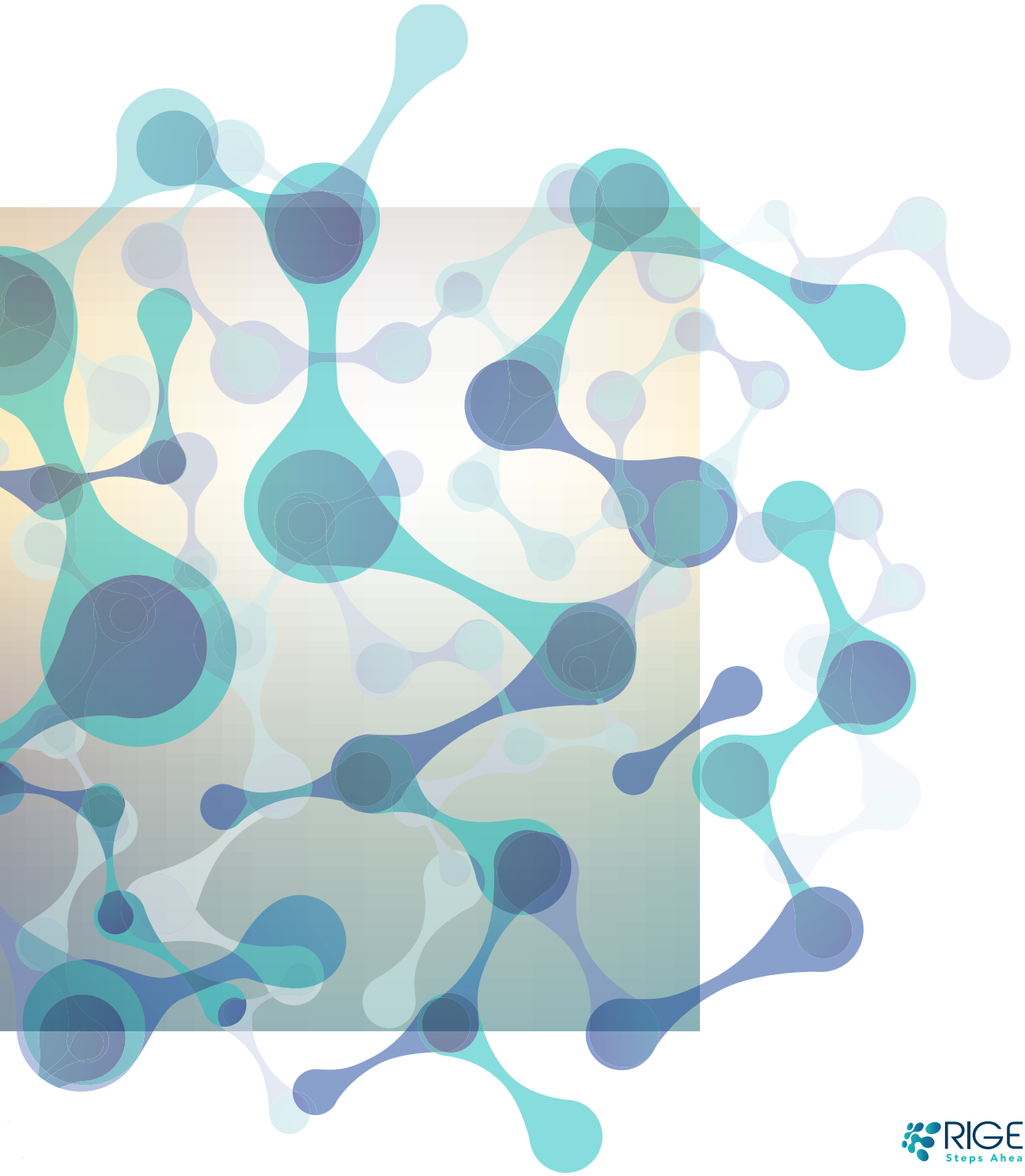
