Supplemental Information:

RNA Isolation and Quantitative real-time RT-PCR

Total RNA isolation and quantitative real-time RT-PCR (qRT-PCR) were performed as previously reported[1]. The primers sets used for the study are shown in the following Table:

Primer Sets for qPCR

Primer Name	Sequence (Forward)	Sequence (Reverse)
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG
ORF73/LANA	CCTGGAAGTCCCACAGTGTT	AGACACAGGATGGGATGGAG
ORF50/RTA	CAAGGTGTGCCGTGTAGAGA	TCCCAAAGAGGTACCAGGTG
ORF74/vGPCR	TGTGTGGTGAGGAGGACAAA	GTTACTGCCAGACCCACGTT
K8.1	CACCACAGAACTGACCGATG	TGGCACACGGTTACTAGCAC
EBNA	TACAGGACCTGGAAATGGCC	TCTTTGAGGTCCACTGCCG
EBER1	AAAACATGCGGACCACCAGC	AGGACCTACGCTGCCCTAGA
LMP1	TATCTTCAGAAGAGACCTTCTC	ATCAACCAATAGAGTCCACCAG
EBV-TK	GTGGGATCCATGGCTGGATT	GCTACCCGGAGAGTTTCCAGT

Viability, cell cycle and apoptosis

Cell proliferation was assessed with CellTiter 96 Aqueous Non-Radioactive Cell Proliferation (MTS) Assay (Promega) following the manufacturer's instructions. For cell cycle studies, $1x10^6$ cells were incubated for 24 hours with brentuximab vedotin or Ig-vcMMAE control at indicated concentrations, collected, washed with PBS and fixed with 70% ethanol at 4°C. Cells were then washed with PBS and incubated with propidium iodide (PI) (50µg/ml) and

0.1mg/ml RNAse (Invitrogen) for 30-45 minutes. Cell cycle analysis was performed on a BD FACS Canto analyzer (BD Biosciences). Apoptosis studies were performed using YO-PRO-1 and PI as reported previously[2].

Cytogenetics

Karyotype of the lymphoma cells was investigated at diagnosis using standard cytogenetic procedures. Chromosomes were identified using G-banding techniques and classified according to ISCN nomenclature[3].

KSHV Immunofluorescence

A total of 1 x 10^6 cells per treatment were cytospun at 800rpm for 3 minutes, and stained as previously described[4]. K8.1 antibody (Advanced Biotechnologies) was used at 1:200 dilutions and LANA antibody (Abcam) was used at 1:100 dilutions. Secondary antibodies for K8.1 (anti-mouse) and LANA (anti-rat) were purchased from Invitrogen. Slides were mounted with ProLong Gold antifade reagent with 4',6'-diamidino-2-phenylindole (Invitrogen).All images were acquired using Zeiss Axiovision 4.8.2 with a Hamamatsu ORCA-R2 CCD camera and Zeiss Axiovert 200M inverted fluorescence microscope.

sCD30 ELISA

sCD30 levels were determined in cell free media collected from culture of 1x10^6 (BC-1, BC-3, UM-PEL-1c, UM-PEL-3c and Karpas-299) and mice ascites harvested from UM-PEL-1 and UM-PEL-3 xenografts by a sandwich enzyme-linked immunosorbent assay (eBioscience) following manufacturer's protocol.

Antigen binding capacity [ABC]

Measurement of ABC units/cell was used to depict CD30 and brentuximab vedotin antigen density on PEL cell lines. ABC values for each cell line was calculated using Quantum

Simply Cellular kit (Bangs Laboratories, Inc.) according to the manufacture's protocol. Briefly, the microbeads used were coated with known quantities of goat antimouse IgG, which when mixed with saturating quantities of mouse anti-hCD30-PE (BD Biosciences) or mouse anti-hIgG-FITC (BD Biosciences), produced a standard curve to measure density of the CD30 and brentuximab vedotin respectively. In a separate reaction, mean fluorescence intensity from BC3, UM-PEL-3c, and Karpas-299 cell lines exposed to brentuximab vedotin at 15μgm/mL for 0, 24 and 48h were measured for both CD30 and brentuximab vedotin antigens by the same protocol. The antigen densities were calculated by extrapolating values from the standard curves.

