DR. CARSTEN KRIEG: In our paper, which was accepted in *Nature Medicine,* we actually initially asked the question whether we can predict response towards anti-PD-1 immunotherapy, because if you look at it, the-- so the most part of the patients do not respond towards immunotherapy. And we asked ourselves whether we can just design a very easy way to look at the proteome in the immune compartment and predict whether a patient responds or not towards immunotherapy.

What we clearly found, and what was known before, is that under therapy, you have a response in the T-cell compartments of the [INAUDIBLE]. It's a T-cell therapeutic. That's what it was designed for initially.

But, surprisingly, what we found is that actually it's monocytes. So the frequencies of monocytes which is enhanced in responders over nonresponders towards immunotherapy, which is a stratifier to give immunotherapy.

It's a very new technology, and what it does is it uses a rare metal conjugated antibodies instead of fluorochrome greater than copper antibodies. So this has the advantage that in normal biological tissues, there are no raw metals, so-- which allows us, first of all, we are more sensitive in detecting our targets and, secondly, we do not have any biologic background.

And then what we use for detection of these metal conjugated antibodies is mass spectrometry, which is a very old and very established, very robust technology in biochemistry, and also pharmacology, to detect new drug targets. And we use, actually, plasma, which-- into which we inject those cells which have the metal tact antibodies on the surface, and where this plasma is caught. So the machine is called the Helios. And the plasma has a temperature of 6,000 degrees. It's also the Helios of the sun, the temperature, and this will disintegrate the cells. So it's a destructive technology. Produce an ion cloud and we detect this ion cloud on the mass spectrometer.

And what we get at the end is it is the standard file you would got also on the flow centimeter. So it's this FCS file, which you, in terms, could analyze by hand. But we use artificial intelligence and bioinformatics. And so a human cannot interpret this data easily.

So what it does, actually, is it makes a two-dimensional visualization, a picture of it, which can

be a heat map, which can be a SPADE tree or which is these instagrams, as we call it, which is two-dimensional dot blots.

You have a picture before therapy, you have a picture during therapy, but you can make many more of these pictures. So you can look after three months, after half a year, after 12 month. So this enables you kind of like to do a Facebook approach. So every-- in the time frame, every time you get a picture of the immune system. So these instagrams pictures get context.

And by that, also together with histology, you can then make this much more networking. So you can tell where was your immune system before we switched the immune system, did it-- which component of the immune system did it interact with? Which tumor started to interact?

And this is something we are very, very excited, because this is a systems biology approach and will, first of all, help us many more like what we can combine an immunotherapy with, but also understand the mechanism and the biology behind it.

The technology which we have here now at MUSC, which is mass cytometry, allows us to, in the end, develop precision medicine more. I mean, if you look back on a couple of decades during the time of our parents, we only had chemotherapy. Then we started to sequence the genome, so we had DNA, we found mutations, and we were able to develop specific inhibitors for this. So we move away from chemotherapy to all its specific inhibitors.

Now, we look at the RNA and the transcriptome. And the exciting thing about this technology is that we now look at the proteome. And this is, actually, what makes the interaction. So where your immune cell interacts with an immune cell or tumor cell, because in the RNA, the transcriptome, you cannot really tell whether this makes a protein or does not make a protein.

And here at MUSC, we hope to help physicians, first of all, in their clinical trial to monitor success or to tell the clinician whether they should switch immunotherapy. And also there's a very exciting biology behind this to understand the mechanism how immunotherapy works and why, of course, interesting like artificial intelligence and to develop programs to analyze this.

I think the important thing also about this work was that this is done in a collaboration. So how it initially-- so a scientific community is kind of small. So this initially started this, that my former boss, [INAUDIBLE], he went to actually Singapore. And when the lab of Evan Newell got to know and learned about mass cytometry, brought this technology back to Zurich.

And then as a second element, we had the translational and immunology program in Zurich, which was headed by the head of dermatology, and this is Lars French, together with Konnie Bassler from the Molecular Life Science.

And that enabled us, together with the clinicians Reinhard Dummer and Mitch Levesque, to get access to melanoma samples, so melanoma patients who were treated with anti-PD-1.

And so there was the biologic side, there was the clinical side, and then we were very fortunate to also find the bioinformaticians with Mike Robinson, who then allowed us to actually develop a generalized bioinformatic workflow, which we show that it works here on anti-PD-1. But this can also be applied to any other disease or any other immunotherapy.