1.1. Cancer and its global impact.

Cancer is a disease which everyone recognises today due to its widespread global impact. It is one of the most prominent non-communicable diseases which affects almost all the nations whether they are under developed, developing or developed. There are no adequate measures taken to curb this disease. In 2018, 18.1 million people were found to be affected from cancer globally out of which 9.6 million people did not survive. It is the leading cause of mortality in 30% of the cancer patients aged around 30-69. As per the latest WHO report on cancer, it is estimated that every one in five people are likely to encounter this disease. The most commonly diagnosed cancer is lung cancer (11.6% of all cases), followed by female breast (11.6%) and colorectal cancers (10.2%). Lung cancer is the leading cause of death from cancer (18.4% of all deaths), followed by colorectal (9.2%) and stomach cancer (8.2%). There are numerous causes of cancer including sedentary lifestyle, diet, and exposure to physical, chemical or biological carcinogens. Genetics also play a major role in precipitation of this disease as per current research findings. Despite of the lethality of this disease, early diagnosis and preventive intervention can help in survival of the patient. It is projected that the cancer rates are to rise 60% in next 20 years if sufficient control measures are not taken (1).

1.2. Treatment of Cancer and barriers to drug delivery.

The conventional approaches for treatment of cancer are use of chemotherapeutic agents of various classes, radioisotopes and surgery. The chemotherapeutic agents and radiotherapy are non-targeted therapies. The delivery of cytotoxic drugs has always been a complicated and a challenging task as the distribution of the drug is highly unpredictable. Mononuclear phagocyte system can alter the pharmacokinetic distribution of drug. The tumour microenvironment can also affect the distribution of drug as various factors like its pH, presence of enzymes, osmotic pressure, endosomal escape, presence or absence of receptors and efflux pumps on its cell membrane determine the kinetics of drug transport into the cancerous cell. Mutation in cancerous cells can alter their biological characteristics which may produce drug resistance (2-4).

The inability of the therapeutics to differentiate between the target and non-target cells and altered pharmacokinetics due to the biological barriers often causes accumulation of the therapeutic agents in non-target organs or regions which results in the toxicity. This collectively results into poor patient compliance (5).

1.3. Brain tumours and its complications

Gliomas are the malignant form of brain tumours which originate in the glial and glial progenitor cells including astrocytoma, glioblastoma, oligodendroglioma, ependymoma, mixed glioma and other cells of the brain. Glioblastoma accounts for about 50% of the brain tumours and survival rate of patients is less than 5% after 5 years of diagnosis. The tumours of the brain are extremely complicated to diagnose and treat owing to the location of the organ and the protection offered by the blood brain barrier which is difficult to penetrate by chemotherapeutic agents. The efflux pumps driven by ATP are located on the tumour cells and also blood brain barrier which make it difficult for drugs to reach the site of action as rapid elimination takes place. (6, 7).

1.4. Drug targeting in cancer with nanotechnology.

Targeted drug delivery is one of the methodologies which is used to deliver the chemotherapeutic agents to the desired site of action.Various carriers like liposomes, nanoparticles, micelles etc. have been rigorously studied for their application in targeted delivery of anticancer drugs. There are numerous advantages of nanoparticulate drug delivery systems. The small size of nanocarriers makes them highly permeable to most of the biological barriers. Another phenomenon known as the “EPR effect” which is enhanced permeation and retention is useful to target the tumours as they are highly vascularised and nanocarriers loaded with therapeutic agents accumulate in these vascularised regions thereby delivering the cytotoxic drugs to cancerous regions. Nanocarriers can be modified in a variety of ways which can alter its distribution and enhance targeting and lowering of toxicity (8).

1.5. Diagnostic tools for cancer.

Diagnosis is the important aspect of early detection of cancer which can help in selection of proper strategy of its treatment. Various techniques have evolved for diagnosis which sometimes utilise "molecular probes" which are commonly known as imaging agents or contrast agents. The selection of the imaging agents depends on the application, technique used and the region under examination(9).

1.5.1. Magnetic resonance imaging in cancer (MRI)

Magnetic resonance imaging has been widely used since the emergence of the technique in the field of medicine for imaging of vital organs and for tumour detection. The main advantage of this technique is it offers a three dimensional overview and high spatial resolution. The principle of this technique is the contrast offered by the relaxation times of the protons of water molecules inside the body. The technique can be used along with specialised contrast agents like chelated iron and gadolinium which give T2 (dark) and T1 (light) contrast respectively. MRI is a useful tool for imaging but it also provides additional important information like vasculature of tumour, oxygen levels, metabolic pathways, expression of biomarkers, metastasis etc. This technique is used for imaging of brain, breast, colorectal, prostrate and other solid tumours (10).

