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FOREWORD

Fludarabine Supplement

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In the last decade, we have witnessed significant advances in our understanding of the biology of chronic lymphocytic leukemia (CLL), and substantial progress has been made in the treatment of this lymphoproliferative disease. The utility of the Binet and Rai staging systems has been complemented by the development of immunological techniques that have helped to define the phenotype of CLL cells and establish CLL as a discrete disease entity. Recently, cytogenetic approaches have led to the identification of more sophisticated prognostic factors, such as *BCL-2* overexpression and p53 gene mutations, which enable particularly aggressive or indolent types of CLL to be predicted. It is expected that this stratification of patients according to risk will permit the 'individualization' of treatment, therefore optimizing the chances of a durable response.

This increased knowledge of the biological and clinical features of CLL has been mirrored by the development of therapeutic agents that are more active than previous approaches (eg chlorambucil- or cyclophosphamide-containing regimens). Of these innovative therapies, the purine analog fludarabine had made the most significant impact on how we manage CLL. The activity of fludarabine in CLL was first demonstrated in the late 1980 s in the treatment of relapsed or refractory patients. Compared to traditional strategies, fludarabine improved remission rates and lengthened response duration, and rapidly became established as the gold standard of care in this setting. Based on these benefits of fludarabine, its use as a front-line CLL therapy was suggested, a promise subsequently borne out in clinical investigation with 60–70% of patients achieving remission. The most recent improvement to fludarabine therapy is the development of an oral formulation with equivalent efficacy and tolerability to the intravenous preparation, coupled with the advantage of improved convenience of administration (for both patient and physician) and potentially superior cost effectiveness. As summarized in this supplement, fludarabine is now an established first-line, second-line and salvage therapy, and also a key component of new therapeutic developments.

With a 30–40% complete response rate when used for first-line therapy, fludarabine brings us considerably closer towards the goal of curing CLL. However, additional therapeutic progress is required to meet this aspiration. With fludarabine providing the foundation on which novel treatment approaches can be built, a number of promising strategies are emerging. In addition to combination regimens of fludarabine and traditional chemotherapeutics or antisense oligonucleotides, a particularly promising treatment option in CLL is the combination of fludarabine and monoclonal antibody (with or without cyclophosphamide). Although survival data are only preliminary, most CLL patients respond to fludarabine plus monoclonal antibody regimens and high rates of complete remission are reported; molecular complete responses and eradication of minimal residual disease are also frequently achieved. The questions that remain unanswered are: can we cure CLL and can we obtain this cure with the combination of fludarabine, cyclophosphamide and monoclonal antibody?

The benefits of fludarabine are not limited to the treatment of CLL. There is a substantial body of evidence supporting the use of fludarabine for a range of other lymphoproliferative disorders, including non-Hodgkin's lymphoma, mantle cell lymphoma, Waldenström's macroglobulinemia and acute myeloid leukemia. Moreover, there is extensive experience with fludarabine-containing conditioning regimens in stem cell transplantation therapies for CLL and other hematological malignancies.

In the following collection of informative articles, it is anticipated that the reader will gain a full appreciation of the biology of CLL and the clinical utility of fludarabine in present and future treatment strategies for this disease. The effectiveness of fludarabine across a wide diversity of cancers and in transplantation approaches is also highlighted in this supplement. With improved understanding of the biological and cytogenic features of CLL, and more effective drug treatments for this disease, we are now in an era where the intent of therapy is cautiously shifting away from palliation towards cure.

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Treatment options in chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is the most common adult hematological malignancy in the Western world and predominantly affects the elderly. The disease encompasses a wide spectrum of clinical symptoms, which translate into variable prognosis and survival. The stratification of patients based on their clinical risk profile has been aided by the recognition of novel prognostic markers, for example, V_H mutations and ZAP-70 expression, and this process is fundamental to assigning the most appropriate treatment strategy on an individual basis. Although CLL remains incurable with standard treatments, important progress in treatment has been made. The discovery of purine analogs such as fludarabine has led to significant improvements in remission rates and freedom from progression but, unfortunately, no significant prolongation in survival. With the success of newer therapeutic approaches, such as the monoclonal antibodies and stem cell transplantation, the focus of current therapy is on using these approaches in combination with fludarabine to produce high rates of molecular complete response, to eradicate minimal residual disease, and to lengthen survival. This paper provides an overview of CLL and discusses how recent therapeutic developments have changed the management of this form of leukemia.
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Overview of chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder characterized by a progressive accumulation of long-lived, immune-incompetent, neoplastic lymphocytes in the blood, bone marrow and lymphoid tissues.[1,2] The disease is the most common adult hematological malignancy in the Western world and, based on recent estimates, has an incidence of 3.5/100 000 in the United States.[3] CLL arises from a malignant clone of B cells with a characteristic phenotype. Although it has been previously suggested that some cases of CLL involve T cells, this notion is now disputed and the term T–cell CLL is no longer used in present classification systems.[4] The etiology of CLL is unknown; in contrast to other leukemias, CLL is not associated with exposure to radiation or other cytotoxic agents. However, genetic influences probably play an important role in CLL as there is evidence of familial aggregation and of a higher risk of having CLL in first-degree relatives of patients with the disease.[5]

CLL is a disease of the elderly with the median age of diagnosis approximately 70 years and an incidence in those >75 years of 27/100 000.[3] Further, the prevalence of CLL is increasing as the expected life-span of the population increases. Although 80–90% of patients are asymptomatic at initial diagnosis,[6] the presentation and clinical course of CLL is highly variable: one-third of patients present with aggressive disease requiring immediate treatment; another third present with an initially indolent phase followed by disease progression; and the remaining third never require therapy and die of causes unrelated to CLL.[7]

Compared with the general population, CLL patients experience an increased frequency of autoimmune disorders (between 10 and 20% are affected), of which hemolytic anemia is the most common.[8] Infection is a frequent complication in patients with advanced CLL and is presumably associated with neutropenia, hypogammaglobulinemia and generally impaired immunity.[2] Hypogammaglobulinemia often occurs late in the clinical course of the disease. The major morbidity and cause of death are associated with infection.[2] In 3 to 10% of cases, CLL may transform into Richter's syndrome, an aggressive, diffuse large cell lymphoma.[8] Transformation of CLL into a 'prolymphocytic' condition (often confused with prolymphocytic leukemia), and into other disorders such as acute lymphoblastic leukemia or multiple myeloma, has been documented but these reports are controversial.[2,8]

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Diagnosis, staging and prognosis of CLL

Diagnosis

Based on criteria established by the International Workshop on CLL (IWCLL)[9] and the National Cancer Institute-Sponsored Working Group Guidelines for CLL (NCI-WG),[10] CLL can be diagnosed whenever there is a sustained and absolute increase in morphologically mature lymphocytes ($>10\times10^9$/l); a bone marrow aspirate containing >30% lymphocytes; and a pattern of expression of cell surface markers consistent with CLL.

CLL is the leukemic counterpart of small lymphocytic lymphoma and should not be confounded with other lymphomas with leukemic expression such as mantle cell lymphoma, Waldenström's macroglobulinemia, splenic lymphoma with villous lymphocytes and follicular lymphoma (Table 1). Other lymphomas that are less frequently confused with CLL include B- and T-cell prolymphocytic leukemias, hairy cell leukemia, a hairy cell variant of CLL, large granular lymphocytic leukemia and small-cell Sézary syndrome.[8] B-CLL cells are characterized by sparse surface immunoglobulin (Ig), and expression of antigens CD5+, CD23+, CD79b$^-$ and FMC7$^-$; although the expression of these antigens within CLL can be heterogeneous, most cases of CLL express four or all of these five markers.

Staging systems

The Binet and Rai classification systems are the two clinically based staging systems widely employed to characterize CLL patient groups with different prognosis.[12,13] The Binet staging system is more commonly employed in Europe, whereas the Rai system has become most uniformly used in the United States. Both systems are useful for estimating prognosis and for stratifying patients into different risk groups (Table 2). For both classification systems, patients are assigned to one of three prognostic groups: 'low-risk' (good), 'intermediate-risk', and 'high-risk' (poor) prognoses.

More recent analyses by other groups have identified an especially benign subpopulation of patients with either Binet stage A or Rai stage 0 disease. In general, these 'smoldering' CLL patients have a lymphocyte count of $<30\times10^9$/l, normal platelet and hemoglobin levels, and a lymphocyte doubling time in excess of 1 year. The risk of progression to symptomatic CLL is low (around 20% at 5 years from diagnosis) and their survival is identical to that of a sex- and age-matched population.[14,15]

Other prognostic factors

The clinical staging systems are useful tools for predicting survival in CLL, however, they cannot predict the individual risk of disease progression in patients that are diagnosed during the early stages of CLL (Binet stage A or Rai stage 0). In addition to these classification systems, there are 3 parameters that may have independent prognostic value in CLL.[2,16] These include the white blood cell count, the degree of the infiltration in the bone marrow, as assessed by bone marrow aspirate or biopsy, and the peripheral lymphocyte doubling time. There are also certain serological parameters which correlate with prognosis, particularly serum thymidin-kinase, sCD23 and beta-2 microglobulin.[16]

A number of biological parameters are gaining increasing importance to evaluate the prognosis of patients with CLL. In a study using the highly sensitive technique of fluorescent *in situ* hybridization (FISH),[17] cytogenetic abnormalities were detected in 268 of 325 CLL patients, the most frequent of which were 13q deletion (55%), 11q deletion (18%), trisomy 12 (16%) and 17p deletion (7%). In this study, patient outcome differed significantly according to the genetic subgroup with median survival times of 32 months (17p deletion), 79 months (11q deletion), 114 months (trisomy 12) and 133 months (13q deletion); the median survival time of patients with normal karyotype was 111 months.

Recent molecular analysis has indicated the existence of two clinical forms of CLL, as distinguished on the basis of Ig V_H gene mutational status: (1) a pregerminal variant originating from B cells lacking somatic Ig V_H mutations and (2) a postgerminal variant originating from B cells displaying somatic mutations.[18,19] There are clear clinical and survival differences between the two patients subsets. In one study,[18] patients with evidence of Ig V_H mutations (55% of all patients) had

Table 1 Immunophenotype of mature B-cell chronic lymphocytic leukemia (B-CLL) and related malignancies[6,8,11]

	Surface Ig	*Surface antigen*							
		CD5	*CD10*	*CD19*	*CD20*	*CD22*	*CD23*	*CD79b*	*FMC7*
B-CLL	Weak	+	−	+	+	±	+	±	−
MCL	High	+	−	+	+	+	−	+	+
WM	High	±	±	+	+	+	−	+	+
FL	High	−	+	+	+	+	−	+	+
SLVL	High	−	−	+	+	+	−	+	+

Abbreviations: +, positive; −, negative; ±, weakly positive or negative; FL, follicular lymphoma; Ig, immunoglobulin; MCL, mantle cell lymphoma; SLVL, splenic lymphoma with villous lymphocytes (also called splenic marginal zone lymphoma); WM, Waldenström's macroglobulinemia.

Table 2 Classification of chronic lymphocytic leukemia as designated by the Binet and Rai staging systems.[7]

System and stage	*Risk*	*Manifestations*	*Patients (%)*	*Median survival (years)*	*Recommended treatment*
Binet					
A	Low	Lymphocytosis ($>10\times10^9$/l)[a], <3 lymphoid areas enlarged[b]	63	>12	Do not treat
B	Intermediate	⩾3 lymphoid areas enlarged	30	5	Treat only with progression
C	High	Anemia (Hb <100 g/l) and/or thrombocytopenia (platelets $<100\times10^9$/l)	7	2	Treat in most cases
Rai					
0	Low	Lymphocytosis only[a]	31	>12	Do not treat
I	Intermediate	Lymphadenopathy	35	8	Treat only with progression
II	Intermediate	Splenomegaly and/or hepatomegaly; lymphadenopathy may or may not be present	26	6	Treat only with progression
III	High	Anemia (Hb <110 g/l) with/without organomegaly	6	2	Treat in most cases
IV	High	Thrombocytopenia platelets $<100\times10^9$/l with/without anemia, organomegaly	2	2	Treat in most cases

[a]Lymphocytosis is present at all stages of the disease.
[b]Each of cervical, axillary, inguinal area (unilateral or bilateral), spleen and liver count as one area.

characteristics that were significantly associated with more malignant disease, including evidence of advance stage disease ($P=0.0009$), progressive disease ($P<0.0001$), atypical cell morphology ($P<0.0001$), trisomy 12 ($P=0.0019$) and 13q14 deletions ($P=0.023$). The absence versus presence of Ig V_H mutations is also significantly correlated with poorer outcome, as demonstrated for Binet stage A patients (95 versus 293 months, $P=0.0008$[18]) or Rai intermediate-risk patients (108 versus 204 months, $P=0.0007$[19]).

Unfortunately, studying Ig V_H mutations is not possible on a routine basis and a good surrogate for Ig V_H mutations is actively being sought. In this regard, CD38 expression correlates, although not absolutely, with Ig V_H mutations; moreover, CD38 expression may vary over time. Recently, it has been demonstrated that ZAP-70 expression, as evaluated by cytofluorometry, strongly correlates with Ig V_H mutations and has important prognostic significance.[20,21] As reported by Crespo *et al.*,[20] the Ig V_H gene was unmutated in 32 of 35 patients in whom ⩾20% of leukemic cells expressed ZAP–70, whereas all 21 patients with Ig V_H mutations had <20% ZAP-70-positive CLL cells. Moreover, an increased proportion of ZAP-70-positive cells (⩾20 versus <20%) was associated with decreased median survival times in Binet stage A patients (90 months versus 'not reached', respectively).

Indications for therapy

The diagnosis of CLL does not necessarily convey the necessity of treatment. Classically, treatment has been indicated in the presence of any of the following features: (1) general symptoms (fever, weight loss, night sweats, extreme fatigue); (2) lymphadenopathy or splenomegaly increasing in size or causing symptoms; (3) decreasing hemoglobin levels or platelet counts due to bone marrow infiltration; (4) autoimmune hemolytic anemia not responsive to corticosteroids; (5) hypogammaglobulinemia and infections.[10] Whether or not CLL should be treated on the basis of biological features that indicate the possibility of rapid disease progression and poor outcome (eg adverse cytogenetic abnormalities, Ig V_H mutational status, ZAP-70 expression) is currently being investigated in clinical trials.

Low-risk cases (Binet stage A or Rai stage 0) may include up to two-thirds of patients; many of these patients are asymptomatic at presentation and will not progress. It is generally accepted that these patients require only observation and should not be treated unless there is evidence of disease progression. This conclusion is based on data from a number of trials that investigated the outcome of immediate versus delayed therapy with chlorambucil, an alkylating agent widely used for the past 50 years for the treatment of CLL. A meta-analysis by the CLL Trialists' Collaborative Group[22] showed no significant difference in 10-year survival rates between immediate (44%) versus delayed (47%) chlorambucil treatment. However, given the availability of newer and more effective treatments it now seems appropriate to reconsider the strategy of early therapy in low-risk CLL patients, particularly (as discussed above) in those with high-risk prognostic factors.

Intermediate-risk patients (Binet stage B or Rai stage I and II) should generally be treated only when there is evidence of disease progression. High-risk patients (Binet stage C or Rai stage III and IV) typically have advanced disease due to bone marrow infiltration and virtually all patients require therapy.

Aims of therapy

With the introduction of new therapies and novel combination approaches, the therapeutic goal of CLL has shifted to that of achieving a molecular complete response, especially in younger patients. Nonetheless, the option of palliation remains an important consideration in elderly patients due to the problems associated with treating this patient group. However, elderly patients that are free from associated diseases that may hamper therapy should be considered as candidates for standard therapy.

Uniform guidelines for defining a response have been established by the NCI-WG[10] and the IWCLL.[9] Although the two groups proposed different criteria, responses can be broadly classified as complete response (CR), partial response (PR), stable disease, or progressive disease; stable or progressive disease should be considered a treatment failure. The goal of modern therapeutic approaches in CLL, however, is to attain a molecular CR by eradicating all malignant B cells and therefore, residual disease. Many methods of detecting CLL cells to assess and quantify minimal residual disease are established; the most sensitive of these techniques—polymerase chain reaction or 4-color flow cytometry—are based on the clonal rearrangement of the Ig H chain or the pattern of expression of CD markers. It is not clear how achieving a molecular CR affects the natural course of the disease, although abolition of minimal residual disease appears to be associated with superior survival in some studies.[23–26]

History of disease treatment

Corticosteroids

The use of corticosteroid monotherapy for CLL was tested over 40 years ago but their activity was limited by low response rates and steroid-induced adverse advents. In one randomized study,[27] 19 stage III and IV patients were treated with a 6-week course of prednisone (0.8 mg/kg for 2 weeks, 0.4 mg/kg for 2 weeks and 0.2 mg/kg for 2 weeks); prednisone was then given once a month at 0.8 mg/kg daily for a 7 day cycle. The overall response (OR) rate to prednisone was 11% (CR 0%) and the median time to relapse was 7 months.

However, corticosteroids may have a role in treating patients with autoimmune hemolytic anemia or thrombocytopenia. For autoimmune complications, prednisone is typically started at 40 mg/m^2 daily for 2 weeks then tapered over another 2 weeks. Further, a pilot study[28] showed that high-dose methylprednisone (250 mg/m^2 daily for 5 days) produced a 30% OR rate (all PRs) in 12 high-risk, refractory CLL patients. In this trial, hemoglobin and platelet counts were normalized, but there was an increased risk of infection.

Chlorambucil

Chlorambucil, a bifunctional alkylating drug, has been the standard first-line treatment for CLL since 1952. The optimal dosing schedule for chlorambucil is not well defined but various daily dosing schedules (eg $\sim$0.1 mg/kg) and intermittent dosing schedules (e.g. $\sim$0.4 mg/kg as a single pulse every 2 weeks, every 28 days or for 5 days every month, with or without dose escalation) have been described.[27,29–31] In intermediate- or high-risk CLL patients, chlorambucil produced OR rates of 47–62% and 29–39% in previously untreated and previously treated cases, respectively; however, complete remissions were rare (8–10%) and the duration of remission was short (7–16 months).

The results of an early randomized, double-blind study involving 26 CLL patients[30] suggested that the addition of prednisone to chlorambucil was more effective than chlorambucil alone (OR: 87 versus 45%; $P<0.05$), although there was no significant improvement in 2 year survival rates (93 versus 54%). A meta-analysis of this study and two additional follow-up trials that included 424 patients could not demonstrate a survival benefit of adding prednisone to chlorambucil.[22] The issue of the dose intensity of chlorambucil has also been addressed in a continuous high-dose therapy trial.[32] In this multicenter, randomized study of 181 previously untreated CLL patients, greater OR (89 versus 50%; $P<0.001$) and CR rates (70 versus 31%), and median survival time (6 versus 3 years; $P<0.01$) were seen for the high-dose schedule (15 mg daily up to CR or to toxicity) versus a weekly dosing regimen (75 mg weekly for 6 weeks). Despite its increased effectiveness, the daily high-dose regimen was also associated with increased toxicity and diminished compliance.

Cyclophosphamide combination regimens

The use of cyclophosphamide as monotherapy (50–100 mg/day) is generally limited to patients who are resistant or intolerant to chlorambucil. Cyclophosphamide is more commonly used in combination with other chemotherapeutic agents, some of which are described in Table 3.

The use of combination chemotherapy (typically cyclophosphamide, vincristine and prednisone [COP], or including doxorubicin [CHOP]) as a first-line treatment generally produces greater response rates than those achieved with chlorambucil at standard doses. However, this effect is not observed consistently and any benefit of the combination regimens does not translate into increased survival times.[33–35,44,46,47] A meta-analysis involving intermediate- to high-risk patients that compared combination regimens (mostly cyclophosphamide-based) with chlorambucil (with or without prednisone),[22] showed no difference in overall survival at 5 years between the chemotherapy and chlorambucil regimens (48% for both). Inclusion of an anthracycline in the combination did not affect outcome. In one of

Table 3 Overview of cyclophosphamide-containing treatments for CLL

Regimen[a]	*Trial*	*Patient stage*	*Overall response rate*
COP (CVP): Cyclophosphamide, vincristine, prednisone	Montserrat *et al.*[33]	Binet C ($n=96$)	1st line: 33 versus 71% with C + P
			2nd line: 28 versus 35% with C + P
	Raphael *et al.*[34]	Rai III/IV ($n=124$)	1st line: 82% (CR 23%) versus 72% (CR 25%) with C + P
	French Cooperative Group on CLL[35]	Binet B ($n=291$)	1st line: no difference between COP and chlorambucil in terms of response, follow-up status, time to progression and survival
	Oken and Kaplan[36]	Rai II–IV ($n=18$)	2nd line: 44% (CR 11%)
	Liepman and Votaw[37]	Rai I–IV ($n=36$)	Overall 1st + 2nd line: 72% (CR 44%)
CAP: Cyclophosphamide, doxorubicin, prednisone	French Cooperative Group on CLL[38]	Binet B/C ($n=196$)	1st line: 60% (CR 17%)
			2nd line: 27% (CR 6%)
	Keating *et al.*[39]	Rai I–IV ($n=46$)	1st line: 62% (CR 26%)
CHOP: Cyclophosphamide, doxorubicin, vincristine, prednisone	Leporrier *et al.*[40]	Binet B/C ($n=938$)	1st line: 72% (CR 30%) versus 58% (CR 15%) with CAP
	French Cooperative Group on CLL[41]	Binet C ($n=70$)	1st line: overall 3-year survival superior to COP (71 versus 28%)
	Hansen *et al.*[42]	Binet B/C ($n=116$)	1st line: 82% (CR 63%) versus 71% (CR 29%) with C + P
POACH: Cyclophosphamide, doxorubicin, cytosine-arabinoside, vincristine, prednisone	Keating *et al.*[43]	Rai I–IV ($n=65$)	1st line: 56% (CR 21%)
			2nd line: 26% (CR 7%)
CMP: Cyclophosphamide, melphalan, prednisone	Montserrat *et al.*[44]	Binet B/C ($n=96$)	1st line: 55% (CR 13%) versus 75% (CR 27%) with C + P
M-2 protocol: Cyclophosphamide, melphalan, vincristine, BCNU, prednisone	Kempin *et al.*[45]	Rai II–IV ($n=63$)	1st line: 81% (CR 30%)
			2nd line: 35% (CR 0%)

[a]Typical dosing schedules: cyclophosphamide (300–750 mg/m^2 p.o. on day 1); vincristine (1 mg/m^2 i.v. on day 1); prednisone (40–100 mg/m^2 p.o. on days 1–5); doxorubicin (15–50 mg/m^2 i.v. on day 1); cytosine-arabinoside (25 mg/m^2 i.v. every 12 h on days 1–5); melphalan (6 mg/m^2 p.o. on days 1–4); BCNU (0.5 mg/kg i.v. on day 1). Schedules were usually repeated every 3–4 weeks.
Abbreviations: C + P, chlorambucil and prednisone; CR, complete response; i.v., intravenously; p.o., orally.
Note. In drug combinations, the dosages of each drug listed in this table are under permanent investigation and vary greatly depending on the investigation center and patient condition.

these trials involving 228 patients,[48] both the OR rate (90 versus 75%) and median overall survival (68 versus 47 months) were greater with high-dose chlorambucil (15 mg/day until toxicity) than with the low-dose French CHOP regimen.

Cyclophosphamide-containing combination chemotherapy regimens have also been used as a second-line treatment option, but their use in this setting is limited. For example, in previously treated CLL patients, low OR rates were reported: 27% with CAP (cyclophosphamide, doxorubicin and prednisone),[38] 28–44% with COP,[33,36] and 26% with COP plus doxorubicin and cytosine–arabinoside (POACH).[43] Although beneficial responses with various combination therapies have been reported in small studies, these findings have generally not been confirmed in prospective trials. As such, no effective salvage therapy for CLL was available until the discovery of the purine analogs.

Purine analogs

Until the late 1980 s, only chlorambucil and cyclophosphamide-containing regimens had shown relatively good activity in CLL, but complete remissions were rare with these agents. Further, no effective therapy was available for patients that were refractory to, or had relapsed after these treatments. The introduction of purine analogs such as fludarabine, cladribine, and pentostatin has renewed research in the treatment of CLL. The most effective and most extensively studied of these agents is fludarabine.

Following initial reports of the activity of fludarabine in relapsed or refractory CLL patients,[49,50] this agent rapidly emerged as the standard second-line treatment in CLL. Because of its high efficacy as single-agent therapy, fludarabine has also recently become an established first-line treatment option in CLL;

Table 4 Recommended dosing schedules for fludarabine in CLL patients

Route of administration	*Dose (mg/m²)*	*Schedule*[a]
Intravenous	25	One dose daily for five consecutive days, every 28 days; fludarabine should be administered until the best response is achieved (eg complete or partial remission, usually six cycles)
Oral	40	

[a]The duration of treatment depends on the treatment success and the tolerability of fludarabine.

fludarabine was registered for this indication throughout Europe in 2003.

In noncomparative trials, fludarabine (typical dose: 25 mg/m² intravenously on days 1–5, every 4 weeks for up to six cycles) produced OR rates of between 12 and 100%. The effectiveness of fludarabine depends on the extent of (and response to) previous therapy. Therefore, the response rate in previously untreated or relapsed patients (~80%) is better than in patients unresponsive to first-line treatment (35–40%).[51–53] In comparative studies in previously untreated CLL patients, fludarabine was more effective than chlorambucil (OR: 63 versus 37%, CR: 20 versus 4%)[54] and CAP or CHOP (OR: 71 versus 58 vs. 72%, CR: 40 versus 15 versus 30%).[40] Likewise, fludarabine was superior to CAP in previously treated patients (OR: 48 versus 27%, CR: 13 versus 6%).[38] However, overall survival times were not significantly different in any of these trials. In these studies, myelosuppression and infection were the most common adverse reactions associated with fludarabine.

The development of an oral formulation of fludarabine has been an important advance as it decreases both the need for intravenous access and hospital visits. Oral fludarabine is as effective and as well tolerated as the intravenous formulation.[55] In one study involving 78 previously treated CLL patients,[55] oral fludarabine (40 mg/m² on days 1–5, every 4 weeks; mean of five cycles) produced OR and CR rates of 51 and 18%, respectively. The recommended dosing schedules for fludarabine given orally or intravenously are presented in Table 4.

Although fludarabine is associated with a higher response rate than traditional therapies, the proportion of CR could be improved. Because of this, many combination therapies based on fludarabine are presently being investigated for the treatment of CLL.[52,56] An overview of the effectiveness of fludarabine-based combination regimens is presented by Hallek in this Supplement.

Experimental therapeutic approaches

Recently, novel agents have been explored for the treatment of CLL. These treatments include immunotherapy with monoclonal antibodies, either naked or conjugated to toxins or radioisotopes.[6] Two such biologicals that have shown particular promise in CLL, and other hematological cancers, are the anti-CD52 antibody alemtuzumab (MabCampath®; Campath®) and the anti-CD20 antibody rituximab (Rituxan®; Mabthera®). Additionally, alemtuzumab and fludarabine given together or sequentially to refractory CLL patients produces high remission rates (83–92%)[57–59] and is an effective conditioning regimen prior to stem cell transplantation techniques.[60]

Other more experimental approaches include the protein kinase C inhibitor UCN-01, the protein kinase C activator bryostatin, the cyclin-dependent kinase inhibitor flavopiridol, the topoisomerase-I inhibitor 9-aminocamptothecin, depsipeptide and bcl-2 antisense oligonucleotides,[8,53] as well as new monoclonal antibodies (eg anti-CD23).[61] Additional approaches that are increasingly being considered for the treatment of some CLL patients are autologous and allogeneic stem cell transplantation.[62] Although there is limited evidence that autotransplantation can cure the disease, allotransplantation (which harnesses the graft-versus-leukemic effect) appears to be curative for a subset of CLL patients. These techniques have traditionally involved myeloablative conditioning regimens prior to transplantation and are associated with excessively high treatment-related mortality; therefore, their use generally has been limited to younger patients or to those with advanced poor-risk disease. However, the development of reduced intensity nonmyeloablative conditioning therapies may extend the usefulness of these transplantation approaches to a greater proportion of CLL patients. These and other novel therapeutic approaches for the treatment of CLL are covered in greater detail by Hillmen and Carella later in this Supplement.

Conclusions

Since chlorambucil and cyclophosphamide-containing regimens became the foundation of CLL therapy, many decades passed without substantial progress in the treatment of this disease. The discovery of fludarabine has rejuvenated research into CLL and has helped to shift the therapeutic goal from palliation to cure, with eradication of minimal residual disease now a realistic prospect in many patients. Considering the heterogeneous nature of CLL patients, the key challenges that remain for treating physicians are to identify accurately the therapeutic strategy most likely to benefit an individual patient. These judgments rely, in part, on accurate patient diagnosis and prognosis. With the development of combination regimens involving fludarabine in conjunction with chemotherapeutic or biological agents, together with promising approaches such as stem cell transplantation, a cure for this hematological malignancy is now nearer than ever.

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Role of fludarabine as monotherapy in the treatment of chronic lymphocytic leukemia

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Fludarabine is a synthetic adenine nucleoside analog that is indicated for first- and second-line treatment of chronic lymphocytic leukemia (CLL). The recommended intravenous (i.v.) dosage regimen is 25 mg/m^2 daily for 5 consecutive days, with treatment cycles repeated every 28 days. In treatment-naïve patients with Binet stage B and C CLL, i.v. fludarabine produces superior responses to established first-line chemotherapies. Fludarabine produces a higher overall remission rate (60–70%) and longer progression-free survival (median $\sim$20–30 months) than standard therapy with chlorambucil $\pm$ prednisone and CAP (cyclophosphamide/doxorubicin/prednisone), and a comparable overall remission rate to CHOP (cyclophosphamide/vincristine/prednisone/doxorubicin). Fludarabine demonstrates high efficacy in both intermediate-risk (Rai stage I or II) and high-risk (Rai stage III or IV) patients. Furthermore, fludarabine is equally effective in younger ($\leqslant$65 years) and older ($>$65 years) patients. Fludarabine has significant activity as monotherapy in previously treated CLL, producing objective response rates of up to 94% in typically small-scale, noncomparative studies, with the majority of studies yielding rates of 30–60%. In a phase III multicenter study, the overall remission rate was significantly higher with fludarabine than with CAP (48 versus 27%) among the subset of treatment-refractory patients ($n=96$). For those patients who are refractory to or have relapsed following conventional chemotherapy (chlorambucil, CAP and CHOP), fludarabine can be considered the treatment of choice for second-line therapy. Moreover, patients with relapsed CLL may benefit from retreatment with fludarabine if they have previously demonstrated sensitivity to the drug. Standard-dose i.v. fludarabine has an established safety profile and comparable tolerability to anthracycline-based regimens (CAP and CHOP) in terms of its myelosuppressive and immunosuppressive effects, and offers the advantage of a markedly lower incidence of gastrointestinal effects (nausea/vomiting) and alopecia.
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Introduction

The purpose of this article is to provide an overview of the clinical profile of the intravenous (i.v.) formulation of fludarabine in the treatment of chronic lymphocytic leukemia (CLL). Fludarabine was initially developed for the second-line treatment of CLL, and soon became the gold standard in this setting. The use of fludarabine rapidly expanded to first-line treatment of CLL during the 1990 s on account of its demonstrated activity. An oral formulation of fludarabine, which offers advantages in the outpatient setting and is recommended in preference to the i.v. formulation by the UK National Institute for Clinical Excellence, has subsequently been introduced. The properties of the oral formulation are considered separately within this supplement in the article by Boogaerts.

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Overview of the pharmacological properties of fludarabine

Fludarabine is a synthetic adenine nucleoside analog that is structurally related to cytarabine (ara-C) and the antiviral agent vidarabine (ara-A). The pyrimidine antimetabolite ara-C has proved to be one of the most effective drugs in the treatment of acute leukemia, but its antineoplastic activity is highly dependent upon nucleoside activating and inactivating enzyme activities, and ara-C resistance is a significant problem.[1] The adenosine analog ara-A inhibits virus-induced DNA polymerases in tumor cells but, being a ready substrate for adenosine deaminase, it undergoes rapid metabolic inactivation and hence lacks clinically significant anticancer activity.[2] Structural modification of vidarabine,

aimed at conferring resistance to degradation by adenosine deaminase while conserving the desirable metabolic and inhibitory properties of the parent compound, subsequently led to the synthesis of 2-fluoroadenosine (F-Ado) and its arabinosyl derivative, 9-β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A).[3] As F-ara-A is relatively insoluble, its 5′-monophosphate has been developed for clinical application. Fludarabine 5′-monophosphate (fludarabine) acts as a soluble prodrug that is rapidly converted *in vivo* to the active drug F-ara-A (Figure 1).

As a hydrophilic anion at physiological pH, fludarabine 5′-monophosphate does not readily cross the cell membrane. Following i.v. administration, however, it undergoes rapid dephosphorylation (presumably by 5′-nucleotidases in erythrocytes and endothelial cells) to yield F-ara-A, which appears to be taken up preferentially by leukemia cells via the nucleoside transport system.[4] Within the cell, F-ara-A is rephosphorylated, via monophosphate and diphosphate intermediaries, to the cytotoxic moiety fludarabine triphosphate (F-ara-ATP)[5–7] (Figure 2).

The cytotoxic action of F-ara-ATP is mediated primarily through inhibition of DNA synthesis,[8] although inhibition of RNA transcription may also contribute to its apoptotic activity against CLL cells[9] (Figure 2). Several specific enzymes involved in DNA synthesis are targets for inhibition by F-ara-ATP, including DNA polymerase, DNA primase, DNA ligase and ribonucleotide reductase.[7] In particular, F-ara-ATP competes directly with the natural deoxynucleotide deoxyadenosine 5′-triphosphate (dATP) for incorporation into DNA, and also depletes cellular deoxynucleotide pools. Once incorporated into the DNA strand, F-ara-AMP serves as an effective chain terminator, thereby inactivating DNA synthesis and triggering subsequent cell apoptosis.[7]

Following i.v. administration of fludarabine, rapid and extensive first-pass metabolism leads to its clearance from the circulation within 2–4 min.[10] Accordingly, the plasma metabolite F-ara-A forms the main focus of pharmacokinetic investigation of fludarabine. This metabolite displays linear kinetics, with no appreciable accumulation occurring after repeated daily administration (i.v. bolus or 30-min infusion) of its prodrug.[11] The tissue distribution of F-ara-A appears to be extensive, as indicated by its relatively large volume of distribution (44–96 l/m^2).[11] Intracellular levels of the cytotoxic moiety F-ara-ATP peak within 3–4 hours of termination of fludarabine infusion and decline monophasically with a median half-life of 23 h.[12] However, there is no clear correlation between intracellular F-ara-ATP level and the clinical response to fludarabine in patients with CLL.[12]

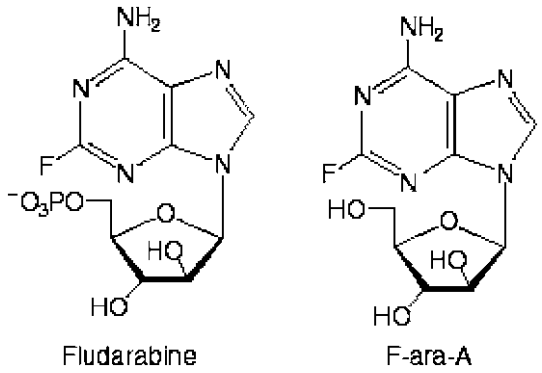

Figure 1 Structures of fludarabine and 9-β-D-arabinosyl-2-fluoroadenine (F-ara-A).

Efficacy of intravenous fludarabine in first-line treatment of CLL

Fludarabine is an approved first-line treatment for CLL, where it achieves superior remission rates to traditional first-line therapies such as chlorambucil and anthracycline-based regimens. The recommended i.v. dosage regimen for fludarabine in the treatment of CLL is 25 mg/m^2 daily for 5 consecutive days, with the cycle repeated every 28 days until a maximal response is obtained (this usually requires six treatment cycles). Clinical trials of fludarabine in the first-line treatment of CLL have typically employed a dosage regimen of 25–30 mg/m^2, administered as a 30-min i.v. infusion on 5 consecutive days, with treatment cycles repeated every month. Treatment responses have been assessed according to the criteria recommended by the National Cancer Institute (NCI) working group on CLL,[13] which define complete remission as disappearance of all palpable disease, neutrophil count $\geqslant$1 500 cells/μl, platelet count $>$100 000 cells/μl, hemoglobin level $>$11 g/dl and a bone marrow relative lymphocyte count of $<$30%. Partial remission is defined as a $\geqslant$ 50% reduction in measurable disease and a $>$50% improvement in abnormal blood counts.

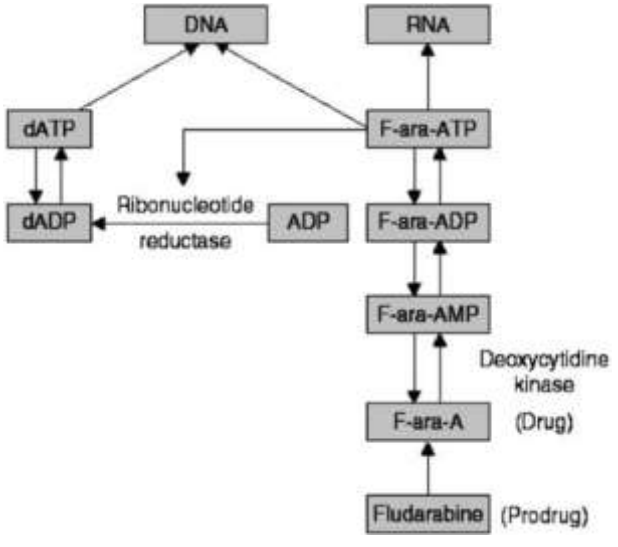

Figure 2 Metabolism and actions of fludarabine. A = adenosine; dA = deoxyadenosine; F-ara-A = 9-β-D-arabinosyl-2-fluoroadenine; MP, DP and TP refer to the nucleoside 5′-monophosphates, diphosphates and triphosphates, respectively.[7] (Reproduced with permission.)

