LOXO-101

Briefing Document for the 29 June 2016 Oncologic Drugs Advisory Committee Pediatric Subcommittee

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Term
AE	adverse event
AIEOP-STSC	Associazione Italiana Ematologia Oncologia Pediatrica–Soft Tissue Sarcoma Committee
ALK	anaplastic lymphoma kinase
ALL	acute lymphoblastic lymphoma
AST	atypical Spitz tumor
ATP	adenosine triphosphate
BDNF	brain-derived neurotrophic factor
BID	twice daily
BRAF	B-Raf proto-oncogene
CDK	cyclin-dependent kinase
CFS	congenital fibrosarcoma
CMN	congenital mesoblastic nephroma
CNS	central nervous system
CR	complete response
CT	computed tomographic
DIPG	diffuse intrinsic pontine glioma
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
EFS	event-free survival
ERK	extracellular signal-related kinase
ETV6	ETS variant gene 6
FISH	fluorescent in situ hybridization
GI	gastrointestinal
hERG	human ether-à-go-go-related gene
HGGs	high-grade gliomas
HPC	hemangiopericytoma
IC_{50}	50% inhibitory concentration
IC_{90}	90% inhibitory concentration
ICCC	International Classification of Childhood Cancer
ID	ifosfamide and doxorubicin
IFS	infantile fibrosarcoma
IND	investigational new drug
LGGs	low-grade gliomas
MAP	mitogen-activated protein
MAPK	mitogen-activated protein kinase
MASC	mammary analogue secretory cancer

Abbreviation	Term
MPRIP	myosin phosphatase-Rho-interacting protein
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
MYCN	neuroblastoma MYC oncogene
NBS-HGG	non-brain stem high grade gliomas
NCI	National Cancer Institute
NCI-MATCH	National Cancer Institute – Molecular Analysis For Therapy Choice
NGF	nerve growth factor
NGS	next generation sequencing
NSCLC	non-small cell lung cancer
NT-3	neurotrophin-3
NT-4/5	neurotrophin-4/5
NTRK	neurotrophic tyrosine kinase receptor gene (referring to family)
OS	overall survival
PD	progressive disease
Ph + ALL	Philadelphia chromosome-positive acute lymphoblastic leukemia
Ph like-ALL	Philadelphia chromosome like acute lymphoblastic lymphoma
PK	pharmacokinetics
PO	*per os*, orally
PR	partial response
PTC	papillary thyroid cancer
QD	once daily
RANO	Response Assessment in Neuro-Oncology Criteria
RAS	rat sarcoma/ras protein
RECIST	Response Evaluation Criteria in Solid Tumors
RET	rearranged during transfection oncogene
RMS	rhabdomyosarcoma
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
SEER	Surveillance of Epidemiology and End Results
SIOP-MMT	International Society of Pediatric Oncology–Malignant Mesenchymal Tumor Committee
STSs	soft tissue sarcomas
TEAE	treatment emergent adverse event
TNK2	non-TRK kinase
TPM3	tropomyosin 3
TRK	tropomyosin-related kinase (referring to family)
TRIM24	tripartite motif containing 24

Abbreviation	Term
VA	vincristine plus actinomycin-D
VAC	vincristine, actinomycin-D and cyclophosphamide (VAC)
WHO	World Health Organization

1.0 EXECUTIVE SUMMARY

LOXO-101 is a potent, highly selective adenosine triphosphate (ATP)-competitive inhibitor of the tropomyosin-related kinases (TRK) TRKA, TRKB, and TRKC. In cancer biology, the neurotrophic TRK (NTRK) genes, which encode for the TRK kinases, are subject to dominant oncogenic activating rearrangements (fusions) that lead to constitutive activation of downstream signaling pathways. Based on results of ongoing clinical studies in adult patients (LOXO-TRK-14001, LOXO-TRK-15002), LOXO-101 is expected to deliver clinically relevant, single-agent efficacy in pediatric patients whose tumors harbor an NTRK gene fusion. However, NTRK gene fusions in pediatric cancer either 1) occur at high frequency in very rare tumors; or 2) occur at low frequency in more common tumors. Therefore, the efficient development of LOXO-101 in pediatric patients will require increased education of pathologists and clinical professionals, as well as the widespread adoption of diagnostic methods with sufficient sensitivity to detect NTRK gene fusions.

NTRK fusions are rare and occur across a number of tumor histologies. The first pediatric NTRK fusion was reported in 1998, with the identification of a fusion of ETS variant gene 6 *(ETV6)* and *NTRK3* as the predominant genetic feature of both congenital fibrosarcoma (CFS) and cellular congenital mesoblastic nephroma (CMN) (Knezevich, Garnett et al. 1998, Knezevich, McFadden et al. 1998). More recent reports have implicated *ETV6-NTRK3* fusions in children with Ph+-like acute lymphoblastic lymphoma (Ph+-ALL) (Roberts, Li et al. 2014). NTRK gene fusions have also been identified in pediatric astrocytomas, high-grade gliomas, sarcomas, and thyroid cancer.

LOXO-101 has low nanomolar potency against all three TRK enzymes, with 100- to 1,000-fold selectivity relative to other kinase and non-kinase targets, and has shown dramatic effects in preclinical cancer models harboring TRK fusion proteins. Given the role neurotrophins play in the developing nervous system, LOXO-101 was designed to have limited distribution into central nervous system (CNS) tissues. Preclinical and clinical evidence suggests that sustained inhibition of TRK signaling in the nervous system can result in adverse events (AEs) such as ataxia, behavior changes, and paresthesia (Hempstead, Rabin et al. 1992, Weiss, Hidalgo et al. 2012), yet anti-tumor efficacy in the nervous system may be achieved with pulsatile exposures that exceed certain inhibitory concentration thresholds. LOXO-101 has shown encouraging preliminary evidence of anti-tumor activity in an adult patient with an NTRK gene fusion cancer with CNS metastases (in Study LOXO-TRK-14001).

LOXO-101 is orally dosed (PO) twice daily (BID) as a capsule or as a liquid. The safety and efficacy of LOXO-101 is currently being evaluated in 3 clinical trials: An adult Phase 1 dose-escalation study in advanced cancer patients (LOXO-TRK-14001), an adult Phase 2 basket study in NTRK fusion-positive tumors (LOXO-TRK-15002), and a Phase 1 dose-escalation study in pediatric advanced cancer patients or patients with primary CNS tumors (LOXO-TRK-15003). In the adult Phase 1 study (LOXO-TRK-14001), LOXO-101 has shown promising efficacy and durability in cancer patients with NTRK gene fusions, with 5 of 6 patients achieving a confirmed partial response (PR) by Response Evaluation Criteria in Solid Tumors (RECIST), and the sixth patient demonstrating regression that did not meet RECIST criteria (Hong, Farago et al. 2016). As of the 16 February 2016 data cutoff, no NTRK gene fusion patient had progressed, with the longest response continuing at

14 months. In addition, the first patient enrolled on the LOXO-101 pediatric Phase 1 study (LOXO-TRK-15003) achieved a rapid and durable tumor response in the setting of infantile fibrosarcoma (Nagasubramanian, Wei et al. 2016).

LOXO-101 appears to be well-tolerated at all dose levels with the most common treatment-emergent adverse events (TEAEs) reported as gastrointestinal system disorders; the most common individual TEAEs include fatigue, constipation, and dizziness.

NTRK gene fusion cancers in adults and children appear to be susceptible to therapeutic TRK inhibition with LOXO-101, regardless of tumor context. In the ongoing Phase 1 adult study (LOXO-TRK-14001), patients with NTRK gene fusions represent five different histologic diagnoses and the published case report of a pediatric infantile fibrosarcoma patient represents a sixth tumor type (Hong, Farago et al. 2016, Nagasubramanian, Wei et al. 2016).

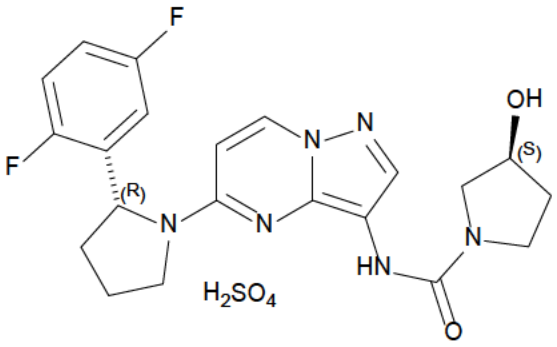
The diversity and rarity of NTRK gene fusion cancers require a systems approach to drug development. Stakeholders who can accelerate or hinder this opportunity include drug sponsors, clinical testing labs, clinical investigators, regulatory agencies, and payors (who control access to clinical testing). While LOXO-101 is well-suited to pediatric drug development—it is easily formulated as a liquid, highly selective, and has favorable tolerability as preliminarily assessed—the widespread identification of patients with NTRK gene fusions represents the most meaningful barrier to the efficient development of LOXO-101. We hope that the many stakeholders mentioned above work together to find creative solutions to democratize comprehensive tumor testing so that all patients can access personalized medicines such as LOXO-101.

2.0 DESCRIPTION OF THE MOLECULE AND MECHANISM OF ACTION

2.1 Description and Mechanism of Action

LOXO-101 is an oral, rationally designed, highly selective, and potent small molecule that blocks the adenosine triphosphate (ATP) binding site of the tropomyosin-related kinases (TRK) TRKA, TRKB, and TRKC. LOXO-101 is a 3-urea-substituted pyrazolo[1,5a]-pyrimidine with the empirical formula of $C_{21}H_{24}F_2N_6O_6S$ and a molecular weight of 526.52 (428.4 as the free base) (Figure 1).

Figure 1. Chemical Structure of LOXO-101



3.0 REGULATORY HISTORY

Loxo Oncology is currently conducting studies under two active investigational new drug (IND) applications. LOXO-101 received orphan drug designation in the US and Europe for the treatment of soft tissue sarcoma (STS).

4.0 RATIONALE FOR THE DEVELOPMENT OF LOXO-101 FOR THE TREATMENT OF ADVANCED PEDIATRIC SOLID OR PRIMARY CENTRAL NERVOUS SYSTEM TUMORS

4.1 Tropomyosin-Related Kinase: Normal Physiology

In normal cells, the TRK family of tyrosine kinase receptors is involved in the regulation of the growth, differentiation, and survival of neurons (Nakagawara 2001). The TRK family of receptors, TRKA, TRKB, and TRKC, are encoded by the NTRK genes *NTRK1*, *NTRK2*, and *NTRK3*, respectively, and are the preferred receptors of the neurotrophin family of growth factors, which includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) (Nakagawara 2001).

Following ligand binding, TRK receptors dimerize, become catalytically active and transphropshorylate specific tyrosine residues within the cytoplasmic-facing region of their dimer counterpart. The propagation of these TRK-induced signals may stimulate growth, survival, and differentiation. Activated TRK receptors signal through many pathways, including the phosphatidylinositol 3-kinase (PI3K) , phospholipase C-ɣ (PLC ɣ), extracellular signal-related kinase 1 and 2-mitogen-activated protein kinase (ERK 1-2 MAPK) and ERK5 MAPK pathways (Rubin and Segal 2001).

