Different effects of gedatolisib versus single-node PI3K/AKT/mTOR pathway inhibitors on breast cancer cell metabolic functions

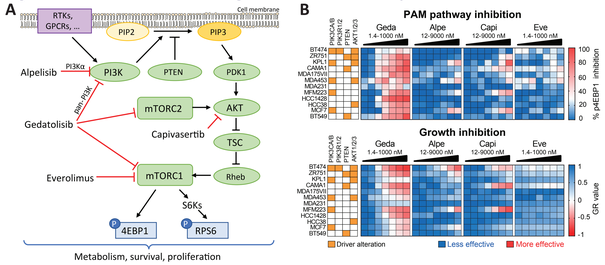
Stefano Rossetti, Aaron Broege, Jhomary Molden, Ross Kopher, Stephen Schulz, Ky McCracken, Igor Gorbatchevsky, Brian Sullivan, Lance Laing
Celcuity Inc., Minneapolis, MN, USA

P2-02-07
Presented at the
San Antonio Breast Cancer Symposium
December 10-13, 2024

BACKGROUND

- The PAM (PI3K-AKT-mTOR) pathway is one of the most commonly activated oncogenic pathways in breast cancer (BC). PAM pathway dysregulation is frequently associated with mutations of PAM pathway genes, e.g., PIK3CA activating mutations and loss of PTEN function [1].
- Dysregulation of PAM signaling in cancer cells can promote cellular functions and metabolic adaptations (e.g., increased glycolysis) that drive cancer progression even in the absence of PAM pathway gene alterations [2,3].
- The metabolic changes induced by dysregulation of PAM signaling can:
  - ✓ Directly impact cancer cells by sustaining their increased demand for energy production and biosynthesis, which are required for cell growth and proliferation [2,4].
  - ✓ Affect the surrounding microenvironment; e.g., low glucose, high lactate (with consequent decrease in pH), and hypoxia can impose critical metabolic restrictions to anti-tumor immune cells and promote immune suppressor cells [5].
- Due to the heavy reliance of tumor cells on the cellular functions and metabolic adaptations controlled by PAM signaling, targeting the PAM pathway is a promising therapeutic strategy for cancer treatment [6,7].
- Currently approved treatment options for patients with HR+/HER2- advanced breast cancer include single-node PAM inhibitors, such as everolimus (mTORC1), capivasertib (AKT), and alpelisib (PI3Kα), in combination with hormonal therapy.
- In a previous study [8], we found that comprehensive inhibition of multiple PAM pathway nodes by gedatolisib, a pan-PI3K/mTORC1/mTORC2 inhibitor, resulted in greater inhibition of PAM pathway and cell growth relative to single-node PAM inhibitors in breast cancer models, regardless of PTEN, PI3K, or AKT mutational status (**Figure 1**).
- The objective of the present study was to test whether gedatolisib exerted a greater effect on key metabolic functions relative to single-node PAM inhibitors in BC cells.

Figure 1. Gedatolisib versus Single-node PAM Pathway Inhibitors



(A) Gedatolisib targets the PI3K-AKT-mTOR (PAM) pathway at multiple nodes, while other PAM inhibitors like alpelisib, capivasertib, and everolimus target single nodes. **(B)** Gedatolisib inhibits the PAM pathway (top) and cell growth (bottom) more effectively than single-node PAM inhibitors, regardless of PAM pathway gene alterations. Flow cytometric analysis of p4EBP1 was used to assess inhibition of PAM activity in response to increasing concentration of PAM inhibitors. Cell viability before and after a 72-hour treatment with PAM inhibitors was used to calculate growth rate (GR) values and assess anti-proliferative (GR values between 0 and 1, blue) and cytotoxic (GR values between 0 and -1, red) effects. Data from Rossetti et al. 2024.

METHODS

Cell lines. The BC cell lines used in this study are shown in **Figure 1B**. Cells were maintained according to American Type Culture Collection (ATCC) recommendations and authenticated by short tandem repeat (STR) profiling. Genetic alterations in PAM pathway genes were identified by cBioPortal (https://www.cbioportal.org) analysis of the Cancer Cell Line Encyclopedia (CCLE). Only driver alterations are shown.

Treatments with PAM inhibitors. Cells were seeded on 96-well plates, allowed to attach for 24-48 hours, and treated with PAM inhibitors (**Table 1**) at the indicated concentrations for the indicated times.

Growth Rate (GR) Assay. After treatment for 72 hours, cell viability was measured by RT-Glo MT assay (Promega) using a luminescence microplate reader. Cell viability assessments were used to calculate GR values as described [9]. The GR approach was used to rule out confounding effects of traditional IC50 metrics, such as the number of cell divisions occurring during the assay (Hafner 2016).

