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REVIEW

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Brentuximab vedotin resistance in classic Hodgkin's lymphoma and its therapeutic strategies: a review

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Abstract

Background Bone marrow cancer has been at the forefront of cancer research. The propensity of cancers to extravasate to the bone makes it a very relevant topic in the topology of this heterogeneous disease. Our narrative review article addresses Brentuximab vedotin (BV) resistance in classic Hodgkin's lymphoma patients and discusses the current trends in the therapeutic process. The data has been collected from the works of well-established researchers and the scientific evidence was abundantly supplemented with clinical and pre-clinical trial data. Although the findings cited are the latest, this review might not be very accurate for every population as the data from which this was derived have a population bias in several instances. The analysis has mostly been qualitative and interpretive, and quantitative evidence has only been used to explain the clinical trial results. We have divided our paper into the mode of action of BV, its probable and proven causes of resistance, and the therapeutic strategies employed to reverse them to ensure a systemic flow of information throughout the text.

Main body Brentuximab vedotin is an antibody–drug conjugate with antineoplastic activity, used to target a novel immunophenotype tumor necrosis factor CD30. This factor is specific to the tumor-causing Reed Sternberg cells in the inflammatory infiltrate. Though the drug had shown promise initially, the cancer was quick to develop resistance against the drug. We have analyzed and represented abundant statistical evidence to back this claim. The paper further discusses the role of the CD30 receptor, MDR1 gene, valine-citrulline linker, and tumor microenvironment in drug resistance. Lastly, we have discussed the possible therapeutics that can be used to overcome this resistance, discussing the well-established and trial-stage approaches taken in the endeavor.

Conclusion The treatment is much better after the pursuit of reversing the drug resistance phenomenon. However, no therapeutic approach has been entirely successful in restricting the neoplastic property of cancer cells once and for all. This paper describes why that is so and how the heterogeneity of the disease complicates troubleshooting. We have tried to approach such problems through this specific example.

Keywords Brentuximab vedotin, Drug resistance, R/r- classic Hodgkin lymphoma, Reed-Sternberg cells, Oncotherapy

Background

Natural selection turns moot when normal human cells in vivo are brought into question. Though free-living unicellular organisms such as bacteria survive based on reproductive fitness, multicellular organisms thrive on collaboration. Somatic cells have a limit to the number of generations they can produce, thus capping their reproductive fitness, and will perish to keep the germ progeny alive and active. If in such a setting, a

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cell assumes a mutation that renders it superior reproductive fitness, it can soon choose to compete, rather than collaborate, thus developing cancer [1]. Extensive research has been performed which has backed seemingly random observations of metastatic behavior (for instance, prostate cancer often spreads to bones) [2]. The bone is a nutritionally enriched organ having an intricate vascular network and different types of cells. When the tumor affects the stem cells of the bone marrow, different forms of blood cancers develop [3].

Bone marrow cancer can broadly be classified under the following categories: Myeloma, Leukemia, Lymphoma, Myeloproliferative disorders, and myelodysplasia [4, 5].

Hodgkin's and non-Hodgkin's are both lymphomas but there are several differences between the two. Hodgkin's lymphoma is localized in a single group of axial lymph nodes and is spread contiguously by the lymph. Though the incidence rate might appear nominal at 3 per 1,00,000 person-years, it is important to note that about 50% of patients are young, within the age bracket of 15–35 years. The disease follows a bimodal age distribution with a 2nd incidence peak in late adulthood (above 55) [6]. Why this is so has not yet been studied in-depth.

The following treatments are available for classic Hodgkin's lymphoma:

- High-dose chemotherapy and radiotherapy cause complete remission of the disease.
- Brentuximab vedotin drug treatment, often supplemented with allo-SCT (allogeneic stem cell treatment) or not.
- Programmed cell death-1 checkpoint inhibitor [7].

On the contrary, non-Hodgkin's lymphoma is localized in multiple peripheral lymph nodes because it spreads through the more extensive blood vessels. Because of this, the homing of the infection often occurs at non-contiguous lymph nodes and thus the disease requires a considerable time before the inflammation is externally perceivable. Diagnosis is thus relatively poor.

Histopathological studies have shown the presence of a special class of cells called the Reed-Sternberg cells which have a very strong correlation with the occurrence of Hodgkin's lymphoma. These cells are transformed B cells from the pre-apoptotic germinal center (GC) (in lymphoid organs) which have undergone extensive genetic reprogramming leading to epigenetic changes that have suppressed the expression of a large fraction (almost all) of the B-cell receptors (BCRs) [8].

Alongside suppressing the native receptor production, **t**he neoplastic Hodgkin's Reed-Sternberg (H-RS) cells have a profuse expression of a type I membrane protein, CD30, which is a member of the tumor necrosis factor (TNF) receptor superfamily. This rare expression profile of CD30 makes it a good immunophenotypic disease marker for Hodgkin's lymphoma. Also, HRS cells constitute a mere 1–2% of the inflammatory cell population and thus CD30 is a highly exclusive target for exterminating the tumor cells. Research has also proposed CD30 involvement in the genesis and proliferation of the H-RS cells [9]. This makes CD30 a good target for antibody-directed therapies [10]. Though risk remains of cross-cellular CD30 targeting, the population of CD30 is so minimal in normal immune cells, that this risk is often ignored [7].

Brentuximab Vedotin as shown in Fig. 1 is an antibody–drug conjugate (ADC). The ADC is synthesized by the chimeric mouse-human IgG1 anti-CD30 monoclonal

