# A Review Paper on Nanotechnology Based Strategies in the Treatment of Cancer

By

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School of Pharmacy

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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/We have acknowledged all main sources of help.

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## Approval

The project titled "A Review Paper on Nanotechnology Based Strategies in the Treatment of Cancer" submitted by Samia Rahman (18146034) of Spring, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons).

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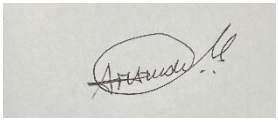

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## Ethics Statement

This study does not involve any human or animal trial.

## Abstract/ Executive Summary

Nanotechnology is a new discipline meeting the requirement for novel methods in cancer treatment. Recently, cancer became very fatal condition that causes death worldwide. Hence, traditional cancer treatment like chemotherapy, radiotherapy and surgery shows higher amount of side effects such as off-target toxicity, tumor resistance and myelosuppression which reduce the effectiveness and patient compliance gradually. Nanotechnology provides characteristics such as bioavailability, biodegradability, controlled release, high target specificity, and co-delivery of the drugs for combination therapy. Current available strategies for nanoparticle mediated cancer treatment includes nanomedicines, immune checkpoint blockade therapy, biomimetic nanoparticles, different types of immunotherapies like CAR-T cell base nano therapy, CRISPR/Cas's nanoparticle mediated therapy, nano chemotherapeutics agents and also nano vaccines. This review discusses, current emergence of nanotechnology mediated cancer treatment studies around worldwide. Therefore, future research will also look into nanoparticles based on smart target therapy or combination therapy, as well as RNA-mediated therapy in the treatment of cancer.

Key words: Nanotechnology, cancer, nanomedicine, immunotherapy, CAR-T cell therapy, CRISPR/Cas's nanotechnology, nanovaccines.

## Dedication

Dedicated to my parents.

## Acknowledgement

First of all, I would like to thank Almighty Allah (SWT) for his uncountable blessing and mercy that provide me the strength to complete the project.

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## List of Acronyms

| | |
|---|---|
| ACT | Adoptive Transfer T-Cell Therapy |
| ICB | Immune Checkpoint Blocker |
| HDL | High Density Lipoprotein |
| CAR T cell | Chimeric Antigen Receptors |
| CRISPR | Clustered Regularly Interspaced Short Palindromic Repeat |
| NK | Natural Killer |
| CD | Cluster Differentiation |
| APC | Antigen Presenting Cell |
| TAA | Tumor Associated Antigen |
| MHC | Major Histocompatibility Complex |
| EPR | Enhanced Permeability and Retention |
| PGA | Polyglycolic Acid |
| HPMA | N-(2-Hydroxypropyl) Methacrylamide |
| PEG | Polyethylene Glycol |
| AuNP | Gold Nanoparticle |
| CNT | Carbon Nanotubes |
| VEGF | Vascular Endothelial Growth Factor |
| PLGA | Poly (D, L-lactic-co-glycolic acid) |

| | |
|---|---|
| ERBB2 | Growth Factor |
| SK-BR3 | Breast Tumor Cell Line |
| RGD | Arginylglycylaspartic Acid |
| FDA | Food and Drug Administration |
| EMA | European Medicine Agency |
| PDT | Photodynamic therapy |
| MPLA | Monophosphoryl Lipid A |
| BPQD | Biomimetic Black Phosphorus Quantum Dot |
| ALL | Acute Lymphoblastic Leukemia |

# Chapter1

## Introduction:

Nanotechnology, which incorporates modifying matter on a 'nano' scale, is regarded as a crucial enabler. Nanomedicine (medical uses of nanotechnology) is anticipated to considerably improve illness diagnostic and treatment methods while also lowering health-care expenditure (Satalkar et al., 2015). This technology can recognize the matter size of 1 to 100 nm. At 100 nm the quality of nanoparticles remains unaffected but when it goes to the lower range, the quality of nanoscale particles starts to deteriorate. The size of the particle is less than human cell mediates target therapy. Nanoparticles have an advantage characteristic in correlating to receptors and enzymes from the inside and outside of the surface of the cell due to their smaller size and proportionally higher surface area than capacity. As mentioned to its characteristic micro-scale size it can easily help to determine diseases and deliver treatment targeted cell (Saravanakumar et al., 2014).

The capacity of nanoparticles to transcend physiological barriers and obtain access to a specific anatomical area, their availability in systemic circulation at the pathogenic site, and safety profile have all been factors that influence their therapeutic success. Recently, it has been immersed in various fields of drug delivery, diagnosis related to viral infection, diabetes condition, immune system, and even in various cancer treatments. It serves immersive drug delivery to the target site without causing harmful effects to adjacent cells/tissues which is also referred to as controlled release by elevating the protection of therapeutic agents (Grodzinski et al., 2019). Nanoparticles can optimize important bioactive compound storage and distribution at the tumor site, improve therapeutic effectiveness, and reduce the severity of adverse effects on surrounding tissues. It is feasible to combine diagnosis and treatment chemicals in a single nanoscale due to the intrinsic properties of nanoparticles. These properties

enable the tracking of agents' bioavailability and deposition at the target location, allowing the release of medications to be observed and measured, thereby allowing for a more effective evaluation of their efficacy of treatment in cancer (Jurj et al., 2017).

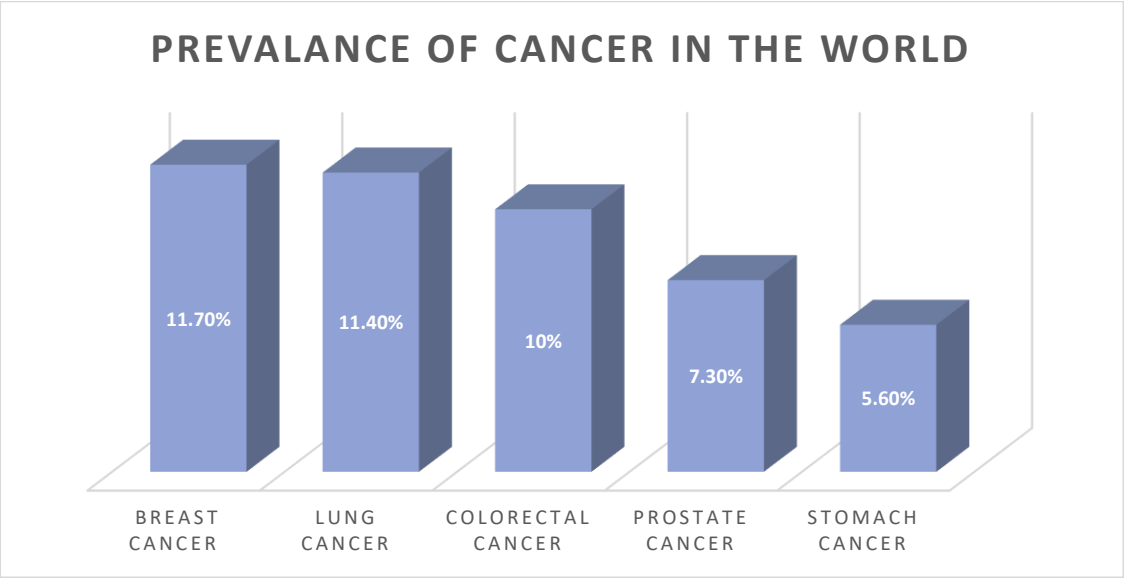


*Figure 1 Prevalence of cancer worldwide in 2020 (Sung et al., 2021)*

From the figure 1 in 2020, an estimated 19.3 million developing incidences regarding cancer would be diagnosed worldwide, with around 10.0 million fatal issues. With an anticipated 2.3 million new cases (11.7 percent), female breast cancer has surpassed lung cancer as the most often diagnosed malignancy, followed by lung (11.4%), colorectal (10.0%), prostate (7.3%), and stomach (5.6%) (Sung et al., 2021).

Cancer refers to a pathologic condition that arises from continuous unregulated proliferation of the cell. Cancer cells proliferate and divide uncontrollably, infiltrating normal tissues and organs and subsequently proliferating to other parts, rather than responding adequately to the signals that control normal cell behavior. Cancer cells demonstrate a broad loss of growth control as a result of accumulated aberrations in various cell regulatory systems, which is

manifested across many features of cell activity that distinguish cancer cells from their healthy equivalents (Cooper, 2000). Owing to variables such as rising pollution, radioactivity, insufficient physical activity, and a balanced nutrition, besides other factors such as heredity, the frequency at which cancer is growing is only expanding over time. This underlying factor causes mutation in the DNA and produces oncogenes which are responsible for various cancers. Conventional treatment of cancer includes three vital methods; chemotherapy, surgery, radiation or the combined therapies of this (Damyanov et al., 2018). Chemotherapy often acts by interacting with DNA synthesis in quickly proliferating malignant cells, resulting in cell death or slowed cell replication. For this reason, it affects the healthy cell of the body which is the main underlying reason for cancer patients' mortality. There are also many drugs that are used in the treatment of cancer like alkylating agents, cytotoxic antibiotics, antimetabolites, topoisomerase inhibitors and microtubule inhibitors. Regardless of its ease, durability, and higher patient acceptability, the oral route of medication delivery is preferred. Nevertheless, leading to inadequate aqueous solubility, limited oral availability, gastrointestinal decomposition (sustainability), the first-pass phenomenon, and high affinity binding to cellular proteins, many cancer medicines cannot be administered orally. All of these cancer therapies are useful to treat cancer but risk of adverse effects minimizes its benefit ratio such as non-specificity, and severe toxicity and so on. Resistance to tumor is another shortcoming of these anticancer-treatments which occur in high dose intake as their bioavailability is comparatively lower. Also, there is a chance of facing non-patient compliance as most of the anticancer therapies are administered through Intravenous infusion which is more common in third world countries like South Asia (Chivere et al., 2020). Novel anticancer agents have been developed that work by disrupting apoptosis in the cell cycle, signaling pathway pathways, gene transcription, and angiogenesis inhibition (Klochkov et al., 2021). Immunological modulation new therapies including adoptive cell transfer (ACT), cytokines,

and immune checkpoint blockers (ICBs) have just been licensed for cancer treatment, and several therapeutic vaccines are now being tested through clinical studies. Thus, nanomedicines can improve the accessibility and efficiency of these treatments by delivering and releasing several biomacromolecules (e.g., antibody, nucleic acids and antigens) to particular tissues and cells in a spatially, temporally, and dosage-controlled manner in responding to extrinsic or intrinsic stimulation (P. Zhang et al., 2019).

The incorporation of nanotechnology in cancer treatment has elevated the safety and effectiveness of anticancer therapies. Nanoparticles filled with versatile medicines and functionalized with identification proteins on their surfaces can be used to target specific cancer cells. The amount of medicine required to provide a therapeutic impact can be considerably lowered, and the concentration of the drug on the cancer site can be boosted without having any negative effects on normal tissues. Doxil, the first Food and Drug Administration approved nanotechnology-based drug which clinically proved the high specificity to the cell and comparatively lesser toxicity. Currently, Nano disks, High density lipoprotein (HDL) nanostructures, gold nanoparticles, and viral nanoparticles are just a few of the nanoparticle-based drug delivery methods that have demonstrated promising outcomes in cancer therapy. Progress has been achieved in understanding the biology characteristics of cancer in order to improve the usage of nanoparticles – such as overcoming biological barriers and distinguishing between healthy and diseased tissue also improved the oral solubility and reduced multidrug resistance of anticancer medication (Ho et al., 2017).

## 1.1 Aim

The main objective of this review on nanotechnology-based strategies to treat cancer treatment.

## 1.2 Objectives

The paper objectives are;

- to evaluate the scope of nanotechnology in cancer treatment
- compare the effectiveness of nanotechnology with conventional treatments
- evolution of future challenges and possible solutions of the strategies.

## 1.3 Rational of Study

Cancer is one of the most fatal diseases worldwide. According to a World Health Organization (WHO) report, approximately 10 million people will die in 2020 from these fatal diseases. The number of cases rises up day by day which is alarming, also the causes are undefined in various cancers (*Cancer*, n.d.).

