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Supplemental Material and Methods

Plasma Epstein–Barr Virus DNA and pCMVd Quantification

Real-time PCR with the CMV and EBV Artus kits (Qiagen Hamburg GmbH, Hilden, Germany) were performed on the ABI 7500 (Applied Biosystems) platform. The Qiagen Artus CMV kit contains reagents and enzymes for the specific amplification of a 105-base pair (bp) region of the CMV major immediate-early gene and the Qiagen Artus EBV kit and reagents targeted a 97-bp region of the Epstein-Barr nuclear antigen 1 gene. DNA extractions were performed using the NucliSens easyMAG (Biomerieux) extractor system. DNA was extracted from 200 μL of plasma and eluted in a final volume of 25 μL. An internal control was added to each primary sample before extraction, and amplification was performed with specific primers and hydrolysis probes contained in the Artus CMV and/or EBV master mix to ensure adequate extraction efficiency and the absence of inhibitors. PCR was carried out in 96-well microtiter plates with a reaction volume of 26 μL (11 μL of DNA extract and 15 μL of master mixture). Reaction mixtures underwent an initial 10 minutes at 95°C, followed by 95°C for 15 seconds, and 55°C for 1 minute (45 cycles). Acrometrix (Life Technologies) intact whole virus quantitative standards were used to establish the standard curve for the CMV after extraction (6 concentrations ranging from 5,160,000, 516,000, 51,600, 5160, 516, and 258 copies/mL) and for EBV (5 concentrations ranging from 10,000,000, 1,000,000, 100,000, 10,000, and 1000 copies/mL), respectively. Additional CMV and EBV low positive, high positive, and negative external controls were included with each run. The CMV and EBV DNA load was calculated from the individual standard curve and expressed as the number of CMV or EBV DNA copies/mL of plasma. The linear range of the CMV assay was 250 to 5×10^6 copies/mL. For CMV plasma, any result between 1 and 250 copies/mL was reported as <250 copies/mL. Results $>5 \times 10^6$ were reported as $>5 \times 10^6$ copies/mL. The linear range of the EBV assay was 2000 to 10,000,000 copies/mL. For EBV plasma, any result between 1 and 1999 copies/mL was reported as <2000 copies/mL. Performance characteristics of these tests have been established by the Clinical Microbiology Laboratory at The OSU Wexner Medical Center. Before August 24, 2008, the lower limit for detectable EBV DNA was 1000 copies/mL. Before August 24, 2008 (EBV) and January 10, 2008 (CMV) these assays were performed and developed at Mayo Clinic. Its performance characteristics were determined by the Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota.

Tissue Specimens

All tissue biopsies were reviewed by 2 independent hematopathologists (A.A.G. and J.A.H.) with one being an expert cutaneous lymphoma pathologist (A.A.G.). LCT was defined as the presence of large cells (≥4 times the size of a small lymphocyte) in 25% or more of tissue (lymph node or skin) or the presence of a nodular aggregate of large cells in an individual with a clinical and pathologic history consistent with MF. For each available case, chromogenic ISH for EBER (EBER-ISH) was performed on formalin-fixed, paraffin-embedded tissue that was sectioned at 4 μm and placed on positively charged slides. Slides with specimens, as well as appropriate controls, were then placed in a 60°C oven for 1 hour, cooled, and loaded onto the Ventana Benchmark XT Autostainer for subsequent deparaffinization, cell conditioning, enzymatic digestion (Protease 3, Ventana Medical Systems, Tucson, AZ), and in situ staining. The ready-to-use probe for EBER (EBER 1 DNP Probe, ASR, Ventana Medical Systems), and an anti-DNP-biotin/streptavidin chromogen system were used in succession. When the probe was dispensed on the slides, and incubated for 60 minutes, the iView Blue Plus Detection Kit (Ventana Medical Systems) was used to produce the chromogenic reaction. Slides were counterstained using Ventana Medical Systems Red Stain II. After removal from the Benchmark XT Autostainer, slides were manually dehydrated through graded ethanol solutions and coverslipped using a nonaqueous mounting media. $EBER^+$ samples were scored from 1+ to 3+ depending on the number of positive cells at 200× (Supplemental Figure 1). Specifically, EBER positivity ($EBER^+$) was defined as cells with nuclear staining and semiquantitated into 3 groups by counting the $EBER^+$ cells in the 200× field with the greatest number of positive cells: 1+ had <10 positive cells per 200× field; 2+ had 10 to 30 positive cells per 200× field; and 3+ had >30 positive cells per 200× field.

