# Nanoconjugate for selective antimetastatic effect in colorectal cancer treatment

NANOLIGENT

UAB Universitat Autònoma de Barcelona

Institut sant pau Investigació Biomèdica | Barcelona

**Madrid, 29 de octubre de 2019**

# The Institutions

## VILLAVERDE CORRALES, ANTONIO - CB06/01/0014

ciber-bbn isciii

- Use of microbial and non-microbial platforms for the production of new generation **protein-based drugs**
- Development of novel protein **nanostructured materials** for cell targeted drug delivery
- Improvement of biofabrication processes

## MANGUES BAFALLUY, RAMON - CB06/01/1031

ciber-bbn isciii

- Development of **disseminated models** of human solid tumors and hematological neoplasias
- Preclinical development of fusion-protein nanoparticles for receptor-mediated **targeted drug delivery** (small drugs and toxins)
- Development of biomarkers for **personalized therapy** in oncology

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MEDICAMENTOS INNOVADORES Plataforma Tecnológica Española

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# XVIII Encuentro de Cooperación Farma-Biotech

NANOLIGENT

Headquarters: UAB Research Park

## Manuel Rodríguez Mariscal

PhD MBA

Experience in biotech investing and pharma companies:

InnoUp Farma

INVEREADY Technology Investment Group

NOVARTIS

Almirall

## Antonio Villaverde Corrales

PhD

University Professor

Coordinator of Nanobiotechnology group

270 publications

UAB Universitat Autònoma de Barcelona

## Esther Vázquez Gómez

PhD

Nanobiotechnology Researcher

120 publications

UAB Universitat Autònoma de Barcelona

## Ramon Mangues Bafalluy

PhD

Coordinator of the Oncogenesis and Antitumor Drug group

100 publications

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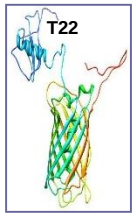
# Product

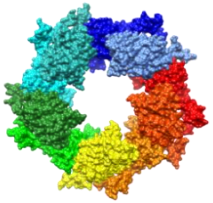
## **NL02: Nanoconjugate** (drug-protein nanoparticle)

**Protein nanoparticle targeting the CXCR4 receptor loaded with a highly potent drug. Three components:**

**Human Carrier Fusion Protein**




**Monomer** (6nm)



**self-assembling nanoparticle** (12 nm)

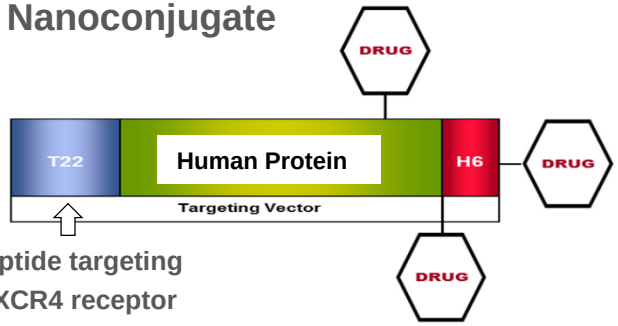
**Payload Drug**

DRUG

**Floxuridine**

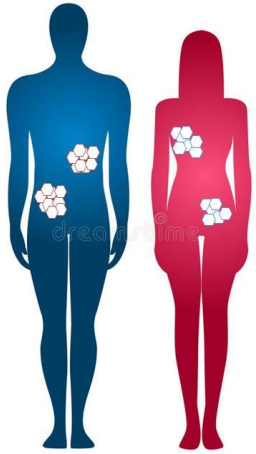
**Covalent Conjugation**

**Nanoconjugate**




**Peptide targeting CXCR4 receptor**

**Classical Drugs**
- **Lack of Selectivity**
- **High Toxicity**

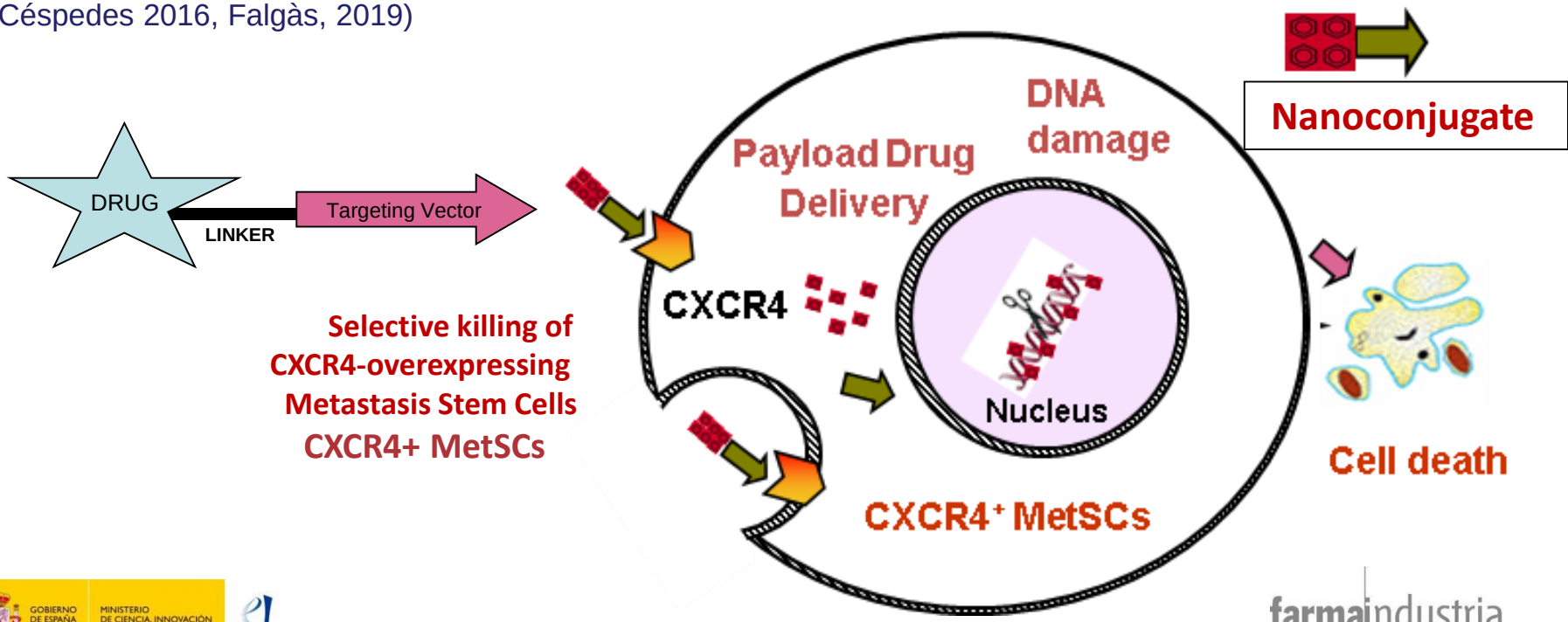


**NL02: Nanoconjugate**
- **Selective Internalization and drug delivery in Target Cells leading to their Selective Killing**
- **Higher Anticancer Potency**
- **Lack of Toxicity (Wide Therapeutic Index)**

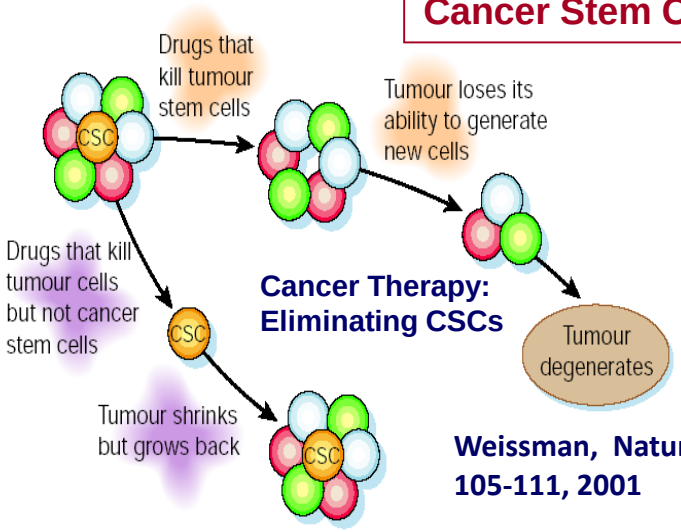
# Innovative mechanisms of action

## DISRUPTIVE TECHNOLOGY: Targeted Drug Delivey through the CXCR4 Receptor

- **CXCR4 chemokine receptor plays a role in embryonic development and homing of HSCs** (Blakwill, 2004)
- **This technology exploits 20-200 higher CXCR4 expression in Target Cancer Cells than in Normal Cells** (Kim 2005, 2006, Nimmagadda 2009)
- **High Antimetastatic Potency of the Nanocojugate by selective elimination of CXCR4+ MetSCs, leading to Metastasis Prevention and induction of Metastases Regression**
- **85 % of NL02 Injected Dose reaches the tumor in animal models of solid and hematological neoplasias** (Céspedes 2016, Falgàs, 2019)



