DISCOVERY OF PI3K DELTA INHIBITORS FOR THE TREATMENT OF INFLAMMATORY AND AUTOIMMUNE DISEASE

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Abstract

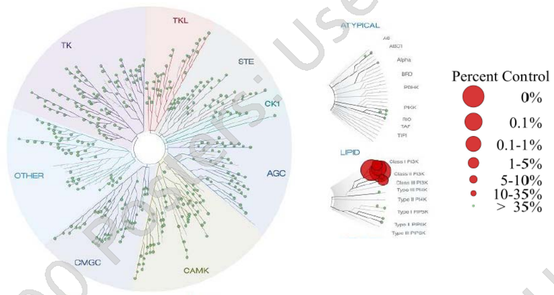
PI3K delta is broadly implicated in inflammation signaling in both B and T cells as well as multiple myeloid cell types, which cooperate in the initiation and progression of various inflammatory and autoimmune diseases. We have discovered a series of potent and highly selective PI3K delta inhibitors that potently inhibit signaling in immune cells and have demonstrated compelling efficacy in rodent models of immune disease. One such molecule, PWT143, inhibits AKT phosphorylation with sub-nanomolar potency in cellular assays reflecting PI3K delta function. This activity translates to robust phenotypic activity in multiple cell types, including inhibition of cytokine release from B and T cells. Importantly, PWT143 inhibits basophil activation (as measured by surface CD63 expression in a whole blood assay) with sub-nanomolar potency in response to anti-IgE stimulation but not in response to fMLP stimulation, suggesting a differential effect on autoimmune compared to bacterial antigen-mediated immune cell activation. In vivo, the cellular activities of PWT143 are reflected by dose-dependent pharmacodynamic activity in a mouse passive cutaneous anaphylaxis model. Furthermore, PWT143 completely prevents disease onset and regresses established disease in a mouse collagen-induced arthritis model, suggesting that inhibition of PI3K delta (without inhibition of PI3K gamma) is sufficient for efficacy in this model. Together, these data support the broad utility of PWT143 and related PI3K delta inhibitors for the treatment of autoimmune disease and other pathologies involving dysregulation of the immune response.

In vitro kinase selectivity

Compound	PI3Kdelta IC_{50} (nM)	PI3Kalpha IC_{50} (nM)	PI3Kbeta IC_{50} (nM)	PI3Kgamma IC_{50} (nM)
PWT143	**5.0**	5022	208	2137
CAL-101	**7.1**	1122	485	89

PWT143 is a potent, selective inhibitor of PI3K delta

DiscoverX Kinomescan (PWT143 @ 1μM)



PWT143 shows no crossreactivity with protein kinases

PI3K Isoform Cellular Assays

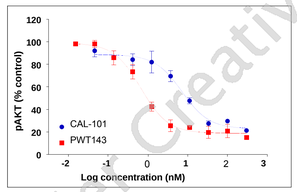
	Cellular IC_{50} (nM)			
	Delta	Alpha	Beta	Gamma
PWT143	**0.8**	1620	18	428
CAL-101	**5.0**	26180	379	1916

- PWT143 was tested in cellular assays reflecting activity of PI3K delta, alpha, beta and gamma isoforms
  - –Delta: pAKT (T308) in anti-IgM-stimulated Raji cells
  - –Alpha: pAKT (T308) in IGF1-stimulated MDA-MB-453 cells
  - –Beta: pAKT (S473) in LPA-stimulated PC-3 cells
  - –Gamma: pAKT (S473) in C5a-stimulated RAW264.7 cells

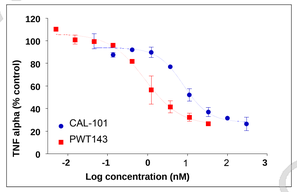
In cells, PWT143 is highly potent towards PI3K delta and highly selective versus PI3K alpha/gamma, with ~30x selectivity versus PI3K beta

Effect on B cells

AKT phosphorylation in Raji B cells (anti-IgM stimulation)



TNFalpha release in Raji B cells (anti-IgM stimulation)

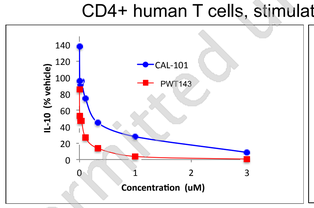


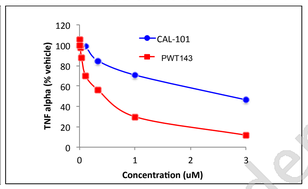
Compound	B cell pAKT T308 IC_{50} (nM)	B cell TNF alpha IC_{50} (nM)
PWT143	0.6	0.6
CAL-101	5.0	3.9

Pathway inhibition translates to phenotypic activity (inhibition of TNF alpha release in Raji cells)

Effect on T cells

CD4+ human T cells, stimulated with anti-CD3 and anti-CD28



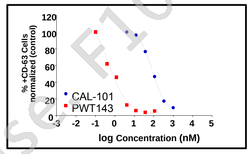


Compound	IL-10 IC_{50} (nM)	IL-4 IC_{50} (nM)	TNF alpha IC_{50} (nM)	IFN gamma (IC_{50} (nM)	IL-2 IC_{50} (nM)
PWT143	<1.4	13	513	<1.4	120
CAL-101	~ 330	~ 100	1540	~ 50	~3000

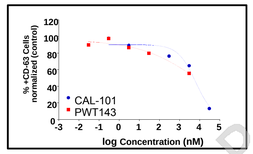
Both Th1-type (IFNγ) and Th2-type (IL-4, IL-10) cytokine production inhibited

Effect on Basophils

CD63 expression in basophils (whole blood assay, **anti-IgE stim.**)



