A Prospective Observational Study to Evaluate a Cytomegalovirus(CMV)–specific T-SPOT® Assay in Hematopoietic Stem Cell Transplant Recipients: The REACT Study Interim Data Review

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ABSTRACT

BACKGROUND:

Cytomegalovirus (CMV) infection causes significant morbidity and mortality after allogeneic hematopoietic cell transplantation (allo-HCT). CMV management after HCT includes risk stratification and mainly a preemptive strategy with CMV viral load serial monitoring. Cell mediated immunity (CMI) plays a role in CMV reactivation and can be assessed by cytokine responses such as T cell production of interferon gamma (IFN-γ). Quantification of CMV CMI may have value in CMV management. This study evaluated the potential of a CMV-specific ELISPOT assay to determine CMI against CMV reactivation in allo-HCT recipients ≤26 weeks post-HCT.

METHODS:

This is an ongoing, multi-center, prospective, observational study of ≥150 adult CMV seropositive allo-HCT recipients. CMV management was according to institutional protocols. Date of CMV reactivation, as defined by each institution, was recorded. T cell responses were serially monitored pre-, and every 2 weeks post-transplantation up to 26 weeks with an ELISPOT assay that uses CMV-specific antigens IE-1 and pp65 (T-SPOT.*CMV*, Oxford Diagnostic Laboratories®, Memphis, TN). Data reviewed include patients reaching ≥12 weeks post-HCT by March 2016.

RESULTS:

Thirty-five patients across 6 sites reached ≥12 weeks post-HCT, and 15 reached ≥22 weeks. Majority of the patients were white (77%), males (54%), with a median age of 57 (25-80) years, and had unrelated (46%) or matched related (46%) HCT (Table 1). CMV reactivation occurred in 13 patients (37%) time to first reactivation occurred ≤10 weeks post-HCT (Table 2). Average immune response as measured by CMV-specific IE-1 and pp65 spot counts (SPC) increased over time post-transplantation (Figure 2).

CONCLUSIONS:

The preliminary analysis of the REACT study showed CMV reactivation occurred early during transplantation when the CMV immune response (measured by a CMV-specific ELISPOT assay) was lower. In contrast, no reactivation was seen later on when immune response was higher. This study may provide insights into the CMV immune response which may guide personalized decisions regarding CMV management.

INTRODUCTION

- Cytomegalovirus (CMV) establishes latent infection after resolution of acute infection
- CMV reactivation is a significant and frequent complication in allogenic hematopoietic cell transplantation (allo-HCT) patients who are CMV seropositive
  - A pre-emptive strategy of monitoring CMV viral load is commonly used to screen for early reactivation
  - Antiviral agents have toxic side-effects and are used with caution
- Cell mediated immunity (CMI) is an important defense mechanism for controlling CMV replication. Understanding the strength of CMI may help identify patients who are protected against CMV reactivation
  - Both CD4+ and CD8+ T cells are implicated in protection against CMV reactivation
  - T cell response to CMV antigens produces cytokines, including interferon gamma (IFN-γ)
  - Measuring IFN-γ serves as an adaptive immune marker
- An enzyme-linked immunospot (ELISPOT) test that measures IFN-γ in peripheral blood mononuclear cells (PBMC) stimulated with CMV-specific peptide antigens IE-1 and pp65, such as the T-SPOT.*CMV* test, can be used to assess CMI (Figure 1)
- REACT[1] is an on-going, multi-site study investigating the relationship between the strength of the T cell immune response and the subsequent protection from, or occurrence of, CMV reactivation

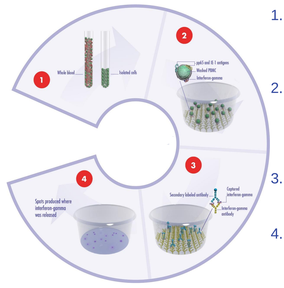
OBJECTIVE

To evaluate the potential of a CMV-specific ELISPOT assay to determine cell mediated immunity against CMV reactivation in allo-HCT CMV seropositive recipients ≤26 weeks post-HCT

METHODS

- On-going, multi-center, prospective observational study
- CMV seropositive allo-HCT patients
- Reached ≥12 weeks post-HCT
- CMV management was according to institutional protocol
- CMI was measured every 2 weeks post-HCT with an ELISPOT assay (T-SPOT.*CMV*, Oxford Diagnostics Laboratories, Memphis, TN)
- **Endpoint: first CMV reactivation**

Figure 1. CMV-specific ELISPOT test method



1. Blood is collected and PBMCs are isolated. Cells are washed, counted and normalized to create a standard cell suspension.
2. A standard number of cells are added into specially designed plates and stimulated with IE-1 and pp65 peptide antigens. Cells responding to these antigens release the cytokine IFN-γ.
3. IFN-γ antibodies directly capture IFN-γ. A secondary labeled antibody is added and binds to the captured IFN-γ.
4. A detection reagent is added and reacts with the secondary labeled antibody. This reaction produces spots which are a footprint of where the IFN-γ was released. Spots are counted.

