# Indotricarbocyanine-loaded AS1411 DNA aptamer- and TGN peptide-modified poly(ethylene glycol)-poly(ε-caprolactone) nanoparticles

DiR-AsTNP

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Created: August 10, 2012; Updated: September 5, 2012.

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| **Chemical name:** | Indotricarbocyanine-loaded AS1411 DNA aptamer- and TGN peptide-modified poly(ethylene glycol)-poly(ε-caprolactone) nanoparticles | |
| **Abbreviated name:** | DiR-AsTNP | |
| **Synonym:** | AsTNP | |
| **Agent Category:** | Nanoparticles | |
| **Target:** | Others | |
| **Target Category:** | Others | |
| **Method of detection:** | Optical imaging | |
| **Source of signal / contrast:** | Indotricarbocyanine (DiR) | |
| **Activation:** | No | |
| **Studies:** | • *In vitro*<br>• Rodents | No structure available. |

## Background

[PubMed]

Indotricarbocyanine (DiR)-loaded AS1411 DNA aptamer- and TGN peptide-modified poly(ethylene glycol)-poly(ε-caprolactone) (PEG-PCL) nanoparticles, abbreviated as DiR-

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NLM Citation: Shan L. Indotricarbocyanine-loaded AS1411 DNA aptamer- and TGN peptide-modified poly(ethylene glycol)-poly(ε-caprolactone) nanoparticles. 2012 Aug 10 [Updated 2012 Sep 5]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

AsTNP, is a cascade delivery system synthesized by Gao et al. for brain glioma imaging and treatment (1).

Effective treatment of brain tumors requires the therapeutic agents to penetrate both the blood–brain barrier (BBB) and the tumor cell barrier. The cascade targeting strategy is designed to meet this requirement, which relies on two specific ligands that are conjugated to a nanocarrier (1, 2). One ligand is designed to penetrate the BBB, and another is designed to target tumor tissues. With this strategy, Gao et al. developed a PEG-PCL cascade targeting delivery system that was modified with an angiopep-2 peptide to penetrate the BBB and an EGFP-EGF1 fusion protein to bind the brain neuroglial cells (2). Because of the promising results of this system, Gao et al. further developed another PEG-PCL system using TGN and AS1411 ligands (AsTNP) (1). TGN is a peptide of 12 amino acids (TGNYKALHPHNG), which was obtained through four rounds of *in vivo* phage display screening from a 12-mer peptide library (3). *In vivo* phage display is a technique that was first introduced by Pasqualini and Ruoslahti in 1996 (4). This technique has been used to screen tissue-specific peptides as targeting moieties for tumors and organs. The three-amino-acid sequence Arg-Gly-Asp is one of the most successful targeting ligands screened with the phage display technique (5). The TGN peptide has been shown to possess a superior BBB-penetrating ability compared with the native phage (3). AS1411 is a G-rich DNA aptamer that exhibits high binding affinity to nucleolin, a highly expressed protein in the plasma membrane of cancer cells including the C6 glioma cells (6, 7). Aptamers are short sequences of DNA or RNA that can bind to specific proteins *via* recognition of their three-dimensional structures (6). Aptamers are good candidates for targeted delivery because of their high stability, easy synthesis, and nonimmunogenicity (7). AS1411 has been used successfully to increase the anti-tumor efficacy of nanoparticles containing chemotherapeutic agents (7). In the AsTNP system, the TGN peptide served as the first-stage targeting ligand to transport the PEG-PCL nanoparticles through the BBB, and the AS1411 aptamer served as the second-stage ligand to deliver the system into the cancer cells after penetrating the BBB. An inhibitor of microtubule depolymerization, docetaxel (DTX), was then encapsulated within the AsTNP particles for therapeutic purpose (1, 8). A fluorescent dye (DiR or coumarin-6) was also encapsulated to track the behavior of AsTNP with optical imaging. The results obtained by Gao et al. showed that presence of both TGN and AS1411 was necessary, and the generated AsTNP had a superior BBB-penetration ability and high tumor accumulation (1). This chapter summarizes the data obtained with DiR-AsTNP.

