ORIGINAL ARTICLE

ALKTERNATE: A Pilot Study Alternating Lorlatinib With Crizotinib in ALK-Positive NSCLC With Prior ALK Inhibitor Resistance

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ABSTRACT

Introduction: ALK-positive lung cancers represent a molecularly diverse disease. With drug exposure, driving selection pressure, and resistance pathways, disease relapse will emerge. There is compelling rationale to investigate novel treatment strategies, informed by dynamic circulating tumor DNA (ctDNA) monitoring.

Methods: The single-arm, pilot study ALKTERNATE investigated fixed alternating cycles of lorlatinib intercalated with crizotinib in individuals resistant to second-generation ALK inhibitors. Dynamic ctDNA explored the correlation with disease response and disease recurrence and defined disease resistance. The primary outcome was time-to-treatment failure, a composite of tolerability, feasibility, and efficacy. Secondary outcomes included standard survival measures, toxicity, pharmacokinetic analysis, and patient-reported outcomes. Tertiary outcomes were proteogenomic analyses of tissue and plasma.

Results: A total of 15 individuals were enrolled; three encountered primary resistance to lorlatinib induction. There were 12 participants who received alternating therapy, and this approach revealed safety, feasibility, and effectiveness. Patient-reported outcomes were maintained or improved on therapy, and toxicity was consistent with previous reports. The pharmacokinetic measures were similar to the single-arm drug experience. Median time-to-treatment failure was 10 months; overall survival was 23 months. ctDNA profiles indicated inferior survival in those with preexistent TP53 mutations and those without clear or cleared ctDNA at trial induction. The study defined a vastly heterogeneous population with an abundance of ALK coexisting with non-ALK resistance variants.

Conclusions: ALKTERNATE revealed feasibility with a novel alternating ALK inhibitor strategy in ALK-positive NSCLC. Results support progressing inquiry into this approach and propose a flexible design with drug(s) selected and alternating time frames, informed by real-time plasma profiling. Moving this concept to treatment naive may also optimize impact.

Keywords: ALK; ALKi; NSCLC; Resistance; ctDNA; Lorlatinib; Crizotinib

Introduction

Since first described in 2007,[1] the *ALK*-rearranged NSCLC community has experienced a revolution in personalized ALK inhibitor (ALKi) therapies—first-, second-, and third-generation ALKis now established in the clinic and fourth-generation ALKis in clinical development.[2–6] Despite median survival of up to 7 years with modern agents,[7] drug resistance remains inevitable. Acquired resistance to ALKis may manifest through "*ALK*-dependent" on-target mutations, and "*ALK*-independent" bypass tract activation,[8,9] or rarely histologic transformation.[10]

Newer generation ALKis promptly superseded first-generation crizotinib as a new standard first-line ALKi. It was shortly thereafter described the array of ALKi disease-resistant phenotypes may be highly variable depending on the unique ALKi exposure(s).[8] This phenomenon is in part due to the unique selection pressure applied by each structurally distinct ALKi in this molecularly heterogeneous disease.[11,12]

Until recently, second-generation ALKis, most frequently alectinib, have been the mainstay first-line therapy globally based on compelling efficacy and superior tolerability.[3,13,14]

Third-generation lorlatinib was next established as an empirical treatment in ALKi-refractory disease.[15] In pretreated individuals, it became apparent those with *ALK*-resistant mutations derived greater benefit and perhaps those who harbored a variant 3 *EML4-ALK* fusion performed favorably compared with variant 1.[1,16] Furthermore, those who had only been exposed to one-line second-generation ALKi had shorter median progression-free survival (PFS) than those who received multiple prior lines.[15]

With exposure to more potent on-target, next-generation ALKis, resistance may more frequently manifest with the emergence of bypass tract, "off target" activation, which has been described through multiple pathways, including *EGFR*, *MET*, *HER2*, *KRAS*, *PIK3CA*, *BRAF*, *NRAS*, and *IGF1-R*. Most often, these activated resistance pathways co-occur with aberrations in tumor-suppressor gene *TP53*.[8,17–21] With latter line lorlatinib, in particular, *MET* dysregulation or compound *ALK* mutations occur frequently.[22] Crizotinib is also an active MET inhibitor. Compound *ALK* mutations, most often including *ALK* G1202R, may be resistant to the established ALKis.[23]

Circulating tumor (ct)DNA has emerged as a valuable minimally invasive tool for temporal genomic analysis in *ALK*-positive (*ALK*+) NSCLC.[24] This modality provides potential to capture intratumoral, intertumoral, and

interindividual heterogeneity in a timely manner. The potential utility in predicting performance on ALKis based on plasma kinetics is reported with ctDNA in *ALK*+ disease.[25–27]

At present, tissue and less commonly blood sampling have been relied on to determine the molecular basis for disease progression, identify phenotypic transformation, and allow selection of the most appropriate therapy.[28,29] Treatment at progression however continues for most to be recommended empirically or "blindly" to the individual's cancer biology.

