Gedatolisib, a well-tolerated pan-PI3K/mTOR inhibitor, exhibits potent therapeutic effects on gynecological cancer models regardless of their PI3K pathway mutational status

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Abstract 4928

BACKGROUND

- The PI3K, AKT, and mTOR (PAM) pathway **(Figure 1)** is one of the most commonly activated oncogenic pathways in gynecological cancers, such as endometrial cancer (EC), ovarian cancer (OC), and cervical cancer (CC) **(Table 1)**. Loss of PTEN function and PIK3CA activating mutations are especially frequent in EC **(Table 1)**.
- Targeting the PAM pathway, in combination with other cooperative oncogenic pathways (e.g., the estrogen pathway), is a promising strategy for cancer treatment, including gynecological cancers[2-4]. Currently, the combination of everolimus, an mTORC1 inhibitor, and letrozole, an aromatase inhibitor, is an NCCN recommended regimen for patients with recurrent or metastatic endometrioid EC[5].
- Most PAM inhibitors (PAMi) selectively spare or weakly inhibit one or more key PAM pathway components, which can lead to drug resistance[2]. To minimize drug resistance, a more comprehensive inhibition of the PI3K isoforms and downstream mTOR complexes may be required.
- We hypothesized that gedatolisib, which potently inhibits all Class I PI3K isoforms, mTORC1, and mTORC2, can more effectively inhibit proliferation of EC and other gynecological cancer cells than other PAM inhibitors targeting individual PAM pathway components.

Figure 1. The PI3K, AKT, and mTOR (PAM) Pathway

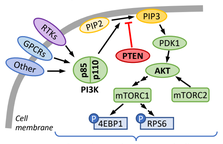


Table 1. Genetic Alterations of Key PAM Pathway Genes in EC, OC, and CC

	Endometrial Cancer (%)	Ovarian Cancer (%)	Cervical Cancer (%)
AKT1	5	4	4
AKT2	7	6	5
AKT3	9	7	2
PIK3CA	37	8	29
PIK3CB	11	8	8
PIK3R1	32	3	4
PIK3R2	7	6	1
PTEN	82	24	34

PIK3CA and PTEN values from Millis et al[1]; other values from cBioPortal analysis of the Cancer Genome Atlas (TCGA).

METHODS

Cell Lines. A panel of 26 well-characterized EC, OC, and CC cell lines were used in this study **(Table 2)**. Cells were maintained according to ATCC recommendations and authenticated by STR profiling. Genetic alterations in PAM pathway genes were identified by cBioPortal (https://www.cbioportal.org/) analysis of the Cancer Cell Line Encyclopedia (CCLE)[6].

Treatments with PAM Inhibitors. Cells were seeded on 24- or 96-well plates coated with collagen-fibronectin-laminin, let attach over night, and treated with PAM inhibitors **(Table 3)** at increasing concentrations to obtain dose response curves (DRCs). The seeding density of each cell line was optimized to ensure untreated cells remained in the growth phase throughout the assay.

Viability and proliferation-normalized inhibition of growth rate (GR) assays. After 72h PAMi treatment, cell viability was measured by RT-Glo MT assay (Promega) using a luminescence microplate reader. The normalized growth rate inhibition (GR) and per-division drug potency (GR50) metrics were calculated using the online GR calculator tool (http://www.grcalculator.org/grcalculator/) and the cell division time for each cell line. The normalized GR inhibition approach was used to rule out confounding effects of traditional IC50 metrics, such as the number of cell divisions occurring during the assay[7-9] (http://www.lincsproject.org).

FACS analysis. After 48h PAMi treatment, cells were harvested from 24-well plates, stained with a fixable viability dye (Zombie), fixed in 1.6% PFA, permeabilized with methanol, stained with antibodies, and analyzed by flow cytometry on the Agilent Novocyte 3005. To quantify cell proliferation, cells were cultured in the presence of 10 µM EdU for 2 hr prior to analysis by FACS using Click-IT chemistry (Invitrogen). Antibodies against pRPS6 and p4EBP1, two markers that integrate PAM signaling pathway outputs from PI3K/mTORC1 and mTORC2/pAKT **(Figure 1)**, were used to assess PAM pathway inhibition by the PAMi[10].

Xenografts. Immunodeficient female mice (nu/nu, SCID, or NOD.SCID) were inoculated subcutaneously in the flank with tumor pieces derived from MFE-280, MFE-296, AN3CA, or RL95-2 cell lines. When tumor size reached ~100–250 mm^3, mice (N = 6–10 per arm) were randomly assigned to either a control vehicle group or a treatment group that received gedatolisib as indicated. Mice were treated every 4th day or twice a week for 12–21 days depending on the xenograft model.