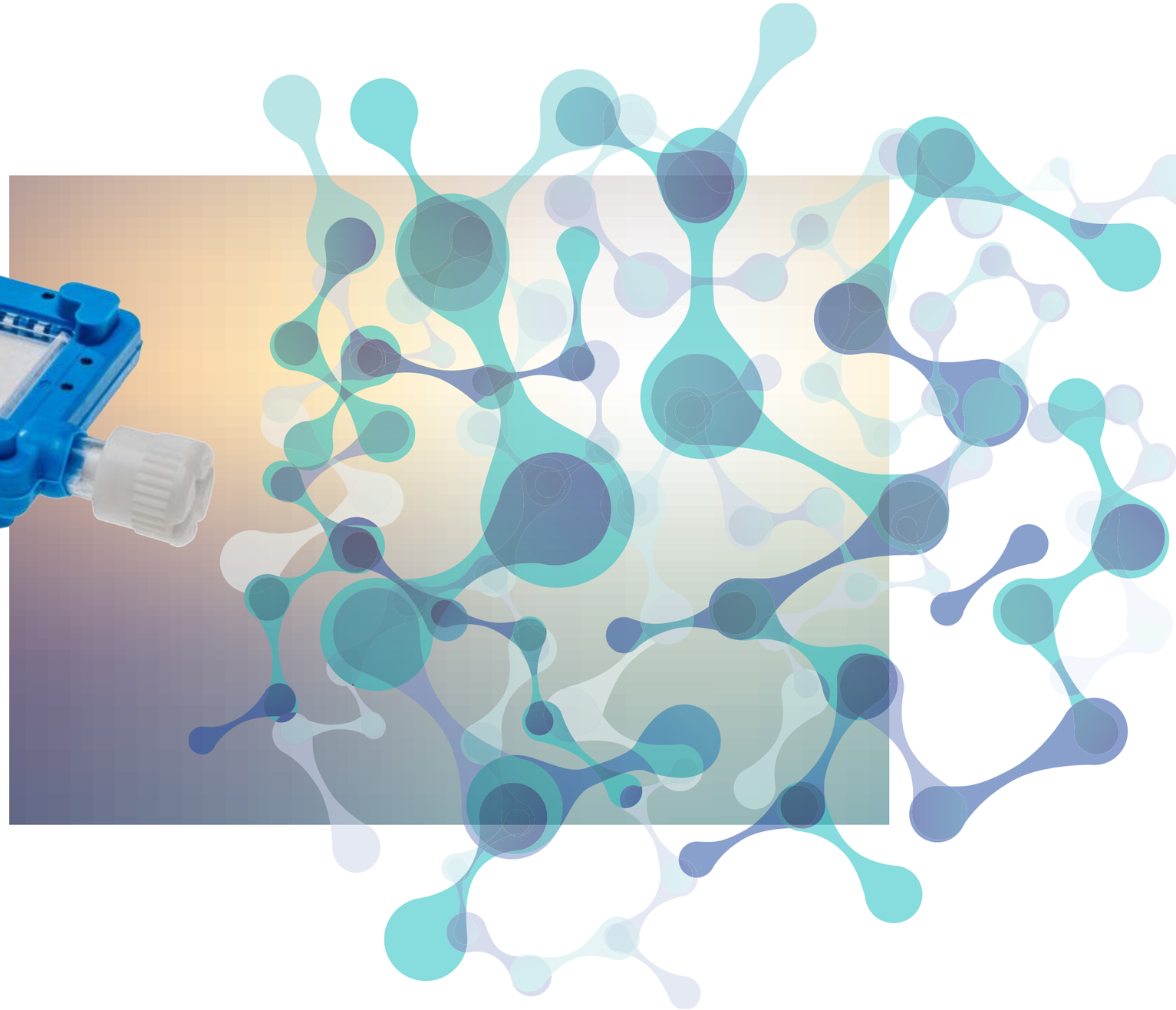
VITVO®

