CELL & GENE THERAPY INSIGHTS

PRECLINICAL/TRANSLATIONAL TOOLS & STRATEGIES

SPOTLIGHT

PODCAST INTERVIEW with:

**Charlie Silver,** Mission Bio

"...for cell and gene therapy, single-cell approaches provide a much greater level of resolution to better characterize the products..."

# On a mission to bring single-cell sequencing to cell and gene therapy

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## Q Can you firstly introduce us to yourself and to Mission Bio?

**CS:** Mission Bio is a very mission-oriented company, and our mission is to help our customers eradicate cancer. We do that by helping drug developers and researchers in translational medicine bring precision drugs to the patients who need them, in the safest and most efficacious way possible. We also support them to reduce the time and cost of bringing these drugs to patients. We are truly very driven by our mission.

Mission Bio is a company we spun out of the University of California, San Francisco (UCSF). We have been on the market for a couple of years now, with the mindset that translational research and clinical spaces really need tools for precision, to understand what is inside cancer biopsies, and to understand with precision how drugs are interacting with patients, and how patients respond to drugs.

We are the only player providing single-cell DNA sequencing. This is a way of assessing the DNA content cell-by-cell, but at high enough throughput that you get a very precise and representative view of every cell in the sample, with great accuracy. Ours is the only platform that does single-cell DNA analysis, and over time we have also added single-cell multiomics, to the point where we can measure both DNA and protein inside every cell that matters.

We started in the space with an offering to cancer centers and drug companies that is used to run large-scale clinical studies, and help assess biomarkers of therapeutic response and resistance by understanding how the clonal architecture of biopsies change in response to treatment. This is particularly applied to clinical trials, where our partners use our technology to understand biopsies on a cell-by-cell basis, and with the depth of resolution and precision of accuracy we provide, they can get exquisite sensitivity to identify every cell in a biopsy that is either responding or resisting therapy.

The platform has come to be adopted by cancer centers everywhere. We sell to the majority of cancer centers here in the US, and many worldwide, as well as drug development companies who use this in support of their clinical trials. We also work closely with CROs and CDMOs that support the whole drug development space.

## Q Why the recent move into cell and gene therapy? What can single-cell sequencing bring to this field?

**CS:** When we came out with the platform a couple of years ago, we had the only product that did single-cell DNA, and we launched with a number of applications in blood cancers and solid tumor profiling.

One of the real advantages that we baked into the platform early on is the ability to build custom content, so we can customize to any gene set that is of interest, for any application. As we were launching the product in the leukemias, it became apparent that there is no one set of content that applies, even within leukemias – and certainly more broadly across cancers. Therefore it made sense to build in this rapid and high-quality customization capability, so we can


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work with our partners and very quickly build in any content, in a way that is purpose-built for their specific application.

Once we had a number of applications across leukemias, we had a lot of partners across the space who were customizing by either adding or subtracting content for their particular disease area or application. Along the way, we had a lot of interest from customers trying to use the platform with its customization capability to assess the quality and underlying characteristics of cell and gene therapy products.

This was not surprising to us. Once you have a technology that is able to measure changes that occur at the genetic level in every single cell that drives cancer, and once you are using that to study biomarkers of disease development within cancer, that technology looks very similar to assessing the gene modifications that we make in these cell and gene therapy drugs. The underlying requirements for the technology are fundamentally the same, just in a different application space.

We kept encountering people who were coming to us and saying "if you have a single-cell approach that does single-cell analysis, we could certainly use that to validate edits and validate integration events for cell and gene therapy products". We recognized that was a clear application set, especially for our customization capability. Over the last year or so we have really started building out applications into that space, to be able to serve the space better.

We know that for cell and gene therapies the burden of characterizing the product comes hand-in-hand with the complexity of manufacturing these drugs. Antibody products on their own are quite complicated to build and manufacture in a robust and repeatable way. When you edit cells, or modify cells, it adds a whole new level of complication when the cell itself is the product.

