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Personalizzazione o grandi volumi di processazione nella raccolta di HPC-A?

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SIdEM
Società Italiana
di Emaferesi
e Manipolazione
Cellulare

Sezione Regione Lombardia

Best practice for peripheral blood progenitor cell mobilization and collection in adults and children: results of a Società Italiana Di Emaferesi e Manipolazione Cellulare (SIDEM) and Gruppo Italiano Trapianto Midollo Osseo (GITMO) consensus process

Luca Pierelli, Paolo Perseghin, Monia Marchetti, Patrizia Accorsi, Renato Fanin, Chiara Messina, Attilio Olivieri, Marco Risso, Laura Salvaneschi, and Alberto Bosi for Società Italiana Di Emaferesi and Manipolazione Cellulare (SIDEM) and Gruppo Italiano Trapianto Midollo Osseo (GITMO)

Question 12: How should an ideal apheresis procedure, for example, flows, volumes, and tailoring, be performed?

RECOMMENDATIONS. *A large-volume apheresis (at least 3 vol) is preferred, excepted for children. A tailored procedure, according to published or local algorithms, is advisable in patients showing a high circulating CD34+ cell count (higher than 80 × 10^6/L) and an increasing trend.*

La procedura di raccolta in aferesi delle HPC-A, leucaferesi – staminoferesi, è fondamentale per la riuscita del programma trapiantologico.

Obiettivi della procedura sono:

1. **raccolta di un numero adeguato di cellule CD34,**
2. **minor numero possibile di procedure,**
3. **prodotto cellulare caratterizzato da ridotta contaminazione in WBC, RBC, PLT.**

Contaminazione cellulare ha effetto negativo:

- **nelle procedure di manipolazione, criopreservazione, e scongelamento del prodotto cellulare;**
- **Per la vitalità cellulare,**
- **Correla con incremento di eventi avversi in corso di reinfusione**

La procedura di raccolta è un fenomeno costante, governata dalla riproducibilità del processo, dall'efficienza del sistema aferetico (CE%), dai parametri del paziente (peso corporeo, ematocrito, conta WBC, % MNC).
Considerate le caratteristiche delle piattaforme aferetiche, **ciò che incide prevalentemente nella resa in cellule CD34+ è il regime di raccolta messo in atto durante la leucoaferesi**: il volume ematico totale processato per singola raccolta. standard leucoaferesi. - large volume leukapheresis (LVL) – very LVL

Regime di raccolta

Standard leucoaferesi: **2 volumi** ematici TBV, (**<200 mL/Kg**);

LVL: 3–4 volumi ematici TBV (**200 mL/Kg**), 15 litri di sangue periferico;

very LVL: 5 - 6 volumi ematici TBV (**>300 mL/Kg**).

La procedura **LVL**, nei pazienti adulti, è **raccomandata**

I regimi di raccolta large volume si caratterizzano:

maggiore resa in cell CD34+, "**CD34+ cells yeld**", > di 2 volte, rispetto alle procedure standard

maggior **volume** ematico processato,

maggior **efficienza** di raccolta

fenomeno reclutamento cellulare intra procedura (**intrapheresis recruitment of PBPC**) .

Smolowicz AG, Villman K, Berlin G, Tidefelt U. Kinetics of peripheral blood stem cell harvests during a single apheresis. **Transfusion 1999;39:403-409**

Obiettivo della procedura LVL, è l'incremento in cells CD34+/Kg per singola procedura, qualunque sia la soglia CD34+ pre aferesi.

Nei pazienti good responder le procedure LVL sono in grado di ottimizzare il programma di raccolta: la dose trapianto è conseguita con il minor numero possibile di raccolte.

Ad un minor numero di leucaferesi corrisponde una minor somministrazione di fattore di crescita, minor assistenza, riduzione dei costi generali della procedura di raccolta .

Guthensohn K, Magens MM, Kuehnl P, Zeller W. Increasing the economic efficacy of peripheral blood progenitor cell collections by monitoring peripheral blood CD34+ concentrations. **Transfusion 2010;50:656-62**

volume ematico processato, 3 volumi o ≥ 15 L, velocità di prelievo elevate, ≥ 70 mL, tempo di procedura, volume anticoagulante (AC ratio 1:12 – 1:14: concentrazione di citrato di 1.99 mg/Kg/minuto) hanno un impatto significativo sulla compliance del paziente:

accesso vascolare

il **sistema cardio circolatorio** deve essere in grado di adattarsi alla velocità di flusso,

il **bilancio** dei liquidi è in positivo,

è presente una riduzione dei valori plasmatici di **K^+**, **Mg^+** (sino – 60%), **Ca^{++}** (ad un flusso di infusione ACD-A di 1.0 – 1.8 mg/Kg/min risulta una riduzione del 25 – 35% del calcio ionizzato, a + 60')

deplezione in eritrociti e piastrine (-50%).

