CD44v6-Targeted Chemotherapeutic Nanosystem Combined with Immunotherapy for Colorectal Cancer

2nd CA17140 STSM Virtual Conference, February 23, 2023

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STSM at Eindhoven University of Technology (TU/e) and the Radboud University Medical Center (Radboudumc) (Netherlands) – Roy Van der Meel, PhD. (Setember-October 2022)

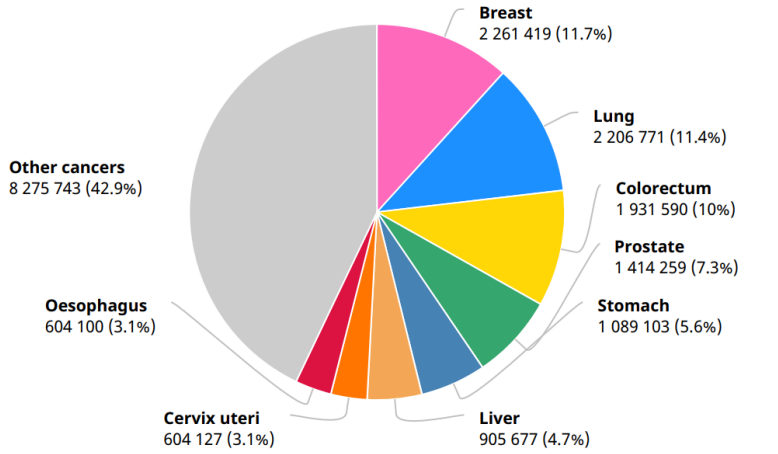
Supervising team: Bruno Sarmento, PhD; Carla Oliveira, PhD; Flávia Castro, PhD (i3S)

Introduction

Colorectal cancer (CRC)

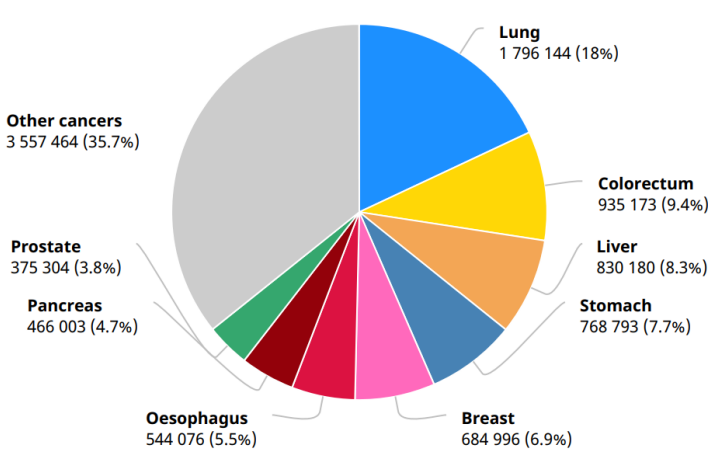
- Third most incident cancer
- Fourth leading cause of dead worldwide

Number of new cases in 2020, both sexes, all ages



Total: 19 292 789 cases

Number of deaths in 2020, both sexes, all ages



Total: 9 958 133 deaths

Source: Globocan 2020

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Introduction

Current CRC therapies

Surgery	Radiotherapy	Chemotherapy/ Immunotherapy

Therapy does not act selectively against tumor cells: **toxicity**

NANOTECHNOLOGY

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Introduction

Current CRC therapies

Surgery	Radiotherapy	**Chemotherapy / Immunotherapy**

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NANOTECHNOLOGY

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Combination therapy

Anti-PD-L1: Atezolizumab

- Humanized IG1 anti-PD-L1 monoclonal antibody

+

Irinotecan

- Chemotherapeutic drug

Few clinical studies evaluating the combination of irinotecan with PD-L1/PD-1 inhibitors