PCR amplification and sequencing of immunoglobulin heavy chain variable (*IGHV*) genes

To amplify the *IGHV* gene sequences, 50–200 ng of DNA extracted from UM-PEL-3 cells using DNeasy kit (Qiagen) were amplified by GoTaq Green Master Mix (Promega, Madison, WI) in a final volume of 50 μl containing 10 pmol of a specific 5′ primer corresponding to one of the 6 human variable immunoglobulin heavy chain family leaders (*IGHV*1 through *IGHV*6) and 10 pmol of 3′ antisense J_H consensus primer[5,6]. *IGHV*1 leader primer also amplifies sequences from the closely related *IGHV*7 family. The PCR conditions were: 96°C for 5 minutes, 55°C for 1 minute, 72°C for 3 minutes, 1 cycle; 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, 30 to 35 cycles; and 72°C for 7 minutes. A control with no added template was used in each PCR reaction to exclude the possibility of contamination. PCR products were analyzed by 2% agarose gel electrophoresis and stained with ethidium bromide. All bands of the appropriate size were excised from the gels and purified by adsorption to a silica matrix (QIAquick Gel Extraction Kit, Qiagen). Direct DNA sequencing of PCR amplicons was performed on a 373 automatic DNA sequencer (Applied Biosystems, Foster City, CA) using the ABI Prism Big Dye Terminator Kit (Perkin Elmer, Foster City, CA), as

recommended by the manufacturer. The same primers used for the PCR were used for sequencing.

Table S1: UM-PEL-3 cells surface antigen expression

Antigen	% positive (UM-PEL-3 cells)
CD30	**85.5%**
CD45	**80.5%**
CD38	**83.1%**
CD138	**22.4%**

UM-PEL-3 cells were stained with antibodies for specific antigen indicated above followed by flow-cytometry to analyze the expression.

Table 2S: Cytogenetic results of primary PEL tumor and UM-PEL-3 cells:

81~91,XY,+add(X)(q28),+Y,-1,-1,der(1)t(1;?)(q21;?),-2,-2,-3,-3,der(3)t(3;?)(q21;?),
+4,+4,-5,del(5)(q33),x2,-6,-6,der(6)t(6;?)(q21;?),+7,+7,del(7)(q32)x2,der(7)t(7;?)(q22;?),
+8,+8,+10,-10,+11,-12,add(12)(p13),der(12)t(12;?)(q13;?),
-13,iso(13)(q10)x2,+14,add(14)(q32)x2,-15,iso(17)(q10),
-18,+19,+21,-22,-22,+5~11mar[cp21]

Supplementary Figures

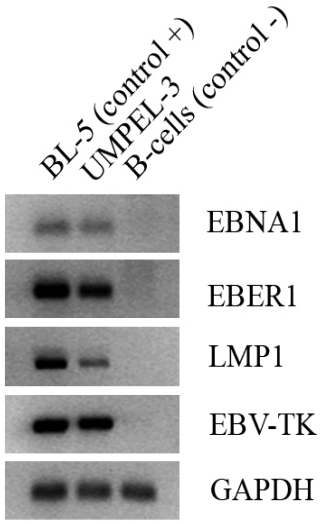

Figure S1: UM-PEL-3 cells are infected with Epstin-Bar Virus (EBV).

DNA isolated from UM-PEL-3 cells was amplified by PCR using primer specific for EBV (EBNA, EBER1, LMP1, EBV-TK) . GAPDH was used as a loading control. BL-5 cell line infected with EBV served as positive control and B-cell from healthy donor served as negative control.

