# CD44v6-Driven Nanoparticles Combined with Immune Therapy for Colorectal Cancer

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NEXT-GENERATION DELIVERY INNOVATIONS

# Colorectal Cancer: barriers to effective treatment

- Colorectal Cancer (CRC): a major cause of cancer-related mortality

**Challenges:**

- Inter- and intratumoral heterogeneity
- Immunosuppressive tumor microenvironment (TME)
- Irinotecan: standard of care, but suffers from poor delivery and efficacy

**OPPORTUNITY**

If we can deliver irinotecan more selectively and simultaneously relieve immune suppression, we may unlock combination potential

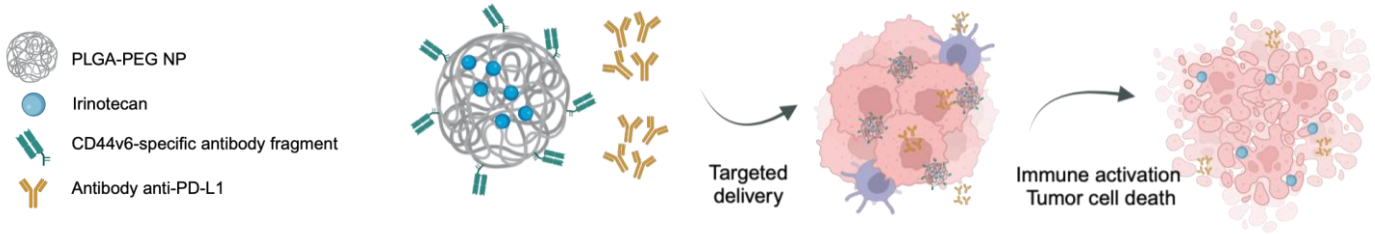
Siegel et al., 2024; Sobral et al., 2022; Buechler, 2021; Gilbert et al., 2012.

# Our hypothesis

## Combining nanomedicines and immunotherapy

- **CD44v6-targeted** PLGA nanoparticles loaded with **irinotecan** (**Iri@NP-v6**) → Enhance irinotecan delivery
- **Anti-PD-L1** immunotherapy → Reprograms the immunosuppressive TME

**A combination approach to improve drug efficacy and immune response**

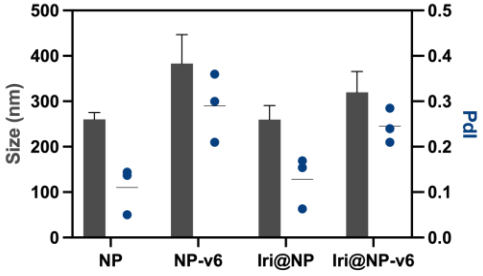


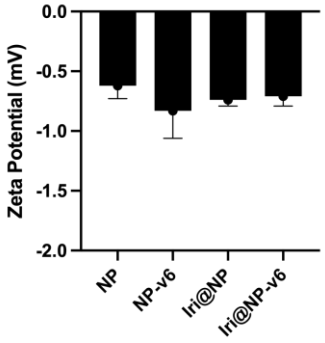

Baião et al., 2020.

# Nanoparticle synthesis and characterization

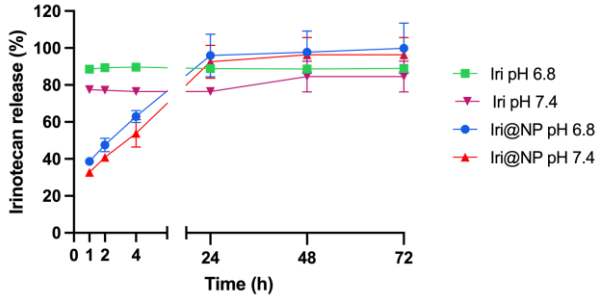
Iri@NP synthesized via single-emulsion and functionalized with a CD44v6-Fab fragment