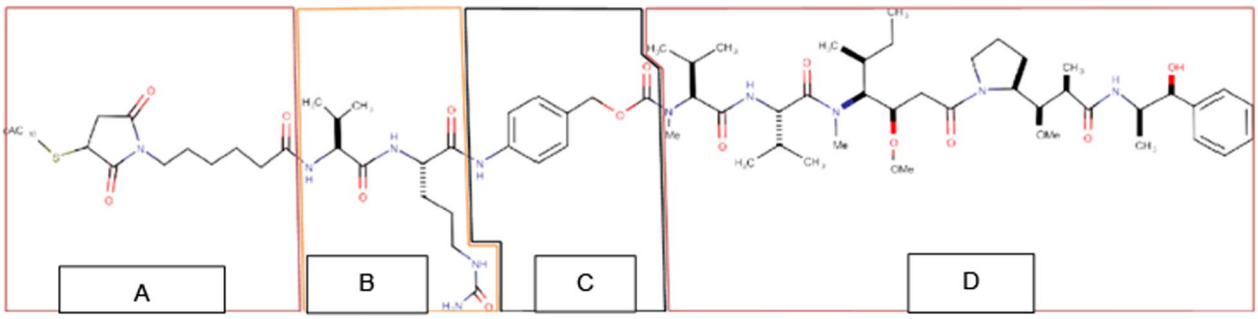

Fig. 1 Chemical Structure of Brentuximab vedotin showing the mAb (cAC10), dipeptide linker, spacer & drug (MMAE). The mAb is used to target the CD30 expressed as the unique immunophenotype on the H-RS cells of the BV infiltrate. The dipeptide linker is cathepsin specific, though specificity to a large number of cathepsins can increase cytotoxicity by promoting non-specific payload release. Scientists are on the lookout for cathepsin-B-specific linkers such that the linker is cleaved only in the cathepsin B-producing tumor cells. The spacer is usually a para-amino benzyl carbamate spacer, which helps maintain stability in the bloodstream and undergoes self-immolated disassembly to release the MMAE

antibody (SGN—30) conjugated to monomethyl auristatin E (MMAE) via a dipeptide linker & it is used in the treatment of Hodgkin's lymphoma [10–12].

Phase I studies showed a significant result with a positive response in patients with relapsed or refractory (R/R) Hodgkin lymphoma upon administration of BV. Phase II trials showed a complete response (CR) rate of 34% and an overall response rate (ORR) of 75% in CD30-positive lymphomas [13]. This outcome resulted in the FDA approving the usage of BV to treat patients with Hodgkin's after the failure of autologous hematopoietic cell transplantation [14]. BV was then started being used as part of initial therapy along with chemotherapy for advanced-stage Hodgkin lymphoma [15].

But soon resistance against the administered drug started registering. Several pharmacokinetic and pharmacodynamic challenges contributed to the gradual resistance to ADCs, due to failure in either antibody, linker, payload, or the poor ADMET properties of the drug [16, 17].

As mentioned above, in the phase II trials, BV demonstrated a CR rate of 34% and an ORR of 75% but patients with Partial Response PR had a very short remission time of medians of 3.5 months [13], therefore patients without a CR will gradually develop a progressive disease even with a continuous BV. Considering BV is the only therapeutic drug prescribed by the FDA in the last 20 years [14], understanding the mechanism of BV resistance & and potential solutions are of great importance.

Main text

Brentuximab vedotin: mode of action

Brentuximab vedotin (BV) (ADCETRIS®; Aptuit [Glasgow] Ltd., Glasgow, United Kingdom), is composed of the chimeric monoclonal IgG1 antibody (cAC10, SGN-30) (derived from mouse xenograft model) that is specific to human CD30. It is conjugated to monomethyl auristatin E (MMAE), which is the payload. The payload is linked to the main body of BV by a protease-cleavable peptide linker containing a valine-citrulline combination [18]. MMAE is a synthetic antineoplastic agent and a very potent anti-mitotic drug (because of tubulin-disrupting properties) against Hodgkin's lymphoma [19]. Each of the antibodies is linked to an average of 4 MMAE groups [18].

As seen in Fig. 2 [20] the ADC is endocytosed upon binding to the extracellular CD30 antigen on the tumor cells. Inside the cells, the proteolytic lysosomal enzymes cleave the dipeptide linker thereby releasing the MMAE drug, which then goes and binds to tubulin leading to the collapse of the microtubular network. As a result, the G2/M-phase cell cycle gets arrested and apoptosis occurs (Francisco et al. [10]). The valine-citrulline peptide linker is highly stable in plasma, which results in comparatively low in-vivo toxicity of the drugs when compared to other linkers [21]. BV also works by mAb-dependent cellular phagocytosis, affecting cell signaling in $CD30^+$ cells, or sometimes even due to free MMAE leaking out of the tumor cells and killing neighboring $CD30^+$ cells [18, 20, 22].

Brentuximab vedotin: efficacy demonstration studies

In 102 Hodgkin lymphoma patients who had relapsed following high-dose chemotherapy and autologous hematopoietic stem cell transplantation, intravenous BV had been linked with an overall ORR (primary endpoint) of 75%. In 94% of patients with cHL, tumor reductions were seen, and the majority of these tumors shrank by almost > 65%. The corresponding predicted 12-month survival rates were 89% [23]. The median duration of response for patients in CR was 20.5 months, and the median progression-free survival time was 5.6 months for all patients. After a median observation period of over 1.5 years, 31 individuals were still alive and had no signs of worsening disease that had been medically verified [13]. These trials showed that BV was generally well tolerated [23].

BV demonstrated cell death induction in CD30-positive cells with $IC_{50} < 10$ ng/ml, but showed around 300-fold inactivity on CD30-negative cells, in patients with Hodgkin's lymphoma [10].

In a study conducted by Chen R., two subsets included a group of six patients who received consolidative allo-SCT (allogeneic stem cell transplantation) and another of 28 patients who didn't [24]. This study analyzes overall survival (OS) and progression-free survival (PFS) based on the Kaplan–Meier methodology. The survival rates help analyze the efficacy of the allo-SCT treatment [24]. From the study, it is seen that stem cell transplantation often prolongs life or at least delays relapse as compared to only BV treatment [25].

CD30 characterization

The CD30 receptor was first identified from a monoclonal antibody (mAb) derived from an HL cell line [26]. Structural analysis has shown that the protein has intracellular, extracellular, and transmembrane domains [27] and sequence similarity has found CD30 to be similar to other TNF (Tumor necrosis factor) receptors in its extracellular sites, thus raising questions regarding the exclusivity of the target of BV. BV might also be useful in treating non-hematopoietic cancers, such as germ cell tumors (testicular embryonal carcinomas) [28].

