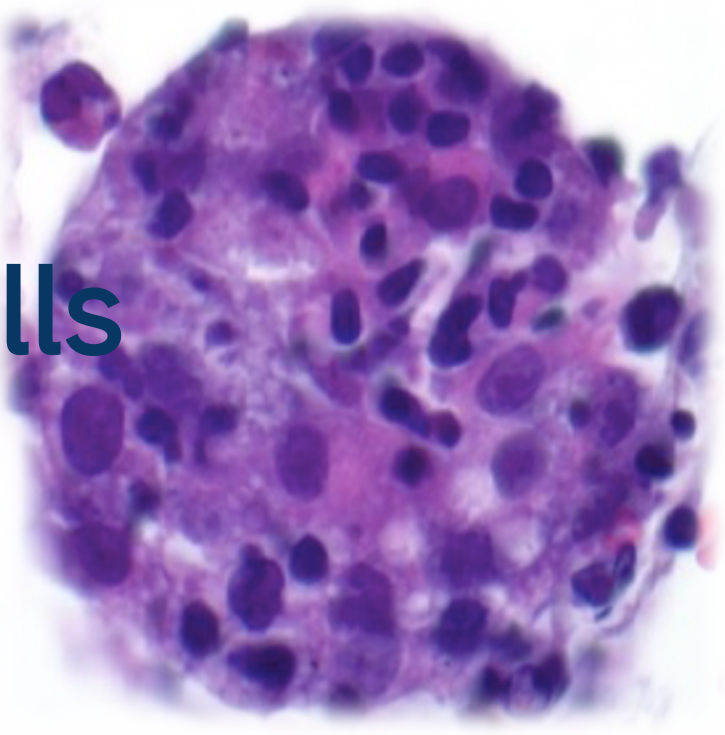
APPLICATION NOTE

Studying the Mechanism of Action of Tumor Immune Cells



This application note outlines how the Mechanism of Action of Tumor Immune Cells can be studied in patient tumors in response to treatment.

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.

The immune compartment

A completely unique approach to enable the detailed analysis of drug effects on the individual cells of the immune compartment. For the first time, Nilogen has brought together it's powerful tumoroid platform with the power of single cell RNA sequencing and single cell proteomics, creating a profoundly new approach to studying and understanding the impact of therapeutic intervention on the individual cells of the immune compartment.

ADDITIONAL CONTENT

Tumoroids - the difference compared to tumor organoids?

LEARN MORE

Bulk vs. single cell

Until recently, the common method for studying proteomics and transcriptomics in cancer has been at the macroscopic level. Bulk DNA/RNA sequencing and Flow Cytometry were the norm, and are still powerful approaches which continue to be used today. However, understanding the impact of therapeutic intervention on, and importance of, immune cells has been severely limited, as it was not practicable to study the impact on individual cells in living tissues due to their low frequency.

The advent of single-cell sequencing (scRNA-seq), has transformed our understanding of complex cellular populations.

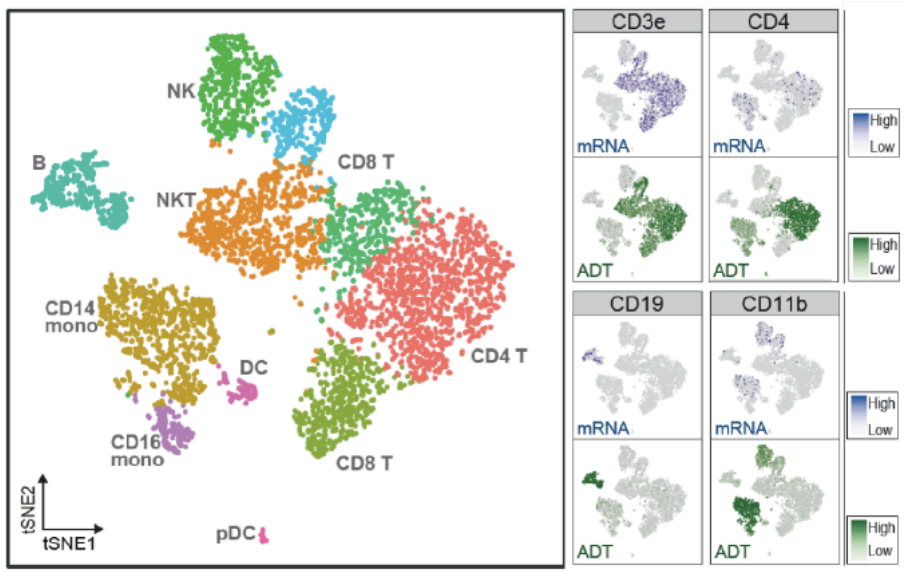
Highly multiplexed phenotypic characterization