Noncomparative studies

Fludarabine has been evaluated as monotherapy in numerous small-scale noncomparative studies conducted in treatment-naïve patients with CLL. The initial study, involving 33 patients (14 with Rai stage III and IV and 19 with stage 0, I or II disease) who were treated with fludarabine (30 mg/m^2 daily for 5 days, repeated every 4 weeks), reported an overall remission rate of 79%, at the time the highest reported for a single agent in CLL, with 33% of patients achieving complete remission and a further 39% complete remission with residual nodules as the only evidence of disease.[14] The response was rapid, usually occurring after 3–6 courses of treatment.[14] These findings were confirmed by subsequent small-scale studies with fludarabine (typically 25–30 mg/m^2 daily for 5 days, repeated every 4 weeks), which reported overall remission rates of 80–100%[15–17] and a median time to disease progression of 33 months[15] (Table 1).

Comparative studies

Several phase III studies conducted in the USA and Europe have compared the efficacy of i.v. fludarabine monotherapy against that of chlorambucil,[20] combination cyclophosphamide/doxorubicin/prednisone (CAP) chemotherapy[18,19] and combination CHOP (cyclophosphamide, vincristine, prednisone, doxorubicin) chemotherapy[19] in previously untreated patients with Binet stage B and C CLL (Table 1). Fludarabine has been shown to induce higher remission rates and longer progression-free survival than standard therapy with chlorambucil,[20] chlorambucil plus prednisone[21] and CAP,[18,19] and a comparable remission rate to CHOP chemotherapy.[19] In addition, findings from the German CLL Study Group trials, involving a total of 98 patients undergoing first-line therapy with standard-dose i.v. fludarabine (25 mg/m^2/day for 5 days, repeated every 4 weeks), indicate that the drug is equally effective in early (Binet stage A) and advanced (Binet stage B and C) disease, and in younger ($\leqslant$65 years) and older ($\geqslant$66 years) patients with CLL.[22]

Pivotal US phase III study of fludarabine versus chlorambucil

The pivotal phase III multicenter study conducted in the USA compared the efficacy of single-agent fludarabine against that of single-agent chlorambucil and the combination of fludarabine plus chlorambucil as first-line therapy in previously untreated CLL patients with high-risk (Rai stage III or IV) or intermediate-risk (Rai stage I or II) disease.[20] For study inclusion, patients with intermediate-risk disease were required to have at least one of the following features: any disease-related

Table 1 Response rates to fludarabine monotherapy in patients with previously untreated CLL

Reference	*No. of patients evaluable*	*Treatment regimen*	*Clinical response (% of patients)*	
			CR	*CR + PR*
Noncomparative studies				
Keating *et al.*[14]	33	Fludarabine 30 mg/m^2/day i.v. for 5 days, every 4 weeks	33	79
Keating *et al.*[15]	35	Fludarabine 25–30 mg/m^2/day i.v. for 5 days, every 4 weeks	74	80
Clavio *et al.*[16]	16	Fludarabine 25 mg/m^2/day i.v. for 5 days, every 4 weeks	31	100
Stelitano *et al.*[17]	17	Fludarabine 30 mg/m^2/day i.v. for 4 days, every 3 weeks, or for 5 days, every 4 weeks	65	94
Comparative studies				
French Cooperative Group on CLL[18]	100	Fludarabine ($n = 52$), 25 mg/m^2 i.v. daily for 5 days, every 4 weeks	23	71
		CAP ($n = 48$), cyclophosphamide 750 mg/m^2 i.v. on day 1 + doxorubicin 50 mg/m^2 i.v. on day 1 + prednisone 40 mg/m^2 p.o. on days 1–5, every 4 weeks	17	60
Leporrier *et al.*[19]	924	Fludarabine ($n = 336$), 25 mg/m^2 i.v. daily for 5 days, every 4 weeks	40***	71***
		CAP ($n = 237$), cyclophosphamide 750 mg/m^2 i.v. on day 1 + doxorubicin 50 mg/m^2 i.v. on day 1 + prednisone 40 mg/m^2 p.o. on days 1–5, every 4 weeks	15	58
		CHOP ($n = 351$), vincristine 1 mg/m^2 i.v. on day 1 + doxorubicin 25 mg/m^2 i.v. on day 1 + cyclophosphamide 300 mg/m^2 p.o. on days 1–5 + prednisone 40 mg/m^2 p.o. on days 1–5, every 4 weeks	30***	72***
Rai *et al.*[20]	509	Fludarabine 25 mg/m^2/day i.v. for 5 days, every 4 weeks ($n = 179$)	20**	63**
		Chlorambucil 40 mg/m^2 p.o., once every 4 weeks ($n = 193$)	4	37
		Fludarabine 25 mg/m^2/day i.v. on days 1–5 + chlorambucil 40 mg/m^2 p.o on day 1, every 4 weeks ($n = 137$)	20**	61**
Spriano *et al.*[21]	115	Fludarabine 25 mg/m^2 by 30-min i.v. infusion on days 1–5, every 4 weeks ($n = 60$)	47	70
		Chlorambucil 30 mg/m^2 p.o. on days 1 and 15 + prednisone 40 mg/m^2 i.m. on days 1–5 and 15–19, every 4 weeks ($n = 55$)	31	65

Abbreviations: CR = complete response; i.m. = intramuscular; i.v. = intravenous; p.o. = oral; PR = partial response.
*** $P < 0.0001$ versus CAP.
** $P < 0.001$ versus chlorambucil.

symptom such as weight loss, extreme fatigue, night sweats or fever without evidence of infection; massive or progressive splenomegaly and/or lymphadenopathy; a >50% increase in peripheral blood lymphocyte count over a 2-month period or an anticipated doubling of the cell count within 12 months.

Of those patients who were randomized to monotherapy (combination therapy was discontinued during the study because of toxicity), 179 patients received i.v. fludarabine (25 mg/m^2/day for 5 days) and 193 patients received oral chlorambucil (40 mg/m^2 once every 28 days). In the absence of either complete remission or disease progression, treatments were repeated every 28 days for a maximum of 12 treatment cycles.

Fludarabine-treated patients had significantly higher ($P<0.001$) overall and complete remission rates than those treated with chlorambucil (63 versus 37% and 20 versus 4%, respectively) (Table 1, Figure 3). Subgroup analysis indicated that fludarabine resulted in superior overall and complete remission rates to chlorambucil in both intermediate-risk (67 versus 46% and 26 versus 6%, respectively) and high-risk (57 versus 23% and 10 versus 1%, respectively) CLL (Table 2).

Fludarabine-treated patients showed a significantly longer median duration of response (25 versus 14 months; $P<0.001$) and median time to disease progression (20 versus 14 months; $P<0.001$) than chlorambucil-treated patients, although overall survival did not differ significantly between the two treatment groups (Figure 4). Subgroup analysis indicated that fludarabine was superior to chlorambucil in delaying disease progression both among intermediate- and high-risk patients (Table 3).

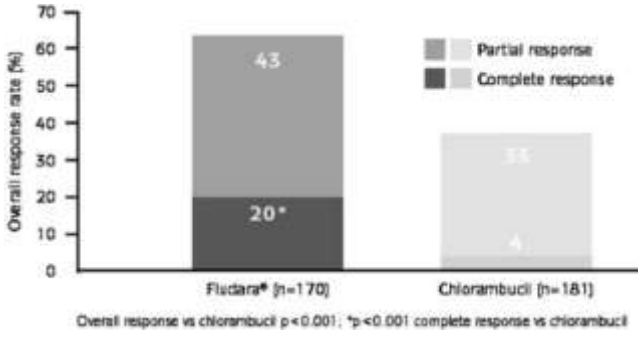

Figure 3 Overall response rates with fludarabine and chlorambucil monotherapy in treatment-naïve patients with CLL.[20]

European phase III study of fludarabine versus chlorambucil plus prednisone

An Italian phase III multicenter study assessed the efficacy of fludarabine versus that of chlorambucil plus prednisone in 147 previously untreated patients with active CLL (Rai intermediate- and high-risk disease).[21] Patients were randomized to receive i.v. fludarabine (25 mg/m^2/day for 5 days; $n=73$) or oral chlorambucil (30 mg/m^2 on days 1 and 15) plus intramuscular

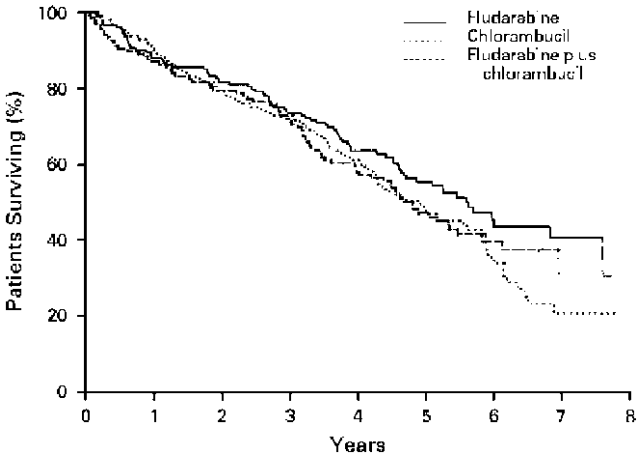

Figure 4 Overall survival in previously treatment-naïve patients with CLL receiving fludarabine, chlorambucil or the combination of fludarabine and chlorambucil.[20] (Copyright © 2000 Massachusetts Medical Society. All rights reserved.)

Table 3 Median duration of response, time to disease progression and overall survival in fludarabine- and chlorambucil-treated patients[20]

Variable	*Fludarabine*	*Chlorambucil*	P-*value*
Median duration of response (months)	25	14	< 0.001
Median time to disease progression (months)			
All patients	20	14	< 0.001
Intermediate-risk group (Rai stage I or II)	23	16	0.02
High-risk group (Rai stage III or IV)	18	12	0.006
Median overall survival (months)	66	56	0.10

Table 2 Response rates to fludarabine and chlorambucil, with patients categorized according to disease severity at presentation[20]

Response	*Intermediate-risk CLL (Rai stage I or II)*			*High-risk CLL (Rai stage III or IV)*		
	Fludarabine (n = 103)	*Chlorambucil (n = 111)*	P-*value*	*Fludarabine (n = 67)*	*Chlorambucil (n = 70)*	P-*value*
OR (%)	67	46	0.002	57	23	< 0.001
CR (%)	26	6	< 0.001	10	1	0.03
PR (%)	41	40	>0.05	46	21	>0.05

Abbreviations: CLL = chronic lymphocytic leukemia; CR = complete response; OR = overall response (CR + PR); PR = partial response.

prednisone (40 mg/m^2 on days 1–5 and 15–19; $n = 74$); treatment cycles were repeated every 4 weeks. An interim analysis of data from 115 evaluable patients (60 fludarabine- and 55 chlorambucil + prednisone-treated) who received at least six cycles of treatment indicated that fludarabine was the more effective of the two treatments, resulting in a higher complete response rate (47 versus 31%), although overall response rates were similar in the fludarabine and chlorambucil + prednisone treatment groups (70 versus 65%, respectively) (Table 1). A subsequent analysis, based on results from 150 evaluable patients, indicated that the treatment response was more durable with fludarabine than with chlorambucil + prednisone (28 versus 21 months).[23]

European phase III study of fludarabine versus CAP

A European phase III study of randomized, open-label design, conducted at 40 centers in France, Germany, Sweden and the UK, compared the efficacy of fludarabine with that of CAP, a combination regimen frequently used for first- and second-line treatment of CLL.[18]

Of the total evaluable study population ($n = 196$), 100 previously untreated patients with advanced-stage Binet B or C CLL received fludarabine (25 mg/m^2 as a 30-min i.v. infusion on days 1–5, repeated every 28 days; $n = 52$) or CAP (cyclophosphamide 750 mg/m^2 i.v. on day 1, doxorubicin 50 mg/m^2 i.v. on day 1, prednisone 40 mg/m^2 p.o. on days 1–5, repeated every 28 days; $n = 48$).

Within this group of treatment-naïve patients, the overall remission rate tended to be higher with fludarabine than with CAP (71 versus 60%; $P = 0.26$) (Table 1; Figure 5), whereas the median duration of disease remission (>1640 versus 208 days; $P < 0.001$) was significantly longer in the fludarabine-treated group than in the CAP-treated group. Likewise, median survival tended to be longer with fludarabine than with CAP (>1800 versus 1580 days; $P = 0.087$) (Figure 6).

Comparison of fludarabine versus CAP versus CHOP

The French Cooperative Group on CLL conducted a randomized clinical trial during the 1990 s to compare the effectiveness of fludarabine with that of two anthracycline-containing regimens – CAP and CHOP – in previously untreated patients with Binet stage B and C CLL.[19] In this multicenter study, a total of 938 patients (n = 651 stage B, n = 287 stage C) were randomized to receive six monthly courses of either fludarabine (25 mg/m^2/day i.v. for 5 days, repeated every 4 weeks; n = 341), CAP (cyclophosphamide 750 mg/m^2 i.v. on day 1 + doxorubicin 50 mg/m^2 i.v. on day 1 + prednisone 40 mg/m^2 p.o. on days 1–5, repeated every 4 weeks; $n = 240$) or CHOP (vincristine 1 mg/m^2 i.v. on day 1 + doxorubicin 25 mg/m^2 i.v. on day 1 + cyclophosphamide 300 mg/m^2 p.o. on days 1–5 + prednisone 40 mg/m^2 p.o. on days 1–5, repeated every 4 weeks; $n = 357$).

The overall response rates with fludarabine (71%) and CHOP (72%) were significantly higher ($P < 0.0001$) than with the CAP regimen (58%). Likewise, complete responses were obtained in significantly ($P < 0.0001$) higher proportions of patients receiving fludarabine (40%) or CHOP (30%) compared with those receiving CAP (15%) (Table 1). The time to disease progression, as indicated by the delay until initiation of second-line therapy, differed significantly ($P < 0.0001$) between the fludarabine (median 45.4 months), CHOP (median 32.2 months) and CAP (median 25.7 months) treatment groups. However, median survival (69, 67 and 70 months, respectively) and estimated 5-year survival rates (58, 57 and 60%, respectively) did not differ significantly between the fludarabine, CHOP and CAP treatment arms. When patients were stratified according to baseline disease severity, overall survival did not differ significantly between the three treatment groups (Figure 7).

A quality-adjusted survival analysis, using the quality-adjusted time without symptoms or toxicity (Q-TWiST) approach, for this patient database indi-

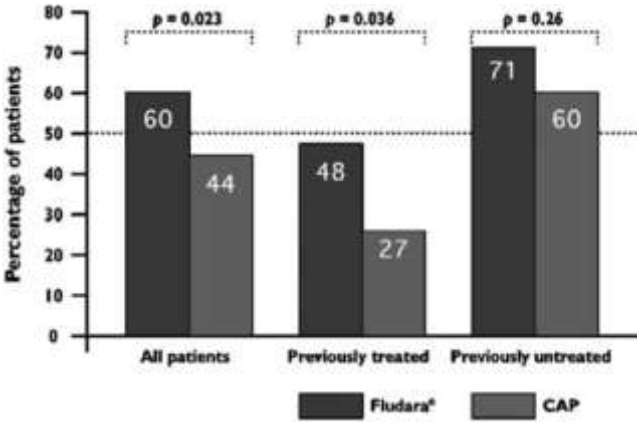

Figure 5 Overall response rates of previously treated and previously untreated patients to fludarabine monotherapy and CAP.[18]

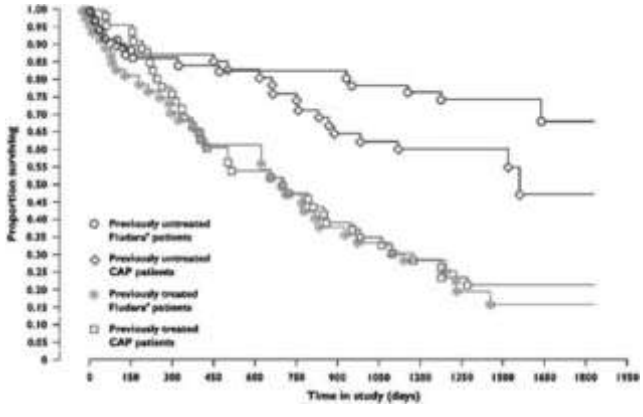

Figure 6 Survival times following fludarabine or CAP therapy of treatment-naïve and previously treated patients with CLL.[18] (Reprinted with permission from Elsevier.)

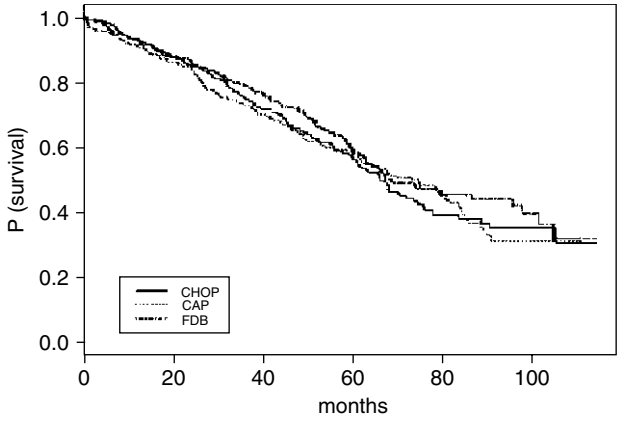

Figure 7 Overall survival in previously treatment-naïve patients with CLL receiving fludarabine, CHOP or CAP.[20] (Copyright American Society of Hematology, used with permission.)

cated that fludarabine provided a moderate quality-of-life advantage over CAP and CHOP.[24] The average time spent in each of four clinical states (toxicity, treatment free of toxicity, no treatment or symptoms, and relapse) was weighted using a utility coefficient (reflecting relative value in terms of quality of life) to generate a Q-TWiST value for each treatment group. Over the 73-month follow-up period, mean Q-TWiST values were higher for fludarabine (32.95 months) than for CAP (31.05 months) or CHOP (27.05 months); fludarabine-treated patients gained a mean of 45 and 61 days, respectively, of toxicity-free survival over CAP- and CHOP-treated patients.[24]

Efficacy of fludarabine in the second-line treatment of CLL

Patients with progressive or advanced CLL may initially respond to chlorambucil therapy, but all will ultimately relapse. Second-line chemotherapy with anthracycline-based regimens can provide good response rates, but the majority of patients eventually succumb to progressive disease.[25]

Fludarabine is currently standard second-line monotherapy for CLL, and can produce notable remission rates in CLL patients who have failed to respond to conventional monotherapy or combination regimens. The ability of fludarabine to induce an antitumor response in these refractory patients suggests that significant clinical cross-resistance does not develop between fludarabine and other commonly used anti-CLL agents.

Noncomparative studies

Numerous noncomparative studies, generally of small scale, have assessed the therapeutic efficacy of fludarabine monotherapy in patients with CLL who have previously received chemotherapy. Following treatment with fludarabine (typically 20–30 mg/m^2/day for 5 days, repeated every 3–5 weeks), objective response rates ranging from 19 to 94% were reported, with the majority of studies yielding rates of 30–60% (Table 4). The large variation in response rates can be attributed, in part, to the small number of patients in some studies and to interstudy differences in disease stage, dose and treatment schedule, and previous chemotherapy.

Several phase II/III trials conducted in the US and Europe have shown fludarabine to have significant activity as monotherapy in previously treated CLL patients.[30,34,35] A retrospective analysis, using NCI diagnostic and response criteria to standardize the results from two early US phase II studies involving a total of 100 CLL patients,[30,35] indicated that complete and overall responses were obtained in 13 and 35–51% of patients, respectively.[45]

A European phase II/III study, conducted in 126 CLL patients (predominantly Binet stage C) who were refractory to or had relapsed after $\geqslant$2 different courses of chemotherapy, including anthracycline- and mitoxantrone-containing regimens, reported complete and partial remission rates of 4.8 and 20.6%, respectively, after six cycles of fludarabine monotherapy (25 mg/m^2/day as a 30-min i.v. infusion for 5 days).[34] Disease stabilization was achieved in a further 18.3% of patients. Overall median survival was 12.7 months, and the median time to disease progression was 9.6 months.

A less aggressive treatment regimen may be appropriate in selected patients (eg, heavily pretreated patients, the elderly and those with comorbidities) when quality of life rather than quality of response is the primary consideration. A recent study in 20 elderly CLL patients (mean age 76 years) noted that a treatment regimen of i.v. fludarabine 30 mg/m^2/day for 3 alternate days, repeated every 28–40 days, provided significant disease control (complete remission rate 20%, overall remission rate 60%), although event-free survival over the 33-month follow-up period was lower than that obtained with a 5-day treatment schedule.[46]

Comparative studies

European phase III study of fludarabine versus CAP A prospective, randomized assessment of fludarabine in the second-line treatment of CLL was provided by the European multicenter phase III study that compared the efficacy of fludarabine with that of CAP in both treatment-naïve and previously treated patients with CLL.[18] Among the per protocol population of 196 patients with CLL, 96 treatment-refractory patients underwent treatment with fludarabine (25 mg/m^2 as a 30-min i.v. infusion for 5 days) ($n=48$) or the combination of cyclophosphamide (750 mg/m^2 i.v. on day 1), doxorubicin (50 mg/m^2 i.v. on day 1) and prednisone (40 mg/m^2 p.o. on days 1–5) ($n=48$). Treatment cycles were repeated every 28 days.

For the per protocol population, which comprised both treatment-naïve and previously treated patients, the overall response rate with fludarabine (60%) was

Table 4 Response rates to fludarabine in non-comparative studies of chemotherapy-pretreated patients with B-cell CLL

Reference	*No. of patients enrolled*	*No. of evaluable patients*	*Fludarabine regimen*	*Clinical response (% of patients)*	
				CR	*CR + PR*
Angelopoulou *et al.*[26]	20	20	25 mg/m^2 30-min infusion daily for 5 days every 4 weeks	33	58
Fenchel *et al.*[27]	61[a]	56	25 mg/m^2 30-min infusion daily for 5 days every 5 weeks	5	73
Gillis *et al.*[28]	10	10	25 mg/m^2 i.v. daily for 5 days every 4 weeks	10	40
Gjedde *et al.*[29]	30	22	25 mg/m^2 30-min infusion daily for 5 days every 4 weeks	5	32
Grever *et al.*[30]	32	21	20 mg/m^2 i.v. daily for 5 days every month	5	19
Hensel *et al.*[31]	46[b]	46	25 mg/m^2 i.v. daily for 5 days every 4 weeks	4	34
Hiddemann *et al.*[32]	20	20	25 mg/m^2 30-min infusion daily for 5 days every 4 weeks	20	55
Hocepied *et al.*[33]	17	17	25 mg/m^2 30-min infusion daily for 5 days every 4 weeks	23	64
Johnson *et al.*[34]	126	126	25 mg/m^2 i.v. daily for 5 days every 4 weeks	5	26
Keating *et al.*[35]	68	68	25–30 mg/m^2 i.v. daily for 5 days every month	13	44
Keating *et al.*[15]	78	78	25–30 mg/m^2 daily for 5 days every 3–4 weeks	38	60
Knauf *et al.*[36]	9	9	25 mg/m^2 i.v. daily for 5 days every month	11	55
Montserrat *et al.*[37]	75	68	20–30 mg/m^2 i.v. for 3 to 5 days every 4 weeks	4	28
Montillo *et al.*[38]	16	13	25 mg/m^2 30-min infusion daily for 5 days every 4 weeks	31	69
O'Brien *et al.*[39]	37[c]	37	25 mg/m^2 i.v. daily for 5 days every 4 weeks	11	43[d]
Puccio *et al.*[40]	49[e]	42	20 mg/m^2 i.v. bolus + 30 mg/m^2/day i.v. infusion for 48 h	0	52
Robertson *et al.*[41]	80	80	30 mg/m^2 i.v. daily for 3 days every 4 weeks	25[f]	46
Sorensen *et al.* [42]	724[c]	703	25 mg/m^2 short infusion daily for 5 days every 4 weeks	3	32
Stelitano *et al.*[17]	15 (relapsed)	15	25 mg/m^2 daily for 4 days every 3 weeks or for 5 days every 4 weeks	20	80
	15 (resistant)	15		13	47
Wijermans *et al.*[43]	17[g]	17	25 mg/m^2 i.v. daily for 5 days every 4 weeks	12	94
Zinzani *et al.*[44]	41[h]	41	25 mg/m^2 i.v. daily for 5 days every 4 weeks	2	41

[a]Includes six previously untreated patients.
[b]Includes one patient with Waldenström's macroglobulinemia.
[c]Includes patients with CLL/PL ($n = 5$), B-cell PLL ($n = 3$), T-cell PLL ($n = 2$).
[d]Partial responses were not reported in patients with T-cell PLL.
[e]Includes two patients with T-cell CLL.
[f]Includes 12 nodular CRs.
[g]Includes four patients with Waldenström's macroglobulinemia or NHL-type immunocytoma.
[h]Includes six previously untreated patients.
Abbreviations: CLL/PL = CLL with increased prolymphocytes; CR = complete response; PR = partial response.

significantly higher than that with CAP (60 versus 44%; $P = 0.023$), and this superior response rate was replicated among the subset of treatment-refractory patients (48 versus 27%; $P = 0.036$) (Figure 6). However, the median survival time of this latter group was virtually identical for those receiving fludarabine (728 days) and those receiving CAP (731 days) (Figure 5).

Patients with refractory or relapsed CLL may benefit from retreatment with fludarabine if they have previously demonstrated sensitivity to the drug. In a study that compared the effect of various salvage therapies in 203 refractory/relapsed CLL patients who had received prior fludarabine therapy, retreatment with single-agent fludarabine resulted in an overall response rate of 85% (complete response rate 22%) among the subset of patients ($n = 142$) who had previously been shown to be sensitive to the drug.[47] Multivariate analysis identified prior fludarabine sensitivity, hemoglobin level, platelet count and number of prior therapies as independent factors determining retreatment outcome.[47]

Safety of i.v. fludarabine in CLL

The most frequent adverse events associated with standard-dose i.v. fludarabine regimens are myelosuppression (neutropenia, thrombocytopenia and anemia) and infection (typically respiratory tract infections and fever). Myelosuppression is the major dose-limiting adverse effect: NCI grade IV hematological toxicity was reported in 43% of patients receiving fludarabine monotherapy for advanced-stage refractory CLL.[42] In large-scale randomized studies, granulocytopenia, thrombocytopenia and anemia (WHO grade III/IV) occurred in 19, 14 and 7% of fludarabine treatment cycles, respectively,[18] and affected 38 15 and 18% of patients, respectively, during the first six treatment cycles.[19] Severe (Grade 3 or 4) neutropenia tended to be more frequent with fludarabine than with chlorambucil (27 versus 19%).[20] Despite this myelosuppressive action, fludarabine does not increase the risk of secondary malignancies beyond that associated with CLL itself.[48]

Fludarabine-associated infection affects ~5% of patients with CLL,[19] is accompanied by a sustained fall in T-cell numbers,[27,43,49] and is exacerbated by coadministration of prednisone.[50] In advanced CLL, the incidence of infection with standard-dose i.v. fludarabine is no greater than that with CAP or CHOP,[18,19] although severe (Grade 3 or 4) infections appear to be more frequent with fludarabine than with chlorambucil (16 versus 9%).[20]

Other adverse events associated with i.v. fludarabine regimens include neurotoxicity (peripheral neuropathy, visual disturbances) and autoimmune hematological complications; however, any causal relationship to the drug is unclear, since many of these effects are features of CLL *per se* and are observed with other chemotherapies.[51] Neurotoxicity was reported in 16% of patients receiving standard-dose fludarabine for hematological malignancies (including CLL), an incidence similar to that seen with other purine analogs.[52] Neurological effects in clinical trials of fludarabine in CLL have generally proved mild and reversible.[27,37,39,49]

Fludarabine exposure appears to be linked to the development of autoimmune hemolytic anaemia (AIHA) in patients with CLL,[53–56] with most cases arising during the first three treatment cycles.[55] The incidence is variable, ranging from 2% to 23%,[19,53,56] with large-scale studies indicating an incidence towards the lower end of this range.[19] Although uncommon, this complication can be severe and fatal, particularly in patients re-exposed to fludarabine after a previous episode of AIHA.[55] Other hematologic complications such as transient pure red cell aplasia are possible,[57] although less frequent.

Nausea/vomiting and alopecia are infrequent sequelae of fludarabine therapy in CLL,[18,19] affecting $<1\%$ of patients during the first six treatment cycles.[19] Importantly, in large-scale randomized trials, nausea/vomiting and alopecia occurred considerably less frequently with fludarabine (4–5 and 2% of treatment cycles, respectively) than with CAP (25 and 65% of treatment cycles, respectively);[18] alopecia was also far less frequent with fludarabine than with CHOP (0 versus 16% incidence) during the first six treatment cycles.[19]

Standard-dose i.v. fludarabine can be safely administered to older patients with CLL, as well as to those with early-stage disease.[22,58] Surprisingly, fludarabine may confer less risk of adverse hematological effects and infection in older ($\geqslant$66 years) patients undergoing first-line therapy for CLL than in their younger ($\leqslant$65 years) counterparts.[22]

Cytoreductive chemotherapy followed by autologous hematopoietic stem cell transplantation has produced encouraging results in younger patients with CLL.[59,60] However, in common with other purine analogs, fludarabine may impair the mobilization and harvest of peripheral blood stem cells (PBSC) in CLL[61,62] and other lymphoid malignancies.[63–65] Successful autologous blood stem cell collection is nevertheless possible in fludarabine-pretreated CLL patients;[61,66,67] factors favoring a good stem cell yield include responsiveness to prior fludarabine chemotherapy[66] and delay of PBSC mobilization until 2 or more months after the last dose of fludarabine.[68]

Conclusions

In treatment-naïve patients with CLL, i.v. fludarabine produces superior response rates (overall and complete response rates) to established chemotherapy (eg, chlorambucil and CAP) and compares favorably with CHOP (similar overall response rate but higher complete response rate). The duration of response and time to disease progression achieved with fludarabine also exceeds that typically obtained with traditional chemotherapeutic regimens. For those patients who are either refractory to or have relapsed following treatment with established chemotherapy (eg, chlorambucil, CAP and CHOP), fludarabine can be considered the agent of choice for second-line therapy of CLL. Moreover, patients with relapsed CLL may benefit from fludarabine retreatment if they have previously demonstrated sensitivity to the drug.

Standard-dose i.v. fludarabine has an established safety profile and the adverse events noted with fludarabine are no more frequent or serious than those seen with traditional chemotherapeutic regimens for CLL. Fludarabine exhibits comparable tolerability to anthracycline-based regimens (CAP and CHOP) in terms of its myelosuppressive and immunosuppressive effects, and offers the advantage over these regimens of a markedly lower incidence of gastrointestinal effects (nausea/vomiting) and alopecia.

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Chemotherapy combination treatment regimens with fludarabine in chronic lymphocytic leukemia

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Fludarabine monotherapy is an established treatment for chronic lymphocytic leukemia (CLL), achieving superior remission rates compared with other treatment regimens containing alkylating agents or corticosteroids. However, CLL remains incurable and research continues into finding new treatments for this, the most common leukemia in the Western world. Recent research has focused on the use of fludarabine in combination with other chemotherapeutic agents. Studies published to date indicate that regimens containing fludarabine plus cyclophosphamide, with or without mitoxantrone, achieve overall response (OR) rates of 64–100% and complete response (CR) rates of up to 50%. Administration of cyclophosphamide at a lower dosage ($\leqslant$300 mg) appears to reduce the risk of myelosuppression without compromising efficacy. Combinations of fludarabine with prednisone or chlorambucil have been shown to be no more effective than fludarabine monotherapy (OR 27–79% with these combinations), while the combination of fludarabine plus cytarabine proved to be less effective than fludarabine monotherapy. Further studies are needed to evaluate the combinations of fludarabine plus doxorubicin and fludarabine plus epirubicin, as results to date have been inconclusive. More trials are also needed to examine a fludarabine, cytarabine, mitoxantrone and dexamethasone combination that has achieved a promising CR rate of 60% in the one trial reported thus far. Taken together, the results obtained so far with fludarabine plus cyclophosphamide suggest that this combination is more potent than fludarabine monotherapy and is able to increase the CR rate, the OR rate, event-free survival and progression-free survival in patients with CLL.
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Overview

Despite some progress in therapy, chronic lymphocytic leukemia (CLL), the most common leukemia in the Western world, remains incurable. Purine analogs, particularly fludarabine, are considered to be the best therapeutic option for patients with CLL. Fludarabine monotherapy produces superior overall response (OR) rates compared with other treatment regimens containing alkylating agents or corticosteroids.[1–3] As detailed in the next section, OR rates of 75–80%[2,4] and $>50\%$[5–8] have been reported in treatment-naïve and previously-treated patients, respectively. Complete response (CR) rates with fludarabine monotherapy are typically 15–20% in previously treated patients and 40–50% in treatment-naïve patients.[2,4–7] However, molecular CR rates are generally low and responses are transient, with all patients eventually experiencing a relapse within a median time of 27 months.[9] Moreover, although fludarabine monotherapy produces higher response rates and longer progression-free survival than other treatments, a trend for longer overall survival can be observed, but is not statistically significant.[3,8,9] Thus, the goal of therapy is to reduce relapse rates, and increase overall survival and the number of molecular CRs.

Efficacy may be increased if fludarabine is combined with other agents. Indeed, fludarabine has been shown to have a biochemical modulating effect on other chemotherapeutic agents *in vitro*, for example cyclophosphamide,[10,11] cytarabine,[12–16] cisplatin[17,18] and mitoxantrone.[10,19] In view of this synergistic/biochemical modulating effect, attempts to improve the CR and relapse rate with fludarabine have been explored with the use of fludarabine in combination with other chemotherapeutic agents.

The purpose of this article is to provide an overview of the efficacy and tolerability of a variety of treatment regimens containing fludarabine in combination with other chemotherapuetic agents in the treatment of CLL.

Efficacy and tolerability of fludarabine-containing combination regimens in CLL

The efficacy and tolerability of fludarabine in combination with other chemotherapeutic agents has been investigated in more than 20 trials to date. There are

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two dosage regimens for fludarabine in combination therapy that have been commonly used in clinical trials: fludarabine 30 mg/m^2 daily for three consecutive days or 10–25 mg/m^2 daily for five consecutive days, with the cycle repeated every 4 weeks for up to eight cycles. Response to treatment was assessed using the criteria recommended by the National Cancer Institute (NCI) Working Group.[20]

The most extensively studied combinations are fludarabine plus cyclophosphamide (10 studies) and fludarabine plus chlorambucil (four studies). Other agents that have been investigated with fludarabine are prednisone, epirubicin, mitoxantrone, cytarabine, cisplatin, doxorubicin, and cytarabine plus mitoxantrone plus dexamethasone.

Fludarabine plus cyclophosphamide

Fludarabine and cyclophosphamide is by far the best investigated fludarabine combination. It has been examined in 10 trials, including two trials with additional filgrastim support[21,22]and another two trials with mitoxantrone or granulocyte colony-stimulating factor (G-CSF) added to the regimen (Table 1).[29,30] The fludarabine/cyclophosphamide combination has been directly compared with fludarabine alone[27] and indirectly with dose-intensified chlorambucil,[28] providing strong evidence of improvements in efficacy. The same fludarabine regimen was used in all five trials of the basic combination of fludarabine plus cyclophosphamide; 30 mg/m^2 daily for three consecutive days every 4–6 weeks.[23–25,27,28] The fludarabine regimen was different in the studies utilizing filgrastim support and mitoxantrone plus G-CSF, where it was 20 mg/m^2 per day for five consecutive days and 25 mg/m^2 per day for three consecutive days, respectively.[29,30]

Noncomparative trials

In four noncomparative trials,[23–26] fludarabine plus cyclophosphamide produced OR rates of 75–88% in all patients except those completely refractory to both fludarabine and an alkylating agent (OR = 38%). In the first study, 93 patients with CLL were stratified into four groups according to pretreatment status, that is untreated, treated with alkylating agents, treated with and responsive to fludarabine with or without alkylating agents but relapsing, and treated with and refractory to fludarabine with or without alkylating agents.[23] Patients received intravenous (i.v.) fludarabine 30 mg/m^2/day for 3 days and decreasing doses of cyclophosphamide for 3 days, repeated every 4–6 weeks. The daily dose of cyclophosphamide was reduced due to myelosuppression, from 500 mg/m^2 for the first 12 patients, to 350 mg/m^2 for the next 27 patients and 300 mg/m^2 for the last 54 patients. A follow-up study including 128 patients using the same treatment regimen reported similar findings.[24] In this trial, an OR rate of 88% and a CR rate of 35% were recorded for previously untreated patients, compared with 85 and 15%, respectively, in patients previously treated with alkylating agents. In patients who were known to be refractory to fludarabine, an OR rate of 39% suggests that the combination of fludarabine with cyclophosphamide may be synergistic in this group. The median time to progression was 12–38 months in patients who had received prior therapy. In previously untreated patients, the median time to progression and survival duration had not been reached after a median follow-up of 41 months.

