84

Identification of STX-721, an EGFR exon 20 mutant inhibitor with superior selectivity and a potential best-in-class profile

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Introduction

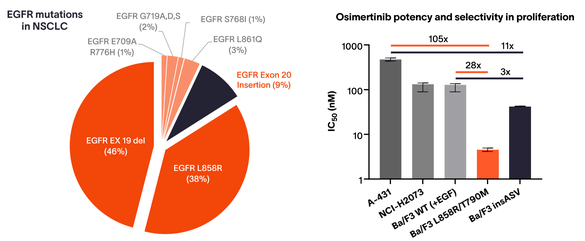
EGFR mutations are well validated clinical targets in NSCLC. Osimertinib, a highly-selective EGFR mutation-targeting drug, achieves an objective response rate of ~80% against cancers with L858R or exon 19 deletion[1]. In contrast, patients with EGFR exon 20 insertion mutations exhibit response rates to currently approved or investigational therapies between 28-52%[2]. The suboptimal efficacy of existing EGFR inhibitors for patients with exon 20 mutation is likely due to poor mutant versus wild-type EGFR selectivity.

The apparent *in vitro* selectivity of EGFR inhibitors is highly dependent on the model systems used, limiting the predictive value of these systems for drug discovery. To address this issue, we optimized drug potency and selectivity across a broad panel of exon 20 mutant cell lines, including engineered Ba/F3 cells as well as NSCLC cells with endogenous or isogenic knock-in exon 20 mutations. The high selectivity of Osimertinib in EGFR L858R/T790M mutant cells was used to benchmark EGFR mutant selectivity, given its exceptional response rate and wide therapeutic index. Compounds were tested in proliferation, cell signaling, and biochemical assays. Finally, we examined inhibitor efficacy, pharmacodynamics, pharmacokinetics, and tolerability in novel patient-derived EGFR exon 20 mutant xenograft (PDX) mouse models.

Herein, we describe a novel EGFR exon 20 inhibitor, STX-721, and characterize its *in vitro* and *in vivo* potency and selectivity vs EGFR WT. To do this comprehensively, we scan across a wide panel of engineered and endogenously mutant EGFR exon 20 model systems.

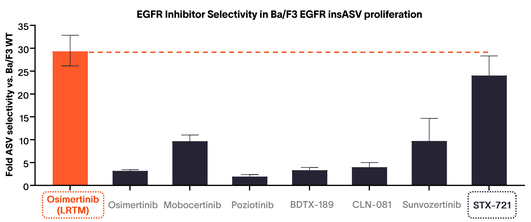
Results

Using Osimertinib to benchmark *in vitro* cellular selectivity assays



- Osimertinib balances strong potency for "classical" EGFR mutants (w/ or w/o T790M) with reduced activity for WT EGFR and other off targets. However, Osimertinib does not achieve selectivity for EGFR exon 20 insertion mutations.
- The apparent EGFR mutant selectivity observed *in vitro* for Osimertinib is dependent upon the EGFR WT control cell line used – Osimertinib selectivity against a "classical" EGFR mutations (orange) sets the *in vitro* selectivity goal for an exon 20 inhibitor, and Osimertinib selectivity against exon 20 mutations (blue) is considered insufficient.

STX-721 is highly selective for a common EGFR exon 20 insertion



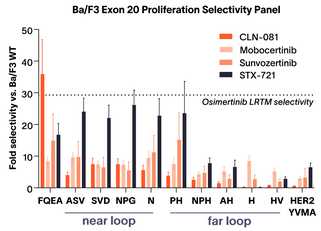
- STX-721 is selective for a common EGFR exon 20 mutation (insASV) in engineered Ba/F3 cells. STX-721 antiproliferative exon 20 selectivity approaches that of Osimertinib for L858R/T790M ("LRTM"), and is superior to other tested EGFR exon 20 benchmark inhibitors in this assay.
- Ba/F3 LRTM, insASV, and WT (+EGF) cells were exposed to varying concentrations of the noted compounds for 72 hr prior to assessment using Cell Titer Glo (Promega), and antiproliferative IC_{50}'s were determined in at least an n=5. Selectivity is determined as the ratio of WT/insASV IC_{50}. Error bars denote 95% confidence intervals.

