Review Article

DENDRIMERS: A NEW DRUG DELIVERY DEVICE FOR TARGETING TO TUMOURS

V.N.L.Sirisha*, P.Kiran kumar, B.Sruthi, I.harika, M.Namrata, Y.Kiran Kumar rao, K.Pranavi,
Anurag Pharmacy College, Affiliated to JNTU-Hyderabad, Kodad, Nalgonda-Dt, Andhra Pradesh, India.
***Corresponding author's E-mail:** sirishavnl@yahoo.com

Accepted on: 29-05-2012; **Finalized on:** 30-06-2012.

ABSTRACT

The discovery, design, and development of anticancer therapeutic agents have proven to be remarkably intractable despite intense efforts at the research and clinical levels over many decades. A brief consideration of the challenges facing anti cancer drug illustrates some of the reasons for frustratingly-slow progress: first the drug must be able to seek out subtle changes that distinguish a transformed cell from the other 200 or so types of healthy cells found in the body and then provide a sufficiently high dose of a toxic agent to kill the cell. The difficulty of this task is amplified by the potential metastasis of cancer cells to widely-spread niches throughout the body, each with unique properties. Furthermore, to successfully cure a patient, each and every cancer cell must be eradicated because even one in a thousand – often harboring latent resistance – can re-grow into a second tumor refractory to therapeutic intervention. Dendrimers are prepared with a level of control not attainable with most linear polymers, leading to nearly monodisperse, globular macromolecules with a large number of peripheral groups. As a consequence, dendrimers are an ideal delivery vehicle candidate for explicit study of the effects of polymer size, charge, composition, and architecture on biologically relevant properties such as lipid bilayer interactions, cytotoxicity, internalization, blood plasma retention time, biodistribution, and tumor uptake. 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. The focus of this review is on dendrimer developments from the last four years for oncological applications.

Keywords: Dendrimer, cancer treatment, drug-conjugates, drug delivery.

INTRODUCTION

Dendrimers are a class of regularly branched mono-dispersed polymer having 5-10 nanometers in diameter with unique structural and topological features whose properties are attracting considerable interest from both scientists and technologists.[1] Dendrimers are just in between molecular chemistry and polymer chemistry. These pertain to the molecular chemistry world by virtue of their step by step controlled synthesis, and they pertain to the polymer world because of their repetitive structure made of monomers. Unlike classical polymers, dendrimers have a high degree of molecular uniformity, narrow molecular weight distribution, specific size and shape characteristics, and a highly- functionalized terminal surface.

STRUCTURE

The word "dendrimer" originated from two words, the Greek word dendron, meaning tree, and meros, meaning part. Dendrimers are different from traditional polymers in that they have a multi-branched, three dimensional architecture with very low poly dispersity and high functionality**.** Typical dendrimer are globular nano scale macromolecule with a particular architecture constituted of three distinct region.[4,5]

1. A central core which is either a single atom or an atomic group.

2. Branches emanating from the core composed of repeating units called generation which is radially in position.

3. Many terminal functional group generally located in the exterior of the macromolecule.

Dendrimers are built from a starting atom, such as nitrogen, to which carbon and other elements are added by a repeating series of chemical reactions that produce a spherical branching structure.

As the process repeats, successive layers are added to form spherical macro molecule. The performance of these dendrimers are dependent upon its size, generation surface functional groups with increase in dendrimer generation the dendrimer, the dendrimer increase linearly while the number of surface group increases exponentially.