RESULTS

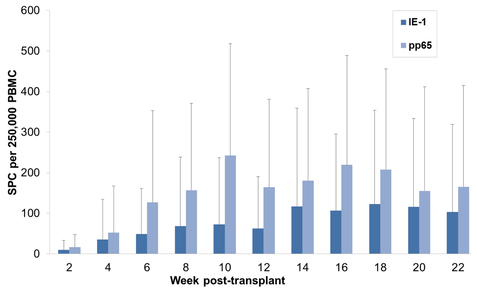
Table 1. Patient characteristics

		All	CMV reactivation	No CMV reactivation
Number		35	13 (37%)	22 (63%)
Age in years (median, range)		57 (25 - 80)	63 (37 - 80)	57 (25 - 71)
Gender	Male	19 (54%)	4 (31%)	15 (68%)
	Female	16 (45%)	9 (69%)	7 (32%)
Race	White	27 (77%)	9 (69%)	18 (82%)
	African American	1 (3%)	1 (8%)	0 (0%)
	Hispanic	1 (3%)	0 (0%)	1 (5%)
	Asian	3 (9%)	2 (15%)	1 (5%)
	Unknown	3 (9%)	1 (8%)	2 (9%)
Type of cancer	Leukemia	25 (71%)	11 (85%)	14 (64%)
	Acute Myelogenous Leukemia	1 (3%)	0 (0%)	1 (5%)
	Lymphoma	4 (11%)	0 (0%)	4 (18%)
	Non-Hodgkin Lymphoma	1 (3%)	0 (0%)	1 (5%)
	Myelodysplastic syndrome	4 (11%)	2 (15%)	2 (9%)
Type of transplant				
	Matched or Mismatched Unrelated Donor	16 (46%)	7 (54%)	9 (41%)
	Matched Related	16 (46%)	3 (23%)	13 (59%)
	Haploidentical	3 (9%)	3 (23%)	0 (0%)
Days from HCT to engraftment (median, range)		12 (8 - 19)	13 (9 - 19)	11 (8 - 14)
Donor CMV serostatus	Positive	20 (57%)	6 (46%)	14 (64%)
	Negative	15 (43%)	7 (54%)	8 (36%)

Table 2. Timing of first CMV reactivation

	Time post-transplant (week)						
	2	4	6	8	10	12	14 – 22
# patients with first reactivation	2	3	4	1	3	0	0
# patients with first reactivation, cumulative	2	5	9	10	13	13	13
% of patients with first reactivation, cumulative	6%	14%	26%	29%	37%	37%	37%

Figure 2. IE-1 and pp65 immune response over time

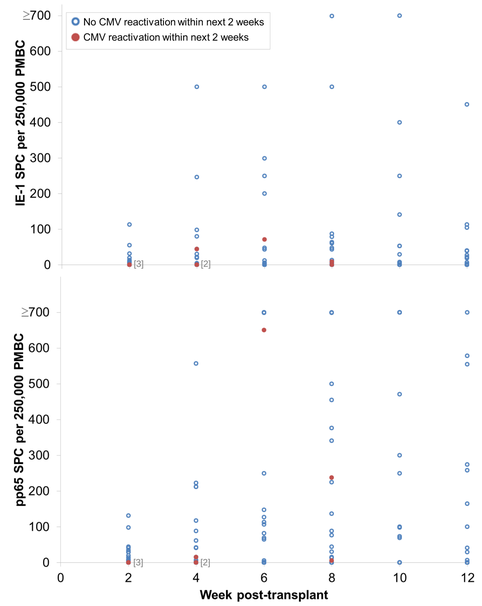


Number of patients with first CMV Reactivation (#)

2	3	4	1	3	0	0	0	0	0	0

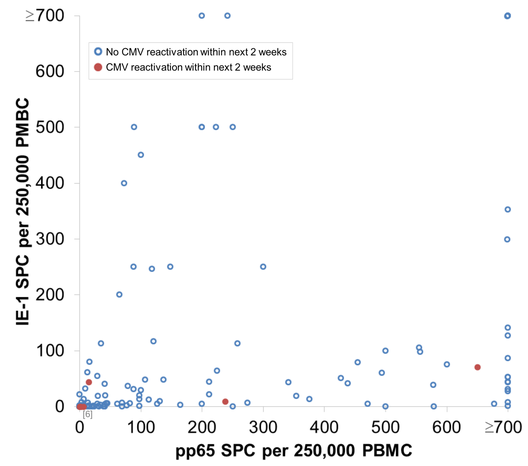
- Spot counts (SPC) of cells responding to CMV-specific antigens and producing IFN-γ were enumerated
- First CMV reactivation occurred when immune response was lower
- Immune response increased over time
- High variability of immune response is a result of the dynamic individual patient response

Figure 3. IE-1 and pp65 immune response and CMV reactivation within next 2 weeks over time



- Post-transplant immune response at each time point and first CMV reactivation within next 2 weeks
  - 9 CMV reactivations with an ELISPOT immune response reading 2 weeks prior to reactivation
  - 4 patients with CMV reactivations did not have an ELISPOT immune response reading within prior 2 weeks

Figure 4. IE-1 and pp65 immune response and CMV reactivation within the next 2 weeks



- Post-transplant immune response for each patient at each post-HCT time point and whether or not the patient had first CMV reactivation within the following 2 weeks
  - Multiple-time points per patient (every two weeks post-transplant)
  - Time points after first reactivation were excluded
  - 9 CMV reactivations with an ELISPOT immune response reading within 2 weeks prior to reactivation
- Higher immune response was associated with protection from reactivation

CONCLUSIONS

- **CMV immune response is dynamic and increased over time following post-HCT**
- **First CMV reactivation occurred ≤ 10 weeks post-HCT when CMV immune response was lower**
- **Monitoring CMV immune response may guide personalized decisions regarding CMV management**

1. A Prospective Observational Trial to Evaluate Hematopoietic Stem Cell Transplantation CMV **R**eactivation **E**vents – **A**ssessed by a **C**MV-specific **T**-SPOT Assay: The REACT Study. US OI 125.

This study is sponsored by Oxford Immunotec, Inc.

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