Dendritic Cell-Targeted Nanoparticles for Dual Enhancement of T Cell Activation and Immune Checkpoint Blockade

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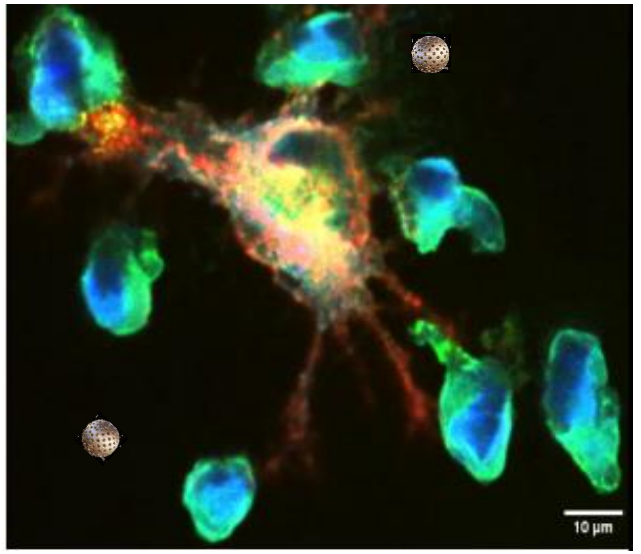
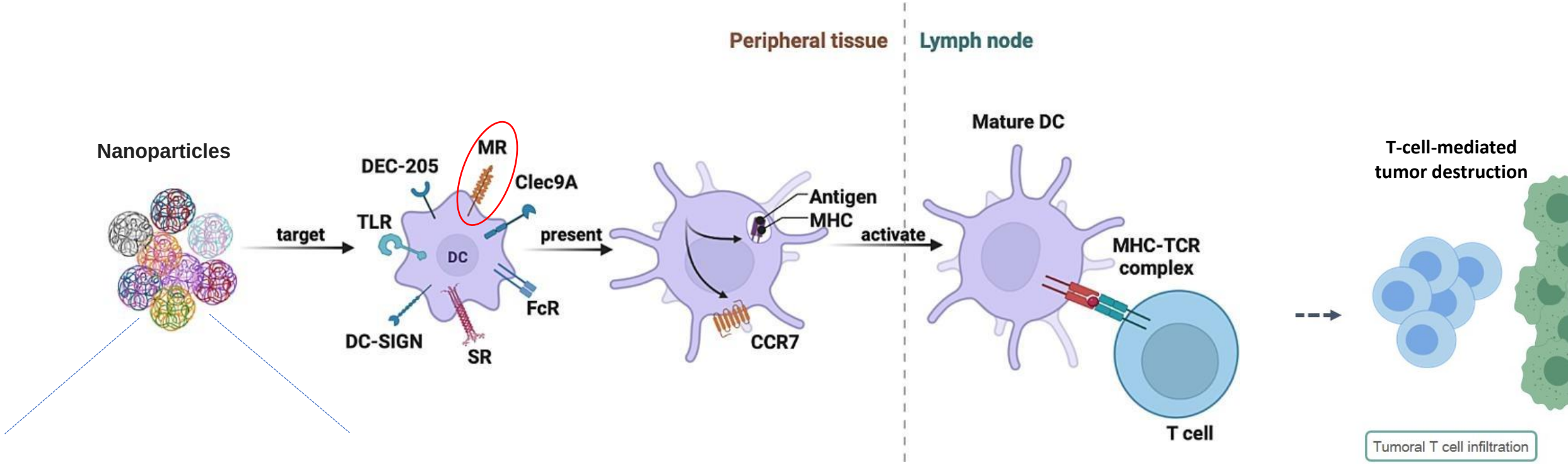
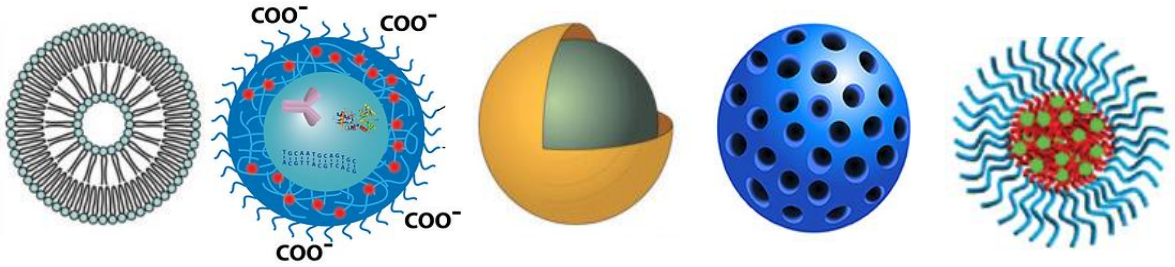


Molecular interactions between stimulated **DC cells** (CD80, red) and EL4 **T cells** (CD28, green). Credit: Gover M. Antonjraj, BGU.

Nanoparticulate-based antigen delivery systems and receptors that target DCs in anticancer immunotherapy



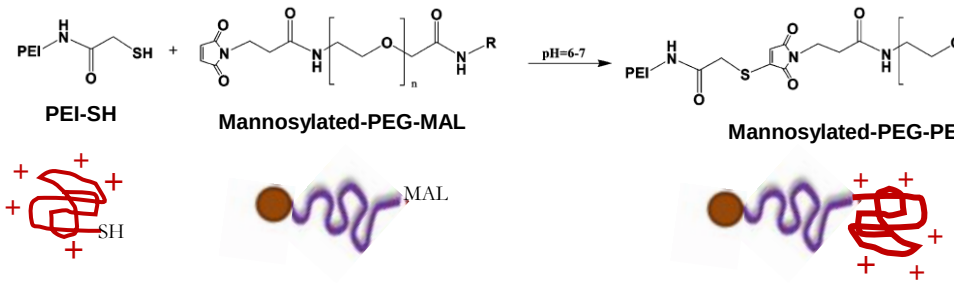


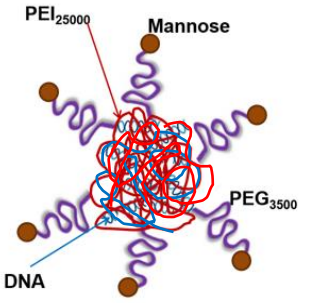
Liposome | PLGA nanoparticle | Gold (Au) | Mesoporous Silica | Polymer micelle

Feature	Description
Delivery System	Often designed from biocompatible organic (liposomes, PLGA, polymeric micelles) or inorganic (Au, MSNP) materials
Payload	Antigens and adjuvants, tumor cell lysate, nucleic acids
Targeting Mechanism	Ligands/antibodies bind DC surface receptors
Immune Activation	Stimulates DC maturation and T cell response
Delivery Route	Systemic or local administration
Advantages	Modular, scalable, efficient immune stimulation, reduced toxicity

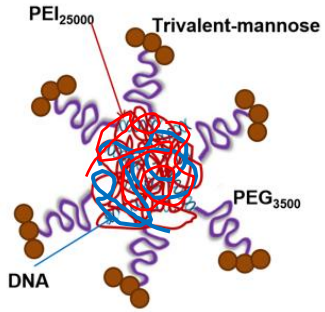
David A. and Golani-Armon A., in Nanomedicine. Advances in Delivery Science and Technology. Springer, New York, NY, 2016

Mannosylated-PEG-b-PEI nanoparticles enhance gene expression in Dendritic Cells of Tumor Draining Lymph Nodes (TDLN)



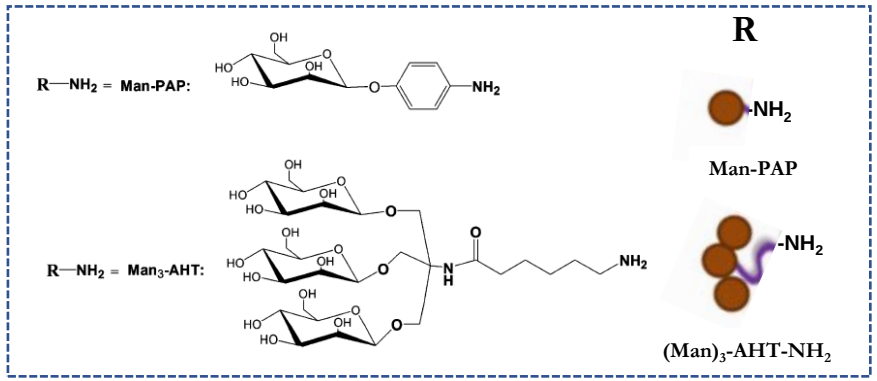


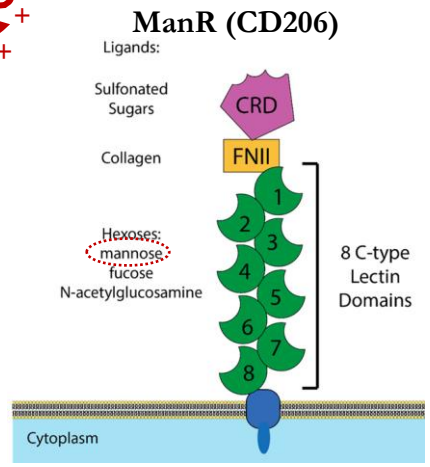
Man-PEG-*b*-PEI



Man_3-PEG-*b*-PEI

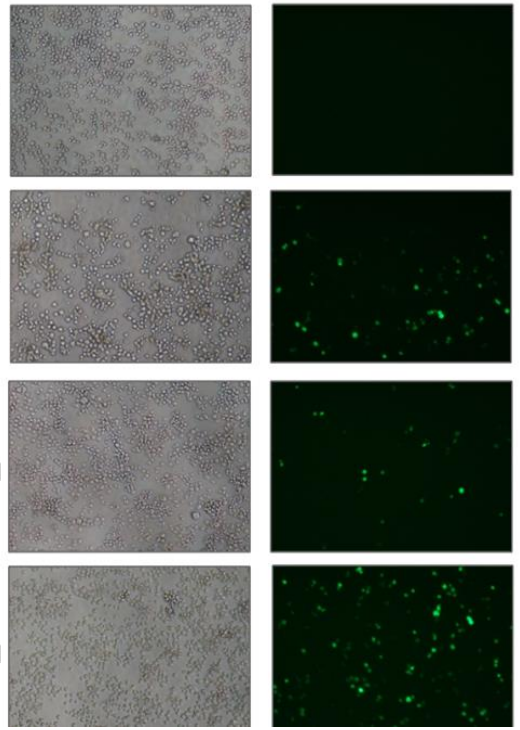
Lior Raviv

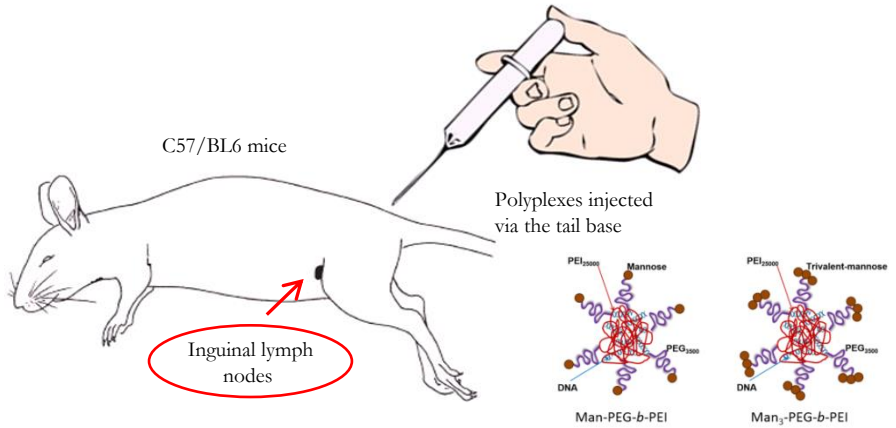




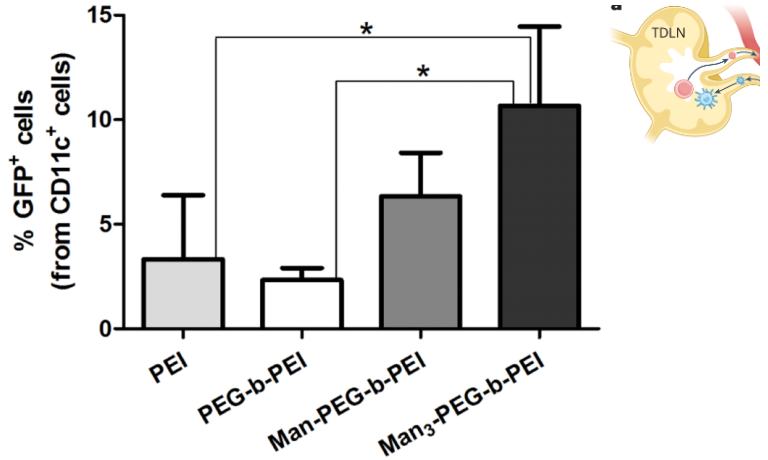
Polyplexes' high cationic charge limits the amounts of gene that can be safely delivered

