TTI-2341: A Novel, Orally Bioavailable, Brain-penetrant, Covalent Epidermal Growth Factor Receptor (EGFR) Inhibitor for Treatment of Glioblastoma Multiforme (GBM) and Brain Metastases of Non-small Cell Lung Cancer (NSCLC)

Zezhou Wang, Peter Dove, David Rosa, Tina Catalano, Andrew Marshall, Bolette Bossen, Jaehyun Choi, Chunlei Wang, Mark Wong, Jeff Winston, Malik Slassi, Penka S. Petrova and Robert A. Uger

Trillium Therapeutics Inc., Mississauga, ON, Canada

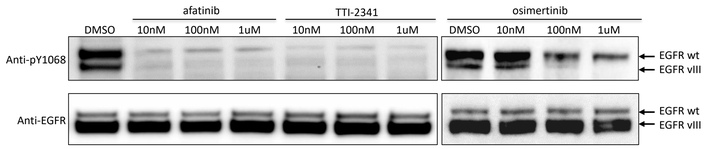
SNO 2017

Abstract # 4329

Introduction

- Aberrant EGFR activity is implicated in the development of central nervous system (CNS) tumors such as GBM, as well as brain metastases of NSCLC
- To date, approved EGFR inhibitors have demonstrated limited therapeutic efficacy against CNS tumors due to insufficient penetration of the blood-brain barrier (BBB) and/or poor activity against resistance-associated EGFR mutants such as T790M and C797S
- Thus there is a strong unmet medical need for the development of novel EGFR inhibitors that effectively access the brain and inhibit EGFR and disease-related EGFR variants
- In the present studies, we report that TTI-2341, a novel, orally bioavailable, covalent EGFR inhibitor, displays potent activity against a broad range of EGFR mutants, excellent PK and BBB penetration, and a favorable preclinical safety profile

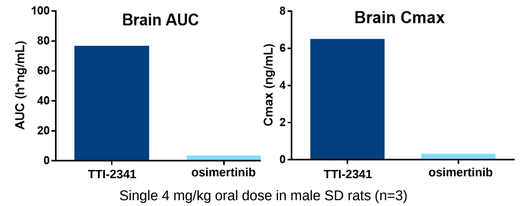
TTI-2341 Potently Inhibits Cell-Based Kinase Activity of EGFR vIII, a Common Driver of GBM



Western blot demonstrating inhibition of EGFR auto-phosphorylation (pY1068) in GBM cell line DKMG expressing EGFR wt and vIII mutant

TTI-2341 Demonstrates Superior Free Drug Exposure in Rat Brain vs. Afatinib and Osimertinib

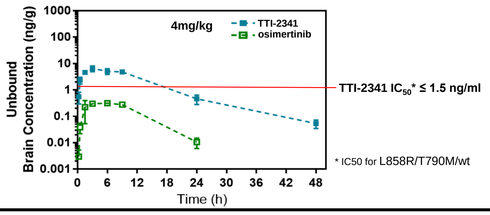
A Comparison of Unbound (Active) Drug Exposure of TTI-2341 and Osimertinib in Rat Brain



Single 4 mg/kg oral dose in male SD rats (n=3)

- TTI-2341 estimated unbound brain exposure (AUC and Cmax) is >20 fold higher than osimertinib and >15 fold higher than afatinib (data not shown)
- TTI-2341 has slightly higher Kpuu (unbound drug brain-to-plasma ratio) than osimertinib and 13-fold higher Kpuu than afatinib

B Estimated Unbound (Active) Drug Level of TTI-2341 in Rat Brain



* IC50 for L858R/T790M/wt

- TTI-2341 is quantifiable in the brain up to 48 hours post 7-day repeat dosing
- Concentrations of TTI-2341 above 1.5 ng/mL (IC_{50} for EGFR L858R, T790M, or wt) are predicted to be efficacious

TTI-2341 Is a Potent and Selective Inhibitor of EGFR and Resistance-Associated EGFR Mutants

A Biochemical Kinase Assay (data presented as nM IC_{50})

Compound	EGFR (wt)	EGFR (T790M)	EGFR (L858R/T790M)	HER2
TTI-2341	<0.5	2.3	2.9	<0.5
erlotinib	<0.5	5,580	4,150	181
afatinib	<0.5	0.6	<0.5	<0.5

