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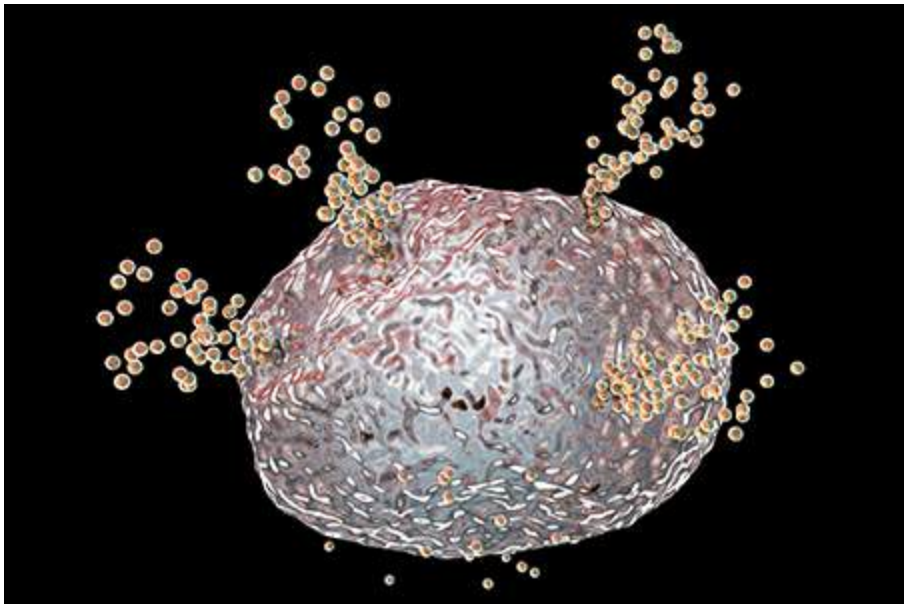
TACONIC BIOSCIENCES

Studying Mast Cell-mediated Allergic Disease Using Humanized Immune System Mice

Aliyah M. Weinstein, PhD

Wednesday, December 15th, 2021



Humanized immune system (HIS) mice have a variety of applications for studying diseases in which the immune system drives pathogenesis. This includes immuno-oncology, autoimmunity, and a less discussed but also crucial subset of immunologic disorders, allergy. Indeed, Taconic's CD34+ hematopoietic stem cell (HSC)-engrafted NOG-EXL (huNOG-EXL) is a model that supports the key myeloid cell populations that mediate allergic responses in humans.

Humanized NOG-EXL mice support key myeloid cell populations

The humanized NOG-EXL mouse, engrafted with CD34+ HSC, was originally characterized in allergic disease. The NOG-EXL mouse transgenically expresses human GM-CSF and human IL-3. These cytokines support the development and maintenance of human myeloid cells after engraftment, including granulocytes, monocytes, mast cells, basophils, neutrophils, and dendritic cells. Like the humanized NOG mouse upon which the huNOG-EXL is based, these mice also support T and B cells. The presence of myeloid cells – especially mast cells, which will be discussed in the case studies outlined below – is what makes the huNOG-EXL a useful model for studying allergic disease.

Human myeloid cells respond to GM-CSF and IL-3 signaling

Mast cells, basophils, eosinophils, and neutrophils are key cell populations driving allergic reactions in humans and humanized immune system mice. All of these populations can be found in the huNOG-EXL, where CD33+ cells comprise 5-20% of the hCD45+ cell population. Human mast cells, eosinophils, and basophils have all been reported to express receptors for both hGM-CSF and hIL-3, which are transgenically expressed by the NOG-EXL[1]. Neutrophils express the receptor for hGM-CSF, and engagement of this receptor upregulates expression of the IL-3 receptor[2].

The impact of IL-3 and GM-CSF on mast cell function *in vivo*

Interestingly, while murine mast cells rely on IL-3 for early differentiation from progenitor cells, this is debatable with regard to human mast cells[3,4]. In humans, IL-3 and GM-CSF typically promote differentiation into granulocytes. Human mast cell progenitors that have seen IL-3 or GM-CSF *in vitro* were shown in one study to retain the ability to differentiate into mast cells but only express low levels of the mast cell marker c-Kit[3]. Instead, IL-3 appeared to specifically support mast cell precursor cell replication and survival[3,5]. Conversely, another study indicated that IL-3 is sufficient for differentiation of myeloid precursors to the pre-mast cell stage expressing c-Kit (Dahlin 2017). One may hypothesize that *in vivo*, such as in HIS mice, other immune cell populations could be responding to hIL-3/hGM-CSF by producing cytokines such as IL-6 to drive terminal mast cell differentiation[6-8].

