IPI-493, a potent, orally bioavailable Hsp90 inhibitor of the ansamycin class

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Abstract

Background:
The cellular chaperone heat shock protein 90 (Hsp90) has emerged as an important target in cancer due to its essential role in several key oncogenic signaling pathways. In several types of cancer (e.g. GIST, NSCLC, breast cancer) inhibition of Hsp90 results in the degradation of key client proteins (e.g. KIT, EGFR, Her2) associated with either disease progression or poor prognosis. Several classes of Hsp90 inhibitors have recently advanced into clinical trials including ansamycin derivatives that are semi-synthetic derivatives of the natural product geldanamycin (e.g. 17-AAG, IPI-504 (retaspimycin hydrochloride), 17-DMAG) or small molecule synthetic derivatives designed from structure-based drug design (e.g. purine derivatives, isoxazoles, pyrazoles). IPI-504 (retaspimycin hydrochloride) is currently in a global Phase 3 registration trial for refractory, metastatic GIST (The RING trial). Geldanamycin derivatives incorporate the advantages of natural products (high affinity and selectivity) but certain derivatives have demonstrated either unacceptable toxicity (Geldanamycin, DMAG) or low solubility/oral bioavailability (17-AAG). We have developed an oral formulation for 17-AG (IPI-493), the primary active, long-lived metabolite of IPI-504 (retaspimycin hydrochloride) and 17-AAG and report herein the *in vitro* and *in vivo* properties of IPI-493.

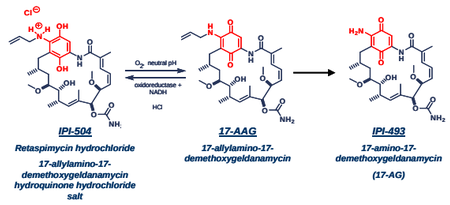
Results:
Multiple formulations of IPI-493 were designed and tested for oral bioavailability. Formulations were identified that led to considerably improved systemic exposure in beagle dogs after oral administration. Similar formulations also led to high IPI-493 exposure in mice following oral dosing. In a mouse xenograft model of TKI resistant NSCLC known to be sensitive to Hsp90 inhibitors (NCI-H1975), this optimal formulation of IPI-493 inhibited tumor growth by 87% at an oral dose of 100 mg/kg, QOD. We have also characterized the biochemical and cellular activity of IPI-493. The high affinity of IPI-493 to purified Hsp90 is not considerably influenced by reduction to the hydroquinone (Ki 17-AG quinone = 21 ± 7.5 nM, Ki 17-AG hydroquinone = 3 ± 1.8 nM). This is in marked contrast to other ansamycin derivatives (e.g. 17-AAG) where the hydroquinone (IPI-504) is approximately 50 times more potent than the quinone derivative. When tested against a panel of normal and cancer cell lines, IPI-493 selectively inhibits the growth of cancer cells over normal cells. Unexpectedly, in a subset of cancer cell lines we find IPI-493 to be notably more potent than 17-AAG.

Conclusion:
We have developed an oral formulation for 17-AG (IPI-493), the major metabolite of IPI-504 (retaspimycin hydrochloride) and 17-AAG. This compound binds tightly to purified Hsp90 and the binding is not considerably dependent on the redox environment. Furthermore, IPI-493 is more potent than 17-AAG and has a longer half-life *in vivo*. To our knowledge, this is the first report of 17-AG as a potential therapeutic as demonstrated by *in vivo* efficacy data. IPI-493 entered Phase 1 clinical development in 2008.

Background & Rationale

17-amino-17-demethoxygeldanamycin (17-AG) is a derivative of the natural product geldanamycin, and is a potent and selective Hsp90 inhibitor. As with many geldanamycin derivatives, 17-AG suffers from poor pharmaceutical properties such as low solubility making it difficult to deliver in pharmaceutically relevant doses. However, as the primary active metabolite of IPI-504 (retaspimycin hydrochloride) and 17-AAG, significant exposure levels of 17-AG have been reported in humans following administration of either compound. As such, an oral formulation for 17-AG that provides good bioavailability would warrant it's clinical development as an anti-cancer therapeutic.

