ENTRECTINIB

BRIEFING DOCUMENT

PEDIATRIC ONCOLOGY SUBCOMMITTEE OF THE ONCOLOGIC DRUGS ADVISORY COMMITTEE

Meeting Date: 29 June 2016

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List of Abbreviations and Definitions of Terms

AE	adverse event
ALCL	anaplastic large cell lymphoma
ALK	anaplastic lymphoma kinase
AUC	area under the (plasma concentration versus time) curve
BSA	body surface area
CLIA	Clinical Laboratory Improvement Amendments
C_{max}	maximal plasma concentration
CNS	central nervous system
CR	complete response
CRC	colorectal cancer
BDNF	brain-derived neurotrophic factor
DLT	dose-limiting toxicity
ECG	electrocardiogram
EIAEDs	enzyme-inducing anti-epileptic drugs
ICH	International Conference on Harmonisation
MASC	mammary analog secretory carcinoma
mCRC	metastatic colorectal cancer
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NSCLC	non-small cell lung cancer
PD	progressive disease
PR	partial response
QD	once daily
QTc	corrected QT interval
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	recommended Phase 2 dose
$t_{1/2}$	plasma terminal half-life
TKI	tyrosine kinase inhibitor
T_{max}	time of maximal plasma concentration

1 INTRODUCTION

Ignyta, Inc. (Ignyta) is participating in the Meeting of the Pediatric Oncology Subcommittee of the Oncologic Drugs Advisory Committee to seek a Written Request from the Agency for pediatric development of entrectinib based on an ongoing Phase 1/1b study RXDX-101-03.

Entrectinib is a potent inhibitor of the receptor tyrosine kinases TrkA (encoded by the gene neurotrophic tyrosine receptor kinase [*NTRK*]*1*), TrkB (encoded by the gene *NTRK2*), TrkC (encoded by the gene *NTRK3*), ROS Proto-Oncogene 1 (ROS1; encoded by the gene *ROS1*), and anaplastic lymphoma kinase (ALK; encoded by the gene *ALK*). Gene rearrangements in each of these genes have been observed in a variety of tumor types, including non-small cell lung cancer (NSCLC), colorectal cancer (CRC), salivary gland cancer, papillary thyroid cancer, melanoma, and sarcoma; overexpression of *NTRK2* and *ALK* and point mutations of *ALK* have also been observed in neuroblastoma. Thus, a pan-Trk, ROS1, and ALK inhibitor like entrectinib may have broad potential therapeutic utility. Three targeted therapies, crizotinib, ceritinib and alectinib, have been approved in the United States for ALK-positive NSCLC, and crizotinib has been approved for ROS1-positive NSCLC. Currently, there are no approved targeted therapies for treatment of *NTRK1*-, *NTRK2*-, or *NTRK3* rearrangement-positive NSCLC, or for *NTRK*-, *ROS1*-, or *ALK* rearrangement-positive cancers outside of NSCLC, and thus, there is an unmet medical need for these patients.

Entrectinib has demonstrated compelling antitumor activity, with acceptable tolerability, in adult patients with locally advanced or metastatic solid tumors whose tumors have an *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangement who are currently enrolled in two ongoing Phase 1 clinical studies (ALKA-372-001 and RXDX-101-01 [STARTRK-1]). As of 7 March 2016, a total of 119 patients have been treated with entrectinib in these studies (54 patients in ALKA-372-001 and 65 patients in STARTRK-1). The most common (>10% incidence) treatment-related adverse events were fatigue/asthenia, dysgeusia, paresthesia, nausea, myalgia, diarrhea, dizziness, arthralgia, vomiting, and constipation; importantly, there was no evidence of cumulative toxicity. Among 24 ALK- or ROS1-inhibitor-naïve patients (1 patient was intolerant to a prior ALK-inhibitor) with an extracranial tumor harboring an *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangement who were treated with entrectinib at or above the recommended Phase 2 dose (RP2D), preliminary response rates of 100% (3 responses/3 patients), 86% (12 responses/14 patients), and 57% (4 responses/7 patients) were observed in the *NTRK1/2/3*, *ROS1*, and *ALK* subgroups, respectively, for an overall objective response rate of 79% (19 confirmed RECIST responses/24 patients). In addition, one patient with a primary brain tumor (astrocytoma) harboring an *NTRK1* gene rearrangement demonstrated 45% tumor regression by volumetric assessment.[1] Furthermore, responses were observed in patients with diverse tumor types: NSCLC (14 patients), metastatic CRC (mCRC) (2 patients), mammary analog secretory carcinoma (MASC) (1 patient), melanoma (1 patient), and renal cell carcinoma (RCC) (1 patient).

Considering the potentially favorable preliminary benefit-risk profile for entrectinib (see Section 5.2) combined with the low incidence of *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangements and their presence across a variety of tumor types, Ignyta is conducting a global Phase 2 basket study, RXDX-101-02 (STARTRK-2), of entrectinib as treatment of adult patients with any solid tumor that harbors an *NTRK1/2/3*, *ROS1*, or *ALK* gene

rearrangement. Patients are being enrolled across multiple solid tumor histology × gene rearrangement "baskets" that will be individually analyzed as separate cohorts. Study STARTRK-2 is intended to demonstrate efficacy and safety of entrectinib in adult patients with solid tumors harboring the target gene rearrangements, including but not limited to (*NTRK1/2/3*- or *ROS1*-rearranged) NSCLC, (*NTRK1/2/3*-, *ROS1*-, or *ALK*-rearranged) metastatic colorectal cancer (mCRC), (*NTRK1/2/3*-rearranged) salivary gland cancer, (*NTRK1/2/3*-rearranged) thyroid cancer, (*ALK*-rearranged) breast cancer, (*ALK*-rearranged) renal cell cancer, (*NTRK1/2/3*- or *ROS1*-rearranged) cholangiocarcinoma, (*NTRK1/2/3* or *ROS1*-rearranged) malignant melanoma, (*NTRK1/2/3* or *ALK*-rearranged) sarcoma, and (*NTRK1/2/3*- or *ROS1*-rearranged) malignant brain tumors. The STARTRK-2 study is currently enrolling in the US and is being activated in an additional 15 countries in the EU and Asia-Pacific region.

In view of the therapeutic potential of entrectinib in the pediatric population with neuroblastoma and the pediatric population with solid tumors harboring the target gene rearrangements (Section 4.1.3), Ignyta is conducting a Phase 1 study RXDX-101-03 in patients age ≥ 2 years and < 22 years with relapsed or refractory extracranial solid tumors (neuroblastoma and non-neuroblastoma) and primary CNS tumors (Section 6.4). The primary objectives are to determine the maximum tolerated dose (MTD), or RP2D of entrectinib in pediatric patients with extracranial solid tumors (Part A); and to determine the MTD or RP2D of entrectinib in pediatric patients with primary CNS tumors (Part B). Secondary objectives are to describe the safety profile; to characterize the pharmacokinetics of entrectinib; and to estimate the tumor response rate in all enrolled patients (Parts A and B), patients with neuroblastoma (Part C) or other non-neuroblastoma, extracranial solid tumors harboring an *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangement (Part D), respectively. The study is currently enrolling at 4 sites in the US.

Ignyta proposes that the ongoing study RXDX-101-03 (Parts A, B, C and D) should be sufficient for a Written Request (Section 8). This study in up to 81 patients with select solid tumors, including those with neuroblastoma and those with non-neuroblastoma solid tumors harboring an *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangement, will provide a robust assessment of the pharmacokinetic (PK), safety and clinical activity of entrectinib in the pediatric population. The results should adequately inform physicians of the safety and activity of entrectinib in children.

2 DESCRIPTION OF THE MOLECULE AND RATIONALE FOR DEVELOPMENT

2.1 Mechanism of Action

Entrectinib is a potent inhibitor of the tyrosine kinases TrkA (encoded by the gene *NTRK1*), TrkB (encoded by the gene *NTRK2*), TrkC (encoded by the gene *NTRK3*), ROS1 (encoded by the gene *ROS1*), and ALK (encoded by the gene *ALK*), with median IC_{50} values for kinase inhibition in the low nanomolar range (1.7, 0.1, 0.1, 0.2, and 1.6 nM, respectively). These kinases are overexpressed or dysregulated in cancer with constitutive activity, making the growth of the cancer cells dependent on or "addicted" to the abnormal kinases. Molecular alterations in kinases (inclusive of rearrangements/fusions; point mutations; deletions and

insertions; splice variants; and variable expression) are found in many types of cancer.[2] Therefore, these kinases represent attractive targets for anticancer therapy.
Gene rearrangements in the target kinases result in activation and dysregulation of the gene's expression and signaling (Figure 1 and Figure 2 for the TrkA/B/C and ROS1, respectively), which can contribute to increased cell proliferation and survival in tumors expressing these proteins. While these enzymes play various roles in normal cellular function, gene rearrangements in these target kinases have the potential to be oncogenic drivers, tend to be mutually exclusive, and are present in small percentages (< 10%)[2, 3] in a variety of tumor types, including NSCLC, CRC, mammary analog secretory carcinoma (MASC), papillary thyroid cancer, melanoma, and sarcoma. As such, the entrectinib clinical development program is evaluating the safety and effectiveness of entrectinib in patients positive for any of the target molecular alterations, irrespective of tumor histology.

Figure 1 TrkA, TrkB, and TrkC Common Downstream Signaling Pathways

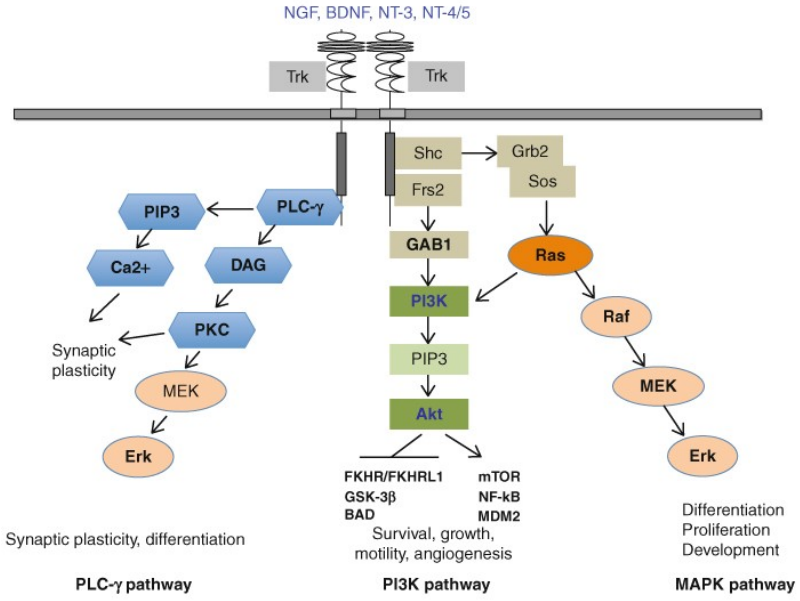
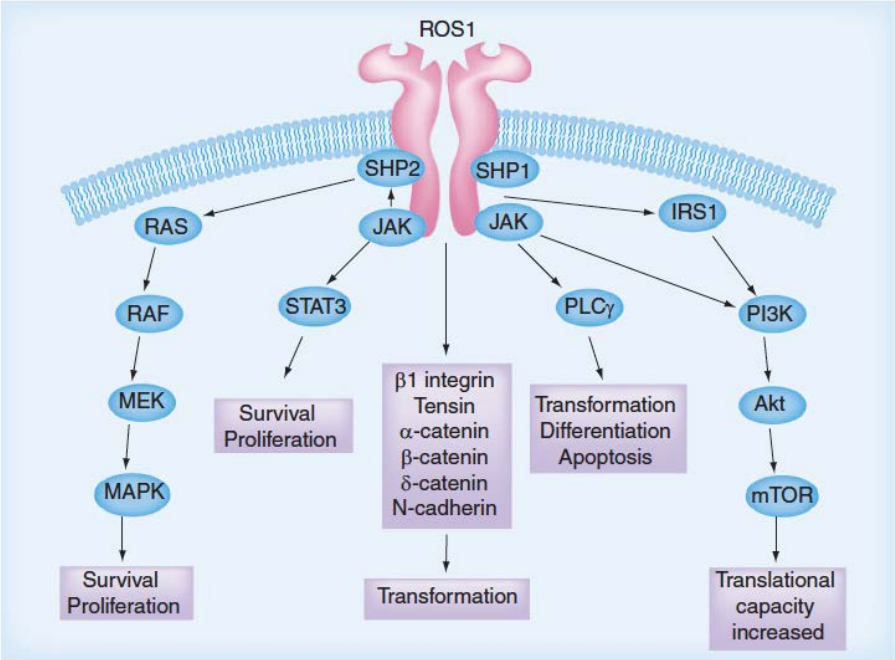


Figure 2 Signaling Pathways Transduced by ROS1



Source: Ou et al., 2012.[4]

2.2 Rationale for Development in Select Adult Indications

As described above, the entrectinib clinical development program is evaluating the safety and effectiveness of entrectinib in patients positive for any of the target (*NTRK1/2/3*, *ROS1*, *ALK*) gene rearrangements, irrespective of tumor histology. Below is outlined the background and rationale for entrectinib therapeutic potential in a few select diseases being studied in the ongoing clinical trials (Section 5).

2.2.1 Rationale for Development in NSCLC

Lung cancer is one of the most common cancers in adult men and women and the leading cause of cancer-related death in developed countries, with a total of 626,600 deaths in 2012.[5] Lung cancer has one of the highest rates of genetic aberrations in cancer and these aberrations have led to molecularly distinct diseases. The frequencies of *NTRK1/2/3* and *ROS1* rearrangements in NSCLC patients are estimated at 1-2% for *NTRK1/2/3* combined and 1-3% for *ROS1*[6]; these frequencies are approximately 5-fold lower than *ALK* rearrangements.

Targeted therapies offer both efficacy and safety advantages over general chemotherapy in the treatment of NSCLC. Clinical studies have been conducted that compared the effectiveness and safety of the orally administered epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) against chemotherapy in NSCLC patients who have tumors that harbor *EGFR* molecular alterations.[7, 8, 9] In these studies, response rates were considerably higher, progression-free survival (PFS) was prolonged, and quality of life parameters were substantially superior with the targeted therapies. Similarly, the ALK TKI, crizotinib, had a

PFS of 19.2 months, a 72% response rate, and a duration of response of 17.6 months in *ROS1*-rearranged NSCLC.[10]

The promising findings with these targeted therapies have resulted in a bifurcation of the treatment approach in NSCLC; first-line therapy is now dependent on whether a relevant molecular driver has been identified. Clinical practice guidelines recommend that those NSCLC patients with advanced tumors that harbor the relevant molecular alteration first be treated with a corresponding EGFR or ALK inhibitor, while those who do not should receive four to six treatment cycles of platinum-based doublet chemotherapy.[11] Chemotherapy usually includes cisplatin or carboplatin paired with another cytotoxic agent such as pemetrexed, gemcitabine, or paclitaxel. Treating beyond four to six cycles of chemotherapy has not been shown to improve overall survival (OS) and few patients can tolerate longer treatment durations.

Currently, there are no approved targeted therapies for *NTRK1/2/3*-rearranged NSCLC, thus making this a life-threatening medical condition with high unmet medical need.

2.2.2 Rationale for Development in mCRC

Colorectal cancer is the third most commonly diagnosed cancer in males and the second in females, with an estimated 1.4 million cases and 693,900 deaths occurring in 2012.[5]

In the past decade, survival of metastatic colorectal cancer patients has approximately doubled. This significant improvement is mainly due to the development of new combinations of standard chemotherapy, including 5-fluorouracil, irinotecan, and oxaliplatin, and also to the introduction of new targeted therapies, such as monoclonal antibodies against EGFR or vascular endothelial growth factor (VEGF). However, the addition of such targeted therapies to standard chemotherapy regimens results in increased toxicity and treatment costs, requiring clinicians to better select patients who are likely to benefit from these novel combination treatments.

The chimeric IgG1 monoclonal antibody cetuximab has shown efficacy in irinotecan-resistant metastatic colorectal cancer expressing EGFR.[12] Cetuximab binds to EGFR with a high specificity and this binding results in activation of intracellular signaling pathways, such as the G protein K-ras, the protein kinase RAF (Ras/mitogen-activated protein kinase (MAPK) pathway), and phosphoinositide 3-kinase (PI3K/Akt pathway). Molecular alterations that activate a protein downstream of the inhibitor-targeted enzyme can greatly reduce that inhibitor's efficacy.[13] As such, it is now known that wild-type *KRAS* mCRC patients treated with cetuximab have a PFS that is twice as long as mutant *KRAS* mCRC patients.[14]

NTRK1, *ROS1*, and *ALK* alterations have been detected in metastatic CRC,[15, 16, 17] at low incidences on the order of 1-2%. Currently, there are no approved targeted therapies for *NTRK1/2/3*-, *ROS1*-, or *ALK*-rearranged mCRC, thus also making these life-threatening medical conditions with high unmet medical need.

2.2.3 Rationale for Development in Salivary Gland Cancer

Salivary gland tumors are a clinically diverse group of rare neoplasms, with an overall incidence in the Western world of approximately 2.5 cases to 3.0 cases per 100,000 per year. Malignant salivary gland neoplasms account for more than 0.5% of all malignancies and approximately 3% to 5% of all head and neck cancers.[18, 19, 20] Although exposure to ionizing radiation has been implicated as a cause of salivary gland cancer, the etiology of most salivary gland cancers cannot be determined.

Most patients with benign tumors of the major or minor salivary glands present with painless swelling of the parotid, submandibular, or the sublingual glands. Neurological signs, such as numbness or weakness caused by nerve involvement, typically indicate a malignancy.

Early-stage low-grade malignant salivary gland tumors are usually curable by adequate surgical resection alone. Large bulky tumors or high-grade tumors carry a poorer prognosis and may best be treated by surgical resection combined with postoperative radiation therapy. Unresectable or recurrent tumors may respond to chemotherapy; however, the use of chemotherapy for malignant salivary gland tumors remains the subject of clinical investigation. There are no approved systemic therapies for salivary gland cancers, making this a disease with a high unmet medical need.

NTRK3 alterations have been detected in salivary gland cancers, specifically the MASC subtype,[21, 22] at low incidences on the order of 2%. Currently, there are no approved targeted therapies for *NTRK1/2/3*-rearranged salivary gland cancer, thus also making these life-threatening medical conditions with high unmet medical need.

3 REGULATORY HISTORY

Nerviano Medical Sciences, the original sponsor for entrectinib (formerly referred to as NMS-1191372), initiated the first-in-human Phase 1 study ALKA-372-001 in Italy in October 2012. The study is currently ongoing in Italy.

Ignyta licensed entrectinib from Nerviano Medical Sciences in November 2013 and initiated clinical development of entrectinib in US with another Phase 1 study STARTRK-1 in July 2014. The Phase 1 expansion portion of STARTRK-1 is currently ongoing in US, Spain, and South Korea.

Entrectinib was granted orphan drug designation and rare pediatric disease designation for neuroblastoma by FDA's Office of Orphan Products Development on 22 December 2014. Entrectinib was also granted orphan drug designation by FDA for TrkA-positive, TrkB-positive, TrkC-positive, ROS1-positive, or ALK-positive NSCLC and TrkA-positive, TrkB-positive, TrkC-positive, ROS1-positive, or ALK-positive CRC on 03 February 2015 and 12 February 2015, respectively.

An initial Pediatric Study Plan (iPSP) was submitted to the FDA on 15 April 2016. No agreement for the iPSP has yet been reached.

A Pediatric Investigational Plan (PIP) will be submitted to the European Medicines Agency in 4Q 2016. No agreement has yet been reached with any regulatory authorities outside of the US.

Entrectinib is not currently approved for marketing in any country.

4 SUMMARY OF PRECLINICAL DATA

The nonclinical testing strategy for entrectinib has followed the ICH S9: Guidance for Industry on Nonclinical Evaluation for Anticancer Pharmaceuticals. Ignyta has determined that the overall nonclinical data package provides sufficient support for the clinical development of entrectinib in pediatric patients from ages ≥ 2 to < 22 years with advanced cancer.

4.1 Nonclinical Pharmacology

Studies were conducted *in vitro* and *in vivo* to investigate the activity, selectivity, and mechanism of action of entrectinib. A kinase screen was performed to identify the target kinases for entrectinib. The antiproliferative effects (*in vitro*) and antitumor effects (*in vivo*) were evaluated in different models dependent on *NTRK1*, *NTRK2*, *NTRK3*, *ROS1*, or *ALK* gene rearrangements or *NTRK2* or *ALK* overexpression.

Secondary pharmacodynamics studies were not conducted.

4.1.1 In Vitro Studies

4.1.1.1 Activity and Selectivity of Entrectinib In Vitro

The selectivity of entrectinib was evaluated in a kinase selectivity panel that included representative members of the tyrosine kinase and serine-threonine kinase subfamilies. Entrectinib inhibited TrkA, TrkB, TrkC, ROS1, and ALK, with IC_{50} values in the low nanomolar range (1.7, 0.1, 0.1, 0.2, and 1.6 nM, respectively).

4.1.1.2 Antiproliferative Activity In Vitro

The antiproliferative activity of entrectinib was evaluated *in vitro* in 1) BaF3 cells transformed with activating *NTRK1*, *NTRK2*, *NTRK3*, *ROS1*, or *ALK* (both wild type and point mutations responsible for crizotinib resistance) gene rearrangements (Section 4.1.1.2.1); 2) human tumor cell lines that expressed an *NTRK1* rearrangement (CRC cell line), *ALK* rearrangements (NSCLC, ALCL, and neuroblastoma cell lines), or overexpressed wild-type *NTRK2* (neuroblastoma cell line) or wild-type *ALK* (neuroblastoma cell line) (Section 4.1.1.2.2); and 3) two different patient-derived CRC cell lines, one with an *NTRK1* gene rearrangement and one with an *ALK* gene rearrangement (Section 4.1.1.2.3).

Collectively, results from these studies suggest that entrectinib may have significant antitumor effects on human tumors that are positive for *NTRK1*, *NTRK2*, *NTRK3*, *ROS1*, or *ALK* gene rearrangements; tumors that overexpress *ALK* or *NTRK2*; and tumors that have developed the

secondary *ALK* point mutations, L1196M and C1156Y, that are known to confer crizotinib resistance.

4.1.1.2.1 BaF3 Transformed Cell Lines

Entrectinib demonstrated potent antiproliferative activity on BaF3 cells dependent on the expression of *NTRK1*, *NTRK2*, *NTRK3*, *ROS1*, or *ALK* gene rearrangements for interleukin-3 independent growth, but had no effect on proliferation of the parental BaF3 cell line (IC_{50} of approximately 2.0 µM) (Table 1). In addition to the rearranged *NTRK1*, *NTRK2*, *NTRK3*, *ROS1*, or *ALK* with wild-type kinase domains, entrectinib also had antiproliferative activity on BaF3 cells expressing two of the crizotinib-resistant *ALK* point mutations (i.e., L1196M and C1156Y) and on BaF3 cells expressing LOXO-101-resistance conferring *NTRK1* mutations V573M, G667A/S/C and F589L.

Table 1 Antiproliferative Activity of Entrectinib on NTRK1-, NTRK2-, NTRK3-, ROS1-, or ALK-Transformed BaF3 Cells

Cell Line-Gene Rearrangement	IC_{50} (µM)
BaF3 parental cell line	2.0
BaF3-*LMNA-NTRK1*	0.0014
BaF3-*TPM3-NTRK1*	0.0025
BaF3-*ETV6-NTRK1*	0.0025
BaF3-*AFAP1-NTRK2*	0.0027
BaF3-*VCL-NTRK2*	0.0043
BaF3-*ETV6-NTRK2*	0.0045
BaF3-*ETV6-NTRK3*	0.0045
BaF3-*TEL-ROS1*	0.005
BaF3-*LMNA-NTRK1 V573M*	0.0242
BaF3-*LMNA-NTRK1 F589L*	0.0097
BaF3-*LMNA-NTRK1 G667A*	0.0051
BaF3-*LMNA-NTRK1 G667S*	0.0146
BaF3-*LMNA-NTRK1 G667C*	0.0690
BaF3-*TEL-ALK* wild-type	0.173
BaF3-*TEL-ALK L1196M*	0.405
BaF3-*TEL-ALK* C1156Y	0.326
BaF3-*TEL-ALK F1174L*	1.174

IC_{50}=median inhibitory concentration.

