Review Article

TARGETED CANCER THERAPY: CAN IT BE THE KEY TO ACHIEVE COMPLETE CURE OF CANCER WITHOUT SIDE EFFECTS? A REVEIW

Sairam Maduri*
Anwar-ul-uloom college of pharmacy, Jawaharlal Nehru technological university, Hyderabad, Andhra Pradesh, India
***Corresponding author's E-mail:** sairamonline2007@gmail.com

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ABSTRACT

In this article we will see recent developments in targeted cancer therapy which includes information about various targets which are identified on cancer cells and are found to be suitable for targeted cancer therapy. These targets include various molecules like receptors, antigens present on cancer cells and tumor angiogenesis. We will also explore various substances and methods developed to target those targets identified on cancer cells, which includes the use of monoclonal antibodies, aptamers, polymer drug conjugates. Along with all these we will also go through the latest trends in targeted cancer therapy and what can be the future developments in the targeted cancer therapy.

Keywords: Chemotherapy, receptors, cancer antigens, angiogenesis, monoclonal antibody, aptamers, liposomes, nanoparticles, polymer drug conjugates.

1. INTRODUCTION

In today's world cancer is considered to be one of the deadliest and most painful diseases. This is mainly because cancer can be cured only if it is identified at a right time i.e.. at a right stage and even after identifying it in right time the treatment which is available at present is very painful and takes a long time to cure cancer. The currently available cancer treatment involves chemotherapy which has lot of side effects and sometimes this chemotherapy is coupled with radiation therapy. The treatment is given in various stages which include initial chemotherapy to shrink the tumors present, to a reasonable size which can then be removed by surgery, followed by more chemotherapy and radiation to avoid reoccurrence. The purpose of the chemotherapy and radiation is to kill the tumor cells. But the chemotherapeutic agents cannot differentiate between normal cells and cancer cells, but cancer cells are more susceptible to the actions of these drugs and methods because of their growth at a much faster rate than healthy cells, this is true at least in case of adults. But here the point to be noted is that the chemotherapeutic agents act both on normal cells and cancerous cells. Because the cancerous cells are rapidly dividing the effect of chemotherapeutic agents is more on cancer cells. But this does not mean that they do not show their effect on normal cells and because of their action on normal cells they cause a lot of side effects. It is due to these side effects that the cancer treatment has become very painful and sometimes these side effects are so severe that further treatment has to be stopped[1]. Now the side effects can be reduced only by giving the action specificity [action only on cancer cells without acting on normal cells] to drugs used in cancer treatment. This can be achieved by targeting the drugs directly on to the cancer cells using various targeting techniques which take the drug directly to the cancer cells and cause the accumulation of drugs in the cancer cells. This phenomenon is called as targeted delivery of drugs. But to achieve this, various targets present on the cancer cells, tumours must be identified first which then can be used as targets for targeted delivery of drugs. The treatment can be more effective if cancer is detected at a early stage which can be achieved by developing new imaging techniques which detect the cancer more efficiently.

2. VARIOUS MOLECULES ON CANCER CELLS THAT ARE FOUND TO BE SUITABLE FOR TARGETED CANCER THERAPY

2.1 Receptors on cancer cells: Generally receptors are present on the cell surface. Various substances(sometimes called as growth factors) which have affinity for the receptors attach to receptors on the surface of normal cells and cancer cells, when these substances attach to receptors they signal the cells to grow by various signaling pathways[2-4]. Certain cancer cells make extra copies of these receptors which makes them grow faster than the normal cells. So if we can block these receptors we can reduce the growth rate of cancer cells.

The drugs which are used in this regard usually block the receptors and prevent the binding of substances to them. By blocking the receptors we can prevent the signaling by the receptor thus can prevent cell division. Table 1 showing various receptors identified so far on cancer cells, type of cancer in which they are over expressed and various drugs used to block these receptors.

2.2 Cancer antigens: Cancer antigens (table 2) are molecules produced by cancer cells that are recognized by components of the body's immune system, and which can act as targets for antibody therapies or cancer vaccines.

