OSPEDALE SAN RAFFAELE

Sistema Sanitario Regione Lombardia

AIBT Associazione Italiana di Immunogenetica e Biologia dei Trapianti

XXX CONGRESSO NAZIONALE

Il chimerismo oltre gli STR nel trapianto di CSE

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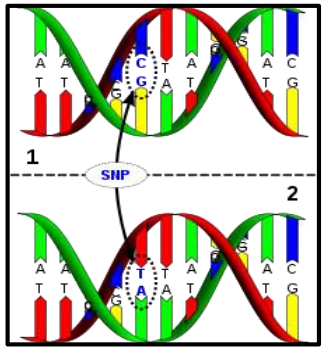
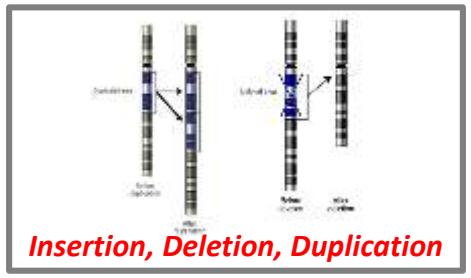
Chimerismo, quali markers?

0.5% variabilità genetica
inter-individuale

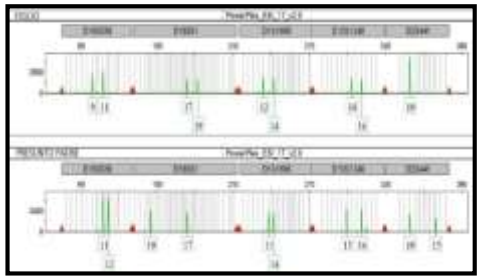
Qualunque differenza genetica tra gli individui può essere usata per analisi di chimerismo



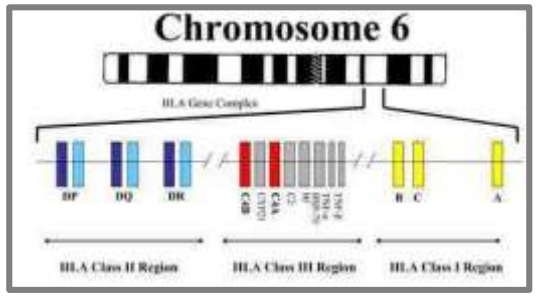
Single Nucleotide Polymorphisms (SNPs)



Copy Number Variations (CNVs)



Short Tandem Repeat (STR)



HLA Polymorphisms

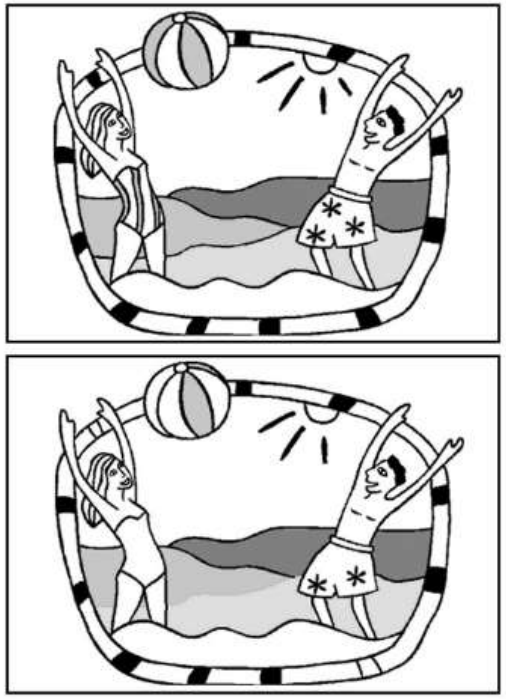
Chimerismo, quali settings?

ogni PERSONA è UNICA …… ogni ALLO-TRAPIANTO di CSE
è un'UNICA COMBINAZIONE di 2 PERSONE
(o più PERSONE per ALLO-TRAPIANTI MULTIPLI)

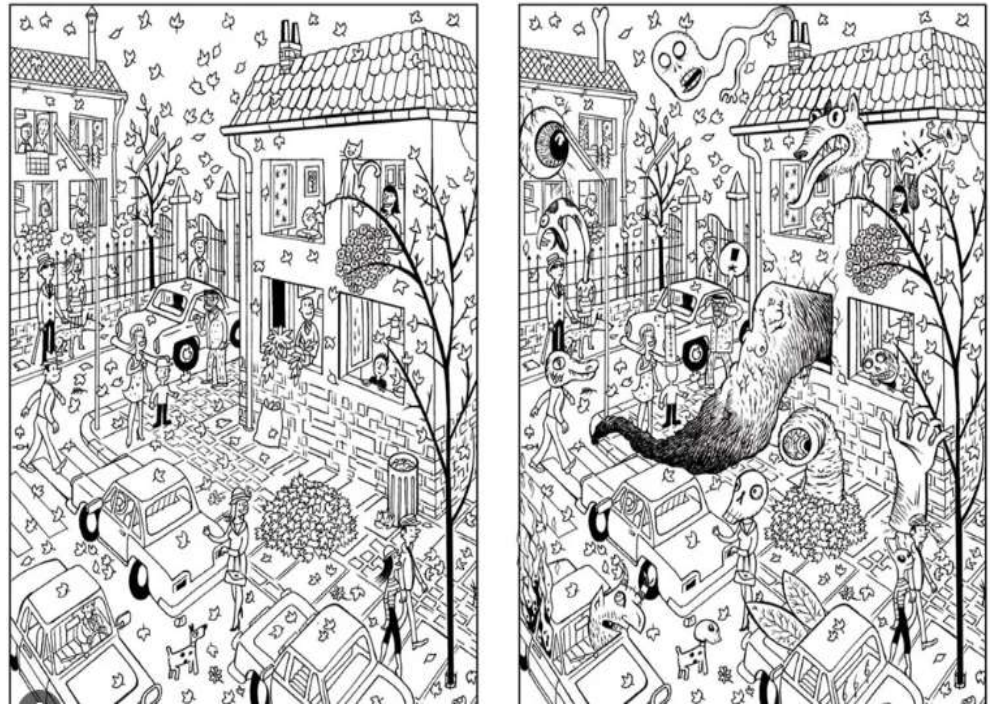
Studio di Chimerismo

Quindi studiare un chimerismo in un paziente trapiantato

vuol dire fare uno **“Studio Personalizzato”** basandosi sulle caratteristiche del paziente pre-trapianto e del/i donatore/i



TROVA LE DIFFERENZE



Chimerismo, quale timing?

