

uSPIO-5D3-DM1 Nano-conjugates as Optical-MRI Theranostics for PSMA-Positive Prostate Cancer Therapy

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**NEXT-GENERATION
DELIVERY INNOVATIONS**

Prostate cancer statistics and 2025 estimation



1 in 7 men will be diagnosed with Prostate Cancer in their lifetime.



Globally, **more than 1.4 million men** are diagnosed with prostate cancer each year.



68%

of men **know someone** who has had Prostate Cancer.



25%

of men said they **never discussed** a Prostate Cancer screening with their doctor.



42%

of men weren't familiar with the **warning signs** of Prostate Cancer.

The American Cancer Society advises you to screen for prostate cancer **starting at age 50** if you're at average risk. If you have a higher risk, begin discussing your options earlier with your healthcare provider.



www.cancerresearch.org

Estimated New Cases

Male		
Prostate	313,780	30%
Lung & bronchus	110,680	11%
Colon & rectum	82,460	8%
Urinary bladder	65,080	6%
Melanoma of the skin	60,550	6%
Kidney & renal pelvis	52,410	5%
Non-Hodgkin lymphoma	45,140	4%
Oral cavity & pharynx	42,500	4%
Leukemia	38,720	4%
Pancreas	34,950	3%
All sites	1,053,250	



Female		
Breast	316,950	32%
Lung & bronchus	115,970	12%
Colon & rectum	71,810	7%
Uterine corpus	69,120	7%
Melanoma of the skin	44,410	4%
Non-Hodgkin lymphoma	35,210	4%
Pancreas	32,490	3%
Thyroid	31,350	3%
Kidney & renal pelvis	28,570	3%
Leukemia	28,170	3%
All sites	988,660	

Estimated Deaths

Male		
Lung & bronchus	64,190	20%
Prostate	35,770	11%
Colon & rectum	28,900	9%
Pancreas	27,050	8%
Liver & intrahepatic bile duct	19,250	6%
Leukemia	13,500	4%
Esophagus	12,940	4%
Urinary bladder	12,640	4%
Non-Hodgkin lymphoma	11,060	3%
Brain & other nervous system	10,170	3%
All sites	323,900	



Female		
Lung & bronchus	60,540	21%
Breast	42,170	14%
Pancreas	24,930	8%
Colon & rectum	24,000	8%
Uterine corpus	13,860	5%
Ovary	12,730	4%
Liver & intrahepatic bile duct	10,840	4%
Leukemia	10,040	3%
Non-Hodgkin lymphoma	8,330	3%
Brain & other nervous system	8,160	3%
All sites	294,220	

- Compared to the 2024 estimation: Increasing new cases and deaths, no cure for mCRPC.
- Developing new, effective treatments is critically important.

Siegel, RL, *et al*, *CA A Cancer J Clinicians*, 2025, 75(1): 10-45

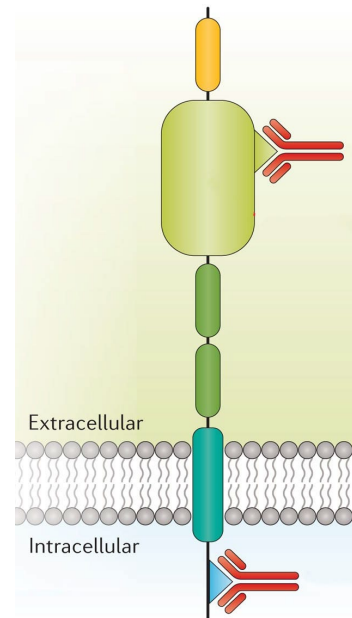
PSMA as a biomarker and nano-scale drug delivery

Prostate-specific membrane antigen

- mCRPC is incurable: Biomarker heterogeneity and drug resistance.
- PSMA: >90% of including advanced PCs.
- For imaging PC and targeted therapy
- Anti-PSMA 5D3 mAb
- 5D3: high binding affinity with PSMA
- 5D3-DM1 and 5D3-MLN8237

