

Novel Agents for the Treatment of Chronic Lymphocytic Leukemia

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Abstract: Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world. Currently, the most effective treatment for CLL consists of a combination of fludarabine, cyclophosphamide, and rituximab. Although this approach has encouraging results, patients with CLL eventually relapse and require additional therapies. Many of the current therapeutic regimens for CLL are myelotoxic, immunosuppressive, and associated with infectious complications. Targeted therapies can often minimize these complications. The US Food and Drug Administration has recently approved 2 agents, bendamustine and ofatumumab, for the treatment of CLL. Emerging therapies ranging from new monoclonal antibodies to small molecules that interfere with vital pathways in signal transduction and cell cycle regulation are currently being developed. This article will focus on novel agents in earlier development phases for CLL, including the immunomodulator lenalidomide; monoclonal antibodies, such as lumiliximab, GA-101, and small molecule immunopharmaceuticals; BCL-2 inhibitors, such as oblimersen, obatoclax, and ABT-263; and protein kinase inhibitors, such as flavopiridol, spleen tyrosine kinase inhibitors, and phosphatidylinositol 3-kinase inhibitors.

Introduction

Chronic lymphocytic leukemia (CLL) is a clonal disorder resulting in the accumulation of B lymphocytes coexpressing CD5, CD19, and CD23.¹ CLL is the most common leukemia in the Western world, with an incidence ranging from 2–6 cases per 100,000 persons per year. In 2008, 15,110 new cases of CLL were diagnosed in the United States, and 4,390 patients died as a result of this disease.² Currently, the most effective treatment for CLL consists of a combination of fludarabine, cyclophosphamide, and rituximab (Rituxan, Genentech; FCR). In previously untreated patients, FCR results in an overall response rate (ORR) of 95%, a complete response (CR) rate of 44%, and a median progression-free survival (PFS) of 52 months.³ Despite these encouraging results, patients

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with CLL eventually relapse and require additional therapies. Many of the current therapeutic regimens for CLL are myelotoxic, immunosuppressive, and associated with infectious complications.³ Targeted therapies can often minimize these complications. Furthermore, patients with unmutated immunoglobulin heavy chain (IgVH) genes as well as del(17p13.1) and del(11q22.3) are more likely to become refractory to conventional therapies⁴ and may benefit from novel agents.

Two agents for CLL were recently approved by the US Food and Drug Administration (FDA). The first, bendamustine (Treanda, Cephalon), is an alkylating agent that resulted in a superior ORR (68% vs 31%) and CR rate (31% vs 2%) compared to chlorambucil in a randomized, controlled trial of 319 patients with previously untreated CLL requiring therapy.⁵ Based on these results, bendamustine was approved by the FDA for use in previously untreated CLL in March 2008, and clinical trials are ongoing to assess where this agent best fits into the therapy of CLL. In particular, the German CLL Study Group is currently conducting a randomized trial comparing FCR to bendamustine plus rituximab in previously untreated CLL patients.

The second newly approved agent, ofatumumab (Arzerra, GlaxoSmithKline), is a fully human anti-CD20 monoclonal antibody that differs from rituximab by binding to a different CD20 epitope and directing more potent complement-dependent cytotoxicity, even against cells expressing lower levels of CD20.⁶ Ofatumumab was evaluated in a study of 138 patients with CLL. The trial incorporated 2 parallel arms: patients refractory to fludarabine and alemtuzumab (Campath, Genzyme; FA-ref arm) and patients with bulky disease refractory to fludarabine and not clinically suitable for alemtuzumab therapy (BF-ref arm). The observed ORR was 58% for the former group and 47% for the latter group. In the FA-ref arm, the median PFS was 5.7 months, and the median overall survival was 13.7 months. In the BF-ref arm, the median PFS was 5.9 months, and the median overall survival was 15.4 months.⁷ Based on this trial, ofatumumab was approved by the FDA in October 2009 for treatment of CLL refractory to fludarabine and alemtuzumab. In-depth discussion of these recently approved agents is beyond the scope of this review. Rather, we will focus our attention on novel agents in earlier development phases for CLL (Table 1).

