

life lab

PRODUCTS, INFORMATION, AND SCIENTAINMENT

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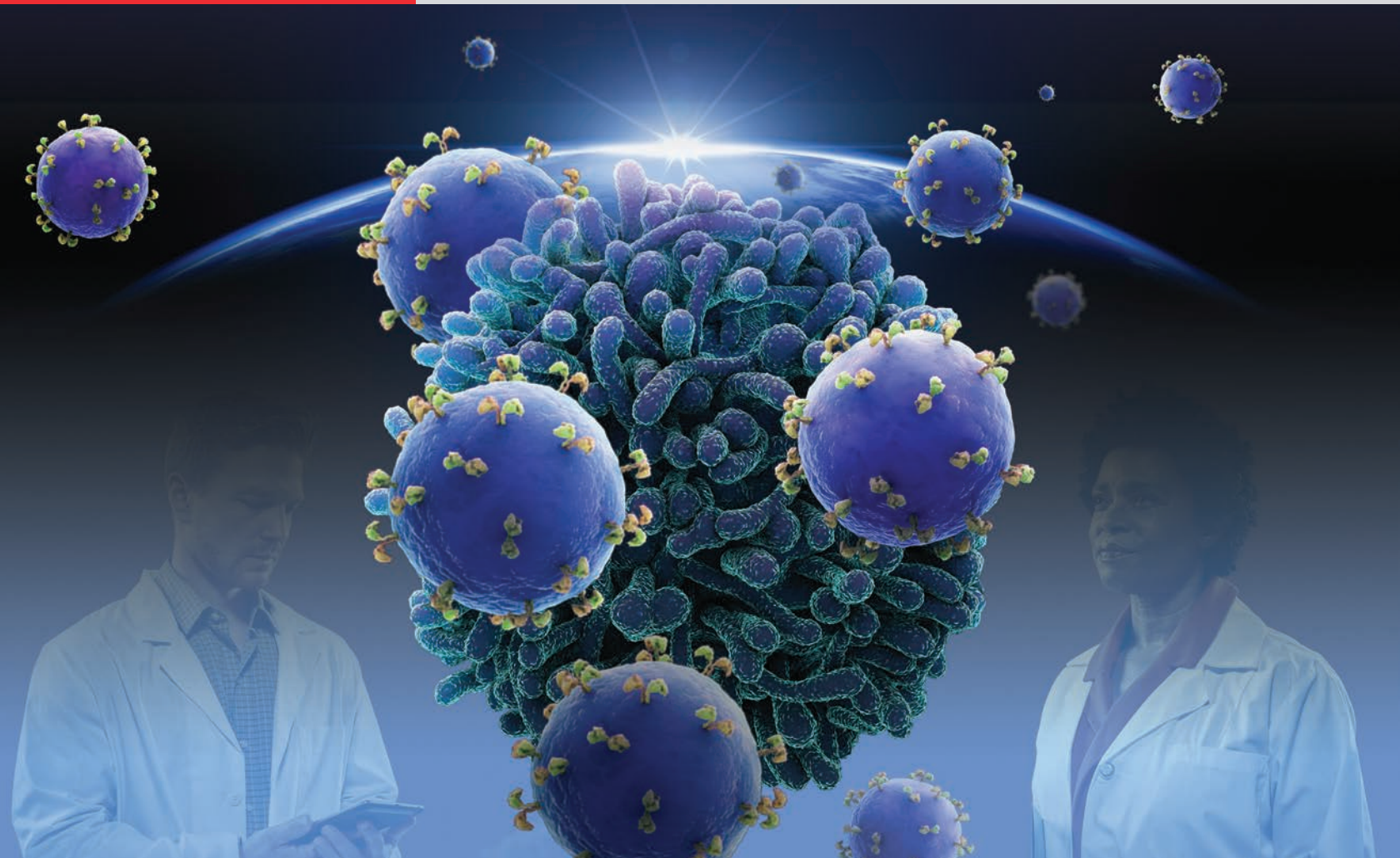
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MISSION (ALMOST) ACCOMPLISHED

Researchers in immunology fight challenges old and new

Investigation of disease states has taken researchers on a complex journey of uncovering the inner workings of the immune system and its responses to pathogens. Throughout this issue we explore both immunology and a variety of diseases, highlighting how continued innovation can improve treatments.

But did you know that some deadly diseases are still lurking around, although they were

thought to have been almost completely eradicated? Recent examples include:

- **Bubonic plague**—over 200 deaths in Madagascar were recorded in 2017
- **Scarlet fever**—it mysteriously resurfaced in England and East Asia
- **Syphilis**—the 90s had the highest rate of infection in 40 years
- **Polio**—while 99% eradicated, it still exists in Afghanistan, Nigeria, and Pakistan

- **Leprosy**—treatable, but it takes three to five years for symptoms to show, making eradication difficult
- **Cholera**—1 million cases in Yemen in 2017, the largest outbreak in recent history

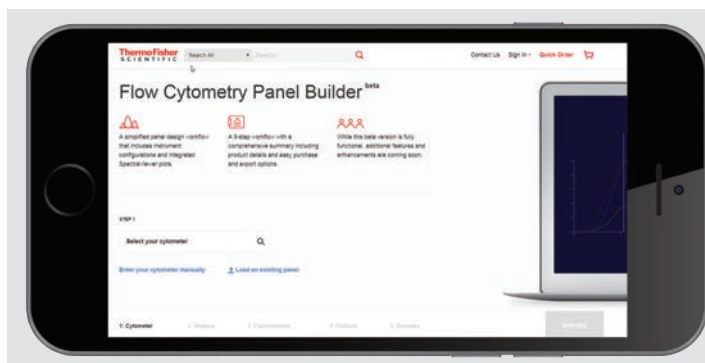
These sobering statistics illustrate the need for continued research and vigilance when it comes to diseases and the immune system.

INVESTIGATING INFLAMMATION

Choosing the right antibodies to understand the complex process of inflammation

Inflammation is the body's response to tissue damage. The accompanying pain, warmth, and swelling are all hallmarks of normal biological processes that remove pathogens in the body. Immune cells swarm to the affected area and release cytokines and other factors to remove damaged tissue and start healing. Even nonpathogenic conditions like touching a hot stove can start the inflammation cascade. Limited inflammation is protective and necessary to keep the body running effectively.

But, what happens when inflammation is not well regulated? This type of inflammation can be found in chronic conditions. A disease like atherosclerosis is accompanied by the production and release of inflammatory factors.¹ Plaques on vessel walls have immune cells and platelets producing molecules that regulate adhesion and lipid uptake. Atherosclerosis can be controlled by diet, activity, and medication; and yet, there is a need to understand the underlying dysregulation of the inflammation process.^{2,3} Greater knowledge of inflammation could lead to better ways to control these types of diseases.



NEW PANEL BUILDER FOR FLOW CYTOMETRY

Dissecting the complexity behind inflammation in chronic diseases requires analysis of markers in immune cells, cytokines, or other proteins.^{4,5} The Invitrogen™ Flow Cytometry Panel Builder makes panel design simpler, helping you design your panel more efficiently regardless of your level of experience. With this tool, you can analyze your markers of interest and get closer to your next discovery.

Learn more at thermofisher.com/order/panel-builder

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2. Libby P (2012) Inflammation in atherosclerosis. *Arter Thromb Vasc Biol* 32:2045-2051.
3. Xue-Qiao Z (2018) Pathogenesis of atherosclerosis. [online] Available at: uptodate.com/contents/pathogenesis-of-atherosclerosis
4. Graves AJ, Padilla MG, Hokey DA (2014) OMIP-022: Comprehensive assessment of antigen-specific human T-cell functionality and memory. *Cytometry A* 85:576-579.
5. Cossarizza A and Cousin D (2015) Overcoming challenges in cellular analysis. *Science* 347: 443 [online] Available at: sciencemag.org/custom-publishing/webinars/overcoming-challenges-cellular-analysis-multiparameter-analysis-rare?_ga=2.88382104.2078857589.1534352862-1479773893.1534352862

BOOST YOUR IMMUNITY WITH THESE TIPS

When it comes to maintaining a healthy immune system, every little bit helps. Below are a few of our favorite methods.

DREAM IN THE DARK



And we mean total darkness, because our circadian rhythm is programmed for two 12-hour periods of light and darkness. By eliminating light pollution

(yes, even from cell phones), you're giving your body better rest and recovery. What's more, research has linked the genes responsible for our internal clock to immune cells.¹ So draw the curtains and nix the night light.

LAUGH IT OFF



A good guffaw or series of chuckles has been found to actually decrease hormones associated with stress.⁶

Regulating your mood with

laughter increases endorphins, which have a positive effect on psychological health as well as the immune system. And that's definitely no joke.

MUNCH ON MICRONUTRIENTS



It's best to naturally ingest micronutrients in your regular meals, but turning to dietary supplements is a popular alternative. Deficiencies in

vitamin B6, vitamin C, vitamin D, vitamin E, magnesium, and zinc have been linked to immune dysfunction,⁴ so make sure you don't skip out on your vitamins.

