International Journal of Pharmacy and Natural Medicines

Journal Home Page: www.pharmaresearchlibrary.com/ijpnm

REVIEW ARTICLE

Review of Type of Nanoparticles, Characterization and Drug Delivery Application

R. Ajitha*, S. Gobinath, R. Ramprasad, Nilsha Anil, N. Venkateshan, J. Amutha Iswarya Devi

Department of Pharmacy, Arulmigu Kalasalingam College of Pharmacy, Anand Nager, Krishnankoil-626126.

ABSTRACT

The most emerging branch in pharmaceutical is "Pharmaceutical nanotechnology "which are expected to have significant applications in disease diagnostics and therapeutics. Pharmaceutical nanotechnology comprised of Nano- sized products which can be transformed in numerous ways to improve their characteristics various pharmaceutical nanotechnology based systems which can be termed as Nano pharmaceuticals like 1. Polymeric Nanopartical biodegradable-excellent carrier for sustained delivery of drug 2.Carbon Nanotubes-unique electric properties-enhance solubility 3.Polymeric Micelles-high drug entrapment-long circulatory. liposome-versatile-active delivery of gene.5.Quantum Dots-narrow emission-receptor mediated endocytosis.6.Metallic Nanoparticle-gold and silver colloids-highly sensitive diagnostic assays, 7.Nano bubbles (NBs) are Nano scaled bubble like structures that are generated in the interface of hydrophobic surfaces in liquids etc. Application like diagnostics, drug transport, free radical scavengers, photosensitizers, cell specificity, internalization, vaccine delivery, gene delivery, gene silencing, reduce toxicity and increase the efficacy etc. Nanoparticle small in size of particles so easily penetration in skin, blood and cardiovascular system and brain etc. These are most important applications and nanoparticles associated health risk related information available till present.

Keywords: Nanotechnology, Nanoparticle, types and application.

ARTICLE INFO

***Corresponding Author**
R. Ajitha
Department of Pharmacy, Arulmigu Kalasalingam College of Pharmacy, Anand Nager, Krishnankoil-626126.
MS-ID: IJPNM3642

PAPER QR-CODE

ARTICLE HISTORY: Received 15 April 2018, **Accepted** 24 May 2018, **Available Online** 15 June 2018

Citation: *R. Ajitha, et al.* Review of Type of Nanoparticles, Characterization and Drug Delivery Application. *Int. J. Pharm. Natural Med*., 2018, 6(1): 44-54.

CONTENTS

1. Introduction

Nanotechnology is the science of material featuring between 10 −9 and 10 −7 of a meter or in another words it's the science of materials and devices whose structures and constituents demonstrate novel and considerably altered

physical, chemical and biological phenomenon due to their nanoscale size [1,2,3]. Thus nanotechnology is defined as the manipulation of matter on an atomic, molecular, and supramolecular scale involving the design, production, characterization and application of different nanoscale materials in different potential areas providing novel technological advances mainly in the field of medicine [4,5,6]. This forms an independent branch of nanostructures, referred as nanomedicine which is specifically utilized for medicines. Nanomedicine involves utilization of nanotechnology for the benefit of human health and well being. Nanomedicine was defined by European Science Foundation as 'the science and technology of diagnosing, treating and preventing disease and traumatic injury, of relieving pain, and of preserving and improving human health, using molecular tools and molecular knowledge of the human body This defi nition was revised by the US NIH as: 'Nanomedicine refers to highly specific medical intervention at the molecular scale for curing diseases or repairing damaged tissues, such as bone, muscle, or nerve'[7,8,9,10].

Specified five sub-disciplines of nanomedicines

- Analytical tools
- Nanoimaging tools
- Nanomaterials and nanodevices
- Clinical and toxicological issues
- Novel therapeutics and drug delivery systems

Potential of nanomedicines in cancer is dependent on passive targeting (due to the enhance of the permeability and retention effect promoted by angiogenic vessels) which can be reinforced by specific targeting (based on multifunctional nanomaterials that bypass the biological barriers and reach cancer cells). Nanoparticles based specific drug targeting and delivery platforms reduce toxicity and other side effects and also improve the therapeutic index of the targeted drug[11,12,13,14]. In the primary objective of nanotechnology especially in cancer therapy is the development of suitable targeting delivery systems which has been taking the lead in what concerns overcoming the MDR problem. Such targeted delivery systems that are based 'Nanosizing' of drugs[15,16,17]:

- Decrease drug resistance
- Decrease toxicity
- Enhance oral bioavailability
- Enhance rate of dissolution
- Enhance solubility
- Increase the stability of drug and formulation
- Increase drug targeting ability
- Increase patient compliance
- Increase surface area
- Reduce the dose

Polymeric Nanoparticle:

The main objective of our book is to explore the recent nano application of wide array of natural polymers obtained from different sources. Natural polymers based nano-conjugates and their advance applications are discussed in this chapter [19,20,21]. In addition various drug delivery and targeting based considerations are also discussed. Natural polymer based nanoparticles are usually biocompatible and non toxic, although often suffer from stability problems when delivered across the various biological membranes. Such delivery exposed nanoparticles against various pH. This variation in pH and certain other problems limit their use sometime. Polymeric nanoparticles consist of a biodegradable polymer which is biocompatible and non toxic. Feature such as biocompatibility is required for potential application in tissue engineering, drug and gene delivery and new vaccination strategies [22,23,24].