To begin with, the conventional treatment used for cancer treatment shows various challenges in current years. These side effects sometimes reduce the efficacy of treatment, so the cancer is not cured effectively. Also, the report showed that traditional treatment in some cancer patients with multiple myeloma causes severe myelosuppression and pulmonary fibrosis after half a session of treatment (Gavas et al., 2021). However, using nanotechnology will be an effective choice as it has particular size, shape, unique control release and high target efficiency which combinedly use to deliver the conventional therapy. As a result, it increases the effect of the therapy and reduces side effect more prominently in comparison with single traditional techniques proved by various trials and researches (Yao et al., 2020b).

Finally, the world has used this technique to serve the cancer patient. Several therapies such as nanomedicine Doxil, Aberexan etc. are on market and most of the strategies are in an ongoing trial phase that are used to treat cancer. Nevertheless, Bangladesh has no significant work in nanotechnology-based treatment of cancer which is the main cause of lagging behind the

developed country in treatment of cancer. Therefore, nanotechnology-based techniques in cancer treatment have been reviewed in this paper to make it easier for development in developing countries like Bangladesh.

# Chapter 2

## Methodology

This review was done by secondary research method using the extensive review of research papers, news articles, academic published articles, review papers related to nanotechnology, cancer treatments and nanotechnology mediated different strategies used in cancer treatment. Articles from renowned journals such as PMCI, Elsevier, Nature and Willey online library etc were analyzed for this review study. Also, the cancer website, government websites and international websites such as WHO, Cancer.net etc. were used to review potential data for study. Data were collected based on the findings on clinical data and other evidence which helped to evaluate the scope of using nanotechnology in cancer treatment.

# Chapter 3

## Nanoparticle Mediated Anticancer Agents

## 3.1 Nanoparticle Mediated Carrier in Cancer Delivery

Nanoparticles are constructed and utilized as medication vehicles because they can transport chemotherapeutics to tumor tissue without harming the healthy organs. The potential of nanotechnology drug delivery systems to have a "controlled-release reservoir," which may effectively distribute therapeutic compounds to damage locations or specific cells, has made massive development in the treatment of cancer. Considering the properties of nanoparticles, it has to be biocompatible in order to be used successfully in treatment. This indicates that they can either be able to assimilate into a biological system without stimulating an immune response or generating severe side effects whether they are actively injected into a malignancy or into the blood. Additionally, it has to provide controlled release in the tumor cell that reduces the risk of death of healthy cells (Wakaskar, 2018).

Therapeutic nanoparticles are frequently made using a controlled bottom-up technique, wherein engineered macromolecular constituents are led by external stimulation to react with one another and self-assemble into complicated structures which might otherwise be impossible. Drugs can be enclosed or connected to the surface of the nanoparticle. Recognizing the synergistic effects of size, shape, surface chemistry, patient-specific information, and other variables are required to design an efficacious NP. Different types of NP carriers are used in the drug delivery of cancer treatment some of which are discussed below (Hwang et al., 2021).

**Organic Nanoparticle:** Organic NPs have indeed been investigated for years and comprise a diverse range of components. Liposome based NP, Polymer based NP and dendrimers are the

types of organic nanoparticles that are used to increase the bioavailability, safety and efficacy of cancer treatment drugs.

Lipid-based nanoparticles (liposomes): Liposomes contain spherical vesicles that are conscious and are mostly made up of phospholipids either through animals or plant sources. It was the first nano-scale drug to be recognized for therapeutic use. Liposomes varied in diameter from 20 nm to ever more than 1 mm. Every tiny vesicle contains a hydrophilic center and a hydrophobic lipid bilayer, allowing both hydrophilic and hydrophobic medications to be encapsulated. Hydrophilic medications are encapsulated inside the hydrophobic phase, while hydrophobic drugs are encapsulated inside the lipid bilayer, which protects the medications from environmental damage throughout the circulatory system (Y. Li et al., 2020). Liposomes have passed through various waves of development and proved the therapeutic efficacy with the drugs like doxorubicin and pactitaxel in the in-vivo delivery in the form of nucleic acids, as much as other chemotherapeutic drugs. It showed an immersive response in the field of breast cancer in recent years. According to many studies, paclitaxel coated with liposomes showed the most efficacy in the tumor cell compared to the free form of the drug. There has been strong evidence of the success of liposomal coated anti-cancer drug doxorubicin which causes very minimal cardiotoxicity than normal doxorubicin doses. Moreover, liposome-based nano systems have established a drug combination option that can boost therapeutic impact and even overcome drug resistance (Colapicchioni et al., 2016). In the present year, lots of liposomal coated drugs have entered to serve as effective treatment of anticancer.

Polymer-Based nanoparticles: A further kind of NP is polymer-based NPs, which have particular institutional structures for drug delivery that are generated by various monomers (Gad et al., 2016). Polymeric nanoparticles are self-assembled monolayer systems with a hydrophobic center and a hydrophilic shell in the nanometer range that find additional under proper conditions such as amphiphilic surfactant concentration, pH, temperatures, and ionic

strength. Polymer–drug addicts through covalent conjugation, polymeric micelles by hydrophobic interactions, and polyplexes or polymerases by containment are three types of polymers–based nanotechnology (Banik et al., 2016). Notices of nanoparticles for cancer treatment that have been scientifically authorized or are in various stages of development. The mechanisms of action of this kind of NP shows a bond that covalently connects with poor aqueous solubility medicines which are in water-soluble natural or synthetic polymeric carriers, which can then slowly accumulate in the tumor cells through the enhanced permeability and retention (EPR) effect. The possible significance of this kind of NPs includes, easy administration of drug with noticeable therapeutic response, minimize side effects thus increase its patient adherence (Ali et al., 2020). Some examples of polymers are albumin, chitosan, and heparin found from natural origin. Albumin obtained from the serum are encapsulated with paclitaxel and currently get a successful therapeutic response in metastatic breast cancer throughout multiple clinical trials. Another widely used biodegradable polymer is polyglycolic acid(PGA) that is used in coagulation with many synthetic polymers. Furthermore, among used most often non-biodegradable synthetic polymers are N-(2-Hydroxypropyl) Methacrylamide (HPMA) and Polyethylene Glycol (PEG). The synthetic polymer-drug conjugate PK1, which would be a conjugation of HPMA with doxorubicin, and the first to be tested in human research as an anticancer agent. Amphiphilic block copolymers are responsible for the functional properties of micelles, which accumulate to form a nanosized core/shell structure in aqueous media (Afsharzadeh et al., 2017).

Dendrimers: Dendrimers are nanometer-sized synthetic polymeric macromolecules made up of numerous highly branching monomers that emanate from a core structure. The structure of the dendrimers involves monomers which have the capacity to attach with 2 or 3 functional groups that generate monomers. Dendrimers have distinctive characteristics including homogeneous size and morphology, globular design, high degree of branching, specified

molecular weight, and functionalized surface, which make them an appealing carrier for drug delivery (Torres-Pérez et al., 2019). Variation in the number of productions controls the size and surface charge density of a particular dendrimer. Polyamidation (PAMAM), which comprises both secondary and tertiary amines, is perhaps the most frequent type of dendrimer employed as a genetic carrier. These dendrimers have four or three branching terminals and are made up of an ethylenediamine or ammonia center. Therefore, considering the many cationic charges on their surfaces improve the contact with target cells, dendrimer-based polyplexes also displayed impressive promise for gene delivery, and its functional groups can be leveraged for subsequent improvements (Amreddy et al., 2018).

**Inorganic NP:** Inorganic NPs have a larger surface area per unit volume than organic NPs. It possesses a versatile surface connection chemistry and is simple to prepare, albeit this generally comes at the cost of biodegradability and biocompatibility. Gold NPs (AuNPs), carbon nanotubes (CNTs), magnet NPs (MNPs), and silica NPs (SNPs) are among the inorganic NPs that have been examined (Montaseri et al., 2020).

Gold Nanoparticle: Although NPs comprised of an array of substances can be used for Photothermal therapy (PTT), 2–4 gold-based nanoparticles (AuNPs), which researchers describe as those formed totally or partially of gold (such as silica core/gold shell 'nanoshells'), have evolved as the leading treatment vehicle because they offer many important advantages. AuNPs provide for efficient gold-thiol bioconjugation chemicals for surface functionalization with medicinal compounds, targeted ligands, or biocompatibility-enhancing passivating agents. Also, its optical characteristics evolve them to use for controlled release. The gold NP targets the active tumor site very selectively so, it reduces the risk of loss of healthy tissue. But for the economic issues, it is not widely appreciable for the cancer patient (Riley & Day, 2017).

Carbon-based NPs: Carbon Nanoparticles (CNTs) are made up of a hexagonal structure of sp2-hybridized carbon and correspond to the heterocyclic group of carbon allotropes75. CNTs have a wall made up of single or several graphene sheets layers. Single-walled carbon nanotubes are created when a single sheet is compressed, and multi-walled carbon nanotubes are generated when multiple sheets are bound up. It has the capacity to penetrate all types of cells including hard-to-transfect types (Zare et al., 2021). Because of their size, unusual shape and structure, and intriguing physicochemical characteristics, carbon nanotubes (CNTs) are being evaluated for possible biological applications. CNT's perspective toxicity has indeed been extensively evaluated *in vitro* and *in vivo* as a novel form of nanocomposites. The biodistribution of the lipid–polymer, phospholipid–PEG (PL-PEG) synthesized SWNT showed that appropriately nanostructured CNTs are safe as they can be eliminated via the hepatic and renal routes following IV administration (Son et al., 2016).

**Hybrid Nanoparticle**: Even though both organic and inorganic NPs have advantages and disadvantages, integrating them in a single cohesive targeted drug delivery offers the multipurpose transporter greater biocompatibility, which can improve treatment potency resistance mechanisms. Lipid-polymer hybrid NPs, organic-inorganic combined NPs and cell membrane coated NP are common hybridized NPs used as carriers in the treatment of cancer. In addition, a multiphase NP delivery system was developed to accomplish greater penetration into tumors by adjusting the size and features of NPs at various stages (R. X. Zhang et al., 2017).

## 3.2 Drug Targeting Strategies of NP Based Anticancer Drugs

Malignant cells are apparently normal cells that have distinctive alterations in genes that regulate growth, allowing cells to proliferate excessively and enabling them to propagate to other organs which is a well-known term ‘metastases. Angiogenesis is a widely infused term

where tumor cells compete effectively with healthy cells for oxygen, glucose, and amino acids in order to split and expand, however a tumor can only develop to about 2 mm to 3 mm without the formation of blood vessels (G. Yan et al., 2017). Most of the conventional treatments show inability to target between malignant cells and normal cells. For this reason, anticancer medications to be efficacious in treatment, must initially be capable of crossing through the body's natural barriers and attain the required tumor tissues with little loss of volume or function in the circulatory system during delivery. Subsequently, drugs ought to be able to specifically kill tumor cells without damaging healthy tissue once they enter the desired location. By raising the cellular uptake of medications while concurrently decreasing dose-limiting toxicities, such basically two techniques have just been linked to increases in patient survival rates. Recently, nanoparticle technology has been shown to have an immersive capacity to work on both of these strategies by targeting the tumor in different ways (Morales-Cruz et al., 2019).