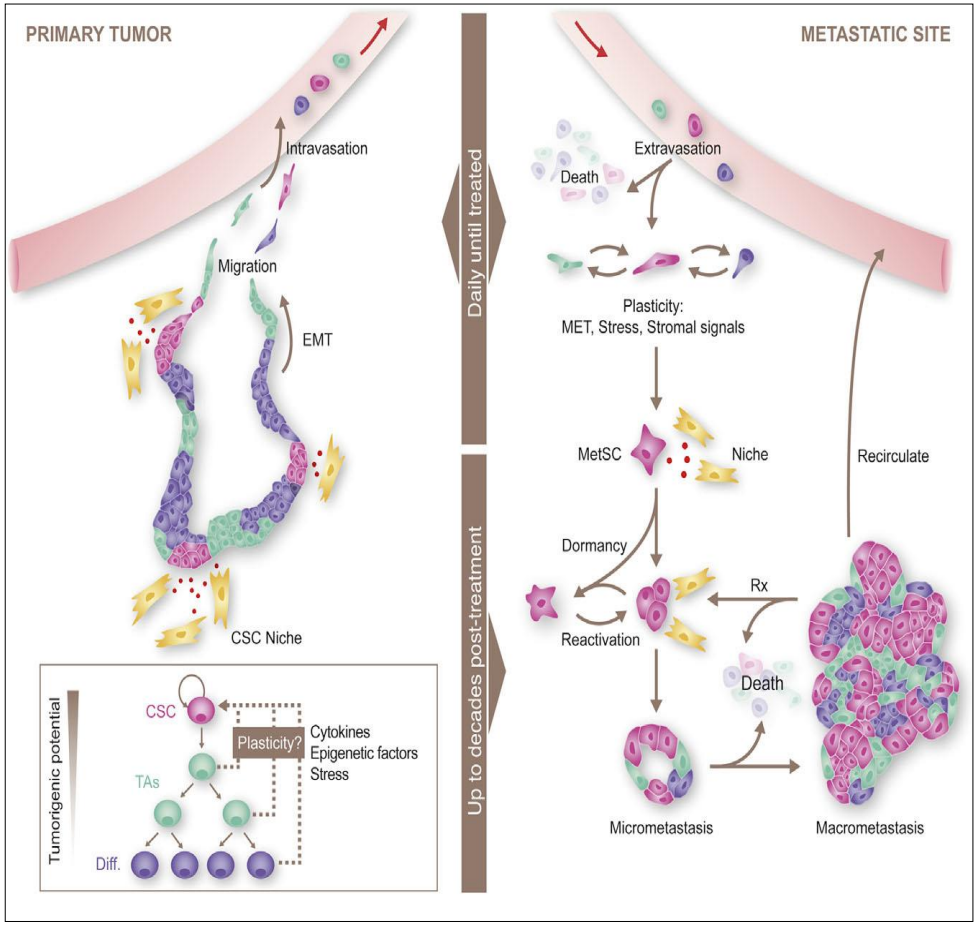
## Cancer Stem Cells




Weissman, Nature 414, 105-111, 2001

## Target Cells: MetsSCs

**Cancer therapy through elimination of MetSCs**




Oskarsson T et al. Cell Stem Cell 2014 Mar 6;14(3):306-21.

## Clinical rationale:

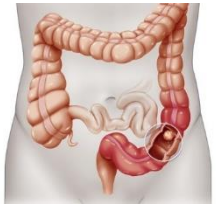
* CXCR4+ cancer cells are Metastasis initiating cells In CRC models (Croker 2008, Zhang 2012)

* CXCR4+ cancer cells associate with metastasis and short survival in CRC patients (Kim 2005, 2006)

# Target Indications

**Main target indication:**

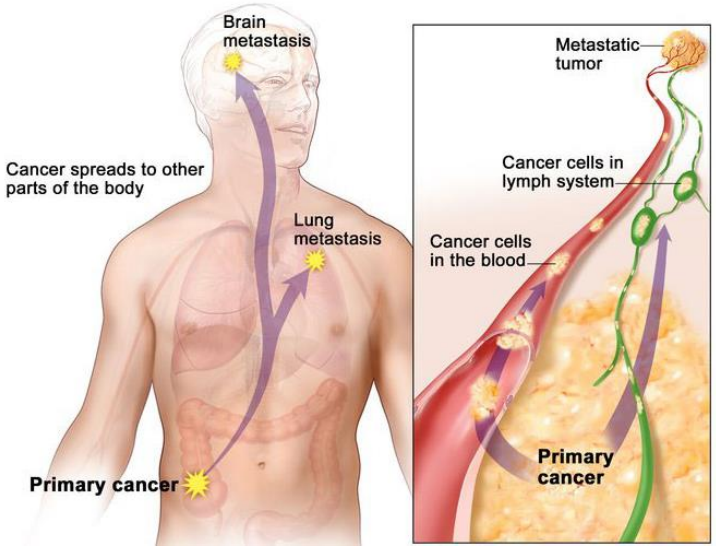
**Colorectal Cancer (CRC) Metastasis Control**



## CXCR4+ MetSCs Elimination

* 3rd most common cancer worldwide
* 20% of patients have metastasis at diagnosis

| $ 8.3 bn | → | $ 9,4 bn |
|---|---|---|
| 2016 | | 2020 |




## Potential development in other Indications

**At least, 23 diferent neoplasias are candidate to nanoconjugate therapy by selective elimination of CXCR4+ MetSCs since their metastasis are mediated by CXCR4**

## CANCER

**Every Year 14 million new cancer diagnoses, worldwide**
**Every Year 8 million deaths**
**Metastasis responsible for 90% cancer of deaths in solid tumors**
**Large Pharmaceutical Market**

# Differential features facing the market

## The NL02 Nanoconjugate is the First Antimetastatic Drug

**Unmet Medical Need:** Metastasis control (90% patients die of metastases (Goldberg 1998)).

**Current Drugs** do not selectively target nor prevent metastases because preclinical drug development is still centered in **primary tumor control** (Steeg 2008).

**CXCR4 antagonists** (peptides, low MW drugs, mAbs): **Mobilization of quiescent CXCR4+ MetScs** by CXCR4 antagonists (hyper-leukocytosis), used in combination with chemotherapy. Some Trials with mAbs (e.g. Ulocuplumab) terminated. They likely have lower selectivity and higher toxicity than cancer cell-targeted drugs (**limited by PK**).

**Antibody drug conjugates (ADCs):** No ADC available targeting the CXCR4 receptor
Lower selectivity for targeting receptor-expressing cancer cells: Only **0.1-1% ID reaches the tumor**
Dose-limiting toxicities in normals organs

**Targeted Nanoparticles** in clinical Trials (None in the market)
Their non-proteic nature limits their targeting capacity because of "**protein corona" formation** in blood (Salvati 2013, Corbo 2015). Protein-based nanoconjugates do not have this problem

**Our technology is Disruptive:** Its modular structure and versatility allows for the incorporation of new domains and functions to address novel PK/PD needs.

# CXCR4 is a target for cancer research

- Cancer treatment based on CXCR4 antagonists is a focus area for Big Pharma.
- Most products are used for hematological tumors, and a few products in solid tumors
- Some products induce apoptosis on cancer cells that control growth of subcutaneous tumors but some of them have a low efficacy in patients even in combination with chemotherapy (e.g. Ulocuplumab).
- None of the products have been tested in colorectal cancer.