Recently research explore some advance modifi cation of natural polymers which consists of synthetic polyesters like polycyanoacrylate and related polymers like poly(lactide-co-glycolide) poly(lactid acid). Among natural polymers the most widely used polymer which is used now days is chitosan. In addition to chitosan many other such as gelatin, and sodium alginate overcome some toxicological problems with the synthetic polymers [25,26,27,28]. Natural polymer based nanoparticles offers a signifi cant improvement over traditional oral and intravenous methods of drug delivery system in terms of effi ciency and effectiveness. The various natural polymers like gelatin, albumin and alginate are used to prepare the nanoparticles. Synthetic polymers used for nanoparticles preparation may be in the form of preformed polymer e.g. polyesters like polycaprolactone (PCL), poly lactic acid (PLA) or monomers that can be polymerized in situ e.g. polyalkyl cyanoacrylate. There are many advantages of using polymeric nanoparticles in drug delivery [29,30,31]:

- Biocompatable and biodregerable
- Increase the stability of any volatile pharmaceutical agents
- Less toxic
- They are easily cheaply fabricated in large quantities by a multitude of methods
- Have engineered specificity, allowing them to deliver a higher concentration of pharmaceutical agent to a desired location
- Nonimmunogenicity and nontoxicity

2. Application

Long-Circulating and Target-Specifi c Nanoparticles:

Nanoparticles are known to be the most successful way of delivering drug at desirable site. They can be used to target tumors which are localized outside mononuclear phagocytic system rich organs. Instantaneous identifi cation of colloidal carriers (liposomes and polymeric nanospheres) from the blood by Kupffer cells, has begin a surge of development for "Kupffer cell-evading" or long-circulating particles. These carriers have major applications in vascular drug delivery and release, site-specific targeting (passive as well as active targeting), as well as transfusion medicine [32,33,34,35]. So much effort has been done to develop so-called "stealth" particles (PEGylated nanoparticles), which are invisible to macrophages or phagocytes [37,38]

Nanoparticles for Oral Delivery of Peptides and Proteins: Exploration of more antigenic substances in biotechnology and biochemistry field persist the production of vaccines. Owing to the recent advancement

biotechnology various bio macromolecules and vaccines are explored [39.40]. Thus there is an urgent requirement of their suitable carriers system which still remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers ofthe gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Polymer based nanoparticles facilitates the encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradatione.g. insulin-loaded polymeric nanoparticles have preserved insulin activity and produced blood glucose reduction in diabetic rats for up to 14 days following the oral administration. It's universally known that the surface area of human mucosa extends to 200 times that of skin [41,42]. Protein or peptide based drug delivery encountered a variety of physiological and morphological barriers:

- Bacterial gut flora
- Mucus layer and epithelial cell lining itself
- Proteolytic enzymes at the brush border membrane (endopeptidases)
- Proteolytic enzymes in the gut lumen like pepsin, trypsin and chymotrypsin

Carbon Nanotubes:

Carbon nano tubes are carbon based tubular structures that are discovered in 1991 These structures are arranged in fashion like a graphite sheet rolled up into a cylinder and capped at one or both ends by a buckyball. These are hexagonal networks of carbon atoms having diameter of one nanometer and length from 1 to 100 nm. These carbon networks are arranged layer of graphite rolled up into a cylinder [43,44]. There are two carbon based confi guration that have received much attention recently .single-walled nanotubes (SWNTs) and multiwalled nanotubes (MWNTs). In addition to these types C 60 fullerenes is also a part of common confi gurations. These are hollow, carbon-based, cage-like architectures (nanotubes and fullerenes), also known as bucky balls, which are differ in the arrangement of their graphite cylinders. The size, geometry, and surface characteristics of these macromolecules make them appealing for drug carrier usage and have remarkable physical proper [44,45].

SWNTs and C60 fullerenes have internal diameters range of 1–2 nm. This dimension is equivalent to about half the diameter of the average DNA helix, whereas MWNTs have diameters ranging from several nanometers to tens of nanometers with 0.36 nm distance between layers of MWCNT, depending on the number of walls in the structure. Size may vary in their length ranging from 1 μm to a few micrometers [46]. As far as their architecture is concerned fullerenes and carbon nanotubes are typically fabricated using laser ablation, chemical vapor deposition, electric arc discharge, or combustion processes. Characterization of these concentric forms is based on their strength and stability so that they can be used as stable drug carriers. Cellular entry of nanotubes may be mediated by endocytosis or by insertion through the cell membrane. Fullerenes have also shown drug targeting capability. Tissue-selective targeting and intracellular targeting of mitochondria have been shown with use of fullerene structures. Furthermore, experiments with fullerenes have also shown that they exhibit antioxidant and antimicrobial behavior [47].

Application:

Cell specificity

Enhancement of cell specifi city by conjugating antibodies to carbon nano tubes with fluorescent or radiolabelling

Internalization

Internalization within mammalian cells can be achieved by surface- functionalized carbon nanotubes

Vaccine delivery

Conjugation with peptides may be used as vaccine delivery structures

Gene delivery

With the advancement in molecular dynamics simulations, the flow of water molecules through surface-functionalized carbon nanotubes has been modeled in such a way so that they can be conveniently utilized as small molecule transporters in transporting DNA, indicating potential use as a gene delivery tool [48,49]. The ability of nanotubes to transport DNA across cell membrane is used in studies involving gene therapy. During this therapy DNA can be attached to the tips of nanotubes or can be incorporated within the tubes. It has been found that gene therapy with β galactosidase marker gene nanotubes showed greater expression compared to transfer of naked DNA. This assures the advantage of non immunogenicity in contrast to viral vectors used for gene transfer [50,51].

Reduced toxicity and increases the effi cacy

Carbon nanotubes enhance drug delivery, efficacy and reduces the toxicity as found in the case of Amphotericin B nanotubes. It has been found that Amphotericin B nanotubes has shown enhanced drug delivery to the interior of cells, increased antifungal effi cacy and reduced toxicity to mammalian cells when compared to amphotericin B administration without nanotubes. The efficacy of amphotericin B nanotubes was also effective on strains of fungi which are usually resistant to amphotericin B alone [52,53,54]

Gene silencing

Highly selective therapy is required for cancer therapy where tumor cells will be selectively modulated. In this case gene silencing has been done with small interfering RNA. This can be achieved by targeting functionalized single walled carbon nanotubes with sRNA to silence targeted gene expression in the targeted cell[55].

In diagnostics

It was reported that compounds that are bound to nanotubes enhance the effIciency of diagnostic methods. This property of functionalization and high length to diameter aspect ratio [56,57].

Toxicity

On a dose per mass basis carbon nanotubes are more toxic then quartz particles which are well known for their lung toxicity [58,59]

Swcnt:

In-vitro incubation of high dose of SWCNT with keratinocytes and bronchial epithelial cells results in ROS generation, oxidative stress, lipid peroxidation, mitochondrial dysfunction and changes in cell morphology;

Platelet aggregation; Intratracheal instillation of high doses of nanotubes causes chronic lung infl ammation, foreign body granuloma formation, interstitial fi brosis; *in vivo* studies SWCNT induce lung granuloma [60].