Binding and uptake by DC2.4 cells





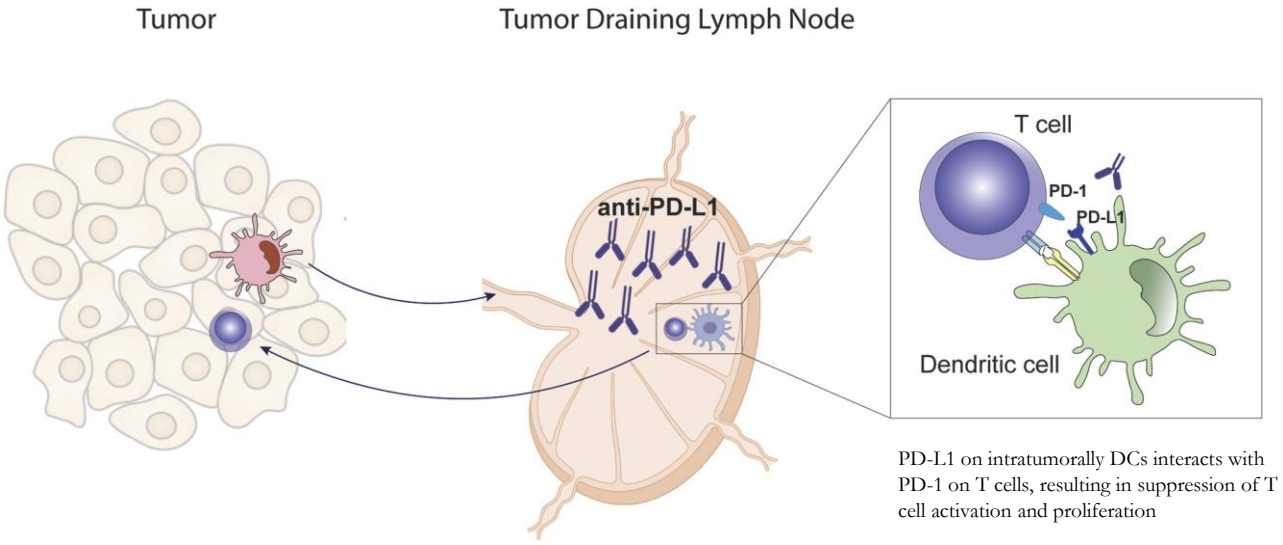
GFP+ cells from DC at draining lymph nodes



Raviv and David., Mol. Pharmaceutics 12, 453, 2015

Challenges for successful DC activation by nanoparticles for cancer immunotherapy

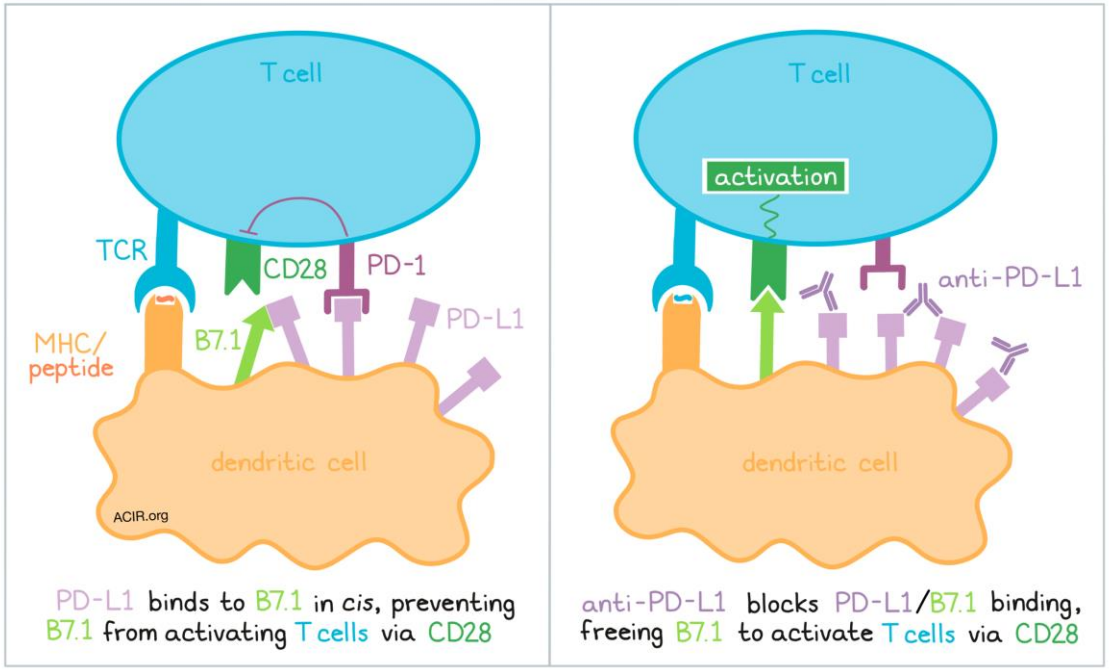
The immunosuppressive TME impairs DC effector functions by altering their phenotype, leading to dysfunction and increased tolerogenicity.



PD-L1 on intratumorally DCs interacts with PD-1 on T cells, resulting in suppression of T cell activation and proliferation

Anti-PD1/PD-L1 antibodies can restore or enhance the activity of DCs in tumors

PD-L1 on DC in the TME dampen the co-stimulatory capacity of DCs, thereby limiting effective T cell activation



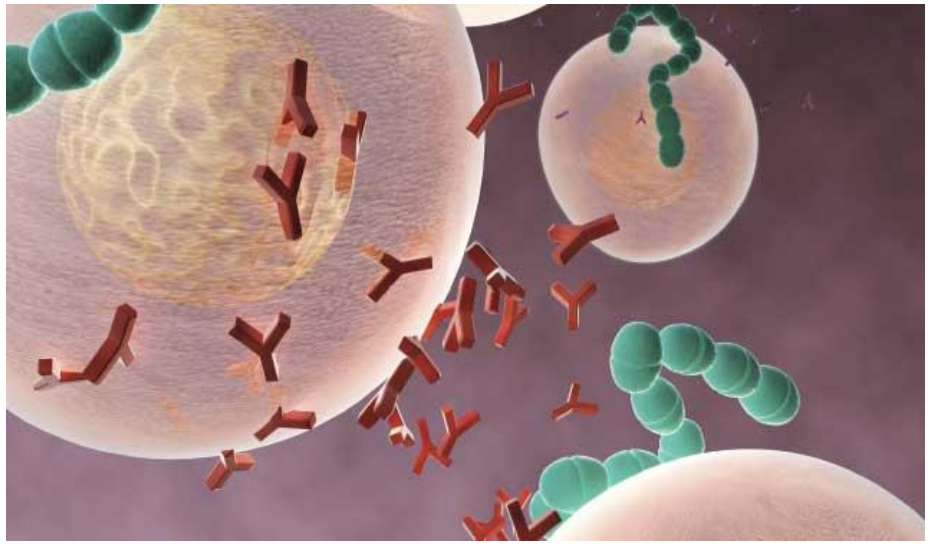
PD-1 = Programmed cell death 1
PD-L1 = Programmed Death ligand-1
B7.1 (CD80) - a costimulatory molecule on APC
CD28 - provides co-stimulatory signals for **T cell** activation

Atezolizumab = inhibits both PD-1/PD-L1 and PD-L1/B7.1 interactions, improves DC function and triggers a potent antitumor T cell immunity

Dammeijer, F. et al., *Cancer Cell.* 2020; 38:685-700

Could nanoparticles be an alternative to antibody immunotherapies?

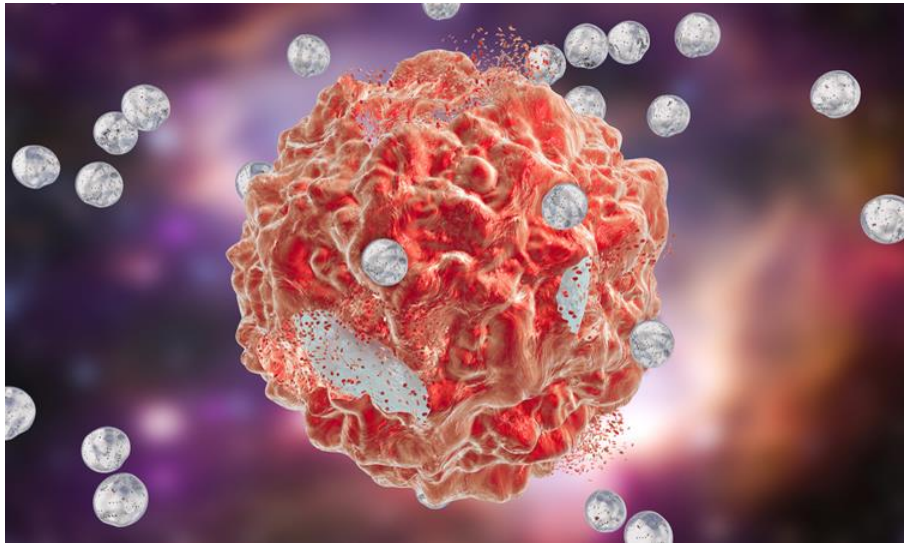
Antibodies which underlie current immunotherapies



Advantages
- Enhanced antitumor immune responses
- Survival benefits in advanced cancers
- Long-lasting remissions
- Outperforming chemotherapy in sustainability and tolerability

Disadvantages
- Primary and acquired resistance
- Immune-related Adverse Events (irAEs)
- Variable response rates
- High cost

Nanoparticles designed to activate immune responses



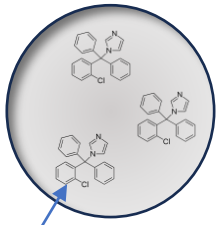
Advantages:
- Biocompatible
- More affordable way to fight cancer
- Can be engineered to target specific DC receptors
- **Co-delivery of multiple stimulatory agents**
- **Controlled drug/antigen release**

Disadvantages:
- Heterogeneous tumor penetration
- Rapid clearance by the mononuclear phagocyte system
- Regulatory and translational barriers

Engineering Dendritic Cell-Targeted Nanoparticles for Dual Enhancement of Antigen Presentation and Immune Checkpoint Blockade

Biocompatible
Can load high amounts of material
Can be easily functionalized with targeting ligands
Enable controlled drug release

Mesoporous silica nanoparticles (MSNPs)



CLT = Clotrimazole

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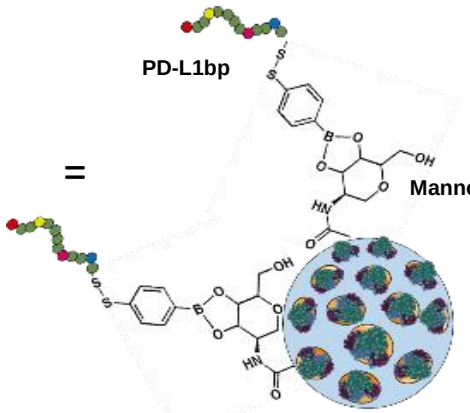
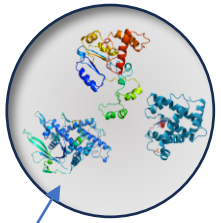


MSNP-MaN-PDL1bp/CLT



TCL = tumor cell lysate

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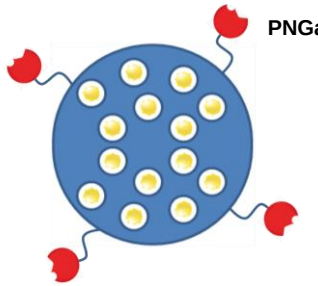
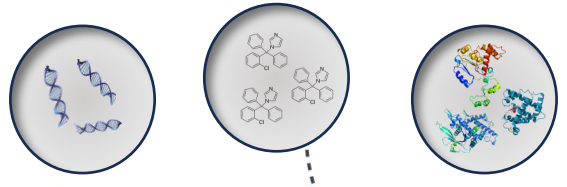
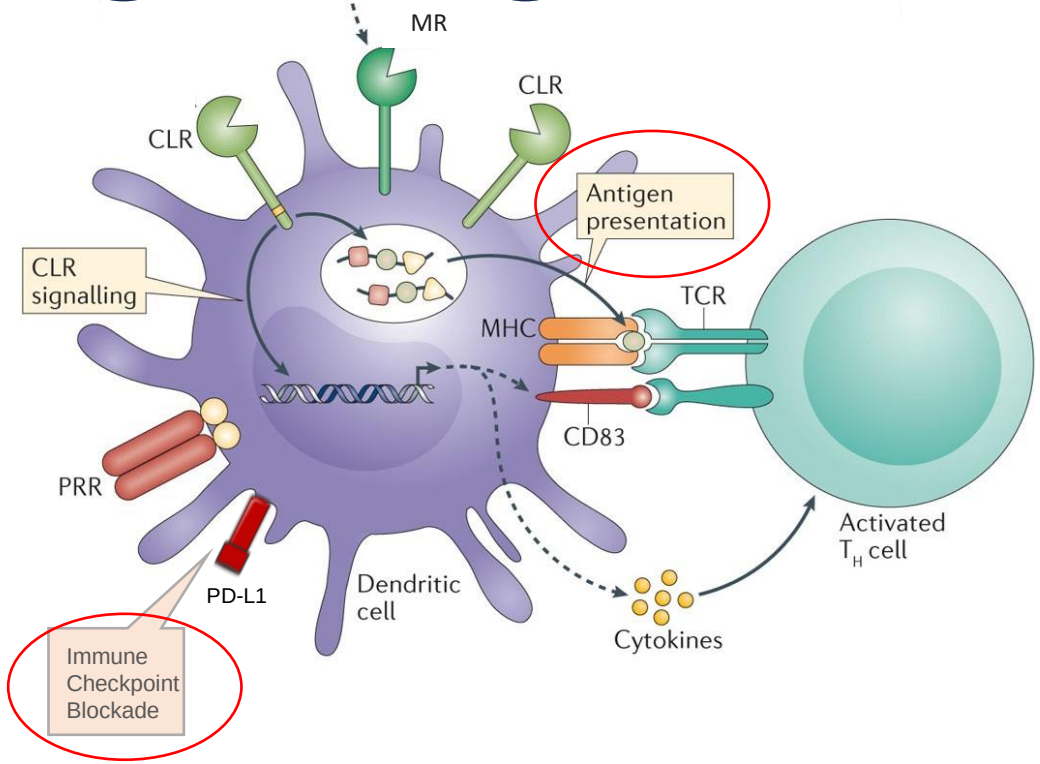


