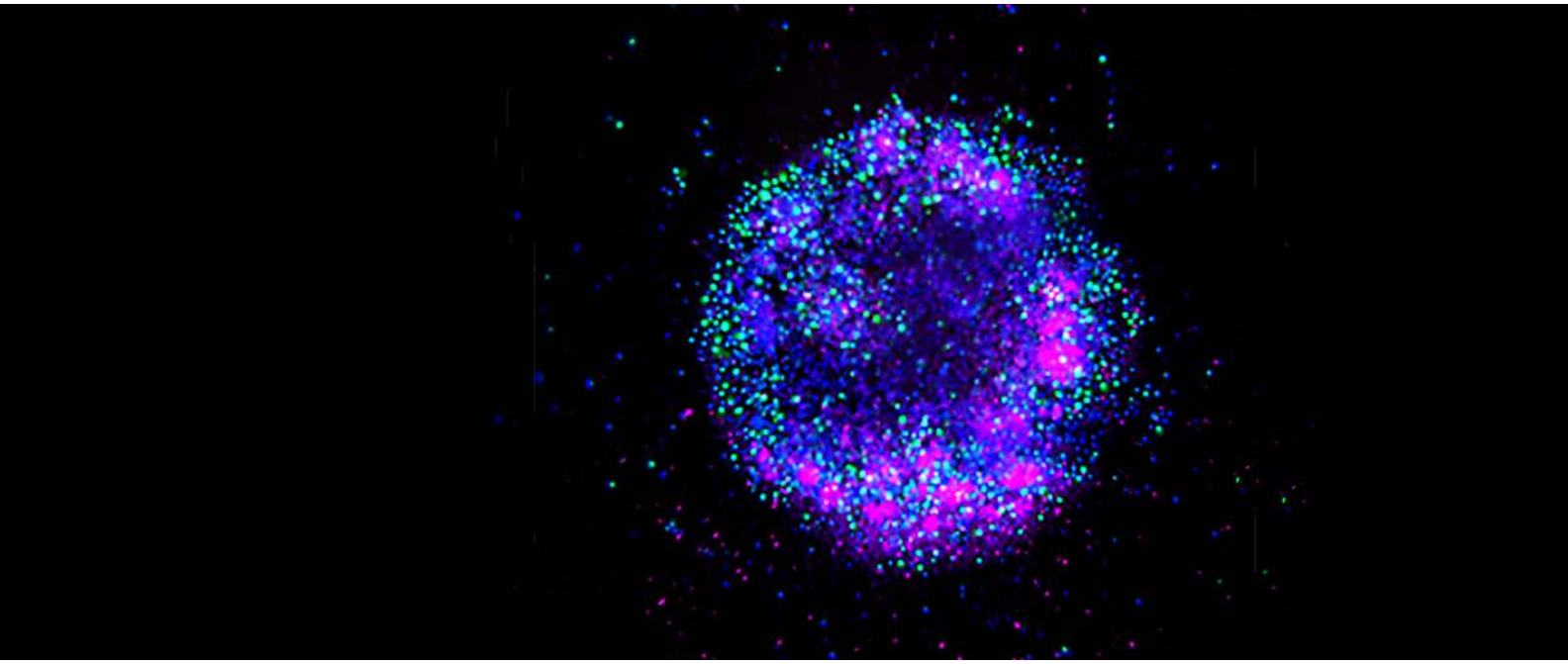

Adoptive cell therapies in immuno-oncology research

Analyze immune cell cytotoxicity in 3D tumor models.

Recent advances in immuno-oncology (I-O) research are paving the way for the discovery of novel classes of cancer therapeutics that enhance or enable antitumor immune responses, overcome tumor evasion mechanisms, and promote conditions that favor immune protection. Two principal areas of research include the development of biotherapeutic antibodies that target immune-checkpoint pathways and the expansion of cellular approaches such as adoptive cell therapies (ACT), which rely on reintroduction of a patient's T cells after genetic modification. Such immunotherapies may offer distinct advantages over standard cancer treatment modalities. For example, tumor-specific immune cells have the ability to migrate to areas of the body that are inaccessible by surgery, as well as to seek out and target microscopic disease and disseminated metastases. In addition, unlike radiation and chemotherapy, the goal of immunotherapies is to act exclusively against the tumor, thereby lowering the risks of damage to surrounding healthy tissue and potentially minimizing other harmful side effects.

