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Commentary

Emerging immunotherapies targeting CD30 in Hodgkin's lymphoma

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ABSTRACT

The immunotherapy of Hodgkin's lymphoma (HL) has been particularly challenging because of the unique features of tumor intrinsic and host mediated factors, interfering with the antitumor activities of therapeutic antibodies. Despite a wide array of compounds tested successfully in preclinical studies, immunotherapy in HL patients resulted in only limited success when compared to the significant improvements in patient survival provided by chemotherapeutic agents. Antibody–drug conjugates (ADCs) may surmount the restrictions posed by the unique pathobiology of HL tumors as they combine the selective tumor targeting of monoclonal antibodies with the potent anti-neoplastic activities of cytotoxic drugs. In early clinical trials, this class of compounds induced robust antitumor effects in patients with relapsed or refractory lymphoproliferative diseases, in the absence of overt toxicities, while naked antibodies failed to induce therapeutic benefit. Here we review some of the unique features of HL tumor biology and the key advantages of ADC-based lymphoma therapies, which may ultimately account for the improved therapeutic benefit provided by ADCs compared to first generation immunotherapeutics tested in HL patients.

1. Introduction

There are an estimated 8000 new Hodgkin's lymphoma (HL) cases diagnosed annually in the United States and Canada. Advances made in the use of combined chemotherapy and radiotherapy in HL over the past half-century resulted in durable remission rates of approximately 80%. However, these multi-agent regimens confer a significant morbidity such as secondary malignancies and infertility. Furthermore, between 20% and 30% of patients with HL will be refractory to initial therapy and will relapse after initial treatment. Overall, effective therapeutic modalities for refractory or relapsed patients are limited and most carry a high morbidity rate. Thus, there continues to be an unmet medical need for this group of patients with a poor prognosis, providing a strong rationale for the development of novel therapies with improved safety and/or efficacy profiles.

Naked antibodies have the potential to lyse tumor cells *via* Fc-mediated effector mechanisms such as antibody-dependent cellular cytotoxicity (ADCC) and were developed successfully in hematologic malignancies. Based on their presence on HL tumors, a large number of cell surface proteins such as CD20, CD30, IL13 receptor, CD40, RANK ligand and DR4 are being considered for potential targets for antibody-based immunotherapy for HL. Several antibodies targeting the above receptors are being developed in HL and are at various stages of preclinical and clinical testing (reviewed in ref. [1]). However, most of the antibodies that advanced to the clinic, including anti-CD20 and anti-CD30 antibodies, displayed only limited efficacy when administered as a single agent to HL patients ([2–4] and Table 1).

To improve on the efficacy of naked antibodies, a variety of approaches targeting HL tumor cells are being considered. For example, technologies to enhance the antitumor activities of naked antibodies, such as radioimmunoconjugates, antibody–cytokine and –toxin conjugates as well as bi-specific antibodies are being developed [5]. In general, these antibody derivatives display superior activities compared to naked antibodies when tested in preclinical and clinical studies. However, a prevalence of anti-therapeutic antibodies (ATA) in patients treated with the first generation of murine antibody-based drug conjugates, limited the utility of this approach for prolonged treatment of HL (Table 1) [6]. Immunoconjugates also pose an additional level of complexity based on the differences in the sensitivity of their target cells towards the various classes of cytotoxics employed, ultimately affecting their therapeutic indexes.

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Table 1
CD30 directed immunotherapy in clinical development.

Year, reference	Drug	Sponsor	Study type	*N*	CR + PR	Comments
1992, [79]	Murine anti-CD30-saporin conjugate (Ber-H2/SO6)		Pilot	4	75%	100% ATA
1997, [86]	Murine anti-CD16/CD30		Phase I/II	15	13%	60% ATA
2001, [85]	Murine anti-CD16/30 combined with IL2, GM-CSF		Pilot	16	25%	38% ATA
2002, [87]	Anti-CD64/CD30		Phase I	10	40%	80% ATA
2002, [94]	Murine anti-CD30-ricin-A conjugate (Ki-4.dgA)		Phase I	17	7%	41% ATA
2005, [88]	Murine anti-CD30-^{131}iodine-conjugate		Phase I	22	27%	18% ATA delayed grade 4 cytopenia in 32%
2002, [88]	Chimerized anti-CD30 mAb (cAC10, SGN-30)	Seattle Genetics	Phase I	13	15%	1–15 mg/kg
2003, [67]	cAC10, SGN-30	Seattle Genetics	Phase I	24	4%	2–12 mg/kg
2004, [3]	cAC10, SGN-30	Seatlle Genetics/NCI	Phase II	35	0%	6 mg/kg, qwkx6
2004, [74]	Humanized anti-CD30-mAb (parental 5F11, MDX-060)	Medarex	Phase I/II	72	8%	Qwkx4
2008, [90]	cAC10-auristatin conjugate (cAC10-vcMMAE, SGN-35)	Seattle Genetics	Phase I	39	45%	0% ATA 1.2–2.7 mg/kg, qwkx $\geq$2
2005, [73]	Humanized, effector cell enhanced anti-CD30 mAb (cAC10, AmAb^{TM} 2513)	Xencor	Phase I initiated in 2008			
2007, [75]	Humanized, effector cell enhanced anti-CD30 mAb (parental MDC-060, MDX-1401)	Medarex	Phase II	72	8%	1–15 mg/kg, qwkx4

This table is modified from ref. [6].
There has been a significant increase in the response rate of HL and ALL patients treated with immunotoxin conjugates such as SGN-35, when compared to unarmed antibodies (i.e. SGN-30, MDX-1401 and AmAb^{TM} 2513). The success of the first generation immunoconjugates targeting HL was limited due to the high incidence of ATA: anti-therapeutic antibodies.

