5D3(CC-MLN8237) as a novel antibody-theranostic conjugate for PSMA(+) prostate cancer therapy

Sudath Hapuarachchige, PhD

Assistant Professor of Radiology and Oncology

Theranostic Research and Applied Therapeutics (TREAT) Lab
Division of Cancer Imaging Research
Department of Radiology and Radiological Science
The Johns Hopkins University School of Medicine
Baltimore, MD, USA.

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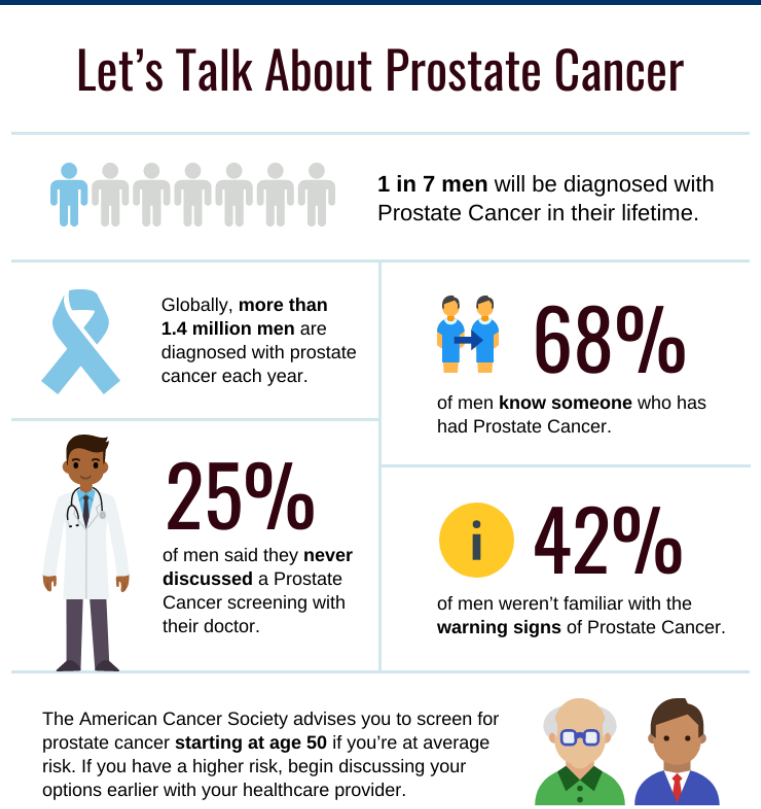
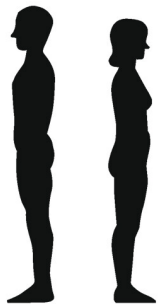
Prostate cancer statistics and 2024 estimation



www.cancerresearch.org

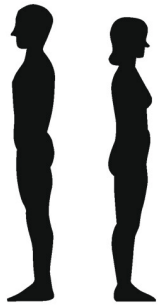
Estimated New Cases

Male			Female		
Prostate	299,010	29%	Breast	310,720	32%
Lung & bronchus	116,310	11%	Lung & bronchus	118,270	12%
Colon & rectum	81,540	8%	Colon & rectum	71,270	7%
Urinary bladder	63,070	6%	Uterine corpus	67,880	7%
Melanoma of the skin	59,170	6%	Melanoma of the skin	41,470	4%
Kidney & renal pelvis	52,380	5%	Non-Hodgkin lymphoma	36,030	4%
Non-Hodgkin lymphoma	44,590	4%	Pancreas	31,910	3%
Oral cavity & pharynx	41,510	4%	Thyroid	31,520	3%
Leukemia	36,450	4%	Kidney & renal pelvis	29,230	3%
Pancreas	34,530	3%	Leukemia	26,320	3%
All sites	**1,029,080**		**All sites**	**972,060**	



Estimated Deaths

Male			Female		
Lung & bronchus	65,790	20%	Lung & bronchus	59,280	21%
Prostate	35,250	11%	Breast	42,250	15%
Colon & rectum	28,700	9%	Pancreas	24,480	8%
Pancreas	27,270	8%	Colon & rectum	24,310	8%
Liver & intrahepatic bile duct	19,120	6%	Uterine corpus	13,250	5%
Leukemia	13,640	4%	Ovary	12,740	4%
Esophagus	12,880	4%	Liver & intrahepatic bile duct	10,720	4%
Urinary bladder	12,290	4%	Leukemia	10,030	3%
Non-Hodgkin lymphoma	11,780	4%	Non-Hodgkin lymphoma	8,360	3%
Brain & other nervous system	10,690	3%	Brain & other nervous system	8,070	3%
All sites	**322,800**		**All sites**	**288,920**	



