Diagnostics WG

Immune Assays Discussion Group

Group Members

- VM will add

Introduction

- Purpose: review how these assays could be used in drug development, and how drug development could help generate the necessary information
- Are we on the way to personalized medicine?
- Describe function and potential role of immune assays in CMV drug development
- Potential role in combined virologic + immunologic endpoints
- Link back to IDSA clinical guidance publication and summary of data for SOT
- Refer to more recent SOT data and data for HSCT

Description of available commercial assays

- Comparison of assay characteristics
- Characteristics to be included
  - Cells – whole blood, PBMCs
  - CMV antigen source
- Ease of use in clinical practice (or at clinical trial sites) – assay stability over time?
- For purposes of drug development, these assay characteristics will be very important

Assay	Approval Status	Sample	Stability
Viracor Eurofins CMV T Cell Immunity Panel		whole blood 10 ml	32 hours at ambient temp
Iophius bioscciences T-Track	EU CE IVD kit	whole blood 3-7.5 ml PBMC	24 hours at room temp
QuantiFeron CMV ELISA	EU	whole blood 3 mls	store 4-25 degrees incubate within 16 hours
Oxford Immunotech T-Spot CMV	EU	PBMC	32 hours

Assay	Assay type	CMV Ag	Assay time
Viracor Eurofins CMV T Cell Immunity Panel	Intracellular cytokine staining	CD4: CMV lysate CD8: pp65 peptide mix	
Iophius bioscciences T-Track	ELISpot	pp65/IE-1 full length proteins	17-21 hours
QuantiFeron CMV ELISA	ELISA	22 CD8+ epitopes restricted through 20 HLA	3 hours after 16-24 incubation
Oxford Immunotech T-Spot CMV	ELISpot	peptide pool pp65; IE-1	

Assay	Intra Assay	Inter Assay	Reporting	Comparisons
Viracor Eurofins CMV T Cell Immunity Panel	< 20% CV	< 23% CV	T-Track CMV Calculator Software	
Iophius bioscciences T-Track			QuantiFeron-CMV Analysis Software	Good w QuantiFeron
QuantiFeron CMV ELISA				
Oxford Immunotech T-Spot CMV				

T CELL COMPANY

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T-Track® CMV Specifications & Performance Characteristics

Summary: June 1st, 2018

RECENT PUBLICATIONS
T-TRACK® CMV

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1. Banas B, Steubl D, Renders L, Chittka D, Banas M, Wekerle T, Koch M, Witzke O, Muehlfeld A, Sommerer C, Habicht A, Hugo C, Huenig T, Lindemann M, Schmidt T, Rascle A, Barabas S, Deml L, Wagner R, Kraemer B, Krueger B. (**2018**). Clinical validation of a novel ELISpot-based *in vitro* diagnostic assay to monitor CMV-specific cell-mediated immunity in kidney transplant recipients: a multicenter, longitudinal, prospective, observational study. Transpl Int 31:436-450
2. Banas B, Böger CA, Lückhoff G, Krüger B, Barabas S, Batzilla J, Schemmerer M, Köstler J, Bendfeldt H, Rascle A, Wagner R, Deml L, Leicht J, Krämer BK. (**2017**). Validation of T-Track® CMV to assess the functionality of cytomegalovirus-reactive cell-mediated immunity in hemodialysis patients. BMC Immunol 18:15.
3. Barabas S, Spindler T, Kiener R, Tonar C, Lugner T, Batzilla J, Bendfeldt H, Rascle A, Asbach B, Wagner R, Deml L. (**2017**). An optimized IFN-γ ELISpot assay for the sensitive and standardized monitoring of CMV protein-reactive effector cells of cell-mediated immunity. BMC Immunol 18:14.
4. Jung J, Lee H-J, Kim S-M, Kang Y-A, Lee Y-S, Chong YP, Sung H, Lee S-O, Choi S-H, Kim YS, Woo JH, Lee J-H, Lee J-H, Lee K-H, Kim S-H. (**2017**). Diagnostic usefulness of dynamic changes of CMV-specific T-cell responses in predicting CMV infections in HCT recipients. J Clin Virol Off Publ Pan Am Soc Clin Virol 87:5–11.
5. Bae S, Jung J, Kim S-M, Kang Y-A, Lee Y-S, Chong YP, Sung H, Lee S-O, Choi S-H, Kim YS, Woo JH, Lee J-H, Lee J-H, Lee K-H, Kim S-H. (**2018**). The Detailed Kinetics of Cytomegalovirus-specific T cell Responses after Hematopoietic Stem Cell Transplantation: 1 Year Follow-up Data. Immune Netw 18:e2.
6. Kim S-H, Lee H-J, Kim S-M, Jung JH, Shin S, Kim YH, Sung H, Lee S-O, Choi S-H, Kim YS, Woo JH, Han DJ. (**2015**). Diagnostic Usefulness of CMV-Specific T Cell Immunity in Predicting CMV Infection after Kidney Transplantation: A Pilot Proof-of-Concept Study. Infect Chemother 47:105–110.
7. Kim T, Park SY, Lee H-J, Kim S-M, Sung H, Chong YP, Lee S-O, Choi S-H, Kim YS, Woo JH, Kim S-H. (**2017**). Assessment of cytomegalovirus and cell mediated immunity for predicting outcomes in non-HIV-infected patients with Pneumocystis jirovecii pneumonia. Medicine (Baltimore) 96:e7243.
8. Kim S-H, Lee H-S, Lee H-J, Kim S-M, Shin S, Park S-H, Kim K-J, Kim Y-H, Sung H, Lee S-O, Choi S-H, Yang S-K, Kim YS, Woo JH, Han D-J. (**2017**). Clinical applications of interferon-γ releasing assays for cytomegalovirus to differentiate cytomegalovirus disease from bystander activation: a pilot proof-of-concept study. Korean J Intern Med 32:900–909.
9. Reuschel E, Barabas S, Zeman F, Bendfeldt H, Rascle A, Deml L, Seelbach-Goebel B. (**2017**). Functional impairment of CMV-reactive cellular immunity during pregnancy. J Med Virol 89:324–331.

