A Review on Nanoparticles Based Drug Delivery in Cancer Treatment: Advantages & Challenges

By

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The project titled "A Review on Nanoparticles Based Drug Delivery in Cancer Treatment: Advantages & Challenges" submitted by Kamrun Nahar (18146043) of Spring, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelors of Pharmacy (Hons.) on June, 2022.

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Ethics Statement

The thesis was completed without doing any unethical acts. This study does not involve with any animal or human trials.

Abstract

Nanoparticles have unique physicochemical and biological properties that make them one of the most promising candidate for targeted cancer therapy. Nanoparticle-based drug delivery has a number of advantages over conventional drug delivery methods, including smaller particle size, enhanced stability and biocompatibility with enhanced permeability and retention effect and specific targeting to the tumor sites enabling selective killing of cancer cells without affecting the healthy cells. Owing to the beneficial properties of nanoparticles, they are increasingly being used to target cancer cells using active targeting and passive targeting mechanisms for both diagnostic and therapeutic purpose of various types of cancer treatment. The present review focuses on the different types of nanoparticles used as a targeted drug delivery system for cancer treatment, highlighting their potential advantages and the challenges associated with the application of these nanoparticles based drug delivery systems with a direction towards future cancer research.

Keywords: Nanoparticles; drug delivery; cancer; tumor targeting; liposome, polymers

Dedication

This paper is dedicated to my dear parents.

Acknowledgement

All praises and glory to Almighty Allah (SWT) who has given me enormous courage, knowledge, wisdom and patience to carry out and complete this thesis. Peace and blessing of Allah be upon last Prophet Muhammad (Peace Be upon Him).

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Table of Contents

List of Tables

List of Figures

List of Acronyms

SCID	Severe combined immunodeficiency disease
HSRs	Hyper sensitivity reactions
IgE	Immunoglobulin E
LV	Left Ventricle
PEG	Polyethylene glycol
EPR	Enhanced permeability and retention
FDA	Food and Drug Administration
PLGA	Polylactic-co-glycolic acid
LDC	Lipid drug conjugates
NLC	Nanostructured lipid carriers
CMC	Critical micelle concentration
PAMAM	Polyamidoamine
ADME	Absorption, distribution, metabolism, excretion
IV	Intravenous
UV	Ultraviolet
AuNPs	Gold Nanoparticles
CNTs	Carbon Nanotubes
SWCNTs	Single-walled carbon nanotubes
MWCNTs	Multi-walled carbon nanotubes

BBB	Blood Brain Barrier
QDS	Quantum Dots
ZnS	Zinc sulfide
CdS	Cadium sulfide
CdSe	Cadium selenide
CdTe	Cadium telluride
InP	Indium phosphide
PbS	Lead sulfide
PbTe	Lead telluride
MSNS	Mesoporous silica nanoparticles
TEOS	Tetraethylorthosilane
LPHNPs	Lipid-polymer hybrid nanoparticles
LSH	Liposome-silica hybrid
EGFR	Epidermal growth factor receptor
CD3	Cluster of differentiation 3
CD4	Cluster of differentiation 4
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
SERS	Surface-enhanced raman spectroscopy
SPR	Surface plasmon resonance

AuNCs	Gold Nanocluster
FFPE	Formalin fixed paraffin embedded tissue
CYP3A4	Cytochrome P450 3A4
5-FU	5-Fluorouracil
AIDS	Aquired immunodeficiency syndrome
APIs	Active pharmaceutical ingredients
PMDI	Pressurized metered-dose inhaler
DPI	Dry powder inhaler
ROS	Reactive oxygen species
siRNA	Small interfering ribonucleic acid
shRNA	Short hairpin ribonucleic acid
miRNA	Micro ribonucleic acid
DNA	Deoxyribonucleic acid
ssDNA	Single-stranded deoxyribonucleic acid
Akt1	Threonine kinase 1
PDT	Photodynamic Therapy
PS	Photosensitizers
LDL	Low-density lipoprotein
NIR	Near-infrared
MRI	Magnetic resonance imaging

MDR	Multi-drug resistance
ABC	ATP-binding cassette
ABCB1	ATP-binding cassette sub-family B member 1
P-gp	P-glycoprotein
NDDSs	Nanoparticle-based drug delivery systems
PK	Pharmacokinetics
MPS	Mononuclear phagocyte
APC	Antigen presenting cell

Chapter 1

Introduction

1.1 Background

Cancer is now one of the major causes of death in the world, accounting for around 7.6 million fatalities each year, or 13% of all deaths globally. By 2030, it is anticipated that cancer-related deaths will increase approximately 13.1 million worldwide (Gmeiner & Ghosh, 2014). Breast cancer is the most common type of malignancy among women. and in case of men, prostate cancer is most common with a prevalence of 29% and 28% respectively for each type of cancer. The exact cause of cancer is still unknown at this time. Despite the fact that, the hereditary aspect is considered to play a role in only 5 to 10% of all cancers, other risk factors such as smoking and eating habits can all cause direct gene damage, which can lead to mutations that are carcinogenic. These mutations can then cause cancer (Hosseini et al., 2016). Finding targeted cancer treatments is a massive challenge around the world, even though a number of novel treatment options has been developed. Chemotherapy is the most commonly used approach to treat cancer. There are many ways that chemotherapy works, but its main goal is to kill quickly growing cells, which includes both tumor and healthy cells. This can cause suppression of bone marrow, hair loss, and also digestive problems (Yao et al., 2020). Radiation therapy is yet another treatment method that employs high radiation to destroy cancer cells and shrink tumors. Similar to chemotherapy, there is a chance that radiation can erase or disrupt the healthy cell growth in the same area as cancer cells. Severe adverse effects can result from the destruction of healthy tissues specifically - hair loss, diarrhea, fatigue, sexual problem (both male & female), skin changes, shortness of breath, fertility problem, edema are the serious side effects from the radio therapy (NIH,2020). As

chemotherapy and radiotherapy both cannot provide targeted treatment and killing of healthy cells along with the cancer cells, therefore, there is an imperative need to search for possible treatment options that can only kill cancer cells without destroying healthy cells. In this context, nanoparticles are promising therapeutic agents for targeted drug delivery for treatment of cancer.

1.2 Rationale

Generally, conventional chemotherapy is the well-known treatment for cancer, through the administration of chemical compounds and regimens which have been used for approximately fifty years. Chemotherapy is the most effective method of cancer control (Mondal J et al., 2014). Due to difficulties in dosage sampling, lack of selectivity, which results in cytotoxicity to normal cells as well as rapid drug metabolism, and intrinsic and obtained drug resistances that vary according to patient status, chemotherapy's success has been limited in terms of its ability to treat cancer (Mondal J et al., 2014). Chemotherapeutic agents are developed on the basis of their potential to kill cells that proliferate at a faster rate, which is the primary characteristic of cancer cells. The major problem associated with chemotherapeutic or cytotoxic drugs is that they also kill normal cells which plays a significant role in our body, such as: bone marrow cells, gut cells, and so forth (Mondal J et al., 2014). Chemotherapeutic drugs are also capable of causing skin toxicity, both locally and systemically. A major cause of concern for both patients and doctors is mainly the long-term consequences of cancer chemotherapy which includes lack of specificity and selectivity, allergic reaction, alopecia (loss of hair), cardiotoxicity, hepatotoxicity etc. Targeted drug delivery methods can solve the complications associated with traditional chemotherapy and radiotherapy. Among the recently explored targeted drug delivery systems, nanoparticles have distinct physical, chemical and biological characteristics that make them attractive candidates for cancer therapy (Nurgali et al., 2018). Nanotechnology has proven to show an immense potential in cancer treatment which

are detected early through enhanced scanning, paired with more aggressive use of conventional screening technologies and may improve treatment outcomes and improve cancer patient care. Nanoparticles are the fundamental component of nanomedicine (nanotechnology in medicine), and they have generated significant interest as promising drug delivery systems for the identification and treatment of cancer (Xin et al., 2017).

1.3 Aim & Objective

The review focuses on the different types of nanoparticles used as targeted drug delivery system for cancer treatment and diagnosis. The numerous advantages of nanotechnology for drug delivery has been highlighted, as well as many other areas of opportunity where nanotechnologies may enable the development of novel therapeutic choices for cancer therapy. Finally, the expected challenges to implement such specialized drug delivery methods in cancer treatment has been discussed in details with an overview on the ways to overcome such challenges along with the future prospects of nanoparticles.

Chapter 2

Nanoparticles in Cancer Therapy

2.1 Problems Associated with Conventional Chemotherapy

Chemotherapy is the main method for treating both localized and metastasized cancers. Chemical anticancer drugs are used commonly and mostly given through IV regimens. Even though chemotherapeutic drugs is the most widely applied treatment approach to treat cancer, they pose a number of problems as discussed below: (Kumari et al., 2016).

- There are major excessive side effects, such as: mucositis, suppression of bone marrow (both immuno and myelosuppression), headache, secondary tumors and infertility due to the lack of specificity and selectivity for malignant tissues. Chemotherapeutic drugs usually target general intracellular pathways shared by both malignant and normal cells, rather than malignant-specific intracellular mechanisms. As a result, the cytotoxic and cytostatic effects of these drugs also harm healthy tissues. This is a major disadvantage of traditional chemotherapy in the treatment of cancer (Kumari et al., 2016). As a result, late-stage cancer patients may be more susceptible to early mortality (Mondal J et al., 2014).
- Chemotherapeutic medicines have been linked to a wide range of acute allergic reactions, ranging from transient febrile episodes to rapid hypersensitivity reactions, which include generalized urticaria, bronchospasm, and systemic anaphylaxis, among other symptoms. Erythematous rash, vesicular or bullous eruptions, erythema multiforme, and exfoliative dermatitis are some of the skin problems that might occur. Acute hypersensitivity reactions to chemotherapeutic drugs are varied in their intensity, ranging from mild flushing to life-threatening anaphylaxis. HSRs are mainly four types: i) Type I, which is mediated by IgE, ii) Type II, which is mediated by antibody, iii)

Type III, which is mediated by immune complex, and iv) Type IV, which is mediated by cell (Syrigou et al., 2010).

- Alopecia is the most often occurring adverse cutaneous symptom of chemotherapy. Chemotherapy has a severe impact on body image, sexuality, and self-esteem. To the point that, up to 8% of patients decide to reject chemotherapy if there is a high possibility of hair loss (Rossi et al., 2017). There are many chemotherapeutic agents that cause alopecia on a regular basis and in a dose-dependent manner. Examples of such chemotherapeutic agents are: doxorubicin, vincristine, cyclophosphamide, 5-FU, bleomycin, and methotrexate. Anticancer drugs are mostly directed at the matrix keratinocytes, which are extremely proliferative. These structures are extremely susceptible to toxins and medications, and exposure to either of these can cause them to undergo fast apoptosis (Rossi et al., 2017).
- Cardiotoxicity is caused by a variety of processes in different chemotherapeutic treatments, including cardiomyopathy, hypercoagulability, arrhythmia, and inflammation (Chang et al., 2018). These include heart problems, angina, (an acute coronary syndrome) and heart attack, a long QT, hypertension, pericardial effusion, cardiac surgery, and pulmonary fibrosis and hypertension, which are all potential risks of cancer chemotherapy, as well as pulmonary hypertension and pulmonary embolism. People who already have heart conditions are more likely to have cardiac problems caused by chemotherapy (Jain & Aronow, 2019). The most severe symptom of chronic cardiotoxicity is asymptomatic systolic or diastolic dysfunction, which can progress to irreversible heart failure and even death if not monitored closely over time by qualified healthcare professionals (Chang et al., 2018).
- Toxic liver damage or hepatotoxicity caused by chemotherapy can cause tissue damage, steatosis, necrosis, liver dysfunction, and vascular damage, among other things (King

& Perry, 2014). Certain chemotherapeutic drugs must be used with great care in patients who have already had liver damage as a result of other treatment. The following are some examples of such chemotherapy agents that can have adverse effects on liver function: anthracyclines, taxanes, vinca alkaloids, imatinib, axitinib, lapatinib, nilotinib, pazopanib, ponatinib, ruxolitin, and ruxolitin (Grigorian & O'brien, 2014). Chemotherapeutic drugs, either alone or in combination, which have the ability, to produce hepatotoxicity, and impaired liver function has the potential to change drug metabolism and increase the risk of non-hepatic toxicity (King & Perry, 2014).

