ANTIBODY DERIVATIVES AS NEW THERAPEUTICS FOR HEMATOLOGIC MALIGNANCIES

Advances in the treatment of hematologic malignancies using immunoconjugates

Maria Corinna Palanca-Wessels[1,2] and Oliver W. Press[1,3,4]

[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA; and [2]Hematology and [3]Oncology Divisions, Department of Medicine, and [4]Department of Bioengineering and Center for Intracellular Delivery of Biologics, University of Washington, Seattle, WA

Monoclonal antibody therapy has revolutionized cancer treatment by significantly improving patient survival both in solid tumors and hematologic malignancies. Recent technological advances have increased the effectiveness of immunotherapy leading to its broader application in diverse treatment settings. Immunoconjugates (ICs) consist of a cytotoxic effector covalently linked to a monoclonal antibody that enables the targeted delivery of its therapeutic payload to tumors based on cell-surface receptor recognition. ICs are classified into 3 groups based on their effector type: immunotoxins (protein toxin), radioimmunoconjugates (radionuclide), and antibody drug conjugates (small-molecule drug). Optimization of each individual component of an IC (antibody, linker, and effector) is essential for therapeutic efficacy. Clinical trials have been conducted to investigate the effectiveness of ICs in hematologic malignancies both as monotherapy and in multiagent regimens in relapsed/refractory disease as well as frontline settings. These studies have yielded encouraging results particularly in lymphoma. ICs comprise an exciting group of therapeutics that promise to play an increasingly important role in the management of hematologic malignancies. (*Blood*. 2014;123(15):2293-2301)

Introduction

A formidable challenge in curing cancer is the difficulty in administering a sufficiently high dose of tumoricidal agents to eradicate systemic disease while minimizing adverse effects on normal tissues. Tumor-targeted delivery can effectively increase the amount of cytotoxic agent that can be safely given and thereby improve patient survival. Development of a therapeutic with the ability to home to a malignant cell based on surface receptors was realized with the advent of monoclonal antibody therapy.[1] Although it required over 20 years from the description of hybridoma technology by Kohler and Millstein to the 1997 US Food and Drug Administration (FDA) approval of rituximab for B-cell non-Hodgkin lymphoma (NHL), unconjugated antibodies have proven to be an essential component of many contemporary treatment regimens for hematologic malignancies.[2,3]

The ascendance of immunotherapy has not been without obstacles. Initial enthusiasm for antibodies as "magic bullets" was quickly tempered by the realization that immunoglobulins of murine origin were highly immunogenic and neutralized by the same tumor immune surveillance system that these agents sought to enhance.[4] Efforts to humanize murine-derived antibodies and create fully human antibodies have largely overcome this impediment.[5,6] Unconjugated antibodies such as rituximab exert antitumor effects through complement- or antibody-dependent cell–mediated cytotoxicity facilitated by Fc binding and by activation of apoptotic pathways by cognate antigen binding. Most antibodies exhibit only modest efficacy as single agents and have generally been used in combination with chemotherapy. Attempts to augment antibody activity have included modifications of the immunoglobulin scaffold to enhance immune activation or trigger direct cell death.[7-9]

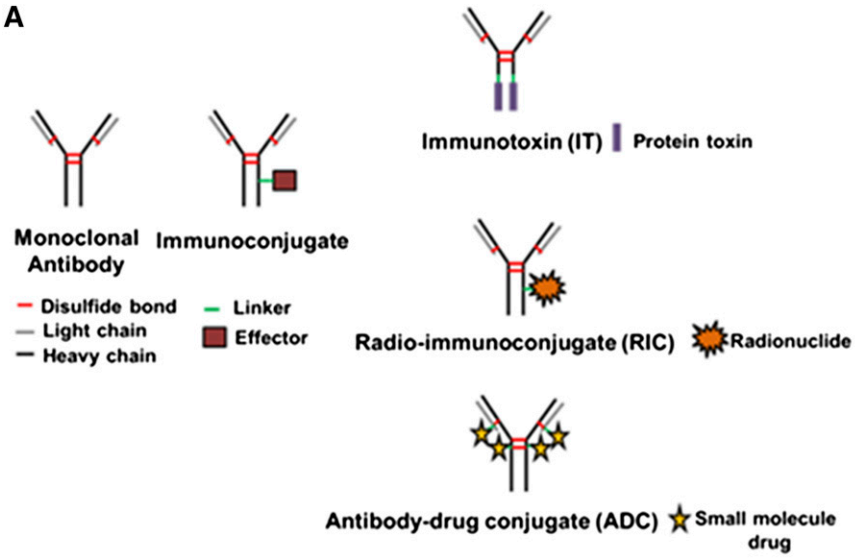
Immunoconjugates (ICs) harness the targeting function of antibodies to specifically deliver a lethal payload to cancer cells.[10-12] ICs rely upon a covalently attached effector moiety for therapeutic activity. The effector type classifies ICs into 3 general groups: immunotoxins (ITs), radioimmunoconjugates (RICs), and antibody drug conjugates (ADCs) (Figure 1A). Antibody targeting focuses higher concentrations of the covalently linked toxin, radionuclide, or small-molecule drug to the tumor while reducing exposure to normal tissues, effectively expanding the therapeutic window. In this review, we emphasize the progress in using RICs and ADCs for the treatment of hematologic malignancies. An accompanying article in this series will focus specifically on ITs.

Features of an IC

An IC consists of: (1) the targeting antibody, (2) the effector molecule, and (3) the linker joining the effector to the antibody. Each part plays an essential role in defining the therapeutic activity of the IC.[10,11]

Several factors are critically important in the selection of an antibody and its cognate cancer antigen or receptor. Ideally, the antigen is preferentially expressed at a high level by neoplastic cells, located on the cell surface with minimal shedding into the surrounding environment and internalizes either constitutively or upon antibody binding (Figure 1B). The latter is critical for ADCs and ITs which carry effectors that inhibit intracellular targets but less so for RICs which emit β- or α-particles that are not restricted by membrane

Submitted October 29, 2013; accepted December 31, 2013. Prepublished online as *Blood* First Edition paper, February 27, 2014; DOI 10.1182/blood-2013-10-492223.



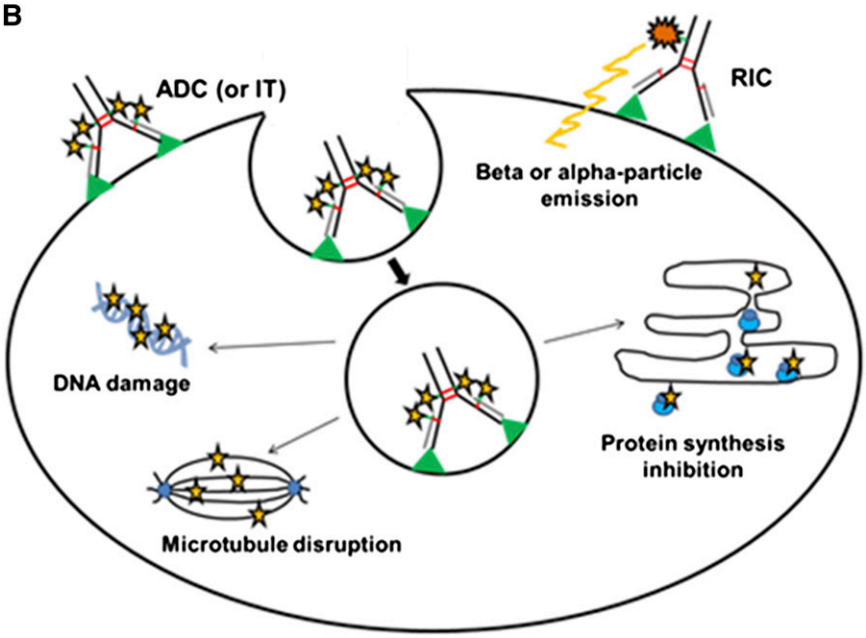


Figure 1. IC structure and mechanism of action. (A) IC types. Schematic diagrams of both a monoclonal antibody and an IC are depicted. An IC consists of a monoclonal antibody, linker, and effector molecule. The 3 general categories of ICs linked to different effector molecules are shown. An IT contains a protein toxin while an RIC possesses a radionuclide. An ADC carries a small-drug molecule. (B) Mechanism of IC activity. The mechanisms of action for the various ICs are illustrated. All ICs recognize and bind to a cognate tumor antigen or receptor. For ITs and ADCs, internalization via receptor-mediated endocytosis is required for entry into the target cell. Subsequent release of the effector moiety from the IC occurs via the conditional cleavage of the linker or protease degradation of the antibody within the endosomal/lysosomal compartment. The released effector toxin or drug diffuses into the cytoplasm and inhibits tumor growth by disruption of microtubules (ADC), damage to DNA (ADC), or inhibition of protein synthesis (IT). For RICs, internalization is not required for cell penetration and damage by the emitted α- or β-particles from the effector radionuclide.

