HHS Public Access

Author manuscript

Leuk Lymphoma. Author manuscript; available in PMC 2017 October 01.

Published in final edited form as:

Leuk Lymphoma. 2016 October ; 57(10): 2359–2369. doi:10.3109/10428194.2015.1135431.

A phase II study of belinostat (PXD101) in relapsed and refractory aggressive B-Cell lymphomas: SWOG S0520

Soham Puvvada[1], **Hongli Li**[2], **Lisa M. Rimsza**[3], **Steven H. Bernstein**[4], **Richard I. Fisher**[5], **Michael LeBlanc**[2], **Monika Schmelz**[3], **Betty Glinsmann-Gibson**[3], **Thomas P. Miller**[1], **Anne-Marie Maddox**[6], **Jonathan W. Friedberg**[4], **Sonali M. Smith**[7], and **Daniel O. Persky**[1]

[1]University of Arizona Cancer Center, Tucson, AZ

[2]SWOG Statistical Center, Seattle, WA

[3]Department of Pathology, University of Arizona, Tucson, AZ

[4]Wilmot Cancer Center, University of Rochester, Rochester, NY

[5]Fox Chase Cancer Center – Temple Health, Philadelphia, PA

[6]University of Arkansas for Medical Sciences, Little Rock, AK

[7]University of Chicago, Chicago, IL

Abstract

Recent advances in diffuse large B-cell lymphomas (DLBCL) underscore the importance of tumor microenvironment in escaping host response. One mechanism is loss of major histocompatibility Class II antigens (MHCII) associated with decreased tumor infiltrating T lymphocytes (TIL) and poor survival. Transcription of MHCII is controlled by CIITA which in turn is regulated by histone acetylation. In this study, we hypothesized that HDAC inhibition with belinostat increases MHCII, CIITA expression, TIL and improves patient outcomes. Primary objective was evaluation of toxicity and response. Twenty-two patients were enrolled. Belinostat was well tolerated with mild toxicity. Two partial responses were observed at 5,13 months after registration for an overall response rate (ORR) of 10.5% (95 % CI 1.3-33.1%), and 3 patients had stable disease for 4.7, 42.3+, and 68.4+ months with minimum 3-year follow-up. Included correlative studies support the hypothesis and serve as the basis for SWOG S0806 combining Vorinostat with R-CHOP.

Keywords

Non-Hodgkin lymphoma; DLBCL; MHC Class II expression; CIITA; HDAC inhibition; SWOG

Corresponding author: Soham Puvvada, MD, University of Arizona Cancer Center, 1515 N Campbell Ave, Rm. 1969, Tucson, AZ 85724, Tel: 520-626-4331; Fax: 520-626-2225, sohampuvvada@email.arizona.edu.

Clinical Trials.gov Identifier: NCT00303953

Declaration of interest: No COI info to disclose: Drs. Bernstein, Li, Maddox, Smith, Leblanc, Miller, Rimsza.
Friedberg: personal fees and other from Eisai, other from Bayer outside of the submitted work
Persky: personal fees from Spectrum Pharmaceuticals, Inc outside of the submitted work
Fisher: other from Johnson & Johnson, other from MorphoSys AG, other from Celgene, outside of the submitted work

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma diagnosed in the United States with exceedingly poor outcomes in transplant ineligible relapsed refractory (R/R) DLBCL [1, 2]. Novel therapies that are well tolerated are an unmet need in R/R DLBCL.

An emerging theme in R/R DLBCL is the tumor's ability to escape immune surveillance; for example, anti-programmed cell death protein 1 (PD1) that inhibits the activity of tumor associated T cells [3]. A prior study demonstrated that the loss of Major Histocompatibility Complex class II (MHCII) expression in DLBCL was associated with a decreased CD8-positive (CD8+) tumor-infiltrating T-lymphocyte (TIL) response (as the final effector cells recruited by CD4+ T cells via MHCII antigen presentation), poor survival, regardless of International Prognostic Index (IPI) or histologic subtype [4]. Further investigations indicated that deletion of MHCII genes was infrequent in MHCII negative cases and altered transcription of the MHCII gene complex was likely under the control of the class II, major histocompatibility complex transactivator, CIITA [5]. CIITA is regulated by acetylation, and modifies H3, H4 histone acetylation at the HLA-DRA promoter in B-cell lines [6, 7]. CIITA is constitutively expressed in antigen presenting cells and controlled by promoters I, III, and IV [8]. Recruitment of histone acetyltransferases (HATs) and chromatin remodeling proteins activates MHCII transcription [6, 9, 10]. CIITA and MHCII can be induced by IFN-y treatment in most cell types [11]. In human cells, CIITA expression is regulated by four promoters referred to as pI, pII, pIII, and pIV, which are cell-type specific [12, 13]. Promoters pI and pIII regulate CIITA expression in myeloid and lymphoid cells, respectively. The CIITA pIV promoter is responsible for the constitutive expression of MHCII genes, and it is the major interferon in non- professional antigen presenting cells [14, 15]. The role of pII is still unknown. Belinostat (PXD101), a hydroxamate pan-histone deacetylase inhibitor (HDACI) active at low micromolar concentration, inhibits deacetylation of both H3 and H4, increasing their acetylation and potentially activating transcription of MHCII genes [16]. This theoretical increase in MHCII expression could reinstitute immune surveillance against lymphoma.

Epigenetic changes are also important in lymphomagenesis. EZH2, a subunit of the polycomb repressive complex 2 (PRC2) represses gene expression by methylating lysine 27 of histone 3. EZH2 mediated methylation is a potential independent mechanism for epigenetic silencing of tumor suppressor genes in cancer. The EZH2 histone methyltransferase usually cooperates with other epigenetic silencing enzymes. In resting naïve B cells EZH2 is suppressed, but is highly upregulated in primary lymphoid follicles during B cell activation and germinal center (GC) formation [17]. EZH2 is overexpressed in GC derived DLBCL [18].

Epigenetic silencing via hypermethylation of DNA and deacetylation of histones leads to loss of tumor suppressor genes. HDACI can reactivate genes associated with tumor suppression, cell cycle arrest, have anti-angiogenic properties, affect tumor microenvironment through changes in T-cell subsets and decrease pro-inflammatory cytokines [19-22]. HDACI effects may be also mediated by deacetylation of non-histone

proteins such as hsp90 and p53 [23]. In a recent study, genes with roles for histone modification were frequent targets for somatic mutations in DLBCL; mutations in histone acetyl transferases (HATs) were one of the most frequent mutations in DLBCL found in >30% of cases [24, 25].

In a phase I study, the MTD of belinostat was 1000 mg/m2 days 1-5 of a 21-day cycle [26]. Nausea, vomiting and fatigue were the main side effects; overall, the drug was well-tolerated, and demonstrated no significant hematologic toxicity [27]. Belinostat is now FDA approved for R/R peripheral T-cell lymphoma (PTCL) [28]. We, therefore, hypothesized that HDAC inhibition with belinostat would increase MHCII expression, CIITA expression, and TIL; this increase in immune surveillance would be correlated with improved clinical outcomes. We also aimed to test this hypothesis with correlates utilizing both cell lines and patient biopsies.