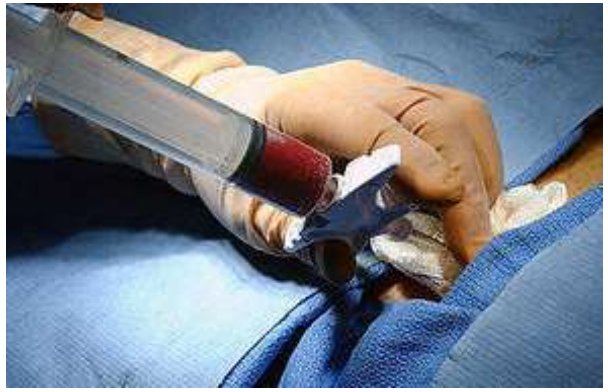
Il Chimerismo post-trapianto è un **FENOMINO DINAMICO**

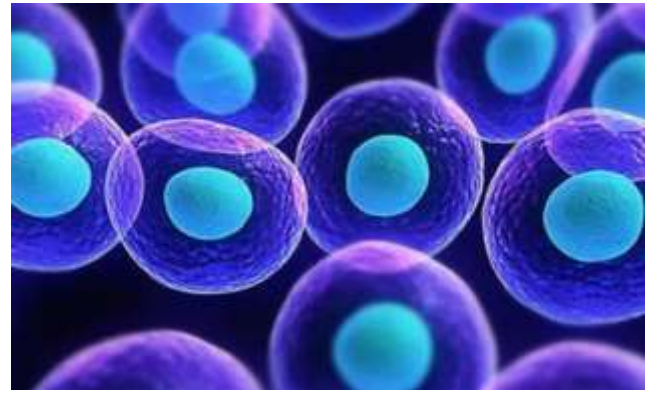
…… **cambia nel tempo**

Studiare la **Cinetica di Chimerismo** e le **Variazioni di Chimerismo**

Chimerismo, quali campioni?

- Sangue periferico
- Sangue midollare
- Sottopopolazioni cellulari (beads, flow)
- Biopsia
- Sciacquo boccale
- Saliva
- ……





Chimerismo, significato clinico

Allo-Trapianto di cellule stamiali

- **Engraftment**
- **Relapse**
- Donor immunological reconstitution on different cell lines
- Graft surveillance for reduced intensity conditionings
- Classification of the relapse: classical or HLA loss
- Monitoring for DLI teatment
- ……

Chimerismo, prospettive e potenzialità

cells

MDPI

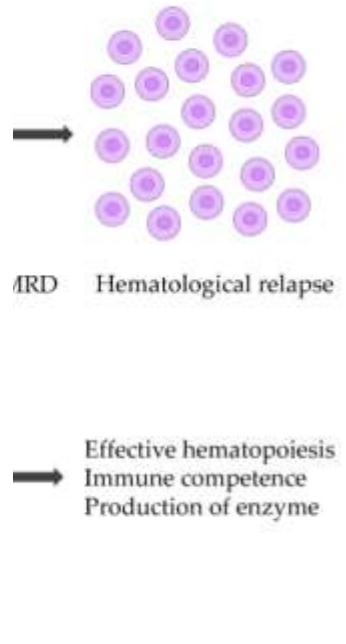
Review

Prospects
Hematopo

Saori Miura [1,2], **K**

Cells **2024**, *13*, 993

Cell Type	Markers	Property	Applications	Advantages	Disadvantages	References
Whole blood	–	PB	Routine use Engraftment confirmation Diagnosis of graft failure	Easy to obtain sample Less manipulation	Low sensitivity Low specificity	[7,8,63]
Bulk marrow	–	BM	Engraftment confirmation Diagnosis and prediction of graft failure	High sensitivity Useful for leukopenic patients	Low specificity	[46,51]
T cells	CD3 CD4, CD8	PB	Surrogate for graft rejection or relapse	High frequency of MC Widely available data	Indirect for hematopoietic reconstitution	[8,47,57,60,61,63–69]
Myeloid cells	CD33 CD14, CD15, CD66b	BM PB	Surrogate for relapse of AML, MDS	Best information of hematopoietic origin	Limited available data	[13,56,63,65,70–72]
HPCs	CD34	BM	Surrogate for relapse of AML, MDS, ALL	High sensitivity for predicting relapse	Difficulty in obtaining sample	[68,73]



Chimerismo, prospettive e potenzialità

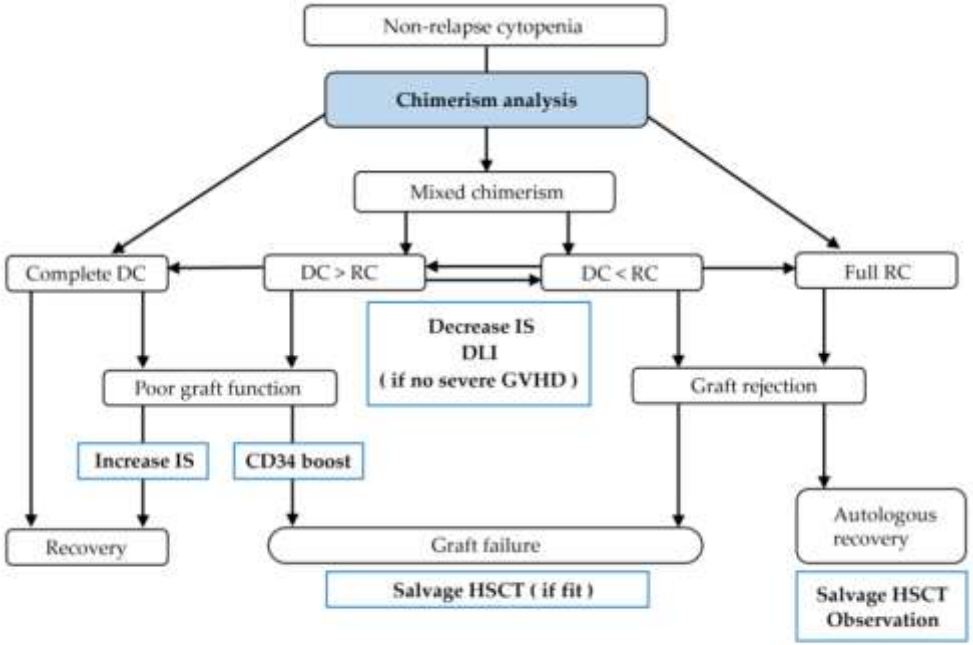


Figure 2. Interventions for non-relapse cytopenias after allo-HSCT. DC, donor chimerism; RC, recipient chimerism; GVHD, graft-versus-host disease; IS, immunosuppressors.