Table 1: Type of receptors

Type of receptor	Type of cancer in which the receptor is over expressed	Drugs used to block receptor	References
Folate receptor	kidney, brain, lung, and breast carcinomas	folate-mitomycin C conjugates,	5, 6
Nicotine receptors	Lung carcinoma	d-tubocurarine/α-Cobratoxin (α-CbT),	7
Cannabinoid receptors	Prostate cancer	Cannabinoids	8
Estrogen receptors	non-small lung cancer (NSCLC)	Anti estogenic drugs like gefitinib and fulvestrant	9
vascular endothelial growth factor receptor (VEGFR)	Colo-rectal cancer	anti-VEGF monoclonal antibody bevacizumab	10
Epidermal growth factor receptor (EGFR).	Most epithelial cancers	anti-EGFR monoclonal antibody cetuximab	10
asialoglycoprotein receptor (ASGP-R, galactose receptor).	Liver cancer	Lactobionic acid, biotin and diamine-terminated poly ethylene glycol	11

Table 2: Antigens expressed in different types of cancers

Type of cancer	Antigens expressed	References
Malignant melanoma	Gangliosides e.g. GD3, GD2 and GM2, Tyrosinase and Melan-A/MART-1	12]
Melanoma, breast, ovary, prostate, bladder, sarcoma, and lung	NY-ESO-1 antigen	13]
Testicular cancer	CRT2 antigen, Melanoma antigen eg-*MAGE1, MAGE2* and *MAGE3*	14
Colon cancer	Thomsen-Friedenreich antigen	15
Paraneoplastic cerebellar degeneration	Purkinje-cell antigens	16
Prostate cancer	six-transmembrane epithelial antigen of prostate (STEAP)	17-20
Colorectal cancer	HLA-ABC antigens, HLA-DR antigens	21

Table 3. Various antiangiogenic drugs[23,24]

Angiostatin	ZD6126	Solimastat
BMS2275291	ZD6474	Suramin
CC4047	Angiozyme	Squalamine
CC5013	Apildine	SU5418
CC7085	Apra	Suradista
CDC801	Avinine	TM
CGP-41251 (PKC412)	BMS275291	Thalidomide
CM101	CA1	TPA + Captopril
Col-3 (Metastat)	CEP-701	Vitaxin
Combretastatin A-4	EMD 121974	RhuMAb anti-VEGF
CP-564,959	Flavopiridol	Avicine
Endostatin	GBC590	Carboxyamidotriazole (CAI)
Flavopiridol	GTE	IM862 (glufanide disodium)
Genistein	IM8862	Interferon alfa
Green tea extract	ImmTher	LDI-200
INGN 241	INFα	Neovastat AE-941
IMC 1C11	IL-12	Octreotide (somatostatin)
NM-3	LDI-200	SU5416
PTK787A (ZK2254)	Metaret	Tetrathiomalyboate (TM)
RO317453	Neovastat	Thalomid (thalidomide)
SMART anti-VEGF	Octeotride	Viraldon
Solimastat	$2ME_2$	
SU6668	Penicillamine	
PI-88	PI-88	
UCN-01	rhuMAb anti-VEGF	

2.3 Tumor angiogenesis: Angiogenesis is the process of formation of tumor vasculature it is vital to the continued development of the tumor mass[22]. By targeting angiogenesis we can stop the growth of tumours, because without the formation of new blood vessels development of tumor mass cannot continue. We can stop the angiogenesis in the tumor by chronically treating the tumour with antiangiogenic drugs to induce a state of prolonged dormancy. Many drugs with antiangoigenic property (table 3) have been discovered/developed and are currently in clinical trials. Most of these anti angiogenic drugs mainly block the receptors which promote tumor angiogenesis.

These targets on cancer cells can be targeted by using various targeting techniques as follows

3. BY USING MONOCLONAL ANTIBODIES

Monoclonal antibodies are monospecific antibodies. All the monoclonal antibodies are same because they are made by one type of immune cell which is a clone of a unique parent cell. Given almost any antigen, it is possible to create monoclonal antibodies that specifically binds to that antigen; because of which they can serve to detect that antigen. Monoclonal antibodies are used in many different ways to combat cancer as follows.

3.1 To mark the cancer cells and make them more visible to the immune system: Immune system detects foreign invaders in our body, but it doesn't always recognize cancer cells as enemies because most of the times cancer cells produce antigens that are very similar to the antigens present on normal body cells. To overcome this problem a monoclonal antibody can be directed to attach to the antigens present on cancer cells and mark the cancer cells and make it easier for the immune system to detect it.

3.2 To block receptors present on cancer cells: Monoclonal antibodies can block the receptors present on the cancer cells and prevent the binding of growth factors to receptors thus preventing signaling by the receptor and thus prevent the growth of cancer cells. Many drugs have been prepared in this regard by using monoclonal antibodies (Table-4).