Polymeric Micelles:

Polymeric micelles contains amphiphilic block copolymers assemble to form nanoscopic supramolecular core-shell structures called as 'polymeric micelles'. These micelles are formed in solution as aggregates in which the component molecules are generally arranged in a spheroidal structure with hydrophobic cores shielded from water by a mantle of hydrophilic groups. There are several examples ofcomponent molecule such as Amphiphilic AB-type or ABA-type block copolymers, where A and B are hydrophobic and Hydrophilic components, respectively. These polymeric micelles are usually <100 nm and are used for the systemic delivery of water-insoluble drugs. Their hydrophilic surface of these dynamic systems protects their nonspecific uptake by reticuloendothelial system. Polymeric micelle carries advantage in trapping drugs or contrast agents physically within the hydrophobic cores or can be linked covalently to component molecules of the micelle. Additionally they are proved as an excellent novel drug delivery system due to their high stability in physiological conditions, high and versatile loading capacity, high accumulation of drug at target site, possibility of functionalization of end group for conjugation of targeting ligands and slower rate of dissolution [61,62].

Application:

Polymer Drug Conjugate:

Polymer drug conjugate formed by the conjugation of low molecular weight drugs with polymer. This interaction/conjugation causes drastic change in pharmacokinetic disposition of drug in whole body and at cellular level. They are designed toincrease the overall molecular weight, which facilitates their retention in cancer cells through enhanced permeation and retention effect using passive delivery approach [63,64].

Liposome:

Polyplexes/ Lipopolyplexes are the assemblies which are used in transfection protocols. These assembies are formed by spontaneous interaction between nucleic acids and polycations or cationic liposomes (or polycations conjugated to targeting ligands or hydrophilic polymers). Usually composition and charge ratio of nucleic acid to that of cationic lipid/polymer determines the shape, size distribution, and transfection potential of these complexes [65]. Current research offers various types of polycations that have been used in gene transfer/therapy protocols:

- Cationic cyclodextrin
- Linear- and branched-poly (ethyleneimine)
- Poly (amidoamine)
- Poly-amino esters
- Poly- L -lysine

Quantum Dots:

Quantumdots (QDs) are nanocrystals of semiconducting materials measuring around 2–10 nm, consisting of a semiconductor inorganic core (CdSe), an aqueous organic coated shell (e.g., ZnS) to improve optical properties, and can be made to fl uorescence when stimulated by light. Quantumdots bear a cap which enables them in improving their solubility in aqueous buffers. They are neither atomic nor bulk semiconductors [66,67]. Core of the quantumdots determines the color emitted and outer aqueous shell is available for conjugation with biomolecules.

Biomolecular conjugation of the quantum dots can be modifi ed according to target various biomarkers. Their properties originate from their physical size, which ranges from 2 to10 nm in radius. Owing to the to their narrow emission, bright fl uorescence, high photostability and broad UV excitation QDs have been adopted for tracking of intracellular process for longer time, for in vitro bioimaging and for real time monitoring. As far as the its applications are concerned QDs covers medical areas as a diagnostic as well as therapeutic tool for *in vitro* and *in vivo* detection and analysis of biomolecules, immunoassays, DNA hybridization, diagnostic tools (magnetic resonance imaging, MRI), time graded fl uorescence imaging of tissue, development of non-viral vectors for gene therapy, labeling of cells, as therapeutic tools for cancer treatment and transport vehicles for DNA, protein, drugs or cells[68].

Application:

Cancer therapy

In one report it was proven that quantum dots are accumulated in prostate cancer developed in nude mice by enhanced permeability and retention [69].

Bioconjugation with polymer

It was reported that its conjugation with polyethylene glycol (PEG) and antibody and targeting to prostate specifi c membrane antigen enhances its accumulation and retention in the tumor tissue [70]

Imaging

They can also be utilized for imaging of sentinel node in cancer patients for tumor staging and planning of therapy [71]. This application assists in detecting suitable therapy and stage for various malignancies like melanoma, breast, lung and gastrointestinal tumors. In addition quantum dot probes provide real time imaging of the sentinel node with Near Infra Red (NIR) fl uorescence system which is having the potential to produce reduced background noise and deeper penetration of rays into the biological sample[72].

Toxicity

QDs utilization in clinical practice is limited since it has serious elimination problems which cause extreme toxicity. After functionalization of the QDs size increases.This size range is greater than the pore size of endothelium and renal capillaries, thus reducing its elimination and resulting in toxicity. Additionally there are very less reports available on the metabolism and excretion of quantum dots making their utilization more diffi cult clinically[73].

3. Metallic Nanoparticles

Currently these nanoparticles are emerging as good delivery carrier for drug and biosensor. For the synthesis of metallic nanoparticles diverse metals have been explored though silver and gold nanoparticles are of prime importance for biomedical use. Surface functionalization on these nanonarticles can easily been done and various ligands have

been decorated onto the surface. Variety of ligands such as sugars, peptide, protein and DNA has been linked to nanoparticles [74,75].

Application

Metallic nanoparticles have been used for active delivery of bioactive, drug discovery, bioassays, detection, imaging and many other applications due to surface functionalization ability, as an alternative to quantum dots.

Nanobubbles:

Nanobubbles (NBs) are nanoscaled bubble like structures that are generated in the interface of hydrophobic surfaces in liquids. These nanobubbles remain stable at room temperature and when heated to physiological temperature within the body coalesce to form microbubbles. The mechanism of NB formation is based on the nucleation of gas at the hydrophobic surface from a supersaturated solution, leading to trap atmospheric gases. However the formation of NBs is thermodynamically forbidden, but the life time of NBs is reached event to the orders of hours. There are four types of nanobubbles: Bulk, interfacial, plasmonic and oscillating nanobubbles. Cancer therapeutic drugs can be incorporated into these nanoscaled bubbles like structures. Nanobubbles potentially exhibit advantages in targeting the tumor tissue and delivering the drug selectively under the infl uence of ultrasound exposure. This may enhance the intracellular uptake of the drug by the tumor cells. Additionally these nanobubbles can be easily visualized in tumor by means of various ultrasound methods [76,77,78].

