CLÍNIC BARCELONA Hospital Universitari

IDIBAPS

Bioluminescence imaging as a tool for the discovery of new therapeutic targets in murine xenograft models of B-cell lymphomas

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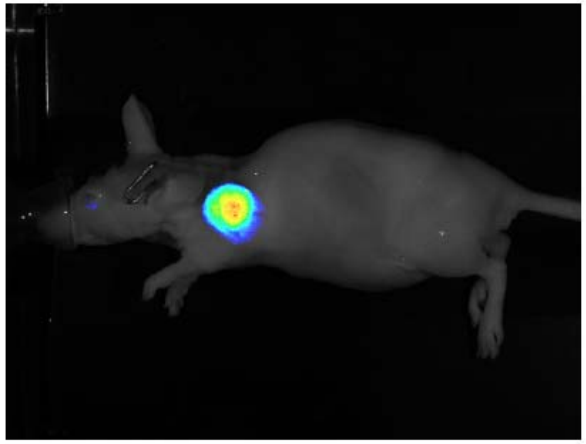
In vivo animal imaging

- Detection of molecular targets in live animals
  - Tumors
  - Cells
  - Processes (inflammation, vascularization, etc.)
  - Enzymes
  - Cellular components



Animal models in oncology

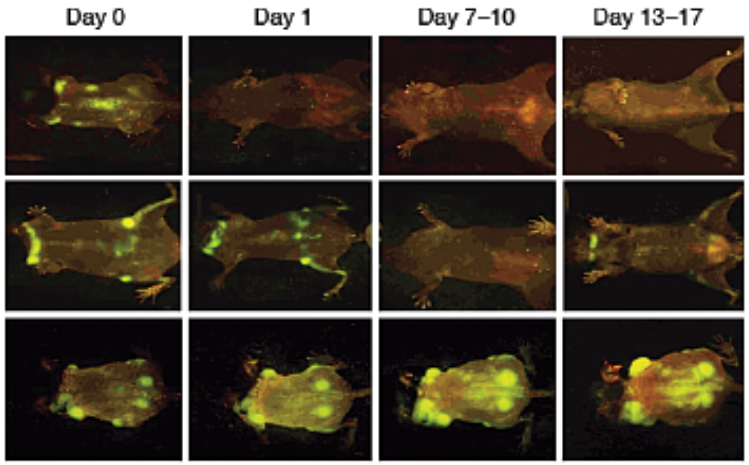
- **Need for animal models with adequate genetic/phenotypic background and closed to what observed in humans.**

  - **to analyze the first steps of tumor development**
  - **to evaluate the relationship between genetic alterations and tumor progression**
  - **to identify new therapeutic targets**
  - **etc…**

Animal models in oncology

- **Tools for evaluation of tumor burden *in vivo* :**
  - **magnetic resonance imaging (MRI)**
  - **positron emission tomography (PET)**
  - **bioluminescence**
  - **optical fluorescence imaging (proteins, probes, near infrared dyes)**

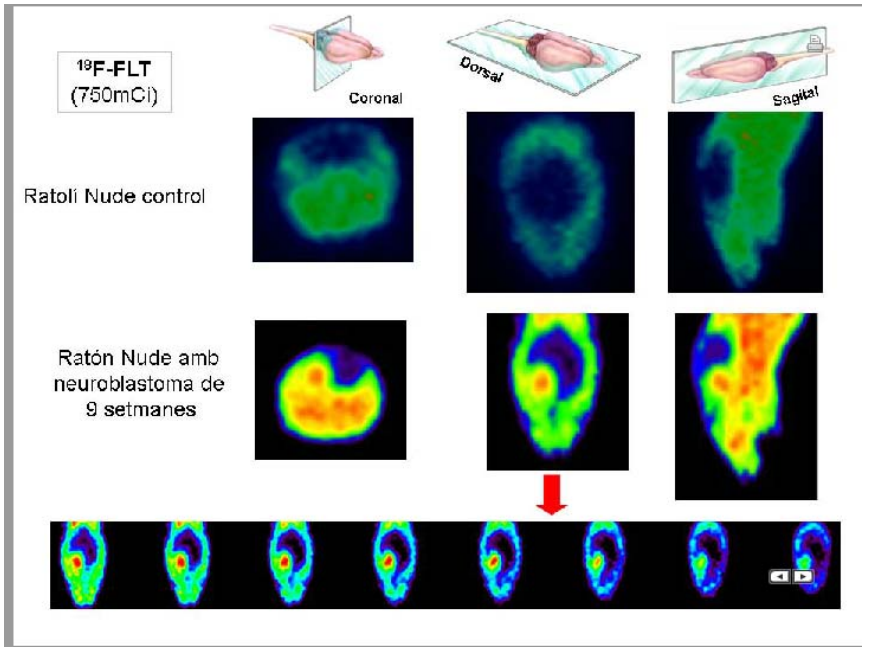
Science 299: 1972, 2002



Drug resistance. In control animals (top row), a chemotherapeutic drug rapidly eliminates lymphoma tumors. But in tumors lacking *p53* (middle), the response is slower and the tumors come back, and when the *Bcl-2* oncogene is overactive (bottom), the tumors show no response at all.

PET for preclinical evaluation of tumor prolifration

[(18)F]-FLT = 3'-deoxy-3'[(18)F]-fluorothymidine

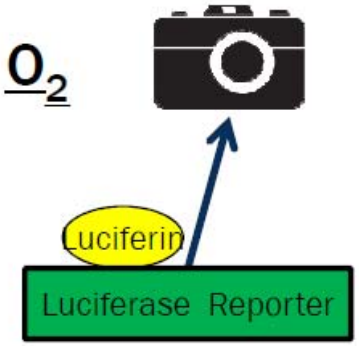


- **Limitations**
  - cannot discriminate moderately proliferative, thymidine salvage-driven tumors from those of high proliferative index that rely primarily upon de novo thymidine synthesis.
  - reflects proliferative indices to variable and potentially unreliable extents (unlike proliferation markers like Ki67)

PLoS One. 2013;8(3):e58938.

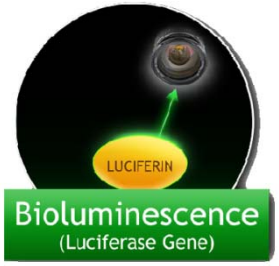
Optical imaging modalities

- Bioluminescence
  - Reporter Genes + Luciferin, ATP and O_2

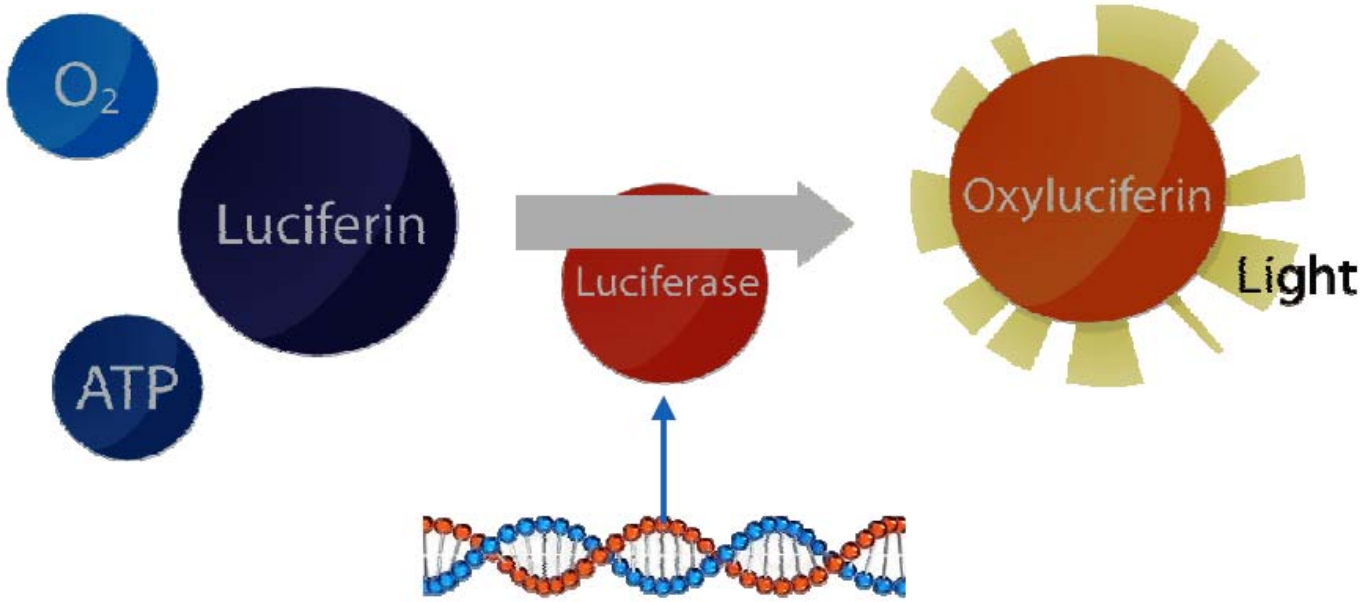


- Fluorescence
  - Fluorescent proteins
    - GFP, iRFP, IFP
  - Fluorescent Probes
    - Injectable dye-labeled molecules

Bioluminescence (BLi) :



Cells are transfected with a luciferase gene that emits photons in the presence of Luciferin, ATP and oxygen



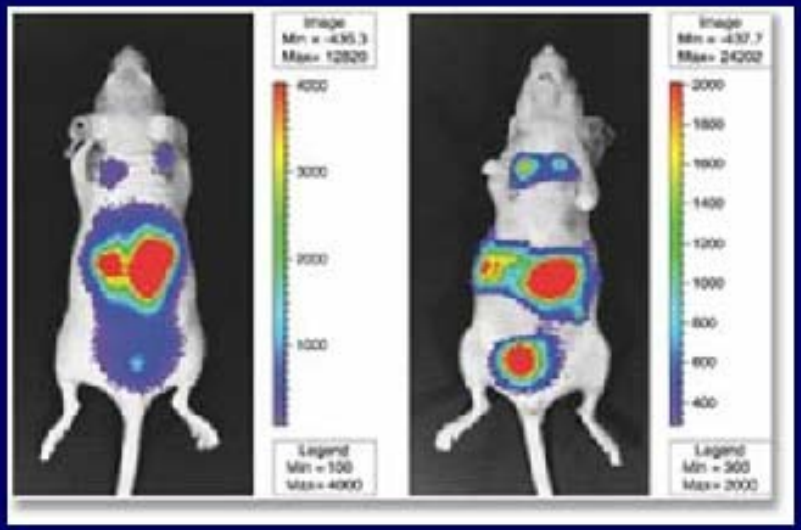
- **Features**
  - Detects the presence of cells expressing a Luciferase gene
  - No need for imaging agents

- **Limitations**
  - Requires the use of transgenic cells
  - Not compatible with clinical studies
  - Limited to imaging growing cells

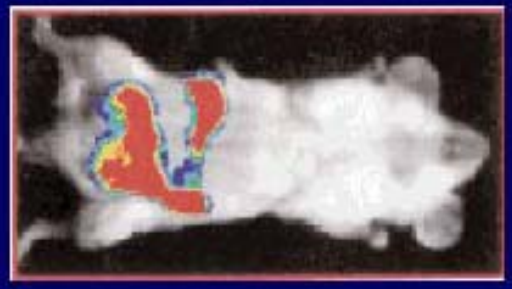
In vivo imaging using transgenic tumoral cells/bacteria

Photinus pyralis

(luciérnaga)

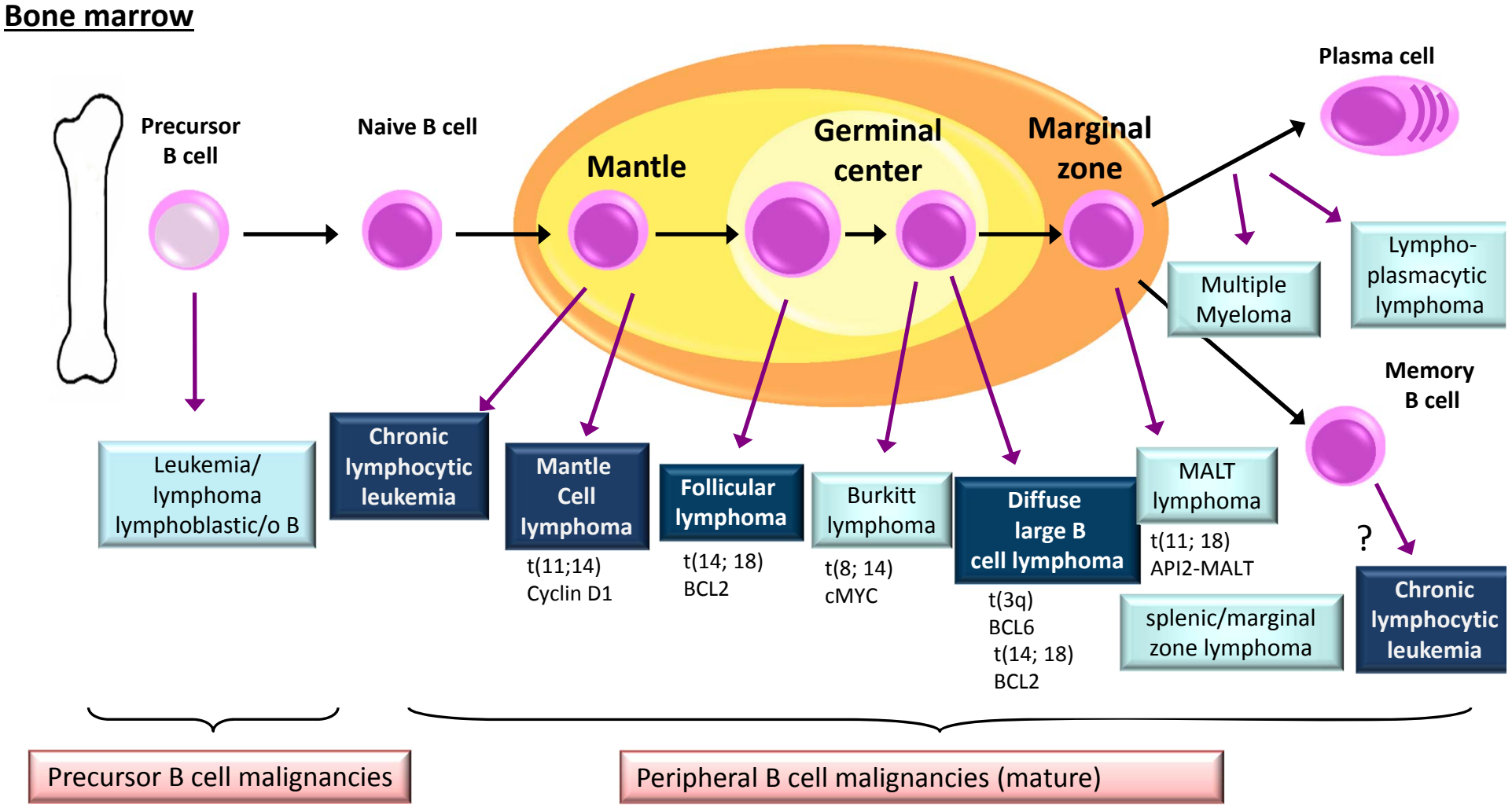


Injecció de cèl·lules de càncer de mama humanes bioluminiscents per via i.v. en ratolí.

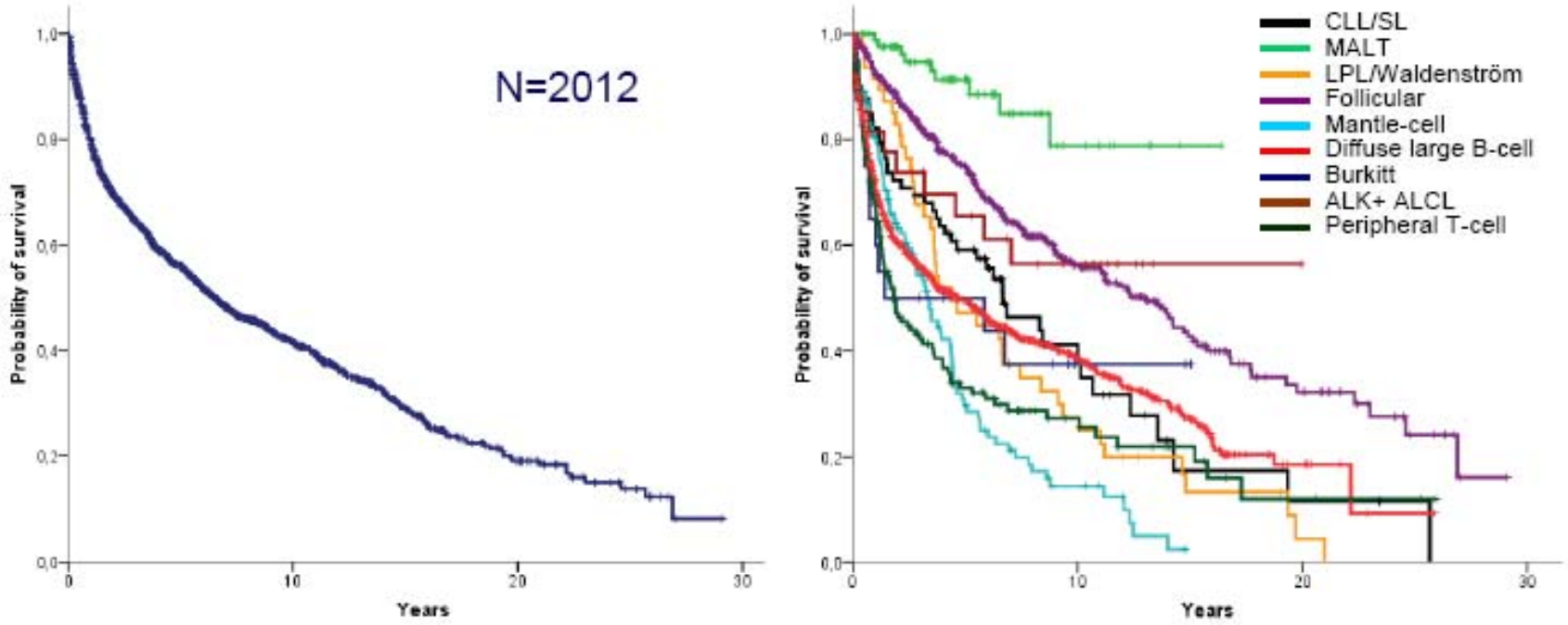


Infecció d'un ratolí amb bacteries (Salmonela) emissores de llum

B cell lymphoid malignancies seem through the lymph node



Overall survival of patients with non-Hodgkin lymphoma (Hospital Clinic, 2012)

