

**XXVI Congresso Nazionale  
Pavia, 3•5 ottobre 2019  
Università degli Studi di Pavia**

# **Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica**

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**Dipartimento di Oncoematologia e Terapia Cellulare e Genica  
IRCCS Ospedale Pediatrico Bambino Gesù**



**Bambino Gesù**  
OSPEDALE PEDIATRICO



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**Rispetto degli standard EFI**

**Ricadute cliniche legate allo status dell'attecchimento dopo HSCT**



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# Version 7.0 - STANDARDS FOR HISTOCOMPATIBILITY & IMMUNOGENETICS TESTING



<b>E4.11</b>	<b>Haemopoietic Chimaerism and Engraftment (HCE) Monitoring</b>
E4.11.1	Standards E4.5.1.1, E4.5.1.3, E4.5.2, E4.5.3.1 and E4.5.3.2 also apply.
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E4.11.4.2	Specificity
E4.11.5	Donor and patient specific allele profiles must be:
E4.11.5.1	Determined using appropriate reference material
E4.11.5.2	Documented
E4.11.6	Optimal ranges of DNA quantity and purity must be:
E4.11.6.1	Defined
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# **E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring**

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**E4.11.1** Standards E4.5.1.1, E4.5.1.3, E4.5.2, E4.5.3.1 and E4.5.3.2 also apply



## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

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E4.5.1 Nucleic acid extraction

**E4.5.1.1 The method used for nucleic acid extraction:**

E4.5.1.1.1 Must be published and documented

E4.5.1.1.2 Must be validated in the laboratory

E4.5.1.2 Purity and concentration of Nucleic Acids:

E4.5.1.2.1 Must be sufficient to ensure reliable test results

E4.5.1.2.2 Should be determined for each sample, or

E4.5.1.2.3 If not determined for each sample, the laboratory must have tested and validated this policy



## **E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring**

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- E4.5.1.3** If the DNA is not used immediately after purification, suitable methods of storage must be available that will protect the integrity of the material





## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

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### E4.5.2 Electrophoresis

- E4.5.2.1 Optimal electrophoretic conditions must be documented
- E4.5.2.2 The laboratory must establish criteria for accepting each slab or capillary gel migration, and each lane of a gel or capillary injection
- E4.5.2.3 When the size of an amplicon is a critical factor in the analysis of data, size markers that produce discrete electrophoretic bands spanning and flanking the entire range of expected fragment sizes must be included in each gel



## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

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- E4.5.3 Analysis
  - E4.5.3.1 Signal intensity**
    - E4.5.3.1.1 Acceptable limits of signal intensity must be specified for positive and negative results
    - E4.5.3.1.1 If these are not achieved, acceptance of the results must be justified and documented
  - E4.5.3.2 The method of allele assignment must be designated**
  - E4.5.3.3 The allele database must be:
    - E4.5.3.3.1 Documented
    - E4.5.3.3.2 Updated at least once a year with the most current version of the IPD-IMGT/HLA database
  - E4.5.3.4 If a manual allele call or interpretation of positive/negative reactions is performed for SSOP or SSP, two independent interpretations of primary data must be performed, except under justified special emergency situations



# Version 7.0

## STANDARDS FOR HISTOCOMPATIBILITY & IMMUNOGENETICS TESTING



...gran parte degli standard relativi a section B, C, D ed F devono essere comunque rispettati.....

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## **E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring**

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**E4.11.2** The polymorphic gene system(s) used for HCE monitoring must be identified and documented with regards to allelic variability



# E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

 <b>Bambino Gesù</b> OSPEDALE PEDIATRICO	PROCEDURA OPERATIVA <b>Monitoraggio Attecchimento</b>	Cod.: PO 17 LIT
		Data: 15/09/2018
		Ed. 3 Rev. 0
		Pagina 1 di 16

Tutti i contenuti (testi, schemi, immagini) delle procedure, istruzioni, modelli ecc., sono di proprietà esclusiva dell'Ospedale Pediatrico Bambino Gesù (OPBG), e non potranno essere fatti propri, copiati, pubblicati, commercializzati, distribuiti, da parte di utenti o di terzi in senso lato, in assenza della preventiva autorizzazione dell'OPBG.

## SOMMARIO

1	SCOPO E CAMPO DI APPLICAZIONE
2	RIFERIMENTI NORMATIVI
3	DEFINIZIONI E SIGLE
4	DESCRIZIONE DELLE ATTIVITA'
5	DOCUMENTAZIONE
6	RESPONSABILITA'
7	ALLEGATI

## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

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**Markers comunemente utilizzati per studiare il chimerismo emopoietico nelle cellule nucleate**

- NGS (?)
- Real Time PCR
- STR
- VNTR
- FISH analysis (sex mismatched)
- HLA

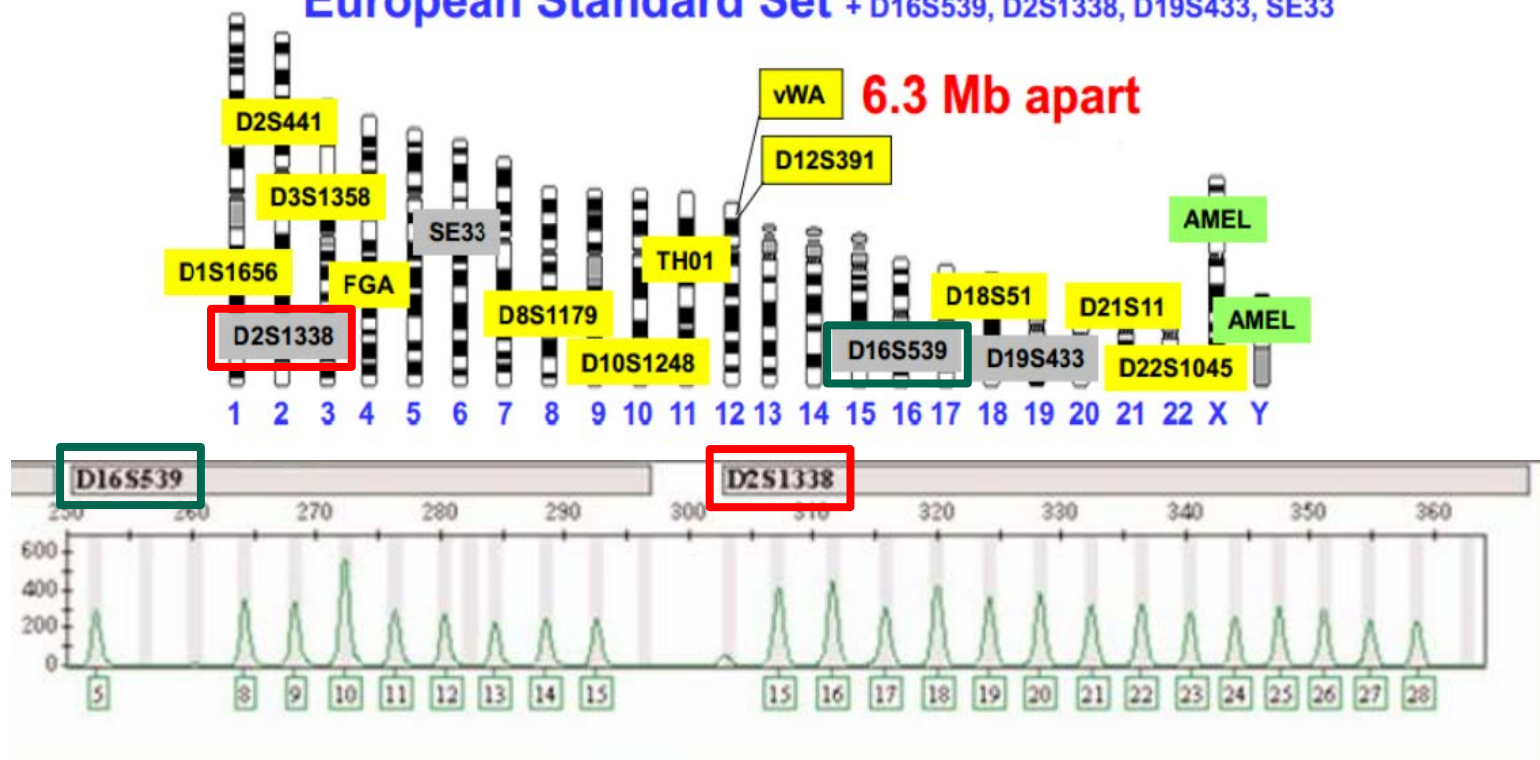


Sensibilità crescente



# E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

European Standard Set + D16S539, D2S1338, D19S433, SE33





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## **E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring**

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## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

 Bambino Gesù OSPEDALE PEDIATRICO	<i>VALIDAZIONE METODICHE E SOFTWARE</i>	Cod.: <i>MD 05 PO 05</i>
		Data: <i>10/01/2010</i>
		Ed. 3 – Rev.
		Pagina 1 di 2

VALIDAZIONE METODICA E DI TIPIZZAZIONE HLA PCR-STR

## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

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Le miscele di DNA sono state eseguite secondo le modalità sotto descritte:

<b>99% DON</b>	99 µl di DNA del DON + 1 µl DNA del PRE
<b>97.5% DON</b>	97.5 µl di DNA del DON + 2.5 µl DNA del PRE
<b>90% DON</b>	90 µl di DNA del DON + 10 µl DNA del PRE
<b>50% DON</b>	50 µl di DNA del DON + 50µl DNA del PRE
<b>10% DON</b>	10µl di DNA del DON + 10 µl DNA del PRE
<b>2.5% DON</b>	2.5 µl di DNA del DON + 97.5 µl DNA del PRE
<b>1% DON</b>	1µl di DNA del DON + 99 µl DNA del PRE



## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

I risultati ottenuti (vedere la tabella 1. allegata) non hanno mostrato differenze significative nei diversi sistemi analizzati.

Il software di interpretazione utilizzato è stato **ABC1.0**  
I kit utilizzati sono stati.