1.6. Concept of Theranostics- A mixed approach of therapy and diagnosis.

The term theranostic was coined by John Funkhouser in year 2002 and it is defined as a system or a material which has bimodal function of diagnosis and therapy (11). In cancer treatment, there is a need of frequent diagnosis and depending on the results of the diagnosis, therapy is initiated. This causes frequent use of diagnostic tools and agents along with therapeutic agents which has high toxicity potential on the patient. In order to avoid the toxicity and undesired biodistribution of the diagnostic agent and cytotoxic drugs to treat cancer, theranostic platforms have been developed which involve use of chemistry and nanotechnology. Prior to 2005, the nanoparticulate drug delivery systems were limited to either diagnosis or drug delivery.

The development of novel nanomaterials aided in the development of organic and inorganic nanoparticles, liposomes, dendrimers, micelles etc. for targeting cancer and its diagnosis simultaneously. The payload for the theranostic nanoparticles included optical imaging agents like Cyanine 5.5, fluorescein isothiocyanate (FITC) etc. Quantum dots can also be loaded into the nanoparticles for fluorescence imaging. Gadolinium based complexes loaded into nanoparticles or fabricated metal nanoparticles have MRI imaging capability which is widely used in theranostics. Radioisotopes such as ^{64}Cu chelated with 1,4,7,10-tetraazacyclododecane-1,4,7-tris-acetic acid are conjugated with chemotherapeutic agents for PET imaging. Carbon based nanomaterials such as carbon nanotubes have near infrared imaging property along with photo acoustic imaging ability (12). The fabrication of the theranostic platforms is a big challenge as the materials used in conventional nanocarriers have to be chemically modified to provide attachment sites for the binding of imaging agent, therapeutic agent and targeting moiety. The targeting moiety consists of ligands which can bind with the overexpressed antigens and aid in the uptake of nanoparticles by cancerous cells (13).

1.7. Polymers used in theranostic applications.

For any material to be qualified for use in clinical applications, it must possess biocompatibility or biodegradability characteristics. Various polymers of natural, semi synthetic and synthetic origins have been used in theranostic platform fabrications. Polymers such as chitosan, human serum albumin, hyaluronic acid, collagen etc. have been used. Chitosan having cationic charge is able to form ionic complexes with DNA which is useful in gene delivery applications while it is also used to encapsulate Gd-complexes, iron oxide nanoparticles and quantum dots for biomedical imaging. Chitosan is also used as a carrier of chemotherapeutic drugs like paclitaxel, docetaxel, 5-fluorouracil etc. Poly lactic co glycolic acid (PLGA) and Poly lactic acid (PLA) are well known polymers used widely in delivery of both hydrophilic as well as hydrophobic drugs like vincristine, doxorubicin and other agents for cancer therapy. The can also be modified with polyethylene glycol (PEG) and c(RGDfK) peptide to target integrin expressing cancer cells. ^{99m}Tc-PLGA nanoparticles are used for imaging and conjugation with diethylenetriaminepentaacetic acid (DTPA) or 1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid (DOTA) for Gd^{+3} labelling is useful for T1 contrast based imaging. Polyaspartic acid nanoparticles labelled with RGD peptide and ^{64}Cu have been developed for imaging and targeting tumours. Human serum albumin has been used as a coating polymer for iron oxide nanoparticles for imaging in MRI. N-(2-Hydroxypropyl) methacrylamide (HPMA) is also used as a conjugating polymer for fabrication of theranostic nanoparticles for prolonged circulation time and delivery of peptides(14, 15).

Polyamidoamine (PAMAM) based polymers have been synthesised using PEG, arginyl-glycyl-aspartic acid (RGD) peptide for delivery of doxorubicin in glioblastoma cells. Polyhedral oligomeric silsesquioxane (POSS) core dendrimers having multifunctional terminals helps in conjugation of various moieties of anticancer drugs and imaging agents(16).

Polyethyleneimine (PEI) is a cationic hydrophilic synthetic polymer having density of primary amines. It is available in branched and linear forms with varying molecular weights. It has wide variety of applications in gene delivery and delivery of cytotoxic drugs and gene transfection. It can also be used to target mitochondria due to its cationic nature. Due to high density of free amines, there are numerous possibilities of its modification which is a requirement for theranostic applications. ^{131}I-labeled polyethylenimine nanoparticles encapsulating gold nanoparticles have been developed for SPECT/CT imaging and cancer therapy (17).

