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Nanocarriers for Anticancer Drug Targeting: Recent Trends and Challenges

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ABSTRACT: Nanocarriers are nanostructured vehicles employed to deliver anticancer drugs to the targeted tumor sites in the body. Nanocarriers have been successfully employed to circumvent certain limitations of conventional anticancer drug delivery while providing greater bioavailability, prolonged circulation time and higher tumor accumulation for enhanced therapeutic outcomes in cancer treatment. Nanocarriers are also responsive to functionalization to tailor their pharmacokinetics and achieve enhanced therapeutic outcomes in cancer therapy. Among organic, inorganic and hybrid type, several nanocarriers have gained approval for use in cancer patients, while many more are under clinical development. For the last two decades, cancer immunotherapy-based advanced targeting approaches such as monoclonal antibodies, antibody drug conjugates and immune checkpoint inhibitors that utilize human immune system functions have vastly developed which furnish better treatment options in several intractable cancers compared with traditional cancer therapies. This review discusses the imperative role of tumor vasculature in passive and active targeting of anticancer drugs using organic and inorganic nanocarriers and the current research efforts underway. The advanced targeting approaches for treatment of various cancers and their most recent clinical development scenario have been comprehensively explored. Further, potential challenges associated with each type of nanocarrier, and their translational obstacles are addressed.

KEY WORDS: cancer targeting, active targeting, passive targeting, advanced targeting, diseased vasculature, monoclonal antibodies, antibody-drug conjugate, nanobodies, immune checkpoint targeting

I. INTRODUCTION

Cancer is a deadly disorder in which some of the body's cells grow abnormally and uncontrollably while spreading to the adjacent organs.[1] Being a major cause of deaths globally, cancer is anticipated to extend 27.5 million new cases and 16.3 million deaths by 2040.[2] Among various types of cancers, lung, prostate, colorectal, stomach and liver cancers are common in men, whereas breast, colorectal, cervical and thyroid cancers are common in women.[1] The widely used treatment for cancer is conventional chemotherapy, which is non-selective and works by interfering with cell DNA synthesis and mitosis to kill rapidly growing cancer cells. Due to non-selectivity, chemotherapy can also damage normal tissues causing severe adverse effects, which is major causes of

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high mortality in cancer patients.[3] On the other hand, conventional drug delivery exhibit poor bioaccessibility, thus requiring higher doses leading to incidence of multiple drug resistance. On the contrary, targeted delivery systems specifically reach to tumor sites, and improve efficacy of anticancer drugs while causing minimal side effects.[4]

Nano drug delivery system utilizes the nanoscale materials or nanocarriers for diagnostic and therapeutic applications which impart unique benefits for treatment of chronic human diseases compared with conventional treatments.[3,4] Nanocarriers are nanostructured vehicles purposed to transport drugs to the targeted sites in the body. In addition to exceptionally improved targeting ability in cancer therapy, different nanocarriers composed of lipid, polymer carbon or metallic nanomaterials are also amenable to surface functionalization to achieve desired pharmacokinetics required to fulfil the clinical needs.[5–7] Various nanocarriers composed of organic or inorganic materials confer distinctive characteristics such as improved pharmacokinetics and cell distribution, reduced toxicities, enhanced drug solubility and stability, as well as desired site-specific delivery of the anticancer drug.[7,8] Thus, design and development of efficient target-oriented anticancer delivery systems are highly desirable to achieve enhanced efficacy and reduced toxicities in cancer treatments. Targeted drug delivery system based on passive targeting modality requires thorough understanding of tumor vasculature and tumor microenvironment, while several advanced targeted therapies based on active targeting modality rely on specific antigens or targets expressed on the tumor cells during tumor progression. Since last couple of decades, cancer immunotherapy using advanced targeting approaches that utilize human immune system functions, such as monoclonal antibodies (mAbs), antibody drug conjugates and immune checkpoint inhibitors have vastly exceeded treatment options in several types of intractable cancers compared with traditional cancer therapies. Many antigen targets have already been recognized and employed for target-specific cancer treatments, however, search for novel molecular target is an area of ongoing cancer research.[9,10] This review discusses imperative role of tumor vasculature in passive and active targeting of anticancer drugs using organic and inorganic nanocarriers and their associated challenges while focusing on immunotherapy-based advanced targeting mechanisms and their recent clinical development.

II. TUMOR VASCULATURE AND TARGETING STRATEGIES FOR NANOCARRIERS

In presence of tumors, vascular abnormalities such as hypervascularisation with abnormal vascular architecture occurs due to irregular shape of endothelium, alongwith higher production and expression of vascular endothelial growth factor (VEGF) which leads to dysregulated endothelial function and blood vessel angiogenesis.[11] Although different cancers produce different forms of pathological vasculature, most cancer forms exhibit similarities in disturbed vessel permeability and disordered vasculature. The diseased vasculature is the cause of decreased or increased endothelial leakiness that allows permeation of nanocarriers across the tumor sites.[12] More specifically, the abnormal vessels mediated through VEGF are formed in solid tumors that show a typical enhanced

permeation and retention (EPR) compared with normal blood vessels. During this condition, an imbalanced angiogenesis occurs, which promotes formation of anatomically and physiologically different abnormal blood vessel network, creating an increased vascular density in solid tumors. Such a diseased vasculature is rich in abnormal blood vessels having large interstitial spaces between endothelial cells. According to an extensive study by Bhavsar et al., the basis of tumor microenvironment is formed through interferences between tumors and its stroma that cause proliferation and invasiveness along with development of metastatic potential and induction of stemness to the tumors.[13] The role of tumor microenvironment and major events taking place in development of a cancer is explicitly shown in (Fig. 1).[14] Further, the understanding of tumor microenvironment helps to determine the suitability and design of targeting modality in terms of passive or active targeting. For instance, the condition of defective vasculature causing the EPR effect is common in most solid tumors. Thus, tumor vasculature abnormalities have been exploited as an effective passive targeting approach in nanocarrier-based targeted anticancer drug delivery.[15]

A. Passive Targeting

The sizes of tumor endothelial cells range from 100 to 700 nm during the EPR effect, which is about 50 to 70 times bigger compared with normal endothelial sizes of about 10 nm, allowing the nanocarriers to move into the interstitial spaces once passing through

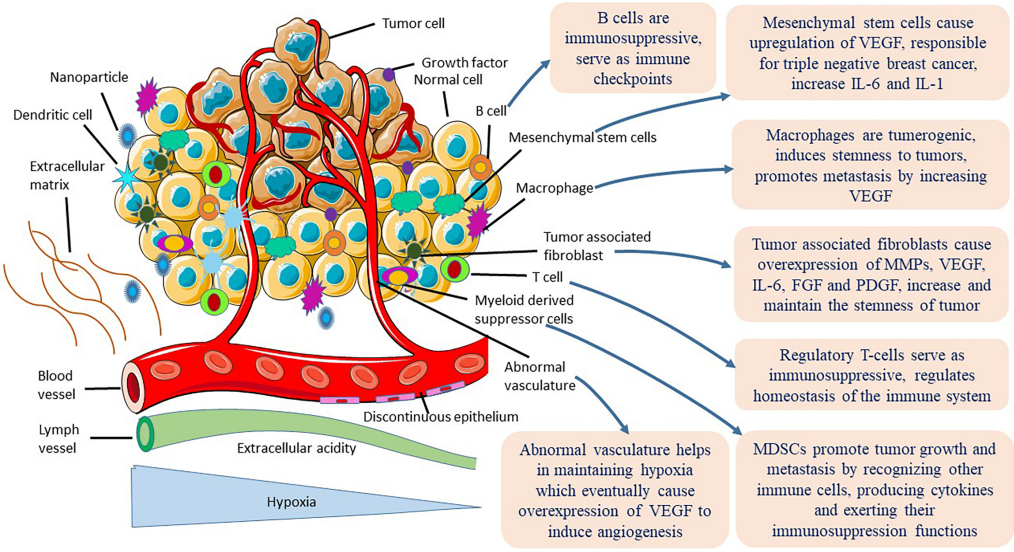

FIG. 1: Tumor microenvironment and its role in cancer development. MMP, matrix metalloproteinase; IL-6, interleukin-6; FGF-2, fibroblast growth factor-2; PDGF, platelet-derived growth factor.

the endothelial barrier (Fig. 2A). Moreover, poor lymphatic drainage of solid tumor causes deficient circulatory regain of extravasated molecules, which leads the nanocarriers to accumulate at tumor site via EPR effect.[15] This does not happen in case of low molecular weight loaded-nanocarriers, as they can return the blood circulation via diffusion, enabling long time stay of nanocarriers at tumor site. Thus, pathophysiological characteristics of the tumor tissues are critical for targeting of low-molecular weight drugs via EPR effect. Organic nanocarriers composed of polymers or lipids utilize the EPR effect to enhance systemic circulation and target tumor cells.[13]

Microenvironment in solid tumor exhibit hypoxia and increased aerobic glycolysis. Cancer cells use glycolysis as a metabolic pathway to generate ATP and gain energy as a fundamental metabolic transformation that occurred during cancer. The inadequate oxygen and nutrients cause the tumor cells to undergo glycolysis for gaining extra energy than normal cells, thus creating an acidic microenvironment.[13] The differences in microenvironment near tumor cells and normal cells also aid passive targeting. In addition, the tumor cells' existence and movement give rise to discharge of uncommon enzymes such as metalloproteases, creating a peculiar tumor microenvironment that can be targeted using liposomes, polymeric nanoparticles, micelles, and antibodies.[16] Since the cancer cells have increased dependence on glycolytic pathway, the pharmacological inhibition of glycolysis to kill cancer cells is an important approach to develop anticancer agents. Plethora of research reports are available in the field of passive targeting with only few product approvals for clinical use. Restricted commercial success of passive targeting may be due to its limitations in tumor targeting including misconceptions in EPR effects, differences among human patients and animal models, and low intracellular penetrability of nanocarriers.

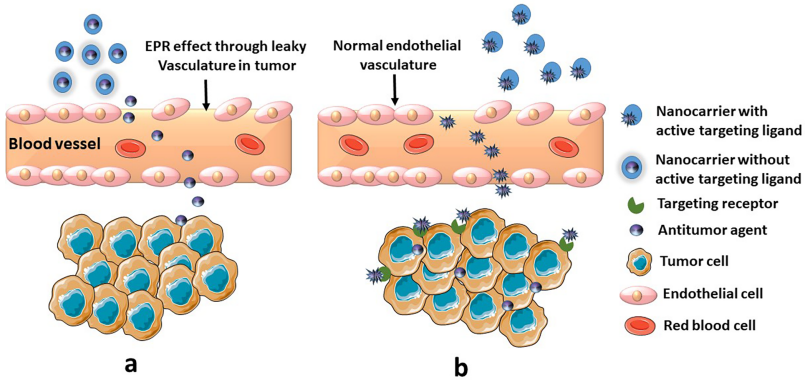

FIG. 2: (a) Angiogenesis-mediated tumor vasculature consisting of abnormal tumor vessels with interstitial spaces between endothelial cells which allows nanocarriers to pass through these spaces for passive targeting (EPR effect). (b) Ligand-attached nanocarrier binds with receptor expressed on target cell via ligand−receptor interactions that leads to nanocarrier internalization causing active targeting.

B. Active Targeting

Active targeting requires nanocarriers' surfaces to be attached to targeting moieties like ligand, antibody, or peptide to recognize and target specific cells expressed during tumor proliferation through the ligand−receptor interactions, followed by the internalization of receptor bound nanocarrier to release the drug inside the cell. Thus, active targeting strategy results in target-specific drug release compared with passive targeting systems (Fig. 2B). In active targeting system, the ligands attached on nanocarrier surfaces possess specificity in recognizing and binding with the protein receptors expressed on tumor cell surfaces. These receptors are either exclusively expressed on tumor cell or either less abundant/absent in normal cells, enabling increased uptake of drug by tumor cells.[17] Targeting moieties used for active targeting include small molecules, antibodies fragments, lipoproteins, hormones, lectins, transferrin, carbohydrates, folic acid, nucleic acids, and growth factors. Nanocarriers possess high surface area-to-volume ratios that enable multiple attachment of targeting moieties to their surfaces for better tumor-specific targeting. The uniform expression of receptors on all tumor target cells and specific binding of nanocarriers' targeting moieties to these tumor cells receptors only, are two important considerations for effectual usage of active targeting strategy. Thus, active targeting offer advantages over passive targeting in terms of preventing non-specific drug delivery and reducing the MDR.[18]

III. NANOCARRIER-BASED ANTICANCER DRUG TARGETING

The purpose of nanocarrier-based drug delivery systems is to solve the critical issues like low selectivity, increased toxicity, and drug resistance exhibited by conventional anticancer delivery. Nanocarrier-based systems with submicron size (typically < 200 nm) can be efficiently utilized for the transport of antitumor drugs enabling the effective delivery of drug or therapeutic agent into the diseased tissues.[19] The nanocarriers for antitumor applications is developed to achieve maximum therapeutic outcomes at minimal level of side effects by exploiting pathophysiology of a diseased microenvironment. Based on composition or materials used, the nanocarriers can be categorized as (i) organic nanocarriers comprising of lipids or polymers, (ii) inorganic nanocarriers comprised of carbon-based inorganic materials or metals, and (iii) hybrid nanocarriers, which are appropriately designed combination of organic and inorganic materials. Some nanocarrier-based systems have gained approval for treatment of various cancers, whereas several others are in clinical pipeline.[20]

A. Organic Nanocarriers

Organic nanocarriers are made up of polymeric or lipidic materials. Organic nanocarriers are versatile in nature, and their surface functionality enables these nanocarriers to show low toxicity, and conjugation ability to a wide range of targeting moieties like ligands, antibodies, or other biomolecules of therapeutic interest for targeted delivery.

Fundamental structures of drug loaded targeted organic nanocarriers are illustrated in Fig. 3.