With the recent introduction of combined single-cell multiplexed proteomics and transcriptomics, the enhanced understanding of cellular processes through the simultaneous analysis of proteins and transcriptome in thousands of individual cells in parallel, has been made possible.

Cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) has huge potential to take single-cell biology to unprecedented new levels. This is achieved using antibody-oligonucleotide conjugates which couple the detection of specific protein markers with unbiased transcriptome profiling, opening up the possibility to identify novel cell types, shedding new light on previously unseen events, which can then be mapped to response to therapeutic interventions in patient's tumors using Nilogen's platform.

Figure 1: CITE-seq of PBMCs

Clustering of 5,000 CITE-seq single-cell expression profiles of PBMCs reveals distinct cell populations based on transcriptome analysis. The left panel shows global gene expression relationships among all cells, and major cell types separated based on gene expression as indicated. The right panels show mRNA (blue) and corresponding Antibody-Derived Tag (ADT, green) signal.



A holistic approach

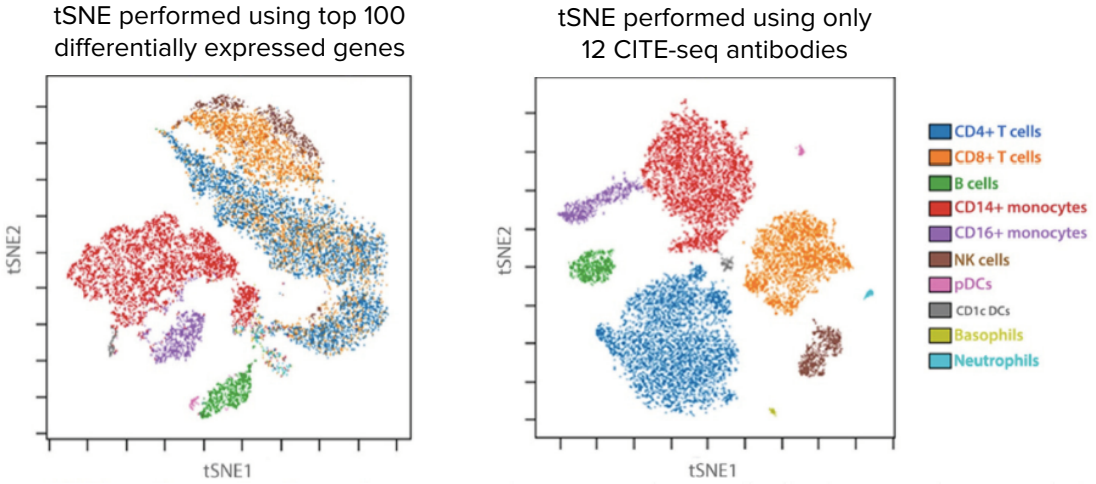
The use of scRNA-seq and CITE-seq in combination with the tumoroid platform, as well as existing analytical methods, takes translational oncology drug discovery to a whole new level. No longer limited to just studying efficacy, but profiling the impact on the immune compartment at a granularity not achievable with bulk RNA analysis. Combined with tumor cell killing, phagocytosis, stromal penetration as well as cytokine or chemokine release, scRNAseq delivers a deep understanding of the impact of drug effect in each patient's tumor.

scRNA-seq in conjunction with CITE-seq reveals additional heterogeneity that was not previously possible to detect with a limited number of antibodies against cell surface markers, permitting the interpretation of transcriptome analyses in the context of functional expression of over 100 protein markers.

