LINE-1 encoded reverse transcriptase (RT) in the generation of new genetic information, embryonic development and tumorigenesis

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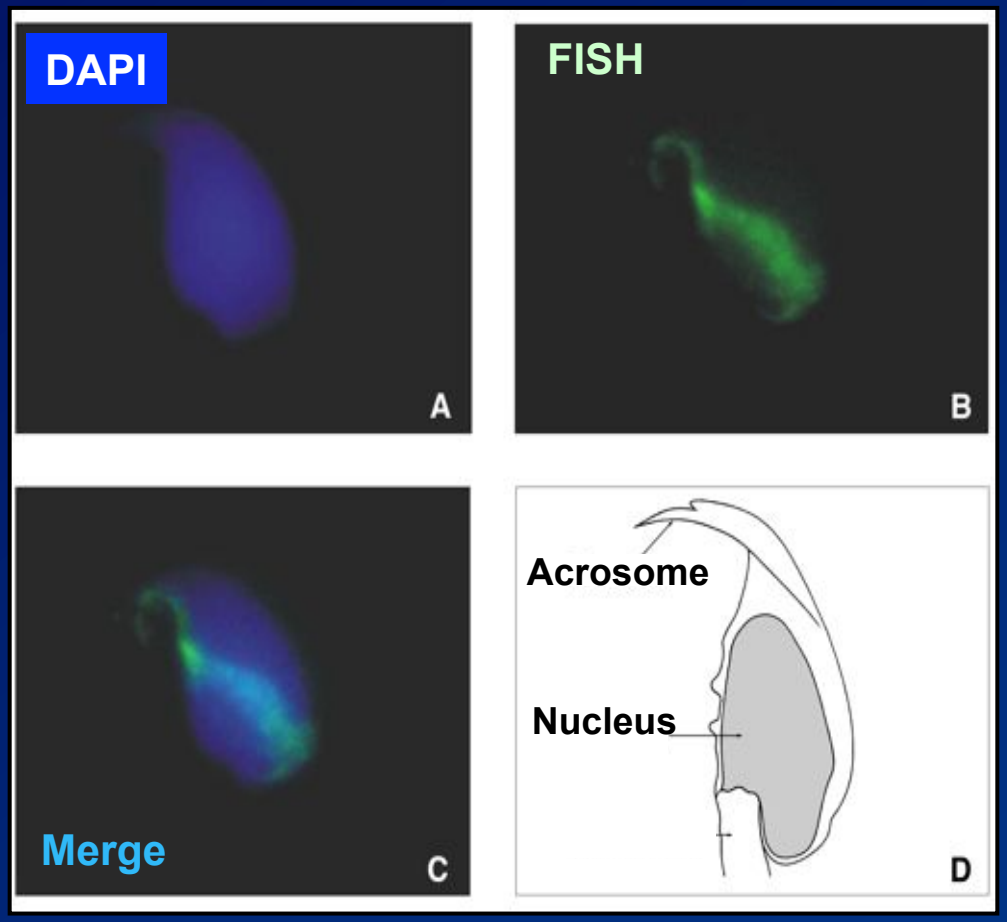
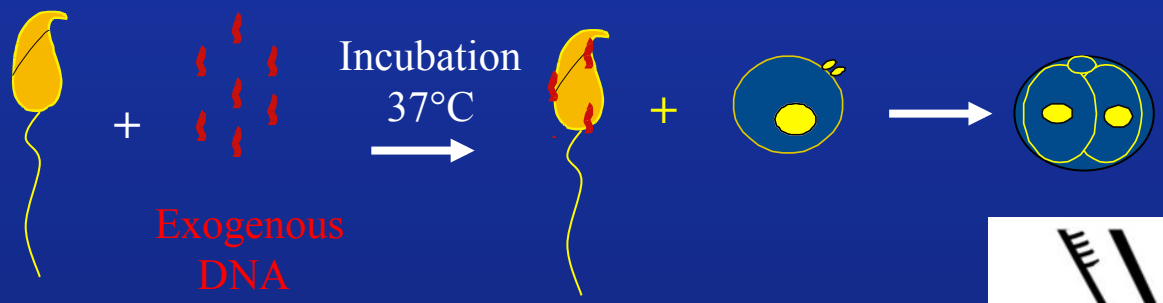
Background: mouse sperm cells can internalize exogenous DNA molecules

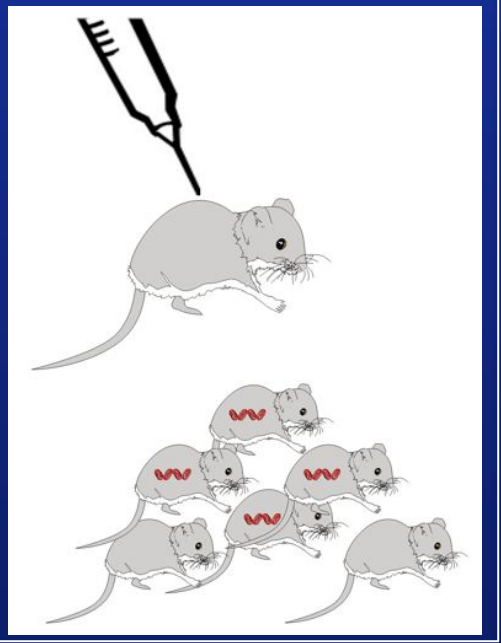


Spadafora, BioEssays, 1998

Sperm-mediated gene transfer: molecular basis



Spadafora, BioEssays, 1998



Sperm-mediated gene transfer: summary features

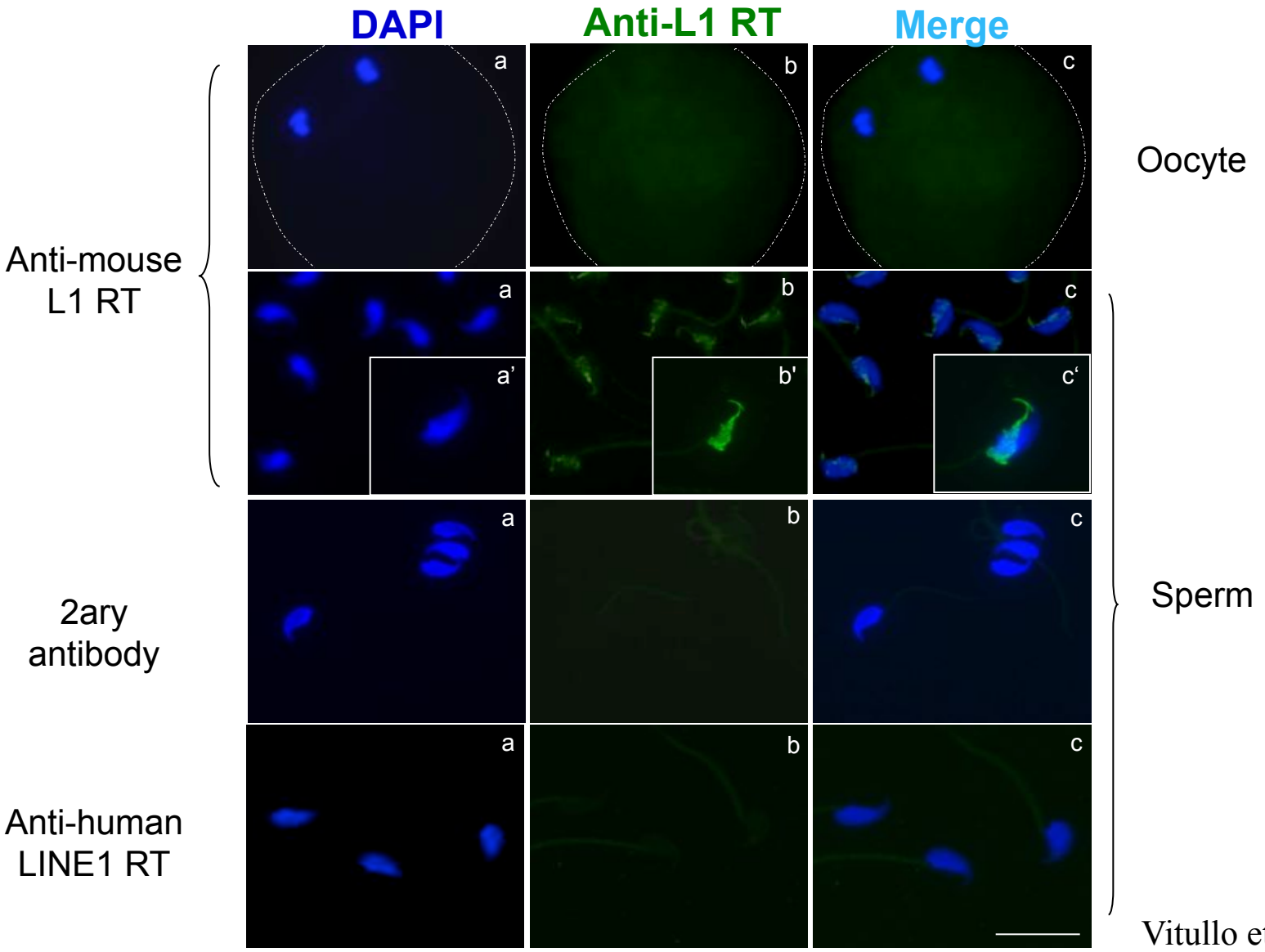
1. Sperm cells can internalize exogenous DNA with which they come in contact

2. The uptake of exogenous DNA is a highly regulated process mediated by specific factors

3. The binding of exogenous DNA activates nuclear functions that are otherwise repressed in spermatozoa

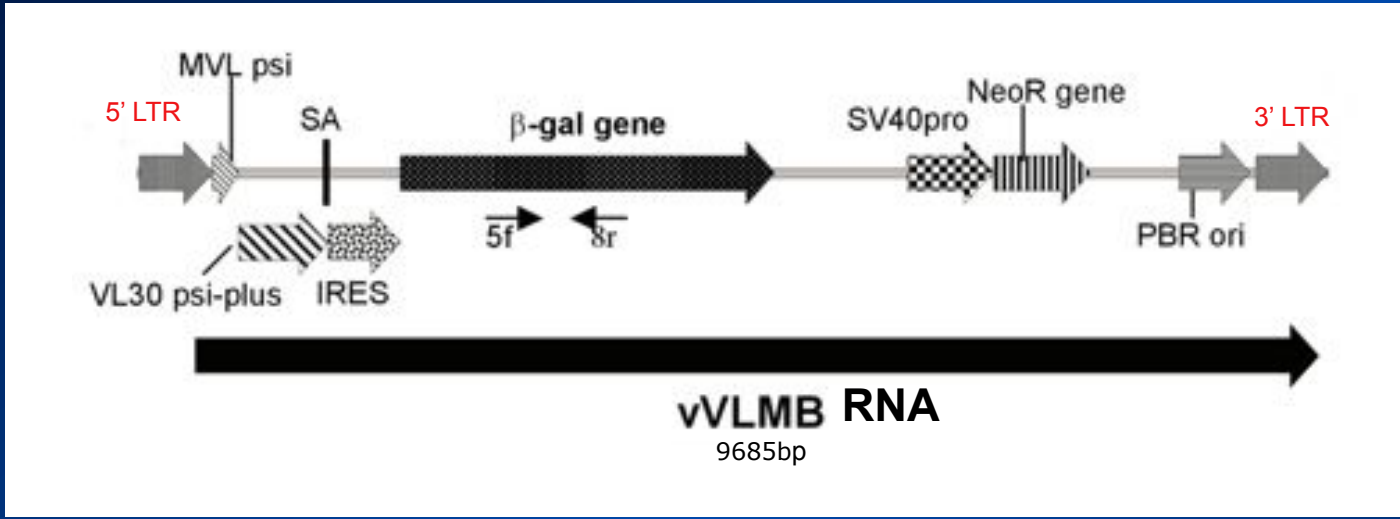
4. One of these activities is an endogenous Reverse Transcriptase (RT)

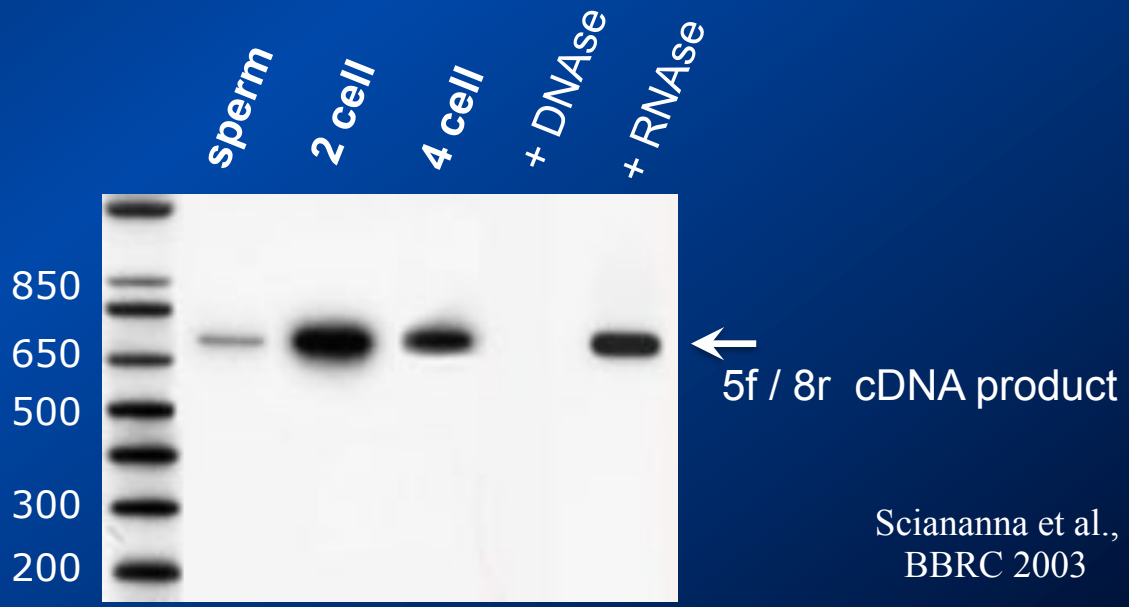
Immunofluorescence detection of LINE-1 encoded RT in mouse sperm cells