**WXY**

La validazione della metodica ha confermato le seguenti indicazioni:

- Nessuna discordanza tra i risultati attesi e quelli ottenuti è stata rilevata
- L'analisi con il software ha prodotto i risultati attesi
- Tutte le componenti del Kit lavorano in maniera adeguata
- Le attrezzature del laboratorio sono idonee all'utilizzo con la metodica stessa
- Il personale del laboratorio è sufficientemente addestrato all'esecuzione della metodica e alla sua interpretazione

SI NO

<input checked="" type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input type="checkbox"/>



Pertanto a partire dal 4-08-2018 tale metodica può essere utilizzata nella routine del laboratorio.

Contestualmente è stato validato l'utilizzo del software di interpretazione ABC1.0  
v. 3.2.1.



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## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

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E4.11.5 Donor and patient specific allele profiles must be:

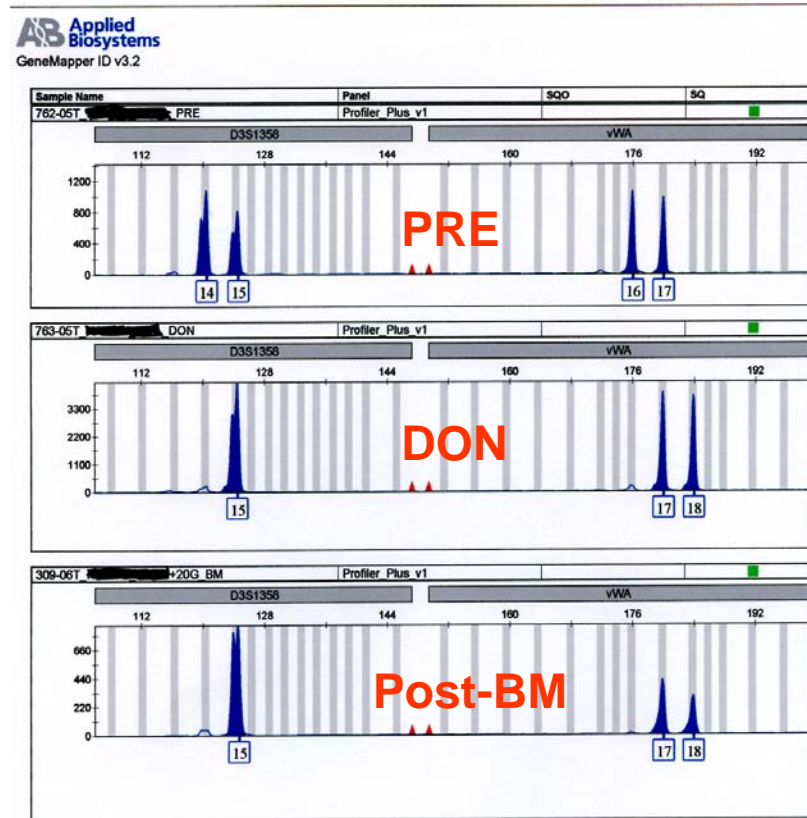
E4.11.5.1 Determined using appropriate reference material

E4.11.5.2 Documented

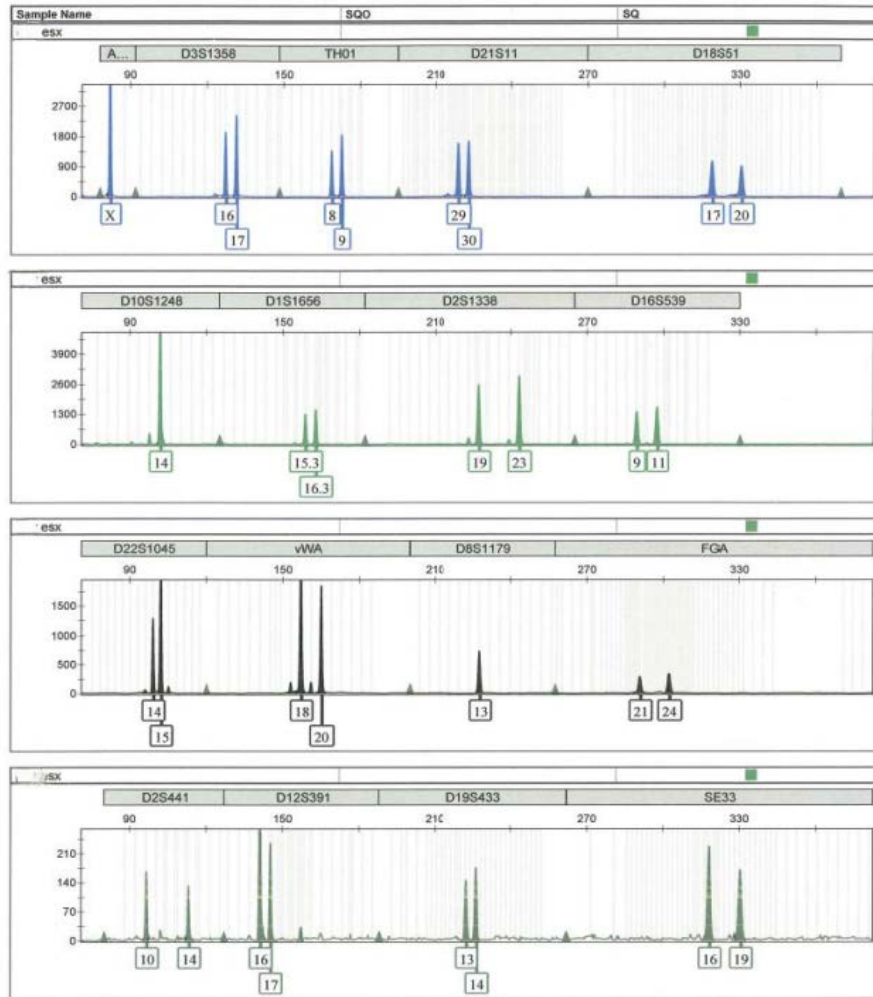




# E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring



# E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring



Locus	Genotype
Amel.	XX
D3S1358	16-17
TH01	8-9
D21S11	29-30
D18S51	17-20
D10S1248	14-14
D1S1656	15.3-16.3
D2S1338	19-23
D16S539	9-11
D22S1045	14-15
vWA	18-20
D8S1179	13-13
FGA	21-24
D2S441	10-14
D12S391	16-17
D19S433	9-18.2
Se33	16-19



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## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

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E4.11.6 Optimal ranges of DNA quantity and purity must be:

E4.11.6.1 Defined

E4.11.6.2 Documented

E4.11.6.3 If a sample falls outside these optimal ranges, a statement must be included in the report



# E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

	PROCEDURA OPERATIVA <b>Monitoraggio Attecchimento</b>	Cod.: PO 31 LIT
		Data: 30/09/2019
		Ed. 3 Rev. 0
		Pagina 1 di 16

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## SOMMARIO

- 1 SCOPO E CAMPO DI APPLICAZIONE
- 2 RIFERIMENTI NORMATIVI
- 3 DEFINIZIONI E SIGLE
- 4 DESCRIZIONE DELLE ATTIVITA'
- 5 DOCUMENTAZIONE
- 6 RESPONSABILITA'
- 7 ALLEGATI

# E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

## 4.3 Validazione della metodica

La metodica di STR-Multiplex è stata validata in laboratorio prima della sua introduzione nella routine. Il processo di validazione è consistito nella valutazione dell'attecchimento con metodica STR-Multiplex in campioni di pazienti post trapianto già analizzati con la metodica FISH. La validazione della metodica fornisce le seguenti indicazioni:

- Tutte le componenti dei kit lavorano in maniera adeguata
- Le attrezzature del laboratorio sono idonee all'utilizzo con la metodica stessa
- Il personale del laboratorio è sufficientemente addestrato all'esecuzione della metodica e a dare una chiara interpretazione dei risultati

La concentrazione ottimale e la purezza del DNA sono state definite nel percorso di validazione

dei test e corrispondono a:

Concentrazione: da 1 a 2.5 ng totali

Purezza: rapporto  $A_{260-280} = 1.6 - 1.9$

Se il campione non rientrasse nel range previsto dalla procedura (esempio sciacquo boccale) ma il test fosse comunque interpretabile (cioè le bande specifiche rientrassero nella distribuzione del Ladder) la variazione dovrà essere segnalata dal report finale.



# Version 7.0 - STANDARDS FOR HISTOCOMPATIBILITY & IMMUNOGENETICS TESTING



Monitoring tests: analysis and reporting	
<b>E4.11</b>	<b>Haemopoietic Chimaerism and Engraftment (HCE) Monitoring</b>
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## **E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring**

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E4.11.7 Criteria for assignment of HCE results, on a qualitative or quantitative basis, must be defined





# E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

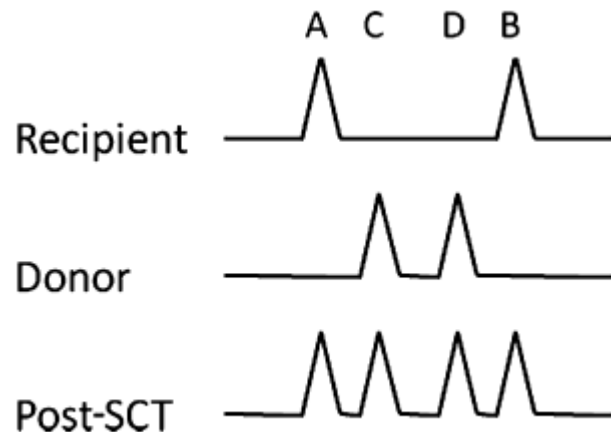
 <b>Bambino Gesù</b> OSPEDALE PEDIATRICO	PROCEDURA OPERATIVA <b>Monitoraggio Attecchimento</b>	Cod.: PO 17 LIT
		Data: 15/09/2018
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## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring



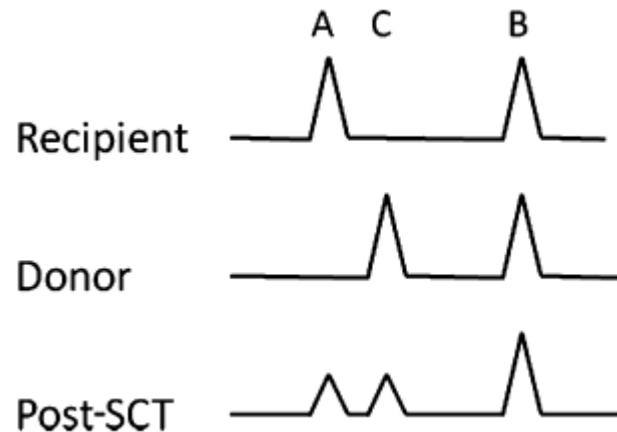
$$\% \text{ chimerism(donor engraftment)} = \frac{(C + D)}{(C + D) + (A + B)} \times 100$$

where the recipient alleles are denoted A and B, and the donor alleles C and D (Fig. 3i). Providing that all other parameters have been accounted for (i.e. stutter, preferential amplification, bleed through, etc.) this constellation is likely to provide the most accurate and reliable result.