To circumvent these limitations, recent clinical trials in Hodgkin's lymphoma employed humanized antibodies or humanized radioimmuno-antibody conjugates, targeting cell surface antigens expressed on HL tumors. In addition, virus specific antigens were targeted by adoptive immunotherapy or vaccine strategies (reviewed in refs. [6,7]) and novel small molecule inhibitors are being developed in HL indications (reviewed in ref. [8]). In this review, we summarize the preclinical and clinical progress made with naked antibodies and antibody–drug conjugates targeting tumor antigens expressed in HL. The main focus is directed towards compounds targeting the CD30 antigen and the response rates reported from early stage clinical trials (Table 1). We also highlight challenges posed by the unique pathobiology of HL (Table 2) and discuss potential ways to overcome some of these limitations by developing the next generation of immunotherapeutics for the treatment of HL patients.

2. Hodgkin's lymphoma, a challenging disease for immunotherapy

A variety of unique biological feature present in HL and summarized in Table 2, may limited the efficacy of first generation biotherapeutics developed for the treatment of HL (summarized in Table 1). Global immunosuppression has long been known to be associated with HL and several mechanisms employed by the tumor itself leading to immunosuppression, were identified previously. Most of the tumor mass in classical HL is comprised of a benign, dense inflammatory infiltrate consisting of B cells, CD4+ T cells, eosinophils, neutrophils, monocytes and macrophages (Fig. 1), surrounding the malignant Hodgkin–Reed–Sternberg (HRS) cells and that is enriched in inhibitory T-regulatory cells [9]. T-regulatory cells induce a profoundly immunosuppressive environment, by targeting effector cell

Table 2
Unique pathobiological features of HL and potential mitigation by therapeutic compounds.

HL pathobiology	Mechanism	Indications	Mitigation	References
Alterations in signaling pathways affecting cell survival	NF-κB activation	HL (RSC), NHL	mAbs/ADCs interfering with NF-κB, combination with NF-κB pathway inhibitors	[35]
	Inhibition of intrinsic and extrinsic apoptotic pathways, alterations in cFLIP, XIAP, BclX_L expression	HL (RSC)	ADCs, combination with targeted compounds activating apoptotic pathways	[36–38]
	MAP/ERK activation	HL (RSC)	ADCs, combination treatment with MAPK inhibitors	[40]
	AP-1 expression	HL, ALCL		[41]
	PI3-K/AKT activity	HL (RSC)	ADCs, combination with PI3-K inhibitors	[42]
Immune evasion	Inhibitory T-regulatory cells	HL	ADCs, chemo combo	[9]
	Th2-type T-cell response	HL (RSC)	ADCs, chemo combo	[95]
	Resistance to CD95	HL (RSC)	siRNA to cFLIP	[12]
	Expression of FAS ligand, RCAS1	HL (RSC)	ADCs	[96,97]
Resistance to chemotherapy	Bcl-2	HL (RSC), NHL	Certain ADCs/mAbs inducing chemo-sensitization, combination anti-Bcl-2 compounds	[98]
Low frequency of RSC, high numbers of inflammatory infiltrates	Insufficient drug delivery	HL	ADCs with bystander activity, combination with cytoreductive agents	[91–93]
Changes in extracellular matrix, solid tumor like appearance	Increased survival of neoplastic cells, pro-angiogenic environment	HL	ADCs, cytoreductive therapy, combination with anti-angiogenic compounds	[15]
Pro-angiogenic environment	Pro-angiogenic cytokine secretion	HL	Combination with anti-angiogenic compounds, chemotherapy	[99]
Low mitotic index of HRS cells	Long G1 phase and expression of abnormally stable cyclin E	HL (RSC)	Prolonged exposure to cytotoxic drugs, including ADCs, mAbs	[13]

A variety of unique biological features of HL may have contributed for the dismal efficacy observed for various biotherapeutic compounds tested in HL and ALL patients. Antibody–drug conjugates may be able to overcome these limitations, as their efficacy is less affected by immune evasion mechanisms, alterations in signaling pathways within HRS cells or changes in the angiogenic phenotype of tumors.

