2.1. Lung Cancer

The lungs are located in the chest. They help you breathe. When you breathe, air goes through your nose, down your windpipe (trachea), and into the lungs, where it spreads through tubes called bronchi. Most lung cancer begins in the cells that line these tubes.(1)

There are two main types of lung cancer:

I Non-small cell lung cancer (NSCLC) is the most common type of lung cancer.(2)

I Small cell lung cancer makes up about 20% of all lung cancer cases.(3)

If the lung cancer is made up of both types, it is called mixed small cell/large cell cancer. If the cancer started somewhere else in the body and spread to the lungs, it is called metastatic cancer to the lung.(4)

2.1.4. Prevention and Treatment

Eliminating tobacco smoking is a primary goal in the prevention of lung cancer, and smoking cessation is an important preventive tool in this process.

- **Surgery**: Positron emission tomography (PET)(1,2) is used to determine whether the disease is localized and amenable to surgery or whether it has spread to the point where it cannot be cured surgically. Video-assisted thoracoscopic surgery (VATS) and VATS lobectomy have allowed for minimally invasive approaches to lung cancer surgery that may have the advantages of a quicker recovery.(1,5)
- **Radiotherapy**: Radiotherapy is often given together with chemotherapy, and may be used with curative intent in patients with non-small-cell lung carcinoma who are not eligible for surgery. This form of high intensity radiotherapy is called radical radiotherapy. A refinement of this technique is continuous hyperfractionated accelerated radiotherapy (CHART), in which a high dose of radiotherapy is given in a short time period. For small-cell lung carcinoma cases that are potentially curable, chest radiation is often recommended in addition to chemotherapy.(6,7) The use of adjuvant thoracic radiotherapy following curative intent surgery for non-small-cell lung carcinoma is not well established and is controversial. Benefits, if any, may only be limited to those in whom the tumor has spread to themediastinal lymph nodes.(1,4,5,8) For both non-small-cell lung carcinoma and small-cell lung carcinoma patients, smaller doses of radiation to the chest may be used for symptom control(palliative radiotherapy. Brachy therapy

(localized radiotherapy) may be given directly inside the airway when cancer affects a short section of bronchus. It is used when inoperable lung cancer causes blockage of a large airway. Patients with limited-stage small-cell lung carcinoma are usually given prophylactic cranial irradiation (PCI).(3) This is a type of radiotherapy to the brain, used to reduce the risk of metastasis. More recently, PCI has also been shown to be beneficial in those with extensive small-cell lung cancer. In patients whose cancer has improved following a course of chemotherapy, PCI has been shown to reduce the cumulative risk of brain metastases within one year from 40.4% to 14.6%. Recent improvements in targeting and imaging have led to the development of extracranial stereotactic radiation in the treatment of early-stage lung cancer. In this form of radiation therapy, very high doses are delivered in a small number of sessions using stereotactic targeting techniques. Its use is primarily in patients who are not surgical candidates due to medical comorbidities.(5,9,10)

- **Chemotherapy**: The chemotherapy regimen depends on the tumor type.
  - **Small-cell lung carcinoma**: Even if relatively early stage, small-cell lung carcinoma is treated primarily with chemotherapy and radiation. In small-cell lung carcinoma, cisplatin and etoposide are most commonly used. Combinations with carboplatin, gemcitabine, paclitaxel, vinorelbine, topotecan, and irinotecan are also used.(11)
  - **Non-small-cell lung carcinoma**: Primary chemotherapy is also given in advanced and metastatic non-small-cell lung carcinoma. Testing for the molecular genetic subtype of nonsmall-cell lung cancer may be of assistance in selecting the most appropriate initial therapy.For example, mutation of the epidermal growth factor receptor gene may predict whether initial treatment with a specific inhibitor or with chemotherapy is more advantageous. Advanced non-small-cell lung carcinoma is often treated with cisplatin or carboplatin, in combination with gemcitabine, paclitaxel, docetaxel, etoposide, or vinorelbine. Bevacizumab improves results in non-squamous cancers treated with paclitaxel and carboplatin in patients less than 70 years old who have a reasonable general performance status. Pemetrexed has been studied extensively in non-small-cell lung cancer, with numerous studies since 1995.(7,12) For adenocarcinoma and large-cell lung cancer, cisplatin with

pemetrexed was more beneficial than cisplatin and gemcitabine; squamous cancer had the opposite results. As a consequence,sub typing of non-small lung cancer histology has become more important.(13,6,14–16) Bronchoalveolar carcinoma is a subtype of non-small-cell lung carcinoma that may respond to gefitinib and erlotinib.

- **Maintenance therapy**: In advanced non-small-cell lung cancer there are several approaches for continuing treatment after an initial response to therapy. Switch maintenance changes to different medications than the initial therapy and can use pemetrexed, erlotinib, and docetaxel, although pemetrexed is only used in non-squamous NSCLC.(6,15)
- **Adjuvant chemotherapy**: Adjuvant chemotherapy refers to the use of chemotherapy after apparently curative surgery to improve the outcome. In non-small-cell lung cancer, samples are taken during surgery of nearby lymph nodes. If these samples contain cancer, the patient has stage II or III disease. In this situation, adjuvant chemotherapy may improve survival by up to 15%. Standard practice has often been to offer platinum-based chemotherapy (including either cisplatin or carboplatin). However, the benefit of platinum-based adjuvant chemotherapy was confined to patients who had tumors with low ERCC1 (excision repair cross-complementing 1) activity.(1,5,12,17) Adjuvant chemotherapy for patients with stage IB cancer is controversial, as clinical trials have not clearly demonstrated a survival benefit. Trials of preoperative chemotherapy (neoadjuvant chemotherapy) in resectable non-small-cell lung carcinoma have been inconclusive.

2.2. Drug Profile

Docetaxel is a clinically well established anti-mitotic chemotherapy medication used mainly for the treatment of breast, ovarian, and non-small cell lung cancer.(14) Docetaxel binds to microtubules reversibly with high affinity and has a maximum stoichiometry of one mole docetaxel per mole tubulin in microtubules.

Indication

For the treatment of patients with locally advanced or metastatic breast cancer after failure of prior chemotherapy. Also used as a single agent in the treatment of patients with locally advanced or metastatic non-small cell lung cancer after failure of prior platinum-based chemotherapy.(16)

2.2.1. Mechanism of Action

Docetaxel interferes with the normal function of microtubule growth. Whereas drugs like colchicine cause the depolymerization of microtubules in vivo, docetaxel arrests their function by having the opposite effect; it hyper-stabilizes their structure. This destroys the cell's ability to use its cytoskeleton in a flexible manner. Specifically, docetaxel binds to the β-subunit of tubulin. Tubulin is the "building block" of mictotubules, and the binding of docetaxel locks these building blocks in place. The resulting microtubule/docetaxel complex does not have the ability to disassemble. This adversely affects cell function because the shortening and lengthening of microtubules (termed dynamic instability) is necessary for their function as a transportation highway for the cell. Chromosomes, for example, rely upon this property of microtubules during mitosis. Further research has indicated that docetaxel induces programmed cell death (apoptosis) in cancer cells by binding to an apoptosis stopping protein called Bcl-2 (B-cell leukemia 2) and thus arresting its function.(18)

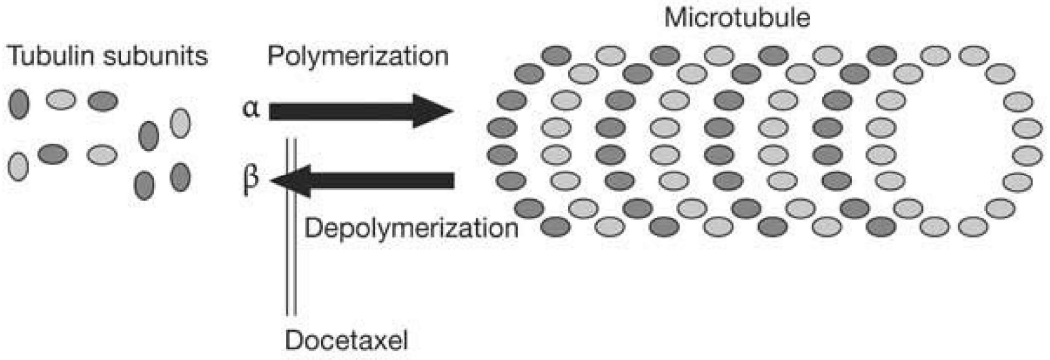


Figure 2.1 Mechanism of Action of Docetaxel

2.2.2. Absorption, Fate, and Elimination(19)

The pharmacokinetics of docetaxel have been evaluated in cancer patients after administration of 5 to 115 mg/m^2 in phase I studies. The kinetic profile of docetaxel is dose independent and consistent with a three compartment pharmacokinetic model with half-lives for the alpha, beta and gamma phases of 4 minutes, 36 minutes and 11.1 hours, respectively. The initial rapid decline represents distribution to the peripheral compartments and the late phase is due, in part, to a relatively slow efflux of docetaxel from the peripheral compartment. Following the administration of a 100 mg/m^2 dose given as a one hour infusion, a mean peak plasma level of 3.7 microgram/mL was obtained with a corresponding area under the curve (AUC) of 4.6 hours.microgram/mL. Mean values for total body clearance and steady-state volume of distribution were 21 L/hour/m^2 and 113 L, respectively.(20)

A study of 14C-docetaxel has been conducted in three cancer patients. Docetaxel was eliminated in both the urine and feces following oxidative metabolism of the tert-butyl ester group; within seven days, the urinary and fecal excretion account for about 6% and 75% of the administered radioactivity, respectively. About 80% of the radioactivity (60% of the administered dose) recovered in feces is excreted during the first 48 hours as one major and three minor inactive metabolites and very low amounts of unchanged drug.

