life IN THE lab

PRODUCTS, INFORMATION, AND SCIENTAINMENT

ISSUE 17 | 2017

Illuminating the immune system

thermo scientific | applied biosystems | invitrogen | gibco | ion torrent

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* See page 22 for full details regarding validation.

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10 STEPS TO FLAWLESS PIPETTING

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2. Work at temperature equilibrium
3. Examine the pipette tip for droplets
4. Select forward or reverse pipetting technique based on liquid speed
5. Pause consistently
6. Remove pipette straight from vessel
7. Minimize handling of pipette and tip
8. Use the correct immersion depth
9. Use the correct pipette tip
10. Use consistent plunger force and speed

View all details in the Good Laboratory Pipetting guide at thermofisher.com/glp

INSIDE IMMUNO-ONCOLOGY RESEARCH

Immuno-oncology (I-O) is considered by some to be one of the most promising anti-cancer therapeutics.[1] In general, I-O manipulates the body's own immune response to mount an attack against tumor cells.

Recent breakthroughs in I-O research translate into a paradigm shift with regards to attacking cancer.[2] Advances in I-O results in long-lasting tumor regression where surgery, radiotherapy, chemotherapy, and targeted therapy had proved less effective previously.[3, 4, 5] Two of the hottest areas for I-O research today are checkpoint inhibitors and chimeric antigen receptor (CAR) T cell therapy. The two models are wildly different and both offer unique advantages.

Regulating immune checkpoints to empower the immune system

Immune checkpoints are cell pathways crucial in maintaining a normal immune response and protecting tissues from damage when the immune system is activated. Tumor cells dysregulate immune checkpoints and use it as a mechanism of immune resistance. Understanding immune checkpoints in natural killer (NK) cells and T cells is a main focus of research as these cells regulate both the adaptive and innate immunity surrounding the tumor (Figure 1).

Immunity is maintained through a combination of cells interacting with their environment. T cell–mediated responses are among the best studied because they:

- are involved in a multitude of immune functions within the tumor environment,
- are highly regulated by both stimulatory and inhibitory signals,
- mediate immunity through membrane receptors and secretory factors that are considered to be easily druggable. Other immune cells such as NK cells and B cells are also being studied for anti-cancer receptor targets.

There has been dramatic progress in recent years in the advancement of checkpoint inhibitors that are able to direct and regulate T lymphocyte proliferation and function. These inhibitors interfere with the signaling pathways that can activate or inhibit cell events to directly or indirectly modulate the activity of immune cells. Only a small fraction of immune checkpoints are being characterized and developed for therapeutic purposes. CTLA-4 was one of the first immune checkpoint pathways to be targeted by therapeutic antibodies and achieved US Food and Drug Administration (FDA) approval. Other checkpoint pathway targets are being developed and include: PD-1, LAG-3, KIR, and OX40. Integration of multiple pathways ondifferent cell types can provide a more successful chance to shrink the tumor size and provide long-term immunity against cancer.

Chimeric antigen receptor (CAR) T cell therapy

Many I-O therapeutics are mass-produced antibody or fusion proteins. Chimeric antigen receptor (CAR) T cell therapy breaks that mold by being a modified patient cell that acts as an anti-cancer therapeutic (Figure 2). CAR T cell therapy utilizes genetically modified T cells to attack and kill the cancer cells. T cells do not normally identify cancer cells as foreign bodies, which would mark them for destruction. For immunotherapy to work, T cells are genetically manipulated to enable them to identify the cancer cells as an enemy and kill them as they would other foreign bodies. These T cells are genetically modified with CARs containing antibody-based recognition domains directed against cell-surface antigens linked to intracellular signaling sequences to overcome the tumor's tolerance.[5] Once created, these modified cells are reintroduced into the patient with either a chemotherapy or other anti-cancer drugs. CTL019 is an example of a successful CAR T cell therapy as it is a patient-derived T cell engineered to express anti-CD19 single-chain variable fragment to shrink indicated B cell malignancies. These findings are encouraging as the field of personalized medicine develops toward mainstream therapy. The question still remains if CAR T cell therapy can be used to effectively treat a wide array of solid tumor types.

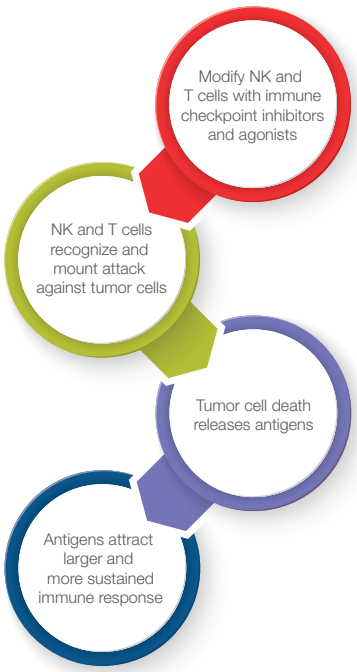


Figure 1. Immuno-oncology therapeutics harness the body's normal immune response to regress tumors.

