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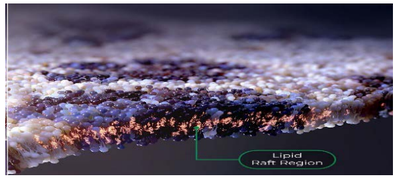
CLR 2000045, a lipid raft targeted phospholipid-drug conjugate, shows potent activity against multiple breast cancer models including triple negative breast cancer

Longcor J, Hoover R, Pinchuk A
Cellectar Biosciences, Inc.

INTRODUCTION

- Lipid rafts (LR) are cell surface microdomains composed of cholesterol, sphingolipids, glycophospholipid and signaling receptors. In normal tissue the presence of LR is limited and transient (~2 nanoseconds). In tumors, they have increased presence and are stabilized (up to 10 days).



- LR have been demonstrated to be highly abundant on nearly all tumor types and 100% of individual cancer cells tested. These features combined with LR providing rapid internalization of phospholipid drug conjugates, makes them an ideal target.

- CLR 2000045 is a phospholipid drug conjugate (PDC) composed of a uniquely designed phospholipid ether conjugated to a novel combretastatin A (CBA) analogue via a cleavable linker. CBAs are potent cytotoxins that inhibit tubulin polymerization within the tumor cell as well as a demonstrated ability to disrupt the local vasculature around/within a tumor.

AIM OF THIS STUDY

Characterization of CLR 2000045's *in vitro* uptake and cytotoxicity in multiple breast cancer cell lines; *in vivo* efficacy and survival benefit in multiple animal models utilizing different types of breast cancer (triple negative and hormone responsive adenocarcinoma).

Materials & Methods

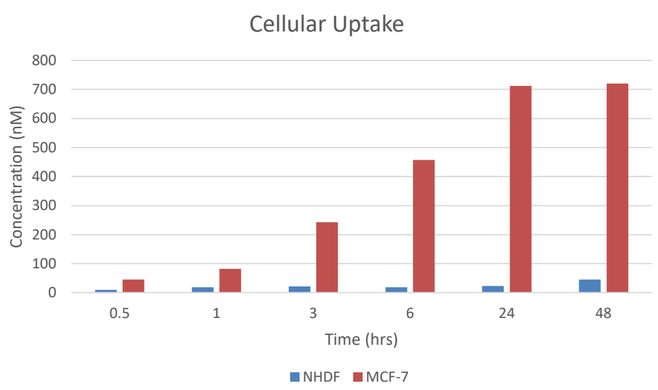
In vitro uptake was assessed using MCF-7 breast cancer cell line and normal human dermal fibroblasts (NHDF) cells and measured via LC/MS/MS. Cells were incubated with 1uM of drug and reported values were the average of triplicate assessments. *In vitro* cytotoxicity was determined by Cell Titer-Glo® assay using MCF-7 breast cancer cells and Hs578T triple negative breast cancer cells.

In an efficacy screening model using chicken embryos, 72uM of CLR 2000045 was administered to determine efficacy against MCF-7 tumors and compared against vehicle control and paclitaxel positive control at 50uM.

In vivo efficacy was further assessed in R2G2 mice bearing HCC70 triple negative breast cancer (TNBC) xenografts. Three doses (1mg/kg given once, twice or 3 times per week for 2 weeks of CLR 2000045 were assessed. Each group contained 10 mice. Tumor volume was monitored for efficacy and body weight for tolerability. Survival was also monitored.

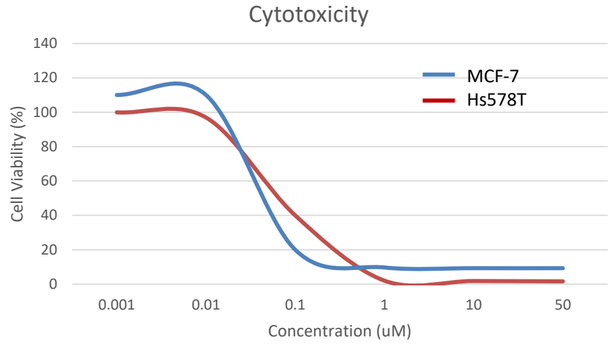
Results

Figure 1: *In vitro* uptake in MCF-7 and NHDF of CLR 2000045



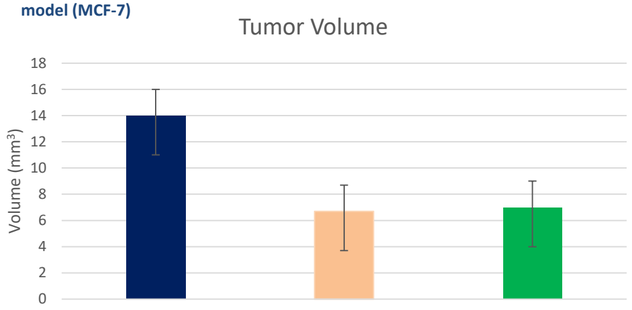
CLR 2000045 shows significant uptake in tumor cells with minimal uptake in normal tissue. Release of the warhead showed approximately 50% release at each timepoint. Between 24 and 48 hours a steady state between uptake and release of the warhead was achieved.