Materials and Methods

SWOG S0520 was a phase II open-label trial of belinostat in patients with R/R aggressive B-cell lymphoma. The primary objective of this study was to assess the probability of response and secondary objectives included toxicity, six-month progression free survival (PFS) and overall survival (OS). The study was approved by the institutional review boards of all participating sites. All patients provided written informed consent before enrollment.

Patient Eligibility

Patients with R/R B-cell lymphoma and aggressive histology of one of the following subtypes: DLBCL, transformed lymphoma, high-grade lymphoma, Burkitt or Burkitt-like lymphoma, or primary mediastinal B-cell lymphoma were eligible. Diagnosis was confirmed on central pathology review of relapsed specimen if available or the original biopsy. Patients registered at first relapse were ineligible for stem cell transplantation. Up-to five prior chemotherapy regimens were allowed, including standard salvage chemotherapy followed by autologous stem cell transplantation (ASCT). Prior lymphoma therapy and valproic acid (HDACI) had to be discontinued at least 2 weeks prior to registration.

Minimum age was 18 years, with performance status of 0, 1 or 2. Patients must have normal, cardiac, hepatic, renal function, and adequate hematologic function as defined by ANC count ≥ 1,500/μL, platelets ≥ 100,000/μL, and leukocytes ≥3,000/μL. Patients with uncontrolled hypertension, clinical evidence of other vascular disease, CNS involvement or HIV-positivity were ineligible.

Treatment

Belinostat was administered in an outpatient setting at 1000 mg/m^2 via 30-minute IV infusion, on days 1-5 of a 21-day cycle for up to 2 years or until disease progression. Dose reductions to 600 mg/m^2 and 300 mg/m^2 were permitted for toxicity. Toxicity was assessed using NCI CTCAE version 3.0. Patients were removed early from the protocol treatment for progressive disease, unacceptable toxicity, delay of treatment for more than three weeks, or patient preference. Initial staging included chest X-ray or chest CT, as well as CT abdomen-pelvis, within 28 days of registration. Bone marrow biopsy and EKG were performed within

42 days of registration. EKG was obtained on day 5 after belinostat infusion of each treatment cycle. Bone marrow biopsy was repeated only if initially positive. Disease assessment with laboratory and radiologic tests occurred after cycle 3 and then every 4 cycles until disease progression. International Working Group (Cheson) criteria were used for response assessment and definition of OS, while PFS was defined as the time from registration until lymphoma progression or death from any cause [29].

Patient Materials for Correlates

Pre-treatment biopsy materials were submitted for expert pathology review. A second core needle biopsy at Cycle 3 day 1-5 was requested for comparison to pre-treatment samples to assess histone acetylation, MHCII expression, CIITA expression, and TIL.

Additional Methods

Immunohistochemistry (IHC) [4, 30], photo microscopy [31], quantitative nuclease protection (qNPA) assays [32], CIITA expression by quantitative RT-PCR [33] and in vitro correlates [34] were all performed as previously described with additional details and modifications available in the supplemental methods, found on *Leukemia Lymphoma* Web site.

Statistics

The primary goal of the study was to assess probability of response. Secondary endpoints included toxicity, PFS and OS. It was assumed that this therapy would be of no further interest if the true response rate probability was 5% or less, and of interest if it was 20% or more.

A two-stage design was used for patient accrual. If at least one response was observed in the first 20 patients, then an additional 20 patients would be accrued to this study. 5 or more responses out of 40 would be considered evidence that this regimen was promising. This overall design had a power of 0.92 when the true response was 20%, and alpha of 0.05. If full accrual had been achieved the design allowed estimation of any particular toxicity to within +/- 16%.

PFS was defined as the time from study registration to the date of lymphoma progression or death due to any cause. Patients last known to be alive and progression-free were censored at the date of last contact. OS was defined as the time from study registration to the date of death or last follow-up. PFS and OS were estimated using Kaplan-Meier method [35].

An exploratory analysis was performed to investigate the relationship between clinical outcomes and baseline of CIITA and HLA-DR mRNA expression. HLA-DR and CD8+ lymphocyte IHC were statistically described. Cox proportional hazard models [36] were fit to assess the relationship between PFS and OS and CD8+ lymphocytes or HLA-DR staining pattern or HLA-DR score. Because of the limited sample size, the objective of this analysis was limited to generating hypotheses to be further investigated in larger cohorts, as there would only be sufficient power to detect very strong relationships.

Results

Patients—Twenty-two patients were registered to this study. Two patients were ineligible due to cardiac abnormalities and inconsistent histology. Another patient who died before receiving any protocol treatment is not analyzable for any endpoint and is excluded from all study tables. Nineteen patients were evaluable for adverse events. Baseline patient characteristics are listed in Table 1.

Safety and Toxicity—Overall toxicity was mild, with most being grades 1-2 (Table 2). There was no grade 5 toxicity. One patient experienced grade 4 lymphopenia; one patient experienced grade 4 myelodysplasia; another experienced grade 4 fatigue and muscle weakness. There was one grade 2 QTc prolongation. All patients have been removed from protocol treatment, 13 due to disease progression. Two patients completed two years of treatment; 2 patients were removed due to toxicities, including nausea, fatigue, and pancytopenia; one patient was removed due to patient's refusal; one patient was removed for non-protocol specified reasons. Median number of treatment cycles was 3 (range 1-31), with 13 (68%) of patients receiving 1-3 cycles.

Efficacy—S0520 was closed as of January 15, 2009, having failed to meet the response requirement for second stage of accrual. Out of the 19 eligible and evaluable patients accrued, 2 PRs were observed at 5 and 13 months after starting protocol treatment for an ORR of (95% CI) of 10.5% (1.3%-33.1%). Incremental change and delayed timing of responses made them hard to detect during disease assessments which were done after cycle 3 and then every 4 cycles until disease progression. The duration of responses was 50.9 and 11.7 months respectively. Furthermore, 3 patients had stable disease, lasting 4.7, 42.3+, and 68.4+ months (with the last two patients having disease stability at the time of censoring). With a minimum follow-up of 3 years on all patients last known alive, median and 6 month PFS are 2.1 months (95% CI: 1.3-3.8) and 21.1% (95% CI: 6.6%-41.0%), respectively (Figure 1A). Median and six-month OS are 13.4 months (95% CI: 2.3-53.8) and 57.9% (95% CI: 33.2% 76.3%), respectively (Figure 1B).

Correlatives

The objectives were to assay the effects of HDAC inhibition (HDACi) on MHCII protein (HLA-DR) expression and on the CD8 infiltration status by IHC in paired pre- and on-treatment tumor samples, and to measure CIITA and HLA-DR mRNA expression using quantitative RT-PCR in DLBCL cell lines.