Given the pace of drug development in *ALK*+ lung cancer, next-generation ALKis have not been compared head-to-head in randomized studies and are unlikely to ever be so. Lorlatinib has recently emerged as the ALKi with the greatest magnitude of impact first line in terms of PFS and central nervous system (CNS) activity.[30] Data describing drug resistance profiles with first-line lorlatinib are awaited. What has been found is that the presence of *TP53* co-mutations leads to inferior survival.[31]

ALKTERNATE hypothesized that alternating ALKi therapy may alter intrinsic ALKi selection pressure and suppress or prevent the expansion of preexistent drug-resistant subclones or the emergence of acquired resistant clones to lorlatinib, thus delaying clinical progression.[32] Furthermore, ctDNA could monitor and detect the molecular mechanisms of resistance before clinical and radiological progression. The study design was informed by two case reports, one revealing the role of serial biopsies in a heavily treated *ALK*+ patient, identifying paradoxical resensitization to crizotinib after lorlatinib resistance,[33] and another revealing the utility of ctDNA in resistance profiling over time.[24] Data emerging regarding *MET* dysregulation as a bypass pathway with lorlatinib further informed the rationale to intercalate the ALKi/MET inhibitor crizotinib.[22]

Materials and Methods

Study Design and Patients

The study design was a proof-of-concept, open-label, single-arm, translational study investigating a fixed schedule of alternating lorlatinib with crizotinib in individuals with *ALK*+ NSCLC who had received any number of prior lines of therapy for advanced disease, provided they had experienced disease progression on at least one second-generation ALKi. Individuals enrolled required extracranial Response Evaluation Criteria in Solid Tumors (RECIST) version (v.) 1.1 measurable disease[34]; CNS and leptomeningeal were eligible if asymptomatic, or treated and stable, and magnetic resonance imaging of the brain mandated. The washout period from previous systemic chemotherapy or radiation treatment was 2 weeks and from ALKi 4 days. Eastern Cooperative Oncology Group performance status 0 to 1 was required. Previous crizotinib therapy was allowed provided it was not the most recent line and there was no previous intolerability.

The study was conducted by the Thoracic Oncology Group of Australasia at two Australian tertiary referral centers—Royal North Shore Hospital (RNSH) and Sydney and Peter MacCallum Cancer Centre, Melbourne, Australia. The trial was approved for by the local ethics review board (NSLHD 2019/ETH00389) and registered with Australian New Zealand Clinical Trial Registry (ANZCTR): ACTRN12619000844145. All patients provided written informed consent.

Treatment and Assessments

At enrollment, patients required plasma with or without tissue sampling for biomarker analysis. Plasma samples for ctDNA assessment occurred at baseline, three monthly on study, and at disease progression. Baseline magnetic resonance imaging of the brain and computed tomography-chest, abdomen, pelvis were performed before patients received 100 mg daily of lorlatinib for 3 months, as induction therapy (cycle 1), and then alternating treatment with 1 month crizotinib 250 mg twice daily, and then 2 months lorlatinib 100 mg daily, with dose interruptions and reductions as clinically indicated.

Patients required at least disease control and no progression after 3 months of lorlatinib (cycle 1), to be eligible to enter the "alternating" phase of therapy.

Given anticipated drug-drug pharmacokinetics, before treatment switch, ALKi was ceased for 48 hours, and at cycle 1 to 2a (lorlatinib switch to crizotinib) and cycle 2a to 2b (crizotinib to lorlatinib), blood samples for pharmacokinetic (PK) analysis were collected.

Alternating therapy was continued until disease progression by RECIST v.1.1, intolerability, study withdrawal, or death.

The trial schema is available in Supplementary Appendix a.Figure 1.

Study End Points and Translational Methods

The primary outcome measure was time-to-treatment failure (TTTF) with alternating ALKi therapy, defined as the time from treatment initiation (post +3 months induction lorlatinib) to treatment discontinuation from any cause. Treatment failure was deemed the appropriate end point given this pilot was assessing feasibility of delivering a novel treatment schedule. Only those who enter alternating therapy were included in the primary outcome analysis. Secondary outcomes included overall survival (OS), PFS on study, and post-progression

(PFS2), including crossover to continuous lorlatinib. Objective tumor response was also assessed.

Toxicity was assessed through standard Common Terminology Criteria for Adverse Events (CTCAE) v.4.03 criteria. Patient-reported outcomes were recorded through Functional Assessment of Cancer Therapy Lung v.4 questionnaires. To focus on the tolerability of lorlatinib with this treatment approach, mental health was assessed through the Beck's Depression Inventory Questionnaire-II and cognitive assessments through the MiniCog quick screening test.

For the tertiary outcome evaluating temporal ctDNA profiles, samples were sequenced with Resolution Bioscience lung ctDx v.8, Resoluton Bioscience, Kirkland, WA, USA. Time points baseline, 6 monthly, and disease progression milestones were analyzed real time, and the remaining time points analyzed retrospectively including 3 months before disease progression on alternating therapy.

For correlative tertiary analysis, tissue core biopsies were obtained if feasible, and formalin fixed, paraffin embedded. Reconfirmation of histologic phenotype and ALK+ immunohistochemistry status were required during screening, with adenocarcinoma-predominant disease required. Tissue was sequenced retrospectively through the Trusight Oncology 500 gene (TSO500) next-generation sequencing assay, in house (Peter MacCallum Cancer Centre).

The PK plasma samples were collected and analyzed, with quantitation at Labcorp Pharmaceutical R&D (Shanghai, People's Republic of China) using validated methods. Blood samples were collected at 1 hour, 2 hours, 4 hours, 6 hours, and 24 hours on cycle 2 (cycle 1-2a and cycle 2a-2b switches).

For plasma proteome exploration, digests were analyzed in random order by liquid chromatography and tandem mass spectrometry in data-independent acquisition mode using a nano-ultrapressure liquid chromatography system (Ultimate 3000 RSLCnano, Thermo Fisher Scientific) coupled to a Q-Exactive HF-X orbitrap mass spectrometer (Thermo Fisher Scientific).[35–37] Further methodology is available in Supplementary Appendix b.

Statistical Analysis

Study sample size for the pilot was not based on a statistical outcome, with a maximum of 20 patients sought.

