XV Encuentro de Cooperación Farma-Biotech

Novel nanoconjugate for the targeted treatment of metastatic colorectal cancer

Universitat Autònoma de Barcelona

Institut santpau
Investigació Biomèdica | Barcelona

Madrid, 15 de noviembre de 2016

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

MEDICAMENTOS INNOVADORES
Plataforma Tecnológica Española

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The Institutions

VILLAVERDE CORRALES, ANTONIO - CB06/01/0014

- 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

- Development of disseminated models of human solid tumors and hematological neoplasias
- Preclinical development of nanoparticles for receptor-mediated targeted drug delivery (low MW drugs, toxins, siRNA)
- Development of biomarkers for personalized therapy in oncology

Nanomedicine Network: http://www.ciber-bbn.es/; Nanomedicine Platform: http://www.nanbiosis.es/

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Target Indications

First antimetastatic drug

* Metastasis control: Unmet medical need

* Metastases rather than primary tumors kill patients

* Colorectal cancer (CRC) patients have micro- or macro-metastasis at diagnosis (DeVita 2001), which are often responsible for recurrence after therapy, so that most patients die because of metastases (Goldberg 1998)

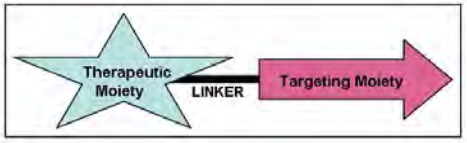
* Lack of drugs in the market that selectively target metastases. Preclinical drug development is centered in control of the primary tumor (Steeg 2008).

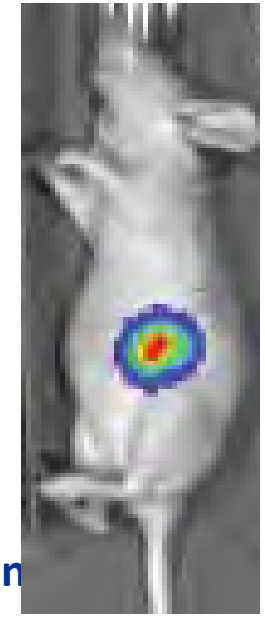
Large Pharmaceutical Market : Cancers with CXCR4-dependent metastasis)

* CXCR4+ cancer cells can be targeted in at least 20 different neoplasias
30-50% of cancer patients in these 20 neoplasias overexpress CXCR4 and are candidates for therapy

Innovative mechanisms of action

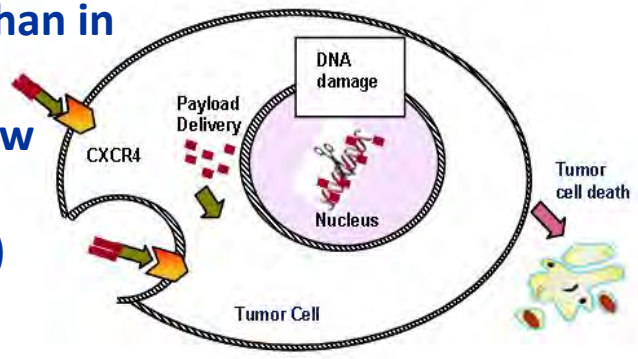
Targeted Drug Delivery





Disruptive Technology (Nanocojugate drug-protein nanoparticle

* Selective killing of tumor cells responsible for metastases
  (CXCR4 membrane receptor overexpressing metastasis stem cells)
* In human CRC, overexpression of CXCR4 in the membrane of tumour cells associates with metastasis, relapse and poor prognosis (Kim 2005).
* The subset of CXCR4 + cells show metastastic stem cell capacity (MetsSCs) in animal models (Croker 2008, Zhang 2012)
* Low toxicity on normal tissues
  CXCR4 receptor overexpression in tumors 5-15 fold higher than in normal tissues (Fischer 2008, Nimmagadda 2009), which increases selective drug delivery and the therapeutic window
* Modular and versatil to address novel needs
  (adaptable to new receptors, new drugs, new PK/PD needs)



Differential features facing the market

* Higher selectivity for targeting membrane receptor-overexpressing cancer cells than Antibody-Drug Conjugates ADCs being used in the clinic (e.g. Kadcyla, Adcetris, 50 more in clinical trials)

* Lower toxicity than ADCs because of low accumulation in normal tissues

* Antibody drug conjugates Biodistribution:
  - Only 0.1-1% of the injected dose biodistriubutes to tumor tissue (Sedlacek 1992)
  - About 80% of the injected dose biodistributes to liver (Lambert 2015)
  - Antibodies interact with the Fc Receptor which mediates their uptake in normal tissues

* Antibody-Drug Conjugates show off-target toxicities that limit dosage: rash, thrombocytopenia, neutropenia, GI toxicity, neuropathy, hand/food syndrome, lymphopenia, ocular toxicity, elevated liver enzymes, mucositis (Hinrichs 2015)

Current status of development

We have developed a prototype of a nanoparticle produced by recombinant protein engineering that we have conjugated to a genotoxic drug

After its intravenous administration of the nanoconjugate to a CXCR4-overexpressing (CXCR4+) disseminated colorectal cancer mouse models we have achieved:

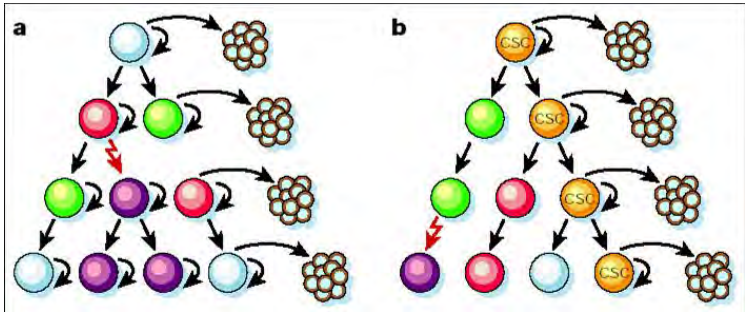
1.- Selective uptake in CXCR4+ cancer cell cytosol with no significant biodistribution in liver, kidney, lung, heart or bone marrow.

2.- Selective elimination of cancer cells responsible for metastasis initiation and maintenance (CXCR4+ metastasis stem cells).

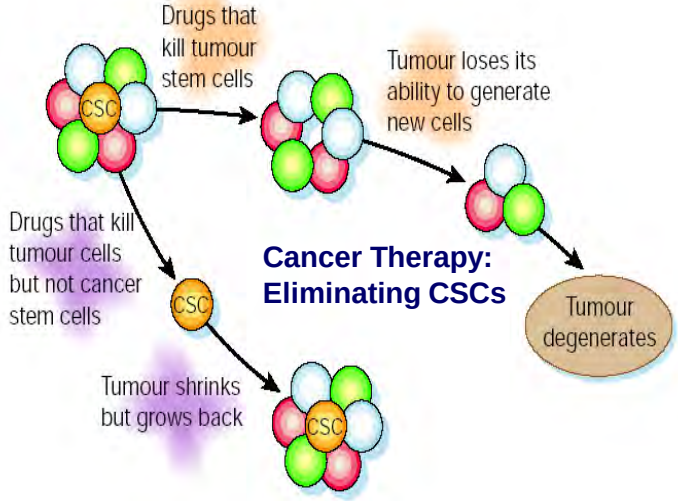
3.- Demonstration of Capacity to prevent and induce the regression of metastasis: Significant reduction in the number of liver, lung and peritoneal metastases in repeated dose protocols for prevention or induction of foci regression protocols

4.- Absence of toxicity in liver, kidney, lung, heart or bone marrow.