Table 2. Cancer Cell Lines Tested

Cell line	Cancer	PIK3CA	PIK3CB	PIK3R1	PIK3R2	PTEN	AKT1	AKT2	AKT3
AN3CA	EC	-	-	Mut	-	Mut	-	-	-
HEC1A	EC	Mut	-	-	-	-	-	-	-
HEC1B	EC	Mut , AMP	AMP	-	-	-	-	-	-
ISHIKAWA	EC	-	-	-	-	Mut	-	-	-
KLE	EC	-	-	-	-	-	-	AMP	-
MFE280	EC	Mut , AMP	-	-	-	-	AMP	AMP	-
MFE296	EC	Mut	-	-	-	Mut	-	-	-
RL952	EC	-	-	Mut	-	Mut	-	-	-
C33A	CC	Mut	-	-	-	Mut	-	-	-
CASKI	CC	Mut	-	-	-	-	-	-	-
DOTC24510	CC	-	-	-	-	-	-	-	-
SIHA	CC	-	-	-	-	-	-	-	-
A2780	OC	Mut	-	-	-	-	-	-	-
CAOV3	OC	Mut	AMP	-	-	-	-	-	-
COV362	OC	-	-	-	-	-	-	-	-
KURAMOCHI	OC	-	-	-	-	-	-	-	-
OV90	OC	-	-	-	HOMDEL	-	-	-	-
OVCAR3	OC	-	AMP	Mut	-	-	-	AMP	-
OVCAR4	OC	-	-	-	-	-	-	-	-
OVKATE	OC	Mut	-	-	-	HOMDEL	-	-	AMP
OVMANA	OC	Mut	-	-	-	-	-	-	-
OVSAHO	OC	-	-	-	AMP	-	-	-	AMP
SKOV3	OC	Mut, AMP	-	-	-	HOMDEL	-	-	-
TOV112D	OC	-	-	-	-	-	-	-	-
TOV21G	OC	Mut	-	-	-	Mut	-	-	-
UWB1289	OC	-	-	-	-	-	-	-	-

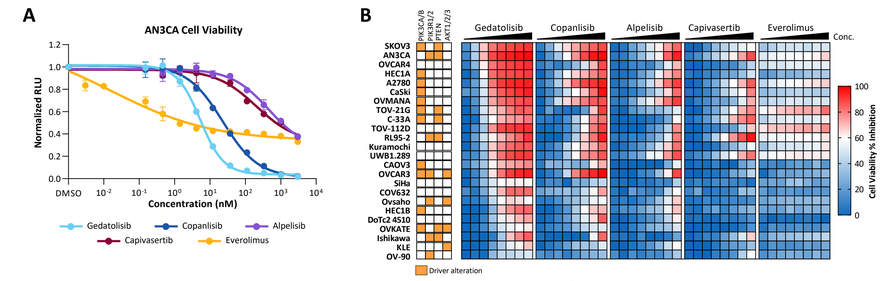
Cell lines with no driver alterations Driver alteration

Table 3. PAM Inhibitors Tested

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

RESULTS

Figure 2. Cell Viability Analysis of EC, OC, and CC Cell Lines Treated With Gedatolisib and Other PAM Inhibitors



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Viability IC50 (nM)

	Geda	Copa	Alpe	Capi	Ever
SKOV3	5.2	13	993	4600	Unstable
AN3CA	5.8	24	1154	917	1.9
OVCAR4	7.9	353	10299	27000*	4.7
HEC1A	10	26	2338	27000*	3000*
A2780	11	18	1169	1458	4
CaSki	12	62	3578	2749	3000*
OVMANA	13	15	667	7736	Unstable
TOV-21G	13	652	18930	1483	0.6
C-33A	16	197	20799	868	0.5
TOV-112D	16	213	11138	13079	0.2
RL95-2	17	164	8345	572	0.7
Kuramochi	18	61	3152	14502	22
UWB1.289	20	64	1379	1245	1.2
CAOV3	23	494	11837	27000*	3000*
OVCAR3	24	42	5589	27000*	3000*
SiHa	29	3000*	27000*	27000*	3000*
COV632	36	384	17675	27000*	3000*
Ovsaho	52	588	9377	27000*	3000*
HEC1B	60	179	6174	27000*	3000*
DoTc2 4510	72	264	12988	27000*	3000*
OVKATE	120	1234	27000	27000*	3000*
Ishikawa	157	918	27000*	8063	3000*
KLE	212	Unstable	11161	27000*	3000*
OV-90	Unstable	3000*	27000*	16900	3000*
Average All (n=24)	41	520	11076	15525	1774
Average PAM wt (n=7)	28	620	11947	19547	1290
Average PAM altered (n=17)	47	477	10717	13869	2001

