A Phase II Clinical Trial of Fludarabine and Cyclophosphamide Followed by Thalidomide for Angioimmunoblastic T-cell Lymphoma. An NCRI Clinical Trial. CRUK number C17050/A5320

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Dear Professor Seymour,

We report here a multi-center, single-arm, phase II, prospective clinical trial assessing the efficacy and tolerability of fludarabine and cyclophosphamide (FluCy) followed by consolidation with thalidomide in previously untreated Angioimmunoblastic T-cell lymphoma (AITL).

AITL is a rare disease accounting for 1-2% of non-Hodgkin lymphomas (NHL) in Europe and North America [1]. The median age at presentation is 62-70 years and 80-90% of patients have advanced stage disease [2-6]. It typically follows an aggressive course with poor prognosis; although complete remission (CR) is achieved in 44-61% of patients, relapses are frequent and the median overall survival (OS) is approximately 3 years with 5-year survival of 32% [2, 3, 6]. Due to its low incidence, there have been few prospective clinical trials in AITL and most data informing treatment strategies are derived from retrospective and registry studies. Most patients are treated with combination chemotherapy containing anthracyclines but in a large retrospective study, no survival advantage was identified for these patients compared to those treated with non-anthracycline-containing regimens [2].

Evidence from case reports and small series indicates that purine analogues are efficacious in AITL [7,8]. The combination of fludarabine and cyclophosphamide (FluCy) is active in other lymphoproliferative disorders but has not been assessed in AITL [9]. The high relapse rate suggests that consolidation or maintenance strategies may be beneficial in the management of AITL; whilst autologous stem cell transplantation (ASCT) is sometimes used in first remission, this is not an option for all patients. Preliminary evidence from case reports supports the use of thalidomide as maintenance therapy but there have been no trials assessing it [10-12].

This trial was designed as a sequential 2-stage trial to evaluate the response rate of previously untreated AITL to FluCy chemotherapy and the incremental response rate to thalidomide maintenance. A Bayesian 2-stage design was used, assuming that a CR rate of <40% would not be of further interest, while a CR rate of ≥60% with FluCy would be of interest to test in further trials. Setting a threshold probability of 0.6 in the first stage and 0.7 overall, the target accrual was 15 patients in the first stage and an additional 22 patients in the second stage if ≥6 first-stage patients experienced a CR. Eligible patients had previously untreated AITL with measurable disease, performance status less than 3, and age >18 years. Baseline investigations included computed tomography scan of neck, chest, abdomen and pelvis, full blood count, biochemistry assessments, viral serology, autoimmune profile, direct antiglobulin test (DAT), bone marrow aspirate and bone marrow biopsy.

Treatment consisted of fludarabine 40mg/m^2 and cyclophosphamide 250mg/m^2, administered orally on days 1-3 of each 28 day cycle. Restaging was performed after 4 cycles. Patients progressing on FluCy came off trial. Patients in CR proceeded to thalidomide maintenance. 2 additional cycles of FluCy could be given to patients with stable disease (SD) or partial remission (PR) prior to thalidomide maintenance. Thalidomide was given as continuous therapy starting 4 weeks after the final cycle of FluCy. The initial dose was 100mg daily increased every 4 weeks to a maximum dose of 300mg daily and could be continued for 6 months or until disease progression.

Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. The protocol was approved by the South West Research Ethics Committee and the trial was conducted in accordance with the Declaration

of Helsinki. The primary endpoint was response rate following FluCy chemotherapy assessed according to standard criteria [13]. Secondary endpoints included response rate to thalidomide, progression-free (PFS), OS, and adverse events. The as-treated population, including all patients who received at least one dose of study treatment, was used for the analysis of all endpoints.

Fifteen patients were recruited from 6 centers between February 2009 and March 2012. The median age was 68 years (range 52 – 91 years), and 13 patients (87%) had advanced stage disease with disease bulk (>10cm in any dimension) in 12 patients (80%). B symptoms were present in 11 (73%) patients. 12 patients (80%) were anaemic with haemoglobin <120g/l, a positive DAT was identified in 6/12 (50%) anaemic patients. Other autoantibodies identified were: anti-smooth muscle antibody (*n*=4), liver kidney microsomal antibody (*n*=2), and anti-mitochondrial antibody (*n*=1). Hypergammaglobulinaemia was present in 10/15 (67%) patients which was polyclonal in 8/10 patients and due to a paraprotein in 2 patients. (Supplementary Table I)

The histological diagnosis of AITL was confirmed by retrospective central review in 11/15 cases (73%). There was insufficient histological material available for central review in one case. In the remaining 3 cases there was discrepancy between local and central histology review. PTCL was confirmed in 1 of these cases but not the specific diagnosis of AITL. In 2 cases, central review indicated a diagnosis of T-cell rich DLBCL but the clinical features, aggressive disease course and, in one case, the findings on repeat biopsy at the time of relapse were considered by the treating center to be in keeping with a diagnosis of AITL.