1. Liposomes

Liposomes are the oldest and most successful natural or synthetic lipid-based nanocarrier employed for anticancer drug targeting. These spherical vesicular systems range from 30 nm to several microns and consist of multiple lipid bilayer which encloses an aqueous core. Liposomes are classified as small unilamellar vesicles consisting of only one bilayer membrane, or multilamellar vesicles consisting of more than one bilayer membranes. Liposomes with single or multiple number of bilayer membranes are achieved by changing composition, preparation method, size, and surface charge.[21]

Liposomes provide multiple advantages including biodegradability, biocompatibility, encapsulation ability for both polar and nonpolar drugs, protection of drug from early degradation via encapsulation, low toxicity, enhanced *in vivo* performance and cost-efficiency.[21] In addition, drugs loaded in liposomes display altered pharmacokinetic and improved biodistribution in comparison to non-encapsulated drugs. Polyethylene glycol (PEG) is used to functionalize liposome surfaces to produce PEGylated or stealth liposomes (Fig. 4), which prolongs the plasma half-life and blood circulation time leading to an increased concentration of drug inside tumor cells while decreasing drug concentrations in normal tissues, thus reducing the side effects.[22] Poly(lactic-co-glycolic acid) can be used to engineer surfaces of liposomes for increased stability and half-life, and their surfaces can also be conjugated with ligands or antibodies for enhanced

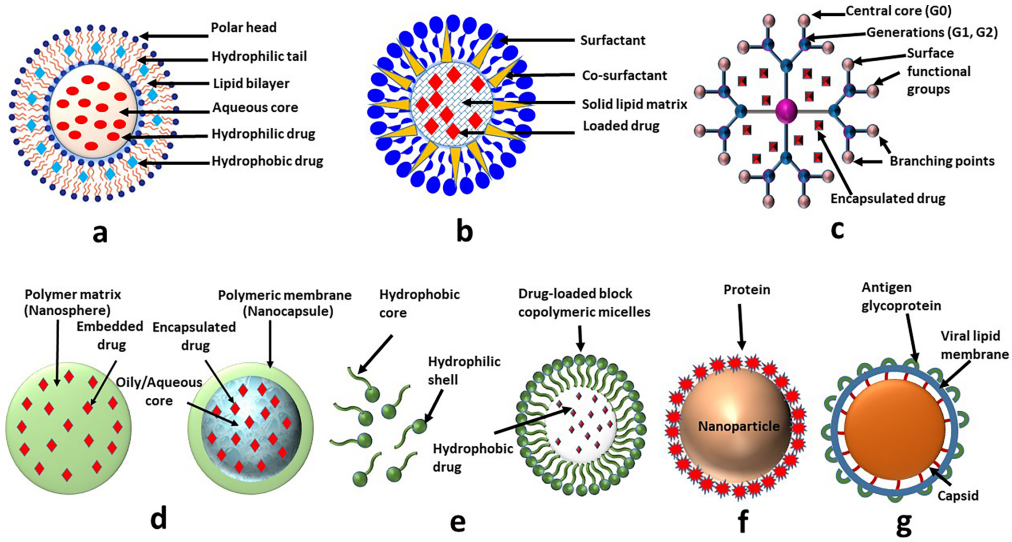

FIG. 3: Various organic nanocarriers for anticancer drug delivery. (a) Liposome, (b) SLN, (c) dendrimer, (d) PNC, (e) PM, (f) VLN, and (g) protein nanocarrier.

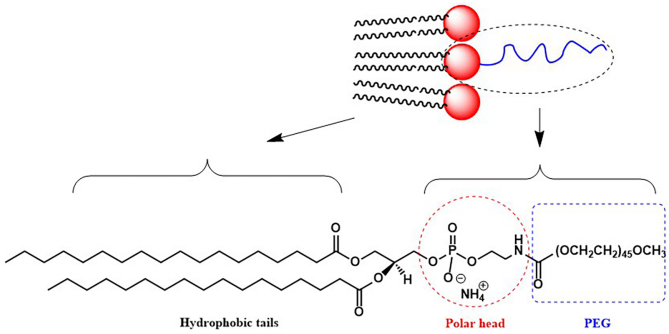

FIG. 4: Covalent linking of PEG chains to liposome to form PEGylated liposome

target-specificity.[21] By exploiting specific features of tumors or tumor microenvironment, liposomal formulations can be functionalized by conjugating targeting moieties onto their surfaces using ligands, antibodies, aptamers, peptides and proteins, or via stimulus-response to facilitate active targeting of tumors. In the active targeting strategy, two major therapeutic targets are the cancer cells and the tumoral endothelium.[23] Recent research outcomes of some significant studies using active liposomal formulations are summarized in Table 1.[24–35]

Despite substantial research efforts and immense potential, the liposome delivery in cancer treatment is challenging and limited as employing liposomes in cancer treatment has not exhibited significant benefits in extending patients' survival. There are several liposome related barriers that play critical role in their *in vivo* performance such as physiological barriers (size, morphology and surface charge, payload design, drug release), vascular-related barriers (EPR effect), tumor-related barriers (tumor interstitial fluid pressure), immune system barrier (liposome- host immune system interactions, low recognition of liposome by immune system) as well as preclinical study challenges. Since experimental models have a crucial role in the evaluation of the efficacy of liposomal formulations, choosing not only most appropriate animal model, but also more than one model is advisable. Currently, subcutaneous tumor models and xenografts models are employed for preclinical evaluation of liposomes, however, they are unable to precisely evaluate the immune response against tumor cells or liposomal systems and have poor prediction of human EPR effect. These challenges associated with liposome nanocarriers have led to the failure of many liposomal formulations in clinical trials despite their excellent performance in preclinical studies.[36]

Moreover, issues related to pharmaceutical manufacturing, high production cost, evaluation and quality assurance especially when the delivery system becomes more complex such as the addition of surface modification with coatings and/or ligands are potential challenges in development of liposome-based nanocarrier systems. To exemplify, in development of functional liposomes, integration of multiple number of ingredients, physicochemical variables and steps involved in their syntheses complicate the assessment of the pharmacokinetics, pharmacodynamics and toxicology of this nanocarrier

TABLE 1: Recent research updates in liposome nanocarriers for active targeting of anticancer drugs

Active targeting strategy	Targeted receptor/ molecule/site	Anticancer agent	Key research outcomes	Ref.
Ligand-receptor	VEGF	Docetaxel	– Anacardic acid-modified docetaxel liposomes possessed enhanced anticancer efficacy, improved pharmacokinetics and lower toxicity to normal cells than marketed formulation Taxotere® in human breast cancer cell lines	24
—	Transferrin receptor	Doxorubicin	– Holo-Lactoferrin modified liposomes were developed for tumor targeting and image-guided combined radio chemotherapy showing ability of catalyzing the conversion of H_2O_2 to O_2 to relieve the tumor hypoxic microenvironment – Holo-Lactoferrin liposomes exhibited excellent cancer treatment application using fluorescence imaging in breast cancer-bearing mice xenograft	25
—	—	Vincristine + Tetrandrine	– Liposome encapsulated with two anticancer drugs – Tetrandrine incorporated into the lipid bilayer showed multi drug resistance reversal by blocking cancer cell invasion and vasculogenic mimicry channels which are major obstacles associated with blood-brain barrier in treatment of glioma – Transferrin-modified vincristine plus tetrandrine increased cellular uptake, enhanced transport across blood brain barrier and prolonged circulation time in brain glioma model	26
—	Folate receptor	Doxorubicin	– Folic acid functionalized liposomal nanocomplex encapsulated both gold nanorods and doxorubicin which facilitated synergistic photothermal and chemotherapy actions to inhibit tumor growth in breast tumor-bearing mice xenograft	27
—	—	5-FU	– Folate-receptor targeting liposomes loaded with 5-FU selectively targeted tumor cells and exhibited higher cellular uptake, increased ROS production, and better tumor inhibition in CT26 colon cancer cell lines without causing tissue abnormalities	28

TABLE 1: (*continued*)

—	—	Bleomycin	– Folate-targeted liposomes loaded with bleomycin demonstrated increased cellular uptake by HeLa (human cervical cancer cell lines) and MCF-7 (breast cancer cell lines) cells compared with folic acid-free liposomes, indicating their potential for targeting folate-receptor positive cancers	29
—	Glycoprotein	Doxorubicin	– Lectin from Lotus tetragonolobus (LTL) was used as targeting ligand to bind glycans containing alpha-1,2-linked fucose, which is found only in highly proliferative cells but not in normally growing cells – Lectin could efficiently direct liposomes to the cytoplasm of tumor cells by a pathway which is different from other lectins showing intracellular delivery of drug after injection to melanoma-bearing mice	30
Peptide and protein	Mitochondria	Antimycin A	– Liposomes coated with a cell penetrating peptide facilitated cellular uptake of a mitochondria-targeting drug antimycin A by its preferentially binding to cytochrome c reductase in mitochondria – Inhibition of mitochondrial respiration and enhanced cellular uptake were showed in A254 human lung cancer cells	31
—	Brain	Doxorubicin	– Cell penetrating peptide R8-conjugated liposomes were further modified with oleic acid to deliver doxorubicin–developed liposomes demonstrated significantly improved biodistribution in brain showing their potential treating glioma	32
Antibody	ER	Doxorubicin	– Dual construct liposome was functionalized with both tumor identification ligand (anti-ER antibody) and an immune targeting ligand (self-peptide) – The functionalized liposome could recognize and bind ER-overexpressed breast cancer cells in distinct manner demonstrating enhanced targeting and reduced phagocytosis by macrophages resulting in enhanced cellular uptake and reduced immune clearance of liposomal nanocarriers	33
—	EGFR	Doxorubicin	– Multifunctional thermal-sensitive liposome with antibody conjugation targeted EGFR-expressing human lung cancer and also found to be an efficient contrast agent as a safer treatment for EGFR-overexpressing human lung cancer in tumor-bearing mice xenografts with no damage to normal tissues	34
Aptamer	BNL 1ME A.7R.1 (MEAR) cells	Cabazitaxel	– Aptamer TLS1c functionalized cabazitaxel-encapsulated liposomes showed improved tumor-targeting efficiency and decreased toxicity in human hepatoma model	35

following its administration. On the other hand, different intellectual property regulations for targeted and non-targeted liposomes, design and composition of liposome and nature of drug encapsulated contribute to weak IP position, which eventually, negatively impact the research, development and commercialization of liposome nanocarriers.[37]

2. Solid Lipid Nanoparticles (SLNs)

SLNs are lipidic nanocarriers in size range 50–1000 nm that have been employed for delivery of lipophilic drugs since the early 1990s. SLNs can be prepared by melting the solid lipid(s) and dispersing in emulsifiers-containing water to stabilize the dispersion using high pressure homogenization or microemulsification. Solid lipids commonly used in SLNs are mono-, di-, and triglycerides, sterols, free fatty acids, fatty alcohols and waxes which create a highly nonpolar lipid matrix that dissolves or dispersed the drug.[38] The drug-loading method into SLNs is selected based on lipid, drug, surfactant, and production conditions. The common drug loading methods include homogeneous drug dispersion in lipid matrix, incorporation of drug in the shell to enclose the lipid core, and incorporation of drug in the core enclosed by a lipid shell.[39]

Unlike nanoemulsion that uses liquid lipids/oils, SLNs employ lipids which are solid at room temperature. SLNs show many drug-delivery benefits such as controlled release, non-toxicity, high drug loading, enhanced bioavailability of nonpolar drugs, high stability and production scalability.[38,39] Traditionally, SLNs incorporate nonpolar drugs, however, recent polymer–lipid hybrid or lipid–drug conjugation approaches have enabled incorporation of hydrophilic and ionic drugs also to achieve their controlled release.

Garanty et al.[40] developed arginine-glycine-aspartate–decorated SLNs (RGD-SLNs) for the selective targeting of an anticancer drug asiatic acid to glioblastoma cells. RGD-SLN employed two different PEG-linker size which showed enhanced anticancer activity of drug via efficient tumor targeting and increased cytotoxicity with 3D tumor spheroids compared with drug alone and nonRGD containing SLNs. RGD-SLNs also exhibited higher cell death and greater uptake toward U87 MG cells, thereby confirming its potential as effective targeting approach in glioma therapy. Moreover, the length of PEG chains that connect RGD to SLN appeared to be of no significant effect on 3D cellular models. The studies can be applied to other integrin-associated cancer forms.

Karamchedu et al.[41] encapsulated poorly water-soluble drug morin hydrate in SLN to improve its poor solubility and oral bioavailability. The drug loaded nanosized SLNs possessed three-fold higher *in vitro* cytotoxicity against human cervical cancer cells and along with improved pharmacokinetic compared with free morin hydrate.

Bhagwat et al.[42] developed transferrin targeted tamoxifen citrate encapsulated SLNs for treatment of breast cancer. The developed SLNs exhibited higher anticancer effect than pure drug on human breast cancer MCF-7 cells. The cellular uptake of developed SLNs proposed that the engineered SLNs could be an effective nanomedicine for the treatment of breast cancer.

Smith et al.[43] investigated the application of SLNs for the targeted delivery of high payload of 5-fluorouracil (5-FU) by utilizing a “Strategic and unique Method to

Advance and Refine the Treatment" (SMART) of colorectal cancer through hot and cold homogenization approach to formulate SLNs employing unique PEGylated lipids and surfactant combination. The pharmacokinetic and tumor efficacy studies were performed on HCT-116 cancer cells, which demonstrated significantly improved inhibition of subcutaneous tumor growth in mice as compared with 5-FU. The studies suggested that developing a smart nano-delivery system could be a powerful strategic approach to optimize the delivery efficiency of anticancer drugs to the tumors.

Despite many advantages, SLNs eliminate quickly from the blood flow by reticuloendothelial system, presenting a challenge for sustaining the drug release. One of the major issues related to SLN manufacturing is related to the polymorphic crystal modification of the lipids during storage leading to drug expulsion and eventual decrease in drug loading of SLNs. In addition, polymorphism induces serious changes in the nanocarrier's size and shape which may give rise to destabilization of the SLN suspension, accelerating the nanoparticle aggregation. Another major challenge concerning SLNs include variability of the EPR effect based on the tumor type, which in turn limits their applications in passive targeting and reinforces the use of active targeting as more attractive targeting strategy.[44]

3. Dendrimers

Dendrimers are hyperbranched, star-shaped, nanocarriers comprising of three structural characteristics: various arms originating from the interior (central core), interior layers (branches), and peripheral surface with functional groups. The dendrimers are produced by stepwise synthesis using natural or synthetic monomers like nucleotides, sugars, amino acids, or carbohydrates to yield highly regular molecules with definite number of surface groups.[45] The step-by-step synthesis processes for dendrimers differ to routine polymerization in terms of highly regular branching patterns and unique architecture compared with traditional polymerization processes which typically results in irregular branching patterns. Moreover, multivalency, monodispersity, distinct shape, nanosize (1.5–14.5 nm diameter), and surface functionality further extends dendrimers' drug delivery applications. During the dendrimer synthesis, branches can be added to the core at each level throughout the synthesis and addition at each synthetic cycle is called a "generation." The dendrimer is functionalized via surface attachment of drugs or targeting ligands to enable its precise contact at active sites, thus reducing the side effects. Drug loading in dendrimer cores can be achieved by hydrogen bonding, chemical linkages, or hydrophobic interactions, however, the internal layers at branching points are not generally used for drug-loading.[45] De Groot et al. investigated a "cascade release" dendrimer that employed an acid-sensitive and reduction-susceptible disulfide linker to attach a drug to the dendrimer surface.[46] The drug molecules released upon single triggering via 4-aminocinnamyl alcohol linker that created slightly acidic tumor environment in which a nitro group underwent reduction to an amino group.

Rompicharla et al.[47] conjugated a poorly soluble anticancer agent paclitaxel on the surface of G4 poly-amido-amine (PAMAM) dendrimer which was then PEGylated and

further tagged with biotin. G4-paclitaxel- PEG-biotin conjugate demonstrated prominent penetration into the monolayers and tumor spheroids, improved cytotoxicity and increased tumor inhibition compared with free drug indicating the potential of biotin-anchored PEGylated dendrimer conjugate to precisely deliver dendrimer nanocarrier in biotin receptor-overexpressed tumors.