Potency: High / Low

Viability % Inhibition at 333 nM

	Geda	Copa	Alpe	Capi	Ever
SKOV3	88%	82%	34%	33%	47%
AN3CA	95%	89%	34%	42%	60%
OVCAR4	90%	49%	13%	8%	53%
HEC1A	88%	72%	21%	14%	36%
A2780	94%	89%	30%	27%	64%
CaSki	89%	70%	17%	22%	42%
OVMANA	85%	80%	45%	24%	48%
TOV-21G	81%	21%	25%	26%	74%
C-33A	75%	59%	2%	35%	51%
TOV-112D	91%	61%	0%	11%	75%
RL95-2	78%	68%	10%	42%	60%
Kuramochi	83%	68%	19%	7%	53%
UWB1.289	88%	77%	28%	34%	63%
CAOV3	74%	28%	-8%	10%	19%
OVCAR3	90%	89%	11%	5%	26%
SiHa	63%	27%	6%	13%	28%
COV632	83%	45%	15%	8%	17%
Ovsaho	67%	43%	12%	9%	31%
HEC1B	78%	58%	23%	24%	30%
DoTc2 4510	65%	53%	11%	2%	7%
OVKATE	55%	44%	16%	1%	15%
Ishikawa	69%	5%	13%	10%	39%
KLE	54%	37%	10%	6%	23%
OV-90	44%	26%	8%	9%	24%
Average All (n=24)	78%	56%	17%	18%	41%
Average PAM wt (n=7)	80%	54%	13%	12%	42%
Average PAM altered (n=17)	77%	56%	18%	20%	40%

Efficacy: High / Low

Driver alteration

*Max concentration tested: IC50 not reached.
Unstable: unstable curve prevented reliable IC50 calculation.

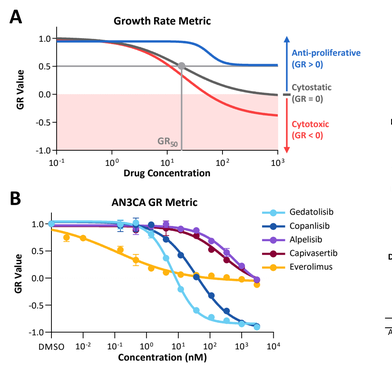
The RT-Glo MT assay was used to obtain cell viability DRCs in a panel of 24 EC, OC, and CC cell lines treated for 72 hours with various PAM inhibitors at increasing concentrations:

- 1.4-3000 nM for gedatolisib, copanlisib, and everolimus
- 12-27000 nM for alpelisib and capivasertib

(A) Dose response curve (DRC) for the AN3CA EC cell line. Data are relative to DMSO-treated cells (set as 1) and represent mean +/- SD. **(B)** Heatmap summarizing the DRCs of all the cell lines tested. **(C)** Summary tables of potency (left) and efficacy (right) based on IC50 values and viability % inhibition for the various PAM inhibitors. The % inhibition values shown in B and C are relative to DMSO-treated cells.

- **The pan-PI3K/mTOR inhibitor, gedatolisib, exhibited superior potency and efficacy in most of the cell lines tested relative to the other inhibitors evaluated, regardless of the PAM pathway mutational status.**
- **Gedatolisib showed similar potency and efficacy in cell lines with wild type or altered PAM pathway genes.**

Figure 3. Normalized Growth Rate Inhibition (GR) Metrics Were Used to Characterize Cytostatic and Cytotoxic Effects of PAM Inhibitors



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GR50 (nM)

