IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
August 2020 Vol.:19, Issue:1

Targeted Drug Delivery System: In Tumour

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Mohammed Hasif MP., K.U Lakshmi*, Nethaji Ramalingam, K.R Vimal

Devaki Amma memorial college of pharmacy, affiliated to Kerala University of Health Science and Approved by AICTE& PCI. Pulliparamba-673634, Malappuram Dt. India.

Submission: 21 July 2020
Accepted: 28 July 2020
Published: 30 August 2020

Keywords: Tumour, Targeted drug delivery, Nano-particles, Active and passive drug delivery, Vehicles

ABSTRACT

A long-standing problem of conventional cancer chemotherapy is the lack of tumour specificity. Tumour-targeting drug-delivery systems have been explored to overcome this problem. Recently, targeted drug delivery systems (TDDSs) have been extensively studied as a promising therapeutic for tumour therapy. In this review, we investigate the typical targeting mechanisms of TDDSs, covering both passively and actively targeting DDSs for tumour therapy. Finally, we present some recent representative TDDSs that are under testing in preclinical trials. Although TDDSs are proving to be promising therapeutic nano-platforms for tumour therapy.

HUMAN JOURNALS
www.ijppr.humanjournals.com

Abbreviations:

TDDS- Targeted drug delivery system

DDS- Drug delivery system

EPR- Enhanced permeability and retention

INTRODUCTION"

The biological effects of a drug in a patient depend upon the pharmacological properties of the drug. These effects arise due to the interaction between the drug and receptors at the site of action of the drug. However, the efficacy of this drug-target interaction stands undermined unless the drug is delivered to its site of action at such a concentration and rate that causes the minimum side-effects and maximum therapeutic effects. Targeted drug delivery aims to achieve the same. Targeted Through the identification of the right gene, the right pathway, and the right target in the pathway, the right drug can be developed to treat the right patients. For such type of process, the foremost thing is the target identification.

Drug delivery, also known as smart drug delivery, is a method of treatment that involves the increase in medicament in one or a few body parts in comparison to others. Therefore, it delivers the medication only to areas of interest within the body. This offers an improved efficacy of treatment and also reduces side effects. Tumor targeting effectiveness can be enhanced by taking into account an altered molecular profile of malignant cells. Here we briefly explain the various tumour targeting drug delivery and their current relevance and also their future challenge.

TARGETED DRUG DELIVERY SYSTEM

Recently, targeted drug delivery systems (TDDSs) have been extensively studied as a promising therapeutic for tumour therapy[1]. The term "targeted drug delivery" (or "drug targeting") used in drug delivery is distinct from "targeted therapy" (or "targeting therapy") that is frequently used in drug discovery.

Target Identification

The conventional drugs used so far are small organic molecules meant to degrade the diseased protein formed as a result of the interference of pathogenic at a cellular level. A high

concentration of these drugs in the cells is necessary for the desired therapeutic effect. This aggravates the problem of toxicity. Ever since the unraveling of the human genome, attempts are being made to target the drugs selectively to the affected sites to increase their efficiency and simultaneously decrease their side effect. There are several approaches to discover new potential drug targets. Two conceptually different approaches are Genomic approaches and Genetic approaches.

Genomic Approach

The genomic approaches involve the differential expression of m-RNA in, for example. normal vs diseased tissue or gene expression changes after a compound has been added to the culture medium of cells or given to an animal. Now many groups are using microarray [2] technology [3] and proteomics to identify the changes in gene expression this approach assumes that the expression of relevant targets will be different in diseased tissues and after treatment.

Genetic Approach

The genetic approach is more long term and labor-intensive but has greater potential. The major problem in facing this approach is that most major diseases are polygenic; for monogenic diseases, many of the underlying genetic alterations have been published [4,5]. Genetic studies in humans are costly in the long run. An interesting complement to human genetic studies is genetic analysis in a more amenable model organism. A challenge is to make sure that the phenotype identifies in the chosen model system is relevant to the human disease and that the biological pathways are similar in humans and the experimental species. [6]. In drug discovery and development model organisms used are mouse, drosophila, zebrafish, worms, flies, and yeast [7,8]. The main advantage genetic approach over genomic approach is that there is a link between a genetic alternation and disease phenotype.