SENSITIVITY
T-TRACK® CMV

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- T-Track® CMV closely mimics the natural immune response to CMV infection (stimulation with T-activated® pp65 & IE-1 proteins, APC processing)
- Enables activation of broad range of CMV reactive immune cells (Th, CTL, NK, NKT-like cells)
- Not limited by HLA restriction

Result:

- **Highly sensitive (comparison to QuantiFERON® CMV and Tetramers)**
- **So far no comparative studies available to T-Spot.CMV**

% positive test results in CMV-seropositive individuals

Cohort	Sensitivity
Healthy donors[1] (n=45)	**97%**
Hemodialysis patients[2] (n=124)	
T-Track® CMV	**90%**
vs. QuantiFERON®-CMV (ELISA)	73%
vs. 6x iTAg™ MHC-I Tetramers (FACS)	77%
Kidney transplant recipients[3] (n=86)	
Pre-transplantation (Tx)	**95%**
Post-Tx (visits 1-7, over 6 months)	**88-92%**

[1]Barabas et al. (2017). *BMC Immunol.* 18:14

[2]Banas et al. (2017). *BMC Immunol.* 18:15

[3]Intermediate risk (D-/R+, D+/R+); Banas et al. (2018). Transpl. Int. 31:436-450

ALLOPROTECT CMV ONGOING STUDY IN HSCT (T-TRACK® CMV)

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Clinical Validation of T-Track® CMV in Allo-HSCT Recipients (AlloProtectCMV): active, not recruiting (ClinicalTrials.gov ID: NCT02156479)

- Official title: „Clinical Validation of an Improved T-Track® CMV Assay to Assess the Functionality of CMV Protein-reactive Cell-mediated Immunity (CMI) and Its Suitability to Determine a Protective Cut-off Value for Recurrent CMV Reactivations in Allo-HSCT Recipients“
- Prospective, observational, longitudinal, multi-center study (anticipated completion in June 2018)
- Intermediate and high risk allo-HSCT patients (D+/R-, D+/R+, D-/R+) (n = 175)
- Pre-emptive antiviral therapy
- Anticipated manuscript submission in 2018

INVESTIGATOR INITIATED TRIALS (IIT)
T-TRACK® CMV

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T-Track® CMV is currently being compared to other CMV-CMI methods in different IITs:

- **Prof. Lindemann**, University Medical Center Essen: application of T-Track® CMV and QuantiFERON-CMV after prophylaxis in kidney Tx (data presented as poster at Eurotransplant Jubilee 2017; manuscript submitted for publication)

- **Prof. Oriol Manuel**, University of Lausanne Hospitals: ClinicalTrials.gov ID: NCT02538172 (recruiting). Official title: „Monitoring of Specific Cytomegalovirus Cell-mediated Immunity (CMV-CMI) for Optimization of Preventive Strategies Against CMV Infection in High-risk Solid-organ Transplant Recipients“
  Open-label randomized controlled trial to adapt the duration of antiviral prophylaxis according to the result of the T-Track® CMV assay, in D+/R- SOT patients and in R+ SOT patients receiving ATG

HOW DOES T-TRACK® CMV DIFFER FROM OTHER CMV-IMMUNOASSAYS?

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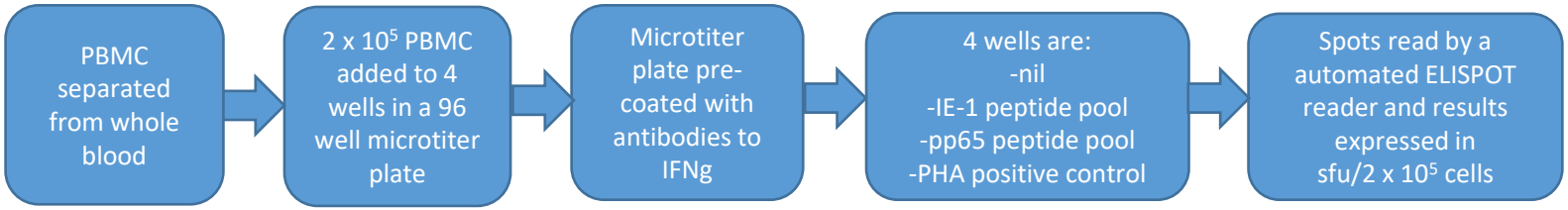
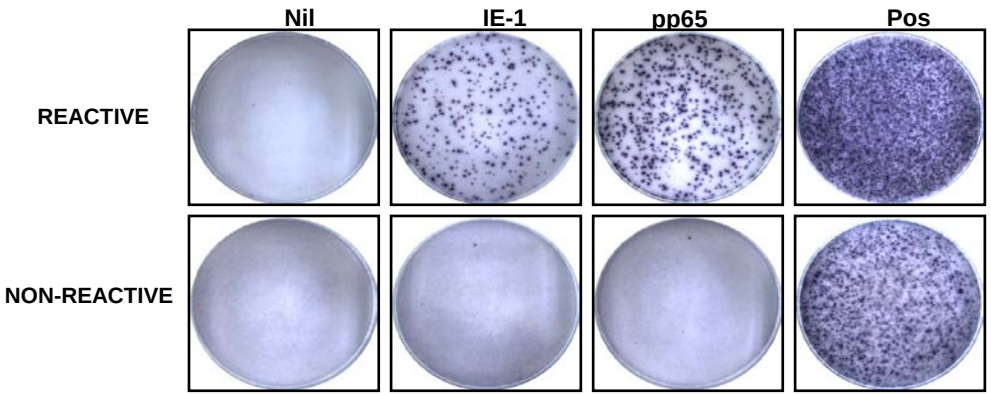
	Method	Stimulus	Quanti-tative	Func-tional	Single cell level	$CD4^+$ T cells	$CD8^+$ T cells	APC function	Starting material	CE	FDA
T-Track® CMV	ELISpot	T-activated® pp65 / IE1 proteins	√	√	√	√	√	√	PBMC	√	x
T-Spot®.CMV	ELISpot	Peptide pool (pp65, IE1)	√	√	√	√?	√	?	PBMC	√	x
QuantiFERON®-CMV (Qiagen)	ELISA	Peptide pool (selected peptides from IE-1, pp65, pp50, IE2, gB)	√	√	x	x	√	x	whole blood	√	x
CMV Tetramer Test (Glycotope)	FACS	6 selected MHC-I-specific CMV tetramers	√	x	√	x	√	x	whole blood	√	x

T-SPOT.*CMV*

- Central Testing of T-SPOT.*CMV* at Oxford Immunotec, Memphis.
- Samples rejected if >32h from collection.