- **Nanoligent nanomedicine** has a potent direct antimetastic effect by selective elimination of CXCR4+ MetSCs because of high uptake and drug delivery in target cancer cells and low toxicity to normal cells

| Product | Type | Company | Phase |
|---|---|---|---|
| AMD3100 Plerixafor (Mozobil) | Small molecule | Genzyme/Sanofi | Launched |
| Ulocuplumab | MAb | Bristol-Myers Squibb | II |
| BL-8040 | Peptide | BioLineR: MSD & Genetech | II |
| LY-2510924 | Cyclic peptide | Lilly | II |
| USL311 | Small molecule | Pusher-Smith | II |
| PF-06747143 | MAb | Pfizer | I |
| POL6326 Balixafortide | Peptide | Polyphor | I |
| GMI-1359 | Small molecule | Glycomimetics | I |
| X4P-001 | Small molecule | X4 Pharmaceuticals | I |
| | | | |

# mCRC therapy

- **Cytotoxic agents** (5-fluorouracil, capecitabine, irinotecan and oxaliplatin): **FOLFOX, FOLFIRI, First line therapy**

- **mAbs/fusion proteins anti-VEGF** (bevacizumab, ramucirumab, ziv-aflibercept) **(Ras mut), anti-EGFR** (cetuximab & panitumumab) **(Ras wt), Fisrt line therapy**

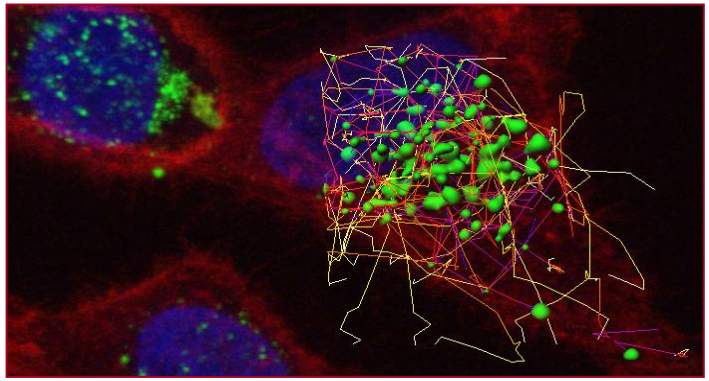
- **Immune checkpoint inhibitors** (anti-PD-1, nivolumab, pembrolizumab), **anti-PD-L1, Atezolizumab, Durvalumab** (anti-PD-L1), **anti-CTLA-4,** ipilimumab) **in h-MSI, Ras mut,** Vemurafenif **in Braf V600E, Second line therapy**

- **Small molecule multikinase inhibitor, Regorafenib,** (mCRC previously treated with chemotherapy, an anti-VEGF therapy and, if Ras wt, an anti-EGFR therapy) **or trifluridine-tipiracil**, **Third line therapy**

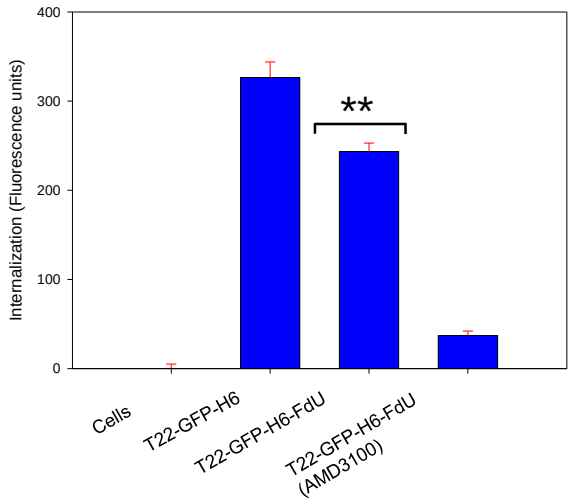
- **Four ADCs in Phase I/II trials with different target receptors and payloads**

- **K-ras mutated and microsatelite-stable mCRC, no available treatment**

# *in vitro* Results



Confocal Microscopy

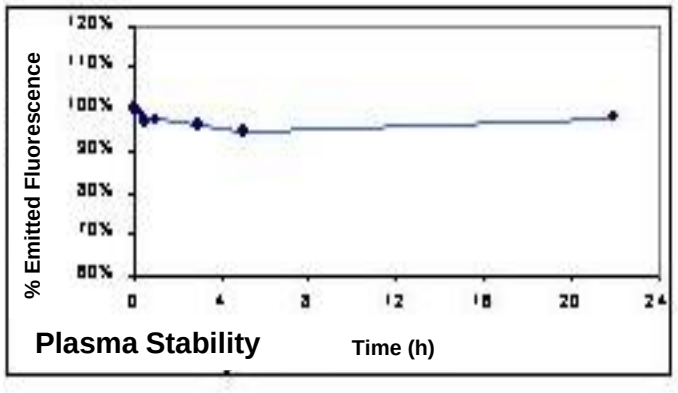



**Internalization in CXCR4+ cancer cells**

**In vitro CXCR4+ SW1417 cell line**

**Competition with ADM3100 (antagonist)**

Unzueta U et al. Int J Nanomedicine. 2012;7:4533-44

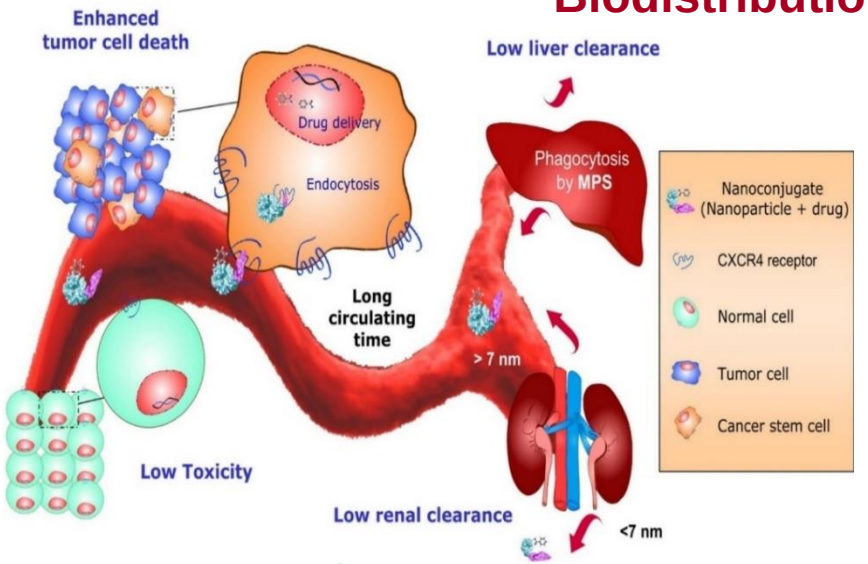



**Nanoconjugate achieves selective internalization in CXCR4+ colorectal cancer cells in vitro**

**Nanoconjugate is stable in plasma for at least 48h**

# *in vivo* Results

## T22-GFP-H6 targeting vector: Selective Tumor Biodistribution *in vivo*




Unzueta U, Céspedes MV, et al. Trends Biotechnol, 2015

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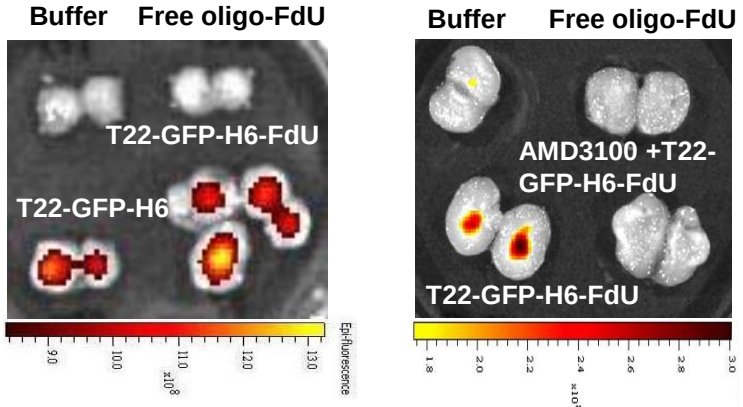
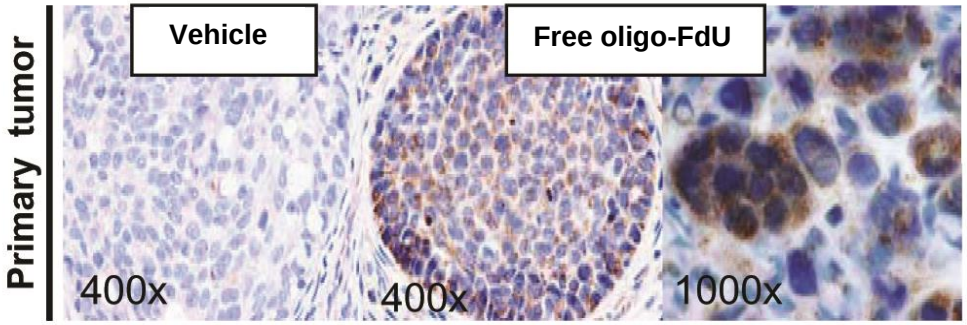