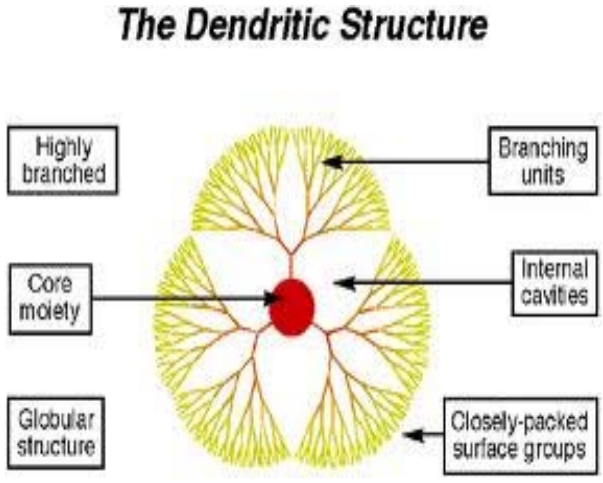

Figure 1: Structure of Dendrimer

APPLICATIONS OF DENDRIMERS IN DRUG DELIVERY

Figure 2: Applications of Dendrimers

The ideal dendrimer carrier should exhibit high aqueous solubility and drug-loading capacity, Biodegradability, low toxicity, favorable retention and bio distribution characteristics, specificity, and appropriate bioavailability. In dendrimer-based drug delivery, a drug is either non-covalently encapsulated in the interior of the dendrimer or covalently conjugated to form macromolecular pro drugs.

Drug-encapsulated dendrimers

Poly (glycerol succinic acid) dendrimers, or PGLSA dendrimers, were investigated as delivery vehicles for camptothecins, a group of naturally-derived hydrophobic compounds with anti- cancer activity. In a preliminary study reported by the Grinstaff group, G4-PGLSA dendrimers with hydroxyl (G4-PGLSA-OH) or carboxylate (G4-PGLSA-COONa) peripheral groups were used to encapsulate 10-hydroxycamptothecin (10-HCPT) for delivery to cancer cells.[6] The G4-PGLSA-OH/10-HCPT solution precipitated upon standing after mixing; the more water-soluble G4-PGLSA-COONa dendrimer was used instead to improve overall solubility and 10-HCPT was successfully encapsulated. Upon exposure to MCF-7 human breast cancer cells, unloaded dendrimer showed no cytotoxic effects, while 10-HCPT-encapsulated dendrimers led to significant cytotoxicity with less than 5% of viable cells at higher concentrations (20 µM). An alternative tri block structure was explored by introducing a 3400 molecular weight PEG core to the G4-PGLSA dendrimer to afford (G4-PGLSA-OH)2-PEG3400.[7] A 20-fold increase in 10-HCPT water solubility was observed following encapsulation. The anti-cancer activity of the macromolecule/drug complex was examined using HT-29human colon cancer cells and similar cytotoxicities were reported for encapsulated and free 10-HCPT. The conclusions drawn from these two studies led to the selection of G4-PGLSA-COONa dendrimer as a delivery vehicle for 10-HCPTand 7-butyl-10-aminocamptothecin (BACPT), a highly potently pophilic camptothecin derivative. Anti-cancer activity was investigated for a human cancer cell line panel includingHT-29 colon cancer, MCF-7 breast carcinoma, NCI-H460 large cell lung carcinoma, and SF-268 astrocytoma. Solubility, cellular uptake, and cellular retention studies were also performed for MCF-7 cells. The release profile of 10-HCPT encapsulated G4-PGLSA-COON a showed full release of the drug within approximately 6 h, suggesting that the delivery system may be best utilized by intratumoral injection. Dendritic delivery of 10-HCPT and BACPT resulted in lowered IC50s for all cell lines tested; for 10-HCPT exposure to HT-29, MCF-7, NCI-H460, and SF-268 cells, IC50s were reduced by 3.5, 7.1, 1.9, and 2.8-fold, respectively, compared to free 10-HCPT dissolved in DMSO. Exposure of BACPT led to IC50reductions of 1.2, 3.2, 1.9, and 5.7-fold for the respective cell lines above. Uptake studies showed that dendrimer-encapsulated10-HCPT was internalized much faster than free drug, with 16-fold intracellular concentrations at 2 h and 8-foldintracellular concentrations at 10 h. Drug delivered via the dendrimers also showed longer retention time in the cell, with 50% of delivered 10-HCPT present in the cell after 30 min, compared to 35% of free drug. Thus, increased toxicity of delivered camptothecins was attributed to enhanced uptake and retention. The cytotoxicity and encapsulation efficiency of staramphiphilic PAMAM block copolymers containing poly (γ-caprolactone) and PEG arms has also been assessed.[9] The polymer forms micelles in solution which were non-cytotoxic. The anti-cancer drugs doxorubicin and etoposide were encapsulated within the micelles. Doxorubicin showed lower capsulation efficiency while the more lipophilic etoposide achieved a loading capacity of approximately 22% by weight.