A population pharmacokinetic analysis has been performed with docetaxel in 577 patients. Pharmacokinetic parameters estimated by the model were very close to those estimated from phase I studies. The pharmacokinetics of docetaxel were not altered by the age or sex of the patient. In a small number of patients (n = 23) with clinical chemistry data suggestive of mild to moderate liver function impairment (ALT, AST greater than or equal to 1.5 times the upper limit of normal, associated with alkaline phosphatase greater than or equal to 2.5 times the upper limit of normal), total clearance was lowered by, on average, 27% (see Dosage and Administration). Docetaxel clearance was not modified in patients with mild to moderate fluid retention. No data are available in patients with severe fluid retention.

Docetaxel is more than 95% bound to plasma proteins. Dexamethasone did not affect protein binding of docetaxel. The effect of prednisone on the pharmacokinetics of docetaxel administered with standard dexamethasone premedication has been studied in 42 patients. No effect of prednisone on the pharmacokinetics of docetaxel was observed.

Phase I studies evaluating the effect of capecitabine on the pharmacokinetics of docetaxel and the effect of docetaxel on the pharmacokinetics of capecitabine showed no effect of capecitabine on the pharmacokinetics of docetaxel (Cmax and AUC) and no effect of docetaxel on the pharmacokinetics of the main capecitabine metabolite 5'DFUR.

The combined administration of docetaxel, cisplatin and fluorouracil in 12 patients with solid tumors had no influence on the pharmacokinetics of each individual medicine.

2.2.3. Therapeutic Uses

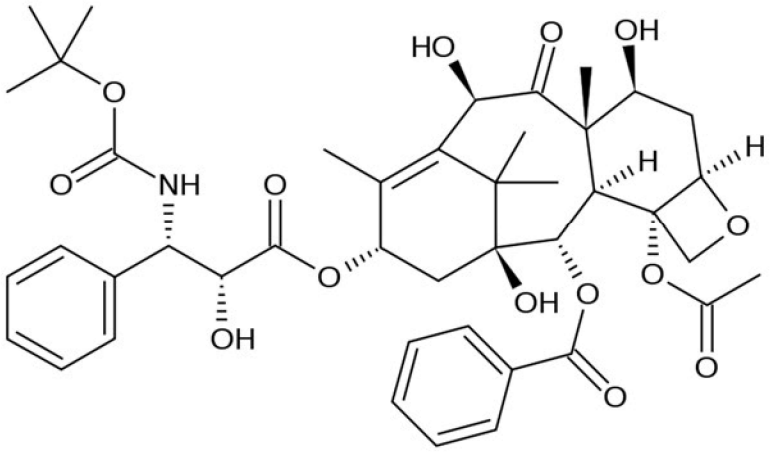
For treatment after failure of prior platinum-based chemotherapy, TAXOTERE was evaluated as monotherapy, and the recommended dose is 75 mg/m^2 administered intravenously over 1 hour every 3 weeks. A dose of 100 mg/m^2 in patients previously treated with chemotherapy was associated with increased hematologic toxicity, infection, and treatment-related mortality in randomized, controlled trials.(16,19,21)

2.2.4. Clinical Toxicities

Oral LD50 in rat is >2000 mg/kg. Anticipated complications of overdosage include: bone marrow suppression, peripheral neurotoxicity, and mucositis. In two reports of overdose, one patient received 150 mg/m^2 and the other received 200 mg/m2 as 1-hour infusions. Both patients experienced severe neutropenia, mild asthenia, cutaneous reactions, and mild paresthesia, and recovered without incident.(19,20)

Table 2.1 Drug Profile(22,23)

Name	Docetaxel
Chemical mane(IUPAC)	1,7β,10β-trihydroxy-9-oxo-5β,20-epoxytax-11-ene-2α,4,13α-triyl 4-acetate 2-benzoate 13-{(2R,3S)-3-[(tert-butoxycarbonyl)amino]-2-hydroxy-3-phenylpropanoate}
Proprietary name	Taxotere
Molecular formula	C43H53NO14
Molecular weight	807.8792
Physicochemical properties	
Physical state and Appearance	White to off white color powder
Melting point	232 °C
Log P	2.4
pka value	pKa (strongest acidic): 10.96

	pKa (strongest basic): -3
Solubility	Soluble in DMSO at 200 mg/mL; soluble in ethanol at 100 mg/mL; very poorly soluble in water
Half life	Dose-dependent. Doses of 70 mg per square meter of body surface area (mg/m^2) or higher produce a triphasic elimination profile. With lower doses, assay limitations precluded detection of the terminal elimination phase. The half-life of the alpha, beta, and gamma phase are 4 minutes, 36 minutes, and 11.1 hours, respectively.
Dose	20mg/mL
Structure	

POLY (D, L-LACTIDE-CO-GLYCOLIDE) PLGA

PLGA or **poly(lactic-*co*-glycolic acid)** is a copolymer which is used in a host of Food and Drug Administration (FDA) approved therapeutic devices, owing to its biodegradability and biocompatibility. PLGA is synthesized by means of random ring opening co-polymerization of two different monomers, the cyclic dimers (1,4-dioxane- 2,5-diones) of glycolic acid and lactic acid. Common catalysts used in the preparation of this polymer include tin (II) 2-ethylhexanoate, tin (II) alkoxides, or aluminum isopropoxide.(24) During polymerization, successive monomeric units (of glycolic or lactic acid) are linked together in PLGA by ester linkages, thus yielding linear, aliphatic polyester as a product.(25,26) Fig. 2.8 depicts the structure of PLGA and its monomers.

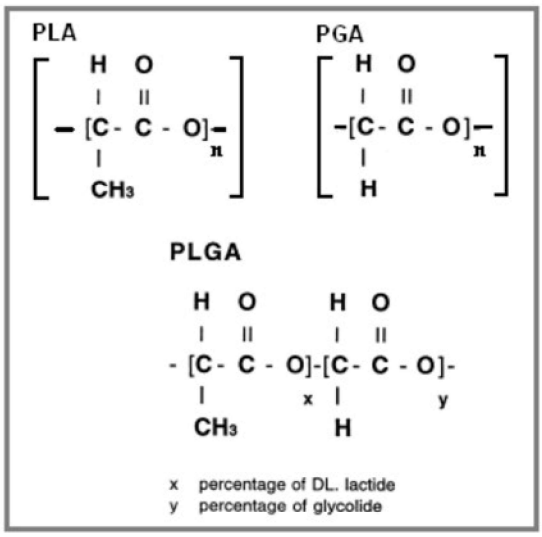


Figure 2.2 Structure of Poly glycolic acid (PGA), Poly lactic acid (PLA) and Poly (lactic-co-glycolic) acid (PLGA)

The understanding of physical, chemical and biological properties of the polymer is helpful before formulating a controlled drug delivery device. Lactic acid is more hydrophobic than glycolic acid and hence lactide-rich PLGA copolymers are less hydrophilic, absorb less water and subsequently degrade more slowly.(24,27–29) The commercially available PLGA polymers are usually characterized in terms of intrinsic viscosity, which is directly related to the molecular weight. The mechanical strength, swelling behavior, capacity to undergo hydrolysis, and subsequently the biodegradation rate are directly influenced by the crystallinity of the PLGA polymer. The crystallinity of the PLGA copolymer is directly dependent on the type and the

molar ratio of the individual monomer components (lactide and glycolide) in the copolymer chain. PLGA polymers containing 50:50 ratios of lactic and glycolic acids are hydrolyzed much faster than those containing a higher proportion of either of the two monomers. The degree of crystallinity and the melting point of the polymers are directly related to the molecular weight of the polymer.The Tg (glass transition temperature) of the PLGA copolymers are above the physiological temperature of 37º C and hence they are glassy in nature. Thus, they have a fairly rigid chain structure which gives them significant mechanical strength to be formulated as drug delivery devices.(30)

Degradation and metabolic pathway of PLGA

The degradation rate of PLGA in water is a function of the molecular weight and the lactide: glycolide ratio. Higher the glycolide content and lower molecular weight increase the degradation rate. For e.g. the degradation time of PLGA 50:50 is 1-2 months while that of PLGA 70:30 and PLGA 85:15 are higher (approx.12-24 months). PLGA show a glass transition temperature in the range of 40-60 °C. The inherent viscosity of PLGA is dependent on their molecular weight as shown in Table 2.2. For e.g. for PLGA 50:50 the molecular weight increases with the increase in its inherent viscosity (Purac biomaterials).(30,31)

Table 2.2: Inherent viscosity and molecular weight for PLGA 50:50 (Purac Biomaterials)

IV [dl/g]	**Mw[g/mol]**
0.2	17,000
0.3	30,000
0.4	44,000
0.5	59,000
0.6	76,000
0.7	94,000
0.8	113,000
0.9	133,000
1.0	153,000
1.1	174,000
1.2	196,000

Unlike the homopolymers of lactic acid (polylactide) and glycolic acid (polyglycolide) which show poor solubilities, PLGA can be dissolved by a wide range of common solvents, including chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate. Degradation of PLA or PLGA occurs by autocatalytic cleavage of the ester bonds through spontaneous hydrolysis into oligomers and d, l-lactic and glycolic acid monomers. Lactate converted into pyruvate and glycolate enter the Krebs' cycle to be degraded into CO_2 and H_2O. The polymer erosion in delivery devices is the degradation of polymers to water-soluble fragments, accompanied by a progressive weight loss of the matrix. Generally, the polymer erosion could be classified into two mechanisms, namely surface or bulk erosion.(32,33)