TUMOUR TARGETING DRUG DELIVERY SYSTEM

Recently, targeted drug delivery systems (TDDSs) have been extensively studied as a promising therapeutic for tumour therapy. The term targeted drug delivery (or drug targeting) used in drug delivery is distinct from “targeted therapy” (or “targeting therapy”) that is frequently used in drug discovery. Targeted drug delivery refers to predominant drug accumulation within a target zone that is independent of the method and route of drug

administration [9]. Drug targeting to specific sites in the body requires different delivery systems depending on the drug delivery route selected. TDDSs are a special form of drug delivery system (DDS) where the therapeutic cargo is selectively delivered to the tumour sites but not to the healthy tissues or cells. This approach not only overcomes the MDR of tumours and improves the efficacy of antitumor drugs but is also capable of minimizing the side effects associated with conventional therapeutics [10].

TUMOUR TARGETING METHODS

The delivery of the drug to the cancer cells can be achieved primarily by two methods - active and passive methods.

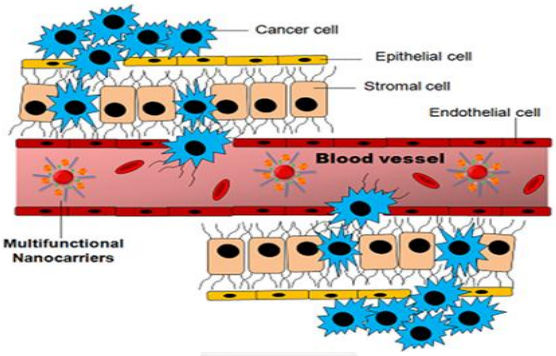

Figure No. 1: Active targeting by conjugating nanoparticle with a targeting moiety

Active Targeting

Active targeting is usually achieved by conjugating the nanoparticle to a targeting moiety where chemotherapeutic agents carried by nanoparticles are designed in such a way as they directly interact with the infected cells in the body. Nanoparticles bear some certain functional characteristics. The surface of the nanoparticles consists of the essential functional group to bind to target cancer cells receptors. In active targeting, the nanoparticles are designed to target the cancerous cells, either by ligand-receptor interaction or antibody-antigen recognition [11-13]. The active targeting is particularly attractive for the intracellular delivery of macromolecular drugs such as deoxyribonucleic acid, small interfering ribonucleic acid, and proteins. The enhanced cellular internalization rather than an increased tumour accumulation is responsible for the anticancer efficacy of actively targeted nanocarriers [14].

Passive Targeting

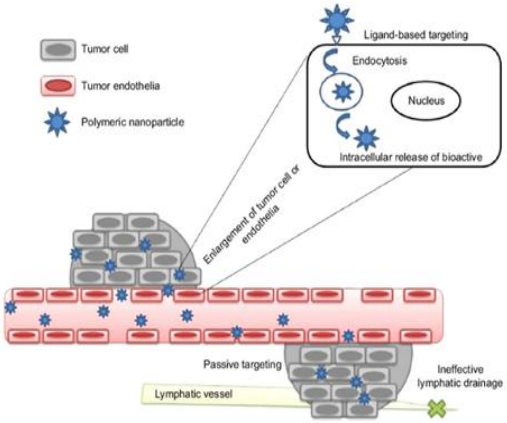

Figure No. 2: Overview of targeting approaches of polymeric nanoparticles in cancer

In passive targeting, nanoparticles accumulate mostly in the neoplastic tissues which result in enhanced permeability and retention (EPR) phenomenon. All nanocarriers use the EPR effect as a guiding principle. The EPR effect is the gold standard in cancer-targeting drug designing. Passive targeting consists of the transport of nanocarriers through leaky tumour capillary fenestrations into the tumour interstitium (composed of a collagen network and a gel-like fluid) and cells by convection or passive diffusion [15]. The EPR effect will be optimal if nanocarriers can evade immune surveillance and circulate for a long period. Very high concentrations of drug-loaded nanocarriers can be achieved at the tumour site, for instance, 10–50-fold higher than in normal tissue within 1–2 days [16]. Some limitations of drugs during passive delivery to the cancer cells can be: The passive targeting depends on the degree of tumour vascularization and angiogenesis [17]. Thus, the extravasation of nanocarriers will vary with tumour types and anatomical sites. The interstitial fluid pressure of solid tumours avoids successful uptake and homogeneous distribution of the drug in the tumour [18].