In another study, Bhatt et al.[48] synthesized transferrin anchored, PEG and α-tocopheryl succinate conjugated G4 PAMAM for the delivery of paclitaxel. The study results demonstrated increased cellular uptake, cytotoxicity and apoptotic potential of paclitaxel compared with its free form and significantly inhibited growth of human cervical epithelial cells spheroids paving the road for targeted delivery of other poorly water-soluble anticancer drugs.

Liu et al.[49] screened and identified a high-affinity peptide, epidermal growth factor (EGF) receptor (EGFR)-binding peptide 1 (EBP-1), as targeting ligand, which conjugated with PAMAM dendrimer to encapsulate doxorubicin. The dual-functional nanocarrier demonstrated improved tumor-targeting efficiency and anti-proliferation effect, which provided higher tumor inhibition against triple-negative breast cancer (TNBC).

In one of the most recent studies by Ouyang et al.,[50] functional theranostic nanoplatform was created utilizing G5 PAMAM dendrimer-encapsulated copper sulfide nanoparticles complexed with plasmid DNA-encoding hypermethylation in cancer 1 to achieve image-directing inhibition of both tumor and tumor metastasis. This functional theranostic system provided an intelligent upgraded design of hybrid theranostic for inhibition of metastasis based on modified zwitterionic-enabled antifouling property for enhanced therapeutic efficacy in TNBC model showing inhibition of both subcutaneous tumor and lung metastasis.

Dendrimer nanocarriers face major challenges in terms of safety and toxicity. Although ambiguous and poorly understood, research efforts have shown dendrimer-associated cytotoxicity and haemolytic activity depending on its generation, chemistry, functionality, as well as concentration, time of exposure, cell type and cell-medium composition under the study.[51] Dendrimer surface groups with electric charge leads to electrostatic interactions with biological structures to cause toxicities. For instance, cationic surface groups show low toxicity whereas anionic surface groups contribute to high toxicity. Likewise, polar surface groups show low toxicity whereas non-polar surface groups show high toxicity. The dendrimer branches show indirect influence on toxicity which is linked to its size, type and number of surface groups while dendrimer core influence toxicity that relates to dendrimer generation. Moreover, the physicochemical properties matters when dendrimer comes in contact with the blood and may interact with plasma proteins, blood cells and biological membranes which could lead to cell disruption and cell death.[52]

4. Polymeric Nanocarriers (PNCs)

PNCs are solid nanosized carriers made up of biodegradable polymers with range of 10–1000 nm. PNCs can be classified as matrix type (nanospheres) and reservoir type (nanocapsules). In nanospheres, drug is loaded via dispersion or entrapment in polymer

matrix, or adsorption or conjugation to the surface. In nanocapsules, drug is loaded via dissolution or dispersion in liquid core (oil or water) which is covered by polymer membrane. The PNCs can be prepared by employing synthetic polymer using solvent evaporation, salting out, nanoprecipitation, dialysis, and supercritical fluid technology. PNCs can also be produced by direct polymerization of monomers using emulsification polymerization, microemulsion polymerization, interfacial polymerization and controlled/living radical polymerization.[53]

Synthetic polymers such as PLGA, polylactic acid, polyglycolic acid, polycaprolactone, N-(2-hydroxypropyl) methacrylamide copolymer, polyaspartic acid, and polyglutamic acid, or natural polymers like albumin, alginate, chitosan, collagen, dextran, gelatin and heparin. PNCs provide advantages such as high drug loading, uniform particle sizes, *in vivo* stability and higher circulation times which are desirable for successful cancer therapy.[54] Tunable physicochemical properties and drug release profiles of PNCs can be achieved via selection of different polymers with varying molecular weight, hydrophobicity, and biodegradability. In addition, surface modification of polymers is used for developing stimuli-sensitive PNCs that change their own physicochemical properties in response to certain stimulus or environmental signals. Stimuli-responsive PNCs may lead to programmable drug delivery in cancer treatment.[53] Zhu et al. synthesized docetaxel-loaded polydopamine-modified nanoparticles which were conjugated to galactosamine for enhanced ligand-mediated endocytosis and improved docetaxel delivery. Studies revealed interactions between PNCs and hepatocellular carcinoma cells occurred via ligand-receptor recognition, which demonstrated high cellular uptake, enhanced tumor inhibition and reduced tumor size on hepatoma-bearing nude mice.[55]

Tangthong et al.[56] synthesized functionalized polymeric nanoparticles via conjugation of water-soluble chitosan, gold nanoparticles and LyslLys3 peptide for the theragnostic applications in prostate cancer. The developed PNC system exhibited specificity toward gastrin-releasing peptide (also known as bombesin) receptors overexpressed in prostate cancer cells while displaying synergistic targeting and cytotoxicity toward PC-3 and LNCaP cancer cells, establishing the template synthesis as a novel approach for the use of functionalized polymeric nanocarrier system in cancer therapy and diagnosis.

Deepika et al.[57] developed rutin and benzamide loaded polyvinyl alcohol stabilized PLGA nanospheres using water-oil-water emulsion method and investigated its inhibitory effects on MDA-MB-231 cells for the treatment of TNBC. The cytotoxic activity of the developed nanospheres were attributed to the disruption of cell cycle and induction of apoptosis while delivering the drugs in sustained manner without inducing genotoxicity in zebrafish.

Zu et al.[58] prepared chondroitin sulfate-functionalized camptothecin loaded actively targeting polymeric nanoparticles. The experimental results proved that the surface functionalization with chondroitin sulfate conferred colon-cancer targeting capacity by improving pro-apoptosis effect against colon cancer cells in colon tumor-bearing mice, demonstrating potential of functionalized PNCs in targeting colon cancer.

Wu et al.[59] developed EGF-functionalized 5-FU and perfluorocarbon-loaded PLGA nanoparticles and investigated their targeting efficiency and *in vivo* performance in

SW620 cells for selective delivery in colon cancer chemotherapy. The functionalized nanoparticles demonstrated higher cellular uptake, suppressed cell viability. and induced apoptosis compared with non-targeted nanoparticles, and the improved therapeutic effects were contributed to oxygen transportation capacity of perfluorocarbons which relieved tumor hypoxia.

PNCs pose challenges related to nanoecotoxicology which must be addressed in the coming years. The first important concern in this context is the determination of physicochemical properties of the PNCs before, during and after the biological experimentation. Secondly, it is challenging to identify the pathway that can examine potential toxic behavior or toxic degradation processes of the polymers, hazards related to toxic monomer aggregation and residual materials of PNCs containing synthetic polymers in the organism or in the food chain. The third challenge relates to the selection of appropriate experimental organisms that can be used in experimentation and testing of anticancer PNCs.[60]

5. Polymeric Micelles (PMs)

PMs are nanosized "core/shell structure" of size range 10–100 nm which are formed by action of insoluble copolymer to form nonpolar core and soluble copolymer to form the polar shell in certain solvents above critical micelle concentration. Nonpolar core in PM entraps insoluble drug which controls its release, whereas polar shell solubilizes PM in aqueous medium and controls drug's pharmacokinetics. Due to the action of shell, higher blood circulation times can be achieved, which prevents recognition and uptake of PMs by reticulo-endothelial system. Block copolymers employed for PMs include poloxamers, PLA, polyaspartic acid, polyglycolic acid, polycaprolactone and PLGA, whereas drug loading is carried out via physical entrapment or chemical attachment using solvent evaporation, oil-in-water emulsion, dialysis, and freeze-drying.[61]

The small size and increased retention time aid preferred accumulation of PMs in cancer tissues through EPR effect for passive targeting. In addition, PMs have been utilized for active targeting by attaching targeting ligands like EGFs, folate, transferrin, and antibody fragments. Further, PMs can be fabricated as "stimuli-responsive," which renders the delivery system sensitive and responsive to the changes in specific stimuli including pH, temperature, heat, and ultrasound, to enhance drug accumulation in tumoral cells. Advanced polymer materials have enabled the use of several novel block copolymers for tailored fabrication of PMs, making them more effective carriers in delivery of various anticancer drugs.[62]

Tesauro et al.[63] synthesized peptide-labelled micelles via anchoring selected synthetic peptide sequences to the poorly soluble drug for active targeting of cancer cells overexpressing EGFR to deliver hydrophilic or hydrophobic payloads or molecular targeted agents such as erlotinib and sorafenib to selectively target tumor cells in various cancer types including hepatocellular carcinoma, which is one of the most common type of primary liver cancer.

Baidya et al.[64] synthesized folate-conjugated pluronic PF127-pluronic F68 mixed micelles by loading a poorly water soluble Biopharmaceutics Classification System class II phytoconstituent chrysin with an objective to enhance its oral bioavailability and cytotoxicity in human breast cancer cell line MCF-7 via active targeting modality. The mixed micelles exhibited 2.5-fold higher but controlled release of chrysin within 24 h and 3-fold higher extent of absorption compared with pure chrysin, suggesting the scope of functionalized mixed micelle nanocarrier as potential platform to deliver hydrophobic anticancer agents in breast cancer therapy via active targeting.

Guan et al.[65] developed a folate-conjugated and pH-responsive doxorubicin-loaded active targeting micellar system which showed a negative-to-positive charged reversal of micelle surfaces to overcome the internalization difficulty associated with negatively charged micelles, while retaining the longevity associated with negatively charges micelle surfaces during active targeted anticancer drug delivery. The study results revealed *in vitro* and *in vivo* potential of conjugated, pH-responsive, and charge-reversal-functionalized micelle nanocarriers for improved tumor targeting and cellular uptake of anticancer agents for effective cancer therapy.

Xu et al.[66] composed hybrid prodrug micelles employing phenyl boric acid-modified F127 as active-targeting group for tumor targeting and doxorubicin-grafted P123 as prodrug groups for acid-sensitivity with an objective to enhance reversal of multi drug resistance. The prodrug micelles were found to augment release via cleavage of β-carboxylic amides bonds under mild acidic condition and demonstrated accelerated cellular uptake and drug accumulation in MCF-7/ADR cells indicating the potential of multifunctional micelles approach in tumor resistant nanocarrier-based therapy.

Although PMs have shown substantial research potential for the poorly soluble drugs, their potential is limited for the water-soluble drugs. The other limitations of PMs such as micellar stability, targeting efficiency and loading capacity can be addressed up to certain extent by surface engineering techniques. However, issues related to effective biodistribution of PMs while maintain their stability and prolonged retention against *in vivo* dilution, pH shifts, change in ionic environment, and plasma protein interactions might lead to premature dissociation and unintentional release of their payloads causing drug accumulation at non-targeted sites and eventual risk of potential toxicity. Biocompatibility and toxicity aspects of materials used for such systems must be considered.[67] In addition, the chemical nature, size, shape, packing parameters and CMC values of the PMs determine the cytotoxic outcomes; although such types of studies are less investigated and ill understood.[68]

6. Virus-Like Nanocarriers (VLNs)

Viruses are rich in functional nucleic acids and proteins, and act as natural carrier to transfer nucleic acid encapsulated by the protein core (capsid proteins). VLNs mimic the authentic viruses, but are non-infectious with inability to replicate due to the lack of viral genome required for their replication.[69] VLNs are composed of self-assembling proteins and exploits the structural characteristics of virus capsids, thus utilizing the

natural biocompatibility and biodegradability of viruses in development of cancer therapeutics, vaccines, and imaging tools.[69] VLNs are powerful platforms to present multivalent antigen glycoproteins on their surfaces and their exterior or interior surfaces can be designed and functionalized with imaging reagents, targeting ligands, and therapeutic molecules based on genetic or chemical protocols via chemical coupling, genetic fusion and engineering or peptide conjugation.[70]

Wang et al.[71] developed cowpea mosaic virus nanoparticles based on use of complete virion and empty virion, which were further compared for their physicochemical properties and immunomodulatory activities after *in situ* vaccination in an ovarian tumor-bearing mouse model. The studies revealed the similarities in both types of nanoparticles with respect to physicochemical properties and cell survival, however, complete virion-based nanoparticles demonstrated promising antitumor efficacy due to presence of virus RNA and were able to promote population of antigen-presenting cells such as neutrophils and dendritic cells, demonstrating the anticancer applications of plant virus with immunomodulatory agents in ovarian and other types of cancers.

Ajabali et al.[72] utilized the internal cavity of the empty plant cowpea mosaic virus to enclose either imaging agent or drug within the capsid of the VLNs. The studies reported that cisplatin-loaded VLNs demonstrated 2.3-fold enhanced cisplatin cytotoxicity against both A549 and MDA-MB-231 cell lines, indicating the potential of VLNs as novel nanoscafold platform for targeted drug delivery and imaging applications.

Gan et al.[73] synthesized hepatitis B VLNs for target-specific delivery of chemically modified 5-FU (5-FU-1-acetic acid) to the EGFR-overexpressed A431, HT29, and HeLa cancer cells which displayed improved cancer cell internalization and killing after conjugating with cell penetrating peptide. The study results demonstrated the potential of VLNs to deliver chemically modified less toxic 5-FU derivative.

VLNs find remarkable applications as *in situ* cancer vaccine to produce potent anticancer response in various types of cancers. However, it is challenging to explore therapeutic VLNs for anticancer drug delivery. The *in vitro* potential of VLNs requires greater insights on their interactions with the host immune system in case of its translation to *in vivo* results which further necessitates determination of pharmacodynamics and pharmacokinetics via biodistribution studies in complex biological environments, *in vivo* fate and immunogenicity before their systemic use. Another challenge is the regulatory burden due to involvement of recombinant sources and nonspecific viral nucleic acids in fabrication of VLN-based nanocarriers.[74]

7. Protein Nanocarriers

"Protein cages" are hierarchical hollow complex architectures derived from viruses or virus-like materials. Caged proteins are usually generated in living hosts like plants, cells or bacteria, which is then heat shocked drug delivery applications.[70] Caged proteins can be functionalized at three surfaces such as internal, external and inter-subunit using protein engineering tools to control surface charge and stability, drug encapsulation, and

ligand display.[75] Being natural biomolecule, protein is an attractive option to synthetic polymers in terms of safety, biocompatibility and biodegradability. However, to be used as a delivery vehicle and perform its activity, therapeutic proteins require modifications. Different animal or plant sources like soy protein, elastin, keratin, collagen, zein, and silk can be used to isolate these proteins.[76] Besides protein cages, other types of protein nanocarriers are protein polymers and charged/amphipathic peptides that involves self-assembling of protein polymers using desolvation method.[76] The primary structure of protein nanocarriers allow surface functionalization via covalently attaching drugs and targeting ligands. Functionalization involves use of genetic engineering techniques under mild preparation conditions by avoiding organic solvents or toxic chemicals, which adds specific benefits to the therapeutic protein delivery.[75] The U.S. Food and Drug Administration (FDA)–approved Abraxane® is a protein nanoparticle drug for delivery of paclitaxel by albumin indicated for advanced-stage breast cancer. This nanocarrier-system is less toxic compared with paclitaxel alone and improves the drug efficacy in tumor via EPR effect.

