Phospholipid drug conjugates (PDC™) show specificity for a broad range of tumor cells and provides a novel approach for targeted or precision therapy.

**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.1,2

**BACKGROUND**

Conjugation of antibodies to toxic moieties 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.1,2

**In vitro Targeting Results (Fluorescent PDCs)**

Presence of Lipid Rafts on Tumor Cells: Using cholera toxin subunit B (A-D), almost every tumor type that was tested demonstrated high lipid raft content in the cell membrane (over 100 cell lines, fresh patient samples, etc tested to date). In image E, A549 cells were co-cultured with normal fibroblast cells for 48 hours and then stained with cholera toxin subunit B, fixed and methyl green stain (Hoescht 33342). CLR 1501 was excited and detected using Alexa-Fluor 488 using standard fluorescein filters. 60% reduction in uptake of CLR 1501 of lipid rafts in A549 cells as compared to untreated (G). As compared to untreated (G), disruption of the majority of lipid rafts in A549 cells resulted in 60% reduction in uptake of CLR 1501 (H) as compared to untreated (G).

**Disruption of Lipid Rafts Reduced Uptake of PDCs: A549 cells were plated overnight into separate wells. The following day cells were either not treated (A) or treated with methyl (β)-cyclopentadienyl (MCP) which has been shown to selectively disrupt lipid rafts. All cells were then incubated for 24 hours with 5uM of CLR 1501. Disruption of the majority of lipid rafts in A549 cells resulted in 60% reduction in uptake of CLR 1501 (H) as compared to untreated (A).

**In vivo Targeting Results (Fluorescent PDCs)**

PDCs Track to Mitochondria: PDC is grade 4, human prostate adenocarcinoma (PC3) cell lines were cultured overnight on micro slide VI (Ibidi, Verona, WI). The next day, the cells were incubated with 5 µM of CLR 1501 for 24 hours at 37°C in complete media. The next day cells were washed and co-stained with nucleic stain (Hoechst 33342), Mitotracker® (Invitrogen, Carlsbad, CA). The cells were observed using Nikon A1R confocal microscope. CLR 1501 was excited and detected using Alexa-Fluor 488 filter, while nucleus stain and mitochondria marker (Mitotracker®) were excited and detected using DAPI filter and Texas-Red filter, respectively. CLR 1501 was co-localized with mitochondria (L-N).

**Cytotoxic PDCs Provide Targeting and Potentially Improved Therapeutic Index:**

**CONCLUSIONS**

1. Phospholipid ether molecules target tumor cells via lipid rafts
2. PDCs show significant uptake into tumor cells versus normal cells even in co-culture
3. Upon entering the tumor cells, PDCs track to the mitochondria and endoplasmic reticulum
4. In vivo, PDCs both target and rapidly accumulate within the tumor
5. Cytotoxic PDCs provide improved targeting and the potential for improved safety

**References**


**In vitro Efficacy with Cytotoxic Payloads**

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**REFERENCES**