Application:

Delivery of drugs

NBs can be potentially utilized in delivery of drugs like doxorubicin *in vitro* and *in vivo* . These NBs reach the tumor and get accumulated which is followed by formation of microbubbles by coalescing of nanobubbles. Disruption of the microbubbles occurs when the site is focused with high intensity focused ultrasound, which ultimately causes release of the drug. This may results in accumulation of higher levels of drug in the target cells and reduced toxicity and increased efficacy. This method needs further exploration for its utility in treatment of variousmalignancies [79,80].

Gene therapy

Liposomal nanobubbles and microbubbles are also being studied for effective non viral vectors for gene therapy. Nanobubbles combined with ultrasound exposure have shown improved transfer of gene in both *in vitro* and *in vivo* studies [81,82].

Thrombolysis

Nanobubbles are also being investigated for removal of clot in vascular system in combination with ultrasound. This process is called as sonothrombolysis. This method is non invasive and causing less damage to endothelium [83,84].

Toxicity

NBs are not that much toxic since the disruption of the microbubbles occurs only at the targeted site when it's being exposed to ultra sound waves. Therefore drug is released at a particular site [85,86].

Health Implication of Nanoparticles:

It's very essential to recognize and differentiate 'free' and 'fixed' nano particles [87]. Free nanoparticles exhibit serious health threat since they are more diffi cult to contain due to airborne and can be inhaled. Nanoparticles can be entered in human body via

- Absorptions by the intestinal tract
- Absorptions by the skin
- Lungs where a rapid translocation through the blood stream to vital organ is possible, including Crossing The BBB and nanoparticles Affect The Following Organs In Several Ways

Lungs:

It has been already observed that titanium dioxide (TiO 2) carbon black and the diesel particles exhibit various adverse effects. Based on previous findings it has been observed that the administration of ultrafi ne nanoparticles to the lung produce more potent adverse effect in the form of infl ammation and subsequent tumors compared with larger sized particles, of identical chemical composition at equivalent mass concentration. Toxicity of these particles is dependent on their surface charachterstics such as surface chemistry [88].

Intestinal Tract:

To facilitate the absorption of the food particles, the epithelium of the small and large intestinal is in close contact with ingested material. These food particles are converted in to a mixture of disaccharides, peptides, fatty acids and monoglycerides generatedhowever digestion in small intestine are further transformed and taken in the villi. Particles having charge (e.g. like carboxylated polystyrene nano particles or those composed of positively charged polymer) exhibit poor oral bioavailability through electrostatic repulsions and means entrapment . Smaller the size of particles facilitate the faster penetration (within 2 mints,); 415 nm particles took 30 mints whereas 1000 nm particles were not capable to translocate through this barrier [89].

Skin:

Particles having size range 500–1000 nm, theoretically afar from the area of nanotechnology can in filtrate and reach the lower level of human skin. Size range smaller than 128 are more likely penetrate deeper in to the skin [90].

Blood and Cardiovascular System:

Cationic nanoparticles including gold and polystyrene have been shown to cause hemolysis and blood clotting while anionic nanoparticle are non toxic. Combustion and model nanoparticles can gain access to blood following inhalation and can enhance the experimental thrombosis. High exposure to DEP by inhalation cause altered heart rate in hypertensive rats. Inhalation of PM causes atheromatous plaque and destablization in rabbits. Recent data showed that Carbon derived nanomaterials induce platelet aggregation [91].

Brain:

High concentrations of anionic and cationic nanoparticles are toxic to brain. Nanoparticles have been shown to produce reactive oxygen species and oxidative stress. This has been confi rmed in the brain after inhalation of MNO 2 nanoparticles [92,93]. Oxidative stress induced by nanoparticles causes various neurodegenerative disease such as Parkinson and Alzheimer. In addition inhalation of

nanoparticles in balb/c mice to particulate matter showed the activation of proinflammatory cytokines in the brain [94,95].