Cancer Stem Cells

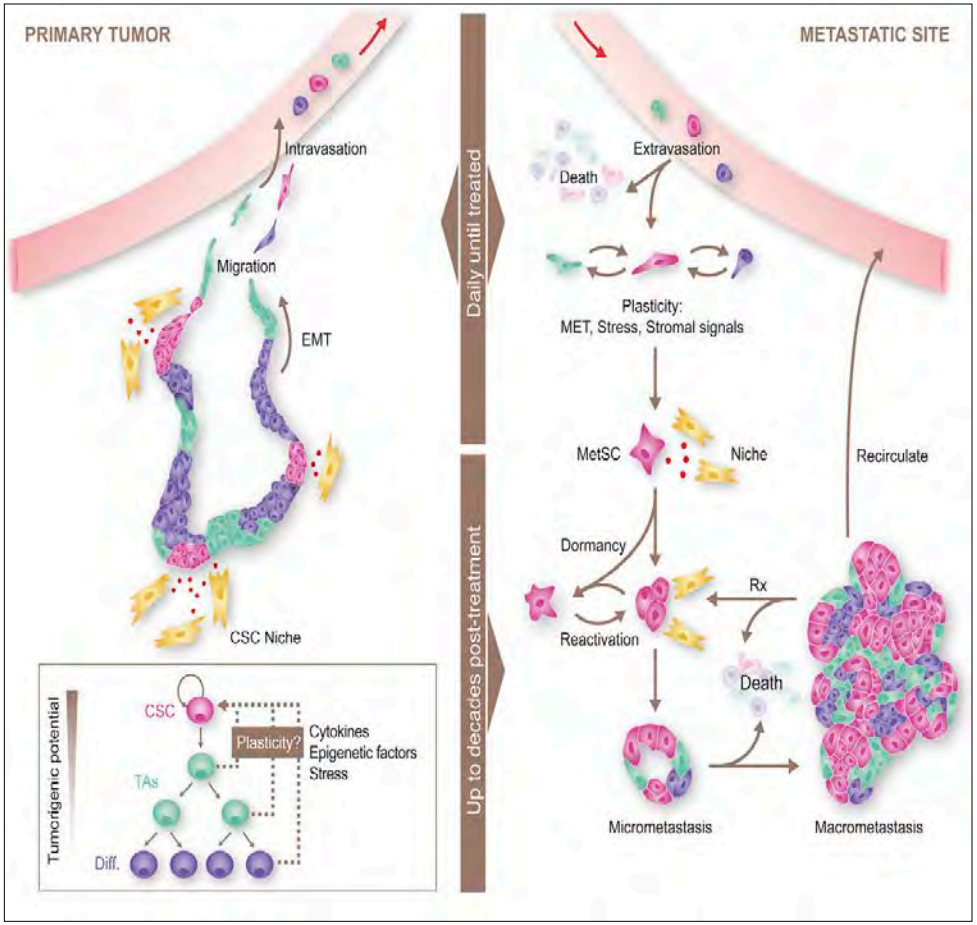


Metastatic Stem Cells



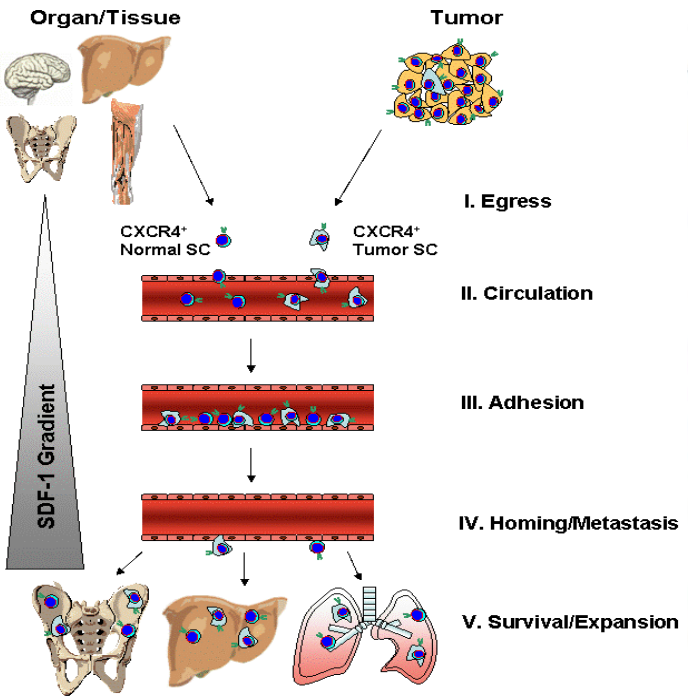
Weissman, Nature 414, 105-111, 2001

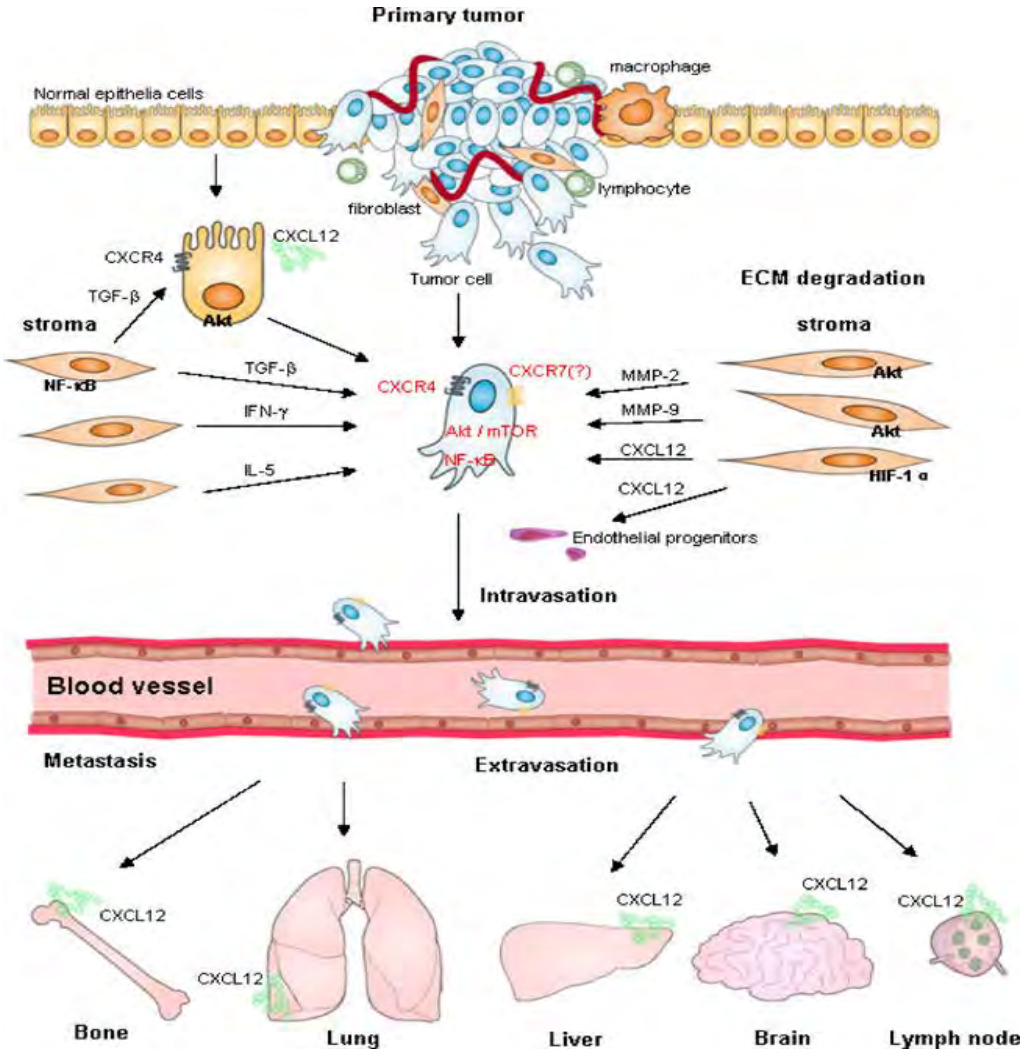
Target Cells: MetsSCs
Cancer therapy through elimination of MetsSCs



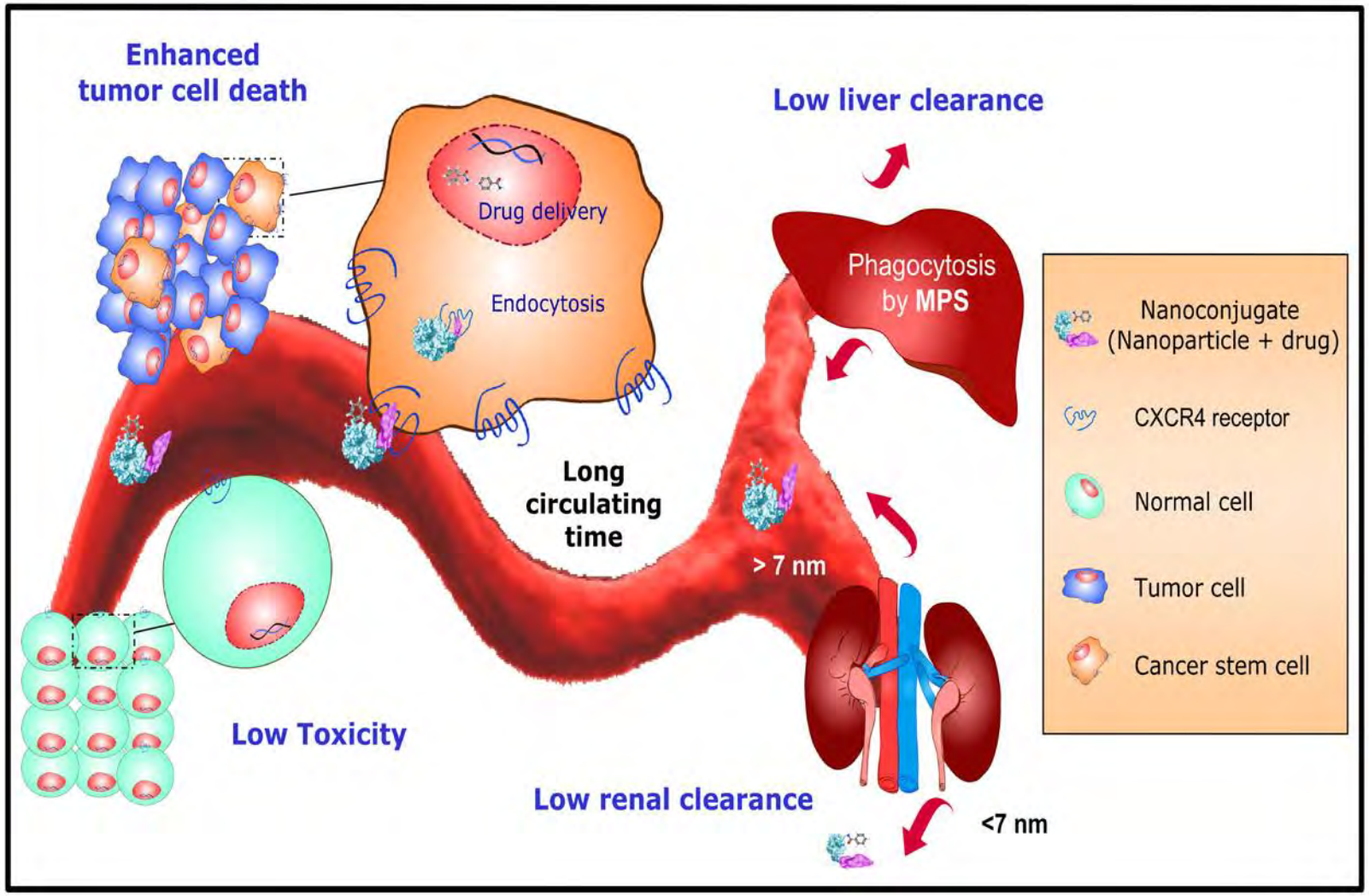
Oskarsson T et al. Cell Stem Cell 2014 Mar 6;14(3):306-21.

