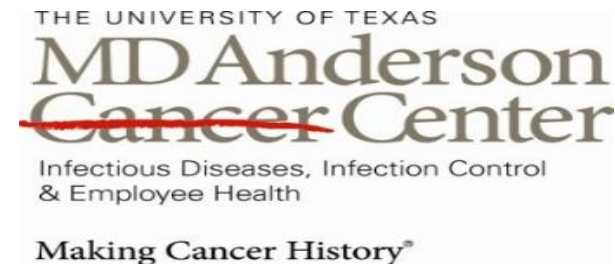

TAVI Meeting October 15, 2024

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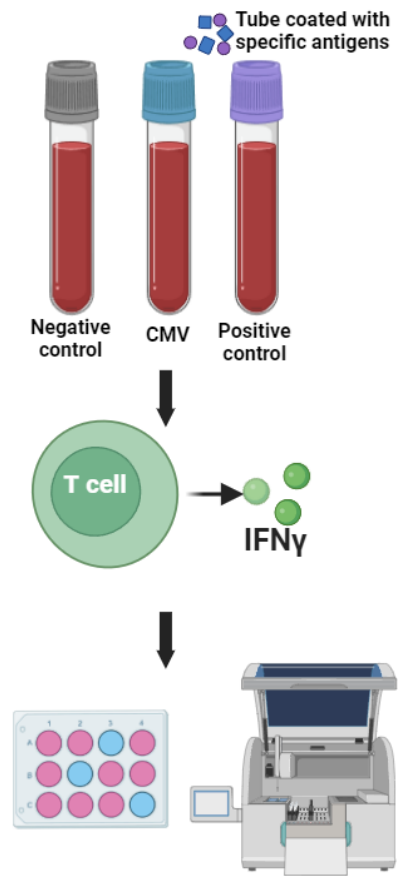
Toward Functional CMV Immune Monitoring for predicting CMV-mediated events in Hematopoietic Cell Transplant Recipients

Agenda

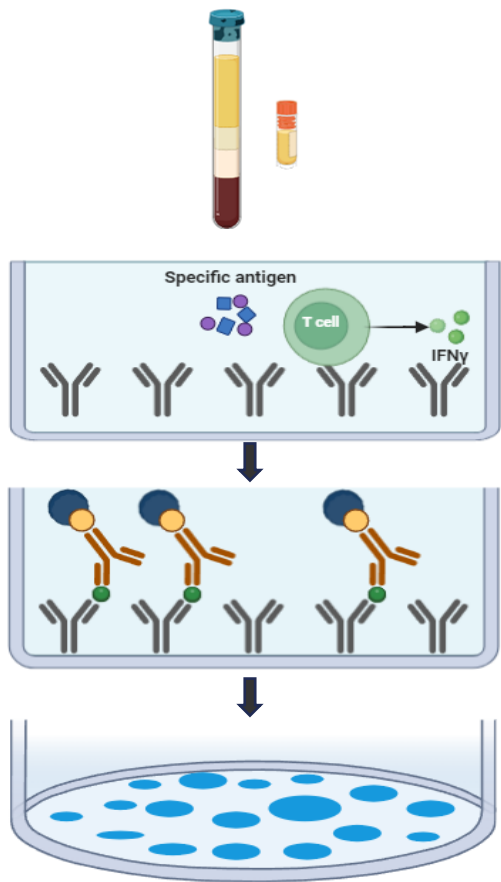
- Introduction
- Prisma guidelines
- Results
- Challenges