### 3.2.1 Passive Targeting

Biodistribution of nanosized medication formulations into malignant tissues by diffusing or convection is referred to as passive drug delivery. The movement of tiny and massive components through the cell membrane of tumor cells is facilitated by passive diffusion. In the cancerous state the endothelium layer of blood vessels become swollen up and become more permeable than normal condition, when subjected to deprivation of oxygen, fast expanding tumors generate new blood vessels or consume preexisting ones. Such spontaneously generated leaky capillaries allow for targeted improved penetration of macromolecules and nanocomposites into the tumor microenvironment known as passive diffusion. Also, the nano composition accumulated into the tumor cell as there is lack of lymphatic drainage (Attia et al., 2019). It depends on the size of nanocarriers, and their pk properties. Most widely used nano carrier used in passive diffusion is Liposomes. An optimal targeting strategy might be realized

by producing liposomes with a size that allows them to extravasate in tumor tissues while preventing the carriers from exiting capillaries in normal tissues (Bao et al., 2018). Improved permeability and retention effect develops in tumor regions in conjunction to improved permeability (EPR) by which this mechanism works. Higher systemic capillary permeability in the afflicted tissues, and even a significantly lower flow of fluids towards the lymphatic circulation, identify the predicament. Additionally, exogenous application of several controlling angiogenesis influencing factors, such as vascular endothelial growth factor (VEGF), can culminate across both disordered tumor vasculature structure and enhanced microcirculation (Ashfaq et al., 2017). Passive drug delivery is controlled by the frequency of tumor metastasis, extravasation, and intratumor response. There are some successful examples of agents that used this mechanism including the commercially available Doxil and Caelyx, which are currently used across treatments, as well as the EPR effect has now become a gold standard in the development of passively tumor-targeted devices. Also, sclareol-SLNs show maximum inhibitory effect in the treatment of lung cancer (Attia et al., 2019).

### 3.2.2 Active Targeting

In increasing uptake specificity, active targeting involves ligands linked to the Surface of NPs. Those ligands have the capacity to converse with target tissues and will frequently try to protect NPs from enzymatic degradation. Active targeting involves utilization of a deep relationship, which also include like ligand-receptor or other molecular detection, which continue providing the delivery mechanism greater selectivity (Falagan-Lotsch et al., 2017). This kind of mechanism involves ligands that functionalize nanoparticles which attach to the molecules and overexpression cancer cells. But difficulty arises with this is that normal cells also release the similar molecule, and while normal cells outweigh tumor cells by a wide factor, majority NPs fail their target (Alavi & Hamidi, 2019). This problem can be overcome by combining several ligands or various varieties of ligands. For instance, Folate targeting is a typical illustration of

targeted medicine delivery in ovarian carcinomas, osteosarcomas, and non-lymphomas, Hodgkin's among other cancers worked by mechanism of overexpression of folate receptors. This receptor became attached to doxorubicin-conjugated poly (D, L-lactic-co-glycolic acid (PLGA) – Polyethylene glycol (PEG) particles which provide higher cellular uptake and longer half-life compared to the single loaded drugs. Due to significant internalization induced by folate receptor active targeting, the drug shows increased cytotoxicity with selective targeting properties (Ahmad et al., 2019).

The establishment of a thorough technique to screening antibodies from diverse phage is another sort of active targeting that may well be performed to select the optimum ligands that fulfill the function of targeting. The Food and Drug administration approved a range of antibodies for use in therapeutic intervention, including rituximab, ipilimumab, and trastuzumab due to its high selectivity and availability (Muhamad et al., 2018). The specific antibodies dendrimer was discovered to attach selectively to PSmA-expressing human prostate cancer (LNCaP) cells. Some scientific evidences have been showed that this approach was predominantly utilized to screen two antibodies (F5 and C1) against the human breast tumor cell line SK-BR3, that interacts to ErbB2, a growth factor upregulated in human breast cancer and many other adenocarcinomas. But antibodies have a very short half-life and are very difficult to conjugate with nano carriers although it is very costly so patient adherence will be least (Wakaskar, 2017).

A preventative alternative to antibody peptides is being used with properties such as smaller size, economical, and show greater stability. Arginylglycylaspartic acid (RGD) is a popular peptide because of its high affinity for v3 integrin receptors. Additionally nucleic acid aptamer can be also a promising active target which combines both antibody and peptide targeting together. Aptamers comprise shorter DNA or RNA nucleotide sequences which can adhere to a ligand but it degrades quickly after going to the circulatory system (Yu et al., 2016).

Active targeting directs a carrier's endogenous circulation pathways to a specific organ. Passive targeting, on the other hand, is based on the drug's natural distribution and the EPR effect. All of those mechanisms are reliant on blood circulation and the primary drug delivery site. Nevertheless, there are yet no commonly marketed actively targeted nanoparticles.

### 3.2.3 Physical Targeting

External signals, like radiation or magnetic fields, are used to guide medications to tumor cells through physical targeting. For instance, Photothermal therapy exploits nanoparticles (NPs) that rapidly transform closer light energy to heat, destroying cancer cells with little negative effects. The inorganic NP gold Nps are used in this technique because they are less toxic and provide controlled release. Despite gold NP, carbon nanotubes are also used in photothermal therapy by inhibiting G2-M cell cycles (Yao et al., 2020a). Graphene oxide conjugated with polymers provide pH sensitivity and trigger the apoptosis process. A drawback of photothermal therapy seems to be that cancer cells are generally resistant to external stress, such as heat shock proteins, which shield the cancer cell from further destruction. Consequently, nanoparticle-free radiation therapy is relatively prevalent. Radiation of strong energy, such as X-rays or gamma rays, are cytotoxic and therefore can destroy cancer cells in key locations (Pasqual-Melo et al., 2018).

## 3.3 NP-Mediated Drug Release

Most of the nano drugs are complex, during measuring the releasing kinetics the physicochemical properties and targeting delivery plays an important role. Factors including size, shape, accessibility into tumor site, flexibility and surface properties play an important role in release kinetics (Jurj et al., 2017). As cancer treatments drugs are strong agents and they need to act slowly, a controlled release manner is ideal for the drug release. In recent years, extended-release mechanism is widely used for NP mediated drug release as it provides a

prolonged time of action to achieve maximum therapeutic response and minimize the possible unwanted effects. Extended-release in a therapeutic setting, NPs contain medications whether on its surface or adsorbed in a matrix that allows for long-term release. The hydrophobic biodegradable polymeric NPs are designed to release in such a pattern. PEGylation, which is the process of conjugating polyethylene glycol (PEG) to a nano polymer, has indeed been widely employed in nanomedicine. This has been demonstrated to improve drug-hydrodynamic radius, extend plasma retention time, reduce proteolysis, minimize renal excretion, and protect antigenic signals from immunodetection (Yao et al., 2020a). Irinotecan pegol for example is an FDA-approved long-acting topoisomerase-1 inhibitor which improves the PK characteristics and tolerance of irinotecan used for breast cancer, showing longer circulation time thus providing maximum therapeutic response. Cancer patients require multiple dosing in each day, which proved very incompliance of the patients. Extended-release gathers patient compliance more effectively by its prolonged release action, and by minimizing the dosing amount (Kalaydina et al., 2018).

## 3.4 Nano-drugs Applied in the Clinic

Nanomaterials are amongst the most intriguing techniques in medicine, neither just for therapeutic applications, as well as for diagnostics and tissue regeneration. Nanodrugs, adhere to the US FDA, are products which typically range from 1 to 100 nm and demonstrate significant variations from bulk counterparts or materials beyond this range that exemplify pertaining dimension-dependent characteristics compact size and large surface area. The prerequisites for advanced nano systems in cancer therapy are constantly changing, as unrealized objectives compel new therapeutic aims and the integration of new principles in the development of personalized medicine. Doxil®, a non-targeted nanotherapeutic drug that has been utilized in the clinic for over two decades, is a prime illustration. This tiny PEG-liposome (100 nm) carrying the cytotoxic chemical doxorubicin was cleared for clinical usage in ovarian

cancer and multiple myeloma compared to free doxorubicin. The following table will demonstrate some FDA approved nanomedicines in the treatment of cancer (Gonzalez-Valdivieso et al., 2021).

*Table 1: Nano drugs used in the clinic*

| **Nano Particle** | **Medication Name** | **Generics** | **Approval date** | **Cancer Type** |
|---|---|---|---|---|
| Liposome NP | Doxorubicin | Doxil (Caelyx) | 1995, FDA | Kaposi sarcoma, ovarian cancer, and multiple myeloma (Wibroe et al., 2016). |
| Liposome encapsulated | Doxorubicin | Myocet | 2000, Approved in Europe and Canada | Breast cancer (Romeo et al., 2019). |
| HER2-targeting liposomal NP | Doxorubicin | MM-302 | Phase II/III | HER2-positive breast cancer (Munster et al., 2018). |
| Liposome encapsulated | Daunorubicin | DaunoXome | 1996, FDA | HIV-related Kaposi sarcoma (Parchekani Choozaki & Taghdir, 2020). |

| | | | | |
|---|---|---|---|---|
| Liposomeinjection | Vincristine | Marqibo | 2012, FDA | Leukemia (Readi & Althubiti, 2019). |
| Liposome | Irinotecan | Onivyde (Merrimack) | 2015, FDA | Metastatic pancreatic cancer (Lamb & Scott, 2017). |
| Liposome | Muramyl tripeptide phosphatidylethanolamine | MEPACT | 2009, EMA | Osteosarcoma (Tsagozis et al., 2020) |
| Liposome | Irinotecan | CPX-1 | 2018, EMA | Advanced colorectal cancer (*ECOA in Clinical Trials - ECOA Services | VeraSci*, n.d.). |
| Liposome | Cytarabine | DepoCyt | 1999, FDA | Lymphomatous meningitis (Crommelin et al., 2020). |
| Liposome | Cisplatin | Lipoplatin | 2009, EMA | Non-small-cell lung cancer (Serinan et al., 2018). |
| EGFR targeting liposome | Doxorubicin | Anti-EGFR immunoliposomes | Phase I | Solid tumours (eCOA in Clinical Trials - eCOA Services | VeraSci, no date ) |

| | | | | |
|---|---|---|---|---|
| | | loaded with doxorubicin | | |
| Albumin Np | Paclitaxel | Genexol-PM | 2007, South Korea | Breast cancer, small cell lung cancer (Maeda et al., 2011). |
| Albumin Np | Paclitaxel | Abraxane | 2005, FDA | Multiple type of cancer (Drusbosky et al., 2020). |
| Lipid NP | siRNA against PLK1 | TKM-080301 | 2017, FDA | Advanced hepatocellular carcinoma (Dika et al., 2019) |
| Colloid gold NP | TNF, several chemotherapies | CYT-6091 AuNPs | Phase I & II | Late-stage cancers (Tamarkin & Kingston, 2017). |
| Polymeric micelle | Paclitaxel | Genexol-PM | 2007, South Korea | Breast cancer and non-small-cell lung cancer (Keam et al., 2019). |

## 3.5 Challenges and Limitations in Developing Anticancer Agents

Although nanotechnology has emerged a wide area of success in cancer treatment by developing anticancer drugs there are also some challenges. Firstly, manufacturing nano-drugs

is a hard process. It is critical to precisely determine and regulate the ideal physicochemical characteristics. Systematic parallel assessment of numerous attributes of nano-drugs becomes challenging because of the problems of consistent and efficient production of nano-drugs with distinct traits. Several existing nanoparticle production technologies are suited for lab-scale synthesizing nanoparticles only, insufficient for the rising case (Meyer et al., 2015). Moreover, dried versions of nanoparticles are easier to agglomerate because of their compact size and huge surface area, rendering these problematic to handle, for instance, Chitosan NP loaded drugs are more possible to influence by the environmental factor which lower the medicinal properties of loaded drug because of its weight variations in different states (Dang & Guan, 2020a). Additionally, the limited rate of drug loading is among the key difficulties for many polymeric nanoparticles loaded drugs which fall under the range of >10% (Hwang et al., 2021). Also, some NP loaded medicines have fast releasing properties after going into circulation which reduce the therapeutic activity in the target organ. Nanoparticles containing various materials tend to show this kind of effect. For instance, dextran sulfate contained in chitosan nanoparticles bursting released 17 percent over 2 hours, and polyethylene oxide-PLGA nanoparticles with an antiangiogenic medicament burst released 40 percent in the initial 3 days (Dang & Guan, 2020b). Nanoparticles transfer medications through the micro vessel wall, extracellular matrix, and cytoplasmic membrane of cells after systemically administration, and each of these barriers limits trans vacuolar, intermittent, and transmembrane movement of nanomaterials. Most of the drugs are developed by passive targeting method with EPR effect, so it will be difficult for the region of lower permeability hence it shows less therapeutic efficacy (Blanco et al., 2015). For instance, Opaxio™paclitaxel-polyglutamic acid conjugate medicine shows great response in females, but poor response in male in clinical trials of multiple cancer. There are also very low responses found in the antibody mediated targeting in many studies (Hare et al., 2017).

