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Dendrimers: Innovative Vehicles for Chemotherapeutic Drug Delivery

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Abstract

Present scenario witnesses a huge scientific interest in dendrimer based drug delivery systems due to the high level of control possible onto the tuning of their size, shape, branching length/density and surface functionalities. Owing to their unique properties, dendrimers have been used in various fields including inorganic, catalytic, synthetic organic and biomedical chemistry, in general and targeted drug delivery, in particular. Their host guest properties have made possible to trap anticancer drugs, imaging agents and other bioactive compounds *via* ionic and hydrophobic interactions and hydrogen bonding to deliver them into their biological sites of interest. Therefore, the controlled spatial and temporal release of the trapped drugs makes them one of the potential candidates in cancer diagnosis and therapy. This review discusses the state-of-the-art of the synthesis and delivery of chemotherapeutic agents *via* dendrimer based polymeric systems. Besides, efforts have been made to discuss the targeting of dendrimer therapeutics *via* both active as well as passive routes. In addition, mechanism of drug loading onto the dendrimer carriers has been discussed with utmost care. Moreover, attempts were made to discuss the recent rocketing developments in dendrimer based drug delivery systems for the efficient and safe future of cancer chemotherapy. Finally, the challenges faced by the medicinal chemists and oncologists and the future perspectives of dendrimers as chemotherapeutic modalities have been discussed.

Keywords: Dendrimers, chemotherapeutic drugs, photodynamic therapy, recent development, future challenges and perspectives.

1. Introduction

Cancer is a leading cause of death within developed nations and part of this morbidity is due to difficulties associated with its treatment. The era of organoplatinum drugs was lead by the discovery of the remarkable biological activity of *cis*-dichlorodiammineplatinum(II) (cisplatin) [1]. The potential of these organoplatinum drugs has been limited because of severe side effects that accompany their administration. Among the most debilitating side effects induced by organoplatinum drugs are severe kidney damage and extreme nausea. In an attempt to identify active but less toxic drugs literally hundreds of platinum compounds in which the structure of the amine ligand has been varied have been synthesized and evaluated for antitumour activity, but on the whole, this has been a fruitless undertaking. While some ligands impart better solubility, activity or toxicity than similar properties associated with compounds derived from simple ammonia ligands, no compound with clearly superior performance have been found. As a result the anticancer drugs used are small cytotoxic molecules that attack both cancerous and noncancerous cells due to limited selectivity of the drugs and widespread distribution of the cytotoxic molecules throughout the body. Attempts are being made towards improvement of chemotherapeutic drugs by employing formulation and particle engineering approaches. Thus, research is now focused on development of drug delivery systems that are more specific in targeting tumor tissue using both passive (such as the enhanced permeation and retention effect) and active (through the use of cancer targeting ligands) modalities, and many polynuclear macromolecules have been evaluated for this purpose [2]. Indeed, the development of multinuclear macromolecular metal-based drugs is an attractive targeted strategy currently attracting considerable attention [3-8]. Dendrimers, a class of hyperbranched polymers are a relatively new class of compounds that are being used to prepare a number of co-ordination compounds. These are being referred to as metallodendrimers. However, the targeted application of metallodendrimer as multinuclear anticancer agents is sparse and a few examples have been reported [9-14]. The first metallodendrimers to be evaluated for their anticancer properties were based on systems derivatised with platinum coordination compounds [10,11]. Over the last several years, substantial progress has been made towards the use of dendrimers for therapeutic and diagnostic purposes for the treatment of cancer, including advances in the delivery of anti-neoplastic and contrast agents, neutron capture therapy, photodynamic therapy, and photothermal therapy. Dendrimers are the systems that can be developed with high structural monodispersity, long plasma circulation times and precise control over surface structure and biodistribution properties. Chemotherapeutic drugs can be associated with dendrimers via covalent conjugation to the surface, or via encapsulation of drugs within the structure. Each of these approaches has demonstrated therapeutic benefit relative to the administration of free drug. The key properties of dendrimers that lend themselves as nano-carriers for biological applications are being identified and reviewed [15-17]. As dendrimer structures have more specialized, improved efficacy, both in-vitro and in-vivo models are a challenge to the research scientist. Various approaches have been used to enhance the therapeutic effect of the dendritic drug with less toxicity. They include improvement of solubility, microspheres, microemulsion, iontophoresis and sonophoresis, liposomes etc. The ability to customize the dendrimers to fit very specific needs makes dendrimers an exciting and promising subject of research. Targeting agents, chemotherapy drugs, and fluorescent markers are just a few of the functional groups that have been conjugated to their branches to

change the function of the dendrimers. In medicinal chemistry, the concept of multinuclearity can be used to improve the potency of chemotherapeutic drugs and dendrimers can also be used to selectively target tumour by exploiting the enhanced permeability and retention effect.

The focus of this review is on dendrimer developments from the last few years towards the treatment of cancer, with emphasis on distinct architectures and the biological responses these structures elicit. More broadly, a comprehensive survey of novel dendrimer structures and updates on existing technologies regarding cancer-specific applications is reported.

3. Synthesis of Dendrimers

Dendrimer consists of three major portions: a core, an inner shell, and an outer shell. A large number of dendrimer syntheses rely upon the traditional reactions such as the Michael reaction or the Wilkinson ether synthesis. The method used to synthesize dendrimer permit almost entire control over the critical molecular design parameters such as solubility, thermal stability and attachment of compounds for particular applications. Synthetic processes can also precisely control the size and number of branches on the dendrimer. The choice of growth reaction dictates the way in which the branching should be introduced into the dendrimers. Following are the methods used to synthesize dendrimers:

3.1 DIVERGENT METHOD:

In chemistry **divergent synthesis** is a strategy which aims to improve the efficiency of chemical synthesis. Often an alternative to convergent synthesis or linear synthesis, in one strategy it aims to generate a library of chemical compounds by first reacting a molecule with a set of reactants. The next generation of compounds is generated by further reactions with each compound of first generation. This methodology quickly diverges to large number of new compounds. For example:

1. A generates A1, A2, A3, A4, A5 in generation 1
2. A1 generates A11, A12, A13 in generation 2 and so on. (fig.1)

Previously two major designs (PPI and PAMAM) have been formulated for the synthesis of dendrimers working in stepwise divergent manner, where dendrimers grow from the reactive core, diverging into space producing higher generation dendrimers. The divergent approach is successful for the production of higher order dendrimers, each step doubling molar mass. Problems occur from side reactions and incomplete reactions of the end groups that lead to structure defects. To prevent side reactions and to force reactions to completion large excess of reagents is required. It causes some difficulties in the purification of the final product.

3.2 CONVERGENT METHOD:

Convergent synthesis aims to improve the efficiency of multi-step chemical synthesis. It is most often used in organic synthesis. Thus the overall yield quickly drops with each reaction step (fig. 2):

$A \rightarrow B \rightarrow C \rightarrow D$

Suppose the yield is 50% for each reaction, the overall yield of D in only 12.5% from A.

In a convergent synthesis

A → B (50%)

C → D (50%)

B + D → E (25%)

The overall yield of E (25%) looks much better.

The convergent approach was developed as a response to the weakness of divergent synthesis [18]. Dendrimers are built from small molecules that end up at the surface of the sphere, and reactions proceeds building inward and eventually attached to a core. This method makes it much easier to remove impurities and shorter branches along the way, so that the final dendrimer is more monodisperse. However dendrimers synthesized this way are not as large as those made by divergent methods because crowding due to steric effects along the core is limiting [19]. Convergent method is applied in the synthesis of complex molecules and involves fragment coupling and independent synthesis. Examples:

1. Convergent synthesis is encountered in dendrimer synthesis where branches (with number of generation preset) are connected to the central core.

2. Proteins of up to 300 amino acids are produced by a convergent approach using chemical ligation.

3. An example of its use in total synthesis is the final step (photochemical [2+2] cycloaddition) towards the compound *Biyouyanagin A*.

Although convergent methodology also suffers from low yields in the synthesis of large structures, it has several advantages:

1. Relatively easy to purify the desired product and the occurrence of defects in the final structure is minimized.

2. Possible to introduce subtle engineering into the dendritic structure by precise placement of functional groups at the periphery of the macromolecules.

3. This approach does not allow formation of high generation dendrimers due to steric interactions during synthesis [20].

3.3 CLICK CHEMISTRY

Click chemistry, employ Diels-Alder reactions [21], thiol-yne reactions [22] and azide-alkyne reactions to synthesize dendrimer [23,24]. Click chemistry was first fully described by K. Barry Sharoless of the Scripps Research Institute in 2001 as chemistry tailored to generate substances quickly and reliably by joining small units together. Click chemistry is not a single specific reaction. It mimics nature and generates substances by joining small modular units (fig. 3). A desirable click chemistry reaction is thus (fig. 3):

(a) Modular, (b) Wide in scope, (c) Gives very high chemical yield, (d) Generates only inoffensive byproducts, (e) Stereospecific, (f) Physicologically stable, (g) Exhibits a large thermodynamic driving force (>84 kJ/mol) to favour a reaction with a single reaction product. A distinct exothermic reaction makes a reactant "spring loaded", (h) Have high atom economy. Click methods are preferred over convergent and divergent approaches because these methods have simple reaction conditions using readily available starting materials and reagents and no use of solvent. If used, the solvent is benign or easily removed providing simple product isolation by non-chromatographic methods such as crystallization or distillation.

3.4 'DOUBLE EXPONENTIAL' AND 'MIXED' GROWTH

The most recent fundamental breakthrough in the field of dendrimer synthesis has come with the concept and implications of 'double exponential' growth. It is similar to rapid growth technique for linear polymers, involving an AB_2 monomer with orthogonal protecting groups for the A and B functionalities. This approach allows the preparation of monomers for both convergent and divergent growth from a single starting material [20]**.** The two products are reacted together to give an orthogonally protected trimer, which may be used to repeat the growth process again. The strength of double exponential growth is more subtle than the ability to build large dendrimers in relatively few steps. In fact, double exponential growth is so fast that it can be repeated only two or perhaps three times before further growth becomes impossible. The double exponential methodology provides a means whereby a dendritic fragment can be extended in either the convergent or the divergent direction as required employing the positive aspects of both approaches without the necessity to bow to their shortcomings [25] (fig.4).

Dendrimers as Chemotherapeutic Drug Delivery Vehicles

There are now more than fifty families of dendrimers, each with unique properties, since the surface, interior and core can be modified to cater to different sorts of application. Many potential applications of dendrimers are based on their unparalleled molecular uniformity, multifunctional surface and presence of internal cavities. These specific properties make dendrimers suitable for a variety of high technology uses including biomedical and industrial applications.