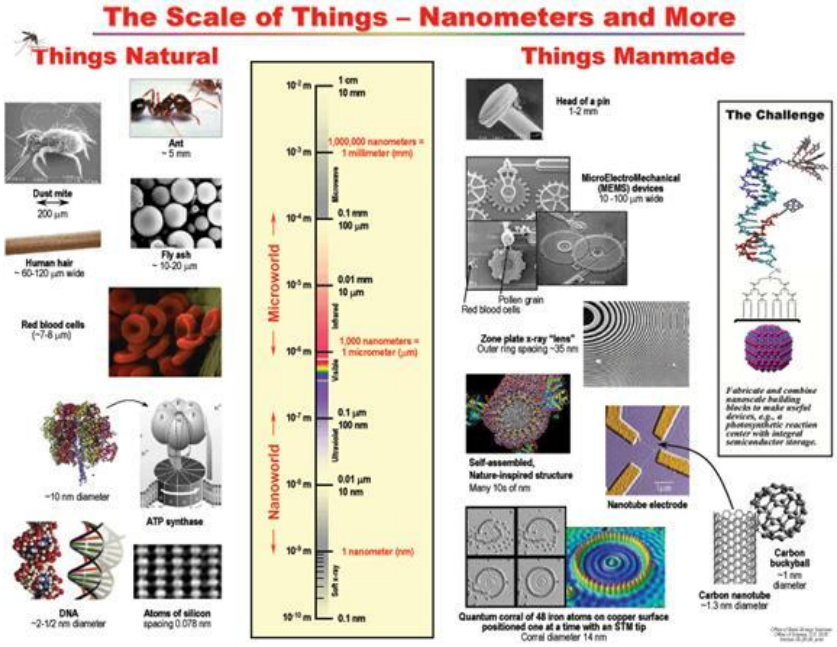

Figure 1: Nano particle scale

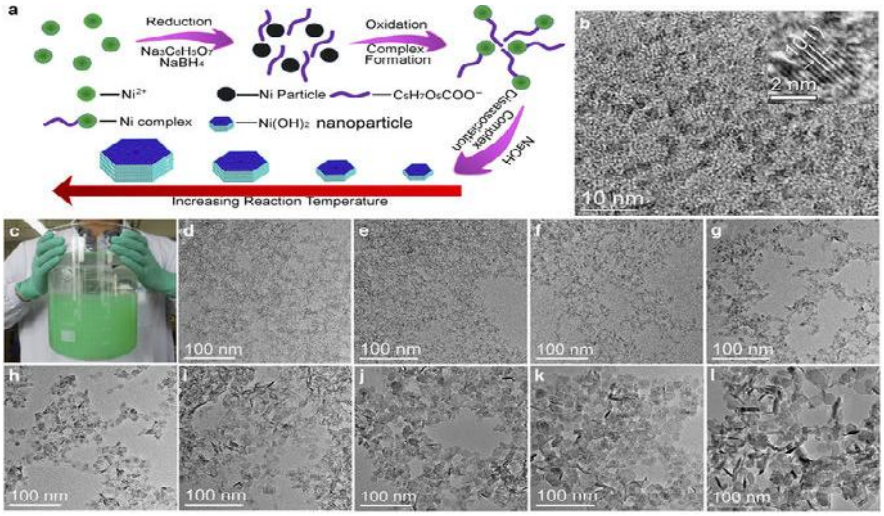

Figure 2: Nanosizing of drugs

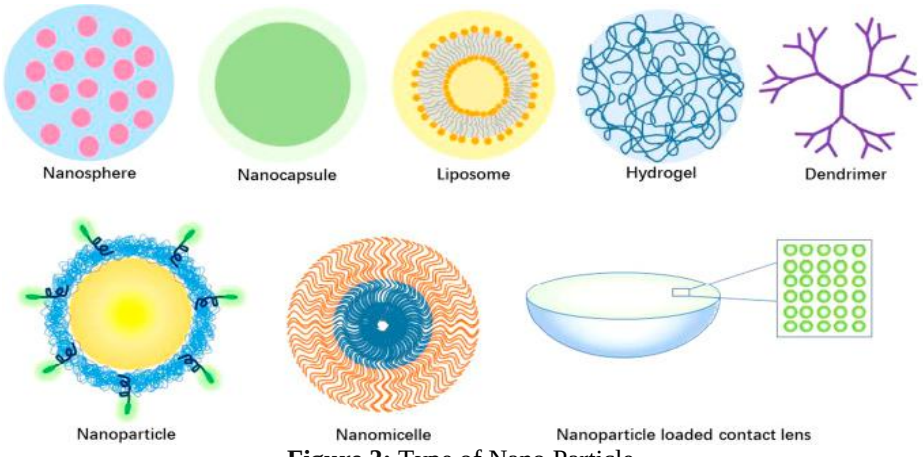

Figure 3: Type of Nano Particle

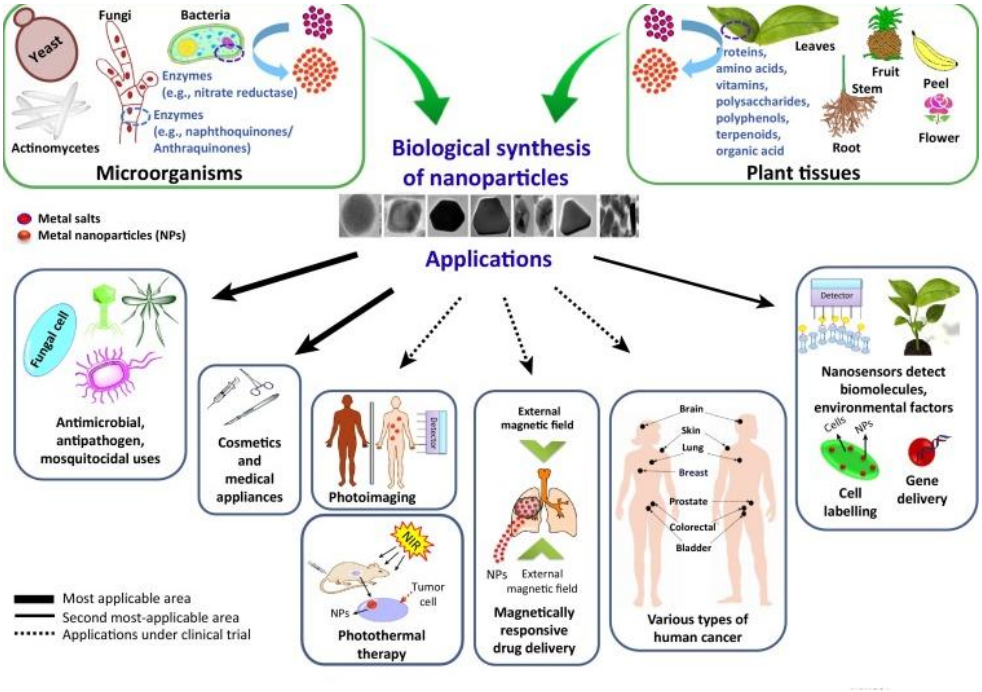

Figure 4: Biological synthesis and application

4. Conclusion

As a result of my study, it is clear that the cancer is trending a new disease and the mortality due to cancer has increased and hence the treatment to cure them also stands first. Hence, my study about cancer stimulates us to conduct programs involving awareness raising component to educate patients, family and community members about the cancer risk factors and the need for taking preventive measure to avoid developing cancer. The treatment for cancer costs high and some people doesn't know about its serious effects. So we should take a oath to provide diagnose and treatment services to all patients presenting with curable cancers such as breast, cervical and oral cancers that can be detected early. This module on diagnosis and treatment is intended to evolve in response to national need and experience. Though there are many treatments to cure cancer, there are also some side effects along with them. Although the scientists have made great strides in understanding the causes of cancer and the developing treatments, there will always be a risk for developing cancer. Finally, my study concludes by saying, as a individual, we should try to aware of the risks of exposure to suspected carcinogens and take appropriate actions to reduce our exposure.