The advantage of using CITE-seq is that antibodies provide enhanced immunophenotyping, especially when analyzing closely related cells. When cells are related, analysis of the transcriptome alone may not provide enough resolution to cluster different cell types as shown in **Figure 2**, where analyzing only gene expression, the resolution between CD8 T cells and NK cells is not optimal. A similar situation can be observed between a subset of CD8 T cells and the CD4 cluster. In contrast, using only 12 CITE-seq antibodies, 10 major cell types are easily distinguishable based on typical surface markers present in these cell types.

Figure 2: Improved cluster resolution

Cell populations clustered based on mRNA of 100 differentially expressed genes are shown in the left panel. In contrast, incorporation of results from CITE-seq antibody binding dramatically enhanced the resolution of the populations as shown in the right panel



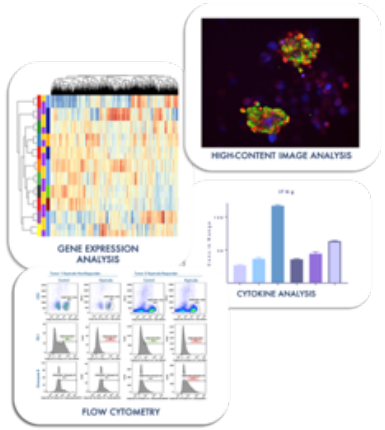
Nilogen's 3D-EXplore platform provides a holistic approach to drug development, enabling a deep understanding and comparison of the mechanism of action of multiple drug therapies in the same patient's tumor.

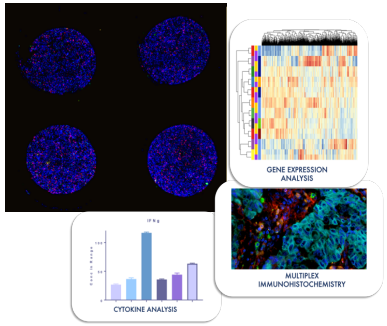
Utilizing leading-edge technologies:

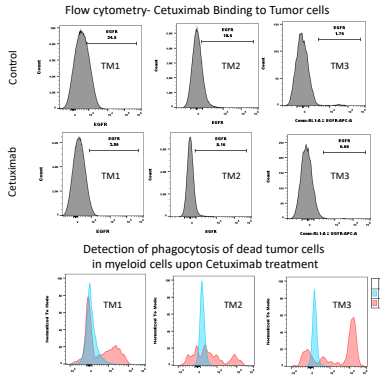
- High content imaging
- Multiplex IF
- Flow cytometry
- NGS/WGS
- RNAseq/Nanostring
- scRNAseq
- CITE-seq
- Cytokines
- Phagocytic activity
- Tumor Cell Killing
- RNA/FFPE prep

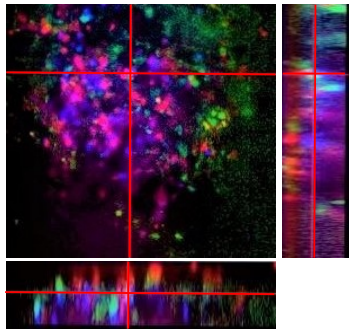
Therapeutic Modalities Validated:

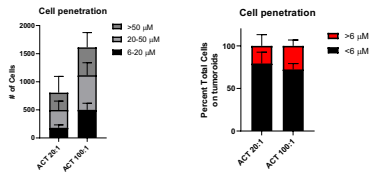
- I-O mAbs/multispecifics
- ACT
- ADCs
- ADCCs
- Oncolytic Viruses
- Small molecules
- Chemotherapies