Table 4: Currently available targeted therapies using antibodies

Generic name of the antibiotic drug	Target	Trade name	Type of cancer in which it is used	References
Rituximab	CD20 protein	RituxanR	Relapsed/refractory CD-20 positive B-call non-Hodgkin's lymphoma and low-grade or follicular-type lymphoma.	25-27
Trastuzumab	Her-2/neu receptor	HerceptinR	Blocks HER2 receptor for HER-2 positive metastatic breast cancer.	25-27
Gemtuzumab ozogamicin	CD33 protein	MylotargR	For relapsed/refractory acute myelogenous leukemia.	25-27
Alemtuzumab	CD52 protein	CampathR	B-call chronic lymphocytic leukemia.	25-27
Ibritumomab tiuxetan	CD20 protein	ZevalinR	For Rituximab-failed non-Hodgkins lymphoma	25-27
Gefitinib	Epidermal growth factor receptor(EGFR)	Iressa	Blocks epidermal growth factor receptors and tyrosine kinase activity for advanced non-small cell lung cancer.	25-27

3.3 To deliver a powerful drug directly into the cancer cells:

This can be achieved in two ways

3.3.1 By conjugating the drug directly to the monoclonal antibody: The drug can be directly conjugated to monoclonal antibody which carries the drug directly to the cancer cells. The drugs remain inactive until they're inside the target cells, lowering the chance of harming other cells because the drug can be released only after the antibody binds to the cancer cell.

3.3.2 By conjugating the monoclonal antibody to a carrier which encloses the drug: The drug carriers carry the drug like how a envelope carries a letter and they only modify various factors like drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance but they cannot take drug directly to the cancer cells, this is achieved by conjugating various targeting molecules like monoclonal antibodies to carriers this process of conjugating monoclonal antibodies to carriers is called as marking. These monoclonal antibodies act like a address on the envelope and take the drug directly to the site of action. Following are the various carriers, which are successfully marked with monoclonal antibodies.

3.3.2.1 Monoclonal antibody marked liposomes: A liposome is a tiny vesicle, made out of phospholipids which are similar to phospholipids present in the cell membrane. Liposomes enclose an aqueous core which can be filled with drugs, and used to deliver drugs for cancer and other diseases. phospholipids have a head group and a tail group. The head is attracted to water (hydrophilic), and the tail, which is made of a long hydrocarbon chain, is repelled by water (hydrophobic).

When phospholipids are disrupted by various methods like sonication, they can reassemble themselves into tiny spheres either as bilayers or monolayers. The bilayer structures are called liposomes in which the arrangement of phospholipids is similar to that of lipid bilayer found in cell membrane that is in the presence of water, the heads are attracted to water and line up to form a surface facing the water. The tails are repelled by water, and line up to form a surface away from the water. In a cell, one layer of heads faces outside of the cell, attracted to the water in the environment. Another layer of heads faces inside the cell, attracted by the water inside the cell. The hydrocarbon tails of one layer face the hydrocarbon tails of the other layer. Due this property liposomes can be easily administered into body fluids which are mostly aqueous in nature. But due to close resemblance of liposomes with that of cell membranes, the immune system can detect liposomes as a foreign substance and destroy it. To overcome this problem, liposomes must be coated with a substance which is inert, which doesn't resemble any biological substance thus is not antigenic in nature, thus will not trigger a immunogenic response. Poly ethylene glycol (PEG) is found to be one such substance and it has been successfully coated as a thin layer on the bilayer surface of liposomes and is found to protect liposomes from destruction by the immune system. This process of protecting liposomes from immune system by coating them with PEG is known as PEGlycolation of liposomes.

Because of all these properties liposomes are useful drug delivery vehicles since they may protect encapsulated drugs from enzymatic degradation and rapid clearance in vivo, or alter biodistribution, potentially leading to reduced toxicities. A major limitation to the development of many specialized applications is the problem of directing liposomes to tissues where they would not normally accumulate. Consequently, a great deal of effort has been made over the years to develop liposomes that have targeting vectors like monoclonal antibodies attached to the bilayer surface. In principle it should be possible to deliver liposomes to any cell type as long as the cells are accessible to the carrier. In practice it is usually not this simple since access to tissue, competition, and rapid clearance are formidable obstacles. Because of these obstacles even though many anti cancer agents have been successfully encapsulated in liposomes and marked with monoclonal antibodies but are very far away from making it to the clinic (table 5).

