

# Long term safety and efficacy of crizotinib in relapsed/refractory ALK+ lymphomas: a monocentric analysis

## **Methods**

ALK positivity was established through immunohistochemical assay on pathological samples using D5F3 staining at first diagnosis and then confirmed in our centre after pathological review of the case. Since ALK expression is highly pathogenic, samples were considered ALK-positive if a significant expression was found in more than 80% of lymphoma cells. We also tested bone marrow sample through fluorescence in situ hybridization (FISH) assay using an ALK break-apart probe, to detect ALK fusion, in 21 of 27 patients, including the two patient affected by diffuse large B-cell lymphoma and plasmablastic lymphoma.

For the detection of Minimal Residual Disease by RT-PCR mononuclear cells from peripheral blood were obtained by density gradient centrifugation. cDNA synthesis and RT-PCRs were performed according to recently described protocols.<sup>1</sup> Briefly, total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. An amount of 1 µg of total RNA was reverse transcribed using SuperScript II reverse transcriptase (Life Technologies, Milan, Italy) and random hexamers. For each sample, ABL expression was assessed as a control for the presence of amplifiable RNA and the efficiency of reverse transcription, using the primers 5'-CGGCCAGTAGCATCTGACTTTG-3' and 5'-CCTTGGCCATTTTGGTTTGG-3'. The primers specific for the chimeric transcript NPM-ALK were TCCCTTGGGGCTTTGAAATAACACC (NPM) and CGAGGTGCGGAGCTTGCTCAGC (ALK). Each reaction mixture contained 10 × buffer, 1.5 mM MgCl<sub>2</sub>, 1.6 mM dNTPs, 400 nM of each primer, 0.2 IU of Taq polymerase and 5% of the RT product in a final 20 µl reaction volume. PCR reaction consisted of initial denaturation at 94 °C for 2 min, followed by 40 cycles of 94 °C for 15 s, 68 °C for 15 s, 72 °C for 30 s and a final extension at 72 °C for 10 min. PCR products were analyzed by 3% agarose gel electrophoresis and visualized under UV illumination after ethidium bromide staining. Ladder 50 (Invitrogen, Milan, Italy) was used as a molecular weight standard. Test assay sensitivity is 10<sup>-6</sup>.<sup>1</sup>

Mussolin L, Pillon M, d'Amore ES, Santoro N, Lombardi A, Fagioli F *et al.* Prevalence and clinical implications of bone marrow involvement in pediatric anaplastic large cell lymphoma. *Leukemia* 2005; 19: 1643–1647.

## **Tables**

Male:Female	15:12
Median age at lymphoma diagnosis, years (range)	24 (15-82)
Lymphoma histotype	25 ALK+ ALCL 1 ALK+ DLBCL 1 ALK+ PBL
IPI at diagnosis	IPI 0: 2 pts IPI 1: 3 pts IPI 2: 5 pts IPI 3: 4 pts IPI 4: 4 pts IPI N.A.: 9 pts
Lymphoma stage at diagnosis	Early (I-II): 6 pts Advanced (III-IV): 21 pts
Previous treatment lines (n°)	1: 6 pts 2: 10 pts

	3: 6 pts 4: 2 pts 5: 2 pts 6: 1 pts
Previous treatment lines (protocols)	CHOP/CHOEP/COMP: 20 pts Platinum-based: 7 pts Autologous SCT: 7 pts Brentuximab Vedotin: 6 pts HD MTX-based: 5 pts Gemcitabine-based: 3 pts AIEOP/ALCL99: 3 pts HYPER-CVAD/HYPER-C-HIDAM: 2 pts IEV: 1 pt VACOP-B: 1 pts BMF: 1 pts MAD: 1 pts MiCMA: 1 pts LLA2000: 1 pts Allogeneic SCT: 1 pts
Median age at crizotinib start, years (range)	24 (15-82)
ECOG PS at crizotinib start	0: 8 pts 1: 9 pts 2: 1 pts 3: 6 pts 4: 3 pts