As a company, our mindset is that it is important to build the toolset that can characterize safety and efficacy of therapy for every patient. When we started with precision medicines, that was very much the mindset – resolution and precision is needed to be able to study the underlying clonality and underlying mechanisms for disease response and resistance for these targeted therapies.

We bring the same mindset to the cell and gene therapy space, where there is an ever-greater need for characterizing the complexity of these products, with a toolset that can untangle the complexity, and characterize it at the most fundamental level. Single-cell analysis is an approach that brings greater resolution in terms of understanding which clones are emerging or evolving as a result of therapy resistance, and understanding at a deep level the clonal heterogeneity of a tumor, and how that changes over time in response to both treatment and other factors of disease evolution.

Similarly, for cell and gene therapy, single-cell approaches provide a much greater level of resolution to better characterize the products, by assessing the critical attributes and characteristics of these products across the whole continuum. It can be applied from R&D development and optimization of the cell and gene product, to the safety and efficacy related to those

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attributes, all the way through to manufacturing release testing and full-on production.

What is really important to this space is the ability to characterize transduction efficiency and vector copy number, which tells you how many integration events have made it into every cell. Then there is the efficiency of that across the entire cell population, hand-in-hand with immune phenotype, which is a way of looking at the surface of the cell and understanding what impact those gene modifications have had on the cell state.

We take the same approach that we have leveraged for biomarkers in the translational space, and apply that toolset to match the complexity of these cell and gene therapies, with an integrated set of measurements that provide greater resolution and greater precision for making these quality control measurements.

**Q** Can you go into more depth on the specific capabilities and any further applications of relevance to cell and gene therapy, and how they can help address the challenges the field faces today?

**CS:** Let me step back and give a view of what the challenges are now, to help appreciate what single-cell can provide in terms of overcoming those challenges.

In characterizing a cell and gene therapy product, whether it is an optimization step as you develop the drug, or in release testing once it is developed and you need to qualify the lot to get it back into the patient, what is really important is understanding with great precision what you have done to change the genetic makeup of the cells.

To do that conventionally for the cell and gene therapy space, drug companies are using a large collection of technologies that run a very diverse set of assays. These are combined into a release test that ultimately characterizes dose and toxicity for the product. These technologies can range from qPCR to digital PCR, to flow cytometry, FISH, plus many other genomic technologies, which are all rolled up together at the bulk level to characterize the product.

Typically, up front of those assays there is a step where you sample the product. You take out a number of cells, then plate it up, typically into a 96-well plate. You grow it for 2 weeks, sample what you have grown in every one of the wells, then run your genomics or flow assays. All of these technologies together are fairly labor intensive to run at scale, because on the bench there are a number of discrete assays to run. They are time consuming due to the number of steps, and also because of that up front culture step required to grow the cells for the downstream analysis. As a result, the turnaround time for characterizing the product is typically in the order of several weeks, and usually more than a month.

Single-cell analysis offers a way of directly characterizing the product, and reduces the time to answer, because you don't have that lengthy cell outgrowth protocol. It also integrates a


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number of these assays together, to provide a much more efficient answer on a much more rapid timescale.

This serves as a drop-in replacement for what is conventionally used to characterize these cell and gene therapies. Ultimately what we are trying to do is get these therapies to the patients who need them as quickly as possible, in the safest and most efficacious way possible.

Reducing these several weeks of characterization time down to less than a week, with a single integrated measurement, offers a much more streamlined, efficient, and cost-effective way of running that characterization, in a way that is much more robust. What I mean by that is that conventionally these assays that are typically used can run in bulk, and because they run in bulk, you are inevitably making inferences back to what is happening at the level of the cell. The cell itself is what matters in this case, because that is the drug. So, conventional bulk approaches where you make these inferences are never going to be as accurate or precise as making a true measurement at the single cell level.

For all these reasons, single-cell gives a much more efficient solution for all of these assays that combine together. Rather than having to interfere or interpolate back to the single cell level, you get a true measurement.