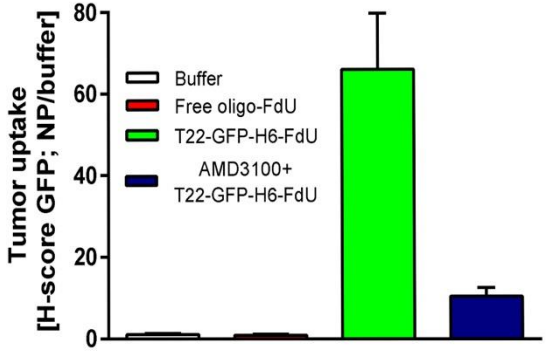

Céspdes MV et al. Nanomedicine. 2016 Oct;12(7):1987-1996

## Internalization in CXCR4+ cancer cells in vivo

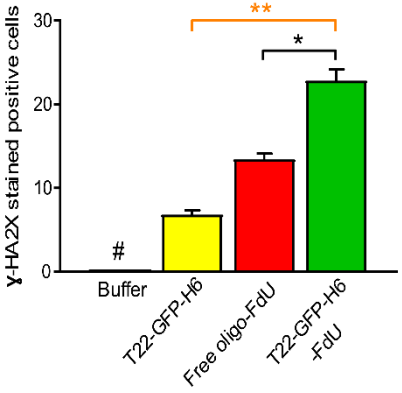



Unzueta U, Céspedes MV et al. Int J Nanomedicine, 2012

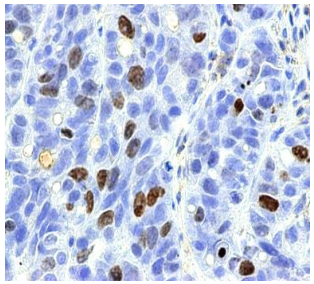
## In vivo CXCR4+ SW1417 subcutaneous colorectal cancer mice model