Riduzione della concentrazione plasmatica di ioni Ca^{++} e Mg^{++} : **tossicità da citrato**

manifestazioni locali 12%,

parestesie generalizzate 0,9%.

Moog R. Adverse events in peripheral progenitor cell collection. A 7 years experience. J Hematother Stem Cell res 2001;10:675-80

Prevenzione reazioni avverese da citrato: Infusione in continuo di Calcio gluconato + Mg

Butcha C, Macher M, Bieglmayer C, Hocker P, Dettyke M. reduction of adverse reactions during autologous large volume PBPC apheresis by continuous infusion of calcium gluconate. **Transfusion 2003;43:1615-1621**

Haddad S, Leitman SF, Wesley RA, Cecco S, Yau YY, Starling J, Rehak NH, Bolan CD. Placebo-controlled study of intravenous magnesium supplementation during large-volume leukapheresis in healthy allogenic donors. **Transfusion 2005;45:934-944**

La presenza nel paziente di un “**rischio relativo**” :

- funzionalità renale - epatica alterata,
- presenza di alterazioni del ritmo cardiaco,
- parametri vitali instabili,
- emometria insufficiente,
- deficit di elettroliti

condizione ad alto rischio

per **eventi avversi** nella procedura LVL.

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PBPC collections: Management, techniques and risks

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In spite of the fact that the standard and LVL collection techniques are used routinely, there may occur special conditions in which the procedures cannot be recommended. Some patients may suffer from serious clinical complications and they cannot tolerate either standard procedures with administration of higher doses of ACD-A, or the high extent of procedure in the course of LVL. We tried to find the safe and efficient collection technique which could help this group of patients to overcome their problems. The "Mixed" collection technique could be such a choice.

Abstract

We evaluated the efficiency, safety and risks of three techniques which were used for autologous PBPC collections: (a) large-volume leukapheresis (LVL), (b) standard collections, and (c) a new modified technique which was named as "Mixed" collections.

In spite of the fact that the standard and LVL collection techniques are used routinely, there may occur special conditions in which the procedures cannot be recommended. Some patients may suffer from serious clinical complications and they cannot tolerate either standard procedures with administration of higher doses of ACD-A, or the high extent of procedure in the course of LVL. We tried to find the safe and efficient collection technique which could help this group of patients to overcome their problems. The "Mixed" collection technique could be such a choice.

The numbers of 136 autologous PBPC collections were performed in 98 patients who suffered from hemato-oncological diseases. We evaluated the results of (a) 93 LVL (more than 3 TBV, total blood volumes of the patients were processed; anticoagulation: ACD-A and Heparin), (b) 16 Standard procedures (less than 3 TBV were processed; anticoagulation: ACD-A), and (c) 27 "Mixed" collections (less than 3 TBV of patients were processed; anticoagulation: ACD-A+ Heparin). Collections were performed by the use of separator Cobe Spectra, Caridian.

In patients (a) with a good effect of mobilization (precollection CD 34+ cells in blood higher than 20×10^3/mL) we prepared almost the same median dose of CD 34+ cells from the standard and "Mixed" collections, 3.8 and 4×10^6/kg, respectively. In LVL the median yield of CD 34+ cells was 8.2×10^6/kg.

In patients (b) who were mobilized weakly (precollection CD 34+ cells in blood lower than 20×10^3/mL), LVL enabled to prepare 1.5×10^6 of CD 34+/kg from one collection, while the median yield of CD 34+ cells from the standard and "Mixed" collections was 0.9 and 1.2×10^6/kg.

All the standard, LVL and "Mixed" procedures were tolerated well without any serious adverse reactions. We detected 22 adverse reactions, but only three reactions were associated directly with the procedure. Mild hypocalcemia (2) and hypotensive reaction (1) were transient and treated efficiently. Procedures could continue and were finished according to the planned programme. Other reactions were related either to the insufficient function of central venous catheter or to the poor clinical condition of the patients.