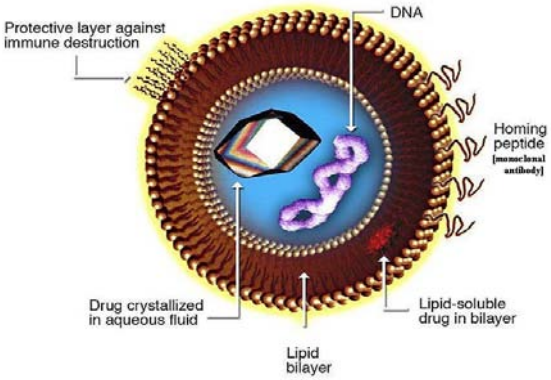

Figure 1: Liposome for targeted drug delivery

Table 5: Various anticancer drugs encapsulated in a liposome and marked with monoclonal antibody

Nature of liposome	Monoclonal antibody used to mark the liposome	Drug encapsulated inside the liposome	Type of cancer in which it is used	References
PEGylated liposomes (PLs).	Monoclonal antibody developed against HER-2	Paclitaxel	Breast cancer	28
PEGylated liposomes (PLs).	Anti disiaioganglioside GD2 antibody	Metaiodobenzylguanidine (MIBG)	neuroblastoma	29
PEGylated liposomes (PLs).	Monoclonal Antibody called anti-CD19	Doxorubicin (DXR)	human $CD19^+$ B lymphomas	30
PEGylated unilamellar liposomes	Antibody rhuMAbHER2	Cisplatin	HER2 (c-erbB-2, neu)-overexpressing cancers like testicular cancers	31
PEGylated liposomes (PLs).	OV-17L3 antibody	Doxorubicin	Ovarian cancer	32
Liposomes made up of dipalmitoyl phosphatidylcholine (DPPC) and cholesterol (CH).	Anti-CD19 antibody	Imatinib	lymphoblastic leukemia (Ph^+ ALL)	33
PEGylated liposomes (PLs).	Anti HLA-ABC antibody	Docetaxel	Colorectal cancer	34

3.3.2.2 Monoclonal antibody marked nanoparticles: Nanoparticles are the substances whose size is in the range of nanometers. Nanoparticles used for targeted cancer therapy are called as nanovectors. These nanovectors are made up of three main constituents which include a core material which acts as the carrier, drug attached to it, a targeting molecule like monoclonal antibody. The use of nanoparticles as carriers for drugs is increasing day by day because a wide range of substances can be used as nanocarriers and their use is also increasing because of their other characteristics like their ease of manufacturing and increased stability. Due to their small size they can easily pass through blood vessels. The drug is bound to the nanoparticles either because of the affinity of nanoparticle to the drug or joined with the help of a linker molecule which has affinity for both the

drug and the nanoparticles. Nowadays carbon nanotubes are mostly used as carriers because of their stability and ease of manufacturing. Along with these FDA has also approved many substances to be used as nanoparticles. Nanoparticles are conjugated with antibody in order to get a selective drug carrier system. Now these carriers can carry the drug directly to the cancer cells (table 6).

Even though monoclonal antibodies have proven their efficacy as anti cancer agents, their use is not considered as the best approach to achieve targeted cancer therapy because they have several disadvantages like

- Monoclonal antibodies are large molecules which cause increase in vehicle size without any added advantage.
- Monoclonal antibodies can become immunogenic.
- Biological development of antibodies is sometimes unpredictable
- Batch to batch variations can occur during the production of monoclonal antibodies

4. BY USING APTAMERS

Aptamers are single-stranded RNA or DNA oligonucleotides 15 to 60 base in length that binds with high affinity to specific molecular targets. Aptamers were not in use earlier because the procedures for producing aptamers were not well established. However due to the development of the systematic evolution of ligands by exponential enrichment SELEX (in this technique the aptamers are selected by incubating the target molecule in a large pool of oligonucleotide usually, the pool size of the oligonucleotide is from 1010 to 1020. The large pool size of the oligonucleotide ensures the selection and isolation of the specific aptamer. Aptamers can distinguish between closely related but non-identical members of a protein family, or between different functional or conformational states of the same protein) process[39], however, made it possible to isolate oligonucleotide sequences (aptamers) with the capacity to recognize virtually any class of target molecules with high affinity and specificity. They also do not pose the problem of becoming immunogenic like monoclonal antibodies. These aptamers can be easily conjugated to many carriers like liposomes and nano particles. Their cost of production is also low on comparison with production cost of monoclonal antibodies. Some aptamers which can target cancer cells have been successfully developed using SELEX process but they are still in early stages of clinical development and are not used clinically (table 7).