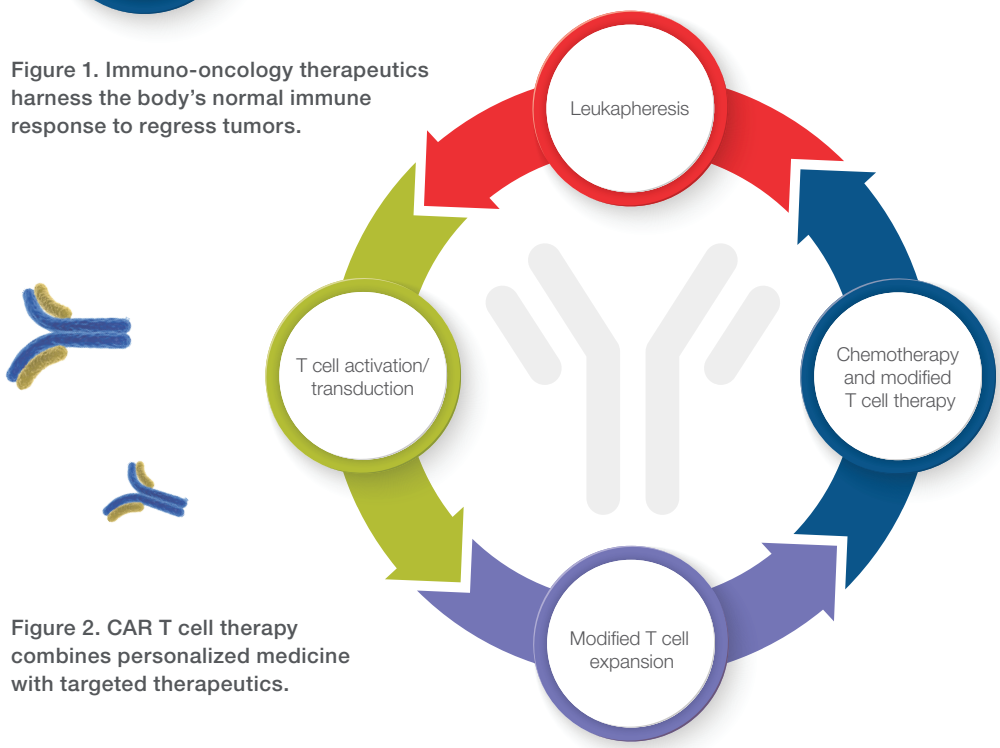
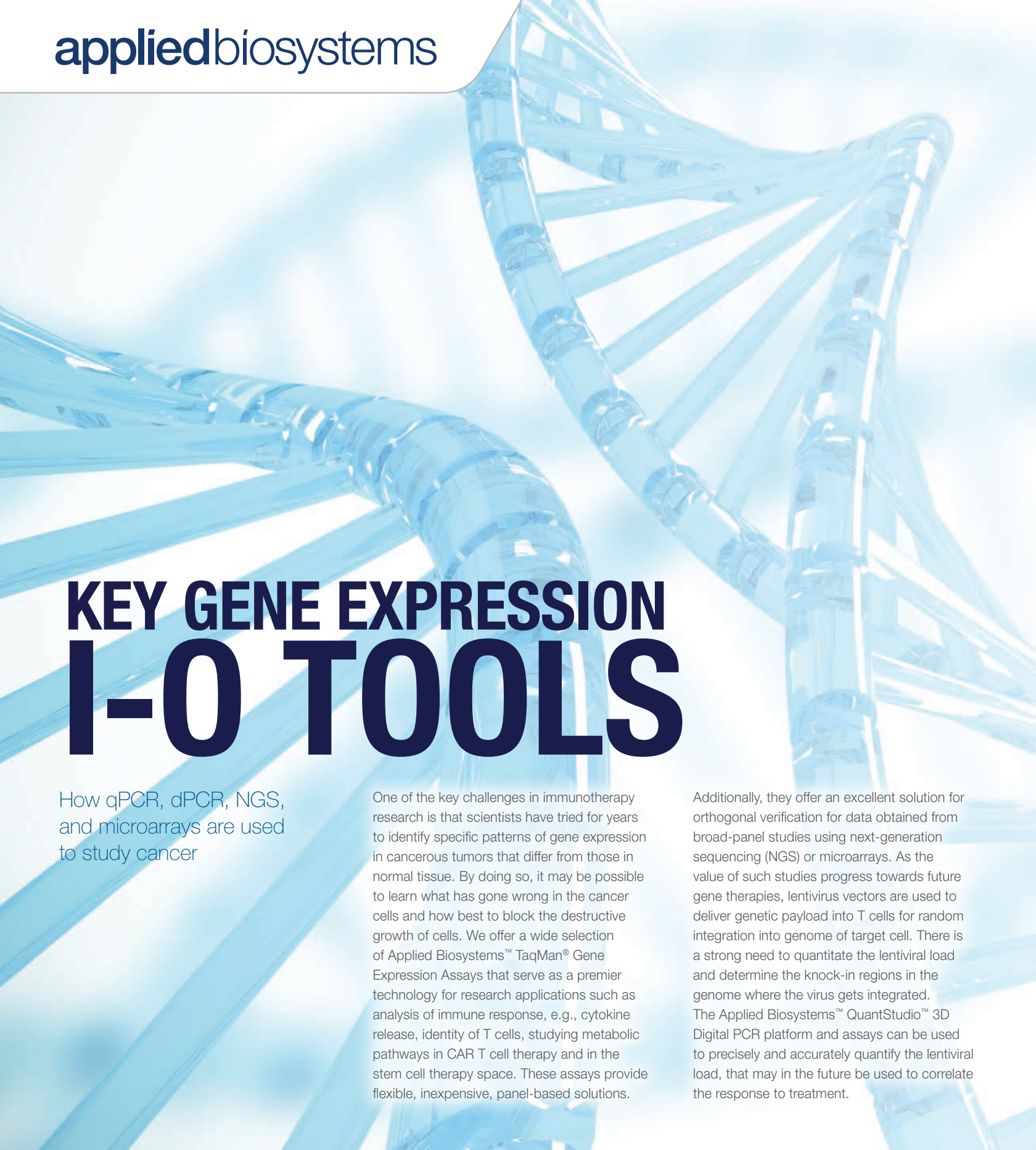


Figure 2. CAR T cell therapy combines personalized medicine with targeted therapeutics.

References:

1. What is cancer immunotherapy? Impacting all cancers / Melanoma. Cancer Research Institute. http://www.cancerresearch.org/cancer-immunotherapy/impacting-all-cancers/melanoma (last updated October 2015).
2. FDA approves Opdivo to treat advanced form of kidney cancer, FDA News Release (November 23, 2015). http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm473971.htm
3. Corrigan-Curay J, Kiem HP, Baltimore D et al. (2014) T-cell immunotherapy: Looking forward. *Mol The* 22:1564–1574.
4. Grupp SA, Kalos M, Barrett D et al. (2013) Chimeric antigen receptor–modified T cells for acute lymphoid leukemia. *N Eng J Med* 368:1506–1518.
5. Batlevi CL, Matsuki E, Brentjens RJ et al. (2016) Novel immunotherapies in lymphoid malignancies. *Nat Rev Clin Oncol* 13:25–40.
6. Seton-Rogers S (2016) Immunotherapy: Two antigens are better than one. *Nat Rev Cancer* 16:128–129.
7. CAR T-Cell Therapy: Engineering Patients' Immune Cells to Treat Their Cancers. (2014) National Cancer Institute.



KEY GENE EXPRESSION I-O TOOLS

How qPCR, dPCR, NGS, and microarrays are used to study cancer

One of the key challenges in immunotherapy research is that scientists have tried for years to identify specific patterns of gene expression in cancerous tumors that differ from those in normal tissue. By doing so, it may be possible to learn what has gone wrong in the cancer cells and how best to block the destructive growth of cells. We offer a wide selection of Applied Biosystems™ TaqMan® Gene Expression Assays that serve as a premier technology for research applications such as analysis of immune response, e.g., cytokine release, identity of T cells, studying metabolic pathways in CAR T cell therapy and in the stem cell therapy space. These assays provide flexible, inexpensive, panel-based solutions.

Additionally, they offer an excellent solution for orthogonal verification for data obtained from broad-panel studies using next-generation sequencing (NGS) or microarrays. As the value of such studies progress towards future gene therapies, lentivirus vectors are used to deliver genetic payload into T cells for random integration into genome of target cell. There is a strong need to quantitate the lentiviral load and determine the knock-in regions in the genome where the virus gets integrated. The Applied Biosystems™ QuantStudio™ 3D Digital PCR platform and assays can be used to precisely and accurately quantify the lentiviral load, that may in the future be used to correlate the response to treatment.

TAQMAN GENE EXPRESSION ASSAYS AND TAQMAN FAST ADVANCED MASTER MIX GENERATE ACCURATE DATA QUICKLY AND MORE EASILY THAN OTHER METHODS

TaqMan Gene Expression Assays are available for many immuno-oncology targets such as CD274, PDCD1, CTLA4, IFNGR2. Experience the convenience of these ready-to-use assays, with no optimization required. Other benefits of TaqMan Assays include:

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Quality—high-quality manufacturing for reproducible results from lot to lot

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I-O SEQUENCING & ASSAYS

Identify new biomarkers—whole transcriptome, targeted panels, individual transcripts

While there have been successes in immunotherapy research, there have also been challenges. One key challenge is the variable responses between different immune systems. This makes identification of new biomarkers associated with response critical for further advancements in immuno-oncology. With Thermo Fisher Scientific you can utilize formalin-fixed, paraffin-embedded (FFPE) research samples for biopsy, perform simple data analysis, and get results in <2 days.

