# A PSMA-targeted Theranostic Nanoplex Combining TRAIL Gene cDNA and Prodrug Enzyme Delivery For Prostate Cancer Treatment

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**Introduction:** We have previously developed a prostrate specific membrane antigen (PSMA)-based platform to deliver a prodrug enzyme and small interfering RNA (siRNA) to suppress a gene of interest for theranostic imaging [1]. Here we have expanded the platform for gene delivery to express tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) cDNA. PSMA is a type II integral membrane protein with abundant expression on the surface of prostate carcinomas. TRAIL has been reported to specifically kill malignant cells but to be relatively nontoxic to normal cells.
Our PSMA-targeted theranostic nanoplex carries a near-infrared (NIR) fluorescent probe Cy5.5 for optical detection, TRAIL cDNA to induce cell apoptosis, and a prodrug enzyme that synthesizes a cytotoxic drug locally from a systemically administered nontoxic drug at the nanoplex accumulation site. The prodrug enzyme bacterial cytosine deaminase (bCD) converts a non-toxic prodrug 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU) that can be detected by $^{19}$F MRS. The pGFP-TRAIL cDNA produces a TRAIL fusion protein containing green fluorescent protein (GFP) to monitor the expression of TRAIL cDNA.

**Methods:** Our prototype nanoplex was synthesized by conjugating three compartments: (i) the prodrug-activating enzyme bCD, (ii) the cDNA (pGFP-TRAIL) delivery vector: PEI (polyethyleneimine)-PEG (polythethyeneglycol) co-grafted-polymer [2] that carries Cy5.5 for optical imaging and, (iii) the linker poly-L-lysine (PLL) between the PEI and bCD. These three compartments were covalently conjugated and TRAIL cDNA was associated with the PEI-PEG co-grafted polymer through electrostatic binding. For PSMA targeting, a low molecular weight urea-based PSMA targeting moiety (2-(3-[1-carboxy-5-[7-(2,5-dioxo-pyrrolidin-1-yloxycarbonyl)-heptanoylamino]-pentyl]-ureido)-pentanedioic acid (MW 572.56) was conjugated to PEI by Maleimide-PEG-NHS (MW ~3,000).
Cell and tumor imaging studies with PSMA targeted nanoplexes were performed with isogenic PSMA-negative (wild type) PC-3 human prostate cancer cells (PC-3 Flu) and cells genetically engineered to overexpress PSMA (PC-3 PIP). PC-3 Flu cells and tumors derived from them were used as controls. Cell imaging was performed with a Zeiss LSM510-Meta single-point laser scanning confocal microscope. Fluorescence imaging of tumors was performed *in vivo* and *ex vivo* with a Caliper IVIS Spectrum system. *In vivo* $^{19}$F MRS of tumors was performed with a Bruker horizontal bore 9.4T MR spectrometer using a home-built RF resonator.

**Results and Discussion:** Higher GFP expression level in PC3-PIP cells indicated more accumulation and incorporation of the cDNA delivered by the nanoplex in cells expressing PSMA (Figure 1). Images obtained with PIP and Flu tumors demonstrated increased uptake in the PSMA overexpressing PIP tumor compared to the PSMA -negative Flu tumor (Figure 2A). In separate studies we performed optical imaging of the nanoplex in tissue slices with PIP and Flu tumors (Figure 2B). Increased uptake of nanoplex was detected by increased concentration of Cy5.5 and higher expression of GFP in the PIP tumor compared to the Flu tumor. The prodrug enzyme bCD converted the prodrug 5-FC to 5-FU at 24 h as shown in Figure 2C. Increased inhibition of PIP tumor growth was demonstrated in Figure 2D.

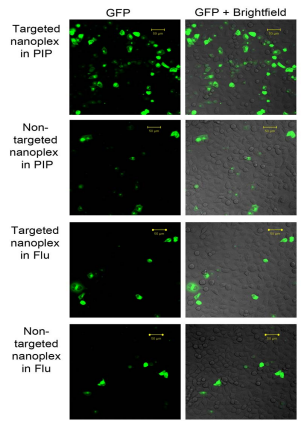


**Figure 1**: Laser confocal fluorescence microscope imaging of PC-3-PIP (PSMA+) and PC-3-Flu (PSMA-) cells. Cells were treated with pGFP-TRAIL cDNA/nanoplex for 6 h (concentration of cDNA: 2 µg/ml; N/P ratio: 50).

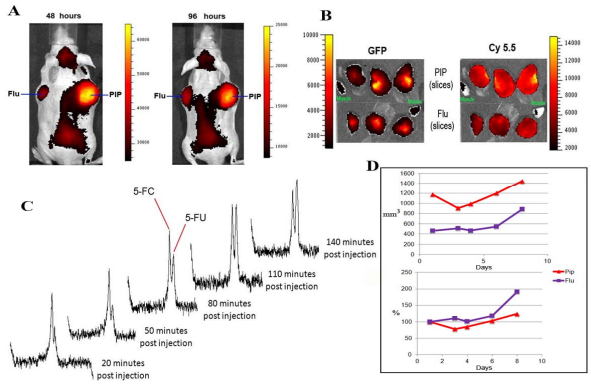


**Figure 2. (A)** *In vivo* optical imaging of a SCID mouse bearing PSMA+ PC-3 PIP and PSMA- PC-3 Flu tumors. **(B)** *Ex vivo* optical imaging of PIP and Flu tumor slices at 96 h . Mouse was injected *i.v.* with nanoplex (150 mg/kg in 0.2 ml). **(C)** *In vivo* $^{19}$FMR spectra acquired from a PC-3-PIP tumor at 24 h after *i.v.* injection of the nanoplex (100 mg/kg) carrying bCD. Spectra were acquired after a combined *i.v.* and *i.p.* injection of 5-FC (450 mg/kg) on a Bruker Biospec 9.4 T spectrometer using a 1 cm solenoid coil tunable to $^{1}$H and $^{19}$F frequency. **(D)** Inhibition of PIP and Flu tumor growth with a combined *i.v.* (200 µL) and *i.p.* (500 µL)) injection of 5-FC (450 mg/kg) at 24 h after *i.v.* injection of the nanoplex (100 mg/kg).

The targeted nanoplex, which carries imaging reporters together with cDNA and a prodrug enzyme, will be useful for theranostic imaging of metastatic PCa. This platform technology can also be extended to many cancer-related targets with the goal of increasing the efficacy, safety, and efficiency of therapy.

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**References:** 1. Chen, Z. *et al., ACS Nano,* 2012. 2. Li, C. *et al., ACS Nano,* 2010.