Increased Survival in Nude Mice Inoculated with MCF7 Breast Cancer (BC) in the Mammary Fat-Pad Achieved via a Single Injection of a Lipid-like Hydrogel Emulsion Containing a New Molecular Entity (NME) and N-allyl Noroxymorphone (NaN) Compared to Tamoxifen (TAM) and Abemaciclib (ABE)

Poster #3638

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Introduction

The breast cancer phenotype and tumor stage at diagnosis dictate which first-line therapies will be deployed. Potential therapeutic interventions include adjuvant/ neo-adjuvant therapy, surgery, and post-surgical radiation. Chemotherapies are well known to result in off-target side effects. Further, systemic targeted therapies, which include endocrine treatments such as tamoxifen (TAM) and CDK4/6 inhibitors like abemaciclib (ABE), also result in significant off-target side effects. Nevertheless, these targeted therapies continue to be investigated for use in patients diagnosed with early-stage breast cancer [**1-5**]. For instance, several recently completed Phase III clinical studies found that the combination of CDK4/6 inhibitors with endocrine therapy significantly increased adverse events compared to treatment with endocrine therapy alone (**Table I**):

Table I: Complications / Adverse Events from CDK4/6 Treatment with and without Endocrine Therapy (ET)

Study Design/ Outcomes	monarchE Study Groups[A,B]		PALLAS Study Groups[A,B]		NATALEE Study Groups[A,B]	
	Verzenio + ET (abemaciclib)	ET	Ibrance + ET (palbociclib)	ET	Kisqali + ET (ribociclib)	ET
Indication	HR+ /HER2- Node+ High Recurrence Risk		HR+ & HER2-		HR+ /HER2- Node+ High Risk Node	
Total Patient Number	5872		5760		5101	
Discontinued Due to AE	38.5%	0.8%	27.2%	2%	19.5%	6.4%
Total TEAE	98.5%	89.9%	99.4%	89.5%	98.0%	87.8%
Diarrhea	86.6%	8.7%	66.4%	8.7%	57.2%	5.6%
Neutropenia	43.6%	0.2%	31.0%	0.1%	21.3%	0.2%
Abdominal Pain	35.7%	9.9%	NR	NR	35.7%	NR
Nausea	29.6%	5.1%	25.3%	5.1%	27.3%	7.4%
Leukopenia	27.7%	0.7%	14.4%	0.3%	12.3%	0.9%
Neutropenia	40.9%	1.5%	32.4%	0.3%	17.1%	0.6%
Anemia	25.5%	3.9%	23.4%	5.4%	NR	NR
Vomiting	19.8%	11.2%	15.3%	11%	17.4%	7.6%
Neutropenia	18.6%	0.2%	11%	0.1%	10.2%	0.2%
Increased AST	17.5%	NR	11.6%	2.6%	7.2%	3.8%
Hypokalemia	12.6%	3.1%	11%	1.5%	NR	NR
Thrombocytopenia	13.4%	1.3%	21.4%	3.3%	56.6%	35.2%
Anemia	14.6%	2%	17.5%	3.4%	15.3%	4.2%
Lymphopenia	34.2%	1.3%	17.9%	8.1%	NR	NR

NR = Not Reported AE = Adverse Event TEAE = Treatment Emergent Adverse Events AST = Aspartate Aminotransferase

Our recent studies in clinical and pre-clinical models has shown that intratumoral (IT) injection can be a highly effective therapeutic approach that reduces tumor burden while also reducing off-target side effects. These previous studies focused on treating metastatic breast cancer in bone [**6**; see **NCT05280067** & **abstract #3549**] and in liver [**7**].

We have developed Zeta-BC-007, a new therapeutic IT neo-adjuvant intervention to treat primary breast cancer. Zeta-BC-007 is designed to reduce or delay the need for for surgical intervention (e.g., mastectomy or lumpectomy) or radiation therapy. We anticipate that IT intervention with Zeta-BC-007 will mitigate off-target, treatment emergent adverse events (TEAE) by replacing the need for more toxic systemic neo-adjuvant therapies or reducing their dose. Further, we expect that the reduction or replacement of conventional neo-adjuvant therapies will reduce the severity of TEAEs, leading to enhanced survival.

