Supplemental Methods

Patient samples and immunoassays: S0816 was a risk-adapted US intergroup trial evaluating FDG-PET after cycle 2 (PET2) of ABVD (Adriamycin, bleomycin, vincristine, dexamethasone) chemotherapy in high stage (III/IV) cHL patients in which patients received two cycles of ABVD and, based on PET2 results, either continued for four more cycles of ABVD (PET2-negative) or intensified chemotherapy (PET2-positive patients). A favorable 2 year progression-free survival (PFS) of 64% in the PET2-positive patients was achieved, albeit at the expense of increased toxicity in the escalated therapy cohort.[1] All patients consented to this study through their local IRBs. Patients who provided informed consent for correlative studies associated with S0816 had serum samples collected prior to therapy, at the time of the interim PET (PET2), and after completion of therapy associated with the end of therapy PET (PET3).

Serum samples were processed at the individual sites, stored centrally at -80 °C in the CALGB/ALLIANCE biorepository, and tested at the Duke University Phase I Biomarker laboratory. Serum levels of MDC and TARC were tested using a multiplex array (Catalog # N05047A-1), while IL-10 was tested using a singleplex kit (Catalog #: L451QUA-1). These kits were purchased from Meso Scale Discovery, Inc. (Rockville, MD). CD163 was tested using predefined DuoSet antibody reagents (Catalog #: DY1607, R&D Systems, Minneapolis, MN) on the Meso Scale Discovery imaging system.

The EBV status of the H-RS cells was determined using digital gene expression of the *EBER2* viral transcript. In brief, *EBER2* mRNA levels were measured on total RNA extracted from the diagnostic formalin-fixed paraffin-embedded biopsy on the NanoString nCounter platform (Seattle, WA). The transcript level was normalized for RNA loading by dividing by the geometric mean of 3 housekeeping genes (*ACTB*, *CLTC* and *RPLP0*), multiplied by 1000 and then $\log_2$ transformed, as previously described.[2] Tumors were designated EBV positive if the resulting *EBER2* mRNA level was greater than 8.8. This threshold had been determined by comparison of *EBER2* mRNA levels to EBER *in situ* hybridization results for 94 classical Hodgkin lymphoma biopsies (data not shown).

Statistical analysis: Statistical analysis was performed at the SWOG statistical center, Seattle WA. Serum levels of biomarkers were normalized by LOG2 transformation. Comparative descriptive statistics and univariable and multivariable Cox proportional hazard models were performed with statistical significance placed at two-sided alpha of 0.05. Survival curves were performed using the Kaplan-Meier method and compared using log-rank testing. Landmark survival analyses (PFS) of patients at post-therapy PET, included patients progressing at that time to assess serum marker association with both future and concurrent disease. For survival analyses concurrent progression at PET3 were coded minimum survival time in the survival analysis. Stepwise regression using the Akaike information criterion (AIC) was used to identify the best biomarker model using significant variables selected from univariable Cox models.[3] All statistical analyses report unadjusted p-values across the 4 serum markers. Analyses of the 4 serum biomarkers both at baseline and after therapy were prespecified in the primary analysis. For EBV status association with biomarker level, the Wilcoxon test was used.

1. Press OW, Li H, Schoder H, et al. US Intergroup Trial of Response-Adapted Therapy for Stage III to IV Hodgkin Lymphoma Using Early Interim Fluorodeoxyglucose-Positron Emission Tomography Imaging: Southwest Oncology Group S0816. *J Clin Oncol*. 2016;34(17):2020-2027.
2. Scott DW, Chan FC, Hong F, et al. Gene expression-based model using formalin-fixed paraffin-embedded biopsies predicts overall survival in advanced-stage classical Hodgkin lymphoma. *J Clin Oncol*. 2013;31(6):692-700.
3. Akaike H. A new look at the statistical model identification. *IEEE Trans Automatic Control*. 1974;19:716-713.

