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Crizotinib in patients with tumors harboring ALK or ROS1 rearrangements in the NCI-MATCH trial


The NCI-MATCH was designed to characterize the efficacy of targeted therapies in histology-agnostic driver mutation-positive malignancies. Sub-protocols F and G were developed to evaluate the role of crizotinib in rare tumors that harbored either ALK or ROS1 rearrangements. Patients with malignancies that progressed following at least one prior systemic therapy were accrued to the NCI-MATCH for molecular profiling, and those with actionable ALK or ROS1 rearrangements were offered participation in sub-protocols F or G, respectively. There were five patients who enrolled on Arm F (ALK) and four patients on Arm G (ROS1). Few grade 3 or 4 toxicities were noted, including liver test abnormalities, and acute kidney injury. For sub-protocol F (ALK), the response rate was 50% (90% CI 9.8–90.2%) with one complete response among the 4 eligible patients. The median PFS was 3.8 months, and median OS was 4.3 months. For sub-protocol G (ROS1) the response rate was 25% (90% CI 1.3–75.1%). The median PFS was 4.3 months, and median OS 6.2 months. Data from 3 commercial vendors showed that the prevalence of ALK and ROS1 rearrangements in histologies other than non-small cell lung cancer and lymphoma was rare (0.1% and 0.4% respectively). We observed responses to crizotinib which met the primary endpoint for ALK fusions, albeit in a small number of patients. Despite the limited accrual, some of the patients with these oncogenic fusions can respond to crizotinib which may have a therapeutic role in this setting.
function of wild-type ROS1 is poorly understood, it is speculated that ROS1 might translate adhesion events to intracellular signaling because of the structural similarities to cell adhesion molecules. In NSCLC, the most common ROS1 fusion partner is CD74. Less common partners include SDC4, EZR, SLC34A2, TPM3, LIMA1, and MSN. The ROS1 proto-oncogene has been identified to be translocated in NSCLC with a frequency of 1.7% (18/1077) to 2.6% (17/656).

While rare, ROS1 rearrangements have also been reported in gastric cancers, glioblastomas, cholangiocarcinomas, ovarian cancers, colorectal cancers, inflammatory myofibroblastic tumors, angiosarcomas, and epithelial hemangiendotheliomas.

Crizotinib is a first-in-class ATP-competitive small-molecule inhibitor of ALK, ROS1, and Met/hepatocyte growth factor receptor (HGF/FR). Crizotinib demonstrated concentration dependent inhibition of ALK kinase activity in biochemical and cell-based assays. In addition, crizotinib demonstrated growth inhibition and increased apoptosis of tumor cell lines with ALK fusion variants (EML4-ALK or NPM-ALK). Mice xenografts with ALK fusion variants also responded to crizotinib treatment in a dose dependent fashion. Based on its significant activity, crizotinib received FDA approval for ALK-positive NSCLC in 2011, for ROS1-positive NSCLC in 2016, and ALK-positive ALCL in 2021. In prior studies, common toxicities related to crizotinib included reversible visual disturbances, gastrointestinal side effects, fatigue, transaminitis and edema.

The NCI-MATCH subprotocols F and G were designed to study the activity of crizotinib in tumors other than NSCLC or ALCL with ALK and ROS1 rearrangements, respectively. Given the common therapeutic option in these subprotocols, the results of both studies were combined for this report.

**RESULTS**

**Patient characteristics**

A total of 5 patients enrolled on sub-protocol F (ALK), with the first patient enrolled on November 24, 2015, and the last on April 24, 2019. One patient was ineligible since treatment was started before a 28-day washout following prior treatment, resulting in four analyzable patients (Supplemental Fig. 1). A total of 4 patients enrolled on sub-protocol G (ROS1), with the first patient enrolled on July 7, 2016, and the last on September 13, 2018 (Supplemental Fig. 2). Due to poor accrual, both sub-protocols were closed. Among both sub-protocols, most patients had gastrointestinal malignancies (Table 1). All but one patient had received two or more prior lines of therapy. Previously reported EML4 and GOPC fusion partners were most frequently identified for ALK and ROS1, respectively. A median of four cycles of crizotinib were administered in both sub-protocols.

