George Van Hook. *Stevens Farm Winter.* Oil on linen, 16″ × 20″.

A variety of approaches are being tested to improve outcomes in diffuse large B-cell lymphoma.

Diffuse Large B-Cell Lymphoma: Current Strategies and Future Directions

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Background: *DLBCL is the most common histology of non-Hodgkin lymphoma, representing 25% to 35% of new cases annually. The incidence of DLBCL has doubled in the past decades, highlighting the need for more effective treatment regimens.*

Methods: *This article reviews the current protocols applicable to this aggressive lymphoma and discusses ongoing research that is focusing on molecular diagnostics, prognostic factors have also been defined for DLBCL.*

Results*: Patients with DLBCL vary in clinical presentation, prognosis, and response to current therapies. While current therapy in the rituximab era has led to improved outcomes with reduced toxicity, novel treatment approaches for localized, advanced, and relapsed/refractory DLBCL are being pursued in clinical trials. Several studies have shown promise, such as trials involving proteasome inhibitors, lenalidomide, and antibody drug conjugates.*

Conclusions: *Recent discoveries in the spectrum of care for patients with DLBCL have prompted a renaissance for personalized cancer medicine and molecularly targeted therapy. Potential targets and novel drug combinations are undergoing continued study in the hope of achieving successful and personalized care of this disease.*

Introduction

Non-Hodgkin lymphoma (NHL) is the most prevalent hematologic malignancy in the United States, representing 4% of all malignancies in both incidence and deaths per year. It is estimated that more than 60,000 people will be diagnosed with NHL this year, and almost 20,000 of these patients will die of lymphoma.[1] Cancer registries have noted that the incidence of NHL has nearly doubled over the course of the last several decades. This increase may be the result of expanded diagnostic techniques as well as increased life expectancies. The peak incidence for diffuse large B-cell lymphoma (DLBCL) occurs in the seventh decade of life.[2]

Several different subtypes are included within NHL. DLBCL is the most common NHL histology, representing 25% to 35% of new cases annually. These disorders are composed of a clinically and pathologically heterogeneous group of lymphoproliferative malignancies, most of which are B-cell origin. The specific clinical, morphologic, and molecular characteristics have been established by the latest World Health Organization (WHO) Classification of Haematopoietic and Lymphoid Tissue to further subtype DLBCL (Table 1).[3]

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Table 1. — World Health Organization Classification of Large B-Cell Lymphomas

DLBCL, not otherwise specified
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system
Epstein-Barr virus-positive DLBCL of the elderly
DLBCL associated with chronic inflammation
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK-positive large B-cell lymphoma
Primary effusion lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

Adapted from Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. *World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues.* IARC, Lyon, 2008. Reprinted with permission.

Patients with DLBCL vary in clinical presentation, prognosis, and response to current therapies. Unlike indolent lymphomas, DLBCL is an aggressive lymphoma, and if left untreated, survival may be measured in weeks to months. Patients often present with a rapidly enlarging mass in a lymphatic region. Extranodal involvement or associated constitutional symptoms are uncommon, although the presence of these symptoms indicates a more aggressive phenotype. Bone marrow involvement is not commonly seen at diagnosis, with only 20% to 30% of patients having evidence of DLBCL in the marrow.[1]

The prognosis is varied among DLBCL patients. Response rates to standard chemoimmunotherapy in the rituximab era range from 80% to 90% in patients with low-risk disease.[4] However, overall survival rates range from 30% to 50% over 5 years for all patients with DLBCL, indicating that there is a clinical spectrum of sensitivity to the standard treatment of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). The International Prognostic Index (IPI) was developed to predict response, based on a retrospective analysis of more than 2,000 patients with aggressive lymphomas (Table 2).[5] Two major modifications to the IPI have been implemented over time and have been validated in the rituximab era: the age-adjusted model for patients aged 60 and younger and a revised IPI that consolidates five prognostic risks into three — tumor stage, performance status, and lactate dehydrogenase (LDH) level.[5,6] Although the age-adjusted model has been accepted into standard practice, the revised IPI continues to be assessed in large prospective trials and has not yet been completely established for general use (Tables 3 and 4).

Table 2. — Variables in the International Prognostic Index (IPI) and the Age-Adjusted IPI

IPI	Age-Adjusted IPI
Age > 60 years	Advanced stage of disease (III, IV)
Advanced stage of disease (III, IV)	Elevated lactate dehydrogenase (LDH)
Extranodal involvement > 1 site	Poor ECOG performance status ≥ 2
Elevated lactate dehydrogenase (LDH)	
Poor ECOG performance status ≥ 2	

ECOG = Eastern Cooperative Oncology Group.