Zeta-BC-007 is a first-in-class biomaterial engineered for IT administration through optimization of solubility and . It is a novel hydrogel-like lipid carrier emulsion optimized for solubility in breast tissue, that carries our new molecular entity (NME) and N-allyl noroxymorphone (NaN) encapsulated in a lipid nanoparticle (LNP).

Zeta-BC-007 was tested in the mouse mammary fat-pad model, which is a pre-clinical approach used to study primary breast cancer treatments. Mice were treated with Zeta-BC-007 and compared to tamoxifen and abemaciclib treatment. In addition, alternative LNP formulations were created that contained abemaciclib with or without N-allyl noroxymorphone. Results demonstrate that Zeta-BC-007 treatment produced superior tumoricidal effects that increased survival after a single IT injection relative to mice treated with systemic therapies. Importantly, show that other hydrophobic drugs, such as abemaciclib, can be incorporated into the lipid nanoparticles and that there is potential for these hydrophobic targeted therapies to be administered via IT as a means of increasing bioavailability and decreasing TEAEs due to off-target drug effects.

Methods

Drug Formulations: The NME – lanthanum (III) tri 9,10-diacetoxy octadecanoate – was developed internally and incorporated with the lipid-like hydrogel emulsion, which contains alginate and linoleic acid. All other drugs were obtained commercially and added to the NME through dissolution. The NME is an intrinsic component of the LPNs (≤ 200-nm) while the drug components were loaded prior to incorporation into the lipid-like hydrogel emulsion. MCF7 cells treated with abemaciclib and N-allyl noroxymorphone were assessed for cell proliferation using the MTT assay and.

Mouse Mammary Fat Pad Study: Female BALB/c mice were inoculated with MCF7 HR$^+$/HER2$^-$ breast cancer cells (N=142 total; N=8/group) and treated when tumors were palpable 150-mm^3 approximately 2-weeks later. Mice were sorted into the following groups: **1) Group 1**: Control – No Treatment; **2) Group 2**: Tamoxifen (1-mg/ mL/ 72-hours) via oral gavage; **3) Group 3**: Abemaciclib (125-mg/ mL/ 72-hours) via oral gavage; **4) Group 4**: Zeta-BC-007 with the NME + 4-mM N-allyl noroxymorphone; **5) Group 5**: Zeta-BC-007 with the NME + 0.625-mg/mL of abemaciclib; and **7) Group 6**: Zeta-BC-007 with the NME + 4-mM N-allyl noroxymorphone + 0.625-mg/mL of abemaciclib. Tumor volumes were measured every 72-hours for 60-days. During necropsy, the excised tumors were grossly evaluated for the relative tumor area versus necrotic and fat tissue within the total tumor mass. H&E histology was then evaluated with histomorphology (e.g., number of mitotic profiles). Terminal blood samples were collected and assessed with standard clinical chemistry and hematology panels.

Statistical Analysis: Tumor volume were analyzed using Malthusian and Gompertz exponential growth models developed using non-linear least-squares regression to estimate the tumor growth doubling time (Malthusian) and the maximum tumor volume (Gompertz). Regression lines were assessed for statistical differences between groups using an Extra-Sum-of-Squares F-test to determine if groups were statistically different or should be modeled using a single regression line (α=0.05). Clinical chemistry, hematology, and histology were assessed with one-way ANOVA for differences between treatment groups.

Results

In vitro, a significant dose-dependent decrease in cell number was seen for the MCF7 cells through 72-hours for N-allyl noroxymorphone and abemaciclib (p<0.0001) (**Figures 1**). N-allyl noroxymorphone (0.25-mM) + abemaciclib resulted in a significant decrease in cell number (p<0.0001) that was synergistic.

