Expanded clinical opportunities for crizotinib identified from an analysis of over 5,000 patient exomes

Abstract C256

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ABSTRACT

Patients with chromosomal rearrangements resulting in fusion proteins are amongst the most responsive to targeted therapy. For example, targeting of the BCR-ABL fusion in chronic myelogenous leukemia (CML) with imatinib and targeting the EML4-ALK fusion in non-small cell lung cancer (NSCLC) with crizotinib has led to dramatic patient responses in these diseases. While crizotinib is approved for use in EML4-ALK positive NSCLC through its inhibition of ALK, the drug also inhibits ROS1, MST1R (RON), MET, and more recently has been shown to inhibit the ALK homolog, LTK. To gain a more comprehensive understanding of the full therapeutic potential of crizotinib, we undertook a genomic survey of ALK, LTK, ROS1, MET and MST1R across thousands of patients subjected to full exome sequencing including patients from The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC), as well as tens thousands of patients from Oncomine™ databases.

We confirmed the presence of EML4-ALK fusions in both lung and colorectal cancer (CRC), identified a PRKAR1A-ALK fusion in CRC, and found evidence of novel recurrent ALK fusions in kidney papillary renal cell carcinoma and thyroid gland carcinoma. ALK hotspot mutations and focal amplifications were confined to neuroblastoma, as previously described. We also report the first instance of an LTK fusion, identified in thyroid gland carcinoma. LTK amplifications were also observed in 1.4% of gastric cancers and rarely in medulloblastoma and breast cancer. LTK was prominently over-expressed in leukemia. In an analysis of over 4,000 leukemia patients, LTK was amongst the most significantly over-expressed genes in PML-RARA fusion positive leukemia. High-level MET amplifications were observed in 1-5% of papillary renal cell carcinoma, the intestinal subtype of gastric adenocarcinoma, oligodendroglioma, glioblastoma and lung adenocarcinoma. Hotspot mutations in MET were frequently observed in head and neck squamous cell carcinoma (HNSCC) (11%), and observed in a third of metastatic HNSCC samples. Additional hotspot mutations were also observed in lung adenocarcinoma (2%) and small cell lung cancer (2%). Aberrations in MST1R were rare.

MATERIALS AND METHODS

To further understand the full therapeutic potential of crizotinib, we undertook a genomic survey of ALK, ROS1, MET, LTK, and MST1R in more than 5,000 patient exomes from The Cancer Genome Atlas (TCGA) and non-TCGA sources as well as in thousands of patient samples found in Oncomine™ Gene Browser, and Oncomine™ Concepts Edition. For total frequencies in cancer types and subtypes, amplification was defined as 4 or more copies of potential crizotinib target genes and genomic events (fusion, mutation, amplification) were assumed to be mutually exclusive.

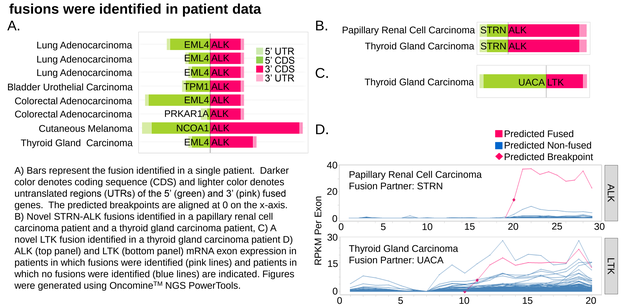
CONCLUSIONS

These results leverage all available genomic profiling data to provide a broadened scope of therapeutic opportunity for inhibitors like crizotinib. Gene expression data suggests crizotinib targets may display elevated activity in a clinically defined subtype of leukemia. With the growing availability of next-generation sequencing data and analyses, such surveys can support hypothesis-driven development of targeted therapies and help expand opportunities for clinical stage therapies.