FIND YOUR CENTER WITH MEDITATION



Over a quarter of the global population is affected by anxiety and depression. In a

2017 study, meditation helped regulate the stress response, suppressing chronic inflammation and helping to maintain a healthy gut microbiome.⁵ Meditation may sound daunting at first, but even five minutes daily can help you de-stress and come back to center. Find a quiet place, get yourself into a comfortable position and just focus on breathing.

VINYASA FLOW YOUR WAY INTO BLISS



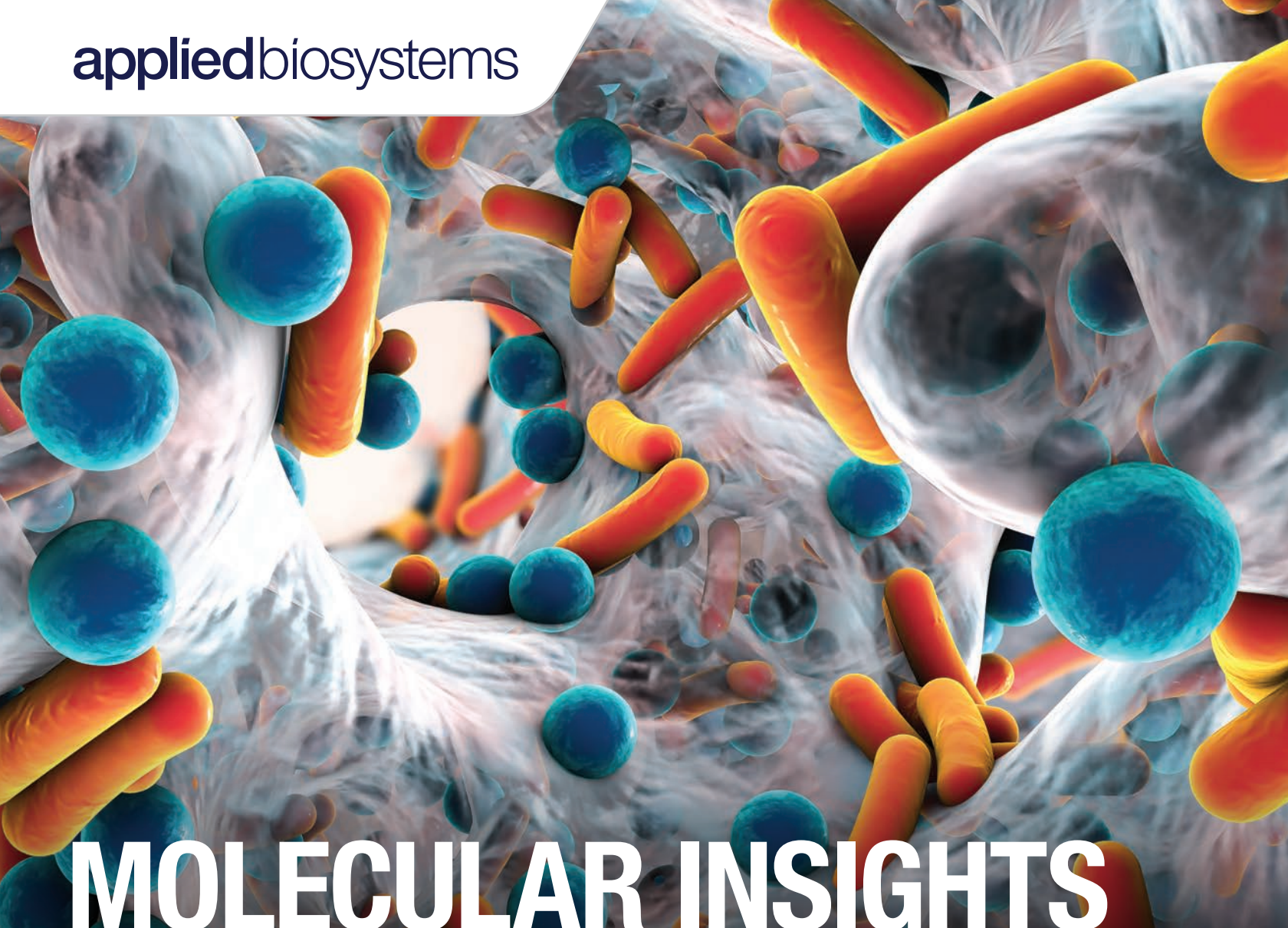
Chronic stress is damaging to the immune system over time.²

It's possible that the balance between the

sympathetic and parasympathetic nervous systems can be positively impacted by yoga. In a study with 60 students, those practicing yoga experienced fewer autonomic and psychologic changes when compared to a control group.² In yet another study, yoga resulted in an increased circulation of immune-related cytokines, which pointed toward a healthier immune system.³

1. Science 342:727 (2013).
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3. J Altern Complement Med 21:530 (2015).
4. Immunol Cell Biol 94:117 (2016).
5. Adv Mind Body Med 3:10 (2017).
6. J Exp Med 239:243 (2016).





MOLECULAR INSIGHTS ON MICROBIAL CHALLENGES

Microbial identification, detection, and role in disease

Genetic analysis technologies have revolutionized microbiology and infectious disease research, expanding our knowledge of microbial diversity and its implication in mechanisms of pathology. Thermo Fisher Scientific offers a broad range of genetic analysis technologies to identify, detect, and characterize microbes faster than traditional microbiological culture techniques. Our optimized platforms for next-generation sequencing (NGS), Sanger sequencing using capillary electrophoresis, real-time PCR, and microarrays offer highly effective approaches to analyzing viruses, bacteria, and fungi. These technologies are also leveraged to study human response to microorganisms. Ultimately, research leading to better understanding of host-pathogen interactions can translate into better prevention and treatment of infectious diseases.

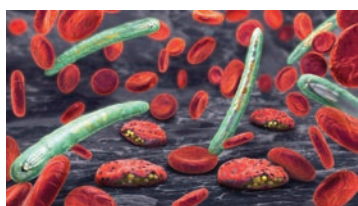


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Find out more at thermofisher.com/microseq

IMPACT OF MALARIA ON BIOLOGICAL AGING—WEBINAR



In the webinar “Infectious diseases accelerate allostatic load and biological aging,” Dr. Muhammad Asghar of the Karolinska Institute Stockholm presents the cellular mechanisms

associated with aging, and how real-time PCR was used to study the impact of chronic asymptomatic malarial infections on telomere degradation.

View the free, on-demand webinar at thermofisher.com/malaria

HUMAN TRANSCRIPTOME PROFILING FROM FECAL SAMPLES—WEBINAR

Transcriptome profiling from clinical research samples is commonly used for disease biomarker discovery. However, blood, FFPE, and feces are challenging samples to work with due to limited extractable RNA. In this webinar, Dr. Mark Manary and Dr. Sean Diehl describe how to measure the human transcriptome from feces (>95% bacterial RNA) with high-performance array technology. They identify biomarkers for environmental enteric dysfunction, a major cause of stunting in the developing world. Overall, this methodology can benefit researchers investigating the effects of the microbiome or microbial infections on gut disease.

View the free, on-demand webinar at thermofisher.com/picowebinar



Analytical validation consulting service aids launch of new laboratory-developed test for women's health. Read the case study at thermofisher.com/aeonavcasestudy

CELLULAR IMMUNOLOGY



Payal Damani-Yokota, PhD
University of Massachusetts, Amherst
Cellular and Molecular Immunology
t @PayalYokota

The scope of her research and the questions she is asking:

For the last 6 years, I have been working toward my PhD at the University of Massachusetts in Amherst under the tutelage of Dr. Cynthia L. Baldwin. The overarching goal of our research is to uncover the signals that drive development and function of gamma delta ($\gamma\delta$) T cells. What makes these cells especially interesting is that they express a family of scavenger receptor cysteine-rich (SRCR) glycoproteins called Workshop Cluster 1 (or WC1) that functions as a hybrid pattern recognition receptor/co-receptor exclusively in conjunction to the $\gamma\delta$ TCR. Our research previously showed that WC1-expressing $\gamma\delta$ T cells are indispensable for response to bacterial antigens such as *Leptospira*; and upon engagement with

ligand, the endodomains can signal via phosphorylation of conserved serines and tyrosines. We have also found that in response to *Leptospira*, one subset of WC1⁺ cells produces IFN γ , while the other does not. While we knew a lot about WC1 function and its importance in recall responses, we did not know how many WC1 molecules a single $\gamma\delta$ TCR⁺ cell could express, or how many of these molecules were required to direct immune responses by $\gamma\delta$ T cells. It was also not known when and where these molecules are first detected during T cell development, or how these functional subsets of $\gamma\delta$ T cells were transcriptionally programmed.

Some answers:

To answer these questions, I flow-sorted purified populations of WC1⁺ $\gamma\delta$ T cell subsets and developed a culture system such that I could expand 78 different WC1⁺ $\gamma\delta$ T cell clones from a single cell origin where primary $\gamma\delta$ T cells could proliferate in vitro for up to 10 weeks. Using [Applied Biosystems™] TaqMan® Assays, I evaluated WC1 gene transcription on each clone to evaluate the WC1 gene expression. The co-transcription of WC1 genes showed that WC1s can be expressed by themselves or in combinations with some overlap between the two subsets. Despite this overlap, the *Leptospira*-responsive WC1⁺ memory $\gamma\delta$ T cell clones showed significantly higher propensity to express WC1 molecules that

are known to bind to the pathogen. I also found that WC1 molecules are exclusively expressed on thymic $\gamma\delta$ T cells and stratify into the mostly nonoverlapping subsets early in development. This is particularly important because unlike conventional $\alpha\beta$ T cells, $\gamma\delta$ T cells bifurcate into functional subsets during thymic development guided by distinct transcriptional programming.