Unloaded dendrimer was non-cytotoxic to porcine kidney epithelial cells, while etoposide-encapsulated dendrimers showed comparable toxicity to free etoposide at similar drug concentrations. Enhanced aqueous solubility of paclitaxel was achieved with poly (glycerol) dendrimer formulations, showing that a hydrophobic dendrimer core is not necessary for encapsulation and solubilization of hydrophobic drugs.[10] Paclitaxel solubilities ranged from 80–128 µg/mL with increasing generations from G3–G5 of poly (glycerol), or three orders of magnitude higher than free paclitaxel. Nuclear magnetic resonance data suggests that the drug is not incorporated within the core of these dendrimers, but instead the methylene groups and aromatic rings of the paclitaxel are surrounded by the dendrimer structure leading to hydrotropic solubilization. Melamine-based dendrimers were used to solubilize the anti cancer drugs methotrexate and 6-mercaptopurine, as well as to reduce drug toxicity.[11] C3H mice received subchronic doses of drug-encapsulated dendrimers and ALT levels were evaluated to determine hepatotoxicity. ALT levels were reduced by 27% for methotrexate-encapsulated dendrimers and by 36% for the 6-mercaptopurine dendrimers compared to animals treated with drug alone. Medium-generation dendrimers (i.e., G4–G6) have been shown to both enhance solubility and increase toxicity (lower IC50) of hydrophobic anti-cancer drugs through non-covalent encapsulation. Therapeutic agents are internalized within the interior core space or

by micellar formation of the dendrimers. A major drawback to these delivery systems is a lack of controlled drug release kinetics, with most systems releasing their payload over the course of several hours. For this reason, drug-encapsulated dendrimer systems may best be utilized via direct intratumoral injection.

Dendrimer-drug conjugates

Dendrimer-drug conjugates generally consist of an anti neoplastic agent covalently attached to the peripheral groups of the dendrimer. This method offers distinct advantages over drug encapsulated systems. Multiple drug molecules can be attached to each dendrimer molecule and the release of the therapeutic molecules is partially controlled by the nature of the linkages.

The Kannan group reported the synthesis of PAMAM-methotrexate conjugates from carboxylic acid-terminated G2.5 PAMAM or amine-terminated G3 PAMAM in order to assess the activity of dendrimer-delivered methotrexate to sensitive and resistant CCRF-CEM human acutelymphoblastoid leukemia and CHO Chinese hamster ovary cell lines.[12] Although both polymers were conjugated to the drug by the formation of amide bonds, the carboxylic acid-conjugated G2.5 PAMAM system showed increased sensitivities of 8- and 24-fold towards the MTX-resistant cell lines CEM/MTX and RII, while amine-conjugated G3PAMAM showed no such increases compared to free methotrexate. The differences in cytotoxicity were attributed to the charge of the dendrimer carrier after cleavage of methotrexate from the peripheral groups. It was proposed that the lysosomotropic effect, in which the displacement of small basic molecules from the lysosome by positively-charged dendrimers is accompanied by an increase in pH and eventually lysosomal disruption, was responsible for a decrease in lysosomal residence time for the cationic PAMAM. As a result, the conjugates experience reduced interactions with proteases and diminished drug release. The results indicate the potential of dendrimer-drug conjugates for the treatment of cancer cells, particularly those that have demonstrate resistance to chemotherapeutics. Paclitaxel was conjugated to PEG or G4-PAMAM to compare the anti-cancer activity of the drug delivered by a linear or dendritic carrier.[13] Both PEG and PAMAM increased the aqueous solubility of paclitaxel (0.3 µg/mL) dramatically to 2.5 mg/mL and 3.2mg/mL respectively. Upon exposure to human ovarian carcinoma A2780 cells, free Paclitaxel accumulated in the cytoplasm near the plasma membrane. The polymer conjugates tended to distribute intracellularly in a more homogenous fashion compared to free drug. PEG-paclitaxel conjugates reduced the efficacy of the drug 25-fold, but the PAMAM-paclitaxel conjugates decreased the IC50 more than 10-fold when compared against free drug, leading to the conclusion that the availability of a drug is dramatically influenced by the architecture of its polymer conjugate. Doxorubicin-G4-PAMAM complexes have been encapsulated into liposomal formulations for potential local delivery to locations such as skin metastasis from breast cancer. The dendrimer-drug complex was incorporated into one of two formulations to modulate release compared to doxorubicin liposomal systems. The first formulation was comprised of egg phosphate dylcholine, stearylamine, and the anti-tumor ether lipid hexadecyl phosphocholine (HePC), while the second formulation was similar except did not include HePC. Incorporation efficiencies were above 90% and slow release was achieved with less than 20% released over 48 h for both systems. Cytotoxicity was assessed based on doxorubicin activity on several cancer cell lines including lung, colon, breast, prostate, and CNS. The doxorubicin-PAMAM liposome formulation with HePC showed the highest activity against most of the cell lines, with enhanced activity towards MDA-MB435breast cells compared to the dendrimers conjugate alone, and high sensitivity towards DMS114 and NCI-H460 lung cancer cells. It should be noted that the dendrimer-liposomal complexesin this study increased in size from approximately 115 nm to2000 nm after 18 weeks in storage at 4°C, and this was attributed to the formation of liposomal aggregates facilitated by hydrophobic forces between dendrimers.