2.2 Nanoparticles

Recent advances in nanomedicine have significantly improved cancer treatment by overcoming the problems associated with conventional chemotherapy treatment. The development of this novel drug delivery system utilizing nanoparticles has resulted in substantial commercialization initiatives around the world, with numerous pharmaceuticals already on the market and others in the development pipeline (Bamrungsap1 et al., 2016). Nanoparticles for drug delivery are often selected for their size, shape, and properties depending on the pathophysiology of the malignancies they are meant to treat (Yao et al., 2020).

Nanoparticles are minuscule particles with dimensions ranging from 1-100 nm (Aghebati-Maleki et al., 2020). They have huge surface area and enhanced reactivity due to their small size range (Ranghar et al., 2014), so that they can circulate more easily within the body than larger materials (Patra et al., 2018). From various range of materials, nanoparticles can be synthesized including polymers (e.g. polymeric nanoparticle vesicles) and lipids (e.g liposomes). Additionally, viral nanoparticles can be synthesized from viruses (viral nanoparticles) or inorganic nanoparticles (Xin et al., 2017). Their unique physical qualities, such as permeability, stability, and the optical characteristics, make them perfect for use in

biological and materials development. They are classified into distinct groups depending on their physical characteristics, as well as their shape and size. Fullerene, ceramic nanoparticles, metal nanoparticles, and polymer nanoparticles are examples of these types of nanoparticles (Aghebati-Maleki et al., 2020). Nanoparticles which are composed of metals and metal oxides have the potential to be effective antibacterial agents against infections that do not develop resistance to them. There are two therapeutic nanocarriers which are FDA-approved for clinical use: liposomes and albumin nanoparticles. A further example of a nanoparticle application based on enhanced permeability and retention (EPR) effect is the liposomal doxorubicin and albumin bound PTX (Abraxane1), which is used to treat breast cancer (Misra et al., 2010).

Nanoparticles based drug delivery system have lots of potential benefits for the treatment of cancer, for instance: improved pharmacokinetics, specific targeting to the tumor cells with minimal adverse effects, and less resistance to drugs (Yao et al., 2020). A further application of nanotechnology is the development of diagnostics that are quick, accurate, and cost-effective in the identification of cancer cells (Ranghar et al., 2014). Besides, nanoparticles can control and sustain drug release at the site of therapeutic action. This can change the distribution and clearance of the drug, which can improve therapeutic effectiveness and reduce toxicity (Bamrungsap1 et al., 2016). Nanoparticles are also called nanovehicles because they are used for conjugated drug delivery. In cancer therapy, tumor cells and the supporting microenvironment are the focus of a drug delivery system based on nanovehicles. Nanovehicles that deliver therapeutic drugs to tumor cells, cancer-initiating cells, and the supportive cancer cell microenvironment are being developed (Chaturvedi et al., 2018). Moreover, the remarkable properties of nanoparticles such as ability to being encapsulated, distributed, absorbed, or combined with other drugs makes them more effective in a wide range of dosage forms. When drug particles are formulated using optimized techniques, they offer numerous advantages such as greater solubility, quick dissolution, and better adhesion to biological

surfaces, which can speed up the start of treatment and improve bioavailability. Importantly, nanoparticles reduce the dose required to elicit the therapeutic action, thus reducing side-effects associated with higher doses of drugs (Bamrungsap1 et al., 2016).

2.3 Classification of Nanoparticles in Cancer Treatment

Drug-carrying nanoparticles are usually classified into two types are: organic and inorganic nanoparticles. These groups may have several subcategories. The first group includes drug carriers primarily formed of organic particles which includes liposomes, dendrimers, solid-lipid nanoparticles and polymers (Aghebati-Maleki et al., 2020). The second group includes drug carriers primarily made of mineral particles, such as: gold nanoparticle, carbon nanotubes, quantum dots, and silica nanoparticle. In addition to these two categories, an additional category is hybrid nanoparticles. Hybrid nanoparticles are combination of organic and inorganic nanoparticles. Hybrid nanoparticles include lipid-polymer, liposome-silica, cell membrane coated, gold-based, and other nanoparticles.

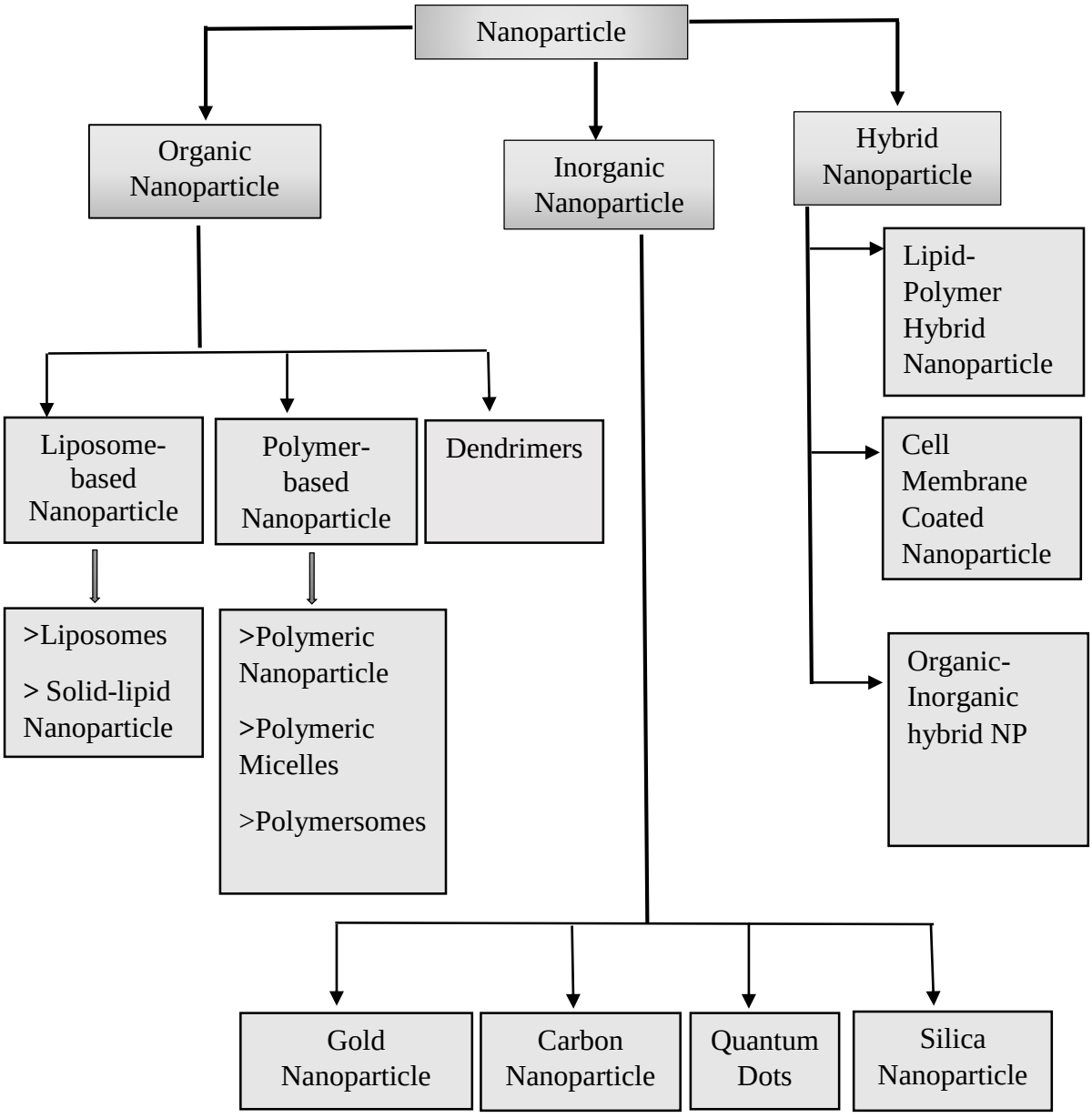

Table 1: Types of nanoparticles used in cancer treatment

2.3.1 Organic Nanoparticles

Over the last few years, organic nanoparticles have been extensively explored and contain many different types of components (Yao et al., 2020). Organic nanoparticles are solid particles that are mostly made of organic molecules, like lipids or polymers, with diameters that range

from 10 nm to 1 m. A number of researches have been done recently to find out more about organic nanoparticles. Organic nanomedicine development has resulted in the development of advanced techniques and the upgrading of existing ones. Organic nanoparticles can be dissolved in water or other liquids, so they won't stay in the environment for a long time. This makes them more environmentally friendly than their inorganic counterparts (Shatrohan Lal, 2014).

2.3.1.1 Liposomes

Liposomes are a well-known clinical nanoparticle used to deliver cytotoxic medicines, antifungal agents, and vaccines (Cheung & Al-Jamal, 2018). Alec D. Bangham made the first discovery of the liposome in 1961 (Aghebati-Maleki et al., 2020). Liposomes are artificial vesicles which are made of amphiphilic phospholipids. Its size ranges from 50 nm to 300 mm and it has a lipid bilayer structure around an aqueous core domain (Bamrungsap1 et al., 2016). Liposomes can perform many functions by changing the structure of their lipid layer. For example, they can copy the biophysical features of living cells, like how they move and change shape, which help them to achieve their target for more successful therapeutic action in drug delivery (Yao et al., 2020). Liposomes are transmitted from the bloodstream to their site of action through extravasation into the interstitial space. Liposomes can target specific cells using both passive and active targeting strategies, depending on the situation (Malam et al., 2009). As a result of decades of research, liposomes have gone through several stages of development. Using liposomes to transport anti-cancer medications to tumors in the body is a useful method in cancer therapy development (Yao et al., 2020). A lot of new liposomal formulations are now on the market, like Doxil (active ingredient: Doxorubicin), DepoCyt (active ingredient: cytarabine liposome injection), and ONCO-TCS (active ingredient: liposomal vincristine. Furthermore, they feature a specific shape that allows them to load hydrophilic medications into the aqueous core while also loading hydrophobic drugs onto the lipid membrane (Hosseini

et al., 2016). Liposomal nanoparticles have shown greater therapeutic efficacy in the treatment of breast cancer and prostate cancer. Breast cancer treatment with liposomal doxorubicin has been shown to be less toxic to the cardiovascular system and have greater efficacy. Liposomal and paclitaxel have demonstrated enhanced anti-tumor efficiency with much greater bioavailability compared to free paclitaxel. A further advantage of liposome-based nanosystems is that, they can be used to combine drugs, which can improve the therapeutic response and even reverse drug resistance (Yao et al., 2020). Moreover, when compared to free medications, liposomal nanoparticles show lower toxicity and higher efficacy retention. It is a potential alternative for numerous delivery systems due to its ease of chemical modification, ability to transport drugs and genes, and versatility of administration (Panahi et al., 2017).