4. Dendrimers as Chemotherapeutic drug delivery vehicle (Fig. 5)

Biological nanodevices based on dendrimers are being developed with the potential to:

a. Recognize Cancer cells
b. Diagnose cause of cancer
c. Delivery of drug to target
d. Report location of tumor
e. Report outcome of therapy (cancer cell death)

Once a nanodevice is synthesized, its effectiveness as a delivery agent must be studied with extensive *in vitro* and *in vivo* testing. Because many cancers are unique, many delivery and targeting options must be explored to determine which targeting agent is most effective for each cell line. The side effects of cancer treatments are largely caused by cytotoxic agents invading healthy cells along with the cancerous tissue. During methods for

targeting cancer tissue specifically may minimize these side effects. Specific and efficient distribution of the therapy allows for a wider range of doses to be administered safely and more effectively. The effectiveness of smaller doses may increase due to the higher percentage of therapy molecules that reach the target cells. Larger doses of targeted therapies can be safely administered because nonspecific distribution is reduced. The enhanced permeability and retention effect (EPR) present in tumor vasculature has allowed for passive targeting of many nanodevice constructs. By taking advantage of the increased "leakiness" of tumor vasculature, nanodevices are able to pass into tumor tissue at a higher rate than healthy tissue. Passive targeting is the simplest, involving no specific targeting moiety, and has been used in many successful studies [26,27], however there are some drawbacks of passive targeting that make it undesirable. The use of passive targeting is ineffective in very young tumors which have not yet developed a vascular system, and against more developed larger tumors [26]. Early studies in lively targeting of nanodevices involved using antibodies to seek out the tumors. One study in particular used a monoclonal antibody, IB16-6, to target Murine B16 Melanoma [28]. The antibody was attached to a boronated dendrimer with an oligopeptide linker. A cysteine residue containing a reactive thiol [29] was able to link to the antibody in a way that did not hinder the antibody's function. Antibodies are being researched and show promise as specific targeting agents. In recent studies experiments are being done with nanodevices conjugated to certain ligands that bind to receptors that are over expressed in some cancers. For example, Herceptin (trastuzamab), has been used to target the HER2 gene which is expressed up to 100 times more in breast cancer tissue than in healthy mammary tissue [30]. Epidermal Growth Factor (EGF) has become a promising targeting agent for severe forms of head and neck cancers. Although using EGF as a targeting agent has worked well *in vitro*, *in vivo* studies showed that the EGF targeted nanodevice accumulated in the liver of mice [28]. Folic acid is also being researched upon as potential target [30-33]. Folic acid is a carbon source for DNA methylation and is required for DNA synthesis. As cancer is a disease of rapid cell division, it requires an excess of folic acid for cell growth. Most types of human epithelial cancers including breast, lung, brain, and ovarian, over express high affinity folic acid receptors [34]. Researchers are exploiting this uniqueness in some cancer types to specifically target nanodevices to those cancer cells using folic acid. Folic acid as a targeting agent includes 1) low cost, 2) high solubility, 3) low immunogenicity, and 4) that it is relatively simple to conjugate. The high affinity (kd = 0.1 to 1 nM) of folic acid to the folic acid receptor (FAR) is not significantly affected by conjugation [35]. The high binding affinity of folic acid-targeted nanodevices, relative to free folic acid, is attributed to the multivalent binding characteristics described by Banaszak Holl *et al*. [32]. Folic acid has served as a targeting agent for several successful bi- and tri-functional nanodevices [31]. Lectins, a class of proteins that are responsible for cell-to-cell recognition, may be another moiety used for drug targeting. In the body, lectins can be found free-floating or embedded within the plasma membrane of cells. Lectins are characteristically sugar-binding proteins that can be both multivalent and highly specific. Lectins are already used as probes for certain markers on tumorogenic cells; it would only be a small jump to utilize natural lectins as targeting moieties. Targeting with lectins is quite different than other targeting strategies in that the nanodevice is actively seeking the cell. Unlike folic acid, for example, where the receptor on the cell is active in the binding process, the binding molecule is part of the nanodevice and is searching for cancer cell signals [36].

4. THERAPY

Several treatment strategies are being incorporated into a wide range of therapeutic nanodevices. In many applications the dendrimer is utilized simply as a vehicle for more efficient distribution of already-established anti-cancer molecules. The applicability of dendrimers is not limited to the encapsulation or conjugation of cytotoxic chemotherapy drugs. Many therapeutic options ranging from thoroughly studied gene transfections to novel applications of Nitric Oxide [37] are being investigated as possible dendrimers based treatments.

4.1 Delivery systems for Anticancer Drugs (fig. 6)

Chemotherapy although the necessity in cancer treatment is currently limited by dangerous side affects that occur with high doses of cytotoxic agents. Thus, the success of a drug depends upon how quickly it races to eradicate the cancerous tissue before causing serious harm to the patient. By decreasing the poisonous effects of chemotherapy, more potent drugs can be approved as aggressive treatment options. The chemotherapy drug Methotrexate (MTX) has been extensively studied as a therapeutic agent, which can be incorporated into a dendrimer-based nanodevice. Methotrexate works by inhibiting the enzyme dihydrofolate reductase (DHFR), an essential enzyme for mitosis. Successful incorporation of Methotrexate into dendrimer-based nanodevices through encapsulation [38] and conjugation [27,33] has been reported. One study comparing encapsulated to conjugated Methotrexate found that the conjugated nanodevices were better suited for drug delivery [39]. Encapsulated Methotrexate performed similarly to free Methotrexate, signifying that it may exhibit premature drug release *in vivo* [39]. The advantage of dendrimer-bound Methotrexate is not attributed to an increase in potency; in fact, one study showed free Methotrexate to be more effective at similar dosages [33]. The study reasoned that the folic acid-targeting moieties of the nanodevice counteracted the Methotrexate by functioning as a competitive inhibitor of the drug. Dendrimer-based nanodevices, utilizing Methotrexate as a therapy agent, are promising because stronger doses can be administered without triggering any serious side effects. A doxorubicin (DOX)-functionalized dendrimer has shown great promise as a novel therapeutic agent [26]. Successful results were found in a trial using DOX-conjugated polyester bow tie dendrimers to treat mice infected with c-26 colon carcinoma [26]. The DOX was conjugated to the dendrimer through a hydrazone formation with a hydrazide linker. A high concentration of the nanodevice in aqueous solution suggested that the POE arms of the bow tie dendrimer successfully sheltered the hydrophobic drug within the core of the dendrimer. Nearly complete disruption of the hydrazone bonds in low pH (pH 5.0) in 48 hours is indicative of a possible drug release mechanism. A safe dosage of this nanodevice was found to induce complete tumor regression in one treatment group of a 60-day experiment [26]. A family of very potent chemotherapy drugs, camptothecins, has previously failed in clinical trials because of poor water solubility. *In vitro* testing using camptothecins encapsulated in polyester dendrimers advocates for the premise that the applicability of this family of cancer drugs should be reconsidered. The cytotoxicity of two dendrimer encapsulated camptothecins on four types of cancer tissue has been investigated by M. Morgan *et al*. [40]. Their study suggested that camptothecins may safely be administered *in vivo* through a dendrimer-based nanodevice. Encapsulation of camptothecins within a dendrimer enhanced the uptake and retention of the therapeutic compound. The functionality of a multifunctional nanodevice, tested *in vitro*, demonstrated the possibility of using dendrimers to administer Taxol is an already established anti-cancer drug. Taxol is conjugated to the dendrimer through a multi-step synthesis of an ester linkage [30] that must be cleaved to activate the drug. Cleavage of the ester linkage occurs in the malignant cell by a reaction that was not described but is most likely an enzymatic

hydrolysis reaction [30]. The trifunctional PAMAM dendrimer conjugate, G5-Ac-FITC-FAOH- Taxol, was targeted by folic acid to KB cells that overexpress the folic acid receptor. Cytotoxicity assays showed that the dendrimer conjugate administered at 50 nM was cytotoxic towards KB (FR+) cells. Respectively, there was no apparent cytotoxicity towards KB (FR-) cells at doses under 100 nM [30]. The FITC moiety of the conjugate served as a means to observe the specificity and distribution achieved by folic acid targeting. The results of this experiment affirm the complete functionality of the multifunctional dendrimer-based nanodevice.

4.2 Agents for Photodynamic Therapy (fig.7)

Utilizing light energy to initiate cell death is just one of several unique treatment methods that can be applied to cancer therapy. Photodynamic therapy (PDT) is currently being applied as an alternative approach for treating superficial tumors. The theory behind this treatment is that energy from photons can excite light-sensitive molecules within a cell to form highly reactive species. These highly reactive species disrupt the delicate balance within a cell, initiating cell death either through necrosis or apoptosis. Researchers are studying at least two applications of dendrimers that could generate more effective methods for administering photodynamic therapy. Utilizing dendrimers as delivery agents has shown many advantages over conventional means of photosensitizer delivery. High molecular weight and hydrophilicity are most favorable qualities of dendrimers that have been cited as advantageous over free photosensitizers [41]. Efficient delivery systems may reduce side effects such as prolonged skin sensitivity and dark toxicity [41]. By having some control over the distribution of the photosensitizers, improvements in the potential applications of PDT have been achieved. For photodynamic therapy to be effective, large quantities of photosensitizers must be delivered into the cell. Experimenting with dendrimers as delivery agents through encapsulation of photosensitive molecules has produced some promising conclusions. A study pertaining to the encapsulation of rose bengal (RB) and protoporphyrin IX (PpIX) within pegylated dendrimers had success with *in vitro* models [42]. In this study, the combination of protoporphyrin IX (PpIX) within a pegylated poly(propylene imine) dendrimer was found to be a very effective photosensitizing agent. The results indicated that success through encapsulation can in part be attributed to the fact that no photosensitizer release is necessary for effectiveness [41]. Light irradiation may still produce the highly reactive species which can escape the dendrimer without a significant loss in cytotoxicity [42]. The unique architecture of dendrimers allows for the dendrimer itself to be applied as a novel photosensitizing agent. Porphyrins from third-generation aryl ether dendrimers perform as an antenna complex to enhance the photodynamic effect of zinc porphyrins [41]. Furthermore, dendrimers with peripheral chromophores double the amount of light energy absorbed with each additional generation because of the doubling of the number of peripheral branches [43]. Analysis after photo-irradiation showed that both higher cytotoxicity against cancer cells and lower damage to healthy cells was achieved by the dendrimer agent over a control of free protoporphyrin IX (PpIX) [41]. Unlike the PpIX, which disrupts membrane organelles [44], it appears that the dendrimer agent functions by hindering Adenosine triphosphate (ATP) production in cells. Passing light energy from the peripheral to the core of the dendrimers complex is comparable to the way that natural photosystems function [45]. Like many novel therapies, improvements to photodynamic therapy can be made that will undoubtedly expand its applicability. One unique barrier to the expansion of photodynamic therapy applications is caused by the transmittance properties of light. Longer wavelengths of light, 750-1000 nm, are more transmissive through organic tissue and

thereby travel deeper into the body [46] than the shorter wavelengths which are often used for photodynamic therapy [41]. Light that is characterized as having shorter wavelengths (300-400) is currently used to promote the maximum absorption by photosensitive agents. The challenge facing researchers is to synthesize novel photosensitizing agents that preferentially absorb longer wavelengths of light, which are more transmissive through organic tissue. A novel strategy of exploiting fluorescence resonance energy transfer (FRET) in conjunction with two-photon absorption (TPA) for photodynamic therapy (PDT) applications is being developed. The role of the dendrimer in this new strategy is similar to other methods in that it serves as a delivery agent and possible amplifier for photosensitizers. In this study a porphyrin and aluminum core was found to be an efficient generator of singlet oxygen, a highly reactive species. These photosensitizers use longer wavelengths of light (750-1000 nm), which are much more transmissive through the human body. This method could serve as a minimally invasive procedure for administering photodynamic therapy to deep tumors [46].

