The Impact of Pre- and Week 2 or 4 Post-transplant CMV-Specific ELISPOT Assay on CMV Reactivation in CMV-seropositive Allo-HCT Recipients

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- Consultant: Oxford Immunotec, Merck, Chimerix, and Astellas

CMV in HCT Recipients

- CMV infection causes significant morbidity and mortality in allo-HCT
- CMV cell-mediated immunity (CMI), assessed by engrafted T cell production of IFN-γ, is a major mechanism to control CMV replication

Ariza E, Nesher L, Chemaly RF, Cancer Letters, 2013

Gap In Knowledge

Novel strategies are needed to further reduce the burden of CMV infection/disease in HCT recipients and improve survival

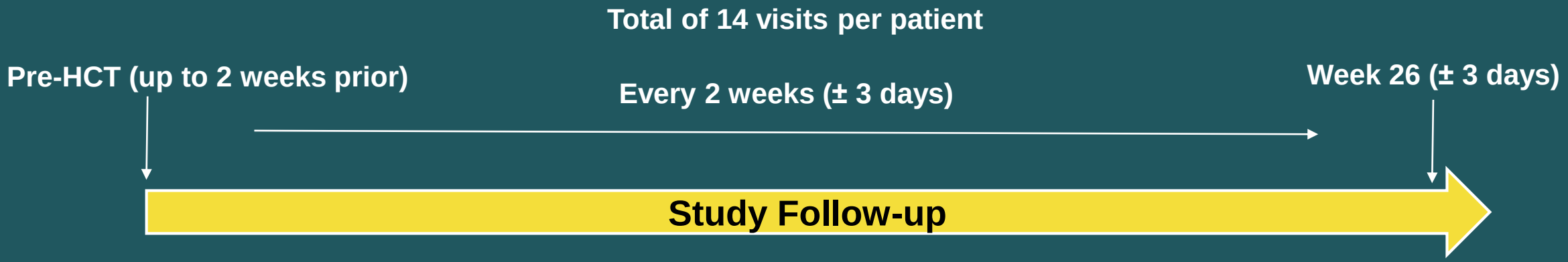
Hypothesis

Measurement of the CMV specific cell mediated immunity can identify the actual risk for the development of CMV infection making it an attractive strategy for personalized management of CMV after transplantation (i.e. prohylaxis)

The Impact of Pre- and Week 2 or 4 Post-transplant CMV-Specific ELISPOT Assay on CMV Reactivation and Survival in CMV-seropositive Allo-HCT Recipients

REACT Study Design

- Ongoing multicenter, prospective, observational study
  - First patient enrolled June 2015; LPLV anticipated April 2017
- 244 CMV seropositive (R+) candidates for allogeneic HCT.
- Donor/Recipient demographics, concomitant medications and transplant related events were collected
- T-SPOT.*CMV* (ELISPOT) assay was used to assess the production of IFNγ following ex-vivo stimulation with CMV specific antigens (IE1 and pp65)
- Serial blood draws (T-SPOT.*CMV* and CMV PCR) were done as follows:



Definition of Events

CMV Event: The first episode of significant CMV reactivation, defined as the detection of CMV in blood via the antigenemia assay or the CMV PCR assay, after which anti-CMV therapy was initiated by the treating physician in accordance with institutional guidelines.

CMV Disease: The first episode of CMV disease, consisting of "end-organ disease" as defined by Per Ljungman *et al**.