**Response assessment and survival outcomes**

For sub-protocol F (ALK), the response rate was 50% (90% CI 9.8–90.2%; Fig. 1) with one, ongoing complete response; the median PFS was 3.8 months, and the 6-month PFS rate was 25% (90% CI 6.0%-100%; Fig. 2). Despite the very low accrual, the null hypothesis of response rate not exceeding 5% can be rejected at the 1-sided .014 significance level, meeting the primary endpoint. The median overall survival was 4.8 months (Fig. 2; Supplemental Table 3). For sub-protocol G (ROS1), with a 25% responses rate (90% CI 1.3–75.1%; Fig. 1), the p-value for testing the null of 5% was 0.186. The median PFS was 4.3 months, and the 6-month PFS rate was 50% (90% CI 22–100%; Fig. 2). The median overall survival was 6.2 months (Fig. 2; Supplemental Table 3).

**Adverse events**

There were very few grade 3 or 4 toxicities at least possibly related to treatment; these included liver test abnormalities, abdominal pain, and acute kidney injury. Less severe edema, hypoalbuminemia, and liver test abnormalities were also observed (Supplemental Table 4). There were two deaths on sub-protocol F attributed to disease progression or a cause that was not otherwise specified. There was one death on sub-protocol G attributed to disease progression.

**Expanded molecular cohort**

In an expanded molecular cohort of subjects compiled by three commercial vendors, the detection of ALK or ROS1 rearrangements outside of NSCLC or lymphoma was rare (Supplemental Table 5). A review of over 30,000 tumors excluding those with NSCLC or lymphoma from two of these commercial vendors identified ALK rearrangements in 1/1,000 specimens and ROS1 rearrangements in 4/10,000 specimens. One external dataset included clinical characteristics of these cases. Whereas there was a similar proportion of females ($n = 18$, 49%) and males ($n = 19$, 51%) with ALK rearrangements, there were more females ($n = 10$, 63%) than males ($n = 6$, 37%) with ROS1 rearrangements. Although there were similar median ages for patients with either rearrangement, there were younger patients with ALK rearrangements than ROS1 rearrangements ($ALK$ median 57 years, range 25–83; $ROS1$ median 60.5 years, range 41–89). ALK rearrangements were most frequently seen in thyroid, colorectal, and soft tissue tumors in this cohort (Supplemental Table 6). ROS1 rearrangements were most frequently seen in glioblastoma multiforme and breast cancer (Supplemental Table 7). The most frequent ALK fusion partners were $EML4$ (38%) and $STRN$ (16%) and the most frequent ROS1 fusion partner was GOPC (44%) (Fig. 3). Of the evaluable tumors, almost all with ALK or ROS1 fusions had low or intermediate TMB (96%; Supplemental Table 8) and were MSI stable (98%; Supplemental Table 9).

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**Table 1.** Patient Characteristics.

<table>
<thead>
<tr>
<th></th>
<th>ALK fusion</th>
<th>ROS1 fusion</th>
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<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
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<tr>
<td>Female</td>
<td>3 (75%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td><strong>Age:</strong> median (range)</td>
<td>59 (52–69) years</td>
<td>54 (47–76) years</td>
</tr>
<tr>
<td><strong>Race:</strong> White</td>
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<td>4 (100%)</td>
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<tr>
<td><strong>Performance Status 0</strong></td>
<td>1 (25%)</td>
<td>1 (25%)</td>
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<tr>
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<td>1</td>
<td>1 (25%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>2</td>
<td>2 (50%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>3</td>
<td>0 (0%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>&gt;3</td>
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<td>2 (50%)</td>
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<td><strong>Weight loss previous 6 months:</strong></td>
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<td>4 (100%)</td>
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<td><strong>Histologies</strong></td>
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<td></td>
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<tr>
<td>Pancreatic adenocarcinoma</td>
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<td></td>
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<tr>
<td>Colorectal adenocarcinoma</td>
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<td>1</td>
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<td>$STRN$</td>
<td>1</td>
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<td>$GOPC$</td>
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DISCUSSION

Accrual to sub-protocols F and G was limited, reducing our ability to assess the efficacy of treatment with any precision. However, we can conclude that the true response rate for sub-protocol F exceeds the null hypothesis rate of 5%, at the 1-sided .014 significance level. We observed responses to crizotinib in patients whose tumors harbored ALK or ROS1 rearrangements outside of the FDA-approved indications for this therapy. However, the median overall survivals of 4.8 and 6.2 months suggest that the anti-tumor activity we observed was modest. Many of the reported toxicities were similar to those reported in prior trials with crizotinib. Furthermore, our rates of detection and analysis of external datasets from three commercial vendors suggest that ALK and ROS1 rearrangements are very rare outside of NSCLC.