Supplemental Table 1: Patient Characteristics

	Total	Correlative cohort
N	336	236
Median Age	32 (18-60)	36 (18-61)
Male	56%	56%
Stage IV	48%	48%
Bulky disease	18%	18%
B symptoms	62%	63%
IPS		
Low (0-2)	49%	50%
High (3-7)	51%	50%

Supplemental Table 2: Serum Biomarker Analysis

	Baseline (n=236)		After Cycle 2* (n=166)		After Completion of Chemotherapy* (n=157)	
Biomarker	**Median**	**(Min – Max)**	**Median**	**(Min – Max)**	**Median**	**(Min – Max)**
CD163 (ng/ml)	521.58	(128.17 – 790.15)	323.02	(95.39 – 943.68)	381.27	(99.43 – 1,063.16)
IL10 (pg/ml)	2.0	(0.12 – 1280)	0.26	(.08 – 15.0)	0.25	(0.03 – 12.0)
MDC (ng/ml)	15.44	(0.31 – 285.55)	1.06	(0.39 – 3.02)	1.31	(0.53 – 7.51)
TARC (ng/ml)	54.43	(0.023 – 8,806.66)	0.47	(0.053 – 6.58)	0.55	(0.051 – 10.69)

*Using ratios of the median values at the different time points:

-CD163 levels decreased from pretreatment levels 1.61 fold after cycle 2 and 1.37 fold after therapy.

-IL10 levels decreased 7.69 fold after cycle 2 and 8.0 fold after therapy.

-MDC and TARC serum levels decreased 14.53 and 115.81 fold after cycle 2 and 11.79 and 98.97 fold after therapy, respectively.

-The median change in the serum levels from baseline to after cycle 2 of chemotherapy and to the completion of chemotherapy is statistically significant for each of these biomarkers using Wilcoxon signed-rank test respectively ($p<.001$).

Supplemental Table 3: EBV Status and Median Baseline Serum Marker Level Correlation

	EBV- (n=132)	EBV+ (n=30)	P value (Wilcoxon Test)
sCD163	513 ng/ml	653 ng/ml	0.025
IL10	1.7 pg/ml	3.8 pg/ml	0.008
TARC	66.2 ng/ml	26.7 ng/ml	0.005
MDC	16 ng/ml	13ng/ml	0.245

Clinical characteristics of the 162 patients with EBV status were similar to the entire study cohort. No difference in PFS or OS was seen between EBV+ and EBV- patients.

Supplemental Table 4. Log2(Post-therapy Serum Marker Levels) and Survival, Multivariable Cox Regression

	Landmark Progression Free Survival		Landmark Overall Survival	
Biomarker	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**
IL10	.025	1.3 (1.03-1.62)	.002	1.7 (1.22-2.45)
TARC	<.0001	1.8 (1.35-2.37)	.021	1.9 (1.10-3.23)

Progression Free Survival: 151 patients, 34 events
Overall Survival 151 patients, 8 events

Supplemental Table 5. Log2 (Post-therapy Serum Marker Levels) and Progression Free Survival, Univariate Cox Regression in PET2-Negative Patients

	Landmark Progression Free Survival*	
Biomarker	**P value**	**HR (95% CI)**
CD163	0.6822	0.9 (0.51 - 1.55)
IL10	0.0288	1.3 (1.03 - 1.75)
MDC	0.0038	3.0 (1.43 - 6.30)
TARC	<.0001	2.2 (1.59 - 2.94)

*Total samples 127, number of events 27
HR=hazard ratio, CI = confidence interval
IL10, MDC and TARC remained significantly associated with PFS after adjusting for IPS