The human TRKA receptor is encoded by the *NTRK1* gene. This kinase is a membrane-bound receptor that, upon activation by one of its ligands such as NGF or NT-3, phosphorylates itself and members of the MAPK pathway. The TRKA receptor is involved in cell differentiation and may play a role in determining sensory neuron subtype. Germline mutations in the *NTRK1* gene in humans can lead to a defective TRKA receptor that has been associated with congenital insensitivity to pain, anhidrosis, self-mutilating behavior, mental retardation, and cancer (Indo 2012).

The human TRKB receptor is encoded by the *NTRK2* gene. This kinase is a transmembrane receptor which binds preferentially to BDNF and neurotrophin-4/5 (NT-4/5) (Skaper 2008). Mice lacking TRKB have drastically reduced numbers of sensory neurons, but the number of sensory neurons is not affected in mice lacking *Bdnf* or NT-4/5 (Skaper 2008). Yeo and colleagues published a case report of a male child with a *de novo* loss-of-function mutation of *NTRK2* who demonstrated global developmental delay, impairment of short-term memory, and impaired nociception, as well as severe early onset obesity (Yeo, Connie Hung et al. 2004).

The TRKC receptor is encoded by the *NTRK3* gene. This kinase is a membrane-bound receptor which binds preferentially to NT-3 (Skaper 2008).

Data suggest that the TRKA receptor is essential for neuronal development, but somewhat dispensable in non-neuronal and post-development tissue in mice (Crowley, Spencer et al. 1994, Smeyne, Klein et al. 1994, Snider 1994, Coppola, Barrick et al. 2004, Chen, Ye et al. 2005, Skaper 2008). Work by Luikart and colleagues suggests that TRKB is required for presynaptic and postsynaptic sites during prenatal development. In homozygous *NTRK2* knockout mice, synapse numbers are significantly reduced (Luikart, Nef et al. 2005). Additional work by Chen and co-workers has confirmed that inhibition of TRK in postnatal mice does not affect nervous system development (Chen, Ye et al. 2005).

In a clinical trial, lestaurtinib (previously known as CEP-701), a multi-kinase inhibitor with pan-TRK family inhibition, was tested in a Phase 1 study in children with refractory neuroblastoma (Minturn, Evans et al. 2011). Forty-seven patients were treated with a median of 2 cycles per patient and a range of 1–28 cycles. The median age was 10.7 years (range 3.5–29.1 years). There were no reports of neurotoxicity or developmental delays in this study (Minturn, Evans et al. 2011).

4.2 Tropomyosin-Related Kinase: Cancer Pathophysiology and NTRK Fusions

In cancer, the *NTRK1, NTRK2,* and *NTRK3* genes are subject to gene rearrangements which may place them adjacent to genes such as *ETV6*, *EML4*, *NPM*, and *TPM*. The first report of an NTRK-fusion was described in colorectal cancer (CRC) in 1982, and more recently has been identified in a wide range of commonly occurring tumors, such as lung cancer, thyroid cancer, and sarcoma, though at low frequency. In very rare tumors, such as infantile fibrosarcoma (IFS), secretory/juvenile breast cancer (SBC) and mammary analogue secretory cancers (MASC) of the salivary glands, NTRK fusions are likely to be the defining genetic feature of these tumors (Vaishnavi, Le et al. 2015).

Oncogenic fusions occur via an intra- or inter-chromosomal rearrangement. The typical gene structures of an oncogenic fusion, such as those involving an NTRK gene, juxtapose the 3′ region of a proto-oncogene (encoding the kinase domain) to the 5′ sequence of an unrelated gene. The resultant novel oncoprotein is both aberrantly expressed and possesses a constitutively active kinase, leading activation of downstream oncogenic pathways.

In 2013, chromosomal rearrangements fusing the *NTRK1* gene with the myosin phosphatase-Rho-interacting protein (*MPRIP*) or *CD74* genes were identified in 2 patients with non-small cell lung cancer (NSCLC) whose tumors tested negative for other, common oncogenic drivers (Vaishnavi, Capelletti et al. 2013). Expression of each fusion oncoprotein in non-transformed fibroblasts led to constitutive TRKA kinase activity *in vitro* and *in vivo* and cellular transformation.

4.3 Tropomyosin-Related Kinase: Pediatric Cancers

TRK fusions were initially reported in pediatric cancers in 1998, with the identification of an *ETV6* - *NTRK3* fusion in the large majority of IFS (also referred to as congenital fibrosarcoma/CFS) and the cellular subtype of CMN (Knezevich, Garnett et al. 1998, Knezevich, McFadden et al. 1998). A recent report identified an *ETV6-NTRK3* fusion in a child with Philadelphia positive acute lymphoblastic lymphoma (Ph+-ALL) (Roberts, Li et al. 2014). Other TRK gene fusions have also been identified in pediatric forms of astrocytoma high-grade glioma, sarcoma and thyroid cancer.

4.4 NTRK Fusion Cancers in Children

4.4.1 Infantile Fibrosarcoma

Fibrosarcomas occur in both pediatric and adult patients. In children, their frequency follows a bimodal distribution, with the first peak in incidence before the first year of age and a second peak in early adolescence. Infantile Fibrosarcoma (IFS) accounts for 5–10% of all sarcoma diagnoses in children less than one year of age (Dillon, Whalen et al. 1995, Orbach, Rey et al. 2005, Orbach, Rey et al. 2010). Overall, the younger the IFS patient the better the

prognosis, with less loco-regional recurrences and metastatic disease than adolescent patients (Stout 1962). Adult fibrosarcoma is a clinically distinct entity with a generally poor prognosis.

IFS is characterized by polysomy of chromosomes 8, 11, 17, and 20, with trisomy of chromosome 11 is the most frequent karotypic change (Knezevich, Garnett et al. 1998, Sandberg and Bridge 2002). Higher resolution profiling of IFS (and CMN) has identified a recurrent (t12;15)(q13;q25) translocation, which generates an *ETV6-NTRK3* gene fusion, in 70 to 100% of tumors (Knezevich, Garnett et al. 1998, Knezevich, McFadden et al. 1998, Rubin, Chen et al. 1998, Bourgeois, Knezevich et al. 2000, Sheng, Hisaoka et al. 2001, Loh, Ahn et al. 2002, Sandberg and Bridge 2002, Russell, Hicks et al. 2009). Recently, Wong and colleagues published a case report of an *ETV6-NTRK3* fusion negative patient with IFS (Wong, Pavlick et al. 2016). Remarkably, the patient's tumor was found to harbor an *LMNA-NTRK1* fusion, providing further evidence that TRK fusions drive tumor biology in the large majority of IFS.

IFS tumors most commonly originate in the extremities, trunk, or head and neck region (Stout 1962, Dillon, Whalen et al. 1995, Bourgeois, Knezevich et al. 2000, Coffin and Fletcher 2002). Less common sites include the oral cavity, bronchus, or spinal meninges (Stout 1962). Overall survival (OS) with local treatment (e.g. surgery) is ≥ 80%, with patients harboring disease limited to an extremity demonstrating the best outcome (Kurkchubasche, Halvorson et al. 2000, Cecchetto, Carli et al. 2001). Definitive surgical resection, when possible, represents the current standard of care (Stout 1962, Chung and Enzinger 1976, Soule and Pritchard 1977, Grier, Perez-Atayde et al. 1985, Dillon, Whalen et al. 1995, Kurkchubasche, Halvorson et al. 2000, Cecchetto, Carli et al. 2001, Loh, Ahn et al. 2002, Russell, Hicks et al. 2009, Orbach, Rey et al. 2010).

In more than one-half of cases, the large size of the tumor relative to the small size of the patient necessitates limb amputation (Kurkchubasche, Halvorson et al. 2000). Radiotherapy is generally avoided due to concerns about toxicity and late effects (Loh, Ahn et al. 2002, Pui, Cheng et al. 2003). Neoadjuvant chemotherapy may be used prior to resection to increase the likelihood of achieving negative surgical margins and improved postsurgical cosmesis and function. Adjuvant therapy is sometimes used empirically in the setting of positive surgical margins or high risk disease. In these settings, combination chemotherapy regimens are adapted from the adult sarcoma experience, and generally include drugs such as ifosfamide, cyclophosphamide, vincristine, actinomycin-D, and etoposide.

The Italian Cooperative Group (now called the Associazione Italiana Ematologia Oncologia Pediatrica–Soft Tissue Sarcoma Committee [AIEOP-STSC]) and the International Society of Pediatric Oncology–Malignant Mesenchymal Tumor Committee (SIOP-MMT) have published the largest case series of IFS patients (Cecchetto, Carli et al. 2001, Orbach, Rey et al. 2005, Orbach, Rey et al. 2010). In the most recent report, 56 patients had been diagnosed and treated (Orbach, Rey et al. 2010). Thirty-three (59%) of patients were diagnosed before the age of 3 months, and tumors from 9 of 13 patients (70%) diagnosed before the age of one month harbored an *ETV6-NTRK3* fusion transcript. Surgical resection was the initial treatment modality for 38/56 (68%) patients, and 22 (39%) patients required some type of chemotherapy due to microscopically incomplete resection (as adjuvant therapy), or the development of progressive disease. Five patients received additional chemotherapy in the second-line setting. With a median follow up of 4 years, 42 (75%) patients remained in their

first complete remission, 5 (9%) patients were in second complete remission, 6 (11%) patients were still requiring ongoing treatment, and 5 (9%) patients had died. The 5- and 10-year event-free survival (EFS) rates were 81% and 72%, respectively, while 5- and 10-year overall survival (OS) rates were both 89%. Five (9%) patients required mutilating surgery, either during initial surgery or after poor response to first-line chemotherapy (two foot amputations each), or during salvage therapy (one foot amputation).

In summary, IFS represents a rare cancer diagnosis, with a good overall long-term prognosis. However, some patients require disfiguring surgery to achieve cure, while others require systemic chemotherapy—with its incumbent short- and long-term risks—to achieve disease control. In addition, the 10-year EFS and OS rates of 72% and 89% indicate that current treatment modalities are not completely effective for all patients. Patients who present with distant metastases or demonstrate primary chemorefractory disease carry a particularly poor prognosis (Punnett, Tomczak et al. 2000, Loh, Ahn et al. 2002, Orbach, Rey et al. 2010). Therefore, the ability to selectively target TRK fusions could dramatically improve patient outcomes while simultaneously minimizing the toxicities of existing therapeutic modalities.

4.4.2 Congenital Mesoblastic Nephroma

Congenital mesoblastic nephroma (CMN) comprises about 5% of childhood kidney tumors but disproportionately affects younger children, accounting for more than 80% of kidney cancers diagnosed before the age of 6 months (Haddad, Haziza et al. 1996).

CMN is divided into three main histologic subtypes: "classical", "cellular", and "mixed" (Knezevich, Garnett et al. 1998). As the name implies, cellular CMN is characterized by a high density of tumor cells, which possess numerous mitoses and cellular pleomorphism, together with minimal tumor-associated stroma (Knezevich, Garnett et al. 1998). Early cellular and cytogenetic studies suggesting a possible relationship between cellular CMN and IFS were subsequently confirmed with the identification of an *ETV6-NTRK3* in 8 of 9 cellular CMN samples analyzed (Knezevich, Garnett et al. 1998). By contrast, no fusions were identified in classical CMN. The high prevalence and specificity of *ETV6-NTRK3* for cellular CMN has been independently confirmed by additional investigators (Rubin, Chen et al. 1998), including a recent report that identified an *ETV6-NTRK3* gene fusion in 8/8 cellular CMN cases, 5/6 mixed CMN cases, and 0/5 classic CMN cases (El Demellawy, Cundiff et al. 2016).