Vitullo et al. 2012

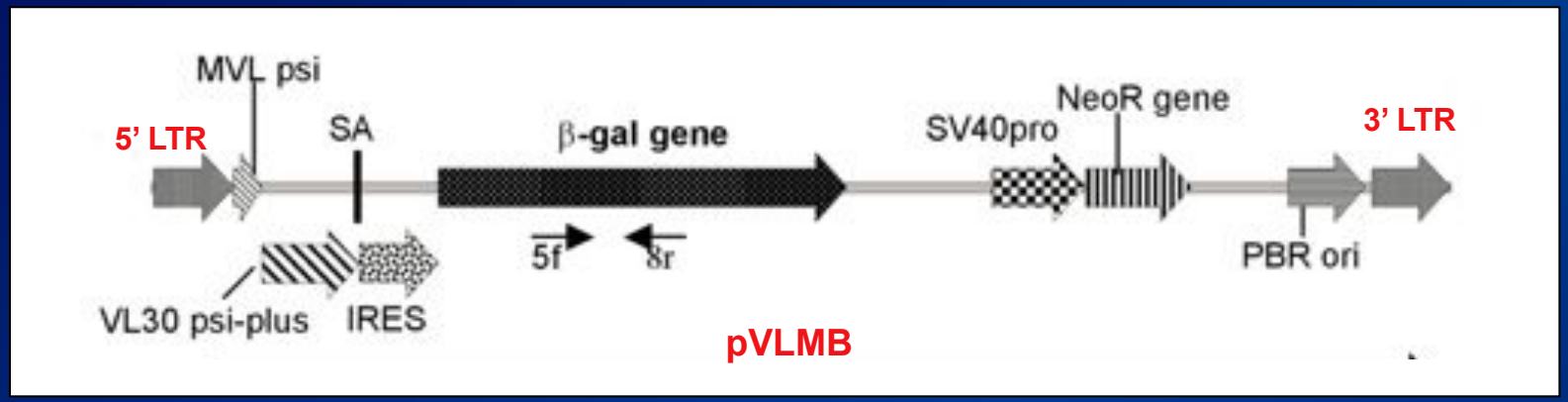
Exogenous β-gal is detected in sperm cells and derived embryos



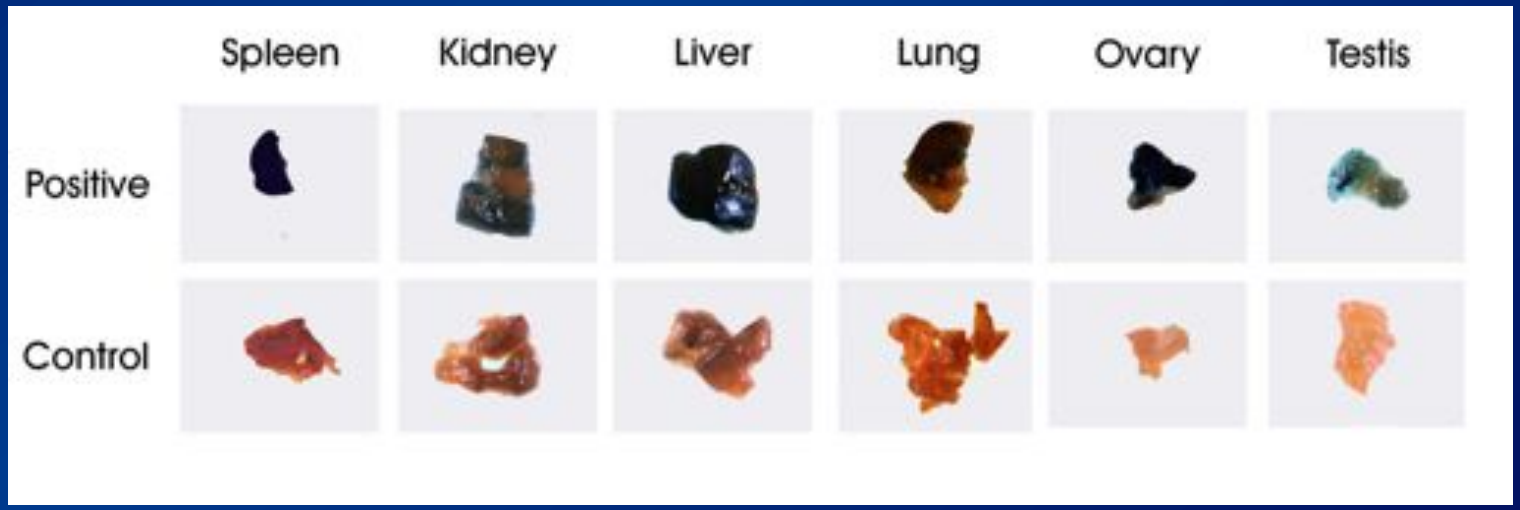


Sciananna et al.,
BBRC 2003

RNA-mediated Sperm-mediated "Reverse" Gene Transfer

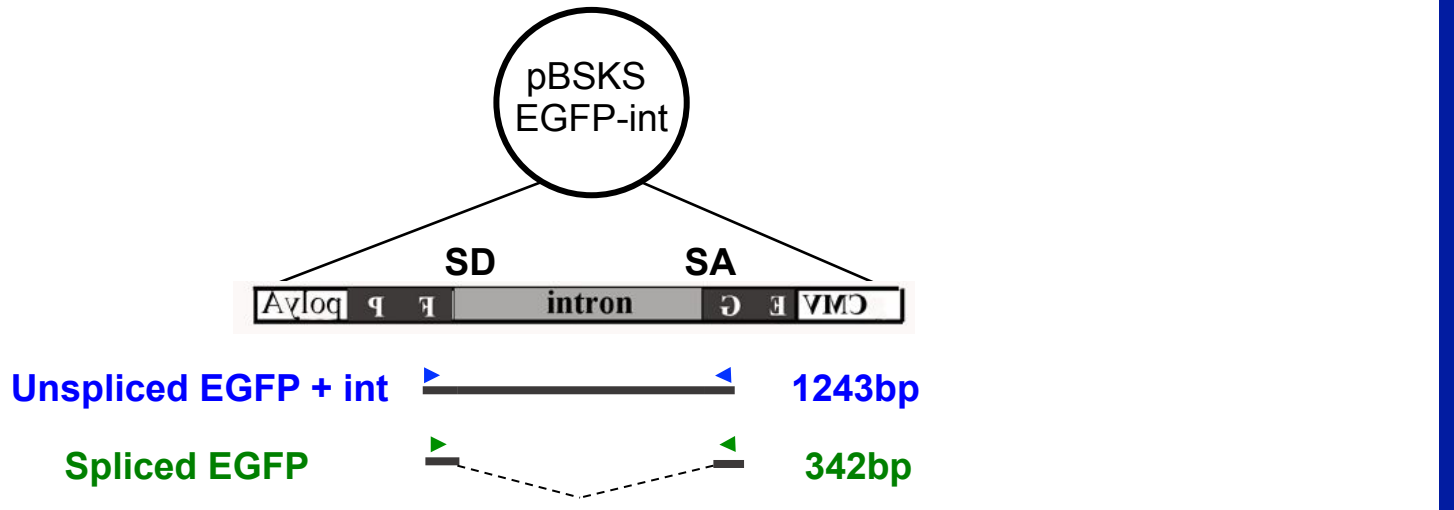


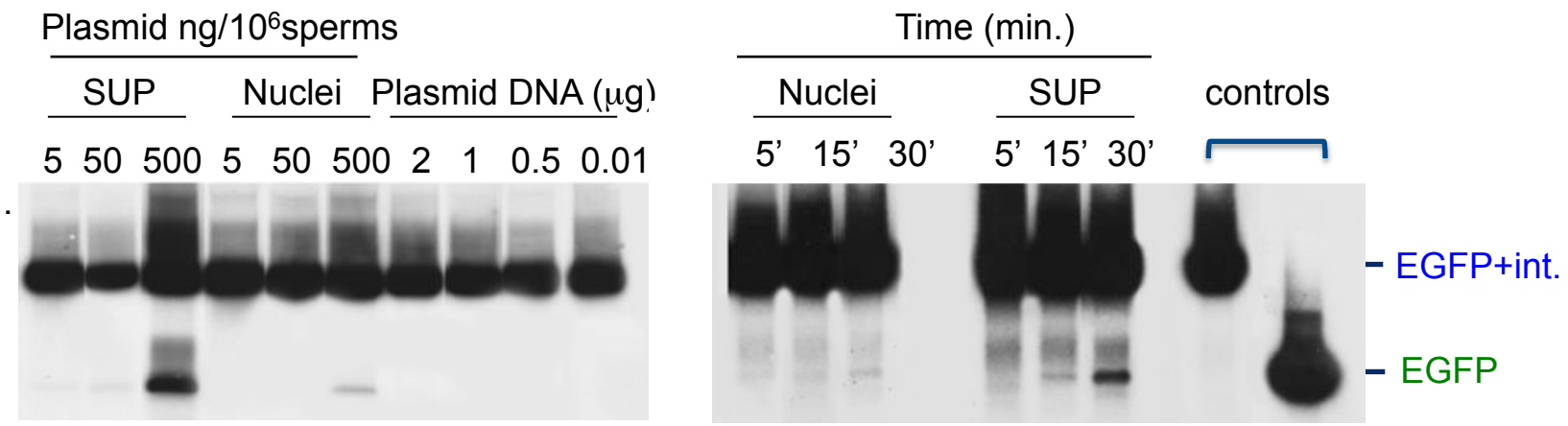
β-gal expression in organs of F0 and F1 pVLMB RNA-transformed animals



Sciananna et al., BBRC 2003

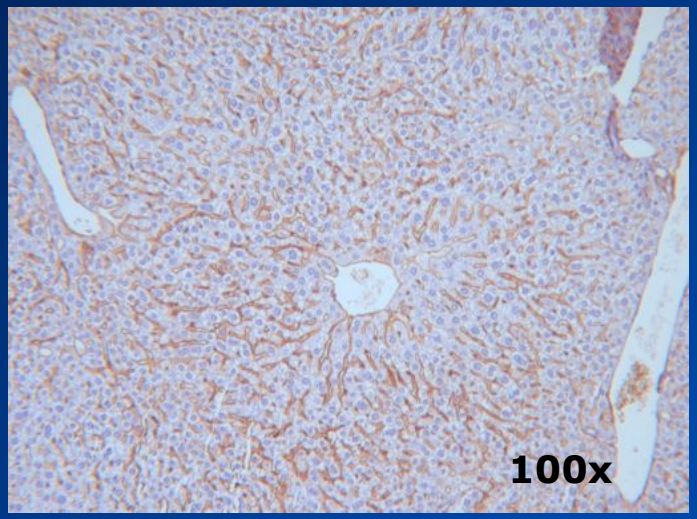
EGFP copies are reverse-transcribed and spliced in sperm cells incubated with pBSKS-EGFP-int



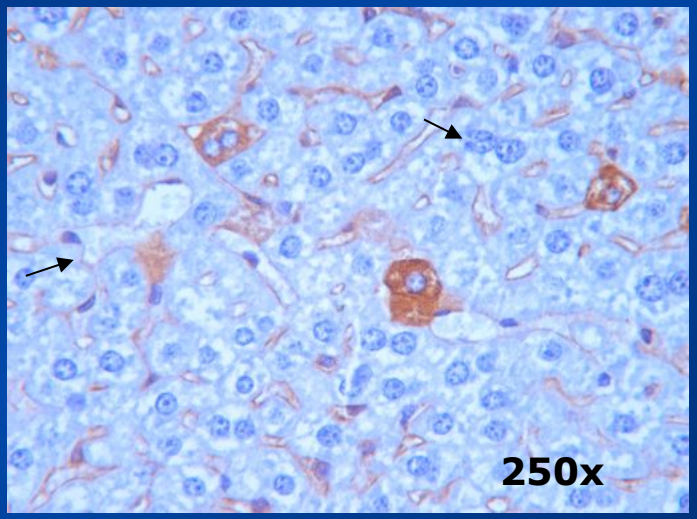


Sperm-derived reverse-transcribed EGFP is expressed in tissues of F0 mice

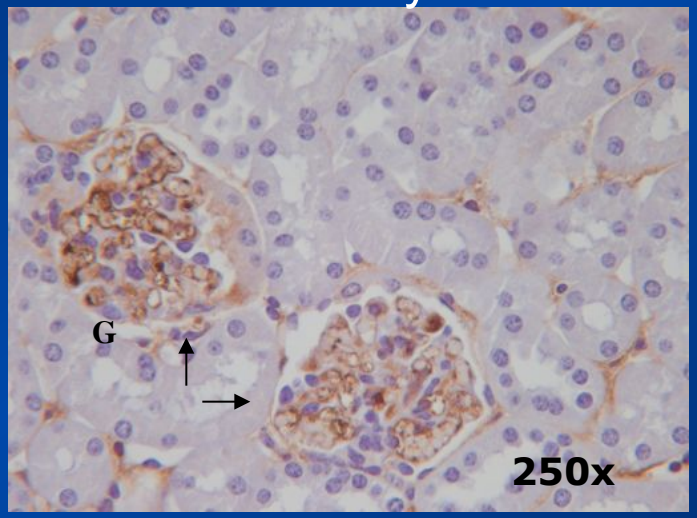
Liver



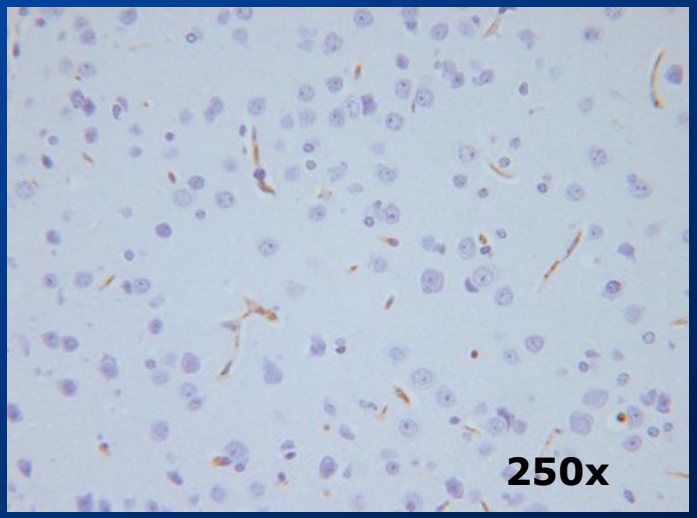
Liver



Kidney



Brain



Conclusions (I)