Globally, there have been additional efforts to target either ALK or ROS1 in rare tumor types. Some of the more successful attempts have been in the pediatric population. For instance, response rates as high as 80–90% with ALK inhibitor therapy have been observed in the treatment of anaplastic large cell lymphomas which harbor ALK translocations. Inflammatory myofibroblastic tumors are another rare malignancy that frequently harbor ALK or ROS1 translocations. In one case, the ALK rearrangement resulted from a complex pattern of chromosomal rearrangements called chromoplexy, and that patient had durable benefit from treatment with the ALK inhibitor ceritinib.

Finally, molecular events involving ALK have also been identified in aggressive thyroid cancers and neuroblastomas. While the role of ALK and ROS1 inhibitors is now established as standard of care among ALK - or ROS1 -positive NSCLC in addition to some of the rare tumors listed above, our data suggest that patients with other tumor types that harbor these mutations may also benefit from targeted therapy. The authors are not aware of other plans to develop crizotinib further for these indications.

There are several potential explanations for the modest impact on response rate and survival noted in both sub-protocols. First, this was a pre-treated population with most patients having received at least 2 lines of prior therapy. As a result, it is likely that these patients had more resistant tumors at the time of treatment. Somatic mutations that confer resistance to crizotinib are now well characterized among series of patients with NSCLC and acquired resistance mutations in ALK include L1196M, G1202R, and S1206Y. Alternatively, these patients’ tumors may have developed bypass pathway signaling or had concurrent mutations as a result of prior therapy which limited the activity of crizotinib. One patient had a concurrent BRAF fusion, which may have been the actual oncogenic driver. Since the approval of crizotinib, more potent ALK and ROS1 inhibitors have received FDA approval such as alectinib, brigatinib, entrectinib, and lorlatinib. Enrollment to basket trials with some of these agents may have competed with enrollment to NCI-MATCH. At this time, it is not known if one of these newer agents would yield improved efficacy in these other tumor types with ALK or ROS1 fusions. Finally, it has been suggested that sequencing RNA might be more appropriate than DNA for the detection of fusions given the difficulties of covering all of the introns from which rearrangements can arise.

The central NCI-MATCH NGS assay did sequence fusions from RNA input, but the assay was specifically targeted for known fusions. The external laboratories use a variety of platforms but many scan introns in DNA. For that reason, there may have been some cases with novel ALK or ROS1 fusions that were not detected for inclusion in NCI-MATCH.
METHODS

Study design and eligibility

The NCI-MATCH trial is an ongoing, nationwide clinical trial with integrated multiple independent single-arm sub-protocols, each addressing an actionable molecular alteration. Each sub-protocol aims to evaluate a single agent or combination treatment for which at least a recommended phase 2 dose has been determined.

Patients with histologically documented solid tumors, lymphomas, or myelomas whose disease had progressed following at least one line of standard systemic therapy or for whom no standard therapy exists were registered on the screening step of the NCI-MATCH protocol to undergo molecular profiling analysis on fresh tumor biopsies. The latter profiling was performed in specific Clinical Laboratory Improvement Amendments (CLIA)-accredited laboratories and consisted of next generation sequencing (NGS) with an investigational targeted gene panel, the Oncomine Ampliseq assay. This assay was validated to detect specific targeted gene fusions with 97.67% sensitivity and 99.99% specificity. In the second phase of the trial, 30 academic and commercial laboratories that perform NGS testing were reviewed and evaluated for concordance of test results with the central NCI-MATCH laboratory assay. These laboratories were then asked to refer patients with an actionable mutation for enrollment. Patients whose tumors were found to harbor actionable ALK or ROS1 rearrangements (Supplementary Tables 1-2) were offered participation in sub-protocols F or G, respectively. The protocol allowed for subsequent treatment on other subprotocols if relevant molecular profiles were present and eligibility criteria were met. The study was initiated after approval from the Central Institutional Review board and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.