Immunomodulators

Lenalidomide

Lenalidomide (Revlimid, Celgene) is an immunomodulatory drug currently approved by the FDA for the treatment of multiple myeloma as well as 5q-deletion

myelodysplastic syndrome. The clinical effects of lenalidomide are likely derived from numerous mechanisms, including antiangiogenic properties, inhibition of tumor necrosis factor alpha (TNF- α), modulation of T-cell- and NK cell-mediated immunologic responses, and induction of apoptosis.⁸⁻¹⁰ Lenalidomide was recently shown to upregulate the expression of costimulatory CD80 on CLL cells leading to T-cell activation, which correlated clinically with the observed cytokine release syndrome.¹¹ Furthermore, lenalidomide induces expression of CD154 on CLL cells, resulting in activation of normal B cells that leads to enhanced immunoglobulin production.¹²

In a phase II trial of 45 patients with relapsed CLL, lenalidomide was administered orally at 25 mg on days 1–21 of a 28-day cycle.¹³ Neutropenia and thrombocytopenia each occurred in 78% of patients. Only 2 patients (5%) developed tumor lysis syndrome (TLS). Fatigue (83%) and flare reaction (58%) were the most common nonhematologic adverse events. Responses were seen in 21 patients (47%), with 4 (9%) achieving a CR and 17 (38%) achieving a partial response (PR). The estimated PFS rate at 12 months was 81%. In another phase II trial of 44 patients with relapsed CLL, a daily 10 mg dose of lenalidomide administered continually resulted in similar efficacy and toxicity profiles.¹⁴ Encouraging response rates, ranging from 31–47%, were also observed among high-risk patients with del(17p13.1) and del(11q22.3).^{13,14}

The use of lenalidomide in CLL has, however, been associated with TLS and flare reaction. The latter is usually manifested by sudden onset of tender, enlarged lymph nodes with low-grade fever and rash, which are generally manageable but can lead to a systemic inflammatory response and even death. These life-threatening tumor flare reactions were highlighted in a published series of 4 patients with relapsed CLL who were treated with lenalidomide at a dose of 25 mg. All patients developed serious adverse events. Three experienced severe tumor flare reactions and 1 patient died.¹⁵

The promising results observed in relapsed CLL, albeit tempered by safety concerns, have led investigators to study the role of lenalidomide in frontline therapy for CLL at a reduced dose. In a phase II study of 25 previously untreated symptomatic CLL patients, lenalidomide was administered at a starting dose of 2.5 mg orally daily with weekly dose escalations up to a target dose of 10 mg/day for 21 days of 28-day cycles.¹⁶ A tumor flare reaction was observed in 88% of patients. Grade 3/4 neutropenia occurred in 18 patients (72%), and febrile neutropenia occurred in 5 (20%). Fourteen patients (56%) achieved a PR, 10 (40%) had stable disease (SD), and 1 patient progressed on treatment, resulting in an estimated 2-year PFS rate of 87%. Interestingly, discontinuation of lenalidomide on days 22–28 of each cycle was associated

Table 1. Novel Agents in the Treatment of Chronic Lymphocytic Leukemia

Category/Agent	Dose and Administration	Response Rates	Response Duration	Toxicity
Immunomodulators				
Lenalidomide ^{13,14,16,17}	2.5–25 mg* orally daily for 21 of 28 days	Upfront single agent: ORR, 53–56%	2-year PFS rate, 87%	Tumor lysis syndrome, tumor flare reaction, myelosuppression
		Relapse single agent: ORR, 32–47%	1-year PFS rate, 81%	
Monoclonal Antibodies				
Lumiliximab ^{21,22}	500 mg/m ² IV every 28 days	Relapse single agent: ORR 0% (reduction in lymph node size and lymphocytosis only)	Not reported	Infusion reactions
		Relapse combination with FCR: ORR, 65%; CR, 52%	Median PFS, 19 months	
GA-101 (obinutuzumab) (anti-CD20) ²⁵	400-2,000 mg IV days 1, 8, and 22, then every 3 weeks for 9 doses	Relapse single agent: ORR, 62%	Response duration ranges from 3.5–8 months	Infusion reactions, neutropenia
SMIP				
TRU-016 (anti-CD37) ²⁸	IV weekly × 4 weeks Dose escalation studies ongoing	Relapse single agent: biologic activity reported	Not reported	Infusion reactions
BCL-2 Inhibitors				
Oblimersen ^{32,33}	3 mg/kg/day IV continuous infusion × 7 days	Relapse combined with FC: CR/nPR, 17%	Median PFS, 27 months	Cytokine release syndrome, tumor lysis syndrome, myelosuppression
Obatoclax ³⁴	28 mg/m ² IV every 3 weeks × 8 doses	Relapse single agent: PR, 4%; biologic activity reported	Not reported	Somnolence, euphoria, ataxia, infusion reactions
ABT-263 ³⁵	100 mg orally daily × 7 days followed by 250 mg orally daily continuous dosing	Relapse single agent: ORR, 33%; CR 0%	Median PFS not reached at 9 months	Thrombocytopenia, myelosuppression, GI side effects, fatigue
AT-101 ³⁸	30 mg orally daily for 21 of 28 days	Relapse combined with rituximab: ORR, 38%; CR, 25%	Not reported	Neutropenia, ileus, fatigue

(Table continues on following page)

with rebound lymphocytosis, suggesting that continuous therapy may be beneficial in patients with CLL.