barriers. Endocytic uptake is in fact detrimental for RICs containing iodine-131 (^{131}I) due to lysosomal degradation and release of free ^{131}I or ^{131}I-tyrosine into the blood.[13]

An ideal antibody penetrates quickly and homogeneously into tumor tissue and is rapidly cleared from systemic circulation after maximal binding of available receptors. The antibody need not possess intrinsic antitumor activity because this is conferred by the effector molecule although affinity maturation can improve antibody binding efficiency and potentiate IC activity.[14] Targets investigated for hematologic malignancies include the internalizing receptors CD19, CD22, CD30, CD33, and CD79b as well as the more surface stable receptors CD20 and CD45.

ICs are differentiated by their effector type: protein toxin (IT), radionuclide (RIC), or small-molecule drug (ADC). Judicious selection, modification, and conjugation of effector molecules can enhance IC efficacy. A potent effector is essential because cellular delivery is limited by the number of surface-bound ICs. Most effector molecules are too toxic to use without conjugation and are delivered by ICs in a prodrug form. Synthetic derivatives of natural compounds with enhanced toxicity such as maytansinoids or auristatins have commonly been used.[15,16] For RICs, ionizing radiation affects not only the bound cell but neighboring cells as well ("crossfire effect"), therefore the use of α-emitting radionuclides with higher energy and shorter path lengths than the more commonly used β-emitters is being investigated.[17,18] Protein engineering can remove immunogenic sequences from ITs that generate neutralizing antibodies. Modification of a drug to a membrane-impermeable form can reduce toxicity stemming from nonspecific uptake of unconjugated effector or premature diffusion out of the target cell after release.[19] The number of effector molecules conjugated and their position within the antibody can affect aggregation, antigen binding, and clearance from the circulation as well as potency and tolerability.[20]

Advances in linker technology have greatly accelerated the development of potent ICs.[16,19,21] An ideal linker prevents premature

Table 1. RICs

Antibody	Target	Isotope	Indication	Stage of development
Anti-Tac antibody (^{90}Y-HAT)	CD25	^{90}Y	T-cell NHL, HL	Phase 1 NCT00001575
BB4 antibody	CD138	^{131}I	MM	Phase 1 NCT01296204
BC8 antibody-streptavidin conjugate	CD45	^{131}I, ^{90}Y	AML, ALL, MDS	Phase 1 NCT00988715
Daclizumab (CHX-A daclizumab)	CD25	^{90}Y	HL	Phase 1/2 NCT01468311
Epratuzumab	CD22	^{90}Y	B-cell NHL, WM	Phase 1/2 NCT01101581, NCT00004107
Ibritumomab tiuxetan	CD20	^{90}Y	B-cell NHL	Approved 2002
Lintuzumab	CD33	^{225}Ac	AML	Phase 1/2 NCT01756677
Tositumomab	CD20	^{131}I	B-cell NHL	Approved 2003; to be discontinued February 2014

HAT, humanized anti-Tac; WM, Waldenstrom macroglobulinemia.

effector release in the circulation yet permits its liberation in the tumor. Unstable linkers lead to nonspecific distribution or rapid clearance accompanied by either intolerable toxicity or reduced potency. ITs and ADCs are typically internalized by receptor-mediated endocytosis and trafficked to the lysosome. Cleavable linkers conditionally release the cytotoxic agent in the presence of a reducing environment (disulfide bond), acid (hydrazone linkage), or lysosomal enzymes (peptide bond) in the endocytic compartment. In contrast, noncleavable linkers (thioether or hindered disulfide bonds) rely upon degradation of the antibody to its constituent amino acids in the lysosome for cytotoxin release. Modification of amino acid residues to control conjugation sites or recombinant DNA technology to generate fusion proteins can overcome difficulties associated with the production of heterogeneous species by traditional chemical conjugation approaches.[22] The latter is an inherent advantage of third-generation recombinant ITs and permits large-scale purification from *Escherichia coli* bulk cultures contributing to reduced complexity of manufacturing and lower production cost compared with chemically conjugated ADCs.[23]

RICs

Radioimmunotherapy (RIT) has proven effectiveness in hematologic malignancies. The most extensive clinical experience has been with RICs containing the β-particle–emitting isotopes ^{131}I or ^{90}yttrium (^{90}Y) which possess advantageous characteristics including favorable emission profiles, availability, and stable antibody attachment (Table 1). Initial studies in the early 1990s used ^{131}I-labeled monoclonal anti-CD20 antibodies for the treatment of NHL.[24,25] The long path length of emitted β-particles produces an advantageous "crossfire effect" on nearby cancer cells not expressing target antigen, though this phenomenon can also produce toxicities in neighboring normal tissues. In contrast, α-particle–emitting radionuclides possess shorter path lengths, exhibit less oxygen dependency for cell killing, and confer a higher linear energy transfer resulting in greater cytotoxicity. However, the limited availability, more difficult radiolabeling chemistry, and short half-lives of most α-emitters have limited their clinical utility to date. Only a few α-emitters, like ^{213}bismuth (^{213}Bi), ^{211}astatine (^{211}At), and ^{225}actinium (^{225}Ac), are practical for clinical use.

To date, RIT has demonstrated the most efficacy in NHL.[26] The only RICs currently approved by the FDA are ^{131}I-tositumomab and ^{90}Y-ibritumomab tiuxetan, which both target CD20, a lineage-specific tetrapass phosphoprotein expressed on normal and malignant B lymphocytes. ^{90}Y-ibritumomab is approved for treatment of relapsed/refractory low-grade B-cell NHL or follicular lymphoma (FL) or previously untreated FL after partial response (PR) or complete response (CR) to initial chemotherapy. ^{131}I-tositumomab is approved for similar indications as well as for transformed and rituximab-resistant or refractory NHL. Targeting CD20 with RICs labeled with either ^{131}I- or ^{90}Y-radioisotopes achieves high overall response rate (ORR) and CR rates (50%-80% and 20%-40%, respectively) in extensively pretreated and refractory patients with low-grade or transformed NHL.[27,28] Toxicity is generally minor, with delayed myelosuppression occurring 4 to 8 weeks later being the dose-limiting toxicity. Delayed myelodysplasia (MDS) and secondary acute myelogenous leukemia (AML) are uncommon but potentially serious late sequelae of RIT. CD22 has also been examined as a target for RIT of NHL. Fractionated doses of ^{90}Y-epratuzumab were administered to patients with relapsed/refractory NHL as a single agent with an ORR of 62% (48% CR) and a median progression-free survival (PFS) of 9.5 months.[29] Dual-targeted RIC and unlabeled antibody has been explored.[30] Combining ^{90}Y-epratuzumab with the anti-CD20 antibody veltuzumab was well-tolerated and yielded an ORR of 53% in relapsed/refractory aggressive NHL.[31]