In the case of surface erosion, the degradation is faster than the water diffusion. Thus the degradation and erosion take place on the surface of the matrix; in contrast, with bulk erosion, the water penetration is faster and the degradation and erosion affect all the polymer bulk (Figure 2.3). PLGA are bulk erosion polymers. The weight loss of the polymer devices doesn't take place at the beginning of the degradation of the PLGA. Accompanying with the produced water soluble oligomers, significant weight loss occurs when the molecular weight of the PLGA reaches certain threshold.(34)

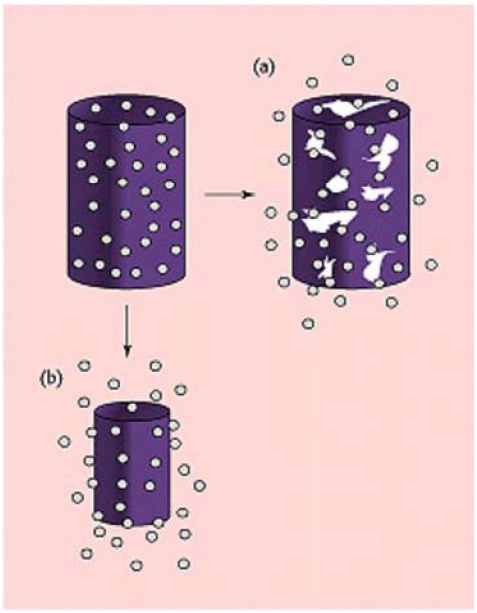


Figure 2.3 Schematic illustration of the changes of polymer matrix during (a) surface erosion and (b) bulk erosion.

Bulk erosion is the main degradation pathway for PLGA copolymer. This occurs by random scission of ester bonds in the polymer backbone proceeding homogenously throughout the device. A three-phase mechanism for PLGA biodegradation has been proposed. Initially, a significant decrease in molecular weight of polymer is observed, with no appreciable weight loss and no soluble monomer products formed after random chain scission. This phase is followed by a decrease in molecular weight with rapid loss of mass and formation of soluble mono and oligomeric products. Finally, soluble monomer products are formed from soluble oligomeric fragments, resulting in complete polymer degradation.(26) It has been reported that the drug(s) having amino functional groups such as amines, basic drugs, protein, and peptides have the potential to interact with polymer pendant groups, accelerating the polymer degradation rates and the release of the drugs incorporated in the polyester matrix.(35)

Degradation rate depends on four basic parameters: hydrolysis rate constant (depending on the molecular weight, the lactic/glycolic ratio, and the morphology), amount of water absorbed, diffusion coefficient of the polymer fragments through the polymer matrix, and solubility of the degradation products in the surrounding aqueous medium.(17) All of these parameters are influenced by temperature, additives (including drug molecules), pH, ionic strength, buffering capacity, size and processing history, steric hindrance etc. Polymer properties such as molecular weight, crystallinity and glass transition temperature also control the degradation rate of polymers.

The biodegradation rate of the PLGA copolymers are dependent on the molar ratio of the lactic and glycolic acids in the polymer chain, molecular weight of the polymer, the degree of crystallinity and Tg of the polymer. The role of enzymes in any PLGA biodegradation has not been well established. Most of the literature indicates that the biodegradation of PLGA does not involve any enzymatic activity and is purely through hydrolysis.(34,36)

Literature data indicate that *in vivo* degradation times for copolymers of lactides and glycolides vary from a few weeks to more than 1 year. The most widely used PLGA copolymer composition of 50:50 has the fastest degradation rate of the d,1- lactide/glycolide materials, with that polymer degrading in about 50-60 days. The 65:35, 77:25, and 88:15 d,l-lactide/glycolides have progressively longer *in vivo* lifetimes, with the 88:15 lasting about 150 days *in vivo*. Poly (d,l-lactide) requires about 12-16 months to biodegrade completely, and poly (1-lactide), being more crystalline and less hydrophilic, can be found *in vivo* even after 1.5-2 years. Poly (D, L-

lactide-co-glycolide), is a polymer of choice for developing an array of micro and nanoparticulate drug delivery systems as it has excellent biocompatibility and predictable biodegradability. (24)

2. 3. Nanoparticles

The term ‘nanoparticle’ may be defined as a submicron drug carrier system which is generally (but not inevitably) composed of polymer. The polymer used may or may not be biodegradable even if the polymer biodegradability appears a main characteristic for drug delivery carriers. As a function of the morphological and structural organization of the polymer, we distinguish the ‘nanosphere’ which is a nanoparticle system with a matrix character and constituted by a solid core with a dense polymeric network, and the ‘nanocapsule’ which is formed by a thin polymeric envelope surrounding an oil or water filled cavity. Nanocapsules may, thus, be considered as a ‘reservoir’ system. Practically, the nanoparticles have a size around 200 nm and the drugs or other molecules may be dissolved into the nanoparticles, entrapped, encapsulated and/or adsorbed or attached. Figure 2.4 depicts various nanocarrier based drug delivery carrier systems used for effective drug delivery research.(37–39)

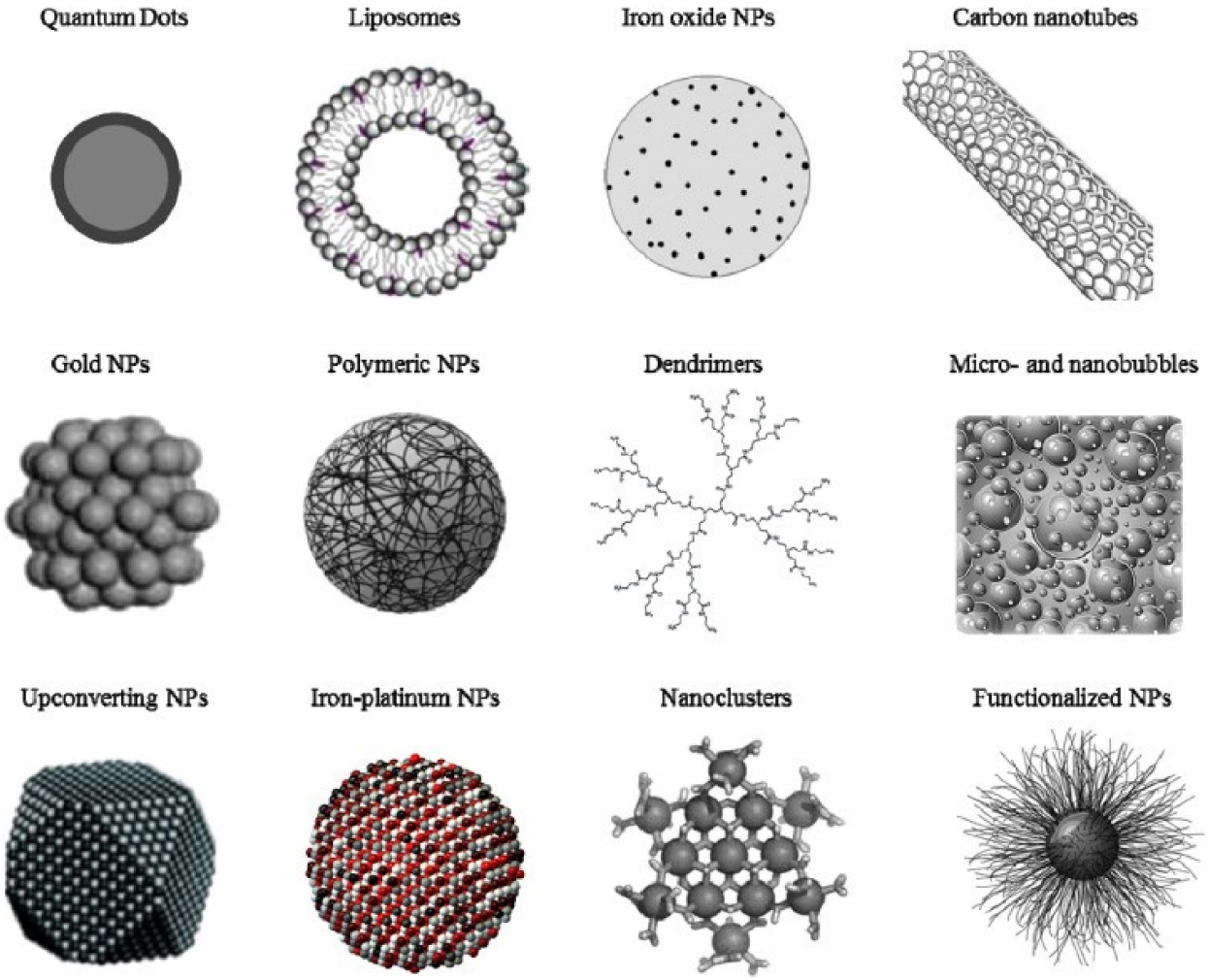


Figure 2.4. Various nanomaterial based drug delivery platforms

2.3.1 PLGA Nanoparticles(24)

Recently, nano-sized drug delivery systems, especially biocompatible and biodegradable polymer nanoparticles have attracted considerable interest since they can offer a suitable means of delivering small molecular weight drugs, proteins or genes to a targeted tissue or organ. Nanoparticles are colloidal systems that have size typically in the range of 10-1000nm in diameter, and drugs can be entrapped in, absorbed or chemically coupled onto the polymer nanoparticle matrix. On the other hand, a number of polymers have been investigated for formulation biodegradable nanoparticles, such as polylactide (PLA), poly(3-caprolactone) (PCL) and poly(lactide-co-glycolide) (PLGA). They are biocompatible and biodegradable polymers approved by the FDA and have been studied extensively. (Figure 2.5)

