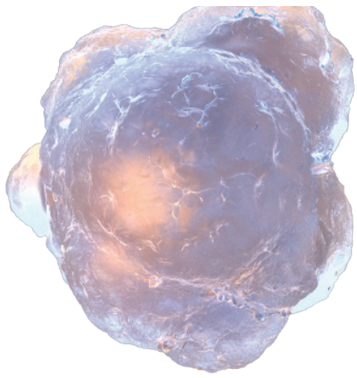
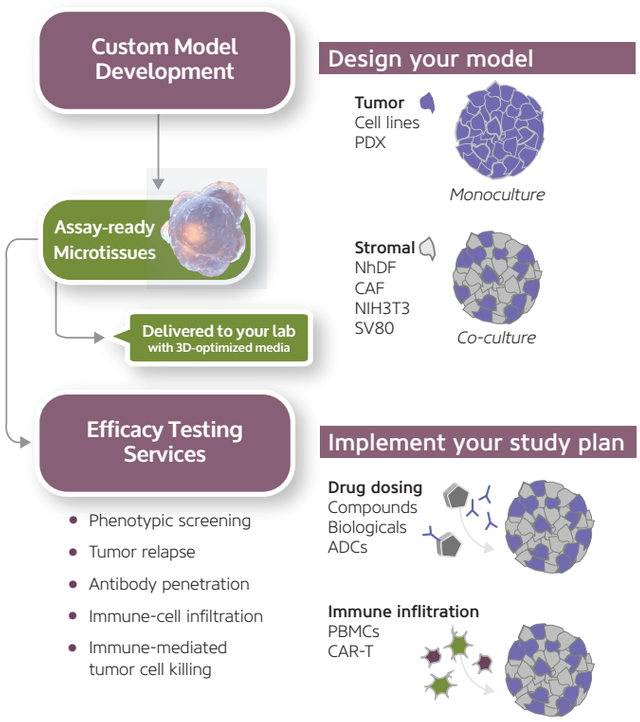
inSphero
Biomimetic Microtissue Technology



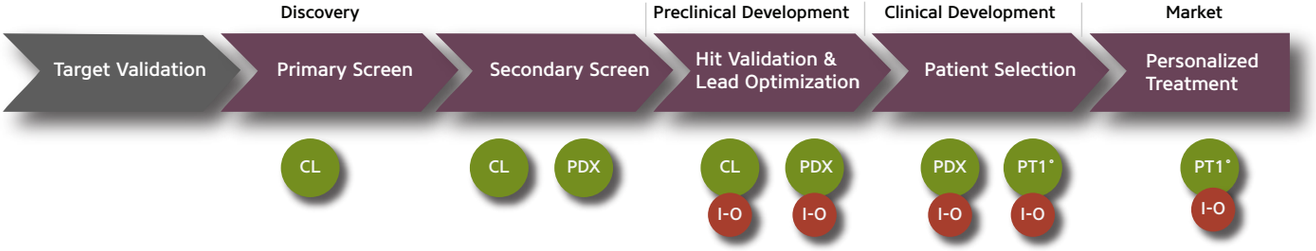
3D InSight™
Oncology Services

InSphero 3D InSight™ Oncology Services for efficacy testing and immuno-oncology applications are designed to accelerate oncology drug discovery and help you gain greater translational insights. When you outsource tumor model development and preclinical testing of anti-cancer drugs and therapeutics to InSphero, we work with you to develop and implement a detailed study plan, and ensure you get robust, reliable results, and comprehensive reporting and analysis.

- **Design a better model** composed of the appropriate ratio of tumor cells, derived from cancer cell line or PDX material, and stromal cell components
- **Test potency and efficacy of drugs** in a physiologically relevant model that better reflects the complex biology of tumor microenvironments
- **Assess single agent or combinatorial efficacy** of small molecule, biological, ADC or immunomodulatory drugs using 3D optimized endpoints
- **Evaluate infiltration of immune cells** or the effect of your drug on immune cell infiltration



Potential Applications for 3D InSight™ Tumor Microtissues in oncology drug discovery



Tumor microtissue models can be used for in vitro *screening, validation, and profiling of anti-tumor drug efficacy using cell lines (CL), patient-derived xenograft (PDX) material as monocultures or in co-culture with fibroblasts. Efficacy testing and optimization of immuno-oncology (I-O) approaches* in vitro *is also possible by addition of immune components during testing.*

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3D InSight™ Oncology Services

based on highly predictive, preclinical 3D oncology models

Tumor Model Development

The first step in any 3D InSight™ Oncology Services project is to choose or develop a tumor model. We offer a broad selection of tumor monoculture and tumor/stromal co-culture models derived from commonly used cancer cell lines. We can also work with you to develop custom monoculture or co-culture models from cell lines or PDX material you provide.