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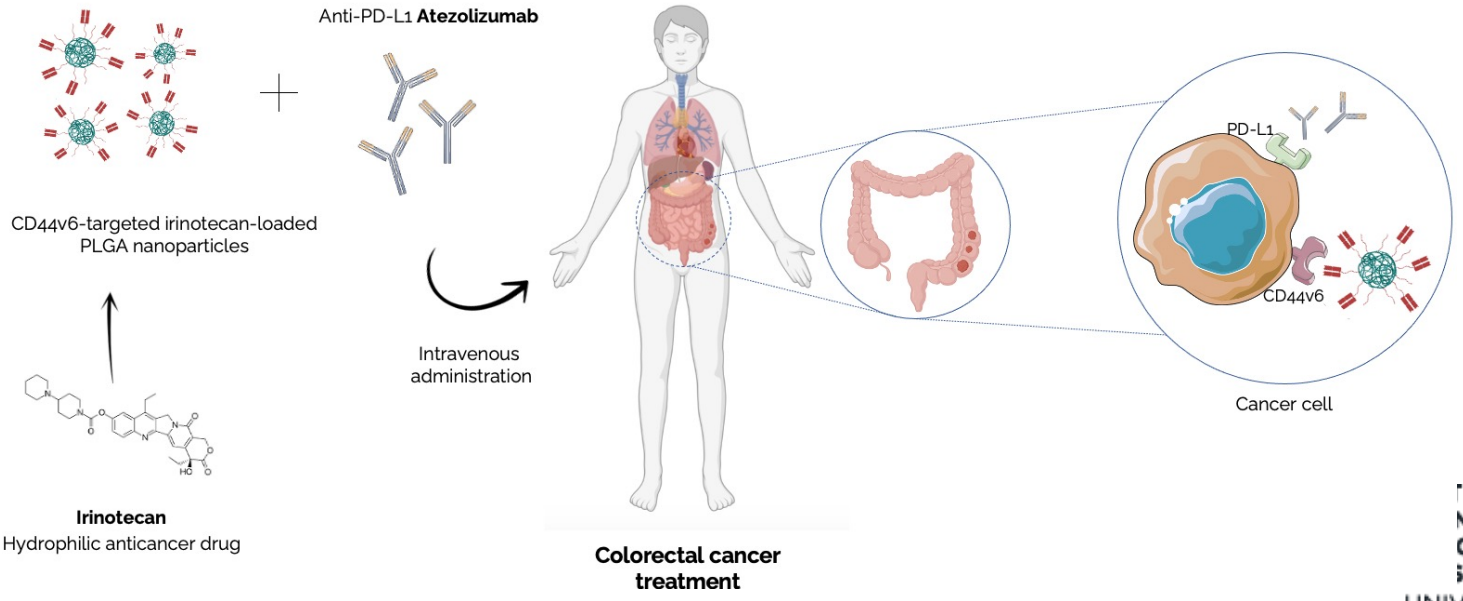
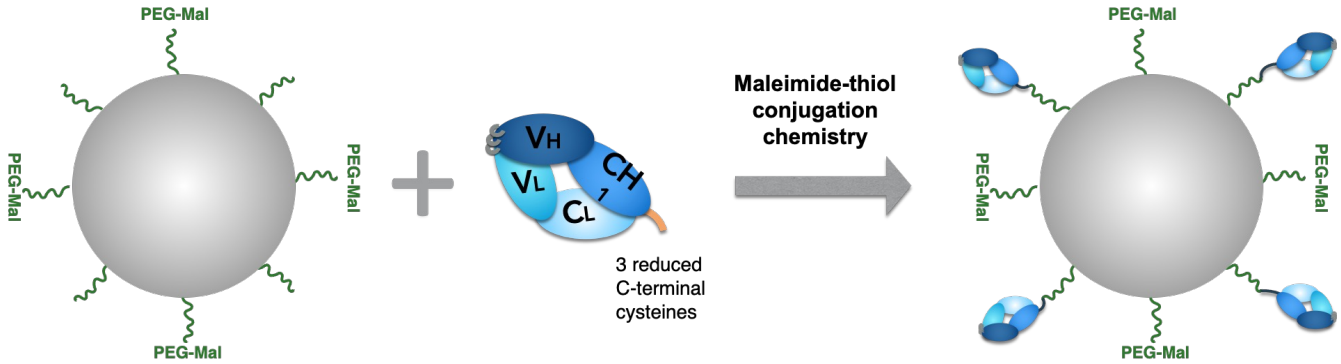
Aim of the project

- Combining chemotherapy and immunotherapy (PD-1/PD-L1 blockade) may have a **strong synergistic effect**
- **Active targeting to CD44v6:** membrane adhesion molecule overexpressed in CRC