RECENT PROGRESS ON NANOPARTICLE-BASED DRUG DELIVERY SYSTEM

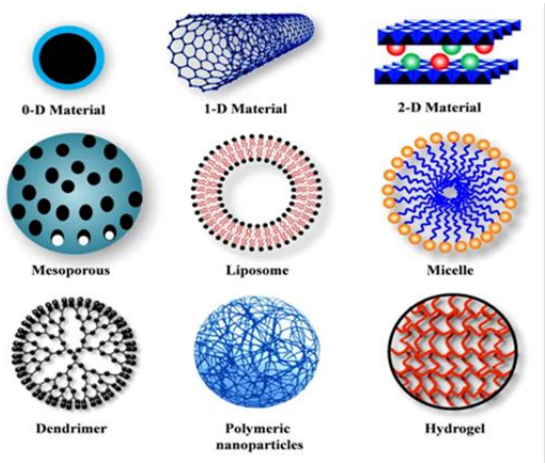

Figure No. 3: Types of nanoparticles used as drug delivery systems

Nanoparticles useful as drug delivery systems are submicron-sized particles (3–200nm), devices, or systems that can be made by using a variety of materials including lipids (liposomes), polymers (polymeric nanoparticles, micelles or dendrimers), viruses (viral nanoparticles) and even organometallic compound. (A) polymeric nanoparticles: polymeric nanoparticles in which drugs are conjugated to or encapsulated in polymers. (B) Polymeric micelles: amphiphilic block copolymers that form to nanosized core/shell structure in aqueous solution. The hydrophobic region serves as a reservoir for hydrophobic drugs, whereas the hydrophilic shell region stabilizes the hydrophobic core and renders the polymer to be water-soluble. (C) Dendrimers: synthetic polymeric macromolecule of nanometer dimensions, which is composed of multiple highly branched monomers that emerge radially from the central core. (D) Liposomes: self-assembling structures composed of lipid bilayers in which an aqueous volume is entirely enclosed by a membranous lipid bilayer. (E) Viral-based nanoparticles: in the general structure are the protein cages, which are multivalent, self-assembles structures. (F) Carbon nanotubes: carbon cylinders composed of benzene rings [19].

FACTORS TO CONSIDER FOR EFFECTIVE TUMOUR TREATMENT

Clinical EPR effect

In experimental animal models, the EPR effect has been shown to differ from tumour to tumour xenografts implanted at the same site, and from site to site following implantation of the same tumour [20]. Further, the tumour growth rate in mice is not comparable to that in

human patients, and not much is known of blood vessel morphology in clinical tumours. The normalized accumulation of stealth liposomes in the clinical breast, head, and neck and bronchus tumours varies from 2.7 to 53% ID/kg [21], reflecting the extremely heterogeneous nature of the EPR effect. The clinical EPR effect could be influenced by numerous tumour biological factors. Overall, there is a definite need for systematic investigation of factors that could affect clinical EPR outcomes.

Extravasation and Intratumoral Distribution

A drug carrier, either in the form of soluble macromolecule or nanoparticle, can meet its target cell among various cell populations in a solid tumour after reaching the tumour vasculature. But the drug carrier has to extravasate through the openings in the blood vessels and penetrate and distribute within the tumour tissue. Translocation from the blood compartment to the tumour tissue is governed by convection and diffusion. The driving force behind the convective flow is the pressure gradient. Drug carrier extravasation by convective fluid flow depends on the difference in pressure between tumour IFP and capillary hydrostatic pressure (10–30 mm Hg) and the difference in colloid osmotic (oncotic) pressures in both compartments [22].

