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BRIEF COMMUNICATION

Crizotinib in Advanced, Chemoresistant Anaplastic Lymphoma Kinase–Positive Lymphoma Patients

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Anaplastic lymphoma kinase (ALK)–positive lymphomas respond to chemotherapy, but relapses, which bear a poor prognosis, occur. Crizotinib inhibits ALK in vitro and in vivo and was administered as monotherapy to 11 ALK+ lymphoma patients who were resistant/refractory to cytotoxic therapy. The overall response rate was 10 of 11 (90.9%; 95% confidence interval [CI] = 58.7% to 99.8%). Disease status at the latest follow-up is as follows: four patients are in complete response (CR) (months >21, >30, >35, >40) under continuous crizotinib administration; 4 patients had progression of disease (months 1, 2, 2, 2); 1 patient obtained CR on crizotinib, received an allogeneic bone marrow transplant, and is in CR; 2 patients (treated before and/or after allogeneic bone marrow transplant) obtained and are still in CR but they have stopped crizotinib. Overall and progression-free survival rates at 2 years are 72.7% (95% CI = 39.1% to 94.0%) and 63.7% (95% CI = 30.8% to 89.1%), respectively. ALK mutations conferring resistance to crizotinib in vitro could be identified in relapsed patients. Crizotinib exerted a potent antitumor activity with durable responses in advanced, heavily pretreated ALK+ lymphoma patients, with a benign safety profile.

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Anaplastic lymphoma kinase (*ALK*) becomes oncogenic when fused to partner genes through chromosomal translocations that activate its kinase domain and drive the expression of ALK, which is normally expressed only in nervous system (1–4). In hematological disorders ALK is expressed in more than 50% of anaplastic large cell lymphomas (ALCLs) as a result of a t(2;5)(p23;q35) translocation that causes the *ALK* gene to fuse with *NPM* on chromosome 5. ALK-positive large B-cell lymphoma (ALK+ DLBCL) is a rare lymphoma with a poor prognosis and a frequent t(2;17)(p23;q23) translocation responsible for Clathrin-ALK fusion protein (5).

ALK+ ALCLs respond to cytotoxic drugs, but relapses, which bear a poor prognosis, can occur (6–9). Crizotinib is the first ALK inhibitor that entered clinical practice; it is an orally bioavailable small molecule of the ALK and MET receptor tyrosine kinases. The activity of crizotinib in ALK+ lung cancer is documented (10); no report on long-term effects of crizotinib in ALK+ lymphomas exists. Impressive short-term therapeutic activity was reported in two patients (11), but no long-term data are available. We report here the long-term follow-up of crizotinib administered to 11 patients with advanced, resistant, ALK+ lymphomas.

Compassionate use of crizotinib was authorized by the institutional review boards of our institutions under a named-patient protocol (see Supplementary Materials, available online). Informed consent was signed by each patient. Data were collected prospectively, and information on centers entering patients was provided by Pfizer, the producer of crizotinib. Patients were diagnosed with ALK+ non-Hodgkin lymphoma by immunohistochemistry and fluorescent in situ hybridization using an *ALK* break-apart probe (12). Patients had a refractory or relapsed disease after at least one prior chemotherapy regimen (typically cyclophosphamide, doxorubicin (adriamycin), vincristine, prednisone (CHOP); see Table 1) and measurable disease. Use of cytotoxic drugs/steroids in the 14 days before crizotinib administration was not permitted (see Supplementary Materials, available online). Patients received crizotinib 250 mg twice daily as monotherapy until disease progression. Response to therapy was assessed according to Response Evaluation Criteria In Solid Tumors (RECIST) criteria (13).

Kaplan–Meier analysis was conducted to assess overall survival (OS) and progression-free survival (PFS) rates. The analysis was conducted accounting for competing risk according to Kalleisch–Prentice estimator (14).

Reverse-transcription polymerase chain reaction (RT-PCR) for *NPM/ALK* was performed as described (15). Eleven patients (7 men, 4 women; median age = 28 years, range = 19–55) were treated. Nine patients had an ALCL histology, the remaining two had a DLBCL histology. The median follow-up for all the patients is 8 months (range = 1–40). The median follow-up for patients still on crizotinib is 32.5 months (range = 21–40). Patients were resistant to one to four (median = 3) lines of treatment, including autologous bone marrow transplant (n = 3 patients) and allogeneic bone marrow transplant (alloBMT; n = 2 patients) (Table 1). All had involvement at multiple sites (nodal and extranodal) and an Eastern Cooperative Oncology Group performance score of one to four.