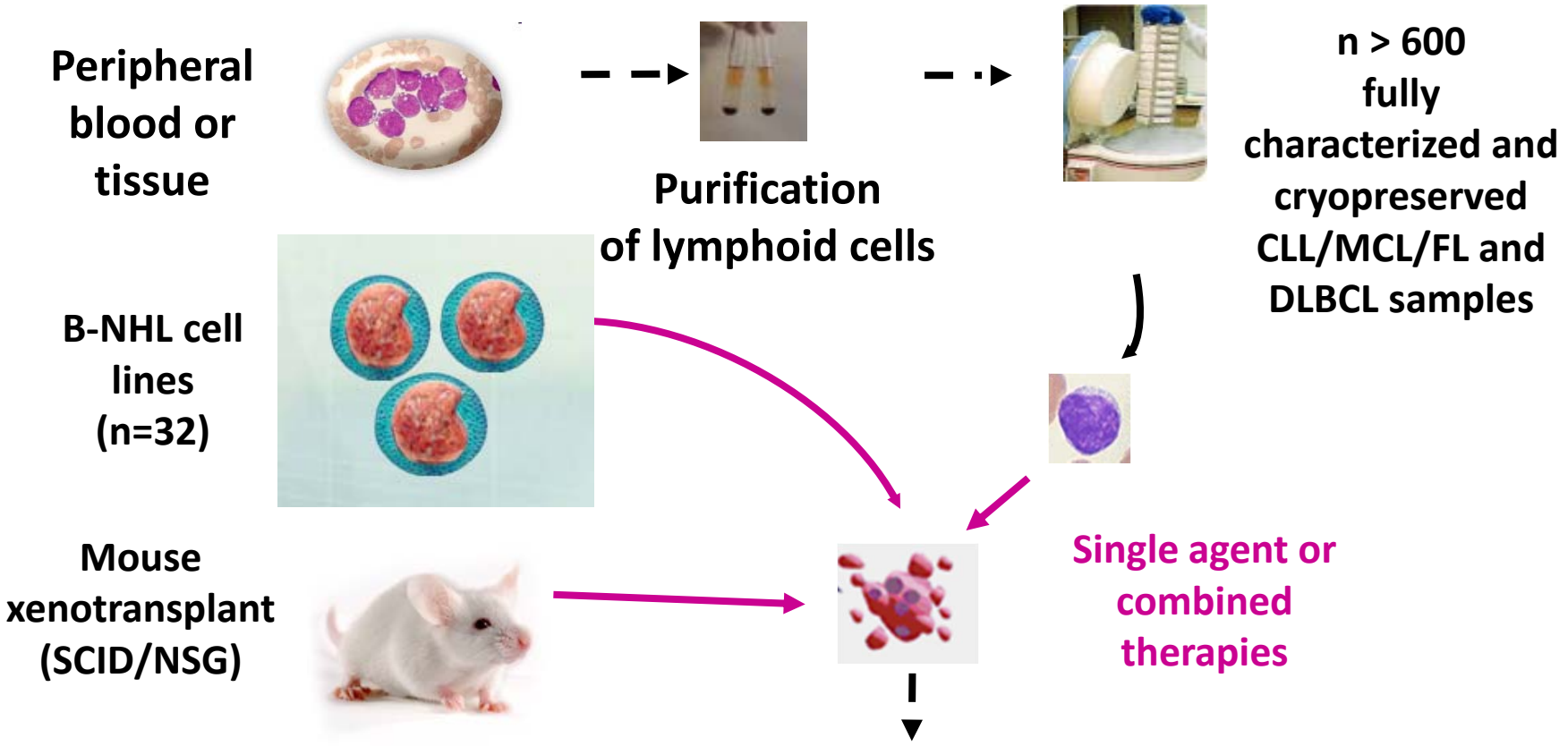


Aggressive B-cell lymphoma study group (IDIBAPS): main goals

- **Genetic, molecular and physiopathological study of various B-NHL subtypes for the design of potent and selective therapies:**

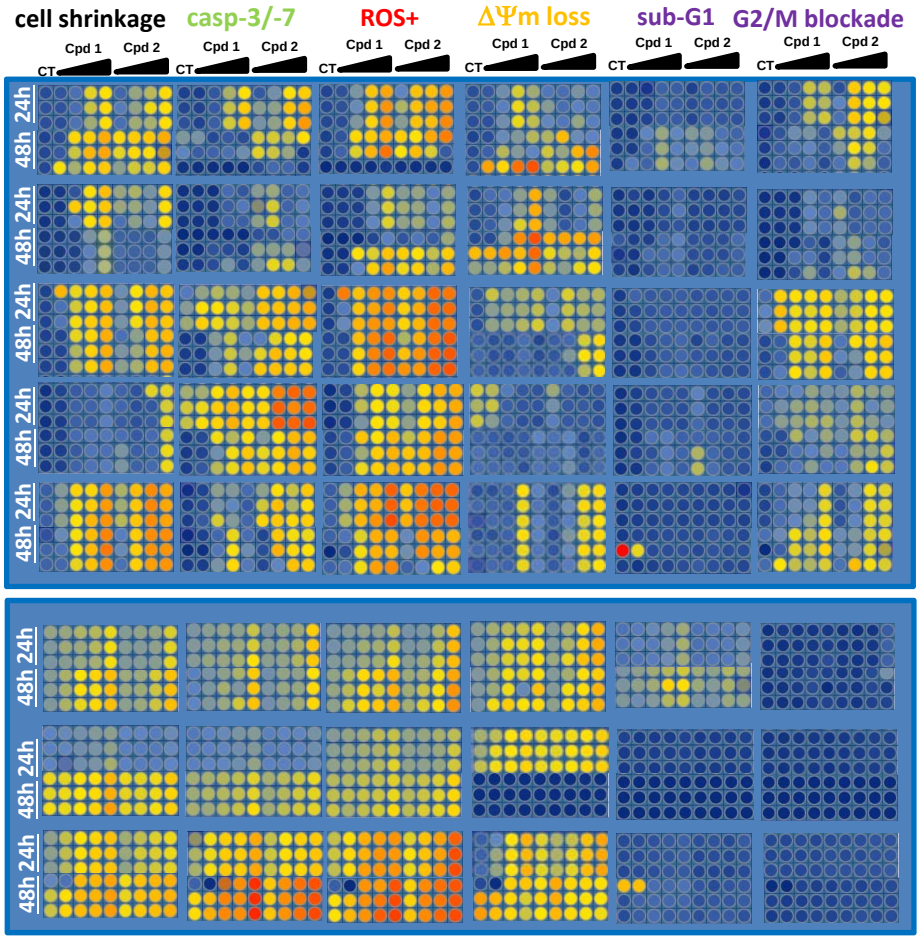
  - Search for selective therapies against deregulated signaling pathways in preclinical models of MCL, FL, DLBCL and DH lymphoma
  - Mechanism of resistance to conventional therapies (mutations, microenvironment, ..)
  - HTS screening of new therapeutic compounds. Molecular bases underlying drug efficacy and selectivity
  - *In vivo* validation in xenotransplant mouse models

Experimental design



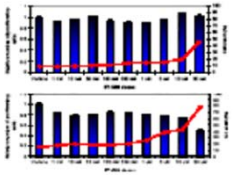
Cytotoxic assays, apoptosis measurement and molecular determination of anti-tumoral signaling

Multiparametic detection of novel putative antitumoral agents by flow cytometry

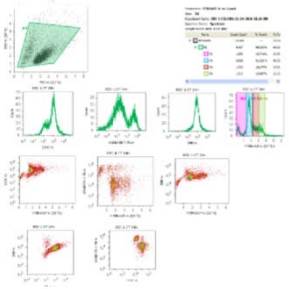


Sample processing and analysis

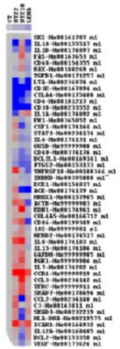
MTT

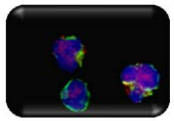


FCM



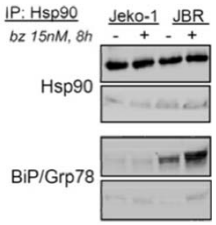
PCR array

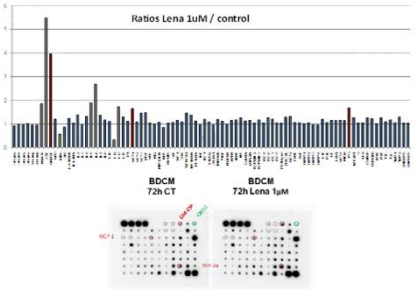




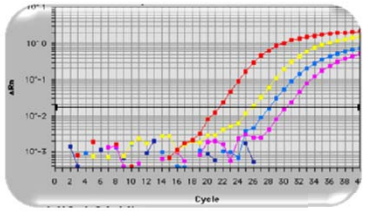
IF

WB, IPP Antibody array

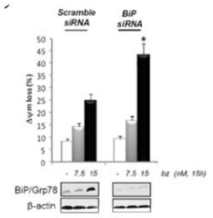




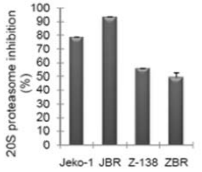
RQ-PCR



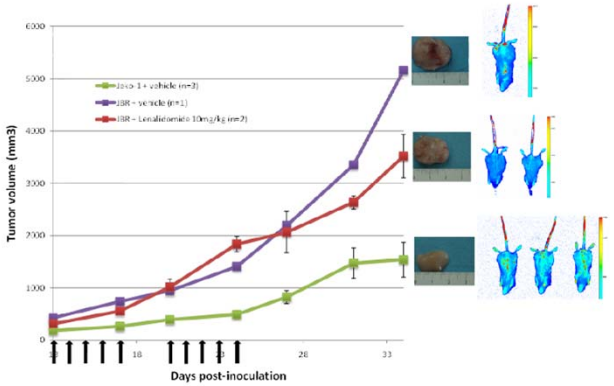
siRNA

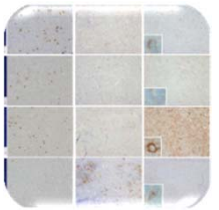


Enzymatic activity



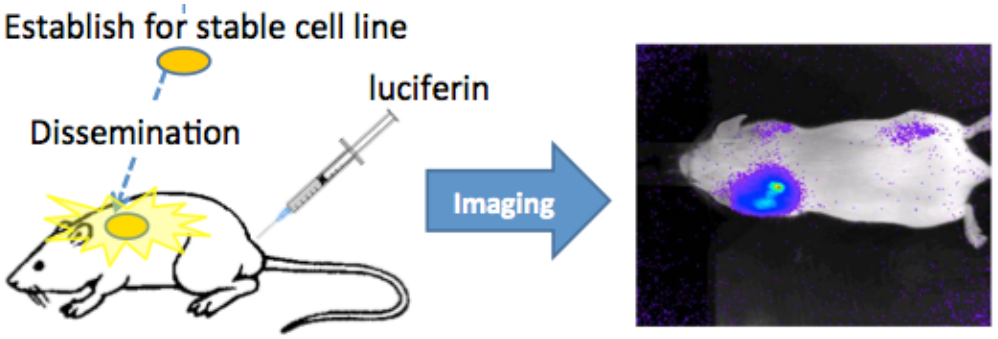
In vivo imaging





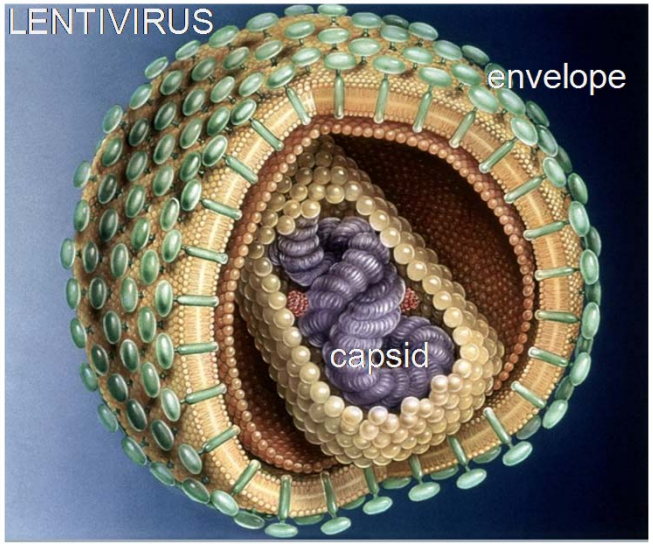
Introduction of LUC-GFP genes in lymphoid cell lines using lentiviral particles

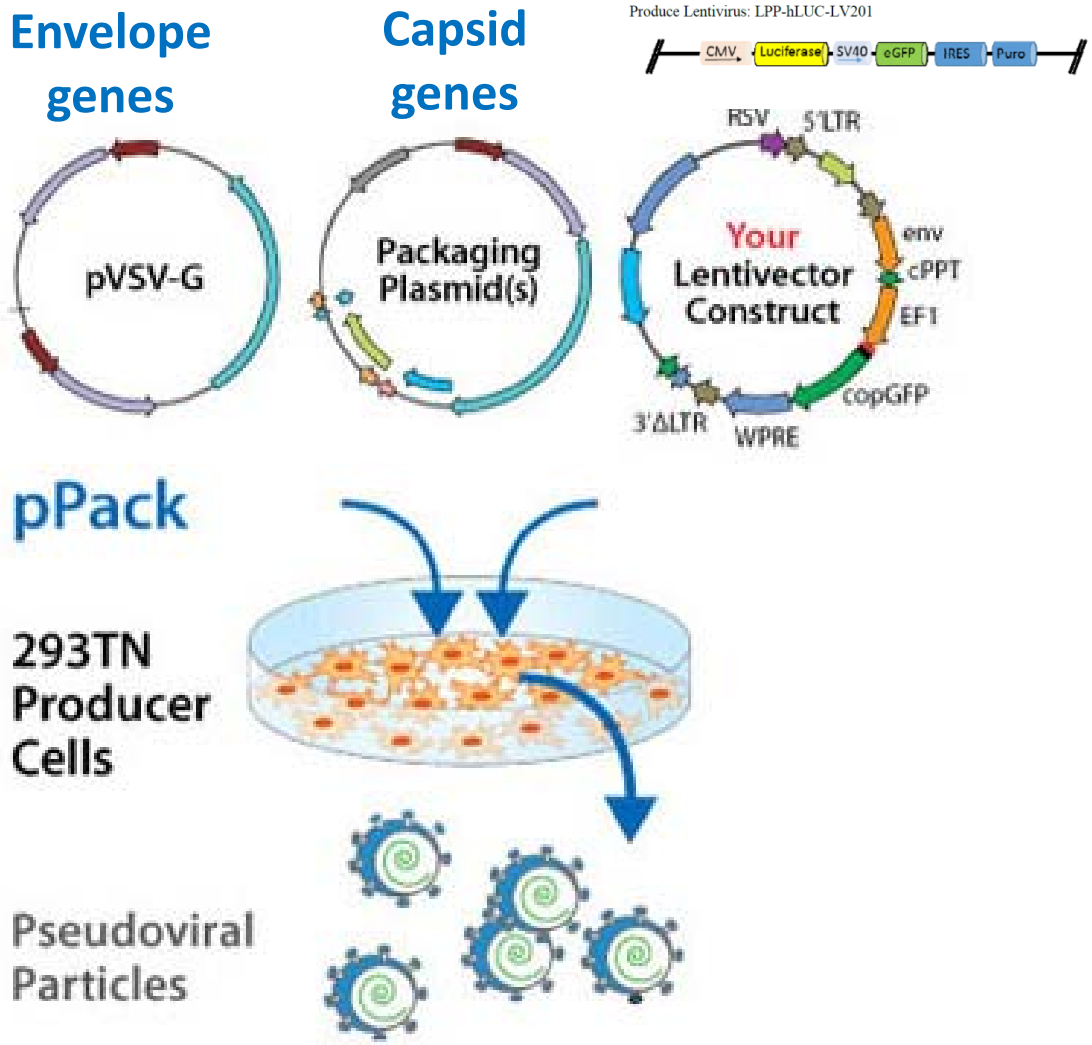
1. Production of GFP-luciferasa Lentivirus
2. Infection of B-NHL cell lines (MOI determination, cell sorting (GFP) and luciferase activity *in vitro*)
3. Subcutaneous (s.c.) or intravenous (i.v.) injection in immunocompromised mice (SCID or NSG) and luminescence detection



Hamamatsu imaging system

3-step protocol for GFP-LUC lentivirus production

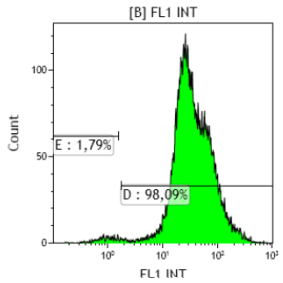




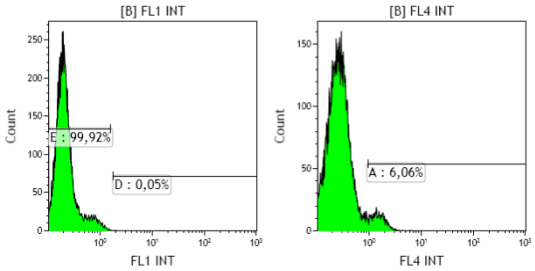
Luciferase activity *in vitro*

GFP/mCherry signal (CMF)