Case Studies

Mast cells mediate Type I hypersensitivity and anaphylaxis

The original publication characterizing the CD34+ HSC-engrafted NOG-EXL described its utility in Type I hypersensitivity reactions using the pollen challenge to model anaphylaxis. The group from the Central Institute for Experimental Animals (CIEA) concluded that the NOG-EXL engrafted with a human immune system supported functional tissue-resident mast cells that can mediate allergic responses following sensitization. These mast cells were characterized as FcεRI+ cells expressing mast cell chymase and tryptase. In humans, these cells are localized to the skin; in the humanized NOG-EXL, they are found in the skin as well as the spleen, lung, and stomach[9]. NOG-EXL engrafted with CD34+ HSC can therefore support tissue-resident human mast cells that can mediate cutaneous allergic reactions.

Cytokines produced by ILC2 and mast cells drive asthma

A follow-up study by the group that initially characterized the humanized NOG-EXL then analyzed its utility as a model for asthma. While there are many murine asthma models available, most do not have all the key characteristics of human disease such as the infiltration and impact of human myeloid cells in the airway. Thus, the huNOG-EXL, with its support for eosinophils, mast cells, and basophils in addition to leukocytes, recapitulates this feature of human asthma in a unique way. Infiltration of human T cells, eosinophils, mast cells, and basophils is observed in the bronchial alveolar lavage fluid (BALF) of IL-33-treated humanized NOG-EXL. This inflammation can be ameliorated by blocking IL-13, a cytokine produced by ILC2, T cells, and mast cells that drives airway inflammation downstream of IL-33[10]. Humanized NOG-EXL animals therefore encompass more features of human asthma than previous HIS models used to study this disease.

Humanized NOG-EXL mice can be sensitized to food allergens

The same group most recently used CD34+ humanized NOG-EXL mice to develop a model of anaphylactic food allergy. Upon challenge with an oral antigen to which they had previously been sensitized, expression of the human cytokines MCP-1, IFNg, IL-6, and IL-8 increase, however, the levels of mouse

cytokines were not impacted. This demonstrates the utility of this model for studying human food allergy without interference from the residual mouse immune system. Humanized NOG-EXL also exhibit a rapid decrease in body temperature and succumb to anaphylaxis within 1 hour following challenge, whereas humanized NOG mice (which do not express hGM-CSF or hIL-3) do not. In parallel, humanized NOG-EXL mice have increased serum histamine and increased expression of the mast cell degranulation marker CD63, both hallmarks of an anaphylactic response mediated by mast cells[11].

Future directions for using huNOG-EXL to study allergy

The Taconic team is eager to expand the use of huNOG-EXL to study allergic diseases. While the proof-of-concept studies have been published for close to a decade, relatively few publications have taken advantage of the expanded myeloid compartment in the huNOG-EXL to study allergy. The presence of functional human mast cells as well as basophils, eosinophils, and neutrophils make this an enticing model for these acute immune responses.

Download the Taconic Biosciences White Paper:

- An Introduction to Humanized Immune System Mouse Models

If you are interested in discussing a potential collaboration with Taconic to generate data in this area, please reach out to schedule a consult with our Field Application Scientist team.

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Taconic Biosciences' model generation team has produced about 5,000 models in the last 15 years, developing a globally-recognized reputation for advancing

MassBio News

MEMBER SPOTLIGHT (HTTPS://WWW.MASSBIO.ORG/NEWS-CATEGORY/MEMBER-SPOTLIGHT/)

Member Spotlight: Q&A with Addgene

MAR 01, 2019

Every month, MassBio spotlights a member company and the great work they're doing to advance the life sciences industry and support the patients we serve. This month we spoke with Aliyah M. Weinstein, PhD, Marketing and Communications Manager at Addgene (https://www.addgene.org/), the nonprofit plasmid repository. In this role, she works to ensure that scientists around the world know about Addgene's materials, services, and educational resources. Aliyah advocates for equitable access to STEM education and careers through writing and community outreach.

