Identification of Pan-Trk Inhibitors for the Treatment of Trk-Driven Cancers

Karyn S. Bouhana, Anna M. Gomez, Steven W. Andrews, Patrice A. Lee, Taylor Alford, Rich Woessner, Ira von Carlowitz and Shannon L. Winski.

Array BioPharma Inc., Boulder, CO

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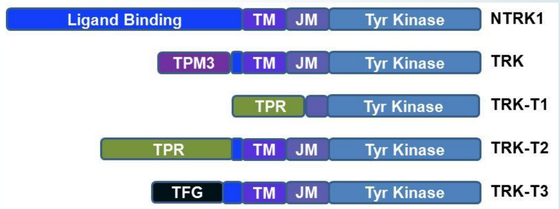
Abstract

The Trk family of genes, which includes trkA/NTRK1, trkB/NTRK2 and trkC/NTRK3, encode the tyrosine kinase receptors for the neurotrophin family of nerve growth factors. Deregulated kinase activity of trk family members is associated with human cancer. Oncogenic translocations involving trkC kinase domain have been identified in AML, salivary gland carcinoma, adult secretory breast cancer, congenital fibrosarcoma, and pediatric nephroma. Oncogenic trkA translocations have been reported in papillary thyroid and colorectal cancers. We have identified orally bioavailable, potent and selective ATP-competitive inhibitors of the trk family of receptor tyrosine kinases and are developing these for the treatment of Trk-driven cancers.

AR523 is a pan-Trk inhibitor, inhibiting TrkA, B and C, and has similar activity against all 3 receptors in a cell based assay (IC_{50} ~10 nM). In a screen at 1 µM against a panel of 230 kinases, AR523 inhibited only three additional kinases more than 50% (TNK2, Bmx and Txk). In cell culture AR523 was found to exhibit selective anti-proliferative activity toward the cell line KM12, which harbors the oncogenic TPM-TrkA translocation, while exhibiting no activity toward HT29, a cell line with no trk gene rearrangements. Studies in mice engrafted with KM12 tumors revealed significant anti-tumor and pharmacodynamic activity of AR523. Administration of a single oral dose of AR523 reduced tyrosine phosphorylation of TrkA by nearly 80% at twelve hours after dosing. Parallel analysis of Akt and Erk revealed reduced phosphorylation of these downstream effectors. Administration of 100 mg AR523 daily for two weeks to mice with KM-12 xenografts produced mean tumor growth inhibition of 86% and a mean of 42% tumor regression. AR523 exhibits dose-dependent inhibition of tumor growth at 10 and 30 mg/kg in KM-12 xenografts with 54 and 72% TGI, respectively. AR523 was well-tolerated, causing no weight loss or deaths compared with vehicle control.

Increasingly Trk mutations have been shown to be activating and the use of Trk inhibitors may provide a new therapeutic strategy for targeted treatment.

TrkA (NTRK1) and TrkC (NTRK3) Oncogenes



Trk Fusion Oncogene Characteristics

- **Cytoplasmic localization (no longer a transmembrane receptor)**
- **Constitutive dimerization resulting in constitutive kinase activity**

ETV6-NTRK3

- **ETV6 (ETS variant gene 6), also known as TEL**
- **TEL-JAK2, TEL-ABL and TEL-PDGFR identified in hematologic cancers**
- **Identified in congenital fibrosarcoma, secretory breast cancer, AML and salivary gland carcinoma**

Indication	Juvenile Breast	Pediatric Nephroma	Congenital Fibrosarcoma	Adult Secretory Breast	Salivary (MASC)	Papillary thyroid carcinoma	CRC	AML
Trk Translocation	ETV6-NTRK3	ETV6-NTRK3	ETV6-NTRK3	ETV6-NTRK3	ETV6-NTRK3	TPM3-NTRK1 TPR-NTRK1 TFG-NTRK1	TPM3-NTRK1	TPM3-NTRK1

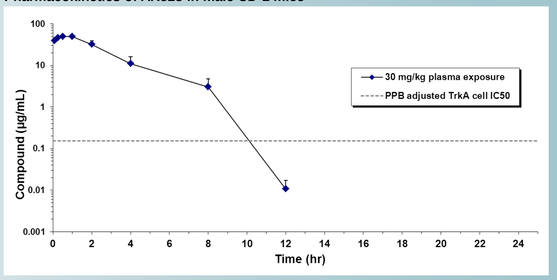
The prevalence of these translocations is largely unknown. Determination of prevalence will be conducted as the information becomes available.

Selected *In Vitro* Properties of Pan-Trk Inhibitor Leads

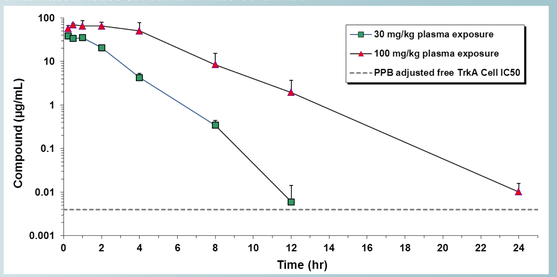
Parameters	AR772	AR523
TrkA Binding / Cell IC_{50} (nM)	1.6 / 2.0	3.3 / 10.4
Hu PPB adj Cell IC_{50} / IC_{90} (nM)	10 / 90	42 / 378
%PPB human / rat / dog / monkey	79.0 / 79.5 / -- / --	75.3 / 89.2 / 67 / 71
MIC ER % human / rat / dog / monkey	67 / 46 / 47 / 82	38 / 30 / 57 / 69
IV dose rat / dog / monkey (mg/kg)	1 / 3 / 1	1 / 1 / 3
IV Cl rat / dog / monkey (mL/min/kg)	17 / 7 / 18	11 / 10 / 21
IV %ER rat / dog / monkey	24 / 19 / 41	16 / 28 / 47
PO dose rat / dog / monkey (mg/kg)	10 / 10 / 10	20 / 10 / 10
PO AUC rat / dog / monkey (µg-hr/mL)	1.9 / 22.1 / 2.0	3.4 / 5.4 / 3.9
PO %F rat / dog / monkey	17 / 83.2 / 22	12 / 31.2 / 45