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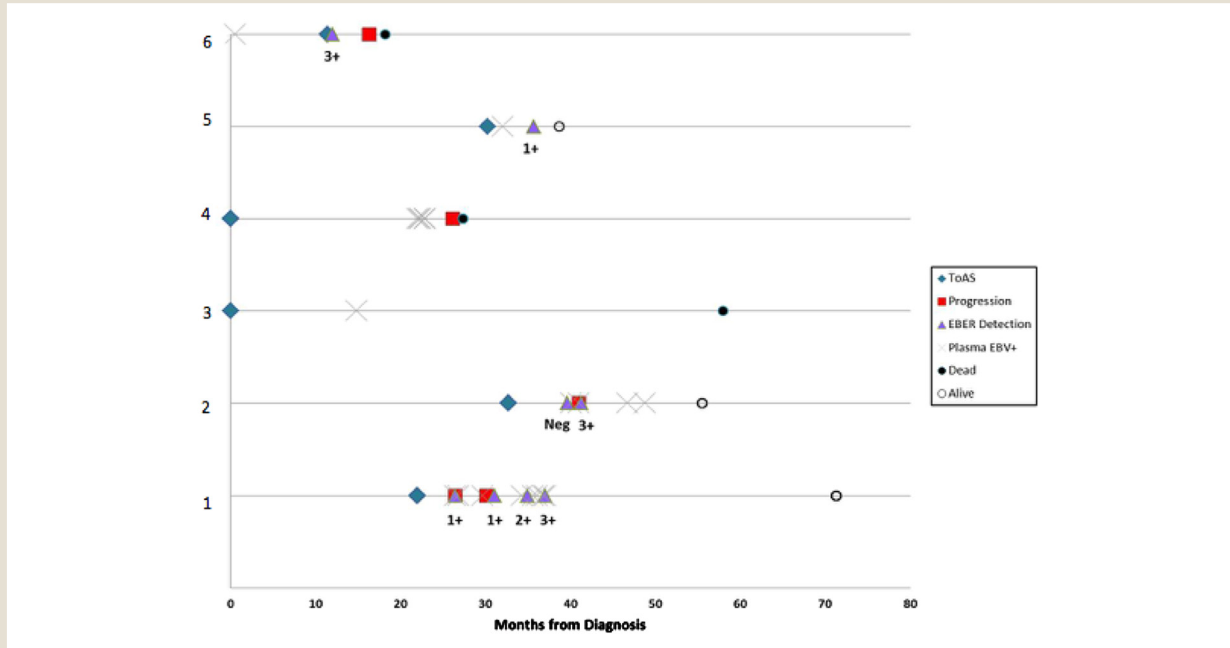
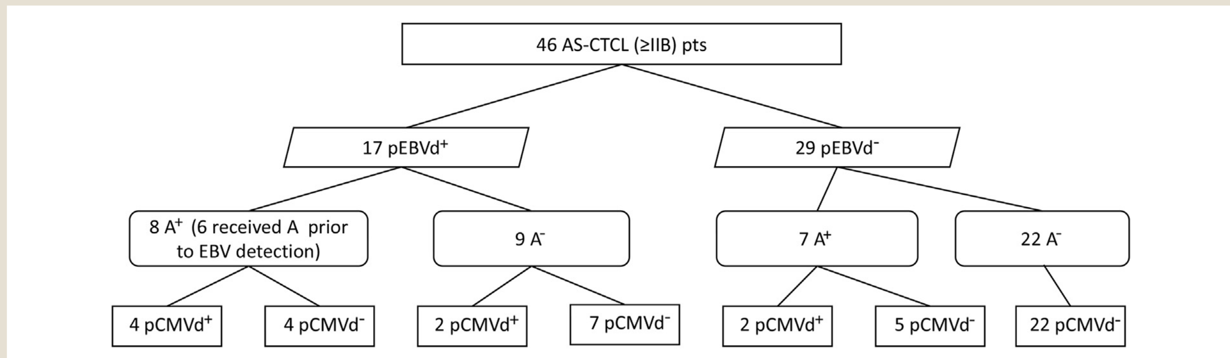
Supplemental Figure 1 Longitudinal Follow-Up in Alemtuzumab and Histone Deacetylase Inhibitor (HDACi)-Naive Cutaneous T-Cell Lymphoma Patients From Time of Diagnosis to Time of Advanced Stage (≥IIB) to Further Progressions Requiring a Change in Systemic Treatment. Longitudinal Time Points When Plasma Epstein—Barr Virus DNA and Epstein—Barr Virus (EBV)—Encoded RNA (EBER) Was Detected Are Highlighted. None of the Patients Received Alemtuzumab or HDACi. Disease Progression Was Defined as the Development of New Cutaneous, Nodal, or Visceral Disease or an Increase in International Society for Cutaneous Lymphomas (ISCL) Blood Stage (ISCL B_{0-2}), Which Also Necessitated a Change in Therapy, With the Exclusion of Local Therapy