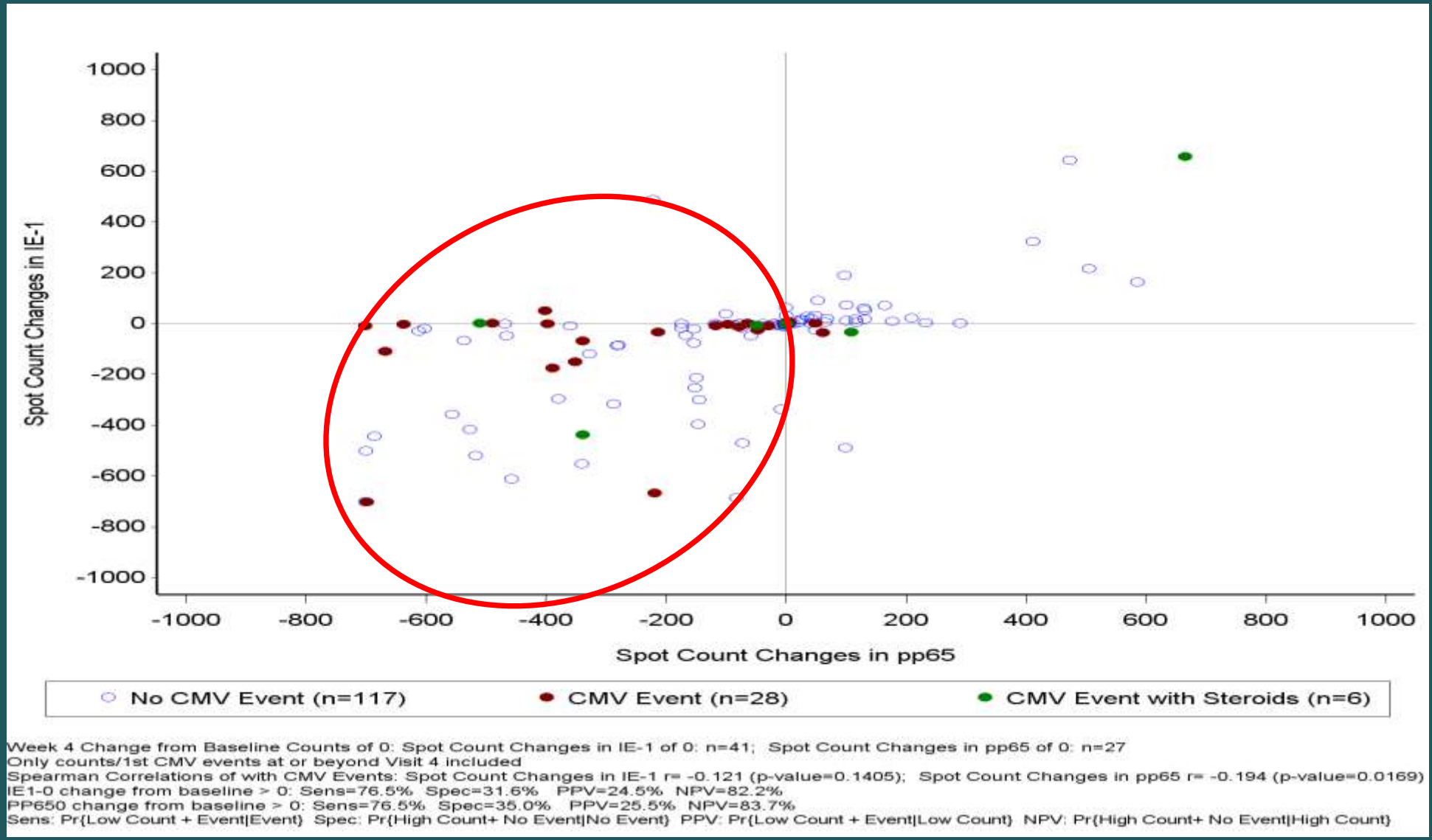
** Definitions of Cytomegalovirus Infection and Disease in Transplant Patients for Use in Clinical Trials 2016 Clin Infect Dis. 2017 Jan 1;64(1):87-91*

Results

Clinical Characteristics of 244 HCT Recipients

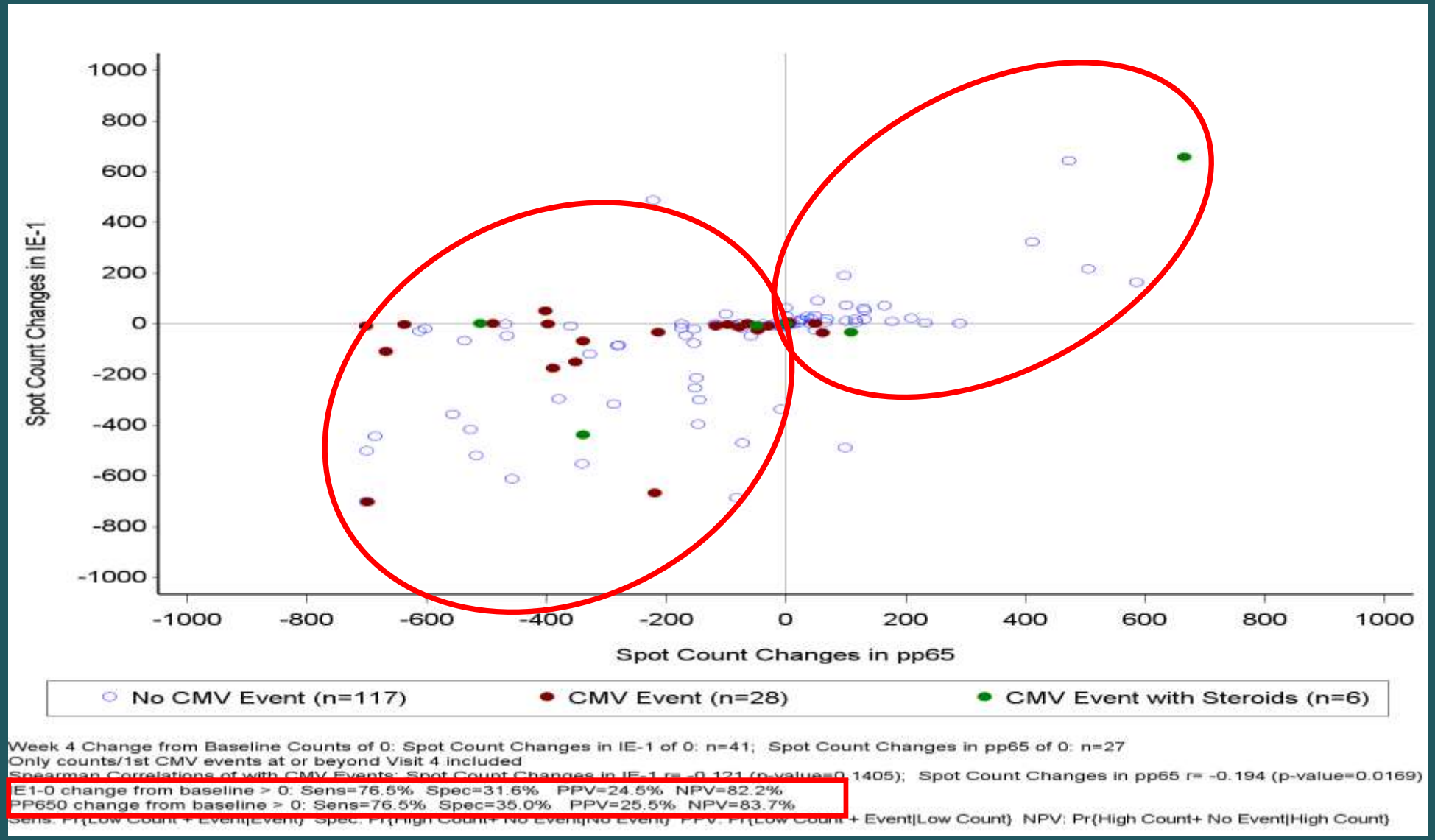
Characteristics		CMV Reactivation (n=59)	No CMV Reactivation (n=185)
Sex		No (%)	No (%)
	Male	29 (49)	108 (58)
	Female	30 (51)	77 (42)
Race			
	White	40 (68)	138 (74)
	African American	3 (5)	13 (7)
	Asian	7 (11)	9 (5)
	Unknown/Other	9 (15)	25 (14)
Type of Transplant			
	Match Related Donor	15 (25)	76 (41)
	Match Unrelated Donor	31 (53)	79 (43)
	Cord Blood	3 (5)	1 (1)
	Haploidentical	9 (15)	27 (14)
	Unknown	1 (2)	2 (1)
HCT donor status			
	CMV +	33 (56)	99 (54)
	CMV -	24 (41)	72 (39)
	Unknown	1 (2)	12 (7)