TARGETED DRUG DELIVERY

Macromolecular delivery of anti-cancer drugs using multifunctional dendritic architectures allows for the conjugation of both drugs and targeting moieties such as folic acid, monoclonalantibodies, and peptides to the dendrimer periphery for increasingly specific delivery. In the field of oncology, the targeted delivery of chemotherapeutics to tumor cells translates to significantly reduced side effects compared to systemic delivery where healthy tissue such as the liver, spleen, kidneys, and bone marrow can accumulate toxic levels of drug. The two general strategies of targeting include the passive targeting of bulk cancerous tissue and the active targeting of unique tumour cells. Non-specific or passive targeting of tumors is usually a achieved by increasing the hydrodynamic radius of the dendrimer though PEGylation, leading to the accumulation of dendrimer in tumor tissue via the enhanced permeability retention (EPR) effect. The EPR effect is a result of tumor induce dangiogenesis leading to neovasculature that is irregular, leaky or defective with disorganized endothelial cells; tumor issues also suffer from poor lymphatic drainage, all leading to the accumulation and retention of macromolecules in the tumor mass. Specific or active targeting relies on the conjugation of one or more targeting moieties to the dendrimer to facilitate cell-receptor-mediated interactions.

Folic acid

Studies have shown that folic acid-conjugated dendrimers preferentially target tumor cells that Over express folic acid receptors.[15-17] A recent study by Hong et al. explicitly quantified the binding avidity of multi-valent targeted G5-PAMAM containing different numbers of folic acid

molecules.[18] Binding avidity to folic acid receptor-over expressing cells increased with each additionally bound FA molecule conjugated to the dendrimer, saturating at 5–6 moieties per dendrimer, though the rate of intracellular internalization was not significantly affected with increased binding. The dendrimers demonstrated a dramatic enhancement of binding avidity of almost 5 orders of magnitude. It was suggested that aggregates of 5–6 FA receptors are pre organized on the membrane and that the key factor in reported tumor reduction is enhanced residence time on the cell and not the rate of endocytosis. In another example, DNA-assembled PAMAM dendrimer clusters were prepared by linking two dendrimer components with single But different functionalities for concurrent delivery of the rapeutic, imaging, and targeting agents.[19,20] Complexes were formed between a folic acid-modified dendrimer and a FITC-modified dendrimer connected by a 34-base-pair long oligonucleotide. Clusters effectively targeted KB cells expressing folic acid receptors and were internalized by the cells.