CD63 expression in basophils (whole blood assay, **fMLP stim.**)

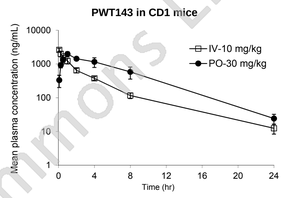


Compound	Anti IgE-mediated basophil activation IC_{50} (nM)	fMLP-mediated basophil activation IC_{50} (nM)
PWT143	1.6	>3000
CAL-101	77	>5000

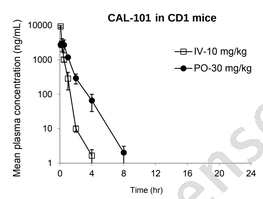
Differential response to anti-IgE vs fMLP: potential to inhibit autoimmune function without inhibiting antibacterial activity

Mouse Pharmacokinetics

PWT143 in CD1 mice



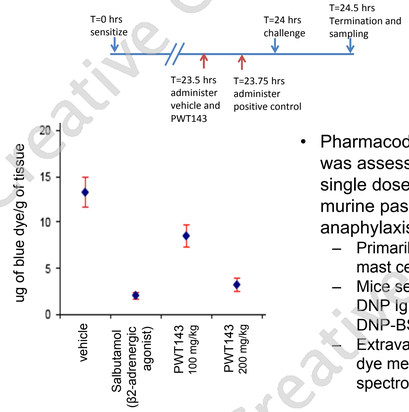
CAL-101 in CD1 mice



	Route	Dose (mg/kg)	Cmax (ng/mL)	Tmax (hr)	AUCinf (ng·hr/mL)	Cl (L/hr/kg)	Vss (L/kg)	$T_{1/2}$ (hr)	MRT (hr)	F (%)
PWT143	IV	10			5860	1.71	6.36	4.29	3.73	
	PO	30	1977	1.0	13987			3.56		80
CAL-101	IV	10			3088	3.24	0.73	0.433	0.225	
	PO	30	2903	0.25	3483			0.829		37.6

Superior half life and exposure profile in mice compared to clinical POC compound CAL-101

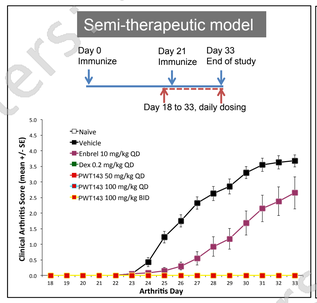
Pharmacodynamics

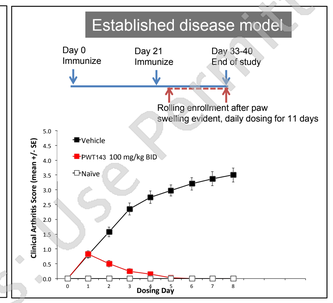


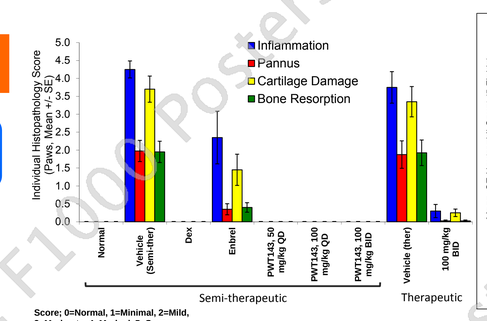
- Pharmacodynamic response was assessed at 1 hour post single dose of PWT143 using a murine passive cutaneous anaphylaxis model
  - Primarily a measurement of mast cell response in vivo
  - Mice sensitized using anti-DNP IgE, challenged using DNP-BSA
  - Extravasation of Evans Blue dye measured spectrophotometrically

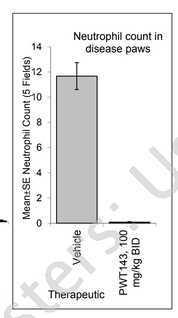
A 200 mg/kg dose of PWT143 elicits a pharmacodynamic response equivalent to the positive control (salbutamol)

Efficacy

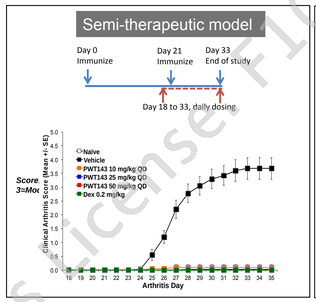


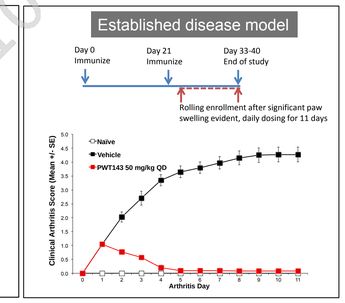






100% prevention of disease in 'semi-therapeutic' model
Dramatic regression of established disease in therapeutic model
Significant impact on histopathological markers of disease
Well-tolerated at efficacious doses





10mg/kg dose is fully efficacious in 'semi-therapeutic' model
Dramatic regression of established disease @ 50mg/kg

Conclusions

- PWT143 is a potent, selective inhibitor of PI3K delta
  - >300x vs alpha/gamma, 30x selective vs PI3K beta
- Superior activity vs CAL-101 in multiple immune cell types
- 100% inhibition of disease onset and resolution of established disease in a mouse CIA model

We acknowledge and thank Chempartner (ShangPharma) for chemical synthesis, in vitro PI3 kinase assays and mouse pharmacokinetics; DiscoverX for kinome profiling; Caliper Life Sciences for T-cell assays; MD Biosciences for the PCA assay, and Bolder Biopath for CIA model studies.