These are the normal immune functions of the CD30 found as of now:

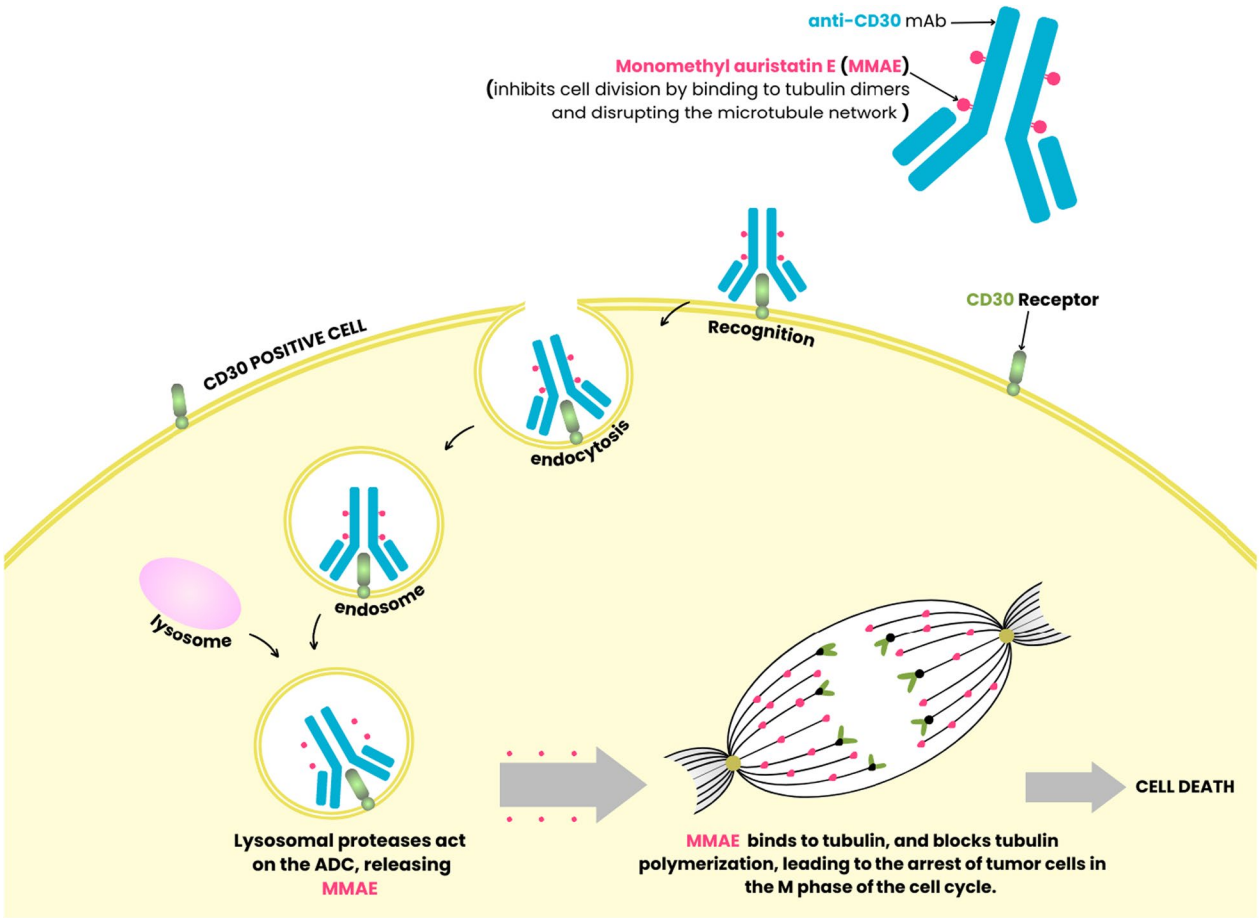

Fig. 2 Mechanism Pathway of BV, displaying its internalization into the cell, and subsequently disrupting the microtubular network. This figure shows how BV is internalized into the cell. The drug, through its CD30-specific mAb, binds to CD30 outside the cell, on the cell surface. It is then internalized by endocytosis. Next, the endosome reacts with lysosomes, leading to the release of lysosomal proteases—the cathepsins in the endosome. Cathepsin-specific cleavage of the di-peptide linker occurs, leading to MMAE release. The MMAE exits the endosome and reaches the nucleus, where it binds to microtubules and triggers its anti-mitotic activity

- Expressed on the surface of CD4 + and CD8 + T lymphocytes which are activated because of an infection and show a propensity to secrete Th2 cytokines (Il-4, Il-5) predominantly, although Th0 and Th1 cytokine production has also been reported [29].
- Negative selection of partially mature T lymphocytes having double positive CD4 + CD8 + antigens is thought to be guided by a transient expression of CD30 in a relatively smaller population of thymic cells that can eliminate T cells with high affinity towards self-peptides via apoptosis, thus preventing potential autoimmune disorders [30, 31].

When analyzing CD30 as a potential cause for BV resistance in cHL patients, it is important to note that several researchers have found CD30 to be an active perpetrator of lymphomagenesis. It is established that CD30 is not downregulated upon and after BV treatment [32]. This rules out the possibility of the lack of a target for BV as a potential cause of drug resistance. However, the involvement of CD30 in continued lymphomagenesis as a potential cause of relapse in BV-treated patients is a glaring possibility. Downstream activation of nuclear factor kappa B (NF-kB) and mitogen-activated protein kinase (or extracellular signal-related kinase) pathways both ultimately lead to the activation of anti-apoptotic and pro-proliferative genes. Contrasting views on the role of CD30 in the normal immune system have led to poor functional characterization [33]. The downstream effects of CD30 stimulation might be exploited when searching for a potential reversal of BV resistance.

Brentuximab vedotin resistance: potential causes

Surface antigen level downregulation

BV utilizes a mAb against the antigen CD30 on the cell surface of tumor cells as a potential drug target. It is thus

possible that the downregulation of CD30 levels in the tumor cells causes BV resistance.

Chen et al. [32] conducted MTS assays to determine the IC50 (Half-maximal inhibitory concentration) of parental cell lines of L428 (HL). The BV-resistant cell models were selected using persistent exposure to sub-IC50 concentrations of BV and cell numbers in culture were kept on track for 3 months. However, the team was unable to obtain resistant L428 cells through constant exposure to the drug. Then a supra-IC50 concentration of BV exposure was applied and cell numbers were tracked & assessed twice a week until cessation of cell proliferation was seen. In the end, it was seen that BV-resistant cell lines were able to grow at supra-IC50 parental line BV concentrations. This was confirmed by cell proliferation assays. At the same concentrations, the parental cell lines quickly died. However, there was absolutely no significant decrease in the percentage of CD30 + cells [32].

