Program in Cellular and Molecular Medicine
at Boston Children's Hospital

HARVARD MEDICAL SCHOOL
TEACHING HOSPITAL

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Greetings, PCMM –

As the weather is getting slightly cooler, we hope everyone's enjoying the last few days of August. We're looking forward to seeing everyone at the PCMM retreat this September 15-17th, at Cape Cod! Please stay tuned for more details to come via PCMM emails.

As always, if you have any suggestions for the newsletter, please contact us at vera.gaun@childrens.harvard.edu and colin.smith@childrens.harvard.edu.

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PCMM Scientific Retreat 2024

Research Highlights

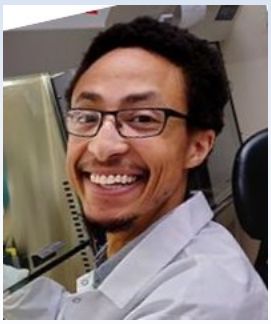
Nanobody-based bispecific antibodies for cancer immunotherapy

Submitted by Vera Gaun on August 26, 2025

Immunotherapy has revolutionized cancer treatment, however it works well only in a specific subset of tumors. In a recent *Nature Biomedical Engineering* article, members of the former Ploegh Lab at PCMM, including first co-authors Dr. Xin Liu, Dr. Camille Le Gall, and Dr. Ryan Alexander, have created an improvement on the type of immunotherapy called immune checkpoint blockade, which uses monoclonal antibodies of one isotype (usually IgG class). The lab used a new strategy by utilizing nanobodies that recruit antibodies of multiple isotypes (IgG, IgA, IgM, and IgE), thus creating the potential to activate more diverse downstream effectors.

Xin Liu

Camille Le Gall



Ryan Alexander

Hidde Ploegh

To create this new treatment, the lab made use of a modified version of an antibody called nanobodies. Nanobodies are created via the process of reducing heavy-chain only antibodies (derived from Camelids) to the variable region V_H domain specifically (figure 1). Advantages of nanobodies include: a) smaller size (one-tenth of a conventional antibody), often with an extended complementarity-determining region 3 (CDR3) loop, thus allowing for better accessibility to harder-to-reach targets, b) low immunogenicity, and c) ability to be mass-produced in *Escherichia coli.*

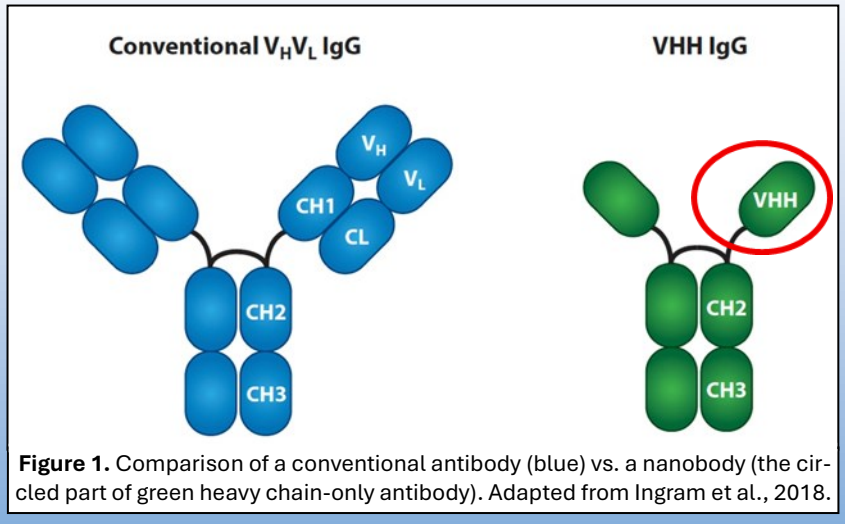


Figure 1. Comparison of a conventional antibody (blue) vs. a nanobody (the circled part of green heavy chain-only antibody). Adapted from Ingram et al., 2018.