The Baker group has investigated several variations of folic acid-conjugated dendrimers for targeted drug delivery. Surface conjugated folic acid G5-PAMAM dendrimers were prepared where the remaining free amine groups were capped with glycidol to neutralize the positive charges, and then further reacted with methotrexate (MTX) to form ester linkages.[21]

A comparison between encapsulated MTX vs covalently bound drug release showed a rapid release for the free drug over 2.5 h(~75%), compared to a much slower release for the bound drug over the same period of time (~5%). Furthermore, encapsulated drug displayed diffusion characteristics similar to free drug. Folic acid-targeted MTX conjugates demonstrated high specificity for KB cells over expressing folic acid receptors. When exposed to these cells, both free drug and dendrimer conjugates show similar cytotoxicity activity, but when the folic acid receptors are blocked or under expressed, the conjugates lose their anti-proliferative effect, indicating receptor-mediated delivery. Improvements in the synthesis and scale-up of PAMAM-methotrexate conjugates have led to high synthetic reproducibility.[22] In a separate study, folic acid, fluorescein and methotrexate were conjugated to PAMAM and examined invitro against KB cells.[23] Anti-proliferative activity was slightly lower for the dendrimer-drug conjugates compared to free methotrexate. Dose-dependent binding to KB cells was demonstrated and compared to fluorescein-modified PAMAM not containing folic acid.

Targeting was diminished yet still significant against KB cells under expressing FA receptors. The drug-dendrimer conjugates became ineffective when the cells were pretreated with free folicacid. A comparable study was performed with folic acid, fluorescein, and paclitaxel conjugate to partially acetylated PAMAM dendrimers.[24] Again, folic acid-targeting occurred, preferentially delivering paclitaxel-conjugated dendrimers to KB cells. Internalization was not detected when dendrimers were exposed to down-regulated KB cells. In a related project, PAMAM dendrimer-based sensors have been targeted to tumor cells to detect the anti-cancer activity of therapeutics. Multi-functional folic acid-targeted PAMAM delivery vehicles were synthesized and covalently bound to the apoptotic sensor PhiPhiLux G1D2 in order to detect the extent of cell killing caused by a delivered anti-proliferative agent.[25] PhiPhiLux G1D2 is a caspase-specific FRET-based agent that responds to the release of the apoptosis-inducing agent, staurosporine. The dendrimers were internalized with in the first 30 min of incubation with Jurkat cells and upon apoptosis, a 5-fold increase in intracellular fluorescence was detected, demonstrating the potential of chemotherapeutic delivery while monitoring cell-killing efficacy *in-vivo*.

Peptides

A doubly cyclized RGD (RGD-4C) peptide and Alexa Fluor488 fluorescent label were conjugated to partially acetylate G5-PAMAM for the targeting of tumor neova sculature via uniquely expressed αVβ3 integrins.[26] The RGD-4C peptide was attached by an acylhexanoic acid spacer to ensure adequate exposure of the conjugate to the target. Binding studies were performed on several cell lines with varying levels of integri receptor expression. Free RGD-4C bound much more rapidly than the RGD-4C-dendrimer complexes, but the Dendrimers disociated approximately 522 times slower, suggesting a synergistic effect of multiple peptide conjugation on binding avidity. Cyclic RGDs have also been attached to DOTA-conjugated mono, di-, and tetravalent dendrimeric alkynes for αVβ3 integrintargeting.[27]

Binding characteristics were evaluated *in vitro* and *in vivo* in mice with human SK-RC-52 tumors and it was shown through bio distribution studies that the tetrameric RGD-dendrimer showed the highest level of tumor targeting.

Monoclonal antibodies

Monoclonal antibody-conjugation to PAMAM has been explored for specific targeting of tumor cells that over express certain antigens. An anti-prostate specific membrane antigen (PSMA), J591, was conjugated to G5-PAMAM and evaluated *in vitro* for binding affinities and internalization.[28] PSMA is over expressed in all prostate cancers, non-prostatic tumor neovasculature, and vascular endothelium in most solid sarcoma and carcinoma tumors.[29] The antibody–dendrimer conjugate was found to specifically bind to PMSA-positive (LNCaP.FGC) cells but not to PMSA-negative (PC-3) cells. Furthermore, the conjugate was internalized as determined by confocal microscopy, while the un conjugated dendrimer was not significantly taken up by cells. A similar study was performed using anti-HER2-G5-PAMAM for the targeting of human growth factor receptor-2, which is often over expressed in breast and ovarian malignancies.[30,31] The conjugates showed binding and internalization into HER2-expressing cells.

