The Role of Fludarabine in the Treatment of Follicular and Mantle Cell Lymphoma

Georg Lenz, M.D.
Wolfgang Hiddemann, M.D., Ph.D.
Martin Dreyling, M.D., Ph.D.

Department of Internal Medicine III, Grosshadern Hospital, Ludwig-Maximilians University, Munich, Germany.

Advanced-stage follicular lymphoma (FL) and mantle cell lymphoma (MCL) cannot be cured using conventional chemotherapy. Fludarabine, the most widely used purine analog, exhibits a particularly high level of activity against small lymphocytic lymphoma and chronic lymphocytic leukemia (CLL). Numerous studies have investigated the efficacy of fludarabine as a single agent or in combination with other cytostatic compounds in the treatment of FL and MCL. Hematologic toxicity is the most commonly observed adverse event in patients treated with fludarabine, but serious infectious complications are relatively rare. Fludarabine monotherapy has proven to be particularly effective in the treatment of FL; however, complete responses (CRs) are observed in only approximately 20–40% of all cases. In contrast, combinations containing fludarabine and anthracyclines or alkylating agents have yielded superior response rates and longer periods of progression-free survival (PFS), and the addition of the anti-CD20 antibody rituximab appears to yield even better results. In a randomized trial, an immunochemotherapy regimen consisting of a fludarabine-containing combination and rituximab resulted in superior remission and survival rates compared with the fludarabine-containing combination alone. In summary, fludarabine has proven to be a safe and effective agent in the treatment of indolent lymphoma. In particular, combinations containing fludarabine, anthracyclines and/or alkylating agents, and rituximab have yielded remarkable CR and PFS rates. Consequently, current research efforts have focused on the use of fludarabine-containing combinations in the first-line treatment of FL and MCL. *Cancer* 2004;101:883–93.
© 2004 American Cancer Society.

KEYWORDS: fludarabine, follicular lymphoma, mantle cell lymphoma, chemotherapy, immunochemotherapy, rituximab.

Introduction

Follicular lymphoma (FL) is the second most common non-Hodgkin lymphoma (NHL), accounting for 20–30% of all cases of NHL.[1] FL typically follows an indolent clinical course and is associated with a median survival of 7–10 years.[2] Only in certain cases of Ann Arbor Stage I or II, FL can be cured using radiotherapy; however, approximately 80% of all patients have Stage III or IV FL at presentation. For these patients, conventional chemotherapy is not curative, nor does it substantially prolong overall survival (OS).[2]

Unlike FL, mantle cell lymphoma (MCL) accounts for only 5–10% of all cases of lymphoma.[3,4] MCL, which is characterized by an aggressive clinical course and a median survival duration of only 3 years, is the lymphoma subtype associated with the poorest long-term outcome.[5] Consequently, treatment should be administered immediately after diagnosis, although conventional chemotherapy is not curative.[6]

Address for reprints: Georg Lenz, M.D., Department of Internal Medicine III, University Hospital Grosshadern, Ludwig-Maximilians University, Marchioninistrasse 15, 81377 Munich, Germany; Fax: (011) 49 89 7095 5550; E-mail: georg.lenz@med3.med.uni-muenchen.de

Recently, the progression-free survival (PFS) of patients with MCL was found to be significantly improved by high-dose therapy followed by autologous stem cell transplantation.[7] Similarly, very encouraging results have been obtained using aggressive regimens (e.g., hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone) as induction chemotherapy or for patients for whom autologous stem cell transplantation is not possible.[8,9] Nonetheless, even after receiving such dose-intensified regimens, the majority of patients will eventually experience recurrence. Thus, novel therapeutic approaches and chemotherapeutic agents developed with the goal of improving clinical outcome for patients with FL and MCL are urgently needed.

Fludarabine, an antimetabolite that inhibits DNA synthesis, currently is the most widely used purine analog. This agent has exhibited high levels of efficacy in the treatment of chronic lymphocytic leukemia (CLL) and Waldenstrom macroglobulinemia, and in combination with cytosine arabinoside, it has also been effective in the treatment of acute myeloid leukemia (AML).[10–12] Various studies have also investigated the use of fludarabine to treat FL and MCL. The purpose of the current review was to summarize the existing body of knowledge regarding the clinical activity of and the toxicities associated with fludarabine in the treatment of these two malignancies. Special attention has been given to recently established immunochemotherapy regimens in which fludarabine is used in combination with the monoclonal anti-CD20 antibody rituximab.