That becomes really important for these cell and gene therapy products, because you are trying to understand on a cell-by-cell basis whether, first of all, you have changed the genetic makeup of each cell. Then you need to know if you have inadvertently edited in or modified in anything that might be harmful – in other words, have you added unintentional toxicity to the product? When you look at that on a cell-by-cell basis, you are getting a truer readout of what is happening in the sample. Instead of looking in bulk, which is what is conventionally done, you are truly assessing what every cell is telling you.

What that means is that your bulk measurement might be the result of a few cells that have a totally different vector copy number, or it might be a result of a uniform integration into every single cell, and you just don't know that answer when you make that bulk measurement. Single-cell gives you a much more definitive and robust readout for the same measurement. This gives the space a much more precise view into what cell and gene therapy products are doing, before the product goes back into the patient.

The need across this space is great, and it is really important to begin standardizing how the space is characterizing these cell and gene therapy products. Ultimately, that standardization is best done at the single cell level, because that gives you a true readout of what the cells are doing.

The more you can add in at the front end of a development process for cell and gene therapy to really robustly characterize it, the more your program is set up for success in making it all the way through to FDA clearance. We have seen a number of examples – Freeline were in the news recently, and we have seen some others like Bluebird Bio, BioMarin, and Voyager, where they have started down the path with

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the best of intentions, and with a set of characterization assays at pilot scale that they then transferred through to clinical trials. Midway through the trials they had to slow down, because they realized there were additional characterization steps that were needed. These types of delays can really have an impact on how long it takes to get these drugs to market.

By reducing the time to answer, by reducing your cycle time in making these measurements, and then by adding in a full suite of characterization attributes, from vector copy number to editing and transfection efficiency through to phenotyping, and providing all of that in an assay up front, you can really enable standardization for these critical attributes that are needed in every stage of the development cycle. This can also alleviate some of the data integration and variability issues that can occur with the plethora of assays that are conventionally being deployed.

We believe that providing the best toolset at the front end of the process, and then enabling that same toolset to carry through to every stage, all the way through to manufacture release testing, gives a much more robust set of measurements to enable these drugs to get into patients within the time that is desired. While also providing a much faster readout, and a much faster characterization cycle time.

**Q** How easily can single-cell sequencing be implemented into cell and gene therapy developers' existing processes? Are there any challenges in achieving this, and how does Mission Bio address those?

**CS:** This a good question, because we are leveraging this toolset to replace existing assays. Ease of integration tends to be really important, because folks are used to using these conventional assays, and it is important to cleanly bridge over that.

The real positive of what we are doing is that NGS is our readout. We are doing single-cell sequencing with NGS as a readout, and this is a datatype that the entire space, and particularly the FDA, is very comfortable with. They have had years of working with this type of assay, especially around NGS applications across precision oncology. Technically anyone using these existing assays has a nice clean bridge over to implementing our assays, because it looks very similar.

The ease of integration is also really important because we replace or supplement a number of these different and various technologies and assays with a single integrated measurement. You get a single definitive result without the challenges of integrating multiple assays with bioinformatics, which can be needed to glue together a lot of different assays. It really simplifies the ability to get to a simple and robust answer quickly, and that really makes a difference in terms of integrating with existing cell and gene therapy pipelines.

Again, because it is single-cell analysis, it tends to be much easier to interpret, because you don't have that interpolation step where you have to take a bulk measurement and then try to apply it to what is fundamentally a single-cell product. You are making a true measurement at the single-cell level straight off. That ease of interpretation tends to be much easier to adopt for cell and gene therapy partners. It also provides a much cleaner measurement as they characterize all of the parameters that ultimately add up to the safety and efficacy of the product.


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This single integrated readout is the hallmark of what single-cell does. It is something that as a company, we have got very comfortable with through our years of biomarker work with the pharma industry, and that translates very cleanly over to existing cell and gene therapy workflows.

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**Q** Could you summarize your chief goals and priorities as a company in cell and gene therapy over the next 1–2 years?

**CS:** As a company we have been working with a lot of partners in the space at the pilot stage, where we will come in and support the front end optimization of their drug development. Again, because of the complexity of these products, that optimization tends to be very important.

