Target therapy for bone metastatic prostate cancer with Micro RNA145 inhibits tumor growth in vivo

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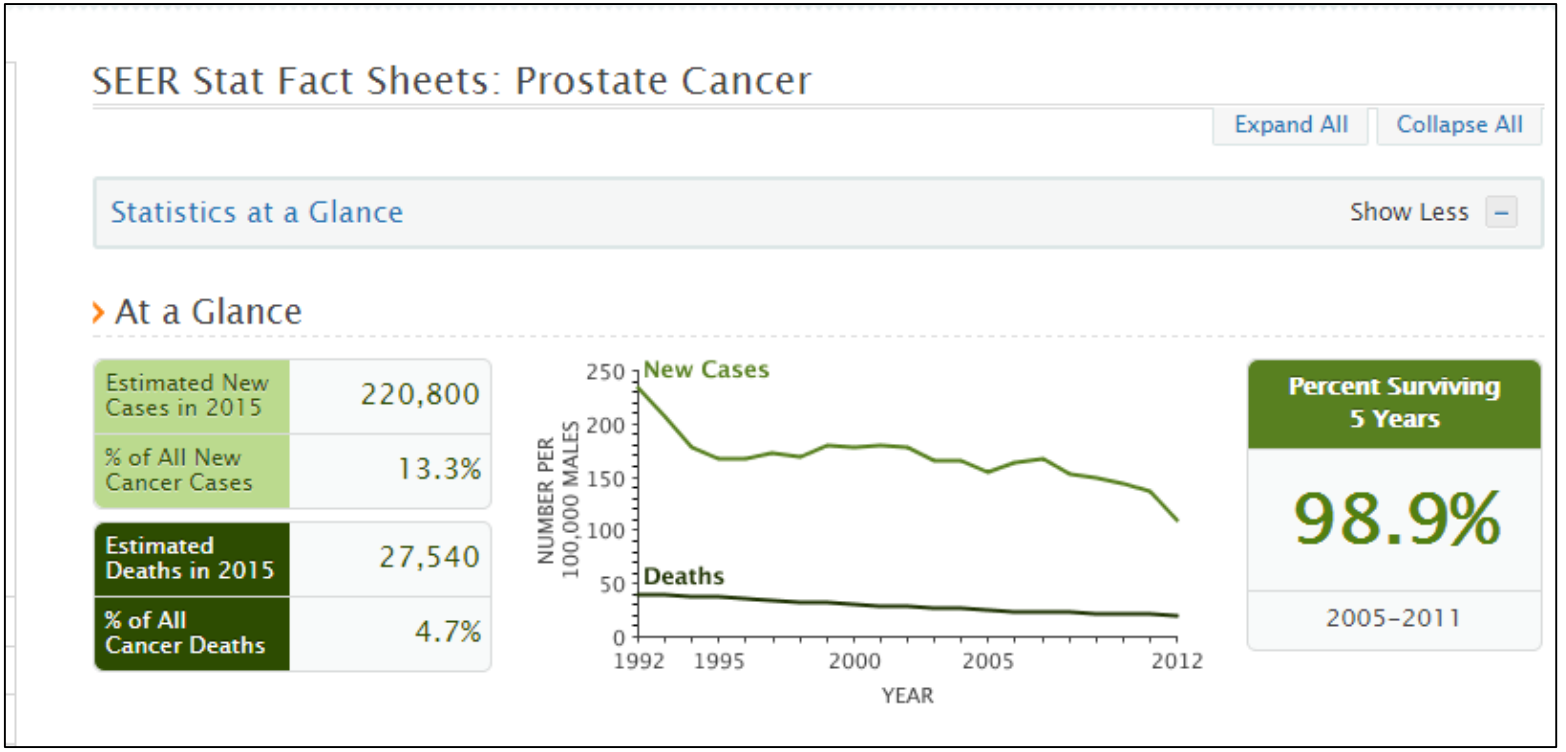
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#2012/21966-8
There are no conflicts of interest to disclose