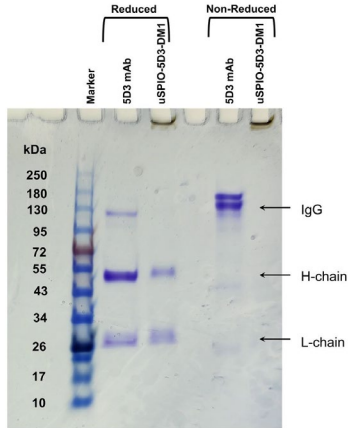
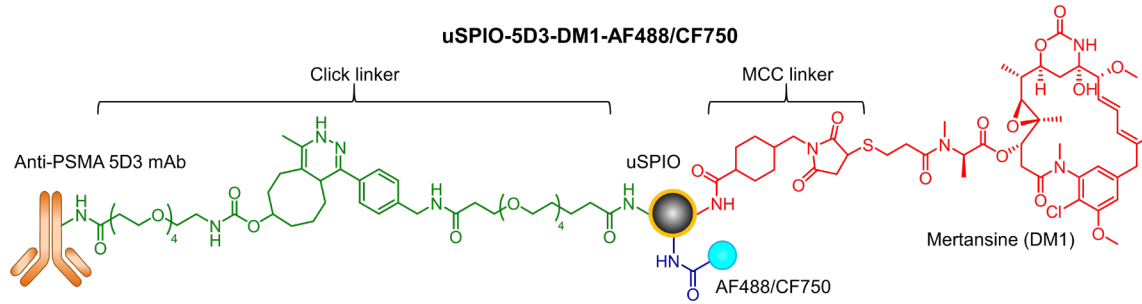
Ultrasmall superparamagnetic iron oxide (uSPIO) nanoparticles

- High loading/conjugation capacity
- Passive and active targeting
- Biocompatibility
- MRI-sensitive/contrast agent
- Ideal platform for clinically-relevant theranostics

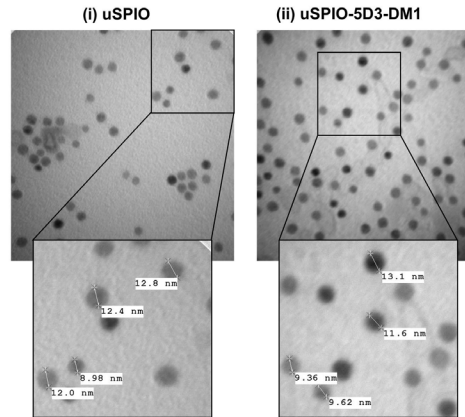


Nature Reviews Urology,
2016; 13, 226–235.

