Phospholipid ether delivery vehicle shows specificity for a broad range of tumor cells and provides a novel and improved approach for targeted therapy.

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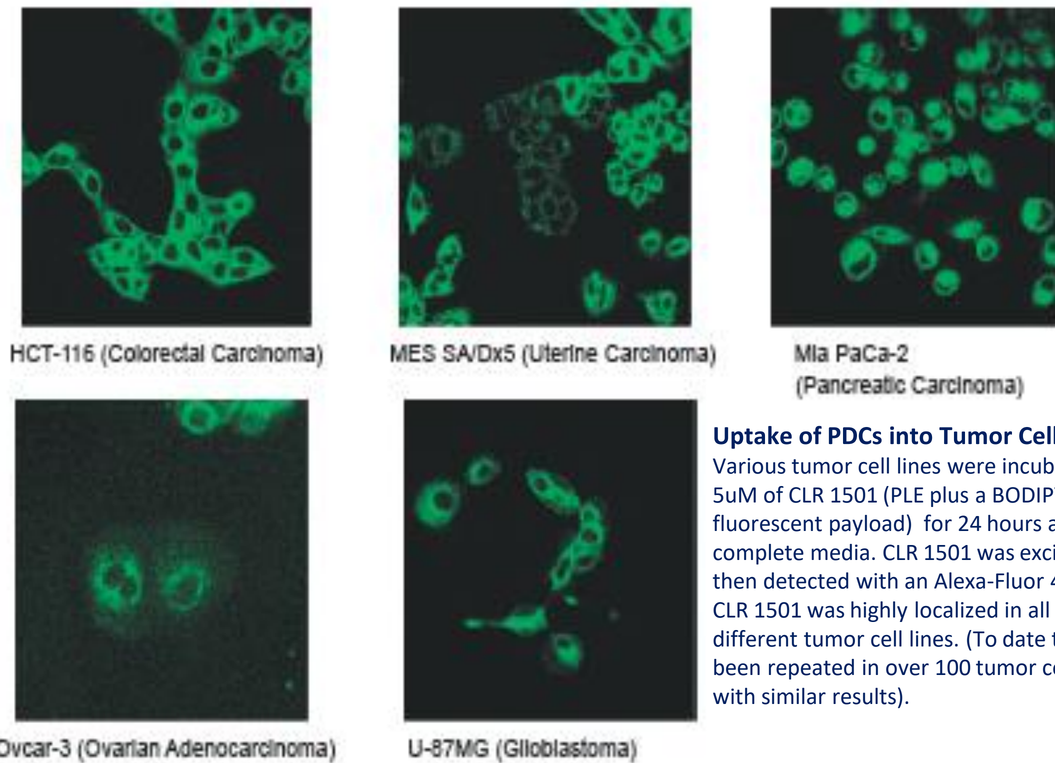
ABSTRACT

The majority of anticancer drugs in clinical use have their utility limited by their toxicity to all proliferating cells and/or the inability to exert their effect on all of the tumor cells. Novel agents continue to be developed with unique mechanisms of action meant to provide increased targeting, however, many of these compounds still lack absolute tumor selectivity and continue to be limited in their therapeutic utilization due to off-target effects. Antibody drug conjugates (ADCs) have been designed to bind to specific epitopes on the surface of tumor cells and have offered an alternative method to target tumor cells in an effort to reduce associated toxicities.[1] Although highly selective, very few antibody drug conjugates are therapeutically useful since they only achieve modest cellular uptake (<1% of infused drug) and limited cell killing activity. Here we describe the development of a novel targeting platform using phospholipid ether (PLE) molecules to provide tumor cell specific targeting. These PLE molecules provide both a novel mechanism to target tumor cells versus normal tissue and a cytosolic release of the cytotoxic payload.