# Chapter 4

## Emergence of Nanotechnology in Different Cancer Therapy

### 4.1 Immunotherapy

Immunotherapy is an immersive treatment that dynamically regulates the immune response to destroy tumor cells in a different number of ways. It is largely implemented to boost the immune system by modulating the immunological microenvironment, permitting immune cells to assault and eliminate tumor cells at numerous different nodes. Human immune system identifies and eliminates aberrant cells in the regular way of incidents, in which most probably prevents or suppresses cancer progression. However, cancer cells have multiple strategies to evade the body's defense system; like down regulating expression of MHC molecules into antigens, sending off signals to T cells by protein expression, or by releasing chemicals that help them to evade work as immunosuppressive chemicals. Immunotherapy works to strengthen the defense system against cancer cells and with higher targeted efficiency than combined chemotherapy. Innovative approaches to block immune checkpoint regulators, overcome immunological tolerance, including such modified T cell treatment, or identify unknown tumor antigens with next sequencing have ushered in a new era of cancer immunotherapy. It includes passive and active pathways to treat cancer. Active immunotherapy attempts to induce the self-immune system to damage tumor cells via vaccination, non-specific immunomodulation, or targeting specific antigen receptors; passive immunotherapy is indeed the administration of substances such as mAbs, lymphocytes, or cytokines that improve extant anti-tumor responses (H. Zhang & Chen, 2018).

### 4.1.1 Immune Checkpoint Inhibitors (ICIS) and Nanomedicine

The potential of cancer cells to elude immune vigilance is among their defining features: they can avoid immunological elimination by standing on immune cell 'brakes' widely known as checkpoints. Hindering the release of these endogenous brakes that hold the immune system in control is a promising method to cancer immunotherapy. A majority of effector T cells would develop into depleted T cells at late stages of illnesses due to immunological checkpoint hyperactivation. On depleted T cells, inhibitory receptors are typically abundantly expressed, and functional cytokine production was also reduced. To enhance effective antitumor immunity, it is really necessary to restore T cells effector activity and counteract the immunosuppressive tumor microenvironment (Chen et al., 2018).

The regulatory molecules PD-1/PD-L1 and CTLA-4, which are abundantly expressed in many solid tumors, are important elements of these pathways. The PD-1/PD-L1 regulatory system delivers an inhibitory signal to T-cell, reducing normal effector T-cell function, enabling tumor-specific T cells to die and cancerous cells to become antiapoptotic. Anti-PD-1/PD-L1 antibodies suppress the PD-1/PD-L1 pathway, reinstating T-cell functionality and expanding cellular proliferation and cytotoxic activity, boosting anticancer immune responses and clinical outcome. Antibodies that suppress immunological checkpoints have already shown exceptional efficacy in tumors such as non-small-cell lung cancer, melanoma, head and neck squamous cell carcinoma, lymphoma, and pancreatic cancers (Kruger et al., 2019).

### 4.1.2 Mechanism of Immune Checkpoint Inhibitors

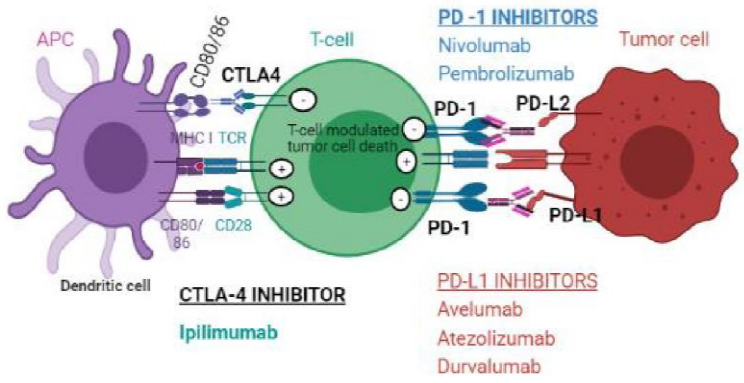


*Figure 2 Mechanism of immune checkpoint inhibitors (Centanni et al., 2019)*

**Immune checkpoint inhibitors targeting CTLA-4**

CTLA-4 is a T cell suppressive receptor found on the surface of activated CD4+ helper T cells as much as other antigen-presenting cells including dendritic cells containing ligands like CD80 and CD86. It is a member of the B7 protein family. CTLA-4's major function is to control immune function by reducing IL-2 synthesis and preventing cell cycle progression to minimize autoimmunity (G Lahori & Varamini, 2021).

Monoclonal antibody-based CTLA-4 inhibition decreases Treg-associated immune suppression and increases CD4+ and CD8+ T cell effector function in anti-tumor immunity investigations in both murine systems and cancer patients. CTLA-4 also controls the functionality of Tregs in the tumor microenvironment (TME), suppressing antitumor immune responses (Osipov et al., 2020). From figure 3 Ipilimumab, a CTLA-4-targeting monoclonal antibody approved by FDA, improves helper T-cell function while decreasing Treg immunosuppressive activity, resulting in a boost in cytotoxic T cells and tumor cell killing use in the treatment of myelomas *(Centanni et al., 2019)*.

**Immune checkpoint inhibitors targeting PD-1**

PD-1, a checkpoint receptor expressed on the surface of activated T cells that inhibits T-cell effector functions within tissues, is another important target in checkpoint suppression immunotherapy. Upon this surface of cancer cells, PD-L1 is a T cell inhibitory ligand that is abundantly expressed. The attachment of PD-L1 to PD-1 on T cells is a major mechanism for tumor immune escape, since it suppresses cytokine release, resulting in impaired antitumor pathways and metastasis (Sasikumar & Ramachandra, 2018). Increased expression of PD-L1 has indeed been linked to a variety of cancers, including leukemia, melanoma, breast cancer, and pancreatic cancer. Figure 3 describes the immune checkpoint drug nivolumab inhibits the PD-1/PD-L1 pathway. It's the first monoclonal antibody to target PD-1, and it's shown to be effective against a variety of epithelial malignancies, including melanoma, non-small-cell lung cancer, kidney cancer, and colorectal cancer*(Centanni et al., 2019).*

*Table 2: FDA approved Immune checkpoint inhibitors (Chen et al., 2018; Martin et al., 2020; Twomey & Zhang, 2021)*

| **Immune Checkpoint Inhibitors** | **Generic Name** | **Expression Site** | **Approval Status** | **Indication** | **Adverse Effects** |
|---|---|---|---|---|---|
| CTLA-4 ICIs | Ipilimumab | Activated T cell, NK cell | FDA approved | Metastatic melanoma | Dermatitis, enterocolitis, hepatitis, colitis, thyroiditis |
| | Avelumab | | FDA approved | Merkel Cell Carcinoma | |
| PD-1 ICIs | Nivolumab | T cell, B cell, Monocytes, | FDA approved | Breast cancer, | Diarrhea, itching, fatigue |

| | | | | | |
|---|---|---|---|---|---|
| | | Dendritic cell, Tumor cell, NK cell | | malignant melanoma, etc | |
| | Toripalimab | | Phase I-III | Multiple myeloma | |
| | Cemiplimab | | Phase I | Multiple cancers. | |
| | Sintilimab | | Phase I-III | Hodgkin's lymphoma, Lymphoma, Bladder cancer, non-small cell lung cancer | |
| | Pembrolizumab | | FDA approved | Non-small cell lung cancer, malignant melanoma, etc | |
| PD-L1 ICIs | Pidilizumab | | Phase I-II | | |

| | | | | | |
|---|---|---|---|---|---|
| | Atezolizumab | Dendritic cell, macrophages, Tumor cell | | Bladder cancer, non-small cell lung cancer | Fatigue, loss of appetite, cough, nausea, musculoskeletal pain and constipation. |

### 4.1.3 Challenges of Immune Checkpoint Inhibitors

There are several challenges and uncertainty faced with immune checkpoint inhibitors. Impoverished responses to checkpoint inhibitor therapy may be attributable to the fact that several late-stage cancer patients have been heavily pre-treated with more conventional treatments, and the progression of aggressive tumors and their possibly bad effect on immune cell populations by the time such patients receive checkpoint inhibitors may necessarily prevent any effective response to immune checkpoint blockade (ICB) therapy. Though PD-1/PD-L1 inhibitors are increasingly selective and thus have minimal side effects, some individuals can endure significant and often life-threatening adverse consequences as a result of their immune system being hyperactive (Hargadon et al., 2018). Because of the absence of PD-L1 expression by tumor cells or the immunosuppressive effect of the TME, most patients have innate resistance to PD-1/PD-L1 blocking medicines. As a result, tumors which are less immunogenic, including such prostate and breast malignancies, have a really poor rate of response. Moreover, patients who were treated with immune checkpoint inhibitors have developed resistance against the drugs that are used in treatment (Jenkins et al., 2018). It could be attributable to the fact that perhaps the resistant phenotypes had genetic abnormalities such as mutations, deletions, or epigenetic modifications, all of which can modify tumor neoantigen expression. Certain

factors that affect patient response to ICB therapy, such as tumor mutational burden/neoantigen availability and the presence of specific baseline gut microbiota at the initiation of therapies, are arising as biological markers of interest, in addition towards this minimum standard for the expression of ICB targets on tumor cells and anti-tumor T cells (G Lahori & Varamini, 2021).

### 4.1.4 Solution of the limitations of Immune Checkpoint Inhibitors

In comparison to immune checkpoint treatment separately it will show several side effects, but integration of immune checkpoint blockade therapy with nanotechnology may show to become a game-changer in hopes of preventing the issues related to poor targeted respondents in a large group of patients. Because it has a number of benefits, including preserving the load from deterioration in vivo, establishing regulated content release, extending the therapeutic effect, optimizing targeting delivery, and lowering side effects. Nanoparticles have a large surface to volume ratio, which provides for greater solubility in the circulation and so therefore easier access to tumor tissue (Martin et al., 2020). For minimizing toxicity, it can also be made from biocompatible and biodegradable substances (Shao et al., 2015). This has been shown that poly (lactic-co-hydroxymethyl-glycolic-acid) polymeric particles may be constructed to pack antibodies efficiently and liberate the antibody in various kinetics. Immune checkpoint drugs embedded in nanoparticles could enhance potential therapeutic responses while simultaneously reducing off-target complications. Inorganic nanoparticles (e.g., silica, gold and metal-based nanoparticles, iron oxide nanoparticles, carbon nanoparticles, and quantum dots), liposomes, dendrimers, micelles, and are also frequently used as carriers due to their passive and active targeting capabilities, low toxicity, and good biocompatibility. Basically, the aim of this combination therapy is to increase the therapeutic window with minimal autoimmune related adverse effects (Deng & Zhang, 2018).

For instance, core shell NP based drug delivery systems are very common to deliver this combination immunotherapy along with Photodynamic therapy (PDT). In PDT, breakdown of tumor vasculature, leads the lesion exposed to a photosensitizing chemical and triggered at a specific wavelength in condition with molecular oxygen, by triggering necrosis or apoptosis in tumor tissues. It enhances the tumor immunogenicity by increasing sensitivity of tumor with immune checkpoint blockade therapy. The core shell NP containing zinc and pyrophosphate helps to encapsulate the photosensitizer pyrolipid and combined with an anti-PD-L1 antibody show greater efficacy in metastatic breast cancer, colorectal cancer and lung cancer under various researches (G Lahori & Varamini, 2021).

Also, the silica-based NP and some chemotherapeutic agents like doxorubicin are used in combination with immune checkpoint inhibitors to improve the therapeutic condition with higher efficacy and less toxicity.

