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76th Meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC)Meeting

Zoom Video Conference

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Call to order

Dr. Ahsan: Great. Good morning. I'm Taby Ahsan, Vice President of Cell and Gene Therapy Operations at the City of Hope. I'm acting chair for today's meeting. I'd like to welcome everyone to the 76th meeting of the Cellular Tissue and Gene Therapies Advisory Committee for the Center for Biologics Evaluation and Research at the Food and Drug Administration.

Today's meeting, we'll meet in open session to discuss and make recommendations on the BLA125787 from Vertex Pharmaceuticals for Exa-cel. The applicant has requested an indication for the treatment of Sickle Cell Disease in patients, 12 years and older with current vaso-occlusive crises. So, I'd like to welcome all the committee members, the participants and the public that's viewing remotely. Again, I want to remind committee members and participants to use the raise your hand feature and turn on your camera when you have a question or comment to make, and then I can recognize you, and then you can be called on to speak. And so, with that, I'd like to introduce Cicely Reese, the designated federal officer for today's meeting to make administrative announcements, conduct roll call and read the conflict of interest statement.

Administrative Announcements

Dr. Reese : Thank you, Dr. Ahsan. Good morning, everyone. I am Cicely Reese, and it is my honor to serve as the designated federal officer for today's 76th Cellular Tissue and Gene Therapies Advisory Committee meeting. On behalf of the FDA, the Center for Biologics Evaluation and Research and the committee, I am happy to welcome everyone for today's virtual meeting. Today, the committee is meeting in open session to discuss and make recommendations on Biologics License Application 125787 from Vertex Pharmaceuticals Incorporated. Today's meeting and topic were announced in the Federal Register Notice that was published on September 7th, 2023.

At this time, I would like to acknowledge and thank my Division Director, Division of Scientific Advisors and Consultants, Dr. Prabha Atreya, my team, whose contributions have been critical for preparing today's meeting. Those persons include Ms. Tonica Burke, Ms. LaShawn Marks, Ms. Joanne Lipkind, and many others from the division who have provided helpful and administrative support in preparation of this meeting.

I would now like to acknowledge CBER leadership, including Dr. Peter Marks, Director of CBER, Dr. Celia Witten, Deputy Director of CBER, Dr. Nicole Verdun, the new Director of CBER's Office of Therapeutic Products, and many other OTP staff who will be serving as speakers and presenters during the day, as indicated on the agenda. On behalf of DSAC, our sincere gratitude also goes to many CBER and FDA staff working very hard behind the scenes to ensure that today's virtual meeting will also be a successful one. I also thank all other FDA staff contributing to today's discussion, some of whom are present and others who may be joining the meeting at other times.

Please direct any press or media questions for today's meeting to FDA's Office of Media Affairs at fdaoma@fda.hhs.gov. I would like to thank the audio-visual team, Ms. Gretchen Carter, Devante Stevenson, and Derek Bonner for facilitating today's meeting. The transcriptionist for today's meeting is Ms. Debbie Dellacroce. And we will begin today's meeting by taking a formal roll call for the committee members and temporary voting members. When it is your turn, please make sure you turn on your video camera and you are unmuted. Then, state your first and last name, organization, expertise, or role, and when finished you may turn off your camera so we may proceed to the next person.

Please see the member roster slides, in which we will begin with the chair. Dr. Ahsan, please go ahead and introduce yourself. Thank you.

Roll Call

Chairperson. Ahsan: Good morning. Thank you, Cecily. So, I'm Tabby Hassan. I'm vice president for cell and gene therapy operations at the City of Hope. My expertise is in biomedical engineering, or I'm a bioengineer by training, particularly in the applications of stem cells, tissue engineering and of late immunotherapies.

Dr. Reese : Thank you. Next, Dr. Breuer.

Dr. Breuer: Morning. My name is Chris Breuer. I'm the director of the Regenerative Medicine Center at Nationwide Children's Hospital in Columbus, Ohio. And my expertise is in translational research and regenerative medicine. Thank you.

Dr. Reese : Thank you, Dr. Crombez.

Dr. Crombez: Hi, I am Eric Crombez, chief Medical Officer at Ultragenyx. I've been working in the field of gene therapy for the past nine years, trained in pediatrics and in genetics, and I'll be serving as the industry representative.

Dr. Reese : Thank you, Dr. London.

Dr. London: Good morning. I'm Wendy London. I'm a biostatistician from Dana-Farber and Boston Children's Hospital. I'm the director of biostatistics within Pediatric HemOnc, and my expertise is in prognostic factors for neuroblastoma, and I've served as a study statistician on many trials for pediatric oncology and Sickle Cell Disease.

Dr. Reese : Thank you. Next slide, please. Dr. Kathleen O'Sullivan-Fortin.

Dr. O'Sullivan-Fortin:Hi, I'm Kathleen O'Sullivan-Fortin. I'm a patient advocate and co-founder of ALD Connect, and I'll be serving as the consumer representative.

Dr. Reese: Thank you. Dr. Ott.

Dr. Ott: Good morning, everybody. My name is Melanie Ott. I'm the director of the Gladstone Institute of Virology and a professor of medicine at UCSF in San Francisco. My expertise is in viral pathogenesis, viral vectors, and delivery. Thank you.

Dr. Reese : Thank you, Dr. Wu.

Dr. Wu: Good morning, everyone. My name is Joe Wu. I'm the director of the Stanford Cardiovascular Institute. I'm a professor of medicine and radiology. My expertise is in cardiac cell therapy, gene therapy, and organoids.

Dr. Reese : Thank you. Next, we will do a roll call of our temporary voting members, starting with Dr. Robert Dracker.

Dr. Dracker: Thank you for letting me attend this meeting. I am currently the chairperson of the Pediatric Advisory Committee for the FDA. I'm a pediatrician, hematologist, oncologist, and transfusion medicine specialist. I am in Syracuse, New York and medical director of Summerwood Pediatrics and Infused Care Medical Services. Thank you.

Dr. Reese : Thank you. Miss Hightower.

Ms. Hightower: Hello. My name is Jasmine Hightower. I am a patient advocate for Sickle Cell. I am also a patient. I have a background and master's in social work and I am currently on the board and on many Sickle Cell and rare disease advisory committees. And I will be your patient representative today.

Dr. Reese : Thank you very much. Dr. Komor.

Dr. Komor: Hi, I'm Alexis Komor. I'm an assistant professor of chemistry and biochemistry as well as the Deputy Director of the Sanford stem cell Innovation Center at the University of California, San Diego. And my expertise is in genome editing.

