A combination vertical inhibition approach with inhibitors of SHP2 and ERK provides improved activity in KRAS-mutant pancreatic and colorectal cancer models

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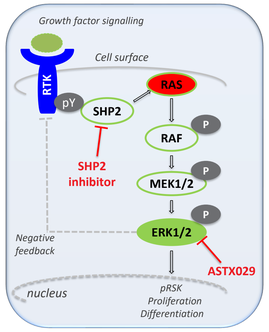
INTRODUCTION

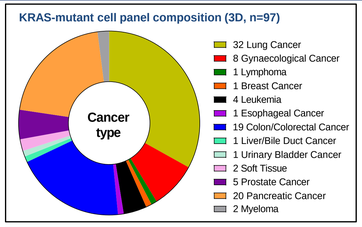
Inhibitors of the MAPK pathway are approved therapeutic agents, however single agent use of MAPK inhibitors in the clinic is often limited by resistance through mechanisms which commonly result in reactivation of MAPK signaling. Vertical pathway combination strategies are therefore of interest for addressing resistance through pathway reactivation.

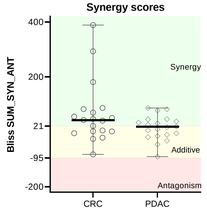
Src homology region 2-containing protein tyrosine phosphatase 2 (SHP2) is a key regulator of the MAPK pathway downstream of RTKs and upstream of RAS, whilst ERK1/2 (ERK) is the final node of the kinase cascade. We hypothesized that the combination of ASTX029[1, 2, 3], a dual-mechanism ERK inhibitor which is undergoing clinical development (NCT03520075), with inhibitors of SHP2 would enhance anti-tumor activity in KRAS-mutant pancreatic and colorectal cancer models.



METHODS

Combination KRAS-mutant cell panel screen analysis





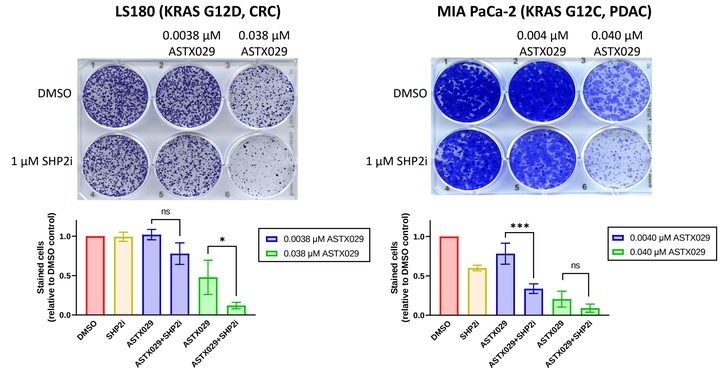
Overall Findings

Cell panel	Indication	Total No. of cell lines	No. of sensitive cell lines (%)		
			SHP2i	ASTX029	Combination
KRAS	All	97	38 (39%)	80 (82%)	91 (94%)
KRAS	CRC	19	10 (53%)	11 (58%)	18 (95%)
KRAS	PDAC	20	4 (20%)	17 (85%)	19 (95%)

We previously presented on a large combination cell panel screen (ChemPartner, China) testing the combination of ASTX029 with an inhibitor of SHP2 developed via structure-guided drug design (SHP2i)[4,5]. We observed an enhanced loss of viability with this combination in KRAS-mutant colorectal cancer (CRC) and pancreatic ductal adenocarcinoma (PDAC) cell lines. Selected cell lines were investigated further *in vitro* and *in vivo*.

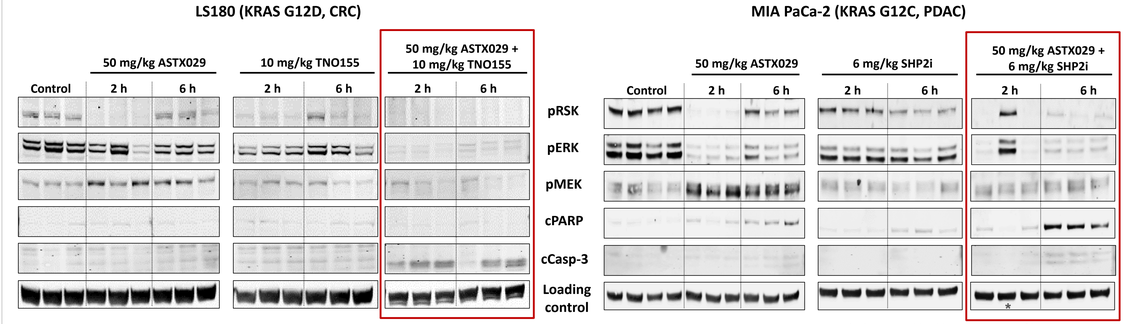
RESULTS

Combination enhances anti-proliferative effects of single agents *in vitro*



Selected CRC and PDAC cell lines were subjected to 14-day colony formation assays in the presence of single agent or combined treatment (ASTX029 at proliferation assay IC_{50} or 10% $IC_{50} \pm 1$ µM SHP2i). Crystal violet stained wells and their quantifications are shown. Combination treatment resulted in significantly decreased cell growth.