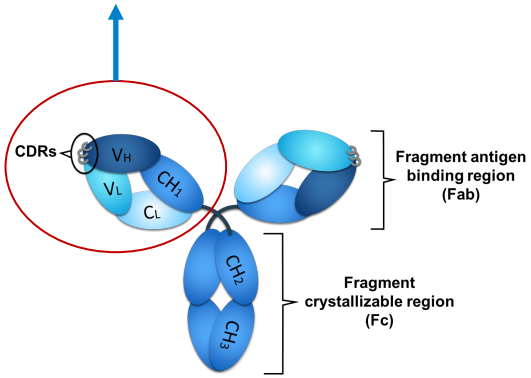


Methods

Polymeric targeted nanosystem



Double emulsion evaporation technique



- ✓ Biodegradable
- ✓ Biocompatible
- ✓ Safe

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Results

Physicochemical characteristics

Formulation	Z-average (size, nm)	Polydispersity Index (PdI)	Zeta Potential (charge, mV)
Bare PLGA-PEG NPs	124.1 ± 0.1	0.098 ± 0.015	-4.5 ± 0.2
	183.5 ± 4.9	0.388 ± 0.044	-6.4 ± 1.1
(-) Fab-PLGA-PEG NPs	167.2 ± 2.5	0.235 ± 0.005	-6.1 ± 0.8
	253.5 ± 1.4	0.353 ± 0.003	-9.8 ± 0.1
v6 Fab-PLGA-PEG NPs	245.4 ± 2.9	0.186 ± 0.013	-8.2 ± 0.5
	345.8 ± 16.4	0.382 ± 0.072	-12.0 ± 0.9

v6 Fab conjugation efficiency (by direct ELISA)
= 86.08 ± 5.53 %

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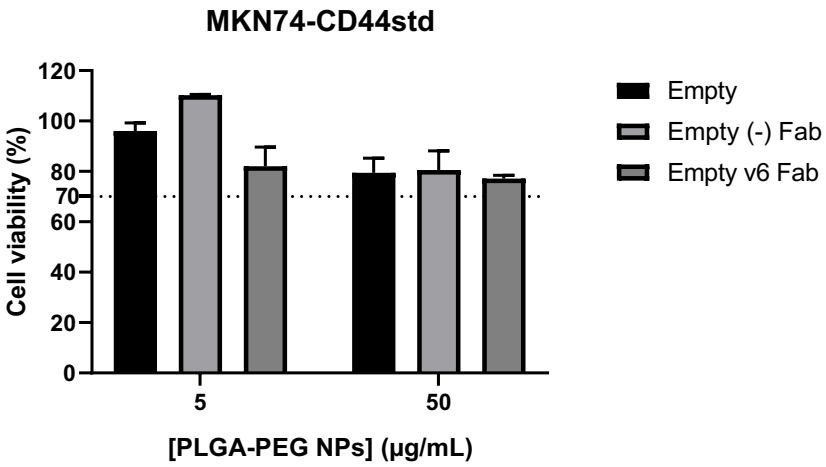
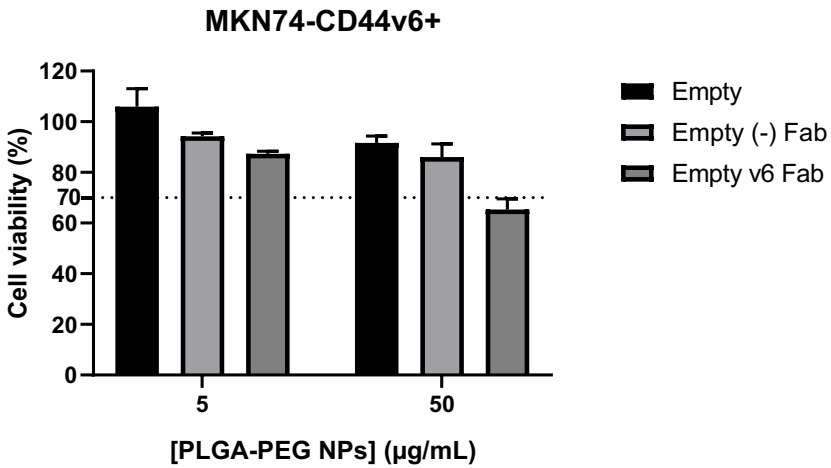
Results
Cytotoxicity