CMV CMI Assays

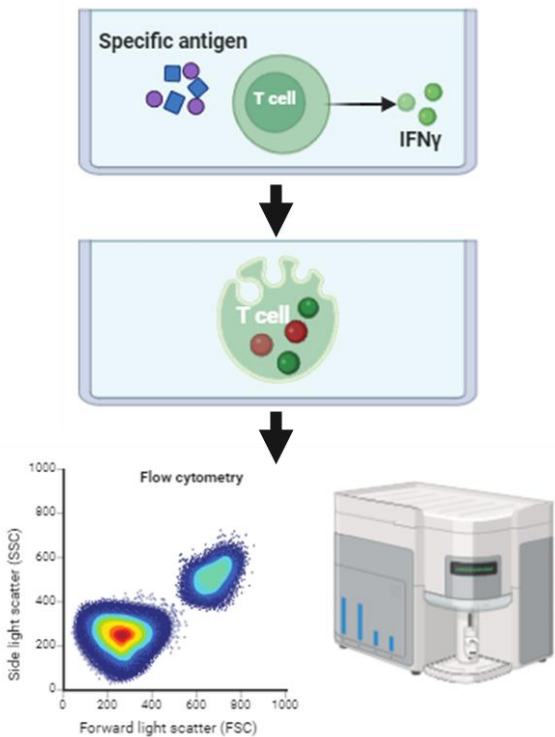
QuantiFERON



T- Spot



Flow cytometry ICS



Dextramer CMV Kit



Commercial assays for detection of CMV CMI

CMV-CMI Assay Type	Commercial Assay(s)	Patient Sample	Required Quantity	Antigen(s)	Required Equipment	Results and Interpretation
ELISA	Quantiferon-CMV (Qiagen Inc, Hilden, Germany)	Whole blood	3 mL	Peptide pool	ELISA microplate reader	Reports IFN- γ production in response to exposure to CMV antigen as a surrogate for CD8+ response Test considered reactive if IFN- γ response in CMV tube is significantly increased over IFN- γ response in nil antigen tube (≥ 0.20 IU/mL and $\geq 25\%$ of nil antigen value)
ELISpot	T.Spot.CMV assay (Oxford Immunotec International, Oxfordshire, England) T-Track CMV assay (Mikrogen GmbH/Lophius Biosciences GmbH, Neuried, Germany)	Whole blood	12 mL ^a 7.5 mL	pp65 and IE-1	ELISpot reader	Measures both CD4+ and CD8+ responses Test reports number of "spot-forming units," regions in test wells that produce IFN- γ Positive cutoff has not been defined, but use of cutoffs ranging from 20 to 40 spot-forming units have been reported
Intracellular cytokine staining	CMV inSIGHT™ T Cell Immunity Panel (Eurofins Viracor, Lenexa, Kansas, USA)	Whole blood	10 mL	CMV peptides	Flow cytometer	Reports both CD4+ and CD8+ responses distinctly as a percentage of cells producing IFN- γ A test is considered positive if $\geq 0.20\%$ of T cells express IFN- γ
MHC multimer staining	CMV CD8 T Cell Immune Competence (Mayo Clinic Laboratories, Rochester, Minnesota, USA)	Whole blood	20 mL	CMV peptides	Flow cytometer	Reports absolute CD3+ and CD8+ T cell counts, as well as CMV-specific CD8 + T cells Total number of CMV-specific CD8+ cells that express IFN- γ and cytotoxic cell surface markers is also reported Reference ranges provided to allow for interpretation of results

Abbreviations: CE, Conformité Européenne; CMI, cell-mediated immunity; CMV, cytomegalovirus; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunospot; FDA, Food and Drug Administration; IFN- γ , interferon gamma; MHC, major histocompatibility complex.

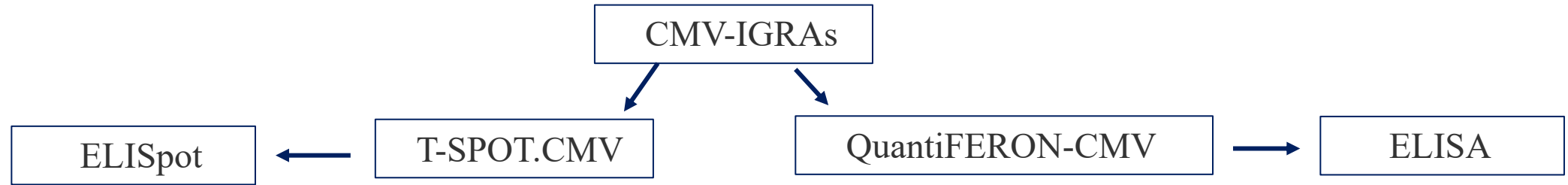
^aCMV peptide pool from IE-1, IE-2, pp65, pp50, and gB; designed to target CD8+ T cells, including HLA Class I haplotypes covering >98% of human population.