The Innovative 3D in Vitro Model Mimicking in Vivo Complexity

VITVO® – Bioreactors Generation



RIGENERAND
Steps Ahead In Cell Technology



VITVO®

Explore the VITVO® experience adding flexibility and versatility to your drug development and pre-clinical assays

3D Life In Vitro Has Advantages

Three dimensional (3D) platforms have potential to provide more physiologic environments for cell culture in drug discovery and toxicology than classical 2D culture systems

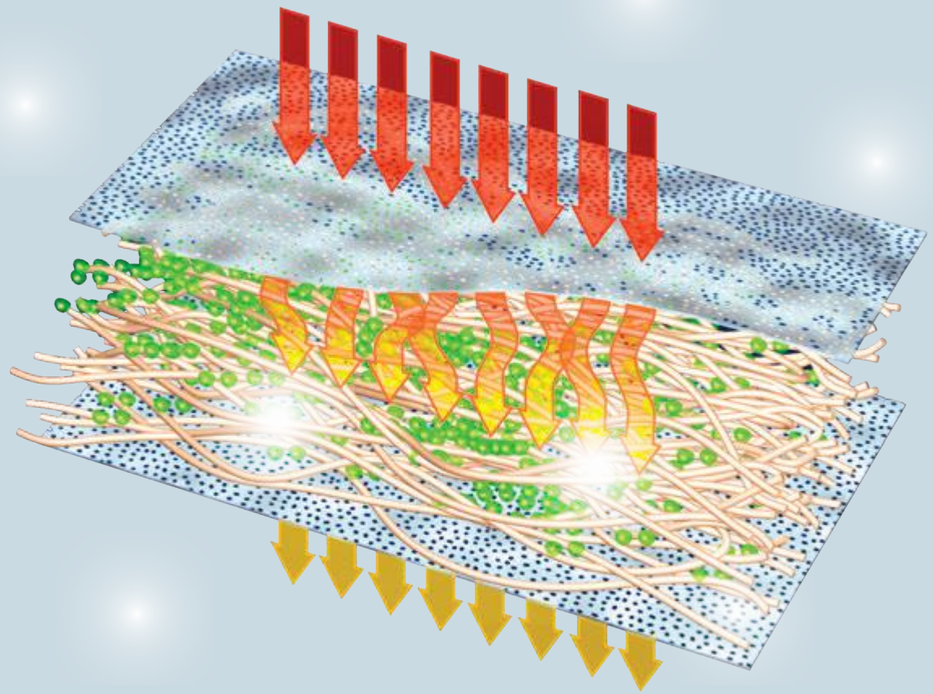
3D culture allows a greater predictivity of efficacy and toxicity before putative drugs move into pre-clinical animal testing and towards clinics, lowering the attrition rate of drug development

3D cultures retain the advantage to mimic an in vivo-like context enabling drug safety and efficacy testing in a human avatar environment

3D culture leads to a different modulation of gene expression, ultimately influencing the antigenic profile of the cells and closely mimicking an in vivo environment

VITVO®
Transferring Vivo to Vitro and Return

VITVO® represents a novel generation of 3D bioreactors that recreates in vitro an in vivo-like environment to grow normal and pathological cells in a 3D manner within a biologically relevant environment



VITVO®

TM PENDING

What's Nice About VITVO®

VITVO® is simple. Usability allows a fast, safe and rapid loading of different cell types in less than 2 ml of culture media

VITVO® is cell friendly. Cells rapidly colonize and repopulate the inner part of VITVO® creating a 3D tissue-like structure

VITVO® is sized to reproduce tissues. Technical features of VITVO® allow hosting a higher number of cells reducing the over-efficacy bias of the miniaturized 2D cultures

VITVO® is fast. Readouts applicable to VITVO® have high reproducibility and can be introduced into the experimental approaches starting from few hours after the cell loading to rapidly generate results

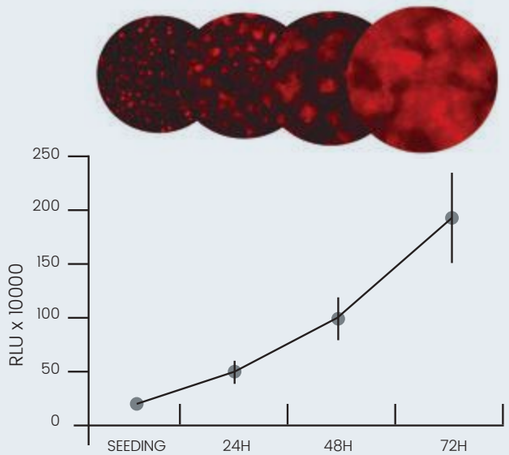
VITVO® is adapting to your reagents. Thanks to a patented technology, the 3D tissue like structure can be easily monitored, quantified by different simple laboratory technologies based on fluorescence (i.e GFP/dsRED/Calcein AM) and/or luminescence (i.e. luciferase/real time GLO™) providing versatility to different R&D strategies