STX-721 is potent and selective across EGFR and HER2 exon 20 mutants

Ba/F3 CTG potency

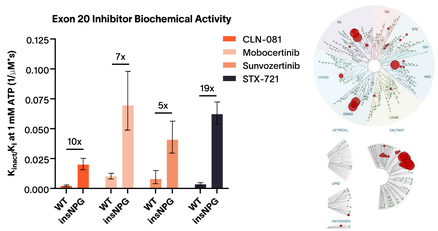
Mutation	Mean IC_{50} (nM)	% of total exon 20 observed*
EGFR WT	136.70	N/A
EGFR A763_V764 insFQEA	7.70	rare
EGFR V769_D770 insASV	5.62	20%
EGFR D770_N771 insSVD	6.22	13%
EGFR D770_N771 insNPG	5.17	~1%
EGFR N771_P772 insN	5.75	1-2%
EGFR H773_V774 insPH	6.01	2-3%
EGFR H773_V774 insNPH	16.70	6%
EGFR H773_V774 insAH	20.71	rare
EGFR H773_V774 insH	590.06	4%
EGFR V774_C775 insHV	45.52	~1%
EGFR L858R	0.32	N/A
EGFR Del 19	0.17	N/A
HER2 A775_G776 insYVMA	19.99	71% (HER2)**

* NCI GENIE database, July 2022. n=260 Exon 20 Mut NSCLC samples analyzed
** Robichaux et al. Cancer Cell 2019
N/A: not applicable
Rare: not observed in this dataset



- Ba/F3 cells with the noted EGFR and HER2 mutants were assessed via Cell Titer Glo and WT selectivity determined as previously noted. Error bars are 95% confidence intervals. The dotted line represents the fold-selectivity for Osimertinib in Ba/F3 cells with EGFR L858R/T790M (LRTM) vs. EGFR WT Ba/F3 cells (+EGF).
- Selectivity trends highest in "near-loop" exon 20 mutants as previously described[3].

STX-721 is a highly selective mutant EGFR binder

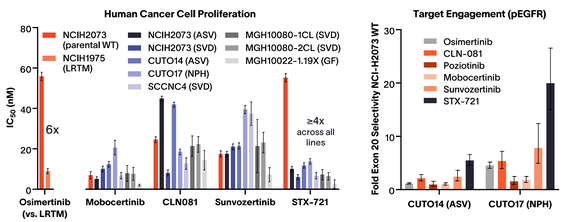


KinomeScan

- 3 μM STX-721
- S(10)=0.027
- Off targets <10x vs. WT at K_d followup: HER2, ERBB4

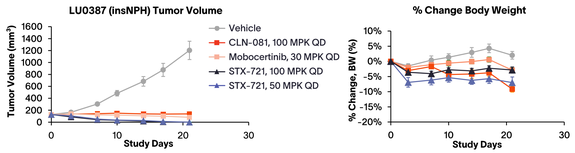
- Left panel: K_{inact}/K_I was determined for the noted inhibitors using a Chelation Enhanced Fluorescence (ChEF) biochemical assay with AQT0001 peptide substrate. Data is geometric mean ± geometric standard deviation.
- Right panel: STX-721 was profiled at 3 μM in KINOMEScan selectivity binding assays (Eurofins) vs. >450 kinases. S(10) = (# nonmutant kinases <10% control)/# nonmutant kinases tested). K_d followup was performed for 9 off-target hits with >95% inhibition at 3μM .

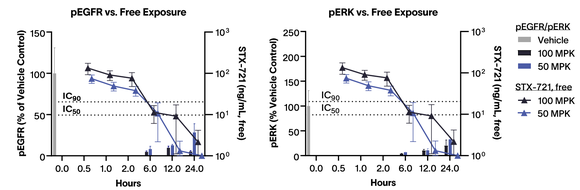
STX-721 exon 20 selectivity extends to human cancer cells



- STX-721 shows strong EGFR exon 20 mutant-selective proliferation and signaling (pEGFR) inhibition across human cancer cell line models. The observed selectivity is superior to other tested benchmarks compounds.
- Left panel: The noted cell lines were assessed in 72 hr CTG assays, and GI_{50} inflection points were determined. Selectivity is for the noted cell lines relative to EGFR pathway WT/dependent NCI-H2073 cells[4]. Homozygous exon 20 mutant derivatives were generated through CRISPR knockin at the endogenous EGFR locus. All cell lines noted are NSCLC, with the exception of SCCNC4, which is a sinonasal squamous carcinoma cell line. Error bars are standard deviation.
- Right panel: pEGFR IC_{50}'s were determined using pEGFR pY1068 AlphaLISA. Noted selectivity is relative to EGFR WT/dependent NCI-H2073. Error bars are 95% confidence intervals.