Landmark Studies for CMV-CMI in HCT recipients

- Two CMV-CMI assays that have been prospectively studied in allo-HCT include the T-SPOT.CMV and the QuantiFERON-CMV
- The multicenter REACT study demonstrated that 94% of allo-HCT patients who developed CS-CMVi had low CMV-CMI according to the T-SPOT.CMV, and that patients with high CMV-CMI were protected from CS-CMVi
- Single-center trials have demonstrated that QuantiFERON-CMV predicted protection from CS-CMVi

Any Difference: T-SPOT. CMV and QuantiFERON-CMV?

Background: CMV-IGRA can quantify CMV cell-mediated immunity by measuring the IFN- γ that is released by CD4+ and CD8+T lymphocytes in the presence of CMV antigens.



Methods: comparison between T-Spot. CMV and QTF-CMV at D+28 and D+100 post Allo-HCT to assess predictive values of both tests for CMV reactivation. Clinical information was captured until D+270.

Results: preventive or therapeutic strategies should be assay-specific; Only moderate agreement between the two tests at D+100. The two tests are not interchangeable.

Screening Methods

- Inclusion

Population

- HCT - Allogeneic
- >10 patients that meet the study criteria
- Prospective observational studies

Intervention

- EliSPOT
- QuantiFERON
- Intracellular cytokine staining and Flow Cytometry
- Dex/Tetramer CMV kit

Control / Outcomes

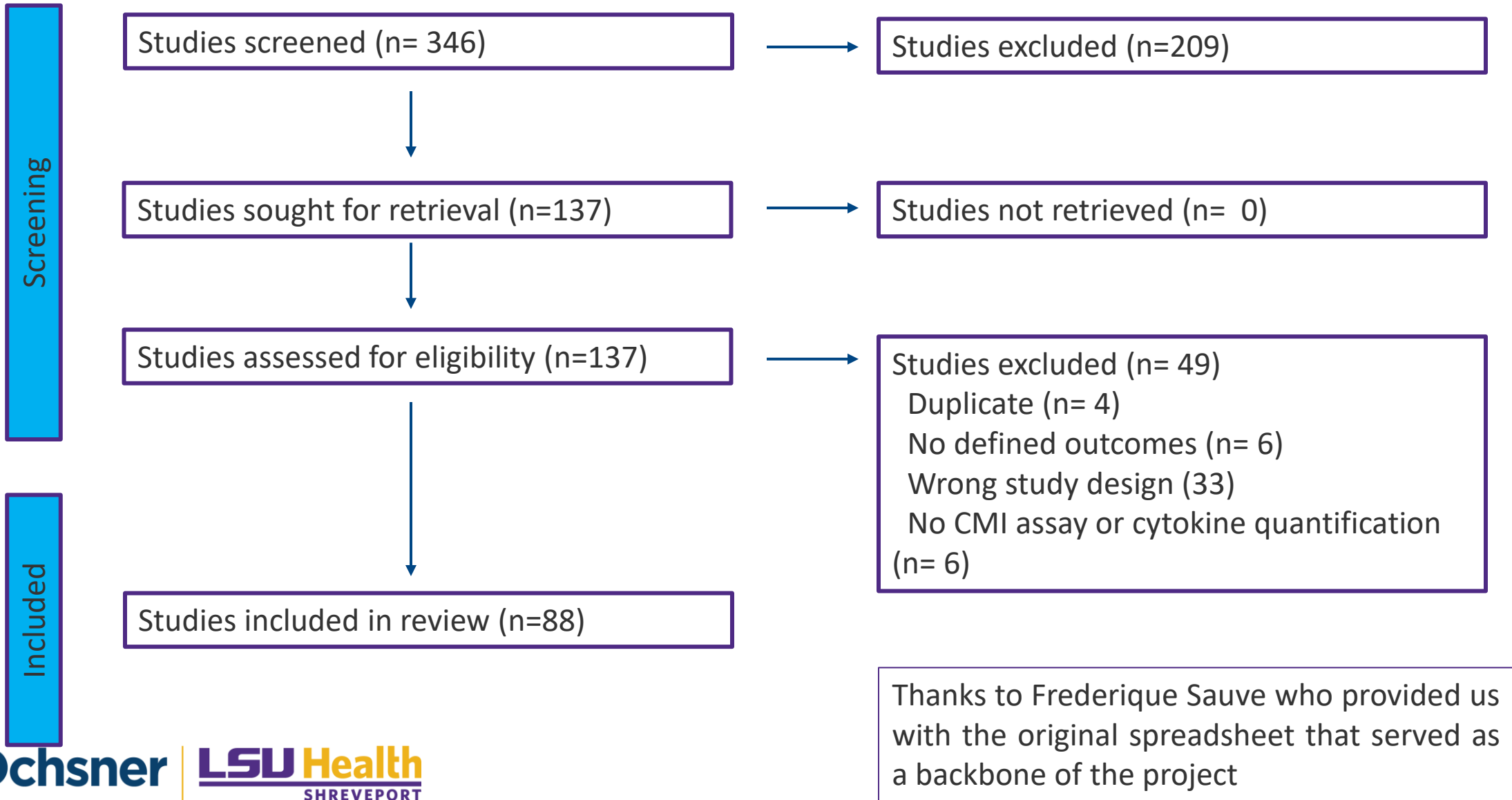
- CMV infection (PP65 antigenemia or DNAemia)
- CS- CMVi/CMV disease

