Combined inhibition of SHP2 and ERK enhances anti-tumor effects in preclinical models

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INTRODUCTION

MAPK signalling is frequently dysregulated in cancer. The pathway can be targeted by inhibition of different nodes and is tightly regulated by feedback mechanisms. Resistance to single-agent therapies frequently occurs through several different mechanisms including upregulation of receptor tyrosine kinases (RTKs), therefore, combination therapies are of interest.

The Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2 (SHP2) is a key regulator of MAPK pathway downstream of RTKs and upstream of RAS, whilst ERK acts at the bottom of the pathway phosphorylating multiple substrates.

We investigated the potential of targeting the MAPK pathway through a combination of SHP2 and ERK inhibition in preclinical models. Using a SHP2 inhibitor (SHP2i) discovered by our structure-based drug discovery programme and ASTX029, an ERK inhibitor in a Phase I-II clinical trial (NCT03520075), we tested panels of cell lines representing various indications and genetic backgrounds *in vitro* and confirmed enhanced tumor growth inhibition by the combination in a xenograft model.

METHODS

Cell line panel screening

Experimental set up

- KRAS-dependent cell lines were grown as 3D cultures and KRAS-independent (non-KRAS) cell lines in 2D (ChemPartner).
- Compounds were added in matrix, incubated for 5 days and viability measured with CellTiter-Glo (Promega).
- All cell lines were annotated according to CCLE.

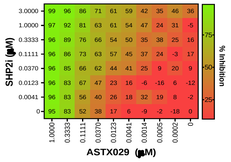
Analysis

- Maximum inhibition was used to define sensitivity.
- Dose-response curves of single-agent treatments and combinations were examined.
- Bliss independence model was used (SynergyFinder R package). The sum of the synergy and antagonism volume across $\log_{10}$-transformed screened drug concentrations (SUM_SYN_ANT) was calculated for each cell line.
- Both Max % inhibition and Bliss scores were used to identify cell lines that responded to the combination in a synergistic or additive manner.

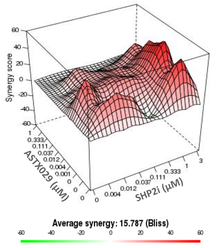
***In vivo* anti-tumor activity**

- MIA PaCa-2 subcutaneous xenograft model in SCID mice was used.

Dose-response matrix



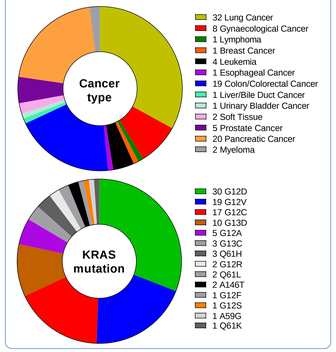
Bliss synergy



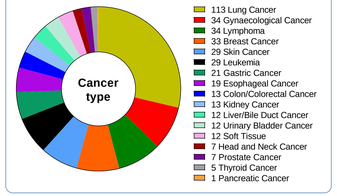
COMBINING SHP2 INHIBITOR AND ERK INHIBITOR ENHANCES RESPONSE *IN VITRO*

Panel composition and the number of responding cell lines

KRAS-mutant cell panel (3D, n=97)



Non-KRAS cell panel (2D, n=394)

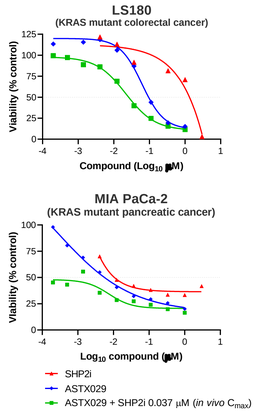


- Sensitivity to SHP2i or ASTX029 as single agent was widely observed.
- Greater number of cell lines showed sensitivity (>50% inhibition) to the combination than to either single agent.

Overall finding

Cell panel	Total No. of cell lines	No. of sensitive cell lines (%)		
		SHP2i	ASTX029	Combination
KRAS	97	38 (39%)	80 (82%)	91 (94%)
Non-KRAS	394	137 (35%)	204 (52%)	280 (71%)
All	491	175 (36%)	284 (58%)	371 (76%)

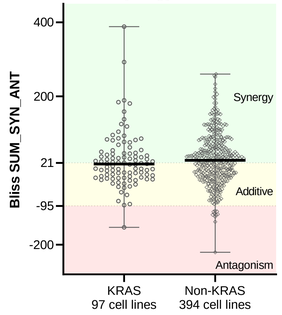
Examples of dose-response curves



- Both agents led to dose-dependent reduction of cell viability and this was enhanced when used in combination.