# Mechanism: Selective depletion of CXCR4+ Tumor Cells in CRC



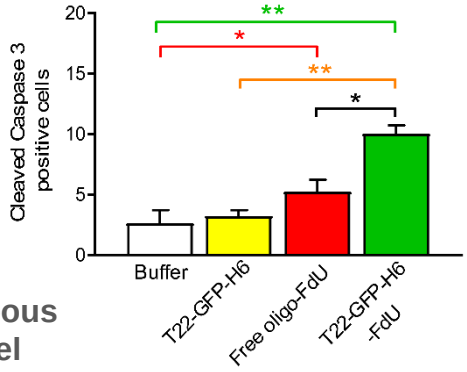

γ-H2AX



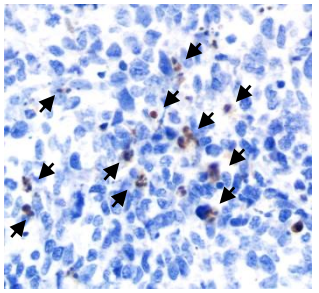
Increase DNA DSBs

CXCR4+ SW1417 subcutaneous colorectal cancer mice model

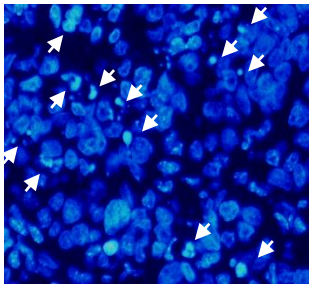
Increased apoptotic signaling



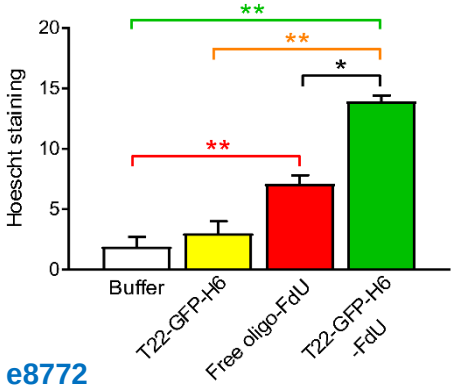

Cleaved Caspase 3



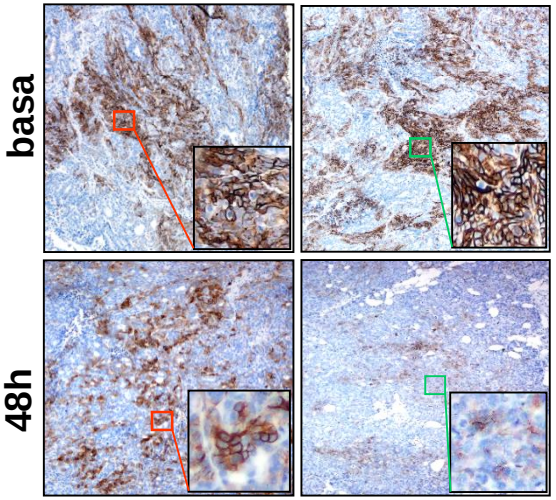
Hoescht

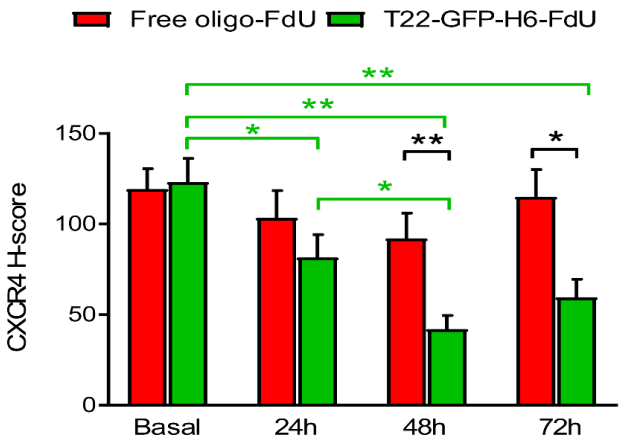


Increased Apoptotic Body number



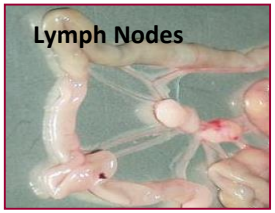

Free oligo- FdU T22-GFP-H6-FdU



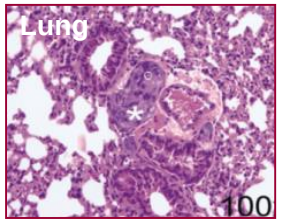




Céspedes MV et al. EMBO Mol Med. 2018 Oct;10(10). pii: e8772

## Metastatic Colorectal Cancer Model

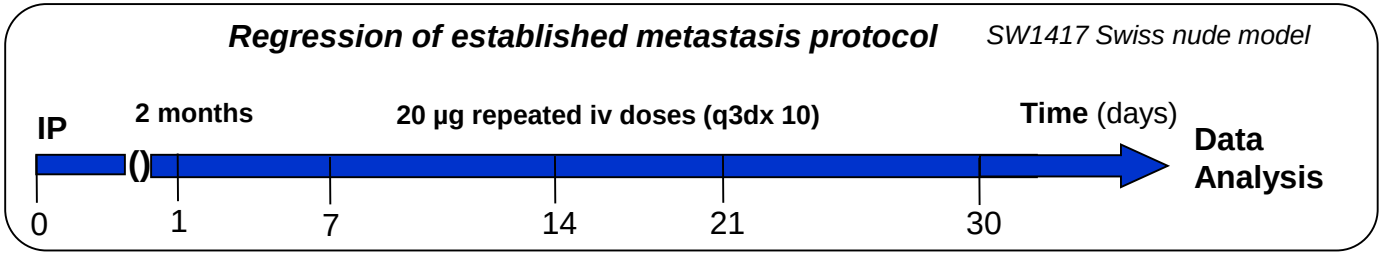


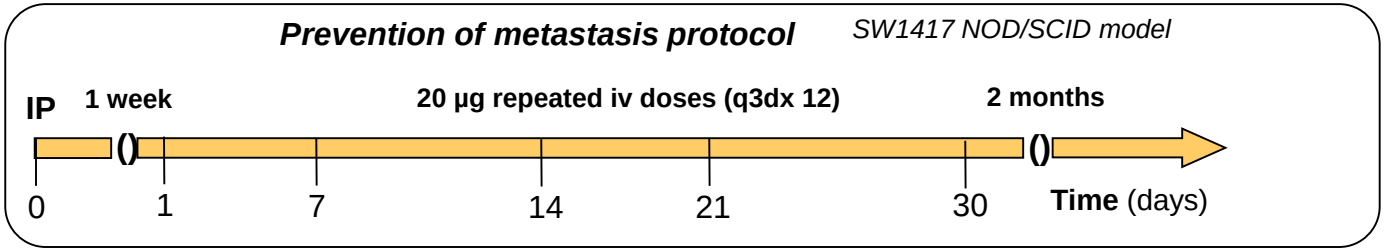






## Nanoconjugate dosage schedule

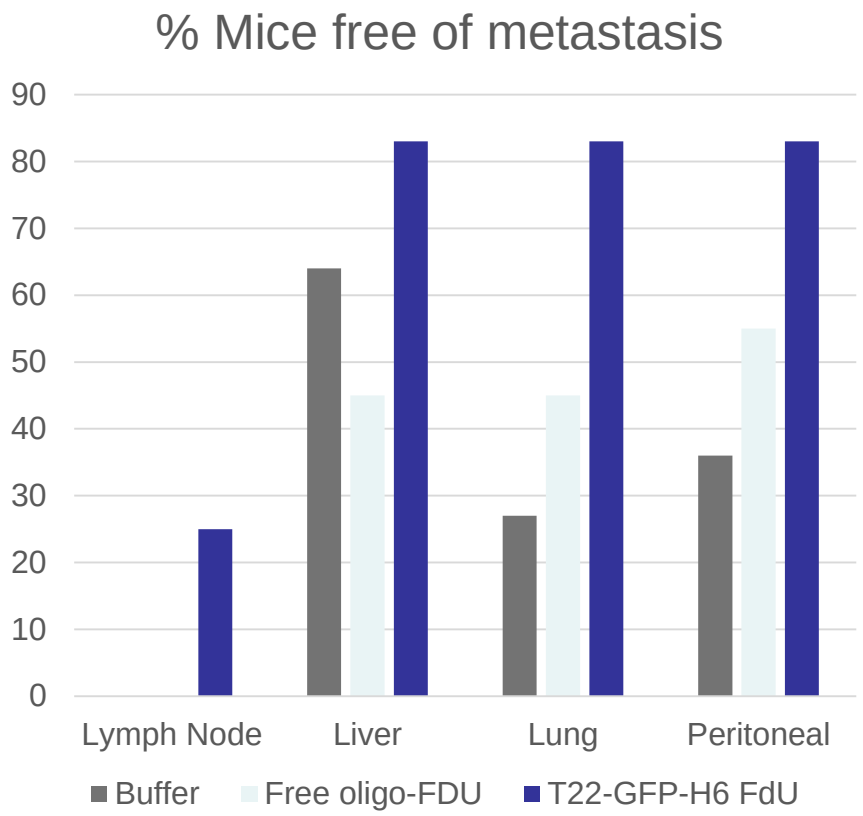



Céspedes MV et al. EMBO Mol Med. 2018 Oct;10(10). pii: e8772

# Prevention of metastasis

## More than 80% of animals free of lung, liver and peritoneal metastasis




**SW1417 cell line-derived orthotopic model**

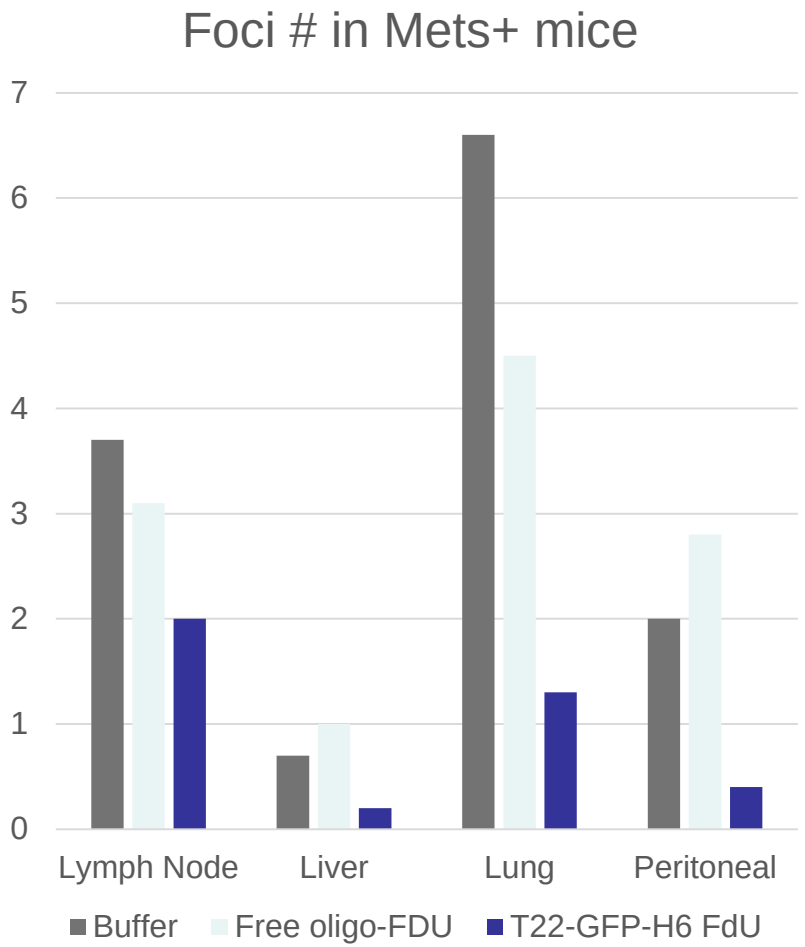
**Starting therapy 1 week after CRC cell intracecal implantation.**

**Dosage: 20mg 3qd x 12 doses.**

Céspedes MV et al. EMBO Mol Med. 2018 Oct;10(10). pii: e8772

# Prevention of metastasis

## Reduction foci number in mice with metastasis

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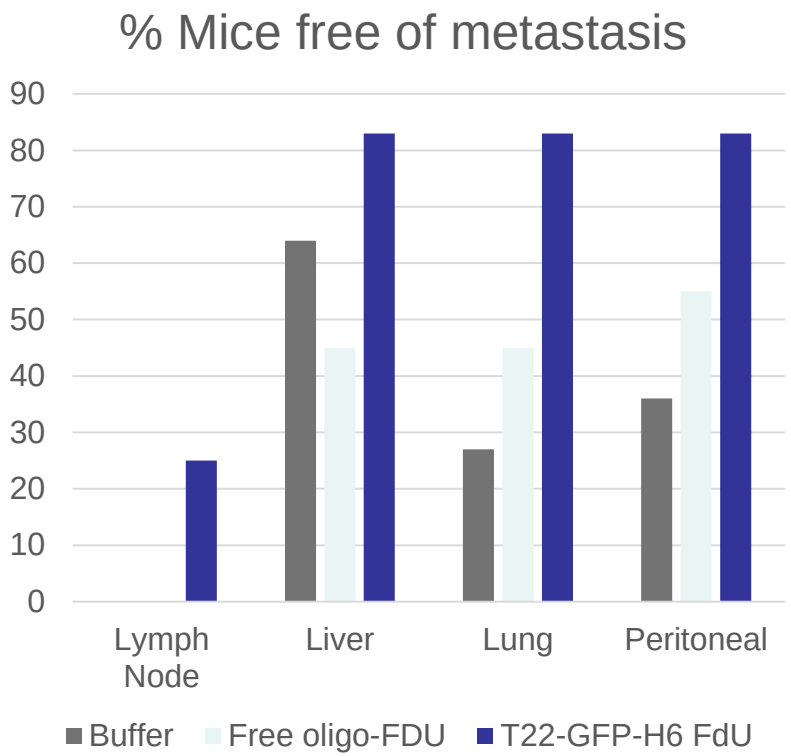
# Prevention of metastasis

