A novel liposomal Irinotecan (CPT-11) formulation with increased therapeutic potential to treat colorectal cancer

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Background & Hypothesis

The use of irinotecan (CPT-11) as first-line chemotherapy drug is associated with side effects like gastrointestinal (GI) toxicity which limits its use, especially in metastatic colorectal cancer (mCRC) [1].

Our lab previously described a liposomal CPT-11 formulation: Irinophore C™ (IrC™), which significantly reduced gastrointestinal (GI) toxicity in a validated rat model compared to free CPT-11. More importantly IrC™ demonstrated improved efficacy in mCRC xenograft models [2].

The preparation method of IrC™ relied on Cu(II) ion that can interact with CPT-11 and retain CPT-11 in its active lactone form (Fig 1a) in the liposomes. However, the method used to prepare IrC™ involved a divalent metal ionophore (A23187) to generate a pH gradient that promoted CPT-11 loading. A23187 addition resulted in 80% loss of the Cu(II) ion from the liposome (Fig 1b) [2].

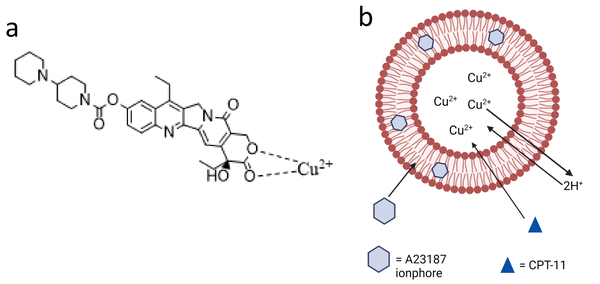

Figure 1. Interaction site between CPT-11 and Cu (II) ion inside liposomes (a) and the preparation method used in IrC™ (b). A23187 is incubated with the liposomes and it incorporates into the lipid bilayer. There it can transport one Cu(II) ion out for two protons to establish pH gradient which can engender CPT-11 encapsulation.

An improved liposomal CPT-11 formulation (**Irinosome High** C) is described here without the use of A23187 to encapsulate CPT-11. This new method resulted in minimal Cu(II) ion loss.

It is hypothesized that this formulation would achieve sustained release rate of CPT-11 from liposomes; extending the circulation of CPT-11 in the blood following intravenous injection. This should improve the formulation's therapeutic potential.

1. Fujita, K. et al; **Irinotecan, a key chemotherapeutic drug for metastatic colorectal cancer**. World journal of gastroenterology *2015*, ***21***(43), 12234–12248.
2. Euan C. Ramsay et al.; **Irinophore C: A Liposome Formulation of Irinotecan with Substantially Improved Therapeutic Efficacy against a Panel of Human Xenograft Tumors**. Clin Cancer Res 2008, **14** (4): 1208–1217.