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Prostate cancer

- Most common malignancy in men
- Second cause of death



http://seer.cancer.gov/statfacts/html/prost.html

Treatment

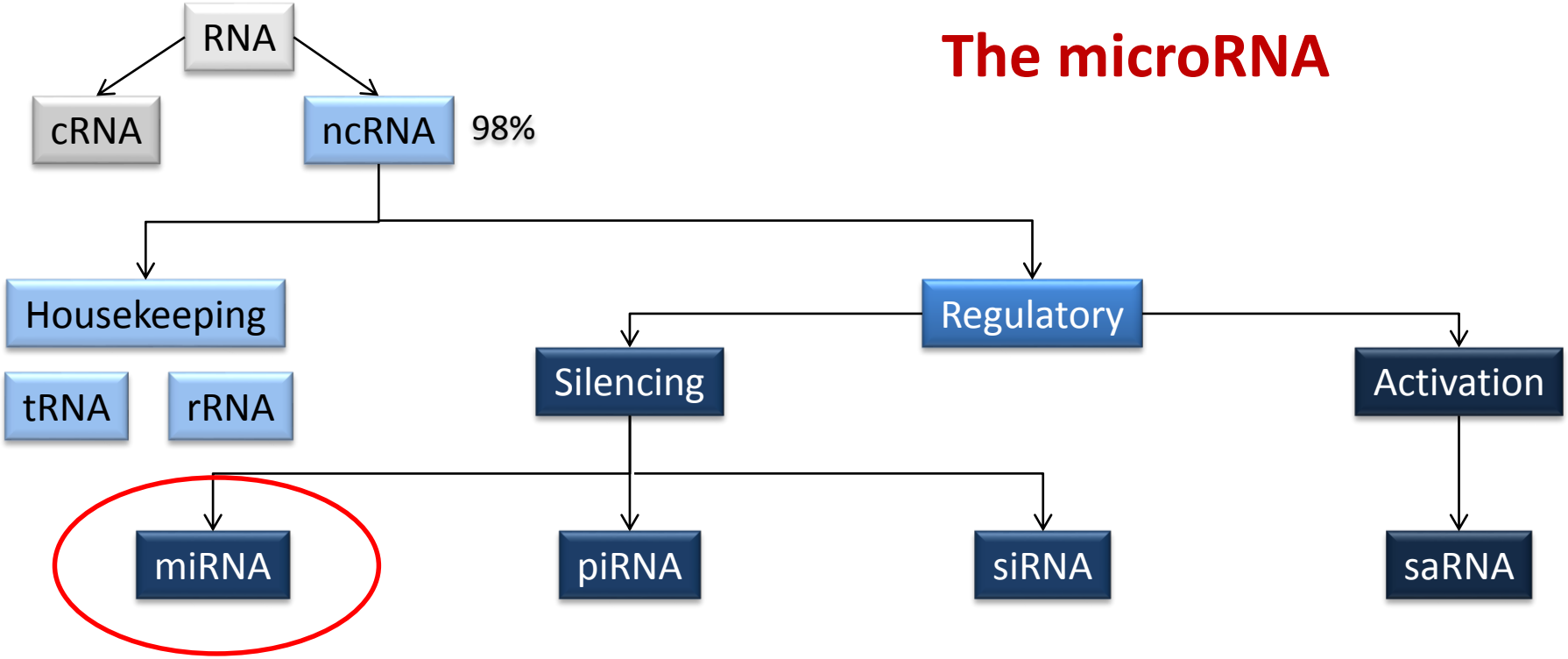
- Active surveillance
- Radical prostatectomy → **Localized**
- Radiotherapy

- Hormone therapy → **metastatic**

LOCALIZED PROSTATE CANCER 5 YEAR SURVIVAL – 100%
METASTATIC PROSTATE CANCER 5 YEAR SURVIVAL – 28%

http://seer.cancer.gov/statfacts/html/prost.html

The microRNA



- 20 – 24 nts
- Endogenous
- Eukaryotes
- Single-stranded
- Dicer dependent
- Ago subfamily
- 3'/5'-UTR/promoter/conding reg/pseudogene
- mRNA degradation/ transcriptional or **<u>postranscriptional silencing (HUMAN</u>**)