Single-agent lenalidomide may also be beneficial in older patients with untreated CLL, who often are not candidates for toxic regimens such as FCR. Sixty patients with untreated CLL who were older than 65 (median age 71 years) were evaluated in a phase II study in which lenalidomide was administered continuously at a starting dose of 5 mg orally, with dose escalations up to 25 mg daily.¹⁷ Treatment was well tolerated, despite the older age of the patients. The most common grade 3/4 toxicities

were hematologic, and mild tumor flare reaction was observed in 50% of patients. The ORR was 53%, including 3 CR (5%), 7 nodular PR (12%), and 22 PR (37%). After a median follow-up of 15.5 months, the median time to treatment failure had not been reached.

Although studies of lenalidomide as upfront therapy in CLL are ongoing, excessive myelotoxicity may preclude its use in combination with other myelosuppressive agents. A recent phase I study of lenalidomide in combination with fludarabine and rituximab for previously untreated CLL patients was stopped early

Table 1. (Continued) Novel Agents in the Treatment of Chronic Lymphocytic Leukemia

Category/Agent	Dose and Administration	Response Rates	Response Duration	Toxicity
Protein Kinase Inhibitors				
Flavopiridol ⁴⁵	30 mg/m ² IV bolus followed by 30 mg/m ² IV infusion day 1 cycle 1, then 30 mg/m ² + 50 mg/m ² IV administered in cycles of 3 of 4 weeks or 4 of 6 weeks × 6 cycles	Relapse: ORR, 53%; CR, 1.6%	Median PFS, 10–12 months	Tumor lysis syndrome, diarrhea, fatigue
SNS-032 ⁴⁶	6-hour IV loading dose followed by 3 weekly doses for each 28-day cycle Dose escalation studies ongoing in CLL	ORR, 0% to date	Not reported	Tumor lysis syndrome
SCH 727965 ⁴⁷	2-hour IV infusion on days 1, 8, and 15 of a 28-day cycle Dose escalation studies ongoing in CLL	Not reported	Not reported	Tumor lysis syndrome, vomiting, diarrhea
Fostamatinib ⁵³	200 mg orally BID	Relapse: ORR, 55%; CR, 0%	Median PFS, 6.4 months	Myelosuppression, leukocytosis, fatigue, diarrhea
CAL-101 ⁵⁶	Orally BID Dose escalation studies ongoing in CLL	Relapse: ORR, 24%; CR, 0%	Not reported	Transaminitis
Everolimus ^{61,64}	5–10 mg orally daily	Relapse: ORR, 14–18%; CR, 0%	Not reported	Myelosuppression, opportunistic infection
Dasatinib ⁶⁸	140 mg orally daily	Relapse: ORR, 20%	Not reported	Myelosuppression, pleural effusion

*There are increased risks of toxicity with the 25 mg dose.

BID=twice daily; CLL=chronic lymphocytic leukemia; CR=complete response; FC=fludarabine, cyclophosphamide; FCR=fludarabine, cyclophosphamide, and rituximab; GI=gastrointestinal; IV=intravenous; nPR=nodular partial response; ORR=overall response rate; PFS=progression-free survival; SMIP=small molecule immunopharmaceutical.

due to significant myelotoxicity and idiosyncratic tumor flare reactions, which prevented 7 of the 9 patients from completing therapy.¹⁸ The sequential use of lenalidomide in combination with other myelosuppressive regimens may ultimately be more tolerable.

Monoclonal Antibodies

Lumiliximab

Lumiliximab (IDEC-152, Biogen Idec) is a monoclonal antibody constructed from macaque variable regions and human immunoglobulin G (IgG1) constant regions. It is directed against CD23, a surface glycoprotein commonly expressed on the surface of CLL cells but rarely found on other cells.¹⁹ Lumiliximab has been

shown to induce apoptosis in CD23-bearing CLL cells and to prolong survival in severe combined immune deficiency (SCID) mice inoculated with CD23-bearing lymphoblastic cell lines.²⁰ Lumiliximab demonstrated a favorable safety profile in a phase I study of 46 patients with relapsed/refractory CLL. Only 2 cases of dose-limiting toxicity were observed and neither prevented dose escalation. Grade 3/4 toxicity occurred in only 7 (15%) patients.²¹ Although no patient achieved a CR or PR, 91% of evaluable patients showed a median 32% reduction in lymphocytosis, and 52% demonstrated a decrease in lymph node bulk at day 29.