Pharmacokinetics Following a Single Oral Dose of Pan-Trk Inhibitors in Male CD-1 Mice

Pharmacokinetics of AR523 in male CD-1 mice



Pharmacokinetics of AR772 in male CD-1 mice



AR Number	Dose	AUC_{last} (µg-hr/mL)	F (%)	T_{max} (hr)	C_{max} (µg/mL)	Hours over PPB adjusted TrkA cell IC_{50}
AR523	30 mg/kg	155	~100	0.5	50	10
AR772	30 mg/kg	94.0	94	0.3	38	12
AR772	100 mg/kg	388	~100	0.5	69	24

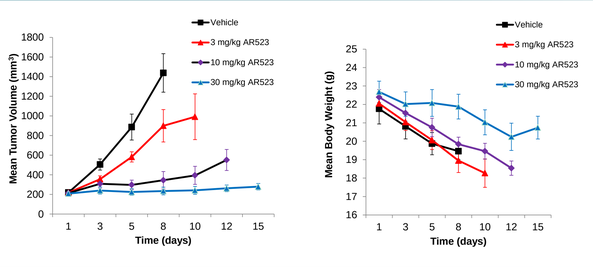
Selective Dependence on Trk *in vitro*

- Cell potency was confirmed in a NGF-driven transfected CHO cell system
- Proliferation of KM-12 colorectal tumor cell line with TPM3-TrkA fusion is potently inhibited by pan-Trk inhibitors
- Tumor cell lines without Trk rearrangements are >100-fold less sensitive

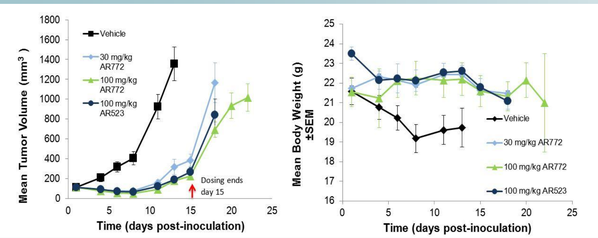
	Cell Line Proliferation EC50 (nM)				Trk EC50 (nM) *		
Inhibitor	KM-12	HT-29	PC-3	MiaPaca-2	TrkA	TrkB	TrkC
AR772	23	>5,000			1.6	1.6	---
AR523	24	>5,000			10.4	7.9	6

* Transfected CHO cells

Dose-Dependent Inhibition of Tumor Growth in KM-12 Xenografts which Harbor a TrkA Translocation

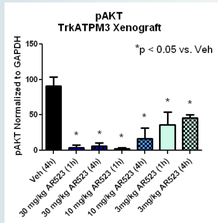


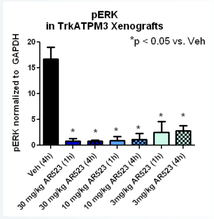
KM-12 human tumor xenografts staged at ~200mm^3 in nude mice were treated with AR523 PO, BID at 3, 10 and 30 mg/kg for 15 days and tumor volumes determined twice per week. Body weights were measured at the time of tumor measurement. AR523 exhibits dose-dependent tumor growth inhibition and improves tumor-induced body weight loss. KM-12 xenografts may be cachectic in nude mice.

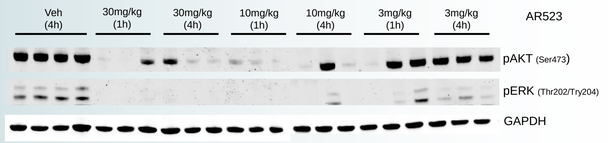


KM-12 human tumor xenografts staged at ~150 mm^3 were treated with AR772 PO, BID at 30 and 100 mg/kg for 15 days, and tumor volumes and body weight determined twice per week. To examine durability of response, dosing was discontinued on day 15 and tumor growth followed for an additional week. Treatment with Trk inhibitor resulted in tumor stasis during the treatment phase with rebound tumor growth observed when Trk inhibitor was withdrawn.

Trk Inhibition Blocks Downstream Signaling in both ERK and Akt Pathways







KM-12 tumor-bearing mice were treated with pan-Trk inhibitors, and tumors excised and processed for Western Blot analysis of phospho-Erk and phospho-AKT at 1 or 4 hours following oral dosing. Flash frozen tumors were processed and analyzed by gel electrophoresis and phospho-protein normalized to GAPDH.

Summary

- Trk is often dysregulated in tumors through translocations which can lead to constitutive activation of the receptor. Trk A expression is also linked to increased tumor aggressiveness in pancreatic cancer and AR-negative prostate cancer.
- Rearrangements in Trk receptors are oncogenic and highly prevalent in some tumor types, including neuroblastoma, head and neck cancer, gastric carcinoma, lung adenocarcinoma, breast cancer, colorectal carcinoma, and glioblastoma.
- Point mutations and fusion proteins for all three receptors are common in many types of cancers. Oncogenic rearrangements of both the TrkA and TrkC genes have been identified, highlighting the potential benefits of a pan-Trk inhibition strategy, and providing a means to identify appropriate target patient populations.
- Our data support the clinical investigation of Trk inhibitors in oncology.

References

1. Greco A, *et al*. *Molecular and Cellular Endocrinology* 321 (2010) 44-49
2. Bamford et al (2004) The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. Br J Cancer, 91,355-358.