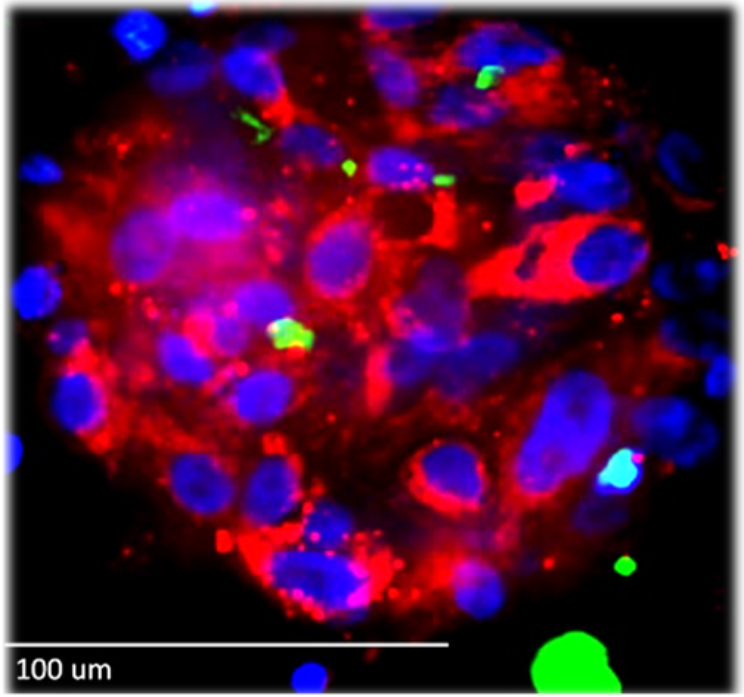
WHITE PAPER

Benefits of Tumoroids for Oncology R&D

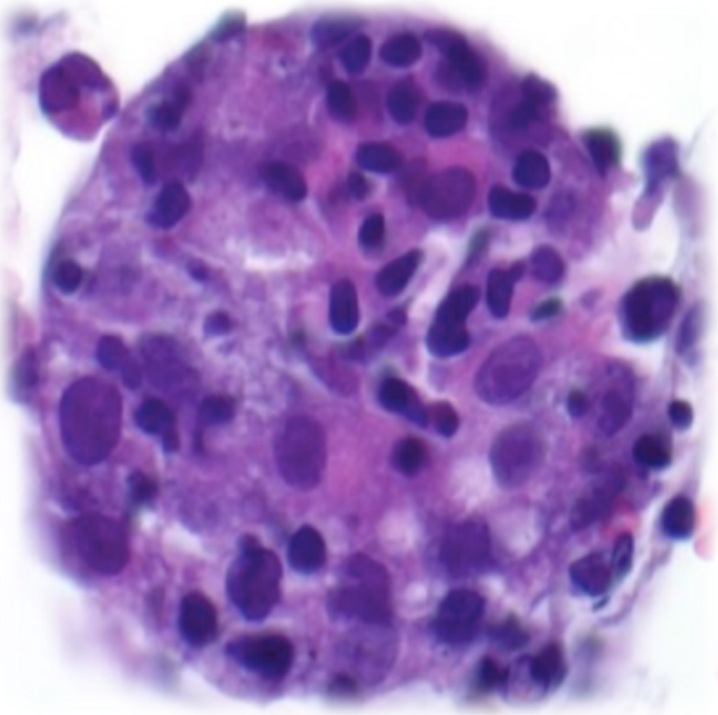
This white paper outlines the benefits of using tumoroids in oncology research and development, and co-clinical studies.



What are tumoroids?

Up to 9,000 tumoroids are created from a patient's tumor. Each tumoroid is spherical and ~150µm in diameter - a true micro-version of the Tumor MicroEnvironment (TME).

Unlike conventional tumor organoids, there are no chemical dissociation processes, no propagation steps using growth factors, no attempt to reassemble the complex mixture of constituents that are the tumor microenvironment.



ADDITIONAL CONTENT

Tumoroids are used to power ADC, ADCC and ACT studies

CLICK LINKS TO LEARN MORE

Why is the TME so important?

