Polydisulfide Gd(III) chelates as biodegradable macromolecular magnetic resonance imaging contrast agents

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Abstract: Macromolecular gadolinium (Gd(III)) complexes have a prolonged blood circulation time and can preferentially accumulate in solid tumors, depending on the tumor blood vessel hyperpermeability, resulting in superior contrast enhancement in magnetic resonance (MR) cardiovascular imaging and cancer imaging as shown in animal models. Unfortunately, safety concerns related to these agents’ slow elimination from the body impede their clinical development. Polydisulfide Gd(III) complexes have been designed and developed as biodegradable macromolecular magnetic resonance imaging (MRI) contrast agents to facilitate the clearance of Gd(III) complexes from the body after MRI examinations. These novel agents can act as macromolecular contrast agents for in vivo imaging and excrete rapidly as low-molecular-weight agents. The rationale and recent development of the novel biodegradable contrast agents are reviewed here. Polydisulfide Gd(III) complexes have relatively long blood circulation time and gradually degrade into small Gd(III) complexes, which are rapidly excreted via renal filtration. These agents result in effective and prolonged in vivo contrast enhancement in the blood pool and tumor tissue in animal models, yet demonstrate minimal Gd(III) tissue retention as the clinically used low-molecular-weight agents. Structural modification of the agents can readily alter the contrast-enhancement kinetics. Polydisulfide Gd(III) complexes are promising for further clinical development as safe, effective, biodegradable macromolecular MRI contrast agents for cardiovascular and cancer imaging, and for evaluation of therapeutic response.

Keywords: biodegradable, contrast agents, gadolinium, MRI, polydisulfide

Introduction
Magnetic resonance imaging (MRI) is a clinical diagnostic modality based on differences in the longitudinal and transverse relaxation rates (1/T1 or 1/T2) of water protons in different tissues (Liang and Lauterbur 2000). MRI signal intensity reflects the value of the relaxation rate of tissues. Contrast agents have been developed and used to enhance MR image contrast and to improve diagnostic accuracy. Paramagnetic metal chelates, eg, Gd(III), Fe(III), and Mn(II) complexes, can alter the relaxation rate of the surrounding water protons to allow for more effective MRI contrast enhancement (Caravan et al 1999). Gadolinium (Gd)(III) ions have seven unpaired electrons and a large paramagnetic moment. Gd(III) chelates with high stability, eg, Gd-DTPA, Gd-DOTA, or their derivatives, have been mainly developed as MRI contrast agents, which increase both 1/T1 and 1/T2 relaxivities (Weinmann et al 1984; Magerstadt et al 1986; Hayne et al 1989). Currently, Gd-containing contrast agents are used in approximately 30% of MRI examinations.
The Gd chelates approved for human use are nonspecific and have unfavorable pharmacokinetics. These low-molecular-weight agents rapidly extravasate from the vasculature and nondiscriminatively distribute in the extracellular space. The agents have a very short blood half-life and a short time window for contrast-enhanced imaging. In many situations it is difficult to estimate the timing for optimal imaging, impeding accurate diagnostic imaging. Macromolecular Gd(III) complexes have been developed to improve contrast-enhanced MR imaging. Macromolecular contrast agents are prepared by the conjugation of Gd(III) DTPA, DOTA complexes, or their derivatives to synthetic polymers (Schuhmann-Giampieri et al 1991; Bogdanov et al 1993; Wiener et al 1994; Kobayashi and Brechbiel 2004; Langereis 2004) or natural macromolecules (Lauffer and Brady 1985; Wang et al 1990; Sirlin et al 2004), or by copolymerization of the low-molecular-weight contrast agents (Kellar et al 1997; Ladd et al 1999; Duarte et al 2001). These agents have a long blood circulation time and can preferentially accumulate in solid tumor tissue depending on the hyperpermeability of the tumor vasculature (Gerlowski and Jain 1986; Hobbs et al 1998). As a result, they provide superior contrast enhancement in the blood pool and in solid tumor tissue in animal models (Gossmann et al 1999; Turetschek et al 2001; de Lussanet et al 2005).