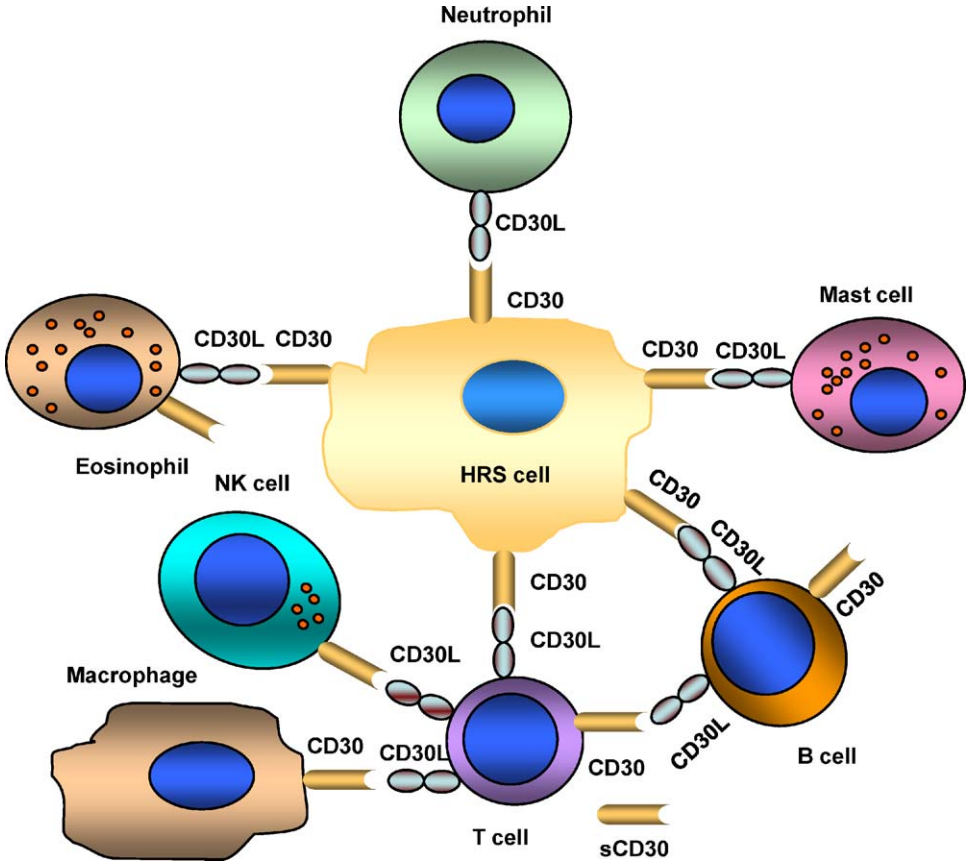

Fig. 1. Expression of CD30 on Reed–Sternberg (RS) cells in HL tumors and expression of CD30 and CD30L on inflammatory cells. SGN-35 blocks ligand–receptor interactions and has the ability to internalize following binding to CD30, leading to the release of the microtubule destabilizing agent monomethyl auristatin E (MMAE). CD30 is present on subsets of activated T cells, B cells, NK cells, eosinophils and monocytes. CD30L expression was found on activated T cells, subsets of activated B cells, neutrophils, eosinophils and mast cells.

immune responses. Their increased presence in HL may provide an explanation for the apparent lack of immune clearance of HRS cells by naked monoclonal antibodies, which are frequently dependent on immune effector cell activation to induce therapeutic effects [10].

Another mechanism potentially contributing to immune evasion are the cytokines and chemokines produced by HL tumors, favoring a Th2-type T cell response. Th2 types of immune responses facilitate evasion from cytotoxic reactions of Th1 type lymphocytes, allowing for immune evasion (reviewed in ref. [11]). Finally, upregulation of immunomodulatory surface receptors, including RCAS1 or Fas ligand on HRS cells can induce death of activated cytotoxic T cells and natural killer (NK) cells (reviewed in ref. [6]). In addition, HRS cell are resistant to CD95 mediated cell death triggered by inflammatory cells [12]. Thus, the presence of several independent immune evasion mechanisms in HL tumors may explain the lack of strong antitumor effects of several experimental therapeutic antibodies developed in early stage clinical trials that engaged effector cell dependent antitumor activities.

A key distinguishing feature of HL is the paucity of malignant HRS cells. Unlike most other malignant tumors, HL is not characterized by high numbers of proliferating tumor cells. HRS cells usually compose only a minor fraction of the tumor mass, with the remainder mainly inflammatory lymphocytes. HRS cells frequently display mitotic defects as well as a high incidence of apoptosis, suggesting that they fail to accumulate due to an intrinsic failure to divide properly [13]. The low mitotic index of HRS cells in HL tumors (0.5%) could at least partially explain the lack of successful tumor cell production [14]. One consequence of these findings is that antibodies directed towards tumor antigens expressed on malignant HRS cells target only between 0.5% and 5% of the cell population. Within HL tumors, the composition of the cell infiltrates is heterogeneous, and the majority of cells are CD4+ lymphocytes. In contrast, chemotherapeutic agents are effective against a variety of mitotic cells, including activated inflammatory lymphocytes within the HL tumor mass. Therefore, the more promiscuous cell targeting properties of cytoreductive agents, targeting infiltrating inflammatory cells, may have contributed to their superior therapeutic effects, mediating tumor de-bulking activities reported to occur in HL patients treated with this class of compounds [7].

Even though HL is classified as a liquid tumor, HL lesions frequently grow within lymph nodes in various organs or tissues. Such disseminated HL lesions have the tendency to form large tumor masses, reminiscent of the structural and morphological characteristics of solid tumors [15]. Certain morphologic features of HL tumors, including the hard packing of cells and the fibrous layers within the nodular tumor mass make HL tumors unique and solid tumor like in appearance and composition. In contrast to small-molecular-weight compounds, macromolecular therapeutics such as antibodies and antibody–drug conjugates, permeate solid tumor masses poorly and unevenly. Typically, only 0.001–0.01% of the injected antibody localizes to the tumor in humans [16]. Such poor tumor distribution is caused by several interrelated factors. First, the dense packing of tumor cells and the fibrous tumor stroma constitute a physical barrier to macromolecular transport. Second, elevation in the interstitial fluid pressure within

tumors hinders extravasation and fluid convection of macromolecular compounds [17,18]. Third, the distribution of antibodies with high binding affinities is often localized to perivascular regions, limiting the amounts of compounds reaching tumor cells at more distant sites and causing poor antitumor efficacy [19]. Therefore, the formation of solid tumor like lesions combined with the low frequency of HRS cells within lymph nodes may cause low exposure of malignant cells to immunotherapeutics, limiting their efficacy in HL patients.