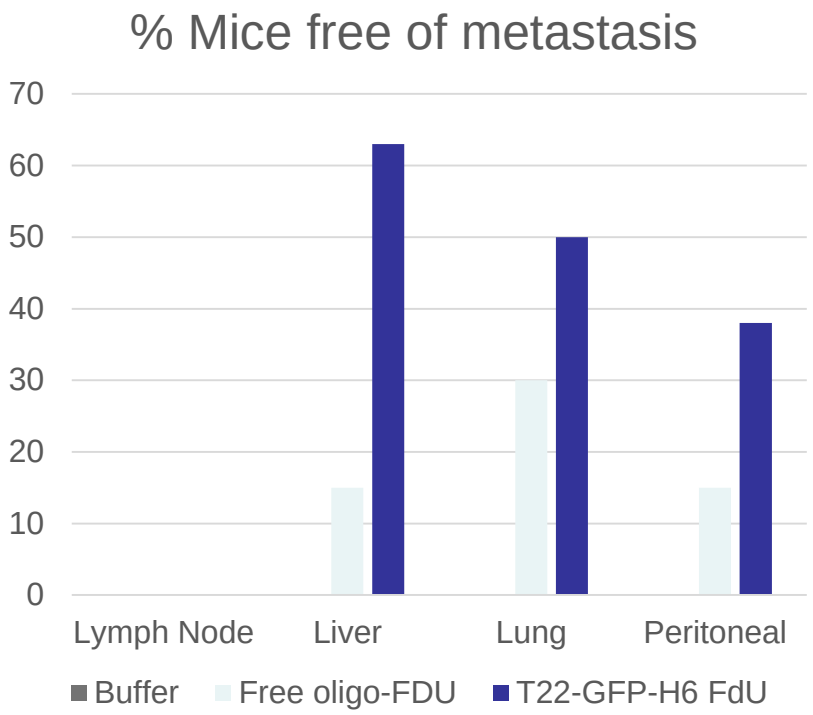
## NL02 prevents hematogenous and transcelomic metastasis

SW1417 cell line-derived orthotopic model




Starting therapy 1 week after CRC cell intracecal implantation.
Dosage: 3 days dose interval, 12 doses.

M5 patient-derived orthotopic model

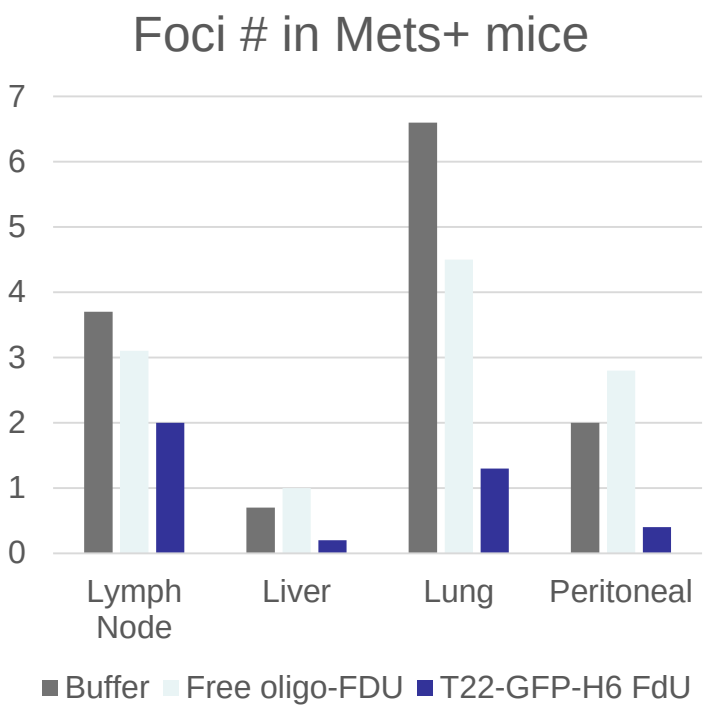



Starting therapy 1 week after CRC cell intracecal implantation.
Dosage: 3 days dose interval, 7 doses.

# Prevention of metastasis

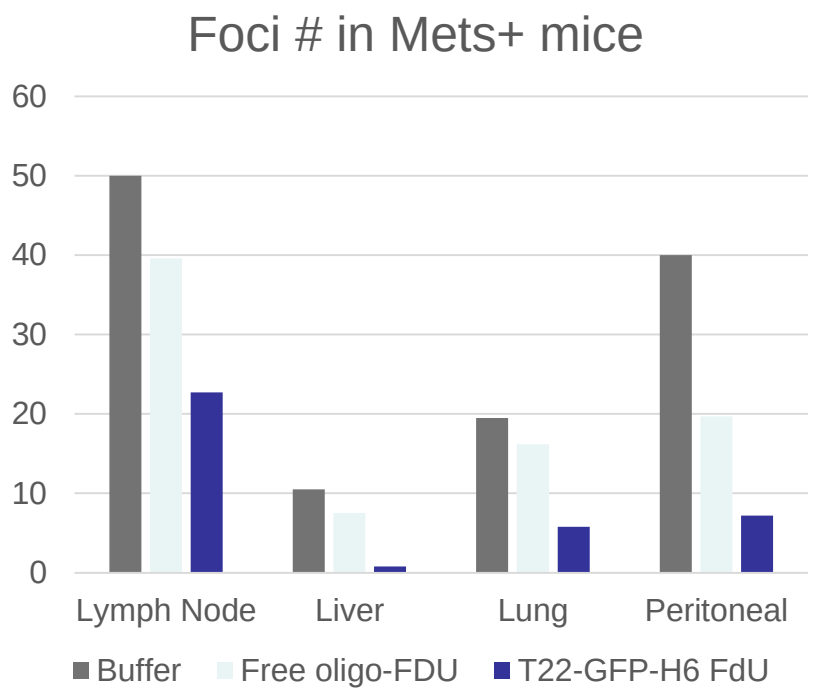
## Nanoconjugate reduction of foci number in mice bearing metastases