- PubMed Searched on 10.07.24

MeSH Terms:

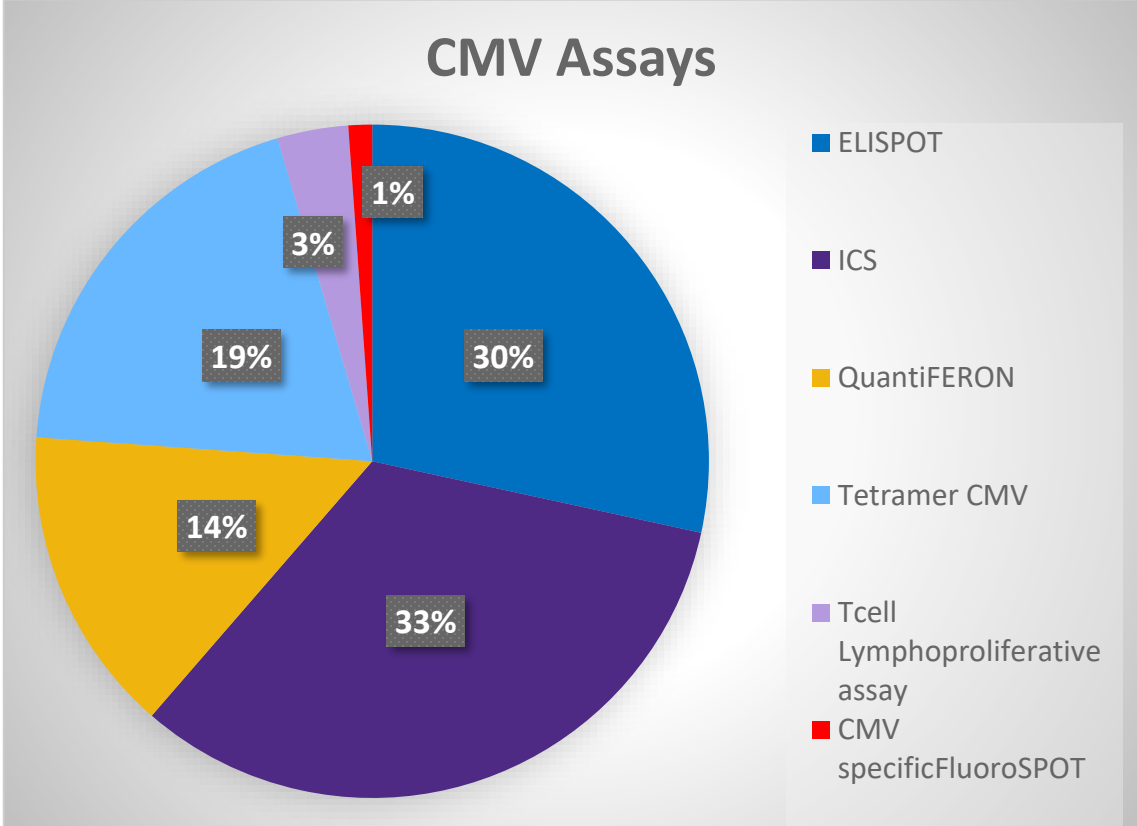
- ("Cytomegalovirus" OR "CMV") AND ("cell mediated immunity" OR "CMI" OR "immunity, cellular" OR ("elisspots" OR "enzyme linked immunospot assay" OR ("enzyme linked" AND "immunospot" AND "assay")) OR "Intracellular cytokine staining" OR "Tetramer" OR "QuantiFERON" OR "interferon-gamma release assay" OR "IGRA" OR "interferon gamma") AND ("bone marrow transplantation" OR "bone marrow transplant" OR ("Hematopoietic Stem Cell Transplantation" OR "Hematopoietic Stem Cell Transplant" OR "transplantation"))
- We also searched abstracts presented at Transplant Cellular Therapy meetings, American Society of Clinical Oncology meetings, and IDWeek meetings

Flow Chart



Type of CMI Assays - Summary

Types of the commercial assay	Number of observational studies
ELISPOT / Tspot-CMV	26
ICS and flow cytometry	29
QuantiFERON – CMV / ELISA	12
Dextramer CMV	17
CMV- T cell Lymphoproliferative assay	3
CMV Specific FluoroSPOT	1



Clinical Scenarios

Clinical Questions

- Pretransplant risk stratification in CMV seropositive transplant candidates
- CMV Reactivation Post HCT
- Risk stratification posttransplant at cessation of prophylaxis or extending prophylaxis period
- Preemptive monitoring with low-level CMV DNAemia
- Post therapy monitoring for CMV infection or disease
- Periods of heightened immunosuppression (treatment of rejection or GVHD)