Patient samples—Ten eligible patients had pre-treatment biopsies with sufficient tissue for IHC and RT-PCR correlates, with 2 of the patients having follow-up fresh frozen core needle biopsies. Results of HLA-DR and CD8+ lymphocyte IHC are listed in Table 3. There was significant heterogeneity in HLA-DR positivity with variable, surface and punctate patterns of expression (Figure 2A-2C); an aberrant cytoplasmic HLA-DR expression pattern in the absence of cell surface expression was observed in 47% of samples in a globular or punctate vesicular pattern in a perinuclear position (Figure 2D). There were no statistically significant effects of CD8+ lymphocytes or staining pattern (surface vs. no surface staining)

on PFS or OS when analyzed by increment of one unit or above/below median. Since our previous work demonstrated that near loss of HLA-DR staining was prognostically unfavorable, HLA-DR score was analyzed at breakpoint of 25th percentile, which showed statistically significant difference (permutation p=0.01) in PFS in favor of higher HLA-DR score, but no difference in OS [4].

There were only 2 patients with pre-treatment and on-treatment biopsies available, making the analysis of results from quantitative Nuclease Protection Assay (qNPA) descriptive, but still instructive. Matched pre- and on-treatment biopsies of patient 1 who responded to therapy showed that percent of malignant cells staining for HLA-DR increased from 75 to 100%, while staining intensity and pattern (surface and punctate cytoplasmic) remained unchanged (Figure 3A,C). Likewise, proportion of CD8+ lymphocytes increased from 2.3% to 10.9% with appearance of dense CD8+ infiltrate of the tumor (Figure 3B,D). Analysis by qNPA showed a 2.5 fold increase in mRNA levels of HLA-DR alpha gene and a 1.8 fold increase in HLA-DR beta gene expression (Figure 3E). HLA-DP gene expression increased by 1.4 fold, while not much change was observed in the level of HLA-DQ mRNA expression (Figure 3E). Quantitative RT-PCR showed up regulation of CIITA promoter regions I (3.5 fold), III (1.8 fold) and IV (1.1 fold); (Figure 3F-H). Conversely, comparison of HLA-DR and CD8 expression levels in matched pre- and on-treatment biopsies of patient 2 who did not respond to therapy demonstrated that HLA-DR staining percentage decreased to 50% tumor cells, staining intensity decreased from 3+ to 2+, while HLA-DR staining pattern remained unchanged as punctate cytoplasmic with absence of surface staining (Figure 4A, C). Proportion of CD8+ lymphocytes decreased from 14.2 % (Figure 4B) to 7.5% (Figure 4D). Analysis by qNPA showed decreased level of mRNA transcripts from HLA-DR genes (Figure 4E). Quantitative RT-PCR showed down-regulation of CIITA promoter regions I, III and IV (Figure 4F-H) in the on-treatment biopsy as well. In order to assess CIITA baseline levels, the CIITA mRNA expression levels of 10 patients were compared in pre-treatment specimens (Figure 5). This revealed that the pre-treatment CIITA expression levels in the responding patient were among the highest (Figure 5, patient 10). Interestingly, the non-responding patient showed the lowest pre-treatment expression levels of CIITA as compared to other patients (Figure 5, patient 6).

In vitro correlates—To study in vitro effects, we chose OCI-Ly 3 and DB DLBCL cell lines. OCI-Ly 3 has reduced MHCII surface expression, while DB is mostly MHCII negative. Cells were incubated in concentrations ranging from 0.2 μM to 20 μM for 1 hour. Drug effect was examined from 1- 24 hours after incubation with the drug. Although changes in marker intensity levels were statistically not significant, a trend was observed. In OcI-Ly3, increase in acetylation of histone 3 (86%), histone 4 (106%), MHCI (46%) and MHCII (65%) was observed at a dose of 0.2 μM after 24 hours post-treatment as compared to untreated control (Supplemental figure 1). In DB cells, a peak of acetylated histone 4 increase was observed after 4 hours post-treatment at 0.02μM. No increase in acetylated histone 3 and MHCII was observed. (Supplemental Figure 2).

Discussion

S0520 was conducted based on the hypothesis that by increasing MHCII expression, HDACi may improve the outcome of patients with DLBCL who have unfavorable features of low MHCII expression. Despite preclinical and early clinical rationale, the study was terminated at the first stage due to inadequate response at first response assessment. Interestingly, 2 late PRs were identified, occurring at 5 and 13 months after treatment initiation. Furthermore, there were 3 patients with stable disease (SD), 2 of which had ongoing stable disease at the time of censoring, lasting 42.3 and 68.4 months. These were elderly patients not eligible for salvage therapy and consolidative ASCT. Despite a short median PFS of 2.1 months, median OS was much longer at 13.4 months. This may have resulted from successful rescue treatment, however, a possibility of HDACI-mediated disease stabilization or sensitization to subsequent therapy cannot be excluded. It is noteworthy that, among our two available post-treatment samples, the responding patient had increased HLA-DR mRNA levels, CIITA, and increased percentage of CD8+ TIL; while the non-responder who relapsed, died despite subsequent aggressive treatment had decreased HLA-DR, CIITA, and TIL. Other T-cell subtypes including PD1/PDL1 expression were not analyzed mainly due to limited availability of tissue. Although PDL1 expression is a promising biomarker in cancer immunotherapy and is markedly upregulated in tumor-infiltrating CD8-positive T cells in Hodgkin's lymphoma, melanoma, hepatocellular carcinoma and gastric cancer [37-40], there still are unsolved problems for its use as a predictive biomarker by immunohistochemistry (IHC): variability in detection between antibodies, differing IHC cutoff, variability in tissue processing, and staining of tumor versus cells involved in immunosurveillance [41].

In both patients, responding and non-responding, the pretreatment tissue shows a higher amount of HLA-DRA mRNA than HLA-DRB. In the on-treatment tissue of the responding patient, both HLA-DRA and HLA-DRB mRNA levels increase 2.5 fold and 1.8 fold, respectively. Accordingly, in the on-treatment tissue of the non-responding patient the mRNA levels decreased by 5.7 fold for HLA-DRA and 5.8 fold for HLA-DRB. The amount of increase in both HLA-DRA and HLA-DRB gene activity is sufficient to increase the amount of functional HLA-DR heterodimer proteins. However, the amount of protein expressed cannot be deduced by measuring RNA concentration. Translation, mRNA decay and protein life time, turn over and degradation determine steady state protein concentrations [42]. The downregulation of CIITA and with it MHCII in the non-responding patient could be due to epigenetic silencing by methylation of CIITA. EZH2 may play a role since, recent studies have shown that there are links between EZH2, DNA methyltransferases [43] and histone acetylases [44, 45].

Trials of HDAC inhibition have shown modest ORRs but a variable duration of response; a phase I trial of belinostat noted 2/9 patients with DLBCL having SD including one case with Richter's transformation after 9 cycles [27]. Another phase I trial of vorinostat (the 1st FDA-approved HDACI) had 12 patients with DLBCL, including 7 who received oral vorinostat, 3 of whom had responses. One patient with DLBCL had PR for 4 months, while both patients with transformed DLBCL responded: one had a CR and one had PR, lasting over 12 and over 5 months, respectively [46]. Another phase II trial of vorinostat in 18 patients with R/R DLBCL showed one CR lasting over 15 months, with time to response of 2.8 months, and

one SD lasting 9.9 months [47]. Mocetinostat was associated with 1 CR and 5 PRs in 41-patient cohort of a phase II trial [48]. These observations coupled with the findings in our study suggest a subset of patients may experience significant benefit from HDAC inhibition.

