Precision Nanomedicine-based Immunotherapy to Overcome Immunosuppression Against Melanoma Brain Metastasis

Helena F. Florindo

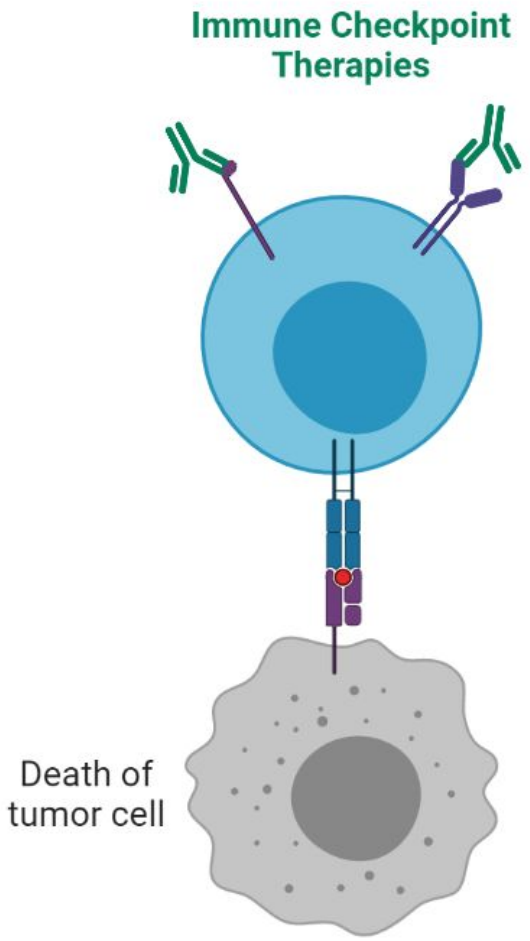
CRS 2022 Annual Meeting & Expo

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

Advanced Delivery Science

The Hurdle – Metastatic Melanoma

Immune checkpoint therapies have become standard of care for **advanced melanoma** patients



Responders

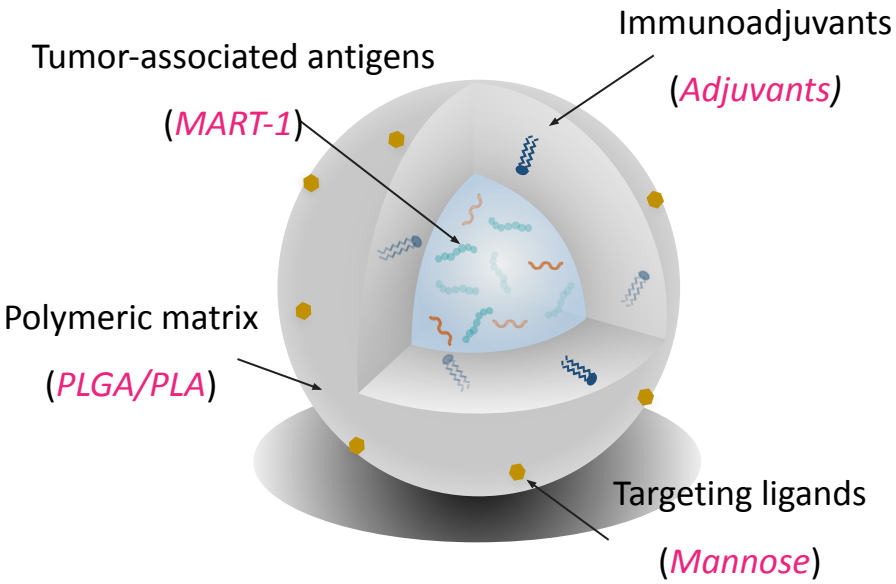
Non-responders

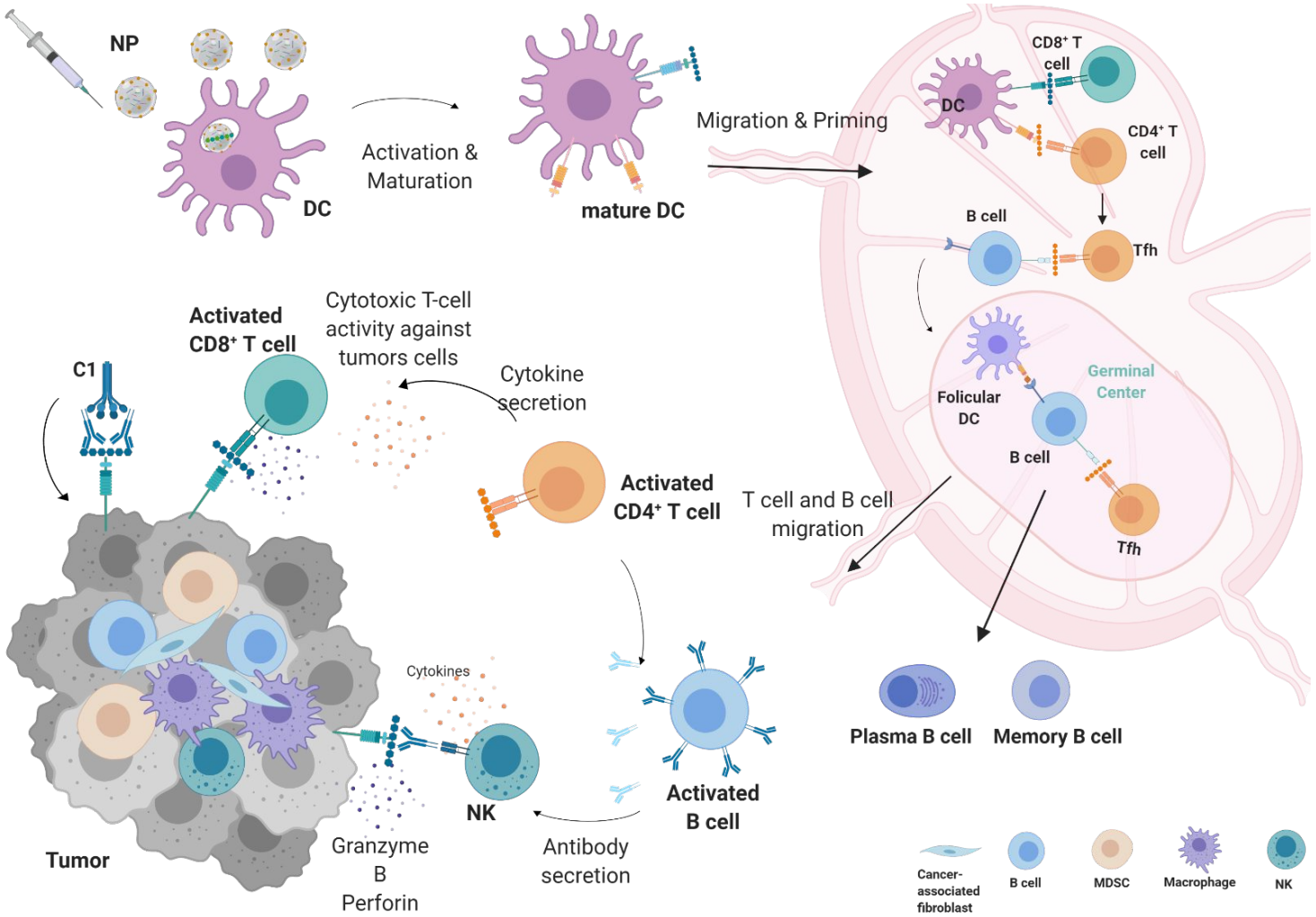
Yet, there is still an urgent, unmet need for **patients** whose **disease does not respond** to these therapies

Our Approach – A Multifunctional Nanovaccine

nature nanotechnology ARTICLES
https://doi.org/10.1038/s41565-019-0512-0

Immunization with mannosylated nanovaccines and inhibition of the immune-suppressing microenvironment sensitizes melanoma to immune checkpoint modulators





Conniot, Scomparin *et al. Nature Nanotechnology*, 2019 Sep;14(9):891-901.

