XX Congresso della Società GITMO

RIUNIONE NAZIONALE GITMO

ROMA, Ergife Palace Hotel, 7-8 Maggio 2026

Ruolo del monitoraggio immunologico della ricostituzione CMV specifica nella gestione delle infezioni da CMV nel trapianto allogenico

Liliana Gabrielli

UOC MICROBIOLOGIA

SERVIZIO SANITARIO REGIONALE EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Bologna
IRCCS Istituto di Ricovero e Cura a Carattere Scientifico

POLICLINICO DI SANT'ORSOLA

ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA

Disclosures of Liliana Gabrielli

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Roche Diagnostics					X		

CMV infection end early onset disease in SOT and HSCT

CMV infection

End Organ Disease

- **CMV syndrome**
- **Gastrointestinal disease**
- **pneumonia**
- **hepatitis**
- **retinitis**
- **encephalitis**

mortality risk

Average Rate of CMV infection and CMV disease by graft type

Graft	CMV infection rate	CMV disease rate
Kidney	8-32%	8%
Heart, heart/lung	9-35%	25%
Liver	22-29%	29%
Pancreas, pancreas/kidney	50%	50%
HSCT transplant	7-37%	2-14%
(HSCT autologous transplant)	(12%)	

Grossi PA, J Clin Virol. 2020;123:104211

STRATEGIE DIAGNOSTICHE utili nella gestione dell'infezione da CMV nel POST-HSCT

- Test virologici

- Test immunologici

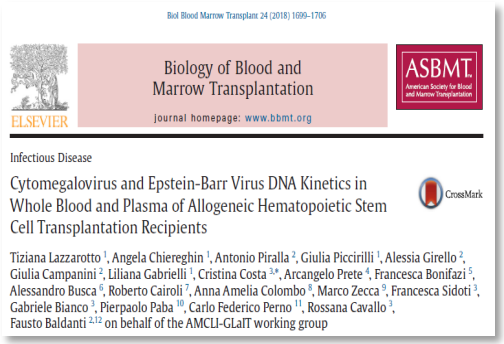
CMV-DNAemia

Il **gold standard** per la diagnosi ed il monitoraggio post-trapianto dell'infezione da CMV è rappresentato dalla determinazione e quantificazione ematica del genoma virale **CMV-DNAemia mediante Real Time PCR**

La recente introduzione di letermovir e maribavir ha evidenziato come la CMV-DNAemia potrebbe non essere un accurato marker di attiva replicazione virale

Prevenzione, diagnosi e gestione dell'infezione di CMV nei trapiantati

sangue intero *vs* plasma



Biol Blood Marrow Transplant 24 (2018) 1699–1706

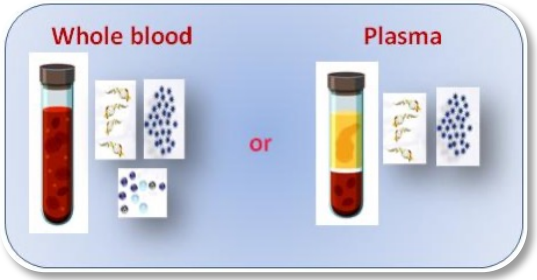
Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Infectious Disease

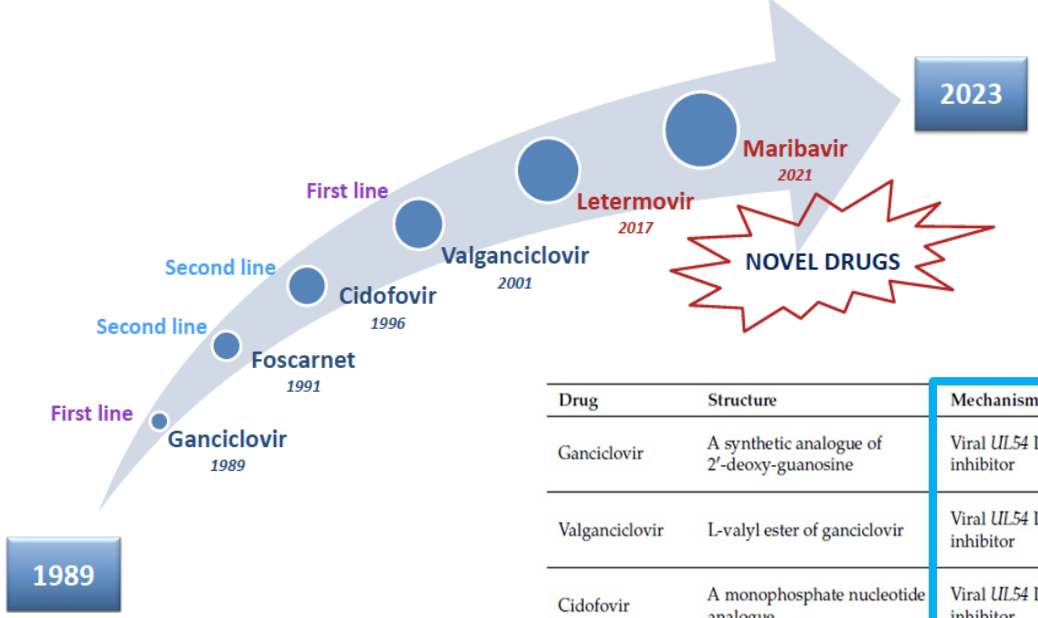
Cytomegalovirus and Epstein-Barr Virus DNA Kinetics in Whole Blood and Plasma of Allogeneic Hematopoietic Stem Cell Transplantation Recipients

Tiziana Lazzarotto [1], Angela Chiereghin [1], Antonio Piralla [2], Giulia Piccirilli [1], Alessia Girello [2], Giulia Campanini [2], Liliana Gabrielli [1], Cristina Costa [3,*], Arcangelo Prete [4], Francesca Bonifazi [5], Alessandro Busca [6], Roberto Cairoli [7], Anna Amelia Colombo [8], Marco Zecca [9], Francesca Sidoti [3], Gabriele Bianco [3], Pierpaolo Paba [10], Carlo Federico Perno [11], Rossana Cavallo [3], Fausto Baldanti [2,12] on behalf of the AMCLI-GLaIT working group



Test CMV-DNAemia su sangue intero e su plasma

1. **La determinazione di CMV-DNA in entrambe le matrici ematiche è in grado di fornire informazioni univoche sulla cinetica di replicazione virale.**
2. **Nel 72,4% dei pazienti sottoposti a terapia antivirale, il monitoraggio della CMV-DNAemia plasmatica rispetto alla CMV-DNAemia del sangue intero potrebbe ritardare l'interruzione del trattamento di 7-14 giorni.**
3. **È fortemente raccomandato utilizzare un solo tipo di campione ematico per il monitoraggio CMV-specifico post-trapianto dei pazienti.**



New antivirals:

Letermovir and Maribavir

Drug	Structure	Mechanism	Main Side Effects
Ganciclovir	A synthetic analogue of 2′-deoxy-guanosine	Viral *UL54* DNA polymerase inhibitor	A potential carcinogen, granulocytopenia, neutropenia, anemia, thrombocytopenia
Valganciclovir	L-valyl ester of ganciclovir	Viral *UL54* DNA polymerase inhibitor	A potential carcinogen, granulocytopenia, neutropenia, anemia, thrombocytopenia
Cidofovir	A monophosphate nucleotide analogue	Viral *UL54* DNA polymerase inhibitor	Nephrotoxicity, neutropenia, nausea, uveitis, iritis, asthenia, alopecia, ocular hypotony
Foscarnet	Pyrophosphate analogue, a structural mimic of the anion pyrophosphate	Inhibitor of the pyrophosphate-binding site on viral DNA polymerase (or reverse transcriptase); Noncompetitive inhibitor of many RNA and *UL54* DNA polymerase	Nephrotoxicity, electrolyte disturbance, genital ulceration, paranesthesia, irritability, hallucination
Letermovir	A non-nucleoside, 3,4-dihydroquinazolinyl acetic acid	Viral terminase complex inhibitor encoded by gene *UL56, UL51, UL89*	Nausea, diarrhea, vomiting, swelling in arms and legs, cough, headache, tiredness, hepatitis, stomach pain
Maribavir	A benzimidazole riboside	Viral protein kinase (*UL97*) inhibitor	Taste disturbance, nausea, diarrhea, vomiting and fatigue

Viral DNA polymerase inhibitors.

Early stage of viral replication.

Inhibitors of viral genome encapsidation stage ⇒ they block the phase of assembly of virions

Late stage of viral replication.

Adapted from Chen SJ et al. Trop Med Infect Dis. 2022

Am J Transplant. 2021;21:1622–1628.