A number of macromolecular Gd(III) complexes have been investigated as blood-pool contrast agents (Schuhmann-Giampieri et al 1991; Bogdanov et al 1993; Shibata et al 1995; Van Beers et al 1995; Roberts et al 1997; Kobayashi et al 2001b; Li et al 2001; Lee 2003; Sirlin et al 2004). These agents show molecular-weight-dependent plasma half-lives (Vexler et al 1994; Kobayashi et al 2001a,b), and agents with higher molecular weights and longer blood half-life produced the better contrast enhanced vascular imaging. Higher-molecular-weight chelates were prepared to increase their intravascular retention time for longer and clearer visualization of the neovascularity of neoplastic tissue (Weissleder and Jain 1986; Hobbs et al 1998). As a result, they provide superior contrast enhancement in the blood pool and in solid tumor tissue in animal models (Gossmann et al 1999; Turetschek et al 2001; de Lussanet et al 2005).

Although macromolecular Gd(III) complexes have clear advantages as blood-pool contrast agents, their slow body clearance is also a disadvantage with respect to toxicity. Free Gd(III) ions are highly toxic, with an LD₅₀ as low as 0.5 mmol/kg in rat models (Weinmann et al 1984). The dissociation of Gd(III) complexes and release of Gd(III) ions can be lethally toxic (Cacheris et al 1990). The longer body retention time increases the probability of metabolism of the macromolecular complexes and release of Gd(III) ions. The clearance rate decreases with the increase of molecular weight of the contrast agents (Kobayashi 2003). It has been reported that a Gd-DTPA polypropyleneimine dendrimer (generation 2) conjugate (7 kDa) resulted in the retention of 45% of injected dose in rats 14 days after injection (Wang 2003). The conjugation of Gd-DOTA to carboxymethyl hydroxylethyl starch resulted in a macromolecular agent (72 kDa) that had about 47% of injected dose detected in rat body 7 days after the injection (Helbich et al 2000). Because of this safety concern, clinical development of macromolecular contrast agents has been limited.

Biodegradable polymers including poly(L-lysine), dextrans, and proteins have been used in the synthesis of macromolecular contrast agents. However, in vivo degradation of these materials takes place primarily in intracellular enzymatic compartments. MRI contrast agents are mostly extracellular agents and have low cellular uptake. In addition, the extensive chemical conjugation on the polymers can significantly reduce their biodegradability (Crepon et al 1991). Consequently, contrast agents prepared from these polymers have longer body retention times and result in significant accumulation of Gd ions in tissues (Bogdanov et al 1993; Franano et al 1995; Helbich et al 2000). Innovative approaches are needed for the design and development of safe, effective macromolecular contrast agents for clinical application.

We have recently designed and developed novel extracellular biodegradable macromolecular Gd(III) complexes based on polydisulfides to facilitate Gd(III) elimination and to minimize its tissue retention (Lu et al 2004). These complexes act as macromolecular agents for MR contrast enhancement and rapidly excrete as low-molecular-weight Gd(III) chelates after the macromolecules are broken down by the endogenous thiols via the disulfide-thiol exchange reaction. Here, we summarize the rationale of the biodegradable macromolecular contrast agents, their
physicochemical and biological properties, and in vivo contrast enhancement in animal models.

**Rationale of polydisulfide macromolecular MRI contrast agents**

The ideal MRI contrast agent would be an agent able to accumulate in the tissue of interest long enough for imaging with conventional or dynamic MRI, then rapidly clear from the body. Currently available MRI contrast agents are primarily low-molecular-weight Gd(III) chelates, which have transient tissue retention and clear rapidly from the body. Macromolecular Gd(III) complexes exhibit long tissue retention and excrete slowly. We hypothesize that the incorporation of biodegradable structures into the backbone of macromolecules containing Gd(III) chelates can result in MRI contrast agents that will provide sufficient blood pool and tissue retention for effective contrast enhanced MR imaging. The structures will then react with biomolecules present in plasma or tissues to break down the macromolecules into smaller Gd(III) complexes that can be readily excreted (Lu et al 2004).