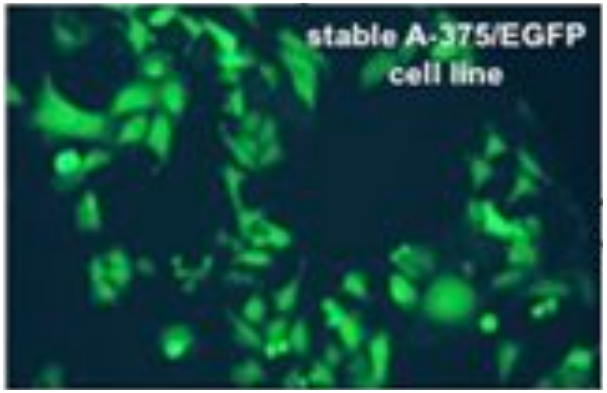
The sperm-mediated reverse gene transfer assays suggest that

- a sperm RT-mediated mechanism is responsible for the genesis of newly reverse-transcribed genetic information,

- that can be transmitted to offsprings, besides that carried by chromosomes

Detection of EGFP from tumours to germ cells: experimental outline

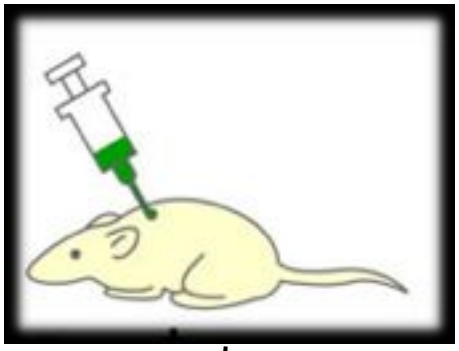
1 A-375 human melanoma cells stably expressing EGFP



whole cells — RNA | DNA | proteins

released exosomes — RNA | DNA | proteins

2 A-375/EGFP cells xenografted in athymic mice



45 days

3 A-375/EGFP xenograft growth

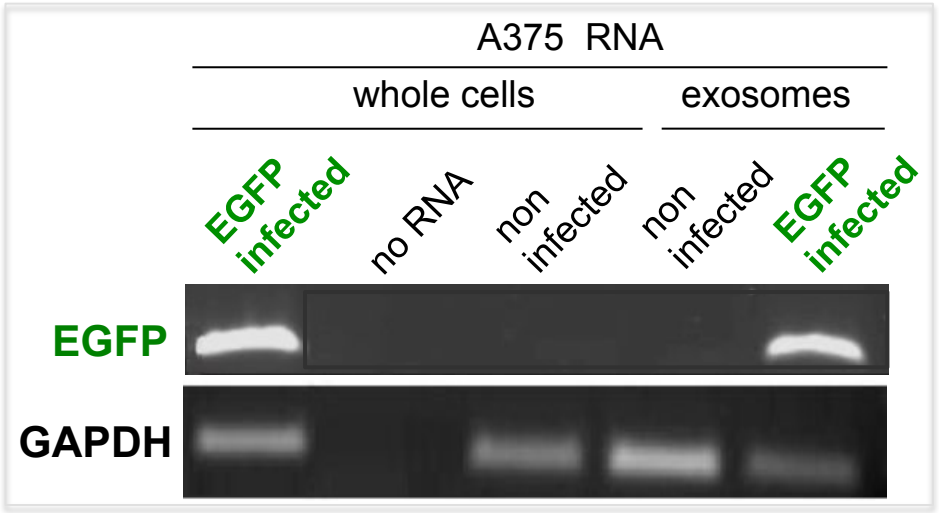
blood exosomes — RNA

mature sperm cells — RNA

Cossetti et al.
Plos One 2014

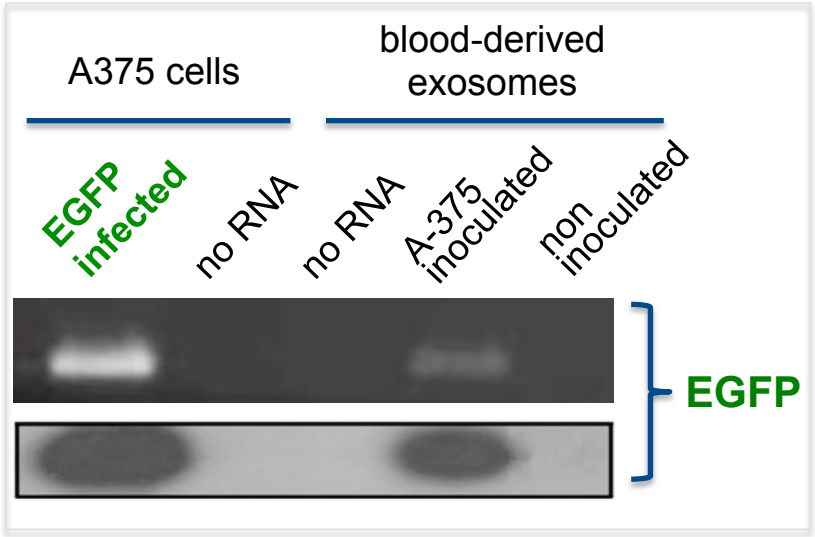
Tumour marker RNAs in circulating exosomes and in germ cells of xenografted mice

A-375 melanoma cell line

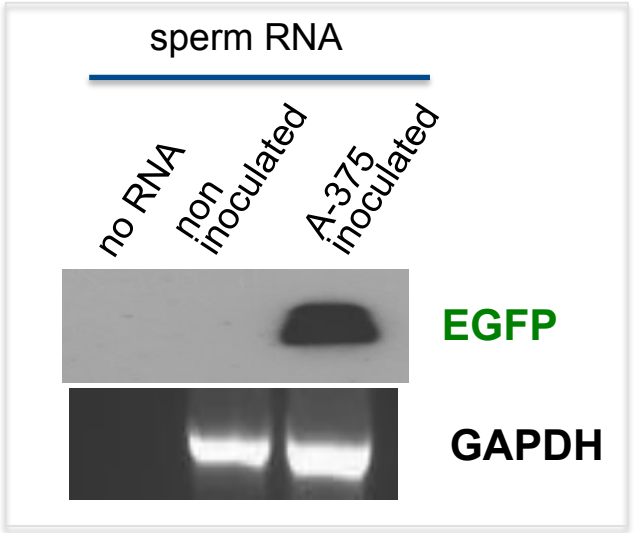


Cossetti et al.
PLoS ONE 2014

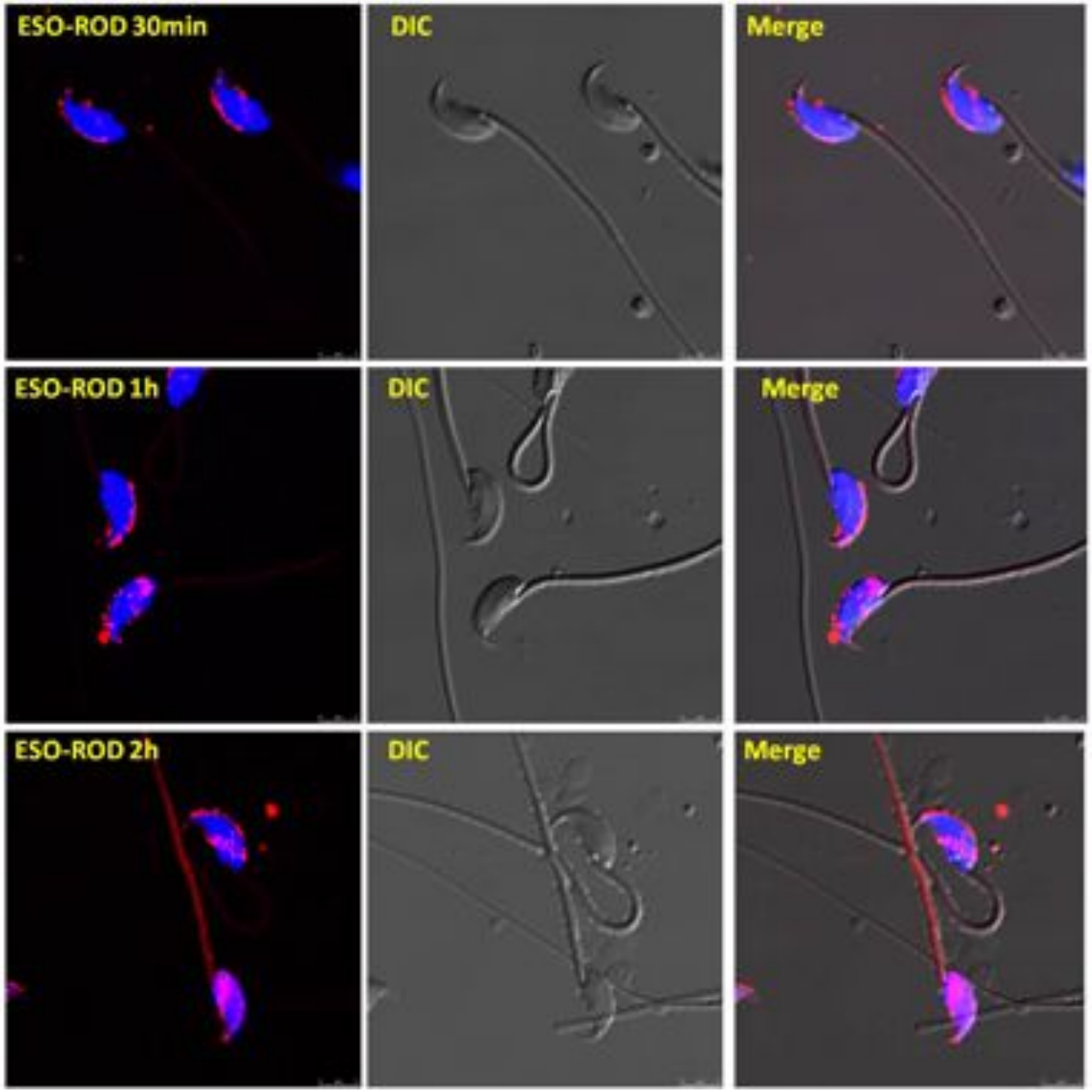
Mice-derived blood exosomes



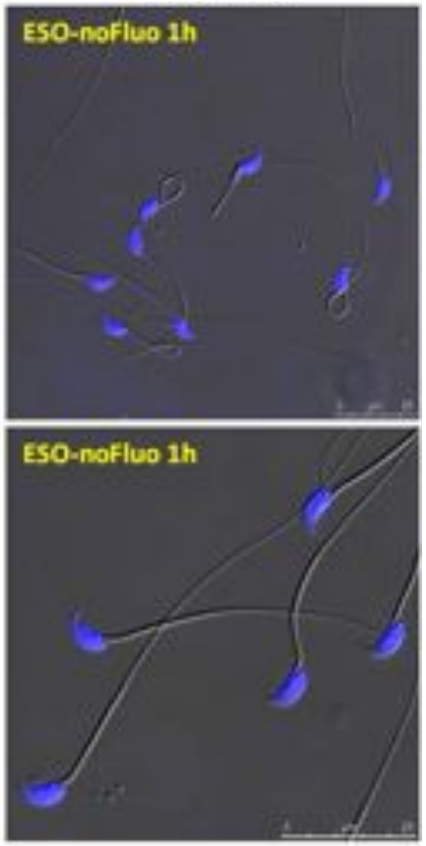
Mice-derived sperm cells



Human exosomes are taken up by murine spermatozoa



Interaction with non labelled exosomes



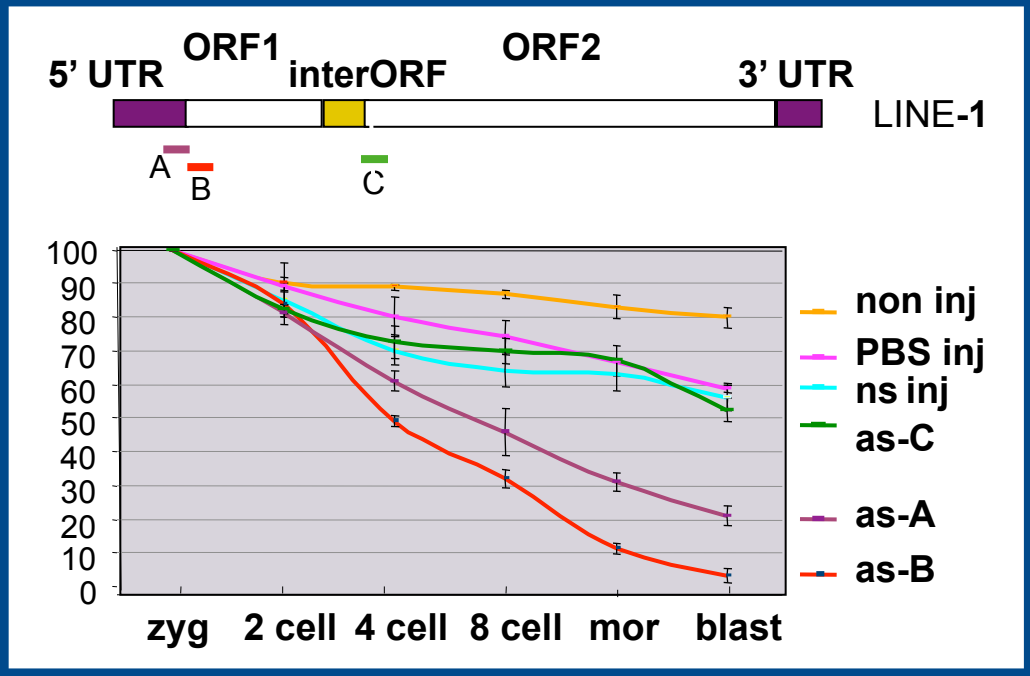
Conclusions (II)

