VITVO: Mimicking In Vivo Complexity By The Innovative 3D Model

A novel 3D In Vitro Platform for Pre-Clinical Investigations in DrugTesting, Gene Therapy, and Immuno-oncology

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INTRODUCTION

3D tumor cultures are a promising tool for in vitro rebuilding the in vivo behavior of cancer cells for the development and validation of anti-tumor therapies. Various 3D cell culture technologies have been created to better represent in vivo biology, however, advantages and limitations exist[1-3]. Therefore, we developed a novel 3D culture system as a tool that contributes to bridge in vitro and in vivo studies. This tool, named VITVO, is a flat, handheld and versatile 3D cell culture bioreactor that can be loaded with tumor and/or normal cells in combination to rapidly recreate in vitro an in vivo-like environment which can be monitored using a variety of read-outs. Herein, the use of VITVO in several assays focusing on oncology is reported and the efficacy of chemotherapy, biologics, and cell-based anti-cancer agents was tested by comparing VITVO with an in vivo preclinical xenotransplant model.

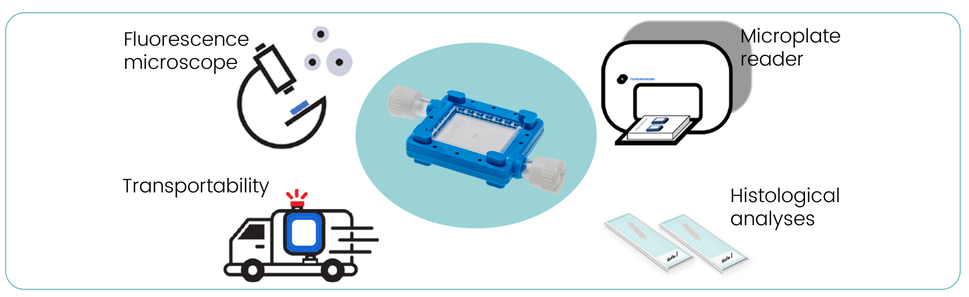

Figure 1. VITVO technology and potentials.

RESULTS

VITVO is biocompatible and allows real-time monitoring of 3D tumor cell growth

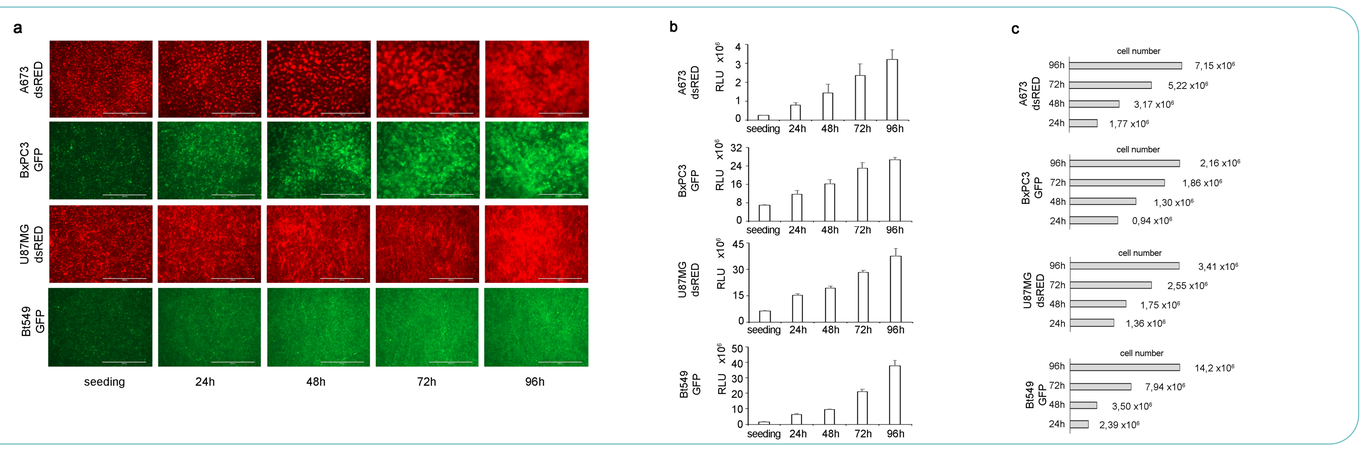

Figure 2. Cell growth in VITVO

(a) In VITVO tumor growth monitoring under a fluorescence microscope. Scale bar 1,000 mm. **(b)** In VITVO cell viability monitoring using Real Time GLO (Promega). Real Time GLO was added at 1X concentration at seeding, at 24h and 48h for A673 dsRED, U87MG dsRED and Bt549 GFP, while it was added at 2X concentration for the 72h and 96h time points. For BxPC3 GFP, Real Time GLO was added 1X concentration at seeding and at 24h, and 2X concentration at 48h/72h/96h. All measurements were performed in triplicate and data are expressed as means ± standard deviation (SD) **(c)** Estimation of tumor cell number in VITVO based on relative light units (RLU; cell number = measured RLU*cell number at seeding/RLU at seeding).