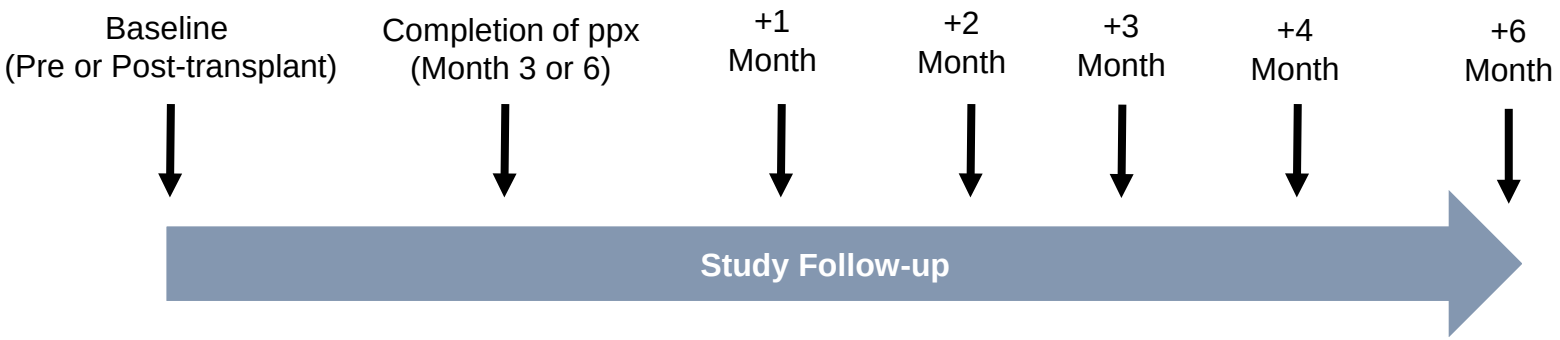


T-SPOT.*CMV* in Kidney Transplant: The PROTECT Study

- **Study rationale**: CMI is an essential pathway to control replication of CMV; therefore by measuring CMV-CMI, we can identify those patients with a robust immune response, capable of controlling the virus without therapy

- **Hypothesis**: CMV antigen sensitivity (high response) may suggest that the subject is subsequently at lower risk of clinical infection.

- Multicenter, prospective, observational study
- 583 kidney transplant subjects followed up to 1 year post-transplant
  - Serial blood draws were conducted as follows:



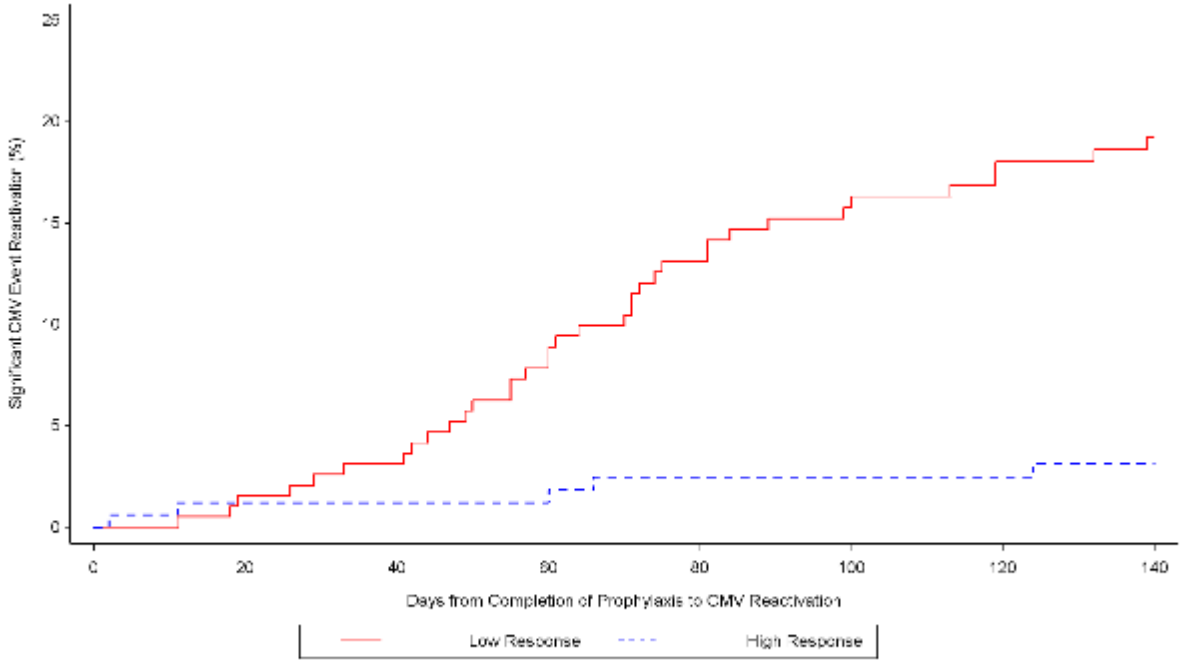
High-level Overview: Spot counts at completion of prophylaxis against occurrence of CMV

pp65 > 50			
Cohort	**NPV**	**% High Count**	**P-value**
All	97.0% (159/164)	41.6% (164/394)	<.0001
D+/R-	86.4% (19/22)	13.1% (22/168)	0.3085
R+	98.6% (139/141)	69.1% (141/204)	0.0181

IE-1 > 50			
Cohort	**NPV**	**% High Count**	**P-value**
All	97.7% (85/87)	21.1% (87/394)	0.0029
D+/R-	75.0% (6/8)	4.8% (8/168)	0.8351
R+	100% (77/77)	37.7% (77/204)	0.0360

pp65 and IE-1 > 50			
Cohort	**NPV**	**% High Count**	**P-value**
All	97.5% (77/79)	20.1% (79/394)	0.0064
D+/R-	71.4% (5/7)	4.2% (7/168)	0.6694
R+	100% (71/71)	34.8% (71/204)	0.0492