Flow cytometry. After treatment, cells were harvested, stained with a viability dye (Zombie), fixed in 1.6% paraformaldehyde, permeabilized with methanol, stained with antibodies, and analyzed by flow cytometry on the Agilent Novocyte 3005. PAM pathway activity was assessed using an Antibody against phospho-4EBP1, a marker that integrates PAM signaling pathway outputs from PI3K/mTORC1 and mTORC2/AKT (**Figure 1**).

Glucose uptake. Cells seeded in 96-well plates and treated with the indicated drugs for 24 hours were analyzed for glucose uptake by using the Glucose Uptake-Glo assay (Promega) as described [10]. For the assay, cells were washed with PBS and incubated with 1 mM 2-deoxyglucose for 10 minutes. Detection reagent was applied to detect 2-deoxyglucose-6-phosphate in the cells with an Infinite M1000 (Tecan) microplate reader. RT-Glo MT luciferase assay was used to normalize glucose uptake to viable cells.

Analysis of glucose and lactate levels. Glucose and lactate levels in the conditioned medium were measured using the Biosen R-line instrument (EKF Diagnostic Holdings). Glucose consumption and lactate production were calculated from baseline glucose and lactate levels in the culture medium. Values were normalized to cell number.

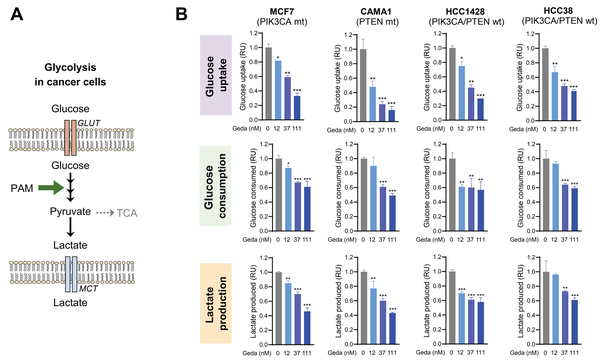
Analysis of oxygen levels and oxygen consumption rate (OCR). After drug treatment, oxygen levels were monitored on a Resipher instrument (Lucid Scientific, Inc.) during a 24h period. Oxygen consumption rates were calculated on the Resipher instrument from the oxygen levels and normalized to cell number.

Table 1. PAM Inhibitors Tested

Drug	PAM specificity	Cell-free Assay Ki (nM)								
		PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ	mTOR	AKT1	AKT2	AKT3	Ref
Gedatolisib	Pan-PI3K, mTORC1/2	0.4	6	8	6	1	-	-	-	[15]
Alpelisib	PI3Kα	5	>1000	250	290	-	-	-	-	[16]
Capivasertib	AKT	-	-	-	-	-	3	8	8	[17]
Everolimus	mTORC1	-	-	-	-	1.6-2.4	-	-	-	[18]

RESULTS

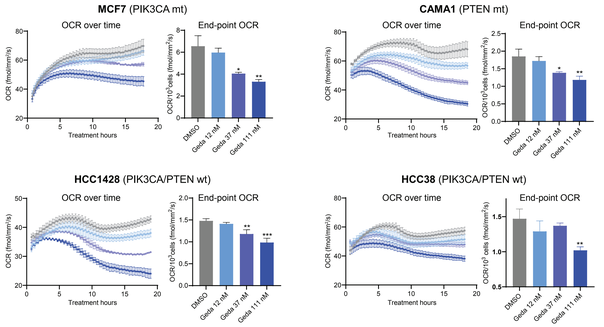
Figure 2. Effect of Gedatolisib on Glucose Metabolism



(A) Elevated glycolysis, even under aerobic conditions, is a hallmark of cancer cells [4]. Glucose enters the cell through glucose transporters (GLUT) and is converted into pyruvate through multiple glycolytic enzymatic reactions. In normal cells, pyruvate is used mostly in the tricarboxylic acid cycle (TCA), while in cancer cells is typically converted into lactate, which can be excreted into the microenvironment via monocarboxylate transporter (MCT). PAM pathway activation promotes glycolysis [2]. **(B)** BC cells with various PIK3CA/PTEN mutational status were treated for 24 hours with vehicle (DMSO) or different concentrations of gedatolisib and analyzed for glucose uptake by Glucose Uptake-Glo luciferase assay. After 24-hour treatment, the conditioned medium was also analyzed for glucose and lactate levels to assess glucose consumption and lactate production. Values were normalized to the cell number at the end of the treatment. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus DMSO-treated cells; mt = mutant; wt = wild type.