	Geda	Copa	Alpe	Capi	Ever
COV362	2.3	15	8495	27000*	3000*
AN3CA	3.3	33	354	197	0.1
OVMANA	3.6	4	45	161	0.2
OVCAR4	4.2	100	3139	12434	0.2
SKOV3	5.1	12	884	1414	13.7
Kuramochi	6.4	13	474	1342	0.2
COV632	7.1	3	1955	27000*	3000*
A2780	7.2	10	502	668	0.9
CaSki	8.5	49	2426	1931	3000*
OVCAR3	8.7	16	1629	27000	3000*
TOV-112D	8.9	84	4327	3126	0.0
SiHa	9.0	192	14120	27000*	Unstable
UWB1.289	9.0	24	257	236	0.06
HEC1A	9.5	29	2138	27000*	3000*
OVKATE	10.9	6	170	27000*	2.5
RL95-2	12.2	121	4021	378	0.1
C-33A	13.2	117	13776	475	0.3
Ovsaho	15.0	29	1078	27000*	1.4
TOV-21G	15.6	603	27000*	1616	0.6
DoTc2 4510	26.1	56	2413	27000*	3000*
OV-90	31.1	388	7103	4184	Unstable
KLE	44.4	38	1470	3310	3000*
HEC1B	59.5	174	5160	27000*	3000*
Ishikawa	158.5	900	27000*	8311	3000*
Average All (n=24)	20	125	5414	11783	1126
Average PAM wt (n=7)	10	67	3812	14020	858
Average PAM altered (n=17)	24	148	6073	10861	1237

Potency: Low / High

GR value at 333 nM

	Geda	Copa	Alpe	Capi	Ever
COV362	-0.5	0.4	1.1	0.8	0.6
AN3CA	-0.8	-0.6	0.5	0.3	0.0
OVMANA	-1.0	-0.9	-0.4	0.1	-0.5
OVCAR4	-0.6	0.2	0.8	0.9	0.1
SKOV3	-0.3	-0.1	0.7	0.7	0.5
Kuramochi	-0.7	-0.5	0.6	0.8	-0.2
COV632	-0.8	-0.1	0.6	0.8	0.6
A2780	-0.7	-0.5	0.6	0.6	0.0
CaSki	-0.3	0.1	0.8	0.8	0.5
OVCAR3	-0.9	-0.8	0.8	0.9	0.4
TOV-112D	-0.7	-0.1	1.0	0.8	-0.4
SiHa	-0.2	0.5	0.9	0.7	0.2
UWB1.289	-0.6	-0.4	0.5	0.4	-0.1
HEC1A	-0.2	0.2	0.8	0.9	0.6
OVKATE	-0.7	-0.5	0.3	0.9	0.3
RL95-2	-0.2	0.1	0.9	0.5	0.2
C-33A	-0.1	0.2	1.0	0.6	0.3
Ovsaho	-0.4	0.1	0.7	0.8	0.3
TOV-21G	0.0	0.8	0.8	0.8	0.2
DoTc2 4510	-0.3	-0.1	0.8	1.0	0.9
OV-90	0.1	0.5	0.8	0.8	0.5
KLE	-0.2	0.2	0.8	0.9	0.5
HEC1B	0.0	0.4	0.8	0.8	0.7
Ishikawa	0.2	1.0	0.9	0.9	0.6
Average All (n=24)	-0.41	-0.01	0.70	0.73	0.30
Average PAM wt (n=7)	-0.58	-0.05	0.75	0.78	0.20
Average PAM altered (n=17)	-0.34	0.01	0.69	0.70	0.35

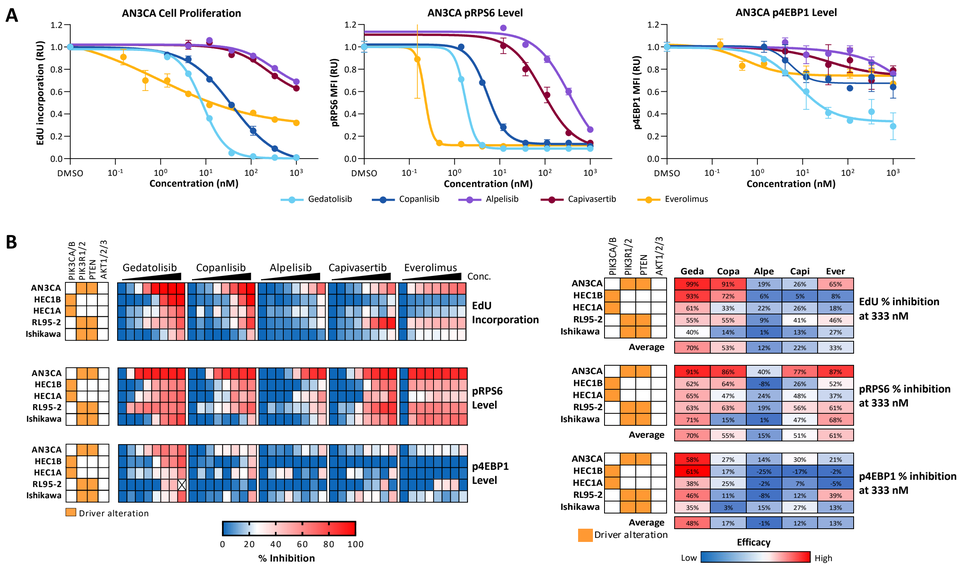
Efficacy: High / Low

*Max concentration tested; GR50 not reached. Unstable: unstable curve prevented reliable GR50 calculation.