Figure 1 (above). Natural killer (NK) cells invading breast cancer spheroids. See details in Figure 3 caption. NK cell penetration and tumor cytotoxicity were evaluated using live-cell whole-spheroid imaging on the Thermo Scientific™ CellInsight™ CX7 LZR HCA Platform using confocal mode with 10 µm Z slicing.

In 1988, autologous T cell adoptive transfer of *ex vivo* expanded cells was used with relative success to treat patients with metastatic melanoma resistant to conventional therapies, and incremental improvements in efficacy have emerged over time [1]. More recently, investigators have realized gains with the development of chimeric antigen receptor (CAR) T cell therapy for treating certain forms of cancer, and two CAR T cell therapies were approved by the US Food and Drug Administration (FDA) in 2017. In CAR T cell therapy, the patient's (autologous) or a healthy donor's (allogeneic) T cells are genetically modified with CARs made up of antibody-based domains that recognize antigens expressed on the surface of the cancer cells [2].

Working with 3D tumor spheroid cultures

Increasingly, ACT research is focusing on the treatment of solid tumors such as those found in lung and breast cancers. Tumors and their associated microenvironments within intact organisms contain highly complex and dynamic sets of interactions between different cell types, as well as multiple chemical gradients and a variety of extracellular matrix components. Unlike their 2D tissue culture counterparts, 3D tumor spheroids can provide physiological and biochemical conditions that more closely resemble the tumor microenvironment in an intact organism. Thermo Fisher Scientific is developing 3D cell culture techniques and low–cell attachment microplates, along with assays and instruments for analyzing cell function, including fluorescent reagents for detecting viability, proliferation, and apoptosis. Here we demonstrate the use of several of these techniques and reagents to study immune cell infiltration in 3D tumor spheroid cultures and the subsequent death, by apoptosis, of spheroid cells (Figures 1–3).

Immune cell cytotoxicity in 3D cell models

Figures 2 and 3 show our investigation of the penetration and potency of cytotoxic T cells and natural killer cells in 3D cell models of lung and breast cancer tumors, respectively. We used the Thermo Scientific™ CellInsight™ CX7 LZR High-Content Analysis (HCA) Platform with Thermo Scientific™ HCS Studio™ Cell Analysis Software to segment and measure fluorescence intensities of individual cells within the 3D spheroid. The ability to conduct this type of detailed analysis of immune cell cytotoxicity may facilitate our understanding of the mechanisms of cytotoxic immune cells and their potency in immunotherapies.

Figure 2 shows the response of lung cancer spheroids (generated with A549 cells) in the presence of increasing numbers of T cells. The T cells were labeled with a far-red–fluorescent cell tracer (Invitrogen™ CellTracker™ Deep Red Dye), and the spheroids were labeled with an apoptosis indicator (Invitrogen™ CellEvent™ Caspase-3/7 Green Detection Reagent). As the T cell dose increased, we observed more A549 cells in a lung cancer spheroid undergoing apoptosis, →