EXPERIENCE THE DIFFERENCE

- Discover new tumor antigens with whole-transcriptome analysis
  - Deep coverage from as little as 100 pg RNA with Applied Biosystems™ Clariom™ D Pico Assays
  - Gene-level coverage with Ion AmpliSeq™ Transcriptome Solution, Applied Biosystems™ Clariom™ S Pico Assays
- Measure the expression of gene panels with targeted analysis
  - Genes involved in tumor-immune system interaction with Ion Torrent™ Oncomine™ Immune Response Research Assay
  - Select your genes of interest with Ion AmpliSeq™ RNA Research Panels
- Potential future routine testing for individual targets
  - Industry gold-standard TaqMan and capillary electrophoresis (CE) assays

SELECT YOUR GENE PANEL IN MINUTES

Ion AmpliSeq RNA Research Panels

Provide a cost-effective alternative to nontargeted whole-transcriptome analysis.

Choose from eight predesigned research panels, or edit the gene list of any panel to create a custom-targeted panel from a database of >20,000 human RefSeq genes.

Ion AmpliSeq Transcriptome Human Gene Expression Kit

Fast and easy gene-level expression analysis starting with as little as 10 ng RNA, FFPE compatible.

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INTERROGATE THE GENES CRITICAL TO IMMUNE RESPONSE

Oncomine Immune Response Research Assay

Enables immunotherapy research with the quantitative evaluation of the expression of markers associated with different leukocyte subsets, antigen presentation, TCR signaling, checkpoint pathways, and tumor progression.

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EXPAND THE POTENTIAL TO FIND NEW, ACTIONABLE BIOMARKERS

Clariom D Pico Assay

Comprehensive coverage of >540K transcripts including lncRNA and alternative splicing events, allowing translational scientists to generate biomarker signature quickly and easily.

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OPTIMIZE ANTIBODIES FROM THE START

Synthetic biology solutions provide successful antibody research

Over the past 40 years, research using monoclonal antibodies (mAbs) have become powerful tools in the fight against cancer. As the interest in using mAbs grows, the need to fully understand the dynamics of cancer cells and the immune system becomes essential. Whether studying overexpression or mutations of potential targets, iterative studies for the selection of antigen targets and optimization of antibodies is becoming more of a necessity. Synthetic biology enables almost any design vision possible to obtain proteins with the variation and characteristics you need for your research advancements.

ENGINEER YOUR ANTIBODY
USING DIRECTED EVOLUTION

Mutation, selection, reproduction—repeat this for millions of generations—every biologist is familiar with how evolution has resulted in the incredible diversity and adaptations found in the natural world. Directed evolution uses a similar strategy to create biomolecules for use in diverse fields spanning industrial production, medical science, and basic research. The crucial difference is that, with directed evolution, results can be achieved much more quickly, in many cases with just a few rounds of mutagenesis and selection.

Take a look at our Behind the Bench blog post on directed evolution methods: thermofisher.com/directedevolutionblog

Invitrogen™ GeneArt™ Directed Evolution services include combinatorial library construction services using trinucleotide mutagenesis (TRIM) technology with full control over amino acid distribution and fewer out-of-frame mutations than traditional randomized libraries.

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EXPRESSION WORLD
ON-DEMAND RECORDING

Did you miss Gibco™ ExpressionWorld? Check out this recording from Stephan Fath, Manager of Manufacturing of Invitrogen™ GeneArt™ Gene Synthesis at Thermo Fisher Scientific.

He describes a holistic approach as the most efficient way to streamline your gene-to-protein workflow for maximal performance, either by purchasing reagents for in-house work or by outsourcing the complete workflow to a highly customized service offered by the Invitrogen™ GeneArt™ Protein Team.

Our proprietary Invitrogen™ GeneArt™ GeneOptimizer gene sequence optimization algorithm improves gene sequence via multiple parameters. To further maximize protein yield, researchers can take advantage of our best-in-class serum-free Gibco™ FreeStyle™ or Expi293™ and ExpiCHO expression host systems.

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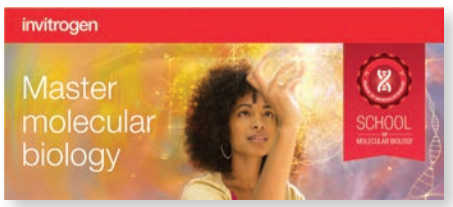

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FREE MOLECULAR BIOLOGY HANDBOOK

Find solutions to help improve critical steps of your molecular biology workflow in immuno-oncology research. Download this free molecular biology handbook—packed with tips, science facts, educational resources, and new product information.

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IT'S ALL IN THE DNA

Nucleic acid purification is an important step in your immunology research. Choosing the right tools provides you with the best outcome.

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KingFisher instruments help reduce your hands-on time while maintaining high yields and excellent reproducibility. This instrument comes in two formats depending on your throughput needs. Each system comes with an easy installation, optimized protocols, and Thermo Scientific™ BindIt™ software for customized protocols. KingFisher instruments are also optimized to work with Applied Biosystems™ MagMAX™ kits, a range of magnetic-bead based reagents that purify high-quality DNA from a range of sample types and input volumes.

KINGFISHER FLEX PURIFICATION SYSTEM

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- High-throughput purification of 96 samples per run
- Four different magnetic head formats for wide volume range (20–5,000 µL)

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KINGFISHER DUO PRIME PURIFICATION SYSTEM

- Flexible configuration includes both 6-pin and 12-pin magnetic heads to process different sample numbers and volumes
- New or edited protocols can be uploaded with a USB drive
- UV lamp for decontamination and optional barcode reader provide enhanced usability

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JUST FOR FUN

WHAT'S YOUR IMMUNE PICK-ME-UP?

While we're focused on the immune system, let's talk about what you can do to give yours a little boost.

Just when you're at your busiest, you get a scratchy throat and it can only mean a virus is on its way to getting in the way of your deadline.

Everyone has their go-to remedy for stopping that in its tracks. These are our favorites and a delicious snack at all times. Share your favorites with us on social media #fueltheimmunesystem

GARLIC—it contains sulfur compounds that aid the immune system.

VITAMIN C—everyone's go-to are citrus fruits like oranges, but did you know kale and red peppers have a high amount of vitamin C?

EGGS—the yolks are a great source of vitamin D, which helps regulate the immune system.

ALMONDS—these popular snacks are packed with vitamin E, which is important in maintaining a strong immune system.

CHICKEN SOUP—this is a favorite when you are sick, unless you're a vegetarian. It contains vitamin B6, which is vital in the production of new blood cells.

UNCOVERING
CHECKPOINT BIOMARKERS

An interview with
Lisa Butterfield, PhD

Professor of Medicine, Surgery and Immunology

Director, UPCI Immunologic Monitoring and Cellular Products Laboratory, University of Pittsburgh

Cancer cells cloak their identity to avoid detection from host defense cells by exploiting genes important to differentiate between self and non-self. Though cells in a tumor mass may go undetected by the immune system, their presence leaves behind molecular clues. Researchers like Lisa Butterfield are sleuthing using archived blood and tumor samples to find biomarkers and capture the presence of these tumor cells.

What is the role of the research laboratory in clinical trials? What is the role of your biomarker core facility in clinical trials?

I direct a research laboratory and a cancer center core facility for the immunologic monitoring and development of cellular products. Initially, we support bench-to-the-bedside development by performing translational research and development. Once the clinical trial is rolling, we process and bank both blood and tissue samples in a standardized fashion. Immunological assays are used to elucidate the drug mechanism of action in these clinical research samples and find potential prognostic and predictive biomarkers.

