

Immune Correlates of Protection against Herpes Zoster (HZ) in People Living with HIV (PLWH)



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Abstract

Background Herpes zoster (HZ) has high morbidity in immune-compromised hosts, including people living with HIV (PLWH). We investigated the relationship of VZV-specific Th1 and cytotoxic T lymphocytes (CTL) and of Treg with the risk of HZ in PLWH.

Methods Peripheral blood mononuclear cells (PBMC) cryopreserved before HZ from PLWH HZ cases on ART with plasma HIV RNA <200 c/mL, and CD4 counts ≥200 cells/μL and non-HZ controls matched 1:1 to cases by CD4 count, age, sex, race and ART were used to measure T cell subsets by flow cytometry after ex vivo VZV- and mock-restimulation and in freshly thawed unstimulated PBMC using the following markers: CD3, CD8, CD25, CD28, CD39, CD57, CD127, CTLA4, FOXP3, IFNγ, IL10, KLRG1, LAG3, PD1, perforin, TGFβ, TIM3 and TNFα. Data were analyzed by paired t-tests.

Results In unstimulated PBMC, 31 HZ cases had higher CD4+FOXP3+CD25+% Treg compared to controls. After VZV-restimulation cases had lower CD8+Perforin+% CTL, and CD4+IL10+% and CD4+TGFβ+% Treg compared with controls. The differences in VZV-CTL and Treg between cases and controls were present for up to 16 months before development of HZ, without being correlated with each other.

Conclusions Persistently low VZV-specific CTL were associated with increased risk of HZ in PLWH. High Treg were associated with the risk of HZ independently of CTL and they decreased over time of ART, suggesting that the risk of HZ may decrease over time, too.

Introduction

Herpes zoster (HZ) used to be a very common complication of HIV infection before the advent of effective antiretroviral therapy (ART) and remains more frequent in people living with HIV (PLWH) than in the general population in spite of the widespread utilization of ART. This indicates that cell-mediated immune (CMI) reconstitution is incomplete. The importance of CMI in preventing HZ is well established, but its mechanism of action is not well defined. VZV reactivates in neurons of the dorsal root ganglia (DRG), travels through axons to the dermal/epidermal junction and replicates in the basal epidermal cell layer to cause HZ21. Clinical observations provide evidence that protective CMI that is sufficient to prevent HZ may allow VZV not only to reactivate, but also to replicate in a limited fashion: VZV DNA can be found in the blood and oropharynx of asymptomatic healthy adults, suggesting that the virus replicates in the DRG and/or skin, and enters the blood stream to seed distant sites without causing symptoms. The factors that allow reactivated VZV to progress to HZ or not are not known.

In the general population, the risk of HZ increases with age ≥50 years. Aging is accelerated in PLWH, which is an additional factor that may contribute to the increased incidence of HZ in ART recipients. PLWH and older individuals share decreased viral-specific Th1 responses and increased exhausted, senescent and regulatory T cells (Treg). CD8+ T cells have increased expression of inhibitory immunologic checkpoints. We have previously shown that 47 children perinatally-infected with HIV (PHIV) that developed HZ had lower VZV-specific CD8+CD107a+% cytotoxic T lymphocytes (CTL) compared with 141 PHIV controls matched approximately 3:1 to the HZ cases by age at enrollment and at primary varicella infection. These differences were independent of CD4+% or plasma HIV viral load, suggesting that VZV-specific CTL were protective against HZ. In contrast, high Treg and activated T cells were associated with increased risk of HZ in PHIV.

Our goals in this study were to validate VZV-specific CTL as a surrogate marker of protection against HZ and to identify additional factors that may predict the risk of HZ in ALWH on stable and effective ART, with undetectable plasma RNA and CD4+ counts ≥200 cells/dL.

Study Design and Methods

Study Design.

This was a matched case-control study that used plasma and peripheral blood mononuclear cells (PBMC) cryopreserved and stored in ALLRT, a prospective cohort study that enrolled subjects as they entered ACTG antiretroviral studies after 2001 and followed them beyond the duration of the parent study. Inclusion criteria were ≥200 CD4+ cells/dL and HIV plasma RNA<200 copies/mL. Main exclusion criteria were the presence of immune suppressant conditions or medications other than HIV infection, recent opportunistic infections and antiviral medication with activity against VZV. Cases were identified by history of HZ and availability of PBMC collected within 6 months before diagnosis. Controls without HZ were matched to the cases by age (±10 years), gender, race, duration of ART (±48 weeks), parent study and CD4+ cell numbers (±250 cells/dL) at the time of PBMC collection.