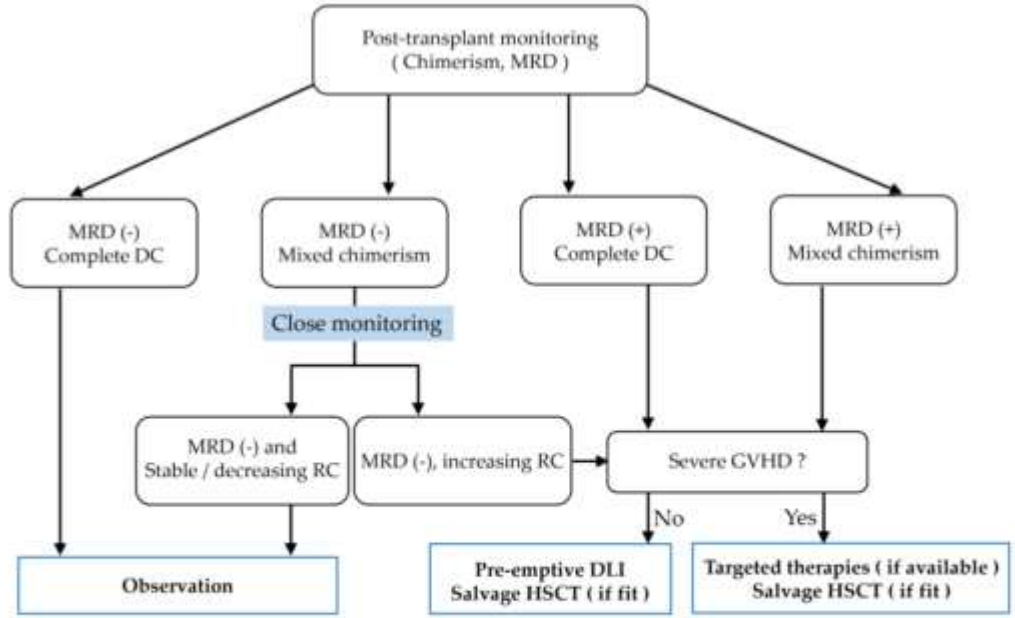


Figure 3. Interventions based on chimerism analysis after allo-HSCT for leukemia in the presence of residual recipient chimerism and/or minimal residual disease (MRD) with a potential to relapse into malignancy. Targeted therapies include tyrosine kinase inhibitors, monoclonal antibodies, and cellular therapies. DC, donor chimerism; RC, recipient chimerism; GVHD, graft-versus-host disease; IS, immunosuppressors; DLI, donor lymphocyte infusion.

水 親 愛 川 山

Chimerismo, quale metodi?

Table 1. Methods of chimerism analysis.

Technique	Applications	Markers	Sensitivity *	Informativity
STR-PCR	Chimerism	STRs	1–5%	=100%
qPCR	Chimerism, MRD	SNPs, indels	=0.1%	90–100%
X/Y FISH	Chimerism after sex-mismatched transplantation	X/Y chromosome	≤5%	≒50%
Digital PCR	Chimerism, MRD	SNPs, indels	0.01–0.1%	90–100%
NGS	Chimerism, MRD	SNPs, indels	0.01–1%	100%

* Detection limits depend on the DNA sample quantity and quality. STR, short tandem repeat; qPCR, quantitative PCR; X/Y FISH, fluorescence in situ hybridization for sex chromatins; NGS, next-generation sequencing; MRD, minimal residual disease.

1. Informativo e specifico
2. Sensibile
3. Quantitativo e accurato
4. Riproducibile

Techniche e Tecnologie

BASATE SU POLIMORFISMI GENETICI

- PCR & elettroforesi capillare
- Real Time PCR
- Droplet digital PCR & Crystal digital PCR
- NGS
- PCR & Spettrometria di massa
- KDM5D mRNA hybridization & flow cytometry

INSTRUMENT, DNA INPUT, THROUGHPUT, TOTAL HAND ON TIME, TOTAL ANALYSIS TIME, ….

Techniche e Tecnologie

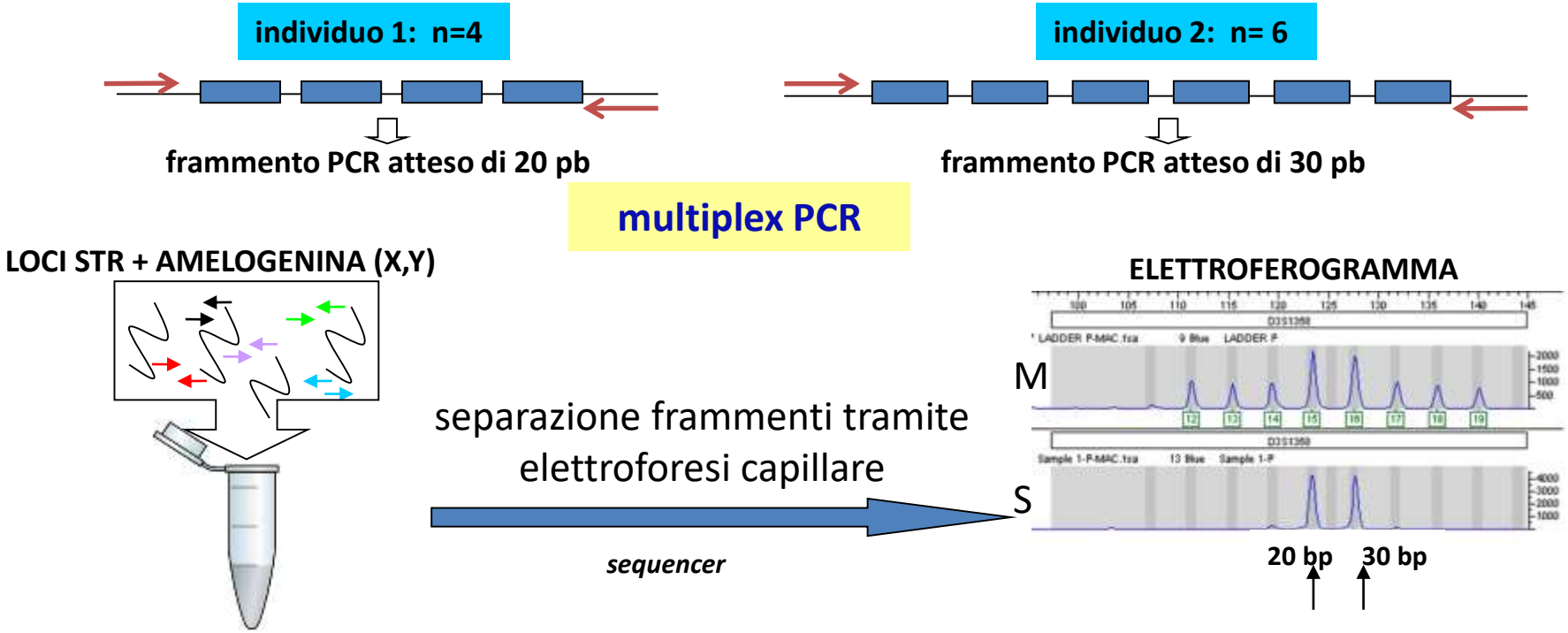
BASATE SU POLIMORFISMI GENETICI

- PCR & Elettroforesi Capillare
- Real Time PCR
- Droplet digital PCR
- NGS

PCR & Elettroforesi Capillare (1)