SW1417 cell line-derived orthotopic model




**Starting therapy 1 week after CRC cell intracecal implantation.**
**Dosage: 3 days dose interval, 12 doses.**

M5 patient-derived orthotopic model

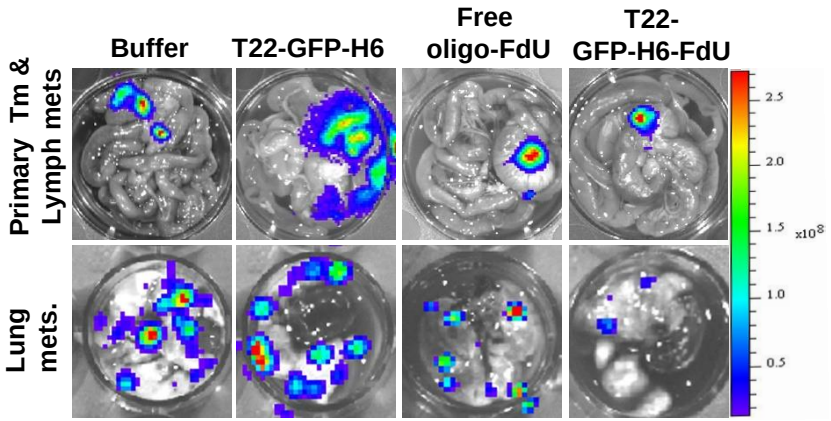



**Starting therapy 1 week after CRC cell intracecal implantation.**
**Dosage: 3 days dose interval, 7 doses.**

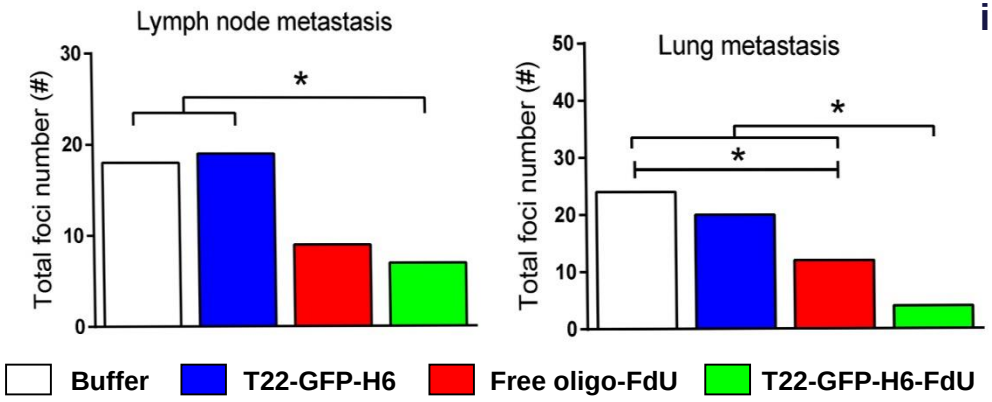
# Regression of established metastasis

**Regression of the number of established metastasis foci**

A




B



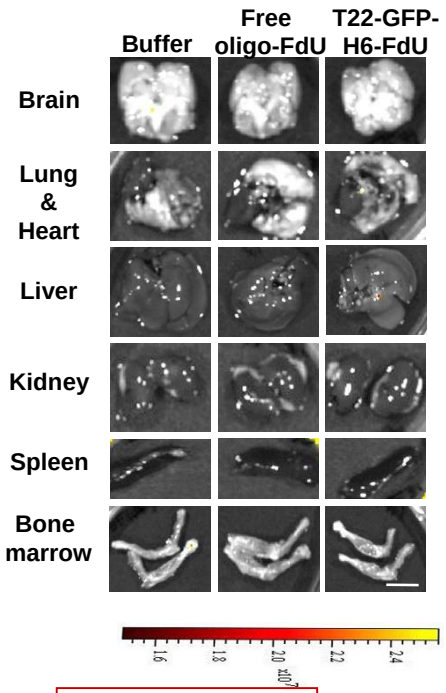

**Orthotopic bioluminescent CXCR4+ SW1417 colorectal cancer mice model.**

**Starting therapy 2 months after CRC cell implantation.**

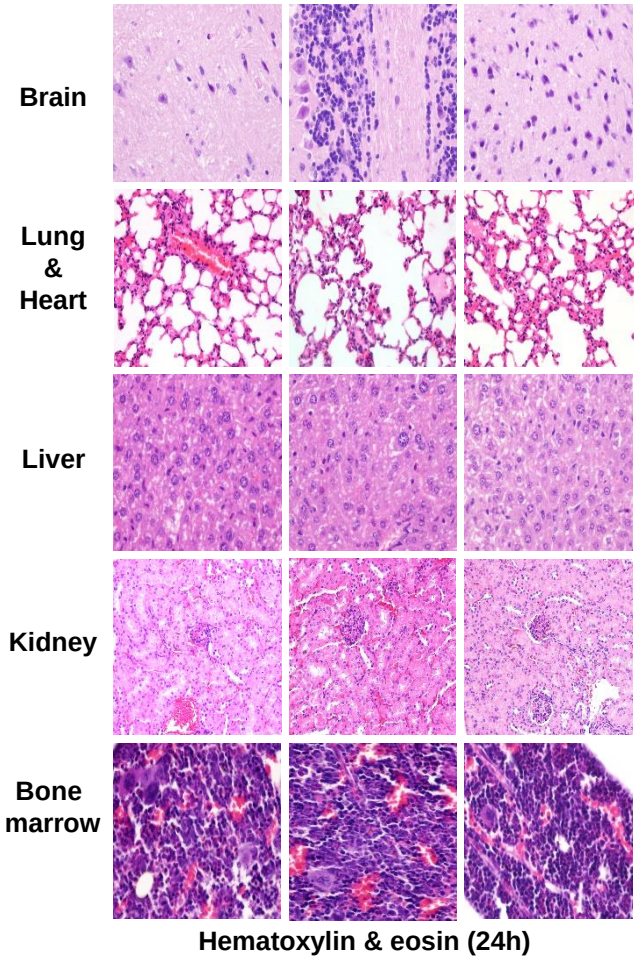
**Dosage: 3 days dose interval, 10 doses.**

Céspedes MV et al. EMBO Mol Med. 2018 Oct;10(10). pii: e8772

## Low toxicity & Improved therapeutic Index

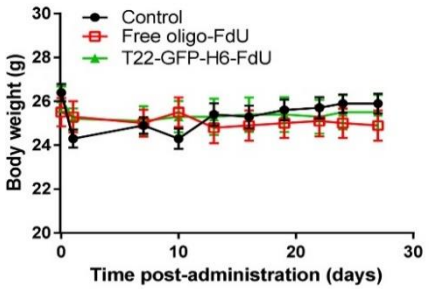


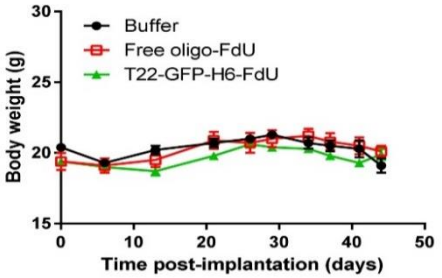

**Negligible Distribution to non-Tumor Tissues**

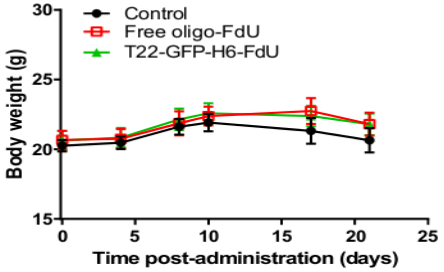



Hematoxylin & eosin (24h)

### Evolution of mouse body weight




- Undetectable accumulation in circulating blood cells
- Undetectable uptake in normal organs (bone marrow, kidney …) after single dose
- No histological alterations in the tested groups in bone marrow, liver or kidney
- No body weight loss and no sign of histological or clinical toxicity

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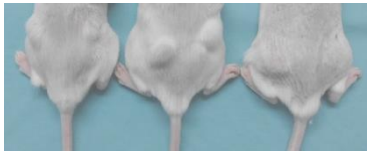
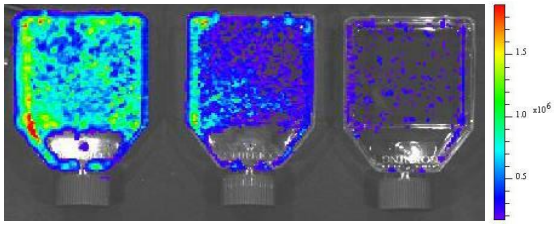
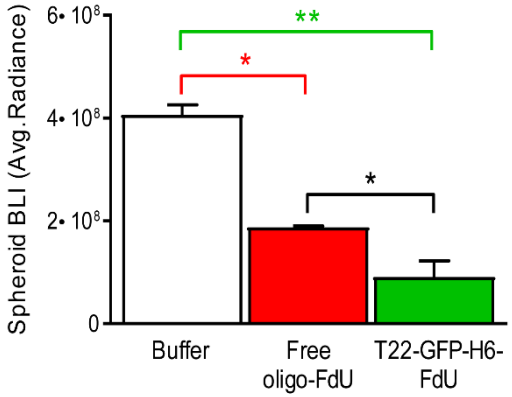
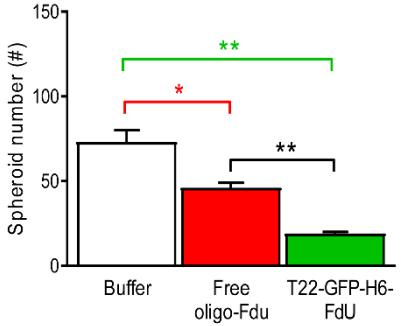
## Mechanism: Block of Tumor Re-Initiation (Elimination of Cancer Stem Cells)



| | Buffer | Free oligo-FdU | T22-GFP-H6-FdU |
|---|---|---|---|
| Positive tumor growth/ total injection points | 4/4 | 3/4 | 1/4 |
| Tumor volume ($mm^3$) | 401±98 | 209 ± 18 | 90 |