4.3 Vectors in Gene Therapy (fig. 8)

The fundamental link between cancer and DNA indicates that the gene therapy will be most effective cancer treatment option of the future. Understanding the correlation between flaws in one's genomes and the occurrence of cancer is a pivotal step in discovering a cure. There are many more milestones between today's technology and an "end-all" cure for cancer. Scientists have the ability to successfully establish reproducible methods for altering the genetics of simple organisms for many years, however, finding a way to safely alter the genetics of more complex subjects, including humans, is one hurdle that is yet to be overcome [47]. Scientists are now pursuing the dendrimers as their vector of choice for administering safe and effective gene therapies. Using dendrimers as a non-viral gene transfection agent had a long and contentious past. A study dating back to 1995 by Kukowska-Latallo *et al*. reported that efficient *in vitro* transfection of several mammalian stem cell lines can be achieved with DNA-dendrimer complexes [48]. Since then several studies have came out both in support of and in opposition to applications of gene therapy. The clinical applicability of a dendrimer-based gene transfection agent is yet to be established; many basic principles of applicability are still highly debated. Establishment of the most efficient dendrimer construct for DNA transfection has been by no means a simple task. Studies have reported mixed results on the relationship between dendrimer generation and the efficiency of DNA complexation. Because the phosphate backbone of DNA is negatively charged, positively charged cationic dendrimers are used to complex with DNA. Despite the fact that low generation dendrimers (G<3) are easiest to produce and least cytotoxic, their small size and limited surface charge prevent efficient DNA complexation thereby limiting their use in gene transfer [49]. The positive correlation between generation size and transfection efficiency shows that higher generation dendrimers are better transfection vectors than their lower generation predecessors. In this study, transfected gene expression increased noteworthy 2-3 orders of magnitude between transfer vectors of generation 4 and 5 [50]. However, due to their dense surface charge, higher-generation PAMAM dendrimers have been found to accumulate into large aggregates that would strictly limit their *in vivo* applicability [48]. Even the most accepted dendrimers transfection agents (G6 and G7 PAMAM) are overshadowed by the disadvantages of the spherical dendritic shape [51]. Nevertheless, research has shown that it is possible for a generation 6 dendrimer to reach a cell's nucleus within a reasonable amount of time, 25-45 minutes, depending on cell type [52]. One simple modification to the dendrimer architecture has

improved the DNA-binding properties of PAMAM dendrimers. The process of activation involves heating the dendrimer in an appropriate solvolytic solvent over a period of time. The hydrolytic cleavage of amido bonds within the PAMAM dendrimer removes branches, reducing the overall molecular weight, and carboxyl groups form in place of the missing branches. Activation of the PAMAM dendrimers increases the flexibility of the dendrimer architecture and thereby improves the DNA complexation efficiency [53,54]. Removal of branches also decreases the overall surface charge, which in turn reduce the amount of aggregation seen in higher-generation dendrimers [51,53,55]. The relatively poor performance of this technology in many experiments has called for the novel strategies to improve dendrimer-mediated DNA transfection. The positive cytotoxic effects of DNA transfection can be improved by using a polypropylenimine dendrimer vector which exhibits natural anti-cancer properties [56]. A Newkome dendritic scaffold composed of spermines, which naturally interact with DNA, demonstrates improved DNA binding [55]. A novel transfection vector described as a poly(amidoamine) dendrimer-capped mesoporous silica nanosphere (G2-MSN) has exhibited promising results *in vitro* [49]. Also, the use of Chloroquine [55] or Tyloxapol [57] has been shown to improve transfection efficiency by increasing the porosity of cellular and endosomal membranes.

4.4 Devices for Boron Neutron Capture Therapy (Fig. 9)

Boron neutron capture therapy (BNCT), a contemporary method for inducing apoptosis, is an effectual alternative to conventional cancer therapies. BNCT invokes a neutron beam, created by either a nuclear reactor or a particle accelerator, to excite boron atoms within a cell. Low-energy thermal neutrons are captured by 10Boron to form 11Boron for a small fraction of a second before separating into a high energy alpha particle and a Lithium ion, both toxic to the cell [28]. However, for this therapy to be effective, it is estimated that 109 atoms of boron per cell [28] or 20 μg of boron per gram of tumor [58] must be delivered. Research is being done to determine if dendrimer-based nanodevices can successfully distribute such large quantities of boron specifically to cancerous tissue. Because BNCT research is primarily focused on high grade gliomas, the need for a very specific delivery agent is of utmost importance. In order for BNCT to be clinically accepted, damage to the delicate neural tissue surrounding gliomas must be kept to a minimum. Targeted dendrimers have shown the level of specificity required for BNCT in many other applications. Researchers are developing dendrimers that fulfill requirements—high boron content and specific delivery—for employment in BNCT. In 1995 a group of scientists from Germany created a poly(lysine) dendrimer conjugated to 80 boron atoms, a sufficient number if specific delivery can be achieved [29]. More recently researchers have conjugated up to at least 1100 boron atoms [59] and estimate the possibility of conjugating 2500 boron atoms to G5 or G6 Starburst PAMAM dendrimers [60]. Such high boron/dendrimer ratios are achieved by conjugation of polyhedral borane to the many terminal groups of the dendrimer. Several tumor-specific targeting strategies are being researched to determine how to properly deliver the high number of dendrimers needed for BNCT to be effective. Accumulations of boronated dendrimers in the liver and spleen have been reduced with the addition of PEO chains to the periphery of the dendrimers [28]. The anti-EGFR mAb cetuximab, already approved for clinical use, has been the targeting moiety of choice for several BNCT studies. A conjugate of highly boronated dendrimer with cetuximab has demonstrated specificity to tumor cells both *in vitro* and *in vivo* towards F98EGFR glioma [59]. In a follow-up study utilizing BNCT to treat fisher rats infected with F98EGFR glioma,

one experimental group exhibited over a 100% increase in lifetime over an untreated control group [59]. An alternative approach to specifically delivering boron using a very similar PAMAM dendrimer construct has also been tested. In these experiments decaborate clusters and a strand of poly(ethylene glycol) [60] were conjugated to a G3 PAMAM dendrimer. These nanodevices exploited the over-expression of the folic acid receptor protein of some brain sarcomas. While this construct did exhibit tumor specific targeting *in vitro, in vivo* biodistribution resulted in a much higher uptake by the hepatic and renal systems than by the tumor—38.8 % ID/g and 62.8 % ID/g vs. 6.0 % ID/g, respectively.

4.5 Apoptosis Sensing Agents (fig. 10)

Monitoring the performance of the nanotherapeutics in real-time would be advantageous in both experimental and clinical applications. Implementing an apoptotic sensor into nanodevices provides a system for which the real-time analysis of a therapy's efficacy can be analyzed. At the Institute of Laser Life Science, part of the South China Normal University, Da Xing *et al*. have been using real-time apoptosis sensing to monitor the effects of photodynamic therapy [61]. These experiments not only supported the applicability of photodynamic therapy (PDT) but also demonstrated apoptosis detection on a single-cell level. Activation of caspase-3 is signaled by an increase in the donor chromophore (enhanced cyan fluorescent protein, ECFP) fluorescence and a decrease in the acceptor chromophore (Venus) fluorescence. The ratio of ECFP/Venus fluorescence in a single cell was analyzed through confocal microscopy. Real-time analysis of the expression of caspase-3 coincides with analysis of the apoptosis pathway [86]. The Michigan Nanotechnology Institute for Medicine and Biological Sciences (MNIMBS) improved apoptosis sensing by using a PAMAM dendrimer to deliver the FRET probe specifically to tumor cells. Both a targeting moiety (folic acid) and a FRET reagent (PhiPhiLux G1G2) were conjugated to a PAMAM dendrimer. Because PhiPhiLux G1G2 is activated by caspase-3, it becomes an ideal detector of apoptosis at the cellular level. The study found that KB cells that expressed the folate receptor actively took in the targeted apoptosis-detecting nanodevice. Internalization of the PhiPhiLux G1G2 by cells not expressing the folate receptor was minimized, providing better contrast between the healthy and apoptotic cells. One current limitation on this technology is that *in vitro* fluorescence analysis is done through flow cytometry, which cannot be used for *in vivo* applications [30]. Development of a two-photon optical fiber fluorescence (TPOFF) probe may allow real-time *in vivo* fluorescence measurements. This technology has been used to detect real time fluorescence of KB cells implanted in mice. The optical fiber was inserted with a 27-gauge needle into anesthetized mice at different positions around the tumor. This method of fluorescence measurement could be used as a minimally invasive technique for analyzing deep tumors [62]. The use of a double-clad photonic crystal fiber demonstrated the ability to detect fluorescence with an order of magnitude greater sensitivity than flow cytometry [63]. A combination of using a TPOFF probe in conjunction with targeted FRET reagents may someday be used to monitor tumor regression in cancer patients[62].

5. Targeting of Dendrimer Therapeutics

5.1 Passive Targeting (fig.11)

Therapeutic macromolecules including dendrimer-based drug delivery systems exploit the pathophysiological patterns of solid tumors, particularly their leaky vasculature, to preferentially extravasate and accumulate in

tumor tissue in a process known as enhanced permeability and retention (EPR) effect [64]. The amount of dendrimer-based drug delivery systems that accumulates in tumor tissue is influenced by their size, molecular weight, and surface charge, which affect their residence time in the systemic circulation, transport across the endothelial barrier, and nonspecific recognition and uptake by RES [65] El-Sayed et al. studied the effect of size, molecular weight, and surface charge on the permeability of fluorescently labeled PAMAM-NH_2 (G0-G4) dendrimers across epithelial and endothelial barriers [66-68]. Their data showed that the increase in dendrimers size/molecular weight results in a corresponding exponential increase in their extravasation time (τ) across the microvascular endothelium of the cremaster muscle preparation of Syrian hamsters [68]. A subsequent investigation by Kobayashi and co-workers studied the biodistribution of Gadolinium-functionalized G2-NH_2 to G10-NH_2 conjugates administered intravenously into normal mice [69]. Results showed that Gadolinium-functionalized G2-G4 dendrimers were quickly excreted in urine after 3 min of their intravenous injection whereas G5 and higher generations displayed limited renal secretion because of their larger hydrodynamic volume [69]. These results clearly indicate the influence of dendrimers size/hydrodynamic volume on their transport across the microvascular endothelium in vivo. Cationic dendrimers show high nonspecific uptake by the RES particularly in the liver and lungs, which reduces their accumulation in tumor tissue [70-75]. Upon comparing the biodistribution of cationic G5-NH_2 dendrimers and their neutral counterparts prepared by partial or full acetylation of the surface amine groups in nude mice bearing melanoma and prostate tumors, results showed that both dendrimers displayed a similar distribution profile to all major organs within 1 h after dendrimers injection with particularly high accumulation in the lungs, kidneys, and liver (27.9-28.6% ID/g) [74] While the cationic and neutral dendrimers displayed similar biodistribution profiles, cationic dendrimers showed higher net accumulation in each organ due to their favorable electrostatic interaction with the negatively charged epithelial and endothelial cell surface. It is interesting to note that all polylysines [70] anionic PAMAM-COOH dendrimers [75] and polyester dendrimers [71] exhibit high distribution to the liver and quick elimination into the urine. This biodistribution profile can be attributed to the dendrimer's small hydrodynamic volumes, which results in less than 5% of the initial dose remaining in the systemic circulation 24 h after administration [71,72]. Attachment of PEG arms to the dendrimer surface increases their size and molecular weight, thus reducing their systemic clearance and improving their biocompatibility [70,76-78]. Specifically, attachment of PEG chains with molecular weight up to 20 kDa to the dendrimer's surface groups increases their plasma half-life to 50 h for G3 polyester dendrimers [70,76] 75.4 h for polylysine dendrimers [70] and 100 h for triazine dendrimers [77]. Bhadra et al. showed that the attachment of PEG (5 kDa) chains to 25% of the surface groups of G4-NH_2 dendrimers results in a 3-fold reduction in their hemolytic activity compared to the parent dendrimers [79]. Another in vivo study showed that intraperitoneal administration of melamine dendrimers into Swiss- Webster mice induces significant hepatic toxicity at doses g10 mg/kg,118 whereas PEGylation of 50% of the surface NH_2 groups would enhance its biocompatibility and increase the tolerated dose to 1 g/kg [80]. These studies clearly indicate the positive effect of surface PEGylation of PAMAM dendrimers by enhancing their plasma residence time and reducing their nonspecific toxicity.