3. CD30 gene expression and function

CD30 is a member of the tumor necrosis factor receptor (TNFR) superfamily that includes TNFR, CD40, Fas (CD95) and OX-40 (CD134), among others (reviewed in ref. [20]). Human CD30 is a type 1 glycoprotein, containing both N- and O-linked sugars, with a molecular weight ranging from 105–120 kDa [21]. The intracellular portion of the protein contains several serine/threonine phosphorylation sites, which regulate cell signaling following receptor ligation. Mature human CD30 is comprised of 577 amino acids, including a 365 amino acid residue extracellular region, a 24 amino acid transmembrane segment, and a 188 amino acid cytoplasmic domain [21]. The pre-processed form of the transmembrane protein includes an additional 18 amino acid signal sequence. Structurally, human CD30 is composed of six cysteine-rich repeats in the extracellular domain, characteristic of this family, interposed with a 60 amino acid partial repeat [22]. An 85 kDa form of CD30, a product of proteolytic cleavage (sCD30) can be found in the blood of patients with CD30 positive lymphomas or autoimmune diseases [23]. An alternatively spliced transcript (isoform 2) was described, encoding a 132 amino acid N-terminal fragment located in the cytoplasm. Between species, the CD30 coding regions are relatively well conserved, with 64%, 58% and 97% sequence identity between human and mouse, rat and chimpanzee, respectively.

In non-pathological conditions, CD30 expression is generally limited to activated B and T lymphocytes and NK cells and generally lower levels of expression were reported for activated monocytes and eosinophils [24]. In addition, CD30 is found on a small percentage of CD8 positive T cells and negligible expression on naïve or resting lymphocytes was described (Table 1) [25]. CD30 expression is induced on T cells following mitogen activation, antigen receptor cross-linking or as a result of viral infection [26]. Histological examination of CD30 expression in normal tissues identified a rare population of large lymphoid cells in sections of lymph node, tonsil, thymus and endometrial cells with decidual changes [27]. Importantly, CD30 expression is absent on most cells outside the immune system (Table 1)

The key biological events regulated by the CD30 signaling pathway include activation of MAP kinases and NF-κB [28,29]. These signals are capable of promoting cell proliferation and survival as well as induction of anti-proliferative responses and cell death, depending on the cell type and the co-stimulatory signals involved [30]. Activation of CD30 as a consequence of CD30 ligand binding or cross-linking by immobilized antibody, induces trimerization of the receptor, recruitment of signaling proteins, ultimately leading to transduction of numerous biological signals such as cell proliferation or apoptosis.

4. Dysregulated CD30 signaling in ALCL and HL

Several types of CD30/CD30L interactions involving HRS cells and benign cells in the tumor microenvironment were postulated to contribute to disease pathobiology (Fig. 1) [31]. Constitutive activation of the NF-κB signaling pathway is a hallmark of HRS cells and critical for cell survival. Evidence in support of this model was provided by studies conducted with HL tumor cell lines, where outcome of the response to CD30 ligation correlated with constitutive activation of the NF-κB [32]. While most of the CD30 antibodies described in the literature triggered direct tumor cell killing against ALCL cells, only two, 5F11 and AC10, triggered cell death against HL cell lines grown in culture [33], and these effects were directly associated with interference with NF-κB signaling. In further support of a critical role for constitutive NF-κB signaling for HL transformation, stimulation of CD30 and concomitant inhibition of NF-κB by bortezomib induced enhanced antitumor effects in both, *in vitro* and *in vivo* model systems of HL [34]. In summary, differences between various anti-CD30 antibodies in their ability to interfere with NF-κB signaling may explain their variability in promoting therapeutic effects when tested against human HL cell lines.

Increasing knowledge of the pathobiology of HL tumor has enabled us to understand that most HRS cells are derived from germinal center B cells and that constitutive activation of nuclear factor kappaB (NF-κB) is a common molecular feature [35]. Therefore, the self-growth-promoting potential of HRS cells caused by constitutive NF-κB signaling may add another layer of complexity when targeting HL. Activation of NF-κB in HRS cells is known to induce expression of anti-apoptotic genes, such as cFLIP [36], XIAP [37] and $BclX_L$ [38], which antagonizes the extrinsic and intrinsic apoptotic pathways in HRS cells. Blockade of the NF-κB signaling pathway may thus be beneficial for therapeutic antibodies to interfere with growth of HL tumors [39]. Cultured and primary HRS cells were also shown to express the active, phosphorylated form of mitogen-activated protein kinase kinase/extracellular signal regulated kinase (MAPK, ERK; p44/42), and inhibition of the upstream kinase ERK was associated with decreased growth of HL cell lines [40]. The AP1 complex is formed by hetero- and homodimers of jun, Fos and other members of the activating transcription factor (ATF) family of proteins. In HL and in ALCL, but not in other lymphoma subtypes, c-jun and junB were found to be aberrantly expressed in the malignant cell population [41]. Finally, inhibitors of the phosphatidylinositol-3 kinase/AKT pathway were shown to induce apoptosis in HRS cells [42]. Combined, such alterations in major signaling pathways within HRS cells and the unique HL biology may have limited the effectiveness of conventional antibody-based immunotherapies and contributed to the persistence of the malignancy (summarized in Table 2). The lack of efficacy of traditional immunotherapeutic approaches in HL emphasizes the need for novel therapeutic strategies that act independently of functional signaling pathways in HRS cells and the dysfunctional immune system within the tumor mass, exemplified by ADCs.