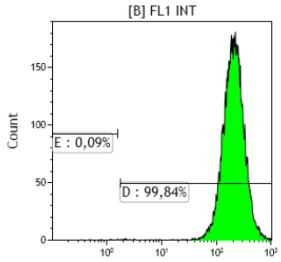
REC-1



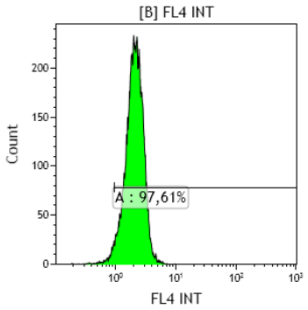
Z-138



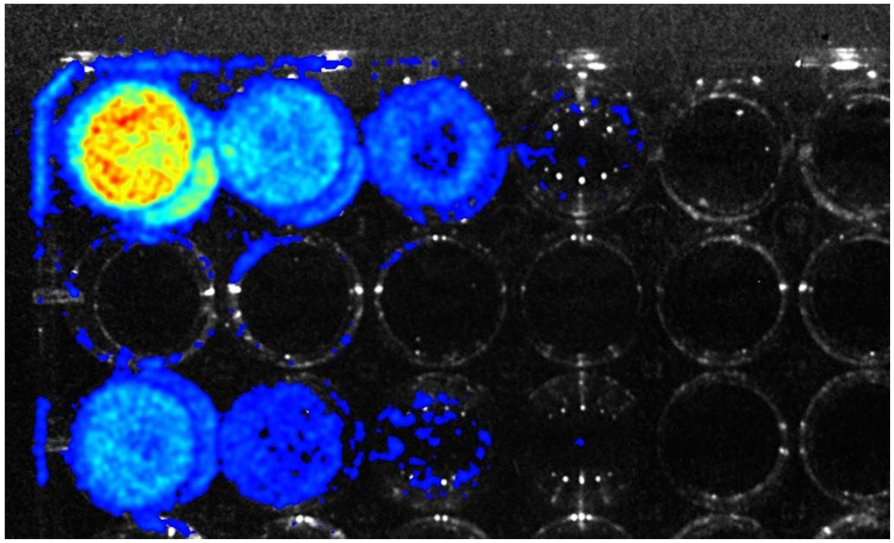
Granta-519

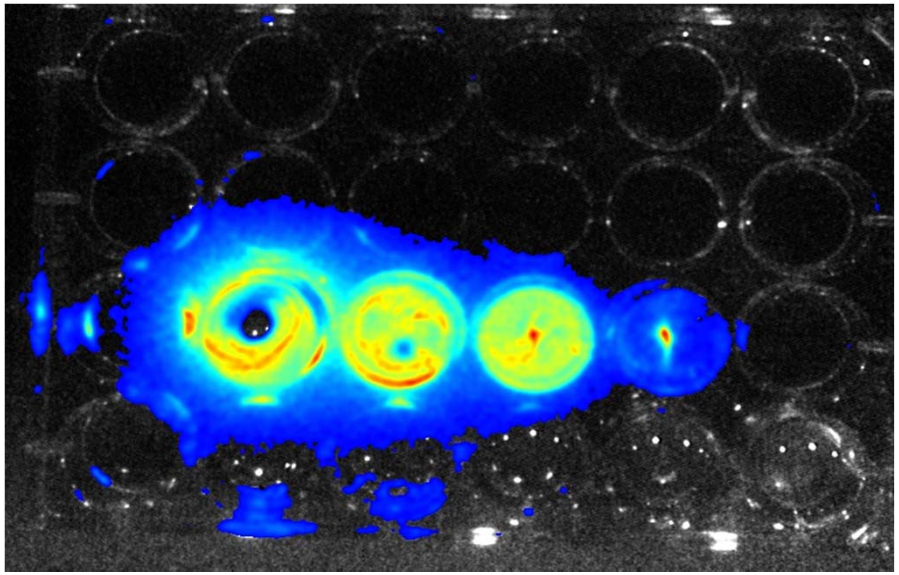


JeKo-1



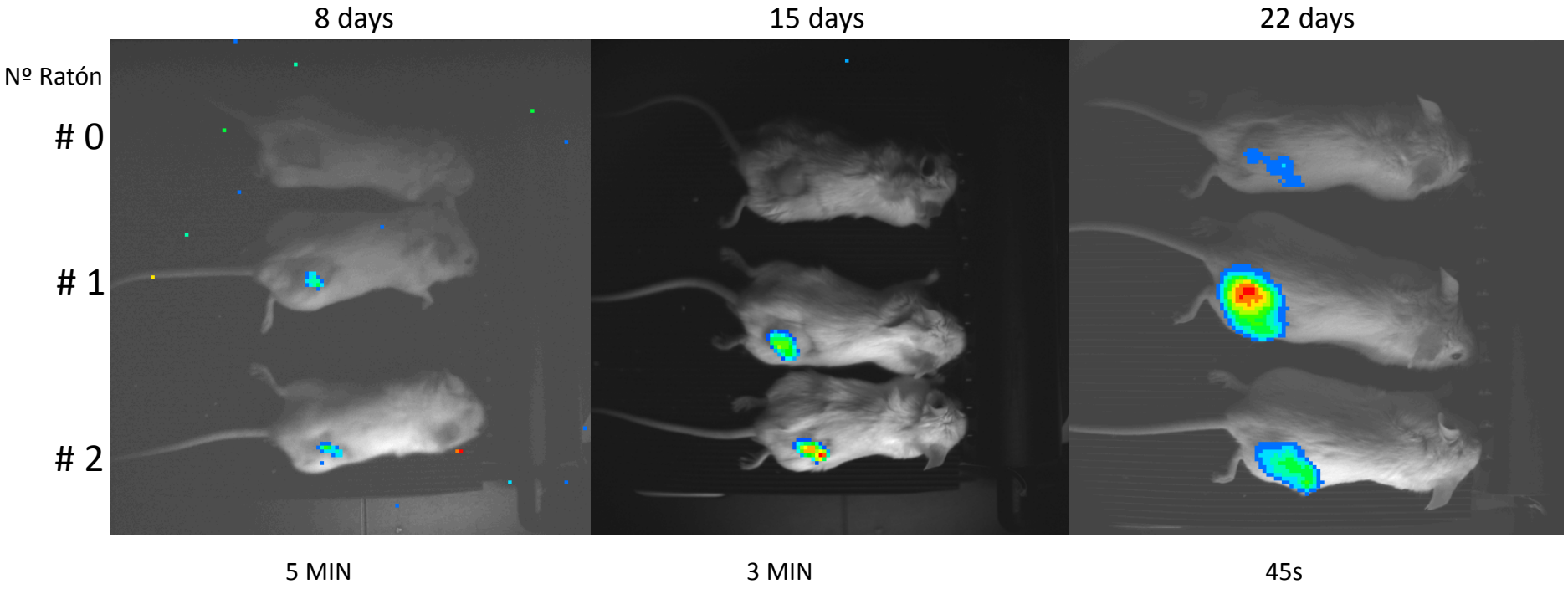
2.5 1 0.5 0.1 nb of cells (x10^6)





Luciferase activity *in vivo*

- Experiment: Z138-Luc
- SCID mice
- 10^7 cells/mouse (s.c.)
- 75mg/kg luciferine (i.p.)



Luciferase activity *in vivo*

Day 1 (5')

FRONT

Mouse

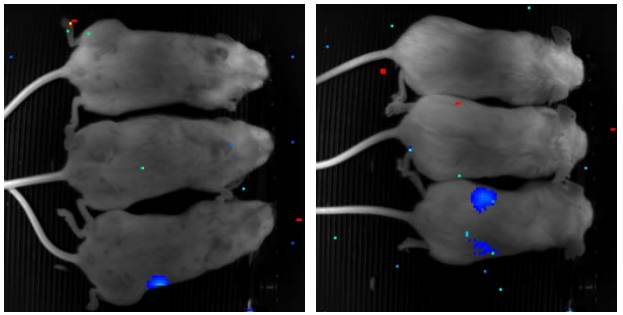
15

17

18

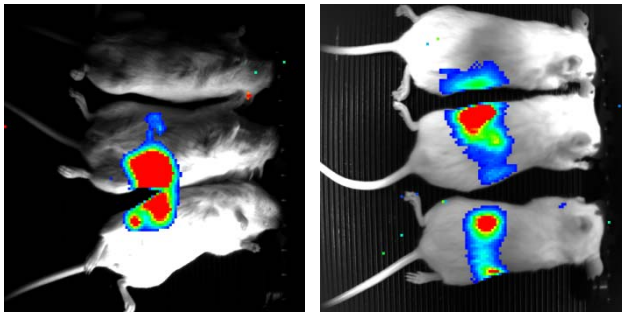
Day 15 (5')

FRONT **BACK**



Day 21 (3')

FRONT **BACK**



Day 28 (2'')

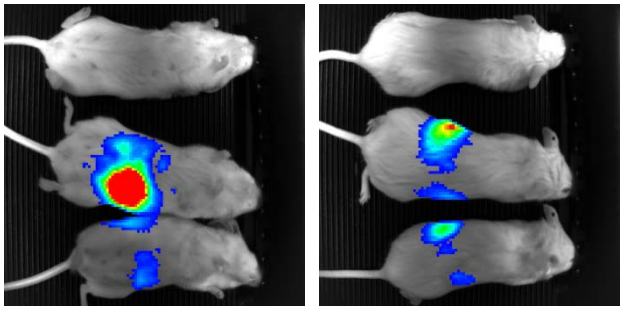
FRONT **BACK**

Mouse

15

17

18

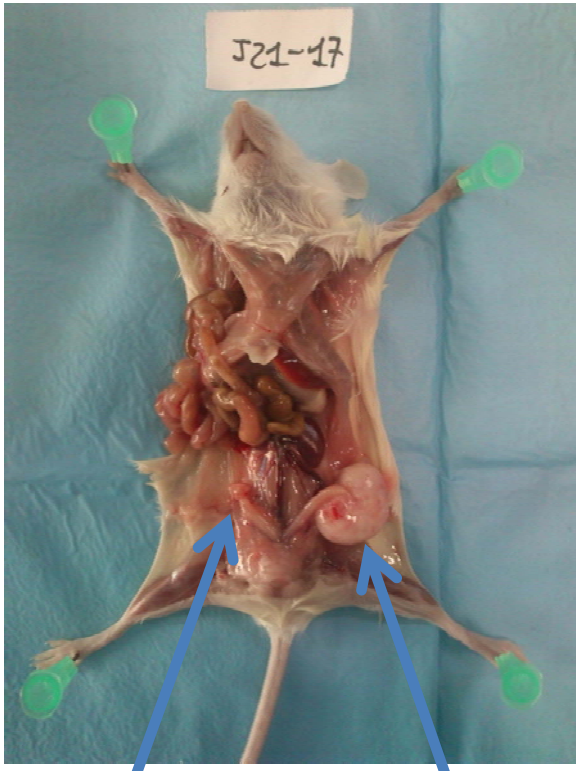


- Experiment: RL-Luc
- NSG mice
- 10^7 cells/mouse (i.v.)
- 75mg/kg luciferine (i.p.)

Luciferase activity *in vivo*

Normal ovary

Enlarged ovary



Normal ovary

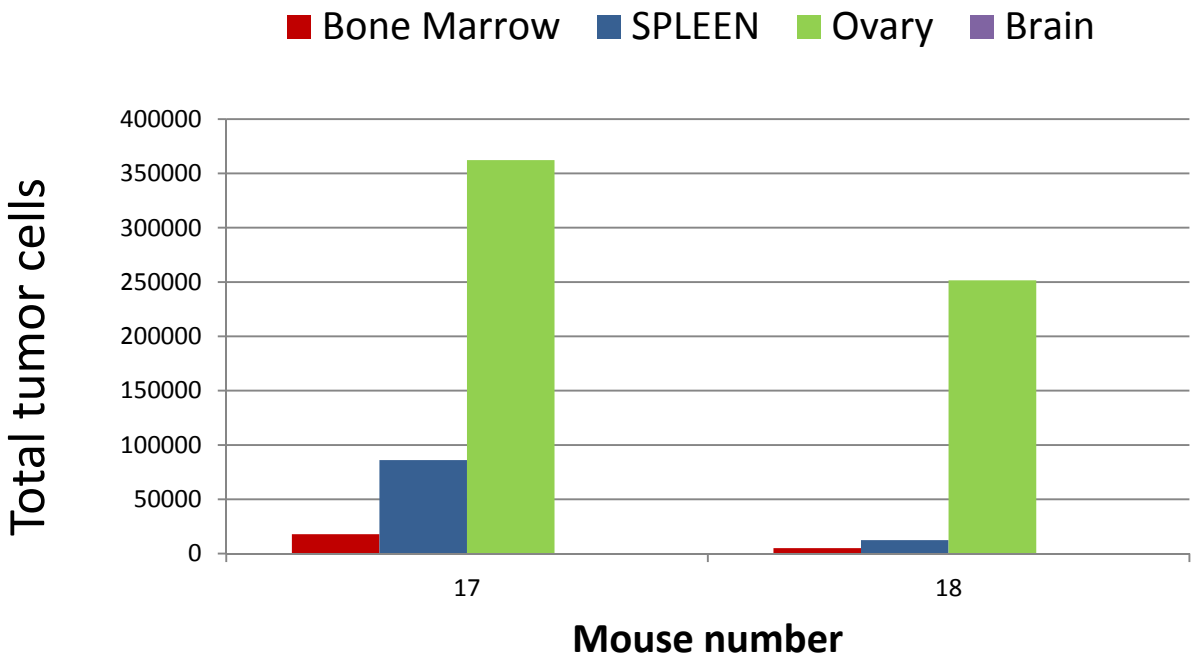
Enlarged ovary

Enlarged ovaries

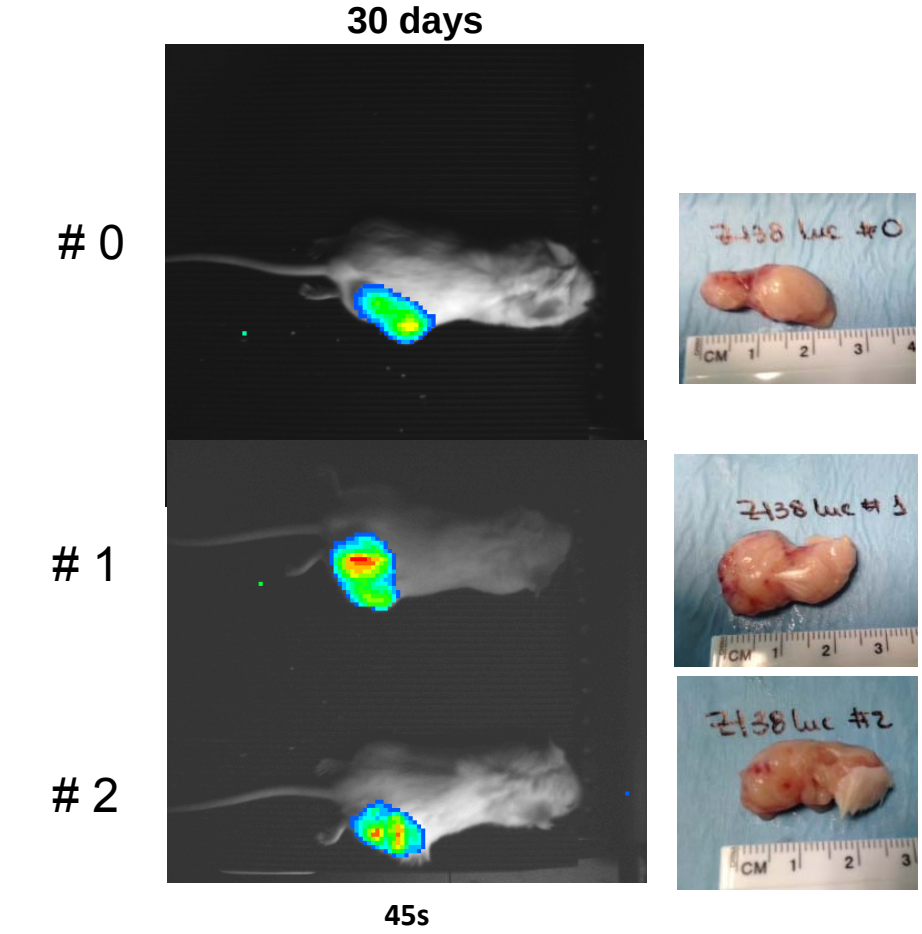
FL cell recount by flow cytometry

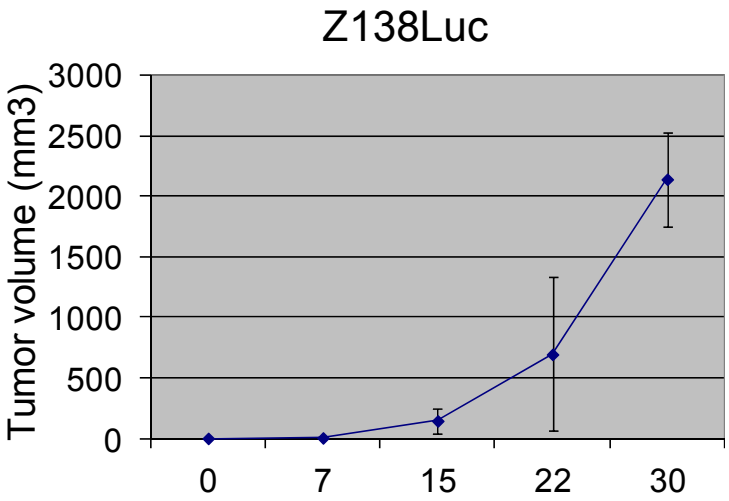
Flow cytometry detection of CD45+/CD20+/CD10+ cells

1. Peripheral blood (PB)
2. Bone marrow (BM)
3. Spleen
4. Brain
5. Ovary



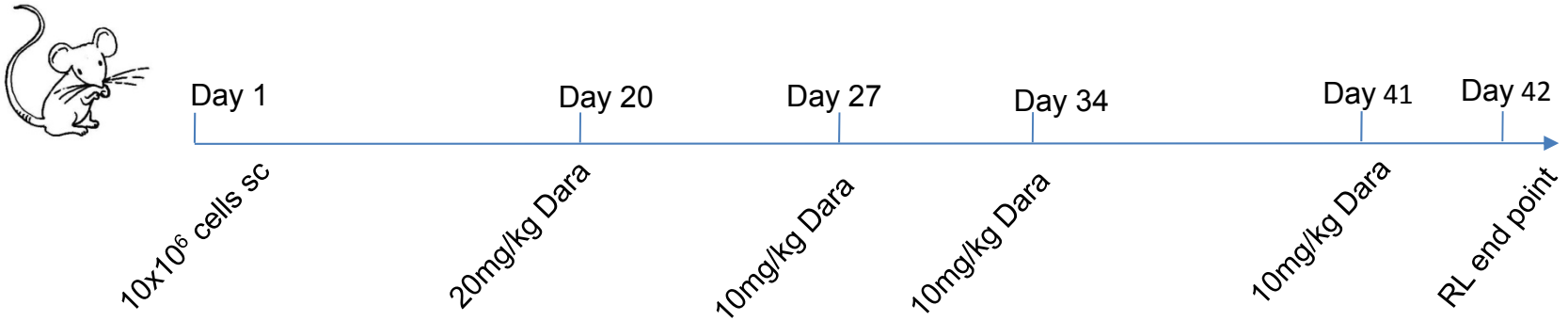
Luciferase activity *in vivo*



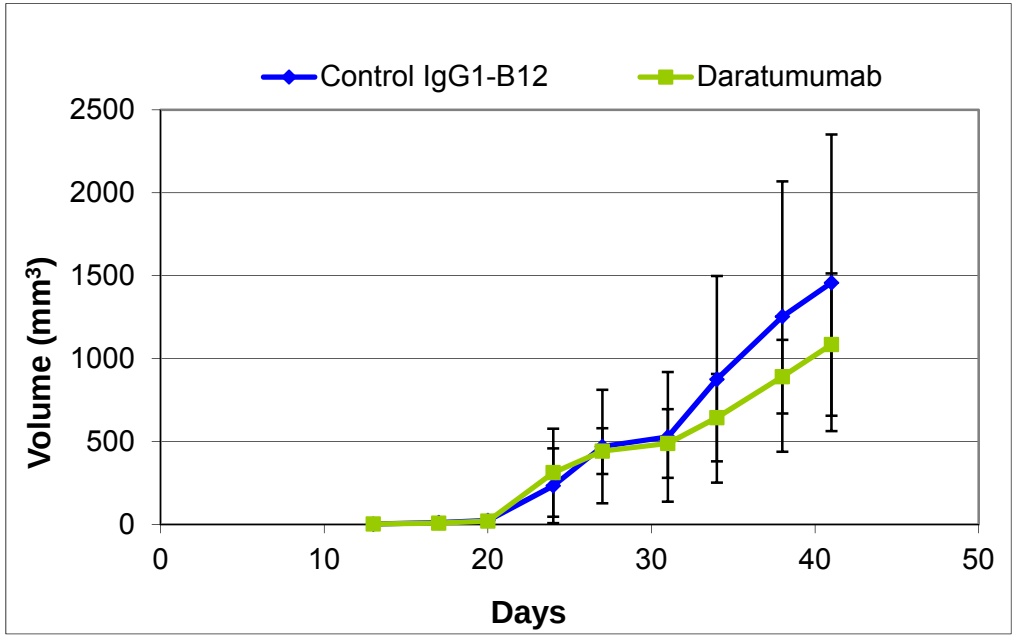


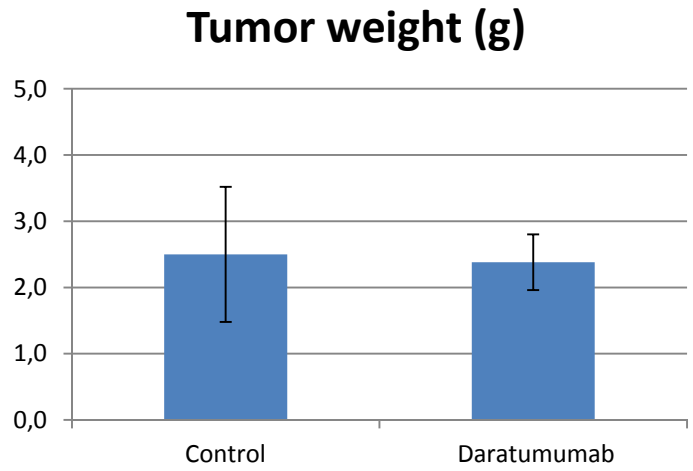
Example#1: anti-CD38 therapy in FL-bearing mice

- Daratumumab (human anti-CD38) activity in subcutunaeous xenograft models (SCID) with the FL cell line RL-Luc
- Cell line shows strong CD38 expression and good Dara-induced ADCC in vitro
- i.p. treatment after tumor growth. Treatment with a loading dose of 20mg/kg to ensure target saturation, followed by weekly dosing of 10mg/kg .