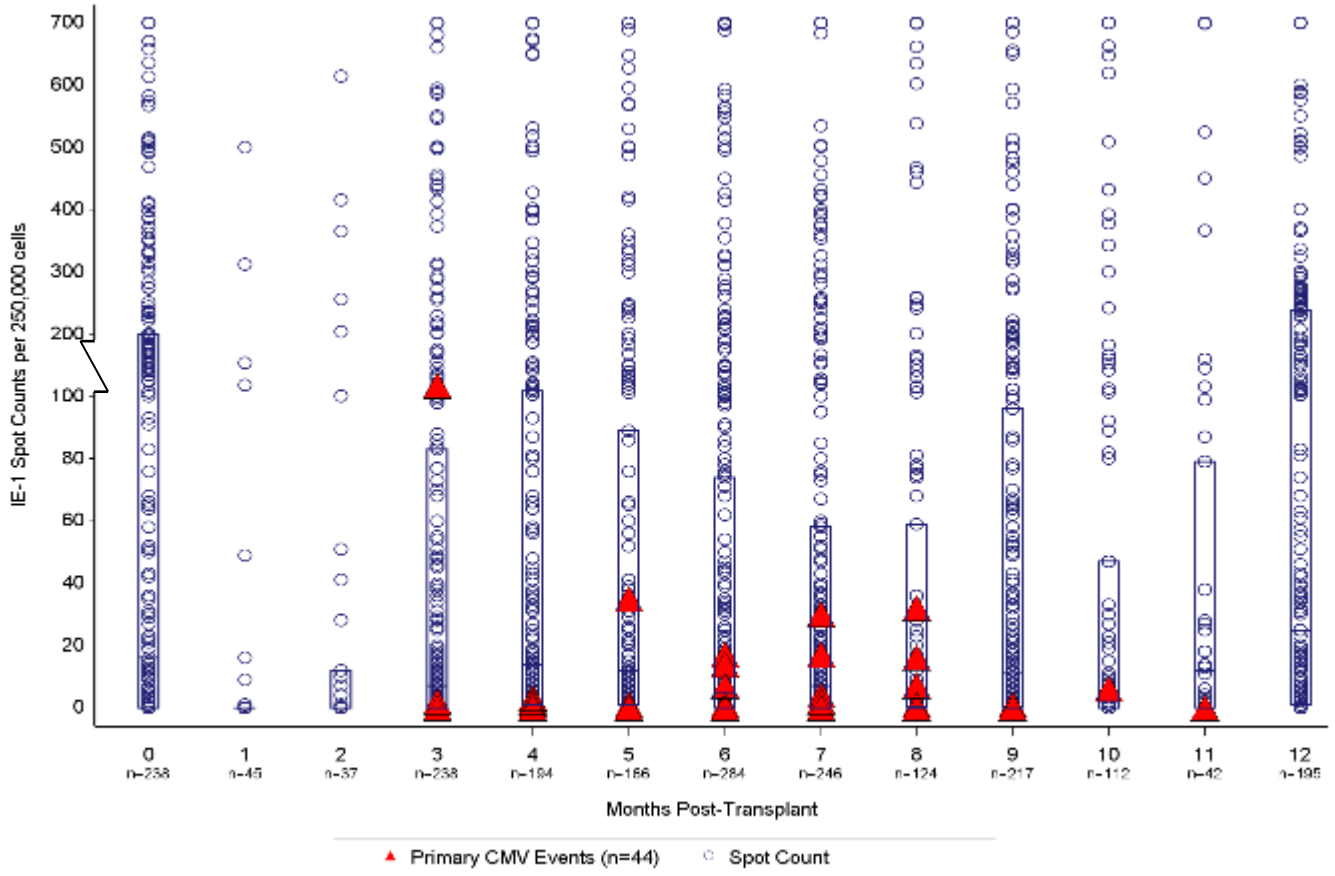
PROTECT: KM Plot – Days from Completion of Prophylaxis to CMV Event (IE-1 OR pp65 > 50)



IE-1 OR pp65 > 50 = High Response / Positive Cellular Mediated Immunity (CMI*)*

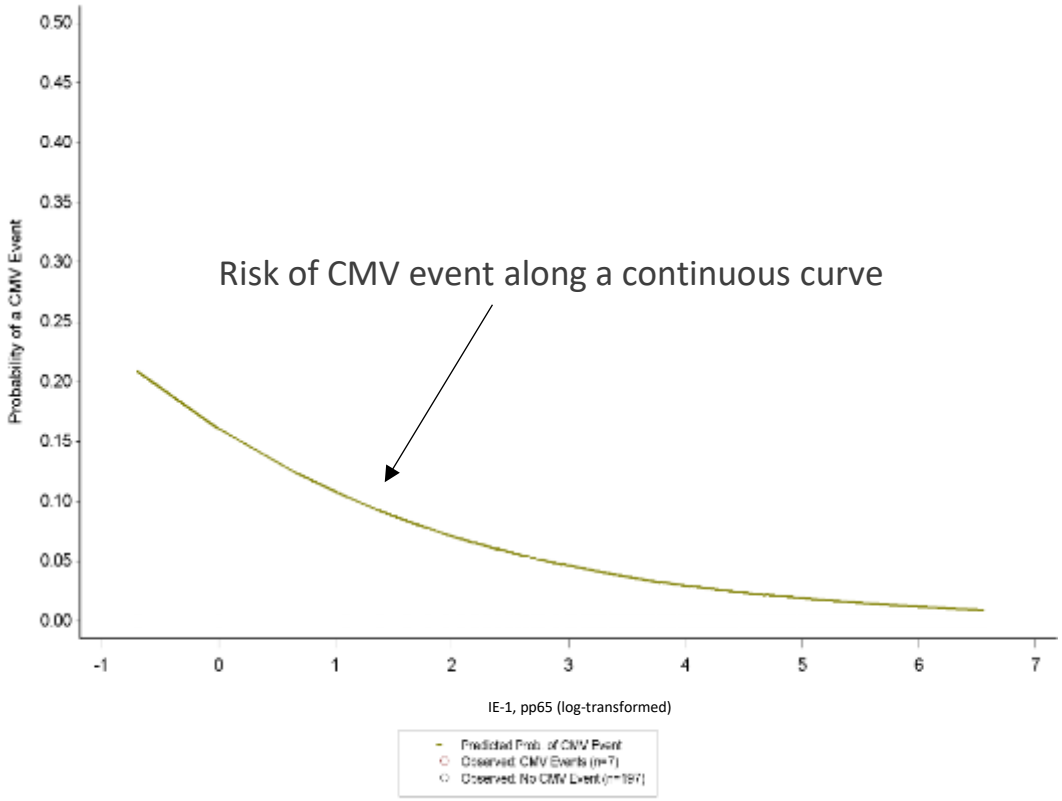
CMV events were significantly lower in CMI positive vs. negative patients (2.9% vs. 17.6%, p = <0.0001).