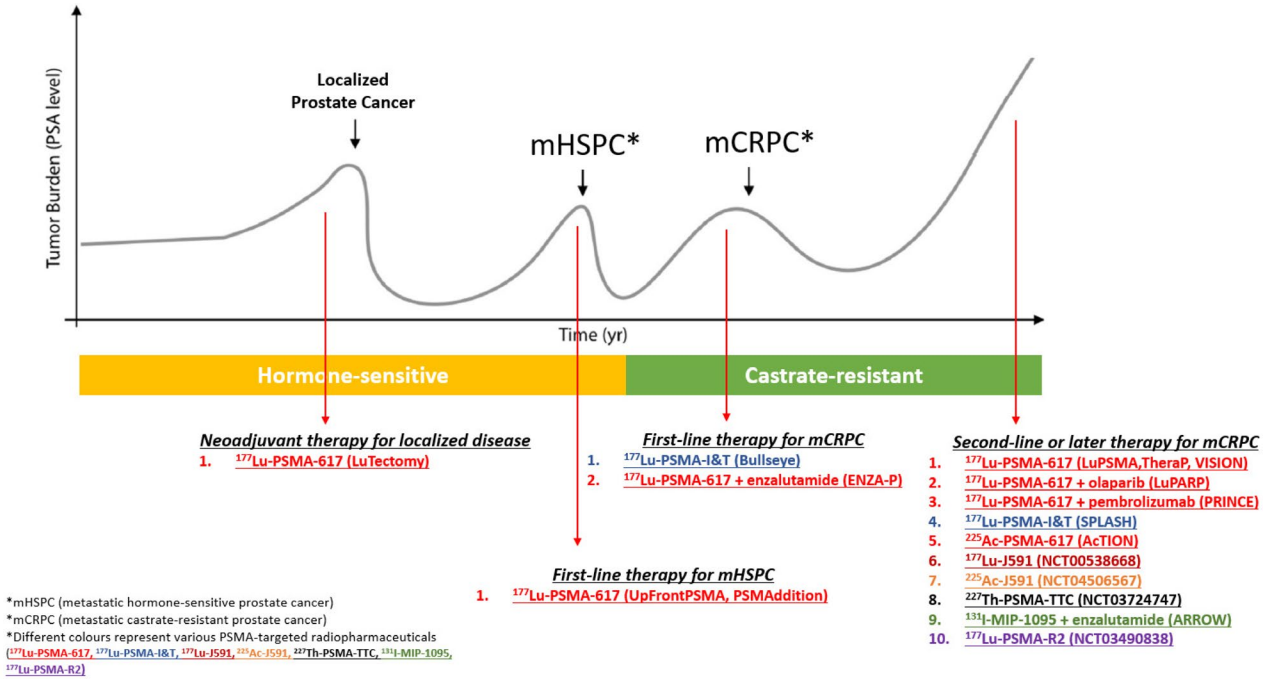
Estimates are rounded to the nearest 10, and cases exclude basal cell and squamous cell skin cancers and in situ carcinoma except urinary bladder. Estimates do not include Puerto Rico or other US territories. Ranking is based on modeled projections and may differ from the most recent observed data.

Siegel *et al.* **2024** *CA: Cancer J. Clin.* Cancer statistics, 2024.

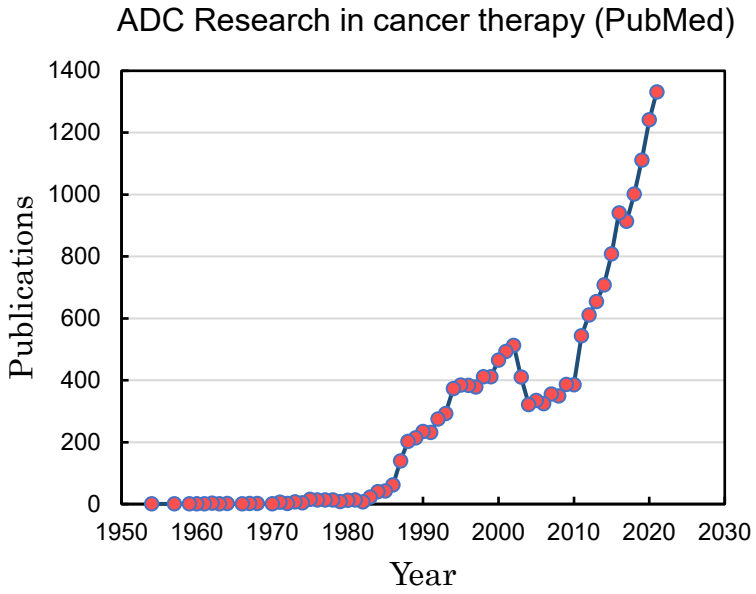
- Androgen deprivation therapy (ADT) is the mainstay of therapy for metastatic and recurrent prostate cancers.
- However, nearly all patients will eventually develop castration resistance.
- Despite significant advances in systemic therapy options, the prognosis for metastatic castration-resistant prostate cancer (mCRPC) is poor, with overall survival under two years.

PSMA theranostics and ADCs in cancer therapy

Schematic overview of selected PSMA theranostics clinical trials.



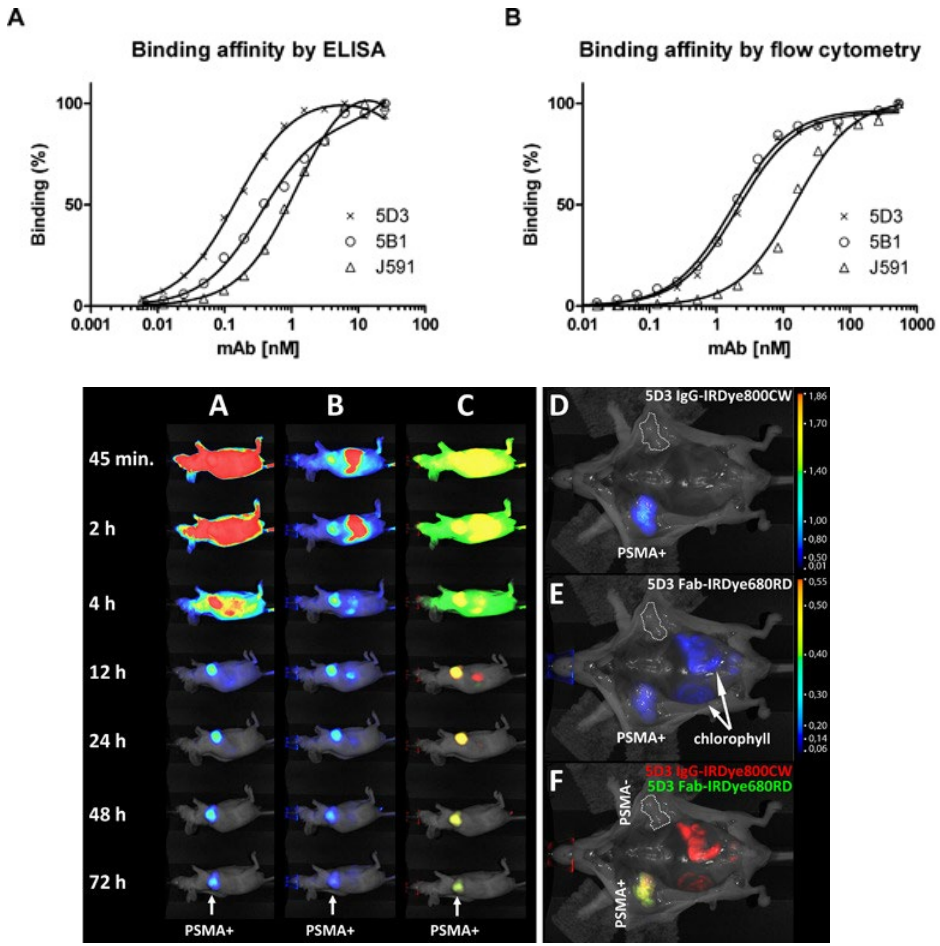
- PSMA is overexpressed (up to 1000 times more than normal prostate cells) in most prostate cancers (>90%).
- The level of expression may increase with tumor dedifferentiation and castration resistance.
- It can serve as a target for imaging and therapy due to its tumoral overexpression.



