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Identification of STX-241, a potential best-in-class, CNS-penetrant, and highly mutant-selective EGFR inhibitor with activity on osimertinib-resistant C797x mutations

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Introduction

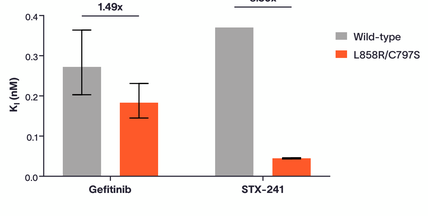
EGFR mutations are well validated clinical targets in non-small cell lung cancer (NSCLC). Osimertinib, a highly-selective EGFR mutation-targeting covalent drug, is increasingly used in the first line setting for patients with NSCLC bearing EGFR L858R mutation or exon 19 deletions (ex19del)[1]. In a subset of these patients, co-occurring L858R/C797x or ex19del/C797x mutations ("double mutants") are emerging as an on-target resistance mechanism, underscoring the need for new therapies, particularly in patients with CNS metastases. The exact proportion of cancers that become resistant to first line osimertinib via C797x mutation is still emerging, with the most recent data analyses suggesting mutation frequencies of up to 12.5%[2-6]

In theory, first-generation reversible inhibitors can retain potency against C797S double mutant EGFR proteins. However, designing a next generation inhibitor with an improved mutant selectivity profile versus wild-type EGFR could further reduce adverse events related to on-target wild-type EGFR inhibition. In addition, the relative lack of brain exposure with first-gen inhibitors may limit durable clinical responses, particularly in patients with brain metastases[7]. We therefore reasoned that a best-in-class next-generation EGFR double mutant inhibitor must improve on the potency/selectivity of first-generation inhibitors and have appreciable CNS penetrance in order to address the unmet need of CNS metastases in patients that progress on osimertinib.

Herein, we describe a novel EGFR C797S-active double-mutant inhibitor, STX-241, and characterize potency and selectivity vs EGFR WT. To do this comprehensively, STX-241 was assessed *in vitro* and *in vivo* across a variety of engineered or endogenously occurring single (ex19del or L858R) or double (ex19del/C797S or L858R/C797S) mutant systems. We also characterize the CNS exposure of STX-241 using a gold-standard rat brain slice method to assess free exposure in brain tissue.

Results

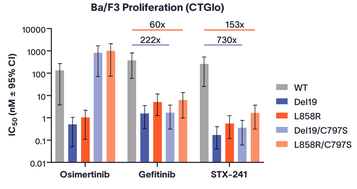
STX-241 demonstrates high selectivity in biochemical assays



- K_i was determined for STX-241 and the benchmark first-generation EGFR inhibitor gefitinib using a Chelation Enhanced Fluorescence (ChEF) biochemical assay with AQT0001 peptide substrate and recombinant EGFR kinase domain proteins. Data is mean ± standard deviation.
- STX-241 demonstrated more potent inhibition of an EGFR L858R/C797S double mutant than gefitinib. STX-241 residence time on the mutant protein was roughly 4.3 hr, relative to 14 min for gefitinib.
- STX-241 selectivity for the L858R/C797S double mutant relative to WT EGFR exceeded that of gefitinib.

STX-241 retains strong potency and selectivity in the presence of C797S

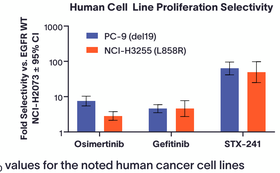
Ba/F3 with Ectopic EGFR	Mean IC_{50} (nM)
Wild-type	253.5
L858R	0.5
Ex19 del 745-750 (ex19del)	0.2
L858R/C797S	1.7
Ex19del/C797S	0.3



- Left panel: STX-241 proliferation inhibition IC_{50} values for Ba/F3 lines expressing the noted EGFR constructs (CellTiterGlo, 72 hr assays)
- Right panel: STX-241, osimertinib (C797 covalent), and gefitinib (1st gen reversible) mutant potency for the noted Ba/F3 cells relative to cells expressing WT EGFR.
- STX-241 retains potent antiproliferative activity and selectivity in the presence of C797S double mutation, and was greater than that observed for the first generation reversible EGFR inhibitor gefitinib (double mutant vs. WT values noted)

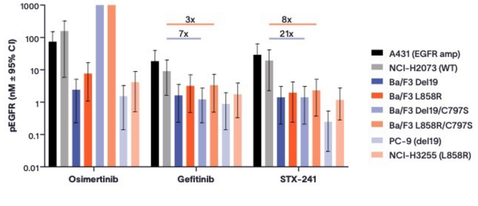
Strong STX-241 potency and selectivity extends to human cancer cells

Cell Line	Mutation	Mean IC_{50} (nM)
A431	Amplified / EGFR-PPARGC1A fusion	2562
NCI-H2073	Wild-type	193.8
NCI-H3255	L858R	3.9
PC-9	ex19del	3.1