- 21 – 23 nts
- Endogenous or exogenous
- Eukaryotes
- Double-stranded
- Dicer dependent
- Ago subfamily
- mRNA or gene promoter
- mRNA degradation/ transcriptional or postranscriptional silencing

Characteristics of miRNAs

- Stable in different specimens
  - Control of at least 30% of human genes.
  - Regulate important cell process (apoptosis, proliferation…)
  - Related to the development and progression of cancer

Mitchell et al. *PNAS 2008;105:10513*

http://microrna.sanger.ac.uk/cgi-bin/sequences/browse.pl

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Tumor suppressor miRs

miR-15a and 16-1

Target – Bcl2, CCND1, CCND3, CCNE1, CDK6, VEGF, FGF2, FGFR1

miR-143/145

Target – **RAS, Myc**, BNIP3, FSCN1, OCT4, SOX2, KLF4

OncomiRs

Cluster miR-17-92

Target – PTEN

miR-221/222

Target – p27, p57, DDIT4, PTEN, TIMP3

miR-21

Target – PTEN, RHOB, RECK, PDCD4, TIMP3

miRNA and prostate cancer

- Volinia et al. (2006)
- Porkka et al. (2007)
- Cancer stem cell maintenance – ↓miR-34a (CD44)

  Bommer et al Curr Biol 2007;17:1298

- Epithelial mesenchymal transition – ↓miR-200b (ZEB1,2)

  Kong et al Stem Cell 2009;27:1712

- Tumor suppressor miRs – miR-15a, 16, 143, 145

  Aqeilan et al. Cell Death Diff 2010;17:215
  Musumeci et al. Oncogen 2011;30:4231

- OncomiRs – miR-221, 222

  Sun et al. Ca Res 2009;69:3356
  Galardi et al. 2007;282:23716
  Zheng et al. Med Oncol 2012

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MicroRNA and treatment

- Mercatelli et al *PLoS One. 2008;3:e4029*
  - Xenograft of prostate cancer
  - anti- miR-221 / 222
  - Impairs tumor cell growth
- Takeshita et al. *Mol Ther 2010;18:181*
  - Xenograft of prostate cancer
  - miR-16
  - Suppression of tumor growth
- Humans
  - Anti-miR-122 - Miravirsen®
  - Phase II trial (NCT01200420)
  - Treatment of hepatite C

Microarray panel

	HGPIN	Favorable	Unfavorable	Metastasis/Cell lines	
	Mean	Mean	Mean	Mean	p-value
Mir100	108.2	14.7	18.0	8.3	0.002
Mir143	71.1	1.6	1.8	**0.2**	<0.001
Mir145	5.0	1.6	3.6	**0.04**	0.001
Mir146a	**2.3**	**0.1**	**0.4**	**0.04**	0.003
Mir15a	1.9	0.1	0.3	1.3	<0.001
Mir16	**2.0**	**0.1**	**0.3**	**0.5**	<0.001
Mir191	**4.5**	**0.4**	**0.5**	**0.2**	<0.001
Mir199a	1.5	0.2	1.1	**0.6**	0.224
Mir206	2.9	2.8	1.2	**0.7**	0.244
Mir218	112.3	1.8	10.9	**0.9**	<0.001
Mir25	**1.9**	**0.2**	**0.6**	**0.9**	0.007
Mir32	6.0	0.3	1.1	6.2	0.004
MirLet7c	31.8	3.1	7.5	**0.9**	<0.001

PCR after transfection

microRNA	Target	LNCAP	DU145
mir145	cMYC	0.71*	0.46*
anti-145	cMYC	1.14	1.70*
mir145	kRAS	0.44*	0.49*
anti-145	kRAS	1.16	1.57