The earliest sign of therapeutic activity detectable by clinical evaluation (symptoms, lactate dehydrogenase values) was observed within a few days of crizotinib (Table 1) (11). The overall response rate was 10 of 11 (90.9%; 95% confidence interval [CI] = 58.7% to 99.8%) and included 9 complete response (CR) (81.8%; 95% CI = 48.2% to 97.8%) and 1 partial remission (Table 1). B symptoms disappeared promptly, and lactate dehydrogenase levels normalized

Table 1. Patient characteristics at the time of crizotinib treatment and response rates*

Patient No.	Age, y	Diagnosis	Stage (Ann Arbor)	ECOG	BM involvement, %†	Previous therapy lines	TTN LDH, days	TTN fever, days	Previous BMT	Response, duration, months
1	26	ALCL	IIIB	2	3-->0	CHOP, DHAP, HD-VP16	29	2	No	CR, >40
2	19	ALCL	IVB	3	8-->0	CHOP, DHAP, BEAM	22	10	ABMT	CR, 2 ‡
3	22	DLBCL	IVB	3	65-->0	BFM, HD-CTX, HD-AraC, Bortezomib	21	Na	ABMT	PR, 2 ‡
4	22	ALCL	IIB	1	0	CHOP, VAD, H-CyVAD	30	30	No	CR, >35
5	39	DLBCL	IVB	3	NA	BEP, CHOP, ICE, H-CyVAD	19	Na	No	SD ‡
6	20	ALCL	IIB	2	15-->0	CHOP, DHAP, BEAM	30	10	ABMT	CR, 2
7	47	ALCL	IIIBe	2	0	IEV, DHAP, CHOP	30	14	No	CR, >30
8	28	ALCL	IIIB	2	0	CHOP, DHAP, mini-BEAM	Na	Na	No	CR, 2
9	34	ALCL	IVBe	2	0	CHOP, ESHAP	Na	30	No*	CR, 3
10	38	ALCL	IVB	4	0	CHOP, DHAP, VIM	28	15	allogeneic	CR, 8
11	55	ALCL	IIIB	1	0	CHOP	30	30	No	CR, >21

* Patients 1 and 2 correspond to the patients whose short-term course was described in our original report (11). Patient 6 progressed after 2 months of crizotinib. Currently the patient is in complete response (CR) on brentuximab vedotin. Patient 8 was treated with crizotinib as a bridge to allogeneic bone marrow transplant (alloBMT). The patient obtained a CR and was subsequently transplanted after 2 months of crizotinib; the patient is now in CR with chronic graft versus host disease (GVHD). Patient 9 was treated both before alloBMT and after a relapse at day 80 after alloBMT; in both cases a CR was obtained. Crizotinib was discontinued at month 10 because of patient requests for gastric motility problems considered by the principal investigator (PI) to be related mainly to the transplant procedure; the patient is in CR with chronic GVHD. Patient 10 was treated for relapse at day 90 after alloBMT and obtained a CR; crizotinib was discontinued after 8 months because of liver function test elevations considered by the PI to be related mainly to the transplant procedure (GVHD); the patient is in CR with chronic GVHD. The different values reported in the 6th column, "x –> y", refer to the percent of positive cells at the two time points. ABMT = autologous bone marrow transplant; BEAM = carmustine, etoposide, cytarabine, melphalan; BEP = bleomycin, etoposide, cisplatin; ALCL = anaplastic large cell lymphoma; BFM = 7-drug combination systemic chemotherapy plus single-agent intrathecal chemotherapy: prednisone, vincristine daunorubicin, asparaginase, cyclophosphamide, cytarabine, mercaptopurine, plus intrathecal methotrexate; BM = bone marrow; BMT = bone marrow transplant; CHOP = cyclophosphamide, doxorubicin (adriamycin), vincristine, prednisone; CR = complete response; DHAP = dexamethasone, cisplatin, cytarabine; DLBCL = large B-cell lymphoma; ESHAP = etoposide, methylprednisolone, cytarabine, cisplatin; H-CyVAD = alternate regimens of 1) cyclophosphamide, vincristine, doxorubicin (adriamycin), dexamethasone; 2) methotrexate and cytarabine; HD-AraC = high-dose cytarabine; HD-CTX = high-dose cyclophosphamide; HD-VP16 = high-dose etoposide; ICE = ifosfamide, carboplatin, etoposide; IEV = Ifosfamide, epirubicin, etoposide; LDH = lactate dehydrogenase; mini-BEAM: carmustine, etoposide, cytarabine, melphalan; NA = not available; PR = partial remission; PRO = progression of disease; SD = stable disease; TTN = time to normalization; VAD = vincristine, doxorubicin, high-dose dexamethasone; VIM = ifosfamide, mitoxantrone, etoposide.