Zhou et al.[77] employed human serum albumin, kolliphor HS 15, and pirarubicin to form albumin-bound complex for the tumor targeted delivery. The developed biomimetic protein nanoparticles demonstrated higher cellular uptake, higher cytotoxicity, longer half-life, greater tumor accumulation, higher tumor penetration and anti-metastasis compared with pirarubicin through gp60- and SPARC-mediated biomimetic transport, suggesting their clinical potential.

Shi et al.[78] constructed albumin-based nanoplatforms for co-delivery of celecoxib and doxorubicin. The combination system provided enhanced anticancer outcomes by showing minimum side effects of doxorubicin and enhanced *in vivo* biodistribution. The improved anticancer efficacy of this combination system was attributed to celecoxib co-delivery that augmented doxorubicin cytotoxicity via glucose transportation into the cells and eventual inhibition of energy metabolism, indicating the potential of smart delivery of targeted protein nanocarriers for anticancer drug delivery.

Choi et al.[79] investigated feasibility of direct pulmonary delivery of self-assembled human serum albumin conjugated, doxorubicin and octyl aldehyde endowed, and apoptotic TRAIL protein-adsorbed inhalable nanoparticles for treatment of drug-resistant lung cancer. The developed protein nanoparticles displayed synergistic cytotoxicity, apoptosis and anti-tumor efficacy in mice bearing H226 cell-induced metastatic lung cancer. The enhanced anti-tumor efficacy was achieved due to synergistic apoptotic activities of doxorubicin and TRAIL, which were gradually released for over 3 days. It was revealed that doxorubicin doses and side effects can be significantly lowered in presence of TRAIL for pulmonary delivery of protein nanocarriers.

Kim et al.[80] fabricated hyperthermal albumin nanoparticles loaded with paclitaxel as anticancer agent, indocyanine green as hyperthermal agent and hyaluronidase as agent that breaks down hyaluronan present in tumor extracellular matrix via modified nanoparticle albumin-bound technique. At severe (> 50°C) and mild (41–42°C) hyperthermal states, cytotoxicity of paclitaxel was significantly enhanced showing deep permeation of nanoparticles in tumor tissues without significantly affecting nanoparticles'

physicochemical and anticancer properties. The results demonstrated scope of hyperthermal protein nanocarriers as potential anticancer agents.

There are several albumin-bound nanocarriers under clinical trials, however, only one product has gained FDA approval at this time. Partial success of protein nanocarriers may be assigned to challenge related to albumin's own endocytosis mediated by albumin receptor gp60, which is located at caveolae. When cells lack or possess limited caveolae, additional ligands are required for the efficient anticancer drug delivery using albumin nanoparticles to the target cells.[81] In addition, non-specific delivery of the drugs to non-targeted cells that have albumin receptor is challenging.[82] Also, maintaining the activity of drug cargo is also challenging due to possible deactivation of enzyme cargo during the preparation process. Development of successful protein nanocarrier system requires extensive small-scale preliminary trials using a wide range of manufacturing conditions which allows to select an appropriate manufacturing process. Moreover, the varying quality of protein from varied sources leads to variations in their final quality and purity which might result in suboptimal product preparation.[81]

Among organic nanocarriers, liposomes have gained highest clinical success to be delivered via passive targeting modality, whereas other types have received either limited or no success.[82] Approved liposomal formulations for cancer treatment are summarized in Table 2.

B. Inorganic Nanocarriers

Inorganic nanocarriers are highly stable, non-toxic, and biocompatible nanostructures with massive surface area to volume ratio, unique optical properties and distinct magnetic properties.[77] The inorganic nanoparticles possess flexible properties which allow their functionalization with specific ligands to increase their binding affinity to target cells. Functionalization also enables imaging, biosensing, cell labelling, and diagnostics applications of inorganic nanocarriers in targeted cancer therapy. Major classes of inorganic nanocarriers such as carbon nanotubes (CNTs), gold nanocarriers (AuNCs), quantum dots (QDs), magnetic nanocarriers (MNs), and mesoporous silica nanocarriers (MSNs) that find applications in cancer therapy are illustrated in Fig. 5.

1. CNTs

The CNTs are monolithic, hollow, tube-like architecture of 1 nm diameter and 1–100 nm length. CNT was discovery by Ijma in 1991, which was then fabricated via rolling a single graphite layer (graphene) at discrete and specific angles to form a seamless cylinder and known as single-walled nanotubes (SWCNTs). Likewise, multi-walled nanotubes (MWCNTs) can be fabricated by several concentric layers of graphene sheets rolled into a seamless cylindrical structure.[83] Due to very small size and axial symmetry, CNTs are attractive inorganic carrier for targeted anticancer drug delivery. The SWCNT possess 1–2 nm internal diameter, 0.4–100 nm cross-sectional diameter and length of about thousand times elongation of diameter. High length-to-diameter ratio (aspect

TABLE 2: Approved marketed organic nanocarriers for anticancer drug delivery

Generic/brand name	Type of nanocarrier	Therapeutic agent	Indication	Company and country of approval	Year of approval
SMANCS	Polymer conjugate	Neocarzinostatin	Liver and renal cancer	Yamanouchi, Japan, approved in Japan	1993
Liposomal-doxorubicin (Doxil™)	PEGylated liposome	Doxorubicin	HIV-associated Kaposi sarcoma, ovarian cancer, and multiple myeloma	Janssen, USA, FDA approved	1995
Liposomal-daunorubicin (DaunoXome™)	Liposome	Daunorubicin	HIV-associated Kaposi sarcoma	NeXstar Pharmaceuticals', USA, FDA approved	1996
Liposomal-doxorubicin (Myocet™)	Liposome	Doxorubicin	Metastatic breast cancer	Elan Corporation, Ireland, approved in Europe and Canada	2000
Nab-paclitaxel (Abraxane™)	Albumin nanoparticle	Paclitaxel	Breast, lung, and pancreatic cancer	American Pharmaceutical Partners, Inc., FDA approved	2005
PM- paclitaxel (Genexol-PM™)	PM	Paclitaxel	Breast cancer and NSCLC	Samyang Corporation, Korea, marketed in Europe	2007
Mifamurtide (Mepact™)	Liposome	Muramyl tripeptide Phosphatidyleth-anolamine	Nonmetastatic, resectable osteosarcoma	Takeda Pharmaceutical Company Limited, Japan	2009
Liposomal vincristine (Marqibo™)	Liposome	Vincristine sulfate	Acute lymphoblastic leukaemia	Talon Therapeutics FDA approved	2012
Liposomal irinotecan (Onivyde™)	PEGylated liposome	Irinotecan	Post-gemcitabine metastatic pancreatic cancer	Merrimack Pharmaceuticals, Inc., USA, FDA approved	2015
DHP 107	Lipid nanoparticles (oral)	Paclitaxel	Gastric cancer	Daehwa Pharmaceutical Co. Ltd., Approved in South Korea	2016
Vyxeox	Liposome	Daunorubicin and cytarabine	High-risk acute myeloid leukemia	Jazz Pharmaceuticals, Ireland, FDA approved	2017
Apealea	Micelle	Paclitaxel	Ovarian, peritoneal, and fallopian tube cancer	Oasmia Pharmaceutical AB, Approved in Europe	2018

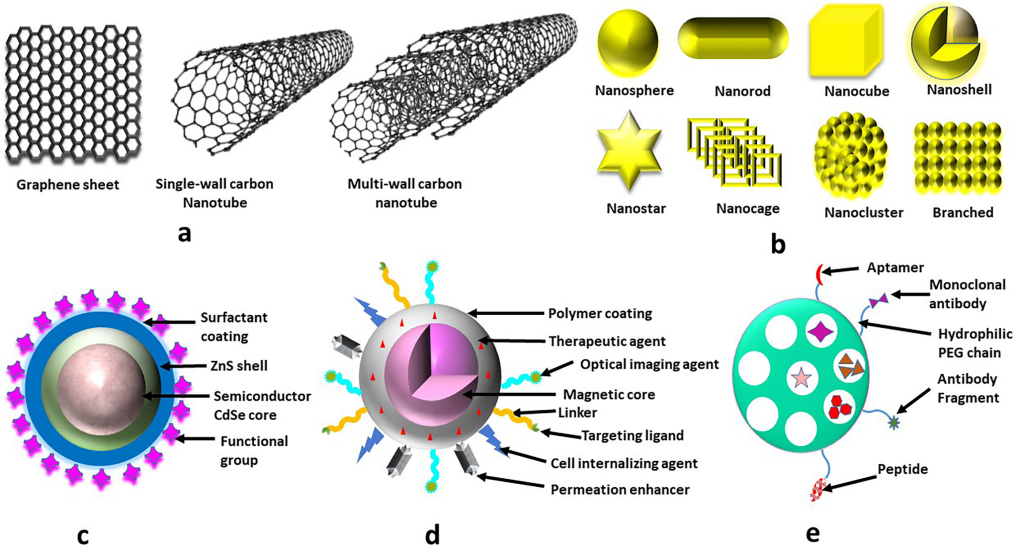

FIG. 5: Various inorganic nanocarriers for applications in cancer therapy. (a) CNTs, (b) AuNCs, (c) QDs, (d) MNs, and (e) MSNs.

ratio), huge surface area, extremely light weight and nanosized structure along with distinguished chemical, thermal, mechanical, and electrical properties enable CNTs to be employed for wider applications in anticancer therapy. However, very high hydrophobic molecular composition requires covalent functionalization of CNTs via attachment of polar –COOH or $–NH_2$ groups; or non-covalent functionalization via attachment of biomolecules, surfactants, or synthetic molecules for loading of cargos.[84] The functionalized CNTs become water-soluble providing high serum longevity compared with the non-functionalized CNTs which are water-insoluble and toxic.[79,80] Functionalized CNTs have been employed for advanced biomedical applications via rapid photothermal delivery,[85] physical loading,[86] or covalent linking,[87] and the CNTs can enter the target cell via endocytosis or cell membrane penetration.

Liu et al.[88] developed hyaluronic acid modified amino SWCNT for targeted delivery of doxorubicin for improved breast cancer treatment. The fabricated SWCNTs exhibited pH-triggered release, which was highest at the pH 5.5, that is the pH of the tumor cell microenvironment, compared with physiological pH 7.4, which was favorable for the intracellular drug release in CD44 overexpressing MDA-MB-231 cells. The doxorubicin-hyaluronic acid-SWCNTs significantly inhibited the tumor growth in breast cancer.

Yan et al.[89] developed pH-responsive, folic-acid-modified, carboxyl-group-treated MWCNTs for active targeting of doxorubicin. The fabricated nanocomplexes demonstrated high drug loading high encapsulation, pH-responsive release in acidic environment, improved suppression of tumor growth on tumor cells overexpressing folic-acid receptors while decreasing the doxorubicin-associated systemic toxic effects.

Yang et al.[90] utilized oxidizing agents for purification of SWCNTs to obtain shortened CNTs which were further conjugated with PEG and polyethylenimine and characterized for drug loading, cytotoxicity, and cellular uptake in breast cancer MCF-7 cells. The study results indicated efficient cell internalization and tumor inhibition for CNTs conjugated with both PEG and polyethylenimine compared with single conjugated CNTs, which might be due to better dispersibility and difunctionalization of CNTs. This study opened up the scope of CNT's for multifunctional anticancer drug delivery for different types of cancers.

One of the main challenges of CNTs is its highly hydrophobic nature which results in lack of solubility in aqueous media and environmental toxicity upon its leakage via water. The ultra-small sizes of CNTs make them more reactive and toxic than larger particles. In addition, constructing material's uniformity with respect to their wide distribution of the CNT diameters, residual metal contents, division of the individual CNT, dispersibility and their sensitivity to gases and other species are challenges that require attention and hinder their use for anticancer drug delivery.[91] Moreover, when CNTs are surface functionalized, their properties change which increases their capacities to bind with heavy metals. Another biggest challenge is the perceived non-biodegradability of CNTs due to their extremely physically and chemically stable nature, which makes the design and development of biocompatible CNTs a difficult process.[92] Further, CNTs pose challenge in form of health hazards after its inhalation and are known to cause respiratory problems upon long term exposure causing potential pulmonary toxicities such as bronchitis, lung cancer, emphysema and asthma, granuloma and fibrosis, as well as gene damage in the lung besides reproductive and developmental toxicity.[93]

2. AuNCs

AuNCs have wide scope in cancer diagnosis and treatment based on their characteristics including inertness, biocompatibility, ease of fabrication and functionalization, remarkable property to absorb and scatter light, optical tunability, high drug loading capacity, low toxicity and stability. Gold as a noble metal with inert nature, has been used to cater various biomedical applications including photoacoustic imaging,[94] chemotherapy,[95] surface-enhanced resonance spectroscopy,[96] gene therapy,[97] and photothermal therapy.[98] AuNCs provide unique optical localized surface plasmon resonance, enabling them to undergo photothermal conversion causing heating up and subsequent killing of targeted tumor cells when concurrent supply of proper wavelength-light as external stimuli was provided along with administration of AuNCs. The LSPR property is tuned for imaging, diagnosis, and photothermal therapy.[99] In addition, AuNCs are used for complementing radiation and thermal therapy, applying the light irradiation at target site to trigger release of cargo, and to improve contrast for *in vivo* imaging for various types of tumors and diseased organs.[99] AuNCs can be used to deliver low-soluble drugs and imaging agents showing poor pharmacokinetics, enzyme susceptibility, and poor intracellular penetration.[95]

Various anisotropes of AuNCs include nanospheres, nanostars, nanorods, nanocages, nanoshells, and nanoprisms. The sizes of these forms range in 1 nm to more than 100 nm, with additional one-, two- or three- dimensional arrays that impart totally new properties to these nanoforms compared with their individual nanoparticles.[100] Currently, the most interested shapes include solid spheres; nanorods which are narrow in two dimensions and approximately 100 nm longer along a third dimension; and nanostars, which are highly symmetric structures. The optical and electrical properties of AuNCs greatly depend on their shape and size.[100] The optical property of AuNCs allow attachment of biomolecules like enzymes, carbohydrates, fluorophores, peptides, proteins, and genes for their effectively transportation across the barriers via noncovalent attachment or covalent bonding. Noncovalent adsorption of drug on AuNCs can efficiently release the drug whereas covalent bonding is possible through either chemically modifying the drug or externally triggering the drug release. Major application of AuNCs include efficient imaging of tumor cells since they can capture three-dimensional images of tissues when used with optical coherence tomography agents.[94–99]

Xiong et al.[101] demonstrated potential of zwitterionic-functionalized dendrimer-entrapped gold nanoparticles for non-viral serum-enhanced gene delivery for inhibition of tumor metastasis. G5 PAMAM was anchored with carboxybetaine acrylamide and lysosome-targeting agent morpholine to encapsulate gold nanoparticles. The fabricated polyplexes demonstrated that the partial zwitterionic functionalization of gold nanoparticles conferred antifouling property to gold nanoparticles enabling them to resist serum protein adsorption, to maintain positive effects in serum environment, and to enhance the lysosome targeting ability and inhibit metastasis.