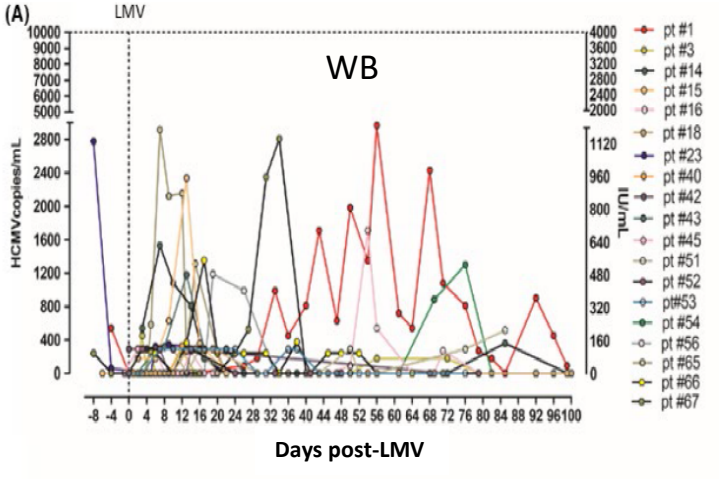
Positive HCMV DNAemia in stem cell recipients undergoing letermovir prophylaxis is expression of abortive infection

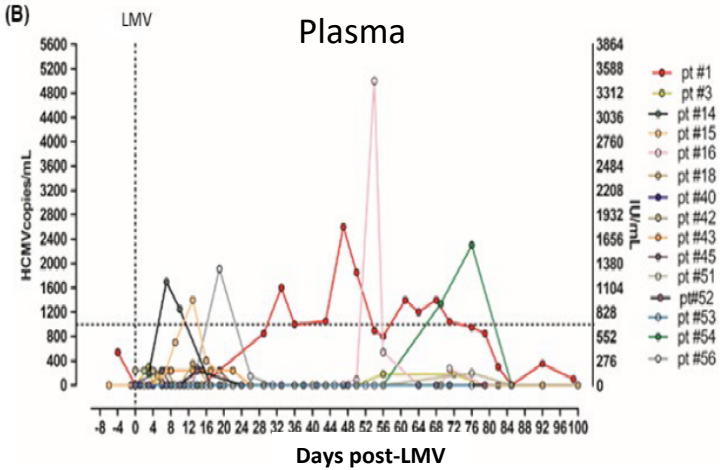
Irene Cassaniti[1] | Anna A. Colombo[2] | Paolo Bernasconi[2] | Michele Malagola[3] | Domenico Russo[3] | Anna P. Iori[4] | Corrado Girmenia[4] | Raffaella Greco[5] | Jacopo Peccatori[5] | Fabio Ciceri[5] | Francesca Bonifazi[6] | Elena Percivalle[1] | Giulia Campanini[1] | Giulia Piccirilli[7] | Tiziana Lazzarotto[7] | Fausto Baldanti[1,8]

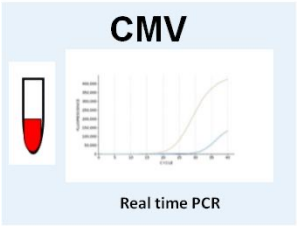
- 75 HSCT recipients in LMV prophylaxis
- 26/75 WB positive CMV-DNAemia
- 21/75 plasma positive CMV-DNAemia

The DNAemia test cannot distinguish between active infection and abortive infection because the DNAemia test identifies both free viral DNA (blips) and encapsidated DNA.

HCMV plasma DNAemia after digestion with **DNase I** was undetectable in all patients, suggesting the absence of replicative HCMV DNA. Similarly, in none of the patients HCMV could be isolated in **shell vial cultures**, further corroborating the finding of abortive HCMV replication during LMV prophylaxis.
For this reason, no patients received GCV-PET.







ROUTINE TEST

Detection and quantification of CMV DNAemia on **WB samples**

NEGATIVE → **STOP**

POSITIVE
< 109 IU/mL in only 1 sample → **STOP**

POSITIVE
< 109 IU/mL in 2 consecutive samples or ≥ 109 IU/mL in one sample → **ADDITIONAL TESTS**

Viremia
prospectively performed

Detection and quantification of positive cells by inoculating **PMNL** in a culture of human fibroblasts

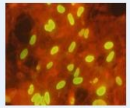
DNase
prospectively performed

Detection and quantification of CMV DNAemia on treated and untreated **plasma samples**

RNAemia
retrospectively performed

Detection and quantification of CMV RNAemia on **plasma samples**

CMV viremia test



CMV DNase test

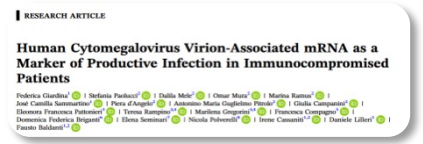
CMV RNAemia target mRNA UL21.5

Journal of Clinical Microbiology

CMV-RNAemia as new marker of active viral replication in transplant recipients

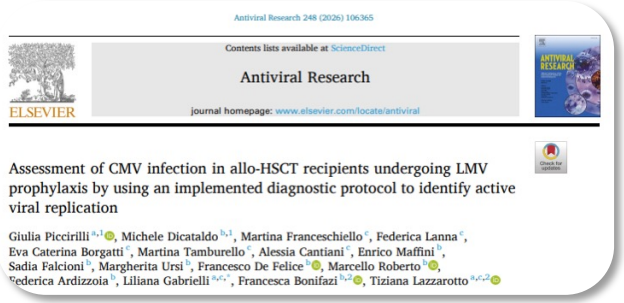
RESEARCH ARTICLE

Cytomegalovirus-RNA Accurately Identifies Clinically Significant Infection Needing Preemptive Therapy in Liver Transplanted Children: A Proof-of-Concept Study



RESEARCH ARTICLE

Human Cytomegalovirus Virion-Associated mRNA as a Marker of Productive Infection in Immunocompromised Patients



Antiviral Research 248 (2026) 106365

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Assessment of CMV infection in allo-HSCT recipients undergoing LMV prophylaxis by using an implemented diagnostic protocol to identify active viral replication

Giulia Piccirilli[a,1], Michele Dicataldo[b,1], Martina Franceschiello[c], Federica Lanna[c], Eva Caterina Borgatti[c], Martina Tamburello[c], Alessia Cantiani[c], Enrico Maffini[b], Sadia Falcioni[b], Margherita Ursi[b], Francesco De Felice[b], Marcello Roberto[b], Federica Ardizzoia[b], Liliana Gabrielli[a,c,*], Francesca Bonifazi[b,2], Tiziana Lazzarotto[a,c,2]

SERVIZIO SANITARIO REGIONALE EMILIA-ROMAGNA Azienda Ospedaliero - Universitaria di Bologna IRCCS Istituto di Ricovero e Cura a Carattere Scientifico

POLICLINICO DI SANT'ORSOLA

ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA

STRATEGIE DIAGNOSTICHE UTILI nella gestione dell'infezione da CMV nel POST-HSCT

- Test virologici

- Test immunologici

Ruolo dei TEST IMMUNOLOGICI CMV-SPECIFICI

Potrebbero contribuire a:

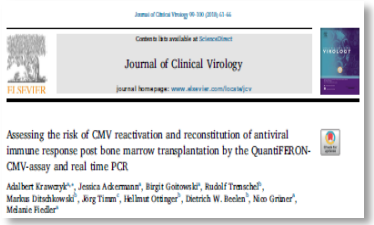
- ✓ **Identificare i pazienti a rischio di infezione clinicamente significativa da CMV (csCMVi)**
- ✓ **Selezionare i pazienti che potrebbero beneficiare di un prolungamento della profilassi antivirale**
- ✓ **Stratificare il rischio nei candidati nel contesto pre-trapianto, soprattutto quando la sierologia è dubbia**

CMV specific cell-mediated immunity (CMI)

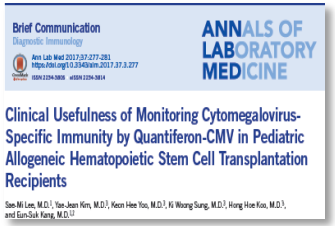

Journal of Clinical Virology

Letter to the Editor

Quantiferon CMV assay in allogenic stem cell transplant patients


Journal of Clinical Virology

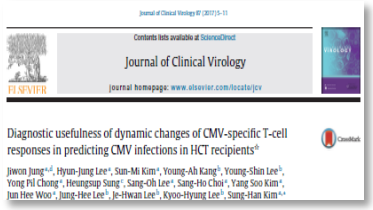
Assessing the risk of CMV reactivation and reconstitution of antiviral immune response post bone marrow transplantation by the QuantiFERON-CMV-assay and real time PCR


Brief Communication

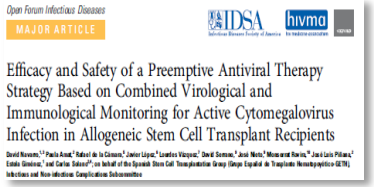
Diagnostic Immunology

ANNALS OF LABORATORY MEDICINE

Clinical Usefulness of Monitoring Cytomegalovirus-Specific Immunity by Quantiferon-CMV in Pediatric Allogeneic Hematopoietic Stem Cell Transplantation Recipients

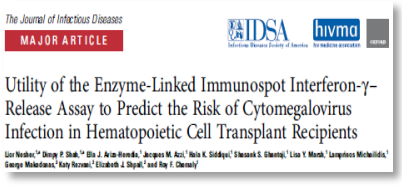

Journal of Clinical Virology

Diagnostic usefulness of dynamic changes of CMV-specific T-cell responses in predicting CMV infections in HCT recipients


Open Forum Infectious Diseases

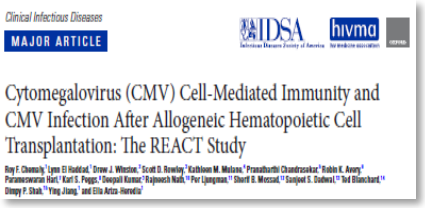
MAJOR ARTICLE

Efficacy and Safety of a Preemptive Antiviral Therapy Strategy Based on Combined Virological and Immunological Monitoring for Active Cytomegalovirus Infection in Allogeneic Stem Cell Transplant Recipients


The Journal of Infectious Diseases

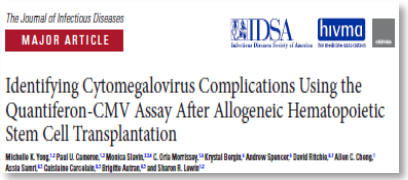
MAJOR ARTICLE

Utility of the Enzyme-Linked Immunospot Interferon-γ–Release Assay to Predict the Risk of Cytomegalovirus Infection in Hematopoietic Cell Transplant Recipients


Clinical Infectious Diseases

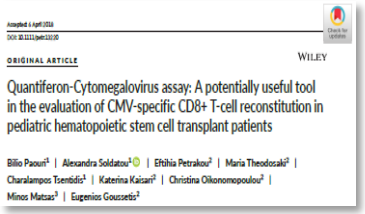
MAJOR ARTICLE

Cytomegalovirus (CMV) Cell-Mediated Immunity and CMV Infection After Allogeneic Hematopoietic Cell Transplantation: The REACT Study


The Journal of Infectious Diseases

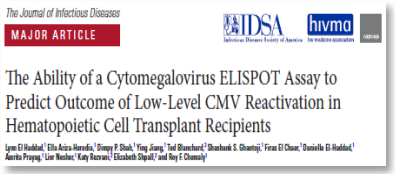
MAJOR ARTICLE

Identifying Cytomegalovirus Complications Using the Quantiferon-CMV Assay After Allogeneic Hematopoietic Stem Cell Transplantation