VITVO® is adapting to your lab equipment. Readout detection takes place in many commercially available laboratory equipments (i.e Victor light, GloMAX Discover)

VITVO® is a histological grade device. The 3D tissue-like structure of VITVO® can be embedded for multi-purpose histological analyses

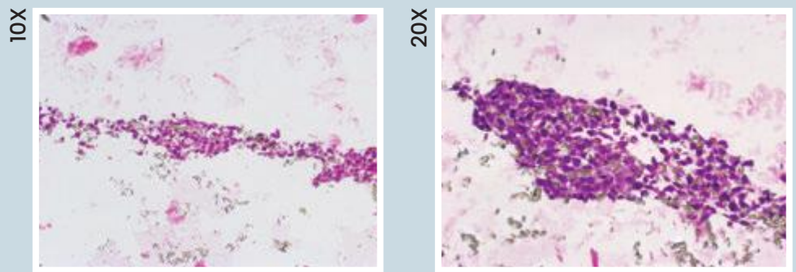
VITVO® is portable. The self-contained technology of VITVO® ensures the transportability which allows shipping from the clinic to the laboratory for testing

Cell Growth by VITVO®



In VITVO® growth monitoring of a dsRED Ewing's Sarcoma cell line. Fluorescent cells were visualized by microscopy and cell growth was in parallel evaluated by a bioluminescent method (RLU=relative light units).

Histology by VITVO®



Haematoxylin and eosin staining of VITVO® loaded with A673 cell line and embedded in methacrylate

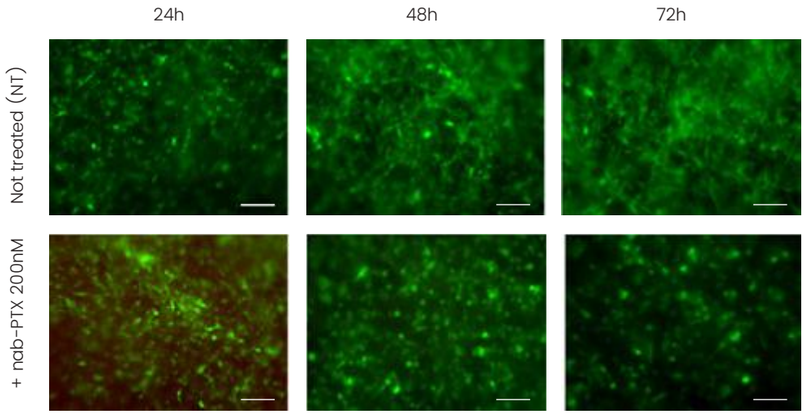
Shipping VITVO®

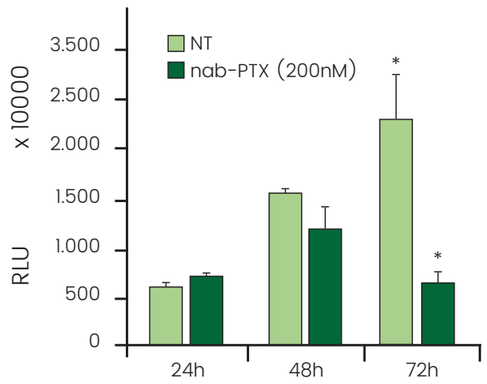
Exploring VITVO® potentials

VITVO® is adapting to your cells. The flexibility of the platform allows to test different cell types (normal/tumoral) for ADMET and for a variety of drug development approaches by challenging them with chemotherapy agents, TKI, biologics and cell-based treatments