5. Advantages of using antibody–drug conjugates for the treatment of HL

Conceptually, the use of antibody–drug conjugates (ADCs) for the treatment of HL appears attractive, since this approach may help to overcome some of the limitations caused by the unique tumor biology of HL tumors. ADCs mediate antitumor effects independently of inflammatory cells and thus may not be affected by the immune evasion mechanisms which are abundant in HL. The limitations in tumor penetration by macromolecular compounds may be compensated by the superior potencies of ADCs, which require lower exposure levels to achieve therapeutic effects when compared with naked antibodies. Alternatively, the limited access of macromolecules to tumor lesions may be mitigated by combining ADCs with cytoreductive chemotherapeutic agents. The combination of highly active cytotoxic drugs with the selective tumor targeting of antibodies may create a local increase of active drug in malignant cells, which can compensate for the low

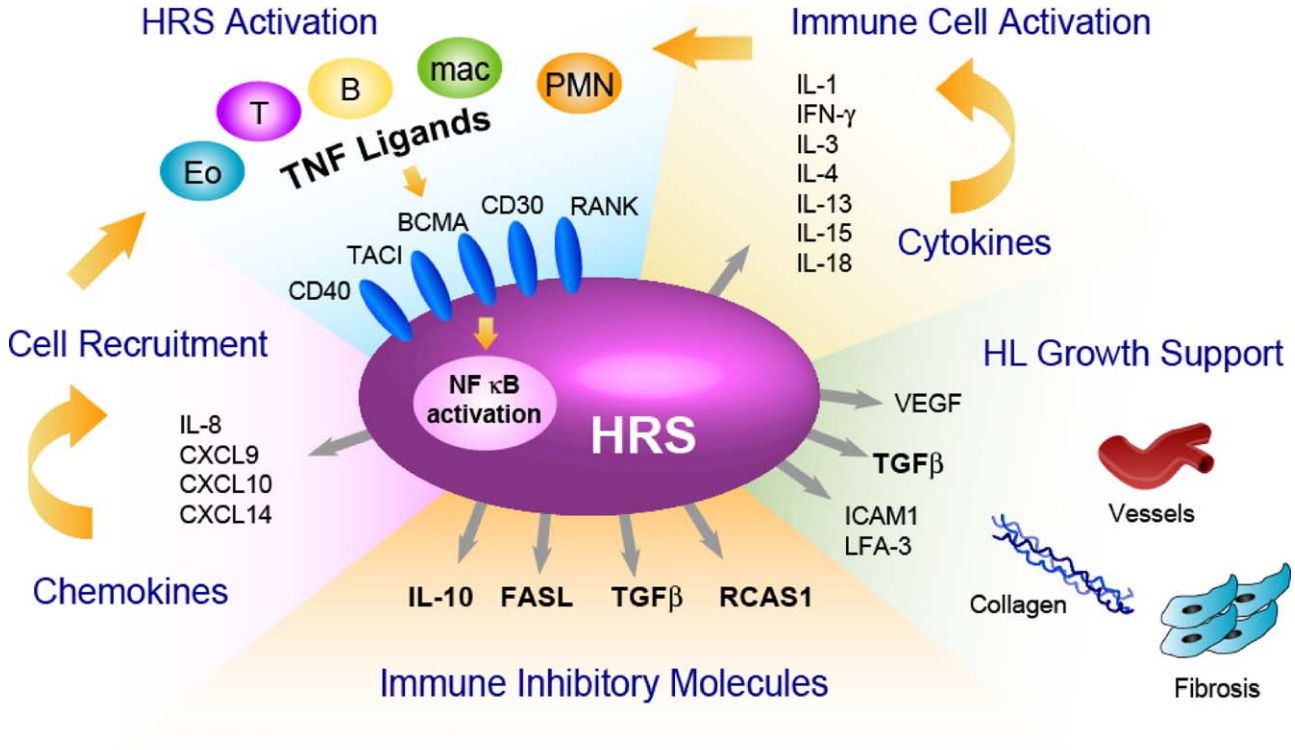

Fig. 2. Complex nature of HL. HRS cells frequently display alteration in signaling pathways associated with increased cell survival and decreased apoptosis and proliferation. As a consequence, HRS cells secrete a variety of chemokines leading to the recruitment of inflammatory cells, which themselves express several members of the TNF family. HRS cells also secrete a variety of cytokines which ultimately induced immune cell activation within the tumor mass. The impaired signaling in evens in HRS cells lead to immune evasion associated with the expression of immune inhibitory molecules and recruitment of stromal cells and matrix depositions, enabling rapid increase in tumor mass.

numbers of malignant HRS cells in HL lesions. Furthermore, interference with NF-κB signaling in HRS cells by the targeting antibody may provide an additional advantage, since constitutive activation of this signaling pathway is associated with malignant transformation of HRS cells. Because of the improved toxicity of ADCs compared to cytotoxic drugs, they can be administered over prolonged periods of time, without the need for treatment holidays. This may be advantageous given that HRS cells display a low mitotic index, and paucity of therapy may allow remaining HRS cells to proliferate during chemo-recovery periods. Finally, the use of a highly cell permeable cytotoxic drug may provide additional bystander effects, which may further reduce the viability of the abundant inflammatory cell infiltrates (Fig. 2).