Flow Cytometry.

Cryopreserved PBMC were thawed and stained with the following markers: CD3 Alexa Fluor 700 (BD Biosciences), CD8 PC5.5 (Beckman Coulter), FoxP3 APC (eBioscience™), PD1 PE efluor610 (eBioscience™), CD28 PE Cy7 (eBioscience™), KLRG1 APC (eBioscience™), CD127 PE Cy7 (BD Biosciences), CD25 APC H7 (BD Biosciences), TIM3 PE (BD Biosciences), LAG3 PE (BD Biosciences), CD57 BV421 (BD Biosciences), CD39 APC Cy7 (Biolegend). Intra-nuclear staining of FoxP3 was performed using eBioscience™ FoxP3 transcription buffer. Freshly thawed cells were also rested overnight in culture media containing RPMI 1640 (Corning), Penicillin/Streptomycin 100 U/ml/100 μg/ml (Gemini), 10mM HEPES (Corning) 10% FBS (Gibco). Rested PBMC were stimulated with VZV OKA-virus at 10⁶pfu or mock-stimulated for 48hrs. At the end of stimulation 2ug/mL of Brefelding A (Sigma-Aldrich) was added to VZV stimulated and mock stimulated cultures for 4 hrs before staining for flow cytometry with the following markers: CD39 FITC (Biolegend), perforin PE (eBioscience™), CTLA-4 PE CF594 (BD Biosciences), IL-10 PE Cy7 (Biolegend), TGFβ APC (Biolegend), TNFα APC Cy7 (Biolegend), IFNγ BV421 (Biolegend). Flow cytometry acquisition was performed in a 10-color, 3 laser Gallios instrument and analyzed using Kaluza and FlowJo software.

Statistical Analysis.

A sample size of 35 case-control sets with evaluable data was estimated as providing around 90% power to detect an effect size of 0.56 for a specific biomarker between cases and controls (two-sided paired t-test with a significance level of 0.05).

For each pre-specified marker, the differences between cases and controls were assessed by paired t-tests. Wilcoxon signed-rank tests were used to assess marker change over time. Results were not adjusted for multiple comparisons. Correlations between markers were assessed using Pearson correlations.

Results

Table 1. Participant characteristics

Variable	Cases (N=31)	Controls (N=31)	P value
Mean years of age (s.d.)	41.3 (10.0)	40.1 (9.4)	
Males	23 (74%)	23 (74%)	
Race/Ethnicity	White Non-Hispanic	14 (45%)	14 (45%)
	Black Non-Hispanic	11 (35%)	11 (35%)
	Hispanic	6 (19%)	6 (19%)
Categorical Year of Index PBMC Sample	2001 - 2005	7 (23%)	7 (23%)
	2006 - 2009	12 (39%)	10 (32%)
	2010 - 2013	12 (39%)	14 (45%)
Mean CD4+ cells/dL (s.d.)	489 (233)	512 (214)	0.24*
Plasma HIV RNA <50 copies/mL	29 (94%)	27 (87%)	0.41**
Mean Weeks on ART (s.d.)	117 (130)	125 (130)	0.046*
Hepatitis C Positive	2 (6%)	7 (23%)	0.06**