Carreira B, et al. Advanced Therapeutics, 2020; 4:1-39

Our Approach – A Multifunctional Nanovaccine

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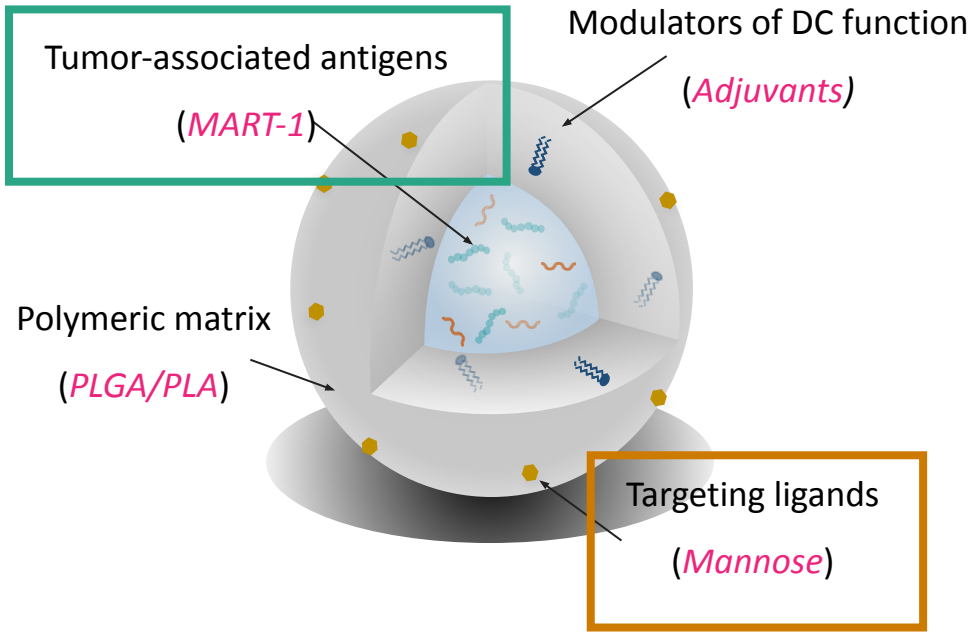
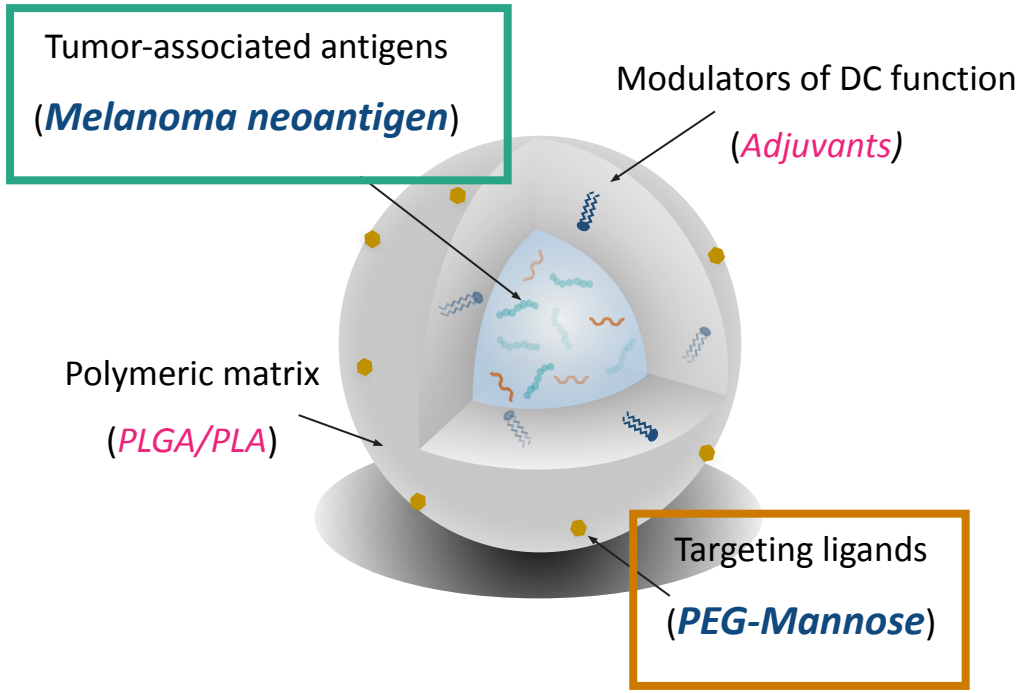
Immunization with mannosylated nanovaccines and inhibition of the immune-suppressing microenvironment sensitizes melanoma to immune checkpoint modulators



Neoantigen – Increased immunogenicity



Mannose-PEG spacer – enhanced active targeting to DC

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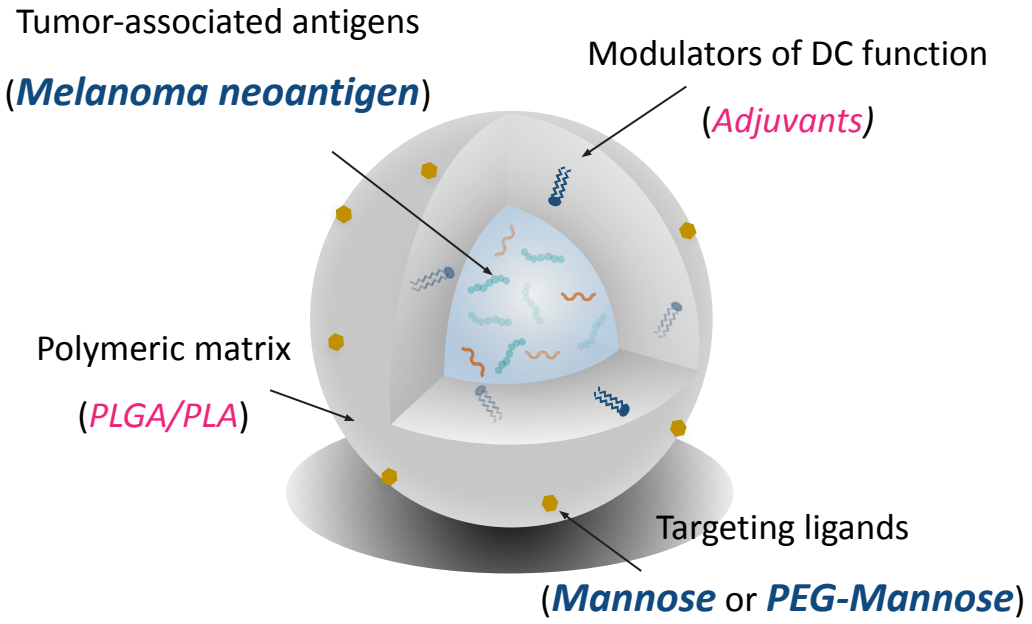
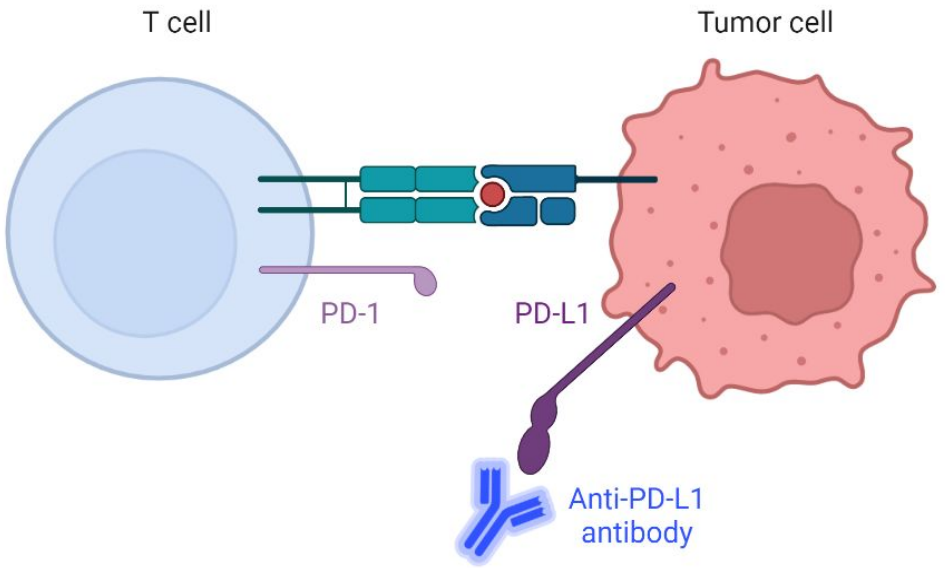
Our Approach – A Multifunctional Nanovaccine + PD-1/PD-L1 axis modulation





Multifunctional Nanovaccine to modulate progression and immune evasion pathways to sensitize metastatic melanoma to immune checkpoint modulators

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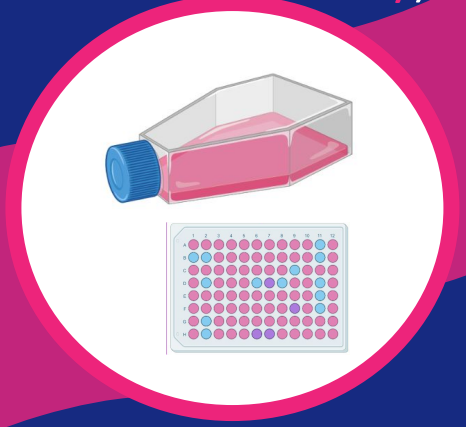
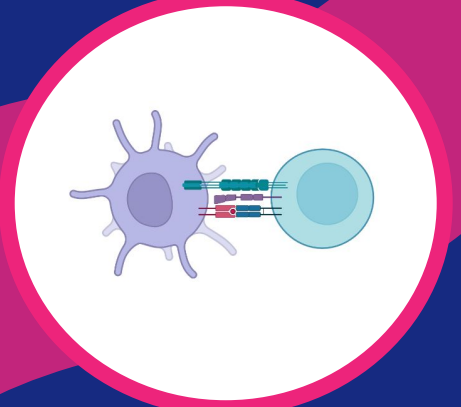
Project Design

Nanovaccine Preparation & Characterization
(Polymeric Nanoparticles)

In vitro Nanovaccine Interaction with DC
(Uptake and Cell Viability)