It is recommended, where possible, that fully informative markers should be used in the calculations of % Chimerism.



## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring



donor are heterozygous and share one common allele. This constellation is defined as informative. In such instances the shared allele may be disregarded in the calculation:

$$\% \text{ chimerism (donor engraftment)} = \frac{C}{A + C} \times 100$$

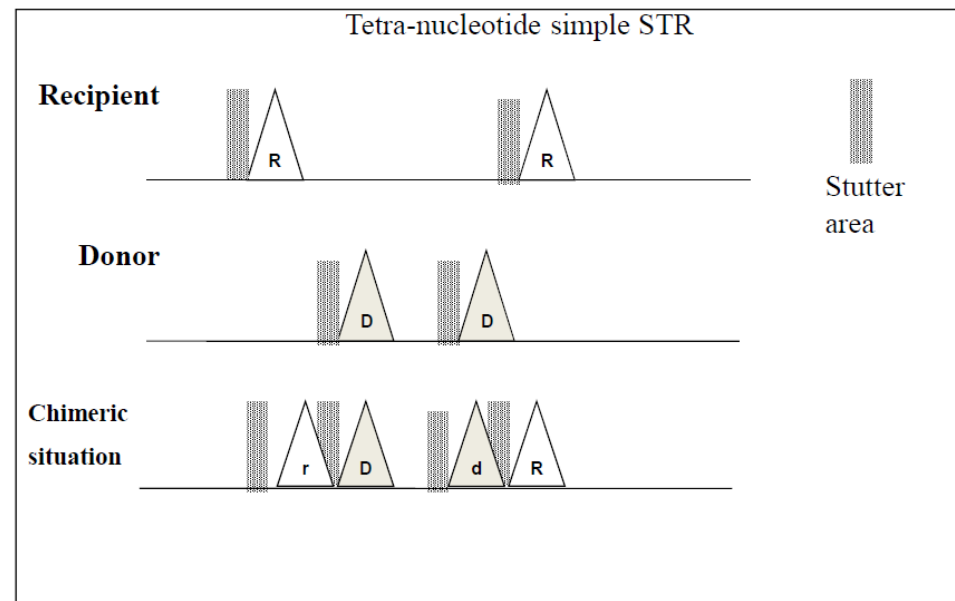


## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

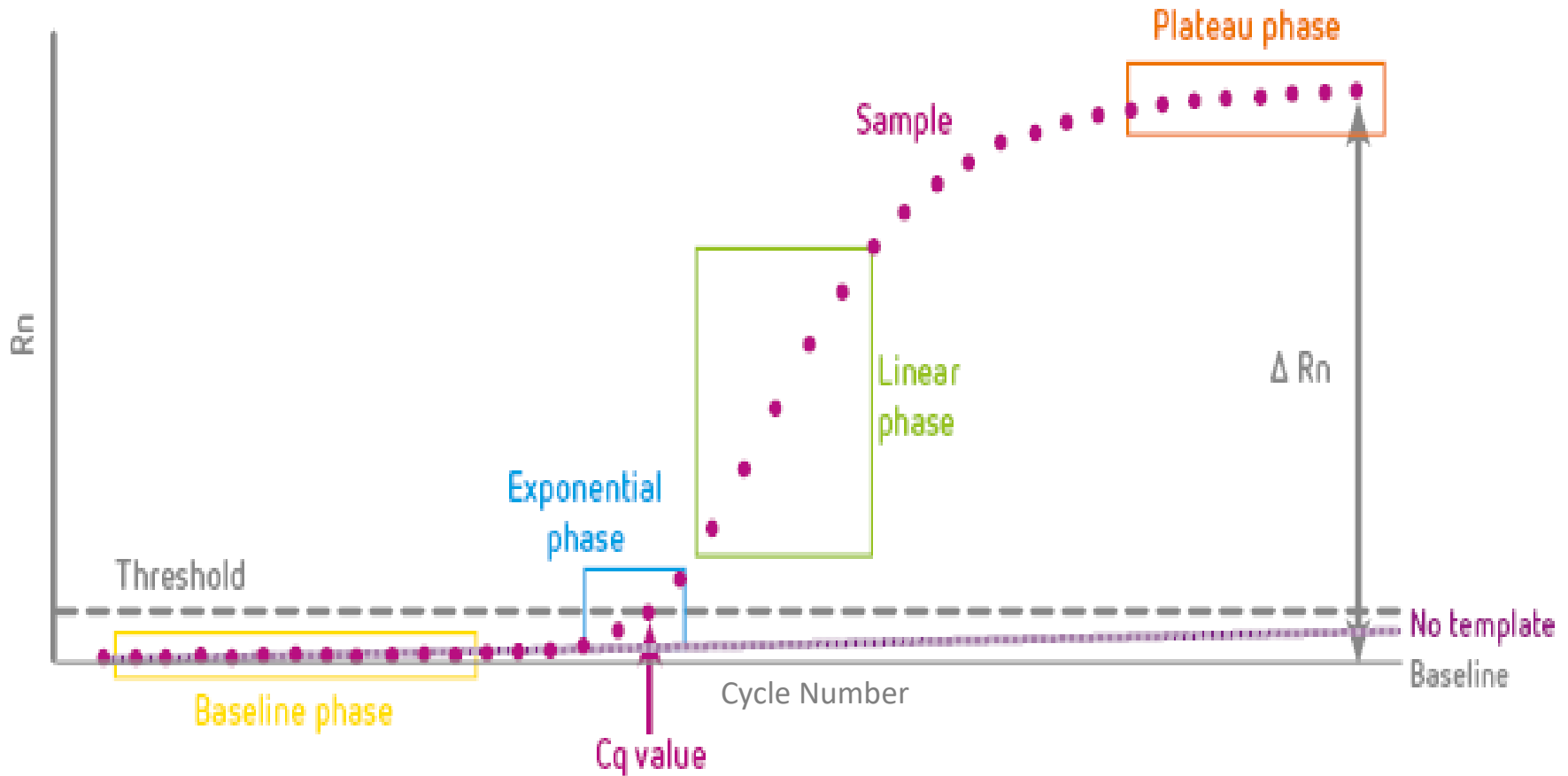
### *Interpretazione di profili STR misti*

Distinguere tra extrabande e alleli: infatti a volte è possibile trovare nell'elettroferogramma di un sistema 3 o più alleli in un locus.

Questo non necessariamente significa che ci si trovi di fronte ad un campione misto, ma gli extrapicchi possono essere causati per esempio dalle “**stutter bands**”, da artefatti non specifici o da errori del software.



## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring



# E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

*Mol Med Rep.* 2016 Oct;14(4):2967-74. doi: 10.3892/mmr.2016.5593. Epub 2016 Aug 4.

## A technical application of quantitative next generation sequencing for chimerism evaluation.

Aloisio M<sup>1</sup>, Licastro D<sup>2</sup>, Caenazzo L<sup>3</sup>, Torboli V<sup>1</sup>, D'Eustacchio A<sup>4</sup>, Severini GM<sup>4</sup>, Athanasakis E<sup>4</sup>.

Table II. List of all main SNPs included in the Ion AmpliSeq custom chimerism panel.

SNP ID	Genome position	Alleles	European heterozygosity	Informativity of recipient allele <sup>a</sup> %
rs12070036	chr1:g.227819514	A/G	0.407	41
rs1234315	chr1:g.173178463	C/T	0.513	37
rs10496711	chr2:g.134516742	C/G	0.407	40
rs12612347	chr2:g.219057338	A/G	0.442	40
rs1984630	chr3:g.134414219	G/T	0.522	36
rs9831477	chr3:g.30693522	A/T	0.483	38
rs10033900	chr4:g.110659067	C/T	0.496	37
rs5335	chr4:g.148463840	C/G	0.492	37
rs983889	chr5:g.15555486	A/C	0.487	38
rs10038113	chr5:g.25902342	C/T	0.469	38
rs552655	chr6:g.13370488	A/G	0.504	37
rs2077163	chr6:g.33636907	C/T	0.460	39
rs39395	chr7:g.103489729	A/G	0.425	40
rs2270188	chr7:g.116140524	G/T	0.496	38
rs10505477	chr8:g.128407443	C/T	0.531	36
rs532841	chr8:g.12957475	C/T	0.549	35
rs2297313	chr9:g.91669362	A/G	0.960	37
rs424539	chr9:g.14442595	C/G	0.467	38

# Version 7.0 - STANDARDS FOR HISTOCOMPATIBILITY & IMMUNOGENETICS TESTING



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## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