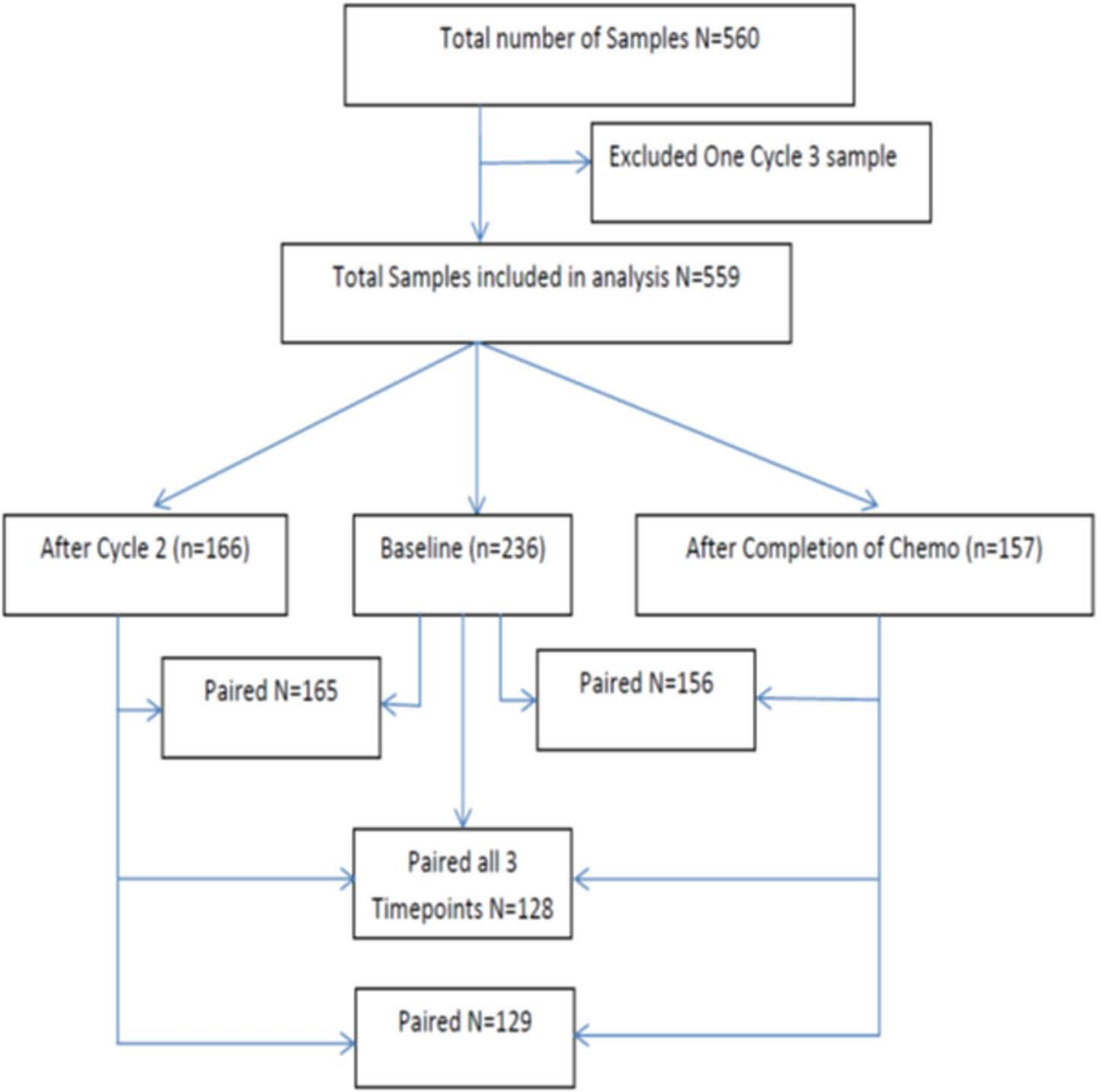
LEGEND:

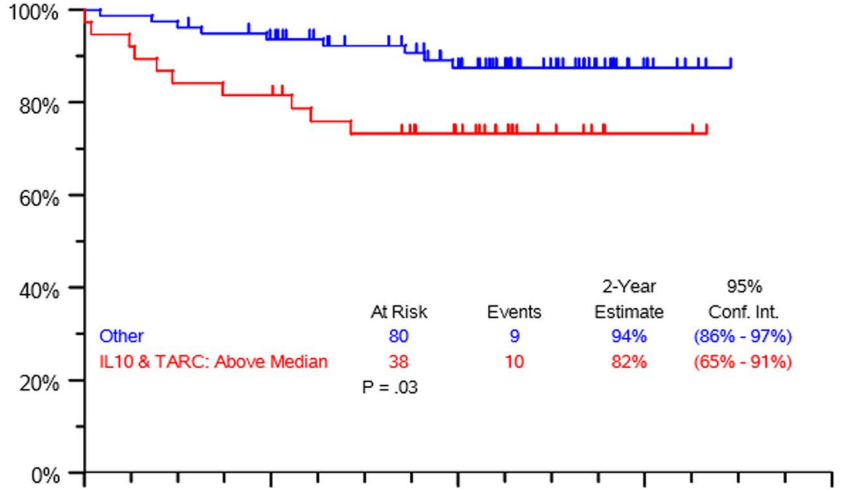
Supplemental Figure 1 –Specimen Makeup

LEGEND:

Supplemental Figure 2. Landmark Kaplan-Meier PFS curves for PET2-negative patients.

These curves exclude patients who were PET3+/progressed at time of PET3 or progressed shortly after PET3 assessment. Patients with post-therapy TARC level above median and those with post-therapy TARC level below median for this cohort are compared (logrank test).


Total number of Samples N=560
Excluded One Cycle 3 sample
Total Samples included in analysis N=559
After Cycle 2 (n=166)
Baseline (n=236)
After Completion of Chemo (n=157)
Paired N=165
Paired N=156
Paired all 3 Timepoints N=128
Paired N=129



# at Risk								
Other	80	78	72	62	52	29	8	0
IL10 & TARC: Above Median	38	32	31	26	19	7	2	0