Specific and increased binding to HER2-expressing tumors was also demonstrated in vivo. A Third study investigated two different antibody-G5-PAMAM conjugates,60bca and J591, for the targeting of CD14 an prostate-specific membrane antigen (PMSA), respectively.[32] Targeting was achieved in vitro using two different antigen-expressing cell models including CD14-expressingHL-60 human myeloblastic leukemia cells and PMSA expressing LNCaP cells.

Methotrexate was covalently attached to G5-PAMAMbioconjugates containing cetuximab, a monoclonal antibody that acts as an epidermal growth factor receptor (EGFR) inhibitor and is currently used as a drug to treat colorectal, head, and neck cancers. The conjugate was designed for targeted delivery to EGFR-positive brain tumors, to build upon the demonstrated successful targeting and delivery of boronated PAMAM cetuximab conjugates to gliomas for neutron capture therapy (discussed in detail later).[33] Approximately 13 methotrexate molecules were attached to each dendrimer as confirmed by UV/vis spectroscopy.

The bio conjugate showed a modest 0.8 log unit reduction in its EC50, though the IC50 was 2.7 log units lower for the conjugate compared to free methotrexate. Unfortunately, tumor-bearing animals did not show a significant response from the bioconjugate compared to free methotrexate, possibly due to a lack of cleavage from the PAMAM scaffold.

Glycosylation

One strategy to selectively deliver drug-conjugates to tumor cells used glycopeptide dendrimers conjugated to the anti-mitotic agent cholchicine. Glycodendrimers are a class of dendrimers that incorporate sugar moieties such as glucose, galactose, mannose,[34] and/or disaccharides[35] into their structure. The dendrimer consists of 2,3-diaminopropionic acid branching featuring amino acids, a cysteine core, and four to eight glycoside groups on the periphery. The conjugates were prepared and evaluated against a cancer cell line (HeLa) and healthy cells (non-transformed mouse embryonic fibroblasts or MEFs).[36] While the glycopeptides dendrimer conjugates were not as anti-proliferative as cholchicine alone, the dendrimers were 20–100 times more effective at inhibiting proliferation of HeLa cells than MEFs whereas non-glycosylated dendrimers showed a selectivity of less than 10-fold for HeLa cells.

CONCLUSION

The culmination of these advances in dendrimer-based delivery systems along with fundamental work performed over the last couple decades has led to the founding of several start-up companies and a large number of patents focused, at least in part, in the development of dendrimer technologies for oncology applications.