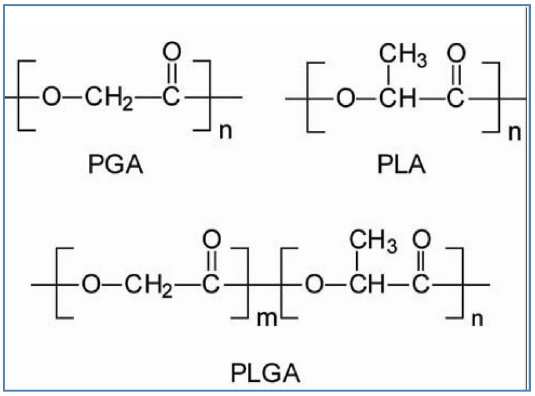


Figure 2.5 Chemical structure of poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA)

PLGA is one of the most successfully used biodegradable material for the development of nanomedicines because it undergoes hydrolysis in the body to produce the biodegradable metabolites monomers, lactic acid and glycolic acid. Since the body effectively deals with these two monomers, there is very minimal systemic toxicity associated by using PLGA for drug delivery or biomaterial applications. PLGA nanoparticles have been mostly prepared by emulsification-diffusion, solvent emulsion evaporation, interfacial deposition and nanoprecipitation method. Briefly, in emulsification-diffusion method, the PLGA polymers are dissolved in organic solvent, poured and separated in aqueous phase having stabilizer and subsequently emulsified by homogenizer.(32) In solvent evaporation method the polymers are dissolved in volatile organic solvent and poured into continuously stirring aqueous phase with or without emulsifier/stabilizer and sonicated. Interfacial deposition methods have been used for the formation of both nanocapsule and nanospheres. The

nanoparticles are synthesized in the interfacial layer of water and organic solvent and finally the nanoparticles are separated by centrifugations. Most commonly used method for the preparation of PLGA nanoparticles is nanoprecipitation. Polymer dissolved in acetone is added drop-wise into continuously stirring aqueous phase with or without emulsifier/stabilizer and consequently organic phase is evaporated under reduced pressure.(32,40,41)

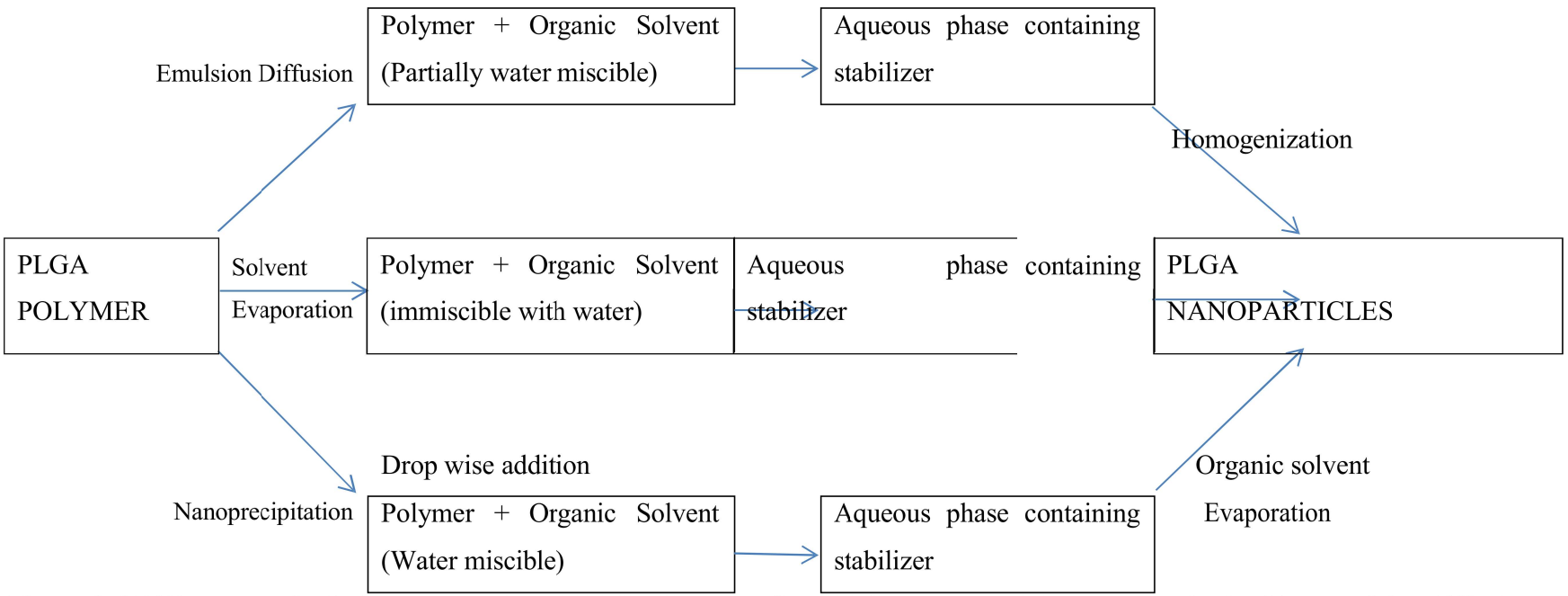


Figure 2.6 Different methods for preparation of PLGA Nanopartiles: PLGA nanoparticles were synthesized by emulsion diffusion , solvent evaporation and nanoprecipitation methods.

PLGA nanoparticles have been used to develop the proteins and peptides nanomedicine, nano-vaccines, nanoparticles based gene delivery system, nano-antigen and growth factor, etc. Surface modification of PLGA, drug encapsulation methods and particle size, additives added during formulation, molecular weight of drug, ratio of lactide to glycolide moieties has strong influence on the release and effective response of formulated nanomedicines.(31,36,42) The acidic nature of PLGA monomers is not suitable for some sensitive drugs or bioactive molecules.85 However, the approaches to overcome these problems have been developed. PLGA nanomedicine formulations are blended with alginate, chitosan, pectin, poly(propylenefumarate), polyvinylacohol, poly(orthoester) etc. The approval of PLGA has been granted by the US Food and Drug Administration (USFDA) for human use and nanomedicines.(27,28,36,43)

PLGA is approved by FDA for therapeutic use in humans. Various preparation methods have been optimized for PLGA nanoparticles synthesis and numerous cancer related drugs have been incorporated in PLGA. These loaded nanoparticles protect poorly soluble and unstable payloads from the biological milieu and are small enough for capillary penetrations, cellular internalization and endosomal escape. Furthermore, their surface is modified for targeted delivery of molecules to tumor or other tissues. The larger size of PLGA nanoparticles is advantageous as multifunctional imaging and probes which incorporate encapsulated cancer drug, release, imaging, and targeting in a single nanoparticles platform.(44–46)

The performance of these nanoparticles is not completely satisfactory and great effort is needed to improve its physiochemical properties and synthesis process. The properties of nanoparticles as precursor of good nanomedicine are particle size, size distribution, surface morphology, surface chemistry, surface charge, surface adhesion, surface erosion, interior porosity, drug diffusivity, drug encapsulation efficiency, drug stability, drug release kinetics and hemodynamic. The surface charge of the nanoparticles is important for the cellular internalization of the NPs, clustering in blood flow, adherence, and interaction with oppositely charged cells membrane.93

PLGA nanoparticles are frequently used for the encapsulation of various drugs and their successful delivery in vivo. PLGA nanoparticles loaded with hydrophobic poorly soluble drugs aremost commonly formulated by nanoprecipitation. Drug release and effective response of PLGA nanoparticles are influenced by (i) the surface modification, (ii) the method of preparation, (iii) the particle size, (iv) the molecular weight of the encapsulated drug and (v) the ratio of lactide to glycolide moieties. The cancer related drug paclitaxel, doxorubicin, 5-

fluorouracil, 9-nitrocamptothecin, cisplatin, triptorelin, dexamethasone, xanthone, etc., have been successfully encapsulated on PLGA nanoparticles.

The CD4+ T lymphocyte is the major target for infection by HIV-1. Cells of the mononuclear phagocyte system also serve as a reservoir for HIV. Macrophages are mature, non-proliferating and immunologically active cells that can be productively infected with HIV-1 and HIV-2. Altered cellular functions in the macrophage population may contribute to the development and clinical progression of AIDS. Evidence has accumulated that cells of the macrophage lineage are vectors for the transmission of HIV-1. The placental macrophage is likely to be the primary cell type responsible for vertical transmission of HIV-1. An important property of HIV-1 for mucosal transmission is the ability to infect macrophages. Because of the important role of cells of the monocytes/macrophage lineage in the pathogenesis of HIV-1, fully effective ARV must react with monocytes/macrophage in addition to other targets.Macrophages possess various receptors such as fucose receptors, mannosyl, galactosyl, and many others. Mannose receptors are present at the surface of monocyte macrophages, alveolar macrophages, astrocytes in the brain, hepatocytes in liver and so on. Therefore, targeting of ARV drugs to HIV infected macrophages could be an attractive approach in improving the therapeutic efficacy and reducing the toxicity of ARV bioactives.(43,47)

Polymeric nanoparticles have been used to target ARVs to (i) macrophages/monocytes and (ii) CNS which act as viral reservoir sites during HIV infection. Macrophages have been reported to be a major cause of dissemination of the infection in the body in the later stages of the disease during which there is a continuous depletion of CD4+ T lymphocytes. During this period, virus production from these mature non-proliferating macrophages/ monocytes is dramatically enhanced without being affected by the lethal effect of the replicating virus. Nanoparticulate mediated targeting of macrophages is well known and has been reported by several authors. Following i.v. administration, nanoparticles are removed from the blood circulation by macrophages. The recognition of particles by macrophages is mediated by a process called opsonization. When the distance between the particles and the opsonins is sufficiently small, they can bind to the surface of particles by any of the interactions such as van der Waals, electrostatic, ionic etc. After binding to the surface, particles become recognizable by macrophages and phagocytosis takes place. PLGA-nanoparticles are internalized in cells partly through fluid phase pinocytosis and also through clathrin-mediated endocytosis. PLGA-

nanoparticles rapidly escape the endo-lysosomes and enter the cytoplasm within 10 min of incubation. This facilitates interactions of nanoparticles with the vesicular membranes leading to transient and localized destabilization of the membrane resulting in the escape of nanoparticles into the cytosol. The body recognizes hydrophobic particles as foreign. The reticulo-endothelial system (RES) eliminates these from the blood stream and takes them up in the liver or the spleen.(27,48) This process is one of the most important biological barriers to nanoparticles-based controlled drug delivery. The binding of opsonin proteins present in the blood serum to injected nanoparticles leads to the attachment of opsonized particles to macrophages and subsequently to their internalization by phagocytosis.

