The PI3K/mTOR inhibitor PWT33597 regresses 786-0 renal xenografts

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Background

- Renal cell carcinoma (RCC) accounts for ~3% of adult malignancies in the US, with an estimated 61,000 new cases and 13,000 deaths in 2011 (seer.cancer.gov/statfacts/html/kidrp.html).
- The 5 year survival rate for metastatic RCC is ~11%, and despite the recent approval of several targeted agents, responses are short-lived and occur only in a subset of patients.
- Approved drugs for advanced RCC include 2 classes of targeted agents:
  - ✧ VEGFR/multi-kinase inhibitors/anti-VEGF-A: sunitinib, sorafenib, pazopanib (first line), axitinib (after failure of 1 prior systemic therapy), bevacizumab (in combination with interferon alpha).
  - ✧ Rapalogs: temsirolimus and everolimus, which partially inhibit TORC1 signaling (second-line, after sunitinib or sorafenib failure).
- PWT33597 is a balanced dual inhibitor of phosphatidylinositol 3-kinase alpha (PI3K) alpha and mTOR, currently in clinical development. PWT33597 is an attractive candidate for testing in RCC based on its target profile:
  - ✧ mTOR inhibition is clinically validated by rapalogs and can be improved on by targeting both TORC1 and TORC2.
  - ✧ PI3K is expected to be inhibited downstream of VEGF signaling by VEGFR inhibitors, also clinically validated.
- Prognostic data support a role for both PI3K and mTOR
  - ✧ Activation of the mTOR pathway is associated with poor outcome in metastatic RCC (Abou Youssif *et al.,* 2011). Blocking TORC2 as well as TORC1 with PWT33597 may increase anti-tumor activity (compared to that observed with rapalogs).
  - ✧ Activation of the PI3K pathway is associated with poor clinical outcome (Merseburger *et al.,* 2008), and PI3Ks are activated downstream of VEGFRs.
  - ✧ High expression of mTOR and PI3K (p85 subunit) are often co-expressed and correlate with decreased survival (Elfiky *et al.,* 2011).

PWT33597 inhibits PI3K and mTOR signaling in RCC cell lines

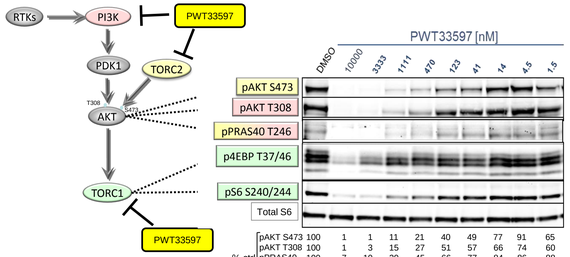

Figure 1. Western blot analysis in 786-0 cells following 2 hour treatment with PWT33597.

PWT33597 inhibits cell proliferation

IC_{50} (nM)	PI3K alpha	PI3K beta	PI3K gamma	PI3K delta	mTOR	Selectivity
PWT33597	86	3234	310	1623	17	PI3K/mTOR
GDC-0941	9.5	101	100	7.1	234	PI3K>mTOR
Rapamycin (Everolimus)	Binds to and inhibits FKBP-12 which inhibits mTOR activity					
Sorafenib (Nexavar)	Inhibitor of RAF, VEGFRs, PDGFRβ, RET, KIT, FLT3					

Table 1. IC50 values were determined using Kinase-Glo (PI3K alpha, delta), ADP-Glo (PI3K beta, gamma) and Lance Ultra (mTOR) assays.

Cell proliferation/viability IC_{50} (nM)			
Cell line	**786-0**	**769-P**	**A498**
Genotype	VHL -/- PTEN -/-	VHL -/- (hyper-CH3) PTEN WT	VHL -/- PTEN WT
PWT33597 (dual PI3K/mTOR)	1116	617	89
GDC-0941 (PI3K)	2455	905	87
Rapamycin (mTORC1)	>30000	>30000	>30000

Table 2. Effects on cell proliferation/viability were determined using a fluorescently-labeled NHS ester assay after a 72 hour culture with compounds in 786-0, 769-P and A498 cell lines.

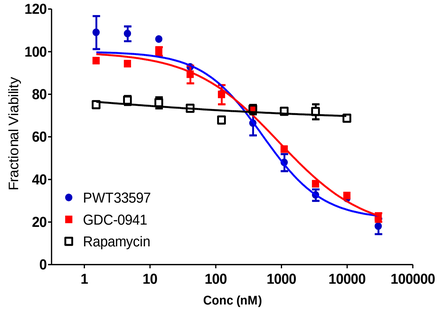

Figure 2. Effects of PWT33597, GDC-0941 and rapamycin on cell proliferation/viability in 769-P cells. Rapamycin consistently shows only partial inhibiton of cell viability in 786-0, 769-P and A498 cells.