Incorporating RIT into frontline therapy has also been investigated. A phase 2 study administering a single therapeutic dose of ^{131}I-tositumomab as initial therapy for advanced FL yielded a remarkable 95% ORR (75% CR) and a median PFS of 6.1 years.[32] A phase 2 study of cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP) chemotherapy followed by ^{131}I-tositumomab (SWOG 9911) showed excellent results with an ORR of 91% (69% CR) in patients with previously untreated FL with 60% of patients remaining progression-free for >10 years.[33] A subsequent phase 3 trial (SWOG S0016) randomized newly diagnosed advanced-stage FL patients to CHOP plus rituximab (CHOP-R) for 6 cycles vs CHOP for 6 cycles followed by consolidation with ^{131}I-tositumomab (CHOP-RIT).[34] There was a trend toward a better 5-year PFS favoring the RIT group (76% CHOP-R vs 80% CHOP-RIT) but it did not reach statistical significance, nor was there an improvement in overall survival (OS) (97% CHOP-R and 93% CHOP-RIT after a median follow-up of 4.9 years). Phase 2 studies have also examined the utility of ^{90}Y-ibritumomab as either a single agent or following chemotherapy in the frontline treatment of FL.[35-37] Frontline monotherapy produced an ORR of 87% (56% CR) with a PFS of 26 months after follow-up of 30.6 months.[38] Results of a phase 3 trial using ^{90}Y-ibritumomab as consolidation after first remission in advanced-stage FL showed an 8-year PFS of 41% for patients receiving RIT consolidation compared with 22% for patients in the control arm not receiving RIT ($P < .001$). The time-to-next treatment was prolonged by 5.1 years in patients receiving RIT, although the OS rates were similar.[39] There was a higher annualized incidence rate of MDS/AML in the ^{90}Y-ibritumomab–treated group (0.50% vs 0.07%; $P = .042$).

RIT has been studied in the setting of hematopoietic stem cell transplantation (HSCT) in hopes of improving durable responses. Early studies using myeloablative doses of ^{131}I–anti-CD20 RICs

Table 2. ADCs

Antibody	Target	Drug	Indication	Stage of development
BV	CD30	Monomethyl auristatin E	HL, ALCL	Approved 2011
BT062	CD138	DM4 (Maytansinoid)	MM	Phase 2 NCT01001442, NCT01638936
Polatuzumab vedotin (DCDS4501A)	CD79b	Monomethyl auristatin E	DLBCL, FL	Phase 2 NCT01691898
GO	CD33	Calicheamicin	AML	Approved 2000; withdrawn June 2010
INO (CMC-544)	CD22	Calicheamicin	B-cell NHL, B-cell ALL	Phase 3 NCT01564784, NCT01232556
IMGN529	CD37	DM1 (Maytansinoid)	B-cell NHL, B-cell CLL	Phase 1 NCT01534715
Milatuzumab-doxorubicin (hLL1-Dox; IMMU-110)	CD74	Doxorubicin	MM, CLL, NHL	Phase 1/2 NCT01101594
PV (DCDT2980S)	CD22	Monomethyl auristatin E	DLBCL, FL	Phase 2 NCT01691898
SAR-3419	CD19	DM4 (Maytansinoid)	DLBCL, B-cell ALL	Phase 2 NCT01472887, NCT01440179
SGN-CD19A	CD19	Monomethyl auristatin F	B-cell NHL, B-cell ALL	Phase 1 NCT01786135, NCT01786096
SGN-CD33A	CD33	Pyrrolobenzodiazepine dimer	AML	Phase 1 NCT01902329

ALCL, anaplastic large cell lymphoma; CLL, chronic lymphocytic leukemia.

(approximately fivefold higher doses of ^{131}I than conventional RIT) followed by autologous HSCT showed objective remissions in 85% to 95% of patients with multiply relapsed/refractory B-cell NHL and demonstrated durable 10- to 20-year remissions in 40% to 50% of patients.[24,40,41] This approach has subsequently been validated using ^{90}Y-ibritumomab with equally promising results.[42-44] However, a recent phase 3 trial adding conventional, low doses of ^{131}I-tositumomab to the BEAM regimen (BiCNU [carmustine], etoposide, cytarabine [Ara-C], melphalan) in the setting of autologous HSCT for relapsed/refractory diffuse large B cell lymphoma (DLBCL) did not improve outcomes compared with the control arm (BEAM-rituximab).[45] Conversely, a randomized phase 2 trial of ^{90}Y-ibritumomab added to BEAM showed a significantly improved OS for patients receiving BEAM-RIT compared with control patients receiving BEAM alone (92% vs 61%, $P = .05$).[46] A confirmatory phase 3 trial is currently under way (NCT00463463).

The utility of RIT for other hematologic malignancies is being actively explored. RICs targeting CD33, CD45, or CD66 for AML have been examined.[47-54] Early-phase clinical trials studying ^{131}I- or ^{213}Bi-conjugated to the humanized anti-CD33 antibody lintuzumab (HuM195) showed tolerability and moderate efficacy in AML patients.[47,53,54] To circumvent the short 46-minute half-life of ^{213}Bi, ^{225}Ac has been used in subsequent phase 1/2 trials of RIT with lintuzumab for AML. A series of phase 1/2 studies combining ^{131}I-BC8 anti-CD45 antibody with allogeneic HSCT for AML, acute lymphoblastic leukemia (ALL), and MDS has demonstrated the feasibility, safety, and efficacy of this approach.[48,49] A phase 1 dosimetry study showed the feasibility of targeting CD138 in multiple myeloma (MM)[55] and an ^{131}I-CD5 antibody has been investigated for cutaneous T-cell lymphoma.[56]

Multistep pretargeted RIT (PRIT) is a strategy to improve tumor-to-organ ratios of absorbed radioactivity compared with conventional 1-step RIT by separating the slow distribution phase of the antibody from administration of the radionuclide. Nonradiolabeled antibody is administered and allowed to bind at tumor sites then followed by the infusion of a radioisotope which has a high affinity for a conjugated adaptor molecule on the antibody. Radiation exposure to normal organs is limited as the small radioisotope can quickly penetrate the tumor while the unbound radiolabeled ligand is rapidly cleared from the circulation through renal excretion. Addition of a "clearing agent" before the second step can further improve specificity by complexation of excess unbound antibody in the bloodstream, which is subsequently removed by hepatic receptors recognizing the complexes. Several preclinical studies have validated the advantages of this approach utilizing the affinity of streptavidin or avidin for biotin.[57-62] Other attractive PRIT strategies use bispecific (antitumor × antiligand) antibodies,[63,64] "dock and lock" methods that exploit binding between the regulatory subunits of cAMP-dependent protein kinase and the anchoring domains of A-kinase anchor proteins,[65,66] complementary hybridization of phosphorodiamidate morpholino oligomers (MORFs),[67] or cyclooctene-modified antibodies with radiolabeled tetrazine ligands.[68] Early trials investigating PRIT have yielded encouraging results in hematologic malignancies.[69,70] Four of 7 patients with advanced NHL who had failed multiple prior therapies including HSCT and were treated with CD20-streptavidin conjugate and ^{90}Y-DOTA-biotin PRIT had objective responses (3 CR and 1 PR).[70] A phase 1 trial of PRIT in AML patients using anti-CD45 antibody (BC8) streptavidin conjugate and ^{90}Y-DOTA biotin prior to total body irradiation and allogeneic HSCT is ongoing (NCT00988715).

