blood® Letters to Blood

TO THE EDITOR:

Next-generation ALK inhibitors are highly active in ALK-positive large B-cell lymphoma

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Anaplastic lymphoma kinase (ALK)-positive large B-cell lymphoma (ALK-LBCL) is a rare lymphoma characterized by morphology and immunophenotype of plasmablasts and driven by oncogenic *ALK* fusions.[1-3] The most frequent abnormality is t(2;17)(p23;q23) fusing *ALK* with the clathrin heavy-chain gene (*CLTC*), although other fusions have been described.[1,4-9] ALK-LBCL is associated with a dismal prognosis, reflecting its chemoimmunotherapy refractoriness and absence of effective targets.[2] Its poor prognosis is pronounced in the relapsed/refractory setting where, to our knowledge, no long-term survivors have been reported.

CLTC-ALK fusion protein drives constitutive ALK activity on which ALK-LBCL appears dependent.[10] ALK inhibitors (ALKi) have shown efficacy in ALK-rearranged anaplastic large cell lymphoma and non-small-cell lung cancer.[11-15] Anecdotal reports of the first-generation ALKi crizotinib with cytotoxic chemotherapy in ALK-LBCL have been discouraging.[16]

Alectinib and lorlatinib are next-generation ALK inhibitors with higher potency than crizotinib and are effective in crizotinib-refractory ALK-positive cancers.[11-13] We generated the first patient-derived xenograft (PDX) models of ALK-LBCL and evaluated the therapeutic activity of a higher potency ALKi. Based on promising efficacy in these models, we tested alectinib in 4 consecutive patients with relapsed/refractory ALK-LBCL.

Portions of tumor biopsies from 2 patients with relapsed/refractory ALK-LBCL were obtained with informed consent and xenografted under Dana-Farber/Harvard Cancer Center institutional review board protocol #13-351. Tumor seeds were engrafted by renal capsule implantation (RCI) into Nod.Cg-*Prkdc*^{SCID}*IL2Rγ*<tm1Wjl>/SzJ (NSG) mice (Jackson Laboratories). Engrafted tumors were repassaged and both subcutaneous implantation (SCI) and RCI PDXs were generated. Mice were handled per Dana-Farber/Harvard Cancer Center Institutional Animal Care and Use Committee-approved protocol #13-034.

RCI and SCI engrafted mice were monitored with ultrasound and caliper measurements respectively. Upon engraftment, mice were assigned to tumor volume-matched groups and dosed daily with lorlatinib, alectinib, crizotinib, or vehicle (acidified water) by oral gavage. Tumor volume was measured daily thereafter.

We identified 4 consecutive patients with relapsed/refractory ALK-LBCL. Alectinib was administered at 600 mg PO twice daily off-label.[2] Responses were assessed per Lugano Criteria.[17] All patients provided written informed consent.

A fresh tumor biopsy was taken from a patient with ALK-LBCL (Figure 1A-E) refractory to 8 therapies including progressive disease on crizotinib (supplemental Material available on the *Blood* Web site).

We created RCI and SCI PDX models of this tumor in NSG mice (Figure 1F-J). Engraftment was detected 4 to 6 weeks after implantation and restricted to the site of implantation in all models. Tumors comprised sheets of large neoplastic cells with morphology and immunophenotype of the original tumor, including strong expression of ALK, absence of other B- and T-cell markers (Figure 1F-H). We also confirmed the PDX and original tumor shared an identical HLA haplotype and clonotype, carried a *CLTC-ALK* fusion accompanied by high expression of ALK (RNA-sequencing), and exhibited very strong concordance of copy number alterations, somatic mutations and associated variant allele frequencies (supplemental Material).

We evaluated lorlatinib in tumor volume-matched cohorts of RCI PDX mice. Lorlatinib resulted in complete abrogation of ALK phosphorylation by western blot (Figure 2D). In a cohort of mice with subrenal capsule engraftment, lorlatinib resulted in markedly reduced mean tumor volume (54.0 mm^3 [range, 5.45-172 mm^3] to 9.1 mm^3 [range, 2.4-16.4 mm^3]) after 14 days of lorlatinib. In contrast, vehicle-treated PDX tumors increased (55.1 mm^3 [range, 8.6-130.6 mm^3] to 398.3 mm^3 [range, 77.1-888.23 mm^3]; $P = .0015$) (Figure 2A-C; supplemental Material). We also evaluated lorlatinib and alectinib in tumor-matched cohorts of SCI PDX mice from 2 separate patients and found similar tumor volume reductions in lorlatinib- and alectinib-treated mice, and tumor volume increases in vehicle-treated mice (Figure 2E-F). After a transient initial response, tumor volume increased in crizotinib-treated mice (Figure 2F).