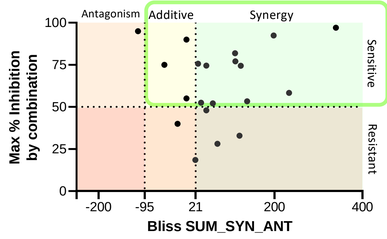
Identifying synergy and additivity within combination-sensitive cell lines

Synergy scores



Box plot showing the median and the range (minimum to maximum) of synergy scores.

Example



Scatter plot illustrating identification of combination-sensitive cell lines showing synergy or additivity, using a subset of cell lines as an example. Each symbol is a cell line. The table below summarises the finding.

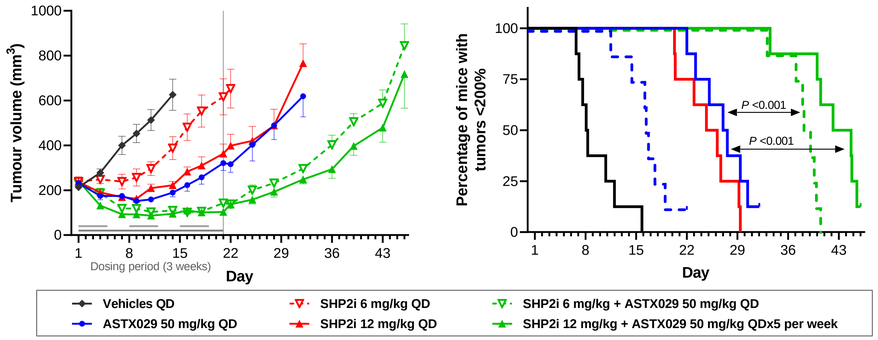
- Synergy and additivity were widely observed within combination-sensitive cell lines in both panels.

Combination-sensitive cell lines showing synergy or additivity

Cell panel	Synergy (%)	Additivity (%)	Total (%)
KRAS	43 (44%)	47 (48%)	90 (93%)
Non-KRAS	159 (40%)	114 (29%)	273 (69%)
All	202 (41%)	161 (33%)	363 (74%)

COMBINATION RESULTS IN MIA PACA-2 TUMOR REGRESSION *IN VIVO*

Anti-tumor activity of SHP2i, ASTX029 and combination in MIA PaCa-2 xenograft



MIA PaCa-2 tumor-bearing mice were orally treated with ASTX029 and/or SHP2i, or their vehicles (n=8). Treatments were given daily for 21 days (QD) or 5 days per week (QDx5 per week). Effects of the treatments were compared by mean tumor volumes (± SEM) (left) and survival with tumor doubling an endpoint (right).
The key findings are summarised below. T/C = treated / control × 100 on Day 22, calculated from median tumor volumes relative to Day 1.

Summary of *in vivo* anti-tumor response

Treatment	T/C (%, Day 22)	Partial regression (>50% reduction)	Median days for tumors to reach 200%
Vehicles QD	100	0/8	8
ASTX029 50 mg/kg QD	35	1/8	27
SHP2i 6 mg/kg QD	67	0/8	17
SHP2i 12 mg/kg QD	43	2/8	25
SHP2i 6 mg/kg + ASTX029 QD	14	8/8	39
SHP2i 12 mg/kg + ASTX029 QDx5	15	8/8	43

- Treatment with SHP2i or ASTX029 as single agents resulted in transient tumor stasis.
- Combination treatment led to partial regression in 100% of mice and significantly prolonged survival.
- No notable health issues were observed.

SUMMARY AND CONCLUSIONS

- SHP2i and ASTX029, as single agents, reduced viability of multiple cell lines in both KRAS-dependent and KRAS-independent cell line panels.
- When the agents were combined *in vitro*, the number of responding cell lines increased compared to single agents. The combination enhanced loss of cellular viability in a synergistic or additive manner.
- Combined treatment with SHP2i and ASTX029 resulted in regression of MIA PaCa-2 tumors and prolonged survival.
- These results demonstrate the therapeutic potential of combining a SHP2 inhibitor with an ERK inhibitor. Clinical investigation of the combination is warranted.

Related posters at ENA 2020

#161 Hearn et al., *Identification of potent small-molecule allosteric inhibitors of SHP2*

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

Poster presented at the 32nd EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics, October 24th-25th 2020