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Identification of potent small molecule allosteric inhibitors of SHP2

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INTRODUCTION

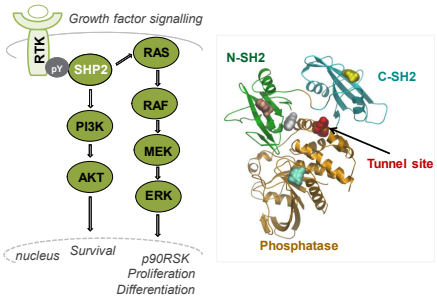
SHP2 is a ubiquitously expressed protein tyrosine phosphatase required for growth factor signalling downstream of receptor tyrosine kinases (RTKs) and plays a role in regulating many cellular processes.

Genetic knockdown and pharmacological inhibition of SHP2 inhibits proliferation of RTK-driven cancer cell lines and suppresses RAS/MAPK signalling.

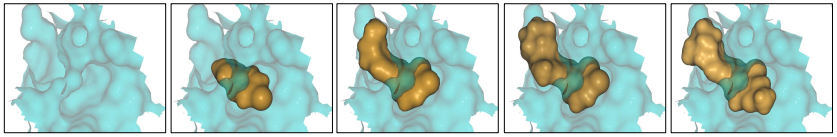
SHP2 inhibitors are a promising therapeutic approach as RTK deregulation often leads to a wide range of cancers and several compounds are being tested in the clinic.

Using our fragment-based screening approach, Pyramid™, we identified fragment hits binding to the *tunnel* region[1] between the phosphatase domain and the C-SH2 domain of SHP2 which were improved using structure-guided design.

Here we describe the optimisation of mM fragment hits into potent SHP2 antagonists with in vitro and in vivo anti-tumour activity.

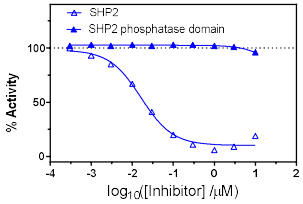


Fragment hit optimisation to lead compound



	Apo tunnel site	Fragment	Early lead	Optimised lead	Advanced lead
SHP2 ITC K_D / IC_{50} (μM)		1000 (ITC K_D)	2.1	0.077	0.010
Ligand Efficiency		0.34	0.37	0.39	0.38

Phosphatase activity- advanced lead



DiFMUP-based fluorescence enzyme assay used to measure inhibition of SHP2

- A fragment screen was carried out on two constructs
  - A closed, autoinhibited C-terminal truncated form having phosphatase catalytic and both SH2 domains
  - A phosphatase domain only form
- Multiple hits were obtained for C-terminal truncated form; >70 structurally validated hits bound in the tunnel-like site. No hits were obtained for the phosphatase-only construct
- Structure-guided growing of the fragment hit into adjacent pockets gave an early lead with increased affinity and further optimisation lead to a more potent compound which inhibited SHP2 enzyme activity with an average IC_{50} of 10 nM
- Optimised leads inhibit activity of SHP2 enzyme with no concomitant inhibition of the phosphatase catalytic domain-only construct

Anti-proliferative activity and MAPK pathway modulation in RTK-driven cells

Proliferation – advanced lead

Cell line	Origin	Background	EC_{50} μM
HCC827 (3D)	NSCLC	EGFR Ex19del	0.47
HCC827	NSCLC	EGFR Ex19del	2.1
MOLM-13	AML	FLT3-ITD	0.41
MV4-11	AML	FLT3-ITD	0.23
A375	Melanoma	BRAF V600E (negative control)	>10

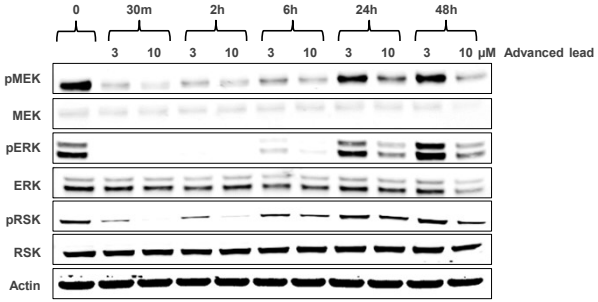
All 2D cultures except where noted using CellTiter-Glo assay (Promega) at 72-144 hrs