Ongoing studies are evaluating the benefit of HDACi in combination with novel targeted agents. Preclinical data have shown synergy between HDACI and proteasomal inhibition via apoptosis in DLBCL [49]. This was tested in a phase I trial of carfilzomib in combination with vorinostat in R/R DLBCL [50]. The combination was well tolerated and 2/20 patients had stable disease. Examples of other combinations under investigation include bortezomib, rituximab, and lenalidomide [51-53].

This underscores the role of the microenvironment in DLBCL. Prior studies have shown prognostically favorable “stromal-1” gene expression signature that reflected extracellular matrix deposition, histiocytic infiltration and an unfavorable “stromal-2” signature reflecting tumor blood vessel density in the microenvironment of DLBCL patients [54]. A “host response” subset in DLBCL has been previously characterized that contained higher number of CD2+/CD3+ TIL [55]. This complex interplay between the microenvironment and lymphoma cells may escape or dampen host anti-tumor responses. Along with reduced MHCII expression, other mechanisms of immune evasion include binding of PD1 on CD4 + T lymphocytes by its ligands PDL1, PDL2 expressed on lymphoma cells leading to T cell exhaustion. Disabling immune tolerance by PD1 blockade is a promising therapeutic strategy in R/R DLBCL [56].

Lastly, the results of correlative studies are hypotheses generating; a higher proportion of patients (47%) had evidence of punctate cytoplasmic HLA-DR staining pattern than what is seen in newly diagnosed DLBCL. In our prior study punctate staining cases were grouped together with those with complete lack of staining and together correlated with inferior outcome [4, 30]. A similar aberrant MHCII expression pattern was also documented in Hodgkin lymphoma, which when grouped with true MHC-negative cases correlated with reduced survival [57]. Since classic MHCII molecules pass through subcellular organelles in their biosynthetic pathway, it is possible that cytoplasmic punctate staining pattern reflects a defect in MHCII processing and trafficking resulting in lack of cell surface expression; lack of MHCII surface expression equals lack of MHCII antigen presentation with the functional consequence of impaired immunosurveillance.

Based on these promising results, we have successfully completed accrual of SWOG S0806 which tests the combination of R-CHOP and vorinostat in newly diagnosed DLBCL. In this study we plan to validate that HDAC inhibition can successfully increase MHCII expression and CIITA levels, provocatively reinstituting immune response that correlates with improved patient outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This investigation was supported in part by National Institutes of Health (NIH)/National Cancer Institute (NCI)/ National Clinical Trials Network (NCTN) grants CA180888, CA180819; and NIH/NCI Community Oncology Research Program (NCORP) grants CA189957, CA189858, CA189872, CA189854, CA189953, CA189817, CA189830. We would also like to specially acknowledge Ruth Heaton for technical assistance in developing and running the RT-PCR assays.

Glinsmann-Gibson: grants from CTEP, grants from Spectrum Pharmaceuticals during the conduct of the study

Schmelz: grants from Spectrum Pharmaceuticals during the conduct of the study.

Puvvada: Institutional research funding from Spectrum Pharmaceuticals outside of the submitted work and after conduct of the study. Institutional research funding from Abbvie and Genentech outside of the submitted work. Consultant and advisory board member to Genentech and Abbvie. Travel funding from Genentech for an investigator's meeting outside of the submitted work.

References

1. Armitage JO, Weisenburger DD. New approach to classifying non-Hodgkin's lymphomas: clinical features of the major histologic subtypes. Non-Hodgkin's Lymphoma Classification Project. J Clin Oncol. Aug.1998 16:2780–95. [PubMed: 9704731]
2. Gisselbrecht C, Glass B, Mounier N, Singh Gill D, Linch DC, Trneny M, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. J Clin Oncol. Sep 20.2010 28:4184–90. [PubMed: 20660832]
3. Andorsky DJ, Yamada RE, Said J, Pinkus GS, Betting DJ, Timmerman JM. Programmed death ligand 1 is expressed by non-hodgkin lymphomas and inhibits the activity of tumor-associated T cells. Clin Cancer Res. Jul 1.2011 17:4232–44. [PubMed: 21540239]
4. Rimsza LM, Roberts RA, Miller TP, Unger JM, LeBlanc M, Braziel RM, et al. Loss of MHC class II gene and protein expression in diffuse large B-cell lymphoma is related to decreased tumor immunosurveillance and poor patient survival regardless of other prognostic factors: a follow-up study from the Leukemia and Lymphoma Molecular Profiling Project. Blood. Jun 1.2004 103:4251–8. [PubMed: 14976040]
5. Rimsza LM, Roberts RA, Campo E, Grogan TM, Bea S, Salaverria I, et al. Loss of major histocompatibility class II expression in non-immune-privileged site diffuse large B-cell lymphoma is highly coordinated and not due to chromosomal deletions. Blood. Feb 1.2006 107:1101–7. [PubMed: 16239429]
6. Beresford GW, Boss JM. CIITA coordinates multiple histone acetylation modifications at the HLA-DRA promoter. Nat Immunol. Jul.2001 2:652–7. [PubMed: 11429551]
7. Lee SC, Bottaro A, Insel RA. Activation of terminal B cell differentiation by inhibition of histone deacetylation. Mol Immunol. May.2003 39:923–32. [PubMed: 12695118]
8. Muhlethaler-Mottet A, Otten LA, Steimle V, Mach B. Expression of MHC class II molecules in different cellular and functional compartments is controlled by differential usage of multiple promoters of the transactivator CIITA. EMBO J. May 15.1997 16:2851–60. [PubMed: 9184229]
9. Boss JM, Jensen PE. Transcriptional regulation of the MHC class II antigen presentation pathway. Curr Opin Immunol. Feb.2003 15:105–11. [PubMed: 12495741]
10. Masternak K, Muhlethaler-Mottet A, Villard J, Zufferey M, Steimle V, Reith W. CIITA is a transcriptional coactivator that is recruited to MHC class II promoters by multiple synergistic interactions with an enhanceosome complex. Genes Dev. May 1.2000 14:1156–66. [PubMed: 10809673]
11. Steimle V, Siegrist CA, Mottet A, Lisowska-Grospierre B, Mach B. Regulation of MHC class II expression by interferon-gamma mediated by the transactivator gene CIITA. Science. Jul 1.1994 265:106–9. [PubMed: 8016643]
12. LeibundGut-Landmann S, Waldburger JM, Krawczyk M, Otten LA, Suter T, Fontana A, et al. Mini-review: Specificity and expression of CIITA, the master regulator of MHC class II genes. Eur J Immunol. Jun.2004 34:1513–25. [PubMed: 15162420]