5. Reference

[1] Klawiter M. The biopolitics of breast cancer: Changing cultures of disease and activism. U of Minnesota Press; 2008.
[2] Streiner DL, Norman GR. PDQ epidemiology. PMPH-USA; 2009.
[3] Greaves M. Cancer: the evolutionary legacy. Oxford University Press on Demand; 2001.
[4] Ezeome ER. Cancer in Nigeria: Surviving the Emerging Epidemic.
[5] McElroy A, Townsend PK. Medical anthropology in ecological perspective. Hachette UK; 2014 Nov 18.
[6] Brown P, Mikkelsen EJ. No safe place: Toxic waste, leukemia, and community action. Univ of California Press; 1997 Oct 10.
[7] Goodwin CJ, Goodwin KA. Research in psychology methods and design. John Wiley & Sons; 2016 Nov 7.
[8] Guengerich FP. Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. Cancer research. 1988 Jun 1;48(11):2946-54.
[9] Bingham S, Riboli E. Diet and cancer—the European prospective investigation into cancer and nutrition. Nature Reviews Cancer. 2004 Mar;4(3):206.
[10] Pearce N. Traditional epidemiology, modern epidemiology, and public health. American journal of public health. 1996 May;86(5):678-83.
[11] Finn OJ. Cancer vaccines: between the idea and the reality. Nature Reviews Immunology. 2003 Aug;3(8):630.
[12] Fishbein L. Potential industrial carcinogens and mutagens. Elsevier; 2011 Aug 30.
[13] Yuspa SH, Shields PG. Chemical Factors. Cancer: Principles and Practice of Oncology.:203-8.
[14] Zamanian A, Hardiman C. Electromagnetic radiation and human health: A review of sources and effects. High Frequency Electronics. 2005 Jul;4(3):16-26.
[15] Eisenbud M. Sources of ionizing radiation exposure. Environment: Science and Policy for Sustainable Development. 1984 Dec 1;26(10):6-33.

[16] McColl N, Auvinen A, Kesminiene A, Espina C, Erdmann F, de Vries E, Greinert R, Harrison J, Schüz J. European Code against Cancer 4th Edition: ionising and non-ionising radiation and cancer. Cancer epidemiology. 2015 Dec 1;39:S93-100.

[17] Kastner J. The natural radiation environment. US Atomic Energy Commission (AEC); 1968 Jan 1.

[18] de Martel C, Franceschi S. Infections and cancer: established associations and new hypotheses. Critical reviews in oncology/hematology. 2009 Jun 1;70(3):183-94.

[19] Dalton-Griffin L, Kellam P. Infectious causes of cancer and their detection. Journal of biology. 2009 Jul;8(7):67.

[20] Morales-Sánchez A, Fuentes-Pananá EM. Human viruses and cancer. Viruses. 2014 Oct 23;6(10):4047-79.

[21] De Flora S, Bonanni P. The prevention of infection-associated cancers. Carcinogenesis. 2011 Mar 24;32(6):787-95.

[22] Hoppe-Seyler F, Hoppe-Seyler K. Emerging topics in human tumor virology. International journal of cancer. 2011 Sep 15;129(6):1289-99.

[23] Lynch HT, De la Chapelle A. Genetic susceptibility to non-polyposis colorectal cancer. Journal of medical genetics. 1999 Nov 1;36(11):801-18.

[24] Choyke PL, Glenn GM, Walther MM, Zbar B, Linehan WM. Hereditary renal cancers. Radiology. 2003 Jan;226(1):33-46.

[25] Barlow WE, Lehman CD, Zheng Y, Ballard-Barbash R, Yankaskas BC, Cutter GR, Carney PA, Geller BM, Rosenberg R, Kerlikowske K, Weaver DL. Performance of diagnostic mammography for women with signs or symptoms of breast cancer. Journal of the National Cancer Institute. 2002 Aug 7;94(15):1151-9.

[26] Wasserstrom WR, Glass JP, Posner JB. Diagnosis and treatment of leptomeningeal metastases from solid tumors: experience with 90 patients. Cancer. 1982 Feb 15;49(4):759-72.

[27] Fijten GH, Starmans R, Muris JW, Schouten HJ, Blijham GH, Knottnerus JA. Predictive value of signs and symptoms for colorectal cancer in patients with rectal bleeding in general practice. Family Practice. 1995 Sep 1;12(3):279-86.

[28] Eddy DM. Variations in physician practice: the role of uncertainty. Health affairs. 1984;3(2):74-89.

[29] Blenkinsopp A, Duerden M, Blenkinsopp J. Symptoms in the pharmacy: a guide to the management of common illnesses. John Wiley & Sons; 2018 Apr 3.

[30] Blenkinsopp A, Duerden M, Blenkinsopp J. Symptoms in the pharmacy: a guide to the management of common illnesses. John Wiley & Sons; 2018 Apr 3.

[31] Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW. Activation of β-catenin-Tcf signaling in colon cancer by mutations in β-catenin or APC. Science. 1997 Mar 21;275(5307):1787-90.

[32] Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R. Identification and expansion of human colon-cancer-initiating cells. Nature. 2007 Jan;445(7123):111.

[33] Ashwin S, Ramesh J, Kumar SA, Gunavathi K. Efficient and reliable lung nodule detection using a neural network based computer aided diagnosis system. InEmerging Trends in Electrical Engineering and Energy Management (ICETEEEM), 2012 International Conference On 2012 Dec 13 (pp. 135-142). IEEE.

[34] Christopher T, Jamera BJ. A study on mining lung cancer data for increasing or decreasing disease prediction value by using ant colony optimization techniques. InProceedings of the UGC Sponsored National Conference on Advanced Networking and Applications 2015 Mar 27.

[35] Aalberts M, Stout TA, Stoorvogel W. Prostasomes: extracellular vesicles from the prostate. Reproduction. 2014 Jan 1;147(1):R1-4.

[36] Jones RE, Lopez KH. Human reproductive biology. Academic Press; 2013 Sep 28.

[37] Kurman RJ, Shih IM. The Origin and pathogenesis of epithelial ovarian cancer-a proposed unifying theory. The American journal of surgical pathology. 2010 Mar;34(3):433.

[38] Kim J, Coffey DM, Creighton CJ, Yu Z, Hawkins SM, Matzuk MM. High-grade serous ovarian cancer arises from fallopian tube in a mouse model. Proceedings of the National Academy of Sciences. 2012 Mar 6;109(10):3921-6.

