PAS-004: A novel macrocyclic MEK inhibitor inhibits cancer cell growth in vitro and tumor growth in mouse xenograft studies.

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Background

MEK 1/2 kinases are central proteins in the mitogen-activated protein kinase or MAPK pathway. Abnormal activation of this pathway results in the formation and progression of tumors, fibrosis and other diseases. MEK inhibitors block phosphorylation (activation) of extracellular signal-regulated kinases ("ERK"), which can lead to cell death and inhibition of tumor growth. MEK inhibitors have been approved to treat several BRAF driven solid tumors as well as plexiform neurofibromas associated with neurofibromatosis (NF1) in pediatric patients.

PAS-004 is the first macrocyclic MEKi and consistent with macrocycle attributes PAS-0004 has a long half-life (52 hours) and excellent oral bioavailability in dogs (96%). We believe may address the limitations and liabilities associated with existing drugs with a similar mechanism of action. PAS-004 is currently in a Phase 1 dose escalation study in patients with advanced solid tumors with rat sarcoma virus (RAS), neurofibromatosis type I (NF1), or rapidly accelerated fibrosarcoma (RAF) mutations (ClinicalTrials.gov Identifier: NCT06299839). Primary endpoint is safety with pharmacokinetic and pharmacodynamic (i.e. pERK measurements) secondary endpoints.

The study reported here compares the anti-cancer efficacy of PAS-004 in NRAS mutant cancer cell lines in vitro and in vivo. PAS-004 was compared with the approved MEKis selumetinib and binimetinib and the PK/PD relationship for PAS-004 was examined using tumor and plasma samples from the NSCLC xenograft study.

Methods

Cell growth assays: Cell viability of 10 NRAS mutant cancer cell lines (Table 1) were evaluated after exposure to the vehicle or the MEK inhibitors PAS-004 (Shanghai Syn-TheAll Pharmaceutical Co., Ltd., Shanghai, CH), selumetinib (Cat # HY-50706, MedChem Express, Monmouth Junction NJ), binimetinib (Cat # HY-15202, MedChem Express, Monmouth Junction NJ) and trametinib (Cat # HY-10999 , MedChem Express, Monmouth Junction NJ). Cells were incubated in 96 well plates and each compound was tested in triplicate at a dose range of 0.001-10 uM. After 3 days viability was assessed using Cell Titer GloTM (Promega, Madison WI, USA).

Xenograft Studies: Nude Balb/C mice (Shanghai Sino-British Sippr / BK Lab Animal Co., Ltd.) were inoculated (SC) with 5x106 NCI-H1299 tumor cells or HepG2 cells (ATCC). Treatments began on day 18 when the average tumor volume reached ~150 mm3. Mice were dosed by oral gavage with vehicle, PAS-004 (2.5, 5, 10 mg/kg) once daily (QD) and selumetinib and binimetinib (2.5, 5, 10 mg/kg) dosed twice daily (BID). Tumor volumes and body weights were measured on Days 0, 2, 6, 9, 13, 16, 20). Tumor biopsies and plasma samples were collected 24 hours post last dose on Day 21.

pERK detection: Tumor samples collected at the end of the NSCLC xenograft study were lysed in RIPA buffer in the presence of protease and phosphatase inhibitors.

Total lysate protein concentrations were measured by BCA and 20 mg of each sample was loaded on an SDS-PAGE gel for Western transfer and analysis. To detect determine the ratio of pERK:total ERK blots were probed with the pERK specific antibody Phospho-ERK1/ERK2 (Thr202, Tyr204) Polyclonal Antibody (Invitrogen, Waltham MA) and the total ERK antibody ERK7D8 (Thermo Fisher, Waltham MA). After incubation and washing the Radience Q ChemiLuminesent (Azure Biosciences, Dublin CA) was added, and images captured using an Azure300 Imager (Azure Biosciences, Dublin CA).

PK analysis: PAS-004 plasma samples were collected 24 hours after the final dose was administered in the NSCLC xenograft study and PAS-004 levels measured by LC-MS (Northeast Bio, MA, USA).

Results

In Vitro Cell Growth Assays

- PAS-004 inhibits the growth of NRAS mutant cell lines.