The next steps:

My insatiable curiosity about where and how $\gamma\delta$ T cells develop and why and how they function has led me to investigate even more. The beauty of innate-like lymphocytes is that they have the clonotypic TCRs as well as accessory receptors that help them to respond early and fast, and to expand despite being tiny populations in blood. $\gamma\delta$ T cells act as bridges between the innate and adaptive immunity, thereby making for an elegant, synchronous, pathogen-combating immune system. On a global note, my research has led me to ask if and where such lateral systems can exist in humans and mice, where PRR/co-receptor molecules partake in recognition of complex antigens and can help in producing functional responses to protect against “damaged/stressed self” as well as “non-self.” So, I am packing up the lessons learned from my PhD and taking them “to go,” as I embark on a venture toward cancer immunology in my next gig as a postdoc.



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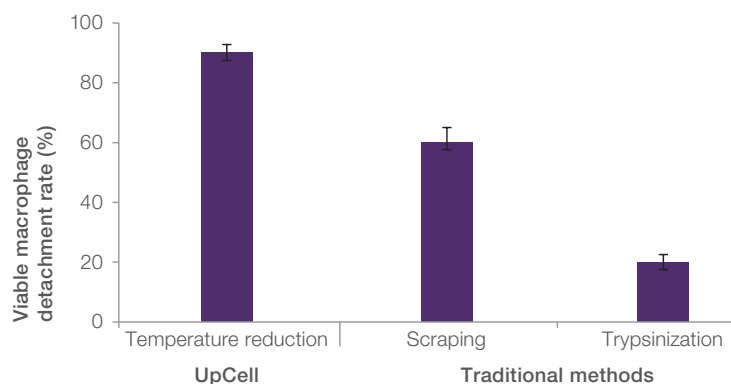
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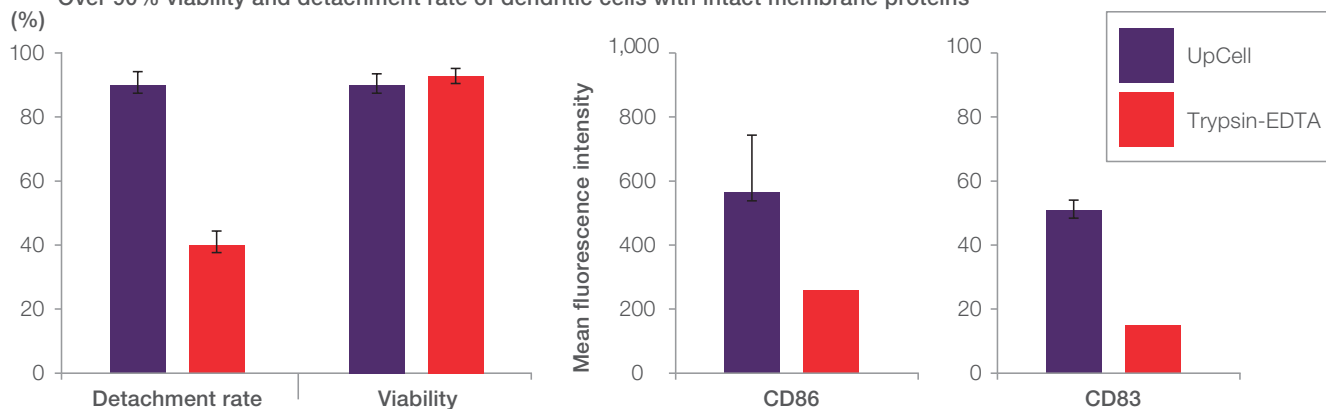
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Step 1: Protein expression



Step 2: Protein isolation & purification



Step 3: Gel electrophoresis



Step 4: Western blotting



Step 5: Protein assays & analysis



Step 6: Mass spectrometry



Step 7: Crosslinking & modification

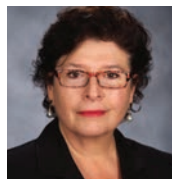
SPEAKERS



Paul Haney, PhD

Senior Product Manager, Protein and Cell Analysis, Thermo Fisher Scientific

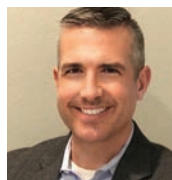
Webinar: Light Up Your Western Blots—Fluorescent Western Blotting Tips, Tricks, and More



Voula Kodoyianni, PhD

Product Marketing Manager—Materials and Structural Analysis, Thermo Fisher Scientific

Webinar: Protein Sample Evaluation Using the Thermo Scientific™ NanoDrop™ One UV-Vis Spectrophotometer



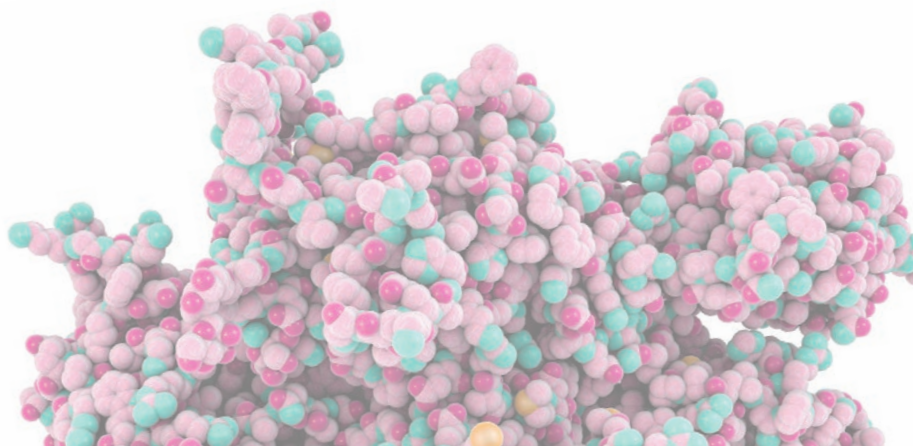
David Bourdon, PhD

Senior R&D Manager, Antibodies and Immunoassays, Thermo Fisher Scientific

Webinar: Invitrogen™ ProQuantum™ High-Sensitivity Immunoassays Offer Minimum Sample Consumption with Maximum Performance



Other presentations and webinars cover topics ranging from detection of multiple autoimmune diseases to analysis of signaling pathways like AKT/mTOR. To see the full list of presentations and webinars, go to thermofisher.com/7steps





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THE COMPLEXITIES OF ADULT-ONSET HEARING LOSS

Forward-looking NGS research enables discovery of connections between lifestyle and genetics



Dr. Pan Zhang
Director of the Sequencing and Microarray Center, Coriell Institute for Medical Research

Distinguishing age-related hearing loss from other environmental factors such as noise, medications, or disease can be difficult. To better understand these differences, the Coriell Personalized Medicine Collaborative™ (CPMC) hearing loss study has been collecting data from thousands of participants for more than a decade.

Dr. Pan Zhang, director of the Sequencing and Microarray Center at the Coriell Institute for Medical Research, spoke to us about using next-generation sequencing (NGS) for adult-onset hearing loss research.

Tell us about your research at CPMC.

Many cases of hearing loss in adults are noise-related. However, we are asking if there is a genetic component. Existing genetic studies are quite extensive on hereditary hearing loss, but not so much focused on adult-onset hearing loss. We are asking questions such as: do some people have a genetic predisposition for hearing loss, resulting in them being more susceptible to noise-induced hearing loss? What is the interplay between that and environmental factors?

The Genome-Wide Association Study (GWAS) gave us a broad overview at the genome level so that we can now combine detailed NGS profiles of genes and regions of interest to enable us to look deeper into the genomic data and identify variants for further study.

How has Thermo Fisher Scientific, and specifically the Ion Torrent™ Ion AmpliSeq™ On-Demand Panels, made an impact on your research with adult-onset hearing loss?

When we decided to move beyond the regions and targets that the GWAS identified, we then needed a tool to identify genetic variants in the genes and regions of interest. For this poorly understood condition, we needed a discovery tool that is both rapid and cost-effective for analyzing multiple genes in depth in many samples. We needed an approach that was both customizable and flexible. Targeted NGS and Ion AmpliSeq On-Demand Panels offered the solution.

While we had target candidates from CPMC and GWAS as well as literature, there are always new genes being proposed. We wanted a way to be able to combine established targets with new research candidates for a more comprehensive, but still targeted study.

The flexibility of Ion AmpliSeq On-Demand Panels allowed high-level customization of panel design, which provided a unique solution.

And how has Ion Torrent™ NGS technology impacted your research?

The CPMC study combines genetic information with environmental factors and personal lifestyle data collected through questionnaires to make better predictions about the risk for disease or the response to prescription drugs. The addition of targeted NGS to the genetic information collected by GWAS helps better define genetic factors underlining hearing loss and identify variants that may have an association with a predisposition to hearing loss.