Chemotherapy agents

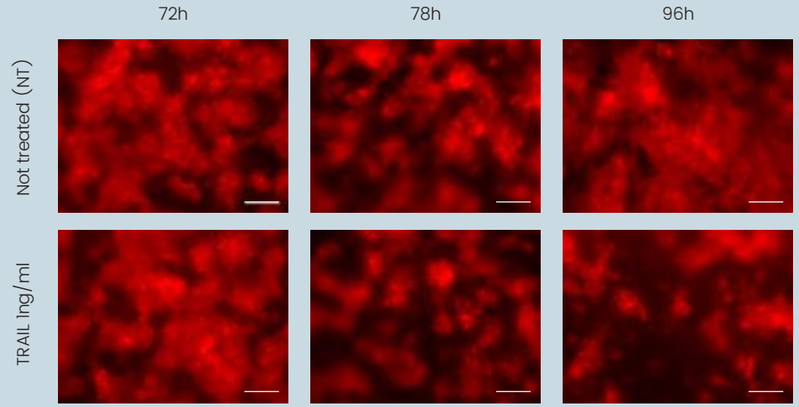
Evaluation of the nab-paclitaxel (nab-PTX, Abraxane®, Celgene) effect against a green fluorescent protein (GFP) positive triple negative breast cancer cell line. Tumor cells were treated with nab PTX starting from 24 hours after seeding. 3D cell growth dynamic was evaluated for up to 72 hours using the reagent Real Time GLO™ (Promega). Luminescence signals expressed as relative light units (RLU) from treated and untreated samples were detected by GloMax® Discover System (Promega); *p<0.005 by t-test. Scale bar 200 µm

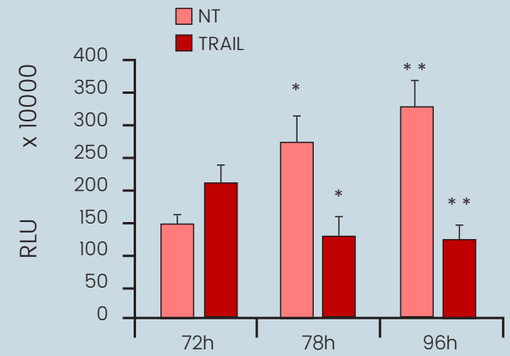




Biologics

TNF-related apoptosis-inducing ligand (TRAIL) is a protein functioning as a ligand that induces apoptosis. Here TRAIL efficacy was evaluated against a dsRED positive Ewing's sarcoma cell line. Tumor cells were grown for 72h inside VITVO® and then treated for 24h with a soluble TRAIL form (1 ng/ml). Viability of treated cells was evaluated by Real Time GLO™ at two time points (6 and 24 hours). The luminescent signal expressed as RLU was detected by GloMax® Discover System and compared with not treated (NT) control; *p, **p<0.01 by t-test. Scale bar 200 µm

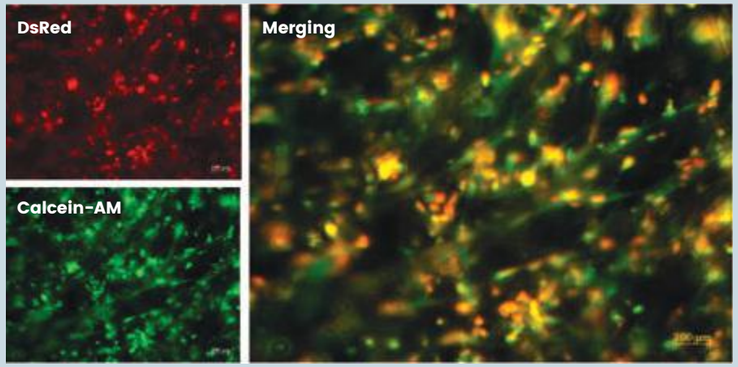




VITVO® can simultaneously host different cell types. Due to their size and proprietary engineering features, VITVO® can host different cell types in combination allowing an in vitro real reconstruction of complex in vivo tissues, like tumor and its immune and/or stromal microenvironment