PNGase decorated H-XL-MSNP

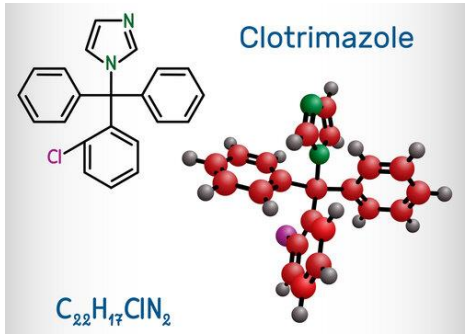
Small molecule,
FDA approved drug



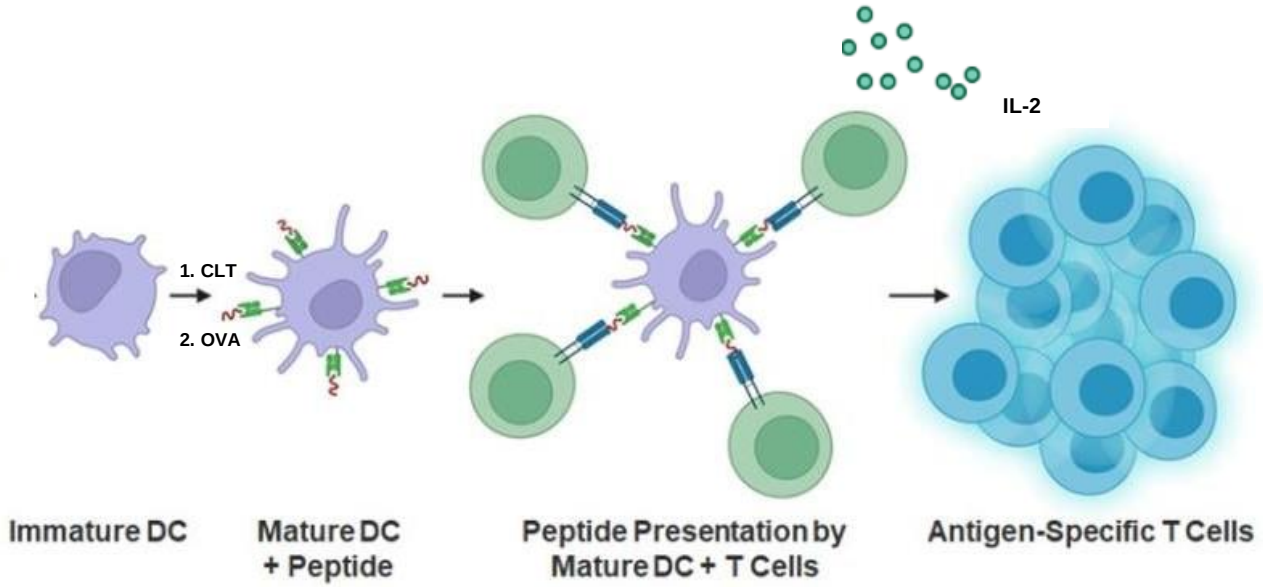
Whole **tumor cell lysate** = provide more comprehensive antigen repertoire

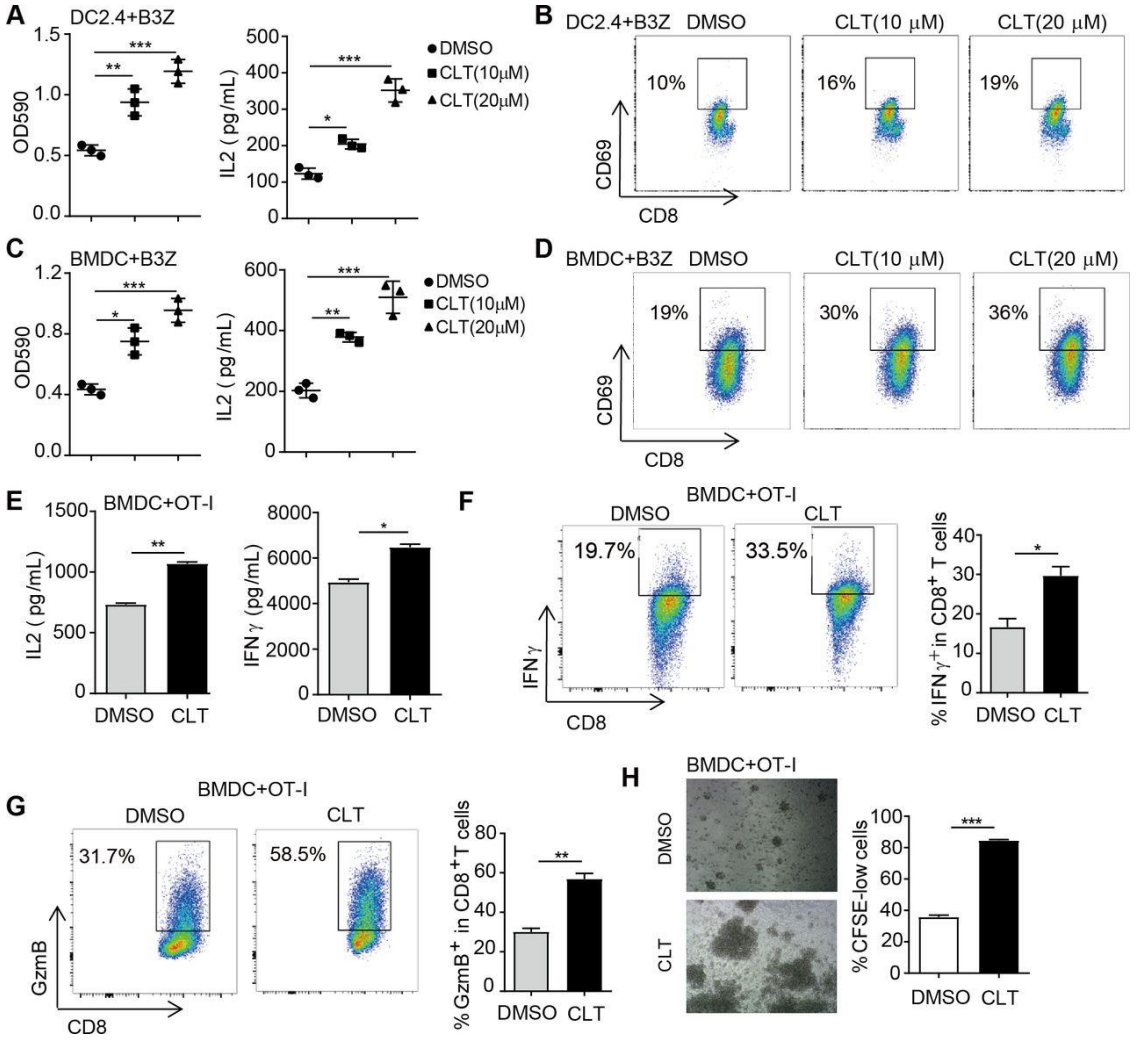


Clotrimazole (CLT) enhanced DC-induced maturation and T cell activation



Clotrimazole (CLT) enhances DC maturation and T cell activation.
Not approved for systemic use



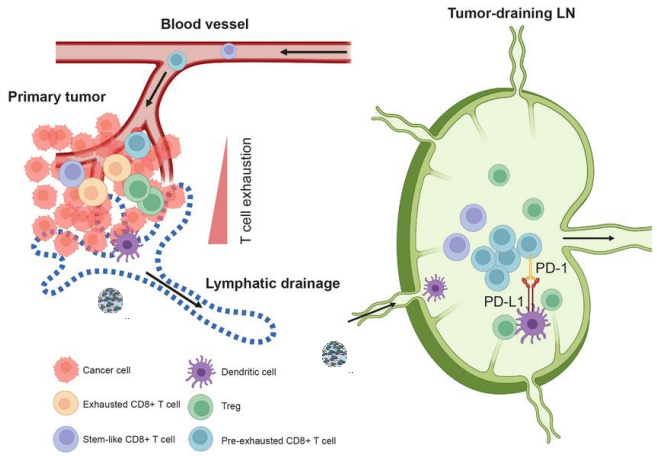


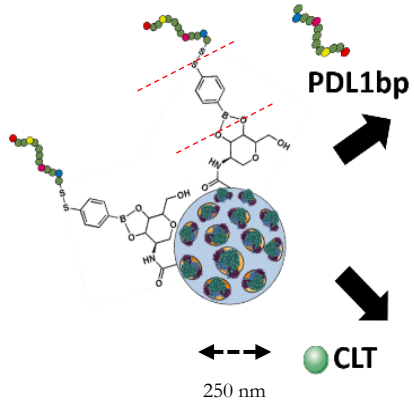
CTL = Clotrimazole
BMDC = bone marrow-derived DCs
B3Z = T cell hybridoma expressing a TCR that specifically recognizes OVA
OT-1 = mice T cells carry a transgenic TCR on $CD8^+$ T cells specific for OVA
CD69 (type II C-lectin receptor) = an early marker of lymphocyte activation
GzmB = serine protease commonly used by natural killer cells, cytotoxic $CD8^+$ T cells, and granulocytes to mediate apoptosis in target cells

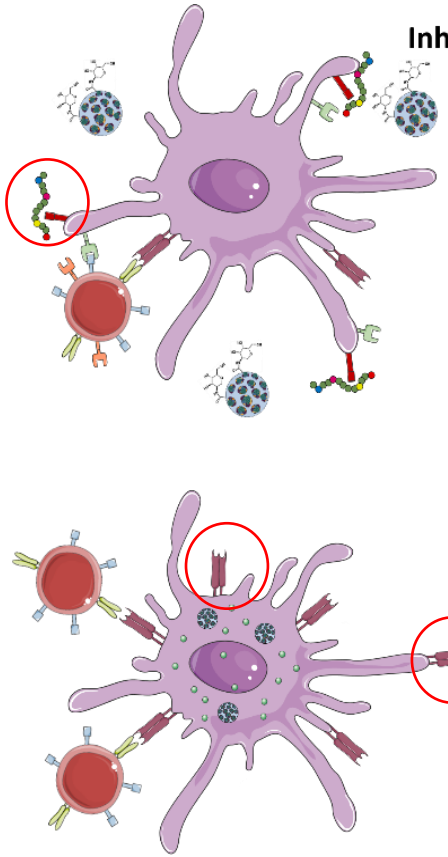
Journal for ImmunoTherapy of Cancer

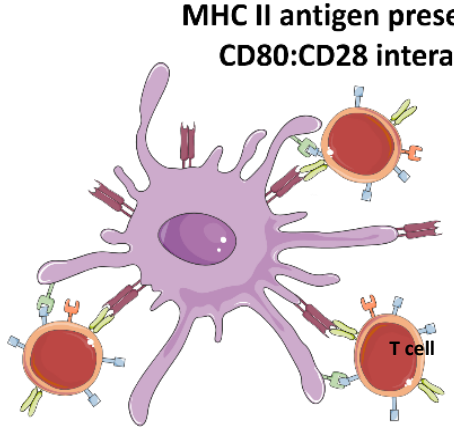
Zining Wang et al. J ImmunoThererapy of Cancer 2021

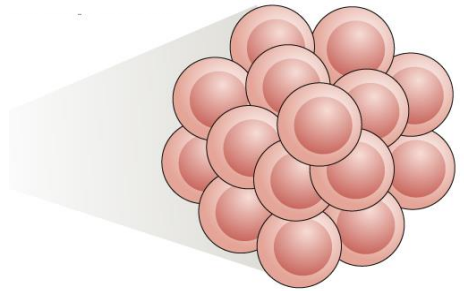
PD-L1-binding Peptide decorated MSNPs for Dual Enhancement of T Cell Activation and Immune Checkpoint Blockade