The tumor microenvironment is not just tumor cells, but immune cells, fibroblasts, signalling molecules, and the extracellular matrix (ECM). The ECM is a three dimensional network of extracellular macromolecules such as collagen, enzymes and glycoproteins, where cell adhesion, cell-to-cell communication and differentiation occur frequently.
Maintaining the TME during drug studies is essential to ensure therapeutic intervention is correctly modelled.

The challenge

The costs of new cancer therapeutics have soared over the past years. The increase in the number of therapeutic targets representing smaller sections of patient populations, and the increased complexity of clinical trials and regulatory requirements have contributed to rising costs.

Meanwhile, the likelihood that a drug will reach market approval after entering phase 1 clinical testing has remained the same, and is significantly lower for cancer therapies compared with other disease areas. Only 1 in 15 cancer drugs that enters clinical development will achieve FDA approval and half of these failures are due to a lack of efficacy in the clinic. The lack of translatability between traditional pre-clinical model systems and patients is a key factor in the attrition rate in the clinic.

The rise of organoids

Over the last decade, many have sought to build on the discovery that tumor cells dissociated and separated from primary tumors could be propagated *in vitro* using various “patterning” factors such as growth factors in a 3D artificial matrix, in order to self-organise into 3D structures owing to their abilities to self-renew and differentiate.
The success rate for establishing organoids varies tremendously between indications, for example >90% for CRC but < 20% for indications such as Prostate and NSCLC.
It is important to also bear in mind that organoids are clones of individual or small groups of cells selected on the basis of their ability to propagate, which would infer “only the strong survive”, and lack the full complement of cells and factors found in a patient’s tumor. Also, organoids are propagated to produce large numbers for screening assays. It has been shown that organoid heterogeneity drifts with time. CRC organoids can reach clonality in 30-40 days, as particular oncogenic mutations provide some tumor cells with a competitive advantage.

Mouse modelling

Mouse models are the staple models for many preclinical cancer therapies before they are translated into development. Whilst there are many varieties, one of the most popular today are Patient Derived Xenograft (PDX) models. Even when PDX are created on a backbone of humanised mice, the mice still lack the full complement of the human immune system, so studies involving cancer immune therapies are extremely challenging.

in vivo modelling, whether subcutaneous or orthotopic, is seen as the gold standard for determining efficacy in a living animal model. Early toxicity and PK/PD studies *in vivo* derive a substantial body of science which has proven valuable in the cycle of drug development. As such, mouse modelling is a valuable tool to build knowledge around drug effect.

However, as is commonly quipped, science has been very successful at curing mice of cancer, but not so humans. There are significant questions as to the translatability of efficacy and even mechanism of action between mice and patients in the clinic.

Traditional pre-clinical models are often seen as a significant achilles heel in successfully translating cancer therapies into the clinic.

The need for greater sophistication in modelling

The explosion of immune therapies in the last decade along with the advent of cell therapies has changed the landscape for drug discovery, development and patient treatment, resulting in a growing need to identify pre-clinical models which can not only improve our ability to infer efficacy, toxicity, etc. in humans, but also significantly improve our ability to understand the impact of drug effect across the whole tumor. Understanding mechanism of action and developing effective biomarker strategies is key to improving our ability to identify patient cohorts who will benefit from therapies.

Enter stage left, tumoroids…

As described previously, tumoroids are significantly different from other modelling systems, and a large body of evidence has been developed to demonstrate their ability to support many approaches to therapeutic intervention from conventional chemotherapies, to checkpoint inhibitors, bi- and tri-specific antibodies, ADCs, ADCCs and cell therapies such as CAR-Ts. Over several years the use of tumor tissue slice technology (*ex vivo* histoculture or

explants) has been investigated for drug development and predicting patient response. Creating and maintaining these slices has proven difficult, but more importantly, they can never represent the complete tumor as they represent limited diversity amongst cells and ECM in a tissue section only 2-300 µm thick. Each slice may vary significantly depending on where the section is cut from the tumor. Significant effort is required to compare and contrast multiple sections from different areas of the same tumor.