Table 6: Monoclonal antibody marked nanoparticles developed for the treatment and imaging of different types of cancers

Composition of nanoparticle	Antibody conjugated	Type of cancer in which it is used	References
Gelatin nanoparticles	NeutrAvidin (NAv). Antibodies specific for the CD3 antigen on lymphocytic cells	leukemia	35
Gold nanoparticles	monoclonal anti-epidermal growth factor receptor (anti-EGFR) antibodies	Imaging of epithelial cancers	36
Super paramagnetic monocrystalline iron oxide nanoparticles	tumor-specific monoclonal antibodies	Used for the imaging of all types of cancers	37
Polylactic-co-glycolic acid (PLGA) immuno-nanoparticles	Monoclonal antibody specific to breast cancer cells	Breast cancer	38

Table 7: Aptamers developed by SELEX process, their targets and types of cancers in which they can be used

Aptamer	Target molecule to which aptamer is selective	Function of target molecule, type of cancer in which the target molecule is expressed	References
2'-fluropyrimidine RNA aptamers named Xpsm-A9 and Xpsm-A10	Prostate specific membrane antigen(PSMA)	Prostatic interepithelial neoplasia, prostatic adenocarcinoma.	40
RNA aptamer called clone 5	Sialyl lewis X	Metastatic cancers	40
35 Base pair,2'-fluropyrimidine modified nuclease stable RNA aptamer called D60	Cytotoxic T-cell antigen-4(CTLA-4)	Reduces immunity to tumors by binding to T-cells	40
TTA1	Tenascin-C	It stimulates tumour growth in many cancers.	40
III.1	YPEN-1 cells	Causes angiogenesis in tumors of Ewings sarcoma family	40
RNA aptamer called A30	Human epidermal growth factor-3 Receptor	HER-3 overexpressing cancers like testicular cancer	40
AS-1411	nucleolin	controls DNA and RNA synthesis, replication in cancer cells	40

5. BY USING DRUG CONJUGATES

Polymer conjugates[41] are becoming established as a new approach towards improved cancer therapy. These water-soluble, hybrid constructs fall into two main categories: polymer-protein conjugates (already available as licensed products), and polymer-drug conjugates (currently in clinical development). Polyethylene glycol conjugation of proteins is accepted as a means to reduce immunogenicity, prolong plasma half-life and enhance protein stability. Polymer-drug conjugation promotes tumor targeting by the 'enhanced permeation and retention' effect, and at the cellular level, allows lysosomotropic drug delivery. In polymer drug conjugates it is envisioned that not only could the pharmacokinetics of the drug attached to the polymeric carrier be modulated but also that active targeting could be achieved if a homing moiety is attached to the same polymeric carrier. Many polymer-drug conjugates have entered clinical development and activity has already been observed in chemotherapy refractory patients. Certain compounds have also demonstrated a marked reduction in drug toxicity. (table 8). Sometimes a drug can be conjugated to a naturally occurring substance that has high affinity for receptors on cancer cells. Eg: Folate-mitomycin C conjugates.

Table 8: Polymer-drug conjugates in clinical trials

Conjugates	Indication	Status	Company	References
HPMA-Doxorubicin(PK1; FCE28068)	Lung and breast cancers	Phase II as of 2002	Research Campaign UK Pfizer; Cancer	42, 43
HPMA-doxorubicin- galactosamine (PK2, FCE28069)	Hepatocellular carcinoma	Phase I/II	Pfizer; Cancer Research Campaign UK	44
HPMA-camptothecin (PNU166148)	Solid tumors	Phase I; discontinued	Pfizer; Cancer Research Campaign UK	45
HPMA-paclitaxel (PNU166945)	Solid tumors	Phase I; discontinued	Pfizer; Cancer Research Campaign UK	46
HPMA-platinate (AP5346, ProLindac)	Ovarian, melanoma, and colorectal cancers	Phase I	Access Pharmaceutical	47
PEG-Camptothecin(Pegamotecan)	Solid tumors	Phase I; discontinued	Enzon	48
PEG-SN38 (EZN-2208)	Solid tumors	Phase I; initiated as of October 2007	Enzon	49
Polymeric Micelles(NK911)	Pancreatic cancer	Phase II	Nippon Kayaku, Japan	50
Cyclodextrin-Based Polymer-CPT (IT-101)	Solid tumors	Phase I	Insert Therapeutics	51
Carboxymethyldextra n-Exatecan (DE-310)	Solid atumors	Phase I	Daiichi Pharmaceuticals, Japan	52
PG-TXL (CT-2103, Xyotax)	Lung, ovarian, colorectal, breast, and esophageal cancers	Phase III	Cell Therapeutics	53
PG-camptothecin(CT2106)	Colorectal, lung, and ovarian cancers	Phase I	Cell Therapeutics	54