Survival, time-to-event Kaplan-Meir plots, and a swimmer plot were calculated and composed using R v.4.1.3 software.

Descriptive analysis of qualitative and quantitative ctDNA profiles are reported, with correlation to survival. Individual patient time point variant allelic frequency (VAF) plots were extracted from the RESOLUTION BIO HUB reporting software.

The PK parameters, including C_{max} and AUC_{0-6}, were calculated by noncompartmental analysis methods using the WinNonlin software package (v.8.3.4 Pharsight Corporation, Sunnyvale, CA) for PK analytes crizotinib and lorlatinib.

Differentially abundant proteins were assessed using two-sided *t* test in Perseus (v.1.6.5.0) with *p* less than 0.05 and presented with GraphPad Prism v.10 (Dotmatics, Boston, MA, USA).

Study Amendment

The study was approved by the local ethics board at lead site RNSH and opened in July 2019. Delays in accrual occurred with recent approval of first-line use of alectinib in Australia delaying disease progression and the coronavirus disease (COVID19) pandemic. The study completed enrollment in May 2023. The trial was amended to allow for remote assessments by telehealth and local drug delivery during the pandemic.

Results

Patients

There were 21 individuals screened, and 15 were enrolled (Fig. 1). Those not pursuing study included two identified through ctDNA to harbor non–*ALK*-driven disease, two did not have RECIST v.1 measurable disease, one withdrew consent during screening, and one had persistent elevated bilirubin level.

Three (20%) experienced primary resistance to lorlatinib and were not eligible for alternating therapy. There were 12 patients who proceeded to alternating ALKi therapy.

Median age was 67 years, 60% male, almost 25% Asian, 50% never-smokers, and 50% were Eastern Cooperative Oncology Group of 0. In addition, 40% harbored CNS metastases at trial inclusion with 66% not having received previous local therapy. The median number of previous lines of therapy was two, with almost 50% receiving multiple previous lines of ALKi. Demographics are detailed in Table 1.

Survival

At data lock on June 30, 2023, median follow-up was 31 months, 83% (N = 10) had experienced "treatment failure" with alternating therapy, and 60% had died.

The median (m) time from diagnosis to trial enrollment was 27 months. The range of previous lines of therapy was one to five. Time on trial ranged from 3 to 42 months. The swimmer plot in Figure 2 illustrates

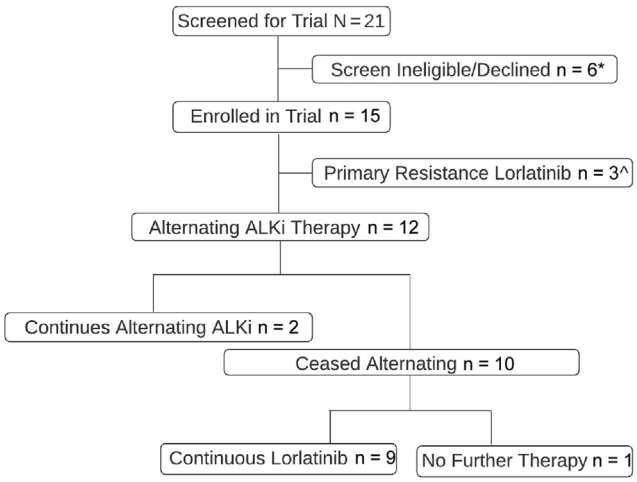

Figure 1. Consort diagram of patient screening, accrual, and progress on the study. *Two patients were deemed trial ineligible at the TMC discretion due to baseline ctDNA indicating disease unlikely *ALK* driven: ALK01-04 plasma, 10 variants detected, no ALK-fusion; ALK01-11 *KRAS* G12C detected, confirmed on tissue biopsy, no *ALK* fusion. ^Three patients with primary resistance to lorlatinib harbored an *EML4-ALK* V1, two with co-occurring *TP53* in the plasma and tissue, and one with *MYC* CNG present in tissue biopsy (ALK01-17). ctDNA, circulating tumor DNA; TMC, trial management committee.

the therapies received and duration on treatment before enrollment, during the study, and after the study, including individuals' TTTF on alternating treatment.

The median TTTF was 11 months (n =12, 8.4 mo–not reached [NR]), 8 months from initiation of alternating therapy (Fig. 3*A*). PFS1, all-comers, was 6.1 months (n = 15, 5.4 mo–NR) (Fig. 3*C*). PFS2, those crossed over to continuous lorlatinib, was 3.1 months (n = 9 2.1 mo–NR). Objective response rate was 53%, all partial responses. Four (27%) achieved stable disease as best response and three (20%) progressive disease. Median OS was 23 months (18 mo–NR) and 26 months in alternating (Fig. 3*C*). Median time from diagnosis to death was 40 months.

In those without baseline *TP53* mutations, mPFS was 27.0 months versus 5.5 months in those with p equals 0.025 (Fig. 3*D*). mPFS in those who had clear plasma (ctDNA) at diagnosis or after 1 cycle of alternating therapy was 24 months versus 5.9 months in those who did not have clear plasma ($p = 0.14$) (Fig. 3*E*). There was no difference in PFS in those with *ALK*+-resistant mutations detected at baseline versus not, nor by *ALK* fusion variant, clot, CNS status, or previous lines of therapy.

A total of 50% on alternating (n = five of 10) progressed in the CNS, two without previous CNS disease. All with CNS progression were managed with local stereotactic radiotherapy.