## Physicochemical characterization




## Release profile



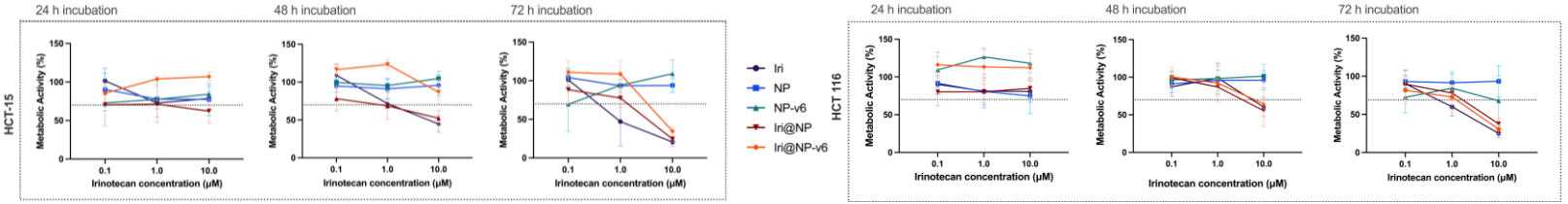

Iri@NP-v6 are **stable**, **efficiently loaded** with irinotecan (AE ≈ 34%), and **release the drug in a sustained manner**

Baião et al., submitted.

# NPs cytotoxicity and specificity in 2D CRC models

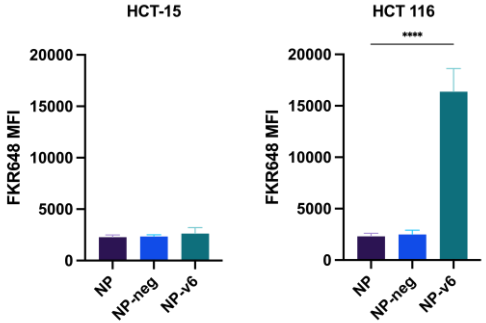
CRC human cell lines: **HCT-15 (CD44v6$^{low}$)** and **HCT 116 (CD44v6$^{high}$)**

## Resazurin metabolic assay

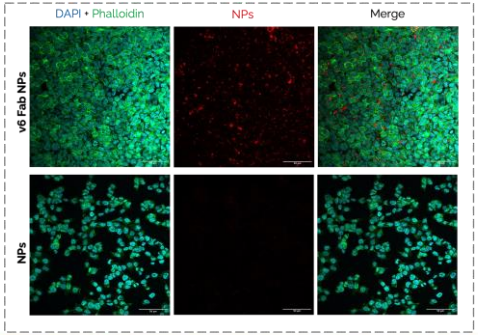



## Cellular internalization

*Flow cytometry*




*Confocal microscopy*

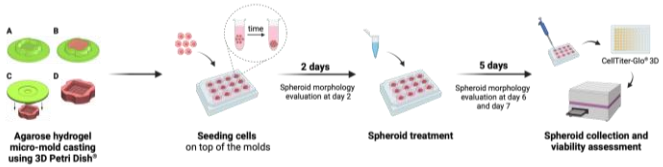



Validation of **specificity and functionality** our CD44v6-targeting strategy

Baião et al., submitted.

# NPs cytotoxicity in 3D CRC models

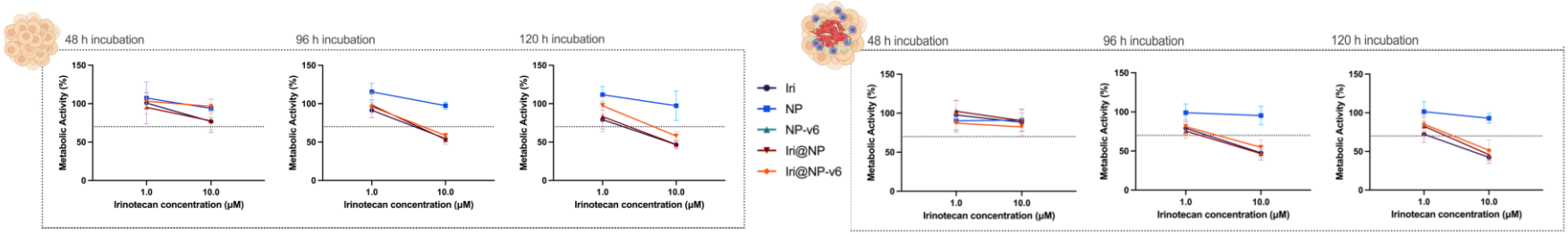
## Experimental setup




**Monoculture** CRC spheroid: HCT 116

**Quadruple** CRC spheroid: HCT 116 + HPMEC (endothelial) + HIF (fibroblasts) + monocytes

CellTiter-Glo® 3D cell viability assay




NPs demonstrate their **therapeutic potential** beyond simple monolayer cultures

Carvalho et al., 2023; Baião et al., submitted.