With tumoroids, around 9,000 tumoroids are created from each patient tumor (**Figure 1**). A homogeneous mixture of tumoroids is created and then around 400 tumoroids are plated per assay well. Sufficient to support various assays such a Flow Cytometry, High Content Confocal Imaging and sequencing, whether Nanostring, conventional RNAseq or single cell RNAseq.

The power of tumoroids is not only the fact they retain the TME, but also the true diversity of TMEs and cells are reflected in every assay well, minimizing well-to-well variation. **Figure 2** shows how reproducible the Flow Cytometry data is across 7 wells of tumoroids derived from the same tumor.

This allows direct comparison of results from multiple assays, to investigate and ask a wide variety of questions from tumor cell killing to penetration of the TME to possible biomarker signatures which differentiate between responder and non-responders, as well as helping to elucidate mechanism of action.

Figure 1: The process of creating tumoroids

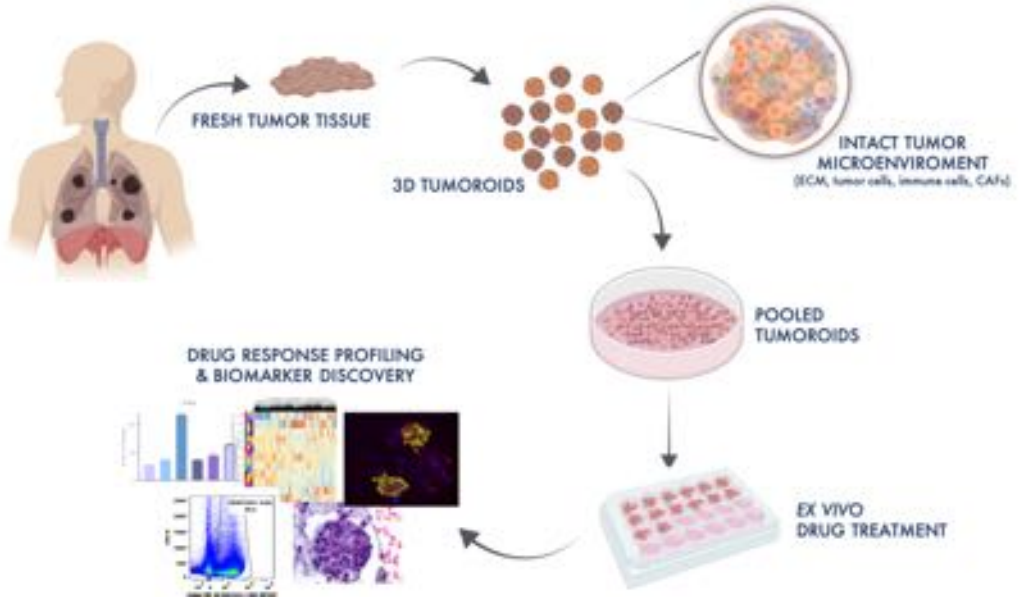
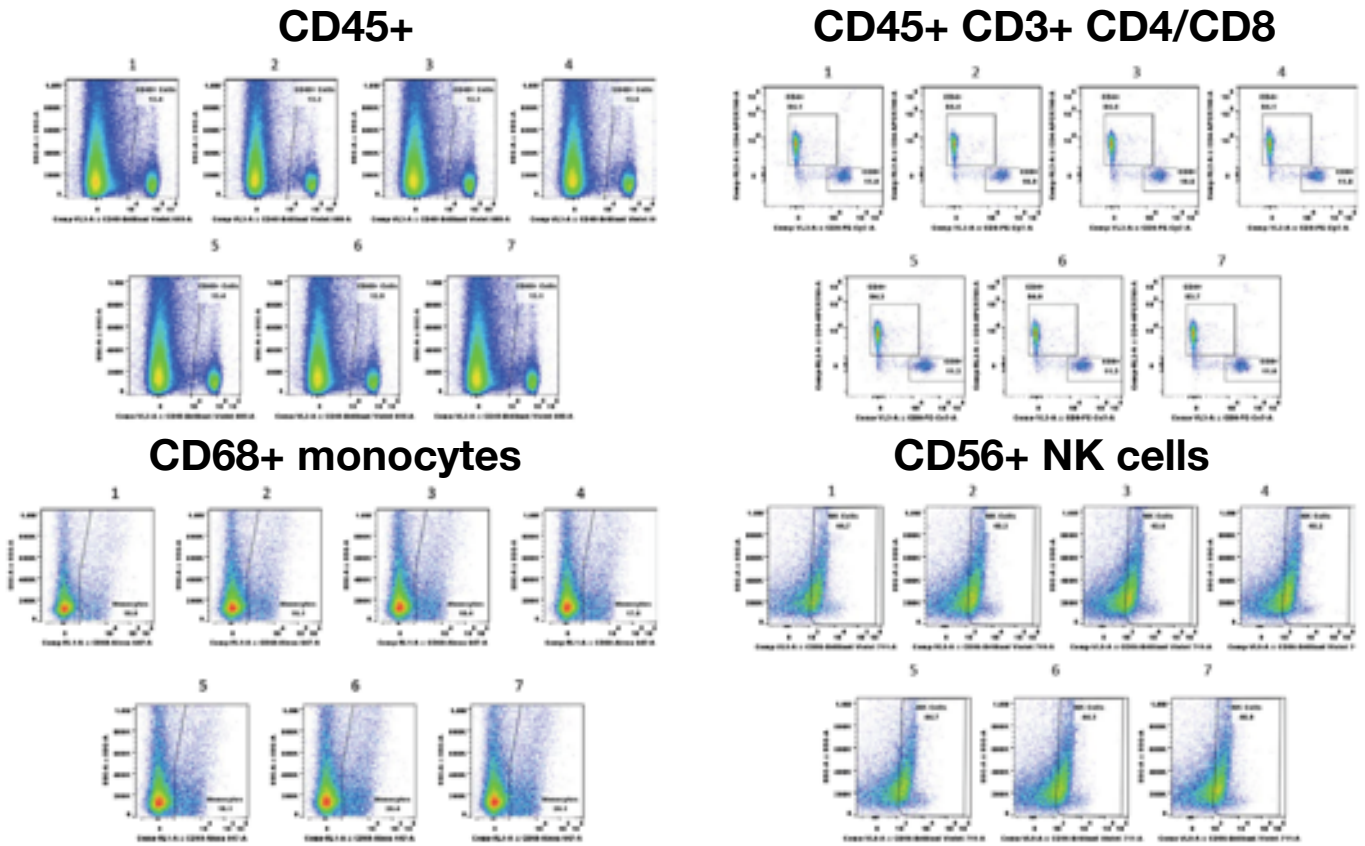