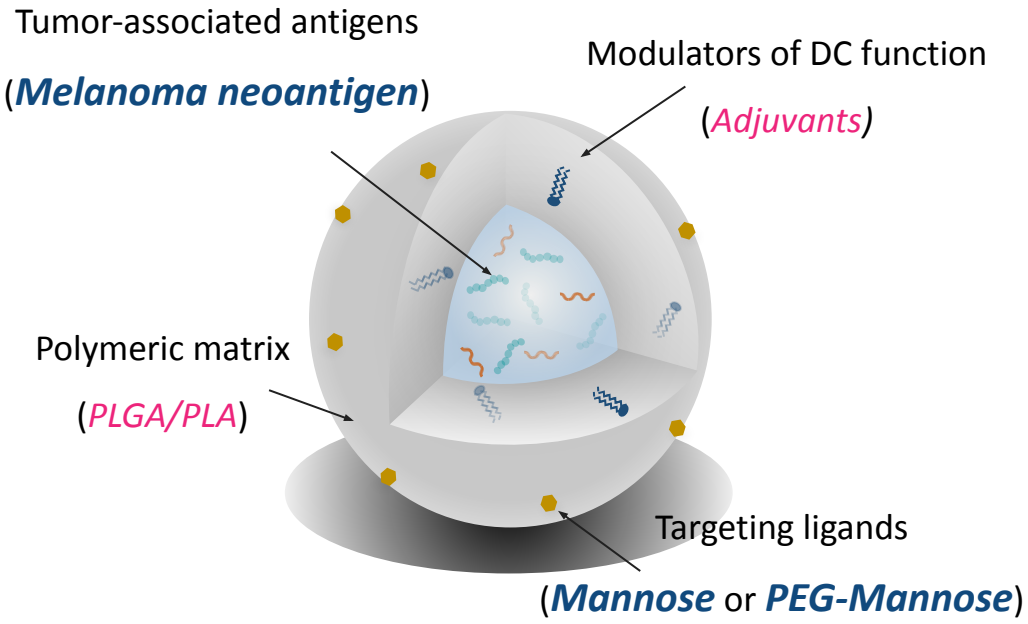
In vivo DC & T cell activation
(Maturation & Activation)

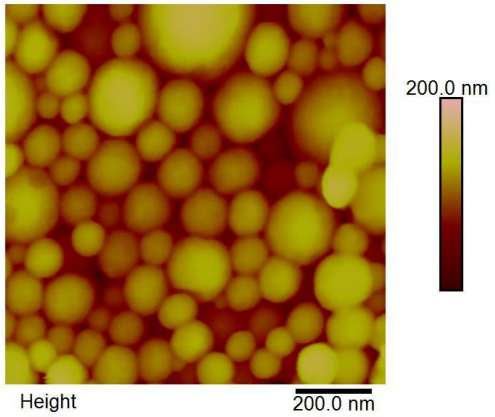


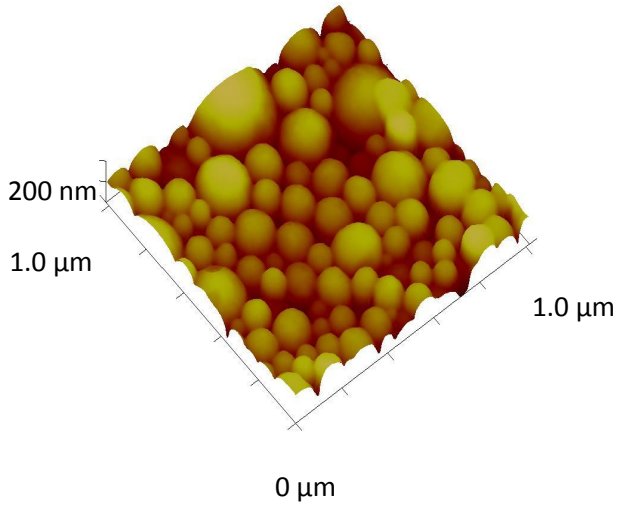
Nanovaccine Efficacy
(Anti-tumor immune response)

No breakthrough is too small

Nanovaccine Physicochemical Characterization







Target product specifications for optimal DC activation

Size: < 200 nm

PDI: < 0.2

Peptides: Moderate entrapment efficiency

Adjuvants: High entrapment efficiency

Table 1. Nanoparticle (NP) size, polydispersity index (Đ), entrapment efficiency (EE) and loading capacity (LC) of antigens into NP.

NP	Size[1] (nm ± SD[2])	PdI ± SD[2]	Z-Potential (mV ± SD[2])	Peptide EE (% ± SD[2])	Peptide LC (µg/mg ± SD[2])	Adj1 EE (% ± SD[2])	Adj1 LC (µg/mg ± SD[2])	Adj2 EE (% ± SD[2])	Adj2 LC (µg/mg ± SD[2])
NP	214 ± 8	0.14 ± 0.03	-5.24 ± 0.7	62.2 ± 4.2	31.1 ± 4.01	87.1 ± 4.2	4.5 ± 0.33	89.9 ± 4.7	10.0 ± 0.6
man-NP	198 ± 2	0.16 ± 0.02	-4.48 ± 0.6	60.8 ± 3.7	30.4 ± 3.27	82.0 ± 5.2	4.6 ± 0.65	93.2 ± 5.2	10.4 ± 0.9
man-PEG-NP	216 ± 2	0.15 ± 0.03	-4.96 ± 0.5	65.4 ± 3.9	32.7 ± 4.61	90.2 ± 3.9	4.6 ± 0.55	90.2 ± 3.9	10.1 ± 0.5

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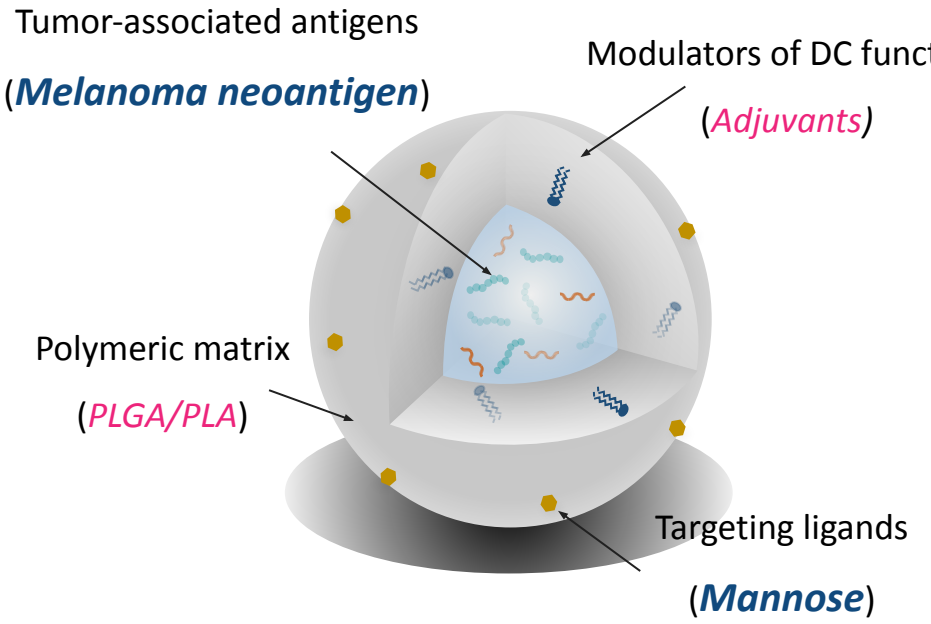
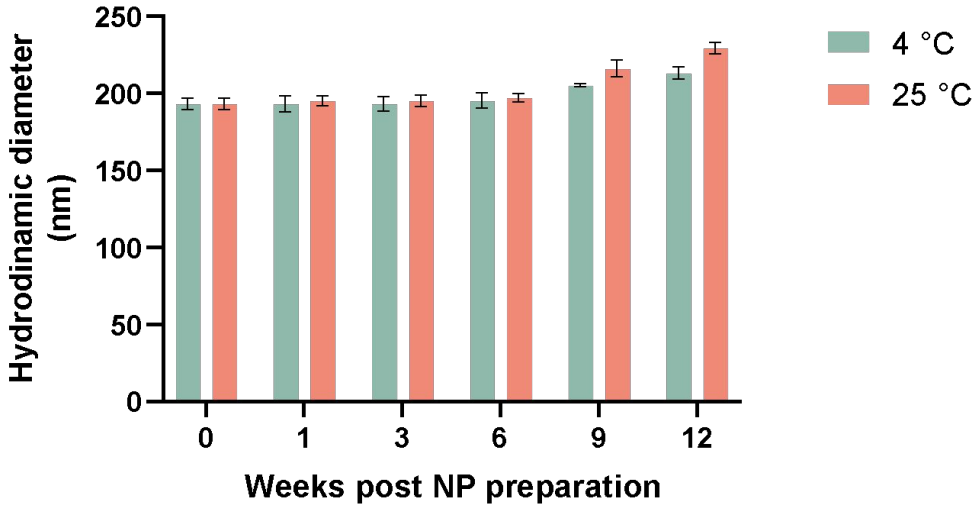
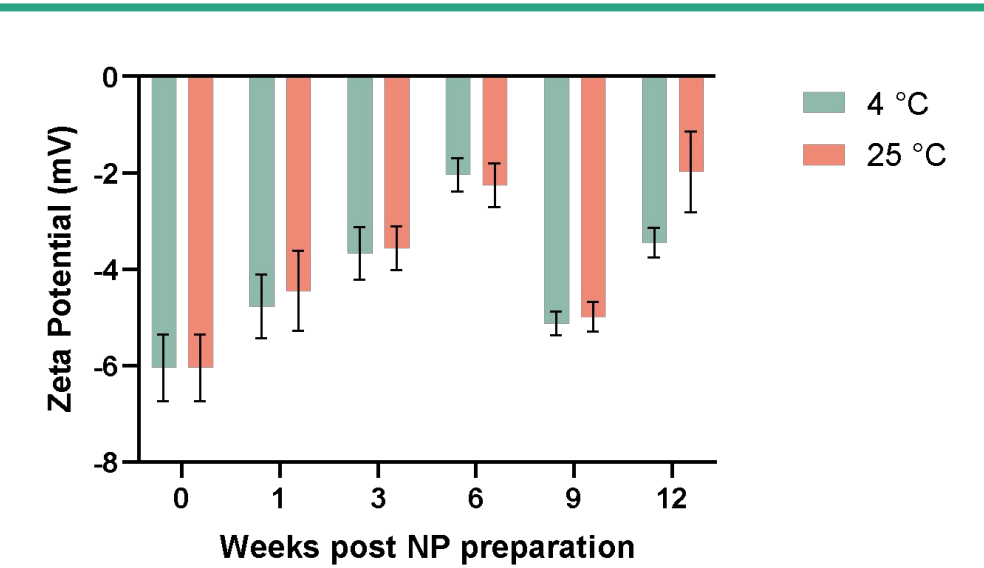
Nanovaccine Physicochemical Characterization