As advances in molecular diagnostics have occurred, prognostic factors have also been defined for DLBCL. Some mutational changes, eg, bcl-6, bcl-2, p53, c-myc, and Ki-67 expression, are associated with poor outcomes.[7] In 2002, Rosenwald et al[8] reported that DNA expression analysis in 160 patients identified three distinct subtypes of DLBCL: (1) germinal-center B-cell type (GCB), (2) activated B-cell type (ABC or non–GBC subtype), and (3) type 3 DLBCL. Outcomes for patients with GCB vs non–GCB subtypes are statistically different — 59% vs 30%, despite current treatment standards.[8] While the addition of therapy to known molecular targets such as CD20 (rituximab, a monoclonal antibody against CD20) has not changed outcomes in each subgroup,[9] isolating specific targets such as NFκB constitutive expression through proteasome inhibition has shown a benefit in early trials for the non–GCB subtype. This is currently being expanded into larger prospective phase III clinical trials. Gene expression profiling is costly and technically difficult to obtain outside of academic centers. However, molecular pathways utilizing a small subset of the products of the genes analyzed may correlate with expression profiles and thus patient outcomes. Hans et al[10] proposed a small set of immunohistochemical (IHC) markers including CD10, bcl-6, and MUM1 (Fig 1) that demonstrated an 80% concordance to DNA expression analysis. Furthermore, Choi et al[11] described an algorithm using markers GCET1, CD10, bcl-6, MUM1, and FOXP1, which closely approximated gene expression profiling with a concordance rate of 93%. Outcomes were also similar to historical data in which 87% of patients with the GCB subtype were alive at 3 years compared with 44% of those with the ABC subtype ($P < .0001$). Several IHC algorithms have been suggested, and concordance with outcomes has been questioned recently. While it may be cost effective to subtype DLBCL patients in both the academic and the community setting, this needs additional study in larger, prospective trials in patients with DLBCL.

Current Treatment Strategies

Localized DLBCL

The approach to localized DLBCL is often curative with combination immunochemotherapy. Certain adverse risk factors including elevated LDH level, bulky disease (ie, lymphadenopathy

Table 3. — Scoring of the IPI and the Age-Adjusted IPI: Associated 5-Year Overall Survival

IPI			
Risk Group	**Number of IPI Factors**	**% of Patients (n = 2,031)**	**5-Year Overall Survival (%)**
Low	0 or 1	35	73
Low-intermediate	2	27	51
High-intermediate	3	22	43
High	4 or 5	16	26
Age-Adjusted IPI ≤ 60 years			
Risk Group	**Number of Age-Adjusted IPI Factors**	**% of Patients (n = 1,274)**	**5-Year Overall Survival (%)**
Low	0	22	83
Intermediate	1	32	69
Intermediate	2	32	46
High	3	14	32

From A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. *N Engl J Med.* 1993;329(14):987-994. Reprinted with permission by Massachusetts Medical Society.

Table 4. — Scoring of the Revised IPI and Associated 4-Year Overall Survival

Risk Group	Number of IPI Factors	% of Patients (N = 365)	4-Year Overall Survival (%)
Standard IPI			
Low	0, 1	28	82
Low-intermediate	2	27	81
High-intermediate	3	21	49
High	4, 5	24	59
Revised IPI			
Very good	0	10	94
Good	1, 2	45	79
Poor	3, 4, 5	45	55

From Sehn LH, Berry B, Chhanabhai M, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. *Blood*. 2007;109(5):1857-1861. *Blood*: Journal of the American Society of Hematology by American Society of Hematology; High-Wire Press Copyright 2012. Reproduced with permission of AMERICAN SOCIETY OF HEMATOLOGY (ASH) in the format Journal via Copyright Clearance Center.