ORIGINAL ARTICLE

WILEY

Quantiferon-Cytomegalovirus assay: A potentially useful tool in the evaluation of CMV-specific CD8+ T-cell reconstitution in pediatric hematopoietic stem cell transplant patients


The Journal of Infectious Diseases

MAJOR ARTICLE

The Ability of a Cytomegalovirus ELISPOT Assay to Predict Outcome of Low-Level CMV Reactivation in Hematopoietic Cell Transplant Recipients

La risposta CMV-specifica delle cellule T ha un importante ruolo per il controllo della progressione dell'infezione da CMV verso la malattia nel periodo post-trapianto

⇒ lo studio della CMI (CMV) predice il rischio di insorgenza dell'infezione da CMV nei pazienti trapiantati

MONITORAGGIO IMMUNOLOGICO
dell'infezione da CMV nel paziente trapiantato

Test diagnostici che permettono di analizzare le diverse proprietà funzionali dei linfociti T CMV-specifici e correlarle al controllo dell'infezione

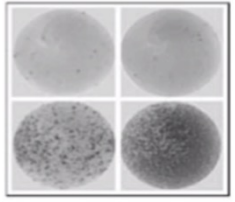
Diversi metodi di laboratorio

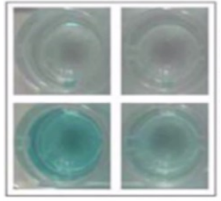
Rilevazione della citochina Interferon-γ secreta dai linfociti T dopo stimolazione *ex vivo* con specifici antigeni virali

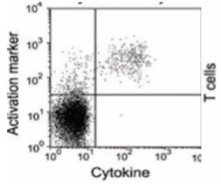
IGRAs
Interferon-Gamma Releasing Assays

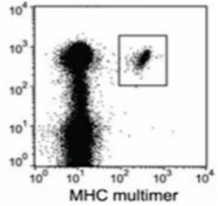
TEST IMMUNOLOGICI CMV-SPECIFICI

	INF-γ-EliSpot	QuantiFERON-CMV	Dosaggio intracellulare INF-γ (ICS)	Test multimetrico MHC
Kit commerciali disponibili	1. **T. Spot** (Oxford Diagnostics) 2. **T-Track** (Lophius Bioscience Mikrogen)	QuantiFERON®- CMV ELISA (QIAGEN)	IFN-γ ICS (Eurofins Viracor)	Test multimerico MHC CMVC8 (Mayo Clinic Laboratories)
Materiale **Tempo di processazione** **Antigeni**	**PBMC** 30-40h Proteine, peptidi, lisati di cellule infette	**Sangue in toto** 24h 22 peptidi	Sangue in toto/PBMC 8-24h Proteine, peptidi, lisati di cellule infette	PBMC 2-3h Peptidi
Vantaggi	Identificazione CD4+ e CD8+	Standardizzato Facile esecuzione 3 ml di sangue	Identificazione CD4+ e CD8+ 1 ml di sangue	1 ml di sangue
Svantaggi	Non standardizzato No distinzione CD4+ e CD8+ Separazione dei PMBC da 10 ml di sangue	Limitato ai CD8+ Risultati falsi negativi in pazienti con HLA non incluso nel saggio	Non standardizzato Necessità di citofluorimetro	Non standardizzato Limitato ai CD8+ Necessario conoscere HLA del paziente Necessità di citofluorimetro



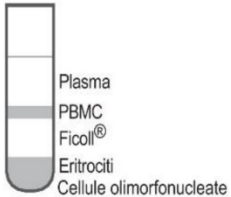






RISPOSTA T-CELLULARE CMV-SPECIFICA - ELISpot-CMV

T-SPOT®CMV



sangue intero raccolto in provette di litio eparina

stimolazione di antigeni CMV specifici
(**IE1, pp65)**

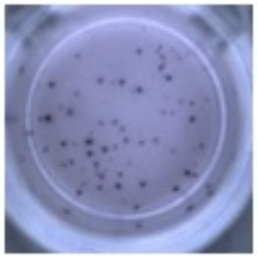
T-Track® CMV

Non esiste una soglia di positività universalmente standardizzata; al contrario, i cut-off sono spesso specifici per singolo centro

Debole attività linfocitaria CMV-specifica: 5 ≤ SFC < 20 /200.000 PBMC

Buona attività linfocitaria CMV-specifica: 20 ≤ SFC < 100 /200.000 PBMC

Ottima attività linfocitaria CMV-specifica: ≥ 100 SFC /200.000 PBMC



IE-1: ≥ 10 SFC/1 milione PBMC

pp65: ≥ 10 SFC/1 milione PBMC

PBMC: peripheral blood mononuclear cell
SFC: spot forming cell

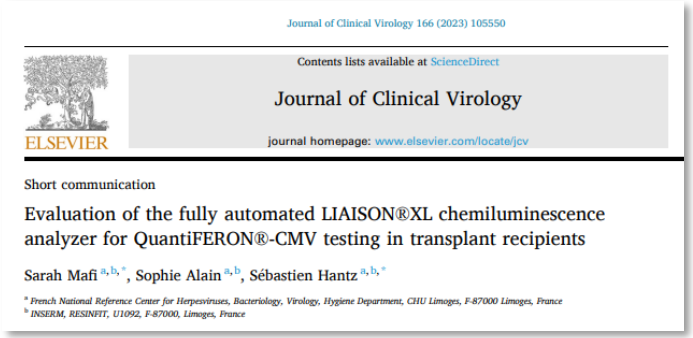
RISPOSTA T-CELLULARE CMV-SPECIFICA - QuantiFERON-CMV

NIL TUBE: negative control

CMV TUBE: pool of 22 peptides → CD8 viral epitopes
pp65, pp50, immediate early antigen-1 (IE-1), IE-2, and glycoprotein B

MITOGEN TUBE: positive control

Three specialized collection tubes > 1 mL whole blood into each tube



Journal of Clinical Virology 166 (2023) 105550

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

ELSEVIER

Short communication

Evaluation of the fully automated LIAISON®XL chemiluminescence analyzer for QuantiFERON®-CMV testing in transplant recipients

Sarah Mafi [a,b,*], Sophie Alain [a,b], Sébastien Hantz [a,b,*]

[a] French National Reference Center for Herpesviruses, Bacteriology, Virology, Hygiene Department, CHU Limoges, F-87000 Limoges, France
[b] INSERM, RESINFIT, U1092, F-87000, Limoges, France

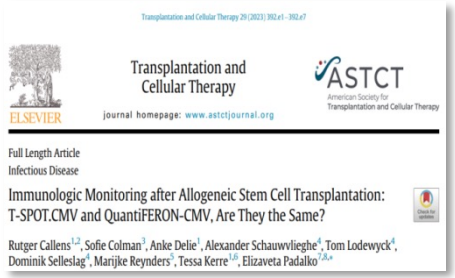
QuantiFERON-CMV: interpretazione dei risultati

CMV meno Nil (UI/mL)	Mitogeno meno Nil (UI/mL)	Risultato del test QF-CMV	Interpretazione
≥ 0,20	Qualsiasi	**Reattivo**	Presenza di CMI-CMV specifica
< 0,20	≥ 0,5	**Non reattivo**	Presenza di CMI globale e assenza di CMI-CMV specifica
	< 0,5	**Indeterminato**	Assenza di CMI

Nil: controllo negativo; CMV: miscela di 22 epitopi sintetici che mimano le proteine di CMV e stimolano i T-CD8+ a produrre IFN-γ; Mitogeno: controllo positivo

EliSpot CMV e QuantiFERON-CMV

- Per ottenere risultati affidabili con i test IGRA, il campione di sangue deve contenere un numero minimo di cellule T CD3+.
  La maggior parte degli esperti riporta che l'uso dei test IGRA ha un limite minimo di 100 linfociti T/mm^3.


Transplantation and Cellular Therapy 29 (2023) 392.e1–392.e7

Transplantation and Cellular Therapy

ASTCT American Society for Transplantation and Cellular Therapy

ELSEVIER

journal homepage: www.astctjournal.org

Full Length Article
Infectious Disease

Immunologic Monitoring after Allogeneic Stem Cell Transplantation: T-SPOT.CMV and QuantiFERON-CMV, Are They the Same?

Rutger Callens[1,2], Sofie Colman[3], Anke Delie[1], Alexander Schauwvlieghe[4], Tom Lodewyck[4], Dominik Selleslag[4], Marijke Reynders[5], Tessa Kerre[1,6], Elizaveta Padalko[7,8,*]

- i risultati di EliSpot-CMV e QuantiFERON-CMV non sono intercambiabili

- **i risultati non devono essere valutati «in modo statico»**

- **La <u>CMI specifica</u> per CMV deve essere monitorata dopo il trapianto non prima di 60 giorni dopo l'attecchimento nei riceventi di trapianto allogenico di cellule staminali ematopoietiche (allo-HSCT).**


Clinical Transplantation

WILEY

Clinical TRANSPLANTATION

REVIEW ARTICLE

Management of Cytomegalovirus Infection in Allogeneic Hematopoietic Stem Cell and in Solid Organ Transplantation: Updated Recommendations by the GITMO, SITO, SIMIT, and AMCLI Italian Societies