Figure 2: Pooling tumoroids helps overcome tumor heterogeneity and minimizes well-to-well variation.

Pooled tumoroids were evenly distributed in seven treatment wells comparing CD45+, CD68+, CD3+ and CD56+ populations using Flow Cytometry.

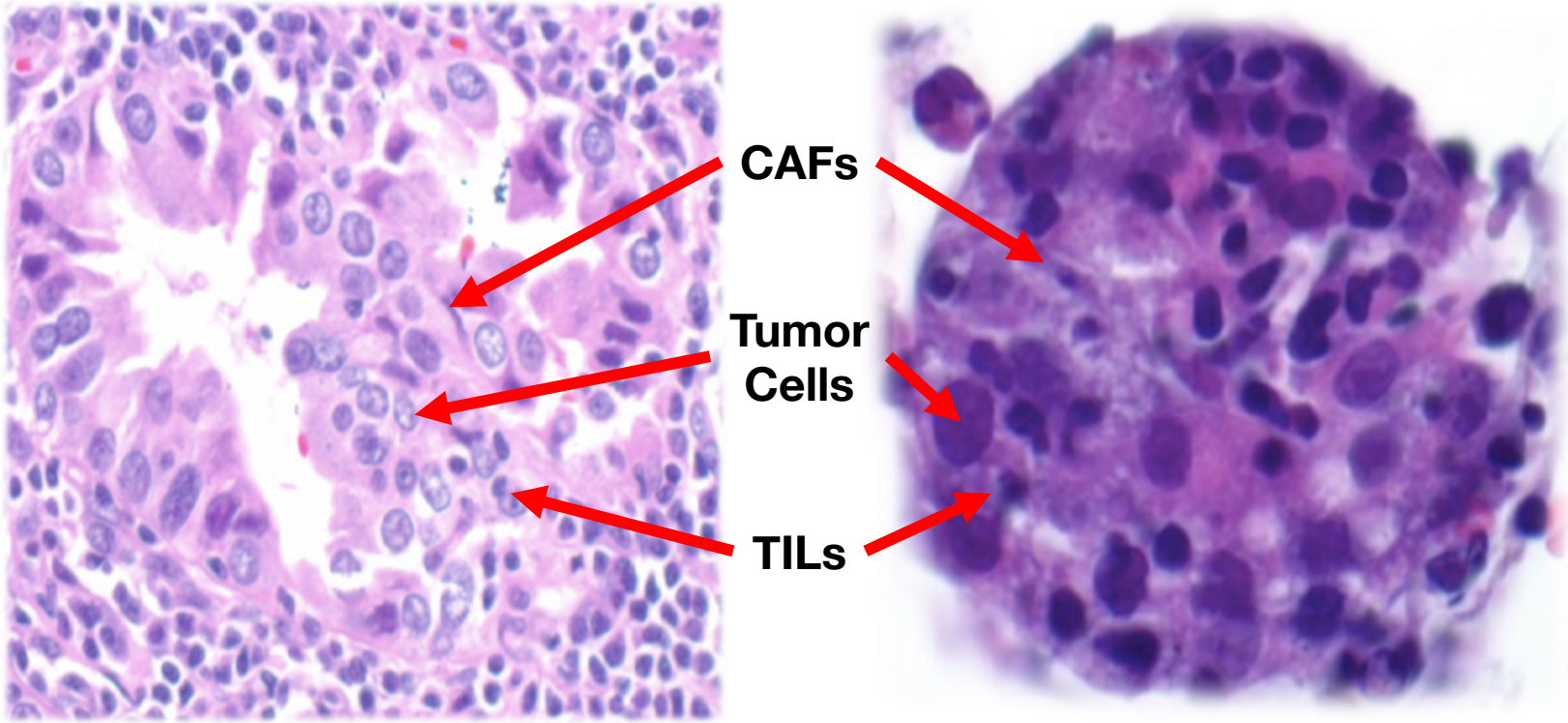


Cell type	Mean Percent	Variance
CD45+	13%	0.2
CD68+ monocytes	18.4%	1.8
CD45+ CD3+ CD4/CD8	84.1%/ 11.11%	0.05/0.12
CD56+ NK cells	44.8%	0.5

The Tumoroid Landscape

Tumoroids have all the components of the original patient tumor and preserve cell-cell and cell-ECM interactions. **Figure 3** shows how the components within the tumoroid match those in the original tumor. **Figure 4** expands a region of a tumoroid to show how the fine structure of the original tumor is retained. Because the ECM and stroma are intact, it is possible to study tumor cell killing, as well as the impact of therapeutic intervention on the resident tumor cells, immune cells and extracellular matrix. Also how, and to what extent, penetration occurs by oncolytic virus and ACTs.