Scatter plot of Week 4 Change-from-baseline IE-1 vs. pp65 and occurrence of a post-Week4 CMV event indicated by a red or green dot

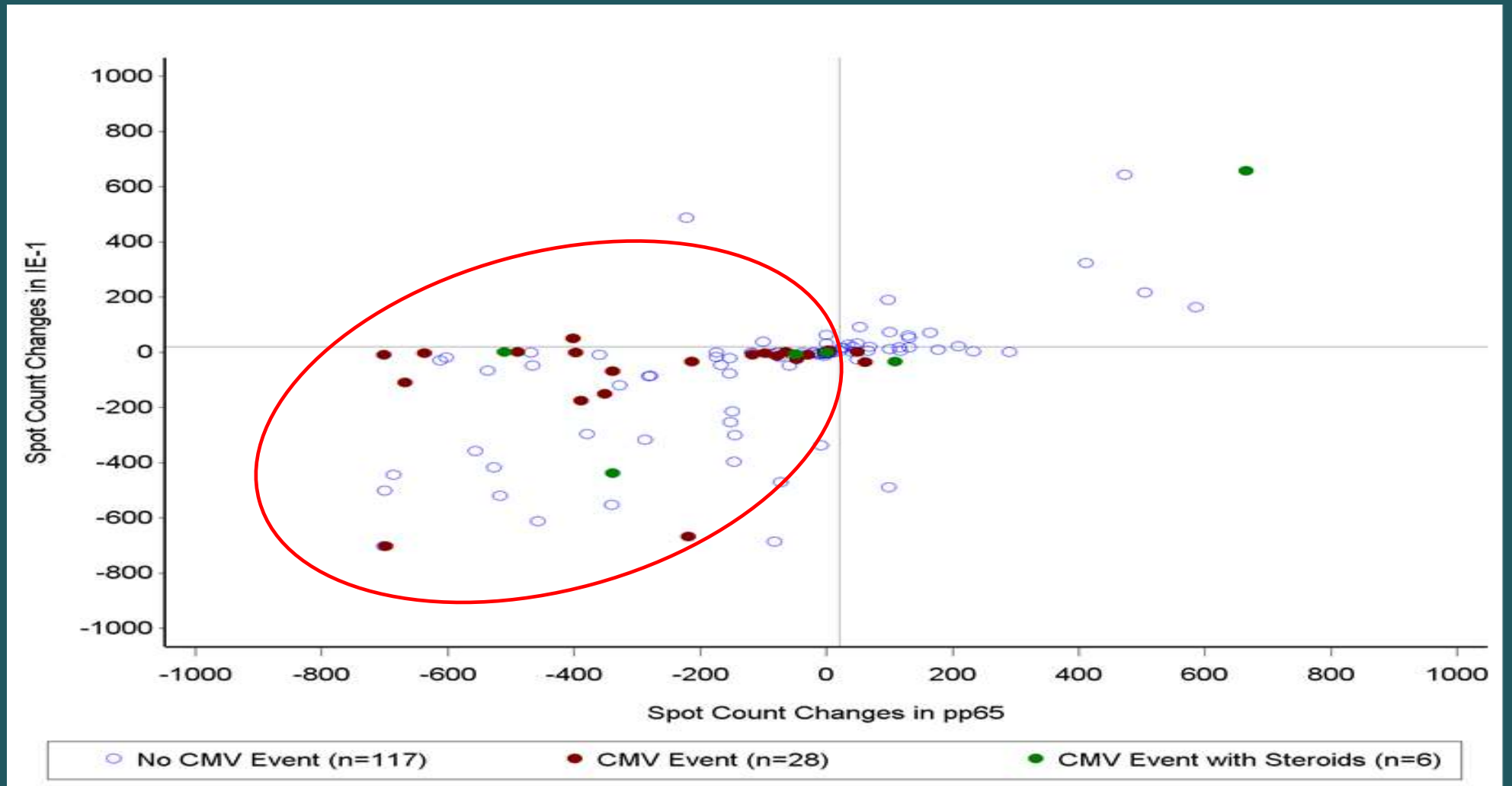


Week 4 Change from Baseline Counts of 0: Spot Count Changes in IE-1 of 0: n=41; Spot Count Changes in pp65 of 0: n=27
Only counts/1st CMV events at or beyond Visit 4 included
Spearman Correlations of with CMV Events: Spot Count Changes in IE-1 r= -0.121 (p-value=0.1405); Spot Count Changes in pp65 r= -0.194 (p-value=0.0169)
IE1-0 change from baseline > 0: Sens=76.5% Spec=31.6% PPV=24.5% NPV=82.2%