NGS and the Ion AmpliSeq On-Demand Panels are allowing us to create customized and comprehensive panels to get more in-depth information about the human genome.

A distinct feature of Ion Torrent NGS technology is providing data analysis as part of a solution. The bioinformatic pipeline is integrated, and analyses such as alignment and variant calling can be performed in a streamlined manner. The Ion Torrent™ Ion Reporter™ Software includes different ways for us to look at the data.

For example, some tools provided by the software allow us to select and cross-compare a specific group of samples and look for common variants and for a genetic signature. We do have to do further analysis; however, to have part of the data analysis just done by the system is still valuable.

What has made the biggest impact on your research?

NGS and the Ion AmpliSeq On-Demand Panels are allowing us to create customized and comprehensive panels to get more in-depth information about the human genome. Technological advancement, an expanded knowledge base, and large-scale data sharing has changed the landscape of medical research and the healthcare industry. The way we think and design our research has changed a lot over the past 5 years. The more we study, the more we will find. We can now examine and study variants in genes and regions, structure variation, copy numbers, and many other genomic abnormalities in a short period of time.

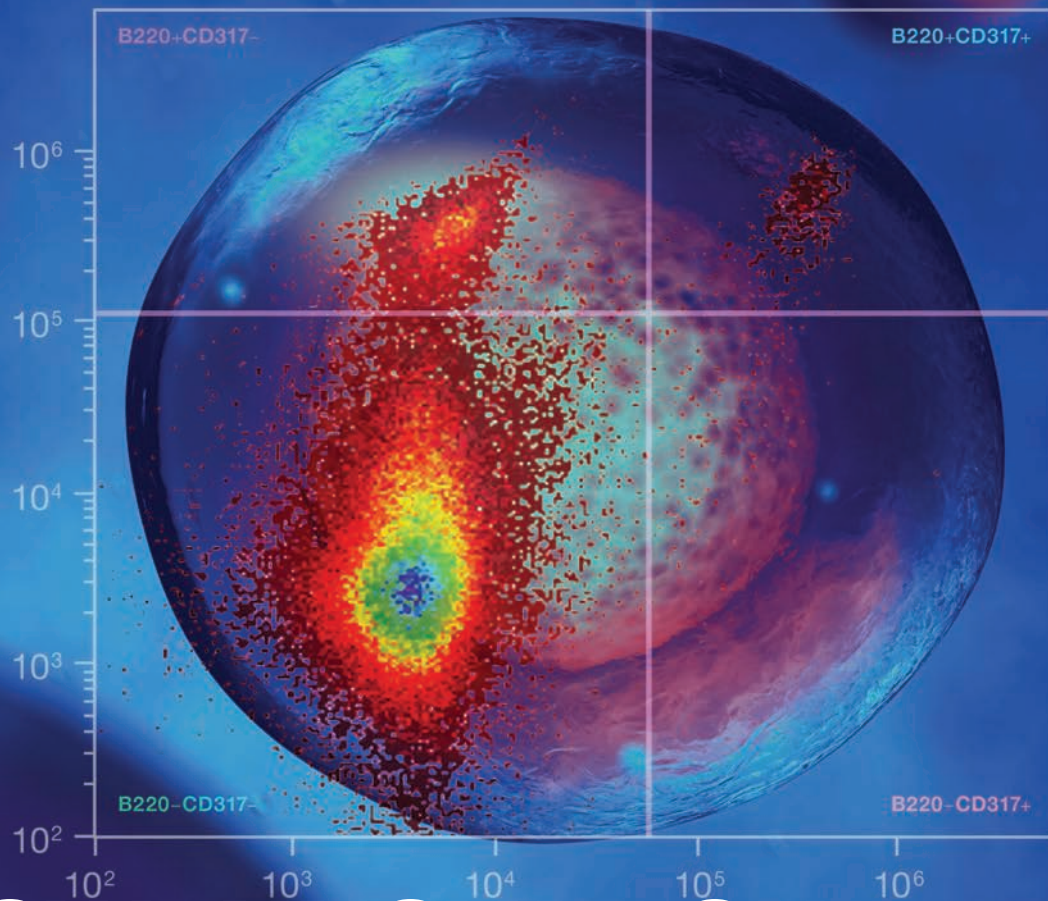
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Ion AmpliSeq HD technology is a new amplicon-based enrichment technology for Ion Torrent targeted next-generation sequencing. It gives you the power to design your own panels and find variants with low limits of detection—down to 0.1%. This revolutionary technology enables researchers to routinely get answers where ultrahigh sensitivity is required, such as detecting low-frequency alleles in circulating tumor DNA or trace pathogenic microbial species in blood.

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FLOW INNOVATION ADVANCES HIV RESEARCH

One researcher's pursuit of rare T cell analysis using flow cytometry



Sara De Biasi, PhD
Department of Life Sciences
University of Modena and Reggio Emilia
(UNIMORE)
Modena, Italy

Immunotherapies transform the body's inherent immune system, elevating its ability to fight cancer. T cells and their mediated responses are central to this transformation. In acquired immunodeficiency conditions such as HIV, T cells are fatally targeted by the virus in a sophisticated effort to hinder the body's immune system. Scientists like Sara De Biasi are achieving immense insights on the activities and characterization of T cells, specifically invariant natural killer T cells (iNKT) in HIV-positive subjects. Using flow cytometry technology to inch closer to uncovering revolutionary immunotherapies, Sara shares her research journey—one driven by natural

curiosity and an early exposure to the mesmerizing interplay between HIV and the immune system.

What drives your passion for science?

I am a very curious person. In every field, I aspire to know how and why something happens—what are the causes, what are the consequences. The more complex it is, the more passionate I become.

What inspired you to pursue this profession?

My childhood dream was to become a medical doctor and perform autopsies. However, I decided to start my studies by

gaining a solid background in biology. Thus, I took a degree program in biotechnology. I was so enthusiastic to understand the molecular and cellular mechanisms responsible for an immunological disorder that I realized I had found my way—to this day, it's still the most appealing aspect of my research.

How did you first become involved in researching acquired immunodeficiencies, such as HIV?

Professor Andrea Cossarizza was my professor of immunology. His enthusiasm was contagious, and I was extremely curious to understand how the immune system works during HIV infection and how HIV kills CD4⁺ T cells. He was looking for students to carry on HIV research; in particular, the effects and side effects of antiretroviral therapy. I applied and started the day after.

You're interested in rare populations of T cells and their role in autoimmune diseases. Can you share more?

I have good training and experience in immunology, particularly in the development and use of new flow cytometric approaches. My longstanding research commitments are centered on identifying the molecular and cellular basis and involvement of the immune system in several diseases and infections, such as HIV/AIDS or hepatitis and physiopathological conditions. I have built expertise in the clinical application of new methods for the identification of rare cellular subsets in patients affected by HIV infection and in patients undergoing liver transplantation, as well as in patients suffering from multiple sclerosis. Such methods are allowing a new characterization of the functional activities of these cells.

What challenges do you face in your research?

Every time I am dealing with a new project, there are at least two big things to think about: how to choose the right methodology and how to deal with huge amounts of data. Communication is crucial to achieving knowledge and results, so it's paramount to feel comfortable reaching out to colleagues for help. Moreover, I work at the university, so one of my activities is to teach students.

With multiparameter capabilities and a very high analysis rate, flow cytometry is, at present, the most potent technology to address rare cell analysis.

I explain how to work in the lab and emphasize that productivity is the most valuable quality a researcher should possess. Teaching has been a challenging endeavor; but in the end, I realize that I have empowered potential new researchers and I'm proud of that.

In your research journey, you've leveraged flow cytometry and continue to be an advocate for this technology. Can you share more?

Flow cytometry is fast and precise, and it allows the study of several dozens of proteins expressed at a single cell level. The study of rare cell populations is of growing importance. It's useful not only to understand disease mechanisms, but also to find novel targets. With multiparameter capabilities and a very high analysis rate, flow cytometry is, at present, the most potent technology to address rare cell analysis. When I first studied iNKT cell populations in HIV-positive patients, I had to deal with a more evident paucity of cells (iNKT cells are rarer in HIV, as HIV-positive people are immunocompromised). More than 20 million cells should be stained to find these rare populations among PBMCs. Until recently, no instrument was able to acquire 20 million events. Then the Invitrogen™ Attune™ NxT Flow Cytometer made my project reliable. The consequence of acquiring 20 million cells was how data should be managed, but powerful computers with many gigabytes solved this problem.

What is ISAC and what is it like being one of their scholars?

The International Society for Advancement of Cytometry (ISAC) aims to advance the impact of cytometry in the sciences. ISAC has a worldwide membership of more than 1,850 scientists. I became a scholar in 2016 and can network with top experts, strengthen my critical thinking and independent approach to science, and develop a solid foundation for my career. Thanks to the scholarship, I obtained the Italian National Scientific Habilitation to become Associate Professor.

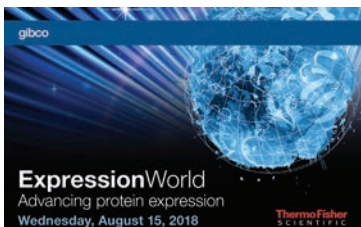
How will the work you've done impact the research community at large? What's the potential of applying flow cytometry technology to other areas of research?