VITVO supports 3D cytotoxicity assay

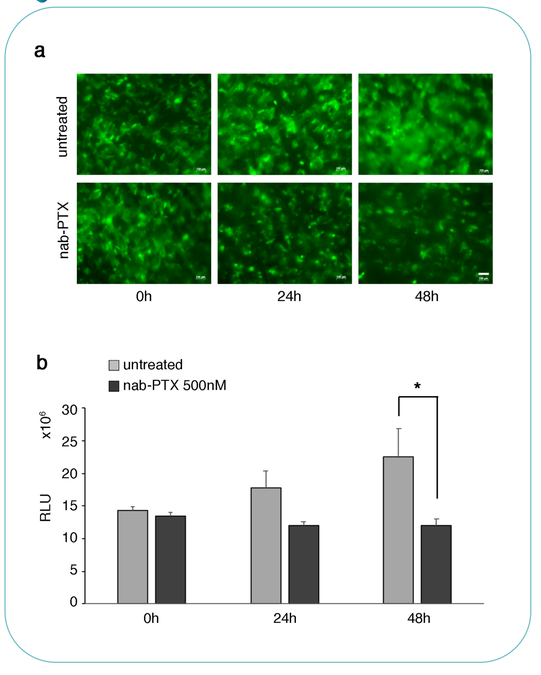

Figure 3. In VITVO efficacy evaluation of a chemotherapy agent

(a) In VITVO visualization of GFP+ BxPC3 under a fluorescence microscope. Scale bar 100 mm. **(b)** In VITVO growth monitoring of tumor cells after treatment with 500 nM nab-PTX using Real Time GLO viability assay. Untreated cells were used as control. All measurements were performed in triplicate and data expressed as means ± SD, *P = 0.015 (t-test).

VITVO hosts a biologic-agent driven pro-apoptotic effect visible using the luciferase assay

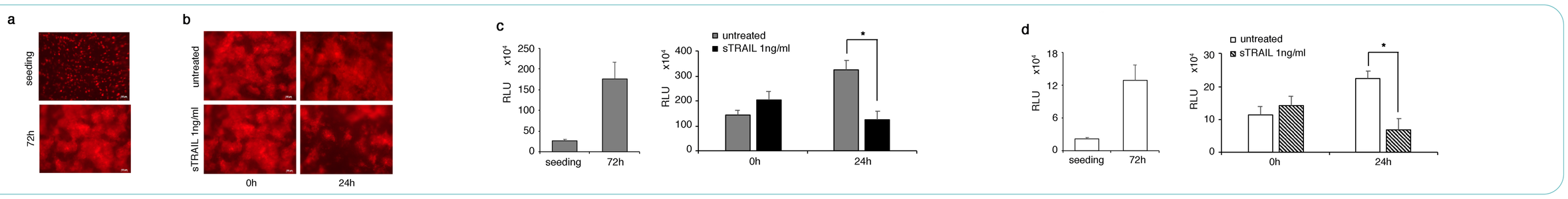

Figure 4. In VITVO evaluation of sTRAIL pro-apoptotic action on Ewing Sarcoma using two different luminometric approaches

(a) In VITVO dsRED+ A673 visualization using fluorescence microscopy at seeding and after 72 hours. Scale bar 100 mm. **(b)** In VITVO monitoring of sTRAIL action against tumor using microscope visualization of fluorescent cells during treatment (0 hour and 24 hours). **(c)** In VITVO measurement of dsRED+ A673 viability based on RealTime-Glo. Cell growth was monitored for 72 hours (left panel), then sTRAIL was added in VITVO and cell viability was measured after 24 hours based on luminescence (right panel). Untreated dsRED+ A673 cells were used as control. All measurements were performed in triplicate and data are expressed as means ± SD *P = 0.003. **(d)** In VITVO measurement of Luc+ A673 viability by the addition of luciferin. Cell growth was monitored for 72 hours (left panel), then sTRAIL was added in VITVO and cell viability was measured after 24 hours based on luminescence (right panel). Untreated Luc+ A673 cells were used as control. All measurements were performed in triplicate and data are expressed as means ± SD, *P = 0.002

VITVO can be loaded with primary lung cancer cells targeted by nivolumab to rapidly identify an immune-based anti-tumor response