For example, for CRISPR edited products, depending on how you set up your CRISPR system, that CRISPR system can have an enormous impact on the efficiency of edits both on-target and off-target, and the functional result of those. So optimizing at the front end tends to become very important. Similarly, for the products that are made using viral transfection, that optimization step in reducing time to answer makes a big difference at the front end of your development pipeline, to make sure you have got the right system to go on to full manufacturing, and ultimately to make sure you don't have hiccups as you scale up your clinical studies. Coming in at the front end tends to be very important with these partners, and that is something we have done a lot of over the last year or so.

Over the next couple of years we are going to continue implementation in our customers' processes, from that early optimization and validation step, through to the chemistry, manufacturing and controls (CMC) process, and ultimately release testing. We will start with the front end, then continue supporting the processes all the way through to release, to help them get their drugs into patients faster.

Our ultimate goal across the space is to move towards a standardized assay that can support every cell and gene therapy player out there. We think that standardization is going to become very important to move the entire field forward. Once we know what we need to test for, and once we have a clean and robust set of measurements that provide both a gold standard but also ground truth for every sample being measured, this will provide a much more accurate characterization method across the entire therapy development pipeline.

There are so many of these drugs in the pipeline right now, and the real bottleneck in implementing them, and getting the therapies to patients, is that characterization time. We believe that by evolving into standards, we will enable the space overall to reduce characterization time, and help get the drugs to market for the right patients, in the right way, as quickly as possible.

A lot of our priorities over the next couple of years are around continuing to move our customers through that pipeline to full-scale implementation at the back end of their clinical

trials. Next, our priority is to support standardization across the space, so that it becomes routine and efficient for every player.

**Q** Finally, how do you see this technology area evolving further in the future, and what new opportunities and applications might this open up in due course?

**CS:** The basic mindset behind Mission Bio is that people are complex, and cancer is complex. It is really important to provide tools that simplify, and help us understand that underlying complexity at the level of the disease.

We are fundamentally single cell creatures. Every one of us is made of 30 trillion or so individual cells, but it only takes a genetic change in one of those cells to cause or to drive cancer. We are truly complex, but even within that one cell that would cause cancer, or within the many single cells that constitute a biopsy, every single molecule is important to the disease, from the DNA all the way through to the protein as the functional result. It is important to match that complexity, in order for us to be able to make a difference in cancer, and ultimately enable our customers to eradicate cancer.

We have started with a single-cell DNA product because that is the assay that is needed in the biggest way for the translational, clinical, and production side of the space for real patient impact. When we started with DNA, we started with single-base resolution, which is important for a lot of cancers. We expanded that into copy number, where we can do gene level copy number, which is the foundation of our cell and gene therapy offering. In addition to single nucleotide changes, we can run copy number at large scales across the chromosomes. Every scale of DNA is important, and we have continued to evolve the platform so that we can support everything that is needed from a DNA perspective.

We have also added capabilities for protein, which was a launch that came out last year, and we now have a full toolset. I think of it as book ending the dogma of biology: you can measure DNA, which is the blueprint of life, all the way through to protein, which is the functional result of it. That is what drug development programs are built around, and now you can characterize that entire pathway from DNA through to protein at the ground truth; at the level of the cell.

Over time, the mindset of the company is to continue to fill out every analyte and every measurement that needs to be made at the single-cell level, in order to support bringing these drugs to market faster, and bringing therapies more quickly to the patients that need them, both in a precision medicine setting and also for cell and gene therapies. Over time we are also going to continue expanding the capability of what we can do from a single-cell multiomics perspective.

## BIOGRAPHY

**Charlie Silver**

Charlie is CEO and co-founder of Mission Bio, where he leads a team dedicated to solving complex biological problems with precision engineering, innovative biochemistry, and supported

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bioinformatics. Charlie has dedicated his career to commercializing next-generation hardware technology and scientific instrumentation at emerging ventures in healthcare and semiconductor industries. Prior to Mission Bio, he led R&D and Product at Novelx (acquired by Agilent) and then served in R&D and Marketing at Agilent. Charlie received a joint MBA from UC Berkeley and Columbia University, an MS in physics from UW Madison, and a BA in physics from Columbia University.