5.2 Active Targeting (fig. 12)

Active targeting of polymer-drug conjugates to cancer cells is commonly achieved by conjugation of tumor-specific targeting ligands such as vitamins, carbohydrate residues, peptides or antibodies, which selectively bind to receptors that are expressed on the surface of cancer cells. Binding of these ligands to the receptors displayed on cancer cell surface triggers receptor-mediated endocytosis and internalization of the whole conjugate into cancer cells. Dendrimers-based drug delivery systems exploit similar targeting strategies to bypass the nonspecific uptake by the RES systems and increase their net accumulation in cancer cells [78,82]. Baker and co-workers have extensively utilized folic acid (FA) as a targeting ligand for their dendrimers-based drug delivery systems [74,82-86]. The rationale behind using FA as a targeting ligand is its affinity to folic acid receptors (FAR) overexpressed by human breast, lung, and brain tumors [82]. Attachment of 5 FA ligands per dendrimers appeared to trigger receptor-mediated endocytosis into cancer cells [87] FA-targeted dendrimers-based drug delivery systems exhibit substantially higher accumulation and toxicity in FAR-positive cancer cells compared to non targeted dendrimers [88,89] and free anticancer drugs [90,91]. Specifically, 100% of FAR-positive KB nasopharnyx cancer cells take up FA-targeted G5-MTX conjugates [88] (G5-FA-MTX) after 30 min of incubation, which is approximately a 20-fold increase in cellular accumulation compared to nontargeted G5-MTX conjugates Nontargeted G5-MTX conjugates are less toxic (IC50 ≈ 1.0 μM MTX equiv) compared to targeted G5-FA-MTX conjugates (IC50 ≈ 0.3 μM MTX equiv) when incubated for 72 h with FAR-positive KB cells.126 Incubation of targeted G5-FA-MTX conjugates (150 nM equivalent concentration of MTX) with KB cells for 24 h resulted in a 40% reduction in cell proliferation, while no significant difference was observed in cell proliferation between the nontargeted G5-MTX conjugates [92] and the control While G5-FA-MTX conjugates were found to be more cytotoxic than G5-MTX conjugates, there was little difference in their therapeutic activity when incubated with FAR-negative cells [89,91], which clearly indicates that FA targeting and the associated internalization process is selective for the FAR-positive cancer cells. It is interesting to note that utilizing FA as a targeting agent in the in vitro efficacy studies by Baker's group was believed to limit the overall anticancer activity of the G5-FA-MTX conjugates since MTX exhibits its anticancer activity through an antifolate pathway. This indicates that the FA incorporated in G5-FA-MTX conjugates can effectively "rescue" the FA-deprived cancer cells from the anticancer effects of MTX and act as an apoptosis reversing agent [88,89]. This was later confirmed by in vitro cytotoxicity results of G5 FA-MTX conjugates incubated with FAR positive cells, which showed a dramatic decrease in conjugate's toxicity in the presence of free FA. Specifically, viability of KB cells treated with the highest concentration of G5-FA-MTX conjugates (225 nM equiv of MTX) went from 50% viability in FA-free medium to 100% viability in the presence of 50 μM free FA [92]. In vivo biodistribution studies of targeted G5-FA conjugates in KB tumor-bearing mice showed a 4-fold increase in their tumor accumulation compared to non-targeted G5 dendrimers 4 days after administration [82]. As a result of this improved tumor localization of targeted conjugates, biweekly doses of G5-FA-MTX conjugates (at MTX equivalent doses of 5.0-7.2 mg/kg) into KB tumor-bearing SCID mice led to survival rates that were roughly 40 days longer than the mice receiving parallel and equal doses of non-targeted G5-MTX conjugates [82]. Other groups used different targeting agents including peptides to direct dendrimers-based drug delivery systems to cancer-specific receptors. Falciani et al. used neurotensin (NT) peptides to develop NT-targeted dendrimers carrying chlorin e6 (Che6) and MTX chemotherapeutic agents to different malignancies expressing the neurotensin receptor, which include colon, pancreatic, prostate, and small-cell lung carcinomas

[93]. Treatment of HT29 tumor-bearing mice with targeted NT-MTX conjugates for 20 days showed reduction in tumor size to approximately one-third the size of the tumors in mice receiving saline, free MTX, or scrambled NT-MTX conjugates at an equal drug concentration, which indicates the therapeutic benefit of the targeting approach [93]. Another example is reported by Hildgen and co-workers, who developed G2 polyether-copolyester (PEPE) dendrimer- MTX inclusion complexes for treatment of brain tumors, which utilized D-glucosamine ligands displayed on the conjugate's surface to target the GLUT-1 transporter highly expressed on the luminal side of the endothelial cells of the blood-brain barrier and glioma cancer cells [94]. In vitro studies showed that targeted PEPE-MTX conjugates exhibited 2-8-fold higher accumulation into glioma cells, which resulted in 2-4.5-fold higher cytotoxicity compared to nontargeted dendrimers [94]. Other targeted dendrimers conjugates utilized tetrameric avidin glycoproteins to target lectins differentially expressed on the surface of ovarian carcinoma cells [95]. Yet another PAMAM construct utilized J591 antibodies to target the prostate-specific membrane antigen (PSMA), which is a glycoprotein expressed by all prostate cancer cells and supporting vasculature [96]. Effective targeting of dendrimers-based drug delivery systems requires the choice of a selective ligand and optimization of the ligand valency to tune the binding and dissociation rates of the targeted conjugates to their specific receptor. Baker and co-workers studied the binding kinetics of G5-NH_2 dendrimers displaying cyclic Arg-Gly-Asp (RGD) ligands that selectively target the Rv_3 integrin receptors expressed solely during angiogenesis and thereby present in high numbers in rapidly growing tumor capillaries [97]. Binding of the targeted G5-RGD conjugates to Rv_3 integrin receptors of human umbilical vein endothelial cells was 150 times slower than free RGD [97], and the associated equilibrium disassociation constant (*K*D) of the targeted conjugates was 522 times lower compared to that of free RGD. Dijkgraaf et al. studied tumor-specific accumulation of RGD-targeted dendrimers displaying mono-, di-, and tetrameric cyclic RGD ligands in nude mice bearing SKRC- 52 renal carcinoma, which showed that tumor accumulation increased linearly with the increase in the number of RGD ligands ranging from 0.46% ID/g for the monomeric RGD conjugates to 1.52% ID/g for the trimeric ones [98]. Baker and co-workers carried out a quantitative and systematic analysis of the effect of FA density on the binding of FA-targeted dendrimers to FAR-positive cells [87]. Incubation of G5-NH_2 dendrimers displaying 2.6-13.7 FA ligands per dendrimer with KB cells for 1 h showed that the association constant (*K*a) increased linearly with the increase in number of FA ligands while the dissociation constant (*K*D) improved exponentially (2 500 - 166 700 fold) with the increase in FA ligand density compared to free FA. This kinetic profile suggests that targeted dendrimer conjugates are preferentially taken up by targeted cancer cells not as a result of any increase in the endocytic rate but rather due to longer residence times of the conjugates on the cell surface [87].

6. Mechanisms of Drug Loading onto Dendrimer Carriers

6.1 Physical Encapsulation of Drug Molecules

The work of Vogtle and co-workers, who looked at entrapment of guest molecules into branched polymers, represents an earlier form of physical encapsulation of poorly soluble drug molecules in dendrimer voids to improve their aqueous solubility and control their release profile [79,84,99-102]. Inclusion of hydrophobic molecules into dendrimers is typically accomplished by simple mixing of the polymer and drug solutions where

the hydrophobic drug associates with the nonpolar core through hydrophobic intereactions [84,99-101]. As a result of this physical interface between the guest molecules and the dendrimer carrier, release of the encapsulated molecules in an aqueous environment is passively controlled by a range of noncovalent interactions including hydrophobic forces, hydrogen bonding, steric hindrance, and electrostatic interactions. To maximize the loading capacity of drug molecules within the dendrimer, one has to carefully consider polymer architecture, specifically the characteristics of the internal voids. Initial computational and experimental studies by Goddard and Tomalia showed that G1-G3-alanine dendrimers exhibit an oblong open structure while G4 and higher generations possess a densely packed surface that is necessary to produce enclosed internal voids that can effectively encapsulate and retain guest molecules [101,103]. Spin-lattice relaxation profiles of acetyl salicyclic acid and 2,4-dichlorophenoxy acetic acid encapsulated within a dendritic carrier displayed a decline in carbon-13 relaxation time with increasing dendrimer's generation number from G0.5-G5.5, thus indicating the shielding of the guest molecules in the polymer network. These findings set the stage for development of different inclusion complexes where dendrimers can encapsulate hydrophobic anticancer drugs to improve their aqueous solubility, control their release rates, and achieve cancer therapy. Kojima et al. reported the encapsulation of DOX and MTX anticancer drugs in PEGylated G3-NH_2 and G4-NH_2 dendrimers with a maximum DOX and MTX encapsulation efficiency of 6.5 mol/mol dendrimer and 25 mol/mol dendrimer, respectively [100]. The encapsulation efficiency of both drugs appeared to increase with the increase in dendrimer generation number and the increase in the molecular weight of the surface-bound PEG chains from 550 Da to 2 kDa. These results were further supported by another study that compared the in vitro and in vivo release of 5-fluorouracil (5FU) encapsulated in non-PEGylated G4-NH_2 dendrimers and PEGylated ones displaying 25% capping of the surface groups using 5 kDa PEG chains [79]. The in vitro data indicates that the PEGylated dendrimers show 12-fold higher loading capacity and 6-fold slower release of 5FU drug molecules compared to non-PEGylated dendrimers with complete drug release from the PEGylated carriers in 6 days [79]. Furthermore, intravenous administration of PAMAM-5FU complexes (1000 μg 5FU equiv) to albino rats showed that the residence time of 5FU in the systemic circulation achieved by the PEGylated complexes was 3 times longer than that for the non-PEGylated derivatives [79]. These results indicate that the attachment of PEG chains to the dendrimer surface not only slows down the release of the encapsulated drug but also modulates the conformation of the internal voids, thereby improving drug loading efficiency. Similarly, another study showed that MTX encapsulation into G2 PEPE dendrimers improved when PEG chains (200-400 Da) were present in the internal cavities and increased with the increase in PEG molecular weight [94]. However, attachment of four glucosamine molecules to the dendrimer surface decreased the encapsulation of MTX molecules [94]. As expected, PEGylation improved MTX loading (20.3-24.5 mg of MTX/mg of dendrimer) and slowed its release through PEG steric effects, whereas attachment of glucosamine ligands to the dendrimers led to a 10%-15% decline in MTX encapsulation, which is possibly due to folding of the conjugated glucosamine molecules into the dendritic structure, causing congestion of the dendrimer surface and limiting the penetration of the MTX molecules. Despite these improvements in the encapsulation and retention of molecules into PEGylated dendrimers, sustained and controlled release of the encapsulated molecules in physiological solutions remains almost inaccessible. Similarly, Baker and co-workers reported 70% release of the MTX loaded into G5-MTX inclusion complexes upon incubation for 2.5 h in phosphate buffered saline (PBS) compared to insignificant

MTX release in water under the same experimental conditions [84]. Grinstaff and coworkers also reported the release of 90% of the anticancer drug 10-hydroxycaptothecin (10HCPT) loaded into G4.5 PGLSA dendrimers upon incubation for 2.5 h in PBS [99]. It is important to note that PEPE-MTX [94] and PGLSA-10HPCT [104] inclusion complexes exhibited 10- and 4-fold higher cytotoxicity against cancer cells compared to equal concentrations of the free drug, respectively. However, this enhanced anticancer activity is simply a result of rapid bolus release of the encapsulated drug due to the interaction of the buffer salts with the dendrimers, thus weakening the ionic forces "holding" the loaded drug, which will happen in vivo upon administration of these inclusion complexes, resulting in premature drug release into the systemic circulation causing nonspecific toxicity. One approach to control the rate of drug release from the inclusion complexes is to encapsulate them in a liposomal envelope forming modulatory liposomal controlled release systems (MLCRS).138 DOX was the drug used in this hybrid system where 3.7 mol of DOX were loaded per 1 mol of G4-NH2 dendrimers. In this study, *N*-tris(hydroxymethyl)- methyl-2-aminoethanesulfonic acid (TES) buffer (pH 7.5) resulted in 96.6% loading of the added DOX compared to 68.9% DOX loading in acetate buffer (pH 4.5), which is possibly due to the limited electrostatic repulsion between the cationic dendrimers and ionized DOX molecules at pH 4.5. Incubation of MLCRS in cell culture medium at 37 °C for 48 h resulted in release of 12% of the loaded DOX, which is a significant improvement compared to the observed rapid drug release with the conventional inclusion complexes. These studies collectively show that loading of therapeutic molecules into different dendrimers depends on dendrimers generation number, internal composition, net surface charge, and type and degree of functionalization of surface groups. These parameters affect the volume of the internal voids and the physical interactions between guest molecules and the dendrimers core, thus controlling the degree of drug loading and the associated release kinetics. Nevertheless, the issue of rapid drug release from dendrimers-based inclusion complexes still remains a significant challenge. While liposomal encapsulated complexes seem promising in terms of controlling the release rates of the encapsulated drugs, their activity against different tumors need to be further evaluated both in vitro and in vivo.