Results

Regression lines (**Figure 2**) were statistically different between groups (p<0.0001). Tamoxifen (**Group 2**) and abemaciclib (**Group 3**) decreased tumor volume (**Table II**) 33.20% and 40.29% vs **Group 1** (Control – No Treatment). In **Group 4** (Zeta-BC-007: NME + 4-mM N-allyl noroxymorphone) tumor volume decreased 65.76% while tumor volume decreased 64.16% in **Group 5** (Zeta-BC-007: NME + 0.625-mg/mL abemaciclib) and 77.05% in **Group 6** (Zeta-BC-007: NME + 4-mM N-allyl noroxymorphone + 0.625-mg/mL abemaciclib).

Table II: Changes in Maximum Tumor Volume (Compared to Tumor Doubling Time in Days Malthusian)

Non-Linear Analysis Models	Group 1 (control – No Treatment)	Group 2 (tamoxifen)	Group 3 (abemaciclib)	Group 4 (Zeta-BC-007: NME + N-allyl noroxymorphone)	Group 5 (Zeta-BC-007: NME + abemaciclib)	Group 6 (Zeta-BC-007: NME + N-allyl noroxymorphone + abemaciclib)
Gompertz Analysis	Maximum Tumor Volume (mm^3)	255.4	170.6	152.5	87.48	90.54	58.65
	% Change in Maximum Tumor Volume		33.20%	40.29%	65.76%	64.16%	77.05%
Malthusian Analysis	Tumor Doubling Time (days)	9.06	10.44	10.94	13.53	13.22	16.09
	% Change in Tumor Doubling Time		15.31%	13.29%	40.60%	36.93%	56.53%

The tumor growth doubling time increased (**Table II**), which relates to survival and tumor volume. By day 60, all mice in the Control – No Treatment (**Group 1**), tamoxifen (**Group 2**), and abemaciclib (**Group 3**) groups were dead (**Figure 2**). A significant decrease in relative tumor areas (**Figure 3**) was observed while Histomorphometric analysis demonstrated a decrease in the number of mitotic cells (**Figure 4**). Clinical chemistry showed a significant increase in Aspartate Aminotransferase (**Figure 5**) in the tamoxifen and abemaciclib groups, consistent with clinical results [**1-5**]. Importantly, an increase in the numbers of circulating monocytes (**Figure 6**), although this increase was not significantly different it does correspond to increased macrophages seen in the histology.

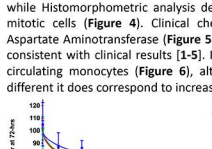


Figure 1: Treatment effects on proliferation for three tumor cell lines with abemaciclib or the abemaciclib + 0.25-mM allyl noroxymorphone. We tested MCF7 (HR+), BT474 (HER2+), BT20 (triple negative) cell lines. Abemaciclib treatment alone was not as effective as treatment with abemaciclib + N-allyl noroxymorphone. All regression lines were significantly different from one another (p<0.0001). The IC_{50} values for the following cell lines decreased after treatment with abemaciclib + 0.25-mM N-allyl noroxymorphone: MCF7 cells IC_{50} decreased 52.8% from 35.8-mM to 16.9-mM; BT474 cell IC_{50} decreased 88.1% from 18.2-nM to 2.2-nM ; and BT20 cells IC_{50} decreased 37.9% from 14.8-nM to 9.2-nM. These changes corresponded with increased cell death. Previous work showed that the IC50 for the N-allyl noroxymorphone was 2.84-mM for the MCF7 cells, 1.06-mM for the BT474 cells, and 1.04-mM for the BT20 cells. While further work is needed, these results suggest that CDK4/6 inhibitor/ N-allyl noroxymorphone combination therapy is synergistic and not additive since the 0.25-mM N-allyl noroxymorphone dose used is 11.36-fold less for the IC_{50} in MCF7 cells, 4.24-fold for the IC_{50} in BT474 cells and 4.16-fold for the IC_{50} in BT20 cells.