Wan et al.[102] investigated the molecular mechanisms of novel Dtxl stacked gold doped apatite nanoparticles in induction of apoptosis via *in vitro* anticancer tests. The results revealed higher cytotoxicity against human liver cancer cells (HepG2) which was attributed to the apoptosis-induced mitochondrial break in HepG2 cells by stacked nanoparticles which hugely widened the cell passing. The research demonstrated the probable dynamic role of nanoparticles in interfering the organic impacts apart from their role as a basic medication carrier.

Del Valle et al.[103] developed an aptamer-conjugated gold nanostar for targeting nucleolin which is present in both tumor cells and tumor vasculature via near-infrared (NIR)–cleavable action to perform drug-resistant cancer treatment. A gold nanostar was assembled with a layer of anti-nucleolin aptamer AS1411. Doxorubicin was conjugated to deoxyguanosine residues employing heat and acid labile methylene linkages. The intravenous injection, upon NIR irradiation, delivered doxorubicin on-demand, providing higher drug accumulation in the nuclei of drug-resistant breast cancer cells resulting in higher anticancer efficacy even at 54 times less equivalent dose of pure doxorubicin. The studies demonstrated that superior tumor inhibition could be attained with simultaneous photothermal and chemotherapeutic actions to defeat drug resistance while achieving much improved tumor inhibition than using either of single action only.

Because various gold nanoforms range in size from 1 to 500 nm, it is not only challenging but also important to establish the relationship of AuNC sizes with their cellular

uptake, organ accumulation, and toxicity. In one important study, it was revealed that AuNCs of 1 nm size could penetrate the cell and nuclear membranes and could attach to DNA without cell injury and cell death, indicating that smaller AuNCs promote their stay in biological systems and also increase their reactivity which in turn leads to changes in biological system. AuNCs can be absorbed based on size and uptake across intestinal cells.[104] In another study, it was demonstrated that AuNCs of 15 nm were absorbed by the intestinal epithelial and spread quickly into cells, whereas those of 50 nm crossed the intestinal epithelium from the apical side while excreting out through the basolateral side of intestine. AuNCs of 100 nm could accumulate in intestinal cells limiting its excretion.[105] Further, AuNCs with sizes up to 240 nm could cross the human placental barrier without affecting the viability of the placental explants.[105] Likewise, it is challenging to address the shape-related toxicity of AuNCs. For instance, gold nanorods demonstrated higher toxicity to human keratinocyte cells compared with spherical AuNCs. Also, the use of AuNCs in drug delivery systems is challenging concerning their biodistribution which is mainly affected by size- and surface charge-related accumulation of gold content in spleen, liver, lungs, heart, and kidney.[105] In a systematic study by Carnovale et al., the effects of size, shape, capping ligand, and biological corona on uptake and toxicity of AuNCs were analyzed. The study reported that serum proteins were found to have limited role in mediating the AuNCs' toxicities, however, serum proteins play role in their cellular uptakes.[106]

3. QDs

QDs are nanoscale colloidal particles with size range 2–10 nm. QDs consist of a semiconducting material that includes Se, Zn, Te, Cd (atoms in the II–VI) or In, As, P (atoms in III–V), or ternary I–III–VI QDs where Cu or Ag from group I, Ga or In from group III, and S or Se from group VI are used. Depending on their sizes, QDs show unusually high surface-to-volume ratio that produces distinctive fluorescence colors which are superior to traditional fluorescent dyes. For instance, different sizes of QDs result in different light emission in ultraviolet or NIR regions. Due to this property, smaller QDs (~ 2 nm) emit blue fluorescence, whereas bigger QDs (~ 5 nm) emit red fluorescence.[107] Deep tissues can detect the fluorescence in NIR region, which makes NIR-QDs a suitable *in vivo* imaging tool for diagnostic applications. Narrow emission spectrum, bright fluorescence, and high photo-stability are unique optical properties of QDs which are utilized for cell tracking or cell imaging of active agents inside cells/tissues.[107] In QDs, core accounts for color emission, and hydrophilic shell is utilized for conjugating peptides, protein, or DNA biomolecules. For example, ZnS shell enclosing cadmium selenide (CdSe) core exhibit enhanced site-specific accumulation and reduced toxicity for efficient delivery of therapeutic agent when a tumor-homing peptide F3 attached on QD surface since this peptide could selectively bind to tumor endothelial cells and showed enhanced cell internalization via nucleolin-mediated transport for diagnostic and drug delivery applications.[108]

Li et al.[109] explored mitochondria-based aircraft system as a delivery platform of carbon QDs for dual purposes of *in vivo* imaging and doxorubicin delivery. Upon

intravenous administration, the mitochondria carriers were found to be compatible with QDs while retaining their optical properties, improving their biodistribution and prolonged their circulation time, demonstrating the mitochondria-based aircraft nanosystem as a potential strategy for imaging and drug delivery applications.

Jia et al.[110] designed and developed high amount of doxorubicin loaded γ-cyclodextrin-based metal-organic framework composite, which was modified by pH-responsive radical polymerization, and then immobilizing the aptamer over it. The nanocomposites displayed pH-responsive doxorubicin delivery, high drug loading (89.1%), sustained release and improved targeting efficiency. The nanocomposites were effective in tumor suppression with negligible side effects both *in vivo* and *in vitro*, demonstrating their potential for anticancer drug delivery.

Li et al.[111] fabricated fluorescent nanoparticles employing supramolecular assembling of carbon dots and dihydroartimisinin to improve its stability, solubility, pH-dependent release and biocompatibility and antitumor efficacy. The fabricated carbon dots suppressed progression of hepatic carcinoma via induction of apoptosis and inhibition of glucose metabolism which were attributed to decreased expression of PKM2 and blocking of Akt/mTOR signaling pathway, supporting the anticancer potential of carbon dots in clinical treatment.

Although QDs have shown considerable interest and potential for their anticancer applications in cells and small animals, their clinical use is ambiguous based on toxicity concerns. CdSe QDs have been found toxic in animal cells, and many researchers extrapolate this toxicity to human cells, which make it challenging to explore QDs in clinical applications, despite immense research efforts in this field. Another challenge for QDs is their non-uniform design that confers distinct but variable physicochemical properties, which in turn influence their biological potential and toxicity, displaying design-dependent cellular internalization for QDs. CdSe QDs may cause cytotoxicity by releasing free Cd and may preferentially accumulate in liver and spleen after intravascular injection.[112] In addition to QD core, their capping materials can be immunogenic and may cause immune reactions in host, making the QDs ineffective *in vivo* due to antibody binding. Further, the size of QD complexes bypass their excretion through kidney, thereby preventing their clearance from the systemic circulation, resulting in liver accumulation, which is particularly susceptible to cadmium toxicity. Thus, it is challenging to fabricate clinically foreseeable QDs using low-toxicity compounds like silicon and carbon, protect them from exposure, and enable their clearance from the body. Meanwhile, it is another primary challenge to maintain therapeutically effective drug concentrations at target sites while attempting toxicity prevention.[113]

4. MNs

Metal nanoparticles made up of nickel, cobalt, Prussian blue, gadolinium, and magnetic iron oxide can be manipulated using magnetic field to exhibit different degrees of magnetism depending on their orbital and spin characteristics under externally applied magnetic field. Among other types, iron oxide nanoparticles have shown promising

diagnostic and theranostics applications. During synthesis of MNs, the drug is attached to the nanocarrier surface via covalent bond or entrapped or adsorbed within nanopores of magnetic carriers. Initially, MNs were fabricated as contrast agents for magnetic resonance imaging.[114] In addition, the application of magnetic field directs drug-loaded MNs to the tumor site, where the drug accumulates, thus decreasing the systemic distribution to decrease side effects. The surface functionalization by polymer coating of iron oxide MNs possessed magnetic resonance that can be selected as contrast agents for cancer diagnosis and passive targeting therapy.[115,116]

Jin et al.[117] developed tumor-targeted magnetic nanobubbles for dual-modal applications of both imaging and ultrasound-triggered delivery of doxorubicin by co-encapsulating it with superparamagnetic iron oxide nanoparticles into PLGA nanobubbles via double emulsion process. Use of focus ultrasound by non-invasive remote-control technique was employed for triggered release of drug from the nanobubbles which generated sonoporation effect resulting in enhanced drug release and cellular uptake along with decreased 4T1 cell viability through folic acid ligand-receptor targeting. The studies demonstrated potential of using combined ultrasound and magnetic resonance for enhanced tissue accumulation to achieve enhanced anti-tumor outcomes.

Attari et al.[118] fabricated biodegradable methotrexate-conjugated iron oxide magnetic nanoparticles with arginine capping via *in situ* one-pot coprecipitation method to achieve controlled drug delivery and magnetic resonance imaging for targeted cancer treatment and diagnosis. Magnetic nanoparticles showed covalent conjugation to methotrexate which was then functionalized with arginine and the nanoparticles were able to target folate receptor-overexpressed tumor cells without producing cytotoxicity.

Khaledian et al.[119] prepared doxorubicin loaded PLA-PEG-folic acid MNs using a double emulsion method and evaluated in the external magnetic field using a hyperthermia device which demonstrated higher cellular uptake and apoptosis level in nanocarrier-treated cells compared with free drug and folic acid free nanocarriers, demonstrating potential of combination therapy in cancer treatment.

Design and selection of magnetic carriers that cross the anatomical barriers and reach the target site safely for specific clinical indications is a key challenge.[115] Clinically successful MNs is limited due to lack of critical processes required to overcome complex challenges of MNs like through knowledge of complex physiological barriers and nano-bio interactions in humans specific to cancer type, escaping the endosome/lysosome system into the cytosol of cancer cells, and long-term toxicity.[120] Magnetic nanoparticles face other challenge in context to biological barriers and obstacles such as clearance by mononuclear phagocyte system, cell internalization, biodistribution that diminish the drug accumulation at target site limiting the efficacious use of MNs for anticancer drug delivery applications. Another challenge is related to cancer-specific targeting using MNs which help to reduce the dose and achieve better treatment outcomes. These challenges together account for limited success of MNs in clinical trials.[120]

5. MSNs

Silica (SiO_2)-based nanocarriers offer considerable advantages in cancer targeting including specific surface characteristics, porosity, capacity for functionalization, simple synthesis, ability to be designed as complex systems and cost-effectiveness. Mesoporous silica is a type of silica-based nanocarriers which have particular importance in therapeutic delivery based on their high drug-loading and high porosity because of its unique honeycomb-like structure consisting of hundreds of pores. MSNs possess controllable pore diameter of 2–50 nm with substantial biocompatibility, high pore volume and high surface area.[121] Also, the polar silanol groups that cover large surface area of these nanocarriers facilitate water adsorption, and thermal/chemical stability of both polar and nonpolar therapeutic agents.[121]

The nanopore sizes and density of MSNs can be tuned while surface functionalization enables controlled and targeted transport of drug for enhanced efficiency and reduced toxicity.[122] Interaction of surface-functionalized nanocarriers with nucleic acids enables their use in non-viral gene delivery platform for *in vivo* targeted brain therapy.[123] MSNs possess high drug loading capacity of anticancer drug via physical adsorption and solvent evaporation, whereas nanosize structure allows their accumulation at tumor site by passive targeting. MSNs have been functionalized with targeting ligands for active targeting in cancer tissues. Moreover, various stimuli-responsive molecules are used to cap the pores of MSNs to trigger the drug release in targeted tumor tissues. For example, cyclodextrin has been used to cap MSNs which released the encapsulated drug at the acidic tumor tissue.[121]

Shen et al.[124] fabricated aptamer-functionalized, pH-sensitive, β-cyclodextrin-capped doxorubicin-loaded mesoporous silica nanoparticles for targeted delivery of doxorubicin to human epidermal growth factor receptor 2 (HER2)-positive cells via a pH-stimuli-responsive release mechanism. The nanoparticles underwent HER2-mediated endocytosis to show better specific uptake and anticancer effect in HER2-positive SKBR3 cells compared with HER2-negative MCF-7 cells, demonstrating the efficacy of novel MNs for HER2-positive cancers.

Ding et al.[125] synthesized complexed mesoporous silica nanoparticles with TAT peptide which carried liver-cancer-specific aptamer TLS11a in which doxorubicin was loaded. The nanosystems showed much faster drug release pH 5 than pH 7.4. *In vivo* cytotoxicity studies in H22 tumor-bearing mice model demonstrated maximum tumor drug accumulation after at 48 h after intravenous injection. The studies emerged as a promising strategy of effective dual targeting of fabricated mesoporous silica nanoparticles to liver cancer tissues and nuclei of liver cancer cells while lowering toxic systemic side effects.

Zhao et al.[126] developed dacarbazine loaded cancer cell membrane camouflaged-mesoporous silica nanoparticles conjugated with aPD1 to improve antitumor efficacy in treatment of melanoma. These biocompatible and tumor acidic environment-responsive nanosystems showed remarkable inhibition of melanoma and also prolonged the survival time, which was attributed to selective tumor killing, tumor-specific T cells activation, and tumor microenvironment regulation while showing lower systemic toxicity.

The study results demonstrated potential of combined dacarbazine chemotherapy and aPD1 immunotherapy for improved melanoma therapy.

In the case of MSNs, it is particularly challenging to achieve consistent quality and characteristics while synthesizing using large amounts. Moreover, it is difficult to obtain desirable concentrations of drug in MSNs as not all loaded drugs can be incorporated in MSNs and actual amount loaded affects the required dose of MSNs.[127] There are only a few reports regarding dependency of MSNs' efficiency and biocompatibility on surface functionalization. Also, studies related to immune response and side effects in functional immunogenic environment are limited and more data are required. Further, the clinical translation of MSNs is challenging concerning their accumulation in liver, spleen and other normal tissues in case of burst release from the MSN systems. Despite the myriad of research in this area, only one iron oxide nanocarrier system, NanoTherm™ is approved by the FDA and indicated for thermal ablation glioblastoma.

Major challenges of inorganic nanocarriers such as carbon-based nanocarriers (e.g., CNTs and QDs) or metallic nanocarriers (e.g., gold, magnetic, and MSNs) face challenges with regard to their design-dependent toxicity based on size, shape, and other related physicochemical properties, as well as type and quality of construction material and synthesis processes involved, which could be induced via various routes of entry such as ingestion, inhalation, or dermal penetration. Based on most recent research efforts in the field, certain nanocarrier have been found to show efficacy in certain types of cancers via targeting receptors overexpressed on tumor cell surfaces which are summarized in Table 3.

