Therapeutic effect of gedatolisib, a pan-PI3K/mTOR inhibitor, on prostate cancer models differing in PI3K or PTEN mutational status

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BACKGROUND

- Adaptive activation of the PI3K/AKT/mTOR (PAM) pathway has been associated with resistance to androgen receptor (AR) inhibitors used for treatment of prostate cancer (PC).
- Co-targeting PAM/AR pathways has, thus, long been considered a promising treatment strategy for metastatic castration resistant prostate cancer (mCRPC), but this approach has been confounded by the feedforward and feedback loops between PI3K isoforms, AKT, and mTOR that cross-activate uninhibited sub-units.
- Due to this compensatory resistance, PAM inhibitors that selectively spare or weakly inhibit one or more key PAM pathway components cannot achieve optimal therapeutic effect when combined with an AR inhibitor (AR-i).
- This likely explains why PAM inhibitors (PAM-i) evaluated to date in mCRPC, primarily AKT inhibitors, have demonstrated modest benefit only in patients with a PAM mutation, including those involving the loss of PTEN (a negative regulator of PI3K).[1]
- We posited that gedatolisib, as a potent inhibitor of all Class I PI3K isoforms, mTORC1, and mTORC2, would be more efficacious in both PTEN-wild type (WT) and PTEN-loss prostate cancer cells than sub-unit-specific PI3K, AKT, and mTOR inhibitors.

METHODS

Cell Lines: A panel of well-characterized prostate cancer cell lines were used in this study **(Table 1)**. Cells were maintained according to ATCC recommendations and authenticated by STR profiling. Note that cell lines are described as PTEN-WT or PTEN-loss in this study based on the status of their PTEN protein expression.

Table 1. Prostate Cancer Cell Lines Tested

	Relevant Characteristics				
Cell Line	**PTEN**	**AR Expression**	**PIK3CA**	**PIK3R1**	**Androgen Inhibitor Sensitivity**
22RV1	+/+	+	MT	WT	Resistant
MDA-PCa-2b	+/+	+	WT	WT	Sensitive
Du145	+/-	-	WT	WT	Resistant
LNCaP	-/-	+	WT	MT	Sensitive
C4-2[1]	-/-	+	WT	MT	Resistant
PC3	-/-	-	WT	Del	Resistant

Abbreviations: **+/+ = homozygous; +/- = heterozygous; -/- = null;** WT = wild type, MT = mutation; Del = deletion.
[1]Androgen-independent.

Viability and proliferation-normalized inhibition of growth rate (GR) assays for drug sensitivity: Cells were dispensed into collagen-fibronectin-laminin coated 96 microwell plates 24 hours prior to dosing with different PAM inhibitors **(Table 2)**. Cell viability was measured in triplicate wells using RT-Glo MT assay (Promega) using a luminescence microplate reader. The seeding density of each cell line was optimized to ensure untreated cells remained in the growth phase throughout the assay. The normalized growth rate inhibition (GR), per-division drug potency (GR_{50}), and efficacy (GR_{max}) metrics were calculated as described, using additional 0 hour (pre-treatment) data collection for RT-Glo viability measurements.[1]

Table 2. PAM Inhibitors Tested

		(Cell-free Assay nM Ki)							
Drug	**PAM Specificity**	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**mTOR**	**AKT1**	**AKT2**	**AKT3**
Gedatolisib	Pan-PI3K/mTOR	0.4	6	5.4	6	1.6	-	-	-
Alpelisib	PI3Kα	5	>1000	250	290	-	-	-	-
Copanlisib	Pan-PI3K	0.5	3.7	6.4	0.7	40	-	-	-
Samotolisib	Pan-PI3K	6	77	23	38	165	-	-	-
Capivasertib	AKT	-	-	-	-	-	3	8	8
Ipatasertib	AKT	-	-	-	-	-	5	18	8
Everolimus	mTOR	-	-	-	-	1.6	-	-	-

FACS analysis: Cells were stained using a viability dye, then fixed in 1.6% PFA, permeabilized with 10% methanol and analyzed by multicolor FACS on the Agilent Novocyte 3005 as described. To quantify cell proliferation, cells were cultured in the presence of 10uM EdU for 2 hr prior to analysis by FACS using Click-IT chemistry (Invitrogen). Cell death was measured using a fixable (Zombie) viable stain. In addition, we measured p4EBP1, a marker that integrates PAM signaling pathway outputs from PI3K/mTORC1 and mTORC2/pAKT.[2-5]

