

# PWT33597 promotes apoptosis in tumor cells through balanced, dual inhibition of PI3K alpha and mTOR

David J. Matthews, Richard Ventura, Albert Tai, Charles Holst<sup>1</sup> and Marie O'Farrell

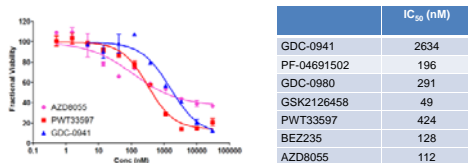
Pathway Therapeutics, San Francisco, CA <sup>1</sup>Current address: Pfizer Inc.

Abstract #B160

## Abstract

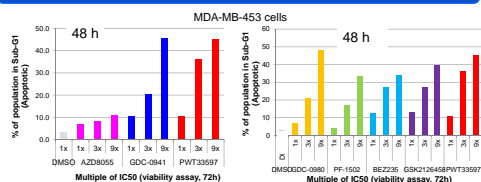
Phosphatidylinositol 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) serve as critical nodes in an intracellular signaling network that regulates cell growth, proliferation and survival, and dysregulation of this signaling network is frequently found in tumor cells. PWT33597 is a novel, balanced, dual inhibitor of PI3K alpha and mTOR currently in clinical development. To dissect the relative contribution of PI3K and mTOR pathways to activity, we explored the effects of various mono versus dual PI3K and mTOR inhibitors on intracellular signaling pathways, proliferation and apoptosis in cancer cell lines harboring PI3K alpha mutations. The panel of inhibitors used includes compounds that are highly selective for PI3K (GDC-0941), molecules that are highly selective for mTORC1/2 (AZD8055), as well as dual inhibitors such as PWT33597, BEZ235, PF-04691502 and GSK2126458. Our results indicate that compounds predominantly inhibiting PI3K have modest effects on phosphorylation of pathway proteins downstream of mTOR (in particular 4EBP), and do not lead to sustained induction of apoptosis markers such as PARP. Conversely, compounds that predominantly target mTORC1/2 produce feedback activation of PI3K signaling, resulting in increased phosphorylation of AKT (T308) and very limited effects on cell death. Dual PI3K/mTOR inhibitors produce more significant inhibition across the pathway, in particular leading to sustained inhibition of pAKT (S473 and T308) and p4EBP (T37/46), decreased release of normal feedback loops within the pathway, and an increased percentage of apoptotic cells as measured by percent population in sub-G1 phase of the cell cycle. In particular, PWT33597 produces potent and sustained pathway inhibition both for PI3K and mTOR-dependent readouts (pAKT S473, pAKT T308 and p4EBP T37/46), and maintains induction of apoptotic markers, resulting in cell death.

## Cell viability assays



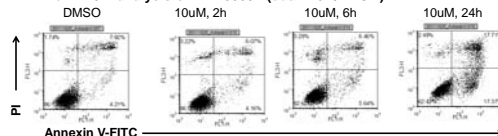
- Effects on cell viability were determined in the MDA-MD-453 cell line (Her2+, PIK3CA H1047R, PTEN E307K) using fluorescently-labeled NHS ester to stain cells (72h incubation)
- Exemplary dose-response curves are shown for PWT33597 (dual PI3K/mTOR), AZD8055 (mTOR) and GDC-0941 (PI3K)

## Cell cycle and annexin V analysis



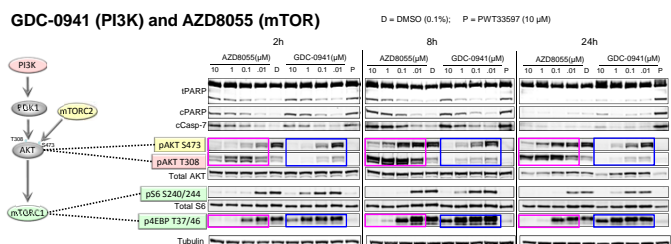
- AZD8055 (mTOR) shows little induction of apoptosis, even at 9x proliferation IC<sub>50</sub>
- GDC-0941 (PI3K) only shows apoptosis at high concentrations, where mTOR is also inhibited (9x IC<sub>50</sub> ~25uM)
- Dual PI3K/mTOR inhibitors all show dose-dependent induction of apoptosis

### Annexin V/PI analysis of PWT33597 (dual PI3K/mTOR)



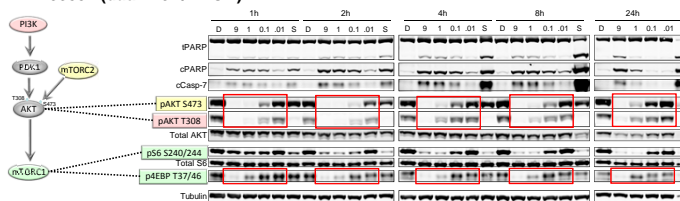
- Untreated cells are largely PI and annexin V negative
- Annexin V positive/PI negative and annexin V positive/PI positive populations evident at 24h post treatment, reflecting cells undergoing apoptosis and dead/late-stage apoptotic cells respectively
- Data confirm that PWT33597 promotes apoptosis in MDA-MB-453 cells