Tell us about your organization, its mission, and current initiatives.

Addgene is a global nonprofit plasmid repository. Our mission is to accelerate research and discovery by improving access to useful research materials and information. We organize the transfer of DNA-based reagents such as plasmids and ready-to-use viral preps between scientists in over 90 countries and have over 71,000 plasmids available in our repository. Over the past few months, Addgene has hit several memorable milestones. We shipped our 1 millionth plasmid in September, relocated to the LINX building in Watertown in November, and celebrated our 15th anniversary in January! With our new space comes the exciting opportunity to grow our team and expand the services that we can offer to the scientific community.

How does your organization's activities help patients now and into the future?

Clinical breakthroughs are fueled by basic science discoveries. The materials that Addgene distributes help basic science researchers easily access the DNA-based tools they need to make new discoveries. For scientists who develop new tools and deposit them at Addgene, we provide support by taking care of quality control, MTA compliance, reagent production and distribution, and record-keeping. Sharing speeds science, and Addgene empowers researchers worldwide to make an impact on the present and future of science and medicine.

What do you see as the biggest challenge facing the life sciences industry today?

The biggest challenge in the industry is the need for inclusive and diverse company cultures. It's the only way to ensure that every employee will feel welcomed, supported, and engaged – and ultimately for the organization to reach its full potential. Addgene is committed to fostering this kind of environment through our philosophy of supportive, radical flexibility; harassment awareness and unbiasing training for all employees; recruiting partnerships with local organizations such as Just a Start and community colleges; and as a founding sponsor of the Massachusetts LGBT Chamber of Commerce. As our CEO, Joanne Kamens, highlighted last year (https://www.massbio.org/discover/diversity/diversity-stories/joanne-kamens-creates-culture-of-diversity-and-inclusion-at-addgene-140346), we are engaged in at least half of the efforts to reach gender parity in the life sciences sector that were recommended in the MassBio report (http://files.massbio.org/file/MassBio-Liftstream-Gender-Diversity-Report-2017.PDF). We encourage other leaders to spend time understanding why taking these kinds of actions are important not only to their company, but to the success of the industry as a whole.

What's next for your organization / what are you focused on in the coming year?

As always, we want to make it easy for scientists around the world to access the reagents they need. This year, Addgene is focused on filling gaps in our collection, expanding our distribution of plasmids and ready-to-use viral preps, and developing new services that further accelerate research. We'll continue working closely with the non-researchers in our pipeline, such as technology

transfer offices and international distributors, to improve our processes for reagent sharing. We are also involved in the growing effort to enable scientific reproducibility. We will continue to provide open access molecular biology protocols and educational resources, and plasmid sequence validation and associated information, to the scientific community.

If you're interested in being featured in MassBio's Member Spotlight, please see guidelines here (https://www.massbio.org/wp-content/uploads/2020/03/Member-Spotlight-Guidelines_2020.pdf).

