Abstract No. 187

The clinical candidate, ASTX029, is a novel, dual-mechanism ERK1/2 inhibitor and has potent activity in MAPK-activated cancer cell lines and in vivo tumor models

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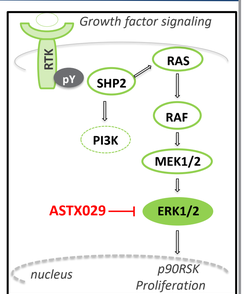
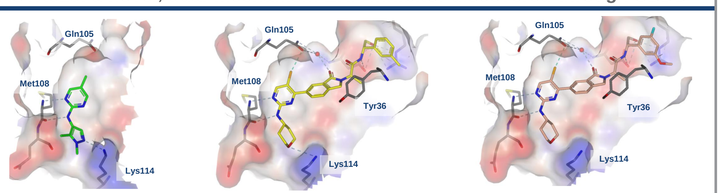
INTRODUCTION

- The MAPK signaling pathway is commonly upregulated in human cancers due to oncogenic mutations of upstream components such as BRAF or KRAS.
- MAPK pathway inhibition has been clinically validated by BRAF and MEK inhibitors.
- As the final node in the MAPK pathway, ERK is an attractive therapeutic target for the treatment of MAPK-activated cancers, including those resistant to upstream inhibition.
- Previously we described the fragment-based discovery of a chemical series targeting ERK[1]. Here we disclose for the first time the structure of the clinical candidate, ASTX029.



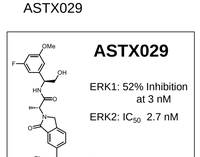
1. ASTX029: a novel, dual-mechanism ERK inhibitor discovered through SBDD

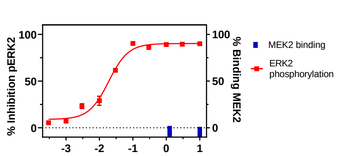


Fragment 5[1] | Compound 27[1] | ASTX029

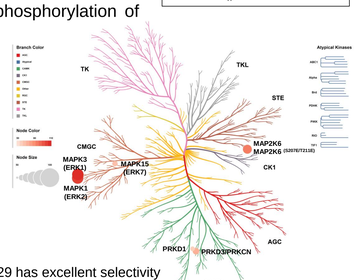
ASTX029 was discovered through fragment screening and subsequent optimisation by structure-based drug design (SBDD). It binds to the active site of ERK2 and adopts an extended conformation, exploiting a pocket which is created by an unusual movement of the P-Loop Tyr36 residue.

ASTX029 has a dual-mechanism, potently and selectively inhibiting both ERK catalytic activity and the phosphorylation of ERK itself.



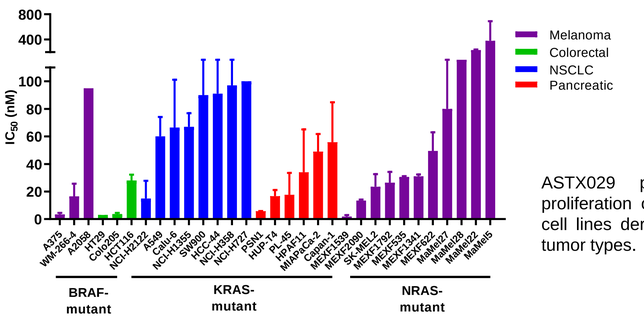


ASTX029 inhibits ERK2 phosphorylation by MEK without directly inhibiting MEK



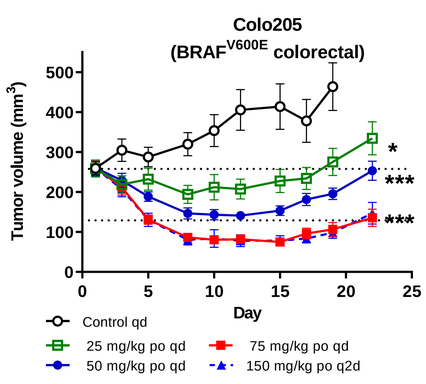
ASTX029 has excellent selectivity across a 462 kinase panel

2. Proliferation of MAPK-activated cell lines is inhibited by ASTX029

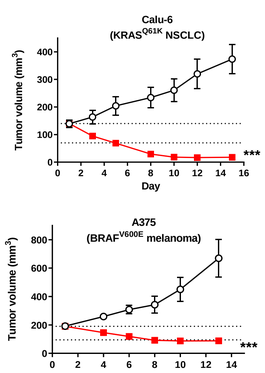


ASTX029 potently inhibited proliferation of MAPK-activated cell lines derived from multiple tumor types.