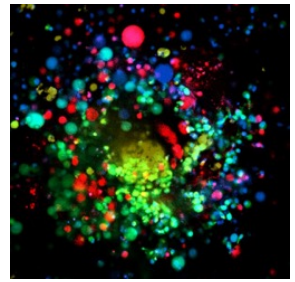


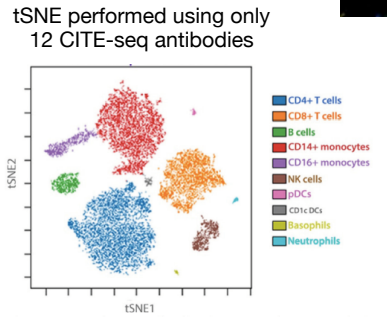












Compare multiple therapies in the same tumor

The power of Nilogen's tumoroid platform comes from the number or arms that can be run simultaneously in the same patient's tumor tissue. Sufficient tumor is obtained to run many treatment arms with multiple readouts per treatment. As many as 9,000 tumoroids are created and pooled from each patient's tumor, with up to 400 tumoroids distributed into each assay well, overcoming the issue of heterogeneity as each assay well is equivalent.

Figure 3 provides as example study design where 10 treatment arms are created from a NSCLC patient's tumor. Each treatment arm is evaluated with Flow Cytometry to determine the percentage of cell types pre and post treatment, as well as scRNA-seq/ TOTALSeq to immuno-profile low abundance immune cells, cytokines, and confocal analysis to quantitate treatment-mediated tumor cell killing (TCK).

Figure 3: Example tumoroid study

Flow, scRNAseq (10X), TOTALSeq (Biolegend), Cytokine and Confocal analysis of treatment-mediated tumor cell killing

Treatment No.	14 color Flow panel	scRNA-seq / TOTALSeq	Up to 100 cytokines	High Content Confocal TCK
1. Untreated Tumor	X			
2. Negative Control	X	X	X	X
3. Drug A dose 1	X	X	X	X
4. Drug A dose 2	X	X	X	X
5. Drug A & SoC	X	X	X	X
6. Drug B dose 1	X	X	X	X
7. Drug B dose 2	X	X	X	X
8.Drug B & SoC	X	X	X	X
9. Positive Control	X	X	X	X

Unprecedented granularity

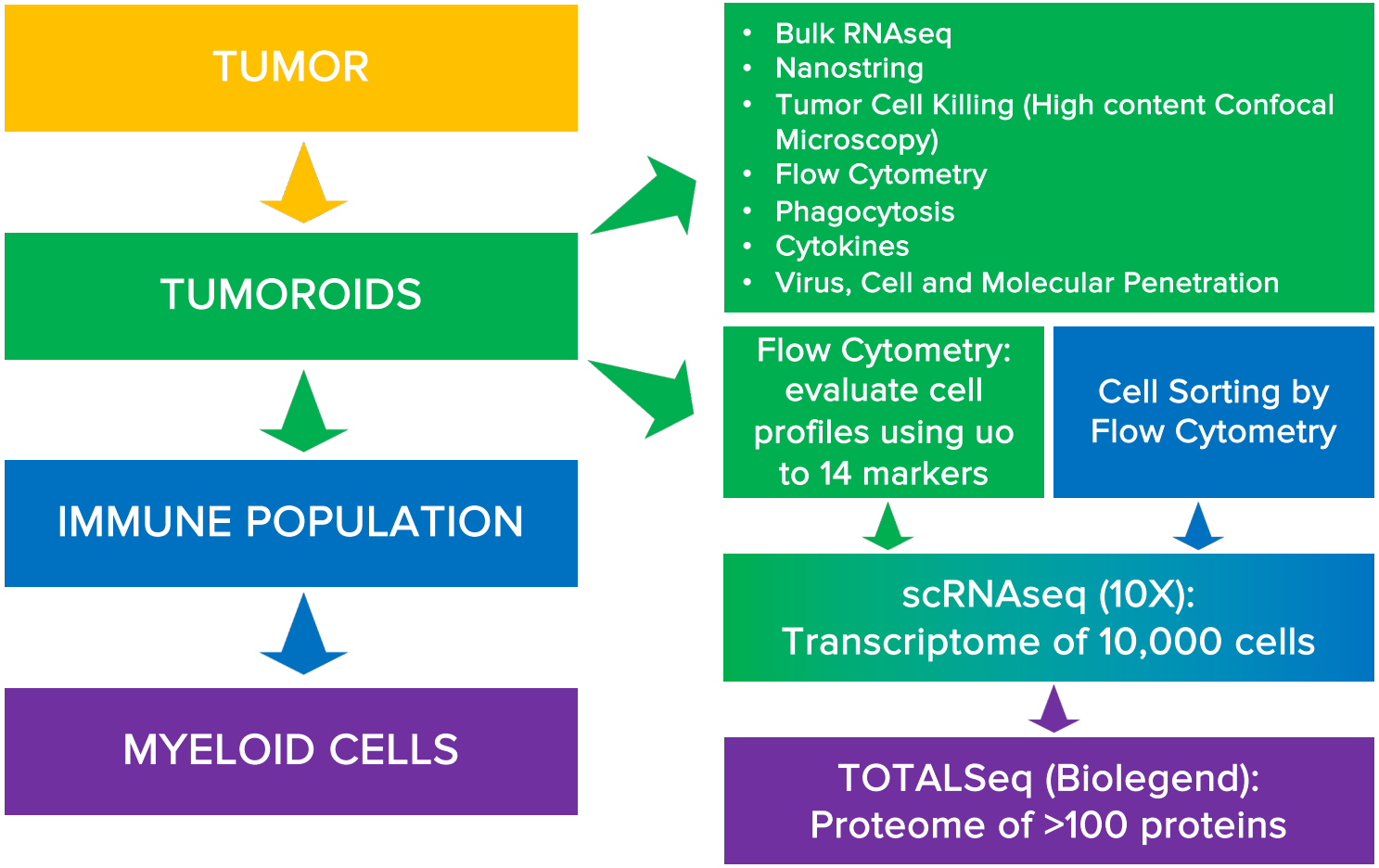
The power of Nilogen's 3D-EXplore platform enables drug development teams to investigate the impact of therapeutic intervention at an unprecedented level of granularity.