Encouraged by these data, we treated 4 consecutive patients with refractory, centrally confirmed ALK-LBCL (supplemental Material; p11) with next-generation ALK inhibitors. None had achieved durable complete response (CR) with prior therapies in the relapsed/refractory setting, and 3/3 were refractory to prior crizotinib. We selected alectinib based on high-potency against ALK and drug access (at the time, lorlatinib was restricted to patients with *ALK*-positive non-small-cell lung cancer with progressive disease after ≥2 ALKi including crizotinib).

Four consecutive patients were treated with alectinib at 600 mg PO twice daily off-label. All 4 responded including 3 CRs and 1 partial responses (supplemental Material). Response is maintained in 2 patients on continued alectinib, including

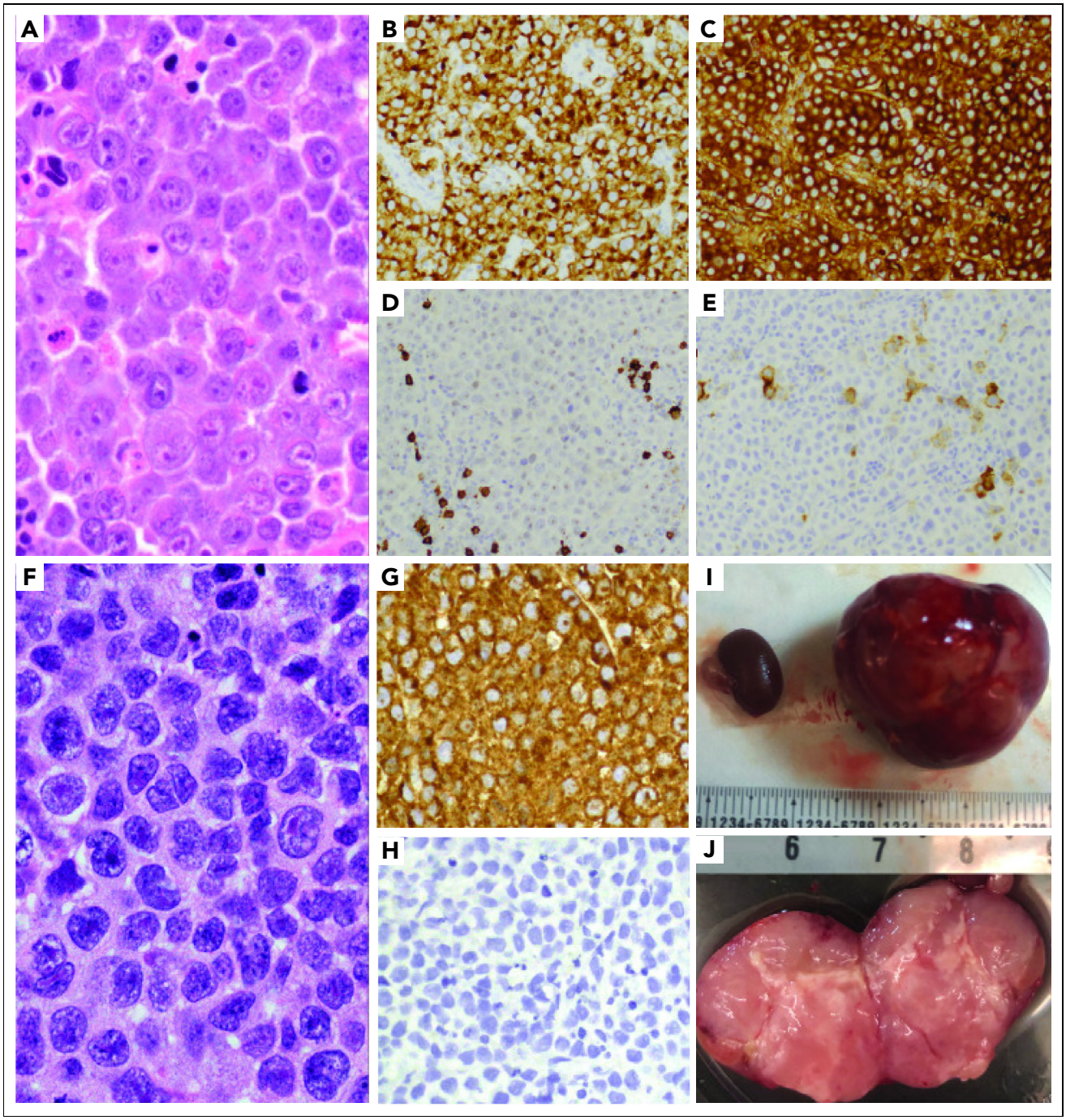

Figure 1. ALK-positive large B-cell lymphoma patient-derived xenografts. (A-E) ALK-positive large B-cell lymphoma morphology shows sheets of large neoplastic lymphoid cells with plasmablastic morphology (A), including moderate to abundant cytoplasm and round to irregular nuclei with prominent nucleoli. The neoplastic cells are positive ALK1 (B) and IgA (C), and negative for CD20 (D) and CD30 (E). (F-J) A PDX model of the ALK-positive large B-cell tumor was generated by implanting a tumor fragment underneath the kidney capsule, which generated a tumor mass after 6 to 8 weeks. The morphology of the PDX model (F) was identical to that of the patient's tumor, with strong ALK1 expression (G) and negative for CD20 (H). (I) PDX tumor replacing the implanted left kidney of mouse (right) and uninvolved right kidney on left (millimeters). (J) PDX bivalved showing tan, soft-cut surface of tumor (centimeters).