C. Hybrid Nanocarriers

Formation of hybrid nanocarriers employs combination of two or more organic and inorganic nanomaterials like organic-inorganic, organic-organic, inorganic-inorganic, or multicomponent, such as lipid-polymer; ceramic-polymer, etc. These bifold hybrid nanocarriers combine benefits of both components and results in enhanced properties. Folate-conjugated polymer-lipid hybrid nanocarriers have been prepared via ionic gelation technique for targeted delivery of letrozole, which is a steroidal anticancer agent with hydrophobic nature.[128] Silica can be preferably combined with various contrast agents such as gold to form silica-gold, silver to form silica-silver, iron oxide to form silica-magnetic, QD to form silica-QD, as well as other hybrids like silica-protein, silica-peptide, and silica-nucleic acid for therapeutic or bioimaging applications.[129] Recently, lipid-polymer hybrid nanoparticles gained interest to overcome liposome-associated limitations like internal solution leakage and low stability, which cause its easy removal from the circulation resulting in low therapeutic efficacy.[130]

The selection of nanocarriers to be combined depends on nature of conjugating drug, desired site of action and physiological barriers during its delivery, as well as stability and solubility of nanocarriers to achieve higher drug bioavailability and minimal side effects.[120] Various therapeutic agents including proteins and nucleic acid are physically adsorbed or entrapped, or attached by covalent bonding to the hybrid system.

TABLE 3: Receptor-specific anticancer efficacy of nanocarriers based on most recent research efforts

Nanocarrier system	Therapeutic agent	Type of cancer	Targeted receptor	Ref.
Liposomes	Docetaxel	Breast cancer	VEGF	24
	Doxorubicin	Breast cancer	Transferrin	25
	Vincristine + Tetrandine	Brain glioma	Transferrin	26
	Doxorubicin	Breast cancer	Folate	27
	5-FU	Colon cancer	Folate	28
	Bleomycin	Breast cancer	Folate	29
	Doxorubicin	Melanoma	Glycoprotein	30
Solid lipid nanocarriers	Asiatic acid	Glioma	Integrin	40
	Tamoxifen citrate	Breast cancer	Transferrin	42
Dendrimer	Paclitaxel	Biotin-overexpressed tumors	Biotin	47
	Paclitaxel	Cervical cancer	Transferrin	48
	Doxorubicin	TNBC	EGFR	49
PNCs	Bombesin peptide	Prostate cancer	Gastrin-releasing peptide	56
	Rutin + Benzamide	TNBC	CD44	57
PMs	Erlotinin, Soratinin	Hepatocellular carcinoma	EGFR	63
	Chrysin	Breast cancer	Folate	64
VLNs	5-fluorouracil-1-acetic acid	Colorectal cancer cervical cancer	EGFR	73
CNTs	Doxorubicin	Breast cancer	CD44	88
MSNs	Doxorubicin	HER2 positive cancers	HER2	124

The zoledronic acid-loaded mesoporous silica-lipid hybrid demonstrated high intracellular delivery and high retention rate of zoledronic acid in breast cancer.[131] This hybrid system prevented the premature drug-release enabling a stimuli-responsive drug-release into the body.[132] Polymer-lipid hybrid nanocarriers can be PEGylated and functionalized with site-specific targeting ligands like peptide, antibody, antibody fragment, aptamer etc. for targeted drug delivery applications.[130–132]

IV. ADVANCED TARGETING APPROACHES IN CANCER TREATMENT

A. mAbs

mAbs are a major treatment approach based on cancer immunotherapy. mAbs are man-made versions of antibodies developed in laboratory either to act as antibodies themselves

or to strengthen body's natural antibodies to fight cancers. mABs act by recognizing and finding specific proteins on cancer cells and block a specific target present on surface of cancer cells (Fig. 6). mAbs are targeted immunotherapy-based cancer treatment which is limited to cancers in which antigens have been identified.[133] mAbs were first created in 1975 using mice hybridoma technique using fusion of myeloma cell lines with B cells to form hybridomas which produced specific antibodies for specific antigens and were further immortalized.[134] In 1997, the mAb rituximab was first approved to treat B cell lymphoma.[124] Since then, the mAb approach is being widely employed as a standard treatment for many haematological and solid tumors. Most of the targeted therapies are either small molecule compounds or mAbs, however, the difference being that the small molecules can target and enter inside the cell unlike mAbs usually cannot enter cells and only targets the cell surface.[135]

mAbs can be of two types: unconjugated (naked) and conjugated. The naked mAb works by themselves without attaching to the radioactive materials.[135] For instance, binding of Alemtuzumab to CD52 antigen expressed on lymphocytes causes the bound antibody to attract immune cells to kill target cells. In conjugated mAbs (known as loaded, tagged, or labeled antibodies), an anticancer agent is attached to radioactive substance, and conjugated mAb works as "homing device" for direct delivery of these substances to tumor cells.[136] Conjugated mAb circulates all over the body until it finds and attaches to target antigen, eventually delivering the toxic substance solely to the target cells, thereby reducing destruction to normal cells.[135,136]

The cell destruction happens through three main mechanisms including direct tumor cell death, immune-mediated tumor cell death, and ablation of vascular and stromal

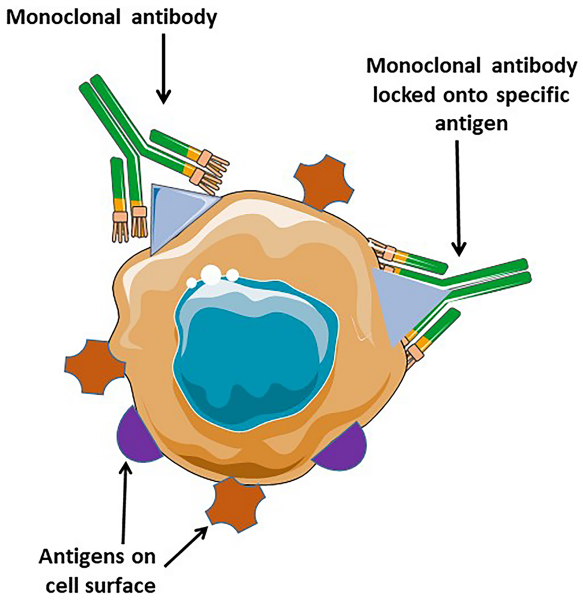

FIG. 6: mAb targeting to specific cell surface antigen

cells.[136] Direct tumour cell death occurs by targeting and inhibiting cell survival signals through action of antibody via receptor blockage, apoptosis, anticancer drug delivery, or radiation modalities.[137,138] Immune-mediated tumour cell killing occurs by regulation of T cell function, antibody-dependent cellular cytotoxicity (ADCC), targeting genetically modified T cells, activation of complement-mediated-cytotoxicity due to specific effects on tumor vasculature, or cellular phagocytosis. Immunostimulatory mAbs work by T cell activation through inhibition of T cell inhibitory receptors.[133] The most successful mAbs cetuximab and trastuzumab act by abrogation of tumor-cell signals, rituximab by ADCC and ipilimumab by immune modulation of T cell function.[123] To date, several mAbs are approved to treat various cancers[135,138] (Table 4).

B. Antibody-Drug Conjugates (ADCs)

ADCs are advanced version of mAbs constructed by conjugation of mAbs with cytotoxic drugs (cytotoxic payload) via a linker to specifically recognize a cell-surface antigen and deliver the cytotoxic payload directly to tumor cells.[139] ADC combines immunotherapy with targeted chemotherapy and delivers the cytotoxic payload directly to the cancer cells while exploiting specificity of mAb to reach cancer cell-expressed target antigen. This strategy effectively reduces the off-target toxicity to normal tissues, widens therapeutic window, and also improves pharmacokinetic and pharmacodynamics of anticancer payloads.[140] Key elements in ADC targeting include selection of antigen target, antibody, cytotoxic payload, linker, and conjugation techniques.

1. Selection of Antigen Target

There are three important considerations for target antigen selection for ADC design. First, the target antigen abundantly expresses on tumor tissues, but less abundant in healthy tissues. Second, the target antigen expresses exclusively over the surfaces of the tumor cells to enable easy access/binding by antibody. Third, the binding of ADC with target cell antigen leads to its effective internalization via receptor-mediated endocytosis, undergo intracellular trafficking and degeneration to allow release of cytotoxic payload[141] (Fig. 7). For instance, FDA-approved ADCs gemtuzumab targets CD30 antigens, and ozogamicin targets CD79 antigen. Both these antigens consistently express on target tumor cell surfaces while secreting minimally from the tumor cells into the circulation. If there is higher secretion of these target antigens into the circulation, ADCs can bind to these secreted antigens in the circulation before reaching to target tumor cells, limiting availability of antibodies to bind with target cells, thus decreasing efficiency of ADCs.[142]

2. Selection of Antibody

In ADCs, antibodies are selected based on their molecular weight, target specificity and binding affinity, as these are critical to achieve the increased accumulation and retention of ADCs in tumor sites. In addition, low reactivity, low immunogenicity, long half-life,

TABLE 4: FDA-approved mAbs

mAb (type)	Target antigen	Approval year	Cancer type indication
Rituximab (Chimeric)	CD20	1997	Non-Hodgkin's lymphoma
Trastuzumab (Humanized)	HER2	1998	Metastatic or nonmetastatic breast cancer; metastatic gastric or gastroesophageal adenocarcinoma
Alemtuzumab (Humanized)	CD52	2001	B-cell lymphocytic leukemia
Tositumomab (Murine)	CD20	2003	Relapsed or refractory; low grade, follicular, or transformed non-Hodgkin's lymphoma
Cetuximab (Chimeric)	EGFR	2004	Metastatic colorectal cancer; metastatic NSCLC
Bevacizumab (Humanized)	VEGF-A	2004	Metastatic colorectal cancer; metastatic NSCLC; metastatic RCC; metastatic cervical cancer; glioblastoma; recurrent ovarian, fallopian tube, or primary peritoneal cancer
Panitumumab (Human)	EGFR	2006	Colorectal cancer
Catumaxomab* (Chimeric mouse-rat hybrid)	EpCAM/CD3 Multi-targeting	2009	Malignant ascites
Ofatumumab (Human)	CD20	2009	Chronic lymphocytic leukemia
Ipilimumab (Human)	CTLA-4	2011	Late-stage metastatic melanoma and cutaneous melanoma
Cetuximab (Chimeric)	EGFR	2011	Late-stage head and neck cancer
Pertuzumab (Humanized)	HER2	2012	Breast cancer
Ado-trastuzumab (Humanized)	HER2	2013	Metastatic breast cancer
Elotuzumab (Humanized)	SLAMF7	2015	Multiple myeloma
Necitumumab (Human)	EGFR	2015	Metastatic squamous NSCLC
Daratumumab (Human)	CD38	2015	Multiple myeloma
Dinutuximab (Chimeric)	GD2	2015	Pediatric high risk neuroblastoma
Ramucirumab (Human)	VEGFR-2	2015	Advanced or metastatic gastric or gastroesophageal adenocarcinoma; metastatic NSCLC; metastatic colorectal cancer
Nivolumab (Human)	PD-1	2015	Unresectable or metastatic melanoma; metastatic squamous NSCLC; metastatic NSCLC; advanced RCC, recurrent or metastatic head and neck cancer

TABLE 4: (*continued*)

mAb (type)	Target antigen	Approval year	Cancer type indication
Olaratumab (Human)	PDGFR-α	2016	Soft tissue sarcoma
Atezolizumab (Humanized)	PD-L1	2016	Urothelial carcinoma
Olaratumab (Human)	PDGFR	2016	Soft tissue sarcoma
Atezolizumab (Humanized)	PD-L1	2016	Locally advanced or metastatic urothelial carcinoma, metastatic NSCLC
Pembrolizumab (Humanized)	PD-1	2017	Unresectable or metastatic melanoma, Metastatic NSCLC; recurrent or metastatic head and neck cancer
Pertuzumab (Humanized)	HER2	2017	HER2-positive metastatic breast cancer; HER2-positive, locally advanced, inflammatory, or early stage breast cancer; approved for cutaneous squamous cell carcinoma in 2020
Gemtuzumab ozogamicin (Humanized)	CD33	2017	Acute myeloid leukemia
Ocrelizumab (Humanized)	CD20	2017	Multiple sclerosis
Inotuzumab-ozogamicin (Humanized)	CD22	2017	Precursor B-cell acute lymphoblastic leukemia
Avelumab (Human)	PD-L1	2017	Metastatic Merkel cell carcinoma
Durvalumab (Human)	PD-L1	2017	Urothelial carcinoma
Rituximab-abbs* (Humanized (90%–95% human)	CD20	2018	Non-Hodgkin lymphoma
Cemiplimab (Human)	PD-1	2018	Advanced squamous cell carcinoma of the skin
Mogamulizumab (Humanized)	CCR4	2018	Mycosis fungoides and Sézary syndrome cell lymphoma (a group of cancers of the immune system)
Polatuzumab vedotin-piiq (Humanized)	CD79b	2019	Diffuse large B-cell lymphoma that has progressed or returned after at least 2 prior therapies
Tafasitamab-cxix (Humanized)	CD19	2020	Relapsed or refractory diffuse large B-cell lymphoma

EpCAM, epithelial cell adhesion molecule; GD2, ganglioside; PDGFR, platelet-derived growth factor receptor; RCC, renal cell carcinoma; SLAMF7, signaling lymphocytic activation molecule family member 7.
*First biosimilar of rituximab approved by FDA.

