Target and Selectivity Profiling of Axitinib in Cell-Based and Biochemical Assays

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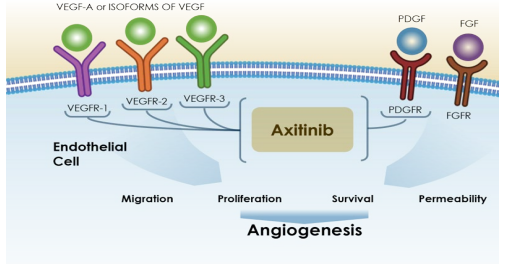
PURPOSE

Axitinib is a highly potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases (VEGFR-1, -2, and -3). The VEGFR and PDGFR pathways are targeted in oncology to inhibit tumor angiogenesis starving the tumors of blood supply.[1] The potency and selectivity of axitinib, sunitinib, and vorolanib was compared in kinome profiling biochemical assays at a physiologically relevant ATP concentration (1 mM) at two drug concentrations. Important kinases were further compared to determine IC_{50} concentrations in cellular phosphorylation assays. Ocular Therapeutix, Inc. is investigating an intravitreal axitinib hydrogel injection (OTX-TKI), designed to deliver axitinib for up to 12 months using our proprietary bioresorbable hydrogel platform (ELUTYX™).

Figure 1. Axitinib Targets VEGFR and PDGFR in Cells



** Adapted from: Zhao Y, Adjei AA. Targeting Angiogenesis in Cancer Therapy: Moving Beyond Vascular Endothelial Growth Factor. Oncologist. 2015;20(6):660-673. doi:10.1634/theoncologist.2014-0465*

METHODS

- Kinase activity was quantified via PhosphoSens® assay (AssayQuant®) at physiological ATP concentration (1 mM).
- Cell-based kinase assays were conducted at Reaction Biology, Germany, to evaluate half-maximal inhibitory concentration (IC_{50}) of axitinib, sunitinib, and vorolanib on VEGFR2, PDGFRβ, FGFR2, and TIE2 kinase activity.
- Tyrosine kinase inhibitor (TKI) drugs were obtained from MedChemExpress

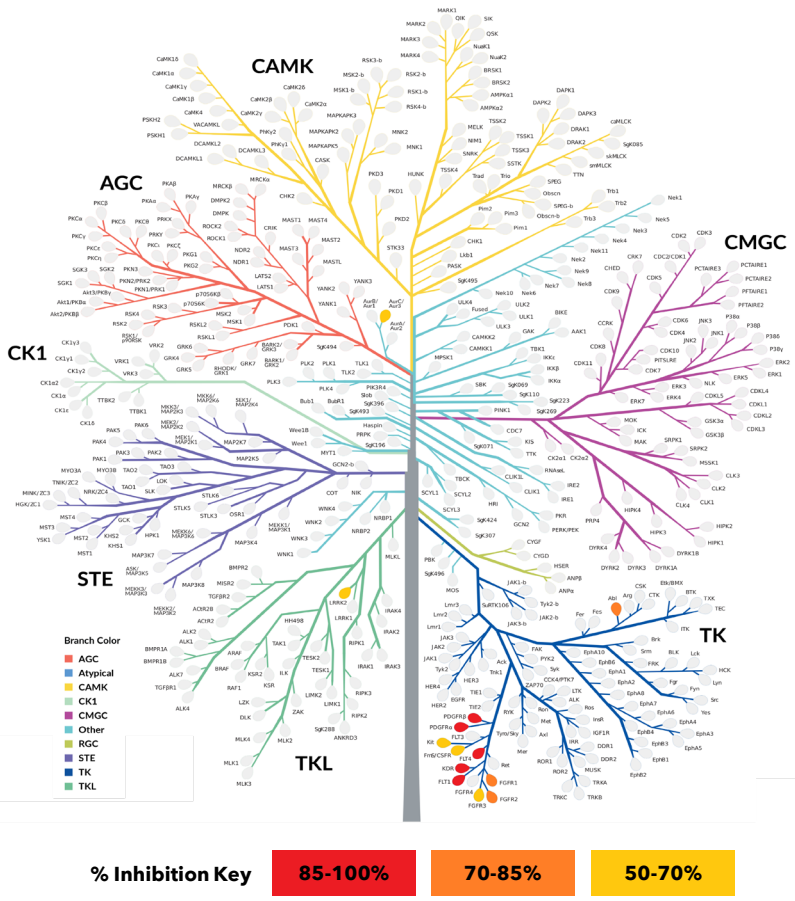
RESULTS

Table 1. Heat Map of Percent Inhibition* from 400 Kinase Panel Screen at 1 mM ATP and Two Drug Concentrations