6.2 Chemical Conjugation of Drug Molecules

Covalent conjugation of anticancer drugs to dendrimer surface groups has been used to achieve controlled spatial and temporal release of the attached drugs. The large number of dendrimer surface groups and the versatility in their chemical structures allow the conjugation of different anticancer drugs, imaging agents, and/or targeting ligands while maintaining the compact spherical geometry of dendrimer in solution.

6.2.1 Direct Coupling

In the early 1990s, Barth and co-workers conjugated boronated monoclonal antibodies to a dendrimer carrier via stable urea linkages and utilized this conjugate for neutron capture therapy where localized neutron ionization would cause necrosis of neighboring cancer cells [73,105]. This conjugate achieved high loading capacities of 250-1000 boron atoms per G4 dendrimer while retaining 82% of the antibodies activity in vitro. A few years later, Duncan and coworkers reported the coupling of cisplatin (Pt), a hydrophobic DNA intercalating agent, to G3.5 PAMAM dendrimers via an ester linkage [106] PAMAM-Pt conjugates carried 20-25 weight % platinum exhibiting 10-fold higher aqueous solubility compared to free Pt and displayed great stability (<1% Pt release)

upon incubation in PBS (pH 7.4) and citrate buffers (pH 5.5) at 37 °C for 72 h [106]. Despite the high aqueous solubility and stability of these conjugates, they failed to produce the desired anticancer activity due to limited drug release. Specifically, PAMAM-Pt conjugates displayed insignificant toxicity towards three cancer cells lines when treated with 0.1 × 10-5-0.01 mg/mL Pt equivalent for 72 h [106]. Similarly, PAMAM-DOX conjugates exhibited 5-fold lower toxicity toward HeLa cells upon incubation with 0.001-1000 μM DOX equivalent for 24 h compared to free DOX, which is a result of insignificant drug release (<5% of DOX is released upon incubation in PBS solution for 24 h) [107]. These observations were further supported by a separate evaluation of the in vitro and in vivo activity of amide-linked PAMAM-MTX conjugates where results showed that PAMAM-MTX has 2.7 log units higher IC50 values on glioma cells compared to free MTX in vitro, and there was no increase in the survival rate of glioma-bearing rats receiving the PAMAM-MTX conjugates compared to those receiving an equal dose of free MTX [104]. Other classes of therapeutic molecules including Che6 and DOX anticancer drugs [71,108,93] natural curcumin derivatives [109], BH_3 pro-apoptotic peptide [91], and photosensitizing agents [110,111] were coupled to a dendritic carrier, which significantly increased the solubility of the loaded drug; however, the associated in vitro and in vivo anticancer activity markedly decreased due to limited release of the loaded drug. Studies also demonstrated that the conformation of the anticancer drug molecules displayed on the dendrimer surface is a critical design parameter for retention of their cytotoxic activity. Gurdag et al. compared the anticancer activity of MTX when coupled through its amine group to the carboxylic acid surface groups of G2.5-COOH dendrimers forming stable amide linkages versus MTX coupling through its carboxylic group to the primary amine groups of G3-NH_2 dendrimers [112]. Results showed that G2.5-MTX conjugates were 3-fold more cytotoxic compared to free MTX toward lymphoblastic leukemia cells, whereas G3-MTX conjugates were 10-fold less toxic than the free MTX. Similarly, Baker and co-workers compared the in vitro anticancer activity of G5-OH and G5-NH_2 conjugates with MTX attached via ester and amide linkages, respectively [83]. Incubation of esterlinked G5-MTX conjugates with KB cells at a concentration of 1-100 nM MTX equiv resulted in a 10-fold lower IC50 value compared to amide-linked G5-MTX conjugates, which is a result of faster hydrolysis of the ester linkages and release of the incorporated MTX drug molecules [83]. This data was further supported by Minko's report showing that ester-linked PAMAM-TAX conjugates release 25% of the loaded TAX upon incubation for 24 h in PBS solution and produce a 10-fold decrease in the IC50 value observed upon incubation with human ovarian carcinoma cells for 24 h compared to the free drug [113].

6.2.2 pH-Sensitive Linkages

In order to achieve cancer cell-specific delivery and release of anticancer drugs, studies were undertaken to develop the dendrimer-drug conjugates with hydrolyzable linkages. Specifically, the linkages in question has to remain intact in the systemic circulation but quickly degrade once internalized into the cancer cell and release the attached drug to produce the desired therapeutic activity. The induction of pH-sensitive linkages into dendrimer-drug conjugates seemed to fit the desired criteria as they remain stable in the systemic circulation (pH 7.4) but quickly hydrolyze in acidic environment (pH 5-6) like the endosomes/lysosomes, thus releasing the incorporated drug inside the target cell. In 2006 Szoka and co-workers reported the synthesis of asymmetric bow-tie polyester G3-G4 dendrimers. DOX was conjugated to the G4 side via either a pH-sensitive hydrazone

(hyd) or a carbamate linkage to yield dendrimer- hyd-DOX and dendrimer-DOX conjugates, respectively. Dendrimer-hyd-DOX conjugates were stable at pH 7.4 as indicated by the release of <10% of the incorporated DOX compared to the release of 100% of the attached DOX upon incubation at pH 5.0 for 48 h. Dendrimer-hyd-DOX conjugates were more cytotoxic toward colon carcinoma cells with an IC50 of 1.4 μg of DOX/ mL compared to carbamate-linked dendrimer-DOX conjugates with an IC50 of 2.0 μg of DOX/mL upon incubation for 72 hr [71,108]. In addition, dendrimer-hyd-DOX conjugates displayed a remarkable anticancer activity in vivo where a single injection at 20 mg/kg of DOX equiv administered 8 days after tumor implantation resulted in complete tumor regression and 100% survival of the treated animals for 60 days.34 Subsequent reports confirmed the higher in vitro and in vivo anticancer activity of dendrimer-hyd-DOX conjugates compared to amide-linked conjugates and the free drug [107,111]. For example, G4-hyd-DOX conjugates (IC50) 8.7 μM) were nearly 7 times more cytotoxic toward HeLa cells compared to G4-amide-DOX conjugates (IC50) 60.2 μM). In addition, G4-hyd-DOX conjugates proved to be equally effective against DOX-sensitive and –resistant cells, whereas free DOX was 58 times less effective in inducing apoptosis in resistant cancer cells [107]. Fluorescence microscopy studies of Ca9-22 cells separately treated with G4.5-hyd-DOX and G4.5-amide-DOX conjugates revealed that the hydrolysis of the hydrazone linkage allows the liberated DOX molecules to enter the nucleus, whereas G4.5-amide-DOX conjugates fail to release the incorporated drug, thus limiting its access to the nucleus and diminishing its therapeutic activity [111]. It is interesting to note that another study showed that smaller G0-DOX conjugates were able to enter the nucleus regardless of the linkage chemistry [107]. While these pH-sensitive linkages represent a significant improvement over noncleavable conjugates for intracellular drug delivery of anticancer drugs, they only sense the acidity of the endosomal compartment but fail to differentiate between cancer cells and normal healthy ones. Therefore, further selectivity of drug release from dendrimer conjugates can be achieved by development of novel chemical linkages that are sensitive to cancer-specific markers such as intracellular enzymes. This will allow the release of the incorporated drug only in response to these enzymes, which are solely expressed by cancer cells. Initial studies showed that incubation of 1,3,5-tris(3-aminopropyl)benzene dendrimers displaying specific amino acids on their surface, which include phenylalanine, methionine, aspartic acid, or diaminopropionic acid, with proteolytic enzymes would selectively cleave these amino acids with cleavage rate dependent on the dendrimer generation number [114]. This initial report suggests the potential of enzyme-sensitive conjugates for cancer cell-specific drug delivery.

7. Recent developments in dendrimer based drug delivery systems

The development of efficient and biocompatible non-viral vectors for gene therapy remains a great challenge, and exploiting the properties of both nanoparticle carriers and cationic polymers is an attractive approach. In this work, gold nanoparticle (AuNP) polyamidoamine (PAMAM) conjugates were developed for the use as non-viral transfection agents. AuPAMAM conjugates were prepared by crosslinking PAMAM dendrimers to carboxylic-terminated AuNPs via EDC and sulfo-NHS chemistry. EDC and sulfo-NHS have been utilized widely and in numerous applications such as amino acid coupling; however, their use in the coupling of PAMAM dendrimers to AuNPs presents new challenges to form effective and stable constructs for delivery that have not yet been examined. Enhanced colloidal stability and DNA condensation ability was established by

probing two critical synthetic parameters: the reaction rate of the PAMAM crosslinking step, and the amine to carboxyl ratio. Based on this work, increasing the amine to carboxyl ratio during conjugation of PAMAM onto AuNPs yielded the optimal vector with respect to colloidal stability and transfection efficiency in vitro. AuPAMAM conjugates present attractive candidates for non-viral gene delivery due to their commercial availability, ease of fabrication and scale up, high yield, high transfection efficiency and low cytotoxicity [115].

8. Conclusion

Dendrimers being relatively new class of compounds offer abundant opportunities for research in the field. The molecules themselves are a challenge as well as solution in cancer drug development research. They can be exploited as nano drug carriers and can themselves be developed as cancer drugs. Metallodendrimers are a promising answer to the search of a versatile molecule which can act as a drug, a drug carrier and a drug delivery system. A lot of work has been done in these fields and a lot more needs to be done in order to harness the full potential of these wonderful molecules. Unlike many diseases which can be attributed to a definitive cause, such as a virus, bacteria, or genetic defect; many simple things that people are exposed to on a daily basis can predispose one to the onset of cancer. Creating a vaccine or a cure for cancer may be impossible because of the great variety of causes and complications with varying types of cancers. The therapies described in this review will not eradicate cancer but will more likely improve the ways in which it can be handled. Improved diagnostic techniques combined with treatments that offer increased potency with less risk will provide for a more optimistic prognosis of cancer patients. A disease that now is a major health threat to all people may someday be thought of simply as a manageable side effect of aging.

References

Chauvin, Y.; Gilbert, B.; Guibard. I. Vib. Spectrosc. **1991**, *1*, 299–304

1.B.A. Howell, D. Fan, proceedings of royal society, 2009, DOI:10.1098/rspa.2009.0359

2.C.G. Hartinger, A.D. Phillips, A.A. Nazarov, Curr. Top. Med. Chem. 2011 **11** 2688–2702.

3.N.P.E. Barry, O. Zava, W. Wu, J. Zhao, B. Therrien, Inorg. Chem. Commun. 2012 **18** 25–28.

4.B. Therrien, Eur. J. Inorg. Chem., 2009 2445–2453.

5.W. Kandioller, C.G. Hartinger, A.A. Nazarov, C. Bartel, M. Skocic, M.A. Jakupec, V.B. Arion, B.K. Keppler, Chem. Eur. J. 2009 **15** 12283–12291.

6.W.H. Ang, E. Daldini, L. Juillerat-Jeanneret, P.J. Dyson, Inorg. Chem. 2007 **46** 9048–9050.

7.M.G. Mendoza-Ferri, C.G. Hartinger, A.A. Nazarov, R.E. Eichinger, M.A. Jakupec, K. Severin, B.K. Keppler, Organometallics. 2009 **28** 6260–6265.

8.T. Gianferrara, A. Bergamo, I. Bratsos, B. Milani, C. Spagnul, G. Sava, E. Alessio, J. Med. Chem. 2010 **53** 4678–4690.

9.P. Govender, B. Therrien, G.S. Smith, Eur. J. Inorg. Chem. 2012 2853–2862.