The most recent immunotherapies use an approach that reshapes the body's own immune system to fight dangerous cancer cells that may have disguised themselves or put the brakes on the immune system. The recent immuno-oncology therapies are revolutionizing treatment in some patients with certain types of cancers. In tumors such as melanoma, the results have been even more impressive, but many patients unfortunately will develop progressive disease. Thus, it is important to understand immune checkpoint inhibitors and the molecular mechanisms that lead to the discovery of biomarkers that are likely to predict an individual's response to therapy.

As for last thoughts to leave us with—what do your potential findings mean in the long term, for those affected by HIV?

HIV patients with a relatively advanced age, e.g., more than 50 years old, can experience pathologies that affect much older non-HIV citizens. Chronic inflammation and immune activation, observed typically in elderly people and defined as “inflammaging,” can be present in HIV patients, who experience a type of premature aging. This relatively new condition is extremely complex. It is important to understand the role of inflammation and immune activation in HIV patients in order to design strategies that can support the most potent approaches.

EDUCATE YOURSELF

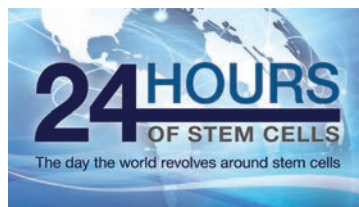


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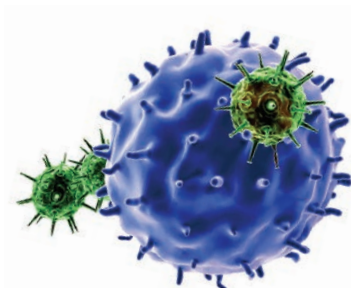
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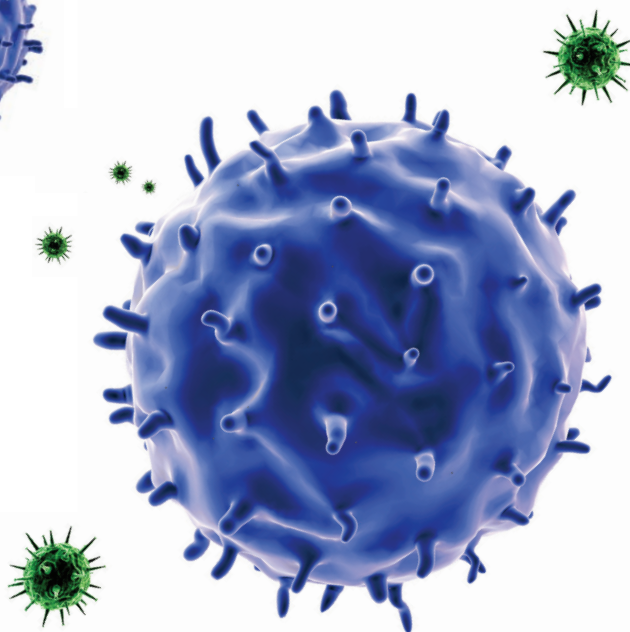
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4 STEPS TO ACTIVATE T CELLS



RETAIN *IN VIVO*-LIKE FUNCTION, WITHOUT FEEDER CELLS

Adoptive cell transfer (ACT) is a rapidly emerging immunotherapy approach in which a patient's own genetically engineered immune cells are used to attack and treat their cancers. The most promising type of ACT comes from chimeric antigen receptors (CARs), where a patient's T cells are harvested and scientists add a receptor that binds to a protein on the cancerous cells. This technique is possible thanks to extensive research performed on activation and expansion of T cells.

Here are four easy steps to activate T cells without using feeder cells, but with results similar to *in vivo* activated cells.

- 1 Count T cells to control the ratio of beads to cells. **Pro tip:** Counting cells is an important step to maintain reproducibility.
- 2 Isolate T cells from mononuclear cells or whole blood cells using a negative isolation method like the Invitrogen™ Dynabeads™ Untouched™ Human T Cells Kit. **Pro tip:** Your cells are not exposed to the stress of being passed through a column when you use a negative isolation method.
- 3 Transfer cells to desired cell culture medium. Add CD3/CD28 beads and mix gently. **Pro tip:** No need for antigen-presenting cells (APCs), mitogens, antigens or feeder cells. This technology outperforms traditional home-brew methods for generic activation (mitogens, ConA, soluble antibodies, etc.) and is well documented in the published literature.
- 4 Transfer to cell culture plate and incubate at 37°C in a cell culture incubator. **Pro tip:** Sit back and relax as your T cells get activated.

[thermofisher.com/activate](https://www.thermofisher.com/activate)

CRISPR IS AN IMPORTANT TOOL FOR T CELL MODIFICATIONS

Several T cell-related therapies are already in the clinic, including checkpoint inhibitors and CAR T cells. Since it allows scientists to precisely edit the genome, CRISPR is a valuable tool for T cell research. With CRISPR technology, T cells can be altered to improve their ability to destroy cancer cells. For best results, start with pure populations of T cells. Invitrogen™ Dynabeads™ bead-based kits highly enrich T cell populations without adding contaminants.

[thermofisher.com/dynabeads](https://www.thermofisher.com/dynabeads)





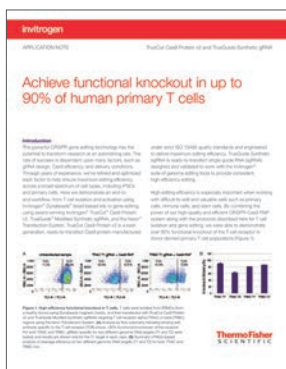
IMPACTING IMMUNITY AT THE GENETIC LEVEL

New ways to unlock the secrets of T cells

T cells are an important part of the adaptive immune system, and highly diverse T cell receptors (TCRs) allow the T cell to specifically recognize a wide range of antigens. Therefore, it is important to study TCRs in order to learn more about T cell diversity and specificity.

Sequencing and analyzing TCR genes, combined with the ability to specifically edit TCRs with CRISPR technology, will enable future T cell research. See how our synthetic biology solutions, from Invitrogen™ GeneArt™ Gene Synthesis to CRISPR-Cas9 genome editing products, are making a real difference with scientists who are trying to push the boundaries of working with T cells.

ACHIEVE UP TO 90% KNOCKOUT



CRISPR gene editing in primary T cells

T cells are emerging as important targets for cancer immunotherapy research. Obtaining high gene editing efficiency with CRISPR technology can be challenging, especially in primary cells. Compounding this challenge, primary cells are precious, and often difficult to obtain and expand. A new application note describes a complete solution for

gene editing in primary T cells from cell isolation to verification of edit. This includes T cell isolation and activation using Dynabeads bead-based kits to gene editing using Invitrogen™ TrueCut™ Cas9 Protein V2, TrueGuide™ Modified Synthetic sgRNA, and the Invitrogen™ Neon™ Transfection System.

Combining Cas9 protein with sgRNA for high editing efficiency

TrueCut Cas9 Protein V2 and TrueGuide Modified Synthetic sgRNA have significant advantages over plasmid-based systems. CRISPR plasmids remain in the cell for up to 72 hours posttransfection compared to less than 24 hours with protein. This leads to fewer off-target effects or instances of nonspecific cutting. Using this method allows researchers to bypass transcription and translation steps, speeding up the process.

thermofisher.com/tcellknockout



Interested in learning more about CRISPR genome editing?

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PARTNERSHIP FOR PROMISING SOLUTIONS



Peter Ebert, PhD
Scientist
Adaptive Biotechnologies

Tell us about your company; what goals you are trying to achieve?

We are, at heart, an immune receptor sequencing company. We do T cell and B cell receptor sequencing at very high throughput and we use the precision of the adaptive immune system to develop therapy for patients. More recently, we have a tie-up with the Microsoft™ Healthcare NEXt initiative to combine high-throughput immunosequencing with artificial intelligence, to map the entire human

immune system. This “antigen mapping” would allow for better diagnosis; for example, if you have a particular sequence in large numbers in your T cell repertoire, it might be an indication that you can have a reaction against a particular antigen, which could be valuable for diagnostic purposes.

How does GeneArt Gene Synthesis help with your research?