2.3.1.2 Solid-Lipid Nanoparticles

Solid-lipid nanoparticles are mainly made up of a matrix of solid lipid nanoparticles including: triglycerides, steroids, lipids, waxes and fatty acids (Aghebati-Maleki et al., 2020). They are developed as a carrier system for water-soluble medications (Yadav et al., 2013). They are less than 1 micron in size (Aghebati-Maleki et al., 2020). Advantages of using solid lipid nanoparticles include suitable physical stability and tolerability, controlled drug release, site-specific targeting and protection of labile drugs from degradation. However, there are certain drawbacks associated with the use of solid lipid nanoparticles such as limited loading capacity and leakage of drugs following crystallization process. Therefore, to overcome these problems modified solid-lipid nanostructures are used such as lipid drug conjugates (LDC) and nanostructured lipid carriers (NLC) (Hosseini et al., 2016). They have a number of favorable characteristics, including high encapsulation efficiency so greater loading of drugs, small diameter with a large surface, with improved stability enabling longer therapy duration (Yadav et al., 2013), (Scioli Montoto et al., 2020).

2.3.1.3 Polymeric Nanoparticles

Polymer-based nanoparticles have particular structural configurations for drug delivery and are made up of several monomers (Yao et al., 2020). Their size ranges from 10-100 nm. They can be in round, branched, or core–shell. They can be found in a variety of shapes and sizes, too. Natural polymers include albumin, gelatin, collagen, alginate, and chitosan while synthetic polymers include: polylactide-polyglycolide copolymers, polyacrylates, polycaprolactones are all biodegradable materials (Bamrungsap1 et al., 2016). Polymer nanoparticles could be a game changer in the treatment option for cancer (Madkour, 2019). They have a number of advantages including the capacity to control release, protect drugs from other chemicals and other biological processes and increase bioactivity and therapeutic efficacy (Zielinska et al., 2020). Other distinguishing characteristics include their distinctive size and shape - properties which makes them easier to get into tissues through passive and active targeting, specific intracellular or subcellular transportation mechanisms (Madkour, 2019). Polymeric nanoparticles can be constructed in various ways, depending on the medicine they contain and how they are delivered (Zielinska et al., 2020). A common polymeric nanoparticle, Polylactic-co-glycolic acid (PLGA), a common polymeric nanoparticle, is formed through the copolymerization of glycolic and lactic acid. Owing to its excellent biodegradability and biocompatibility, including the EPR effect, PLGA is often utilized as a carrier for drugs (Yao et al., 2020).

2.3.1.4 Polymeric Micelles

Polymeric micelles are a type of nanoscale drug delivery systems that are made up of blocks of amphiphilic surfactants or co-polymers that spontaneously self-assemble in aqueous medium to form core-shell structures. The hydrophobic inner core of the micelle is surrounded by a shell composed of hydrophilic polymers such as polyethylene glycol (PEG) (Ghezzi et al., 2021). The hydrophobic core enables loading of insoluble and amphiphilic anti-cancer drugs while the hydrophilic shell add stability to the inner core, thus prolonging circulation time in

blood and subsequent accumulation in tumor tissue. The critical micelle concentration (CMC), the total number of micelles, length of the polymer chains, the formation and size of the structural system are critical factors that affect the formation of these polymeric micelles. These micelleshave a lower CMC, which means that polymer micelles with a lower CMC are better at dissolving drugs and keeping the micelles together than their higher CMC counterparts (Aghebati-Maleki et al., 2020). Polymer micelle structure design is becoming increasingly advanced in order to optimize drug-loading capacity, tumor-specific absorption, and anticancer efficacy (Y. Zhang et al., 2014). Prevention of the drug from the harsh GI environment, controlled release at target sites, prolonged gut residence time via mucoadhesion, and efflux pumps inhibition to increase drug absorption are all advantages of using polymeric micelles (Xu et al., 2013). Polymeric micelles can encapsulate or covalently attach different medicines (Bamrungsap1 et al., 2016). Two polymer micelles, NK911 and NK105, are now available in market. They contain doxorubicin and paclitaxel, both have been approved by FDA (Aghebati-Maleki et al., 2020).

2.3.1.5 Polymerosomes

Polymersomes are water-soluble amphiphilic copolymers with a bi-layer structure. They will become a three-layer structure if three block copolymers are present. Unlike liposomes, these structures are less cell-permeable (liposomes have a phospholipid visceral structure). The hydrophobicity of the copolymer increases with length. The main advantage of using polymerosomes is controlled release of drugs. Unlike liposomes, copolymers have greater mechanical and biological stability than liposomes due to a much lesser extent of visceral and macrophage interference with co-polymers, thus enabling greater protection of the anticancer drug (Aghebati-Maleki et al., 2020).

2.3.1.6 Dendrimers

Dendrimers are monodispersed nanoscale systems composed of branched macromolecules with a tree-like structure of specific shape and size (Abbasi et al., 2014). Examples of molecules used to synthesize dendrimers including poly (propylene imine), poly(glycerol-co-succinic acid), poly(L-lysine), poly (glycerol), poly(2,2-bis(hydroxymethyl)propionic acid) (Hosseini et al., 2016). They are very uniform, have minimal polydispersities, and are commonly generated in approximate nanometer steps from 1 to 10 nanometers in size. As these macromolecules have globular forms and internal voids, drugs can be encased within them and released slowly from the inner core. The architecture and branching of dendrimers allow anti-cancer drugs to be loaded onto the structure's outer surface by covalent or electrostatic interactions (Bamrungsap1 et al., 2016). They have shown enhanced physicochemical and rheological properties compared to linear polymers, however they are non-biodegradable and therefore can accumulate within the cells and cause cytotoxicity and hemolysis. Changing the size and surface functionality of the dendrimers using polymers like polyethylene glycol (PEG) can increase the drug loading and enable prolonged circulation in blood and escaping removal by the reticulo-endothelial system, overall making them an attractive candidate for cancer therapy (Abbasi et al., 2014). The recently developed PEGylated polyamidoamine (PAMAM) is the dendrimers that has been explored for the delivery of the anti-cancer drug 5-fluoruracil (5-FU) that showed controlled release of the drug with reduced hemolytic toxicity (Chauhan, 2018). These studies show the substantial potential of dendrimers in nanotechnology based cancer therapy.

2.3.2 Inorganic Nanoparticles

Inorganic nanoparticles are non-toxic, biocompatible, hydrophilic, and chemically stable drug-delivery systems that has been utilized for cancer therapy. (Sharma, 2010). Inorganic nanoparticles have the advantage of having a larger surface area to the ratio of the volume than

organic nanoparticles, thus allowing broad and easily modifiable surface conjugation chemistry. They are also relatively simple to prepare but may result in weaker biological and biodegradable properties which again requires surface modification (Yao et al., 2020). Gold, silica and iron all have been utilized to manufactured nanostructured based materials for cancer therapeutic administration and imaging. They can be manufactured in many sizes, dimensions, and geometries depending on their use (Mitchell et al., 2021).

2.3.2.1 Gold Nanoparticles

Gold nanoparticles, often termed as colloid gold, are most stable metal nanoparticles and so play a vital role in the constantly increasing fields of nanotechnologies (Sharma, 2010). AuNPs are the most extensively explored inorganic nanoparticles for cancer therapy and diagnosis, with a mixed monolayer-protected clusters inside the gold core as an anti-cancer drug delivery systems. The gold core is not reactive, non-toxic, and surface-functionalized AuNPs has demonstrated increase drug concentration within tumors while also overcome drug resistance (Yao et al., 2020). In addition to physical methods like microwave and UV radiation, sputtering has also been used to create gold nanoparticles with specified size and shape. Cancer research and cell biology have both benefited from the usage of AuNPs, which are available in a variety of forms including gold nanorods, nanocages, nanostars, nanostructures and nanospheres. Its favorable optical characteristics and physical properties makes it a promising candidate for cancer therapeutic applications, that can be utilized in cancer diagnostics and therapy (P. Singh et al., 2018). Hyperthermia can be used to eliminate tumors when AuNPs are focused to the disease location. Additionally, AuNPs can be further explored for multi-modal treatment of cancer using gene therapy, photothermal therapy and immunotherapy (Yao et al., 2020).

2.3.2.2 Carbon Nanotubes

Carbon nanotubes (CNTs) are a type of tubular substance with unique physiological, structural, and chemical compositions that have shown promising potential in the delivery of drugs for cancer treatment (Yao et al., 2020). CNTs are generally divided into two categories according to their layer number: i) Single-walled carbon nanotubes (SWCNTs), which using a single layer of cylindrical graphene ,, and ii) Multi-walled carbon nanotubes (MWCNTs), which are made up of many layers of graphene sheets (Guo et al., 2017). A lot of the physical and chemical characteristics of CNTs are linked with their configuration, surface area, good physical strength, metallic conductivity, and light weight. CNTs have a variety of physical and chemical characteristics that make them appropriate for a wide range of biomedical uses (Chaturvedi et al., 2018). The cylindrical shape of CNTs makes it easier for them to cross the blood brain barrier (BBB). This allows intracellular CNTs to get through the BBB. Along with the property of being a photo-thermal conductor, CNTs are also capable of absorbing light, photoluminescence, and making strong Raman signals. These features make CNTs a suitable candidate to treat cancer cells with photothermal or photoacoustic methods. CNTs also have a lot of surface area, which enables them to load higher amount of drugs into them. CNTs also have high surface area and which makes it easier for them to be chemically functionalized (Guo et al., 2017). The cell penetrating characteristics of CNT allow for the usage of f-CNT as drug delivery vehicles (Bianco et al., 2005). Anticancer drugs, including: methotrexate, doxorubicin, and paclitaxel are delivered to cancer patients using CNTs (Yao et al., 2020).

2.3.2.3 Quantum Dots

Quantum dots (QD) are semiconducting nanoparticles or nanomaterials discovered by Ekimov and others in 1980. They range within the size from 2-10 nm (Chaturvedi et al., 2018). These materials are often created by combining binary combinations of a variety of semiconductors (ZnS, CdS, CdSe, InP, CdTe, PbS, PbTe) (Delehanty et al., 2009). Many QD technologies have

evolved throughout time, such as modified QD conjugates and QD immunostaining. The enhanced multiplexing capabilities of modified QDs conjugates allow for faster and cheaper tests than single color assays. Moreover, compared to typical immunochemistry experiments, QD immunostaining is more precise at reducing protein expression and has less background. It can identify tumor biomarkers such as cell proteins or additional components in various tumor patterns. QDs can deliver cancer drugs to specific body regions (Chaturvedi et al., 2018). The benefits of QDs as a delivery of drug and targeting technique in the treatment of cancer is an attractive option. The use of QDs for targeted drug administration has generated a lot of interest. Their remarkable features have led to a variety of research (Badıllı et al., 2020). Paclitaxel, a widely used anti-cancer drug used to treat many types of human cancer, was mixed with CdS, CdTe and ZnS in nanostructured lipid carriers to achieve a therapeutic and diagnostic effect in cancer therapy (Matea et al., 2017). Anti-cancer drugs doxorubicin and 5-FU can also be delivered by incorporating them into the QDs. Furthermore, QDs have the potential to be used as molecular probes to detect various types of cancer early such as pancreatic cancer (Badıllı et al., 2020). The transferring receptor, antigen claudin-4, and urokinase plasminogen activator receptor are all used as QD-based imaging probes (Chaturvedi et al., 2018). Surface functionalization with optical property enables construction of various types of QDs that offer advantages of greater drug loading, targeting, controlled release and monitoring of pharmacokinetics and biodistribution (Badıllı et al., 2020). QDs are also cross-linked with amino-functionalized immune liposomes for cancer diagnostics and targeted drug delivery for the treatment of HER2 overexpressing human breast carcinoma cells, SK-BR-3 and MCF7-C18 (Chenthamara et al., 2019). As QDs have wide application in anti-cancer drug delivery, detection, and bioimaging, they are continually being combined with other nanoparticles and physiologically active compounds to provide new therapeutic and diagnostic platforms for cancer treatment (Matea et al., 2017).