PP650 change from baseline > 0: Sens=76.5% Spec=35.0% PPV=25.5% NPV=83.7%
Sens: Pr{Low Count + Event|Event} Spec: Pr{High Count+ No Event|No Event} PPV: Pr{Low Count + Event|Low Count} NPV: Pr{High Count+ No Event|High Count}

Scatter plot of Week 4 Change-from-baseline IE-1 vs. pp65 and occurrence of a post-Week4 CMV event indicated by a red or green dot

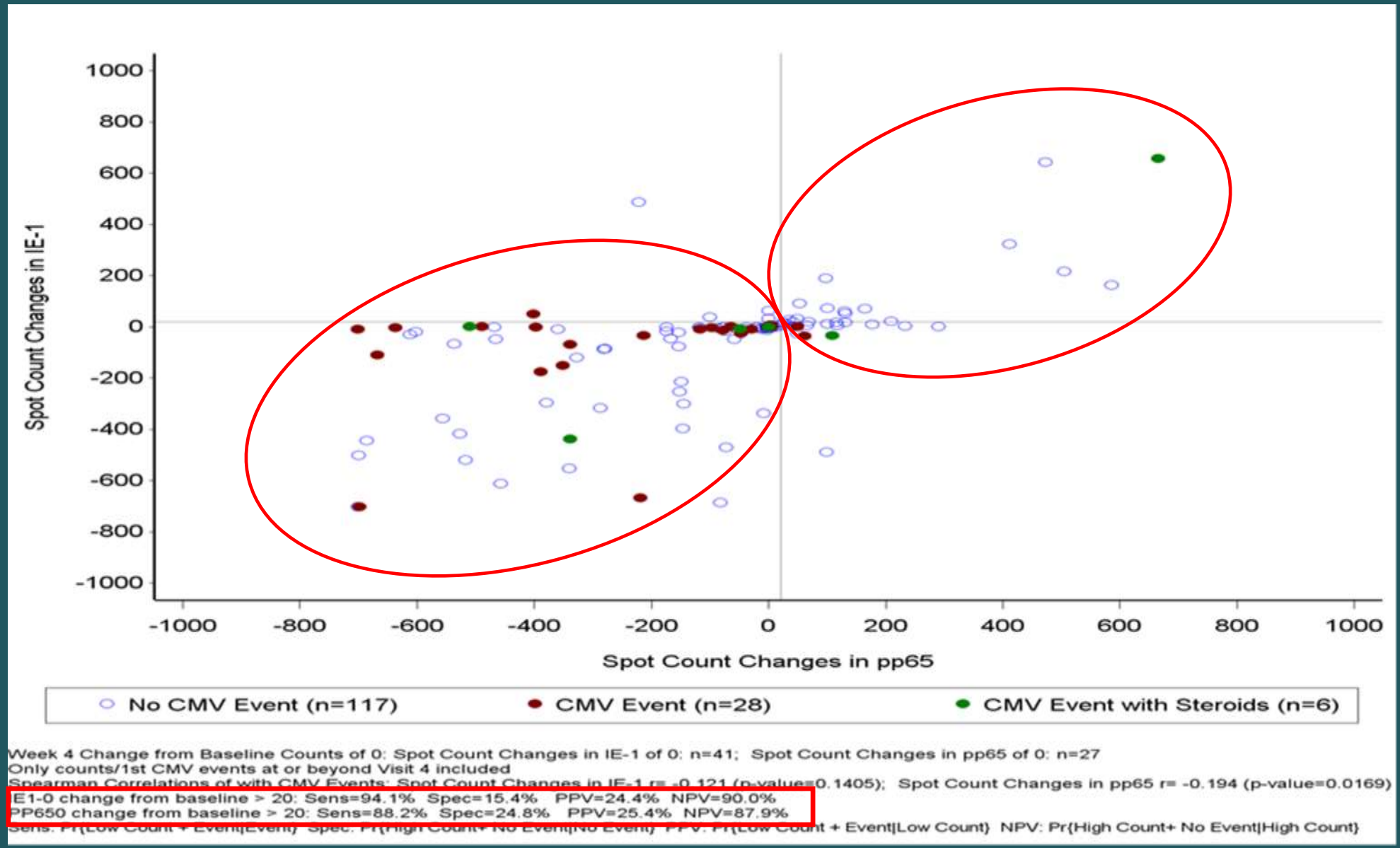


Scatter plot of Week 4 Change-from-baseline IE-1 vs. pp65 with patient's experiencing a post-Week4 CMV event indicated by a red or green dot