## AUTHORSHIP & CONFLICT OF INTEREST

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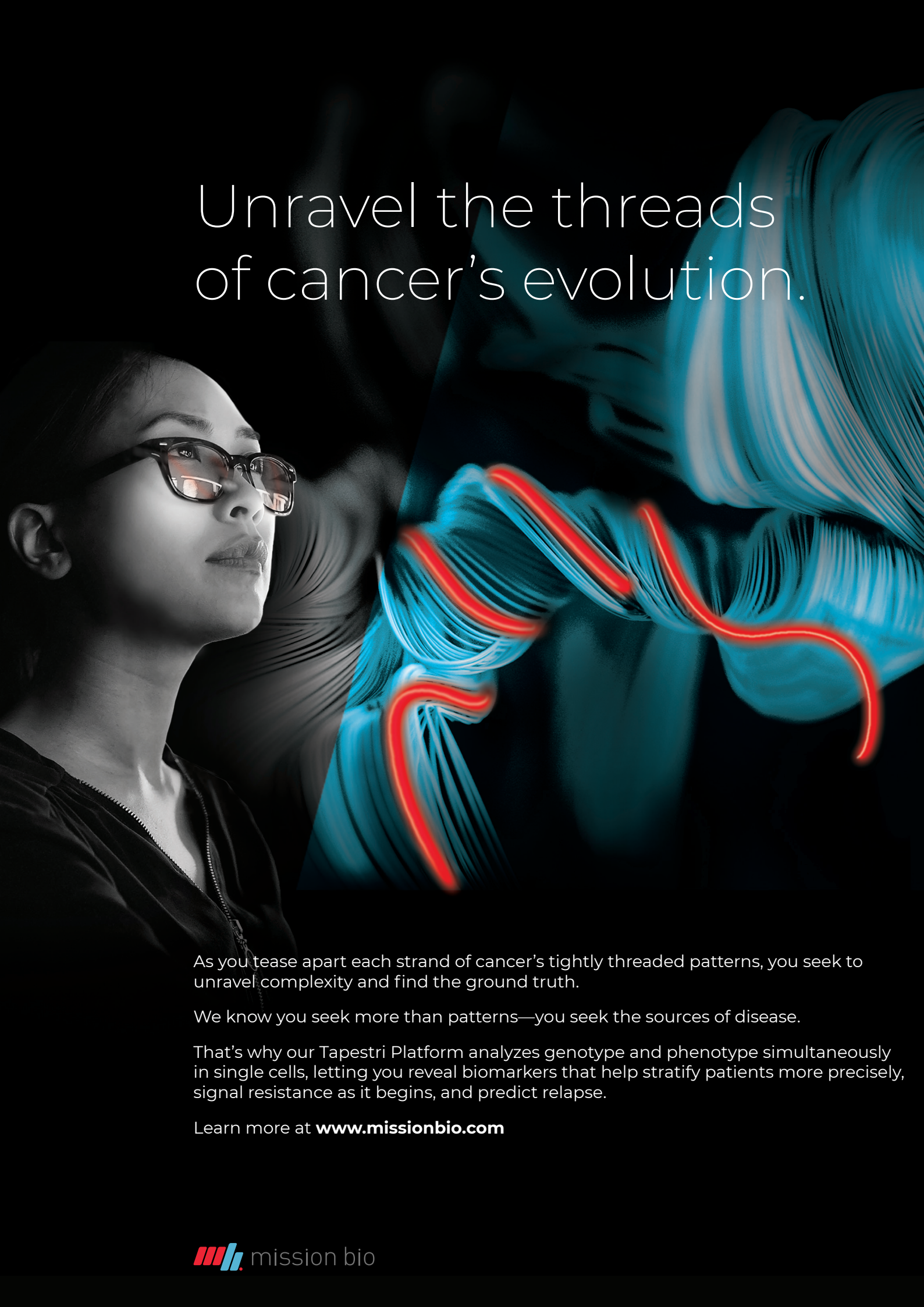


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