Standard treatment options for CMN include nephrectomy and adjuvant chemotherapy. The risk for recurrence is closely associated with stage III disease (e.g. histologically positive surgical margins), cellular subtype, and age 3 months or older at diagnosis. A large, international collaborative study of pediatric kidney cancer diagnoses in the first 7 months of life included a detailed description of CMN (van den Heuvel-Eibrink, Grundy et al. 2008). CMN frequency was shown to decrease with increasing age, 74% of patients had early stage disease, 5-year EFS was 93.8% and 5-year OS was 96.1%. No patient presented with metastatic disease and most patients were cured with immediate nephrectomy. However, despite this favorable overall prognosis, rare patients have developed locally recurrent and/or metastatic disease after primary treatment and have required sytemic chemotherapy and radiotherapy to achieve long-term disease-free survival (Steinfeld, Crowley et al. 1984). In one case, an infant who underwent radical nephrectomy for IFS at birth developed brain

metastases at the age of 7 months and ultimately succumbed to progressive disease despite two regimens of multiagent salvage chemotherapy (Heidelberger, Ritchey et al. 1993).

In summary, CMN is rare in children as a whole, but represents the most common form of kidney cancer diagnosed in children less than 6 months of age. Patients are usually cured with nephrectomy, although recurrences occur, and these require aggressive salvage approaches with radiation, multiagent chemotherapy or both. An *ETV6-NTRK3* gene fusion has been identified in virtually all cases of cellular (and mixed CMN), which are associated with a higher risk of recurrence than the classical CMN subtype. Therefore, NTRK fusions may represent a potential therapeutic target for CMN patients who require systemic treatment.

4.4.3 Papillary Thyroid Cancer

Papillary thyroid cancer (PTC) accounts for approximately 4% of all pediatric tumors (Siegel, King et al. 2014). The application of next generation sequencing (NGS) approaches has greatly increased the understanding of the genetic landscape of PTC in adults, with 60% harboring B-Raf proto-oncogene *(BRAF)*-activating mutations, 13% activating mutations in ras protein (*RAS)* genes, 6% rearranged during transfection oncogene *(RET)* fusions, and 2.3% fusions involving *NTRK1* or *NTRK3*.

The natural history of NTRK gene fusion PTC, in both adults and children, has been difficult to characterize, due to variability in the molecular methods used for fusion detection across studies and the inclusion of NTRK gene fusion and RET gene fusion positive patients together in early studies examining clinicopathologic features. The advent of NGS technologies has overcome several of the inherent limitations of traditional molecular approaches, resulting in more comprehensive and accurate assessment of the presence of NTRK gene fusions. For example, NGS confirmed previous findings demonstrating an increased prevalence of fusion oncogenes (including *NTRK1* fusions) in post-Chernobyl radiation-induced thyroid cancers, and identified *ETV6-NTRK3* fusions with novel breakpoints in additional cases (both radiation-induced and radiation-naïve) (Ricarte-Filho, Li et al. 2013).

More recently, *NTRK1* and *NTRK3* fusions were identified at a surprisingly high frequency in sporadic pediatric PTC patients in the Northeastern United States. Among 27 tumors from patients between the ages of 6 and 18, 7 (26%) were found to harbor an NTRK gene fusion, one *TPR-NTRK1*, one *NTRK3* with unknown partner gene, and 5 *ETV6-NTRK3*. Four of 7 patients presented with multi-focal disease, 6 (86%) patients demonstrated microscopic tumor lymphatic invasion, 5 (71%) patients had overt lymph node metastases, and one developed distant metastasic disease. Of the remaining patients, 13 patients had a $BRAF^{V600E}$ mutation, 6 patients had a *RET* fusion, and one patient was negative for the analyzed mutations. Of note, no patient in this study had a history of prior radiation exposure.

Compared with adults, differentiated thyroid cancer (DTC, which includes both PTC and follicular thyroid cancer/FTC) presents at more advanced stages in children and is associated with higher rates of recurrence (Rivkees, Mazzaferri et al. 2011). Although randomized trials have not been applied to pediatric PTC patients, given the aggressive nature of pediatric PTC and the young age of affected patients, aggressive treatment strategies have been recommended, including total thyroidectomy, central compartment lymph node dissection and postoperative radioactive iodine therapy to eradicate residual disease. In addition,

long-term follow up is essential, because disease recurrence has occurred decades after initial diagnosis and treatment. Although multikinase inhibitors (e.g. sorafenib, levnatinib) are emerging for adult patients with recurrent, radioactive, iodine-refractory disease (Brose, Nutting et al. 2014), (Schlumberger, Tahara et al. 2015), treatment with a multikinase inhibitor may not be appropriate for all patients after weighing the possible risks against the potential for benefit, and this may be especially true for children, in whom the long-term toxicities of multikinase inhibitors have not been fully investigated. Cytotoxic chemotherapy is not effective for patients with radioactive iodine-refractory PTC (Shimaoka, Schoenfeld et al. 1985).

In summary, NTRK gene fusions may explain a significant proportion of pediatric papillary thyroid cancers. Though preliminary, outcomes data suggest that these tumor may be associated with adverse prognostic features. Although mutlikinase inhibitors in the setting of recurrent, radioactive iodine-refractory disease have clear benefit in adult PTC patients, their utility for pediatric PTC patients is not yet known, and their potential for toxicity may be significant.

4.4.4 Spitzoid Tumors

Spitzoid neoplasms comprise a class of melanocytic tumors, distinct from melanomas, that occur more frequently in younger patients. They are classified into three main types: benign Spitz nevi, frankly malignant Spitzoid melanomas and atypical Spitz tumors (ASTs). ASTs have histologic features and clinical features overlapping those of naevi and melanomas. Despite possessing a high propensity for regional lymph node metastasis, both Spitzoid melanomas and ASTs typically have a favorable prognosis, though some patients whose tumors possess aggressive features (e.g. severe atypia, positive lymph node metastasis) require systemic treatment (e.g. with interferon) (Sepehr, Chao et al. 2011).

The genetic landscape of Spitzoid neoplasms has recently been characterized (Wiesner, He et al. 2014). In addition to less common *BRAF* and *HRAS* gene mutations, more than one-half of Spitzoid neoplasms harbored activating kinase fusions, including *ALK* (10%), *RET* (2.9%), *ROS1* (17.1%), *BRAF* (5%) and *NTRK1* (16.4%). *NTRK1* fusions were distributed across all three histologic subtypes: Spitz naevi (nevi (8/75=10.7%), ASTs (8/32=25%), and Spitzoid melanomas (7/33=21.2%), and tumors harboring *NTRK1* fusions showed strong expression of NTRK1 by immunohistochemistry. In addition, expression of one of the identified fusions (*LMNA-NTRK1)* in melanocytes led to dramatic activation of downstream signaling pathways (e.g. AKT, ERK) that was sensitive to a TRK inhibitor, as well as rapidly growing tumors when the cells were injected subcutaneously into immunocompromised mice. The high frequency of *NTRK1* gene rearrangements in Spitzoid neoplasms has been subsequently validated in an independent study, in which two of six Spitzoid tumors from pediatric patients that were successfully analyzed by ribonucleic acid (RNA) sequencing harbored *TMP3-NTRK1* gene fusions (Wu, Barnhill et al. 2016).

In summary, while the majority of Spitzoid neoplasms behave in an indolent fashion, some metastasize and require systemic therapy. Although it is not yet known whether the presence of an NTRK gene fusion correlates with more aggressive behavior, the high frequency of fusion kinases represent potential therapeutic targets for Spitzoid neoplasms that require systemic treatment.

4.4.5 Pediatric High Grade Gliomas and Other Central Nervous System Tumors

Pediatric high-grade gliomas (HGG), which include diffuse intrapontine glioma (DIPG) and non-brainstem high-grade glioma (NBS-HGG), constitute a significant unmet clinical need with an especially poor prognosis. The anatomic location of these tumors poses particular challenges for treatment (both surgical resection and radiation), and makes biopsy for molecular genetic characterization difficult. Pediatric HGGs as a whole have a median survival of only 12 to 15 months, and less than 20% of patients survive 2 years or more from diagnosis. DIPG is uniformly fatal, with median overall survival of less than one year (Taylor, Vinci et al. 2014). No treatment has so far been shown to alter the natural history of DIPG—surgical resection is not possible due to their anatomic location, and no clinical trials have demonstrated any benefit for systemic therapies (Warren 2012).

Recent literature has shed light on the genetic landscape of pediatric HGGs. A recent comprehensive whole genome sequencing analysis of 127 pediatric HGGs identified recurrent somatic mutations in the *ACVR1* gene, encoding a receptor tyrosine kinase, in one-third of DIPG, in addition to previously reported frequent somatic mutations in genes encoding components of histone H3, as well as *TP53* and *ATRX*. Although therapeutic targeting of these alterations is challenging due to the nature of the encoded proteins (histone H3 genes, *TP53, ATRX)* or lack of available therapies (*ACVR1*), recurrent oncogenic fusions of the *NTRK1*, *NTRK2*, and *NTRK3*, identified in 40% of NBS-HGG occurring in children under the age of 3, and in 4% of DIPG, represent potential therapeutic targets. Notably, with the exception of histone H3 variants, *NTRK* fusions are mutually exclusive with other potential HGG drivers. Furthermore, the fusions are oncogenic *in vivo* when introduced into TP53-null astrocytes that were implanted into mouse brain, and induced high levels of MAPK and PI3K pathway activation (Wu, Diaz et al. 2014).

Pediatric low-grade gliomas (LGGs) are the most common pediatric brain tumors. In contrast to their high-grade counterparts, pediatric LGGs (inclusive of astrocytomas, gangliogliomas and oligodendrogliomas) have a much better prognosis. However, tumors that cannot be surgically resected cause significant morbidity and mortality (Whittle 2004). Thus, as with HGGs, there is a critical need to identify new therapeutic modalities, particularly for the subset of LGG patients who cannot be treated surgically.

A recent comprehensive whole genome sequencing analysis of 151 pediatric LGGs confirmed the presence of frequent alterations in genes comprising the MAPK pathway, including *FGFR1, BRAF* and *NF1,* as well as the transcription factors *MYB* and *MYBL1* (Zhang, Wu et al. 2013). In addition, 2% of tumors harbored oncogenic *NTRK* gene fusions that were mutually exclusive of other driver mutations. This observed frequency is consistent with a separate analysis of 96 pediatric pilocytic astrocytomas, among which two *NTRK2* gene fusions were identified (Jones, Hutter et al. 2013).

Together, these summarized studies identify NTRK gene fusions as potential therapeutic targets in a fraction of high-grade and low-grade pediatric gliomas.

4.4.6 Sarcomas

There are approximately 100 histologically defined STS subtypes, which together comprise less than one percent of all adult malignancies, but as many as 12% of pediatric cancers, with

a prevalence of 12.01 per one million children in the United States (Miller, Young et al. 1995, Coffin and Fletcher 2002, Merchant and Mackall 2009, Schoffski, Cornillie et al. 2014, Siegel, King et al. 2014, Siegel, Miller et al. 2016).