CXCR4+ Metastasis Initiating Cells





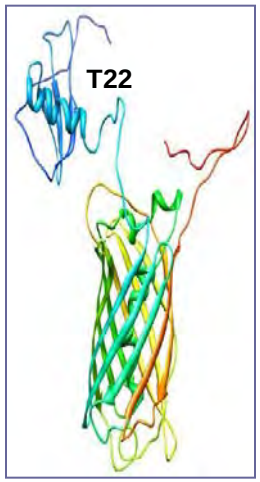
Kucia et al. Stem Cells 2005, 23:879-894



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

Ligand-directed Therapy

Design of the Mutlimeric Protein Nanoparticle as Targeting Vector



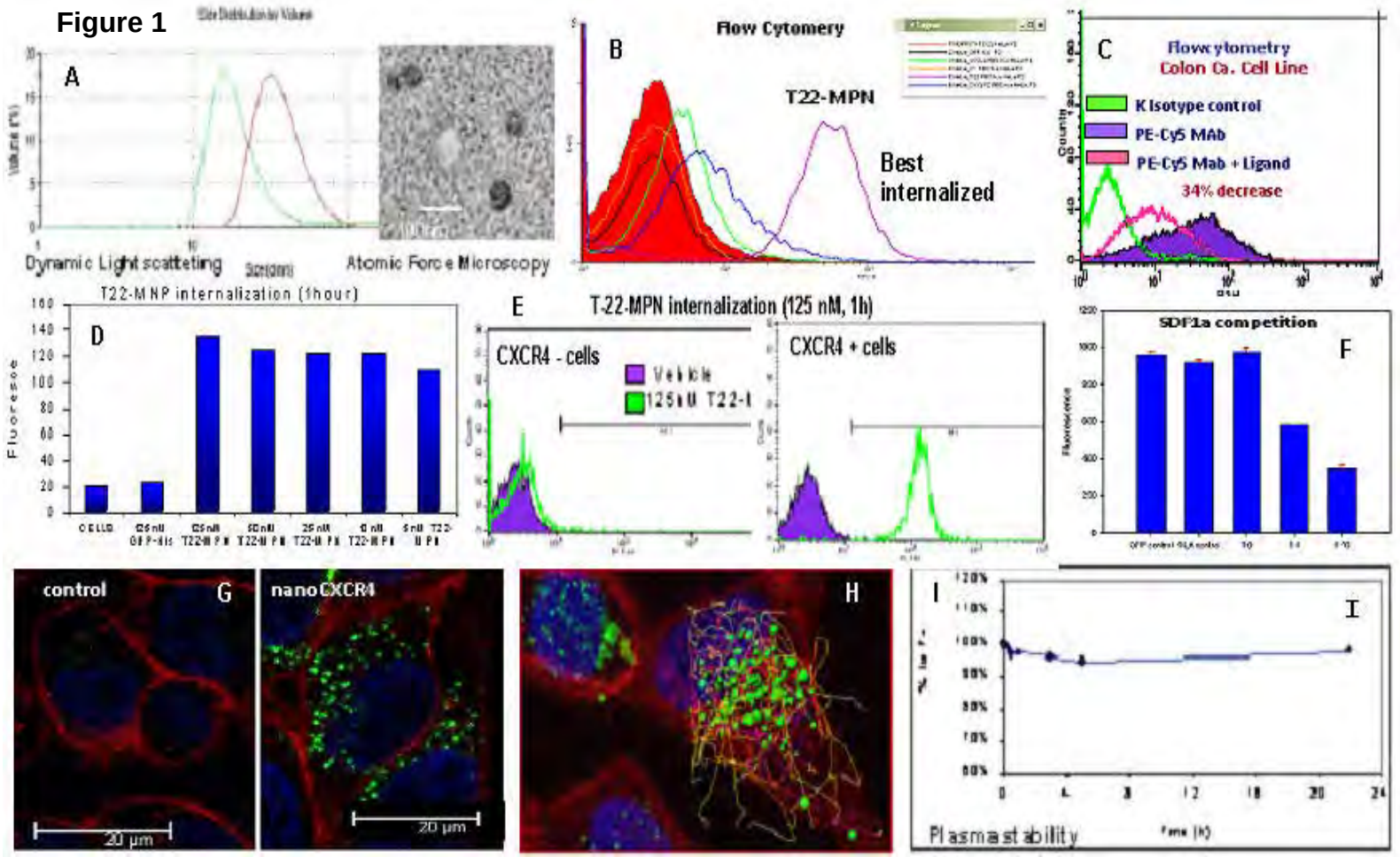
T22-GFP-H6

GS-HMRRWCYRKCYKGYCYRKCRGGSSRSSSKGEELFTGVVPILVELDGDVNGHKF
SVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYP
DHMKRHDFFKSAMPEGYVQERTISFKDDGNYKTRAEVKFEGDTLVNRI
ELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIED
GSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLL
EFVTAAGITHGMDELYKHHHHHH

CXCR4 Ligands	
CXCL12 (SDF-1 alpha)	Derivatives: CTCE-0214,KPVSLSYR, RFFESH, 1-9, 5-14
GP120 del VIH	Domain V3, SPC3
vCCL2	Peptide V1, DV1, 1-11
Polyphemusine II	Derivatives: T22, T140, T134, CGPG4222, ALX40-4C