In vitro, lumiliximab also enhances the effects of fludarabine and rituximab, providing a rationale for their combined use in CLL. A recent phase I/II multicenter

study treated 31 patients with relapsed CLL with lumiliximab in combination with FCR for up to six 28-day cycles.²² All patients completed therapy, and lumiliximab did not appear to result in additional toxicities; 23 (74%) patients reported a grade 3 or 4 event, a rate similar to that previously reported with FCR alone. The ORR was 65%, with 52% CR and 13% PR. After a median follow-up of 16.8 months, the median PFS was 19.3 months for all patients, 23.4 months for patients who achieved a response, and 30.4 months for patients who achieved a CR. These encouraging results led to a randomized phase II registration trial comparing FCR with and without lumiliximab in patients with relapsed CLL. This trial was stopped early in the fall of 2009, and further updates are not yet available.

GA-101

GA-101 (obinutuzumab, Roche/GlycArt/Genentech) is a third-generation, fully humanized anti-CD20 IgG1. The Fc-region of GA-101 is glycoengineered to result in higher affinity binding to the CD20 type II epitope. As a result of this characteristic, the ability of GA-101 to induce antibody-dependent cellular cytotoxicity is enhanced 5–50-fold compared to other standard anti-CD20 monoclonal antibodies, such as rituximab.²³ Furthermore, GA-101 demonstrated superior dose-dependent antitumor activity in aggressive non-Hodgkin lymphoma (NHL) xenograft models in SCID mice compared to rituximab.²⁴ The safety and tolerability of GA-101 was recently reported in a phase I trial of 13 patients with relapsed/refractory CLL in which 62% of patients had prior exposure to rituximab.²⁵ GA-101 was administered at a dose ranging from 400–2,000 mg given intravenously on days 1, 8, and 22, and subsequently every 3 weeks for a total of 9 infusions. The most common adverse events were grade 1/2 infusion-related reactions occurring for the most part at the time of the first infusion. Grade 3/4 transient neutropenia occurred in 69% of patients. The ORR was 62%, with 1 CR with incomplete blood count, 7 PR, and 5 SD. Response durations have so far ranged from 3.5–8 months. Based on these results, studies of GA-101 in CLL and indolent lymphomas are ongoing; for example, it is being studied as combination therapy with bendamustine.

Small Molecule Immunopharmaceuticals

Small molecule immunopharmaceuticals (SMIPs) are peptides designed to contain the variable region from a specific antibody and a constant region encoding IgG-1 domains. TRU-016 (Trubion Pharmaceuticals) is engineered to contain a variable region from anti-CD37 antibodies. CD37 is a glycoprotein strongly expressed on the surface of normal B cells as well as B-CLL and B-NHL

cells. It mediates signal transduction for cell growth and development. The expression of CD37 is particularly strong on CLL cells compared to CD20.²⁶ TRU-016 has shown significant antitumor activity by NK-mediated antibody-dependent cellular cytotoxicity against human B cell neoplasms in xenograft SCID mouse models. Furthermore, TRU-016 also appears to be effective in tumors harboring resistance to anti-CD20 monoclonal antibodies.²⁷ TRU-016 was administered to 10 patients with relapsed/refractory CLL with high-risk genomic features as part of an ongoing phase I study.²⁸ TRU-016 has so far been well tolerated, with minimal infusional toxicity. Six patients had reductions in peripheral lymphocyte counts, ranging from 27–94%, while taking low doses of TRU-016. Reduction in lymphadenopathy, clearing of leukemia cutis, and improvements in hematopoietic functions were also observed. TRU-016 has shown activity as a single agent, and synergy has been observed when TRU-016 has been combined with rituximab in xenograft models.²⁹ Combination therapy may be considered in the future.

BCL-2 Inhibitors

The majority of CLLs overexpress the antiapoptotic BCL-2 family members BCL-2, BCL-XL, and MCL-1.³⁰ These proteins sequester the proapoptotic proteins BAX and BAK, thereby preventing apoptosis. Modulation of the BCL-2 pathway has therefore been intensively studied as a possible therapeutic modality in CLL. A good candidate inhibitor would ideally affect both BCL-2 and MCL-1 because both are often highly expressed, particularly in refractory CLL.