The disulfide-thiol exchange reaction plays a crucial role in biological systems. Free thiols including cysteine, glutathione (GSH), cysteinylglycine (Cys-Gly), and homocysteine are important endogenous biomolecules, with a total concentration of 15 µM in human blood plasma and about 10 mM in the cytoplasm (Andersson et al 1995; Deneke 2000). We have incorporated disulfide bonds into the backbone of macromolecular Gd(III) complexes to prepare biodegradable macromolecular MRI contrast agents. The disulfide bonds in the polymer chains can be readily cleaved by the thiols via disulfide-thiol exchange reaction to break down the macromolecules into smaller Gd(III) complexes, which can be excreted through renal filtration. Since Gd(III) chelates are mostly extracellular agents and the plasma thiol concentration is low, the degradation of the macromolecular agents is a slow process, which allows the agents to reside for an acceptable period in the circulation for effective contrast-enhanced MR imaging.

The concept of extracellular biodegradable macromolecular MRI contrast agents was validated with the first polydisulfide agent, Gd-DTPA cystamine copolymers (GDCC) (Lu et al 2004). GDCC was gradually degraded into smaller Gd(III) complexes by the cleavage of the disulfide bonds in the polymer backbone via the disulfide-thiol exchange reaction in the presence of 15 µM cysteine (Figure 1). The structures of degradation products were verified by MALDI-TOF mass spectrometry. GDCC resulted in more significant and prolonged contrast enhancement in the cardiovascular systems in rat than a clinical agent, Gd-(DTPA-BMA), and excreted rapidly via renal filtration. Metabolic degradation products were identified by mass spectrometry in the rat urine samples.

The structure of the polydisulfides can be readily modified to alter the physicochemical properties, pharmacokinetics, and in vivo contrast enhancement of the biodegradable MRI contrast agents. Functional groups have

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**Figure 1** Degradation of Gd-DTPA cystamine copolymers (GDCC) in the presence of cysteine.

**Figure 2** Structure of Gd-DTPA cystine copolymers (GDCC) and modified GDCC.

**Abbreviations:** OEt, ethoxy group; PEG, poly(ethylene glycol); GDCEP, Gd-DTPA cystine diethyl ester copolymer.
been introduced around the disulfide bonds by replacing cystamine with cystine. The chemistry of cystine is more versatile and the carboxylic groups in cystine can be further modified to design and prepare biodegradable macro-molecular contrast agents with different properties (Mohs et al 2004; Zong et al 2005). Different substituents have been attached to cystine (Figure 2), and the modified agents demonstrated different degradability, pharmacokinetics, and in vivo contrast enhancement. The chemistry, physicochemical and pharmaceutical properties, and in vivo contrast enhancement of the polydisulfide agents are discussed in the following sections.

**Synthesis of polydisulfide Gd(III) complexes**

The synthetic procedure of polydisulfide Gd(III) complexes is described in Figure 3 with GDCC as an example. DTPA dianhydride is first copolymerized with a disulfide-containing diamine, e.g., cystamine or cystine, by condensation copolymerization under basic conditions to give a polymeric ligand. Different solvents and bases can be used according to the solubility of disulfide monomers. Dimethyl sulfoxide was used as the solvent and triethylamine as the base for the monomers soluble in organic solvent. For cystine, which is not soluble in organic solvent, the copolymerization was performed in aqueous solution of sodium carbonate or sodium hydroxide. Polydisulfide Gd(III) complexes are then prepared by the complexation of the polymeric ligands with GdCl$_3$ or Gd(OAc)$_3$ at pH 5–6. The pH of the reaction mixture with GdCl$_3$ decreases significantly during the complexation because of the release of HCl, and NaOH is added to maintain the pH at 5–6. There is no significant change of pH in the reaction with Gd(OAc)$_3$.

The molecular weight of polydisulfides from the condensation copolymerization can be as high as 100 000 Da. There is a significant decrease of apparent molecular weight of copolymers after complexation with Gd(III) because of the change of hydrodynamic volume of the copolymers after complexation. The molecular weights of some paramagnetic copolymers are listed in Table 1. The polydisulfides are negatively charged and have a large hydrodynamic volume. Once the polydisulfide ligands chelate with Gd$^{3+}$, the polydisulfide Gd(III) complexes are neutral or less charged and have reduced hydrodynamic volume.