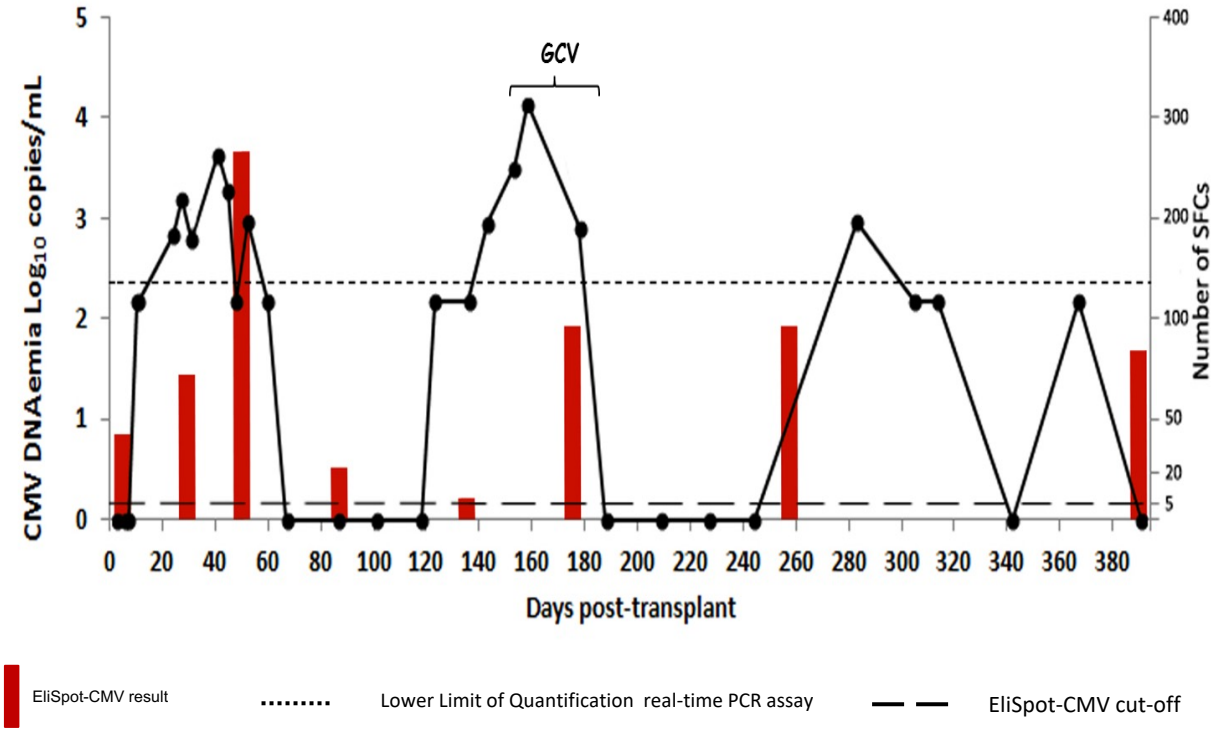
Corrado Girmenia[1] | Tiziana Lazzarotto[2,3] | Massimo Martino[4] | Francesca Bonifazi[5] | Fausto Baldanti[6,7] | Pierangelo Clerici[8] | Franco Citterio[9] | Luciano De Carlis[10,11] | Giovanni Barosi[12] | Paolo Antonio Grossi[13]

EliSpot-CMV → i risultati non devono essere valutati «in modo statico»

Trapianto di cuore – 46 anni

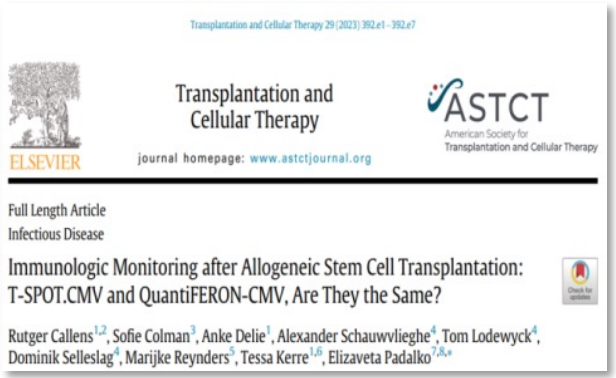
CMV D+/R+

Strategia pre-emptive → Test da eseguire alla risoluzione dell'episodio infettivo



EliSpot-CMV e QuantiFERON-CMV

Quando?



Transplantation and Cellular Therapy 29 (2023) 392.e1 – 392.e7

Transplantation and Cellular Therapy

journal homepage: www.astctjournal.org

ASTCT American Society for Transplantation and Cellular Therapy

ELSEVIER

Full Length Article

Infectious Disease

Immunologic Monitoring after Allogeneic Stem Cell Transplantation: T-SPOT.CMV and QuantiFERON-CMV, Are They the Same?

Rutger Callens[1,2], Sofie Colman[3], Anke Delie[1], Alexander Schauwvlieghe[4], Tom Lodewyck[4], Dominik Selleslag[4], Marijke Reynders[5], Tessa Kerre[1,6], Elizaveta Padalko[7,8,*]

27 patients were included in a bicentric prospective trial

All patients received letermovir prophylaxis up to day +100

Aims

- **Comparison** between T-SPOT.CMV and QuantiFERON-CMV in post allo-HSCT at:

  28 days post-allo-HSCT

  100 days post-allo-HSCT

- **assess predictive values of both tests for CMV reactivation**

Monitoraggio immunologico dopo il trapianto allogenico di cellule staminali: T-SPOT.CMV e QuantiFERON-CMV

Transplantation and Cellular Therapy 29 (2023) 392.e1 - 392.e7

Transplantation and Cellular Therapy

ASTCT American Society for Transplantation and Cellular Therapy

ELSEVIER

journal homepage: www.astctjournal.org

Full Length Article
Infectious Disease

Immunologic Monitoring after Allogeneic Stem Cell Transplantation: T-SPOT.CMV and QuantiFERON-CMV, Are They the Same?

Rutger Callens[1,2], Sofie Colman[3], Anke Delie[1], Alexander Schauwvlieghe[4], Tom Lodewyck[4], Dominik Selleslag[4], Marijke Reynders[5], Tessa Kerre[1,6], Elizaveta Padalko[7,8,*]

I test QuantiFERON-CMV e T-SPOT.CMV sono risultati positivi al giorno +28 solo in una piccola minoranza di campioni (rispettivamente 2 e 3 su 26 campioni).

Il valore clinico aggiunto di un test IGRA al giorno +28 dopo un trapianto allogenico di cellule staminali ematopoietiche è discutibile.

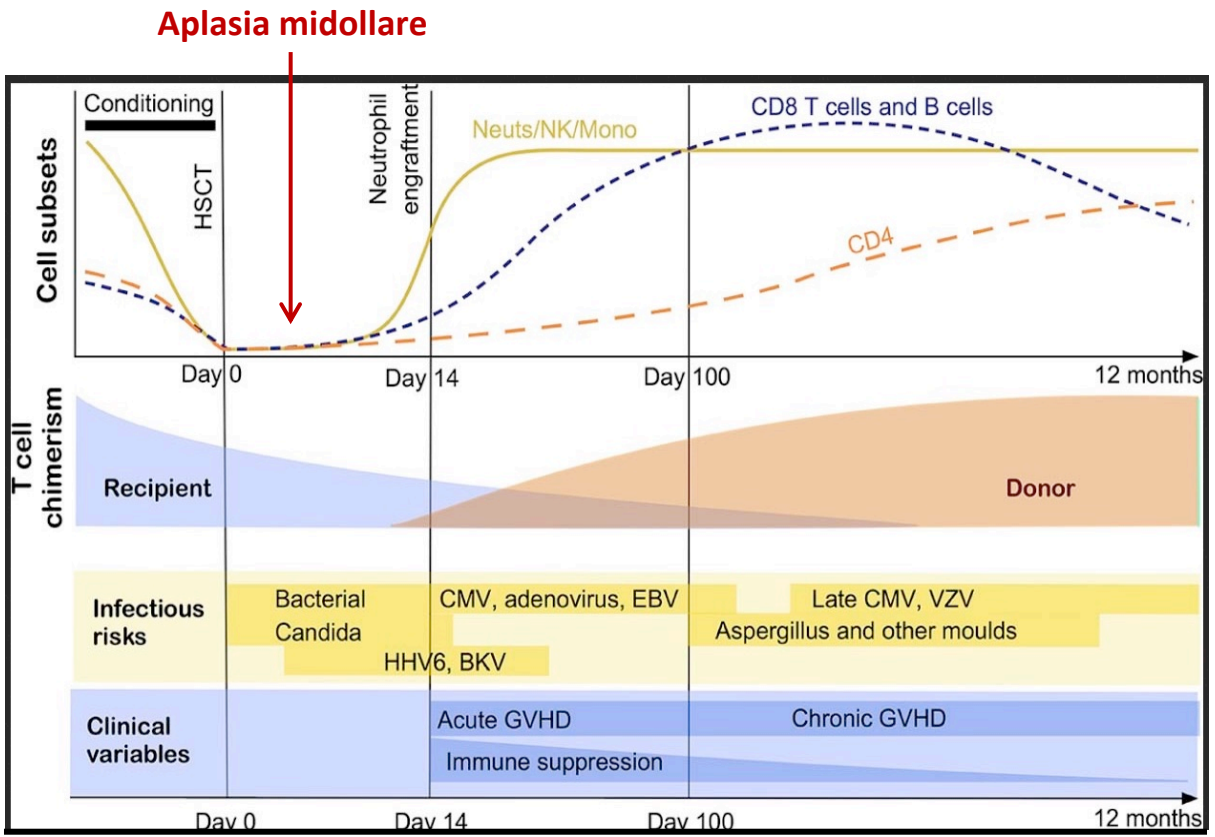
Predictive Values of T-SPOT.CMV and QuantiFERON-CMV for Predicting CS-CMVi between Day +100

Test	Value
T-SPOT.CMV, day +100	
Sensitivity, %	100
Specificity, %	35
NPV, %	100
PPV, %	23.50
QuantiFERON-CMV, day +100	
Sensitivity, %	100
Specificity, %	45
NPV, %	100
PPV, %	26.70

		CS-CMVi	
		Yes	No
T-SPOT.CMV on day +100	–	4	13
	+	0	7
QuantiFERON-CMV on day +100	–	4	11
	+	0	9

CS-CMVi: infezione clinicamente significativa

CMV specific cell-mediated immunity



Sutrave G,et al. Cytotherapy. 2017;19(11):1284-1301.