PLGA nanoparticles can act as potential drug carriers to improve the delivery of ARV agents to the mononuclear phagocyte system in-vivo, overcoming pharmacokinetic problems and enhancing the activity of drugs for treatment of HIV infection and AIDS. PLGA nanoparticles are worth investigating in this area of research.

2. 3.2. Characteristic of nanoparticles

Characterization of the nanoparticle carrier systems to thoroughly understand the properties is essential before putting them to a pharmaceutical application. After preparation, nanoparticles are characterized at two levels. The physicochemical characterization consists of the evaluation of the particle size, size distribution, and surface properties (composition, charge, hydrophobicity) of the nanoparticles. The biopharmaceutical characterization includes measurements of drug encapsulation, in vitro drug release rates, and in vivo studies revealing biodistribution, bioavailability, and efficacy of the drug. (49)

There are many sensitive techniques for characterizing nanoparticles, depending upon the parameter being looked at; laser light scattering (LLS) or photon correlation spectroscopy (PCS) for particle size and size distribution; scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) for morphological properties; X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR) for surface chemistry; and differential scanning calorimetry (DSC) for thermal properties. Parameters such as density, molecular weight, and crystallinity affect the release and degradation properties, whereas surface charge, hydrophilicity, and hydrophobicity significantly influence interaction with the biological environment.

2.3.2.1. Particle size and morphology

Nanoparticle size is critical not only in determining its release and degradation behaviour but also in determining the efficacy of the therapeutic agent by affecting tissue penetration or even intracellular uptake. Particle size can be determined by PCS, SEM, TEM, AFM. PCS is a technique employed to determine the mean particle size (PCS diameter) and size distribution (polydispersity index, PI) in Malvern Zetasizer Nanoseries ZS.(31,45,50,51) It is a light scattering experiment in which the statistical intensity fluctuations in light scattered from the particles are measured. These fluctuations are due to the random brownian motion of the particles. Brownian motion is the random movement of particles due to the bombardment by the solvent molecules that surround them. The parameter calculated is defined as the translational diffusion coefficient (usually given as D). The particle size is then calculated from the translational diffusion coefficient by the Stokes-Einstein equation.(52)

PCS diameter gives information about the average particle size. The measured PCS diameter is based on the intensity of scattered light and therefore is not identical to the numeric diameter except in case of monodisperse particle suspensions. For polydisperse samples, PCS diameter is larger because it is based on the scattering intensity of the particles.

2.3.2.2. Crystallinity

The physical state of both the drug and the polymer are determined because this will have an influence on the in vitro and in vivo release characteristics of the drug. The crystalline behavior of polymeric nanoparticles is studied using XRD and thermoanalytical methods such as DSC and differential thermal analysis (DTA). DSC and XRD techniques are often combined to get useful information on the structural characteristics of both drugs and polymers.(35)

2.3.2.3. Surface charge

Zeta potential is a measure of the surface charge of the nanoparticles. The zeta potential value can influence particle stability and mucoadhesion as well as intracellular trafficking of nanoparticles as a function of pH. High zeta potential values, either positive or negative, should be achieved to ensure stability and to avoid aggregation of the particles. The extent of surface hydrophilicity can then be predicted from the values of zeta potential. Surface charge is generally determined by the well-known electrophoresis method with the help of Zetasizer.(53,54)

Commercial product based on Poly(lactic acid) (PLA), Poly(glycolic acid) (PGA), and PLGA The first FDA-cleared PLGA product was the Lupron Depot drug-delivery system (TAP Pharmaceutical Products, Lake Forest, Illinois), a controlled release device for the treatment of advanced prostate cancer that used biodegradable microspheres of 75:25 lactide/glycolide to administer leuprolide acetate over a period of 4 months (replacing daily injections).

2.4. Methods of Preparation of Nanoparticles

Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors, including: (a) size of nanoparticles required; (b) inherent properties of the drug, e.g., aqueous solubility and stability; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and toxicity.(24,31,55)

2.4.1 Nanoparticles Prepared by Polymerization Process of Monomers:

In this method, monomers are polymerized to form nanoparticles in an aqueous solution. The drug is incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed. The Nanoparticle suspension is then purified to remove various stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium. Nanocapsules formation and their particle size depend on the concentration of the surfactants and stabilizers used. Two types of polymerization processes have been adopted to prepare polymeric nanoparticles.(56)

a) Dispersion Polymerization: Dispersion polymerization starts with monomer, an initiator, solvent in which the formed polymer is insoluble, and a polymeric stabilizer. Polymer forms in the continuous phase and precipitates into a new particle phase, which is stabilized by the polymeric stabilizer. Small particles are formed by aggregation of growing polymer chains precipitating from the continuous phase as these chains exceed a critical chain length. Coalescence of these precursor particles with themselves and with their aggregates results in the formation of stable colloidal particles, which occurs when sufficient stabilizer covers the particles.(57)

b) Emulsion Polymerization: In this technique the monomer is emulsified in nonsolvent containing surfactant, which leads to the formation of monomer swollen micelles and stabilized

monomer droplets. The polymerization is performed in the presence of initiator. Emulsion polymerization may be performed using either organic or aqueous media as continuous phase. Poly (methyl methacrylate), poly (alkyl cyanoacrylate), acrylic copolymer, polystyrene, poly (vinyl pyridine) and polyacrolen nanoparticles are prepared by emulsion polymerization technique.(58)

2.4.2 Nanoparticles Prepared from Dispersion of Preformed Polymers:

Several techniques have been suggested to prepare the biodegradable polymeric nanoparticles from preformed polymers such as poly (D,L-lactide) (PLA), poly (D,L-glycolide) (PLG) and poly (D,L-lactide-co-glycolide) (PLGA). The basic methodologies of the commonly used preparation methods are as follows:

a) Emulsion/evaporation

This is one of the most frequently used methods. The preformed polymer and drug are first dissolved in a water-immiscible organic solvent, which is then emulsified in an aqueous solution containing stabilizer. The emulsification is brought about by subsequent exposure to a high-energy source such as an ultrasonic device, homogenizer, or colloid mill. The organic phase is evaporated under reduced pressure or vacuum, resulting in the formation of the aqueous dispersion of nanoparticles. The nanoparticles are collected by ultracentrifugation and washed with distilled water to remove stabilizer residues or any free drug and lyophilized for storage. This method involves preparation of a coarse emulsion, which is then subjected to homogenization under high-pressure followed by overnight stirring to remove organic solvent. This method has the advantage of obtaining small, monodispersed nanoparticles with high encapsulation efficiency and reproducibility.(14,32) The emulsion evaporation method can be used for preparation of particles with sizes varying from a few nanometers to micrometers by controlling the stirring rates and conditions. A diagrammatic representation of this method is shown in Figure. 2.7.

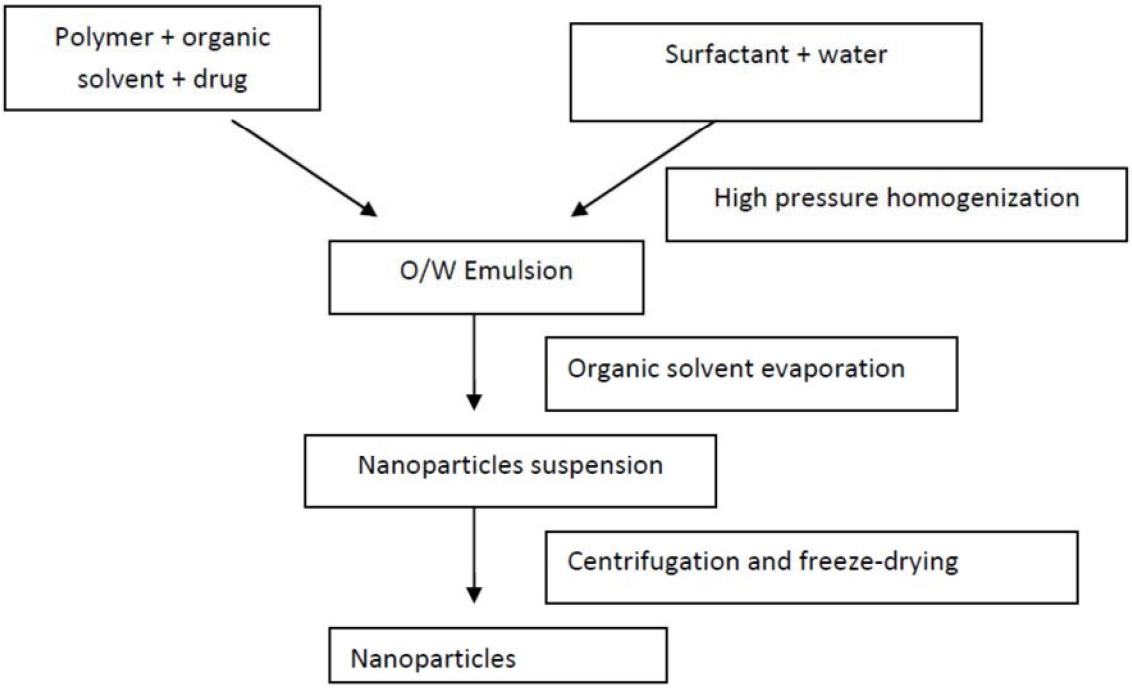


Figure 2.7 Schematic diagram of o/w emulsion method for preparation of Nanoparticles