Similar results were seen in a phase II trial by the German CLL Study Group, who treated 36 CLL patients with fludarabine 30 mg/m^2/day and cyclophosphamide 250 mg/m^2/day for 3 days every 28 days for a maximum of six cycles (Table 1).[25] In all, 21 patients had received between one and three different treatment regimens prior to the study, while the remaining 15 patients had no prior treatment. In the 32 patients evaluable for response, the OR was 91%, including partial response (PR) 75% and CR 16%. There was no significant difference in response rate between untreated and pretreated patients with an OR of 86% and 94%, respectively (Figure 1).

Another study evaluated the combination of oral fludarabine (30 mg/m^2 days 1–5) plus oral cyclophosphamide (200 mg/m^2 days 1–5) every 28 days for six courses in treatment-naïve patients with CLL ($n = 75$). In a preliminary analysis of data from this study, OR and CR rates of 75 and 49%, respectively, were reported.[26]

Comparative trials

Fludarabine plus cyclophosphamide versus fludarabine. In a phase III trial of 207 treatment-naïve patients with advanced CLL, who were available for response, both fludarabine alone and fludarabine plus cyclophosphamide were effective.[27] In total, 190 patients were randomized to receive IV fludarabine 25 mg/m^2/day, and 185 patients were randomized to receive IV fludarabine 30 mg/m^2/day plus cyclophosphamide 250 mg/m^2/day, on days 1–3 every 28 days. Of 207 patients evaluable for response, patients treated with fludarabine plus cyclophosphamide ($n = 102$) had significantly higher OR (94 versus 85% with fludarabine alone; $P = 0.041$) and CR (21 versus 9%, respectively; $P = 0.009$). After a median observation time of 11.7 months, the median progression-free survival was 18.6 months in patients treated with fludarabine alone and has not yet been reached in patients treated with fludarabine plus cyclophosphamide (more than 28 months; $P = 0.0062$).

Among the studies described here, the most common adverse events associated with the combination of fludarabine plus cyclophosphamide were myelosuppression (neutropenia, thrombocytopenia and anemia) (Table 2). Although the incidence of myelosuppression was high (up to 69%), the rate of infection, especially severe infection, was always comparatively low (less than 25%). This pattern was particularly apparent in the

Table 1 Efficacy of fludarabine plus cyclophosphamide combination regimens in patients with CLL

Reference (study design)	*No. of evaluable patients*	*Histology*	*Prior therapy*	*Treatment regimen*	*Clinical response*		*Survival/duration of response*
					CR(%)	CR + PR(%)	
Fludarabine + cyclophosphamide							
O'Brien (1998)[23]	93	—	Yes (some)	FLU 30 mg/m^2 × d1–3 CYC 300–500 mg/m^2 × d1–3	—	69	n/a
O'Brien *et al.* (2001)[24]	128	Rai III/IV 47%	Yes (some)	FLU 30 mg/m^2 × d1–3 q4–6wk CYC 300–500 mg/m^2 × d1–3 q4–6wk	17 (3–35)	74 (39–88)	Median survival 12–38 mo 12 mo for FLU refractory 21 mo for prior FLU/ALK 38 mo for prior ALK
Hallek *et al.* (2001)[25] (Phase II)	32	Binet A 3% Binet B 50% Binet C 47%	Yes (some)	FLU 30 mg/m^2 × d1–3 q4wk CYC 250 mg/m^2 × d1–3 q4wk	16	91	Median DoR had not been reached after 14.4 mo of follow-up
Cazin *et al.* (2002)[26] (Phase II)	75	Binet B 79% Binet C 21%	No	FLU 30 mg/m^2 × d1–5 q4wk CYC 200 mg/m^2 × d1–5 q4wk[a]	49	75	n/a
Eichhorst *et al.* (2003)[27] (Phase III; COMP, RAND)	209	Binet A, B or C	No	FLU 30 mg/m^2 × d1–3 q4wk CYC 250 mg/m^2 × d1–3 q4wk	21	94	Median PFS had not been reached (>28 mo) after median 11.7 mo follow-up
Hallek *et al.* (1999)[28] (Phase II; COMP)	25	Binet B or C	Yes (some)	FLU 30 mg/m^2 × d1–3 q4wk CYC 250 mg/m^2 × d1–3 q4wk	12	88	n/a
Fludarabine + cyclophosphamide + GM-CSF/G-CSF/filgrastim							
Flinn *et al.* (2001)[22] (Phase II)	36	—	No	FLU 20 mg/m^2 × d1–5 q4wk CYC 600 mg/m^2 × d1 q4wk G-CSF 5 μg/kg × d8–18/22 q4wk	42	64	1 year DFS 92.9% Median DoR 27 mo
Flinn *et al.* (2000)[21] (Phase II)	17	Rai I 29% Rai II 35% Rai III 18% Rai IV 18%	No	FLU 20 mg/m^2 × d1–5 q4wk CYC 600 mg/m^2 × d1 q4wk G-CSF 5 μg/kg × d8–18/22 q4wk	47	100	n/a
Fludarabine + cyclophosphamide + mitoxantrone ± G-CSF							
Bosch *et al.* (2002)[29]	60	Binet A 7% Binet B 58% Binet C 35%	Yes	FLU 25 mg/m^2 × d1G–3 q4wk CYC 600 mg/m^2 × d1 or 200 mg/m^2 × d1–3 q4wk MIT 6–8 mg/m^2 × d1 q4wk	50	78	Median DoR 19 mo (responders)
Schmitt *et al.* (2002)[30] (Phase III)	27	Binet B or C	Yes (some)	FLU 25 mg/m^2 × d1G–3 q4wk CYC 200 mg/m^2 × d1–3 q4wk MIT 8 mg/m^2 × d1 q4wk ±G-CSF 5 μg/kg × d6 q4wk	4	78	Median RFS 18 mo

[a]Fludarabine and cyclophosphamide were administered orally.

Abbreviations: CHL = chlorambucil; COMP = comparative; CYC = cyclophosphamide; DFS = disease-free survival; DoR = duration of response; FLU = fludarabine; MIT = mitoxantrone; PFS = progression-free survival; RFS = relapse-free survival; d = days; wk = weeks; mo = months; RAND = randomised

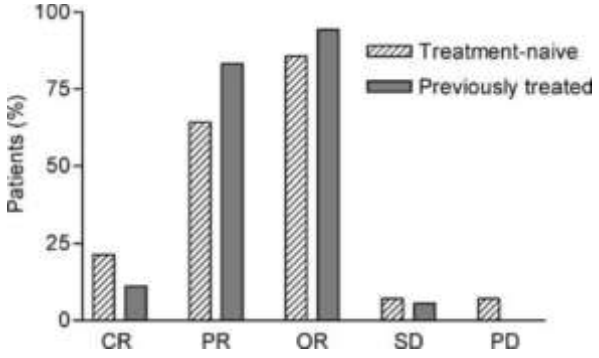

Figure 1 Response to treatment with fludarabine 30 mg/m^2/day and cyclophosphamide 250 mg/m^2/day for 3 days every 28 days for a maximum of 6 cycles in 32 patients evaluable for response. CR = complete response; PR = partial response; OR = overall response; SD = stable disease; PD = progressive disease.[25]

German CLL Study where 69% of patients had severe neutropenia, but only 17% of patients reported minor infections, and no severe infections were recorded.[25] The impact of cyclophosphamide dose on the incidence of myelosuppression was highlighted by O'Brien *et al*.[24] The cyclophosphamide dose needed to be reduced in 60–63% of patients treated with the 350 or 500 mg/m^2 dose, but only 29% of patients receiving 300 mg/m^2 required a dose adjustment. Interestingly, in the one study that compared fludarabine plus cyclophosphamide with fludarabine alone, the incidence of thrombocytopenia was significantly lower in the fludarabine monotherapy group (14.2 versus 23.6% of all courses; $P<0.0001$).[29]

Fludarabine plus cyclophosphamide with filgrastim support

Two phase II studies have investigated the combination of fludarabine plus cyclophosphamide plus filgrastim support.[21,22] In both these studies, previously untreated CLL patients received i.v. fludarabine 20 mg/m^2/day for 5 days and cyclophosphamide 600 mg/m^2/day on day 1, followed by filgrastim 5 μg/kg for 10–14 days starting around day 8. Treatment was repeated every 28 days for a maximum of six cycles (Table 1). In the smaller of the two studies, 17 patients with previously untreated CLL were evaluated. The OR was 100%, including 47% CR and 53% PR (Table 1).[21] In the second study, the response rate was evaluable in 36 treatment–naïve patients. The OR was 64%, with a 42% CR and a 22% PR. The 1–year disease-free survival was 93%. The duration of response for responding patients was 27 months.[22] An interesting finding in one of these studies that requires further investigation is the reduced incidence of leukocytopenia, and the increased incidence of thrombocytopenia and anemia in patients receiving G-CSF in addition to the fludarabine plus cyclophosphamide combination.[21]

Fludarabine plus cyclophosphamide and mitoxantrone

The combination of fludarabine with cyclophosphamide and mitoxantrone has been assessed in one clinical trial to date,[29] in which two slightly different treatment regimens were used (Table 1). In all, 23 patients received i.v. fludarabine 25 mg/m^2/day on days 1–3, cyclophosphamide 600 mg/m^2 on day 1 and mitoxantrone 8 mg/m^2 on day 1, at 4-week intervals for up to six courses. A further 37 patients received the same fludarabine regimen plus cyclophosphamide 200 mg/m^2/day on days 1–3 and mitoxantrone 6 mg/m^2 on day 1. The OR was 78%, including 50% CR and 28% PR. Negative minimal residual disease was detected in 17% of patients by cytofluorometric and molecular methods. The median duration of response was 19 months and the actuarial median survival duration was 41 months.

The incidences of myelosuppression and infection were noticeably higher in this study and in another study (described below) where mitoxantrone with or without G-CSF support was added to the fludarabine/cyclophosphamide regimen.[29,30] Myelosuppression (neutropenia or leukocytopenia) was recorded in 90–100% of patients in these two studies, with corresponding infection rates of 23 and 35% (Table 2).

Fludarabine plus cyclophosphamide and mitoxantrone ± G-CSF support

As G-CSF was shown to reduce the rate of infections in fludarabine-treated patients in a previous study,[43] the German CLL Study Group initiated a phase III trial evaluating the efficacy of fludarabine plus cyclophosphamide and mitoxantrone with or without G-CSF support.[30] Patients with relapsed CLL were randomized to fludarabine 25 mg/m^2/day on days 1–3, cyclophosphamide 200 mg/m^2/day on days 1–3 and mitoxantrone 8 mg/m^2 on day 1, every 28 days with ($n=31$) or without ($n=32$) G-CSF 5 μg/kg on day 6. Among 27 evaluable patients, the OR was 78% (CR 4% and PR 74%). The median relapse-free survival duration was 18.2 months.

Therefore, considered collectively, data from the 10 studies examining the combination of fludarabine plus cyclophosphamide with or without mitoxantrone or filgrastim indicate that this combination provides a potentially useful alternative treatment option for both untreated and previously treated patients with CLL, achieving OR rates of 64–100% and CR rates of up to 50%. Notably, the combination of fludarabine with cyclophosphamide, with or without filgrastim support, was generally well tolerated, particularly when cyclophosphamide dosages of $\leqslant 300$ mg/m^2 were used. However, the incidences of myelosuppression and infection were higher when mitoxantrone was added to the fludarabine/cyclophosphamide regimen (Table 2).

Table 2 Tolerability of combination regimens comprising fludarabine plus other chemotherapeutic agents in patients with CLL

Reference (study design)	*No. of evaluable patients*	*Treatment regimen*	*Myelosuppression/infection*	*Other events*
Fludarabine + cyclophosphamide				
O'Brien (1998)[23]	93	FLU 30 mg/m^2 × d1–3 CYC 300–500 mg/m^2 × d1–3	CYC dose was reduced due to high rate of myelosuppression	
O'Brien *et al.* (2001)[24]	128	FLU 30 mg/m^2 × d1–3 q4-6wk CYC 300–500 mg/m^2 × d1–3 q4-6wk	Neutropenia: 48% Sepsis or pneumonia: 25%	Fever: 25%
Hallek *et al.* (2001)[25] (Phase II)	32	FLU 30 mg/m^2 × d1–3 q4wk CYC 250 mg/m^2 × d1–3 q4wk	Severe neutropenia: 69% Minor infection: 17% Anemia: 17% Thrombocytopenia: 17%	
Eichhorst *et al.* (2003)[27] (Phase III, COMP, RAND)	102	FLU 30 mg/m^2 × d1–3 q4wk CYC 250 mg/m^2 × d1–3 q4wk	Myelosuppression: 33% Thrombocytopenia: 58%	
Hallek *et al.* (1999)[28] (Phase II, COMP)	25	FLU 30 mg/m^2 × d1–3 q4wk CYC 250 mg/m^2 × d1–3 q4wk	Myelotoxicity: 22% Thrombocytopenia: 6%	
Fludarabine + cyclophosphamide + GM-CSF/G-CSF/filgrastim				
Flinn *et al.* (2001)[22] (Phase II)	36	FLU 20 mg/m^2 × d1–5 q4wk CYC 600 mg/m^2 × d1 q4wk G-CSF 5 μg/kg × d8–18/22 q4wk	Severe infections: 13.9% Anemia: 25% Thrombocytopenia: 23%	
Flinn *et al.* (2000)[21] (Phase II)	17	FLU 20 mg/m^2 × d1–5 q4wk CYC 600 mg/m^2 × d1 q4wk G-CSF 5 μg/kg × d8–18/22 q4wk	Thrombocytopenia: 17%	
Fludarabine + cyclophosphamide + mitoxantrone ± G-CSF				
Bosch *et al.* (2002)[29]	60	FLU 25 mg/m^2 × d1–3 q4wk CYC 600 mg/m^2 × d1 or 200 mg/m^2 × d1–3 q4wk MIT 6–8 mg/m^2 × d1 q4wk	Infections: 23% Neutropenia: 90% Thrombocytopenia: 56% Anemia: 53%	Nausea/vomiting: 33%
Schmitt *et al.* (2002)[30] (Phase III)	27	FLU 25 mg/m^2 × d1–3 q4wk CYC 200 mg/m^2 × d1–3 q4wk MIT 8 mg/m^2 × d1 q4wk ± G-CSF 5 μg/kg × d6 q4wk	Leukocytopenia: 100% Infections: 35% Severe infections: 4%	
Fludarabine + prednisone				
O'Brien *et al.* (1993)[32]	264	FLU 30 mg/m^2 × d1–5 q4wk PRE 30 mg/m^2 × d1–5 q4wk	Minor infections: 12% Severe infections: 3–13% (correlated with Rai stage)	
Keating *et al.* (1998)[1] (Phase I/II)	103	FLU 30 mg/m^2 × d1–5 q4wk PRE 30 mg/m^2 × d1–5 q4wk	Infections: 69%	
Fludarabine + chlorambucil				
Weiss *et al.* (1994)[33] (Phase I/II)	15	FLU 10–20 mg/m^2 × d1–5 q4wk CHL 20 mg/m^2 × d1 + 15 q4wk	Infections: 53% Severe thrombocytopenia: 73% Anemia: 87%	
Elias *et al.* (1993)[34] (Phase I)	17	FLU 10–20 mg/m^2 × d1–5 q4wk CHL 15–20 mg/m^2 × d1 q4wk	Infections: 100% Thrombocytopenia: 100%(led to reduction of CHL dose)	
Rai *et al.* (2000)[3] (RAND, COMP)	509	FLU 20 mg/m^2 × d1–5 q4wk CHL 20 mg/m^2 × d1 q4wk	Infections: 28% Neutropenia: 43% Thrombocytopenia: 43%	
Morrison *et al.* (2001)[35] (RAND, COMP)	518	FLU 20 mg/m^2 × d1–5 q4wk CHL 20 mg/m^2 × d1 q4wk	Major infections: 45% Neutropenia: 19%	
Fludarabine + other chemotherapeutic agents				
Rummel *et al.* (1999)[36] (Phase II)	38	FLU 25 mg/m^2 d1–5 EPI 25 mg/m^2 d 4–5	Severe granulocytopenia: 40% Severe thrombocytopenia: 6% Infections: 24% Anemia: 41%	

Table 2 (*continued*)

Reference (study design)	*No. of evaluable patients*	*Treatment regimen*	*Myelosuppression/infection*	*Other events*
Rummel *et al.* (2002)[37] (Phase III, RAND, COMP)	117	FLU 25 mg/m^2 d1–5 q4wk $\pm$EPI 25 mg/m^2 d4–5 q4wk	n/a	n/a
O'Brien *et al.* (1996)[38]	88	FLU 30 mg/m^2 d1–3 MIT 10 mg/m^2 d1	Sepsis/pneumonia: 13%	Nausea/vomiting: 33% Fatigue/weakness: 17%
Gandhi *et al.* (1994)[39]	21	FLU 30 mg/m^2 d1 q4wk CYT 500–1000 mg/m^2 d1 q4wk	Severe neutropenia: 73% Infections: 38% Severe thrombocytopenia: 43%	
Giles *et al.* (1999)[40] (Phase II)	41	FLU 30 mg/m^2 d4 CIS 25 mg/m^2 d1–4 with or without CYT 500 mg/m^2 d4	Severe myelosuppression: 63% Severe neutropenia: 55% Severe thrombocytopenia: 76% Minor infections: 12%	
Robertson *et al.* (1995)[41]	29	FLU 30 mg/m^2 d1–3 or 25–30 mg/m^2 d1–4 q4wk DOX 50 mg/m^2 d1 q4wk $\pm$PRE 30 mg/m^2 d1–5 q4wk	Severe neutropenia: 56% Severe infections: 13% Minor infections: 17% Thrombocytopenia: 32%	
Mauro *et al.* (2002)[42]	31	FLU 25 mg/m^2 d1–3 q4wk CYT 1000 mg/m^2 d1or d1–2 q4wk MIT 10 mg/m^2 d1 q4wk DEX 20 mg d1–3 q4wk	Severe granulocytopenia: 69% Major infections: 12%	

Abbreviations: COMP = comparative; CYC = cyclophosphamide; CHL = chlorambucil; CIS = cisplatin; CYT = cytarabine; DEX = dexamethasone; DOX = doxorubicin; EPI = epirubicin; FLU = fludarabine; MIT = mitoxantrone; PRE = prednisone; RAND = randomized; d = days; wk = weeks; mo = months.

Fludarabine plus prednisone

Two studies involving more than 300 patients with CLL have evaluated the efficacy of fludarabine plus prednisone (Table 3).[1,31] An identical treatment regimen was used in both studies with patients receiving fludarabine 30 mg/m^2 daily for 5 days and prednisone 30 mg/m^2 daily for 5 days, every 4 weeks.

In the first study of 264 patients with CLL, fludarabine plus prednisone achieved an OR rate of 79% as first-line therapy and 52% as second-line.[32] These results were comparable to those reported for a historical cohort of patients treated with fludarabine alone, in which the OR was 80% first-line and 58% second-line (Table 3). The median time to progression in responding patients was 22 months in the fludarabine plus prednisone recipients. The survival curves for fludarabine plus prednisone and fludarabine alone, paralleled each other during 2 years of follow-up.

Using data from two different studies, Keating *et al.*[1] reported similar OR rates for fludarabine plus prednisone (77%) and fludarabine alone (80%) as first-line treatment in 103 patients with CLL. However, the CR rate for fludarabine plus prednisone (23%) was significantly lower than for fludarabine alone (38%; $P = 0.04$). No differences in overall survival were seen. The results from these studies therefore indicate that fludarabine plus prednisone is no more effective than fludarabine alone for the first- and second-line treatment of patients with CLL (Table 3).

Information on the tolerability of fludarabine plus prednisone is limited (Table 2). However, it appears that the combination is associated with a similar prevalence of infection as fludarabine plus cyclophosphamide (25–69%). O'Brien *et al.*[31] and Anaissie *et al.*[43] found that the rate of severe infections was correlated with the Rai stage of disease, previous therapies and serum creatinine level.

Fludarabine plus chlorambucil

Fludarabine plus chlorambucil was evaluated in four clinical trials, comprising two small phase I/II trials and two large, randomized, comparative phase III trials (Table 3).[3,32–34] The treatment regimens employed in the four studies were similar. Fludarabine was administered at a dose of 10–20 mg/m^2 per day for five consecutive days, and the chlorambucil regimen was 15–20 mg/m^2 daily on day 1 of each cycle. The smaller of the two phase III studies randomized 509 previously untreated patients with CLL to treatment with chlorambucil, fludarabine or fludarabine plus chlorambucil. The OR was 61% for fludarabine plus chlorambucil, 63% with fludarabine alone, and 37% for chlorambucil alone. The overall survival was 55, 66 and 56 months, respectively.[3] The other phase III study did not report efficacy results,[35] and focused on infection rates which will be discussed later in this section. Collectively, the results from these four studies suggest that the combination of fludarabine plus chlorambucil does not produce a

Table 3 Efficacy of fludarabine plus prednisone and fludarabine plus chlorambucil combination regimens in patients with CLL

Reference (study design)	*No. of evaluable patients*	*Histology*	*Prior therapy*	*Treatment regimen*	*Clinical response*		*Survival/duration of response*
					CR(%)	CR + PR(%)	
Fludarabine + prednisone							
O'Brien *et al.* (1993)[32]	264	Rai I/II 53% Rai III/IV 47%	Yes (some)	FLU 30 mg/m^2 × d1–5 q4wk PRE 30 mg/m^2 × d1–5 q4wk	63% 1st line 37% 2nd line	79% 1st line 52% 2nd line	Median TTP 22–30 mo
Keating *et al.* (1998)[1] (Phase I/II)	103	Rai I/II 62% Rai III/IV 38%	No	FLU 30 mg/m^2 × d1–5 q4wk PRE 30 mg/m^2 × d1–5 q4wk	23	77	Median TTP 31 mo
Fludarabine + chlorambucil							
Weiss *et al.* (1994)[33] (Phase I/II)	15	Rai II 20% Rai III 13% Rai IV 67%	Yes	FLU 10–20 mg/m^2 × d1–5 q4wk CHL 20 mg/m^2 × d1 + 15 q4wk	6	27	Median TTP 4–30 mo
Elias *et al.* (1993)[34] (Phase I)	17	—	Yes	FLU 10–20 mg/m^2 × d1–5 q4wk CHL 15–20 mg/m^2 × d1 q4wk	6	53	n/a
Rai *et al.* (2000)[3] (RAND, COMP)	509	Rai I/II 61% Rai III/IV 39%	No	FLU 20 mg/m^2 × d1–5 q4wk CHL 20 mg/m^2 × d1 q4wk	20	61	n/a
Morrison *et al.* (2001)[35] (RAND, COMP)	518	Rai I/II 62% Rai III/IV 38%	No	FLU 20 mg/m^2 × d1–5 q4wk CHL 20 mg/m^2 × d1 q4wk	n/a	n/a	1-year OS 84%

Abbreviations: CHL = chlorambucil; COMP = comparative; FLU = fludarabine; OS = overall survival; PRE = prednisone; RAND = randomized; TTP = time to progression; d = days; wk = weeks; mo = months.

significant improvement in response rate or survival compared with fludarabine alone.

Furthermore, treatment with fludarabine plus chlorambucil appeared to be associated with a higher incidence of adverse events compared with either fludarabine or chlorambucil alone, in the majority of studies reviewed (Table 2). Grade 3–4 thrombocytopenia, neutropenia and infection were significantly more common with fludarabine plus chlorambucil than fludarabine alone in the study of 509 previously untreated patients, and as a consequence the combination therapy arm was discontinued early.[3] Moreover, significantly more infections were reported with fludarabine plus chlorambucil than either agent alone ($P = 0.008$).[34] In the two phase I/II trials, chlorambucil 15–20 mg/m^2 reduced the maximum tolerated dose of fludarabine to 15–20 mg/m^2.[32,33]

Fludarabine plus other chemotherapeutic agents

Seven trials have investigated fludarabine in combination with six other chemotherapeutic agents. The majority of these studies have been small, with fewer than 50 evaluable patients, and all but one of the combinations have only been investigated in a single study. Details are summarized in Tables 2 and 4, with the key outcomes highlighted below.

O'Brien *et al.*[37] found that the addition of mitoxantrone (10 mg/m^2 on day 1) to fludarabine (30 mg/m^2 on days 1–3) did not markedly increase the response rate to fludarabine in 88 patients with CLL. In previously untreated patients ($n = 35$), the OR was 77% and CR was 20%, while in previously treated patients the response rates were 40 and 9%, respectively.

Fludarabine plus doxorubicin (with or without prednisone) was administered to 30 patients with CLL in a study by Robertson *et al.*[40] Fludarabine was administered as 30 mg/m^2 per day for three consecutive days to 10 patients, 25 mg/m^2 day for 4 days to three patients, and to 17 patients as 30 mg/m^2 per day for 4 days. A 50 mg/m^2 dose of doxorubicin was given to all patients. The first 17 patients also received prednisone 30 mg/m^2 for 5 days, but this was discontinued when data became available, demonstrating no therapeutic benefit and increased rates of opportunistic infections with coadministration of corticosteroids with fludarabine. For the 29 patients included in the response analysis, the OR was 55%; however, the CR was only 3%.

A higher response rate was achieved in a phase II study of 25 previously untreated patients and 13 patients in first relapse treated with fludarabine (25 mg/m^2 for 5 days) plus epirubicin (25 mg/m^2 for 2 days).[35] Previously untreated patients had an OR of 92% and a CR of 40%, whereas the pretreated patients had an OR of 62% and a CR of 15%. Hematologic toxicity was relatively mild (40% severe granulocytopenia, 6% severe thrombocytopenia) in this study and no grade 4 infections were noted. In a phase III, randomized trial, fludarabine was compared with fludarabine plus epirubicin in untreated

Table 4 Efficacy of combination regimens comprising fludarabine plus other chemotherapeutic agents in patients with CLL

Reference (study design)	*No. of evaluable patients*	*Histology*	*Prior therapy*	*Treatment regimen*	*Clinical response*		*Survival/duration of response*
					CR(%)	CR + PR(%)	
Fludarabine + epirubicin							
Rummel *et al.* (1999)[36] (Phase II)	38	Binet B 53% Binet C 47%	Yes (some)	FLU 25 mg/m^2 d1–5 EPI 25 mg/m^2 d 4–5	32	82	Median survival 33 mo
Rummel *et al.* (2002)[37] (Phase III, randomised)	117	—	Yes (some)	FLU 25 mg/m^2 d1–5 q4wk ± EPI 25 mg/m^2 d4–5 q4wk	—	89	n/a
Fludarabine + mitoxantrone							
O'Brien *et al.* (1996)[38]	88	Rai III/IV 43%	Yes (some)	FLU 30 mg/m^2 d1–3 MIT 10 mg/m^2 d1	20% 1st line 7% 2nd line	77% 1st line 60% 2nd line	n/a
Fludarabine + cytarabine							
Gandhi *et al.* (1994)[39]	21	Rai III 33% Rai IV 67%	Yes	FLU 30 mg/m^2 d1 q4wk CYT 500–1000 mg/m^2 d1 q4wk	0	5	Median survival 9 mo
Fludarabine + cisplatin ± cytarabine							
Giles *et al.* (1999)[40] (Phase II)	41	Rai I/II 24% Rai III/IV 76%	Yes	FLU 30 mg/m^2 d4 CIS 25 mg/m^2 d1–4 with or without CYT 500 mg/m^2 d4	0	19	Median survival 6 mo
Fludarabine + doxorubicin ± prednisone							
Robertson *et al.* (1995)[41]	29	Rai O 7% Rai I/II 63% Rai III/IV 30%	Yes (some)	FLU 30 mg/m^2 d1–3 or 25–30 mg/m^2 d1–4 q4wk DOX 50 mg/m^2 d1 q4wk ± PRE 30 mg/m^2 d1–5 q4wk	3	55	Median survival time had not been reached at 19 mo follow-up
Fludarabine + cytarabine + mitoxantrone + dexamethasone							
Mauro *et al.* (2002)[42]	31	Active disease Rai II 39% Rai III/IV 35% Partial remission 26%	Yes	FLU 25 mg/m^2 d1–3 q4wk CYT 1000 mg/m^2 d1or d1–2 q4wk MIT 10 mg/m^2 d1 q4wk DEX 20 mg d1–3 q4wk	60	70	5.5-year survival 68% (responders)

Abbreviation: CIS = cisplatin; CYT = cytarabine; DEX = dexamethasone; DOX = doxorubicin; EPI = epirubicin; FLU = fludarabine; MIT = mitoxantrone; PRE = prednisone; d = days; wk = weeks; mo = months.

patients and patients in first relapse.[37] Preliminary results for 117 patients showed that the combination was superior to fludarabine alone, with an OR of 89% versus 72%. In previously untreated patients, the OR was 85% and CR 26%. In previously treated patients, the OR was 72% and CR 8%.

There are three other fludarabine combination regimens which have proven to be less effective than monotherapy with fludarabine. In 21 fludarabine-refractory patients, fludarabine plus cytarabine yielded only 1 PR (OR 5%).[38] The addition of cisplatin to this regimen improved the OR slightly to 19% in a phase II study of 41 patients.[39] Notably, the combination of fludarabine with cytarabine[38] or cisplatin[39] was associated with particularly high toxicities. Almost three-quarters of the patients treated with fludarabine plus cytarabine developed severe neutropenia, 38% had infections and 43% met the criteria for severe thrombocytopenia.[38] Severe myelosuppression was recorded for 63% of patients receiving fludarabine plus cisplatin, and 76% had severe neutropenia.[39]

Better results were achieved with the combination of fludarabine, cytarabine, mitoxantrone and dexamethasone in 31 previously treated patients (OR 70%, CR 60%), but further studies are required to confirm these findings.[41] However, severe granulocytopenia occurred in 69% of courses, although major infections were seen in only 12% of these courses. This low incidence is probably explained by the infection prophylaxis with fluconazole, acyclovir, trimethoprim/sulfamethazole and G-CSF.

Conclusions

The data reviewed here indicate that fludarabine administered in combination with other chemotherapeutic agents may produce higher response rates, including CRs and molecular CRs, compared with fludarabine monotherapy or other treatment regimens in both treatment-naïve and previously treated patients with CLL. In particular, the combination of fludarabine with cyclophosphamide (with or without filgrastim) has demonstrated the most promise. OR rates of 69–91% were achieved in CLL patients treated with fludarabine plus cyclophosphamide.[23–25,28] Studies using a lower cyclophosphamide dosage (up to 300 mg/m^2) were associated with a reduced incidence of severe myelosuppression, suggesting that this combination may be better tolerated.[23,25] When the fludarabine plus cyclophosphamide combination was administered with filgrastim support, OR rates of 64 and 100% were achieved in treatment-naïve patients.[21,22] Further studies, particularly in treatment-experienced patients, are required to determine if this combination is more effective than fludarabine plus cyclophosphamide.

It remains to be determined whether the combination of fludarabine plus epirubicin is more efficacious than fludarabine plus cyclophosphamide, as study results to date have been inconclusive. A potential disadvantage of fludarabine plus epirubicin is that the accumulation of anthracycline in relapsed patients must be considered.[44] Phase III trials comparing the two different regimens are needed to determine which is best.

However, not all combination therapies that include fludarabine appear to be more useful than fludarabine alone. There was no difference in OR rates or overall survival in patients treated with fludarabine alone compared with those treated with a combination of fludarabine plus prednisone.[1,31] Indeed, the CR rate for fludarabine plus prednisone was significantly lower than that seen with fludarabine alone in a study involving treatment-naïve patients.[1] Fludarabine in combination with chlorambucil also appears to offer no advantage over fludarabine alone in patients with CLL, and was also associated with an increased incidence of adverse events.[3,32,33]

More recently, promising results were obtained in preliminary trials of combination regimens comprising fludarabine, cyclophosphamide and mitoxantrone,[29] and fludarabine, cytarabine, mitoxantrone and dexamethasone.[41] The combination of fludarabine, cyclophosphamide and mitoxantrone produced an OR rate of 78% (including 50% CR) in resistant or relapsed CLL,[29] while the fludarabine, cytarabine, mitoxantrone and dexamethasone combination had an OR of 70% and a CR of 60% in treatment-experienced patients.[41] Further trials are needed to establish the optimal combinations of these regimens, and to investigate their effectiveness as initial treatment for CLL.

In conclusion, the administration of fludarabine with other chemotherapeutic agents has the potential to increase OR rates in both the first- and second-line treatment of CLL and is well tolerated. Further comparative studies are needed to confirm if such combinations will also increase CR rates and survival time beyond that which can be achieved with fludarabine alone.

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Oral fludarabine therapy in chronic lymphocytic leukemia - increased convenience

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Intravenous fludarabine is a well-established therapy for the first-line treatment of chronic lymphocytic leukemia and the standard of care for second-line treatment. More recently, an oral formulation of fludarabine has been developed, with equivalent efficacy and tolerability to the intravenous formulation, but with improved convenience of administration and potentially greater cost effectiveness. In previously treated patients receiving oral fludarabine monotherapy, overall response rates of 46–51% were achieved, depending on the response criteria used. Oral fludarabine is also an effective first-line treatment, both as monotherapy (overall response 72–80%) and in combination with cyclophosphamide (overall response 80%). Infusion-related adverse effects are eliminated with oral administration. Importantly, WHO performance status is maintained or improved in more than 50% of patients. As oral fludarabine can be taken at home, administration costs are greatly reduced due to fewer physician and nursing interventions and less time spent in hospital. Oral fludarabine was approved first in the UK as second-line therapy for chronic lymphocytic leukemia and, based on its ease of administration and potentially greater cost effectiveness, is recommended in preference to the intravenous formulation by the UK National Institute for Clinical Excellence. The oral formulation is also now available in the majority of European countries. Therefore, with equivalent efficacy and tolerability to the intravenous preparation, oral fludarabine gives the hematologist an important new option in the management of chronic lymphocytic leukemia.
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Overview of oral fludarabine

Chronic lymphocytic leukemia (CLL) is the most common hematological malignancy in the Western world, with an incidence of more than 20 new cases per 100 000 inhabitants per year after the age of 60.[1,2] Standard therapy for this disease consists of chlorambucil, anthracycline-containing regimens such as CAP (cyclophosphamide, doxorubicin and prednisone) or CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone), and the purine analog fludarabine (Figure 1). Intravenous (IV) fludarabine is indicated for the treatment of patients with B-cell CLL who have not responded to, or whose disease has progressed during or after, treatment with at least one standard alkylating agent-containing regimen. It is also indicated for the first-line treatment of patients with advanced disease. Indeed, several clinical trials have confirmed the effectiveness of IV fludarabine as a second-line agent for CLL, with objective response rates of 32% achieved with monotherapy and 52–85% achieved in combination with prednisone or cyclophosphamide.[3–5] IV fludarabine has also been compared with CAP in treatment-naïve and previously treated CLL patients.[1] Overall, significantly more patients responded to fludarabine than to CAP (60 versus 44%; $P = 0.023$), with a similar trend seen in the subgroup of previously treated patients (48 versus 27%; $P = 0.036$). IV fludarabine has also been compared with chlorambucil in treatment-naïve patients, with fludarabine therapy resulting in higher response rates (63 versus 37%; $P < 0.001$) and longer progression-free survival (20 versus 14 months; $P < 0.001$).[6]

While IV fludarabine is extremely effective, such treatment requires frequent outpatient visits or hospital admission, as a typical treatment cycle is infused over 30 min once daily for 5 days every 4 weeks. Thus, an oral formulation would be more convenient for healthcare workers and patients, the majority of whom are elderly, often with poor venous access. Furthermore, due to potentially lower administration costs, oral fludarabine may be more cost effective than IV therapy.

An oral formulation of fludarabine has been developed, comprising 10 mg fludarabine in an immediate-release tablet. Oral fludarabine is indicated as second-line therapy in patients who have not responded to, or whose disease has progressed during or after treatment with, at least one standard alkylating agent-containing regimen. Oral fludarabine is typically given at a dosage

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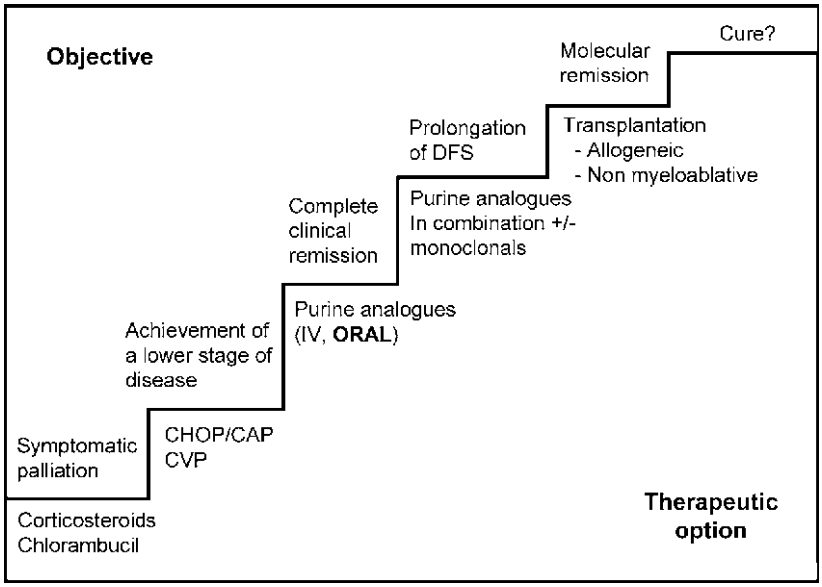

Figure 1 Therapy objectives in chronic lymphocytic leukemia. DFS, disease-free survival; CAP, cyclophosphamide, doxorubicin and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; CVP, cyclophosphamide, doxorubicin, vincristine and prednisone; IV, intravenous.

of 40 mg/m^2 (7–8 tablets) once daily for 5 days, repeated every 4 weeks for up to six cycles.[7] In September 2001, the UK National Institute for Clinical Excellence (NICE) endorsed the second-line use of oral fludarabine for the treatment of CLL.[8] NICE recommends oral fludarabine in preference to IV fludarabine on the basis of more favorable cost effectiveness and states that IV fludarabine should only be used when oral fludarabine is contraindicated.