- Human cancer cells xenografted in mice release tumor-specific RNA-containing nanovesicles (exosomes) in the circulating blood

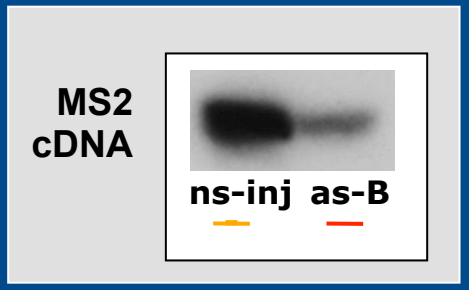
- RNA-mediated information flows from the soma to the germline, crossing the Weissman barrier

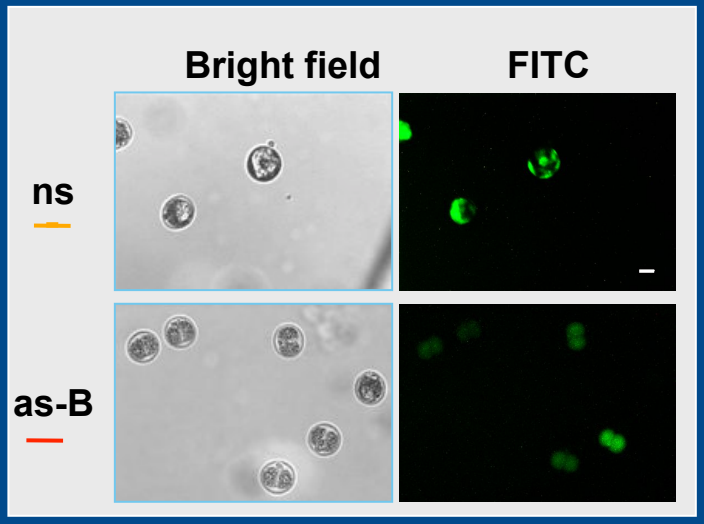
Does the endogenous Reverse Transcriptase play a role in embryogenesis?

Antisense oligonucleotides targeting active LINE-1/L1 arrest early embryo development



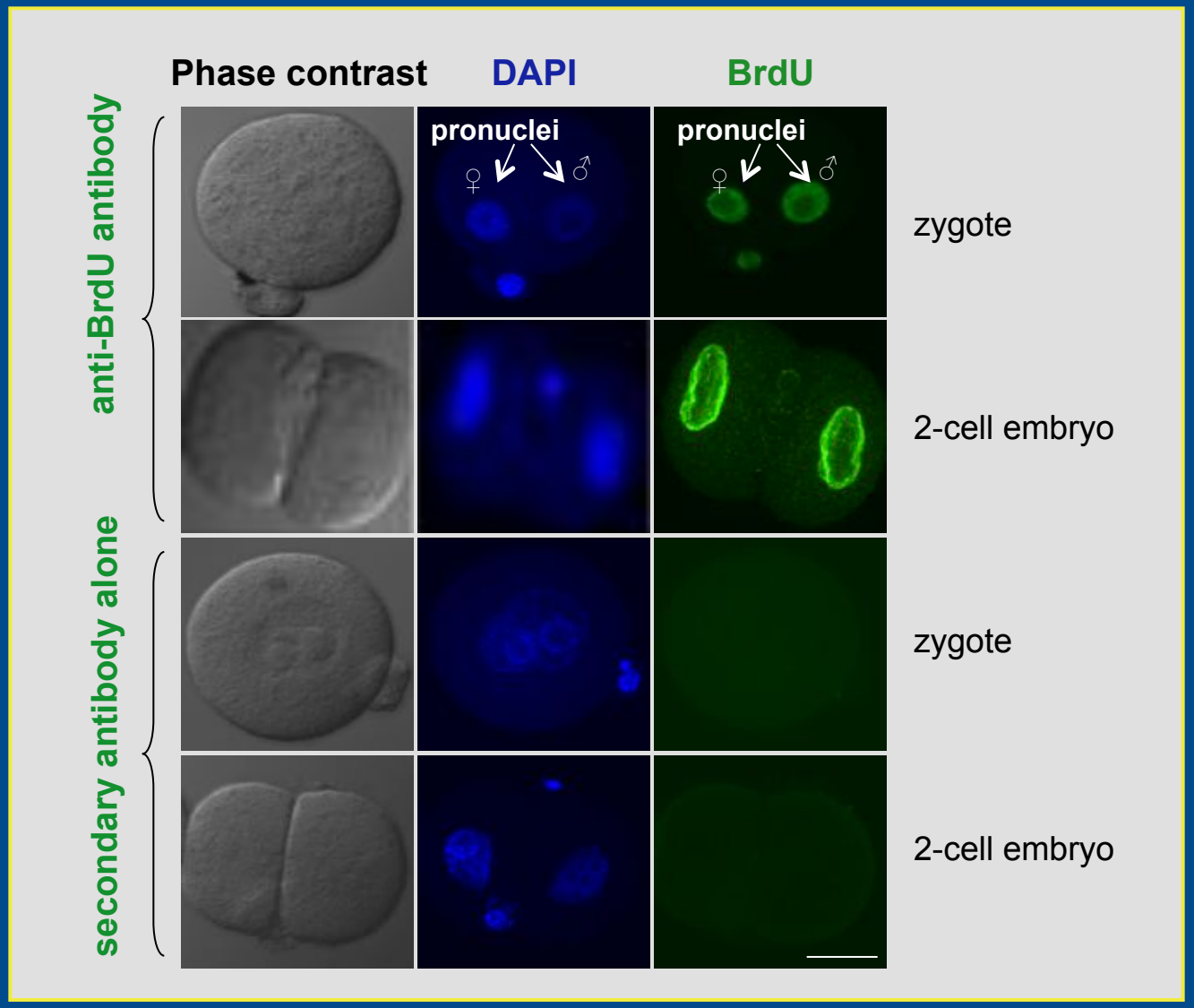
RT activity assay





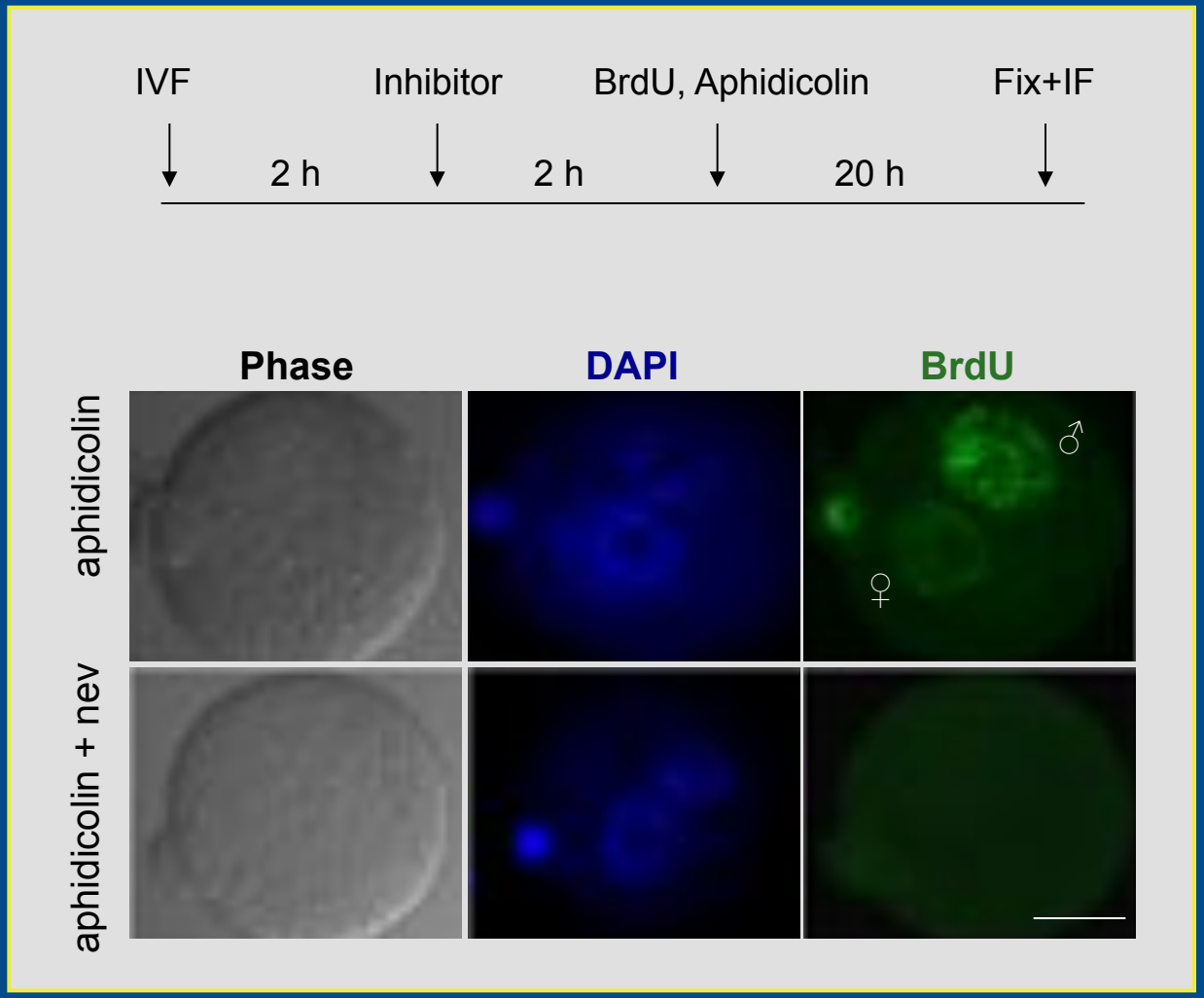
Beraldi et al Mol Repr Dev 2006

BrdU incorporation in early mouse embryos



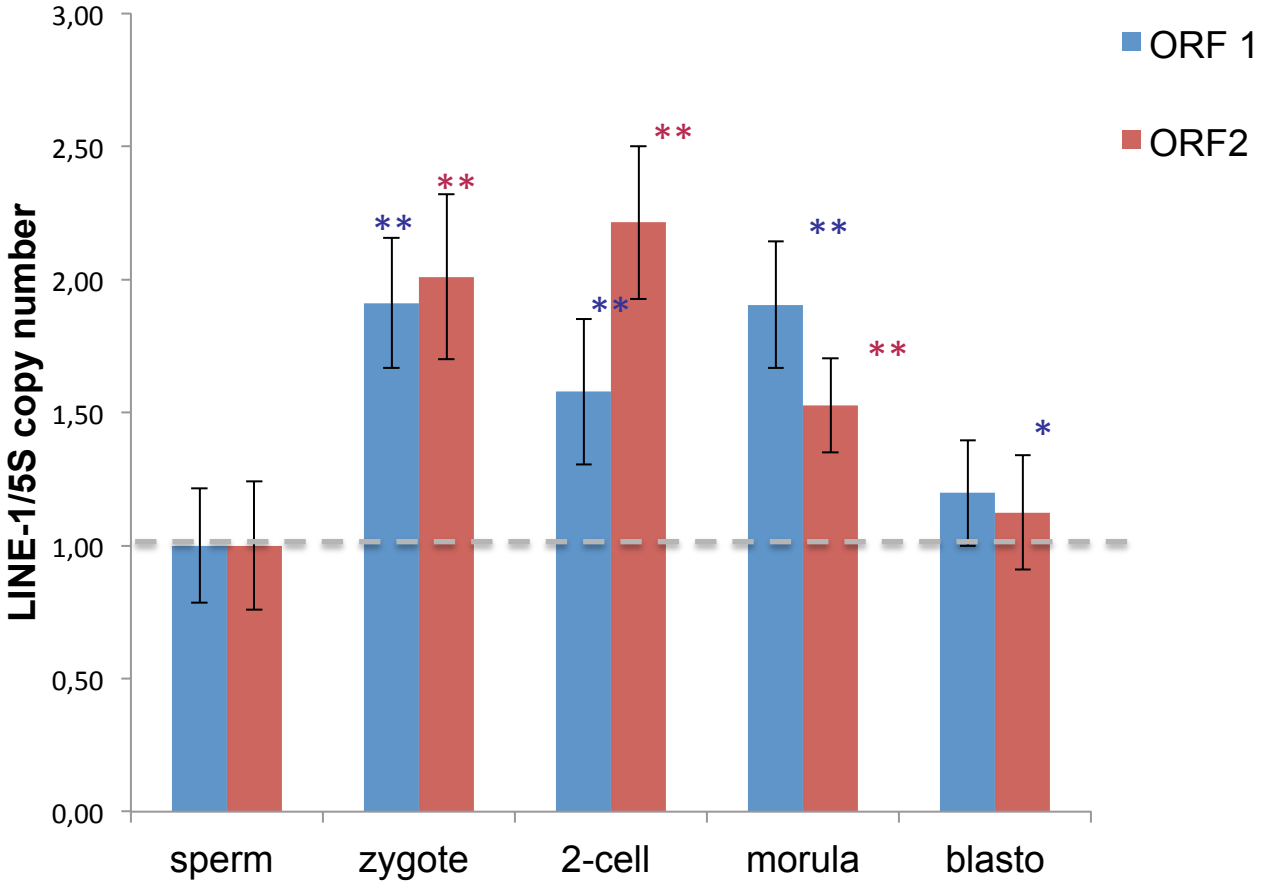
Vitullo et al. Mol Repr Dev 2012

Nevirapine (nonnucleoside RT Inhibitor) abolishes aphidicolin-resistant BrdU incorporation