Short Tandem Reapeats

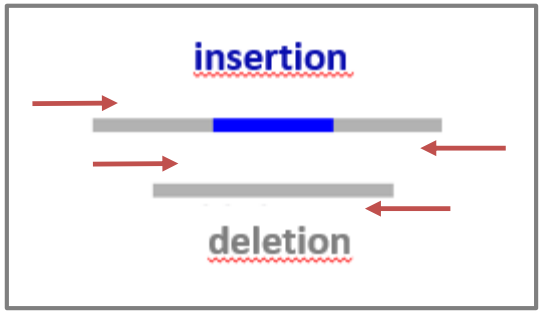
- markers: microsatelliti, sequenze di DNA ripetute altamente polimorfiche + amelogenina (range n. ripetizioni 4-50)
- dimensione frammento ripetuto: 3-7 pb (tetra- & penta nucleotidi, less stutter peaks)
- sensibilità 1-5%
- test di screening & quantificazione



PCR & Elettroforesi Capillare (2)

IN/DEL Polymorphisms

- markers: inserzioni/delezioni + amelogenina
- sensibilità 2-5%
- test di test di screening & quantificazione



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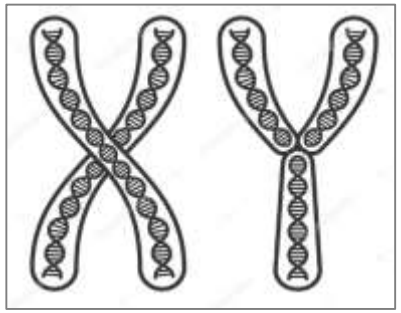
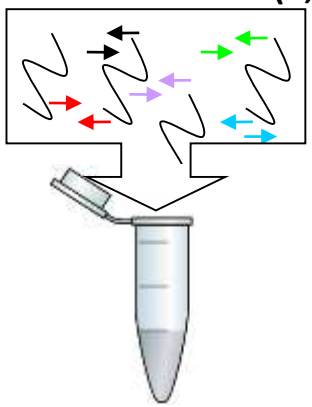


multiplex PCR

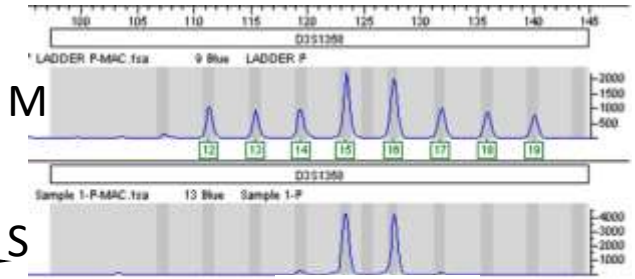
IN/DEL + AMELOGENINA (X,Y)



separazione frammenti tramite elettroforesi capillare

sequencer

ELETTROFEROGRAMMA



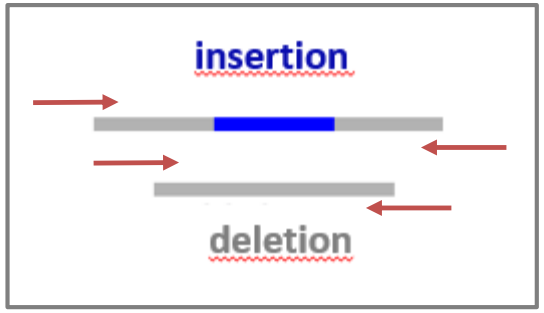
Techniche e Tecnologie

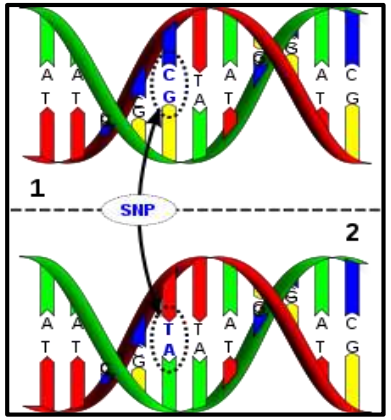
BASATE SU POLIMORFISMI GENETICI

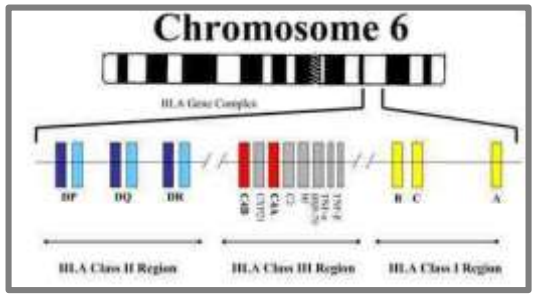
- PCR & Elettroforesi Capillare
- Real Time PCR
- Droplet digital PCR
- NGS

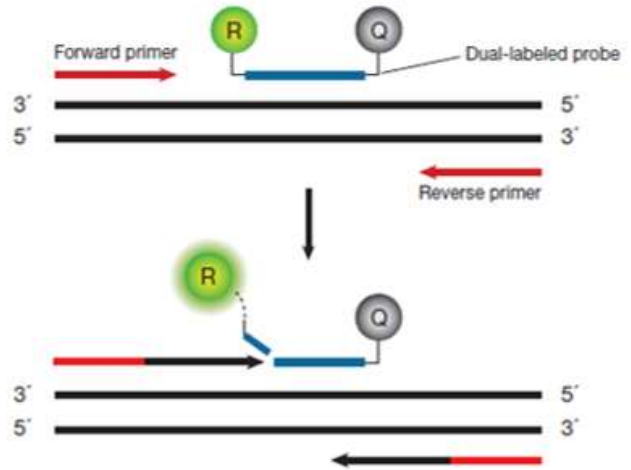
Real Time PCR

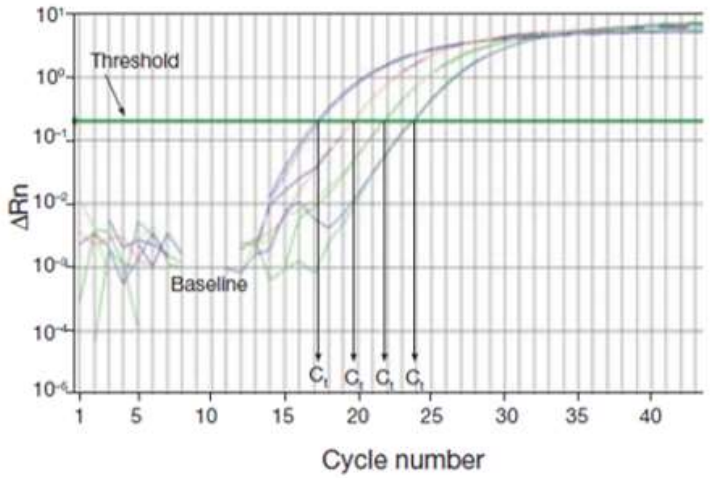
- markers: inserzioni/delezioni & SNPs & HLA
- sensibilità 0.05% in funzione di: input & quality of DNA
- test di test di screening (multiplex and monoplex) & quantificazione (monoplex only)
- quantificazione relativa: $\% = 2^{-\Delta\Delta Ct} \times 100$ (Livak et al. Methods, 2001)