LVL enabled to get a higher yield of CD 34+ cells than the Standard and "Mixed" collections in well mobilized patients as well as in weakly mobilized patients. We observed the similar efficiency in standard and "Mixed" collections in well mobilized and weakly mobilized patients. We can recommend LVL in all patients who can tolerate it due to a greater chance of collecting higher yields of progenitor cells. In the weakly mobilized patients LVL offers a greater chance of collecting at least a minimum amount of CD 34+ cells needed for transplantation. "Mixed" collections may be used as an alternative technique under the circumstances in which standard or LVL cannot be recommended – like in patients who do not tolerate a high amount of citrate or a high extent of the procedure, e.g. patients with cardiac arrhytmia, impaired liver or renal function or unstable vital signs.

In spite of the fact that the standard and LVL collection techniques are used routinely, there may occur special conditions in which the procedures cannot be recommended. Some patients may suffer from serious clinical complications and they cannot tolerate either standard procedures with administration of higher doses of ACD-A, or the high extent of procedure in the course of LVL. We tried to find the safe and efficient collection technique which could help this group of patients to overcome their problems. The "Mixed" collection technique could be such a choice.

Regime alternativo: «**mixed collection**»

3 volumi TBV processati,

velocità di prelievo < 60 mL/min,

ACD-A + eparina (eparina 10 UI x ml ACD-A, ratio Ac 26–30:1)

CD34+ yelds: Mixed Collection = LVL

Minore tossicità da citrato,

Maggior deplezione in PLT

allungamento aPTT

assenza segni di sanguinamento.

LVL vs Mixed Collection

Group 1°: LVL – ACD-A				Group 2°: Mixed – ACD-A/Heparin			
44 patients				105 patients			
MM	8	m/f	8/1	MM	57	m/f	31/26
nHL-HL	24	m/f	7/17	NHL-HL	33	m/f	20/13
AL	12	m/f	8/4	AL	15	m/f	3/12

Separatore cellulare
COBE Spectra, Caridian v. 6.1,

protocollo di raccolta «**semi automatic mononuclear cell collection protocol**» regolazione manuale dell'interfaccia sangue plasma a **3%** Hct, colorgram,
volume di raccolta 1.0 mL/minuto.

TBV/procedura, 200 mL/Kg (13 – 15 L.), + 200 mL di plasma autologo.

LVL

anticoagulante ACD-A, ratio 14:1

Mixed Collection

anticoagulante ACD-A + Eparina (10 ui x mL ACD-A), ratio 30:1.

LVL profilassi tossicità da citrato

infusione in continuo, pompa di infusione, di calcio gluconato (4 fiale calcio gluconato diluito in 500 mL di soluzione salina, velocità di infusione 1 mL/min;

via di infusione è stata connessa, nella linea di ritorno, prossimale al sito di venipuntura.

Mixed Collection:

- PLT valore soglia pre aferesi ≥ 40 x 10^9/L
- profilassi PLT.

All procedure, pre e post
K+, Na+, Ca+, BUN, creatinine, aPTT e PT, emocromo e conta CD34+.
Threshold CD34+: ≥ 15 cell/µL.
CD34+ pre < 15 cell/µL : scarsi o cattivi mobilizzatori (21% dei pazienti arruolati nel programma)

Risultati.
Mixed Collection vs LVL

volume Ac (949 vs 505 mL, P<0,001),
volume prodotto (240 vs 220 mL, P<0.001),
Hct prodotto (5 vs 3%; P=0.001),
TC - WBC (244 vs 190x10^3, P=0.001).

CD34+cells x10^6 e **CD34+cell/Kg x10^6**:
P=0,02 / P=0,04
Mixed Collection: **yeld CD34+ > 2 volte (6.9 vs 3.6).**

Risultati.
Mixed Collection vs LVL

Pur in assenza di profilassi per ipocalcemia, nel regime mixed non sono stati registrati manifestazioni cliniche riconducibili a tossicità da citrato.