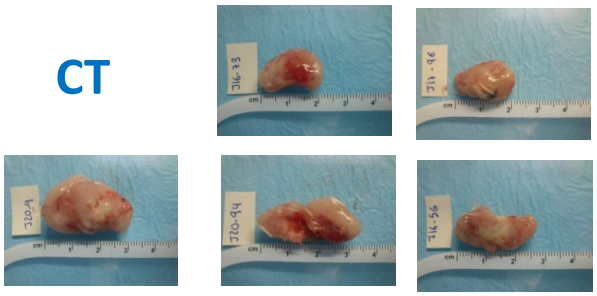


Example#1: anti-CD38 therapy in FL-bearing mice

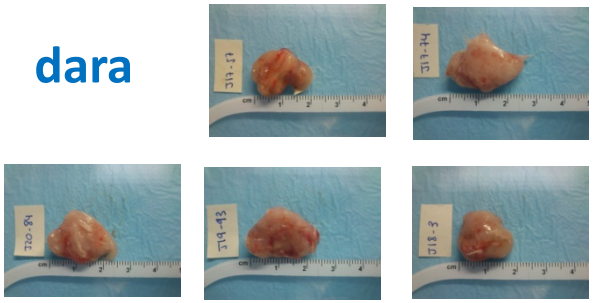


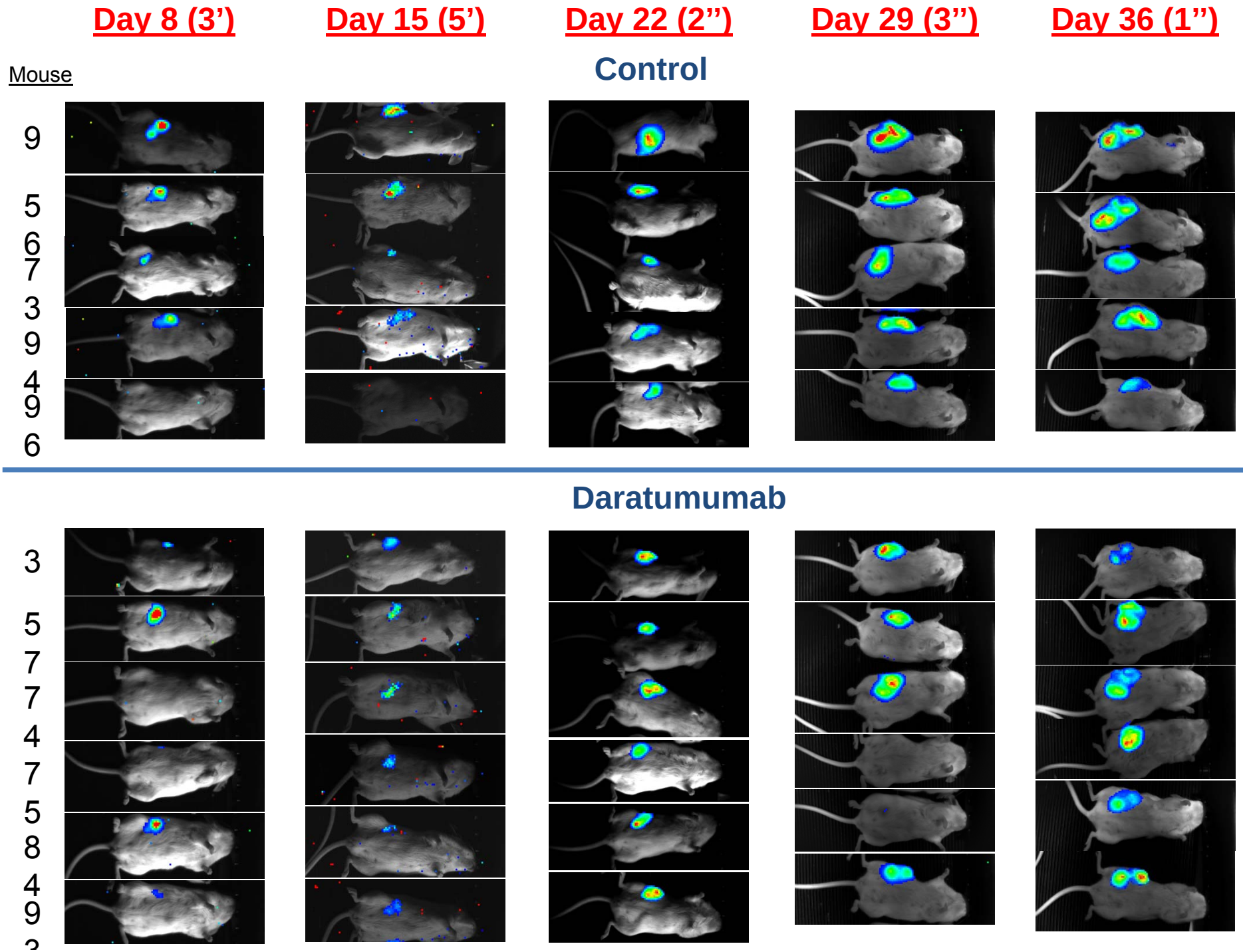


CT



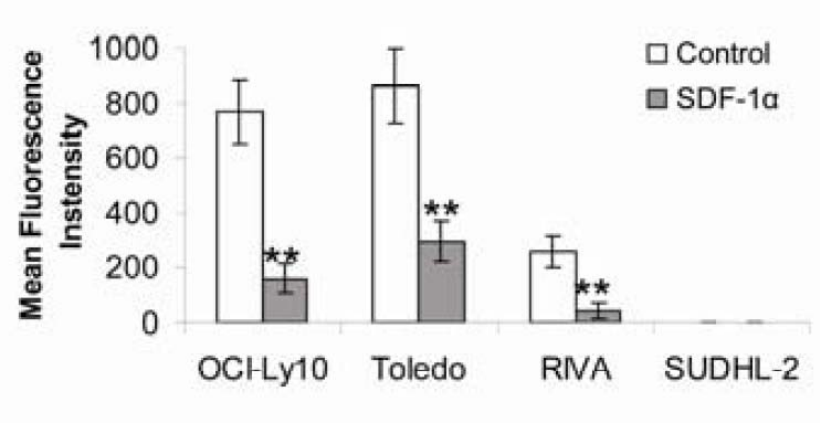
dara

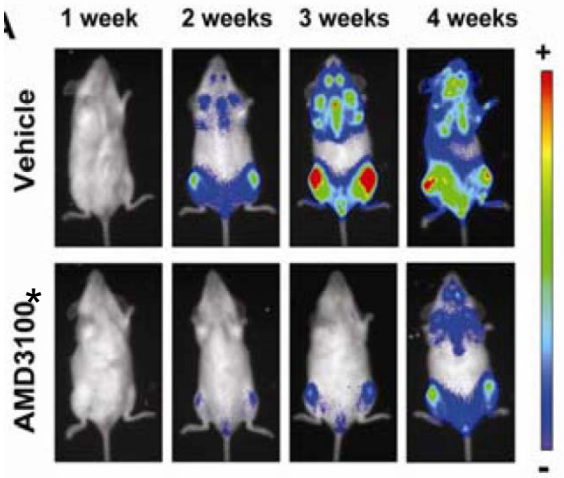


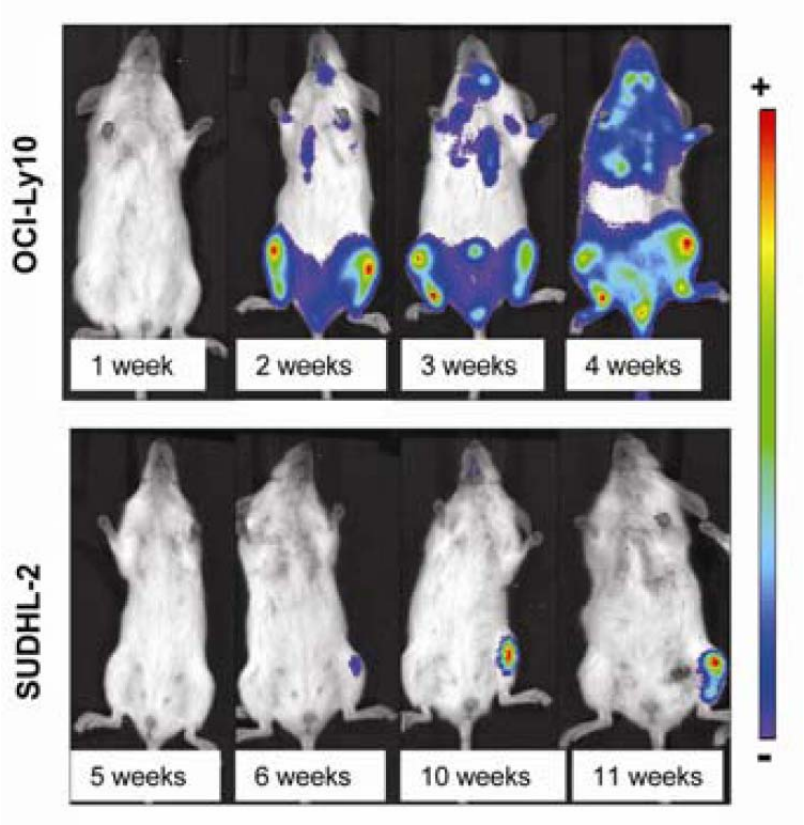

Day 8 (3')
Day 15 (5')
Day 22 (2")
Day 29 (3")
Day 36 (1")
Mouse
Control
9
5
6
7
3
9
4
9
6
Daratumumab
3
5
7
7
4
7
5
8
4
9

Example#2: Role of surface CXCR4 in DLBCL

Levels of CXCR4 surface expression correlate with aggressiveness after injection into mice.







Moreno et al, J Pathol , 2014

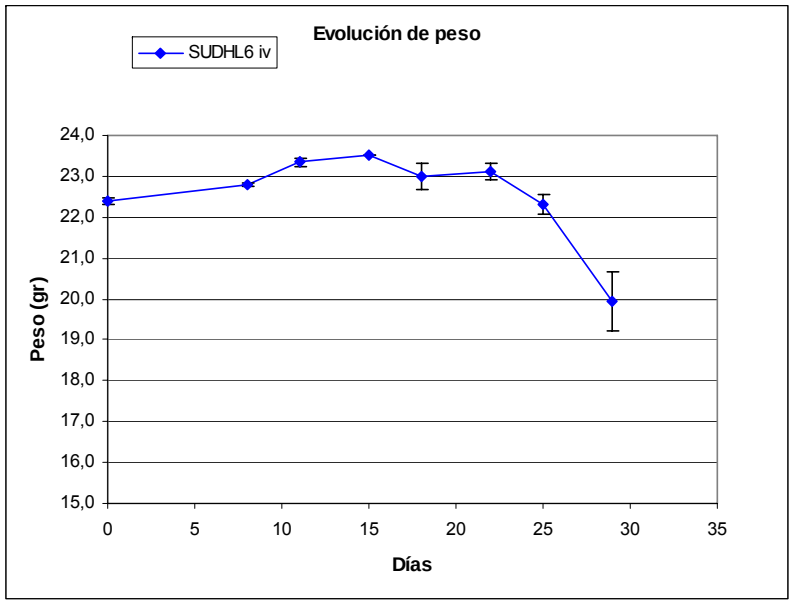
CXCR4 inhibition through Plerixafor (AMD3100) rescues mice from DLBCL agressiveness

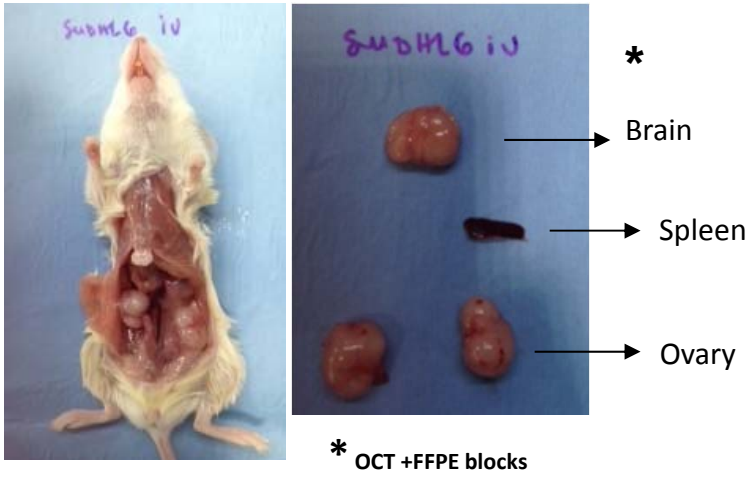
Bioluminescence (BLi): applications for drug discovery in lymphoma research

- **Limitations:**
  - Requires the use of transgenic cells
  - Not compatible with clinical studies
  - Limited to imaging growing cells

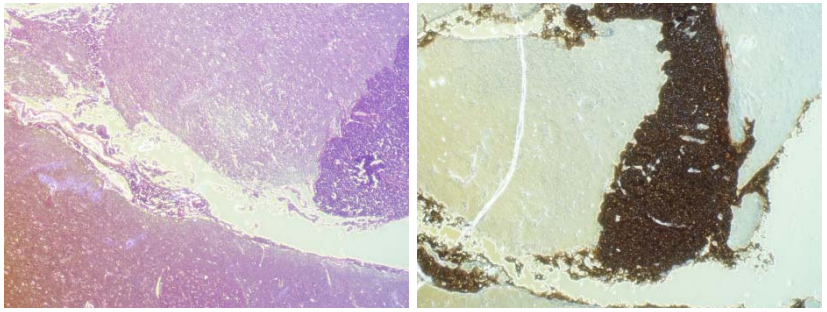
How to monitorize tumor burden while using hard-to-transfect malignant B cells?

SUDHL-6 (DLBCL cell line), 8x10^6 cells by i.v.