Regrettably, despite encouraging clinical results, RIT has not been widely embraced as a treatment modality. The recent decision to discontinue manufacture and distribution of ^{131}I-tositumomab in February 2014 was based on the anticipated decline in its use as a result of the recent emergence of multiple other alternatives for relapsed/refractory NHL, including bendamustine, ibrutinib, idelalisib, and ABT-199. Logistical issues involving the transfer of care from the treating oncologist/hematologist to the nuclear medicine physician, economic concerns about insufficient reimbursement and expense, and an exaggerated emphasis on delayed effects such as marrow damage and secondary malignancies have contributed to the limited use of RIT.[71] Importantly, the inability to administer RIT locally at community practice sites with the resultant need for referral to distant centers has been a major economic disincentive. Although the development of strategies to further improve RIT efficacy and extend its use to other hematologic malignancies is continuing, reducing the logistic hurdles to RIT administration will be essential for more widespread adoption of the next generation of RICs.

ADCs

ADCs are inarguably the most active current area of IC development. Although the voluntary withdrawal in 2010 of the first approved ADC for the treatment of a hematologic malignancy (gemtuzumab ozogamicin [GO]) transiently diminished the enthusiasm for ADCs, the approval of brentuximab vedotin a year later, as well as adotrastuzumab emtansine for metastatic breast cancer in early 2013, has buoyed the ADC field. Multiple ADCs are in clinical development (Table 2). Targets include CD19, CD22, CD33, and CD79b. Several

recent clinical trials have demonstrated the therapeutic promise of ADCs for a variety of malignancies.[11]

GO retains the dubious distinction of being both the first ADC approved under an accelerated approval program in May 2000 and the first withdrawn 10 years later. It is composed of a humanized anti-CD33 antibody linked to calicheamicin via an acid-labile hydrazone linker. It was approved on the basis of multicenter phase 2 trials demonstrating its efficacy and safety in 141 AML patients in first relapse with an ORR of 30% (16% CR).[72] A confirmatory phase 3 trial in 2004 was initiated to determine whether addition of GO to induction and postconsolidation therapy improved OS in newly diagnosed younger AML patients. The trial was halted after no clinical benefit was demonstrated and more deaths were observed due to liver toxicity in the GO plus chemotherapy arm than in the arm with chemotherapy alone.[73] Although GO was withdrawn in 2010, subsequent studies have strongly suggested a benefit in a defined AML patient population raising hope that this ADC may be resurrected for use in the future.[74]

Brentuximab vedotin (BV; SGN-35) was approved in 2011 for treatment of relapsed/refractory Hodgkin lymphoma (HL) and systemic anaplastic large cell lymphoma (sALCL). It is composed of a chimeric anti-CD30 antibody linked to the microtubule inhibitor monomethyl auristatin E (MMAE) via a protease-cleavable linker. The development of BV was recently reviewed.[75] The parental unconjugated anti-CD30 antibody (SGN-30) exhibited modest efficacy in phase 2 studies with clinical responses observed in 7 of 41 sALCL patients and 0 of 38 HL patients.[76] In contrast, the pivotal phase 2 studies administering BV demonstrated impressive clinical activity, including an ORR of 80% (57% CR) in patients with relapsed/refractory sALCL[77] and an ORR of 75% (34% CR) in relapsed/refractory HL.[78] Common adverse events ($\geq$10%) reported in both studies included peripheral sensory neuropathy, nausea, fatigue, neutropenia, pyrexia, diarrhea, emesis, pruritis, myalgia, and alopecia. The most common grade $\geq$3 toxicities included neutropenia (20%-21%), peripheral sensory neuropathy (8%-12%), and thrombocytopenia (14% in sALCL). Addition of BV to frontline chemotherapy regimens is the subject of ongoing clinical trials in HL and sALCL.[79,80] Phase 1 study results of 26 previously untreated sALCL patients receiving BV at the standard 1.8 mg/kg dose combined with standard dose CHP (cyclophosphamide, doxorubicin, and prednisone) yielded an ORR of 100% (88% CR).[79] Interim phase 1 study results combining BV with ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) or AVD (doxorubicin, vinblastine, dacarbazine) in newly diagnosed advanced stage HL patients showed tolerability up to 1.2 mg/kg of BV.[80] Pulmonary adverse events were observed in 7 of 25 patients on the combination ABVD arm leading to omission of bleomycin from subsequent cycles of therapy, though 5 of the 7 were able to safely continue treatment with BV plus AVD. All 10 patients who had completed therapy achieved CR. Phase 3 studies investigating frontline use of ABVD vs BV combined with AVD in advanced classical HL (NCT01712490) or combined with CHP vs CHOP in CD30-positive mature T-cell lymphomas (NCT01777152) are ongoing. Additional studies have suggested utility in other settings including relapse after allogeneic HSCT.[81] CD30 expression identifies a unique subset of DLBCL[82] and BV is being explored both as monotherapy in relapsed/refractory DLBCL (NCT01421667) and as frontline therapy with R-CHOP (NCT01925612).

Another promising ADC in clinical development is inotuzumab ozogamicin (INO; CMC-544). INO consists of a humanized IgG4 anti-CD22 monoclonal antibody attached to calicheamicin via an acid-labile linker and showed favorable antitumor activity in mouse xenograft models of B-cell NHL and ALL.[83,84] A phase 2 study demonstrated encouraging results in both adults and children with relapsed/refractory ALL who were treated with single-agent INO at a dose of 1.8 mg/kg every 3 weeks with an ORR of 57%, with 28 of 49 patients achieving either CR (18%) or marrow CR (39%).[85] The most common nonhematologic adverse events reported were drug-related fever (59%), elevated aminotransferase (57%), elevated bilirubin (29%), drug-related hypotension (27%), and nausea (49%). A phase 3 trial investigating single-agent INO compared with the investigator's choice of chemotherapy (FLAG [fludarabine and cytarabine], high-dose cytarabine, or cytarabine and mitoxantrone) in relapsed/refractory adult ALL is ongoing (NCT01564784). Phase 2 study results in relapsed/refractory pediatric ALL patients receiving INO as a single agent at 1.8 mg/kg or as split weekly doses reported 3 of 5 responses (1 CR in bone marrow and normal peripheral counts and 2 with morphologic remissions in the bone marrow but with platelets $<$100 000).[86] Although toxicities included fever, sepsis, and liver function abnormalities, the ADC was generally well tolerated. A phase 3 trial of pediatric ALL patients is planned.

Results of INO in NHL have been mixed. A phase 1 study of single-agent INO enrolling 79 patients with relapsed/refractory B-cell NHL yielded an ORR of 68% in FL and 15% in DLBCL at a dose of 1.8 mg/m^2 given every 3 to 4 weeks.[87] A phase 1/2 study of INO combined with rituximab showed impressive ORRs of 87%, 74%, and 20% for relapsed FL, relapsed DLBCL, and refractory aggressive NHL, respectively, with a 2-year PFS of 68% for FL and 42% for DLBCL.[88] Toxicities were manageable and included thrombocytopenia, neutropenia, hyperbilirubinemia, and transaminitis. However, a phase 3 study (NCT01232556) of monthly 1.8 mg/kg INO with rituximab vs investigator's choice chemotherapy (bendamustine or gemcitabine) with rituximab in relapsed/refractory aggressive CD22$^+$ B-cell NHL was halted in May 2013 after an independent data monitoring committee concluded that the ADC experimental arm would not meet the primary objective of improving OS compared with the chemotherapy arm. Another phase 3 study comparing INO with rituximab vs R-CVP or R-FND (fludarabine, mitoxantrone, dexamethasone) in FL had previously been discontinued due to slow accrual (NCT00562965).