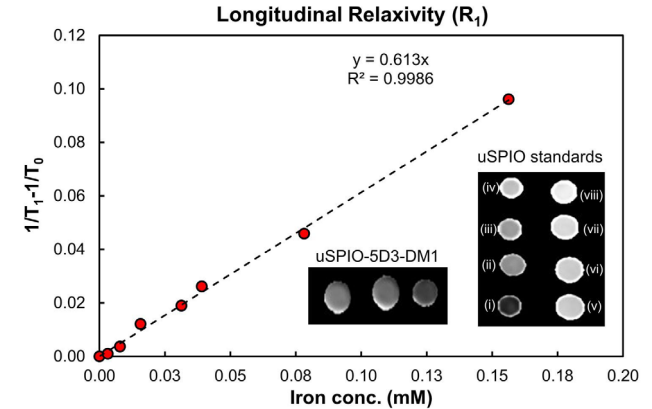
uSPIO-5D3-DM1-AF488/CF750



SDS-PAGE



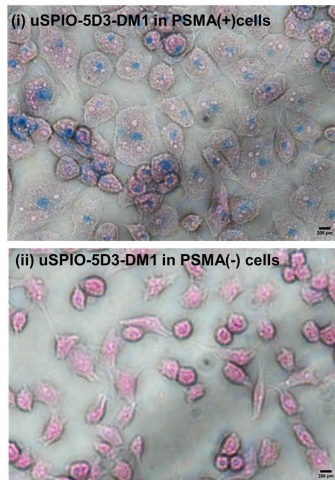
TEM



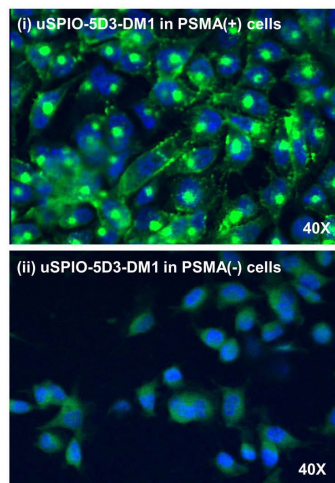
MRI phantom

In vitro evaluation of uSPIO-5D3-DM1-AF488

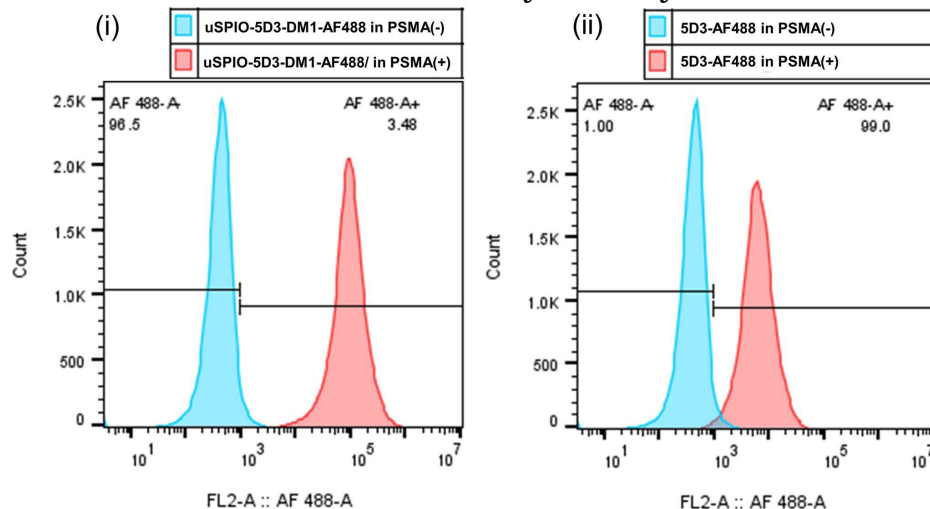
Prussian blue



Confocal fluorescence



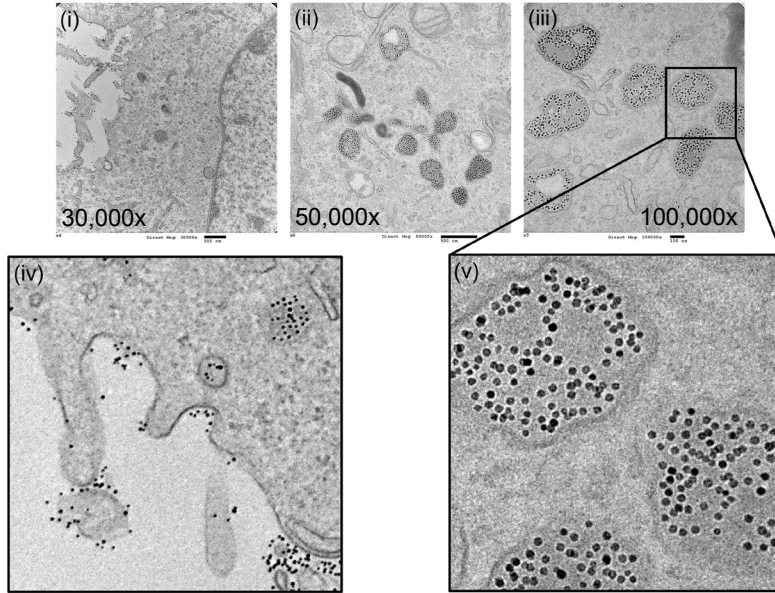
Flow cytometry



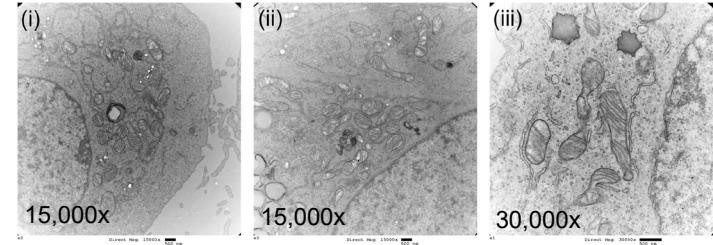
- PSMA(+) PC3-PIP and PSMA(-) PC3-Flu cells.
- PSMA(+/-) cells treated with uSPIO-5D3-DM1-AF488 (20 μ g/mL at 37 $^{\circ}$ C for 2h).
- Prussian blue staining and fluorescence imaging: Uptake by PSMA(+) cells
- Flow cytometry: Better cellular uptake than anti-PSMA mAb.