GR metrics were used to rule out confounding effects of individual cell line proliferation rates on drug responses. **(A)** The normalized GR inhibition approach allows to identify both cytostatic and cytotoxic drug effects. GR values below zero reveal cytotoxicity of drugs not evident from the IC50 metric. **(B)** Example of GR value DRCs in response to PAMi in the AN3CA EC cell line. **(C)** GR-based metrics showing GR50 (potency) and GR values (efficacy) for gedatolisib and other PAMi in the panel of EC, OC, CC cell lines.

- **The pan-PI3K/mTOR inhibitor, gedatolisib, exerted potent anti-proliferative and cytotoxic effects, regardless of PTEN or PI3K status**
- **On average, the cytostatic and cytotoxic effects of gedatolisib were superior to the other PAM inhibitors evaluated.**

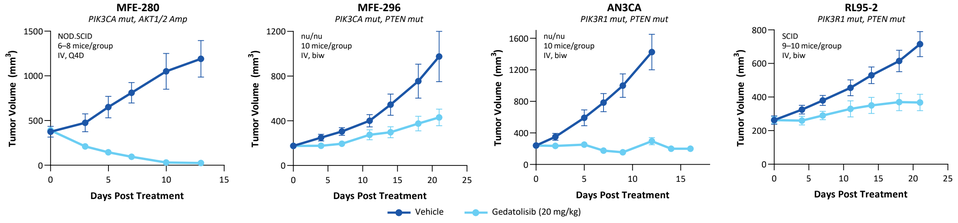
Figure 4. Assessment of DNA Replication and PAM Signaling Markers in EC Cell Lines



A panel of five EC cell lines was treated for 48 hours with escalating doses of PAM inhibitors and analyzed by flow cytometry for markers of DNA replication (EdU incorporation) and PAM signaling activity (pRPS6 and p4EBP1). **(A)** Examples of PAMi DRCs in AN3CA cells. Data are relative to DMSO-treated cells (set as 1) and represent mean +/- SD. **(B)** Heatmaps summarizing PAMi DRCs (1.4–1000 nM for gedatolisib, copanlisib, and everolimus; 12–9000 nM for alpelisib and capivasertib) in the five EC cell lines tested. **(C)** Table comparing the efficacy of gedatolisib and the other PAMi at inhibiting cell proliferation (EdU) and PAM signaling (pRPS6 and p4EBP1). The % inhibition shown in B and C is relative to DMSO-treated cells.

- **The pan-PI3K/mTOR inhibitor, gedatolisib, suppressed DNA replication and PAM signaling in EC cells independent of their PAM pathway status more effectively than the other PAM inhibitors evaluated.**

Figure 5. In Vivo Efficacy of Gedatolisib in Endometrial Cancer Xenograft Models



Immunodeficient mice were subcutaneously xenografted with MFE-280, MFE-296, AN3CA, or RL95-2 EC cell lines and treated with gedatolisib as described in the methods. Tumor growth inhibition (TGI) was assessed at the end of the treatment.

- **In the MFE-280, MFE-296, AN3CA, or RL95-2 xenograft models, gedatolisib induced 145%, 68%, 95%, and 77% TGI respectively.**

SUMMARY AND CONCLUSIONS

- The pan-PI3K/mTOR inhibitor, gedatolisib, exhibited superior potency and efficacy across EC, OC, and CC cell lines with different PAM pathway mutational status *in vitro* relative to other PAM inhibitors.
- Potent anti-proliferative and cytotoxic effects of gedatolisib were seen in EC, OC, and CC cell lines regardless of PTEN, PI3K, or AKT mutational status.
- Gedatolisib demonstrated robust tumor growth inhibition *in vivo* in EC xenograft models with different PAM pathway mutational status.
- These findings provide a strong rationale to evaluate gedatolisib in gynecologic cancer clinical studies.

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