Our core is useful for clinical trials as we have standard operating procedures in place. Competency training is paramount for everyone who touches clinical research samples. This means all samples are processed the same way to minimize variables, reduce error and noise, and to maximize meaningful signal. We also have a large bank of healthy donor serum. While each subject can serve as their own control when we are looking for a change over baseline during time course studies, we also have a big collection of healthy, noncancer donors that are used as a comparison to cancer subjects, giving us an accurate baseline to know what is normal and what is not.

How does a group start the process for biomarker validation?

After discovering a potential biomarker, there is a roadmap to standardization and validation. The Society for Immunotherapy

Cancer (SITC) released open-access white papers outlining the analytical and clinical validation of immunotherapy biomarkers. There are 5 white papers and a meeting summary report, all found here on the society's website: **sitcancer.org/research/biomarkers**

How did your core facility become involved with the SARC028 clinical trial?
SARC028 is the first clinical study of PD-1 blockade in sarcomas. The SARC cooperative group and their clinical leads, Drs. Hussein Tawbi and Melissa Burgess, were responsible for starting and co-ordinating this clinical trial. Our collaboration formed to standardize handling and banking of blood samples in order to look for mechanisms of action and biomarkers of the immune-tumor response.

Let's set the stage for the SARC028 clinical trial. Can you give us the background for the study?
In a sense, PD-1 upregulation on lymphocytes is an activation marker. T cells upregulate their expression of PD-1 when exposed to antigen, and that includes tumor antigen. Tumors across a spectrum can upregulate PD-ligand 1 (PD-L1) and express it on the surface of both the tumor cell and myeloid cells in the tumor. PD-1 upregulation and binding to PD-L1 induces T cells to reduce their activity or shut them off. Antibodies that block the PD-1 pathway are designed to interfere with downregulation of T cell activity and allow them to do a better job of eradicating the tumor.

What role did PD-L1 play in the SARC028 trial?
The role of PD-L1 had not yet been investigated in sarcomas. However, it has been hypothesized that patients with higher levels of PD-L1 in tumors are more likely to respond to therapy. The investigators recruited a diverse set of sarcoma patients to test which subset would most likely respond to PD-1 therapy. They collected and analyzed tumor samples for the correlation between PD-L1 expression and positive response to therapy. My group was responsible for testing serum samples to look for generation of tumor antigen–specific immunity and novel prognostic markers to better understand which patients would respond to therapy.

> "Our goal is to identify a robust biomarker, then create a panel to test for which patients would benefit from PD-1 blockade."

SARC028 found some subgroups of sarcoma patients did not benefit particularly well from the PD-1 pathway blockade. However, there were subsets of patients who did benefit. We found from our biomarker testing that there were novel correlations between soluble PD-1 and PD-L2, and clinical outcomes. Some of our other investigations found possible soluble factors markers, such as IL-15, which correlated with a positive anti-tumor kind of immune milieu in the serum. Our goal is to identify a robust biomarker, then create a panel to test for which patients would benefit from PD-1 blockade.

Can you talk about liquid biopsy and what are the challenges with liquid biopsy?
Finding checkpoint biomarkers from tumors is difficult because the biopsy is an invasive procedure. Ideally, the best tumor samples are taken at points where standard of care biopsies and surgical resections occur. Repeatedly taking a solid tumor sample at different times in the trial is a challenge. Blood samples are more attractive as collection is minimally invasive, can be drawn at any time point and in a reasonable amount. Serum can be aliquoted and easily stored at –80˚C for future testing.

How are the ProcataPlex panels* for the Luminex platform applied in the SARC028 trial?

Multiplex measurement of soluble forms of the immune checkpoint receptors and ligands is novel. There have been only a few preliminary suggestions that the soluble forms play a role and this is the first evidence that measuring these soluble factors correlates with treatment in these sarcoma patients, and may have clinical utility to potentially predict who might benefit from a given therapy.

Detection of 65 cytokines, chemokines, and growth factors in a single Luminex® assay was also a favorable trait as it combines many analytes into a single assay and with small sample volumes. This diversifies the use of Luminex-based immunoassays for broad biomarker discovery and validation rather than only for testing a specific hypothesis.

What do you think is the future of biomarkers in immuno-oncology research?
An important evolution is taking place. Traditionally, we only had easy access to blood and not the solid tumor, and we were also investigating very small numbers of good clinical responders. After years of looking in the blood, there were not many robust biomarker signals from those methods and samples, from the few clinical responders. Advancements in biotechnology and pharmaceutical fields provided new technologies and research is to now examine both tumor and blood samples for markers from the much higher number of clinical responders who are receiving these more effective drugs. We can now better understand what is happening in the tumor and this can then be applied to finding those signals and biomarkers in the blood.

* For research use only. Not for use in diagnostic procedures. To learn about Immuno-Oncology Checkpoint 14-Plex and Immune Monitoring 65-Plex Human ProcartaPlex™ Panels, go to thermofisher.com/procartaplex

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Optimisation of UCB's Transient Expression Platforms

To meet the challenge of developing antibody therapeutics, UCB utilizes several different transient platforms. Through continuous optimization, streamlining, and automation of component parts, the UCB panel of platforms (utilizing both HEK293 and CHO host cells) has the capability to produce microgram to gram quantities of panels of purified antibodies and antibody fragments in as little time as 4 weeks from receipt of plasmid DNA.

Christina Gordon
Scientist, Protein Services
UCB Pharma

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Stabilization of Native & Functional Membrane Proteins for Drug Discovery

CALIXAR has developed an innovative approach to isolate and stabilize therapeutic membrane protein targets such as GPCRs, ion channels, and transporters. They partnered with Thermo Fisher Scientific to help tackle the production and characterization of these very promising and druggable targets to provide a new hope for the development of more accurate drug discovery.

Anass Jawhari
Chief Scientific Officer
CALIXAR

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EVERYDAY HERO Ameet Chimote, PhD

Department of Internal Medicine,
Division of Nephrology,
University of Cincinnati

The challenge: I have been working as a postdoctoral research associate in Dr. Laura Conforti's laboratory (Department of Internal Medicine, University of Cincinnati) for the past 7 years. The main research focus of our laboratory is to study the role played by the K^+ ion channels in the function of T lymphocytes and their implications in cancer. Previously, I had shown *in vitro* that exposure to hypoxia and adenosine (pathological conditions in solid tumors) inhibited these ion channels. The next logical step for me was to evaluate the expression and function of these ion channels in solid tumors. I was fortunate to avail the resources of University of Cincinnati Cancer Institute's Tumor Bank in procuring surgically resected solid tumor samples from head and neck cancer patients. However, I had no experience in isolating T cells from solid tumors; furthermore, another challenge was that I could not isolate the tumor-infiltrating lymphocytes (TIL) by enzymatic dissociation because I needed to preserve the T cell function.

The solution: Try, try, and try, till you succeed! Under the guidance of my mentor, I "troubleshooted" protocols for isolating TILs based on published procedures in literature and consulted with technical support from companies until I could successfully isolate fully functional T cells from surgically resected head and neck tumors without using any enzymatic dissociation. The cell number I would isolate would vary from patient to patient; but for all of the samples the cell number of the infiltrated lymphocytes was much lower than what I expected. I learned to work with that small cell number and I was able to measure Ca^{2+} fluxes in these cells by flow cytometry and also phenotype them. To complete the "story", I did immunofluorescence staining for ion channel expression and functional markers in sections from these tumors.