Nanoplatform stable as a powder.
NP mean diameters and Đ
at **different time-points** and **storage temperatures**.





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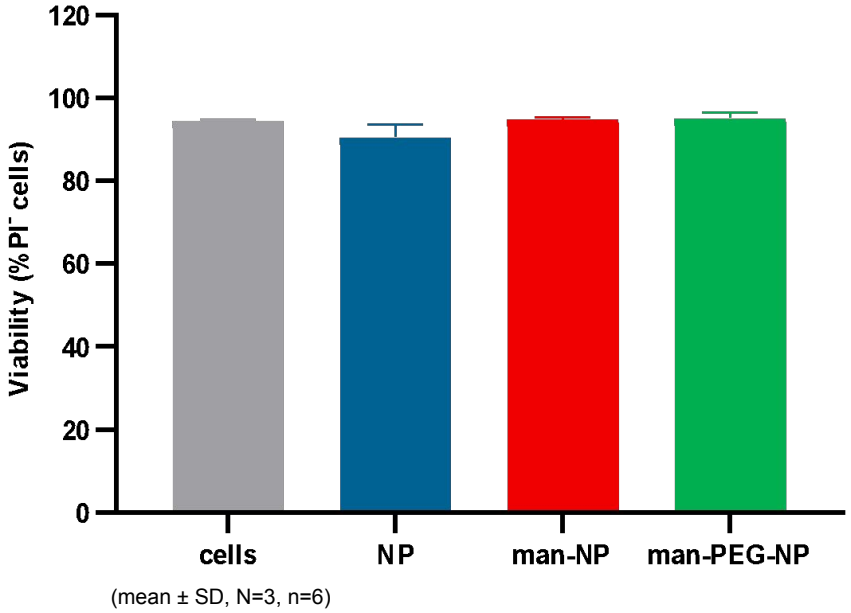
Nanovaccine Impact on DC – *in vitro studies*

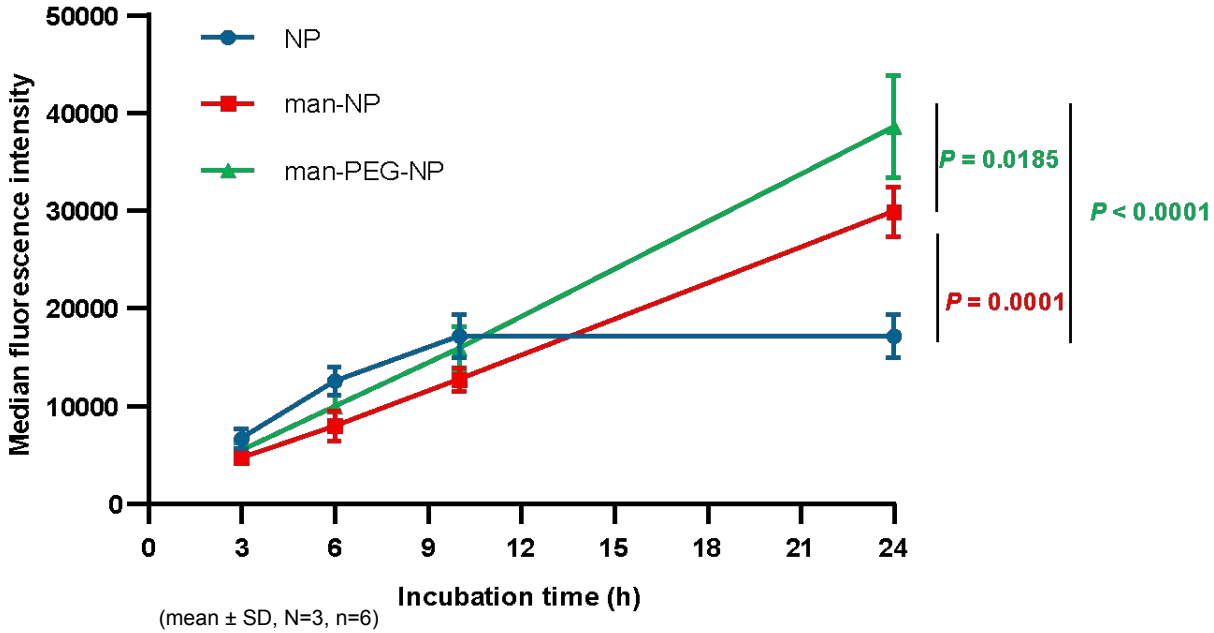
Cellular Viability



(mean ± SD, N=3, n=6)

All Nanovaccines did not change cellular viability

Cellular Internalization



(mean ± SD, N=3, n=6)

All Nanovaccines were **efficiently internalized** by **iDC,** with higher extent for **mannosylated Nanovaccines**