- For the targeted drug delivery.
- Era of precision medicine.
- New robust mAb with high binding affinity.
- Smart conjugations for narrow DAR.
- Development of targeted/potent drugs.

Zang *et al. Cancers* **2021**, 13(16), 4023; DOI: 10.3390/cancers13164023

Anti-PSMA 5D3 mAb



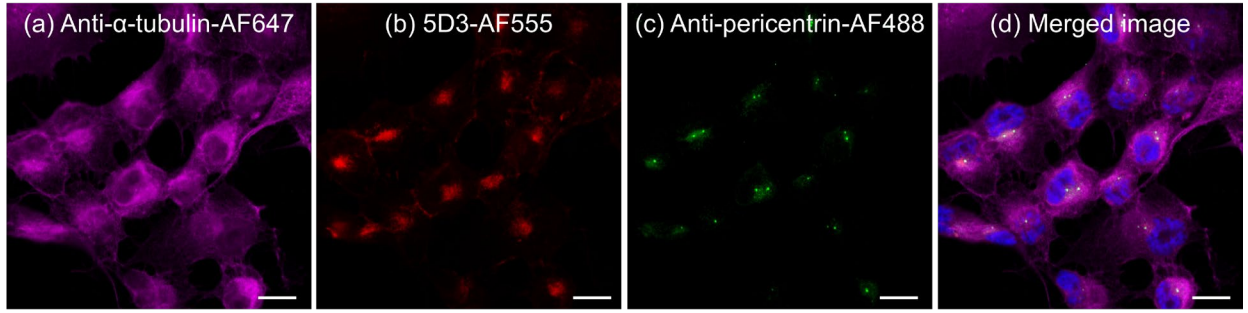
- 5D3 mAb shows higher binding affinity with PSMA than 5B1 and J591.
- *In vivo* NIR fluorescence imaging of the 5D3 IgG and Fab fragment.
- A mouse was co-injected with the IgG-IRDye800CW (A) and the Fab-IRDye680RD (B) with overlay shown in (C).
- A: High tumor contrast is achieved 12 h post-injection. By 24 h, whole-mouse background uptake is also low.
- B: High tumor contrast is achieved already 2 h post-injection [gastrointestinal (GI) signal is chlorophyll] and continues till 48 h post-injection.
- C: A high degree of co-localization from 12 h onwards.
- D/E: The 72 h uptake without skin of the IgG and Fab, respectively. Both are selective for the PSMA-positive tumor at 72 hr.
- F: Yellow co-localization of both antibody formats within the PSMA-positive tumor.
- 5D3-DM1 ADC: The first 5D3-based antibody-drug conjugate.

Nováková Z. *et al. The Prostate*, **2017**; 77(7): 749-764.

Huang CT *et al. Mol Pharm.* **2020**; 17(9): 3392-3402.