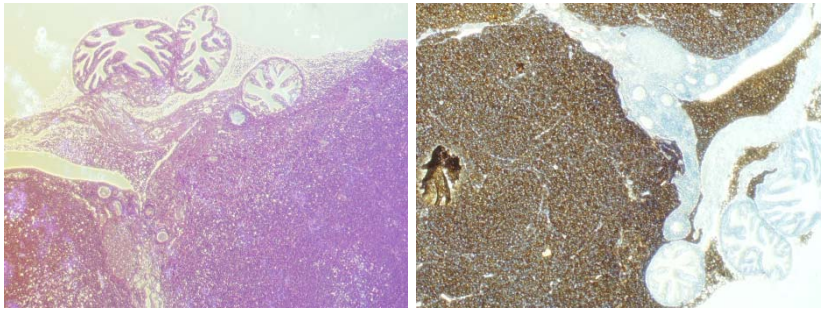


* OCT +FFPE blocks



H&E CD20

Brain



H&E CD20

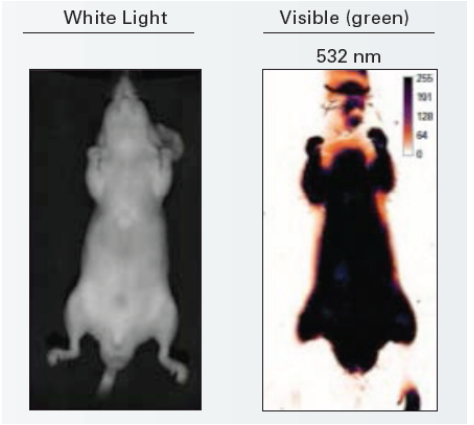
Ovary

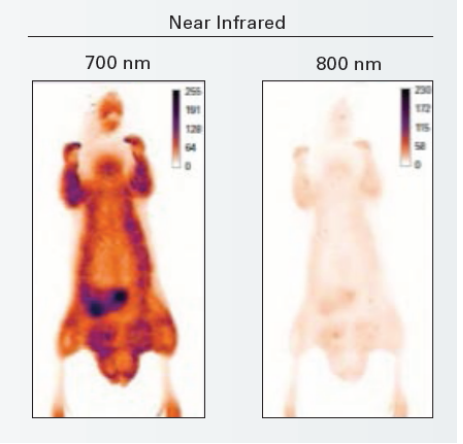
Recasens *et al,* unpublished data

Use of GFP-expressing cells or fluoresent probes

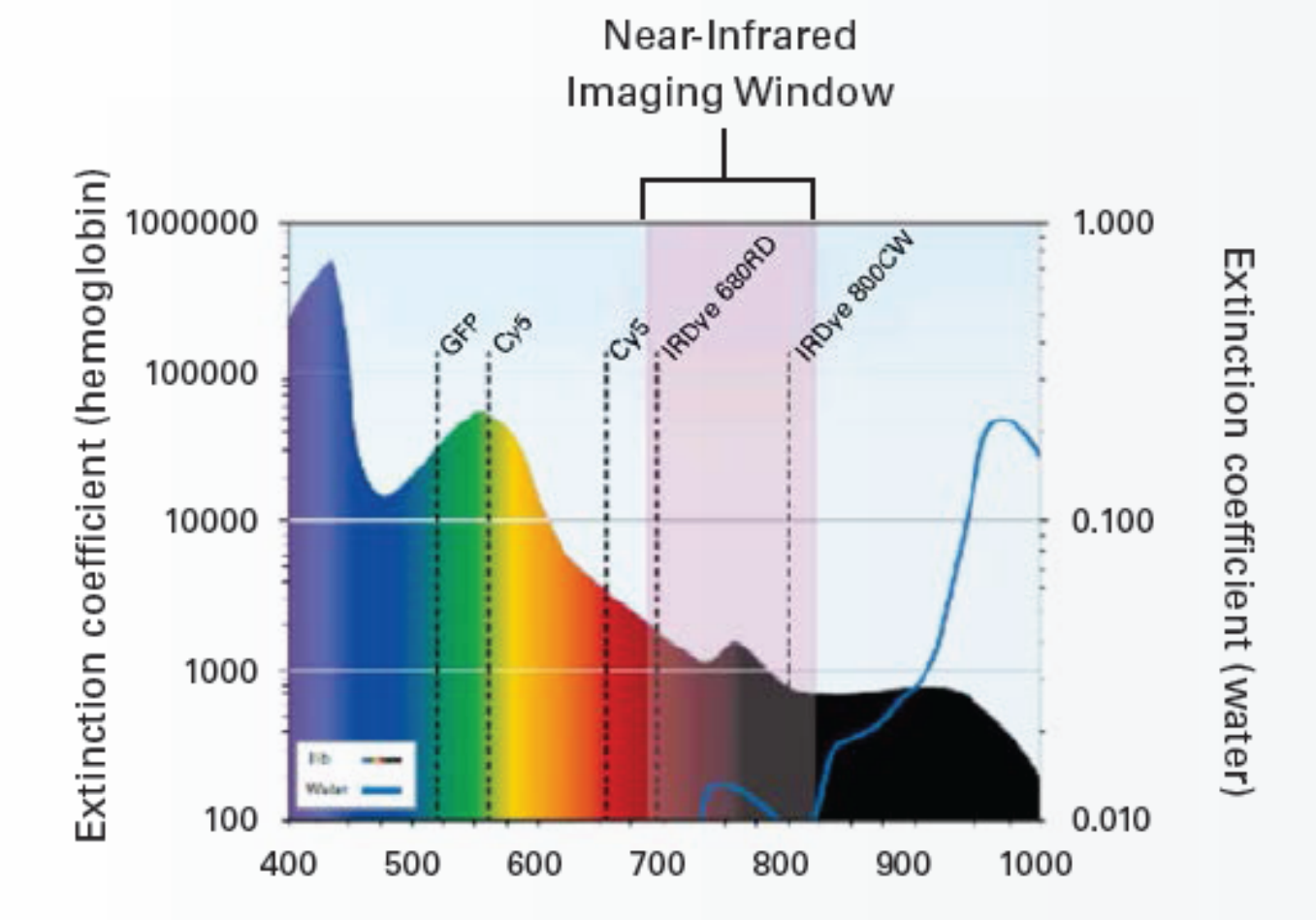
- **Limitations:**
  - visible fluorophores do not offer optimal performance for all applications
  - cells, animal tissue, plasticware, blotting membranes, and chemical compound libraries all possess intrinsic autofluorescence that can interfere with detection

- **Solution:**
  - in the near-infrared (NIR) spectral region (700-900 nm), autofluorescent background is dramatically reduced.





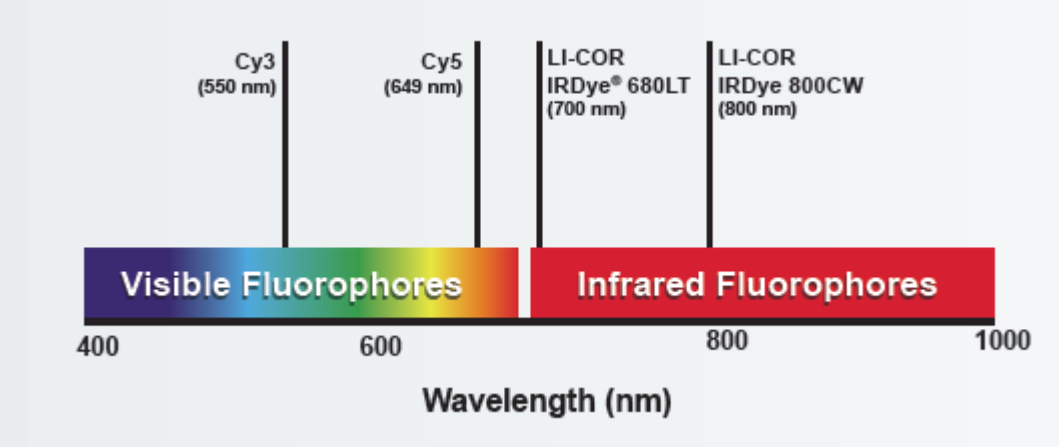
Animal tissue absorbs visible light



- Hemoglobin (Hb) and other tissue components strongly absorb visible light.
- In the NIR region, where IRDye agents are detected, tissue absorbance is dramatically reduced.
- Above 820 nm, light absorbance by water increases and can affect performance.

Near-infrared (NIR) fluorescence detection system using NIR dyes

- IRDye® infrared dyes: first commercialized in 1993 by LI-COR Biosciences:
  - reduced autofluorescent background
  - high sensitivity
  - enhanced signal-to-noise ratios
  - wide dynamic range
  - fast
  - easy to conjugate to various molecules
  - native cell lines may be used
  - potential for clinical translation



- **Applications:** Western Blotting, Multiplex Phosphorylation Analysis, Protein Microarrays, In-Cell Western™ Immunofluorescent Assay, Electrophoretic Mobility Shift Assays (EMSA), IRDye FRET Assays for Protease Activity, Mycroscopy, **In vivo Optical Imaging**

IRDye fluorophore and characteristics

Dye	Exmax (nm)	Emmax (nm)	Reactive Group	Recommended for labeling	LI-COR channel
IRDye 800CW	778	794	NHS ester Maleimide	Proteins Peptides	800 nm
IRDye 800RS	770	786	NHS ester	Nucleic acids	800 nm
IRDye 680RD	680	694	NHS ester Maleimide	Proteins Peptides	700 nm
IRDye 680LT	680	694	NHS ester Maleimide	Proteins Peptides	700 nm
IRDye 700DX	680	687	NHS ester	Proteins Peptides	700 nm
IRDye 750	766	776	NHS ester Maleimide	Proteins Peptides	–
IRDye 650	651	668	NHS ester Maleimide	Proteins Peptides	–
IRDye 700	685	705	Phosphoramidite	Oligos	700 nm
IRDye 800	795	819	Phosphoramidite	Oligos	800 nm

NIR imaging systems

MousePOD® *in vivo* Imaging Accessory for the Odyssey CLx Infrared Imaging System.

Odyssey® FcSystem

FieldBrite™ XT optical system

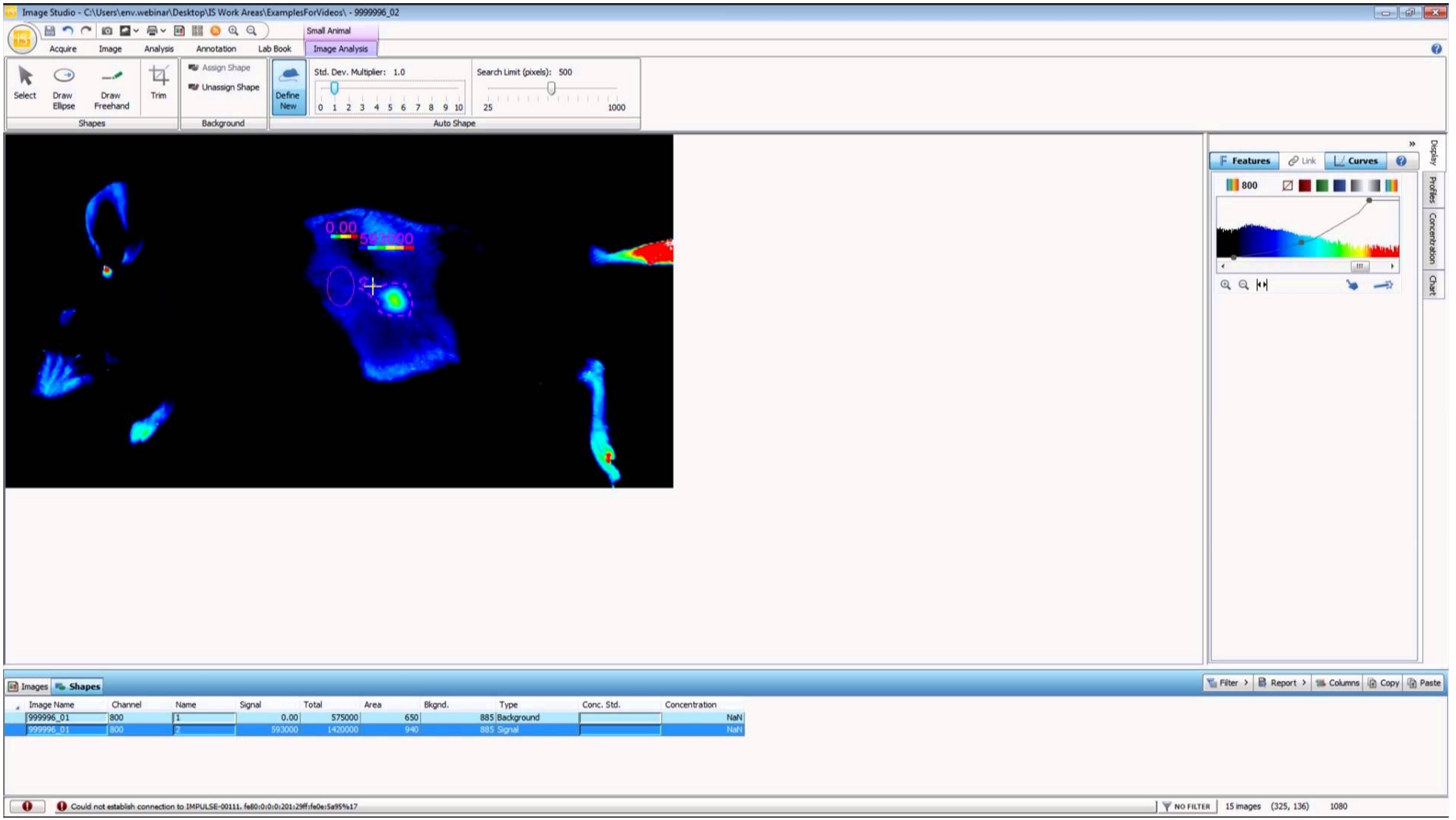
- Systematic approach
- Exceptional reagents
- Highly specific
- in vivo and in vitro
- Excellent binding activity
- Efficient clearance
- Research versatility

Pearl Impulse

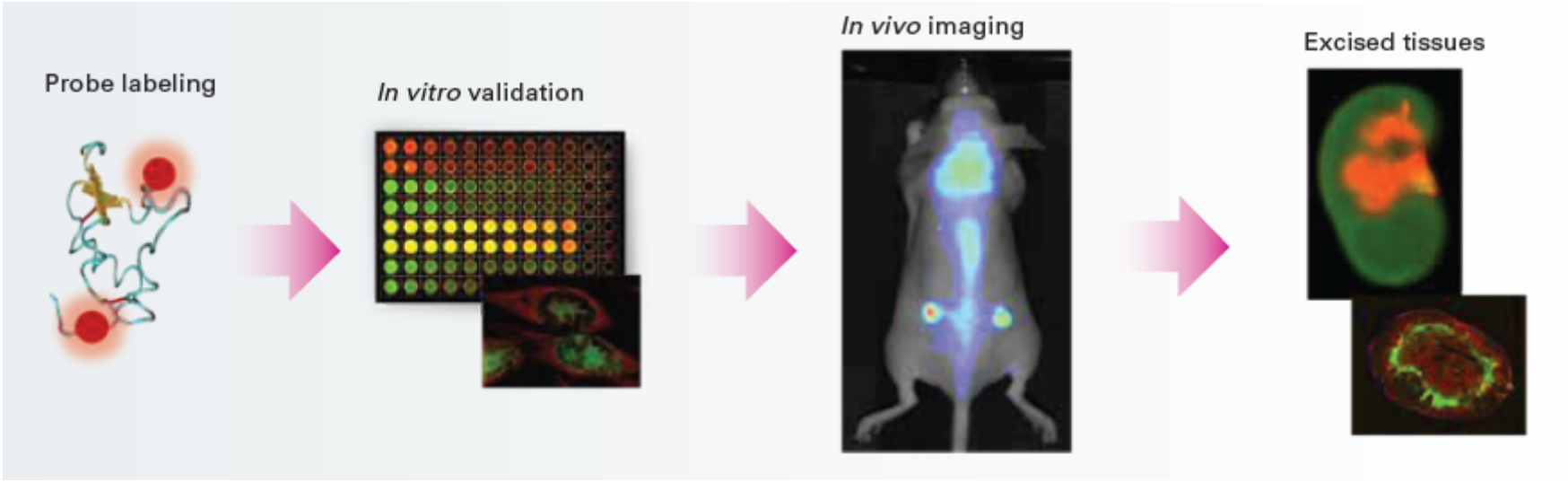
FieldBrite™ Xi CCD-based optical system

- Affordable benchtop system
- Best NIR detection: FieldBrite Xi
- Simplicity – No adjustments.
- Dynamic Range – No saturation, long term studies
- Speed – Fluorescent images in 30 seconds
- Sensitivity – NIR allows detection of deeper targets
- Reliability – The system simply WORKS!
- Designed to work with IRDye 800CW

Acquisition and analysis: Image Studio v5.0

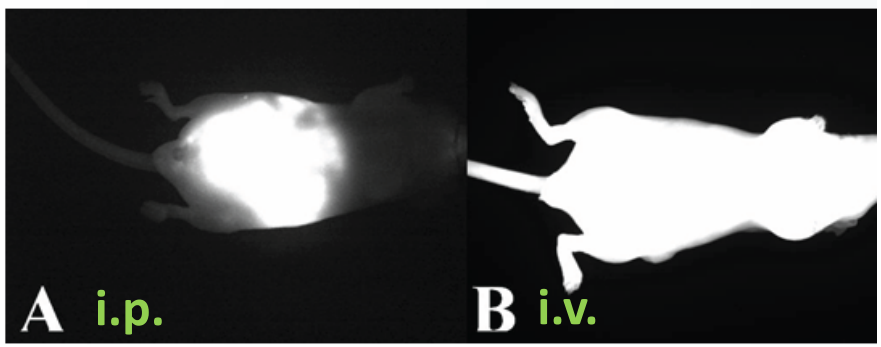


IRDyes: workflow



- NIR optical imaging: non-invasive study of molecular targets inside the body of the living animal to:
  - follow the progression of disease
  - evaluate the effects of drug candidates on the target pathology
  - analyze the pharmacokinetic behavior of drug candidates
  - to develop biomarkers indicative of disease and treatment outcomes.

IRDyes: administration site and clearence



15min post-injection

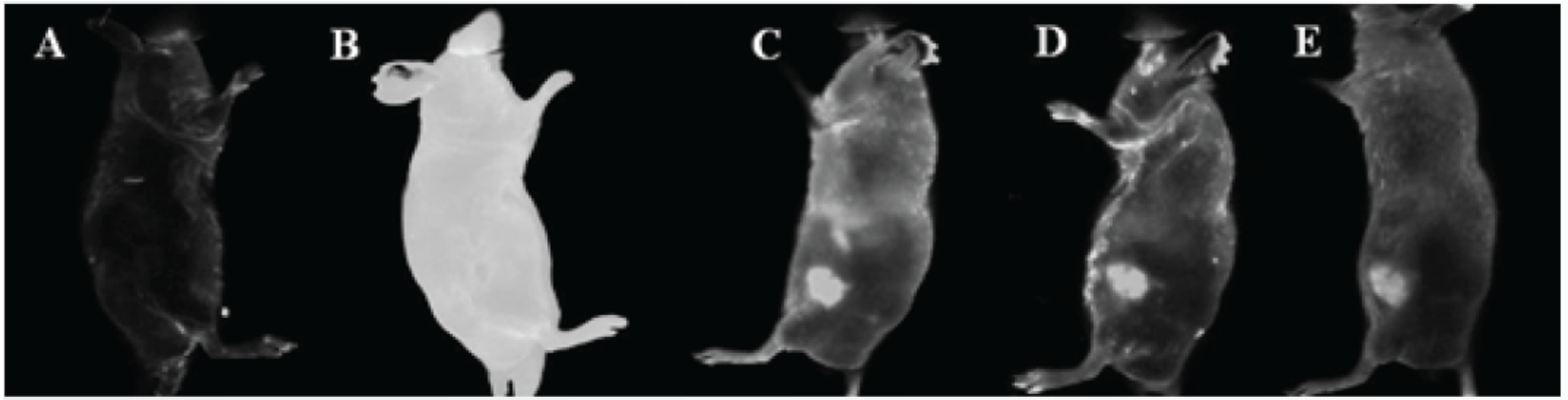
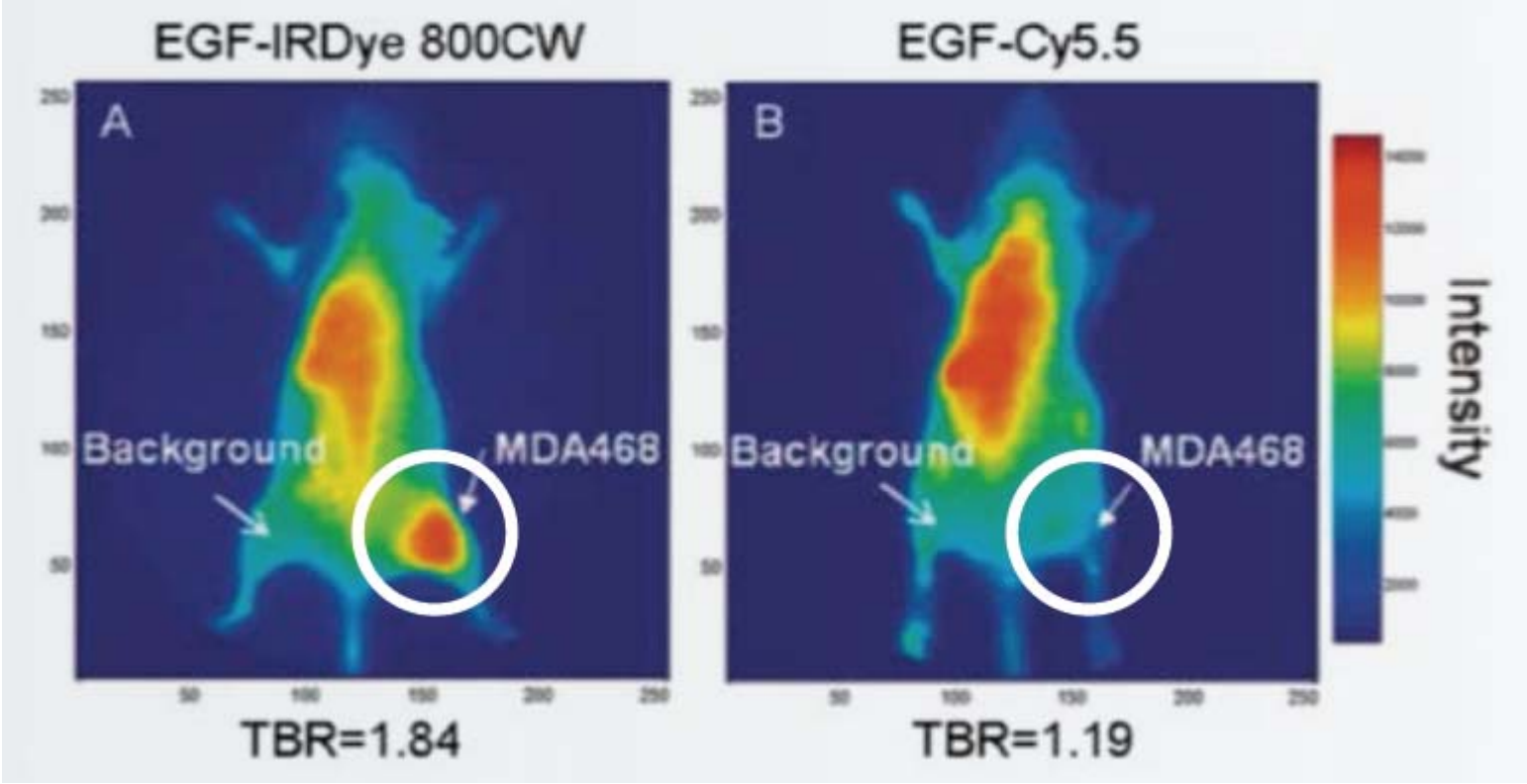


Figure 8. Time course for accumulation of IRDye® 800CW EGF in a subcutaneous tumor. Images of a nude mouse were collected prior to injection (A), or at 20 min (B), 24 h (C), 48 h (D), and 72 h (E) following intravenous injection of the animal with 1 nmol of IRDye 800CW EGF.