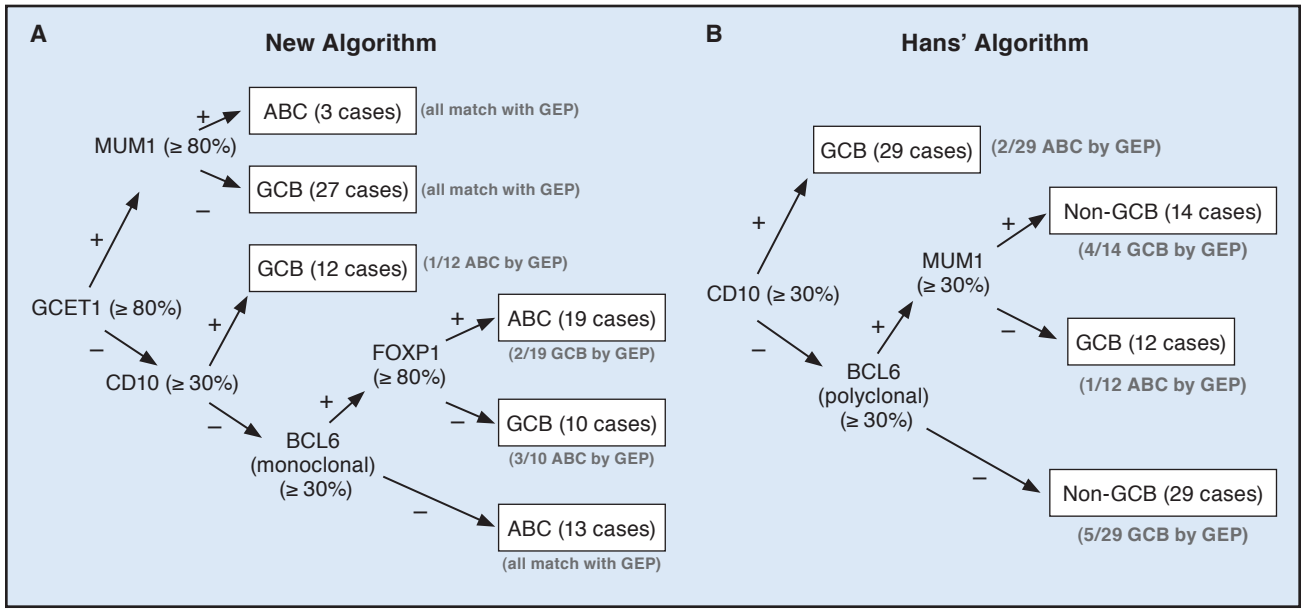

Fig 1. — The new algorithm (A) and the Hans' algorithm (B). The new algorithm uses five markers, with 78 of 84 cases concordant compared with the GEP classification, whereas the Hans' algorithm had 72 of 84 cases concordant in the same set. Reproduced with permission of American Association for Cancer Research from Choi WW, Weisenburger DD, Greiner TC, et al. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. *Clin Cancer Res*. 2009;15(17):5494-5502, permission conveyed through Copyright Clearance Center, Inc.

measuring more than 10 cm or more than 1/3 of the mediastinum), and extranodal presentation help to stratify patients according to risk. Prospective evaluation of patients with stage I and II disease has demonstrated that radiotherapy alone is insufficient. However, multimodal therapy utilizing both immunochemotherapy and radiation may minimize the effective dose and thus the toxicity associated with these approaches. Miller et al[12] reported on 400 patients who were randomized to CHOP chemotherapy alone for 8 cycles vs a shortened course of CHOP for 3 cycles plus external beam radiation. Patients receiving the shortened course of CHOP followed by radiation demonstrated a significantly better progression-free survival (PFS) compared with those receiving multimodal therapy. Long-term toxicities were statistically similar in both groups, although cardiac-related toxicity was higher in the chemotherapy-alone arm.

The Groupe d'Etudes des Lymphomes de 1'Adulte (GELA) prospectively evaluated this scenario in two further studies. GELA LNH 93-1 stratified patients under 61 years of age with localized aggressive lymphomas and low-risk IPI (0) into two arms.[13] Patients in arm 1 received chemotherapy alone with an intensive regimen of doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone plus consolidation with methotrexate/ifosfamide/cytarabine. Patients in arm 2 received a less intensive regimen of CHOP plus involved-field radiation therapy. The 5-year overall survival rate in arm 1 was statistically higher at 90% vs 81% (P = .003). In addition, toxicity was substantially higher for those in arm 1. The GELA LNH 93-4 trial evaluated older patients (60 to 80 years of age) with early-stage good-risk aggressive lymphomas treated with CHOP (4 cycles) alone compared with those treated with CHOP (4 cycles) plus involved-field radiotherapy.[13] There was no statistical difference in efficacy or 5-year overall survival in both arms (72% in the CHOP-only arm and 68% in the CHOP plus radiotherapy arm).

There are few to no formal prospective evaluations of the efficacy of the multimodal therapy in the rituximab era. Consolidating the data above, the toxicity of intensive chemotherapy for localized stage I/II disease should be considered in relation to the use of CHOP alone vs multimodal therapy. There continues to be advances in decreasing toxicity for external beam radiation therapy have decreased both long- and short-term toxicity. Although the impact of these factors is unknown, standards in the United States for localized DLBCL patients recommend risk stratification in order to delineate therapy.[1] Patients and physicians should discuss the effects of a shortened course of immunochemotherapy (R-CHOP) plus radiation therapy or a longer course of immunotherapy alone, taking into consideration each patient's presentation, comorbidities, and risk stratification. Patients with bulky disease benefit from the addition of external beam radiation when possible.