## 4.2 CRISPR-Cas's Nanoparticles for Cancer Immunotherapy

Genetic factors play an important role in causing cancer, most of the cancer occurs due to the influence of TP53, EGFR, KRAS, BRAF, HER2, and MET. Conventional treatments target this mutation in the treatment of cancer but this kind of gene delivery cannot show effective results on solid tumor-based cancer. The development of CRISPR/Cas9 delivery has changed this scenario as it highly suppresses the tumor cell growth. CRISPR/Cas9 is also typically utilized to induce precise mutations in genetic markers in order to investigate their potential causal involvement in pathogenicity. CRISPR barcoding technology can also be utilized to look into tumor heterogeneity. Furthermore, genome-wide CRISPR/Cas9 screening is often employed in many malignancies to identify possible therapeutic targets (Song et al., 2021).

### 4.2.1 Mechanism of Action of CRISPR-Cas9 Nanoparticles

CRISPR/Cas9 systems consist of three components;

1) Cas9 protein with DNA endonuclease activity,

2) a single guide RNA (sgRNA), which is specific to a target sequence of DNA

and

3) a tracrRNA that connects with Cas9.

It mostly binds into the G-protein binding site which increases the activity. G protein contains 10-20-fold of nucleotides that is important for gene delivery through CRISPR. The' seed sequence,' which is the first 10–12 nucleotides at the 3 ends of gRNA located close to a protospacer adjacent motif (PAM), binds to the intended sequence and determines specificity. gRNAs with fewer complementary nucleotides (20) can lower off-target impacts approximately 5000 times while retaining on-target effectiveness. Furthermore, increasing the length of the gRNA duplex by 5 base pairs may boost knockout efficiency considerably. The target sites are different according to the cancer subtype. In generalize, CRISPR/Cas in human cell lines with single or multiple gene(s) deletions has become simple and practicable, such as CRISPR-based mediated silencing of MELK, a cancer treatment target. The CRISPR-mediated silencing of MELK is still responsive to target and has no effect on the efficiency of cancer-derived cell lines. CRISPR is also used to knock in or knock out functional alleles in the experiment to generate drug resistance. CRISPR allows researchers to swiftly assess prospective genes or particular mutations linked to therapy resistance. CRISPR technology targets the specific gene with vectors targeting tumor and causing specific alteration of mutant cells which reduce the cell division and growth of tumor (Yadollahpour et al., 2021).

### 4.2.2 Delivery of CRISPR- Cas's through NP

The conventional mode of delivery used three methods to deliver CRISPR- Cas into cancer cells. The first one is gRNA ribonucleoprotein (RNP) method which forms a complex that provides transient genome editing mode by reducing off target effect, lower toxicity and huge stimulation to the immune system. Nevertheless, this method has derived various shortcomings including less efficiency due enlarged protein and mostly the endotoxin contamination.

Another type of delivery is plasmid-based delivery of CRISPR- Cas9 to a malignant cell but it shows least onset action into the target cell because of the large size protein and causes difficulties in delivery. Additionally, the mRNA-based delivery became the alternative of all these methods with minimal off-target effect. Although research of (Huang et al., 2018) shows that, the instability of mRNA in target cells proved very less efficacy of the system.

To overcome all these problems, nanoparticle-based delivery of CRISPR-Cas 9 is available to ensure safe and effective delivery to the target cell. Nanoparticles have some unique capability like limited packaging, less immunogenicity and genetic mutation, specific size and shape, and their control release properties deliver the active protein into genetic materials such as DNA and RNA to an efficient target site (J. Yan et al., 2021).

Some nano carriers show effective delivery of CRISPR-Cas 9 to the cancer proved by many studies.

Lipid based NP delivery of CRISPR-Cas 9: Lipid based nano carrier is the first choice for the delivery which resolves the instability of the protein, shows greater efficacy in immune response and renal clearance. Combining negatively charged nucleic acids with positively charged lipids is a common technique for forming lipid/DNA complexes. Furthermore, because

Cas9 is a very positively charged protein, it is challenging to directly electrostatically bind Cas9 with positively charged carriers. For this reason, the lipid surface must be cationic during delivery of hydrophobic substances like cargos. Some market developed liposome-based carriers such as AmBisome®, Doxil®, and Myocet® (J. Yan et al., 2021).

Lipid NP can provide 97% of knock out efficiency by combining with other delivery systems to show less off target effects. Some lipid-based delivery can be done through nano vesicles which can escape from phagocytosis and can pass through the biological barrier very easily because of their tiny size and shape. Additionally, it can combine with tissue specific promoter and non-viral vector-based delivery showed prolong delivery action and minimal off target effects. Lipid-based carriers can offer as a conceivable replacement to viral vectors for in vivo activities because lipids are less immunogenic than viruses. Some market developed liposome-based carriers such as AmBisome®, Doxil®, and Myocet® (J. Yan et al., 2021). Despite the advancements stated, low endosomal escape and distribution efficiency are limiting considerations for this strategy's in vivo deployment (Wan et al., 2019).

Polymer-based delivery of CRISPR/Cas9: Polymer-based delivery carriers have exceptional encapsulation capabilities, as well as spectacular characteristics in stabilizing pDNA (plasmid DNA containing Cas9 and sgRNA) from serum-induced aggregation and selective tissue or organ targeting. For instance, Hela cells were efficiently modified at distinct genome loci termed hemoglobin subunit beta and rhomboid 5 homolog 1 (RHBDF1) after in vitro distribution of CRISPR/cas9 controlled by the cationic polymer polyethyleneimine—cyclodextrin (PC). Targeting HPV16, E7 created an innovative nanostructure consisting of poly (b-amino ester) (PBAE) to transmit CRISPR/short hairpin RNA (shRNA) into HPV16 transgenic mice, which demonstrated high transfection efficiency, tumor growth inhibition, low toxicity, excellent biocompatibility, and quick onset action in vivo. PEGylation with hydrophilic polymers is usually used to limit opsonization and minimize clearance by the

reticuloendothelial system (RES), and it can also be used to distribute CRISPR/Cas9 using nonviral vectors to avoid the immunogenic reactions. Also, the chitosan polymer pegylated with other polymers provide efficient efficacy in the delivery of CRISPR/Cas9 (Aghamiri et al., 2020).

DNA Nanostructure-Based delivery of CRISPR/Cas9: In current studies, nanoparticle-based delivery uses DNA to develop smart nanoparticles with the properties of small uniform size, biocompatibility, and spatially addressability. It's been discovered that substantially complementary sequences between the sgRNA guide sequence and the nano clew sequence can enhance genome editing. Smart DNA nanostructures, could stimulate the emergence of new nanoparticle drug delivery systems customized with diverse particular ligand configurations for CRISPR/Cas9 delivery, and thus could be effective in treating cancer.

Therefore, there are many rigid nanoparticles like gold, carbon, iron oxide, calcium carbonate and black phosphorus-based nanoparticle proved to be efficient nanocarriers for delivering CRISPR/Cas9 for cancer treatment with high uptake capacity, specific size and shape, greater surface area and maximum stability in physiological system (Aghamiri et al., 2020).

*Table 3: Different NPs to deliver CRISPR/Cas9 (D. Kim et al., 2020; Wan et al., 2019)*

| **Material** | **Target gene** | **CRISPR/Cas9 Target site** | **Diseases** | **Advantages** | **Disadvantages** |
|---|---|---|---|---|---|
| Liposome | PLK1<br>Iduronidas<br>HPV16E6, E7 | Cas9 mRNA and sgRNA; Cas9 plasmid | Cervical cancer, Melanoma, | Simple to prepare; minimal side effects | Low delivery efficacy |

| | | | | | |
|---|---|---|---|---|---|
| | | Cas9 protein and minicircle DNA or sgRNA | | | |
| Polymer | MTH1 HPV16E6, E7 | Cas9 protein and sgRNA Cas9 plasmid | Ovarian cancer | Preparation is simple and safe. All CRISPR/Cas9 forms are compatible. | Efficiency is lower |
| Gold nanoparticles | mGluR5 | Cas9 protein and sgRNA | fragile X syndrome | High delivery efficiency | Show toxic effect at higher concentration |
| DNA nanostructure | cargos | Cas9 protein and sgRNA | Under research | Controllable size and architecture | Difficult to develop |
| Black phosphorus nanosheet | MCF-7 A549/EGFP | Cas9 protein and sgRNA | Breast Cancer Lung carcinoma | Less toxic and biocompatible | Degrade rapidly |

| | | | | | |
|---|---|---|---|---|---|
| Peptides | EMX1, DDX3, Tyrosinase, HPD Dystrophin | Cas9 protein and sgRNA | Hereditary tyrosinemia type I (HT1) Duchenne muscular dystrophy | Can be deliver to brain, rapid onset action | Low efficiency |

### 4.2.3 Challenges of CRISPR-Cas's Nanoparticles

Despite having immersive effects by the NP based delivery of CRISPR there are some challenges faced during subsequent trial procedures. The polymeric nano carriers which have higher molecular weight show higher cytotoxicity and impose toxic effects on healthy tissue. Also, there is lack of biodistribution of nanoparticles in some cases and as it is a new technique the studies are ongoing. Additionally, nanoparticles provide greater safety and prolong action but it also depends on the cancer type and prognosis and the delivery process (Wan et al., 2019).

### 4.2.4 Solution of Challenges of CRISPR-Cas's Nanoparticles

Nanoparticles are the best choice for reducing the limitation of CRISPR / Cas's delivery system in the treatment of cancer. Hence, there are slight limitations which can be solved by

developing smart nanocarriers for the delivery. For instance, Selective organ-targeting (SORT) NPs are a new class of NPs capable of delivering mRNA and the CRISPR/Cas9 genome editing tool to specific tissues. Overall, the lipid composition of conventional lipid NPs was precisely optimized to create SORT NPs (LNP). Incorporating different quantities of anionic (18BMP, 14PA, and 18PA), cationic (DDAB, EPC, and DOTAP), and ionizable cationic (5A2-SC8, DODAP, and C12-200) lipids into LNPs (C12-200 LNPs, mDLNP, and SORT NPs) is the main technique in developing SORT NPs. SORT NPs have been used to deliver specific mRNA and CRISPR/Cas9 to lung, spleen, and hepatic tissues proved by much research. The gene-editing machinery of smart NPs will stay dormant unless activated by endogenous or external stimuli. Smart NPs with multimodal features that respond towards both external and endogenous signals may have advantages over single stimulus-responsive delivery methods that provide great efficiency of CRISPR/Cas9 gene delivery in treatment of cancer (Naeem et al., 2021).

## 4.3 Biomimetic NPs for Cancer Immunotherapy

Conventionally developed nanocarriers such as lipid, polymers, gold and carbon-based NPs are currently ruling the world for their fruitful effect in cancer treatment. Recently, many studies have found that these nano carriers have some inherent drawbacks like, fast clearance from systemic circulation, poor permeabilities against biological barriers, EPR retention effects, these arise toxicity related to the NP in tumor specific sides. The enhanced release of vascular endothelial growth factors (VEGFs), hypervascularity, abnormal vascular architecture, and inability of lymphatic drainage are all characteristics of the EPR effect. Owing to the lack of lymphatic outflow, nanoparticles can passively target the tumor and preferentially expand

macromolecule and NP penetration to the tumor stroma, yet remaining in the tumor and produce lots of unwanted effect related to the therapy (Jin et al., 2019).

To overcome these difficulties of vasculature systems cell membrane-coated (CMC), NPs are developed with greater permeability and less EPR related side effects. These types of NPs developed to exhibit the cell-like behaviors and show biomimetic functionality. The surface of NPs is modulated by cell membranes in the area of oncology to promote bio interfacing capabilities as well as provide efficient drug delivery. This overlay of the cell layer mimics the parent cells' antigenic diversity, permitting the parent cells to fulfill a variety of roles such as immune evasion, extended circulation, efficient drug administration, and active targeting. Diverse bio interfacing functions can be performed by NPs that are enveloped in the cell membrane using non-nucleated cells (erythrocytes and platelets), prokaryotes, and eukaryotes (leukocytes) are known as cell ghost. Co-extrusion, extrusion/sonication, freeze-thaw/sonication, and extrusion/sonication are all methods for coating NPs. The derived cell NPs exhibit a physiologically intact bilayer membrane by simulating the surfaces of the parent cells, with the opportunities to enhance nanocarrier bioavailability and achieve and extended circulating in vivo, along with accomplishment of targeted aims (A. Li et al., 2021).