In VITVO® co-culture

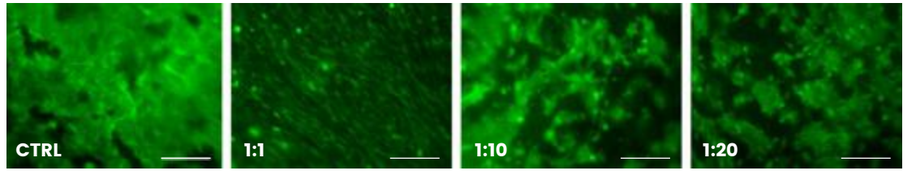
DsRED positive-Ewing's Sarcoma cell line (red) 3D co-cultured in VITVO® with Calcein-AM labeled (green) human stromal cells. The merging allows the assessment of the interaction between two distinct populations.

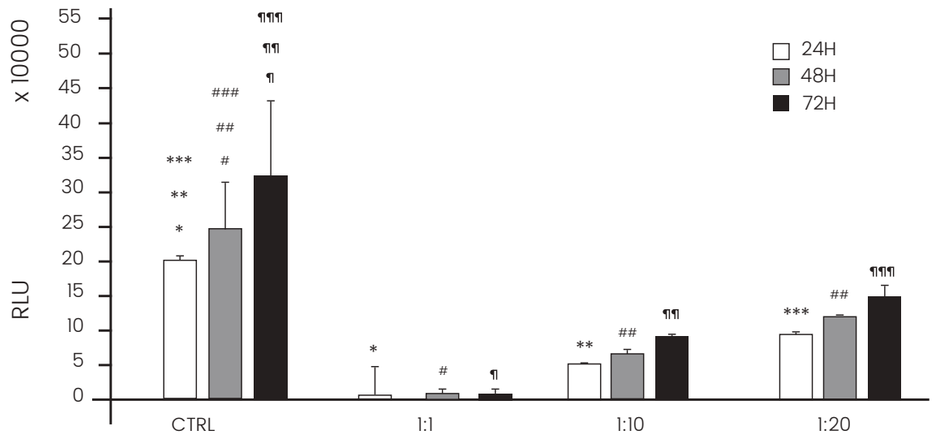


VITVO® suits cell-based therapies product developments. The 3D tissue like structure can be introduced within cell-based therapeutic strategies during pre-clinical development, for efficay prediction and safety studies

In VITVO® evaluation of cell based therapy approach

MSC modified to express the molecule TRAIL were challenged against a luciferase positive pancreatic ductal adenocarcinoma cell line that was labeled by Calcein-AM. 24h after tumor cells loading in VITVO®, MSC-TRAIL were 3D co-cultured at different effector: tumor ratios. Cell viability was monitored adding luciferin (PerkinElmer) and bioluminescent signal expressed as relative light units (RLU) was detected by GloMax® Discover System comparing treated and contols (CTRL). All calculated p<0.01 by t-test. Scale bar 200 µm





VITVO®
3Rs approach in animal testing

Replacing, **Reducing** and **Refining** principles are increasingly becoming relevant in mandatory animal testing due to evolving ethical and cultural needs

The 3Rs approach is going to change the paradigm of toxicity testing.
This change has been slow to be embraced so far due to the lack of radical technological breakthroughs that could make some of the “3 Rs” bigger and more relevant

3R

REDUCING	REPLACING	REFINING
as many trials as required, as few as possible	replace animal studies with other methods	minimize stress of study animals

VITVO®
A concrete solution for the 3Rs

VITVO® thanks to its peculiarities can host human and animal cells to at least reduce and replace small animal studies with both ethical and economic impacts in drug development