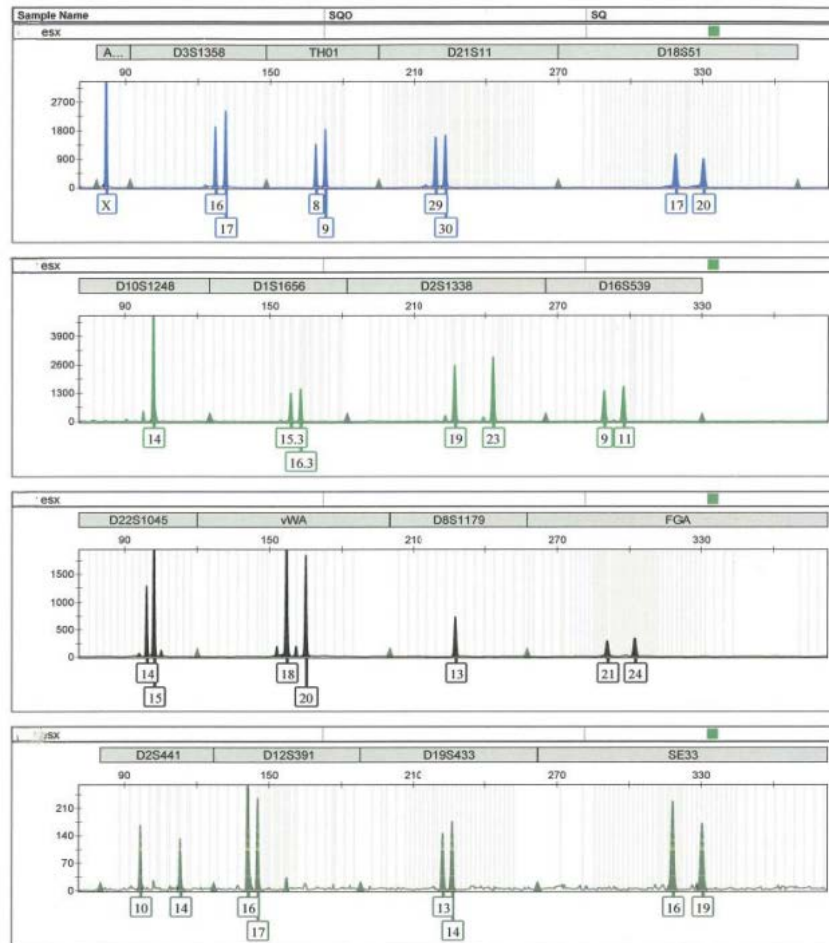
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- E4.11.8 When multiple PCR primers are used in the same tube (multiplex PCR), results must take into account possible amplification bias





# E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring



Vanno eseguiti dei test che tengano conto della possibile amplificazione preferenziale di alcuni alleli rispetto ad altri. Le possibili distorsioni (bias) dovranno essere tenuti in considerazione quando si esegue la quantificazione del chimerismo.



# Version 7.0 - STANDARDS FOR HISTOCOMPATIBILITY & IMMUNOGENETICS TESTING



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## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

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**E4.11.9** When HCE testing is performed on cellular subsets isolated by cell sorting, the purity of the sorted population:

**E4.11.9.1** Must be documented and

**E4.11.9.2** Taken into account in the analysis of the results

**E4.11.9.3** **If this is not possible it must be clearly stated in the report**



# E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

 Bambino Gesù OSPEDALE PEDIATRICO	<i>VALIDAZIONE METODICHE E SOFTWARE</i>	Cod.: <i>MD 05 PO 05 LIT</i>
		Data: 15/09/2018
		Ed. 3 – Rev. 0
		Pagina 1 di 2

VALIDAZIONE PROCEDURA DI SEPARAZIONE DELLE SOTTOPOPOLAZIONI  
LINFOCITARIE CON KIT EASYSEP®

## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

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Nel giorno 04/10/2018 è stata eseguita la validazione su un prelievo di un campione del quale si è proceduto alla separazione delle seguenti sottopopolazioni linfocitarie:

1 campioni: T

1 campione: B

1 campioni: NK

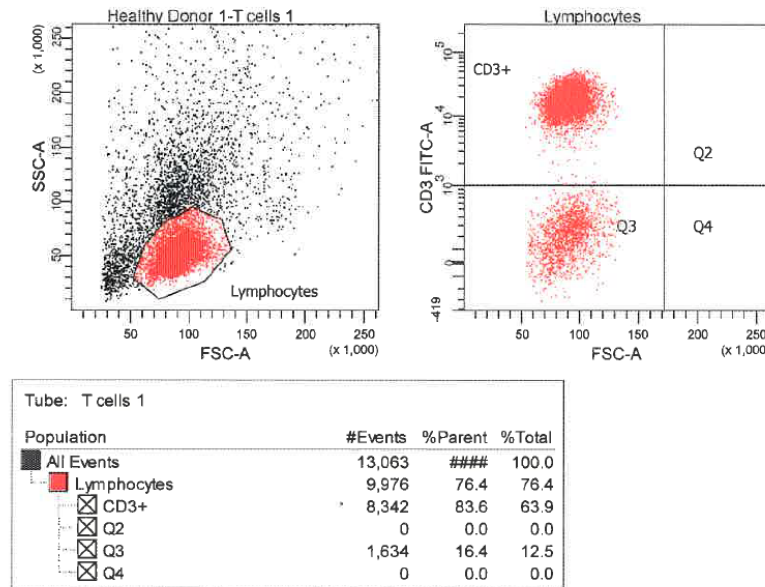
**Il materiale ottenuto è stato poi analizzato mediante Citofluorimetria.**

**I risultati ottenuti sono i seguenti (documentazione allegata):**



# E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

Test purezza popolazioni T cells separate RosetteSep



note, il limite di sensibilità del metodo impiegato è circa il 2%. Nel referto viene segnalato che la purezza delle sottopopolazioni non è valutata nel test in esame, ma dedotta dalla validazione del kit e varia dal 75 al 99%.



## E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

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Tecnica utilizzata:	Analisi STR mediante Sequenziatore Automatico ABI3130xl
Loci analizzati	AMEL,D8S1179,D21S11,D7S820,CSF1PO,D3S1358,TH01,D13S317,D16S539,D2S1338,D19S433,vWA,TPOX,D18S51,D5S818,FGA,
Loci informativi	D21S11,D2S1338,D19S433,vWA,TPOX,D5S818,
Risultato:	<b>100% di cellule del donatore</b>
Sottopopolazioni:	<b>Cellule CD19: 100%</b> <b>Cellule CD3: 100%</b> <b>Cellule CD56: 100%</b> <b>Cellule PMN: 100%</b>
Tecnica:	EasySep, ditta: StemCell Technologies
Note:	La sensibilità del metodo impiegato è circa 2%. La purezza delle sottopopolazioni non è valutata nel test in esame, ma dedotta dalla validazione del kit e varia dal 75% al 99%.



# Version 7.0 - STANDARDS FOR HISTOCOMPATIBILITY & IMMUNOGENETICS TESTING



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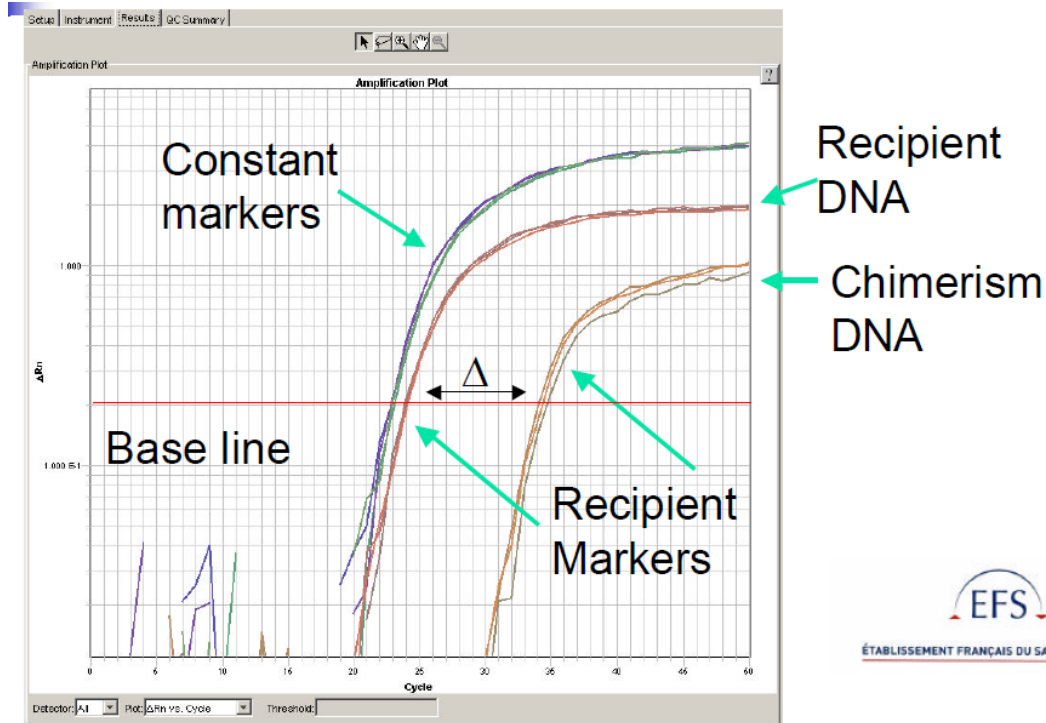
# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica

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# E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring



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E4.11.6.2	Documented
E4.11.6.3	If a sample falls outside these optimal ranges, a statement must be included in the report
E4.11.7	Criteria for assignment of HCE results, on a qualitative or quantitative basis, must be defined
E4.11.8	When multiple PCR primers are used in the same tube (multiplex PCR), results must take into account possible amplification bias
E4.11.9	When HCE testing is performed on cellular subsets isolated by cell sorting, the purity of the sorted population:
E4.11.9.1	Must be documented and
E4.11.9.2	Taken into account in the analysis of the results
E4.11.9.3	If this is not possible it must be clearly stated in the report
E4.11.10	For quantitative HCE monitoring by quantitative PCR (Q-PCR), the following must be defined
E4.11.10.1	Chemistry used
E4.11.10.2	Internal control gene
E4.11.10.3	Thresholds for positive and negative results of each reaction
E4.11.11	All steps of locally developed Q-PCR assays must be validated
E4.11.12	In addition to the requirements from standard F3.5.7, the report must contain
E4.11.12.1	A description of the specimen used for testing (bone marrow, peripheral blood, cellular subsets isolated by cell sorting etc.)
E4.11.12.2	The date of transplant
E4.11.12.3	Other information if deemed relevant for HCE interpretation (i.e. limited informative markers or clinical condition of the patient)



# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica

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E4.11.12 In addition to the requirements from standard **F3.5.7**, the report must contain

**F3.5.7** Information regarding the condition and disposition of specimen that did not meet the laboratory's criteria for acceptability



# **Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica**

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- E4.11.12.1 A description of the specimen used for testing (bone marrow, peripheral blood, cellular subsets isolated by cell sorting etc.)
- E4.11.12.2 The date of transplant
- E4.11.12.3 Other information if deemed relevant for HCE interpretation (i.e. limited informative markers or clinical condition of the patient)



# E4.11 - Haemopoietic Chimaerism and Engraftment (HCE) Monitoring



**Ospedale Pediatrico Bambino Gesù**  
ISTITUTO DI RICOVERO E CURA A CARATTERE SCIENTIFICO  
Dipartimento di Oncoematologia e Terapia Cellulare e Genica  
Direttore : Professor Franco Locatelli  
Laboratorio di Immunogenetica dei Trapianti  
Responsabile: Dr Marco Andreani



Laboratorio accreditato dall'European Federation for Immunogenetics (EFI) N° 07-IT-052.948

Richiedente Referto PTV Tor Vergata di Roma

Data di Stampa: 12/09/2019

Pag: 1 di 1

Provenienza : 11601 S. Paolo - Ambulatorio

ID: 01563942

Data prelievo: 10/09/2019

N. Caso: 1912-2019

Data ricezione Campione 10/09/2019

ID: Famiglia 7862 PAZIENTE

Richiesta: 09101497 del 10/09/2019

## Valutazione di attecchimento

**Materiale Biologico:**

**Sangue Periferico**

**Tecnica Utilizzata**

Analisi STR mediante Sequenziatore Automatico ABI3130xl

**Loci analizzati**

AMEL,D8S1179,D21S11,D7S820,CSF1PO,D3S1358,TH01,D13S317,D16S539,D2S1338,D19S433,vWA,TPOX,D18S51,D5S818,FGA  
D8S1179,D7S820,CSF1PO,D3S1358,TH01,D13S317,D16S539,D2S1338,D2S433,vWA,TPOX,D18S51,D5S818,FGA

**Loci Informativi**

**Risultato**

100% di cellule del donatore

**Note**

La sensibilità del metodo impiegato è circa il 2%

**Data TMO**

18/04/2019

**gg POST TMO**

145

A description of the specimen used for testing (bone marrow, peripheral blood, cellular subsets isolated by cell sorting etc.)

The date of transplant

Other information if deemed relevant for HCE interpretation (i.e. limited informative markers or clinical condition of the patient)



# **Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica**

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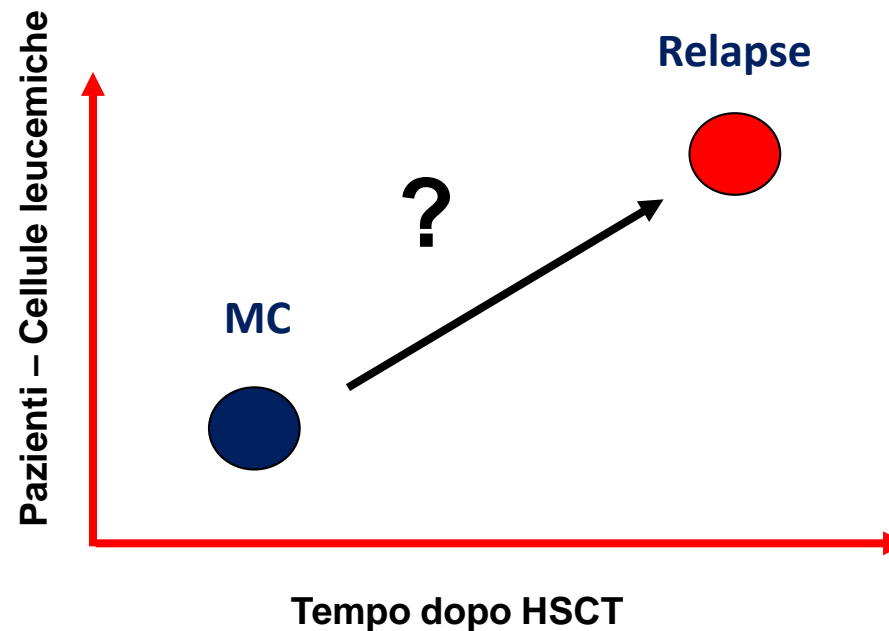
Rispetto degli standard EFI

**Ricadute cliniche legate allo status dell'attecchimento dopo HSCT**





# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica

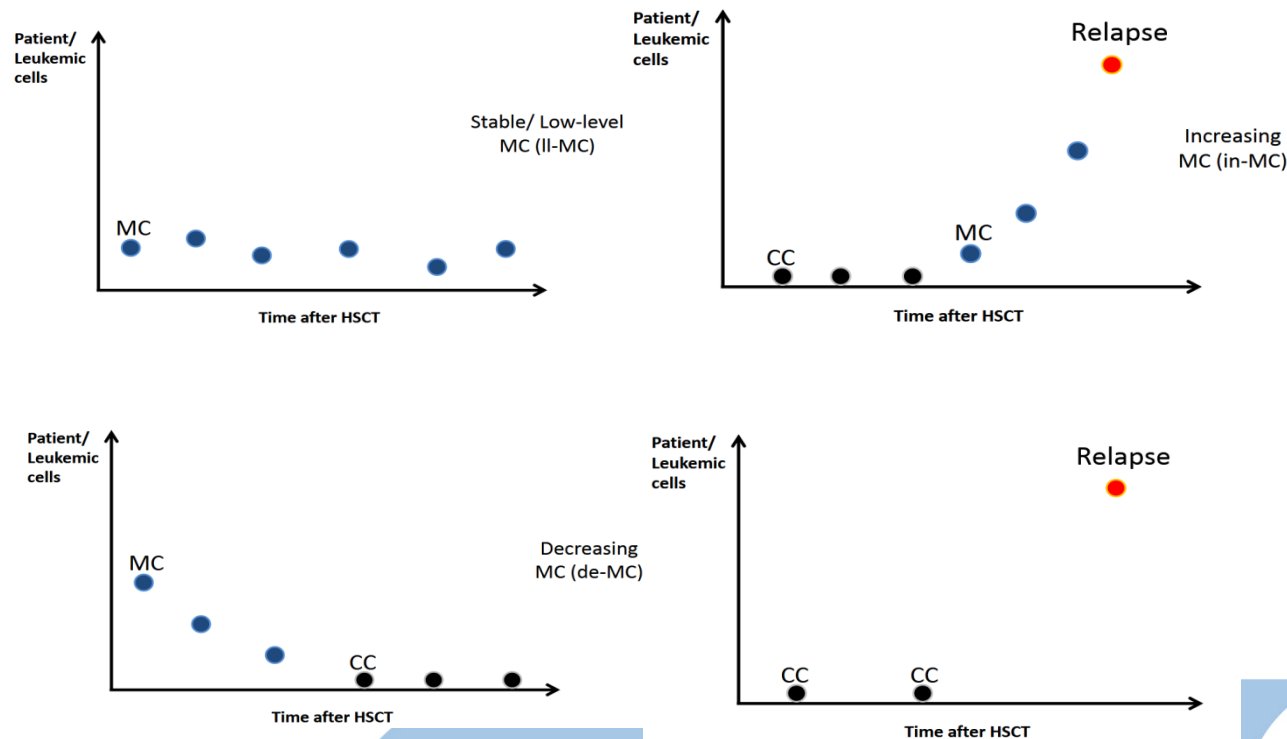


# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica

[Bone Marrow Transplant](#), 2007 Mar;39(5):285-91. Epub 2007 Jan 29.

**Chimerism analysis within 6 months of allogeneic stem cell transplantation predicts relapse in acute myeloid leukemia.**

[Huisman C<sup>1</sup>](#), [de Weger RA](#), [de Vries L](#), [Tilanus MG](#), [Verdonck LF](#).