### 4.3.1 Mechanism of Action of Biomimetic NPs

Cancer cells can interact via homotypic aggregation, blocking metastatic cells from being cleared. CMC-NPs work by the homotypic aggregation-based targeting to achieve efficient results. For instance, core-shell PLGA NPs coated with cell surface adhesion motifs present on MDA-MB-435 cells were reported to have a significant homotypic attraction with the tumor cells of origin, resulting in increased cellular uptake. Additionally, when combined with an adjuvant, NPs can enhance T-cell maturation. In summary, these biomimetic cancerous cells NPs were loaded with the adjuvant chemical monophosphoryl lipid A (MPLA), and interaction

with mice dendritic cells resulting in overexpression of the dendritic maturation biomarkers CD40, CD80, and CD86, culminating in a strong immunological response. As a result, biomimetic cancer cell membrane-coated NPs can produce tumor-associated antigens (TAAs) on its surface, stimulating dendritic cells to activate immune responses towards TAAs (B. Li et al., 2018).

Leukocyte membrane covered NP mechanism: White blood cells, also referred as leukocytes, are the greatest blood cells, with diameters ranging from 7 m (small lymphocytes) to 20 m (large lymphocytes known as monocytes). Leukocytes are susceptible to amoeboid motions and crossing through blood arteries in order to accomplish their functions, which involve migrating to inflamed extravascular locations and eliminating infections. Because of their adhesion qualities, they can interact with tumor cells immediately in the tumor microenvironment or in blood circulation. Active therapeutic molecules may defy phagocytic uptake and target the desired region, bypassing any vascular barriers to enter the designated tissue, in addition to carrying out their functions effectively. For illustration, a study of (B. Li et al., 2018) found that, WBC membrane-covered NPs were made using J774 cell membranes. These doxorubicin (DOX)-loaded WBC-coated NPs were taken up by J774 cells at a frequency of approximately 75%. Such NPs could also bind directly to inflamed areas, enabling for drug transport throughout the vasculature and ensuring effective DOX administration to the tumor location.

Another mechanism of leukocyte coated NP involve natural killer cell (NK), where it used to develop DOX-loaded liposomes coated with membranes In vitro and in vivo, the resultant "NKsomes" showed a stronger and enhanced affinity for cancer cells, as well as a prolonged circulation half-life.

Platelet membrane covered NP mechanism: Platelet membranes have effective vehicles for cancer targeting because of their capacity to alleviate vascular damage and engage with circulatory cancer cells. Platelet-biomimetic NPs have longer blood circulation durations and less normal tissue involvement. Platelet membranes, in particular, provide possible benefits for NP coating attributed to the prevalence of certain ligands on their surface, such as CD47, which allows immunological elusion, and CD55/59, which can prevent complement activation. Platelet membrane-coated nanovesicles (PMNVs) encapsulating DOX as well as the ligand Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and potential of inducing apoptosis in target cells are recently created. Their ability to transport TRAIL to MDA-MB-231 cell membranes and cause apoptosis has been demonstrated. Also, the surface of magnetic beads was changed with antibodies targeting circulating tumor cells (CTCs) after they were coated with platelet (PLT) and WBC membranes. Owing to the cancer cell binding capabilities of PLTs, these PLT–WBC hybrid membrane-covered immunomagnetic beads were employed for the selective isolation of CTCs (B. Li et al., 2018).

RBC Membrane covered NP mechanism: RBCs can alter shape as they move through to the body and are easily separated from the blood. RBCs are thus a potentially great supply of cell membranes that are ideally suited for in vivo flow via patients' blood arteries. Whenever NPs like mesoporous silica coated with RBC membranes were injected into mice, they displayed a lengthy circulating period and regulated drug release of the enclosed medicines, such as DOX, with an enhanced LC50. Because of the RBC membrane, allowing immune evasion, and a regulated release of DOX from Nanoparticles, such NPs observed a significant improvement in circulation time (B. Li et al., 2018).

The employment of biomimetic black phosphorus quantum dots (BPQDs) encapsulated with RBC membrane in association with NIR irradiation and PD-1 antibody injection also has been found to suppress primary and secondary tumors. As a result, the drug combination of biomimetic NPs coated with RBC membranes, NIR, and PD-1 antibodies significantly slowed the progression of remaining and metastatic cancer in animals. Because of their long time in bloodstream in vivo, the existence of CD47, which enables them to avert phagocytosis by the immune system, and their semi-permeable membrane, that also permits for controlled sustained release, RBC cells provides a potential strategy for targeting therapeutic agent precise utilizing biomimetic NPs with major applications in cancer therapy.

### 4.3.2 Challenges of Biomimetic NPs

Membrane-coated NPs have the capacity to solve complications linked with the administration of free biologically active molecules, such as poor solubility in aqueous environments, non-specific target for tumor cells, and subsequently side effects on healthy cells. Hence, the biocompatibility is the main issue to address about the biomimetic NPs. Such nano-bio hybrid NPs are engineered to have a longer circulation duration and escape RES filtration, making them more likely to cause adverse reactions. To address this possible constraint and accelerate their use in clinical trials, the experimental techniques used to make biomimetic NPs must be carefully standardized across laboratories in order to create repeatable nanostructures. The laboratory processes needed for the formation of biomimetic NPs may modify the biochemical characteristics of the used membrane by altering membrane protein stability, orientation, compositions and glycosylation, posing a danger of an unexpected immune response and negative side effects. Indeed, research has shown that biomimetic NPs' toxicity rises in conjunction with their structural alterations of membrane proteins (J. Ma et al., 2020).

### 4.3.4 Solution of Challenges of Biomimetic NPs

As the challenges are related to the production process, that leads to serious immune related adverse effects. So, it is necessary to develop a standardized production process which can posses' characteristics such as biodegradable, biocompatible, and incredibly safe, with properties in regard to size, surface charge, and membrane in general that permit it to engage with the specified target while evading immune system recognition as "not self." Modifications in the stability, composition, orientation, and glycosylation of membrane proteins decrease the chances of an unanticipated immunological response and negative side effects. This emerging field of nanotechnology opens up the possibility of treating serious, widespread diseases in novel ways. Novel strategies include lipid insertion, metabolic engineering, hybridization of membrane and the most emerging technique genetic engineering (Guido et al., 2020).

## 4.5 CAR-T Cells and Cancer Nano Immunotherapy

CAR-T immunotherapy refers to Chimeric antigen receptors (CAR) that are designed to regenerate T lymphocytes for targeting specific tumor cells. Through innate immunity of the human defense system, it can identify the self and nonself-substance which include foreign pathogens and cancer cells. Tumor cells are identified by their gained antigenicity and immunogenicity, which is determined by the production of foreign antigens. T cells have functionality to destroy the tumor cell by suppressing the tumor cell growth (Srivastava & Riddell, 2018). CAR-T cell therapy is the process to induce the function of T cells by external supply of T cells from outside. CAR-T cells can identify particular tumor ligands and kill the targeted tumor cells selectively post genetic engineering, hence the therapeutic outcomes to CAR-T cells in individuals with chronic treatment alternatives have been exceptional in several trials. In phase 1 trials, for illustration, CAR T-cell treatment showed full response rates of 69-90 percent in young patients with recurrent or refractory acute lymphoblastic leukemia (ALL) (S. Ma et al., 2019). The advancement of CAR T-cell treatment has already progressed above phase 1 studies and into phase two multi-site trials (NCT02435849, NCT02228096), and

institutions of higher learning and industry are grappling with ways to scale up CAR T-cell generation in an efficient and productive way. CAR T cells' shows great efficacy in larger phase 1/2 trials at a number of centers, notably in ALL, where complete and total remission (CR) rates of 70–93% were reached. After CD19 CAR T cell treatment, CRs have been found to last up to 56 months. Moving on from this, the Food and Drug Administration recently approved CD19 targeted CAR-T therapy for the treatment of individuals with specific B-cell malignancies. The FDA has approved two medications, including Novartis' Kymriah and Kite Pharma's Yescarta. Patients with B-ALL and lymphoma can receive Kymriah, whereas those with large B-cell lymphoma can receive Yescarta. Optimizing CAR T cell manufacturing techniques, target selection, and clinical considerations could lead to the development of a number of genetically modified therapies for various malignancies, particularly solid tumors (Elahi et al., 2018).

*Table 4: The Approved CAR-T cell therapies (S. Ma et al., 2019; Styczyński, 2020)*

| **CAR-T cell therapies Generics** | **Brand Name** | **Approval Authorities** | **Date of approval** | **Indications** | **Target Patient** |
|---|---|---|---|---|---|
| Tisagenlecleucel | Kymriah | US Food and Drug Administration | August 30, 2017 | B Cell lymphomas (large) | Children and young people up to the age of 25 are eligible. |

| | | | | | |
|---|---|---|---|---|---|
| Axicabtagene ciloleucel | Yescarta | US Food and Drug Administration | October 18, 2017 | Acute lymphoblastic leukemia & B Cell lymphomas | Adult patients |
| Brexucabtagene autoleucel | Tecartus | US Food and Drug Administration | July 24, 2020 | Mantle cell lymphoma (MCL) (refectory or relapse state) | Adult patients |
| Lisocabtagene maraleucel | Breyanzi | US Food and Drug Administration | February 5, 2021 | B-cell lymphomas (refectory or relapse state) | Adult patients |

*Table 5: Significant Advance CAR-T cell therapy (Abdalla et al., 2020)*

| **CAR-T cell therapy** | **Response Rate** | **Response duration** | **Remission Rate** | **Target Onco Diseases** |
|---|---|---|---|---|
| Kymriah (CTL019) | 90% | 5 years | 82% | Acute lymphoblastic leukemia |

| | | | | |
|---|---|---|---|---|
| Kymriah (CD19) | 80% | 6 months | 43% | Diffuse large B-cell lymphoma |
| BCMA CAR-T | 31% | Less than 6 months | 94% | Non-Hodgkin lymphoma |
| Yescarta (ZUMA-1) | 40% | 9 months | 54% | Multiple myeloma |

Note: BCMA, B-cell maturation antigen

### 4.5.1 Mechanism of Action CAR-T Cells Therapy

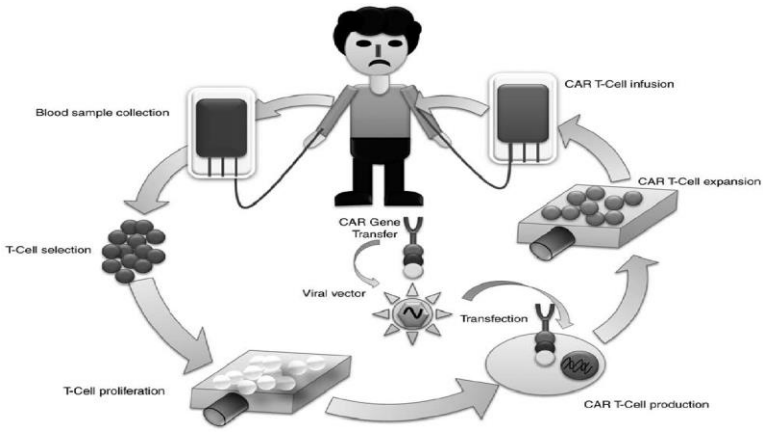


*Figure 3 The adoptive process of CAR-T cell therapy (S. Ma et al., 2019)*

CAR-T is a novel type of immunotherapy that can be used to treat a variety of cancers. Figure 3 describes, the treatment which is an investigational form of gene therapy in which T lymphocytes are redirected to kill malignant cells in an adoptive process. A tumor-associated antigen (TAA) binding domain (typically derived from the scFv fraction of the monoclonal antibodies antigen-binding region), an extracellular hinge domain, a transmembrane domain,

and an intracellular signal domain make up a basic CAR (S. Ma et al., 2019). To begin with, leukapheresis, or the isolation of a patient's peripheral blood, is the first stage in this treatment. Apheresis is a technique for isolating blood from patients and separating it into its constituent parts, that are then genetically engineered before being reinjected back into the patient's body. After transfecting to the human body, it will activate the CAR-T cell. Then it will reach the tumor active site. The process is known as trafficking and infiltration of T cells into tumor sites. The method entails combining scFv fragments in the joint area that splits scFv from the cellular membranes to create an engineered chimeric receptor for T cells. The inclusion of scFv on the cell surface, along with other tiny functional molecules, promotes the induction of the modified T cell's cytolytic capability. By this it can recognize the tumor cell and successfully destroy them as the anticancer functionally T cell does. Yet, in vivo, the anti-tumor effectiveness of the first generation of CAR engineered T cells is minimal, and reduced T cell proliferation leads inevitably to apoptosis. A second generation of CAR presents a distinct intracellular costimulatory signal that expands on the initial "signal I" produced from TCR/CD3 complexes; these signals are modified by the new category of CAR-T cell which is referred to as third and fourth generation. Furthermore, CAR T cells can function as long-term memory cells in the body for several years. In the event of a relapse, this trait permits them to locate and eliminate cancer cells in the bloodstream (Mohanty et al., 2019).