Dr. Reese : Thank you. Dr. Lee.

Dr. Lee: Good morning. My name is Lisa Lee. I serve as the associate vice president for research and innovation at Virginia Tech, where I also serve as a professor of public health. I'm trained in epidemiology and public health ethics, and I am serving today as the bioethicist for the panel.

Dr. Reese : Thank you. Dr. Shapiro.

Dr. Shapiro: Good morning. My name is Amy Shapiro. I'm a pediatric hematologist oncologist. I am CEO and medical director of the Indiana Hemophilia and Thrombosis Center. My area of expertise is hemostasis, thrombosis, classical hematology, including Sickle Cell and clinical research. Thank you.

Dr. Reese : Thank you. Dr. Tisdale.

Dr. Tisdale: Hi, I am John Tisdale. I am Chief of the Cellular and Molecular Therapeutics branch at NHLBI, and I've been working on transplant and gene therapy for Sickle Cell disease for now almost 30 years.

Dr. Reese : Thank you. Dr. Wolfe.

Dr. Wolfe: Good morning. I'm Scott Wolfe. I'm a professor at UMass Chan Medical School. My lab focuses on genome editing and off-target analysis. Excited to be here.

Dr. Reese : Thank you very much. Thank you to everyone. There are a total of 14 participants, 13 voting members and one non-voting member. Thank you very much for your introductions.

Conflict of Interest

Now I will move to the conflict of interest statement. Before I begin reading the conflict of interest statement, I would just like to briefly mention a few housekeeping items related to today's virtual meeting format. For members, speakers, FDA staff, and anyone else joining us in the Zoom room, please keep yourself on mute, unless you are speaking, to minimize feedback. If you have raised your hand and are called upon to speak by the chair, Dr. Ahsan, please turn on your camera, unmute, state your name, and speak slowly and clearly so that your comments are accurately recorded for transcription and captioning. Thank you. I will now proceed with reading of the Conflict of Interest Statement for the Public Record. Thank you.

Dated October 31st, 2023. FDA Conflict of Interest Disclosure Statement. Read for the Public Record by Cicely Reese, Designated Federal Officer, Division of Scientific Advisory Consultants, DFO for this committee meeting. The Food and Drug Administration is convening virtually today, October 31st, 2023, the 76th meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee under the authority of the Federal Advisory Committee Act of 1972. Dr. Taby Ahsan is serving as the acting chair for today's meeting. Today, October 31st, 2023, the committee will meet an open session to discuss and make recommendations on Biologics License Application BLE125787 from Vertex Pharmaceuticals Incorporated for Exagamglogeme Autotemcel or Exa-Cel. The applicant has requested an indication for the treatment of Sickle Cell Disease in patients 12 years and older with recurrent vaso-occlusive crises. This topic is determined to be a Particular Matter Involving Specific Parties, or PMISP. With the exception of the industry representative member, all standing and temporary voting members of CTGTAC are appointed as special government employees or regular government employees from other agencies and are subject to federal conflicts of interest laws and regulations.

The following information on the status of this committee's compliance with federal ethics and conflict of interest laws include, but are not limited to, 18 USC section 208, which is being provided to participants in today's meeting and to the public. Related to the discussions at this meeting, all members and RGE and SGE consultants of this committee have been screened for potential financial conflicts of interest of their own, as well as those imputed to them, including those of their spouse or minor children, and for the purposes of 18 US Code section 208, their employers. These interests may include investments, consulting, expert witness testimony, contracts and grants, Cooperative Research and Development Agreements, also called CRADAs. Teaching, speaking, writing, patents, and royalties, and primary employment. These may include interests that are current or under negotiation. FDA

has determined that all members of this advisory committee, both regular and temporary members, are in compliance with federal ethics and conflict of interest laws. Under 18 US Code section 208, Congress has authorized FDA to grant waivers to special government employees who have financial conflicts of interest when it is determined that the agency's need for a special government employee's services outweighs the potential for a conflict of interest created by the financial interest involved. Or when the interest of a regular government employee is not so substantial as to be deemed likely to affect the integrity of the services, which the government may expect from the employee.

Based on today's agenda and all financial interests reported by committee members and consultants. One conflict of interest waiver was issued under 18 US Code section 208 in connection with this meeting. We have the following consultants serving as temporary voting members, Dr. Robert Dracker, Dr. Lisa Lee, Dr. Amy Shapiro, Dr. John Tisdale, Dr. Scott Wolfe, Dr. Alexis Komor, and Ms. Jasmine Hightower. The following member has been issued a conflict of interest waiver for participation in today's meeting, Dr. Wendy London. The waiver is posted on the FDA website for public disclosure.

Ms. Kathleen O'Sullivan-Fortin is serving as the consumer representative for this committee meeting. Consumer representatives are appointed special government employees and are screened and prior to their participation in the meeting. They are voting members of the committee.

We have one patient representative, namely Ms. Jasmine Hightower. Patient representatives are special government employees and are screened and cleared prior to their participation in the meeting. They are temporary voting members of the committee.

Dr. Eric Crombez will serve as the industry representative for this meeting. Industry representatives are not appointed as special government employees and serve as non-voting members of the committee. Industry representatives act on behalf of all regulated industry

and bring general industry perspective to the committee. Disclosure of conflicts of interest for guest speakers follows applicable federal laws, regulations, and FDA guidance.

FDA encourages all meeting participants, including open public hearing speakers, to advise the committee of any financial relationships that they may have with any affected firms, its products, and if known, its direct competitors. We would like to remind members, consultants, and participants that if the discussions involve any other products or firms not already on the agenda for which an FDA participant has a personal or imputed financial interest, the participant needs to inform the DFO and exclude themselves from such discussions and their exclusion will be noted for the record.

This concludes my reading of the conflict of interest statement for the public record. At this time, I would like to hand over the meeting to Dr. Taby Ahsan. Thank you.

FDA Introduction

Dr. Ahsan: Thank you, Cicely. To start off the meeting today, we're going to have a short FDA introduction and that will be from Dr. Nicole Verdun the Director of Office of Therapeutic Products. Dr Verdun, could you turn on your camera and unmute yourself, please?