In vitro evaluation of uSPIO-5D3-DM1 by TEM

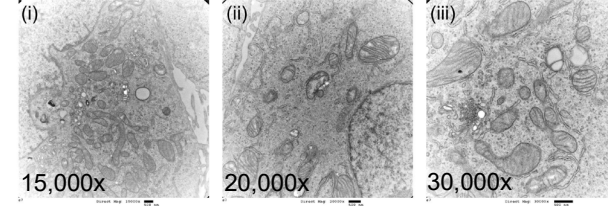
uSPIO-5D3-DM1 in PSMA(+) PC3-PIP cells



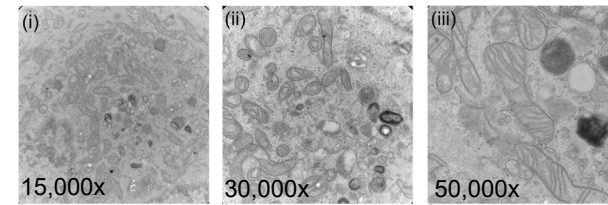
uSPIO-5D3-DM1 in PSMA(-) PC3-Flu cells



uSPIO in PSMA(+) PC3-PIP cells

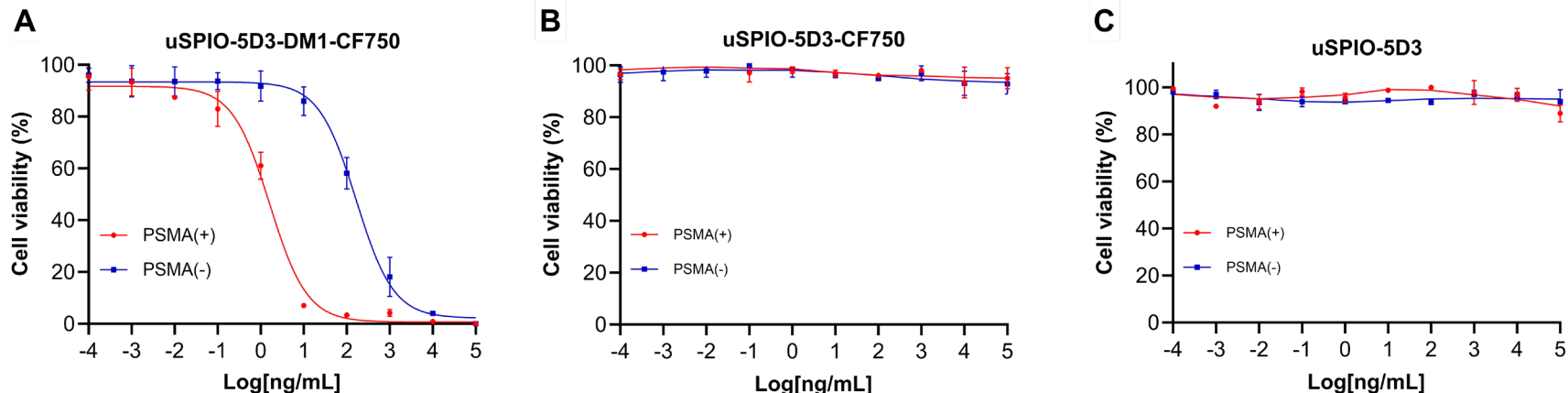


uSPIO in PSMA(-) PC3-Flu cells



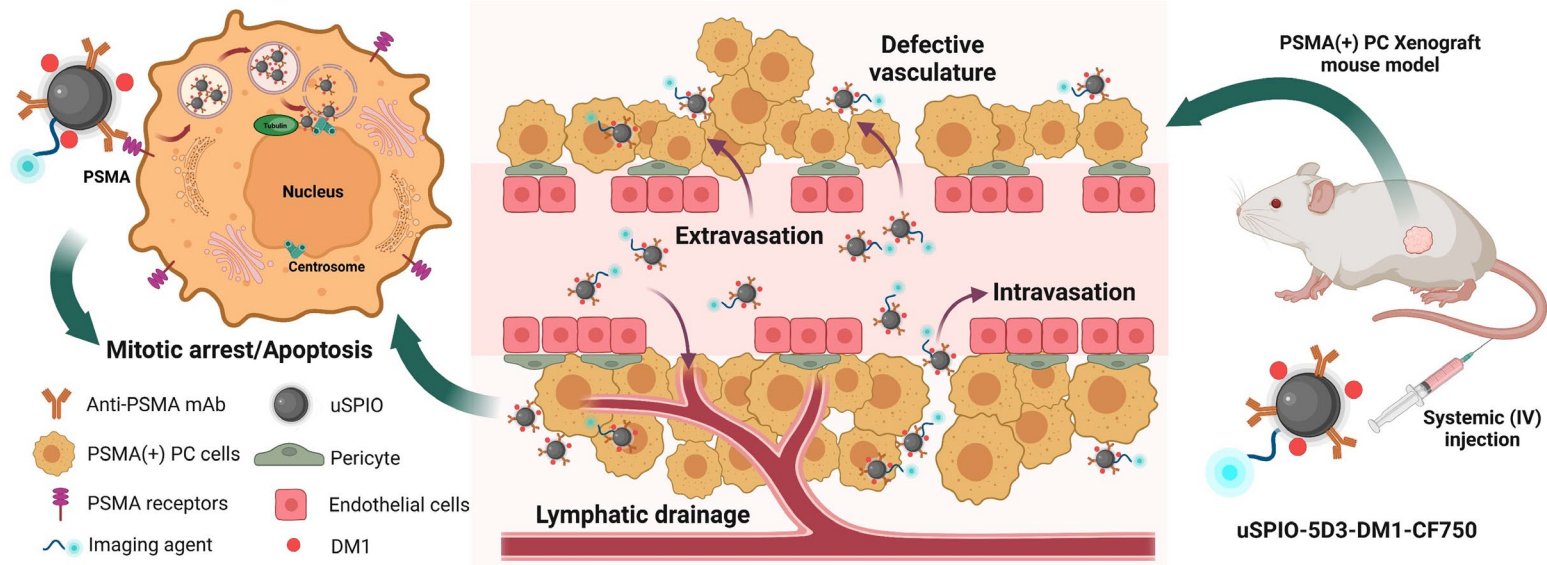
- uSPIO-5D3-DM1 (50 μ g/mL at 37°C for 5 h).
- Philips CM120 TEM.
