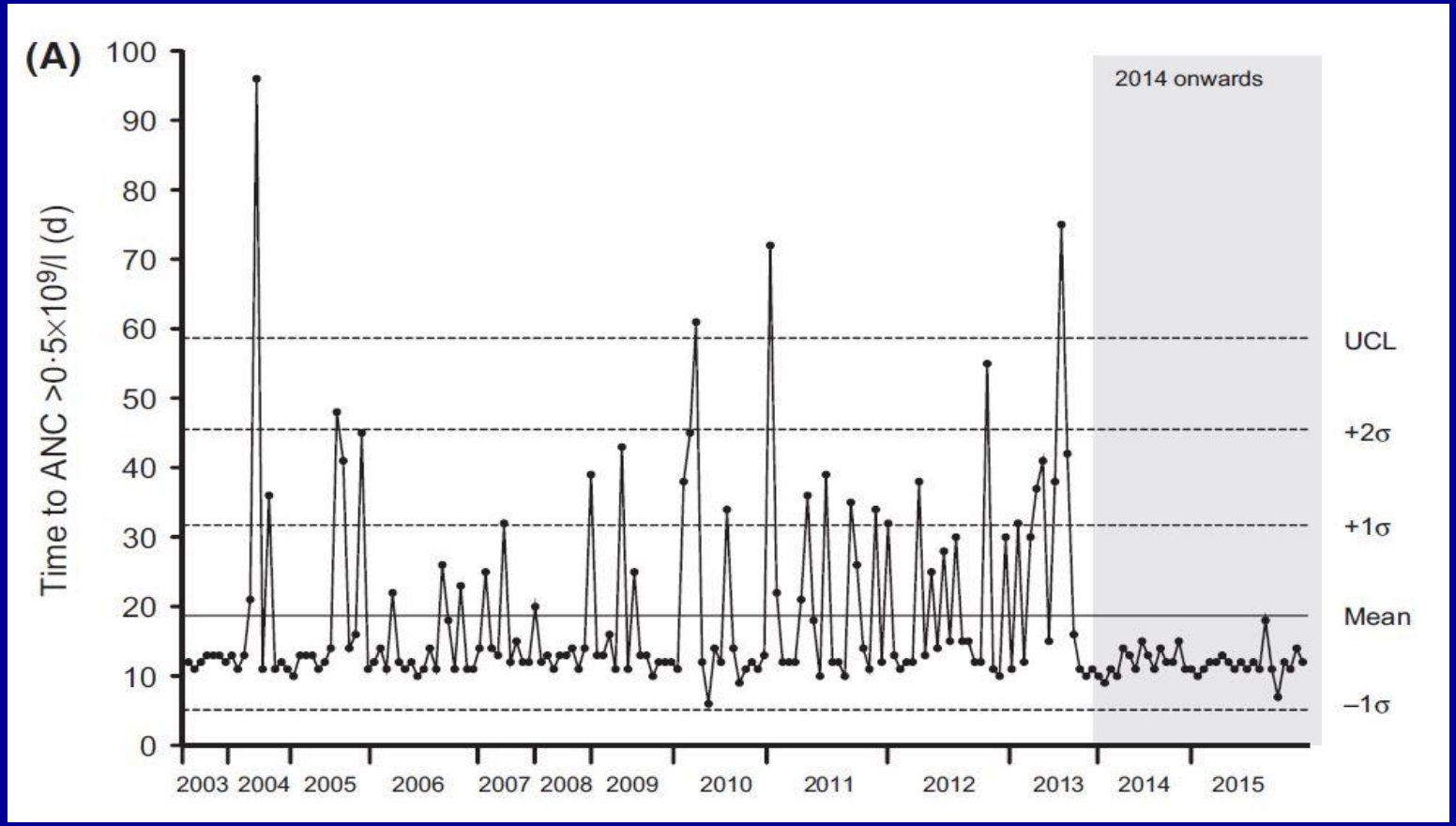
Morgenstern *et al* 2016 BJH





ANC recovery using ‘Passive Freeze’ for HPC at -80^{0}C

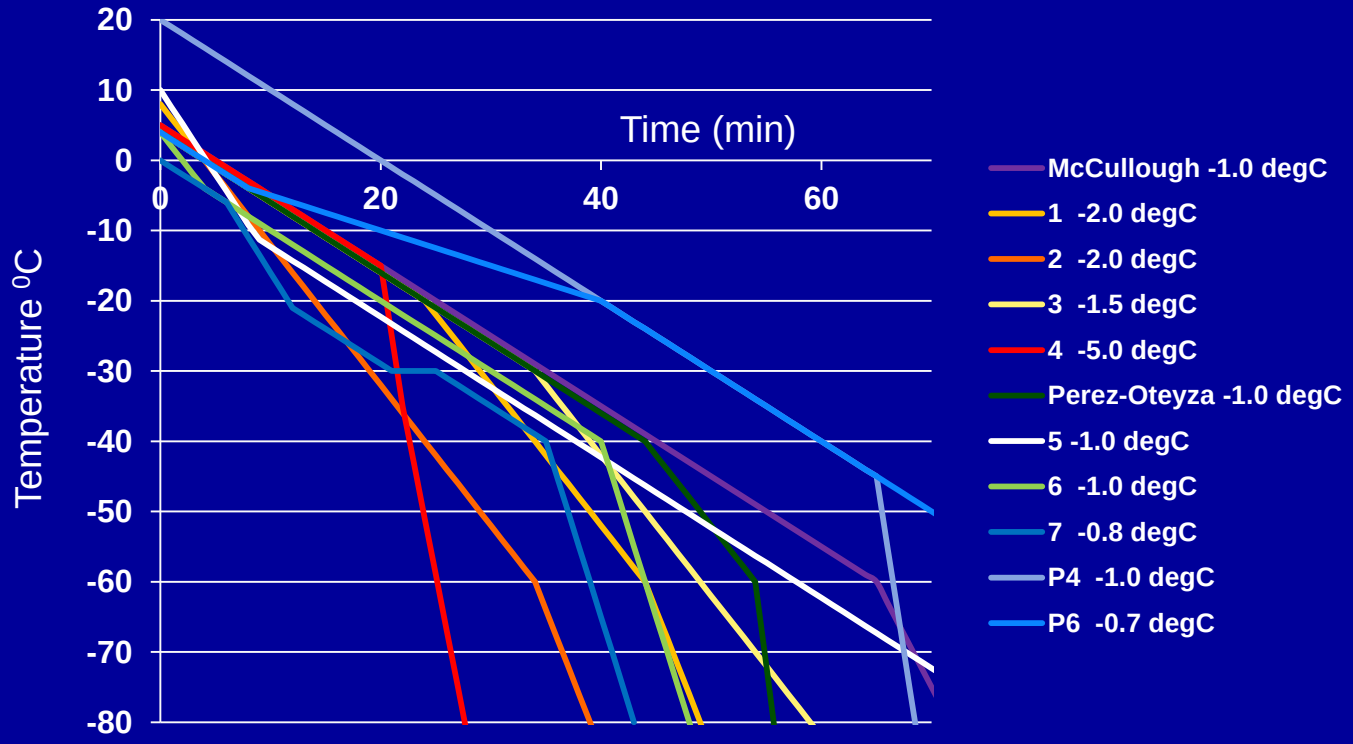
Morgenstern *et al* 2016 BJH



What is the ‘Standard Controlled Rate Freeze’ profile?

Variability in HPC Freeze Profiles in Clinical Use

Seven UK centres (1-4 and 5-7), two published and two 'pre-set' on CRF machine
Freeze rate/min from -20^{0}C to -40^{0}C shown