13. Wright KL, Ting JP. Epigenetic regulation of MHC-II and CIITA genes. Trends Immunol. Sep. 2006 27:405–12. [PubMed: 16870508]
14. LeibundGut-Landmann S, Waldburger JM, Reis e Sousa C, Acha-Orbea H, Reith W. MHC class II expression is differentially regulated in plasmacytoid and conventional dendritic cells. Nat Immunol. Sep.2004 5:899–908. [PubMed: 15322541]
15. Waldburger JM, Suter T, Fontana A, Acha-Orbea H, Reith W. Selective abrogation of major histocompatibility complex class II expression on extrahematopoietic cells in mice lacking promoter IV of the class II transactivator gene. J Exp Med. Aug 20.2001 194:393–406. [PubMed: 11514597]
16. Plumb JA, Finn PW, Williams RJ, Bandara MJ, Romero MR, Watkins CJ, et al. Pharmacodynamic response and inhibition of growth of human tumor xenografts by the novel histone deacetylase inhibitor PXD101. Mol Cancer Ther. Aug.2003 2:721–8. [PubMed: 12939461]
17. Velichutina I, Shaknovich R, Geng H, Johnson NA, Gascoyne RD, Melnick AM, et al. EZH2-mediated epigenetic silencing in germinal center B cells contributes to proliferation and lymphomagenesis. Blood. Dec 9.2010 116:5247–55. [PubMed: 20736451]
18. van Kemenade FJ, Raaphorst FM, Blokzijl T, Fieret E, Hamer KM, Satijn DP, et al. Coexpression of BMI-1 and EZH2 polycomb-group proteins is associated with cycling cells and degree of malignancy in B-cell non-Hodgkin lymphoma. Blood. Jun 15.2001 97:3896–901. [PubMed: 11389032]
19. Li N, Zhao D, Kirschbaum M, Zhang C, Lin CL, Todorov I, et al. HDAC inhibitor reduces cytokine storm and facilitates induction of chimerism that reverses lupus in anti-CD3 conditioning regimen. Proc Natl Acad Sci U S A. Mar 25.2008 105:4796–801. [PubMed: 18347343]
20. Qian DZ, Kato Y, Shabbeer S, Wei Y, Verheul HM, Salumbides B, et al. Targeting tumor angiogenesis with histone deacetylase inhibitors: the hydroxamic acid derivative LBH589. Clin Cancer Res. Jan 15.2006 12:634–42. [PubMed: 16428510]
21. Richon VM, Sandhoff TW, Rifkind RA, Marks PA. Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. Proc Natl Acad Sci U S A. Aug 29.2000 97:10014–9. [PubMed: 10954755]
22. Tao R, de Zoeten EF, Ozkaynak E, Chen C, Wang L, Porrett PM, et al. Deacetylase inhibition promotes the generation and function of regulatory T cells. Nat Med. Nov.2007 13:1299–307. [PubMed: 17922010]
23. Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene. Aug 13.2007 26:5541–52. [PubMed: 17694093]
24. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature. Aug 18.2011 476:298–303. [PubMed: 21796119]
25. Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. Nature. Mar 10.2011 471:189–95. [PubMed: 21390126]
26. Steele NL, Plumb JA, Vidal L, Tjornelund J, Knoblauch P, Rasmussen A, et al. A phase 1 pharmacokinetic and pharmacodynamic study of the histone deacetylase inhibitor belinostat in patients with advanced solid tumors. Clin Cancer Res. Feb 1.2008 14:804–10. [PubMed: 18245542]
27. Gimsing P, Hansen M, Knudsen LM, Knoblauch P, Christensen IJ, Ooi CE, et al. A phase I clinical trial of the histone deacetylase inhibitor belinostat in patients with advanced hematological neoplasia. Eur J Haematol. Sep.2008 81:170–6. [PubMed: 18510700]
28. Lee HZ, Kwitkowski VE, Del Valle PL, Ricci MS, Saber H, Habtemariam BA, et al. FDA Approval: Belinostat for the Treatment of Patients with Relapsed or Refractory Peripheral T-cell Lymphoma. Clin Cancer Res. Jun 15.2015 21:2666–70. [PubMed: 25802282]
29. Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol. Apr.1999 17:1244. [PubMed: 10561185]
30. Wilkinson ST, Vanpatten KA, Fernandez DR, Brunhoeber P, Garsha KE, Glinsmann-Gibson BJ, et al. Partial plasma cell differentiation as a mechanism of lost major histocompatibility complex

class II expression in diffuse large B-cell lymphoma. Blood. Feb 9.2012 119:1459–67. [PubMed: 22167754]

31. Schmelz M, Montes-Moreno S, Piris M, Wilkinson ST, Rimsza LM. Lack and/or aberrant localization of major histocompatibility class II (MHCII) protein in plasmablastic lymphoma. Haematologica. Oct.2012 97:1614–6. [PubMed: 22689685]
32. Roberts RA, Sabalos CM, LeBlanc ML, Martel RR, Frutiger YM, Unger JM, et al. Quantitative nuclease protection assay in paraffin-embedded tissue replicates prognostic microarray gene expression in diffuse large-B-cell lymphoma. Lab Invest. Oct.2007 87:979–97. [PubMed: 17700562]
33. Lossos IS, Czerwinski DK, Wechser MA, Levy R. Optimization of quantitative real-time RT-PCR parameters for the study of lymphoid malignancies. Leukemia. Apr.2003 17:789–95. [PubMed: 12682639]
34. Chang H, Blondal JA, Benchimol S, Minden MD, Messner HA. p53 mutations, c-myc and bcl-2 rearrangements in human non-Hodgkin's lymphoma cell lines. Leuk Lymphoma. Sep.1995 19:165–71. [PubMed: 8574164]
35. Kaplan, EaM; P. Nonparametric estimation from incomplete observations. Journal of the American Statistical association. 1958; 53:457–481.
36. Cox DR. Regression models and life tables (with discussion). J Roy Statist Soc Serv. 1972; 34:187–220.
37. Ahmadzadeh M, Johnson LA, Heemskerk B, Wunderlich JR, Dudley ME, White DE, et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood. Aug 20.2009 114:1537–44. [PubMed: 19423728]
38. Fourcade J, Kudela P, Sun Z, Shen H, Land SR, Lenzner D, et al. PD-1 is a regulator of NY-ESO-1-specific CD8+ T cell expansion in melanoma patients. J Immunol. May 1.2009 182:5240–9. [PubMed: 19380770]
39. Gehring AJ, Ho ZZ, Tan AT, Aung MO, Lee KH, Tan KC, et al. Profile of tumor antigen-specific CD8 T cells in patients with hepatitis B virus-related hepatocellular carcinoma. Gastroenterology. Aug.2009 137:682–90. [PubMed: 19394336]
40. Yamamoto R, Nishikori M, Kitawaki T, Sakai T, Hishizawa M, Tashima M, et al. PD-1-PD-1 ligand interaction contributes to immunosuppressive microenvironment of Hodgkin lymphoma. Blood. Mar 15.2008 111:3220–4. [PubMed: 18203952]
41. Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. Mol Cancer Ther. Apr.2015 14:847–56. [PubMed: 25695955]
42. Vogel C, Abreu Rde S, Ko D, Le SY, Shapiro BA, Burns SC, et al. Sequence signatures and mRNA concentration can explain two-thirds of protein abundance variation in a human cell line. Mol Syst Biol. Aug 24.2010 6:400. [PubMed: 20739923]
43. Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, et al. The Polycomb group protein EZH2 directly controls DNA methylation. Nature. Feb 16.2006 439:871–4. [PubMed: 16357870]
44. Tie F, Furuyama T, Prasad-Sinha J, Jane E, Harte PJ. The Drosophila Polycomb Group proteins ESC and E(Z) are present in a complex containing the histone-binding protein p55 and the histone deacetylase RPD3. Development. Jan.2001 128:275–86. [PubMed: 11124122]
45. van der Vlag J, Otte AP. Transcriptional repression mediated by the human polycomb-group protein EED involves histone deacetylation. Nat Genet. Dec.1999 23:474–8. [PubMed: 10581039]
46. O'Connor OA, Heaney ML, Schwartz L, Richardson S, Willim R, MacGregor-Cortelli B, et al. Clinical experience with intravenous and oral formulations of the novel histone deacetylase inhibitor suberoylanilide hydroxamic acid in patients with advanced hematologic malignancies. J Clin Oncol. Jan 1.2006 24:166–73. [PubMed: 16330674]
47. Crump M, Coiffier B, Jacobsen ED, Sun L, Ricker JL, Xie H, et al. Phase II trial of oral vorinostat (suberoylanilide hydroxamic acid) in relapsed diffuse large-B-cell lymphoma. Ann Oncol. May. 2008 19:964–9. [PubMed: 18296419]
48. Boumber Y, Younes A, Garcia-Manero G. Mocetinostat (MGCD0103): a review of an isotype-specific histone deacetylase inhibitor. Expert Opin Investig Drugs. Jun.2011 20:823–9.