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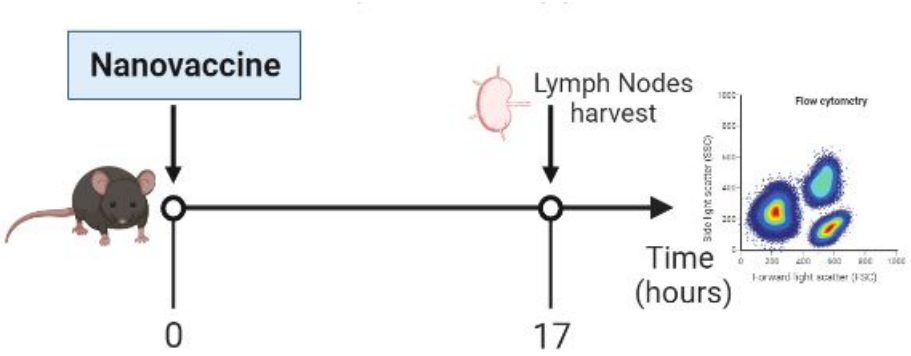
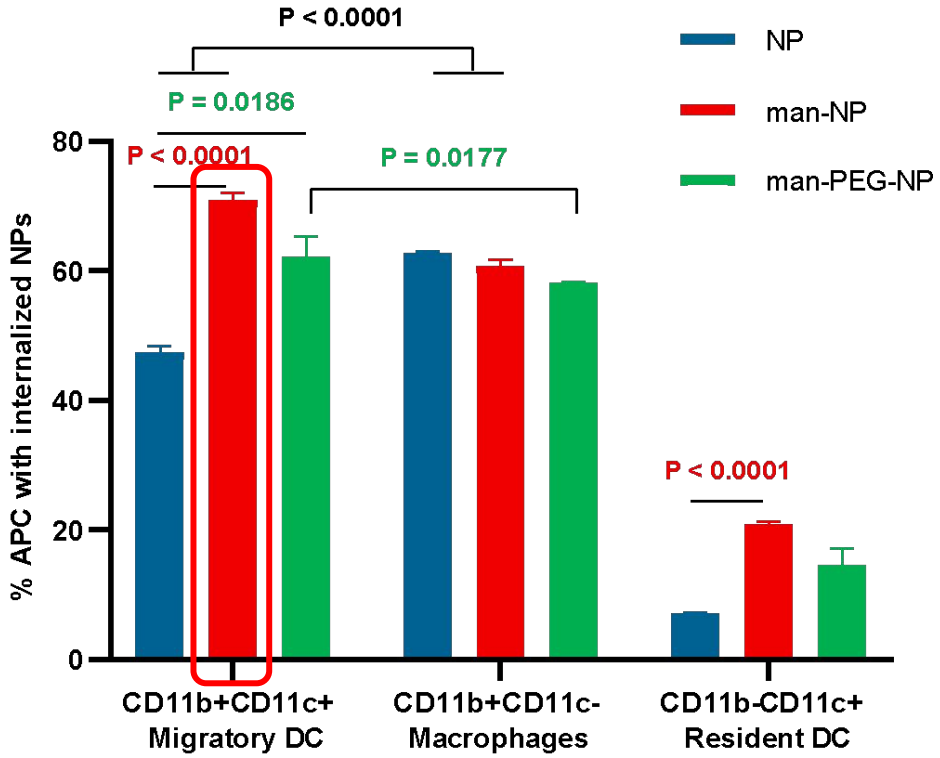
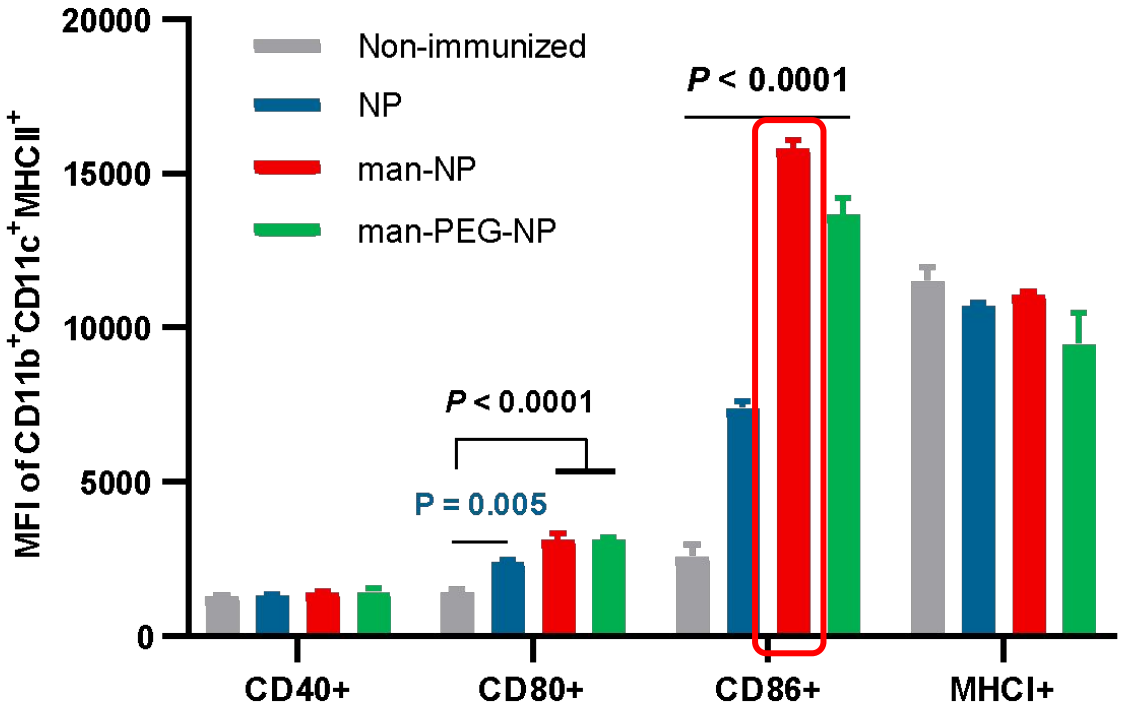
Nanovaccine Impact on DC – *in vivo studies*



Nanovaccines were **preferentially internalized** by **migratory DC**

Nanovaccines triggered the **activation of migratory DC,** in particular **man-NP**





(mean ± SD, N=1, n=3)

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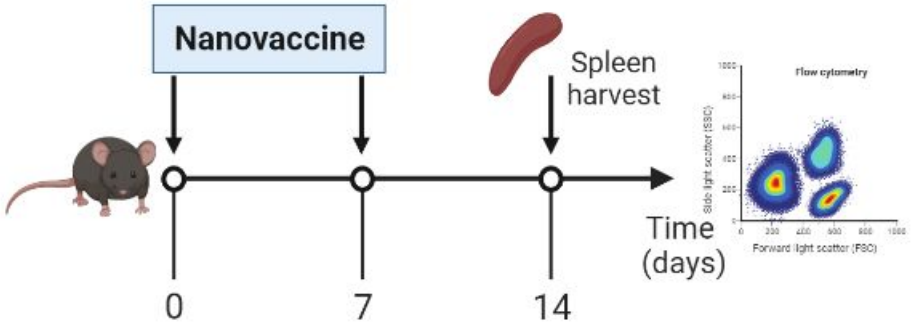
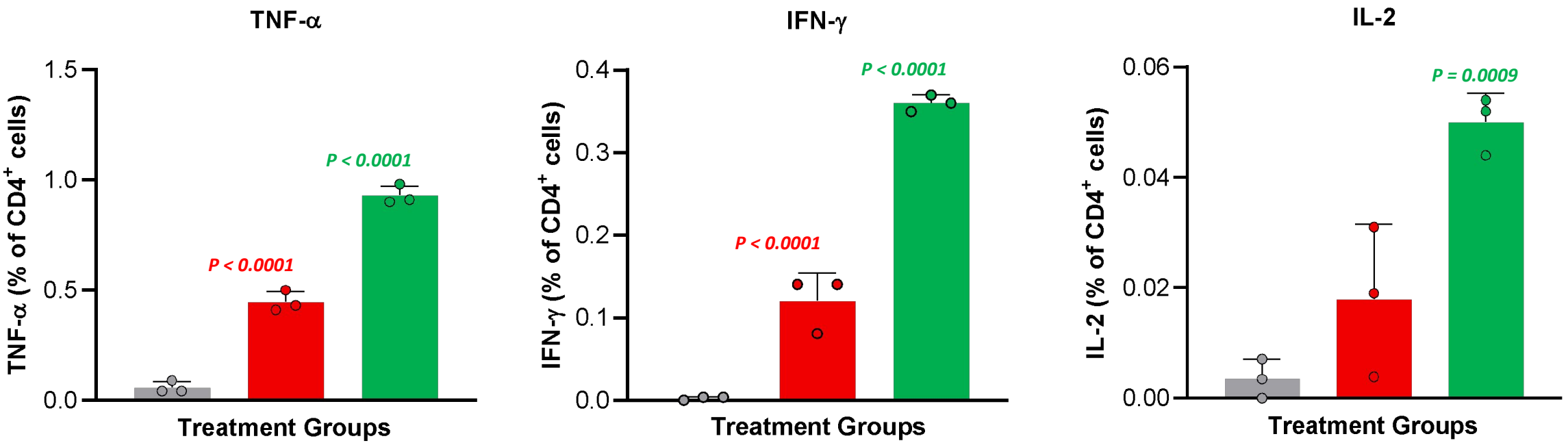
Nanovaccine Impact on T-cell Activation – *in vivo studies*



Nanovaccine elicits **antigen-specific T-cell responses**, by the significant increase in the expression of **TNF-α, IFN-γ and IL-2 (T_H1-guided response)**



(mean ± SD, N=1, n=3)