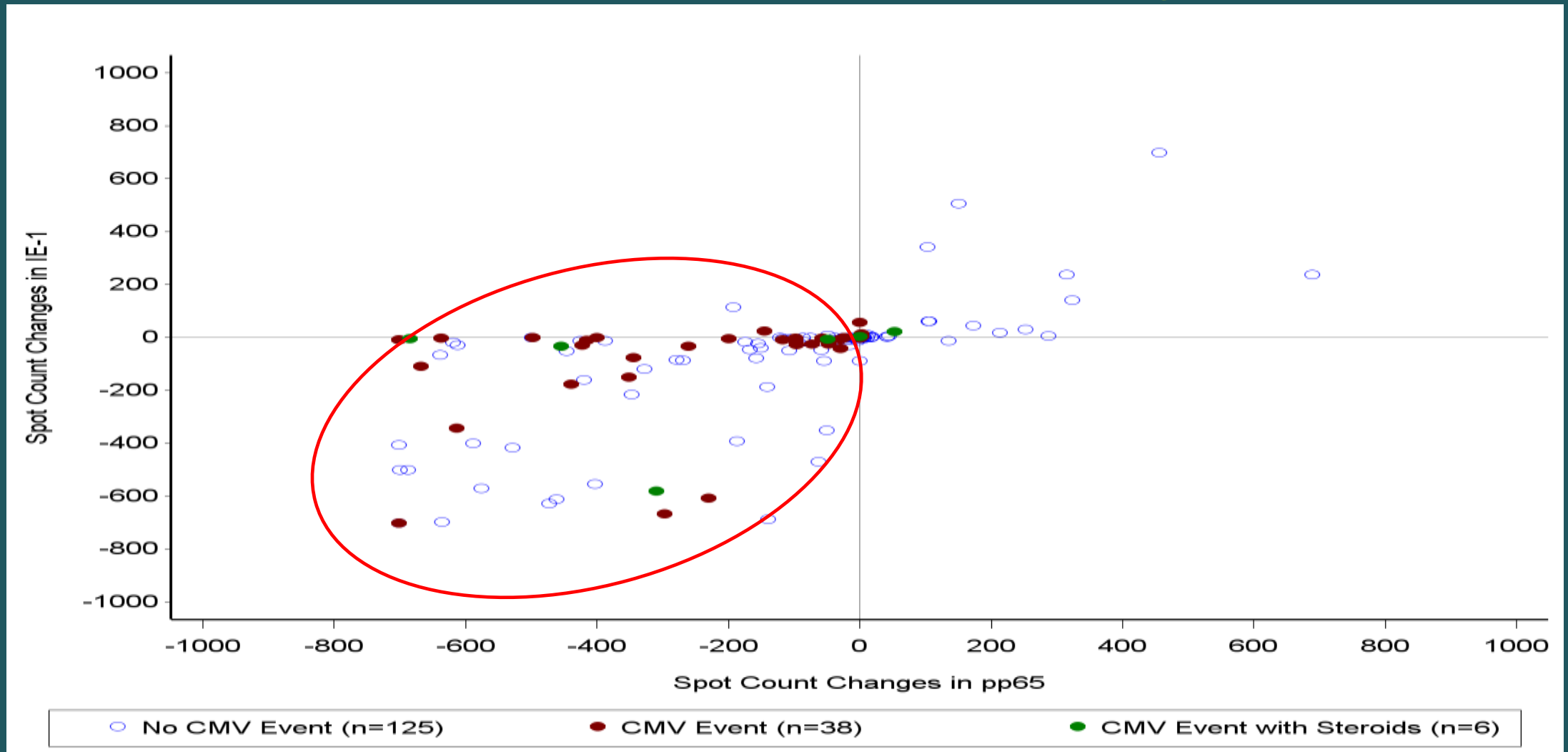


Week 4 Change from Baseline Counts of 0: Spot Count Changes in IE-1 of 0: n=41; Spot Count Changes in pp65 of 0: n=27
Only counts/1st CMV events at or beyond Visit 4 included
Spearman Correlations of with CMV Events: Spot Count Changes in IE-1 r= -0.121 (p-value=0.1405); Spot Count Changes in pp65 r= -0.194 (p-value=0.0169)
IE1-0 change from baseline > 20: Sens=94.1% Spec=15.4% PPV=24.4% NPV=90.0%
PP650 change from baseline > 20: Sens=88.2% Spec=24.8% PPV=25.4% NPV=87.9%
Sens: Pr{Low Count + Event|Event} Spec: Pr{High Count+ No Event|No Event} PPV: Pr{Low Count + Event|Low Count} NPV: Pr{High Count+ No Event|High Count}

Scatter plot of Week 4 Change-from-baseline IE-1 vs. pp65 with patient's experiencing a post-Week4 CMV event indicated by a red dot