Rhabdomyosarcoma (RMS) accounts for nearly half of all STS diagnosis in children, with the remaining fraction divided among several subtypes, including desmoplastic small round cell tumor, Ewing's sarcoma family tumors, and other undifferentiated, non-rhabdomyosarcomas (Merchant and Mackall 2009). In general, STS presenting with localized disease is treated with surgical resection, with postoperative radiation and often chemotherapy added for tumors with high-risk features (Linch, Miah et al. 2014). Systemic chemotherapy for STS consists primarily of combination regimens containing actinomycin-D, doxorubicin, cyclophosphamide, etoposide, ifosfamide, or vincristine.

As with IFS, childhood localized STS is usually curable (Merchant and Mackall 2009). However, for the 30–50% of patients who experience relapse, long-term survival is significantly decreased, with less than 25% of patients surviving for 5 years from the time of relapse (Merchant and Mackall 2009). Irinotecan, temozolomide, vinorelbine, topotecan, gemcitabine, and docetaxel have activity in the salvage setting (Merchant and Mackall 2009).

While the frequency of NTRK gene fusions in childhood STS is low, certain morphological features may increase the likelihood of an underlying *NTRK* fusion. In a recent case series of NTRK fusion-positive STS patients, Haller and colleagues described four STSs that harbored *NTRK1* gene fusions (Haller, Knopf et al. 2016). Two of these four cases affected children (ages 11 months and 2 years). All four tumors possessed histological features of hemangiopericytoma (HPC) and myopericytoma (both rare spindle-cell tumors), but lacked the *NAB2-STAT6* gene fusion previously shown to be associated with these STS subtypes (Haller, Knopf et al. 2016).

Recently, Foundation Medicine published a targeted mutational analysis of pediatric cancers (Chmielecki, Bailey et al. 2016). Among 1,215 pediatric tumors, 4 harbored NTRK gene fusions as the driving oncogenic lesion: 2/6 fibrosarcomas (one *SQSTM1-NTRK1* fusion and one *LMNA-NTRK1* fusion), 1/19 STS (*TFG-NTRK3* fusion), and 1/6 hemangiomas (*ETV6-NTRK3* gene fusion). The low incidence implied by this study may be negatively biased because the comprehensive genomic panel employed was not optimized for NTRK gene fusion detection. Nevertheless, these data are notable for the apparent clustering of NTRK gene fusion calls in the setting of pediatric STS.

4.4.7 Other Pediatric Malignancies

A molecular analysis of 154 Philadelphia chromosome-like acute lymphoblastic leukemia (Ph-like ALL) tumors identified a single (pediatric) case harboring an *ETV6-NTRK3* gene fusion, that was mutually exclusive with all other oncogenic drivers in this disease (Roberts, Li et al. 2014).

4.4.8 Neuroblastoma

Neuroblastoma is the most common tumor in infants younger than one year and accounts for 7–10% of all childhood cancers, with 700 new cases diagnosed each year (Maris 2010, Ward, DeSantis et al. 2014). Neuroblastoma is typically risk-stratified into low-, intermediate- or high-risk disease, based on age at diagnosis, histology, deoxyribonucleic acid (DNA) ploidy, the presence of neuroblastoma MYC oncogene *(MYCN)* gene amplification and disease extent. While outcomes have improved over time for patients with low- and intermediate-risk disease, there has been little improvement in the management of patients with high-risk disease since the introduction of intensive, high-dose chemotherapy regimens and autologous stem cell transplantation (Maris 2010).

While neuroblastoma is thought to derive from precursor cells of the sympathetic nervous system, where normal TRK expression is expected, clinical outcomes in patients with neuroblastoma may be related to specific features of TRK expression, including the particular family member and even the specific splice variant expressed. High TRKA expression is a positive prognostic factor that correlates inversely with *MYCN* amplification, which itself is a poor prognostic factor (Brodeur, Minturn et al. 2009). In preclinical studies, low-grade neuroblastomas that overexpresses TRKA can be induced to undergo terminal differentiation (and cessation of growth) with exposure to NGF, the endogenous ligand for TRKA (Brodeur, Minturn et al. 2009). Conversely, overexpression of TRKB and its endogenous ligand, BDNF, are associated with drug resistance and angiogenesis, and are correlated with decreased survival in patients (Brodeur, Minturn et al. 2009). Further complicating the picture, a specific splice variant of TRKA, TRKA-III, which is missing the extracellular protein sequences corresponding to exons 6, 7, and 9 (and as a result signals in a ligand-independent, constitutive fashion), is associated with increased tumor vascularity, aggressive tumor behavior, and the development of bone metastases (Tacconelli, Farina et al. 2004, Cao, Liu et al. 2010, Farina, Cappabianca et al. 2012).

Associations between TRKB expression, the splice variant TRKA-III, and adverse prognosis have led clinical investigators to explore the role of the anti-TRK and multikinase inhibitor lestaurtinib (Teva Pharmaceutical Industries, Petah Tikvah, Israel) in a Phase 1 trial in patients with refractory, high-risk neuroblastoma (Minturn, Evans et al. 2011). Forty-six patients were evaluable for disease response. Two patients had PRs. An additional 3 patients had mixed responses and 6 patients had stable disease (range 5–13 cycles). Given that lestaurtinib inhibits many kinases in addition to TRK, it not known whether the observed anti-tumor activity can be attributed to TRK inhibition (Brodeur and Bagatell 2014). Furthermore, the authors did not report whether the more encouraging clinical responses were associated with specific TRK alterations.

In a recent publication by Iyer and colleagues, the investigational agent entrectinib (Ignyta, San Diego, CA), a multi-kinase inhibitor potent against TRK, ROS1, ALK, JAK2, and ACK1, was investigated preclinically in a neuroblastoma model (Iyer, Wehrmann et al. 2016). Using a human neuroblastoma cell line with enhanced expression of TRKB, these authors demonstrated that entrectinib caused potent inhibition of BDNF-stimulated TRK phosphorylation and downstream AKT and ERK phosphorylation *in vitro* and caused modest tumor growth inhibition (though not regression) *in vivo*, when given to mice as monotherapy or alternating with chemotherapy. The authors concluded that entrectinib was more potent than lestaurtinib in this model system. However, given entrectinib's significant activity

against ALK, and the presence of an endogenous activating mutation in the *ALK* gene in the cell line used for this study, it is not possible to exclude potential antitumor effects from concomitant inhibition of both TRKB and ALK.

In summary, available clinical data as well as preclinical models, suggest a limited role for anti-TRK monotherapy in the management of neuroblastoma. While significant unmet need exists for patients with high-risk and recurrent disease, developing TRK inhibitors in this setting would likely require the empiric clinical development of combination regimens in randomized, add-on trial designs focused on differences in progression-free and overall survival. The recent approval of dinutuximab (United Therapeutics, Research Triangle Park, NC) illustrates the complexities of drug development in this setting. The trial that secured FDA approval of dinutuximab required the development of the drug in the background of 13-cis-retinoic acid, granulocyte macrophage-colony stimulating factor and interleukin-2 and took 7 years to enroll (Yu, Gilman et al. 2010). The ability to direct clinical development of a TRK inhibitor in the context of a predictive biomarker, such as TRKB expression or the splice variant TRKA-III, might reduce the time and risk of drug development. However, to our knowledge, neither preclinical nor clinical data exist to support the predictive value of a TRK biomarker in neuroblastoma beyond the retrospective prognostic evidence cited above.

5.0 NONCLINICAL STUDIES WITH LOXO-101

5.1 Potency of LOXO-101

LOXO-101 blocks the ATP binding site of the TRK family of receptors, with enzyme affinities in the low nanomolar range. LOXO-101 has a similar nanomolar potency against TRKA, TRKB, and TRKC in cellular assays (Vaishnavi, Capelletti et al. 2013, Doebele, Davis et al. 2015) (data on file, Loxo Oncology), with no effect on cell lines that do not harbor an NTRK fusion as shown in Table 1.

Table 1. Potency of LOXO-101 in TRK-Driven and Non-TRK-Driven Cell Lines

Cell Line	TRK Family Alteration	IC_{50} (nM)
Ba/F3 cells expressing *MPRIP-NTRK1*	*MPRIP-NTRK1*	8.0
Ba/F3 cells expressing *CD74-NTRK1*	*CD74-NTRK1*	1.9
Cuto3.29	*MPRIP-NTRK1*	59
KM12	*TPM3-NTRK1*	3.5, 6.0
MO-91	*ETV6-NTRK3*	1.0
H1299, A549, HCC78, H3122, H1650, HCT15, HCT116, HT29, and SW837	None	> 1000

Abbreviations: *ETV6* = ETS variant gene 6; IC_{50} = 50% inhibitory concentration; *MPRIP* = myosin phosphatase Rho interacting protein; NTRK = neurotrophic tyrosine kinase receptor gene (referring to family); *TPM3* = tropomyosin 3; *TRIM24* = tripartite motif containing 24; TRK = tropomyosin-related kinase (referring to family).

5.2 Selectivity of LOXO-101

LOXO-101 was designed with a structural biology-based approach to obtain high selectivity for TRKA, TRKB, and TRKC, with little or no interaction with other kinase and non-kinase targets. LOXO-101 was evaluated for off-target kinase enzyme inhibition against a panel of 226 non-TRK kinases at a compound concentration of 1000 nM and ATP concentrations near the K_m for each enzyme. In the panel, LOXO-101 was highly selective and demonstrated only weak inhibition of one non-TRK kinase (TNK2, with 50% inhibitory concentration [IC_{50}] of 1188 nM; data on file, Loxo Oncology). The selectivity of LOXO-101 for TRKA, TRKB, and TRKC is unique among clinical-stage multi-kinase inhibitors with anti-TRK activity, such as crizotinib and entrectinib, as shown in Table 2 (Menichincheri, Ardini et al. 2016).

Table 2. Off-Target Kinase Selectivity of LOXO-101 Versus Other Clinical-Stage TRK Inhibitors

Potency (IC_{50}, nM)	LOXO-101	Crizotinib	Entrectinib
1–10	TRK	HGFR	TRK, ROS1
11–100	-	ALK	ALK, JAK2, ACK1
101–1000	-	TIE2, TRKA,TRKB, AXL, RON	IGFR1, JAK1, FAK, FLT3, BRK, IR, AUR2, JAK3, RET
1001–10000	TNK2	LCK	FGFR1, VEGFR2, VEGFR3, LCK, KIT, AUR1, ABL, PKCβ, CDK2, CYCA, SYK

Source reference: (Menichincheri, Ardini et al. 2016)

Abbreviations: ALK = anaplastic lymphoma kinase; CDK = cyclin-dependent kinase; IC_{50} = 50% inhibitory concentration; TNK2 = non-TRK kinase; TRK = tropomyosin-related kinase (referring to family).

Furthermore, in a broad screen against 82 receptors, enzymes, and nuclear targets, LOXO-101 had no relevant human ether-à-go-go-related gene (hERG) inhibition (IC_{50} >100 μM) and there was no finding of prolonged QT in any preclinical species tested.

5.3 Important Role of Neurotrophins and their Receptors in the CNS

All three TRK receptors appear to play an important role in neuronal development. Inhibiting signaling by any of the receptors leads to direct apoptosis of cultured neurons in vitro. Conversely, treatment of neurons with individual neurotrophins promotes neuronal survival (reviewed in (Kalb 2005)).

The TRKA receptor is involved in cell differentiation and may play a role in determining sensory neuron subtype. Mice with global knockout for *Ntrk1* or *Ngf* genes (the murine orthologs of human *NTRK1* and *NGF*, respectively, encoding TRKA and its endogenous ligand NGF) can develop to birth, but are smaller and die early, possibly because of their defective neuronal development (Crowley, Spencer et al. 1994, Smeyne, Klein et al. 1994).