MTT reduction assay

24h

Empty PLGA-PEG nanoparticles





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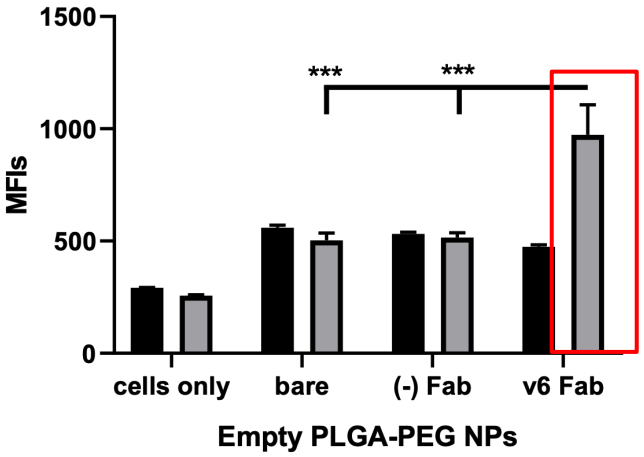
Results

Selectivity of v6 Fab to CD44v6

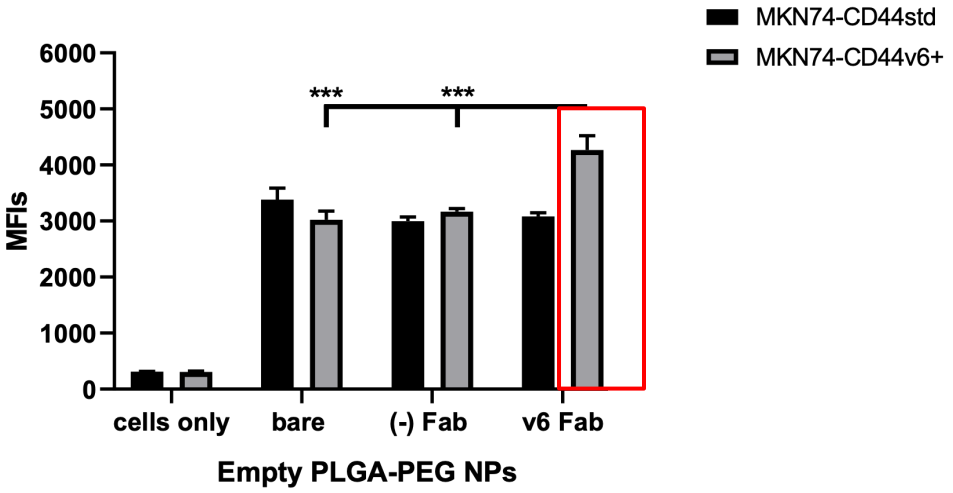
Flow cytometry

Nanoparticles at 50µg/mL for 24 h

(A) Surface binding



(B) Cell uptake



*** $p < 0.001$

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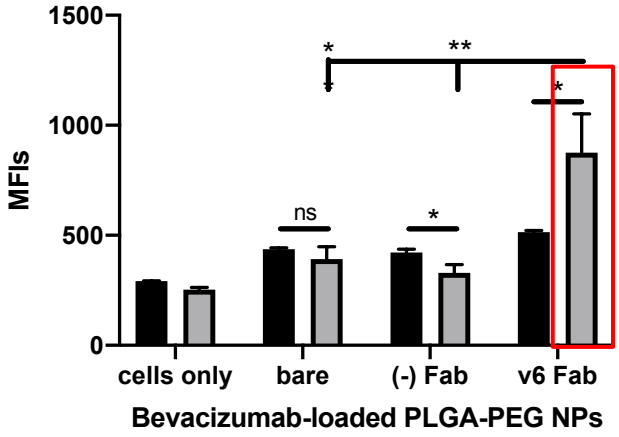
Results