### 4.5.2 Challenges and Limitations of CAR T Cell Therapy

The recent fourth generation of T cells which are effective against multiple antigens are produced with armor protein which is an immunomodulatory substance that induces the T cell cytotoxicity. But the challenge arises with the production of this $4^{th}$ generation version. The protein coated structure causes huge expenses, and so it is difficult to manufacture and also limits the large-scale trials. The method of developing CAR-T cells is complex and time-consuming, putting a significant economical and physical strain on patients and their families.

Some of the delivery approaches of this therapy like nonviral vector through CRISPR has less permeability to the plasma cell, so it restricts to the board application in lots of carcinomas. In the treatment of solid tumors, very less amount of engineered T cells reaches the target site because of lots of ongoing processes such infiltration and lymphatic drainage. So, it should be counted as poor efficacy of the therapy. Additionally, in most of the cases the tumor resistance occurs which limits the effect of the therapy (Abdalla et al., 2020).

### 4.5.3 Side Effects of CAR-T Cell Therapy

Neurotoxicity: In certain trials, neurotoxicity was found, which might be due to T-mediated inflammation, higher cytokine concentrations in the CNS, or cerebral edema or due to unknown. According to the report of Sermer & Brentjens, 2019, women with acute lymphoblastic leukemia had neurological symptoms after six hours and three days after receiving therapy with CAR-T cells. Also defined the symptoms associated with neurotoxicity like, confusion, myoclonus, seizures and aphasia. Although some fatal cases are produced by cerebral edema, this scenario is very rare.

On-Target Off-Tumor Toxicity: One of the most evident possible design concepts in terms of CAR-T therapy efficacy is the detection of non-tumor cells that display the epitope targeted by the CAR-T therapy. Rather than being unique to tumor cells, tumor antigens are frequently chemicals that are abundantly expressed on tumors. In the treatment of metastatic renal cell carcinoma, it showed toxicity due to the epitope-target effect. Due to the on target and off target toxicity, the destruction of B cell occurs and leads to B cell aplasia with CD19 therapy. Since cardiac and pulmonary epithelial cells display the HER antigen, HER2 CAR T cells utilized to treat breast cancer may induce cardiopulmonary damage.

Cytokine Release Syndrome (CRS): The occurrence of cytokine release syndrome linked to the infused T cells' rapid activation, and that in turn stimulates other immune cells, culminating

in the production of a large number of cytokines, leading in a cytokine storm. It involves multiple organs like renal, cardiovascular, hepatic and hemolytic systems. The possible symptoms of CRS include hypotension, myalgia, vascular leakage, fever, nausea and fatigue. The risk of CRS increases with high predisposition of CAR-T cells and may lead to fatal issues in some cases.

Other toxicities include tumor lysis syndrome (TLS), anaphylactic shock, sickle cell anemia and several CNS problems (Shah & Fry, 2019).

### 4.5.4 Solution of CAR-T Cell therapy Limitation

The huge challenges in manufacturing and several toxicities like off-targeting effects, tumor lysis syndrome etc. led to the reduction of the effectiveness of CAR-T cell therapies in their current condition. For this reason, the new technique of CAR-T cell therapy is combined with nanotechnology (Miliotou & Papadopoulou, 2018). Nanotechnology has the capability to deal with the problems of CAR-T treatment described previously. Therapeutic chemicals are loaded into NPs in a method which leads to achieving their target areas despite getting impeded by physiological systems. Nanoparticles has the potential to ameliorate the challenges by producing low DNA carriers. As they migrate within the patient, these carriers can selectively implant tumor-recognizing skills into cells of the immune system. The nanocarrier developed for T cell therapies must follow some distinct characteristics such as; i) it must be stable and safe while being in circulation, and must not react with biomolecules in order to generate an immune response; ii) design should be done by effective way that induce the transfection efficacy. iii) it should be biocompatible and biodegradable in the physiological system, iv) it must migrate the off-target effect and other severe adverse effects (Nawaz et al., 2020). Nanoparticles have the potential to increase the effectiveness of genetically modified T cell therapy for cancer by targeting immune cells and stimulating innate immunity through the toll-

like receptor (TLR) pathway Provided intrinsic features of NPs, it could be used to boost immune modulator administration, limit tumor relapse, and track the treatment activity to cancer therapy. NPS work by boosting the production of CARs, enhancing intrinsic activity of CAR-T cell, CAR-T cell trafficking can be modified, eliminating tumor-associated cells and vasculature that inhibit the immune system. CAR-T cells are being monitored for their therapeutic potential. There are some NP based strategies that combine with CAR-T cell therapies to increase the efficacy and toxicity of the system (Abdalla et al., 2020).

*Table 6: NP mediated CAR-T cell therapies (Abdalla et al., 2020)*

| **Approaches** | **Type of nanoparticles** | **Mechanisms of action** | **Advantages** |
|---|---|---|---|
| Cationic liposomes | Lipid-based | Delivers nucleic acids to T cells in vivo. | Proliferation and cytolytic activity of T cells are unaffected. |
| Cationic polymers | PHEMA-g-PDMAEMA | T cells were effectively transfected with mrna Molecule and plasmid DNA with minimal toxicity. | The main T cell transfection conditions were improved. |
| Phenotypic changes | mRNA mediated NP | Improve the activity of T cell | Immune response was effective. |

| | | | |
|---|---|---|---|
| CRISPR-CAS9 editing | CRISPR/Cas9-RNP | Gene repair of the delivery lead efficiency. | Less mutation |
| Electroporation-based method | mRNA in polymeric PGA NPs | Targeting specific cell subtypes, enhancing receptor-mediated endocytosis, and increasing the therapeutic effects of programmed T cells | Successful removal of TRAC region |
| Transposon-based integration | Polymeric NPs | CAR expression enabled, and in vivo growth of CAR-T cells aided by efficient delivery of DNA payload into T cells | Higher efficacy rate |

## 4.6 Np Mediated Cancer Chemotherapy

Chemotherapy relies on a specific chemical agent which was first used to fight cancer in the 1940s, and with substantial side effects, it has now become a cornerstone in the oncology sector. With the breakthroughs in genomes and proteomics, it has become obvious that cancer is the outcome of a network of interrelated pathological pathways that demonstrate

heterogeneity and diversity. As a result, monotherapy that inhibits a particular target pathway generally results in limited treatment efficacy, substantial adverse reactions, and the formation of drug resistance, owing to the activation of compensatory mechanisms in tumor cells. Combination treatments can more efficiently exhibit maximal anticancer effects with acceptable side effects, while minimizing the possibility of adaptive drug resistance, because tumor cells/tissues are less able to compensate for the simultaneous action of numerous drugs (E. S. Kim, 2016).

### 4.6.1 Challenges of Cancer Chemotherapy

The chemotherapeutic drugs have cytotoxic ability to kill the tumor cell. These medications work by targeting cellular DNA or RNA, as well as their metabolism, to stop cells from proliferating. Chemotherapy is linked to a variety of serious complications, including both acute and subsequent indicators of chronic toxicity. According to the WHO classification, there are 4 grades of chemotherapeutic side effects based on their intensity; mild (grade 1), moderate (grade 2), severe (grade 3), or life-threatening (grade 4). Skin and hair, bone marrow and blood, the digestive tract, and the kidneys all exhibit immediate effects. In grade 2, most organs of the body, particularly vital organs like the heart, lungs, and brain, might be impacted. Furthermore, chemotherapy led to off target effects which caused severe adverse effects to the patients (Schirrmacher, 2019).

### 4.6.2 Solution of the Challenges (Nano chemotherapeutic agent)

To reduce the severe life-threatening adverse effects the chemotherapeutic drug has been loaded with nanoparticles in recent years. Nanoparticles (NPs) are anticipated to provide effective drug encapsulation, small drug administration, increased drug concentration in tumor tissues, increased therapeutic efficacy, and reduced side effects. A platinum (IV) [Pt (IV)]

offering a wide range to a hydroxyl group-appended polylactide (PLA) to produce PLA-Pt, a combined treatment (IV). Using microscopic channels, the polymer has been used to make nanoparticles (NPs) encapsulating the poly (lactic-co-glycolic acid)-block-poly (ethylene glycol) copolymer (PLGA-PEG) and the anticancer drug docetaxel (Dtxl). This combined drug shows greater response compared to the single loaded drug. The adverse effects are reduced by up to 65% compared to the single drug used in the chemotherapy (Xiao et al., 2017).

Therefore, the NP- mediated chemotherapy will be the better option to reduce off target toxicity and high adverse reaction in several types of cancer.

## 4.7 Nano Vaccine in Treatment of Cancer

Vaccines for cancer treatment have been studied for over a decade, although in comparison to vaccines against infectious diseases, it brings more hope than efficacy. This type of vaccine is designed for only disease treatments (Thomas & Prendergast, 2016). Cancer vaccines are divided into two categories: preventative and therapeutic. To begin with, preventive vaccinations can limit tumorigenesis by controlling infections via carcinogenic microorganisms, although if they don't have direct therapeutic potential on cancer. A very well of these vaccines are the human papillomavirus (HPV) and hepatitis B virus vaccines, which have also been licensed by the Food and Drug Administration (FDA) and have already been broadly applied, leading in dramatically lower rates of cervical cancer and liver cancer. On the other hand, therapeutic vaccinations can destroy tumor cells by generating specific autoimmune responses. For example, melanoma-associated antigen 1 (MAGE-1) is a human tumor antigen that is found in melanoma tissues but not in normal tissues or cells that works as antitumor antigen for cancer vaccine (Qin et al., 2018).

### 4.7.1 Mechanism of Action of Cancer Vaccines

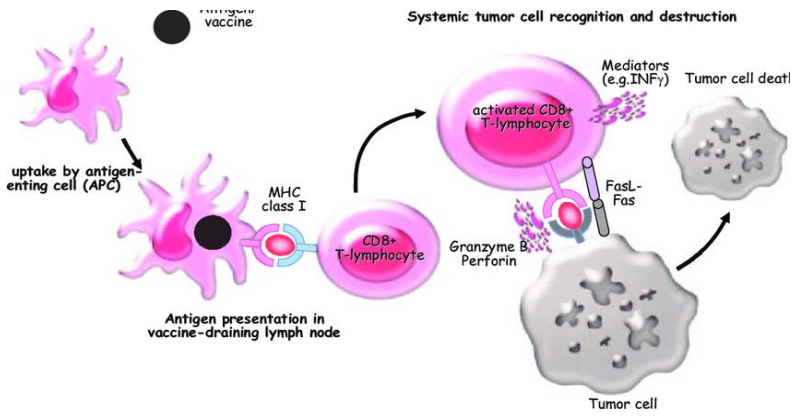


*Figure 4 Mechanism of action of cancer vaccine (Rusch et al., 2018)*

Cancer vaccines are administered through the intradermal route into patients. According to figure 4, after administration the antigen presenting cell uptake the tumor antigen which is in a form of peptide, proteins or tumor cell. Most of the antigen presenting cells are also known as dendritic cells, these cells bind to the antigen in lymphatic drainage nodes for the proliferation and stimulation of CD4 and CD8 cells. These specific cells are responsible for recognition of tumor cells and help to destroy them by direct cell-cell interaction mechanism or using interleukin pathways. Also, these antigens can be presented by MHC complexes which help in identification of tumor cells by the T cell to give antitumor cytotoxic responses against cancer (Rusch et al., 2018).