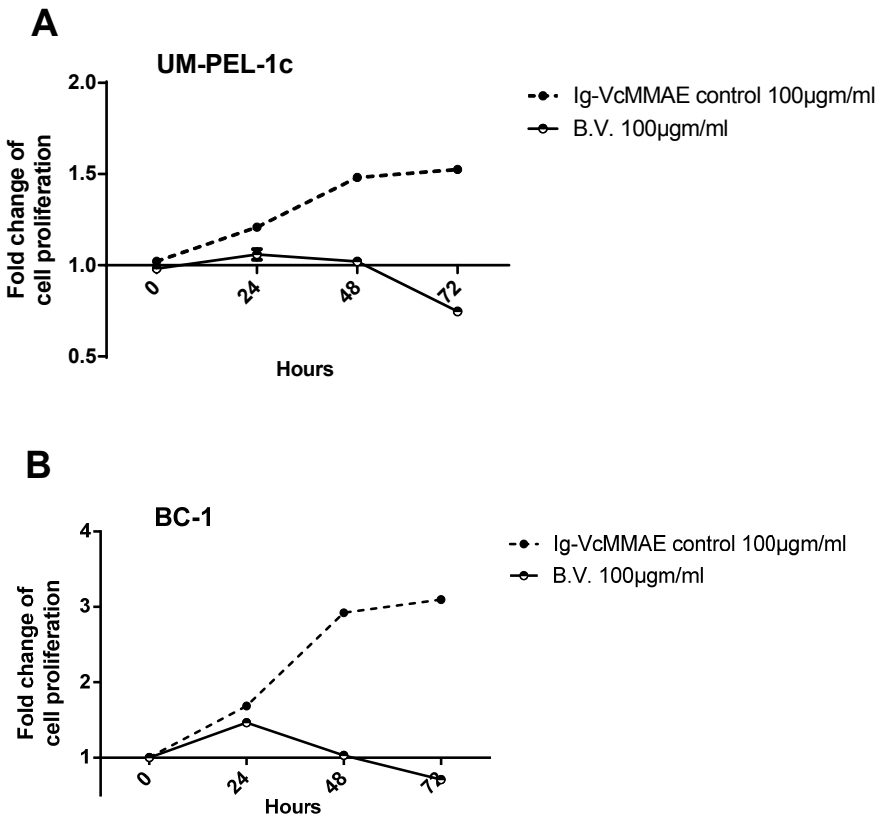

Figure S2. Treatment with irrelevant isotype Ig-VcMMAE dose not affect the proliferation of PEL cells.

Human PEL cell lines UM-PEL-1c (A) and BC-1 (B), were treated with Ig-VcMMAE or brentuximab vedotin at 100µgm for 0, 24, 48 and 72hours. Proliferative response at each time point was measured by MTS assay. Results are shown as fold change of proliferation compared to time 0 hours. All experiments were repeated thrice independently in triplicate. Representative data from one experiment is shown. Error bars corresponds to standard error of mean in all graphs.