Cell line/Tumor type	Compound	RelIC$_{50}$ (μM)	Max Inhibition (%)
NCI-H2135	PAS-004	0.098	50.44
	AZD6244	0.101	54.57
	Trametinib	0.005	55.27
	Binimetinib	0.082	55.46
HepG2/Hepatocellular	PAS-004	0.024	76.72
	AZD6244	0.036	81.25
	Trametinib	0.001	89.67
	Binimetinib	0.023	80.47
HT1080/Colorectal	PAS-004	0.306	88.44
	AZD6244	0.109	63.63
	Trametinib	0.012	87.81
	Binimetinib	0.073	64.07
NCI-H1573/Non-small cell lung carcinoma	PAS-004	0.053	58.28
	AZD6244	0.082	59.25
	Trametinib	0.003	63.74
	Binimetinib	0.055	59.92
HCC15/Non-small cell lung carcinoma	PAS-004	0.044	46.78
	AZD6244	0.031	35.78
	Trametinib	0.023	50.29
	Binimetinib	0.021	36.54
NCI-H1155/ Non-small cell lung carcinoma	PAS-004	25.820	44.44
	AZD6244	0.084	21.39
	Trametinib	19.890	48.88
	Binimetinib	0.028	23.00
NCI-H1299/Non-small cell lung carcinoma	PAS-004	1.698	65.29
	AZD6244	0.202	43.64
	Trametinib	0.019	67.83
	Binimetinib	0.119	49.36
NCI-H1876/ Small cell lung carcinoma	PAS-004	n.a.	13.23
	AZD6244	n.a.	4.60
	Trametinib	n.a.	20.34
	Binimetinib	n.a.	-2.48
SW1271/Small cell lung carcinoma	PAS-004	4.059	57.81
	AZD6244	n.a.	39.44
	Trametinib	n.a.	50.35
	Binimetinib	n.a.	40.50
SNU-387/Hepatocellular	PAS-004	5.956	64.56
	AZD6244	0.044	18.75
	Trametinib	4.408	63.56
	Binimetinib	0.031	20.59

Table 1. PAS-004 inhibition of NRAS mutant cancer cell lines

- PAS-004 inhibition of NRAS lines does not plateau with activity similar to trametinib and greater inhibition than selumetinib and binimetinib.

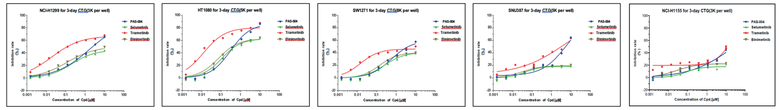

Figure 1. Dose response of MEK inhibitors in NRAS mutant cell line growth assay.

In Vivo Xenograft Studies

- PAS-004 has more potent anti-tumor activity than selumetinib and binimetinib in NRAS mutant tumor xenografts (QD vs. BID).

Hepatocellular tumors (HepG2) NSCLC tumors (NCI-H1299)

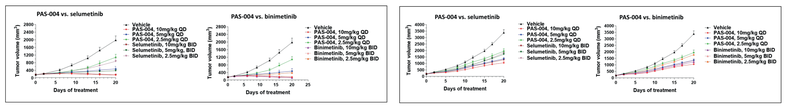

Figure 2. Tumor volume.

- PAS-004 has similar maximal tumor reduction as selumetinib and binimetinb when administered once daily vs twice daily (QD vs. BID).

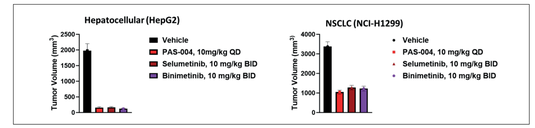

Figure 3.
Terminal xenograft tumor volume.

- PAS-004 did not result in any body weight loss.

Hepatocellular tumors (HepG2) NSCLC tumors (NCI-H1299)

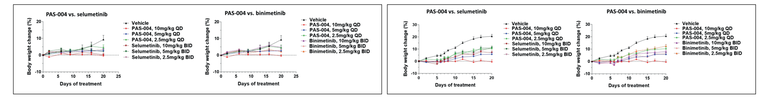

Figure 4. Body weight change

- PAS-004 (10 mg/kg) reduces final NSCLC tumor volume by 70% with 79% tumor pERK inhibition.

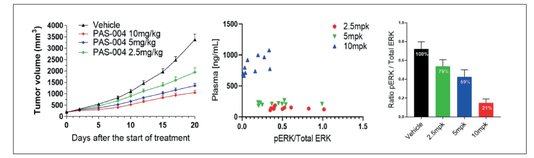

Figure 5.
PAS-004 PK/PD relationship.

Conclusions & Future Directions

- PAS-004 exhibit a dose-dependent anti-tumor efficacy in the liver cancer xHepG2 and the lung cancer NCI-H1299 cell-line-derived xenograft model.
- PAS-004 inhibition of in vitro NRAS mutant cell lines is greater than selumetinib and binimetinib in 5/10 cell line tested and is comparable to trametinib.
- PAS-004 activity in NRAS mutant lines does not plateau at highest levels tested versus other MEK inhibitors tested.
- PAS-004 has superior activity versus selumetinib and binimetinib in NSCLC xenograft studies and similar to that of binimetinib and superior to that of selumetinib in hepatocellular xenograft cancer model.
- In the NSCLC xenograft study, PAS-004 exhibit a dose-dependent tumor pERK suppression, reaching up to 75% at the highest dose tested.
- PAS-004 is a potential new treatment option with a more convenient dosing regimen for patients with advanced cancer and patients with NF-1.