Dr. Verdun: Yes. Good morning. My name is Nicole Verdun, and I'm the Super Office Director in the Office of Therapeutic Products in CBER, and I'm happy to be leading the office at such an exciting time. On behalf of FDA, CBER, and the office of therapeutic products, I would like to welcome you to the 76th meeting of the Cell Tissue and Gene Therapies Advisory Committee. I would like to start by welcoming our committee members. Thank you for the time you have taken to review the materials provided in advance of the meeting in order to participate in the discussion today. I would also like to thank our invited speakers for sharing their expertise in the area of genome editing and associated genetic modifications in the morning session. I would like to thank members of the public who will

be participating in the open public hearing and those that have submitted comments to the docket.

Vertex Pharmaceuticals has submitted an application for Exa-cel for the treatment of Sickle Cell Disease in patients 12 years and older with recurrent vaso-occlusive crises, developed using CRISPR Cas9 gene editing technology to result in increased levels of fetal hemoglobin in recipients. We are here to discuss, specifically, the study and analysis of potential off-target genome editing with Exa-cel and additional recommendations.

As many of you know, Sickle Cell Disease is a debilitating hemoglobinopathy with significant unmet medical need and can carry a reduction in overall survival for those affected. In addition, curative options are significantly limited. I've had the pleasure of taking care of several Sickle Cell patients and admire the courageous and resilient patient community. I'm also reminded of the Sickle Cell Disease patient focused drug development program at FDA, in which we heard directly from patients and their caregivers, which highlighted the significant unmet need in this disease. It is an exciting time in cell and gene therapy that we are beginning to address some of this unmet need in a variety of diseases. Exa-cel has been studied for treatment of Sickle Cell Disease with severe vaso-occlusive crises and has shown efficacy and safety in this population. In today's discussion, we would like to focus the discussion specifically on the off-target analyses for genome editing for Exa-cel. We appreciate the committee's review and for the discussion today, and I would like to turn it back over to Dr. Ahsan to start us off. Thank you, everyone.

Dr. Ahsan: Great. Thank you, Dr. Verdun. So, at this point, we're going to have two guest speaker presentations, one on genetic editing and one on the off-targets of genetic editing at the end of those two presentations will then take questions from both speakers. So, at this point, I'd like to introduce Dr. Fyodor Urnov. Professor of the Department of Molecular and Cell Biology at UC Berkeley, as well as the Director of Technology and Translation at the

Innovative Genomics Institute in Berkeley, California. Dr. Urnov if you could turn on your camera and unmute yourself please.

Introduction: Genetic Editing – Dr. Fydor Urnov

Dr. Urnov: Good morning. I'm honored to provide a survey of the Scientific Foundations of Human Genome Editing for you today.

My disclosures, which as Dr. Reese just mentioned, were reviewed by the FDA prior to this meeting are shown here. I note my work as a paid consultant to Vertex Pharmaceuticals on the Exa-cel program.

I need to frame the state of our field of gene editing today by stepping 20 years back. So, at the time, the sole method for targeted genetic engineering in human cells was an approach called gene-targeting, a schematic of which you can see here. And when used in cancer cells, it was inefficient. Now, one in 400 cells acquired the desired gene knockout. It was also genotoxic. The knockout cells acquired a bunch of extra chromosomes in the process, and you can see them in the skiagram on the right. Most importantly, it just didn't work in primary human cells, so there were no therapeutic applications. They couldn't even be imagined.

Well folks, I can say that here we are in 2023 and we are proverbially in a whole new world. There are 27,000 references with the word Cas9 and PubMed and genome editing with Cas9 and other tools has been shown to work on every basic and applied research setting we've been tried, as well as in clinical trials in blood stem cells, T cells, the liver, and the eye.

And we owe this remarkable exponential scale up in the use of editing to the 2012 discovery by Jennifer Doudna here at UC Berkeley, and Emmanuel Charpentier of how a remarkable bacterial enzyme, Cas9, is naturally routed to its target and how it can be reprogrammed. Now, since 2012, the toolbox of editing has been expanded by invention of new ways to change DNA in living cells, for example, repairing point mutations, such as the

work on base editing from the lab of David Liu and that has markedly accelerated the growth of editing as well.

Now, while celebrating the truly magnificent impact that this work has had, I want to note that genome editing has a three-decade history. Its core principle was established by Maria Jasin at Memorial Sloan Kettering in 1994. And then extensive work in the 2000s built a toolbox of editing of native human genes using earlier generation programmable nucleases. My own work on human genome editing for therapeutic purposes began 21 years ago. And in my remarks today, I will use that extended perspective to showcase key scientific features of editing that have stood the test of time and remain relevant for the discussion today.

In 2005, in an important collaboration with Matt Porteus, my colleagues at Sangamo Therapeutics and I demonstrated the efficient repair of a point mutation at a mutation hotspot in a native gene in a human cell. So, we then proposed the term genome editing to highlight the fact that the method requires an engineered enzyme, a genome editor, which binds the DNA target in the cell in an investigator-specified way, and then drives an enzymatic reaction that results in genetic change at that target. Two enduring concepts emerged from that work.

First, as all enzymes, genome editors follow biochemical principles that can be studied, understood, and that inform their in-cell action. However, in contrast to enzymes reacting with substrates and test tubes, genome editors act on the genome in its living form. The biology of the cell is the prism through which genome editors act. And look, this fact is not surprising. The most widely-used genome editor, the enzyme Cas9, from a bacterial adaptive immune response system, evolved to function in bacteria and work by Doudna and Charpentier and followed by that of others, described principles of repurposing it for genome editing in eukaryotic cells, which, as we all know, it's a very different biophysical environment than the bacterial one.

Now, let me showcase for you the human genome editing relevant differences that emerged by focusing on Cas9 itself. So, this remarkable enzyme uses an RNA molecule it carries shown in orange in the crystal structure up on the upper left to recognize and distort a DNA double helix, which is shown here in blue and black. And then it forms a striking intermediate before creating a double strand break in the DNA. Now its key feature was discovered by Martin Jinek here at UC Berkeley when he was in Jennifer Doudna's lab. And they found that this recognition mechanism is driven by a Boolean logic using an AND operator. The protein Cas9 has to bind to a specific DNA sequence that has an awkward name and, I'm sorry, some of the nomenclature in my field is not user-friendly. So, this specific motif is called the Protospacer Adjacent Motif, or PAM, and you can see it in gray on the lower left. So, once that happens, the RNA component of the complex takes over. A 20 nucleotide stretch of that RNA then pairs with just one strand in the DNA double helix and in the structure in the upper left, it's shown in blue, and it uses pairing rules for that, which we've known since 1953. And so, once this complex forms, Cas9 can cleave both strands of the DNA target and creates a double strand break and then releases it.