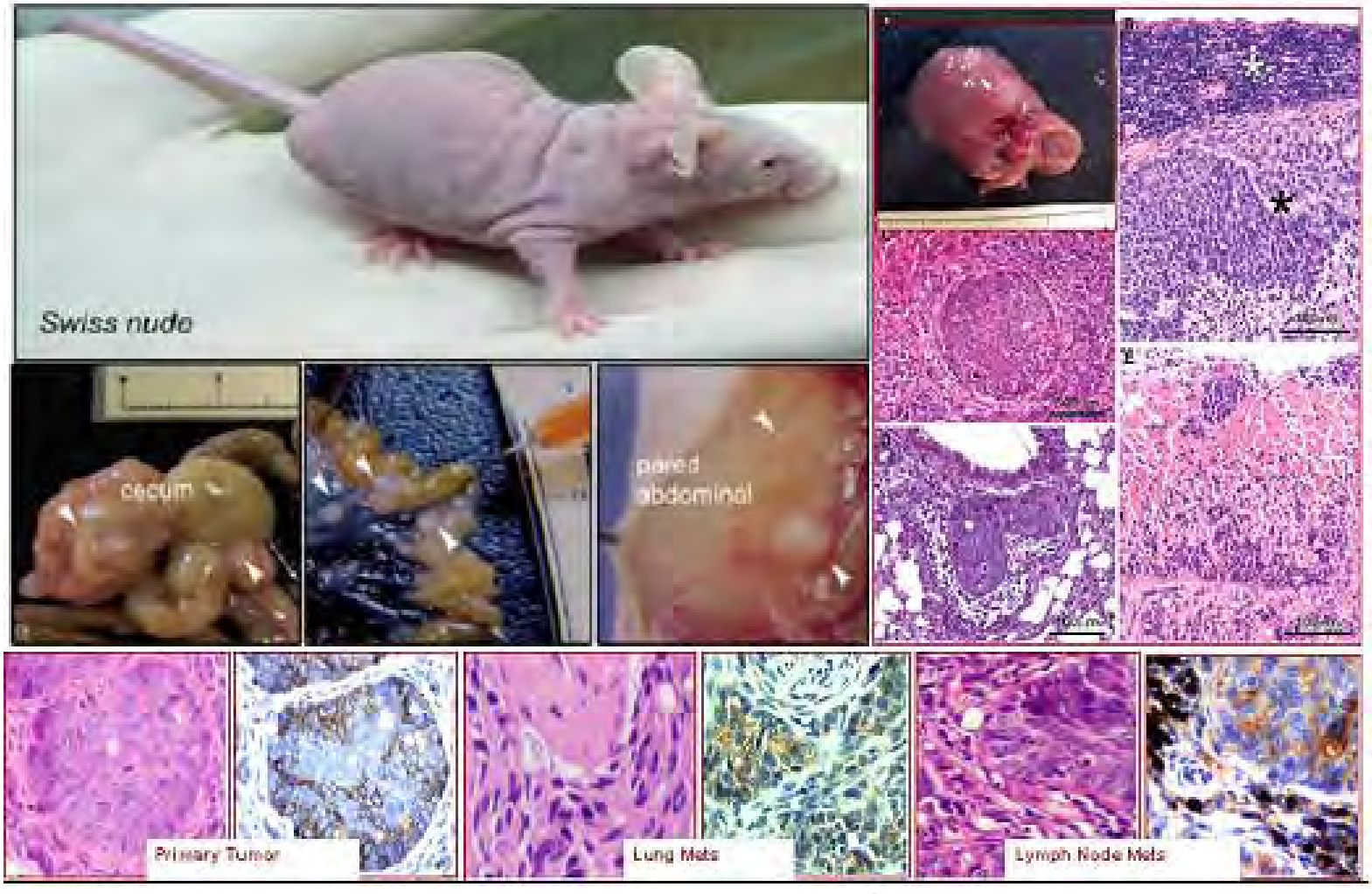
T22-GFP-H6 *in vitro* evaluation

Figure 1



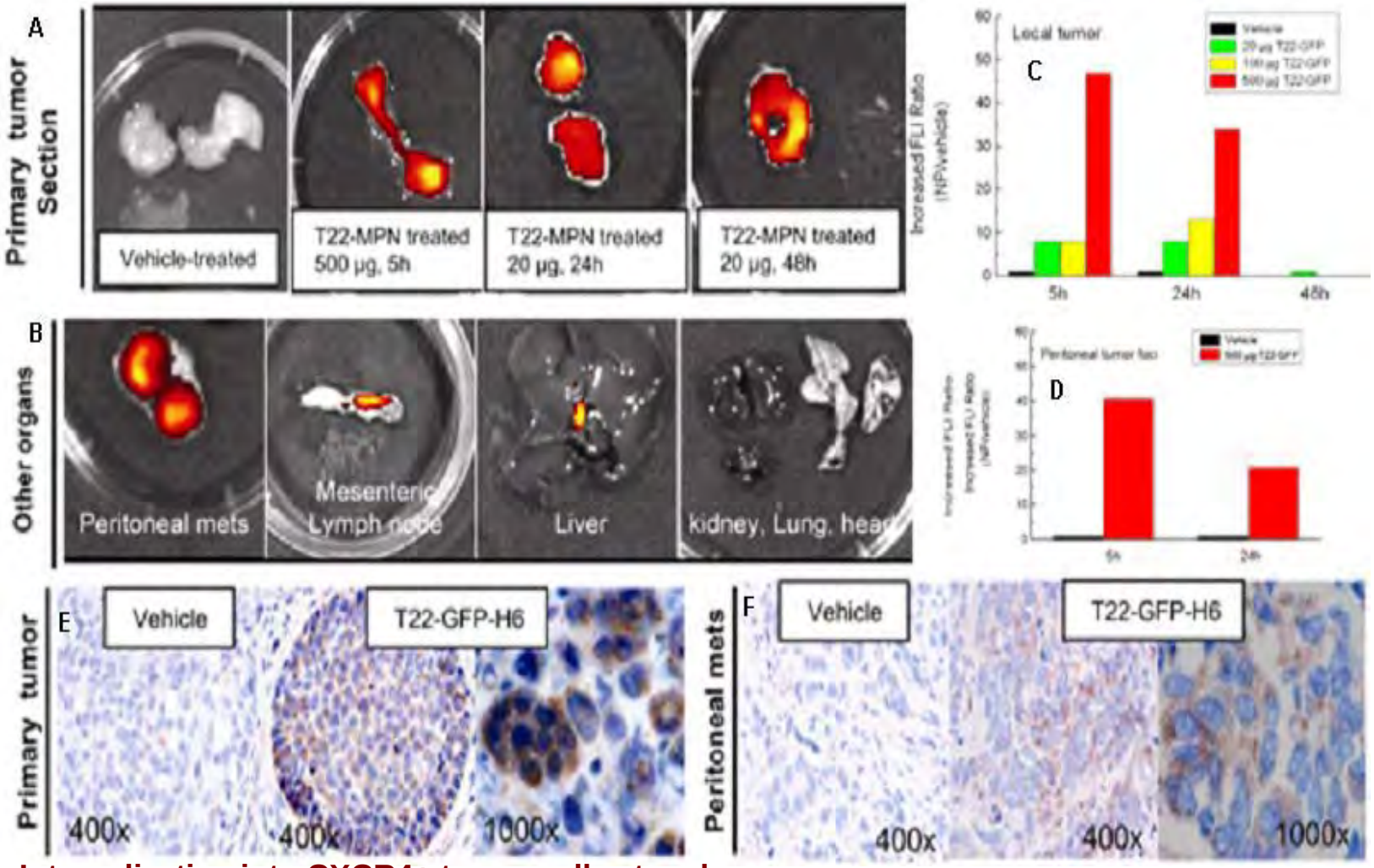
Unzueta U, Céspedes et al. Int J Nanomecine 2012

CXCR4+ SW1417 CRC model (LN, liver lung and peritoneal metastases)



CXCR4 expression in tumor tissues (Céspedes et al. Am J Pathol, 2007)

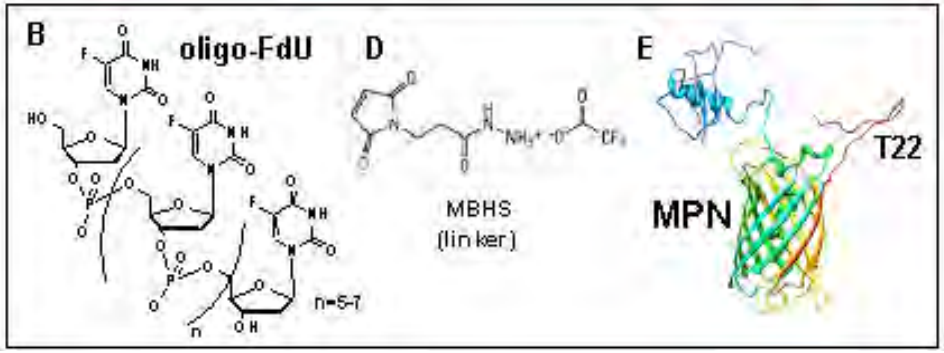
Selective biodistribution to tumour tissue

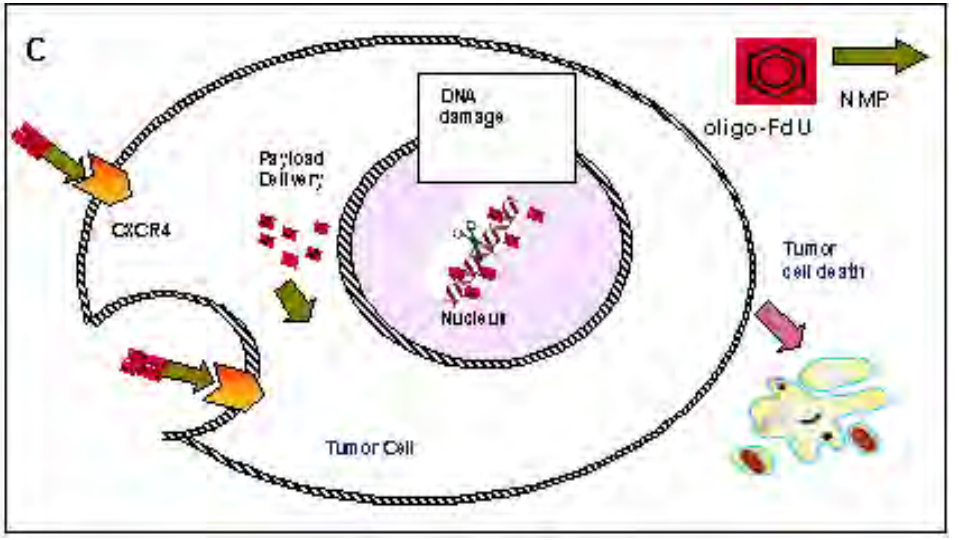


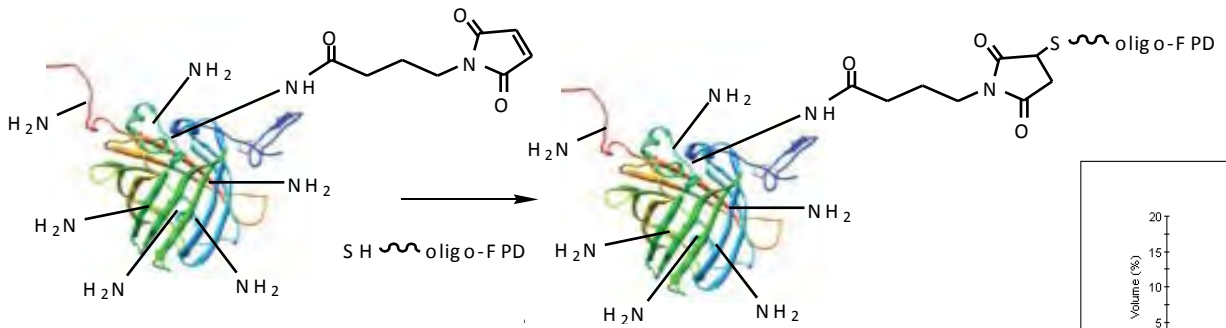
Internalization into CXCR4+ tumor cell cytosol

(Unzueta U et al. Int J Nanomedicine, 2012)

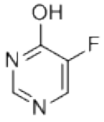
Nanoconjugate for selective depletion of MetSCs