1 who underwent myeloablative allogeneic stem cell transplant (ASCT; matched related donor [MUD]), in ongoing CR at 22.3 month; one who received consolidation with 1 cycle of daratumumab/hyaluronidase-fihj in combination with alectinib, then underwent myeloablative ASCT (MUD), in ongoing CR at 7.2 months. In both patients undergoing ASCT, we did not observe acute/chronic graft versus host disease or other transplant-related complications.

Of 2 patients who developed PD, 1 subsequently received lorlatinib 100 mg PO daily off-label.[4] The patient's lymphoma symptoms resolved within days and positron emission tomography-computed tomography-confirmed CR (supplemental Material).

Of 3 patients with prior crizotinib refractoriness, post-crizotinib/pre-alectinib tissue was available for targeted DNA sequencing in 2, *ALK* fluorescence in situ hybridization in 1, and *ALK* and *KRAS* copy number analysis in 1 (supplemental Material). We found no *ALK* or *KRAS* amplification or ALKi resistant mutations.

In summary, ALK-LBCL has a dismal prognosis, particularly in the relapsed/refractory setting, where no long-term survivors have been reported in the literature. We encourage clinical trial participation for all patients with ALK-LBCL given no established standard of care, but clinical trials have generally been unfeasible because of the rarity of the disease, and most patients are treated with off-label therapies. Our data support use of alectinib or lorlatinib as off-label therapeutic options in relapsed/refractory ALK-LBCL.

We found that increasing ALKi potency at least partially overcomes ALKi resistance in ALK-LBCL. Additionally, lorlatinib induced CR in 1 patient who progressed on alectinib, indicating that transitioning between high-potency ALK inhibitors might also be beneficial. We did not find *ALK* resistance mutations or gene amplifications to be associated with crizotinib-resistant tumors. We hypothesize that crizotinib resistance in ALK-LBCL might occur via upregulation of bypass signaling pathways possibly engaged by the tumor microenvironment, and that this can