Pharmacokinetics

Pharmacokinetic studies in patients with cancer confirm that single doses of oral fludarabine results in dose-dependent increases in maximum plasma concentration (C_{max}) and 24-h area under the concentration–time curve ($AUC_{0-24\,h}$).[9] Similar mean $AUC_{0-24\,h}$ values were achieved with a 90 mg oral dose of fludarabine as with a 50 mg IV dose, but mean C_{max} values were approximately 20–30% lower compared with the corresponding values for the IV formulation.[10,11] The time to reach C_{max} is independent of dose.[10] The bioavailability of oral fludarabine is approximately 51–55% following single- and multiple-dose administration, with low intraindividual variation.[10,12] Systemic bioavailability, C_{max} and time to C_{max} are increased slightly (<10%) with concomitant food intake; the terminal half-life is unaffected.[13] These, and other pharmacokinetic studies,[14–16] have shown that a once-daily oral fludarabine dose of 40 mg/m^2 would provide a similar systemic exposure to fludarabine 25 mg^2/day IV (Figure 2). A summary of the different pharmacokinetic values for oral fludarabine *versus* IV is shown in Table 1.

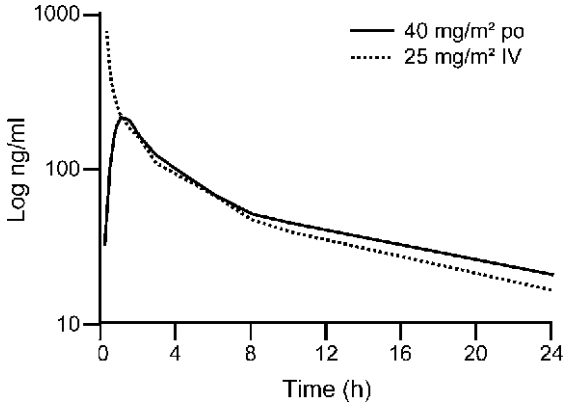

Figure 2 Plasma level time profile of fludarabine adminstered orally (po) or intravenously (IV).[12]

A small number of clinical studies have been conducted with oral fludarabine in CLL. The activity of oral fludarabine in patients with CLL who are resistant to, or have relapsed after treatment with a standard alkylating agent-containing regimen has been demonstrated in a prospective, multicenter, open-label, phase II trial.[17] A total of 78 patients with previously treated B-cell CLL received oral fludarabine 40 mg/m^2/day for 5 days, repeated every 4 weeks, for a total of 6–8 cycles. The primary end point was response to therapy, measured 3–5 weeks after the last treatment cycle. Complete response (CR) was defined as the absence of disease according to National Cancer Institute (NCI)[18] and International Workshop on CLL (IWCLL)[19] criteria. Partial response (PR), stable disease (SD) and progressive disease (PD) were also defined according to

these criteria. The patients had a mean age of 63 years and 96% had a WHO performance status of 0–1 at baseline. Nearly all of the patients had received prior chlorambucil therapy, either alone or in combination with prednisone. The majority of patients who failed this regimen were treated with cyclophosphamide alone or in combination with vincristine and prednisone. Efficacy and tolerability data were compared with data from a historical control group comprising 48 patients with previously treated CLL who received IV fludarabine as part of a comparative study with CAP.[1]

Efficacy

Response rates indicated that the clinical efficacy of oral fludarabine as second-line therapy is comparable to that of IV fludarabine (Figure 3).[17] According to IWCLL criteria, the OR rate was 46.2%, with 20.5% of patients achieving a CR and 25.6% achieving a PR. The comparative figures using NCI criteria were 51.3, 17.9 and 33.3%, respectively. These differences in response rate according to IWCLL and NCI are due to more

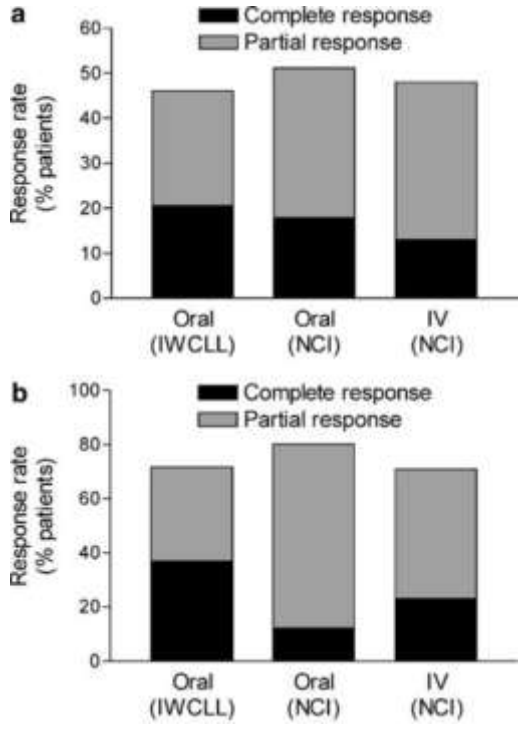

Figure 3 Clinical response rates with oral fludarabine in chronic lymphocytic leukemia (CLL) when used as (a) second-line therapy (78 patients)[17] or (b) first-line therapy (81 patients).[20] In both panels, responses to oral fludarabine are compared results on intravenous (IV) fludarabine from an historical control group (48 patients).[1] Oral fludarabine (40 mg/m^2/day) and IV fludarabine (25 mg/m^2/day) were adminisitered for five days every 28 days for six cycles (with provision for up to two or four additional cycles, respectively). The criteria for complete response and partial response was defined according to the International Workshop on CLL (IWCLL)[19] or the National Cancer Institute (NCI).[18]

Table 1 Pharmacokinetic profile of IV and oral fludarabine in patients with cancer[10–12,15]

Pharmacokinetic parameter	IV	Patient number	*Reference*	Oral	Patient number	*Reference*
Elimination $t_{1/2\alpha}$ (min)	4.97	30	Malspeis *et al.* (1990)[11]	NS		
Elimination $t_{1/2\beta}$ (h)	1.38	30	Malspeis *et al.* (1990)[11]	NS		
Elimination $t_{1/2\gamma}$ (h)	10.41	30	Malspeis *et al.* (1990)[11]	NS		
CL (l/h/m^2)	4.08	30	Malspeis *et al.* (1990)[11]	NS		
V_{ss} (l/m^2)	44.2	30	Malspeis *et al.* (1990)[11]	NS		
Terminal $t_{1/2}$ (h)	21.8	38	Klein *et al.* (1997),[12] O'Rourke *et al.* (1997)[15]	22.5	21	Klein *et al.* (1997)[12]
C_{max} (ng/ml)	1539	15	Foran *et al.* (1999)[10]	306–485	18	Foran *et al.* (1999)[10]
t_{max} (h)	NA			1.1–1.6	17	O'Rourke *et al.* (1997)[15]
$AUC_{(0-24\,h)}$ (ng h/ml)	3060	15	Foran *et al.* (1999)[10]	1760–3016	18	Foran *et al.* (1999)[10]
Bioavailability (%)	100			51–55	36	Klein *et al.* (1997)[12], Foran *et al.* (1999)[10]

Abbreviations: $AUC_{(0-24\,h)}$ = area under the concentration-time curve from 0 to 24 h; CL = total body clearance; C_{max} = maximum plasma concentration; NA = not applicable; NS = not stated; $t_{1/2\alpha}$ = alpha disposition phase half-life; $t_{1/2\beta}$ = intermediate phase half-life; $t_{1/2\gamma}$ = terminal half-life; t_{max} = time to reach C_{max}; Vss = volume of distribution at steady state.

stringent CR criteria in IWCLL (eg nodular bone marrow response). In the historical cohort of patients treated with IV fludarabine, the OR rate was 48%, with a CR of 13% (Figure 3).[1] Response to oral fludarabine was strongly correlated with disease stage; patients with low- or intermediate-risk disease (Rai stage 0–II) had higher OR rates according to IWCLL criteria than patients with high-risk disease (Rai stage III–IV) (66.7 and 61 versus 26.5%). A similar trend was observed for Binet staging; according to IWCLL and NCI criteria, respectively; the OR rates were higher for patients with Binet stage A (56.5 and 65.2%) or B disease (58.3 and 62.5%) at baseline, relative to those with Binet stage C disease (29 and 32.2%). These results were comparable with data from the historical cohort.[1] While patients in the oral and IV fludarabine studies were recruited using similar eligibility criteria, the mean number of previous treatments was slightly higher in the oral study (2 versus 1.2) and there was a greater proportion of Binet stage A patients in the oral group (29% versus 2%).

WHO performance status improved in 12 patients (15.4%) and remained unchanged in 43 patients (55.1%) treated with oral fludarabine. Of the 12 patients with improved performance, three achieved a CR, one achieved a PR, five had SD and three had PD. In this elderly population with relapsed and refractory disease, the fact that around 15% of patients had an improvement in WHO performance status and more than half maintained their performance status, suggests that the benefits of oral fludarabine extend beyond blood cell responses and have a positive impact on quality of life (QoL). This advantage, together with the additional convenience of treating CLL on an outpatient basis, emphasizes the potential value of oral fludarabine in the palliative setting, where QoL is an important consideration.

Tolerability

The tolerability profile of oral fludarabine in the second-line treatment of CLL was comparable to that of the IV formulation, with the exception of more frequent gastrointestinal (GI) toxicity.[17] Nausea/vomiting and diarrhea each occurred in 38.5% of patients, although no patient stopped treatment as a result of these complications (Figure 4). However, the majority of these events were WHO grade 1 or 2, with only a single case of grade 3 nausea and three reports of grade 3 diarrhea. GI toxicity is important when evaluating oral agents because vomiting or diarrhea may prevent accurate dosing, thereby affecting drug serum levels. The most common adverse effect during oral fludarabine therapy was myelosuppression; WHO grade 3 or 4 myelosuppression included granulocytopenia, leukocytopenia, thrombocytopenia and anemia (Figure 4). A total of 20 patients (25.6%) required dose reductions due to hematological toxicity. Autoimmune hemolytic anemia (AIHA) was noted in four patients (5.1%), with three requiring hospitalization; however, all of these

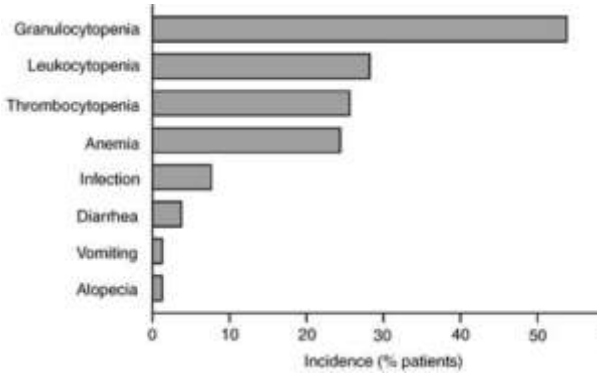

Figure 4 Incidence of WHO grade 3 or 4 adverse events with oral fludarabine (25 mg/m^2/day for 5 days every 28 days for 6–8 cycles) as second-line therapy therapy in patients with chronic lymphocytic leukemia.[17]

patients responded to corticosteroids. This serious adverse event has also been observed after IV fludarabine administration, demonstrating a need to monitor patients for this event during purine analog therapy. Infection was also common, occurring in 44.9% of patients; 7.7% of cases were of WHO grade 3 severity. Grade 3 or 4 alopecia, commonly reported with chlorambucil and cyclophosphamide, was only seen in one patient treated with oral fludarabine. Similarly, skin toxicity was seen in fewer patients than is seen with chlorambucil, with only four cases of rash (5.1%) and three cases of pruritus (3.8%). Four patients died during the study, and all had been withdrawn early from therapy; two because of disease progression and two because of pulmonary infection.

This key study confirms that oral fludarabine is as effective and well tolerated as IV fludarabine in the second-line treatment of CLL. GI toxicity, although higher with the oral formulation, does not generally require treatment, and infusion-related adverse events are eliminated. Importantly, performance status is maintained or improved, which is a vital consideration in elderly, heavily pretreated patients with refractory disease.

Oral fludarabine as first-line treatment for CLL

Oral fludarabine has also been evaluated in treatment-naïve CLL patients. The key study demonstrating the effectiveness of oral fludarabine as monotherapy was conducted by Rossi *et al*.[20] In this multicenter, open-label study, 81 patients with previously untreated CLL (median age 64 years) received oral fludarabine 40 mg/m^2/day for 5 days every 4 weeks, for 6–8 cycles. The main objectives were to assess OR rate (NCI and IWCLL criteria), safety and QoL. At baseline, all patients had a WHO performance status of 0–1 and were classified as Binet stage A progressive (18.5%), stage B (63%) or stage C (18.5%).

Efficacy

The OR rates were 71.6% according to IWCLL criteria (CR 37%; PR 34.6%) and 80.2% (CR12.3%; PR 67.9%) using NCI criteria (Figure 3). The OR rate was comparable with that achieved in a similar historical cohort who received first-line therapy with IV fludarabine (71%).[1] CR and PR rates were 23 and 48%, respectively, in this cohort. As in the previous study, response to oral fludarabine was related to disease stage: the OR rate according to IWCLL criteria was 80% for patients who had Binet stage A progressive disease at baseline, 75% for patients with stage B, and 53% for patients with stage C disease.

At the end of therapy, WHO performance status improved in 13.6% of patients and remained unchanged in 77.8%. Importantly, assessment of QoL (using the EORTC (European Organization for Research and Treatment of Cancer) QLQ-30 questionnaire and Spitzer's QoL Index) showed that treatment with oral fludarabine had no negative impact on patients' QoL; significant improvements from baseline were achieved in the emotional ($P = 0.0001$), insomnia ($P = 0.0022$) and health ($P = 0.03$) domains.

Tolerability

The majority of side effects were of mild-to-moderate intensity, manageable and reversible; the most common WHO grade 3–4 adverse events were granulocytopenia (32.1% of patients), leukocytopenia (18.5%), anemia (9.9%), diarrhea (6.2%) thrombocytopenia (4.9%), infection (4.9%), and nausea/vomiting (1.2%). Once again, GI toxicity was more common with the oral formulation than previously reported with the IV formulation, but did not require treatment in the majority of cases. While half of the patients had infections, severe infection was rare, with only 4.9% of patients reporting infections of WHO grade 3 or 4 severity. Dose reductions were required by 14 patients, mainly due to myelosuppression. There were three cases of AIHA, which resolved in all three patients on treatment withdrawal. Four patients died, one due to septicemia during therapy and three between 7 and 14 months after therapy as a result of myocardial infarction, Richter's transformation, and deterioration due to progressive disease, respectively.

These findings are supported by an additional but smaller study.[21] A total of 15 patients (median age 51 years) with previously untreated CLL received six cycles of oral fludarabine 40 mg/m^2 on days 1–5. After six cycles, three patients (20%) achieved a CR, nine (60%) achieved a PR, one (6.7%) had SD and two (13.3%) had PD (OR 80%; response criteria not stated). As expected, toxicity was predominantly hematological, with one case of severe febrile neutropenia. Mild transient rash was seen in seven of 15 patients (46.7%).

Oral fludarabine combination therapy as first-line treatment for CLL

The effectiveness of oral fludarabine plus cyclophosphamide combination therapy has also been investigated in patients with previously untreated CLL.[22] The rationale for combining fludarabine with cyclophosphamide was based on the findings of preclinical studies suggesting that these two agents have synergistic activity.[23,24] In an open-label study, 75 treatment-naïve patients with CLL (median age 54 years) received oral fludarabine 30 mg/m^2/day plus oral cyclophosphamide 200 mg/m^2/day, on days 1–5 every 28 days for 6 cycles. A total of 59 patients had Binet stage B disease and 16 had Binet stage C disease.

After a mean of 5.3 cycles, the OR rate (NCI criteria) was 79.9%, including CR 49.3%, nodular PR 5.3% and PR 25.3%. SD was achieved in 8% of patients and 2.6% had PD. Response duration and time to treatment failure, which were included in the efficacy endpoints, had not been reached at the time of trial reporting.

Toxicity was mainly hematological and included NCI grade 3–4 lymphopenia (79% of cycles), neutropenia (52%), thrombocytopenia (6%) and anemia (3%). AIHA was noted in five patients and peripheral thrombocytopenia in three patients. The incidence of infection was low, with grade 1–2 infection noted during 13% of cycles and only three cases of grade 3–4 infections. GI disturbances were mild and included nausea (grade 1–2, 75% of patients; grade 3–4, 7%), and vomiting (grade 1–2, 37% of patients; grade 3–4, 3%). Only one patient withdrew from therapy because of GI toxicity. Two patients died during treatment (unexplained ($n = 1$), neutropenia ($n = 1$)) and 2 patients died during follow up (Richter's syndrome ($n = 1$), neuroendocrine carcinoma of the liver ($n = 1$)).

These three trials demonstrate that the efficacy and tolerability of oral fludarabine is essentially similar to the IV formulation in the first-line treatment of CLL, either alone or as part of combination therapy. One of the studies also indicates that oral fludarabine has a beneficial effect on certain aspects of QoL.[20] Further studies of this agent in previously untreated patients with CLL are warranted. Fludara® received European marketing approval as first-line treatment for B-cell chronic lymphocytic leukemia in February 2003.[25]

Cost effectiveness

As mentioned previously, NICE recommends oral fludarabine for the second-line treatment of CLL on the basis of greater cost effectiveness than IV fludarabine.[8] In particular, administration costs were higher with IV fludarabine than with oral fludarabine, mainly because of the need for hospitalization with the IV formulation. However, the institute warns that its assessment of cost effectiveness is subject to considerable uncertainty because reliable data on the costs of managing adverse effects are not available. NICE used

three sources of data to examine the cost of managing side effects associated with oral and IV fludarabine and CHOP. The first (described as the low estimate) was submitted by the manufacturer and was based on limited patient numbers, and thus is subject to a high sampling error. The second (described as the high estimate), was submitted by a different manufacturer for a different type of lymphoma and also involved limited patient numbers. The higher cost estimates derived from this source seemed to be attributable to advanced disease. The third source was from the Medical Research Council trial CLL3; this yielded similar costs to the high estimate, and may also have included costs of disease as well as side effects.

Conclusions

The above studies show that oral fludarabine has similar efficacy and tolerability to IV fludarabine in patients with CLL. This efficacy has been demonstrated for both first- and second-line treatment, with OR rates of 72–80%, and 46–51%, respectively. Consequently, oral fludarabine has recently become available in the majority of European countries for the second-line treatment of CLL, with NICE recommending oral fludarabine in preference to the IV formulation. The benefits of oral fludarabine versus the IV formulation are several-fold and include ease of administration, improved patient QoL, absence of infusion-related adverse events, and potential for reduced medical costs because of fewer hospital visits and fewer healthcare worker interventions. In particular, the convenience of oral administration has the potential to significantly improve patients' QoL. Indeed, the ability to receive fludarabine orally at home is a major advantage for patients. In light of the extensive use of fludarabine across a broad range of hematological malignancies, and promising results seen in combination with cyclophosphamide, an oral formulation has potential advantages. Therefore, with comparable efficacy and a similar tolerability profile to the IV formulation, oral fludarabine is fast becoming established as a second-line treatment for CLL.

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Clinical experience with fludarabine in indolent non-Hodgkin's lymphoma

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Fludarabine, a purine nucleoside analog, is currently indicated for the first-line treatment of chronic lymphocytic leukemia and is also licensed for the management of indolent non-Hodgkin's lymphoma (NHL) in countries such as Switzerland and Canada. Clinical evidence from studies in patients with NHL suggests that fludarabine monotherapy is at least as effective, if not better, than conventional therapies such as cyclophosphamide, vincristine, prednisone (CVP) for the first- and second-line treatment of NHL, achieving objective response rates of 31–84%. The combination of fludarabine with other chemotherapeutic agents such as cyclophosphamide or mitoxantrone also provides the clinician with additional useful treatment options in this setting. Objective response rates of 70–100% have been reported with fludarabine-containing combination regimens, often exceeding those reported with CVP. Furthermore, beneficial effects on overall and progression-free survival have been reported with fludarabine or fludarabine-containing combination regimens in a number of studies, including a significant survival benefit with the combination of fludarabine, cyclophosphamide, mitoxantrone and rituximab. While adverse events such as granulocytopenia, neutropenia and anemia and, less frequently, infectious complications have been reported with fludarabine, its adverse event profile generally compares favorably with that of other available treatment options. Available clinical data therefore indicate that fludarabine has an important role to play in the treatment of patients with indolent NHL. Further, studies are warranted to identify the optimal fludarabine regimen for this patient group.
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Introduction

The non-Hodgkin's lymphomas (NHL) are a heterogeneous group of lymphomas predominantly involving malignant monoclonal populations of B lymphocytes, although T or natural killer (NK) lymphocytes may be involved. The disease most commonly presents as lymphadenopathy, usually with abdominal lymph node involvement; splenomegaly and hepatomegaly may also be present early on in the disease together with nodular infiltration of the skin. Clinical investigation usually reveals a picture of normochromic normocytic anemia with raised erythrocyte sedimentation rate, abnormal liver function tests, lymph node involvement and infiltration by lymphoid tissue (as seen on bone marrow biopsy).[1]

In the US, NHL is the sixth most common cause of cancer death and accounted for 5% of all cases of invasive cancer in 2001 (56 000 of 1.27 million new cases).[2] The incidence of NHL in the UK is estimated to be approximately 16/100 000. The incidence of NHL has increased steadily over the past 30 years, with an estimated annual increase in incidence of 3–4% across the US and Europe.[3,4] In particular, the incidence of NHL has increased in patients aged >65 years, and is a particular burden in this older population.[1]

The development of an internationally acceptable histopathological classification system for NHL has proved to be a significant challenge during the last two decades. Until recently, the Working Formulation was the most commonly used system in the US and the Kiel classification was used predominantly in Europe.[5] However, the two systems were not compatible making it difficult to compare the results of studies conducted in the US and Europe. Subsequently, in 1994, the Revised European–American Lymphoma (REAL) classification system was proposed by the International Lymphoma Study Group[6] and this was closely followed by the WHO classification system.[7] The WHO classification is based on the REAL classification with some minor changes and represents the first generally accepted classification system for lymphoid malignancies. Classification of the B-cell

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Table 1 World Health Organization (WHO) classification system for B-cell lymphomas[5]

Category
Precursor B-cell diseases
Precursor B-lymphoblastic lymphoma/leukemia
Mature B-cell disease
Chronic lymphocytic leukemia/small lymphocytic lymphoma
Prolymphocytic leukemia
Lymphoplasmacytic lymphoma, Waldenström's macroglobulinemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Plasma cell neoplasms
Extranodal marginal zone B-cell lymphoma of MALT type
Nodal marginal zone B-cell lymphoma
Follicular lymphoma (variants: grades 1, 2, 3a and b)
Mantle cell lymphoma (variant: blastic)
Diffuse large B-cell lymphoma (variants: centroblastic, immunoblastic, T-cell or histiocyte rich, anaplastic large cell)
Mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
Primary effusion lymphoma
Burkitt's lymphoma (variants: Burkitt's lymphoma with plasmacytoid differentiation, atypical Burkitt/Burkitt-like)
Lymphomatoid granulomatosis

Abbreviations: MALT = mucosa-associated lymphoid tissue.

lymphomas according to the WHO system is presented in Table 1.

For practical purposes, the NHL neoplasms in the WHO classification can also be divided into prognostic groups, namely indolent and aggressive forms of NHL. Indolent lymphomas account for 25–40% of NHLs, of which follicular lymphomas are the most common.[8,9] Although most patients with indolent NHL have a relatively good prognosis, with a median survival of up to 10 years, the disease generally follows a continuous remitting course and is usually incurable.[10] Aggressive lymphomas, which include diffuse large B-cell lymphomas and some peripheral T-cell lymphomas, progress rapidly without therapy but are usually responsive to intensive combination chemotherapy. A 5-year survival rate of 50–60% has been reported in patients with aggressive NHL, with up to 30–60% of patients being cured.[10]

In addition to prognostic categorization (indolent or aggressive), the survival of patients with NHL is also dependent on the disease stage, which is often determined using the Ann Arbor staging system. This system categorizes the disease into four stages (I–IV) according to number, size and location of involved lymph nodes and extralymphatic organs and the presence or absence of systemic symptoms.[11] Originally developed for the staging of Hodgkin's lymphoma, this system does have some limitations and has led investigators to attempt to identify clinical prognostic factors that more accurately predict the survival of patients with NHL. Significant risk factors that have been identified for NHL include age at diagnosis (<60 versus >60 years), systemic B symptoms, performance status, serum lactate dehydrogenase (LDH) and serum β2-microglobulin levels (normal versus elevated) and number of extranodal sites ($\leqslant 1$ versus >1 site).[2,12]

Treatment options for indolent NHL

Although a wide range of treatment options are available for indolent NHL, the disease invariably follows a continuous clinical course of regression and progression, regardless of any treatment. Furthermore, there has been no demonstrable improvement in overall survival for patients with advanced disease over the last 30 years, even in patients who have achieved a complete response to treatment.[13] As a consequence, the treatment of indolent NHL remains controversial and no single or combination therapy is currently considered to be the gold standard.[10,14] For the small number of patients who present with localized stage I NHL, the standard treatment option is radiotherapy.

Despite these shortcomings, the National Cancer Institute in the US currently recommends several first-line treatment options for stages II–IV indolent follicular NHL.[10] The standard option frequently offered to newly diagnosed asymptomatic patients with low tumor burden is 'watchful waiting.' Several studies have reported similar survival rates for patients in whom treatment was deferred and for those who received early chemotherapy.[15] Other treatment options for stages II–IV indolent NHL include monotherapy with purine nucleoside analogs (fludarabine and cladribine) or oral alkylating agents (cyclophosphamide or chlorambucil with or without steroids), and combination chemotherapy such as cyclophosphamide, vincristine and prednisone (CVP) or cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP). Other newer treatment options include the anti-CD20 monoclonal antibody rituximab (either alone or with combination chemotherapy), radiolabeled monoclonal antibodies (eg 90Y ibritumomab tiuxetan) and myeloablative chemo/radiotherapy followed by autologous or allogeneic bone marrow or peripheral stem cell transplantation (the reader is referred to the article by Goldman, also in this supplement, for a more detailed account of the use of transplant techniques in NHL).[10] Importantly, the current goal of therapy in NHL is to increase the complete response rate and achieve minimal residual disease, thereby increasing the chance of cure or at least prolonging remission/progression-free survival.

Although remissions can be achieved after relapse in patients with indolent NHL, a second relapse and even multiple relapses are common, sometimes with a more aggressive histology. Treatment options for indolent disseminated lymphomas such as B-cell small lymphocytic lymphoma (SLL) and lymphoplasmocytic lymphoma/immunocytoma, are similar to those for chronic lymphocytic leukemia (reviewed by Leporrier and Hallek elsewhere in this supplement). The indolent extranodal marginal zone B-cell lymphomas (MALT; mucosa-associated lymphoid tissue) can involve several areas, including the gastrointestinal tract, salivary glands and breast, but usually remain localized for a period of time prior to systemic spread. For this reason, local treatment involving surgery or local/regional radiotherapy is often effective.[1]

Efficacy of fludarabine in indolent NHL

Of the treatment options currently recommended for stages II–IV indolent NHL, the purine nucleoside analog fludarabine has emerged as one of the most effective, when used either as a single-agent or in combination with other chemotherapeutic drugs such as cyclophosphamide and/or mitoxantrone.

Numerous studies have been conducted to evaluate the efficacy of fludarabine in indolent NHL in both previously untreated patients and in relapsed or refractory patients. Definitions of response used in the studies were predominantly resolution of all symptoms and signs of lymphoma for $\geqslant$28 days for a complete response, and a $\geqslant$50% reduction in measurable disease for $\geqslant$28 days for a partial response. The objective response rate was obtained by combining the complete and partial response rates.

Single-agent fludarabine as first-line treatment

Good objective response rates of 47–84% have been reported in studies evaluating single-agent fludarabine for the first-line treatment of indolent NHL (Table 2). Available data also suggest that when used in this setting fludarabine is at least as effective, if not better, than conventional therapies such as CVP and may also offer benefits in terms of delayed disease progression.

The largest ($n = 309$) study to evaluate single-agent fludarabine for the first-line treatment of NHL was conducted by the EORTC Lymphoma Cooperative Group and the British National Lymphoma Investigation (BNLI).[20] Patients in this phase III, multicenter trial had stage III or IV low-grade NHL and were randomized to receive either fludarabine or conventional CVP chemotherapy for eight courses (Table 2); treatment was initiated either immediately after diagnosis or after a wait-and-see period. Objective response rates (complete plus partial) were 68 and 51% in the fludarabine and CVP groups, respectively. After a median follow-up period of 69 months, there was a trend towards a longer median time to progression for responders in the fludarabine group (21 versus 15 months).

In a smaller study, single-agent fludarabine was evaluated in 49 patients with previously untreated advanced follicular lymphoma.[21] Total and complete response rates were 65 and 37%, respectively, and 30 of 32 responding patients achieved at least a partial response after three treatment cycles. After a median follow-up time for surviving patients of 14.8 months, the median progression-free survival interval was 13.6 months and the median time-to-treatment failure was 9 months.

Another study compared the efficacy of fludarabine versus fludarabine plus idarubicin in patients with indolent or mantle cell lymphoma ($n = 199$) (Table 2).[16] In the indolent NHL group ($n = 170$), which included patients with follicular and small lymphocytic lymphoma and immunocytoma, complete and partial response rates of 47 and 37% were reported for fludarabine and 39 and 42% for fludarabine plus idarubicin. The study investigators also conducted a detailed analysis of the complete response rate according to histological subtype and found fludarabine to be superior to the combination in treating follicular lymphomas (60 versus 40%), whereas the combination of fludarabine plus idarubicin was more effective in treating nonfollicular lymphomas (small lymphocytic 43 versus 29%; immunocytoma 38 versus 23%).

Table 2 Published studies evaluating the efficacy of single-agent fludarabine in the first-line treatment of patients with indolent non–Hodgkin's lymphoma

Reference	*Study design*	*No. of evaluable patients*	*Treatment regimen*[a]	*Clinical response*	
				CR (%)	*CR + PR (%)*
Zinzani *et al.*[16]	Phase III, mc, r	199[b]	FLU 25 mg/m^2 × d1–5q4wk versus	47	84
			FLU 25 mg/m^2 × d1–3q4wk + IDA 12 mg/m^2 d1 q4wk	39	81
Clavio *et al.*[17]	Phase II	16	FLU 25 mg/m^2 × d1–5q4wk	31	75
Zinzani *et al.*[18]	Phase II	8	FLU 25 mg/m^2 × d1–5q4wk	38	75
Pigaditou *et al.*[19]	Phase II	16	FLU 25 mg/m^2 × d1–5q3–4wk	38	69
Hagenbeek *et al.*[20]	Phase III, mc, p, r	309	FLU 25 mg/m^2 × d1–5q4wk versus	38	68
			CYC 750 mg/m^2 iv d1 + VINC 1.4 mg/m^2 iv d1 + PRED 40 mg/m^2 po d1–5q4wk	15	51
Solal-Céligny *et al.*[21]	Phase II, mc	49	FLU 25 mg/m^2 × d1–5q4wk	37	65
Dumontet *et al.*[22]	Phase II	21	FLU 25 mg/m^2 × d1–5q4wk	n/s	52
Rohatiner *et al.*[23]	Phase II	36	FLU 25 mg/m^2 × d1–5q4wk	14	47

[a]FLU administered by short iv infusion.

[b]Includes 29 patients with mantle cell lymphoma.

Abbreviations: CR = complete response; CYC = cyclophosphamide; d = days; FLU = fludarabine; IDA = idarubicin; iv = intravenous; mc = mul-multicenter; n/s = not stated; p = parallel group; po = oral; PR = partial response; PRED = prednisone; q4wk = every 4 weeks; q3–4wk = every 3–4 weeks; r = randomized; VINC = vincristine.

Table 3 Published studies evaluating the efficacy of single-agent fludarabine in the second-line treatment of patients with indolent non-Hodgkin's lymphoma

Reference	*Study design*	*No. of evaluable patients*	*Treatment regimen*[a]	*Previous therapy (no. regimens)*	*Clinical response*	
					CR	*CR + PR*
Gillis *et al.*[24]	n/s	14	FLU 25 mg/m^2 × d1–5q4wk	1–3	21	71
Tondini *et al.*[25]	Phase II, r, o	54	FLU 25 mg/m^2 × d1–5q4wk versus	⩾1	48	68
			CDA 0.14 mg/kg iv d1–5 q4wk		38	72
Dumontet *et al.*[22]	Phase II	29	FLU 25 mg/m^2 × d1–5q4wk	2 (1–5)	n/s	66
Klasa *et al.*[26]	Phase III, mc, r, o	91	FLU 25 mg/m^2 × d1–5q4wk versus	1–4	9	64
			CYC 750 mg/m^2 + VINC 1.2 mg/m^2 iv d1 + PRED 40 mg/m^2 po d1–5q3wk		7	52
Falkson *et al.*[27]	Phase II	21	FLU 25 mg/m^2 × d1–5q4wk	1–2	33	62
Zinzani *et al.*[18]	Phase II	13	FLU 25 mg/m^2 × d1–5q4wk	1–3 ($n=9$) >3 ($n=4$)	0	62
Redman *et al.*[28]	Phase II	38	FLU 25 mg/m^2 × d1–5q3–4wk	3 (1–>4)	13	55
Hochster *et al.*[29]	Phase II	25	FLU 18 mg/m^2 × d1–5q4wk	1–2	20	52
O'Brien *et al.*[30]	n/s	48	FLU 25 mg/m^2 × d1–5q4wk	1–8	10	50
Moskowitz *et al.*[31]	Retro	32	FLU 25 mg/m^2 × d1–5q 4wk	2	6	50
Pigaditou *et al.*[19]	Phase II	45	FLU 25 mg/m^2 × d1–5q3–4wk	3 (1–7)	9	44
Whelan *et al.*[32]	n/s	34	FLU 25 mg/m^2 × d1–5q3–4wk	3 (1–7)	18	38
Leiby *et al.*[33]	Phase II	25	FLU 20 mg/m^2 + 30 mg/m^2 q3–4wk[b]	2.6	4	32
Hiddemann *et al.*[34]	Phase II	38	FLU 25 mg/m^2 x d1–5q4–5wk	3 (1–11)	13	31

[a]FLU administered by short iv infusion.
[b]20 mg/m^2 loading dose + 30 mg/m^2/day continuous iv infusion for 48 h.
Abbreviations: CDA = cladribine; CR = complete response; CYC = cyclophosphamide; d = days; FLU = fludarabine; IDA = idarubicin; iv = intravenous; mc = multicenter; n/s = not stated; o = open-label; po = oral; PR = partial response; PRED = prednisolone; q3–4wk = every 3–4 weeks; q4wk = every 4 weeks; q3wk = every 3 weeks; r = randomized; retro = retrospective; sc = single center; VINC = vincristine.

Single-agent fludarabine as second-line treatment

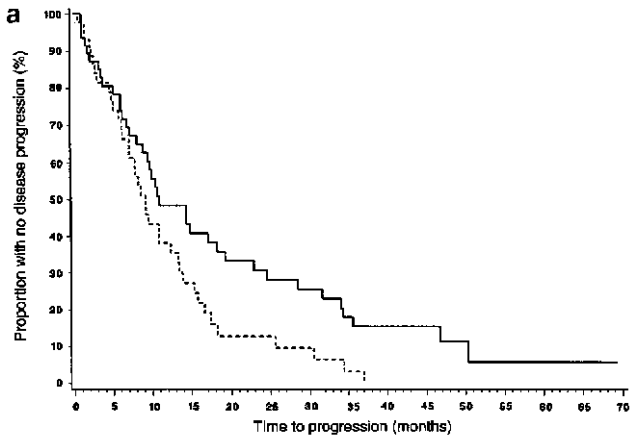
The efficacy of fludarabine as second-line treatment for relapsed or refractory indolent NHL has been evaluated in several clinical trials, with promising results (Table 3). Objective response rates ranged from 31 to 71%, and complete responses were observed in up to 48% of patients, most of whom had far advanced disease and had received extensive prior chemotherapy. In two studies that used lower doses of fludarabine, the objective response rates were considerably lower.[29,33] As a second-line treatment, single-agent fludarabine also appears to be at least as effective as cladribine, and to offer benefits over conventional therapy such as CVP in terms of progression- and treatment-free survival.

In the largest study conducted to date in this setting ($n=91$), Klasa *et al.*[26] compared the efficacy of fludarabine with that of conventional CVP combination therapy (Table 3). Although there were no significant differences in the objective response rate (fludarabine 64%, CVP 52%) or median overall survival (fludarabine 57 months, CVP 44 months) between the two treatment groups, fludarabine significantly improved progression-free survival (10.9 versus 9.2 months; $P=0.03$), treatment-free survival (15.3 versus 11.2 months; $P=0.02$) and social function scores ($P=0.008$) compared with CVP (Figure 1).