6. Success of current standard of care therapies for HL and opportunities for improvements

There are four stages of Hodgkin's lymphoma diagnosis, which depend on how far the cancer has spread through the body. Stage I involves the lymph node region, stage II involved two or more lymph node regions on the same side of the diaphragm, stage III involves lymph nodes on both side of the diaphragm and stage IV involves other organs. The ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) chemotherapy regimen is currently a standard of care in the first line treatment of HL [43]. When administered either alone or combined with irradiation therapy, ABVD treatment results in a 2 year survival rate of >90% (reviewed in ref. [7]). The relapse rates after first line ABVD treatment range from 5% in early stage disease to 35% in patients with advanced disease. Patients with relapsed and refractory HL typically receive platinum-based combination chemotherapy salvage regimens followed by high dose chemotherapy and autologous stem cell transplantation [44].

Gemcitabine, a pyrimidine antimetabolite that inhibits DNA synthesis, is an active and reasonably well tolerated therapy for the salvage treatment of HL and is increasingly used for the treatment of relapsed and refractory HL patients due to its favorable safety profile at comparable activity. In relapsed or refractory transplant-naïve patients receiving gemcitabine, a 39% response rate with an overall survival time of 10.7 months in the absence of any overt toxicities was reported [45]. However, the long-term complications of these regimens vary by treatment and modality and include infertility, cardiopulmonary toxicity and secondary malignancies, such as beast cancer and lymphomas [43]. Therefore, novel treatment options with improved therapeutic indices are needed for the treatment of HL, particularly for relapsed or refractory patients. In addition, ADCs keep great promises to reduce systemic toxicity due to their highly selective tumor targeting properties, suggesting that improved toxicity profiles and as a consequence, favorable therapeutic indexes, may be achievable.

7. CD30 a rational target for immunotherapeutic approaches

Although a variety of cell surface targets expressed on HRS cells are being considered for antibody-based immunotherapy [1], the lack of CD30 expression on most cells outside the human immune system makes this surface antigen a particularly attractive target for immunotherapeutic approaches for HL and ALCL, including ADCs. CD30 was originally identified based on its presence as a cell surface antigen that is selectively expressed on HRS cells, the malignant cell type in HL [46]. CD30 is also abundantly expressed on ALCL tumors, on about 10% of non-Hodgkin lymphomas (NHL)

and on certain solid tumors, including embryonal carcinomas and seminomas [47] (reviewed in refs. [48–50]). In non-pathological conditions, CD30 is a marker for cell activation and its expression is limited to activated natural killer (NK) cells, monocytes, eosinophils, small percentages of B and T cells and to a rare population of large lymphoid cells in sections of lymph node, tonsil, thymus and endometrial cells [27]. The ligand for CD30, CD30L, is expressed on a variety of hematopoietic cells, including activated T cells, resting B cells, granulocytes, medulla of the thymus and various leukemia cells [51]. A proteolytic fragment defined as soluble CD30 (sCD30), was identified in the blood of patients with CD30 positive lymphomas or certain autoimmune diseases [23]. Several reports identified correlations between serum sCD30 levels and poor disease prognosis, such as in ALCL [52] and HL [46,53]. Therefore, it was suggested that high serum levels of sCD30 represent an independent predictor of disease progression and poor prognosis for patients with CD30 positive lymphomas.

8. Preclinical development of therapeutic antibodies targeting CD30 in hematological malignancies

Several monoclonal antibodies targeting CD30 were tested for efficacy in preclinical tumor models (Table 1, [54–57]). It is important to note that antibodies targeting members of the TNF family can exhibit both, antagonistic or agonistic signaling functions, depending on their individual abilities to induce oligomerization of the ligands and/or receptors and/or to block ligand–receptor interactions. In addition to triggering direct cell killing, naked therapeutic antibodies can mediate cell death indirectly, *via* ADCC, complement dependent cytotoxicity (CDC) or antigen dependent cellular phagocytosis (ADCP). Importantly, differences in the physicochemical properties of various anti-CD30 antibodies were described, including epitope recognition, binding affinities and effector cell activation characteristics. Therefore, it is likely that each anti-CD30 compound induces a unique set of pharmacodynamic responses. In support of this notion, cross-blocking competition studies conducted with various anti-CD30 antibodies demonstrated that AC10 recognizes a domain on CD30 distinct from that recognized by Ki-1, 5F11, M67 or Ber-H2 [54,58]. These circumstances may help to explain why most of the therapeutic anti-CD30 antibodies interfere potently with growth of ALCL cells, while only two antibodies, AC10 and 5F11, blocked growth of human HL cell lines grown in culture [32,59–61].

The hybridoma derived anti-human CD30 antibody (5F11) directly inhibits the growth of CD30 expressing cell lines *in vitro*, *via* induction of growth inhibitory cell signaling and by engaging antibody-dependent cellular cytotoxicity (ADCC) [54]. *In vivo*, administration of 5F11 to mice implanted with solid or disseminated HD tumor cells (L540cy) resulted in reduced tumor volumes and increased survival of tumor bearing mice [54]. Furthermore, 5F11 displayed increased activity when combined with conventional cytotoxic drugs against a variety of lymphoma cell lines *in vitro*. Most combination treatments revealed at least additive effects and combinations with gemcitabine and etoposide resulting in the most pronounced antitumor effects [57].