*** p<0,05**

NE: not expressed

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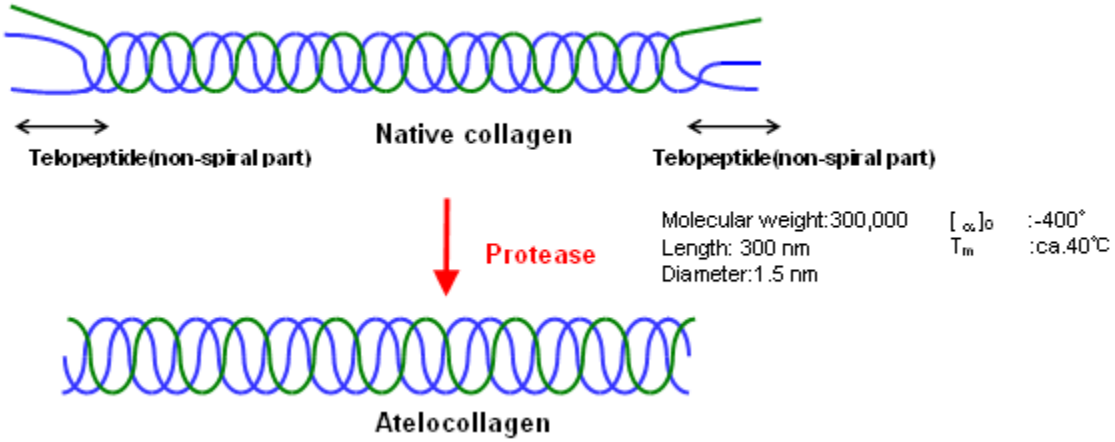
Purpose

Study the effects of treatment with intravenous miRNAs 145 in a pre-clinical model of disseminated bone metastatic prostate cancer.

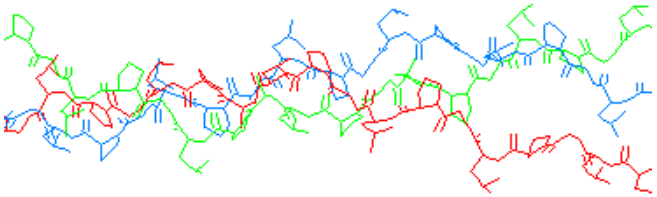
Methods

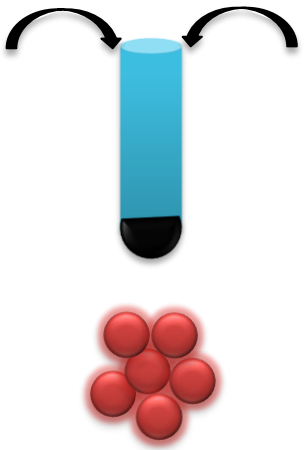
- Balb/c NUDE mice – 9-11 weeks (n=8)
  - Intraventricular injection of PC-3M-luc-C6
  - IVIS® Spectrum (Caliper)
  - miRNA and atelocollagen





Atelocollagen
300 kD – 300 nm (c) – 1.5 nm (d)





miR145 ou scramble

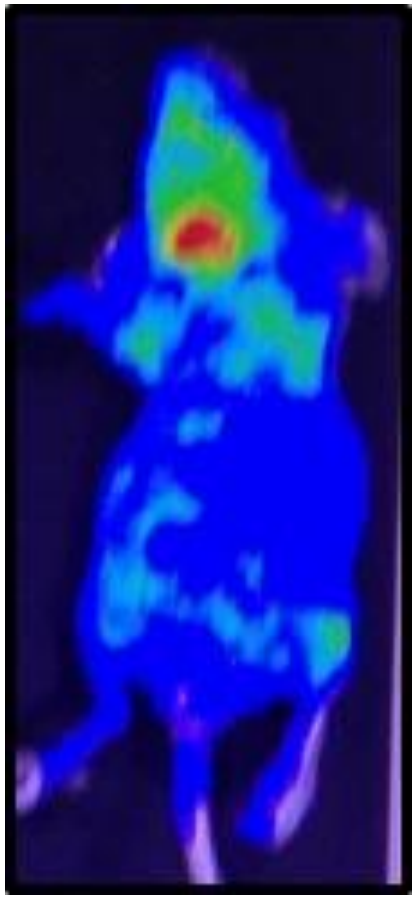




Minacuchi et al, Nucleic Acids Res, 2004; 32: e109
Takeshita et al. PNAS USA 2005;102:12177