In another research conducted by Nathwani et al. [34] two patients: a 27-year-old woman (with relapsed cHL in IVA stage following prior 6 cycles of ABVD, 2 cycles of ICE, and a cycle of ACT) and a 19-year-old man (with relapsed cHL in IIIA stage following ABVD, ICE & additional treatments including rituximab, gemcitabine, vinorelbine, liposomal doxorubicin, MOPP, and palliative radiation) were enrolled in a study to find out more about the role that CD30 plays in BV resistance. In both cases, the patients achieved a significant reduction in tumor sizes following the BV treatment through 8 and 10 cycles respectively. Here too, a persistent level of CD30 throughout the treatment demonstrated that a reduction in levels of CD30 does not appear to be a potential cause of BV resistance [34].

Drug transporter protein overexpression

The same study conducted by Chen et al. [32] with L428-R (resistant) and L428-P (parental) cells showed a decrease in the amount of intracellular MMAE in L428-R as compared to L428-P cells. The R cells accumulated 6.7-fold (± 3.4 fold) more MMAE compared to the L428-P cells within 48 h when the cells were incubated with 20 µg/ml of BV [32]. To confirm this, they performed an additional test with rhodamine-123 dye for two days (Rhodamine is a substrate for the transporter responsible for MMAE efflux), and L428-R cells showed tenfold lesser fluorescence than L428-P cells. Subsequent qRT-PCR was done to see mRNA levels for *MRP1*, *MDR1,* and *MRP3*, in sets of resistant and parental cell lines [32]. Results showed increased MDR1 levels (ATP-dependent translocase ABCB1) and protein levels of P-glycoprotein (who preferentially exports hydrophobic cargo like MMAE out of cells) in L428-R cells relative to L428-P cells, though *MRP1* or *MRP3* mRNA levels in both cells lines were same [32].

In another study conducted by Chen et al. [11] they used another BV-resistant HL cell model KMH2-R. They again showed that CD30 expression was unchanged in KMH2-R compared with KMH2-P. And gradually re-confirmed the overexpression of the gene *MDR1* by qPCR in KMH2 cells, where *MDR1* presence was threefold higher in KMH2-R cells compared with KMH2-P cells [11]. *MDR1* RNA expression was threefold greater in KMH2-R cells & sevenfold greater in L428-R cells compared to respective KMH2-P and L428-P cells [11]. This displayed how drug transporters can play such a crucial role in BV drug resistance in HL.

Defective linker-payload processing

There are two types of linkers, cleavable and non-cleavable. An appropriate linker is not only required to prevent degradation during systemic circulation but also to facilitate the quick and efficient release of the drug inside the tumor cells [35]. Non-cleavable linkers release their conjugated drug only after antibody degradation. For example, for Kadcyla which has a non-cleavable thioether linker, lysosomal membrane proteins are required to first transport the drug catabolite out of the lysosomal compartment & then exert a therapeutic effect [36]. Barok et al. [37] established that non-cleavable linkers are more susceptible to ADC resistance in tumor cells because any faulty linker degradation will not lead to functional drug release.

BV is composed of protease-cleavable valine-citrulline peptide, where the MMAE does not depend on the degradation of the antibody backbone. Thus, it leads to much faster payload release after ADC internalization compared to a non-cleavable linker [38]. However, a faulty linker cleaving or non-cleaving can lead to an ineffective payload discharge. As per a study conducted by Caculitan et al. [39], valine-citrulline (Val-Cit) linker showed broad-spectrum specificity to different types of cathepsins, including cathepsin B, cathepsin L, cathepsin K, etc. [39]. Since only cathepsin B is postulated to be highly expressed in HL cells, and normal cells have other kinds of cathepsins, this phenomenon could be very deleterious as it would induce toxic side effects on other normal cells [40].

A defective linker thereby affects the cytotoxic drug delivery in many ways from the drug not reaching the target, to off-target toxicity and the drug not being able to dissociate from the mAb and the linker, thereby overall promoting resistance.

Tumor microenvironment

In cHL tumor, the entire cellular infiltrate contains only infrequent neoplastic HRS cells (about 1%) and is surrounded mostly by a characteristic tumor microenvironment (TME) composed of several benign immune and extracellular matrix stromal cells, including different types of T and B cells, eosinophils, fibroblasts, macrophages (M1 and M2). By contrast, NLPHL (nodular lymphocyte predominant HL) differs from cHL1 based on specific histopathological characteristics [41].

Extensive crosstalk mediated by a large network of cytokines and chemokines between tumor cells and immune cells, acting in an autocrine and paracrine manner, suggesting the existence of an entire pro-malignant cancerous ecosystem present around the tumor cells has been established [41]. As Fig. 3 shows, the CD30 ligand (CD30L), as well as neutrophils and eosinophils are commonly mixed with HRS cells [42].

TME-mediated development of drug resistance occurs by multiple mechanisms quite different from one another [43–45]:

- Metabolic reprogramming, leading to altered drug delivery and various tumor proliferation strategies.
- ECM remodeling through changes in the matrix-forming heterogeneous class of stromal cells.
- Development of cancer stem cell phenotype (CSC) through expression of various immuno-phenotypic markers such as CD44, CD24, and CD133 and the development of the conserved Nodge and Hedgehog pathways involved in cellular differentiation.
- Angiogenesis plays a major role by determining the level of development of the surrounding vasculature which provides the tumor with oxygen, nutrients, etc., and removal of metabolic wastes.
- Immune suppression mechanisms.
- Exosome-mediated trapping of therapeutic antibodies.