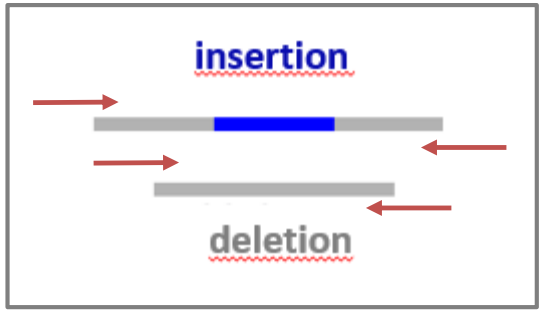
Techniche e Tecnologie

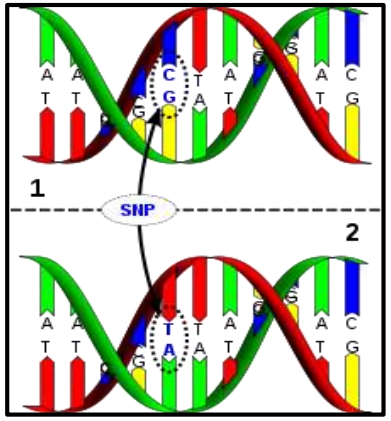
BASATE SU POLIMORFISMI GENETICI

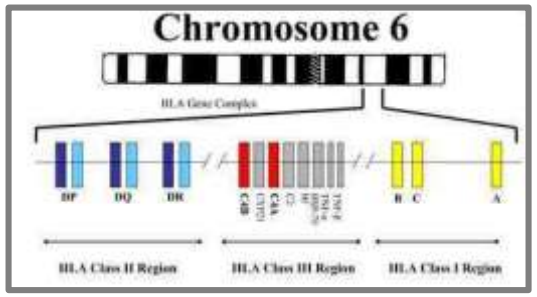
- PCR & Elettroforesi Capillare
- Real Time PCR
- Droplet digital PCR
- NGS

Droplet Digital PCR

- markers: inserzioni/delezioni & SNPs & HLA
- sensibilità 0.05-0,1% in funzione di: input (minore di RT-PCR; maggiore di STR) & quality of DNA
- test di test di screening (ddPCR e STR) & quantificazione (ddPCR)
- quantificazione assoluta con reference gene (duplex PCR), end point PCR – no curva standard







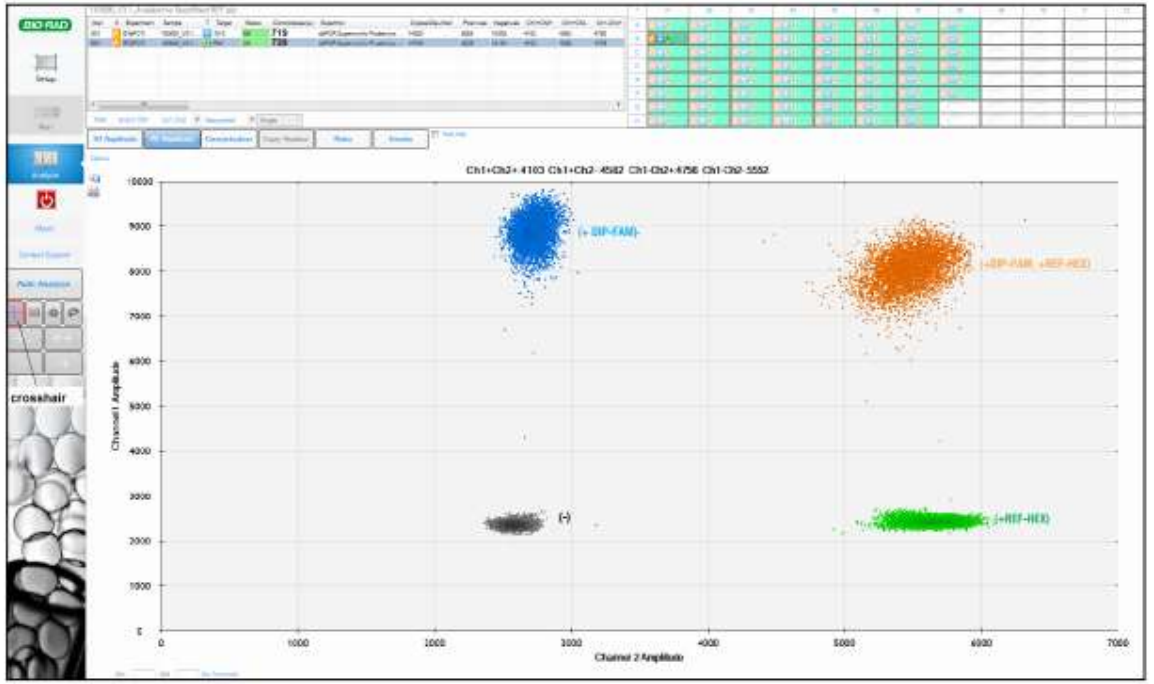


Figure 4 2D amplitude view (scatter plot) of the droplet fluorescence

Poisson statistics

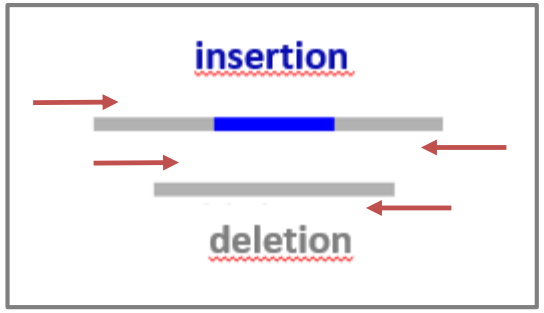
Techniche e Tecnologie

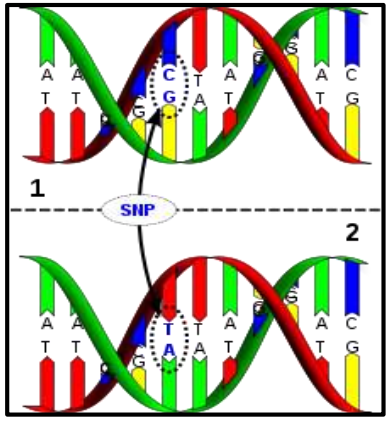
BASATE SU POLIMORFISMI GENETICI

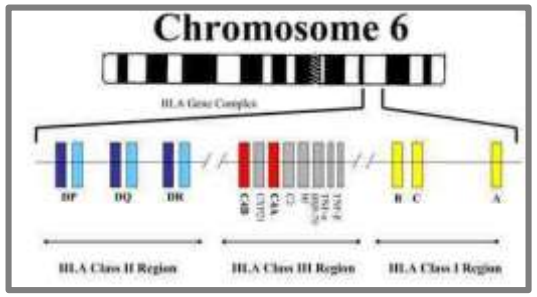
- PCR & Elettroforesi Capillare
- Real Time PCR
- Droplet digital PCR
- NGS