Mechanism of Action

Fludarabine, a prodrug, is converted to the free nucleoside 9-beta-D-arabinosyl-2-fluoroadenine, which enters the cells and accumulates as the 5′-triphosphorylated compound 9-beta-D-arabinosyl-2-fluoroadenosine triphosphate (F-ara-ATP). F-ara-ATP inhibits ribonucleotide reductase, as well as DNA ligase and DNA primase. In addition, F-ara-ATP is incorporated into DNA, with this incorporation resulting in the repression of further DNA polymerization. In cell lines, the incorporation of F-ara-ATP into RNA and the subsequent inhibition of transcription has also been demonstrated.[13]

Fludarabine has also been used in combination with other chemotherapeutic agents. In patients with indolent lymphoma, the combination of fludarabine and cyclophosphamide, an alkylating agent that induces DNA damage, resulted in increased treatment efficacy,[14] which may have been attributable to the inhibition of interstrand DNA crosslink removal by fludarabine.[15] Similarly, synergy between fludarabine and the anti-CD20 antibody rituximab has been demonstrated in vitro. Rituximab acts primarily by stimulating antibody-dependent as well as complement-dependent cytotoxicity.[16,17] Fludarabine is capable of down-regulating the complement inhibitor CD55, which is partially responsible for the decreased activity of rituximab in therapy-resistant NHL. Thus, fludarabine and rituximab exert synergistic effects, leading to increased response rates.[18]

Toxicity

The toxicity of single-agent fludarabine is considered to be moderate. In a number of nonrandomized Phase II studies, the most commonly observed adverse effect was myelosuppression leading to neutropenia, thrombocytopenia, and anemia. In a recent randomized trial, Hagenbeek et al.[19] compared fludarabine with cyclophosphamide, vincristine, and prednisone (CVP) in patients with newly diagnosed indolent lymphoma and found that granulocytopenia and thrombocytopenia were significantly more common in the fludarabine arm (28% vs. 12% and 8% vs. 1%, respectively). This observation has been confirmed in various other studies; in those studies, 0–4% of all patients receiving fludarabine experienced severe anemia, 0–8% experienced thrombocytopenia (incidence rate in previously treated patients, 11–13%), and 11–41% experienced neutropenia (incidence rate in previously treated patients, 11–21%).[20–23] Nonetheless, the duration of myelosuppression is short, and blood cell support is only rarely required.[24] Immunomodulation due to an altered CD4-to-CD8 ratio and changes in the T cell repertoire has also been observed following fludarabine therapy.[25,26] Thus, infectious events occur relatively frequently in patients receiving fludarabine. Klasa et al.,[27] who compared fludarabine with CVP in patients with recurrent low-grade lymphoma, reported that 36% of patients in the fludarabine arm developed infectious complications, with 11% experiencing World Health Organization Grade 3 or 4 infection.

Nonhematologic toxicities are uncommon and are generally mild in patients receiving fludarabine. Nausea and emesis are observed in approximately 20–30% of such patients. Neurologic side effects, alopecia, and cardiac, pulmonary, and renal toxicities are also relatively rare.[22,24,27]

Chemotherapy combinations containing fludarabine and alkylating agents or anthracyclines are generally well tolerated. Various Phase II studies have demonstrated the feasibility of such combinations, with myelosuppression being identified as the most serious toxic event in those studies (Table 1).[28–31] In a study conducted by Velasquez et al.,[32] patients with

TABLE 1
Grade 3/4 Hematologic Toxicity in Patients Receiving Fludarabine-Containing Regimens

Study	Regimen	No. of patients	Disease status	Anemia (%)	Thrombocytopenia (%)	Neutropenia (%)
Zinzani et al., 1997[64]	Fludarabine 25 mg/m² per day × 3; mitoxantrone 10 mg/m² per day × 1; prednisone 40 mg per day × 5	30	Recurrent	n.a.	0	17
Flinn et al., 2000[45]	Fludarabine 20 mg/m² per day × 5; cyclophosphamide 600 mg/m² per day × 1	43	Untreated	9	2	40
Cohen et al., 2001[28]	Fludarabine 20–25 mg/m² per day × 3; cyclophosphamide 600 mg/m² per day × 1	30	Untreated or recurrent	36	37	50
Tsimberidou et al., 2002[37]	Fludarabine 25 mg/m² per day × 3; mitoxantrone 10 mg/m² per day × 1; dexamethasone 20 mg per day × 5	73	Untreated	n.a.	12	81
Velasquez et al., 2003[32]	Fludarabine 25 mg/m² per day × 3; mitoxantrone 10 mg/m² per day × 1	78	Untreated	4	8	35
Montoto et al., 2002[63]	Fludarabine 25 mg/m² per day × 3; cyclophosphamide 200 mg/m² per day × 3; mitoxantrone 6 mg/m² per day × 1	53	Untreated	n.a.	0.5	7
Spriano et al., 2002[39]	Fludarabine 25 mg/m² per day × 3; cyclophosphamide 300 mg/m² per day × 3; mitoxantrone 10 mg/m² per day × 1	54	Untreated	25[a]	27[a]	42[b]
Dreyling et al., 2003[52]	Fludarabine 25 mg/m² per day × 3; cyclophosphamide 200 mg/m² per day × 3; mitoxantrone 8 mg/m² per day × 1	57	Recurrent	5	11	41