CMV CMI (IE-1) at completion of ppx. Overall cohort (n=368)



Note: Boxplots of spot counts provided for each month; Box is 25th -75th percentile with line at 50th percentile

Predictive algorithm to determine risk of CMV event



Interpretation*:*

- Consistent with our understanding of cellular immunity, results will not fall into a simple positive/negative delineation, but rather will provide a response continuum expressed as risk correlated with clinical outcomes

Utility of T-SPOT.*CMV**:***

- Helps determine immune competence against CMV infection
- Assists with patient risk stratification
- May help determine if antiviral therapy should be initiated or stopped

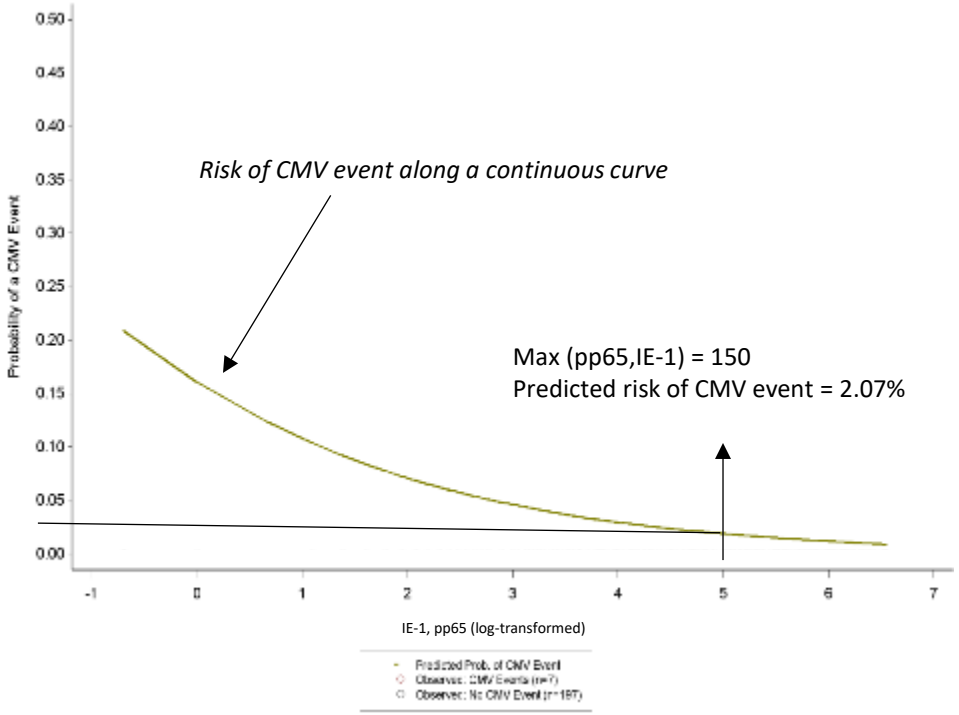
Predicted probability of a CMV event – algorithm examples

Example 1:

Max count of IE-1 or pp65 = **150**

Parameter	**Max(pp65,IE1)**
Values	**150**

Prob Event	**2.07%**



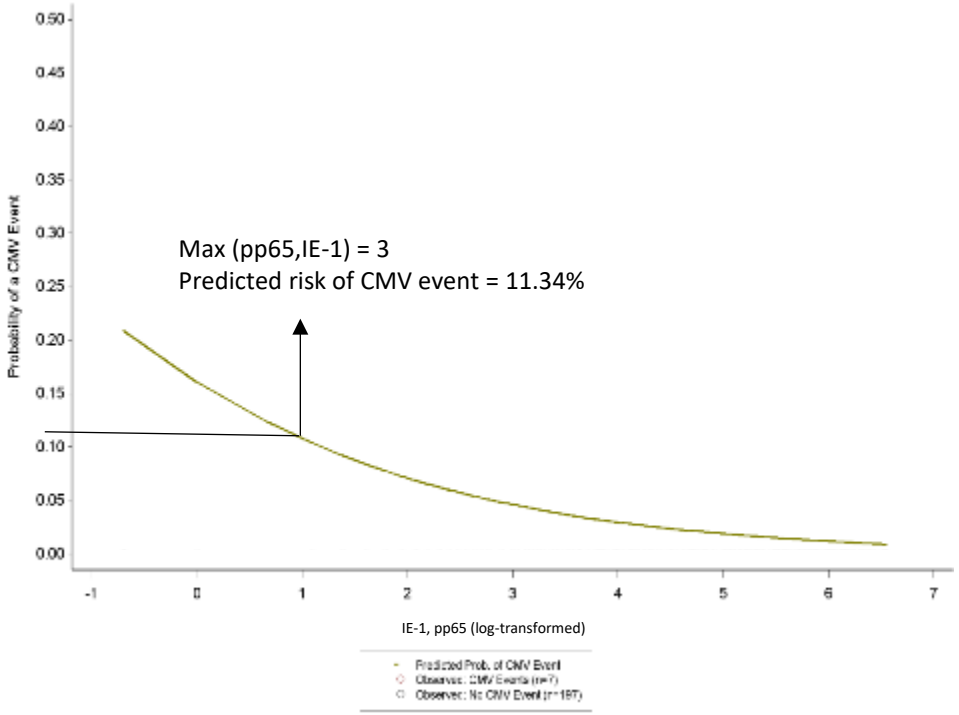
Predicted probability of a CMV event – algorithm examples

Example 1:

Max count of IE-1 or pp65 = **3**

Parameter	**Max(pp65,IE1)**
Values	**3**

Prob Event	**11.34%**



2018 T-SPOT.*CMV* publications and presentations

- SOT: 5 publications planned for submission prior to end of Q3 2018

Location	Enrollment	Planned Submission or Publication Date	Objective
UK	108	Published March 20th	Prognostic utility in kidney transplant
US	583	Submission Q2	PROTECT: Relationship of T-cell response to CMV antigens and risk of progressive CMV infection
US	N/A	Submission Q3	Analytical validity of T-SPOT.*CMV*
Spain	160	Submission Q3	Investigation of patient response randomized to pre-emptive vs prophylactic therapy
Spain	317	Submission Q3	Pre-transplant T-cell immunity is an additional independent variable predicting CMV infection