1.8. Iron oxide based nanoparticles.

The iron oxide nanoparticles are the magnetic nanoparticles which is one of the most researched theranostic platform for various medical applications including cancer. The types of iron oxide nanoparticles include super paramagnetic iron oxide nanoparticles (SPIOs) and ultra-small super paramagnetic iron oxide nanoparticles which consist of particles having diameter less than 50 nanometres (18). Iron oxide nanoparticles are considered safe due to their biocompatibility and biodegradability. Most of the iron oxide nanoparticles except some are metabolized by lysosomal enzymes into elemental iron which is utilized in the body or excreted by ferritin proteins which regulate the iron metabolism in the body (19). Iron oxide nanoparticles are widely used in targeted delivery of drug molecules, gene therapy agents and also act as contrast agents in magnetic resonance imaging owing to their magnetic nature (20). The iron oxide nanoparticles show T2 contrast in MRI but doping of iron oxide with other metals of lanthanide group can alter the magnetic properties of the iron oxide and also provide T1 contrast capability to the nanoparticles.

1.9. Anti angiogenic drugs in treatment of glioblastoma and challenges to delivery.

One of the prominent features of glioblastoma is overexpression of vascular endothelial growth factor(VEGF) which is responsible for angiogenesis and enhanced proliferation(21). Anti-vascular endothelial growth factor drugs such as lenalidomide are potent inhibitors of angiogenesis and promote apoptosis on cancerous cells (22). The drug is in clinical trials for treatment of glioblastoma (23). The main drawback associated with the delivery of lenalidomide is its low solubility as the drug belongs to BCS class II and is responsible for low oral bioavailbility (<33%) (24). The half-life of drug is also 3-4 hrs. which requires frequent administration of drug to maintain the therapeutic window concentration (25). In case of glioblastoma the drug delivery is a challenge as the barriers like blood brain barrier and tumour are difficult to penetrate and require strategies involving use of nanotechnology. Also the efflux transporters show rapid removal of drug in case of the chemoresistance which reduces the available concentration of drug at the site which gives poor treatment outcomes. Thus it is necessary to have a nanocarrier system which addresses all the drawbacks and delivers the drug to the tumours in a safe and effective manner and also has diagnostic capabilitites to monitor the treatment outcomes.

1.10. Aims, Objectives, Rationale and hypothesis.

Aim: To formulate lanthanide doped iron oxide based theranostic nanoparticles with modified polyethylenimine coating to deliver lenalidomide to brain tumours for improvement of the therapeutic outcomes monitored by the dual contrast imaging ability of theranostic nanoparticles using MRI.

Objectives

1. Synthesis of magnetic metallic core with Gd doping.
2. Coating of magnetic metallic core with lenalidomide to treat glioblastoma by inhibition of angiogenesis.
3. Modification of PEI for targeting mitochondria and enhanced cellular uptake.
4. Secondary coating of modified PEI on drug loaded core.
5. Evaluation of final formulation by *in vitro*, *ex vivo* and *in vivo* studies.

Rationale

The formulated theranostic nanoparticles will have the following advantages:

- The metallic core will be magnetic in nature due to iron oxide which will be useful in targeting by external magnetic fields.
- The lanthanide doping will provide diagnostic capabilities by T1 and T2 contrast.
- The metal core will show hyperthermia in presence of magnetic field which is useful for treatment of cancer.
- The drug coating on the metal core will provide anti-cancer activity.
- Modified PEI coating will assist in enhanced cellular uptake and mitochondrial targeting.

Hypothesis

It was hypothesized that the formulated PEI coated drug loaded iron oxide nanoparticles would provide diagnostic capabilities in MRI imaging and also provide therapeutic benefit from hyperthermia and drug loaded on the nanoparticles with mitochondrial targeting.

1.11. Plan of work.

1st Year

- Literature review.
- Procurement of materials.
- Preformulation studies.
- Development of analytical method
- Synthesis of metallic core by different method.
- Selection of feasible method for synthesis of metallic core.

2nd Year

- Optimization of process for metallic core.
- Selection of method for drug coating on metallic core.
- Optimization of method for drug coating on metallic core.
- Synthesis of modified PEI and its characterization.

3rd Year

- In vitro studies , toxicity analysis and cell line studies for theranostic nanoparticles
- Radiolabelling of the nanoparticles and biodistribution studies.
- Compilation and interpretation of results.
- Thesis writing and publication of research work.