Vitullo et al. Mol Repr Dev 2012

LINE-1 copy number is amplified in preimplantation embryogenesis



Vitullo et al. Mol Repr Dev 2012

Conclusions (III)

- RT inhibition causes a drastic arrest of embryo development (2-4 cell stages)

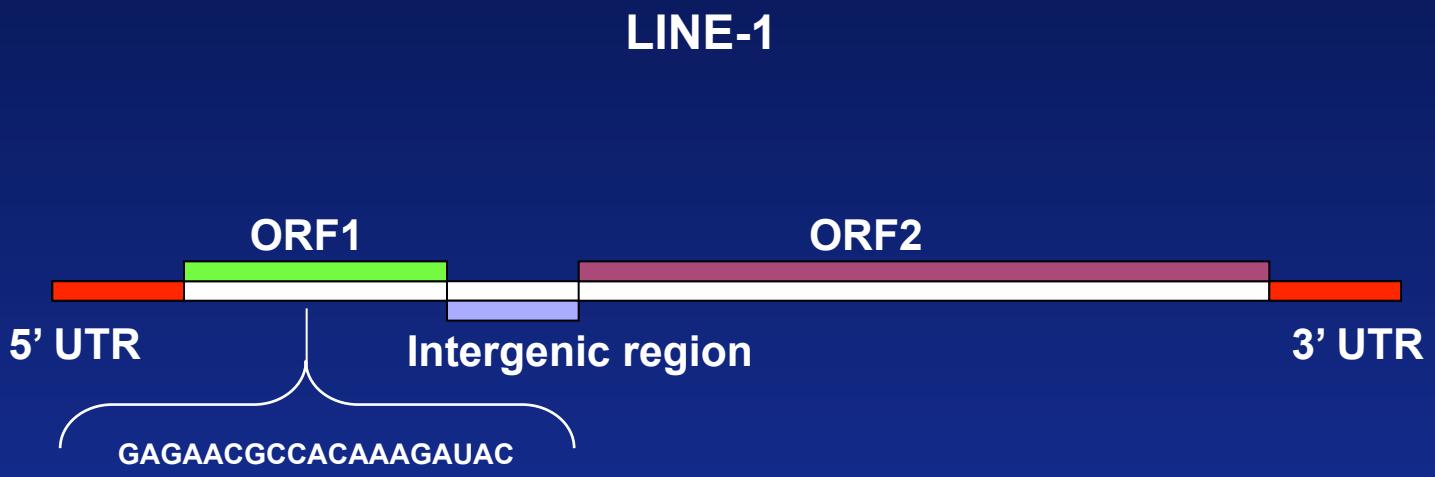
- Reverse transcription takes place in both male and female zygotic pronuclei soon after fertilization

-LINE-1s are amplified throughout preimplantation development

RT activity is strictly necessary for preimplantation development

Does the endogenous Reverse Transcriptase play a role in cell proliferation and tumor growth?

Targeting human active LINE-1 retroelements by RNA interference

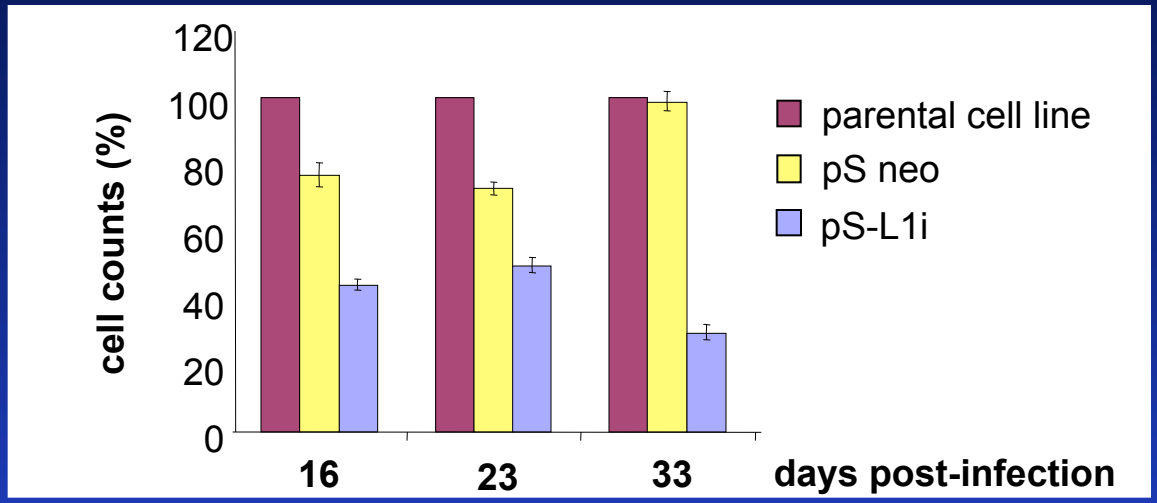


Oricchio et al., Oncoegne 2007

LINE1-TARGETED RNAi REDUCES CELL PROLIFERATION AND STIMULATES DIFFERENTIATION

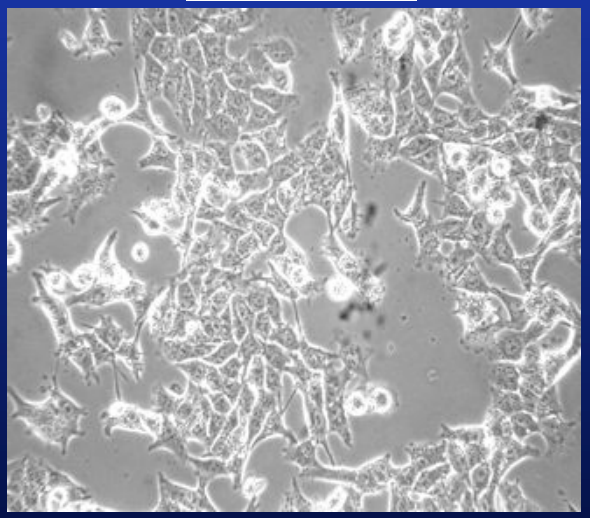
A375 MELANOMA CELL CULTURES

PROLIFERATION

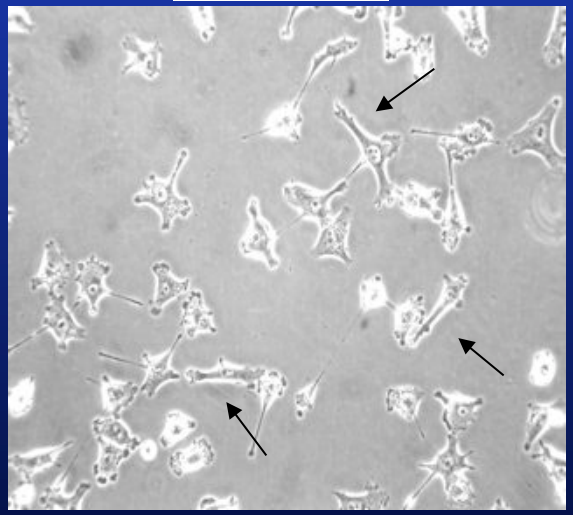


MORPHOLOGICAL DIFFERENTIATION

pS neo

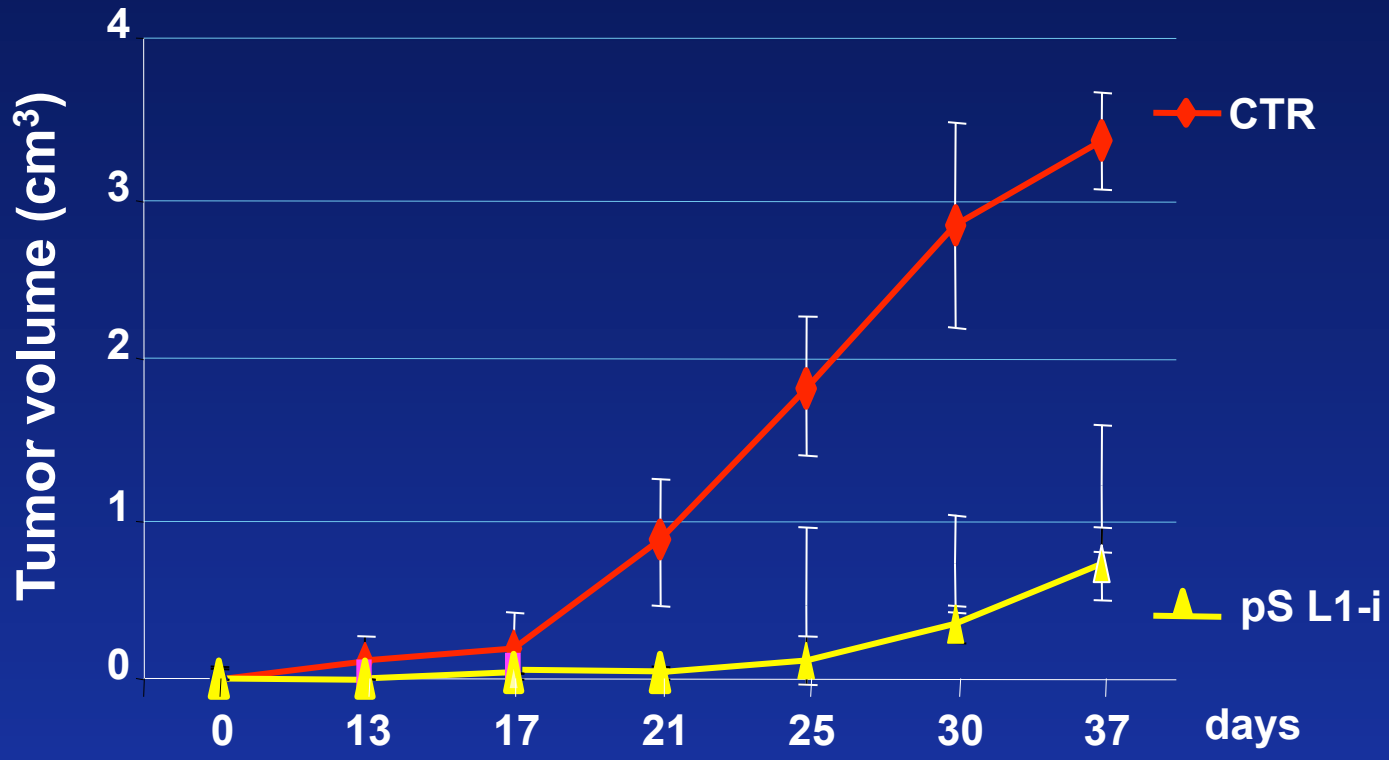


pS-L1i



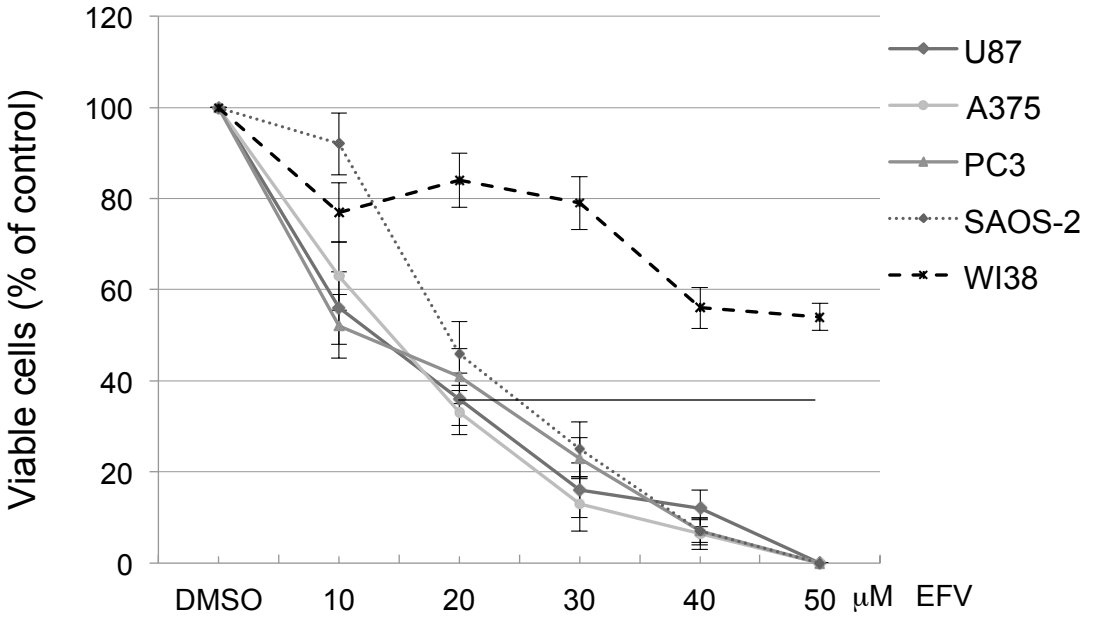
Oricchio et al.,
Oncoegne 2007

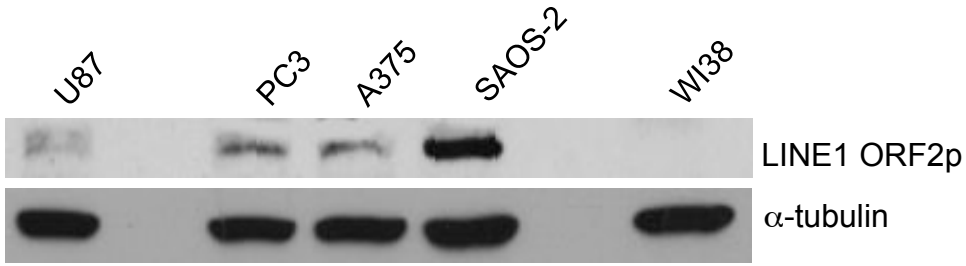
A375 cells interfered for LINE-1 exhibit reduced tumorigenicity *in vivo*