In 1994, Gruss et al. generated a monoclonal anti-CD30 antibody termed M67, which inhibited growth of ALCL tumor cells *in vitro*. However, this antibody stimulated the growth of HD cell lines *in vitro* and had no effects on the growth of human HD cell lines grown in mice [55,56]. While most of the therapeutic compounds targeting CD30 were active in models of ALCL, some of them failed to induce cell death when tested on human HD tumor cell lines [55]. A correlation between the antitumor effects of the M67 antibody and the differences in the constitutive NF-κB signaling in ALCL and HD cell lines was demonstrated [32]. Data from *in vitro* studies suggested that ALCL cells undergo apoptosis following exposure to immobilized M67, a finding that was attributed to the inability of these cells to activate the transcription factor NF-κB. In contrast, HD cell lines (L428, KM-H2, L591), which constitutively expressed NF-κB, were not sensitive to M67 treatment.

The chimeric-AC10 has a typical structure of the human IgG_1 subclass and was shown to induce direct antitumor effects by promoting growth arrest and DNA fragmentation of CD30 positive tumor cells [10,62–64]. In addition, ADCP, mediated by macrophages is critical for antitumor activity of cAC10, as revealed from *in vitro* experiments and effector cell ablation studies conducted in a disseminated model of HD (L540cy) *in vivo*. In these experiments, depletion of macrophages almost completely abolished the therapeutic effects of SGN-30, demonstrating that macrophages contribute significantly to SGN-30 activity [10]. Importantly, treatment of HL cell lines with SGN-30 sensitized these cells to conventional chemotherapeutics, including bleomycin, etoposide, and cytarabine, and enhanced antitumor activity was noticed when cAC10 was combined with bleomycin in HL xenografts [33].

Cross-blocking competition studies with AC10 and other anti-CD30 antibodies revealed that AC10 recognizes a domain on CD30 distinct from that recognized by Ki-1, 5F11 or Ber-H2 [54,58]. The AC10 binding domain, designated cluster C, encompasses at least a portion of the CD30L binding site [59]. Cluster A, also involved in the interaction of CD30 and CD30L, was bound by Ber-H2 and 5F11, and a third distinct cluster, Cluster B, was recognized by mAb Ki-1. Combined, these findings suggested that the differences in the pharmacodynamic effects of these CD30 targeting antibodies may be explained by variations in their epitope recognition, binding affinities, differential interference with signaling pathways and other physicochemical properties that are not yet fully understood.

9. Clinical development of anti-CD30 mAbs

Safety and tolerability of the chimeric anti-CD30 antibody (cAC10, also termed SGN-30) was assessed in phase I and phase I/II dose escalation studies in patients with refractory or relapsed CD30+ hematologic malignancies, including HD and NHL [65,66]. SGN-30 was tested at doses of up to 15 mg/kg (single dose) and up to 12 mg/kg (multiple dose) and both regiments were well tolerated in most patients and a maximum tolerated dose was not reached [67]. SGN-30 was also tested in a single agent phase II study in patients with HD [3] and ALCL [68]. Encouraging response rates were achieved in patients with CD30 positive ALCL. Of the 42 patients with systemic ALCL, 39 were evaluable with an overall response rate of 18% (2 complete responses (CRs), 5 partial responses (PRs) and 5 stable diseases (SDs)) [68–70]. More pronounced responses to SGN-30 treatment were reported in patients with cutaneous ALCL. In a phase II trial, 19 patients were enrolled, and 17 were evaluable with an overall response rate of 58% (5 CRs, 5 PRs and 1 SD) [70,71]. Phase II studies combining SGN-30 with chemotherapy have been initiated in HD and systemic ALCL. The National Cancer Institute (NCI) is currently sponsoring a phase II trial of SGN-30 in combination with GVD chemotherapy (gemcitabine, vinorelbine and doxil) in relapsed/refractory HD patients. The NCI is also sponsoring a phase II trial of SGN-30 plus CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) in newly diagnosed patients with systemic ALCL [70].

Two different anti-CD30 antibodies are currently being developed in the clinic, 5F11 (MDX-060, Medarex [72]) and XmAb2513 (Xencor [73]). MDX-60 is a fully human IgG1k monoclonal antibody that mediates killing of HD and ALCL cell lines *in vitro* and xenograft models *in vivo* [54]. MDX-060 was tested in phase I/II studies in patients with relapsed or refractory HD and ALCL and other CD30 positive lymphomas. Dosing of MDX-060 in 4 weekly

doses of 0.1–10 mg/kg did not induce dose limiting toxicities. However, two drug related serious adverse events including one liver transaminase elevation and a patient with respiratory distress syndrome were reported. Among 48 HD patients treated with MDX-060 at dose levels between 10 and 15 mg/kg, three patients displayed objective clinical responses [7,74,75]. These preliminary results suggested that MDX-060 is well tolerated and has clinical activity (Table 2).

10. Improved antibody–drug conjugates targeting CD30 offer promise for HL

SGN-35 is an ADC consisting of the chimeric anti-CD30 antibody cAC10 chemically conjugated to the tubulin destabilizer monomethylauristatin E (MMAE), a synthetic analog of the naturally occurring cytotoxic agent dolastatin 10. One of the critical features of SGN-35 is the dipeptide valine-citrulline linker, which is cleaved by lysosomal enzymes upon conjugate internalization to the lysosomal compartment. The stability of the resulting ADC is improved compared to disulfide and hydrazone based linkers described previously, in that the half life of drug elimination for circulating cAC10-vcMMAE in mice and monkeys is 6 and 10 days, respectively [76]. SGN-35 combines at least 4 key mechanisms of action contributing to antitumor activity: (1) interference with NF-κB cell signaling, (2) sensitization of tumor cell to chemotherapy, (3) cytotoxic activity of the microtubule destabilizing agent monomethyl auristatin E (MMAE), (4) targeting of malignant HL tumors cells and potentially inflammatory infiltrates, *via* bystander activity of the released drug.