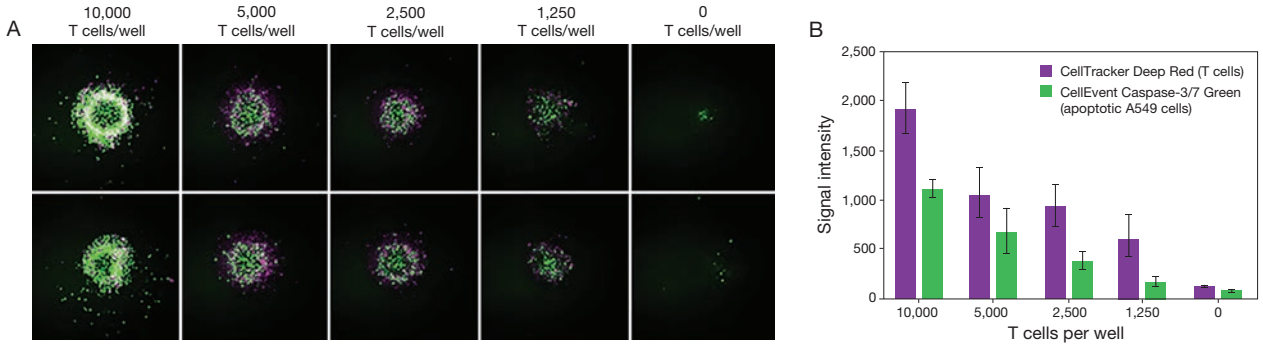

Figure 2. T cell–dependent killing of lung cancer spheroids. T cells were isolated from human peripheral blood and expanded for 5 days with Gibco™ Dynabeads™ Human T-Activator CD3/CD28 (Cat. No. 11131D) in Gibco™ CTS™ OpTmizer™ T Cell Expansion SFM (Cat. No. A1048501). Cells were analyzed with the Invitrogen™ Countess™ II FL Automated Cell Counter to determine cell viability and concentration, and cell concentration was adjusted to 1 x 10^6 cells/mL in Gibco™ PBS, pH 7.2. T cells were then labeled with 2 µM Invitrogen™ CellTracker™ Deep Red Dye (purple, Cat. No. C34565) for 15 min and washed with CTS OpTmizer medium. For spheroid formation, A549 (adenocarcinomic human alveolar basal epithelial) cells were plated in a Thermo Scientific™ Nunclon™ Sphera™ 96-well microplate at a density of 7,500 cells/well, incubated overnight in a cell culture incubator with 5% CO_2 at 37°C, and analyzed on the Invitrogen™ EVOS™ XL Core Imaging System to confirm spheroid formation. Invitrogen™ CellEvent™ Caspase-3/7 Green Detection Reagent (2 µM final concentration) (green, Cat. No. C10723) and the indicated number of labeled T cells were added to each well, and the mixture was incubated for 4 hr at 37°C. **(A)** Two examples of the response of a lung cancer spheroid to 4 different T cell concentrations are shown, each imaged on the Thermo Scientific™ CellInsight™ CX7 LZR HCA Platform using confocal mode with 10 µm Z slicing. **(B)** Apoptosis in A549 spheroids and the T cell dose response were quantified using the CellInsight CX7 LZR platform and Thermo Scientific™ HCS Studio™ Cell Analysis Software. The spheroids were segmented as single objects based on the brightfield image using HCS Studio software, and cells were counted within the object. Mean signal intensities of cells within the spheroid were used to quantify immune cell cytotoxicity.

which was quantified using the mean signal intensities of cells within the spheroid.

Figure 3 shows the results of an investigation of antibody-dependent cell killing in breast cancer spheroids by natural killer (NK) cells. The NK cells were labeled with the far-red–fluorescent CellTracker Deep Red Dye, and the SKBR3 cells in the breast cancer spheroids were labeled with Hoechst 33342 nucleic acid stain and the CellEvent Caspase-3/7 Green reagent. While the addition of NK cells alone to the spheroids induced moderate cytotoxicity, the combination of NK cells and trastuzumab (a monoclonal antibody used to treat breast cancer) resulted in increased apoptosis and degradation of the spheroid structure.