† Evaluated by fluorescent in situ hybridization, using an ALK break apart probe (12), before treatment and after 30 days of crizotinib.

‡ Indicates a death.

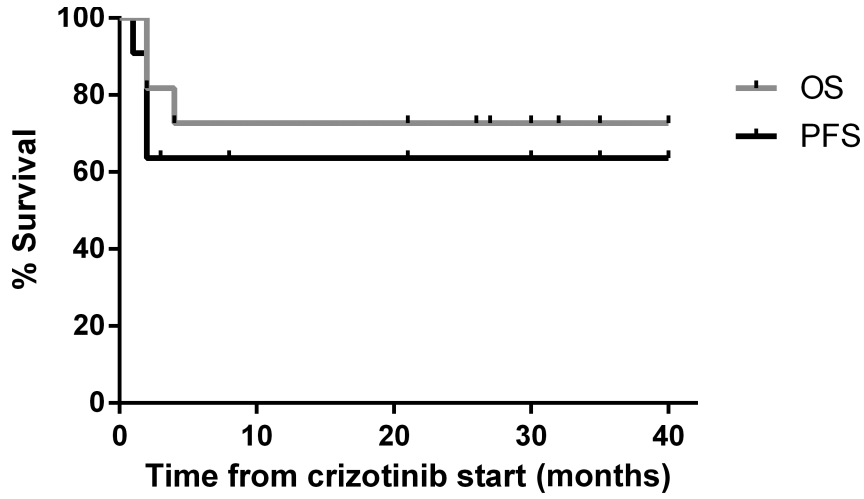
within 30 days after the start of crizotinib. Bone marrow aspirates were *NPM/ALK*+ by fluorescent in situ hybridization analysis in four patients and became negative within the initial first month of therapy (Supplementary Figure 1, available online).

Disease status at the latest follow-up (October 2013) is as follows (details in Table 1): Four patients (patients 1, 4, 7, and 11 at months >21, >30, >35, >40, respectively) are in CR under continuous crizotinib treatment; they also test negative by RT-PCR for *NPM/ALK* (15). Four patients (two with DLBCL [patients 3 and 5] and two with ALCL [patients 2 and 6]) progressed (months 1, 2, 2, and 2, respectively). Three patients (patients 8, 9, and 10) received crizotinib before/after alloBMT: one patient obtained CR on crizotinib, received an alloBMT and is in CR; two patients (treated before and/or after alloBMT) obtained and are still in CR but they have stopped crizotinib.

In the ALCL group, nine of nine patients obtained CR (100%; 95% CI = 71.7% to 100%) with a rate of CR loss of 0.13/year (Supplementary Figure 2, available online). There were no obvious differences between responses and type of cytotoxic drugs used. The 2-year PFS and OS rates are 63.7% (95% CI = 30.8% to 89.1%) and 72.7% (95% CI = 39.1% to 94.0%), respectively, with a plateau in the curve after the initial 2 months (Figure 1).

Crizotinib-related toxicities observed were mild and included ocular flashes (n = 10 patients), peripheral edema (n = 3 patients), skin rash (n = 1 patient), and erectile dysfunction (n = 1 patient). Laboratory abnormalities included neutropenia (n = 2 patients), thrombocytosis (n = 1 patient), and liver function tests elevation (n = 1 patient). All toxicities were graded I or II. No patient died from a cause related to treatment. No treatment-related event led to treatment discontinuation.