# NPs immune modulation in 3D CRC models

**Quadruple** CRC spheroid

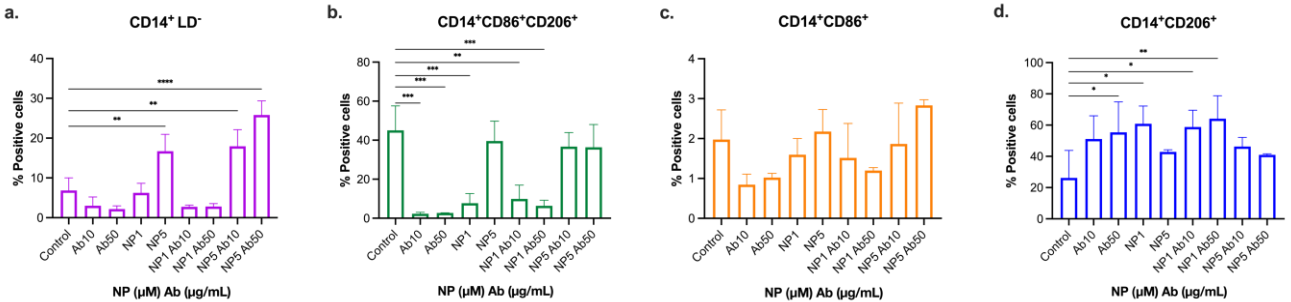
**Treatment: NP** (CD44v6-targeted irinotecan-loaded nanoparticles) and **Ab:** anti-PD-L1 antibody

Flow cytometry

**CD14:** monocytes

**CD86:** M1-like

**CD206:** M2-like

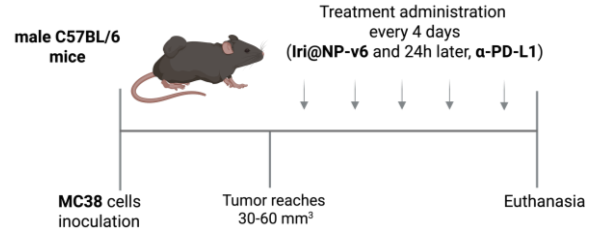


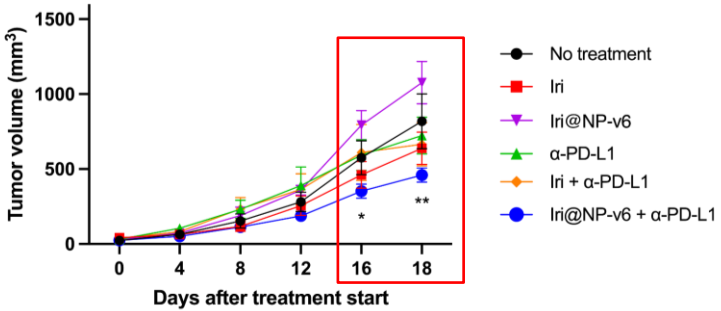

Combination therapy promoted monocyte retention and shifted the macrophage profile toward a less suppressive, more inflammatory phenotype (**suggesting immune reprogramming within the tumor-like microenvironment**)

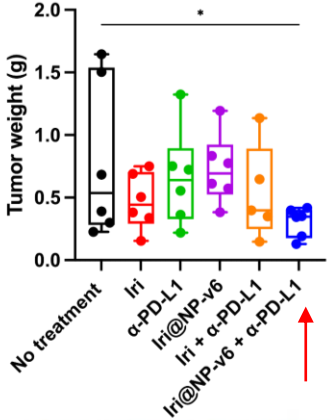
Baião et al., submitted.