The structure of polydisulfide Gd(III) complexes have been modified by grafting PEG of different lengths to the carboxylic groups in Gd-DTPA cystine copolymers (GDCP) (Mohs et al 2004, 2005). Monomethoxy-PEG amine

![Figure 3 Synthesis of the polydisulfide agent Gd-DTPA cystamine copolymers (GDCC).](image)

**Table 1** Physicochemical parameters of some polydisulfide Gd(III) complexes

<table>
<thead>
<tr>
<th>Polymeric contrast agent</th>
<th>Molecular weight (kDa)</th>
<th>Relaxivity mM$^{-1}$sec$^{-1}$</th>
<th>PEGylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$M_w$</td>
<td>$M_n$</td>
<td>$M_w/M_n$</td>
</tr>
<tr>
<td>GDCC</td>
<td>17.7</td>
<td>15.0</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>35.0</td>
<td>27.0</td>
<td>1.3</td>
</tr>
<tr>
<td>GDCP</td>
<td>10.1</td>
<td>10.0</td>
<td>1.0</td>
</tr>
<tr>
<td>GDCEP</td>
<td>10.2</td>
<td>9.4</td>
<td>1.1</td>
</tr>
<tr>
<td>PEG$_{1000}$GDCP</td>
<td>24.5</td>
<td>21.0</td>
<td>1.2</td>
</tr>
<tr>
<td>PEG$_{550}$GDCP</td>
<td>22.9</td>
<td>19.9</td>
<td>1.2</td>
</tr>
<tr>
<td>PEG$_{1000}$GDCP</td>
<td>37.7</td>
<td>28.5</td>
<td>1.3</td>
</tr>
<tr>
<td>PEG$_{1000}$GDCP</td>
<td>37.8</td>
<td>31.1</td>
<td>1.2</td>
</tr>
<tr>
<td>PEG$_{550}$GDCP</td>
<td>33.7</td>
<td>30.8</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Abbreviations: PEG, polyethylene glycol; GDCC, Gd-DTPA cystamine copolymer; GDCP, Gd-DTPA cystine copolymer; GDCEP, Gd-DTPA cystine diethyl ester copolymer; $M_w$, averaged molecular weight; $M_n$, averaged molecular number.
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(MPEG-NH<sub>2</sub>) was attached to GDCP in the presence of a coupling agent, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, in aqueous solution (Figure 4). The grafting degree can be readily manipulated by altering the ratio of mPEG-NH<sub>2</sub> to GDCP. The modification increases apparent molecular weights of the copolymers at a low grafting degree depending on the size of PEG. Further increase of grafting degree with PEG of the same size does not further increase apparent molecular weight of the copolymers.

The coordination feature of Gd(III) in the polydisulfide complexes is the same as that in Gd-(DTPA-BMA), a clinical MRI contrast agent. The Gd(III) is coordinated to 3 tertiary amine nitrogen atoms, 3 carboxylic groups, and 2 carbonyl groups of the amides of the DTPA bisamide. Gd-(DTPA-BMA) is a nonionic agent and has low acute toxicity in mice (LD<sub>50</sub> = 14.8 mmol/kg) because of high stability and low transmetallation with Ca<sup>2+</sup> (Cacheris et al 1990).

**Relaxivity of polydisulfide Gd(III) complexes**

The paramagnetic chelates decrease both T<sub>1</sub> and T<sub>2</sub> relaxation times, or increase the relaxation rates of surrounding water protons, resulting in increased signal intensity in T<sub>1</sub>-weighted imaging and decreased signal intensity in T<sub>2</sub>-weighted imaging. The chelates have a more significant effect on the relaxation rate of water protons in the coordination sphere of Gd(III) ions, less effect on water molecules immediately surrounding the chelate, and little direct effect on the bulk water molecules. Therefore, an effective Gd(III) chelate contrast agent should have at least one coordination site for water molecules. The complexed water molecule rapidly exchanges with bulk water, resulting in efficient relaxation of bulk water. The net change of the relaxation rate of water protons depends on the concentration of Gd(III) chelates and water exchange rate at the coordination site. Relaxivity is the measurement of the efficiency of a contrast agent to change the relaxation rate and can be determined from the equation 

\[(1/T_{\text{obs}}) = (1/T_{\text{d}}) + r_i[Gd] (i = 1, 2),\]