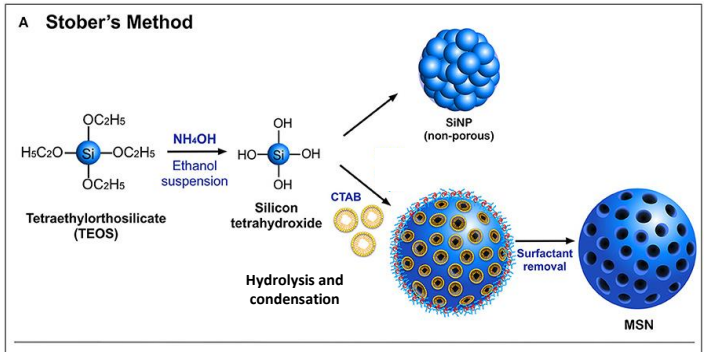




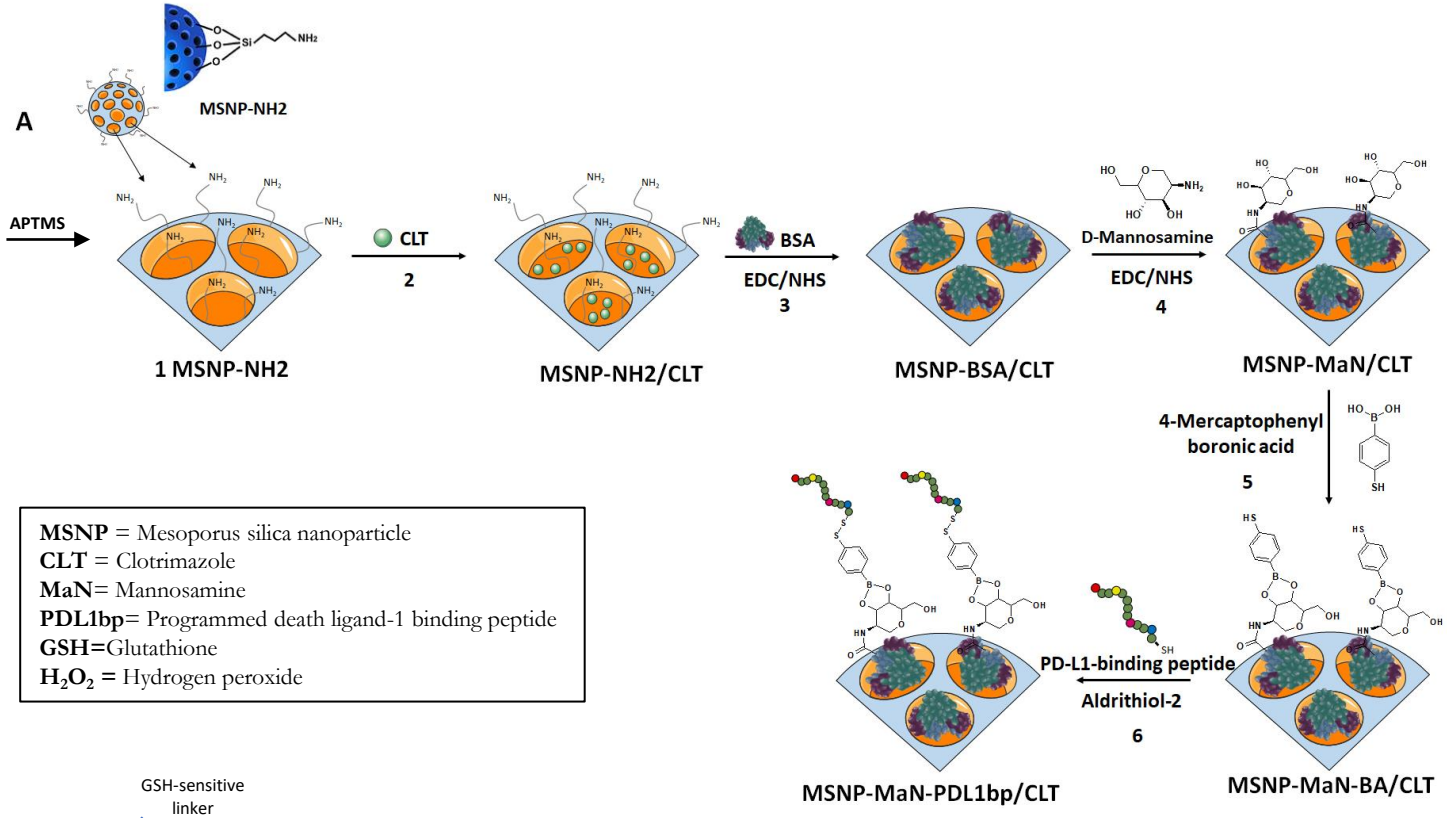


Immunogenic Cell Death

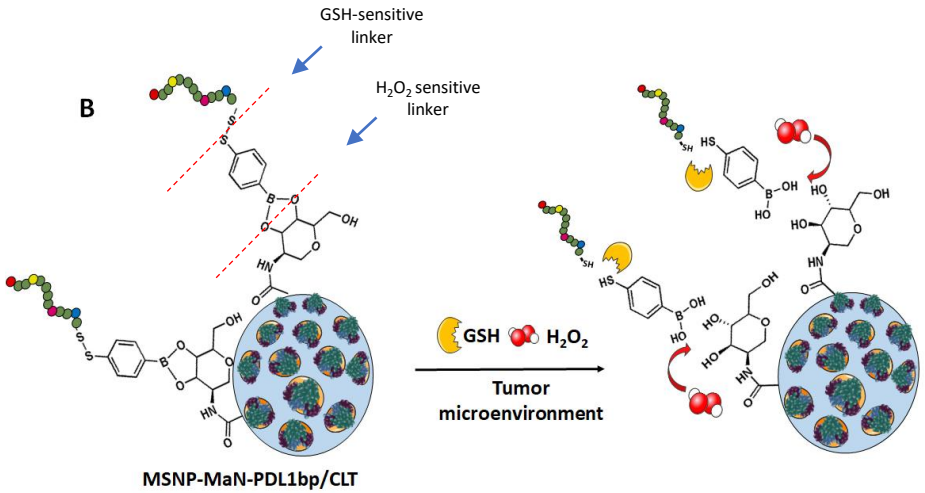
Preparation of DCs-targeted MSNPs to promote DC activation and antigen presentation



CTAB = Cetyltrimethylammonium bromide
TEOS = tetraethyl orthosilicate
APTMS = 3-(aminopropyl)trimethoxysilane



MSNP = Mesoporus silica nanoparticle
CLT = Clotrimazole
MaN= Mannosamine
PDL1bp= Programmed death ligand-1 binding peptide
GSH=Glutathione
H_2O_2 = Hydrogen peroxide



PD-L1bp sequence:
C-NYSKPTARQYHF

Effectively blocks the PD-1/PD-L1 interaction
K_D value of 0.51 μm for the binding to PD-L1.

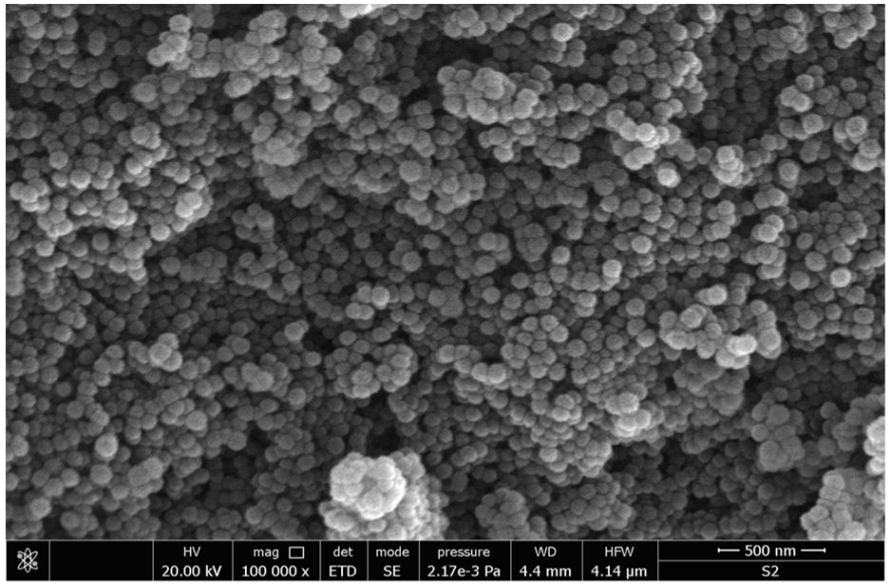
Chang H.N. *et al.*, Angew.Chem. Int.Ed. 2015

Prateek Srivastava

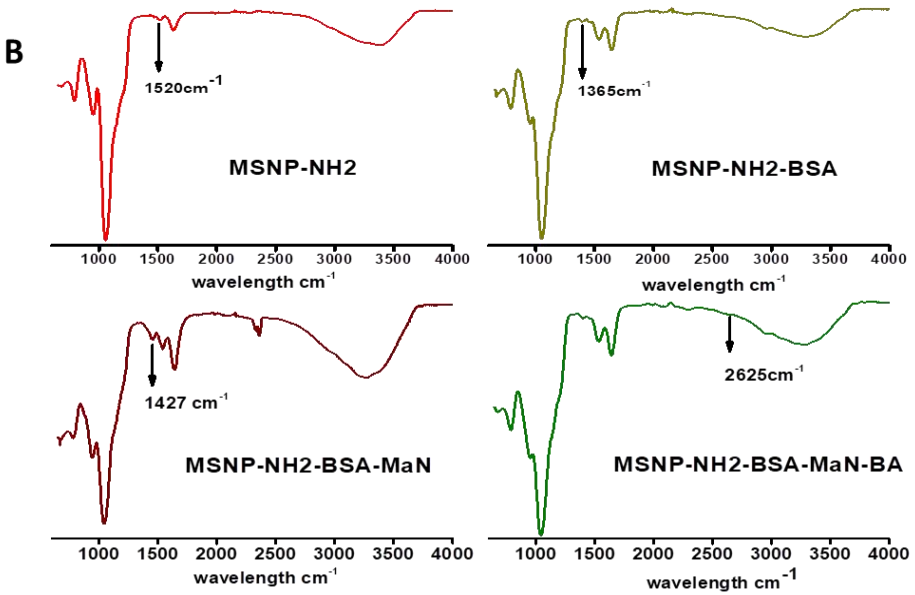
Marie Ruetter

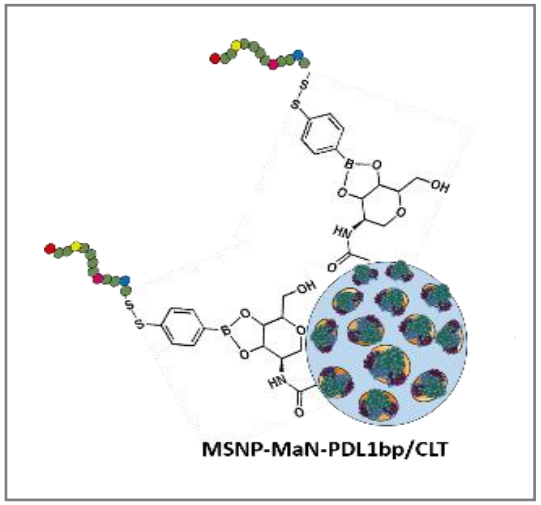
Physicochemical characteristics of functionalized MSNPs