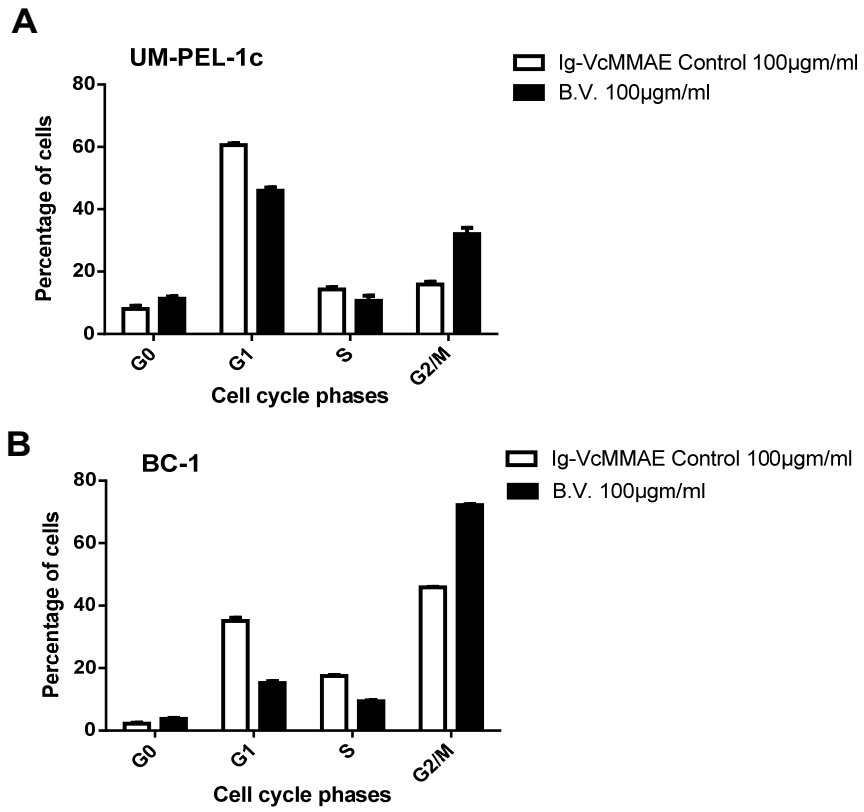

Figure S3. Treatment with irrelevant isotype Ig-VcMMAE dose not affect cell cycle profile of PEL cells

Human PEL cell lines UM-PEL-1c (A) and BC-1 (B), were treated with Ig-VcMMAE or brentuximab vedotin at 100µgm. At 24hours after treatment cells were stained with propidium iodide to measure DNA content and analyzed by flow cytometry for cell cycle distribution. Bar graphs indicate the percentage of cells in different phases of cell cycle (G0, G1, S, G2/M). All experiments were repeated thrice independently in triplicate. Representative data from one experiment is shown. Error bars corresponds to standard error of mean in all graphs.