 Patients with NSCLC were excluded from both sub-protocols, given that the FDA previously approved crizotinib for patients with NSCLC harboring ALK or ROS1 rearrangements. Patients who had already received ALK or ROS1 inhibitors were also excluded. Patients were treated with crizotinib 250 mg twice daily on 28-day cycles. Dose reductions to 200 mg twice daily or 250 mg daily were allowed for treatment-related adverse events. Responses were assessed using revised RECIST (Response Evaluation Criteria in Solid Tumors) version 1.1. Chosen criteria for patients with lymphoma, and RANO criteria for patients with glioblastoma multiforme.

Statistical considerations

The primary objective of these NCI-MATCH sub-protocols was to evaluate the proportion of patients who had objective response, defined as complete or partial response, to a targeted study agent. This proportion, termed the objective response rate and expressed as a percentage, was compared against a null benchmark value of 5%. If the observed objective response rates were ≥ 5/31 (16%), it would then be concluded that the agent is promising and worthy of further investigation. Allowing for a 10% ineligibility rate, 35 patients were to be accrued to each sub-protocol, to obtain 31 eligible patients per sub-protocol. With this design, the power was 91.8% to conclude an agent is promising if its true response rate is 25%, and the type 1 error (one-sided) was 1.8% of its true response rate is 5%. Secondary objectives included the proportion of patients who were progression-free at 6 months (PFS6), progression-free survival, and toxicity assessment.

Expanded molecular cohort

Three NGS vendors provided data on the detection of ALK and ROS1 rearrangements in the tumors of patients excluding NSCLC and lymphoma as part of their commercial testing services in an effort separate from NCI-MATCH. We used these data to see how the frequency of ALK and ROS1 rearrangements in this expanded molecular cohort compared to that detected by NCI-MATCH. Two of these vendors provided the total number of cases tested, and one vendor provided additional data on the clinical
characteristics of these tumors, microsatellite instability (MSI) status, and tumor mutation burden (TMB). These cases were not necessarily included in NCi-MATCH. MSI was examined using over 7,000 target microsatellite loci and compared with the reference genome hg19 from the University of California, Santa Cruz (UCSC) Genome Browser database. The status was defined as MSI-high (MSI-H) or MSI-low/microsatellite stable (MSI-S). The number of microsatellite loci that were altered by somatic insertion or deletion were counted for each sample. Only insertions or deletions that increased or decreased the number of repeats were considered. Genomic variants in the microsatellite loci were detected using the same depth and frequency criteria used for mutation detection. MSI-NGS results were compared with results from over 2,000 matching clinical cases analyzed with traditional PCR-based methods. The threshold to determine MSI by NGS was determined to generate a sensitivity of >95% and specificity of >90%. TMB was measured by counting all nonsynonymous missense mutations found per tumor that had not been described previously as germline alterations (592 genes and 1.4 megabases (MB) sequenced/tumor). Potential germline mutations were excluded by comparing data against dbSNP 137 full and 1000 Genomes Phase 3. The threshold to determine MSI by fragment analysis in colorectal cancer cases, based on reports of TMB having high concordance with MSI-H in colorectal cancer. TMB-intermediate was defined as ≥ 7 but <17 mutations/megabase, and TMB-low was defined as ≤ 6 mutations/megabase. These data were summarized and the donut charts were created with R Studio (version 1.2.5033) and tidyverse packages.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

DATA AVAILABILITY
The data generated or analysed from these clinical trials are included in this published article. The expanded molecular datasets generated or analysed for the current study are available from the corresponding author on reasonable request. The de-identified sequencing data from Caris Life Sciences are owned by Caris Life Sciences. Qualiﬁed researchers can apply for access to these summarized data by contacting Joanne Xiu, PhD and signing a data usage agreement. Strata Oncology will provide de-identified molecular results upon request for Strata-referred samples included in this analysis. More speciﬁcally, they will provide all prioritized variants for the ALK/ROS1 + patients, including for the fusions 5′/3′ partners, junctions and read support, as well as cancer type, age range, etc. Requests should be addressed to Dan Hovelson, PhD. Tempus has provided summary statistics, including the number of screened patients for this cohort and the count of the speciﬁc positives for post-publication replication and veriﬁcation purposes (Supplemental Table 10). The protocols are available as supplemental materials.

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REFERENCES

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