TEM image

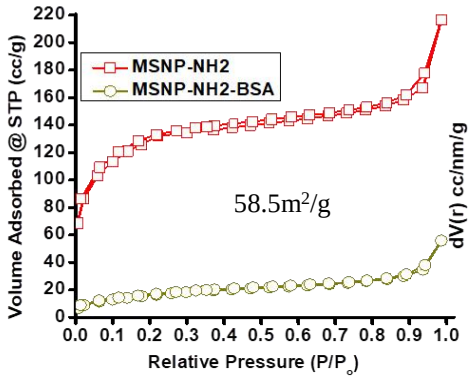


FTIR analysis

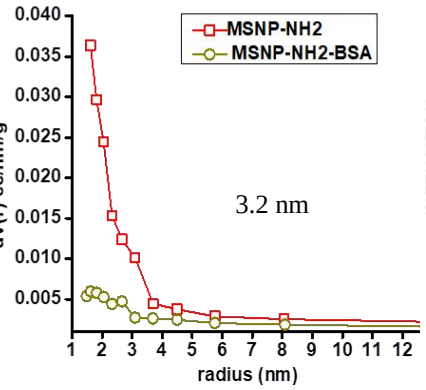




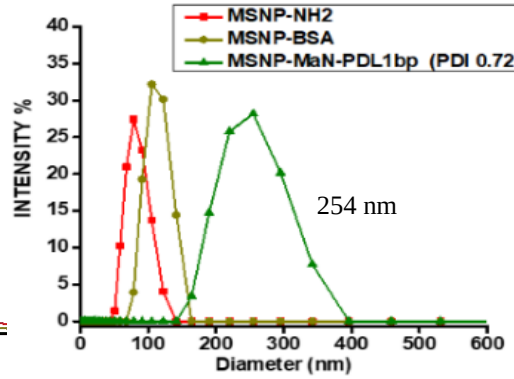
C BET calculated surface area



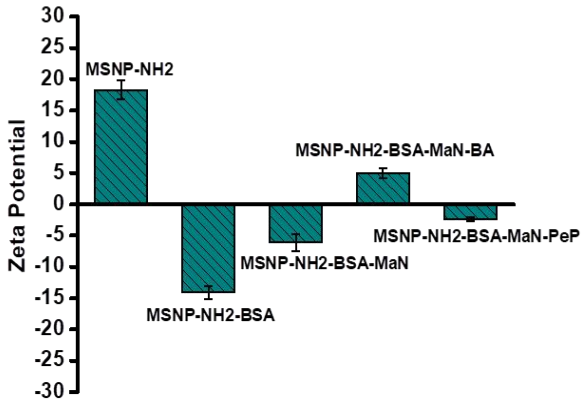
D BJH calculated pore size



E DLS analysis

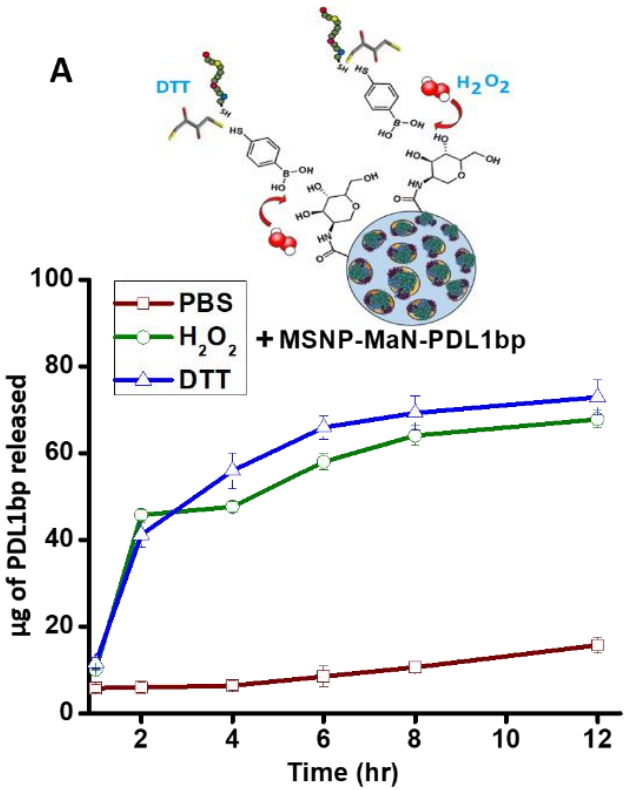


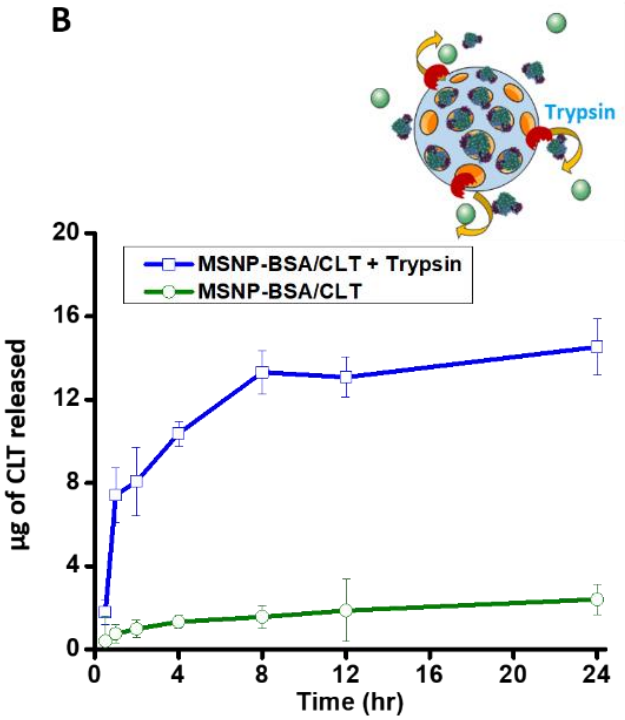
F Zeta Potential analysis

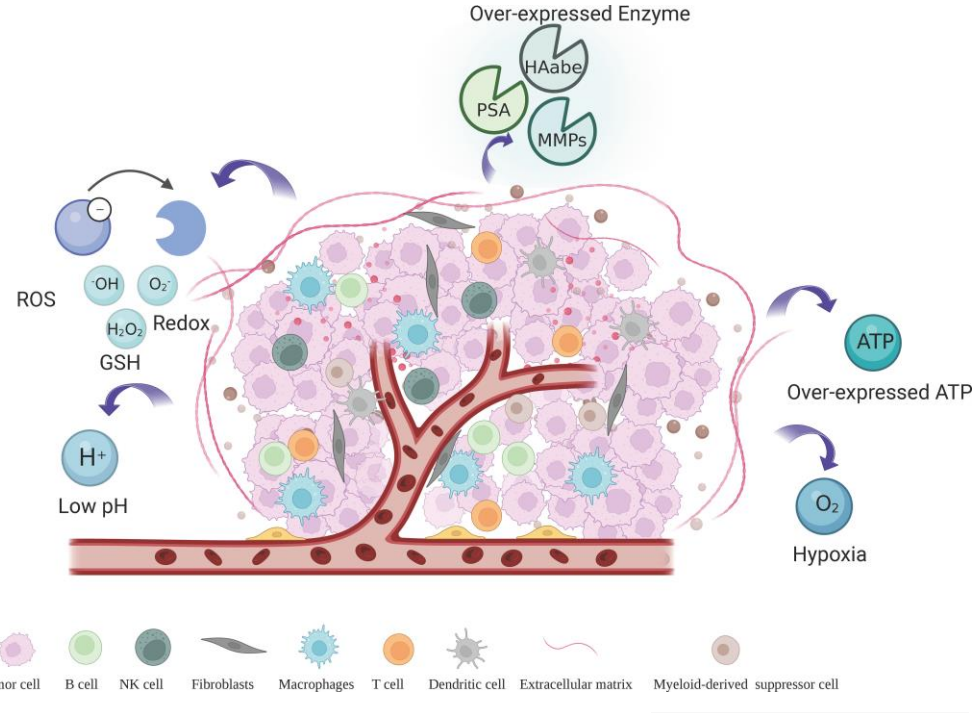


Srivastava P and Rutter M., ACS Applied Materials and Interfaces, 2024

PDL1bp and CLT were released from the MSNPs under conditions simulating the TME



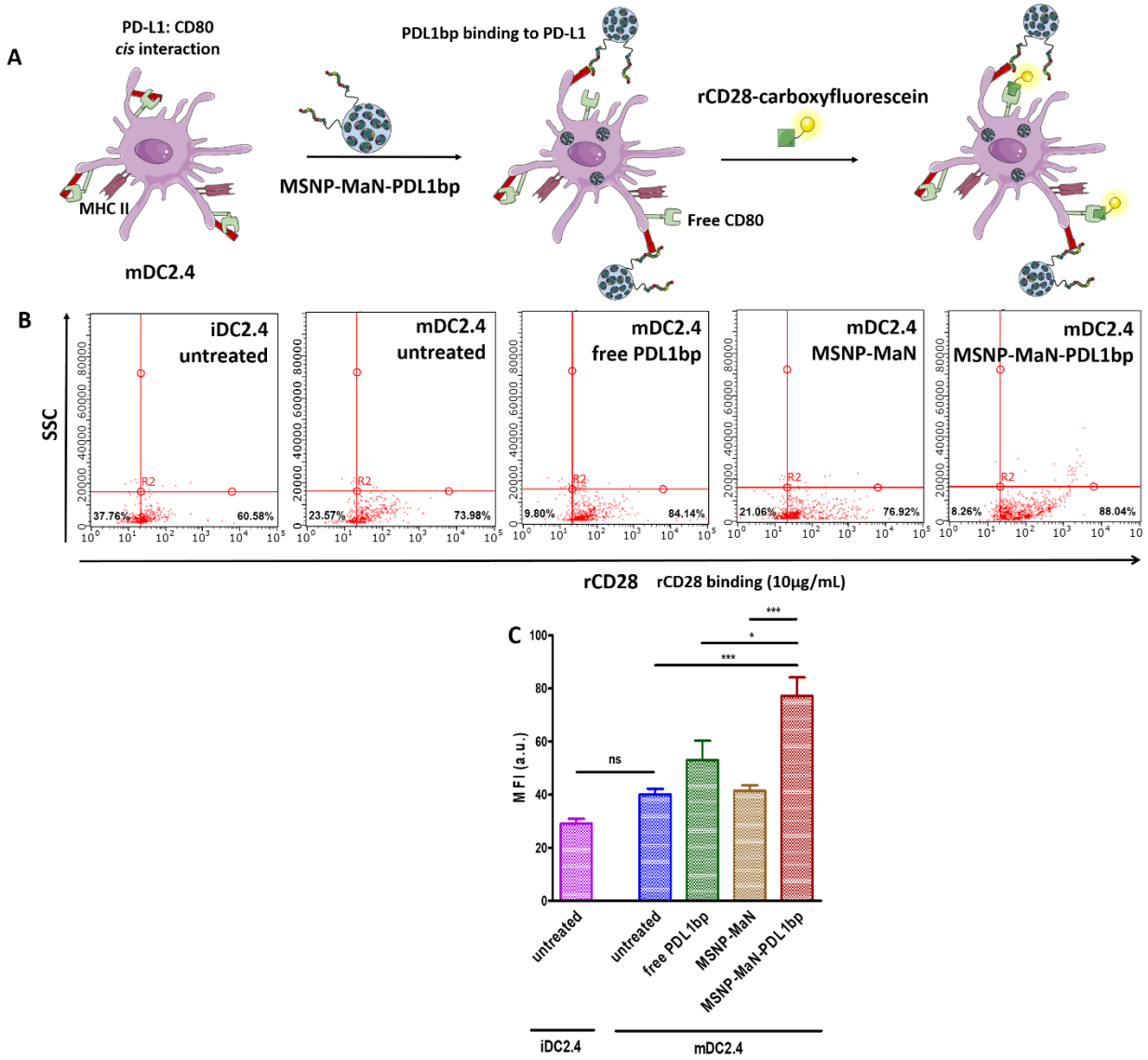




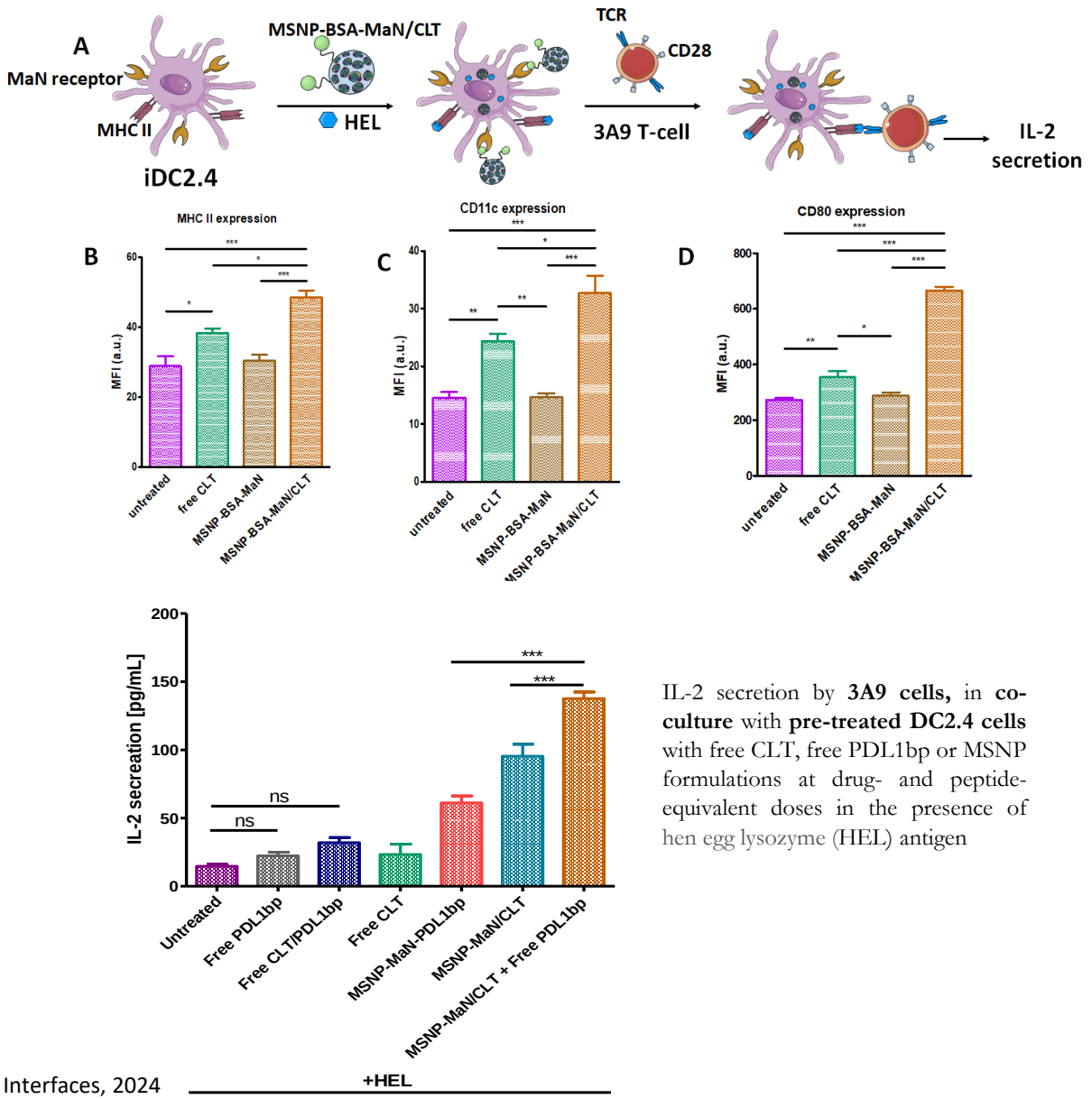
From Yang R., Front. Immunol., 2023

Dithiothreitol (DTT) or H_2O_2, 10mM

PD-L1bp-decorated MSNPs disrupt PD-L1/CD80 interactions and enhance CD28 recognition