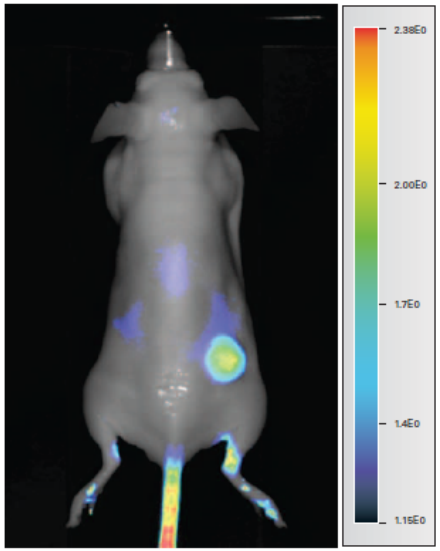
IRDyes: improved target-to-background ratio

Comparison of target-to-background ratio (TBR) with IRDye® 800CW and Cy5.5 fluorescent conjugates.

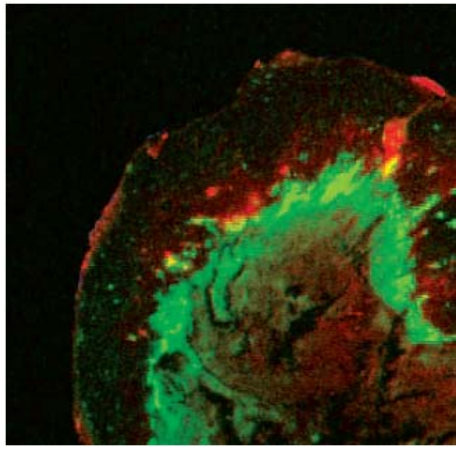


Adapted from Adams, KE et al. *J Biomed Optics 12: 024017 (2007)*

Example#1: use of IRDye 2-DG optical probe for the monitoring of tumor outgrowth and validation of novel therapeutic agents in B-cell lymphoma

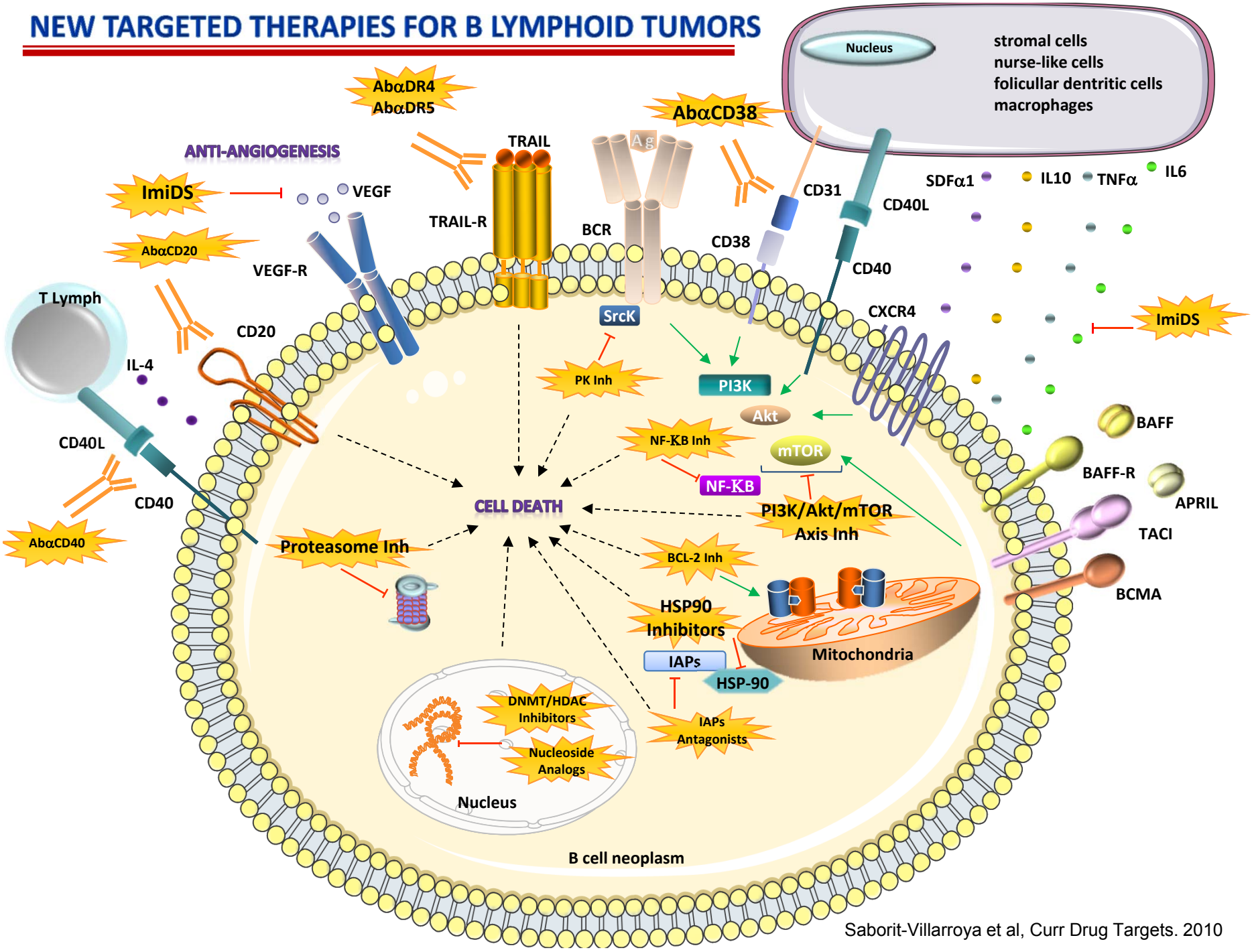


Increased glucose metabolism imaged with IRDye® 800CW 2-DG. Subcutaneous A431 tumor was detected. Image acquired with Pearl® Impulse



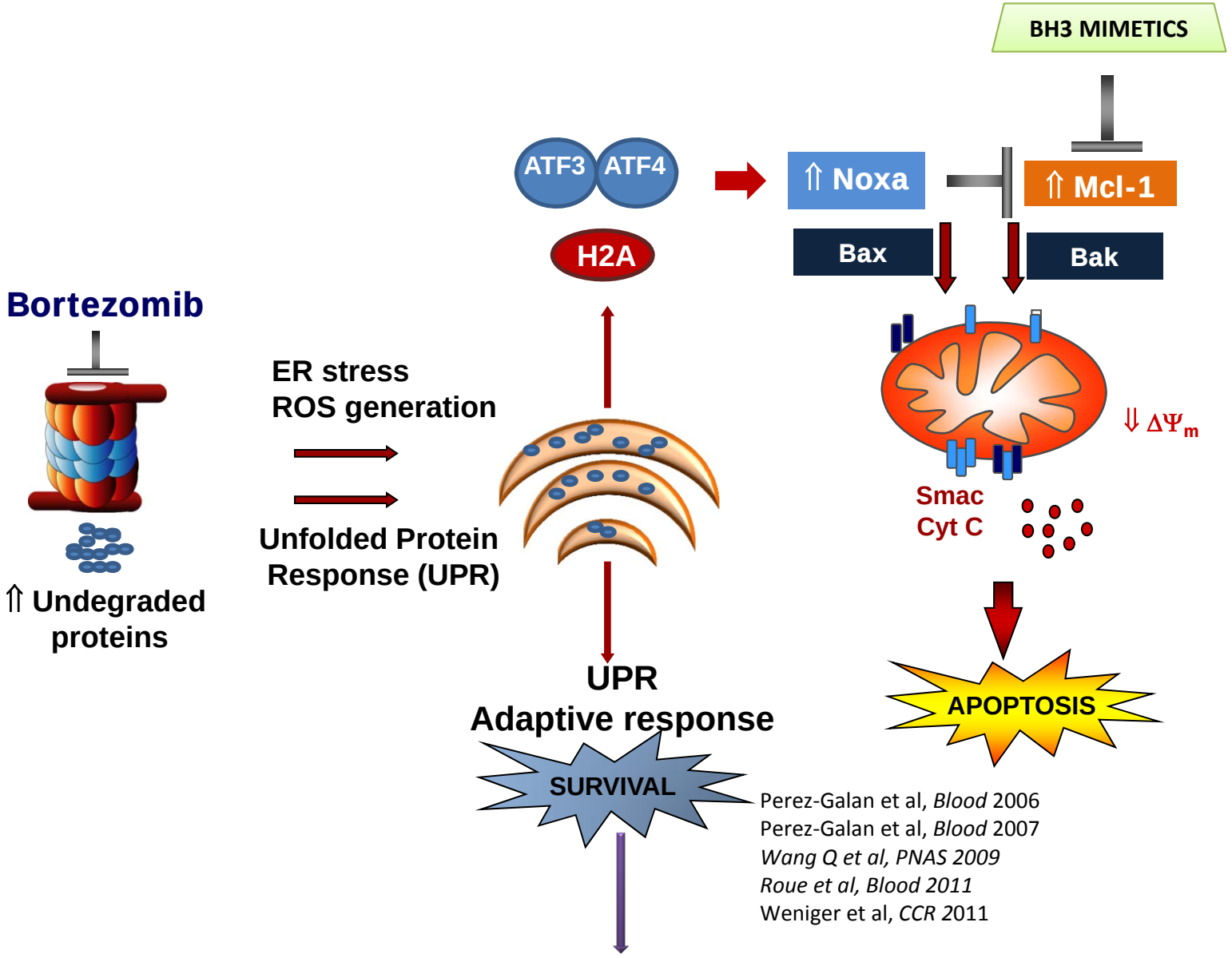
Uptake of IRDye® 800CW 2-DG probe by hypoxic tissue surrounding the necrotic center of a tumor. A431 tumor was excised, fixed, and embedded. Tissue sections were then imaged at 21 µm resolution with Odyssey® Classic Imager. Green indicates probe fluorescence (800 nm) and red indicates tissue autofluorescence

NEW TARGETED THERAPIES FOR B LYMPHOID TUMORS



Saborit-Villarroya et al, Curr Drug Targets. 2010

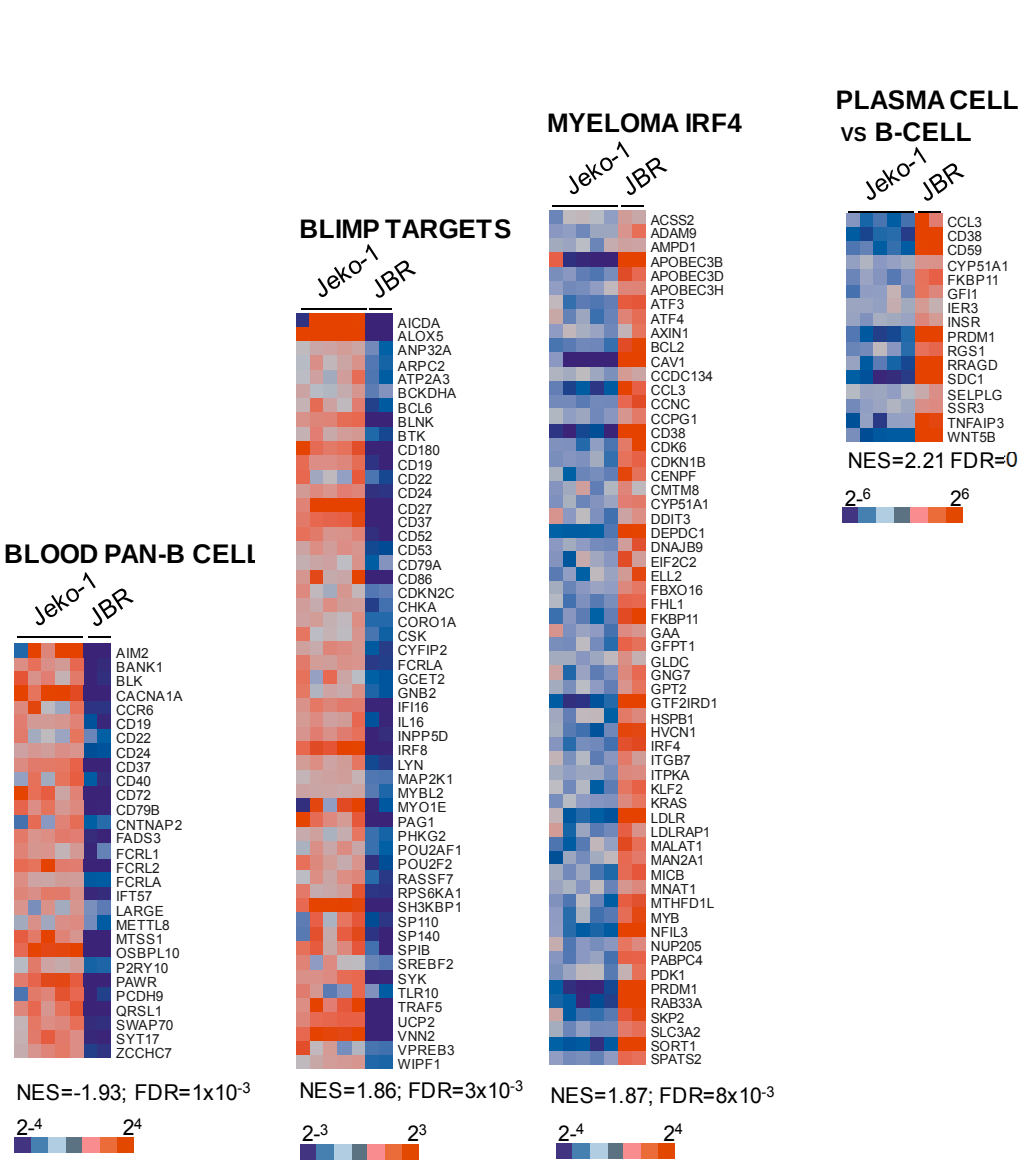
Bortezomib in B-NHL: proposed mechanism of action

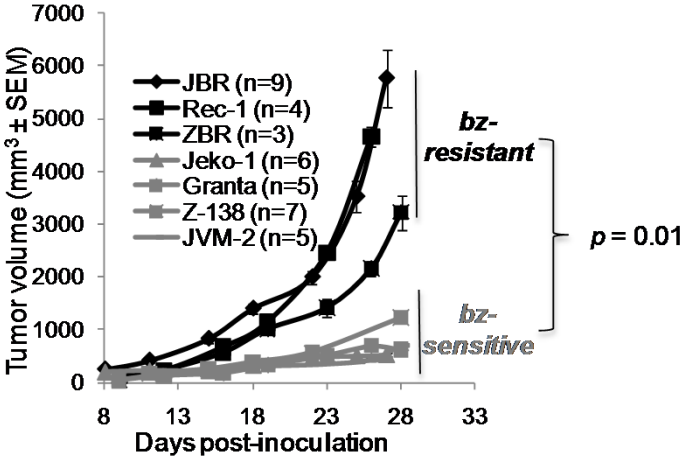


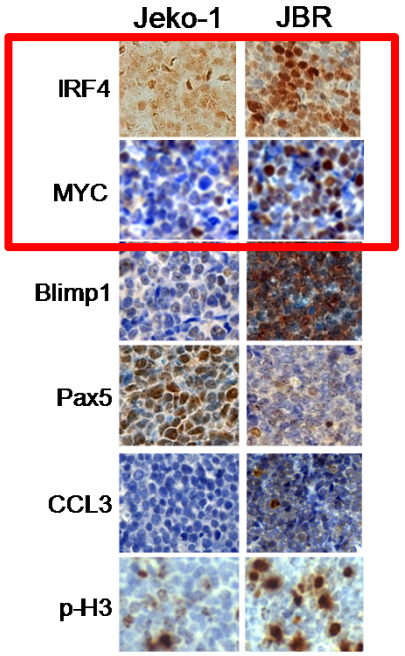
Perez-Galan et al, *Blood* 2006
Perez-Galan et al, *Blood* 2007
Wang Q et al, PNAS 2009
Roue et al, Blood 2011
Weniger et al, *CCR* 2011

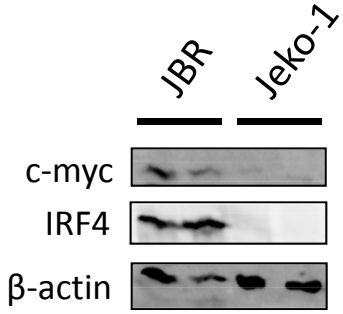
GX15-070 MCL (Bortezomib) phase I/II Protocol ID GEM012