4.1.1.2.2 Human Tumor Cell Lines

Entrectinib was tested for antiproliferative activity *in vitro* following 72 hours of continuous exposure to a panel of 154 human tumor cell lines and 6 normal cell lines. As summarized below, antiproliferative activity was seen only against cell lines that expressed either *NTRK1* or *ALK* gene rearrangements; human tumor cell lines without these gene rearrangements were insensitive to entrectinib.

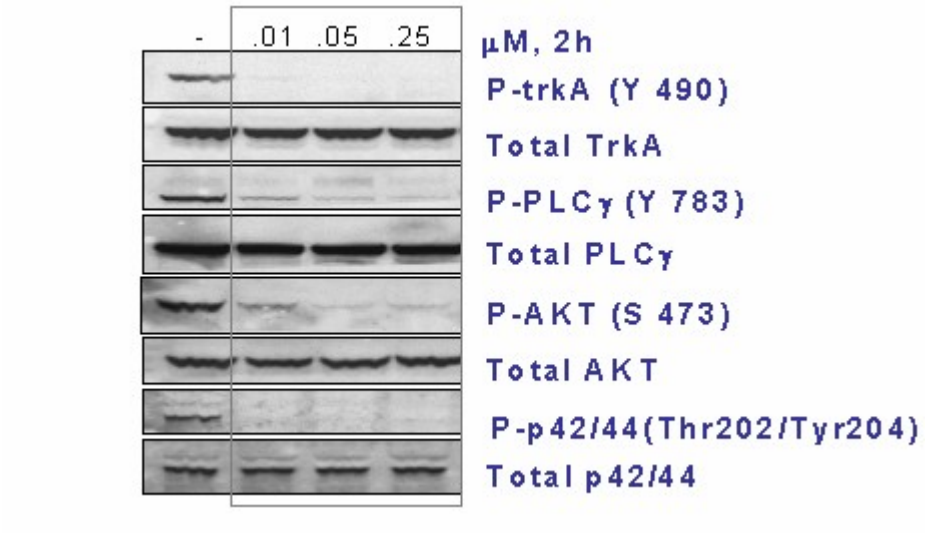
In the CRC cell line KM12, which expresses the *TPM3-NTRK1* gene rearrangement, entrectinib inhibited cell proliferation with an IC_{50} value of 17 nM, and completely inhibited the phosphorylation of TrkA at concentrations of 0.01 µM and higher. Concomitant inhibition of the phosphorylation of key downstream transducers of TrkA signaling, including PLC-γ, AKT, and p42/44 (MAPKs), was also seen (Table 2 and Figure 3, respectively). Cell cycle distribution analysis of KM12 cells treated with entrectinib doses of 10, 50, and 250 nM for 24, 48, and 72 hours was also evaluated by flow cytometry. All entrectinib doses induced a G1 arrest at 24 hours and an increase in subG1 DNA content at 48 hours, consistent with cell death from apoptosis induction.

Table 2 Antiproliferative Activity of Entrectinib in Various Cell Lines

Cell Line	Cancer Type	IC_{50} (µM)
KM12	adenocarcinoma colon *TPM3-NTRK1*+	0.017
SU-DHL-1	anaplastic large cell lymphoma *NPM1-ALK*+	0.020
KARPAS-299	anaplastic large cell lymphoma *NPM1-ALK*+	0.031
SUP-M2	anaplastic large cell lymphoma *NPM1-ALK*+	0.041
NCI-H2228	non-small cell lung cancer *EML4-ALK*+	0.068
SR-786	anaplastic large cell lymphoma *NPM1-ALK*+	0.081
MV-4-11	biphenotypic B myelomonocytic leukemia	0.081
MOLM-13	acute myeloid leukemia	0.237
IGROV-1	lymphoma	0.352
SK-N-MC	neuroblastoma	0.424
KU812	acute myeloid leukemia	0.471
SET-2	thrombocythemia leukemic	0.477
Saos-2	osteosarcoma	0.571
SH-SY5Y	neuroblastoma	0.615
KG-1	acute myeloid leukemia	0.626
SW-612	adenocarcinoma colon	0.868
SN12C	adenocarcinoma kidney	0.884
EVSA-T	adenocarcinoma mammary	0.960

IC_{50}=median inhibitory concentration.

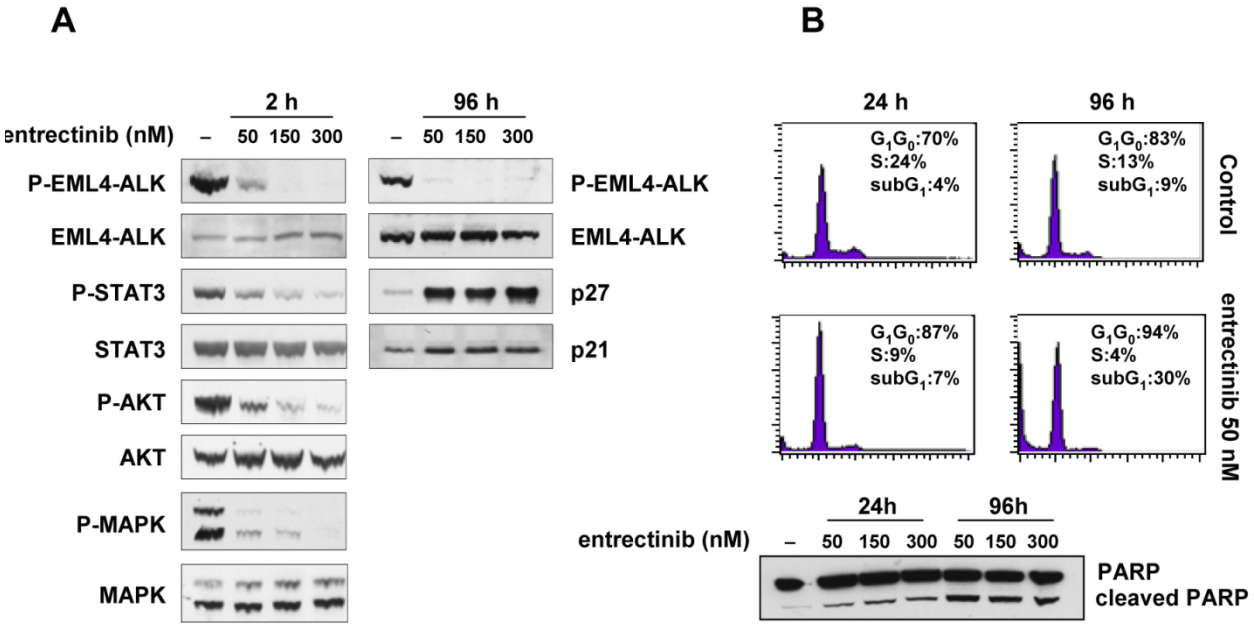
Figure 3 Phosphorylation of TrkA and Downstream Transducers of TrkA Signaling in KM12 Cells (*TPM3-NTRK1* Gene Rearrangement-Positive CRC Line)



High antiproliferative activity was also seen with entrectinib in cell lines known to express constitutively activated forms of ALK, such as the NSCLC cell line; NCI-H2228, which expresses the *EML4-ALK* gene rearrangement (IC_{50} = 0.068 µM); and four different ALCL cell lines that express *NPM1-ALK* gene rearrangements, including Karpas-299 (IC_{50} = 0.031 µM), SR-786 (IC_{50} = 0.081 µM), SU-DHL-1 (IC_{50} = 0.020 µM), and SUP-M2 (IC_{50} = 0.041 µM) (Table 2).

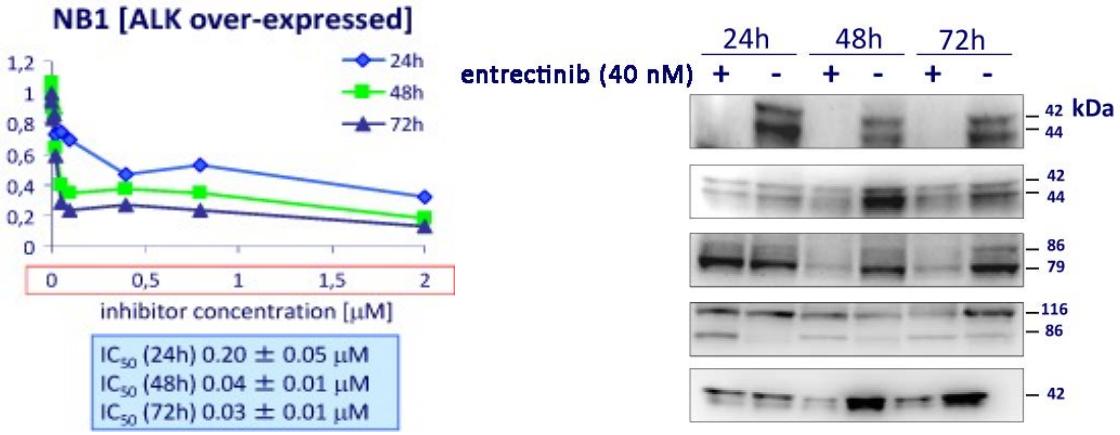
Additionally, entrectinib induced dose-related inhibition of ALK phosphorylation and the phosphorylation of one of its main downstream signal transducers, STAT3, in NCI-H2228 cells (Figure 4, Panel A), and the four ALCL cell lines with ALK rearrangements (NMS Study N-0026079; NMS Study N-0026076). Dose-related inhibition of the phosphorylation of MAPK and AKT was also seen in NCI-H2228 cells after 2 hours of entrectinib treatment (Figure 4, Panel A). Additionally, a G1 block was seen in NCI-H2228 cells after 24 hours of entrectinib treatment, and a subG1 DNA content was seen after 96 hours of entrectinib treatment with concomitant increases in markers of apoptosis, including cyclin D1, p27, and p21 levels and PARP cleavage (Figure 4, Panel B). In Karpas-299 cells that were treated with entrectinib for 48 hours, ALK and STAT3 phosphorylation remained completely inhibited 6 days after entrectinib removal. An accumulation of cells in G1 and decreased expression of the anti-apoptotic markers, survivin and Mcl-1, were also seen in entrectinib-treated Karpas-299 cells (data not shown).

Figure 4 NSCLC Cell Line NCI-H2228



The antiproliferative activity of entrectinib was also assessed in an *ALK*-overexpressing neuroblastoma cell line, NB1, exposed to varying concentrations of entrectinib for 24, 48, and 72 hours. NB1 cells were highly sensitive to entrectinib treatment with calculated IC_{50} values of 30 nM (72 hours), 40 nM (48 hours), and 200 nM (24 hours) (Figure 5, left panel). Entrectinib treatment (40 nM) of NB1 cells led to significant down-regulation of two of the main ALK kinase pathways, ERK1/2 and STAT3, and increased apoptosis as indicated by PARP cleavage (Figure 5, right panel). In an *NTRK2*-overexpressing neuroblastoma cell line, SY5Y-TrkB, entrectinib was also shown to dose-dependently inhibit phosphorylation of TrkB and its downstream signal transducers, including MAPK and ERK.

Figure 5 Effects of Entrectinib on *ALK*-overexpressing Neuroblastoma Cell Line NB1



Note: IC_{50} values are the means from triplicate experiments.

4.1.1.2.3 Patient-Derived Cell Lines

The antiproliferative activity of entrectinib was tested in two patient-derived CRC cell models, one with a *TPM3-NTRK1* gene rearrangement and one with an *EML4-ALK* gene rearrangement. Both cell lines were KRAS and BRAF wild-type.

The antiproliferative effect of 1 µM entrectinib or vemurafenib was examined using the *TPM3-NTRK1* rearrangement CRC model. The antiproliferative IC_{50} was 0.0082 µM for entrectinib; vemurafenib did not show a significant antiproliferative effect even at a concentration of 10 µM (Figure 6). Entrectinib potently inhibited phosphorylation of TrkA and key downstream transducers of the signaling pathway, including PLC-γ and AKT (Figure 7).

The antiproliferative effect of 1 µM entrectinib or crizotinib was examined using the *EML4-ALK* rearrangement cell model (data not shown). In this model, both entrectinib and crizotinib significantly inhibited cell proliferation compared with control ($p<0.0001$ for entrectinib and crizotinib). Entrectinib had significantly greater ($p<0.0001$) antiproliferative activity compared with crizotinib.

Figure 6 Antiproliferative Effects of Entrectinib and Vemurafenib in a Patient-Derived CRC Cell Model with an Activating *TPM3-NTRK1* Gene Rearrangement

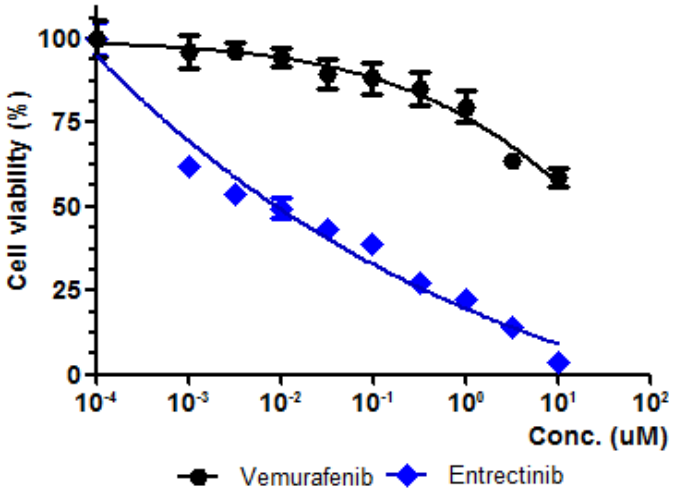
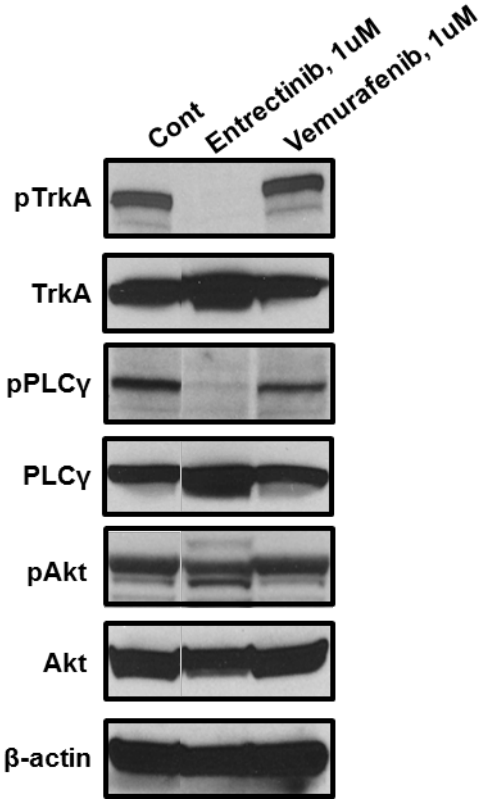


Figure 7 Effect of Entrectinib and Vemurafenib on TrkA Phosphorylation and Downstream Signaling Pathways in a Patient-Derived CRC Cell Line with an Activating *NTRK1* Rearrangement



4.1.2 In Vivo Studies

The antitumor activity of entrectinib was assessed in mouse xenograft models of CRC (*NTRK1* gene rearrangement), neuroblastoma (*NTRK2* overexpression), NSCLC (*ALK* gene rearrangement), and ALCL (*ALK* gene rearrangement) and an allograft model with a *ROS1* gene rearrangement.

4.1.2.1 NTRK- and ROS1-Dependent Models

The *in vivo* activity of entrectinib was assessed in a patient-derived CRC xenograft model with an *LMNA-NTRK1* gene rearrangement, a cell line-derived CRC xenograft model, KM12 with a *TPM3-NTRK1* gene rearrangement, and an *NTRK2*-overexpressing neuroblastoma xenograft established with SY5Y-TrkB cells. Collectively, the data from these studies demonstrate that entrectinib induces tumor regression in tumors driven by the constitutive activation of TrkA or TrkB.

In the patient-derived CRC xenograft, groups (n=10 per group) of mice were treated with 15 mg/kg entrectinib, 60 mg/kg entrectinib, or vehicle control once daily (QD) for 5 weeks in a 4-day on/3-day off schedule, with treatment started when the average tumor size was 400 mm^3. Statistically significant tumor growth inhibition (TGI) was seen in both entrectinib dose groups ($p<0.001$) compared with the vehicle control group.

In the KM12 CRC xenograft model, groups of mice (n=10 per group) were treated with entrectinib doses of 15, 30, or 60 mg/kg or vehicle control twice daily (BID) either on a continuously daily dosing basis for 21 days, or daily for 4 consecutive days followed by 3 days off treatment for a total of 3 cycles. Maximal tumor inhibition was achieved with all three entrectinib doses and both dose schedules (Table 3). Maximum tumor inhibition was 96% and 95% at Day 20 for the continuous and intermittent dosing schedules, respectively.

In the *NTRK2*-overexpressing SY5Y neuroblastoma xenograft model, groups of mice (n=10 per group) were treated with vehicle, 60 mg entrectinib alone (BID), chemotherapy (irinotecan 0.63 mg/kg/day and temozolomide 7.5 mg/kg/day) alone, and entrectinib in combination with chemotherapy. Entrectinib demonstrated significant TGI and a survival benefit as a single agent. When combined with an irinotecan/temozolomide regimen, entrectinib demonstrated a superior benefit compared with chemotherapy regimen alone (Table 3). Entrectinib, either as single agent or in combination with chemotherapeutic agents, was well tolerated.

The *in vivo* activity of entrectinib was assessed in an allograft tumor model established by subcutaneous injection of BaF3 cells expressing an *ETV6-ROS1* gene rearrangement in female severe combined immunodeficiency mice. Groups of mice (6 per group) were treated with either vehicle control or 60 mg/kg entrectinib BID for 10 days with treatment initiated when mean tumor was approximately 0.14 cm^3. Complete regression was observed in all entrectinib-treated mice (Table 3).

Table 3 Summary of Entrectinib Activity in *NTRK*- and *ROS1*-Dependent Models

Model	Entrectinib Treatment	Efficacy
LMNA-NTRK1 rearrangement-dependent patient-derived xenograft model (in NOD-SCID mice)	15 mg/kg QD PO × 5 weeks (4 days on, 3 days off per week cycle)	Regression
	60 mg/kg QD PO × 5 weeks (4 days on, 3 days off per week cycle)	Regression
TPM3-NTRK1 rearrangement-dependent cell line-derived xenograft model (KM12 in nude mice)	15 mg/kg BID PO × 21 days (continuous)	96% TGI (day 20)
	30 mg/kg BID PO × 21 days (continuous)	96% TGI (day 20)
	60 mg/kg BID PO × 21 days (continuous)	96% TGI (day 20)
	15 mg/kg BID PO × 21 days (4 days on, 3 days off per week cycle)	95% TGI (day 20)
	30 mg/kg BID PO × 21 days (4 days on, 3 days off per week cycle)	95% TGI (day 20)
	60 mg/kg BID PO × 21 days (4 days on, 3 days off per week cycle)	95% TGI (day 20)
NTRK2 overexpression-dependent cell line-derived xenograft model (SY5Y-TrkB in nude mice)	60 mg/kg BID PO × 5 weeks	65% TGI (day 26)
	60 mg/kg BID PO × 5 weeks+ I/T QD, PO, 5×/week for 5 weeks	81% TGI (day 26)
	60 mg/kg BID PO × 5 weeks+ I/T QD, PO, 5×/week for weeks 1, 3 and 5	82% TGI (day 26)
ETV6-ROS1 rearrangement-dependent allograft model (BaF3 transformed cells in SCID mice)	60 mg/kg BID PO × 10 days	Regression

NOD-SCID=non-obese diabetic-severe combined immunodeficiency; PO=orally; QD=once daily; TGI (tumor growth inhibition) = (1-T/C) × 100%; where T is the average tumor volume of drug-treated tumors, C is the average tumor volume of vehicle-treated tumors; I/T=irinotecan/temozolomide.

4.1.2.2 ALK Gene Rearrangement-Dependent Models

Entrectinib caused dose-dependent TGI in a variety of *ALK* gene rearrangement-positive models, including allografts expressing crizotinib-resistant *ALK* mutations, with doses of ≥ 15 mg/kg BID resulting in significant antitumor activity (Table 4). Additionally, in the Karpas-299 xenograft model, which expresses an *NPM1-ALK* gene rearrangement, 30 mg/kg and 60 mg/kg entrectinib resulted in complete inhibition of phosphorylation of ALK and STAT3, which is ALK's major downstream signal transducer in ALCL.

Table 4 Summary of Entrectinib Activity in *ALK* Rearrangement-Positive Models

Model	Entrectinib Treatment	% TGI
EML4-ALK rearrangement-dependent xenograft (NCI-H2228-bearing Balb Nu/Nu mice)	15 mg/kg BID PO × 10 days	87%
	30 mg/kg BID PO × 10 days	98%
	60 mg/kg BID PO × 10 days	98%
NPM1-ALK rearrangement-dependent xenograft (Karpas-299 bearing SCID mice)	10 mg/kg BID IV × 5 days	43%
	30 mg/kg BID PO × 10 days	94%
	60 mg/kg BID PO × 10 days	99%
	80 mg/kg BID PO × 10 days	100%
	120 mg/kg BID PO × 10 days	100%
	30 mg/kg BID PO × 10 days	92%
	30 mg/kg BID PO × 20 days	92%
	50 mg/kg BID PO × 20 days	84%
	60 mg/kg BID PO × 20 days	100%
	240 mg/kg QD PO in 3-days on, 7-days off schedule × 5 cycles	99%[a]
NPM1-ALK rearrangement-dependent xenograft (SR-786-bearing SCID mice)	15 mg/kg BID PO × 10 days	67%
	30 mg/kg BID PO × 10 days	99%
TEL-ALK wild-type rearrangement-dependent allograft (BaF3 transformed cells in SCID mice)	120 mg/kg BID PO × 10 days	97%
BaF3 *TEL-ALK L1196M* (BaF3 transformed cells in SCID mice)[b]	120 mg/kg BID PO × 10 days	83%
BaF3 *TEL-ALK* C1156Y (BaF3 transformed cells in SCID mice)[b]	120 mg/kg BID PO × 10 days	94%

[a] On Day 26 (i.e., 17 days after the first dose of entrectinib).
[b] Crizotinib-resistant point mutations.
TGI=tumor growth inhibition; SCID= severe combined immunodeficiency; BID=twice daily; PO=orally; QD=once daily.

4.1.2.3 Intracranial Model

Because NSCLC and other solid tumor patients frequently develop brain metastases or because certain primary brain malignancies may harbor the target gene rearrangements, entrectinib was also tested in an experimental model of intracranial tumor growth. The NSCLC tumor cell line, NCI-H2228 (*EML4-ALK* gene rearrangement), was intracranially injected in male Balb Nu/Nu mice. Groups of mice (6 to 7 animals per group) were treated with either vehicle control, 60 mg/kg entrectinib, or 120 mg/kg entrectinib BID by oral gavage for 10 days, with treatment initiated when mean tumor volume for all groups was between 0.015 and 0.018 cm^3. Both doses of entrectinib inhibited tumor growth. Additionally, there was a dose-dependent increase in survival time, with a median survival time of 31, 36,

and 43 days for the control, 60 mg/kg entrectinib, and 120 mg/kg entrectinib groups, respectively.

4.1.3 In Vitro and In Vivo Studies in Pediatric Models

Entrectinib has been tested for its ability to inhibit TrkB autophosphorylation in response to its ligand, brain-derived neurotrophic factor (BDNF, Figure 8). *In vitro*, no cytotoxicity was seen in Trk-nonexpressing neuroblastoma cells at doses up to 200 nM, whereas inhibition of growth was seen in TrkB-expressing neuroblastoma cells at 10-20 nM (Figure 9).

Entrectinib has also been tested to determine inhibition of growth of TrkB-expressing neuroblastoma cells growing as xenografts in a nude mouse model. Entrectinib was compared to vehicle as a negative control and lestaurtinib (CEP-701) as a positive control. Entrectinib exhibited significantly greater tumor control when compared to lestaurtinib in the mouse xenograft model of neuroblastoma (Figure 10). Based on life-table analysis, entrectinib was significantly better than control ($p < 0.0001$) and lestaurtinib ($p = 0.0069$) (Figure 11), with all entrectinib treated animals surviving for at least 4 weeks, all control animals had to be sacrificed by 18 days, and more than half the lestaurtinib-treated animals were sacrificed by 21 days. There was no apparent toxicity of entrectinib during or after therapy, including by behavior, weight, blood counts or other measures. These results suggest that entrectinib is a very potent and selective inhibitor of Trk-expressing tumors.