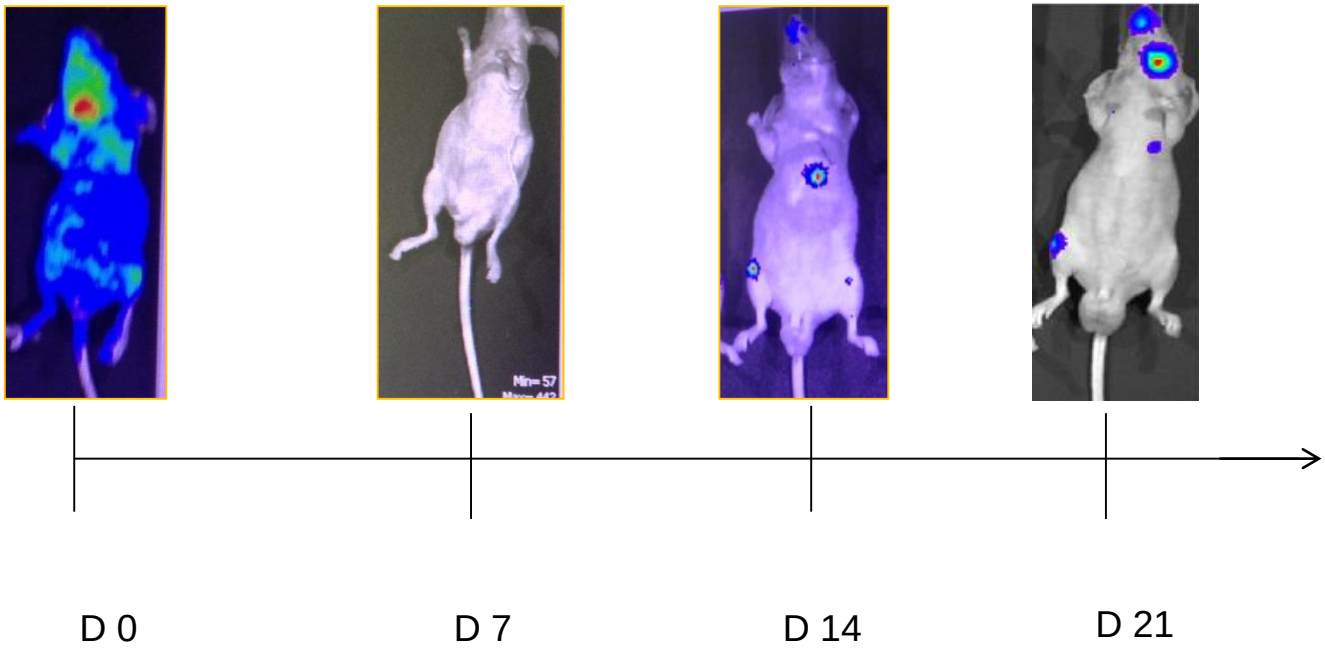
A



B

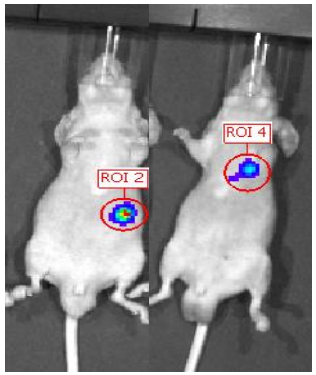
In vivo studies - *Xenograft*

PC-3M-luc-C6 $2x10^6$



RESULTS

D21
Begin of treatment



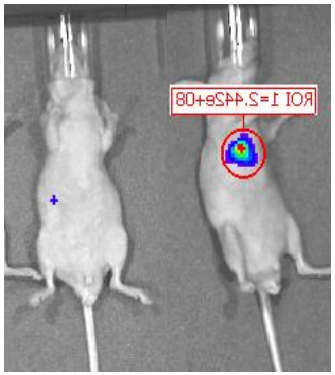
Mir145 Control

D27
End of treatment