n.a.: not available.
[a] Grade 1–3.
[b] Leukopenia.

previously untreated advanced-stage, low-grade NHL received a combination of fludarabine and mitoxantrone. Fifteen percent of those patients developed Grade III neutropenia, and 19% developed Grade IV neutropenia. In contrast, fever was observed in only 18 of 78 patients (23%) in that study.

Immunosuppression due to prolonged T lymphocytopenia is another major side effect associated with fludarabine-containing regimens. Accordingly, the reactivation of latent Epstein–Barr virus (EBV) infections as well as EBV-positive lymphoproliferative disorders has been observed.[33,34] The addition of corticosteroids to fludarabine-containing regimens significantly increases the risk of opportunistic infection and therefore should be avoided.[35] McLaughlin et al.[36] observed that a significant number of patients developed opportunistic infections, including *Pneumocystis carinii* pneumonia, herpes zoster virus infection, and various mycobacterial infections, after receiving an FND regimen (fludarabine 25 mg/m² daily for 3 days, mitoxantrone 10 mg/m² per day for 1 day, and dexamethasone 20 mg per day for 5 days). In another trial conducted at the M. D. Anderson Cancer Center (Houston, TX), 2 of 73 patients developed *P. carinii* pneumonia after receiving FND.[37] Thus, although randomized trials confirming the superiority of prophylactic antibiotics have not been performed, all patients receiving fludarabine and concomitant steroid therapy should also receive trimethoprim sulfamethoxazole as a prophylactic measure against *P. carinii* pneumonia.[38]

The incidence of toxic side effects depends heavily on chemotherapy dose levels, with slight increases resulting in significant increases in the incidence of Grade 3/4 hematologic toxicity.[39] This finding was confirmed by Hochster et al.,[40] who conducted a Phase I trial involving previously untreated patients with low-grade lymphoma. In that study, the administered cyclophosphamide dose was increased from 600 mg/m² to 1000 mg/m² (Day 1), and fludarabine was administered at a dose of 20 mg/m² (Days 1–5). Treatment cycles initially were repeated every 21 days, but due to the observation of Grade 4 hematologic toxicity in 50% of all patients, these cycles eventually were extended to 28 days. In addition, prophylaxis for *P. carinii* pneumonia and herpes zoster infection were required. Nineteen percent of all patients developed Grade 3 or 4 interstitial pneumonia, and 11% of patients developed other infectious toxicities (Grade 4 fungal sepsis, lobar pneumonia, and venous port infection in 1 case each). These data confirm that increasing the doses of chemotherapeutic agents in fludarabine-containing regimens significantly increases the risk of infectious complications. Thus, when rela-

tively dose-intense regimens are used, prophylactic growth factor support and *P. carinii* prophylaxis are strongly recommended.

Reviews of various trials have suggested that herpes simplex and herpes zoster infections occur rather frequently during and after fludarabine-based chemotherapy in patients with CLL.[41,42] In contrast, the incidence of these infections has not been investigated in large series of patients with FL or MCL. Thus, at present, it is unclear as to whether a subgroup of patients with FL or MCL and high-risk characteristics (e.g., depressed CD4 counts) might benefit from prophylaxis involving acyclovir or valganciclovir; this issue warrants attention in future studies.

One major side effect of fludarabine in patients with CLL is autoimmune hemolysis[43,44]; however, this phenomenon has not been described in larger studies involving patients with FL or MCL. This finding suggests that autoimmune hemolysis is not related exclusively to fludarabine use but might also depend on the subtype of lymphoproliferative disease being treated.