Tumour Heterogeneity

Clinically, tumours are classified as one of the multiple potential subtypes using histological and genetic profiling. As an example, the tumour in the central nervous system can be categorized into one of 120 subtypes [23,24]. It is well-appreciated that, due to the mutable nature of cancer cells, their nature and characteristics can change both spatially and temporally with a tumour. clonal evolution and cancer stem cell theories support intra-tumoral heterogeneity arising from genetic variation as well as epigenetic modification. The cancer stem cell theory predicts the presence of hierarchical heterogeneity of cell populations as observed in blood cells derived from hematopoietic stem cells. A tumour is not a monoculture or collective mass of a single cell type. Even a cultured cell line presents its heterogeneity in cell population including cancer stem-like cells with a different set of surface markers from other bulk cells which can survive from standard cytotoxic treatments[25] Tumours can thus be viewed as a community of various subpopulations that can respond to environmental cues induced by soluble factors and interaction with non-cancerous endothelial cells, pericytes, cancer-associated fibroblasts, immune-inflammatory cells, and

stem and progenitor cells within the tumour stroma. These cells together can develop an extremely complex tumour microenvironment [26].

CURRENT UNDERSTANDING ON DRUG TARGETING OF I.V. ADMINISTERED SYSTEM

Our current understanding of drug targeting to tumours is based on a combination of several independent concepts, involving events associated with the EPR effect, nanoparticle properties and design, increased retention in the circulation due to PEGylation, and ligand-receptor type interactions, as shown in fig.4.

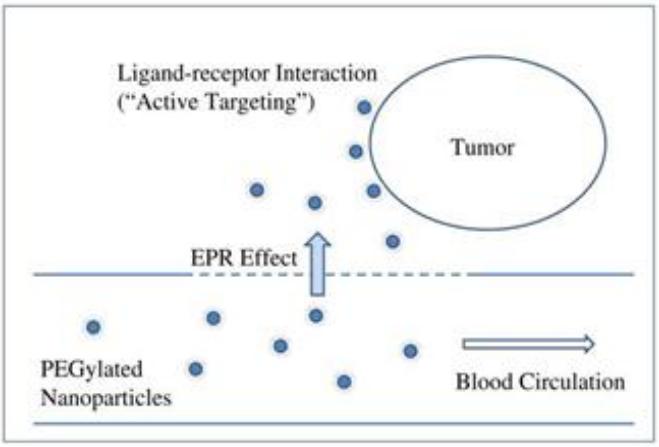

Figure No. 4: Drug targeting to tumour-combination of several independent events

(A) EPR Effect: The key observation of the role played by EPR started with the enhanced antitumor effect of arterially infused high-molecular-weight antitumor agent SMANCS dissolved in lipid lymphographic agent (Ethiodol®) in man [27]. (B)Nanoparticle Property: macromolecules in the molecular weight range of 15,000–70,000 g/mol, with certain additional properties, can effectively accumulate in a solid tumour. This accumulation occurred faster with smaller molecules compared with larger, but the larger molecules were retained longer within the tumour. Thus, size is an important factor for controlling tumour accumulation kinetics and for preventing diffusion back into the systemic vascular bed [28], (C)PEGylation has been used extensively to modify the pharmacokinetics of the drug itself and/or the nanoparticles. PEGylated nanoparticles have increased systemic circulation times, and this appears to be dependent upon the molecular weight of PEG used. When PEG of different molecular weights was i.v. injected into mice, it was found that the circulation half-life of PEG 6000 (molecular weight of 6000 g/mol) was only less than 30 min, while it was

extended to a day with PEG 190,000[29] (D) Ligand–receptor interaction may help explain observations such as the lack of improved uptake of folate-targeted liposomes relative to non-targeted liposomes by tumour cells [30].

NEED FOR TARGETED DRUG DELIVERY

The quest for the specificity of therapeutic agents is implicit in all treatment modalities. In cancer treatment, where chemotherapeutic and radiotherapeutic options are designed to kill cells, the specificity of drug action gains paramount importance. These strategies are based on the basic principle of preferentially killing cancer cells, without having any significant toxic effect on normal cells. It is necessary that all the cancer cells must be killed, either directly as a result of drug effect or indirectly due to bystander effect of the therapy to achieve complete remission in patients presenting a disseminated disease (4). Chemotherapy regimens alone are not entirely satisfactory in aggressive carcinomas and often produce only transient responses. Combination therapy, which involves a high dose of radiation (~60-70 Gy) with continuous infusion of chemotherapeutic agents (like paclitaxel) has been investigated for the management of unresectable locally advanced tumours (5). Paclitaxel radio sensitizes tumour cells, and hence the combination therapy is more effective than drug or radiation therapy alone. Targeting drugs with specially designed drug delivery systems offers a lucrative option to enhance the therapeutic efficacy and to reduce the event of systemic toxicity of anti-cancer agents. Thus, the need for developing specifically targeted drug delivery systems arises from not only the clinical perspective but can also help in eradicating cancer from the patient before it kills the patient [31].