- HSCT: 3 publications planned for submission prior to end of Q3 2018

Location	Enrollment	Planned Submission or Publication Date	Objective
US	60	Submission Q2	Test utility in the management of HCT patients with low viral loads
US	244	Submission Q2	REACT: Overall observations
US	244	Submission Q3	REACT: Test utility at week 2, week 4 and delta change from baseline

Accepted as an oral presentation at:

1) **American Transplant Conference** (Seattle - June 4th)
2) **The Transplantation Society** (Madrid - July 2nd)

Oxford Immunotec

QuantiFeron

Intended Use

QuantiFERON-CMV ELISA (QF-CMV) is an in vitro assay using a peptide cocktail simulating human cytomegalovirus (CMV) proteins to stimulate cells in heparinized whole blood. Detection of interferon-gamma (IFN-γ) by Enzyme-Linked Immunosorbent Assay (ELISA) is used to quantify in vitro responses to these peptide antigens that are associated with immune control of CMV infection. Loss of this immune function may be associated with development of CMV disease. The intended use of QF-CMV is to monitor a patient's level of anti-CMV immunity.

QF-CMV is not a test for determining CMV infection and should not be used to exclude CMV infection.

Expected Values

Expected IFN-γ values using QuantiFERON-CMV were obtained from testing 591 samples from healthy subjects — 343 samples tested seropositive and 248 samples tested seronegative to CMV IgG. CMV serology status was unknown at the time of QF-CMV testing. In the 248 samples from CMV-seronegative subjects, 100% (248/248) of samples tested were nonreactive by QF-CMV ELISA producing IFN-γ responses of <0.2 IU/ml to the CMV Antigen tube (Nil subtracted). The distribution of IFN-γ responses to CMV Antigen tube (Nil subtracted) for the 343 CMV seropositive subjects is shown (Figure 2).

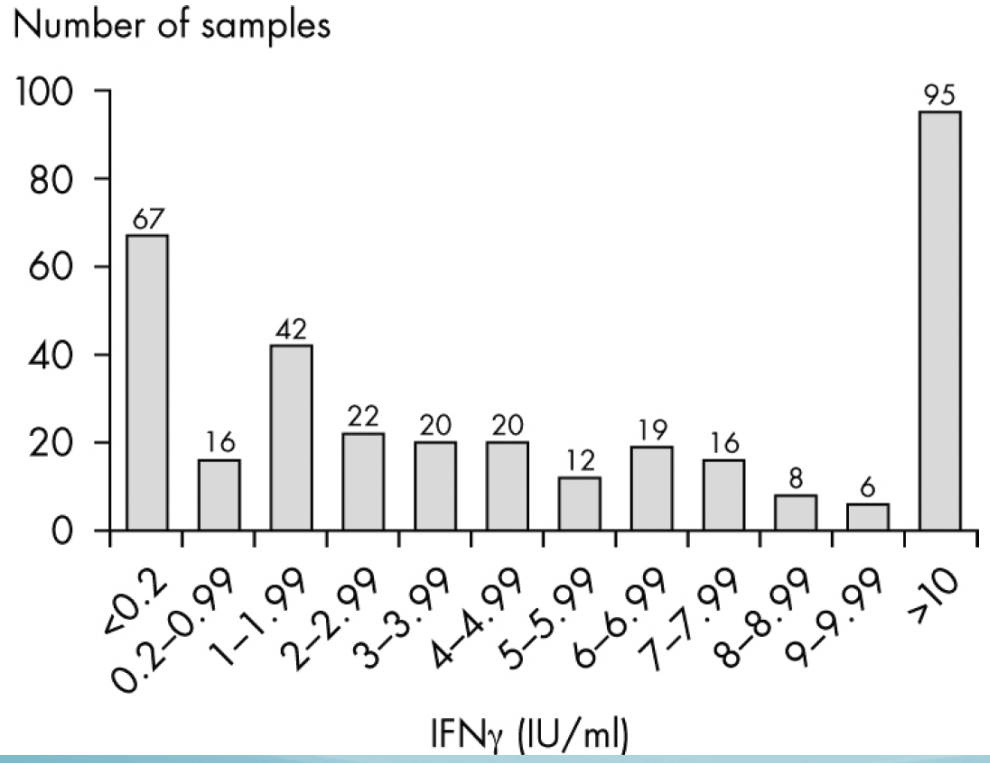


Table 2. Agreement between QuantiFERON-CMV and CMV IgG serology test in healthy subjects.

		CMV Serology		
		Positive	**Negative**	**Total**
QuantiFERON-CMV	**Reactive**	145	0	145 (46.8%)
	Non-reactive	16	149	165 (53.2%)
	Total	**161** (51.9%)	**149** (48.1%)	**310** (100%)

Specificity

In a study of 591 samples from healthy subjects, no false-positive QF-CMV results were detected in individuals testing seronegative for CMV IgG with 248/248 samples testing nonreactive by QF-CMV ELISA and negative by CMV IgG serology test. Therefore, the results obtained using QF-CMV and the CMV IgG serology test showed 100% concordance.

In all other specificity evaluations conducted in recipients of solid organ transplants (1, 3, 4, 8, 12, 14–16), recipients of hematopoietic stem cell transplants (7, 13) and HIV-infected patients (2), the concordance between QF-CMV and CMV IgG serology has also been shown to be 100%.

Sensitivity

In a study conducted in 343 samples from healthy subjects testing seropositive for CMV IgG, the level of agreement between QF-CMV responses and CMV IgG serology results was 80.5% with 275/343 samples testing reactive to QF-CMV and positive to the CMV IgG serology test. The observed discordance may be due to false-positive CMV serology, or the absence of responsive HLA types in the individuals tested.

The levels of agreement in sensitivity evaluations conducted in solid organ transplant recipients (1, 3, 4, 8, 12, 14–16), hematopoietic stem cell transplant recipients (7, 13) and HIV-infected patients (2), have been shown to be lower and may be due to false-positive CMV serology, the absence of responsive HLA types in the individuals tested, or the absence of reactive T cells in these patients due to their immunosuppression.

