Leukemia (2010) 24, 2056–2062

www.nature.com/leu

ORIGINAL ARTICLE

Therapy-related myelodysplastic syndrome and acute myeloid leukemia following fludarabine combination chemotherapy

DA Carney[1,2], DA Westerman[1,2], CS Tam[3], A Milner[4], HM Prince[1,2], M Kenealy[1], M Wolf[1,2], EH Januszewicz[1], D Ritchie[1,2], N Came[1,2] and JF Seymour[1,2]

[1]Department of Haematology and Medical Oncology, Peter MacCallum Cancer Centre, St Andrews Place, East Melbourne, Victoria, Australia; [2]Department of Medicine, University of Melbourne, Parkville, Victoria, Australia; [3]Department of Haematology, St Vincent's Hospital, Fitzroy, Victoria, Australia and [4]Centre for Biostatistics and Clinical Trials, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia

Fludarabine combination chemotherapy achieves high response rates in chronic lymphocytic leukemia (CLL) and indolent lymphoma. The aim of this study was to investigate the incidence and characteristics of treatment-related myelodysplasia and acute myeloid leukemia (t-MDS/AML) after treatment with fludarabine in combination for lymphoproliferative disorders and identify risk factors for its development. In all, 176 patients treated with fludarabine combination were followed for a median of 41 months (range 6–125 months). In all, 19 cases of t-MDS/AML have been identified for an overall rate of 10.8%. Median overall survival post-t-MDS/AML diagnosis was 11 months. Patients developing t-MDS/AML included 11/54 with follicular lymphoma (FL) (crude rate 20.4%), 5/82 with CLL (6.1%) and 3/24 with Waldenstrom macroglobulinemia or marginal zone lymphoma (12.5%). Most patients had other cytotoxic treatments (median 4, range 0–7) but three with FL had fludarabine combination as their only line of treatment. Of the eleven patients (6.3%) who received mitoxantrone with their first fludarabine combination, four (36.4%) developed t-MDS/AML ($P=0.007$). There was a trend toward prior cytotoxic therapy increasing the risk for t-MDS/AML ($P=0.067$). Fludarabine combination chemotherapy is associated with a moderate risk of t-MDS/AML particularly when combined with mitoxantrone. This complication should be considered when evaluating the potential benefit of this treatment in lymphoproliferative disorders.
Leukemia (2010) **24,** 2056–2062; doi:10.1038/leu.2010.218; published online 21 October 2010
Keywords: fludarabine; myelodysplasia; toxicity

Introduction

Advances in the treatment of indolent non-Hodgkin lymphoma and chronic lymphocytic leukemia (CLL) have resulted in higher response rates and more durable remissions. Chemoimmunotherapy has become the standard of care with the addition of rituximab to combination chemotherapy associated with improved outcomes.[1,2] As a result, the late toxicities of treatment particularly therapy-related myelodysplasia and acute myeloid leukemia (t-MDS/AML) are becoming a more important concern. It is established that up to 10% of patients with indolent non-Hodgkin lymphoma treated with either standard alkylator-based chemotherapy or high dose chemotherapy and autologous stem cell transplantation (SCT) will develop t-MDS/AML within 10 years of primary therapy.[3] The evaluation of new therapies such as radioimmunotherapy[4] and fludarabine-based combination chemotherapy and chemoimmunotherapy regimens should include an assessment of the risk of t-MDS/AML.

Therapy-related myeloid neoplasms are a distinct entity in the 2008 WHO classification of tumors of hematopoietic and lymphoid tissues.[5] Cytotoxic agents associated with this complication include alkylating agents, topoisomerase II inhibitors, ionizing radiation, antimetabolites and antitubulin agents.[5,6] The WHO classification considers t-MDS/AML as a unique clinical syndrome even though some cases may satisfy the morphological or cytogenetic criteria for other entities. Indeed, particular cytotoxic agents are associated with t-MDS/AML with characteristic biological and clinical features. Alkylating agents and/or radiation tend to be associated with MDS/AML, with a gradual dysplastic clinical onset and a latency period of 5–10 years, with unbalanced chromosomal aberrations mainly involving chromosomes 5 and 7.[7] Topoisomerase II inhibitors have been associated with overt AML without a preceding myelodysplastic phase with a shorter latency period and balanced chromosome translocations frequently involving 11q23 (*MLL*) or 21q22 (*RUNX1*).[5] The prognosis of t-MDS/AML is generally poor, with a median survival <1 year.[7]