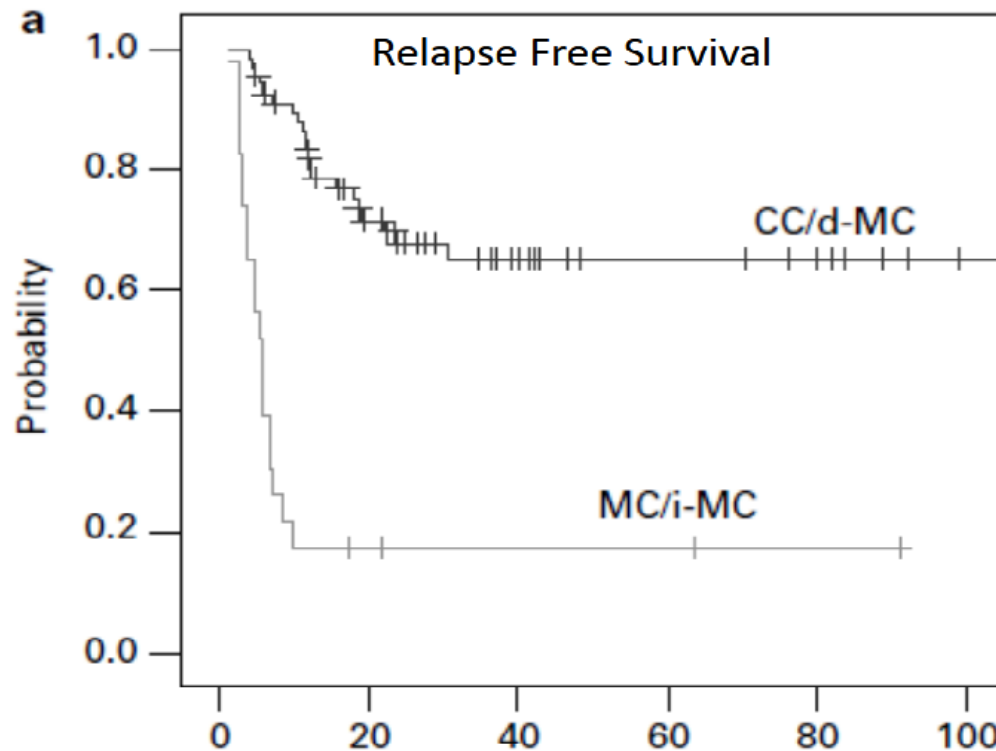


Huisman C et al. Bone Marrow Transplant. 2007



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OSPEDALE PEDIATRICO

# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica



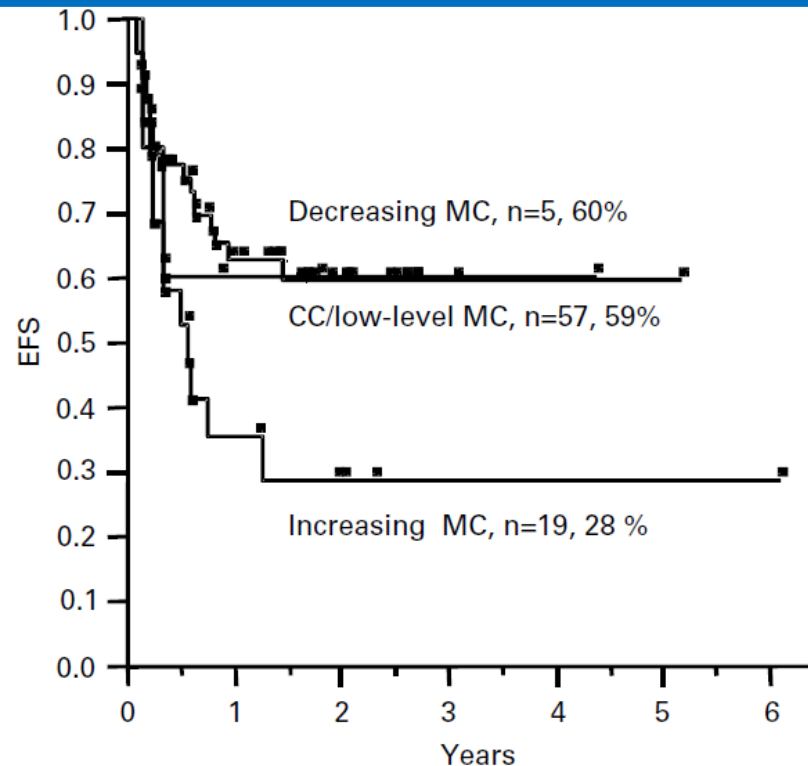
In campioni biologici prelevati ad 1 e 6 months dopo HSCT, la presenza di CC/decreasing MC era significativamente correlata ad un ridotto rischio di realapse e di mortalità rispetto a pazienti con MC/increasing MC.

Huisman C et al. Bone Marrow Transplant. 2007



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# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica



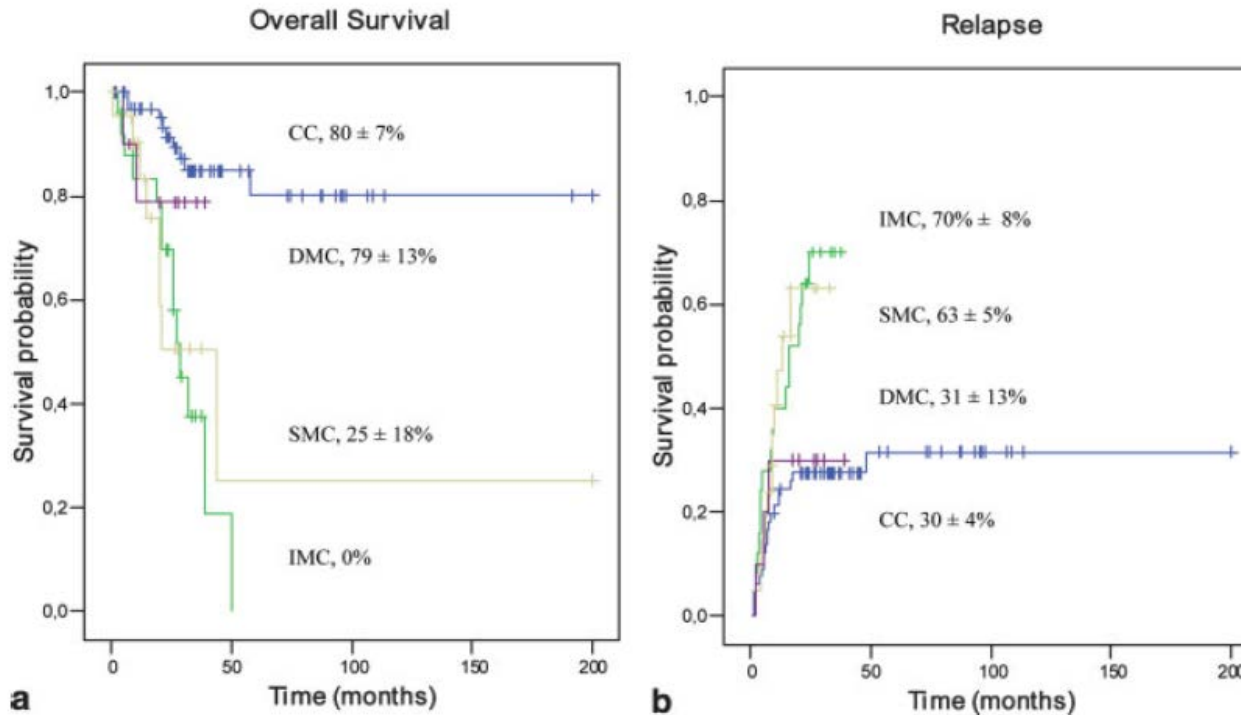
Bader P et al. Bone Marrow  
Transplant. 2004 Apr;33(8):815-21.

# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica

*Am J Hematol*. 2006 Oct;81(10):735-46.

**Quantitative analysis of chimerism after allogeneic stem cell transplantation by real-time polymerase chain reaction with single nucleotide polymorphisms, standard tandem repeats, and Y-chromosome-specific sequences.**

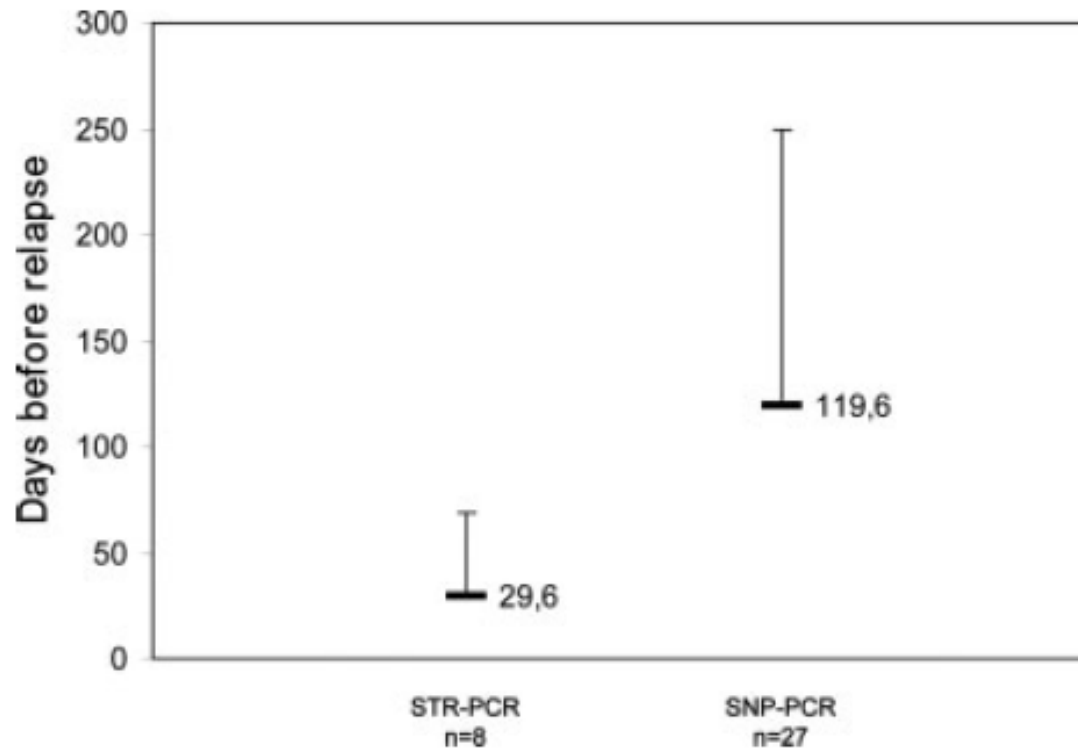
Koldehoff M<sup>1</sup>, Steckel NK, Hlinka M, Beelen DW, Elmaagacli AH.



Overall survival probability (a) and relapse probability (b) in the complete donor chimerism (CC), increasing mixed chimerism (IMC), stable mixed chimerism (SMC), and decreasing mixed chimerism groups (DMC),



# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica



Subsequently, the higher sensitivity of SNP-PCR resulted in an earlier detection of MC in 42 patients ( $P < 0.0001$ ), many of whom relapsed to a later point in this study. Using realtime SNP-PCR we detected a MC in patients 119.6 days (mean) prior to the occurrence of relapse compared to 29.6 days prior to the time point of relapse by STR-PCR using agarose or polyacrylamide gel resolution.

Koldehoff M et al. Am J Hematol.. 2006  
Oct;81(10):735-46.

# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica



Biology of Blood and  
Marrow Transplantation

journal homepage: [www.bbmt.org](http://www.bbmt.org)



Clinical Utility of Quantitative PCR for Chimerism and  
Engraftment Monitoring after Allogeneic Stem Cell  
Transplantation for Hematologic Malignancies



Müberra Ahci <sup>1</sup>, Karin Stempelmann <sup>1</sup>, Ulrike Buttkeireit <sup>2</sup>, Pietro Crivello <sup>1</sup>, Mirko Trilling <sup>3</sup>,  
Andreas Heinold <sup>4</sup>, Nina Kristin Steckel <sup>2</sup>, Michael Koldehoff <sup>2</sup>, Peter A. Horn <sup>4</sup>,  
Dietrich W. Beelen <sup>2</sup>, Katharina Fleischhauer <sup>1,5,\*</sup>

They retrospectively studied commercial qPCR and STR chimerism with respective positivity thresholds of .1% and 1% in **359 peripheral blood (PB) and 95 bone marrow (BM) samples from 30 adult patients after first HLA-matched SCT** for myeloid malignancies or acute lymphatic leukemia.

qPCR predicted all **8/8 relapses** with samples in the 6 months before onset by sustained positivity in both PB and BM compared with **1/8 relapses** predicted by STR mainly in BM.

Biol Blood Marrow Transplant  
23 (2017) 1658–1668



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# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

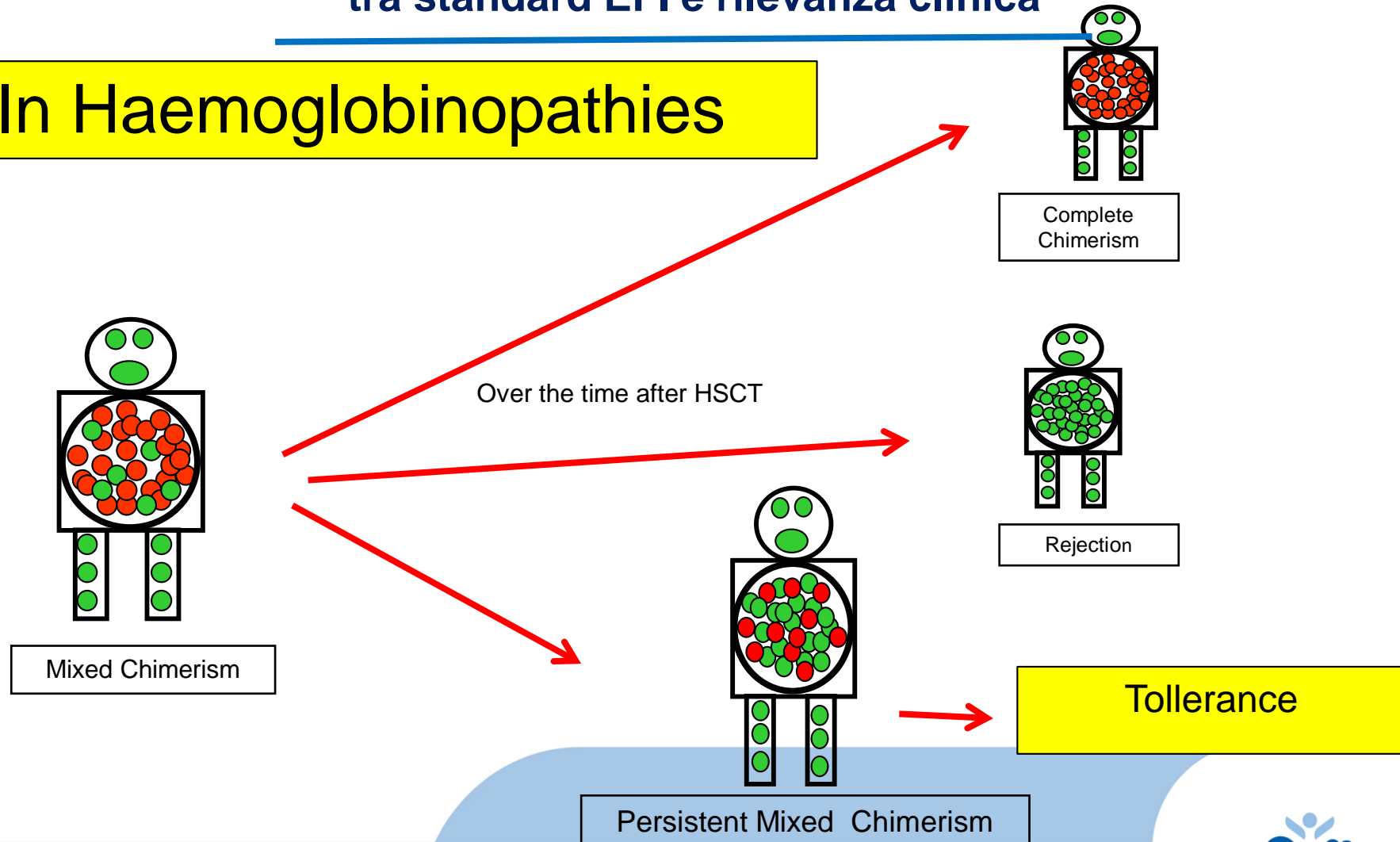
## Loss of Mismatched HLA in Leukemia after Stem-Cell Transplantation

Luca Vago, M.D., Ph.D., Serena Kimi Perna, M.D., Monica Zanussi, B.Sc.,  
Benedetta Mazzi, B.Sc., Cristina Barlassina, B.Sc., Maria Teresa Lupo Stanghellini, M.D.,  
Nicola Flavio Perrelli, B.Sc., Cristian Cosentino, B.Sc., Federica Torri, B.Sc.,  
Andrea Angius, Ph.D., Barbara Forno, M.D., Monica Casucci, B.Sc.,  
Massimo Bernardi, M.D., Jacopo Peccatori, M.D., Consuelo Corti, M.D.,  
Attilio Bondanza, M.D., Ph.D., Maurizio Ferrari, M.D., Silvano Rossini, M.D.,  
Maria Grazia Roncarolo, M.D., Ph.D., Claudio Bordignon, M.D.,  
Chiara Bonini, M.D., Fabio Ciceri, M.D., and Katharina Fleischhauer, M.D.



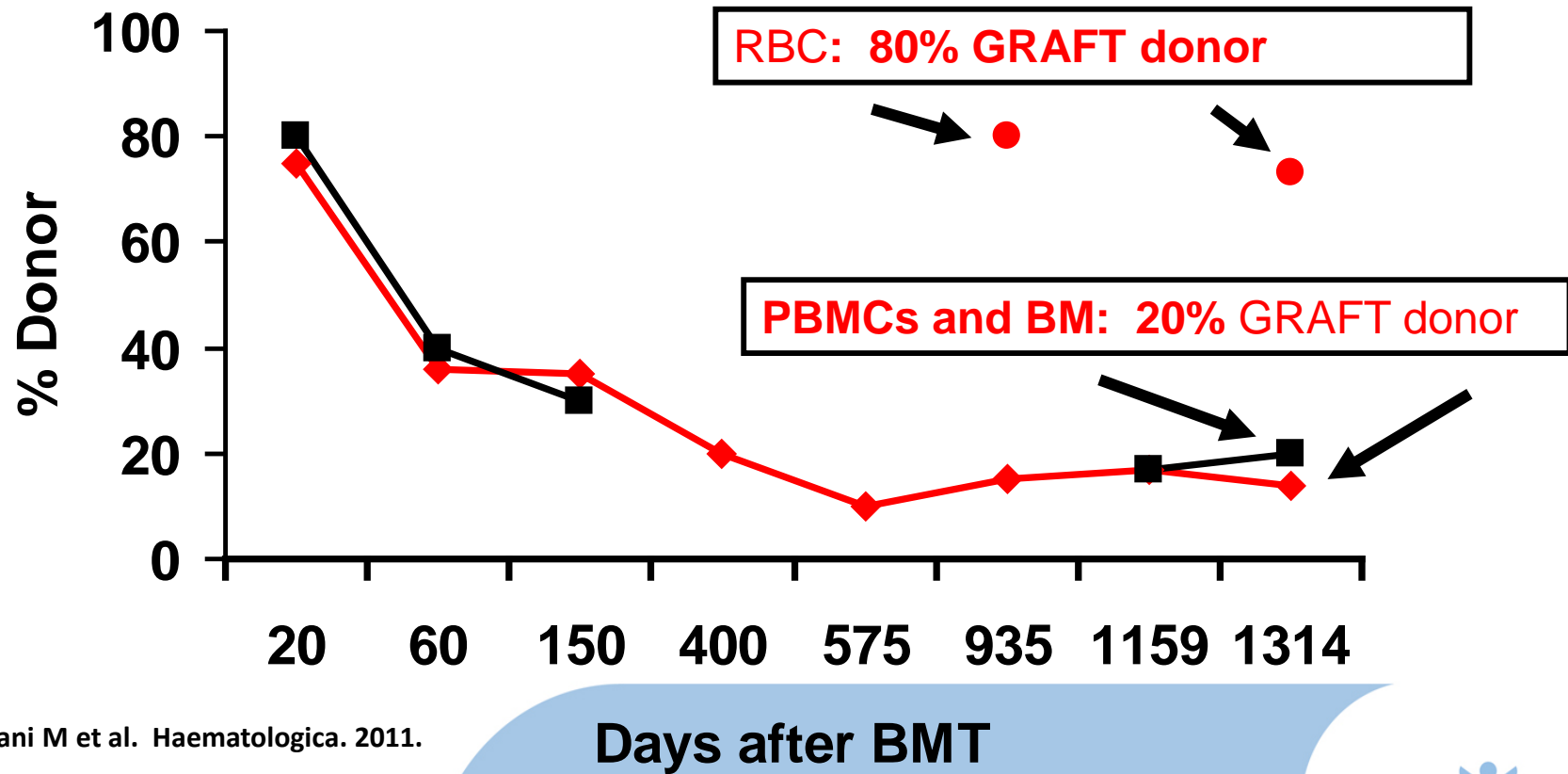
# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica

## In Haemoglobinopathies



## Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica

GH- HSCT: 15-12-2005



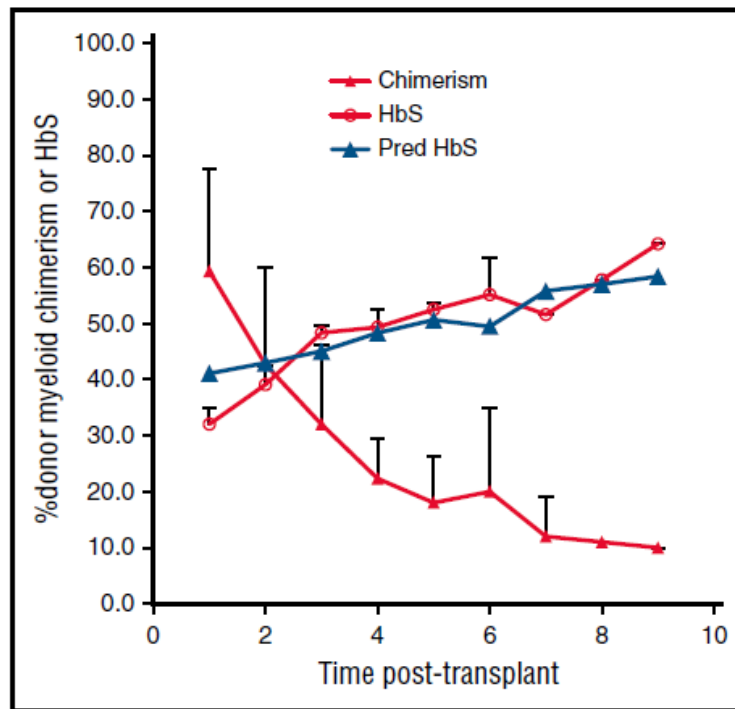
Andreani M et al. Haematologica. 2011.

# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica

Blood. 2017 Oct 26;130(17):1946-1948. doi: 10.1182/blood-2017-03-772392. Epub 2017 Sep 8.

**At least 20% donor myeloid chimerism is necessary to reverse the sickle phenotype after allogeneic HSCT.**

Fitzhugh CD<sup>1,2</sup>, Cordes S<sup>3</sup>, Taylor T<sup>2</sup>, Coles W<sup>2</sup>, Roskom K<sup>1</sup>, Link M<sup>2</sup>, Hsieh MM<sup>2</sup>, Tisdale JF<sup>2</sup>.



At least 20% donor myeloid chimerism is necessary to reverse the sickle phenotype after allogeneic HSCT



# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica



**Extensive multilineage analysis in patients with mixed chimerism after allogeneic transplantation for sickle cell disease: insight into hematopoiesis and engraftment thresholds for gene therapy**

*by Alessandra Magnani, Corinne Pondarré, Naïm Bouazza, Jeremy Magalon, Annarita Miccio, Emmanuelle Six, Cecile Roudaut, Cécile Arnaud, Annie Kamdem, Fabien Touzot, Aurélie Gabrion, Elisa Magrin, Chloé Couzin, Mathieu Fusaro, Isabelle André, Jean-Paul Vernant, Eliane Gluckman, Françoise Bernaudin, Dominique Bories, and Marina Cavazzana*

*Haematologica 2019 [Epub ahead of print]*



## **Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica**

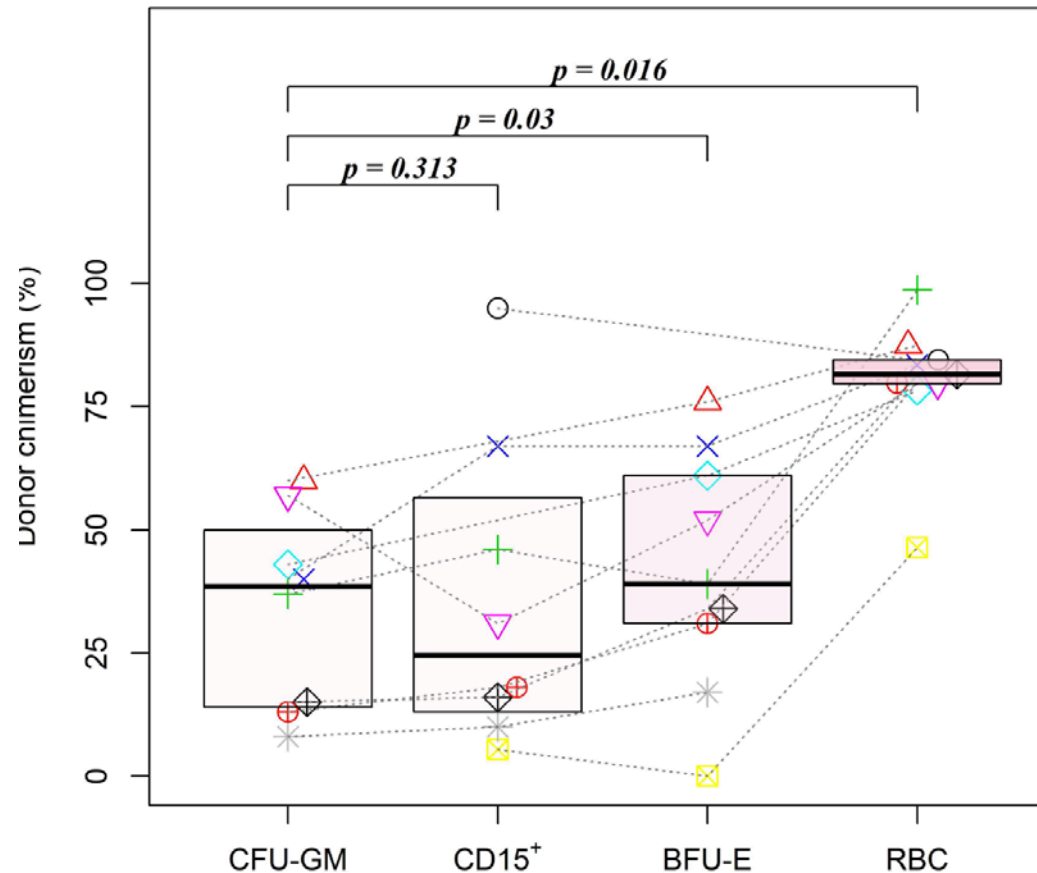
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We analyzed chimerism simultaneously in peripheral erythroid and granulomonocytic precursors/progenitors, highly purified B and T-lymphocytes, monocytes, granulocytes and red blood cells.

Thirty-four patients with mixed chimerism and  $\geq 12$  months of follow-up were included. A selective advantage of donor red blood cells and their progenitors/precursors led to full chimerism in mature red blood cells (despite partial engraftment of other lineages), and resulted in the clinical control of the disease. Six patients with donor chimerism  $< 50\%$  had



# Il monitoraggio dell'attecchimento dopo il trapianto di cellule staminali emopoietiche: tra standard EFI e rilevanza clinica





# Controlli di qualità ISS



MINISTERO DELLA SALUTE  
*Istituto Superiore di Sanità*  
*Centro Nazionale Trapianti*

## Controllo di Qualità 2017

Monitoraggio del chimerismo

Provider: Centro Nazionale Trapianti  
Manager: Francesca Quintieri



Bambino Gesù  
OSPEDALE PEDIATRICO

# Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

**bjh** guideline

**Monitoring of chimerism following allogeneic haematopoietic stem cell transplantation (HSCT): Technical recommendations for the use of Short Tandem Repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group**

Jordan R. Clark,<sup>1</sup> Stuart D. Scott,<sup>1</sup> Andrea L. Jack,<sup>1</sup> Helena Lee,<sup>2</sup> Joanne Mason,<sup>3</sup> Geoffrey I. Carter,<sup>4</sup> Laurence Pearce,<sup>4</sup> Tony Jackson,<sup>5</sup> Hazel Clouston,<sup>6</sup> Anne Sproul,<sup>7</sup> Leigh Keen,<sup>8</sup> Karen Molloy,<sup>9</sup> Najeem'deen Folarin,<sup>10</sup> Liam Whitby,<sup>1</sup> John A. Snowden,<sup>11</sup> John T. Reilly<sup>1</sup> and David Barnett<sup>1</sup>

<sup>1</sup>UK NEQAS for Leucocyte Immunophenotyping, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, <sup>2</sup>Transplantation Laboratory, Manchester Royal Infirmary, Central Manchester University Hospitals NHS Foundation Trust, Manchester, <sup>3</sup>West Midlands Regional Genetics Service, Birmingham's Women's NHS Foundation Trust, Birmingham, <sup>4</sup>Molecular Diagnostics and Immunophenotyping, Nottingham University Hospitals NHS Trust, Nottingham, <sup>5</sup>Northern Genetics Service, Institute of Genetic Medicine, The Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, <sup>6</sup>Sheffield Diagnostic Genetics Service, Sheffield Children's NHS Foundation Trust, Sheffield, <sup>7</sup>Department of Haematology, Western General Hospital, Edinburgh, <sup>8</sup>Histocompatibility and Immunogenetics Laboratory, NHS Blood and Transplant, Filton, UK, <sup>9</sup>St James Hospital, Dublin, Ireland, <sup>10</sup>Haematological Medicine, Rayne Institute, King's College Hospital NHS Foundation Trust, London, and <sup>11</sup>Department of Haematology, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK





# Linee Guida

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## **“INDICAZIONI TECNICHE” PER LO STUDIO DEL CHIMERISMO POST-TRAPIANTO DI CSE**

**ver.1.1 - 2016**

**a cura del Gruppo di Lavoro per il Chimerismo AIBT**

**AIBT** ASSOCIAZIONE ITALIANA  
DI IMMUNOGENETICA  
E BIOLOGIA DEI TRAPIANTI

### **Partecipanti alla stesura:**

**Loredana Elia**

**Benedetta Mazzi**

**Marcella Margiotta**

**Marco Andreani**

**Carla Cervelli**

### **Coordinatore:**

**Franco Papola**



**Bambino Gesù**  
OSPEDALE PEDIATRICO

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**Tiziana Galluccio**  
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**Annalisa Guagnano**  
**Giuseppe Testa**  
**Andrea Di Luzio**  
**Martina Mangione**

