#476

CLR 180099, a lipid raft targeted phospholipid-drug conjugate, shows potent improved safety and efficacy against colorectal tumors

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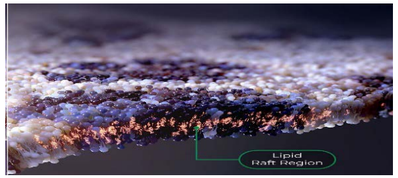
INTRODUCTION

- 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 all cancerous cells tested. These features combined with LR providing rapid internalization of phospholipid drug conjugates, makes them an ideal target.
- CLR 180099 is a phospholipid drug conjugate (PDC) composed of a uniquely designed phospholipid ether conjugated to a flavagline (FLV) analogue via a cleavable linker. FLVs are potent cytotoxins that inhibit translation, cell cycle progression and induce apoptosis.

AIM OF THIS STUDY

Characterization of the *in vitro* uptake, release and cytotoxicity, *in vivo* efficacy, maximum tolerated dose (MTD) and pharmacokinetics (PK) of CLR 180099.

Materials & Methods

In vitro uptake and release was assessed using A549 tumor 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.

CLR 180099 was administered intravenously (IV) to healthy C57BL/6 mice to determine the maximum tolerated dose (MTD) as compared to the FLV molecule alone. Each group contained 5 mice.

In vivo efficacy was assessed in athymic nude mice bearing HCT 116 xenografts. Two doses (2mg/kg given 2 times or 2 mg/kg given 3 times) of CLR 180099 were assessed. Each group contained 10 mice. Tumor volume was monitored for efficacy and body weight for tolerability.

Total conjugated CLR 180099 and free FLV were determined via mass spectrometry.

Results

Figure 2: *In vitro* uptake in A549 and NHDF of CLR 180099A and CLR 180099B

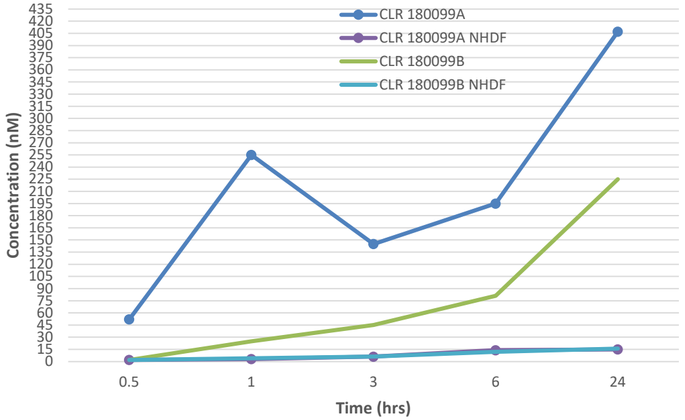


Figure 3: *In vitro* Release of Payload in A549

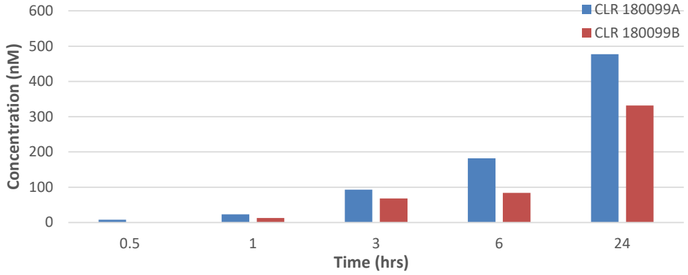
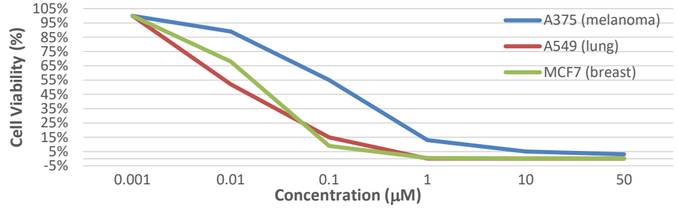
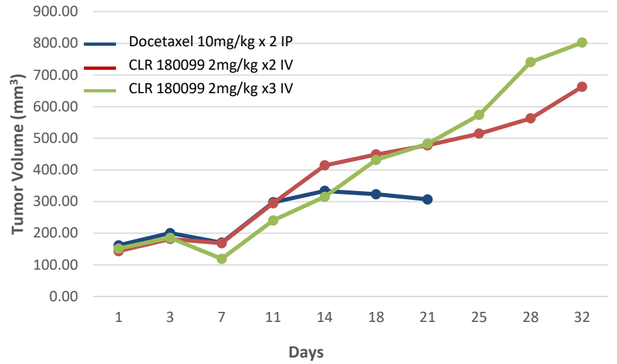