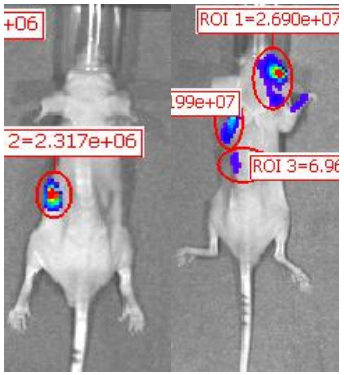
Mir145 Control

D34

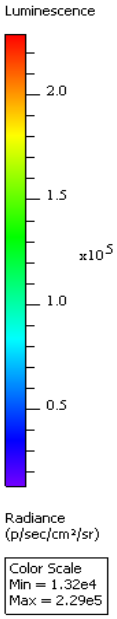


Mir145 Control

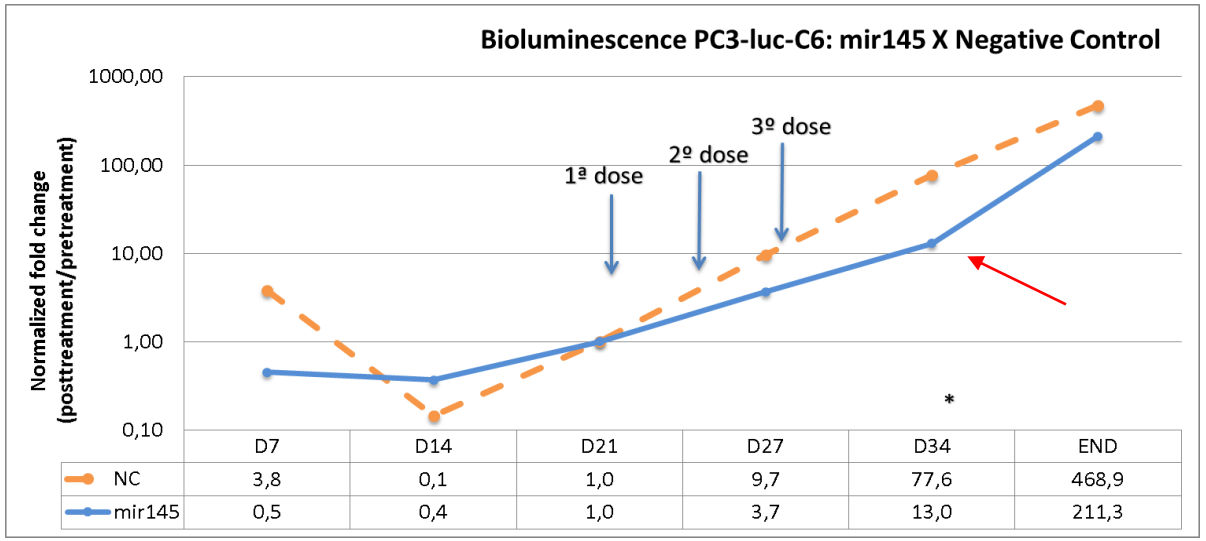
D48
End of experiment



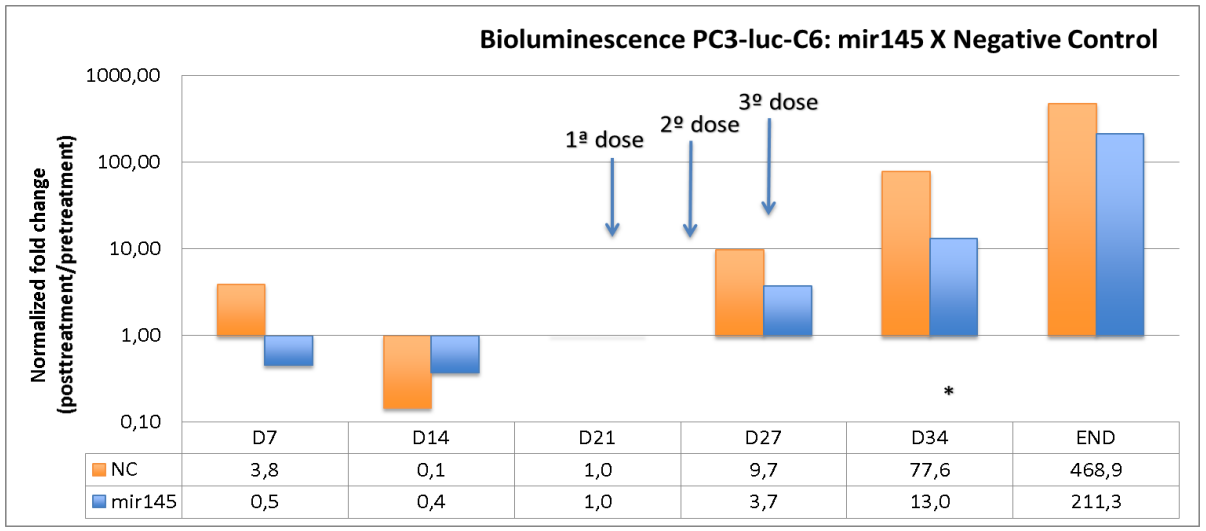
Mir145 Control



RESULTS



	D7	D14	D21	D27	D34	END