- Internalization by receptor-mediated endocytosis.

Cytotoxicity of uSPIO-5D3-DM1-CF750



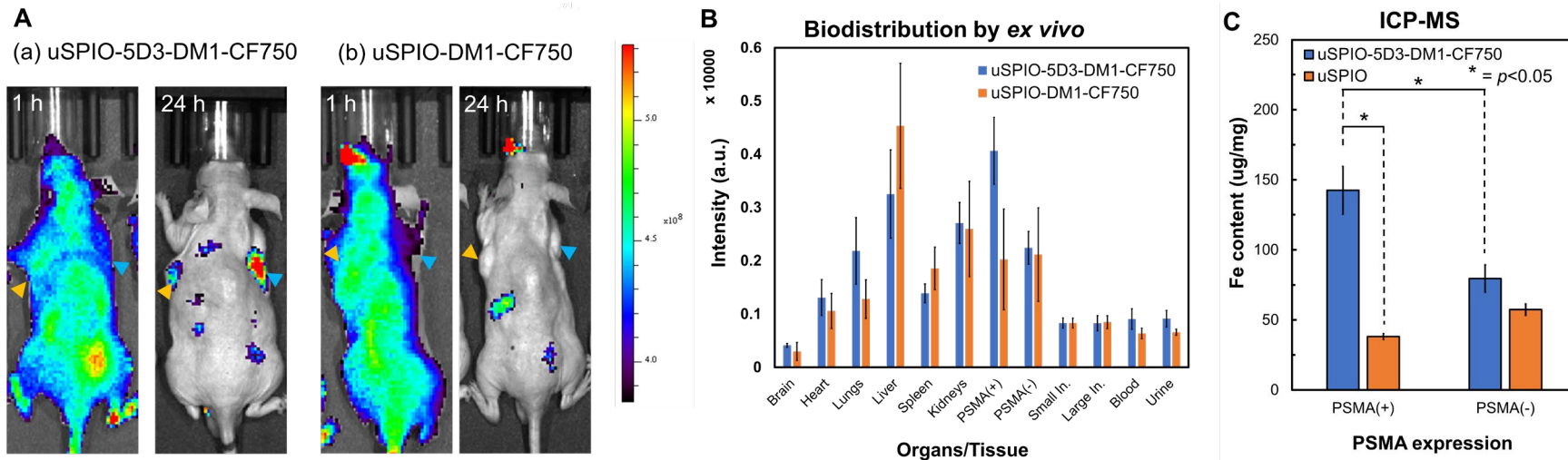
- (A) Cytotoxicity of uSPIO-5D3-DM1-CF750 in PSMA(+) PC3-PIP ($IC_{50} = 1.62$ ng/mL, $R^2 = 0.9896$) and PSMA(-) PC3-Flu ($IC_{50} = 166.2$ ng/mL, $R^2 = 0.9898$) cells.
- (B) uSPIO-5D3-CF750: Change in cell viability in PSMA(+) and PSMA(-) cells.
- (C) uSPIO-5D3: Change in cell viability in PSMA(+) and PSMA(-) cells.