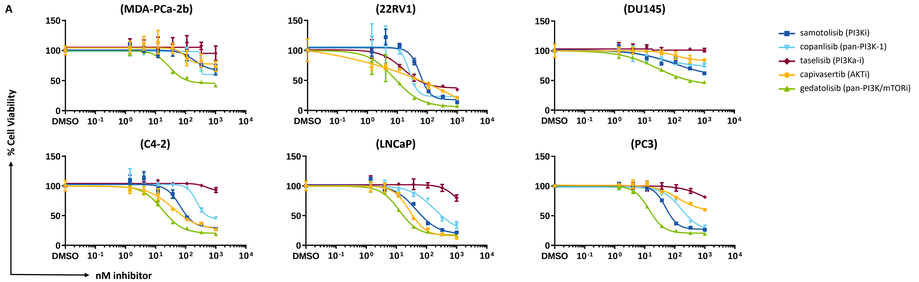
Xenografts: Six-to-eight-week-old male castrated BALB/c nude mice were inoculated subcutaneously with either 1 x 10^7 22RV1 or 5 x 10^6 PC3 cells or tumor pieces of C4-2 per mouse. When tumor size of 22RV1 or PC3 reached ~120 mm^3, mice (N=7–10 per arm) were randomly assigned to either a control vehicle saline group or a treatment group that received gedatolisib (15 mg/kg, Q4D IV) or enzalutamide (10 mg/kg PO). C4-2 tumors began treatment at ~500 mm^3 with gedatolisib (10 mg/kg, Q4D IV), enzalutamide (10 mg/kg, PO) or a combination of the two drugs (N=10 per arm).

References

1. Thorpe LM, et al. *Nat Rev Cancer.* 2015; 15, 7-24.
2. Earwaker P, et al. *PLoS One*. 2018; 13, e0191890.
3. Frosi Y, et al. *BMC Biol*. 2019; 17,40.
4. She QB, et al. *Cancer Cell*. 2018; 18, 39-51.
4. Cho DC, et al. *Clin Cancer Res*. 2010; 16, 3628-3638.
5. Hafner M, et al. *Nat Methods*. 2016; 13 (6): 521-527.
6. Hafner M, et al. *Curr Protoc Chem Biol*. 2017; 9 (2): 96-116.
7. Niepel M, et al. *Curr Protoc Chem Biol*. 2017; 9 (2): 55-74.

RESULTS

Figure 1. RT-Glo Viability Marker for PC Cell Lines Treated With Gedatolisib and Other PAM Inhibitors



B

Potency (IC50) (nM)

	PTEN-WT				PTEN-loss			
	MDA-PCa-2b	22RV1	DU145	Average	C4-2	LNCaP	PC3	Average
Samotolisib	174	66	68	103	68	48	54	57
Taselisib	>1000*	19	>1000	673	441	640	1650	910
Copanlisib	291	28	30	116	231	186	173	196
Capivasertib	81	2063	121	755	45	31	147	74
Gedatolisib	23	13	27	21	20	14	16	17

*>1000 – poor fit.

C

Efficacy (% Inhibition at 111 nM)

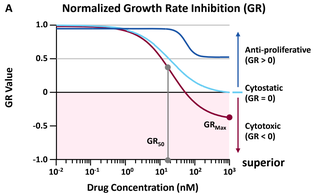
	PTEN-WT				PTEN-loss			
	MDA-PCa-2b	22RV1	DU145	Average	C4-2	LNCaP	PC3	Average
Samotolisib	9%	68%	15%	31%	53%	63%	63%	60%
Taselisib	0%	55%	1%	19%	0%	0%	3%	1%
Copanlisib	0%	66%	18%	28%	4%	23%	23%	17%
Capivasertib	13%	59%	8%	27%	56%	72%	19%	49%
Gedatolisib	45%	88%	44%	59%	71%	77%	76%	75%

% inhibition of RT-Glo signal at 111 nM drug compared to DMSO controls.

Viability of PC cell lines treated with PAM inhibitors. (A) The RT-Glo assay dose response curves were used to measure the viability of a panel of PC cell lines treated for 72 hours with different PAM inhibitors. Error bars are +/- standard deviation of triplicate cell culture wells. **(B-C)** Summary tables of potency and efficacy based on viability for the drugs and cell lines shown in **(A)**. The column "Average" represents the average metrics for the individual drugs within a PC sub-type.