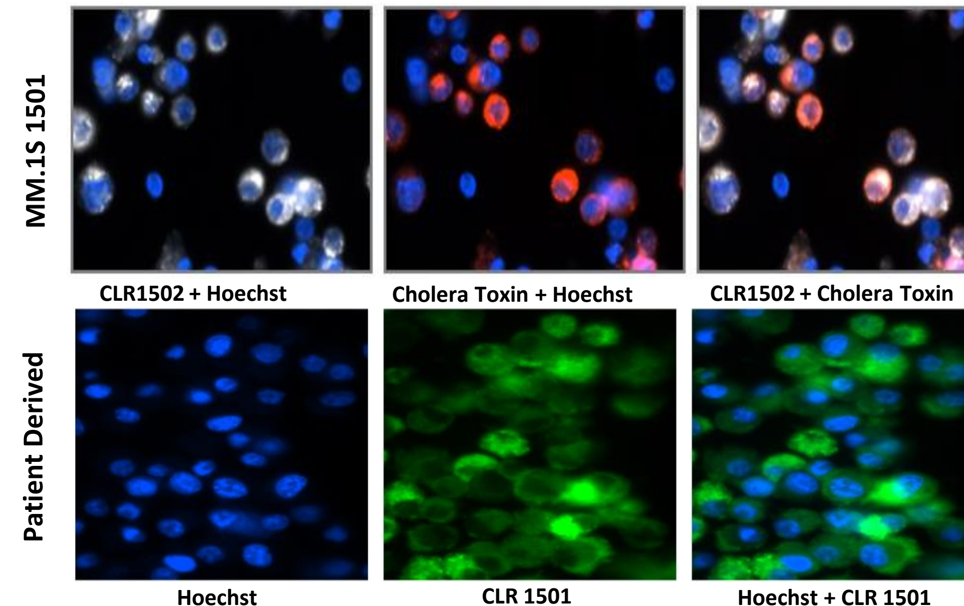
BACKGROUND

Novel agents continue to be developed with unique mechanisms of action meant to provide increased targeting, however, many of these compounds still lack absolute tumor selectivity and continue to be limited in their therapeutic utilization due to off-target effects. Phospholipid ether (PLE) molecules take advantage of the metabolic shift that tumors cells undergo in order to generate the energy necessary for the rapid cell division. Tumors enhance the utilization of the beta oxidative pathway to convert long chain fatty acids (LCFA) into energy. In order to increase the uptake of LCFA, tumor cells alter the cell membrane forming specialized microdomains known as lipid rafts. These lipid rafts are highly organized regions which are composed of various signaling molecules, sphingolipids, glycosphingolipids and cholesterol.[2] Within tumor cells these regions have become overabundant and stabilized allowing them to be potential tumor specific targets. Cellectar's PLE analogs are LCFA mimetics. The PLEs have undergone extensive structure activity relationship (SAR) analysis related to targeting lipid rafts on tumor cells and have been shown to specifically bind to these regions.[3,4] The PLE molecules provide entry directly into the cytoplasm and transit to the endoplasmic reticulum and mitochondria along the golgi-apparatus-network within the cell cytoplasm.

In vitro Targeting Results (Fluorescent PDCs)