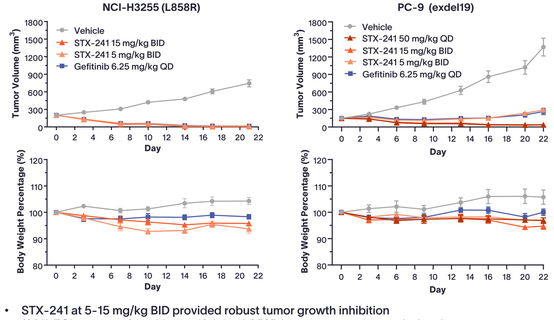
- Left panel: STX-241 cell proliferation IC_{50} values for the noted human cancer cell lines (CellTiterGlo).
- Right panel: STX-241, osimertinib, and gefitinib mutant cell line selectivity versus the EGFR wild-type and EGF-dependent cell line NCI-H2073.
- STX-241 selectivity for commonly occurring EGFR mutant cell lines was greater than that observed for either osimertinib or gefitinib.

STX-241 is potent and selective in blocking pEGFR target engagement



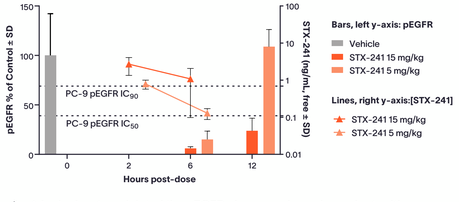
- STX-241, osimertinib, and gefitinib IC_{50} values for pEGFR (phosphorylated EGFR, Tyr 1068) inhibition (AlphaLISA). STX-241 potency was on-par with, or better than, that of benchmarks
- STX-241 selectivity in double mutant Ba/F3 vs. EGFR WT NCI-H2073 WT cells exceeded that of gefitinib

STX-241 is efficacious and well tolerated *in vivo*



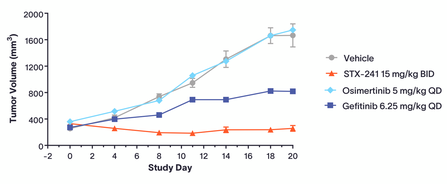
- STX-241 at 5-15 mg/kg BID provided robust tumor growth inhibition (90% TGI or greater) in either ex19del or L858R human tumor xenografts in mice
- All doses were well tolerated
- Antitumor activity was comparable or superior to a published clinically relevant dose of gefitinib

STX-241 suppresses pEGFR in PC-9 (ex19del) PK/PD studies



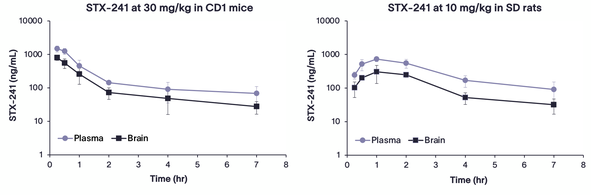
- 5-15 mg/kg (single dose) modulated the pEGFR pharmacodynamic readout, with 15 mg/kg demonstrating >50% pEGFR inhibition over a 12 hour BID dosing window

STX-241 retains *in vivo* activity in C797S double mutant xenografts



- A PC-9 derivative with an ex19del/C797S *in cis* double mutation was engineered by CRISPR knock-in.
- STX-241 at 15 mg/kg BID retained *in vivo* tumor growth inhibition in the presence of C797S

STX-241 CNS penetrance is on-par with osimertinib



Mouse Brain $K_{p,uu}$

STX-241	osimertinib[8]
0.4	0.2

Rat Brain $K_{p,uu}$

STX-241	osimertinib[8]
0.2	0.2

- Upon oral dosing in blood brain barrier-intact rodents, STX-241 showed good brain distribution with no delay in distribution compared to plasma.
- STX-241 demonstrates high *in vitro* permeability (Caco-2 ER 1.76 at 1 µM) and low efflux (MDCK-MDR1 ER 0.95, MDCK-BCRP ER 1.17 at 1 µM)
- Using a rat brain slice method[9] to calculate free fraction in brain, STX-241 demonstrates rodent brain exposure $K_{p,uu}$ values similar or superior to values obtained for osimertinib using the same method.

Conclusions

- **STX-241 demonstrates strong biochemical inhibition of EGFR double mutant kinase activity, and a wider selectivity than the first generation reversible compound gefitinib.**
- **STX-241 is potent and selective for C797S double mutants relative to WT EGFR across proliferation and target engagement assays. The observed *in vitro* potency and selectivity exceeds that of gefitinib in double mutant cell lines.**
- **STX-241 displays strong *in vivo* antitumor activity across tested single and double mutant CDX models, and retains *in vivo* activity in the presence of C797 mutation.**
- **With low efflux and good unbound exposure in brain tissue, STX-241 demonstrates brain exposure on-par with that of osimertinib, providing potential to address CNS metastases.**
- **STX-241 preclinical data indicates it is a potential best-in-class 4th generation double-mutant EGFR inhibitor, and IND-enabling studies are completing for a 1H 2024 submission.**

References / Acknowledgements

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