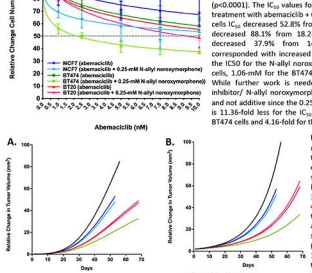


Figure 2: (**A**) Gompertz & (**B**) Malthusian non-linear regression analyses showed that treatment groups were significantly different (p<0.0001). Gompertz analysis showed that maximum tumor volumes (**Table II**) decreased in **Group 2** and **Group 3**, 33.20% and 40.29%, vs **Group 1**. In **Group 4** tumor volume decreased 65.76% while tumor volume decreased 64.16% in **Group 5** and 77.05% in **Group 6**. Malthusian analysis showed that tumor growth doubling time (**Table II**) increased in **Group 2** and **Group 3**, 10.12% and 13.23%, vs **Group 1**. In **Group 4** the tumor growth doubling time increased 40.03%, 36.82% in **Group 5**, and 66.53% in **Group 6**.

Conclusion

Treatment of breast cancer tumors via IT is a novel therapeutic approach that promises to increase drug bioavailability while eliminating negative off-target effects. **Importantly, IT can be used alongside existing systemic therapies**. Abemaciclib and N-allyl noroxymorphone appear to have synergistic treatment effects while the NME also induced significant cytotoxicity. Zeta-BC-007 showed significant reductions in tumor volume via increased necrosis that relates to increased cytotoxicity in the tumor. This increase tumoricidal effect from Zeta-BC-007 led to increased survival. Further work is needed; however, these initial results point to a new treatment paradigm.

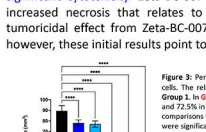


Figure 3: Percent tumor volume measured in mice inoculated with the MCF7 breast cancer tumor cells. The relative means tumor volume decreased in **Group 2** and **Group 3**, 12.7% and 13.9%, vs **Group 1**. In **Group 4** tumor volume decreased 75.3% while tumor volume decreased 76.6% in **Group 5** and 72.5% in **Group 6**. A one-way ANOVA was used to assess tumor volumes, with Dunnet's multiple comparisons test was used to test between-group significant differences (α=0.05). The treated groups were significantly different when compared to **Group 1** (Control – No Treatment group) mice (******** = p<0.0001). There was no significant difference between **Group 2** (tamoxifen) and **Group 3** (abemaciclib). Further, there was no significant difference between the Zeta-BC-007 treatment groups (**Group 4**, **Group 5**, and **Group 6**).

Figure 4: Mitotic cells (i.e., those that are actively going through cell division) were assessed with histomorphometry. The means mitotic cell number decreased in **Group 2** and **Group 3**, 78.2% and 57.8%, vs **Group 1**. In **Group 4** mitotic cell number decreased 83.5% while mitotic cell number decreased 69.9% in **Group 5** and 91.7% in **Group 6**. A one-way ANOVA was used to assess mitotic cell number, with Dunnet's multiple comparisons test was used to test between-group significant differences (α=0.05). The treated groups were significantly different when compared to **Group 1** (Control – No Treatment group) mice (******** = p<0.0001). There was no significant difference between **Group 2** (tamoxifen) and **Group 3** (abemaciclib). Further, there was no significant difference between the Zeta-BC-007 treatment groups (**Group 4**, **Group 5**, and **Group 6**).

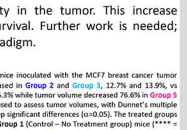


Figure 5

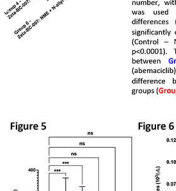


Figure 6

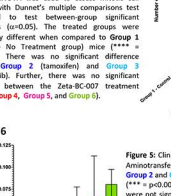


Figure 5: Clinical chemistry identified that Aspartate Aminotransferase was significantly elevated in **Group 2** and **Group 3**, 96.7% and 107.8%, vs **Group 1** (******* = p<0.0002). Aspartate Aminotransferase levels were not significant different between Group 1 and **Group 4**, **Group 5**, and **Group 6** (NS = not significant).

Figure 6: Hematology assessment identified that the numbers of circulating monocytes was increased in the three Zeta-BC-007 treatment groups, but these differences were not significant. Some of the samples were lost to coagulation. As such, we may not have been able to detect significant differences. Further work will be needed to determine if this outcome is biologically meaningful.

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2. Charles River Labs Clinical Research Organization)

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