Abbreviation: ToAS = time of progression to advanced stage.

Supplemental Figure 2 Incidence of Cytomegalovirus (CMV) Detection Stratified According to Plasma Epstein—Barr Virus (EBV) DNA (pEBVd) Detection and Alemtuzumab (A) Exposure



Abbreviations: A^+ = treated with alemtuzumab; A^- = never treated with alemtuzumab; AS CTCL = advanced stage cutaneous T-cell lymphoma; $pCMVd^+$ = CMV-DNA ≥250 copies per mL in plasma; $pCMVd^-$ = <250 copies per mL in plasma; $pEBVd^+$ = EBV-DNA ≥1500 copies per mL in plasma; $pEBVd^-$ = <1500 copies per mL in plasma; pts = patients.

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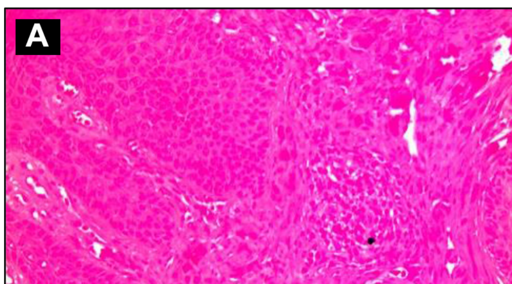
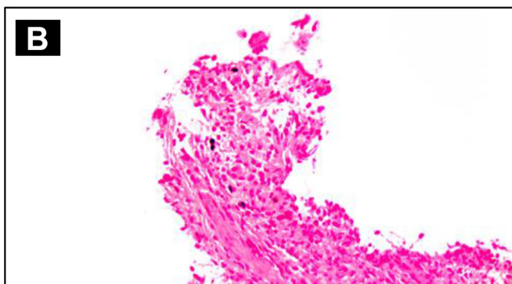
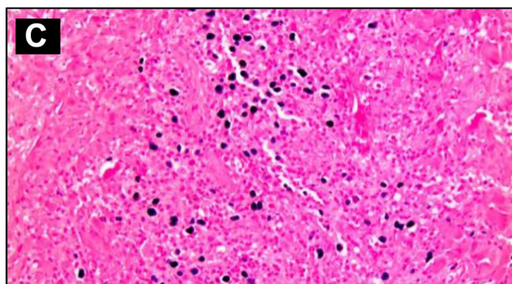
Supplemental Figure 3 Representative Degrees of Positive In Situ Hybridization Epstein—Barr Virus RNA (EBER) Staining in Skin Biopsies. (A) EBER 1+ = <5 Positive Cells per 200× Field. (B) EBER 2+ = 5-30 Positive Cells per 200× Field. (C) EBER 3+ = >30 Positive Cells per 200× Field