Combination more effectively inhibits MAPK signaling and induces markers of cell death *in vivo*



* The animal had low exposure of both compounds.

CB17 SCID mice bearing xenografts of the indicated CRC or PDAC cell lines were treated with a single dose of each single agent or the combination as indicated. Tumours were collected at the indicated timepoints and protein levels assessed by western blotting. Combination treatment resulted in enhanced inhibition of MAPK signaling. Combination treatment also resulted in increased levels of apoptotic markers (cleaved PARP, cleaved caspase-3). TNO155: Published SHP2 inhibitor (Novartis)[6].

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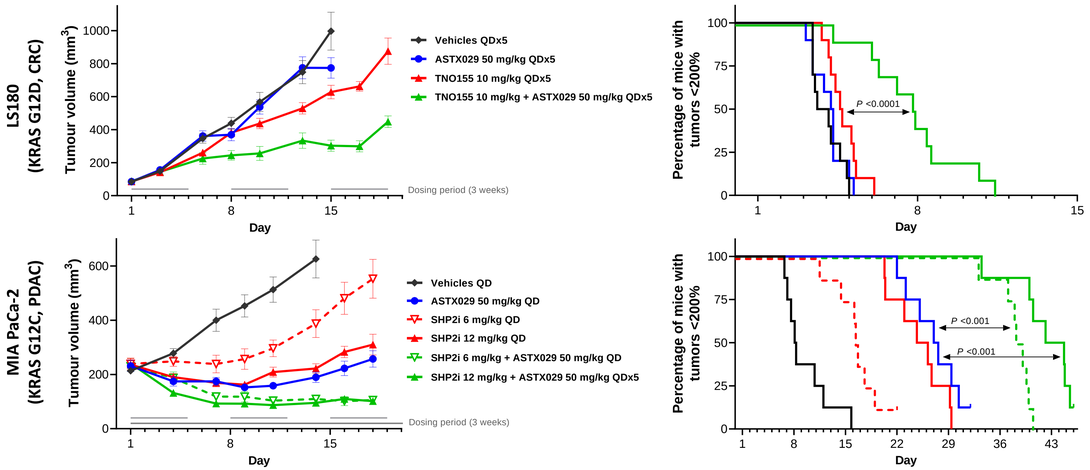
Combined inhibition of ERK and SHP2 led to enhanced anti-tumor response in CRC and PDAC xenograft models



CB17 SCID mice bearing xenografts of the indicated CRC or PDAC cell lines were orally treated with ASTX029 and/or SHP2i, or their vehicles (n≥8). Treatments were given at the indicated schedules (QD: Once a day; QDx5: Once a day for 5 consecutive days per week). Mean tumor volumes ± SEM (left) and survival time with tumor doubling as an endpoint (right) were compared. No notable health issues were observed. TNO155: Published SHP2 inhibitor (Novartis)[6].

SUMMARY AND CONCLUSIONS

- The combination of SHP2i and ASTX029 synergistically reduced viability of multiple CRC and PDAC cell lines in a large scale KRAS-mutant cell line panel screen
- The combination of both agents *in vitro* resulted in enhanced anti-proliferative effects in long-term colony formation assays in KRAS-mutant CRC (LS180) and PDAC (MIA PaCa-2) cell lines
- Combined treatment with a single dose of SHP2i or TNO155 and ASTX029 resulted in decreased MAPK signaling and an increase in apoptotic markers in LS180 and MIA PaCa-2 xenograft tumors
- The combination resulted in increased anti-tumor response in both xenograft models
  - In LS180, survival benefit was observed only with the combination treatment
  - In MIA PaCa-2, multiple combination dose schedules led to an enhanced survival benefit
- These data provide a strong rationale for the use of a vertical inhibition approach with inhibitors of SHP2 and ERK in KRAS-mutant PDAC and CRC and warrants further investigation in the clinic

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