Mice lacking Trkb have drastically reduced numbers of sensory neurons, but the number of sensory neurons is not affected in mice lacking *Bdnf* or NT-4/5 (Skaper 2008). Trkb (-/-) knockout mice do not feed, and typically die within 24–48 hours of birth (Snider 1994). Knockouts for *Bdnf* (-/-) have a milder phenotype, exhibiting head bobbing, spinning and hind limb extension during locomotion. These animals typically survive a few weeks. Vestibular ganglia in *Bdnf* (-/-) knockouts have a diminished number of neurons, typically a > 80% reduction (Snider 1994). Heterozygote *Bdnf* knockouts develop hyperphagia and obesity (Yeo, Connie Hung et al. 2004). Yeo and colleagues published a case report of a male child with a *de novo* mutation of TRKB. The child was found to have a loss-of-function mutation in *NTRK2* and demonstrated global developmental delay, impairment of short-term memory, and impaired nociception, as well as severe early onset obesity (Yeo, Connie Hung et al. 2004).

Mice bred with a Trkc (-/-) knockout exhibit abnormal movements and postures (Snider 1994). Due to the similarity of these movements to "pseudoathetosis," exhibited by humans with large fiber sensory neuropathies, these animals most likely are deficient in

reduction in muscle spindles, while homozygous knockouts lack development of proprioception related sensory end organs and muscle spindles (Snider 1994).

Even a partial reduction in the levels of the individual neurotrophins may cause profound neurological effects in animals. Heterozygous mice, with neurotrophin levels reduced by ~50%, while viable, show a range of neurological deficits, including severe problems with the formation and retention of new memories, and atrophy of cholinergic neurons (Ngf/Trka)(Chen, Nishimura et al. 1997), decreased numbers of peripheral nervous system neurons (Ngf/Trka, Bdnf/Trkb and Nt3/Trkc) (Crowley, Spencer et al. 1994, Ernfors, Lee et al. 1994), decreased neuronal plasticity (Bdnf/Trkb) (Korte, Carroll et al. 1995), and altered epileptic seizure threshold (Bdnf/Trkb) (Elmer, Kokaia et al. 1997).

Although each of the above studies involves constitutive deletion of the individual NTRK or neurotrophin genes during prenatal development, deletion/decreases after birth may have significant effects as well. Conditional deletion of *Bdnf* in mice after birth leads to hyper-aggressive and hyperphagic behavior, hyperactivity, anxiety, and obesity (Rios, Fan et al. 2001). Prolonged stress also decreases the levels of neutrophins and their receptors in the brains of adult rats, contributing to the pathogenesis of stress-induced disturbances in mood, memory and learning (Ueyama, Kawai et al. 1997). Finally, direct infusion of BDNF into the brain has anti-depressant affects comparable to pharmacological antidepressants (Shirayama, Chen et al. 2002).

Defects in neutrophin/TRK receptor levels or activity may even be linked to overt neuropathology in humans. Naturally occurring polymorphisms in the *BDNF* gene have been linked to bipolar disorder, depression, memory deficits, and Alzheimer's disease in humans (Ernfors, Lee et al. 1994, Neves-Pereira, Mundo et al. 2002, Ventriglia, Bocchio Chiavetto et al. 2002, Sen, Nesse et al. 2003).

Given the likely role of TRK receptors in the developing brain, an understanding of the potential effects of inhibiting these receptors in the brain is important for the pediatric use of a TRK inhibitor.

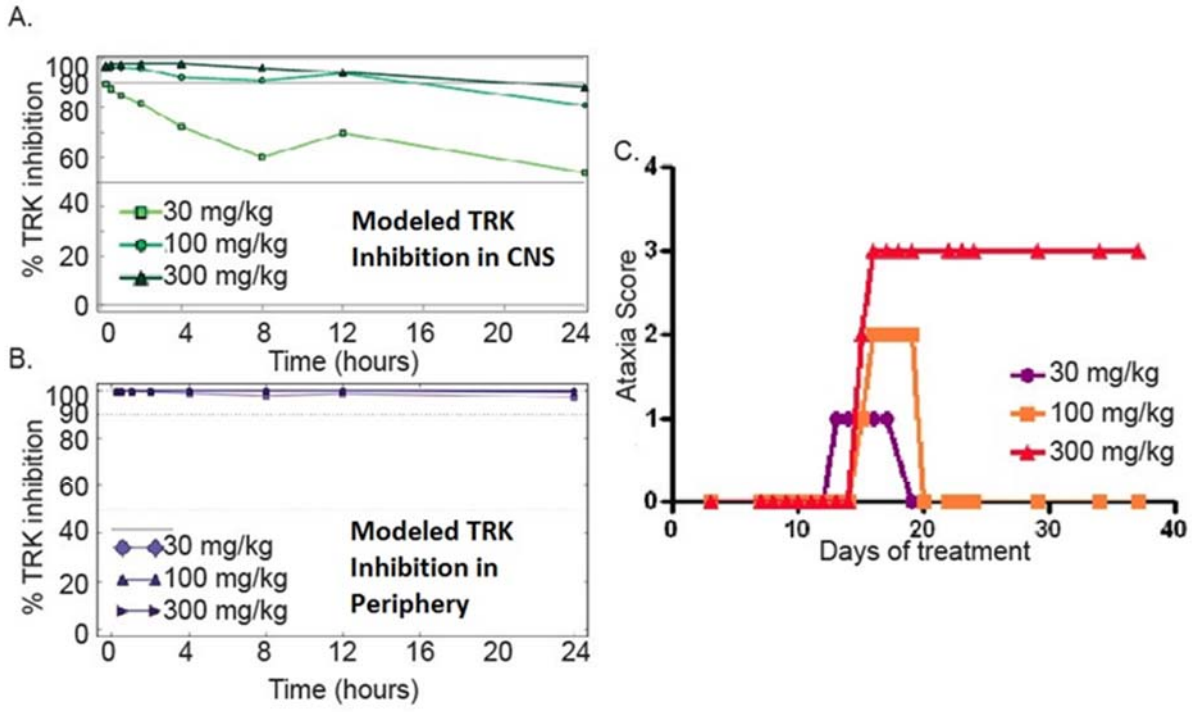
Early evidence in patients indicates that tonic inhibition of TRK signaling in the CNS may cause neurologic toxicity that is reversible with drug cessation. For example, in a Phase 1 study of a dual TRKA and cyclin-dependent kinase (CDK) inhibitor PHA-848125AC (Nerviano Medical Science, Nerviano, Italy), 3 patients exhibited ataxia and 2 patients exhibited tremors. The single patient treated with the highest dose of drug experienced Grade 4 ataxia (Weiss, Hidalgo et al. 2012). In a recent update of the Phase 1 clinical trial experience with entrectinib (a multi-kinase inhibitor potent against TRK, ROS1, ALK, JAK2, and ACK1), Grade 3 cognitive disturbances comprised one of the two dose-limiting toxicities (DLTs); treatment-related paresthesias were observed in 28% of patients (Grade 1), and dizziness and peripheral sensory neuropathy occurred in 13% and 9% of patients treated at the recommended Phase 2 dose, respectively (Drilon, Hong et al. 2016).

The lack of significant neurological AEs in patients treated with crizotinib or lestaurtinib, which possess some anti-TRK activity, is consistent with their limited target coverage of TRK at clinically achievable concentrations and limited CNS penetration (Tang, Nguyen et al. 2014, Mathias, Natarajan et al. 2015).

5.4 Drug Exposure and Timing and Onset of CNS Side Effects

Factors other than a simple brain-to-plasma ratio may be important to the CNS effects of TRK inhibitors. In the selection of a clinical candidate, the Sponsor evaluated TRK inhibitors with similar pharmaceutical properties and potencies as LOXO-101 at TRKA, TRKB, and TRKC, but varying degrees of CNS penetration, and scored their ataxia based on observations of behavior, gait, and balance in rats. Ataxia scores were higher following treatment with highly brain-penetrant TRK inhibitors than peripherally restricted TRK inhibitors. As can be seen in Figure 2C, the onset of CNS effects, such as ataxia, were not apparent until after 10 to 14 days of continuous treatment. Further analysis of correlation of ataxia scores with estimated CNS TRK inhibition based on unbound brain concentrations showed that continuous TRK inhibition (24 hours/day) of > 90% led to severe ataxia (Figure 2), while strong yet intermittent inhibition (14 hours/day) led to less ataxia, and a more pulsatile profile, oscillating between 50% and 90% calculated occupancy caused no significant ataxia. These studies raised concern that highly brain penetrant compounds would have limited therapeutic index where there is a goal of 90% inhibitory concentration (IC_{90}) coverage of TRK signaling peripherally in the setting of cancer. These findings suggest that in addition to CNS tissue levels, other drug properties such as half-life and target on/off time may contribute to the development of CNS-related AEs of TRK inhibitors.

Figure 2. Relationship Between Sustained TRK Inhibition in the Brain and Onset of Ataxia Following Repeated Dosing with a Representative Selective TRK Inhibitor in the Rat



Ataxia worsens with increased brain plasma levels. (A and B) Dose-dependent increases in calculated TRK receptor occupancy based on unbound brain (A) and plasma (B) levels of a representative selective TRK inhibitor (not LOXO-101) in rats. (C) As the dose increases, and brain and plasma levels increase, there was an increase in the severity of ataxia, as scored by a visual observation score.

Abbreviations: TRK = tropomyosin-related kinase.

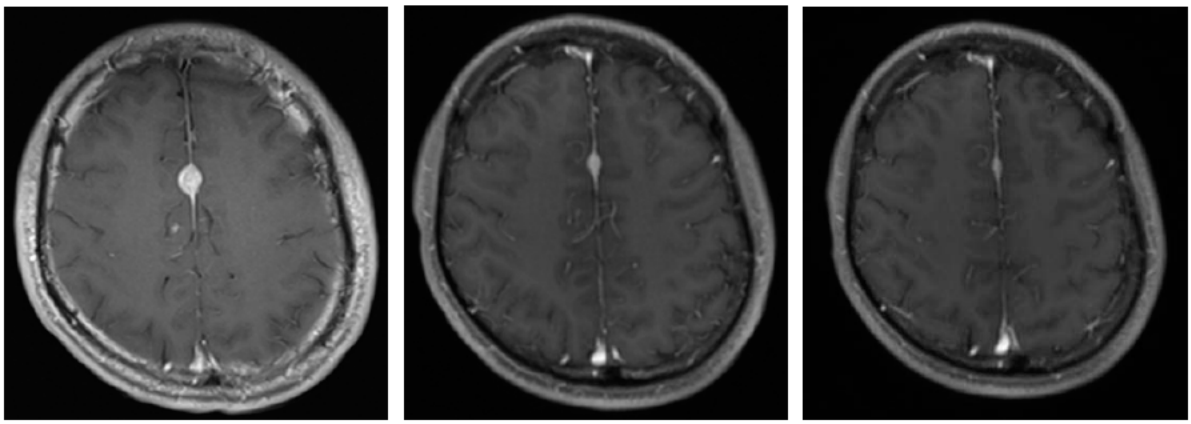
5.5 Selection of LOXO-101 to Minimize CNS Side-Effects

In addition to the selectivity criterion, LOXO-101 was designed to have limited brain distribution to provide robust inhibition of peripheral TRK receptors and to balance activity against CNS tumors with the potential toxicity associated with inhibition of TRK receptors in the brain. Satisfaction of this design criterion of modest CNS penetration was demonstrated preclinically by a brain microdialysis study in rats that showed free brain levels of LOXO-101 are lower than free plasma levels. Furthermore, CNS effects such as ataxia were minimal for LOXO-101. Despite a potentially reduced fraction of LOXO-101 that may be able to access CNS tissues relative to peripheral tissues, it is possible that even these lower CNS levels will be sufficient to provide benefit for NTRK-fusion tumors in the CNS. The plasma exposures achieved in the clinic for LOXO-101 suggest meaningful target coverage for much of the dosing period, while still allowing for periods of time with unbound brain levels < IC_{50}, allowing recovery time in the CNS and therefore creating less potential for CNS toxicity. Kinase inhibitors against other oncogenic targets are able to deliver objective responses in the brain at levels that achieve IC_{50} coverage (Togashi, Masago et al. 2010).