REFERENCES

1. Tomalia, D.A., Baker, H., Dewald, J., Hall, M.,Kallos, G and Martin, S.,. A newclass of polymers: starburst-dendritic macromolecules, 3, 1985, 23-31.
2. Polymers in Drug Delivery. Indian J. Pharm. Educ.Res., 40 (3), 2003, 153-158.
3. Sakthivel, T and Florence, A.T., Adsorption of Amphipathic Dendrons on PolystyreneNanoparticles, Int. J. Pharm., 254, 2003, 23-26.
4. Kitchens, K.M., El-Sayed M and Ghandehari H. Transepithelial and endothelial transport of poly (amidoamine) dendrimers.,Adv Drug Deliv Rev., 57, 2005, 2163-2176.
5. M.T. Morgan, M.A. Carnahan, C.E. Immoos, A.A. Ribeiro, S.Finkelstein, S.J. Lee, M.W. Grinstaff, Dendritic molecular capsules for hydrophobic compounds, J. Am. Chem. Soc. 125, 2003, 15485–9.
6. M.T. Morgan, M.A. Carnahan, S. Finkelstein, C.A. Prata, L. Degoricija, S.J. Lee, M.W. Grinstaff, Dendritic supramolecular assemblies for drug delivery, Chem. Commun. (Camb.) (2005) 4309–4311.
7. 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,Dendrimer-encapsulated camptothecins: increased solubility, cellular uptake, and cellular retention affords enhanced anticancer activity invitro, Cancer Res. 66, 2006, 11913–21.
8. F. Wang, T.K. Bronich, A.V. Kabanov, R.D. Rauh, J. Roovers, Synthesis and evaluation of a star amphiphilic block copolymer from poly(epsiloncaprolactone) and poly(ethylene glycol) as potential drug delivery carrier, Bioconjug. Chem. 16, 2005, 397–405.
9. T. Ooya, J. Lee, K. Park, Hydrotropic dendrimers of generations 4 and 5:synthesis, characterization, and hydrotropic solubilization of paclitaxel, Bioconjug. Chem. 15, 2004, 1221–1229.
10. M.F. Neerman, H.T. Chen, A.R. Parrish, E.E. Simanek, Reduction of drug toxicity using dendrimers based on melamine, Mol. Pharm. 1, 2004, 390–393.
11. S. Gurdag, J. Khandare, S. Stapels, L.H. Matherly, R.M. Kannan, Activity of dendrimer-methotrexate conjugates on methotrexate-sensitive and -resistant cell lines, Bioconjug. Chem. 17, 2006, 275–283.
12. J.J. Khandare, S. Jayant, A. Singh, P. Chandna, Y. Wang, N. Vorsa, T.Minko, Dendrimer versus linear conjugate: influence of polymeric architecture on the delivery and anticancer effect of paclitaxel, Bioconjug. Chem. 17, 2006, 1464–1472.
13. A. Papagiannaros, K. Dimas, G.T. Papaioannou, C. Demetzos, Doxorubicin-PAMAM dendrimer complex attached to liposomes: cytotoxic studies against human cancer cell lines, Int. J. Pharm. 302, 2005, 29–38.
14. 14.A.K. Iyer, G. Khaled, J. Fang, H. Maeda, Exploiting the enhanced permeability and retention effect for tumor targeting, Drug Discov. Today 11, 2006, 812–818.
15. E.C. Wiener, S. Konda, A. Shadron, M. Brechbiel, O. Gansow, Targeting dendrimer-chelates to tumors and

tumor cells expressing the high-affinity folate receptor, Invest. Radiol. 32, 1997, 748–754.