Table No. 1: Currently approved nanomedicines in cancer therapy

Year approved	Name	Type	Active drug	Diameter	Type of cancer
Japan (1994)	Zinostatin stimalamer	Polymer protein con‐ jugate	Styrene maleic anhy‐ dride neocarzinostatin (SMANCS)	*	Renal cancer
FDA (1995) EMA (1996)	Doxil/caelyx	Liposome (PEGylated)	Doxorubicin	80–90 nm	HIV‐associated Kaposi's sarcoma, ovarian cancer, metastatic breast cancer, multiple myeloma
FDA (1996)	DaunoXome	Liposome (non‐PEGylated)	Daunorubicin	45 nm	HIV‐associated Kaposi's sarcoma
Taiwan (1998)	Lipo‐Dox	Liposome	Doxorubicin	180 nm	Kaposi's sarcoma, breast, and ovarian cancer
FDA (1999)	DepoCyt	Liposome	Cytosine arabinoside (cytarabine)	10–20 μm	Neoplastic meningitis
EMA (2000)	Myocet	Liposome	Doxorubicin	190 nm	Neoplastic meningitis
FDA (2005) EMA (2008)	Abraxane	Nanoparticle albumin-bound	Paclitaxel	130 nm	Advanced non‐small‐cell lung cancer, metastatic pancreatic cancer, metastatic breast cancer
FDA (2006)	Oncaspar	PEG protein conjugate	l‐Asparaginase	50–200 nm	Leukemia
South Korea (2007)	Genexol‐PM	Genexol‐PM PEG‐PLA polymeric micelle	Paclitaxel	20–50 nm	Breast cancer, Lung cancer, Ovarian cancer
EMA (2009)	MEPACT	Liposome (non‐PEGylated)	Mifamurtide	*	Osteosarcoma
EMA (2010)	Nano Therm	Iron oxide nanoparticle	-	20 nm	Thermal ablation glioblastoma
FDA (2012)	Marqibo	Marqibo Liposome (non‐PEGylated)	Vincristine	100 nm	Philadelphia chromosome-negative acute lymphoblastic leukemia
FDA (2015)	MM‐398 (Onivyde)	Liposome (PEGylated)	Irinotecan	80–140 nm	Metastatic pancreatic cancer (2nd line)

Table: 1 shows the list of recently approved targeted medicines.

CHALLENGES IN DRUG DELIVERY

For the majority of pharmaceuticals currently in use, the activity against certain diseases or disease sites is not based on their ability to accumulate selectively in the pathological organ, tissue, or cell. Usually, the pharmaceutical agent is rather evenly distributed within the body. Moreover, to reach the site of action, the drug has to cross many biological barriers, such as other organs, cells, and intracellular compartments, where it can be inactivated or express the undesirable influence on organs and tissues that are involved in the pathological process. As a result, to achieve a required therapeutic concentration of a drug in a certain body compartment, one has to administer the drug in large quantities, the great part of which is just wasted in normal tissues. In addition, under these circumstances, cytotoxic and/or antigenic drugs can become the cause of many negative side effects. The challenge of modern drug therapy is the optimization of the pharmacological action of drugs, coupled with the reduction of their toxic side effect in vivo. Under such conditions, the local concentration of the drug at the disease site(s) should be high, while its concentration in other non-target organs and tissues should be below a certain minimal level to prevent any negative side-reactions. Drug targeting can achieve the goal of this challenge [32].

FUTURE OF TARGETED DELIVERY

True targeted drug delivery is still beyond our grasp, but it is probably the single most important property that drug delivery systems should acquire for treating cancers and certain other diseases where it will be important to place a drug selective at a specific site of the body. The information necessary to achieve effective drug targeting may already exist, and we simply are not able to extract the answers from all information currently available. By understanding our current misunderstandings on targeted drug delivery, we will be in a better position to discover the solutions for true drug targeting. The current concept of ligand-modified PEGylated nanoparticles as a “magic bullet” needs to be modified. It simply presents an inaccurate picture of a very complicated problem.