IPI-493 is the primary active metabolite of IPI-504 and 17-AAG



IPI-504
Retaspimycin hydrochloride

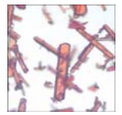
17-AAG
17-allylamino-17-demethoxygeldanamycin

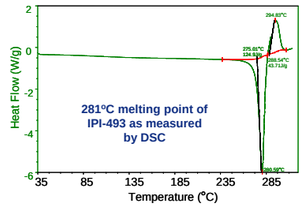
IPI-493
17-amino-17-demethoxygeldanamycin (17-AG)

17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride salt

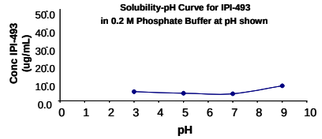
Poor pharmaceutical properties of IPI-493

IPI-493 drug substance





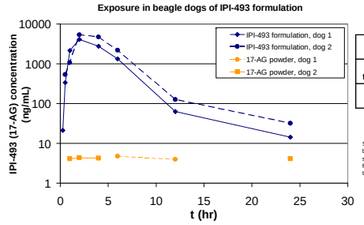
281°C melting point of IPI-493 as measured by DSC



Results: Formulation

Multiple formulations of IPI-493 were designed and tested for oral bioavailability, as well as physical and chemical stability. Formulations were identified that led to significantly improved systemic exposure in beagle dogs and CD-1 mice after oral administration. The formulations also provide dose responsive exposure across a therapeutic dose range.

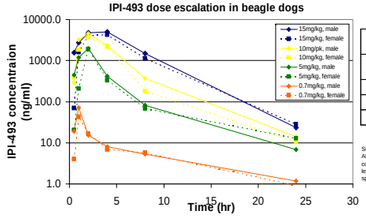
Greatly improved exposure following oral dosing of IPI-493 formulation in beagle dogs



Dose	C_{max} (ng/mL)	AUC_{0-last} (h*ng/mL)
Preferred formulation	4763	24475
17-AG powder	6	NA

Single oral dose, HPMC capsules 15mg/kg in beagle dogs. At selected time points, plasma samples were collected, processed, and analyzed for IPI-493 levels by liquid chromotography-tandem mass spectrometry (LC-MS/MS).

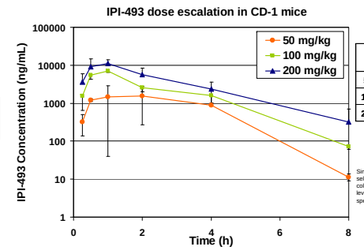
Exposure following single oral dose escalation of IPI-493 in beagle dogs



Dose Level	C_{max} (ng/mL)	AUC_{0-last} (h*ng/mL)
0.7 mg/kg	62	150
5 mg/kg	1998	5748
10 mg/kg	4220	17740
15 mg/kg	5623	35758

Single oral dose, HPMC capsules in beagle dogs. At selected time points, plasma samples were collected, processed, and analyzed for IPI-493 levels by liquid chromotography-tandem mass spectrometry (LC-MS/MS).

Exposure following single oral dose escalation of IPI-493 in CD-1 mice

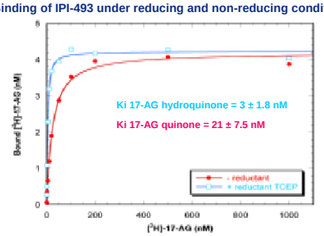


Dose Level	C_{max} (ng/mL)	AUC_{0-last} (h*ng/mL)
50 mg/kg	1530	6295
100 mg/kg	7060	19661
200 mg/kg	10062	28929

Single oral dose by gavage in CD-1 mice. At selected time points, plasma samples were collected, processed, and analyzed for IPI-493 levels by liquid chromotography-tandem mass spectrometry (LC-MS/MS).

Results: *in vitro* activity

Binding of IPI-493 under reducing and non-reducing conditions



Affinities determined using a binding assay with [3H]17-AG and Hsp90 purified from Hela cells under reducing (+TCEP) or non-reducing (-TCEP) conditions at 37°C

Cell growth inhibition of cancer and normal cell lines by IPI-493

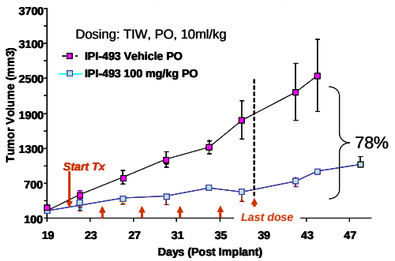
cells	cancer	GI_{50}% (nM)
1650	NSCLC	24
1975		17
HCC-827		16
H460		9
H4006		30
A549		11
HCT-116	CRC	34
H129		2
SW480		30
LXSs1	PaCa	2
LXSs1		7
AsPC1		4
MCF7	Breast	51
MDA-MB-468		174
BT-474		9
JIMT-1		13
SKBr3		17
MDA-MB-231		20

cells	cancer	GI_{50}% (nM)
A2780	ovarian	30
SKOV3		24
IGROV-1		226
RPMI-8226	MM	56
KMS-12		29
H929		13
MM1.1S	AML	13
K562	CML	7
DaudI	lymphoma	218
RL		58
Toledo		84
PBL	normal human lymphocytes	20000
hepatocytes		20000