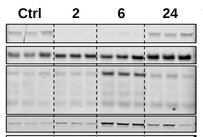
3. ASTX029 inhibits tumor growth in MAPK-activated tumor xenograft models



Mean ± SEM and * = *P* <0.05, *** = *P* <0.001 vs control



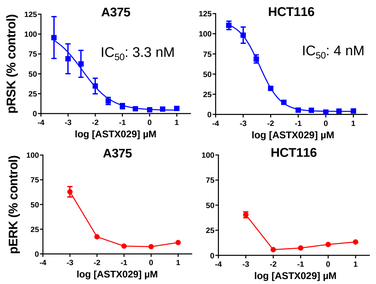
ASTX029 treatment resulted in tumor regressions in Colo205, A375 and Calu-6 xenografts in mice. ASTX029 was well tolerated, with median body weight loss of up to 7% at high doses and no other notable adverse effects observed.



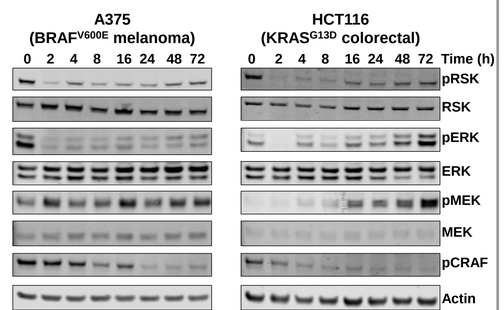
Induction of apoptotic markers was observed in Colo205 xenograft tissue following treatment with a single 75 mg/kg oral dose of ASTX029.

4. ASTX029 has a dual-mechanism in cells and in vivo xenograft tumors

Cell lines



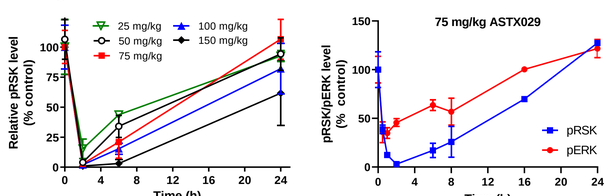
2 h treatment with ASTX029



A375 and HCT116 cells treated with 20 nM and 100 nM ASTX029, respectively .

Phosphorylation of the ERK substrate, RSK, was inhibited in a dose dependent manner for up to 72 hours in A375 and HCT116 cells on treatment with ASTX029. Inhibition of ERK phosphorylation was also observed in these cell lines.

Xenograft Tissue



The catalytic activity and phosphorylation of ERK were inhibited in Colo205 tumor tissue after single, oral doses of ASTX029. Maximal inhibition was observed 1-2 hours after dosing, with levels of pRSK and pERK returning to control by 24 h.

	$T_{\frac{1}{2}}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC (h*ng/mL)
Plasma	2.2	0.5	24000	43000
Tumor	4.5	0.5	2100	12000

Pharmacokinetic parameters for ASTX029.

SUMMARY AND CONCLUSIONS

- Using SBDD, a novel dual-mechanism ERK inhibitor, ASTX029, was identified, which potently inhibited cell and tumor growth in MAPK-activated models.
- The dual-mechanism of ASTX029 was demonstrated in vitro and in vivo where it inhibited both the catalytic activity and phosphorylation of ERK itself in cells and tumor tissue.
- These data support the ongoing clinical development of ASTX029 (NCT03520075).

1. Heightman et al (2018). J Med Chem 61; 4978

Related poster ENA 2020: #162 Smyth et al., *Combined inhibition of SHP2 and ERK enhances anti-tumor effects in preclinical models*

Poster presented at EORTC-NCI-AACR Molecular Targets and Cancer Therapeutics Conference. October 24 - 25, 2020. (Poster available to download from Astex's website at www.astx.com)