Tolerability

Safety. There were no grade 4 or 5 toxicities encountered, 60% experienced a grade 3 treatment-related toxicity, and 40% grade 2 as their highest-grade adverse event (AE). Dose interruption for grade 3 AEs ranged from 1 day to 17 days. Dose reduction was required in four (27%) on lorlatinib due to delirium, mood disturbance, word-finding difficulties, and unconfirmed seizure (later attributed to a hemorrhagic stroke on anticoagulation) and one (7%) on crizotinib due to esophagitis, prompting an early protocol amendment to mandate medication with food (Table 2). The highest-grade and most frequent toxicities are detailed in Table 2. Most toxicities in the study were grade 1; the most common are weight gain, peripheral edema, and hypercholesterolemia. Weight gain ranged from 5% to 25%. In addition, syncope encountered by two patients in the study was potentially attributed to a drug, but it is not confirmed.

Table 1. Patient Demographics

Characteristic	N = 15
Age, y	n (%)
Median	67
Range	34-77
Sex	
Male	9 (60)
Female	6 (40)
Race	
Asian	4 (27)
Non-Asian	11 (73)
Smoking	
Never	7 (47)
Former	8 (53)
Current	0
ECOG performance status	
0	7 (47)
1	8 (53)
CNS metastases, no. (%)	
Yes	6 (40)
No	9 (60)
Prior CNS treatment	
Stereotactic radiosurgery	1 (17)
Whole brain radiotherapy	1 (17)
No treatment	4 (66)
Previous systemic therapies	
Median (range)	2 (1-5)
Second-generation ALK inhibitor only	7 (46)
>1 prior ALK inhibitors	7 (46)
Previous PE/VTE	
Yes	3 (20)
Detectable ctDNA at baseline	
Yes	13 (87)
No	2 (13)

CNS, central nervous system; ctDNA, circulating tumor DNA; ECOG, Eastern Cooperative Oncology Group; PE, Pulmonary embolus; VTE, Venous thromboembolism.

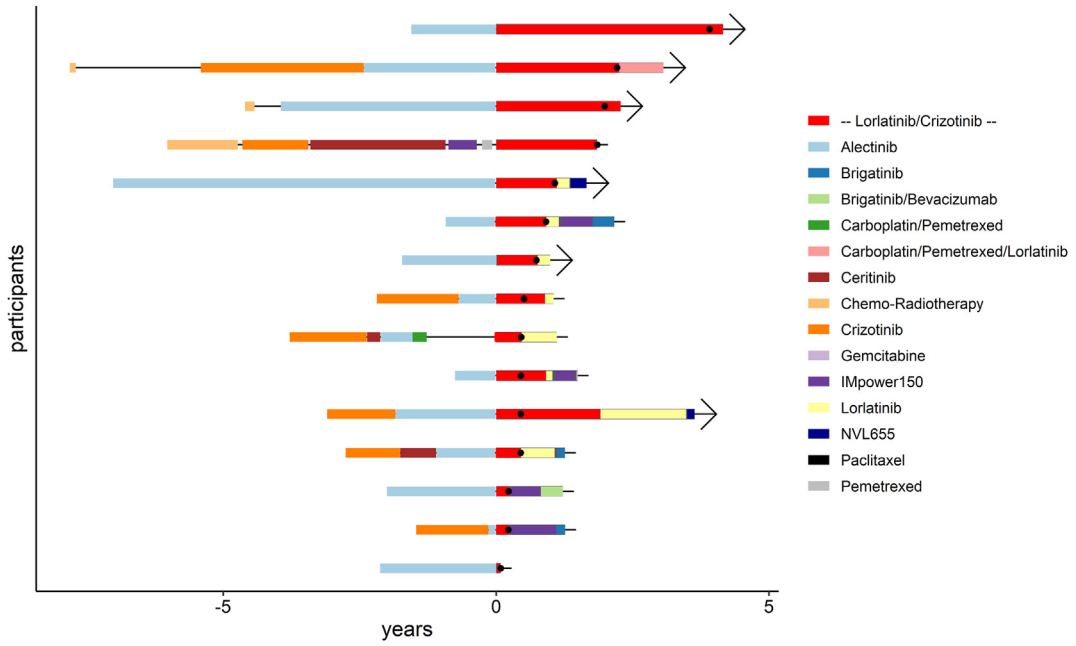

Figure 2. Swimmer plot of participants and treatments received and duration of treatment benefit before and after study commencement (y = 0 is ALKTERNATE trial commencement).

Pharmacokinetics. Plasma AUC_{0-6}, C_{max}, and T_{max} for both crizotinib and lorlatinib were similar compared with historical single-dose data. Other PK parameters could not be reliably estimated because sampling was limited to 6 hours (the 24-h sample was inconsistently collected pre-dose, which is a study oversight).

Mean and SD plasma concentrations (ng/mL) of crizotinib and lorlatinib from 0 to 6 hours post-drug switch