Figure 3: Lung Adenocarcinoma tumoroid compared to original H&E section

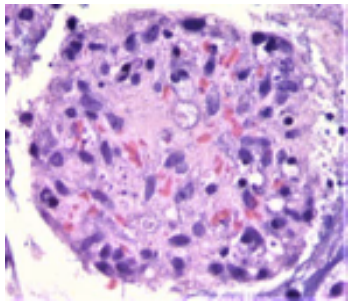


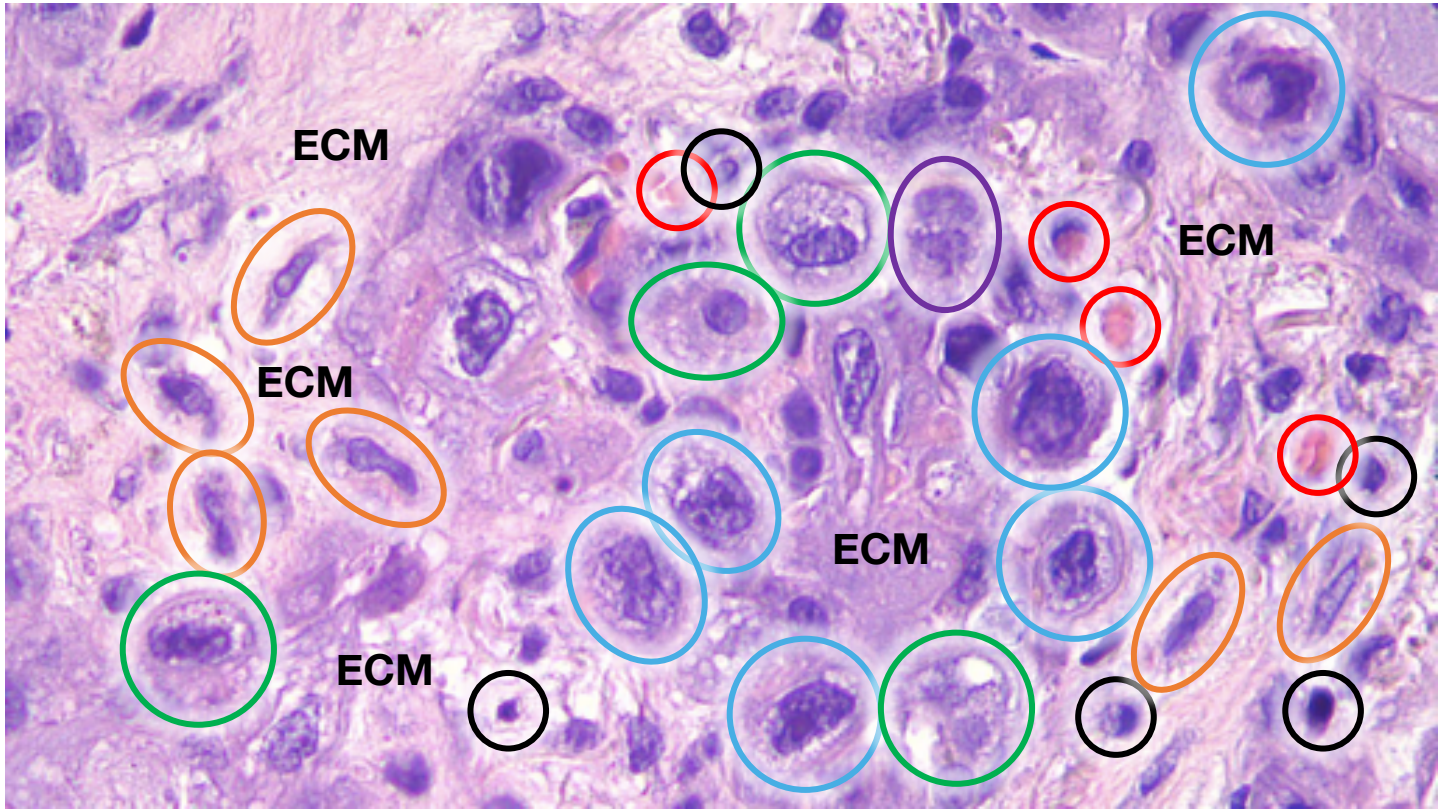
Tumoroid has the same composition as the original patient tumor – tumor cells, cancer associated fibroblasts, stromal elements, and all tumor resident inflammatory cells including tumor infiltration leukocytes.

