In vivo models for oncology

Lentiviral vectors expressing luciferase or fluorescent reporters are a good tool for **noninvasive analyses of animal model tumors**. They can efficiently be used through direct injections into adult animals and through *in vitro* transductions of any tumoral cells, then reimplanted in cancer models.

Our data shows that lentiviral vectors have to be highly **concentrated** and **purified** to efficiently transduce cells of interest, to maintain the original cell phenotype, proliferation, and viability and to achieve an effective transgene expression. These criterias combined with the lentiviral vector properties are required to ensure the development of **trustworthy predictive cancer models**, and thus build the link between *in vitro* assays and *in vivo* results to ultimately be translated into therapeutic applications.

The genetic modification of tumoral cells

Introduction

Relevant predictive cancer models must be conceived through the use of a tool ensuring both the original phenotype of the cells to be strictly maintained as well as a stable expression of the sequence of interest in all target cells.

Vectalys has developed an innovative and efficient system to allow the use of viral vectors for the design of animal models. Here, we present the results obtained with some oncogenic models, using concentrated lentiviral suspensions for the transduction of tumoral cell lines before their transfer into recipient mice.

Material and methods

Vectalys offers to carry out the preliminary bioinformatic evaluation stage of your gene expression or silencing project. Our team of experts will help you define, analyze target gene(s), provide a detailed gene study (alternative transcripts, SNP...) and design the best shRNA sequences when gene silencing is required.

We have constructed self-inactivating (SIN) vectors by deleting a segment in the U3 region of the 3' LTR, resulting in the transcriptional inactivation of the LTR in the proviruses. These lentiviral vectors also contain a cPPT/CTS sequence resulting in an increased reverse transcription.

cDNA or shRNA of the gene of interest are cloned into an expression plasmid containing an ubiquitous promoter or the specific promoter of your choice, a fluorescent marker and/or an antibiotic resistance gene.

Lentiviral vectors are produced and subsequently concentrated (1E9 TU/ml) and purified (PP/TU<200).

Cell lines or primary cells are then transduced with our lentiviral vectors at their optimal MOI (multiplicity of infection), before being finally injected into recipient mice.

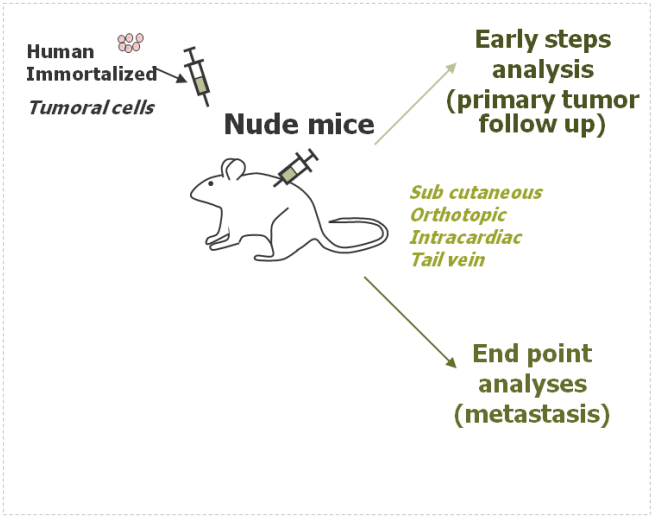
Results

In vitro transduction of tumor cell lines

This chart shows a non-exhaustive list of some tumor cells commonly used for predictive cancer models.

The transduction efficiency always reached 100%, depending on the MOI.

Cancer model	Organism	Cell line	Transduction efficiency
Breast	Human	MCF7	100 %
	Mouse	4T1	
CNS	Human	SHSY-5Y, DAOY	
	Mouse	N1E-115	
Colon	Human	HCT116, Caco2	
	Mouse	MC38	
Kidney	Human	HEK	
	Mouse	Renca	
Leukemia	Human	THP1, Jurkat	
Liver	Human	HuH7	
Lung	Human	A549	
	Mouse	LL2	
Lymphoma	Human	U937, RAJI	
	Mouse	EL4	
Melanoma	mouse	B16F1, B16F10	
Osteosarcoma	Human	U2OS	
Ovary	Human	OVCAR3	
Pancreas	Mouse	Min6	
	Rat	INS1	

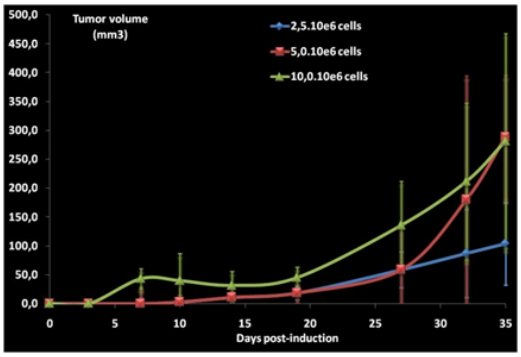


Validation of the *in vivo* expression

The following results illustrate the *in vivo* injection of tumoral cell lines transduced with lentiviral vectors expressing DsRed or Luciferase reporters or carrying cDNA or shRNA against specific genes.