Methods & Results

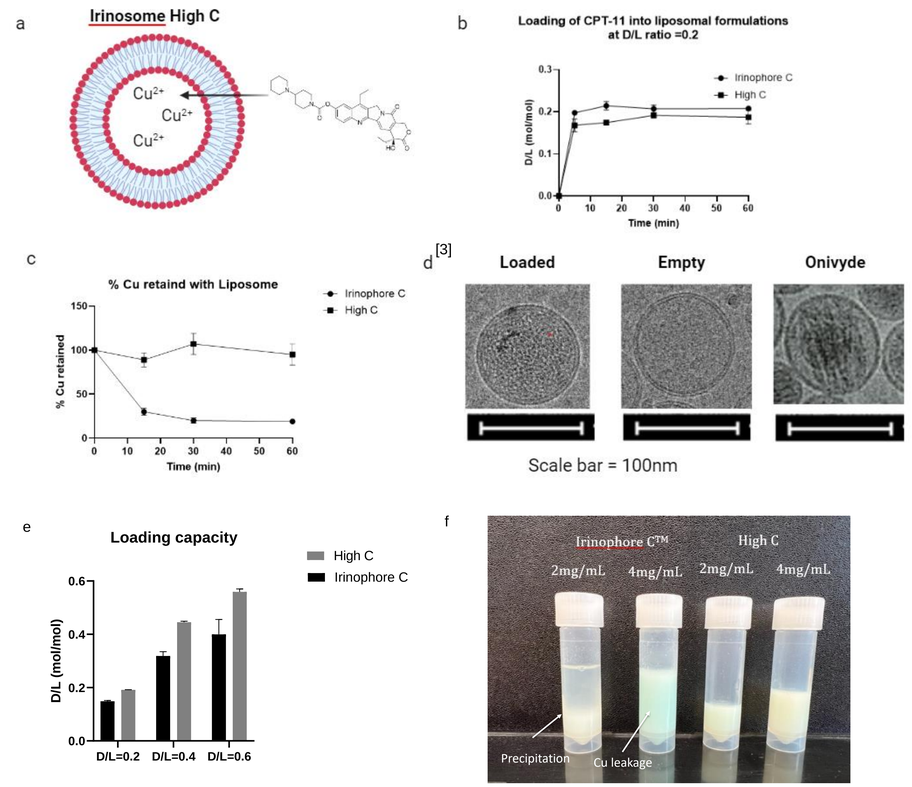

Figure 2. Preparation method used in Irinosome High C formulation candidates (a) The internal acid (AH^+) generates a pH gradient which alone can promote CPT-11 loading. **Loading curve comparing Irinophore C and High C formulation candidates (b).** The loading rate of two formulations are fast and very similar at 50ºC. **The entrapped Cu(II) ion was retained during loading for Irinosome High C (c). Cryo-Transmission Electron Microscopy (TEM) image of Irinosome High C (d).** The Cryo-TEM image shows electron dense pattern inside the liposomes suggesting CPT-11 precipitation. The Onivyde Cryo-TEM image was reproduced from literature [3]. **The Irinosome High C has higher loading capacity than Irinophore C (e). High C formulation is more stable when stored at 4ºC compared to Irinophore C over 3 months (f)**

	$t_{1/2}$ (h)
Irinophore C	5.5 ±0.7
High C	5.5 ±0.3
Free CPT-11	~ 30mins

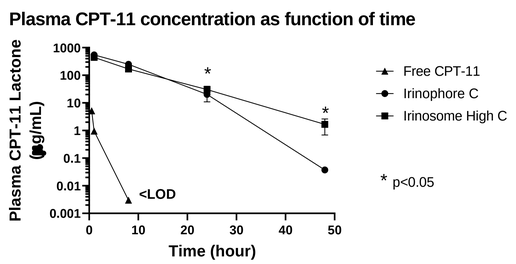

Figure 3. Pharmacokinetic study comparing CPT-11 elimination following i.v injection of Irinophore C, High C formulations and free CPT-11. Mice (n=4 at each time point) were injected i.v. with either the test articles and at each time point the mice were sacrificed through cardiac puncture to collect blood. High C formulation has higher plasma CPT-11 concentration at 24 and 48h time point compared to Irinophore C

3. Wenqian Yang et al.; **The influence of trapping agents on the antitumor efficacy of irinotecan liposomes: head-to-head comparison of ammonium sulfate, sulfobutylether-β-cyclodextrin and sucrose octasulfate.** Biomater. Sci. 2019, **7**: 419-428

Methods & Results

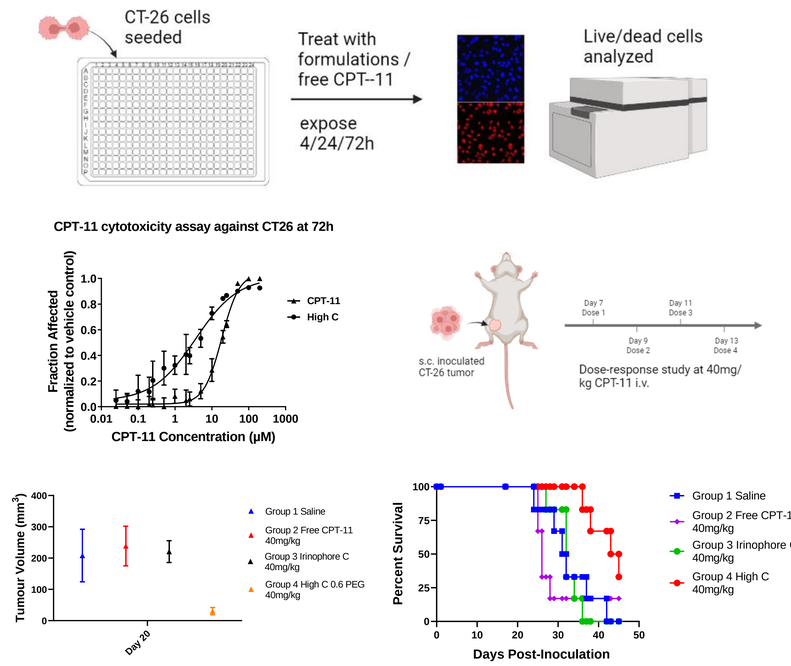

Figure 4. *In vitro* cytotoxicity assays comparing Irinosome High C formulation candidates and native CPT-11. CT-26 cells were seeded in 384-well plate and exposed to different formulations or free CPT-11. IC_{50} value of High C is significantly lower than free CPT-11 72h exposure time. **Efficacy study result of Irinosome High C formulation, Irinophore C and native CPT-11 in subcutaneous inoculated CT-26 immunocompetent BALB/c mice.** Delayed tumor growth was observed in High C treated group compared to Irinophore C, free CPT-11 and control. High C treated group also has improved Kaplan-Meier survival curve.

Conclusion

This work describes a novel preparation of liposomal CPT-11. **Irinosome High C** to IrC™, the new formulation has demonstrated:

- Higher loading capacity and storage stability
- Improved CPT-11 retention in vivo
- Superior activity than native CPT-11 *in vitro* against CT26 CRC cell lines
- Superior therapeutic activity in the CT26 s.c. syngeneic mice model.

Acknowledgement

The authors would like to thank the funding sources indicated below and the animal care team at PharmaCore (Investigational Drug Program) and the Animal Resource Facility at BCCRI.