Hi, I'm James Barker, an Associate Publisher from F1000.

and I'm joined today by Dr. Dr. Carl Laflamme, a Research Associate from McGill University and Head of the Antibody Characterization Group.

Today we are talking about antibodies.

Antibodies are a vital component of our immune system and also a
Antibodies in our body will be produced in response to infections and our body engineers them to be highly specific through a process of trial and error, creating more and more antibodies until they find one that binds perfectly to the target. That specific binding to a specific target is something vital component of research.
that we now exploit in the lab.

Most classically, this is done through techniques such as Western Blots, where we will produce commercial antibodies or might produce them in the lab ourselves, and these will be raised to a specific target.

And then we can use them to identify those specific proteins in complex mixtures that we might extract from things like
cell lines or from tissue samples.

Now while there are thousands of these antibodies available, not all of them work in the way that we expect them to, either binding to incorrect targets or just not binding to anything at all.

And this is obviously a massive issue when it comes to research.
in terms of resources and in terms of wasted time, but it's something that is very rarely addressed.

So Carl, what would be great is if you could explain a bit about this issue that we're having with antibodies and their ability to actually work in the experiments we're using them for and a bit about the work that you're doing to address this.

So thank you.
Thank you, James.

It's actually a pleasure to be here today.

There are various kinds of antibodies and today we'll be discussing the research antibodies, the ones that we use in the lab, as you said, in contrast to therapeutic antibodies, when selective, as you said, antibodies are great.
They can detect one protein in a complex cell mixture for diseases.

For example, we can look at the protein that will go up or down in a certain disease.

And if it goes up and it needs to be down, we can develop a drug that targets against the protein and so on.

And when selective antibodies can give us this information.
But unfortunately they don't necessarily behave as advertised by the manufacturers.

So there are articles in the literature that used underperforming antibodies leading to bad hypotheses, bad conclusions etcetera.

When I was in the lab, we were looking at cancer cells and
extracting the proteins there to
look at up regulation of certain areas

where it might be binding sites
or messenger proteins.

So what are some of the examples
of diseases where you know this,

these antibodies are used to
classify those proteins and

what essential issues where they,
when they don’t work, what that

might lead to?

Sure.
So we are at the Montreal Neurological Institute at The Neuro and we do focus on proteins involved in neurodegenerative diseases.

And what's quite tremendous or really fun is that we've been able to characterize antibodies and identify key successful antibodies for all 25 proteins linked to amyotrophic lateral
sclerosis or ALS and for many proteins involved in Parkinson's
disease and many actually for Alzheimer's disease.

So first, that was a big, a great breakthrough.

Yeah.

And I guess the key problem there is where you might have that unspecific binding to those proteins, particularly on something like a Western blot when you're looking at
the relative expression so, I should maybe explain it.

When you do a Western blot, you usually use some sort of what we call a housekeeping protein, so usually gap DPH or something more like actin or something like that where you can know what the expression should be in a cell of what that would be. So then you can compare that to say a disease protein, so TP53
for example in cancer cells where you see the up regulation

or down regulation of that, you can look at that difference in

expression.

But if you have, for example, unspecific binding to proteins that particularly if one might be on a similar molecular weight, so that's what you're using is that comparison, that inappropriate binding could mean
that when you look at that block, you know you're seeing more expression or less.

expression or expression in other areas, which is not telling you the true picture of what's happening in vivo.

But on the, you know on the page that you're seeing that and that obviously could be a massive issue down the line because you could be making assumptions on this protein,
does this, this protein does that.

And actually it's completely unfounded.

And while you've done everything perfectly for yourself, and I'm sure you of course do all your processes perfectly, when you're in the lab, then it's actually the antibody which is the crucial problem.
And I guess another thing that should be talked about is that these antibodies aren't always cheap as well.

Some of them are very reasonable, but if you're in research limited settings that can be a large issue as well so to jump on your point about having great antibodies to evaluate protein expression.

So we’re working at The Neuro
but in close collaboration with

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the Structural Genomic
Consortium, the SGC.

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And one goal of the SGC is to
explore the unknowns, to explore

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these proteins that we don't
know anything about.

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And to be honest 90% of human
proteins, we don't know much

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about them, but most of these
proteins are involved in neuro-

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degenerative diseases or in
cancer.
So with our good collection of high quality antibodies, we're now able to say for example in the ALS, in the Amyotrophic Lateral Sclerosis, where are these protein express. And what we found is even though in ALS motor neuron dies, not all ALS disease genes are expressed in motor neurons, some are in other types of brain cells and that really guides
therapeutic roots and that is actually very important and

that's something we're really excited about.

So the community needs one or two potent selective renewable antibodies for every single human proteins.