1.12. References

1. Organization WH. WHO report on cancer: setting priorities, investing wisely and providing care for all. 2020.
2. Choudhury H, Gorain B, Pandey M, Kaur R, Kesharwani P. Strategizing biodegradable polymeric nanoparticles to cross the biological barriers for cancer targeting. International journal of pharmaceutics. 2019.
3. Nizzero S, Ziemys A, Ferrari M. Transport barriers and oncophysics in cancer treatment. Trends in cancer. 2018;4(4):277-80.
4. Maeda H, Islam W. Overcoming barriers for tumor-targeted drug delivery: the power of macromolecular anticancer drugs with the EPR effect and the modulation of vascular physiology. Polymer-Protein Conjugates: Elsevier; 2020. p. 41-58.
5. Mondal J, Panigrahi A, Khuda-Bukhsh A. Conventional chemotherapy: problems and scope for combined therapies with certain herbal products and dietary supplements. Austin Journal of Molecular and Cellular Biology. 2014;1:1-10.
6. Schwartzbaum JA, Fisher JL, Aldape KD, Wrensch M. Epidemiology and molecular pathology of glioma. Nature clinical practice Neurology. 2006;2(9):494-503.
7. Agarwal S, Sane R, Oberoi R, Ohlfest JR, Elmquist WF. Delivery of molecularly targeted therapy to malignant glioma, a disease of the whole brain. Expert reviews in molecular medicine. 2011;13.
8. Stirland D, Nichols J, Denison T, Bae Y. Targeted drug delivery for cancer therapy. Biomaterials for Cancer Therapeutics: Elsevier; 2013. p. 31-56.
9. Janib SM, Moses AS, MacKay JA. Imaging and drug delivery using theranostic nanoparticles. Advanced drug delivery reviews. 2010;62(11):1052-63.
10. Gore JC, Manning HC, Quarles CC, Waddell KW, Yankeelov TE. Magnetic resonance in the era of molecular imaging of cancer. Magnetic resonance imaging. 2011;29(5):587-600.
11. Funkhouser J. Reinventing pharma: the theranostic revolution. Curr Drug Discov. 2002;2:17-9.
12. Lim E-K, Kim T, Paik S, Haam S, Huh Y-M, Lee K. Nanomaterials for theranostics: recent advances and future challenges. Chemical reviews. 2015;115(1):327-94.
13. Kelkar SS, Reineke TM. Theranostics: combining imaging and therapy. Bioconjugate chemistry. 2011;22(10):1879-903.
14. Fernandez-Fernandez A, Manchanda R, McGoron AJ. Theranostic Applications of Nanomaterials in Cancer: Drug Delivery, Image-Guided Therapy, and Multifunctional Platforms. Applied Biochemistry and Biotechnology. 2011 2011/12/01;165(7):1628-51.
15. Wang Z, Niu G, Chen X. Polymeric Materials for Theranostic Applications. Pharmaceutical Research. 2014 2014/06/01;31(6):1358-76.
16. Sk UH, Kojima C. Dendrimers for theranostic applications. Biomolecular concepts. 2015;6(3):205-17.
17. Sun N, Zhao L, Zhu J, Li Y, Song N, Xing Y, et al. (131)I-labeled polyethylenimine-entrapped gold nanoparticles for targeted tumor SPECT/CT imaging and radionuclide therapy. Int J Nanomedicine. 2019;14:4367-81. PubMed PMID: 31354266. eng.
18. Lee N, Yoo D, Ling D, Cho MH, Hyeon T, Cheon J. Iron Oxide Based Nanoparticles for Multimodal Imaging and Magnetoresponsive Therapy. Chemical Reviews. 2015 2015/10/14;115(19):10637-89.
19. Saeed M, Ren W, Wu A. Therapeutic applications of iron oxide based nanoparticles in cancer: basic concepts and recent advances. Biomaterials Science. 2018;6(4):708-25.
20. Thomas R, Park I-K, Jeong YY. Magnetic iron oxide nanoparticles for multimodal imaging and therapy of cancer. International journal of molecular sciences. 2013;14(8):15910-30.
21. Batchelor TT, Reardon DA, De Groot JF, Wick W, Weller M. Antiangiogenic therapy for glioblastoma: current status and future prospects. AACR; 2014.

22. Wang N, Jain RK, Batchelor TT. New directions in anti-angiogenic therapy for glioblastoma. Neurotherapeutics. 2017;14(2):321-32.
23. Segler A, Tsimberidou A-M. Lenalidomide in solid tumors. Cancer chemotherapy and pharmacology. 2012;69(6):1393-406.
24. Dai X-L, Chen J-M, Lu T-B. Pharmaceutical cocrystallization: an effective approach to modulate the physicochemical properties of solid-state drugs. CrystEngComm. 2018;20(36):5292-316.
25. Chen N, Zhou S, Palmisano M. Clinical pharmacokinetics and pharmacodynamics of lenalidomide. Clinical pharmacokinetics. 2017;56(2):139-52.