Fludarabine is a purine analogue with marked efficacy in indolent lymphoproliferative disorders.[8,9] As part of combination chemotherapy, fludarabine achieves high response rates in CLL and indolent lymphoma.[10–12] Fludarabine inhibits DNA repair and augments the cytotoxic effect of DNA damaging agents such as cyclophosphamide.[13] This enhancement of DNA damage may also affect marrow progenitor cells. Such a mechanism could explain the observed impairment of peripheral blood progenitor cell collection after prior fludarabine treatment and could predispose to an increased risk of t-MDS/AML.[14]

The aim of this study was to investigate the incidence and characteristics of t-MDS/AML after treatment with fludarabine in combination with other cytotoxic agents for lymphoproliferative disorders and identify risk factors for its development. It updates our previously reported experience of 137 patients treated with fludarabine combination chemotherapy where 10 patients had developed t-MDS/AML (crude rate 7.3% at a median follow-up of 40 months).[15]

Materials and methods

Review of the Peter MacCallum Cancer Centre Pharmacy database from 1996 to 2008 identified 176 patients with indolent lymphoproliferative disorders treated with fludarabine

Correspondence: Dr DA Carney, Department of Haematology and Medical Oncology, Peter MacCallum Cancer Centre, Locked Bag 1 A'Beckett Street, Melbourne, Victoria 8006, Australia.
E-mail: dennis.carney@petermac.org
Received 4 January 2010; revised 7 August 2010; accepted 18 August 2010; published online 21 October 2010

combined with cyclophosphamide (C) and/or mitoxantrone (M) ± rituximab (R) as initial (57 patients) or salvage therapy (119 patients). Details of treatment protocols have been previously reported.[12,16] All patients had at least 6 months follow-up since commencing treatment and were reviewed at Peter MacCallum Cancer Centre with clinical assessment and disease-appropriate investigations. Bone marrow examinations were performed to assess disease response or to investigate unexplained cytopenias or abnormal peripheral blood smears. Institutional board review and patient consent were not required, as this was a retrospective quality-assurance activity assessing complications of standard therapy at our institution.

Kaplan–Meier analysis was used to estimate time-to-t-MDS/AML (TTMDS), defined as the time from first exposure to fludarabine combination therapy to onset of t-MDS/AML, censored at date of last follow-up or by death. The Mantel–Cox log-rank test was used to assess the effects of patient characteristics and other variables on TTMDS, including age, gender, disease type, treatment with anthracyclines, alkylating agents or radiation therapy at other times, treatment with high dose chemotherapy and autologous SCT, number of fludarabine containing treatment episodes, number of prior lines of cytotoxic treatment, and the type of fludarabine combination therapy (Table 1). It was not possible to use a study close-out (censor) date in the analysis and so results should be treated with some degree of caution.

Results

In all, 176 patients treated with fludarabine combination were followed for a median of 41 months (range 6–125 months). Patients had at least 6 months follow-up from commencement of treatment and one third (56 patients) have now been followed for >5 years. In all, 112 patients (63.6%) were males, and the median age of all patients was 59 years (range 26–85 years) at the time they were first treated with fludarabine in combination with another cytotoxic agent. Underlying disease was CLL in 82 patients (46.6%), follicular lymphoma (FL) in 54 patients (30.7%), Waldenström macroglobulinemia or marginal zone lymphoma in 24 patients (13.6%) and mantle cell lymphoma in 16 patients (9.1%).

To date, 19 cases of t-MDS/AML (Table 2) have been identified for an overall crude rate of 10.8% (13 refractory cytopenia with multilineage dysplasia, 2 chronic myelomonocytic leukemia, 1 refractory anemia with excess blasts and 3 AML with multilineage dysplasia). The diagnosis was made at a median of 42 months (range 5–99 months) following the first treatment with fludarabine in combination with another cytotoxic agent. The estimated t-MDS/AML rates at 3 and 5 years were 5.6% (95% confidence interval = 2.8–10.9%) and 10.5% (95% confidence interval = 5.9–18.2%), respectively (Figure 1a). Median overall survival post-t-MDS/AML diagnosis was 11 months.

Patients developing t-MDS/AML included 11 with FL (crude rate 20.4%), 5 with CLL (6.1%), 3 with Waldenström macroglobulinemia or marginal zone lymphoma (12.5%) and no patients with mantle cell lymphoma ($P = 0.221$). Most t-MDS/AML patients had received additional treatments, but three with FL had fludarabine combination as their only treatment. There was a median number of 2 prior cytotoxic treatments (before the first fludarabine combination—range 0–6) and a median of 4 (range 0–7) cytotoxic treatments other than the first fludarabine combination prior to the development of t-MDS/AML. Excluding the first fludarabine combination, 15 of the 19 patients who developed t-MDS/AML had been treated with alkylators, 8 with anthracyclines and 6 with radiotherapy including one with radioimmunotherapy. Treatment with steroids, rituximab and interferon as single agents was not included in this assessment. The type and number of lines of other cytotoxic treatments were not statistically significant risk factors for the development of t-MDS/AML when total patient cohort was assessed, although prior cytotoxic therapy was associated with a trend toward increasing the risk of t-MDS/AML ($P = 0.067$) (Figure 1b).