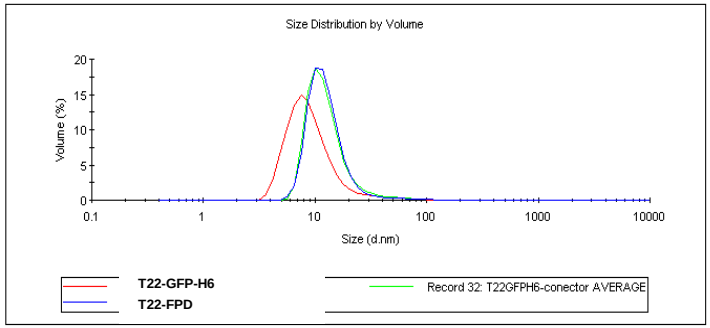




Fluoropyrimidine Derivative

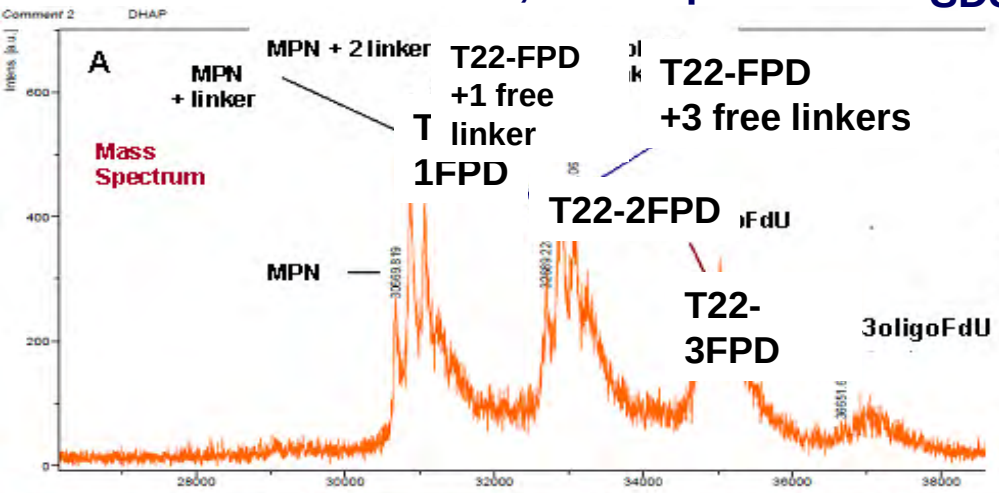


Genotoxic Nanoconjugate

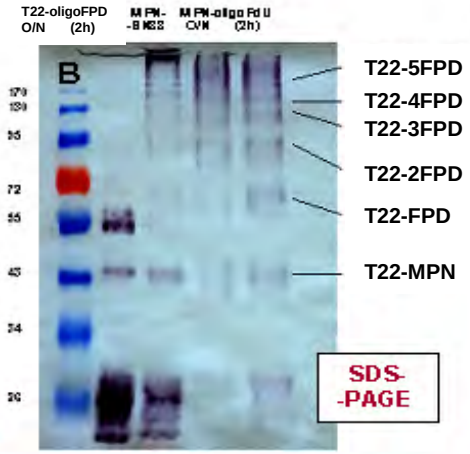


Effect of T22-FPD internalization and cytotoxiciy in CXCR4+ Cells

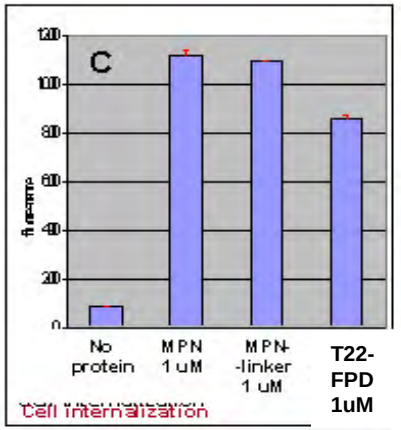
MALDI-TOFF, Mass spectra

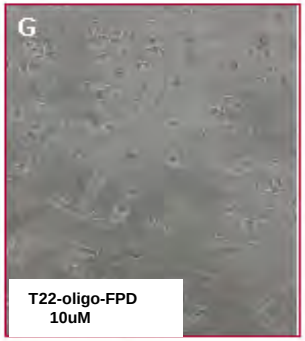


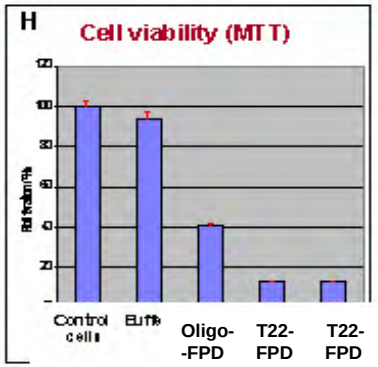
SDS-PAGE

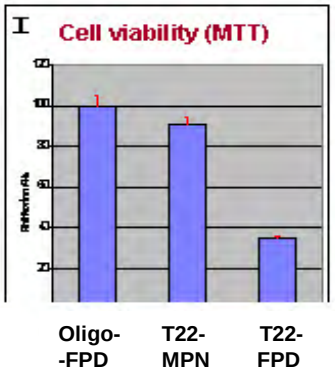


Cell internalization



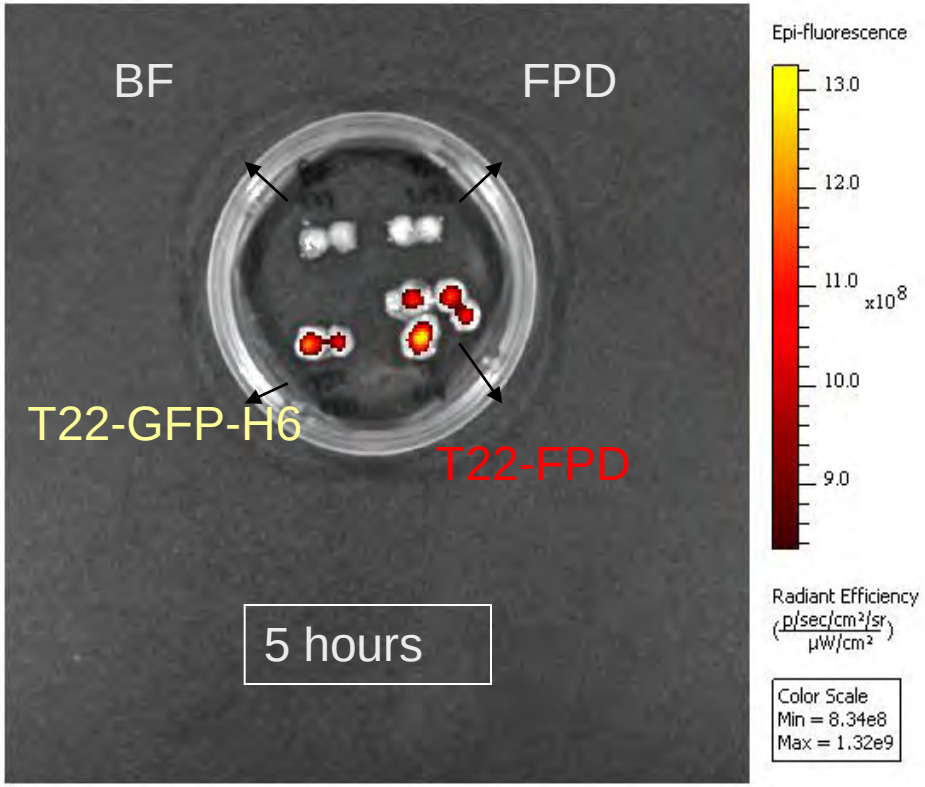


