Scientific and Technical Progress Report (STPR) [*]

(DST-SERB)

Section-A : Project Details

A1. Project Title: **SERB/F/7471/2020-2021, 05 Feb, 2021 & "Folate decorated pH responsive combinatorial nanocarriers mediated co-delivery of dual drugs and siRNAs for reversal of multidrug resistance and synergistic breast cancer therapy: Development and Characterization"**

A2. SERB Sanction Order No. & Date**: SERB/F/7471/2020-2021, 05 Feb, 2021**

A3. Date of Project Initiation **(If this date is different than date mentioned in A2, please clarify the reason): NA**

A4. Date of project completion**: March 2023**

A5. Name of Project Coordinator/ Principal Investigator/Co-PI/Co-Investigator:

PI

Dr. Anita Kamra Verma

Professor

A6. Institute(s): Kirori Mal College, University of Delhi, Delhi-110007

A7. Address with Contact Nos. (Landline & Mobile) & Email of Project Co-coordinator/ PIs and Co-PIs/ Co-Is:

Nanobiotech Lab, Department of Zoology, Kirori Mal College, University of Delhi, Delhi-110007;
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A8. Total Approved Cost (including additional cost, if any): **Rs. 3,35,000/- per year**

A9. Approved Duration: The duration of the project is **3 year**s from the date of this sanction order.

Anita K. Verma

PRINCIPAL INVESTIGATOR
(Signed and stamped)

V. Chauhan.

HEAD OF THE INSTITUTE
(Signed and stamped)

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A10. Rationale and background information of project (in brief Maximum 500 words):

Breast cancer is the most frequent cancer among women, impacting 2.1 million women each year, and also causes the greatest number of cancer-related deaths among women. Although various drug combinations either alone or as nanomedicines are often used to prevent drug resistance in cancer patients, the ability of the cancer cells to adapt and develop one or more drug resistance pathways ultimately leads to treatment failure in most cancers therefore novel nanocarriers that can co-deliver siRNA and chemotherapeutics optimally through receptor mediated endocytosis resulting into many folds higher intracellular concentration into the cellular tropics (i.e cytosol) to accomplish efficient and effective transfection that knocks down drug resistance genes with simultaneous restoration of drug sensitivity are urgently needed to decrease the side effects as well as toxicity and frequency of administration of existing drugs, to overcome MDR and to increase the survival rates of breast cancer cases.

However, clinical success has been limited due to nucleic acid biodegradation and lack of target tissue- or cell-specificity of the delivery system. Another key challenge of gene therapy is the development of safe and effective delivery systems. Both viral and nonviral vectors have been used to deliver nucleic acids; but there are substantial challenges limiting viral vectors use, including safety and immunogenicity issues. In contrast, nonviral vectors have lower apparent immunogenicity and diverse nonviral vectors have been used to deliver therapeutic nucleic acids to the bone microenvironment. However, these vectors have off-target effects, and are often not biodegradable or biocompatible, or have low-encapsulation efficiency. Consequently, improved and more efficient osteoblast-targeted nonviral delivery systems are required. Modification of the nanoparticle by targeting ligand, can improve distribution. Key attributes can be modified such as specificity and stability. Peptides are attractive targeting ligands due to potentially low immunogenicity, high avidity, easy bioconjugation and synthesis. Small molecule-targeting moieties such as endogenous folate, sugar, and carbohydrates have high receptor binding affinity, but their receptors are widely expressed; thus, they are nonselective and nonspecific in targeting tissues. Exogenous ligands such as proteins and monoclonal antibodies are potentially immunogenic and are rapidly cleared, further compounded by engineering challenges for scale-up and manufacturing. Additionally, aptamer stability may be affected by heat, exonuclease or endonuclease degradation, and other environmental factors, so their efficacy and applicability are limited too. Moreover, nanoparticles of 20−200 nm offer prolonged nucleic acid encapsulation and circulation as they are too large for renal filtration and evade phagocytic clearance, reducing non-targeted drug distribution. The purpose of study to develop combinatorial innovative nanocarriers, liposomes and emulsomes. Emulsomes, having the characteristics of both liposomes and emulsions, provide the advantages of high hydrophobic drug loading in the internal solid lipid core and the ability to encapsulate water-soluble medicaments/nucleic acids in the aqueous compartments of surrounding phospholipid layers.4 Similarly liposomes provide the advantage of encapsulating hydrophilic cargos in aqueous compartment and hydrophobic cargos in the surrounding phospholipid bilayers. Furthermore, both nanocarriers can be modified with target specific ligands and PEG to promote accumulation of selected cargos at the target site. Also, lipid bilayer destabilization under acidic conditions may provide for pH triggered cargo release from the endosome wherein simultaneous burst release of siRNA and small chemotherapeutics from the aqueous compartments of surrounding phospholipid layers and lipid layers themselves respectively will be followed by controlled release of chemotherapeutics from the apolar/polar core within the cytosol of breast cancer cells.