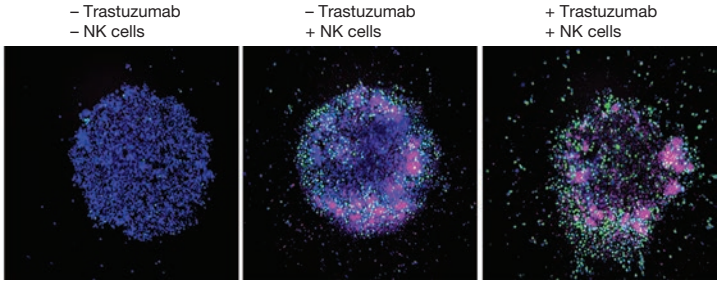

Figure 3. Antibody-dependent cell killing in breast cancer spheroids. Natural killer (NK) cells were isolated from human PBMCs using the Invitrogen™ Dynabeads™ Untouched™ Human NK Cells Kit (Cat. No. 11349D) and labeled with Invitrogen™ CellTracker™ Deep Red Dye (purple, Cat. No. C34565). These cells were added to SKBR3 breast cancer spheroids labeled with Invitrogen™ NucBlue™ Live ReadyProbes™ Reagent (blue, Cat. No. R37605) and Invitrogen™ CellEvent™ Caspase-3/7 Green Detection Reagent (green, Cat. No. C10723), with or without 30 nM trastuzumab, and the mixture was incubated for 2 hr at 37°C. NK cell penetration and tumor cytotoxicity were evaluated using live-cell whole-spheroid imaging on the Thermo Scientific™ CellInsight™ CX7 LZR HCA Platform using confocal mode with 10 µm Z slicing. The spheroids were segmented as single objects based on the brightfield image using Thermo Scientific™ HCS Studio™ Cell Analysis Software, and the cells were counted within the object. Mean signal intensities of cells within the spheroid were used to quantify immune cell cytotoxicity (data not shown). The addition of NK cells induced moderate cytotoxicity (middle), whereas the addition of NK cells and trastuzumab resulted in substantial apoptosis and degradation of the spheroid structure (right).

High-content imaging and analysis

The CellInsight CX7 LZR HCA Platform is an ideal platform for high-resolution imaging and analysis of live 3D cell cultures. This integrated benchtop system offers widefield, confocal (critical for axial resolution in 3D acquisition), and brightfield imaging, with extremely bright illumination to penetrate thick samples, and microscope objectives from 2x to 60x. It also provides fast image acquisition with shorter exposure times and laser autofocus capabilities.

With seven fluorescence detection channels on the CellInsight CX7 LZR system, and expanded excitation options provided by the near-infrared (785 nm) laser, it is possible to conduct multiplex analysis using a combination of structural and functional probes to investigate complex biological processes in both the immune cells and the individual cancer cells that make up the tumor spheroids. Live-cell imaging and analysis also benefit from advanced instrument features that allow you to control the amount of light reaching the sample, helping to minimize photobleaching and phototoxicity. The CellInsight CX7 LZR HCA Platform is compatible with a broad range of plate formats and types and offers optional onstage incubation and robotic plate handling, while the HCS Studio software allows access to all instrument configuration and control functions. This intuitive, icon-driven tool helps to manage the experimental design and workflow, starting with plate maps and protocol setup, all the way through image acquisition and data analysis.

Explore 3D culture and analysis

Thermo Fisher Scientific offers a suite of culture media, cultureware, cell analysis reagents, and fluorescence instrumentation for 3D cell culture. Learn more at **thermofisher.com/spheroid**. ■

References

1. Rosenberg SA, Restifo NP, Yang JC et al. (2008) *Nat Rev Cancer* 8:299–308.
2. Batlevi CL, Matsuki E, Brentjens RJ et al. (2016) *Nat Rev Clin Oncol* 13:25–40.

Product	Quantity	Cat. No.
CellEvent™ Caspase-3/7 Green Detection Reagent	25 µL 100 µL	C10723 C10423
CellInsight™ CX7 LZR High-Content Analysis Platform	1 each	CX7A1110LZR
CellInsight™ CX7 LZR HCA Platform with HCS Studio™ Cell Analysis Software Extended Warranty Package	1 each	A37014
HCS Studio™ 2.0 Cell Analysis Software	1 each	SX000041A
CellTracker™ Deep Red Dye	20 x 15 µg	C34565
CTS™ OpTmizer™ T Cell Expansion SFM, bottle format	1,000 mL	A1048501
Dynabeads™ Human T-Expander CD3/CD28	10 mL	11141D
Dynabeads™ Human T-Activator CD3/CD28 for T Cell Expansion and Activation	0.4 mL 2 mL 5 x 2 mL	11161D 11131D 11132D
Dynabeads™ Untouched™ Human NK Cells Kit	1 kit	11349D
EVOS™ XL Core Imaging System	1 system	AMEX1000
NucBlue™ Live ReadyProbes™ Reagent	1 kit	R37605