5D3 mAb in PSMA(+/-) cells

PSMA(+)
PC3-PIP



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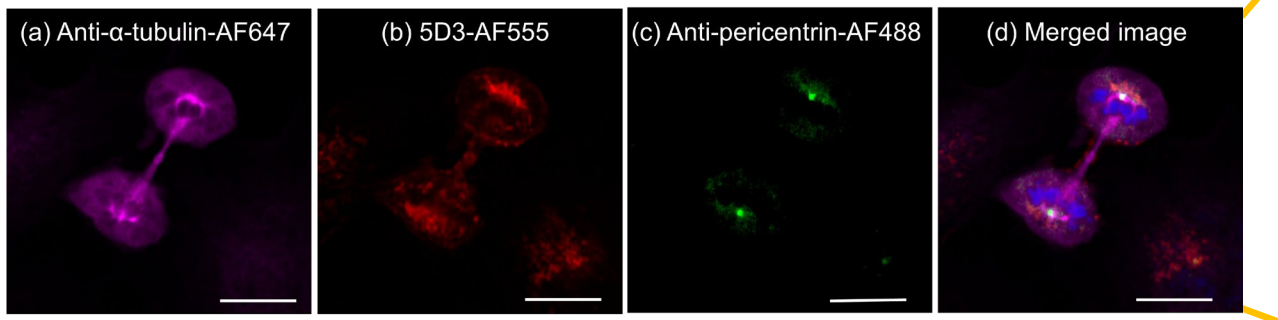
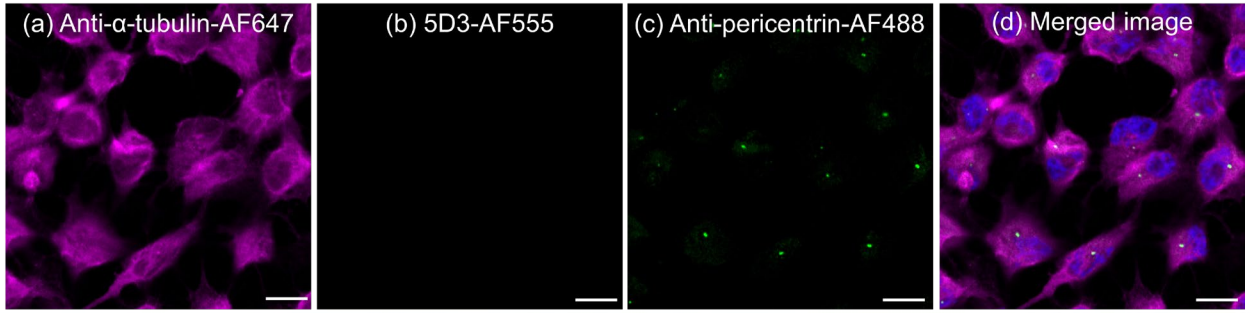
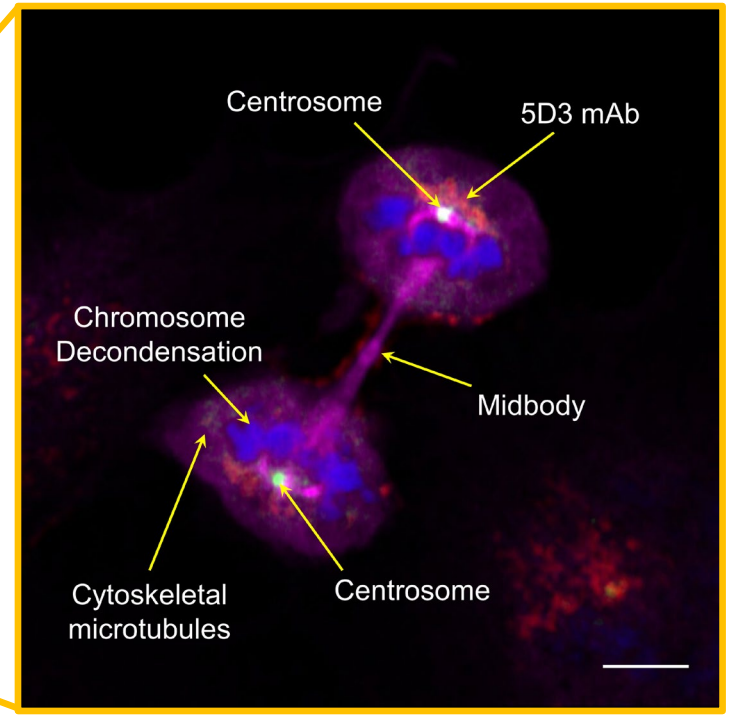
PSMA(+)
PC3-PIP