10. B.A. Jansen, J.v.d. Zwan, J. Reedijk, H.D. Dulk, J. Brouwer, Eur. J. Inorg. Chem. 1999 **9** 1429–1433.

11. N. Malik, E.G. Evagorou, R. Duncan, 1999 **10** 767–776.
12. T. Kapp, A. Dullin, R. Gust, J. Med. Chem. 2006 **49** 1182–1190.
13. X. Zhao, A.C.J. Loo, P.P.-F. Lee, T.T.Y. Tan, C.K. Chu, J. Inorg. Biochem. 2010 **104** 105–110.
14. T. Ahamad, S. F. Mapolie, S. M. Alshehri, Med. Chem. Res. 2010 **21** 2023-2031.
15. C.C. Lee, J.A. MacKay, J.M. Fréchet, F.C. Szoka, Nat. Biotechnol. 2005 **23** 1517–1526.
16. H. Yang, W.J. Kao, J. Biomater. Sci. Polym. Ed. 2006 **17** 3–19.
17. S. Svenson, D.A. Tomalia, Adv. Drug Deliv. Rev. 2005 **57** 2106–2129.
18. C.J. Hawker, J.M.J. Frechet, J. Am. Chem. Soc. 1990 **112** 7638–7647.
19. H. Paul; C.R. Vas, T. Harper, Dendrimers: Technology White Papers, Cientifica, 2003.
20. S. Sonke, D.A Tomalia, Advanced Drug Delivery Reviews. 2005 **57** 2106 – 2129.
21. G. Franc, A.K. Kakkar, Chem. Eur. J. 2009 **15** 5630-5639.
22. K.L. Killops, L.M. Campos, C.J. Hawker, J. Am. Chem. Soc. 2008 **130** 5062–5064.
23. K. Noda, Y. Minatogawa, T. Higuchi, Jpn. J. Psychiatry Neurol. 1991 **45** 107–8.
24. J.P. Machaiah, Indian J. Exp. Biol. 1991 **29** 463–7.
25. K.Barbara, B. Maria, Acta Biochimica Polonica, 2001 **48** 199–208.
26. C.C. Lee, E.R. Gillies, M.E. Fox, S.J. Guillaudeu, J.M.J Fréchet, E.E. Dy, F.C. Szoka, Proc. Natl. Acad. Sci. U.S.A. 2006 **103** 16649-16654.
27. S. Gurdag, J. Khandare, S. Stapels, L.H. Matherly, R.M. Kannan, Bioconjug. Chem. 2006 **17** 275-283.
28. E.R. Gillies, J.M.J. Fréchet, Drug Discov. Today. 2005 **10** 35-43.
29. B. Qualmann, M.M. Kessels, H.J. Musiol, W.D. Sierralta, P.W. Jungblut, L. Moroder, Angew. Chem. Int. Ed. 1996 **35** 909-911.
30. I.J. Majoros, A. Myc, T. Thomas, C.B. Mehta, J.R. Baker, Biomacromolecules. 2006 **7** 572-579.
31. I.J. Majoros, T.P. Thomas, C.B. Mehta, J.R. Baker, J. Med. Chem. 2005 **48** 5892-5899.
32. S. Hong, P.R. Leroueil, I.J. Majoros, B.G. Orr, J.R. Baker, M.M. Banaszak Holl, Chem. Biol. 2007 **14** 107-115.
33. T.P. Thomas, I.J. Majoros, A. Kotlyar, J.F. Kukowska-Latallo, A. Bielinska, A. Myc, J.R. Baker, J. Med. Chem. 2005 **48** 3729-3735.
34. Y. Choi, J.R. Baker, Cell Cycle. 2005 **4** 669-671.
35. I.J. Majoros, A. Becker, T. Thomas, R. Shukla, X. Shi, in: Majoros, I. J; Baker (eds.), Pan Stanford Publishing, 2008 103-158.
36. R.S. Singh, A.K. Tiwary, J.F. Kennedy, Crit. Rev. Biotechnol. 1999 **19** 145-178.
37. B. Bonavida, S. Khineche, S. Huerta-Yepez, H. Garban, Drug Resist. Updates. 2006 **9** 157-173.
38. D.A. Tomalia, L.A. Reyna, S. Svenson, Biochem. Soc. Trans. 2007 **35** 61-67.
39. A.K. Patri, J.F. Kukowska-Latallo, J.R. Baker, Adv. Drug Deliv. Rev. 2005 **57** 2203-2214.
40. M. T. Morgan, Y. Nakanishi, D.J. Kroll, A.P. Griset, M.A. Carnahan, M. Wathier, N.H. Oberlies, G. Manikumar, M.C. Wani, M.W. Grinstaff, Cancer Res. 2006 **66** 11913-11921.
41. N. Nishiyama, H.R. Stapert, G.D. Zhang, D. Takasu, D.L. Jiang, T. Nagano, T. Aida, K. Kataoka, Bioconjug. Chem. 2003 **14** 58-66.
42. C. Kojima, Y. Toi, A. Harada, K. Kono, Bioconjug. Chem. 2007 **18** 663-670.

43. A. Adronov, S.L. Gilat, J.M.J. Fréchet, K. Ohta, F.V.R. Neuwahl, G.R. Fleming, J. Am. Chem. Soc. 2000 **122** 1175-1185.
44. D. Kessel, Proc. SPIE. 2005 **5689** 1-8.
45. A. Adronov, J.M.J. Fréchet, Chem. Commun. 2000 1701-1710.
46. M.A. Oar, W.R. Dichtel, J.M. Serin, J.M.J. Fréchet, J.E. Rogers, J.E. Slagle, P.A. Fleitz, L.S. Tan, T.Y. Ohulchanskyy, P.N. Prasad, Chem. Mater. 2006 **18** 3682-3692.
47. S. Akhtar, Non-Viral cancer gene therapy: beyond delivery, Gene Therapy. 13 (2006) 739-740.
48. J.F. Kukowska-Latallo, A.U. Bielinska, J. Johnson, R. Spindler, D.A. Tomalia, J.R. Baker, Proc. Natl. Acad. Sci. USA. 1996 **93** 4897-4902.
49. R.R. Daniela, C.Y. Lai, K. Jeftinija, E.W. Rowe, S. Jeftinija, V.S.Y. Lin, J. Am. Chem. Soc. 2004 **126** 13216-13217.
50. J. Haensler, F.C. Szoka, Bioconjug. Chem. 1993 **4** 372-379.
51. M. Mannisto, S. Vanderkerken, V. Toncheva, M. Elomaa, M. Ruponen, E. Schacht, A. Urtti, J. Contr. Release. 2002 **83** 169-182.
52. P. Ruenraroengsak, K.T. Al-Jamal, Ni. Hartell, K. Braeckmans, S.C. De Smedt, A.T. Florence, Int. J. Pharm. 2007 **331** 215-219.
53. J. Dennig, E. Duncan, Rev. Mol. Biotechnol. 2002 **90** 339-347.
54. M. Tang, C.T. Redemann, F.C. Szoka, Bioconjug. Chem. 1996 **7** 703-714.
55. J.G. Hardy, M.A. Kostiainen, D.K. Smith, N.P. Gabrielson, D.W. Pack, Bioconjug. Chem. 2006 **17** 172-178.
56. C. Dufes, W.N. Keith, A. Bilsland, I. Proutski, I.F. Uchegbu, A.G. Schaetzlein, Cancer Res. 2005 **65** 8079-8084.
57. J.F. Kukowska-Latallo, C. Chen, J. Eichman, A.U. Bielinska, J.R. Baker, Biochem. Biophys. Res. Commun. 1999 **264** 253-261.
58. R.F. Barth, J.A. Coderre, M.G.H. Vicente, T.E. Blue, Clin. Cancer Res. 2005 **11** 3987-4002.
59. G. Wu, W. Yang, R.F. Barth, S. Kawabata, M. Swindall, A.K. Bandyopadhyaya, W. Tjarks, B. Khorsandi, T.E. Blue, A.K. Ferketich, M. Yang, G.A. Christoforidis, T.J. Sferra, P.J. Binns, K.J. Riley, M.J. Ciesielski, R.A. Fenstermaker, Clin. Cancer Res. 2007 **13** 1260-1268.
60. S. Shukla, G. Wu, M. Chatterjee, W. Yang, M. Sekido, L.A. Diop, R. Mueller, J.J. Sudimack, R.J. Lee, R.F. Barth, W. Tjarks, Bioconjug. Chem. 2003 **14** 158-167.
61. Y. Wu, D. Xing, S. Luo, Y. Tang, Q. Chen, Cancer Lett. 2006 **235** 239-247.
62. T.P. Thomas, J.Y. Ye, C.S. Yang, M. Myaing, I.J. Majoros, A. Kotlyar, Z. Cao, T.B. Norris, J.R. Baker, Proc. SPIE. 2006 **6095**. DOI: 10.1117/12.645084
63. M.T. Myaing, J.Y. Ye, T.B. Norris, T.P. Thomas, J.R. Baker, Proc. SPIE. 2004 **5317** 151-157.
64. H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, Controlled Release. 2000 **65** 271-284.
65. R. Haag, F. Kratz, Angew. Chem., Int. Ed. 2006 **45** 1198.
66. M. El-Sayed, M. Ginski, C.A. Rhodes, H. Ghandehari, J. Bioact. Compat. Polym. 2003 **18** 7-22.
67. M. El-Sayed, C.A. Rhodes, M. Ginski, H. Ghandehari, Int. J. Pharm. 2003 **265** 151-157.
68. M. El-Sayed, M.F. Kiani, M.D. Naimark, A.H. Hikal, H. Ghandehari, Pharm. Res. 2001 **18** 23-28.

69. H. Kobayashi, M.W. Brechbiel, AdV. Drug Delivery ReV. 2005 **57** 2271.
70. L.M. Kaminskas, B. Boyd, P. Karellas, G.Y. Krippner, R. Lessene, B. Kelly, C.J.H. Porter, Mol. Pharm. 2006 **3(5)** 614-627.
71. O.L. Padilla De Jess, H.R. Ihre, L. Gagne, J.M.J. Frechet, F.C. Szoka, Bioconj. Chem. 2002 **13** 453-461.
72. T. Okuda, S. Kawakami, N. Akimoto, T. Niidome, F. Yamashita, M. Hashida, J. Controlled Release. 2006 **116** 330-336.
73. R.F. Barth, D.M. Adams, A.H. Soloway, F. Alam, M.V. Darby, Bioconj. Chem. 1994 **5** (58-66.
74. S.S. Nigavekar, L.Y. Sung, M. Llanes, A. El-Jawahri, T.S. Lawrence, C.W. Becker, L. Balogh, M.K. Khan, Pharm. Res. 2004 **21** 476-483.
75. N. Malik, R. Wiwattanapatapee, R. Klopsch, K. Lorenz, H. Frey, J.W. Weenerc, E.W. Meijerc, W. Paulusd, R. Duncan, J. Controlled Release. 2000 **65** 133-148.
76. E.R. Gillies, J.M.J. Frechet, Drug DiscoVery Today. 2005 **10** 35-43.
77. J. Lim, Y. Guo, C.L. Rostollan, J. Stanfield, J.T. Hsieh, X. Sun, E.E. Simanek, Mol. Pharm. 2008 **5** 540-547.
78. V. Gajbhiye, P.V. Kumar, R.K. Tekade, N.K. Jain, Curr. Pharm. Des. 2007 **13** 415-429.
79. D. Bhadra, S. Bhadra, S. Jain, N.K. Jain, Int. J. Pharm. 2003 **257** 111-124.
80. H.T. Chen, M.F. Neerman, A.R. Parrish, E.E. Simanek, J. Am. Chem. Soc. 2004 **126** 10044-10048.
81. S. Battah, S. Balaratnam, A. Casas, S. O'Neill, C. Edwards, A. Batlle, P. Dobbin, A. MacRobert, Bioconj. chem. 2001 **12(6)** 980-988.
82. J.F. Kukowska-Latallo, K.A. Candido, Z. Cao, S.S. Nigavekar, I.J. Majoros, T.P. Thomas, L.P. Balogh, M.K. Khan, J.R. Baker, Cancer Res. 2005 **65** 5317-5324.
83. D.A. Tomalia, A.M. Naylor, W.A. Goddard, Angew. Chem., Int. Ed. 1990 **29** 138-175.
84. A. Quintana, E. Raczka, L. Piehler, I. Lee, A. Myc, I. Majoros, A.K. Patri, T. Thomas, J. Mule, J.R. Baker, Pharm. Res. 2002 **19** 1310-1316.
85. Y. Choi, T. Thomas, A. Kotlyar, M.T. Islam, J.R. Baker, Chem. Biol. 2005 **12** 35-43.
86. A. Myc, I.J. Majoros, T.P. Thomas, J.R. Baker, Biomacromolecules. 2007 **8** 13-18.
87. S. Hong, P.R. Leroueil, I.J. Majoros, B.G. Orr, J.R. Baker, M.M.B. Holl, Chem. Biol. 2007 **14** 107-115.
88. A. Quintana, E. Raczka, L. Piehler, I. Lee, A. Myc, I. Majoros, A.K. Patri, T. Thomas, J. Mule, J.R. Baker, Pharm. Res. 2002 **19** 1310-1316.
89. A.K. Patri, J.F. Kukowska-Latallo, J.R. Baker, Adv. Drug Delivery Rev. 2005 **57** 2203.
90. I.J. Majoros, A. Myc, T. Thomas, C.B. Mehta, J.R. Baker, Biomacromolecules. 2006 **7** 572-579.
91. A. Myc, A.K. Patri, J.R. Baker, Biomacromolecules. 2007 **8** 2986-2989.
92. T.P. Thomas, I.J. Majoros, A. Kotlyar, J.F. Kukowska-Latallo, A. Bielinska, A. Myc, J.R. Baker, J. Med. Chem. 2005 **48** 3729.
93. C. Falciani, M. Fabbrini, A. Pini, L. Lozzi, B. Lelli, S. Pileri, J. Brunetti, S. Bindi, S. Scali, L. Bracci, Mol. Cancer Ther. 2007 **6** 2441-2448.
94. R.S. Dhanikula, A. Argaw, J.F. Bouchard, P. Hildgen, Mol. Pharm. 2008 **5** 105-116.
95. H. Xu, C.A.S. Regino, Y. Koyama, Y. Hama, A.J. Gunn, M. Bernardo, H. Kobayashi, P.L. Choyke, M.W. Brechbiel, Bioconj. Chem. 2007 **18** 1474-1482.