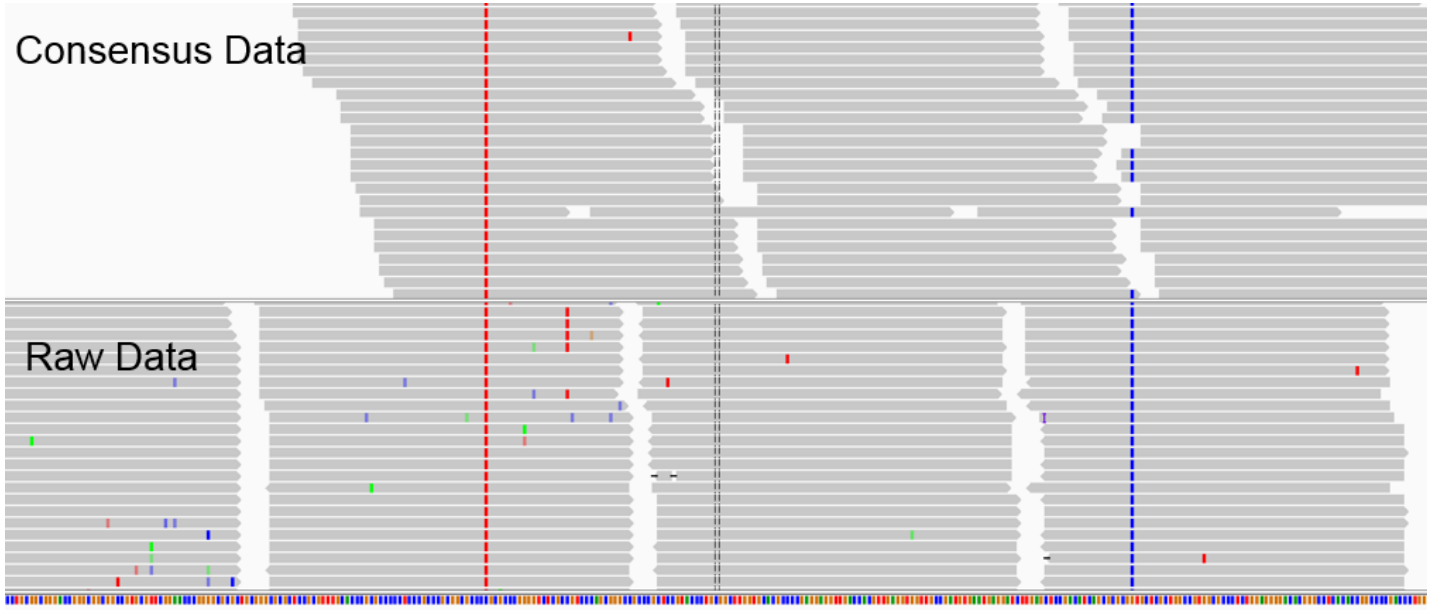
Next Generatio Sequencing

- markers: inserzioni/delezioni & SNPs & HLA
- sensibilità 0.1%
- test di quantificazione (non serve screening test)
- algoritmi di quantificazione,depth of coverage based









Chimerismo e Terapie avanzate

Bone Marrow Transplantation (2020) 55:1229–1239
https://doi.org/10.1038/s41409-020-0822-8

REVIEW ARTICLE

Beyond chimerism analysis: methods for tracking a new generation of cell-based medicines

Joaquim Vives[1,2,3] · Aina Casademont-Roca[1] · Lluís Martorell[1,2] · Núria Nogués[3,4]

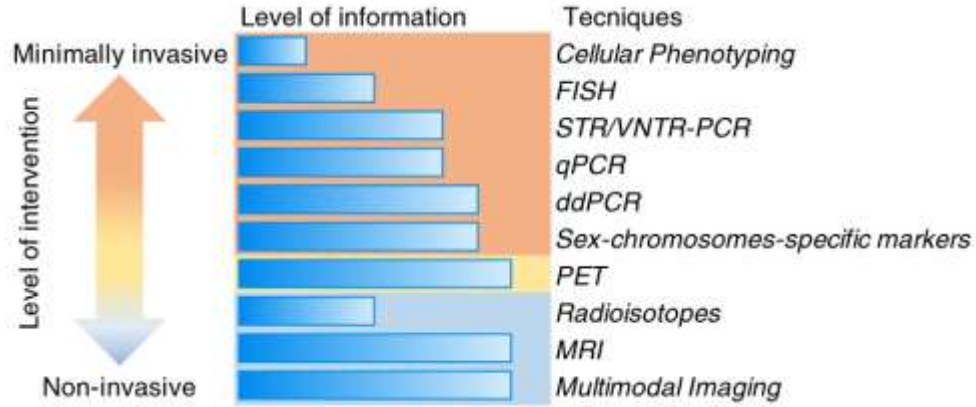
Table 1 Classification of advanced therapy medicines products (ATMP).

Somatic cell therapy medicinal products	Cells that have been subject to substantial manipulation in order to alter their characteristics in a way that can be used for treating, preventing or diagnosing specific clinical conditions.
Gene therapy medicinal products	Insertion, alteration, or removal of genes within individual cells and biological tissues to treat a disease.
Tissue engineered products	"Engineered" cells or tissues having properties for, or used in, or administered to human beings with a view to regenerating, repairing or replacing a human tissue.

According to their composition, ATMP fall in either one or in a combination of any of the following groups or combination thereof. Based on Regulation (EC) no. 1394/2007 and Directive 2001/83/EC.

Table 2 Summary of methodologies available for tracking persistence and biodistribution of advanced therapy pharmaceuticals in the clinical setting.