Table 1 Prognostic factor analysis for time-to-t-MDS/AML

	No. patients	*Kaplan–Meier analysis*		
		Estimated t-MDS/AML rate at 5 years		
		%	*95% CI*	*P-value log-rank test*
All patients	176	10.5%	5.9–18.2	
Disease group				0.221
CLL	82	1.3%	0.2–8.9	
FL	54	27.1%	15.2–43.4	
MCL	16	0.0%	—	
MZL/WM	24	0.0% (All events occurred after 5 years)	—	
Gender				0.115
Male	112	6.3%	2.3–15.9	
Female	64	18.3%	8.9–33.8	
Age (years)				0.581
<60	92	12.8%	6.3–24.3	
⩾60	84	6.7%	2.5–16.9	
Fludarabine with mitoxantrone				0.007
No (FC/FCR)	165	7.8%	3.9–15.0	
Yes	11	40.0%	13.1–74.6	
Prior lines of cytotoxic treatment				0.067
0	61	6.4%	2.0–18.4	
>0	115	13.1%	6.6–24.4	
Lines of fludarabine treatment				0.826
1	138	12.7%	6.8–22.5	
>1	38	4.0%	0.6–23.5	
Other cytotoxic treatment				
Anthracycline				0.413
No	108	6.6%	2.7–15.3	
Yes	68	18.8%	8.5–36.4	
Alkylating agents				0.595
No	50	7.8%	2.5–22.2	
Yes	126	11.8%	5.9–21.9	
Radiotherapy				0.659
No	109	9.2%	4.2–18.7	
Yes	67	13.5%	5.4–29.8	
Autologous SCT				0.416
No	153	9.3%	4.9–16.9	
Yes	23	14.9%	3.7–44.1	

Abbreviations: CI, confidence interval; CLL, chronic lymphocytic leukemia; FCR, fludarabine in combination with cyclophosphamide and rituximab; FL, follicular lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; SCT, stem cell transplant; t-MDS/AML, treatment-related myelodysplasia and acute myeloid leukemia; WM, Waldenström macroglobulinemia.

Table 2 Characteristics of patients developing treatment-related MDS and secondary AML following fludarabine combination therapy

Patient	*Age*	*Sex*	*Disease*	*Prior treatment*	*F regimen*	*WHO classification*	*Cytogenetics*	*Time to t-MDS/AML (months)*	*Outcome (months since t-MDS/AML diagnosis)*
1	62	F	CLL	Cyclophosphamide CHOP Etoposide Radiotherapy	FND ×3 FC ×3 FCR ×3	Refractory anemia with excess blasts (I)	38∼44, XX, ?dic(5;17)(q?11;p?13), del(7)(q22), add(8)(p13), −13[cp8]/46, XX[13]	75	Died 9 months
2	46	M	CLL	Chlorambucil	FC ×5 FCR ×6	Refractory anemia with multilineage dysplasia	No mitoses	96	Died 11 months
3	51	F	CLL	Rituximab Chlorambucil + Prednisolone R-CHOP	FCR ×6	Refractory anemia with multilineage dysplasia	46, XX[20]	12	Alive 5 months (4 months post-AlloSCT)
4	72	M	SLL/CLL	CVP R-CVP	FC ×3	Refractory anemia with multilineage dysplasia	46, XY, del(13)(q12)[5]/47, XY, add(4)(q35), +12[3]/46, XY[15]	60	Died 12 months
5	67	M	CLL	Chlorambucil	FC ×6	AML with multilineage dysplasia	44–47, XY, der(5;17)(5pter→5q11::5q31→5q33::17p11→17pter), add(9)(q21), −13, add(15)(p11), −18, add(21)(q22), −22, +2–4r, +mar1[cp16]/46, XY[3]	64	Died 2 months
6	51	F	FL	CHOP Chlorambucil and Prednisolone Radiotherapy Rituximab	FC ×3	Refractory anemia with multilineage dysplasia	46, XX, t(4;12)(q21;p11)[8]/46, XX[19]	5	Died 13 months (5 months post-AutoSCT)
7	65	M	FL	Radiotherapy CHOP	FCR ×6	AML with multilineage dysplasia	44, XY, −3, del(5)(q13q34), −7[5]/43, idem, −del(5), del(7)(q31q36), −13, add(19)(q13.4), +mar1[6]/46, XY[9]	29	Died 11 months (8 months post-AutoSCT)
8	53	F	FL	CHOP Rituximab	FCMR ×4	Refractory anemia with multilineage dysplasia	46, XX, −7, +mar1 [1]/46, XX, −7,−21, +mar2, +mar3 [6]/45, XX, −7,−20, −21, + mar2, +mar3 [2]/46, XX [24]	28	Died 21 months (5 months post-AutoSCT)
9	54	M	FL	Chlorambucil	FCMR ×4 (followed by radiotherapy)	Chronic myelomonocytic leukemia (I)	45, XY, del(2)(p11;p14), dic(3;7)(p11;p13), del(7)(q22), −15, der(?)t(?;3)(?;p13)[11]/44∼46, idem, −10, add(12)(p13), +mar1, +mar2, +mar3[cp20]	46	Died 15 months
10	69	M	FL	Chlorambucil I^{131}anti-CD20	FCR ×3	Refractory anemia with multilineage dysplasia	46, XY, del(7)(q22)[12]/46, XY[18]	16	Died 17 months
11	51	F	FL	0	FCR ×6	Refractory anemia with multilineage dysplasia	Not performed	40	Alive 17 months (5 months post-AlloSCT)
12	53	M	FL	Chlorambucil CHOP AutoSCT	FM ×3 FC ×3 (followed by CNOP, AutoSCT and radiotherapy)	Refractory anemia with multilineage dysplasia	Not performed	42	Died 8 months
13	39	F	FL	CVP CNOP BEAM-AutoSCT	FC ×4 (followed by R-CVP and radiotherapy)	Refractory anemia with multilineage dysplasia	46, XX, +1, der(1;7)(q10;p10)[17]/46, XX, del(5)(q21q31)[7]/46, XX[14]	55	Died 25 months (post AlloSCT)
14	51	F	FL	0	FCR ×6	Refractory anemia with multilineage dysplasia	Trisomy 8	13	Alive 11 months