2.3.2.4 Silica Nanoparticles

Nanocarriers that are made of silica, like MSNs, have been shown to be excellent drug delivery methods for tumors due to their numerous benefits. Valeri and coworkers first discovered this in the early 2000s (Yang & Yu, 2016). Pure alkoxysilanes, such as TEOS, are used to make silica nanoparticles, while. Wide range of compounds, like: polymers and fluorescent dyes, which can be used to enhance the capabilities of these silica nanoparticles for drug administration and tracking, making them more efficient (Ways et al., 2020). Recently, the nano-form of MSN has attracted the curiosity of many researchers who are interested in its possible application in pharmaceuticals as a nano-drug delivery system. Many various designs have been implemented for MSNs as nano-drug delivery system in order to optimize their performance. Due to the fact that MSNs can be modified to make them more effective by altering their shape, size of pore, and the characteristics of the pores and the surface functions that help them at carrying drugs. There are numerous advantages to using MSNs as drug carriers, including the following: biocompatibility of the carrier material, the ability of targeting the specific cell types, and the ability to achieve effective local concentrations of drugs via controlled release of drug molecules (Karaman & Kettiger, 2018). Hollow MSNs are being considered for use in chemotherapy because of their great drug-loading ability and simplicity of surface functionalizing. Because these MSNs include hollow interstitial spaces and mesopores on the outer shell, they may accommodate large amounts of bioactivities (Poonia et al., 2018).

2.3.3 Hybrid Nanoparticles

Hybrid drug delivery systems combine the advantages of both organic and inorganic nanoparticles, boosting therapeutic efficacy and reducing drug resistance, even though both nanoparticles have their own advantages and disadvantages (Yao et al., 2020). Hybrid nanostructures are made up of minimum two different materials. Unlike single-component

nanoparticles, multi-component nanocarriers may perform several roles and overcome the limits of single-component nanoparticles (Ma, 2018). Hybrid nanoparticles can perform multiple functions at the same time such as detection and diagnosis of diseases, like cancer. These theranostic hybrid nanoparticles would make it easier for the doctor to keep track of the progress of cancer and the success of cancer treatment (Sailor & Park, 2012). Hybrid nanoparticles may be able to help fight cancer by changing their formulations, surface properties, and pharmacokinetic patterns. In the field of nanomedicine, hybrid nanoparticles are thought to have a great future (He et al., 2015).

2.3.3.1 Lipid-Polymer Hybrid Nanoparticles

Lipid–polymer hybrid nanoparticles (LPHNPs) are a new type of core–shell nanostructures that are based on liposomes and polymer nanoparticles. A polymer core is inside a lipid layer (Mukherjee et al., 2019). LPHNPs have significant advantages, such as low production costs and better ability to encapsulate therapeutic agents. LPHNPs also have a high level of biocompatibility, which means that the whole nanovector can be broken down and used by the body. Since the structure of the core and lipid shell can be changed, these particles can change in shape and surface properties. This can make them more efficient at capturing drugs and releasing them at the right time. Furthermore, LPHNPs tend to be very durable both when they are stored and when they are in the bloodstream. They are difficult for the immune system to locate due to their polymeric core cytoskeleton and PEG-coated lipid coating and therefore can escape clearance by reticulo-endothelial system thus can be retained by the cancer cells indicative of prolonged therapeutic action (Persano et al., 2021). LPHNPs have shown promising applications for the following cancers treatment: pancreatic, breast, and metastatic prostate cancer (Yao et al., 2020).

2.3.3.2 Cell Membrane Coated Nanoparticles

Cell membrane nanoparticles have been recently getting a lot of attention because they can copy many of the natural features shown by the cells that made them, which is why they are now so promising (Fang et al., 2014). This method could make nanoparticles that have biological characteristics by trying to coat them with cell membranes that are made by naturally occurring cells. This could make nanoparticles that have biological features more potent and safe mimicking the natural environment of the body. Surfaces are coated with membranes made by cells like leukocytes and red blood cell membranes. Platelets, cancerous cells, and even microorganisms are also used. Putting a cell membrane made from leukocytes on top of nanoporous silicon particles can prevent elimination by phagocytes, and this hybrid particle allows the drug to circulate in the blood for a prolonged time, which leads to greater amount of drug accumulation within the tumor, enabling longer duration of action of the anti-cancer drug. Several studies have used mesoporous silica nanoparticles that have been coated with cancer cell membranes to treat cancer. This enhances nano-carrier's durability and selectivity, as well as their targeting ability (Yao et al., 2020).

2.4 Current Trend of Nanoparticles in Cancer Therapy

Cancer is the world's second largest leading cause of death. With any cancer treatment, the most important factor is achieving maximum concentration of the therapeutic substance in the tumor site while causing the least amount of damage to normal cells. The development of anticancer drugs in a single drug delivery system with multi-functional potential of cancer detection and treatment using nanoparticles has been a major advancement in the area of cancer therapeutics (Misra et al., 2010). Nanoparticles were first discovered about 35 years ago and have since become widely used. The first use for these vehicles were as carriers for vaccinations and cancer chemotherapeutic drugs (Kingsley et al., 2006). The unique optical, electromagnetic and structural features of nanoparticles, such as: semiconductors, quantum dots, and iron-oxide

nanomaterials that cannot be found in biomolecules or bulk material, are exploited in cancer research for diagnostic and therapeutic purposes. There are many different cytotoxic drugs that can be mixed with nanoparticles so that they can target cancer cells with a high affinity and specificity. This combination is particularly attractive at screening cancer cells at the very beginning stages of cancer (Chaturvedi et al., 2018). Nanocarriers alter the pharmacokinetic parameters of drugs, allowing them to be more effective while also having less adverse effects (Aghebati-Maleki et al., 2020). Nanoparticles possess different chemical and physical characteristics and allows surface functionalization which makes it a favorable candidate for treating cancer (Hosseini et al., 2016). Size of the nanoparticles is one of the key factors that modulates the development of nanoparticle based drug delivery system for cancer therapy. Nanoparticles with diameters between 10 and 100 nm are usually used to treat cancer as they can effectively deliver drugs with enhanced permeability and retention (EPR) effect. Particles less than 1–2 nm in diameter, can easily leak out of the normal vasculature and destroy normal cells and smaller particles less than 10 nm in diameter can also be easily eliminated via filtration by the kidneys. Larger particles (over 100 nm in diameter) are more likely to be cleared from the systemic circulation by phagocytosis. Modification of surface properties of nanoparticles can significantly alter the bioavailability and half-lives of the anti-cancer drugs (Yao et al., 2020), (Fukumori & Ichikawa, 2006). A classic example of such surface modification of nanoparticles for cancer therapy is nanoparticles coated with hydrophilic compounds like polyethylene glycol (PEG) that has shown to inhibit opsonization and thus immunological clearance. Therefore, hydrophilic nanoparticles are widely used owing to the advantages of extended drug circulation and enhancing tumor penetration and accumulation resulting in greater therapeutic efficacy and targeted killing of cancer cells (Yao et al., 2020).

Furthermore, the nanoparticles can be developed to be pH- or temperature-sensitive carriers, depending on the application. Due to the defect in the lymphatic system drainage in cancer,

drugs that entered the tumor affected area after crossing the systemic circulation accumulate within the tumor area for a prolonged period of time thus eliciting enhanced therapeutic effect for a longer time. The different types of nanoparticles namely liposomes, polymers, polymeric micelles discussed earlier all utilize this phenomenon for targeted delivery of anti-cancer drugs to tumor tissues. This physiological state in cancer disease changes the environmental conditions of cancer cells making the temperature of the tumor infected area greater than 40°C and pH lower than 5.4 (Aghebati-Maleki et al., 2020). This paves a way for the use of temperature-sensitive and pH-sensitive system to carry and release drugs in response to changes in temperature and pH in the tumor area and thus allows for combined therapies like chemotherapy and hyperthermia to be used (Nguyen, 2011). The EPR effect allows nanoparticles to target tumors more precisely, opening up new cancer treatment options. In addition, these nanoparticles are capable of carrying a wide range of medications, allowing the nanocarriers to transfer the medicines to tumor site without harming the normal healthy cells. Nanoparticles that have already been approved for cancer treatment are shown in Table 2 and the nanoparticles based drug delivery systems that are undergoing clinical trials are shown in Table 3 to highlight the current trend of nanoparticles in cancer treatment.

Nanoparticles Types	Drug	Name	Therapeutic Impact	Status	Company
Polymeric (PLGA-PEG)	Docetaxel	BIND-014	Solid tumor and metastatic cancer	Phase I	BIND Bioscience
Liposome	Oxaliplatin	MBP-426	GI tract and esophagus	Phase Ib/ II	Calando Pharmaceuticals
Glutathione PEGylated Liposome	Doxorubicin	2B3-101	Solid malignancies, brain cancers	Phase I/ IIa	Pharmaceuticals, Inc BBB Therapeutics B.V
Polymeric	siRNA	CALAA-01	Solid malignancies	Phase I	Calando Pharmaceuticals
Liposome	Dexamethasone	CD74-IL-DEX	B-cell tumor	Preclinical	Immunomedics, Inc.
Polymeric micelle	Doxorubicin	NK911	Pancreatic cancer	Phase II	Nippon Kayaku
Colloidal gold	TNF-α	Aurimmune (CYT-6091)	Head cancer, neck cancer	Phase I	CytImmune Sciences, Inc.
Polymer	Docetaxel	Docetaxel-PNP	Solid malignancies	Phase I	Samyang Pharmaceuticals
Polymeric micelle	Paclitaxel	NK-105	Breast cancer	Phase III	Nippon Kayaku

Table 2: Nanoparticles currently in clinical trials (Hosseini et al., 2016)

There are some nanoparticles based drug is already in the market which are approved by FDA and other countries heath care institutions. These drugs are extensively used to treat cancer.

Nanoparticles Types	API	Trade Name	Therapeutic Indications	Advantages	Approval
Liposome	Doxorubicin	Doxil	Sarcoma, ovarian Cancer and multiple myeloma	Greater efficacy; less the systemic toxicity	FDA-approved (1995)
Liposome	MTP-PE	Mepact	Non-metastatic osteosarcoma	Less poisonous; longer the half-life	Europe-approved (2009)
Polymer-Based	Interferon alpha-2a	Pegasys	Hepatitis B and Hepatitis C	Prolonging retention time; enhancing protein stability	FDA-approved (2002)
Liposome	Daunorubicin	DaunoXome	Karposi's sarcoma	Greater efficacy; less the systemic toxicity	FDA-approved (1996)
Inorganic	Iron-oxide	Nanotherm	Glioblastoma	Generated by magnetic field	Europe-approved (2010)
Polymer-Based	Paclitaxel	Opaxio	Head cancer, neck cancer	Glioblastoma polyglutamate enzymatic release	FDA-approved (2012)
Inorganic	Iron oxide	Feraheme	Iron deficiency and anemia	Improved release stability, safe in kidney disease	FDA-approved (2009)
Polymer-Based	T-DM1	Kadcyla	Breast cancer metastasis	Improved delivery	FDA-approved (2013)
Liposome	Irinotecan	Onivyde	Pancreas cancer	Improved delivery; less toxicity	FDA-approved (2015)
Inorganic	Iron-oxide	Feridex	Contrast agent for MRI	uperparamagnetic characteristics	FDA-approved (1996)
Liposome	Verteporfin	Visudyne	Age-related macular degeneration	Good for photosensitive drugs	FDA-approved (2000)

Table 3: Some approved nanoparticles on the market (Chatterjee et al., 2008)

Chapter 3

Drug Targeted Approaches by Nanoparticles

Targeted drug delivery utilizing nanoparticles has been shown to be an useful strategy for addressing the lack of specificity of drug delivery in chemotherapy treatments (Hosseini et al., 2016). There are numerous advantages of targeted drug delivery system, include: protection of healthy cells from harmful substances, reducing adverse effects and fighting drug-resistant cancerous cells (Attia et al., 2019). Besides, understanding tumor biology and how nanocarriers interact with tumor cells is critical to successfully targeting and controlling tumors. Methods of targeting cancer cells fall into two main categories: (1) passive targeting and (2) active targeting (Yao et al., 2020).