For cancer therapies, the ideal targeted drug delivery system is the one that delivers the drug only to the target tumour. The reality, however, is far away from that ideal scenario. The amount of drug delivered to tumour targets is much less than 5% at most. Our efforts instead may have to be focused on how to better exploit this moderate amount of the drug delivered to the target tumour. As tumours may not be eradicated by just aiming at one target, it may

also be necessary to simultaneously aim at multiple targets. Thus, it may be worthwhile to develop "magic shotgun" strategies that deliver multiple drugs, and/or deliver the drug to multiple targets [33] .

Current targeted drug delivery approaches are all prepared by scientifically sound rationale. The limited success of current nanoparticles is mainly because these materials are constructed according to engineering and biochemical principles alone. While the known current nanoparticles can increase the blood circulation time and facilitate partitioning into tumours via the EPR effect, potentially improving their ability to interact with target cell receptors, these promising materials do address issues such as the dynamic changes of cancer cells and tumour heterogeneity. It is time to take these factors into account for developing better-targeted drug delivery systems. Dynamic changes in cellular events cannot be described by mathematical equations yet, and thus, it is difficult to predict the cellular behavior or responses to drug delivery systems. This, however, should not mean that we can ignore these important factors in the design of targeted drug delivery systems. Recognizing what we are missing is the first step toward moving in the right direction to solving the many problems that remain [34].

CONCLUSION:

Targeted drug delivery in tumour therapy is now developing fast due to its potential to deliver drugs at specific sites. The application of nanotechnology in drug delivery has particularly enhanced the delivery of drugs. Numerous nanoparticles have been approved for clinical use and, although they are still in their development stages, they hold the key to the future of drug-targeting. Several other approaches have also been developed with similar results. They all outline the bright future of targeted drug delivery in the treatment of tumour.

CONFLICT OF INTESREST:

The authors confirm that this article content has no conflict of interest.

REFERENCES:

1. Torchilin VP. Drug targeting. Eur J Pharm Sci. 2000;11: S81–S91.
2. Lockhart D J & Winzerler E A, Genomics, gene expression and DNA- arrays, Nature, 405 (2000) 827-836.
3. Miles M F, Microarrays: lost in a strom of data? Nat Rev Neurosci, 2 (2001) 441-443.
4. Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett L M, Haugen-Strano A, Swensen J, Miki Y et al, BRCAL mutation in primary breast and ovarian carcinomas, Science, 266 (1994) 120-122.