Oblimersen

Oblimersen (Genasense, Genta) is an antisense, 18-base, single-stranded DNA oligonucleotide that can bind to the first 6 codons of BCL-2, thereby inhibiting its transcription and impairing tumor cell viability.³¹ Updated results of a phase III randomized trial of fludarabine and cyclophosphamide with or without oblimersen in 241 patients with relapsed/refractory CLL were recently reported.^{32,33} In this study, oblimersen was administered at 3 mg/kg/day as a 7-day continuous infusion. A superior CR/nodular PR rate was achieved in the oblimersen group (17% vs 7%; $P=.025$), and the median time to progression in patients achieving a CR/nodular PR was significantly longer in the oblimersen arm. After 5 years, these benefits did not translate into a survival advantage for the oblimersen arm, although responders in that arm did have a longer median survival (56 months vs 38 months; $P=.04$). In March 2009, the FDA announced that it would not support the approval of oblimersen

for the treatment of patients with relapsed or refractory CLL until evidence from confirmatory trials became available.

Obatoclox

Obatoclox (GX15-070, Gemin X Pharmaceuticals) is a small molecule mimicking the BH3 peptidic domain that acts as a pan-inhibitor of BCL-2 family proteins, resulting in apoptosis. In a phase I study, obatoclox was administered to 26 patients with relapsed CLL.³⁴ The maximum tolerated dose was established at 28 mg/m² intravenously over 3 hours every 3 weeks for up to 8 cycles due to dose-limiting toxicities that consisted of somnolence, euphoria, ataxia, and infusion reactions. One patient (4%) achieved a PR. However, reductions in circulating lymphocyte counts were observed in 18 of 26 patients, with a median reduction of 24%. Furthermore, improvements in hematopoietic function resulted in increased hemoglobin in 3 of 11 anemic patients and increased platelet counts in 4 of 14 thrombocytopenic patients. Despite its modest single-agent clinical activity, obatoclox may provide benefits in combination therapy regimens with minimal added toxicity. A single-center phase I study of obatoclox in combination with FR and FCR is currently ongoing at the Dana-Farber Cancer Institute, in Boston, MA.

ABT-263

ABT-263 (navitoclax, Abbott) is a second-generation, orally available small molecule that binds preferentially to antiapoptotic BCL-2 family proteins BCL-XL, BCL-2, and BCL-W but not MCL-1 or A1. Preliminary results of a phase I/II study of ABT-263 in 29 patients with relapsed/refractory CLL were recently reported.³⁵ The most common adverse events were gastrointestinal reactions followed by fatigue, thrombocytopenia (20%), and neutropenia (12%). The dose-limiting toxicity was thrombocytopenia occurring 3–5 days following drug administration and resulting from decreased survival of circulating platelets due to inhibition of BCL-XL. This initial drop in platelet count is usually followed by partial recovery due to a compensatory rise in megakaryopoiesis. In order to minimize this problem, a 100 mg 7-day lead-in dose followed by 250 mg/day of continuous dosing was the recommended phase II dose for ABT-263; this regimen resulted in an average circulating platelet count drop of 63% from baseline. Of 21 evaluable patients, 7 had PR, 8 had SD, and 2 had progressive disease. The median PFS was not reached after a median follow-up of 9 months. Phase I studies combining ABT-263 with FCR and with bendamustine plus rituximab are currently under way, in addition to a randomized phase II trial of ABT-263 alone or in combination with rituximab.

AT-101

AT-101 (Ascenta Therapeutics) is an orally bioavailable small molecule BH3 mimetic that inhibits the antiapoptotic functions of BCL-2, BCL-XL, and MCL-1. AT-101 induces apoptosis in CLL cells³⁶ and demonstrates synergistic effects with cyclophosphamide and rituximab in SCID mouse xenograft models of B-cell NHL.³⁷ In a phase II study, 12 patients with relapsed/refractory CLL received AT-101 in combination with rituximab.³⁸ AT-101 was administered at a dose of 30 mg orally daily during 21 or 28 days for three 28-day cycles. Rituximab was administered at 375 mg/m² on days 1, 3, and 5 in the first week of each cycle and weekly thereafter for 2 months. The main reported toxicities consisted of nausea, vomiting, and fatigue. In 8 patients evaluable for efficacy endpoints, the ORR was 38% with 2 unconfirmed CR and 1 PR. Three patients had SD, and 2 progressed. Significant reductions in peripheral lymphocyte counts and splenomegaly occurred in 50% and 63% of patients, respectively. AT-101 is currently being studied in combination with lenalidomide for relapsed/refractory CLL at Roswell Park Cancer Institute in Buffalo, New York.