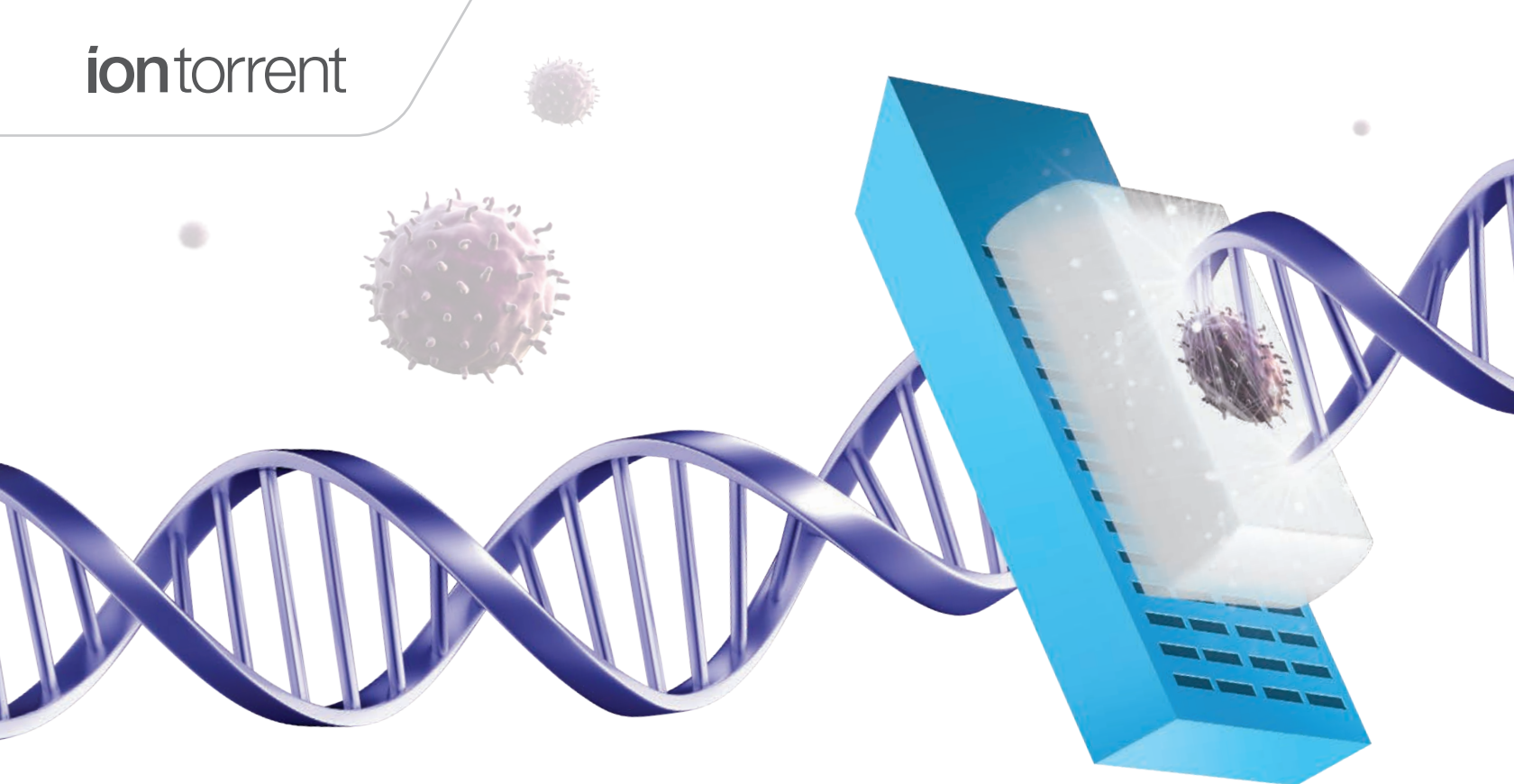
We do immune receptor sequencing at very high throughput and discover millions of sequences at a time, and the biggest challenge was validating these sequences at a pace that would not be a hold-up. We found a great partner in the GeneArt Gene Synthesis team, which gave us the ability to have those constructs made efficiently. We just make a list of sequences, hand it over to them, and then in about three weeks we get the final product. In addition, we also use the GeneArt subcloning services, as we need to shuttle

these sequences between series of vectors depending on the function. We then test the sequences in different systems, so that the sequences can eventually be transferred to the cells of interest. We have now evolved our bottleneck and therefore, having this convenient partner is really valuable.

What do you expect this partnership to bring in the near future?

Keeping up with the new technologies like genome engineering, TCR manipulating, and CRISPR reagents, is important to us. We have been pretty focused on the core set of technologies, but with an eye toward the future, we do dip our toes in newer technologies—however, these are all exploratory at this point. But then again, having a partner like GeneArt [Gene Synthesis] that brings the newer technologies to us and helps us to do something new in a quick time frame is quite valuable to us.





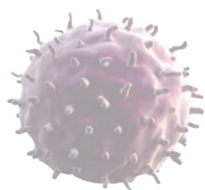
MULTIDIMENSIONAL NGS FOR IMMUNO-ONCOLOGY

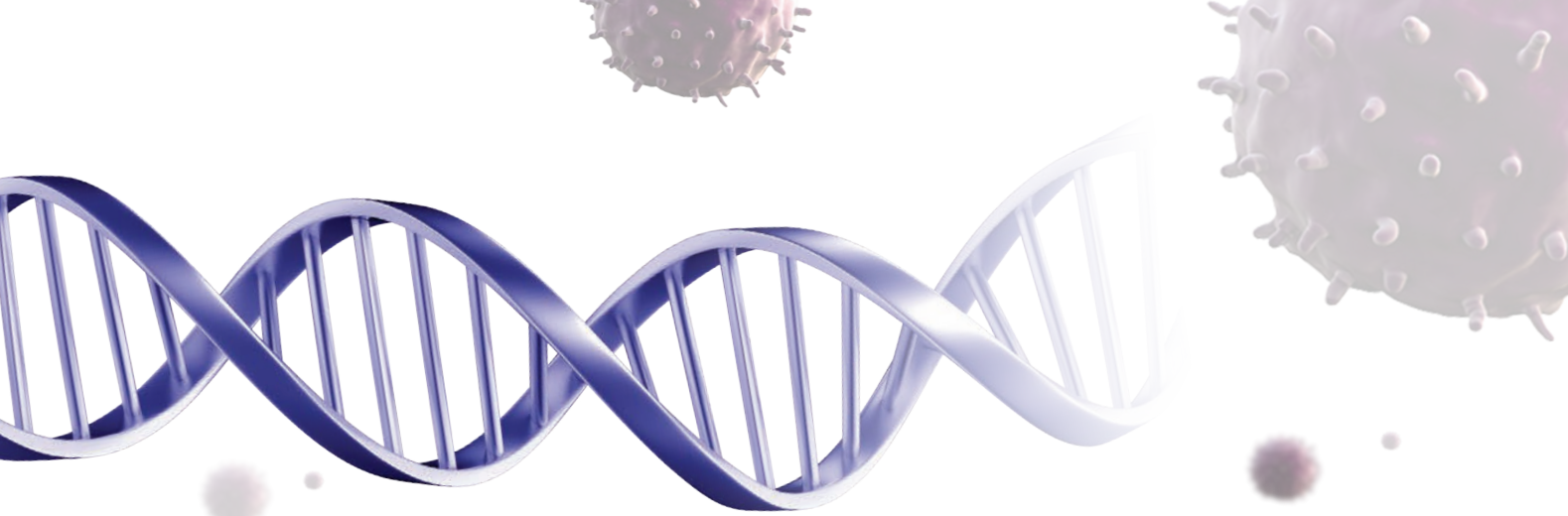
The latest technologies
for interrogating the tumor
microenvironment

Immunotherapy is now widely accepted as a key component of therapeutic strategies in oncology. Utilizing the body's own immune system to recognize, control, or potentially eliminate cancers is revolutionizing therapeutic concepts and changing the standard of care for cancer treatment. Moreover, immunotherapy has the potential to effect a "clinical cure," i.e., to convert cancer from a fatal disease into a non-life-threatening or chronic disease.¹ However, despite the current success of these therapies, not all patients respond to immunotherapy; and even those that do, often experience toxicities. Thus, there is a growing need to enhance our understanding of the mechanisms underlying the complex interactions between the immune system and cancer, and to identify predictive and

prognostic biomarkers of immune toxicity and immune response.

Tumors are now understood to be comprised of heterogeneous cell populations, including transformed cells and untransformed cells (such as stromal, endothelial, and immune cells), all with critical functions in the tumor microenvironment (TME). The complexity and heterogeneity of the interactions between the immune system and tumor cells, particularly in the TME, influences the clinical outcome for each individual tumor for every patient. The immune contexture, which is determined by the density, composition, functional state, and organization of the T cell infiltrate of the tumor, can yield information that is relevant to prognosis, prediction of a treatment response, and various other pharmacodynamic parameters.^{2,3}





Recent technological advances in high-throughput techniques, reviewed¹ by the Society for Immunotherapy of Cancer (SITC), are facilitating the discovery of novel biomarkers and are enhancing our understanding of the molecular mechanisms underlying cancer immunotherapies. Such research has the potential to refine our strategy for practical clinical applications of biomarker research and help guide the development of future personalized cancer immunotherapies.

The most studied biomarker in immunoncology is PD-L1 and its protein expression in the tumor microenvironment. Immunohistochemical (IHC) assays for PD-L1 are used as predictors of response to anti-PD-1/PD-L1 therapy. However, there are well-recognized limitations to PD-L1 as a predictive biomarker. Although PD-L1 expression is typically predictive of response to anti-PD-1/PD-L1 therapy, patients lacking PD-L1 expression may still demonstrate a clinical response. In addition, patients who are positive for PD-L1 expression often do not respond to therapy, further underscoring the limitations of the single-marker approach.²

Given the complexity of tumor-immune interactions, a multidimensional approach is required to get a comprehensive picture of the immune status of the TME. Thus, it may be optimal to perform a combination of assays in order to obtain different types of protein and molecular data for biomarker discovery.