29 ALCL: anaplastic large cell lymphoma. DLBCL: diffuse large B-cell lymphoma. PBL: plasmablastic lymphoma. IPI: international  
30 prognostic index. ECOG PS: Eastern Cooperative Oncology Group Performance Status. SCT: stem cell transplantation. HDMTX: high  
31 dose methotrexate. HYPER-CVAD: hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone. HYPER-C-  
32 HIDAM: Hyperfractionated cyclophosphamide with high-doses of arabinosylcytosine and methotrexate. IEV: ifosfamide, epirubicine  
33 and etoposide. VACOP-B: etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin. BMF: Berlin-Munster-  
34 Frankfurt. MAD: high dose cytarabine, mitoxantrone and dexamethasone. MiCMA: mitoxantrone, carboplatinum,  
35 methylprednisolone and cytarabine.

<b>Table S2. Crizotinib response, molecular and radiological assessment (n. 27)</b>		
	<b>CT/PET or CT scan</b>	<b>RT-q-PCR for ALK transcript</b>
At crizotinib start	PD: 27/27	Positive: 18/27 Negative: 7/27 N.A.: 2/27
+ 4 weeks	CR: 14/27 PR: 6/27 SD: 1/27 PD: 4/27 N.A.: 2/27	Positive: 7/18 Negative: 10/18 N.A.: 1/18*
+12 weeks	CR: 13/27 PR: 2/27 SD: 1/27 PD: 11/27	Positive: 4/18 Negative: 9/18 N.A.: 5/18*
<b>Disease status at latest follow-up, n</b>	CR: 16/27 <sup>†</sup> PR: 0/27 SD: 0/27 PD: 11/27	Negative: 16/27 <sup>†</sup>

<b>Median follow-up, months (range)</b>	All patients: 31 (0-140)	Alive patients: 61 (6-140)
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36 CT/PET: computed tomography/positron emission tomography. RT-q-PCR: real time quantitative polymerase chain reaction. Data on  
37 molecular response was evaluated after 1 and 3 months of therapy in patients with positive RT-PCR at baseline. CR: complete  
38 remission. PR: partial remission. SD: stable disease. PD: progressive disease. \*N.A.: not applicable, patients in overt progressive  
39 disease in which RT-PCR was not evaluated. Pts: patients. †15 patients currently on treatment with crizotinib, 1 patient received  
40 allogeneic transplantation after 2 months of therapy and thus is censored from survival analysis.

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<b>Table S3. Therapy-related adverse events (only if &gt; 10% pts)</b>			
<b>Type</b>	<b>Any grade</b>	<b>G1/2</b>	<b>G3/4</b>
<b>Haematological AEs</b>			
Neutropenia (ANC < 1500/ul)	10/27 (37%)	3/27 (11%)	7/27 (26%)
<b>Non-haematological AEs</b>			
Diarrhoea	14/27 (52%)	13/27 (48%)	1/27 (4%)
Visual disturbances	13/27 (48%)	13/27 (48%)	0/27 (0%)
Epigastric pain/burning	13/27 (48%)	11/27 (41%)	1/27 (4%)
Nausea and vomiting	10/27 (37%)	8/27 (30%)	2/27 (7%)
Peripheral oedema	9/27 (33%)	8/27 (30%)	1/27 (4%)
Asthenia	9/27 (33%)	8/27 (30%)	1/27 (4%)
Aspecific abdominal pain	6/27 (22%)	5/27 (19%)	1/27 (4%)
Paraesthesia	5/27 (19%)	4/27 (15%)	0/27 (0%)
Muscle cramps	5/27 (19%)	4/27 (15%)	1/27 (4%)
Transaminase elevation	4/27 (15%)	1/27 (4%)	3/27 (11%)
Creatinine kinase elevation	3/27 (11%)	0/27 (0%)	3/27 (11%)
Headache	3/27 (11%)	3/27 (11%)	0/27 (0%)
<b>Crizotinib dose at last contact and dose-reduction list</b>			
500 mg/die: 4 pts 400 mg/die: 5 pts 250 mg/die: 6 pts Median dose at last contact: 400 mg/die			

42 AEs: adverse events. ANC: absolute neutrophil count.