TRADEMARKS/LICENSING

RESULTS

Figure 1. EML4-ALK fusions were confirmed and novel STRN-ALK and UACA-LKT fusions were identified in patient data



A) Bars represent the fusion identified in a single patient. Darker color denotes coding sequence (CDS) and lighter color denotes untranslated regions (UTRs) of the 5' (green) and 3' (pink) fused genes. The predicted breakpoints are aligned at 0 on the x-axis. B) Novel STRN-ALK fusions identified in a papillary renal cell carcinoma patient and a thyroid gland carcinoma patient, C) A novel LTK fusion identified in a thyroid gland carcinoma patient D) ALK (top panel) and LTK (bottom panel) mRNA exon expression in patients in which fusions were identified (pink lines) and patients in which no fusions were identified (blue lines) are indicated. Figures were generated using Oncomine™ NGS PowerTools.

Figure 2. Recurrent mutations in ALK were identified in ganglioneuroblastoma, lung adenocarcinoma, and neuroblastoma

A) Summary of the ALK mutation landscape across cancer. The y-axis indicates the number of patients with ALK mutations at a given amino acid position (denoted on the x-axis). B) Pie charts indicate the distribution of mutations across cancer subtypes (e.g. 33% of recurrent T1151 mutations were observed in lung adenocarcinoma).

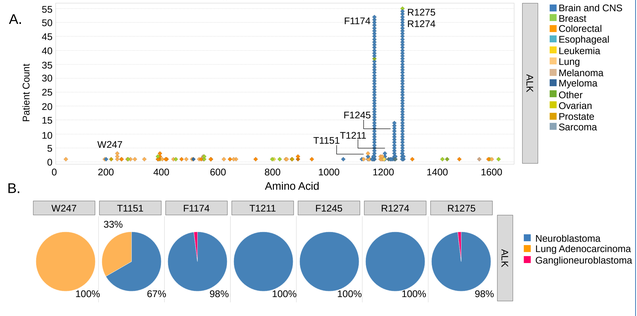
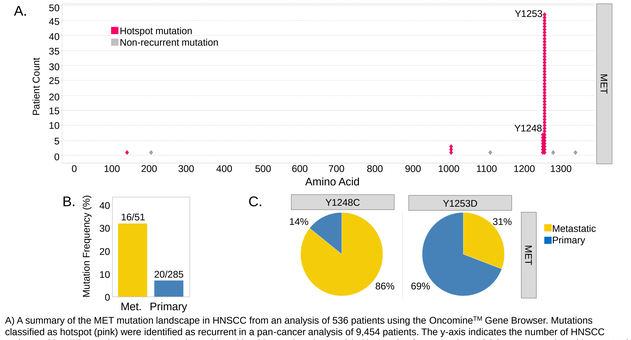
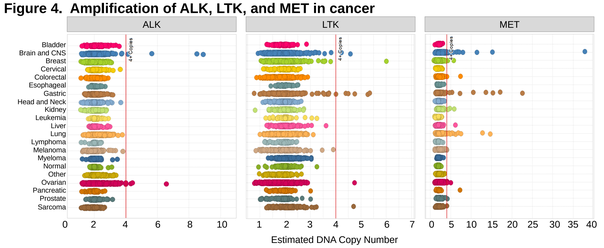


Figure 3. High frequency of recurrent mutations in MET were observed in metastatic head and neck squamous cell carcinoma (HNSCC)



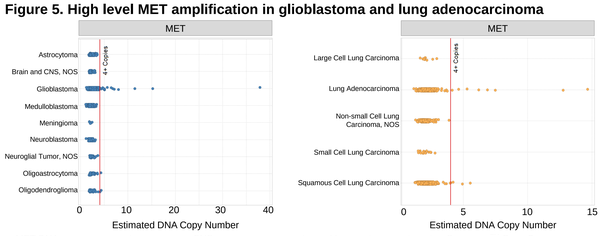
A) Summary of the MET mutation landscape in HNSCC from an analysis of 536 patients using the Oncomine™ Gene Browser. Mutations classified as hotspot (pink) were identified as recurrent in a pan-cancer analysis of 9,454 patients. The y-axis indicates the number of HNSCC patients with MET mutations at a given amino acid position (denoted on the x-axis). B) Mutation frequency in HNSCC tumor samples with metastatic (yellow) and primary (blue) tumor type information. The number of patients assessed for mutation of MET is indicated at the top of each bar. C) Pie charts indicate the distribution of Y1248C and Y1253D hotspot mutations in metastatic (yellow) and primary (blue) HNSCC.