Advanced DLBCL

The standard of care for advanced-stage DLBCL (stage III-IV) is combination immunochemotherapy with R-CHOP.[4] Several small studies have reported mixed results when using more intensive regimens for advanced-stage patients with an aggressive phenotype or higher IPI. Data from the Southwest Oncology Group (SWOG) compared CHOP with regimens such as (1) M-BACOD, consisting of intermediate-dose methotrexate with leucovorin rescue, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone, (2) ProMACE-CytaBOM, consisting of prednisone, methotrexate, doxorubicin, cyclophosphamide, and etoposide followed by cytarabine, bleomycin, vincristine, and methotrexate with leucovorin rescue, and (3) MACOP-B, consisting of methotrexate with leucovorin rescue, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin).[14] No long-term benefit was seen in utilizing more intensive regimens. Almost 900 patients were treated in each group, with no significant difference between the subgroups for all outcomes including response rate, time to treatment failure, and overall survival (Fig 2).[15] CHOP remains a time-tested standard of care.

The Rituximab Era

Rituximab is a monoclonal antibody targeting CD20, a common B-cell marker that is present on the majority of malignant lymphoma cells in DLBCL. Although its mechanism of action has not been completely elucidated, several actions have been postulated, including complement-mediated toxicity.[16] Outcomes from the MabThera International Trial (MInT) demonstrated promising results for rituximab in DLBCL.[17] In this

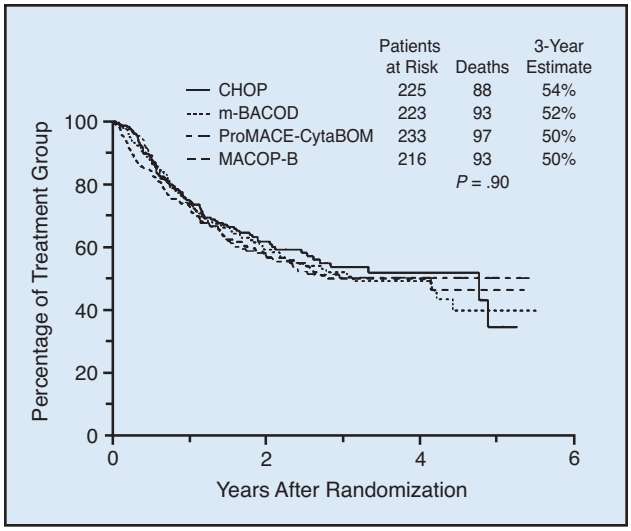

Fig 2. — Overall survival in patients who received CHOP vs intensive chemotherapy (SWOG experience). Fisher RI, Gaynor ER, Dahlberg S, et al. Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. *N Engl J Med*. 1993;328(14):1002-1006. Reprinted with permission by Massachusetts Medical Society.

large multicenter trial, patients from 18 to 60 years of age with good-risk DLBCL (low IPI), stage II–IV, received 6 cycles of CHOP-like chemotherapy with or without rituximab. Over 800 patients were treated, and a 6-year event-free survival demonstrated a favorable response in patients who received rituximab (74.3% vs 55.8%; $P = .0001$). Rituximab was well tolerated by patients with low overall toxicity outside of infusion reactions and with little to no long-term toxicity to date.[4,17] The GELA group added rituximab to CHOP in a prospective trial of 399 patients who were 60 through 80 years of age with aggressive lymphomas.[4,18] A 10-year update of this GELA study demonstrated survival at 43.5% for the rituximab cohort and 20% for patients treated with CHOP alone.[4,18] Thus, rituximab continues to be an integral treatment component of patients with DLBCL.

Relapsed/Refractory DLBCL

Although a percentage of patients with DLBCL achieve durable remissions (primarily those with a low to intermediate IPI score), patients who relapse or who are refractory (relapse to therapy within 6 months of completion of therapy or no appreciable response during therapy) have a poorer outlook. Salvage chemotherapy options are often inadequate; response rates range from 30% to 60%, with frequent relapses. These therapies involve combination chemotherapy with or without rituximab. They can be tailored to fit a patient's clinical presentation and toxicity profile from prior chemotherapies.[1] Gisselbrecht et al[19] reported a study in which a comparison of two of the most commonly used regimens — R-ICE (rituximab, ifosfamide, carboplatin, and etoposide) and R-DHAP (rituximab, dexamethasone, high-dose cytarabine, and cisplatin) — demonstrated no difference in efficacy or overall toxicity in the CORAL trial (Collaborative Trial in Relapsed Aggressive Lymphoma), a prospective evaluation of salvage therapy and transplant in patients with varied front-line exposure of rituximab.[19] This trial also demonstrated that patients without prior exposure to rituximab had better outcomes than those who were exposed to rituximab in the front-line setting. It may be extrapolated that patients who present with rituximab refractory or resistant disease have a negative prognostic risk and may represent a patient population with chemotherapy-insensitive disease (Table 5).