INCREASED TUMORIGENECITY OF BZ-RESISTANT MCL CELL LINES IS ASSOCIATED WITH PLASMACYTIC DIFFERENTIATION





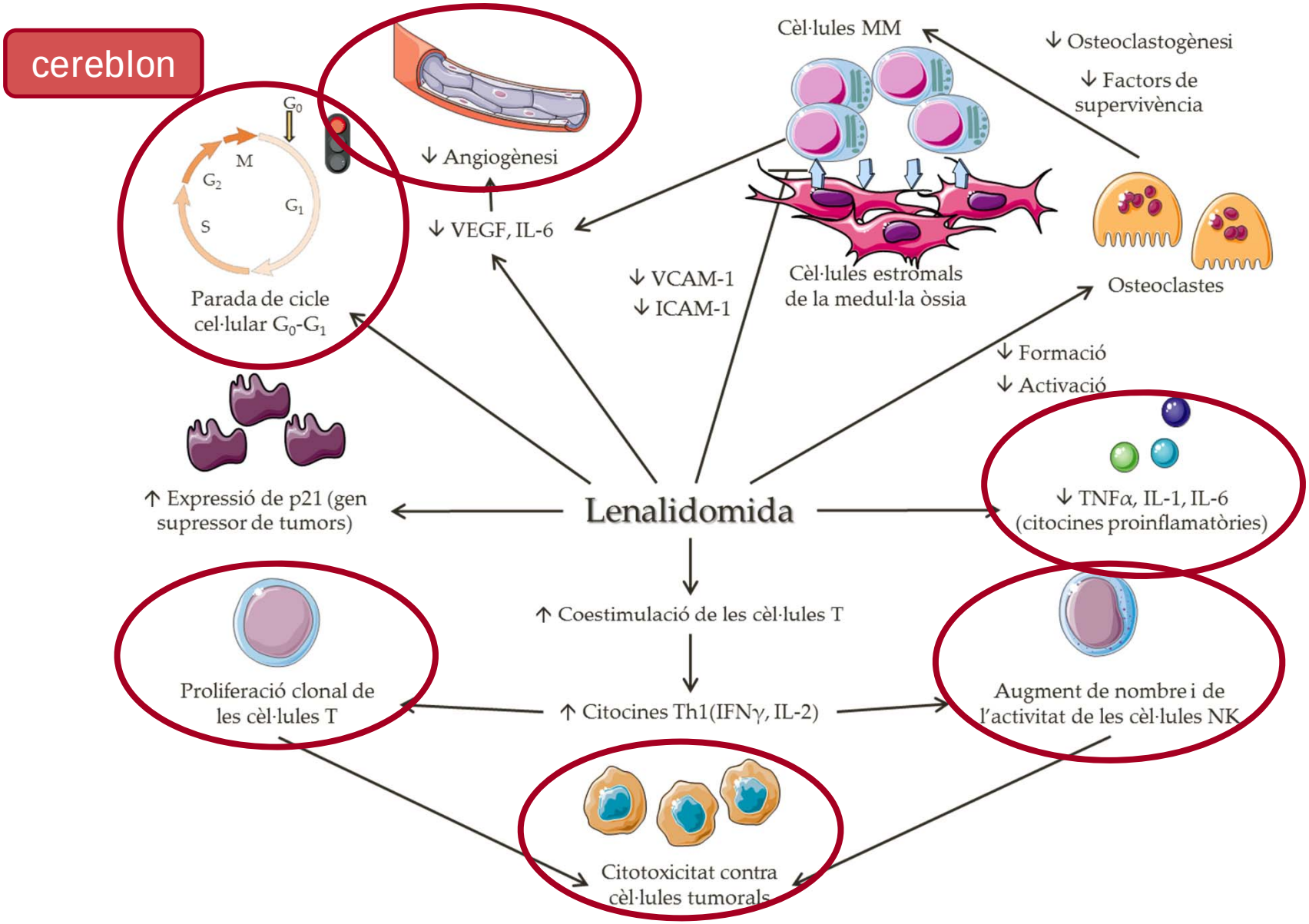




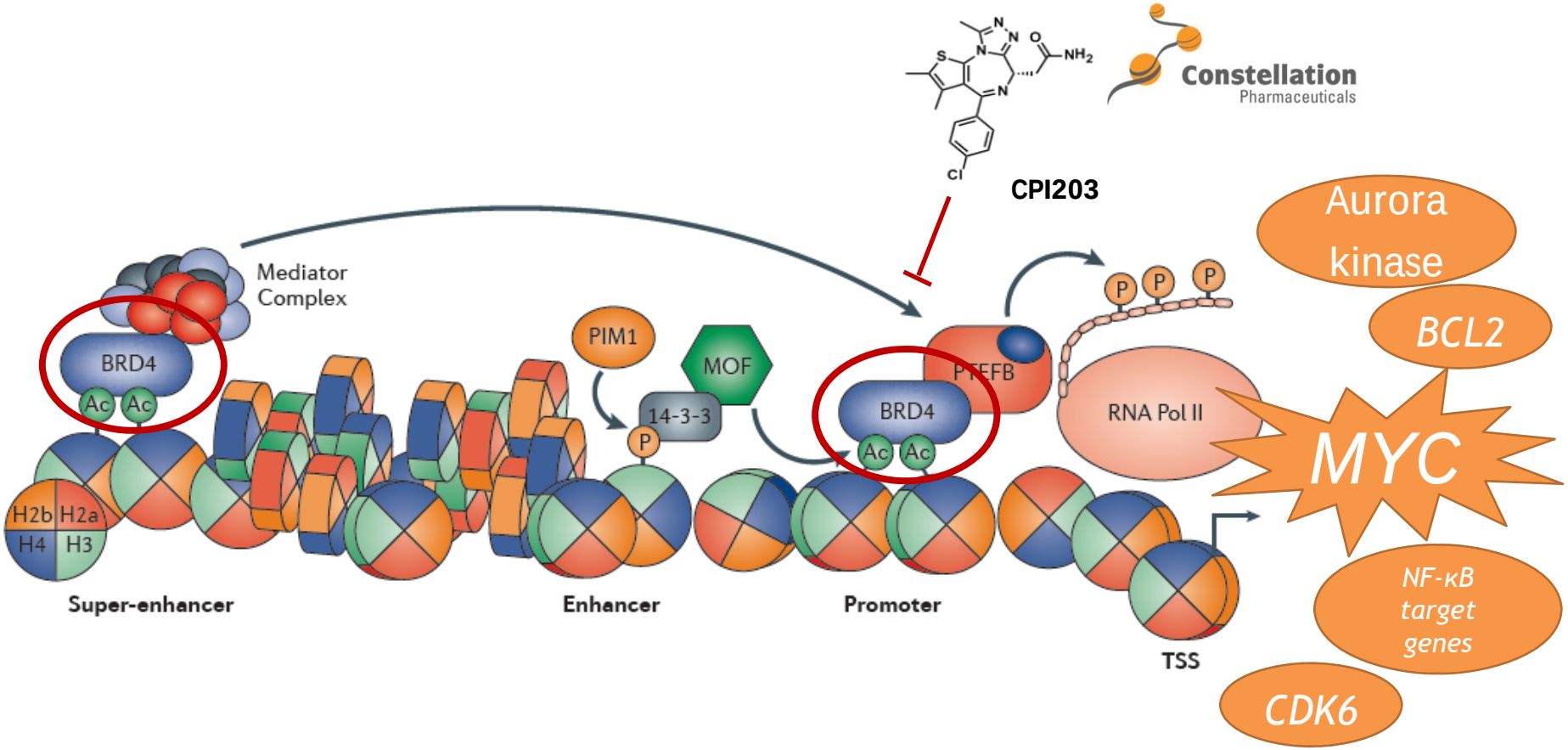
Moros A *et al*. Leukemia. 2014 Oct;28(10):2049-59.

Lenalidomide: a post-translational inhibitor of IRF4

cereblon

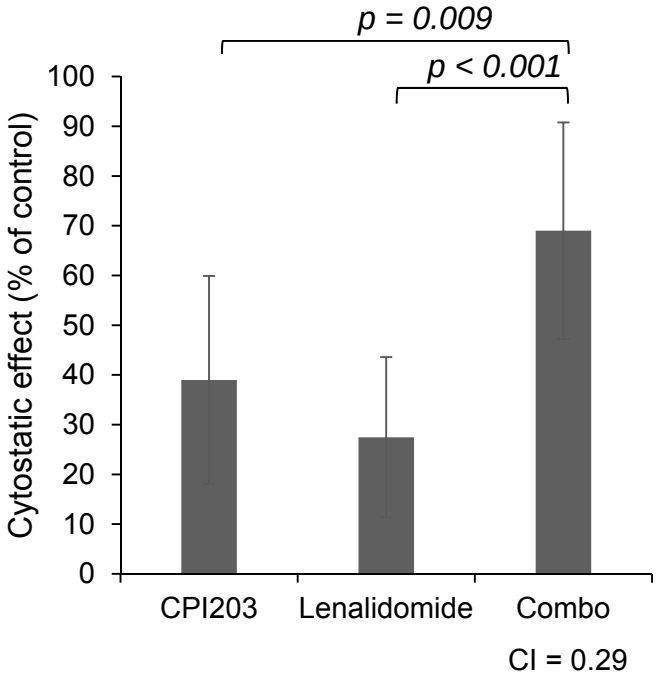


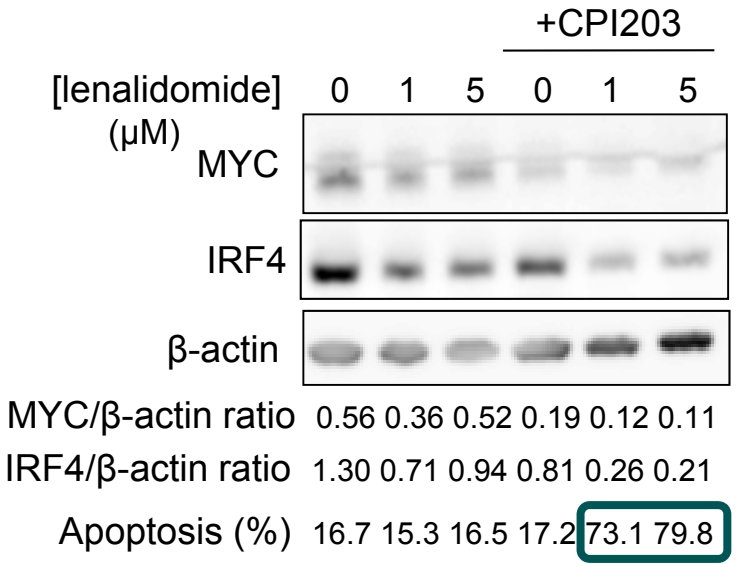
CPI203 (BETi): interferes with BRD4 and MYC transcription



Adapted from Filippakopoulos P, Knapp S. Nat Rev Drug Discov. 2014 May;13(5):337-56.

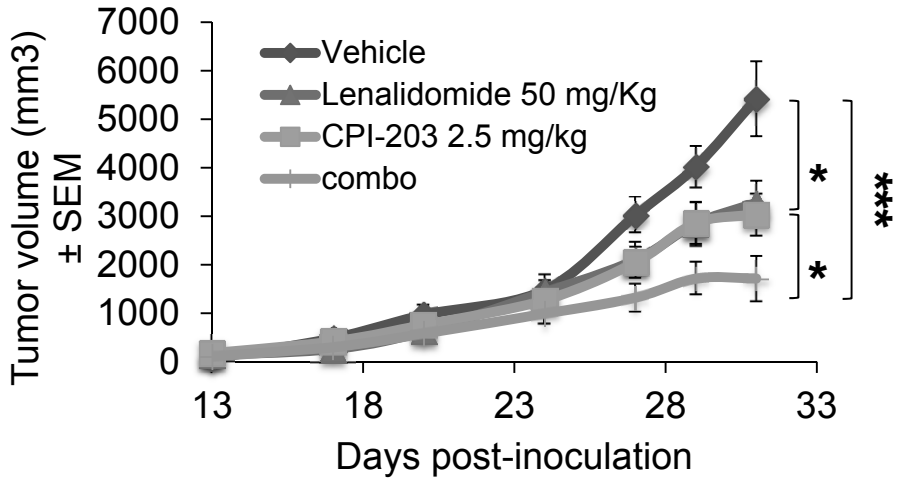
Synergistic activity of lenalidomide and CPI203 in bz-resistant MCL (*in vitro*)

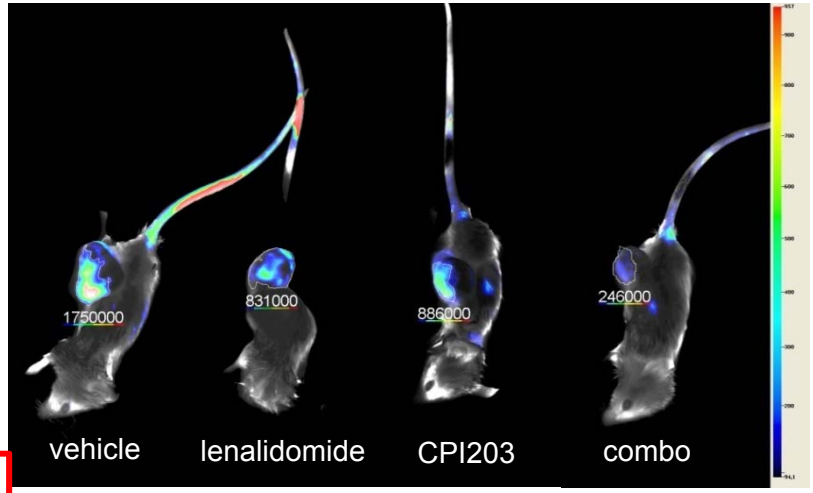


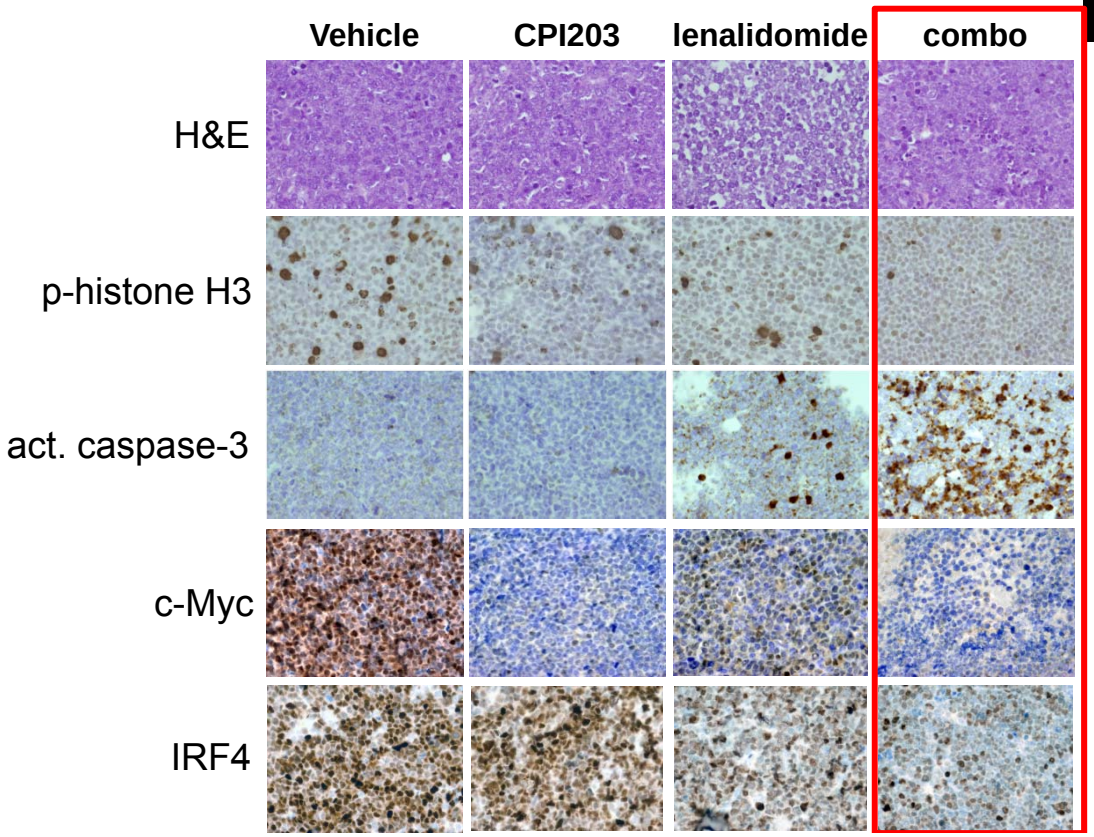


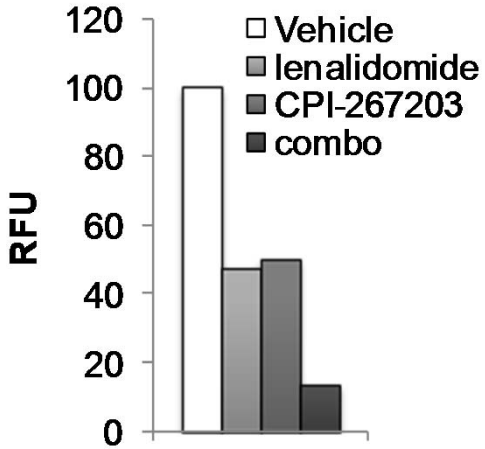
Moros A *et al*. Leukemia. 2014 Oct;28(10):2049-59.