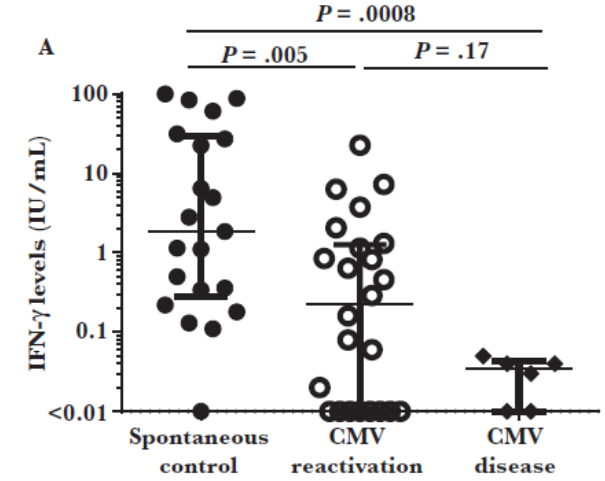
The Journal of Infectious Diseases
MAJOR ARTICLE

Identifying Cytomegalovirus Complications Using the Quantiferon-CMV Assay After Allogeneic Hematopoietic Stem Cell Transplantation

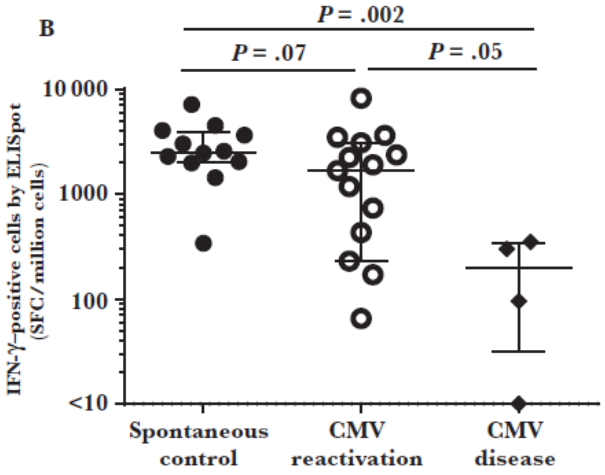
Michelle K. Yong,[1,2] Paul U. Cameron,[1,2] Monica Slavin,[2,3,4] C. Orla Morrissey,[1,5] Krystal Bergin,[5] Andrew Spencer,[5] David Ritchie,[6,7] Allen C. Cheng,[1] Assia Samri,[8,9] Guislaine Carcelain,[8,9] Brigitte Autran,[8,9] and Sharon R. Lewin[1,2]

Pazienti NON in profilassi con LTV

At 3 months after HSCT, participants who developed CMV disease compared with those with CMV reactivation or spontaneous viral control had a significantly lower magnitude of IFN-γ production in the **Quantiferon-CMV assay**
(median IFN-γ = 0.04 vs 0.23 vs 1.86 IU/mL, respectively; Kruskal–Wallis *P* = .0008)



Similar results were observed at 3 months using the CMV ELISpot assay, whereby participants with CMV disease compared with those with CMV reactivation or spontaneous viral control had significantly lower **ELISpot IFN-γ**
(median = 198 vs 1670 vs 2513 SFC/106 cells; Krukal-Wallis test *P* = .002)

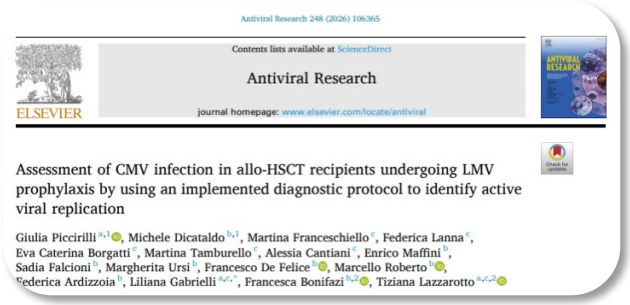


Ruolo dei TEST IMMUNOLOGICI CMV-SPECIFICI

Potrebbero contribuire a:

- ✓ Identificare i pazienti a rischio di infezione clinicamente significativa da CMV
- **Selezionare i pazienti che potrebbero beneficiare di un prolungamento della profilassi antivirale**
- ✓ Stratificare il rischio nei candidati nel contesto pre-trapianto, soprattutto quando la sierologia è dubbia

LETERMOVIR



Antiviral Research 248 (2026) 106365

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Assessment of CMV infection in allo-HSCT recipients undergoing LMV prophylaxis by using an implemented diagnostic protocol to identify active viral replication

Giulia Piccirilli [a,1], Michele Dicataldo [b,1], Martina Franceschiello [c], Federica Lanna [c], Eva Caterina Borgatti [c], Martina Tamburello [c], Alessia Cantiani [c], Enrico Maffini [b], Sadia Falcioni [b], Margherita Ursi [b], Francesco De Felice [b], Marcello Roberto [b], Federica Ardizzoia [a], Liliana Gabrielli [a,c,*], Francesca Bonifazi [b,2], Tiziana Lazzarotto [a,c,2]

1. Riduzione significativa dei pazienti con risultati positivi per la CMV DNAemia nei primi 100 giorni dopo il trapianto (39,1% contro 70%). Sviluppo di **infezioni da CMV clinicamente significative** nel 3,6% dei casi nel gruppo LMV rispetto al 39,1% dei casi nel gruppo di controllo.

2. Estendendo l'analisi a 200 giorni dopo il trapianto, l'incidenza delle infezioni da CMV clinicamente significative è aumentata nel gruppo LMV, ma è rimasta significativamente inferiore rispetto al gruppo di controllo (**16,4**% contro 39,1%).

Russo D et al. Lancet Haematol 2024

→ l'estensione della durata della profilassi con LMV fino al giorno +200 è stata efficace nel ridurre l'incidenza di csCMVi nei pazienti ad alto rischio (19% gruppo placebo *versus* **3%** gruppo letermovir).

Estensione della durata della profilassi con LMV fino al giorno +200

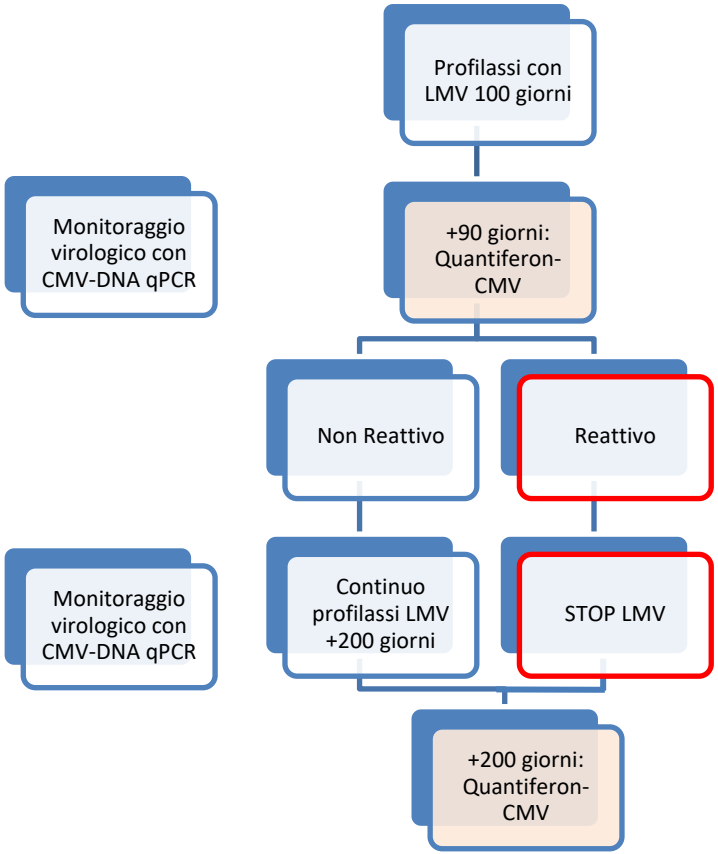
Quali pazienti?

1. **Variabili cliniche**: l'uso di donatori aploidentici, non compatibili o non imparentati, trapianto di cellule staminali ematopoietiche da sangue cordonale, deplezione dei linfociti T ex vivo o in vivo e presenza di GVHD.

2. **I test IGRA** (Interferon Gamma Release Assay) potrebbero essere un ulteriore strumento utile per identificare i pazienti che dopo la fine della profilassi sono a maggior rischio di infezioni da CMV clinicamente significative ? Possono costituire una guida preziosa per strategie preventive personalizzate che evitino trattamenti non necessari?

STUDIO CLINICO MULTICENTRICO (BS, PV, TO e BO) → P.I. Russo Domenico

STUDIO CLINICO MULTICENTRICO (BS, PV, TO e BO) → P.I. Russo Domenico



Study Population

patients who:

- have received allo-HSCT for haematological diseases;
- have completed prophylaxis with LET from day 0 to day +100;
- are CMV-IgG positive pre-transplant;
- have at least one risk factor (mismatched, haploidentical, cord blood, GVHD with steroids, etc.);
- have a positive CMV-specific immune response (Quantiferon-CMV positive test) at day +90 .

These patients will be followed in the +100/+200 period without continuation of prophylaxis with LET and monitored for the occurrence of CMV-csi.