96. A.K. Patri, A. Myc, J. Beals, T.P. Thomas, N.H. Bander, J.R. Baker, Bioconj. Chem. 2004 **15** 1174-1181.

97. R. Shukla, T.P. Thomas, J. Peters, A. Kotlyar, A. Myc, J.R. Baker, Chem. Commun. 2005 5739-5741.

98. I. Dijkgraaf, A.Y. Rijnders, A. Soede, A. Dechesne, G.W.V. Esse, A.J. Brouwer, F.H.M. Corstens, O.C. Boerman, D.T.S. Rijkers, R.M. Liskamp, J. Org. Biomol. Chem. 2007 **5** 935-944.

99. M.T. Morgan, Y. Nakanishi, D.J. Kroll, A.P. Griset, M.A. Carnahan, M. Wathier, N.H. Oberlies, G. Manikumar, M.C. Wani, M.W. Grinstaff, Cancer Res. 2006 **66** 11913-11921.

100. C. Kojima, K. Kono, K. Maruyama, T. Takagishi, Bioconj. Chem. 2000 **11** 910-917.

101. M.T. Morgan, M.A. Carnahan, C.E. Immoos, A.A. Ribeiro, S. Finkelstein, S.J. Lee, M.W. Grinstaff J. Am. Chem. Soc. 2003 **125** 15485-15489.

102. A.S. Chauhan, S. Svenson, L. Reyna, D. Tomalia Mater. Matters. 2007 **2** 24-26.

103. A.M. Naylor, W.A. Goddard, G.E. Keifer, D.A. Tomalia, J. Am. Chem. Soc. 1989 **111** 2339-2341.

104. G. Wu, R.F. Barth, W. Yang, S. Kawabata, L. Zhang, K. Green- Church, Mol. Cancer Ther. 2006 **5** 52-59.

105. F. Alam, A.H. Soloway, R.F. Barth, N. Mafune, D.M. Adams, W.H. Knoth, J. Med. Chem. 1989 **32** (1989) 2326.

106. N. Malik, E.G. Evagorou, R. Duncan, Anticancer Drugs. 1999 **10** (767-776.

107. K. Kono, C. Kojima, N. Hayashi, E. Nishisaka, K. Kiura, S. Watarai, A. Harada, Polymer. 2008 **49** 2832-2838.

108. C.C. Lee, E.R. Gillies, M.E. Fox, S.J. Guillaudeu, J.M.J. Frechet, E.E. Dy, F.C. Szoka, Proc. Natl. Acad. Sci. 2006 **103** 16649-16654.

109. W. Shi, S. Dolai, S. Rizk, A. Hussain, H. Tariq, S. Averick, W. L'Amoreaux, A.E. Idrissi, P. Banerjee, K. Raja, Org. Lett. 2007 **9** 5461-5464.

110. S. Battah, S. Balaratnam, A. Casas, S. O'Neill, C. Edwards, A. Batlle, P. Dobbin, A. MacRobert, Bioconj. chem. 2001 **12(6)** 980-988.

111. P.S. Lai, P.J. Lou, C.L. Peng, C.L. Pai, W.N. Yen, M.Y. Huang, T..H. Young, M.J. Shieh, J. Controlled Release. 2007 **122** 39-46.

112. S. Gurdag, J. Khandare, S. Stapels, L.H. Matherly, R.M. Kannan, Bioconj. Chem. 2006 **17** 275-283.

113. J.J. Khandare, S. Jayant, A. Singh, P. Chandna, Y. Wang, N. Vorsa, T. Minko, Bioconj. Chem. 2006 **17** 1464-1472.

114. T. Kapp, P. Francke, R. ChemMedChem. 2008 **3** 635-641.

115. R.E. Figueroa, A.Y. Lin, J. Yan, L. Luo, A.E. Foster, R.A. Drezek, Biomaterial 2014 **35** 1725-1734.

Figure1: Divergent Growth Method [advances in the chemistry of dendrimers, Marta Sowinska and Zofia Urbanczyk-Lipkowska, *New J. Chem.*, 2014, **38**, 2168-2203]

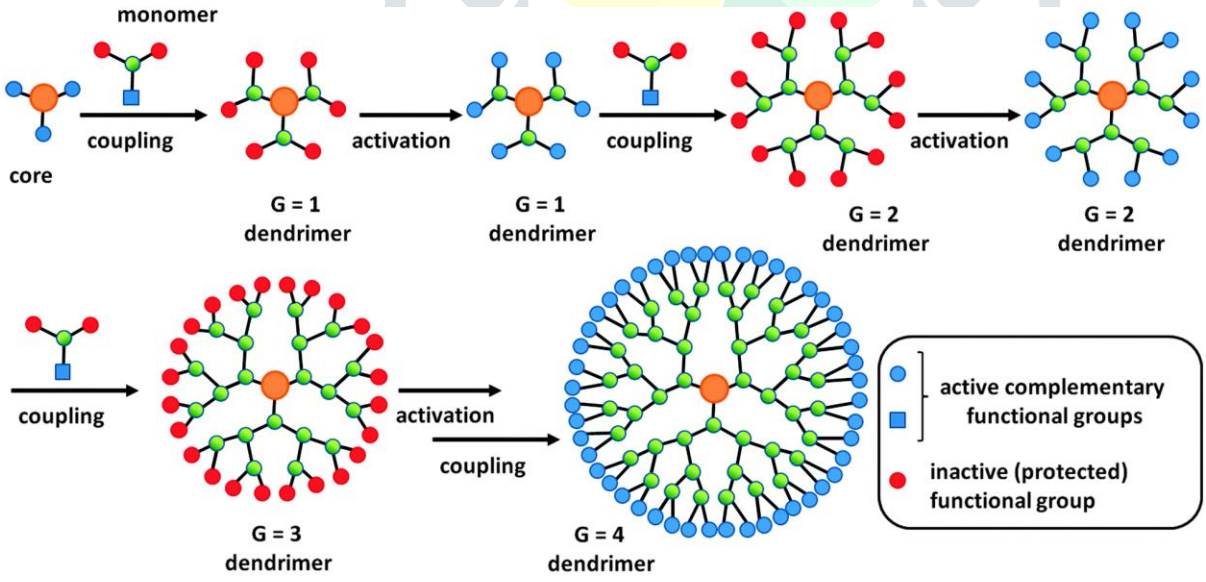

Figure2: Convergent Growth Method [advances in the chemistry of dendrimers, Marta Sowinska and Zofia Urbanczyk-Lipkowska, *New J. Chem.*, 2014, **38**, 2168-2203]