In 1991 the Parma international trial demonstrated that lymphoma patients with relapsed or refractory disease could be induced into a durable remission after high-dose chemotherapy followed by autologous stem cell rescue.[20] Twenty percent of the patients who entered the pilot study are long-term survivors without disease progression. Further prospective studies have validated these data by demonstrating that approximately 50% of patients undergoing transplant achieved a durable remission after 3 years. Thus, a hematopoietic stem cell transplant (HSCT) is the only option for a durable remission at this point for patients with relapsed or refractory DLBCL. In many cases, HSCT availability is limited by patient age, treatment-related morbidities, and poor performance status.[21] Following is a discussion of some novel targeted therapies that may help improve patient outcomes.

Table 5. — Candidates for Salvage Chemotherapies

Candidate for Hematopoietic Stem Cell Transplant*	
Therapy	**Agents**
DHAP	Dexamethasone, cisplatin, cytarabine
ESHAP	Etoposide, methylprednisolone, cytarabine, cisplatin
Dose-adjusted EPOCH	Etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin
GDP	Gemcitabine, dexamethasone, cisplatin
GemOx	Gemcitabine, oxaliplatin
ICE	Ifosfamide, carboplatin, etoposide
MINE	Mesna, ifosfamide, mitoxantrone, etoposide
Not a Candidate for Hematopoietic Stem Cell Transplant**	
Therapy	**Agents**
CEPP	Cyclophosphamide, etoposide, prednisone, procarbazine
Dose adjusted EPOCH	Etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin
CEOP	Cyclophosphamide, etoposide, vincristine, prednisone
GDP	Gemcitabine, dexamethasone, cisplatin
GemOx	Gemcitabine, oxaliplatin
Lenalidomide	
Rituximab	

* Rituximab can be added to all regimens at clinician discretion.
** Each agent can be given as a single agent for palliative intent.

Future Directions

Monoclonal Antibodies

As first in its class to be approved by the US Food and Drug Administration (FDA), rituximab has revolutionized treatment strategies in NHL and specifically in DLBCL. Several mechanisms of action for monoclonal antibodies in malignancies have been proposed, including (1) complement-mediated lysis and phagocytosis, (2) antibody-dependent cell-mediated cytotoxicity (ADCC), (3) physiologic activation/deactivation of target receptor, (4) potentiation of chemotherapy, (5) induction of secondary immune reactions, and/or (6) delivery method for targeting radioisotopes, other drugs, or toxins.[16] The advent of rituximab has heralded a wealth of monoclonal antibodies that have entered the armamentarium in lymphoma management, against CD20 and a multitude of other cellular extracellular and intracellular markers. Although uncommon, loss of CD20 positivity does occur as well as de novo CD20-negative lymphomas allowing for the development of other monoclonal antibody use in DLBCL. This loss of CD20 positivity may be a rationale for rituximab failure in the salvage setting.

Anti-CD20 Antibodies

There are key differences among the various anti-CD20 antibodies available for the treatment of DLBCL. Distinguishing between type I and type II antibodies is important to elucidate mechanisms of action. A type I antibody such as rituximab is integral in complement-dependent cytotoxicity and is less important in stimulating pathways attached to its target receptor, whereas type II antibodies are able to initiate and potentiate apoptotic pathways in vitro with more efficacy than type I antibodies.[16]

CD20 is a monoclonal antibody that binds to the small loop of CD20. It is currently approved in the treatment of patients with fludarabine- and alemtuzumab-refractory chronic lymphocytic leukemia. Ofatumumab is currently being studied in DLBCL. Coiffier et al[22] described a study that included heavily pretreated patients with relapsed or refractory lymphoma. The majority of patients (96%) had received prior rituximab. All patients were given 8 weekly infusions of ofatumumab (dose 1 = 300 mg; doses 2–8 = 1,000 mg). Overall response rates reached 11% (3 complete responses and 6 partial responses). Patients tolerated the drug well, with the most common adverse events being fatigue, gastrointestinal distress, and hematologic toxicity. Studies have shown that a durable response may be difficult to achieve with single-agent ofatumumab. However, evaluation of ofatumumab is ongoing in relapsed DLBCL in combination with traditional chemotherapy regimens.