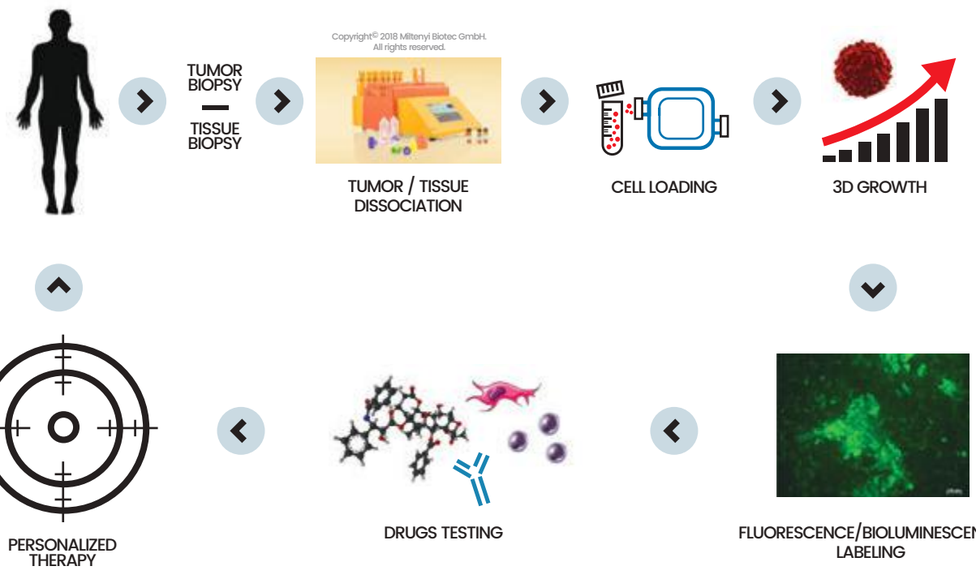
VITVO® positioning between the in vitro high throughput screening and the in vivo models will reduce costs of drug screening improving the reliability of pre-clinical testing for safety (i.e normal hepatocytes) and efficacy (i.e cancer cell lines or primary tumor cells)

3V

IN VITRO HIGH THROUGHPUT SCREENING	IN VITVO®	IN VIVO ANIMAL MODEL
drug discovery and develompent	drug development and pre-clinical assay	pre-clinical assay

VITVO®
A novel tool for personalized medicine

VITVO® can be loaded with human primary tumor cells dissociated after biopsies or surgery to grow them into their recreated 3D microenvironment and test them with different drugs for predictive purposes



VITVO®
Technical specifications

CLAIM	CONFORMITY
Raw material	Traceability in DMR for each lot Fulfils the criteria of USP grade VI Free of leachable chemical toxic compound according to ISO 10993-18:09
Quality management system	Clean room assembly ISO 8 or above according ISO14644-1 ISO9001:15 certification (TUV SUD) in progress
Biological safety	Non-cytotoxic based on ISO 10993-5 Free from detectable DNA/RNA Free from detectable DNAse/RNAse Non-pyrogenic LAL level < 0.06 EU/ml based on EU Ph. 2.6.14 Sterility based on EU Ph. 2.6.1
Sterilization	Product intended for single use only Beta ray irradiaton, validation method based on ISO 11137-1/3:15 in progress
Storage and warehousing	Two years shelf life Product packaging QC passing

RUO (research use only)

References

Duval, K., Grover, H., Han, L.-H., Mou, Y., Pegoraro, A.F., Fredberg, J., and Chen, Z. (2017). Modeling Physiological Events in 2D vs. 3D Cell Culture. Physiology 32, 266–277.

Horvath, P., Aulner, N., Bickle, M., Davies, A.M., Nery, E.D., Ebner, D., Montoya, M.C., Östling, P., Pietiäinen, V., Price, L.S., et al. (2016). Screening out irrelevant cell-based models of disease. Nat. Rev. Drug Discov. 15, 751–769.

Fitzgerald, K.A., Malhotra, M., Curtin, C.M., O' Brien, F.J., and O' Driscoll, C.M. (2015). Life in 3D is never flat: 3D models to optimise drug delivery. J. Controlled Release 215, 39–54.