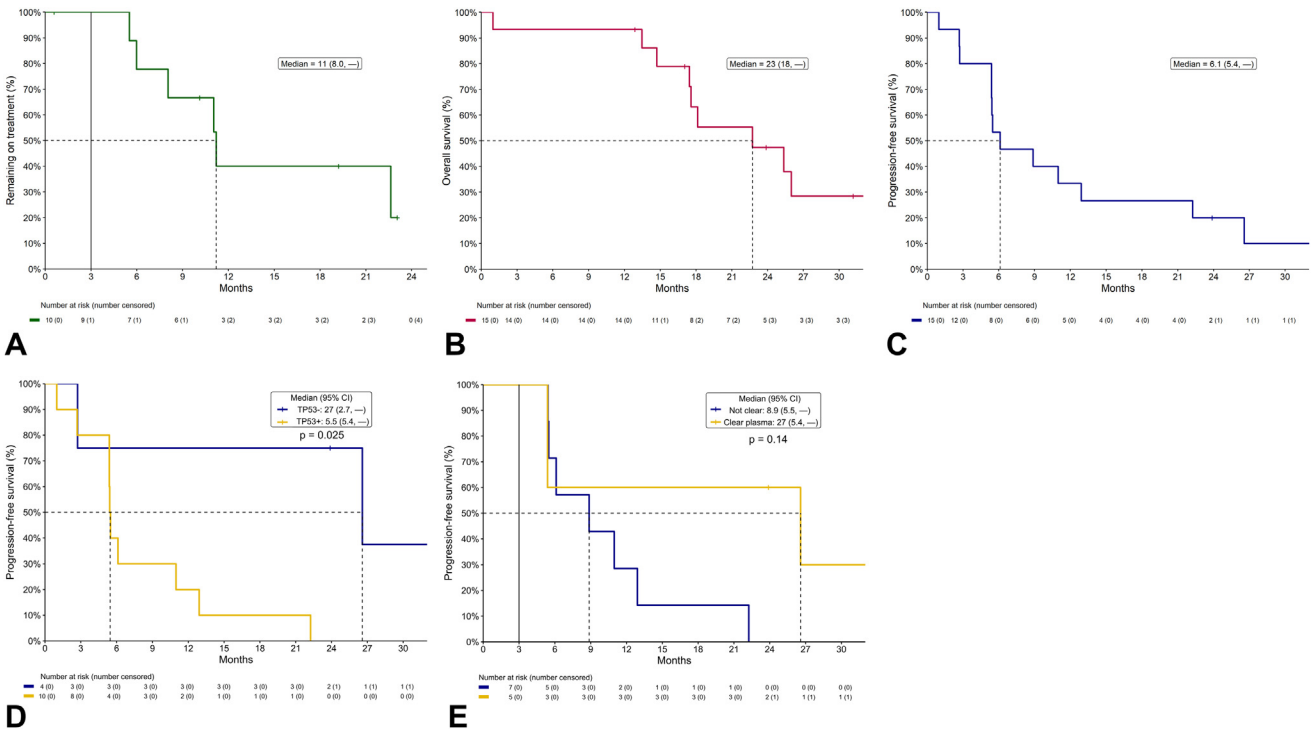

Figure 3. Kaplan-Meir survival plots. (*A*) Time-to-treatment failure for alternating therapy; (*B*) overall survival in all enrolled; (*C*) progression free survival in all enrolled; (*D*) progression-free survival based on *TP53* trial entry status; and (*E*) progression-free survival in those with clear ctDNA at trial entry or after one cycle of alternating treatment versus those with persisting detectable ctDNA. ctDNA, circulating tumor DNA. ctDNA, circulating tumor DNA.

Table 2. Safety, Tolerability, and Treatment-Related Adverse Events

Safety and Tolerability	N = 15		
Worst-grade toxicity, n (%)			
2	6 (40)		
3	9 (60)		
Dose discontinuation	0 (0)		
Dose reduction			
Lorlatinib	4 (27)[a]		
Crizotinib	1 (7)[b]		
Potential treatment-related adverse events—most frequent adverse events (no. participants)			
Event		Grade	
	1	2	3
Syncope			2
Paresthesia	5		1
Hypercholesterolemia	4	5	1
Weight gain		3	1
Dyspnea	3	3	1
Pneumonia			2
Esophagitis			1
Hypertension		3	
Bone pain	4	2	
Rash	4	2	
Upper respiratory tract infection	3	2	
Cough	2	2	
Dysgeusia	1	2	
Urinary tract infection		2	
Peripheral neuropathy	7	1	
Diarrhea	7	1	
Nausea	6	1	
Arthralgia	5	1	
Fatigue	4	1	
Peripheral edema	11		
Dizziness	5		
Transaminitis	4		
Vomiting	3		
Mood lability	3		
Word finding difficulty	3		
Constipation	3		
Flatulence	3		
Anorexia	3		
Abdominal pain	3		

[a]Dose reduction on lorlatinib due to delirium, mood disturbance, word-finding difficulties, and unconfirmed seizure (later attributed to a hemorrhagic stroke while on direct oral anticoagulation, unprovoked).
[b]One patient was dose reduced on crizotinib due to esophagitis and re-educated to take with food.

are available in Supplementary Appendix c.Table 1. and d.Figure 2.

Patient-Reported Outcomes. Quality of life was maintained or improved throughout and compared consistently with the adult population reference value.[38] Complete results of the Functional Assessment of Cancer Therapy Lung assessments are available in the Supplementary Appendix e.Figure 3.i.–vii.

One patient scored in the depression range on questionnaire assessment, including at baseline. The trial reporting intervals did not allow capture of fluctuations in mood around drug switches after the first 6 months. Anecdotally, multiple individuals volunteered their mental state felt "better," both mood and cognition, on their month of crizotinib, and this was noted with minor improvements in scoring reported in the first crizotinib switch.

Cognition screening revealed that all but one experienced a degree of cognitive impairment, mild to moderate, which revealed fluctuation on the study and a degree of improvement on crizotinib in the first 6 months when assessed. This observation was neither captured under the standard CTCAE formal reporting nor able to be evaluated with statistical significance.