Therapeutic strategies to overcome BV resistance

Usage of different linker-payload combinations

MMAE is uncharged, and hence non-polar. This often leads to the death of cells near cancerous tumor cells known as the "bystander effect". As seen in Fig. 4 MMAE

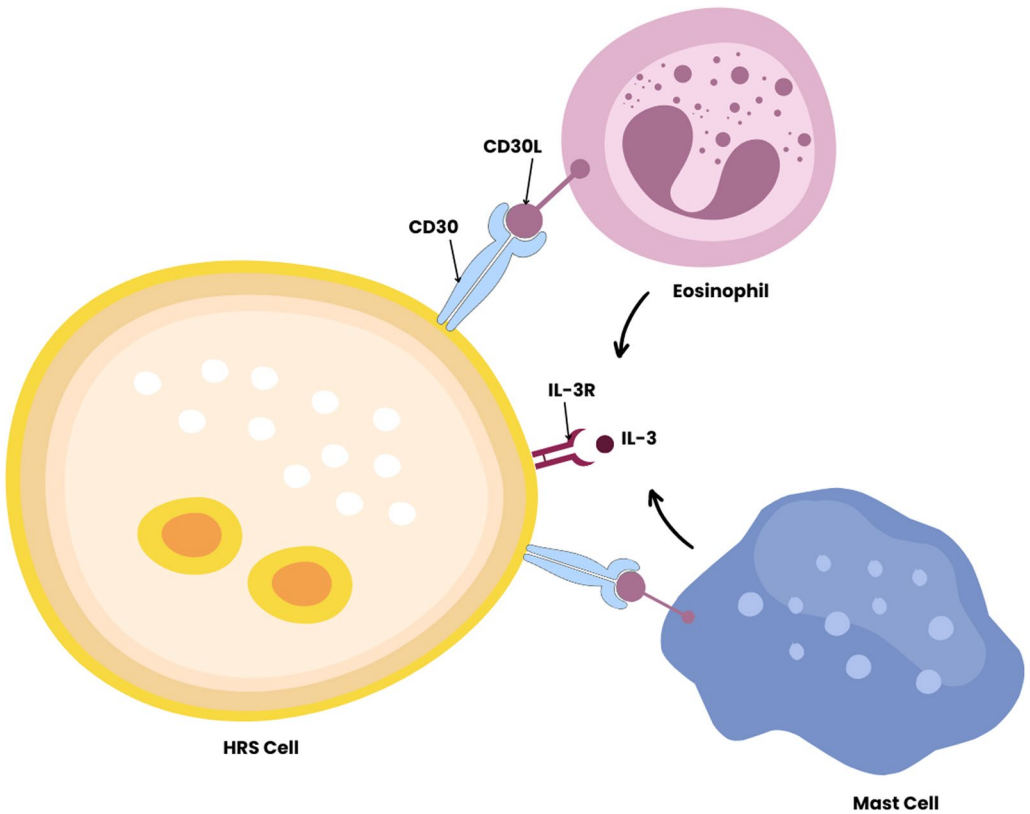

Fig. 3 Different interactions of cell surface receptors of HRS cells with the tumor microenvironment describe the way the HRS cells influence the microenvironment. Such influence is the driving cause behind sustained tumor growth and unchecked proliferation. In the figure, we can see the CD30 binding eosinophils and mast cells and also interacting with mast cell-produced interleukins

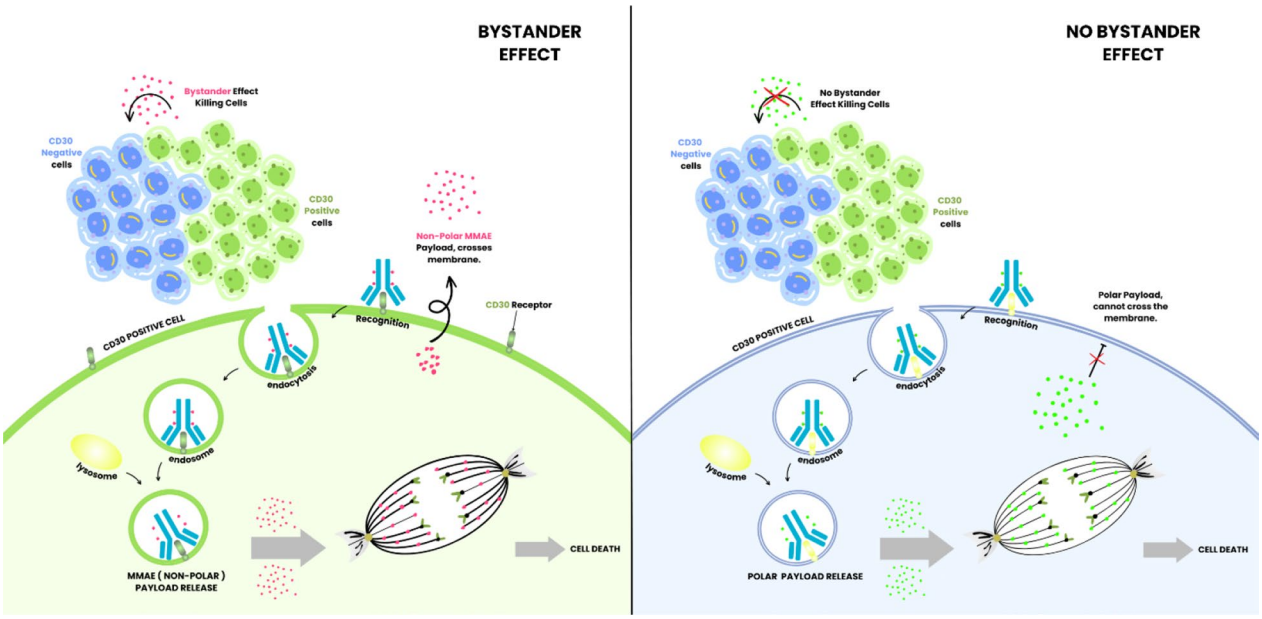

Fig. 4 Schematic of the bystander effect shown by non-polar conjugates showing cytoplasmic leakage shows the cytotoxicity difference between a polar and a non-polar payload because of the permeability exhibited by the non-polar payload in crossing the cell membrane into the nearby cell causing non-specific toxicity. This phenomenon can be positive if the nearby cell is toxic too but that is seldom the case and this kind of effect mostly kills healthy cells

internalization leads to the destruction of cancer cells and when these cells lyse, they release the MMAE [46]. Because of its hydrophobicity, it can easily pass through the phospholipid bilayer of the surrounding cells, causing antimitotic effects in them [46]. While it can have a positive effect if the surrounding cells are cancerous too, on the flip side, the cells develop BV resistance through MDR1 upregulation very quickly. To overcome this resistance, often the MMAE is replaced by a charged payload/linker combination, which is impermeable to the nearby cells upon lysis of the initially targeted cell. This keeps the bystander effect in check, helping in minimizing resistance [46].

A linker that used a cyclobutane-1,1-dicarboxamide (cBu) structure was designed by Wei et al. which was specific to cathepsin B cleavage. This was proven by intracellular cleavage studies in which a cathepsin B inhibitor stopped drug release from cBu-Cit-containing linkers by over 75%, while a cathepsin K inhibitor did not have an appreciable effect [40].