3.1 Passive Targeting

Passive targeting is based on the distinguishing characteristics between tumor and normal tissue (Yao et al., 2020). Passive targeting therapeutic approaches involves a development of a drug carrier complex that prevents the drug from being eliminated from the body via various physiological functions, such as: metabolic activities, excretion, opsonisation, and phagocytosis, thus enabling the drug to remain in circulation for a prolonged period of time and is subsequently delivered to its target site (Kumar Khanna, 2012). Direct drug injection or catheterization exemplify such types of passive drug targeting.

The rapid rate of cancer cell proliferation makes the endothelium of the blood vessels more permeable than the healthy cells, therefore enabling the fast growing tumor cells to recruit new blood vessels or utilize the existing blood vessels (Attia et al., 2019). These newly formed leaky vascularization allows macromolecules of 400 nm or larger to leak from the blood vessels that supply the tumor and thus move into tumor mediated area to give a therapeutic effect (Bamrungsap1 et al., 2016). Furthermore, there is an absence of normal lymphatic drainage in

tumor tissues and this enables retention of NPs and eventual release of the nanocarrier content to the tumor cells (Attia et al., 2019). All these processes summarizes the enhanced permeability and retention (EPR) effect which is a key driver of passive targeting (Kumar Khanna, 2012). The EPR process relies on the nanoparticle range of sizes and two important features of cancerous tissues: leaky vasculature and inadequate lymphatic drainage (BAZAK et al., 2014). Thus, passive targeting allows drugs to be transported to the target site to elicit a therapeutic effect with minimum adverse effects.

Another important factor that contributes to passive targeting of nanoparticles for cancer treatment is the environment around the cancerous cells known as the tumor microenviroment which is different from the healthy cells which makes it easier for passive targeting to operate. Tumor cells need more oxygen and nutrients because they are growing so quickly. As a result, glycolysis being one of the distinguishing feature of cancerous cells is stimulated to get more energy, which leads to an acidic environment. As such, pH-sensitive liposomes have been developed so that they can stay stable at regular pH of 7.4, but break down at an acidic pH so that the drug molecules can be released (Bamrungsap1 et al., 2016).

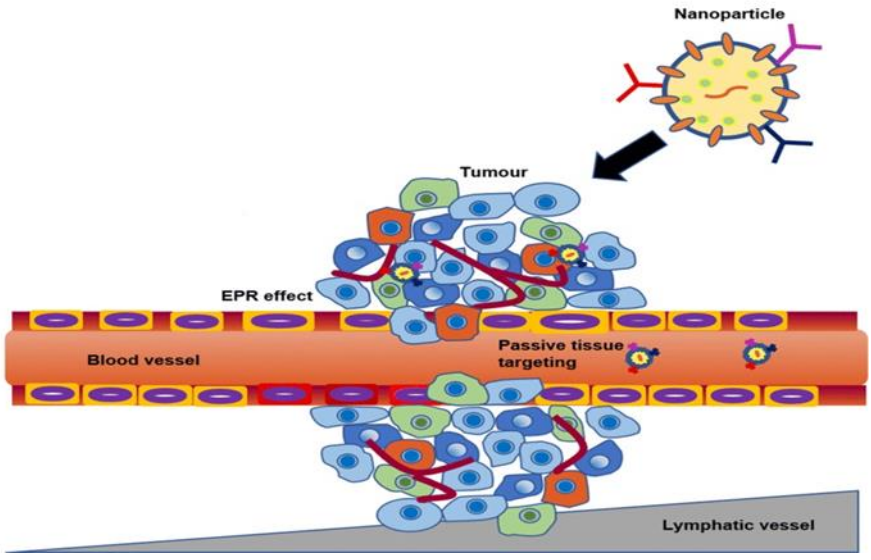

Figure 1: Passive Targeting Drug Delivery (Adapted from Subramaniam et al., 2020)

However, there are certain drawbacks associated with passive targeting. Nanoparticles are easily recognized as foreign entities leading to opsonization by plasma proteins in the blood stream and they move to the reticulo-endothelial system, eventually causing rapid clearance from the systemic circulation (Talekar et al., 2011). Therefore, in order to increase the circulation time of the nanoparticlies, hydrophilic polymers such as polyethylene glycol (PEG) or PEG containing copolymers (poloxamers, poloxamines, and polysorbates) are attached to the surface of the nanoparticles. Utilizing these polymers, passive targeting has beensuccessfully utilized in clinics with nanocarriers among them DaunoXome (liposomal daunorubicin; Gilead Sciences, Foster City, California, USA) and Doxil (PEG-coated liposomal doxorubicin; Centocor Ortho Biotech, Raritan, New Jersey, USA) are the first two nano-particles based drug delivery systems against cancer that were approved by the US Food and Drug Administration (FDA) (Yao et al., 2020). Although passive targeting can target the anti-cancer drugs directly into the tumors, the drug has to internalized to elicit a therapeutic effect which is achieved by active targeting.

3.2 Active Targeting

The development of disease-specific biomarkers in the nanomedicine field has paved the way for an active targeting strategy to circumvent some of the drawbacks of passive targeting. Active targeting requires a component being attached to the nanocarrier that recognizes the malignant cells organ, tissue, or intracellular organelle as a target (Talekar et al., 2011). Active targeting can significantly increase drug delivery to target cells compared to passively targeted nanosystems (Attia et al., 2019).

Active targeting targets cancerous cells by directly binding ligands to receptors. To distinguish between targeted and healthy cells, nanoparticles' ligands are designed to selectively target molecules overexpressed on the surface of the cancer cells (Yao et al., 2020). Nanocarriers detect and bind target cells via ligand–receptor interactions via surface receptors or epitopes.

The receptors must be expressed overly on tumor cells but not on the normal cells. The ligands should be produced consistently so that it does not leak into the blood vessels. After binding with target cells, targeting conjugates can undergo receptor mediated endocytosis, allowing for drug release inside the cells. The plasma membrane incorporates the ligand–receptor combination to form an endosome. Acidic pH or enzymes may release medicines from the newly created endosome (Bamrungsap1 et al., 2016). Active targeting is preferred for macromolecular drugs like proteins and siRNAs. Amino acids, monoclonal antibodies, peptides, vitamin supplements, and carbohydrates are all targeting moieties. These ligands only bind with receptors on target cells. The glycoproteins, transferrin receptor, folate receptor, and EGFR are among the most recognized receptors (Yao et al., 2020).

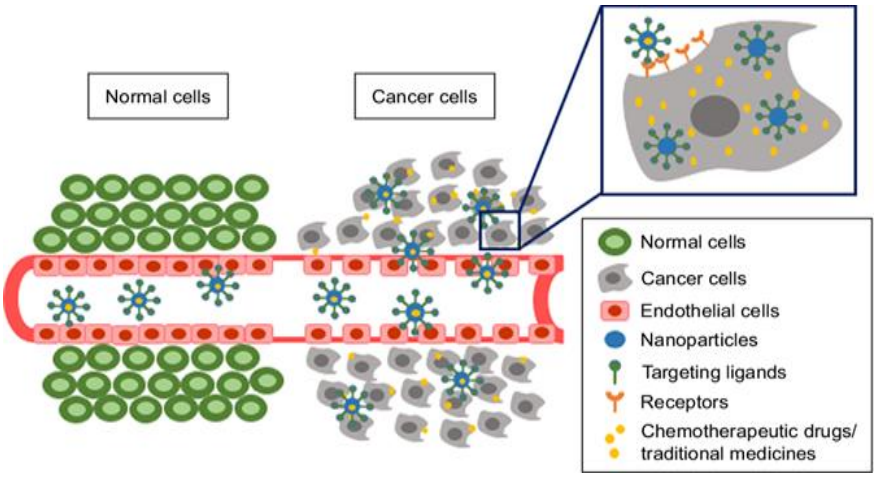

Figure 2: Active Targeting Drug Delivery (Adapted from Muhamad et al.,2018)

Chapter 4

Nanoparticles for Cancer Biomarkers Dictation

Identifying and detecting chemicals in blood and other physiological liquids which are associated with the appearance of cancer is one potential strategy in the early cancer cell detection. Biomarkers are chemicals that are used to identify the cancer (Chinen et al., 2015). In the body, changed levels of tissues, post-translational adjustments, peptides, metabolites, nucleic acids, and variances of gene can all help assess individual risk factors, prognosis, and medication response. In addition to guiding treatment, tracking treatment response can identify molecular alterations (Combes et al., 2021). Tumor biomarkers should preferably have a high sensitivity (more than 75%) as well as specificity (almost 99.6%). Biomarkers derived from blood, urine, or saliva specimans are now utilized to test patients for cancer risk. However, the markers are not enough for cancer screening. For this, several researchers are studying abnormally developed proteins, peptide particles, glycoproteins, and antibodies in cancer patients' blood, urine, and/or tissues (Jin et al., 2020). The development of cancer biomarker assays has been hampered by both fundamental and technical problems. Assays with high sensitivity and selectivity can be made using nanotechnology because specific nanoparticle probes have unique features. Researchers use nanoparticle-based technologies to detect biomarkers that are separated by analyte detection (Chinen et al., 2015).

4.1 Cancer Biomarker Detecting by Fluorescence with Quantum Dots

The high quantum efficacy, huge molecular extinction coefficients, and great emission peaks of quantum dots make them ideal for identifying cancer biomarkers by the use of fluorescence. The sandwich-type assay is the most common way to look for biomarkers. It consists of a substrate, a capturing antibody, the analyte of concern, a second capturing antibody, and a secondary antibody, which is normally labeled with a fluorescent probe. Using a surface-bound

primary antibody, an assay can detect biomarkers. Then a second biomarker antibody is placed between the target and the first antibody. As a result of the secondary antibody binding to the second primary antibody, a fluorescent signal is produced, which can be viewed by microscope or spectrophotometer. Using quantum dots to mark the secondary antibody with a fluorescent dye makes them very selective and sensitive. As gold nanoparticles (AuNPs) have a higher absorbance than organic fluorophore quenchers, they are suited for use in biosensors and have wide application for cancer diagnosis (Chinen et al., 2015).

4.2 Cancer Biomarker Detecting by Metallic Nanoparticles

There are a lot of ways to find metallic nanoparticles that are bound to molecular targets. These methods depend on the nanomaterial's properties and the type of nanomaterial-labeled biosample, which could be a cell line or a whole organism. While certain nanomaterials, like AuNCs are fundamentally luminous in the visible to NIR region, others require functionalization to provide the appropriate detection approach. Fluorescence microscopy can be used to do this or less common procedures including: ICP-MS, ICP-AES, auto-metallography, and SERS. These techniques can be applied with labeled liquid biopsies (human serum and urine). Finally, xenografted mice can be used to image nanomaterials in vivo using MRI, nuclear imaging, Fluorescence imaging in the NIR, or photoacoustic waves image capturing. They must contain or be covered with a heavy metal, like: gadolinium and iron-oxide for MRI. But nuclear imaging requires nanomaterial radioisotope tagging. The high imaging depth of photoacoustic imaging makes it one of the most promising approaches (up to 1 cm). Few nanomaterials designed for cancer detection have reached clinical testing. Currently, CTC.gov mentions 27 nanoparticle cancer trials. Despite their medical promise, there are challenges to overcome (Combes et al., 2021).