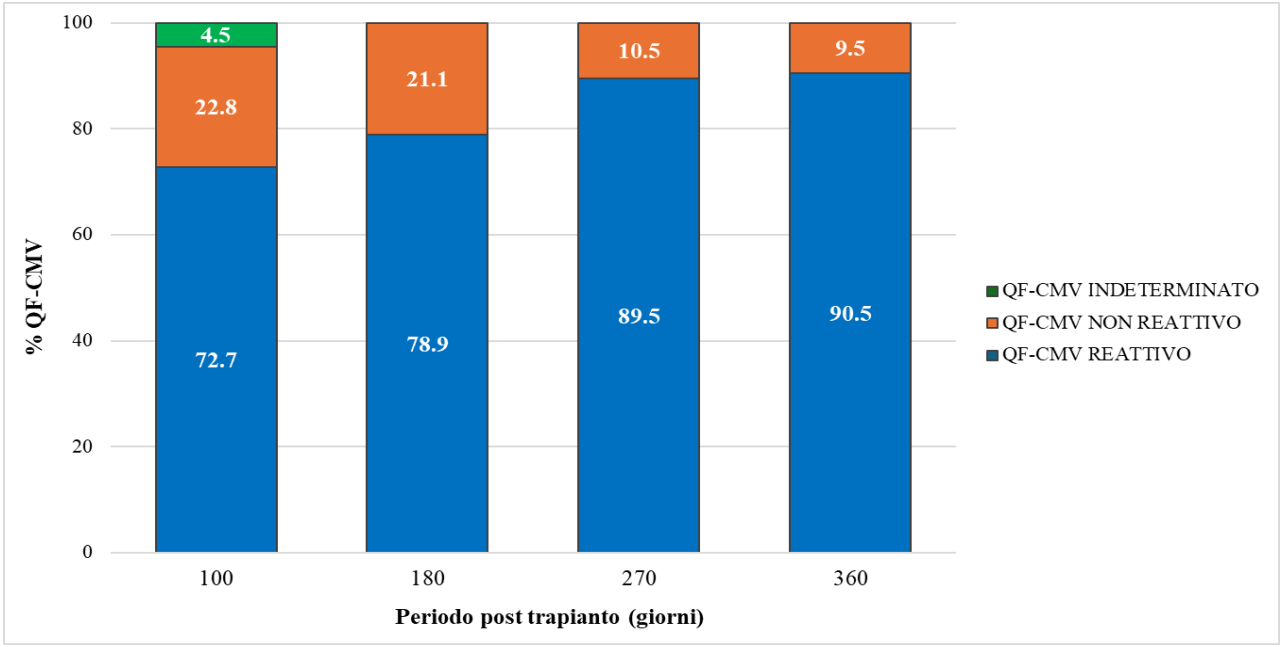
The primary objective of this study is to assess the incidence of csCMVi in high-risk patients with positive Quantiferon CMV test at day +90, not extending the prophylaxis with LET from day +100 to day +200

Historical reference data show an incidence of CMV-CSI of 3% in high-risk patients treated with LET up to day +200.

Monitoraggio immunologico post LTV profilassi

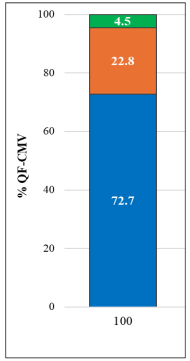
22 pazienti
100 giorni post-TX STOP LTV

Aumento della ricostituzione della CMI-CMV specifica nel tempo



2026 Author's unpublished data

Monitoraggio immunologico post LTV profilassi



22 pazienti
100 giorni post-TX STOP LTV

16/22 (72.7%)
QuantiFERON®-CMV positivo

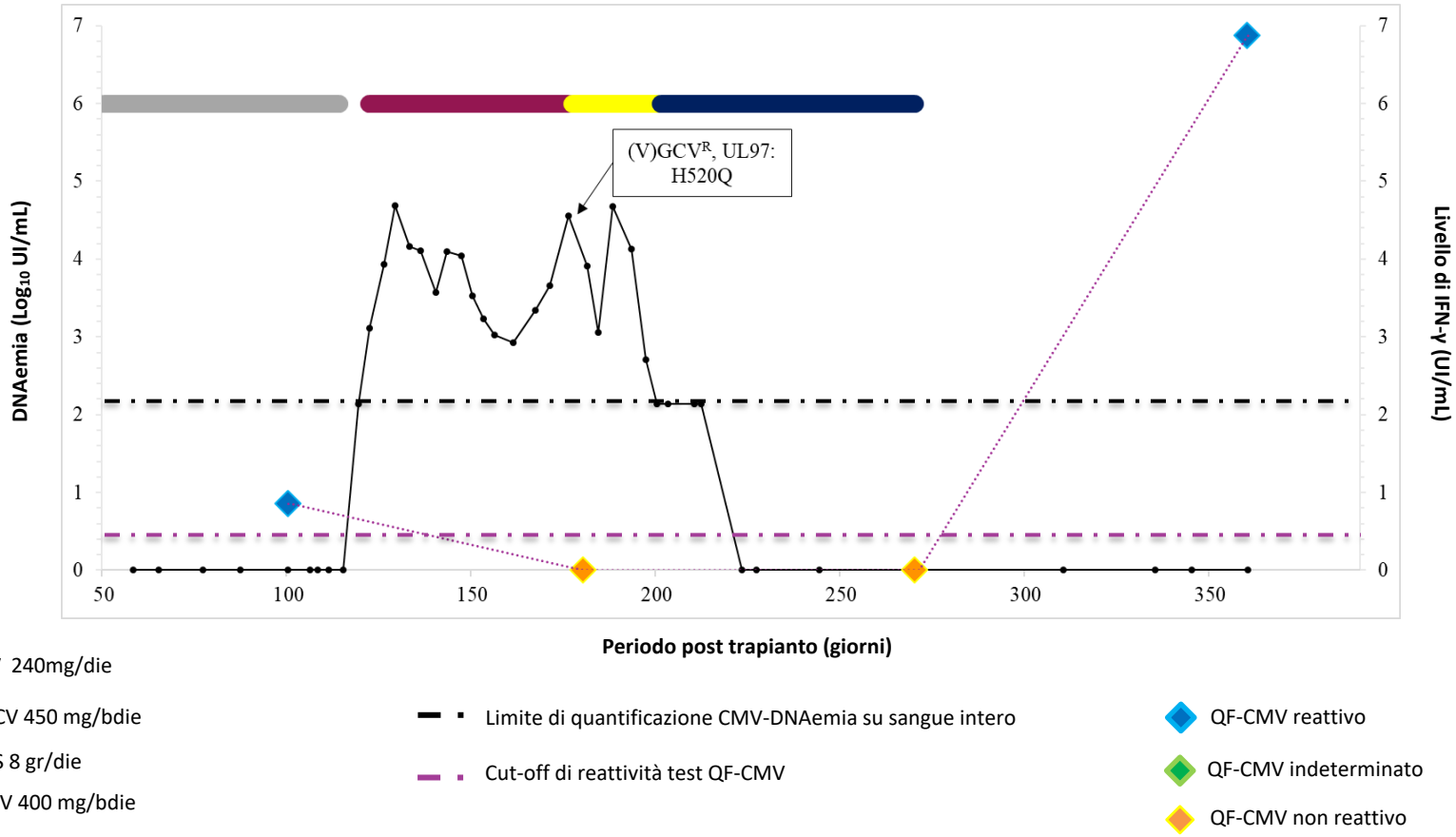
5 pazienti ⇒ infezione da CMV al termine della LTV-profilassi.
3/5 ⇒ debole CMI-CMV
valori vicino al cut-off (mediana: 0.5 UI/ml) e quindi non stabili.
Uno di questi ha sviluppato un'infezione severa da CMV con resistenza a ganciclovir (mutazione H520Q - UL97)

ID paziente	Valore QF-CMV (UI/mL)	Picco CMV-DNAemia (UI/mL)	PET Sì/No
BO_213	0.25	2.5×10^3	No
BO_152	0.65	4.8×10^4	Sì
BO_155	1.62	2.0×10^3	No
BO_217	0.50	2.9×10^3	No
BO_173	4.70	1.9×10^3	No

Cut-off: 0.2 UI/ml

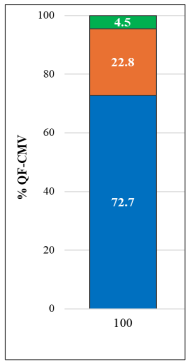
Monitoraggio virologico e immunologico - Paziente BO_152

TX-CSE a dicembre 2023, maschio, 68 anni; patologia di base: Leucemia mieloide acuta; CMV-sierostato: D-/R+



TX-CSE: trapianto di cellule staminali emopoietiche; LTV: letermovir; VGCV: valganciclovir; FOS: foscarnet; MBV: maribavir; QF-CMV: QuantiFERON®-CMV, Qiagen