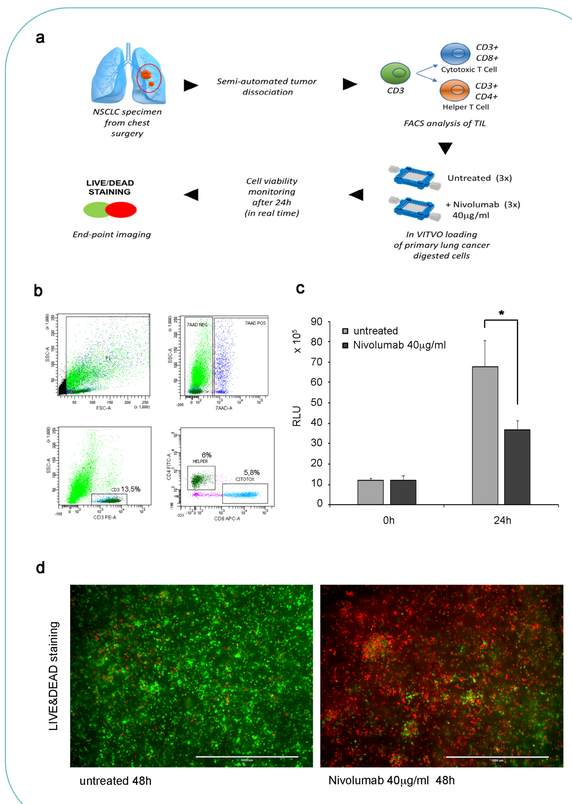

Figure 5. In VITVO testing of the effect of nivolumab on lung primary tumor.

(a) Experimental scheme. **(b)** Immunostaining of unfractionated tumor cells dissociated from lung cancer specimen with anti-CD3, anti-CD4, and anti-CD8 to quantify the percentage of immune cells (CD3+) and the cell subpopulations of lymphocyte T helper (CD4+) and lymphocyte T cytotoxic (CD8+). **(c)** In VITVO cell viability evaluation based on RealTime-Glo at seeding and after 24 hours of treatment with nivolumab. Untreated samples were used as control. All measurements were performed in triplicate and data are expressed as means ± SD, *P = 0.01. **(d)** In VITVO live and dead staining after nivolumab treatment (48 hours) to visualize live (green) and dead (red) cells. Representative images of untreated sample (left pic) and treated sample (right pic) are shown. Scale bar 1,000 mm.

In VITVO dose finding study for a gene therapy-based approach

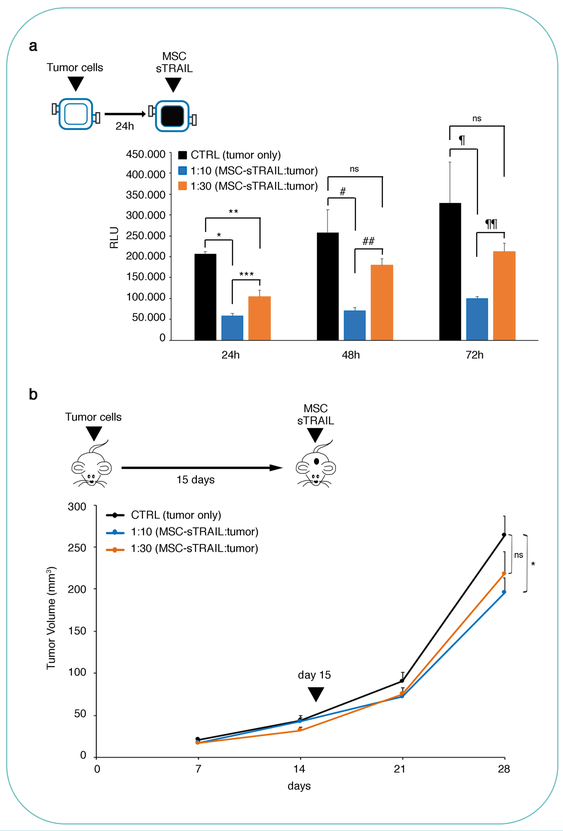

Figure 6. In VITVO dose finding for cell-based therapy.

(a) In VITVO cell viability of Luc⁺BxPC3 was evaluated by the addition of luciferin after treatment with MSC-sTRAIL at different effector:tumor ratios (E:T 1:10 and 1:30) at 24-, 48-, and 72-hour time points. Untreated Luc⁺ BxPC3 cells were used as control. All measurements were performed in triplicate and data are expressed as means ± SD. *P = 0.000003, **P = 0.0004, ***P = 0.007, #P = 0.004, ##P = 0.004, ¶P=0,015, ¶¶P = 0.0006, based on ANOVA and multiple comparisons. **(b)** *In vivo* xenotransplant of BxPC3 and evaluation of tumor inhibition by a single subcutaneous intratumoral injection of MSC-sTRAIL at different effector:tumor ratios (E:T 1:10 and 1:30). Untreated mice were used as control. Values are expressed as means ± SEM; *P = 0,04 based on ANOVA and multiple comparisons.

CONCLUSION

The novel features as well as the flexibility offered by this platform within the different approaches suggest the potential of VITVO as an **innovative 3D *in vitro* model** for **pre-clinical testing** with a possible relevant application in other areas beside oncology field. VITVO may contributes to fill the gap between in silico hypothesis/in vitro results and in vivo settings, also significantly impacting on the **3D rules**.

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