Oricchio et al., Oncoegne 2007

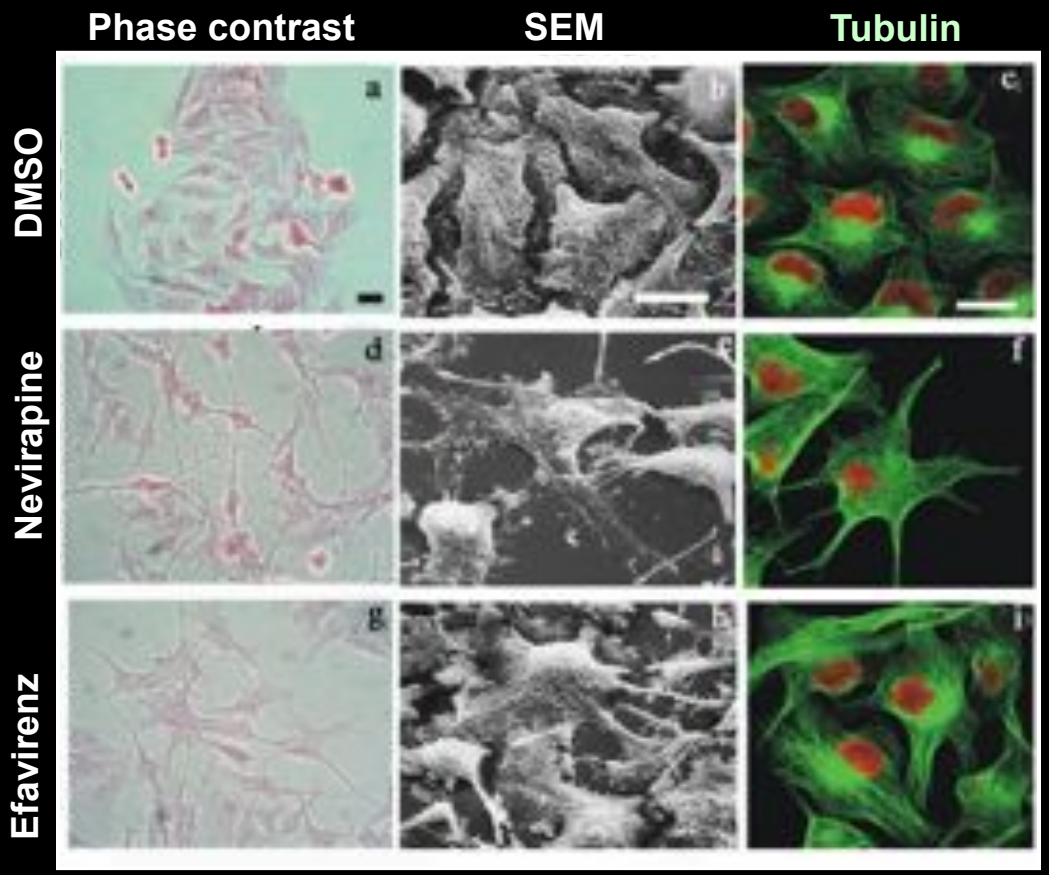
Efavirenz inhibits proliferation in human transformed cell lines





Sciamanna et al. Oncotarget 2013

RT inhibitors induce morphological differentiation of melanoma cells



A-375 melanoma cells exposed to RT inhibitors acquire:

- dendritic-like extensions
- flattened shape
- high adhesion

These features are typical of melanoma cells induced to differentiate

In vivo anti-tumor effectiveness of RT inhibitors

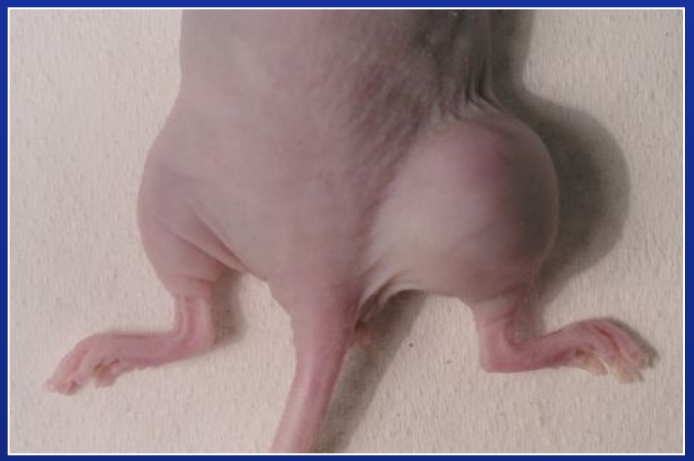
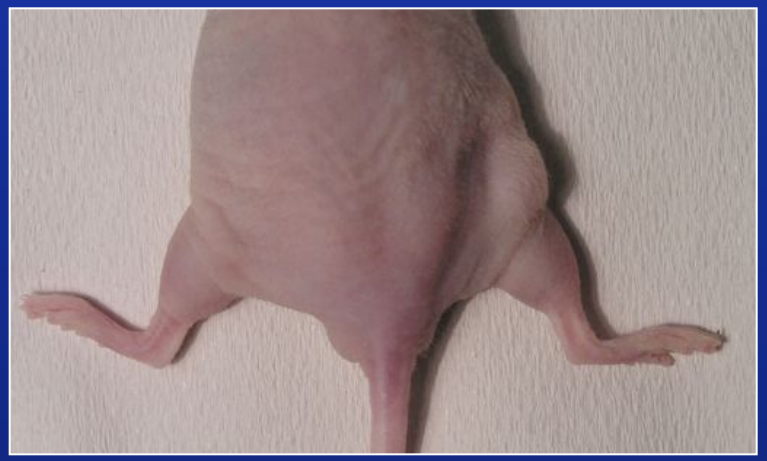
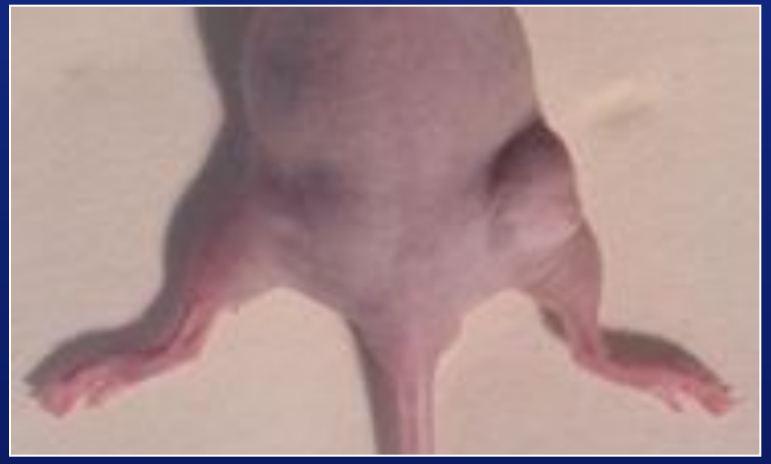
Assaying RT inhibitory treatments in animal models

Human tumor cell lines xenografted in nude mice:

- **PC3 prostate carcinoma**
- **HT29 colon carcinoma**
- **A375 melanoma**
- **H69 small cell lung carcinoma**

Treatment with RT inhibitors started one day, or one week, after tumor xenograft

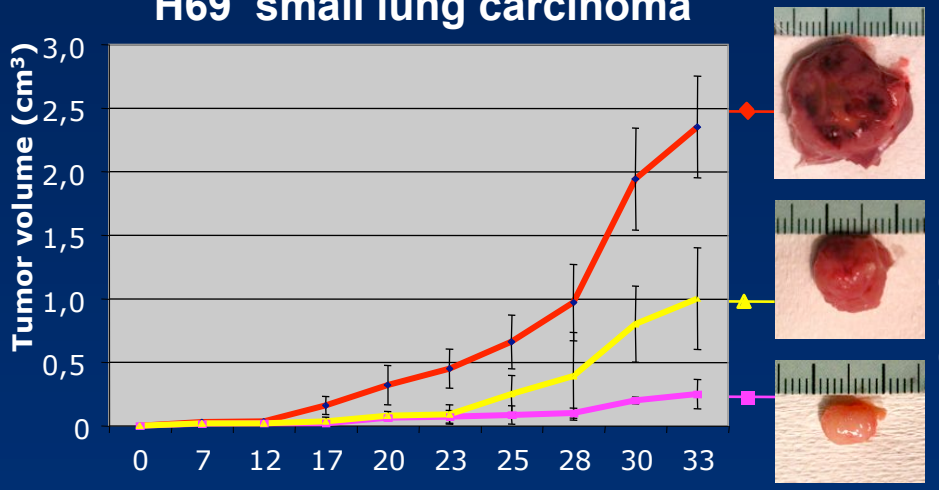
Efavirenz inhibits the growth of H69 small cell lung carcinoma in nude mice

	Untreated	Efavirenz-treated
25 days		
40 days		

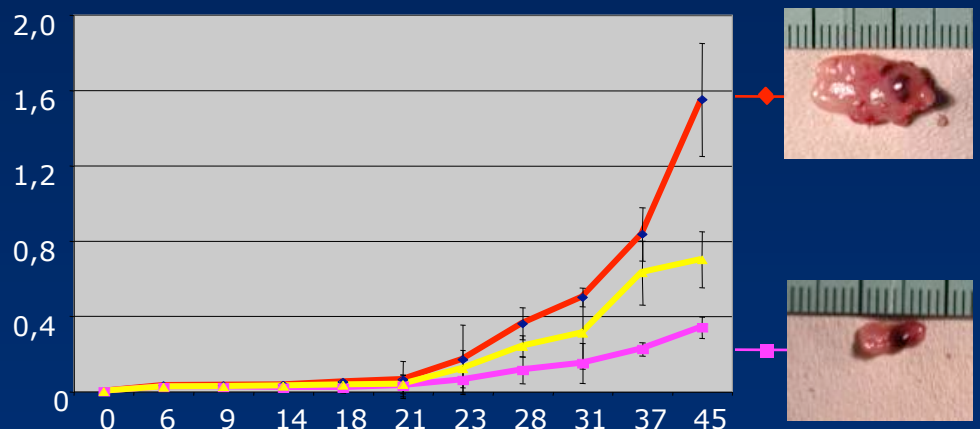
RT inhibitors reduce the growth of tumor xenografts *in vivo*

Sciamanna et al., Oncogene 2005

H69 small lung carcinoma

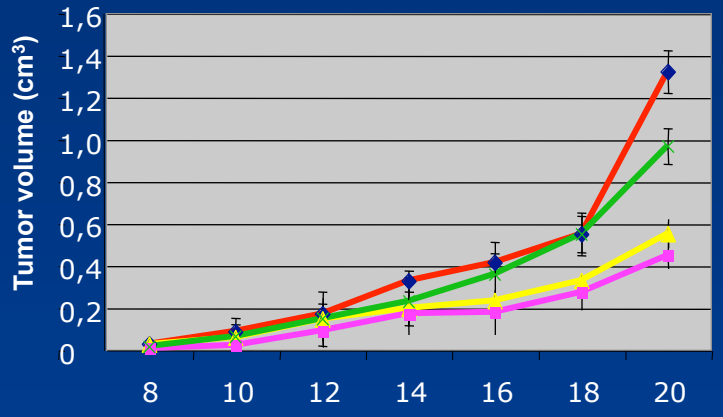


A375 melanoma

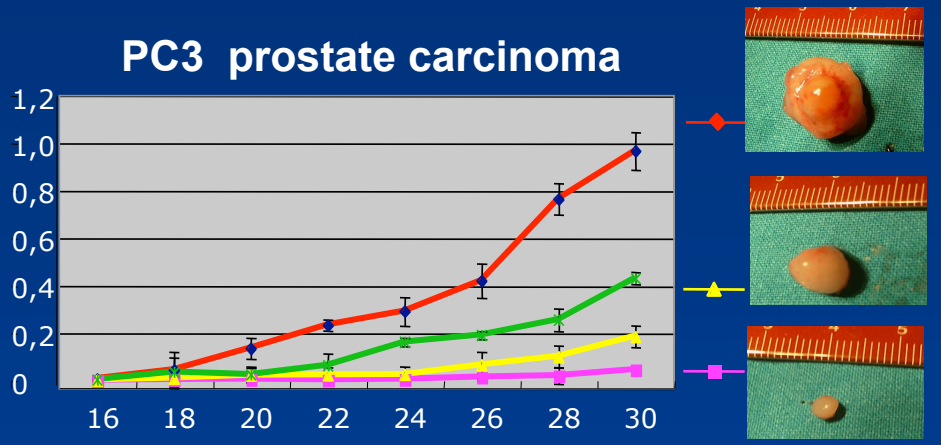


ctrl
Efavirenz starting 1 day after tumor cell inoculation
Efavirenz starting 1 week after tumor cell inoculation
Efavirenz interrupted after 14 days