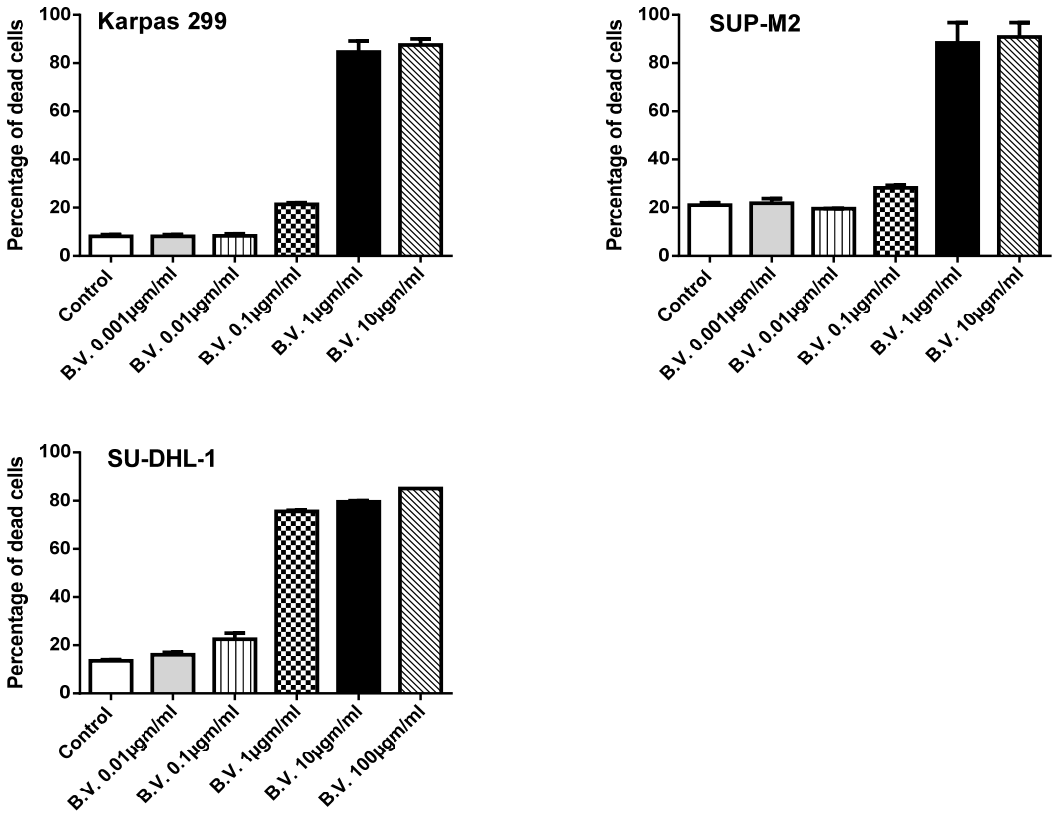

Figure S4. Brentuximab vedotin induces cell death of Anaplastic large cell lymphoma cells

ALCL cell lines expresing CD30 were treated with increasing concentrations of brentuximab vedotin (B.V.). At 72 hours after treatment cell viability was determined by flow-cytometry following YO-PRO and Propidium Iodide staining. Error bars correspond to standard error of mean in all graphs.

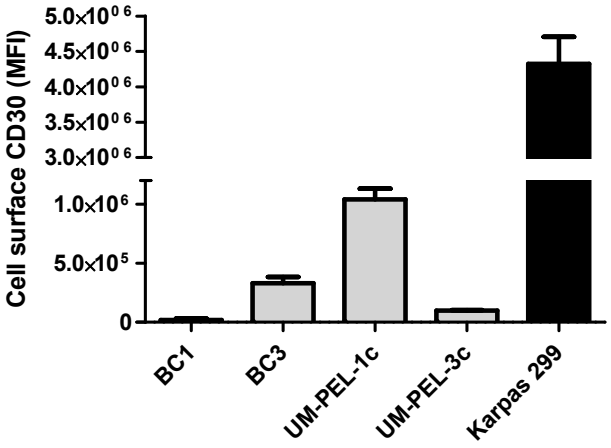

Figure S5. Cell surface CD30 expression levels in PEL cell lines.

Indicated PEL (BC-1, BC-3, UM-PEL-1c and UM-PEL-3c) and ALCL (Karpas 299) cell lines were stained with the anti-CD30-FITC antibody and analyzed by flow-cytomerty to obtain mean fluorescence intensity (MFI). Background fluorescence was omitted by staining with matching isotype control staining. Data is representative of 3 independent experiments and error bars correspond to the standard error of the mean in all graphs.