So, that's the structural part, but the reason we're here today is this amazing 2012 experiment shown on the right. An analysis of this pairing mechanism led Martin and Jennifer to propose that if you change this 20-nucleotide stretch to match a given such stretch in a DNA sequence, every time flanked by the famous PAM, of course, you can create a double strand break on demand. And this worked beautifully. So, when Cas9 was armed with one of five different such guide RNAs, each matching a different stretch on a piece of naked DNA, then incubated with that DNA and analyzed on a gel, a pattern emerged that proved the notion that Cas9 can be programmed to induce a double strand break on demand using pairing rules that are simple. I can explain them to my seven-year old daughter and that simplicity was incredibly empowering.

Because you see, this discovery supercharged 20 previous years in developing double strand break genome editing I alluded to earlier. So, at the level of the introductory biology class, such breaks in human cells are resolved by one of two pathways. End-joining that puts the two ends back together, and homology directed prepare, which uses unidirectional transfer of genetic information from a related DNA molecule, typically a sister chromatid, to heal the break.

Genome editing is a collaboration with these two pathways. In the absence of the repair template, repeated cycles of cleavage by the editor result in small insertions and deletions at the target. If a repair template is provided, in certain cell types, under some circumstances, a mutation can be repaired, or an entire transgene can be inserted at the site of the double strand break.

It is key to appreciate that this schematic is a simplification. The cellular machinery for end joining and for homology directed repair is elaborate, putting it mildly, and it imposes a lot of context-specifics, so dependent on DNA sequence, on cell type, and cell cycle state, rules on the outcomes of editing.

However, complex the machinery, in practical terms, this end-joining based genome editing approach gives us small insertions and deletions, and in certain settings, other arrangements, including larger deletions. And you will hear a lot more about this shortly from Dr. Bauer.

But let us examine these small indels first. As you see on the left, a key finding is whether using finger nucleases on the CCR5 gene or Cas9 on the same gene, the resulting alleles do not span every imaginable placement and size, but instead form a distinct pattern. On the right, as shown in this paper from Caribou, genome editing using Cas9 armed with a guide RNA carrying a match to this protospacer, and I waited all this time to introduce yet more terminology.

The bit in the chromosome that gets cut is called the protospacer, and the bit in the RNA that Cas9 carries that matches that bit is called the spacer. Again, I realize this is challenging, but here we are. So, if you have a Cas9 armed with a guide RNA, carrying a match to this protospacer, and then use it on living cells, you get a one-base pair insertion as a dominant allele, and then a three base pair deletion, and a one base pair deletion, and others in decreasing order. The pattern will differ from gene to gene and cell type to cell type, and this really provides a magnificent example of how the biology of the cell is the prism through which editors act. Remember that in in vitro, the double strand break is either a blunt one or a one base pair stagger, whereas look at what's happening in living cells.

So, the pattern will differ from gene to gene and cell type to cell type. And this provides an example of how the biology of the cell is the prism through which editors act. Now, such indels can form in living cells at genomic positions that are only a partial match to the guide RNA spacer, the so-called off-target sites. And these, of course, deserve a very careful look. So, what I will do is give you a preview of the structural biology, biophysics, and biochemistry. I will touch on the in-cell activity briefly, and Dr. Bauer will speak in a lot more depth to all about this.

So, as Martin Jinek and colleagues write, the target DNA specificity of the CRISPR associated genome editor nucleus Cas9 is determined by a complementary 20 nucleotide segment in its guide RNA. However, Cas9 can bind and cleave partially complimentary of target sequences, which raises safety concerns for use in clinical applications. In this work, the UNIC lab identified off-target sites for Cas9 armed with a guide RNA for the FANCF gene, and the sequences in this multicolored stretch on the upper right and determined a high resolution atomic structure of the enzyme guider in a complex, bound to the target shown at the top, and then to two off-targets shown here.

For off-target number two, there is a clear structural explanation for why Cas9 binds it. The A to C mismatch you see on the left is accommodated by a wobble interaction, which we all learned in Bio 1 in terms of tRNA anticodon interactions. So, it's somewhat of a base pair. And this somewhat of a base pair happens between the other mismatch, between this U and this G. And I say because as you can see in the thermodynamic analysis below, this imperfect pairing severely penalizes the enzyme. Look at the numbers. The off-rate is different by two orders of magnitude. So, the enzyme just loves falling off the off-target. And the KD is nearly a hundred times higher for the off-targets. So, this is why most of targets which require such mis-pair driven-binding are cleaved much less efficiently. Before I leave this slide, I want to slow down and state very clear. It is not the case that you can sprinkle in any number of mismatches anywhere in the target and the enzyme will cut there to some extent. In fact, rigorous data have shown that accommodation of mismatches is guide-RNA specific and for each guide, only some, but not other mismatches are tolerated. At a high level, this means knowing whether a given person's genome has a partial match or a given guide RNA spacer is only the first step towards understanding whether or not that exact off-target will be cut by that enzyme in cells from that person.

There is more to the story. You will recall these beautiful data from the Doudna lab to arm Cas9 in a test tube with five different guide RNAs that will efficiently cut a plasmid DNA using each one at a perfect match. That's not what happens in cells.

Instead, Cas9 efficiency varies dramatically from target to target, even if each one is a perfect match. And this finding dates back to the earliest days of editing before Cas9. We first sold this with zinc thinkers 20 years ago. But here's a 2012 dataset from Shengdar CAI. In this experiment, Cas9 was armed with one of 14 guide RNAs, each perfectly matched to a sequence in the same small genetic region. And when you test each one in cells and measure the efficiency, the result is pretty striking. Some guide RNAs just don't work. Most are

mediocre, and for some, for instance, guides number 10, 11, 12, and 14, they're pretty highly active. A cell imposes its own rules on what the enzyme can do, and it's going to get more interesting.

Shengdar CAI and Keith Jung developed a way to identify off-target sites that are actually cut in living cells. The method is called GUIDE-seq, and in brief, you expose the cells to the editor, capture an oligo-adapter into each break, map where the adapter has landed, and when you do this exact analysis for all 14 guides, something striking emerges. Yet again, guides vary, this time in terms of specificity, but note how the correlation of activities are a poor one. For example, please compare guides 10 and 11. Both are equivalently potent but guide 10 is a champ. It has zero off-target sites. It only cuts the target. And in striking contrast, guide 11 is one divided by champ. It has nearly 300 of target sites.