Next steps: This was my first foray in the realm of "Translational Research" and I enjoyed the experience very much. Currently there is a lot of interest in studying ion channels in cancer T cells as potential targets for cancer immunotherapy. I want to build up on my experience and technical expertise gained from this project and continue to investigate ion channels in cancer T cells and decipher whether any "defects" in these ion channels can lead to the lack of immune response in cancers.

Learn more about the Conforti Lab:
med2.uc.edu/conforti/

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GO BEYOND LIMITATIONS

Tools and technologies that revolutionize the way you pipette

A connected lab is a workplace that leverages modern enhancements, like cloud technology, to improve the way scientists do everyday tasks. The pipette, the lab's familiar workhorse, has evolved and risen to the challenge, alongside tools and technologies that help you to do more, better, faster, and with reproducible results. These upgrades empower researchers to extend the value of their time and expertise by swiftly and securely sharing work and insights with colleagues around the world.

A growing number of labs are crossing the chasm from manual to electronic pipettes, opening up a host of exciting opportunities to "get connected" and drive increased efficiency, consistency, and collaboration among researchers. From innovative pipetting systems to web-based apps that connect us to a realm of new possibilities, to advancing pipetting skills and techniques for improved accuracy, revolutionizing pipetting is a tall order to fill. Get started now.

A NEW ERA OF PIPETTING–GETTING CONNECTED WITH MY PIPETTE CREATOR

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THE MY PIPETTE CREATOR PROTOCOL LIBRARY

To further extend the benefits to your pipetting process, My Pipette Creator features access to the Protocol Library, an ever-expanding collection of validated protocols designed specifically for our assays and reagent kits. These pre-programmed protocols are available in the app for downloading and sharing with connected pipettes and colleagues.

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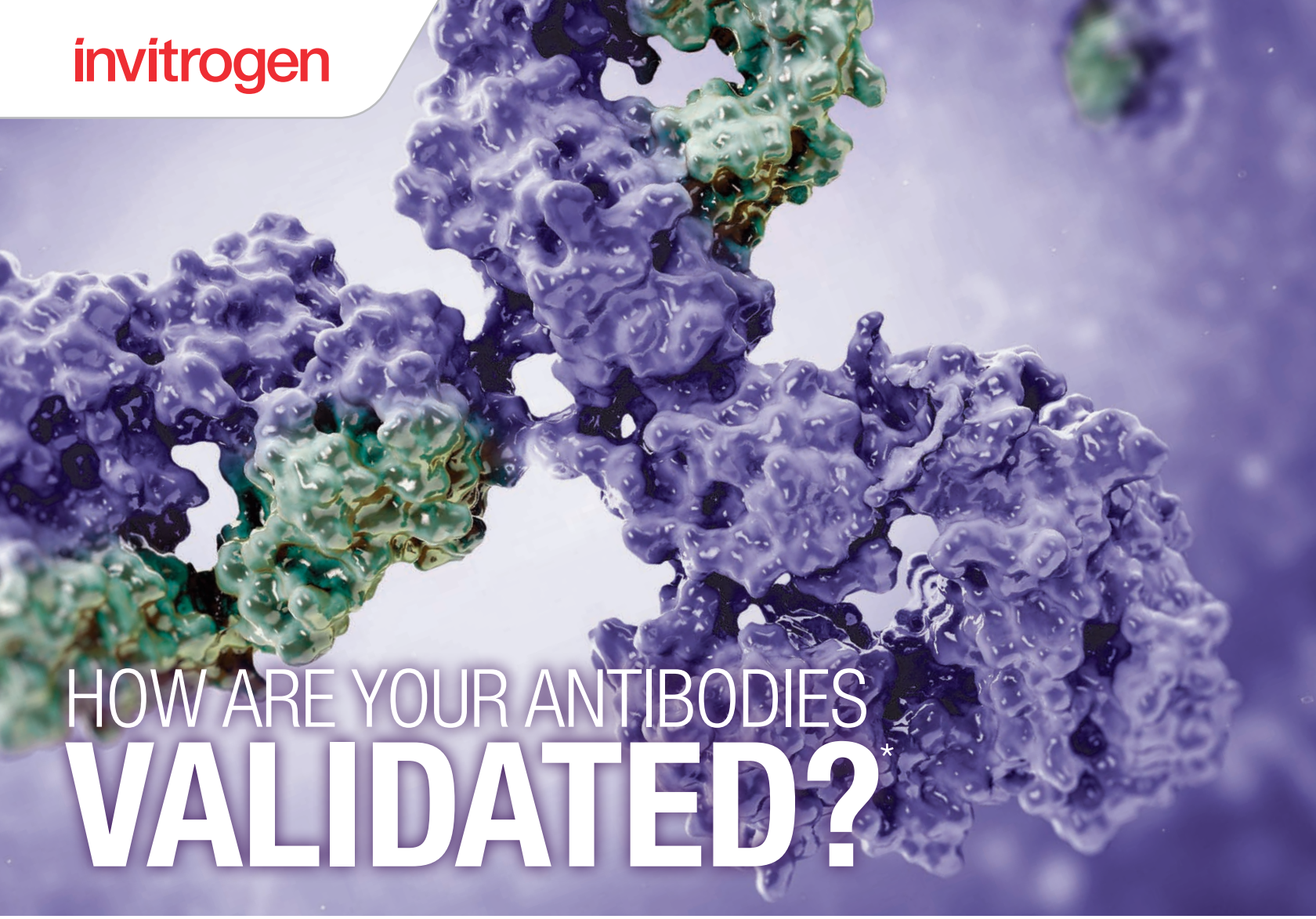

HOW ARE YOUR ANTIBODIES VALIDATED?*

Every year, researchers waste valuable time and money using antibodies that do not perform as intended

In September 2015, Thermo Fisher Scientific helped organize and supported a group of leading researchers from institutions throughout the world to draft a set of proposed standards for the best ways to validate* antibody specificity.

The International Working Group for Antibody Validation (IWGAV) published their proposed strategies in *Nature Methods* in September 2016. In the fall of 2016, many of the IWGAV members, as well as representatives from antibody producers, pharmaceutical companies, and leading academic research labs, met at an event coordinated by the Global Biological Standards Institute (GBSI) to further study this topic. This meeting reviewed and validated the IWGAV's proposed standards and discussed ways to implement the standards across the industry.

As a result of our active discussions and key participation in these forums, we have seen a clear consensus develop around several impactful practices to help further advance antibody validation. These new guidelines will add to the extensive application validation* already performed for Invitrogen™ antibodies and will focus on confirming antibody specificity. Testing antibodies for application validation* is critical and must be carried out with the utmost rigor, but it can be a further challenge to confirm antibody specificity. By combining both application validation* and specificity testing, we aim to ensure that antibodies meet the highest possible standards of overall antibody validation.*

* 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.