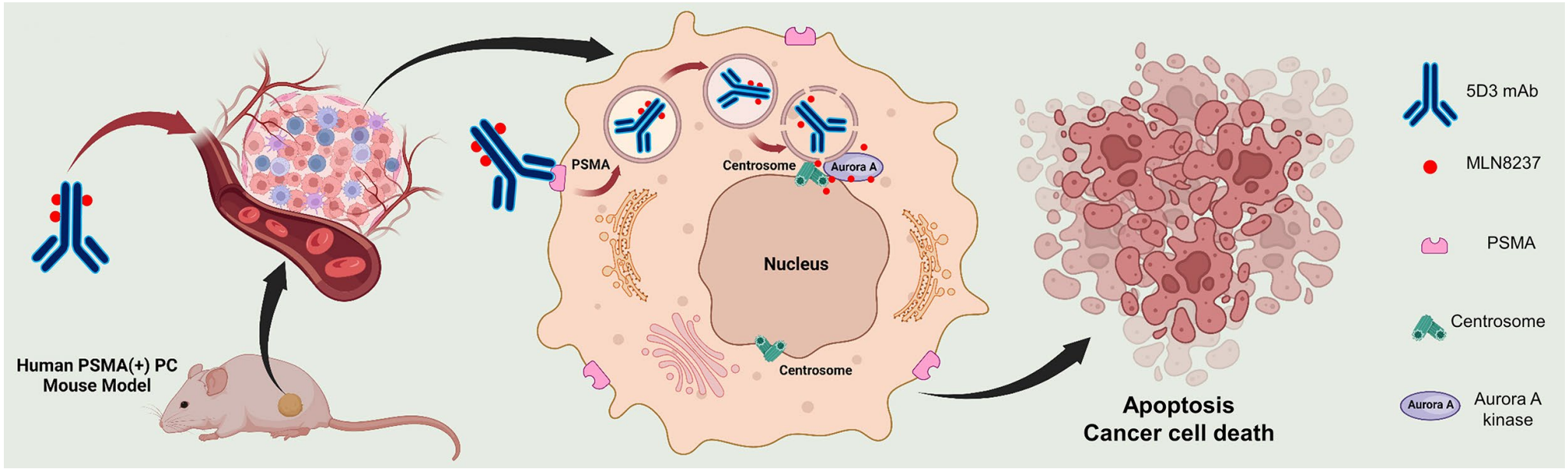


PSMA(-)
PC3-Flu





Anti-PSMA 5D3 antibody-Aurora A kinase inhibitor conjugate



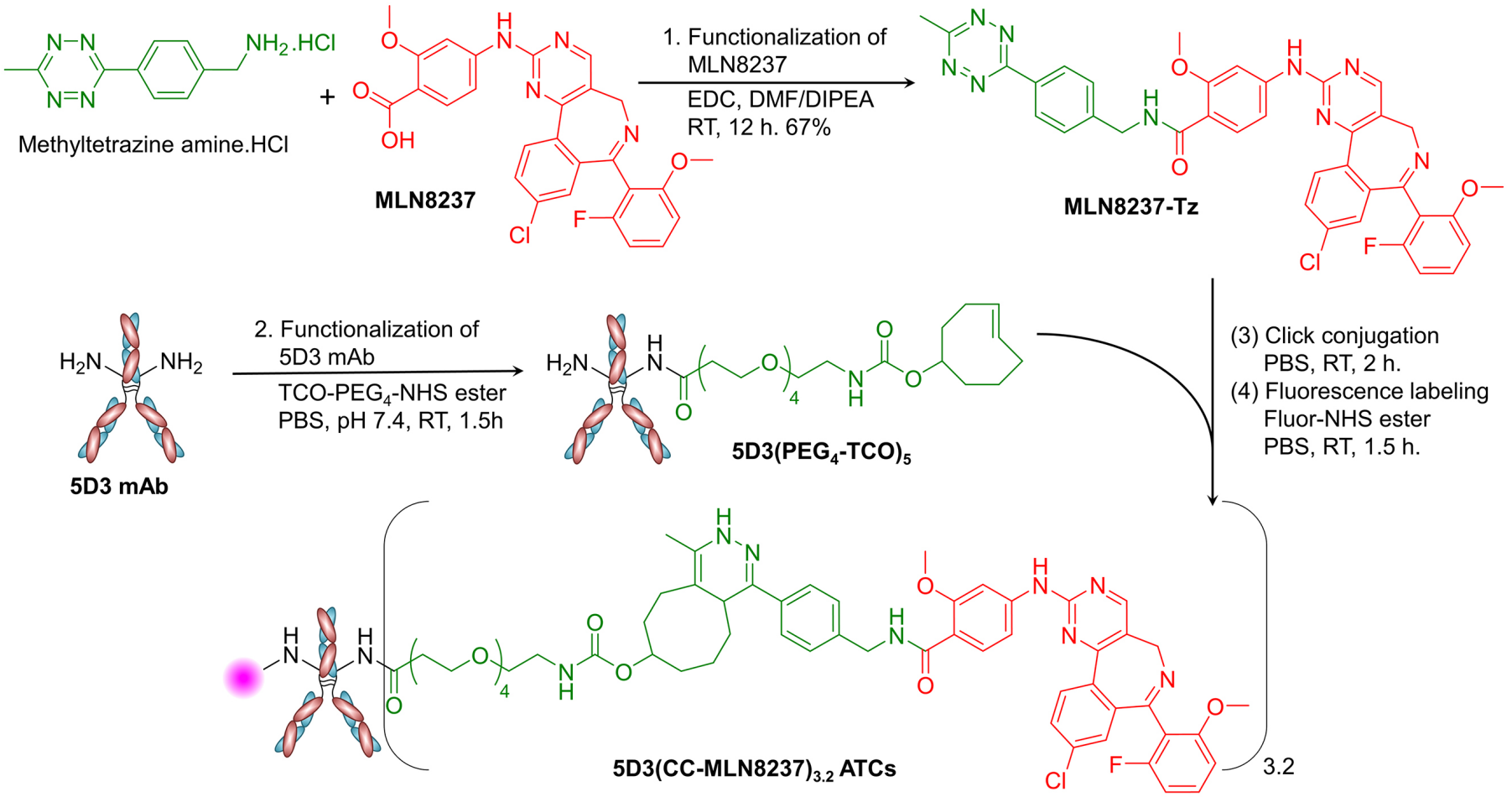
Aurora A kinase

- Centrosome maturation
- Chromatin condensation
- Spindle assembly
- Bipolar spindle formation
- Chromosome duplication

5D3-MLN8237 conjugation

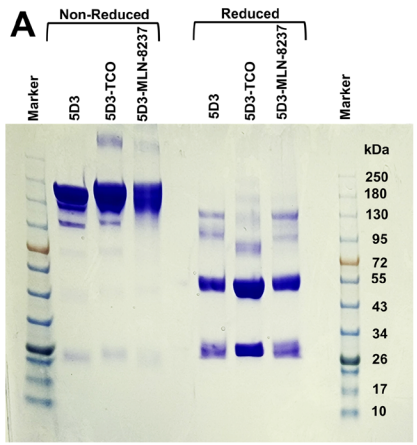
- PSMA specific and fast internalization
- Delivery of Aurora A kinase inhibitors at centrosomes
- Inhibition of Aurora A kinase
- Mitotic catastrophe
- Apoptosis/Cancer cell death