Obinutuzumab (GA-101) is a type II humanized anti-CD20 antibody currently being studied in lymphomas. Compared with rituximab, obinutuzumab is thought to increase signaling in the target cells, thereby activating the apoptotic pathway. In fact, in vitro studies have demonstrated superiority of this antibody in inducing cell death compared to rituximab. Obinutuzumab binds CD20 in a different orientation than rituximab does, allowing an increased affinity to the CD20 molecule. Phase I studies have demonstrated a well-tolerated toxicity profile similar to that of other monoclonal antibodies, including infusion reactions, neutropenic fever, and/or thrombocytopenia. Cartron et al[23] investigated obinutuzumab in relapsed/refractory aggressive lymphoma in which 40 patients were given the drug on days 1, 8, and 22 for 3 weeks for 6 doses from 400 mg to 1,600 mg. Endpoints included response rates, which for DLBCL patients were 30% in the low-dose arm (3 patients), 27% in the high-dose arm (4 patients), and 29% overall (7 patients). Forty-five percent of all patients had completed all 9 infusions, and the phase I data shows a tolerable toxicity profile. Currently, a clinical study is comparing obinutuzumab in combination with CHOP vs rituximab and CHOP in patients with DLBCL (NCT01287741).

Veltuzumab binds to a similar epitope as does rituximab; however, the backbone of the molecule is identical to that of epratuzumab (an anti-CD22 monoclonal antibody). The rationale for this combination is due to significantly reduced infusion-related reactions. In phase I/II studies by Morshhauser et al,[24] veltuzumab was well tolerated, with a 34% overall response rate in nonfollicular lymphoma histologies (2 complete responses in marginal-zone patients). The most frequent adverse events were fatigue, pruritus, fever, headache, asthenia, dyspnea, and cough. Studies in more aggressive lymphomas are pending.

Non–Anti-CD20 Antibodies: Targeting Tumor Cells and Stroma

Epratuzumab is a humanized monoclonal antibody to CD22, a highly restricted B-cell marker. CD22 has a key role in B-cell receptor signaling, internalization, and homing and is expressed in high concentrations on large B cells. In phase I trials, epratuzumab was well tolerated as a single agent and in combination with rituximab plus chemotherapy (R-CHOP).[5,25] Although single-agent efficacy was low (10% overall response), activity was enhanced with rituximab in the indolent lymphoma setting; overall responses were 66% in the relapsed/refractory aggressive lymphoma setting. Micallef et al[25] reported on 107 patients treated with standard R-CHOP with the addition of epratuzumab 360 mg/m^2 intravenously for 6 cycles. Responses were high, with 96% in eligible patients and a complete response rate of 74%. The event-free survival rate for patients was 70% at 8 years, with an overall survival at 80%, thus demonstrating epratuzumab as an effective and well-tolerated addition that will continue to be aggressively investigated in combination with other targeted agents and with traditional chemotherapy.

CD40 is a transmembrane protein in the tumor necrosis factor receptor (TNFR) super family that is primarily expressed on B cells, including DLBCL. Dacetuzumab is a humanized anti-CD40 antibody that has demonstrated activity as a single agent through binding of the ligand and subsequent growth inhibition of the cell. As with most antibodies, it may also have action in the ADCC pathway. A phase I study conducted by Advani et al[26] demonstrated the safety and tolerability of this agent in lymphomas, specifically DLBCL. The maximum tolerated dose was not reached in this study; the drug was given at 8 mg/kg per week. The most common toxicity was headache, which occurred following the first infusion. Responses were seen in 6 patients (12%), 4 of whom were patients with DLBCL. Dacetuzumab has also been combined with traditional salvage regimens such as single-agent gemcitabine to produce response rates up to 54% and with ICE followed by autologous stem cell transplant with some success.[27,28]

Blinatumomab is a bi-specific antibody with specificity for CD19 (a common B-cell marker) and CD3 (a T-cell engager). Upon binding and engaging both cells, the B cell is stimulated to growth arrest and apoptosis as the T cell is stimulated to proliferate. A phase I study by Nagorsen et al[29] involving 12 lymphoma patients receiving 60 μg/m^2 per day of blinatumomab infused for 4 to 8 weeks has demonstrated a good risk profile. Dose-limiting toxicity was primarily neurologic and consisted of a reversible encephalopathy, cerebellar toxicity, and speech impairment that occurred with patients with a B-cell to T-cell ratio of < 1:8. However, 11 of the 12 patients in this study demonstrated an objective response. At least half of the responders continue to respond at 1 year out from therapy. This interesting molecular warrants further study, specifically in DLBCL.

In recent years, the tumor stroma has become an important consideration as a target for lymphoma therapy. As gene expression microarrays have been used in determining poor-risk subtypes in DLBCL, the microenvironment has also been examined. Lenz et al[30] described the use of gene expression arrays to profile 181 patients with DLBCL by their stroma after treatment with CHOP (n = 181) and R-CHOP (n = 233). Three signatures were obtained: (1) germinal-center B cell, (2) stromal-1 (consistent with extracellular matrix deposition and histiocytic infiltration), and (3) stromal-2 (consistent with tumor blood vessel density). The stromal-2 pattern was determined to be poorer risk compared with the other stromal patterns. Technology advances in the future may help to define individual targets within these signatures, leading to the development of novel targeted therapies and improvements in patient outcomes.