Another controversial issue involves stem cell mobilization following the administration of fludarabine-containing chemotherapy. Flinn et al.[45] did not observe reduced stem cell mobilization after the administration of several cycles of a combined regimen containing fludarabine and cyclophosphamide, whereas other studies have reported that stem cell harvesting is significantly impaired following fludarabine-containing chemotherapy.[46–48] Thus, the issue of stem cell mobilization in this setting has not been fully resolved.

A recent analysis performed by Morrison et al.[49] revealed another potential adverse effect associated with fludarabine. In patients with CLL, those investigators observed an increased incidence of secondary myelodysplastic syndromes (t-MDS) following treatment with fludarabine (either alone or in combination with chlorambucil). In contrast, Cheson et al.[50] did not detect a significant increase in the incidence of secondary malignancies. The incidence of secondary malignancies has not been described in larger series of patients with FL or MCL; to date, only individual case reports of t-MDS following fludarabine use have been published.[51] Thus, it currently is unclear as to whether patients with malignant lymphoma have an increased risk of developing secondary neoplasia following treatment with fludarabine.

The use of fludarabine-containing combinations in conjunction with rituximab has proven to be feasible in various studies.[52–56] Hematologic toxicity is the primary side effect associated with such treatment, whereas nonhematologic toxicity is rare. In a study conducted by Cohen et al.,[56] patients were treated with the FCR chemotherapy regimen (4–6 cycles of fludarabine 25 mg/m^2 per day for 3 days, cyclophosphamide 250 mg/m^2 per day for 3 days, and rituximab 375 mg/m^2 weekly); hematologic toxicity was noted in 30% of all patients, with 6% of patients developing Grade 3 or 4 neutropenia. It is noteworthy that despite the expectation of immunosuppression due to the elimination of the B cell compartment and changes in the T cell repertoire, only 1 of 33 patients (3%) in that study developed neutropenic fever. The addition of rituximab to fludarabine-containing regimens leads to myelosuppression in a larger number of cases. Byrd et al. found that patients with CLL who were treated with fludarabine and rituximab experienced significantly more hematologic toxicity (especially Grade 3/4 neutropenia) compared with patients who did not receive rituximab.[57] This finding was confirmed in a recent trial conducted by McLaughlin et al.,[58] in which patients receiving a combined immunochemotherapy regimen (rituximab, fludarabine, mitoxantrone, and dexamethasone) experienced neutropenia slightly more frequently compared with patients in the chemotherapy-only arm (27% vs. 16%). Similarly, in a randomized trial conducted by Dreyling et al.,[52] lymphocytopenia was significantly more common among patients receiving rituximab, fludarabine, cyclophosphamide, and mitoxantrone compared with patients in the chemotherapy-only arm.

In summary, the use of fludarabine as a single agent or in combination with other cytostatic drugs or the anti-CD20 antibody rituximab is feasible. Nonetheless, hematologic toxicities must be closely monitored, especially following the administration of combination regimens, so that therapy-associated infections can be prevented.

Efficacy of Fludarabine
Fludarabine as a single agent

Various Phase II studies have investigated the efficacy of fludarabine as a single agent in the treatment of previously untreated FL. Fludarabine exhibits a high level of efficacy in this setting, with overall response rates of approximately 60–70% and complete remission (CR) rates of approximately 30% (Table 2).[21,59] Encouraging data regarding PFS have also been obtained; Coiffier et al.[59] reported a 2-year PFS rate of 49% in patients receiving fludarabine monotherapy. In a randomized trial, Hagenbeek et al.[19] compared fludarabine with CVP in patients with low-grade NHL and found that the overall response rate associated with fludarabine use was significantly higher (68% vs. 51% [$P = 0.001$]; CR rate, 38% vs. 15%); however, the observed median time to progression did not differ significantly between the two treatment arms (21

TABLE 2
Efficacy of Fludarabine as a Single Agent[a] in Patients with Follicular Lymphoma

Study	No. of patients	Disease status	CR/OR (%)	Median PFS (mos)
Whelan et al., 1991[73]	23	Recurrent	22/48	n.a.
Redman et al., 1992[23]	28	Recurrent	n.a./68	n.a.
Solal-Celigny et al., 1996[21]	54	Untreated	37/65	13.6
Coiffier et al., 1999[59]	61	Untreated	33/59	49%[b]
Zinzani et al., 2000[24]	60	Untreated	60/87	n.a.
Hagenbeek et al., 2001[19]	n.a.	Untreated	38/68	21

CR: complete remission; OR: overall response; PFS: progression-free survival; n.a.: not available.
[a] Fludarabine 25 mg/m^2 per day for 5 days.
[b] Two-year progression-free survival rate.