Figure 4. Amplification of ALK, LTK, and MET in cancer



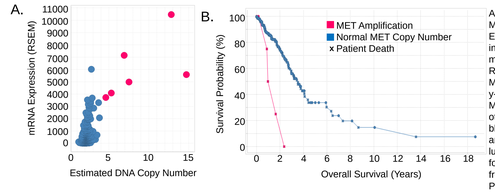
DNA copy number data in major cancer types. Visualizations are from the Oncomine™ Gene Browser across 15,763 clinical cancer samples and 7,211 normal samples. Cancer types are indicated on the y-axis and estimated gene copies on the x-axis.

Figure 5. High level MET amplification in glioblastoma and lung adenocarcinoma



MET DNA copy number data in brain and CNS and lung cancer subtypes. Visualizations are from the Oncomine™ Gene Browser. Cancer subtypes are indicated on the y-axis and estimated gene copies of MET on the x-axis.

Figure 6. MET amplification led to increased mRNA expression and associated with poor outcome in lung adenocarcinoma



A) DNA-RNA correlation of MET in lung adenocarcinoma. Estimated copy number is indicated on the x-axis and mRNA expression (RSEM, RNA-Seq by Expectation-Maximum) is indicated on the y-axis. Samples with amplified MET are colored pink, all other samples are colored blue B) Kaplan-Meier survival analysis of MET amplified lung adenocarcinoma. Data for figures was downloaded from Oncomine™ NGS PowerTools.

Figure 7. Genomic aberration and cancer type frequency summary of crizotinib targets

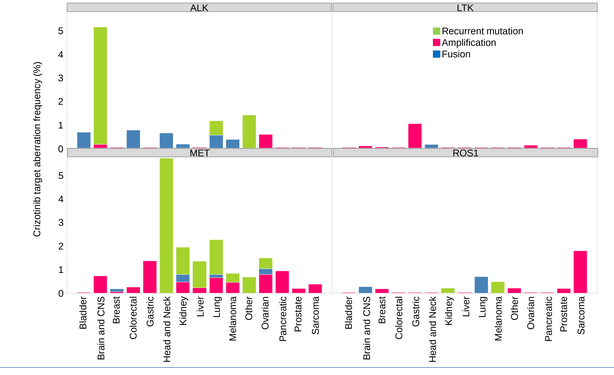


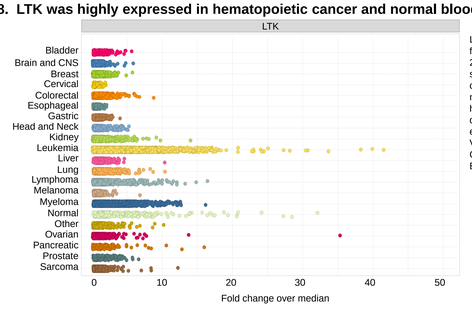
Table 1. Clinical implications of findings

Cancer subtypes in which aberrations in ALK, LTK, MET, ROS1, and/or MST1R were identified are indicated. Patients with cancer subtypes below the blue line are being recruited in clinical trials testing the efficacy of crizotinib. Clinical trial information was identified in the Trialtrove® database and is considered current as of September 15, 2013.

Cancer Type	Aberration Frequency
Liposarcoma	3.6%
Gastric Intestinal Type Adenocarcinoma	3.3%
Gastric Tubular Adenocarcinoma	3.2%
Small Cell Lung Carcinoma	2.8%
Thyroid Gland Follicular Carcinoma	2.6%
Cutaneous Melanoma	2.3%
Hepatocellular Carcinoma	1.9%
Endometrial Carcinoma	1.9%
Pancreatic Carcinoma	1.6%
Gastric Adenocarcinoma	1.3%
Thyroid Gland Carcinoma	1.0%
Thyroid Gland Undifferentiated (Anaplastic) Carcinoma	1.0%
Colorectal Adenocarcinoma	0.9%
Bladder Urothelial Carcinoma	0.7%
Prostate Carcinoma	0.5%
Thyroid Gland Papillary Carcinoma	0.5%
Endometrial Endometrioid Adenocarcinoma	0.3%
Clear Cell Renal Cell Carcinoma	0.2%
Ganglioneuroblastoma	18.2%
Papillary Renal Cell Carcinoma	11.6%
Head and Neck Squamous Cell Carcinoma	11.2%
Neuroblastoma	7.6%
Lung Adenocarcinoma	5.2%
Oligodendroglioma	3.4%
Non-Small Cell Lung Carcinoma, NOS	2.6%
Glioblastoma	2.5%
Ovarian Serous Adenocarcinoma	2.2%
Oligoastrocytoma	1.5%
Ovarian Carcinoma	1.5%
Squamous Cell Lung Carcinoma	1.0%
Breast Carcinoma	0.4%
Medulloblastoma	0.3%
Ductal Breast Carcinoma	0.3%
Invasive Breast Carcinoma	0.1%