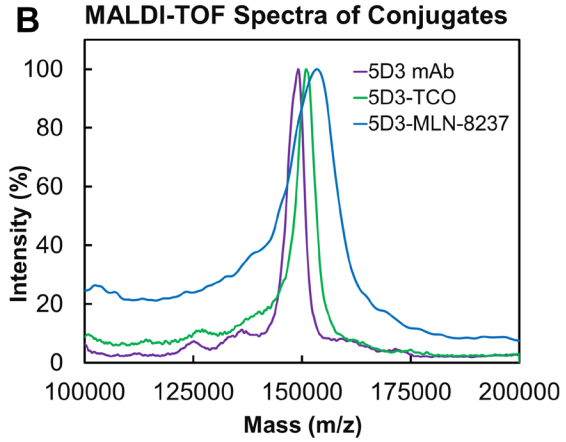
Synthesis of 5D3(CC-MLN8237)$_{3.2}$

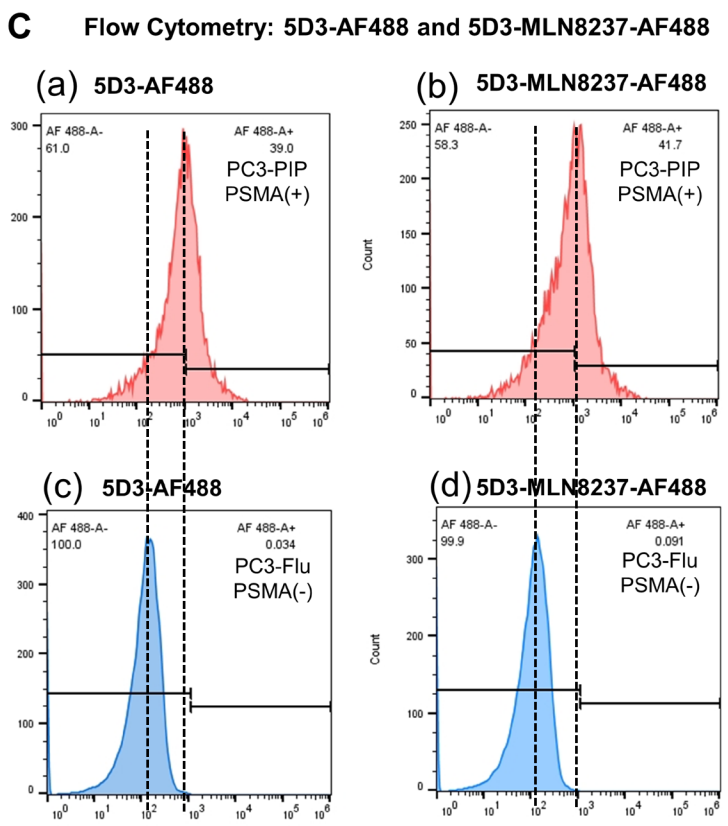


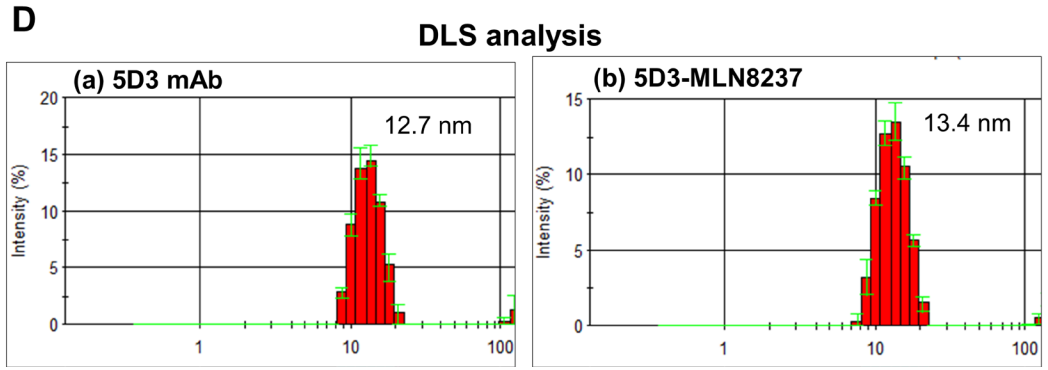
Liatsou I *et al. Front. Pharmacol.* **2024**; 5: 1-14.

Characterization of 5D3(CC-MLN8237)$_{3.2}$



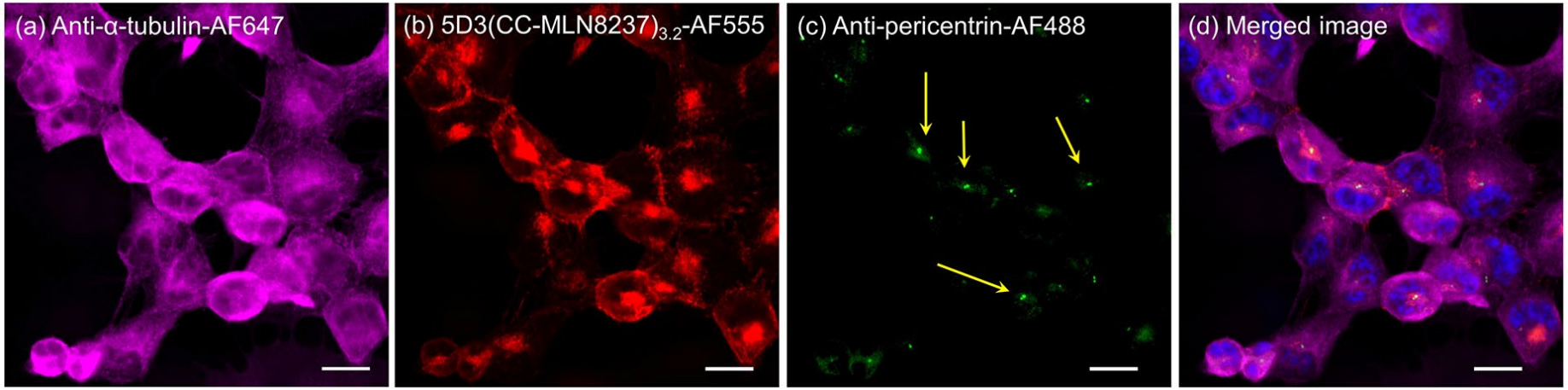






(A) SDS-PAGE analysis of 5D3 mAb, intermediate 5D3-TCO, and 5D3(CC-MLN8237)$_{3.2}$ under non-reducing and reducing conditions.
(B) MALDI-TOF spectra of 5D3 mAb, intermediate 5D3-TCO, and 5D3(CC-MLN8237)$_{3.2}$.
(C) Flow cytometry histogram showing unchanged affinity of 5D3-AF488 and 5D3(CC-MLN8237)$_{3.2}$-AF488 in PSMA(+/-) PC3-PIP cells.
(D) Dynamic light scattering analysis (DLS): Size distribution of (a) 5D3-AF488 and (b) 5D3(CC-MLN8237)$_{3.2}$ ATC.