6. LATEST TRENDS IN TARGETED CANCER THERAPY

6.1 Delivering radiation to cancer cells from inside: Ibritumomab tiuxetan is a drug in which the radioactive components like yttrium-90 or indium-111 are conjugated with monoclonal mouse IgG1 antibody and this is used in the treatment of some forms of B cell non-Hodgkin's lymphoma this drug delivers radiation to the cancer cells from inside by delivering radioactive component directly to the cancer cell[55].

6.2 Destroying cancer cells by heating cells from inside: Chimeric L6 (ChL6) monoclonal antibody linked iron oxide nanoparticle (bioprobes) when administered are taken up by the cancer cells then these bioprobes[56] can be inductively heated by externally applied alternating magnetic field (AMF)

6.3 Killing cancer cells by using RNA interference: RNA interference works through molecules called small interfering RNA (siRNA). Each specific molecule prevents the expression of a particular gene by mediating the destruction of its product by a cellular apparatus called RNA induced silencing complex (RISC). siRNA can be potentially directed against any target gene. Once a gene responsible for disease is identified, sequence specific siRNA molecules can be designed to silence that specific gene. Synthetic siRNA can be easily manufactured in therapeutic quantity and quality. These siRNA molecules when enclosed in nanospheres (sometimes called as nanobots) of 70nm can be administered safely without the fear of damage to siRNA molecules. When these nanospheres are absorbed into the cell, the nanosphere splits apart, releasing the RNA. These are still in the early stages of development and at present their efficacy

cannot be conformed because they are still in the phase I clinical trials[57].

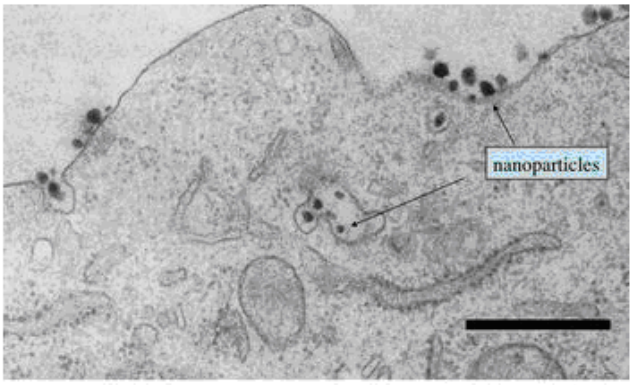

Figure 2: Nanobots showing their effect on cancer cells by RNA interference[57]

6.4 Imaging cancer by using nanoparticles: The anti-EGFR antibody conjugated gold nanoparticles specifically and homogeneously binds to the surface of the cancer type cells with 600% greater affinity than to the noncancerous cells. Which can be detected using surface plasmon resonance (SPR) scattering images and SPR absorption spectra[58]. Thus cancer cell imaging can be done more efficiently.

6.5 CONCLUSION

Research activity aimed at achieving targeted cancer therapy has expanded tremendously in recent times leading to the development of new techniques for delivering drugs directly to tumors. Some of these techniques have made it to the clinic and some are under clinical development, but there is still a long way to go before we can achieve complete treatment of cancer by targeted delivery of drugs. Finding solutions to problems like the development of immunogenicity by monoclonal antibodies is an important research area on which more emphasis should be laid. Development of targeted drug delivery systems by which complete localization of drug within the tumour can be achieved and side effects of chemotherapy can thus be completely eliminated will remain the greatest clinical goal for the future. More emphasis should be laid on development of superior detection techniques by which cancer can be detected at an early stage where treatment proves to be more effective.

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