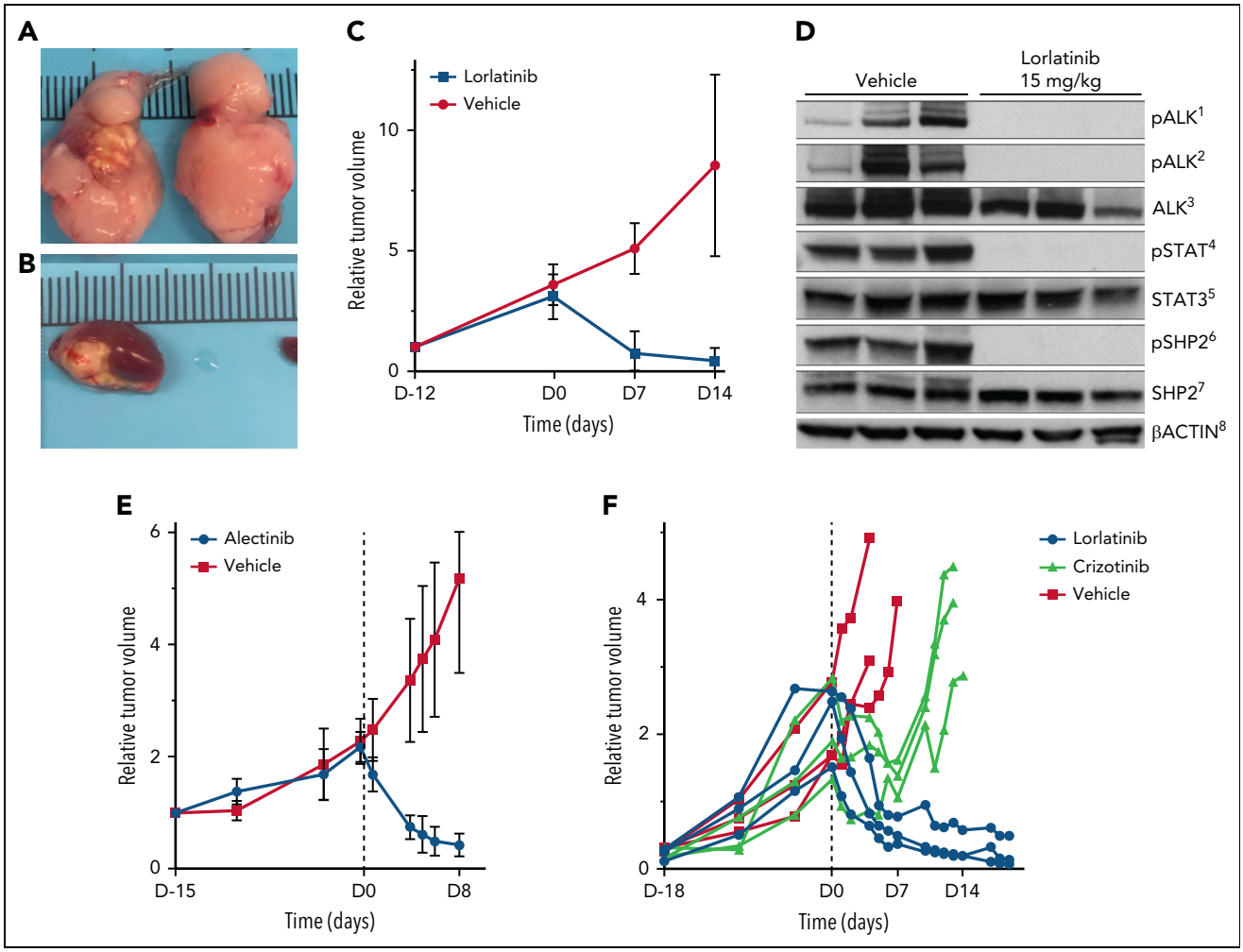

Figure 2. Lorlatinib, alectinib, and crizotinib activity in ALK-positive large B-cell lymphoma patient derived xenografts. (A-B) PDX tumors after 4 days of treatment with vehicle show tumor growth in the engrafted left kidney as well as extension and growth into the right kidney (A). (B) PDX tumors treated with lorlatinib show marked regression of tumor in the engrafted kidney in mice after 4 days of treatment with lorlatinib. (C) Lorlatinib induced rapid tumor regression in vivo in RCI PDX tumors. (D) Lorlatinib induced a complete block of ALK phosphorylation, in turn disrupting activation of downstream signaling pathways, including abrogation of STAT3 and SHP2 phosphorylation. (E) Alectinib induced rapid tumor regression in vivo in SCI PDX tumors. (F) Lorlatinib induced rapid tumor regression and crizotinib induced only transient tumor regression followed within 7 days by rapid tumor growth in vivo in SCI PDX tumors. 1, pALK^{Tyr1604}; 2, pALK^{Tyr1278}; 3, total ALK and ALK fusion proteins; 4, pSTAT3^{Tyr705}; 5, total Stat3 protein; 6, pSHP2^{Tyr542}; 7, total SHP2 protein; and 8, levels of total β-actin.

be overcome with higher potency ALK inhibitors. Further studies are necessary to determine whether bypass mechanisms similar to those reported in ALKi-resistant anaplastic large cell lymphoma[18] may mediate ALKi resistance in ALK-LBCL. Because crizotinib is a P-glycoprotein substrate whereas alectinib/lorlatinib are not, further studies are also necessary to determine if drug efflux via P-glycoprotein contributes to crizotinib resistance in ALK-LBCL.