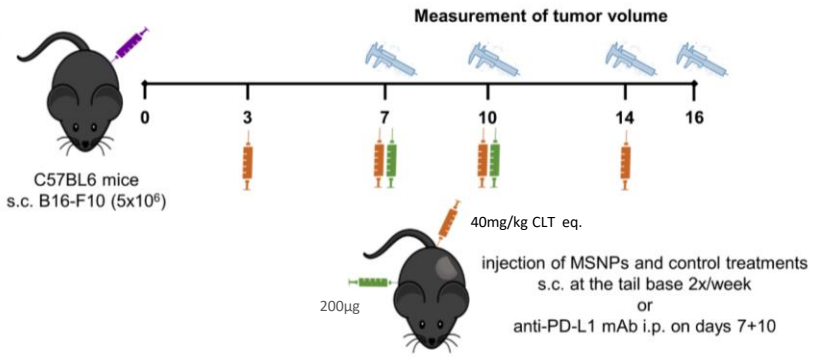
CLT-loaded MSNPs enhance DC activation, antigen presentation and subsequent 3A9 T-cell priming

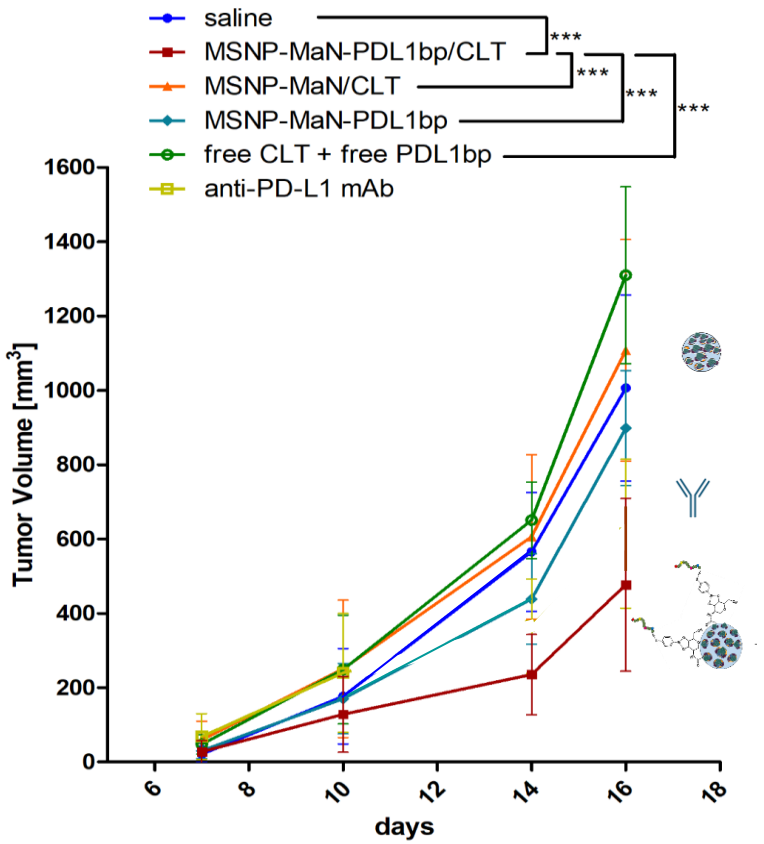


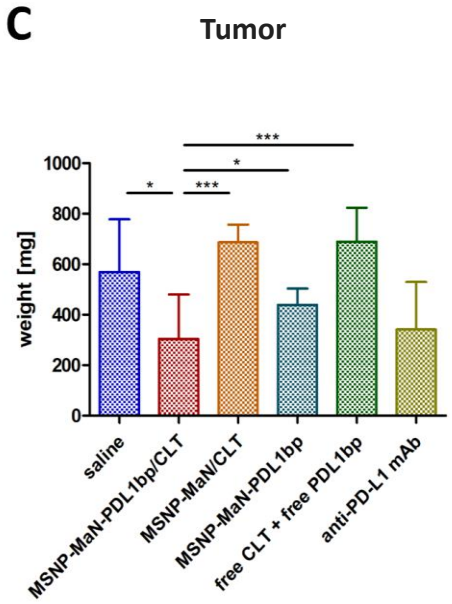
IL-2 secretion by **3A9 cells,** in **co-culture** with **pre-treated DC2.4 cells** with free CLT, free PDL1bp or MSNP formulations at drug- and peptide-equivalent doses in the presence of hen egg lysozyme (HEL) antigen

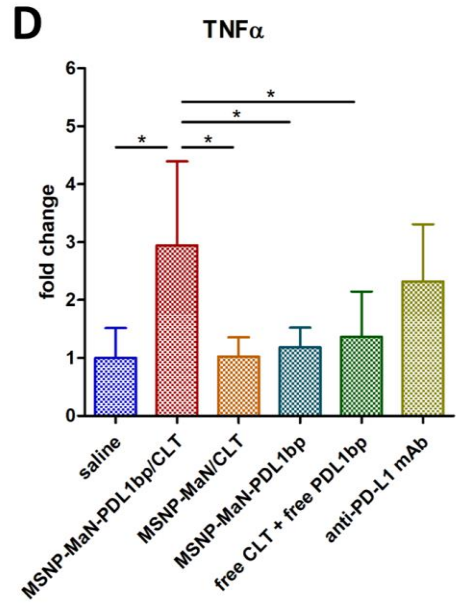
Srivastava P. and Rutter M., ACS Applied Materials and Interfaces, 2024

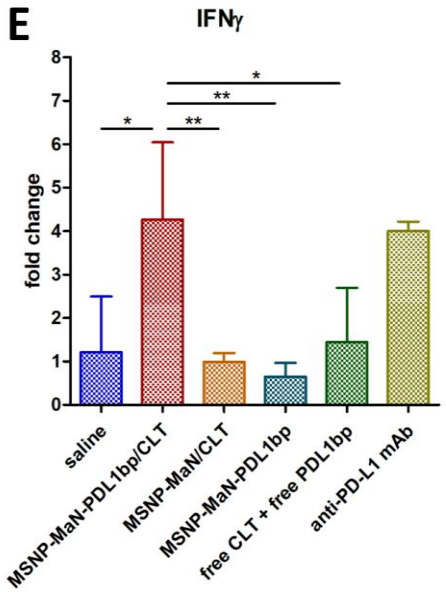
MSNP-MaN-PDL1bp/CLT significantly inhibit tumor growth and enhance T cell activation in a mouse melanoma model

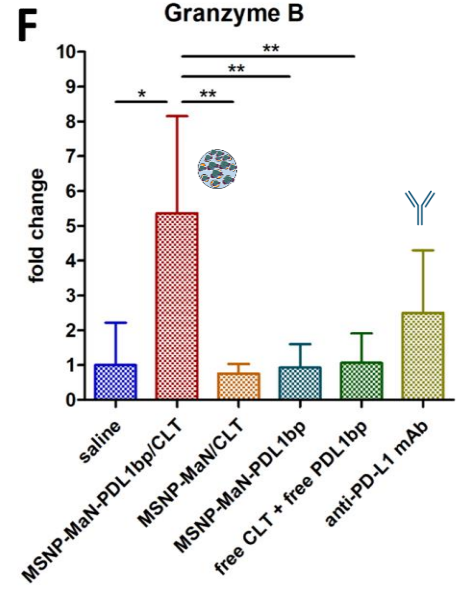






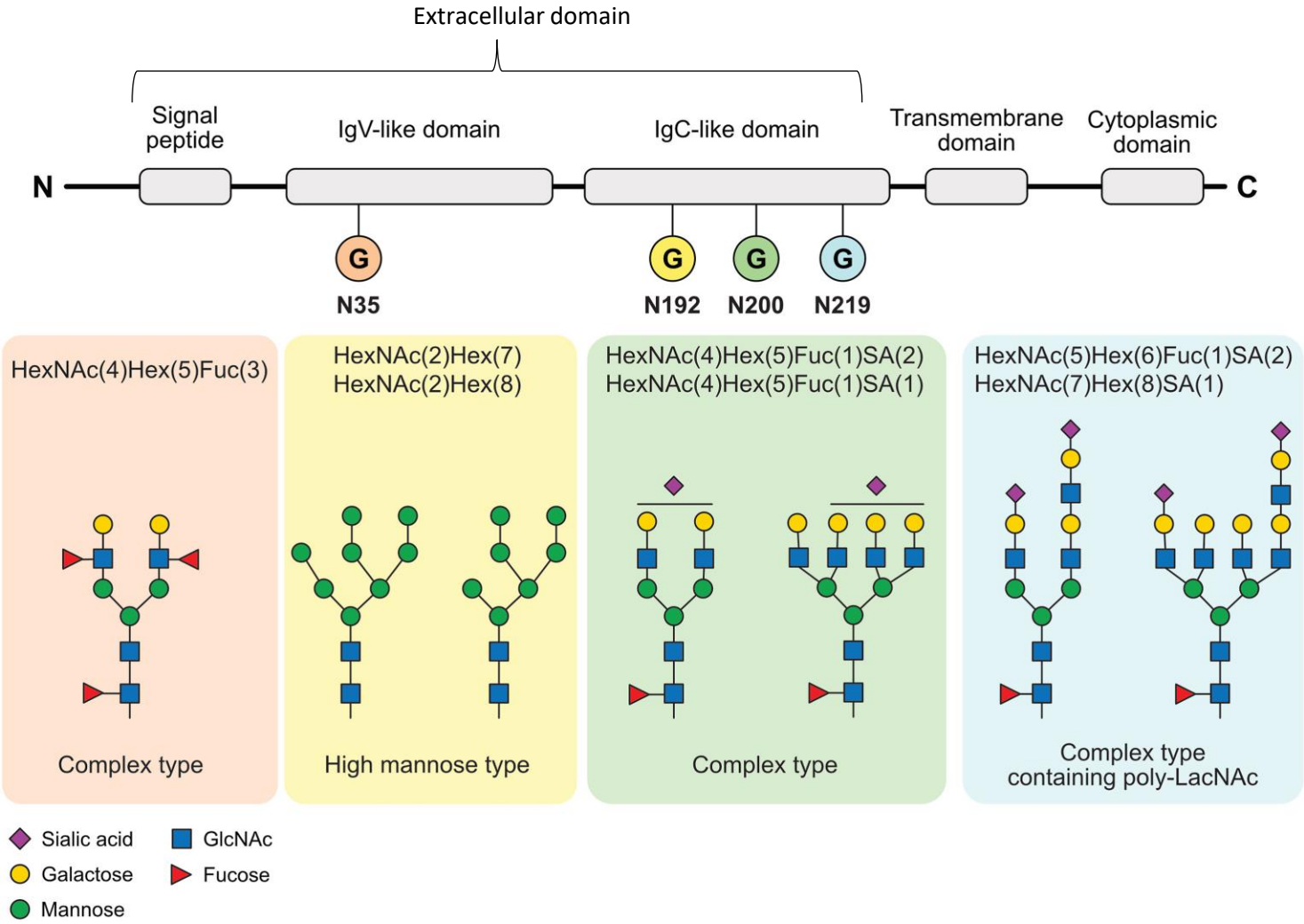


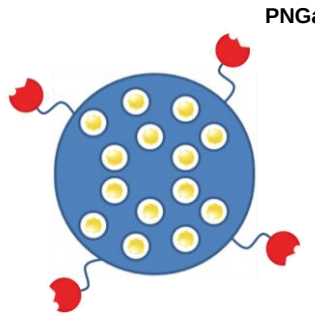




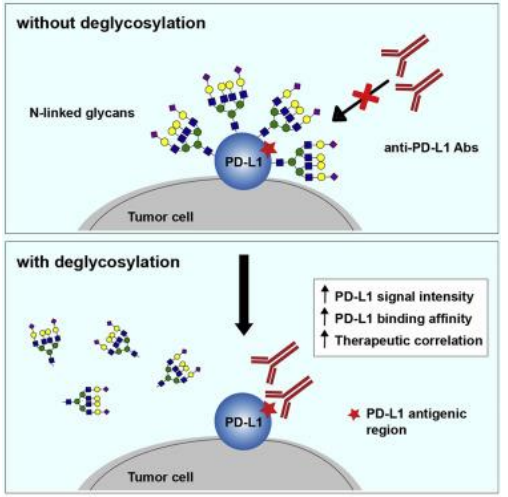
Srivastava P and Rutter M., ACS Applied Materials and Interfaces, 2024