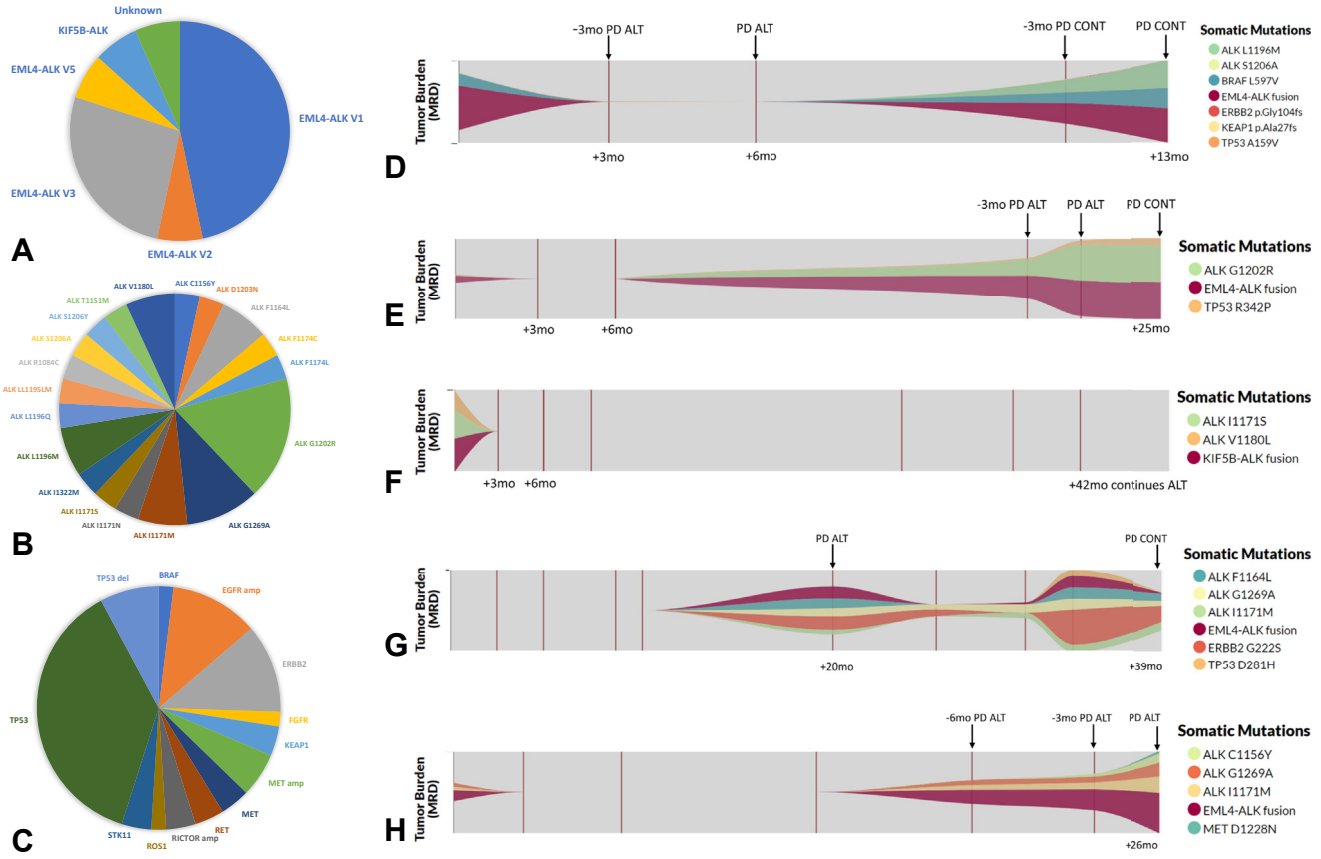

Figure 4. Qualitative ctDNA and the frequency of variant capture (*A*) for the ALK fusion variant, (*B*) ALK mutations detected in the blood and frequency across individuals, (*C*) non-ALK variants detected in the blood and frequency across individuals, and (*D–H*) five individual patient temporal ctDNA allelic frequency plots revealing variant quantitatively. CNG is not captured in VAF plots presented, (*D*). Harbored *MET* CNG at baseline (6 copies) and PD continuous lorlatinib (four copies). ctDNA, circulating tumor DNA.

Biomarker Analysis

Circulating Tumor DNA. A total of 89 samples were analyzed with a reporting success rate of 97%.

There were 13 individuals (87%) who harbored detectable ctDNA at baseline. Two screen ineligibilities were based on the ctDNA profile, one reported a *KRAS* G12C mutation with no *ALK* fusion (confirmed then on biopsy); the second patient harbored 12 variants in ctDNA with no *ALK* fusion, in retrospect the standard-of-care testing deemed falsely positive.

The three patients (20%) with primary resistance to lorlatinib harbored an *EML4-ALK* variant 1 fusion, two with co-occurring *TP53* mutations and no further cause for resistance in ctDNA.

The *ALK*-fusion variant was defined in 14 of 15 through ctDNA (Fig. 4*A*). There were 19 differing *ALK*-resistant mutations detected throughout, with overlapping variants detected in five, five harboring *ALK* G1202R, three *ALK* G1269A, and two *ALK* F1164L, I1171M, L1196M, and V1180L. The range of *ALK* mutations in an individual sample was zero to five (Fig. 4*B*). A spectrum of non-*ALK* variants were also detected across the cohort (Fig. 4*C*). One individual harbored a germline *TP53* P72A mutation.

Two patients had low-level *MET* copy number gain (CNG) at trial entry, none at progression on alternating therapy, and one encountered *MET* CNG on crossover continuous lorlatinib (17 copies). This same individual and one other had a new *MET* point mutation at alternating progression disease (PD). Four (40% of those with PD) developed compound *ALK*-resistant mutations at progression on alternating therapy.

Five (42%) had clear plasma at entry or cleared their detectable ctDNA in plasma on treatment (Fig. 4*D–H*). The remaining on alternating therapy experienced a decrease in VAF with therapy. Two individuals with primary resistance to lorlatinib did not have a decrease in VAF, and the third did not undergo a progression sample collection.