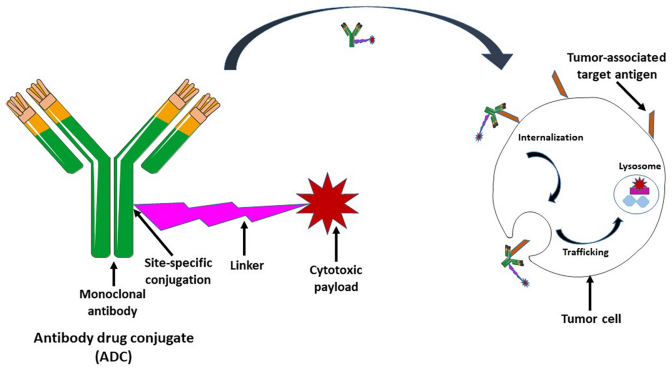

FIG. 7: An ADC construct composed of mAb and cytotoxic payload through a linker via site-specific conjugation. ADCs internalize and degenerate in lysosome to release cytotoxic payload.

and efficient internalization are desirable characteristics.[143] Generally, mAb with two heavy and two light chains possess molecular weight of around 150 kDa and binding affinities with K_D values 0.1 to 1.0 nmol/L are used in ADCs. The very high binding affinity of antibody affects its delivery in solid tumor, causing the binding-site barrier.[144]

Antibodies in human beings are categorized as immunoglobulin A (IgA), IgD, IgE, IgG, and IgM based on their heavy chain structures and effector functions. IgA further subdivides in IgA1 and IgA2. IgG further subdivides in IgG1 to IgG4. Of these, IgG has remained in focus due to presence of two Fab (antigen binding fragments) and two Fc (constant fragments). Fab mediates antigen recognition while Fc mediates antibody binding with effector cells of immune system, interacts with FcRn, (neonatal Fc receptor for IgG), and regulates circulation half-life of antibody.[142] The serum stability and strong binding affinity for Fc receptor are characteristics to IgG1 subtype, hence, IgG1 is commonly preferred in ADCs. However, Fc binding may lead to undesirable effects, and its suitability is determined on individual basis. For instance, an ADC, LOP628, comprised of anti-cKit humanized IgG1/κ antibody conjugated to a cytotoxic drug maytansine induced hypersensitivity reaction in phase I trial due to FcR1 and FcRII binding on mast cells.[142]

3. Selection of Cytotoxic Payload

The first important criterion to select payload for ADCs is its potency or high cytotoxicity. In general, a payload with half maximal inhibitory concentration (IC_{50}) in nanomolar and subnanomolar range is preferred, because only about 2% of intravenously administered ADCs distribute into tumors. Second, the cytotoxic agent should either possess a functional group or is able to be conjugated with the antibody. However, the payload structure should be non-interruptive to the internalization of ADCs administered parentally. Third, the payloads should be stable in physiological conditions. In addition, other desirable characteristics of ADCs include *in vivo* stability, long half-life, low immunogenicity, and low molecular weight.[144]

Initially, ADCs employed traditional anticancer agents including doxorubicin, methotrexate, mitomycin, 5-FU and vinca alkaloids, which exhibited lack of potency and selectivity, alongwith decreased target accumulation, resulting in poor efficacy. Over the time, potent payloads with subnanomolar IC_{50} values have been utilized.[142,144] Only few cytotoxic agents have been employed in currently ongoing clinical trials. Most of these payloads target either DNA or microtubulins. DNA-targeting payloads include calicheamicin, which induces double-strand DNA breakage (e.g., duocarmycin), which causes DNA alkylation, and pyrrolobenzodiazepine dimers, which cause cross-linking with DNA.[145] An ADC trastuzumab duocarmazine which incorporates duocarmycin as the cytotoxic payload is undergoing phase II/III trials in HER2-positive endometrial cancer patients. The microtubule inhibitors like auristatins and maytansinoids works by inducing cell cycle arrest at G_2/M phase, that causes DNA damage, blocks cell division and prevents tumor cell growth. To date, nine ADCs received FDA-approval, out of which four products use auristatins payloads. FDA-approved ADCs brentuximab vedotin, polatuzumab vedotin and enfortumab vedotin employed monomethyl auristatin E. Phase III trials of depatuxizumab mafodotin incorporating monomethyl auristatin F has been ceased due to poor results. Other cytotoxic payloads like RNA polymerase II inhibitors, crytophycin-based tubulin inhibitors, novel anthracyclines, and RNA spliceosome inhibitors are still in preclinical stage.[142–145]

4. Selection of Linker

Linker plays a significant role in ADCs' pharmacokinetics as it links mAb and cytotoxic payload. The most important property required for the linker is its stability in the blood circulation which is needed to reach the ADC to tumor cell in its intact form.[146] If the linker is unstable in circulation, it may release cytotoxic payload in the blood circulation before reaching the tumor site, leading to toxicity and lowering the therapeutic index. On the contrary, linker must be cleaved to release the payload once the ADC is internalized into the tumor cell.

Linkers can be of two types based on their mechanisms of payload release. First type is non-cleavable linkers in which cytotoxic payload is released via lysosomal degradation of amino acid residues present in mAb.[145] The formation of non-reducible bonds between amino acid moieties and non-cleavable linker imparts higher circulation stability, longer plasma half-lives and low toxicity to the ADC. For example, thioether linkers which upon internalization in tumor cells, undergo lysosomal degradation to produce metabolites. These metabolites maintain linker attachment to amino acid lysine while releasing the intact cytotoxic payload.[147] In an ADC Brentuximab vedotin, the maytansinoid payload was linked to the HER2 antibody through succinimidyl trans-4-(maleimidylmethyl) cyclohexane-1-carboxylate, which is a non-cleavable linker.[148]

The second type is cleavable linker which possesses a particular structural location between payload and mAb. The cleavage at a particular structural site to release the payload relies on physiological conditions inside the lysosomes. Depending on this environment, cleavable linkers can be of three types.[146] First type is acid-sensitive

cleavable linkers, which are cleaved inside lysosomes due to acidic pH conditions. For example, the ADCs gemtuzumab ozogamicin and inotuzumab ozogamicin, incorporate cleavable acid-sensitive linker 4-4′-acetylphenoxy butanoic acid to link anti-CD33 and anti-CD22, respectively, to payloads calicheamicin, which causes hydrazide (of calicheamicin) to form hydrazone. This hydrazone undergoes hydrolysis at lysosomal pH to release calicheamicin. This acid-sensitive linker exhibited nonspecific release leading to low plasma half-life of ADC.[149] Second type is protease-sensitive peptide linkers, which are cleaved by intracellular proteases inside the lysosomes. For example, in an ADC brentuximab vedotin, a lysosomal protease cathepsin B is used for linking anti-CD30 antibody to payload monomethylauristatin E, which cleaves the valine-citrulline dipeptide linkage after internalization in the tumor cells.[150]

Third type is redox-sensitive linkers, which are cleaved due to high glutathione concentration in tumor microenvironment. The example of this type is disulfide linkers whose steric hindrance is optimized to restrict premature intracellular cleavage. For example, in ADC lorvotuzumab mertansine, the cleavable disulfide linker N−succinimidyl−4-(2-pyridyldithio)pentanoate is used for linking anti-CD56 antibody to cytotoxic payload maytansinoid DM1, in which disulfide linker first cleaves to release a thiol compound DM4, followed by its S−methylation by the action of cellular methyltransferase.[144]

5. Conjugation Techniques

The conjugation of average number of drug molecules to each antibody is referred as drug to antibody ratio (DAR), which is a key factor in design of an ADC because conjugation of only few drug molecules on each antibody results in low efficacy, whereas a very high DAR results in reduced pharmacokinetics of ADCs.[151] In most ADCs in clinical trials, DAR is in the range of 2–4.[144]

In ADCs, site-specific conjugation is desirable due to its ability to generate products without modifying the antigen binding affinity.[144] There are three advanced site-specific conjugation techniques available for ADCs.[152] The first technique is insertion of engineered cysteine via introducing ultra-nucleophilic thiol group in cysteine side chains. Engineered cysteine is obtained via modifying cysteine side chain by introducing highly nucleophilic thiol group in this chain. The ADCs created using this technique possessed DAR 2 to 4 and showed higher *in vivo* efficacy and improved tolerance compared with conventional conjugation techniques.[153] For example, vadastuximab talirine is the first site-specific ADC which incorporated anti-CD33 antibodies and pyrrolobenzodiazepine dimer through a cleavable dipeptide linker (valine-alanine) using engineered cysteines technique.[154,155] Second technique uses insertion of unnatural amino acids with biorthogonal groups, which is then incorporated to engineer tRNA synthetases. This leads to recognition of genetic coding of unnatural amino acids for the site-specific conjugation. The third technique is enzymatic conjugation, which uses several enzymes such as transglutaminases, glycotransferases, bacteria-derived formyl glycine-generating enzyme and sortases that can specifically react with analogous functional groups of amino acid.[155]

6. ADCs in Clinical Development

Presently, nine ADCs gained FDA approval for treatment of various types of cancers, and above 80 are under clinical development for various types of cancers including breast cancer, bladder cancer, glioblastoma, Hodgkin's and non-Hodgkin lymphoma, ovarian cancer, and leukaemia.[142,144,156] FDA-approved ADCs for cancer treatments are summarized in Table 5. The potential ADCs undergoing phase III/IV trials are summarized in Table 6. Significant results of recent clinical trials for TNBC are briefly discussed.

a. Sacituzumab Govitecan

Sacituzumab govitecan is an anti-trophoblast cell-surface antigen (Trop-2) antibody conjugated with SN-38, via a cleavable pH-sensitive hydrolysable linker. SN-38, an active metabolite of irinotecan, is a potent DNA damaging agent which shows reversible binding with topoisomerase 1 cleavage complex of DNA and retards DNA replication causing cell death. All subtypes of breast cancer cells express Trop-2, but TNBC tumors show abundant expression of Trop-2. Initially, 108 patients with metastatic TNBC who had received at least two prior therapies (anthracycline or taxane, and platinum chemotherapy) were enrolled in a multi-center, open-label phase I/II study. These patients further received 10 mg/kg of Sacituzumab govitecan on 1st and 8th day of a 21-day cycle. Patients found to be benefited from the drug irrespective of number of prior treatments or age, with overall survival of 13 months. Sacituzumab govitecan-treated patients showed grade 3 or 4 adverse events including neutropenia (42%), anemia (11%), hypophosphatemia (9%), diarrhea (8%), and fatigue and asthenia (8%). Phase I/II clinical trial results demonstrated that sacituzumab govitectan is a breakthrough therapy for the treatment of metastatic TNBC.[144,157]

Currently, the international, open-label, randomized, confirmatory phase III ASCENT trial is ongoing to verify the early-stage study findings. This trial enrolled 488 patients with refractory or metastatic TNBC who had previously received at least two chemotherapies in the metastatic setting including a taxane. Patients are assigned treatment with either sacituzumab govitecan or to physician's choice of single-agent chemotherapy (eribulin, capecitabine, gemcitabine, or vinorelbine) [ClinicalTrials.gov Identifier: NCT02574455].[144,158]

b. Trastuzumab Deruxtecan

An ADC, trastuzumab deruxtecan is constructed using a humanized anti-HER2 antibody conjugated with a cytotoxic topoisomerase I inhibitor, exatecan derivative DS-8201a by a cleavable tetrapeptide-based linker. About 15–20% of TNBC tumors express HER2. DS-8201a was found 10 times more potent than SN-38. The response to trastuzumab deruxtecan by most of advanced HER2-positive breast cancer patients (median response duration, 20.7 months) require confirmation, for which open-label, single-group,

TABLE 5: FDA-approved ADCs

ADC name (trade name)	Cytotoxic payload	Antibody	Target antigen	Linker	Indication	Approval year
Brentuximab vedotin (SGN-35, Adcetris)	Monomethyl auristatin E	Chimeric IgG1	CD30	Protease-cleavable linker	Non-Hodgkin's lymphoma	2011
Trastuzumab emtansine (T-DM1, Kadcyla)	Maytansine 1 (DM1)	Humanized IgG1	HER2	Non-cleavable thioether linker	Metastatic breast cancer	2013
Gemtuzumab ozogamicin (Mylotarg)	Calicheamicin derivative	Humanized IgG4	CD33	Acid-labile hydrazone-based linker	Newly-diagnosed acute myeloid leukemia	2000 (withdrawal 2010, reapproval 2017)
Inotuzumab ozogamicin (Besponsa)	Calicheamicin derivative	Recombinant humanized IgG4	CD22	Acid-labile hydrazone-based linker	Relapsed or refractory B-cell precursor acute lymphoblastic leukemia	2017
Polatuzumab vedotin (Polivy)	Monomethyl auristatin E	Humanized IgG1	CD79	Protease-cleavable linker	Diffuse large B-cell lymphoma	2019
Enfortumab vedotin (ASG-22ME, Padcev)	Monomethyl auristatin E	Humanized IgG1	Nectin-4	Cleavable valine-citrulline linker	Metastatic urothelial cancer	2019
Trastuzumab deruxtecan (DS-8201a, Enhertu)	DX-8951 (Exatecan derivative)	Humanized IgG1	HER2	Cleavable peptide linker	Metastatic breast cancer	2020
Belantamab mafodotin (GSK2857916, Blenrep)	Monomethyl auristatin F	Humanized IgG1	B cell maturation antigen	Non-cleavable maleimidocaproyl (mc) linker	Refractory or relapsing multiple myeloma	2020
Sacituzumab govitecan (IMMU-132, Trodelvy)	SN-38	Humanized IgG1	TROP2	Cleavable CL2A linker	Metastatic TNBC	2020

TABLE 6: ADCs in ongoing phase III/IV clinical trials

ADC name	Cytotoxic payload	Target antigen	Indication	Sponsor	ClinicalTrials.gov Identifier
DS-8201a (Trastuzumab deruxtecan)	Topoisomerase I inhibitor	HER2	Breast cancer	Daiichi Sankyo, Inc.	NCT03734029
SYD985 (trastuzumab valine-citrulline-seco-DUBA)	Duocarmycin-hydroxybenzamide-azaindole	HER2	Metastatic breast cancer	Byondis B.V.	NCT03262935
(SGN-D33A) (Vadastuximab talirine)	Pyrrolobenzodi-azepines dimer	CD33	Acute myeloid leukemia	Seagen Inc.	NCT02785900
Brentuximab Vedotin	Monomethyl auristatin E	CD30	Relapsed or refractory Hodgkin lymphoma	Millennium Pharmaceuticals, Inc.	NCT01990534
Inotuzumab ozogamicin	Calicheamicins	CD22	Leukemia, B-cell lymphoblastic leukemia, acute lymphoblastic leukemia	Pfizer	NCT03677596
Brentuximab vedotin	Monomethyl auristatin E	CD30	Anaplastic large-cell lymphoma, non-Hodgkin lymphoma, T-cell lymphoma lymphoma	Seagen Inc.	NCT01777152
ABT-414 (Depatuxizumab mafodotin)	Monomethyl auristatin F	EGFR	Newly diagnosed glioblastoma with EGFR amplification	AbbVie	NCT02573324
SGN-35 (Brentuximab vedotin)	Monomethyl auristatin E	CD30	High risk of residual Hodgkin Lymphoma following stem cell transplant	Seagen Inc.	NCT01100502

TABLE 6: (*continued*)

Brentuximab vedotin	Monomethyl auristatin E	CD30	Relapsed or refractory systemic anaplastic large cell lymphoma	Millennium Pharmaceuticals, Inc.	NCT01909934
Depatuxizumab mafodotin (ABT-414)	Monomethyl auristatin F	EGFR	Pre-treated HER2 breast cancer that cannot be surgically removed or has spread	Daiichi Sankyo, Inc.	NCT03523585
Brentuximab vedotin	Monomethyl auristatin E	CD30	DS-8201a versus investigator's choice for HER2-low breast cancer that has spread or cannot be surgically removed	Daiichi Sankyo, Inc.	NCT03734029
Enfortumab vedotin	Monomethyl auristatin E	Nectin-4	Ureteral cancer, urothelial cancer bladder cancer	Astellas Pharma Global Development, Inc.	NCT03474107
IMGN853 (Mirvetuximab soravtansine)	Maytansine 4 (DM4)	FRα	Folate receptor alpha (FRα)-positive advanced ovarian cancer, primary peritoneal or fallopian tube cancer	ImmunoGen, Inc.	NCT02631876

multicenter, phase II study was undertaken in which 184 patients who had undergone a median of six previous treatments received the recommended dose of 5.4 mg/kg trastuzumab deruxtecan.[159,160]