In a large study of 108 solid organ transplant recipients (4), patients with a QF-CMV reactive result at the completion of anti-CMV prophylaxis had a significantly lower rate of subsequent CMV disease (3.3% or 1/30; using an 0.2 IU/ml threshold) compared to patients having a QF-CMV nonreactive result (21.8% or 17/78, p=0.044) (Figure 5).

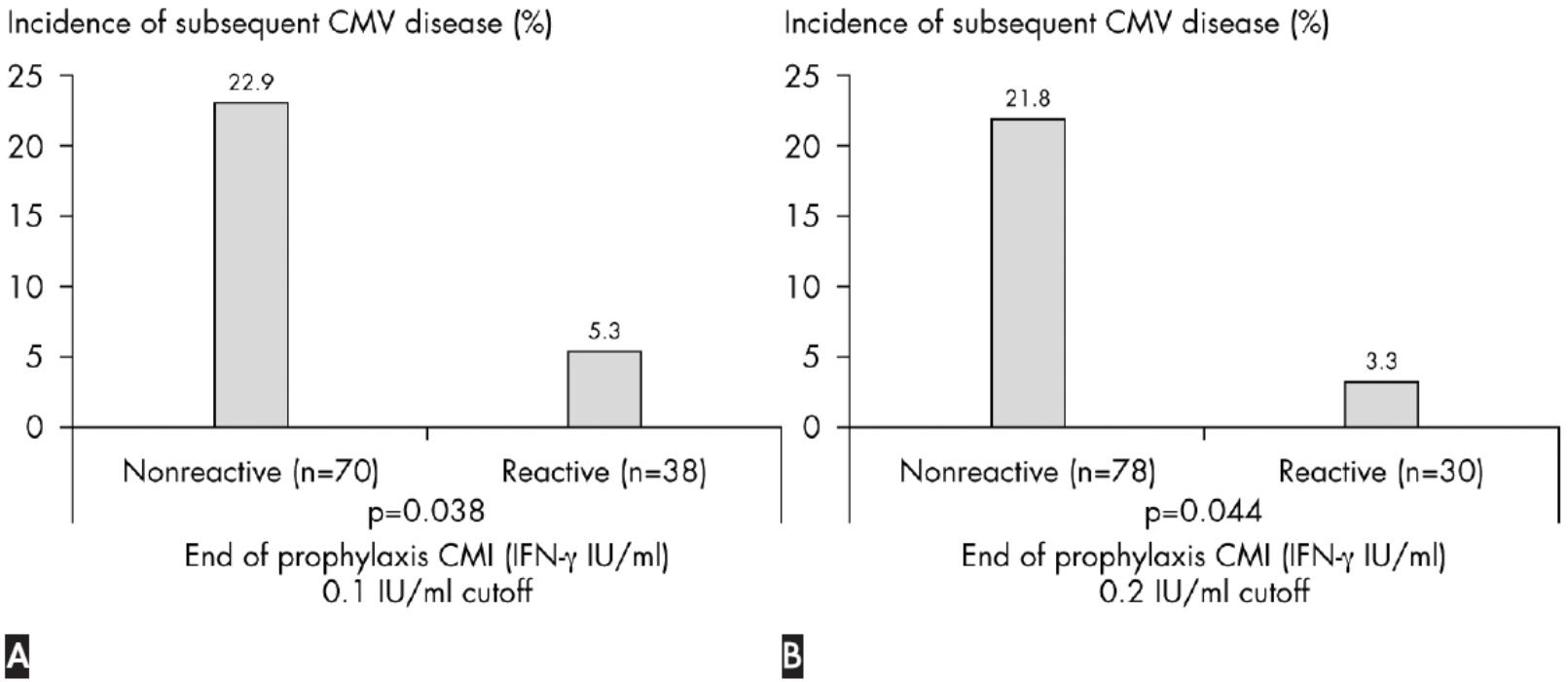


Figure 5. Rates of late onset CMV disease in patients with a QuantiFERON-CMV reactive result vs. a QuantiFERON-CMV nonreactive result at the end of prophylaxis. Data reproduced from Kumar et al.(4)

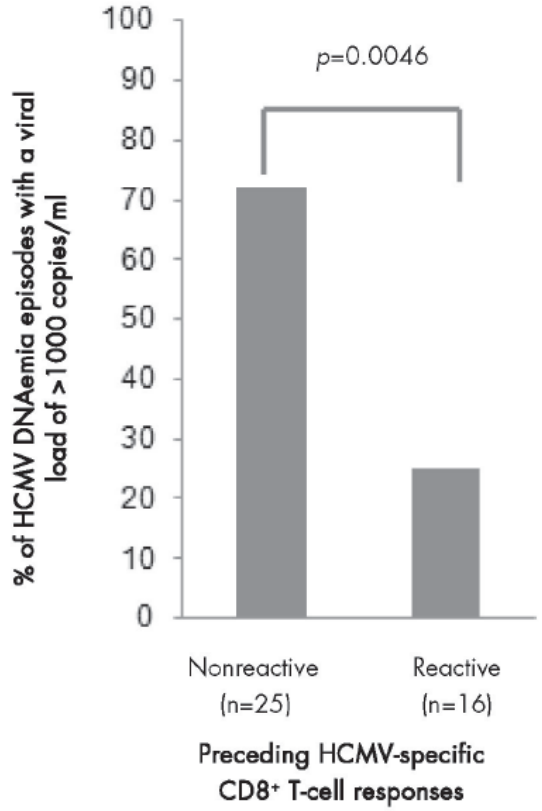


Figure 6. Statistical analysis of CMV-specific CD8+ T- cell responses as detected by QuantiFERON-CMV and the development of CMV viremia (Fisher's exact test, *p*=0.0046). Data reproduced from Weseslindtner et al (14).