The art and craft of genome editor design involves finding the equivalent of guide 10. Maximally potent. Maximally specific. By screening for such guides and the relevant cell type. And I want to emphasize in cell analysis for the following reason. There is an additional method to identify a candidate of target sex. It was also invented by Shengdar CAI. And it involves digesting naked genomic DNA and finding where the enzyme cuts. So, when Shengdar and CICERO at Toronto in his lab did this experiment for the same set of 14 guides, you find something that's frankly not surprising. The number of DNA targets that a given Cas9 guide RNA can cut in the naked human genome is, oh, sorry, I managed to, in an egregious mistake, use the wrong word in the title. It should say is much greater than of what it actually cuts in the cell. Sorry about that. So, the number of sites that Cas9 cuts in naked DNA is much greater, apologies, I should have proofed my own slides better, than what it cuts in the cell. But I guess I'm glad I made this mistake because it lets me hammer in the point. We'll look at our champion guide. It has no measurable off-target, but it's still cuts 176 targets in naked genomic DNA, but not in cells. And look at the dirty guide. Guide number

11 cuts about 295 of targets and cells. But it cuts more than 9,000 in naked DNA. So, this means yet again that the cell imposes its own rules on what the enzyme can do inside the cell so that the number of sites in naked DNA is much, much bigger than the number of sites in the chromosome. So, this bottom line, it means that in-silico and on naked DNA analysis are only the initial step in determining whether a given candidate off-target site will be cut inside the cell. And if yes, to what efficiency.

So, wrapping up for my last piece of primary research data on the science of editing, I want to showcase a recent finding that aligns with my own experience in studies of this type dating back 15 years and of the field more broadly. The key takeaway from this is this, how you handle the cells during genome editing provides critical input to the outcome. In this recent study, the lab of Jennifer Doudna here at the Innovative Genomics Institute investigated what happens to primary human T cells following genome editing. In a research setting, they found, and other studies agree with this, that double strand breaks induced by the nucleases lead to chromosome loss in primary human T cells. The scientists then collaborated with the group of Carl June at Penn and did the same analysis on T cells manufactured in an optimized clinical scale process using a modified protocol. And the finding was, quote, Undetectable chromosome loss above average. Let me be clear. I am not, repeat, not saying that any clinical manufacturing scheme will leave the genome of the target cell in pristine shape. But what I am saying that how you make the cells will have critical input into what the genome of the cell will look like. And that is also a key part of the art and craft of editing. Finding those conditions where you love the cells, I guess, when you gene edit them.

In conclusion, the presence in the human genome of a perfect sequence match or a partial match to a guide RNA spacer that Cas9 can carry is of modest utility being diplomatic in determining the potency or the outcome spectrum of genome editing using that Cas9 guide RNA in a living human cell.

Context is critical in determining the outcome of genome editing. What does that mean, context? What Cas9 did you use? In what form? What was the guide RNA? What was the spacer? What is the chemical composition of both? What sequence were they targeted to? How were they delivered? To what amount? Into what kinds of cells? How did you handle the cells before and after editing? So again, the art and craft of genome editing involves permuting endlessly. This is really labor-intensive work, permuting all of these variables until you find sort of the Goldilocks conditions of maximum potency and least genetic system. But my own experience, and that of our entire field is, at the end of the day, the only way to truly determine what the functional consequences are of editing on the cells in the near and long term. The only way to do that is actually transplant the cells into a subject on a clinical trial and see what happens. Now, all the ex vivo studies in this regard are important, but the trial data, objectively speaking, more so.

In the last minute, let me close by offering this perspective. As I look at the progress of our field, in the two decades that I've had the honor of being part of it, progress by every objective criterion has exceeded our wildest expectation. In 2005, if you'd ask Maria Jasin, Dana Carroll, Matt Porteus, my colleagues at Sangamo or pretty much anybody else, are we going to get to a world in 2023 when there'll be 27,000 references on Cas9 that will have genome editors, which knock out 100% of the target with no measurable of target sites. I don't know that we would have believed you. And yet here we are. It is certain that a decade from now, the field will grow further in ways that we can predict only to a certain extent.

But that said, as I think about the maturity of this technology, like where are we in the overall trajectory? My personal perspective is we have completed our period of exponential growth in terms of, if you will, genome editing quality score. I personally think that we are in a more linear stage of growth, having established methods for editor design, deployment, and de-risking. And this, to me, means that genome editing is ready for prime time, which of

course is why we're here today. Again, I'm honored for this opportunity to speak with you today, and I will turn the floor over to Dan Bauer.

Dr. Ahsan: Thank you, Dr. Urnov for both the historical and technical context. They're very important for us to understand and so now we'll hold off questions for Dr. Urnov until we have the presentation by Dr. Bauer. Daniel Bower is Principal Investigator and Staff Physician at Dana Farber Boston Children's Cancer and Blood Disorder Center in Boston, Massachusetts. Dr. Bauer if you could turn on your camera and go off mute, please.

Off-Targets of Genetic Editing – Dr. Daniel E. Bauer

Dr. Bauer: Good morning. I'm delighted to present today regarding comprehensive evaluation of genome editing associated genetic modifications.

So, my disclosures also reviewed by FDA include that I'm a co-inventor of patents related to therapeutic genome editing for blood disorders, and I hold a license patent that's related to BLA125787 from Vertex Pharmaceuticals, and it's possible that I could receive future-related royalties.

Today, I will discuss the therapeutic genome editing can produce genetic modifications both away from and at the genomic target site that is off-target and on-target edits. Off-target edits may be influenced by human genetic diversity. On-target edits may include short indels and structural variants and the edit distribution reflects the clonal composition of the hematopoietic graft.

First, I'll discuss that off-target edits may be influenced by human genetic diversity. Here, I'm considering off-target effects as genomic modifications away from the intended target locus. As introduced by Professor Urnov, based on its biochemical properties, Cas9 may bind and cleave genomic sites with sequence similarity to the target locus. Current methods to nominate candidate off-target sites are mainly based on two approaches. First, in-

silico approaches based on sequence homology. And second, cell-based and/or in vitro assays that empirically assess modification of genomic DNA.

For the BCL11A+58 enhancer targeting guide RNA originally called number 1617 and now referred to by the sponsor as SPY 101, both of which names indicate the same space or sequence. The published off-target analysis using combined in-silico and empiric approaches nominated 24 and 223 off-target sites in publications from 2019 and 2021, respectively. Validation by deep sequencing of the candidate sites in edited cells identified no off-target sites with significant editing at pre-specified detection thresholds of 0.1% or 0.2%.