Figure 8 Inhibition of Trk Phosphorylation and Downstream Signaling by Entrectinib in Neuroblastoma Cell Line SY5Y-TrkB

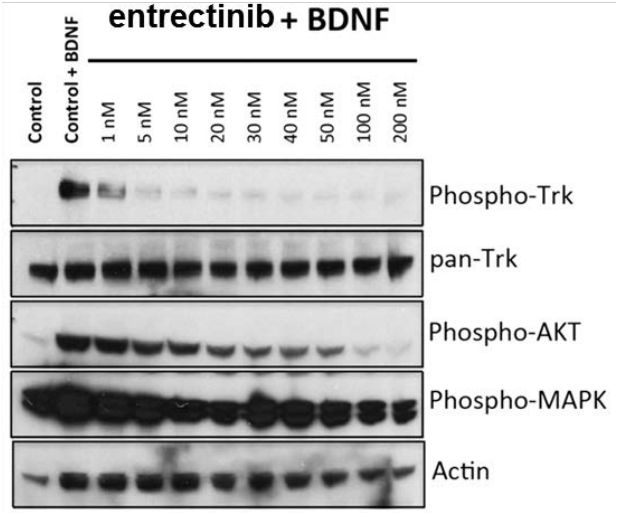


Figure 9 Inhibition of Cellular Proliferation by Entrectinib in Neuroblastoma Cell Line SY5Y-TrkB

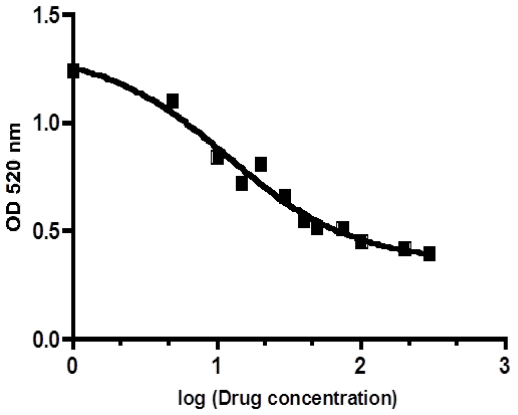


Figure 10 Inhibition of *In Vivo* Tumor Growth by Entrectinib and CEP-701 in Neuroblastoma Cell Line SY5Y-TrkB

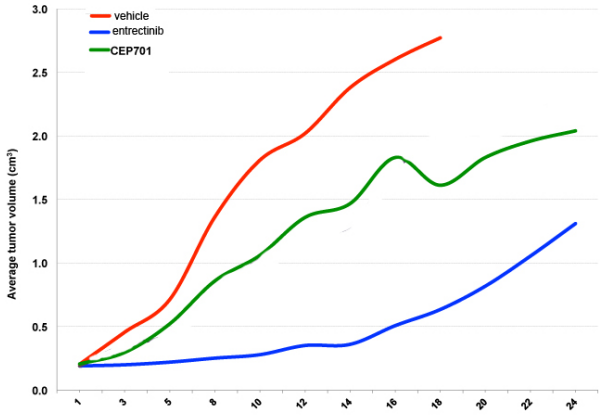
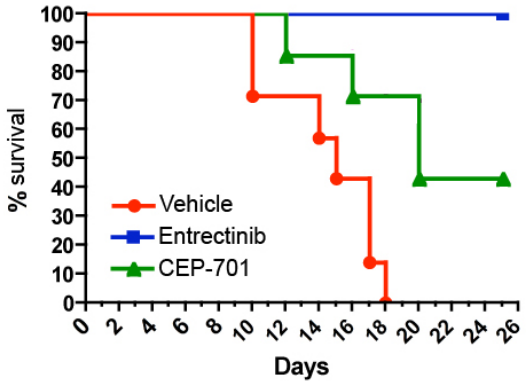


Figure 11 Improvement of *In Vivo* Host Survival by Entrectinib and CEP-701 in Neuroblastoma Cell Line SY5Y-TrkB



4.2 Nonclinical Drug Metabolism and Pharmacokinetics

Entrectinib was readily absorbed in mice, rats, and dogs following single dose and exhibited dose-dependent exposure increase following multiple doses in rats and dogs without apparent accumulation.

Entrectinib is highly protein bound and distributed to multiple tissues, including the brain. Concentration of entrectinib was detected in brain homogenates in mice, rats, and dogs given either single or multiple oral doses, suggesting that entrectinib crossed the blood/brain barrier.

The absorption, distribution, metabolism, and excretion (ADME) profiles of entrectinib in animals have supported clinical trials of entrectinib in patients.

4.3 Nonclinical Safety

In vivo safety pharmacology studies in rats and dogs did not reveal biologically adverse effects in central nervous system (CNS), respiratory or cardiovascular systems at entrectinib plasma concentrations exceeding the anticipated efficacious concentration. However, CNS signs (rats and dogs) and QT/QTc prolongation (dogs) were observed in repeat dose toxicity studies.

Entrectinib was concluded to be nongenotoxic. Entrectinib was not irritating to the skin, but induced transient and reversible irritant reactions to eyes. An *in vitro* phototoxicity study indicated phototoxic potential of entrectinib.

Repeat-dose cycling studies (intermittent or continuous daily dosing) in rats and dogs produced dose-limiting and systemic toxicities similar to those reported for other TKIs and are consistent with the pharmacology of entrectinib. Target organ toxicities were observed in skin, gastrointestinal tract, CNS, ECG, liver and hemolymphopoietic system. CNS clinical signs (incoordination, staggering, abnormal gait, tremors, hypoactivity, and depression) were associated with appreciable entrectinib levels present in the brain of both species without any histopathological findings. In dogs, ECG evaluations showed slight to moderate QT/QTc prolongation following repeat dosing. The CNS effect and QT/QTc prolongation occurred at

plasma concentrations above the anticipated efficacious concentration in patients. All observed effects exhibited reversibility. Findings identified at exposures that approximate the anticipated therapeutic range are considered clinically manageable (e.g., skin, diarrhea), monitorable (e.g., CNS, hematological parameters, liver function, ECG) and reversible.

5 CLINICAL DEVELOPMENT IN ADULTS

5.1 Overview

Four clinical studies are ongoing (Table 5): Phase 1 Study ALKA-372-001 at two sites in Italy; Phase 1 Study RXDX-101-01 (STARTRK-1) at eight sites in the US, one site in Spain, and one site in South Korea; Phase 2 Study RXDX-101-02 (STARTRK-2) at thirty-six sites in the US; and Phase 1/1b Study RXDX-101-03 (pediatric study) at four sites in the US.

Table 5 Overview of Entrectinib Clinical Studies

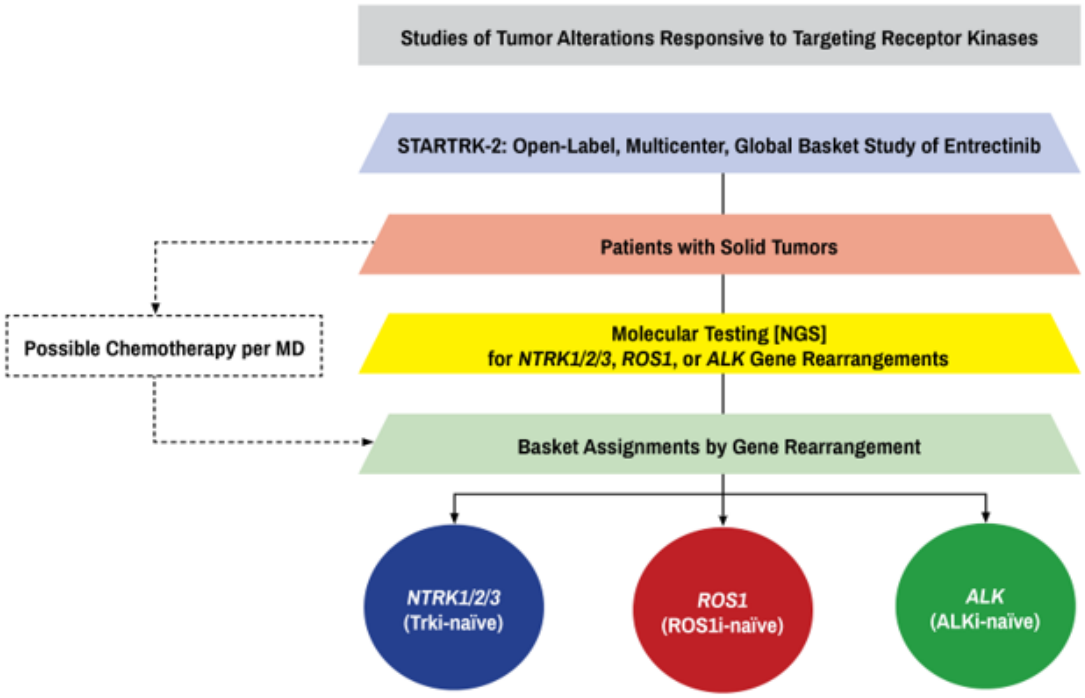
Protocol No.	Phase, Design	Patient Population	Entrectinib Regimen
ALKA-372-001	First-In-Human Phase 1, multicenter, open-label, ascending-dose study with dose escalation according to a standard 3 + 3 scheme	Advanced/metastatic solid tumors, including patients with TrkA/B/C, ROS1, or ALK molecular alterations	Schedule A: once daily (fasted) 4-days on, 3-days off schedule × 3 weeks followed by 7-day rest[a] Schedule B: continuous once daily (fed)[b] Schedule C: once daily (fed) in a continuous 4-days on, 3-days off schedule[c]
RXDX-101-01 (STARTRK-1)	Phase 1/2, multicenter, open-label, ascending-dose study with dose escalation according to a standard 3 + 3 scheme	Solid tumors with *NTRK1/2/3*, *ROS1*, or *ALK* molecular alterations	Continuous once daily (fed) on 28-day (i.e., 4-week) cycles
RXDX-101-02 (STARTRK-2)	Pivotal Phase 2, global, multicenter, open-label, basket study	Patients (≥ 18 years of age) with advanced or metastatic solid tumor that harbors an *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangement	600 mg PO, QD on 28-day (i.e., 4-week) cycles
RXDX-101-03 (see Section 6.4)	Phase 1/1b, open-label, dose escalation and expansion study	Children and adolescents (2-22 years of age) with recurrent or refractory solid tumors and primary CNS tumors	PO, QD Dosing nomogram based on BSA, ranging from 250 mg/m^2 to 750 mg/m^2

[a] Terminated at 1600 mg/m^2/day because of a plateau in entrectinib exposure above 800 mg/m^2/day.
[b] Dose escalation up to 400 mg/m^2/day, subsequently transitioned to fixed dose 600 mg/day.
[c] Terminated at 800 mg/m^2/day, the highest dose evaluated as of 1 May 2015.
PK= pharmacokinetic; RP2D= recommended Phase 2 dose (i.e., 600 mg/day), PO = oral; QD = daily; M = male; F = female.

The global Phase 2 STARTRK-2 study of entrectinib as treatment of patients with any solid tumor that harbors an *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangement is ongoing (Figure 12). Patients are being enrolled across multiple solid tumor histology × gene rearrangement "baskets" that will be individually analyzed as separate cohorts. Study STARTRK-2 is

intended to demonstrate efficacy and safety of entrectinib in adult patients with the target gene rearrangements, such as (*NTRK1/2/3*- or *ROS1*-rearranged) NSCLC, (*NTRK1/2/3*-, *ROS1*-, or *ALK*-rearranged) metastatic colorectal cancer (mCRC), (*NTRK1/2/3*-rearranged) salivary gland cancer, (*NTRK1/2/3*-rearranged) thyroid cancer, (*ALK*-rearranged) breast cancer, (*ALK*-rearranged) renal cell cancer, (*NTRK1/2/3*- or *ROS1*-rearranged) cholangiocarcinoma, (*NTRK1/2/3* or *ROS1*-rearranged) malignant melanoma, (*NTRK1/2/3* or *ALK*-rearranged) sarcoma, and (*NTRK1/2/3*- or *ROS1*-rearranged) malignant brain tumors.

Figure 12 Study STARTRK-2 Schema



5.2 Clinical Safety and Efficacy in Cancer Patients

At the data cutoff of 7 March 2016, 119 patients had been treated with entrectinib in the two Phase 1 studies (54 patients in ALKA-372-001 and 65 patients in STARTRK-1), and the two studies are both ongoing. The global Phase 2 STARTRK-2 study is currently enrolling in the US and is being activated in an additional 15 countries in the EU and Asia-Pacific region.

The two Phase 1 studies were designed to determine the MTD and/or RP2D of entrectinib in patients with advanced or metastatic solid tumors harboring *NTRK1/2/3*, *ROS1*, or *ALK* molecular alterations. The following dosing regimens were tested:

- Schedule A (ALKA-372-001 study): once daily (fasted) 4 days on, 3 days off for 3 weeks followed by 1 week of rest. Doses tested ranged from 100 mg/m^2 to 1600 mg/m^2 without dose-limiting toxicity (DLT).
- Schedule B (ALKA-372-001 study): continuous daily dosing (fed) in 4-week cycles. Doses tested ranged from 100 mg/m^2 to 400 mg/m^2 and 600 mg fixed, without DLT.

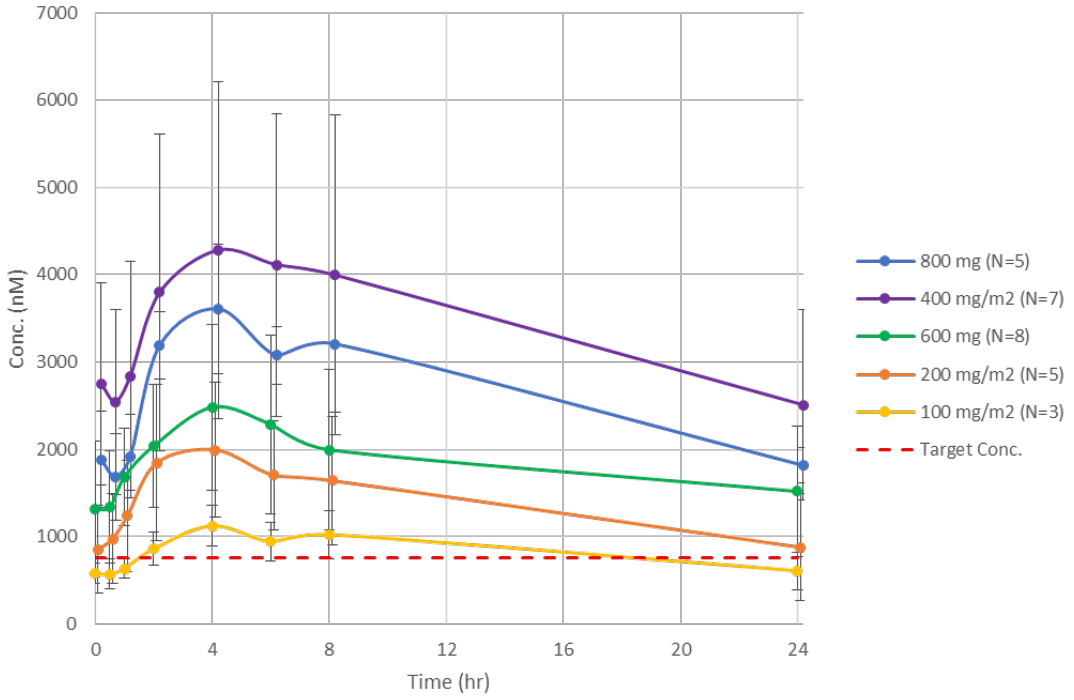
- Schedule C (ALKA-372-001 study): once daily (fed) 4 days on, 3 days off in 4-week cycles. Doses tested ranged from 400 mg/m^2 to 800 mg/m^2 without DLT.
- Continuous Daily Dosing (STARTRK-1 study): once daily (fed) in 4-week cycles. Doses tested were 100 mg/m^2, 200 mg/m^2, 400 mg/m^2, 600 mg fixed, and 800 mg fixed.

Exposures of entrectinib administered on a continuous daily dosing regimen increased in a dose-proportional manner and reached steady-state within a week of dosing, with a C_{max} of 2.7 µM and AUC_{0-24} of 50.1 µM·hr (RP2D of 600 mg/day in Study STARTRK-1). Based on accumulation, the plasma half-life was estimated to be approximately 20 to 24 hours, consistent with that observed in the healthy subjects, supporting a once daily dosing (QD) regimen.

Two DLTs were observed at the flat-fixed 800 mg/day dose: one event of Grade 3 cognitive impairment and one event of Grade 3 fatigue. Both events were reversible upon study drug interruption.

Based upon these two DLTs, 400 mg/m^2 administered once daily on a fed regimen was selected as the BSA-based RP2D. Exploratory correlations revealed that BSA and BMI were not associated with exposure variability, so a flat-fixed dose regimen was determined to be more appropriate. Subsequently, additional expansion cohorts of patients were enrolled to further explore a fixed daily dosing regimen, and 600 mg/day was selected as the RP2D for future Phase 2 studies. At both the BSA-based RP2D of 400 mg/m^2 and fixed RP2D of 600 mg, the plasma protein binding-corrected mean C_{trough} values are multiples above the concentrations required for complete tumor growth inhibition in animal models (Figure 13).

Figure 13 Steady State Entrectinib Pharmacokinetics (continuous daily dosing)

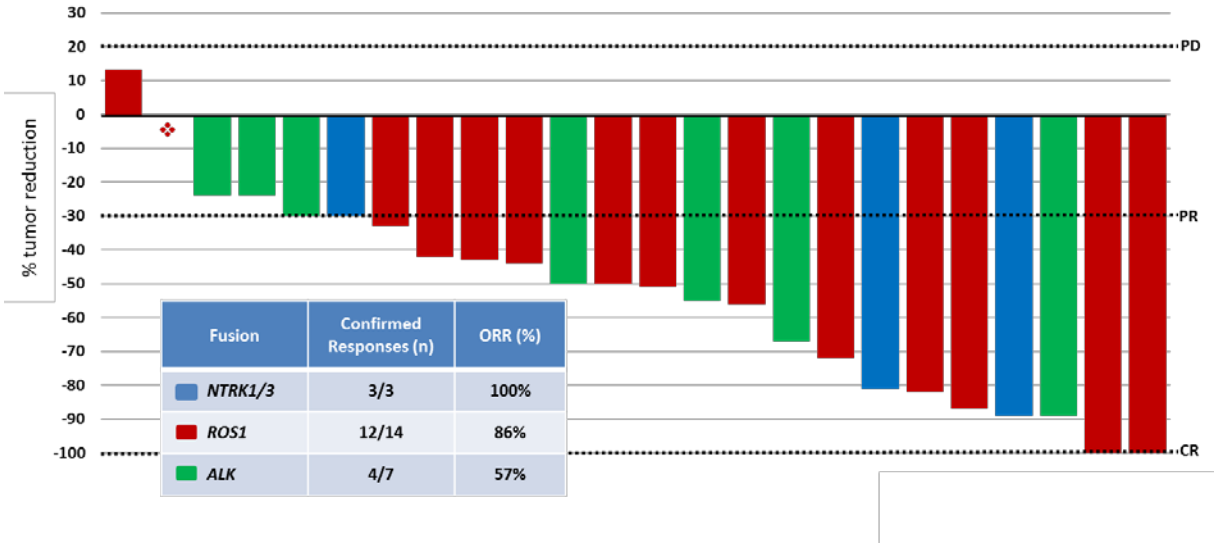


At doses ≥ RP2D, 25 patients have been treated at 400 mg/m^2, 45 patients at 600 mg, and 12 patients at 800 mg. In total, across both studies, 19 patients have been treated > 6 months: of those, 11 have been treated > 1 year, including 3 patients > 2 years.

The most common (≥ 10% incidence), treatment-related, adverse events (AEs) observed across both studies were fatigue/asthenia (44%), dysgeusia (41%), paresthesia (28%), nausea (24%), myalgia (22%), diarrhea (19%), dizziness (16%), arthralgia and vomiting (15% each), and constipation (12%); importantly, there was no evidence of cumulative toxicity, hepatic or renal toxicity, or QT prolongation. All AEs were reversible with dose medication.

Tumor assessments were performed at the end of Cycle 1 (or Cycle 2, depending on earlier protocol version) and every 8 weeks thereafter. Among 24 Trk-, ROS1-, or ALK-inhibitor-naïve patients (1 patient was ALK-inhibitor intolerant) with an extracranial tumor harboring an *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangement who were treated at or above the RP2D, preliminary response rates of 100% (3 responses/3 patients), 86% (12 responses/14 patients), and 57% (4 responses/7 patients) were observed in the *NTRK1/2/3*, *ROS1*, and *ALK* subgroups, respectively (Figure 14), for an overall objective response rate of 79% (19 confirmed RECIST responses/24 patients). In addition, one patient with a primary brain tumor (astrocytoma) harboring an *NTRK1* gene rearrangement demonstrated 45% tumor regression by volumetric assessment. Furthermore, responses were observed in patients with diverse tumor types: NSCLC (14 patients), metastatic CRC (mCRC) (2 patients), mammary analog secretory carcinoma (MASC) (1 patient), melanoma (1 patient), and renal cell carcinoma (RCC) (1 patient).

Figure 14 Antitumor Activity in ALK and ROS1 Inhibitor-naïve Patients (n=24) with Extracranial Solid Tumors Harboring *NTRK1/2/3*, *ROS1*, or *ALK* Gene Rearrangements



Refer to Appendix 1 for details of the Phase 1 data summary.

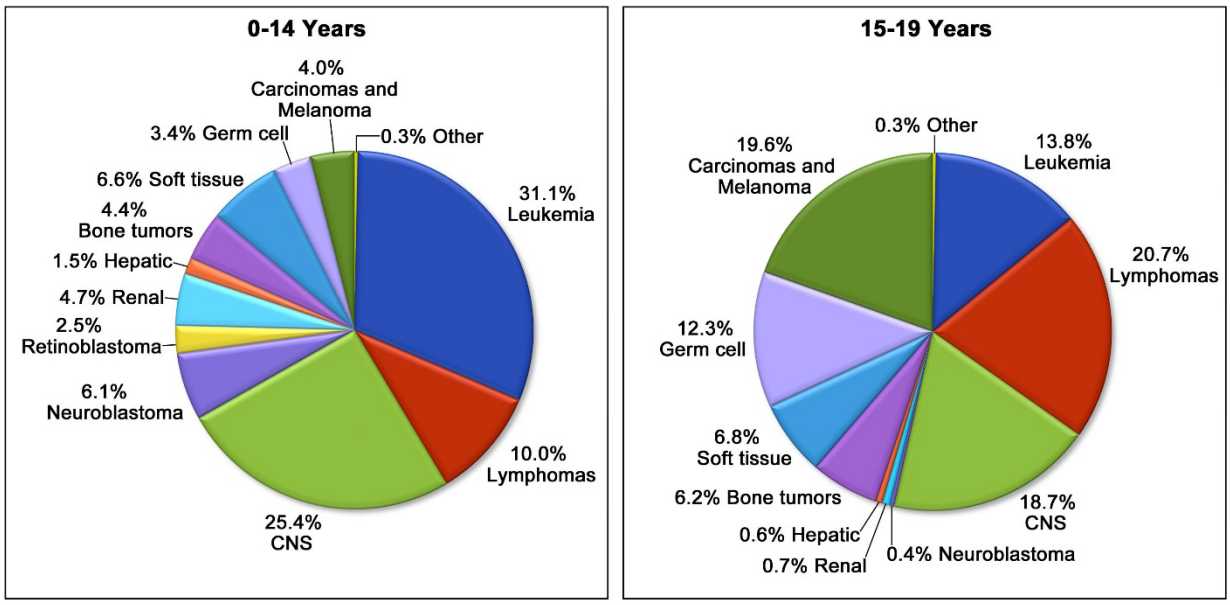
6 CLINICAL DEVELOPMENT IN PEDIATRICS

6.1 Disease State Overview – Childhood Cancer

In 2014, an estimated 15,780 United States children younger than 20 years of age were diagnosed with cancer.[23]

Childhood cancer is composed of a spectrum of various malignancies that may also differ in histology and site of disease origin. The distribution and incidence of childhood cancer varies by age, sex, and race. The Surveillance, Epidemiology and End Results (SEER) Program of the NCI conducted a 20-year study to determine the incidence of various cancer types by pediatric age groups. A summary of these results is illustrated in Figure 15.