49. Dasmahapatra G, Lembersky D, Kramer L, Fisher RI, Friedberg J, Dent P, et al. The pan-HDAC inhibitor vorinostat potentiates the activity of the proteasome inhibitor carfilzomib in human DLBCL cells in vitro and in vivo. Blood. Jun 3.2010 115:4478–87. [PubMed: 20233973]
50. Holkova B, et al. Phase I trial of Carfilzomib in combination with vorinostat (SAH) in patients with relapsed/refractory B cell lymphomas. Blood. 2013; 122:4375.
51. Barnes JA, et al. Panobinostat in Combination with Rituximab in Heavily Pretreated Diffuse Large B-Cell Lymphoma: Results of a Phase II Study. Blood. 2014; 124:3055.
52. ClinicalTrials.gov. A Phase II Trial of Panobinostat and Lenalidomide in Patients With Relapsed or Refractory Hodgkin's Lymphoma. [Last accessed 05-25-15] ed, NCT01460940.
53. Kikuchi J, Wada T, Shimizu R, Izumi T, Akutsu M, Mitsunaga K, et al. Histone deacetylases are critical targets of bortezomib-induced cytotoxicity in multiple myeloma. Blood. Jul 22.2010 116:406–17. [PubMed: 20351311]
54. Lenz G, Wright G, Dave SS, Xiao W, Powell J, Zhao H, et al. Stromal gene signatures in large-B-cell lymphomas. N Engl J Med. Nov 27.2008 359:2313–23. [PubMed: 19038878]
55. Monti S, Savage KJ, Kutok JL, Feuerhake F, Kurtin P, Mihm M, et al. Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response. Blood. Mar 1.2005 105:1851–61. [PubMed: 15550490]
56. Armand P, Nagler A, Weller EA, Devine SM, Avigan DE, Chen YB, et al. Disabling immune tolerance by programmed death-1 blockade with pidilizumab after autologous hematopoietic stem-cell transplantation for diffuse large B-cell lymphoma: results of an international phase II trial. J Clin Oncol. Nov 20.2013 31:4199–206. [PubMed: 24127452]
57. Diepstra A, van Imhoff GW, Karim-Kos HE, van den Berg A, te Meerman GJ, Niens M, et al. HLA class II expression by Hodgkin Reed-Sternberg cells is an independent prognostic factor in classical Hodgkin's lymphoma. J Clin Oncol. Jul 20.2007 25:3101–8. [PubMed: 17536082]

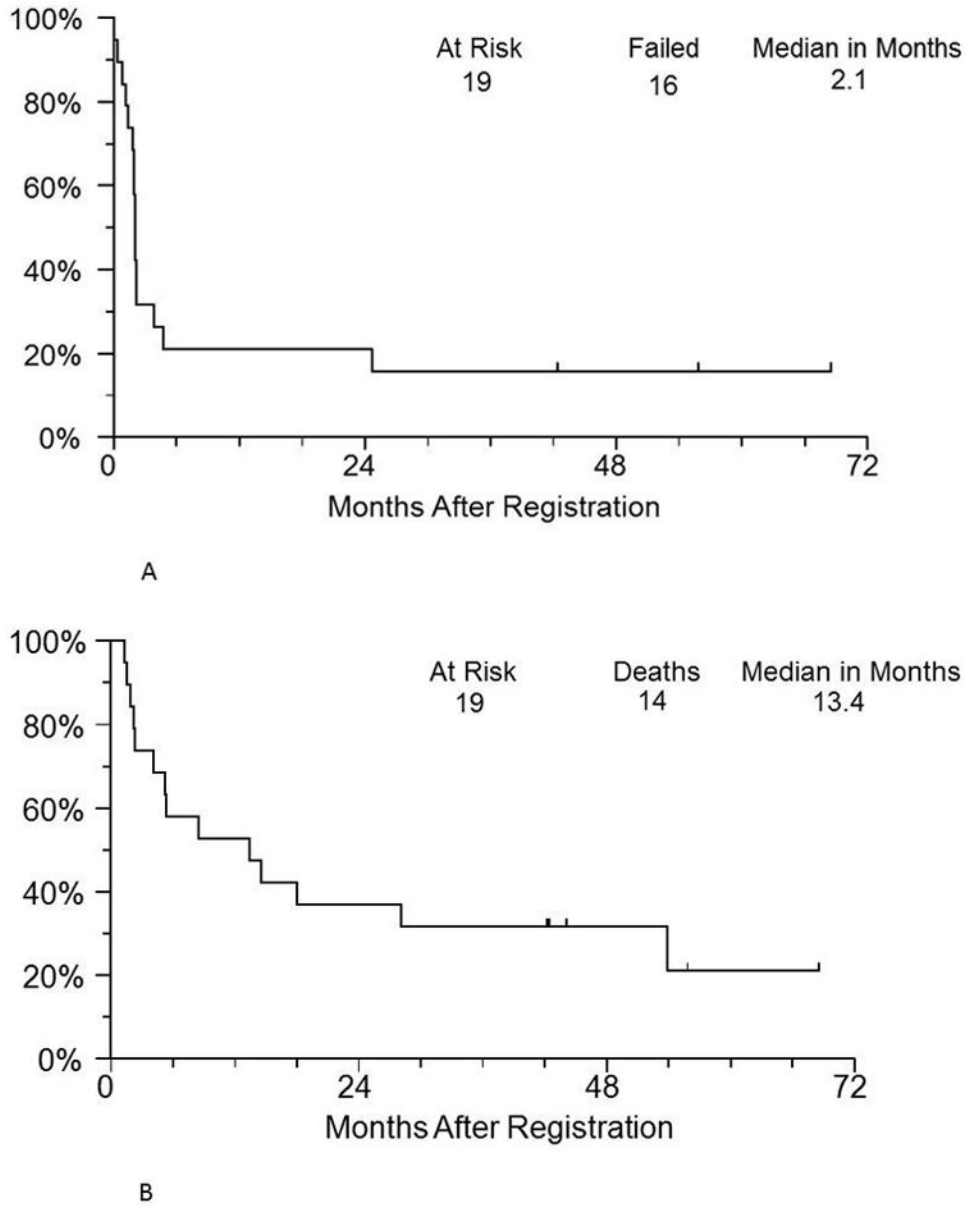


Figure 1.

a: Progression free survival of patients in S0520.

b: Overall survival of patients in S0520.