Advanced verification testing methods

Immunoprecipitation-mass spectrometry (IP-MS)

IP-MS: testing using immunoprecipitation followed by mass spectrometry to identify atibody targets

Genetic modification

Knockout: expression testing using CRISPR-Cas9 cell models

Knockdown: expression testing using RNAi to knock down gene of interest

Independent antibody verification (IAV)

IAV: measurement of target expression is performed using two differentially raised antibodies recognizing the same protein target

Biological verification

Cell treatment: detecting downstream events following cell treatment

Relative expression: using naturally occurring variable expression to confirm specificity

Neutralization: functional blocking of protein activity by antibody binding

Peptide array: using arrays to test reactivity against known protein modifications

Orthogonal method: correlation using both antibody dependent and independent method

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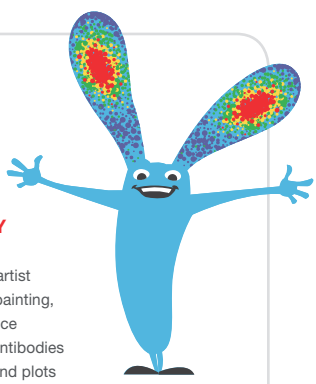


PRIMARY CONJUGATED ANTIBODY
DOB: 1972
Personality: Strong-willed, an artist
Hobbies: Going with the flow, painting, and dreaming of beautiful science
Seeking: Compatible primary antibodies for designing beautiful panels and plots
Statistics:
- Over 10,000 primary conjugated antibodies for flow cytometry
- Over 40 different fluorophores available
- Fully verified conjugates for flow cytometry, with performance guarantee**

** For details, go to thermofisher.com/antibodyperformanceguarantee

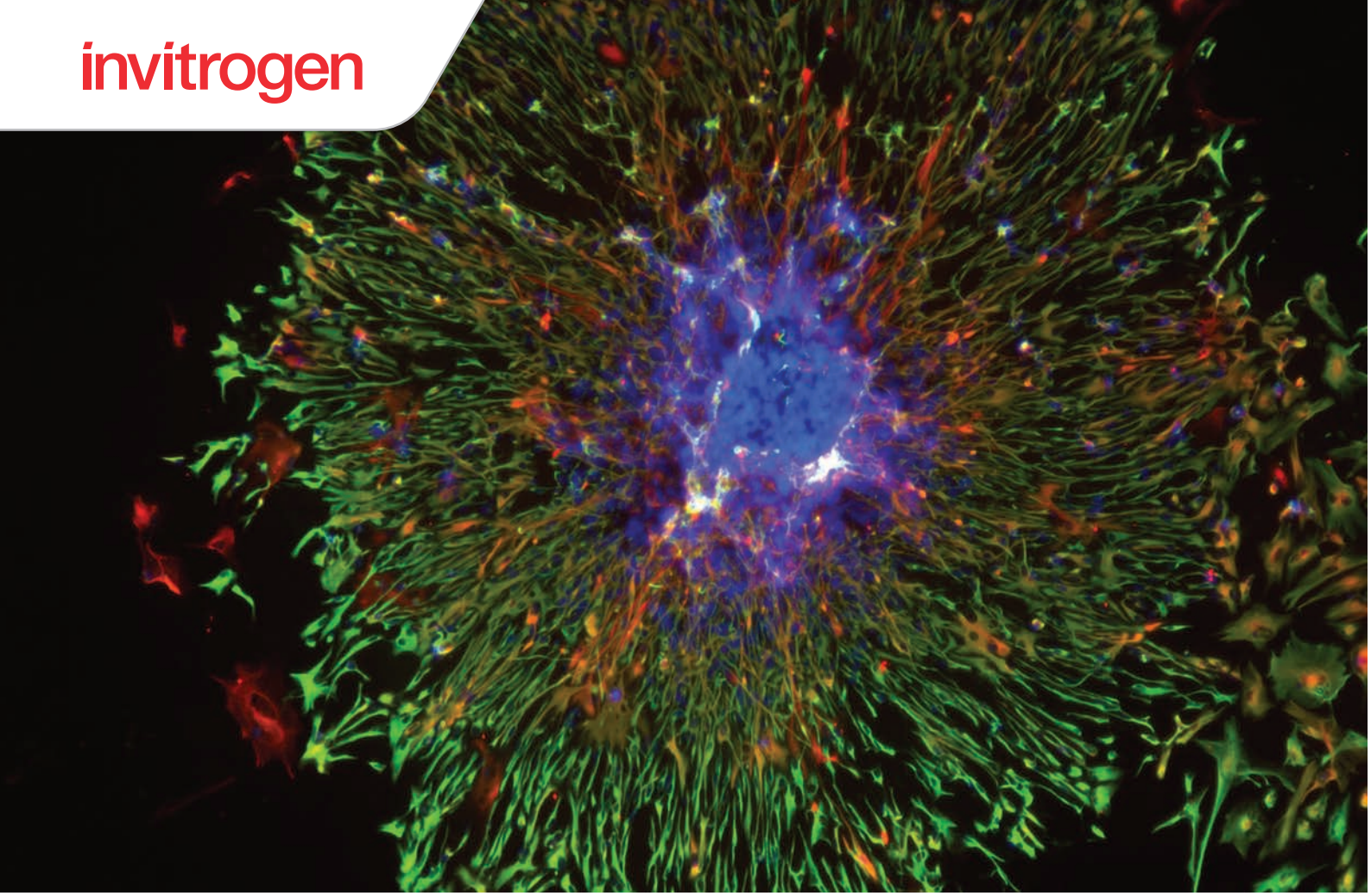
PRIMARY ANTIBODY
DOB: 1975
Personality: Born leader, the center of attention
Hobbies: Binding, purifying, and detecting antigens in over 50 research areas
Seeking: A truly binding relationship
Statistics:
- Over 200,000 antibody citations
- Over 63,000 primary antibodies available
- Over 50 diverse target species

Meet the rest of the characters on page 30.

These specificity verification methods are being used to systematically validate the Invitrogen™ antibody portfolio. In addition, Invitrogen™ antibodies have more than 120,000 supporting images and more than 60,000 reference citations. Stay tuned as we continue to establish new standards in antibody testing and verification.

To find out more, go to thermofisher.com/antibodyvalidation

* 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.



IT'S CLEARLY INTERNALIZED

Antibody drug conjugates (ADC) represent a novel method to deliver a drug to diseased cell using the specific antigen-binding capability and internalization (Figure 1). Antibodies that do not internalize efficiently will not have good therapeutic results. Our reactive chemistries, bright and versatile fluorophores, and detection platforms help save time and dollars, all the while delivering actionable data in screening for efficient internalization.

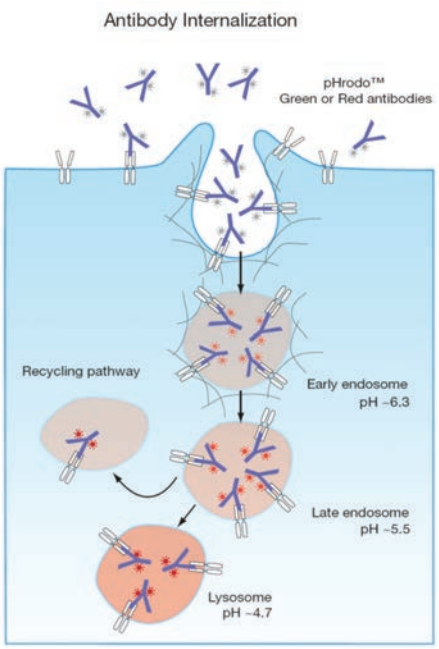


Figure 1. Antibody internalization process.