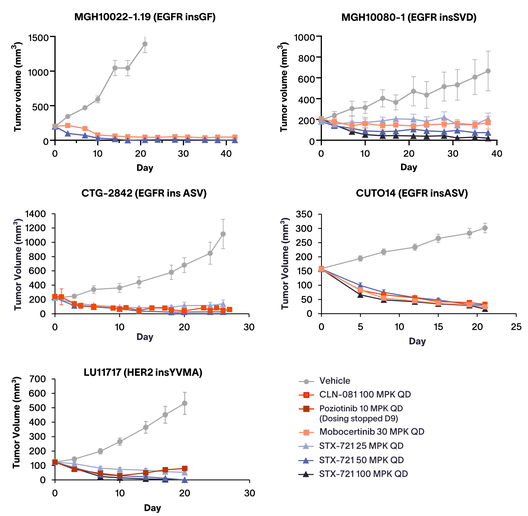
STX-721 Efficacy, Tolerability, and PK/PD in LU0387 (insNPH PDX)





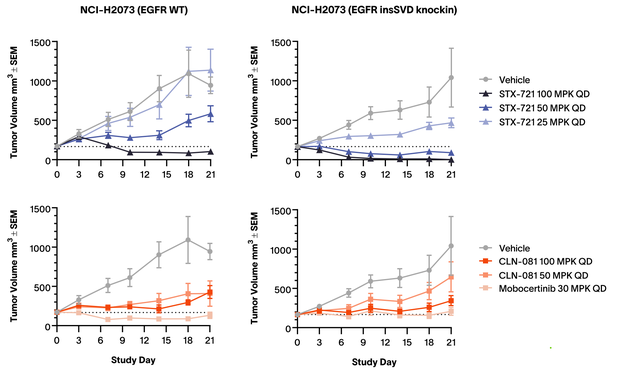
- LU0387 contains an NPH insertion "far loop" mutation, which is relatively less sensitive to selective exon 20 inhibitors, and was therefore chosen for this reason to establish the PK/PD/efficacy relationship.
- STX-721 at 50-100 mg/kg (MPK) QD regresses LU0387 tumors equivalently to benchmark exon 20 inhibitors, and was well tolerated throughout the study.
- STX-721 at 50-100 MPK QD demonstrates strong 24 hr suppression of pEGFR (Y1068)/pERK1/2 (T202/Y204) signaling in single dose PK/PD experiments.

STX-721 Regression Across Exon 20 Mutant PDX/CDX models



- STX-721 demonstrated tumor regression doses of 25-100 MPK across a variety of EGFR exon 20 and HER2 exon 20 mutant models.
- All models except CUTO14 are PDX lines not cultured *in vitro*.
- All doses shown were well tolerated.

STX-721 is EGFR exon 20 selective *in vivo*



- STX-721 at 25-50 MPK demonstrates strong antitumor activity (25 MPK) to regression (50 MPK) in SVD knockin xenografts. These doses show substantially weaker to no activity in EGFR WT parental isogenic control tumors, demonstrating EGFR exon 20 selective growth inhibition *in vivo*.
- Mobocertinib and CLN-081 at therapeutically relevant doses show equivalent efficacy *in vivo* regardless of EGFR exon 20 genotype.
- All doses used in this study were well tolerated.
- Work ongoing to profile additional benchmark EGFR exon 20 inhibitors in this EGFR exon 20 isogenic model system.

Conclusions

- **STX-721 demonstrates strong EGFR exon 20 mutant potency and selectivity in isogenic Ba/F3 and human cancer cell line proliferation assays.**
- **STX-721 potency and exon 20 selectivity is also observed in signaling (pEGFR) and biochemical assays.**
- **Exon 20 selectivity for STX-721 exceeds that of key clinical competitor benchmarks in these model systems, and approaches the selectivity of Osimertinib against "classic" EGFR mutations.**
- **STX-721 displays strong *in vivo* antitumor activity across a variety of EGFR and HER2 exon 20 mutant CDX and PDX models, and *in vivo* selectivity in human isogenic cancer cell line xenografts.**
- **STX-721 has the potential to provide a best-in-class profile to improve outcomes in patients harboring cancers with EGFR/HER2 exon 20 mutations and is currently in IND enabling studies.**

References / Acknowledgements

[1] Soria JC et al 2018, NEJM 378, 113
[2] IASLC 2021, ASCO 2021, ASCO 2022, ESMO 2022
[3] Robichaux et al 2021 Nature 597, 732; and Elamin et al 2022, Cancer Cell 40, 754
[4] Floc'h et al 2018, Mol Cancer Ther 17, 885; and Scorpion data on file

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