Technique	Advantages	Concerns	AUTO	ALLO	Informative
Cellular phenotyping	Simple, accurate and very sensitive technique. Validated flow cytometry protocols based on cellular phenotyping are employed to monitor the persistence of CAR-T cells as the efficacy of the treatment is assessed.	Less informative and blood transfusion can lead to confusion. Detects only one cell type.	✗/✓[a]	✓	Low
Digital droplet PCR	Absolute, precise, ultrasensitive quantitation of specific DNA sequences.	Variability of sample preparation and validation of the assay.	✗/✓[a]	✓	High
FISH	Allows for screening of large number of cells with high sensitivity and specificity. Useful in gene-corrected autologous cells.	Labour intensive and requires manual time-consuming counting of cells. Restricted to sex-mismatched HSCT and needs large amount of sample.	✗/✓[a]	✓	High
Sex-chromosome markers	Highly informative in sex mismatch HSCTs.	Useful in case of female donor, cannot be used for sex-matched HSCTs.	✗	✓	High
STR/VNTR	Highly informative and polymorphic regions. Neither sex mismatch nor HLA mismatch dependent.	Low sensitivity due to same primer competition for both minor and major cell population.	✗	✓	High
qPCR	Rapid, robust and quantitive. Highly sensitive technique. Useful in gene-corrected autologous cells.	Costly and efficient mainly with biallelic markers. False positive results possible in SNP-based procedures.	✗/✓	✓	High
NGS	Produces 100-fold more data and at a lower cost compared with Sanger based capillary sequences. Sequences millions of DNA molecules simultaneously.	High complexity of workflow and results. Selection of genes for the NGS panel. Informatics challenges for analysis and clinical reporting.	✓	✓	High
Radioisotopes	Non-invasive technique. Visualisation of tracking and biodistribution of stem cells post-HSCT.	Tracking of stem cells for a limited time, as they have short-half lives. Leakage of radionuclides into non-target cells. Emission of ionising radiation may damage stem cell proliferation and survival.	✓	✓	High
Multimodal imaging	Improve early detection and localisation of chimerism. Enables examining more than one molecule at a time, so that cellular events may be examined simultaneously or the progression can be followed in real time.	The combination of the aforesaid imaging techniques can have the potential to affect each other's performance in their current form.	✓	✓	High
MRI	High spatial resolution. Does not need the use of ionizing radiation.	Cytotoxicity of specified labelling agents. Transference of contrast agents from dead or apoptotic MSCs to macrophages, leading to false positives.	✓	✓	High
PET/CT	Very sensitive technique to evaluate cell viability and accurately quantify both autologous and allogenic cells.	Labelling agents can be expelled by renal and hepatobiliary pathways. Needs the delivery of ionizing radiation.	✓	✓	High

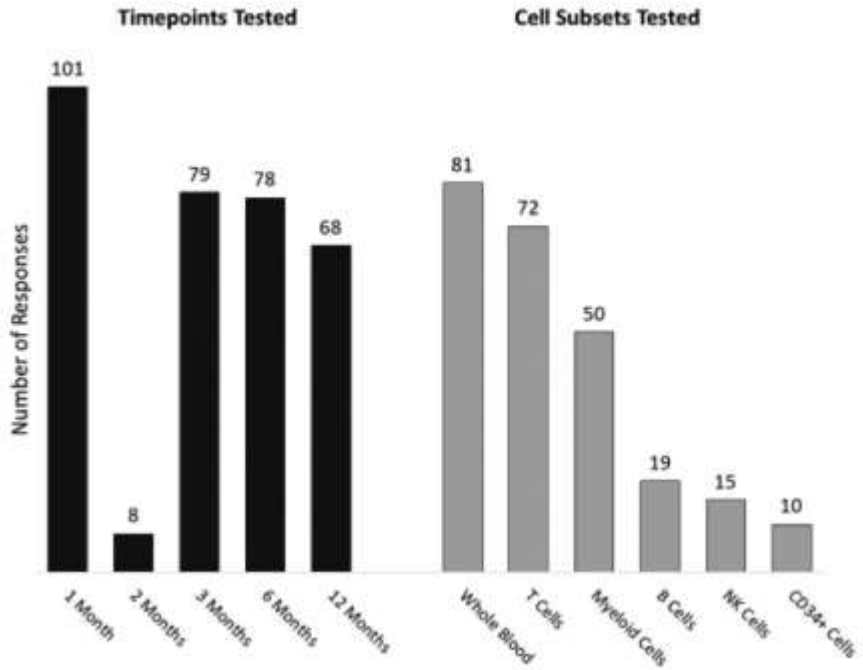


Chimerismo e Clinica: US survey

Bone Marrow Transplant. 2022 March ; 57(3): 347–359. doi:10.1038/s41409-022-01579-9.

Chimerism Analysis for Clinicians: A Review of the Literature and Worldwide Practices

Amanda G. Blouin, MD, PhD[a], Medhat Askar, MD, PhD[a,b,c,d]



Demographics of survey-respondent HCT programs.

Country of Practice	%	N
Europe & UK	19%	20
North America	48%	52
OTHER	33%	36
Patient Population	**%**	**N**
Adults	50%	54
Pediatrics	22%	24
Both	28%	30
Program Size	**%**	**N**
<=50	51%	55
51–100	36%	39
>100	13%	14
Types of Transplants Performed	**%**	**N**
HLA identical	100%	108
HLA haploidentical	99%	107
HLA matched unrelated donor	93%	100
HLA mismatched unrelated donor	75%	81
Single cord blood donor	52%	56
Double/multiple cord blood donors	44%	47
Chimerism Testing Laboratories	**%**	**N**
University Hospital/Academic Institute-based	79%	85
Other hospital-based	13%	14
Private/reference laboratory	6%	7
Government	5%	5

Chimerismo e Clinica: US survey

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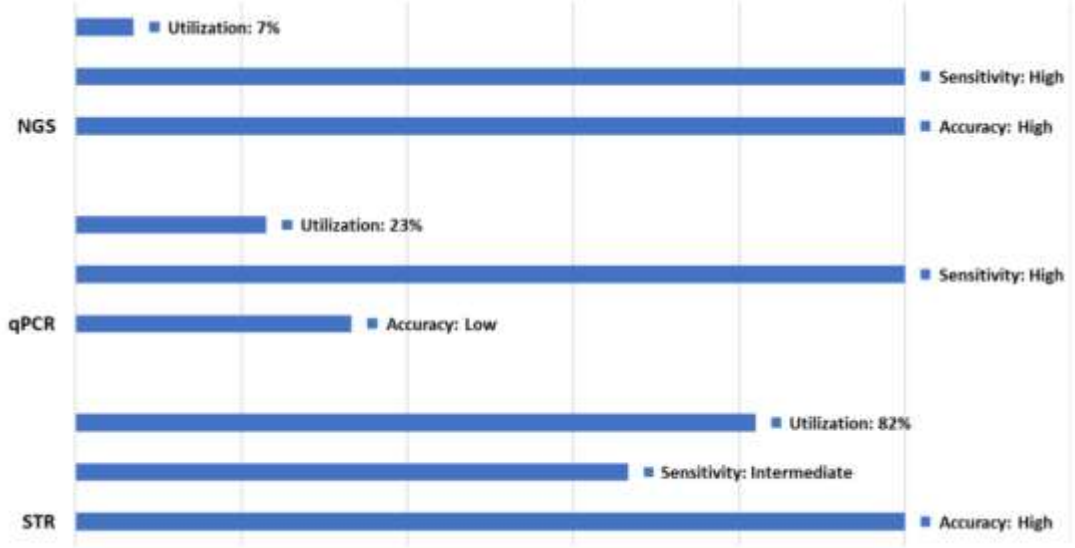


Summary of Chimerism Testing Guidelines.