USING PH CHANGE TO DETECT AND MEASURE INTERNALIZATION

Invitrogen™ pHrodo™ iFL dyes dramatically increase fluorescence as pH changes from basic to acidic (Figure 2). Attachment of an iFL pHrodo dye to your antibody makes for a fast, effective, and unambigious signal to determine antibody internalization.

Clear internalization of Herceptin™ antibody in breast cancer cells

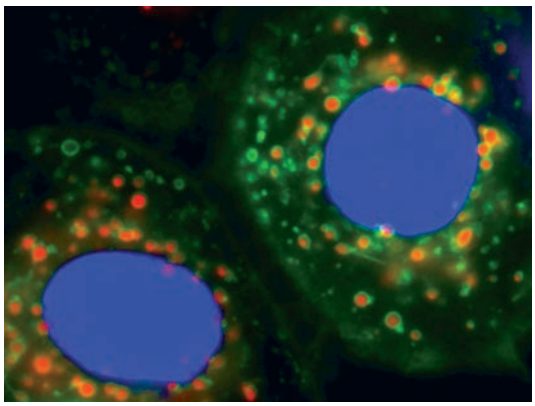


Figure 2. Herceptin antibody conjugates of amine-reactive pHrodo iFL Red dye traffic into SKBR3 cells overexpressing Her2 protein (Image from Invitrogen™ EVOS™ FL Auto Imaging System).

SITE-SPECIFIC BINDING WITH MINIMAL DISRUPTION TO YOUR ANTIBODY

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EFFICIENT INTERNALIZATION OF HERCEPTIN™ ANTIBODY

Figures 3 and 4 show binding and internalization using flow cytometry and high-content image anlaysis.

Specific binding and internalization of Gazyva™ obinutuzmab in CD20+ cells

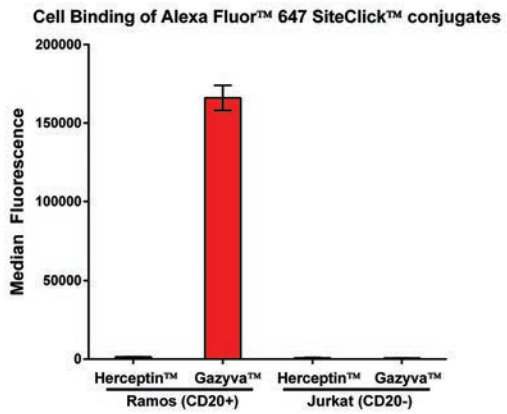

Figure 3. Cells from triplicate samples were analyzed on the Invitrogen™ Attune™ NxT Flow Cytometer with AutoSampler to show binding specificity and internalization of Gazyva™ obinutuzmab to CD20+ cells. Nonspecific binding and internalization was not in observed in CD20- cells.

Efficient tracking of internalization events

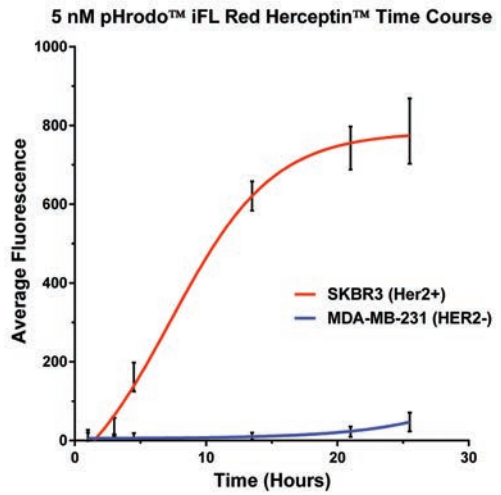

Figure 4. Conjugated Herceptin™ antibody internalizes into Her2+ cells over time. Her2- cells provide minimal signal. Cells from triplicate samples were analyzed on the Thermo Scientific™ CellInsight™ CX5 platform.

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PROTEOMICS:
INSIGHTS INTO CANCER SIGNALING

Quantitative analysis of AKT/mTOR pathway using western blotting and IP-MS techniques

Because the AKT/mTOR pathway plays a central role in tumor progression and anti-cancer drug resistance, the quantitative measurement of this pathway's protein expression and posttranslational modifications (PTMs) is vital to cancer research.[1] A major limitation when measuring the protein levels in the AKT/mTOR pathway is the lack of rigorously verified methods and reagents. Because many biologically relevant proteins are present in very limited quantities, immunoprecipitation (IP) is commonly used as a tool for enriching protein targets before detection and quantification by western blotting and mass spectrometry (MS).[2,3] Western blotting is a foundational proteomics research application, yet the potential of the application to determine subtle differences in protein expression and protein modification states is limited by shortcomings in detection reagent selection and instrumentation. The IP-MS method enables the enrichment of signaling pathway proteins, revealing protein-protein interactions and PTMs.[4] Multiplex IP coupled with MS (mIP-MS) further enhances this workflow by simultaneously quantifying multiple proteins and their phosphorylation states in a specific signaling pathway.* Here we demonstrate improved western blot detection using the new Invitrogen™ iBright™ FL1000 Imaging System and compare the results to mIP-MS methodology by analyzing a specific set of protein targets in the AKT/mTOR pathway. The iBright FL1000 Imaging System is a CCD-based western blot imaging instrument, featuring advanced automated features, a powerful 9.1 megapixel camera, and Smart Exposure™ optimized exposure technology.

Invitrogen™ iBright™ FL1000 Imaging System (Cat. No. A32752)

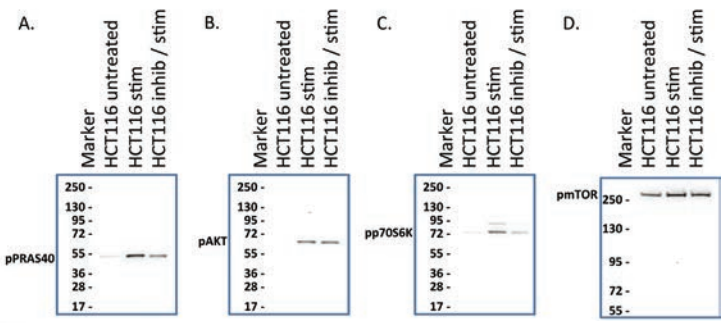

Figure 1. Western blotting of phospho AKT/mTOR pathway proteins; (A) pPRAS40, (B) pAKT, (C) pp70S6K, (D) pmTOR.

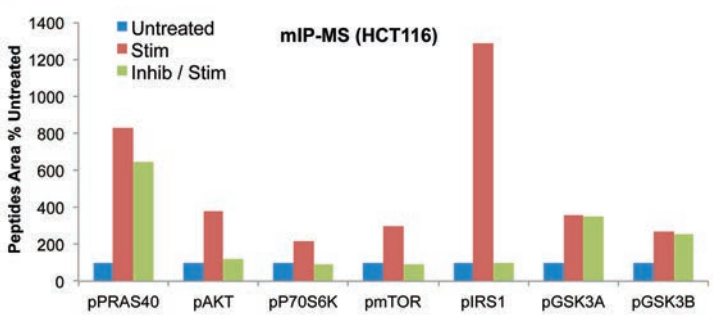

Figure 2. mIP-MS assays allows quantitation of low abundant phosphorylated AKT/mTOR pathway proteins. All 7 phospho targets were quantified across all three lysates conditions.