NC	3,8	0,1	1,0	9,7	77,6	468,9
mir145	0,5	0,4	1,0	3,7	13,0	211,3



	D7	D14	D21	D27	D34	END
NC	3,8	0,1	1,0	9,7	77,6	468,9
mir145	0,5	0,4	1,0	3,7	13,0	211,3

(n=8)

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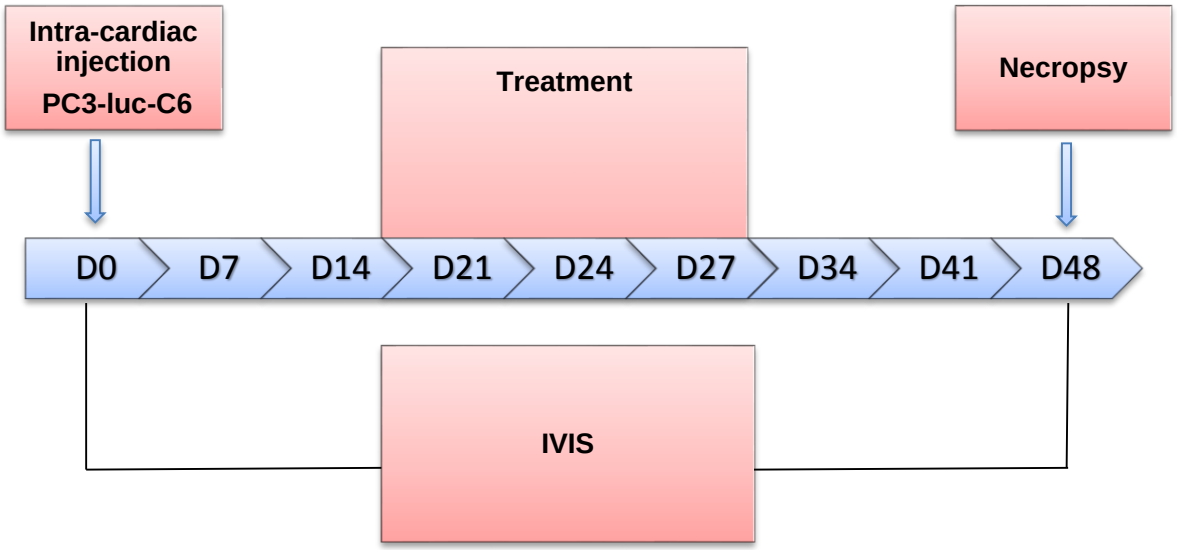
CONCLUSION