Table 2 (*Continued*)

Patient	Age	Sex	Disease	Prior treatment	F regimen	WHO classification	Cytogenetics	Time to t-MDS/AML (months)	Outcome (months since t-MDS/AML diagnosis)
15	61	F	FL	Chlorambucil CVP Rituximab R-CHOP R-CVP Cyclophosphamide	FCR × 4	Refractory anemia with multilineage dysplasia	46, XX, del(13)(q12q22)[2]/46, XX[47]	9	Alive 11 months (10 months post-AutoSCT)
16	60	F	FL	0	FCR × 6	Refractory anemia with multilineage dysplasia	46, XX [16]	13	Alive 3 months
17	57	M	WM	Fludarabine alone	FC × 4	Chronic myelomonocytic leukemia (l)	46, XY, del(13)(q12q22)[20]	76	Alive 49 months
18	60	F	WM	Chlorambucil CVP	FC × 4	AML with multilineage dysplasia	39–40, XX, ?add(1)(q32), −3, add(5)(q31), add(7)(p22), −10, add(12)(p11), ?der(13;21)(q10;q10), −15, add(16)(q24), add(17)(p13), −21, −22[cp2]	61	Died 7 months
19	60	M	WM	CVP	FC × 4 FCR × 3 FCR × 3	Refractory anemia with multilineage dysplasia	46, XY[20]	99	Alive 20 months

Abbreviations: AML, acute myeloid leukemia; Auto/AlloSCT, autologous/allogeneic stem cell transplant; BEAM, carmustine, etoposide, cytarabine and melphalan conditioning; C, cyclophosphamide; CEP, cyclophosphamide, etoposide and prednisolone; CHOP, cyclophosphamide, adriamycin, vincristine and prednisolone; CLL, chronic lymphocytic leukemia; CNOP, cyclophosphamide, mitoxantrone, vincristine and prednisolone; CVP, cyclophosphamide, vincristine and prednisolone; E, etoposide; F, fludarabine; FL, follicular lymphoma; FND, fludarabine, mitoxantrone and dexamethasone; M, mitoxantrone; MDS, myelodysplasia; MZL, marginal zone lymphoma; R, rituximab; R-VIC, rituximab, etoposide, ifosfamide and carboplatin; SLL, small lymphocytic lymphoma; WM, Waldenström macroglobulinemia.

However, the type of fludarabine combination therapy was significantly associated with the risk of t-MDS/AML. Eleven patients (6.3%) received mitoxantrone (M) with their first fludarabine (F) (FM ± steroids in 3, fludarabine, cyclophosphamide and mitoxantrone (FCM) in 1 and FCMR in 7) combination treatment and four of those (36.4%) developed t-MDS/AML. Median TTMDS was significantly shorter for those patients treated with fludarabine with mitoxantrone (F + M) ($P = 0.007$), being 6.3 years compared with >10.1 years without mitoxantrone (Figure 1c). The rate of t-MDS/AML at 5 years was 40.0% for F + M compared with 7.8% without mitoxantrone.