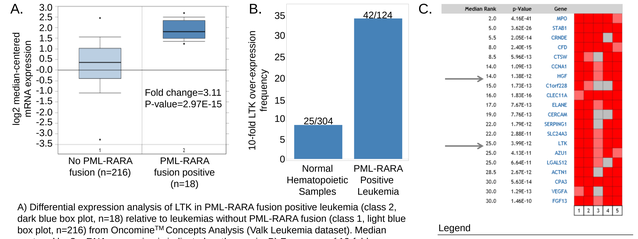
- Integrative analysis of all genomic aberrations across a broad spectrum of cancer types allowed the maximal clinical potential of crizotinib to be evaluated
- In several cases the identified cancer subtypes have not been previously described as clinical opportunities for crizotinib and related drugs
- Potentially new clinical opportunities with high unmet need include:
  - **Melanoma:** ALK fusions and recurrent mutations in MET
  - **Pancreatic carcinoma:** High level of MET amplification (result needs to be confirmed in a larger sample set)
  - **Hepatocellular carcinoma:** Amplification of and recurrent mutations in MET (results need to be confirmed in a larger sample set)

Figure 8. LTK was highly expressed in hematopoietic cancer and normal blood samples



LTK mRNA expression from an analysis of 25,798 clinical cancer samples across all major cancer types and 304 normal tissue samples of hematopoietic origin in a quantile normalized expression environment. Visualization is taken from Oncomine™ Gene Browser.

Figure 9. mRNA expression of LTK and the MET ligand, HGF was prominently elevated in PML-RARA fusion positive leukemia



A) Differential expression analysis of LTK in PML-RARA fusion positive leukemia (class 2, dark blue box plot, n=18) relative to leukemias without PML-RARA fusion (class 1, light blue box plot, n=216) from Oncomine™ Concepts Analysis (Valk Leukemia dataset). Median centered log2 mRNA expression is indicated on the y-axis. B) Frequency of 10-fold over-expression of LTK in PML-RARA fusion positive leukemia relative to normal hematopoietic samples. Sample counts meeting the over-expression threshold and total samples for the analysis are indicated atop each bar graph segment. Over-expression was defined as expression 10-fold above the global median as determined in an analysis of 26,507 cancer samples and 2,657 normal tissue samples. C) Oncomine™ Concepts Edition meta-analysis of genes over-expressed in PML-RARA fusion positive leukemia patients from five datasets with 4,304 total patients. Genes are sorted by median over-expression rank across the five datasets and median p-values for over-expression in PML-RARA positive leukemia vs. leukemia samples without PML-RARA fusion are also given. The arrows indicate HGF and LTK and the red coloration indicates whether a given gene was in the top 1, 5, 10, or 25% of over-expressed genes within the analysis listed in the legend.

Legend

1. Childhood Acute Myeloid Leukemia – PML-RARA Gene Fusion, *Balgobind Leukemia, Haematologica, 2011*
2. Acute Myeloid Leukemia – PML-RARA Gene Fusion, *Haferlach Leukemia, J Clin Oncol, 2010*
3. Acute Myeloid Leukemia – PML-RARA Gene Fusion, *Haferlach Leukemia, J Clin Oncol, 2010*
4. Acute Myeloid Leukemia – PML-RARA Gene Fusion, *Valk Leukemia, N Engl J Med, 2004*
5. Acute Myeloid Leukemia – PML-RARA Gene Fusion, *Wouters Leukemia, Blood, 2009*