Bi-specific antibodies were created via conjugation of anti-CTLA-4 or anti-PD-L1 nanobodies to anti-kappa light chain nanobodies, thereby allowing for engagement of endogenous antibodies bearing kappa light chains (>95% in mice and ~60% in humans), regardless of isotype or specificity, and allowing for multiple mechanisms of downstream action (figure 2). In one version of treatment, drug adducts or immune system agonists were attached to the bi-specific antibodies to enable targeted delivery to tumors (figure 2).

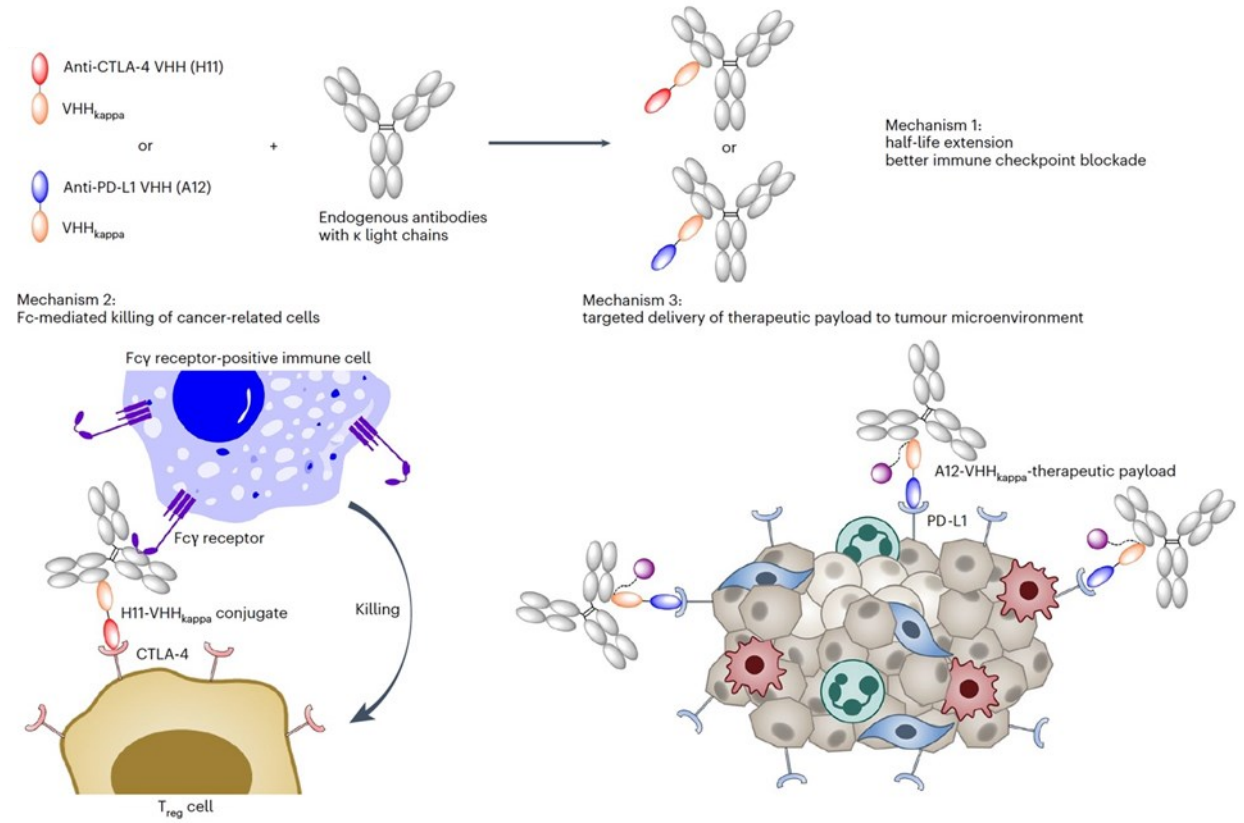


Figure 2. Multiple potential mechanisms of action of bispecific antibodies.

The lab then tested the anti-CTLA-4 bispecific antibody in mice engrafted with a colorectal carcinoma cell line and showed that it is superior to the anti-CTLA-4 monoclonal antibody conventionally used in immunotherapy in this mouse model (figure 3).