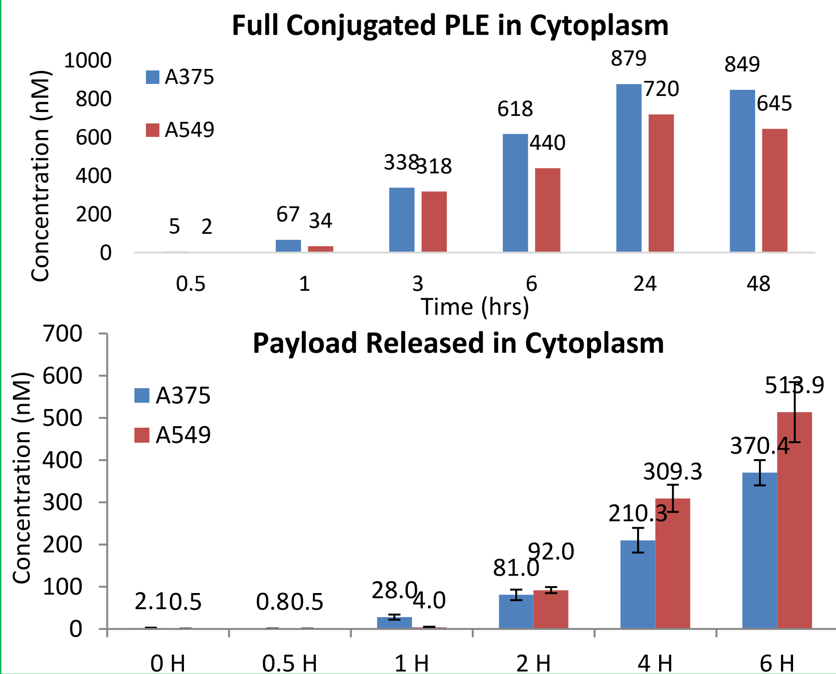


Uptake of PDCs into Tumor Cell Lines: Various tumor cell lines were incubated with 5uM of CLR 1501 (PLE plus a BODIPY (green) fluorescent payload) for 24 hours at 37^{0}C in complete media. CLR 1501 was excited and then detected with an Alexa-Fluor 488 filter. CLR 1501 was highly localized in all the different tumor cell lines. (To date this has been repeated in over 100 tumor cell lines with similar results).



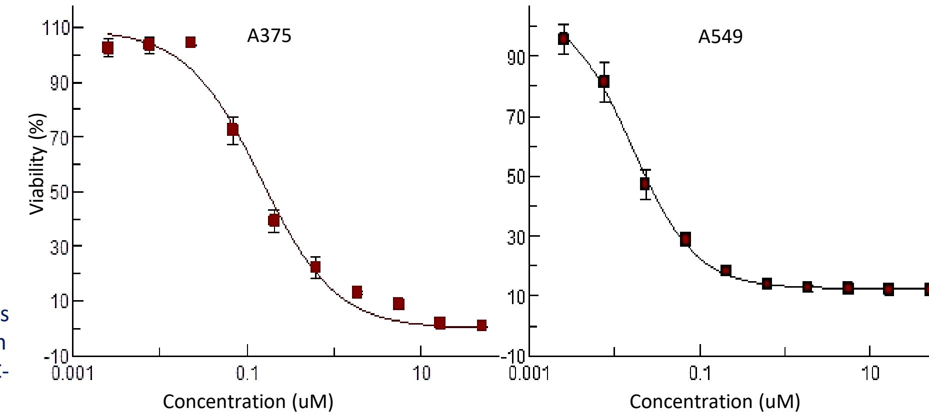
Uptake via Lipid Rafts on Tumor Cells & Primary Tumor Samples: Multiple myeloma cells were incubated with CLR 1502 (near infrared molecule bound to the PLE; white) for 24 hours at 37^{0}C. The next day cells were washed and co-stained with nucleus stain (Hoescht 33342; blue). Using cholera toxin subunit B, they were further stained for the presence of lipid rafts (red). Additionally, patient derived multiple myeloma cells were stained with Hoescht 33342 and incubated with CLR 1501 to demonstrate uptake in patient derived tumor cells.