Cell lines were plated subconfluently according to ATCC conditions and exposed to IPI-493 over a range of concentrations. GI50 values were determined using luciferase cell viability assay (Promega CellTiter-Glo) 72h after addition of compound

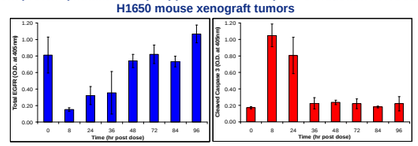
Results: *in vivo* efficacy & activity

Efficacy of IPI-493 in a mouse xenograft model of NSCLC cell line H1650 [EGFR (del E746-A750)]



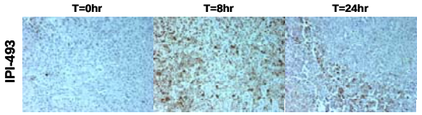
Subcutaneous xenografts tumors of the NSCLC cell line H1650 [EGFR (del E746-A750)] were established in athymic mice. Dosing schedule was TIW at 10ml/kg. Animals received either vehicle or IPI-493 at tested dose levels. Tumor dimensions were measured twice weekly

Client protein (mutant-EGFR) suppression and caspase-3 induction in NSCLC H1650 mouse xenograft tumors



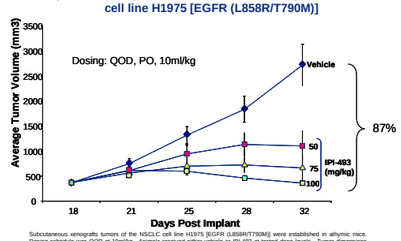
Following a single oral dose of IPI-493 at 100mg/kg, xenografts tumors of the NSCLC cell line H1650 [EGFR (del E746-A750)] were harvested at various time points, and the effect of IPI-493 on total EGFR and caspase-3 levels measured over time by ELISA. Data and sample loading normalized to Actin levels measured by ELISA.

IHC of caspase-3 levels in NSCLC H1650 mouse xenograft tumors



Following a single oral dose of IPI-493 at 100 mg/kg, xenograft tumors of the NSCLC cell line H1650 [EGFR (del E746-A750)], were harvested at various time points and fixed with 10% Neutral Buffered Formalin. Xenograft tumors were subsequently embedded in paraffin, sectioned, and the amount of endogenous cleaved caspase-3 was measured via immunohistochemistry.

Dose response efficacy of IPI-493 in a mouse xenograft model of NSCLC cell line H1975 [EGFR (L858R/T790M)]



Subcutaneous xenografts tumors of the NSCLC cell line H1975 [EGFR (L858R/T790M)] were established in athymic mice. Dosing schedule was QOD at 10ml/kg. Animals received either vehicle or IPI-493 at tested dose levels. Tumor dimensions were measured twice weekly

Conclusion

- We have developed an oral formulation for 17-AG (IPI-493), the major active metabolite of IPI-504 (retaspimycin hydrochloride) and 17-AAG
- With this formulation, we are able to achieve therapeutically relevant, dose responsive exposure *in vivo* across multiple species
- IPI-493 binds tightly to purified Hsp90 and is not significantly dependent on the redox environment with Ki values of 3 ± 1.8 nM and 21 ± 7.5 nM in reducing and non-reducing conditions, respectively
- IPI-493 demonstrates potent cell killing across a panel of cancer cell lines (Median GI_{50}= 27nM), but not normal cells (GI_{50}>20uM)
- We have demonstrated efficacy in the NSCLC xenograft model H1650 [EGFR (del E746-A750)] with 78% reduction in tumor volume and dose responsive efficacy in NSCLC xenograft model H1975 [EGFR (L858R/T790M)] with a maximum reduction of 87% in tumor volume
- To our knowledge, this is the first report of 17-AG as a potential oral cancer therapeutic as demonstrated by *in vivo* efficacy data
- IPI-493 is currently in Phase 1 clinical development

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