Protein Kinase Inhibitors

Flavopiridol

Flavopiridol (Alvocidib, Sanofi-Aventis) is a pan-inhibitor of cyclin-dependent kinases, including CDK9, and can induce apoptosis in primary human CLL cells.³⁹ This process occurs through p53-independent pathways by downregulation of antiapoptotic proteins such as MCL-1 and X-linked inactivator of apoptosis.⁴⁰ Although flavopiridol has shown remarkable preclinical activity, initial clinical studies of this agent in patients with relapsed CLL failed to show clinical benefits^{41,42} due to higher than anticipated binding to human serum, resulting in insufficient drug concentration to achieve clinical activity. A pharmacokinetically derived dosing schedule consisting of flavopiridol administered by 30-minute intravenous bolus followed by 4-hour continuous intravenous infusion subsequently resulted in encouraging efficacy in a phase I trial. However, hyperacute TLS was identified as a dose-limiting toxicity, particularly in patients with pretreatment leukocyte counts exceeding $200 \times 10^9/L$.^{43,44} In a subsequent phase II study, flavopiridol was initially administered at a dose of 30 mg/m² by 30-minute intravenous bolus followed by 30 mg/m² by 4-hour continuous infusion.⁴⁵ Patients who did not develop severe TLS subsequently underwent a dose escalation to 30 mg/m² intravenous bolus followed by 50 mg/m² continuous infusion for all subsequent treatments. A cycle consisted of 3 weekly infusions of flavopiridol every 4 weeks for up

to 6 cycles. Supportive care included the administration of prophylactic dexamethasone and pegfilgrastim, as well as antimicrobial prophylaxis with ciprofloxacin, valganciclovir, and trimethoprim/sulfamethoxazole. TLS prevention therapy—with intravenous hydration, urine alkalization, allopurinol, and prophylactic rasburicase, phosphate binder, and hourly monitoring of serum potassium—was administered with each dose of flavopiridol. All patients were treated in a facility with rapid access to bedside hemodialysis. Among 64 patients enrolled, 34 (53%) achieved a response, including 1 CR (1.6%), 3 nodular PR (5%), and 30 PR (47%). A particularly high ORR was observed in high-risk patients with del(17p13.1) (12/21) and del(11q22.3) (14/28). Furthermore, flavopiridol was shown to be efficacious in patients with bulky lymphadenopathy. The median PFS among responders was 10–12 months and did not vary significantly across cytogenetic risk groups. Despite the prophylactic measures, 42% of patients developed at least biochemical TLS, and 5% required dialysis. Other toxicities were generally transient and related to cytokine release syndrome; they included electrolyte and liver function abnormalities, fatigue, diarrhea, and cytopenias leading to nonopportunistic infections. A large, international phase II registration trial of single-agent flavopiridol in fludarabine-refractory CLL is expected to complete accrual in 2010.

SNS-032 (Sunesis Pharmaceuticals) is another small-molecule inhibitor of CDK2, CDK7, and CDK9 currently being studied in a multicenter phase I trial in relapsed CLL and multiple myeloma. Among 17 patients with CLL, TLS has been observed as a dose-limiting toxicity at doses up to 75 mg/m², and no objective responses have been reported.⁴⁶

SCH 727965 (dinaciclib, Merck) is a selective small-molecule inhibitor of CDK1, CDK2, CDK5, and CDK9. SCH 727965 exhibits a superior therapeutic index compared to other CDK inhibitors. Preclinical studies demonstrate potent induction of apoptosis in CLL cells irrespective of prior drug exposure, IgVH gene mutational status, or the presence of high-risk cytogenetic abnormalities. In an ongoing phase I study, SCH 727965 has shown promising activity, including objective responses in patients with relapsed/refractory CLL.⁴⁷

Spleen Tyrosine Kinase Inhibitors

Signaling through the B-cell receptor (BCR) expressed on many NHL cells appears to be important in tumor pathogenesis.⁴⁸ BCR signals are transduced by the nonreceptor spleen tyrosine kinase (Syk).⁴⁹ Activation of Syk leads to the activation of phosphatidylinositol 3-kinases (PI3K) and AKT, and the phosphorylation of multiple signaling proteins including RAS, PLC-gamma, and MAP kinases, resulting in cell survival.⁵⁰ Using a transgenic mouse model, researchers have been able to demonstrate that

Syk expression is required for the proliferation of certain lymphomas and that pharmacologic inhibition of its kinase activity results in tumor regression *in vivo*.⁵¹ Syk is expressed mainly in hematopoietic cells, and its expression is upregulated in CLL,⁵² making it a potential target for CLL treatment.