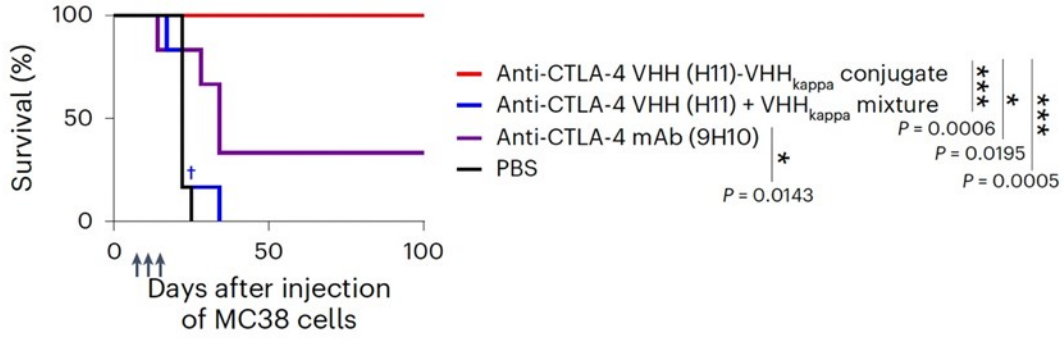


Figure 3. Anti-CTLA-4 bispecific antibody enhances survival as compared to the conventional monoclonal anti-CTLA-4 antibody in a mouse model of colorectal carcinoma (when treated 7, 10, and 13 days post tumor injection).

The anti-PD-L1 bispecific antibody didn't perform as well in comparison to anti-CTLA-4 (but still performed better than the monoclonal anti-PD-L1 counterpart), so the lab conjugated this anti-PD1-L1 bispecific antibody to a drug adduct or a STING agonist, which resulted in an improved response. The suggested mechanisms include: reduction of T_{reg} cells (involved in suppressing T cell responses) specifically in the tumor environment (but not in the surrounding tissue), an increase in

total T cell numbers in the tumor, increased neutrophil infiltration (involved in suppression of autoimmunity), activation of $CD4^+$ and $CD8^+$ T cells, and depletion of tumor-associated macrophages.

These bispecific antibodies were thoroughly characterized and showed high specificity and affinity. No systemic side effects were observed (as tested for liver and kidney function, systemic inflammation, and weight loss). However, the possibility of side effects should be considered for each disease/treatment context, because in some FDA-approved immune checkpoint blockade therapies, immune-related adverse events have developed as side effects.

Overall, these results demonstrate a new strategy for improved immunotherapeutics. Dr. Liu has now joined the Wu lab at PCMM and is working on another bispecific antibody, conjugated to a gasdermin D agonist, an innate immune response inducer.

Dr. Le Gall and Dr. Liu conclude:

"One of the most surprising aspects of this study was how robust our platform turned out to be. The nanobody fusions recruit endogenous immunoglobulins, repurpose them as checkpoint blocking antibodies, and achieve potent checkpoint inhibition via targeting either PD-L1 or CTLA-4. In addition, the fusions can recruit immunoglobulins of any isotype, which diversifies Fc-effector mechanisms to maximize immune cell activation and deplete checkpoint-overexpressing immune suppressive cells. This simple strategy rivals traditional monoclonal antibodies in efficacy, manufacturability, and stability. We were pleasantly surprised by how well the bispecific nanobody fusions performed *in vivo*. They outperformed the benchmark therapy in multiple mouse cohorts. That level of robustness was an eye-opening demonstration of the design elegance and potential for clinical translation. Looking ahead, we want to take advantage of the unique features of our bispecific nanobody fusions. They are very small, highly stable, and well-suited for aerosolization, mucus penetration, and alveolar transport. Our goal is to develop inhaled formulations for lung cancer that deliver them straight to the tumor-bearing lung, achieving higher local concentrations, reducing systemic side effects, and making treatment easier for patients."

Xin Liu, PhD, Camille Le Gall, PhD*, and Ryan Alexander, PhD*, were the first co-authors of the article, and Hidde Ploegh, PhD, (now appointed as the Jon van Rood Professor in chemistry of the immune system University of Leiden Medical Center) was the senior author. Other authors include Ella Borgman and Thomas Balligand, MD PhD.*

Xin Liu and Hidde Ploegh have filed for a patent on the bispecific antibodies mentioned. The rest of the authors declare no conflict of interest.