In 112 patients, the median duration of follow-up was 11.1 months, the median response duration was 14.8 months, and the median duration of progression-free survival was 16.4 months showing durable antitumor activity. The most common adverse events of grade 3 or higher included a decreased neutrophil count (20.7%), anemia (8.7%), and nausea (7.6%). Currently, this ongoing, open-label, multicenter phase III study is recruiting patients with HER2-low, unresectable and/or metastatic breast cancer. Approximately 540 patients will be randomized in a 2 to 1 ratio to trastuzumab deruxtecan (5.4 mg/kg every 3 weeks) versus physician's choice of chemotherapy to evaluate responses, survival, and safety [ClinicalTrials.gov Identifier: NCT03734029].[161]

c. Trastuzumab Duocarmazine

An ADC is composed of the recombinant humanized anti-HER mAb linked to duocarmycin prodrug via a cleavable linker that cleaved at the dipeptide valine-citrulline by proteases inside the tumor cell and releases the antineoplastic payload duocarmycin, that binds to the minor groove of DNA, alkylates adenine at the N3 position, and induces cell death. In addition, trastuzumab induces ADCC against tumor cells that overexpress HER2.[162]

Currently, a randomized, active-controlled, superiority study in ongoing in 436 patients with unresectable locally advanced or metastatic HER2-positive breast cancer with either progression during or after minimum two HER2-targeting treatment regimens or progression during or after (ado-) trastuzumab emtansine treatment who are randomly assigned (2:1) to receive trastuzumab duocarmazine or physician's choice treatment until disease progression, unacceptable toxicity, or study termination. Patients are needed to visit the clinical site during treatment to enable assessment of efficacy, safety, and quality of life.[163]

C. Immune Checkpoint Inhibitors

Immune checkpoint inhibitor exploits one's own immune system in attempt to destroy cancer cells by blocking proteins called as checkpoints.[164] These checkpoints are made by immune system lymphocytes such as T cells, and B cells, which performs specific functions in immune system. These checkpoints keep immune responses away from becoming very strong and also keep the T cells away from killing tumor cells. Blocking these checkpoints free up T cells to kill cancer cells in better way.[165] Thus, checkpoint immunotherapy targets and blocks inhibitory immune checkpoints to restore immune system function.[166]

Usually, co-stimulatory and co-inhibitory ligands and receptors that regulate T cell activation do not express on tumor tissues compared with normal tissues, however, those

which regulate T cell effector functions usually express on tumour cells in the tumour microenvironment,[156] Therefore, antibodies that block immune checkpoints do not directly target tumour cells, but they target lymphocyte receptors or their ligands, showing improved anticancer effect.[155,156] Antibodies blocking two immune checkpoint receptors named programmed cell death protein 1 (PD1) or its ligand PD-L1, or cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) gained FDA approval for clinical use[167,168] (Table 7).

D. Nanobodies

Despite huge clinical success, mAbs suffer from problems such as possible immunogenicity, large size (~ 150 kDa) and incomplete tumor penetration which are major limitations to their therapeutic efficacy.[169] This led researchers to develop "heavy-chain only" antibodies comprising only two heavy chains and named as "nanobodies."[170] Nanobodies are antibodies with one antigen-binding fragment sourced from naturally occurring heavy-chain-only (VHH) antibodies of camelids serum and lack a VL domain causing detrimental effect to antigen binding.[171] Antigen specificity of nanobodies is determined through three CDRs (complementarity determining regions) CDR1, CDR2, and CDR3.[171] (Fig. 8). Nanobodies have abundant CDR3 loop, which is quite longer than CDR1 and CDR2 and forms finger-like epitopes which are major contributors to their specificity, diversity, and interactions with target antigens.[172,173]

Nanobodies combine specificity of mAbs and targeting potential of nanoscale to enhance tumor penetration.[170] In addition, nanobodies possess superior features such as natural origin, aqueous solubility, high stability, and strong antigen-binding affinity to deliver biodrugs in advanced cancer treatments.[159] Moreover, nanobodies possess relative ease of large-scale production than costly manufacture of mAbs.[171] However, the small size of nanobodies are prone to fast renal clearance limiting their therapeutic lifetime, which needs their doses to be increased to achieve clinically efficacy. This limitation is combatted by modifying nanobodies through extending their half-life via strategies like PEGylation, binding to anti-albumin nanobodies or fragment crystallisable (Fc) domains, and multimerization.[172,173] The clinical status of camelid-based VHH domains is prominent with one product approval and eight products under clinical development, among which only two products are currently undergoing clinical evaluation for cancer treatment[173] (Table 8).

E. Cell-Penetrating Peptides (CPPs)

CPPs are small and diverse polypeptides with 5–30 amino acids that can undergo cell internalization and uptake.[174] Among polypeptide chain, amino acids are positively charged such as lysine or arginine, or a pattern of alternating polar and nonpolar amino acids exist.[175] Positively charged CPPs have shown to target and penetrate tumor cells independent of receptors. Cationic CPPs with minimum eight positive charges entered the cell through endocytic pathways.[176] Although CPPs can cross

TABLE 7: FDA-approved checkpoint inhibitors for cancer immunotherapy

Name (tradenName)	Target	Type and source	Cancer type	Company	Approval year
Ipilimumab (Yervoy)	CTLA-4	Whole antibody, human	Metastatic melanoma	Bristol Myers Squibb, US	2011
Nivolumab (Opdivo)	PD-1	Whole antibody, human	Metastatic melanoma, NSCLC, renal cell carcinoma, Hodgkin's lymphoma, head and neck cancer, urothelial carcinoma	Ono Pharmaceuticals Co. Ltd., Japan	2014
Pembrolizumab (Keytruda)	PD-1	Whole antibody, humanized (from mouse)	Metastatic melanoma, NSCLC, head and neck squamous cell cancer, classical Hodgkin's lymphoma	Merck & Co. Inc., US	2014
Atezolizumab (Tecentriq)	PD-L1	Whole antibody, humanized	NSCLC, bladder cancer	Genentech, US	2016
Avelumab (Bavencio)	PD-L1	Whole antibody, human	Urothelial carcinoma, Merkel cell carcinoma	Merck KGaA, Germany and Pfizer Inc., US	2017
Durvalumab (Imfinzi)	PD-L1	Whole antibody, human	Urothelial carcinoma	AstraZeneca, UK	2017
Cemiplimab (Libtayo)	PD-1	Whole antibody, human	Cutaneous squamous cell carcinoma	Regeneron Pharmaceuticals, Inc., US	2018

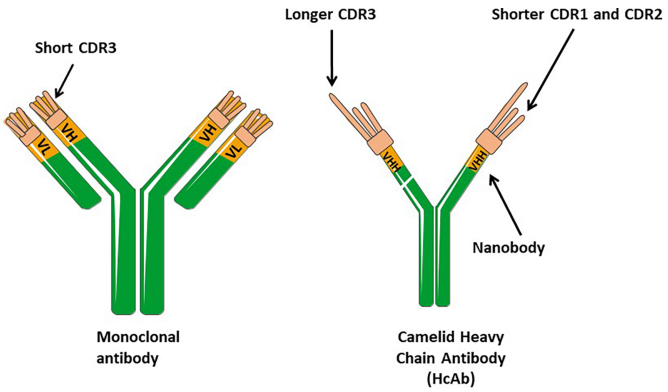

FIG. 8: Structural difference between mAb and heavy chain antibody (HcAb). Compared with VH-VL domain in mAbs, the VHH/nanobody lacks the VL domain and possess a longer CDR3 loop than mAb, enabling stronger antigen affinity.

TABLE 8: Clinical status of tumor-targeting nanobodies

Drug	Target	Indication	Status	Sponsor
LCAR-B38M	B cell maturation antigen	Multiple myeloma	Phase II	Legend/Janssen
BI 836880	VEGF, angiopoietin-2	Solid tumors	Phase I	Boehringer Ingelheim (Ablynx)

the cellular membrane unlike common peptides, they still suffer similar limitations of conventional peptides such as short half-life, rapid renal clearance and low bioavailability.

Homing peptides have been developed to target tumor tissues in which therapeutic agent can accumulate via tumor-specific markers, specifically present in tumor vasculature.[177] Homing peptides are linked to CPPs to add specificity to the CPPs allowing the transportation of cargo through the cell membrane, which is otherwise absent in CPPs by their own. For instance, attachment of the trans-acting activator of transcription (TAT) sequence (GRKKRRQRRRPPQ) could achieve cargo-internalization from the surrounding media. In second type, homing peptide bind directly to a tumor marker causing the internalization of the cargo.[178] One important study using human breast cancer xenografts in nude mice demonstrated improved efficacy and low toxicity using doxorubicin coupled to two peptide motifs. It indicated that RGD (arginine/glycine/aspartic acid) or NGR (asparagine/glycine/arginine) sequences in peptides possess inherent tumor targeting capacity.[179] TAT-like and RGD-like sequences in homing peptides can be linked with cytotoxic agent to form hybrid anticancer systems to efficiently deliver cargo to tumors.[179] Cancer targeting CPPs under clinical development are summarized in Table 9.

TABLE 9: CPPs under phase I clinical development for treatment of tumors

Active	CPP	Cargo	Indication	Sponsor	ClinicalTrial.gov ID
p28	Azurin-derived CPP p28	Non-HDM2-mediated peptide inhibitor of p53	Central nervous system tumors	Pediatric Brain Tumor Consortium	NSC745104
p28	p28	P28	Solid tumors that resist standard treatment	CDG Therapeutics, Inc.	NCT00914914
AVB-620	Activatable cell penetrating peptide	Cy5, Cy7	Tumor imaging	Avelas Biosciences, Inc.	NCT02391194

F. Tumor-Associated Fibroblast (TAF) Targeting

The vastly heterogeneous and multicellular tumor microenvironment consists of tumor cells, non-tumor stromal cells like fibroblasts, mesenchymal stem cells, endothelial cells and cells of hematopoietic origins such as immune cells in the extracellular matrix.[180] TAF is a dominant components in the tumor stroma which is found in almost all cancers, but abundant in breast, prostate, colorectal, pancreatic, and non-small-cell lung cancer (NSCLC), while less abundant in ovarian, renal, brain, and head and neck cancers.[181] The spindle-shaped can synthesize, deposit and rebuild the structure of extracellular matrix to secrete cytokines and growth factors that facilitate tumor growth by promoting angiogenesis, and inflammation while increasing the risk of metastasis in breast, pancreatic and lung cancer.[182–184]

Stromal fibroblasts have been deactivated via β-catenin signalling which reduced the production of diffusible proteins and extracellular matrix proteins. The tumor-stroma interaction suppressed tumor microenvironment and resulted in improved melanoma treatment.[185] Although the clear role of TAFs in tumor microenvironment makes them promising targets for immunotherapy, drug delivery strategies which use high-specificity TAFs are still limited.[186] A list of therapeutic targets of TAFs and their clinical development are shown in shown in Table 10.

V. TRANSLATIONAL CHALLENGES AND EMERGING PROSPECTS

The cancer targeted drug delivery via nanocarriers has progresses enormously. However, there are several challenges including toxicity, manufacturing, and regulatory issues, which pose obstacles in the pathway of clinical translation of nanocarrier-based products for cancer-targeted therapies. Current nanocarrier-based anticancer drug targeting suffer

TABLE 10: Clinical status of therapeutic systems targeting TAF

Therapeutic target	Function of therapeutic target	Therapeutic agent	Status
VEGF	Angiogenesis	Bevacizumab	Phase III
Janus kinase 2	Promotes progression	SAR302503	Phase III
—	—	—	Phase III
Fibroblast activation protein	Tumor angiogenesis	PT-100	Phase I
—	—	Sibrotuzumab	Phase I
Matrix metalloproteinases	Cell proliferation	Mamrimastat	Phase III
—	—	Tenomastat	Phase III

from limitations such as low targeting efficiency, rapid plasma clearance, and tissue permeability.[187] To deal with these issues, it is necessary to understand thoroughly about the nanoparticle transport in complex biological network, so that the drug or carrier properties can be manipulated to resolve these issues. Recently, "Particle Replication in Non-wetting Template" (PRINT) has emerged as reproducible technology to synthesize nanocarrier batches with strict control over size, shape, surface properties, composition, and drug loading. The chemistry, manufacturing and control of nanoparticles become more challenging for complex nanocarrier system as it transitions from preclinical to clinical development followed by its regulatory requirements for market approval.[188] To promote feasibility of clinical translation of nanocarriers, the research and development costs, and cycle time should also be reduced.[179] To assess pharmacokinetics, biodistribution, efficacy and safety of nanocarriers using animal models; different xenografts such as orthotopic, patient-derived and genetically engineered mouse models have been developed. Despite, any single animal model is currently not available which can fully mimic human malignancy and EPR effect.[188]

In designing cancer targeting nanosystems, cancer complexity and dynamics that require appropriate studies to understand tumor microenvironment are crucial.[189] In this milieu, it is critical to consider the complex heterogeneity associated with various types of tumors and focus on the personalized cancer treatment via designing precision nanomedicines which requires the study of individualized molecular interventions and treatment tailoring based on the tumor stage. Molecular targeted therapy that precisely targets and blocks cancer-specific genes including signal transduction inhibitors, gene expression modulators, angiogenesis inhibitors, and hormone therapy, are foreseen as emerging prospects against several cancers. For instance, uncommon and actionable EGFR mutations detected by molecular profiling in advanced NSCLC has transformed treatment criteria for this malignancy. In addition, genetic heterogeneity of cancers limits accurate detection of druggable molecular targets, whereas limited studies related to biocompatibility, long term toxicity and genotoxicity of nanomaterials is another challenge and prospective area of study for cancer targeting nanosystems.

Development of peptidic ligands can be envisaged by developing methods to select and generate target-specific peptides with improved *in vivo* stability. Cell-derived microvesicles to construct cell-based nanosystems are foreseen as one of the emerging strategies to enhance delivery efficiency in treatment of metastatic cancers. Such cell-based nanosystems can be obtained by camouflaging nanoparticles with diverse cell membranes or loading nanoparticles in living carrier cells for anti-metastasis therapy.[190] Thorough understanding of tumor microenvironment and tumor metastasis will lead efforts in development of new therapies with improved functionality in treatment of anti-metastatic cell-based nanosystems in future.

VI. CONCLUSIONS

Organic, inorganic, and hybrid nanocarrier-based systems exploiting tumor vasculature via passive or active targeting modalities have been developed for several types of cancers, however, with limited clinical and commercial success, demanding better targeting modalities in treatment of metastatic cancers. Advanced immunotherapy-based targeting approaches including mAbs, antibody drug conjugates and checkpoint inhibitors have emerged as highly efficient treatment options demonstrating revolutionized success in immunotherapy-based cancer treatments. Continuing advancements in cancer research have provided other potential targeting systems based on nanobodies, CPPs, and TAFs in efforts toward achieving better target specificity for enhanced anticancer outcomes while reducing toxic side effects. A few products based on these strategies are approved to market, while some others are under clinical pipeline. Despite huge success, manufacturing complexities and nanotoxicity are major challenges for clinical translation of complex nanostructured targeting systems. Search of new molecular targets, anti-metastasis therapy, advanced immunotherapy, and signal-transduction therapy are foreseen as emerging prospective strategies that will lead the future research efforts in pursuit of "maximum-effect-minimal-side-effect" nanomedicines for treatment of intractable cancers.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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