Utilizing a novel luciferase labeling technique to establish and validate preclinical models of pancreatic cancer

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Jenni Bernoulli[1], Matthias Bozza[2], Katja M. Fagerlund[1], Leila Lukin[1], Johanna Tuomela[1], Mari I. Suominen[1], Suzanne Dilly[3], George Morris[3], Jussi M. Halleen[1] and Richard Harbottle[2]
[1]Pharmatest Services Ltd., Turku, Finland, [2]German Cancer Research Center, Heidelberg, Germany, [3]ValiRx Plc, London, United Kingdom
Email correspondence to jenni.bernoulli@pharmatest.com

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Introduction

Bioluminescent-labeling allows sensitive non-invasive sequential imaging of tumor development and early metastasis. However, current methods for the genetic modification of cells typically use integrating genotoxic viruses that can potentially disrupt the molecular behavior of cancer cell lines due to their random nature of integration. Here, we utilized a DNA vector that comprises a S/MAR (Scaffold/Matrix Attachment Region) element to stably modify cells that can be subsequently used in xenograft studies providing robust and long term expression without adversely affecting cellular behavior or function.

Aim of the Study

Our aim was to establish an improved, cost efficient and simple method to genetically modify human cancer cells with a bioluminescent reporter gene that can be utilized for *in vivo* drug development. Secondly, our aim was to study effects of VAL401 (formulation of Risperidone in Rumenic Acid) *in vitro* and *in vivo* in pancreatic cancer models.

Materials and Methods

Human Capan-1, Panc-1, MiaPaca-2 and BxPC-3 pancreatic cancer cells (ATCC, DSMZ) were stably transfected with UBC-LUC-S/MAR and cultured for 4 weeks under selection. Colonies were isolated, expanded and evaluated for luciferase expression and molecular integrity of the DNA vector.

For *in vitro* proliferation assay, parental and luciferase-labeled cells were cultured in 96-well plates. A known chemotherapy drug, 0.1 µM gemcitabine (Eli Lilly), was used as reference compound. The cells were cultured for 6 days and the effects of the gemcitabine was identified by measuring the cells at days 0, 3 and 6 using a WST-1 proliferation kit (Roche Diagnostics). Effects of VAL401 on Capan-1 proliferation was determined using CellTiter-Glo® Luminescent Cell Viability Assay (Promega, WI, USA).

For *in vivo* studies, 3 x 10^4 parental BxPC3 and BxPC3-luc cells were inoculated into the pancreas of athymic nude mice (Envigo, the Netherlands). Tumor-bearing mice were treated with vehicle, gemcitabine (60 mg/kg, q3dx4 i.p, one week pause, q3dx4 i.p) or VAL401 (risperidone in rumenic acid doses 1 and 2 mg/kg, p.o daily). Tumor growth was followed by bioluminescence imaging (BLI) once a week (IVIS Lumina 2, Caliper Life Sciences). After sacrifice, orthotopic tumors were characterized using histology (H&E staining) and immunohistochemistry (Polyclonal Goat IgG Human/Firefly Luciferase antibody, Novus Biologicals). Stained slides were scanned using Pannoramic slide scanner (3D Histech).

Efficient Genetic Modification of Cells

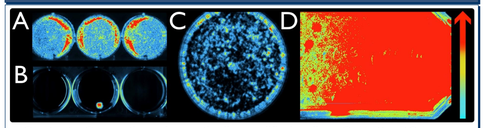
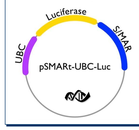


FIGURE 1. Generation of genetically modified cells. pSMARt-UBC-Luc was introduced into BxPC3 cells using PeqFect reagent (PeqLab/VWR).

This figure represents the results of a typical experiment where cells can be imaged at each stage of the procedure. (A) Cells imaged 24 hours after transfection in a 6-well plate. (B) Within weeks, colonies of cells expressing luciferase can be isolated and seeded into 10 cm petri dishes (C) and expanded ad infinitum. (D) Illustrates a confluent flask of BxPC3 cells robustly expressing the transgene luciferase. The arrow represents the increasing intensity of bioluminescence (from blue to red) of transgenic Luciferase expression.



Three months following the initial transfection procedure, total DNA was isolated from the BxPC3-Luc cell line by plasmid rescue, Southern Blot analysis and PCR demonstrated that the DNA vector remained episomal and the expression cassette remained intact.