b) Double emulsion process

The emulsion evaporation method suffers from the limitation of poor entrapment of hydrophilic drugs because of their diffusion and partitioning from the dispersed oil phase into the aqueous continuous phase. Therefore, to encapsulate hydrophilic drugs and proteins, the double-emulsion technique is employed, which involves the addition of the aqueous drug solution to organic polymer solution under vigorous stirring to from a w/o emulsion. This w/o emulsion is added into a second aqueous phase containing more stabilizers with stirring to form the w/o/w emulsion. The emulsion is then subjected to solvent removal by evaporation. A number of hydrophilic drugs like the peptide leuprolide acetate, a lutenizing hormone-releasing agonist, vaccines, proteins/peptides and conventional molecules have been successfully encapsulated by this method. After evaporation of organic solvent under reduced pressure, the polymer precipitates and nanoparticles can be isolated by centrifugation at high speed. The formed nanoparticles must be thoroughly washed before lyophilization.(30,35,59–61)

2.5. Formulation Optimization

An experimental approach to Design of Experiment (DoE) optimization of drug delivery system comprises several phases. Broadly, these phases can be sequentially summed up in seven salient steps. (62,63,63)

The optimization study begins with

- **Step I**, where an endeavor is made to ascertain the initial drug delivery objective(s) in an explicit manner. Various main response parameters, which closely and pragmatically epitomize the objective(s), are chosen for the purpose.
- In **Step II**, the experimenter has several potential independent product and/or process variables to choose from. By executing a set of suitable screening techniques and designs, the formulator selects the ―vital few influential factors among the possible ―so many input variables. Following selection of these factors, a factor influence study is carried out to quantitatively estimate the main effects and interactions. Before going to the more detailed study, experimental studies are undertaken to define the broad range of factor levels as well.
- During **Step III**, an opposite experimental design is worked out on the basis of the studyobjective(s), and the number and the type of factors, factor levels, and responses being explored.Working details on variegated vistas of the experimental designs, customarily required toimplement DoE optimization of drug delivery, have been elucidated in the subsequent section.Afterwards, response surface modeling (RSM) is characteristically employed to relate a responsevariable to the levels of input variables, and a design matrix is generated to guide the drugdelivery scientist to choose optimal formulations.
- In **Step IV**, the drug delivery formulations are experimentally prepared according to the approved experimental design, and the chosen responses are evaluated.
- Later in **Step V**, a suitable mathematical model for the objective(s) under exploration is proposed, the experimental data, thus obtained are analyzed accordingly, and the statistical significance of the proposed model discerned. Optimal formulation compositions are searched within the experimental domain, employing graphical or numerical techniques. This entire exercise is invariably executed with the help of pertinent computer software.

- **Step VI** is the penultimate phase of the optimization exercise, involving the validation of the response prognostic ability of the model put forward. Drug delivery performance of some studies, taken as the checkpoints, is assessed vis-a-vis that predicted using RSM, and the results are critically compared.
- Finally, during **Step VII**, which is carried out in the industrial milieu, the process is scaled up and set forth, ultimately for the production cycle.The niceties of the significance and execution of each of these seven steps are discussed in greater detail below. The foremost step while executing the systematic DoE methodology is to understand the deliverables of the finished product. This step is not merely confined to understanding the process performance and the product composition, but it usually goes beyond to enfold the concepts of economics, quality control, packaging, market research, etc.

The term objective has been used to indicate either the goal of an optimization experiment or the property of interest. The objectives for an experiment should be clearly determined after discussion among the project team members having sound expertise and empiricism on product development, optimization, production, and/or quality control. The group of scientists contemplates the key objectives and identifies the trivial ones. Prioritizing the objectives helps in determining the direction to proceed with regard to the selection of the factors, the responses, and the particular design. This step can be very time consuming and may not furnish rapid results. However, unless the objectives are accurately defined, it may be necessary to repeat the entire work that is to follow. The response variables, selected with dexterity, should be such that they provide maximal information with the minimal experimental effort and time. Such response variables are usually the performance objectives, such as the extent and rate of drug release, or are occasionally related to the visual aesthetics, such as chipping, grittiness, or mottling.

The word “optimize” simply means to make as perfect, effective, or functional as possible. The term optimized has been used in the past to suggest that a product has been improved to accomplish the objectives of a development scientist. However, today the term implies that DoE and computers have been used to achieve the objective(s). With respect to drug formulations or pharmaceutical processes, optimization is a phenomenon of finding the best possible composition or operating conditions. Accordingly, optimization has been defined as the

implementation of systematic approaches to achieve the best combination of product and/or process characteristics under a given set of conditions.

2.5.1. Experimental Designs

The conduct of an experiment and the subsequent interpretation of its experimental outcome are the twin essential features of the general scientific methodology. This can be accomplished only if the experiments are carried out in a systematic way and the inferences are drawn accordingly. An experimental design is the statistical strategy for organizing the experiments in such a manner that the required information is obtained as efficiently and precisely as possible. Runs or trials are the experiments conducted according to the selected experimental design. Such DoE trials are arranged in the design space so that the reliable and consistent information is attainable with minimum experimentation. The layout of the experimental runs in a matrix form, according to the experimental design, is known as the design matrix. The choice of design depends upon the proposed model, the shape of the domain, and the objective of the study.(62–64)

Primarily, the experimental (or statistical) designs are based on the principles of randomization (i.e., the manner of allocations of treatments to the experimental units), replication (i.e., the number of units employed for each treatment), and error control or local control (i.e., the grouping of specific types of experiments to increase the precision). DoE is an efficient procedure for planning experiments in such a way that the data obtained can be analyzed to yield valid and unbiased conclusions.

An experimental design is a strategy for laying out a detailed experimental plan in advance to the conduct of the experimental studies. Before the selection of experimental design, it is essential to demarcate the experimental domain within the factor space - i.e, the broad range of factor studies. To accomplish this task, first a pragmatic range of experimental domain is embarked upon and the levels and their number are selected so that the optimum lies within its realm. While selecting the levels, one must see that the increments between them should be realistic. Too wide increments may miss finding the useful information between the levels, while a too narrow range may not yield accurate results.(64)

There are numerous types of experimental designs. Various commonly employed experimental designs for RSM, screening, and factor-influence studies in pharmaceutical product development are

Factorial Designs

a) Fractional Factorial Designs
b) Plackett-Burman Designs
c) Star Designs
d) Central Composite Designs
e) Box-Behnken Designs
f) Center of Gravity Designs
g) Equiradial Designs
h) Mixture Designs
i) Taguchi Designs
j) Optimal Designs
k) Rechtschaffner Designs
l) Cotter Designs

For a three-factor study, an experimental design can invariably be envisaged as a "cube," with the possible combinations of the factor levels (low or high) represented at its respective corners. The cube, thus can be the most appropriate representation of the experimental region being explored. Most design types discussed in the current article are, therefore, being depicted pictorially using this cubic model, with experimental points at the corners, centers of faces, the centers of the edges, and so forth. Such depiction facilitates easier comprehension of various designs and comparisons among them. For designs in which more than three factors are adjusted, the same concept is applicable, except that a hypercube represents the experimental region. Such cubic designs are popular because they are symmetrical and straightforward for conceptualizing and envisioning the model.

2.5.1.1. Factorial Designs

Factorial designs (FDs) are very frequently used response surface designs. A factorial experiment is one in which all levels of a given factor are combined with all levels of every other factor in the experiment. These are generally based upon first-degree mathematical models. Full

FDs involve studying the effect of all the factors (k) at various levels(x), including the interactions among them, with the total number of experiments being xr. FDs can be investigated at either two levels (2k FD) or more than two levels. If the number of levels is the same for each factor in the optimization study, the FDs are said to be symmetric, whereas in cases of a different number of levels for different factors, FDs are termed asymmetric.

2.5.1.2. Design Augmentation

In the whole DoE endeavor, a situation sometimes arrives in which a study, conducted at some stage, is found to be inadequate and needs to be investigated further, or when the study carried out during the initial stages needs to be reused. In either situation, more design points can be added systematically to the erstwhile design. Thus, the erstwhile primitive design can be enhanced to a more advanced design furnishing more information, better reliability', and higher resolution. This process of extension of a statistical design, by adding some more rational design points, is known as design augmentation.(62) For instance, a design involving study at two levels can be augmented to a three-level design by adding some more design points. A design can be augmented in a number of ways, such as by replicating, adding center points to a two-level design, adding axial points (i.e., design points at various axes of the experiment domain), or by folding over.

2.5.2. Response Surfaces

During this crucial stage in DoE, one or more selected experimental response is recorded for a set of experiments carried out in a systematic way to develop a mathematical model. These approaches comprise the postulation of an empirical mathematical model for each response, which adequately represents a change in the response within the zone of interest. Rather than estimating the effects of each variable directly, response surface modeling (RSM) involves fitting the coefficients in the model equation of a particular response variable and mapping the response over the whole of the experimental domain in the form of a surface.(63)

Principally, RSM is a group of statistical techniques for empirical model building and model exploitation. By careful design and analysis of experiments, it seeks to relate a response to a number of predictors affecting it by generating a response surface, which is an area of space defined within upper and lower limits of the independent variables depicting the relationship of these variables to the measured response.