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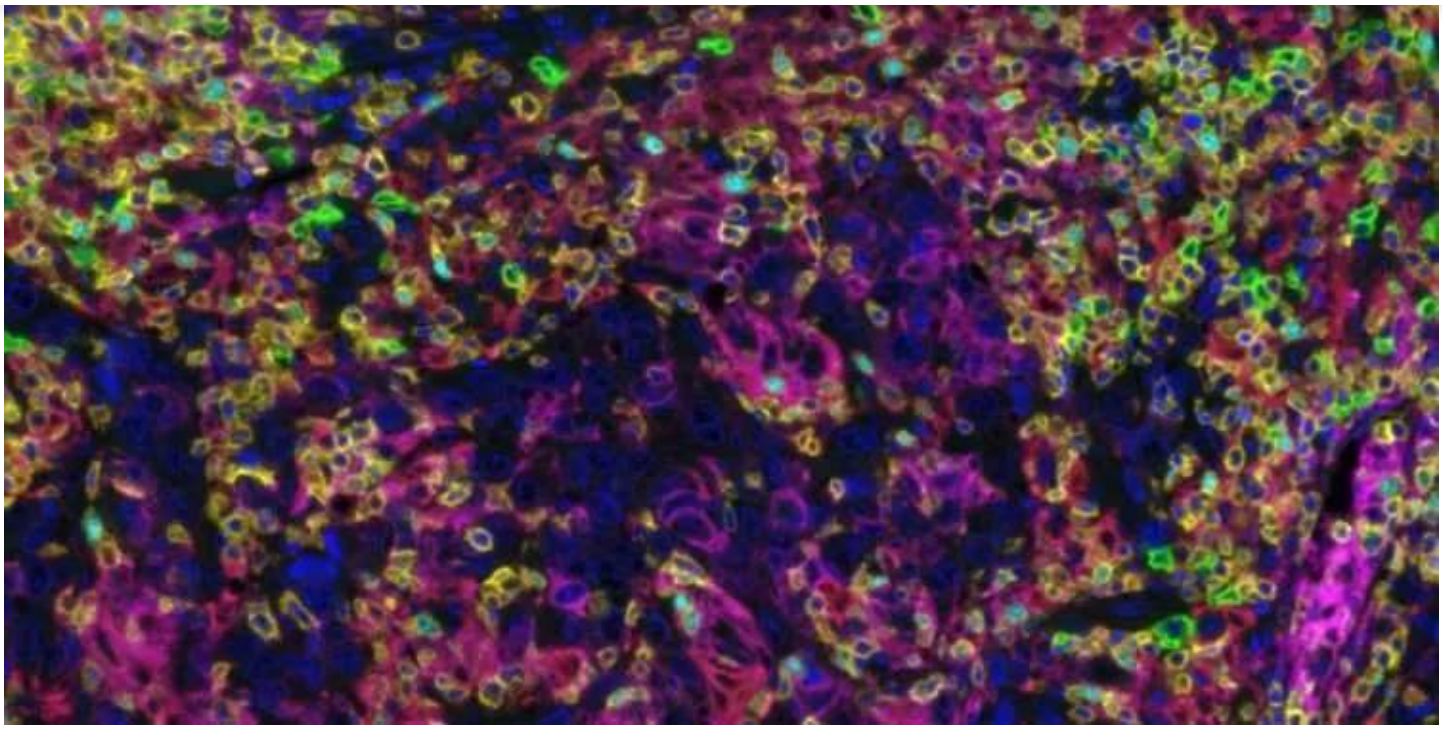
Why choose Bethyl antibodies for your R&D



10 January 2023

Posted in News | Tagged Antibodies, Antibody, Antibody discovery workflow, Antibody-based therapeutics, Bethyl Laboratories, Drug discovery

This article is sponsored by Bethyl Laboratories.

For over 50 years, Bethyl Laboratories (now a Fortis Life Sciences brand) has been dedicated to improving lives by supporting scientific discovery through qualified antibody products and custom services. We are vertically-integrated from immunisation through manufacturing and validation. All of our antibodies are highly validated using six pillars of validation, meaning that our antibodies do what we say they'll do. Our catalog of over 6,500+ primary antibodies, 1,200+ secondary antibodies, and 50+ ELISA kits includes reagents for multiplex IF, flow cytometry, and more in fields including immunology, oncology, and neuroscience. Or, you can collaborate with our scientists to develop a custom monoclonal or polyclonal antibody, or a custom IHC or multiplex IF assay.

Antibody validation

fundamental to ensuring the rigor and reproducibility of data generated by immunoblotting, immunohistochemistry, ELISA, and other assays that rely on antibodies.

Bethyl antibodies are highly regarded for passing strict validation testing before arriving in customer hands. The principles that have guided our rigorous validation practices for decades preceded a publication (Uhlen et al., A proposal for validation of antibodies. *Nat Methods* 2016 Oct;13(10):823-7) that describes five conceptual pillars for validating antibodies in an application – and context-specific manner. Multiple pillars are used in a complementary fashion to validate each antibody, and the pillars can be variable based on the unique biology of each target protein, or the reagents available for that project. At Bethyl, we have adapted many of the principles laid out in that publication to capture our expertise and experiences validating antibodies here, as listed below.