The pharmacokinetic (PK) properties of LOXO-101 may also balance CNS efficacy versus side effects. LOXO-101 is rapidly absorbed, leading to high peak plasma levels and likely robust engagement of both peripheral and central TRK receptors. Due to the 2–3 hour half-life of LOXO-101, coupled with a reduced CNS exposure, the extent of TRK inhibition in the CNS is anticipated to be below a level, which if sustained continually, would lead to increased risk to the developing brain or contribute to CNS-related AEs.

Early data suggest the potential for clinical activity for LOXO-101 in patients with CNS disease. An adult patient (in Study LOXO-TRK-14001) with *TPR-NTRK1* fusion metastatic NSCLC with radiographic abnormalities in the brain (not included in the RECIST determination) demonstrated regression during treatment with LOXO-101 (Figure 3).

Figure 3. Diagnostic Imaging of the Brain of a NSCLC Patient with a *TPR-NTRK1* Fusion



Study Baseline **Study Cycle 3 Day 1** **Study Cycle 7 Day 1**

6.0 FORMULATION

6.1.1 Drug Product

LOXO-101 is provided for clinical investigation in two formulations, capsules and liquid.

6.1.1.1 Capsules

LOXO-101 capsules are provided in 2 strengths: 25 mg and 100 mg.

6.1.1.2 Liquid

LOXO-101 liquid drug product consists of a 20-mg/mL LOXO-101 solution.

7.0 CLINICAL TRIAL EXPERIENCE WITH LOXO-101 IN ADULTS

Loxo Oncology has designed a clinical development program that will investigate the safety and efficacy of LOXO-101 in pediatric and adult patients with NTRK gene fusion cancers. LOXO-101 is currently being evaluated in three Sponsor-initiated clinical studies and has been included in one National Cancer Institute (NCI)-sponsored study expected to open soon (refer to Table 3).

Table 3. Overview of Ongoing and Planned Safety and Efficacy Clinical Studies of LOXO-101

Study ID	Phase	Country	Study Title	Dosing Regimen	Study Population
LOXO-TRK-14001 (NCT-02122913)	1	US	A Phase 1 Study of the Oral TRK Inhibitor LOXO-101 in Adult Patients with Solid Tumors	Escalation Phase: Dosing schedule: Continuous, 28-day cycles until PD or intolerability Dose levels: 50 mg QD 100 mg QD 100 mg BID 150 mg BID 200 mg QD Expansion Phase: Dose TBD	Adult patients with advanced solid tumors that have progressed or are non-responsive to available therapies and for which no standard or available curative therapy exists. Accelerated access for patients with tumors known to harbor NTRK gene fusions.
LOXO-TRK-15002 (NCT-02576431)	2	US/Asia/EU	A Phase 2 Basket Study of the Oral TRK Inhibitor LOXO-101 in Subjects with NTRK Fusion-Positive Tumors	Dosing schedule: Continuous, 28-day cycles until PD or intolerability Dose: 100 mg BID	Adult patients with advanced solid tumors harboring a fusion of *NTRK1*, *NTRK2*, or *NTRK3*.
LOXO-TRK-15003 (NCT-02637687)	1	US	A Phase 1 Study of the Oral TRK Inhibitor LOXO-101 in Pediatric Patients with Advanced Solid or Primary Central Nervous System Tumors	Dosing schedule: Continuous, 28-day cycles until PD or intolerability Escalation Phase: Adult equivalent doses: 100 mg BID 150 mg BID 200 mg BID 300 mg BID Expansion Phase: Dose TBD	Pediatric patients with advanced solid or primary CNS tumors.
EAY131	2	US	NCI-Molecular Analysis for Therapy Choice (NCI-MATCH) Trial	Dosing schedule: Continuous, 28-day cycles until PD or intolerability Dose: 100 mg BID	Adult patients with cancers that have advanced on standard therapy and /or for whose disease no standard treatment exists that has been shown to prolong survival

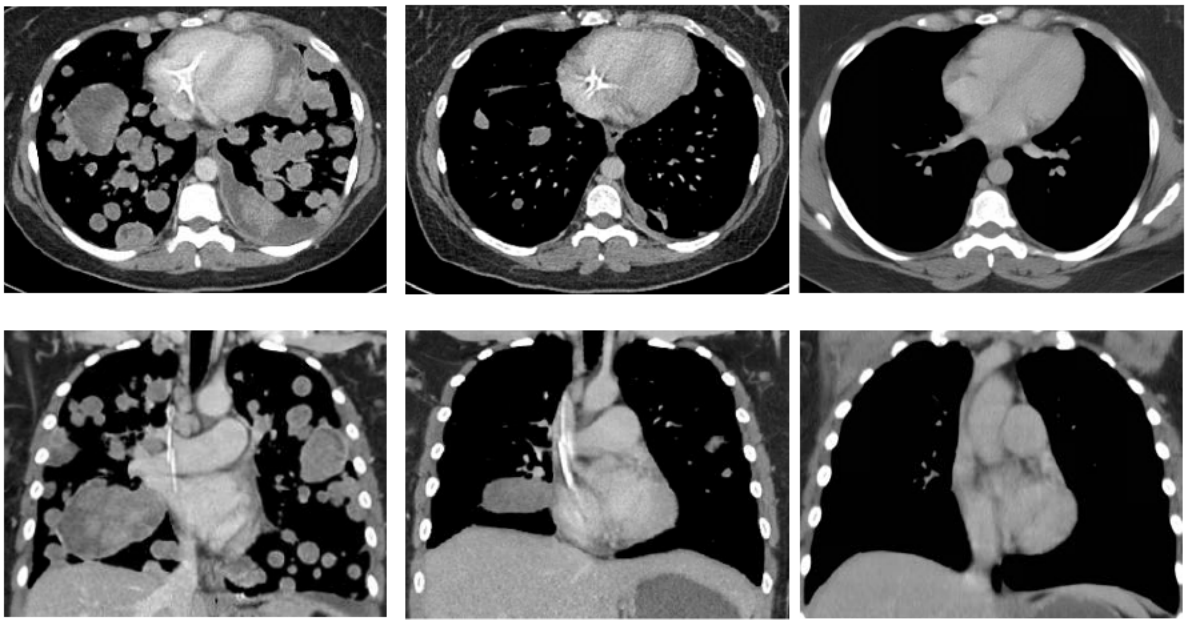
Abbreviations: BID = twice daily; CNS = central nervous system; NCI-MATCH = National Cancer Institute Molecular Analysis for Therapy Choice; NTRK = neurotrophic tyrosine kinase receptor gene (referring to family); PD = progressive disease; QD = once daily; TBD = to be determined; TRK = tropomyosin-related kinase; US = United States.

7.1 LOXO-TRK-14001

A first-in-human Phase 1 study (Study LOXO-TRK-14001) entitled "*A Phase 1 Study of the Oral TRK Inhibitor LOXO-101 in Adult Patients with Solid Tumors*" was initiated in April, 2014. This study is a multicenter, open-label, dose-escalation study in adult patients with advanced solid tumors. The primary objectives are to determine the safety and PK of oral LOXO-101, and to identify the maximum tolerated dose (MTD) and/or the appropriate dose of LOXO-101 for further clinical investigation.

As of the data cutoff of 16 February 2016, 41 patients with solid tumors refractory to available therapies have been enrolled, including 7 patients with NTRK fusions across 5 different tumor types: sarcoma (1), PTC (1), MASC of the salivary glands (3), NSCLC (1), and gastrointestinal (GI) stromal tumor (1). Collectively, these 7 patients harbor gene fusions involving both *NTRK1* and *NTRK3*, with a variety of fusion partners. Six of the 7 patients with NTRK fusions were evaluable for response evaluation at the time of data cutoff, and all 6 have demonstrated a clinical response to LOXO-101. Five of the 6 patients have achieved confirmed PRs by standard RECIST version 1.1 criteria (representative response shown in Figure 4), while the sixth patient has exhibited a 21% tumor regression. All 7 patients remain on study with no evidence of progressive disease. For these 7 patients, duration of therapy ranges from 1 to 14 cycles. No objective responses have been observed in treated patients without NTRK fusions (Hong, Farago et al. 2016).

Figure 4. Diagnostic Imaging of the Thoracic Cavity of a Soft Tissue Sarcoma Patient with an *LMNA-NTRK1* Fusion



Study Baseline **Study Cycle 3 Day 1** **Study Cycle 13 Day 1**

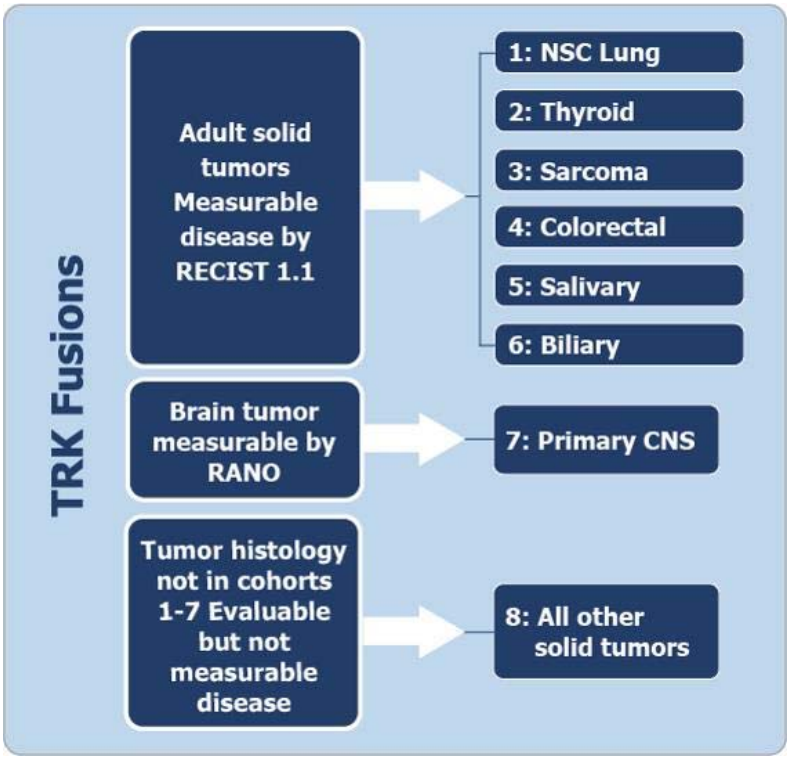
Forty two-year-old female with metastatic undifferentiated sarcoma treated with 100 mg BID LOXO-101. The patient was observed to have metastatic disease only in the lungs. Computed tomography (CT) scan images show axial (top) and coronal (bottom) images focusing on the thoracic cavity. The images demonstrate a marked tumor response with decreased size and/or resolution of the numerous pulmonary metastases (Doebele, Davis et al. 2015)

In total, 41 patients have been treated in the Phase 1 study, across five dose levels (50 mg QD, 100 mg QD, 200 mg QD, 100 mg BID, and 150 mg BID). Maximum plasma concentrations of LOXO-101 were reached 30 to 60 minutes following dosing. The unbound drug levels of LOXO-101 appear sufficient for approximately 98% inhibition of TRKA, TRKB, and TRKC at peak concentrations at all dose levels. LOXO-101 has been well tolerated. The MTD has not been reached, and the most common AEs are Grade 1 and 2 fatigue (29%), dizziness (24%), and nausea (20%). As the 100 mg BID dose has been well tolerated, with adequate LOXO-101 plasma exposure, this dose was selected for use in the Phase 2 study (LOXO-TRK-15002) in patients with NTRK fusion-positive tumors. This Phase 1 study (LOXO-TRK-14001) is currently ongoing (Hong, Farago et al. 2016).