Schematic for uSPIO-5D3-DM1-CF750 *in vivo*



Schematic of drug administration, tumor microenvironment, and subcellular activity depicting the extravasation, PSMA-driven cell surface binding, cellular uptake, and therapeutic mechanism of uSPIO-5D3-DM1-AF488/CF750.

Biodistribution of uSPIO-5D3-DM1-CF750

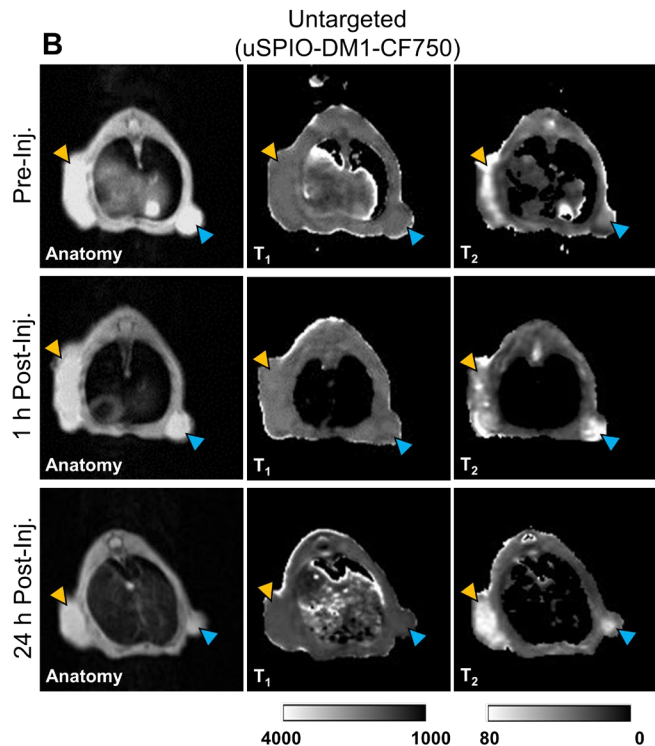
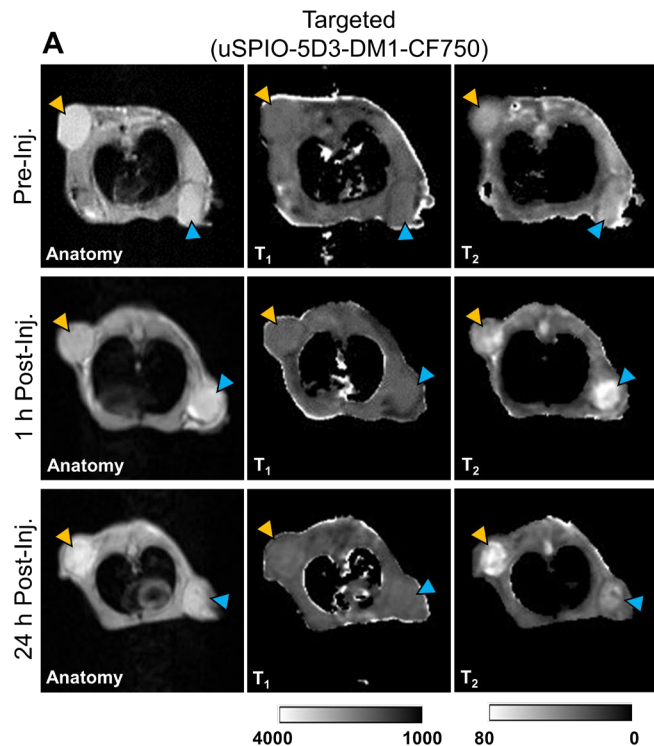