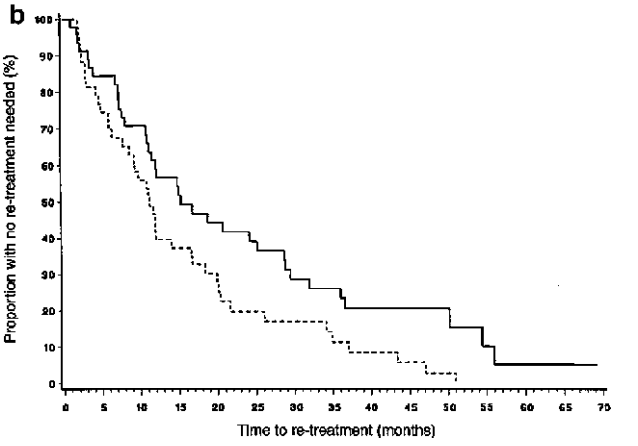
In another comparative study, Tondini *et al.*[25] compared the efficacy of fludarabine and cladribine in 54 patients with relapsed or refractory indolent NHL (Table 3). Upon treatment failure, eligible patients were crossed over to the other study drug. Objective response rates of 68 and 72% were reported for fludarabine and cladribine, respectively. The actuarial 3-year progression-free survival among responding patients was 58% for fludarabine and 52% for cladribine. Seven patients who became refractory to therapy failed to respond to treatment after crossover. There was greater toxicity with cladribine than with fludarabine; significantly more patients in the cladribine group than in the fludarabine group withdrew due to hematological toxic effects (15 patients (47%) versus two patients (8%); $P=0.001$). A greater proportion of patients treated with cladribine initially, compared to fludarabine initially, experienced Grade 3–4 neutropenia (66 versus 50%) or thrombocytopenia (22 versus 4%).

Fludarabine-based combination therapy

The discovery of potential synergy between fludarabine and other chemotherapeutic agents (eg mitoxantrone or cyclophosphamide) *in vitro* led to the design of numerous studies to evaluate the efficacy of fludarabine-containing combination regimens, initially in CLL and more recently in NHL.[35–37] To date the most widely investigated fludarabine combination regimens for the treatment of NHL are those combining the drug with mitoxantrone, cyclophosphamide or mitoxantrone plus cyclophosphamide. Other less widely investigated regimens are those combining fludarabine with epirubicin, idarubicin or rituximab. Objective response rates typically ranging from 70 to 100% have been reported with fludarabine-based combination therapy in NHL, with the combination of fludarabine, mitoxantrone and cyclophosphamide potentially the most promising option.



	12 mos	18 mos	24 mos	30 mos	36 mos	42 mos	48 mos
FLU Estimated progression-free	48%	38%	30%	25%	15%	15%	11%
No. at risk	20	15	12	10	6	5	3
CVP Estimated progression-free	38%	16%	13%	10%	3%	0%	0%
No. at risk	14	5	4	3	1	0	0



	12 mos	18 mos	24 mos	30 mos	36 mos	42 mos	48 mos
FLU Estimated treatment-free	59%	47%	42%	29%	24%	20%	20%
No. at risk	25	19	16	11	9	7	5
CVP Estimated treatment-free	40%	33%	20%	17%	11%	8%	3%
No. at risk	17	13	7	6	4	3	1

Figure 1 Kaplan–Meier analysis of progression-free survival (a) and treatment-free survival (b) for patients with non-Hodgkin's lymphoma treated with fludarabine (FLU) versus cyclophosphamide, vincristine and prednisone (CVP).[26] Reprinted with permission from the American Society of Clinical Oncology.

Fludarabine plus mitoxantrone

The combination of fludarabine plus mitoxantrone (with or without dexamethasone/prednisone) is the most widely studied fludarabine-containing combination regimen in indolent NHL. The largest study to evaluate this combination enrolled 81 patients with previously untreated advanced indolent NHL[38] (Table 4). Overall, 91% of patients responded to treatment with 43% achieving a complete response. After a median follow-up of 31 months, 36% of patients progressed after achieving a response. The estimated 2-year progression-free survival was 63% for all patients.

Despite a poor prognosis, a high-objective response rate (90%) was also reported with fludarabine plus mitoxantrone in 22 patients with low-grade lymphoproliferative disorders who had received a median of two prior therapies.[53] The median remission duration was 39 weeks and the 2-year actuarial survival rate was $65 \pm 15\%$. A high objective response rate (89%) was also reported in 27 previously untreated patients with indolent NHL following treatment with fludarabine plus mitoxantrone.[54]

Fludarabine plus mitoxantrone combination therapy has also been compared with doxorubicin, cyclophosphamide, vindesine and prednisone (CHEP) in a phase II trial in patients with newly diagnosed stage II bulky or stage III/IV indolent NHL.[55] At 1 year, the complete response, partial response and failure rates were significantly different between the two treatment groups in favor of fludarabine plus mitoxantrone (fludarabine plus mitoxantrone: 44, 40, 15% versus CHEP: 22, 26, 52%, respectively; $P = 0.023$). Moreover, in another study involving 93 previously untreated patients with stages II–IV follicular lymphoma, the rate of complete remissions was significantly higher with fludarabine plus mitoxantrone (68%; $P = 0.003$) than with CHOP (37%); similar objective response rates were achieved for the two treatments (fludarabine plus mitoxantrone: 94% versus CHOP: 93%).[50]

Overall, the addition of prednisone or dexamethasone to fludarabine plus mitoxantrone combination therapy does not appear to confer any additional advantage in terms of efficacy and may even increase the incidence of infection[51,56–58] (Table 4). However, good activity was reported even in elderly patients, those with high LDH levels and in those who had received multiple prior treatment regimens following treatment with fludarabine, mitoxantrone and dexamethasone in one study.[51] Furthermore, evaluation of this regimen in a comparative study in 142 patients with previously untreated stage IV indolent lymphoma, suggested that it was at least as effective as an alternating triple therapy regimen (ATT) (objective response rate 97% in both groups; 5-year survival rate 84% with fludarabine, mitoxantrone and dexamethasone, 82% with ATT).[49] Notably, maintenance interferon/dexamethasone was also given to both treatment groups for 1 year in this study.

Fludarabine plus cyclophosphamide

The combination of fludarabine plus cyclophosphamide has frequently been investigated, and the results compare favorably with those reported with fludarabine plus mitoxantrone. A range of fludarabine (20–30 mg/m^2)

and cyclophosphamide dosages (250–600 mg/m^2) have been used (most commonly fludarabine/cyclophosphamide 25/250 or 30/300 mg). Some investigators have also used this combination in conjunction with dexamethasone or granulocyte/macrophage-colony stimulating factors (G-CSF, GM-CSF) (Table 4).

Santini *et al*.[39] treated 22 patients with recurrent low-grade NHL with fludarabine plus cyclophosphamide and reported an objective response rate of 95%.[39] Furthermore, 58% of patients who achieved a complete response had clinical and instrumental disappearance of disease after three cycles of treatment. Similar objective response rates (80–100%) have been achieved with fludarabine plus cyclophosphamide in conjunction with filgrastim or G-CSF/GM-CSF.[41–43]

In another study, 25 pretreated patients with advanced indolent lymphoma received a combination regimen comprising fludarabine, cyclophosphamide and dexamethasone (FLUCYD).[40] The objective response rate was 72% and the median failure-free survival time was 21 months for responders and 9 months for nonresponders. All of the patients with a complete response remained stable for periods of 5–21 months after their initial response.

Fludarabine plus cyclophosphamide and mitoxantrone

Fludarabine has also been combined with both cyclophosphamide and mitoxantrone, with particularly promising results. Santini *et al*.[39] evaluated this combination in 31 patients with recurrent low-grade NHL. The objective response rate was 84%. After three cycles of treatment, the disease had completely disappeared in 90% of the patients who achieved a complete response. At 3 years the overall survival rate was 71% and the failure-free survival rate was 57%. Two other studies that evaluated this combination as first-line therapy in patients with NHL reported objective response rates of 91 and 95% (complete response rates 61 and 75%)[59,60] (Table 4).

Recent data from a study in patients with relapsed or refractory lymphoma also suggest that the addition of rituximab to this regimen may produce a significant survival benefit.[63] Median progression-free survival was 483 days with the addition of rituximab compared with 213 days with fludarabine, cyclophosphamide and mitoxantrone alone ($P = 0.0484$).

Fludarabine plus other agents

A small number of trials have examined the combination of fludarabine and the anthracyclines epirubicin or idarubicin (Table 4). Objective response rates of up to 85% have been reported with the fludarabine plus epirubicin and cyclophosphamide (FLEC) regimen in patients with relapsed or refractory indolent lymphoma,[44,45] with an overall survival of 70% at 27 months reported in one study.[45]

One of the largest studies to evaluate fludarabine combination therapy in NHL compared a regimen comprising fludarabine plus idarubicin with fludarabine monotherapy in patients with newly diagnosed stages II–IV indolent NHL ($n = 199$).[16] The complete response rate was higher in patients treated with fludarabine alone than in those receiving the combination (47 versus 39%). Furthermore, fludarabine alone appeared to be more effective than the combination against follicular lymphoma (complete response rate 60 versus 40%). However, after a median follow-up of 19 months, 62% of patients in the fludarabine group compared with 84% in the combination therapy group maintained a complete response, suggesting that fludarabine plus idarubicin therapy may confer a longer-lasting complete response. A similar level of efficacy was reported with a combination of fludarabine plus idarubicin with either prednisone or dexamethasone in two pilot studies in NHL.[46,47]

Promising results have also been reported with the combination of fludarabine plus rituximab in patients with low-grade and/or follicular NHL ($n = 30$). An objective response rate of 93% was reported (complete response rate 80%), with a median duration of response of >14 months (ongoing).[9]

Safety of fludarabine

As with all chemotherapeutic agents, patients treated with fludarabine may experience adverse events; however, as monotherapy or as a component of a combination regimen, fludarabine is generally a well-tolerated treatment option for the management of indolent NHL, with an adverse event profile that generally compares favorably with those of other available treatment options.

The most common adverse events associated with fludarabine therapy are hematological, and usually reversible. Infectious complications (mostly respiratory tract infections or unexplained fever) have been reported with fludarabine but tend to occur predominantly in patients treated with fludarabine plus a corticosteroid.[40,51,64] Severe neurotoxicity has been reported with fludarabine but this is clearly dose-related and is minimal with standard doses of the drug.[64]

In a large study ($n = 309$) comparing fludarabine with CVP in patients with NHL, WHO grade >2 granulocytopenia (28 versus 12%; $P < 0.0005$) and thrombocytopenia (8 versus 1%; $P = 0.001$) were more common with fludarabine; however, the frequency of severe infections was low and similar in the two groups (2 versus 3%). Marked alopecia was limited to the CVP group.[20] Another study also comparing fludarabine with CVP ($n = 91$) reported essentially similar adverse event profiles for the two treatment groups, although CVP was associated with a higher incidence of nausea and vomiting (18–30 versus 83–95%) and neurotoxicity (any degree 36 versus 75%; $P = 0.0005$), and fludarabine was associated with more lymphopenia ($P = 0.005$).[26] In a comparison of fludarabine with fludarabine plus

Table 4 Published studies evaluating the efficacy of fludarabine-based combination chemotherapy regimens in patients with indolent non-Hodgkin's lymphoma

Reference (study design)[a]	*No. of evaluable patients*	*Histology*	*Prior therapy*	*Treatment regimen*[b]	*Clinical response (%)*		*Survival/duration of response*[a]
					CR	*CR + PR*	
Fludarabine + cyclophosphamide							
Santini *et al.*[39] (mc, c)	22	FU ($n=13$), LL ($n=8$), MCL ($n=1$)	Yes	FLU 25 mg/m^2 x d1–3 + CYC 300 mg/m^2 d1–3 q4wk	54	95	3-year OS 42.8%, 3-year FFS 31.1%
Lazzarino *et al.*[40]	25	FU ($n=14$), MCL ($n=3$), LL ($n=3$), other ($n=5$)	Yes	*FLUCYD*: FLU 25 mg/m^2 x d1–3 + CYC 350 mg/m^2 d1–3 + DEX 20 mg d1–3 q4wk	32	72	Median FFS 21mo (responders)
Fludarabine + cyclophosphamide + GM–CSF/G–CSF/filgrastim							
Lossos *et al.*[41] (phase II)	6	SLL ($n=1$), FSCCL ($n=3$), FML ($n=2$)	Yes (some)	FLU 30 mg/m^2 x d1–3 + CYC 300 mg/m^2 d1–3 ±G-CSF 5 μg/kg q4wk	67	100	
Flinn *et al.*[42] (phase II, mc)	30	FCC ($n=20$), MCL ($n=10$)	No	FLU 20 mg/m^2 x d1–5 + CYC 600 mg/m^2 d1 + FILG 5 μg/kg sc d8–18/22 q4wk	60 (FCC) 40 (MCL)	92 (FCC) 80 (MCL)	
Gregory *et al.*[43] (phase II, r)	12	n/s	Yes	FLU 30 mg/m^2 x d1–3 + CYC 300 mg/m^2 d1–3 ±G-MCSF 250 μg/m^2 d4 q4wk	41	82	Median duration of response 12.3mo
Fludarabine + epirubicin + cyclophosphamide							
Bocchia *et al.*[44]	20	MCL ($n=8$), MZL ($n=3$), FU ($n=6$) LL ($n=3$)	Yes ($n=10$)	*FLEC*: FLU 15 mg/m^2 x d1–4 + EPI 30 mg/m^2 d1 + CYC 200 mg/m^2 d1–4 q4wk	30	85	OS 48%, PFS 45% at 4years
Bocchia *et al.*[45]	30	BL ($n=11$), FU ($n=11$), MCL ($n=6$), MZL ($n=1$), MALT ($=1$)	No ($n=20$) Yes ($n=10$)	*FLEC*: FLU 15 mg/m^2 x d1–4 + EPI 60 mg/m^2 d1 + CYC 250 mg/m^2 d1–4 q3–4wk	43 50 (NPT) 30 (PT)	79 85 (NPT) 70 (PT)	OS at 27mo 70%; PFS at 24mo 79%
Fludarabine + idarubicin							
Caracciolo *et al.*[46]	16	Working formulation A ($n=7$), B ($n=1$), C ($n=8$)	No	*FLIDA*: FLU 25 mg/m^2 x d1–3 + IDA 10 mg/m^2 d1 + PRED 150 mg/m^2 po d1–5 q4wk	44	94	OS 85%, FFS 75% at 2years
Zinzani *et al.*[16] (mc, r, c)	198	FU ($n=102$), SLL ($n=38$), MCL ($n=29$), IMM ($n=29$)	No	FLU 25 mg/m^2 x d1–5 q4wk ($n=89$) versus FLU 25 mg/m^2 d1–3 + IDA 12 mg/m^2 d1 q4wk ($n=80$)	47 39	84 81	OS ~72% at 42mo for both groups
Tedeschi *et al.*[47] (phase I)	12	n/s	Yes	FLU 30 mg/m^2 x d1–3/5 + IDA 8–10 mg/m^2 d1 + DEX 20 mg/m^2 d1–3/5 q4wk	17	67	
Fludarabine + mitoxantrone							
Jain *et al.*[48]	46	FU ($n=41$), MZL ($n=5$)	n/s	FND alternating with CHOP (regimen n/s)	80 (molecular 62)	100	
Tsimberidou *et al.*[49] (r, c)	142	FL ($n=112$), SLL ($n=30$)	No	FLU 25 mg/m^2 x d1–3 + MITOX 10 mg/m^2 d1 + DEX 20 mg po d1–5 q4wk versus alternating triple therapy regimen. Maintenance interferon/DEX given for 1 year in both groups	79 87	97 97	5-year OS 84% and 82% (triple regimen)
Zinzani *et al.*[50] (r, c)	93	FL	No	FLU 25 mg/m^2 x d1–3 + MITOX 10 mg/m^2 d1 versus CHOP: DOXO 50 mg/m^2 d1 + CYC 750 mg/m^2 d1 + VINC 1.4 mg/m^2 d1 + PRED 100 mg po d1–5	68 37	94 93	

McLaughlin *et al.*[51] (phase II)	51	SLL ($n=13$), FSCCL ($n=26$), FML ($n=4$), FLCCL ($n=3$), MCL ($n=5$)	Yes	*FND*: FLU 25 mg/m^2 x d1–3 + MITOX 10 mg/m^2 d1 + DEX 20 mg iv/po d1–5 q4wk	47	94	Median FFS 21mo (CR), 9mo (PR)
Fludarabine + mitoxantrone							
Emmanouilides *et al.*[52] (phase II)	25	FU ($n=8$), SLL/CLL ($n=11$), MCL ($n=1$), other ($n=5$)	No ($n=12$) Yes ($n=13$)	FLU 25 mg/m^2 x d1–3 + MITOX 10 mg/m^2 d1 q4wk	42 (NPT) 23 (PT)	92 (NPT) 77 (PT)	
Velasquez *et al.*[38]	81	FU ($n=67$), SLL ($n=14$)	No	FLU 25 mg/m^2 x d1–3 + MITOX 10 mg/m^2 d1 q4wk	43	91	2-year PFS 63%, OS 93%
Seymour *et al.*[53]	22	n/s	Yes	FLU 25 mg/m^2 x d1–3 + MITOX 10 mg/m^2 d1 q4wk	11	90	2-year OS 65%
Zinzani *et al.*[54]	27	FU ($n=17$), SLL ($n=6$), IMM ($n=4$)	No	FLU 25 mg/m^2 x d1–3 + MITOX 10 mg/m^2 d1 q4wk	67	89	OS 92% at 32mo, RFS 83% at 2years (estimated)
Foussard *et al.*[55] (mc, r)	53 at 1y	n/s	No	FLU 20 mg/m^2 x d1–5 + MITOX 10 mg/m^2 d1 versus CHEP: DOXO 25 mg/m^2 d1 + CYC 750 mg/m^2 d1 + VIND 3 mg/m^2 d1 + PRED 50 mg/m^2 po d1–5	44 22	84 48	
Zinzani *et al.*[56] (phase II)	48	b1 ($n=18$), b2 ($n=22$), b3 ($n=8$)	Yes	FLU 25 mg/m^2 x d1–3 + MITOX 10 mg/m^2 d1 + PRED 40 mg po d1–5 q3wk	35	83	OS 67% at 33mo
Crawley *et al.*[57] (phase II)	54	FU	Yes ($n=44$)	FLU 25 mg/m^2 x d1–3 + MITOX 10 mg/m^2 d1 + DEX 20 mg po d1–5 q4wk	20	69	
Pott *et al.*[58] (phase II)	34 (cycle 2) 23 (cycle 4) 13 (cycle 6)	b4 ($n=13$), b5 ($n=9$), b1 ($n=12$), T–CLL ($n=1$), monocytoid B-cell lymphoma ($n=1$)	Yes	FND: FLU 25 mg/m^2 x d1–3 + MITOX 10 mg/m^2 d1 + DEX 20 mg po d1–5 q4wk	9 (cycle 4)	57 (cycle 4)	
Fludarabine + mitoxantrone + cyclophosphamide							
Montoto *et al.*[59]	40	FL	No	FLU 25 mg/m^2 x d1–3 + MITOX 6 mg/m^2 d1 + CYC 200 mg/m^2 d1–3 q4wk	75	95	18-mo FFS 90%
Spriano *et al.*[60]	54	FL	No	FLU 25 mg/m^2 x d1–3 + MITOX 10 mg/m^2 d1 + CYC 300 mg/m^2 d1–3 q4wk	61	91	3-year OS (estimated) 91%, FFS 74%
Santini *et al.*[39] (mc, c)	31	FU ($n=21$), LL ($n=4$), MCL ($n=4$), MZL ($n=2$)	Yes	FLU 25 mg/m^2 x d1–3 + MITOX 10 mg/m^2 d1 + CYC 300 mg/m^2 d1–3 q4wk	61	84	3-year OS 71%, 3-year FFS 57%
Fludarabine + paclitaxel							
Younes *et al.*[61] (phase I)	19	SLL ($n=6$), FSCCL ($n=5$), FML ($n=2$), FLCCL ($n=4$), FU ($n=1$), MALT ($n=1$)	Yes (all CHOP)	FLU 12, 15, 20 mg/m^2 d1–5 + PAC 25, 30, 60 mg/m^2 d1–4 q4wk	11	50	
Abbasi *et al.*[62] (phase I)	13	SLL ($n=6$), FML ($n=2$), MCL ($n=2$), Other ($n=3$)	Yes ($n=8$)	FLU 25 mg/m^2 d1–3 + PAC 125/150/175 mg/m^2 d3 q4wk	0	15	

Table 4 (*Continued*)

Reference (study design)[a]	*No. of evaluable patients*	*Histology*	*Prior therapy*	*Treatment regimen*[b]	*Clinical response (%)*		*Survival/duration of response*[a]
					CR	*CR + PR*	
Fludarabine plus rituximab							
Czuczman *et al.*[9]	30	n/s	No ($n = 18$)	FLU 25 mg/m^2 d1–5 q4wk + RIT 375 mg/m^2 d1 and d5 during wk 1 and 26. Single RIT infusions given 72 h prior to cycles 2, 4 and 6 of FLU	80	93	DOR 14mo (ongoing)

[a]Information provided where available.
[b]All doses are daily doses administered iv unless specified otherwise.
Abbreviations: b = according to Kiel classification; b1 = lymphoplasmocytoid immunocytoma; b2 = centroblastic/centrocytic follicular; b3 = centroblastic/centrocytic follicular and diffuse; b4 = centrocytic/centroblastic NHL; b5 = centrocytic NHL; BL = B lymphocytic; c = comparative; CR = complete response; CYC = cyclophosphamide; d = days; DEX = dexamethasone; DOR = duration of response; DOXO = doxorubicin; EPI = epirubicin; FCC = follicular center cell lymphoma; FFS = failure-free survival; FILG = filgrastim; FL = follicular; FLCCL = follicular large cleaved cell lymphoma; FLU = fludarabine; FML = follicular mixed lymphoma; FSCCL = follicular small cleaved cell lymphoma; FU = follicular unspecified; G-CSF = granulocyte colony-stimulating factor; G-MCSF = granulocyte macrophage-colony stimulating factor; IDA = idarubicin; IMM = immunocytoma; LL = lymphocytic lymphoma; MALT = mucosa-associated lymphoid tissue; mc = multicenter; MCL = mantle cell lymphoma; MITOX = mitoxantrone; mo = month; MZL = marginal zone lymphoma; NPT = no previous therapy; n/s = not stated; OS = overall survival; PAC = paclitaxel; PFS = progression-free survival; po = oral; PR = partial response; PRED = prednisone; PT = previous therapy; RFS = regression-free survival; RIT = rituximab; SLL = small lymphocytic lymphoma; q3–4wk = every 3–4 weeks; q4wk = every 4 weeks; r = randomized; VINC = vincristine; VIND = vindesine.

idarubicin combination therapy ($n = 199$), hematological adverse events (ECOG scale $\geqslant 3$) were observed during just 3.8 and 3.7% of treatment courses, respectively, and both granulocytopenia and thrombocytopenia were generally of short duration.[16] Infectious episodes were reported in a total of five patients.

Conclusions

Other than in a few patients with localized disease, indolent NHL is still incurable and no single or combination chemotherapy regimen can be considered the standard treatment option for the disease.[10] Despite this, several treatment options are available that have been shown to offer benefit to patients with NHL. Included among these is fludarabine, for which there is now a wealth of clinical data demonstrating it's efficacy in the management of patients with newly-diagnosed or relapsed/refractory NHL. When used as monotherapy for the first- and second-line treatment of indolent NHL, fludarabine achieves objective response rates (31–84%) that are at least, if not better than conventional therapies such as CVP. As a component of combination therapy in this setting even higher objective response rates have been achieved (70–100%), often exceeding those reported with conventional chemotherapeutic regimens such as CVP and CHEP. Fludarabine is also generally well tolerated, with a tolerability profile that generally compares favorably with that of other treatment options.

Although significant survival benefits are notoriously difficult to achieve in patients with NHL, fludarabine has been shown to have a beneficial effect on overall and progression-free survival in certain studies, and was also associated with a significant improvement in survival when used in combination with cyclophosphamide, mitoxantrone and rituximab. These encouraging data therefore suggest that fludarabine should be useful in a variety of settings for the treatment of indolent NHL. Further clinical studies will hopefully clarify its optimal role whether used as monotherapy or as a component of combination therapy.

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Use of fludarabine in the treatment of mantle cell lymphoma, Waldenström's macroglobulinemia and other uncommon B- and T-cell lymphoid malignancies

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After initial efforts using fludarabine as a single agent in the treatment of acute leukemia, its activity at lower and safer doses was demonstrated in chronic lymphocytic leukemia (CLL) patients who were refractory or had relapsed from traditional chemotherapies, representing a highly effective therapy for this condition. Fludarabine was also rapidly shown to be beneficial as first-line therapy in CLL. There is now considerable evidence that fludarabine is an effective agent in non-Hodgkin's lymphoma and in combination therapy for acute myeloid leukemia. Further, good responses are achieved when fludarabine-based approaches are used as conditioning regimens prior to transplantation procedures. The actions of fludarabine are not restricted to these settings and its potential role in the treatment of a range of uncommon T- and B-cell lymphoid malignancies is slowly emerging. This review will focus on the characteristics and treatment options for two B-cell disorders, mantle cell lymphoma and Waldenström's macroglobulinemia, with emphasis on the clinical activity of fludarabine. Additionally, the advantages of using fludarabine-containing regimens for a range of other lymphoproliferative conditions will also be discussed. These include B-cell neoplasms such as the CLL variant prolymphocytic leukemia, hairy cell leukemia and mucosa-associated lymphoid tissue-derived lymphomas; the T-cell disorders cutaneous T-cell lymphoma, angioimmunoblastic lymphadenopathy and other rarer T-cell diseases; and aggressive variants of non-Hodgkin's lymphoma including Richter's syndrome.
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Introduction

The development of fludarabine over a decade ago was an important stimulus to research into treatments for B-cell chronic lymphocytic leukemia (CLL) and other hematological disorders. The activity of this purine analog was established when it was used as salvage therapy in relapsed or refractory CLL patients.[1,2] In chemotherapy-pretreated patients, fludarabine typically produces objective response (OR) rates of 30–60%[3,4] and is more effective than CAP (cyclophosphamide, doxorubicin, prednisone) (OR 48 versus 27%; $P=0.036$).[5] Indeed, fludarabine is widely regarded as the gold-standard second-line therapy for CLL.

Fludarabine also demonstrates good activity as first-line therapy for CLL, with significantly higher OR rates than traditional chemotherapy with chlorambucil (63 versus 37%; $P<0.001$)[6] or CAP (71 versus 58%; $P<0.0001$;[7] but also 71 versus 60%; $P=0.26$[5]), and a higher proportion of clinical remissions than CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone; 40 versus 30%; $P<0.004$).[7] Remission rates of 60–70% have been demonstrated with fludarabine as first-line therapy for CLL.[3,4] A recently developed oral formulation of fludarabine has been shown to be as effective and well-tolerated as intravenous fludarabine,[8,9] and is preferred on the basis of fewer hospital admissions and more favorable cost-effectiveness.

Fludarabine is an effective therapy for other lymphoid cancers, including indolent non-Hodgkin's lymphoma (NHL). As reviewed by Zinzani earlier in this supplement, first- or second-line treatment of indolent NHL with fludarabine monotherapy achieves OR rates of 50–84% in most studies; better response rates (80–100%) can be achieved when fludarabine is used in combination with other cytotoxics. Furthermore, there is good evidence to support the effectiveness of fludarabine-based combinations in acute myeloid

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leukemia and in conditioning regimens prior to transplantation procedures (discussed by Jackson and Carella, respectively, later in this supplement).

In addition to CLL and NHL, there is substantial information on fludarabine for the treatment of other, less common lymphoid malignancies. A moderate amount of evidence exists for the activity of fludarabine in mantle cell lymphoma (MCL) and Waldenström's macroglobulinemia (WM), and a review of this experience will be presented here. This article will also discuss the use of fludarabine in a range of other uncommon B- and T-cell disorders, and aggressive variants of NHL.

B-cell disorders

Mantle cell lymphoma

The disease MCL is now recognized as a distinct histological and clinical entity on the basis of morphologic, immunophenotypic, and cytogenetic and molecular genetic features by the revised REAL[10] and WHO[11] classification systems. MCL is a rare lymphoma, accounting for a small proportion of NHLs in Europe (7–9%), although lower incidences are found in the USA (2.5–4%).[12] Tumor cells in MCL originate from immunologically naïve B cells of the follicle mantle of lymph nodes and, on histopathologic examination, appear as atypical small lymphoid cells. Immunophenotypically, these lymphocytes express a distinctive pattern of cell-surface markers, notably CD19, CD20, CD22, surface immunoglobulin IgM and IgD, and commonly CD5; expression of CD10 and CD23 are usually absent.[12,13] In the majority of MCL cases, the hallmark cytogenetic feature is the translocation t(11;14)(q13:q32), which is thought to underlie the pathogenesis of this disorder.

An accurate diagnosis of MCL is often difficult and requires both histologic and phenotypic evidence to distinguish this disease entity from other related lymphoid disorders. The median age at diagnosis is 60–65 years, with male patients consistently affected more frequently than female patients (up to 6.5 : 1).[14] At diagnosis, 80–90% of cases are stage III/IV according to the Ann Arbor classification system, with most patients (75–100%) presenting with generalized nodal involvement.[15] Other common clinical features of MCL include infiltration of the bone marrow (60–87% of cases), spleen (33–75%), blood (20–58%) or liver (35%), multiple gastrointestinal involvement (15–40%) and B symptoms (25–50%).[13,15] Infiltration of the central nervous system has also been reported (4% in one study).[16]

The prognosis of MCL patients is generally poor, with a median survival of 36 months.[15] However, a recent review of 68 cases[17] reported that overall survival rates varied according to pathologic features. In this study, patients with the diffuse lymphocytic variant had the worst median survival (16 months) in contrast to those with the blastic or nodular types (55 and 50 months, respectively). Other adverse prognostic factors include age >65 years, advanced stage, high lactic dehydrogenase levels, bone marrow involvement, poor performance status and B symptoms.[13,15,17] A full account of the prognostic factors, clinical features, diagnosis and genetics of MCL is reviewed elsewhere.[12,15,18,19]

Treatment options Patients with MCL typically have only a moderate outcome in terms of survival with conventional chemotherapies despite the fact that treatment achieves a relatively high first-line remission rate (up to 90%). In a minority of patients, specifically those at low risk (stage I/II), radiotherapy has proved to be effective.[13] However, relapses are frequent and the median time to treatment failure can be as short as 14 months.[20] Salvage regimens are generally unsuccessful, producing low response rates and shorter remission times.[19] When considering these issues, the continued development of new therapeutic approaches for the management of MCL is justified.

Conventional chemotherapy: When used first-line, conventional chemotherapy achieves response rates of 50–90%, with complete response (CR) rates $>50\%$ frequently reported.[13] The standard therapy for MCL has been combination chemotherapy with COP (cyclophosphamide, vincristine, prednisone) or the CHOP regimen. A randomized multicenter investigation found no significant difference between COP and CHOP regimens in terms of CR (41 versus 58%), partial response (PR; 43 versus 31%) and overall survival (32 versus 37 months).[21] Although anthracycline-containing therapies may result in better survival in low-risk patients[22] and have a tendency for higher CR rates, the advantages of such combination regimens remain unclear.[13,15,17]

Other combination chemotherapy regimens tested in MCL include PmM (prednimustine, mitoxantrone),[23] CHVmP-VB (cyclophosphamide, doxorubicin, vincristine, prednisone, teniposide, bleomycin) and ProMACE-MOPP (cyclophosphamide, doxorubicin, etoposide, mechlorethamine, vincristine, procarbazine, prednisone).[24] However, none of these therapies consistently improve the natural history of MCL. Furthermore, although interferon-α demonstrates activity when used in combination with anthracycline-containing regimens in first-line treatment,[25] it is of limited use as maintenance therapy.

Fludarabine: The activity of fludarabine in MCL has been demonstrated in recent phase II clinical investigations. The standard dosing regimen for intravenous fludarabine is 25 mg/m^2/day for 5 consecutive days every 28 days for 4–8 cycles. When used first-line in 17 MCL patients with advanced-stage disease, fludarabine monotherapy achieved a remission rate of 41% (CR 29%) with median response and survival times of 1.2 and 1.9 years, respectively.[26] These findings are consistent with those of a retrospective study of 121 patients, which reported an OR rate of 33% and a CR rate of 25% in 12 patients given fludarabine as primary treatment.[27] In a

cohort of MCL patients who were mostly pretreated (13 of 15), fludarabine monotherapy produced an OR rate of 33% with no CRs and a short response duration (4–8 months), results that underline the relative insensitivity of relapsed patients to subsequent therapies.[28] Treatment with fludarabine was well-tolerated in these studies; adverse advents were typically related to myelosuppression and infection, mostly of WHO grade I/II severity.

Based on the improved effectiveness of fludarabine when used in combination with other chemotherapeutic agents in patients with CLL and other lymphoid malignancies, fludarabine-combination chemotherapy regimens have been tested in MCL. The FC regimen is the most successful investigated to date and there is reported experience with the Eastern Cooperative Oncology Group schedule of fludarabine (20–25 mg/m^2/day on days 1–5) with cyclophosphamide (500–1000 mg/m^2 on day 1) repeated every 28 days for up to six cycles. One prospective, multicenter study involving 10 high-risk MCL cases reported an OR rate of 80% (CR 40%) in response to first-line FC.[29] A single-center retrospective analysis of Ann Arbor stage IV MCL patients reported a similar observation; FC achieved a 100% OR rate (CR 70%) in 10 previously untreated patients, with a 45% OR rate (CR 10%) when tested in 20 previously treated cases (Figure 1).[30] The major toxicities associated with FC therapy were hematological (eg neutropenia, thrombocytopenia and anemia) with infections also commonly reported.

Another fludarabine-based chemotherapy regimen tested in MCL is fludarabine plus idarubicin, as evaluated by Zinzani *et al*.[31,32] In these trials involving treatment-naïve MCL patients, no apparent advantage was conferred by the addition of idarubicin to fludarabine (OR 61%, CR 28–33%) over fludarabine alone (OR 64–72%, CR 27%); relapse-free and progression-free survivals were not significantly different between the two regimens. Additionally, the combination of fludarabine with bendamustin has been assessed in a study of 14 relapsed or refractory low-grade lymphoma patients (including five patients with MCL) with good results (OR 64%, CR 21%).[33] A summary of the experiences of fludarabine-containing regimens in MCL is presented in Table 1.

Immunotherapy: In MCL, a strongly CD20-positive tumor, the anti-CD20 antibody rituximab has demonstrated clear activity. In the most important prospective, multicenter trial conducted to date, rituximab (administered at the standard intravenous regimen of 375 mg/m^2 once weekly for 4 weeks) achieved similar response rates (OR, CR) in patients who were treatment-naïve (38, 16%) or previously treated (37, 14%).[34] The median duration of response was 1.2 years. Apart from the toxicities commonly associated with rituximab infusion, the most frequent adverse events in this study were hematological events and infection. Remission rates of 20–33% have been reported in other smaller investigations of rituximab in MCL.[35–37]

Considering the potential synergy between cytotoxic and biological agents, clinical trials have been initiated in MCL patients to assess the effectiveness of combining rituximab with traditional (CHOP) or newer (fludarabine) chemotherapeutic agents. In a phase II study in previously untreated MCL patients, rituximab plus CHOP produced a response rate of 96%.[38] Finally, a UK-wide phase II randomized study in treatment-naïve MCL patients is currently underway to evaluate the response of fludarabine plus cyclophosphamide, with or without the addition of rituximab. Further information on this trial can be obtained through the National Cancer Research Network (www.ncrn.org.uk).

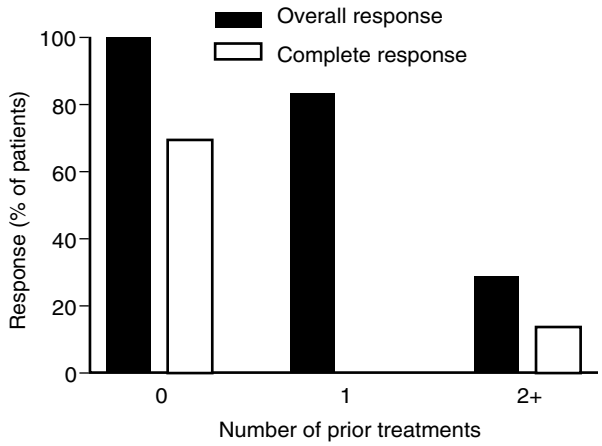

Figure 1 Responses to fludarabine + cyclophosphamide in previously untreated ($n = 10$), primary refractory ($n = 6$; one prior treatment) or refractory relapse ($n = 14$; $\geqslant 2$ prior treatments) patients with MCL.[30]

Waldenström's macroglobulinemia (WM)

The disease WM is a rare, chronic lymphoproliferative disorder characterized by the presence of monoclonal B lymphocytes that produce monoclonal immunoglobulin M (IgM) paraprotein. According to the revised REAL[10] and WHO[11] classification of lymphoid neoplasms, WM represents cases that are included under the diagnosis of immunocytoma/lymphoplasmacytoid lymphoma. The disease accounts for $<2\%$ of hematological cancers, and age-adjusted incidences are 3.4 and 1.7 per million person-years at risk in men and women, respectively.[39] The incidence of WM increases with age (median age at diagnosis: 60–65 years).

The diagnosis of WM should be made on the combined findings of the presence of an IgM monoclonal gammopathy and infiltration of the bone marrow with cells of typical immunophenotype and morphology. The pattern of marrow infiltration may be interstitial or diffuse, but is often characterized by a mixture of small lymphocytes, lymphoplasmacytoid cells and mature plasma cells.[40] In the majority of cases, lymphocytes show a strong expression of the cell-surface markers IgM, CD19 and CD20, but not CD5, CD10, CD23 or IgD.[41] At present, no cytogenetic abnormalities characteristic of WM have been reported.