Several other ADCs are undergoing phase 1/2 studies. Two of these use the same protease-cleavable linker to MMAE as BV but replace the anti-CD30 antibody with antibodies targeting either the internalizing receptor CD22 (pinatuzumab vedotin [PV]; DCDT2980S) or CD79b (polatuzumab vedotin; DCDS4501A), a component of the B-cell receptor. A phase 2 study randomizing patients with relapsed/refractory FL or DLBCL to either DCDT2980S or DCDS4501A in combination with rituximab is ongoing (NCT01691898). Results from the prior phase 1 studies suggested possible greater efficacy of the anti-CD79b ADC than the anti-CD22 ADC with an ORR of 55% vs 30% as a single agent and 78% vs 33% when combined with rituximab.[89,90] Toxicities observed included neutropenia and peripheral neuropathy which were not unexpected given the previous clinical experience with BV. Two other ADCs use maytansinoids as effectors and target either CD19 (SAR-3419) in DLBCL or ALL or CD138 (BT-062) in MM.[91,92] In a phase 1 study enrolling relapsed/refractory B-cell NHL patients, single-agent SAR3419 was found to have a maximum tolerated dose of 160 mg/m^2 with 6 of 35 patients (17%) achieving an objective response.[93] The notable dose-limiting toxicity in this trial was reversible bilateral corneal epitheliopathy, which has also been observed with other ADCs incorporating DM4. ADCs in phase 1 testing include an

Table 3. Small-molecule drug effectors

Effector drug	Ozogamicin	MMAE	Maytansinoid DM1 (mertansine)	Maytansinoid DM4
Origin	Semi-synthetic derivative of γ-calicheamicin (*Micromonospora echinospora calichensis*–Actinomycete soil bacterium)	Synthetic derivative of dolastatin 10 (*Dolabella auricularia*–Sea hare)	Synthetic derivative of maytansine (*Maytenus serrata*–Ethiopian shrub)	Synthetic derivative of maytansine (*M serrata*–Ethiopian shrub); DM1 with 2 additional methyl groups
Class of molecule	Enediyne-containing antibiotic	Linear cytotoxic pentapeptide	Ansamycin macrolide antibiotic	Ansamycin macrolide antibiotic
Mechanism of action	Intercalates in the minor groove of DNA causing double-stranded breaks	Binds tubulin and inhibits normal microtubule polymerization causing mitotic arrest	Binds tubulin and inhibits normal microtubule polymerization causing mitotic arrest	Binds tubulin and inhibits normal microtubule polymerization causing mitotic arrest
Example ADCs (target antigen)	GO (CD33)	BV (CD30)	Ado-trastuzumab emtansine (Her2/neu)	SAR3419 (CD19)
	INO (CD22)	PV (CD22) Polatuzumab vedotin (CD79b)	IMGN529 (CD37)	BT062 (CD138)
Major toxicities including phase 1 study DLTs	Thrombocytopenia (DLT); neutropenia (DLT); hepatotoxicity	Thrombocytopenia (DLT); neutropenia (DLT); hyperglycemia (DLT); peripheral neuropathy; pulmonary toxicity	Thrombocytopenia (DLT); hepatotoxicity; interstitial lung disease; peripheral neuropathy	Ocular/corneal toxicity (DLT); peripheral neuropathy (DLT); neutropenia; thrombocytopenia

DLT, dose-limiting toxicity.

anti-CD74 antibody conjugated to doxorubicin,[94] an anti-CD37 antibody conjugated to maytansinoid,[95] an anti-CD19 antibody conjugated to monomethyl auristatin F,[96] and an anti-CD33 antibody conjugated to pyrrolobenzodiazepine.[97] Antibodies fused to a cell-signaling molecule (immunocytokines) comprise another increasingly recognized group of ICs with activity in hematologic malignancies.[98] A tetrameric interferon-α construct attached to veltuzumab showed promising activity in a lymphoma mouse xenograft model.[99]

Drug and linker affect the efficacy and toxicity profile of ADCs. Microtubule inhibitors such as maytansine and dolastatin derivatives and DNA damaging agents including calicheamicin and pyrrolobenzodiazepine comprise the majority of small-molecule drug effectors currently incorporated into ADCs (Table 3). Maytansinoids and auristatins, like the vinca alkaloids and taxanes, cause neuropathy by virtue of a common mechanism of tubulin disruption. However, the relative membrane permeability of the released drug can impact severity. Hydrophobic effectors such as DM4 produce less neuropathy than hydrophilic effectors like DM1 and MMAE which diffuse across the cell membrane to affect bystander cells. Membrane permeability can be modulated by linker attachment. Intracellular processing of specific linker-drug combinations result in charged metabolites preventing drug escape and uptake by neighboring cells. However, local bystander effects can prove beneficial in tumors heterogeneously expressing the targeted cell-surface antigen. Free MMAE released by intracellular processing of BV by rare CD30-expressing Reed-Sternberg cells is believed to enhance the tumoricidal efficacy in HL.[100] Bystander effects may also prove beneficial when intact ADCs have difficulty penetrating deep into bulky tumors. Selection of ADCs targeting the same antigen may depend upon the side effect profile conferred by the linker drug. In choosing between CD22-targeted ADCs PV and INO, for example, preexisting severe neuropathy would exclude PV whereas prior HSCT may conversely prohibit INO use. Emerging trial data clarifying the advantages and disadvantages of individual ADCs should provide further guidance to the clinician.

Conclusions

ICs represent an exciting class of biologics that have increasingly established a place in the treatment of hematologic malignancies. RIT has proven to be an effective although underutilized modality in the treatment of NHL and is being studied for other neoplasms. Several ADCs are in clinical development for a variety of indications and may soon be incorporated into frontline treatment regimens. Continued research to improve components of ICs including linker optimization and development of more potent and specific effector molecules may further expand their use in a variety of hematologic cancers.

Acknowledgments

This work was supported by the National Institutes of Health, National Cancer Institute grants: K08CA163603 (M.C.P.-W.), P50 CA083636, (M.C.P.-W.), R01EB002991 (O.W.P.), R01 CA076287 (O.W.P.), R01 CA109663 (O.W.P.), R01 CA154897 (O.W.P.), R01 CA136639 (O.W.P.), and P01 CA044991 (O.W.P.); Washington State Life Sciences Discovery Fund #2496490 (O.W.P. and M.C.P.-W.); the Wayne D. Kuni & Joan E. Kuni Foundation, and the Kuni family through the 3725 Fund of the Oregon Community Foundation (M.C.P.-W.).

Authorship

Contribution: M.C.P.-W. and O.W.P. wrote the manuscript.

Conflict-of-interest disclosure: O.W.P. and M.C.P.-W. have received research funding from Roche/Genentech. O.W.P. was a consultant for Roche/Genentech.

Correspondence: Oliver W. Press, Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; e-mail: press@uw.edu.