Validated tumor cell lines

Tumor	Cell Line
Breast	MCF-7
	T47D*,R
	MDA-MB-231R
	MDA-MB-361*,R
	BT474
Colorectal	HT-29
	DLD-1*
	LS174T*,G
	HCT-116*,G
	Lovo
Stomach	NCI-N87*,G
Lung	A549*,G
	H292
	NCI-H460
	NCI-H2170
	NCI-H1975
	HCC-827

Tumor	Cell Line
Kidney	A498*
	786-O*
	COLO-205
Prostate	22RV1
	DU-145*
	LnCap*
	PC-3
Brain	SNB-19
	LN-18
Pancreas	PANC-1*,G
Ovarian	A2780
	SKOV-3
	HEY*,G
Liver	HEP-G2*

* Available for delivery to your lab
R Available with RFP label
G Available with GFP label

Service Description

For custom model development, we assess:

- Feasibility and optimization of 3D microtissue formation
- Size-based growth kinetic over 10 days in culture
- ATP after tissue formation and 10 days in culture
- Histology at day 4 and 10 (H&E plus 3 markers)*
- Full written report and consultation

*Histological characterization varies depending on cell sources

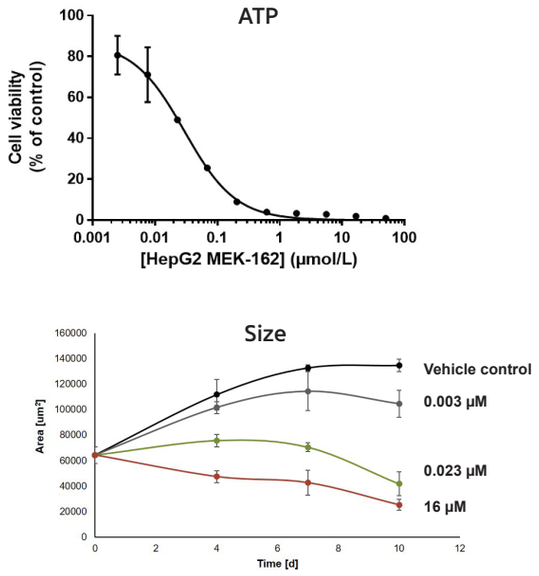
Catalog #	Description
SP-01-040-01	3D InSight™ Custom Tumor Microtissue Development (1 model)

Drug Efficacy Testing

Test your compounds or biologics of interest to assess their potency (EC_{50}) and efficacy (maximum response) on tumor microtissues using 3D-optimized biochemical (ATP) and phenotypic (size) endpoints.

Standard protocol

- Choose a 3D model and supply ≥ 2 test compounds
- Choose controls (e.g., Staurosporine, MEK162, Ipatasertib, Sorafenib/Sunitinib, DMSO, etc.)
- 9-point dose-response curve, in quadruplicate
- 11-day exposure with dosing at day 0, 4, and 7



ATP-based (top, day 10) dose-response curve and dose-dependent size-based growth kinetics (bottom) displaying potency and efficacy of the MEK inhibitor Binimetinib (MEK-162) in HepG2 microtissues.

Data delivered include

- ATP-based dose-response curve at day 11 using Promega CellTiter-Glo® assay
- Size-based dose-response curve at day 11 and growth kinetics over time
- Efficacy (IC_{50}) values

Catalog #	Description
SP-01-041-02	3D InSight™ Efficacy Testing Service

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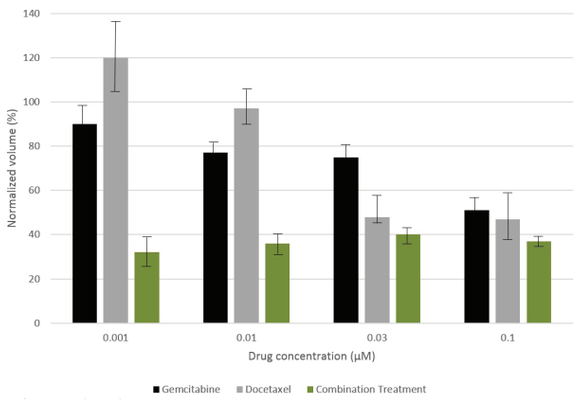
Combinatorial Drug Testing

Test your drug of interest in combination with other therapeutics to identify pairs with synergistic, additive, or antagonistic effects. We determine potency (EC_{50}), efficacy (maximum response) and growth kinetics of 2 compounds alone and in combination, using size and ATP (viability) endpoints over a 10 day dosing period.

Standard protocol

- Range finding for 2 compounds alone (ATP and size after 10 day exposure), plus Staurosporine control
- Individual and combinatorial testing at selected doses with analysis by Chou-Talalay method[1]



Simultaneous treatment of MX-1 (breast)/NIH3T3 tumor/fibroblast co-culture spheroids with Gemcitabine and Docetaxel reveals synergistic combinatorial growth inhibition compared to treatment with either compound alone.

Data delivered include

- ATP-based dose-response curve
- Size-based growth kinetics over time
- Potency (EC_{50}), efficacy (max response) and combinatorial analysis

1. Chou, T.C. (2010) Drug combination studies and their synergy quantification using the Chou-Talalay method. Cancer Res. Jan 15; 70(2):440-6.