Figure 15 Age-Adjusted and Age-Specific Cancer Incidence Rates for Patients 0-19 Years of Age (SEER 2005-2009)



Cancer incidence rates for patients aged 0 to 14 years and 15 to 19 years in the SEER program from 2005 to 2009. Incidence rates are age-adjusted and age-specific and are shown for leukemia, lymphoma, central nervous system (CNS) tumors, neuroblastoma, retinoblastoma, renal tumors, hepatic tumors, bone tumors, soft tissue tumors, germ cell tumors, carcinomas and melanomas, and other cancers. Retinoblastoma occurs infrequently in adolescents aged 15 to 19 years. ("Unusual Cancers of Childhood Treatment." NCI Website. 31 July 2015.)[24]

The most common tumor types in patients aged 0 to 14 years are leukemia, CNS tumors and lymphomas, representing 31.1%, 25.4%, and 10% of all childhood cancers, respectively. Other cancer indications in this age group are estimated to occur at a rate of <7% of all cancer cases.

As patients age, cancer incidence increases. Tumor types are generally more diverse in patients aged 15 – 19 years and include lymphomas, carcinomas and melanoma, CNS tumors,

leukemia and germ cell, occurring at rates of 20.7%, 19.6%, 18.7%, 13.8%, and 12.3%, respectively. Other tumors in this age group occur at a rate of <7% of all cancers.

Table 6 shows the relative distribution of various cancer diagnoses in pediatric patients by age category.

Table 6 Percent distribution of childhood cancers by ICCC category and age group, all races, both sexes, SEER, 1975 – 95

	Age					
	<5	5-9	10-14	15-19	<15	<20
All Sites - Number of cases	9,402	5,024	5,419	9,814	19,845	29,659
	%	%	%	%	%	%
All Sites	100.0	100.0	100.0	100.0	100.0	100.0
I(total) - Leukemia	36.1	33.4	21.8	12.4	31.5	25.2
Ia - Lymphoid Leukemia	29.2	27.2	14.7	6.5	24.7	18.7
Ia - excl. Acute Lymphoid	0.2	0.3	0.2	0.1	0.2	0.2
Acute Lymphoid	29.0	27.0	14.5	6.4	24.5	18.5
Ib - Acute Leukemia	4.6	4.1	5.4	4.1	4.7	4.5
Ib - excl. Acute Myeloid	1.9	0.9	1.6	0.9	1.5	1.3
Acute Myeloid	2.8	3.2	3.8	3.2	3.2	3.2
Ic - Chronic myeloid leukemia	0.6	0.7	0.9	1.2	0.7	0.9
Id - Other specified leukemias	0.2	0.2	0.1	0.1	0.2	0.2
Ie - Unspecified leukemias	1.4	1.2	0.8	0.5	1.2	1.0
II(total) - Lymphomas and reticuloendothelial neoplasms	3.9	12.9	20.6	25.1	10.7	15.5
IIa - Hodgkins' disease	0.4	4.5	11.4	17.7	4.4	8.8
IIb - Non-Hodgkins' Lymphoma	2.0	5.2	6.1	6.0	4.0	4.6
IIc - Burkitt's lymphoma	0.8	2.4	1.9	0.6	1.5	1.2
IId - Miscellaneous lymphoreticular neoplasms	0.4	0.2	0.3	0.2	0.3	0.3
IIe - Unspecified lymphomas	0.3	0.7	0.9	0.7	0.6	0.6
III(total) - CNS and miscellaneous intracranial and intraspinal neoplasms	16.6	27.7	19.6	9.5	20.2	16.7
IIIa - Ependymoma	2.6	1.3	1.1	0.5	1.9	1.4
IIIb - Astrocytoma	6.7	14.2	11.8	6.0	10.0	8.7
IIIc - Primitive neuroectodermal tumors	4.3	6.3	3.1	1.0	4.5	3.3
IIId - Other gliomas	2.2	5.0	2.9	1.5	3.1	2.6
IIIe - Miscellaneous intracranial and intraspinal neoplasms	0.2	0.3	0.3	0.3	0.3	0.3
IIIf - Unspecified intracranial and intraspinal neoplasms	0.5	0.6	0.4	0.2	0.5	0.4
IV(total) - Sympathetic nervous system	14.3	2.7	1.2	0.5	7.8	5.4
IVa - Neuroblastoma and ganglioneuroblastoma	14.0	2.6	0.8	0.3	7.5	5.1
IVb - Other sympathetic nervous system tumors	0.3	0.1	0.3	0.1	0.3	0.2
V(total) - Retinoblastoma	6.3	0.5	0.1	0.0	3.1	2.1
VI(total) - Renal tumours	9.7	5.4	1.1	0.6	6.3	4.4
VIa - Wilms' tumor, rhabdoid and clear cell sarcoma	9.7	5.2	0.7	0.2	6.1	4.2
VIb - Renal carcinoma	0.1	0.1	0.4	0.4	0.2	0.2
VIc - Unspecified malignant renal tumors	0.0	0.0	0.0	0.0	0.0	0.0

(Ries et al., 1999)[25]

Table 6 Percent distribution of childhood cancers by ICCC category and age group, all races, both sexes, SEER, 1975 – 95 (continued)

	Age					
	<5	5-9	10-14	15-19	<15	<20
All Sites - Number of cases	9,402	5,024	5,419	9,814	19,845	29,659
	%	%	%	%	%	%
VII(total) - Hepatic tumors	2.2	0.4	0.6	0.6	1.3	1.1
VIIa - Hepatoblastoma	2.1	0.2	0.1	0.0	1.0	0.7
VIIb - Hepatic carcinoma	0.1	0.3	0.5	0.5	0.3	0.3
VIIc - Unspecified malignant hepatic tumors	0.0	0.0	0.0	0.0	0.0	0.0
VIII(total) - Malignant bone tumors	0.6	4.6	11.3	7.7	4.5	5.6
VIIIa - Osteosarcoma	0.2	2.2	6.6	4.4	2.4	3.1
VIIIb - Chondrosarcoma	0.0	0.1	0.6	0.6	0.2	0.3
VIIIc - Ewing's sarcoma	0.3	2.1	3.7	2.3	1.7	1.9
VIIId - Other specified malignant bone tumors	0.1	0.1	0.3	0.3	0.2	0.2
VIIIe - Unspecified malignant bone tumors	0.0	0.1	0.1	0.1	0.1	0.1
IX(total) - Soft-tissue sarcomas	5.6	7.5	9.1	8.0	7.0	7.4
IXa - Rhabdomyosarcoma and embryonal sarcoma	3.4	4.2	2.8	1.9	3.4	2.9
IXb - Fibrosarcoma, neurofibrosarcoma and other fibromatous neoplasms	1.0	1.4	3.1	3.1	1.7	2.1
IXc - Kaposi's sarcoma	0.0	0.1	0.0	0.1	0.0	0.1
IXd - Other specified soft-tissue sarcomas	0.7	1.2	2.2	2.1	1.3	1.5
IXe - Unspecified soft-tissue sarcomas	0.4	0.7	1.0	0.9	0.6	0.7
X(total) - Germ-cell, trophoblastic and other gonadal tumors	3.3	2.0	5.3	13.9	3.5	7.0
Xa - Intracranial and intraspinal germ-cell tumors	0.2	0.8	1.3	0.9	0.7	0.7
Xb - Other and unspecified non-gonadal germ-cell tumors	1.7	0.1	0.5	1.4	1.0	1.1
Xc - Gonadal germ-cell tumors	1.4	1.1	3.0	9.4	1.7	4.2
Xd - Gonadal carcinomas	0.0	0.0	0.4	1.9	0.1	0.7
Xe - Other and unspecified malignant gonadal tumors	0.0	0.1	0.1	0.3	0.1	0.1
XI(total) - Carcinomas and other malignant epithelial neoplasms	0.9	2.5	8.9	20.9	3.5	9.2
XIa - Adrenocortical carcinoma	0.2	0.1	0.1	0.1	0.1	0.1
XIb - Thyroid carcinoma	0.1	1.0	3.5	7.4	1.2	3.3
XIc - Nasopharyngeal carcinoma	0.0	0.1	0.7	0.8	0.2	0.4
XId - Malignant melanoma	0.4	0.7	2.0	6.8	0.9	2.9
XIe - Skin carcinoma	0.0	0.0	0.1	0.1	0.0	0.0
XIf - Other and unspecified carcinomas	0.2	0.7	2.5	5.7	1.0	2.5
XII(total) - Other and unspecified malignant neoplasms	0.5	0.3	0.6	0.8	0.5	0.6
XIIa - Other specified malignant tumors	0.1	0.1	0.1	0.3	0.1	0.1
XIIb - Other unspecified malignant tumors	0.4	0.3	0.5	0.5	0.4	0.4

(Ries et al.,1999)[25]

6.1.1 NTRK overview

The Trk family of tyrosine kinase receptors, which include TrkA, TrkB, and TrkC, are activated by neurotrophins, a family of nerve growth factors (e.g., nerve growth factor, brain-derived neurotrophic factor, neurotrophins). The Trk family members play a key role in normal central and peripheral neuronal cell development and differentiation. They regulate survival (or prevention of programmed cell death) and maintain the function of neuronal cells throughout the body. Trk receptors are found on a number of different cell types, and many non-neuronal cells also produce neurotrophins. Deregulated kinase activities of Trk family members occur because of gene mutations, overexpression, splice variants, and gene rearrangements, and are associated with a number of human cancer types.[26, 27, 28]

Oncogenic *NTRK1* gene rearrangements have been reported in papillary thyroid cancer, spitzoid melanoma, CRC, and NSCLC. These gene rearrangements result in a constitutively active kinase that provides the driving force for transformation and tumor progression via the relay of growth and survival signals within cancer cells. Oncogenic *NTRK2* gene rearrangements have been identified in pilocytic astrocytoma, and overexpression of TrkB in neuroblastoma and pancreatic cancer is associated with poor outcome.[29, 30, 31] Oncogenic gene rearrangements involving the *NTRK3* kinase domain have been identified in acute myeloid leukemia, salivary gland carcinoma, adult secretory breast cancer, congenital fibrosarcoma, and pediatric nephroma.[32]

A survey of two pediatric cancer databases, St Jude pediatric cancer database (PeCan; total n=1,604) and the University of Michigan database (Peds-MiOncoSeq; total n=91) resulted in the identification of three gene rearranged-cancers, one each involving *NTRK1*, *NTRK2*, and *NTRK3* in a sarcoma, a low grade glioma, and a B-cell ALL, respectively. In addition, according to a literature survey, the following tumor types largely confined to the pediatric patient population, are also known to harbor gene rearrangements of the *NTRK* family of genes: congenital or infantile fibrosarcoma, secretory breast cancer, mesoblastic nephroma, and intrinsic pontine gliomas.

6.1.2 ROS1 overview

ROS1 belongs to the insulin-receptor superfamily. Like other tyrosine kinase receptor molecules, it plays a role in relaying growth signals from the environment outside the cell into the cell's nucleus. It is 1 of 2 orphan receptor tyrosine kinase family members with no known binding ligand. Genetic changes in *ROS1*, such as gene rearrangements, mutations, or copy number increases, create oncogenes that can lead to cancer.[33] ROS1 was discovered in NSCLC patients in the form of a fusion protein (6 different partners for ROS1) and is found in approximately 2% of patients with NSCLC.[34, 35] *ROS1* gene rearrangements have been detected in a variety of other cancers, including glioblastoma multiforme, cholangiocarcinoma, ovarian cancer, gastric adenocarcinoma, CRC, inflammatory myofibroblastic tumor, angiosarcoma, and epitheloid hemangioendothelioma and malignant melanoma.[1, 2, 35, 36, 37]

Similar to *NTRK* and *ALK* (see below) gene rearrangements, *ROS1* gene rearrangements create fusion proteins with constitutively active kinase domains that activate downstream signaling pathways leading to oncogenic properties in cells, including uncontrolled proliferation and resistance to cell death with prolonged tumor cell survival. These pathways include Ras-ERK for cellular proliferation and the JAK-STAT and phosphatidylinositol 3-kinase/AKT pathways, which regulate cell survival (anti-apoptosis) and proliferation. ROS1 fusion proteins may also activate the mammalian target of the rapamycin pathway, which is critical for the regulation of protein translation. Cancers that have these pathways activated tend to be more aggressive, with invasion and metastasis leading to poor patient survival.[37]

The currently available ROS1 inhibitor drug, crizotinib, has demonstrated clinical benefit in NSCLC.[38] However, the eventual emergence of resistant tumors, and the poor penetration of crizotinib into the brain for treating brain metastases support the need for the development of improved ROS1 inhibitors with the ability to penetrate the blood-brain barrier and activity against TKI-resistant *ROS1* mutations.[39]

6.1.3 ALK overview

ALK also belongs to the insulin-receptor superfamily and is related to ROS1. ALK was first identified in anaplastic lymphomas, a distinct subset of non-Hodgkin's lymphoma. Molecular changes in *ALK* through gene rearrangements, mutations, and overexpression lead to the formation of at least 14 *ALK* oncogenes.[40, 41] Aberrant ALK fusion proteins spontaneously form molecular structures that lead to self-activation and constitutive activity within cancer cells via activation of signal transduction pathways and intracellular kinases that drive uncontrolled tumor cell growth, metabolism, and survival. In addition to anaplastic lymphomas, *ALK* oncogenes are found in a number of cancers such as NSCLC, diffuse large B-cell lymphoma, neuroblastomas, inflammatory myofibroblastic tumors, and possibly subsets of esophageal/gastric and renal cell cancers.[40, 42, 43] The currently available ALK inhibitor drugs, crizotinib, ceritinib and alectinib, have demonstrated clinical benefit in NSCLC.[38, 44, 45] However, the eventual emergence of resistant tumors, and the poor penetration of crizotinib into the brain for treating brain metastases support the need for the development of improved ALK inhibitors with the ability to penetrate the blood-brain barrier and activity against TKI-resistant *ALK* mutations.[41, 46, 47] Alectinib, recently approved by the FDA in late 2015, is a second-generation ALK inhibitor with proven activity in the CNS and in the crizotinib-refractory population.[48]

6.2 Disease States Being Explored in the Pediatric Population

As described in Section 6.4, Phase 1 study RXDX-101-03 is evaluating the safety, PK and activity of entrectinib in the pediatric population with relapsed or refractory extracranial solid tumors (neuroblastoma and non-neuroblastoma) and primary CNS tumors, including in pediatric patients positive for any of the target (*NTRK1/2/3*, *ROS1*, *ALK*) gene rearrangements, irrespective of tumor histology. Below is outlined the background and rationale for entrectinib therapeutic potential in a few select disease states being explored in the ongoing study RXDX-101-03.

6.2.1 Neuroblastoma

There is abundant evidence supporting a role for activated receptor tyrosine kinases in human cancers. Furthermore, there is growing evidence for activation of Trk family receptors (TrkA, B, C [encoded by the genes *NTRK1*, *NTRK2*, *NTRK3*, respectively]) in pediatric and adult tumors. The Trk neurotrophin receptors play critical roles in development and maintenance of the central and peripheral nervous system. TrkA and TrkB have also been implicated in the clinical and biological behavior of favorable and unfavorable neuroblastomas (NBs), respectively.[28, 29, 49-58] TrkB is co-expressed with its ligand, BDNF, and this autocrine pathway leads to invasion, angiogenesis and drug resistance.[59-62] TrkB expression and activation also suppresses anoikis, favoring the development of metastasis.[63-65] Mutations in or translocations involving *NTRK* genes have not been found in NBs, but there is autocrine activation of the TrkB/BDNF pathway in 50-60% of high-risk NBs, making this one of the most attractive targets for biological therapy of this tumor. TrkB and BDNF are co-expressed in most high-risk NBs, especially those with MYCN amplification,[54] and blocking TrkB with inhibitors leads to apoptosis and sensitivity to chemotherapy.[60, 66-69]

Many other pediatric and adult cancers have rearrangements or aberrant expression of *NTRK* genes (Table 7). In general, TrkA, TrkB and TrkC have occasionally been activated by translocation, but they are more commonly activated by autocrine overexpression. However, deep sequencing of glial tumors, ranging from diffuse intrinsic pontine gliomas to glioblastomas, has identified a surprising number of translocations involving *NTRK* genes (Table 7). Indeed, because of its frequency of activation, TrkB has been proposed as a promising target for cancer therapy.[70] However, in any given tumor type, one or more different members of the Trk family may be activated. Thus, targeting Trk receptors with a pan-Trk inhibitor would be of benefit for many cancers in children and adults.

Table 7 TRK Gene Activation in Pediatric Cancers

Pediatric cancer	TrkA/ NTRK1	TrkB/ NTRK2	TrkC/ NTRK3	Alteration	TRKI tested	References
Neuroblastoma	X	X	X	No	Inhibited	28, 29, 49-58, 60, 66-69
Medulloblastoma	X	X	X	No	inhibited	67, 71-74
Retinoblastoma	X	X	X	NT	inhibited	75
Wilms tumor (anaplastic)	X	X	X	NT	NT	76
Congenital fibrosarcoma	–	–	X	*ETV6-NTRK3*	NT	77-79
Mesoblastic nephroma	–	–	X	*ETV6-NTRK3*	NT	77-79
Ewing sarcoma	X	X	X	NT	NT	80
Glial tumors	X	X	X	*TPM3-NTRK1* *BTBD1-NTRK3* *ETV6-NTRK1* *VCL-NTRK2* *AGBL4-NTRK2* *QK1-NTRK2* *NFASC-NTRK1* *BCAN-NTRK1*	NT	30, 81, 82

X= Rearrangements or aberrant expression of *NTRK* genes; NT=not tested.

6.2.2 Thyroid Cancer – Medullary and Well-Differentiated Non-medullary

Approximately 1.8% of new thyroid cancer cases occur in patients less than 20 years of age.[83] According to a literature search, incidence of thyroid cancer in the pediatric patients is 8.9 per 1,000,000 age-adjusted person-years. This incidence is highly age-dependent, however and when divided into age groups the incidence is not measured in pediatric patients less than 5 years of age, and is 1.2, 7.1, and 26.7 per 1,000,000 age-adjusted persons, in pediatric patients between 5 – 9 years, between 10 and 14 years and between 15 and 19 years, respectively.[83]

Thyroid cancer is commonly divided into four subtypes: papillary, follicular, medullary, and anaplastic. Papillary is the most common type of thyroid cancer and occurs in 83% of children diagnosed with thyroid cancer. Follicular cancer occurs in 10% of cases and medullary thyroid cancer occurs in 5% of cases.[84] There is a good prognosis for pediatric patients diagnosed with thyroid cancer, and patients younger than 20 years of age make up 0.2% of all deaths from thyroid cancer. Thirty-year survival rates were 91%, 92%, and 15% for papillary thyroid cancer, follicular thyroid cancer and medullary thyroid cancer, respectively.[84]

Although the prognosis of papillary and follicular carcinomas is generally good, a subset of patients will develop relapsed and refractory disease after available therapies have been exhausted. Therefore, patients with well-differentiated papillary and follicular as well as non-differentiated or differentiated medullary thyroid cancers could see benefit from the use of a novel targeted agent.

Current adult studies with entrectinib will be enrolling patients with any type of thyroid malignancy harboring an *NTRK1/2/3*, *ROS1* or *ALK* gene rearrangement. Although to date, *NTRK* gene rearrangements have been identified only in papillary thyroid cancer; other subtypes of thyroid cancer, including other well-differentiated follicular as well as non-differentiated or differentiated medullary thyroid cancers would be eligible if they harbor one of the necessary molecular alterations.

6.2.3 Malignant Melanoma

Pediatric melanomas are rare and account for approximately 0.5% of new melanoma cases.[83] Although rare, melanoma is the most common skin cancer in children[85] and accounts for 1 – 3% of childhood malignancies.[86] For individuals younger than 20 years, the annual incidence of melanoma is 5.6 per million individuals.[83]

The most common melanomas in pediatric patients are conventional (CM), congenital nevi (CMN), and spitzoid (SM). The precise incidence and distribution of melanoma subtypes is largely unknown due to low incidence. However, studies have estimated that 30 to 75% of pediatric melanomas have originated in giant CMN, and the estimates of lifetime risk of malignant transformation of a giant CMN range from 2 to 20%. Overall, the incidence of pediatric melanoma is seven-times more common in the 10 to 20-year-old age group compared to children less than 10 years of age, with adolescents aged 15 to 19 years accounting for 73 – 79% of pediatric melanoma cases.[86]

In pediatric patients, the 5-year survival for *in situ* disease was 100%, 96.1% for localized disease, 77.2% for regional metastatic disease, and 57.3% for distant metastatic disease. Overall survival was 88.9% for children less than 10 years of age and 91.5% for pediatric patients 10 to 20 years of age.[86]

The primary source of pathophysiology understanding comes from adult data, where the relative prevalence of actionable targets and/or known molecular oncogenic drivers in melanomas are known and include: *BRAF* mutations (45%), *KIT* mutations (1 – 20% of CM, 15 – 20% of acral lentiginous melanoma, and 15 – 20% of mucosal melanoma), and *NRAS* mutations (18%).[87] A recent analysis of childhood and adolescent melanoma showed that molecular pathogenesis of CM is largely similar between adults and pediatrics, while CMN and SM pediatric melanomas appear to be distinct. Similar to adults, the molecular pathogenesis of CM in pediatric patients often contains a *BRAF* mutation. CNMs tend to contain *NRAS* mutations and SMs lacked both *BRAF* and *NRAS* mutations.[88]

As previously mentioned, *NTRK1* mutations have been identified in SM and *ROS1* rearrangements have been implicated in malignant melanoma. Due to the relatively high incidence of this disease state in pediatric patients and unmet medical need for patients with metastatic disease harboring these mutations, entrectinib is currently being investigated for this indication in pediatric patients.