T-cell regulation is important in tumor surveillance and response. One such pathway that reduces antitumor immune responses is the cytotoxic T lymphocyte antigen-4.[31] Blockade of this signal serves to increase T-cell activity against tumor tissue. Ipilimumab (MDX-010) is an antagonist anti–CTLA-4 antibody.[31] It is approved by the FDA for the treatment of relapsed or refractory melanoma. Ansell et al[31] reported a phase I trial of this agent in relapsed/refractory B-cell lymphomas. Among 18 patients treated, 2 had clinical responses. One of them, a patient with DLBCL, has had an ongoing complete response of longer than 31 months. Based on these results, ipilimumab is an agent to pursue in larger phase II trials in DLBCL.

Stimulation of the B7 receptor, found on lymphocytes, leads to stimulation of natural killer cell activity and survival of T memory cells.[32] Cross-linking of CT-011 (a humanized monoclonal antibody) to its ligand PD-1 (a member of the B7 receptor) activates this cascade and stimulates a T-cell–mediated immune response. This activity may be beneficial in many tumor types. Berger et al[32] undertook a phase I study with CT-011 in patients with advanced hematologic malignancies. The maximum tolerated dose was not defined in the study. An objective overall response was seen in 33% of the patients, with 1 complete response in a patient with DLBCL. Larger multicenter phase II trials with CT-011 maintenance following auto–HSCT have demonstrated a PFS rate of 70%, an improvement compared with historical controls.[33] This agent needs further validation and may be combined with other targeted agents, traditional chemotherapy, and vaccine therapy in the future.

Antibody-Drug Conjugates

Antibody-drug conjugates deliver targeted cytotoxic therapy and utilize immune responses unlike naked antibody analogs. Furthermore, the toxicity profile of the cytotoxic compound is limited due to the drastic reduction of active agent being delivered to the target cell. Several compounds are being investigated for the treatment of lymphomas.

Inotuzumab ozogamicin is an antibody against CD22 conjugated with calicheamicin. This molecule is internalized, thereby delivering the calicheamicin directly to the cell. This agent demonstrated high levels of cytotoxic activity in B-cell lines. Advani et al[34] reported a maximum tolerated dose of 1.8 mg/m^2 administered every 28 days. Toxicity included thrombocytopenia, asthenia, nausea, and neutropenia, but overall the agent was well tolerated. Responses were 39% for all 79 eligible patients and 15% for patients with DLBCL at the maximum tolerated dose. Dang et al[35] reported using combination rituximab and inotuzumab ozogamicin at standard dosing in patients with recurrent DLBCL. Patients had a 1-year overall survival rate of 77%, an overall response rate of 80%, and a PFS of 15.1 months. The overall response rate for patients in the rituximab-refractory arm was much lower (20%), with a PFS of 2 months. There was no worsening of toxicity with combination therapy.

Inotuzumab ozogamicin has also been evaluated as a salvage regimen for use prior to autologous HSCT.[35] Wagner-Johnston et al[36] reported on 34 patients treated with inotuzumab ozogamicin plus rituximab in an ongoing clinical trial in which 11% of patients had durable responses. Seven patients proceeded to peripheral blood stem collection and 5 patients underwent transplant, demonstrating successful mobilization following treatment with inotuzumab ozogamicin.

Lenalidomide

Lenalidomide is an immunomodulatory agent whose mechanism of action is not quite understood but may affect tumor microenvironment rather than the tumor itself. One possible advantage of this agent is the ability to enhance the cytotoxic activity of T and NK cells. Lenalidomide may also act by decreasing proliferation and angiogenesis by upregulating tumor suppressor genes. It has demonstrated activity both in vitro and in vivo in patients with NHL, specifically DLBCL. In the original pilot studies, 23% of lymphoma patients achieved an objective response when treated with lenalidomide.[37] Responses were durable in a highly refractory population, with a median response of 16.5 months. Witzig et al[38] reported on a larger phase II study in which 217 patients received lenalidomide 25 mg given orally daily for 21 days in a 28-day cycle. The overall response rate was 35% (13% complete responses, 22% partial responses, and 21% stable disease), with a median response duration for responders of 10.6 months. Toxicity was well tolerated and similar to that seen in other tumor types.

Further studies have investigated the efficacy of lenalidomide on the different subtypes of DLBCL. A retrospective review of patients treated with salvage lenalidomide at four academic institutions found 40 patients with relapsed/refractory DLBCL treated with lenalidomide who were classified as germinal center B-cell-like or non–GCB subtype based on the Hans' algorithm.[39,40] The overall response rate in the patients with non–GCB subtype was 52.9% vs 8.7% in the GCB cohort (P = .006), and the complete response rate was 23.5% vs 4.3%. A statistical difference was found in the PFS of the non–GCB cohort (6.2 months vs 1.7 months), although overall survival was not statistically different.