The discovery of RNAi raises the possibility to explore new approaches for many incurable and difficult to treat breast malignancy. The advantage of siRNA as therapeutics is that siRNA can target many undruggable genes. A siRNA-based drug may target any mRNAs of interest, regardless of their cellular locations or structures of the translated proteins. Therefore, Combinational therapy of siRNA and chemo- therapeutics shows promises to meet these challenges and has emerged as new generation bio-

drugs under intensive investigation. The combination of these nanocarriers following their optimization in a single dose cocktail will address the problem related with multidrug resistance (MDR) since the release shall be implicated through biodigestion of the carrier; it can be further modified through pH dependent gelling or material specific characters. The same systems could alternatively be used for parentral administration as powder dose ready for reconstitution. The target specific treatment will certainly curtail down the side effects with maximum therapeutic promises. The same formulation may also be used for dual sequential treatments that first target a known drug resistance mechanism (e.g., overexpression of the multi-drug resistance P-glycoprotein MDR117) by means of siRNA-containing nanocarrier targeted to cancer cells. After permitting for the adequate time to accomplish considerable knockdown of the drug-resistance mediating protein, it may be followed with a second in line therapy involving intravenous (i.v.) administration of targeted nanocarriers encapsulating a cytotoxic multidrug combination. Additionally, a range of siRNAs in appropriate doses may be incorporated to bombard and eradicate a range of drug resistant genes thus the present system as proposed would essentially be a multidrug combination therapy with abilities to address the multi drug resistance related problems. It will have excessively high commercial viability, patient compliance and low cost of treatment with possibilities of radical cure. The mechanism of formulation development and their linkage with the performance will offer patentability to such formulations with defined state of art attributed to the performance.

A11. (i) Approved Objectives of the Project:

1. **Preparation and optimization of long circulatory folate targeted nanocarriers using different drugs (docetaxel and doxorubicin) and/or siRNAs (MRP1 and Bcl2) separately (liposomes and emulsomes).**
2. **Characterization of nanocarriers (size, zeta potential, cargo capacities, *in-vitro* pH dependent release of drugs and siRNAs etc.)**
3. **Stability studies of developed nanocarriers at different temperatures/pH**
4. **Preparation and optimization of combinatorial nanocarriers encapsulating different siRNAs/drugs.**
5. **Intracellular localization and endosomal escape of nanocarriers**
6. **Development of resistant cell lines**
7. **Effect of codelivery on cell proliferation (cytotoxicity assay)**
8. **Apoptosis assay**
9. **Determination of in vivo anticancer activity**
10. **Gene expression and western blot assay**

Work Plan 1: (A-2) Hydrodynamic diameters and zeta potential

Hydrodynamic diameters and zeta potentials to be measured by laser light scattering using a Malvern Zetasizer Nano ZS at 25°C following dilution with distilled water

Objective 2. (A) *In-vitro* characterization of siRNA formulation. (In Progress)

SiRNA have been ordered and few preliminary experiments are in progress.

Objective 3. Stability studies of developed nanocarriers at different temperatures/pH (To be Done)

Objective 4. Stability studies of developed nanocarriers at different temperatures/pH (To be done)

Objective 5. Preparation and optimization of combinatorial nanocarriers encapsulating different siRNAs/drugs.

Objective 7. Development of resistant cell lines (In progress)

(A) *In vitro* cell culture studies: i) **Human breast cancer cells (MCF-7)**

(B) To develop paclitaxel and docetaxel resistant MCF-7 cells. (In progress)

Objective 6. Intracellular localization and endosomal escape of nanocarriers

Objective 8. Effect of codelivery on cell proliferation (cytotoxicity assay) (to be done)

Objective 9. Apoptosis assay (to be done)

Objective 10. Determination of *in vivo* anticancer activity (to be done)

Objective 11. Gene expression and western blot assay (to be done)

Signature of the grantee
Date: 31/03/2022

Signature of the Mentor
Date: 31/03/2022

Signature & Seal of Principal
Date 31/03/2022

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