The type of lymphoproliferative disease did not effect TTMDS when all four diseases were considered but when the two largest groups were compared, FL had a higher t-MDS/AML risk than CLL ($P = 0.05$) (Figure 1d). The increased risk in FL patients may have been influenced by the greater proportion of these patients with prior cytotoxic treatment (78% compared with 59%). Indeed, the CLL patients who developed t-MDS/AML had all received prior cytotoxic therapy. A higher proportion of patients with FL were treated at other times with anthracyclines (61%), alkylating agents (81%) and radiotherapy (48%) compared with those with CLL (24, 67 and 32%, respectively) although overall, these other treatments did not feature as statistically significant risk factors for t-MDS/AML.

Karyotypic analysis of t-MDS/AML was typically complex (Table 2). Results are available in 16 of the 17 cases assessed. Thirteen of the 16 had cytogenetic aberrations. Abnormalities of chromosome 7 were observed in 7 patients, chromosome 13 was involved in 7 patients and chromosome 5 in 5 patients.

Autologous SCT was used as treatment for t-MDS/AML in three patients. Durable responses were not achieved in these patients (survival 5, 5 and 8 months post-autologous SCT). Allogeneic SCT was performed in three patients with t-MDS/AML. One patient died from early transplantation-related complications and the other two patients are alive at 4 and 5 months post-allogeneic SCT.

Discussion

There is growing recognition of the leukemogenic potential of purine analogue therapy, particularly when combined with other DNA damaging agents.[17] Assessment of the risk of this complication is often confounded in patients with indolent lymphoproliferative disorders by the frequent exposure to multiple lines of cytotoxic therapy. Rates of t-MDS/AML will also vary with the length of follow-up. Our median follow-up of 41 months is still relatively early in view of the median time of 42 months to the development of t-MDS/AML. Furthermore, our overall crude rate of 10.8% is also likely to be an underestimate because bone marrow examinations were only performed in patients with either cytopenias or marked morphologic dysplastic changes in the peripheral blood. Conversely, estimations may be falsely high if early reversible dysplastic features and cytopenias associated with cytotoxic treatment are misinterpreted.[18] In our study, the diagnosis of t-MDS/AML was not made within 6 months of cytotoxic treatment unless the diagnostic features were confirmed at a later time or a clonal cytogenetic abnormality was detected. An abnormal karyotype occurs in the leukemic cells in over 90% of t-MDS/AML cases and therefore the diagnosis should be made with caution in the absence of this feature.[5]

Other published trials of fludarabine combination therapies allow a comparison to be made with the rates of t-MDS/AML we