[39] Fahim RB, McDonald JR, Richards JC, Ferris DO. Carcinoma of the gallbladder: a study of its modes of spread. Annals of surgery. 1962 Jul;156(1):114.

[40] Wistuba II, Gazdar AF. Gallbladder cancer: lessons from a rare tumour. Nature Reviews Cancer. 2004 Sep;4(9):695.

[41] Melicow MM. Tumors of the urinary bladder: a clinicopathological analysis of over 2500 specimens and biopsies. The Journal of urology. 1955 Oct 1;74(4):498-521.

[42] Ravich A, Stout AP, Ravich RA. Malignant granular cell myoblastoma involving the urinary bladder. Annals of Surgery. 1945 Mar;121(3):361.

[43] Papanicolaou GN, Traut HF. Diagnosis of uterine cancer by the vaginal smear. New York. 1943;46.

[44] Macnab JC, Walkinshaw SA, Cordiner JW, Clements JB. Human papillomavirus in clinically and histologically normal tissue of patients with genital cancer. New England Journal of Medicine. 1986 Oct 23;315(17):1052-8.

[45] Cooper, D.S., Doherty, G.M., Haugen, B.R., Kloos, R.T., Lee, S.L., Mandel, S.J., Mazzaferri, E.L., McIver, B., Pacini, F., Schlumberger, M. and Sherman, S.I., 2009. Revised American Thyroid Association management guidelines for patients

with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association (ATA) guidelines taskforce on thyroid nodules and differentiated thyroid cancer. *Thyroid*, *19*(11), pp.1167-1214.

[46] Singer PA, Cooper DS, Daniels GH, Ladenson PW, Greenspan FS, Levy EG, Braverman LE, Clark OH, McDougall IR, Ain KV, Dorfman SG. Treatment guidelines for patients with thyroid nodules and well-differentiated thyroid cancer. Archives of internal medicine. 1996 Oct 28;156(19):2165-72.

[47] Walther MM, Lubensky IA, Venzon D, Zbar B, Linehan WM. Prevalence of microscopic lesions in grossly normal renal parenchyma from patients with von Hippel-Lindau disease, sporadic renal cell carcinoma and no renal disease: clinical implications. The Journal of urology. 1995 Dec 1;154(6):2010-5.

[48] Rankin EB, Tomaszewski JE, Haase VH. Renal cyst development in mice with conditional inactivation of the von Hippel-Lindau tumor suppressor. Cancer research. 2006 Mar 1;66(5):2576-83.

[49] Enzinger PC, Mayer RJ. Esophageal cancer. New England Journal of Medicine. 2003 Dec 4;349(23):2241-52.

[50] Al-Sarraf M. Esophageal Carcinoma: It is Time to Move on. Oncol. 1997 Jan 1;15(1):277-84.

[51] Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. The Lancet. 1997 May 24;349(9064):1498-504.

[52] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International journal of cancer. 2010 Dec 15;127(12):2893-917.

[53] Sankaranarayanan R, Esmy PO, Rajkumar R, Muwonge R, Swaminathan R, Shanthakumari S, Fayette JM, Cherian J. Effect of visual screening on cervical cancer incidence and mortality in Tamil Nadu, India: a cluster-randomised trial. The Lancet. 2007 Aug 4;370(9585):398-406.

[54] Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA: a cancer journal for clinicians. 2007 Jan 1;57(1):43-66.

[55] Murray CJ, Lopez AD, World Health Organization. The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020: summary.

[56] Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. Cancer statistics, 2003. CA: a cancer journal for clinicians. 2003 Jan 1;53(1):5-26.

[57] Ezzati M, Lopez AD. Estimates of global mortality attributable to smoking in 2000. The Lancet. 2003 Sep 13;362(9387):847-52.

[58] Aaronson NK, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez NJ, Filiberti A, Flechtner H, Fleishman SB, de Haes JC, Kaasa S. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. JNCI: Journal of the National Cancer Institute. 1993 Mar 3;85(5):365-76.

[59] Foley KM. The treatment of cancer pain. New England Journal of Medicine. 1985 Jul 11;313(2):84-95.

[60] Wust P, Hildebrandt B, Sreenivasa G, Rau B, Gellermann J, Riess H, Felix R, Schlag PM. Hyperthermia in combined treatment of cancer. The lancet oncology. 2002 Aug 1;3(8):487-97.

[61] Schuhmacher C, Gretschel S, Lordick F, Reichardt P, Hohenberger W, Eisenberger CF, Haag C, Mauer ME, Hasan B, Welch J, Ott K. Neoadjuvant chemotherapy compared with surgery alone for locally advanced cancer of the stomach and cardia: European Organisation for Research and Treatment of Cancer randomized trial 40954. Journal of clinical oncology. 2010 Dec 10;28(35):5210

[62] Boulis-Wassif S, Gerard A, Loygue J, Camelot D, Buyse M, Duez N. Final results of a randomized trial on the treatment of rectal cancer with preoperative radiotherapy alone or in combination with 5-fluorouracil, followed by radical surgery trial of the european organization on research and treatment of cancer gastrointestinal tract cancer cooperative group. Cancer. 1984 May 1;53(9):1811-8.

[63] Lissoni P, Tancini G, Barni S, Paolorossi F, Ardizzoia A, Conti A, Maestroni G. Treatment of cancer chemotherapy-induced toxicity with the pineal hormone melatonin. Supportive Care in Cancer. 1997 Mar 1;5(2):126-9.

[64] Jacobs EM, Reeves WJ, Wood DA, Pugh R, Braunwald J, Bateman JR. Treatment of cancer with weekly intravenous 5-fluorouracil. Study by the Western Cooperative Cancer Chemotherapy Group (WCCCG). Cancer. 1971 Jun 1;27(6):1302-5.

[65] Bartelink, H., Roelofsen, F., Eschwege, F., Rougier, P., Bosset, J.F., Gonzalez, D.G., Peiffert, D., Van Glabbeke, M. and Pierart, M., 1997. Concomitant radiotherapy and chemotherapy is superior to radiotherapy alone in the treatment of locally advanced anal cancer: results of a phase III randomized trial of the European Organization for Research and Treatment of Cancer Radiotherapy and Gastrointestinal Cooperative Groups. *Journal of clinical oncology*, *15*(5), pp.2040-2049.