*Paired T-test

**McNemar's Test

Gating Strategy

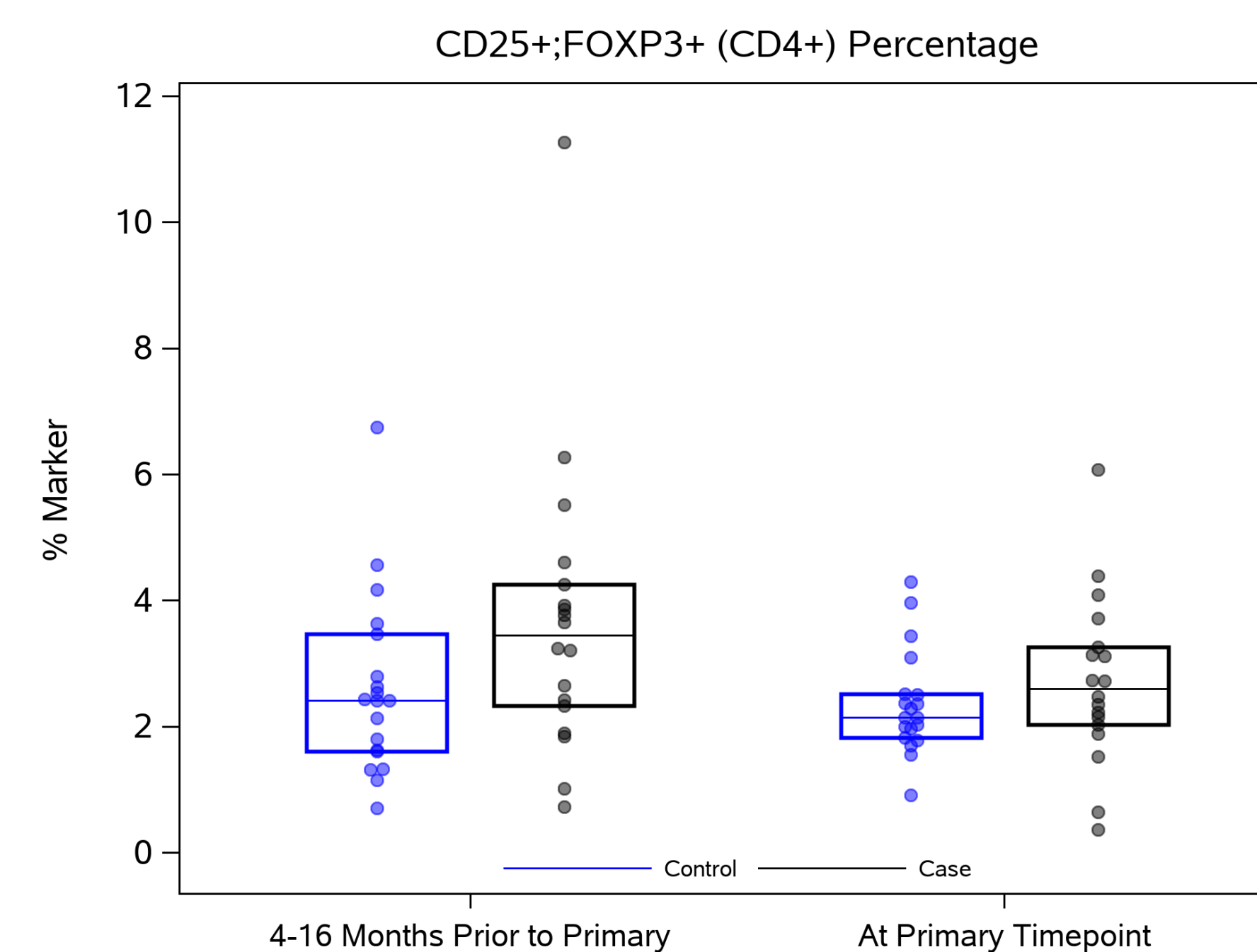
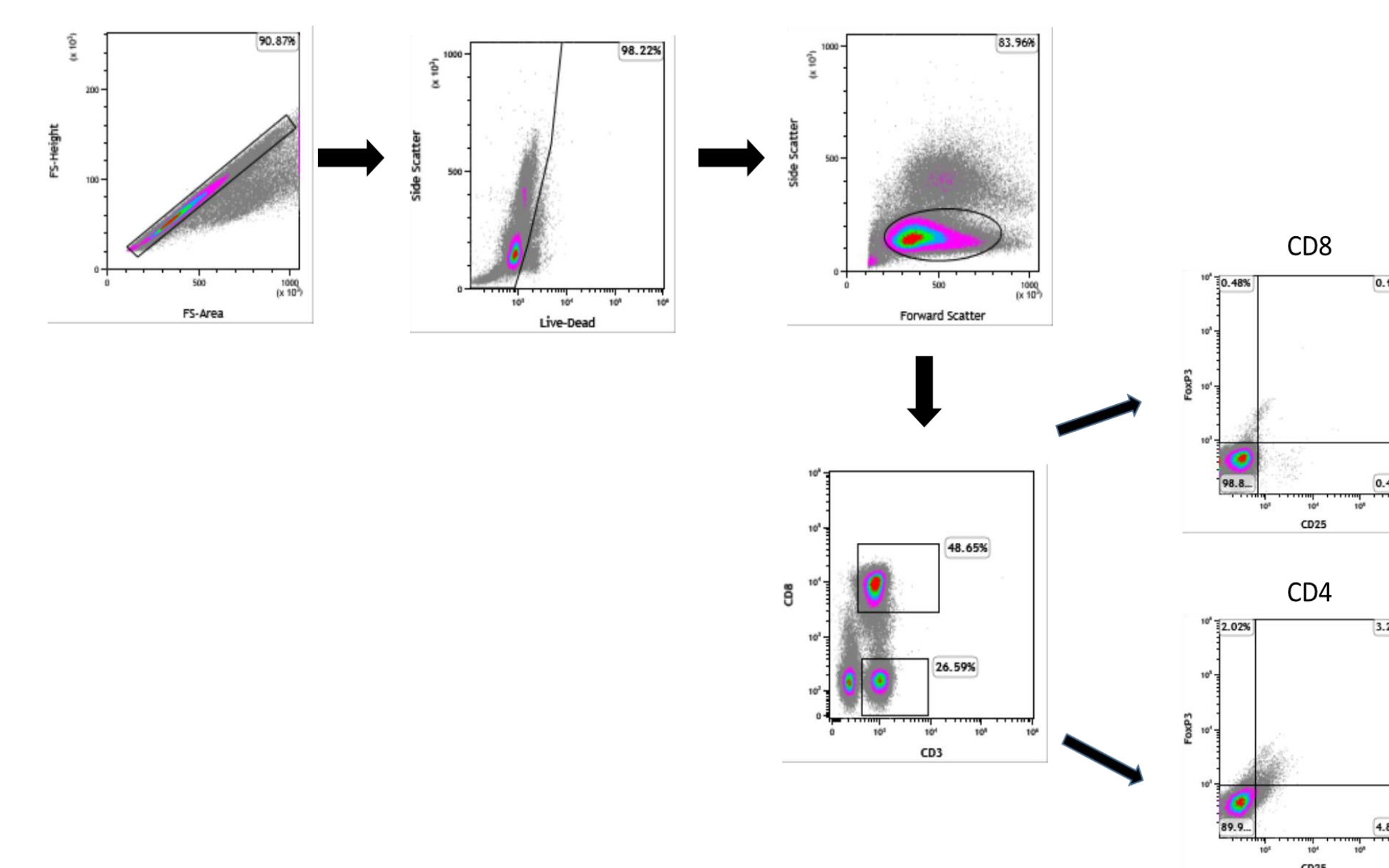