# Combination therapy inhibits tumor growth *in vivo*

Mouse MC38 CRC cell line inoculated subcutaneously in C57BL/6 mice



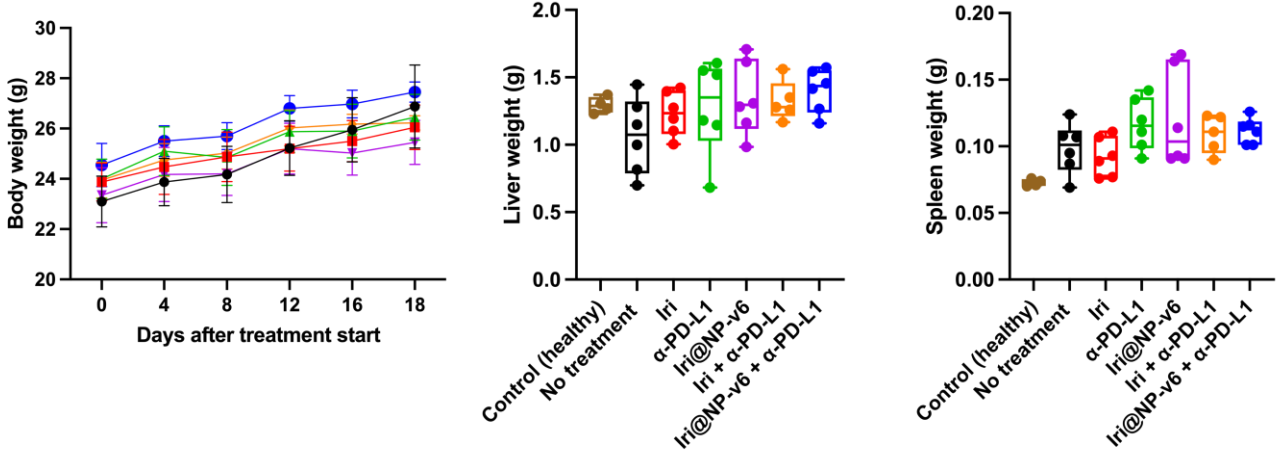




Tumor volume and weight were significantly reduced in mice treated with **Iri@NP-v6 + αPD-L1**, highlighting a **clear combination effect**

Baião et al., submitted.

# Favorable safety profile and systemic tolerability *in vivo*

Mouse MC38 CRC cell line inoculated subcutaneously in C57BL/6 mice

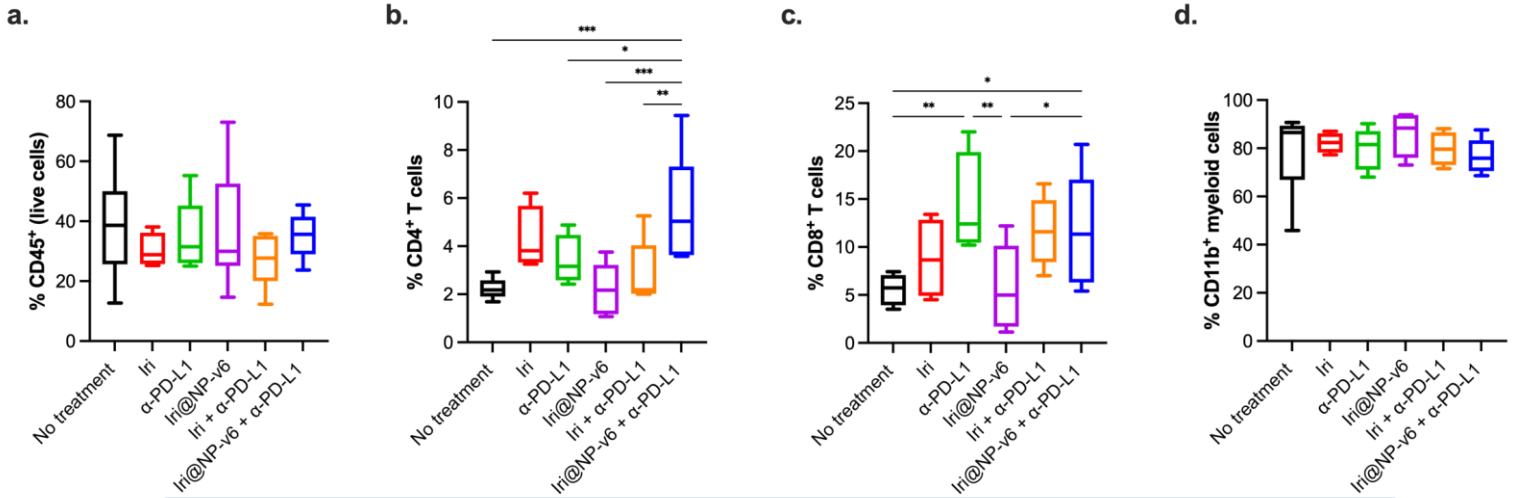



No significant changes in body weight or organ weight, confirming the **systemic safety of the nanoparticle-based combination therapy**

Baião et al., submitted.