6.2.4 Sarcoma

Pediatric soft tissue sarcomas (STS) are a heterogeneous group of malignant tumors[24] and account for 7% of all childhood tumors.[89] Soft tissue sarcomas are more common in the general population compared to bone and joint cancers, however, they occur less frequently in pediatric patients compared to adults. Between 2008 and 2012, the incidence of soft tissue sarcomas in the US population was 3.4 per 100,000 persons per year (with estimated new cases in 2015 of 11,930), and approximately 8.9% of soft tissue sarcomas occur in patients less than 20 years of age. The incidence of soft tissue sarcomas in the pediatric population is approximately 1.1 persons per 100,000 persons ages 0 to 19 years.[83]

Rhabdomyosarcoma, a tumor of striated muscle, is the most common STS in children aged 0 to 14 years and accounts for 50% of STS tumors in this age group.[83] The remaining STS cases include neoplasms of connective tissue (e.g., desmoid fibromatosis, liposarcoma), the peripheral nervous system (e.g., malignant peripheral nerve sheath tumor), smooth muscle (e.g., leiomyosarcoma), and vascular tissue (blood and lymphatic vessels, e.g., angiosarcoma).[90] In children, outside of rhabdomyosarcoma, synovial sarcoma, fibrosarcoma, fibrohistiocytic tumors, and malignant peripheral nerve sheath tumors are the most frequent extraosseous STS.[91]

Bone and joint cancers are more frequently diagnosed in pediatric patients than in adults. The estimated number of new cases in the overall US population in 2015 is 2,970 and approximately 27.6% of those newly diagnosed bone and joint cancers occur in patients less than 20 years of age.[83] The age-adjusted SEER incidence lists bone and joint cancer as 0.9 per 100,000 persons, ages 0 to 19 years, with osteosarcomas and Ewing sarcomas being the most

frequent subgroups, with incidence rates of 4.4 and 2.5 pediatric patients per 100,000 age-adjusted persons per year in the United States, respectively.[83]

The molecular pathogenesis of sarcomas involves gene rearrangements (approximately 33%), point mutations (undefined prevalence), or nonspecific complex unbalanced karyotypes (undefined prevalence). A number of potential oncogenic molecular targets have been identified in various subsets of patients with STS and bone and joint cancers. Multiple actionable targets have been identified for various sarcoma subtypes. Estimates of the prevalence of genetic mutations for sarcomas has shown that *ALK* translocations have approximately 50% prevalence in inflammatory myofibroblastic tumor types. Clinical experience with the ALK/ROS1 inhibitor, Crizotinib, has demonstrated the potential for benefit of molecular targeting in this patient population.[87]

Although the prevalence of *NTRK* fusions in sarcomas is unclear at this time, recent data has shown that patients with soft tissue sarcomas with oncogenic *NTRK* fusions may have a clinical response to tyrosine kinase inhibitors that target this genetic alteration.[3]

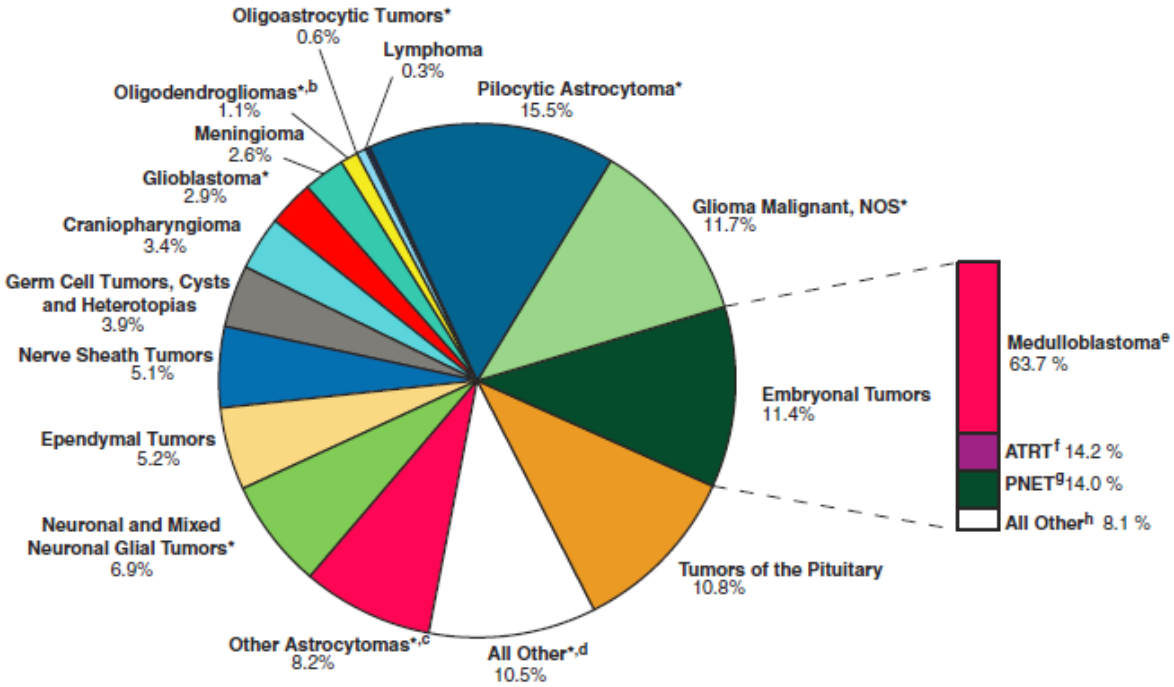
6.2.5 Malignant Brain Tumors (e.g., Astrocytoma and Glioblastoma Multiforme)

CNS tumors are the second most common childhood malignancy and the most common type of solid tumor in children. Between 2008 and 2012, about 7% of brain and CNS tumors occurred in children and adolescents. According to the Central Brain Tumor Registry of the United States (CBTRUS)[93], the incidence of non-malignant and malignant CNS tumors is 5.57 cases per 100,000 person-years for pediatric patients less than 20 years of age with an estimated 66 - 73.6% 5-year survival rate for malignant brain and CNS tumors in this age group. An estimated 26,000 children in the United States are living with a brain tumor.[93, 94]

The CBTRUS estimates incidence rates of brain and CNS tumors across further divided age groups in children (< 1 year, 1 – 4 years, 5 – 9 years, and 10 – 14 years, 15 – 19 years) ranges between 5 and 6.22 per 100,000 age-adjusted person years, with the highest incidence in infants less than 1 year of age (6.22 per 100,000 persons) and adolescents (6.19 per 100,000 persons).[95]

Nervous system tumors are comprised of various subsets of diseases. Figure 16 illustrates the distribution of brain and CNS tumors in children and adolescents, however, individual histology distributions vary widely within age sub-groups.

Figure 16 Distribution in Children and Adolescents (Age 0 – 19 years) of Primary Brain and CNS Tumors by CBTRUS Histology Groupings and Histology (N = 23,113), CBTRUS Statistical Report (2008 – 2012).



*All or some of this histology are included in the CBTRUS definition of gliomas, including ICD-O-3 histology codes 9380-9384, 9391-9460 (Table 2a).
a. Percentages may not add up to 100% due to rounding. b. ncludes oligodendrogliomas and anaplastic oligodendrogliomas (Table 2a).
c. Includes diffuse astrocytoma, anaplastic astrocytoma, unique astrocytoma variants (Table 2a). d. Includes choroid plexus tumors, other neuroepithelial tumors, tumors of the pineal region, other tumors of cranial and spinal nerves, mesenchymal tumors, primary melanocytic lesions, other neoplasms related to the meninges, other hematopoietic neoplasms, hemangioma, neoplasm unspecified, all other (Table 2a). e. ICD-O-3 histology codes: 9470/3, 9471/3, 9472/3,9474/3. f. ICD-O-3 histology code: 9508/3. g. ICD-O-3 histology code: 9473/3. h. ICD-O-3 histology codes: 8963/3, 9364/3, 9480/0, 9480/3, 9490/0 , 9490/3, 9500/3, 9501/3, 9502/3.

(Ostrom, et al., 2016.) [95]

Brain tumors account for about 90% of all CNS tumors, with the remaining 10% occurring in the spinal cord and cranial nerves. Gliomas, which include tumors derived from astrocytes, oligodendrocytes, and ependymal cells, account for almost 75% of all primary childhood CNS tumors and occur at a rate of 3.7 per 100,000 person-years.[94] Astrocytomas are the most common type of brain tumor in children, with an incidence of 1.3 per 100,000 person-years. Most of these are comprised of low-grade astrocytomas such as pilocytic astrocytoma with an incidence of 0.84 per 100,000 person-years.[94] Ependymomas have an incidence of 0.28 per 100,000 person-years and account for 10% of intracranial tumors and 40 – 60% of spinal cord tumors in children and adolescents.[94]

Other brain and CNS tumors occur at rates of less than 0.75 per 100,000 person-years each and include embryonal tumors, neuronal and mixed neuronal-glial tumors, tumors or the sellar region, tumors of the cranial and paraspinal nerves, germ cell tumors and cysts, meningiomas, lymphomas and hemopoietic neoplasms and unclassified tumors.

As with adults, the therapy for children with supratentorial high-grade astrocytoma includes surgery, radiation therapy, and chemotherapy. Despite this initial therapy, overall survival

rates are poor. Gene rearrangements of *NTRK2* have been identified in adult astrocytomas.[30] In addition, Drilon et al. described one patient with an astrocytoma that harbored an *NTRK1* gene rearrangement.[1] The patient received entrectinib and experienced a 45% tumor regression based upon volumetric assessment. These results show potential therapeutic benefit of Trk inhibitors in this patient population.

6.3 Pediatric Clinical Experience to Date

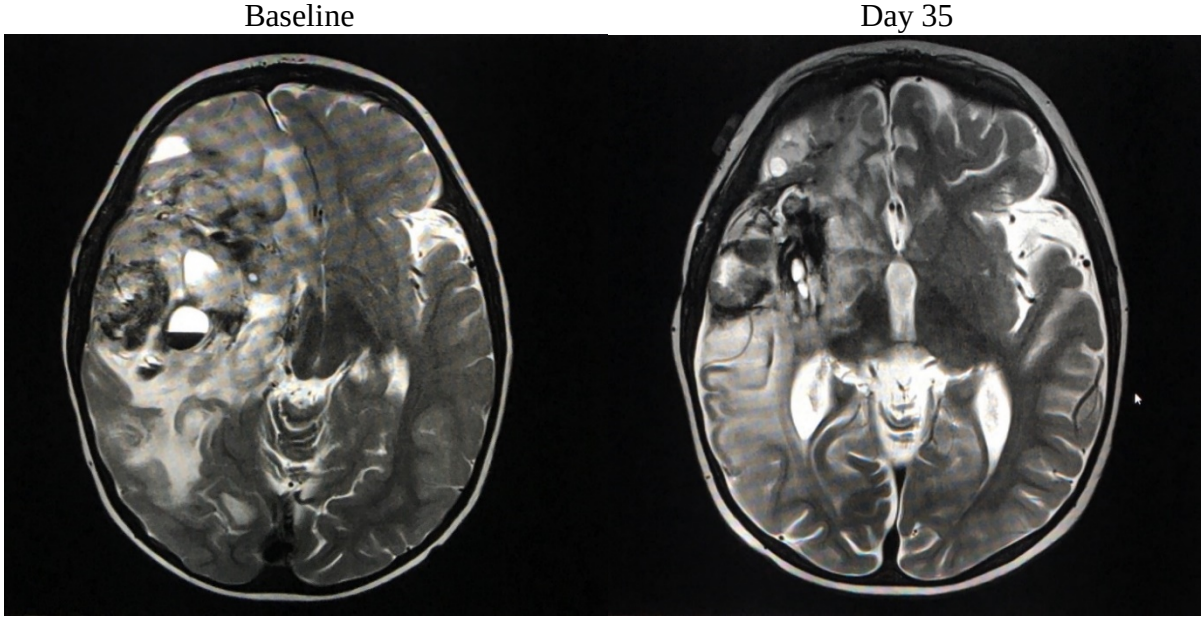
Phase 1/1b Study RXDX-101-03 (Part A) is currently enrolling at 4 sites in the US; no data have been publicly disclosed to date.

In addition to the Phase 1/1b study, a 20 month-old male patient diagnosed at birth with infantile fibrosarcoma has been receiving entrectinib treatment.

Infantile fibrosarcoma is known to frequently harbor oncogenic fusion protein drivers, specifically due to a high prevalence of the *ETV6-NTRK3* gene rearrangement. This patient's tumor was tested using Ignyta's *NTRK1/2/3, ROS1, ALK* gene rearrangement test service (companion diagnostic assay) and the results were positive for an *ETV6-NTRK3* gene rearrangement. Due to his age, the baby was not eligible for the pediatric Phase 1 study RXDX-101-03, so he instead received entrectinib under an Individual Patient Expanded Access – compassionate use.

Dosing with entrectinib was initiated at a starting dose of 200 mg/m^2 (absolute dose 100 mg), and after 14 days, increased as per protocol to 400 mg/m^2 (absolute dose 200 mg), which is the adult BSA-based RP2D. Baseline and on study (Day 35; Figure 17) brain MRIs showed decreased tumor and edema, accompanied by clinical improvement. The patient continues on an entrectinib dose of 400 mg/m^2 (absolute dose 200 mg) once daily.

Figure 17 20 month-old baby brain MRI images (Day 35)



6.4 Ongoing Clinical Trial in Pediatrics, Adolescents and Young Adults: RXDX-101-03

6.4.1 Study Design

This is a 4-part, Phase 1/1b multicenter, open-label dose escalation study in pediatric subjects with relapsed or refractory solid tumors (Part A) and pediatric subjects with primary CNS tumors (Part B), with 2 expansion cohorts (Phase 1b) for subjects with neuroblastoma (Part C) and other non-neuroblastoma, extracranial solid tumors harboring *NTRK1/2/3, ROS1*, or *ALK* gene rearrangements (Part D). Treatment schema is shown in Figure 18.

Part A will determine the MTD or the RP2D, PK, and safety profile of entrectinib in children and adolescents with relapsed or refractory extracranial solid tumors. Entrectinib will be administered orally once daily (QD) in repeated 4-week cycles. The starting dose in Part A will be 250 mg/m^2. Up to four dose levels will be evaluated. All dose levels (mg/m^2) will be based on the participants' actual body surface area (BSA) measured within 7 days of initiating therapy for each cycle. Assigned dose and dose reductions, if necessary, will be specified for each participant using a protocol specific dosing nomogram for each dose level. Up to 2 dose reductions due to treatment-related toxicity will be permitted in individual participants. A "3+3" subject enrollment scheme will be followed during the dose escalation.

The RP2D will be determined from dose-limiting toxicity (DLT) derived from clinical and laboratory observations in the first treatment cycle (28 days). Subjects will be evaluable for the primary endpoint in Part A if they discontinue drug due to toxicity during Cycle 1 or experience DLT at any time during Cycle 1. Subjects who do not experience DLT will be considered evaluable for toxicity if they have received at least 75% of the prescribed dose during Cycle 1 ($\geq$ 21 of 28 doses). Subjects who discontinue entrectinib treatment due to progressive disease (PD) or other reason not related to toxicity will be replaced if they have not received 75% of the prescribed dose during Cycle 1.

Subjects will receive additional cycles of entrectinib provided the toxicities from the previous cycle have resolved to Grade $\leq$ 2 or baseline and there is no evidence of disease progression.

The RP2D will be based upon an overall assessment of entrectinib from Part A. This assessment will include the safety profile, the PK profile, and any clinical evidence of efficacy. The RP2D may be determined to be less than or equal to the MTD based upon this assessment. In addition, the RP2D may be determined before an MTD is established because of compelling demonstration of adequate exposure or clinical evidence of activity.

The MTD is defined as the dose level immediately below the dose level at which $\geq$ 2 subjects from a cohort of 3 to 6 subjects experience a DLT. Up to 9 subjects, $\geq$ 2 years and < 22 years of age, will be treated at the MTD, including at least 3 of 9 subjects < 12 years of age and at least 3 of 9 subjects who are $\geq$ 12 years of age. For cohorts in which 3 or more participants receive at least 75% of the prescribed dose, the MTD will be considered to have been exceeded if > 33% of participants at a given dose level experience a DLT during the initial 28 days of drug administration (Cycle 1). Intra-subject dose escalations will be permitted once a RP2D is established. Individual subject enrolled at dose levels below the established RP2D

may escalate one dose level every 2 cycles to reach the RP2D, as tolerated. Toxicity and DLT beyond Cycle 1 (enrollment dose level) will be tabulated and considered in determining the RP2D but will not be used to determine the MTD. When the RP2D is established, subjects being treated above the RP2D can elect to de-escalate their dose to the RP2D or they may remain at the same dose level if they are tolerating their treatment.

Part B, Part C and Part D will be opened after determination of the RP2D in Part A.

Part B will determine the RP2D, PK, and safety profile of entrectinib in children and adolescents with relapsed or refractory primary CNS tumors.

A "3+3" subject enrollment scheme will be followed during the dose escalation and DLT assessment will be completed as described in Part A. Entrectinib will be administered orally once daily (QD) in repeated 4-week cycles. The starting dose (mg/m^2) in Part B will be one dose level below the RP2D determined in Part A; the maximum dose level will be the RP2D for extracranial solid tumor subjects in Part A.

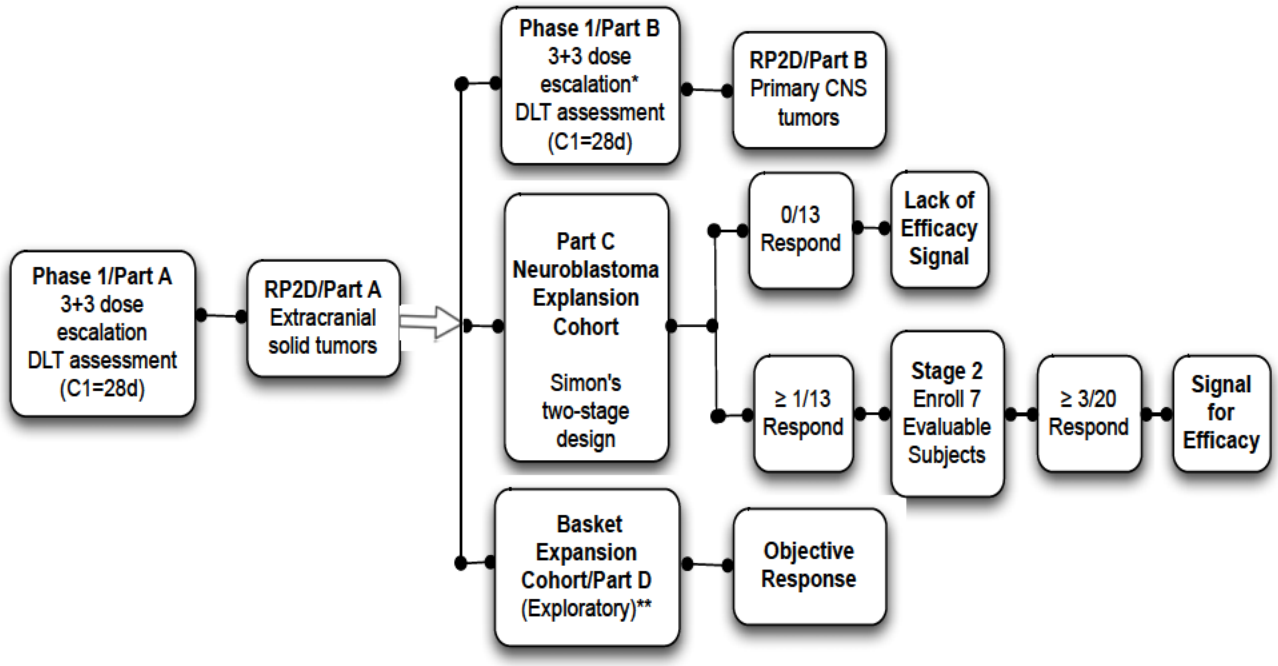
Part C (neuroblastoma expansion cohort) will evaluate tumor response (objective response rate) in children and adolescents with relapsed or refractory neuroblastoma receiving entrectinib at the RP2D determined in Part A. Overall best response (CR or PR) will be evaluated by RECIST v1.1 in subjects with measurable disease, ± (radiolabeled metaiodobenzylguanidine) MIBG avid bone/bone marrow lesions, ± bone marrow metastasis. Overall best response (CR or PR) will be evaluated using Curie Scale for response in children and adolescents who do not have measurable disease but have MIBG avid bone ± bone marrow disease.

Part D (basket expansion cohort) will evaluate tumor response (objective response rate) in 10 children and adolescents with relapsed or refractory non-neuroblastoma, extracranial solid tumors harboring *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangements receiving entrectinib at the RP2D determined in Part A. This cohort is considered exploratory. All subjects must have measurable disease and documented gene rearrangements by a CLIA lab. Overall best response (CR or PR) will be evaluated by RECIST v1.1.

All subjects will have tumor assessments performed during Screening and at the end of Cycles 2, 4, and 6; then every 3 cycles × 2 (i.e., after Cycles 9 and 12); then every 4 cycles × 3 (i.e., after Cycles 16, 20, and 24); and then every 6 cycles thereafter. Assessments may include magnetic resonance imaging (MRI) or computed tomography (CT) scans, MIBG, +/- bone marrow aspirates and biopsies.

Subjects may continue entrectinib until clinical, laboratory or radiographic evidence of progressive disease, development of unacceptable toxicity, or discontinuation at the discretion of subject/parent/guardian or Investigator. Subjects who discontinue entrectinib will be encouraged to complete relevant safety procedures.

Figure 18 RXDX-101-03 Treatment Schema



RP2D=recommended phase 2 dose, DLT=dose limiting toxicity
*Starting dose level= RP2D/A-1 dose level
**Presence of *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangement documented by a CLIA lab.

6.4.2 Subject Selection

6.4.2.1 Inclusion Criteria

1. Body surface area (BSA): subjects must have a body surface area ≥ 0.45 m^2 at the time of the study enrollment
2. Disease status: subjects must have measurable or evaluable disease, by RECIST v1.1 ± Curie Scale Criteria
3. Tumor type: **Part A**: Relapsed or refractory extracranial solid tumors; **Part B**: Relapsed or refractory primary CNS tumors; **Part C**: Relapsed or refractory neuroblastoma; **Part D**: Relapsed or refractory non-neuroblastoma, extracranial solid tumors with *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangements documented by a CLIA lab prior to enrollment
4. Histologic/molecular diagnosis of malignancy at diagnosis or the time of relapse
5. Archival tumor tissue from diagnosis or, preferably, at relapse
6. Age: male or female age ≥ 2 years and < 22 years
7. Performance status: Lansky or Karnofsky score ≥ 60%
8. Patient's cancer must have relapsed after or failed to respond to frontline curative therapy or there must not be other potentially curative treatment options available

9. Adequate organ and neurologic function
10. Females of child bearing potential must have a negative serum pregnancy test within 14 days prior to entrectinib dosing

6.4.2.2 Main Exclusion Criteria

1. Receiving other experimental therapy
2. Known congenital long QT syndrome
3. Known active infections
4. Receiving Enzyme Inducing Antiepileptic Drugs (EIAEDs) within 14 days of first dose.
5. **Parts C and D only**: Prior treatment with approved or investigational Trk, ROS1, or ALK inhibitors
6. **Part C only:** Neuroblastoma subjects with bone marrow space-only disease
7. Incomplete recovery from acute effects of any surgery prior to treatment
8. Active gastrointestinal disease or other malabsorption syndromes that would impact drug absorption
9. Other severe acute or chronic medical or psychiatric condition or lab abnormality that may increase the risk associated with study participation, drug administration or may interfere with the interpretation of study results

6.4.3 Study Objectives

Primary Objectives

1. To determine the MTD, or RP2D, of entrectinib in pediatric subjects (children and adolescents) with relapsed or refractory extracranial solid tumors
2. To determine the MTD or RP2D of entrectinib in pediatric subjects with relapsed or refractory primary CNS tumors

Secondary Objectives

1. To describe the safety profile of entrectinib as characterized by AE type, severity, timing and relationship to entrectinib treatment, as well as ECG and laboratory abnormalities in the first and subsequent treatment cycles
2. To characterize the pharmacokinetics (PK) of entrectinib in plasma
3. To estimate the tumor response rate in all enrolled subjects (Parts A and B) and, at the RP2D determined in Part A, estimate response rate in subjects with relapsed or refractory neuroblastoma (Part C) or other non-neuroblastoma, extracranial solid tumors harboring *NTRK1/2/3, ROS1*, or *ALK* gene rearrangements (Part D), respectively
4. To estimate the PFS in subjects receiving entrectinib

6.4.4 Statistical Considerations

Evaluability

Intention-to-Treat (ITT) population is defined as all subjects who received at least one dose of entrectinib.

Safety population: Participants will be evaluable for the analysis to address the primary objective if they discontinue drug due to toxicity during Cycle 1 or experience DLT at any time during Cycle 1. Subjects who do not experience DLT will be considered evaluable for toxicity if they have received at least 75% of the prescribed dose during Cycle 1. Participants enrolled in Parts A and B who discontinue entrectinib treatment due to progressive disease (PD) or other reason not related to toxicity will be replaced if they have not received 75% of the prescribed dose during Cycle 1.

Efficacy population: Efficacy population is defined as all subjects who received at least one dose of entrectinib in Parts C and D and received the dose level ultimately selected as the RP2D in Part A.

Antitumor Activity and Progression-Free Survival (Secondary Objectives)

The Efficacy population (Parts C and D expansion cohorts of up to 20 evaluable subjects with neuroblastoma and up to 10 evaluable subjects with non-neuroblastoma extracranial solid tumors harboring *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangements, respectively) will be used to estimate the tumor response rate, as a secondary objective.

Part C: A Simon's two-stage design will be conducted to attempt to identify an efficacy signal.

Stage 1 – Enroll 13 evaluable subjects. If there are no responders, then accrual to Part C will be halted due to lack of evidence to support efficacy. If there is one or more responder, then accrual can continue to Stage 2.

Stage 2 – Enroll 7 additional subjects for a total of 20 evaluable subjects. If there are 2 or fewer responders, then there is insufficient evidence of efficacy. If there are 3 or more responders, then there is sufficient evidence of efficacy to warrant further study.

This rule has 90% power, with alpha=0.074, to reject a null hypothesis that the response rate is < 5% compared to an alternative hypothesis that it is >25%. The expected sample size is 14.82, and the probability of early termination is 0.599.

The proportion of subjects with an objective response (CR or PR) will be tabulated with 95% confidence intervals.

Part D is considered exploratory. Objective response will be described and summarized by gene rearrangement.

PFS, as a secondary objective, will be addressed within the Efficacy population. Progression-free survival curves will be presented using the methods of Kaplan-Meier, with standard errors according to Peto.

6.5 Age-Appropriate Formulation Development Plan

The design and development of age-appropriate pediatric formulations present a number of challenges in balancing and addressing acceptability of and preference among potential different pediatric dosage forms. Ignyta's initial approach is to focus on a minimum number of acceptable dosage forms that are capable of meeting the needs of the majority of the children in the different target age groups.