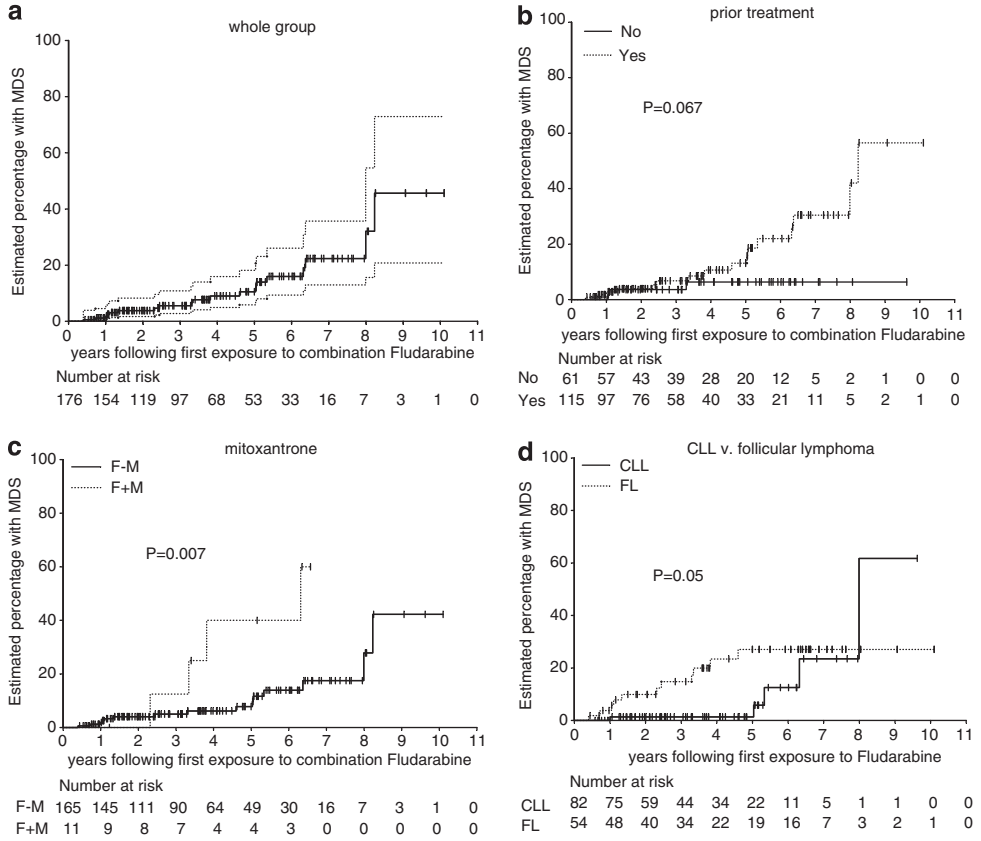

Figure 1 Kaplan–Meier plots of time-to-t-MDS/AML following first exposure to fludarabine combination therapy. Patients with censored times are shown by tick marks. (**a**) Whole group—95% confidence intervals are shown by dotted lines. (**b**) Effect of prior cytotoxic treatment. (**c**) Effect of mitoxantrone (M) when included as part of fludarabine (F) combination therapy. (**d**) Comparison between CLL and follicular lymphoma.

have observed. In the CALGB 9011 study of frontline CLL treatment, the rate of t-MDS/AML was 3.5% for the combination of fludarabine and chlorambucil compared with 0.5 and 0% for patients randomized to receive fludarabine or chlorambucil alone.[19] More recently, fludarabine in combination with cyclophosphamide and rituximab has demonstrated remarkable activity in CLL.[2] Evaluation of 300 patients receiving this regimen as initial therapy, with a median follow-up of 6 years, revealed eight patients who developed MDS for an actuarial risk of 2.8% at 6 years. With a median follow-up of 42 months, fludarabine in combination with mitoxantrone and dexamethasone has been associated with a similar rate of MDS (4%) as initial therapy of indolent non-Hodgkin lymphoma.[20] Another trial assessing FCM as initial treatment in 120 patients with advanced FL reported no late toxicity after a median follow-up of 3.9 years.[21] In our study, there was a high rate of t-MDS/AML in patients who received mitoxantrone as part of their first fludarabine combination treatment. This was the most highly significant risk factor for t-MDS/AML identified in our cohort of patients. In contrast to the fludarabine, mitoxantrone and dexamethasone and FCM studies cited above, all patients in our series had prior cytotoxic treatment. Mitoxantrone dose may also play a role in the risk of t-MDS/AML with our patients generally receiving 8–10 mg/m^2 with each cycle. The fludarabine, mitoxantrone and dexamethasone trial also used 10 mg/m^2 whereas the FCM trial used 6 mg/m^2.

Prior cytotoxic therapy is likely to contribute to the risk of t-MDS/AML following fludarabine treatment. Bowcock *et al*.[22] assessed 41 patients with indolent lymphoproliferative disorders treated with fludarabine alone or with cyclophosphamide. In this study, t-MDS/AML developed in eight patients (crude incidence 20%) and all had received prior alkylator therapy and fludarabine in combination with cyclophosphamide. The risk of MDS was also associated with the total dose of fludarabine. Fludarabine may also play a role in the development of t-MDS/AML following subsequent cytotoxic therapy. In a review of 746 patients treated with yttrium-90 (^{90}Y) ibritumomab tiuxetan (radioimmunotherapy) 19 patients (2.5%) developed t-MDS/AML at a median of 4.4 years follow-up.[4] FL histology and prior purine analogue therapy were the only significant risk factors identified for the development of t-MDS/AML.

In our study, the effect of both previous and subsequent cytotoxic treatment in relation to the initial fludarabine combination therapy were assessed. Treatment at other times with alkylators, anthrayclines, autologous SCT and radiotherapy did not significantly influence TTMDS although there was a trend toward prior cytotoxic therapy increasing the risk of t-MDS/AML ($P=0.067$). However, the development of t-MDS/AML in three patients following fludarabine combination therapy as their only line of treatment has demonstrated a risk independent of other cytotoxic therapy. These three patients comprise 7% of 43 patients who had no other cytotoxic therapy. Overall, gender did not reach statistical significance ($P=0.115$), but notably the three patients who developed t-MDS/AML following fludarabine combination therapy as their only line of treatment were all females with FL.