References

1. Strebhardt K, Ullrich A. Paul Ehrlich's magic bullet concept: 100 years of progress. *Nat Rev Cancer.* 2008;8(6):473-480.
2. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature.* 1975;256(5517):495-497.
3. Maloney DG. Anti-CD20 antibody therapy for B-cell lymphomas. *N Engl J Med.* 2012;366(21): 2008-2016.
4. Hwang WY, Foote J. Immunogenicity of engineered antibodies. *Methods.* 2005;36(1): 3-10.
5. Morrison SL, Johnson MJ, Herzenberg LA, Oi VT. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc Natl Acad Sci USA.* 1984; 81(21):6851-6855.
6. Jones PT, Dear PH, Foote J, Neuberger MS, Winter G. Replacing the complementarity-determining regions in a human antibody with those from a mouse. *Nature.* 1986;321(6069): 522-525.
7. Herter S, Herting F, Mundigl O, et al. Preclinical activity of the type II CD20 antibody GA101 (obinutuzumab) compared with rituximab and ofatumumab in vitro and in xenograft models. *Mol Cancer Ther.* 2013;12(10):2031-2042.
8. Weiner LM. Building better magic bullets—improving unconjugated monoclonal antibody therapy for cancer. *Nat Rev Cancer.* 2007;7(9):701-706.
9. Brekke OH, Løset GA. New technologies in therapeutic antibody development. *Curr Opin Pharmacol.* 2003;3(5):544-550.
10. FitzGerald DJ, Wayne AS, Kreitman RJ, Pastan I. Treatment of hematologic malignancies with immunotoxins and antibody-drug conjugates. *Cancer Res.* 2011;71(20):6300-6309.
11. Sievers EL, Senter PD. Antibody-drug conjugates in cancer therapy. *Annu Rev Med.* 2013;64:15-29.
12. Teicher BA, Chari RV. Antibody conjugate therapeutics: challenges and potential. *Clin Cancer Res.* 2011;17(20):6389-6397.
13. Press OW, Shan D, Howell-Clark J, et al. Comparative metabolism and retention of iodine-125, yttrium-90, and indium-111 radioimmunoconjugates by cancer cells. *Cancer Res.* 1996;56(9):2123-2129.
14. Alderson RF, Kreitman RJ, Chen T, et al. CAT-8015: a second-generation pseudomonas exotoxin A-based immunotherapy targeting CD22-expressing hematologic malignancies. *Clin Cancer Res.* 2009;15(3):832-839.
15. Chari RV, Martell BA, Gross JL, et al. Immunoconjugates containing novel maytansinoids: promising anticancer drugs. *Cancer Res.* 1992;52(1):127-131.
16. Doronina SO, Toki BE, Torgov MY, et al. Development of potent monoclonal antibody auristatin conjugates for cancer therapy. *Nat Biotechnol.* 2003;21(7):778-784.
17. Orozco JJ, Bäck T, Kenoyer A, et al. Anti-CD45 radioimmunotherapy using (211)At with bone marrow transplantation prolongs survival in a disseminated murine leukemia model. *Blood.* 2013;121(18):3759-3767.
18. Haro KJ, Scott AC, Scheinberg DA. Mechanisms of resistance to high and low linear energy transfer radiation in myeloid leukemia cells. *Blood.* 2012;120(10):2087-2097.
19. Doronina SO, Mendelsohn BA, Bovee TD, et al. Enhanced activity of monomethylauristatin F through monoclonal antibody delivery: effects of linker technology on efficacy and toxicity. *Bioconjug Chem.* 2006;17(1):114-124.
20. Hamblett KJ, Senter PD, Chace DF, et al. Effects of drug loading on the antitumor activity of a monoclonal antibody drug conjugate. *Clin Cancer Res.* 2004;10(20):7063-7070.
21. Doronina SO, Bovee TD, Meyer DW, et al. Novel peptide linkers for highly potent antibody-auristatin conjugate. *Bioconjug Chem.* 2008; 19(10):1960-1963.
22. Axup JY, Bajjuri KM, Ritland M, et al. Synthesis of site-specific antibody-drug conjugates using unnatural amino acids. *Proc Natl Acad Sci USA.* 2012;109(40):16101-16106.
23. Pastan I, Hassan R, FitzGerald DJ, Kreitman RJ. Immunotoxin treatment of cancer. *Annu Rev Med.* 2007;58:221-237.
24. Press OW, Eary JF, Appelbaum FR, et al. Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. *N Engl J Med.* 1993;329(17): 1219-1224.
25. Kaminski MS, Zasadny KR, Francis IR, et al. Radioimmunotherapy of B-cell lymphoma with [131I]anti-B1 (anti-CD20) antibody. *N Engl J Med.* 1993;329(7):459-465.
26. Palanca-Wessels MC, Press OW. Improving the efficacy of radioimmunotherapy for non-Hodgkin lymphomas. *Cancer.* 2010;116(suppl 4): 1126-1133.
27. Kaminski MS, Zelenetz AD, Press OW, et al. Pivotal study of iodine I 131 tositumomab for chemotherapy-refractory low-grade or transformed low-grade B-cell non-Hodgkin's lymphomas. *J Clin Oncol.* 2001;19(19): 3918-3928.
28. Witzig TE, Flinn IW, Gordon LI, et al. Treatment with ibritumomab tiuxetan radioimmunotherapy in patients with rituximab-refractory follicular non-Hodgkin's lymphoma. *J Clin Oncol.* 2002; 20(15):3262-3269.
29. Morschhauser F, Kraeber-Bodéré F, Wegener WA, et al. High rates of durable responses with anti-CD22 fractionated radioimmunotherapy: results of a multicenter, phase I/II study in non-Hodgkin's lymphoma. *J Clin Oncol.* 2010;28(23): 3709-3716.
30. Sharkey RM, Press OW, Goldenberg DM. A re-examination of radioimmunotherapy in the treatment of non-Hodgkin lymphoma: prospects for dual-targeted antibody/radioantibody therapy. *Blood.* 2009;113(17):3891-3895.
31. Tomblyn M, Witzig T, Himelstein A, et al. Anti-CD22 radioimmunotherapy (RIT) combined with anti-CD20 immunotherapy in aggressive non-Hodgkin lymphoma (NHL): phase I results [abstract]. *J Nucl Med.* 2013;54(suppl 2): Abstract 1368.
32. Kaminski MS, Tuck M, Estes J, et al. 131I-tositumomab therapy as initial treatment for follicular lymphoma. *N Engl J Med.* 2005;352(5): 441-449.
33. Press OW, Unger JM, Braziel RM, et al; Southwest Oncology Group. Phase II trial of CHOP chemotherapy followed by tositumomab/iodine I-131 tositumomab for previously untreated follicular non-Hodgkin's lymphoma: five-year follow-up of Southwest Oncology Group Protocol S9911. *J Clin Oncol.* 2006; 24(25):4143-4149.
34. Press OW, Unger JM, Rimsza LM, et al. Phase III randomized intergroup trial of CHOP plus rituximab compared with CHOP chemotherapy plus (131)iodine-tositumomab for previously untreated follicular non-Hodgkin lymphoma: SWOG S0016. *J Clin Oncol.* 2013;31(3): 314-320.
35. Zinzani PL, Tani M, Pulsoni A, et al. Fludarabine and mitoxantrone followed by yttrium-90 ibritumomab tiuxetan in previously untreated patients with follicular non-Hodgkin lymphoma trial: a phase II non-randomised trial (FLUMIZ). *Lancet Oncol.* 2008;9(4):352-358.
36. Jacobs SA, Swerdlow SH, Kant J, et al. Phase II trial of short-course CHOP-R followed by 90Y-ibritumomab tiuxetan and extended rituximab in previously untreated follicular lymphoma. *Clin Cancer Res.* 2008;14(21):7088-7094.
37. Hainsworth JD, Litchy S, Morrissey LH, et al. Rituximab plus short-duration chemotherapy as first-line treatment for follicular non-Hodgkin's lymphoma: a phase II trial of the minnie pearl cancer research network. *J Clin Oncol.* 2005; 23(7):1500-1506.
38. Scholz CW, Pinto A, Linkesch W, et al. (90) Yttrium-ibritumomab-tiuxetan as first-line treatment for follicular lymphoma: 30 months of follow-up data from an international multicenter phase II clinical trial. *J Clin Oncol.* 2013;31(3): 308-313.
39. Morschhauser F, Radford J, Van Hoof A, et al. 90Yttrium-ibritumomab tiuxetan consolidation of first remission in advanced-stage follicular non-Hodgkin lymphoma: updated results after a median follow-up of 7.3 years from the International, Randomized, Phase III First-LineIndolent trial. *J Clin Oncol.* 2013;31(16): 1977-1983.
40. Press OW, Eary JF, Appelbaum FR, et al. Phase II trial of 131I-B1 (anti-CD20) antibody therapy with autologous stem cell transplantation for relapsed B cell lymphomas. *Lancet.* 1995; 346(8971):336-340.
41. Liu SY, Eary JF, Petersdorf SH, et al. Follow-up of relapsed B-cell lymphoma patients treated with iodine-131-labeled anti-CD20 antibody and autologous stem-cell rescue. *J Clin Oncol.* 1998; 16(10):3270-3278.
42. Winter JN, Inwards DJ, Spies S, et al. Yttrium-90 ibritumomab tiuxetan doses calculated to deliver up to 15 Gy to critical organs may be safely combined with high-dose BEAM and autologous transplantation in relapsed or refractory B-cell non-Hodgkin's lymphoma. *J Clin Oncol.* 2009; 27(10):1653-1659.
43. Devizzi L, Guidetti A, Tarella C, et al. High-dose yttrium-90-ibritumomab tiuxetan with tandem stem-cell reinfusion: an outpatient preparative regimen for autologous hematopoietic cell transplantation. *J Clin Oncol.* 2008;26(32): 5175-5182.
44. Nademanee A, Forman S, Molina A, et al. A phase 1/2 trial of high-dose yttrium-90-ibritumomab tiuxetan in combination with high-dose etoposide and cyclophosphamide followed by autologous stem cell transplantation in patients with poor-risk or relapsed non-Hodgkin lymphoma. *Blood.* 2005;106(8):2896-2902.
45. Vose JM, Carter S, Burns LJ, et al. Phase III randomized study of rituximab/carmustine, etoposide, cytarabine, and melphalan (BEAM) compared with iodine-131 tositumomab/BEAM with autologous hematopoietic cell transplantation for relapsed diffuse large B-cell lymphoma: results from the BMT CTN 0401 trial. *J Clin Oncol.* 2013;31(13):1662-1668.
46. Shimoni A, Avivi I, Rowe JM, et al. A randomized study comparing yttrium-90 ibritumomab tiuxetan (Zevalin) and high-dose BEAM chemotherapy versus BEAM alone as the conditioning regimen before autologous stem cell transplantation in patients with aggressive lymphoma. *Cancer.* 2012;118(19):4706-4714.