Table 1 Efficacy of fludarabine in patients with MCL

Reference (study design)	*Treatment regimen*	*Median age (years)*	*Prior therapy*	*Number of patients evaluable*	*Clinical response (% of patients)*		*Survival*
					OR	*CR*	
Fludarabine monotherapy							
Foran *et al.* (1999)[26] (phase II)	25 mg/m^2 i.v. for 5 days, every 4 weeks; maximum eight cycles	64	None	21	41	29	TTP 1.1 years
Samaha *et al.* (1998)[27] (retrospective)	NS	NS	None	12	25	8	NS
Decaudin *et al.* (1998)[28] (phase II)	25 mg/m^2 i.v. for 5 days, every 4 weeks; maximum cycles NS	62	None Prior therapy	2 13	50 31	0 0	RD: 7 months RD: 4–8 months
Fludarabine + cyclophosphamide (CYCLO)							
Cohen *et al.* (2001)[30]	FLU 20–25 mg/m^2 i.v. for 4–5 days + CYCLO 500–1000 mg/m^2 i.v. for 1 day, every 3–4 weeks; median three cycles	64	None Primary refractory Refractory relapse	10 6 14	100 83 29	70 0 14	OS: 42+ months; FFS: 28 months OS: 18 months; FFS: 5 months OS: 16 months; FFS: 3 months
Flinn *et al.* (2000)[29] (phase II)	FLU 20 mg/m^2 i.v. for 5 days + CYCLO 600 mg/m^2 i.v. for 1 day, every 4 weeks; maximum six cycles	NS	None	10	80	40	NS
Fludarabine + idarubicin (IDA)							
Zinzani *et al.* (2000)[32] (COMP)	FLU 25 mg/m^2 i.v. for 5 days *versus* FLU 25 mg/m^2 i.v. for 3 days + IDA 12 mg/m^2 i.v. for 1 day; both regimens repeated every 4 weeks, maximum six cycles	NS	None	11 18	72 61	27 33	No significant differences in terms of both RFS + PFS
Zinzani *et al.* (1999)[31]	FLU 25 mg/m^2 i.v. for 5 days *versus* FLU 25 mg/m^2 i.v. for 3 days + IDA 12 mg/m^2 i.v. for 1 day; both regimens repeated every 3 weeks, maximum six cycles	57	None	11 18	64 61	27 28	For both groups combined: OS: 20 months RFS: 15 months
Fludarabine + bendamustin (BEND)							
Koenigsmann *et al.* (2001)[33] (phase I/II)	FLU 30 mg/m^2 i.v. + BEND 30–50 mg/m^2 i.v. for 3 days, every 4 weeks; maximum six cycles	62	Prior therapy	14 (5 MCL; 9 FL)	64	21	NS

Abbreviations: BEND, bendamustin; COMP, comparative; CR, complete response rate; CYCLO, Cyclophosphamide; FFS, failure-free survival; FL, follicular lymphoma; FLU, fludarabine; IDA, idarubicin; i.v., intravenously; NS, not stated; OR, overall response rate; OS, overall survival; PFS, progression-free survival; RD, response duration; RFS, relapse-free survival; TTP, time to progression.

The clinical manifestations associated with WM are highly variable. Many patients are asymptomatic at presentation, while others have nonspecific symptoms (weakness, anorexia, weight loss) or more advanced, specific features related to direct tumor infiltration and IgM paraprotein properties. These features include hepatomegaly, lymphadenopathy, splenomegaly, hematological abnormalities (anemia) or symptoms due to increased serum viscosity.[42] Hyperviscosity may lead to mucosal hemorrhage, visual impairment and neurological symptoms. The physicochemical properties of the IgM paraprotein may also result in cryoglobulinemia, while IgM antibody activity may produce cold-agglutinin hemolysis or neuropathy. In addition to clinical features, the identification of prognostic factors has improved the stratification of patients according to risk and treatment suitability. Advanced age and number of cytopenias have consistently been identified as significant factors associated with poor outcome, as reviewed by Johnson.[43]

Treatment options Patients without symptoms should not be treated. For patients in whom treatment is indicated, a number of options are available.

Plasmapheresis: Standard therapy for patients with WM who have evidence of hyperviscosity is based initially on reducing plasma viscosity by plasmapheresis followed by combination chemotherapy to reduce the abnormal cell population. Plasmapheresis is indicated in patients with severe constitutional symptoms or significant bulky disease related to the level or viscosity of the IgM paraprotein. This supportive therapy is an effective strategy for patients with hyperviscosity, cryoglobulinemia and IgM-related neuropathy. This treatment offers rapid, but limited (4–6 weeks) benefit to most patients, and is therefore typically used only as a short-term measure until concomitant cytotoxic therapy becomes effective (as reviewed by Johnson).[43] However, prolonged plasmapheresis may be suitable for patients who are older and who are unlikely to tolerate or who are resistant to chemotherapy, or whose symptoms are specific to the paraprotein rather than tumor burden.[44]

Alkylating agents: Chlorambucil with or without prednisone is the most commonly used initial therapy in WM. The response rate is 50–60% with CRs only rarely reported; median survival is approximately 5 years.[45] Responses to chlorambucil are usually slow and may take up to 18 months. A recent prospective, randomized study comparing intermittent versus continuous dosing of chlorambucil showed no difference in response rate (68 versus 79%); median survival was 5.4 years with no difference between the two regimens.[46] Cyclophosphamide-based combination therapies as primary treatment in WM have been investigated in several phase II studies with higher remission rates and median survival than those reported for chlorambucil (see Johnson[43] for a review).

Fludarabine: Fludarabine was one of the first effective therapies for patients who relapse from, or are resistant to, initial alkylator therapy. When used in this setting, responses to single-agent fludarabine are achieved in 30–40% of patients, although most are PRs (Table 2).

In the largest retrospective study published to date, a maximum of nine courses of standard-dose fludarabine were administered to 71 pretreated WM patients (63 refractory relapse patients; eight primary refractory patients).[49] The remission rate was 30% with no CRs; the overall median survival time was 23 months. The results of a multicenter, randomized comparative study demonstrated a significantly higher response rate for fludarabine versus CAP in 92 WM patients in first relapse or with resistance to first-line therapy with alkylating agents (30 versus 11%; $P=0.019$). The median duration of response (19 versus 3 months; $P<0.01$) and event-free survival rate (Figure 2), but not median survival time (41 versus 45 months), were significantly longer after fludarabine therapy.[48] Fludarabine monotherapy may also be appropriate for patients pretreated with another purine analog, cladribine.[52] Finally, the results of a small trial involving 11 patients (only two were treatment-naïve) suggest a potential benefit of combining cyclophosphamide and fludarabine (OR 55%; all PRs).[53]

In addition to its activity as salvage therapy, fludarabine is also an effective first-line treatment for WM (Table 2). Initial results from a small retrospective analysis of 19 previously untreated patients reported a remission rate of 79%.[26] A subsequent phase II trial by the Southwest Oncology Group involving 182 patients evaluated the role of fludarabine in both previously untreated and previously treated WM. Similar results were achieved in treatment-naïve and pretreated cases in terms of OR (38 versus 33%), but not 5-year rates of overall survival (62 versus 36%) and progression-free survival (49 versus 30%).[47] In a follow-up report by this group, progression-free survival was reported to be 59 and 30 months in previously untreated and pretreated patients, respectively.[54]

A number of case reports have indicated that single-agent fludarabine may have activity in a variety of other rare disorders that are related to WM by the production of paraprotein (often IgM). High response rates have been achieved by fludarabine in patients with splenic lymphoma with villous lymphocytes (SLVL), as reported by case studies on four patients (four CRs)[55] and a retrospective analysis of 10 patients (seven CRs, three PRs).[56] Single case reports of CRs to fludarabine indicate that this agent has activity in γ-heavy chain disease,[57] cold hemagglutinin hemolysis[58] and type II mixed cryoglobulinemia[59,60] (but see also Zaja;[61] none of four patients responded to fludarabine). Furthermore, since fludarabine monotherapy was first tested in multiple myeloma and found to be inactive (0/31 patients responded),[62] the benefits of fludarabine used in combination with vincristine, doxorubicin and dexamethasone (VAD) have been demonstrated (OR 90 versus 56% for VAD alone).[63]

Other treatment options: A range of other therapies are also available for WM, as reviewed elsewhere.[39,43] The purine analog cladribine has been assessed in a number of small studies, with response rates of 40–85%;

Table 2 Efficacy of fludarabine in patients with WM

Reference (study design)	*Treatment regimen*	*Median age (years)*	*Prior therapy*	*Number of patients evaluable*	*Clinical response (% of patients)*		*Survival*
					OR	*CR*	
Monotherapy							
Foran *et al.* (1999)[26] (phase II)	25 mg/m^2 i.v. for 5 days, every 4 weeks; maximum eight cycles	64	None	19	79	5	TTP 3.4 years
Dhodapkar *et al.* (2001)[47]	30 mg/m^2 i.v. for 5 days, every 4 weeks; 4–8 cycles	66	None Prior therapy	118 64	38 33	3 0	OS 5-year: 62% OS 5-year: 36%
Leblond *et al.* (2001)[48] (COMP)	25 mg/m^2 i.v. for 5 days, every 4 weeks; maximum six cycles	64	Primary refractory or primary relapse	46	30	0	MRD: 19 months; MS: 41 months
	versus CAP[a]	65		46	11	0	MRD: 3 months; MS: 45 months
Leblond *et al.* (1998)[49]	25 mg/m^2 i.v. for 5 days, every 4 weeks; maximum nine cycles	68	Primary refractory Refractory relapse	8 63	30	0	MS: 23 months MRD: 32 months
Dimopoulos *et al.* (1993)[50]	30 mg/m^2 i.v. for 5 days, every 4 weeks; until maximum response (median five cycles)	65	None Primary refractory Refractory relapse	2 14 12	100 43 17	NS NS NS	NS NS NS
Zinzani *et al.* (1995)[51]	25 mg/m^2 i.v. for 5 days, every 3–4 weeks; maximum six cycles	56	Primary refractory Refractory relapse	4 8	50 37	NS NS	Response duration: 6–15 months
Lewandowski *et al.* (2002)[52]	25 mg/m^2 i.v. for 5 days, every 28–45 days; median two cycles	67	Primary refractory (all to cladribine)	6	33	0	NS
Combination therapy							
Dimopoulos *et al.* (2003)[53]	FLU 25 mg/m^2 and CYCLO 250 mg/m^2 i.v. for 3 days, every 4 weeks; four cycles	73	None Primary refractory Refractory relapse	2 7 2	55 (overall)	0	Overall MRD: 24 months

[a]CAP (750 mg/m^2 cyclophosphamide and 25 mg/m^2 doxorubicin given i.v. on day 1, and 40 mg/m^2 prednisone given orally on days 1–5; repeated every 4 weeks with a target of six courses).
Abbreviations: COMP; comparative; CR, complete response rate; CYCLO, cyclophosphamide; FLU, fludarabine; i.v., intravenously; MRD, median response duration; MS, median survival; NS; not stated; OR, overall response rate; OS, overall survival; TTP, time to progression.

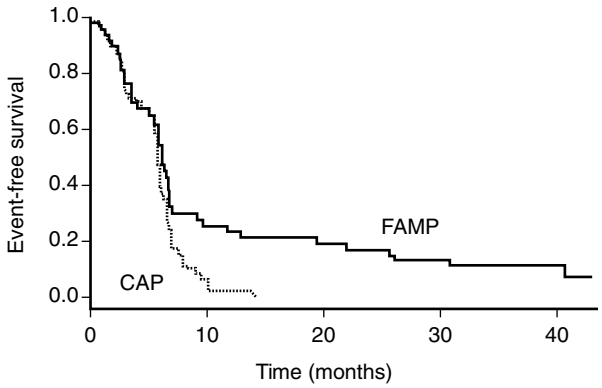

Figure 2 Treatment with fludarabine (FAMP) produced significantly longer ($P<0.01$, log-rank test) event-free survival in WM patients compared with CAP.[48] Copyright American Society of Hematology, used with permission.

CRs to cladribine are rarely reported. Following the success of thalidomide in multiple myeloma, this agent has been tested in a small cohort of WM patients and achieved a remission rate of 25%. As for MCL, interferon-α also appears to be of limited use in WM; although a response rate of 50% (median duration of response 27 months) to interferon-α was reported in an early study on a small series of patients, no confirmatory studies have been conducted. As the cells of macroglobulinemia strongly express CD20, it is not unexpected that response rates of 23–57% (all PRs) have been reported for the anti-CD20 antibody rituximab. Finally, good responses have been achieved with high-dose chemotherapy followed by autologous or allogeneic stem cell transplantation techniques, but this approach is typically limited to younger patients.

CLL variants

Prolymphocytic leukemia B-cell prolymphocytic leukemia (B-PLL) is a rare lymphoproliferative disorder related to CLL, but is now recognized as a separate disease entity by the WHO classification system.[10] The presence of circulating prolymphocytes in the peripheral blood and marrow, most of which are precursors to B lymphocytes, is the characteristic morphology of B-PLL. The immunophenotype of these cells is typically strong surface Ig, CD22, CD79b and FMC7, but negative for CD23 and (usually) CD5.[64] The rarity of this disorder has precluded the firm identification of prognostic features, although clonal abnormalities such as t(11;14) and deletions of 13q, 11q or p53 have been reported.[65,66]

Patients usually present with massive splenomegaly, leukemic infiltration of the marrow and liver, and white cell count $>100\times10^9$/L, but with little or no lymphadenopathy. Fatigue, weight loss and sweating are also common. Patients with B-PLL are usually over 70 years of age. It has been proposed that the presence of >55% prolymphocytes defines B-PLL, with 11–55% prolymphocytes considered a CLL prolymphocytic variant (CLL/PLL).[67] Compared to CLL, B-PLL is generally less responsive to therapy and more aggressive; most patients die from hematological complications. In one series of 38 cases of B-PLL, the median survival was 3 years.[67]

There is no standard therapy for B-PLL and conventional chemotherapy is ineffective. There are few data regarding the usefulness of salvage therapy in treatment-resistant patients. As such, other therapies such as fludarabine have been evaluated in a limited number of studies. In the largest study to date on fludarabine (±prednisone), its activity was assessed in patients with B-PLL ($n=5$) or CLL/PLL ($n=12$). An OR rate of 50% was achieved (CR 25%) in CLL/PLL patients, but no ORs were achieved in B-PLL patients.[68] More anecdotally, case reports indicate that fludarabine can induce CR in chlorambucil-resistant B-PLL,[69] including one account of a long-lasting response (5 years).[70] Finally, fludarabine also achieved a CR in one patient with B-PLL complicated by leukemic pleural effusion.[71]

Hairy cell leukemia Hairy cell leukemia (HCL), previously known as leukemic reticuloendotheliosis, is an indolent disorder that accounts for about 2% of all leukemias. The B-cell lineage in HCL is supported by immunophenotypic expression of moderate surface Ig, intense CD22 and CD20, and coexpression of CD19 with either B-ly7, or CD11 and CD25; CD5 and CD10 are usually absent.[72] The clinical course of HCL is variable. Common features include splenomegaly, palpable liver, thrombocytopenia, neutropenia, varying degrees of anemia, fatigue and fever; lymphadenopathy is less frequent.

Splenectomy was the mainstay of therapy for HCL until the early 1980 s, but has now been replaced by a number of cytotoxic agents. Two agents with demonstrated activity in HCL are fludarabine and cladribine. Anecdotal evidence from a number of case reports suggests that fludarabine may be an effective treatment in HCL patients who are refractory or have relapsed from previous chemotherapy with interferon-α or 2′-deoxycoformycin.[73,74] The actions of cladribine have been documented much more extensively, for example in one study involving 50 previously untreated or treated HCL patients with a high rate (80%) of durable CRs was achieved.[75] More recently, the anti-CD22 immunotoxin BL22 has demonstrated activity in 16 cladribine-resistant patients (OR 81%, CR 69%).[76] The reader is referred to Mey *et al*.[77] for a more comprehensive review of current treatment options in HCL.

MALT lymphoma

Mucosa-associated lymphoid tissue (MALT)-derived lymphomas are the most common category of lymphomas occurring in extranodal organs. The occurrence of MALT is rare, accounting for 7–8% of all B-cell lymphomas. The median age of diagnosis is approximately 60 years. MALT lymphomas arise by the

recruitment of autoreactive lymphoid tissue at sites where this tissue is normally absent; this process is likely to be antigen-induced following an autoimmune or possibly environmental challenge. The most frequently involved site is the stomach, but other organs have been described, including lung, skin, thyroid, head and neck, orbit, salivary gland and other intestinal locations.[78] MALT is typically a low-grade disease that may relapse either locally within the site of origin (localized) or in other MALT sites (disseminated).

The clinical manifestations of MALT patients are highly variable, possibly reflecting the diversity of organs involved. However, patients typically present with indolent disease characterized by good performance status, no weight loss or B symptoms, and normal levels of lactate dehydrogenase or β2-microglobulin. The outcome of MALT patients is generally good with long overall survival (e.g. 5- and 10-year survival rates of 86 and 80%, respectively).[79] Recommended treatment options for MALT depend on the extent of the disease (localized versus disseminated).[78] In MALT-localized lymphomas, surgery or local radiation may be suitable, with chlorambucil preferred for advanced disease. For patients with disseminated MALT, chemotherapy with fludarabine or chlorambucil is recommended and achieves good remission rates, while CHOP is most appropriate for patients with a large tumor mass.[78]

T-cell disorders

Cutaneous T-cell lymphoma

Cutaneous T-cell lymphoma (CTCL) comprises a spectrum of disease resulting from proliferation of predominately helper T-cells. The most common form of CTCL is mycosis fungoides (MF), an indolent disorder involving skin manifestations such as cutaneous patches and plaques. Progression to erythroderma or development of tumors are poor prognostic factors; the median survival in tumor-stage MF is <40 months.[80] The Sézary syndrome (SS) is a leukemic form of CTCL characterized by diffuse erythroderma, the presence of circulating abnormal lymphocytes (Sézary cells), and frequent lymph node and visceral involvement.[81] Response to standard therapies is poor and transformation of SS into a more aggressive large-cell lymphoma is common; the median survival of patients with SS is 30 months.[80]

The most common therapeutic approaches in MF/SS are photochemotherapy and topical chemotherapy. However, patients with advanced disease are usually refractory to these conventional approaches. A number of alternative treatments, including fludarabine, have been assessed in advanced MF/SS. In early evaluations, fludarabine monotherapy demonstrated activity in advanced stage MF[82] and achieved a CR in a case study involving transformation of SS to large-cell lymphoma.[83] Based on these findings, encouraging results (including CRs) have been achieved in patients with advanced refractory MF/SS when fludarabine is given with cyclophosphamide (OR 45%)[84] or interferon-α2a (OR 51%).[85] Finally, the potential therapeutic role of the anti-CD52 antibody alemtuzumab in these high-risk patients (OR rates of up to 55%)[86,87] requires further investigation.

Angioimmunoblastic lymphadenopathy

Angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) is characterized by generalized lymphadenopathy, fever, hepatosplenomegaly, hemolytic anemia, polyclonal hypergammaglobulinemia, autoantibodies and pruritic skin rash.[88] In most cases, AILD follows an aggressive course with a short median survival; 75% of patients die within 2 years of presentation, despite receiving treatment. Standard combination chemotherapy is unsatisfactory in AILD, with complete and sustained remissions seen in only 25% of patients. There is anecdotal evidence for the activity of fludarabine in five patients with this disease: CRs were reported in each case, which included two treatment-naïve and three CHOP-relapsed patients.[89–92]

Other T-cell disorders

As detailed by the case reports that follow, responses to fludarabine-based regimens have been reported for the following T-cell disorders for which, owing to the rarity of most, optimal treatments have not been defined:

- Subcutaneous panniculitis-like T-cell NHL – characterized by subcutaneous tumor and plaques with accompanying hemophagocytic syndrome in many cases; rare involvement of lymph nodes.[93,94]
- T-cell large granular cell lymphocyte leukemia (T-LGL)[95] or T-cell CLL[96] – characterized by neutropenia and anemia, and also recurrent infection and transfusion dependence in T-LGL.
- Familial hemophagocytic lymphohistiocytosis – an aggressive disease characterized by T-cell and macrophage proliferation due to a perforin gene defect.[97]
- Immunoproliferative small intestinal disease (IPSID) – a MALT subtype characterized by high production of α-heavy chains.[98]
- Adult T-cell leukemia/lymphoma (ATLL) – an aggressive disease associated with human T-cell lymphotropic virus type 1 and characterized by subacute or chronic leukemia, skin lesions, lymphadenopathy, hepatosplenomegaly and hypercalcemia.[99]
- Hodgkin's disease – originates from neoplastic Hodgkin–Reed–Sternberg cells and characterized by involvement of lymph nodes.[100]

High-grade NHL/transformed low-grade NHL

The outcome of patients with relapsed/refractory aggressive NHLs is particularly poor. In one report

involving primary refractory aggressive NHL, a 15% response rate to conventional salvage chemotherapy was achieved with a median survival of 7 months (no patient survived beyond 2.5 years).[101] The combination of fludarabine with cisplatin and cytosine has been used for the treatment of aggressive NHL malignancies, including mantle cell NHL. Salvage therapy with this treatment regimen in 44 patients with refractory, histologically aggressive or mantle cell NHL achieved an OR rate of 48%, with 7% CRs. Patients with mantle cell NHL ($n=8$) were considerably more sensitive to this regimen than those with other histologies (OR 88 versus 39%) and survived longer (28 versus <4 months; $P=0.008$).[102] In another study involving (mostly) refractory NHL patients, this combination chemotherapy given with dexamethasone (FLUDAP) achieved a 39% remission rate, with 15% CRs;[103] the estimated median survival time was 34 months for responders and <5 months for nonresponders.

Richter's transformation of CLL

The development of aggressive NHL in the context of CLL is termed Richter's syndrome (RS). The NHL is typically diffuse large-cell lymphoma or its immunoblastic variant and develops in approximately 3% of CLL cases. The onset of RS is usually abrupt, with rapid clinical deterioration, tumor growth and/or extranodal involvement.[104] Much of the experience in RS has been reported by the MD Anderson Cancer Center.[104,105] Evidence from these retrospective reviews indicates that about 40% of patients with RS respond to CHOP-like therapy, but response duration and survival are generally poor ($\sim$5 months). The use of higher-intensity fludarabine/cisplatin/cytosine-based chemotherapy does not improve remission rates or extend survival.[106,107] However, a highly intensive regimen with cyclophosphamide-based combination therapy may double overall survival time to 10 months.[108]

Conclusions

The activity of fludarabine as second-line therapy in CLL is unquestionable, while its first-line effectiveness is now well established. In addition to CLL, fludarabine and fludarabine-based chemotherapy regimens are now recognized as useful therapeutic approaches for the treatment of NHL and acute myeloid leukemia. As discussed in this paper, fludarabine is emerging as an effective treatment option to manage a host of less common B-cell neoplasms such as MCL, WM (and related disorders), B-PLL, HCL and MALT. Further, evidence from case studies and anecdotal reports suggests that fludarabine is active against many rare T-cell cancers, including cutaneous T-cell lymphoma (MF/SS) and angioimmunoblastic lymphadenopathy with dysproteinemia, among others. Moreover, the potential benefits of fludarabine-based regimens have been suggested for aggressive variants of NHL, including those that develop in the context of other conditions (eg Richter's syndrome in CLL). Although these promising results with fludarabine for the treatment of rare or aggressive hematological lymphomas are largely anecdotal, such observations should encourage future study of the application of fludarabine in a variety of uncommon lymphoid malignancies.

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Use of fludarabine in the treatment of acute myeloid leukemia

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Acute myeloid leukemia (AML) is a disease, which when left untreated, is invariably fatal. The disease is more common in elderly people, who also fare worse than younger patients with AML due to a higher rate of unfavorable prognostic factors, such as poor performance status, multiple comorbidities, reduced tolerance to treatment, 'unfavorable' chromosomal abnormalities and multidrug resistant protein-1 expression. While many patients achieve a complete remission, the rate of relapse is high and prognosis after relapse very poor. Promising results have been published in recent years using fludarabine-containing combination therapy for AML, most commonly fludarabine + cytarabine + granulocyte colony-stimulating factor (G-CSF) [FLAG], FLAG + mitoxantrone (FLANG), or FLAG + idarubicin (FLAG-Ida). Such combinations maximize favorable cytotoxic interactions between cytarabine and G-CSF, and between cytarabine and fludarabine. In small studies, such combinations used as second-line therapy have resulted in complete response (CR) rates of 36–59%. Early retrospective analyses suggested higher CR rates in patients with refractory AML than in those with relapsed AML, but this observation has not been confirmed in recent prospective trials. Fludarabine-containing combinations have also been evaluated as first-line therapy in high-risk patients and resulted in CR rates of 34–70%, with median survival from 7 to 16 months. The current large MRC randomized high-risk study will provide further data on the use of fludarabine-containing regimens in patients with poor prognosis AML. Further studies are investigating the use of fludarabine in combination with other agents, such as gemtuzumab ozogamicin and gemcitabine, in patients with AML.
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Overview of acute myeloid leukemia

The acute leukemias are notable for a rapid clinical course, which untreated, is invariably fatal. These leukemias can be broadly categorized as lymphoblastic or myelogenous. Acute myeloid leukemia (AML) is a disease that is more common in older adults, with a median age at presentation of 65 years.[1] The overall incidence of AML is around 2.5 per 100 000 persons in the US, where it affects approximately 9000 individuals per year.[1] The incidence increases with increasing age, from around 1 per 100 000 at age 40 to more than 15 per 100 000 at age $\geqslant$75 years.[1]

AML can develop as a consequence of chromosomal abnormalities induced by prior chemotherapy, including epidophyllotoxins, anthracyclines or alkylating agents, or radiotherapy. Such cases of therapy-related leukemia (t-AML) are particularly difficult to treat. However, for the majority of patients with AML, the underlying cause of disease is unknown.

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Known prognostic factors for poor treatment response in AML are poor performance status, presence of pre-existing trilineage myelodysplasia, multidrug resistance protein (MDR1) positivity, elevated serum lactate dehydrogenase levels, CD34 positivity and 'unfavorable' cytogenetics. Older patients have a remarkably worse prognosis than younger patients. The poorer performance status, presence of multiple comorbidities and reduced tolerance to treatment in older patients is thought to contribute to their dismal prognosis. In addition, older patients tend to present with intrinsically different disease than is seen in younger patients, with a higher rate of 'unfavorable' chromosomal abnormalities. Various chromosomal abnormalities have been associated with better or worse disease prognosis. The t(15;17) translocation, t(8;21) and inv(16) are associated with a better survival than is seen in patients without these abnormalities, whereas the −5, del(5q), −7 karyotype, as well as abnormal (3q) and complex >4 abnormalities are associated with an unfavorable prognosis.[2,3] The presence of the so-called 'favorable' cytogenetics has been found to be more common among younger patients and decreases with increasing age.[4] Older AML patients are also more likely to have leukemic cells overexpressing MDR-1. An analysis of 211 AML

patients aged >55 years found that 71% had MDR1 expression.[5]

If left untreated, AML is inevitably fatal due to complications associated with bone marrow failure, namely infection or hemorrhage. The prognosis of treated AML patients is also alarmingly poor. Overall, only 30–40% of adults younger than 60 years can currently expect to be cured of disease. An Eastern Cooperative Oncology Group review of the long-term survival of 2882 AML patients treated between 1973 and 1996 (including all patients irrespective of whether they completed protocol therapy) found an overall median survival of 11 months, which dropped to 6 months when patients aged >55 years were considered.[6] Five-year survival in this older patient group was particularly poor (7.6%).

History of disease treatment and treatment considerations in AML

Current treatment options for AML patients are:

- Watch and wait/supportive care,
- Palliative chemotherapy, and
- Intensive chemotherapy.

Intensive chemotherapy is suitable for patients who are fit and have good performance status, while supportive care ± palliative chemotherapy may be the most appropriate option for patients with pre-existing medical conditions and a poor performance status.[7] The 'watch and wait' approach with supportive care is reserved for the small minority of patients who have clinical problems associated with pancytopenia rather than hyperleukocytosis. For these patients, it may be necessary to provide transfusion support, and delay chemotherapy until their white cell count rises.[7]

Cytarabine (Ara-C) has long been the cornerstone of chemotherapy in AML patients. Since the 1970 s, investigators have focused on increasing the effectiveness of this agent by modulating the dose or treatment schedule and through combining it with other agents. Current remission induction therapies involve combining cytarabine with an anthracycline such as daunorubicin or idarubicin. Typically, cytarabine 100 mg/m^2 is administered by continuous infusion for 7 days, while the anthracycline is administered for 3 days – hence these regimens are commonly referred to as 3 + 7.

Hematopoietic growth factors have been investigated in older patients as a means of ameliorating the toxicity of chemotherapy but have no effect on survival rates. Another treatment approach in younger patients is the use of bone marrow transplantation: after patients undergo myeloablative chemotherapy, hematopoiesis is restored by infusion of bone marrow cells from an HLA-matched donor (allogeneic bone marrow transplant) or from the patients own bone marrow obtained while in CR (autologous bone marrow transplant).

Treatment fails in the majority of patients with AML, and may be related to resistance of the leukemic cells to chemotherapy or treatment-related morbidity/mortality. The high rate of relapse is a significant problem in the treatment of AML, with around 50% of patients in CR eventually relapsing.[8] The majority of relapses occur within 1 year of CR. Prognosis subsequent to relapse is very poor, with a 2-year survival rate in retreated patients of only around 20%.[8]

Clearly, it is essential to find ways to optimize first-line therapy in order to reduce relapse rates and improve the overall survival in AML patients. Recently, fludarabine has been evaluated in the second-line treatment of AML, as well as for first-line therapy in high-risk patients.

Treatment of AML with fludarabine-containing regimens

Refractory/relapsed AML

Several key studies in recent years have reported promising results with fludarabine combination therapy in patients with relapsed/refractory or secondary AML. While neurotoxicity issues preclude the use of high-dose fludarabine monotherapy in AML, fludarabine combination therapy has been found to be better tolerated.

The FLAG chemotherapy regimen consists of fludarabine plus cytarabine and granulocyte colony-stimulating factor (G-CSF). The regimen typically involves fludarabine 30 mg/m^2/day plus cytarabine 2 g/m^2/day (administered 4 h after fludarabine) for 5 days, with G-CSF ~300 μg/day given 12–24 h before chemotherapy starts and continued until complete neutrophil recovery. This regimen was developed following the observation that coadministration of fludarabine with cytarabine results in increased intracellular retention of cytarabine's active metabolite, cytosine arabinoside 5′ triphosphate, producing a synergistic antitumor effect.[9] In addition, by increasing cell cycling, hematopoietic growth factors are thought to improve treatment response by rendering dormant leukemic cells more sensitive to cytotoxic drugs.[10–13] Also, G-CSF potentiates the effects of cytarabine by increasing its incorporation into DNA.[10,12,13]

Other variations on the FLAG regimen include FLANG, in which mitoxantrone 10 mg/m^2/day is given at the end of the cytarabine infusion, cytarabine dose is reduced to 1 g/m^2/day and treatment is given for only 3 days, and FLAG-Ida, in which 3 days' idarubicin is added to the standard FLAG protocol. These fludarabine-based regimens have been evaluated in a number of noncomparative studies in patients with treatment-refractory, relapsed or other high-risk forms of AML (Table 1). In a small early trial of fludarabine + cytarabine in 59 patients with refractory or relapsed AML, Estey *et al.*[14] reported a CR rate of 36%, with responses lasting for a median duration of 39 weeks. Adding G-CSF to the regimen produced overall CR rates of 48–62% in several small studies involving patients with primary refractory or relapsed AML, confirming the efficacy of this regimen in these difficult-

Table 1 Fludarabine-based combination therapy in the treatment of refractory or relapsed AML

Reference	*Study design*	*Regimen*	*Setting*	*CR*	*Median DFS*	*Median OS*
Estey *et al.* (1993)[14]	Non-comparative study	Fludarabine 30 mg/m^2/day for 5 days + cytarabine 0.5 g/m^2/h for 2–6 h/day for 6 doses	Refractory or relapsed AML	21/59 (36%)	39 weeks	
Clavio *et al.* (1996)[15]	Retrospective, non-comparative study	FLAG or FLANG	Refractory AML Relapsed AML Poor prognostic factors* All patients	8/10 (80%) 3/10 (30%) 19/31 (61%) 30/51 (59%)		9 months
Gobbi *et al.* (1997)[36]	Retrospective, non-comparative study	FLAG, FLANG or FLAG-Ida	Relapsed AML Refractory AML	34% 7/11 (64%)		
Nokes *et al.* (1997)[16]	Retrospective, non-comparative study	FLAG[a]	Relapsed AML Refractory AML	13/19 (68%) 4/4 (100%)		
Montillo *et al.* (1998)[17]	Prospective, open-label, non-comparative study	FLAG[b]	Relapsed AML Refractory AML All patients	14/22 (64%) 7/16 (44%) 21/38 (55%)	13 months	9 months
Ferrara *et al.* (1999)[18]	Noncomparative study	FLAG	AML in relapse after ASCT	13/26 (50%)	13 months	6 months
Jackson *et al.* (2001)[19]	Phase II, open-label, noncomparative study	FLAG[c]	Late relapse** AML Early relapse*** or refractory AML All patients	17/21 (81%) 13/44 (30%) 46%	8.2 months 0	>16.2 months 3.0 months
Hänel *et al.* (2001)[22]	Randomized, pilot study	FLANG[b]	Relapsed or refractory AML	17/29 (59%)	3.2 months	6.8 months
Pastore *et al.* (2003)[21]	Open-label, prospective study	FLAG-Ida[b]	Refractory AML Relapsed AML All patients	5/10 (50%) 19/36 (53%) 24/46 (52%)	12 months	11 months
Steinmetz *et al.* (1999)[20]	Phase II, open-label, non-comparative study	FLAG-Ida[d]	Refractory AML Relapsed AML	1/14 (7%) 12/15 (80%)		
Kern *et al.* (1999)[37]	Prospective, randomized comparison	Sequential high-dose cytarabine ± fludarabine	Refractory or relapsed AML	21/46 (46%)		

CR = complete response; OS = overall survival; DFS = disease-free survival; AML = acute myeloid leukemia; ASCT = autologous stem cell transplantation; FLAG = fludarabine + cytarabine + G-CSF; FLANG = fludarabine + cytarabine + mitoxantrone + G-CSF; FLAG-Ida = fludarabine + cytarabine + idarubicin + G-CSF.* First-line treatment of secondary AML or *de novo* AML with poor prognostic factors (advanced age and high leukocyte count).** Relapse ⩾6 months after completing chemotherapy.*** Relapse <6 months after completing chemotherapy.

[a]Cytarabine dose varied between 1 and 3 g/m^2/day; G-CSF dose also varied, the original dose used was 400 μg/m^2.

[b]G-CSF dose of 5 μg/kg.

[c]G-CSF dose of 30 MU/day, continued until 1 day after chemotherapy, with additional G-CSF given to support neutrophil recovery given if needed.

[d]Fludarabine dose of 25 mg/m^2/day and filgrastim dose of 400 μg/m^2/day.

to-treat patient groups.[15–19] CR tended to be higher in patients with relapsed disease than in those with treatment-resistant disease,[16] and one study demonstrated that patients with late relapse, that is ccurring >6 months after completing chemotherapy, had markedly better responses to FLAG than those with early relapse or refractory disease (81% versus 30%).[19]

Overall survival of 6–9 months has been reported in patients with refractory or relapsed disease treated with FLAG,[15,17,18] and is greater in patients with late relapse than in those with early relapse or refractory disease (>16.2 months versus 3.0 months).[19]

FLAG-Ida produced CR rates of 80 and 7% in patients with relapsed AML and refractory AML, respectively, in a phase II open-label trial.[20] As expected, median overall survival at 20 weeks was higher among patients with relapsed disease (55%) than in those with refractory disease (7%). In contrast to this study, Pastore *et al.*[21] recently reported similar responses to FLAG-Ida in patients with primary refractory AML (50%) and relapsed AML (53%). The median overall survival in this study was 11 months.

Another fludarabine-based regimen that has been evaluated in relapsed or refractory AML is FLANG. In a study in 29 patients with relapsed refractory AML, 17 patients (59%) achieved a CR and the median overall survival was 6.8 months.[22]

Patients who have failed to respond or have relapsed after multiple lines of chemotherapy have a dismal prognosis. Encouragingly, adding fludarabine to intermittent-sequential high-dose cytarabine plus mitoxantrone produced a CR in 5/16 (31%) heavily pretreated AML patients; patients had received at least two lines of intensive first- and second-line chemotherapy.[23]

First-line treatment in high-risk patients

Fludarabine-based combination therapy has also been evaluated in a number of small, noncomparative studies for the first-line treatment of high-risk AML patients, including those with unfavorable cytogenetics or AML with trilineage myelodysplasia (Table 2).

AML with multilineage dysplasia (concomitant dysplastic abnormalities in $\geqslant 2$ hematopoietic cell lineages) may appear as a *de novo* event, or may evolve following a previously diagnosed myelodysplastic syndrome. Historically, standard intensive chemotherapy has produced lower CR rates and survival in this setting than is seen with AML without multilineage dysplasia.[24–26] This may reflect the fact that AML with multilineage dysplasia is frequently associated with older patient age and unfavorable cytogenetics.