7.2 LOXO-TRK-15002

In September 2015, Loxo Oncology initiated Study LOXO-TRK-15002, entitled *"A Phase 2 Basket Study of the Oral TRK Inhibitor LOXO-101 in Subjects with NTRK Fusion Positive Tumors"* (refer to Figure 5). The primary endpoint of this study is overall response rate, as defined by RECIST version 1.1. This multinational study includes 7 anatomically-defined cohorts: 1) locally advanced or metastatic NSCLC; 2) thyroid cancer; 3) sarcoma; 4) CRC; 5) salivary gland cancer; 6) biliary cancer; and 7) primary CNS tumors. An eighth cohort is designed to capture other patients with NTRK fusions with tumor types other than those listed above, as well as those who do not have measurable disease. Patients are treated with LOXO-101 as a single agent at 100 mg PO BID. This Phase 2 study (LOXO-TRK-15002) is currently ongoing (Drilon, Hong et al. 2016).

Figure 5. Study Schema: Study LOXO-TRK-15002



Abbreviations: CNS = central nervous system; NSC = non small cell; RANO = Response Assessment in Neuro-Oncology Criteria; RECIST = Response Evaluation Criteria in Solid Tumors; TRK = tropomyosin-related kinase.

7.3 NCI-MATCH

On 28 October 2015, Loxo Oncology announced that LOXO-101 was selected to be the TRK inhibitor treatment for the NCI's Molecular Analysis for Therapy Choice (NCI-MATCH) trial. As of the submission of this briefing book, the study arm containing LOXO-101 has not yet been opened by the NCI.

7.4 Clinical trial Experience of LOXO-101 For Pediatric Patients

LOXO-TRK-15003

In December 2015, Loxo Oncology initiated a multi-center Phase 1 pediatric trial (Study LOXO-TRK-15003, entitled: *A Phase 1 Study of the Oral TRK Inhibitor LOXO-101 in Pediatric Patients with Advanced Solid or Primary Central Nervous System Tumors*) with LOXO-101 to assess the preliminary safety, tolerability, and clinical activity in pediatric patients with advanced solid tumors and primary CNS tumors.

The starting dose level used in this pediatric study was based on the 100 mg BID Phase 2 dose being developed in adults. Pharmacokinetic modeling (SimCyp®) was used to select a dose for pediatric patients that is predicted to provide exposure in terms of area under the plasma concentration-versus-time curve (AUC) that is equivalent to the exposure achieved in adult patients taking a dose of 100 mg BID. The modeling takes into account body size differences and ontogeny of the enzymes that metabolize LOXO-101, which varies by patient age.

Study LOXO-TRK-15003 is designed to determine the safety of oral LOXO-101, including DLT, in pediatric patients with advanced solid or primary CNS tumors. It is also designed to characterize PK and to identify the MTD and/or the appropriate dose of LOXO-101 for further clinical investigation, to describe antitumor activity of LOXO-101, and pain and health related quality of life (HRQoL) in this patient population. At the starting dose in pediatrics, LOXO-101 is administered by a weight and age-adjusted dose algorithm to approximate adult exposures achieved with a 100 mg PO BID dose. Key eligibility criteria include: patients between 1 and 21 years old on Cycle 1 Day 1 with locally advanced or metastatic solid tumor or primary CNS tumor that has progressed, or was nonresponsive to available therapies, and for which no standard or available curative therapy exists, *OR* patients ≥ 1 month old with a diagnosis of infantile/ congenital fibrosarcoma, with a documented NTRK fusion that has progressed, or was nonresponsive to available therapies and for which no standard or available curative therapy exists; Karnofsky (those 16 years old or older) or Lansky (those younger than 16 years) performance score of at least 50; and adequate hematologic, hepatic, and renal function. The expected sample size in this study is up to 36 patients in order to define the MTD and up to 30–60 additional patients in the dose expansion cohorts.

The first patient treated in Study LOXO-TRK-15003 was an otherwise healthy female born with a right-sided neck/face mass initially diagnosed and treated as a hemangioma. By the time the patient reached 6 months of age, the mass grew rapidly and surgical resection revealed a diagnosis of IFS confirmed by an *ETV6* translocation by fluorescent in situ hybridization (FISH). Post-operatively, the tumor rapidly progressed, encroaching on the oral cavity. Chemotherapy with vincristine, actinomycin-D and cyclophosphamide (VAC) was started but disease progression was documented. An alternative chemotherapy regimen comprising ifosfamide and doxorubicin (ID) was initiated. Two additional courses of ID and 4 courses of ifosfamide and etoposide had minimal impact on the tumor, which had come to involve the base of skull, mastoids, and cervical vasculature. An additional surgical resection was attempted in (b) (6), but clear surgical margins could not be achieved.

Tumor tissue from the final surgical resection was re-evaluated by reverse transcription polymerase chain reaction (RT-PCR), revealing an *ETV6-NTRK3* gene fusion, consistent with the diagnosis of IFS. Five weeks following surgical resection, a magnetic resonance imaging (MRI) scan of the brain and neck showed a 20 mm × 19 mm × 18 mm hyper-enhancing mass involving the skull base of the middle cranial fossa, just anterior and inferior to the inner ear structures. No additional surgery was feasible. In (b) (6), at the age of 16 months, the patient enrolled on this Phase 1 study. At the end of Cycle 1, the patient underwent MRI scan with a ≥ 90% decrease in the MRI enhancing mass from baseline. At the end of Cycle 2, there was a further reduction in MRI enhancement, confirming the patient's PR. The investigator has reported that the patient has been rapidly achieving

previously delayed developmental milestones, such as speaking and walking. As of April 19, 2016, the patient remains on study with a PR reported as a continued ≥ 90% decrease from baseline (Nagasubramanian, Wei et al. 2016).

7.5 Summary of LOXO-101 Clinical Efficacy

In summary, preliminary data from Loxo Oncology's clinical development program has shown robust and durable anti-tumor activity for LOXO-101 in patients with NTRK gene fusion cancers across a diversity of anatomically-defined cancers. Seven patients with NTRK gene fusions enrolled on the Phase 1 study of which 6 were available for response evaluation and all 6 have demonstrated a clinical response to LOXO-101. Five of the 6 patients have achieved confirmed PRs by RECIST 1.1 criteria, while the sixth patient has exhibited a 21% tumor regression. Furthermore, the first patient enrolled on the pediatric Phase 1 study achieved a ≥ 90% tumor reduction, qualifying as a confirmed PR. All 8 NTRK gene fusion patients described remain on study with the longest patient in response for great than 14 cycles, as of the data cut-off, and no patient has demonstrated evidence of progressive disease. Given this promising evidence of efficacy and the safety profile for LOXO-101, the Loxo Oncology is currently enrolling a Phase 2 basket study with registration intent. Loxo Oncology is also committed to working with investigators and regulatory agencies on positioning LOXO-101 for rapid development in pediatric patients with NTRK gene fusion cancers.

8.0 POTENTIAL CHALLENGES FOR THE CLINICAL DEVELOPMENT OF LOXO-101 FOR PEDIATRIC PATIENTS

8.1 Rarity of Fusions by Cancer Type

An estimated 15,780 new cancer diagnoses occurred in children 0 to 19 years of age in 2014, with an estimated 1,960 deaths (Ward, DeSantis et al. 2014). The most common of these diseases are ALL, brain and CNS tumors, neuroblastoma, lymphoma, and thyroid cancer. Although 5, 10, and 15-year disease-related mortality have improved in recent decades with better therapies and supportive care, many patients still relapse and eventually succumb to their disease. Additionally, patients who respond to intensive treatment regimens and ultimately survive their disease face long-term morbidity and the risk of early-mortality as a result of their treatment (Mitby, Robison et al. 2003, Pui, Cheng et al. 2003, Armstrong, Pan et al. 2010, Kirchhoff, Leisenring et al. 2010, Yeh, Nekhlyudov et al. 2010).

As summarized in Section 4.3, NTRK fusions have been commonly identified in rare pediatric tumors, and rarely in more common tumors (refer to Table 4).

Table 4. Overall Incidence of Tumors with NTRK Fusions

Tumor Type	Estimated 2013 Incidence*	Metastatic/Locally Advanced/Recurrent Potential	Percentage of NTRK fusion
Infantile Fibrosarcoma (IFS)	0.1 per 1 million	Extremely low	> 75%
Cellular Subtype Congenital Mesoblastic Nephroma (CMN)	0.08–0.4 per 1 million	Extremely low	> 90%
Pediatric Papillary Thyroid Carcinoma (PTC)	5.3 per 1 million	Low	~ 25%
Pediatric Spitzoid Tumors	0.005–0.02 per 1 million	Extremely low	~ 25%
Pediatric Primary Central Nervous System (CNS) Disease	30.8 per 1 million	Extremely high	~ 10%
Pediatric Soft Tissue Sarcoma (STS)	9.6 per 1 million	High	< 1%

Sources: (Cotignola, Reva et al. 2007) (Forman, Ferringer et al. 2008) (Wang, Li et al. 2014) (Sebire and Vujanic 2009) (Furtwaengler, Reinhard et al. 2006) (Santos, Carvalho Jde et al. 2011) (Swetter, Boldrick et al. 2005).

*The incidence and prevalence of various tumors were investigated using data from the SEER from the NCI (SEER, released April 2016) and the scientific literature. Appropriate SEER codes were available from the International Classification of Childhood Cancer (ICCC) Recode ICD-O-3/ WHO 2008 classification system (http://seer.cancer.gov/iccc/iccc-who2008 html). Incidence calculations were made using the SEER*STAT program Surveillance Research Program, National Cancer Institute (SEER*Stat software (www.seer.cancer.gov/seerstat; version 8.3.2.). However, not all tumors could be identified in SEER*STAT so literature was used to aid in estimating the incidence and prevalence. Incidence figures were age-adjusted to the 2000 US standard Population (single ages to 84; Census P25-1130) standard.