Fludarabine is able to target quiescent as well as cycling cells.[23] This property contributes to its efficacy in the treatment of indolent lymphoproliferative disorders but may also produce

hematopoietic stem cell toxicity. This is evident in the prolonged cytopenias and impaired ability to harvest stem cells associated with fludarabine-based regimens,[2,18,24] and may also be manifest by t-MDS/AML. The t-MDS/AML associated with fludarabine combination therapy has similar cytogenetic abnormalities to those seen with alkylating agents.[19,20] This may reflect the role of fludarabine in preventing repair of DNA damage initiated by the other cytotoxic agent. However, it is interesting to note the presence of chromosome 7 abnormalities in case reports of t-MDS/AML associated with single agent fludarabine, suggesting that it may also exert a direct mutagenic effect.[25,26] Our results also show an association of fludarabine with chromosome 7 abnormalities although chromosome 13 and 5 abnormalities were also prominent. Three of the four cases of t-MDS/AML associated with F + M had cytogenetic assessments and all demonstrated deletions of chromosome 7 in addition to multiple other aberrations.

Fludarabine may also cause undetected genetic damage or deplete lymphocytes involved in immunosurveillance of malignant cells. This may play a role in the increased risk of non-hematopoietic malignancies reported after fludarabine treatment.[27]

Our results confirm the poor prognosis of t-MDS/AML and highlight the importance of prevention through identification of risk factors prior to fludarabine combination therapy. The susceptibility of some patients could result from defects in drug metabolism or DNA repair and these factors should be assessed in the future. Early detection of MDS in patients at risk is also worthwhile as more effective interventions, particularly azacitidine, are becoming available.[28]

Conclusion

Fludarabine combination chemotherapy is associated with a moderate risk of t-MDS/AML. Our results suggest that fludarabine combined with mitoxantrone as salvage therapy has a greater risk of t-MDS/AML and if this observation is confirmed in other series, such combinations should be used with caution outside the setting of a clinical trial. Fludarabine combination regimens are highly effective in lymphoproliferative disorders but further research is required to identify those patients at a higher risk for the development of t-MDS/AML to enable more selective application of this therapy.

Conflict of interest

The authors declare no conflict of interest.

References

1 Hiddemann W, Kneba M, Dreyling M, Schmitz N, Lengfelder E, Schmits R *et al*. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. *Blood* 2005; **106**: 3725–3732.
2 Tam CS, O'Brien S, Wierda W, Kantarjian H, Wen S, Do KA *et al*. Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. *Blood* 2008; **112**: 975–980.
3 Armitage JO, Carbone PP, Connors JM, Levine A, Bennett JM, Kroll S. Treatment-related myelodysplasia and acute leukemia in non-Hodgkin's lymphoma patients. *J Clin Oncol* 2003; **21**: 897–906.
4 Czuczman MS, Emmanouilides C, Darif M, Witzig TE, Gordon LI, Revell S *et al*. Treatment-related myelodysplastic syndrome and acute myelogenous leukemia in patients treated with ibritumomab tiuxetan radioimmunotherapy. *J Clin Oncol* 2007; **25**: 4285–4292.
5 Vardiman JW, Arber DA, Brunning RD, Larson RA, Matutes E, Baumann I *et al*. Therapy-related myeloid neoplasms. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, *et al*. (eds). *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, 4th edn. International Agency for Research on Cancer: Lyon, 2008, pp 127–129.
6 Seymour JF, Juneja SK, Campbell LJ, Ellims PH, Estey EH, Prince HM. Secondary acute myeloid leukemia with inv(16): report of two cases following paclitaxel-containing chemotherapy and review of the role of intensified ara-C therapy. *Leukemia* 1999; **13**: 1735–1740.
7 Smith SM, Le Beau MM, Huo D, Karrison T, Sobecks RM, Anastasi J *et al*. Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the university of Chicago series. *Blood* 2003; **102**: 43–52.
8 Keating MJ, O'Brien S, Lerner S, Koller C, Beran M, Robertson LE *et al*. Long-term follow-up of patients with chronic lymphocytic leukemia (CLL) receiving fludarabine regimens as initial therapy. *Blood* 1998; **92**: 1165–1171.
9 Keating MJ, O'Brien S, McLaughlin P, Dimopoulos M, Gandhi V, Plunkett W *et al*. Clinical experience with fludarabine in hemato-oncology. *Hematol Cell Ther* 1996; **38** (Suppl 2): S83–S91.
10 Zinzani PL, Pulsoni A, Perrotti A, Soverini S, Zaja F, De Renzo A *et al*. Fludarabine plus mitoxantrone with and without rituximab versus CHOP with and without rituximab as front-line treatment for patients with follicular lymphoma. *J Clin Oncol* 2004; **22**: 2654–2661.
11 Keating MJ, O'Brien S, Albitar M, Lerner S, Plunkett W, Giles F *et al*. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. *J Clin Oncol* 2005; **23**: 4079–4088.
12 Tam CS, Wolf M, Prince HM, Januszewicz EH, Westerman D, Lin KI *et al*. Fludarabine, cyclophosphamide, and rituximab for the treatment of patients with chronic lymphocytic leukemia or indolent non-Hodgkin lymphoma. *Cancer* 2006; **106**: 2412–2420.
13 Yamauchi T, Nowak BJ, Keating MJ, Plunkett W. DNA repair initiated in chronic lymphocytic leukemia lymphocytes by 4-hydroperoxycyclophosphamide is inhibited by fludarabine and clofarabine. *Clin Cancer Res* 2001; **7**: 3580–3589.
14 Morgan SJ, Seymour JF, Grigg A, Matthews JP, Prince HM, Wolf MM *et al*. Predictive factors for successful stem cell mobilization in patients with indolent lymphoproliferative disorders previously treated with fludarabine. *Leukemia* 2004; **18**: 1034–1038.
15 Tam CS, Seymour JF, Prince HM, Kenealy M, Wolf M, Januszewicz EH *et al*. Treatment-related myelodysplasia following fludarabine combination chemotherapy. *Haematologica* 2006; **91**: 1546–1550.
16 Tam CS, Wolf MM, Januszewicz EH, Prince HM, Westerman D, Seymour JF. Fludarabine and cyclophosphamide using an attenuated dose schedule is a highly effective regimen for patients with indolent lymphoid malignancies. *Cancer* 2004; **100**: 2181–2189.
17 Rai KR, Peterson BL, Appelbaum FR, Kolitz J, Elias L, Shepherd L *et al*. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. *N Engl J Med* 2000; **343**: 1750–1757.
18 Gill S, Carney D, Ritchie D, Wolf M, Westerman D, Prince HM *et al*. The frequency, manifestations, and duration of prolonged cytopenias after first-line fludarabine combination chemotherapy. *Ann Oncol* 2010; **21**: 331–334.
19 Morrison VA, Rai KR, Peterson BL, Kolitz JE, Elias L, Appelbaum FR *et al*. Therapy-related myeloid leukemias are observed in patients with chronic lymphocytic leukemia after treatment with fludarabine and chlorambucil: results of an intergroup study, cancer and leukemia group B 9011. *J Clin Oncol* 2002; **20**: 3878–3884.
20 McLaughlin P, Estey E, Glassman A, Romaguera J, Samaniego F, Ayala A *et al*. Myelodysplasia and acute myeloid leukemia following therapy for indolent lymphoma with fludarabine, mitoxantrone, and dexamethasone (FND) plus rituximab and interferon alpha. *Blood* 2005; **105**: 4573–4575.