Peptide linkers have been seen to be easily optimizable by minimal structural changes, including the types and stereochemistry of the amino acids. For instance, valine-alanine (Val-Ala) has better hydrophilicity and stability than Val-Cit [47].

Ward and his coworkers [48] from Texas University have developed an innovative approach to solving drug resistance. They developed such a targeting moiety of the ADC (antibody–drug conjugate) such that its binding affinity was two orders lower in the endolysosomal lumen (pH < 6.5; $[Ca^{2+}] \sim 2$ μM) than it was in the extracellular space (pH > 6.8; $[Ca^{2+}] \sim 2$ mM) [48]. Since the drug is more easily accessible to its intracellular substrate because of faster dissociation from the antibody target, it gives a two-fold advantage: recycling of the target to the cell surface to sequester more ADC, and faster downstream signaling from the drug binding to its substrate, which in the case of the anti-tubulin activity of MMAE is cessation of cell proliferation. This diminishes the cytosolic payload of the drug [48].

"Component switch" mechanism

The "component switch" mechanism is difficult to implement because the prognosis will change radically based on the stage of cancer and/or the treatment [46]. Age, ethnicity, and other variable factors will also bear a sensitive relationship to the treatment because targeting multiple pathways or events in an already heterogeneous disease can trigger severe physiological imbalances, resistant phenotypes, and immune weakening events. Think about the different variables involved with the single treatment pathway of ADC delivery: antibody target identification, internalization, payload release, binding of MMAE to tubulin, and the fate of tubulin after cell lysis–problems at a single step can jeopardize the entire treatment [46].

There are many problems with medical and healthcare procedures too. Analysis of resistant mechanisms is limited by the want of systematic and routine pre-and post-treatment biopsies and the problem of setting up standardized clinical assays for quantifying protein levels of clinical biomarkers [46].

Combination of ADCs with immune checkpoint blockade

Immune suppression of cancer is bypassed by the resuscitation of effector T-cells which helps in infection response and memory. Antibodies designed to inhibit immune checkpoints help in this. Immune checkpoint blockade has proven promise in many long-lasting cures [46].

The use of such antibodies however has disadvantages. In tumors that have not developed any anti-tumor T-cell response, usually comprised of "immune desert" or "immune excluded" types (the former means that the immune system is not recognizing the tumor as an infection, resulting in no T cell production against it, whereas the latter means that T cells are formed against the tumor and are present in the extracellular stromal matrix but are unable to penetrate the core tumor mass), the ICI (immune checkpoint inhibitor) antibodies don't work [49].

Anti-tumor immunity is conferred by ADCs and chemotherapeutics through the following mechanisms:

- release of tumor antigens from dying cells allows these antigens to be taken up by the dendritic cells, macrophages, or B cells, which phagocytose the antigens and present them to the T cells via the MHCs (major histocompatibility complexes) for immune activation [46].
- Maturation and activation of the antigen-presenting dendritic cells are largely influenced by the free payload that comes conjugated with the ADC. If the payload is PAMPs (pattern-associated molecular patterns) and DAMPs (damage-associated molecular patterns) (like Toll receptors), it can directly be presented by the APCs (antigen-presenting cells, here dendritic cells) for immune activation. Free payload can also lead to co-stimulatory molecule release (like CD40, and CD80) or cytokine release [46].
- Triggering cell death, often with the aid of antimitotic factors [46].

Combining ADCs with ICI antibody treatment can help immunologically "cold" tumors to get converted to tumors with an active T cell pool by the methods described above. Since T cell activation leads to an overall increase in adaptive immune response, a global mechanism to eliminate the tumors is undertaken without the dependency on surface antigen gene regulation [46].

Ongoing clinical trials involving BV and ICI are given below [46]:

- BV and Nivolumab block PD1
- BV and Pembrolizumab block PD-1 too
- BV and Nivolumab + / − Ipilimumab blocks PD-1 and CTLA-4

Overcoming MDR1-mediated resistance by using a modified linker

MDR1 transporters use maytansinoids as substrates to be transported across the cell membrane against their gradients. DM1 (Mertansine) is a thiol-containing maytansinoid that is actively pumped out by overexpressed MDR1 receptors as a form of response to DM1 treatment in HER-2-positive breast cancer. Kovtun and colleagues designed an ADC in which the DM1 was linked to an antibody using a hydrophilic linker, PEG4Mal, which was a replacement for the initial SMCC linker. This resulted in the release of lysine-PEG4Mal-DM1 instead of lysine-MCC-DM1 upon cathepsin cleavage. MDR1 doesn't recognize lysine-PEG4Mal-DM1 as a substrate for transmembrane transport, thus solving the problem of efflux of the payload drug. This method prevented MDR1-mediated resistance in both in-vivo xenografts and in-vitro cells expressing MDR1 [37]. This can be extended to cHL treatment by BV administration by modifying the dipeptide linker.

Using PD1 inhibitors

Cancer often leads to immunosuppression by inactivating the activated B and T cells. PD1 discovery, which was awarded the Nobel in 2018, led to the dawn of a new direction in cancer therapy. PD1 acts as a negative regulator on T cells. PD1 inhibitors such as pembrolizumab and nivolumab have led to promising clinical results because of the reactivation of immunosuppressed T cells [42]. This has led to the abatement of refractory cancers such as the HL249, 250 cell lines. PD1 inhibitors are now often administered with ADC, as forms of combination therapy. Though some have shown severe autoimmune complications, most patients have been able to accept the treatment, some even being fully cured. Preliminary results of patients diagnosed with advanced-stage Hodgkin's lymphoma administered with a combination therapy of nivolumab and AVD have shown good results [42].

Brentuximab vedotin plus Ibrutinib

BTK (Bruton's tyrosine kinase), an important oncogenic non-receptor tyrosine kinase is active in various subtypes of non-Hodgkin lymphoma and is also expressed in malignant Reed-Sternberg cells. Ibrutinib (Ibr) is a Bruton's tyrosine kinase (BTK) inhibitor which can also use a Th1-based response to inhibit IL-2-inducible kinase (ITK). This can promote immunogenic cell death in combination with BV [50].