where \((1/T_{\text{obs}})\) refers to the measured relaxation rate, \((1/T_{\text{d}})\) the relaxation rate without a contrast agent, and \(r_i\) the T<sub>1</sub> or T<sub>2</sub> relaxivity. Gd(III) chelates are primarily used in T<sub>1</sub>-weighted, contrast-enhanced MRI. High \(r_1\) of Gd(III) chelates will result in strong relaxation or contrast enhancement. Polydisulfide Gd(III) complexes have higher T<sub>1</sub> relaxivity than Gd-(DTPA-BMA) and the relaxivity varies based on the structure of the complexes (Table 1). GDCC, GDCP, and Gd-DTPA cystine diethyl ester copolymer (GDCEP) have similar relaxivity in the range of 4.5–6.5 mM<sup>−1</sup>s<sup>−1</sup>. It appears that the degree of PEGylation in poly(ethylene glycol) (PEG)-modified GDCP has a more significant impact on its relaxivity than the PEG chain length. PEG-GDCP copolymers with different PEG lengths have similar relaxivity at the same grafting degree (Mohs et al 2005). For PEG-GDCP copolymers with the same PEG length (2000 Da), the increase of grafting degree significantly decreases relaxivity. The T<sub>1</sub> relaxivity of PEG<sub>2000</sub>-GDCP with grafting ratios of 0.33, 0.76, and 1.2 mM<sup>−1</sup>s<sup>−1</sup> decreases from 16.3 to 12.7, and 8.73 mM<sup>−1</sup>s<sup>−1</sup>, respectively. A high density of PEG on the backbones of the copolymers may interfere with the interaction of water molecules to the Gd(III) complexes because PEG can associate with water molecules via hydrogen bonding (Mohs et al 2004).

**Degradability of polydisulfide Gd(III) complexes**

Polydisulfide Gd(III) complexes with different structures demonstrate different degradation characteristics in the presence of cysteine. There was no degradation of GDCP in the incubation with 15 µM cysteine for 6 hours at pH 7.4 and 37°C. GDCC gradually degraded under the same conditions and its molecular weight decreased approximately 28%, 33%, and 50% at 5, 15, and 60 minutes.
in the reaction (Lu et al 2004). The degradation of GDCEP was relatively slow in the first 60 minutes of incubation compared with GDCC, and its molecular weight decreased approximately 6%, 11%, and 24% at 5, 15, and 60 minutes. Both GDCC and GDCEP degraded into smaller oligomers and complexes at 6 hours (Zong et al 2005). Figure 5 shows size exclusion chromatograms of GDCEP in the incubation with 15 µM cysteine at pH 7.4 and 37°C. All three macromolecular agents degraded more rapidly at higher cysteine concentration (150 µM) and completely degraded into the smallest repeat units in the incubation with an excess of cysteine, which was verified by mass spectrometry. GDCP and GDCEP are more sterically hindered around the disulfide bonds than GDCC, resulting in slower degradation. GDCP also has negative charges around the disulfide bonds at neutral pH, which further impede the access of the negatively charged cysteine to the disulfide bonds (Hupe and Wu 1980). Therefore, little reduction of the disulfide bonds in GDCP occurs at low cysteine concentrations.

The modification of GDCP also resulted in macromolecular agents with different degradability based on the length of PEG chains (Mohs et al 2004, 2005). The PEG-modified GDCP was stable at low cysteine concentration (circa 10 µM), similar to GDCP, and degraded with increased cysteine concentration. It appears that GDCP modified with short PEG chains degraded more rapidly than that with long chains. The structural modification resulted in macromolecular agents with different degradability towards the disulfide-thiol exchange reaction, which may have different pharmacokinetic properties and in vivo contrast enhancement.