Experimental designs, which allow the estimation of main effects, interaction effects, and even quadratic effects, and, hence, provide an idea of the (local) shape of the response surface being investigated, are termed response surface designs. Under some circumstances, a model involving only main effects and interactions may be appropriate to describe a response surface. Such circumstances arise when analysis of the results reveals no evidence of "pure quadratic" curvature in the response of interest - i.e., the response at the center approximately equals the average of the responses at the two extreme levels, +1 and - 1.

Conduct of DoE trials, according to the chosen statistical design, yields a series of data on the response variables explored. Such data can be suitably modeled to generate mathematical relationships between the independent variables and the dependent variables. Graphical depletion of the mathematical relationship is known as a response surface. A response surface plot is a 3-D graphical representation of a response plotted between two independent variables and one response variable. The use of 3-D response surface plots allows us to understand the behavior of the system by demonstrating the contribution of the independent variables.

2.5.3. Mathematical Models

The mathematical model, simply referred to as the model, is an algebraic expression defining the dependence of a response variable on the independent variable(s). Mathematical models can either be empirical or theoretical. An empirical model provides a way to describe the factor/response relationship. It is most frequent, but not invariably, a set of polynomial equations of a given order.

2.6. Antibody Structure and Function

Antibody is involved in the humoral branch of the adaptive immunity. Antibodies,produced by B cells, recognize pathogens or foreign molecules through specific binding to the antigen. This specific interaction can neutralize the antigen or trigger effector functions of the immune system to eliminate the antigen. Examples of the effector functions are opsonization, activation of complement and antibody-dependent cell mediated cytotoxicity (ADCC). (65–67)

Antibody consists of two identical light (L) chains and two identical heavy (H)chains, as shown in Figure 2.8. The molecular weight of the light and heavy chain are about 25 and 50OkDa respectively. Each light chain is bound to a heavy chain by a disulfide bond and a combination of noncovalent interactions such as salt bridges,hydrogen bonds and hydrophobic interaction. The amino-terminal regions of light and heavy chains, which vary greatly among antibodies with different specificities, are called variable (V) regions, VL for light chain and VH for heavy chain. The regions of relatively constant sequences beyond the variable regions are called constant (C) regions, CL for light chain and CH for heavy chain. Within the V regions, sequence variability is concentrated in several hyper variable regions. These hyper variable regions, which constitute the antigen-binding site of an antibody, are called complementarity determining regions (CDRs). The remaining domains of VL and VH, which exhibit far less variation, are called the framework regions (FW).

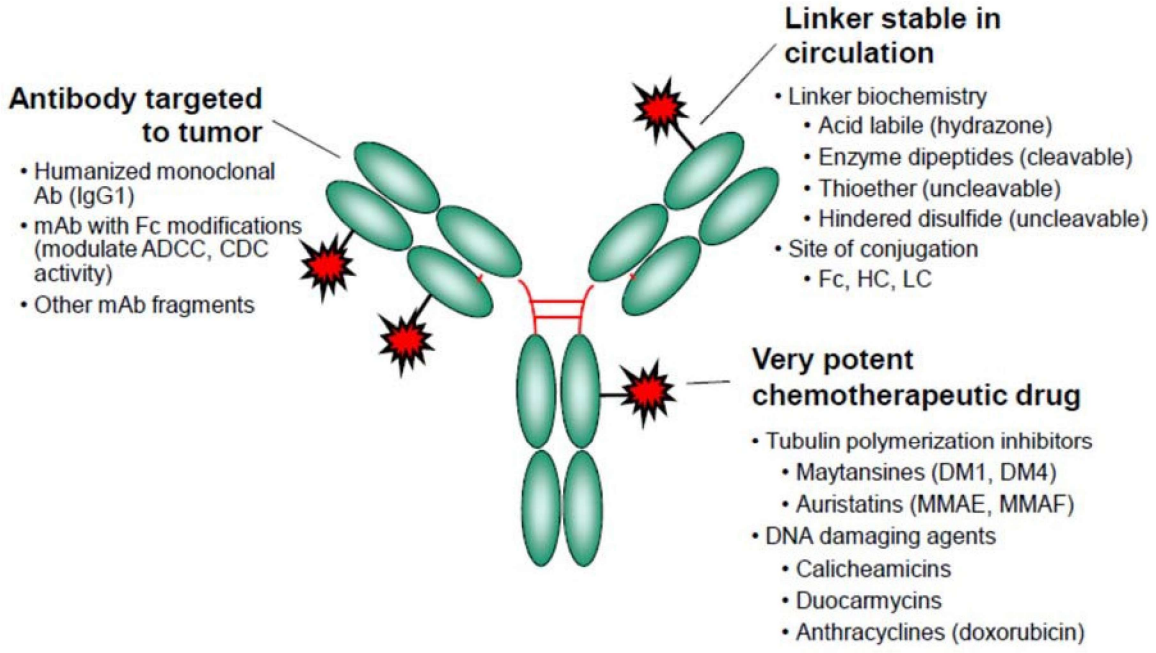


Figure 2.8 Antibody Structure

Epidermal Growth Factor Receptor

Epidermal growth factor receptor (EGFR) is a cell membrane growth factor receptor characterized by tyrosine kinase activity that plays a crucial role in the control of key cellular transduction pathways in both normal and cancerous cells. EGFR is over-expressed in a variety of human tumours, including head and neck, breast, lung, colorectal, prostate, kidney, pancreas, ovary, brain and bladder cancer.(68–70)

The 170 kDa protein function depends either on the formation of EGFR – EGFR homodimers or heterodimers – that comprise the three members of the EGFR [human epidermal receptor 1 (HER1)] family of growth factor receptors (HER2, HER3 and HER4) following binding of an EGFR-selective ligand. The activating ligands include the epidermal growth factor (EGF), transforming growth factor-α (TGF-α), amphiregulin or neuregulin. The binding EGFR/ligand results in conformational changes that allow the activation of EGFR tyrosine kinase and the phosphorylation of specific tyrosine residues within the EGFR intracellular carboxyl- terminal domain. Phosphorylated tyrosine residues serve as docking sites for several signalling proteins finally stimulating cell proliferation, loss of differentiation, invasion, angiogenesis and blocking of apoptosis. Within a few hours of activation, receptors are internalized into cytoplasm, where they are either degraded or recycled back to the membrane.(71–73)

EGFR homodimers undergo degradation, whereas EGFR and HER2 heterodimerization is associated with recycling upon endocytosis, which enhances mitogenic signalling. Homodimers are weaker effectors compared with heterodimers: EGFR and HER2 is the most common heterodimer; HER2:3 plus neuregulin is the most potent combination; HER2 decelerates the internalization of HER1; HER1 requires ligand binding before dimerization, while HER2 does not require a ligand to dimerize and is often expressed at a 100-fold higher concentration compared with HER1. The complex signalling network generated by triggering EGFR includes the ras- and mitogen-activated protein kinase (MAPK) pathway that leads to cell proliferation, the phosphatidylinositol-3 kinase (PI3K) and protein kinase B (Akt) pathway driving cell cycle progression and cell survival. There is also evidence that EGFR can translocate to the nucleus, where it acts as a transcription factor (Figure. 2.9).

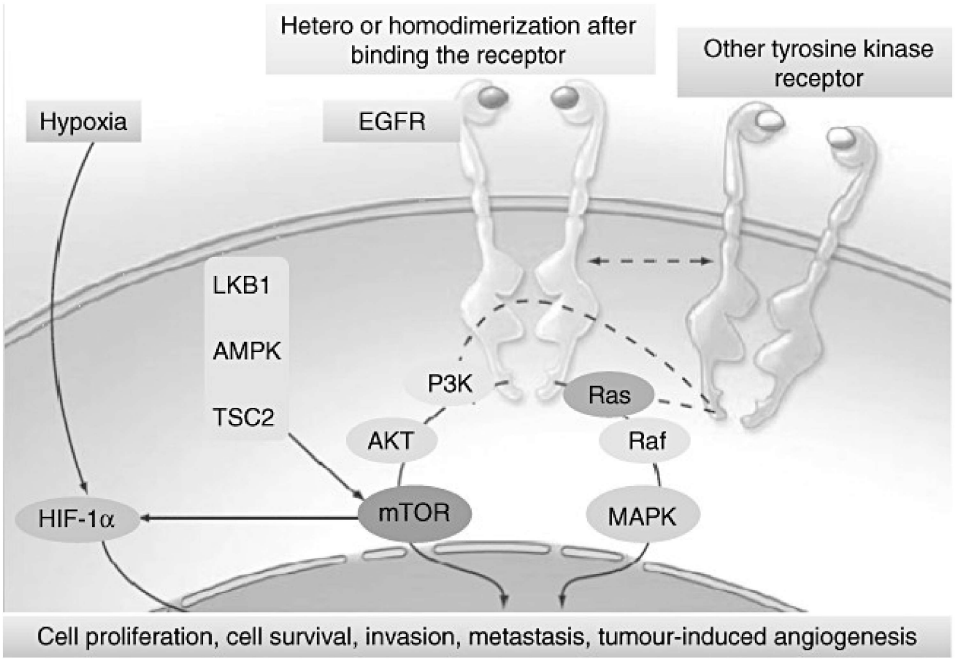


Figure 2.9 Signal transduction pathway mediated by epidermal growth factor receptor (EGFR).