16. A. Quintana, E. Raczka, L. Piehler, I. Lee, A. Myc, I. Majoros, A.K. Patri, T. Thomas, J.Mule, J.R. Baker Jr., Design and function of a dendrimer based therapeutic nano device targeted to tumor cells through the folate receptor, Pharm. Res. 19, 2002, 1310–1316.
17. K. Kono, M. Liu, J.M. Fréchet, Design of dendritic macromolecules containing folate or methotrexate residues, Bioconjug. Chem. 10, 1999, 1115–1121.
18. S. Hong, P.R. Leroueil, I.J. Majoros, B.G. Orr, J.R. Baker Jr., M.M.Banaszak Holl, The binding avidity of a nano particle-based multivalent targeted drug delivery platform, Chem. Biol. 14, 2007, 107–115.
19. Y. Choi, T. Thomas, A. Kotlyar, M.T. Islam, J.R. Baker Jr., Synthesis and functional evaluation of DNA-assembled polyamidoamine dendrimer clusters for cancer cell-specific targeting, Chem. Biol. 12, 2005, 35–43.
20. Y. Choi, J.R. Baker Jr., Targeting cancer cells with DNA-assembled dendrimers: a mix and match strategy for cancer, Cell Cycle 4, 2005, 669–671.
21. A.K. Patri, J.F. Kukowska-Latallo, J.R. Baker Jr., Targeted drug delivery with dendrimers: comparison of the release kinetics of covalently conjugated drug and non-covalent drug inclusion complex, Adv. Drug Deliv. Rev. 57, 2005, 2203–2214.
22. I.J. Majoros, T.P. Thomas, C.B. Mehta, J.R. Baker Jr., Poly(amidoamine) dendrimer-based multifunctional engineered nanodevice for cancer therapy, J. Med. Chem. 48, 2005, 5892–5899.
23. T.P. Thomas, I.J.Majoros, A. Kotlyar, J.F. Kukowska-Latallo, A. Bielinska, A. Myc, J.R. Baker Jr., Targeting and inhibition of cell growth by an engineered dendritic nanodevice, J. Med. Chem. 48, 2005, 3729–3735.
24. I.J. Majoros, A. Myc, T. Thomas, C.B. Mehta, J.R. Baker Jr., PAMAM dendrimer-based multifunctional conjugate for cancer therapy: synthesis, characterization, and functionality, Biomacromolecules 7, 2006, 572–579.
25. A. Myc, I.J. Majoros, T.P. Thomas, J.R. Baker Jr., Dendrimer-based targeted delivery of an apoptotic sensor in cancer cells, Biomacro molecules 8, 2007, 13–18.
26. R. Shukla, T.P. Thomas, J. Peters, A. Kotlyar, A. Myc, J.R. Baker Jr, Tumor angiogenic vasculature targeting with PAMAM dendrimer-RGD conjugates, Chem. Commun. (Camb.) (2005) 5739–5741.
27. I. Dijkgraaf, A.Y. Rijnders, A. Soede, A.C. Dechesne, G.W. van Esse, A. J. Brouwer, F.H. Corstens, O.C. Boerman, D.T. Rijkers, R.M. Liskamp, Synthesis of DOTA-conjugated multivalent cyclic-RGD peptide dendrimers via 1,3-dipolar cycloaddition and their biological evaluation: implications for tumor targeting and tumor imaging purposes, Org. Biomol. Chem. 5, 2007, 935–944.
28. A.K. Patri, A. Myc, J. Beals, T.P. Thomas, N.H. Bander, J.R. Baker Jr., Synthesis and in vitro testing of J591 antibody–dendrimer conjugates for targeted prostate cancer therapy, Bioconjug. Chem. 15, 2004, 1174–1181.
29. S.S. Chang, D.S. O'Keefe, D.J. Bacich, V.E. Reuter, W.D. Heston, P.B. Gaudin, Prostate-specific membrane antigen is produced in tumor associated neovasculature, Clin. Cancer Res. 5, 1999, 2674–2681.
30. R. Shukla, T.P. Thomas, J.L. Peters, A.M. Desai, J. Kukowska-Latallo, A.K. Patri, A.Kotlyar, J.R. Baker Jr., HER2 specific tumor targeting with dendrimer conjugated anti-HER2 mAb, Bioconjug. Chem. 17, 2006, 1109–1115.
31. D.J. Slamon, W. Godolphin, L.A. Jones, J.A. Holt, S.G. Wong, D.E. Keith, W.J. Levin, S.G. Stuart, J. Udove, A. Ullrich, et al., Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer, Science 244, 1989, 707–712.
32. T.P. Thomas, A.K. Patri, A. Myc, M.T. Myaing, J.Y. Ye, T.B. Norris, J.R.Baker Jr., In vitro targeting of synthesized antibody-conjugated dendrimer nanoparticles, Biomacromolecules 5, 2004, 2269–2274.
33. G. Wu, R.F. Barth, W. Yang, S. Kawabata, L. Zhang, K. Green-Church,Targeted delivery of methotrexate to epidermal growth factor receptor positive brain tumors by means of cetuximab (IMC-C225) dendrimer bioconjugates, Mol. Cancer Ther. 5, 2006, 52–59.
34. E.K. Woller, M.J. Cloninger, Mannose functionalization of a sixth generation dendrimer, Biomacromolecules 2 (2001) 1052–1054.
35. R. Roy, M.G. Baek, Glycodendrimers: novel glycotope isosteres unmasking sugar coding. case study with T-antigen markers from breast cancer MUC1 glycoprotein, J. Biotechnol. 90, 2002, 291–309.
36. D. Lagnoux, T. Darbre, M.L. Schmitz, J.L. Reymond, Inhibition of mitosis by glycopeptide dendrimer conjugates of colchicine, Chemistry, 11, 2005, 3941–3950.

****