## Synthesis

[PubMed]

Gao et al. described the synthesis of AsTNP and relevant nanoparticles in detail (1). The methoxy PEG-PCL (MPEG-PCL; molecular weight (MW), 3–15 kDa), R-carboxyl PEG-PCL (HOOC-PEG-PCL; MW, 3.4–15 kDa), and maleimide PEG-PCL (MAL-PEG-PCL; MW, 3.4–15 kDa) diblock copolymers were synthesized with ring-opening polymerization of the ε-caprolactone in dry toluene with MPEG, HOOC-PEG, or

maleimide-PEG as the initiator and stannous octoate as the catalyst. The chemical structures of the three diblock copolymers were confirmed with $^{1}$H NMR. The MW of the MPEG-PCL, HOOC-PEG-PCL, and MAL-PEG-PCL was 14,947.6, 14,975.5, and 16,866.8 Da, respectively.

The PEG-PCL nanoparticles (designated as NP) were prepared with an emulsion/solvent evaporation method (1). The carboxyl unit of NP was then activated and conjugated with AS1411 through reaction for 4 h in the dark (designated as AsNP). Conjugation of TGN was achieved by reaction with NP (designated as TNP) or AsNP (designated as AsTNP) for 6 h in the dark. Sepharose CL-4B column was used to remove the free AS1411 and TGN. For imaging and therapeutic purposes, DTX-, coumarin-6-, and DiR-loaded nanoparticles were also prepared separately with similar procedures.

Gao et al. characterized the size, morphology, and zeta potential of this system (1). Dynamic light scattering revealed that NP, AsNP, AsTNP, and TNP had diameters of 151.6 nm, 162.8 nm, 170.6 nm, and 153.6 nm, respectively. Conjugation with AS1411 or TGN slightly increased the particle sizes, and encapsulation of DTX, coumarin-6, or DiR did not affect the particle size. Transmission electron microscopy showed that these particles were generally spheroid in shape. The zeta potential was approximately −4.45 mV, −8.91 mV, −8.79 mV, and −2.31 mV for NP, AsNP, AsTNP, and TNP, respectively. The negative charge of the AS1411 lowered the zeta potentials of the AsNP and AsTNP compared to that of the NP. The information on how many TGN or AS1411 were bound per NP was not provided.

Release of encapsulated coumarin-6 and DiR from the particles was evaluated with a dialysis method, and the results showed that the accumulated release of coumarin-6 and DiR from the particles was <0.1% of their total amounts within three days. The DTX encapsulation efficiency (DTX encapsulated/DTX total × 100%) and drug-loading capacity (DTX encapsulated/materials × 100%) were 47.8% and 1.59%, respectively.

## *In Vitro* Studies: Testing in Cells and Tissues

[PubMed]

*In vitro* cell uptake of the nanoparticles was evaluated with C6 glioma cells and bEnd.3 cells after incubation for 1 h with coumarin-6-loaded NP, AsNP, TNP, or AsTNP (1). bEnd.3 cells are an immortalized mouse brain endothelial cell line exhibiting endothelial properties. This cell line is widely used as a model of BBB due to its rapid growth, maintenance of the BBB characteristics over repeated passages, formation of functional barriers, and amenability to molecular interventions (9). Fluorescent microscopy showed that the uptake by both cell types was dependent on the particle formulations. For the bEnd.3 cells, the cellular uptake for AsNP was only slightly higher than that for NP, suggesting that AS1411 did not enhance the uptake by bEnd.3 cells. Conjugation with TGN markedly increased the cellular uptake for AsTNP and TNP, indicating that TGN effectively mediated the uptake of the nanoparticles by endothelial cells. For the C6 cells, the uptake of TNP was almost the same as that of NP, but the uptake of AsNP and AsTNP

was higher than that of TNP and NP, which was considered to be due to the AS1411-mediated endocytosis. These results demonstrated that TGN mediated the first-stage targeting and AS1411 mediated the second-stage targeting.