A pediatric drug product formulation comprising granules meant to be sprinkled over soft food ("sprinkle formulation") that can be swallowed without chewing is being progressed to support the initial clinical development plan in children age $\geq$ 2 years and $\leq$ 17 years. It is recognized that mixing with food and/or drinks may possibly affect biopharmaceutical performance and this will be taken into account when making any dosing administration recommendations.

Ignyta plans to test the sprinkle formulation in healthy adults in order to verify the *in vivo* performance (pharmacokinetic exposure) before introducing it into pediatric patients. Based on *in vivo* performance combined with the formulation development and stability data, the pediatric sprinkle formulation will be selected for further clinical testing.

For children who are able to swallow conventionally-sized solid dosage forms, there will be the option to use the adult capsule formulation. The form of the active drug substance entrectinib already in use in the adult capsule solid dosage forms currently in clinical studies has been selected for the development of the pediatric formulation.

7 CURRENT OR POTENTIAL CHALLENGES FOR CLINICAL DEVELOPMENT IN PEDIATRICS

The low incidence of pediatric cancer and the scarcity of potentially eligible patients, particularly pediatric patients with non-neuroblastoma, solid tumors harboring the target (*NTRK1/2/3*, *ROS1*, or *ALK*) gene rearrangements, is likely to lead to a very slow recruitment period, and thus, a protracted study timeline to data readout. Furthermore, in addition to the scarcity of the target pediatric patient population, the high level of clinical activity seen with entrectinib in the Phase 1 adult studies against the same molecular targets undermines the equipoise necessary to conduct a randomized, controlled study against best available therapy to assess efficacy and safety of entrectinib in pediatric patients.

8 IGNYTA'S PROPOSAL FOR A WRITTEN REQUEST

Ignyta is of the view that the ongoing Study RXDX-101-03 (Section 6.4) should be sufficient for a Written Request. This study in up to 81 patients with select solid tumors, including those with neuroblastoma and those with non-neuroblastoma solid tumors harboring an *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangement, will provide a robust assessment of the pharmacokinetic (PK), safety and clinical activity of entrectinib. It is believed that Study RXDX-101-03 will generate meaningful safety and efficacy data for inclusion in the entrectinib prescribing information and that such data should adequately inform physicians of

the safety and activity of entrectinib in children. Table 8 provides an overview of data to be generated from this study.

Table 8 Data to be Generated from Study RXDX-101-03

Tumor Types	Numbers of patients
Relapsed or refractory solid tumors (Part A)	6 – 30
Primary CNS tumors (Part B)	6 – 15
Neuroblastoma (Part C)	Up to 24 (up to 20 evaluable subjects)
Non-neuroblastoma, extracranial solid tumors (e.g., sarcoma, astrocytoma, glioblastoma multiforme, malignant melanoma) harboring an *NTRK1/2/3*, *ROS1*, or *ALK* gene rearrangement (Part D)	Up to 12 (up to 10 evaluable subjects)

Ignyta looks forward to participating in the meeting of Pediatric Oncology Subcommittee of the Oncologic Drugs Advisory Committee in June and the opportunity to discuss the pediatric development of entrectinib with the subcommittee.

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10 APPENDICES

Appendix 1

Entrectinib, an oral pan-Trk, ROS1, and ALK inhibitor in TKI-naïve patients with advanced solid tumors harboring gene rearrangements

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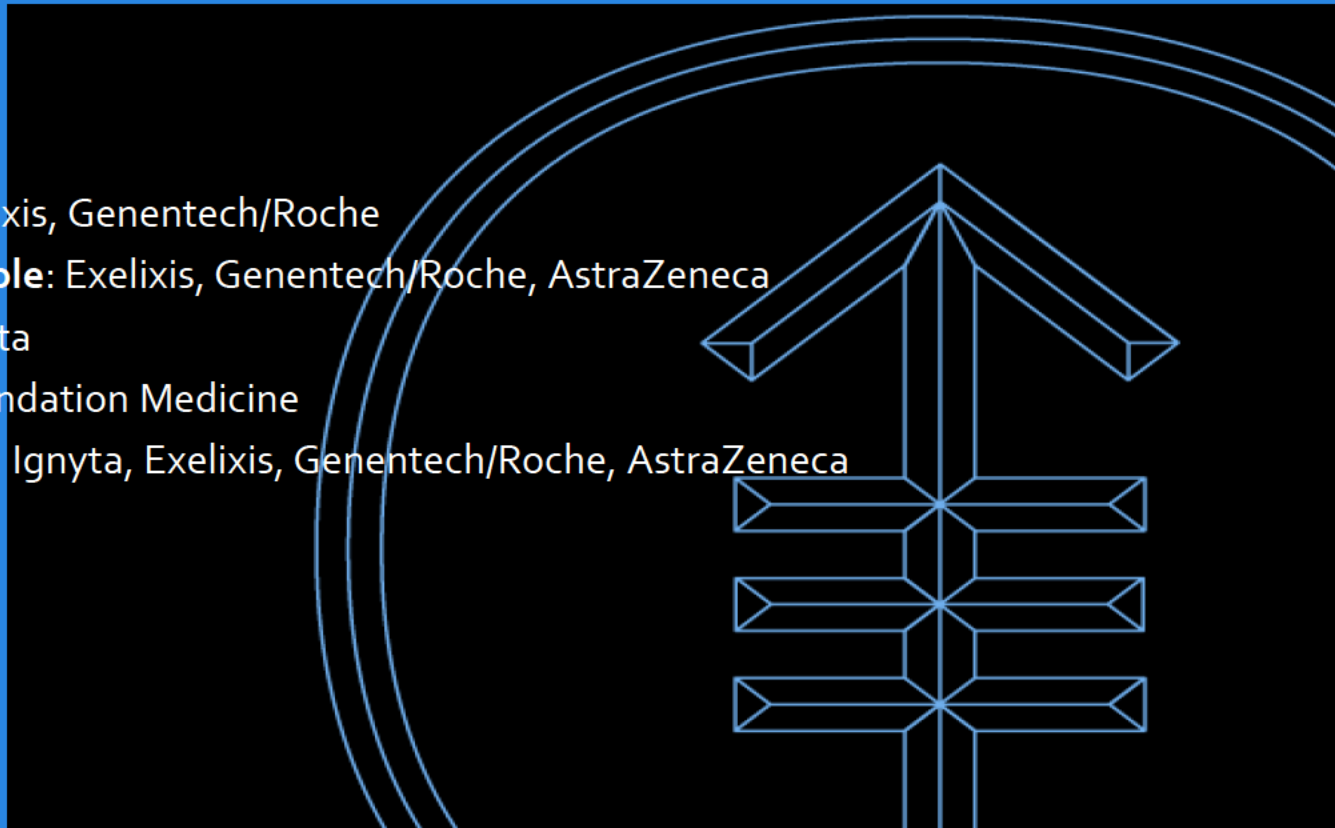
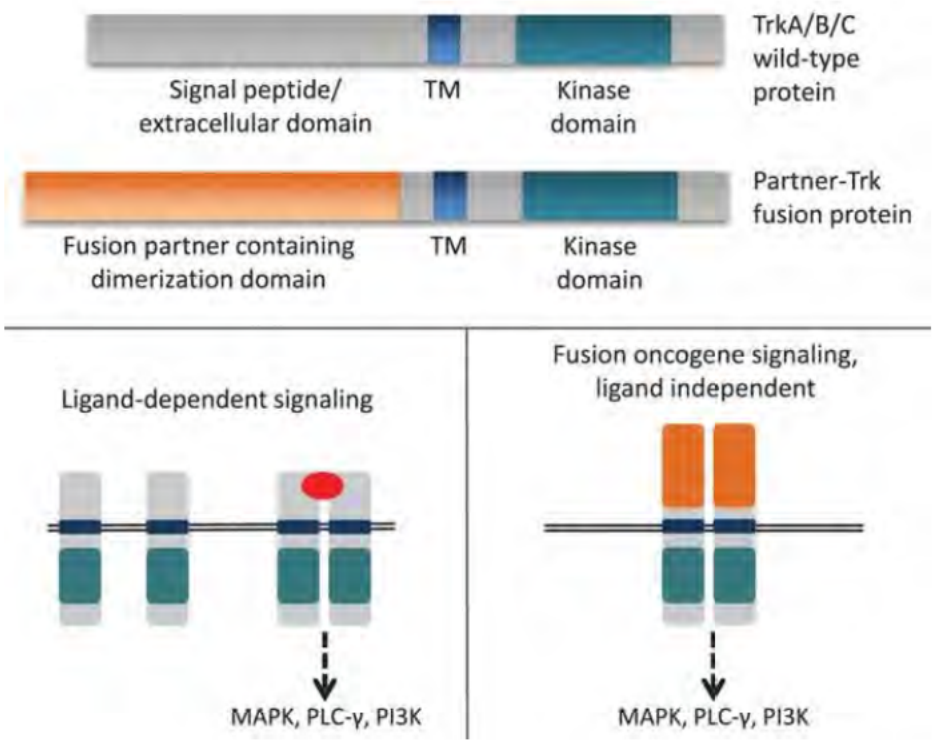
Disclosures

- **Honoraria**: Ignyta, Exelixis, Genentech/Roche
- **Consulting/Advisory Role**: Exelixis, Genentech/Roche, AstraZeneca
- **Speaker's Bureau**: Ignyta
- **Research Funding**: Foundation Medicine
- **Travel/Accomodations**: Ignyta, Exelixis, Genentech/Roche, AstraZeneca



Recurrent Gene Rearrangements

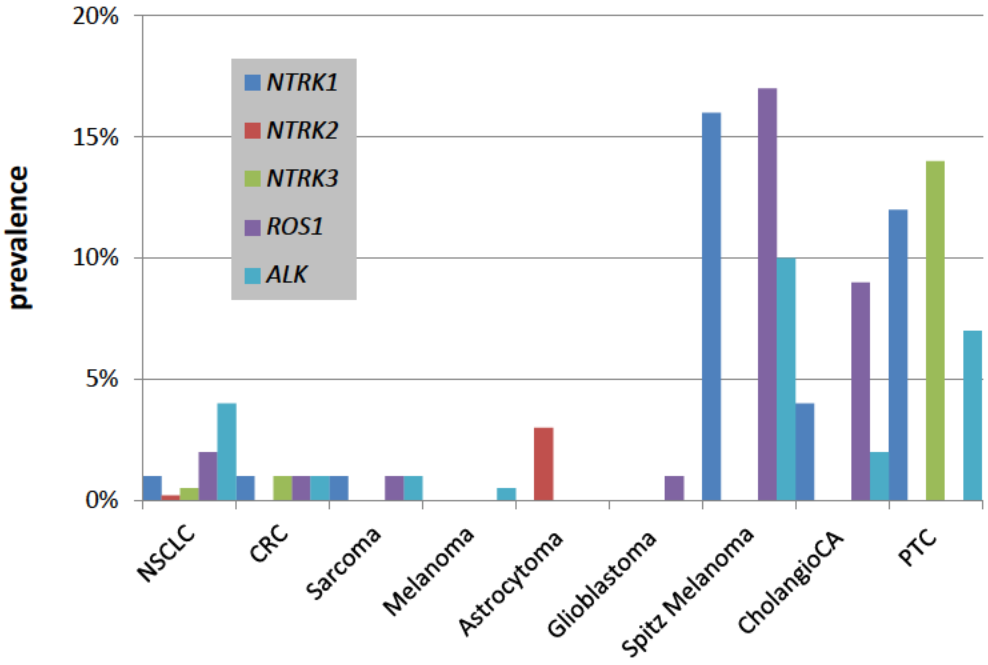


- **Oncogenic drivers across a variety of cancers**
  - Upstream partner can provide dimerization domains → ligand-independent signaling
  - Activation of downstream pathways
- **Detectable in the clinic**
  - FISH
  - RNAseq
  - DNA-based NGS
- **Select fusions are clinically-actionable**
  - responses to targeted therapy can be dramatic and durable

Farago *et al*, J Thorac Oncol. 2015 Dec;10(12):1670-4.

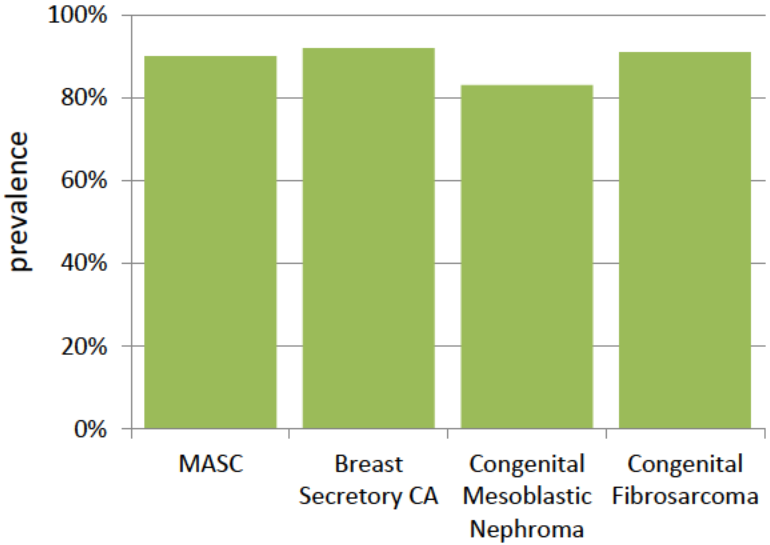
Recurrent Gene Rearrangements

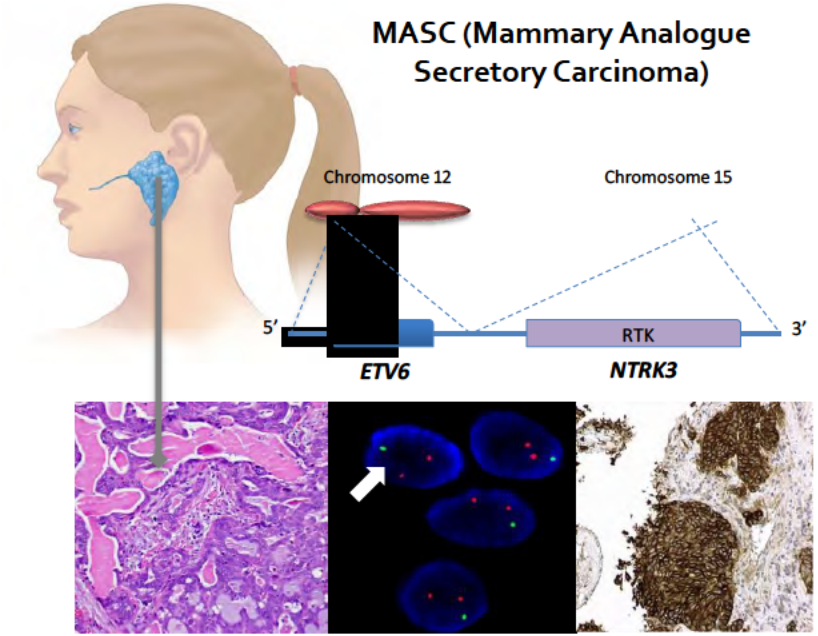
- ***NTRK1/2/3*, *ROS1*, and *ALK* fusions are identified across multiple cancers**
  - lower prevalence in more common cancers



Recurrent Gene Rearrangements

- ***NTRK1/2/3*, *ROS1*, and *ALK* fusions are identified across multiple cancers**
  - high prevalence events in rare adult and pediatric cancers





Drilon *et al*, Ann Oncol, 2016 Feb 15. PMID: 26884591

Entrectinib (RXDX-101)

- **Highly-potent, orally-available, ATP-competitive tyrosine kinase inhibitor**
  - Low to sub-nanomolar efficacy against 5 kinases
  - Results in decreased downstream effector activity
    - PLCγ, MAPK and PI3K/AKT pathways

- **Active *in vitro* and *in vivo***
  - *NTRK1/2/3*-rearranged cancers
  - *ROS1*-rearranged cancers
  - *ALK*-rearranged cancers

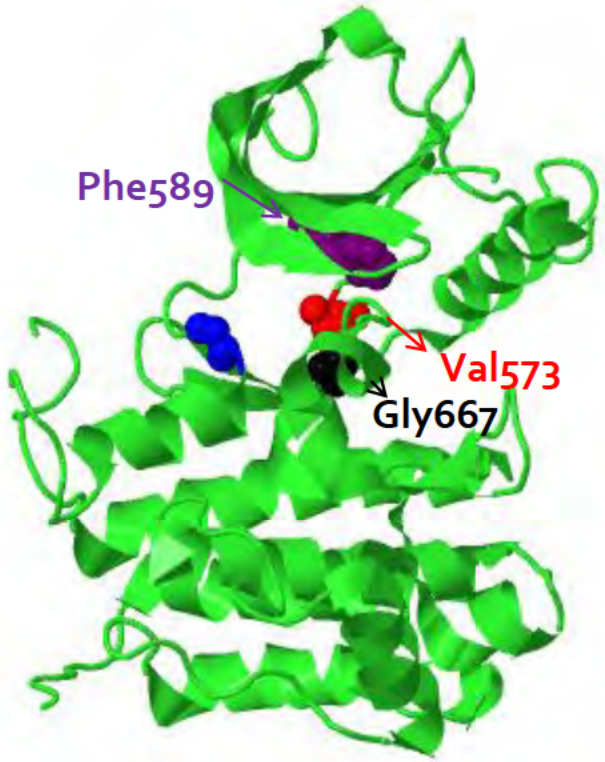
Kinase	IC50 (nM)
TrkA	1.7
TrkB	0.1
TrkC	0.1
ROS1	0.2
ALK	1.6

Entrectinib (RXDX-101)

- **Active against potential Trk inhibitor resistance mutations at clinically achievable exposures**
  - *NTRK1* F589L (gatekeeper)
  - *NTRK1* V573M
  - *NTRK1* G667S

Mutation in TrkA	LOXO-101 IC_{50} (nM)	Entrectinib IC_{50} (nM)	Entrectinib Human Exposure Equivalent (nM)
F589L	959.4	9.7	58.2
V573M	534.5	24.2	145.2
G667S	185.3	14.6	87.6
Wildtype	15.0	2.3	13.8

AACR Abstract 2136, Data generated by Ignyta



TrkA Kinase domain

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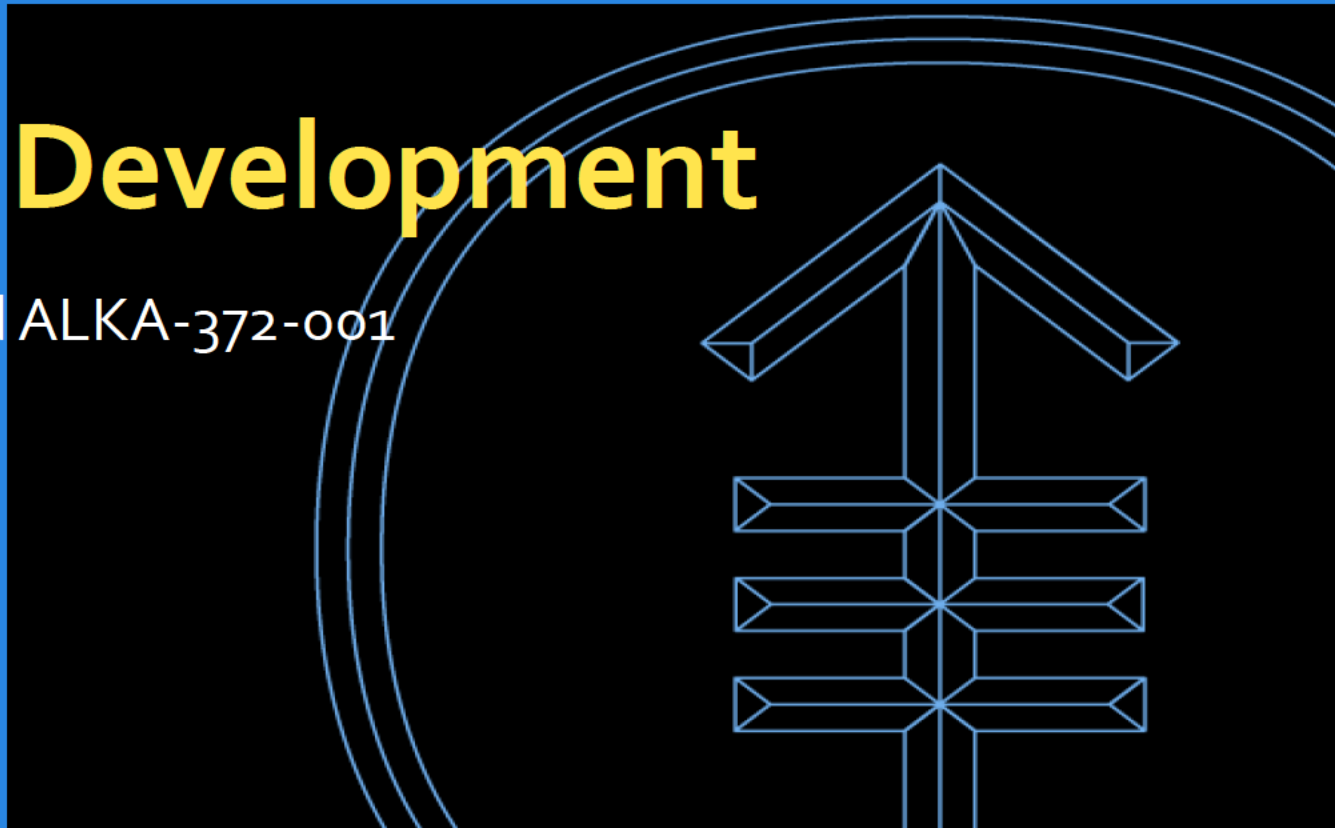
Phase I Development

STARTRK-1 and ALKA-372-001



Entrectinib: Phase I Studies

ALKA-372-001 (n=54)

- Dosing: intermittent & continuous
- *NTRK/ROS1/ALK* alterations
- Italy
  - FIH study: Nerviano Medical Sciences in October 2012 → Ignyta assumed responsibility in November 2013

STARTRK-1 (n=65)

- Dosing: continuous
- *NTRK/ROS1/ALK* alterations
- US, EU, Asia
  - Ignyta initiated in July 2014

- RP2D: 600 mg PO once daily, continuous
- Total clinical experience: **n=119 patients**
  - Updated safety and efficacy data
  - Data cut-off: March 7, 2016

Baseline Characteristics

	ALKA-372-001 (n=54)	STARTRK-1 (n=65)	TOTAL (n=119)
Age, years, median (range)	53 (46-63)	57 (46-66)	55 (46-66)
Sex, male/female (%)	44/56	48/52	46/54
ECOG performance status, n (%)			
0	30 (56)	22 (34)	52 (44)
1	21 (39)	41 (63)	62 (52)
2	2 (4)	2 93)	4 (3)
Unknown	1 (2)	0	1 (1)
Prior Cancer Therapies, n (%)			
0	0	6 (9)	6 (5)
1 -2	0	15 (23)	15 (13)
3 - 4	3 (6)	25 (39)	28 (24)
> 4	51 (94)	19 (29)	70 (59)
Tumor type, n (%)			
NSCLC	35 (65)	36 (56)	71 (60)
Gastrointestinal Tract	9 (17)	9 (14)	18 (15)
CNS	4 (7)	1 (2)	5 (4)
Head & Neck	1 (2)	4 (6)	5 (4)
Other (e.g., sarcomas, breast, melanoma, RCC, ovarian)	5 (9)	15 (23)	20 (17)

