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**Research Plan:** Nanoparticle Retinoid Delivery: A Novel Functional Method for Inducing Cytotoxicity in Cancer

**Category:** Biomedical & Health Sciences

**Rationale:**

Cancer is the second leading cause of death in the United States today, affecting roughly 1.7 million Americans annually. Although our understanding of molecular and cancer biology has furthered, there has yet to be a treatment that specifically targets cancer cells. Recent research has indicated that retinoids–skin care drugs used for acne or psoriasis—have the potential to promote cell differentiation and apoptosis. Retinoid delivery, however, remains inefficacious as they’re metabolized prior to reaching the cancer cells. To overcome this obstacle, nanotechnology can be considered. Previous studies have demonstrated the ability of iron oxide nanoparticles to carry drugs within their polymeric coating, allowing the drugs to travel unimpeded throughout the body. This study seeks to determine the efficacy of loading iron oxide nanoparticles with retinoids to combat the delivery limitation. It also seeks to ascertain the cytotoxicity of All trans retinoid acid, as well as three additional retinoid drugs—Tamibarotene, Adapalene, and Tazarotene—that have not been mentioned in previous research, in leukemia and breast cancer cell lines.

**Purpose:**

The purpose of this study is threefold:

1. Optimize the non-covalent loading of FDA-approved retinoids into Feraheme iron oxide nanoparticles.
2. Determine if All-trans retinoic acid, Adapalene, Tamibarotene, or Tazarotene can be used as a treatment for retinoid-sensitive cancers.
3. Determine if retinoid-loaded Feraheme iron oxide nanoparticles can elicit cytotoxicity in retinoid-sensitive cancers.

**Hypotheses:**

1. Retinoids can be successfully be loaded in Feraheme iron oxide nanoparticles.
2. All-trans retinoic acid, Adapalene, Tamibarotene, and Tazarotene can exhibit cytotoxic effects in leukemia and breast cancer cells.
3. Retinoid loaded Feraheme iron oxide nanoparticles can elicit cytotoxicity in leukemia and breast cancer cells.

**Methodology:**

Role of Mentor: Purchased cell lines, drugs, and equipment.

Role of Student Research: Performed all subsequent methods

1. *Cell Culture*

Mouse leukemia virus-induced tumor cells (RAW 264.7) and breast cancer cells (T47D), purchased from the ATCC (Manassas, VA), will be cultured in a tissue culture flask and passaged every three days. Cell passaging will consist of washing with Phosphate Buffer Saline (PBS), replacing the media (Dulbecco’s Modified Eagle Medium and RPMI-1640), gently detaching cells with a cell scraper, transferring cell suspension into centrifuge tubes, centrifuging at 4000 rpm for 3-5 minutes, adding cell suspension into a new flask, and then being incubated at 37°C. Following incubation, cells will be counted using the Thermo Fisher Scientific Countess Automated Cell Counter and will be seeded in 96-well tissue culture plates based on number of live cells.

2. *Drug Loading*

The retinoid drugs All-trans retinoic acid, Tazarotene, Tamibarotene, and Adapalene will be purchased from Sigma-Aldrich (St. Louis, MO). Drugs will be diluted with dimethyl sulfoxide (DMSO) to the following two-fold concentrations: 10 μg/mL, 5 μg/mL, 2.5 μg/mL, 1.25 μg/mL, and 0.0625 μg/mL; each concentration will recieve their own group. In addition, Feraheme iron oxide nanoparticles, purchased from AMAG Pharmaceuticals (Waltham, MA), will be diluted with 500 μl of phosphate buffer saline (PBS) and then combined with 200 μl of the drugs each separately via dropwise addition while vortexing. The samples will then be run through the magnetic columns for purification and then washed with 300 μl of PBS (Figure 3). Iron particles are attracted to the magnets on the column therefore any unloaded drug will wash away. The nanoparticles are eluted by the removal of the magnetic field. All samples will be stored at 4°C.

3. Absorbance Spectrum

Each retinoid as well as a DMSO blank will be placed into a 96 well-plate and then into a SpectraMax iD5 spectrophotometer to determine the best wavelength to detect them in after they have been loaded into the nanoparticles. The absorbance spectrum will be measured between 350nm and 1000nm.

Lyophilization

All retinoid-loaded nanoparticle samples, as well the unloaded nanoparticles, will be placed into vials (200μl per sample) and then into the lyophilization cannister and left overnight in the FreeZone 4.5 Liter Cascade Benchtop Freeze Dry System lyophilizer (Figure 3). This will freeze-dry the samples to remove water so they can then be resuspended in DMSO to determine the drug concentration in the nanoparticles.

*5. Drug Detection*

The dried-out samples will be removed from the lyophilizer and then diluted with 200μL of DMSO. From there, they will be spun down in a centrifuge at 1400 rpm for 5 minutes, transferred into new 100μL tubes and then spun down again at 1400 rpm for 5 minutes**.** They will then be put in a SpectraMax iD5 spectrophotometer and absorbance will measured at the wavelength determined by the absorbance spectrum. The iron quantification assay will also be completed to measure the amount of iron present in the samples. From there, the ratio of drug to iron can be mathematically computed using the values generated from drug absorbance and the iron quantification assays.

6. *Dynamic Light Scattering*

All retinoid-loaded nanoparticle samples, as well the unloaded nanoparticles**,** will be diluted with 1000 μL of PBS and placed into a cuvette, to which will then placed in a Malvern Zeta Sizer to perform Dynamic Light Scattering. This will measure the hydrodynamic radius of nanoparticles, from which we can determine whether or not aggregation/clumping occurred as a result of the loading process to judge the particle stability and solubility.

7. *Experimental Trials*

Two separate 96-well tissue culture plates will be seeded, one with the T47D breast cancer cells and the other with the RAW 264.7 leukemia virus-induced tumor cells. The All-trans retinoid acid loaded nanoparticles, Adapalene loaded nanoparticles, Tamibarotene loaded nanoparticles, and Tazarotene loaded nanoparticles will placed into their own respective wells in a 96 well plate (Figure 5). Additionally, each concentration of the free All trans retinoic acid, Adapalene, Tamibarotene, and Tazarotene alone will be placed into their own respective wells (Figure 5). A non-loaded nanoparticle group will be added as a control, and untreated cells will not receive any form of treatment (Figure 5). All groups will have duplicates. The plate will be left to incubate at 37°C for 24 hours following treatment.

8. *Cell Titer Glo Cell Viability Assay*

All media will be aspirated from the wells and then replaced with 100 μL of the respective media. 100 μL of CellTiter-Glo reagent will be added to each well and the plate will be put into the SpectraMax iD5 spectrophotometer and luminescence will be measured at 450nm. The addition of the CellTiter-Glo reagent will result in cell lysis and the generation of a luminescent signal proportional to the amount of ATP present. From there, the amount of ATP is directly proportional to the number of cells present, allowing us to determine the cell viability; relative fluorescence will be used as a measurement of cell viability.

9. *Risk and Safety*

All methods will be performed according to safety regulations and caution will be carried out. All hazardous substances will be disposed of separately through methods established by institutional standards.

10. *Data Analysis*

All data will analyzed in Microsoft Excel 2016 and GraphPad Prism 8. Differences between treatment conditions will be measured using ANOVAs and Post Hoc Tukey tests. Statistical significance will be set at p≤0.05.