We know without doing any work that at the moment there's 1 million or so commercial available antibody for roughly 70% of the human proteome.
So what we decided to do, we created YCharOS.

YCharOS is a program to characterize the available antibodies in collaboration with leading antibody manufacturers, granting agencies, participating academics, to characterize side by side each of these antibodies for three research application and release quickly and openly all the data to benefit the
At the moment, we characterized more than 700 antibodies against roughly 70 human proteins. And what’s really exciting is that for most of these proteins we could find at least one renewable antibody for each of these protein. And this is spectacular.
Yes, there's some antibodies that failed and that's correct.

We report all negative data, but in these reports, they can be used as a guide.

You can see that there's one potentially one key renewable antibody for all three applications for most of these proteins.

So as part of this and as we've mentioned several times, these
antibodies that are being used in mainly commercial antibodies and one of the large parts of working on YCharOS is the fact you’re working directly with the people who are producing these antibodies. So maybe you could talk a bit about that commercial partnership that you have and how that is helping with this
process of characterizing and validating these antibodies,

right.

So these partnerships are very important.

The very first step and that was key was to develop trusted protocols because as you say, there's no standardized protocols for testing antibodies.

So that was a gradual process, but we decided to use strictly
knockout cell lines to characterize these antibodies.

A knockout cell line is a cell line where we removed only one gene and that gene produces the protein of interest.

So briefly we have the parental line and then the knockout cell line which there's only one protein missing and that's a protein to be targeted by the antibody.
So in theory, if the antibody is specific, we will have signal in the parental cell and absence of signal in the knockout cell line. And that would be our general process for all applications which we're testing antibodies. So this is a great example of commercial and research groups coming together to address a problem. But considering that these
commercial bodies are producing

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you’re saying you’re finding

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in the way that they’re

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question is what is the benefit

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partners that are helping you on

00:09:59.673 -- 00:10:00.250 YCharOS? Right.

00:10:00.450 -- 00:10:01.370 That’s a great question.
So we work in complete open science setup.

We're completely transparent in our antibody characterization reports.

We show both the positive results, the great antibodies as well as the nonspecific antibodies.

We do not remove any data.

Companies do like it and our partnerships involves companies
that are really, that really care about the issue.

So for example, 20% of all antibodies that we tested that are nonspecific have been removed from the catalog.

So no one will ever use these non selective antibodies.

And James, we do see for some diseases and I'll take the example of Parkin which is a protein involved in Parkinson's
Most of the antibodies that were used previously to our report have been used, antibodies that were specific but not perfectly outstanding.

And one company saw that opportunity and developed brand new recombinant antibodies, perfectly renewable that are performing spectacularly both for Western Blot and Immuno-
precipitation.

And now the field have been using these two antibodies for, for a little while now.

And so we’re publishing these data notes.

So data notes are articles which describe the methodology and how you’ve produced a set of data, but don't provide any
interpretation or effectively any sort of results alongside.

It’s literally the data how you collected it and how you went about doing that process.

What do you feel are the advantages to presenting these characterization reports in that format where it is quite agnostic without that level of interpretation about the
antibodies, just providing the data about how they work.

So we presented the YCharOS initiative many times and the feedback we received was that, oh, this is great work, it's easy to interpret.

What we found is a researcher that is looking for an antibody for Western Blot will be more than able to interpret our
characterization data for 10 different antibodies on the single figure.

The problem with scoring an antibody would be that that score would be accurate in the experimental settings that we've been using for these reports.

Outside these very precise experimental conditions, the antibody might be subperforming, might recognize
other proteins.

Hard to tell.

So we provide a guide so that scientists can select the most promising antibodies for their need.

And it would be important that scientists be careful and acknowledge that the antibody might behave differently in their setup.
Great.

And I think as well we were just discussing earlier the fact that these are now being incorporated into RRID.

So research resource identifiers and the fact that these reports are now attached to those identifiers where people might then go away and use these antibodies and and cite that
identification that we at F1000 encouraged authors to do and

other journals do it as well.

The fact that these reports are now being associated with those IDs and providing that characterization alongside that I think is vitally important and something that really should be becoming much more universal across publications to provide

that peer reviewed,
But also that I really do think that agnostic side of it where you were just presenting the facts without any interpretation and just being like this is the information we provided.

You can go and interpret it as you said, not every condition is going to be the same and a good researcher should realize that's the case and realize just doing an experiment following the same protocol doesn't mean you'll get
the same outcome every single time.

But providing that report alongside it is not only great for you to get the views on it, but I think also to provide that information where it's not previously been available.

I think is a really good step towards addressing this replication crisis and I think YCharOS is a great contributor to
that.

And the RRID components is important.

You know we characterize 100 of antibodies and what I've noticed is most of the antibodies are called MAB1112 and MAB1112 could correspond to an antibody against protein X at company A, but could correspond to another protein to another company.