Pillars of validation:

1. Pillar 1: Independent Antibodies. This pillar requires that two or more antibodies directed against different epitopes of a protein generate similar results.
2. Pillar 2: Complementary Assays. This pillar requires that multiple, antibody-dependent assays produce complementary results.
3. Pillar 3: Orthogonal Characteristics. This pillar requires that antibody-independent and antibody-dependent assays produce results that are correlative.
4. Pillar 4: Biological Characteristics. This pillar takes advantage of the unique biology associated with some protein targets.
5. Pillar 5: Protein OE/Epitope Tags. This pillar uses over-expressed (OE) proteins to validate antibodies against targets where we cannot identify a natively expressing cell line, or the protein is expressed at levels insufficient for detection.
6. Pillar 6: Genetic Strategies. This pillar uses gene knockout or knockdown to reduce the levels of target protein available for detection.

Custom antibody development

For many of our clients, we realise that cutting-edge research requires custom solutions. Therefore, we are pleased to offer custom antibody development services, where our team of experienced scientists can ensure a successful outcome to projects across a wide range of applications.

goals and requirements. We can also help you smoothly transition from polyclonal to monoclonal antibodies at any point in the project.

Recombinant monoclonal antibodies can be custom made to meet your project-specific requirements by our expert team. We generate numerous candidates for you to test in your assays and workflows ensuring the antibodies are effective in whatever application they're being used. Our robust monoclonal antibody generation platform delivers large, diverse clone sets, enabling our partners to find fit-for-purpose monoclonal antibodies to suit their applications for 90% of projects.

We also offer many high-quality custom polyclonal antibody packages that are designed to suit a range of scientific needs within your budget. We specialise in custom peptide synthesis, immunisation, and polyclonal antibody production/purification to generate high-quality antibodies.

Our areas of expertise:

- Cell surface, secreted and intracellular targets
- Anti-idiotype antibodies
- Post-translational modifications (phospho-specific antibodies)
- Small molecules

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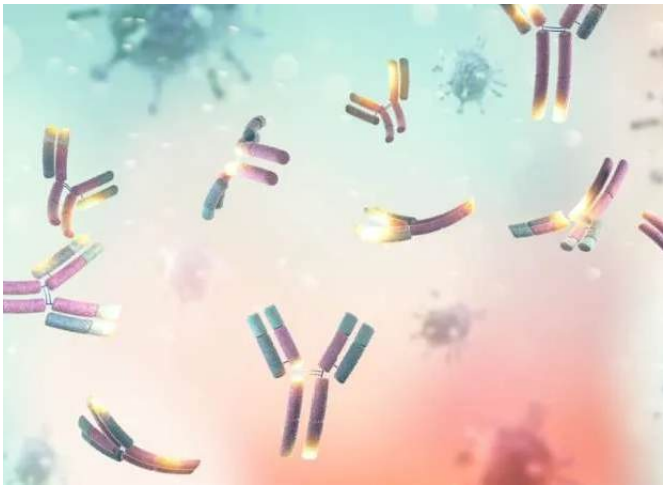

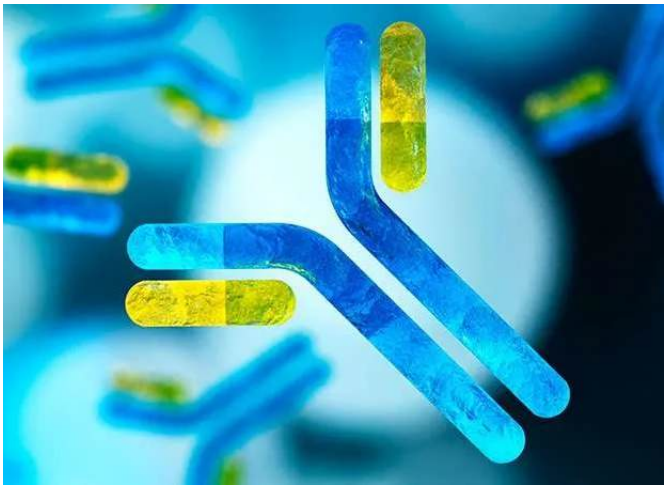

The Sentinel

THE OFFICIAL BLOG OF THE SOCIETY FOR IMMUNOTHERAPY OF CANCER (SITC).