Supplemental Table 1 Tumor-Node-Metastasis-Blood (TNMB) Classification of Cutaneous T-Cell Lymphoma

Type	Description
Skin	
T1	Limited patches, papules, and/or plaques covering <10% of the skin surface
T2	Patches, plaques, or plaques covering ≥10% of the skin surface
T3	One or more tumors ≥1 cm in diameter
T4	Confluence of erythema covering ≥80% of body surface area
Node	
N0	No clinically abnormal lymph nodes
N1	Clinically abnormal lymph nodes; histopathology Dutch Grade 1
N1a	Clone negative
N1b	Clone positive
N2	Clinically abnormal lymph nodes; histopathology Dutch Grade 2
N2a	Clone negative
N2b	Clone positive
N3	Clinically abnormal lymph nodes; histopathology Dutch Grade 3-4
Nx	Clinically abnormal lymph nodes; no histologic evaluation
Visceral	
M0	No visceral organ involvement
M1	Visceral involvement (must have pathology confirmation)
Blood	
B0	No significant blood involvement: ≤5% Sezary cells. Also defined as <250/µL Sezary cells; $CD4^+CD26^-$ or $CD4^+CD7^-$ cells or $CD4^+CD26^-$ and $CD4^+CD7^-$ cells <15% using flow cytometry
B0a	Clone negative
B0b	Clone positive
B1	Low blood tumor burden: does not meet criteria of B0 or B2
B1a	Clone negative
B1b	Clone positive
B2	High blood tumor burden: positive clone plus 1 of the following: ≥1000/µL Sezary cells; CD4+/CD8+ cells ≥10; $CD4+CD7^-$ cells ≥40%; or $CD4^+CD26^-$ cells ≥30%

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Supplemental Table 2 Clinical Staging System for Mycosis Fungoides

Clinical Stage	Tumor-Node-Metastasis-Blood (TNMB) Classification			
IA	T1	N0	M0	B0 or B1
IB	T2	N0	M0	B0 or B1
IIA	T1 or T2	N1 or N2	M0	B0 or B1
IIB	T3	N0-N2	M0	B0 or B1
IIIA	T4	N0-N2	M0	B0
IIIB	T1-T4	N0-N2	M0	B1
IVA1	T1-T4	N0-N2	M0	B2
IVA2	T1-T4	N3	M0	B0-B2
IVB	T1-T4	N0-N3	M1	B0-B2

Supplemental Table 3 Systemic Therapies Before EBV Detection Compared With Cohort Without Detectable pEBVd

Systemic Therapy	Before First $pEBVd^{+}$	$pEBV^{-}$
Single-Agent[a]	1 (6)	10 (34)
Multiagent[b]	7 (41)	9 (31)
Alemtuzumab	6 (35)	7 (24)
Nucleoside Analogue[c]	3 (18)	2 (7)
ECP	1 (6)	5 (17)
Histone Deacetylase Inhibitor[d]	6 (35)	11 (38)
Cytokine Therapy[e]	7 (41)	13 (45)
Other[f]	1 (6)	2 (7)

Data are presented as n (%).

Abbreviations: EBV, Epstein—Barr virus; ECP = extracorporeal photopheresis; pEBVd = plasma Epstein—Barr virus DNA; $pEBVd^{+}$ = EBV-DNA $\geq$1500 copies per mL in plasma; $pEBVd^{-}$ = <1500 copies per mL in plasma.

[a]Single-agent: doxorubicin, cyclophosphamide, vincristine, etoposide, flavopiridol, methotrexate, pralatrexate.

[b]cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP), etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin (EPOCH), hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate, cytarabine (HyperCVAD), carmustine, etoposide, cytarabine, melphalan (BEAM), gemcitabine/doxorubicin, fludarabine/busulfan, fludarabine/cyclophosphamide.

[c]Single-agent: pentostatin, gemcitabine, cytarabine, fludarabine.

[d]Single-agent: vorinostat, romidepsin, AR-42, LBH-589.

[e]Single-agent: denileukin diftitox, interferon.

[f]Enzastaurin, bortezomib/azacitidine.