Figure 1. Longitudinal analysis of CD4+FOXP3+CD25+% Treg in PBMC from HZ cases and controls. Data were derived from 18 HZ cases and 19 controls. The primary time point indicates the closest time prior to development of HZ in cases and also corresponds to the matching time point between cases and controls. The data show that the higher CD4+FOXP3+CD25+% Treg in cases compared with controls could be detected up to 16 months before development of HZ.

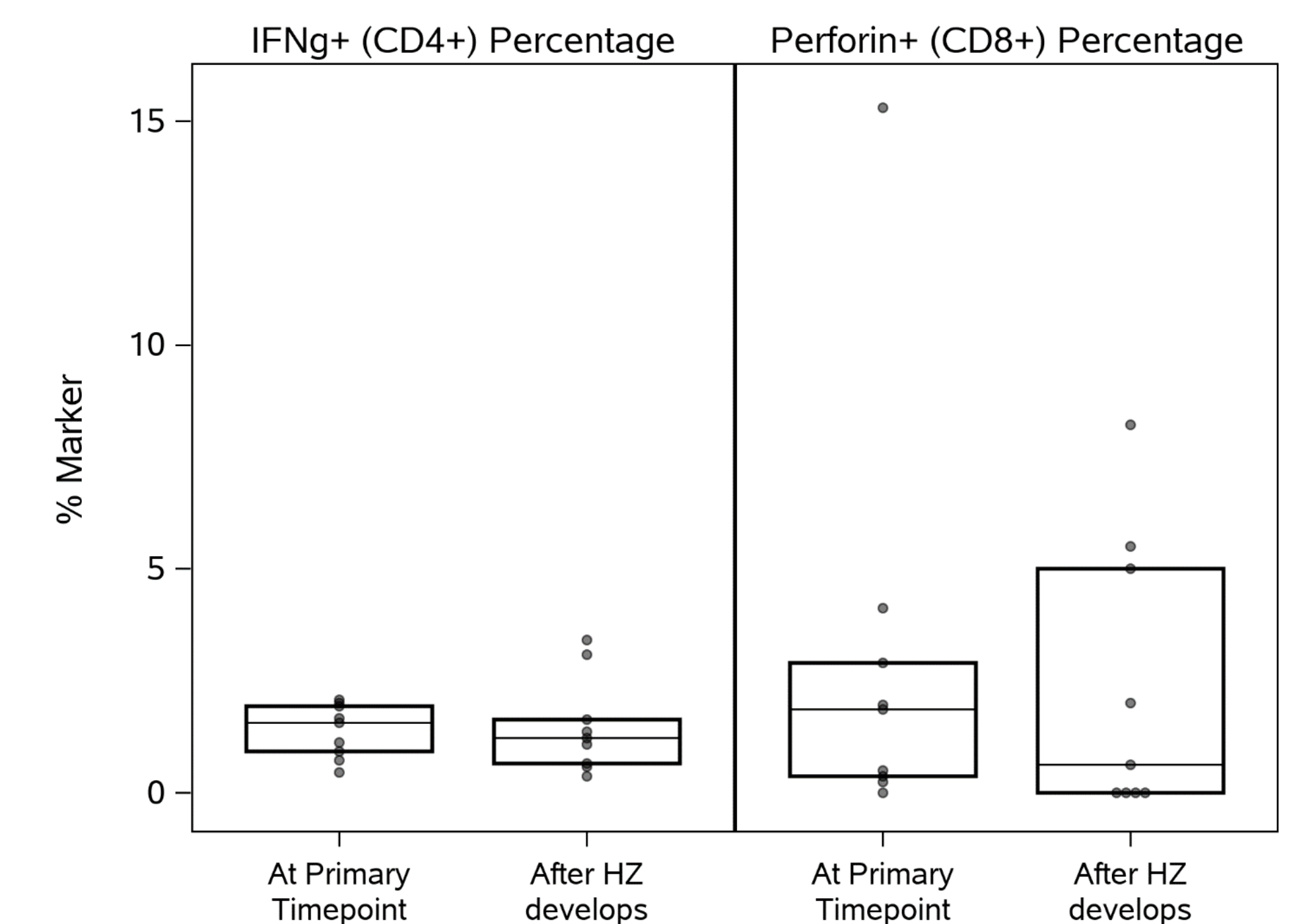


Figure 2. VZV-specific CD8+perforin+% CTL and CD4+IFNγ+% Th1 before and after HZ. Data were derived from 9 HZ cases. The primary time point indicates the closest time prior to development of HZ. The data show the lack of VZV CTL reconstitution after HZ.

Table 2. Correlation analyses of circulating Treg with Th1 and CTL responses

Marker		Circulating Treg	VZV-specific Th1	VZV-specific CTL
Circulating Treg	Correlation	1	-0.23	-0.08
	P-value	-	0.08	0.57
	N	61	60	60
VZV-specific Th1	Correlation	-0.23	1	0.28
	P-value	0.08	-	0.03
	N	60	60	60
VZV-specific CTL	Correlation	-0.08	0.28	1
	P-value	0.57	0.03	-
	N	60	60	60

Conclusions

- This study showed absence of VZV-specific CMI reconstitution in PLWH who developed HZ, before and after HZ
- PLWH who developed HZ had marginally higher proportions of circulating Treg compared with controls. However, Treg had a decreasing frequency in HZ cases (but not in controls).
- The correlation analyses showed significant positive associations of VZV-specific CD4 Th1 and CTL before HZ and marginal negative associations of circulating Treg with VZV-specific CD4+ Th1 responses. VZV-specific CTL did not correlate with circulating Treg.
- Collectively, these observations suggest that Treg may dampen the generation of Th1 responses to VZV, thereby allowing viral reactivation to progress to HZ.
- Our study was limited by the sample size that fell below our a priori estimation of the number of participants necessary to generate significant results.
- We propose that PLWH develop HZ because of a slower decrease in circulating Treg over time of ARV that may negatively affect the ability of the host to mount robust VZV-specific Th1 responses when the virus reactivates.