Seven (70%) had an increase in the abundance of detectable preexisting ctDNA variants or new variants detected preceding radiological progression on

alternating therapy (Fig. 4*A*, *B*, and *D*–*H*). Nine (90%) identified new non-*ALK* variants on alternating treatment and five (50%) new *ALK*-resistant variants.

The ctDNA profiles and kinetics were unique to each case. Figure 4*D* to *H* illustrates the utility of ctDNA profiling in defining the molecular phenotype, monitoring disease response, and determining disease resistance, before radiological disease progression in case examples.

The complete set of ctDNA results is available in Supplementary Appendix f.Table 2.

Tissue Biopsy Evaluation. Ten of 15 individuals (66%) had a tissue biopsy at inclusion and seven of 13 (54%) at disease progression (54%). All were confirmed to remain *ALK*+ and adenocarcinoma-predominate phenotype.

The tissue genomic sequencing failure rate was 53% due to insufficient sample. Notable findings complementing ctDNA results in those analyzable included an *STRN3-NTRK2* fusion, co-occurring with an *EML4-ALK* (V1) fusion in a resected progressing CNS metastasis, not detected in matched time point ctDNA. A participant with primary systemic progression on lorlatinib harbored *EML4-ALK* (V1) and *MYC* CNG (10 copies), not identified in ctDNA.

One individual harbored mixed large cell neuroendocrine histology with adenocarcinoma at entry and, then, at disease progression, new squamous differentiation and an emergent *ALK* G1202R, not present at baseline tissue. Matched plasma detailed an abundance of additional variants, including *ALK* G1202R, to be preexistent, *ALK* V1180L, *EGFR* amplification, and four *TP53* variants, also confirming *EML4-ALK* variant 3. At progression, the ctDNA VAFs were lower in abundance. The squamous differentiation deemed likely driving disease.

One individual exhibited radiological progression in the left side of chest wall axilla ipsilateral to the site of definitive radiation years prior. Fluorodeoxyglucose-positron emission tomography confirmed avid disease. Core biopsy revealed necrosis and abscess material, with no evidence of malignancy. The individual remained on study treatment for a further 13 months given disease control.

Tissue sample status and next-generation sequencing results are available in Supplementary Appendix g.Table 3.

Plasma Proteome Analysis. Plasma proteome data across the treatment continuum were obtained to explore novel biomarkers. A yield of 379 proteins could be used for quantitative analyses which confirmed feasibility of this method. Proteome signatures mostly clustered by individual with variability mapped over time (Supplementary Appendix h.Fig. 4).

Exploratory analyses were conducted in clinically meaningful groups of interest. The baseline level of coagulation factor IX (FA9) was significantly elevated throughout ($p = 0.02$), most abundant at progression in those who experienced a shorter TTTF (below median). Coagulation factors vitamin K-dependent protein C and S (PROC, $p = 0.02$, PROS, $p = 0.03$) were also predictive of TTTF with higher levels experienced in lower survival. Similarly, in the *TP53*+ group (significantly lower survival), platelet-activating factor acetylhydrolase (PLA2G7) was more abundant ($p = 0.01$) (Supplementary Appendix i.Fig. 5.i–ii).

Individuals with an *ALK*-dependent resistance profile at trial entry identified unique proteins to those with an *ALK*-independent co-mutational signature. The *ALK*-dependent plasma proteome was enriched with extracellular matrix (ECM) proteins ($p < 0.05$) including vinculin (VCL), vitronectin (VTN), osteonenctin (SPARC), periostin (POSTN), collectin (COLLEC11), olfactomedin (OLFM1), and tenascin X (TNXB) (Supplementary Appendix i.Fig. 5.iii–vii) and in *TP53*+ ECMs nidogen-1 (NID1, $p = 0.02$) and fibulin-5 (FBLN5, $p = 0.02$) (Supplementary Appendix i.Fig. 5.viii–xii).

Discussion

This pilot study reveals the feasibility, safety, and activity of alternating ALKi therapy with lorlatinib and crizotinib in an ALKi-refractory population. Crizotinib was used for its potential to maintain and prolong sensitivity to lorlatinib and suppress the activation of MET signaling as a prevalent resistance pathway.

The primary outcome measure being the composite outcome of tolerability, feasibility, and efficacy, TTTF supported this being a deliverable strategy. Survival was comparable with the single-arm lorlatinib registrational data, but not superior. Alternating ALKi therapy did not compromise quality of life with the pattern and frequency of toxicities observed in keeping with those expected. PK concentrations were not compromised with drug switching.

This proof-of-concept translational study illustrates the potential impact of personalized care with both tissue and liquid biopsy, including the feasibility of ctDNA in routine care. Collating this approach also confirmed the heterogeneity of "*ALK*+ resistance". The ctDNA signatures helped inform optimal recruitment and correlated with performance on trial, whereas assessment of kinetics predicted superior performance in the study and altered preceding a relapse in disease. Unlike previous reports, *MET* was not an identified resistance mechanism in ALKTERNATE, as has been the

experience with continuous lorlatinib.[22] This may in part be due to intercalated crizotinib exposure. A small series has reported modest clinical activity and general tolerability in adding crizotinib to lorlatinib in those identified to have *MET* dysregulation.[39] In addition, there were no notable unifying non-*ALK* variants emergent on ALKTERNATE, except for *TP53* mutations frequent before the study and dominant at progression. The alternating ALKi method did not diminish in the frequency of *ALK*-compound resistance mutation found with lorlatinib monotherapy, experienced in 50% in the study.