PD-L1 on cancer cells and intratumoral DCs is heavily N-glycosylated





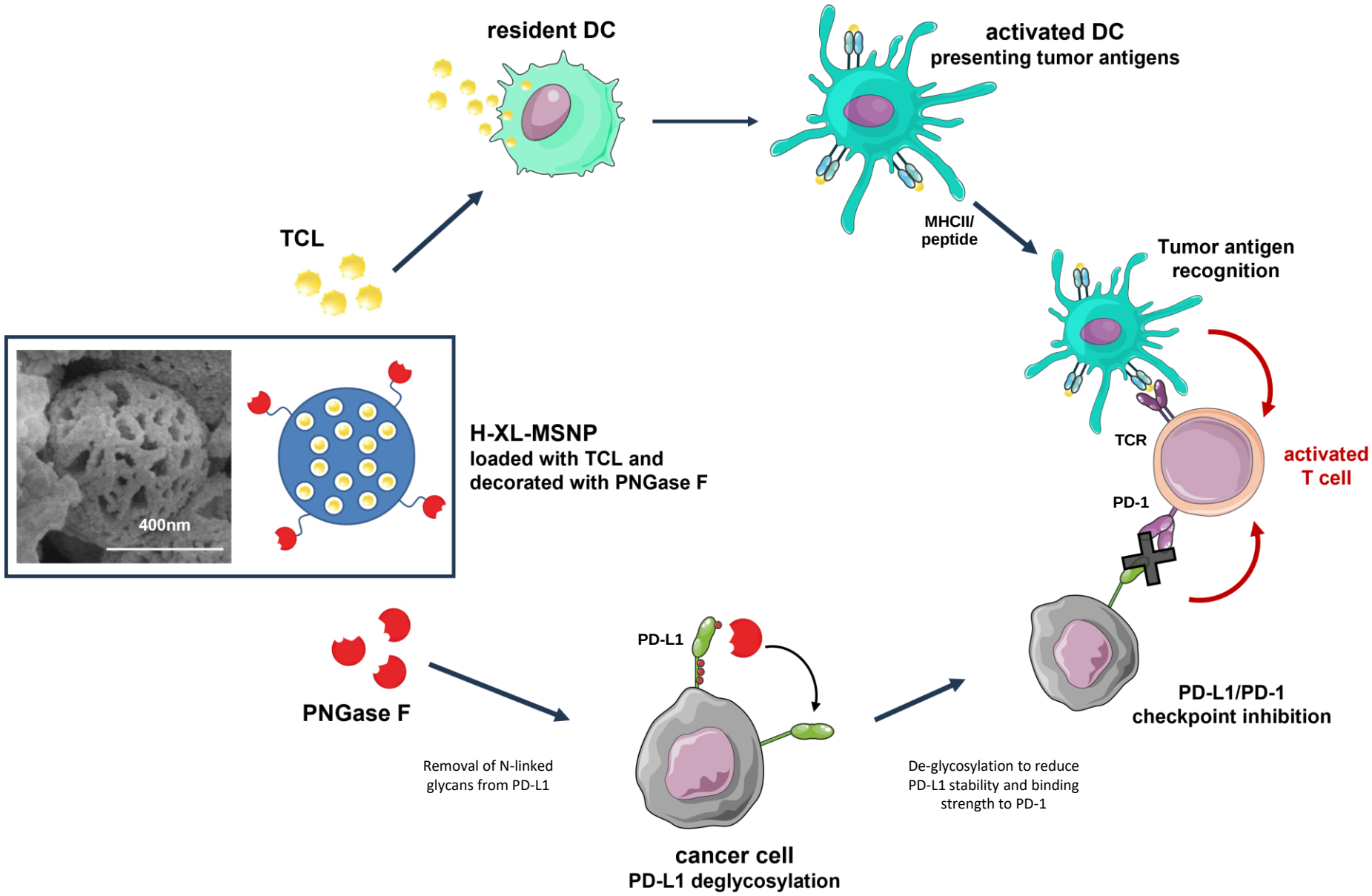
PNGase decorated H-XL-MSNP



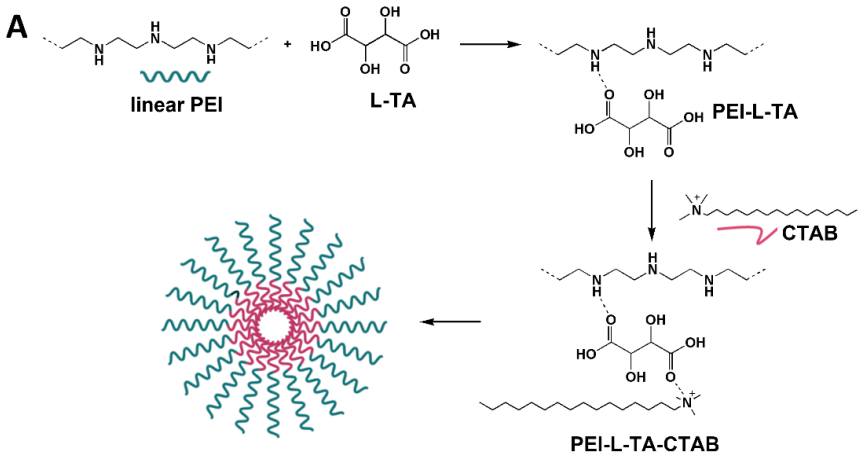
Deglycosylation improves the efficacy of immune checkpoint blockade therapies

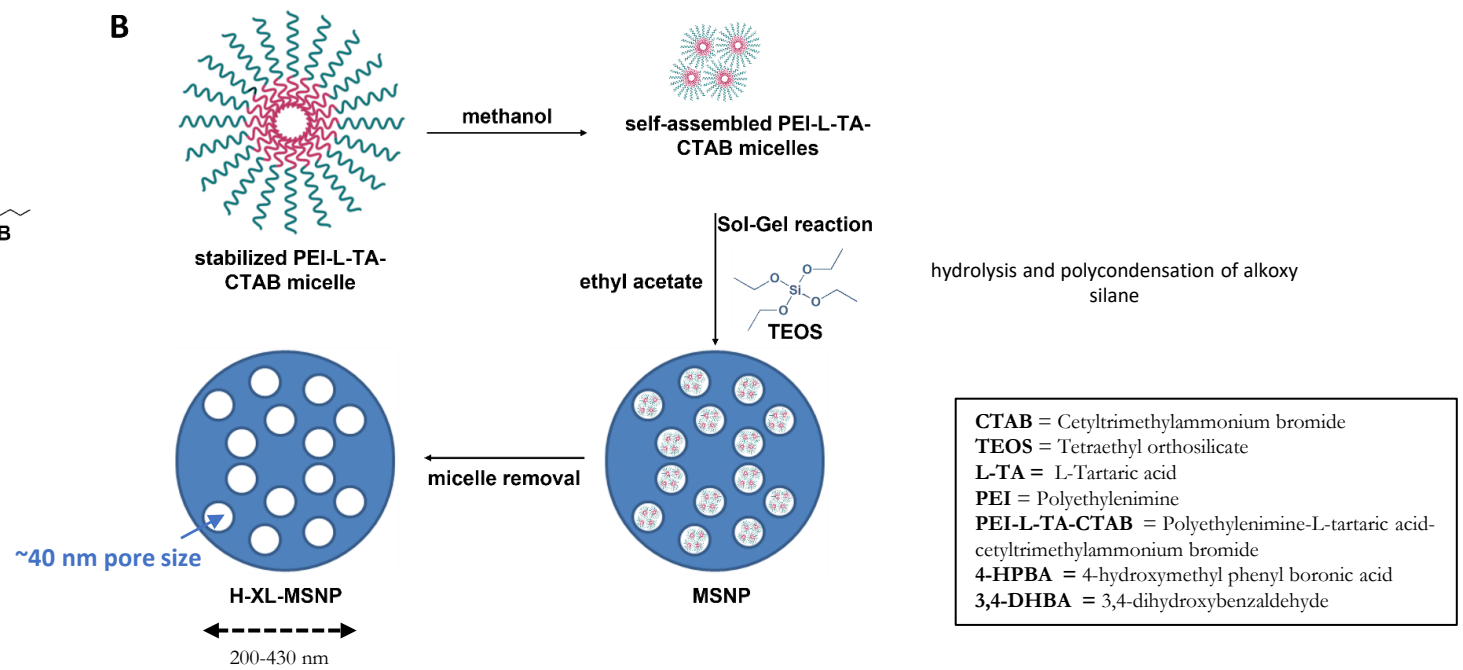
Glycosylation at all four sites (N35, N192, N200, N219) modulates PD-L1's ability to interact with PD-1 and respond to ICBs, with the **N35** site being especially critical for the efficacy of immune checkpoint blockade therapies

Hollow, Extra Large Mesoporous Silica Nanoparticles (H-XL-MSNP) for Efficient Antigen Delivery and PD-L1 Deglycosylation in Cancer Immunotherapy

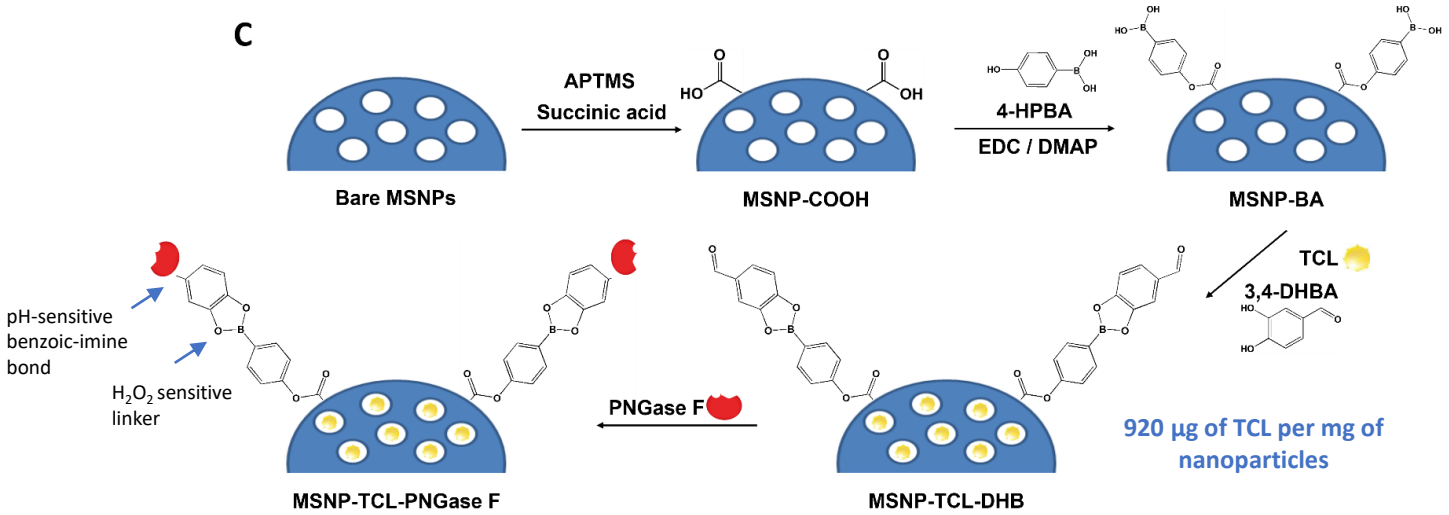


Synthesis scheme for the functionalization of H-XL-MSNP with PNGase F attached and TCL loading

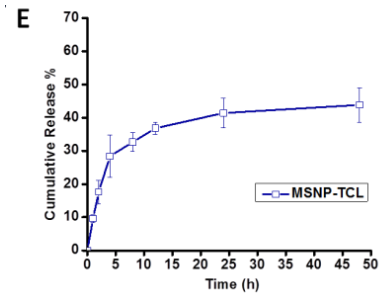




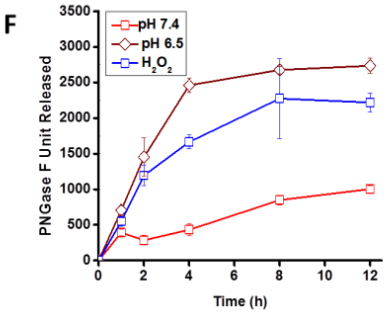
CTAB = Cetyltrimethylammonium bromide
TEOS = Tetraethyl orthosilicate
L-TA = L-Tartaric acid
PEI = Polyethylenimine
PEI-L-TA-CTAB = Polyethylenimine-L-tartaric acid-cetyltrimethylammonium bromide
4-HPBA = 4-hydroxymethyl phenyl boronic acid
3,4-DHBA = 3,4-dihydroxybenzaldehyde



Cumulative TCL release from MSNP-TCL at physiological pH 7.4.