In vitro Uptake & Release with Cytotoxic Payload



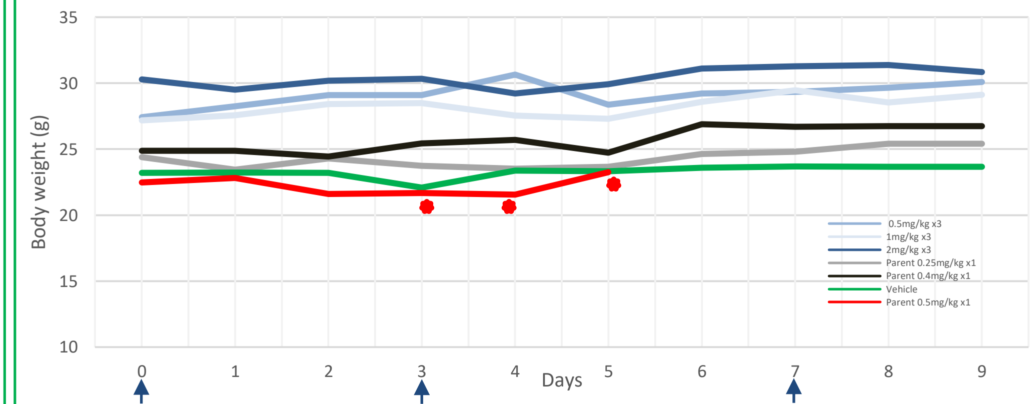
Uptake of PDCs into Tumor Cell Lines: A375 and A549 cell lines were incubated with 2uM of a cytotoxic small molecule PDC with semi-stable linker (PLE plus a small molecule payload (PDC-SM1)) for 48 hours at 37^{0}C in complete media. Uptake of PDC-SM1 was measured by LC/MS/MS. PDC-SM1 demonstrated uptake initiating within 30 mins. 20 – 40% of conjugate exposed to cells was measured in the tumor cell cytoplasm within 24 hrs. PDC-SM2 (same as PDC-SM1 except with a cleavable linker) was then utilized to measure release of payload within tumor cells. Measurable release of the small molecule payload occurred between 1 to 2 hours post incubation. Negligible release of payload occurred in media (<1nM) (data not shown).

In vitro Efficacy with Cytotoxic Payloads



In vitro Efficacy: PDC-SM2 demonstrated sub-micromolar activity (concentration measured based on full conjugate concentration incubated on cells) against melanoma (A375) and lung cancer (A549) cells. PDC-SM2 showed less activitive against melanoma than lung cancer (IC50s 0.131 vs 0.016) but was more potent (0% vs 12% viable cells remaining). PDC-SM2 also showed similar activity and potency against colorectal cancer (HCT-116) cells as lung cancer with no activity against normal fibroblast (data not shown).

In vivo Tolerability with Cytotoxic Payloads



Cytotoxic PDCs Shown to be Tolerated *In vivo*: C57BL/6 mice were dosed in the following manner; PDC-SM2 was dosed on day 0, 3 and 7 at dose levels of 0.5mg/kg, 1.0mg/kg or 2.0mg/kg (blue lines); Payload alone was dosed on day 0 only at 0.25mg/kg, 0.4mg/kg or 0.5mg/kg (grey, black and red lines); vehicle was dosed on day 0, 3 and 7 (green line). PDCs and vehicle control showed no toxicity or adverse events during repeat dosing as measured by changes in weight (no weight loss). Payload doses of 0.25 and 0.4mg/kg were tolerated although there was some toxicity noted to the mice's skin and coat. Payload dose of 0.5mg/kg was not tolerated, two mice died by day 4 following single infusion and all mice were sacrificed on day 5 (red stars).

Compound ID	Human	Mouse
	$t_{1/2}$ (min)	$t_{1/2}$ (min)
PDC-SM2	>400	199
PDC-SM3	>400	>400
Propantheline	54	85

Plasma Stability Assessment: Both PDCs showed good plasma stability in human plasma. PDC-SM2 showed some instability in mouse plasma which could result in some toxicity.

CONCLUSIONS

- Phospholipid ether molecules demonstrate ability to target a wide range of tumors
- PDCs show ability to achieve uptake of 20-40% of exposed drug into tumor cell lines
- PDC uptake has been confirmed to be linked to lipid rafts on tumor cell membrane
- PDCs show release of payload and strong nanomolar activity against tumor cells
- PDCs are shown here to be well tolerated *in vivo*
- PDCs offer a novel and unique approach to targeting small molecules to tumor cells

References

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2. Ando, K. *et al. J. of Pharma & Exp. Thera.* **334(3),** 809- 819
3. Pinchuk, A. N. *et al. J Med Chem.* **49,** 2155-2165
4. Weichert, J. P. *et al. Sci. Transl. Med.* **6**, 240ra75-240ra75