Figure 4: Pancreatic Tumoroid. The ECM is preserved in addition to cell-cell, and cell-ECM interactions

ECM Extracellular Matrix

- Capillary vessel
- Dendritic cell
- Tumor cell
- Macrophage/myeloid
- Lymphoplasmacytic cell
- Cancer associated fibroblast





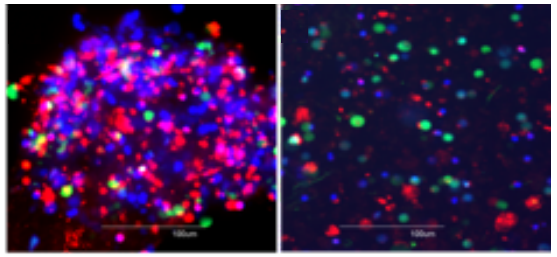
3D with TME vs 2D

At the AACR Annual meeting in 2019, Nilogen presented a poster demonstrating why retaining an intact TME in tumoroids is essential to measure true drug response. 3D tumoroid and 2D cell suspensions were derived from the same tumor tissue. For 2D, tumor was dissociated using standard protocols. Both 2D and 3D tumoroid cultures were treated with the anti-PD1 inhibitor, Keytruda. Treatment mediated changes in immune cell populations were monitored by multiplexed flow cytometry, differential release of cytokines and chemokines were analysed, and tumor cell killing was evaluated using Nilogen's high throughput, high content technology (**Figure 5**).

The results clearly demonstrate that removal of the extracellular matrix by digestive enzymes significantly affects the TME as shown by the Cytokine release data.

Figure 5: 3D Tumoroid vs 2D

Confocal analysis of treatment-mediated tumor cell killing



Live
Dead
Nucleus

Tumoroid | 2D cell suspension

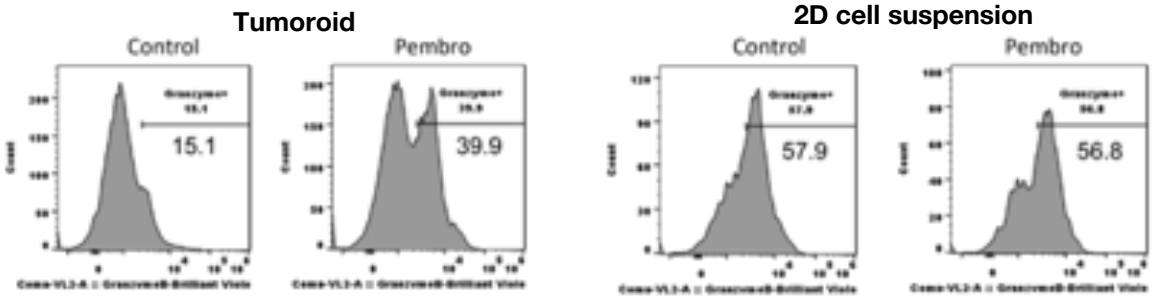
Flow cytometry

Cell Types	3D	2D
CD4+	25.8%	42.3%
CD8+	29.2%	17.5%
B-cells	34.7%	7.87%
Macrophages	9.35%	4.99%
NK cells	41.3%	44.4%

Multiple analysis of Cytokines

Cytokines	3D	2D
IL-2	1.735865	0.123196
IL-4	0.551055	nd
IL-6	192.9861	1.241953
IL-8	776.3713	42.58602
IL-10	2.824051	nd
GM-CSF	9.608017	4.710322
IFNg	17.61097	nd
TNFa	19.26498	nd