Lenalidomide is being brought to the front line in DLBCL management as an addition to R-CHOP in large prospective clinical trials. It is also being used in combination with salvage chemotherapies and as maintenance following autologous transplant. Investigations using combinations with other targeted agents such as histone deacetylase (HDAC) inhibitors and proteasome inhibitors are ongoing.

Proteasome Inhibitors

The non–GCB subtype of DLBCL has been identified as having overexpression of a proapoptotic molecule known as NF-κB. The constitutive activation of this pathway allows the cell to remain immortal and foster resistance against traditional chemotherapeutic agents. In a study of 49 patients with relapsed or refractory DLBCL who were treated with single-agent bortezomib and intensive chemotherapy, bortezomib had no efficacy as a single agent.[9] However, when combined with chemotherapy, it improved outcomes in patients with the non–GCB subtype of DLBCL in greater magnitude than that of patients with the GCB subtype. Bortezomib as an inhibitor of NF-κB may offer insight into the improved responses in the poorer risk group. A large phase III trial is being conducted to evaluate the efficacy of adding bortezomib in the relapsed/refractory setting as well as in the front line in combination with R-CHOP.[41]

B-Cell Receptor Pathway

In addition to monoclonal antibodies, proteasome inhibitors, and agents such as lenalidomide, the B-cell receptor pathway is another new therapeutic target. The activation of this pathway plays a role in proliferation and survival in B-cell NHL. Therapeutic targets in this pathway include splenic tyrosine kinase (Syk) and Burton's tyrosine kinase (Btk). Drugs have been developed to target Syk and Btk and appear promising for refractory or relapsed B-cell NHL.

Syk

Since Syk is needed for survival of mature B cells, there is constitutively active Syk in B-cell lymphomas. Fostamatinib disodium is a tyrosine kinase inhibitor that is an oral prodrug of R406, which has been shown to downgrade B-cell receptor signaling via Syk inhibition, leading to apoptosis in B-cell lymphomas.[42] Fostamatinib disodium has shown promise in a phase I/II trial in which 68 heavily pretreated patients were enrolled into three cohorts, including one for DLBCL.[43] In the phase II study, 22 patients enrolled in the DLBCL cohort had an overall response rate of 22% and a median PFS of 2.7 months. One patient in the DLBCL arm had a complete response. Toxicity included grade 3/4 diarrhea, neutropenia, anemia, and thrombocytopenia in the phase II trial. Larger studies are needed to further assess Syk inhibitors and their use in DLBCL.

Btk

Btk is a cytoplasmic protein mainly expressed in hematopoietic cells. Btk is required for B-cell receptor signaling and plays an important role in B-cell maturation. It is overexpressed in multiple B-cell malignancies.[44] The ABC subtype of DLBCL is driven by activated B-cell receptor, and it has been shown that Btk is an essential kinase for survival.[45] PCI-32765 is a selective and irreversible Btk inhibitor, preventing B-cell activation and the growth of malignant B cells that overexpress Btk as well as inhibiting Btk activity. Two clinical trials have

been completed using PCI-32765. The first trial, which is ongoing, enrolled 78 patients with chronic lymphocytic leukemia at the time of presentation.[46] At a median of 4 months, 39% of patients had a partial response and 5% had a complete response The second trial studied PCI-32765 in relapsed aggressive NHL.[47] Of the 29 patients enrolled, 4 had DLBCL. The overall response rate was 42%, and toxicity was less than grade 2. Staudt et al[48] recently reported on 8 patients with relapsed/refractory ABC-type DLBCL who were treated with PCI-32765. Two patients had a complete response, lasting 11 months and 5 months. Three patients had stable disease for 2 to 4 months, and 3 had progressive disease. A second selective covalent Btk inhibitor, AVL-292, is currently being evaluated in a phase Ib clinical trial.[49] PCI-32765 also continues to be tested in multiple clinical trials to determine its role in treating refractory/relapsed DLBCL.

Conclusions

Recent advances have ushered in a renaissance for personalized cancer medicine and molecularly targeted therapy. Ongoing research is uncovering important clues regarding the etiology of diffuse large B-cell lymphomas and also targeted drug therapy in the management of patients who are not cured in the rituximab era. Further prospective investigations utilizing these targeted agents in the appropriate patient cohorts and in combination with traditional chemotherapy and other novel agents are key in understanding this diverse group of diseases. In addition, correlatives must be included to further identify potential biomarkers in diffuse large B-cell lymphomas to further elucidate the mechanisms of actions and the biology of tumor cells and their interaction with the microenvironment. Such advances will provide the potential to fully personalize our methodology in addressing these malignancies and lead to a more uniform approach to treatment.

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