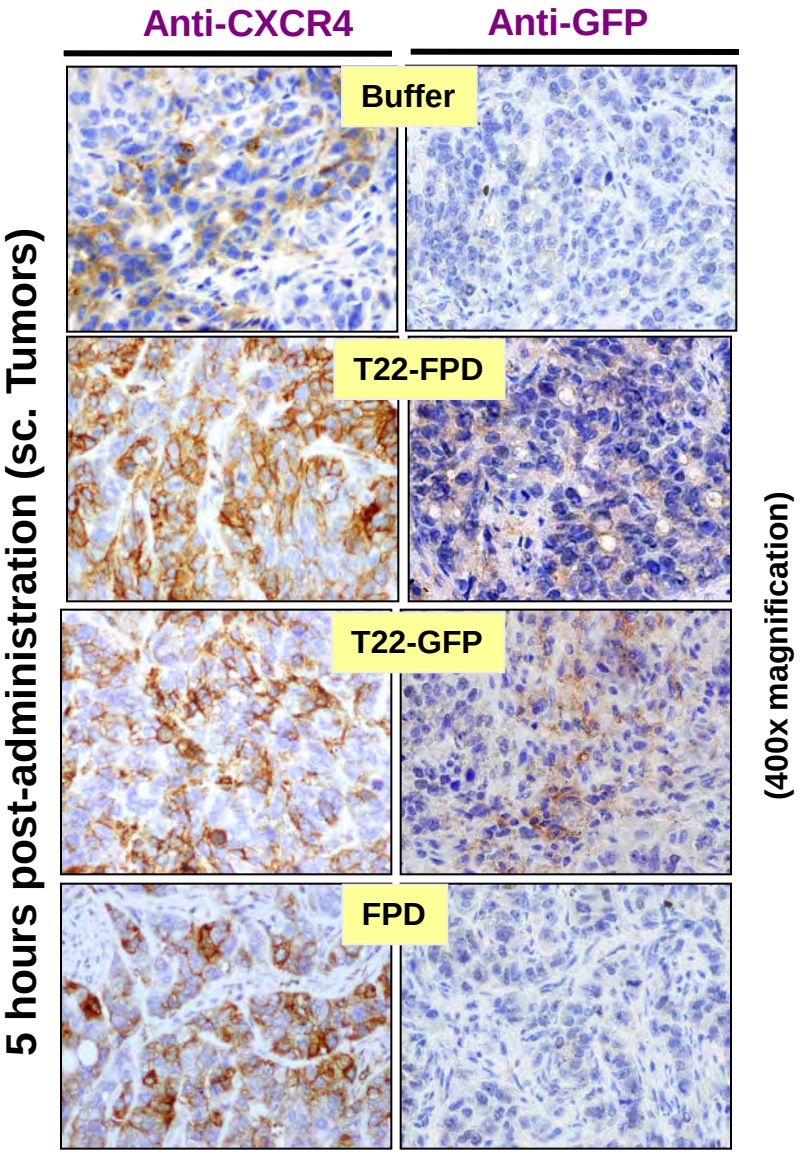




Cytotoxity in CXCR4+ cells

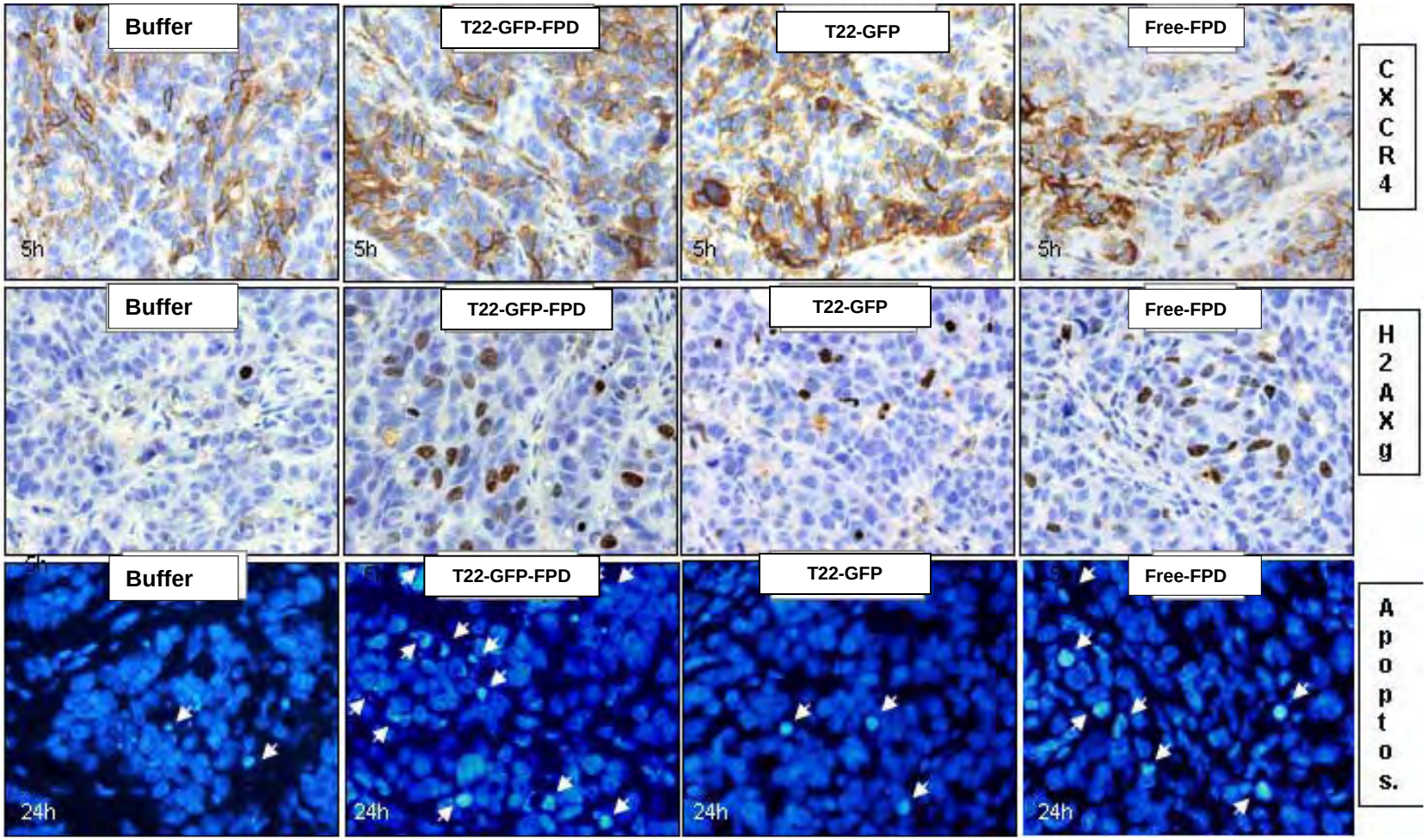
T22-FPD Biodistribution



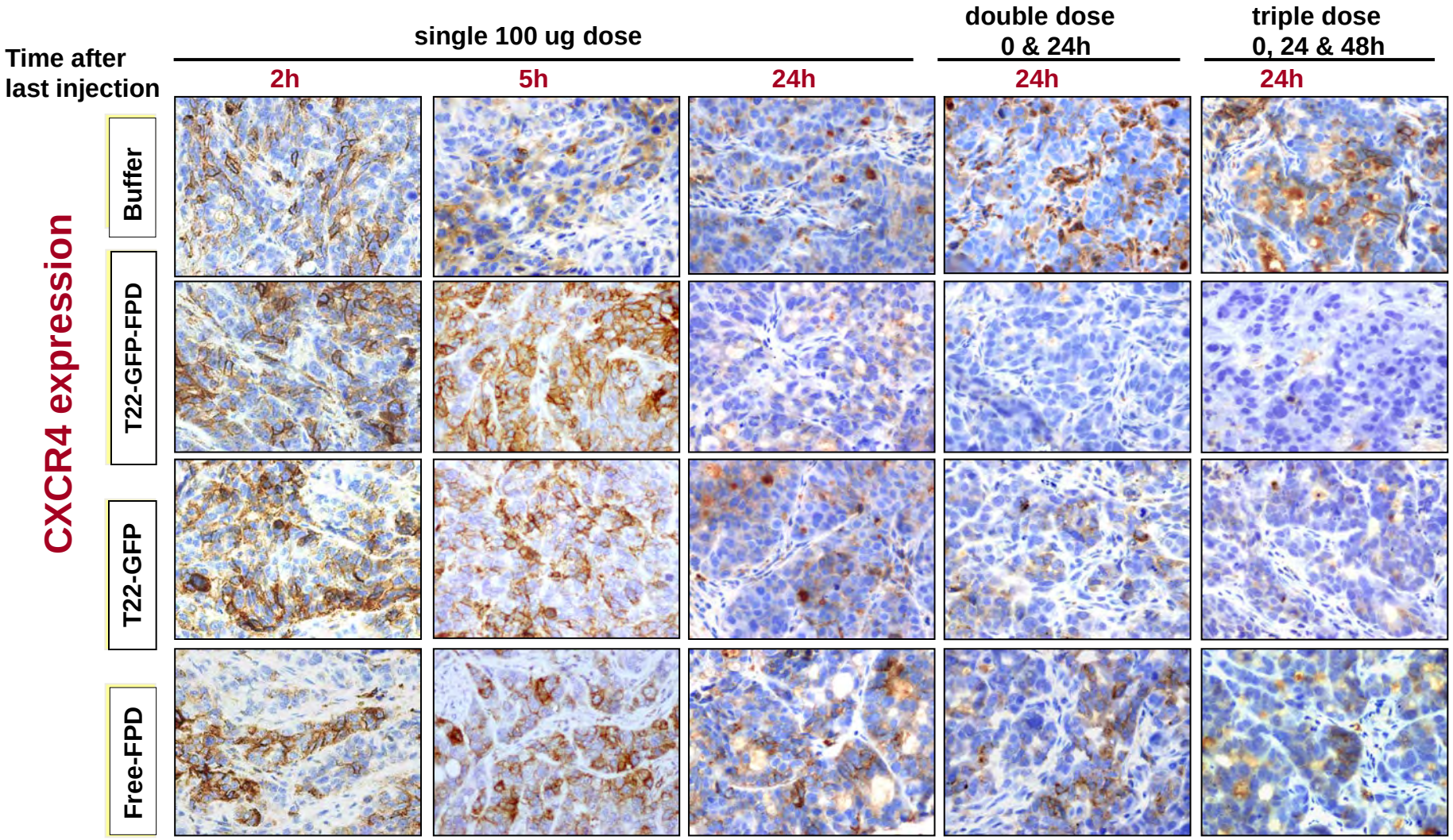


T22-FPD internalization into CXCR4+ Tumor cells

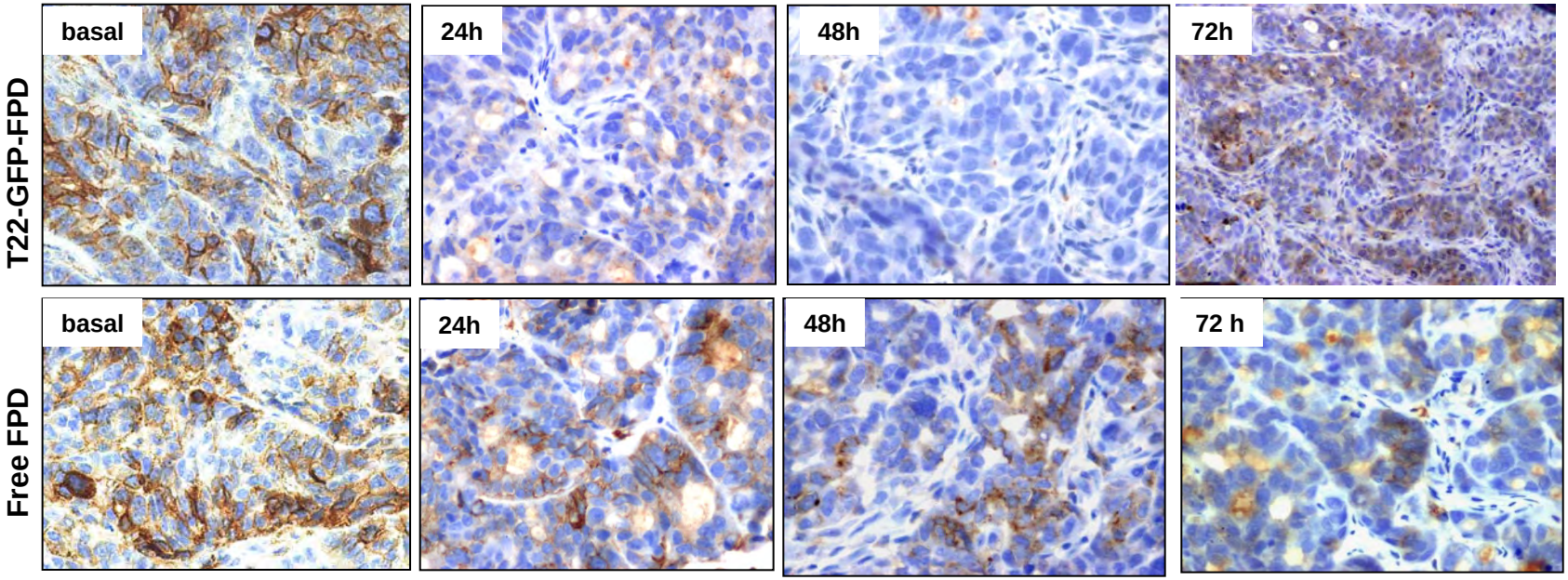
The T22-FPD Nanoconjugate induces higher level of DNA Damage and Apotosis than Free-FPD



Selective elimination of CXCR4+ Tumor cells



Optimal T22-GFP-FdU dosage interval regime (q3d) as a function of CXCR4 re-expression i tumors