- **The pan-PI3K/mTOR inhibitor, gedatolisib, exhibited superior potency and efficacy in all PTEN-WT and PTEN-loss cell lines tested relative to the other inhibitors evaluated.**
- **The AKT inhibitor, capivasertib, was 10-fold less potent in PTEN-WT compared to PTEN-loss PC cell lines.**

Figure 2. Normalized Growth Rate Inhibition (GR) Metrics Were Used to Characterize Cytostatic and Cytotoxic Effects of PAM Inhibitors on PC Cells



(A) Schematic of key features of the normalized growth rate (GR) inhibition approach. This approach is robust to confounders of traditional IC50 dose-response metrics, such as the number of cell divisions occurring during the assay (http://www.lincsproject.org).[6-8] **(B-C)** Presentation of GR-based GR_{50} (potency) and GR_I (efficacy) metrics that describe the effect of gedatolisib and other PAM inhibitors. GR_{max} values below zero reveal cytotoxicity of drugs not evident from the IC50 metric. The column "Average" represents the average metrics for the individual drugs within a PC sub-type.

- **GR metrics were calculated to rule out confounding effects of individual cell line proliferation rates on drug responses.**
  - **The pan-PI3K/mTOR inhibitor, gedatolisib, exerted potent and cytotoxic effects, regardless of PTEN or PI3K status, that were superior to the other PAM inhibitors evaluated.**
  - **The AKT inhibitor, capivasertib, was the only inhibitor that was less potent or efficacious in PTEN-WT compared to PTEN-loss cell lines.**

B

Potency (GR_{50}) (nM)

	PTEN-WT				PTEN-loss			
	MDA-PCa-2b	22RV1	DU145	Average	C4-2	LNCaP	PC3	Average
Samotolisib	167	58	131	119	59	29	49	46
Taselisib	>1000*	27	>1000	676	>1000	>1000	>1000	>1000
Copanlisib	276	27	605	303	258	116	150	175
Capivasertib	130	18	>1000	383	28	19	543	197
Gedatolisib	16	6	17	13	12	8	12	11

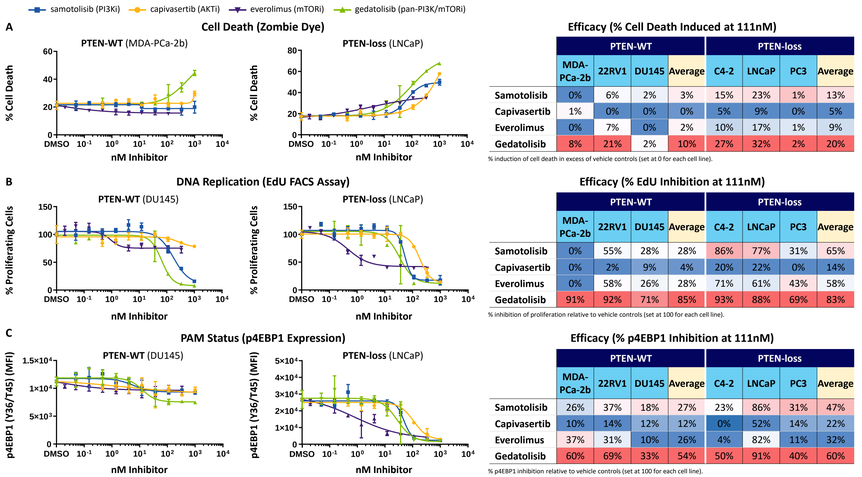
*>1000 – poor fit.

C

Efficacy (GR_{Max})

	PTEN-WT				PTEN-loss			
	MDA-PCa-2b	22RV1	DU145	Average	C4-2	LNCaP	PC3	Average
Samotolisib	-0.03	-0.35	0.22	-0.05	-0.25	-0.43	-0.17	-0.28
Taselisib	0.84	0.1	0.99	0.64	0.89	0.66	0.75	0.77
Copanlisib	-0.24	-0.26	0.47	-0.01	0.1	-0.17	-0.05	-0.04
Capivasertib	0.11	-0.16	0.67	0.21	-0.27	-0.65	0.43	-0.16
Gedatolisib	-0.63	-0.53	-0.08	-0.41	-0.44	-0.55	-0.32	-0.44

Figure 3. Cell Death, DNA Replication, PAM Signaling (p4EBP1) Markers for Assessment of PC Cell Functions



Efficacy (% Cell Death Induced at 111nM)

	PTEN-WT				PTEN-loss			
	MDA-PCa-2b	22RV1	DU145	Average	C4-2	LNCaP	PC3	Average
Samotolisib	0%	6%	2%	3%	15%	23%	1%	13%
Capivasertib	1%	0%	0%	0%	5%	9%	0%	5%
Everolimus	0%	7%	0%	2%	10%	17%	1%	9%
Gedatolisib	8%	21%	2%	10%	27%	32%	2%	20%

% induction of cell death in excess of vehicle controls (set at 0 for each cell line).