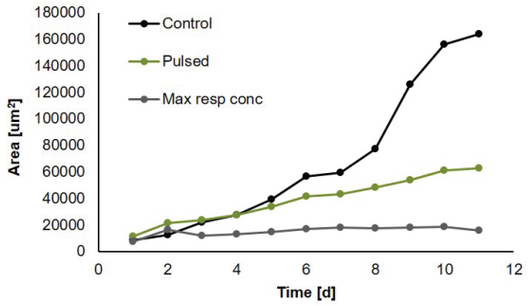
Catalog #	Description
SP-01-042-01	3D InSight™ Combinatorial Drug Testing Service (2 compounds, 1 controls, 1 model)

Tumor Relapse Assay

Simultaneously determine the response of a tumor to your drug as well as the kinetics of tumor re-growth (relapse) following removal of the drug *in vitro*. We use phenotypic (size-based) assessment of spheroid growth by bright-field imaging to allow continuous, non-lytic monitoring of spheroid growth over time.

Standard protocol

- Determination of EC_{20} and EC_{50} using ATP and size after 5 day exposure (test drug & TAX, DOX, STA)
- Relapse assay comparing pulsed dosing (day 0 only) at EC_{50} and EC_{20} to repeat dosing (day 0, 3, 7) at max response concentration over 10 days
- Relapse classification



Example tumor relapse kinetics for Doxorubicin showing recovery of HCT-116 tumor spheroid growth following single treatment (pulsed-green line) at the EC_{50} dose, compared to repeat-dosing over 10 days at the max response dose (gray line) or vehicle control (black line). Growth constants are used to determine relapse classification values at EC_{20} and EC_{50} values.

Data delivered include

- ATP-based dose-response curves
- Size-based dose-response curve of test drug and controls at day 5, and growth kinetics over time
- Potency (EC_{20} and EC_{50}) values, efficacy (max response), growth constants, and relapse classification value at EC_{20} and EC_{50} dosings

Catalog #	Description
SP-01-043-01	3D InSight™ Tumor Relapse Assay Service (1 compound, 3 controls, 1 model)

3D InSight™ Oncology Services

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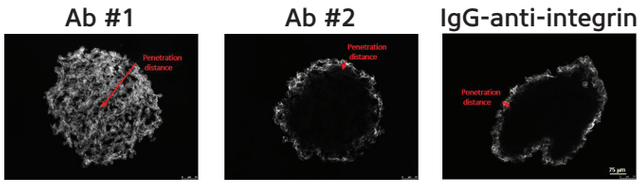
Antibody Penetration Assay

Penetration and distribution of biologics can depend on a variety of factors, such as tissue density, antibody affinity, antigen density per cell and physico-chemical properties. The spherical, 3D structure and more native ECM and biology of 3D InSight™ tumor microtissues makes them ideal for assessing penetration of antibodies and engineered antibody fragments *in vitro*.

Standard protocol

- ***Phase 1:*** **Model Characterization**
  assessment of model feasibility for antibody penetration assay using beta integrin antibody
- ***Phase 2:*** **Biologic Detection**
  optimization of detection protocol for biologics of interest by immunofluorescence (IF)
- ***Phase 3:*** **Kinetic Penetration Assay**
  assessment of antibody penetration kinetics over 3 time points



IF images of 3D InSight™ Tumor Microtissue sections stained to reveal antibody penetration (white) following 1 hour incubation with Fcab fragment, full-size human IgG, or control (beta-integrin) full-size mouse IgG.

Data delivered include

- Feasibility report for Phase 1 assessment
- Method description and imaging results for IF detection of target antibody
- Penetration kinetic based on IF for target antibody and control (anti-beta integrin)

Catalog #	Description
SP-01-044-02	3D InSight™ Antibody Penetration Assay (1 model, 1 antibody, 1 concentration)

Immuno-Oncology Services

3D InSIght™ Tumor microtissues provide a highly predictive *in vitro* platform for testing the ability of activated immune cells components to effectively penetrate and mediate tumor-specific cell killing in a culture environment that more closely reflects human tumor biology. We currently offer two standard I-O services:

- ***CAR-T Cell Efficacy Testing***
  Compares the efficacy of different CAR-T modifications/E:T ratios/donors alone and in combination with biologicals, using fluorescence (tumor viability), morphology and cytokine secretion, and histology characterization
- ***Immuno-modulatory Antibody Efficacy Testing***
  Compares efficacy of immune-modulatory antibodies in co-cultures of tumor models with immune cells using fluorescence (tumor viability), morphology, cytokine secretion and histology characterization endpoints

For more information, please refer to the 3D InSight™ Immuno-Oncology Services brochure or contact your InSphero representative.

Custom Oncology Services

If the tumor model systems or services described in this document or on our website do not meet your requirements, or if you want to test additional endpoints beyond those offered as part of our standard services, contact one of our 3D oncololgy experts, and we will work with you to design a custom study plan and project deliverables.

Visit www.insphero.com for more information InSphero Oncology solutions.

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