## Dose and time-dependent effects on pathway signaling

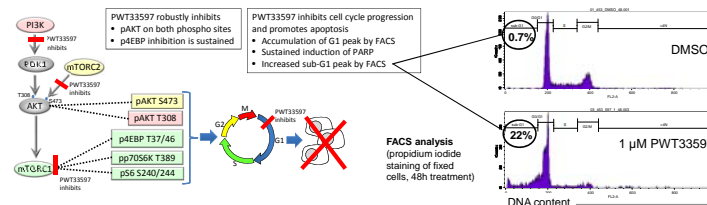


- GDC-0941 shows consistent inhibition of pAKT (S473, T308); little activity vs p4EBP
- AZD8055 shows upregulation of pT308; consistent inhibition of p4EBP
- Transient induction of apoptotic markers for both compounds, not sustained to 24h

### PWT33597 (dual PI3K/mTOR)



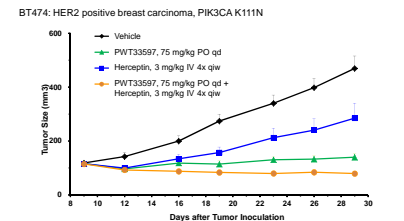
- Consistent inhibition of pAKT (S473, T308) at all timepoints
- Consistent inhibition of p4EBP at all timepoints; time-dependent increase in pS6 inhibition
- Pathway inhibition correlates with induction of apoptotic markers as early as 1h post-treatment; sustained to 24h



- PWT33597 robustly inhibits
  - pAKT on both phospho sites
  - p4EBP inhibition is sustained
- PWT33597 inhibits cell cycle progression and promotes apoptosis
  - Accumulation of G1 peak by FACS
  - Sustained induction of PARP
  - Increased sub-G1 peak by FACS

FACS analysis (propidium iodide staining of fixed cells, 48h treatment)

## In vivo efficacy



Treatment	TGI %*	Regression %
PWT33597, 75 mg/kg	93	-
Herceptin, 3 mg/kg	52	-
PWT33597, 75 mg/kg PO qd + Herceptin, 3 mg/kg IV 4x qw	>100	30

\* TGI% = 100x(1 - (T<sub>100</sub>/T<sub>0</sub>)) where T<sub>100</sub> is treated and vehicle tumor volumes respectively, at the first and last day of study as indicated by the subscript.

- In vivo antitumor activity was assessed using a BT474 (Her2+, PI3K mutant) xenograft model
- PWT33597 produces ~100% tumor growth inhibition at a well-tolerated dose
- PWT33597 combination with Herceptin produces significant regression in this model at a well-tolerated dose

## Conclusions

- Selective inhibition of PI3K produces robust inhibition of AKT phosphorylation, but has less impact downstream of mTOR
- Selective inhibition of mTORC1/2 produces robust inhibition downstream of mTOR, but can upregulate PI3K signaling
- Neither PI3K nor mTOR inhibition alone produces sustained induction of apoptotic markers
- Dual inhibition of PI3K and mTOR produces sustained inhibition of downstream targets (in particular pAKT and p4EBP), leading to sustained induction of apoptotic markers and cell death.
- Apoptotic markers (caspase 7, cPARP) are evident as early as 1-2h post treatment, but cell death is not evident until 24h (as determined by PI/Annexin V staining of live cells)
- PWT33597 produces robust, multi-nodal inhibition of signaling and promotes cell death in vitro, translating to potent antitumor activity in a breast cancer xenograft model and regression in combination with Herceptin.

We thank and acknowledge Chempartner (Shang Pharma) for providing PI3K and mTOR enzyme inhibition data, and Crown Bio for performing the BT474 xenograft study.

## In vitro biochemical activity

IC <sub>50</sub> , nM	PI3K alpha	PI3K beta	PI3K gamma	PI3K delta	mTOR	Selectivity
PWT33597	86	3234	310	1623	17	PI3K/mTOR
PF-04691502	5.0	11	1.7	3.6	11	PI3K/mTOR
GDC-0980	7.4	29	18	6.5	11	PI3K/mTOR
GSK2126458	0.76	0.92	0.20	1.7	0.41	PI3K/mTOR
BEZ235	40	598	85	84	1.1	mTOR-PI3K
AZD8055	400	7228	516	607	0.47	mTOR
GDC-0941	9.5	101	100	7.1	234	PI3K-mTOR

- Compounds tested included PI3K-selective (GDC-0941), mTOR-selective (AZD8055), and dual PI3K/mTOR inhibitors

PWT33597 Ki values	PI3K alpha	PI3K beta	PI3K gamma	PI3K delta	mTOR
K <sub>i</sub> (nM)	105	1595	210	1654	9.6
K <sub>i</sub> (ATP)	67	121	74	257	5.8

- PWT33597 produces balanced inhibition of PI3K and mTOR relative to the relative K<sub>i</sub>(ATP) for each target