There's really a lot of
confusion.

So I believe yes, it is very important to use RRIDs in published papers and through the RRID protocol, a scientist can identify available characterization data directly through the RRIDs.

So yes, RRIDs are a key component in this adventure.
So it's amazing having these resources available in this open format where anyone can access them.

I think, I guess the question is what do you see these resources that you're providing achieving and what are the next steps after that with the YCharOS program and providing these characterization reports?
So what we've noticed in the past year is that some antibodies that we characterized 2 to 3 years ago that we showed to be completely nonspecific are still being used in research articles today. And this really annoys me. We hope that these reports can really help researchers for their selection of antibodies, and I'm happy to work with you.
with F1000 to have them better exposed to the community.

But we need potentially another mechanism to ensure that only good antibodies are being used right now in the literature, so that the ALS Society, the Parkinson's Society, do generate only reproducible data.

And we're having a collaboration with Harvinder Virk at the
University of Leicester who have used antibodies on human samples

and figure out a year after that the antibody he was using were non specific and now he's pushing addressing key antibody stakeholders so that we can try to find the mechanisms to really, really help.

The researchers to use only good antibodies.

No, yeah, I think that's it's definitely the aim that we
need to be going towards.

And I think also a very important part of these data notes and the way they're being presented is that open side of them and obviously open science is at the heart of YCharOS. Also the fact that it's not just that they are available to
everyone, it’s also that the data is available to everyone.

And we mentioned before how these antibodies can be very expensive.

So in those resource limited settings where they might be less likely to have the subscriptions to paywall journals where that information might be available by having these as an open resource that anyone can access, it removes that
So hopefully that research waste isn't just designated to certain regions who can access this information, it's available for everyone.

I guess what we've been talking about today is some of the sort of fundamental issues that underlying biomedical research right now.
And we've seen these with other areas, things like Open Access, the sharing of open data, reproducibility in general.

And I think one of the issues that we always see in these with these key fundamental problems is that people sort of go off into their own little sections.

Researchers, they're the ones doing the work.
So they’re like there’s responsibility of funders or

Publishers will say researchers, the ones doing the research,

that’s their responsibility to sort it out.

We’re too late in the process.

And that creates that stagnation where nobody wants to take

accountability, where realistically everyone is
accountable for the whole process throughout.

It’s a symbiotic relationship where it requires grant funders to mandate certain things, whether that be taking open data, for example, a funder mandates it, a publisher provides a venue that supports that structure and then researchers will do it because they’re mandated to do it and it creates a nice little cycle which it changes,
And then as more people do that, that changes the research ecosystem.

And I think that YCharOS provides a great example of this, but with that commercial and research partnership.

So maybe you could discuss how you think that process is addressing this fundamental issue we're seeing with these
antibodies?

It's funny cuz when I present YCharOS, I have this slide, it's a triangle.

And at each side you have publishers, scientists and antibody manufacturers and everyone saying it's your responsibility to do it.

No, it's you.
But at the end of the day, there's a stagnation.

No one's doing anything.

So yes, it's been three years that we're actively characterizing antibodies and what we're seeing is antibody manufacturers are really proactive.

They are removing antibodies from their catalog.

They are changing recommendation for their different antibodies.
But what we’re seeing is researchers are still using antibodies that are not specific, even though there are great alternatives.

So antibody stakeholders such as publishers like F1000 researchers, funders, needs to develop a mechanism where they could see which antibodies are available and are of high
And we need a way to make sure that these nonspecific antibodies aren't being used in the literature. I don't have the answer, but we'll need to sort it out. Maybe we need to organize a major symposium about this issue. Yeah, but it's really the issue after the antibody
characterization data, after these studies have been done,

what do we do with this data to ensure that everyone is taking advantage of them.

Well, I think just to round it all off, I think this is a really important discussion.

I think as someone who's worked in the lab and as someone who's, you know, studied in biomedical sciences, everyone
has almost certainly done a Western Blot at some point in

And while it's seeing, everyone has their story, right?

Yes, exactly.

Everyone's tried to make a gel when it's fell out through the

Everyone's tried to use an antibody and nothing's turned up
on the film when they've spent hours doing it.

I think it's a point of frustration when you're in the lab.

But then when you look at it at this higher level, when you're looking at the problem it's having with the academic record and the public and the actual published articles, it's
becomes less of a personal frustration to a fundamental

issue that needs to be addressed.

And the work that YCharOS is doing in partnership with these commercial partners and then publishing them through the gateway, I think is a great first step and one of the many first steps I hope we’ll see with this to address that
underlying issue.

So thank you Carl for joining us and thank you for providing your input.

All of these reports are available on the YCharOS Gateway, which is now available on the website F1000Research.com /YCharOS.