Smart Exposure technology rapidly determines optimal exposure time, which helps minimize the potential for over- or underexposed images and the need to repeat exposures to get the desired signal. Together, the system's high sensitivity camera and Smart Exposure technology enables the acquisition of subtle differences in protein expression.

For the western blot and mIP-MS analysis, HCT116 cells were grown and serum-starved for 24 hours prior to the following treatments: untreated, stimulated (15 min in 12.8 nM hIGF-1), inhibited, then stimulated (1 hour in 50 µM LY294002 plus 15 min in hIGF-1). Subsequent to treatments, cells were lysed with Thermo Scientific™ Pierce™ IP-Lysis buffer (Cat. No. 87788) supplemented with 1X HALT™ Protease and Phosphatase inhibitor cocktail (Cat. No. 78440). Protein concentration of lysates was determined with the Thermo Scientific™ Pierce™ BCA Assay (Cat. No. 23225).

For the western blot detection and quantification, 20 µg of each cell lysate was then diluted in electrophoresis reducing sample buffer and run on Invitrogen™ Novex™ Tris-Glycine 4–20% gel (Cat. No. XP04200BOX). After electrophoresis, proteins were transferred to nitrocellulose membrane (Cat. No. 88018) using the Thermo Scientific™ Pierce™ Power Blotter (Cat. No. 22824) and Thermo Scientific™ Pierce™ 1-Step Transfer Buffer (Cat. No. 84731). Membranes were blocked in Thermo Scientific™ StartingBlock™ (TBS) Blocking Buffer (Cat. No. 37542) for 1 hour at room temperature and then incubated with primary antibodies overnight at 40°C, rocking gently. Antibody was washed in TBST and protein was detected using Invitrogen™ Goat anti-rabbit Horseradish Peroxidase Conjugate (Cat. No. 32460) and Thermo Scientific™ SuperSignal™ West Dura Extended Duration Substrate (Cat. No. 34076). Images were acquired using the iBright FL1000 Imaging System (Cat. No. A32752). For the mIP-MS sample preparation and analysis, please refer to the article in *BioProbes* Vol 75; pp. 19–22. Figures 1 and 2 show the correlation between western blotting and mIP-MS.

References

1. Logue JS, Morrison DK (2012) Complexity in the signaling network: insights from the use of targeted inhibitors in cancer therapy. *Genes Dev* 26:641–650.
2. Gingras AC, Gstaiger M, Raught B, Aebersold R (2007) Analysis of protein complexes using mass spectrometry. *Nat Rev Mol Cell Biol* 8:645–654.
3. Carr SA, Abbatiello SE, Ackermann BL et al. (2014) Targeted Peptide Measurements in Biology and Medicine: Best Practices for Mass Spectrometry-based Assay Development Using a Fit-for-Purpose Approach. *Mol Cell Proteomics* 13:907–917.
4. Ackermann BL (2012) Understanding the role of immunoaffinity-based mass spectrometry methods for clinical applications. *Clin Chem* 58:1620.

* Original article published in *BioProbes 75 Journal of Cell Biology Applications.*

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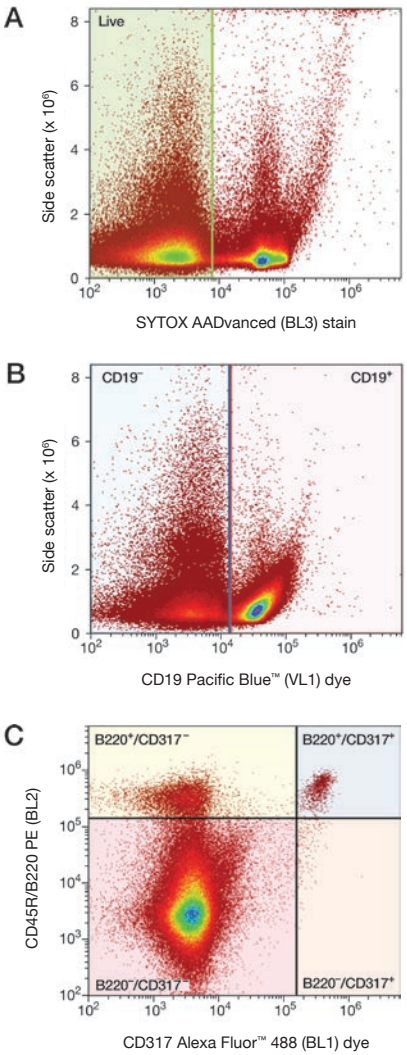

Figure 1. Mouse plasmacytoid dendritic cell (pDC) gating and analysis. (A) a gate was made on live cells using Invitrogen™ SYTOX™ AADvanced™ Dead Cell Stain (Cat. No. S10274; channel BL3, 640 nm longpass fi lter), (B) live cells were then gated on CD19 cells (channel VL1, 450/40 nm bandpass (BP) filter). (C) a 2-parameter plot of CD45R/B220 vs. CD317 was used to detect pDCs (channel BL1, 530/30 nm BP filter; and channel BL2, 574/26 nm BP filter); pDCs were identified as dual B220⁺/CD317⁺ (upper right quadrant) and comprise 0.851% of live CD19 cells, which is 0.194% of total splenocytes. A collection rate of 500 µL/min was used to acquire 1.3 million total cells; total acquisition time was 23 minutes—3x faster than the same sample run on a traditional hydrodynamic focusing cytometer.

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GET TO KNOW OUR ANTIBODY CHARACTERS

HRP SECONDARY ANTIBODY
DOB: Supercentenarian
Personality: Shy, sensitive
Hobbies: Conjugating, catalyzing, and amplifying
Seeking: A reliable primary antibody to ride off with into the western sunset
Statistics:
- Over 280 conjugates available for western blotting
- Over 15 target species
- IgG, IgM, and 11 other immunoglobulin targets

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FOR HIGHEST # OF ANTIBODY CITATIONS

ALEXA FLUOR SECONDARY ANTIBODY
DOB: 1997
Personality: Colorful
Hobbies: Fluorescing and amplifying
Seeking: An outstanding primary antibody match for fluorescence imaging
Statistics:
- Over 250 antibodies available for imaging and western blot applications
- Over 24 different Invitrogen™ Alexa Fluor™ antibody dyes
- Over 30,000 citations

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by sandraculliton
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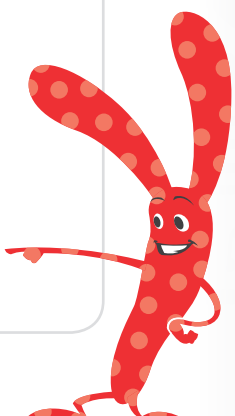
ANTIGEN
DOB: Age is just a number
Personality: Capricious
Hobbies: Playing hard to get
Seeking: An open-minded antibody match
Statistics:
- Our antibodies cover over 85% of the proteome
- Protein expression service available using a choice of 3 expression systems
- Custom peptide synthesis service available for 4 to 75 amino acids

CUSTOM ANTIBODY
DOB: TBD
Personality: Ever-changing, adaptable
Hobbies: Extreme binding
Seeking: A unique antigen match
Statistics:
- Over 18,000 custom antibody projects completed to date
- Bioinformatics-driven peptide design based on titer data from over 15,000 peptides
- Choice of monoclonal or polyclonal antibody production with over 40 labeling options



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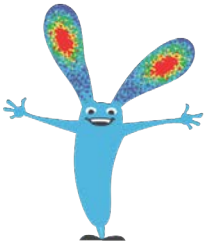

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