4.3 Cancer Biomarker Detecting by Fluorescent Nanoparticles

Fluorescent nanoparticles have been utilized to identify cancer biomarkers subsequently. As a quantitative or qualitative tumor cell detection tool, nanoparticles can be used. They increase the sensitivity of the analysis by concentrating and shielding a marker from deterioration. With streptavidin coated fluorescent nanospheres (Fluospheres®, the green color fluorescence, and TransFluospheres®, the red color fluorescence), the EGFR on human epidermoid carcinoma cells A431 was discovered (Brigger et al., 2012). The fluorescent nanospheres had a 25-fold increase in sensitivity over streptavidin–fluorescein conjugate, according to the research. Conjugates of single dyes were not as brilliant or concentrated as those that were encased in fluorescent markers. To identify both CD3 and CD4 receptors on the JURKAT cells (acute T-cell lymphoma) in multicolor flow cytometry, fluorescent nanoparticles were coupled with R-PE (a reagent which flow cytometry). Quantitative analysis would be impossible with these devices despite their high sensitivity and precision (Brigger et al., 2012).

Cancer Biomarker	Type of Cancer	Specimen Type	Diagnosis
Alpha fetoprotein L3	Hepatocellular cancer	Serum	Risk analysis
Human epididymis secretory protein 4	Ovarian cancer	Serum	Therapy assessment
Nuclear mitotic apparatus protein	Bladder cancer	Urine	Malignancy prediction
Cancer antigen (CA 15-3)	Breast cancer	Serum	Monitoring plasma
Fibrinogen degradation product	Colorectal cancer	Serum	Benign cancer
Ovalbumin	Ovarian cancer	Serum	Malignancy prediction
Human epidermal growth factor receptor 2	Breast cancer	FFPE tissue	Fetal occult blood detection
Pro2- Prostate specific antigen	Prostate Cancer	Serum	Distinguish between cancer and benign disease
Proto-oncogene	GIT cancer	FFPE tissue	Prognosis and therapeutic response
Prostate specific antigen (PSA)	Prostate Cancer	Serum	Free PSA: complete checking PSA testing and monitoring
Cancer antigen 19-9	Pancreatic Cancer	Serum, Plasma	Observing
Thyroglobulin	Thyroid	Serum, Plasma	Observing

Table 4: Cancer Biomarkers Approved by FDA (Chinen et al., 2015)

Chapter 5

Potential Advantages of Nanoparticles Based Drug Delivery

5.1 Advantages of Nanoparticles

In the recent years, the utilization of nanoparticles as a drug delivery carrier has grown rapidly. The special size and unique properties of nanoparticles has turned them into a specialized and efficient class of drug delivery system. As mentioned previously that, anti-cancer drugs are commonly administered orally or by injection, and they circulate throughout the body, potentially harming all types of cells, tissues, and organs. In this circumstances nanoparticles are the potential drug carrier for the administrated drug that are able to recognize the cancerous cells with their targeting moieties, resulting in selective killing of cancerous cells. Using nanoparticles in drug delivery systems has various advantages for cancer treatment which are outlined below.

5.1.1 Targeting Drug to Specific Site

In order to achieve site-specific drug delivery, several sequential but independent steps must be completed, such as: localization of the drug and its carrier within the desired targeted organ, recognition and interaction of the carrier with specific targeted cells, and therapeutic dose delivery of drugs to targeted cells with minimal absorption by non-targeted cells (Klausner, 1987). Nanoparticles are recently used widely as drug delivery carrier to deliver drug to the specific cells. Targeted delivery has two choices: actively or passively. Active targeting of therapeutic agents requires conjugation to a tissue- or cell-specific ligand. An active therapeutic substance is embedded in a macromolecule or nanoparticle that goes to the target organ by passive targeting method. The EPR effect allows drugs contained in nanoparticles or linked to macromolecules to target tumors passively. Catheters can deliver nanoparticles to specific organs or tissues. Localized medication release at specific locations on the arterial wall, such

as vascular restenosis, may be advantageous. Nanoparticles can also transfer medications across biological barriers. Liposomal delivery of drug has been effective in several cases. “Contact-facilitated drug delivery” methods involves binding or interacting with the membranes of targeted cells (R. Singh & Lillard, 2009).

5.1.2 Controlled Drug Release

Controlled drug release from dosage form basically identifies controlled drug delivery systems. By reducing dose and dosing frequency, controlled drug delivery systems can decrease the adverse effects, enhance patient compliance, and maintain medication concentration in systemic circulation. The basic goals of nanoparticle delivery methods are to manage particles size range, surface features, and drug release to produce specific site activity at therapeutically suitable rates and doses. This method also improves the stability of drugs and proteins while allowing for controlled release. It prolongs the therapeutic range of the drug concentration, improves the efficacy-dose relationship, and minimizes the need for frequent dose administration (D. Desai & Shat, 2013). Additionally, the drug is released in controlled mannered during transportation and at the site where it is used. This changes the drug's organ distribution and it is quickly removed from the body, so that it maximizes therapeutic effect and minimize the side effects (S. Singh et al., 2011).

5.1.3 Enhanced Bioavailability

Bioavailability is the percentage of a dose that can be found at the site where it works in the body. This term is interpreted as the proportion of the dose that enters the bloodstream for the majority of oral doses to elicit a therapeutic effect (Shahed-Al-Mahmud, 2018). Use of nanomedicine allows effective bioavailability, drug delivery, and therapy. In general, maintaining effective drug bioavailability at the action site of the body for an acceptable duration of time in order to elicit the desired pharmacological response is a major challenge in

cancer therapies. Nanoparticles help to reduce side effects and improve drug absorption. Some poorly soluble and bioavailable medicines have been formulated by using nanosuspensions, solid-lipid nanoparticles, and liposomes to make them easier to get into the body. Furthermore, for the majority of medications, including anticancer agents, antibiotics, peptides, and macromolecular pharmaceuticals, the blood-brain barrier (BBB) is an impassable barrier and therefore nanoparticles are designed in such a way that the drug is able to cross BBB to kill the cancer cells (Acosta, 2009).

5.1.4 Improved Solubility of Drug

Approximately 40% of the active substances found through combinatorial screening programs are difficult to formulate into medicines because they are very poorly soluble in water (Merisko-Liversidge & Liversidge, 2008). Drugs with low solubility and bioavailability can be formulated in numerous ways using nanoparticles based technology. Micronization, fatty solution use, penetration enhancer or co-solvent use, surfactant dispersion, salt formation, and precipitation are some of the more common procedures used to increase solubility, however they are not always effective (Agrawal & Patel, 2011). When these difficulties arise, a nanoparticle formulation strategy has proven to be very effective and useful at all stages of drug development process, as well as opening up potential for rejuvenating commercial drug with inadequate delivery. In order to create drug nanoparticles, surface area is increased in comparison to particulates larger than 1 micron. This increase in surface area and surface interactions is be able to improve dissolution rate and provide a platform for modifying the dosage form's pharmacokinetic characteristics (Merisko-Liversidge & Liversidge, 2008). It increases drug half-life in circulation by decreasing immunogenicity, allows sustained drug release, and reduces administration frequency for cancer therapy (S. Singh et al., 2011). Additionally, it is excellent for hydrophilic drugs, allowing for higher drug loading, dose reduction, and enhanced physical and chemical stability – thus fulfilling the criteria required

for an effective anti-cancer therapy utilizing passive and active drug targeting for targeted drug therapy to selectively kill the cancer cells without harming the normal, healthy cells (Agrawal & Patel, 2011).

5.1.5 Crossing Blood Brain Barrier

Blood brain barrier is a very significant obstacle between brain tissue and the blood that moves through it, and it helps to keep them separate. BBB is responsible for controlling brain's and controls how ions and molecules move in and out of the brain, too. Failure to keep any part of this specialized multicellular structure in place causes it to break down and cause brain inflammation and brain degeneration in people (Saraiva et al., 2016). With the development of nanomedicine, produced customizable devices in the billionths of meters have been proposed as a solution to the unmet problem of boosting medication transport across the BBB. Nanoparticles technology is rapidly advancing as single-unit objects with diameters ranging from 1-100 nm (Masserini, 2013). Nanoparticles are more accessible for their precise size and surface area and these characteristics allowing them to cross the BBB, enter into the lungs, and penetrate the skin via the tight endothelial cell connections, thus serving as potential treatment options for brain cancer, lung cancer and skin cancer and various other types of cancers as well (Rizvi & Saleh, 2018).

5.1.6 Preventing Tumor Drug Resistance

The main cause of chemotherapy failure is multi-drug resistance (MDR), which includes both innate and acquired resistance. MDR is characterized by decreased drug absorption, increased drug efflux, and cellular alterations. In particular, MDR is connected to increased drug efflux via ABC transporters like P-gp or ABCB1 New techniques have been discovered to address MDR in malignancies. McCormick and colleagues produced Salmonella-like nanoparticles. SipA, a Salmonella effector protein that suppresses P-gp activity, was coated on gold

nanoparticles to create Salmonella mimics. The bacterial mimics lower the P-gp levels and maximized doxorubicin sensitivity in cancerous cells. A new ON/OFF molecular nanoswitch probe embedded in a hydrogel has been developed by Artzi and others to detect and defeat MDR. MDR can be reversed by nanoparticle based drug-delivery system (NDDS) in certain cases, although it is unknown if resistance develops after repeated doses. As it is still unclear which subpopulation of cancer cells is responsible for the resistance, tumor heterogeneity further complicates the situation. However, NDDSs may be able to reverse medication resistance (Wang et al., 2017).

5.1.7 Delivery of Hydrophobic Drugs

Hydrophobic therapeutic compounds are still a big problem for the pharmaceutical industry because they cannot be easily or safely delivered. Using harmful surfactants and solvents, like: Tween and Cremophor in hydrophobic drugs makes them difficult to absorb and causes adverse reactions. For instance, the excipient Cremophor-EL®, the solvent used in Taxol®, can cause potentially lethal hypersensitivity, anaphylaxis, and long-term peripheral neuropathy. This is why Cremophor-EL® is used in Taxol® (D. Desai & Shat, 2013). In addition, Cremophor can put paclitaxel in micelles, which makes the drug more toxic and prolongs the systemic exposure. People who use polysorbate, another common solvent for hydrophobic drugs, can also be allergic to it. In this context, nanomedicines serve as a potential alternative as they do not need to be mixed with toxic solvents and can overcome the problems of toxicity with the use of such solvents. The first authorized protein nanotechnology-based chemotherapy is nab-paclitaxel. In this case, Paclitaxel nanoparticles have a mean diameter of 130 nm. Instead of Cremophor EL/ethanol vehicle is used in Taxol, nab-paclitaxel can be utilized. Shorter infusion times and no premedication is required to avoid solvent hypersensitivity responses as nanotechnology can enhance the effectiveness of nab-paclitaxel. Since it does not form

micelles like solvent-based paclitaxel, nabpaclitaxel exhibits a linear PK and also works with regular IV tubing and bags (N. Desai, 2012).

5.2 Strategies of Nanoparticles for Killing Targeted Cancer Cells

It has become increasingly desirable for cancer treatment to focus on killing cancer cells while avoiding harming healthy cells. Nanotechnology has introduced novel materials and therapy options for cancer patients (Misra et al., 2010). Most nanoparticles used or generated for cancer treatment are not hazardous. To be lethal, nanoparticles must accurately modify the chemical and physical environment surrounding the cancer cell. When nanoparticles reach the tumor, they trigger killing of cancer cells in a variety of ways: (i) drug release, (ii) hyperthermia or thermal ablation, and (iii) reactive-oxygen species (ROS)-mediated killing. These cancer therapy techniques can be used individually or in combination as part of a multimodality approach to cancer treatment (Gmeiner & Ghosh, 2014). Recent progress in the nanotechnology have extended the scope of its application in conventional cancer therapies, for example: gene therapy, photodynamic therapy, photothermal therapy, radio therapy, and so on (Chaturvedi et al., 2018).