Thermo Fisher Scientific offers next-generation assays for translational research to interrogate

the TME. These research solutions provide a detailed molecular portrait of tumors and their microenvironment.

The Ion Torrent NGS portfolio for immunoncology research encompasses four distinct assays that together offer a multimarker approach for better predictive modeling for stratification and selection of potential candidates for clinical trials.

ION TORRENT™ ONCOMINE™ IMMUNE RESPONSE RESEARCH ASSAY

This gene expression assay was designed for superior performance in the detection of low-expressing genes from minimal RNA input from FFPE samples. It targets 395 genes associated with immune response biomarker research.

ION TORRENT™ ONCOMINE™ TUMOR MUTATION LOAD ASSAY

This robust, targeted NGS assay provides clinical researchers with an accurate quantification of somatic mutation counts to assess tumor mutation load with very limited FFPE samples.

ION TORRENT™ ONCOMINE™ TCR BETA-LR ASSAY

This long-amplicon NGS assay is designed to

efficiently capture all three complementarity-determining regions of the TCR beta chain (CDR1, CDR2, CDR3) with high accuracy.

ION TORRENT™ ONCOMINE™ TCR BETA-SR ASSAY

This short-amplicon NGS assay specifically interrogates the CDR3 region of the TCR beta chain for characterization of the TME.

Learn more at

thermofisher.com/ngs-immuno-oncology

Our Analytical Validation Consulting Services portfolio has recently expanded to include Oncomine assays. View the list of panels at thermofisher.com/avconsultingservices

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1. Yuan J, Hegde PS, Clynes R et al. (2016) Novel technologies and emerging biomarkers for personalized cancer immunotherapy. *J Immunother Cancer* 4:3.
2. Taube JM, Galon J, Sholl LM et al. (2018) Implications of the tumor immune microenvironment for staging and therapeutics. *Mod Pathol* 31:214–234.
3. Fridman WH, Zitvogel L, Sautès-Fridman C et al. (2017) The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol* 14:717–734.

FIVE TIPS



Custom HRP Secondary Antigen Primary Alexa Fluor Secondary Primary Conjugated

FOR FINDING YOUR RIGHT ANTIBODY

Antibodies are used in a wide variety of research areas, and their performance and consistency have a direct effect on your results. Set yourself up for success by using these five tips to streamline your antibody selection.

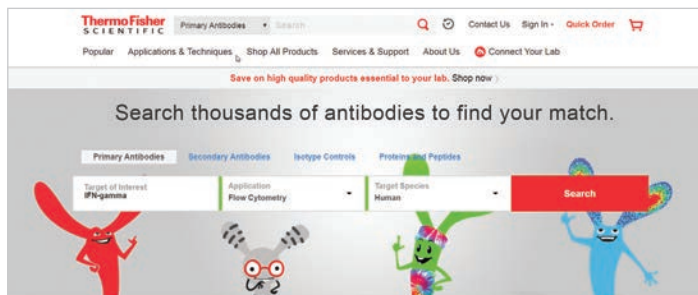
Explore over 85,000 antibodies at [thermofisher.com/antibodies](https://www.thermofisher.com/antibodies)

*The use or any variation of the word "validation" refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic uses.

TIP 1

BOOKMARK THE ANTIBODY SEARCH ENGINE

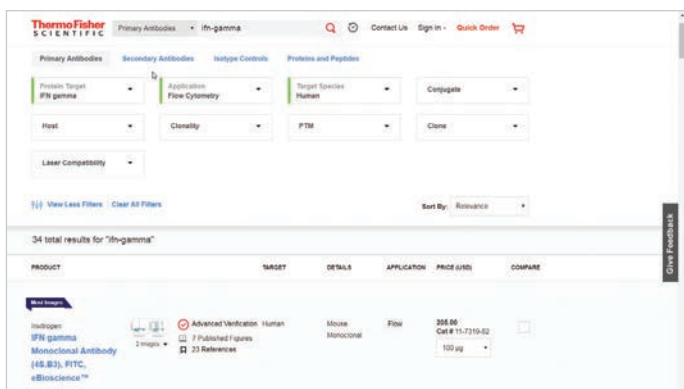
Enter your target of interest and narrow your results by application and target species based on your experimental needs.



TIP 2

REFINE SEARCH RESULTS WITH FILTERS

Modify and narrow results by antibody host species, clonality, posttranslational modification specificity, and other filters.



TIP 3

CONSIDER ANTIBODIES THAT HAVE BEEN VALIDATED*

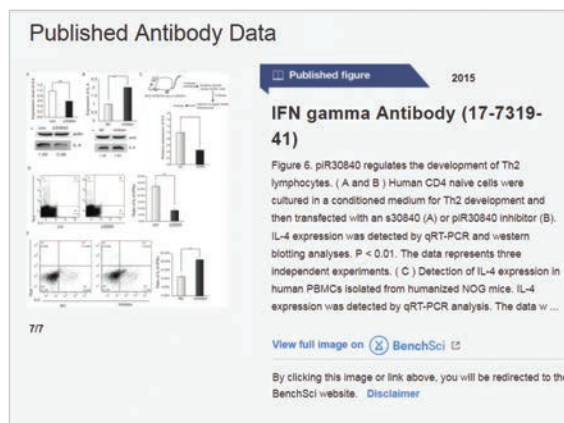
The Advanced Verification icon indicates that the antibody has been tested for both target specificity and for use with a particular application.



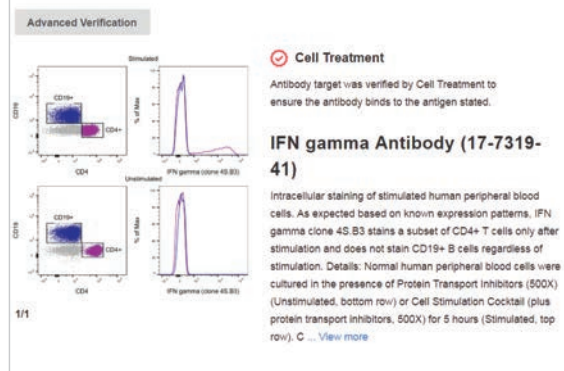
TIP 4

REVIEW ANTIBODY APPLICATION DATA FROM MANY SOURCES

Our published antibody data page, through a partnership with the BenchSci™ platform, provides over 50,000 images showing application figures from peer-reviewed journals, along with our own extensive antibody testing data.



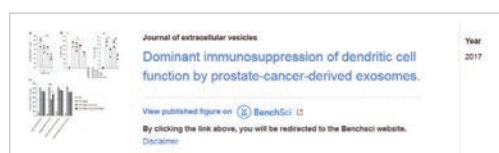
Antibody Testing Data



TIP 5

VERIFY YOUR ANTIBODY PERFORMANCE IN PUBLISHED ARTICLES

References, organized by application, provide links to citations in scientific journals.



FLUORESCENT DETECTION IN 3D CELL MODELS

Simple tools for studying complex biology in a physiologically relevant context

Traditional two-dimensional (2D) cell culture models lack many of the environmental conditions that cells experience within an organism. Their physical and biochemical setting is drastically different. For this reason, researchers have been turning to cell models grown in three dimensions (3D). These are commonly referred to as either organoid or spheroid systems. Organoids and spheroids show great potential in many applications, including drug discovery, toxicology, and disease modeling. These 3D cell models offer

opportunities to better understand complex biology in a physiologically relevant context.

Cells grown in 3D typically exist in a more biochemically relevant state and differ in morphology and function, but growing them is a large investment in time and resources.

We provide analysis tools that researchers already use with confidence in 2D culture. These tools can now be easily applied to the 3D model system to advance discovery at a faster pace.

ANTIBODY DETECTION

Immunofluorescence is an essential technique for the study of any disease state. It is typically done using a primary antibody to detect the desired protein within cells and a labeled fluorescent secondary antibody to provide detection via a fluorescent signal. Three-dimensional models are more dense than 2D models. They present new challenges to labeling and may need careful optimization. Common problems are increased background fluorescence and labeling heterogeneity. A solution to these issues is to label primary antibodies directly. Direct labeling reduces the complexity of optimization; multiple primary antibodies can be used together without problems of cross-reactivity, and nonspecific background issues are often reduced.

Your favorite primary antibody markers can be easily and rapidly labeled with Invitrogen™ Zip Alexa Fluor™ Antibody Labeling Kits (Figure 1).

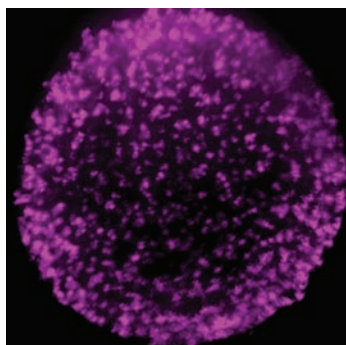


Figure 1. HeLa spheroid cells were stained with Ki67 antibody labeled with Alexa Fluor 647 (using the Zip Alexa Fluor 647 rapid labeling kit). Cells were imaged on a Thermo Scientific™ CellInsight™ CX7 LZR high-content analysis (HCA) instrument using confocal mode.