Close CNS surveillance was performed to monitor for CNS disease progression, but it accounted for 50% of the progression events on alternating therapy. None were found to progress in CNS on a crizotinib cycle and all were asymptomatic. Four patients with CNS PD were successfully treated with stereotactic radiotherapy and all crossed over to continuous lorlatinib with a period of CNS disease control. With continuous lorlatinib latter line, the probability of the first event being CNS progression was consistently lower than the first event being non-CNS progression.[40] Managing the CNS is paramount in *ALK*+ disease, and the crizotinib cycles were designed to be short (1 mo) given inferior CNS penetration. The progression of this study would be alternating in more CNS-penetrant, next-generation ALKis, while also moving this treatment strategy to investigation in the frontline setting to increase impact.

An interest with ALKTERNATE is the potential to mitigate drug AEs, especially the chronic low-grade toxicities given the inbuilt individual drug "holidays." For example, the cognitive effects of lorlatinib are reported to be experienced in at least 23%.[41] The trial design did not enable robust objective assessment of this; however, there was a trend for improvement in cognition and mood in those assessed with drug switch off lorlatinib. It is intriguing that this cognitive impairment was not captured in the CTCAE reporting, as not volunteered by the reporting individual or adequately captured in the survey. Four patients (33%) were dose reduced on lorlatinib in alternating therapy, each with neurologic adverse events. Future optimized objective assessment of the impact of therapy "switches" enabling drug "holidays" as a strategy to mitigate toxicity could add appeal to this strategy. This would be particularly of interest with lorlatinib and chronic low-grade neurocognitive toxicities or weight gain as important examples.

The exploratory plasma proteome analysis found almost 400 proteins quantifiable in the clinically meaningful groups analyzed, which is substantial in the small sample set. Many of the putative biomarkers are suitable for immunoassay in future studies which may move the field beyond genomics. Altered protein profiles in the coagulation pathway, ECM including inflammation and angiogenesis, and apolipoproteins involved in lipid trafficking and proliferation were reported in clinically distinct subgroups. Cancer portends a hypercoagulable state, and indeed *ALK*+ lung cancers are found to have increased clotting rates, including venous thromboembolism.[42,43] Such an approach may enable future prediction of events informed by the proteomic signature.

The main shortcoming of this study is its small sample size with the reported observations being exploratory and hypothesis generating. The initial findings of ALKTERNATE in approaching this novel treatment strategy will serve as a foundation to inform future expansion of this approach.

Recently, lorlatinib has become the standard of care for the frontline treatment of all first-line *ALK*+ diagnoses, shifting the field from investigating latter line lorlatinib treatment strategies. There is compelling scientific rationale to test bespoke combination escalation and de-escalation drug strategies informed by real-time ctDNA qualitative and quantitative signatures with a proposed approach to this "ALKTERNATE 2.0" presented in Supplementary Appendix j.Figure 6. For example, *TP53* mutations are reported in 37% of patients with *ALK*+ de novo[44] and in 73% in the present pretreated study, which has also revealed a clinically and statistically significant inferior survival in those with preexisting *TP53* mutations.[45] This supports the mounting biological rationale and evidence to expand exploration in a combination approach with ALKi given with antiangiogenic therapy as there is an up-regulation of vascular epithelial growth factor pathways in TP53-activated pathways, with or without chemotherapy, as monotherapy is simply insufficient.[46,47]

ALKTERNATE strongly suggests a "one size fits all" universal therapeutic monotherapy approach, even with fixed switch therapy, delivers unpredictable efficacy, and requires ongoing biomarker-informed exploration in pretreated *ALK*+ disease. In the study, there was an array of unique molecular phenotypes, particularly at disease relapse. To elaborate with case examples, one individual harbored the preexisting *ALK* fusion with an emergent *NTRK* fusion in a progressing resected brain metastasis, one encountered histologic squamous transformation, and one compound *ALK* mutations *ALK* C1156Y, *ALK* G1269A, and *ALK* I1171M. These three unique cases suggest three different optimal next therapeutic pathways, such as an ALKi plus NTRKi, chemotherapy, and a fourth-generation ALKi trial versus ALKi plus chemotherapy, respectively. At present, these people would all be treated with the same therapy under "standard of care". We support an N = 1 biomarker-informed treatment approach in ALKi drug resistant

cases to progress the field in this molecularly distinct group of NSCLCs, and across oncogene-positive disease.[48]

Conclusion

The ALKTERNATE study revealed a positive signal for safety, preserved QOL, and comparable survival to historical data with fixed alternating ALKi therapy in treatment-refractory disease, while revealing ctDNA to be therapeutically informative and a harbinger of disease progression. Expanded exploration of this novel treatment approach is supported, including in treatment naive. A future proposed study would use real-time ctDNA profiling in the first-line setting to inform the drug selection of alternating brain-penetrant ALKi monotherapies or combination treatment, including non-ALKis, such as chemotherapy, in a dynamic biomarker-driven manner.

CRediT Authorship Contribution Statement

Malinda Itchins: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

Shirley Liang: Validation, Formal analysis, Investigation, Resources, Data curation, Writing - Review & Editing, Visualization, Project administration.

Chris Brown: Software, Validation, Formal analysis, Resources, Data curation, Writing - Review & Editing, Visualization.

Tristan Barnes: Resources.

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Antony J. Mersiades: Methodology.

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Mark Molloy: Resources, Writing - review & editing, Visualization.

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Ben J. Solomon: Conceptualization, Investigation, Resources, Writing - review & editing, Funding acquisition.

Nick Pavlakis: Conceptualization, Methodology, Validation, Investigation, Resources, Writing - review & editing, Supervision, Funding acquisition.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2024.100703.

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