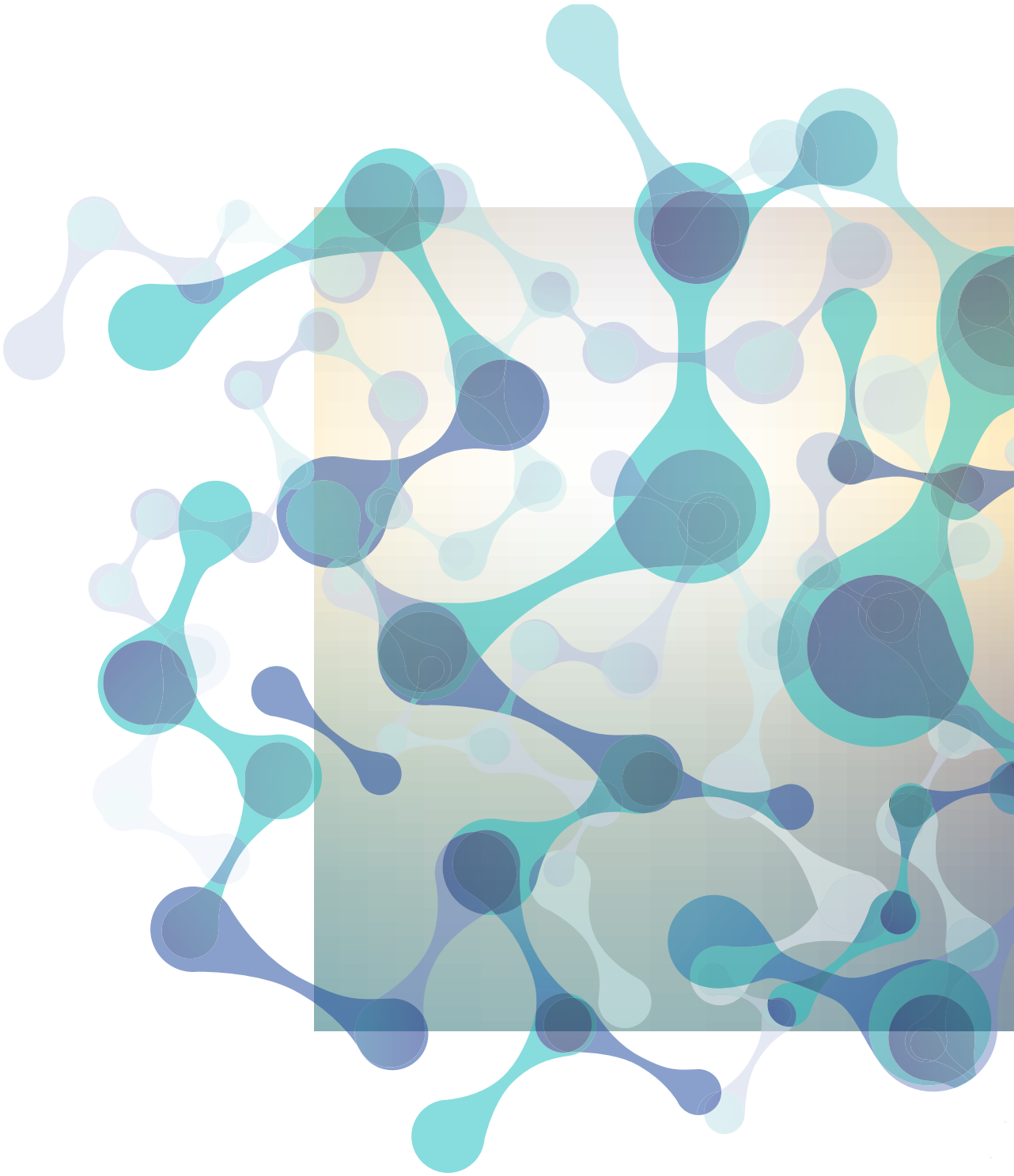
Antoni, D., Burckel, H., Josset, E., and Noel, G. (2015). Three-Dimensional Cell Culture: A Breakthrough in Vivo. Int. J. Mol. Sci. 16, 5517–5527.

Edmondson, R., Broglie, J.J., Adcock, A.F., and Yang, L. (2014). Three-Dimensional Cell Culture Systems and Their Applications in Drug Discovery and Cell-Based Biosensors. ASSAY Drug Dev. Technol. 12, 207–218.

VITVO®

Find out more at www.rigenerand.it

RIGENERAND Srl
Via Maestri del Lavoro, n. 4
41036 Medolla (MO) ITALY
Tel. +39 0535 187.61.72
Fax +39 0535 187.20.53
C.F. / P.IVA (VAT) 03281630362
info@rigenerand.it



www.rigenerand.it