According to the research conducted in a phase II trial of Ibr plus BV in patients with r/r HL, 39 patients were enrolled; 67% were male and the median age was 33. Of 36 evaluable patients, the CR rate was 33%, ORR 64%, and the median DOR (Diagnostic Odds Ratio) was 25.5 months (range) [50].

Results showed that Ibr imparted additional toxicity in comparison to BV monotherapy, and even showed no signs of increased efficacy in patients treated with both BV and Ibr, thus ruling Ibr out as a potential treatment [50].

MDR1 inhibition with CsA and VrP

Effects of CsA (cyclosporine) and VrP (vorinostat, a histone deacetylase inhibitor) were studied on the IC_{50} (measures potency of inhibiting tumor growth) of BV in L428-R (missense mutation involving resistant colon cancer cell) and KMH2-R cells [11]. Neither of the drugs had any effect on the viability of the parental and BV-resistant types of the two cell lines mentioned above in the absence of BV. PgP protein expression and *MDR1* mRNA expression were also unchanged on CsA treatment [11]. It was then seen that competitive MDR1 inhibition increased intracellular MMAE levels and resensitized the two-BV-resistant cHL cell lines. It was also seen that overexpressing exogenous MDR1 in the L428-P led to BV resistance, which was nullified on treatment with CsA [11]. These results conclusively support the hypothesis that resistance to BV in Hodgkin's lymphoma is brought about because of the cytosolic loss of MMAE by the ABC drug transporter MDR1/PgP, whose overexpression is triggered by rising MMAE levels [11].

It was previously reported that the addition of VrP led to a 3.9-fold reduction in BV IC_{50} [32] into L428-R cells and a sixfold reduction in that of KMH2 $IC_{50.}$ Compared to this, this reduction was 10,000-fold for L428-R cells and 600-fold in KMH2-R cells [11].

CRISPR-Cas9 system to reverse drug resistance targeting MDR1

It was reported that the expression of P-glycoprotein, which helps the ATP-mediated transfer of antimitotic drug against its gradient with the help of the transmembrane MDR1 protein, could be efficiently blocked using the CRISPR-Cas9 system. Inhibiting ABCB1 (another name for P-glycoprotein) in osteosarcoma MDR cell lines (U-2OSR2 and KHOSR2) helped in combating MDR against doxorubicin [51].

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats and it uses Cas9, which can form a complex with single guide RNA (which shows Cas9 its point of activity on the DNA). The sgRNA-Cas9 complex then cleaves the DNA 3–4 base pairs upstream of a protospacer adjacent motif (PAM). Cas9 then helps generate DSBs (double-strand breaks) [52], which when repaired by NHEJ (non-homologous end joining) generates small insertions or deletions at the point of Cas9 activity. This is because NHEJ is very erroneous. These point mutations can inactivate genes or genomic elements [52].

Similar CRISPR-based editing can be used for classic Hodgkin's lymphoma to target a lot of proteins: ABCB1 can be targeted to prevent efflux pump overexpression, so cytosolic MMAE levels are maintained. ADAM10 and ADAM17 inhibitors can help keep the cell surface concentration of CD30 nearly constant so that BV gets a docking site and no drug loss happens over the lack of a target molecule [53].

Nanoparticle-based MMAE targeting

MMAE is an anti-tubulin agent and can be detrimental to the normal cells of the body. Though BV is quite a specific drug because of its target being the distinct cancer immunophenotype CD30, cross-reactivities because of partial similarity with other F_{ab} segments (hypervariable regions can be assumed to be unique, but similarities in the variable region are possible) of other CD markers is not only possible but also expected [53]. Also, activated B and T cells often express CD30 as a normal phenotype (as previously discussed), so BV can often be led astray. This raises a two-fold possibility:

- Effective drug delivery to the Reed-Stenberg cells decreases.
- Normal cells are prevented from proliferating (mitosis prevented). This decreases the population of the already strained normal cells of the body, thus helping tumor proliferation (The citrulline-valine linker of BV is specific for a lot of cathepsins, including the ones with abundant expression in normal cells) [53].

Another factor causes reduced drug delivery. CD30 is often cleaved in active Hodgkin's lymphoma patients, which leads to a high concentration of sCD30 (soluble CD30) in the plasma. This ectodomain cleavage is caused

by members of the ADAM group of proteins (ADAM 10 and ADAM 17), appropriately called sheddases [53].

Nanoparticles containing FRRG (Phenylalanine-Arginine- Arginine- Glycine) conjugated with MMAE (MMAE conjugated to C-terminus of FRRG) can be obtained by EDC-NHS coupling at 37 °C for 24 h. The self-assembled nanoparticle stabilized with the intermolecular hydrophobic interactions and needed no further carrier materials. These nanoparticles (prodrugs) showed vigorous uptake in in-vitro breast cancer cells (4T1) [54]. FRRG has proven to be the minimal chain of peptides required to selectively trigger cathepsin B without requiring additional lipids or polymers for nanoparticle assembly [54].

Such nanoparticles can be used as a potential replacement for BV because of the specificity of its cleavable peptide linker and its independence of CD30 presence on the cell surface. The FRRG peptide is a much more specific linker than the citrulline-valine linker in BV. FRRG is specific to cathepsin B, the cathepsin with the most overexpression in tumor cells. Normal cells will not have high enough levels of cathepsin B to trigger the release of MMAE from the prodrug and hence the anti-mitotic action of MMAE will not be active in normal cells, minimizing collateral damage. Cleavage of CD30 ectodomains will also not cause inefficacy in drug delivery [55].

Epigenetic modifications to modulate resistance

Epigenetic modifications often lead the cancer cells to survive and proliferate in the face of subsequent rounds of chemotherapy and since epigenetic changes are defined by their transience, changes call for a lesser strain on the cellular machinery than a genetic change would. Nucleic acid methylation has been the best-characterized epigenetic process contributing to chemoresistance [56]. Methylation rates were studied in FL (Follicular lymphoma) and DLBCL (diffuse large cell B-lymphoma) and compared to that of normal B cells [56]. It was seen that increased dissimilarity in methylation patterns led to faster death of the cells, a fact held even between FL grades. Abnormal methylation patterns had a propensity to be targeted toward promoters of key regulatory factors such as *MYC, BCL6,* and *EZH2.* Upon investigating for similar methylation in DLBCL patients who showed different fates to treatment (durable vs. relapsed) [57], enriched promoter sites with differently hypermethylated regions were found. For instance, *CTCF*, a trans factor involved with DNA methylation (through interactions with histone acetylases and deacetylases) was differentially hypermethylated at its promoter.