Synergistic activity of lenalidomide and CPI203 in mouse model of bz-resistant MCL



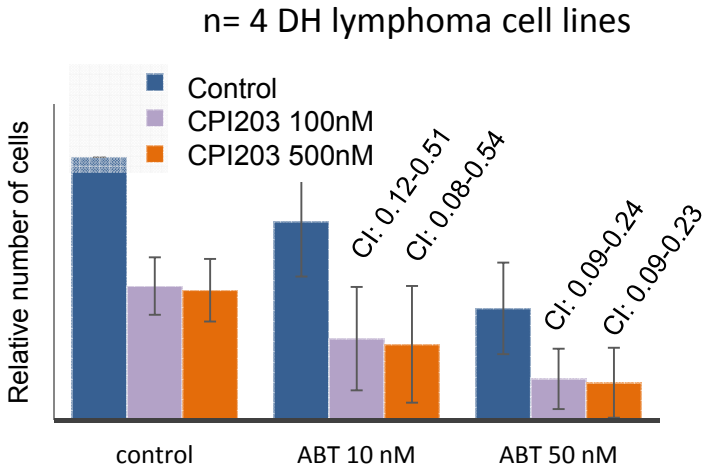


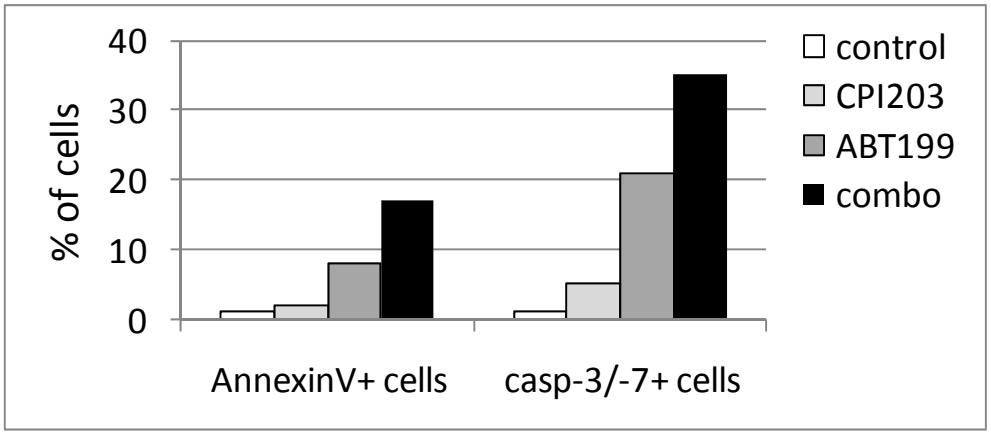


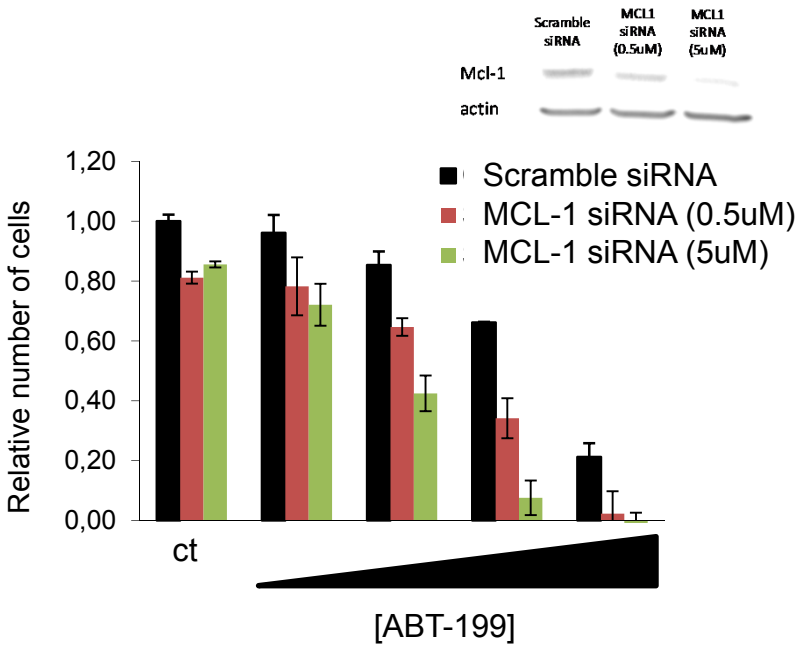


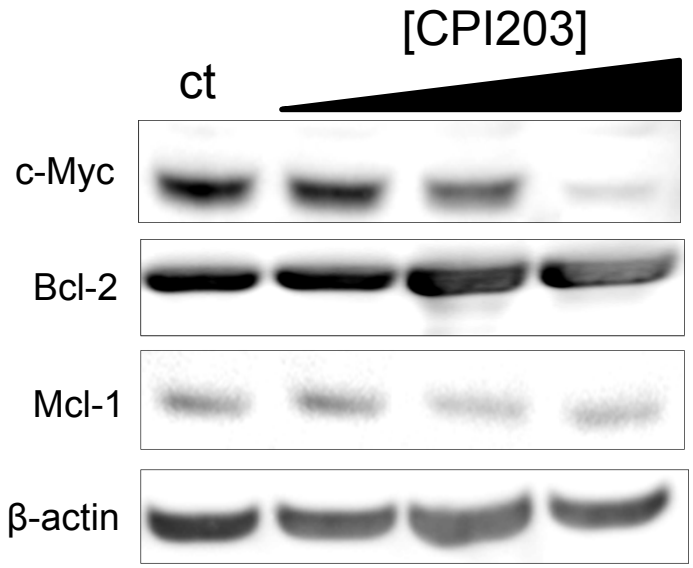
Moros A *et al*. Leukemia. 2014 Oct;28(10):2049-59.

ABT-199 and CPI203 combo in *MYC+/BCL2+* double hit lymphoma



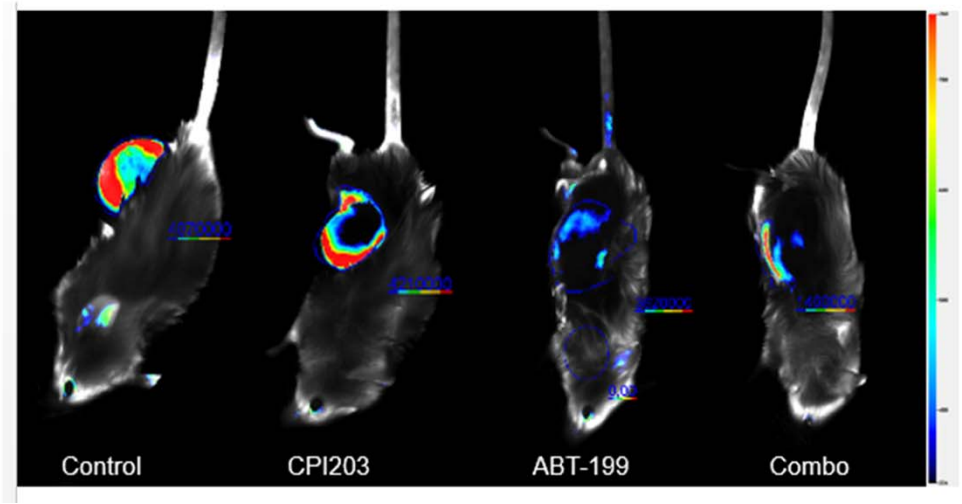


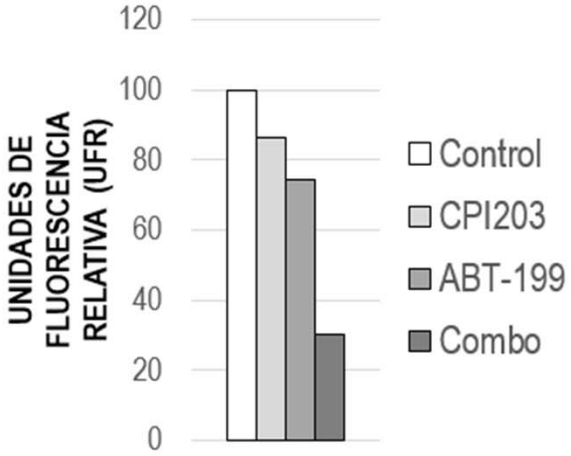


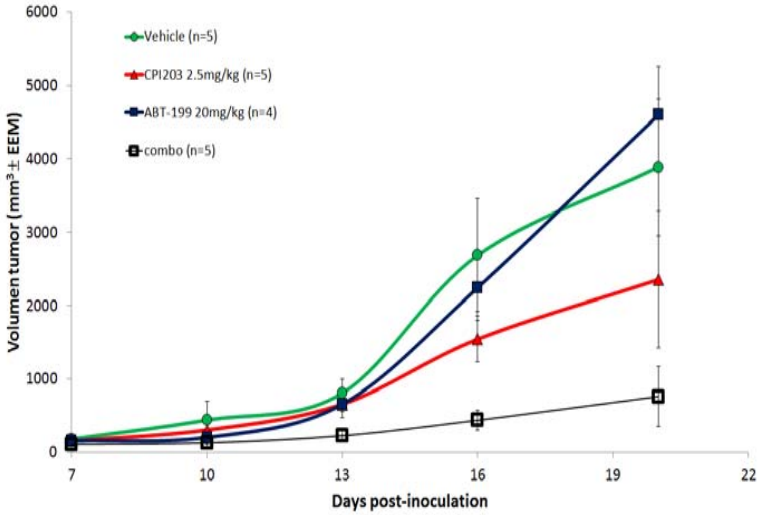


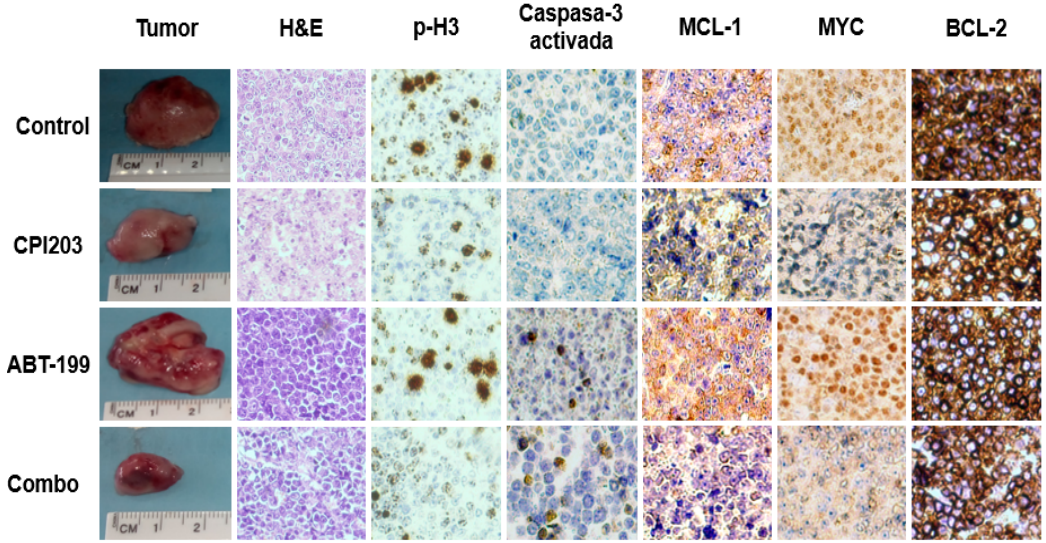
Juan Garcia, Anna Esteve, David Gonzalez, 2015

Synergistic activity of ABT-199 and CPI203 *in vivo*







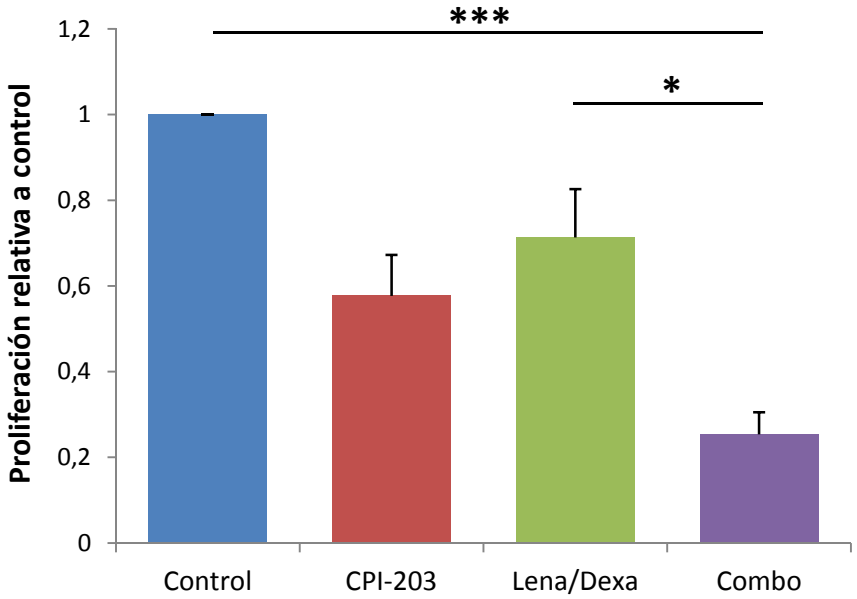


Juan Garcia, Anna Esteve, David Gonzalez, 2015

CPI203 and lenalidomide/dexamethasone combo in multiple myeloma

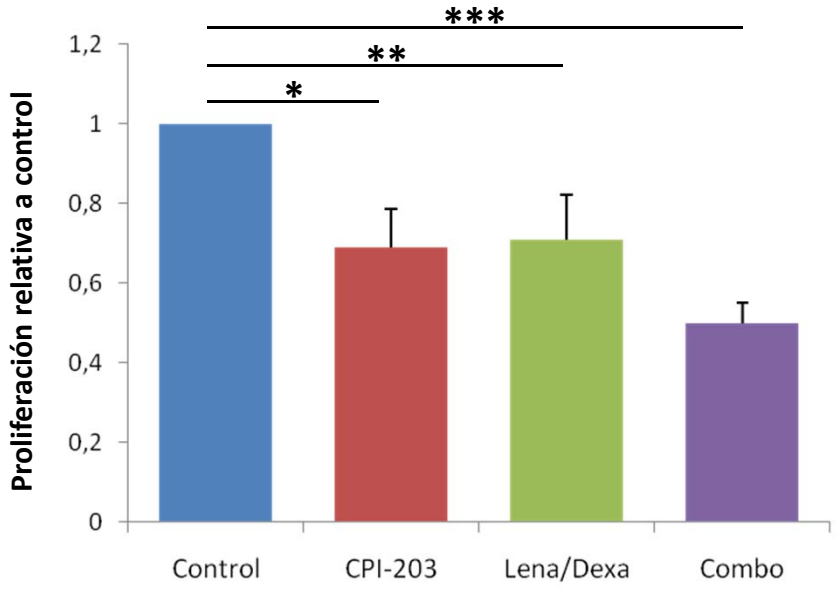
Cell lines(n=7)

ANOVA test: **p=0,0003**



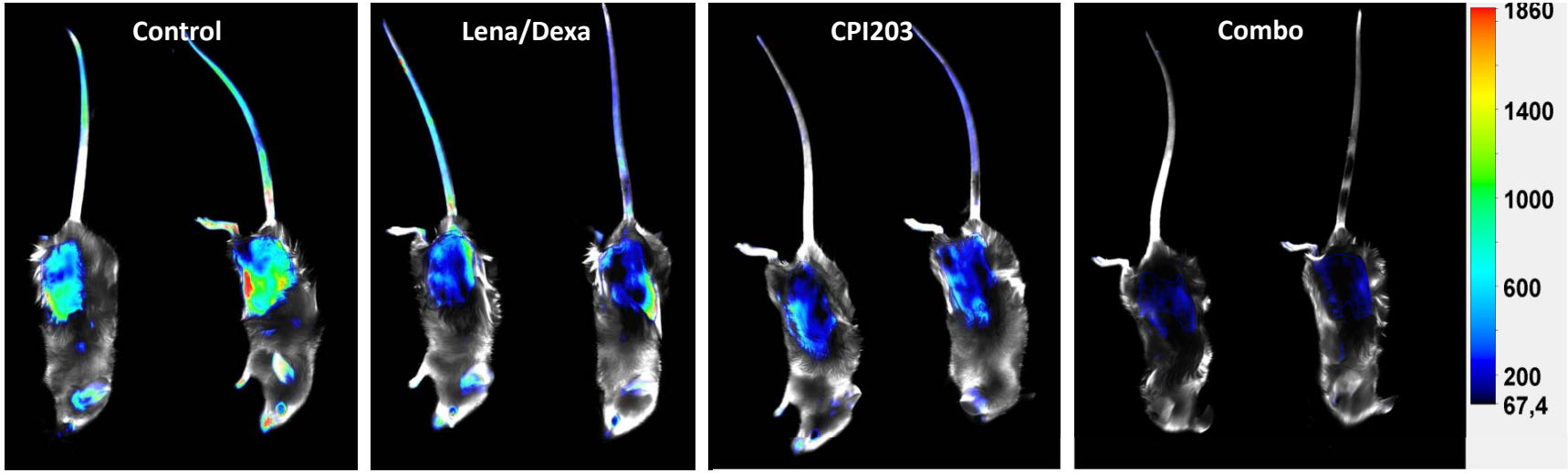
MM primary cultures (n=9)

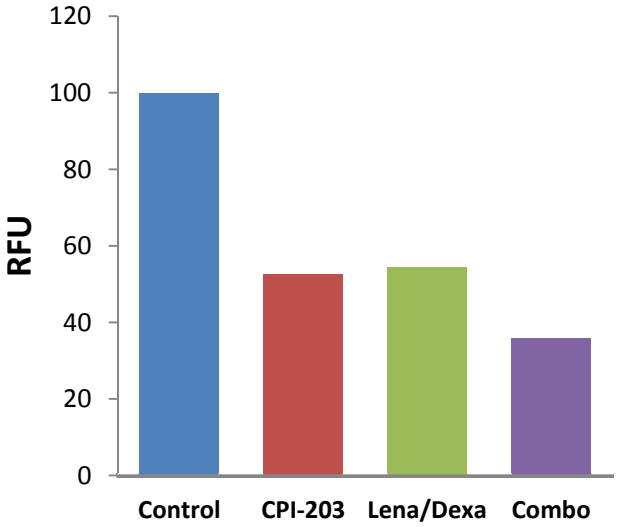
ANOVA test: **p<0,0001**

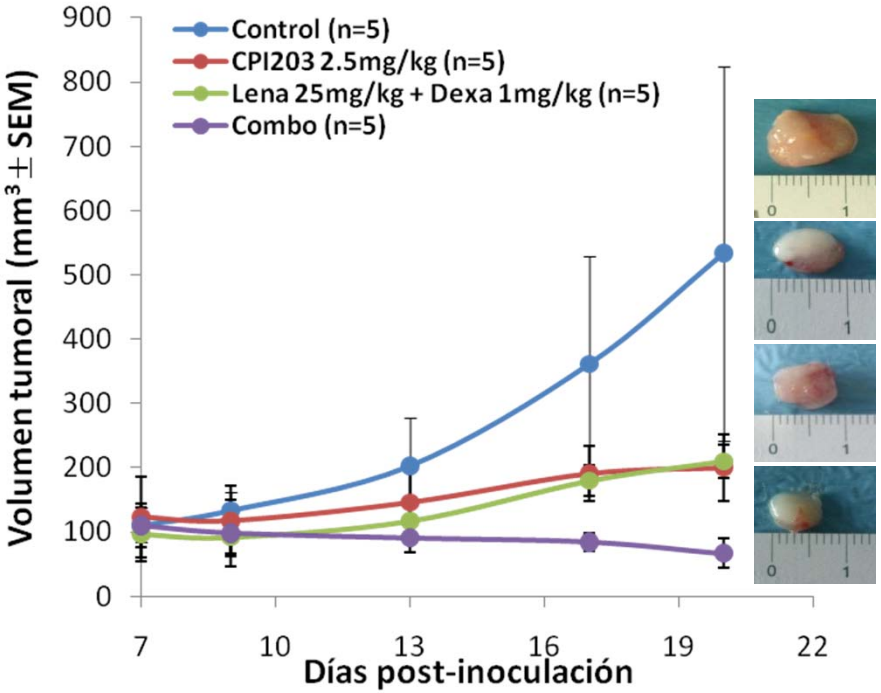


Tania Diaz, 2015

Synergistic activity of CPI203 and Len/Dex therapy MM xenostransplant







Tania Diaz, 2015

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Hemato-Oncology Dpt

Tania Diaz

Carlos Fernandez

Hospital Clínic i Provincial de Barcelona,

Hematopathology Unit

Antonio Martinez

Elias Campo

Dolors Colomer

Hematology Department

Armando Lopez-Guillermo

Constellation Pharmaceuticals

Emmanuel Normant

Peter Sandy

Celgene Corporation

Antonia Lopez-Girona

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