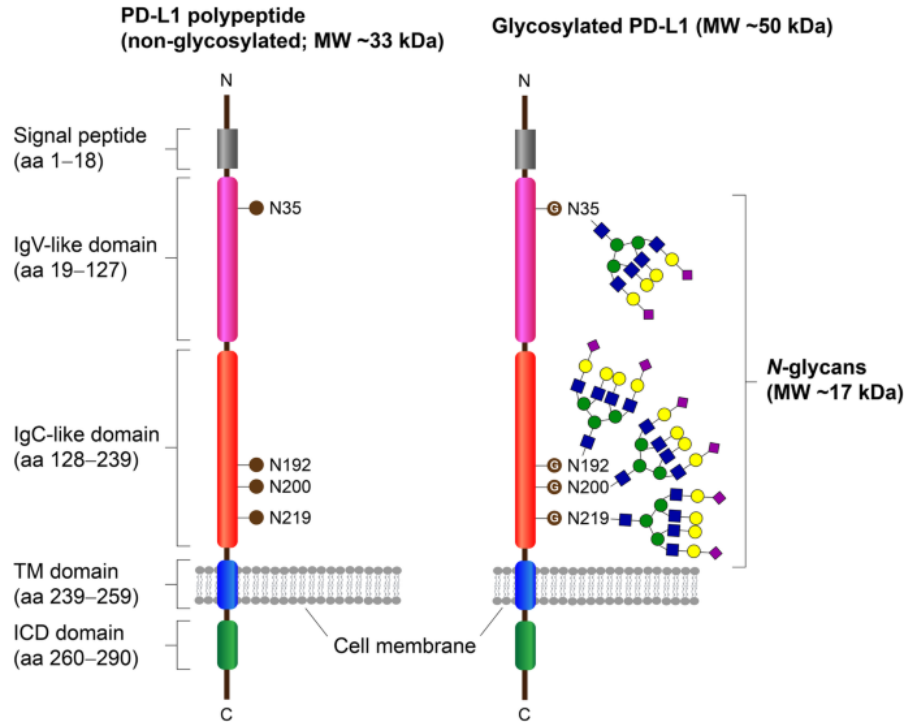


PNGase F release profile at TME mimicking conditions



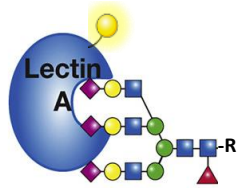
Srivastava P. and Rutter M., Unpublished data. Please do not post

Pretreatment of MDA-MB-231 cells with H-XL-MSNPs-PNGase F enhances T Cell Activation in Co-culture



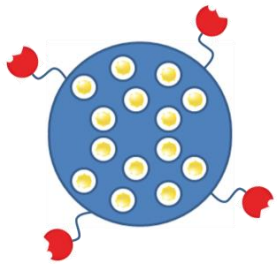
Structures of PD-L1 and N-linked glycosylated PD-L1

5(6)-carboxyfluorescein-labeled ConA binding to MDA-MB-231 cells

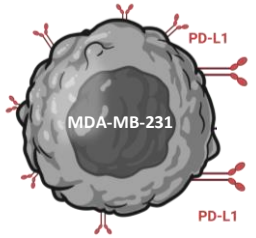


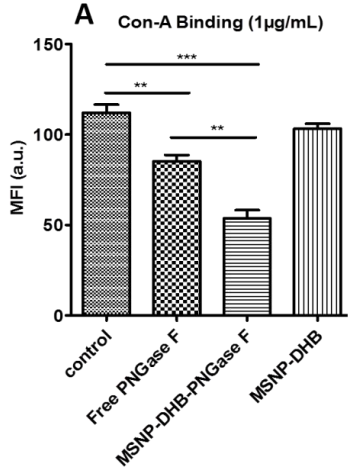
Con-A = concanavalin A lectin, binding to α-D-mannosyl and α-D-glucosyl residues found on glycoproteins and glycolipids

Free PNGase F =

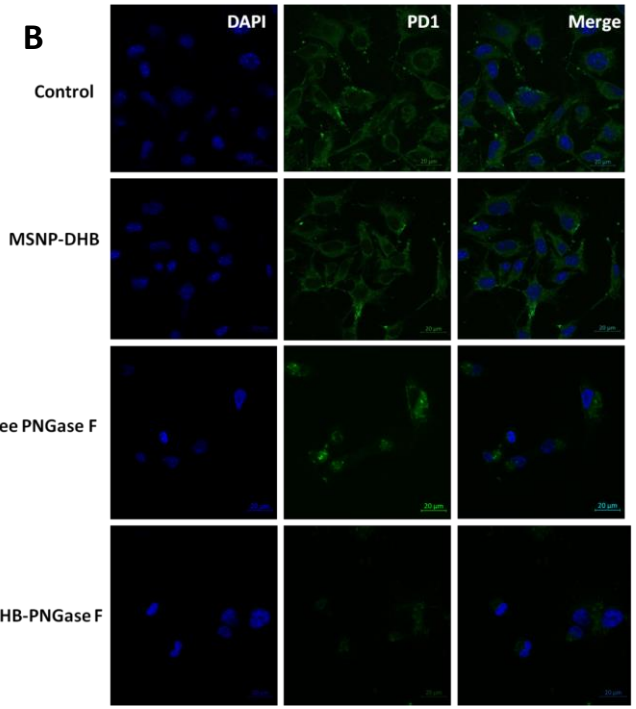


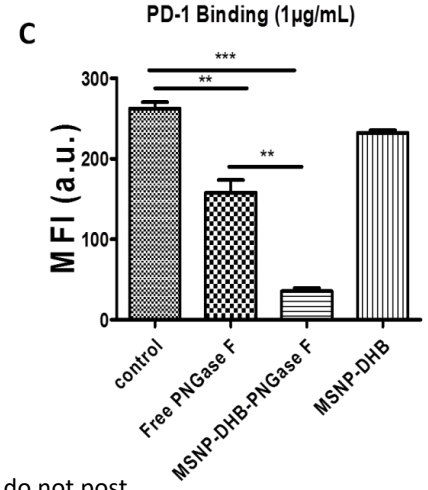
MSNP-DHB-PNGase F

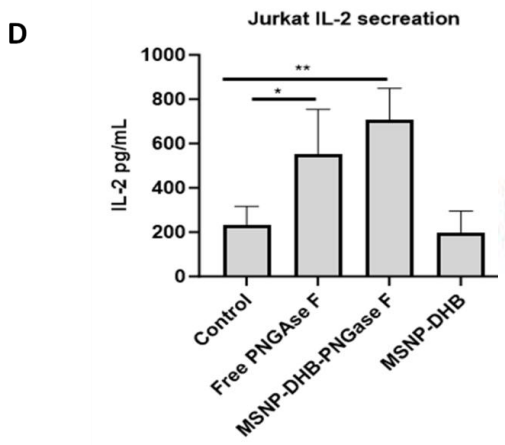


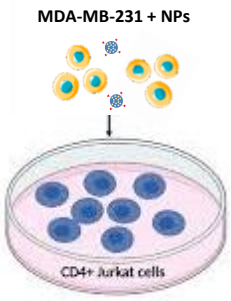


PD1 binding on MDA-MB-231 cells





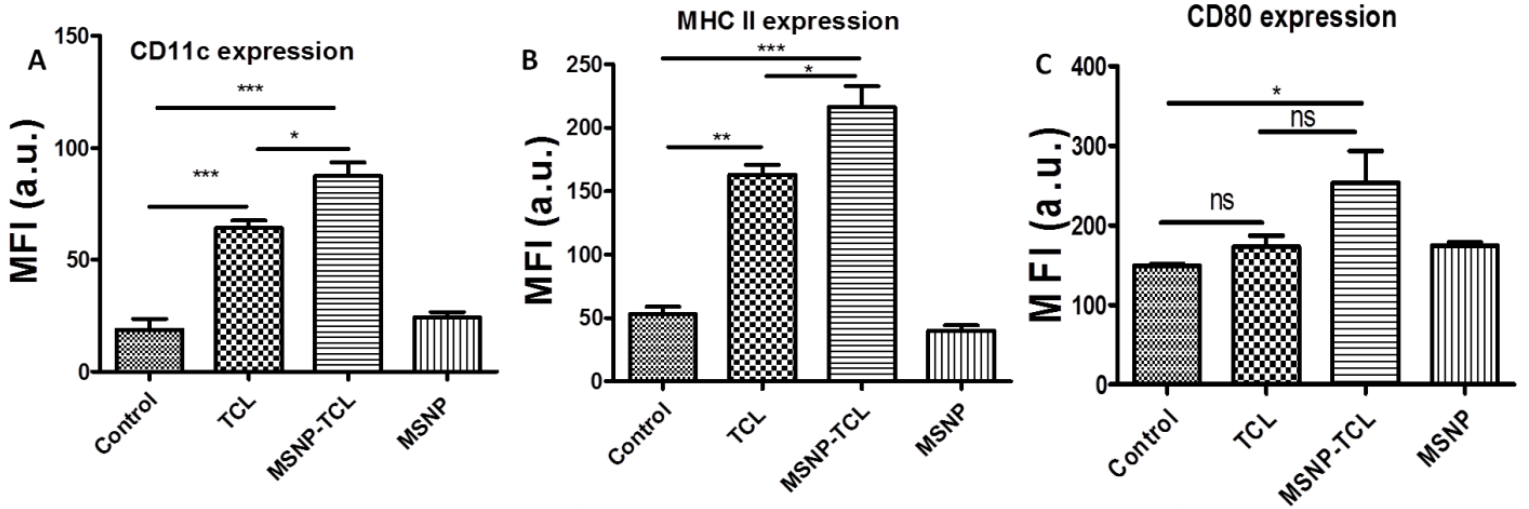




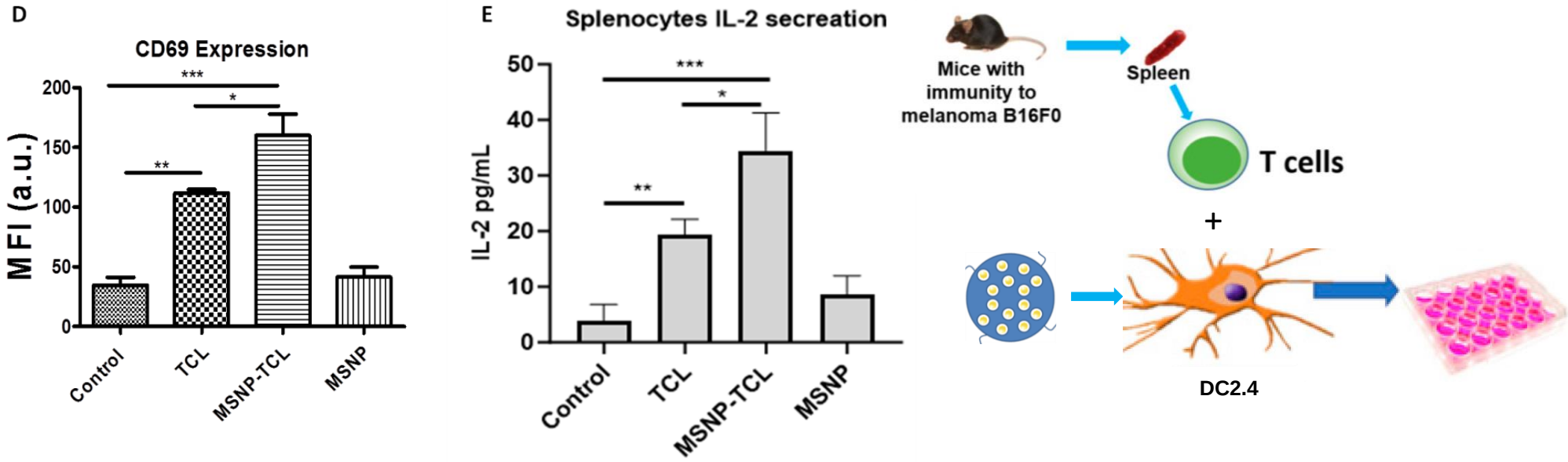
Human T lymphocyte in coculture with MDA-MB-231 that were treated with MSNP-DHB-PNGase F

Immunological activation of DC2.4 using TCL-loaded H-XL-MSNPs

DC2.4 activation



T cell activation in primary splenocytes from B16 tumor-bearing mice that had been sensitized against a B16 antigen



Srivastava P. and Rutter M., Unpublished data. Please do not post

In conclusion,

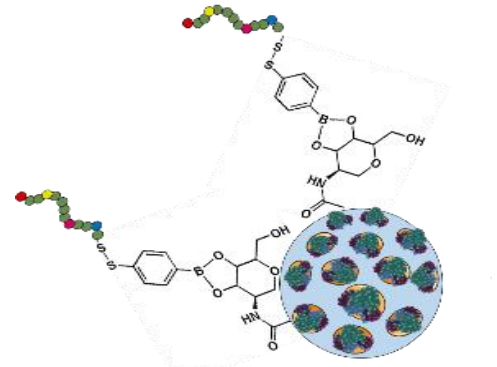
- PD-L1bp-decorated CLT-loaded NPs demonstrate a remarkable **ability to activate DCs** and **initiate a robust anti-tumor immune response**, leading to significant suppression of tumor growth.

- The nanoparticle-based strategy could serve as a **promising alternative** or complement to existing **immune checkpoint blockade** therapies

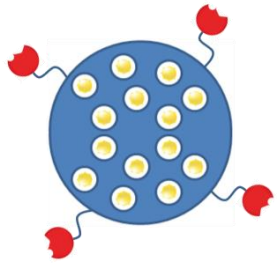
- The activation of DCs by CLT-loaded NPs occurs **independently of antigen delivery**, eliminating the need for additional adjuvant agents and highlighting a major advancement in immunotherapy strategies.

- H-XL-MSNPs exhibit **exceptional TCL loading capacity**, which is crucial for maximizing therapeutic payloads and enhancing treatment efficacy.

- H-XL-MSNPs/TCL improve DC antigen presentation, disrupt PD-1/PD-L1 immune checkpoint interactions, and **amplify T cell-mediated immune responses**.



MSNP-MaN-PDL1bp/CLT



PNGase decorated H-XL-MSNP

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