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

Longcor J[1], Banach M[1], Hoover R[1], Longino M[1], Stehle N[1], Pinchuk A[1], Lange K[1], Nair S[1], Larrabee J[1], Wolf M[2], Betzenhauser M[2]

[1]Cellectar Biosciences, [2]AMRI

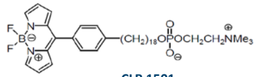
ABSTRACT

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 and limited cell killing activity. Based upon the finding that numerous animal and human tumors contained much higher concentrations of naturally occurring ether lipids than normal tissue[2], it was hypothesized that phospholipid ether (PLE) molecules could provide a novel tumor targeting platform.

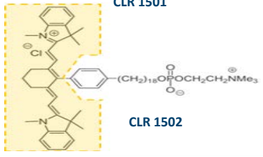
BACKGROUND

Cellectar's PLE analogs have undergone extensive structure activity relationship (SAR) analysis related to targeting tumor cells and tissue distribution.[3, 4] These molecules have been shown to result in increased uptake versus normal tissue. Phospholipid drug conjugates (PDCs) have demonstrated the ability to conjugate a wide range of molecules to them via unique and novel linkers. Uptake experiments have been conducted in over 100 different tumor cells, including fresh human tumor samples.

Structures of PDCs CLR 1501 and CLR 1502: These represent fluorescent versions of our therapeutic PDC molecules. CLR 1501 has the fluorescent moiety, BODIPY, stably linked to the molecule. CLR 1502 utilize a near infrared molecule, IR-775, stably linked to our PLE.

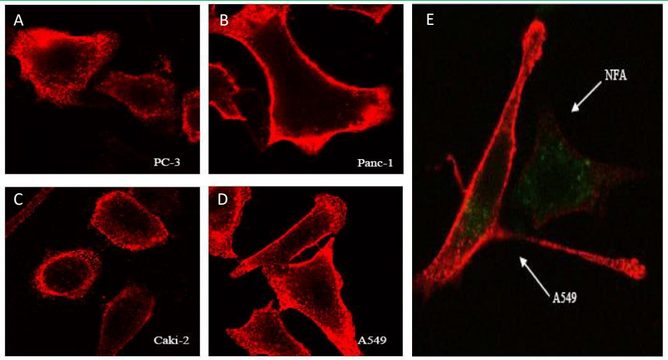


CLR 1501

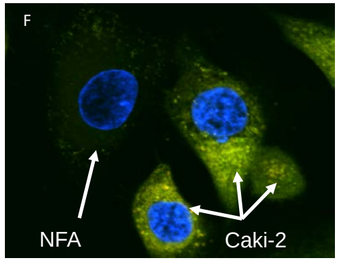


CLR 1502

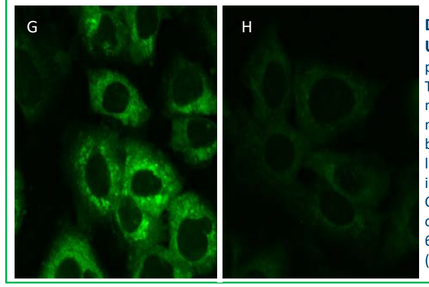
In vitro Targeting Results (Fluorescent PDCs)



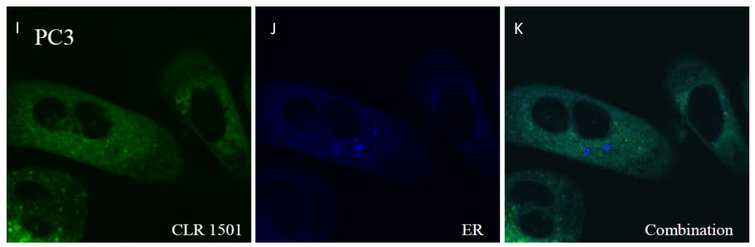
Presence of Lipid Rafts on Tumor Cells: Using cholera toxin subunit B (A-D), almost every tumor type that was tested demonstrated having high lipid raft concentration in the cell membrane (over 100 cell lines, fresh patient samples, etc. tested to date). In image E, A549 cells were co-culture with normal fibroblast cells for 48 hours and then stained with cholera toxin subunit B, fixed with 4% formaldehyde and stained with filipin III for 30 minutes. These results demonstrate that tumor cells possess a higher concentration of lipid rafts than on normal cells.



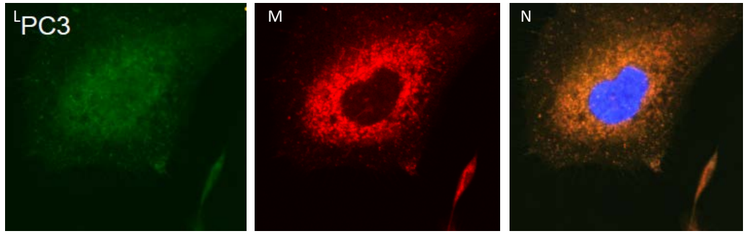
Selective Uptake of PDCs into Tumor Cells: Normal fibroblast and Caki-2 tumor cells (human clear cell renal cell carcinoma) were plated and co-cultured overnight (F). Cells were then incubated with 5uM of CLR 1501 for 24 hours at 37°C in complete media. The next day cells were washed and co-stained with nucleus stain (Hoescht 33342). CLR 1501 was excited and then detected with an Alexa-Fluor 488 filter. CLR 1501 was highly localized in the Caki-2 cells and minimally in the normal fibroblast cells.