The interaction with ligand that occurs in the extracellular portion of the EGFR family induces the formation of a functionally active EGFR–EGFR homodimer or of an EGFR–human epidermal receptor 2 (HER2), EGFR–HER3 or EGFR–HER4 heterodimer. Conformational changes that allow the EGFR tyrosine kinase to be activated with the phosphorylation of specific tyrosine residues within the EGFR intracellular carboxyl-terminal domain. Phosphorylated tyrosine residues trigger a complex programme of intracellular signals to the cytoplasm and then to the nucleus that stimulates cell proliferation, loss of differentiation, invasion and angiogenesis, and blocks the apoptosis.(74)

Inhibition Of The Target

Two pharmacological approaches have been used successfully to inhibit EGFR functions in cancer treatment: neutralizing monoclonal antibodies and small-molecule tyrosine kinase inhibitors. Anti-EGFR monoclonal antibodies bind to the extracellular domain of EGFR in its inactive state; they compete for receptor binding by occluding the ligand-binding region, and thereby block ligand-induced EGFR tyrosine kinase activation. Small-molecule EGFR tyrosine kinase inhibitors compete reversibly with Adenosine 5′ triphosphate to bind to the intracellular catalytic domain of EGFR tyrosine kinase and, thus, inhibit EGFR auto-phosphorylation and downstream signalling.(75–78) In addition, various small-molecule EGFR tyrosine kinase

inhibitors can block different growth factor receptor tyrosine kinases, including other members of the EGFR family, or the vascular endothelial growth factor receptor. Anti-EGFR monoclonal antibodies recognize EGFR exclusively and are therefore highly selective to this receptor. Nevertheless, an intrinsic or acquired resistance to the EGFR inhibitor that limits the use of these drugs in cancer therapy has been evidenced. This could be related to constitutive activation of downstream mediators or over-expression of other tyrosine kinase receptors. Moreover, the increase of angiogenesis caused by up-regulation of the vascular endothelial growth factor in human cancer cells by EGF and TGF-α could promote resistance to EGFR inhibition. To date, two anti-EGFR monoclonal antibodies, panitumumab and cetuximab, are currently in widespread use in cancer treatment.(71,75,76,79)

CETUXIMAB(65,68,80,81)

Cetuximab (C225, Erbitux™) is an immunoglobulin (Ig) G1 human–murine chimeric counterpart of the murine monoclonal antibody M225. It binds to the EGFR with a 2-log higher affinity compared with the natural ligands TGF-α and EGF. Binding of cetuximab to the EGFR promotes receptor internalization and subsequent degradation without receptor phosphorylation and activation. This results in receptor down-regulation, reducing the availability of EGFR on the cell surface and preventing activation of EGFR-associated, downstream signalling pathways. Cetuximab also binds to the mutant receptor EGFRvIII, inducing internalization of 50% of antibody-receptor complexes after 3 h, and an 80% reduction in phosphorylated EGFRvIII.

Binding of cetuximab to EGFR inhibits the progression of the cell cycle at the G0/G1 boundary, increases expression of the cell cycle regulator p27KIP1 and induces apoptosis by increasing expression of pro-apoptotic proteins (e.g. Bax and caspase-3, caspase-8 and caspase-9) or by inactivation of anti-apoptotic proteins (e.g. Bcl-2) inducing decreased expression or phosphorylation. Cetuximab has also been reported to inhibit the production of pro-angiogenic factors such as vascular endothelial growth factor, interleukin-8 and the basic fibroblast growth factor; inhibition of these factors is associated with a decrease in new blood vessel formation and the development of distant metastases in orthotopic cancer models.

2.6.1 Antibody Therapeutics for Cancer Therapy

2.6.1.1 Tumor Antigens

Malignant transformation of the cell is usually associated with alteration in the surface antigenic composition. Tumor antigens can result from a genetic mutation in tumor cells that generate

altered cellular proteins; these kinds of antigens are unique to the tumor cells. These genetic mutations can be induced chemically, physically or virally (i.e.Epstein-Barr virus and Human Papilloma virus). In addition, tumor cell surface antigen scan also are aberrantly glycosylated (Tag-72 and Mucins), rendering them different from those on the normal cells. However, there are also tumor antigens that are not unique to the cancer cells; these tumor antigens are normally present on healthy cells. When the cells become malignant, the expression profile or level of these antigens change dramatically. One example of aberrant expression profiles is carcinoembryonic antigen (CEA). Tumor cells express CEA over the entire surface of the cells, while in normal cells, CEA is only present on the apical surface of the cells. Meanwhile, tumor antigens can also be over-expressed when cells become cancerous. Examples are CD20 over expression in non-Hodgkin lymphoma and CD44 over expression in lung cancer. When these tumor-associated antigens are expressed at a level (higher density) that can significantly distinguish their malignant phenotypes from the normal healthy phenotypes, this makes them (antigens) excellent targets for antibody-targeted therapy. (82–84)

2.6.1.2 Mechanism of Tumor Killing

Antibody can block tumor growth or mediate the killing of tumor cell by several different mechanisms. First, antibody can bind to the growth factor receptors or other signaling molecules on the cancer cells, leading to apoptosis or inhibition of the cell growth. Examples are Bevacizumab, which binds vascular endothelial growth factor (VEGF), and Cetuximab, which block the binding of EGF or TGF-a against EGFR.(72,82,85) Another way to mediate killing is to recruit the natural immune system to kill tumor cells. Antibody bound to the cancer antigen can activate the complement components, leading to opsonization of cancer cells by complement receptors-expressing phagocytic cells, direct lysis of tumor cells and inflammation with recruitment of inflammatory cells. In addition, the bound antibody can bind to the activating Fc receptors on the effector cells like macrophages and NKcells, leading to antibody-dependent cellular cytotoxicity (ADCC) or release of cytokines.Examples are Rituxan® in the treatment of non-Hodgkin's lymphoma and Herceptin in the treatment of metastatic breast cancer. Besides using naked antibody to cure cancer,antibodies or antibody fragments can be conjugated with different cytotoxic moieties to kill the tumor cells.(65,86,87) These cytotoxic moieties include a variety of entities, ranging from radionuclide molecules to a virus carrying therapeutic genes or a liposome carrying loads of drugs, toxins, or enzymes. For example, antibody BR96-doxorubicin

conjugate was used to target LeY-related tumor-associated antigen expressed on most human carcinoma. Antibody-directed drug delivery can improve the therapeutic efficacy of cytotoxic moieties by targeting tumor cells specifically, while reducing the potential systemic toxicities of the drugs. However, the success of a targeted cell-killing function is predicated on the existence of tumor associated antigens.

2.6.1.3 Current Antibody Therapeutics(88)

Antibodies targeting various tumor-associated antigens have been developed successfully to treat cancer. Currently, there are eight monoclonal antibodies approved by the Food and Drug Administration (FDA) for cancer therapy. Antibody-based therapeutics against cancer are highly successful and currently enjoy unprecedented recognition of their potential; 13 monoclonal antibodies (mAbs) have been approved for clinical use in the European Union and in the United States. Bevacizumab, rituximab, and trastuzumab had sales in 2010 of more than $5 billion each. Hundreds of mAbs, including bispecific mAbs and multispecific fusion proteins, mAbs conjugated with small-molecule drugs, and mAbs with optimized pharmacokinetics, are in clinical trials.(89) However, deeper understanding of mechanisms is needed to overcome major problems including resistance to therapy, access to targets, complexity of biological systems, and individual variations. (90–94) Table 2.3 summarizes these FDA-approved antibodies and their targets. These approved antibodies can be divided into two types, naked and conjugated. Naked antibodies are those without any moiety attached to it; while conjugated antibodies have either toxin, radioactive material or a cytotoxic drug attached to them. Most of the approved antibodies, except Bexxar, are either chimeric or humanized, minimizing the neutralization effect of HAMA (human anti-murine antibody). In addition to these approved antibodies, there are hundreds of clinical trials worldwide involving the use of antibodies to treat cancer (www.clinicaltrials.gov). In general, antibody has been proven to be a successful molecule for targeted cancer therapy.(84,95–98)

Table 2.3 Human mAbs approved or under FDA review (99)

Human mAb (trade name; company name)	**Description**	**Indication of first US approval**	**Date of first US (EU) approval**
Adalimumab (Humira; Abbott)	TNF-specific, IgG1κ	Rheumatoid arthritis	31 Dec 2002 (8 Sep 2003)
Cetuximab (Erbitux)	EGFR-specific	Recurrent or metastatic head and neck cancer, Lung cancer, Colorectal cancer	06 July 2012
Panitumumab (Vectibix; Amgen)	EGFR-specific, IgG2κ	Colorectal cancer	27 Sep 2006 (3 Dec 2007)
Golimumab (Simponi; Centocor)	TNF-specific, IgG1	Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis	24 Apr 2009 (1 Oct 2009)
Canakinumab (Ilaris; Novartis)	IL-1β-specific, IgG1κ	Cryopyrin-associated periodic syndromes	18 Jun 2009 (23 Oct 2009)
Ustekinumab (Stelara; Johnson & Johnson)	IL-12/IL-23 p40-specific, IgG1	Plaque psoriasis	25 Sep 2009 (16 Jan 2009)
Ofatumumab (Arzerra; Genmab)	CD20-specific, IgG1	Chronic lymphocytic leukaemia	26 Oct 2009 (19 Apr 2010)
Denosumab (Prolia; Amgen)	RANKL-specific, IgG2	Treatment of postmenopausal osteoporosis‡	1 Jun 2010 (26 May 2010)
Raxibacumab	PA-specific, IgG1	Inhalation anthrax	Under review by the FDA
Belimumab	B lymphocyte stimulator-specific, IgG1	Systemic lupus erythematosus	Under review by the FDA and the EMA
Ipilimumab	CTLA4-specific, IgG1	Metastatic melanoma	Under review by the FDA and the EMA

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