47. Jurcic JG, Larson SM, Sgouros G, et al. Targeted alpha particle immunotherapy for myeloid leukemia. *Blood.* 2002;100(4):1233-1239.

48. Pagel JM, Appelbaum FR, Eary JF, et al. 131I-anti-CD45 antibody plus busulfan and cyclophosphamide before allogeneic hematopoietic cell transplantation for treatment of acute myeloid leukemia in first remission. *Blood.* 2006;107(5):2184-2191.

49. Matthews DC, Appelbaum FR, Eary JF, et al. Phase I study of (131)I-anti-CD45 antibody plus cyclophosphamide and total body irradiation for advanced acute leukemia and myelodysplastic syndrome. *Blood.* 1999;94(4):1237-1247.

50. Matthews DC, Appelbaum FR, Eary JF, et al. Development of a marrow transplant regimen for acute leukemia using targeted hematopoietic irradiation delivered by 131I-labeled anti-CD45 antibody, combined with cyclophosphamide and total body irradiation. *Blood.* 1995;85(4):1122-1131.

51. Bunjes D, Buchmann I, Duncker C, et al. Rhenium 188-labeled anti-CD66 (a, b, c, e) monoclonal antibody to intensify the conditioning regimen prior to stem cell transplantation for patients with high-risk acute myeloid leukemia or myelodysplastic syndrome: results of a phase I-II study. *Blood.* 2001;98(3):565-572.

52. Zenz T, Glatting G, Schlenk RF, et al. Targeted marrow irradiation with radioactively labeled anti-CD66 monoclonal antibody prior to allogeneic stem cell transplantation for patients with leukemia: results of a phase I-II study. *Haematologica.* 2006;91(2):285-286.

53. Rosenblat TL, McDevitt MR, Mulford DA, et al. Sequential cytarabine and alpha-particle immunotherapy with bismuth-213-lintuzumab (HuM195) for acute myeloid leukemia. *Clin Cancer Res.* 2010;16(21):5303-5311.

54. Scheinberg DA, Lovett D, Divgi CR, et al. A phase I trial of monoclonal antibody M195 in acute myelogenous leukemia: specific bone marrow targeting and internalization of radionuclide. *J Clin Oncol.* 1991;9(3):478-490.

55. Rousseau C, Ferrer L, Supiot S, et al. Dosimetry results suggest feasibility of radioimmunotherapy using anti-CD138 (B-B4) antibody in multiple myeloma patients. *Tumour Biol.* 2012;33(3):679-688.

56. Rosen ST, Zimmer AM, Goldman-Leikin R, et al. Radioimmunodetection and radioimmunotherapy of cutaneous T cell lymphomas using an 131I-labeled monoclonal antibody: an Illinois Cancer Council Study. *J Clin Oncol.* 1987;5(4):562-573.

57. Press OW, Corcoran M, Subbiah K, et al. A comparative evaluation of conventional and pretargeted radioimmunotherapy of CD20-expressing lymphoma xenografts. *Blood.* 2001;98(8):2535-2543.

58. Pagel JM, Hedin N, Subbiah K, et al. Comparison of anti-CD20 and anti-CD45 antibodies for conventional and pretargeted radioimmunotherapy of B-cell lymphomas. *Blood.* 2003;101(6):2340-2348.

59. Pantelias A, Pagel JM, Hedin N, et al. Comparative biodistributions of pretargeted radioimmunoconjugates targeting CD20, CD22, and DR molecules on human B-cell lymphomas. *Blood.* 2007;109(11):4980-4987.

60. Axworthy DB, Reno JM, Hylarides MD, et al. Cure of human carcinoma xenografts by a single dose of pretargeted yttrium-90 with negligible toxicity. *Proc Natl Acad Sci USA.* 2000;97(4):1802-1807.

61. Goldenberg DM, Sharkey RM, Paganelli G, Barbet J, Chatal JF. Antibody pretargeting advances cancer radioimmunodetection and radioimmunotherapy. *J Clin Oncol.* 2006;24(5):823-834.

62. Zhang M, Zhang Z, Garmestani K, et al. Pretarget radiotherapy with an anti-CD25 antibody-streptavidin fusion protein was effective in therapy of leukemia/lymphoma xenografts. *Proc Natl Acad Sci USA.* 2003;100(4):1891-1895.

63. Barbet J, Kraeber-Bodéré F, Vuillez JP, Gautherot E, Rouvier E, Chatal JF. Pretargeting with the affinity enhancement system for radioimmunotherapy. *Cancer Biother Radiopharm.* 1999;14(3):153-166.

64. Sharkey RM, Goldenberg DM. Advances in radioimmunotherapy in the age of molecular engineering and pretargeting. *Cancer Invest.* 2006;24(1):82-97.

65. Rossi EA, Goldenberg DM, Cardillo TM, McBride WJ, Sharkey RM, Chang CH. Stably tethered multifunctional structures of defined composition made by the dock and lock method for use in cancer targeting. *Proc Natl Acad Sci USA.* 2006;103(18):6841-6846.

66. Goldenberg DM, Rossi EA, Sharkey RM, McBride WJ, Chang CH. Multifunctional antibodies by the Dock-and-Lock method for improved cancer imaging and therapy by pretargeting. *J Nucl Med.* 2008;49(1):158-163.

67. Liu G, Dou S, Chen X, et al. Adding a clearing agent to pretargeting does not lower the tumor accumulation of the effector as predicted. *Cancer Biother Radiopharm.* 2010;25(6):757-762.

68. Zeglis BM, Sevak KK, Reiner T, et al. A pretargeted PET imaging strategy based on bioorthogonal Diels-Alder click chemistry. *J Nucl Med.* 2013;54(8):1389-1396.

69. Forero A, Weiden PL, Vose JM, et al. Phase 1 trial of a novel anti-CD20 fusion protein in pretargeted radioimmunotherapy for B-cell non-Hodgkin lymphoma. *Blood.* 2004;104(1):227-236.