5.2.1 Gene Therapy

Gene therapy involves directly injecting naked DNA plasmids into cells to deliver and express a gene construct. The use of nanoparticles for medication and DNA delivery has been extensively studied worldwide. Drugs or physiologically active compounds can be dissolved in, entrapped in, or absorbed from nanoparticles. The formulation condition determines nanoparticle internal structure, and the drug-loaded nanoparticle generation technique is dependent on the polymer's physiochemical properties (Kumar et al., 2004). Gene therapy can treat cancer by modifying tumor gene expression, transferring therapeutic protein-producing genes, or transforming a non-toxic chemical into a toxic medication. As a result, new cancer

gene therapy strategies have emerged, such as siRNA/shRNA gene silencing, miRNA-based gene therapy, and suicidal gene therapy, which uses a transgene to prevent cancerous cell development (Chaturvedi et al., 2018). Despite this, the structure and connection between complementary strands of DNA limit its biological significance. Aside from these physiological functions, nanotechnology is discovering DNA's hidden potential. Short-stranded DNA (ssDNA) sequences could be employed to dissolve hydrophobic nanoparticles, like: cNTs for in vivo application. DNA sequences can process data in biological experiments. Its self-assembling structure made it perfect for scaffolding nanoparticles in biochip and biosensor fabrication (Gmeiner & Ghosh, 2014). Previous gene transfer methods used viral vectors, which could trigger severe immune and inflammatory responses in the host. Toxicity, immunological and inflammatory reactions, gene regulation, and targeting are problems with viral vectors. The virus could possibly reactivate and spread sickness. Non-viral gene transfer approaches are gaining prominence. In addition to being non-viral, non-viral vectors are low-cost and immune-suppressive Liposomal cationic polymers and nanoparticles are nonviral vectors. Nonviral gene delivery nanoparticles' efficacy depends on their form, colloidal durability, size and charge intensity among other factors. Akt1 the small interfering RNA-loaded biodegradable nano-polymeric carrier reduces cancer cell lifespan, growth, malignancy, and metastasis (Misra et al., 2010).

Moreover, over the recent years, various researchers developed the potential of nanotechnology to improve gene therapy and successfully translate their findings into clinical studies. In this way, nanoparticle based cancer gene therapy is clearly a promising treatment option (Chaturvedi et al., 2018)

5.2.3 Photodynamic Therapy

Photodynamic therapy (PDT) is currently widely used as a clinical treatment technique for a variety of disorders, including cancer and, in particular, superficial tumors (e.g. oesophagus,

bladder, melanoma) (Bechet et al., 2008) PDT is a type of therapy that uses light and a drug called photosensitizers (PS)to kill unhealthy cells and tissues. A sufficient amount of molecular oxygen must also be present in the tissue. When these components are combined at a proper dose and concentration, they produce lethal oxygen-based molecular species, which are tolerated by sick cells individually. It has three phases: drug molecule excitation, hazardous oxygen production, and cell death (mechanically) (Chatterjee et al., 2008).

PDT is a two-stage technique when a PS is delivered locally or intravenously. PS increases in cancer cells and is stimulated by light at the affected area. Solid tumor accumulation is crucial after intravenous injection and is connected to PS physical-chemical properties. PSs possibly interact with malignancies via low density lipoprotein(LDL)receptors. These attach to LDL, while hydrophilic organisms link to albumin and globulins. As cancer cells have more LDL receptors, they prefer endocytosis of the LDL–PS complex. PS solubility impacts PS diffusion and positioning within tumor cells. The sensitizer's charge influences PS accumulation in cell organelles. These compounds are found in mitochondria and lysosomes. Anionic dye sensitizers concentrate in the perinuclear region, cellular vesicles, and lysosomes. Because PS can travel to other organelles when illuminated, subcellular localization can change. PDT triggers cell death by stimulating apoptosis, necrosis, and autophagy. Other approaches assist in minimizing or eliminating malignancies after PDT treatment (Sortino, 2016).

5.2.3 Photothermal Therapy

A safe and successful way of treating cancer is photothermal therapy. Here a photothermal chemical is used to selectively heat the target cancerous area, destroying it thermally. This includes: transition metal nanoparticles, organic chromophores, and light-absorbing compounds, like: indocyanine green, porphyrin and naphthalocyanine (Chaturvedi et al., 2018). Nanoparticles capable of generating heat under laser illumination have recently attracted much attention. In the past, photothermal therapy was only used for superficial malignancies due to

large attenuation coefficients in human tissues. This will reduce the heat supply within the tumor and increase non-specific tissue damage (Jaque et al., 2014). Photo thermal agents, such as drug-carrying nanoparticles, absorb light and convert it to heat. AuNPs, CNTs, and nanorods have high NIR absorption between 650 and 900 nm. The ability of 10-100nm nanoparticles to convert light into heat reduces the energy required to kill tumor cells. Beyond cellular damage, heating metal nanoparticles for example, gold nanoparticles make bubble production and deformation, which causes mechanical and cellular damage. By accumulating nanoparticles in malignant cells, photothermal treatment works. Since these tissues are sensitive to optical rays, a lower threshold laser is required to raise the heat around them, causing cell death (Chaturvedi et al., 2018). Furthermore, photothermal therapy is getting a lot of attention these days because it is possible to control the incorporation of light-activated heating nanoparticles (L-HNPs) into tumors. This makes it possible for high heat deposition in the tumor area at low laser light intensities, which hurts less of the healthy tissue around it. So to develop nanoparticle-based specific and effective photothermal therapies, the L-HNPs must have to be fulfill several requirements, including: i) Large absorption cross sections for light wavelengths within the two biological windows have to be maintained. This would enable excellent optical radiation absorption and, in combination with high light-to-heat conversion efficiency, allow low-power laser thermal therapy, ii) Toxic-free insertion must be ensured. L-HNP toxicity should only be activated by optical light. L-HNPs should not harm normal or cancerous cells. For a targeted treatment with minimal side effects, iii) Make it easy to use. The ability to treat tumors would also allow for more targeted therapy options, iv) Biocompatible liquid solubility. With lengthy circulation periods (half-lives), cancer tumors would be easily accessible even at low circulation flows. ii) minimum toxicity, iii) easy functionalization, and iv) good solubility in biocompatible solutions (Jaque et al., 2014).

5.2.4 Radiotherapy

Radiotherapy is one of the best ways to treat cancer. Ionizing radiation is delivered to the tumor through an outside beam or by inserting radionuclide-based embryos into the tumor to kill cancer cells. As radiation is not very specific, healthy cells around the tumor area is often damaged by it. A beam or brachytherapy can be used to give radiation to cancer cells either outside or inside, using a radiation source that has been surgically implanted. Due to molecular ionization, ionizing radiation causes damage to various intracellular components, including DNA, resulting in a free radical cascade. Even though ionizing radiation is extremely successful at killing tumor cells, it also has a negative impact on the healthy tissue in the surrounding area. For this, it is important to keep an eye on the radiation dose that is given to healthy tissues in order to keep radiation from harming normal tissues, and different ways to target cancerous cells are being looked into in order to get tumor-specific radiotherapy (Pallares & Abergel, 2020).

Chemotherapy combined with radiotherapy is one of the most effective strategies to treat locally advanced tumors. The idea came after fluorouracil (5-FU) was discovered. Nanotechnology has the potential to make chemo radiotherapy more effective in two ways. Owing to the radiosensitizing action of numerous chemotherapy treatments, including cisplatin, doxorubicin, and paclitaxel, one strategy is to transfer chemotherapeutics utilizing nanoparticles in combination with outside irradiation. Second, it is conceivable to combine chemotherapeutic agents with radioisotopes or radiosensitizers in a single nanoparticle, allowing for simultaneous delivery of agents to the lesion while retaining exact ratio control. Both nanotechnology techniques have advantages in terms of reduced toxicity in the healthy tissues and preferred agglomeration in tumors as a result of the characteristics discussed above (Mi et al., 2016) Radioactivity can be increased by particles with high atomic number (Z) and this has been a long-term study. There is evidence that putting high Z materials into a tumor

increases photoelectric absorption and thus the dose supplied to the tumor during radiation therapy. Radiation dosage enhancers and radiosensitizers should be freely available, easy to use, and harmless. Au (Z = 79) and AuNPs enhance the quantity of the active in cell culture by a murine model. For their bioactivity and ease of conjugation, they are presently actively researched in biological applications. Chang et al. studied gold nanoparticles and single-dose therapeutic electron beams on B16F10 melanoma tumor-bearing mice. Radiofrequency ablation is routinely used to treat various types of cancer. It is most commonly used to treat non-resectable liver cancer (Misra et al., 2010)**.** Traditionally, radiofrequency ablation required the insertion of probes into malignancies, but nanotechnology is enabling noninvasive radiofrequency ablation. The ability of gold nanoparticles to increase cancer cell killing in a noninvasive radiofrequency field has identified the potential usage gold nanoparticles for cancer cell targeting. A noninvasive radio wave machine with gold nanoparticle enhancer solutions was used to thermally ablate tissue and cancer cells in vitro and in vivo (Cardinal, J. et al.,2008)

Chapter 6

Challenges in the Development of Nanoparticles Based Drug Delivery

There has been lots of development in the use of nanotechnology to deliver drugs, as evidenced by the number of nanodrugs on the market today. This field still has many major challenges to overcome (Bamrungsap1 et al., 2016). Nanoparticles have the potential to interact with biomolecules in unprecedented ways, both on the surface and within the body's cells. It may appear that nanoparticles have no harmful effects. They are also more chemically reactive because of their volume ratio to huge surface area. This leads to more ROS being formed as a result. Because ROS are made, they can cause oxidative problem and infection, which can lead to damage to the body's cells. That is one of the reasons for nanoparticle toxicity. Nanoparticles also have unique characteristics in compared to bulk materials because of their volume ratio to huge surface area and the quantum size effect, which makes them different from other materials in many ways. They have showed toxicity in a way that is unexpected and has not been observed with bulk materials. Even gold nanoparticle, which is inert at the bulk level, becomes very active at the nanometric scale (Prabhakar & Banerjee, 2020) Nanoparticles' pharmacodynamics, PK, and safety profiles depend on how carefully they are chosen, how they are made, and how they are made. Both in-process and post-process quality inspections require many orthogonal analysis approaches. The safety and effectiveness of a nanomedicine could be at risk if important nanoparticle characteristics and processes change (Mitchell et al., 2021).

6.1 Biological Barrier

Drugs must overcome a number of biological barriers in order to reach their target disease areas to produce the desired therapeutic action (Bamrungsap1 et al., 2016). Orally delivery drugs must be stable in the gastrointestinal tract with the ability to penetrate the intestinal epithelium in order to reach the systemic circulation to exert their medicinal effect. Likewise, skin, nasal,

and drug delivery to the lungs poses similar biological barriers that the drugs need to cross the epithelium of these organs to reach the site of action (Mitchell et al., 2021). Blood-brain barrier (BBB) is another important factor that restricts the movement of large or hydrophilic molecules into the cerebrospinal fluid and stands as a major obstacle in the delivery of anti-cancer drugs to the brain. A number of nanoparticles based drug delivery systems such as liposome, nanosphere, cationic albumin nanoparticles are under clinical development to enable them to surpass the BBB.