Monitoraggio immunologico post LTV profilassi



22 pazienti
100 giorni post-TX STOP LTV

5/22 (22.7%)
QuantiFERON®-CMV negativo

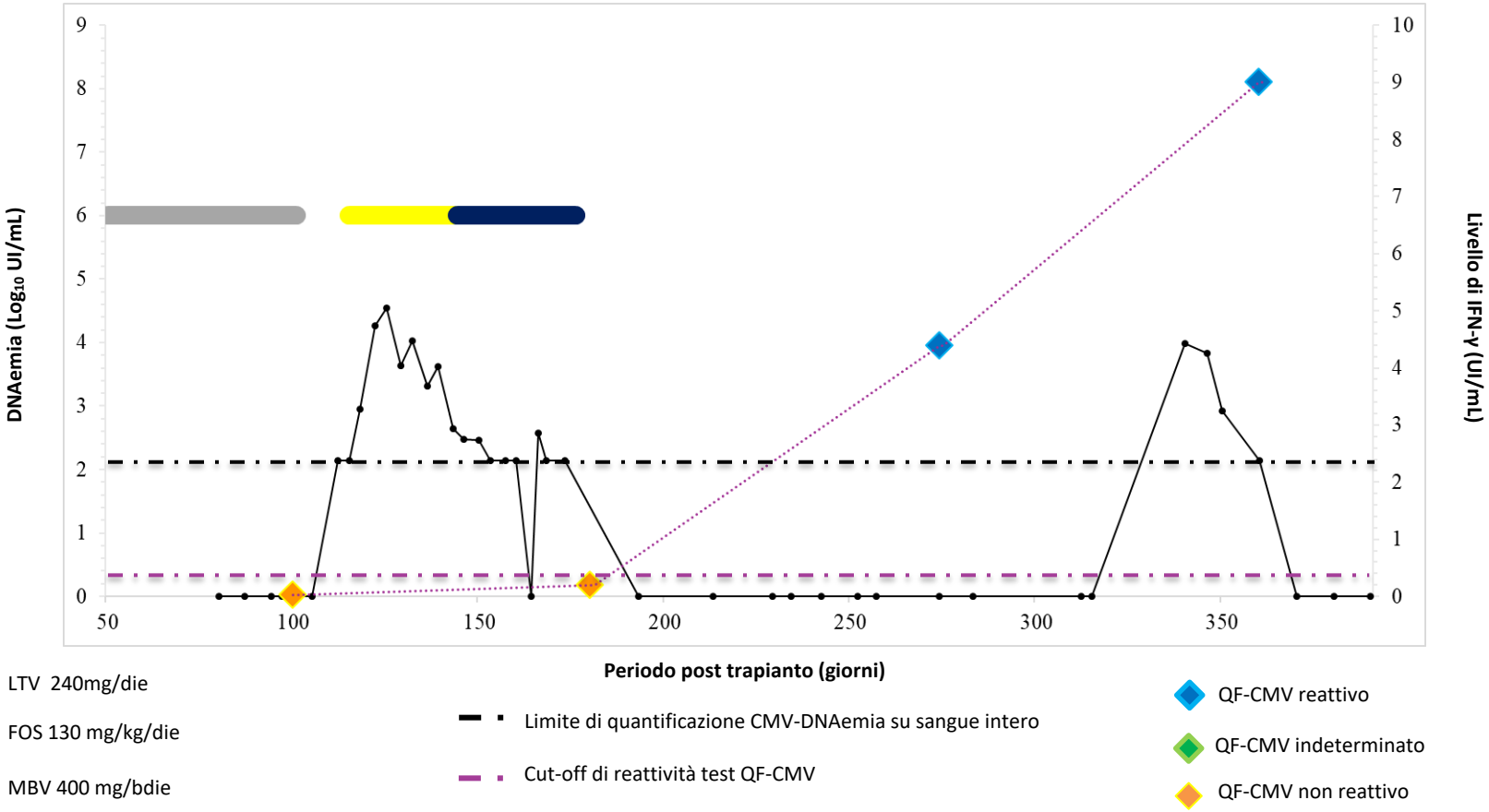
4 pazienti hanno sviluppato un'infezione da CMV dopo lo stop della LTV-profilassi (mediana: 22 giorni; range: 11-64) raggiungendo un picco mediano di CMV-DNAemia pari a 2.1x104 UI/ml e necessitando in 3 casi della PET.

ID paziente	Valore QF-CMV (UI/mL)	Picco CMV-DNAemia (UI/mL)	PET Sì/No
BO_148	0.01	3.0×10^4	Sì
BO_93	0.02	1.2×10^4	Sì
BO_165	0.01	2.5×10^3	No
BO_164	0.01	3.5×10^4	Sì

Cut-off: 0.2 UI/ml

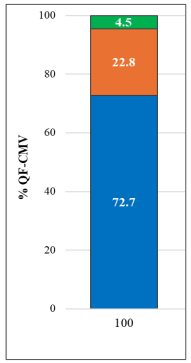
Monitoraggio virologico e immunologico - Paziente BO_164

TX-CSE a maggio 2024, maschio, 54 anni; patologia di base: leucemia acuta linfoblastica; CMV-sierostato: D+/R+



TX-CSE: trapianto di cellule staminali emopoietiche; LTV: letermovir; FOS: foscarnet; MBV: maribavir; QF-CMV: QuantiFERON®-CMV, Qiagen

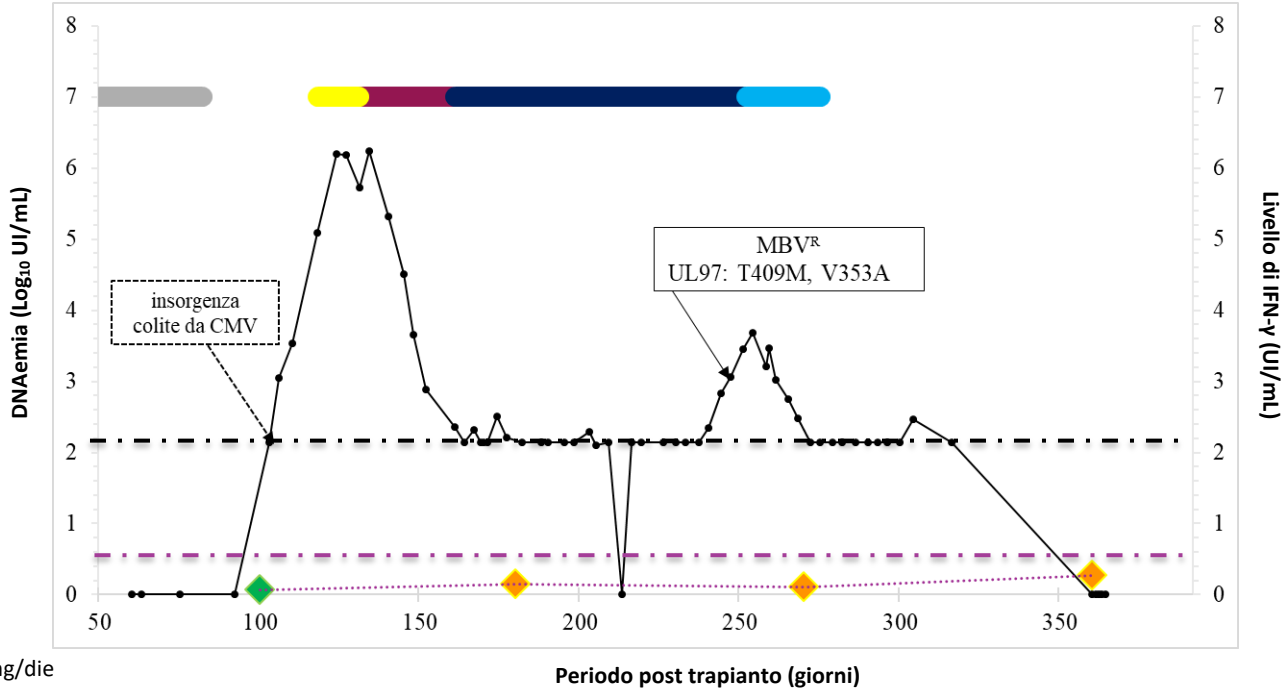
Monitoraggio virologico e immunologico - Paziente BO_174



1/22 (4.6%)
QuantiFERON®-CMV indeterminato

Sospensione precoce (82gg) LTV per nausea
14 giorni dopo STOP LTV
colite da CMV trattata con ganciclovir e foscarnet, sospesi per mielotossicità, poi maribavir e in seguito con cidofovir per l'insorgenza di resistenza a MBV (mutazioni T409M, V353A, UL97).

TX-CSE a maggio 2024, maschio, 24 anni; patologia di base: Linfoma di Hodgkin; CMV-sierostato: D+/R+



TX-CSE: trapianto di cellule staminali emopoietiche; LTV: letermovir; FOS: foscarnet; VGCV: valganciclovir; MBV: maribavir; CID: cidofovir; QF-CMV: QuantiFERON®-CMV, Qiagen

Monitoraggio immunologico post LTV profilassi

Risultati preliminari
l'incidenza delle infezioni da CMV clinicamente significative è risultata pari al 22.7% (5/22).
La risposta cellulo-mediata CMV-specifica a 100 gg è risultata: indeterminata (1 paz), negativa (3 paz) e positiva con con valore vicino al cut-off (1 paz).

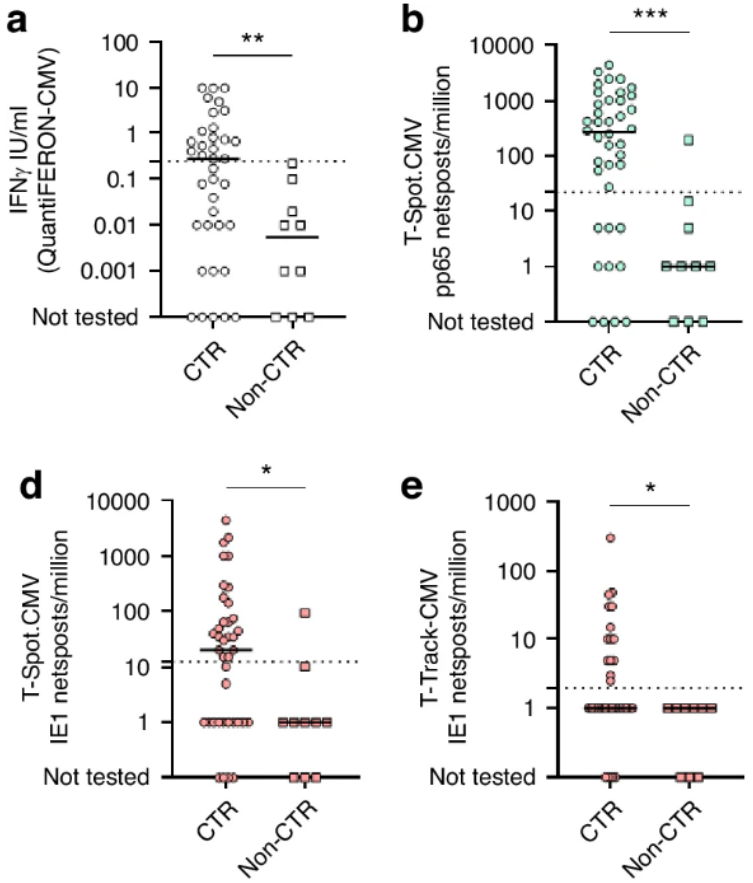
Il test QF-CMV può aiutare a identificare i pazienti a rischio di sviluppare infezioni da CMV clinicamente significative al termine dei 100 giorni di profilassi con LTV.