5. Wooster R, Neuhausen S L, Mangion J, Qqirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D et al., Localization of a breast cancer susceptibility gene, BRACA2 to chromosome Bq 12-13, Science, 256 (1994) 2088-2090.
6. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard J-P, Belaiche J, Almer S, Tysk S, O'Morain C A, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Pug P, Gowe-Rousseac C, Macry J, Colombel J-F, Sahbatou M & Thomas G, Association of NoD2 leucine-rich repeat variants with susceptibility to Crohn's disease, Nature, 411 (2001) 599-603.
7. Parijis L V, Genetic analysis of immunological disease in the mouse by RNAi, Drug Discovery Ser; IBC's Second Annu Model Organism Drug Discovery, (Hynes Convention Center, Boston M A) (12 August 2003).
8. Nehls MC, Deductive Genomes: Industializing discovery biology and target validation using the mouse models, Drug Discovery biology and target validation using the mouse models, Drug Discovery Ser; IBC's Second Annu Model Organism Drug Discovery, (Hynes Convention Center, Boston M A) (13 August 2003).
9. Glynn R, Development & Characterisation of mouse models for complex diseases, Drug Discovery Ser; IBC's Second Annu Model Organism Drug Discovery, (Hynes Convention Center, Boston M A) (13 August 2003).
10. Mills JK, Needham D. Targeted drug delivery. Expert Opin Their Patents. 1999; 9:1499–1513.
11. Malam Y, Loizidou M, Seifalian AM. Liposomes and nanoparticles: Nanosized vehicles for drug delivery in cancer. Trends Pharmacol Sci 2009; 30:592-9.
12. Sutradhar KB, Amin ML. Nanoemulsions: Increasing possibilities in drug delivery. Eur J Nanomed 2013; 5:97-110.
13. Praetorius NP, Mandal TK. Engineered nanoparticles in cancer therapy. Recent Pat Drug Deliv Formul 2007; 1:37-51.
14. Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K, Nielsen UB, et al. Antibody targeting of long-circulating lipidic nanoparticles does not increase tumour localization but does increase internalization in animal models. Cancer Res 2006; 66:6732-40.
15. Haley B, Frenkel E. Nanoparticles for drug delivery in cancer treatment. Urol Oncol 2008; 26:57-64.
16. Iyer AK, Khaled G, Fang J, Maeda H. Exploiting the enhanced permeability and retention effect for tumor targeting. Drug Discov Today 2006; 11:812-8.
17. Bae YH. Drug targeting and tumor heterogeneity. J Control Release 2009; 133:2-3
18. Heldin CH, Rubin K, Pietras K, Ostman A. High interstitial fluid pressure - an obstacle in cancer therapy. Nat Rev Cancer 2004; 4:806-13
19. Dadwal Ankita, Baldi Ashish, Narang KumarRaj. Nanoparticles as carriers for drug delivery in cancer. Artificial Cells, Nanomedicine, and Biotechnology an International Journal, 2018,46(sup2), 295-305.
20. Jain RK, Safabakhsh N, Sckell A, Chen Y, Jiang P, Benjamin L, Yuan F, Keshet E. Endothelial cell death, angiogenesis, and microvascular function after castration in an androgen-dependent tumor: role of vascular endothelial growth factor. Proc Natl Acad Sci USA. 1998; 95:10820–1082
21. Harrington KJ, Mohammadtaghi S, Uster PS, Glass D, Peters AM, Vile RG, Stewart JS. Effective targeting of solid tumors in patients with locally advanced cancers by radiolabeled pegylated liposomes. Clin Cancer Res. 2001; 7:243–254.
22. Guyton AC, Hall JE. Textbook of Medical Physiology. Elsevier; Philadelphia, PA: 1996. p.192
23. Dexter DL, Calabresi P. Intraneoplastic diversity. Biochim Biophys Acta. 1982; 695:97–112
24. Welch DR, Tomasovic SP. The implication of tumor progression on clinical oncology. Clin Exp Matast. 1985; 3:151–188
25. Charafe-Jauffret E, Ginestier C, Iovino F, Wicinski J, Cervera N, Finetti P, Hur M-H, Diebel ME, Monville F, Dutcher J, Brown M, Viens P, Xerri L, Bertucci F, Stassi G, Dontu G, Birnbaum D, Wicha MS. Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. Cancer Res. 2009; 69:1302–1313
26. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144:646
27. Courtice FC. The origin of lipoprotein in lymph. In: Mayersen HS, editor. Lymph and the Lymphatic System. C. C Thomas Publisher; Springfield, IL: 1963. pp. 89–126

28. Guan J, He H, Yu B, Lee LJ. Polymeric nanoparticles and nanopore membranes for controlled drug and gene delivery. In: Gonsalves K, Halberstadt C, Laurencin CT, Nair L, editors. Biomedical Nanostructures. Wiley-Interscience; Hoboken, NJ: 2007. pp. 115–137
29. Hydrophobic molecules from polymer micelles into cell membranes revealed by Förster resonance energy transfer imaging. Proc Natl Acad Sci USA. 2008; 18:6596–6601
30. Leamon CP, Cooper SR, Hardee GE. Folate-liposome-mediated antisense oligodeoxynucleotide targeting to cancer cells: evaluation in vitro and in vivo. Bioconjugate Chem. 2003; 14:738–747
31. M. Gupta and V. Sharma, "Targeted drug delivery system: A review," Research Journal of Chemical Sciences, vol. 1, 2011 Torchilin VP (2000) Drug targeting. Eur J Pharm Sci 11: S81-S91.
32. Orive G, Hernández RM, Rodríguez Gascón A, Domínguez-Gil A, Pedraz JL Drug delivery in biotechnology: present and future, 2003; vol. 14, 659-664
33. K. Rani and S. Paliwal, "A review on targeted drug delivery: Its entire focus on advanced therapeutics and diagnostics," Scholars Journals of Applied Medical Sciences, 2014
34. J. Agnihotri, S. Saraf, and A. Khale, "Targeting: new potential carriers for targeted drug delivery system," International Journal of Pharmaceutical Sciences Review and Research, vol. 8, 2011