In entrambi i regimi di raccolta non sono stati osservati significative alterazioni nei parametri considerati

	LVL	MIXED	Sign.99% P=<0.01	Sign.95% P=<0.05
CD34+ cells/µL	38	60	P=0.22	
Processed volume (mL)	13.472	13.559	P=0.83	
Processed volume (mL/Kg)	206	202	P=0.38	
AC Volume (mL)	949	505	P<0.01	
Collection time (min)	247	246	P=0.7	
Flow rate (mL/min)	55	58	P<0.01	
Graft volume (mL)	240	220	P<0.01	
Graft Hct (%)	5	3	P<0.01	
Total graft Cell:WBC count	244.500	190.540	P<0.01	
CD34+x10^6	533	771	P=0.04	P=<0.05
CD34/Kg x10^6	3.5	6.9	P=0.02	P=<0.05
CE%	78	71	P=0.133	

Tailored Procedure

Poor Mobilizer

Bone Marrow Transplantation (2012) 47, 342–351

www.nature.com/bmt

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ORIGINAL ARTICLE

Proposed definition of 'poor mobilizer' in lymphoma and multiple myeloma: an analytic hierarchy process by *ad hoc* working group Gruppo ItalianoTrapianto di Midollo Osseo

A Olivieri[1], M Marchetti[2], R Lemoli[3], C Tarella[4], A Iacone[5], F Lanza[6], A Rambaldi[7] and A Bosi[8] on behalf of the Italian Group for Stem Cell Transplantation (GITMO)

Tailored Procedure

Poor Mobilizer

LVL o vLVL, obiettivo: raccolta di cell CD34+ in dose utile al trapianto

Nuove strategie di mobilizzazione cellulare

Large-volume leukapheresis yields more viable CD34+ cells and colony-forming units than normal-volume leukapheresis, especially in patients who mobilize low numbers of CD34+ cells

J.F. Abrahamsen, S. Stamnesfet, K. Liseth, T. Hervig, and O. Bruserud

TRANSFUSION 2005;45:248-253.

Tailored Procedure

Super Mobilizer

Sp Conta CD34+ > 80 - 100x10^6/L

Yeld CD34+ ≥ 8x10^6/Kg

Bone Marrow Transplantation (2007) 40, 437–441

www.nature.com/bmt

ORIGINAL ARTICLE

Patients mobilizing large numbers of CD34+ cells ('super mobilizers') have improved survival in autologous stem cell transplantation for lymphoid malignancies

BJ Bolwell[1], B Pohlman[1], L Rybicki[2], R Sobecks[1], R Dean[1], J Curtis[1], S Andresen[1], A Koo[1], V Mineff[1], M Kalaycio[1] and JW Sweetenham[1]

Tailored Procedure

- Super Mobilizer
  - LVL, ???
    - Eccesso di risultato
      - in cellule CD34/Kg
      - in volume
      - in numero di unità satelliti
      - stoccaggio sovranumerario

Tailored Procedure

Normal Mobilizer

Sp Conta CD34+ > 20 < $80x10^6$/L

Yeld CD34+ ≥ 2 ≤ 8 $x10^6$/Kg

1° LVL

2° Standard

3° Tailored

Parametri

Patologia di base

Programma trapiantologico

- Singolo trapianto
- Tandem

Parametri antropometrici

Stato clinico – fase recovery

- Supporto trasfusionale

Quando?

Monitoraggio **CD34+ / WBC**

Definizione ottimale della finestra di raccolta, timing of collection

cells CD34+ **threshold level**: **$20x10^6/L$**

single platform ISHAGE protocol for absolute enumeration of CD34+ cells.

Keeney M, Chin-Yee I, Weir Karin, Popma Jan, Nayar Rakash, Sutherland DR. Single platform flow cytometric absolute CD34+ Cell count based on the ISHAGE Guidelines. Cytometry 1998;34:61-70

PBPC count

Question 9: Which is the optimal method for peripheral CD34+ cell count?

RECOMMENDATIONS. *An International Society of Hematotherapy and Graft Engineering (ISHAGE) double- or single-platform protocol should be used to count peripheral blood CD34+ cells. In the double-platform protocol, the hematology analyzer should be a last-generation counter with proper linearity, producing accurate WBC differentials and preferentially endowed with a dedicated analytical option: it should be maintained daily and calibrated.*

A viability test should be performed in samples from the harvested products.

All centers routinely performing CD34+ cell enumeration should undergo externally driven quality control (QC) programs.

Quanto?

Question 7: Which is the target PBPC dose?