Figure 4: *In vitro* cytotoxicity of CLR 180099A in lung cancer, breast cancer and melanoma



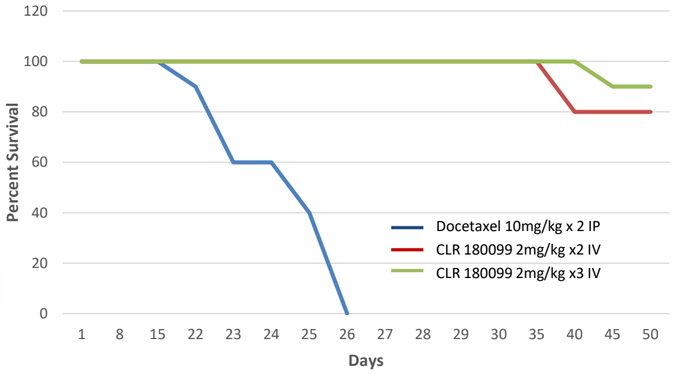
CLR 180099 shows excellent activity and potency against breast cancer and lung cancer with IC50s of 0.024 and 0.011, respectively. The molecule also demonstrated activity against several other solid tumors, including melanoma and colorectal cancer.

Figure 5: *In vivo* antitumor efficacy in implanted colorectal cancer xenograft model



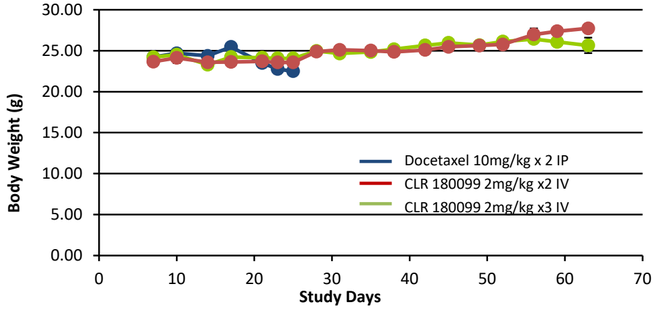
Study was initiated when group mean tumor volume reached ~120mm³ (Day 1). CLR 180099 was dosed IV at 2mg/kg on either day 1 and 4 or day 1, 3 and 5. Docetaxel was dosed at 10mg/kg on day 1 and 4. CLR 180099 demonstrated similar or better reduction in tumor volume than docetaxel and demonstrated dose dependent effect. Docetaxel arm experienced multiple deaths starting day 18 and ending day 26.

Figure 6: Kaplan-Meier survival curve in colorectal cancer xenograft model



Kaplan-Meier curve shows that treatment with CLR 180099 at 2mg/kg day 1 and 4 or day 1, 3 and 5 resulted in significant increase in survival as compared to docetaxel (log-rank test, $p \leq 0.001$).

Figure 7: *In vivo* tolerability



As measured by body weight loss, all mice treated with CLR 180099 (both doses) demonstrated normal body weight growth through-out the study.

Table 1: *In vivo* tolerability

(mg/kg)	0.1	0.5	1	5	10
FLV	5	0	0	0	0
CLR 180099A	5	5	5	5	5
CLR 180099B	5	5	5	5	5

Five mice per group were dosed at each dose level. Both PDCs were tolerated up to dose of 10 mg/kg with all mice alive and showing no end organ toxicities. The payload alone was not tolerated at doses above 0.5mg/kg (all mice died at 0.5mg/kg).

CONCLUSIONS

- CLR 180099 demonstrates significant uptake and release of payload (20 -40% of exposed drug) in tumor cell lines while minimal uptake occurs in normal cells.
- CLR 180099 shows potent *in vitro* activity against various solid tumors, including lung cancer (A549), breast cancer (MCF7), and melanoma (A375), as well as other tumor types (data not shown).
- *In vivo* two or three doses of CLR 180099 showed similar or better activity to docetaxel in colorectal cancer. Additionally, CLR 180099 demonstrated significantly improved survival benefit at both doses as compared to docetaxel.
- Tolerability assessment demonstrated that CLR 180099 was well tolerated in both tumor bearing and normal animals. The FLV payload was toxic in both normal and tumor bearing mice.
- CLR 180099 showed no toxic affects as compared to the FLV analogue payload alone demonstrating that this payload would benefit from targeted delivery with a phospholipid ether (PLE).
- Together these data demonstrate the potent in vitro and in vivo activity of CLR 180099 against a series of solid tumors and warrants the continued development of this PDC.