NTRK fusions occur across a broad range of histologically and/or anatomically-defined cancer types. Given the relative rarity of NTRK gene fusions, and the diversity of contexts in which they occur, a system-wide commitment to comprehensive genomic profiling in pediatric cancer patients is the best approach for maximizing the number of identified patients.

8.2 NTRK Gene Fusions Are Common in Very Rare Cancers Where Metastatic Disease is Uncommon

As previously noted, IFS tumors most commonly occur in the extremities, trunk, or the head and neck (Stout 1962, Dillon, Whalen et al. 1995, Knezevich, McFadden et al. 1998, Bourgeois, Knezevich et al. 2000, Coffin and Fletcher 2002). The standard of care is defined by surgery, though chemotherapy may play a role in downstaging patients prior to excision or re-excision. Metastatic disease is uncommon, though presents a management challenge when it does occur. The Sponsor has initiated conversations with experienced clinicians who have managed metastatic patients. Their collective clinical experience suggests more modest efficacy for combination chemotherapy than reported in the clinical literature. Locally advanced disease is also problematic because the size of the tumor relative to the patient often leads to cosmetic and functional deficits, and even limb amputation. For extremity disease, primary and secondary amputation rates exceed 50% (Kurkchubasche, Halvorson et al. 2000). Radiotherapy is traditionally not used due to long-term toxicity concerns in these very young patients (Loh, Ahn et al. 2002, Pui, Cheng et al. 2003).

Much like IFS, CMN is a rare pediatric tumor that occurs in very young patients. It most commonly presents as unilateral disease that can be cured through surgical intervention (van den Heuvel-Eibrink, Grundy et al. 2008). However, on occasion, patients relapse and develop and metastatic disease. The largest reported case series of 330 children included 17 patients with relapse and 8 with metastatic disease (Beckwith 1993). By implication, LOXO-101 could address an unmet need in these patients.

Pediatric Spitz tumors were historically categorized as a separate entity from melanomas in part due to their more common propensity for benign presentation. Published literature does not comment on the degree to which Spitz tumors with NTRK fusions behave differently than Spitz tumors overall. Therefore, we assume most NTRK fusion Spitz tumors will have a benign presentation and metastatic disease will be uncommon. To the extent that Spitz tumors with NTRK fusions present with metastatic disease, LOXO-101 could be developed in that population.

8.3 Metastatic Pediatric Tumors with Infrequent NTRK Gene Fusions

Pediatric papillary thyroid cancer (PTC), much like adult PTC, can often be managed with surgery and radioactive iodine therapy. Metastatic disease is uncommon, though this presents a potential opportunity for LOXO-101 clinical development. A recently published study reported 7 of 27 (26%) cases of NTRK1 or NTRK3 fusion PTC in pediatric patients in the Northeastern United States. Together with RET fusions, NTRK fusion-positive tumors were associated with larger size, adverse histologic subtypes, local invasion and metastasis (Prasad, Vyas et al. 2016). While several published studies have demonstrated a significant correlation between the presence of a BRAF mutation and the development of iodine-refractory disease, no specific studies analyzing the effect of NTRK fusions and radioactive

iodine uptake have yet been reported. However, a MAPK gene expression signature indicative of mutant BRAF-like signaling was recently demonstrated to be present in primary tumors harboring NTRK3 fusions, and NTRK-fusion PTCs were shown to possess a thyroid differentiation gene expression pattern in between less differentiated BRAF-like and more differentiated RAS-like PTCs (Ricarte-Filho, Li et al. 2013, Cancer Genome Atlas Research 2014). These data could suggest that NTRK fusion positive PTCs behave similarly to BRAF-mutant PTCs with respect to dedifferentiation, MAPK-mediated downregulation of iodine metabolizing genes, and relative resistance to radioactive iodine.

CNS tumors and Ph-like ALL represent more common clinical situations with much lower frequencies of NTRK fusions. Though these clinical situations present with more unmet clinical need due to frequent occurrence in pediatrics and poor outcomes, they present other unique challenges for LOXO-101 clinical development. In DIPG, NBS-HGG, pilocytic astrocytoma, and LGG, the location of these tumors may render biopsies to confirm NTRK gene fusion status unsafe or unappealing to patients and families. Therefore, given the low frequency of NTRK gene fusions in this population, it may be difficult to prospectively identify patients most likely to derive benefit from LOXO-101. In the case of Ph-like ALL, only one case of an NTRK gene fusion has ever been identified in the literature, so it may be premature to assess the clinical development feasibility of LOXO-101 in that setting.

8.4 A Needle In A Haystack

The most difficult challenge for Loxo Oncology to develop LOXO-101 successfully in pediatric patients is the identification and enrollment of NTRK fusion patients.

Even though TRK was one of the first oncogenes identified (Pulciani, Santos et al. 1982), NTRK is not routinely screened for in the pediatric clinical setting. However, there are precedents in oncology where the availability of an active drug alters awareness, and in turn, testing algorithms in productive ways. For example, gene fusions involving *ALK* in adults were first described in anaplastic large-cell lymphoma (1994) though routine testing for *ALK* rearrangement in non-small cell lung cancer only began once crizotinib began exhibiting unequivocal activity in the clinic (Vaishnavi, Capelletti et al. 2013, Vaishnavi, Le et al. 2015).

Our understanding of the clinical disease burden of *NTRK3*-driven pediatric cancers is in part a fortuitous artifact of scientific interest in *NTRK3*'s common gene partner *ETV6*. *ETV6* encodes an ETS family transcription factor, and is involved in a large number of chromosomal rearrangements including ABL in leukemia. Pathology labs that have committed to testing for *ETV6* by fluorescent in situ hybridization (FISH) may inadvertently pick up *NTRK3* events (Knezevich, Garnett et al. 1998, Knezevich, McFadden et al. 1998, Argani, Fritsch et al. 2000).

By implication, we do not know about the frequency or presence of NTRK gene fusions that we are not routinely looking for, such as non-ETV6 *NTRK3* events, *NTRK2* events, and *NTRK1* events. If clinicians and pathologists believe there is no therapeutic actionability to a diagnostic answer, or that the rarity of the genetic event does not justify the exhaustion of biopsy tissue or the cost of assay implementation, emerging novel targets such as NTRK gene fusions will be difficult to exploit for the clinical benefit of patients.

The adoption of comprehensive genomic profiling in the routine clinical care of pediatric patients with cancer upends the inertia that would otherwise work against new single-plex assays, and offers a new opportunity for detecting NTRK fusions. Next-generation sequencing (NGS) assays allow for the testing of 1000s of diagnostic questions in a hypothesis-free setting. NTRK gene fusions could be identified while simultaneously assaying for many other genetic markers in the cancer. This approach stands in contrast to hypothesis-driven, single-plex approaches such as FISH and reverse transcription polymerase chain reaction (RT-PCR), where NTRK fusions, with a priori defined fusion partners, must be assessed individually by deliberate intent.

Next-generation sequencing testing for NTRK gene fusions poses two key challenges, one operational and one technical. First, NGS assays remain uncommonly utilized in routine clinical practice. Technical complexity and third-party payor reimbursement issues have limited their uptake. Second, as complex structural chromosomal abnormalities, gene fusions in general, and NTRK gene fusions in particular, pose unique design challenges to DNA-based NGS assays. NGS assays have poor sensitivity against NTRK gene fusions because 1) there are three different NTRK genes (*NTRK1*, *NTRK2*, *NTRK3*); 2) gene breaks often occur within intronic regions of the DNA; 3) gene breaks may occur in different introns; 4) NTRK introns are unusually large; and 5) once broken, the NTRK portion of the gene can fuse to one of many fusion partner genes (Table 5). Developers of NGS assays must consider these disparate challenges when designing probes and allocating "sequencing bandwidth" to a finite set of genetic questions across the human genome.

Table 5. Selection of NTRK Gene Fusions and Related Pediatric Tumors

5' Partner Gene	3'NTRK Gene	Tumor Type
TPM3	*NTRK1*	Glioma, Sarcoma
TPR	*NTRK1*	Papillary Thyroid Cancer
LMNA	*NTRK1*	Spitzoid neoplasm
LMNA	*NTRK1*	Infantile Fibrosarcoma
TP53	*NTRK1*	Spitzoid neoplasm
VCL	*NTRK2*	Pediatric Glioma
AGBL4	*NTRK2*	Pediatric Glioma
AFAP1	*NTRK2*	Low Grade Glioma
SQSTM1	*NTRK2*	Low Grade Glioma
ETV6	*NTRK3*	Infantile Fibrosarcoma
ETV6	*NTRK3*	"Cellular" Congenital Mesoblastic Nephroma
ETV6	*NTRK3*	Secretory Breast Cancer
BTB1	*NTRK3*	Pediatric Glioma
RBPMS	*NTRK3*	Thyroid Cancer

Adapted from: (Vaishnavi, Le et al. 2015).

RNA-based NGS assays avoid some of the technical issues mentioned above, and may be more sensitive for NTRK fusion detection. However, RNA-based assays have more limited use in routine clinical practice.

In both adults and pediatric patients, the routine use of molecular and specifically NGS testing is still in its infancy. Multiplex testing has become more routine in certain adult cancer settings, such as lung cancer, where there are FDA-approved medications available for specific genetic alterations. However, the Centers for Medicare & Medicaid Services and private payors do not routinely cover the broad molecular profiling of tumors to drive treatment decisions. Today, access to comprehensive genomic profiling is generally limited to patients who present to comprehensive cancer centers/centers of excellence for their care where the costs of testing are assumed by the institution, or limited to patients who assume personal financial liability for third-party commercial lab costs.

The NCI, as part of the Obama Administration's Precision Medicine Initiative, recently launched an umbrella trial called the NCI-MATCH trial that links specific actionable molecular abnormalities with rationally chosen therapeutic agents. A pediatric version, Pediatric MATCH, will be launched soon by the NCI and the Children's Oncology Group. Two recent papers reporting on the feasibility of sequencing pediatric tumors demonstrated that between 30% and 40% of tumors have an actionable mutation (Harris, DuBois et al. 2016, Parsons, Roy et al. 2016).

The efficient development of LOXO-101 in pediatric patients requires encouraging the broader use of NGS assays in routine clinical practice, and encouraging providers of NGS assays to improve sensitivity for NTRK gene fusion detection. As a drug Sponsor, we can encourage these behaviors by publishing LOXO-101 clinical data that support the actionability of NTRK gene fusion identification. However, payors can further accelerate this goal by guaranteeing access to cutting edge genetic testing resources for children with cancer.

8.5 Summary

NTRK gene fusion cancers may be among the first truly genetically-defined cancers, where tumor site of origin is a minor variable in the pathologic description of these cancers, and in the choice of systemic treatment for advanced disease. In pediatrics, NTRK gene fusion cancers have been described in a broad range of tumors, where there are varied standards of care and varied clinical unmet needs. LOXO-101 is a potent and selective inhibitor of TRKA, TRKB, and TRKC kinases that has shown encouraging evidence of efficacy in both adult and pediatric settings, with a high rate of objective tumor response in NTRK gene fusion cancers and favorable tolerability. The Sponsor believes a single risk remains to the efficient development of LOXO-101 in pediatrics—patient identification. Widespread adoption of comprehensive genomic profiling in pediatrics, with methods sensitive for NTRK gene fusion detection, could identify patients with sarcoma, papillary thyroid cancer, and other cancers who might benefit from a precision medicine approach to their care. This approach would also stimulate research and drug development interest in other molecular targets identified through multiplex testing approaches.

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