RECOMMENDATIONS. *The minimum PBPC dose to be collected and infused to assure a low transplant-related morbidity is 2×10^6/kg/body weight CD34+ cells per planned transplant.*

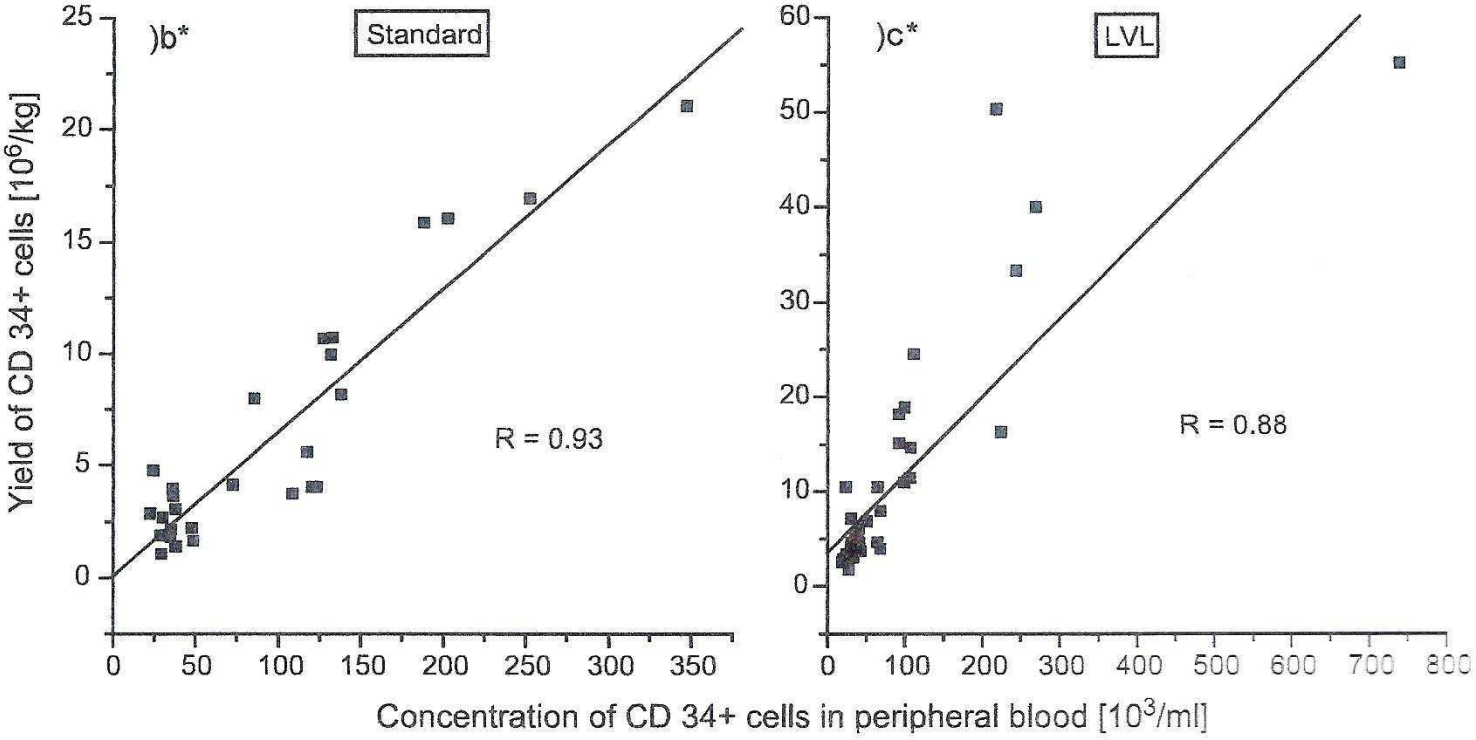
The optimal PBPC dose to be collected and infused to assure a prompt hematopoietic recovery is 5×10^6/kg/body weight CD34+ cells per planned transplant.

The highest PBPC dose to be infused in patients with acute myeloid leukemia is 7×10^6/kg/body weight CD34+ cells, due to a reduced event-free survival at higher doses.