Disruption of Lipid Rafts Reduced Uptake of PDCs: A549 cells were plated overnight into separate wells. The following day cells were either not treated (G) or treated with methyl β-cyclodextrin (H) 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 (G).

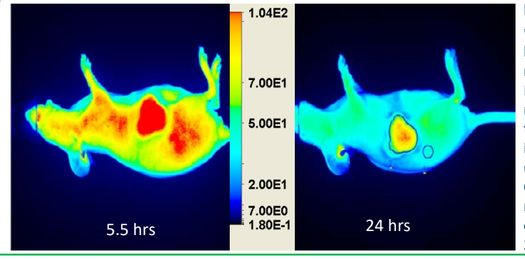


PDCs Track to Endoplasmic Reticulum: Human prostate adenocarcinoma cells (PC3) were plated overnight on the microplate VI (Ibidi, Verona, WI) and then incubated with 5μM of CLR 1501 for 24 hours at 37°C in complete media. After washing, the cells were co-stained with ER-tracker® per protocol and imaged using Nikon A1R confocal light microscope. CLR 1501 and ER were excited and detected using Alexa-Fluor 488 using standard fluorescein filters. CLR 1501 co-localized with ER in malignant (I-K) but not with normal cells (not shown).



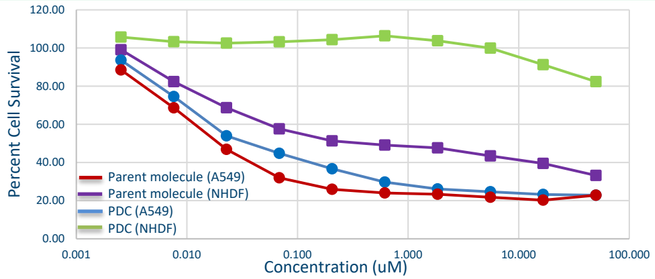
PDCs Track to Mitochondria: PC3 (grade IV, human prostate adenocarcinoma) cell lines were cultured overnight on micro slide VI (Ibidi, Verona, WI). The next day, the cells were incubated with 5 μM of CLR 1501 for 24 hours at 37°C in complete media. The next day after washing with PBS, the cells were co-stained with nucleus stain (Hoechst 33342) and mitochondria marker (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 stain were excited and detected using DAPI filter and Texas-Red filter, respectively. CLR 1501 was co-localized with mitochondria (L-N).

In vivo Targeting Results (Fluorescent PDCs)



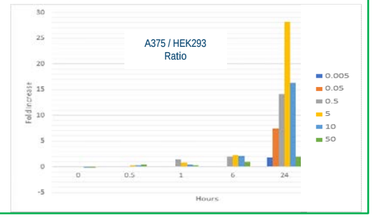
PDCs Provide Targeted Delivery *In vivo:* Colorectal (HCT-116) tumor bearing nude mouse was injected with 1 mg of CLR 1502 and imaged on Pearl Infrared Imaging System. Different color reflects the intensity of CLR 1502 over time. Approximately 5.5 hours post injection, the tumor still shows red color (reflecting the highest distribution of CLR 1502). Within 24 hours, the maximum distribution of CLR 1502 was obtained. Initial targeting noted within 30 minutes (not shown).

In vitro Efficacy with Cytotoxic Payloads



Cytotoxic PDCs Provide Targeting and Potentially Improved Therapeutic Index: A549 (human lung adenocarcinoma) cells and normal human dermal fibroblasts (NHDF) were plated in 96 well dishes overnight. All cells were treated with increasing concentrations of either the parent cytotoxic compound alone or the PDC (parent molecule conjugated to PLE moiety with novel cleavable linker). Parent molecule shows near equal potency to the A549 cells as it does to the NHDF cells. However, the PDC molecules shows selectivity for the A549 cells. The PDC molecules shows almost no effect on NHDF cells until the highest concentrations and near similar potency to the parent molecule in A549 cells. The difference between the cytotoxicity of the PDC molecule for tumor cells and that of the normal cells could result in a potential to improve the parent molecules therapeutic index.

Cytotoxic PDCs Provide Targeting: Evaluating the uptake of PDCs in A375 (human melanoma) and HEK293 (human embryonic kidney) cells, it was shown that the tumor cells possessed anywhere from a 6 to 28 fold increase in PDCs compared to the normal cells within 24 hours of treatment.



CONCLUSIONS

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

References

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2. Synder, F. *et al. Cancer Res.* **28,** 972- 978
3. Pinchuk, A. N. *et al. J Med Chem.* **49,** 2155-2165
4. Weichert, J. P. *et al. Sci. Transl. Med.* **6**, 240ra75-240ra75