Access validated protocols and educational Resources to enable 3D model success!

[thermofisher.com/organoid](https://www.thermofisher.com/organoid)



VIABILITY, PROLIFERATION, AND HEALTH

Functionally, organoids and spheroids differ from 2D models because there are zones with varied levels of oxygen, nutrients, and metabolites. Apoptotic or necrotic regions are typically observed at the core, while zones of proliferative cells can be detected along the periphery. Simple live/dead assays can be performed to monitor the formation and health of organoids and spheroids (Figure 2).

Mitochondria play a central role in cell life and cell death. An increasing number of studies place mitochondrial dysfunction at the heart of disease. It is essential to be able to monitor mitochondrial health in the context of cell death in model systems (Figure 3).

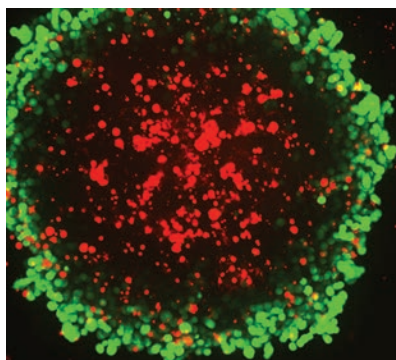


Figure 2. Confocal image of live spheroids of Alexa Fluor 594 cells stained with the Invitrogen™ LIVE/DEAD™ Cell Imaging Kit. Imaged on the CellInsight CX7 LZR HCA platform. Areas of dead cells are typically observed at the center of the 3D culture.

Confirming that 3D cell structures are maintaining the appropriate physiological morphology is paramount to reaching successful research outcomes. Our powerful imaging and high-content analysis platforms combined with our reagents are ideal for analyzing organoid and spheroid cultures (Figure 4).

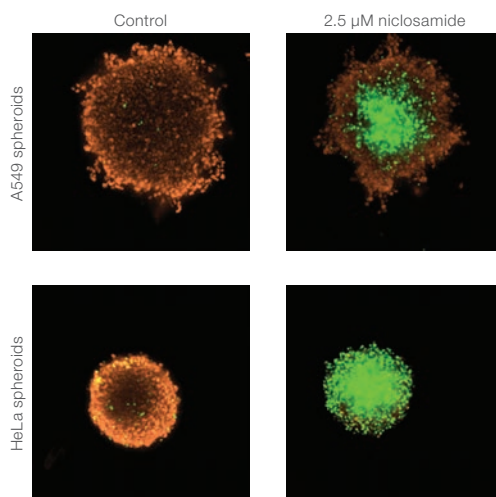


Figure 3. Monitoring mitochondrial health and apoptosis in spheroids. Spheroids were treated with different doses of niclosamide for 24 hrs, stained with Invitrogen™ MitoTracker™ Orange dye and Invitrogen™ CellEvent™ Caspase-3/7 Green Detection Reagent, and imaged with the CellInsight CX7 LZR HCA instrument. Increasing doses of niclosamide lead to depolarization of mitochondria (orange) and increase in apoptosis (green).

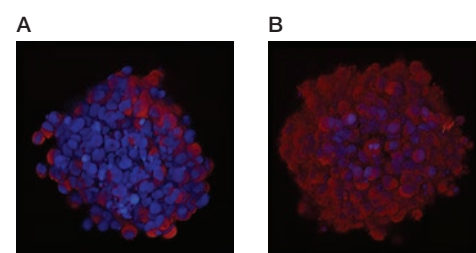


Figure 4. Spheroid staining using Invitrogen™ CellROX™ Deep Red Reagent. HeLa spheroids were (A) left untreated or (B) pretreated with 10 μ M menadione. Cells showing oxidative stress are stained red and live-cell nuclei are stained blue.

Imagine the possibilities - increased sensitivity and speed plus live-cell capabilities

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BUILDING IMMUNITY WITH THE RIGHT TOOLS

Worldwide, researchers are searching for ways to boost the immune system's ability to fight cancer; one such way is by developing novel therapeutics within the pharmaceutical/biopharmaceutical industry.

Technologies such as liquid chromatography and mass spectrometry (LC-MS) can provide insights into drug structure and activity at the cellular level, providing greater confidence in drug discovery, quality, and purity.

Recent developments in LC-MS hardware, software, columns, and sample prep offer a new paradigm in drug discovery—and LC-MS

techniques are being applied to unravel complex cascades of protein interactions that underlie disease states.

Peptide mapping is one way to measure critical quality attributes (CQA) required for the characterization of biotherapeutic proteins.



SMART SAMPLE PREPARATION

Characterize and quantitate proteins more quickly, easily, and reproducibly than ever before with simple sample preparation kits. Offering a significant improvement over in-solution protein digestion, Thermo Scientific™ SMART™ Digest Kits can typically digest monoclonal antibodies in less than an hour and are compatible with automation.

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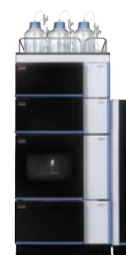


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WRANGLING THE MICROBES

Every day we are exposed to microbes that can cause infection and illness. The most frequent offenders are the germs that cause the common cold, food poisoning, and strep throat.

So how does the immune system keep these critters from invading our bodies and rendering us in a constant state of ill health? A high intake of Vitamin D helps modulate our immune responses. Antibodies are produced in response to an infection, seeking out and binding to an antigen on the offending microbe and preventing further replication. Our white blood cells (leukocytes) help protect us against infection too, with different types battling different targets. Our antimicrobial army is on the job.



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BEHIND THE LAB COAT:

MEET IMMUNOLOGY RESEARCHER CASSANDRA LYBECK



Cassandra Lybeck,
Master of Science in
Biochemistry candidate
at Ottawa Hospital
Research Institute

How did you end up here? Why did you become a scientist? What drew you to this field?

Growing up in a small town in rural Saskatchewan, I didn't really know what a scientist was, so it wasn't always what I pictured myself doing. Every year in grade school we attended the annual science fair. One year my partner and I came up with a research-based project looking into what the difference was between gas and diesel engines. We ended up making it to regionals and winning a Bright Minds award. This was probably the first experience that drove me in this direction.

Choosing a major in university was a challenge because I'm interested in almost everything—so rather than jumping right in after high school, I joined the military as a signal operator. My favorite subject was radio theory, and I really enjoyed creating improvised antennae to propagate different radio frequencies. I knew at this point that I was going to be a scientist, but I was not sure what field I wanted to study.

What study path did you take to get into this field?

My path was not very typical; I changed my mind many times. After coming out of the military, I enrolled in geology. It seemed like a great choice at first, but I didn't find it very interesting and did horribly. It was very discouraging, until I started taking biology and chemistry classes. Eventually, I transferred into biomedical sciences and this led into graduate studies in biochemistry. Now I study a specific adenovirus protein, and how it is regulated during infection.

What is a typical day like in your lab?

Our lab studies the biology of adenoviruses, as well as adenovirus-based vectors for gene therapy. We explore oncolytic applications, as well as investigating potential treatments for neuromuscular dystrophies. To study how adenovirus replicates in cells, we infect them and use a variety of different molecular tools to measure its growth (or lack thereof). We create adenovirus-based vectors by removing the genes that are responsible for efficient replication, and we use different cellular or animal models (depending on the application) to look at the therapeutic impact.

Do you have a memorable breakthrough moment or favorite memory in the lab?

At the start of my project, I had to create and optimize a protocol that would allow us to use affinity purification to isolate an adenovirus protein

that contained an epitope tag. The whole process revolved around infecting cells, lysing them in such a way to preserve protein–protein interactions, and then determining how much bait protein was needed to visualize this on a silver stain. Using this protocol, we were able to purify this adenovirus protein and identify interacting partners using mass spectrometry. Once we identified novel targets, I was able to look into epigenetic regulation, and this is what I'm working on now.

Why is your research important? What are the possible real-world applications?

Studying basic science in a hospital setting has its challenges, primarily because it can be hard to see the clinical relevance. I study a very specific adenovirus protein and what is happening to it during late infection times: is it being modified? Are there important novel interactions taking place? Looking at the epigenetics can tell us how this protein is being regulated and what that means in terms of virus replication. Adenovirus is common and ubiquitous in nature; it's found worldwide and infects almost every vertebrate. Since it usually results in the common cold, it doesn't pose a threat to most healthy individuals; but certain serotypes can cause debilitating illness and mortality, especially in individuals whose immune system is compromised or developing. Understanding how the virus works gives us a better insight into how to treat it. In addition, we can potentially take this knowledge and apply it to other pathogens that may behave in the same way.



You obviously enjoyed science right from the beginning, but you must have met challenges along the way. Do you have any advice for junior scientists facing challenges?

I think it's common for young scientists to doubt what they have accomplished, especially if they compare themselves with others. Imposter syndrome is such a real thing, and something that I used to struggle with (and sometimes still do). My advice would be that we are all good enough and worth it. Give yourself a goal and try to follow through. You can only do your best, so if you are having a hard time with your project or a difficult experiment is bringing you down—don't let it discourage you. Failure is an important part of learning, but it doesn't mean that we are inadequate. Just remember, we are all trying to make sense of this amazing, complex, beautiful world we live in.

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