Comparison of Genetic Modification Protocols

Integrating Vector Xenografts	Isogenic S/MAR DNA Xenografts
Construction of gene transfer vectors is typically lengthy, intensive, costly and generally requires	Construction of S/MAR DNA vectors is versatile and simple
Generation of stable cell lines using viral vectors are lengthy and costly	Generation of stable cell lines using S/MAR DNA is cost-efficient and typically takes only a month
Integration of viral vectors/pDNA alter genetic background of tumor cells	S/MAR DNA Vectors remain episomal and therefore do not affect genetic information of the cells
Viral vectors have a small insert capacity	S/MAR DNA has an unlimited insert capacity
Potential interaction of oncolytic viral vector with host's immune system	S/MAR DNA is non-immunogenic
Integrating Vectors can influence the expression of endogenous genes	Non-integrating S/MAR DNA Vectors have no influence on the expression levels of endogenous genes
Integrated vectors typically provide only variable transgene expression which can skew measurements and affect quantitation	S/MAR DNA Vectors provide robust, consistant and persistent transgene expression following engraftment
Integrated Vectors are prone to epigenetic silencing	The transgene expression driven by S/MAR DNA Vectors is resistant to epigenetic silencing

In vitro analyses of pancreatic cancer cell lines

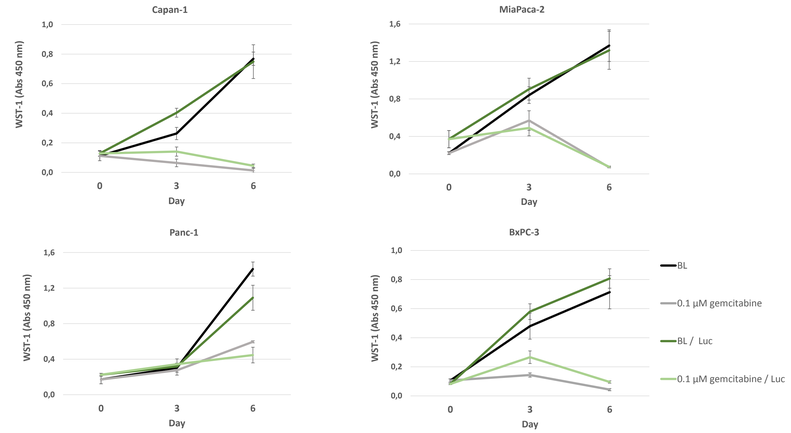


FIGURE 2. Proliferation was determined by measuring number of viable cells. Control compound gemcitabine inhibits proliferation of both parental and luciferase-labeled cells compared the with baseline control (BL).

Luminescence and cell number

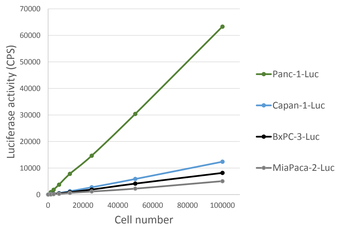


FIGURE 3. Cell number correlates with luminescence output. Serial dilutions of Capan-1-Luc, Panc-1-Luc, MiaPaca-2-Luc and BXPC3-luc cells were made. Luminescence was recorded 5 minutes after reagent addition. Values represent the mean of six replicates for each cell number. There is a linear relationship between the luminescence signal and the number of cells from 0 to 100.000 cells per well.

In vitro VAL401

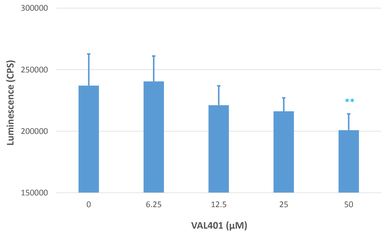


FIGURE 4. Effects of VAL401 on Capan-1 viability on day 3. The results are shown as luminescence (CPS) measured in the CellTiter-Glo viability assay. Two asterisks (**) indicate a statistically significant effect with a p-value < 0.01 compared with the group with no added compound.

Orthotopic pancreatic *in vivo* tumor model

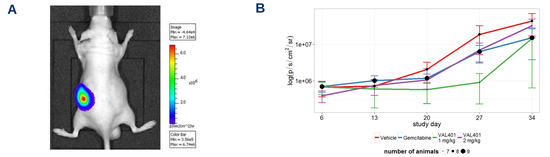


FIGURE 5. (A) BxPC3-luc tumor growth was monitored once a week with IVIS Lumina 2. Images were taken 10 minutes after substrate injection (luciferin 3 mg/mouse ip). (B) Effect of reference compound (gemcitabine) and study compound (VAL401, 1 and 2 mg/kg) on tumor growth.

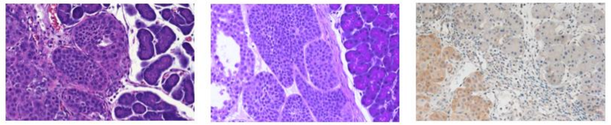


FIGURE 6. To demonstrate that the genetically modified cells generated representative xenografts whilst still retaining robust transgene expression an histopathological assessment of parental BxPC3 and the genetically modified BxPC3-luc xenografts were performed. (A) BxPC3 xenografts, (B) BxPC3-luc xenograft (H&E- staining) and (C) IHC staining of BXPC3-luc xenograft luciferase-staining.

Conclusions

S/MAR DNA vectors are able to generate genetically modified cells without the limitation of random genomic integration, whilst providing extra-chromosomal mitotic stability and robust and sustained transgene expression. When utilized in orthotopic xenograft studies, these luciferase-expressing cells form a reliable non-invasive imaging platform that improves substantially efficacy testing of anticancer drug candidates. VAL401, formulation of Risperidone in Rumenic Acid, inhibited Capan-1 pancreatic cancer cell proliferation *in vitro*.

Acknowledgements

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