Four or more cycles of FluCy were administered to 8 patients (53%). There were frequent dose reductions and delays due to toxicity. Treatment was delayed in 26% of cycles, the dose of fludarabine was reduced in 32% of cycles and cyclophosphamide dose reductions were made in 29% of cycles. Reasons for early cessation of treatment were hematological toxicity (*n*=2), death (*n*=2) and failure to respond (*n*=3). The 3 patients who terminated treatment early due to failure to respond were withdrawn from the trial in order to escalate therapy off protocol. Grade 3 or 4 hematological toxicity occurred in 8 (53%) patients, grade 3 or 4 non-hematological toxicity was experienced in 9 (60%) patients.

Responses were achieved in 9 patients (60%): CR *n*=5 (33%), CR unconfirmed *n*=1 (7%), Partial Response *n*=3 (20%). Progressive Disease occurred in four patients (27%). Two patients only received 1 cycle of FluCy and response was not assessable in these patients. Of the 9 patients with at least stable disease after FluCy, 7 proceeded to thalidomide maintenance with a median dose of 150mg (range 100 – 600mg); thalidomide was continued for a median of 2 cycles (range 1-6). Two eligible patients did not continue to thalidomide maintenance due to hematological toxicity and disease progression before starting thalidomide respectively. Remission status did not improve in any patients receiving thalidomide maintenance and 4 patients progressed during (n=3) or within 3 months (n=1) of completing thalidomide. One patient remains in remission 20 months after thalidomide. After a median follow-up of 22.6 months, 2 patients (13%) are alive without progression, 2 patients (13%) are alive having progressed and 11 patients (73%) have died. Median PFS is 5.5 months and median OS is 14.9 months. The causes of death in the 11 deceased patients were lymphoma (*n*=8), pneumonia (*n*=1), Crohn's disease (*n*=1), and neutropenic sepsis (*n*=1).

The overall response rate to FluCy was 60%, with CR in 33%. The trial did not proceed to the 2nd stage of recruitment as the CR rate was less than the pre-defined threshold of 40%. The CR rate was inferior to that reported with CHOP with or without rituximab [2, 6] and remissions were short lived with a median PFS of 5.5 months.

Disruption of the interaction between the malignant cells and their microenvironment may explain some of the effect seen in patients with AITL treated with immunomodulatory drugs and we hypothesised that thalidomide may augment conventional chemotherapy through this mechanism [14, 15]. In this small trial, no patients experienced an improvement in remission status with thalidomide maintenance, however, the number of patients receiving sustained courses of thalidomide was too low to draw conclusions about its efficacy in this setting.

One reason for the poor outcomes observed in this trial may have been frequent treatment delays and dose reductions consequent on the high toxicity rate in a predominantly elderly patient population. The trial design did not permit ASCT in first remission and recruiting centers may therefore have chosen not to enrol patients who were suitable for transplant and this may have skewed the population. The slow recruitment reflects the low incidence of AITL and highlights the difficulties in conducting prospective trials in this rare disease.

The histological diagnosis of AITL can be difficult as demonstrated by the discrepancy between local and central pathology reports in 3 cases in this trial. When only the 11 patients with centrally confirmed AITL are considered, the CR rate (36%) and ORR (64%) are not different from the trial population as a whole. In future trials, real-time prospective

central histology review should be used with additional patients recruited where there is diagnostic discrepancy.

Due to the poor outcomes observed in this trial, we do not recommend FluCy as first-line treatment in AITL, and the current standard of care should be CHOP with or without rituximab, with enrolment into clinical trials assessing novel approaches wherever possible.

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The authors have no conflicts of interest to declare.

C.R. and P.S. designed the study.

W.T., R.J., B.T., N.C., P.S., H.C., K.E., C.W., and C.R. analysed the data

W.T. wrote the initial draft of the manuscript, all authors contributed to, and agreed the final version.

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Variable	***N* = 15**	
	median (range)	
Age at registration, years	68 (52 – 91)	
	No.	**(%)**
Gender		
Female	5	(33)
Male	10	(67)
Performance status		
0	2	(13)
1	9	(60)
2	4	(27)
Clinical stage at study entry		
II	2	(13)
III	4	(27)
IV	9	(60)
B symptoms		
Absent	4	(27)
Present	11	(73)
Bulky disease		
Absent	12	(80)
Present	3	(20)
Bone marrow infiltration		
Involved	6	(46)
Not involved	7	(54)
Missing	*2*	
Haemoglobin		
<120g/l	12	(80)
≥120g/l	3	(20)
Platelets		
$<150x10^9$/l	3	(21)
$\geq 150x10^9$/l	11	(79)
Missing	*1*	
Paraprotein		
Absent	8	(80)
Present	2	(20)
Missing	*5*	
Hypergammaglobulinaemia		
No	5	(33)
Yes	10	(67)
Direct antiglobulin test (DAT)		
Negative	7	(54)
Positive	6	(46)
Missing	*2*	
Antimitochondrial antibody (AMA)		
Negative	7	(88)
Positive	1	(13)
Missing	*7*	
Smooth muscle antibody (SMA)		
Negative	4	(50)
Positive	4	(50)
Missing	*7*	
Liver/kidney/microsomal antibody (LKM)		
Negative	7	(78)

Positive	2	(22)
Missing	*6*	
Serum Lactate Dehydrogenase (LDH)		
<400IU/l	7	(47)
≥400IU/l	8	(53)

Supplementary Table I Baseline characteristics of patients