MAPK pathway modulation by advanced lead

Cell line	Origin	Background	EC_{50} μM
HCC827 pERK	NSCLC	EGFR Ex19del	0.11
HCC827 pRSK	NSCLC	EGFR Ex19del	0.043
A375 pERK	Melanoma	BRAF V600E (negative control)	>10

pERK ELISA and pRSK MSD assays at 0.5 hr

Time course of MAPK pathway modulation in HCC827 cells

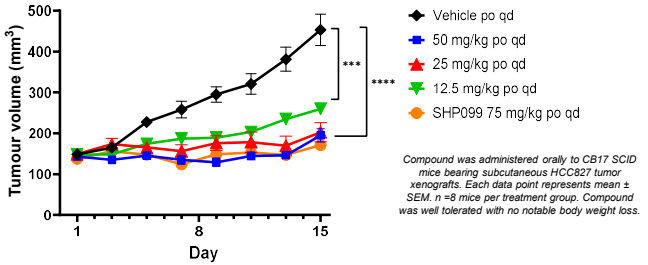


- The compound inhibited the proliferation of RTK-driven human cancer cell lines with potencies ranging from 230 – 2100 nM. No activity was observed in control A375 cells
- Potent MAPK pathway modulation (pERK EC_{50} 110nM and pRSK EC_{50} 43nM) was observed with no activity in control A375 cells
- Signalling time course western blots confirm decreases in pMEK, pERK and pRSK from as early as 30 minutes after compound treatment

HCC827 cell line was established at University of Texas Southwestern Medical Center and deposited at ATCC

Anti-tumour and pharmacodynamic activity in HCC827 tumour xenografts

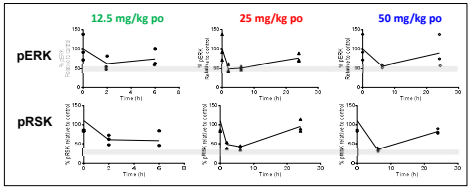
Anti-tumour activity of advanced lead



Compound was administered orally to CB17 SCID mice bearing subcutaneous HCC827 tumor xenografts. Each data point represents mean ± SEM. n =8 mice per treatment group. Compound was well tolerated with no notable body weight loss.

- Once daily oral dosing of the compound conferred significant anti-tumor activity in HCC827 xenografts at all doses tested ($P < 0.001$ or 0.0001)

Inhibition of pERK and pRSK in tumour tissue



A single dose of the compound was administered orally to CB17 SCID mice bearing subcutaneous HCC827 tumor xenografts. Animals were sacrificed and tumors removed at indicated time points. Tumor lysates analysed by pERK ELISA and pRSK MSD.

- Treatment with the advanced lead caused pharmacodynamic effects in HCC827 xenografts
- A single dose of 12.5, 25 or 50 mg/kg po inhibited pERK and pRSK at 2 and 6 hours after treatment

SUMMARY AND CONCLUSIONS

- Inhibition of SHP2 is a promising therapeutic strategy downstream of RTKs to suppress RAS/MAPK signalling and can be used universally to overcome RTK-driven adaptive resistance in various cancers
- Our fragment screen identified several hits in the *tunnel region* which were good starting points for optimisation
- Fragment-based drug discovery was used to optimise weak fragment hits into potent small molecule SHP2 inhibitors
- In vitro, the compound inhibited the proliferation of RTK-driven human cancer cell lines as well as modulated MAPK pathway markers pMEK, pERK and pRSK
- In vivo, significant anti-tumour activity was observed in HCC827 xenografts as well as a pharmacodynamic effect
- For more information on targeting the MAPK pathway through a combination of SHP2 and ERK inhibition, please visit **POSTER #162** Smyth et al., *Combined inhibition of SHP2 and ERK enhances anti-tumour effects in preclinical models*

Reference: 1. Chen et al., (2016) *Nature*, 535, 148–152

DO NOT POST

Poster presented at EORTC-NCI-AACR Molecular Targets and Cancer Therapeutics Conference, October 24 - 25, 2020.