### 4.7.2 Recent Status of Cancer Vaccine

Cancer vaccines promise to deliver nontoxic antitumor responses by active stimulation of the patient immune system. The current studies have shown that it has 5% efficacy to elicit the anti-cancer immune response. Some vaccines that have shown effective results are approved by a US regulatory agency. Sipuleucel-T (PROVENGE) the therapeutic vaccine approved by FDA works by targeting tumor specific antigen found in the surface of tumor use in metastatic

prostate cancer. Also, several vaccines are in clinical trial phases which claim to provide greater efficacy in the treatment of cancer.

*Table 7: Anti-cancer vaccine status based on the clinical trial (Current trials) (Liu et al., 2020)*

| **Vaccine** | **Targeted Approach** | **Type of cancer** | **Efficacy result (percentage of survival rate vs control)** |
|---|---|---|---|
| BiovaxID | Idotype vaccine with autologous tumor cell fusion with murine/human type antibody. | Prostatic adenocarcinoma (metastatic and adrogen independent) | 37.1% |
| Sipuleucel-T (PROVENGE) | Dendritic cell-based target | Follicular lymphoma<br>Metastatic prostate cancer. | 31.5% |
| Montanide ISA with gp 100 antigen | Modified GP peptide | | 22.1% |

*Table 8: Anti-cancer vaccine status based on the clinical trial (Ongoing trials) (Liu et al., 2020)*

| **Vaccine** | **Targeted Approach** | **Type of cancer** | **Efficacy result (percentage of survival rate vs control)** |
|---|---|---|---|
| Belagenpumatucel-L (Lucanix) | Allogenic cell lines and TGF-beta antisense | Non-small cell lung cancer in stage iii and iv | Pending |
| recMAGE-A3 | Melanoma antigen A3 | Melanoma | Pending |
| Stimuvax | Mucin 1 | Non-small cell lung cancer | Pending |

### 4.7.3 Challenges of Cancer Vaccine

Cancer vaccines have some promising effects to reduce the toxicity related to off target and adverse side effects of other conventional therapy. Hence it also shows some drawbacks such as low antigenicity of the targeted tumor antigen. This scenario occurs due to lack of MHC expression into the tumor surface which in turn reduces the ability of T cells to destroy tumor cells. Additionally, some of the vaccines have a short duration of action after administration

so, lower effectiveness is the major issue for these vaccines. Furthermore, cancer vaccines possess some infusion related side effects at the site of infection (Sambi et al., 2019).

### 4.7.4 Solution to the Limitation: (Nano vaccines)

The subsequent limitation of cancer vaccine can be overcome by delivering it into nanoparticles. Nanoparticle's technology has several advantages over the conventional anticancer vaccines such as it uses the ligand to target dendritic cells, it prevents the degradation of vaccines, provide controlled release and distribution, increase antitumor response by co-deliver vaccine with adjuvants and other, and stimulation of CLTs by increasing cross presentation. Encapsulation of vaccine materials has been proven to improve immunogenicity by shielding the molecules' stability from physiological enzymes like nucleases, proteases, and phosphatases. Nanocarriers like lipid, polymers, peptides and cell membrane coated NPs are used to deliver cancer vaccines and show antitumor response. Some gel-like or polymeric nanoparticle systems can serve as depots for adjuvants and antigens, releasing them during a longer period. Immune cells and immune-rich tissues can be better targeted with nanoparticles (Hu et al., 2018).

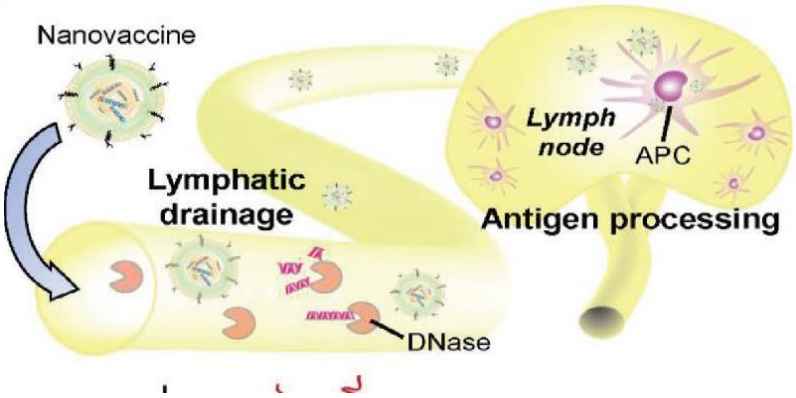


*Figure 5 Nanoparticle based mechanism of anticancer vaccine (Kroll et al., 2019)*

According to figure 5, nano vaccines can easily migrate into the lymphatic drainage which is the largest source of immune cells because of their unique size, shape and surface charge etc properties. Thus, it induces intracellular localization and higher immune response against cancer cells. Nanovaccine characteristics can be tweaked to ensure that their payloads are delivered efficiently for optimal immune activation. Nanomaterials, for instance, can be tailored to target specific subgroups of immune cells. Nanoparticles can also be supplied to precise intracellular compartments; whereby immune pathway receptors can be activated (Kroll et al., 2019). FDA has approved one nano vaccine into the treatment of cancer which shows effective response to reduce the adverse effect and enhance the efficacy compared to conventional vaccine (Beg et al., 2020).

*Table 9: FDA approved nano vaccine in the treatment of cancer (Beg et al., 2020)*

| **Brand Name** | **Type of vaccine** | **Manufacturer company** | **Targeted diseases** | **Current status** |
|---|---|---|---|---|
| Gardasil | Virosome loaded | Merck & Co. | HPV cervical cancer | Developed and marketed |

# Chapter 5

## Recent Progression of NP Mediated Cancer Treatment Worldwide

The treatment of cancer using nanotechnology is still in the development phase or in the trial phase in many developed country-like the United States, European Union, United Kingdom and in some Asian countries. Nanomedicines like Doxil, Abraxane etc are now in the market and used for various purposes like chemotherapy, radiotherapy or as combinational therapy in the treatment of cancer. However, the interest of researchers of different developed country for such medications are increasing amazingly.

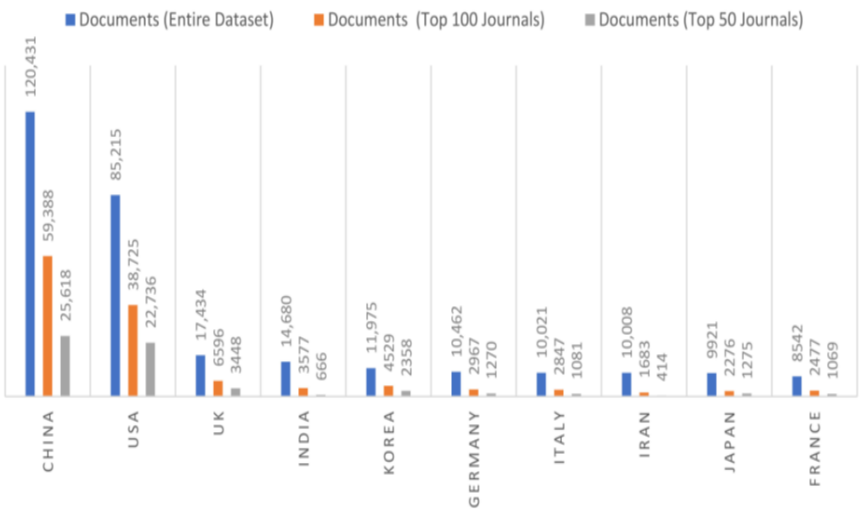


*Figure 6 Recent progression of research in nanotechnology-based cancer treatment over 2000-2020 (Gedara et al., 2021)*

According to the research of (fig 6) (Gedara et al., 2021), the United States and China have been the most productive countries in the world of cancer nanotechnology researches in 2020, and the number of publications spread across most countries in Europe, South Asia, and East Asia over the previous decade period of 2010–2021. Also, countries like Korea, Germany, Italy, Japan and India have potential to develop nanotechnology-based research (Gedara et al., 2021).

Developing countries like Bangladesh use nanotechnology in agriculture and food or in the textile industry. The use of nanotechnology in treatment of certain diseases like cancer are not still widespread in this country. Still now conventional cancer treatments like chemotherapy, radiotherapy or surgery-based treatment are more popular in Bangladesh. For this reason, the remission rate of cancer patients is very low in this country. Hence, in current years the research organizations of Bangladesh like Atomic energy commission, Bangladesh University of Engineering and Technology (BUET) take initiative of collaboration with the government for nanotechnology-based research in cancer treatment (Iftakher & Ahaduzzaman, 2017).

# Chapter 6

## Future Prospects

Nanotechnology has enabled scientists to look for new insights in the diagnosis and treatment of a variety of diseases, including cancer. Nanomedicine reduces the severity of conventional treatments, reduces adverse effects and increases the remission rate of patients. However, there are several nano treatments that do not progress into clinical trials due to some challenges. To begin with, the route of administration of nanomedicines are mostly intravenous which means directly given to blood, so after migration through different organs it cannot be able to reach the target site and may not show the desired therapeutic responses. Magnetic NPs, on the other hand, can be utilized to solve this, as various in vivo and in vitro experiments have shown that 3D magnetic fields can be employed to regulate the migration of NPs against blood circulation (Lv et al., 2021). Another problem arises with the nanoparticle mediated toxicity, some of the nanoparticles containing synthetic materials that are not well biocompatible or generate free radicals under individual factors. It contributes to damage to the major organs like kidney, liver, heart and lung etc. There are also other parameters contributing to NP related toxicity such as shape, size, agglomeration and solubility. Manufacturing NPs from biocompatible materials like chitosan and materials that dissolve when exposed to near-infrared light could be a viable approach. Several NPs utilized in vivo and in vitro investigations are made in small batches, and scale-up for large amounts is sometimes not possible due to apparatus as well as other factors. Also, the strongest clinical candidates in animal models are not systematically planned and optimized. Hence, for effective trials advanced tests in large scale have to be employed in the efficient manner (Xia et al., 2020). Additionally, the immune system sometimes causes phagocytosis of nanoparticles, it is very difficult to evade. Though the nanoparticles are designed to evade this mechanism by coating with protein corona, it does not work as expected.

For this reason, researchers have involved a novel pathway known as CD47-SIRPα which targets the macrophage to reduce its requirements and depleting them. Immunotherapy related to nanoparticles have been shown immunotoxicity, or neurotoxicity during ongoing in vitro tests. However, "nanovaccines" and "artifi-cial APCs" have shown to be more effective than traditional immunotherapy in ongoing clinical studies. Furthermore, there are other difficulties in technological areas or in study design of nanoparticle-based cancer treatment. Therefore, the new advanced research based on nanotechnology can mitigate all the difficulties (Gavas et al., 2021).

# Chapter 7

## Conclusion

Recent advancements in cancer therapeutics are increasing day by day, and nanotechnology is one of the useful techniques with many successful studies. Use of nanoparticles with multiple functions in the treatment of cancer is a growing field because of its competitive advantage over traditional techniques in terms of efficacy and safety. Also, nanotechnology contributes in imagining, diagnosis besides the treatment therapy. It combined with the conventional therapy to ease the delivery into the target side, provide greater safety, stability and non-specific target effects with increasing rate of efficacy. Nano technology mediated cancer treatment provide wide area to treat these fatal diseases including nanomedicines, nano-immune checkpoint blocker, CRIPR/Cas's nanoparticle-based cancer treatment, biomimetic Nps, CAR-T cell therapy and the nano chemotherapeutic agents and nano vaccines with their mechanism, delivery system and side effects also scope of further studies have been addressed in this review article. Finally, nanoparticles based on smart target therapy or combination therapy and RNA mediated therapy are also explored in the future research.

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