Thursday, January 18, 2018

INTRATUMORAL LYMPHOID AGGREGATES MAY BE TERTIARY LYMPHOID STRUCTURES

by Aliyah Weinstein

It's well established in the field of immuno-oncology that in nearly all cancers, a subset of patients presents with an immune infiltrate. In many of these tumors, the infiltrating immune cells appear to form aggregates that are especially noticeable at the tumor periphery. Indeed, when presenting my research on these aggregates at scientific meetings, I've often been approached by other scientists or clinicians who indicate that they've seen lymphoid aggregates in tumor tissue from their patients, but didn't know to investigate them further.

This brief overview may change some of those minds.
These lymphoid aggregates have been termed tertiary (or "ectopic") lymphoid organs, often referred to as TLS. First described about a decade ago, TLS develop at sites of chronic inflammatory responses, including cancer as well as chronic infection and autoimmune disease. They are now a growing area of investigation in immuno-oncology due to their prognostic value in numerous types of cancer; except in hepatocellular carcinoma, they predict extended progression-free and/or overall survival.

While multiparameter imaging can distinguish the features of TLS, they can more easily be appreciated by H&E staining. (Interestingly, a genetic signature based on chemokine expression can also be used to predict the presence of TLS within tumors.) TLS resemble secondary lymphoid organs based on the presence of a germinal center-like B cell aggregate and/or PNAd+ high endothelial venules surrounded by a zone of T cells and dendritic cells. Lymphocytes are recruited into TLS via these high endothelial venules, as PNAd binds L-selectin/CD62L, which is found on the surface of both T cells as well as B cells. TLS are thought to be a site of local T cell priming, independent of lymph nodes due to the ability of APC to recover antigen within the tissue and present it to nearby T cells within the TLS.

Besides their prognostic value, TLS may provide a novel avenue for therapeutic development. A recent publication from my lab demonstrated in a murine model of colon cancer that TLS can be induced therapeutically by the introduction of Type 1-polarized dendritic cells into the tumor microenvironment, and this is associated with delayed tumor growth. Others have postulated that TLS-positive tumors may be more responsive to immunotherapy because of the high density of T cell infiltrate in these tumors, including regulatory T cells.

An understanding by scientists and clinicians that lymphoid aggregates observed in tumors may have the hallmarks of TLS should help the field better appreciate in which tumor types they are found, and what their prognostic value may be. Simultaneously, while research into the benefit of therapeutically targeting TLS is still in its early stages, a basic understanding of these structures should help the field develop novel ways to target them therapeutically, while taking into account other characteristics such as tumor location, mutational status, and standard-of-care therapies for various cancers.

By Society for Immunotherapy of Cancer at January 18, 2018 (2018-01-18T09:00:00-06:00)

Labels: Aliyah Weinstein, chemotaxis, ectopic lymphoid structures, lymphocytes, prognosis, tertiary lymphoid organs

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The importance of broadening science communication

21 Nov 2016 | 13:00 GMT | Posted by Jack Leeming | Category: #ScientistOnTheMove, Academia, Blog, Career paths, Communication, Faculty, In the news, Publishing, Research

Though well-equipped with scientific training and expertise, scientists need to be aware of the resources available to help them share their work with a public audience, learns Aliyah Weinstein.

Recently, more and more emphasis has been put on scientists to communicate their research to public audiences. National scientific organizations such as the American Society for Biochemistry and Molecular Biology and the American Society for Cell Biology encourage their members to reach beyond the bench, and many blogs help scientists describe their research to wider audiences. At the University of Pittsburgh, where I'm working on my PhD, graduate student and postdoc organizations on campus share their excitement about science with the public through partnerships with local museums and school districts.

Unfortunately, while many scientists are interested in sharing their work with scientists in other fields or with lay audiences, these opportunities are not always supported by universities or PIs. To showcase the importance of scientists sharing science, I spoke with Dr. Lisa Girard, Director of Scientific Communications at the Broad Institute of MIT and Harvard in Cambridge, Massachusetts. Here, she explains why science communication is important, her path into the field, and how scientists can expand the reach of their work both within and outside of the scientific community.

How did you get into your career in science communication?