However, in-silico methods traditionally have been based merely on the human reference genome. In cell-based and in vitro empiric methods usually interrogate a limited set of human donor genomes.

Therefore, we wondered about off-target sites that are not found in the human reference genome, but may be found in specific populations or individual patients. This question motivated our research group, working with computational biology colleagues listed below, to develop a publicly available in-silico tool called CRISPRme. Which takes as input a guide RNA spacer sequence, plus flexible sets of genetic variants, such as from the 1000 Genomes Project, Human Genome Diversity Project, or any other source, at flexible homology thresholds, to nominate variant aware off-target sites, and to associate them with genomic, variant, and guide RNA related annotations.

When we tested the guide RNA 1617, that is the guide RNA used in the editing therapy, that is the topic of today's discussion, we found that the top hit candidate off-target site was related to a single nucleotide polymorphism, SNP, called RS114518452 on the Y-axis is the cutting frequency determination score, which is based on the number and position of mismatches of a target sequence with respect to a guide RNA. For the top hit site on the far left shown in red, there was a very low score suggesting a negligible likelihood of cleavage

for the reference allele site. While in blue, with an arrow pointing to it, is shown the non-reference allele site that had a predicted likelihood of cleavage similar to that of the on-target site.

In this case, the variant changes the C to G on the bottom strand, which produces an NGG PAM sequence, shown in bold, which enables the binding of Cas9. The off-target site then just has three PAM distal mismatches, shown in lowercase, for which a high likelihood of cleavage is predicted. This variant is present at different frequencies in different human populations, with 4.5% minor allele frequency in African ancestry populations. This suggests that about 10% of a target population of African ancestry Sickle Cell Disease patients would be expected to carry a risk variant for this off-target effect.

To test the variant specific off-target potential, it is essential to conduct the test in cells carrying the risk allele. We identified a CD34 positive hematopoietic stem and progenitor cell donor heterozygous for the SNP and perform gene editing. Above, deep sequencing showed off-target short indel gene edits exclusively on the non-referenced G allele and never on the reference C allele. Below shows off-target editing was never observed on the reference allele, either in this heterozygous donor or in homozygous donors carrying only the reference allele. In contrast, the non-reference allele showed 5% to 10% short indel off-target gene edits.

Since the BCL11A target sequence is on the P arm of chromosome 2 and the SNP is on the Q arm of chromosome 2, we hypothesized that simultaneous cleavage at both positions could lead to pericentric inversions of approximately 150 megabases. To test this, we designed droplet digital PCR assays to specifically detect and quantify each of the pericentric inversion junctions.

We validated that indeed allele specific pericentric inversions were produced by gene editing at about one in 600 allele frequency in the heterozygous donor but were undetectable

in cells lacking the risk allele. It's important to note that the biological significance of these off-target indels and pericentric inversions is uncertain and may be negligible.

The second point I will discuss is that on-target edits may include short indels and structural variants. After Cas9 cleaves a target site, endogenous DNA repair mechanisms repair the cleavage. When this leads to a genomic modification, this is what we call an on-target gene edit. The edits at the on-target locus may include short indels, which are the easiest edit to identify since they can be amplified and sequenced by conventional short-range PCR and short-read sequencing. However, as shown in panels B through G, there are a range of other possible DNA repair outcomes at the on-target site, which collectively are known as structural variants, including long deletions, translocations, insertions, inversions, and translocations, copy neutral loss of heterozygosity and chromothripsis, or chromosome shattering and repair. Standard short amplicon sequencing cannot capture these structural variant types of on-target gene edits. Again, it's important to note the biological significance of any individual structural variant is often uncertain and may be negligible.

There are a range of alternative methods besides short amplicon PCR that can capture these structural variant types of on-target gene edits. This includes long read sequencing, which is shown on top indicates numerous repair alleles with deletions of hundreds to thousands of base pairs may be frequent on-target edits. On the bottom, a pie chart is shown that sometimes after gene editing up to 40% of the alleles are comprised of intermediate or long deletions that may escape conventional short amplicon PCR detection.

Another set of methods is based on single primer amplification. Where binding of an expected primer on one side of a cleavage, the so-called bait side, can capture edits with unexpected sequence on the opposite side, the so-called prey side. One such method, PEMSEEK, is shown below.

Droplet digital PCR approaches by placing a probe far enough from a cleavage site to be unaffected by short indels can comprehensively capture structural variants as missing alleles. In this experiment, about 15% of the alleles after gene editing were some kind of structural variant.

The point here is that numerous assays exist to detect on-target structural variant gene edits, and that these can be frequent occurrences, although multiple assays may be needed to fully characterize these gene edits.

The final point is that in ex vivo hematopoietic gene editing, the edit distribution within engrafting cells reflects the clonal composition of the hematopoietic graft. Although therapeutic gene editing of hematopoietic cells is relatively new, integrating vector gene therapy has been studied for more than 20 years as a treatment approach for a variety of inherited blood disorders. Unlike genetic therapies targeting other tissues, it is straightforward to measure the distribution of genetic modifications in the blood system of treated patients. Similar to therapeutic gene editing, hematopoietic cells are collected from a patient, ex vivo gene modification is performed, in this case by treatment with an integrating vector. Here, individual stem cells are shown as distinct colors, so there's a red cell, a purple cell, a green cell, and so forth. These cells are marked by unique vector insertion sites. Then, blood or bone marrow samples are routinely collected from the patient over time after cell infusion. The distribution of the vector insertion sites in these samples reflects the contribution to blood cells of hematopoietic stem cells marked by a given insertion site. In this way, measuring vector insertion sites can indicate the evenness of clonal diversity. In the illustrated example, an alert is raised when there's evidence of a decline in diversity suggesting clonal dominance. In this way, monitoring clonal composition may inform the approach's safety by detecting clonal dominance and clonal dynamics, and efficacy by detecting the level of potentially therapeutic gene edits. Gene editing therapy has a strong

analogy to integrating vector gene therapy, although gene edits may not be as diverse as vector integrations, since different clones may share the same edits.

So, is it possible to evaluate the clonal diversity and dynamics after gene editing? Here is a recently published study suggesting the answer is yes. This study was of CRISPR Cas9 mediated gene editing of the BCL11A enhancer for pediatric beta 0, beta 0 transfusion dependent beta thalassemia. Two patients were treated with Cas9 plus guide RNA number 1617 with ex vivo gene editing. The edit distribution was tracked in cell products and in serial patient samples from the blood.