PBS MHCII-ag/CpG/polyI:C man-NP MHCII-ag/CpG/polyI:C

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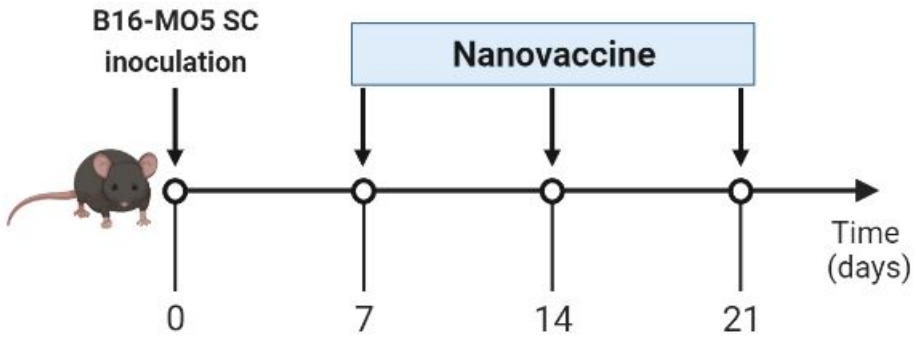
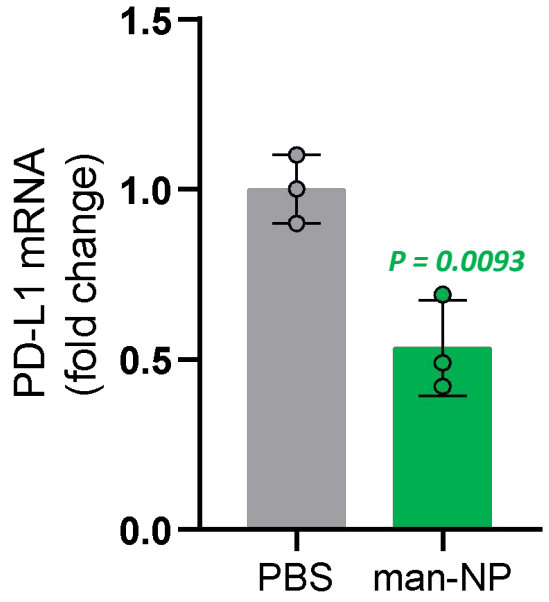
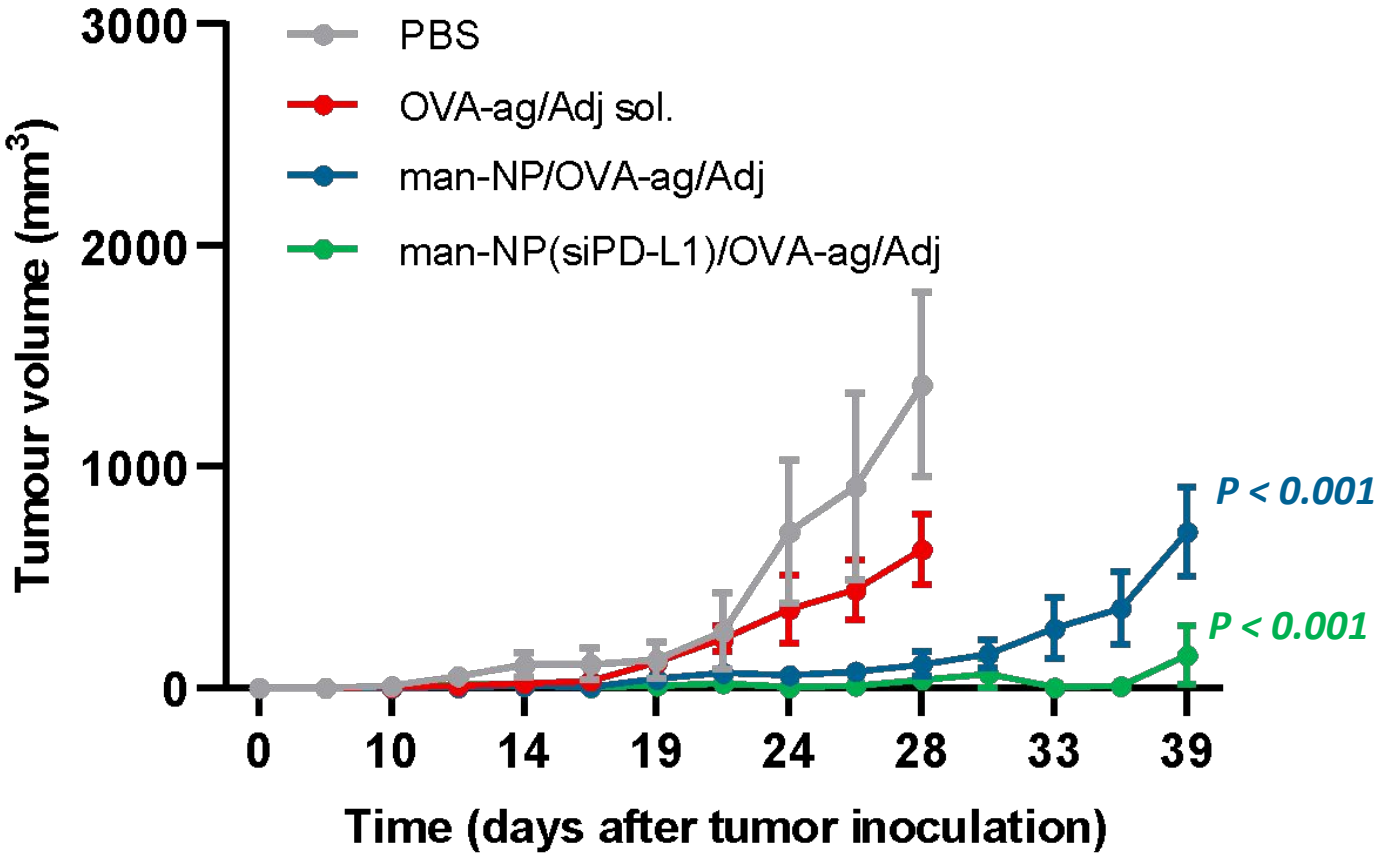
Proof-of-concept – *Therapeutic efficacy of man-NP in combination with PD-L1 modulation*







(mean ± SD, N=1, n=7)

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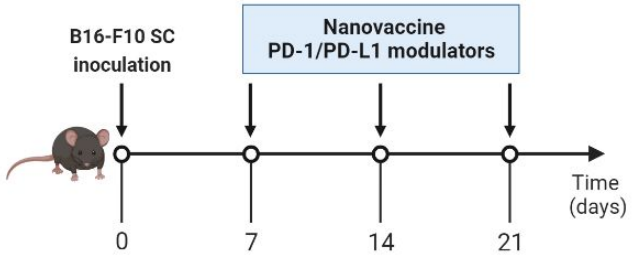
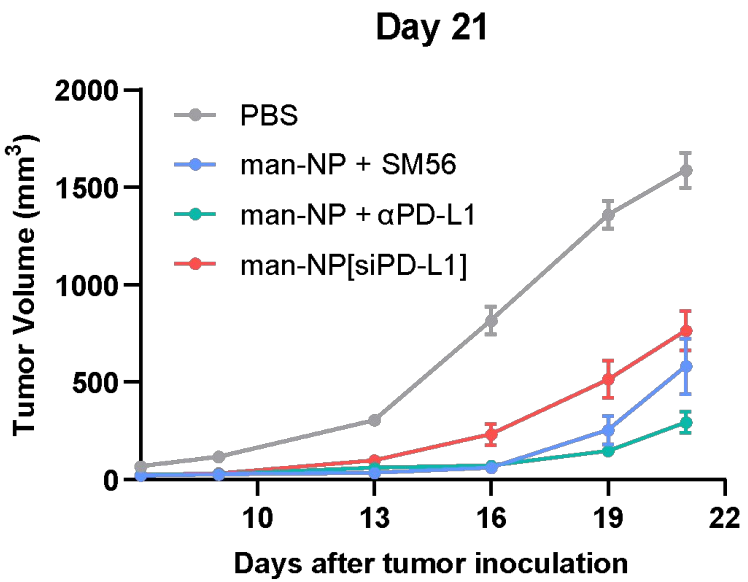
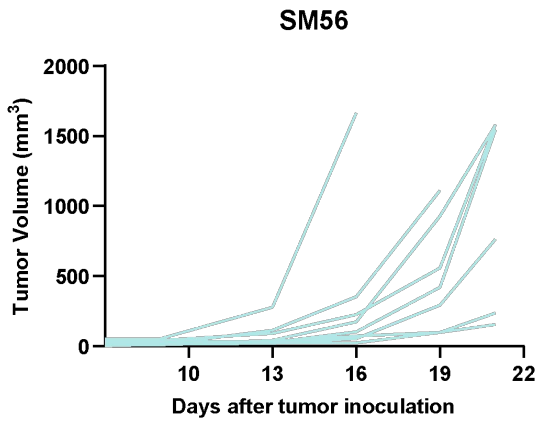
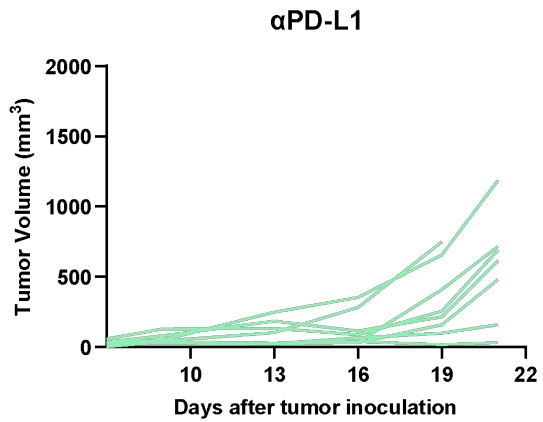
Proof-of-concept – *Therapeutic efficacy of man-NP in combination with PD-L1 modulation*

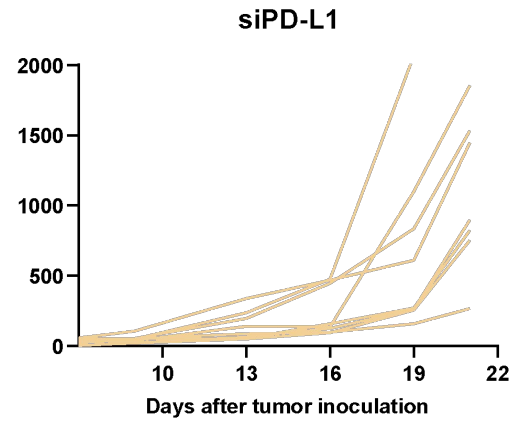


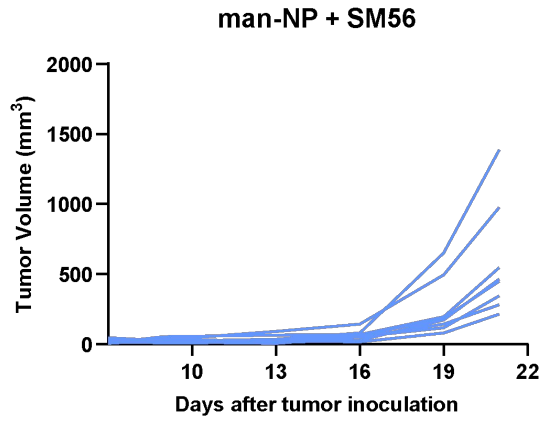


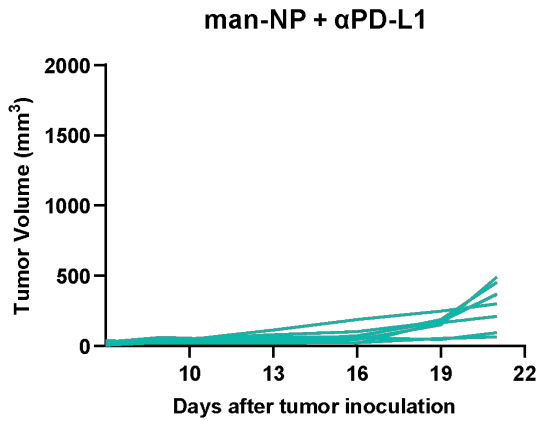
(mean ± SD, N=1, n=8)