**Pharmacokinetics and excretion of polydisulfide Gd(III) complexes**

Polydisulfide Gd(III) complexes should be able to circulate in the vasculature for an acceptable time window for effective contrast-enhanced MR imaging and then clear from the body with minimal long-term tissue retention of toxic Gd(III) ions. Pharmacokinetic data are the quantitative measurement of the circulation, excretion, and retention of the biodegradable macromolecular contrast agents in the body. A pharmacokinetic study showed that the low-molecular-weight agent Gd-(DTPA-BMA) rapidly extravasated from the vasculature, while GDCC had a higher plasma concentration in the first few minutes (approximately 5–15 minutes) post injection at the same dose (0.1 mmol-Gd/kg) in rats (Figure 6). The size of the biodegradable macromolecular agent affected the initial plasma concentration of the agent, but not the clearance from the vasculature. GDCC with high molecular weight (Mw = 60 000 Da) had a higher plasma concentration in the initial period (circa 5 minutes) after the injection than GDCC with low molecular weight (Mw = 18 000 Da). The plasma concentration of GDCC with both molecular weights reached the same level at 20 minutes post injection. Because the breakdown of the macromolecules is gradual, GDCC of the high molecular weight may produce relatively large molecules and remain in the blood at high concentration in the early stages of degradation. The large molecules eventually degrade into smaller complexes that are readily cleared via renal filtration.

Most of the degradation products were cleared in the urine via renal glomerular filtration and more than 50% of injected GDCC was excreted in the first 4 hours post-
Paramagnetic polydisulfides

In vivo MRI contrast enhancement

The polydisulfide Gd(III) complexes present superior contrast enhancement in the cardiovascular systems and tumor tissue compared with the low molecular-weight agent, Gd-(DTPA-BMA), as shown in animal models. Figure 7 shows the 3D maximum-intensity-projection (MIP), contrast-enhanced MR images of rats with Gd-(DTPA-BMA) and GDCC with molecular weights of 18,000 and 60,000 Da (0.1 mmol-Gd/kg). GDCC of both molecular weights resulted in strong contrast enhancement in rat heart and vasculature at 2 minutes post injection and the signal intensity gradually decreased. Gd-(DTPA-BMA) was rapidly extravasated from the vasculature and the contrast enhancement was much less at 2 minutes post-injection than with GDCC. GDCC with higher molecular weight resulted in more significant contrast enhancement in the small blood vessels than GDCC with lower molecular weight. Strong contrast enhancement was observed in rat kidneys and then the urinary bladder with GDCC, indicating that the biodegradable macromolecular agent was eliminated from renal filtration and accumulated in the urinary bladder. The contrast enhancement in the cardiovascular system returned to background levels at 15 minutes post-injection while the signal intensity in the bladder significantly increased.

Structural modification of polydisulfide agents altered in vivo contrast enhancement. PEGylation of GDCP resulted in more significant and prolonged contrast enhancement in the blood pool than GDCP. Both grafting ratio and PEG size have a significant impact on contrast enhancement profile. PEG2000-GDCP at a grafting ratio of 0.76 produced...
of tumor-bearing mice contrast enhanced with GDCEP (0.1 mmol-Gd/kg) after the mice were treated for a period of 21 days with saline, a cationic polymer, anti-VEGF siRNA, and siRNA-polymer complex. Significant contrast enhancement was observed in the tumor rim of the mice, which clearly defined tumor size in the mice in different treatment groups. Mice treated with the siRNA and siRNA-polymer complexes had much smaller tumor sizes than mice treated with saline and cationic polymers as shown by contrast-enhanced MRI.

Conclusion

Polydisulfide Gd(III) chelates have been designed and synthesized as extracellular biodegradable macromolecular MRI contrast agents. The disulfide bonds in the polymer
Paramagnetic polydisulfides can be readily cleaved by endogenous thiols into small and excretable Gd(III) complexes. The degradability of the polydisulfides can be altered by structural modification of the agents to achieve different in vivo properties. A pharmacokinetic and biodistribution study demonstrated that GDCC has a relatively longer plasma retention time for effective contrast-enhanced MRI and is rapidly excreted as low molecular-weight Gd(III) complexes, resulting in minimal long-term Gd(III) tissue accumulation comparable to Gd(DTPA-BMA). The structural modification of polydisulfide Gd(III) complexes can result in biodegradable macromolecular contrast agents with various enhancement profiles in the blood pool. The agents are effective for cardiovascular and cancer MR imaging. The polydisulfide Gd(III) complexes have a great potential to be developed as safe, effective, biodegradable macromolecular MRI contrast agents for clinical applications.

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Abbreviations
DTPA, diethylenetriaminepentaacetic acid; DTPA-BMA, diethylenetriaminepentaacetate bismethyl amide; DOTA, 1,4,7,10-tetraazadecanetetraacetic acid; anti-VEGF, anti-vascular endothelial growth factor; siRNA, small interfering RNA.

References