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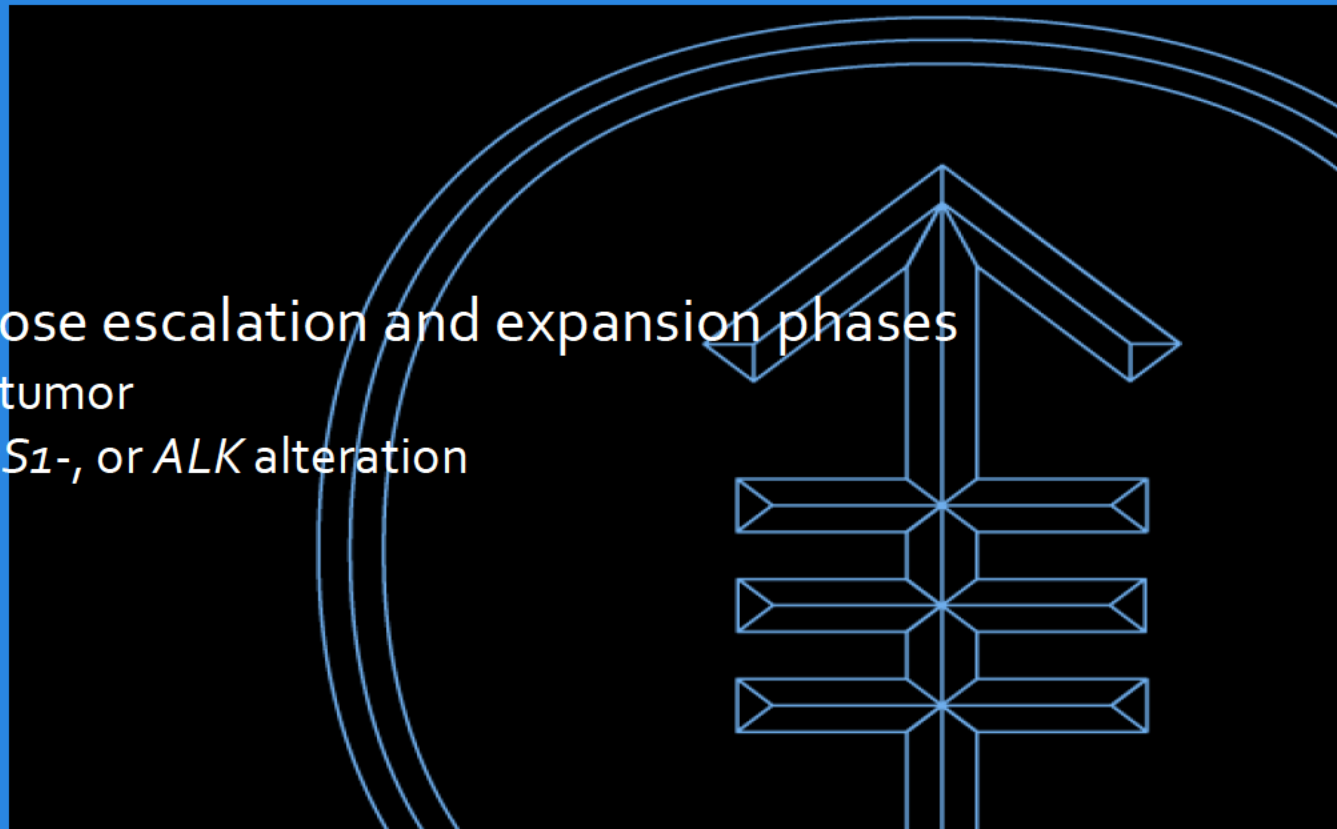
Safety

All patients in dose escalation and expansion phases

-advanced solid tumor

-*NTRK1/2/3*-, *ROS1*-, or *ALK* alteration



Treatment-Related Adverse Events

Adverse Events (AEs) in >10% of Patients at Any Dose Level (n=119)

Adverse Event Term, n (%)	Grades 1-2	Grade 3	Total
Fatigue/Asthenia	47 (40)	5 (4)	52 (44)
Dysgeusia	49 (41)		49 (41)
Paresthesia	33 (28)		33 (28)
Nausea	29 (24)		29 (24)
Myalgia	26 (22)		26 (22)
Diarrhea	22 (19)	1 (1)	23 (19)
Dizziness	19 (16)		19 (16)
Arthralgia	17 (14)	1 (1)	18 (15)
Vomiting	18 (15)		18 (15)
Constipation	14 (12)		14 (12)

- AEs were classified via CTCAE v4.0; all reversible with dose modifications
- No evidence of cumulative hepatic or renal toxicity, or QTc prolongation
- Only 2 DLTs occurred (STARTRK-1): grade 3 cognitive disturbance, grade 3 idiopathic eosinophilic myocarditis

Treatment-Related Adverse Events

Adverse Events (AEs) in >10% of Patients at the RP2D (n=45)

Adverse Event Term, n (%)	Grades 1-2	Grade 3	Total
Dysgeusia	21 (47)		21 (47)
Fatigue/Asthenia	17 (38)	1 (2)	18 (40)
Constipation	10 (22)		10 (22)
Weight Increased	8 (18)	1 (2)	9 (20)
Diarrhea	7 (16)	1 (2)	9 (18)
Nausea	8 (18)		8 (18)
Myalgia	7 (16)		7 (16)
Paresthesia	7 (16)		7 (16)
Dizziness	6 (13)		6 (13)
Peripheral Sensory Neuropathy	4 (9)	2 (4)	6 (13)
Anemia	2 (4)	3 (7)	5 (11)
Dysphagia	4 (9)	1 (2)	5 (11)
Vomiting	5 (11)		5 (11)

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Efficacy

Phase 2-Eligible Population

Population

"Phase 2-Eligible Population" (n=25)

- *NTRK1/2/3*-, *ROS1*-, or *ALK*-rearranged solid tumor
- TKI treatment-naïve
- treated at or above RP2D

Molecular Testing: local testing performed

- FISH
- next-generation sequencing

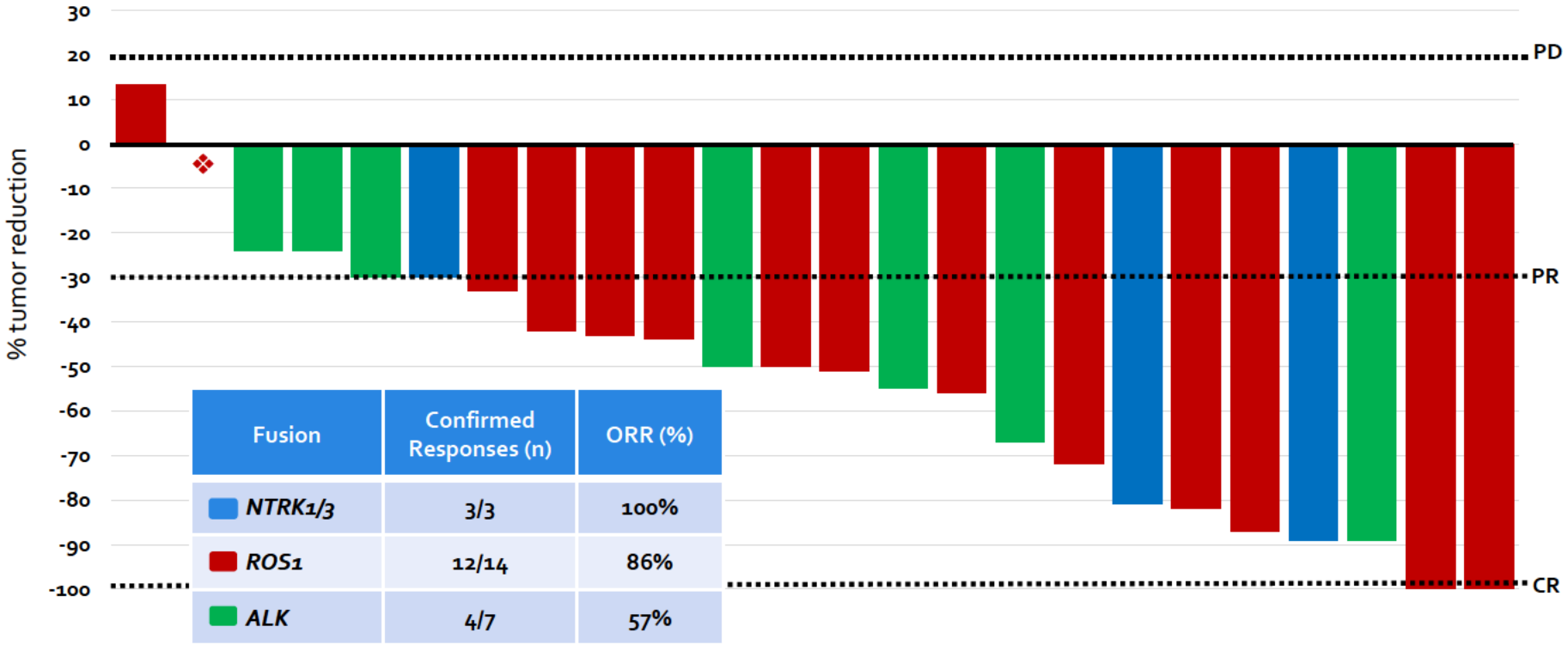
Response Evaluation

- RECIST v1.1, locally assessed and confirmed (n=24)
- volumetric assessment (n=1; primary brain tumor*)

* RECIST criteria not validated in primary brain tumors (FDA-AACR Brain Tumor Endpoints Workshop 2006)

Antitumor Activity

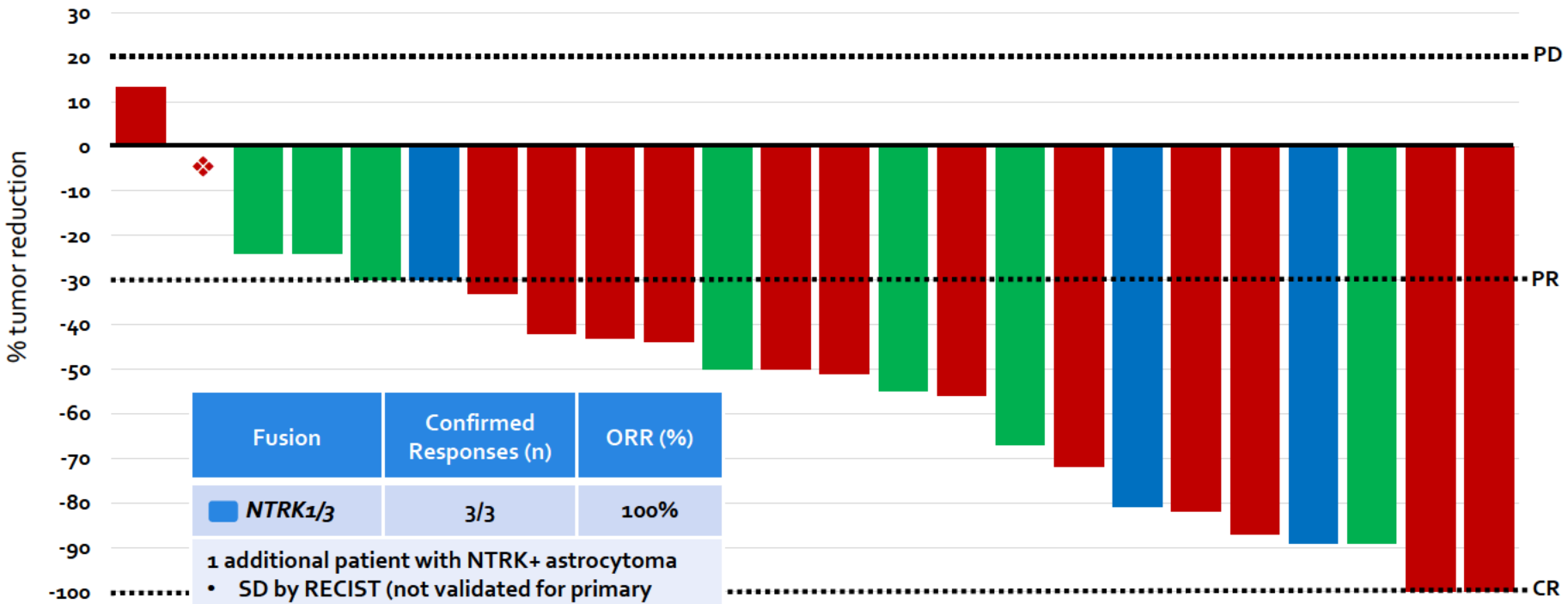
Best Response in TKI Treatment-Naïve *NTRK*-, *ROS1*-, and *ALK*-rearranged Tumors (n=24)



Fusion	Confirmed Responses (n)	ORR (%)
NTRK1/3	3/3	100%
ROS1	12/14	86%
ALK	4/7	57%

Antitumor Activity

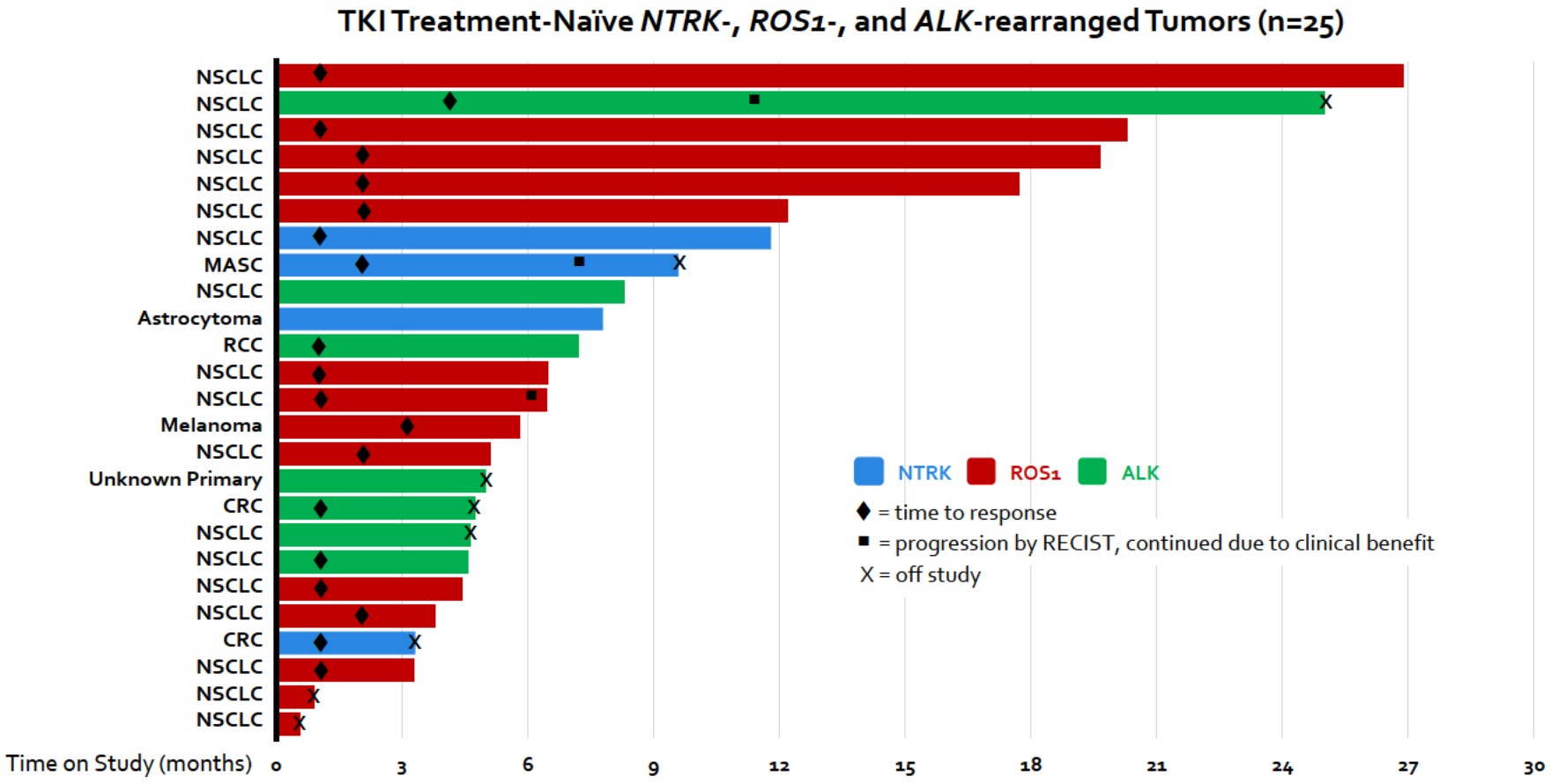
Best Response in TKI Treatment-Naïve *NTRK*-, *ROS1*-, and *ALK*-rearranged Tumors (n=24)



Fusion	Confirmed Responses (n)	ORR (%)
NTRK1/3	3/3	100%

1 additional patient with NTRK+ astrocytoma
- SD by RECIST (not validated for primary brain tumors)
- 45% by exploratory 3-D volumetric assessment

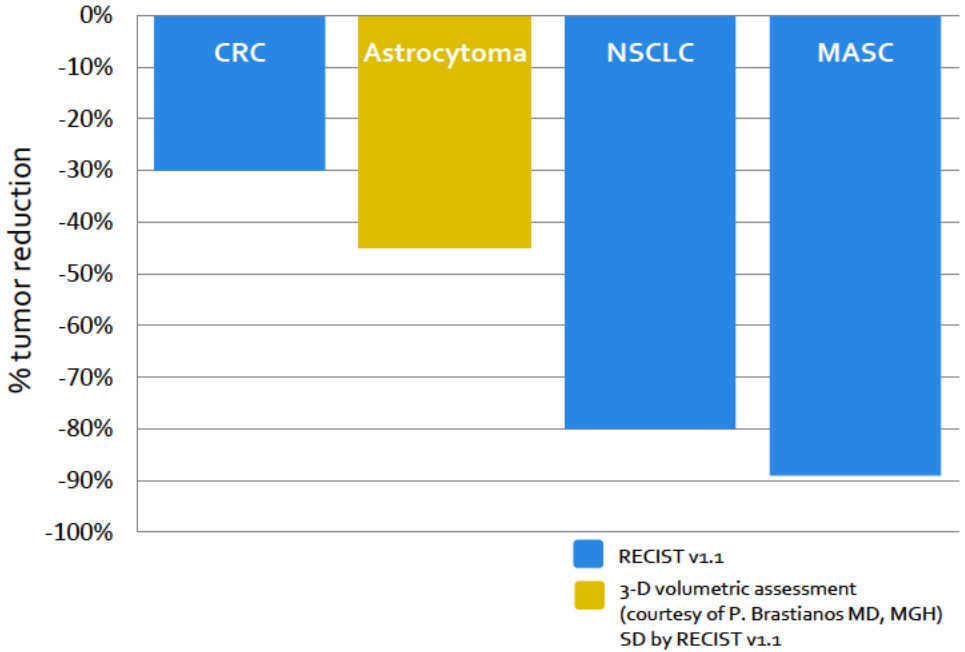
Duration of Clinical Benefit



NTRK-Rearranged Cancers

Best Response in TKI Treatment-Naïve *NTRK*-rearranged Tumors (n=4)

- Response achieved in 100% of tumors
  - Rapid (within 1 month of treatment) and prolonged (~1 year, ongoing) responses were observed
- Response achieved in a variety of histologies and fusion types
  - CRC: *LMNA-NTRK1*
  - Astrocytoma: *BCAN-NTRK1*
  - NSCLC: *SQSTM1-NTRK1*
  - MASC: *ETV6-NTRK3*



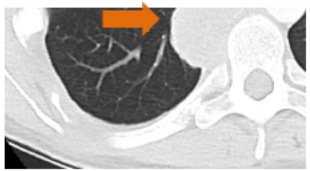
Response to Entrectinib

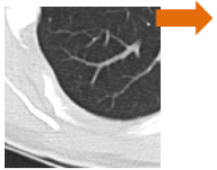
34/F with metastatic *ETV6-NTRK3*-rearranged MASC

- Resected stage III disease and post-operative RT in 2006
- Recurred in 2011 and treated with 3 lines of cytotoxic chemotherapy and RT
- NGS revealed an *ETV6-NTRK3* rearrangement
- Enrolled onto STARTRK-1 in 2015 → **durable PR**, 10 months of entrectinib treatment

baseline

9 weeks on therapy





Images: Drilon, MSKCC

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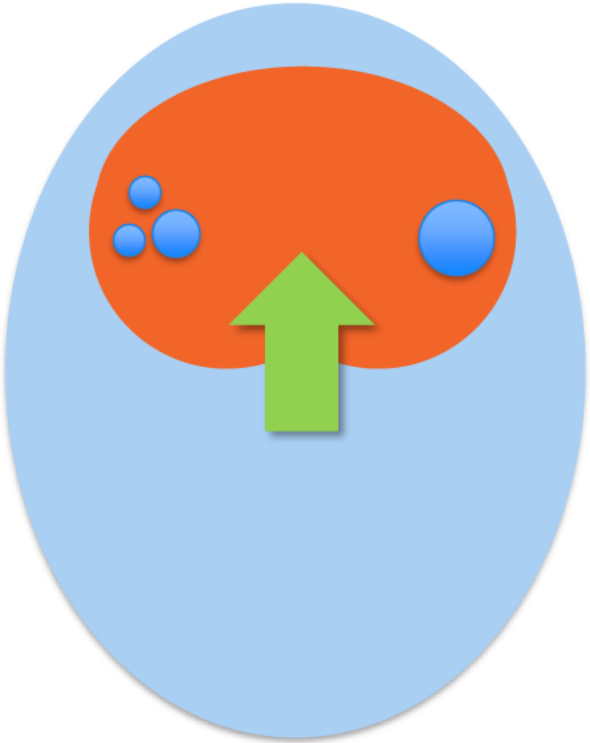
CNS Activity

CNS Disease in Cancer

Brain metastases
-20-40% of all patients with cancer
  -lung (up to 50%)
  -breast
  -melanoma



Primary brain tumors
-astrocytoma (*NTRK2* fusions: 3%)
-glioblastoma (*NTRK1* fusions: 1-2%)
-pediatric gliomas (*NTRK3* fusions: 7%)

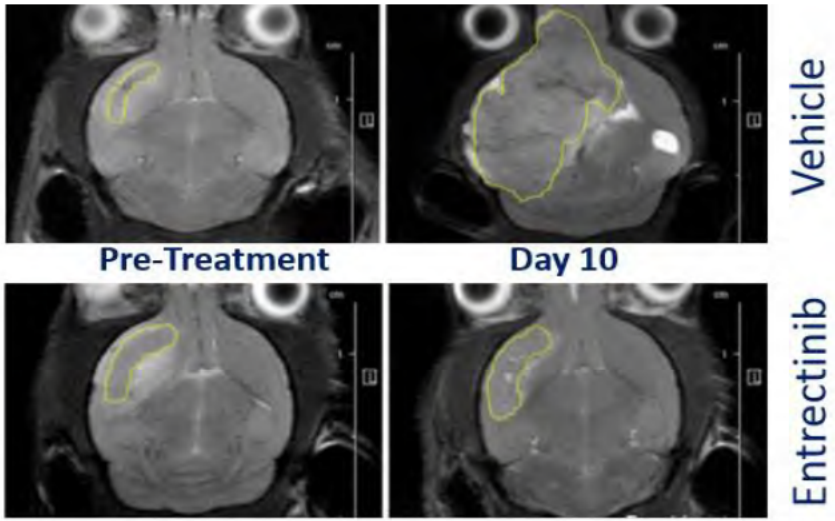
Optimal therapy would address both systemic and CNS disease

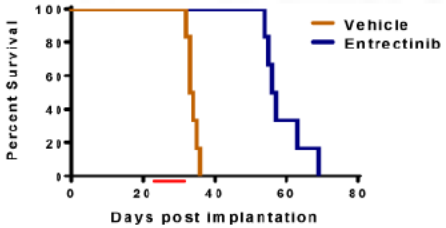
CNS Activity of Entrectinib

- **Entrectinib was designed to cross the blood brain barrier**
  - Brain/Blood ratio

Mouse	0.4
Rat	0.6-1.0
Dog	1.2-1.4

- **Preclinical CNS activity**
  - *EML4-ALK*-rearranged NCI-H228 cells injected intracranially
  - treated with entrectinib orally vs vehicle for 10 days



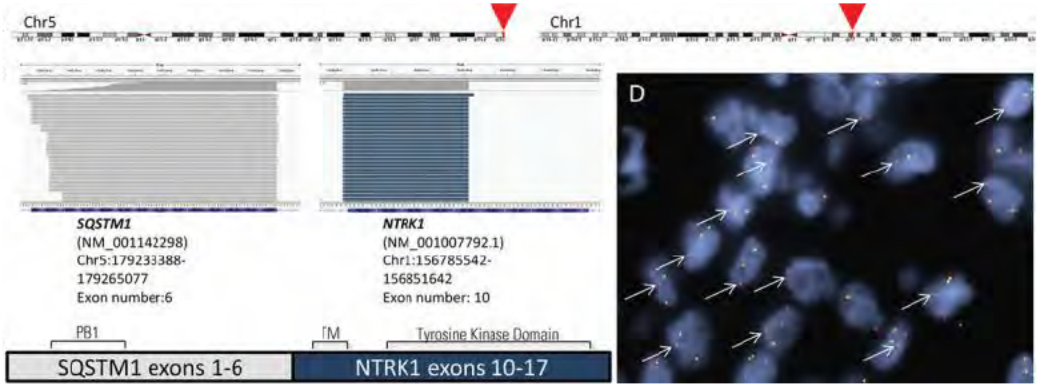


	Median Survival
Vehicle	33.5 days
Entrectinib	56.5 days
P-value:	$<5x10e^{-4}$

Response to Entrectinib

46/M with metastatic *SQSTM1-NTRK1*-rearranged NSCLC

- Diagnosed in November 2013 with widely metastatic disease
- 4 prior therapies including anti-PD-1 therapy: carboplatin/pemetrexed, pembrolizumab, docetaxel, vinorelbine

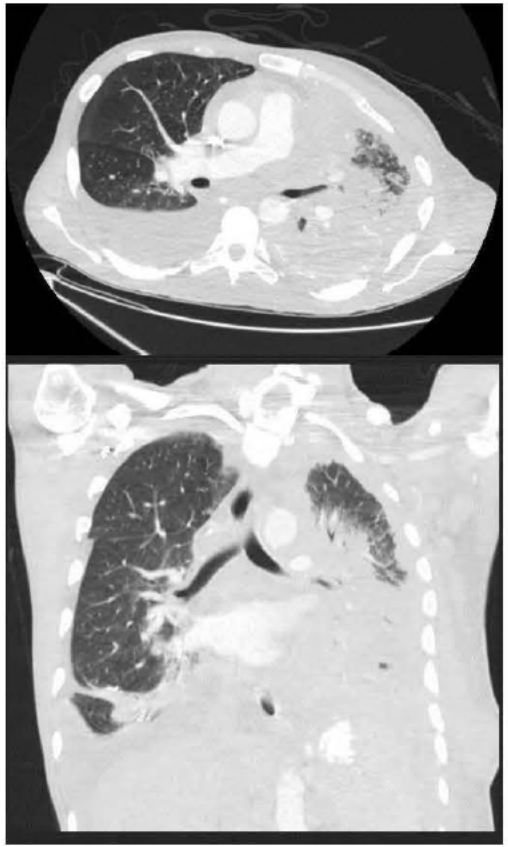


- Poor baseline performance status (ECOG 2), on supplemental O_2 and in hospice
- Enrolled in STARTRK-1 March 2015

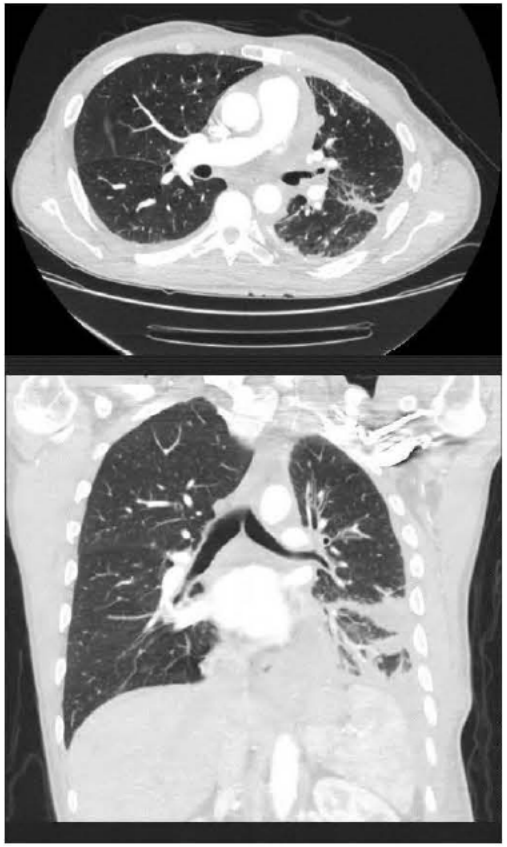
Farago *et al*, J Thorac Oncol. 2015 Dec;10(12):1670-4.