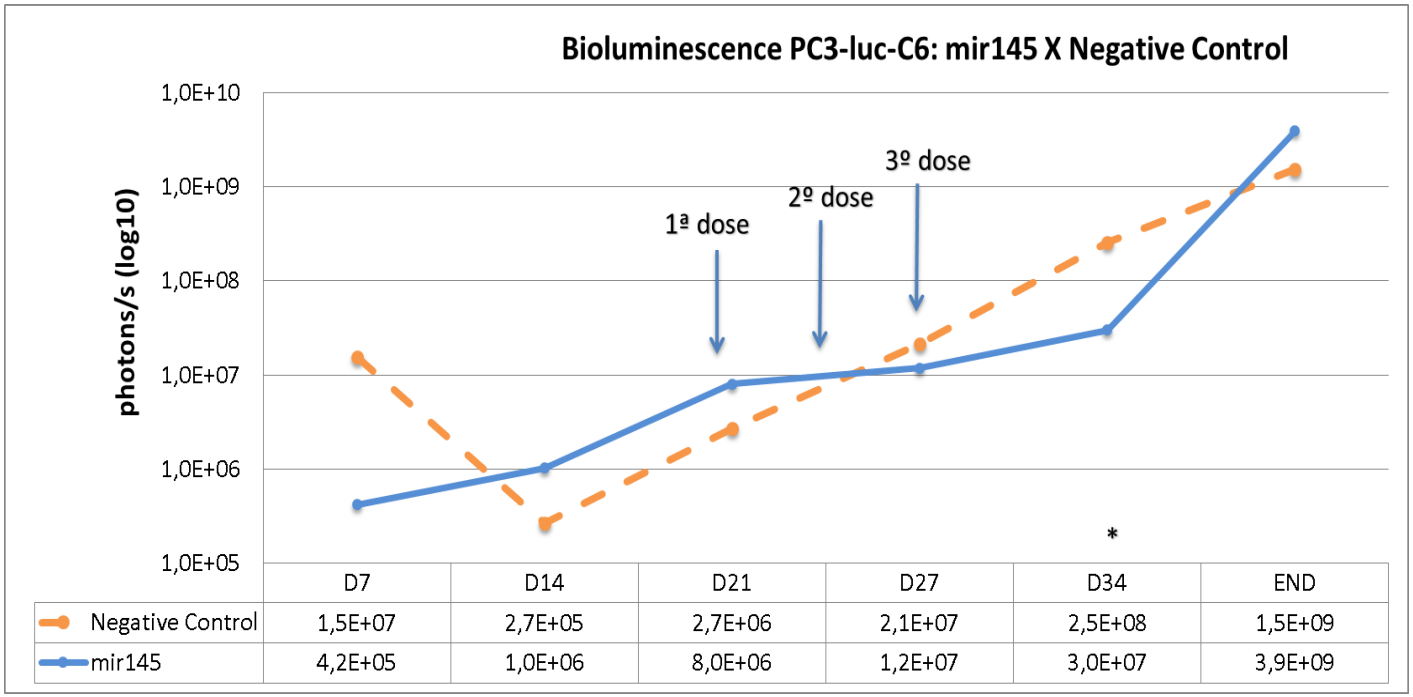
- **In animals with diffuse metastatic disease, the treatment with mir145 leads to a temporary response due to a fast degradation and to cancer cells mechanisms of scape and resistance.**

- **Further studies with this purpose and design will permit the development of novel target drugs based on microRNAs to suppress the metastatic prostate cancer growth.**

THANK YOU




Intra-cardiac injection
PC3-luc-C6
Treatment
Necropsy
D0
D7
D14
D21
D24
D27
D34
D41
D48
IVIS



	D7	D14	D21	D27	D34	END
Negative Control	1,5E+07	2,7E+05	2,7E+06	2,1E+07	2,5E+08	1,5E+09
mir145	4,2E+05	1,0E+06	8,0E+06	1,2E+07	3,0E+07	3,9E+09