Anti-cancer drug delivery

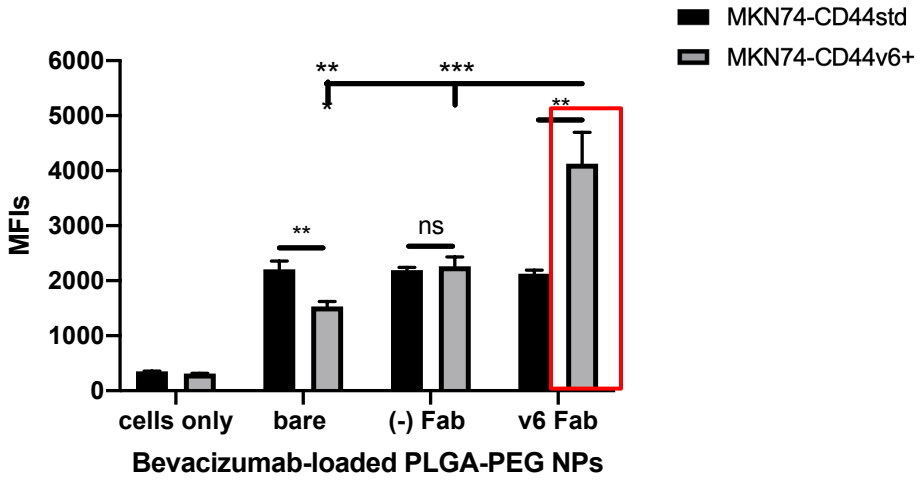
Flow cytometry

Bevacizumab-loaded nanoparticles at 50µg/mL for 24 h

(A) Surface binding



(B) Cell uptake



** p < 0.01; *** p < 0.001

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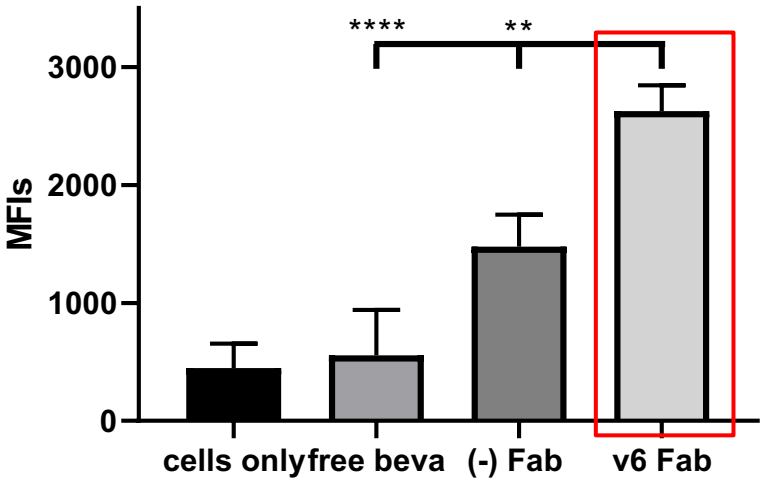
Results

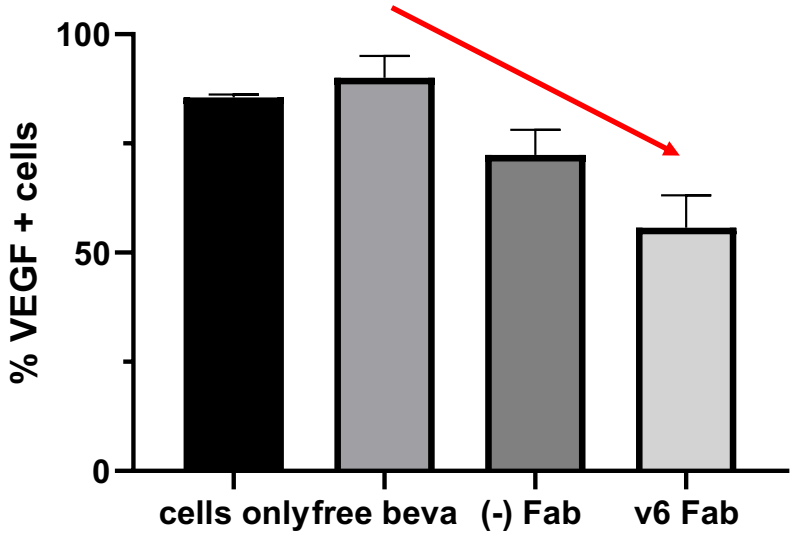
Anti-cancer drug delivery

Flow cytometry

50µg of bevacizumab-loaded nanoparticles for 24 h
MKN74-CD44v6+ cell line



** p < 0.01; **** p < 0.0001



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Conclusions

- v6 Fab functionalized PLGA-PEG NPs demonstrated adequate physical and technological characteristics
- Efficacy of the cell uptake was higher with NPs targeted to CD44v6 expressing cancer cells
- Intracellular delivery of bevacizumab was efficiently achieved with NPs

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Future work

Encapsulate the **chemotherapeutic drug** irinotecan in PLGA-PEG NPs

Combination with **immunotherapy**

Evaluate ***in vitro* anticancer efficacy** in CRC cell models

Expertise of Host Institution on nanoimmunotherapeutic development and their evaluation using primary cells and animal models

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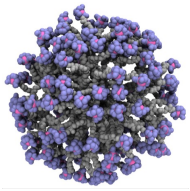

COST ACTION CA 17140
NANO2CLINIC
CANCER NANOMEDICINE - FROM THE BENCH TO THE BEDSIDE

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