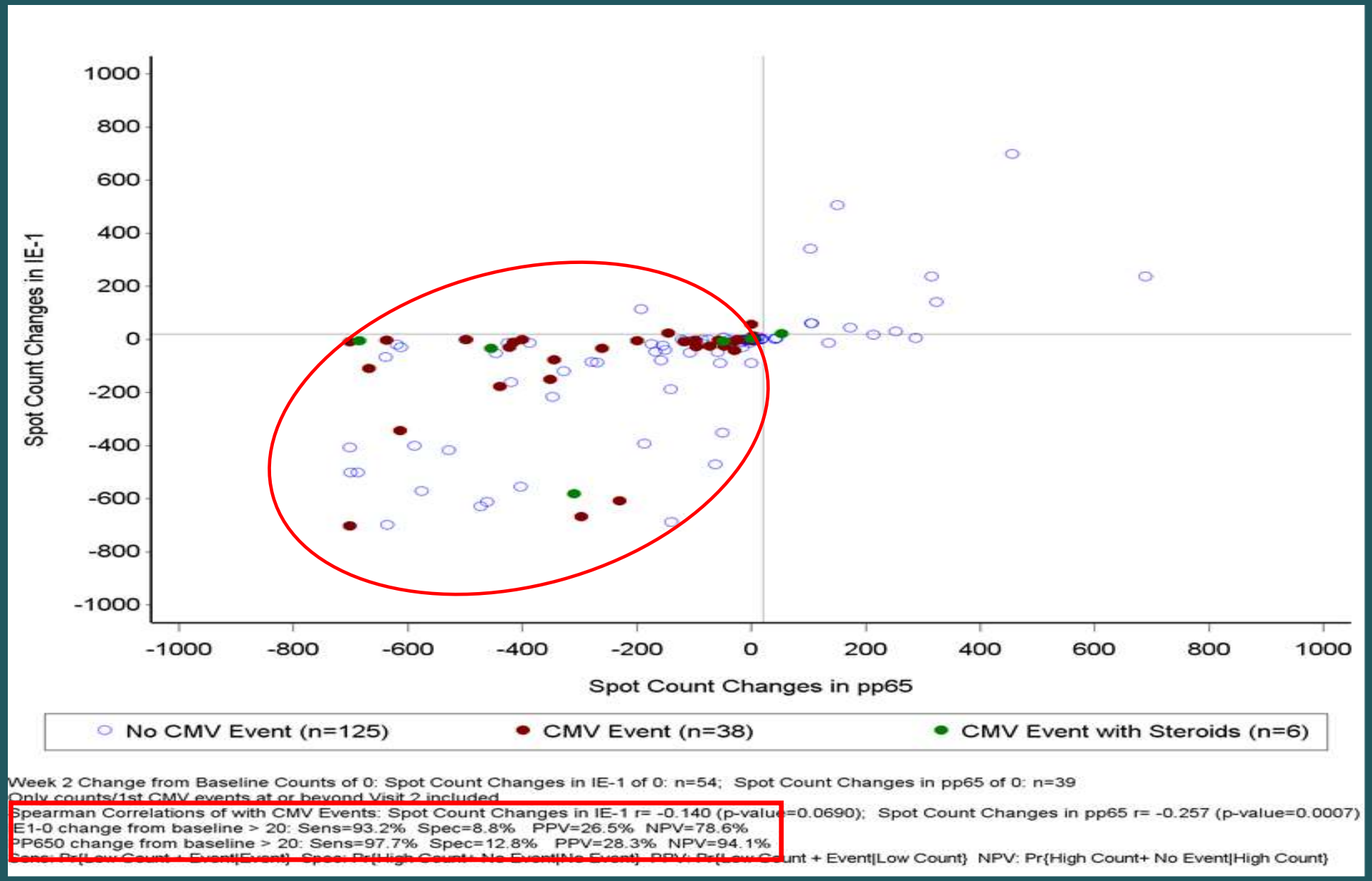


Scatter plot of Week 2 Change-from-baseline IE-1 vs. pp65 with patient's experiencing a post-Week2 CMV event indicated by a red dot or green dot



Week 2 Change from Baseline Counts of 0: Spot Count Changes in IE-1 of 0: n=54; Spot Count Changes in pp65 of 0: n=39
Only counts/1st CMV events at or beyond Visit 2 included
Spearman Correlations of with CMV Events: Spot Count Changes in IE-1 r= -0.140 (p-value=0.0690); Spot Count Changes in pp65 r= -0.257 (p-value=0.0007)
IE1-0 change from baseline > 0: Sens=84.1% Spec=20.8% PPV=27.2% NPV=78.8%
PP650 change from baseline > 0: Sens=90.9% Spec=25.6% PPV=30.1% NPV=88.9%
Sens: Pr{Low Count + Event|Event} Spec: Pr{High Count+ No Event|No Event} PPV: Pr{Low Count + Event|Low Count} NPV: Pr{High Count+ No Event|High Count}