HT29 colon carcinoma



PC3 prostate carcinoma



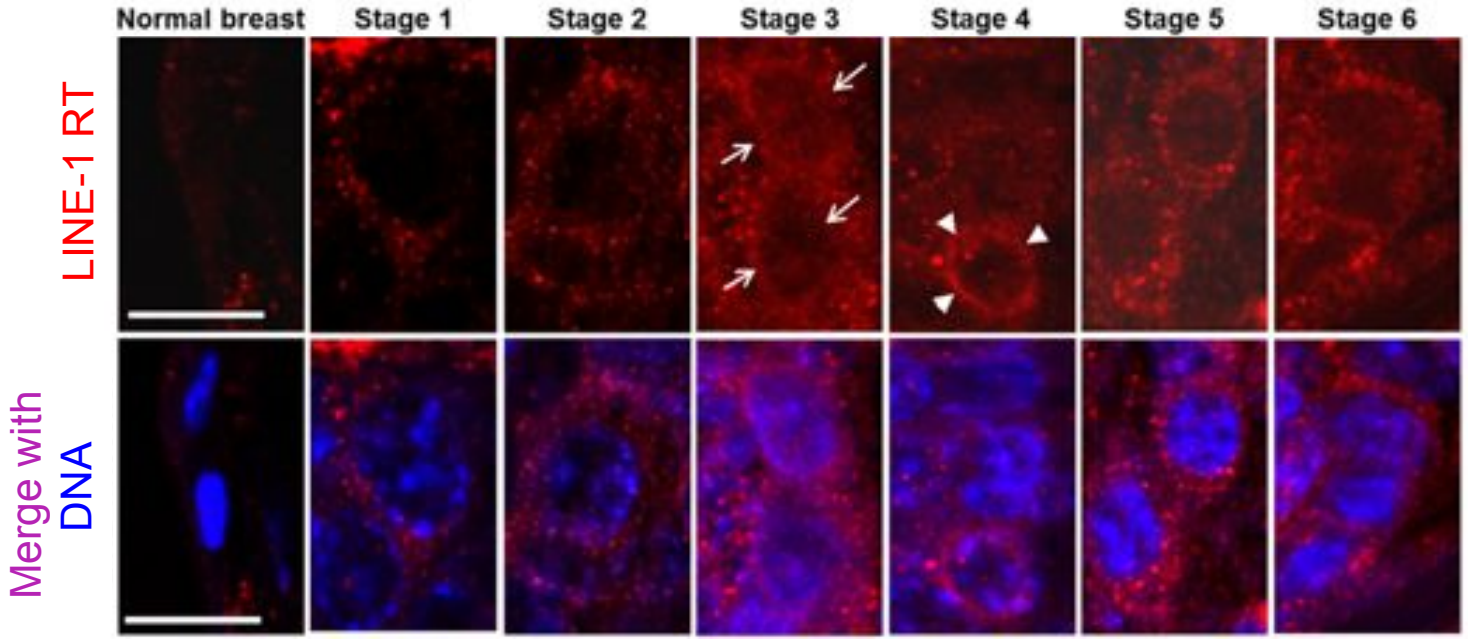
TESTING OF RT INHIBITORS: SUMMARY

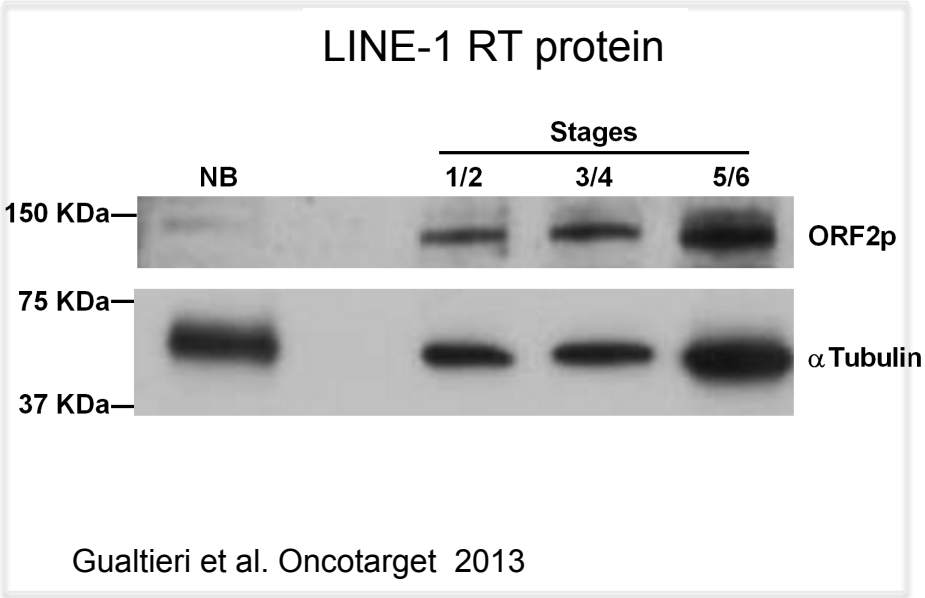
The results with animal models suggest that RT can be regarded as a target in a novel cancer differentiation therapy.

A phase II trial with the RT inhibitor Efavirenz on patients with bone metastasis of primary prostate carcinoma is ongoing (Institut Bergoniè, Bordeaux, France)

A “junk DNA”-based anticancer therapy?

RT expression and activity during breast cancer progression





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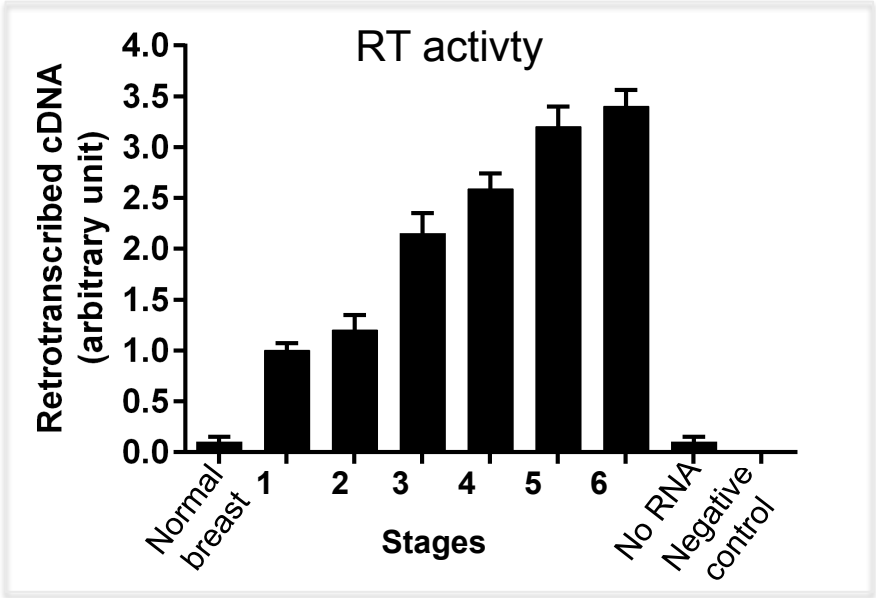
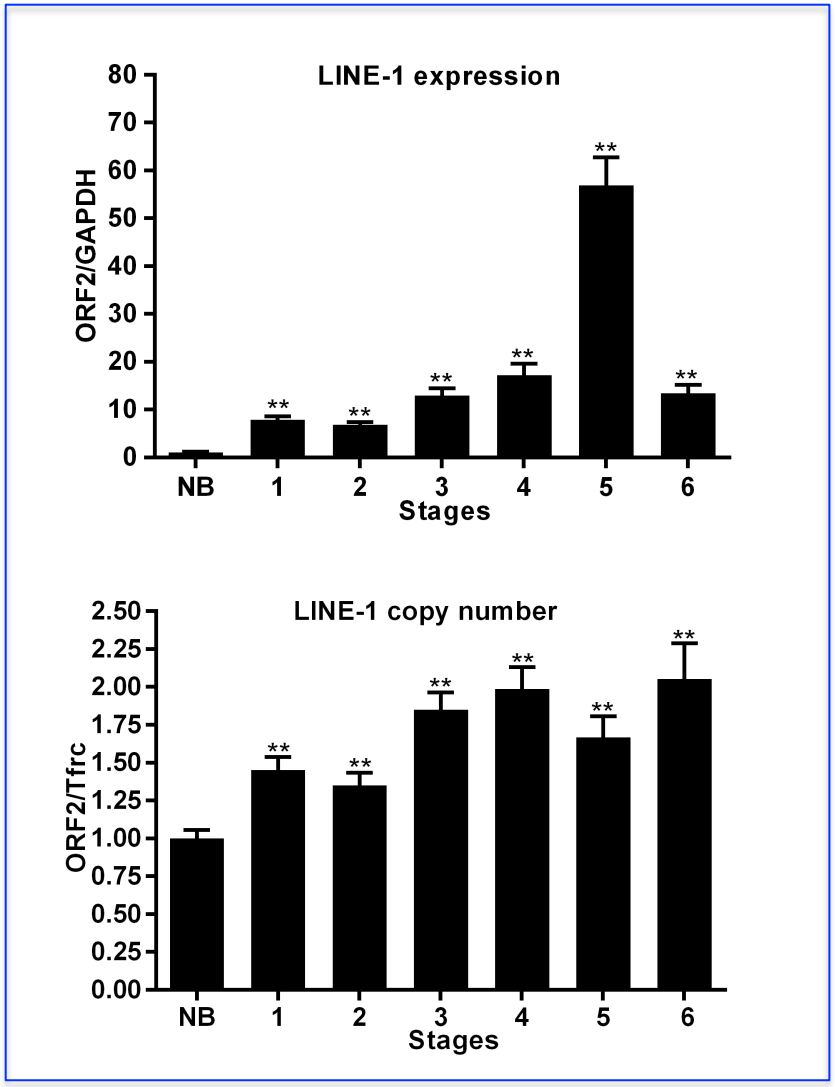
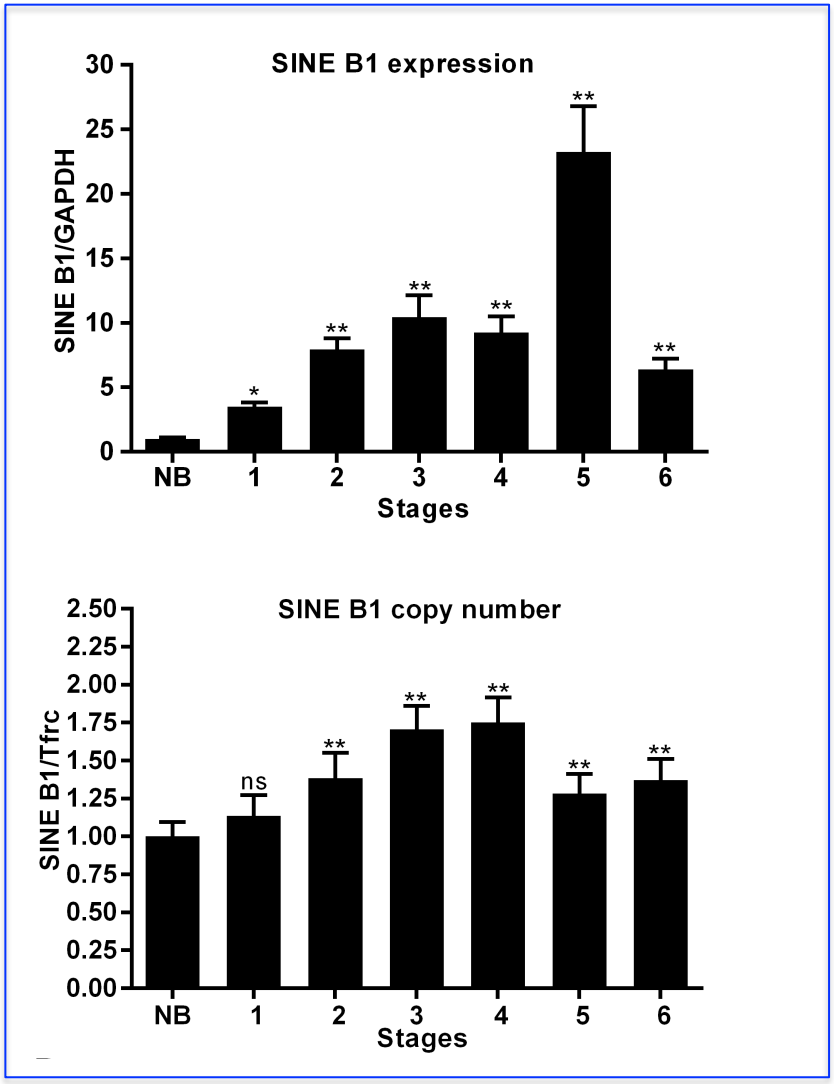


Enhanced transcription and copy number amplification of LINE-1 and SINE B1 during breast cancer progression





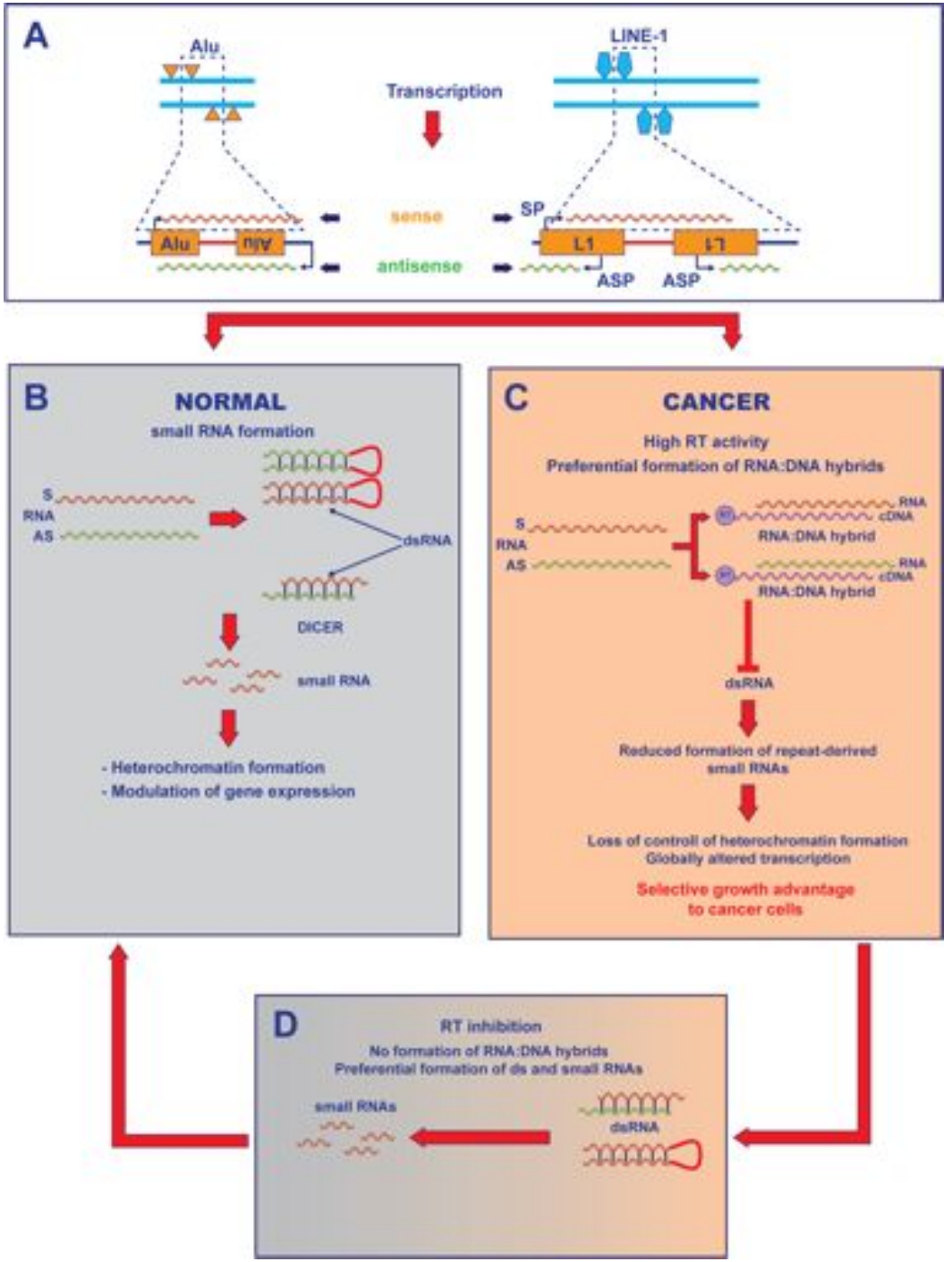
Gualtieri et al. Oncotarget 2013

Efavirenz-modulated metastamiRs, miRNAs promoting tumor progression, invasiveness and metastasis