Fostamatinib disodium (AstraZeneca/Rigel Pharmaceuticals) is the first clinically available oral Syk inhibitor. In a phase II study of 68 patients with relapsed NHL, fostamatinib was administered at a dose of 200 mg orally twice daily.⁵³ The most frequent adverse events were fatigue, hypertension, nausea, and reversible cytopenias. Among 11 patients with CLL, 6 had an objective response—although response evaluation was based on lymphoma criteria in this study and therefore did not take into account changes in the white count. The median PFS for CLL patients was 6.4 months. Interestingly, all patients with CLL experienced an increase in circulating lymphocyte count during the first 29 days of therapy, even while their lymph nodes were shrinking. This finding suggests that the inhibition of Syk may cause disruption of the nodal microenvironment, resulting in increased trafficking of CLL cells out of nodal tissues. Additional development of fostamatinib for use in CLL or other B-cell malignancies has been on hold, and its future in these diseases is unclear.

Phosphatidylinositol 3-Kinase Inhibitors

Class I PI3Ks are a family of intracellular signaling proteins that are essential components of migratory, proliferative, survival, and differentiation pathways in many cell types, including those of hematopoietic origin.⁵⁴ Upon PI3K activation, the p110 catalytic subunit generates the lipid second messenger phosphatidylinositol (3,4,5)-trisphosphate, which acts as a binding site for recruitment and activation of numerous intracellular signaling enzymes. Sustained activation of the PI3K pathway can occur following BCR stimulation and has been shown to have a pivotal role in the survival of CLL.⁵⁵ CAL-101 (Calistoga Pharmaceuticals) is an oral small-molecule inhibitor of the p110 δ isoform of PI3K, which has an expression pattern largely restricted to cells of hematopoietic origin. In a phase I study, CAL-101 was administered orally twice daily at increasing doses to 43 patients with select hematologic malignancies.⁵⁶ Asymptomatic elevations in hepatic enzymes and routine infections were reported. Among the 17 patients with CLL enrolled in this trial, 4 achieved a PR. Interestingly, more than 90% of CLL patients had a greater than 50% reduction in lymphadenopathy, yet in many cases, this reduction was associated with a concurrent increase in peripheral blood lymphocytosis to more than 50% of the baseline value. Despite this increased lymphocyte count, patients derived significant

clinical benefits as well as improvements in anemia and thrombocytopenia. This finding supports the notion that the observed progression of lymphocytosis is more likely the result of redistribution of CLL cells from lymph nodes or bone marrow to peripheral blood. This apparent displacement of CLL cells from the protective micro-environment may render the combination of CAL-101 with other active agents that can clear peripheral blood particularly active.

mTOR Kinase Inhibitors

Although CLL has traditionally been regarded as the result of gradual accumulation of cells unable to undergo apoptosis, a strong proliferative component has recently been recognized.⁵⁷ Mammalian target of rapamycin (mTOR) is a kinase involved in cellular growth and proliferation that transduces signals from the PI3/AKT pathway, which is commonly activated in hematologic malignancies.⁵⁸ CLL cells exposed to the mTOR inhibitor rapamycin have reduced expression of cyclin D3, cyclin E, and cyclin A.⁵⁹ Inhibition of this pathway is currently the focus of numerous research efforts. Rapamycin, also known as sirolimus (Rapamune, Wyeth) acts as an immunosuppressive agent as well as a growth inhibitory agent. Moreover, proapoptotic effects have been observed in lymphocytes.⁶⁰ Everolimus (Afinitor, Novartis) is a more readily bioavailable derivative of sirolimus.⁶¹ Although an initial trial of everolimus in hematologic malignancies yielded encouraging results with minimal toxicity,⁶² results of a phase II study in 7 patients with advanced CLL who had received at least 2 prior lines of therapy were not as encouraging.⁶¹ In this study, everolimus was administered at a dose of 5 mg orally daily. One patient achieved a PR, 3 patients had SD, and 3 developed progressive disease. More importantly, however, an alarming rate of infectious complications was observed, including 1 case of pneumocystis jiroveci pneumonia, 1 case of herpes zoster ophthalmicus complicated by meningitis, and 1 fatal case of Epstein-Barr virus-associated high-grade lymphoma, which eventually led to closure of the trial. Whether these infectious complications were directly related to everolimus or to prior therapies with purine analogs remains unclear. Although everolimus was shown to be safe in patients with solid organ transplants on immunosuppressive regimens comprising cyclosporine A and corticosteroids,⁶³ the degree of immunosuppression experienced by advanced, heavily pretreated CLL patients may mandate the use of antimicrobial prophylaxis. However, a similar degree of toxicity was not observed in a recently reported phase II trial of everolimus in indolent hematologic malignancies.⁶⁴ Among 22 patients with relapsed/refractory CLL treated with 10 mg/day of everolimus, 14 patients experienced grade 3–5 hematologic toxicity, and 2 patients died of infections. Among