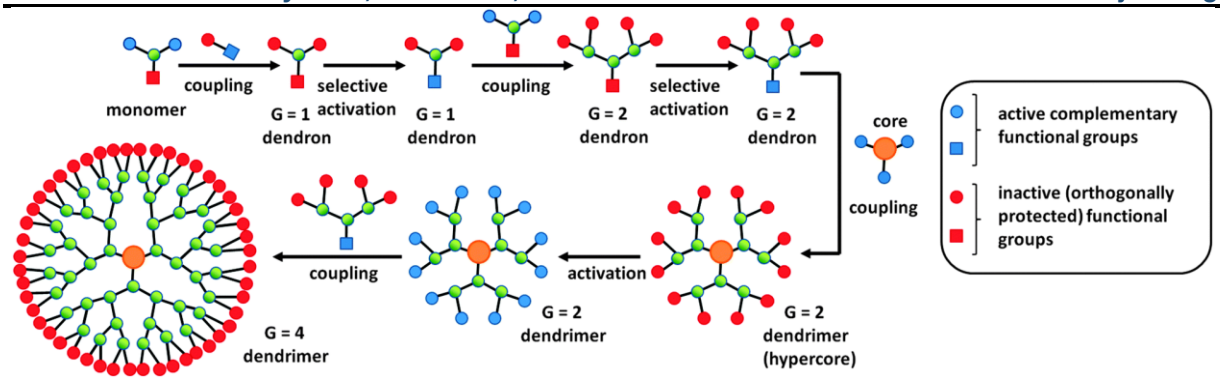

Figure3: Click Chemistry route

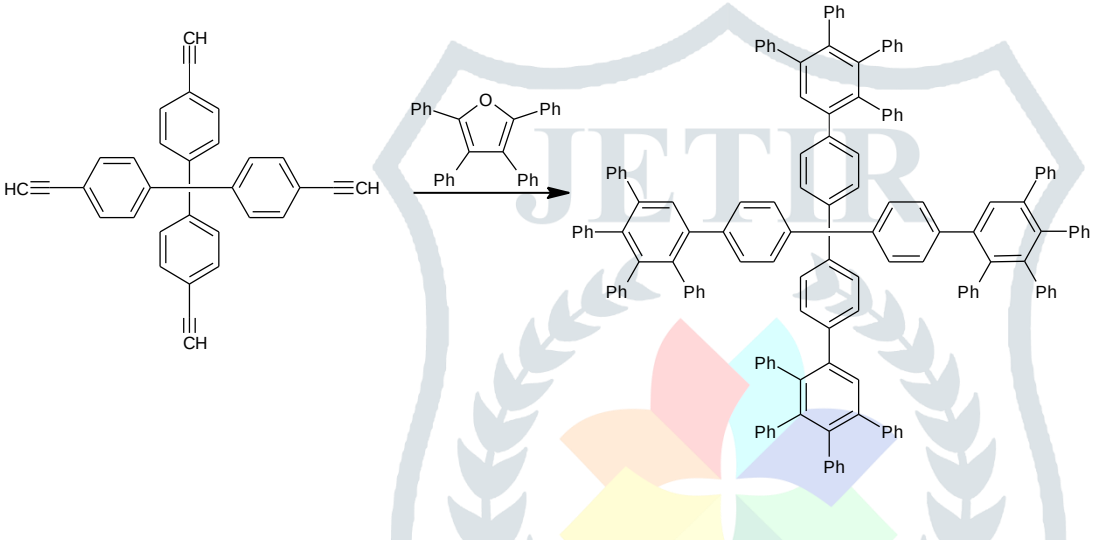

Figure4: Double exponential and mixed growth

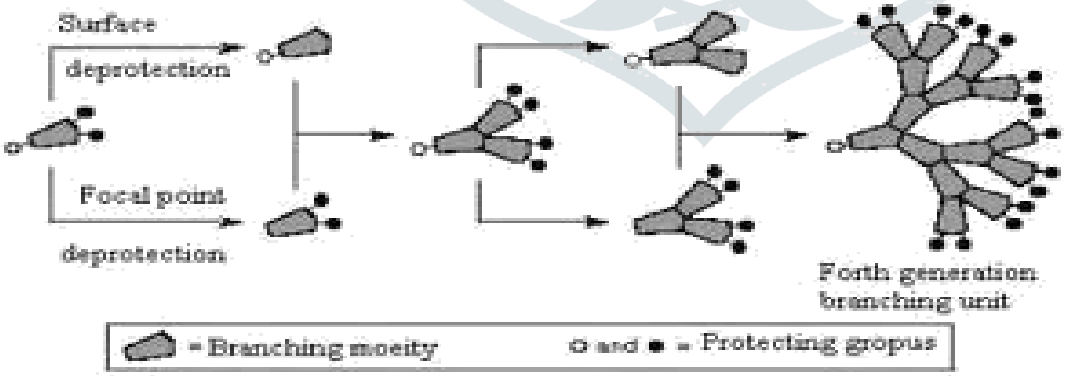

Figure5: Dendrimers as Chemotherapeutic drug delivery vehicle

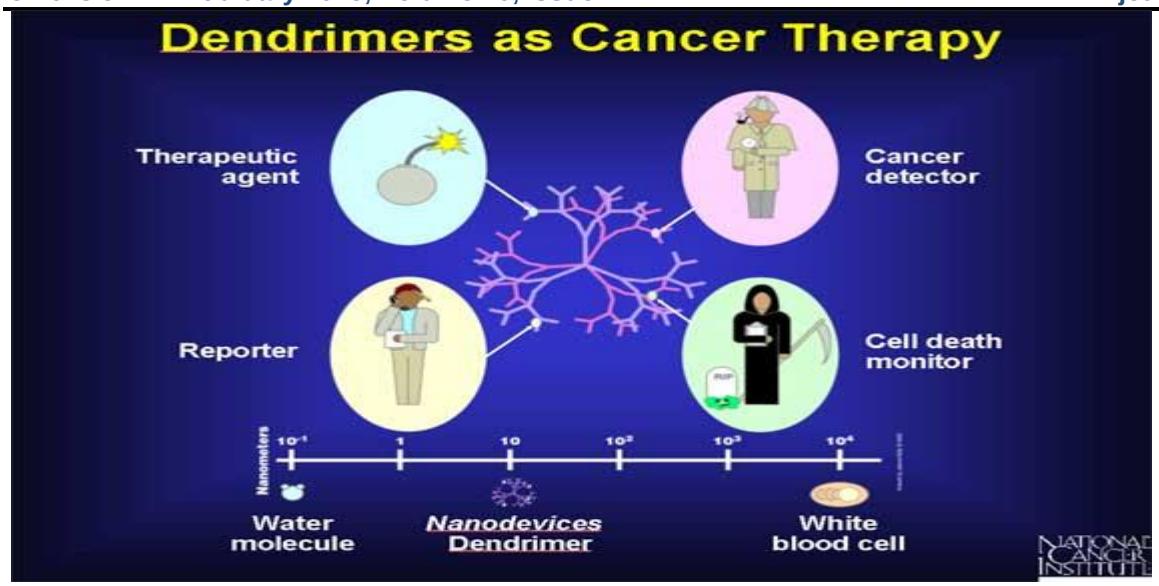

Figure6: Delivery systems for Anticancer Drugs

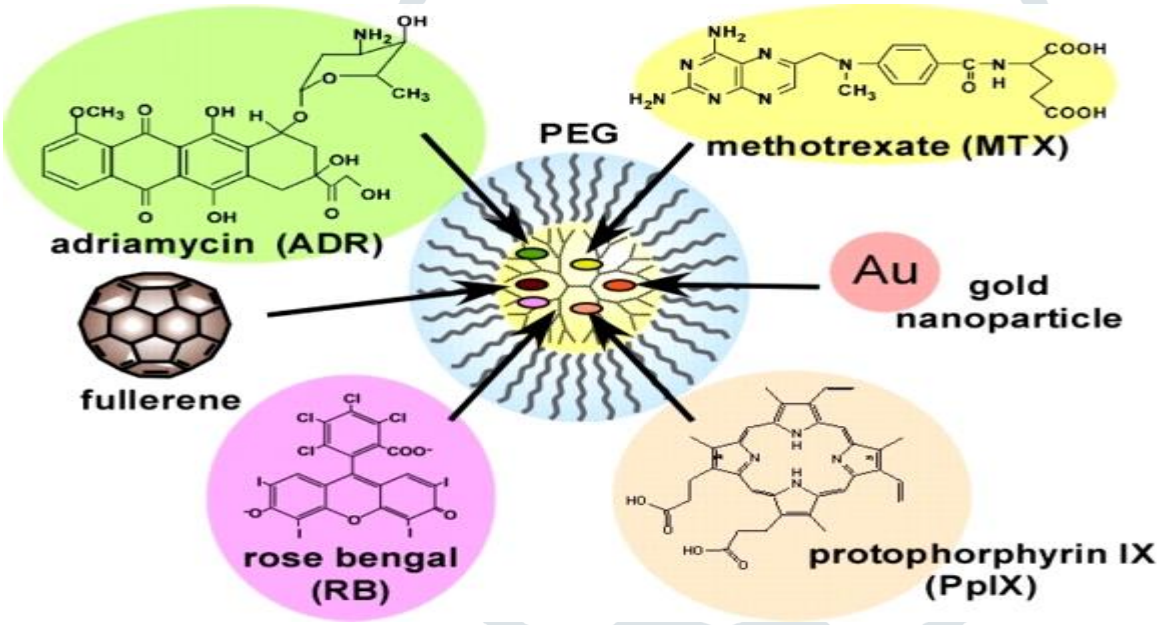

Figure7: Agents for Photodynamic Therapy

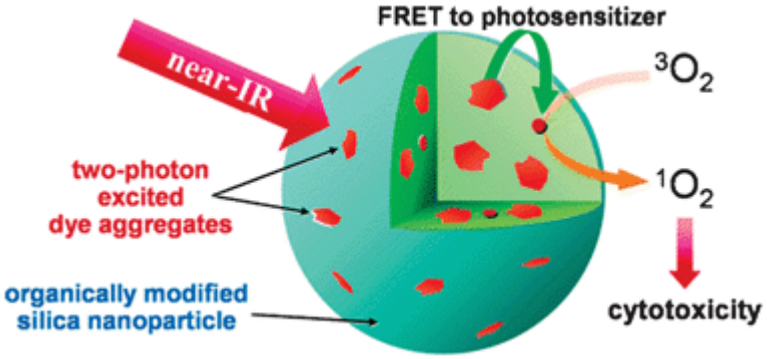

Figure8: Vectors in Gene Therapy

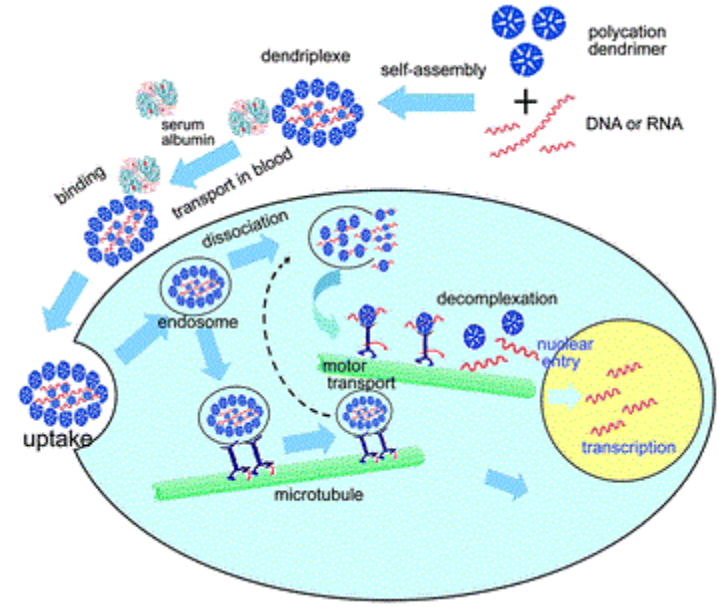

Figure9: Devices for Boron Neutron Capture Therapy

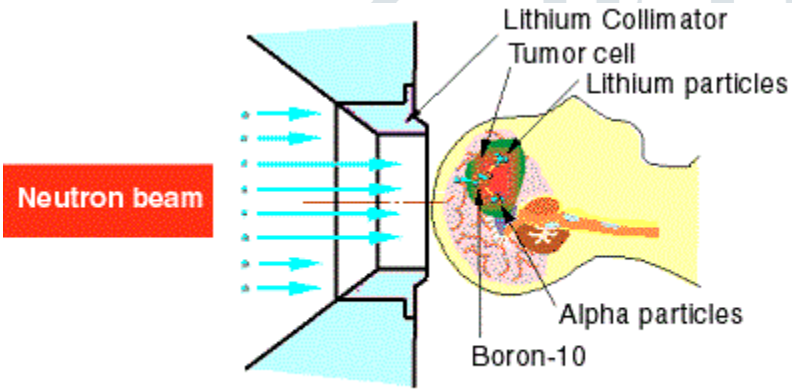

Figure10: Apoptosis Sensing Agents

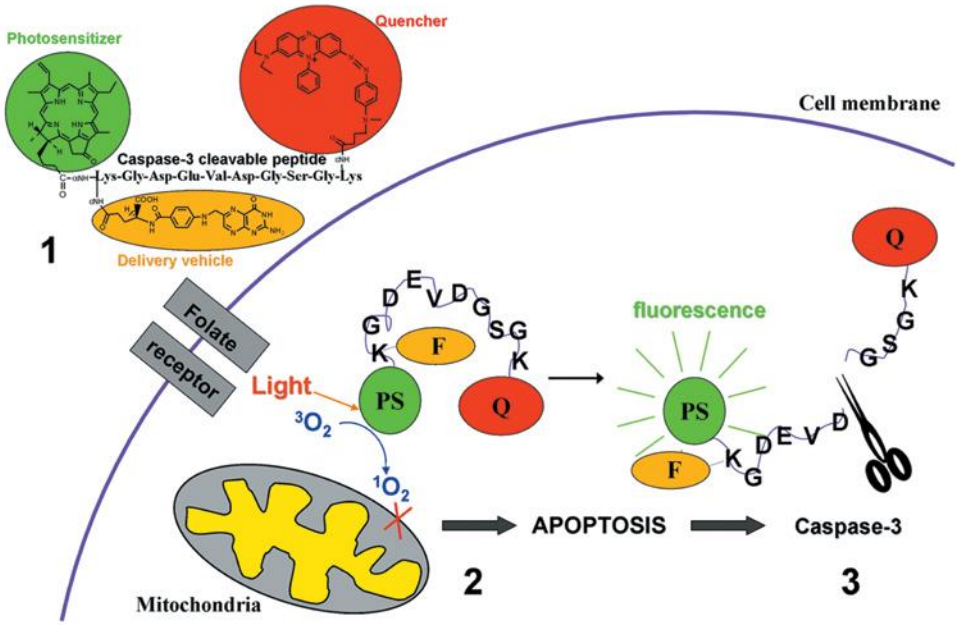

Figure11: Passive Targeting

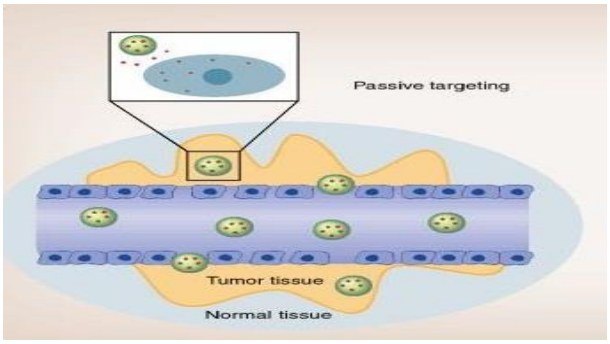

Figure12: Active Targeting