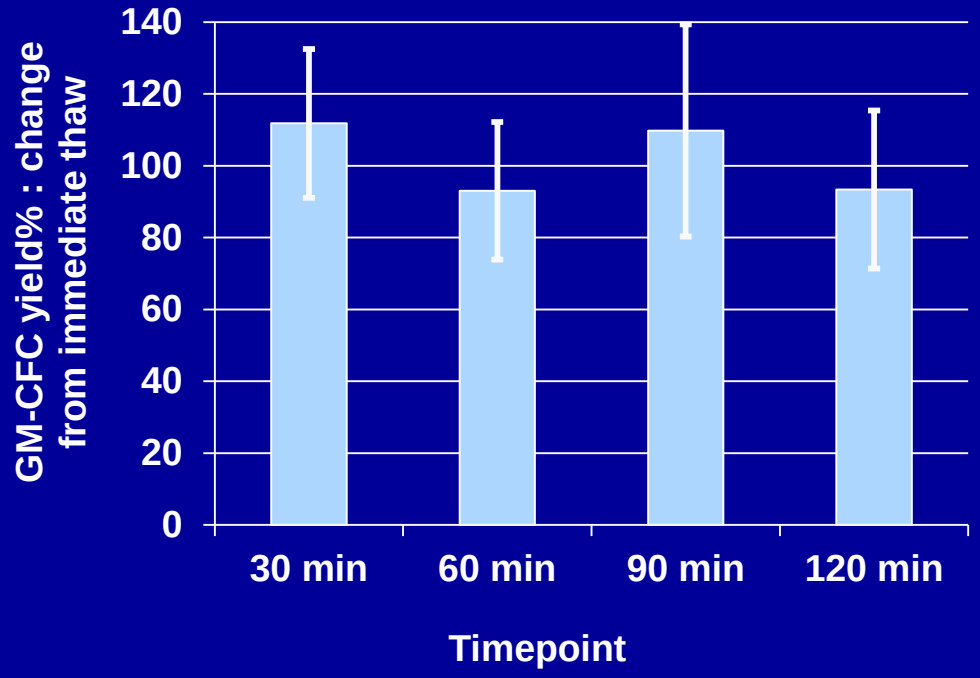
**Two published HPC CRF profiles validated by thaw CFC*
McCullough *et al* Transfusion 2010; 50:808
Perez-Oteyza *et al* Haematologica 1998; 83:1001

Laboratory Validation of HPC Cryopreservation

JACIE v6 standards

‘Viability, potency & stability’	**D9.2.3**
‘Representative pilot vial’	**D8.1.2.2**

Cryopreservation Potency Audit and GM-CFC stability of thawed PBSC harvests at room temperature UCLH (n=8)



GM-CFC yield mean (±SD) from fresh harvest = 68 ± 27%
Yields over time shown normalised to immediate thaw results

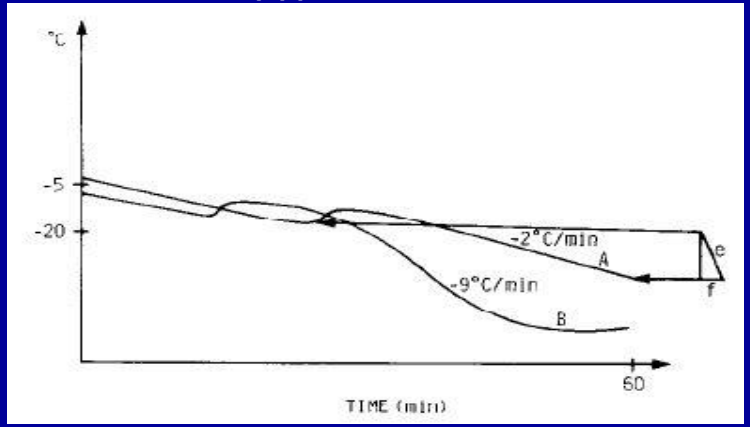
Years stored mean (range) 6 (0.9-10.5) years

Test Vials are an unreliable indicator of Clinical Harvest GM-CFC

Douay 1986 *et al* Cryobiology 1986:23:296 (fig.1 below)

	Thaw GM-CFC (mean ± SD)	>50%	<30%
Douay (BM=11)			
Test vial	48 ± 38%	5/11	4/11
Paired harvest	84 ± 15%	11/11	0/11
UCLH* (PBSC=12)			
Test vial	43 ± 54%	8/12	2/12
Paired harvest	65 ± 24%	10/12	0/12

Fig 1. Douay *et al* Cooling curves of clinical sample (A) and (B) paired test vial



Other reasons for poor pilot vial CFC

- Final step in HPC processing
- Storage: Stays with product?
- Same freezer as product?
- Same temperature as product?
- Rack storage and TWEs?

Conclusions : Quality Systems Check

Shipping/Storage

- Avoid risk: Cryopreserve immediately
- Control risk: 2-10^{0}C, WBC <200 x10^{9}/l (‘safe’ WBC threshold for cryopreservation?)

Monitoring / Validation

- Determine benchmark engraftment kinetics, alert and action limits
- Case by case engraftment monitoring by lab and clinical team
- Colony assays to validate potential functional processing damage for HPC

Unresolved

- Pilot vial samples poorly representative of harvest for functional tests
- Need for a rapid assay to replace CFC – eg flow ‘metabolic viability’ ALDH?