11. Therapeutic effects of immunoconjugates administered as single agent and in combination with chemotherapy in preclinical models of HL and ALCL

SGN-35 was tested in models representing ALCL and HL and improved efficacy relative to the unconjugated antibody cAC10 was demonstrated [77–79]. In both low and high burden tumor models, complete regressions were obtained following SGN-35 administration at doses as low as 1 mg/kg [80,81]. When SGN-35 was combined with ABVD at doses close to the maximal tolerated dose (MTD), additive therapeutic effects were noticed [82]. In a preclinical model of HL, the combination of SGN-35 with gemcitabine, when administered close to MTD greatly enhanced the antitumor effects and induced durable responses in all animals [82]. Complete responses in models of HL and ALCL were also obtained when treatment was initiated at a tumor size of 300 mm^3. These preclinical data suggest that SGN-35 administered as single agent or in combination with chemotherapy, represents a promising new therapeutic strategy to improve efficacy and to reduce long-term toxicity of current standard of care treatment modalities in HL and ALCL.

12. Clinical development of anti-CD30 immunoconjugates

In 1992, Falini et al. tested the murine anti-CD30 mAb Ber-H2 in early stage clinical trials in HD patients [83]. Despite the successful *in vivo* targeting of malignant tumor cells as assessed by immunohistological analysis of tumor biopsies and immunoscintigraphy, there was no evidence of tumor regression in response to Ber-H2 treatment. To enhance the efficacy in HD and ALCL, modified murine anti-CD30 mAbs were developed with improved potency to kill tumor cells. These approaches included the covalent conjugation of saporin (SO6), a type-1 ribosome-inactivating protein, to Ber-H2 [79], Ki-4.dgA [84], or bi-specific constructs HRS-3/A9 [85,86] and H22xKi-4 [87]. Alternatively, murine MAbs were conjugated with radionucleotides to generate radioimmunoconjugates such as ^{131}I-Ki-4 and tested in HD patients [88]. The pharmacological effects of these compounds were only transient ($\leq$2 months), and no objective responses were reported. These early trials were conducted using murine antibodies and high levels of human anti-mouse antibodies (HAMA) were identified in the sera of most patients. Bi-specific antibodies targeting two cell surface antigens (anti-CD16/anti-CD30) were also tested in early stage trials involving 15 patients with HD. Four out of nine patients displayed a clinical response, among them, one complete response, one partial response and two patients with stable disease were reported. However 9 out of 15 patient sera were positive for HAMA [86,89]. Overall, these early attempts to target CD30 positive hematologic malignancies in clinical trials failed, possibly due the induction of neutralizing HAMA antibodies.

A total of 39 patients with relapsed or refractory hematologic malignancies were treated in a phase I trial with increasing doses of SGN-35. Outpatient infusions of SGN-35 were generally well tolerated. Among 22 evaluable patients treated at doses $\geq$1.2 mg/kg, a clinical benefit (CR + PR + SD) was observed in 19 patients (86%). Objective responses (CR + PR) were observed in 10 patients (45%), with complete responses (CR) in 5 patients (23%). The high frequency in objective responses obtained in heavily pretreated lymphoma patients provided indirect evidence of selective tumor targeting by SGN-35 [90]. A phase II study with the goal to evaluate MTD and response evolution in patients treated every 3 weeks is currently ongoing and a second phase I trial was initiated, testing weekly dosing schedules.

13. Conclusions

In contrast to most neoplastic malignancies, the pathology of HL is not caused by rapid accumulation of malignant cells, but rather by the accumulation of reactive lymphocytes and perturbation of cellular and humoral immunity, which may have limited the therapeutic activities of conventional immunotherapeutics in this indication. By design, ADCs appear ideally suited to overcome several limitations posed by the complex biology of HL tumors. ADCs combine highly potent cytotoxic molecules with the tumor selectivity of monoclonal antibodies. Importantly, ADCs function independently of immune effector cells, which may be critical to overcome the immune evasive environment of HL lesion. Furthermore, ADCs allow for prolonged drug exposure compared to chemotherapeutic regiments, which frequently require recovery periods between treatment cycles. Such prolonged, continuous exposure may be required for efficient eradication of transformed HRS cells, which display a low mitotic index and fail to accumulate within tumors [13].

The tumor antigen CD30 is selectively expressed on HRS cells and both, cAC10 and 5F11 antibodies were shown to bind to CD30 and to interfere directly with NF-κB signaling in malignant cells, sensitizing tumors to chemotherapeutic agents [33,82]. In addition, the bystander effect provided by certain drug linker combinations such as the vc-MMAE compound, which is applied for SGN-35, may provide additional benefit by targeting inflammatory cell infiltrates after the release of active drug by the tumor cells. The favorable tolerability and promising activity of the anti-CD30 ADC SGN-35 observed in phase I dose escalation studies in HL patients generated a strong rational for future clinical development as single agent or combined with chemotherapy for the treatment of first line, relapsed or refractory HL and ALCL patients. Pending the outcome of ongoing clinical studies, it is possible that approximately 30 years after the original identification of CD30 as a diagnostic marker in HL [91–93], an antibody–drug conjugate targeting CD30 positive HRS cells may help to improve the survival of HL patients.

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