PWT33597 induces autophagic-like vacuoles

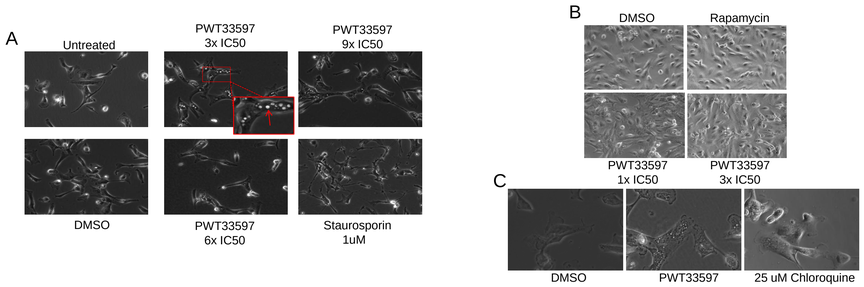

Figure 3. A) Bright field microscopy shows presence of cytoplasmic vacuoles following 72 hour culture. PWT33597 was tested at 1x, 3x or 9x the cellular IC_{50} concentration. B) Rapamycin was tested at 500 nM. C) With and without chloroquine (25 uM), an autophagy inhibitor. Acridine orange staining showed positive staining following PWT33597 treatment (data not shown).

Superior xenograft efficacy than standard of care agents

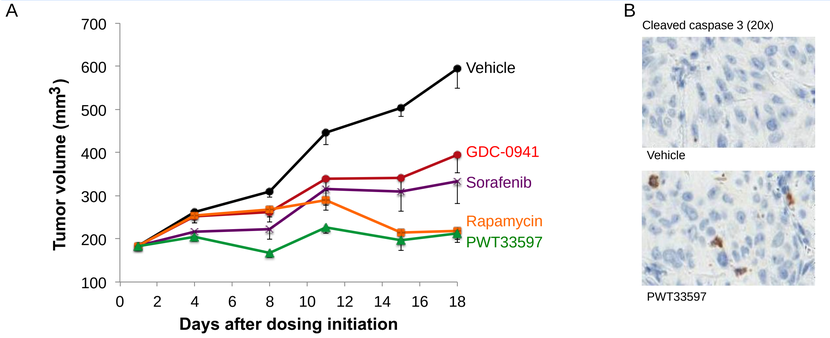

Figure 4. A) Mice bearing 786-0 tumor xenografts were treated with compounds at the following dose levels; GDC-0941 (100 mg/kg QD, PO), sorafenib (80 mg/kg QD, PO), rapamycin (10 mg/kg QD, IP) and PWT33597 (75 mg/kg QD, PO).
B): IHC for cleaved caspase 3 performed on xenograft tumors 12 hr after dosing on day 18. Four-fold increase in H score observed.

Regresses RCC xenografts

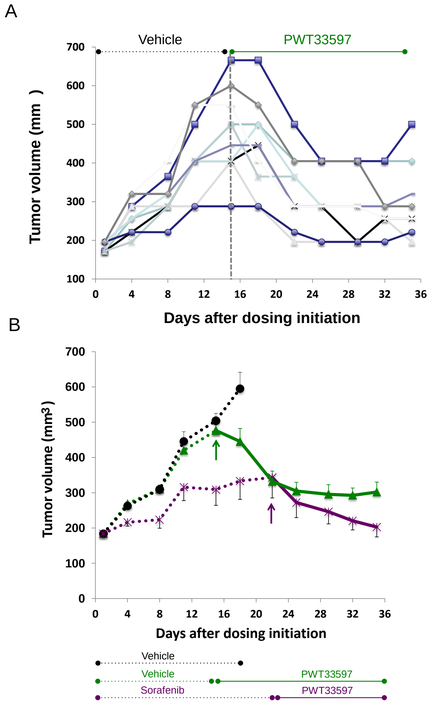

Figure 5. A) Mice with large 786-0 tumors (mean 500 mm^3) were treated with PWT33597 (75 mg/kg QD, PO), 10 individual animals shown. B) Mice with 786-0 xenografts were treated with either vehicle or sorafenib (80 mg/kg QD, PO) for 14 or 21 days respectively, followed by PWT33597 (75 mg/kg QD, PO) until day 35. A separate group was treated with vehicle only for 18 days.

Summary

- PWT33597 is a balanced dual inhibitor of PI3K alpha and mTOR, and inhibits in vitro RCC proliferation and mTOR/PI3K signaling.
- In the renal 786-0 xenograft model, tumor growth inhibition by PWT33597 was superior to that of approved agents rapamycin or sorafenib, or the PI3K inhibitor GDC-0941.
- PWT33597 rapidly regressed large 786-0 xenografts, and evidence of increased apoptosis was apparent by IHC.
- To assess a potential clinical treatment paradigm, PWT33597 was administered after sorafenib treatment, and xenograft tumor regression was observed.
- These data provide rationale to test PWT33597 in patients with RCC.

References; Abou Youssif et al., Cancer 2011, 117:290; Elfizy et al., J Translational Medicine 2011, 9:133; Merseberger et al., Urol Int 2008, 80:372.

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