Another major challenge of nanoparticles based anticancer drugs is the delivery of these anti-cancer drugs to solid tumors. Even though the tumor vasculature is highly heterogenous in distribution with increased permeability, there are certain regions of the tumor reprenting poorly perfused tissues. Increased interstitial fluid pressure (IFP) due to impaired lymphatic drainage is a significant barrier to nanoparticles based anti-cancer drug delivery, leading to limited extravasation and transvascular transport of the larger macromolecules, thus restricting the transport of anticancer molecules in tumor interstitial space. Furthermore, high tumour cell density and dense tumor stroma can hinder the passage of drug molecules in tumor interstitial space (Prabhakar & Banerjee, 2020). The drug biodistribution is a major biological challenge for nanoparticle development. The clinical utility of nanoparticle is determined by the ability of the nanoparticles to cross the epithelial barrier. Specifically, collagen present in tumor extracellular matrix is a primary factor limiting the penetration of drug in the tumor interstitium as observed in pancreatic cancer (Netti PA et al., 2000). There are many ways to improve drug distribution in anti-cancer drug development. Surface modification such as attachment of PEG to the nanoparticles known as PEGylation of nanoparticles can change how drugs are distributed in the body by increasing the circulation time in the blood. Adding targeting ligands and changing the design of nanoparticles have also been used to change the biodistribution of drugs. Knowing the fact that interactions with the biological barriers affect biodistribution, both

on the outside (epidermis, organs) and inside (cell) means that the transport and targeting of nanoparticles on their safety and effectiveness of the drug on patients must be considered during development of nanoparticles based formulations of anti-cancer drugs (C. Zhang et al., 2020). Moreover, other factors including, immunological clearance, limited targeting ability, and difficulties of penetrating biological barriers to produce the desired therapeutic effect must be further researched to maximize therapeutic efficacy of anti-cancer nanoparticulate drug delivery systems (Bamrungsap1 et al., 2016).

6.2 Immunological Challenges

Immunotoxicity or immune responses with an adverse effect can be generated by a variety of factors. The interaction of the drug and the carrier might cause structural changes that may promote immunogenicity and the nanoparticles itself may act as an antigen resulting in immunotoxicity. Factors that affect the immunogenicity of nanoparticles include size, surface characteristics, charge, hydrophobicity, and solubility. Based on these properties, some nanoparticles may be opsonized by plasma proteins and identified as foreign molecules, triggering the complement system, resulting in phagocytosis and clearance by macrophages of the MPS system in the liver or spleen (reticulo-endothelial system) as evidenced by the superparamagnetic iron-oxide nanoparticles, ferumoxytol and Ferumoxtran-10 (C. Zhang et al., 2020). This can lead to fatal life-threatening consequences such as allergic, anaphylactic and hypersensitivity reactions, with activation of humoral and cellular immune responses against the nanoparticles (Zolnik et al., 2010). Another example of nanoparticle induced immune reaction is Paclitaxel's interaction with albumin, observed in mice models. Although addition of PEG and other synthetic polymers can protect the nanoparticles from producing immunological reactions, however studies have shown that PEG-coated liposomes have resulted in production of antibodies that caused rapid clearance of the PEG-coated liposomes, therefore changing the biodistribution and pharmacokinetic properties of the drug

(Dobrovolskaia et al., 2008). Nanoparticles are also related to hemolysis and thrombogenicity. Non-immunogenic hemorrhage occurs when nanoparticles interact with erythrocytes. As demonstrated above, the complexity of nanoparticle systems can contribute to a variety of toxicities. These nanoparticle toxicity issues complicate the safety assessment of nanoparticles-based treatments. To achieve a good safety profile, manufacturers must carefully modify components and settings (N. Desai, 2012).

6.3 Formulation Challenges

Nanomedicines are expected to be multi-component three-dimensional structures with a preference for specific spatial configurations. Subtle changes in procedure or composition might have a deleterious impact on the complex superposition of the components. By conducting extensive physicochemical and functional studies on nanomedicine components, it may be possible to support highly reproducible manufacturing processes. Before a nanomedicine may be used in a clinical setting, it must first be well characterized and successfully manufactured. For therapeutic purposes, the ideal nanoparticle system or nanomedicine may include the following features in addition to the standard criteria for acceptable safety and efficacy and desirable pharmaceutical characteristics (e.g. stability, ease of administration, etc.) that are applicable to most drugs:

- Key components and their interrelationships are thoroughly studied.
- Understanding what factors are important to success and how they relate to each other

reproducibility in manufacture of essential features

- Sterile production is simple, and it has the ability to accumulate at the intended location of action despite biological obstacles that hinder its movement.
- Stable in usage, simple to store and administer (N. Desai, 2012).

Finding the “right” nanoparticle settings is critical. Moving in one way may alleviate one problem but often causes another. In this regard, Doxil® and Myocet® are good examples. Non-stealth liposomes have significant affinity for mononuclear phagocyte system (MPS) and are swiftly eliminated from circulation. However, pegylated stealth liposomes can drastically reduce macrophage absorption. This medicine has a longer half-life, higher tumor drug concentration, superior antitumor activity, and fewer side effects than doxorubicin (O’Brien et al., 2004). While Doxil's PEG coating reduces cardiotoxicity, it also increases concentrations in the skin, causing palmar plantar erythrodysesthesia, or hand–foot syndrome. The pace of infusion can be slowed or premedicated to prevent idiosyncratic non-IgE-mediated hypersensitivity in people. It is licensed in Europe and Canada for treating metastatic breast cancer in conjunction with cyclophosphamide. Because of this, Myocet has less cardiotoxicity but no hand-foot syndrome (Chan et al., 2004).

At the moment, there are no useful in vivo models that can predict how the different types of nanoparticles being studied will behave, so the progress of nanoparticles with desirable characteristics has to rely on real-world evidence and a lot of testing on animals before they are used in humans (N. Desai, 2012).

6.4 Safety Concern

Nanoparticles have numerous safety concerns. For clinical translation, a thorough examination of the safety of nanoparticles is required, but there are currently no standards for methodology or tests to accurately determine the toxicity of nanoparticles. Nanoparticle based medicine cannot be evaluated using the same methods that are used for regular medications. Size, shape, surface area, and aggregation may alter bioavailability then interacts with tissues and biomolecules, affecting safety considerations (C. Zhang et al., 2020). Nanotoxicology is a new field that has grown along with the development of nanomedicine. Nanotoxicology is the study of how interactions between nanomaterials and living things could have adverse effects on

patients. People who have looked at nanotoxicity in the past think nanomaterials might make free radical, damage brain cells, get into body parts that are more sensitive to toxic effects, and worsen the patient's condition (Bamrungsap1 et al., 2016). Even well-known drugs like paclitaxel and doxorubicin have demonstrated complicated toxicity problems in combination with new nanoparticles and nanocarriers. Additionally changes to the biosynthetic pathway, solvents, manufacturing process, or method of administration can make the drug more toxic (C. Zhang et al., 2020). Nanoparticles can be breathed, eaten, or absorbed via the skin. If the particles are small enough, they can enter the nucleus and come into close touch with genetic material. As a result, the toxic effects of nanomaterials should be assessed both in terms of the patient population and the manufacturing and disposal process itself. Achieving a consensus on testing procedures and standards is critical for addressing safety problems (Bamrungsap1 et al., 2016).

6.5 Scale-Up Production and Cost

To assure uniform and repeatable manufacturing and commercialization of nanoparticles on a large scale, it is necessary to scale up laboratory or pilot technology. Depending on the nature of the formulation and the materials used, some nanodrug delivery systems may not be suited for large-scale production. Scaling up is problematic due to low nanomaterial concentration, agglomeration, and chemical procedure. It is significantly easier to change the size or composition of nanomaterials in the laboratory than it is at the broad scale. The biomedical community should reconsider control requirements while working with nanomaterials. Statistical classification of nanoparticles by material model, size, particular ratio, and standard deviation may be achievable without full control of nanomaterials' physical properties. It is impossible to evaluate the toxicological effects of nanomaterials of every size or particular ratio, as such a toxicology database would be appropriate (Bamrungsap1 et al., 2016). Moreover, scaling up nanotherapeutics often requires large costs, limiting their success. Pre -

clinical and clinical development costs are growing. Obtaining regulatory approval for innovative nanotherapeutics is tough. Most accepted nanotherapeutics are modified versions of already approved conventional drugs. This strategy is less costly than manufacturing whole advanced nanotherapeutics. Exorbitant nanomedical device costs can limit nanodrug development and usage. Aspects of synthesis and cost friendly analysis should be thoroughly investigated early in nanodrug development (C. Zhang et al., 2020).

Chapter 7

Conclusion & Future Perspective

7.1 Conclusion

The use of nanotechnology in cancer treatment has opened up a new era for cancer treatment and continues to be a topic of extensive research in the field of cancer therapy. Compared to conventional anti-cancer drugs, nanoparticles based drug delivery systems offer numerous advantages such as improved pharmacokinetics, biocompatibility, tumor targeting and stability along with playing a key role in reducing systemic toxicity while overcoming drug resistance. Owing to these advantages, nanoparticles based drug delivery vehicles are extensively utilized in cancer treatment as chemotherapy, targeted therapy, radiotherapy, hyperthermia and gene therapy. Importantly, nanoparticles based technology paves a way for combination therapy that enables to overcome mechanisms of drug resistance, including efflux transporter overexpression, defective apoptotic pathway, and hypoxia tumor microenvironment. In this context, different types of nanocarriers with distinct properties have been designed for targeted delivery of anti-cancer drugs to tumor tissues namely, organic and inorganic nanoparticles. Currently, different types of hybrid nanoparticles combining the properties of organic and inorganic nanoparticles have shown to have better delivery properties and are researched upon for efficient delivery of nanoparticles for cancer treatment.

Therefore, understanding both the advantages and challenges in the development of nanoparticles based drug delivery systems for cancer treatment is critical for formulation scientists, drug developers and regulatory bodies and ultimately patients who will benefited from nanoparticles based drugs for cancer treatment. Aside from the fundamental technical problems involved with nanoparticles based drug delivery, such as: overcoming biological barriers, interaction with cells, manufacturing problems and scale-up costs, nanoparticles have

the potential to play a key role in bringing about a revolutionary change in clinical practice that could result in a life-saving strategy for cancer patients.

7.2 Future Perspective

Drug delivery to tumor tissues using nanotechnology has been utilized successfully for different types of cancer diagnosis and treatment. There are already two therapeutic nanoparticles used in clinical practice, such as: liposomes and albumin nanoparticles and many other nanoparticles undergoing trials in the preclinical stages for further development. In spite of advances in nanoparticle based technology, only 15 passively targeted nanoparticles have been approved for clinical use and none of the actively targeted nanoparticles have crossed the clinical trials. Understanding how nanoparticle biodistribution influences the body's complex biological network and mass movement across compartmental boundaries is crucial for translating nanotechnologies from fundamental research into clinical applications. A toxicological database is also needed for nanoparticles to support safety determinations and risk management. In addition, various types of nanoparticles are still in the design stage for both imaging and therapeutic purposes. A combination of active or passive drug carrier with an imaging or a diagnostic agent could generate ‘intelligent’ theranostics system capable of monitoring disease progression and evaluate therapeutic efficacy of anti-cancers drug in real time. In this scenario, nanoparticles are followed across tumors using imaging techniques such as MRI and in combination with NIR lasers, heat and ultrasound, nanoparticles can be degraded or "melted" to release their active components locally (i.e. chemotherapy). These combination treatments utilizing hyperthermia and anticancer medicines can quickly kill tumor cells and this strategy avoids surgery and long-term chemotherapy. Future prospects of nanoparticles may also include personalized treatments for cancer therapy. More research should focus on addressing the challenges associated with crossing the physiological barriers (i.e. tumor heterogeneity, penetration, hypoxia and endosomal escape) and overcoming the regulatory

hurdles and the relatively complex scale-up of the manufacturing process of actively targeted nanoparticles in order to develop nanoparticles with controllable/predictable biological identity for accelerating the clinical translation of nanoparticles for cancer therapy.

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