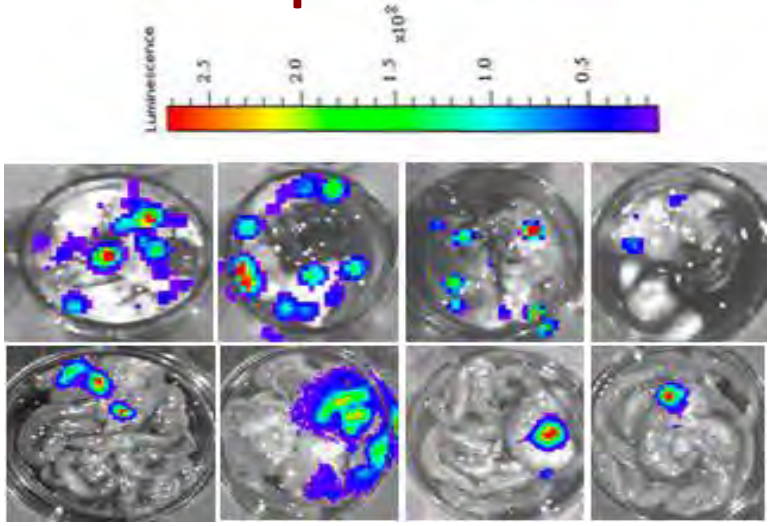
Regression of Metastasis protocol

T22-FPD

ORT SW1417 Swiss Nude Model

t=0 Orthotopic Implantation
t=2 month Start Treatment
Dosage:
20μg T22-FPD q3d x 12 doses
t= 3.2 month Necropsy

T22-FPD Antimetastatic effect significantly higher than Free-FPD effect



(Bioluminiscence emission *ex vivo*)

Antimetastatic Effect of T22-GFP-FPD in the ORT SW1417 model*

Luminescent Foci #			Histological Analysis (Foci #: mean+SE)				
	Lymph Node	Lung		LN Foci	Total	Lung Foci	Total
Buffer	9	18	Buffer	1.0±0.3 [a]	18	4.0±1.1 [c,d]	24
T22-GFP	14	15	T22-GFP	1.1±0.2 [b]	19	1.7±0.6 [e]	20
Free-FPD	2	6	Free-FdU	0.8±0.2	9	2.0±0.7 [c,f]	12
T22-GFP-FPD	4	2	T22-GFP-FdU	0.4±0.1 [a,b]	7	0.7±0.4 [d,e,f]	4

* 6 sections count

a: p=0.03; b: p=0.01; c: p=0.04; d:0.03; e=.3, f: p=0.04 (Mann-Withney)

Prevention of Metastasis protocol

T22-FPD

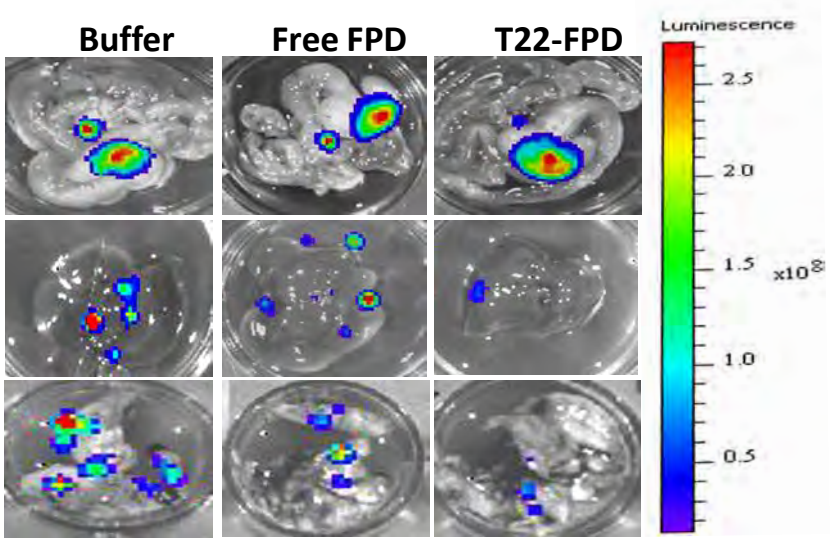
SC+ORT
CXCR4+
SW1417
NOD/SCID
mouse
model

t=0
Orthotopic implantation

t= 10 days
start administration

20μg T22-FPD
x 10 doses

t= 16 weeks
Necropsy



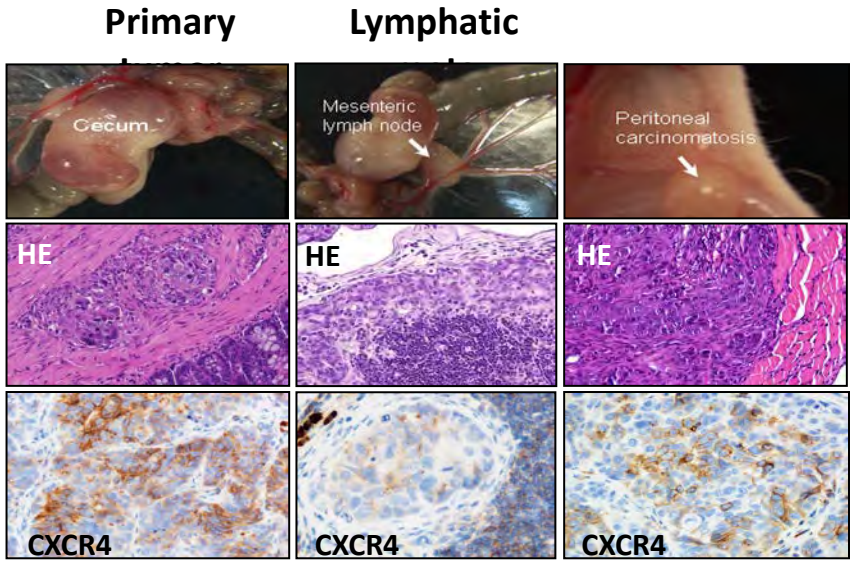
T22-FPD Antimetastatic activity in the CXCR4+ SW1417-derived SC+ORT model

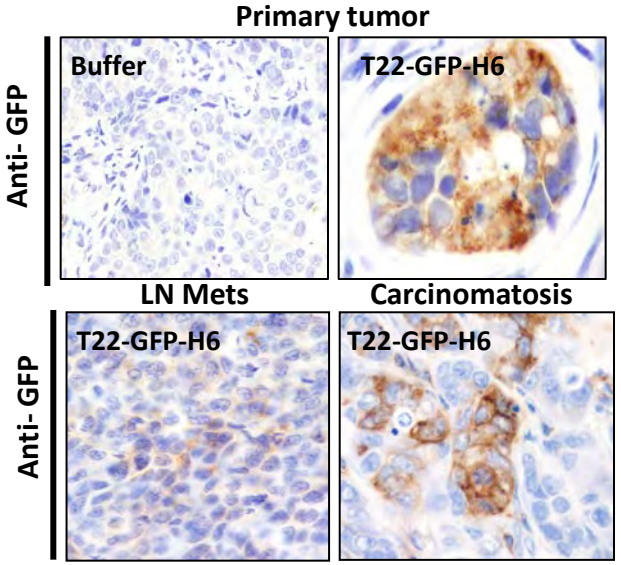
	Primary Tumor		Metastasis											
			Lymph Nodes			Liver			Lung			Peritoneal		
Group	positive mice	%	positive mice	%	# foci**	positive mice	%	# foci**	positive mice	%	# foci**	positive mice	%	# foci**
Buffer	11/11	100%	11/11	100%	36[a]	4/11	36%	8[c]	8/11	73%	72[e]	7/11	64%	22[g]
Free-FPD	11/11	100%	11/11	100%	35[b]	6/11	55%	11[d]	6/11	55%	50[f]	5/11	45%	31[h]
T22-FPD	12/12	100%	9/12	75%	27[a,b]	2/12	17%	3[c,d]	2/12	17%	16[e,f]	2/11	17%	3[g,h]

** total number of foci in 3 tissue sections

[a]p=0.047, [b]p=N.S., [c]p=0.001, [e]p=0.002, [f]p=0.006, [g]p=0.001, [h]p=0.0001

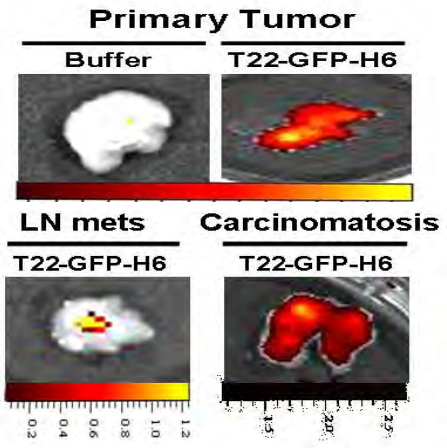
Percent of CXCR4+ tumor cells as marker of response: Therapy personalization