Post treatment, flow cytometry and cell sorting can be simultaneously performed in order to define and understand immune cell populations as a whole while selecting specific cell types on which to perform scRNAseq.

The use of 10X scRNAseq enables the sequence of each of those selected cells to further identify subtypes at a transcriptome level, and in combination with TOTALSeq from Biolegend provides a further level of granularity to understand protein expression of >100 markers, directly correlating genotype and phenotype.

Each level of granularity can then be brought together to understand both the population and impact of therapeutic intervention from single cell to complete tumor.

Mapping out the impact of Therapeutic Intervention from single cell to complete tumor.



The importance of fresh

At Nilogen, tumor tissue is transported from our source sites and is processed into our platform within 24 hours of surgery.
Fresh is key to ensure the loss of viability is minimized. All tissues are recruited under IRB approval with patient consent.
Additionally, all incoming tissues are evaluated using Flow Cytometry for viability to ensure the quality of tissues used in our client's studies, and the frequency of immune cells is quantified to identify hot versus cold tumors. At this stage tissues can also be selected based on the need for certain markers to be present such as HLA typing.
At the end of each assay, the importance of processing fresh tumoroids using Flow Cytometry, or using Cell Sorting and either scRNA-seq or CITE-seq is key to ensure viability is maintained as well as the expression of protein markers. It has been well documented how the use of freeze-thaw cycles can significantly impact the viability of cells, especially more fragile immune cells, creating a bias in the population of cells. Simultaneously the expression of protein markers can be reduced by 60% or more.
If you are considering using third parties to process frozen samples for cell viability, sequencing or protein expression, it's worthwhile asking how many cells they require, which is a strong indication of the success rate in recovering viable cells and protein expression.
Consider that as 10X scRNA-seq can only process up to 10,000 cells, why would you need significantly higher numbers post cell sorting?
At Nilogen, no tissues are frozen for such assays – we only use those cells freshly collected from the study wells.

"60% or more of the expression of protein markers can be lost during the freeze/thaw processing of samples, and cell viability can be significantly reduced."

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?

NILOGEN ONCOSYSTEMS

SOLUTIONS THAT POWER TRANSLATIONAL ONCOLOGY

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

info@nilogen.com

+1 813 440 3588