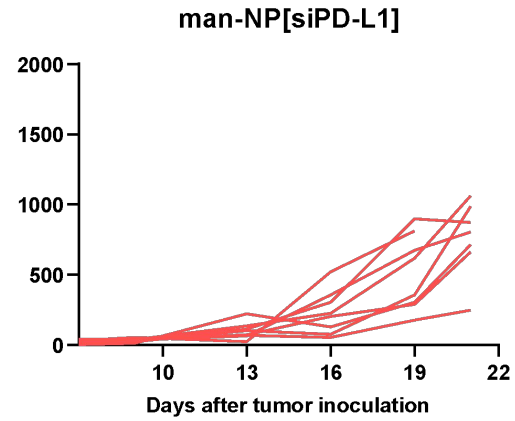












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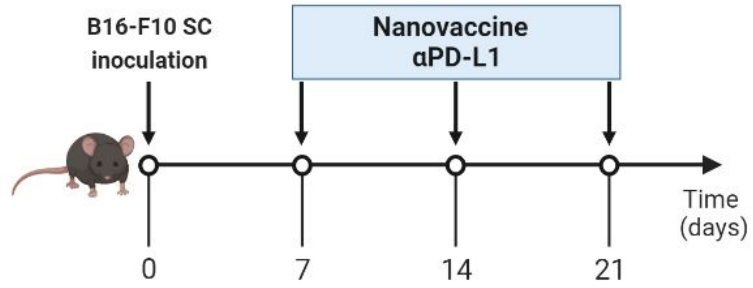
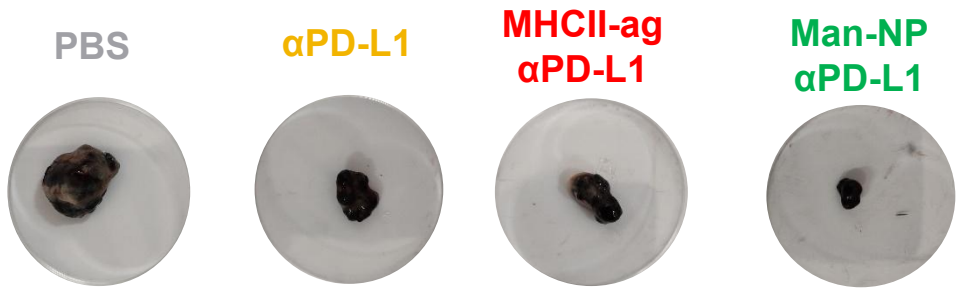
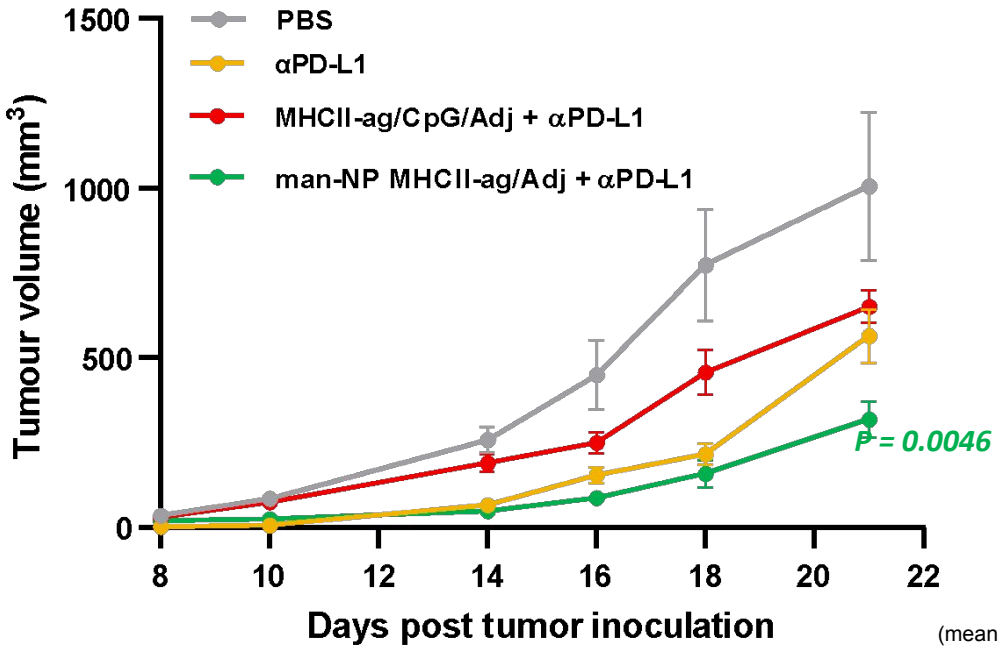
Effective immune-mediated anti-tumor response induced by the combination of man-NP with anti-PD-L1







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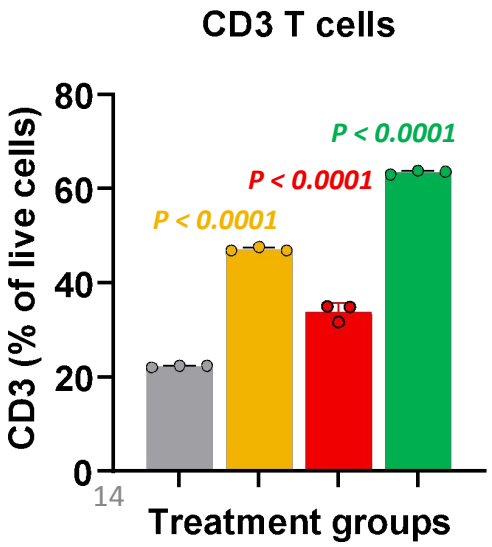