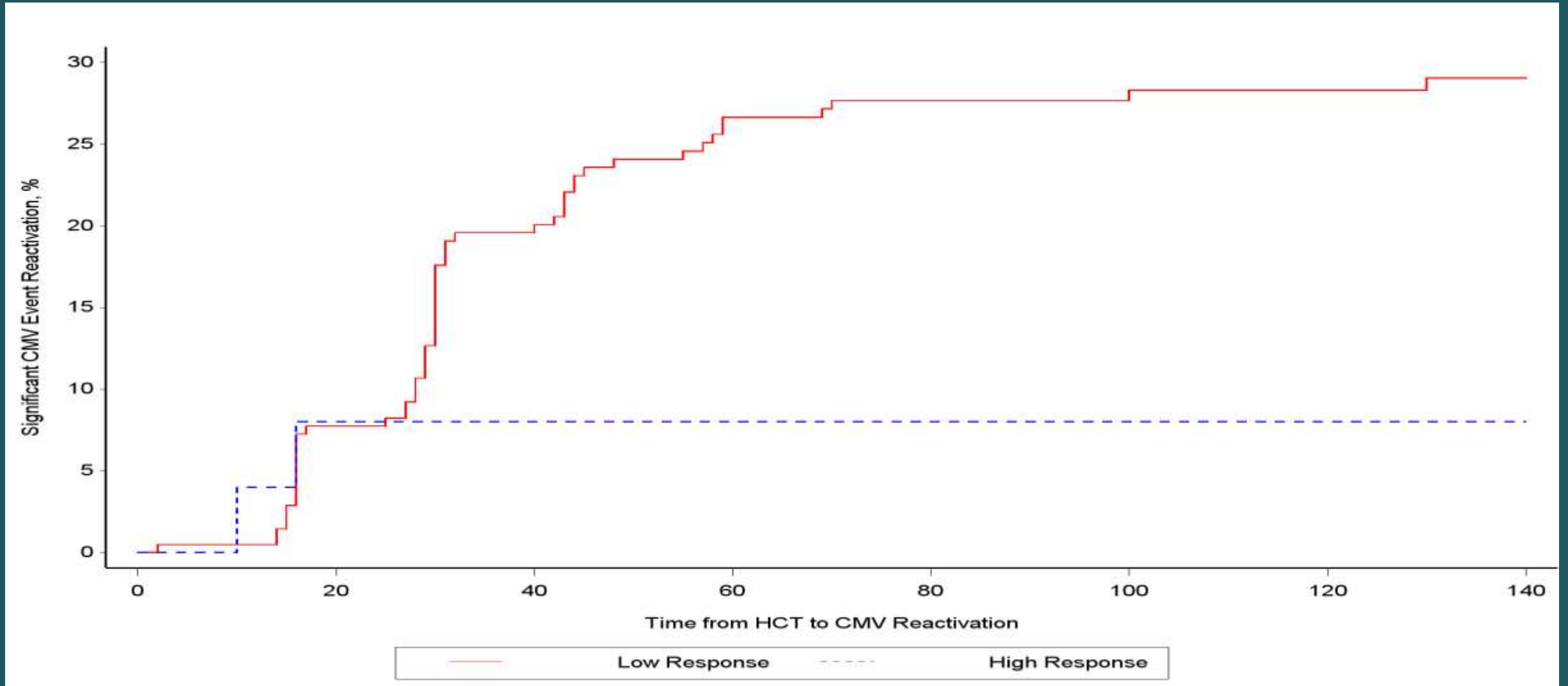
Scatter plot of Week 2 Change-from-baseline IE-1 vs. pp65 with patient's experiencing a post-Week2 CMV event indicated by a red dot or green dot



Sensitivity, Specificity, PPV, NPV for various CMV Event endpoints

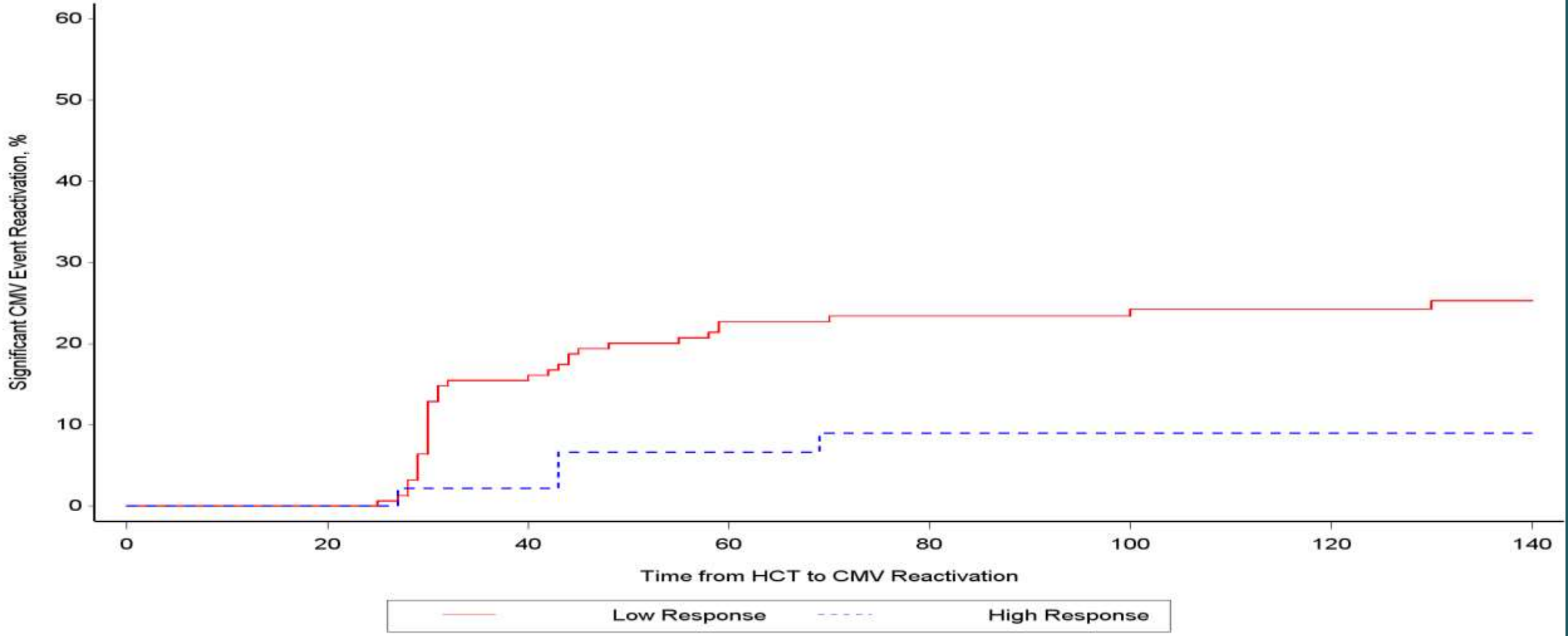
Obs	Count Criteria	Sensitivity	Specificity	PPV	NPV
1	Week 2 PP65 >100	96.6% (56/58)	13.1% (23/176)	26.8% (56/209)	92.0% (23/25)
2	Week 2 IE1 > 50	89.7% (52/58)	8.0% (14/176)	24.3% (52/214)	70.0% (14/20)
3	Week 2 PP65 >100 and IE-1>50	98.3% (57/58)	6.8% (12/176)	25.8% (57/221)	92.3% (12/13)
5	Week 4 PP65 >100	92.5% (37/40)	26.2% (43/164)	23.4% (37/158)	93.5% (43/46)
6	Week 4 IE1 > 50	90.0% (36/40)	17.1% (28/164)	20.9% (36/172)	87.5% (28/32)
7	Week 4 PP65 >100 and IE-1>50	97.5% (39/40)	15.2% (25/164)	21.9% (39/178)	96.2% (25/26)

