Dactolisib-loaded Reconstituted High Density Lipoproteins (rHDL): A Candidate for Glioblastoma Therapy

Trentyn Shaw, **Ethan Berney**; B.Sc., and **Andras Lacko**; PhD

ABSTRACT

Despite substantial chemotherapeutic advances in the 21st century, toxicity remains a prevailing obstacle in cancer treatment. Previously, the Lacko Lab has shown that scavenger receptor B1 (SR-B1) overexpression is a hallmark of several cancers. The natural ligand of this receptor is circulating HDL, whose wildtype action is the receptor-mediated delivery of cholesterol in an apolipoprotein A1-dependent manner (1). In this study, a dual P13K/mTOR inhibitor was incorporated into reconstituted high density lipoprotein (rHDL) nanoparticles, and subsequently tested against a panel of glioblastoma multiforme (GBM) cell lines. The mean diameter of the nanoparticles were 15.7 nm with a standard deviation of 4.5 nm and a polydispersity index of 0.160, and drug concentration of 73.35 uM/mL. These nanoparticles provided an appreciable protective effect against astrocytes while having an IC50 value of 103 nM against GBM line LN229.

INTRODUCTION

Problem: Gliomas are cancers that arise from oncogenic mutations residing in glial cells, which further differentiate into neurons, astrocytes, oligodendrocytes or ependymal cells within the central nervous system (CNS). Primary glioblastoma presents rapidly due to loss of function which abate cell growth; namely, mammalian target of Rapamycin (mTOR) and P13K (Phosphatidylinositol-3-Kinase. The initiation of the PI3K/Akt/mTOR pathway brings about a significant unsettling influence of control of cell development and survival, which at last prompts a focused development advantage, angiogenesis, and treatment resistance (2). The current therapeutic approach to patients that present with GBM is Temozolomide (TMZ), a DNA-alkylating drug, concomitant with radiotherapy (RT). TMZ, however, has enjoyed limited success in combating GBM's fatal nature—although it was shown clinically in 2005 to increase the median survival of patients by 2.5 months[1] (14.6 months with Temozolomide + RT vs. 12.1 months with RT only), minimal advancements toward a better prognosis have been made in the 12 years since. In addition, 49% of patients experienced severe side-effects, suggesting that improvements in the selectivity of the drug delivery system are needed (3).

Purpose: The Lacko Lab has previously shown that scavenger receptor B1 (SR-B1) is overexpressed in many cancers. The protein Apo A1 that is found in HDL molecules is the ligand for the SR-B1 receptor (1). rHDL nanoparticles containing the chemotherapeutic Dactolisib were prepared using cholate dialysis. These nanoparticles were subsequently characterized and tested against a high-expressing SR-B1 GBM cell line, LN229, as well as astrocyte spheroids with the anticipation of mitigating off-target cytotoxicity, as normal tissues, with the exception of the liver, have low native SR-B1 expression relative to cancerous tissue.

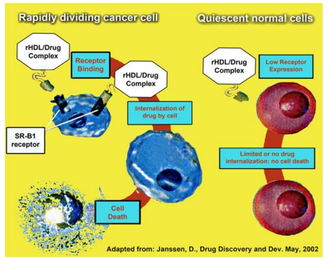
METHOD

- rHDL/Dactolisib nanoparticles were prepared via cholate dialysis as previously described by Lacko et al. (0.50 mg/ml Dactolsisb prep)
- Incorporation percent was calculated using spectrophotometric measurements at ƛ360nm
- Particle characterization was done using phospholipid, cholesterol, and BCA assays
- CCK monolayer assays were performed on astrocytes and GBM cell line LN229, as well as astrocyte spheroids

Nanoparticle Creation ➡Incorporation ➡Characterization ➡CCK

RESULTS

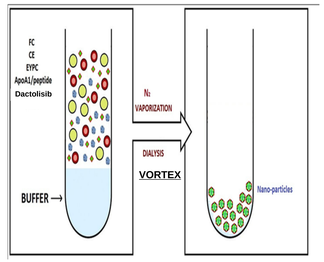
PROPOSED MECHANISIM



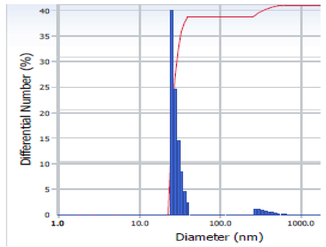
General Overview:

- Drug incorporated nanoparticles are released/injected into cancer cells
- The nanoparticles bind to the SR-B1 receptor in an ApoA1-mediated mechanism
- Receptor mediated delivery of core components
- Via 'Trojan horse' strategy, cell apoptosis is the expected response

PARTICLE SYNTHESIS



DYNAMIC LIGHT SCATTERING



Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	15.7	4.5
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	15.7	4.5