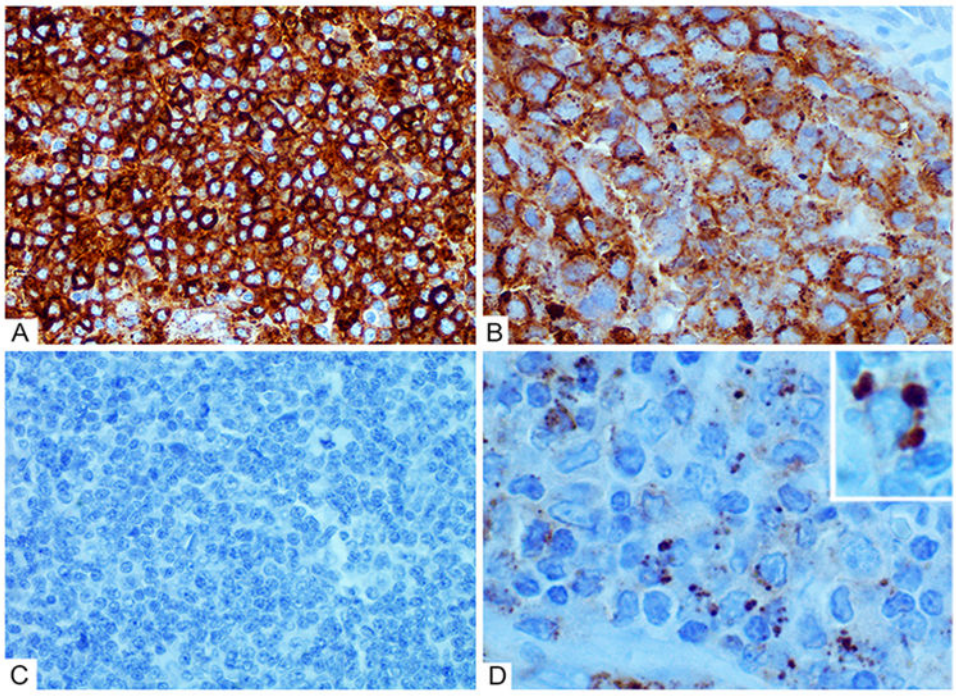


Figure 2.

MHCII expression patterns in DLBCL patients shown by immunohistochemistry using the mAB specific for HLA-DR (clone LN3).

(A) Case exhibiting normal MHCII cell surface expression in 100% of tumor cells (intensity 3+).

(B) Shows a representative area for aberrant cytoplasmic MHCII expression. In this case 100% of the tumor cells are positive for MHCII (intensity 2+) with a mixed expression pattern (either cell surface or aberrant cytoplasmic expression). Note the aberrant cytoplasmic expression pattern in the center of the image, while tumor cells in the periphery express MHCII on the cell surface.

(C) Shows a MHCII-negative case.

(D) Shows a representative area with 50% of the tumor cells expressing aberrant cytoplasmic MHCII (intensity 2+). The insert in (D) shows a tumor cell of the same case with aberrant MHCII expression at higher magnification appearing as globular or punctate-vesicular pattern in a perinuclear position.

Magnification A, C (×400); B, D (×600); D-insert (×800).

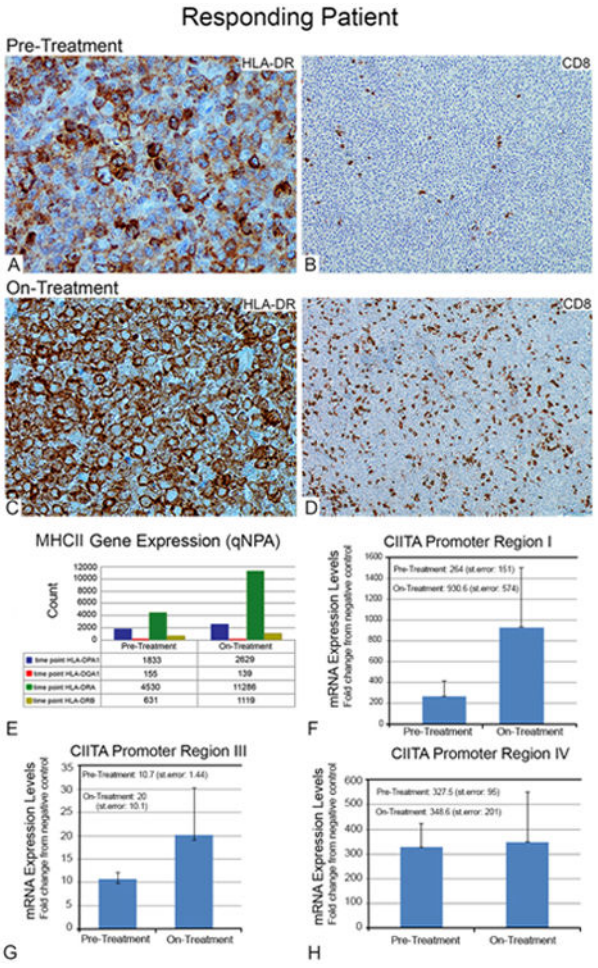


Figure 3.

Analysis of specimens in one responding patient diagnosed with GBC-type DLBCL obtained prior to treatment and after 43 days of treatment.

(A) and (B) are matching fields in sequential sections of pre-treatment tumor showing HLA-DR expression (A) and the amount of CD8 positive T cells present (B).

(C) and (D) are matching fields in sequential sections of on-treatment tumor showing HLA-DR expression (C) and the amount of CD8 positive T cells present (D).

(E) MHCII gene expression analysis in pre-treatment tumor compared to post-treatment tumor by qNPA

(F-H) CIITA mRNA expression levels in pre-treatment and on- treatment tissue. CIITA expression levels were normalized to GAPDH levels.

Magnifications: A, C (×600), B, D (×400).