The cellular uptake of the nanoparticles was further evaluated with tumor spheroids that were prepared by seeding C6 cells in low-melting-temperature agarose for 7 days (1). Confocal microscopy showed that AsNP and AsTNP distributed through the whole spheroids with high fluorescent intensity. In contrast, TNP and NP were only observed at the surface of C6 spheroids with weak fluorescence. These results indicate that AS1411 effectively increased the tumor cell uptake, but TGN did not.

Analysis with co-culture of the C6 spheroids with bEnd.3 monolayers showed that the co-culture significantly decreased the spheroid uptake of AsNP but not the uptake of AsTNP. Of the four formulations, AsTNP had the highest uptake and NP had the lowest. These results suggested that TGN enhanced the transport of nanoparticles across the bEnd.3 monolayer but did not increase their uptake by the C6 spheroids; both AS1411 and TGN were necessary for effective barrier penetration and cell uptake.

# Animal Studies

## Rodents

[PubMed]

*In vivo* imaging was performed in C6 orthotopic glioma-bearing BALB/c nude mice after injection of the DiR-loaded NP, AsNP, TNP, or AsTNP (no description for the number of mice used) (1). NP and AsNP without TGN conjugation showed similar weak fluorescence in the mouse brain, although the tumor fluorescence intensity obtained from AsNP was 0.65-fold higher than that from NP, indicating that AS1411 did not enhance the brain uptake of the particles. On the contrary, TGN-conjugated TNP and AsTNP showed strong fluorescence in the brain, which reached the highest intensity at 12 h after injection. The fluorescent intensity in the brain for TNP and AsTNP was 3.76-fold and 1.38-fold higher, respectively, than that for NP. However, *ex vivo* imaging of the brain at 24 h after injection showed that TNP distributed throughout the whole brain without selectivity, while AsTNP mainly accumulated in the glioma of the brain, indicating effective tumor targeting with AS1411. The tumor uptake for AsTNP and TNP was 3.91-fold and 2.95-fold higher, respectively, than that for NP. The highest tumor/brain ratio was obtained with AsTNP, but this ratio obtained with TNP was lower than that with NP due to high brain fluorescence for TNP. These results indicated that conjugation with TGN and AS1411 enhanced the glioma uptake of the nanoparticles and increase the tumor/brain ratio *in vivo*.

*In vivo* anti-glioma efficacy of DTX-loaded AsTNP, AsNP, TNP, or NP was evaluated in C6 orthotopic glioma-bearing BALB/c nude mice after injection of 6 mg/kg of each formulation every three days for three times ($n$ = 6 mice/group) (1). Saline and DTX were used as controls. Log-rank tests showed that the median survival times for the mice

treated with AsNP, AsTNP, and TNP were all significantly longer than those of the mice treated with saline and DTX (Table 1). Treatment with AsTNP resulted in the longest survival time. In this study, AsNP also significantly increased the median survival time compared with NP, although the second-stage targeting ligand TGN was not conjugated. DTX and NP had no anti-glioma effect, similar to saline, which was most likely due to the low dose of DTX used in the experiment. These results demonstrated that combined TGN and AS1411 conjugation could enhance the anti-tumor efficacy of the nanoparticles.

Table 1: The median survival time of the mice bearing orthotropic glioma after treatment.

| Group | Median (day) | 95% Confidence Interval | Significant | IST |
|---|---|---|---|---|
| Saline | 17.0 | 15.5–18.5 | | |
| DTX | 18.0 | 14.4–21.6 | | 6% |
| NP | 17.0 | 13.8–20.2 | | 0% |
| AsNP | 25.0 | 20.2–29.8 | a, b, c | 47% |
| AsTNP | 32.0 | 26.0–38.0 | a, b, c, d, e | 88% |
| TNP | 25.0 | 13.0–37.0 | a, b | 47% |

[a]$P < 0.05$ *vs* saline, [b]$P < 0.05$ *vs* DTX, [c]$P < 0.05$ *vs* NP, [d]$P < 0.05$ *vs* AsNP, [e]$P < 0.05$ *vs* TNP. IST, increase in survival time compared with saline group.

## Other Non-Primate Mammals

[PubMed]

No references are currently available.

## Non-Human Primates

[PubMed]

No references are currently available.

# Human Studies

[PubMed]

No references are currently available.

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