Polydispersity Index (P.I.) : 0.160

Particle size was measured via Dynamic Light Scattering with a Delsa^{tm}Nano

PARTICLE CHARACTERIZATION

Fig. 1

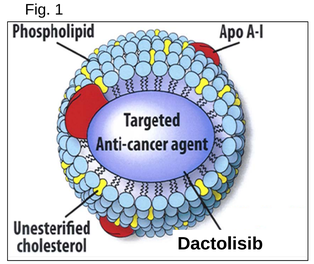


Fig. 2

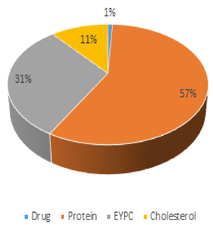
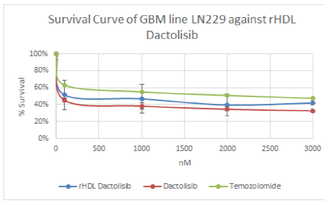
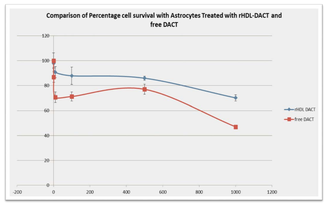


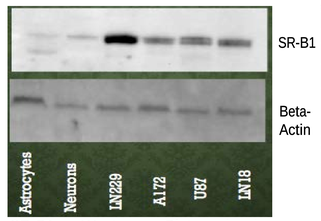
Figure 1 displays the general shape and structure of the lipoprotein nanoparticles after they are constructed. Figure 2 displays the percentages of the different components that made up the nanoparticles that were created in this lab. These percentages were found via BCA/phospholipid/cholesterol assays.

CYTOTOXICITY DATA

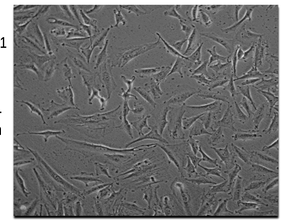




LN229 GBM CELL LINE



SR-B1 Western Blot showing overexpression in GBM line 229 relative to healthy CNS tissue (Astrocytes and Neurons)

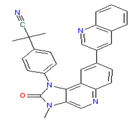


LN229 morphology

DRUG OF STUDY

Dactolisib (NVP-BEZ235)

- XLogP3: 5.2
- "Dual ATP-competitive P13K and mTor inhibitor (4)"



$C_{30}H_{23}N_{5O}$

CONCLUSIONS

In this study, the dual P13K/mTOR inhibitor Dactolisib incorporated and subsequently characterized into rHDL nanoparticles. These nanoparticles, made of components found in native circulating HDL, target the SR-B1 receptor, which the Lacko lab has found to be up-regulated in several cancers (1). Following cholate dialysis, these nanoparticles had a drug concentration of 73.35 uM/mL. DLS concluded that the average diameter of the nanoparticles were 15.7 nm with a polydispersity index of 0.160, suggesting little variance in particle size, and were thus comparable to the average diameter of native HDL particles, which range between 7- 14 nm (5).
Because the P13K/mTOR/AKT pathway is dysregulated in GBM, this formulation was anticipated to be effective against a GBM cell line with robust SR-B1 expression. Subsequently, Dactolisib rHDL nanoparticles were tested against GBM cell line LN229, with a computed IC50 value of 103 nM. An appreciable protective effect of this preparation against healthy astrocytes was also observed relative to the drug in free form. Future studies to expand upon the current data include include optimizing the nanoparticle formulation process to increase Dactolisib's incorporation, and evaluating the cytotoxicity of the rHDL preparation against other high-expressing SR-B1 GBM cell lines to construct a data portfolio of compelling proof to extend the drug delivery system into animal models.

REFERENCES

1. Lacko AG, Sabnis NA, Nagarajan Band McConathy WJ (2015) *HDL as a drug and nucleic acid delivery vehicle*. Front. Pharmacol. 6:24. doi: 10.3389/fphar.2015.00247
2. Porta, C., Paglino, C., & Mosca, A. (2014). Targeting PI3K/Akt/mTOR Signaling in Cancer. *Frontiers in Oncology*, *4*, 64. http://doi.org/10.3389/fonc.2014.00064
3. Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. N Engl J Med 2005; 352:987-996
4."Dactolisib (BEZ235, NVP-BEZ235)." *Dactolisib (BEZ235, NVP-BEZ235) | PI3K Inhibitor | Read Reviews & Product Use Citations*. N.p., n.d. Web. 21 June 2017.
5. Kontush, A. (2015). HDL particle number and size as predictors of cardiovascular disease. *Frontiers in Pharmacology*, *6*, 218. http://doi.org/10.3389/fphar.2015.00218

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