Figure 2: *In vitro* cytotoxicity of CLR 2000045 in breast cancer



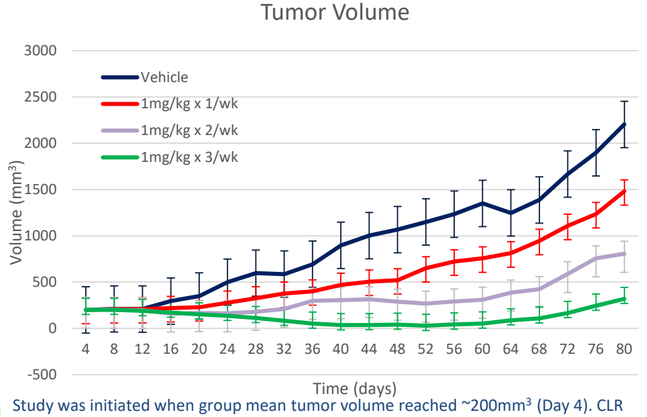
CLR 2000045 shows excellent activity and potency against two breast cancer cell lines (MCF-7 and Hs578T) with IC50s 76 and 51nM, respectively. The molecule also demonstrated activity against several other solid tumors, including lung cancer, melanoma and colorectal cancer.

Figure 3: *In vivo* antitumor activity in chicken embryo chorioallantoic membrane model (MCF-7)



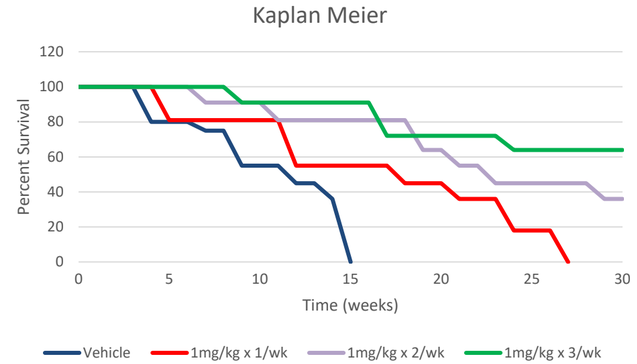
Fertilized White Leghorn chicken eggs (20/dose group) were incubated at 37.5° C for 9 days. MCF-7 cells were cultured under standard conditions prior to implanting. An inoculum of 3.10^6 MCF-7 cells were added to the chorioallantoic membrane on day 10. Eggs were then randomized to treatment groups and treated 4 times (day 11, 13, 15 and 17) under the following conditions; vehicle, paclitaxel 50uM per dose, and CLR 2000045 72uM per dose. CLR 2000045 similar activity to paclitaxel in this screening model.

Figure 4: *In vivo* antitumor efficacy in implanted TNBC (HCC70) xenograft model



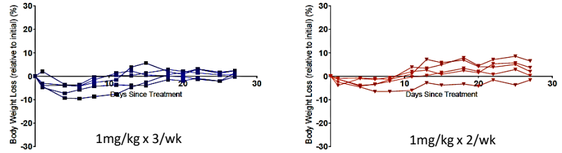
Study was initiated when group mean tumor volume reached ~200mm³ (Day 4). CLR 2000045 was dosed IV at the following doses 1mg/kg on either day 5 and 12 or day 5, 8, 12 and 15 or day 5, 7, 9, 12, 14, and 16. CLR 2000045 demonstrated a dose response reduction in tumor volume from dose group 1 to dose group 3 (3 times per week for 2 weeks) and at the highest dose tested showed near 100% eradication of the tumor. The 2 highest dose groups showed statistically significant reduction in tumor volume as compared to the vehicle control ($p \leq 0.05$ and $p \leq 0.01$ respectively).

Figure 5: Kaplan-Meier survival curve in TNBC (HCC70) mouse xenograft model



Kaplan-Meier curve shows that treatment with CLR 2000045 at 1mg/kg three times per week for 2 weeks resulted in significant increase in survival as compared to vehicle and 1 time per week dosing,($p \leq 0.001$, $p \leq 0.05$) respectively. 1mg/kg twice a week for two weeks resulted in a significant increase as compared to vehicle ($p \leq 0.05$).

Figure 6: Changes in body weight post treatment (HCC70) mouse xenograft model



CONCLUSIONS

- As with other PDCs tested (data not shown), CLR 2000045 demonstrates significant uptake and release of payload (20 -40% of exposed drug) in tumor cell lines while minimal uptake occurs in normal cells.
- CLR 2000045 shows potent *in vitro* activity against multiple breast cancer cell lines.
- CLR 2000045 demonstrated potent *in vivo* activity against a triple negative breast cancer model (HCC70) and a metastatic adenocarcinoma breast cancer model (MCF-7).
- CLR 2000045 provided a statistically significant survival benefit in the TNBC (HCC70) model and the two highest doses were shown to be well tolerated as measured by body weight loss.
- Together these data demonstrate the potent in vitro and in vivo activity of CLR 2000045 against a variety of breast cancer cell lines and animal models and warrants the continued development of this PDC.