My path from bench scientist to scientific communications was actually very serendipitous. All through school I had always loved to write and edit — but I also loved science and doing research. During graduate school, I looked forward to any opportunities I had to write, or help someone write, pretty much anything about science. I even loved writing my thesis! Once I was a postdoc, I still planned to have a career in research, but I was also doing some freelance writing and editing. When I finished, my advisor offered me a role creating an online review for *C. elegans* biology. That review became WormBook, and that was my transition from the bench.

Why is it important for scientists to communicate their research outside of traditional publishing channels?

Scientific journals are aimed almost exclusively at a scientific audience. Beyond that community, there's a significant audience that wants to understand research that's happening, both for its own importance, as well as to consider how

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findings could inform fields such as medicine, climate change, agricultural policy, and more.

How does your science communications office at the Broad work with researchers? How do you decide what to focus on?

Broad Communications creates a range of different types of content, including press releases, blogs, news stories, profiles, and videos to tell the story of the science happening here. We try to talk regularly with researchers around the institute so we know what's going on. They also reach out to us if, for example, they're publishing a paper that they think is interesting. Additionally, our office works with reporters to let them know about stories they might like to cover, and then connects them with the appropriate individuals for interviews.

What are some ways that researchers in the lab can directly share their work with people who wouldn't normally see it?

There are many ways to share your work beyond the confines of your weekly lab meeting! Look for opportunities to speak at less-specialized conferences, see what your university or institute does in terms of partnering with local schools, look to become involved with a speaker series in your town or city, or see if your university has a publication (such as a newspaper or magazine) to which you could contribute.

Give me three tips to ensure that researchers communicate their science effectively.

First, skip the jargon. Scientists spend a lot of time talking with other researchers whose work is very similar to their own, so it's easy to forget that much of what they consider everyday vocabulary is pretty close to gibberish for a non-expert who wants to hear about your work. Use accessible language. If you must use a technical term, explain what it means.

Second, get to the point. When people are reading what you've written or are listening to you, you should frame what you are presenting so your audience can quickly have a handle of what you are intending to convey.

Third, don't overstate your conclusions. Do not try and make your topic or findings seem more impactful than they might actually be.

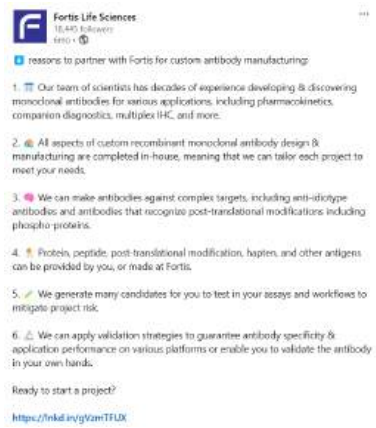
Lisa Girard

Lisa Girard, PhD *is Director of Scientific Communications at the Broad Institute of MIT and Harvard in Cambridge, Massachusetts where she works to create content that translates findings from Broad scientists for the interested non-expert. Previously, Lisa was the science editor at the Harvard Stem Cell Institute and a scientific editor at the California Institute of Technology. Lisa received her PhD from the University of California, Berkeley and completed a postdoc in C. elegans genetics at the California Institute of Technology.*

Aliyah Weinstein *is a graduate student in immunology at the University of Pittsburgh, where her research focuses on the pathways controlling anti-tumor immunity. Outside of the lab she enjoys learning languages, trying to achieve her goal of visiting all 50 U.S. states, and eating at all of Pittsburgh's delicious restaurants. You can find Aliyah on LinkedIn, Twitter, and her blog.*

Suggested posts

Visual Science Communication

	I led art direction and branding for a tradeshow booth at the Society for Immunotherapy of Cancer annual meeting in November 2024. As our antibody manufacturing site (Bethyl) and the show itself are both located in Houston, TX, we opted for a “hometown” themed booth inspired by local street art.
	One of my best-performing social media posts this year combined factual scientific information with emojis to draw visual attention to this post in an otherwise cluttered LinkedIn feed. This post served double the number of impressions than the next most successful post that month.
	For Halloween, I designed a thematic infographic to creatively highlight antibodies from the Fortis portfolio that had spooky-sounding names. This infographic stood out as a way to highlight our catalog products in a thematic yet professional way that aligned with the brand voice.