(A) IVIS optical imaging: Mice with PSMA(+) tumors (▲) and PSMA(-) tumor (▲).

(B) *Ex vivo* BD: Uptake by PSMA(+) tumors > PSMA(-) tumors.

(C) ICP-MS analysis: Uptake of uSPIO-5D3-DM1-CF750 by the PSMA(+) tumor > PSMA(-) tumor.

Anatomical MRI and quantitative T_1 and T_2 maps



- uSPIO-DM1-CF750 and uSPIO-DM1-CF750
- Dose: 10 mg/kg
- Anatomical, T_1 , and T_2 MRI images
- 1 h and 24 h post-inj time points.
- PSMA(+) tumors (▲) and PSMA(-) tumors (▲).
- uSPIO-5D3-DM1-CF750 in PSMA(+): 20.6% reduction in T_1 , PSMA(-) only a 9.7% reduction.
- A 42% reduction of T_2 in PSMA(+) tumors compared to a 16.7% reduction in PSMA(-) tumors.

Toxicological evaluation of uSPIO-5D3-DM1-CF750

Analyte/Blood test	Targeted group	Untargeted group	Healthy group	Standard range
1. Total serum protein (T-Pro, g/dL)	6.85 (± 0.19)	7.77 (± 0.43)	7.65 (± 0.15)	6.00-10.0
2. Blood urea nitrogen (BUN, mg/dL)	53.3 (± 2.9)*	30.3 (± 3.93)	19.5 (± 4.5)	20.0-40.0
3. Alkaline phosphatase (ALP, IU/L)	62.5 (± 26.9)	63.3 (± 17.02)	90.0 (± 9.0)	35.0-96.0
4. Creatinine (Cre, mg/dL)	1.05 (± 0.09)	1.03 (± 0.07)	0.85 (± 0.15)	0.60-2.72
5. Alanine transaminase (ALT, IU/L)	60.8 ± 13.4)	35.3 (± 4.1)	37.0 (± 1.0)	25.0-60.0
6. Red blood cell count (RBC, K/ μ L)	5.93 (± 0.79) $\times 10^6$	6.63 (± 0.58) $\times 10^6$	5.03 (± 2.13) $\times 10^6$	3.8-7.9 $\times 10^6$
7. White blood cell count (WBC, K/ μ L)	15.3 (± 1.9)*	5.87 (± 2.5)	1.2 (± 0.2)	2.0-10.0
8. Platelet count (PLT, K/ μ L)	871 (± 71)	1040 (± 187)	1707 (± 747)	600-1500

- T-Pro, BUN, ALP, Cre, ALT: Spotchem EZ Vet Chem analyzer.
- CBC: Scil Vet abc Plus hematology analyzer.
- uSPIO-5D3-DM1-CF750: T-Pro, ALP, Cre, ALT, RBC, and PLT healthy range.
- BUN and WBC levels are changed, but no life-threatening issues were observed.

Conclusions

- uSPIO was conjugated with anti-PSMA 5D3 mAb and DM1 anti-tubulin agent to make uSPIO-5D3-DM1 nano-theranostic.
- uSPIO-5D3-DM1 shows higher binding affinity with PSMA(+) PC cells and internalizes faster than regular anti-PSMA mAb.
- uSPIO-5D3-DM1-CF750 is used as a theranostic and imaged-by optical and MRI
- It demonstrated high and homogeneous tumor uptake in PSMA(+) tumors and significant control of tumor growth in preclinical settings.
- uSPIO-5D3-DM1-CF750 exhibits low toxicological effects in PC tumor mouse models.
- uSPIO-5D3-DM1 as PET-MRI image-guided theranotics for evaluation of early treatment response and tumor metabolism.

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