- Gedatolisib affected glucose metabolism and glycolysis in BC cells by inhibiting:
  - ✓ Glucose uptake
  - ✓ Glucose consumption
  - ✓ Lactate production
- The effects of gedatolisib were independent of PIK3CA/PTEN mutational status.

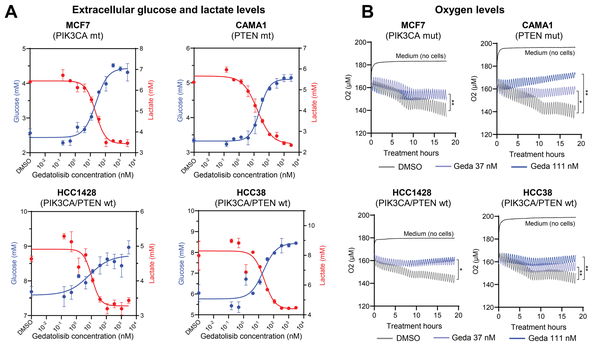
Figure 3. Effect of Gedatolisib on Oxygen Consumption Rate



Oxygen consumption is a rate limiting step for cancer cell proliferation [11] and can be increased by activation of the PAM pathway [12]. BC cells with various PIK3CA/PTEN mutational status were treated with vehicle (DMSO) or different concentrations of gedatolisib and monitored for oxygen consumption rate (OCR) overnight using a Resipher instrument. Real-time OCR measurements throughout the treatment time are shown in the left plots of each cell line. Cell number-normalized, end-point OCR is shown in the right plots of each cell line. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus DMSO-treated cells; mt = mutant; wt = wild type

- Gedatolisib induced significant inhibition of OCR in BC cell lines, regardless of their PIK3CA/PTEN mutational status.

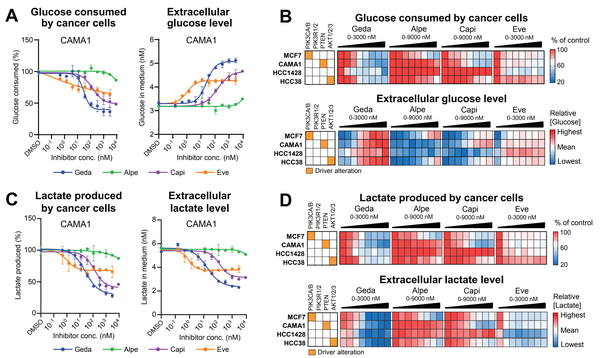
Figure 4. Effect of Gedatolisib on Cancer Cell Microenvironment



(A) BC cell lines with different PIK3CA/PTEN status were treated with increasing concentrations of gedatolisib for 24 hours. The levels of glucose (blue curve) and lactate (red curve) were analyzed in the conditioned medium using the Biosen R-line instrument. **(B)** The same cell lines were also monitored in real time for dissolved oxygen concentration in the conditioned medium using the Resipher instrument. * $P < 0.05$, ** $P < 0.01$.

- The metabolic changes induced by gedatolisib in BC cells affected the cells' microenvironment:
  - ✓ Decreased intracellular glucose consumption led to increased extracellular levels of glucose
  - ✓ Decreased intracellular lactate production led to decreased extracellular levels of lactate
  - ✓ Decreased intracellular OCR led to increased extracellular levels of oxygen

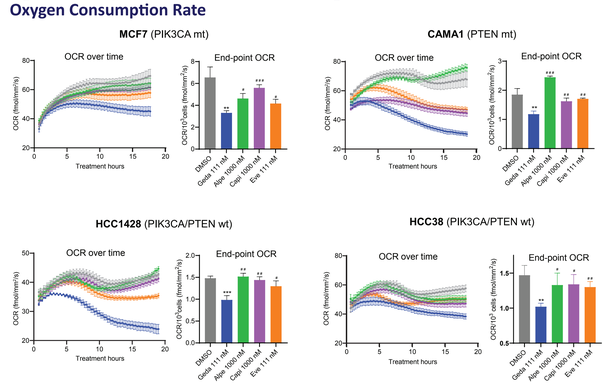
Figure 5. Comparison of Gedatolisib and Single-Node PAM Inhibitors Effects on Glucose Metabolism



(A-B) BC cell lines were treated with increasing concentrations of gedatolisib or single-node PAM inhibitors for 24 hours and analyzed for glucose consumption and glucose levels in the conditioned medium. An example of dose-response curves is shown for CAMA1 **(A)**, while a summary of the response in all cell lines is shown as a heatmap **(B)**. Glucose consumption is relative to DMSO-treated cells (set as 100%). The glucose levels shown in the heatmap are conditionally formatted for each cell line. **(B-C)** The same cell lines were analyzed in parallel for lactate production and lactate levels in the conditioned medium. Inhibitor concentrations tested = 0-4.1-12-37-111-333-1000-3000 nM (gedatolisib and everolimus); 0-12-37-111-333-1000-3000-9000 nM (alpelisib and capivasertib).