TABLE 1 Summary of select clinical studies in solid organ transplant recipients assessing clinical utility of QFN-CMV assay

Transplant recipient cohort	CMV serological status of transplant recipients	QFN-CMV assay time point(s)	Clinical study conclusion	Reference
SOT[a] (heart/lung and kidney) (n = 25)	D^+/R^+ = 17, D^+/R^- = 8	Various	All seropositive transplant recipients showed positive reactivity in QFN-CMV assay, while seronegative recipients showed negative reactivity	15
SOT (lung) (n = 39)	D^+/R^+ = 18, D^+/R^- = 8, D^-/R^+ = 6, D^-/R^- = 7	0.5, 1, 2, 3, 6, 9, 12, and 18 mo posttransplant	QFN-CMV assay accurately tracks the development of *de novo* CMV immunity; a striking decrease was seen in the QFN-CMV reactivity prior to the episode of CMV reactivation	35
SOT (kidney, pancreas, lung, heart, liver, and other) (n = 108)	D^+/R^+ = 39, D^-/R^+ = 34, D^+/R^- = 35	Monthly for 4 mo after completion of prophylaxis	Monitoring of CMV T cell immunity using QFN-CMV assay may be useful for predicting late-onset CMV disease	34
SOT (kidney) (n = 14)	D^-/R^+ = 1, D^+/R^+ = 11, D^+/R^- = 2	Various	QFN-CMV assay is a sensitive and specific test to detect a virus-specific T-cell response; this assay, in combination with viral DNA load estimates, may prove to be useful to stratify patients at risk of CMV disease	33
SOT (kidney, lung, heart, liver, and combined) (n = 37)	D^+/R^+ and D^-/R^+ = 30, D^+/R^- = 7	Monitoring initiated at the onset of CMV viremia	Monitoring of CMV T cell immunity using QFN-CMV assay after the onset of CMV viremia may be useful to predict progression vs spontaneous viral clearance, thereby helping guide in determining the best antiviral therapy and refining current preemptive strategies	8
SOT (lung) (n = 67)	D^-/R^+ = 11, D^+/R^+ = 28, D^+/R^- = 17, D^-/R^- = 11	Monitoring monthly for 1 year	A standardized measurement of $CD8^+$ T cell immunity using QFN-CMV assay might contribute to monitoring the immune status of lung transplant recipients	27
SOT (kidney, pancreas, lung, heart, liver, and other) (n = 127)	D^+/R^- = 127	3–6 mo (at completion of prophylaxis) and 1 and 2 mo after completion of prophylaxis	QFN-CMV assay may be useful to predict if patients are at low, intermediate, or high risk for the development of subsequent CMV disease after prophylaxis	28
SOT (lung and kidney) (n = 113 [55 evaluated])	D^+R^+ = 33, D^-R^+ = 11, D^+R^- = 8, D^-R^- = 3	Pretransplant and posttransplant	Monitoring of CMV T cell immunity using QFN-CMV assay prior to transplantation is useful in informing the risk of posttransplant CMV replication in SOT recipients	36
SOT (lung, liver, kidney) (n = 114)	R^+ = 114, R^- = 27	Various	QFN-CMV assay assessment is recommended for non-HLA A1- and HLA A2-seropositive transplant recipients	37
SOT (liver, lung, kidney) (n = 68)	D^+/R^- = 68	Not specified	Transplant recipients with positive reactivity in QFN-CMV assay had a higher percentage of late-differentiated $CD8^+$ T cells than patients lacking this response	24
SOT (kidney) (n = 25)	D^+/R^+ = 13, D^+/R^- = 9, D^-/R^- = 1, D^-/R^+ = 2	4.38 ± 2.73 mo posttransplant	An indeterminate result of QFN-CMV assay seems to be related to impaired immunity; the QFN-CMV assay appears to be useful in identifying the transplant recipients with increased risk of infectious complications who may benefit from immunosuppression reduction and maintenance of antiviral prophylaxis	23
SOT (kidney) (n = 124)	D^+/R^+ = 124	Pretransplant and 1 mo and 3 mo posttransplant	QFN-CMV assay reactivity is not associated with DNAemia	18
SOT (kidney and lung) (n = 55)	D^+/R^+ = 33, D^+/R^- = 8, D^-/R^+ = 11, D^-/R^- = 3	Pretransplant and 3 or 6 mo and 12 mo posttransplant	D^-/R^- recipients remained nonreactive in QFN-CMV assay both at pretransplant and posttransplant; D^+/R^- recipients showed lower reactivity in QFN-CMV assay than D^+/R^+ or D^-/R^+ patients	22
SOT (kidney, liver, lung, and combined) (n = 27)	D^+/R^- = 12, R^+ = 13, D^-/R^- = 1, unknown = 1	Every 2 wks until 3 mo after completion of prophylaxis	QFN-CMV assay can be used to guide changes to the management of CMV infection	20

[a]SOT, solid organ transplant.

- Regulatory perspective on assay parameters – not sufficient data yet
  - Central vs. local lab will be important
    - Are assays sensitive in terms of time
      - Things have improved but not yet perfect
    - Quantiferon should be OK to send out
    - Clinical labs have to verify stability
- Impact of immunosuppression on assay performance
  - Challenge bc of difference center to center, organ to organ
  - Whether using T cell depleting induction therapy
    - What is the role of lymphocyte depleting therapies in lymphocyte dependent assays?
      - E.g. Liver transplant vs kidney transplant
    - Important to report outcomes in patients in whom assay could not be done (assay failed)
      - Need more systematic approach to reporting/publishing

Role in CMV drug development

- When could an immune assay be a standalone assay vs adjunct to VL?
  - If highly predictive for duration of prophylaxis
- Avoid unnecessary prophylaxis/treatment
- Two decision points
  - When to start treatment?
  - When is it safe to stop prophylaxis/treatment?
- Regulatory perspective – have not seen the data yet
  - Will need to see a lot more data before understanding how to use immune assays as endpoints
- Divide by risk populations for SOT and HSCT (D/R)
  - Lots of small trials, some larger trials
  - Reasonable data on use seropositive SOT recipient

How to best use these assays

- Review of existing data for question i (when to start)
  - SOT
    - Divide by risk group
  - HSCT
    - Divide by risk group
- Review of existing data for question ii (when safe to stop)
  - SOT
    - Divide by risk group
  - HSCT
    - Divide by risk group
- Review level of evidence
  - Is the level of evidence sufficient for i: when to start treatment
    - For what patients?
  - Is the level of evidence sufficient for ii: when is it safe to stop prophylaxis/treatment
    - For what patients?
- Identify research gaps
- Opportunities for filling the gaps