KM for CMV Events stratified by maximum pp65 count >100/≤100 at week 2 (Only pp65 counts occurring after HCT and prior to the CMV event were included)



p-value = 0.0440 (log-rank test); High Response is defined as Week 2 PP65 > 100
Percent of Low Response Patients with a CMV Event: =27.8% (58/209); Percent of High Response Patients with a CMV Event: =8.0% (2/25)
Sens=96.7% Spec=13.2% PPV=27.8% NPV=92.0%
Sens: Pr{Low Count + Event|Event} Spec: Pr{High Count+ No Event|No Event} PPV: Pr{Low Count + Event|Low Count} NPV: Pr{High Count+ No Event|High Count}

KM for CMV Events stratified by maximum pp65 count >100/≤100 at week 4 (Only pp65 counts occurring after HCT and prior to the CMV event were included)



p-value = 0.0236 (log-rank test); High Response is defined as Week 4 PP65 > 100
Percent of Low Response Patients with a CMV Event: =24.1% (38/158); Percent of High Response Patients with a CMV Event: =8.7% (4/46)
Sens=90.5% Spec=25.9% PPV=24.1% NPV=91.3%
Sens: Pr{Low Count + Event|Event} Spec: Pr{High Count+ No Event|No Event} PPV: Pr{Low Count + Event|Low Count} NPV: Pr{High Count+ No Event|High Count}

Cox Regression for CMV Events using maximum pp65 as a covariate, retaining only covariates with a p-value <0.15 via stepwise selection

- Endpoint:
  - Time to CMV Event
- The set of predictor variables were:
  - Maximum pp65 count > 100
  - Recipient's Age
  - GVHD (Yes/No)
  - Transplant Type (4 categories: Cord Blood, Haploidentical, Matched or Mismatched unrelated donor, Unknown)
  - Receipt of systemic corticosteroids (Y/N)
  - Donor CMV sero-status (Positive/Negative)
  - Time to engraftment

Likelihood of CMV events

Analysis of Maximum Likelihood Estimates				
Parameter	P-value	Hazard Ratio	95% Hazard Ratio CI	
Wk2 pp65 > 100	0.0487	0.137	0.019	0.989
Steroid Use	0.0004	8.148	2.544	26.093

Cox Regression Analysis for CMV Events with All Variables Remained in the Model

Analysis of Maximum Likelihood Estimates					
Parameter		**P-value**	**Hazard Ratio**	**95% Hazard Ratio CI**	
Wk2 pp65 > 100		**0.0570**	**0.144**	**0.020**	**1.059**
Age Recipient		0.4760	1.008	0.987	1.029
GVHD		0.9158	0.970	0.548	1.717
Trans Type	**CORD BLOOD**	0.4517	2.244	0.273	18.421
Trans Type	**HAPLOIDENTICAL**	0.1860	1.834	0.746	4.509
Trans Type	**MATCHED OR MISMATCHED UNRELATED DONOR**	0.1427	1.627	0.849	3.121
Steroid Use	**Y**	**0.0005**	**13.283**	**3.086**	**57.171**
Donor CMV Sero-Status	**POSITIVE**	0.1880	1.473	0.827	2.623
Time to Engraftment		0.1567	1.030	0.989	1.074

Summary

- Negative changes in IE-1 and pp65 counts between pre- and week 4 post-transplant are significant predictors of CMV reactivation

- IE1 and pp65 > 100 at week 2 and week 4 correlated with 92% to 96% protection against CMV reactivation, respectively

- After adjusting for different risk factors, Week 2 pp65 > 100 was an independent predictor of protection against CMV reactivation while the use of systemic steroids was an independent predictor for CMV reactivation

Future Directions: CMV immune monitoring Are we there yet?

Clinical Scenarios	Potential clinical management
As part of pre-emptive strategy	Result may help guide frequency of viral load monitoring and thresholds for initiating antiviral therapy
Post-therapy for GVHD	For negative assay, viral load monitoring; For positive assay, no further intervention
Recent completion of therapy for CMV disease or viremia (Prediction of recurrence of viremia)	For negative assay, consider secondary prophylaxis- close monitoring For positive assay, no further therapy
Risk stratification in patients pre-transplant	For positive assay, assume true positive CMV status

Thank you!