T790M mutation is associated with clinical resistance to erlotinib

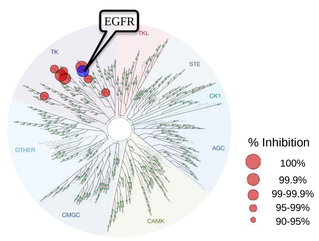
Compound	EGFR (C797S)	EGFR (d746-750/C797S)	EGFR (d746-750/T790M/C797S)	EGFR (T790M/C797S/L858R)
TTI-2341	<0.5	<0.5	62	389
osimertinib	5,810	1,160	584	3,890
afatinib	<0.5	<0.5	40	176

C797S mutation is associated with clinical resistance to osimertinib

B KINOMEscan® with TTI-2341 (300 nM)

EGFR variants	% Inhibition
EGFR WT	100
EGFR(E746-A750del)	100
EGFR(G719C)	100
EGFR(G719S)	100
EGFR(L747-E749del, A750P)	100
EGFR(L747-S752del, P753S)	100
EGFR(L747-T751del,insS)	99
EGFR(L858R)	100
EGFR(L858R,T790M)	98
EGFR(L861Q)	100
EGFR(S752-I759del)	99
EGFR(T790M)	98

Selectivity Profile: KINOME Tree Spot



Non-EGFR family kinase targets include DDR1, EPHA6, BLK, LCK, LYN, and SRC

TTI-2341 Exhibits Superior ADME and PK Properties vs. Afatinib and Osimertinib

A *In Vitro* ADME Studies

Parameter	TTI-2341	Afatinib	Osimertinib	Conclusion
Permeability[1] (Caco-2)	2.43	0.81	0.82	**TTI-2341 has 3x higher cell permeability**
PgP Efflux Ratio[2] (MDCK-MDR1)	1.61	5.02	13.4[3]	**TTI-2341 is not a PgP substrate (efflux ratio < 2)**
Brain Tissue Binding (Rat)	95.7%	99.4%	>99.99%	**TTI-2341 is not highly bound to brain tissue**

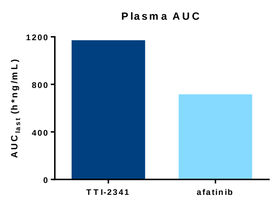
[1]Papp (A-B) 10^{-6} cm/s [2]Papp (B-A) / Papp (A-B) [3]Ballard et al., 2016

Other key ADME parameters of TTI-2341 were comparable or superior to benchmarks

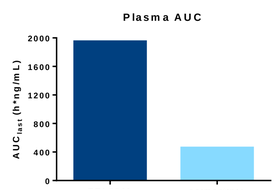
B *In Vivo* PK Studies (Rat)

Parameter	TTI-2341	Afatinib	Osimertinib
Bioavailability (F %)	85.6	43.3	45*
Plasma Half-life (T1/2, h)	> 8 hours (20 mg/kg PO)	> 8 hours (20 mg/kg PO)	2.81 hours (16 mg/kg PO)
Plasma Cmax (ng/ml)	601 (20 mg/kg PO)	400 (20 mg/kg PO)	296 (16 mg/kg PO)

* Finlay et al., 2014



Single 10 mg/kg oral dose in male SD rats (n=4)
Time points: 0-8 hrs



Single 4 mg/kg oral dose in male SD rats (n=3)
Time points: 0-24 hrs

TTI-2341 Is Well Tolerated After Repeat Oral Dosing

- Toxicokinetic/toxicology studies of 7-day daily repeat dosing of TTI2341 were performed in male Sprague-Dawley rats (n=5, per group) at 4 mg/kg, 8 mg/kg, and 16 mg/kg
- TTI-2341 was well tolerated at a dose level of 4 mg/kg/day, with clinical signs of poor tolerability at higher dose levels, which were most prominent at 16 mg/kg/day
- No meaningful test article-related toxicological and/or histopathological changes (adrenals, heart, liver, spleen, lung, kidneys, brain, GI, pancreas, testes and thymus) at up to 8 mg/kg/day
- No prominent CNS observations at doses up to 8 mg/kg/day
- Of note, systemic exposure of TTI-2341 at 4 mg/kg/day is similar to that of approved drug afatinib at 16 mg/kg/day (i.e. approximately 4 x approved clinical equivalent dose)

Conclusions

- TTI-2341 is a novel, covalent, EGFR inhibitor with potent activity against a broad range of EGFR variants, including disease-relevant mutants T790M, C797S, and vIII
- TTI-2341 is BBB-penetrant and demonstrates superior ADME properties, oral bioavailability, and free drug brain exposure to afatinib and osimertinib
- TTI-2341 is well tolerated at dose levels expected to be efficacious
- TTI-2341 has potential to be a best-in-class, CNS-permeant, EGFR inhibitor