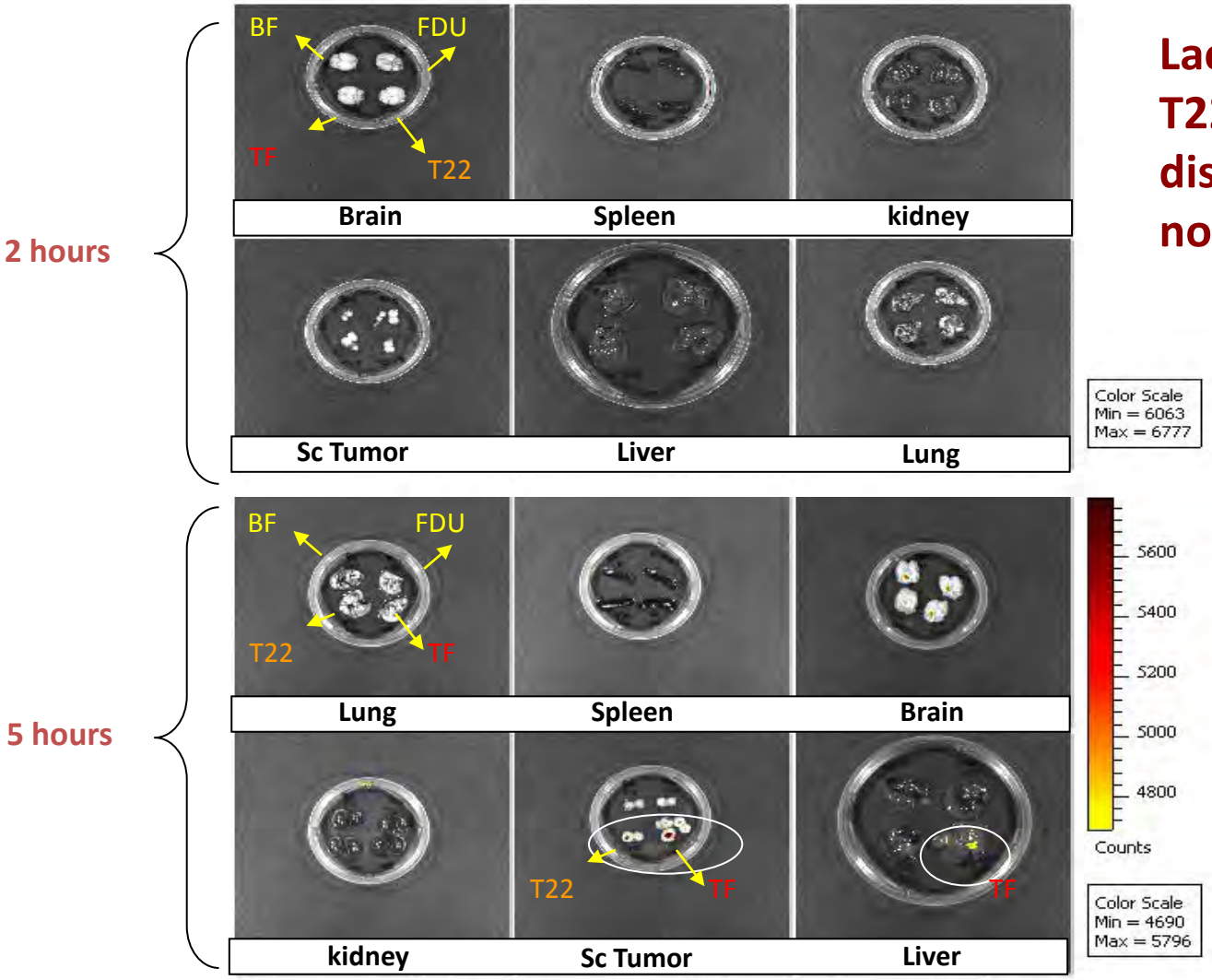




Remaining % of CXCR4+ cells in tissues after T22-GFP-FPD therapy				
	LN Mets	SC Tumors	Lung Mets	Carcino.
BF (control)	12%	19%[a]	17%[b]	20%[c]
T22-GFP-FPD	13%	8%[a]	8%[b]	5%[c]
Sensitivity	Resistant	Medium	Medium	High

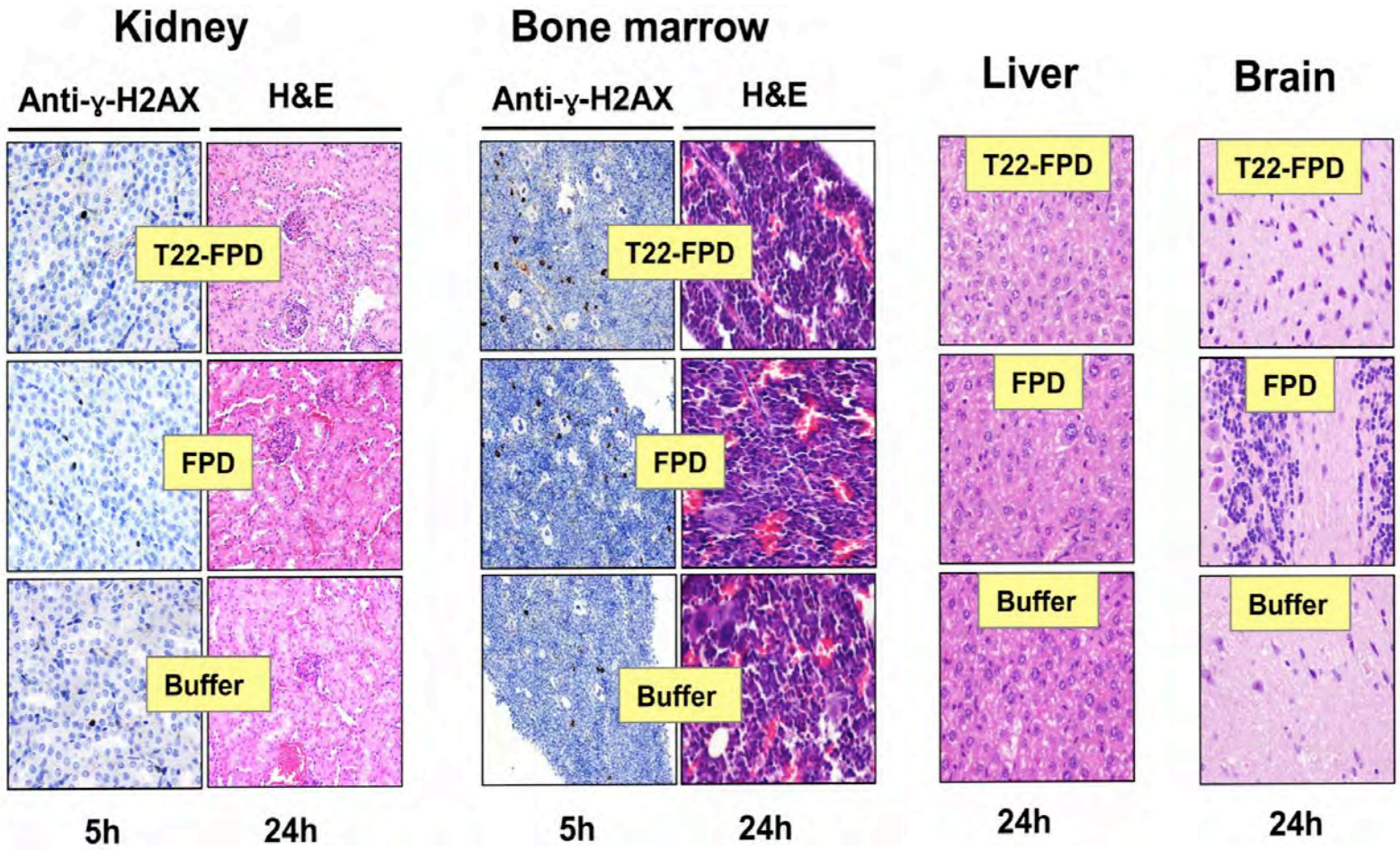
(a) p= 0.047; (b) p= 0.039;(c) p= 0.023; Carcino.=peritoneal Mets





Lack of T22-GFP-H6-FPD distribution to normal tissues

Lack of T22-GFP-H6-FPD induced toxicity on normal tissues



Lack of histopathological damage by T22-FPD in normal tissues despite induction of some induction of DNA damage in the bone marrow

EXPLORING THE POTENTIAL MARKET: CXCR4 association with relapse and poor outcome

- Kim J et al. CXCR4 expression in colorectal cancer increases risk for recurrence and poor survival. J Clin Oncol. 2005 Apr 20;23(12):2744-53.
- Akashi T et al. CXCR4 expression and prognosis in patients with metastatic prostate cancer. Cancer Sci. 2008 Mar;99(3):539-42.
- Scala S et al. Expression of CXCR4 predicts poor prognosis in patients with malignant melanoma. Clin Cancer Res. 2005 Mar 1;11(5):1835-41.
- He H et al. Overexpression of CXCR4 is an independent prognostic predictor in gastric cancer patients. PloS One 2013 8(8):e71864.
- Andre F et al. CXCR4 expression in early breast cancer and risk of distant recurrence. Oncologist 2009, 14(12):1182–1188.
- Hiller DJ et al. CXCR4 level in primary tumors independently predicts outcome in locally advanced breast cancer. Surgery 2011, 150(3):459–465.
- Holm NT et al. Elevated CXCR4 expression in primary tumors following neoadjuvant chemotherapy predicts poor outcomes for patients with locally advanced breast cancer. Breast Cancer Res Treat. 2009 Jan;113(2):293-9.
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Pitfalls & Risks to be considered

* Bone marrow toxicity because of expression of CXR4 by hematopoiteic stem cells

Solution: Selectivity in the delivery

Low molecular weight drugs CXCR4 inhibitors are showing toxicity in clinical trials because of unselective biodsitriubtion

* Immunogenicity: Green fluorecent protein is exogenous and could give rise to an immunogenic response

Solution: substitute GFP in the targeting vector by a human protein

IPR protection

Main patent covering conjugates comprising T22 and therapeutic agents, for any kind of indication.

European Patent recently granted

US Patent recently granted

Australia and Israel, patent pending

Next steps:

Enlargement of IP portfolio covering different types of nanocarriers:

- **T22 nanoparticles built with a specific scaffold protein, etc,**
- **or association with specific therapeutic agents**

Partnering Opportunities

The Tech Transfer Office is looking for:

- **Industrial partners to further develop the technology trough a license and co-development agreement. Our target are Pharma/Biotech companies within oncology field willing to load the carrier with their own drugs of interest.**

- **Once PoC has been preclinically demonstrated, we are looking for funding to further develop the product towards the regulatory preclinical phase (toxicity studies, etc) through companies, patient associations, public research programmes.**