Numero di cellule **CD34+ sp pre** aferesi correla con il numero di cellule **CD34+ raccolte** nella singola leucoaferesi

Correlazione lineare tra CD34+ yeld e CD34+ threshold: R=0.93 – 0.88 (Standard - LVL)

CD34+ sp $20x10^6$/L cell CD34,
volume ematico processato 150 – 200 mL /Kg,
raccolta CD34+ ≥ $1.5x10^6$/Kg



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TRANSFUSION AND APHERESIS SCIENCE

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PBPC collection techniques: standard versus large volume leukapheresis (LVL) in donors and in patients

Zdenka Gašová [a,*], Iuri Marinov [b], Šárka Vodvářková [c], Martina Böhmová [a], Zdeňka Bhuyian-Ludvíková [a]

Graft, CD34+/Kg: **$4\text{-}6 \times 10^6$**

dose soglia (CD34+/Kg body weight ideale): $\geq$ **2×10^6**.

Dosi cellulari inferiori correlano con un tempo di recovery (neutrofili $\geq 500 \times 10^9$/L, piastrine $\geq 20 \times 10^9$/L) superiore alle 2 settimane

dose massima: **8×10^6**

Algoritmi di previsione:
variabili differenti
conta CD34+ sp,
CE%,
valore di emoglobina,
tempo di procedura,
flusso
differenti gradi di accuratezza (60% - 80%)
differenti variabili

previsione delle resa in CD34+
per la singola raccolta

Humpe A, Rigger J, Meineke I, Kurz M, Eil A, Storkebaum B, Binder C, Munzel U, Funke I, Hocker P, Wiesneth M, Kholer M.
A cell-kinetic model of CD34+ cell mobilization and harvest: development of a **predictive algorithm** for CD34+ cell yeld in PBPC collections.
Transfusion 2000;40:1363-1370

Delamain MT, Marques J, A. de Souza C, Lorand-Metze I, Metze K.
An **algorithm** based on peripheral CD34+ cells and hemoglobin concentration provides a better optimization of apheresis than the application of a fixed CD34 threshold.
Transfusion 2008;48:1133-1137

Accurate prediction of autologous stem cell apheresis yields using a double variable-dependent method assures systematic efficiency control of continuous flow collection procedures

L. Pierelli,[1] M. Maresca,[2] N. Piccirillo,[2] S. Pupella,[3] M. Gozzer,[3] M. L. Foddai,[4] M. Vacca,[5] G. Adorno,[6] U. Coppetelli[7] & U. Paladini[8]

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Vox Sanguinis (2006) **91**, 126–134

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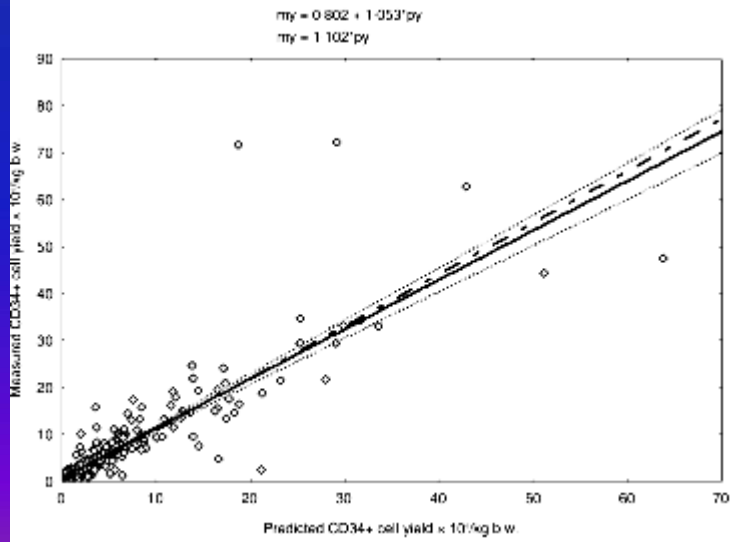

Hence,

$$\text{CD34+ cells per kg b.w. collected} = \frac{\text{CD34+ cells per ml of PB} * [40] * \text{(ml of PB processed per kg b.w.)}}{[100]}$$

$$\text{CD34+ cells per kg b.w. collected} = \text{CD34+ cells per ml of PB} * 0{\cdot}4 * \text{(ml of PB processed per kg b.w.)}$$

Definiscono

QUANDO / QUANTO

Obiettivo TARGET CD34+/Kg

RISULTATO ATTESO
Procedure di raccolta rispondenti ai criteri clinici, procedurali con un buon rapporto costo / efficacia

integrazione tra componenti clinical – collection – manipolation

Personalizzazione della raccolta di HPC-A

Focus dalla PROCEDURA al PAZIENTE

CLINICAL

COLLECTION

MANIPOLATION