22 patients with CLL, 4 achieved a PR. Interestingly, in 8 patients (36%), everolimus resulted in a significant increase in the peripheral lymphocyte count by a median of 4.8-fold, which was associated with a decrease in lymphadenopathy by a median of 76%. Like fostamatinib and CAL-101, everolimus may have the ability to mobilize CLL cells from their protective niche in lymph nodes and bone marrow to the peripheral blood, thereby rendering them more susceptible to chemotherapy. This hypothesis is currently being tested in a recently opened phase I/II trial of everolimus and alemtuzumab for the treatment of relapsed/refractory CLL at the Mayo Clinic.

Dasatinib

Dasatinib (Sprycel, Bristol-Myers Squibb) is a tyrosine kinase inhibitor approved by the FDA for the management of all phases of CML and adults with Philadelphia chromosome-positive acute lymphoblastic leukemia with resistance or intolerance to prior therapy. In addition to inhibition of BCR-ABL, dasatinib has inhibitory activity on SRC family kinases, including LYN, which is often unregulated and constitutively activated in CLL cells.⁶⁵ Dasatinib can induce apoptosis in CLL cells in vitro, with preferential effects on IgVH unmutated CLL.⁶⁶ Dasatinib can also sensitize CLL cells to other chemotherapy agents by inhibiting the antiapoptotic program induced by CD40 stimulation.⁶⁷ In a phase II study in relapsed/refractory CLL, dasatinib resulted in a 20% response rate but was difficult to administer, with significant myelosuppression.⁶⁸ Trials are examining the role of dasatinib in combination with numerous agents, including rituximab, fludarabine, and lenalidomide.

Other Agents

Heat shock protein 90 (Hsp90) is a chaperone protein involved in the proper folding, assembly, transport, and function of important mediators of cell signaling and cell cycle control, such as tyrosine kinase ζ -associated protein of 70 kD (ZAP-70). The Hsp90 inhibitor BIIB021 (Biogen Idec) is currently being evaluated in a phase I trial in relapsed/refractory CLL.

The histone deacetylase inhibitor valproic acid was recently shown to induce apoptosis by modulating antiapoptotic and proapoptotic genes. Furthermore, valproic acid also appears to increase the chemosensitivity of CLL cells to fludarabine, flavopiridol, bortezomib (Velcade, Millennium Pharmaceuticals), thalidomide, and lenalidomide in vitro.⁶⁹ Clinical trials of valproic acid in patients with relapsed CLL are ongoing in India, Canada, and Puerto Rico.

Lastly, the SDF-1/CXCR4 axis has been shown to play an important role in CLL cell trafficking and survival. CXCR4 antagonists may impair migration of CLL

cells to the microenvironment and result in increased susceptibility to chemotherapeutic agents.⁷⁰ A clinical trial of plerixafor (Mozobil, Genzyme), a CXCR4 antagonist, in combination with rituximab in patients with relapsed CLL has begun.

Conclusion

Although CLL remains an incurable disease, purine analog-based chemotherapy in combination with monoclonal antibodies has resulted in prolonged remissions. Novel therapeutics are the result of our improved understanding of the biology of CLL. Emerging therapies ranging from new monoclonal antibodies to small molecules that interfere with vital pathways in signal transduction and cell cycle regulation are currently being developed. Drug synergy studies may provide rationale for the use of novel agents in combination with other emerging drugs and/or current chemotherapeutics. Furthermore, patients with high-risk CLL, such as those with del(17p13.1), who have so far benefited little from combination therapies may derive the most gain from novel agents like flavopiridol. Although a cure for CLL still remains elusive, emerging targeted therapies are showing great promise for the future.

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