Extracranial Response to Entrectinib

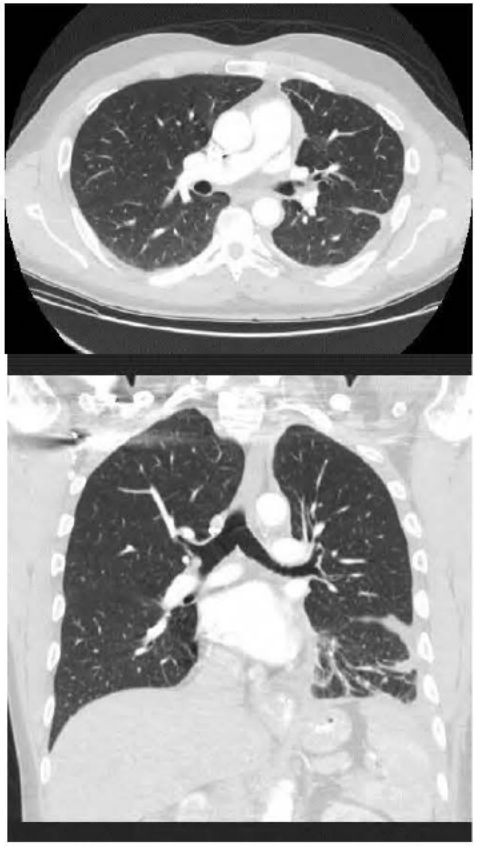
Baseline



Day 26: - 47% response



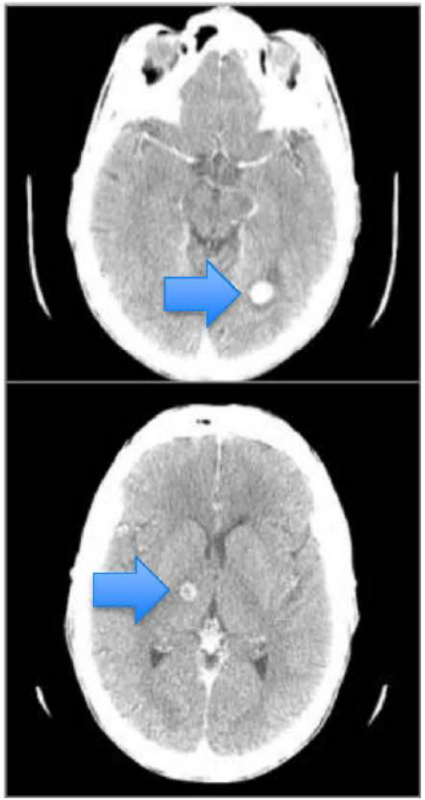
Day 317: - 79% response



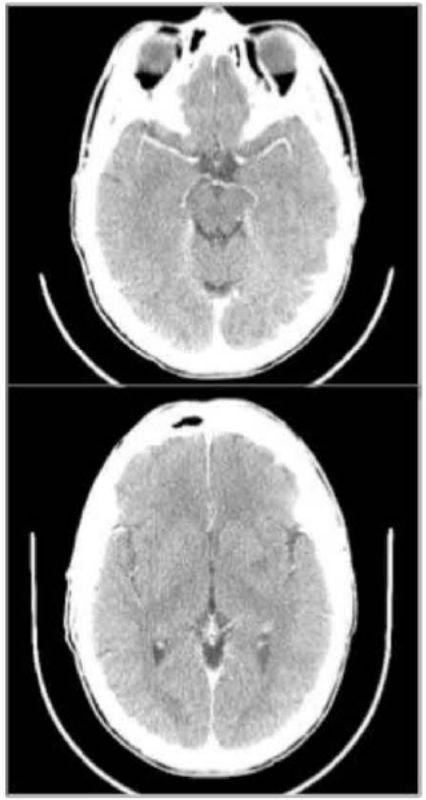
Images: Farago and Shaw, MGH

Intracranial Response to Entrectinib

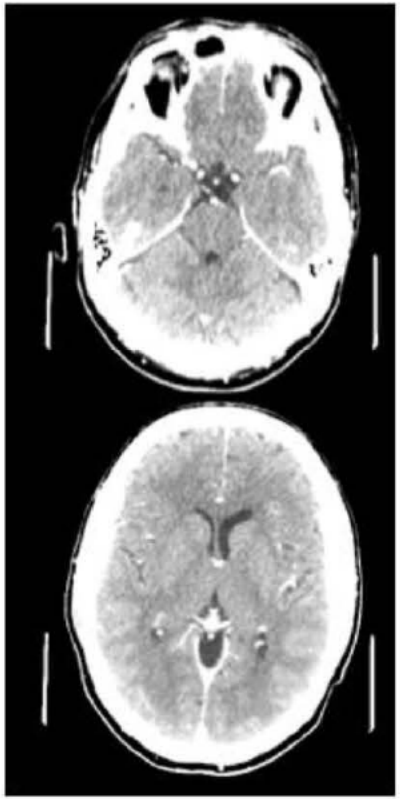
Baseline



Day 26: CR



Day 317: CR

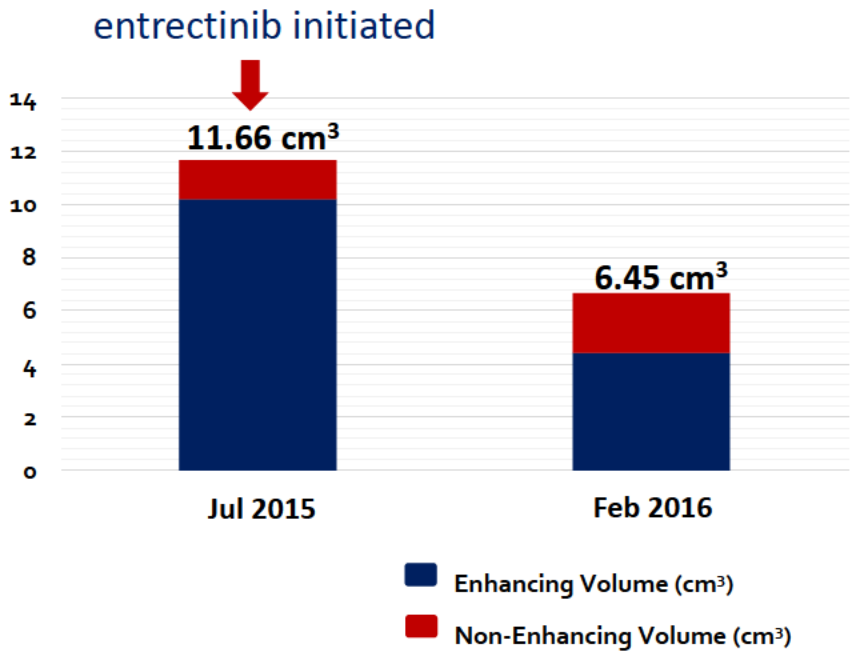


Remains on entrectinib and clinically progression-free at >12 months

Images: Farago and Shaw, MGH

Primary Brain Tumor Response to Entrectinib

- **57/M with low-grade astrocytoma harboring a *BCAN-NTRK1* gene rearrangement**
  - Unresectable pontine tumor
  - SD by RECIST (not validated for primary brain tumors)
  - Exploratory 3-Dimensional volumetric tumor assessment performed showed a 45% decrease in tumor burden
  - Improvements in clinical symptoms of ataxia and diplopia



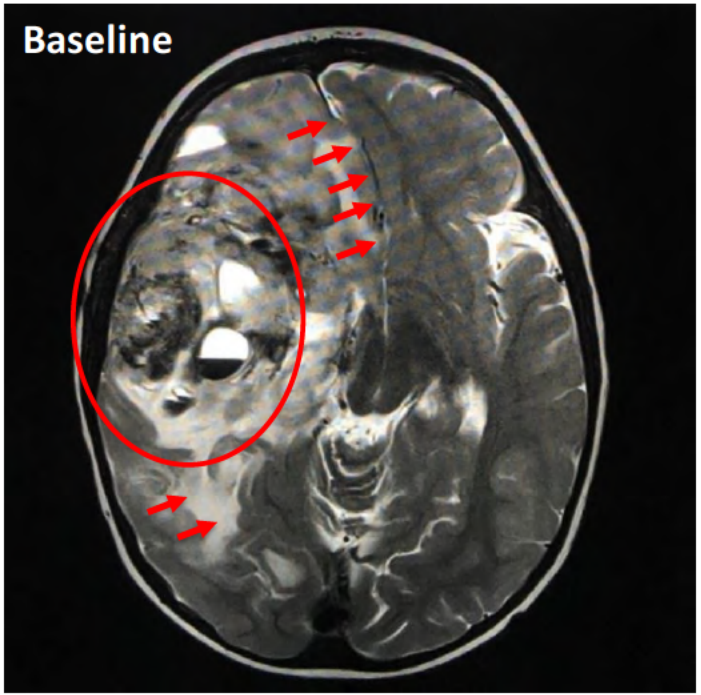
Tumor 3-D volumetric assessments courtesy of P. Brastianos MD (MGH)

Response to Entrectinib

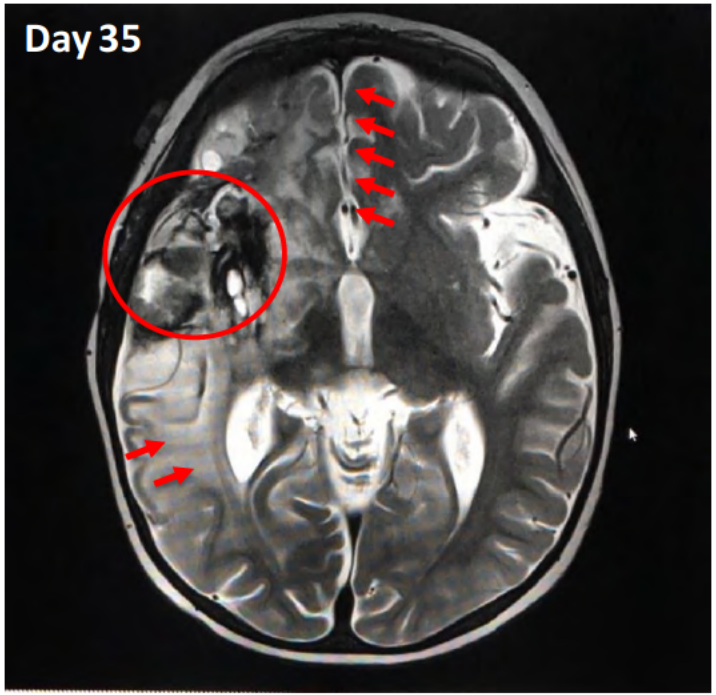
- **20 month-old boy with recurrent, metastatic infantile fibrosarcoma harboring an *ETV6-NTRK3* gene rearrangement**
  - Presented at birth with left leg mass, requiring through-the-knee amputation
    - Age 4 months, large metastases to left lung identified → 24-weeks of chemotherapy
    - Age 12 months, large right frontal intracranial tumor identified → resected, followed by 5 cycles of salvage chemotherapy
    - Recurrent CNS disease with lesions in the right frontal and temporal lobes, as well as leptomeningeal involvement
  - Received entrectinib starting February 2016

Response to Entrectinib

ETV6-NTRK3 gene rearranged metastatic fibrosarcoma in 20-month old



massive peritumoral edema, midline shift, transtentorial herniation, progressive lethargy



decreased tumor and edema, patient with increased alertness, resumed eating and crawling

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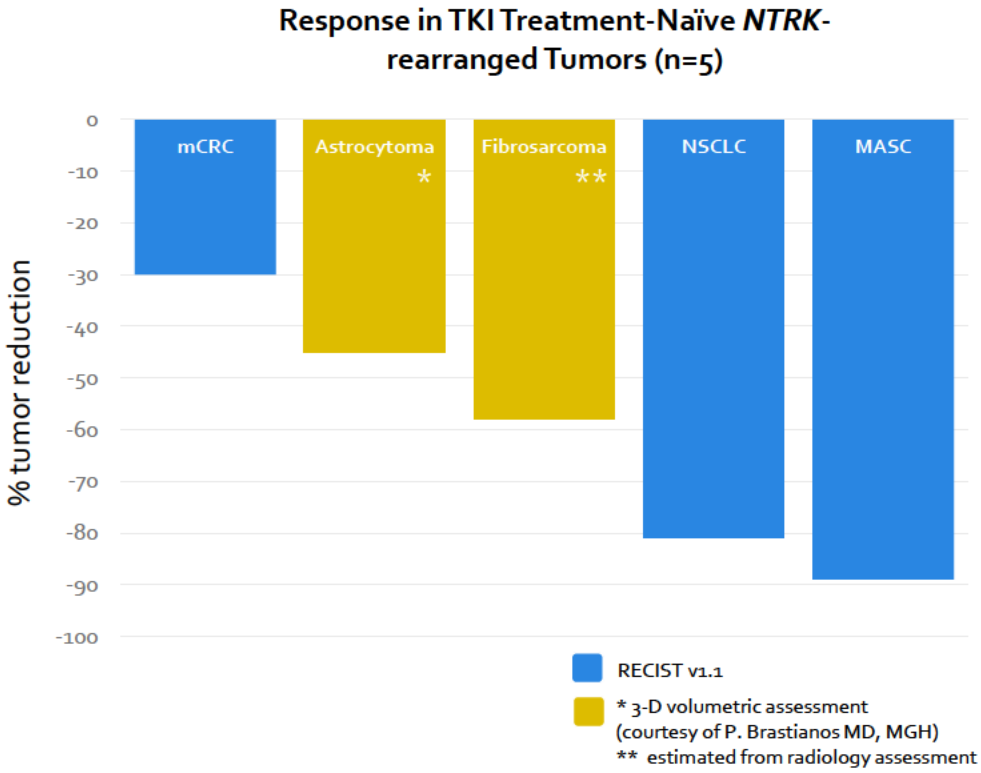
Summary

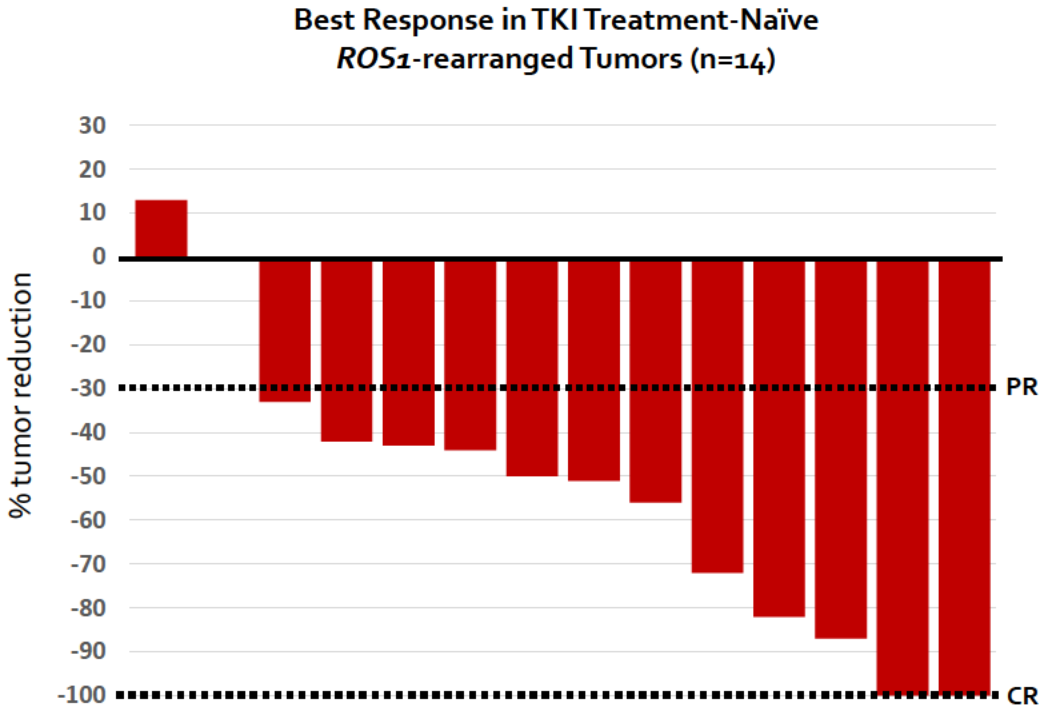
NTRK-Rearranged Cancers

- **Entrectinib is a potent TrkA/B/C Inhibitor**
  - Large safety experience (119 patients)
  - Rapid and durable responses
- **Response in 100% (5/5) of patients achieved in a variety of histologies and fusion types**
  - CRC: *LMNA-NTRK1*
  - Astrocytoma: *BCAN-NTRK1*
  - Infantile fibrosarcoma: *ETV6-NTRK3*
  - NSCLC: *SQSTM1-NTRK1*
  - MASC: *ETV6-NTRK3*
- **Dramatic intracranial activity in 100% of patients with CNS disease (3/3)**
  - 3/5 of patients treated in Phase 1 setting had primary or metastatic CNS disease
  - only Trk inhibitor with demonstrated CNS activity thus far



ROS1-Rearranged Cancers

- Response achieved in 86% (12/14) of TKI-naïve tumors
  - Two complete responders
  - Rapid (within 1 month of treatment) and prolonged responses were observed
    - In NSCLC, ORR of 85% (11/13 patients)
    - One additional response in melanoma
  - Longest ongoing response approaching 2 years and 3+ months



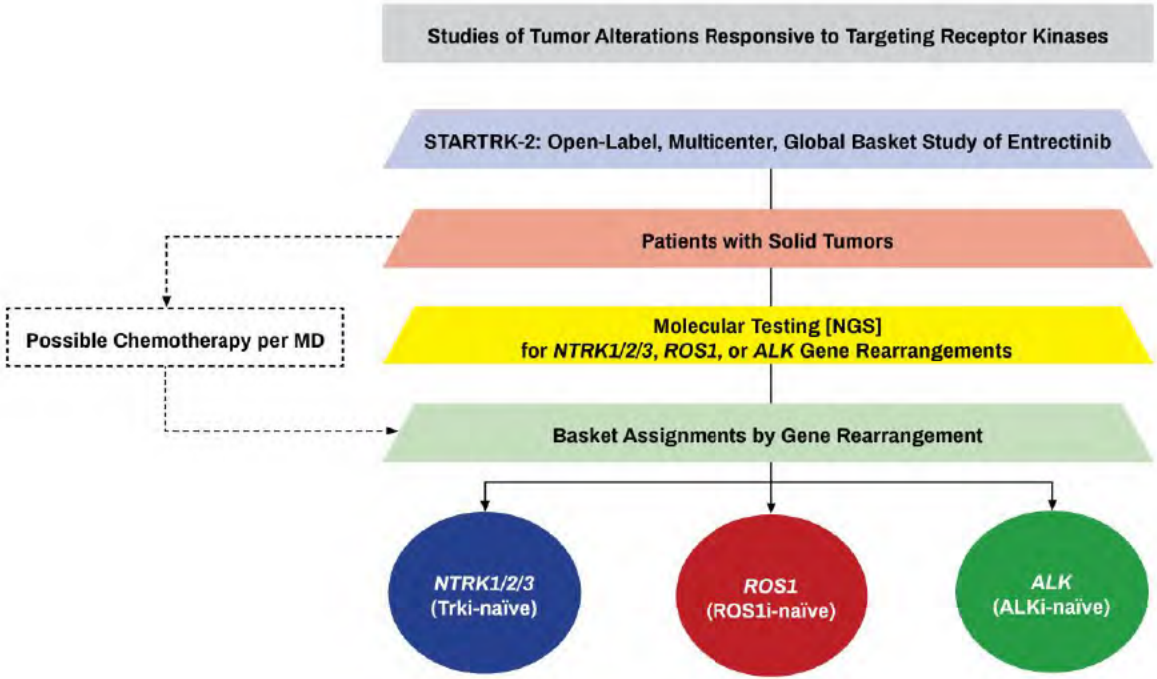
Conclusions

- **Entrectinib is safe and well-tolerated.**
  - 119 patients have been treated: 45 patients at the RP2D of 600 mg daily
  - therapy duration: 19 patients > 6 months (11 patients > 1 year, including 3 patients > 2 years)
- **Entrectinib is an active targeted therapy for *NTRK*-, *ROS1*-, and *ALK*-rearranged cancers.**
  - confirmed responses observed in 19/24 (79%) patients with extracranial solid tumors; in addition, evidence of tumor shrinkage observed in a patient with *NTRK*+ astrocytoma
  - brisk (within 4 weeks) and durable (up to 2 years and 3+ months) responses were achieved
  - *NTRK*-rearranged tumors
    - response achieved in 5 different histologies in both adult and pediatric patients
- **Entrectinib is highly CNS-penetrant.**
  - durable responses in both primary brain tumors and metastatic disease
  - complete response observed in the CNS

Current Directions

STARTRK-2

An Open-Label, Multicenter, Global Phase 2 Basket Study of Entrectinib for the Treatment of Patients with Locally Advanced or Metastatic Solid Tumors that Harbor *NTRK1/2/3*, *ROS1*, or *ALK* Gene



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Thank You