Positive correlation between ELISPOT and respective Clinical Scenarios

Studies	Year of publication	HCT Recipients	Gender (Male, M; Female, F)	Patient age (median)	Clinical Scenario
Sacre et al.	2008	23	M:14, F:9	49	CMV reactivation risk post HCT
Jung et al.	2017	84	M:50, F: 34	46	CMV reactivation risk post HCT
Kiener et al.	2019	69	?	?	Post therapy monitoring for CMV infection or disease
Nesher et al.	2016	63	M:37,F:26	56	CMV reactivation risk post HCT
Shah et al.	2016	25	M:17, F:8	58	Preemptive monitoring with low level CMV viremia
Chemaly et al.	2020	241	M:136, F:105	56	CMV reactivation risk post HCT
Avetisyan et al.	2007	58	M: 32, F:26	46.9	Post therapy monitoring for CMV infection or disease
Gabanti et al.	2022	70	M: 36, F: 25	58.5	Post therapy monitoring for CMV infection or disease
Liang et al.	2021	47	M:23, F:24	35	Risk stratification post HCT at cessation of ppx
Ohnishi et al.	2005	37	M:26,F:11	58	CMV reactivation risk post HCT
Wagner-Drouet et al.	2021	175	M:88, F:66	58	Post therapy monitoring for CMV infection or disease
Hebart et al.	2002	23	M:11, F:12	44	CMV reactivation risk post HCT
Mohty et al.	2004	54	?		Post therapy monitoring for CMV infection or disease
Aubert et al.	2001	11	M:7, F: 4	35	CMV reactivation risk post HCT
Tassi et al.	2023	84	?	56	CMV reactivation risk post HCT
El Haddad et al.	2019	55	M:31, F:24	56	Preemptive monitoring with low level CMV viremia
Abate et al.	2012	31	?	8	CMV reactivation risk post HCT
Bae et al.	2018	51	M:32, F:19	41	CMV reactivation risk post HCT
Gabanti et al.	2019	36	M:23, F:13	58	CMV reactivation risk post HCT
Nemeckova et al.	2016	30	M:17, F:13	53	CMV reactivation risk post HCT
Seo et al.	2021	52	M:34, F:18	10.2	CMV reactivation risk post HCT and Pretransplant risk stratification
Ganepola et al.	2007	38	M:21, F: 17	49	CMV reactivation risk post HCT
Ariza-Heredia et al.	2023	226	M:127; F:99	57	CMV reactivation risk post HCT and Pretransplant risk stratification
Zavaglio et al.	2023	66	M:37, F:29	58	Risk stratification post HCT at cessation of ppx
Xia et al.	2022	189	M:97, F:92	41	CMV reactivation risk post HCT
Bejanyan et al.	2021	26	?	?	CMV reactivation risk post HCT

Correlation between QuantiFERON-CMV and given Clinical Scenarios

Studies	Year of Publication	HCT Recipients	Gender (M, F)	Patient age (median, y)	Clinical Scenarios
Douglas et al.	2020	40	M:27, F:13	47	CMV reactivation risk post HCT
Thompson et al.	2022	46	M: 31, F:15	55	CMV reactivation risk post HCT
Krawczyk et al.	2018	34	M:22, F:12	48	CMV reactivation risk post HCT
Lee et al.	2017	25	M:19, F: 6	33 Mo	Post therapy monitoring for CMV infection or disease
Paouri et al.	2018	37	M:23, F: 14	8.5	Pretransplant risk stratification and CMV reactivation risk post HCT
Soldatou et al.	2014	37	?	?	CMV reactivation risk post HCT
Tey et al.	2013	41	M:23, F:18	51	CMV reactivation risk post HCT
Yong et al.	2017	94	M:44, F:50	50	CMV reactivation risk post HCT
Yoon et al.	2024	81	M:53, F:28	50.5	CMV reactivation risk post HCT
Giménez et al.	2023	12	M:9, F:3	53	Immune recovery of CMV-specific T cell while on LMV?
Valle-Arroyo et al.	2022	81	M:51, F:30	57	Pretransplant risk stratification
Fleming et al.	2010	43	?	?	Immune recovery of CMI

Challenges - Heterogeneity of the data and complex immune protection

- Numerous type of assays for measuring CMV-CMI activity
- Cell-mediated immunity affected by the activity of CD4+ T cell and CD8+ T cell
- Assessing the different role of CMI against Immediate-early (IE-1) or phosphoprotein 65 (pp65) or other key viral antigens
- Release of cytokines to protect the host against CMV infection
- Role of interferon-gamma and prediction of CMI
- The impact of antiviral prophylaxis on the development of CMV-CMI
- Timing and duration of antiviral ppx
- Prediction of late CMV reactivation
- The impact of donor CMV serostatus and CMI
- The use of IS agents to control or prevent GVHD and their impact on CMI (MRD # MUD # Haplo # CB)

Future Plans

- Finalize the data collection with details on GVHD, immunosuppression, and underlying primary cancer disease before the stem cell transplant
- Perform Meta-analysis and sub-analysis for homogenous data based on specific CMV cell-mediated immunity assay and type of stem cell grafts

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- Forum for Collaborative Research Team