**T22-FdU-induced Reduction in Tumor Reinitiation Capacity**

**SW1417 spheroids**


Céspedes MV et al. EMBO Mol Med. 2018 Oct;10(10). pii: e8772

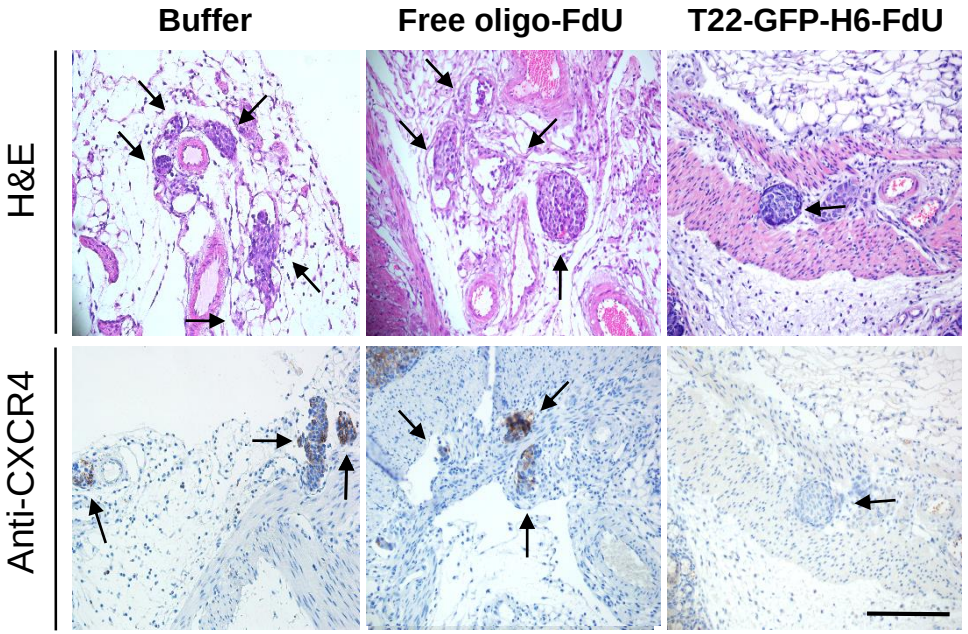
## Mechanism: Blocks CXCR4+ MetSCs trafficking functions

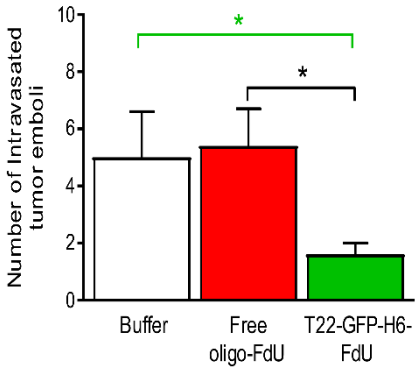
**Nanoconjugate block of tumor emboli intravasation in primary tumor peri-tumoral vessels**

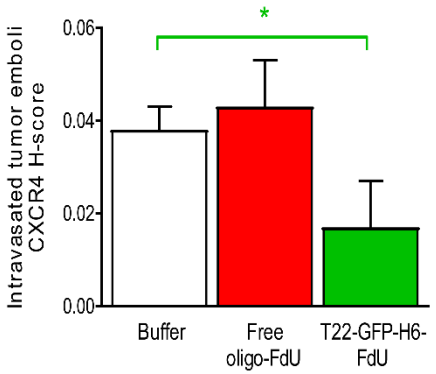
**M5 patient-derived orthotopic colorectal cancer mice model.**

**Starting therapy 7 days after CRC cell implantation.**

**Dosage: 2 doses, consecutive days.**




Céspedes MV et al. EMBO Mol Med. 2018 Oct;10(10). pii: e8772

# Current status of development

**ACCOMPLISHED:**

- Development of a humanized nanoconjugate
- Demonstration of high selectivity in tumor biodistribution (85%) of injected dose and selective uptake in CXCR4+ cells
- Optimization of nanoconjugate dosage schedule 20ug q3d 12 doses for repeated i.v. dose administration
- Demonstration of antitumor effect in subcutaneous CRC models after repeated dose administration
- Demonstration of potent antimetastatic effect in cell-line and patient-derived CRC models, both prevention of metastases and regression of established metastases
- Low distribution to normal tissues and absence of toxicity in all described therapeutic experiments

**ONGOING:**

- Scale-up production of the Humanized Nanoconjugate
- Preclinical regulatory toxicology and toxicokinetic studies in two species

# Current status of development

## Public funds obtained: 1.3 mil €

**NEOTEC:**

- One year project
- Total project: 382.780 €
- **Grant: 212.500 €**
- Financial need: 170.280

**RETOS 2017:**

- Three year project
- Total project: 1.323.320 €
- **Grant: 425.460** €
- **Loan: 583.609**
- Financial Need: 314.251 €
- **TOTAL**: **1.003.069** €

**TORRES QUEVEDO:**

- Three year project
- Total Project: 142.074
- **Grant: 99.451 €**
- Financial need: 42.623

**CIBER:**

- One year project
- Total project: 35.000 €
- **Grant: 17.500** €
- Financial Need: 17.500 €

# IPR Protection

The T22-based protein nanoconjugates and nanoparticles for targeted drug delivery are protected by three patents licensed to Nanoligent:

**METHODS AND REAGENTS FOR EFFICIENT AND TARGETED DELIVERY OF THERAPEUTIC MOLECULES TO CXCR4 CELLS**
Pariority Patent: EP20110382005, Priority Date: 13.01.2011
Active in USA US9580468B2 (Approved); USA US2017209590A1 (pending divisional Patent)
EP2663336B1 (approved patent): Validated in Netherlands, Germany, France, Luxemburg, Spai, Portugal, Sweden, Irland, Denmark, Belgium, Switzerland, Austria, Italy, Poland, United Kingdom
Israel Application Number 227442 (Submitted patent)
Australia AU2012206533B2 (approved patent)

**NANOSTRUCTURED PROTEINS AND USES THEREOF**
Priority Patent: EP17169722.0, Priority Date: 5-05-2017
Aplication No./Patent No.: EP17169722.0 - 1401; Date of filing: 05.05.2017
PCT/EP2018/061732, P14505PC00, 08.08.2018

**THERAPEUTIC NANOCONJUGATES AND USES THEREOF**
Priority Patnetg: EP17382461.6; Priority Date: 14.07.2017
P14353PC00, European Union PCT/EP2018/069303, 16 July 2018; WO2019012157

## Risks to be considered

- **Nanoconjugate Scale-up:** Partnership with companies that produce protein-drug conjugates currently in clinical assays or in the market (e.g. developers of ADCs)

- **Development of an immunogenic response:** We have already developed a humanized nanoconjugate that we expect to highly reduce immunogenicity. In any case, this effect would be detectable early in preclinical drug development

- **Translation of antimetastatic efficacy from results observed in animal models to human patients.** We do not expect translational problems because the nanoconjugate shows potent antimetastatic activity in patient-derived models. In addition, in clinical trials, we will only recruit patients with high expression of CXCR4 in tumor tissue ($Ga^{68}$-pentixafor imaging), with is likely to induce cancer responses, even in advanced cancer patients.

# Partnering Opportunities

- **We have obtained public funds to initiate preclinical regulatory studies (RETOS/MINECO).**
- **Additional private investment is needed to complete preclinical requirements tasks and the initiation of a Phase I trial in CXCR4+ CRC patients.**
- **We are willing to explore a collaboration with Pharma companies: co-development, licensing or partnership.**
- **There is also the opportunity to develop our technology in additional tumor types among the 23 cancers showing CXCR4-dependent metastatization. In fact, we have a new nanoconjugate candidate for the treatment of AML with a potent anticancer effect**

# Anti-metastatic drug for colorectal cancer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