[66] Bijker N, Meijnen P, Peterse JL, Bogaerts J, Van Hoorebeeck I, Julien JP, Gennaro M, Rouanet P, Avril A, Fentiman IS, Bartelink H. Breast-conserving treatment with or without radiotherapy in ductal carcinoma-in-situ: ten-year results of European Organisation for Research and Treatment of Cancer randomized phase III trial 10853—a study by the EORTC Breast Cancer Cooperative Group and EORTC Radiotherapy

Group. Journal of Clinical Oncology. 2006 Jul 20;24(21):3381-7.

[67] Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. The Lancet. 2003 Aug 9;362(9382):419-27.

[68] Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. The Lancet. 2003 Aug 9;362(9382):419-27.

[69] Ascioglu S, Rex JH, De Pauw B, Bennett JE, Bille J, Crokaert F, Denning DW, Donnelly JP, Edwards JE, Erjavec Z, Fiere D. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clinical infectious diseases. 2002 Jan 1;34(1):7-14.

[70] Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G, Varadi G, Kirschbaum M, Ackerstein A, Samuel S, Amar A. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood. 1998 Feb 1;91(3):756-63.

[71] Fisher M, Yang LX. Anticancer effects and mechanisms of polysaccharide-K (PSK): implications of cancer immunotherapy. Anticancer research. 2002;22(3):1737-54.

[72] Blattman JN, Greenberg PD. Cancer immunotherapy: a treatment for the masses. Science. 2004 Jul 9; 305(5681):200-5.

[73] Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shak S, Lieberman G, Slamon DJ. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. Journal of clinical Oncology. 1999 Sep;17(9):2639-.

[74] Weiner LM, Surana R, Wang S. Monoclonal antibodies: versatile platforms for cancer immunotherapy. Nature Reviews Immunology. 2010 May;10(5):317.

[75] Mathé G, Amiel JL, Schwarzenberg L, Schneider M, Cattan A, Schlumberger JR, Hayat M, De Vassal F. Active immunotherapy for acute lymphoblastic leukaemia. The Lancet. 1969 Apr 5;293(7597):697-9.

[76] Till BG, Jensen MC, Wang J, Chen EY, Wood BL, Greisman HA, Qian X, James SE, Raubitschek A, Forman SJ, Gopal AK. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. Blood. 2008 Sep 15;112(6):2261-71.

[77] Keilholz U, Goey SH, Punt CJ, Proebstle TM, Salzmann R, Scheibenbogen C, Schadendorf D, Liénard D, Enk A, Dummer R, Hantich B. Interferon alfa-2a and interleukin-2 with or without cisplatin in metastatic melanoma: a randomized trial of the European Organization for Research and Treatment of Cancer Melanoma Cooperative Group. Journal of clinical oncology. 1997 Jul;15(7):2579-88.

[78] Kirkwood JM, Ernstoff MS. Interferons in the treatment of human cancer. Journal of Clinical Oncology. 1984 Apr;2(4):336-52.

[79] Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson CN, Lee RE, Rubin JT, Seipp CA. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. New England Journal of Medicine. 1987 Apr 9;316(15):889-97.

[80] Keilholz U, Punt CJ, Gore M, Kruit W, Patel P, Lienard D, Thomas J, Proebstle TM, Schmittel A, Schadendorf D, Velu T. Dacarbazine, cisplatin, and interferon-alfa-2b with or without interleukin-2 in metastatic melanoma: a randomized phase III trial (18951) of the European Organisation for Research and Treatment of Cancer Melanoma Group. Journal of Clinical Oncology. 2005 Sep 20;23(27):6747-55.

[81] Garber K. China approves world's first oncolytic virus therapy for cancer treatment. Journal of the National Cancer Institute. 2006 Mar 1; 98(5):298-300.

[82] Aghi M, Chou TC, Suling K, Breakefield XO, Chiocca EA. Multimodal cancer treatment mediated by a replicating oncolytic virus that delivers the oxazaphosphorine/rat cytochrome P450 2B1 and ganciclovir/herpes simplex virus thymidine kinase gene therapies. Cancer research. 1999 Aug 15; 59(16):3861-5.

[83] Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, Kammula US, Royal RE, Sherry RM, Wunderlich JR, Lee CC. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood. 2009 Jul 16; 114(3):535-46.

[84] June CH. Adoptive T cell therapy for cancer in the clinic. The Journal of clinical investigation. 2007 Jun 1;117(6):1466-76.

[85] Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nature medicine. 2004 Sep;10(9):909.

[86] Gilboa E. DC-based cancer vaccines. The Journal of clinical investigation. 2007 May 1;117(5):1195-203.

[87] Bamias A, Kastritis E, Bamia C, Moulopoulos LA, Melakopoulos I, Bozas G, Koutsoukou V, Gika D, Anagnostopoulos A, Papadimitriou C, Terpos E. Osteonecrosis of the jaw in cancer after treatment with bisphosphonates: incidence and risk factors. J Clin Oncol. 2005 Dec 1;23(34):8580-7.

[88] Diel IJ, Solomayer EF, Bastert G. Treatment of metastatic bone disease in breast cancer: bisphosphonates. Clinical breast cancer. 2000 Apr 1;1(1):43-51.

[89] Lee MV, Fong EM, Singer FR, Guenette RS. Bisphosphonate treatment inhibits the growth of prostate cancer cells. Cancer research. 2001 Mar 3;61(6):2602-8.

[90] Huber BE, Richards CA, Krenitsky TA. Retroviral-mediated gene therapy for the treatment of hepatocellular carcinoma: an innovative approach for cancer therapy. Proceedings of the National Academy of Sciences. 1991 Sep 15;88(18):8039-43.

[91] Cross D, Burmester JK. Gene therapy for cancer treatment: past, present and future. Clinical medicine & research. 2006 Sep 1;4(3):218-27.

[92] Zhou XD, Tang ZY. Cryotherapy for primary liver cancer. In Seminars in surgical oncology 1998 Mar 1, Vol. 14, No. 2, pp. 171-174. John Wiley & Sons, Inc..