Granzyme B expression in NK cells



3D with TME vs 2D (continued)…

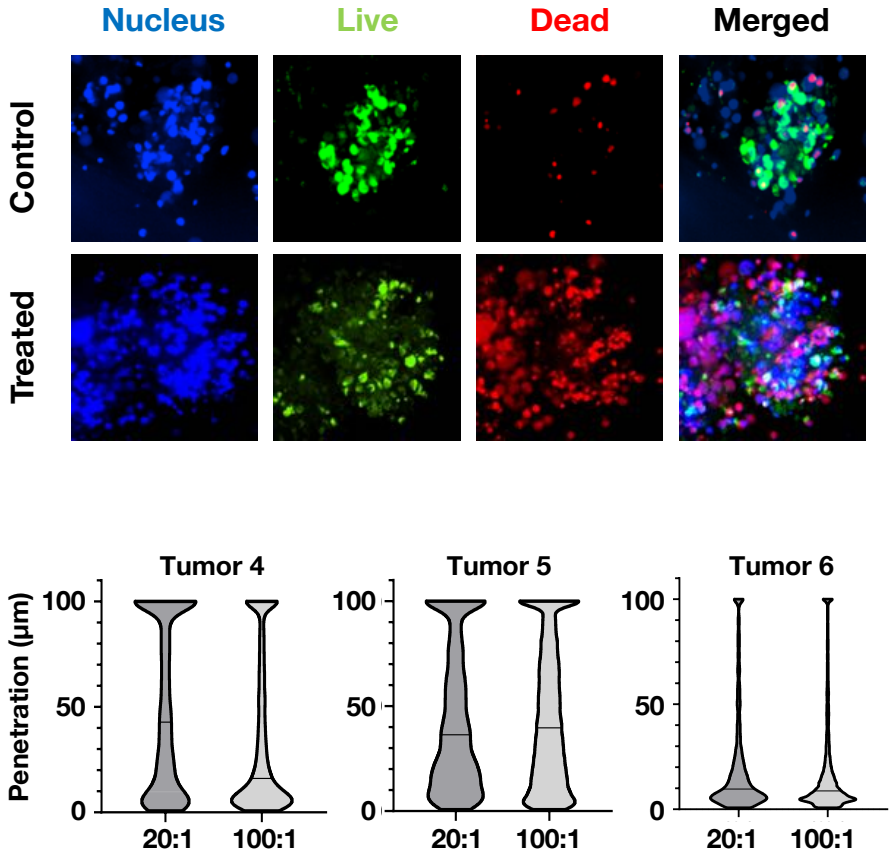
The flow cytometry results demonstrate that whilst the number of T-cells and NK-cells remain similar between 3D tumoroids and 2D cell suspensions, their activation upon anti-PD1 treatment is considerably different, as shown by the level of Granzyme-B expression in NK cells with a more than 100% increase seen in the tumoroids with intact tumor microenvironment, whereas the 2D cell suspension saw no change. Significantly, the untreated control study arms also showed a 4 fold increase in Granzyme B expression in the 2D culture compared to tumoroids, indicating that the immuno suppressive mechanism is largely overcome with the loss of extra cellular matrix, calling into question whether preclinical models which do not contain the original tumor microenvironment can be used to evaluate immune modulatory agents.

"The loss of extra cellular matrix in preclinical models which do not retain the tumor microenvironment has a significant impact on the response of immune cells to checkpoint inhibitors."

Penetration

For oncolytic viruses, Antibody Drug Conjugates, Antibody-Dependent Cellular Cytoxicity, and Adoptive Cell Transfer therapies, not only is tumor cell killing a key consideration, but also penetration of the tumor. Tumoroids retain an intact tumor stroma. High content confocal microscopy combined with image analysis is used to detect tumor cell death within the tumoroids and to identify treatment-induced tumor cell killing. Additionally, penetration of cell tracker labeled therapeutics is detected and quantified in the 3D tumoroids (**Figure 6**).
Effective therapies rely on these therapeutics being able to penetrate the tumor, bind to target cells, and promote killing within the diverse tumor microenvironment – something that is is not achievable in a 2D system or simpler 3D systems which do not contain the full tumor microenvironment.

Figure 6: Tumor cell killing quantified using Confocal Microscopy



Confocal analysis of treatment-mediated tumor cell killing

Penetration distance of cell tracker-labeled cell therapy. The heterogeneity of the tumor microenvironment heavily influences the effectiveness of the therapy with varying levels of penetration and ACT-mediated tumor cell killing

At Nilogen, our vision is to improve the treatment and quality of life for cancer patients by helping companies developing the oncology therapies of the future, improving their chance of succeeding in clinical trials through the application of game-changing human tumor tissue assays which provide a comprehensive analysis of drug impact on the immune compartment as well as tumor cells.

What will you discover today?

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Nilogen Oncosystems
3802 Spectrum Blvd., Suite 207
Tampa, FL 33612, USA

info@nilogen.com

+1 813 440 3588