VALUTAZIONE DELLA RISPOSTA CELLULO MEDIATA CMV SPECIFICA NEI TRAPIANTATI DI CELLULE STAMINALI EMOPOIETICHE DOPO I 100 GIORNI DI PROFILASSI CON LETERMOVIR

E.C. Borgatti[1], F. Lanna[1], M. Franceschiello[1], M. Tomaiuolo[2], F. Bonifazi[3], L. Gabrielli[2], G. Piccirilli[2], T. Lazzarotto[1,2]

[1]DIMEC, Università di Bologna, Bologna; [2]UOC di Microbiologia, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna;
[3]Trapianto e Terapie cellulari in Ematologia, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna

Preliminary results – Part of clinical trial NCT06339892



CORRESPONDENCE

Human cytomegalovirus-specific T-cell responses and immune-guided strategies as predictors of clinically significant infection in hematopoietic stem cell transplant recipients after letermovir prophylaxis

Bone Marrow Transplantation 2026

Dalila Mele[1], Michele Malagola[2], Federica Zavaglio[1], Giulia Grassia[1], Elizabeth Iskandar[1,3], Valentina Zoboli[4], Gloria Vaira[2], Antonio Bianchessi[4], Irene Defrancesco[4], Nicola Polverelli[4], Irene Cassaniti[1,5✉], Domenico Russo[2], Fausto Baldanti[1,5] and Daniele Lilleri[1]

f

	CUT-OFF	AUC	Sensitivity (%)	95% CI	Specificity (%)	95% CI	PPV (%)	NPV (%)
T-Spot.CMV pp65	>20	0.838	90	60-99%	75	60-86%	96.77	47.3
T-Spot.CMV IE1	>10	0.733	90	60-99%	58	42-72%	92.3	33.3
T-Track-CMV pp65	>15	0.811	100	70-100%	61	44-75%	100	40.2
T-Track-CMV IE1	>2	0.727	100	70-100%	42	27-59%	100	32.1
QuantiFERON CMV	>0.25	0.763	100	70-100%	51	36-67%	100	34.48

CTR = controllers: relicaz controllata/CMV PCR neg

Non-CTR: trattati per csCMVi

Between day 100 and 180

50 patients. At day 100 (end of LTV prophylaxis) HCMV-specific T-cell response was higher in CTR with most of the assays used.

Clinical Transplantation

2025

WILEY

Clinical TRANSPLANTATION

REVIEW ARTICLE

Management of Cytomegalovirus Infection in Allogeneic Hematopoietic Stem Cell and in Solid Organ Transplantation: Updated Recommendations by the GITMO, SITO, SIMIT, and AMCLI Italian Societies

Corrado Girmenia[1] | Tiziana Lazzarotto[2,3] | Massimo Martino[4] | Francesca Bonifazi[5] | Fausto Baldanti[6,7] | Pierangelo Clerici[8] | Franco Citterio[9] | Luciano De Carlis[10,11] | Giovanni Barosi[12] | Paolo Antonio Grossi[13]

TABLE 2 | Recommendations on posttransplant CMV status and specific immunological monitoring.

- In both allo-HSCT and SOT, monitoring of CMV infection after transplant should be performed by assaying CMV DNAemia with real-time PCR. Whole blood is preferable to plasma, and results should be preferably reported in International Units (IU)/mL rather than in copies/mL.
- In allo-HSCT, CMV DNAemia monitoring is recommended in all types of transplant and conditions. In standard risk allo-HSCT (i.e., patients with negative CMV DNAemia and not receiving immunosuppressive therapy for GVHD), DNAemia should be determined once a week in the first trimester after allo-transplant, and then at least once a month, until GVHD prophylaxis is discontinued. In high-risk allo-HSCT (i.e., patients with previous CMV DNAemia positivity or receiving immunosuppressive therapy for any cause), intensification of the monitoring schedule (i.e., twice a week) should be applied along the whole high-risk period.
- In SOT, CMV DNAemia monitoring is recommended in patients not receiving antiviral prophylaxis in the context of a preemptive strategy. In standard-risk SOT recipients (i.e., patients with negative CMV DNAemia and not receiving treatment for acute graft rejection), not receiving antiviral prophylaxis, DNAemia should be determined at least once a week in the first trimester post-transplant, once every other week in the second trimester, and once every month up to 1 year in the absence of clinical indications. In high-risk SOT recipients (i.e., with primary infection, during treatment for acute graft rejection, or in the presence of additional infection risk factors, such as T-cell depletion), not submitted to prophylaxis and undergoing a preemptive approach, intensification of the monitoring schedule (twice a week) should be applied.
- Interferon-gamma release assays (IGRAs) should be used for monitoring CMV-specific T cell-mediated immunity (CMI). ELISPOT provides combined CD4+ and CD8+ T cell results, while QuantiFERON provides CD8+ T cell results.
- CMV-specific CMI should be monitored after transplantation in SOT (starting at least 4 weeks after transplantation) and no earlier than 60 days after engraftment in allo-HSCT recipients.
- Measuring CMV-specific CMI in allo-HSCT receiving letermovir prophylaxis is useful in predicting risk and helps identify patients who may benefit from extended surveillance or prolonged prophylaxis.
- In allo-HSCT patients not receiving letermovir prophylaxis, CMV-specific CMI monitoring is suggested starting at least 60 days after transplant. The frequency of the post-transplant CMI determination has not yet been defined.
- In SOT, CMV-specific CMI monitoring is suggested at specific time points posttransplant. After the resolution of the first episode of active CMV infection, CMV-specific CMI monitoring is useful to identify patients at higher risk of viral relapses.
- CMV-specific CMI monitoring is suggested in both allo-HSCT and SOT after CMV prophylaxis discontinuation in view of the possible delay in CMV-specific immunological recovery in patients receiving prophylaxis, particularly when letermovir is used.

Ruolo dei TEST IMMUNOLOGICI CMV-SPECIFICI

Potrebbero contribuire a:

- ✓ Identificare i pazienti a rischio di infezione clinicamente significativa da CMV
- ✓ Selezionare i pazienti che potrebbero beneficiare di un prolungamento della profilassi antivirale
- **Stratificare il rischio nei candidati nel contesto pre-trapianto, soprattutto quando la sierologia è dubbia**

Clinical Transplantation

2025

REVIEW ARTICLE

WILEY

Clinical TRANSPLANTATION

Management of Cytomegalovirus Infection in Allogeneic Hematopoietic Stem Cell and in Solid Organ Transplantation: Updated Recommendations by the GITMO, SITO, SIMIT, and AMCLI Italian Societies

Corrado Girmenia[1] | Tiziana Lazzarotto[2,3] | Massimo Martino[4] | Francesca Bonifazi[5] | Fausto Baldanti[6,7] | Pierangelo Clerici[8] | Franco Citterio[9] | Luciano De Carlis[10,11] | Giovanni Barosi[12] | Paolo Antonio Grossi[13]

TABLE 1 | Recommendations on the assessment of pretransplant CMV status of transplant donor and recipient.

- In allo-HSCT and SOT, both donor and recipient should be evaluated for anti-CMV serological status prior to transplant. CMV-specific IgG should be utilized, as serologic tests measuring IgM or a combination of IgG and IgM exhibit lower specificity and may yield false-positive results.
- When either the donor or recipient results seronegative in the pretransplant phase, serology should be re-evaluated at the time of transplant (no later than 1 week before starting the conditioning regimen).
- For allo-HSCT recipients with equivocal IgG antibody results, assessing CMV-specific cell-mediated immunity (CMI) assays can help to define the true CMV serologic status. For SOT recipients with equivocal CMV-IgG antibody results due to low IgG antibody levels or recent transfusion of intravenous immunoglobulins or other blood products, evaluating CMV-specific CMI assays can help define the true CMV serologic status.
- In allo-HSCT, if equivocal CMV serologic status remains either in the donor or recipient, the transplant should be considered at high risk for CMV infection. In SOT, if equivocal CMV serological status remains in the recipient, the transplant should be considered at high CMV risk.
- In allo-HSCT, the donor evaluation should include anti-CMV IgM testing, and anti-CMV IgM-positive candidate donors should be temporarily excluded from donation while waiting for CMV DNAemia.
- In allo-HSCT, if the recipient is seronegative, a seronegative donor is recommended, and, if possible, selected, while, if the recipient is seropositive, a seropositive donor is recommended, and, if possible, selected.
- In SOT, the CMV serological status of the recipient and donor does not represent a criterion of donor selection but should be used to define the CMV risk for posttransplant preventive strategy.

CONCLUSIONI – test IGRA

In grado di rilevare l'INF-γ nel sangue o nelle cellule dopo stimolazione *ex vivo* con antigeni di CMV.

Vari test, tra cui QuantiFERON-CMV e EliSpot-CMV.

Uso clinico:

Indicatore del rischio di malattia da CMV dopo trapianto.

«Potenziale guida per le strategie di prevenzione e trattamento».

Limiti:

Mancanza di standardizzazione.

Mancanza di una thresholds di positività.

Numero limitato di studi prospettici e sperimentali che utilizzano questi test per la gestione della malattia.

TAKE-HOME message

Le strategie di monitoraggio immunologico virus-specifiche, oggi dovrebbero essere incluse nella pratica clinica per la gestione dei pazienti trapiantati.

Combinando il monitoraggio virologico e immunologico, è possibile realizzare terapie personalizzate per il controllo dell'infezione da CMV dopo il trapianto e cioè realizzare strategie di intervento clinico-assistenziale per ogni singolo paziente trapiantato.

E’ utile condurre studi nazionali/internazionali multicentrici che includano pazienti con diversi profili di rischio per ottenere una valutazione più appropriata dei test immunologici virus-specifici.

SERVIZIO SANITARIO REGIONALE EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Bologna
IRCCS Istituto di Ricovero e Cura a Carattere Scientifico

POLICLINICO DI SANT'ORSOLA

ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA

Unità Operativa Complessa di Microbiologia – Prof. ssa T. Lazzarotto
SS di Virologia – Dott.ssa L. Gabrielli

G. Piccirilli, S. Venturoli, E. Petrisli,
EC. Borgatti, F. Lanna, A. Balboni,
A. Liberatore, M. Tomaiulo, V. Ferraro, T. Longobardi,
F. Baiesi Pillastrini, S. Michelini, F. D'Adamo

Trapianto e Terapie cellulari in Ematologia

F. Bonifazi, M. Dicataldo, E. Maffini, S. Falcioni,
M. Ursi, F. De Felice, M. Roberto, F. Ardizzoia

Unità Operativa di Malattie Infettive

M. Giannella, R. Pascale