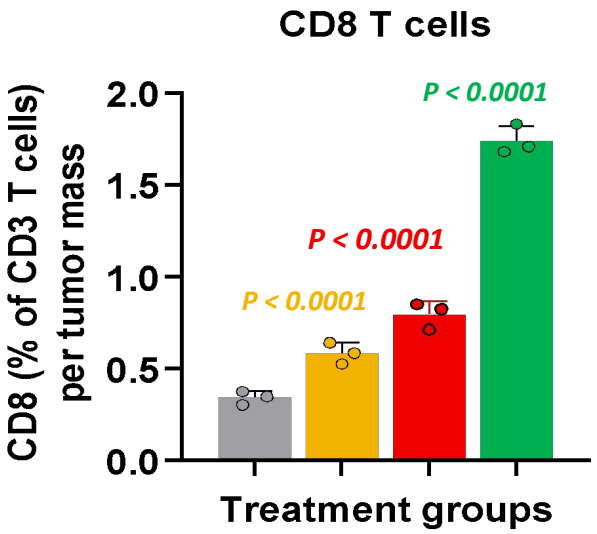

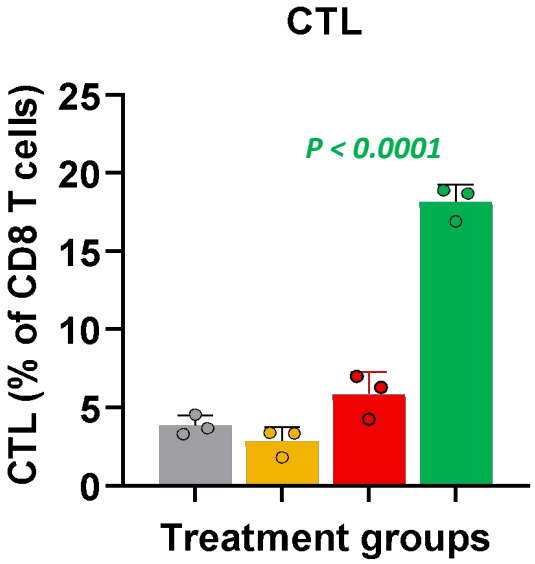

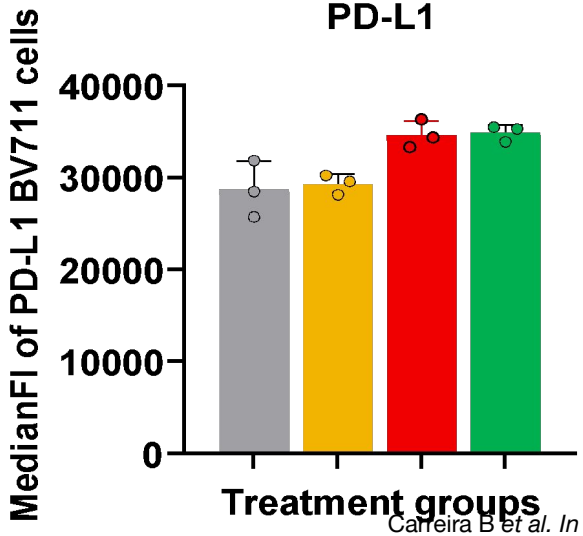

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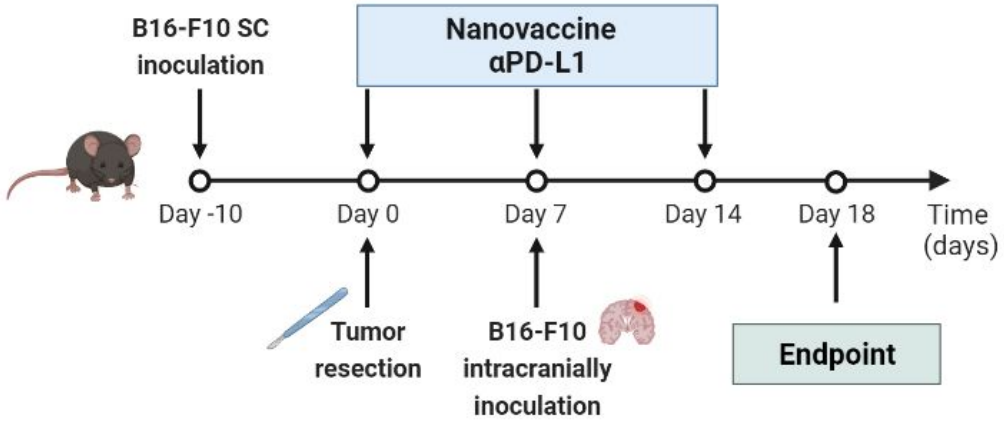
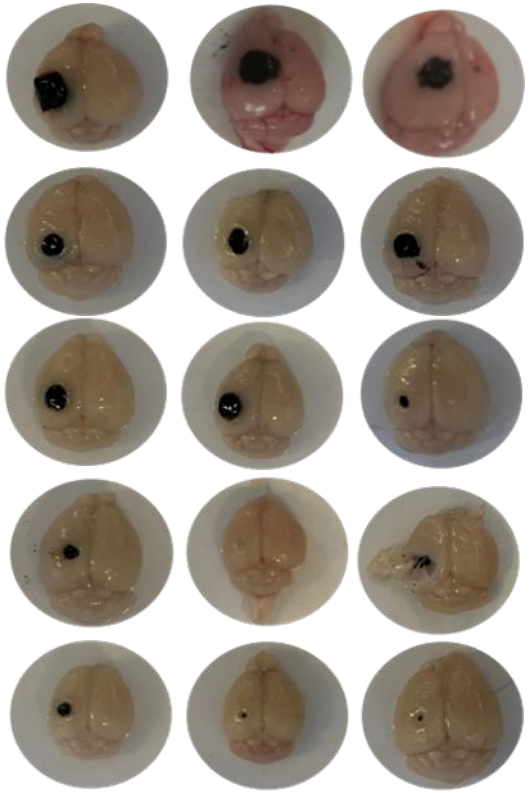
man-NP strongly restricts melanoma brain metastasis when combined with anti-PD-L1





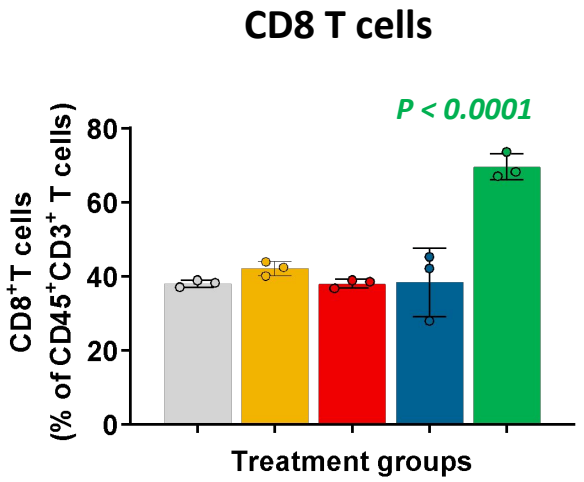
PBS

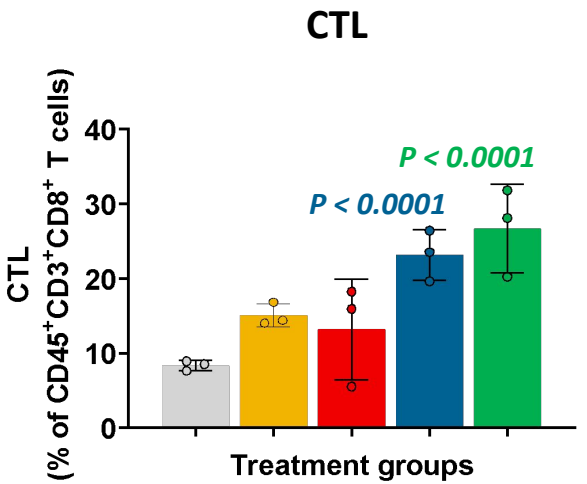
αPD-L1

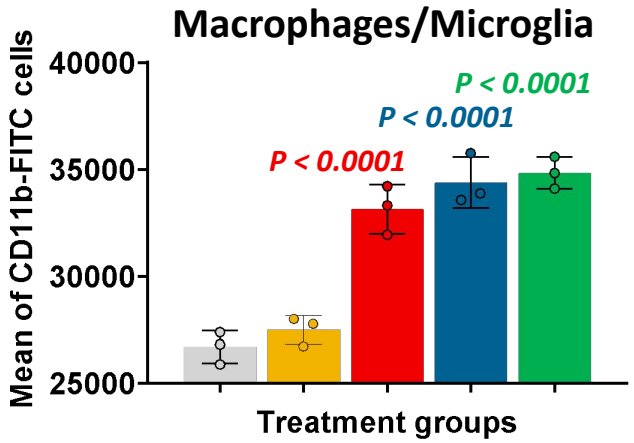
MHCII-ag/Adj sol

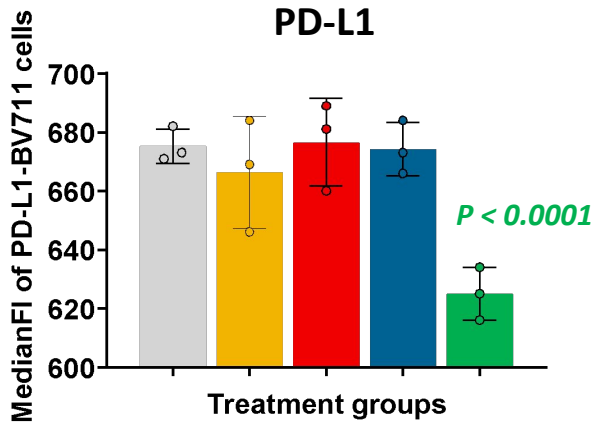
man-NP/MHCII-ag/Adj

man-NP/MHCII-ag/Adj + αPD-L1









(mean ± SD, N=1, n=5)

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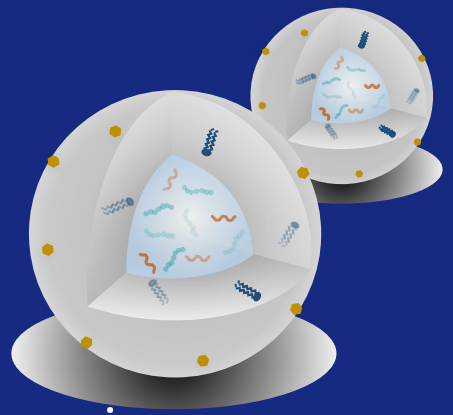

- **man-NP does not affect DC viability and were internalized by iDC.**

- **man-NP is preferentially taken-up *in vivo* by migratory DC**, increasing the expression of activation markers.

- The combination of **man-NP with aPD-L1 strongly inhibits tumor growth** in both **primary** and **metastatic melanoma** mouse models.

- **man-NP modulated DC function** triggering a **strong anti-tumor immune response** while **regulating the PD-L1 expression** within tumor milieu.

Acknowledgments

BioNanoSciences – Drug Delivery & Immunoengineering

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- Rita Acúrcio
- Liane Moura
- Ana I. Matos
- Carina Peres

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PI: Ronit Satchi-Fainaro

- Sabina Pozzi
- Ron Kleiner
- Daniella Vaskovich

PTDC/BTM-SAL/4350/2021
UTAP-EXPL/NPN/0041/2021
EXPL/MED-QUI/1316/2021

LCF/TR/CD20/52700005
LCF/PR/HR19/52160021

No breakthrough is too small

Thank you

CRS 2022 Annual Meeting & Expo

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

Advanced Delivery Science