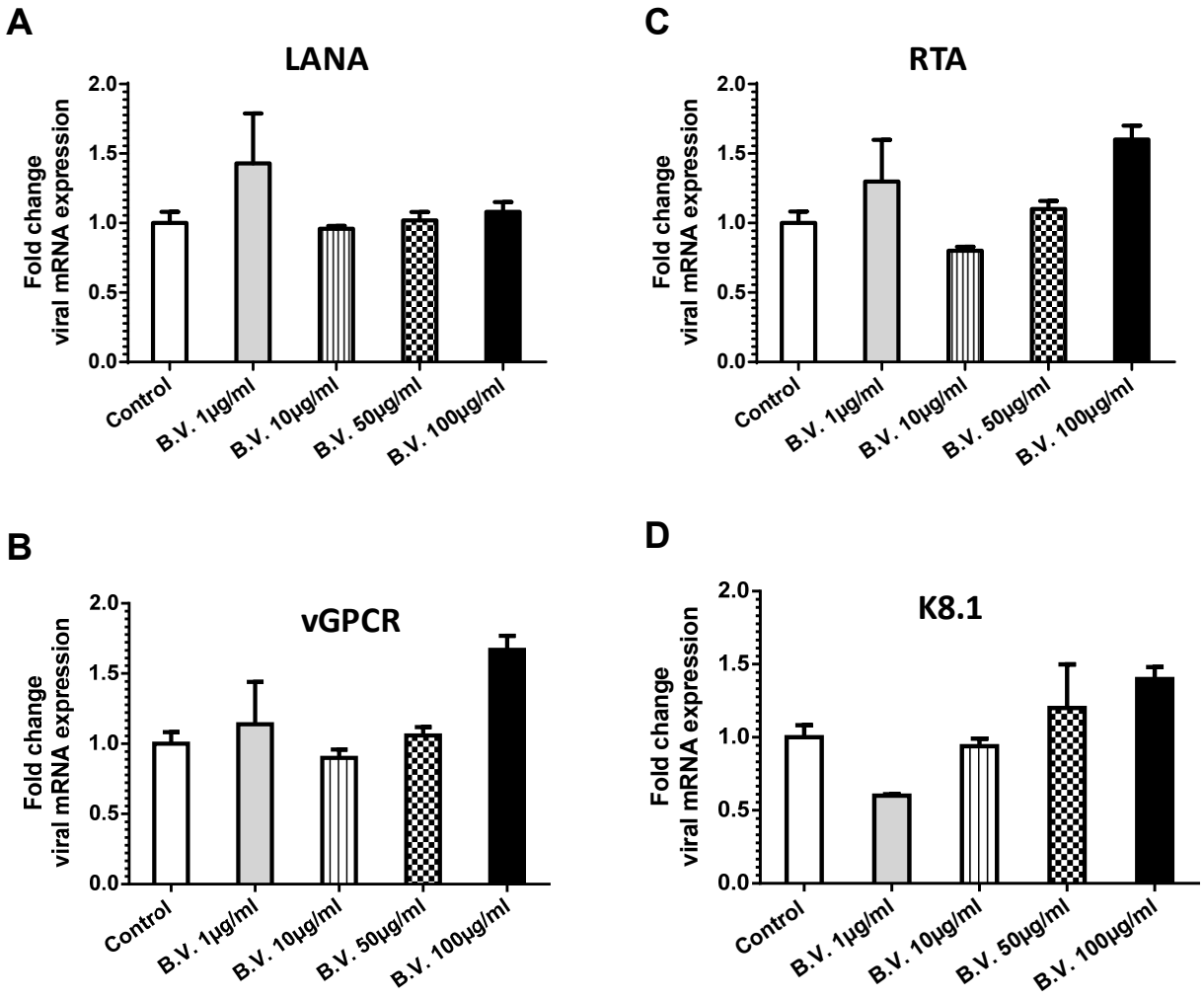

Figure S5. Brentuximab vedotin does not promote lytic replication of KSHV.

UM-PEL-1c cells were treated with indicated concentration of brentuximab vedotin (B.V.) and cells were harvested at 48 hours after treatment. Total RNA was extracted for qRT-PCR analysis of latent (LANA), immediate early lytic (RTA), delayed early lytic (vGPCR) and late lytic (K8.1) viral genes. Values of triplicate wells are represented as fold mRNA expression with respect to the control cells. Data is representative of 3 independent experiments. Error bars correspond to the standard error of the mean in all graphs.

Supplemental References

1. Francisco JA, Cerveny CG, Meyer DL, et al. cAC10-vcMMAE, an anti-CD30-monomethyl auristatin E conjugate with potent and selective antitumor activity. *Blood*. 2003;102(4):1458-1465.
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3. Brothman AR, Persons DL, Shaffer LG. Nomenclature evolution: Changes in the ISCN from the 2005 to the 2009 edition. *Cytogenetic and genome research*. 2009;127(1):1-4.

4. Mutlu AD, Cavallin LE, Vincent L, et al. In vivo-restricted and reversible malignancy induced by human herpesvirus-8 KSHV: a cell and animal model of virally induced Kaposi's sarcoma. *Cancer cell*. 2007;11(3):245-258.
5. Lossos IS, Okada CY, Tibshirani R, et al. Molecular analysis of immunoglobulin genes in diffuse large B-cell lymphomas. *Blood*. 2000;95(5):1797-1803.
6. Campbell MJ, Zelenetz AD, Levy S, Levy R. Use of Family Specific Leader Region Primers for Pcr Amplification of the Human Heavy-Chain Variable Region Gene Repertoire. *Molecular Immunology*. 1992;29(2):193-203.