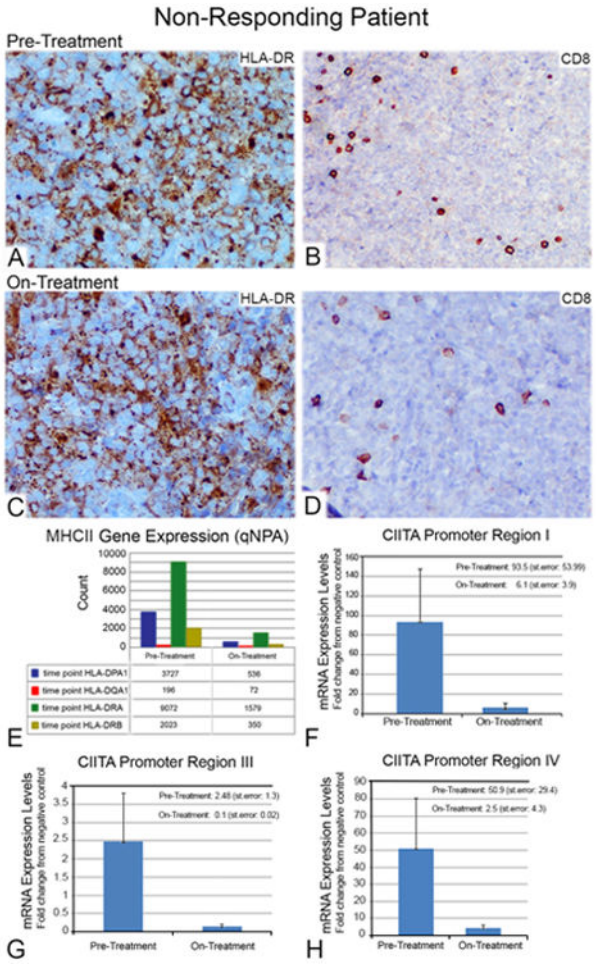


Figure 4.

Analysis of specimens in one non-responding patient diagnosed with non-GBC-type DLBCL obtained prior to and after 43 days of treatment.

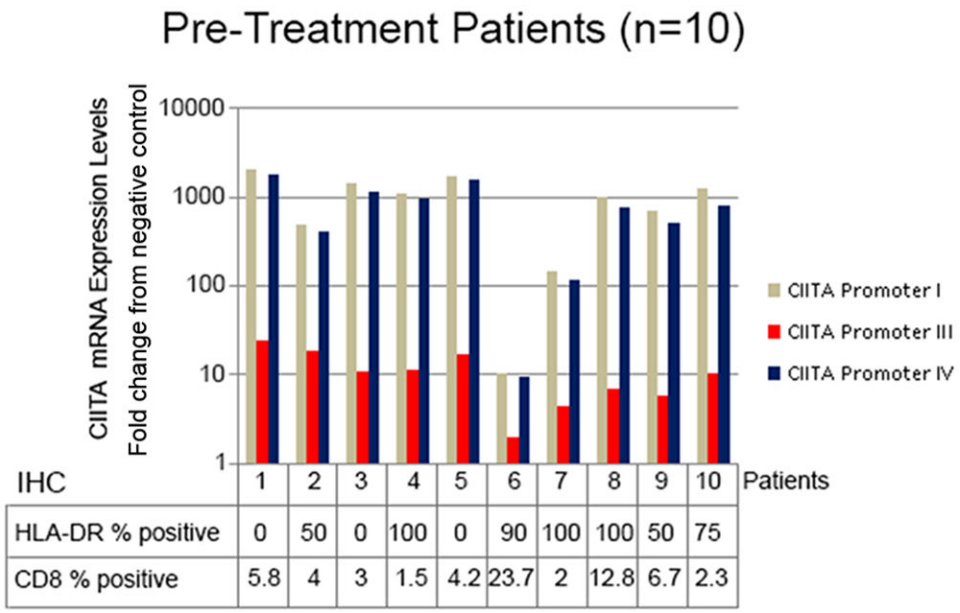
(A) and (B) are matching fields in sequential sections of pre-treatment tumor showing HLA-DR expression (A) and the amount of CD8 positive T cells present (B).

(C) and (D) are matching fields in sequential sections of on-treatment tumor showing HLA-DR expression (C) and the amount of CD8 positive T cells present (D).

(E) MHCII gene expression analysis in pre-treatment tumor compared to on-treatment tumor by qNPA

(F-H) CIITA mRNA expression levels in pre-treatment and post – treatment tissue. CIITA expression levels were normalized to GAPDH.

Magnifications: A-D (×400)



IHC	1	2	3	4	5	6	7	8	9	10	Patients
HLA-DR % positive	0	50	0	100	0	90	100	100	50	75	
CD8 % positive	5.8	4	3	1.5	4.2	23.7	2	12.8	6.7	2.3	

Figure 5.

CIITA mRNA expression levels by RT-PCR were correlated with HLA-DR expression and intensity levels with presence of CD8 T-cells by immunohistochemistry (IHC) in specimens of DLBCL patients obtained prior to treatment. No correlations were observed with CIITA mRNA expression levels by RT-PCR and HLA-DR/CD8 expression levels by IHC. CIITA mRNA expression levels were normalized to GADPH. This cohort includes one responding patient (patient 10), one non-responding patient (patient 6), and one with stable disease (patient 2). All others progressed.

Table 1

Patient characteristics on S0520

Characteristic	Patients (n=19)
Age, median (range)	69 years (52-83)
Male, number (%)	10 (53)
Histology, number (%)	
Diffuse large B-cell lymphoma	18 (95)
B-cell lymphoma, unclassifiable	1 (5)
Elevated LDH, number (%)	12 (63)
Stage at diagnosis, number (%)	
I	1 (5)
II	5 (26)
III	4 (21)
IV	9 (47)
Performance status	
0	12 (63)
1	5 (26)
2	2 (11)
IPI	
0-1	0
2	8 (42)
3	6 (32)
4-5	1 (5)
Missing	4 (21)
Median number of prior chemotherapy (range)	3 (1-4)
Refractory to prior treatment, number (%)	5 (26)
Prior autologous transplant, number (%)	4 (21)

Table 2

Treatment-related toxicity on S0520

Adverse Event (treatment-related), n=19	All grades	Grade 3	Grade 4
Constitutional			
Fatigue	8	0	1
Fever	1	0	0
Hematologic			
Neutropenia	1	1	0
Lymphopenia	2	1	1
Anemia	8	0	0
Thrombocytopenia	5	1	0
Myelodysplasia	1	0	1
Gastrointestinal			
Anorexia	3	1	0
Nausea	8	1	0
Vomiting	5	0	0
Diarrhea	4	1	0
Constipation	2	0	0
Dehydration	1	1	0
Infection (UTI)	2	0	0
Prolonged QTc	1	0	0
Muscle weakness: whole body	1	0	1
Maximum Grade Any Adverse Event	18	3	3

Table 3
Results of immunohistochemical stains for HLA-DR and CD8 (n=15)

Stain characteristic	Number (%)
HLA-DR, % positive	
0%	4 (27)
<5%	1 (7)
50-74%	3 (20)
75-100%	7 (47)
HLA-DR, intensity	
0	4 (27)
1	4 (27)
2	3 (20)
3	4 (27)
HLA-DR pattern	
No stain	4 (27)
Punctate	4 (27)
Surface + punctate	3 (20)
Surface	4 (27)
HLA-DR Score	
0	4 (27)
1.5	1 (7)
4	3 (20)
6	3 (20)
7	4 (27)
CD8+, percent (range)	4.2 (1.50-14.2)