- Gedatolisib inhibited glucose consumption and lactate production more effectively than single node PAM inhibitors.
- As a consequence, the microenvironment of cells treated with gedatolisib had higher glucose and lower lactate levels than the microenvironment of cells treated with single-node PAM inhibitors.

Figure 6. Comparison of Gedatolisib and Single-Node PAM Inhibitors Effects on Oxygen Consumption Rate



BC cells with various PIK3CA/PTEN mutational status were treated with gedatolisib or single-node PAM inhibitors and monitored for OCR overnight using a Resipher instrument. Real-time OCR measurements throughout the treatment time are shown in the left plots of each cell line. Cell number-normalized, end-point OCR is shown in the right plots of each cell line. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus DMSO-treated cells; # $P < 0.05$, ## $P < 0.01$, #### $P < 0.001$ versus gedatolisib-treated cells; mt = mutant; wt = wild type.

- Gedatolisib inhibited oxygen consumption rate more effectively than single-node PAM inhibitors.

CONCLUSIONS AND DISCUSSION

- Gedatolisib is a pan-PI3K/mTOR inhibitor previously shown to inhibit the PAM pathway and PAM-controlled functions in BC cells, resulting in potent anti-proliferative and cytotoxic effects [8].
- The present study shows that gedatolisib inhibited multiple metabolic functions critical for cancer cell survival and proliferation, such as glucose uptake, glucose consumption, lactate production, and oxygen consumption rate. The inhibition of these metabolic functions may contribute to gedatolisib growth-inhibitory effects.
- As a consequence of these metabolic alterations, the microenvironment of BC cells treated with gedatolisib was also affected, showing decreased glucose, increased lactate, and increased oxygen levels. These microenvironmental changes could contribute to promote anti-tumor immune response, as previously shown in a syngeneic mouse model of mammary carcinoma [13].
- Gedatolisib inhibited PAM pathway activity and induced cell growth inhibition more effectively than single-node inhibitors in BC cells, regardless of PTEN, PI3K, or AKT mutational status.
- The greater growth-inhibitory effects of gedatolisib relative to single-node PAM inhibitors were paralleled by greater inhibition of cancer cell metabolic activities, such as glycolysis and oxygen consumption rate.
- Greater inhibition of the PAM pathway and PAM-controlled functions, including metabolic activities critical for cancer cell survival and proliferation, differentiate gedatolisib from single-node PAM inhibitors and may provide a mechanistic explanation for its superior growth-inhibitory action in BC cells.
- Gedatolisib has previously demonstrated promising preliminary clinical efficacy and safety data in patients with HR+/HER2- ABC in combination with hormonal therapy [14]. A phase 3 study (VIKTORIA-1, NCT05501886) evaluating gedatolisib plus fulvestrant with and without palbociclib is underway in patients with HR+/HER2- ABC whose disease progressed while on treatment with a CDK4/6 inhibitor.

REFERENCES:
[1] Millis SZ, et al. *JAMA Oncol*. 2016;2:1565-1573.
[2] Hoxhaj G and Manning BD. *Nat Rev Cancer*. 2020;20:74-88.
[3] Glaviano A, et al. *Mol Cancer*. 2023;22:138.
[4] Pavlova NN, et al. *Cell Metab*. 2022;34:355-377
[5] DePeaux K and Delgoffe GM. *Nat Rev Immunol*. 2021;21:785-797.
[6] Vanhaesebroeck B, et al. *Nat Rev Drug Discov*. 2021;20:741-769.
[7] Vasan N and Cantley LC. *Nat Rev Clin Oncol*. 2022;19:471-485.
[8] Rossetti S, et al. *NPJ Breast Cancer*. 2024;10:40.
[9] Hafner M, et al. *Nat Methods*. 2016;13:521-527.
[10] Sen A, et al. *Mol Oncol*. 2024.
[11] Chen Y, et al. *PLoS One*. 2009;4:e7033.
[12] Nogueira V, et al. *Cancer Cell*. 2008;14.
[13] Yan C, et al. *Int J Mol Sci*. 2021;22.
[14] Layman RM, et al. *Lancet Oncol*. 2024;25:474-487.
[15] Mallon R, et al. *Clin Cancer Res*. 2011;17:3193-3203.
[16] Fritsch C, et al. *Mol Cancer Ther*. 2014;13:1117-1129.
[17] Addie M, et al. *J Med Chem*. 2013;56:2059-2073.
[18] Sedrani R, et al. *Transplant Proc*. 1998;30:2192-2194.