CR rates of 54–64% have been reported in patients with AML secondary to myelodysplastic syndromes or *de novo* AML with multilineage dysplasia treated with the fludarabine-based regimens, FLAG, FLANG or FLAG-Ida.[15,20,27] Ferrara *et al*.[27] concluded that multilineage dysplasia *per se* is not an adverse prognostic factor in patients treated with the FLAG regimen. In their study, 64% of patients achieved CR. Notably, the CR rate was significantly lower in patients with unfavorable cytogenetics than in those with intermediate-risk karyotypes (37 versus 79%; $P = 0.01$). A similar effect of cytogenetics was reported in a small study examining first-line treatment with fludarabine-based regimens in patients with *de novo* AML (CR 38% with unfavorable cytogenetics versus 67% with intermediate karyotypes) or secondary AML (CR 21% and 56%, respectively).[28] Therapy-related AML is also associated with poor responses to standard chemotherapy. Clavio *et al*.[29] reported an overall CR rate of 33% among 42 patients with AML evolving from myelodysplastic syndromes or secondary to prior chemoradiotherapy; 50% of patients with intermediate karyotypes achieved a CR.

Keating *et al*.[30] evaluated the FLAG-Ida regimen as first-line treatment of AML patients with high-risk cytogenetics. CR was achieved by 27/52 (52%) patients

Table 2 Fludarabine-based combination therapy in the first-line treatment of high-risk AML

Reference	*Study design*	*Regimen*	*Setting*	*CR*	*Median DFS*	*Median OS*
Clavio *et al*. (1996)[15]	Retrospective, non-comparative study	FLAG or FLANG	Secondary AML[a] or *de novo* AML with poor prognostic factors[b]	19/31 (61%)		
Gobbi *et al*. (1997)[36]	Retrospective, non-comparative study	FLAG, FLANG or FLAG-Ida	*de novo* non-M3 AML	19/26 (73%)	7 months	8 months
Steinmetz *et al*. (1999)[20]	Phase II, open-label, non–comparative study	FLAG-Ida	AML with signs of trilineage myelodysplasia at diagnosis or the history of a myelodysplasia or myeloproliferative disorder	17/28 (61%)	20 weeks	
Keating *et al*. (1998)[30]	Retrospective study	Fludarabine FLAG FLAG-Ida	AML with high-risk cytogenetics	12/30 (40%) 27/49 (55%) 27/52 (52%)		7 months
Clavio *et al*. (1999)[28]	Retrospective study	FLAG, FLANG or FLAG-Ida	*de novo* AML: intermediate karyotype unfavorable karyotype	 22/33 (67%) 5/13 (38%)		
de la Rubia *et al*. (2002)[38]		FLAG-Ida	High-risk AML or myelodysplastic syndromes	24/45 (53%)		
Ferrara *et al*. (2002)[27]	Prospective, open-label non–comparative study	FLAG	*de novo* AML with multilineage dysplasia	28/44 (64%)	22 months	16 months
Estey *et al*. (1999)[31]	Randomized, phase II study	FAI FAI + G-CSF FAI + ATRA FAI + G-CSF + ATRA All patients	High-risk[c] AML or myelodysplastic syndrome	21/53 (40%) 29/53 (55%) 28/55 (51%) 32/54 (59%) 51%	36 weeks	28 weeks
Clavio *et al*. (2001)[29]	Retrospective study	FLAG, FLANG or FLAG-Ida	Secondary AML (evolving from a myelodysplastic syndrome or secondary to chemoradiotherapy)	14/41 (34%)	Mean 16 months (at a mean of 12 months of follow-up)	Mean 11 months (at a mean of 12 months of follow-up)
Ferrara *et al*. (2002)[32]	Phase II, prospective	Continuous infusion of fludarabine + cytarabine[d]	Non M3 AML	14/20 (70%)		

CR = complete response; OS = overall survival; DFS = disease-free survival; AML = acute myeloid leukemia; FLAG = fludarabine + cytarabine + G-CSF; FLANG = fludarabine + cytarabine + mitoxantrone + G-CSF; FLAG-Ida = Fludarabine + cytarabine + idarubicin + G-CSF; FAI = fludarabine + cytarabine + idarubicin.

[a]AML preceded by a myelodysplastic syndrome lasting >6 months.

[b]Advanced age and high leukocyte count.

[c]Patients were aged >71 years, had an antecedent hematologic disorder, AML or myelodysplastic syndrome secondary to prior chemotherapy, or had failed to respond to previous cytarabine + anthracycline therapy

[d]Fludarabine loading dose 10 mg/m^2 over 15 min on day 0 followed by continuous infusion of 20 mg/m^2/24 h for 72 h; cytarabine loading dose 390 mg/m^2 over 15 min 3 h after fludarabine, then continuous infusion of 1440 mg/m^2/24 h for 96 h.

and the median overall survival was 6.5 months. A comparison of the CR rate in patients treated with FLAG (55%) suggested the FLAG-Ida regimen did not provide any further benefit in these patients.

A randomized, phase II trial in patients with newly diagnosed high-risk AML or myelodysplastic syndromes found that adding all-*trans*-retinoic acid, with or without G-CSF, to chemotherapy with fludarabine + cytarabine + idarubicin, had no significant effect on CR, disease-free survival or overall survival (Table 2).[31] In this study, high risk was defined as any of the following: age >71 years, an antecedent hematologic disorder, AML or myelodysplastic syndrome secondary to chemotherapy for prior cancer, failure to respond to prior cytarabine + anthracycline induction therapy.

As noted earlier, prognosis of AML in elderly patients is particularly poor. Ferrara *et al*. investigated the efficacy of continuous sequential application of fludarabine + cytarabine in elderly (aged >60 years) non-M3 AML patients in a phase II study. Preliminary results were encouraging, with 14/20 (70%) patients achieving CR, all after one course of the continuous infusion regimen.[32]

First-line treatment in patients with favorable prognostic factors

Very promising results were seen in a small study assessing first-line treatment with FLAG-Ida in patients with *de novo* AML and good prognostic factors.[33] Patients were aged <60 years and had WHO performance status ⩽3; those with secondary AML were excluded. Overall, the CR rate was 35/42 (83%) patients. Among patients in CR, 66% went on to receive autologous or allogeneic bone marrow transplantation. At a median follow-up of 24 months, the median CR duration was 17 months and the median survival was 20 months.

New fludarabine-based combinations

The encouraging results seen with fludarabine-based regimens outlined above have led investigators to evaluate other agents in conjunction with fludarabine. Combination therapy with gemtuzumab ozogamicin, a monoclonal antibody that binds to the CD33 antigen of myeloid progenitor cells, plus fludarabine, cytarabine and cyclosporine produced a CR in 9/32 (28%) of patients with relapsed or refractory AML in a phase II study, with an overall median survival of 5.3 months.[34] Following the observation of *in vitro* synergistic antileukemic effect with fludarabine, gemcitabine is also being investigated as a potential partner for fludarabine in the treatment of relapsed or refractory AML.[35]

Conclusions

In addition to its established role in the management of CLL, fludarabine is emerging as a potentially useful treatment option in patients with other hematological malignancies, such as AML. There is an accumulating body of data showing that fludarabine, in combination with other chemotherapeutic agents, can provide important benefits in terms of survival and response rates in patients with relapsed or refractory AML. The same combinations (eg with cytosine arabinoside, idarubicin and G-CSF) may also be useful as first-line treatment, although comparatively fewer data are available in this indication. Other potential combinations, for example, with monoclonal antibodies or gemcitabine, are also under investigation. Ongoing research will further clarify the role of fludarabine in the treatment of this complex and deadly disease. The MRC-HR trial and AML 15 trial will also provide data from randomized studies that may provide further information on the role of regimens containing the combination of fludarabine and high-dose cytosine arabinoside.

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Treatment of hematological malignancies with allogeneic nonmyeloablative stem cell transplantation: conditioning regimens with fludarabine

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Allogeneic stem cell transplantation (alloSCT) is an accepted therapeutic option for various hematological malignancies. For many years, alloSCT was based on the concept that a myeloablative dose of chemoradiotherapy was necessary to allow successful donor stem cell engraftment. These high-dose regimens cause considerable toxicity in graft recipients and even the most intensive conditioning regimens do not reliably eliminate all malignant cells. During the last decade, it became clear that the curative potential of alloSCT was not solely due to the conditioning regimen but also to an immune response of donor cells against the malignancy, termed the graft-versus-leukemia (GVL) effect. The increasing evidence that the GVL effect is essential for the eradication of host tumor cells has led to the development of a new concept in alloSCT: the use of reduced intensity, nonmyeloablative conditioning regimens that allow exploitation of the GVL effect without the toxicity of myeloablative therapy. The purine analog fludarabine is immunosuppressive and has activity against many hematological malignancies. The introduction of nonmyeloablative fludarabine-based conditioning regimens has facilitated alloSCT, while limiting regimen-related morbidity and mortality in patients with susceptible hematological malignancies. This potentially curative approach extends the use of alloSCT to older patients and to those with comorbidities that preclude high-dose chemoradiotherapy. The purpose of this review is to summarize the results obtained with fludarabine-based nonmyeloablative conditioning regimens and alloSCT in patients with malignant hematological disorders.
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Introduction

Allogeneic stem cell transplantation (alloSCT) is used for the treatment of various hematological malignancies. Traditionally, this approach has involved the use of a conditioning regimen, comprising myeloablative doses of chemoradiotherapy, to eradicate the underlying malignancy and eliminate the host's bone marrow in preparation for the allogeneic graft, which functions primarily as a bone marrow rescue product.[1,2] However, myeloablative chemoradiotherapy followed by alloSCT is associated with considerable mortality, morbidity and graft-versus-host disease (GVHD), and this has limited the procedure to medically fit patients generally no older than 50 years.[3] Since most patients with hematological malignancies are aged >50 years, they are not candidates for myeloablative conditioning regimens; consequently, the impact of alloSCT in these conditions is relatively low (Table 1).[4–6]

Furthermore, many hematological malignancies cannot be fully eradicated by myeloablative therapy.[7] It has been suggested that the complete eradication of tumor cells is largely mediated by an immune-mediated destruction of malignant cells by donor lymphocytes, termed the graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) effect. The most compelling evidence for the GVL effect is that many patients who relapse after allogeneic transplantation can be reinduced into remission by infusing additional donor lymphocytes.[8,9] The efficacy of GVL induction to treat relapses suggests that it may be possible to use the GVL effect as a primary therapy for hematological malignancies without the need for myeloablative therapy.[10] Replacing high-dose myeloablative therapy with a nonmyeloablative conditioning regimen would allow treatment of those patients who are too old or medically unfit to qualify for conventional alloSCT.

The aim of nonmyeloablative alloSCT is to use a low-intensity preparative regimen to induce sufficient immunosuppression in the recipient to allow engraftment of allogeneic stem cells. The nonmyeloablative regimen does not completely eliminate host-derived cells, but over a period of time allogeneic lymphocytes act to eliminate residual hematopoietic and malignant cells. This process can take months to complete and

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Table 1 Incidence and outcome of therapy for common hematological malignancies in the United States[4] (adapted with permission from Elsevier)

	Incidence per 100 000	*Median age at diagnosis (years)*	*5-year survival with conventional therapy (%)*	*Patients >50 years undergoing alloSCT (%)*	*5-year survival with alloSCT (all patients) (%)*[a]
CLL	15	65	50	40	30–40
CML	1	55	50	15	70
Multiple myeloma	6	65	30	1	30
HD	2	30	50–80	1	<20
NHL	13.7	50	49	2–10	20–40
AML	2.3	60	30	8	50
Myelodysplastic syndromes	1	60	30	1	40–50
ALL	1.5	35	30	3	50–60

Abbreviations: alloSCT = allogeneic stem cell transplantation; ALL = acute lymphoid leukemia; AML = acute myeloid leukemia; CLL = chronic lymphoid leukemia; CML = chronic myeloid leukemia; HD = Hodgkin's disease; NHL = non-Hodgkin's lymphoma;
[a]Data reflect survival of patients transplanted in remission or during first salvage.

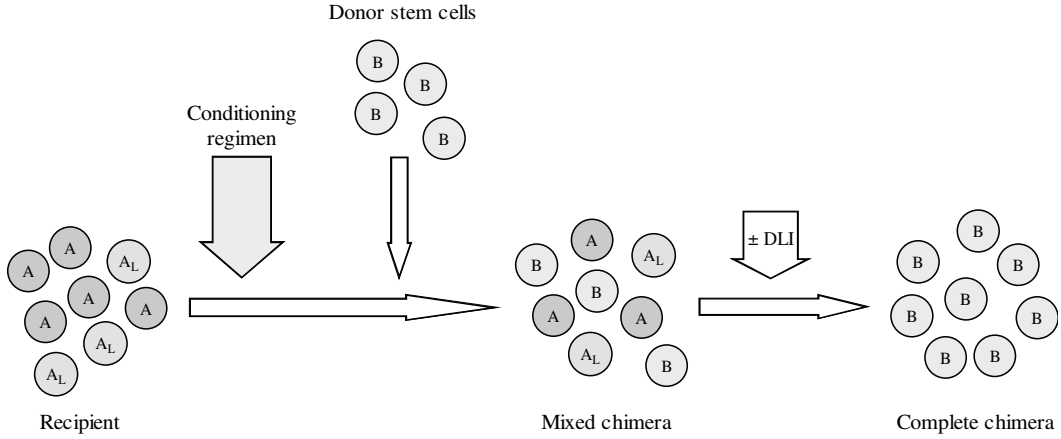

Figure 1 Scheme for nonmyeloablative allogeneic stem cell transplantation. The recipient has normal (A) and malignant (A_L) cells. The patient receives a conditioning regimen designed to achieve engraftment of allogeneic donor stem cells (B). Initially a mixed chimera is present, with coexistence of (A), (A_L) and (B) cells. Donor (B) cells act to eradicate residual host cells leading to complete chimerism. Donor lymphocyte infusions (DLI) can be administered to augment the elimination of host cells.[11] (Copyright American Society of Hematology, used with permission.)

patients may be administered graded doses of donor lymphocyte infusions (DLI) to augment the GVL effect and to achieve full chimerism (Figure 1).[11] Preliminary results using such nonmyeloablative protocols in animal models confirm that durable engraftment of allogeneic stem cells can be achieved with a marked reduction in the immediate toxicity of transplantation.[12]

The drugs used in nonmyeloablative conditioning regimens are generally chosen because they have some activity against the target malignancy and also provide sufficient immunosuppression to prevent graft rejection. The purine analog, fludarabine, has been widely used in nonmyeloablative conditioning regimens. It is immunosuppressive and has antitumor activity against a wide range of hematological malignancies.[13–15] Fludarabine is often combined with a variety of other cytotoxic agents, such as melphalan, cyclophosphamide, cytosine arabinoside and busulfan, or with low-dose total body irradiation, with the aim of inducing enough immunosuppression to allow successful engraftment and to exert some pretransplant anti-tumor activity.

This article reviews the effectiveness of alloSCT after reduced-intensity fludarabine-based conditioning regimens in treating patients with hematological malignancies.

Fludarabine-based nonmyeloablative alloSCT regimens

Nonmyeloablative combination regimens with fludarabine and other cytotoxic agents have been used in patients with various hematological diseases, including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic lymphoid leukemia (CLL), non-Hodgkin's lymphoma (NHL), Hodgkin's disease (HD), acute lymphoid leukemia (ALL) and multiple myeloma. The use of alloSCT was previously limited in these disorders, because they typically affect older and/or debilitated patients. There are major differences among malignancies in their susceptibility to GVL effects. CML appears most sensitive, followed by AML, CLL, low-grade lymphoma and myeloma; ALL

Table 2 Fludarabine-containing conditioning regimens for patients with hematological malignancies

	Number of patients	*Median age in years (range)*	*Diagnosis*	*Conditioning regimen(s)*	*Overall survival (median follow-up)*
Giralt *et al.*[6]	15	59 (27–71)	AML, MDS	FLU (30 mg/m^2/day for 4 days) + IDA (12 mg/m^2/day for 3 days) + ARA-C (2 mg/m^2/day for 4 days) or MEL(140 mg/m^2/day); 2-CDA (12 mg/m^2/day for 3 days) + ARA-C (1 mg/m^2/day for 5 days)	40% (3 months)
Giralt *et al.*[17]	78	52 (22–70)	AML, MDS, CML, ALL, NHL	FLU (25 mg/m^2/day for 5 days) + MEL (180 or 140 mg/m^2)	28% (24 months)
Slavin *et al.*[18]	26	31 (1–61)	AML, ALL, CML, MDS, NHL, MM	FLU (30 mg/m^2/day for 6 days) + BUS (4 mg/kg/day for 2 days) + ATG (10 mg/kg/day for 4 days)	85% (8 months)
Childs *et al.*[19]	50	51 (23–68)	Lymphoid and myeloid malignancies, solid tumors	FLU (25 mg/m^2/day for 5 days) + CYCLO (60 mg/kg/day for 2 days)	70% (4.1 months)
Khouri *et al.*[20]	15	55 (45–71)	CLL, low-grade lymphoma	FLU (90–150 mg/m^2) + CYCLO (900–2000 mg/m^2); FLU (30 mg/m^2/day for 2 days) + ARA-C (500 mg/m^2/day for 2 days) + cisplatin (25 mg/m^2/day for 4 days)	50% (6 months)
Schetelig *et al.*[21]	30		CLL	FLU + BUS + ATG	77% (24 months)
Carella *et al.*[22]	15	(19–60)	HD, NHL	FLU (30 mg/m^2/day for 3 days) + CYCLO (300 mg/m^2/day for 3 days)	66% (11 months)
Khouri *et al.*[23]	20	51 (31–68)	Indolent lymphoma	FLU (25 mg/m^2/day for 5 days) or FLU (30 mg/m^2/day for 3 days) + CYCLO (1 g/m^2/day for 2 days) or CYCLO (750 mg/m^2/day for 3 days)	85% (21 months)
Nagler *et al.*[24]	23	41 (13–63)	NHL, HD	FLU (30 mg/m^2/day for 6 days) + BUS (4 mg/kg/day for 2 days) + ATG (10 mg/kg/day for 4 days)	40% (37 months)
Anderlini *et al.*[25]	6	29 (22–30)	HD	FLU (25 mg/m^2/day for 5 days) + CYCLO (1 g/m^2/day for 3 days) + ATG (20 mg/kg/day for 3 days); FLU (25 mg/m^2/day for 5 days) + MEL (90 mg/m^2/day for 2 days); FLU (30 mg/m^2/day for 4 days) + ARA-C (2 g/m^2/day for 4 days) + IDA (12 mg/m^2/day for 3 days)	50% (9 months)
Mohty *et al.*[26]	11	49 (40–62)	NHL, HD, MM	FLU (25 mg/m^2/day for 5 days) + BUS (2 mg/kg/day for 2 days) + ATG (2.5 mg/kg/day for 4 days)	27% (16.7 months)
Giralt *et al.*[27]	22	51 (45–64)	MM	FLU (30 mg/m^2/day for 4 days) + MEL (140 mg/m^2); FLU (25 mg/m^2/day for 5 days) + MEL (90 mg/m^2/day for 2 days)	30% (24 months)
Kroger *et al.*[28]	21	50 (32–61)	MM	FLU (150 mg/m^2) + MEL (100–140 mg/m^2) + ATG (10 mg/kg for 3 days)	74% (13 months)

Abbreviations: ALL = acute lymphoid leukemia; AML = acute myeloid leukemia; ARA-C = cytosine arabinoside; ATG = antithymocyte globulin; BUS = busulfan; CLL = chronic lymphoid leukemia; CML = chronic myeloid leukemia; CYCLO = cyclophosphamide; FLU = fludarabine; HD = Hodgkin's disease; IDA = idarubicin; MEL = melphalan; MDS = myelodysplastic syndrome; MM = multiple myeloma; NHL = non-Hodgkin's lymphoma; 2-CDA = 2-cholorodeoxy-adenosine.

is least affected by GVL.[16] Depending on the aggressiveness of the underlying malignancy, a range of nonmyeloablative conditioning regimens have been used in alloSCT. Table 2 summarizes some of the results obtained with fludarabine-based conditioning regimens in patients with hematological malignancies. These regimens are only useful in malignancies susceptible to the GVL effect; furthermore, the approach appears to be less effective in patients with very aggressive malignancies where higher doses are required.

Myeloid malignancies: AML and CML

Many patients with AML are ineligible for high-dose chemoradiotherapy as a result of age or medical problems other than leukemia. Therefore, nonmyeloablative conditioning regimens raise the possibility of extending the therapeutic benefit of alloSCT to patients with AML who are not present candidates for transplantation. An initial phase I study evaluated whether fludarabine-containing nonmyeloablative therapy was sufficiently immunosuppressive to allow alloSCT in 15

patients with AML ($n=13$) or myelodysplastic syndrome ($n=2$).[6] The median age was 59 years; 12 of the patients were either refractory to therapy or beyond first relapse. Patients without prior exposure to fludarabine received fludarabine (30 mg/m^2/day for 4 days) with idarubicin (12 mg/m^2/day for 3 days) and cytosine arabinoside (2 mg/m^2/day for 4 days) ($n=7$) or melphalan (140 mg/m^2/day) ($n=1$). Those with prior exposure to fludarabine received 2-chloro-deoxyadenosine (12 mg/m^2/day for 3 days) and cytosine arabinoside (1 mg/m^2/day for 5 days) ($n=7$). The regimen was well tolerated in 13 of the 15 patients. Following alloSCT, eight patients achieved complete remission. Overall survival was poor, with a median survival time of 78 days. However, this study did prove that donor engraftment could be achieved using a fludarabine-based nonmyeloablative regimen. Giralt *et al.*[29] also demonstrated successful donor engraftment in seven patients with recurrent or residual CML who underwent alloSCT following nonmyeloablative fludarabine-based conditioning.

In order to improve outcomes in patients with advanced disease, the same investigators subsequently developed a more intensive nonmyeloablative conditioning regimen of fludarabine and melphalan.[17] The rationale for this combination was to provide greater myelosuppression for better short-term leukemia control to allow successful engraftment. In all, 78 patients (median age 52 years) with advanced hematological malignancies (predominantly AML and CML) received fludarabine (25 mg/m^2/day for 5 days) in combination with melphalan 180 mg/m^2 ($n=66$) or 140 mg/m^2 ($n=12$). Eight patients with prior exposure to fludarabine received 2-chloro-deoxyadenosine (12 mg/m^2 continuous infusion for 5 days) with melphalan 180 mg/m^2. Successful engraftment was consistently achieved, with $>90\%$ of patients showing 80–100% donor cell engraftment by day 30. The 100-day nonrelapse mortality rate for the fludarabine/melphalan combination was 37%. In contrast, the combination of 2-chloro-deoxyadenosine and melphalan was considerably more toxic (100-day nonrelapse mortality rate of 87%). Disease-free survival at 1 year was 57% for patients in first remission or chronic phase and 49% in patients with more advanced disease. GVHD was the most important cause of treatment failure.[17] These results are similar to those achieved with ablative preparative regimens in younger patients.[5] In addition, these observations indicate that the more intensive fludarabine/melphalan regimen produces better outcomes than the low-intensity fludarabine/cytosine arabinoside regimen used in the initial studies.

Based on observations in experimental animals, Slavin *et al.*[18] proposed the use of a reduced toxicity conditioning regimen of fludarabine (30 mg/m^2/day for 6 days), busulfan (4 mg/kg/day for 2 days) and antithymocyte globulin (10 mg/kg/day for 4 days), for the routine treatment of hematological malignancies. Preliminary data from 26 patients with various malignancies, including AML ($n=9$) and CML ($n=8$), indicated that this conditioning regimen was extremely well tolerated, with no severe procedure-related toxicity. Indeed, the low incidence of procedure-related complications led the authors to suggest that nonmyeloablative alloSCT may eventually become an outpatient procedure. All patients underwent alloSCT; 85% were alive and 81% were disease-free at a median follow-up of 8 months. The actuarial probability of disease-free survival at 14 months was 77%. The results of this study were expanded to include data from a larger group of patients, including six additional patients with myeloid malignancies. Trends remained similar to those reported in the initial cohort.[30]

Childs *et al.*[19] used a sequential conditioning regimen of fludarabine (25 mg/m^2/day for 5 days) and cyclophosphamide (60 mg/kg/day for 2 days) in 50 patients with various hematological and solid tumor malignancies, including 16 patients with myeloid disorders. Cyclosporine was used as prophylaxis for GVHD and was tapered to enhance donor engraftment and a GVT effect. Patients with disease progression following cyclosporine withdrawal received graded doses of DLI. The regimen was well tolerated and early donor engraftment was detected in 49 patients. At a median follow-up of 126 days, the overall survival was 70% and tumor responses were seen in 50% of the patients with myeloid malignancies.

CLL, NHL and HD

CLL is a lymphoproliferative disorder characterized by a progressive accumulation of neoplastic lymphocytes in the blood, bone marrow and lymphoid tissues.[31] NHL and HD are malignant tumors of the lymphatic system. There are indolent (low-grade) and aggressive (high-grade) forms of NHL. Indolent lymphomas account for 25–40% of NHLs, of which follicular lymphomas are the most common.[32,33] The treatment of CLL and indolent NHL is a challenge for hematologists, as the long physical history and the persistent nature of these malignancies do not support the choice of aggressive treatment.

In patients with advanced CLL, alloSCT has been associated with a high early mortality rate, largely due to myeloablative conditioning and GVHD.[34] Based on the encouraging results with reduced-intensity fludarabine-based conditioning for allografting of patients with myeloid malignancies, preparative regimens have been developed for patients with CLL. Some success had previously been achieved with fludarabine as a standard chemotherapy agent against CLL, providing a rationale for its use as a conditioning regimen for alloSCT in this disorder.[14,35]

Data for 77 patients with CLL were collected from 29 European transplant centers.[36] Reduced-intensity conditioning regimens (mainly fludarabine–cyclophosphamide combinations) were administered to 56% of patients, whereas the remainder received more intense conditioning of fludarabine–busulfan or melphalan combinations. Universal donor cell engraftment and best response took a median of 3 months to develop. Event-free and overall survival at 24 months was 56 and 72%, respectively. Khouri *et al.*[20] used a nonmyeloablative regimen of fludarabine (90–150 mg/m^2) plus

cyclophosphamide (900–2000 mg/m^2) or fludarabine (30 mg/m^2/day for 2 days) plus cytosine arabinoside (500 mg/m^2/day for 2 days) plus cisplatin (25 mg/m^2/day for 4 days) to treat 15 patients with CLL or low-grade lymphoma. All patients were heavily pretreated and had failed to respond, or their disease recurred after primary chemotherapy. Durable engraftment was achieved in 11 of the 15 patients, with 50–100% of donor cells at 1 month post-transplant, typically converting to 100% over the next 2 months, either spontaneously or after DLI. Increasing the intensity of the regimen appeared to increase the rate of engraftment without increasing treatment-related morbidity. All 11 patients responded, with eight achieving complete remission over the following year. For the whole study population, the probability of being alive at 1 year was 50%. The results indicate the feasibility of using fludarabine-based nonmyeloablative regimens to ensure engraftment and a GVL effect against CLL.[20] These data compare favorably with the results achieved with standard intensive cyclophosphamide total body irradiation regimens, in which treatment-related mortality rates are high.

Recently, a study by German investigators provided evidence of a GVL effect in CLL patients after reduced-intensity conditioning and alloSCT.[21] In total, 30 patients with advanced CLL underwent stem cell transplantation following a preparative regimen of fludarabine, busulfan and antithymocyte globulin. In all, 12 patients achieved a complete remission and 16 patients achieved a partial remission; late complete remission occurred up to 2 years after transplantation, providing evidence of a GVL effect. Acute GVHD was observed in 17 patients. A total of 23 patients were alive after a median follow-up of 2 years.

Indolent NHLs, although initially responsive to a variety of therapeutic regimens, have a continuous relapsing nature and are essentially incurable. In the absence of a cure, the therapeutic options available for patients with indolent NHL range from 'watchful waiting' to high-dose therapy with alloSCT.[37] As with the disorders previously discussed, the effectiveness of high-dose alloSCT has been limited by conditioning-related toxicity and patient age.[22] The success of fludarabine-based nonmyeloablative regimens in the engraftment of allogeneic stem cells in other hematological malignancies has renewed interest in allografting for the treatment of indolent NHL and in identifying a graft-versus-lymphoma effect.

In a pilot study by Carella *et al.*,[22] the feasibility of a dual transplantation approach (autografting followed by fludarabine-based conditioning and alloSCT) in very high-risk patients with low-grade NHL and HD was studied ($n=15$). All patients underwent autografting and when clinically stable (at a median of 61 days) were readmitted for alloSCT. The conditioning regimen consisted of fludarabine (30 mg/m^2/day for 3 days) and cyclophosphamide (300 mg/m^2/day for 3 days). A total of 13 patients achieved 100% donor cell engraftment. In all, 11 patients were in complete remission after alloSCT and five remained in remission at a median of 270 days postprocedure. A total of 10 patients were alive between 210 and 700 days (median of 337 days) after transplantation. Thus, the regimen permitted engraftment of allogeneic stem cells and the generation of a graft-versus-lymphoma effect. The conditioning regimen was extremely well tolerated (only transient nausea occurred in two patients); this low toxicity confirms previous reports of nonmyeloablative alloSCT outcome[6,18,20] and should be viewed in the context of a patient population with a high risk of transplantation-related mortality.

Khouri *et al.*[23] assessed the potential benefits of a nonmyeloablative conditioning regimen of fludarabine and cyclophosphamide in the treatment of 20 patients with indolent follicular or small-cell lymphocytic lymphoma. Patients received fludarabine (25 mg/m^2/day for 5 days or 30 mg/m^2/day for 3 days) and intravenous cyclophosphamide (1 g/m^2/day for 2 days or 750 mg/m^2/day for 3 days). All patients achieved engraftment of donor cells and a complete response. None of the patients had relapsed at a median follow-up period of 21 months.

For aggressive NHL, high-dose chemotherapy followed by autologous peripheral blood stem cell transplantation is an accepted treatment option. However, the relapse rate following this procedure is high.[38] The potential benefits of nonmyeloablative alloSCT in patients with aggressive NHL were evaluated in a study by Nagler *et al.*[24] In all, 19 patients with aggressive NHL and four with HD received a low-intensity fludarabine-based conditioning protocol as a preparative regimen for alloSCT. All 23 patients engrafted and 16 had full donor chimerism. Transplant-related toxicity was moderate and four patients developed GVHD. Disease-free survival at 37 months was 40%. However, a report on the outcome of reduced-intensity allogeneic progenitor cell transplantation for 188 patients with lymphoma was less encouraging.[39] Those with high-grade NHL had a poor outcome and the authors concluded that while reduced-intensity conditioning followed by transplantation may control low-grade NHL, this treatment regimen may not be appropriate for aggressive disease.

Most patients with HD are successfully treated with chemotherapy and/or radiotherapy. For those who relapse, further salvage can be achieved with reinduction of chemotherapy or by alloSCT. Anderlini *et al.*[25] explored the feasibility of reduced-intensity conditioning followed by alloSCT in six patients with advanced HD. Conditioning regimens were fludarabine–cyclophosphamide–antithymocyte globulin ($n=4$), fludarabine–melphalan ($n=1$) and fludarabine–cytosine arabinoside–idarubicin ($n=1$). Chimerism data at day 30 post-transplant indicated 100% donor cell engraftment in 4/5 evaluable patients. Half (3/6) of the patients were alive and progression-free with a median follow-up of 9 months.

To clarify the role of fludarabine-based reduced-intensity conditioning and alloSCT in patients with lymphoma, and to assess the true impact of this treatment strategy on patient outcome, prospective studies with long-term follow-up and larger numbers of patients are needed. In addition, controlled clinical trials are necessary to compare fludarabine-based reduced-intensity alloSCT with alternative treatment strategies in this group of malignant diseases.

ALL

Outcomes following the use of nonmyeloablative regimens in patients with ALL have been disappointing. A retrospective analysis from the European Bone Marrow Transplant Registry (EBMTR) reported an 18-month disease-free survival of only 21% in remission patients and 13% in refractory patients.[4] Patients with ALL have a high relapse rate; therefore, the malignancy may recur rapidly after nonmyeloablative conditioning, outpacing the generation of a GVL effect. For this reason, nonmyeloablative approaches may not be optimal in this patient population.

Multiple myeloma

Multiple myeloma is a hematological malignancy with a median survival of only 3 years. The development of conventional chemotherapy regimens has had minimal impact on the survival of patients with this condition.[26] Consequently, the strategy of performing alloSCT after fludarabine-based reduced-intensity conditioning regimens has also been applied in multiple myeloma to harness a graft-versus-myeloma effect.

Recently, several groups have reported successful engraftment following nonmyeloablative conditioning regimens and alloSCT in patients with multiple myeloma.[26,27,40,41] The feasibility and efficacy of a fludarabine-based, nonmyeloablative conditioning regimen in patients with multiple myeloma was determined in a long-term study by Giralt *et al*.[27] From August 1996 to December 2000, 22 patients received a reduced-intensity conditioning regimen of fludarabine and melphalan. A total of 18 patients received fludarabine 30 mg/m^2/day for 4 days with melphalan 140 mg/m^2 as a single dose and four patients received fludarabine 25 mg/m^2/day for 5 days with melphalan 90 mg/m^2/day for 2 days. Treatment-related morbidity was low and the combination resulted in universal donor cell engraftment. The overall survival for all patients was 30 ± 11% at 2 years; disease recurrence was the primary cause of treatment failure. Thus, despite achieving donor cell engraftment, the graft-versus-myeloma effect did not result in long-term disease control. One possible explanation for this is that most patients in the study were treated for chemorefractory relapses. In order to optimize the results of fludarabine-based nonmyeloablative conditioning in multiple myeloma, it may be necessary to treat patients earlier in the course of their disease when the malignancy is more sensitive to chemotherapy.[27] Indeed, in a study by Lokhorst *et al*.,[42] chemosensitivity was an important predictor of response to DLI in patients with multiple myeloma. Data from the EBMTR confirm the efficacy of nonmyeloablative alloSCT in myeloma patients with chemosensitive disease.[43]

Some encouraging results have recently been reported by a German group who investigated the feasibility of alloSCT in 21 patients with advanced stage II/III multiple myeloma after a reduced-intensity conditioning regimen consisting of fludarabine (150 mg/m^2), melphalan (100–140 mg/m^2), and antithymocyte globulin (10 mg/kg for 3 days).[28] At day 40, complete donor chimerism was detected in all patients. After alloSCT, 40% of the patients achieved complete remission and 50% achieved partial remission, resulting in an overall response rate of 90%. After a median follow-up of 13 months, the 2-year estimated overall and progression-free survival rates were 74% (95% CI 54–94%) and 53% (95% CI 29–87%), respectively. The authors concluded that alloSCT after reduced-intensity conditioning provides durable engraftment, substantially reduces the risk of transplant-related toxicity and induces high remission rates.

Conclusions

AlloSCT is a curative procedure in a number of hematological malignancies. However, due to intensive, myeloablative conditioning regimens, transplant-related mortality, morbidity and GVHD are high, especially in older patients or those with concurrent disease. Consequently, alloSCT has generally been limited to younger medically fit patients.

The antileukemia effect seen with DLI in patients relapsing after alloSCT suggests that if engraftment of donor stem cells can be achieved after low-intensity chemotherapy, it may be possible to exploit a GVL effect without the potential morbidity associated with myeloablative therapy.

The purine analog fludarabine is immunosuppressive and is active against a wide range of hematological malignancies. Nonhematological side effects of fludarabine are mild, making it a potentially useful component of low-intensity conditioning regimens for alloSCT. Clinical data in humans have shown that fludarabine in combination with other agents can cause sufficient immunosuppression for successful alloSCT and the generation of a GVL effect. Indeed, fludarabine has become accepted as the most important drug in these combination regimens for successful nonmyeloablative alloSCT in leukemias and other malignancies.

The objective of achieving donor engraftment using a fludarabine-based nonmyeloablative conditioning regimen has been achieved in all of the studies reviewed. Hematological and nonhematological toxicity was mild in most studies and the regimens were well tolerated by the majority of patients. However, although alloSCT after reduced-intensity conditioning allows the engraftment of donor stem cells with a low spectrum of toxicity, GVHD remains a significant problem. Drugs such as cyclosporine are often used as prophylaxis for GVHD, with the dose being tapered to enhance donor engraftment and a GVT effect.[19] More recently, the addition of the monoclonal antibody alemtuzumab to a fludarabine-based protocol has been shown to reduce the incidence of GVHD, warranting further investigation in a randomized trial;[44,45] moreover, this drug could be effective in steroid-resistant aGVHD.[46]

Fludarabine-based nonmyeloablative alloSCT appears to be most effective in slowly proliferating malignancies, which give time for a GVL effect to

develop. However, the optimal conditioning regimen for different indications remains to be determined. Comparative studies between different fludarabine-based conditioning regimens are needed to determine whether any regimen is superior or better tolerated than another. Furthermore, the median follow-up for the majority of the studies reported to date is less than 1 year; therefore, the evidence for a sustained antitumor effect is limited. In future studies, longer observational periods are required to assess whether fludarabine-based nonmyeloablative transplants will result in better event-free survival compared with conventional myeloablative alloSCT.

In summary, fludarabine-based nonmyeloablative conditioning regimens are active against a wide range of hematological malignancies and have dramatically reduced the acute toxicity of alloSCT, even among patients who would otherwise have been excluded from conventional alloSCT because of age or associated comorbidities. Many other potential applications exist for a fludarabine-based nonmyeloablative regimen that can consistently achieve engraftment of allogeneic stem cells. These include selected malignant nonhematological disorders, such as metastatic melanoma and renal cell carcinoma, and autoimmune diseases.