70. Weiden PL, Breitz HB, Press O, et al. Pretargeted radioimmunotherapy (PRIT) for treatment of non-Hodgkin's lymphoma (NHL): initial phase I/II study results. *Cancer Biother Radiopharm.* 2000;15(1):15-29.

71. Schaefer NG, Ma J, Huang P, Buchanan J, Wahl RL. Radioimmunotherapy in non-Hodgkin lymphoma: opinions of U.S. medical oncologists and hematologists. *J Nucl Med.* 2010;51(6):987-994.

72. Sievers EL, Larson RA, Stadtmauer EA, et al; Mylotarg Study Group. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J Clin Oncol.* 2001;19(13):3244-3254.

73. Petersdorf SH, Kopecky KJ, Slovak M, et al. A phase 3 study of gemtuzumab ozogamicin during induction and postconsolidation therapy in younger patients with acute myeloid leukemia. *Blood.* 2013;121(24):4854-4860.

74. Rowe JM, Löwenberg B. Gemtuzumab ozogamicin in acute myeloid leukemia: a remarkable saga about an active drug. *Blood.* 2013;121(24):4838-4841.

75. Senter PD, Sievers EL. The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. *Nat Biotechnol.* 2012;30(7):631-637.

76. Forero-Torres A, Leonard JP, Younes A, et al. A phase II study of SGN-30 (anti-CD30 mAb) in Hodgkin lymphoma or systemic anaplastic large cell lymphoma. *Br J Haematol.* 2009;146(2):171-179.

77. Pro B, Advani R, Brice P, et al. Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large-cell lymphoma: results of a phase II study. *J Clin Oncol.* 2012;30(18):2190-2196.

78. Younes A, Gopal AK, Smith SE, et al. Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. *J Clin Oncol.* 2012;30(18):2183-2189.

79. Fanale MA, Shustov AR, Forero-Torres A, et al. Brentuximab vedotin administered concurrently with multi-agent chemotherapy as frontline treatment of ALCL and other CD30-positive mature T-cell and NK-cell lymphomas [abstract]. *Blood (ASH Annual Meeting Abstracts).* 2012;120(21):Abstract 60.

80. Younes A, Connors JM, Park SI, Hunder NH, Ansell SM. Frontline therapy with brentuximab vedotin combined with ABVD or AVD in patients with newly diagnosed advanced stage Hodgkin lymphoma [abstract]. *Blood (ASH Annual Meeting Abstracts).* 2011;118(21):Abstract 955.

81. Gopal AK, Ramchandren R, O'Connor OA, et al. Safety and efficacy of brentuximab vedotin for Hodgkin lymphoma recurring after allogeneic stem cell transplantation. *Blood.* 2012;120(3):560-568.

82. Hu S, Xu-Monette ZY, Balasubramanyam A, et al. CD30 expression defines a novel subgroup of diffuse large B-cell lymphoma with favorable prognosis and distinct gene expression signature: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. *Blood.* 2013;121(14):2715-2724.

83. DiJoseph JF, Armellino DC, Boghaert ER, et al. Antibody-targeted chemotherapy with CMC-544: a CD22-targeted immunoconjugate of calicheamicin for the treatment of B-lymphoid malignancies. *Blood.* 2004;103(5):1807-1814.

84. Dijoseph JF, Dougher MM, Armellino DC, Evans DY, Damle NK. Therapeutic potential of CD22-specific antibody-targeted chemotherapy using inotuzumab ozogamicin (CMC-544) for the treatment of acute lymphoblastic leukemia. *Leukemia.* 2007;21(11):2240-2245.

85. Kantarjian H, Thomas D, Jorgensen J, et al. Inotuzumab ozogamicin, an anti-CD22-calecheamicin conjugate, for refractory and relapsed acute lymphocytic leukaemia: a phase 2 study. *Lancet Oncol.* 2012;13(4):403-411.

86. Rytting M, Triche L, Thomas D, O'Brien S, Kantarjian H. Initial experience with CMC-544 (inotuzumab ozogamicin) in pediatric patients with relapsed B-cell acute lymphoblastic leukemia. *Pediatr Blood Cancer.* 2014;61(2):369-372.

87. Advani A, Coiffier B, Czuczman MS, et al. Safety, pharmacokinetics, and preliminary clinical activity of inotuzumab ozogamicin, a novel immunoconjugate for the treatment of B-cell non-Hodgkin's lymphoma: results of a phase I study. *J Clin Oncol.* 2010;28(12):2085-2093.

88. Fayad L, Offner F, Smith MR, et al. Safety and clinical activity of a combination therapy comprising two antibody-based targeting agents for the treatment of non-Hodgkin lymphoma: results of a phase I/II study evaluating the immunoconjugate inotuzumab ozogamicin with rituximab. *J Clin Oncol.* 2013;31(5):573-583.

89. Advani R, Chen A, Lebovic D, et al. Phase I study of the anti-CD22 antibody-drug conjugate (ADC) DCDT2980S with or without [abstract]. *Hematol Oncol.* 2013;31(S1):Abstract 39.

90. Palanca-Wessels MC, Salles G, Czuczman M, et al. Phase I study of the anti-CD79b antibody-drug conjugate DCDS4501A in relapsed or refractory (R/R) B-cell non Hodgkin's lymphoma (NHL) [abstract]. *Hematol Oncol.* 2013;31(S1):Abstract 40.

91. Blanc V, Bousseau A, Caron A, Carrez C, Lutz RJ, Lambert JM. SAR3419: an anti-CD19-Maytansinoid immunoconjugate for the treatment of B-cell malignancies. *Clin Cancer Res.* 2011;17(20):6448-6458.

92. Lutz RJ, Whiteman KR. Antibody-maytansinoid conjugates for the treatment of myeloma. *MAbs.* 2009;1(6):548-551.

93. Younes A, Kim S, Romaguera J, et al. Phase I multidose-escalation study of the anti-CD19 maytansinoid immunoconjugate SAR3419 administered by intravenous infusion every 3 weeks to patients with relapsed/refractory B-cell lymphoma. *J Clin Oncol.* 2012;30(22):2776-2782.

94. Sapra P, Stein R, Pickett J, et al. Anti-CD74 antibody-doxorubicin conjugate, IMMU-110, in a human multiple myeloma xenograft and in monkeys. *Clin Cancer Res.* 2005;11(14):5257-5264.

95. Deckert J, Park PU, Chicklas S, et al. A novel anti-CD37 antibody-drug conjugate with multiple anti-tumor mechanisms for the treatment of B-cell malignancies. *Blood.* 2013;122(20):3500-3510.

96. Borate U, Fathi AT, Shah BD, et al. A first-in-human phase 1 study of the antibody-drug conjugate SGN CD19A in relapsed or refractory B-lineage acute leukemia and highly aggressive lymphoma [abstract]. *Blood (ASH Annual Meeting Abstracts).* 2013;122(21):Abstract 1437.

97. Kung Sutherland MS, Walter RB, Jeffrey SC, et al. SGN-CD33A: a novel CD33-targeting antibody-drug conjugate using a pyrrolobenzodiazepine dimer is active in models of drug-resistant AML. *Blood.* 2013;122(8):1455-1463.

98. Singh H, Serrano LM, Pfeiffer T, et al. Combining adoptive cellular and immunocytokine therapies to improve treatment of B-lineage malignancy. *Cancer Res.* 2007;67(6):2872-2880.

99. Rossi EA, Goldenberg DM, Cardillo TM, Stein R, Chang CH. CD20-targeted tetrameric interferon-alpha, a novel and potent immunocytokine for the therapy of B-cell lymphomas. *Blood.* 2009;114(18):3864-3871.

100. Okeley NM, Miyamoto JB, Zhang X, et al. Intracellular activation of SGN-35, a potent anti-CD30 antibody-drug conjugate. *Clin Cancer Res.* 2010;16(3):888-897.