Few patients achieve durable remissions/cures following frontline chemotherapy (eg, cyclophosphamide, hydroxydaunorubicin, oncovin, and prednisolone; rituximab, etoposide, prednisolone, oncovin, cyclophosphamide; hydroxydaunorubicin). Our data warrant multicenter efforts to determine if adding alectinib/lorlatinib to first-line chemotherapy improves outcomes, and to investigate novel combinations to overcome ALKi resistance (eg, with a novel ALK degrader) through dual inhibition of ALK and key mediators of downstream signaling implicated in ALKi resistance in lymphoma (eg, PI3K-AKT,[19] SHP2,[18] RAS-MAPK,[20] JAK-STAT effector pathways).[21] Numerous PDX models of diffuse large B-cell lymphoma (DLBCL) have been reported. However, ALK-LBCL shares few biologic features of DLBCL and, to our knowledge, none of the DLBCL PDX models capture the unique biology driving ALK-LBCL. The newly generated PDX models will be key to testing novel therapies in ALK-LBCL to overcome ALKi resistance.

These data support use of off-label alectinib or lorlatinib in patients with relapsed/refractory ALK-LBCL, and allogeneic stem cell transplant should be considered for patients who achieve an adequate response. In addition, we have illustrated the potential of PDX models to inform therapeutic options, particularly for patients with rare malignancies.

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Authorship

Contribution: J.D.S. and A.L. had full access to the data and were responsible for data collection, data analysis, and data interpretation, wrote the first draft of the manuscript, and were responsible for manuscript submission; A.R., A.D.Z., Y.W., R.K.G., A.R.E.-J., S.H., J.D., C. Mecca, A.D., H.L., C. Menard, L.Y., L.R., K.B., H.M., M.L., S.M., A.K., O.K., E.P., and R.C. were responsible for data collection, data analysis, and data interpretation, and reviewed and approved the manuscript; and M.L., V.N., and J.S.A. were responsible for data analysis, data interpretation, and reviewed and approved the manuscript.

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Footnotes

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There is a *Blood* Commentary on this article in this issue.

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TO THE EDITOR:

A model of painful vaso-occlusive crisis in mice with sickle cell disease

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Sickle cell disease (SCD) is the most common hemoglobinopathy and is caused by a point mutation in the β-globin chain that is susceptible to polymerization, giving red blood cells (RBCs) a rigid sickle shape and causing vaso-occlusion.[1] Several factors enhance vaso-occlusion,[2,3] which lead to a vaso-occlusive crisis (VOC) that is accompanied by severe acute pain often requiring hospitalization and opioid treatment.[4] Because pain itself maintains VOC,[2,3] effective pain management is of particular importance for the relief of VOC. To better understand the mechanisms underlying acute pain during VOC, a model is needed that mirrors the clinical features of VOC. Considering that low ambient temperature can be a trigger for painful VOC,[3] we developed a model of acute painful VOC caused by exposure to cold in mice with SCD. We investigated whether exposure to cold causes acute pain in mice with SCD and whether this reflects the clinical features of VOC.

Male and female transgenic Berkeley mice[5] (5-9 months old) that express human sickle hemoglobin S (HbSS) or normal human hemoglobin A (HbAA) were used. Mechanical hyperalgesia was defined as a decrease in paw withdrawal threshold using calibrated von Frey monofilaments according to the up-down method.[6] Heat hyperalgesia was defined as a decrease in paw withdrawal latency in response to a radiant heat stimulus.[7,8] Deep tissue hyperalgesia was defined as a decrease in grip force measured by a grip force meter.[7,9] Baseline (BL) measurements were taken over 3 days before each experiment. Paw withdrawal threshold and paw withdrawal latency were measured for both hind paws and averaged. Different modalities of hyperalgesia were studied in different groups of mice. HbSS mice that exhibited BL mechanical threshold and withdrawal latency less than 2 standard deviations (SDs) and grip force less than 1 SD of the average value for HbAA mice were considered nonhyperalgesic HbSS (HbSS[nh]). Consistent with our previous report,[10] the number of HbSS(nh) mice was ~15% of all HbSS mice, and this decreased with age. HbSS(nh) mice were exposed to cold to evoke acute hyperalgesia, an experimental analogue of pain in patients.

For exposure to cold, mice were placed in a plastic cage without bedding in a room with a controlled ambient temperature of 10°C (50°F) for 1 hour and then returned to room temperature (22°C). Hyperalgesia and measures of blood oxygen saturation (SpO_2) were determined before, immediately