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Future prospects for fludarabine-containing regimens in the treatment of hematological cancers

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Developments in the understanding of disease biology and in therapeutic approaches during the last decade have failed to alter the natural history of many human hematological malignancies, including chronic lymphocytic leukemia (CLL). Despite better appreciation of the molecular foundations and phenotypic characteristics of these cancers, improvements to disease classification and prognosis, and the discovery of more effective cytotoxic and biological agents, these disorders remain largely incurable. The development of a new class of cytotoxic agents in the 1980 s, namely the purine analogs, heralded a period of renewed optimism in the treatment of indolent lymphoid leukemias and lymphomas. More recently, monoclonal antibodies that selectively target cell-surface proteins, and agents that target cell-cycle or apoptotic pathways, have been developed and their use alone or in combination promise to have a major impact on the treatment of these conditions. Of these, the anti-CD52 antibody alemtuzumab and the anti-CD20 antibody rituximab have demonstrated the most potential for the treatment of CLL. Further, because of their different mode of action in comparison to conventional cytotoxic agents, combination regimens involving fludarabine and these antibodies are showing particular promise in early trials. The encouraging findings with these combination therapies are moving the intent of therapy from palliation towards cure. This paper reviews the therapeutic application of present and future fludarabine-containing approaches. The strategies that are discussed include fludarabine given with alemtuzumab, rituximab, or other monoclonal antibodies, and the potential benefits of combining fludarabine with more experimental agents such as antisense oligonucleotides, immunotoxins, or radioimmunoconjugates. The successful application of fludarabine plus alemtuzumab, or fludarabine in combination with other cytotoxic agents, as preparative regimens for stem cell transplantation techniques is also covered.
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Introduction

Despite decades of clinical trials and research into treatments for human lymphoproliferative malignancies, this broadly defined class of cancers has remained largely incurable. Traditionally, therapies for hematological cancers were restricted to corticosteroids or alkylating agents such as chlorambucil or cyclophosphamide, given either as monotherapy or in combination regimens. These approaches are considered to be mainly palliative, as they do not produce high rates of compete remissions and have not noticeably improved the prognosis of the indolent lymphoproliferative disorders. Moreover, the therapeutic options for retreatment were considerably limited if patients were refractory to or relapsed after these standard front-line therapies. However, better molecular and genetic understanding of neoplastic disorders and advances in therapeutics have enabled patients to be treated with more risk-adapted approaches and with a curative intent.

Advances in biology

Greater insight into the biological and clinical diversity of the lymphoproliferative disorders has led to more accurate classification, staging and prognosis, and, as a result, therapies that are tailored on an individual basis. This has been achieved by a combination of various techniques including immunophenotyping, cytogenetic analysis, and molecular and gene-profiling approaches that have identified useful prognostic factors predictive of particularly indolent or aggressive forms of cancer.[1–3]

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Some of these advances are beginning to explain the primary and secondary chemotherapy resistance seen in a proportion of cases. For example, dysfunction of p53 in chronic lymphocytic leukemia (CLL) predicts for resistance to alkylating agents and purine analogs as these agents lead to DNA damage, which then triggers apoptosis through a p53-dependent pathway.[4,5] It is likely that DNA-damaging agents are hazardous in cases with p53-dysfunction as, although the DNA is damaged, the cell cannot apoptose. As a result of the genetic damage, the malignancy could become more aggressive or resistant. It is therefore more logical in these cases to use agents that do not depend on the p53 pathway for their efficacy, such as steroids or monoclonal antibodies. This type of information allows the 'personalization' of therapy for an individual patient, therefore optimizing the chances of a better response. Finally, more sensitive methods for detecting and quantifying the level of minimal residual disease (MRD) are currently being developed. This allows for the modification of the duration and type of therapy in individual patients in order to attempt to achieve much more profound remissions. Eradication of MRD is associated with improved survival rates, is fundamental for durable remissions, and will have to be achieved in the majority of patients before a cure for CLL becomes a realistic possibility.[6–9]

Advances in therapy

The discovery of the purine analog fludarabine has been a significant therapeutic advance for the treatment of many of these hematological malignancies. Fludarabine alone or in combination with other treatments was the first effective second-line and salvage therapy for a range of conditions such as CLL, indolent non-Hodgkin's lymphoma (NHL), mantle cell lymphoma (MCL), acute myeloid leukemia (AML), Waldenström's macroglobulinemia, and systemic marginal zone lymphoma. When used in this second-line setting, these regimens usually improve overall response (OR) and complete response (CR) rates, response duration, and progression-free survival time, compared to historical series.[7,10–12] Further, fludarabine is typically more effective at achieving CRs when given as a front-line treatment.

A recent improvement to fludarabine therapy is the development of an efficacious and well-tolerated oral formulation that makes the administration of the drug much more convenient for both the patient and his/her hemato-oncologist. Further information pertaining to fludarabine oral is reviewed comprehensively by Boogaerts earlier in this supplement.

Building on the successes of fludarabine, the search for additional treatment modalities for hematological cancers has continued, with the intention of improving response rates (including molecular CRs by eliminating MRD), and extending overall survival time. Although a number of patients achieve CR with fludarabine monotherapy, molecular remissions are uncommon; most patients will ultimately relapse and have disease progression. Furthermore, current treatment modalities are limited in fludarabine-refractory patients, a group with particularly poor prognosis and reported median survival times of 10–11 months.[13,14]

The increased knowledge of the pathogenetic and molecular mechanisms of cancers has facilitated the development of targeted molecular therapy in the form of chimeric monoclonal antibodies (either 'naked' or conjugated) that are selective for intracellular or cell-surface targets specific to malignant cells.[1,15–17] A range of other experimental agents with suspected activity in certain lymphoproliferative disorders have also been described,[7,18] some of which will be covered later in this review. Finally, in combination with fludarabine, some of these novel therapies represent effective preparative regimens for subsequent transplantation approaches (as reviewed by Carella earlier in this supplement).

Even today, standard treatment strategies for many human hematological cancers are not straightforward and cures do not often exist. For example, for patients with indolent or less aggressive CLL the 'watch and wait' approach remains the most appropriate treatment option, whereas for other patients with poorer risk CLL this approach, particularly in view of the newer more effective therapies, can legitimately be questioned. Therefore, one of the biggest challenges currently facing clinical hematologists when treating indolent lymphoproliferative disorders is deciding who to treat, when to treat, and how to treat. The discovery of much more robust molecular prognostic variables is likely to make this problem more acute. Based on the reported efficacy and tolerability of fludarabine, this nucleoside analog is likely to play a major role in the therapy of these conditions. In this review, the advantages of combining fludarabine with some of the newer treatment options for lymphomas and leukemias will be considered.

Combination therapy with fludarabine and monoclonal antibodies

The development of monoclonal antibodies is a key example of how an improved understanding of the fundamental mechanisms of hematological malignancies has led to an emergence of therapeutic opportunities. Of these agents, alemtuzumab is highly effective in CLL, but also active in other diseases such as mycosis fungoides/Sézary symdrome, T-cell prolymphocytic leukemia, and peripheral T-cell lymphoma, while the benefits of rituximab have been clearly demonstrated in NHL and are currently being investigated in CLL.

Alemtuzumab monotherapy

Alemtuzumab (MabCampath®; Campath®) is a humanized monoclonal antibody directed against the CD52 protein, a cell-surface marker present on most normal and malignant peripheral blood B- and T-lymphocytes at high density (~500 000 molecules per cell).[19] The

mechanisms of action of alemtuzumab are incompletely understood, but are thought to include complement-mediated cytolysis and antibody-dependent cell-mediated cytotoxicity.[7] Alemtuzumab is the only monoclonal antibody licensed for the treatment of CLL in patients who have received alkylating agents and who have not responded to fludarabine. However, the efficacy and clinical benefit of alemtuzumab have also been demonstrated for a range of hematological cancers.[17,20]

The largest trial that led to the approval of alemtuzumab by the FDA for CLL was an international, noncomparative phase II study involving 93 heavily pretreated, high-risk fludarabine-refractory patients (76% Rai stage III–IV).[21] Alemtuzumab was given intravenously (i.v.), initially at 3 mg and dose escalated to 30 mg, thrice weekly for a maximum of 12 weeks. In this heavily pretreated population, objective responses to alemtuzumab were seen in 33% of patients (CR 2%) with an overall median survival of 16 months (32 months for responders). In fact, a number of patients in this study who were classified as partial remissions had no detectable CLL by sensitive techniques, but were not classified as CR due to persisting cytopenias. Responses to alemtuzumab were rapid, with an overall median response time of 1.5 months. Of note, a high proportion of patients showed responses in the blood (83%) and bone marrow (26%), whereas nodal responses were absent in the 17 patients who had lymph nodes greater than 5 cm. The toxicities of alemtuzumab are predictable and can be successfully managed; the most common toxicities in this study related to infusion, including rigors (90%), fever (85%) and nausea (53%), but these were generally grade 1 or 2 in severity and occurred mainly in the first week or two of therapy. As expected for refractory CLL,[22,23] infectious complications were common, occurring in 55% of patients. Further, use of this alemtuzumab regimen in fludarabine-refractory CLL patients produced good rates of molecular remissions (eg MRD was eradicated in 19% of 77 patients).[24] In addition, alemtuzumab used as consolidation following conventional fludarabine-based chemotherapy converts the majority of patients with partial remissions into CR and is effective at eradicating detectable MRD in a proportion of patients.[25]

Alemtuzumab has also proven to be effective when given as a front-line agent in CLL. The key study of alemtuzumab in untreated CLL was a multicenter phase II trial by Lundin *et al*.[26] In this investigation, patients with advanced disease (69% Rai stage III–IV; median age 66 years) were given alemtuzumab subcutaneously at a dose of 30 mg thrice weekly for a maximum of 18 weeks. Of 38 evaluable cases, alemtuzumab produced an OR rate of 87% (CR 19%). The median time to treatment failure had not been reached at 18 months. One major advantage of this route of administration was the lack of systemic reactions, which were replaced by transient skin reactions at the site of injection occurring in 90% of patients, but these were usually mild and only occurred with the first two or three injections. Infections were rare and the only specific problem was that 10% of patients developed cytomegalovirus reactivation, which can be managed successfully. A summary of trials investigating alemtuzumab as first- or second-line CLL therapy is presented in Table 1 and reviewed elsewhere.[6,31]

Alemtuzumab has good activity in other lymphoid malignancies such as mycosis fungoides/Sézary syndrome and T-cell prolymphocytic leukemia (Table 2). Moreover, alemtuzumab is also effective for patients with refractory CLL or T-cell prolymphocytic leukemia with biological high-risk features, such as unmutated V_H genes, 17p or 11q deletions, and p53 gene mutations.[35]

Rituximab monotherapy

The monoclonal antibody rituximab (Mabthera™; Rituxan®) is directed against the CD20 protein, a cell-surface marker found on CLL cells at a much lower density compared to CD52 (~8 000 molecules/cell).[19] Single-agent and combination therapy with rituximab has been used in CLL but is most effective in a variety of B-cell NHLs, including low-grade or follicular NHL.[15,20,36]

The efficacy of rituximab monotherapy in pretreated CLL patients has been tested in a number of relatively small clinical trials. The dosing schedules of rituximab in these studies were variable, ranging from 375 mg/m^2 i.v. once weekly for 4 weeks[37,38] to 375 mg/m^2 i.v. thrice weekly for 4 weeks (after an initial dose of 100 mg/m^2).[39] Responses to rituximab were achieved in 23–45% of patients, with 0–3% CRs (Table 3). Results from a rituximab dose-escalation study showed a significant correlation between OR rate and dose ($P = 0.007$), with a 75% OR rate reported for the maximum dose (2 250 mg/m^2).[40] The median response duration in these trials was 3–10 months. Rituximab toxicity was most frequently related to the first dose (consisting of flu-like symptoms, fever, chills, or hypotension), and typically of mild-to-moderate severity; myelosuppression and infection were uncommon.

Experience with rituximab in previously untreated CLL is more limited (Table 3). When used in this first-line setting, rituximab (375 mg/m^2 once weekly for 4 weeks) tested on 44 high-risk patients (70% Rai stage III–IV) achieved an OR rate of 58% (CR 9%).[42] In a second study involving 21 treatment-naïve Rai stage 0–II CLL patients who did not have an indication for therapy by National Cancer Institute criteria, the OR rate with rituximab (375 mg/m^2 once weekly for 8 weeks) was 86%, with 19% CRs.[41]

In sum, although rituximab monotherapy has some efficacy in CLL the responses are almost all partial remissions and are not durable. However, the efficacy and acceptable safety profile of rituximab have encouraged the development of combinations of rituximab with cytotoxic chemotherapeutic agents.

Table 1 Clinical trials of alemtuzumab in previously treated or treatment-naïve patients with CLL

Study	*Patients*	*Previous therapy*	*Regimen details*	*Response rate: OR (CR + PR) (%)*	*Survival or response duration*
Keating *et al.*[21]	CLL ($n = 93$)	Up to seven regimens, including one with alkylating agents; all fludarabine-refractory	i.v., 30 mg thrice weekly for up to 12 weeks	33 (2 + 31)	Median overall survival 16 months; 32 months for responders
Kennedy *et al.*[24]	CLL ($n = 77$)	Refractory to a median of three prior therapies; 76 fludarabine-refractory patients	i.v., 30 mg thrice weekly to maximum response (median 12 weeks)	44 (25 + 19) (19% had no detectable MRD)	75% actuarial survival at median follow-up of 23 months for responders
Österborg *et al.*[27]	CLL ($n = 29$)	Eight relapsed after chemotherapy; 21 refractory to $\geqslant 1$ alkylating-containing regimen	i.v., 30 mg thrice weekly for up to 12 weeks	42 (4 + 38)	Median response duration 12 months
Rai *et al.*[28]	CLL ($n = 23$) (T–PLL ($n = 1$))	All refractory to fludarabine and other chemotherapy agents	i.v., 30 mg thrice weekly for up to 16 weeks	33 (0 + 33)	Overall median survival 28 months; 36 months for responders
Lundin *et al*[26]	CLL ($n = 38$)	None	SC, 30 mg thrice weekly for up to 18 weeks (median 15 weeks)	87 (19 + 68)	Median time to treatment failure not reached at 18 months
Österborg *et al.*[29]	CLL ($n = 9$)	None	SC ($n = 4$) or i.v. ($n = 5$), 30 mg thrice weekly until CR or up to 18 weeks (median 13 weeks)	SC: 100 (25 + 75) i.v.: 80 (40 + 40)	At follow-up, response duration ranged from 8+ to 24+ months (median response not yet reached)
Bowen *et al.*[30]	CLL ($n = 6$) (B-PLL ($n = 1$))	Refractory to ($n = 4$) or relapsed from ($n = 3$) fludarabine	SC, 30 mg thrice weekly for 6–12 weeks	57 (14 + 43)	Overall median survival 11 months

Abbreviations: B-PLL, B-cell prolymphocytic leukemia; CR, complete response; i.v., intravenous; MRD, minimal residual disease; OR, overall response; PR, partial response; SC, subcutaneous; T-PLL, T-cell prolymphocytic leukemia.

Table 2 Clinical trials of alemtuzumab in previously treated or treatment-naïve patients with lymphoid neoplasms

Study	*Patients*	*Previous response to therapy*	*Regimen details*	*Response rate: OR (CR + PR) (%)*	*Survival or response duration*
Dearden *et al.*[32]	T-PLL ($n = 38$)	62% of 36 patients receiving prior therapy were refractory; two patients treatment-naïve	i.v., 30 mg thrice weekly until maximum response (median 5 weeks)	First line: 100 (100 + 0) Second line: 75 (58 + 17)	Median overall survival 10 months; 16 months for responders versus 4 months for nonresponders
Keating *et al.*[33]	T-PLL ($n = 76$)	100% of 72 patients receiving prior therapy were refractory; four patients treatment-naïve	i.v., 30 mg thrice weekly for 4–12 weeks (median 6 weeks)	First line: 75 (75 + 0) Second line: 50 (38 + 12)	Median overall survival 8 months; 15 months for responders
Lundin *et al.*[34]	MF/SS ($n = 22$)	All had received five or fewer systemic treatments	i.v., 30 mg thrice weekly for up to 12 weeks (median 10 weeks)	55 (32 + 23)	Median time to treatment failure in responsive patients 12 months (range 5–32 months)

Abbreviations: CR, complete response; i.v., intravenous; MF/SS, mycosis fungoides/Sézary syndrome; OR, overall response; PR, partial response; T-PLL, T-cell prolymphocytic leukemia.

Fludarabine plus alemtuzumab combination therapy

The rationale for combining fludarabine and alemtuzumab is two-fold. Firstly, fludarabine has activity against 'bulky' disease (ie lymphadenopathy) but MRD persists; in contrast, alemtuzumab may be most effective when used for the elimination of MRD as it preferentially clears disease from the blood, bone marrow, and spleen.[15] Secondly, the two agents have nonoverlapping mechanisms of actions and therefore lack cross-resistance.[48] This suspected benefit of administering alemtuzumab with fludarabine, either in combination or sequentially, has been borne out in clinical investigations of various hematological cancers, including CLL.

Although results from large-scale trials are not yet available, preliminary studies show that the combination of fludarabine plus alemtuzumab is an effective therapy for heavily pretreated CLL cases (Table 4).[49,50] In one of these studies, six CLL patients refractory to fludarabine alone (median eight courses) and to alemtuzumab alone (median 13 weeks) were cotreated

Table 3 Clinical trials of rituximab monotherapy and combination therapies in CLL

Study	*Regimen and doses (mg/m²)*	*Number of patients*	*Response rate (%)*		
			OR	*CR*	*PR*
Monotherapy					
Byrd *et al.*[39]	R 100, then 375 thrice weekly for 4 weeks	27 PT + 6 UT[a]	45	3	42
O'Brien *et al.*[40]	R 375, then 500–2250 1 × weekly for 3 weeks	39 (mostly PT)	36	0	36
Itala *et al.*[38]	R 375 1 × weekly for 4 weeks	23 PT	35	0	35
Huhn *et al.*[37]	R 375 1 × weekly for 4 weeks	28 PT	25	0	25
Thomas *et al.*[41]	R 375 1 × weekly for 8 weeks	21 UT	86	19	67
Hainsworth *et al.*[42]	R 375 1 × weekly for 4 weeks	44 UT[a]	51	4	47
Combination therapy					
Byrd *et al.*[43] (F + R)	F 25 d1–5 every 4 weeks for six cycles plus R 375 d1 + 3 on cycle 1 and d1 on cycles 2–6 (concurrent)	51 UT	90	47	43
	F 25 d1–5 every 4 weeks for six cycles, then R 375 1x weekly for 8 weeks (sequential)	53 UT	77	28	49
Schulz *et al.*[44] (F + R)	F 25 d1–5 every 4 weeks for four cycles plus R 375 on cycles 3 + 4, then R 375 on d113 + 151	20 UT + 11 PT	87	33	55
Keating *et al.*[45] (FRC)	F 25 d1–3 plus C 250 d1–3 plus R 375–500 d1, repeated every 4 weeks for six cycles	202 UT	95	68	27
Lamanna *et al.*[46] (FRC)	F 25 d1–5 every 4 weeks for six cycles, then HDC 3000 every 3 weeks for three cycles, then R 375 weekly for 4 weeks	24 UT	88	53	35
Wierda *et al.*[47] (FRC)	F 25 d1–3 plus C 250 d1–3 plus R 375–500 d1, repeated every 4 weeks for six cycles	179 PT	73	25	48

[a]Study also included patients with small lymphocytic lymphoma (SLL); results for CLL and SLL are reported together.
Abbreviations: C, cyclophosphamide; CR, complete response; d, days; F, fludarabine; HDC, high-dose cyclophosphamide; OR, overall response; PR, partial response; PT, previously treated; R, rituximab; UT, untreated.

Table 4 Clinical trials of fludarabine and alemtuzumab combination therapy in CLL

Study	*Regimen*[a]	*Number of patients*	*Response rate (%)*			*Patients without MRD (%)*
			OR	*CR*	*PR*	
Elter *et al.*[49]	Flu (i.v.) + Alem (i.v.)	14[b]	86	64	22	36
Kennedy *et al.*[50]	Flu (i.v.) + Alem (i.v.)	6[b]	83	17	66	33
Rai *et al.*[51]	Flu (i.v.) → Alem (i.v.)	36[c]	92	42	50	ND
Dyer *et al.*[52]	Flu (NR) → Alem (i.v.)	6[b]	100	83	17	83
Montillo *et al.*[53]	Flu (oral/i.v.) → Alem (SC)	12[b]	100	92	8	25
Montillo *et al.*[25]	Flu (oral/i.v.) → Alem (SC)	9[b]	100	91	9	33
Tedeschi *et al.*[54]	Flu (NR) → Alem (SC)	11[b]	100	91	9	ND[d]

[a]Fludarabine (typical dosage: 25–30 mg/m² i.v. or 40 mg/m² orally given for 3–5 days every 28 days for 2–4 cycles) and alemtuzumab (typical dosage: 10–30 mg i.v. or SC given thrice weekly for 4–10 weeks) were administered concomitantly (Flu + Alem) or sequentially (Flu → Alem; alemtuzumab was given 4–8 weeks after the last course of fludarabine).
[b]Patients had received previous treatment with chemotherapy.
[c]Patients were previously untreated.
[d]Successful harvesting of mobilized CD34+ peripheral blood stem cells for autologous SCT was achieved using granulocyte colony-stimulating factor and immediate-dose cytarabine in all patients.
Abbreviations: Alem, alemtuzumab; CR, complete response; Flu, fludarabine; i.v., intravenous; MRD, minimal residual disease; ND, not determined; NR, not reported; OR, overall response; PR, partial response; SC, subcutaneous.

with fludarabine (25 mg/m² i.v., days 1–3 every 28 days for 2–4 cycles) plus alemtuzumab (30 mg i.v., thrice weekly). Five patients responded, including one CR; MRD was undetectable in two patients.[50] This combination was similarly active when tested on a reduced-intensity regimen of fludarabine (30 mg/m² i.v., days 1–3) plus alemtuzumab (30 mg i.v., days 1–3) given every 28 days for four cycles.[49] Of 14 evaluable patients, most of which were Binet stage C (86%), 12 patients responded, with nine CRs (Figure 1). This combination was generally well tolerated, although transient grade 3–4 leukopenia ($n = 9/14$) and thrombocytopenia ($n = 3/14$) were reported.[49]

In another series of studies, sequential application of fludarabine for debulking followed by alemtuzumab to purge residual disease demonstrated impressive results.

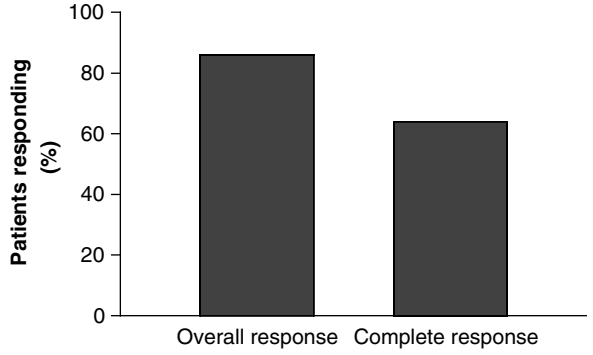

Figure 1 Responses to concomitant application of fludarabine (30 mg/m²/day, days 1–3) and alemtuzumab (30 mg absolute, days 1–3) repeated every 28 days for four cycles in high-risk relapsed/refractory CLL patients.[49]

In the largest of these trials, alemtuzumab (30 mg i.v., thrice weekly) was given to 36 CLL patients after first-line treatment with fludarabine (25 mg/m^2 i.v., days 1–5 every 28 days for four cycles).[51] The OR rate with this regimen was 92% (CR 42%). Most patients experienced alemtuzumab infusion-related adverse events (typically infections). In other more preliminary investigations on CLL patients, i.v. or oral fludarabine followed by i.v. or subcutaneous alemtuzumab eradicated MRD (undetectable by flow cytometry or polymerase chain reaction) and permitted mobilization of uncontaminated stem cells for autografting (Table 4).[25,52–54] In these studies, subcutaneous alemtuzumab was associated with fewer adverse events than when given i.v., without loss of efficacy.[25,53,54] Although the results of these preliminary studies investigating the combination of fludarabine plus alemtuzumab are encouraging, longer follow-up periods are essential to determine if these improved response rates translate into prolonged survival. However, in one such recent study patients who had responded to fludarabine (with or without cyclophosphamide) were randomized to receive either consolidation with intravenous alemtuzumab or no further therapy. At a median follow-up of 15.5 months in this series, none of the 11 patients treated with alemtuzumab had progressed compared with a mean progression-free survival of 21.6 months for the 10 control patients (Figure 2).[55]

Fludarabine plus rituximab combination therapy

Based on the effectiveness of fludarabine in CLL, the observation of some activity of rituximab as a single agent in CLL, and in particular the absence of expected overlap in the toxicity profile of the two agents, trials have been initiated to investigate the combination of fludarabine and rituximab, with or without cyclophosphamide (Table 3).

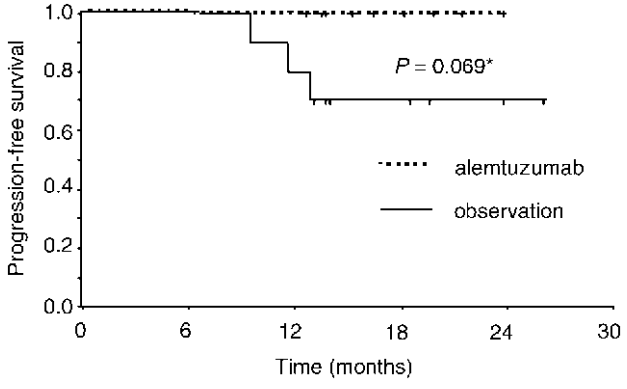

Figure 2 Progression-free survival of patients with CLL who responded to first-line treatment with fludarabine or fludarabine plus cyclophosphamide who then received alemtuzumab consolidation therapy (i.v.; 3 to 10 to 30 mg dose escalation over the first 3 days, then 30 mg thrice weekly for 12 weeks) or no further treatment (observation arm).[55] Reproduced with permission. *A follow-up analysis has revealed a significant effect of alemtuzumab on progression-free survival ($P = 0.036$).[56]

In a trial involving 179 relapsed or refractory CLL patients (50% Rai stage III–IV), the combination of fludarabine (25 mg/m^2, days 1–3) and rituximab (375 mg/m^2, day 1 of cycle 1 (each cycle 28 days) and 500 mg/m^2, day 1 of cycles 2–6) with cyclophosphamide (250 mg/m^2, days 1–3) (FCR) achieved an OR rate of 73% and a CR rate of 25%.[47] As expected, better remission rates were produced by this FCR regimen when tested in 202 treatment-naïve low-risk CLL patients (67% Rai stage I–II), with an OR rate of 95% (CR 68%).[45] The most common toxicities associated with FCR in this report were mainly hematological and gastrointestinal; infectious complications were not reported. In another study involving 24 previously untreated CLL patients, a three-stage sequential regimen consisting of fludarabine (25 mg/m^2, days 1–5 every 4 weeks for six cycles), then high-dose cyclophosphamide (3 g/m^2 versus 250 mg/m^2 standard dose, every 3 weeks for three cycles) and then rituximab (375 mg/m^2, every week for four cycles) produced similarly high response rates (OR 88%, CR 53%).[46]

The extent of clinical benefit provided by cyclophosphamide in the FCR regimen is uncertain as combination therapy with fludarabine plus rituximab, without cyclophosphamide, produced good response rates when given first-line (OR 84–85%, CR 25–38%)[43,44] or second-line (OR 90%, CR 45%)[44] (Table 4). The most frequent adverse events reported in these studies were myelosuppression, infusion-related toxicity and infection. The results of a recent study have also questioned the advantages of combining cyclophosphamide with fludarabine, over fludarabine monotherapy. Interim analysis of a randomized phase III trial showed that first-line fludarabine plus cyclophosphamide did not produce significantly more ORs (92 versus 87%) or CRs (25 versus 12%), compared with fludarabine alone; however, myelosuppression, leucocytopenia, and gastrointestinal symptoms were significantly more frequent (all $P \leq 0.002$) with the combination therapy.[57] The precise role of combinations of fludarabine and rituximab in CLL is currently unclear, but is the subject of several randomized controlled trials that are currently in progress.

Other potential fludarabine and monoclonal antibody combination therapies

Many other monoclonal antibodies that target cell surface or intracellular proteins are also under investigation for the treatment of various leukemic and lymphocytic malignancies.[1,15,16,20] Some of these antibodies, along with their target diseases, include anti-CD22 (CLL, NHL, hairy cell leukemia), anti-CD23 (CLL), anti-CD33 (AML), anti-1D10 (CLL, NHL, follicular lymphoma), anti-HM1.24 (multiple myeloma), and antivascular endothelial growth factor (AML). The growing number of active antibodies for the treatment of hematological cancers provides encouraging evidence that the therapeutic approach to these diseases may be shifting from nonspecific cytotoxics to more specific

biological agents. Finally, based on the promising results of treatment involving fludarabine plus the anti-CD52 antibody alemtuzumab in CLL or plus the anti-CD20 antibody rituximab, it is anticipated that other fludarabine–antibody combination regimens will be of therapeutic benefit for a range of lymphoproliferative disorders.

Fludarabine in combination with other experimental treatments

In addition to the identification of novel genetic, molecular, and phenotypic markers of malignant cells, the recognition of intracellular molecules that regulate processes such as cell apoptosis or cell proliferation has enlarged the therapeutic spectrum of treatments for neoplastic disorders. Two of these experimental agents that have entered the clinic for the treatment of CLL or other lymphoproliferative diseases are discussed.

Fludarabine plus bcl-2 antisense therapy

The proto-oncogene *bcl-2* is a major apoptosis-regulatory gene that is highly expressed in various hematological malignancies and solid tumors. By inhibiting apoptosis, the bcl-2 protein confers resistance to standard treatments involving cytotoxic agents, radiotherapy, or monoclonal antibodies.[58] Antisense oligonucleotides directed against bcl-2 (eg G3139) have been tested in multiple phase I and II studies involving patients with CLL, NHL, acute leukemia, and multiple myeloma, with suspected potential in Waldenström's macroglobulinemia.[59,60] Moreover, G3139 demonstrates synergistic effects when combined with cytotoxic agents.[59] Based on this finding, a number of trials are now underway to assess the activity of G3139 in combination with cytotoxic chemotherapy. Of note, these include two phase III trials of fludarabine plus G3139 (plus cyclophosphamide or cytarabine) in CLL[59,60] and a phase I trial of G3139 plus FLAG (fludarabine with cytarabine and granulocyte colony-stimulating factor) in acute leukemia.[61]

Fludarabine plus modulators of cyclin-dependent kinase

Abnormalities in the cell cycle and of proliferation are the basis of many human lymphoproliferative disorders. Key regulators of these processes are a family of serine/threonine kinases termed cyclin-dependent kinases, and their inhibition may be a useful therapeutic approach. Two such kinase inhibitors that have entered clinical trials are UCN-01 and flavopiridol.[62]

UCN-01 is a hydroxylated derivative of staurosponine with pharmacological properties that include protein kinase C inhibition, promotion of cell-cycle arrest, and induction of apoptosis independently of p53.[62] This inhibitor also sensitizes human tumor cells, including leukemia and lymphoma cells, to the cytotoxic effect of fludarabine and other chemotherapeutic agents.[63–66] These findings suggest that fludarabine and UCN-01 may act synergistically. Phase I/II evaluations of UCN-01 (alone or in combination with cytotoxics) are ongoing in patients with refractory CLL, NHL, or large-cell lymphoma.[62,67]

The second of these cyclin-dependent kinase inhibitors is flavopiridol, which antagonizes several cell-cycle proteins and induces p53-independent apoptosis.[62] Flavopiridol has considerable toxicity against CLL cells *in vitro*[7] and potently inhibited cell growth in a 60-cell-line anticancer drug screen.[62] Phase II/III trials of flavopiridol in refractory CLL, NHL, and MCL are underway,[62] and the potential benefits of combining flavopiridol with cytotoxics like fludarabine are being examined by several ongoing phase I investigations.

Importance of fludarabine as a conditioning regimen for stem cell transplantation

Transplantation techniques involving autologous or allogeneic bone marrow or peripheral blood stem cell engraftment are accepted therapies for a variety of hematological disorders. These approaches involve systemic chemotherapy or radiotherapy conditioning regimens that eliminate the patients' underlying malignancy and also suppress their immune system.[68] However, as many of these conditioning regimens are myeloablative, they are associated with high toxicity that limits their use to young patients (generally less than 50 years) without comorbidities.[69] A recently developed, and now preferred, curative approach is to precondition patients using nonmyeloablative techniques prior to allogeneic stem cell transplantation (SCT). These reduced-intensity preparative regimens are relatively nontoxic and reduce transplant-related mortality. Moreover, the technique of allogeneic engraftment harnesses the alloreactivity of donor immune cells against host cancer cells – the so-called graft-versus-leukemia effect.[69]

The optimal nonmyeloablative conditioning agent should have good antitumor activity and provide sufficient immunosuppression to prevent graft rejection. Based on its established characteristics and nonhematological toxicities, fludarabine has been used extensively in this setting with encouraging results. In these preparative regimens, fludarabine is commonly used in combination with one or multiple chemotherapy agents, such as melphalan, cyclophosphamide, or cytarabine. Nonmyeloablative transplants have been reported in a variety of hematological cancers, including CLL, NHL, MCL, multiple myeloma, and acute leukemias, with complete engraftment (chimerism) of donor and host cells reported in a high proportion of cases.[70–75] Furthermore, the addition of alemtuzumab to fludarabine plus melphalan nonmyeloablative conditioning regimens limits graft-versus-host disease in patients with various leukemic or lymphoid disorders following

allogeneic SCT from sibling HLA-identical or matched unrelated donors.[76–78]

These initial findings with fludarabine-containing preparative regimens for allogeneic SCT are promising, but the long-term antitumor activity of these approaches has not yet been determined. This experimental technique has good potential to increase the rate and duration of complete remissions in some hematological cancers.

Future therapeutic directions

In addition to those strategies already discussed, a variety of experimental compounds and therapeutic approaches have shown promise for the treatment of CLL and other lymphoproliferative disorders. Many of these newly developed agents demonstrate synergistic activity with fludarabine, at least *in vitro*; whether these laboratory findings translate into therapeutic effectiveness will be the subject of future clinical research.

Of the chemotherapy-based cytotoxic compounds, the protein kinase C modulator bryostatin differentiates CLL cells to resemble the morphology of hairy cell leukemia and sensitizes leukemic cells to the effects of cladribine and interferon-α.[79] Owing to these properties, bryostatin has been tested in preliminary trials involving patients with not only CLL or hairy cell leukemia but also NHL,[80] acute leukemias,[81] and multiple myeloma.[82]

Depsipeptide shows selective activity against CLL cells, presumably by inhibiting the enzyme histone deacetylase and altering apoptosis-related proteins.[83] Depsipeptide is currently under clinical investigation. A variety of additional agents with potential activity in various hematological cancers include, among others, nelarabine (a methoxy derivative of guanine arabinoside) and IDEC-132 (a topoisomerase-I inhibitor).[18,79]

A novel approach that is already an established cancer therapy utilizes the synergistic interaction between monoclonal antibodies and toxins or the effects of radiation.[15–17] The successful use of several of these immunotoxins and radioimmunoconjugates has been reported. An anti-CD22 immunotoxin (BL22) has good activity in chemotherapy-resistant hairy cell leukemia patients[84] and an anti-CD19 immunotoxin has antitumor activity in NHL.[20] The two most extensively studied radioimmunoconjugates for NHL are anti-CD20 antibodies linked to either yttrium-90 (ibritumomab) or iodine-131 (tositumomab).[15,20] Although other radioimmunotherapies have been developed for many leukemias and lymphomas, their use in CLL is limited by the extent of bone marrow involvement in this disease.

The immunological efficacy of naked and conjugated antibodies may be further improved by the development of bispecific[85] or anti-idiotypic[86] antibodies that selectively target leukemic cells rather than healthy ones. Finally, active immunization with replication-defective adenovirus vectors (eg encoding CD40 ligand)[87] or manipulation of a patient's post-SCT immune defense following nonmyeloablative chemotherapy may also play roles in future therapies for lymphomas and leukemias.

Conclusions

Increased understanding of the genetic, molecular, and phenotypic characteristics of CLL and other neoplastic disorders has led to the development of therapies that are more effective and more malignancy specific. In spite of these improvements, finding a cure for these hematological cancers remains the greatest challenge faced by those who treat these diseases. Fludarabine is established as a fundamental component of modern treatment strategies, when given either singly or in combination with other traditional chemotherapeutics, monoclonal antibodies, or newer, more experimental agents. In CLL, fludarabine has produced greater objective response rates, including the number of molecular complete remissions. Further, clinically relevant effects are often observed when fludarabine is given as a component of combination therapies.

A particularly promising approach to the treatment of CLL and other lymphoproliferative malignancies appears to be the combination of fludarabine with alemtuzumab or rituximab. Although survival data in trials investigating this strategy are only preliminary, alemtuzumab is an effective consolidation treatment associated with impressive rates of overall and complete responses, and eradication of MRD, even when given to heavily pretreated patients with relapsed or refractory disease. Fludarabine plus alemtuzumab, and other fludarabine-containing combination therapies, have also proved to be useful conditioning regimens prior to SCT techniques. Given the pivotal role that fludarabine plays in established therapies for CLL and other disorders of the blood and lymph system, future regimens for the treatment of these cancers will likely incorporate this purine analog in combination with chemotherapeutic or biological agents.

The development of such a wide variety of novel 'targeted' therapies for CLL and the other chronic lymphoproliferative disorders promises to make the goal of achieving MRD-negative remissions a reality for a large proportion of patients. If this can be achieved, then we can be cautiously optimistic that this will translate into a beneficial impact on survival and that we are moving towards a cure of these disorders.

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