In two patients (patients 2 and 6), the kinase domain of *NPM/ALK* could be amplified from peripheral blood samples obtained at the time of relapse. Deep sequencing of these products (16) revealed the presence of *ALK* mutations (17,18).(Supplementary Materials and Supplementary Figure 3, available online).



	Months	0	1	2	3	4	8	21	26	27	30	32	35	40
No. of subjects at risk	PFS	11	11	10	6		5	4			3		2	1
	OS	11		11		9		8	7	6	4	3	2	1

Figure 1. Progression-free survival (PFS) and overall survival curves. **Gray line** shows overall survival and **black line** shows PFS in anaplastic lymphoma kinase (*ALK*)–positive lymphoma patients treated with crizotinib. PFS for patient 8, who was treated before allogeneic bone marrow transplant (alloBMT), was censored at the date of bone marrow transplant. PFS for patient 9, who was treated both before and after alloBMT, was censored at end of the first course of crizotinib, before alloBMT. PFS for patient 10, who was treated after bone marrow transplant, was censored at the end of crizotinib administration.

These positive results extend our initial observation on two patients (patients 1 and 2) (11) and provide long-term follow-up data. Crizotinib was well tolerated, with objective responses observed within 30 days after starting treatment in 10 of 11 patients. Nine patients obtained CR. Within 2 months of treatment, four patients relapsed, three of whom died shortly after, whereas one (patient 6) obtained a durable response to brentuximab. The remaining patients showed durable responses up to more than 40 months, including three patients that were transplanted or treated after alloBMT (patients 8, 9, and 10). Crizotinib showed activity also in patients failing alloBMT (patients 9 and 10), although in this setting the toxicities associated with the transplant procedure prevented a long-term administration of the drug (Table 1).

Previous studies in advanced relapsed ALK+ ALCL demonstrated a poor outcome (6,9), with the exception of brentuximab-vedotin (19), which showed a 57% CR rate (20) and a median PFS of 14.6 months (17,18). Although the number of patients treated in our study is small, it is noteworthy that no patients with previous autologous bone marrow transplant showed durable responses; this fact could indicate that an early treatment with crizotinib after the failure of first-line treatment could produce better results than when administered later.

In our study, patients in CR also tested negative for *NPM/ALK* when assayed by RT-PCR while their pretreatment samples were positive. These data further reinforce the depth of the response reached and suggest that PCR could represent an effective method to follow minimal residual disease in adult ALK+ lymphoma, similarly to what has been observed in pediatric patients (15). A recent publication described the effects of crizotinib on a pediatric population with ALK+ tumors, which included 9 patients with ALCL (21), with an overall response rate of eight of nine patients and a maximum follow-up of 27 months. Our data extend this and our original report (11) toward an adult population with a longer maximum follow-up (40 vs 27 months). In addition, we also showed the activity of crizotinib in patients who relapsed after alloBMT, the potential usefulness of a PCR-based assay to monitor MRD, and the identification of the molecular nature of resistance to crizotinib.

These patients are probably among the ones with the longest exposure to crizotinib and are testimony to its long-term safety. Although lung cancer patients have a median duration of responses of 10 to 12 months (10), our patients did not show further relapses after the first 2 months of treatment. In future studies, crizotinib could be used in combination with other agents to improve the overall response rates without exposing patients to long-term toxicities. It is interesting to note that brentuximab and crizotinib seem to be non–cross-resistant, as documented by the prolonged response to brentuximab after the failure of crizotinib in patient 6.

Limitations are present in our study: the small number of patients precludes an in-depth analysis of the relationship between factors such as ethnicity or sex and response to crizotinib. However, our results were substantially confirmed in a recent registration trial of crizotinib in 15 additional ALK+ lymphoma patients (22).

In conclusion, these data indicate that ALK+ lymphoma patients have a high chance of responding to crizotinib even when heavily pretreated, with approximately half of them not relapsing within the first months and then enjoying long-lasting responses; however, no available pretreatment parameter is able to predict durable CRs. These data will be useful for the management of this aggressive disease.

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Notes

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