# Enhanced immune cell infiltration *in vivo*

Flow cytometry of mice tumors

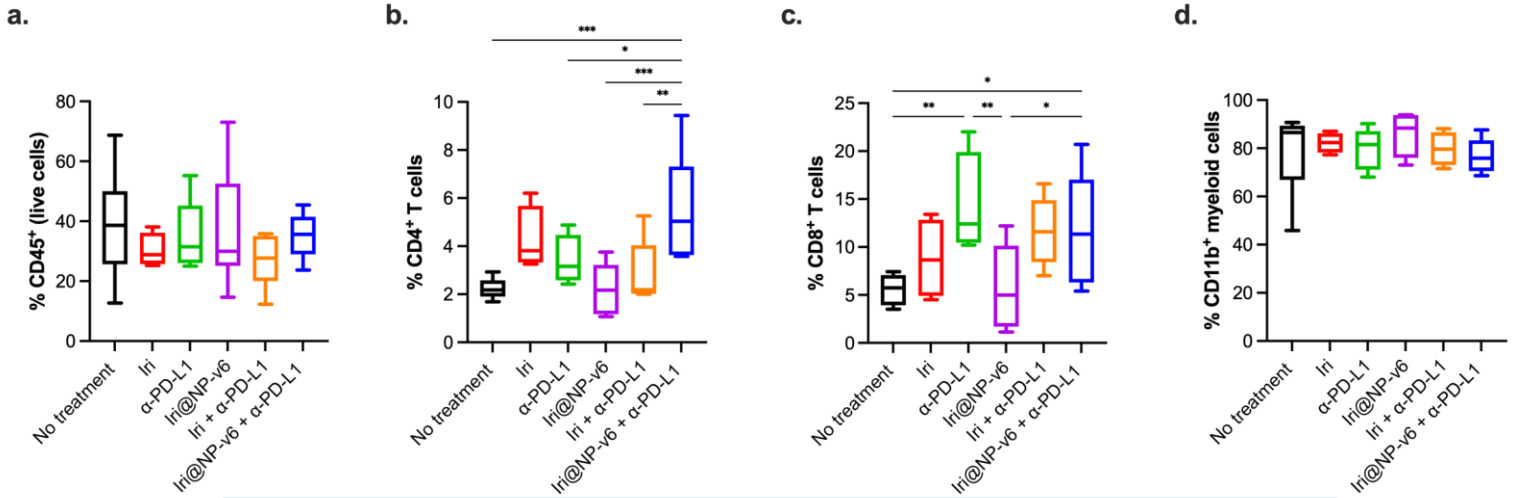



**Combination therapy increased CD4⁺ and CD8⁺ T cell infiltration into tumors, suggesting a more immunologically active tumor microenvironment**

Baião et al., submitted.

# Enhanced immune cell infiltration *in vivo*

Flow cytometry of mice tumors




**Iri@NP-v6 may induce immunogenic cell death and enhance antigen presentation, while PD-L1 blockade restores T cell effector function**

Baião et al., submitted.

## Conclusions and outlook

- CD44v6-targeted irinotecan-loaded NPs enhance drug delivery and immune activation *in vitro* and *in vivo*
- Combination with anti-PD-L1 enhances antitumor activity and increases immune infiltration
- The therapy shows good tolerability and induces immune modulation in the tumor microenvironment

## Future directions

- Evaluate long-term effects and immune memory generation *in vivo*
- Apply the strategy to orthotopic and organoid CRC models for better clinical translation

# Acknowledgments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Flávia Castro, PhD (co-supervisor)

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Animal Facility

Histology and Electron Microscopy

Translational Cytometry



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## Thank you for your attention!

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