21 Montoto S, Moreno C, Domingo-Domenech E, Estany C, Oriol A, Altes A *et al*. High clinical and molecular response rates with fludarabine, cyclophosphamide and mitoxantrone in previously untreated patients with advanced stage follicular lymphoma. *Haematologica* 2008; **93**: 207–214.
22 Bowcock SJ, Rassam SM, Lim Z, Ward SM, Ryali MM, Mufti GJ. High incidence of therapy-related myelodysplasia and acute leukaemia in general haematology clinic patients treated with fludarabine and cyclophosphamide for indolent lymphoproliferative disorders. *Br J Haematol* 2006; **134**: 242–243.
23 Sandoval A, Consoli U, Plunkett W. Fludarabine-mediated inhibition of nucleotide excision repair induces apoptosis in quiescent human lymphocytes. *Clin Cancer Res* 1996; **2**: 1731–1741.
24 Herbert KE, Morgan S, Prince HM, Westerman DA, Wolf MM, Carney DA *et al*. Stem cell factor and high-dose twice daily filgrastim is an effective strategy for peripheral blood stem cell mobilization in patients with indolent lymphoproliferative disorders previously treated with fludarabine: results of a Phase II study with an historical comparator. *Leukemia* 2009; **23**: 305–312.
25 Astrow AB. Fludarabine-related myeloid leukemia. *J Clin Oncol* 2003; **21**: 3709 author reply 3709–3710.
26 Lam CC, Ma ES, Kwong YL. Therapy-related acute myeloid leukemia after single-agent treatment with fludarabine for chronic lymphocytic leukemia. *Am J Hematol* 2005; **79**: 288–290.
27 Sacchi S, Marcheselli L, Bari A, Marcheselli R, Pozzi S, Luminari S *et al*. Secondary malignancies after treatment for indolent non-Hodgkin's lymphoma: a 16-year follow-up study. *Haematologica* 2008; **93**: 398–404.
28 Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A *et al*. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. *Lancet Oncol* 2009; **10**: 223–232.