Kinase	Axitinib		Sunitinib		Vorolanib	
	1 μM	*0.05 μM*	*1 μM*	*0.05 μM*	*1 μM*	*0.05 μM*
FLT1 (VEGFR1)	100	83	59			
KDR (VEGFR2)	98	85	51		53	
PDGFRα	94		70		65	
FLT4 (VEGFR3)	92				51	
PDGFRβ	88	57	78		64	
FGFR1	82					
FGFR2	77				55	
ABL1	75					
FGFR3	70					
SCFR	65					
AURKC	62					
LRRK2	60		69		73	
CSF1R	57					
FLT3			98	93	97	86
CAMKK1			51		54	
MAP3K11					88	
PHKG1			80			
MERTK			60			
ALK			70			
MYLK4			50			
GAK			62		52	
NTRK1			73		63	
AXL			75			
MAST3					54	
STK33			65			
NTRK2			53			
PRKAA2-B1-G1			53			
CDK7/CycH1			57			
NUAK1			69			
MAPK7					52	
CLK4			69			
MUSK			77			
EPHB2			52			
BCR RET			52			
MYLK					53	
MAP4K3			72			
PRKAA2-B2-G1			52			
DDR1			63			
MAP4K1			68			
RET			51			

***only inhibition >50% is shown**

Figure 2. Kinase Tree of Axitinib Targets
(performed at 1 μM axitinib and 1 mM ATP)



% Inhibition Key 85-100% | 70-85% | 50-70%

Table 2. IC_{50} Determination using Cell Phosphorylation

Compound	IC_{50} (nM)			
	VEGFR2	**PDGFR-β**	**FGFR2**	**TIE2**
Axitinib	0.032	1.6	72	1500
Sunitinib	2.900	11.0	>10000	1400
Vorolanib	3.300	16.0	1400	>10000
Cell line	HUE	NIH3T3	Kato-III	CHO
[ATP]	Physiological	Physiological	Physiological	Physiological

- Kinome profiling demonstrated VEGFR-1, -2, -3 and PDGFR- α, -β are the top 5 axitinib kinase targets exhibiting >85% inhibition.
- Axitinib demonstrates kinase selectivity in a 400 kinase kinome profile screen.
- Cellular assay IC_{50} data demonstrated that axitinib is ~100x more potent for VEGFR2 and ~10x more potent for PDGFR- β compared to sunitinib and vorolanib.
- Cellular assay data demonstrated no TIE2 inhibition within physiological drug solubility range at cell ATP levels via the gold standard cell phosphorylation assay.

CONCLUSIONS

Axitinib is a highly selective and more potent inhibitor of VEGFRs and PDGFRs compared to sunitinib and vorolanib.

- Axitinib, within its physiological solubility range, doesn't inhibit TIE2 at relevant ATP concentrations in a biochemical kinome panel screen and in cell phosphorylation assays.
- Axitinib and sunitinib VEGFR2 IC_{50} values in cell phosphorylation assays are consistent with literature.[2,3,4]

Support: Ocular Therapeutix. **Study Disclosures:** The following presentation discusses an investigational drug, OTX-TKI, in development. OTX-TKI's efficacy and safety profiles have not been established, and it has not been approved for marketing by the FDA or any other health agency. Good Laboratory Practice for Nonclinical Laboratory Studies, and as accepted by Regulatory Authorities throughout the European Union (OECD Principles of Good Laboratory Practice), Japan (MHLW), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement. **Author Disclosures**: **Chintan Patel**: Code E (Employment): Ocular Therapeutix | **Charles Blizzard**: Code E (Employment): Ocular Therapeutix | **Peter Jarrett**: Code E (Employment): Ocular Therapeutix. **References: 1.** Attwood, M.M., et al., 2021. Trends in kinase drug discovery: Targets, indications and inhibitor design. Nature Reviews Drug Discovery, 20(11), pp.839-861. **2.** McTigue M *et al. Proc Natl Acad Sci U S A.* 2012;109(45):18281-18289**. 3.** INLYTA NDA 2022324. **4.** SUTENT NDA 021938