TABLE 3
Efficacy of Fludarabine as a Single Agent[a] in Patients with Mantle Cell Lymphoma

Study	No. of patients	Disease status	CR/OR (%)
Decaudin et al., 1998[61]	15	Untreated or recurrent	0/33
Foran et al., 1999[22]	17	Untreated	29/41
Zinzani et al., 2000[24]	11	Untreated	27/73

CR: complete remission; OR: overall response.
[a] Fludarabine 25 mg/m^2 per day for 5 days.

months vs. 15 months [$P = 0.24$]), and the median OS had not yet been reached at the time of that report. In patients with recurrent or refractory disease, although fludarabine remains an active chemotherapeutic agent, response rates generally are lower. In this setting, overall response rates of 40–65% and noteworthy OS rates have been obtained in a number of Phase II studies.[20,32,37,60] In a recent Phase III trial, Klasa et al.[27] found that the response rate associated with fludarabine and the response rate associated with CVP were similar (64% vs. 52% [$P = 0.72$]); unlike Hagenbeek et al.,[19] however, those investigators reported that the median PFS was significantly longer in the fludarabine arm (11 months vs. 9.1 months [$P = 0.03$]), although there was no detectable difference between the two treatment arms in terms of median OS.

In summary, fludarabine is effective in the treatment of FL, and it is particularly active when used as first-line therapy. Furthermore, fludarabine may be especially useful for patients who are ineligible for more aggressive therapeutic approaches, such as high-dose chemotherapy followed by autologous stem cell transplantation.

Fludarabine monotherapy possesses moderate efficacy in patients with MCL. For the most part, the utility of fludarabine as first-line therapy in this setting has been investigated only in small Phase II studies.[22,24,61] Reported overall response rates have been relatively low (40–50%), with accompanying CR rates ranging from 20% to 30% (Table 3). Remission periods tended to be short, ranging from 4 to 8 months.[61] In patients with recurrent or refractory disease, responses to fludarabine monotherapy are even less favorable.[20] Thus, fludarabine monotherapy possesses limited activity against MCL and should be administered only to heavily pretreated patients when other therapeutic options are not available.

Fludarabine-containing regimens

Based on the finding of synergistic effects in vitro, fludarabine has been combined with other chemotherapeutic agents, particularly anthracyclines (e.g., mitoxantrone or idarubicin) and alkylating agents (e.g., cyclophosphamide). In various studies, fludarabine-containing combinations have yielded encouraging results in the first-line treatment of FL. In a recently published Phase II study, Velasquez et al.[32] evaluated the FM regimen (fludarabine 25 mg/m^2 per day for 3 days and mitoxantrone 10 mg/m^2 per day for 1 day) in 78 evaluable patients with low-grade lymphoma; an overall response rate of 94% and a CR rate of 44% were reported in that study (median follow-up, 5.5 years). Those investigators reported a 4-year PFS rate of 38% and a 4-year OS rate of 88%. These results confirmed the findings of previous studies in which similarly high response rates were reported (Table 4).[31,53] Likewise, in various small, nonrandomized trials, high overall response rates have been yielded by the combination of fludarabine and cyclophosphamide (FC). In a study conducted by Flinn et al.,[45] FC (fludarabine 20 mg/m^2 per day for 5 days and cyclophosphamide 600 mg/m^2 per day for 1 day) resulted in an overall response rate of 90%.

The addition of dexamethasone to either FC or FM did not significantly improve response rates or PFS rates.[29,37,60] In a number of studies, an FCM regimen (fludarabine 25 mg/m^2 per day for 3 days, cyclophosphamide 200–300 mg/m^2 per day for 3 days, and mitoxantrone 6–8 mg/m^2 per day for 1 day) yielded noteworthy results.[39,52,62,63] Montoto et al.[63] reported an overall response rate of 95% (CR rate, 75%); in addition, 69% of all patients in that study achieved molecular remission, and the 1.5-year failure-free survival rate was 90%. These results were confirmed by Spriano et al.,[39] who reported the occurrence of molecular remission in 74% of all patients. In contrast, in the only randomized trial to compare single-agent fludarabine with a fludarabine-containing combination, Zinzani et al.[24] found that fludarabine mono-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.

fastcase®
Smarter legal research.