Introductions: Pengxin Chai of the Springer Lab

Submitted by Colin Smith on August 27, 2025

Photo: courtesy of Pengxin Chai

Tell us about your role here at BCH.

I'm currently a Post-doc Research Fellow in Tim Springer's Lab! My previous background is in structural biology but here in the Springer Lab I wanted to receive some training in other areas of biology – cell biology and single molecule biophysics.

Where were you before you joined the Springer Lab?

Before joining the Springer Lab, I was working on my PhD at Yale in the Molecular Biophysics and Biochemistry program. I worked in Dr. Kai Zhang's lab, who is an expert in cryo-electron microscopy. During my PhD, I developed some single particle cryo-EM methods, including different ways of preparing samples, and also some of the imaging processing methods to help us to solve the structure of microtubule systems.

What's your favorite thing to do in the lab?

I mainly worked on cryo-electron microscopy (cryo-EM) previously, and cryo-EM is highly modular, it has several steps, and my favorite step is actually the glow discharge. For cryo-EM, or any EM experiment, you need to deposit your samples on an EM grid and the EM grid needs to be glow discharged before you apply the samples, because if you don't the sample in the buffer won't be spread out. So you use a glow discharge machine, and I like that most because you will see some purplish shining during the glow discharge process.

What's the best piece of advice you've ever gotten?

The best piece of advice I've ever gotten actually wasn't from my mentor, but from my interviewer during my PhD program, Tony Koleske. One piece of advice he gave me that I've thought about for a long time is that every talk is a job-talk. You need to treat every talk as a job-talk, including your Research in Progress talk. His reasoning was that when you reach a final or formal job talk, you will be well prepared.

What are your hobbies outside of work?

I'm a sports fan! And while I do a lot of sports, I'm not really that good at any one of them. When I came to Boston, I started playing a lot of frisbee in the quad. If you see people playing frisbee, I'm probably one of them. I also do rock climbing, I'm a little afraid of heights though! I'm still working on that.

What made you want to pursue a career in science?

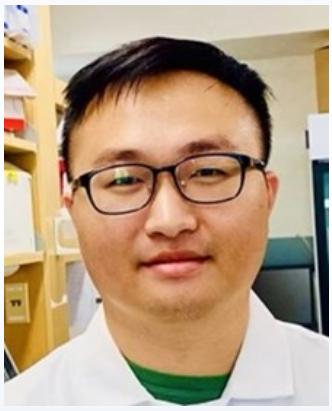
Doing science, and exploring new things, is fun. In general, in the Springer Lab, I'm doing a lot of cell biology and fluorescence microscopy. I think the nature of microscopy is to look at things under a microscope, kind of

exploring new things, because you never know what you'll see under the microscope, and I think during my entire PhD, a lot of my projects came about in a very surprising way. You can never predict what you'll end up getting. Learning from these previously unknown surprises encouraged me to pursue a career in science.

If you had unlimited funding, what kind of research would you do?

Previously, during a meeting, I met a group from Europe, and part of their research is to drive an RV, in which they have a cryo-EM sample preparation lab. Basically, they drive the RV to different areas and collect samples from nature and look at cells under the microscope, which I think is really fun, so if I had unlimited funds, I think I would purchase an RV and make a sample preparation room in it, and drive the RV around the world collecting samples. I think I would do the national parks first, because there's a lot of interesting biology there!

PCMM Researcher Wins a Prestigious Fellowship



Le Xiao, PhD, (Wu Laboratory) has been awarded an NIH K99/R00 Pathway to Independence Award from NIH. The NLRP3 inflammasome is a vital sensor in the innate immune system, linked to numerous inflammatory and chronic diseases. Dr. Xiao uses cutting-edge cryo-electron microscopy and biochemical techniques to uncover how danger signals such as nigericin drive NLRP3 from an inactive form into its active, signaling state, and how post-translational modifications (PTMs) fine-tune this process. The research further identifies novel protein partners that regulate NLRP3 function. This work deepens understanding of immune regulation and provides a structural basis for developing targeted anti-inflammatory therapies.