In vitro imaging and cytotoxicity of 5D3(CC-MLN8237)$_{3.2}$



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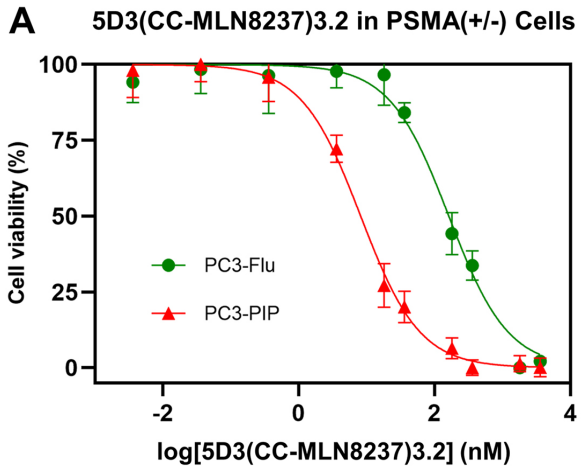
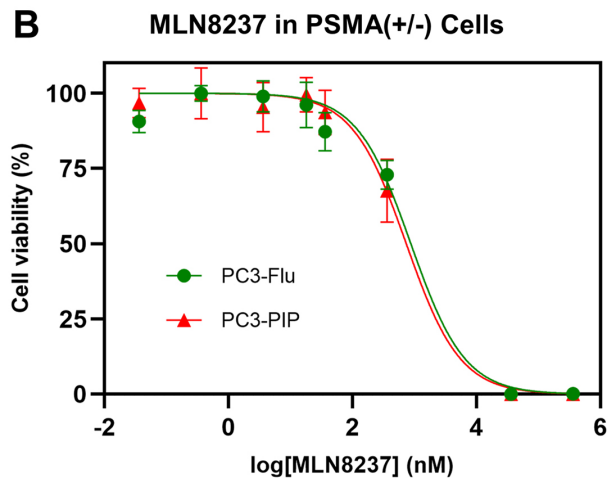
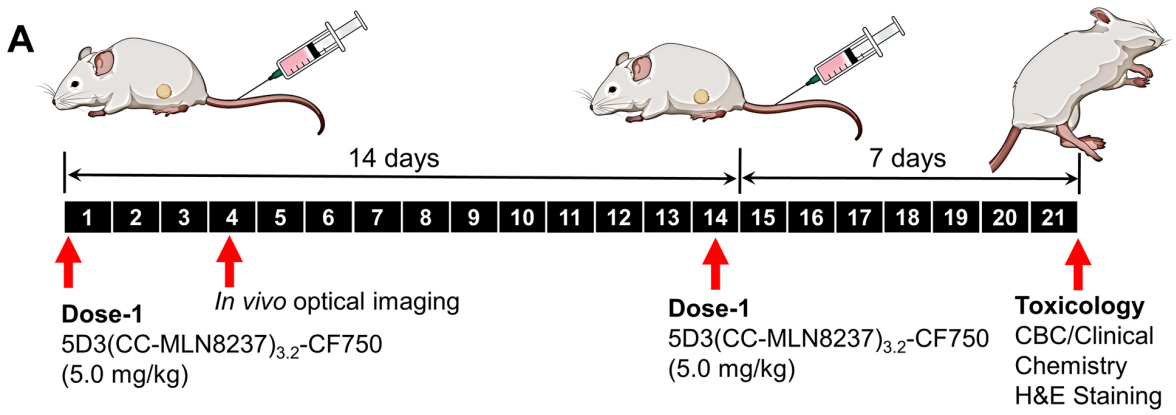
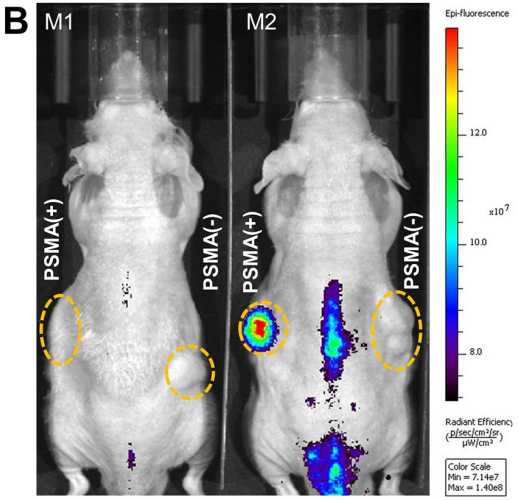


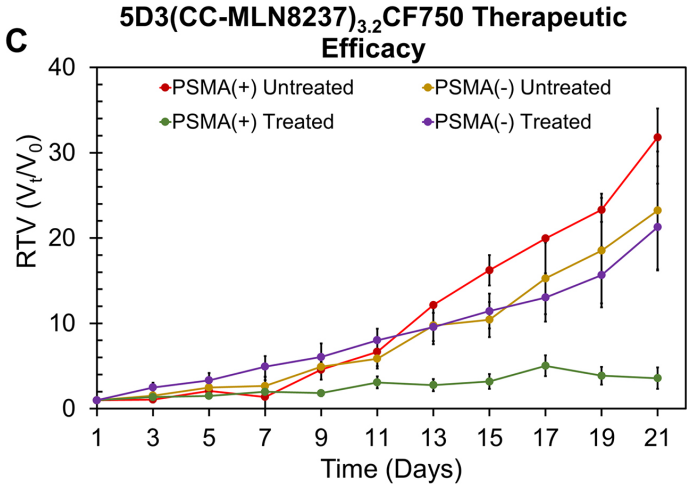


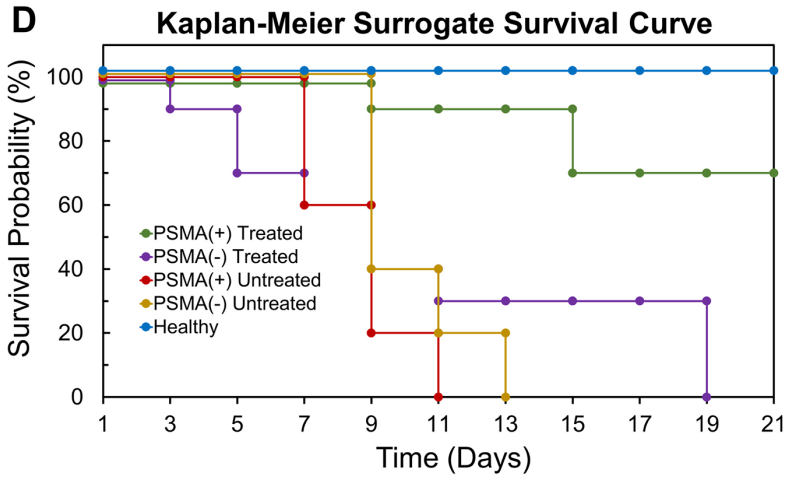
- 5D3(CC-MLN8237)$_{3.2}$-AF488 in PSMA(+) PC3-PIP cells (IC_{50}=8.17 nM) and PSMA(-) PC3-Flu cells (IC_{50}=161.9).
- Free MLN8237 in PSMA(+) PC3-PIP cells (IC_{50}=736.9 nM) and PSMA(-) PC3-Flu cells (IC_{50}=873.4 nM).
- 5D3(CC-MLN8237)$_{3.2}$-AF488 is 20 fold more efficacious in PSMA(+) cells than in PSMA(-) cells and 90 fold more efficacious than free MLN8237 drug in PSMA(+) cells.