Using this powerful approach, the investigators made some important observations. First, they found that the frequency of gene-edited alleles based on microhomology-mediated end-joining repair, MMEJ, was substantially lower in engrafting cells in the blood as compared to the input cell product, while the frequency of edits showing non-homologous end-joining repair, NHEJ, was reciprocally increased. This shows that the edit distribution may differ substantially between cell products and engrafting cells.

In addition, the investigators tracked the top 20 short indel edit alleles over time in the blood. And reassuringly found a stable edit distribution suggesting polyclonal hematopoiesis. This study illustrates how tracking edits enables monitoring of clonal dynamics in treated patients.

In conclusion, therapeutic genome editing can produce genetic modifications both away from and at the genomic target site that is off-target and on-target edits. Off-target edits may be influenced by human genetic diversity. In general, genomic diversity is most pronounced in African ancestry populations. For the 1617 guide RNA targeting the BCL11A+58 enhancer, there's a likely off-target site due to the RS114518452 variant. With about 5% minor allele frequency in African ancestry populations, including a risk of a rearrangement that is a pericentric inversion between the on-target and off-target site. This

off-target can only be detected in cells carrying the risk allele. A risk assessment could include uncertainty about the biological relevance of indels or rearrangements at the off-target site. Patients could be screened and/or patient samples could be monitored to gather information about the frequency and consequence of such events.

On-target edits may include short indels and structural variants. Short amplicon PCR with short read sequencing will miss structural variants. Assays exist to characterize and quantify structural variants, although more than one assay may be needed for comprehensive measurement of these on-target edits. A risk assessment could include uncertainty about the biological relevance of structural variants.

The edit distribution reflects the clonal composition of the hematopoietic graft. The distribution of edits in the cell product may not mimic the distribution of edits in engrafting cells over time, which could impact safety and/or efficacy. Gene edits that do not impact cell fitness, that is passengers, nonetheless mark engrafting stem cells and their progeny, clones, to offer opportunity to track clonal dynamics. Gene edits that do impact cell fitness, if any exist, that is drivers, would be expected to cause clonal loss or expansion, which might be detected by tracking the edit distribution. Tracking gene edit distribution over time is akin to vector integration site analysis in integrating vector gene therapy studies. Thanks for the opportunity to participate in today's discussion.

Q & A

Dr. Ahsan: Thank you, Dr. Bauer. And so, now we have a period of question and answers. So, questions from the committee. I do want to encourage you while Dr. Urnov will be available in the afternoon to answer some questions during our discussion period, if needed. Dr. Bauer will not be. So, now is our opportunity to ask our questions of these two guest speakers. If you can raise your hand, if you have questions, committee members, this would be the time to do so. Dr. Ott, can you turn on your camera and go off mute?

Dr. Ott: Yes. Hello. Thank you very much for these excellent talks. I wanted to ask In-cells and the relationship or the chance of off-target effects, especially when it comes to the applied method, I believe, which is an RNP, guide RNA Electroporation. I would welcome any comments on this. Thank you.

Dr. Urnov: Dr. Bauer. You want to go first? Should I start?

Dr. Bauer: Sure, I'm happy to. So, that's a great question and has been well studied as a main modifier of the risk of off-target potential that the longer the duration of exposure to the editor, the more there's risk of off-targets. Someone could imagine, once the on-target effect has been achieved, there's no more potential benefit of exposure to that editing reagent and that any continued exposure might only incur more off-target risk. And that's been shown many times in the field that delivery methods that have long-term or permanent expression have much greater off-target risk than a short pulse. And the RNP, Ribonucleo Protein Delivery Method is generally the shortest pulse that can be achieved with Cas9 gene editing and is expected to have, thereby, the shortest degree of off-target risk.

Dr. Ott: So, the numbers that you have shown, are they done with long-term or with short-term expression of enzymes, or a mixture of both?

Dr. Bauer: No, the experiments that I showed were all with RNP delivery in a therapeutically relevant delivery context. So, I think that kind of distinguishes the two-step approach of nomination, followed by validation for off-target effects. So, in the nomination step, often one is very broad, one might have relaxed thresholds to try to find, many possible sites, knowing that it's going to be a much larger list than the real edited sites will be. And then the validation can occur in a clinically relevant delivery context in a relevant cellular context to look for those edits. And so, for example, that SNP associated edit that we observed was in a CD34 cell donor cellular context with RNP delivery. So, similar to what would be expected for the therapeutic delivery.

Dr. Ott: Thank you.

Dr. Urnov: If I may step in for just a second. Everything Dr Bauer said is absolutely true. The additional sort of almost hydrodynamic thing to consider is when you make a million cells in a small cuvette. Whether you make 250 million cells in a baggie the parameters through which the RNP then enters the cell and then stays in the cell and leaves the cell are actually different, which is why, a major part of what we do in our field as we go from the research bench to developing a product for potential in human use. You spend a lot of time basically doing exactly what you just alluded to, Dr. Ott, what my colleague at the IGI, Ross Wilson, calls that the area under the curve. The basic idea is you want to have a short pulse that looks like this rather than a pulse that looks like this. But critically, even for the same RNP and for the same cells, the shape of that, will depend on the scale at which you do the experiment.

Dr. Ott: Thank you.

Dr. Ahsan: Great. Thank you very much for that complete answer. Who I have next is Dr. Lisa Lee. Please, if you could go on camera and get off mute.

Dr. Lee: Thank you so much. Thank you for these extremely helpful presentations. Really well done. I want to take us from the baggie of cells to a higher level of view of patients and even higher-level view of that of families. And I wonder, Dr. Bauer, if you could talk a little bit about if you were talking to a family about this kind of treatment, how would you characterize the consequences of off-target edits both cellularly and clinically. If I were concerned about what does this mean if they're off-target, if things get cleaved at the wrong place, what does this mean for a patient?

Dr. Bauer: Yeah, I think it's a great question and I would emphasize to patients that there's often an uncertainty about the functional significance of off-target edits that only a small part of the human genome actually codes for genes. Most of the human genome is non-

coding. Its functional importance could be regulatory, but it's likely that many places in the human genome can tolerate an off-target edit and not have a functional consequence. The challenge is we just don't know for sure and the only way to know that is careful follow-up, I would say. What I would emphasize to patients is what's obvious to them. The known risks of the disease that this is a terrible disease that that has to play in. And then the risks of the therapy, which are known, which include things like the Busulfan conditioning that's used or whatnot.

So, I would say this, my guess is it's a relatively small risk in the scheme of this risk benefit. But it's new, it's unknown, but it's easily measurable. And that's one of the goals I would say of doing this under very careful circumstances is to try to learn what that risk is so that we can continually improve those therapies.