Indication	Relevance	High Sensitivity Method	Time Points	Testing for HLA Loss Relapse
Engraftment monitoring	Particularly in non-myeloablative transplants and those with increased risk of delayed or failed engraftment	Not necessary	+30 / +60 (for delayed engraftment)	Not relevant
Detection of Relapse	Particularly in diseases with high risk of relapse	Advantageous	When clinically suspected	Advantageous particularly in haplo-transplants

Chimerismo e Linee Guida

Transplantation and Cellular Therapy 27 (2021) 642–649

ELSEVIER

Transplantation and Cellular Therapy

journal homepage: www.tctjournal.org

ASTCT
American Society for Transplantation and Cellular Therapy

Guideline

Standardizing Definitions of Hematopoietic Recovery, Graft Rejection, Graft Failure, Poor Graft Function, and Donor Chimerism in Allogeneic Hematopoietic Cell Transplantation: A Report on Behalf of the American Society for Transplantation and Cellular Therapy

Chimerismo e indicazioni Tecniche

AIBT ASSOCIAZIONE ITALIANA DI IMMUNOGENETICA E BIOLOGIA DEI TRAPIANTI

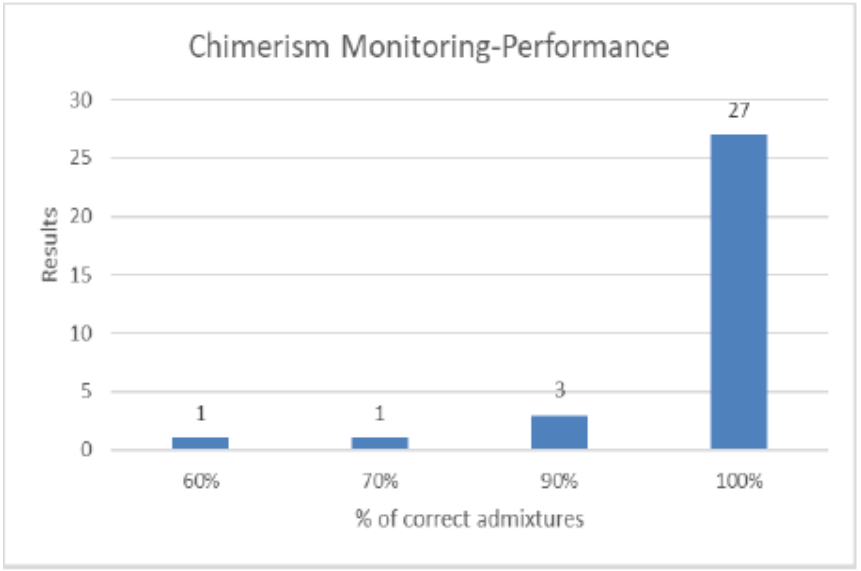
"INDICAZIONI TECNICHE" PER LO STUDIO DEL CHIMERISMO POST-TRAPIANTO DI CSE

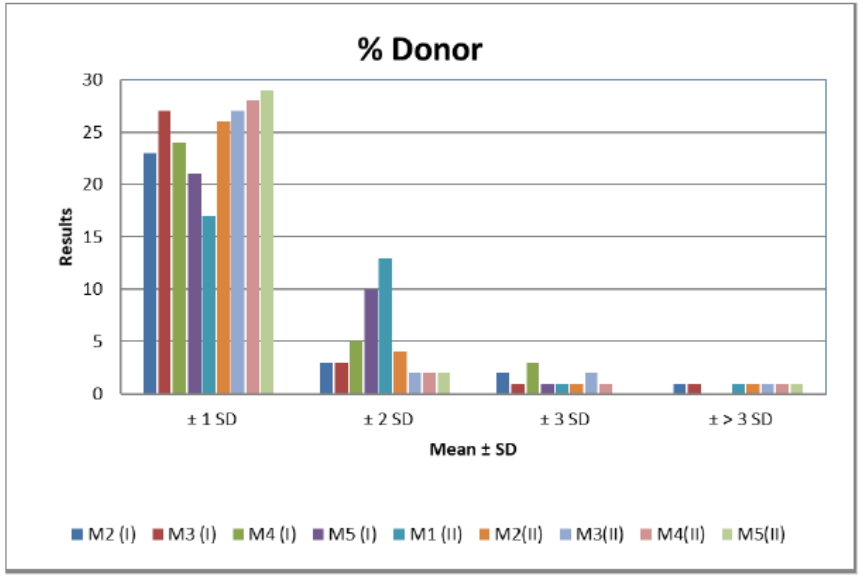
ver.1.1 - 2016

a cura del Gruppo di Lavoro per il Chimerismo AIBT

Chimerismo, Controlli di Qualità e Accreditamento EFI

Laboratorio	Qualità	Metodo di analisi
ALESSANDRIA	Buona	STR-PCR
L'AQUILA	Buona	STR-PCR
BARI	Buona	qPCR
BARI	Buona	STR-PCR; DIP Multiplex PCR
BOLOGNA	Buona	STR-PCR
CAGLIARI	Buona	qPCR
CAGLIARI	Buona	DIP Multiplex PCR
CATANIA	Buona	qPCR
CUNEO	Buona	STR-PCR
FIRENZE	Buona	NGS
MILANO I.TUMORI	Buona	STR-PCR
MILANO Policl.	Buona	STR-PCR
MILANO S.Raff.	Buona	qPCR
NAPOLI Paus.	Buona	STR-PCR
PALERMO	Buona	STR-PCR
PARMA	Buona	STR-PCR
PAVIA	Buona	STR-PCR
PESARO	Buona	STR-PCR
PISA	Buona	STR-PCR
PRAGA	Good	STR-PCR; DIP Multiplex PCR
ROMA Elia	Buona	STR-PCR
ROMA Gemelli	Buona	STR-PCR
ROMA Gra	Buona	STR-PCR
ROMA OPBG	Buona	STR-PCR
S.G.ROTONDO	Buona	STR-PCR
SOFIA	Good	STR-PCR
THESSALONIKI	Good	STR-PCR
TORINO	Buona	NGS
TORINO	Buona	STR-PCR
TREVISO	Buona	NGS
VICENZA	Buona	STR-PCR
ZAGABRIA	Good	qPCR
MILANO S.Raff.	Buona	SSO semi-quantitative





ISTITUTO SUPERIORE DI SANITÀ

CENTRO NAZIONALE TRAPIANTI

European Federation for Immunogenetics

Ringraziamenti

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Alice Oggioni

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Letizia Musolino

Cinzia Candiotto

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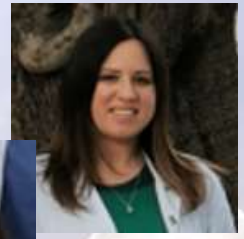




Immunogenetics Lab, HLA & Chimerism

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UOE-TMO-ESTERNI

Beyond Chimerisms ……

GRAZIE PER L'ATTENZIONE