5D3(CC-MLN8237)$_{3.2}$-CF750 therapeutic study





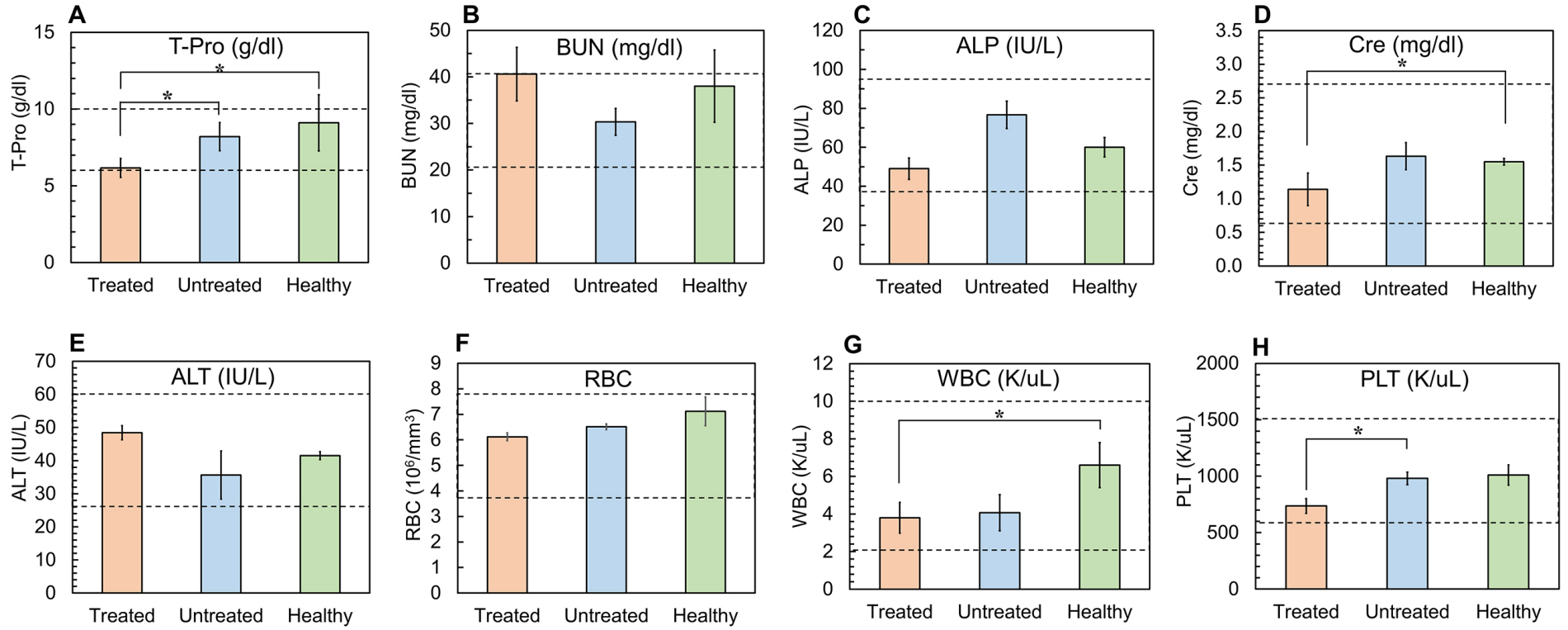




Athymic nu/nu male mice.
PSMA(+/-) bilateral (dual) tumor xenograft mouse models.
Change of relative tumor volume (V_t/V_0) against the time of treatment period.
Significant control of PSMA(+) tumor growth by 5D3(CC-MLN8237)$_{3.2}$-CF750.
Kaplan-Meier surrogate survival curves show higher overall survival of mice bearing PSMA(+) tumors and treated by 5D3(CC-MLN8237)$_{3.2}$-CF750 (Green, 70%).

- There was no significant change in body weight.

5D3(CC-MLN8237)$_{3.2}$-CF750 toxicological study



Analyses of clinical chemistry profile and CBC.
(A) Total serum protein (T-Pro, normal range: 6.0–10.0 g/dL), **(B)** Blood Urea Nitrogen (BUN, normal range: 20–40 mg/dL), **(C)** Alkaline Phosphatase (ALP, normal range: 35–96 IU/L), **(D)** Creatinine (Cre, normal range: 0.60–2.72 mg/dL), **(E)** Alanine Transaminase (ALT, normal range: 25–60 U/L), **(F)** Red Blood cell Count (RBC, normal range: 3.8-7.9 ×10^6/μL, **(G)** White Blood cell Count (WBC, normal range: 2–10 K/μL) **(H)** Platelet Count (PLT, normal range: 600–1500 K/μL). Gray area: normal range of analytes in healthy mice. (Plotted with standard error bars, $n = 5$, $^*p < 0.05$).

Summary

- 5D3 has high binding affinity and fast internalization with centrosomal localization in PSMA(+) PC cells.
- Ideal drug delivery platform to deliver Aurora A kinase inhibitors.
- 5D3(CC-MLN8237)$_{3.2}$ ATC shows higher cytotoxicity in PSMA(+) cells than PSMA(-) cells.
- *In vivo* studies, 5D3(MLN8237)$_{3.2}$ demonstrated high and homogeneous tumor uptake in PSMA(+) tumors and efficient control of tumor growth in PC tumor mouse models.
- Toxicological evaluation in preclinical settings reveals that 5D3(CC-MLN8237)$_{3.2}$ ATC has no significant toxicological effects/systemic toxicities after the treatment.
- These studies establish a solid foundation to develop highly effective 5D3-based theranostics for PSMA(+) PC therapy.

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Thank you


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