Chemoresistance is much attributed to epigenetic modifications since DNA methylation status can affect a broad range of housekeeping cellular activities like cell cycle, autophagy, protein degradation, immune response, apoptosis, and DNA damage. It also affects signaling pathways involving small molecular targets. For instance, Bruton's tyrosine kinase (BTK), which is used as an immuno-target to curtail mantle-cell lymphoma has undergone epigenetic modification to gain resistance to the inhibitor (of BTK) ibrutinib, which was a general treatment for the disease [58]. Among the major drugs involved with epigenetic changes, post-translational modifications (PTM) in many are assumed to be the major cause of activating chemo-resistant pathways. Among these, an important one is the impaired p53 activity, in which p53 acetylation activates the tumor suppressor and protects it from degradation. To induce the acetylation back, often class III HDAC inhibitors are used. Scientists are also looking at ways in which the epigenetic regulation of the tumor microenvironment immune surveillance cells can help in circumventing chemoresistance. To exemplify this, it has been seen that programmed cell death 1 (PD-1) and programmed death ligand 1 (PD-L1) are involved in chemoresistance, both of which are under tight epigenetic control [59, 60].

Epigenetic modulating drugs could be supplemented with essential tumor shrinkage agents with the aim of rewiring pathways causing drug resistance, especially tightening checkpoint inhibitors. Toxicity problems limit the use of epigenetic drugs to minute concentrations. If proper precision medicine data can be obtained to classify patterns of epigenetic derangements, specific epigenetic drugs can be coupled with chemotherapy and immunotherapy to overcome drug resistance.

Conclusions

This narrative review discusses the various ways in which drug resistance can occur in cHL, analyses the existing methods to reverse it, and proposes new ones.

HRS cells are responsible for tumor proliferation in cHL. CD30 is backed by statistical evidence to be proven as the predominant cause of tumor spread and many subsets of patients were shown to have grown resistant against the ADC that was employed to tackle cHL. The potential causes of resistance were hypothesized, some of which were incorrect. For instance, it was seen that BV had an antibody derived from a mouse xenograft which was specific to the CD30 marker on the HRS cells. BV treatment might have led to the downregulation of CD30, thus robbing BV of docking sites to gain entry into the cell. However, clinical data reveals no such happening. This effect was later explained by the action of ADAM proteins, which led to the discovery of the shedding of ectodomains of CD30 in advanced cHL patients. That shedding caused the loss of docking sites

and non-specific docking with the sCD30, both aiding in improper drug delivery.

Another hypothesis, the overexpression of MDR1 which pumped out the active payload of BV, and MMAE out of the cells was proved to be correct. Numerous alterations in the ADC were done to prevent the efflux (such as modifying the payload so that it was no more a substrate for MDR1). Changing MMAE to a charged substrate has often been thought to limit the bystander effect. The linker is often changed to ensure its stability under the treatment of non-tumor-specific cathepsins. This prevents collateral damage and reduces tumor proliferation by not killing normal cells and freeing up space and nutrients for the tumor. The antibody part is also engineered to make it more specific to CD30, as CD30 belongs to the class of TNF receptors, which have quite similar ectodomains, which can result in cross-reactivity. Immune checkpoint blockades such as PD1 inhibitors are thought to be more effective than ADCs because this leads to the global activation of the immune response of the body to attack the tumor. This review has therefore been successful in identifying the causes of BV resistance but the remedies for such resistance are under debate.

The use of CRISPR is being hypothesized to mutate MDR1-producing genes so that cytosolic loss of MMAE decreases or ADAM-producing genes to preserve BV docking sites. Nanobiotechnology has made nanoparticles very important drug-delivery molecules. FRAG-MMAE-containing nanoparticles are employed to deliver the MMAE, instead of the use of an antibody interaction. This increases efficacy by removing the loss of drug due to lack of docking and also limits bystander effect because of the specificity of FRAG to cleavage only by cathepsin B (the one specific to cHL tumor). Finally, epigenetic modifications are being studied, employing the component switch strategy on various levels. These treatment options are relatively new and many of these are still under thorough investigative research or clinical trial phases. However, BV remains the most promising drug in public use until better solutions are discovered.

Further research should focus on a systems biology approach to investigate the individual causes of resistance, so we have an idea of the effect of each of the causes in the broader in-vivo context.

Abbreviations

ABVD	Adriamycin-bleomycin-vinblastine-dacarbazine regimen
ADC	Antibody drug conjugate
ADME	Absorption, digestion, metabolism, excretion
Allo-SCT	Allogeneic stem cell transplantation
APC	Antigen-presenting cells
BCR	B-cell receptor
BTK	Bruton's tyrosine kinase
BV	Brentuximab vedotin
cBu	Cyclobutane-1,1-dicarboxamide
cHL	Classic Hodgkin's lymphoma
CD	Cluster of differentiation
CR	Controlled response
CRISPR	Clustered regularly interspaced short palindromic repeats
CSC	Cancer stem cells
CsA	Cyclosporine
DAMP	Damage-associated molecular patterns
DOR	Diagnostic odds ratio
DSB	Double strand break
EDC	Ethyl(dimethyl aminopropyl) carbodiimide
FDA	Food and Drug Administration
FRRG	Phenylalanine-Arginine-Arginine-Glycine
GC	Germinal Centre
HL	Hodgin's lymphoma
HRS cells	Hodgkin's and Reed Stenberg cells
Ibr	Ibrutinib
ICI	Immune checkpoint inhibitor
IC50	Half maximal inhibitory concentration
Il	Interleukin
MMAE	Monomethyl auristatin E
MDR1	Multidrug resistance 1
MHC	Major histocompatibility complex
NFkB	Nuclear factor kappa B
NLPHL	Nodular lymphocyte-predominant Hodgkin lymphoma
ORR	Objective response rate
OS	Overall survival
PAMP	Pathogen-associated molecular patterns
PD1	Programmed cell death protein 1
PR	Partial response
PFS	Progression-free survival
qRT-PCR	Quantitative reverse transcription-polymerase chain reaction
RR	Relapsed/refractory
sCD	Soluble CD
Th	T-helper cells
TME	Tudor microenvironment
TNF	Tumor necrosis factor
VrP	Vorinostat

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