Name	EFV modulation	Modulation in cancer	Biological function	Cancer type
miR-21	down	up	Correlated with invasion and metastasis	lung, colorectal
miR-33a	down	up	Dysregulated in bone metastasis from primary prostate cancer	prostate
miR-181a	down	up	Related with shortened disease-free survival, highly upregulated in osteosarcoma	osteosarcoma
miR-199b	down	up	Dysregulated in metastasis	brain
miR-34b	up	down	Downregulated in metastasis, reactivated upon drug treatment inhibits tumor growth and lymph node metastasis	colorectal, melanoma, head and neck
miR-125b	up	down/up	Downregulated in breast and upregulated in i cancer, association with cancer metastasis	breast, colorectal
miR-146a	up	down	Inversely correlated expression with cancer progression and metastasis	prostate, breast
miR-148a	up	down	Downregulated in metastasis, acts as metastasis suppressor inhibiting tumor growth and lymph node metastasis	colorectal, melanoma, head and neck
miR-193b	up	down	Inversely correlated expression with cancer progression, invasion and metastasis	breast
miR-204	up	down	Highly reduced expression in cancer progression; overexpression suppresses invasiveness and acts as metastasis suppressor	head and neck

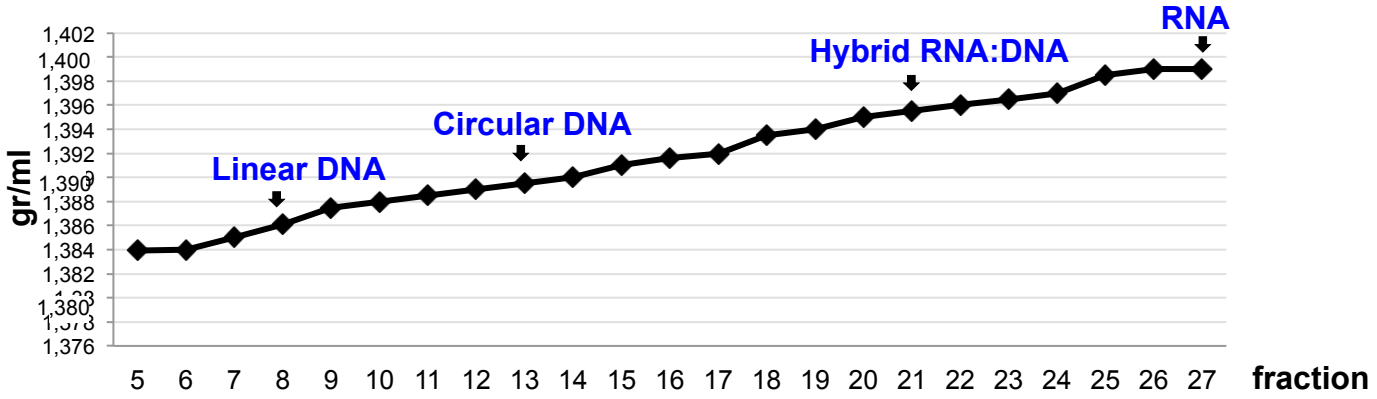
Sciamanna et al. Oncotarget 2013

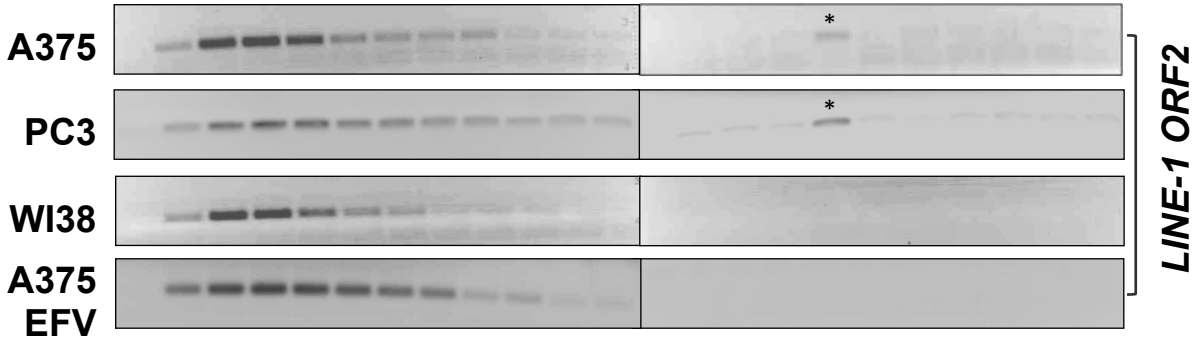
RT control of the cancer cell transcriptome: a model

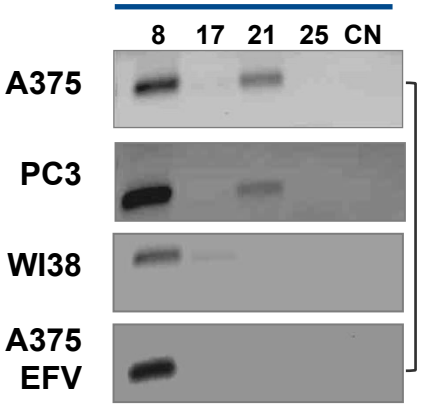


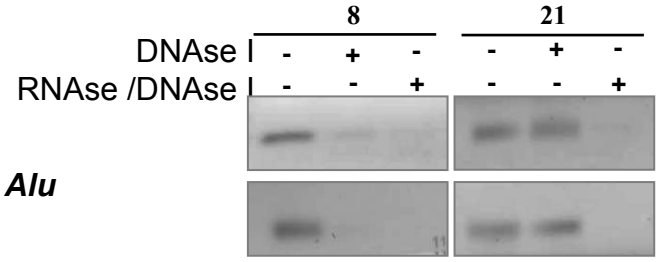
Sciamanna et al. Oncotarget 2013

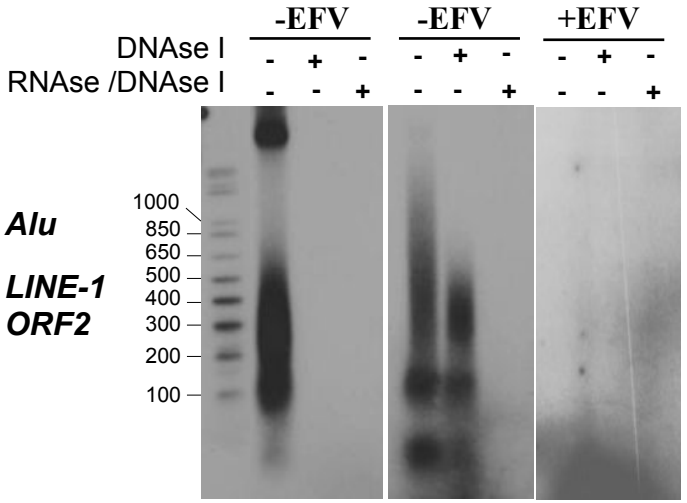
Identification or RNA:DNA hybrid structures in cancer cells through CsCl density gradient centrifugation (Sciamanna et al. 2013)











Conclusions (IV)

- LINE-1 and Alu elements are up-regulated, both in expression and in copy number, during tumor progression

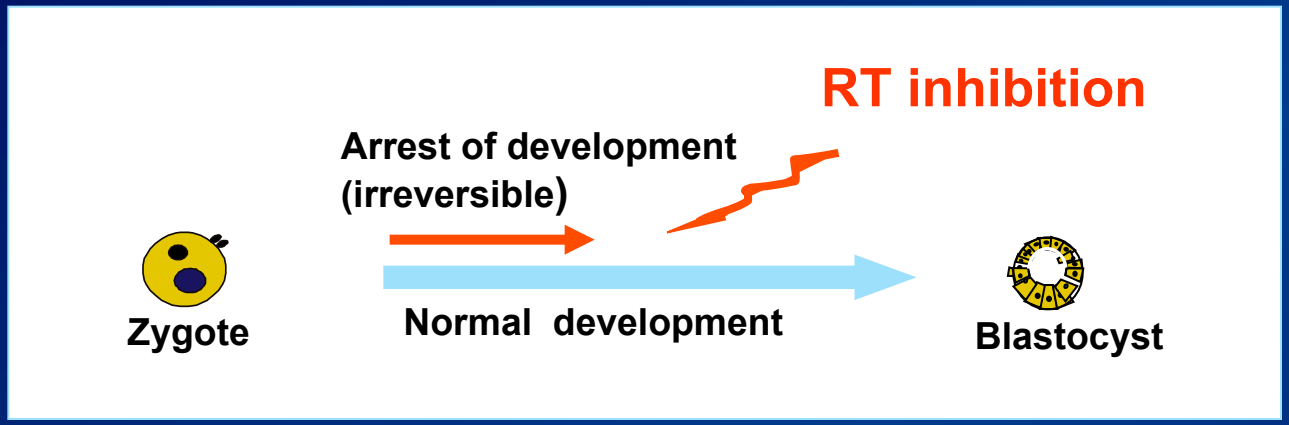
- LINE-1-encoded ORF2 protein, hence RT, increases during tumor progression

- RT inhibition reduces cancer cell proliferation and promotes differentiation; also antagonizes cancer progression in animal models in vivo

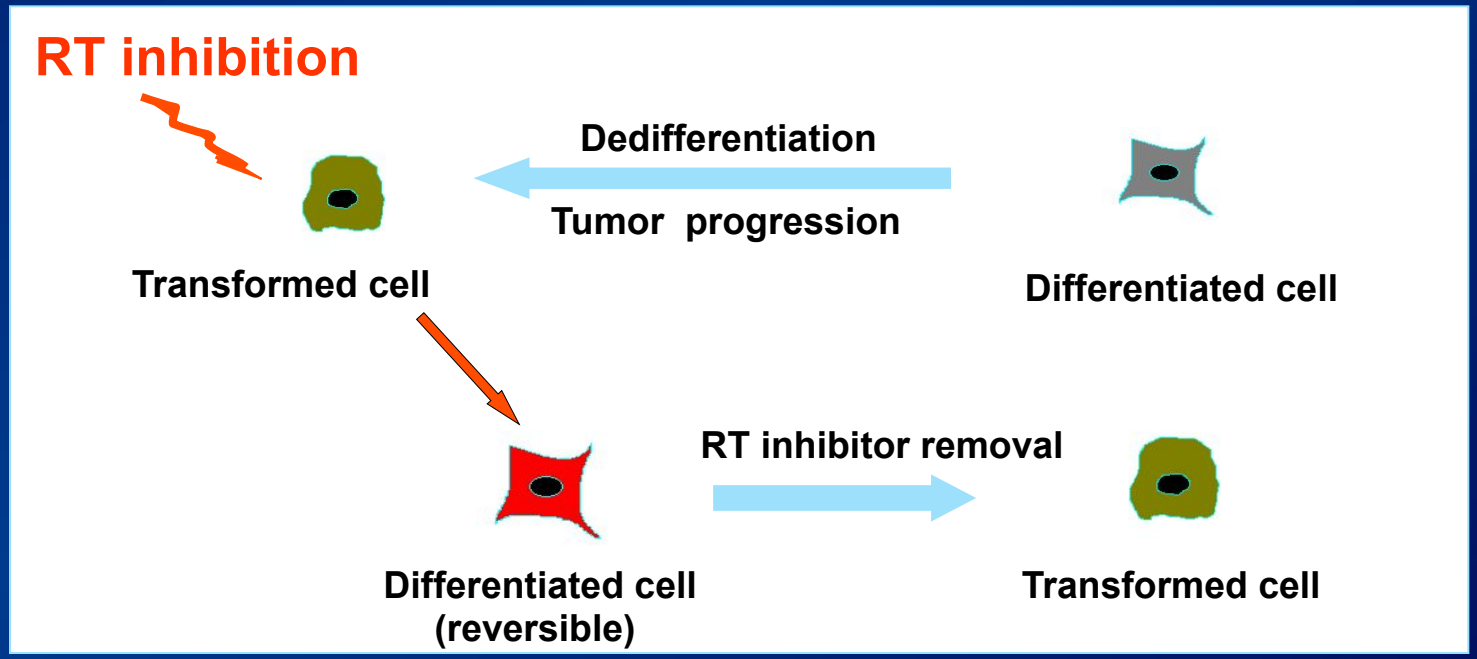
- RT inhibition globally reprogrammes the expression profile in cancer cells

- An RT-dependent cancer-promoting mechanism plays a causative role in cancer onset and progression

A. Inhibition of endogenous RT in early embryos



B. Inhibition of endogenous RT in transformed cells



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