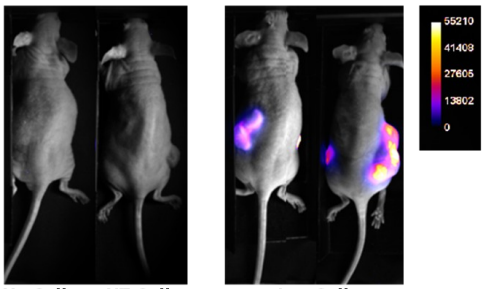
1– Follow up of the primary tumor

The figure below shows the dose response assessment of a PC3 primary tumor development: various amount of PC3 cells have been sub-cutaneously reimplanted into Nude recipient mice.

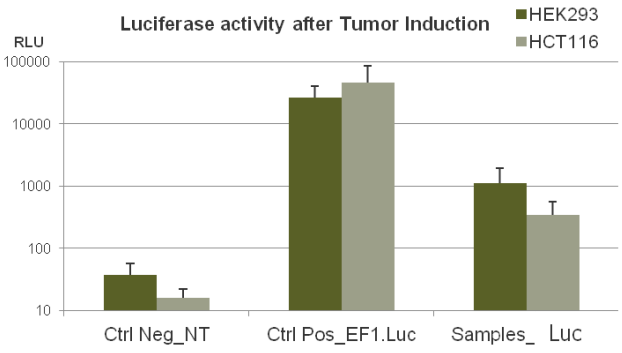


Dose response assessment of a PC3 primary tumor development (s.c.)

The pictures and the graph below show some data obtained after reimplantation of tumor cells expressing a cDNA coupled with the Luciferase reporter.



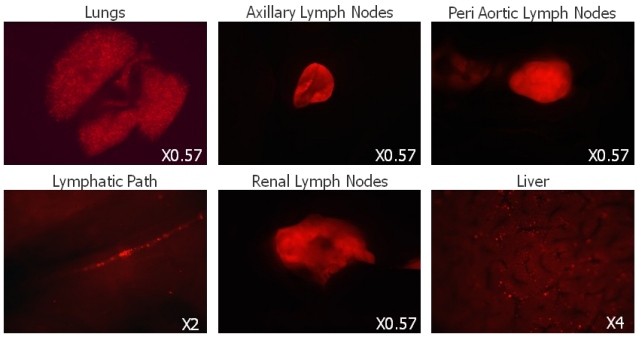
In vivo analyses day 28 post tumor induction (ImageJ Software)
left flank: 2E6 HCT 116 cells , right flank: 5E6 HEK293.



Negative Control value corresponds to background noise.Values expressed as maximum luminescence value/cm²/min

2– End point analyses : metastasis development

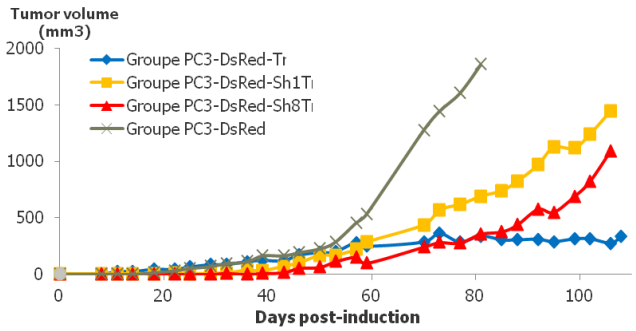
Data shown here illustrate some macroscopic analyses of metastatic development after PC3 cells reimplantation.



Macroscopic fluorescence analyses: Data obtained from one representative mouse injected with 5E6 cells (Day 80)

3– Transfer of PC3 cells transduced with shRNA-DsRed lentivectors into Nude recipient mice.

Data below show the tumor development after reimplantation of cells transduced with lentiviral vectors expressing an anti-tumoral candidate gene cDNA or various shRNA designed against oncogenes. All constructions carry the DsRed reporter.



Time course assessment of PC3 primary tumor development (s.c.)

Conclusion

The use of **highly concentrated and purified lentiviral vectors** enables efficient and safe transduction. These approaches illustrate that highly concentrated and purified lentiviral vectors are ideal engineering tools to study tumor and metastasis developments, without interfering on the cell phenotype.

Contact us to discuss your project and take advantage of the Vectalys team's expertise and custom value-added services .

Tender Loving Care :
Our protocols are animal friendly and approved by an ethics committee.