Efficacy (% EdU Inhibition at 111nM)

	PTEN-WT				PTEN-loss			
	MDA-PCa-2b	22RV1	DU145	Average	C4-2	LNCaP	PC3	Average
Samotolisib	0%	55%	28%	28%	86%	77%	31%	65%
Capivasertib	0%	2%	9%	4%	20%	22%	0%	14%
Everolimus	0%	58%	26%	28%	71%	61%	43%	58%
Gedatolisib	91%	92%	71%	85%	93%	88%	69%	83%

% inhibition of proliferation relative to vehicle controls (set at 100 for each cell line).

Efficacy (% p4EBP1 Inhibition at 111nM)

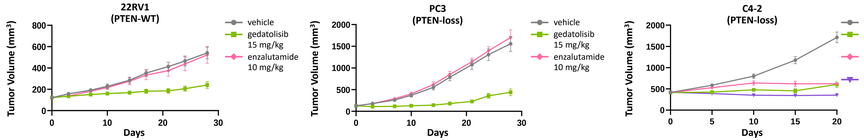
	PTEN-WT				PTEN-loss			
	MDA-PCa-2b	22RV1	DU145	Average	C4-2	LNCaP	PC3	Average
Samotolisib	26%	37%	18%	27%	23%	86%	31%	47%
Capivasertib	10%	14%	12%	12%	0%	52%	14%	22%
Everolimus	37%	31%	10%	26%	4%	82%	11%	32%
Gedatolisib	60%	69%	33%	54%	50%	91%	40%	60%

% p4EBP1 inhibition relative to vehicle controls (set at 100 for each cell line).

A panel of six PC cell lines was seeded on 96-well plates and then treated with escalating doses of either target selective PI3K/AKT/mTOR inhibitors, or gedatolisib, for 48 hours. Each drug was evaluated at 8–10 doses. Cells were harvested and analyzed for the different markers shown by flow cytometry. Error bars are +/- standard deviation of triplicate cell culture wells. The columns labeled "Average" represent averaged metrics for individual drugs within a PC sub-type.

- **The pan-PI3K/mTOR inhibitor, gedatolisib, dose-dependently induced cell death and suppressed DNA replication and p4EBP1 in PC cells independent of their PTEN status more effectively than all the other PAM inhibitors evaluated.**

Figure 4: *In Vivo* Efficacy of Gedatolisib in Treatment of Subcutaneous 22RV1, PC3, and C4-2 PC Xenograft Models



Castrated nude mice with subcutaneously xenografted PTEN-WT (22RV1) or PTEN-loss (PC3) PC cells, or PTEN-loss (C4-2) tumors, were treated with either gedatolisib, enzalutamide, or the combination, as described in the methods. Tumor growth inhibition analysis for the C4-2 model was initiated at day 33 post-implantation.

- **In the 22RV1 and PC3 xenograft models, gedatolisib induced 86% and 80% tumor growth inhibition (TGI), respectively. Enzalutamide induced no effect in either model.**
- **In the C4-2 xenograft model, gedatolisib, enzalutamide, and gedatolisib + enzalutamide induced TGI of 86%, 84% and 116%, respectively. Gedatolisib + enzalutamide induced 32% greater TGI than enzalutamide alone (*p<0.0061*).**

SUMMARY AND CONCLUSIONS

- The pan-PI3K/mTOR inhibitor, gedatolisib, exhibits superior potency and efficacy across different PTEN prostate cancer genotypes *in vitro* relative to other PAM inhibitors.
- Gedatolisib as a single agent demonstrated robust tumor growth inhibition *in vivo* in PTEN-WT and PTEN-loss xenograft models that were insensitive to enzalutamide.
- In a PC xenograft that was sensitive to enzalutamide, gedatolisib + enzalutamide induced significantly greater TGI than enzalutamide alone.
- Potent and cytotoxic effects of gedatolisib were seen in PC cell lines regardless of PTEN or PI3K status.
- The AKT inhibitor, capivasertib, was the only PAM inhibitor that was significantly less potent and less efficacious in PTEN-WT vs. PTEN-loss PC cell lines.
- These findings indicate that gedatolisib may help overcome or prevent development of resistance to AR therapy, which provides a strong rationale to evaluate gedatolisib in mCRPC clinical studies.