Dr. Lee: Theoretically, is it catastrophic?

Dr. Bauer: In theory, as Dr. Urnov said, these cell products. Have lots of cells in them, hundreds of millions of cells, and any one cell that goes awry, could cause leukemia. Now, has that ever been shown that an off-target effect of gene editing causes leukemia? No. Theoretically, could it? Yes. Is there any evidence to suggest that it does, I would say no. But this hasn't really been done very much. We need to be humble and open to learning from these brave patients who are participating.

Dr. Lee: Right. Thank you so much.

Dr. Ahsan: Thank you, Dr. Bauer. Dr. Joseph Wu if you could go on camera and unmute yourself, please.

Dr. Wu: Yeah, so I want to thank Dr. Bauer and Dr. Urnov for two great presentations. My question is, I guess this is for Dr. Bauer, with regard to the off-target papers that you showed for example, the as I understand it, the paper that you have prior off-target assessment, BCR11A, and the guide RNA for 1617. That one was Nature Medicine and then

there's another one in Frangoul in New England Journal. It says the number of donor samples is n equals four. And then the subsequent, the follow up slides, you have the CRISPRme software that you have. I think it's an assessment tool and it's a prediction model. A prediction model, not an actual cell that you validated. So, I just wonder what is the scale in the field that people have done to do the actual editing at the cell samples and to see what our target is on the scale of things so that has somebody done a scale in which you take 100 patients, 200 patients you measure the codex cells, tested it, and just see what the prevalence is and what the consequences are? What is the scale? How much has been tested? And has a sample size.

Dr. Bauer: Yeah, that's a good question. As Dr. Urnov pointed out, there's great variability from a guide RNA to a guide RNA. So, you could have what we call like a clean guide RNA where we can't find any off-target and what we call dirty guide RNA where you could find thousands of off-targets. So, if those experiments were done, the findings might be dramatically different, depending on the guide RNA. So, it's hard to answer when you say what's the scale that's been done. I guess it depends if you mean with just one given guide RNA, like the guide RNA.

Dr. Wu: Yes, so has this guide RNA 1617 been tested on 100, 200 different patients' donor hematopoietic cells just to see what happens in the bigger population rather than n equals for on the New England Journal paper.

Dr. Bauer: Yeah, I think the sponsor has done a few more donors, so it's probably greater than four at this point they could answer. In our studies we've done on the order of fewer than 10, five to 10 different donors. But the problem is when you validate off-targets, you generally find what you're looking for in the sense that you're doing amplicon sequencing of sites that were nominated in the first step. So, if you did hundreds of donors, you would need to do it in a way where you were looking at the relevant sites in those donors. For the

reference genome-related sites, these off-target results are very reproducible. If you do it in one donor, another donor, you get very similar effects. So, I think it would be unlikely at a reference genome off-target site that you'd find a substantial difference if you tested it in many more donors. We haven't seen that, I don't think anyone in the field has seen that. I think where the risk really comes from is when there's genetic variants and those donors might carry different genetic variants, then the number of mismatches for that guide RNA may differ substantially and the likelihood of cleavage may be different.

So, just to clarify, the comment on the CRISPRme tool, you're right, it's a prediction tool. But then what we did was we found a sample, a CD 34 cell sample that carried that risk variant and we validated. In fact, there was off-target editing and I think that's the key point that when one tests donors, unless one knows the variants that they carry, it's hard to interpret what are the off-target sites that they may be at risk for.

Dr. Wu: So, based on what you just mentioned, when do you want to test on many more samples instead of just on less than 10? If you're pushing for this product, or any product in general?

Dr. Bauer: Like I said, testing on many samples, unless you did it in a very focused way, I don't know that it would be of high value because if you're just looking at the reference genome sites, I don't think you'd find new information. If there's genetic variant sites, you could learn something from those samples. But, depending on the allele frequency of that variant, it could be one in 1000. It could be one in 10,000. So, doing 100 samples, you still might not find it. What the tool allows you to do is understand both the allele frequency of the variant and the likelihood of cutting or the other genomic annotations and then prioritize what are the most key variants to consider. There're other ways that one can minimize the risk of off-targets, and that's by the genome editing procedure itself. And what we talked about that a few minutes ago that limiting the duration of exposure can do that using certain Cas

variants or guide RNA modifications or other methods can limit the risk of that off-target. But I think saying we should do 100, then you could say we could do 1000, then you could say we would do 10,000. I'm a little concerned that without some statistical rigor, that study might be ambiguous in terms of what's its power and what's its goal.

Dr. Urnov: Dr. Wu, just add one sentence, if I may. First, let me just emphasize the extent to which the overall thrust of your question is completely sensible. I want to compare and contrast CRISPR Cas with, let's say, a standard small molecule for which we can study the pharmacogenomics, in introductory by chemistry, we'll teach our students about Tamoxifen and how it's metabolized, by CYP2D6 and there are alleles of CYP2D6 that cause differential metabolism of that to the actual active drug, which is Endoxifin. And if a physician knows what the patient's genotype is for CYP2D6, they can route guide the patient's care. The challenge with editing is, we do that in the experiment just discussed, we sequence 10,000 people and we identify, seven additional of target sites. In most cases, if perhaps not all, it's going to be incredibly difficult to look at that potential off-target site and say that off-target site is gives us actionable data. And again, this is because most of these are, not the overwhelming majority, intergenic.

So, I want to be clear. I don't want to throw my hands up in like agnostic paralysis. There's nothing we can do. But I think we should also be mindful of the objective limitations of what we can and cannot do in terms of de-risking the editor. And as Dr. Bauer said, the level of statistical rigor and functional analysis we would need to make sense of this larger scale sequencing, we might not be there yet. But again, I don't want to understate the fact that your overall line of question is completely sensible.

Dr. Ahsan: Thank you. That's a great point of how we take that theoretical information and use it on a per patient basis. It's very challenging. Dr. Komor, can you go on camera and unmute yourself, please?

Dr. Komor: Yeah. Hi. Yeah, I just, Fyodor's right on the nose. Each person has several million genetic variants in their genome, so it's a question of what's reasonable. But I had two quick questions. I just want to clarify is this new off-target that you identified due to the genetic variant. There seemed to be some yes or no in terms of did vertex identify that as a putative off-targets through their in-silico analysis because of the threshold that they used. Was that one of the off-targets that they had identified?

Dr. Bauer: I just read the public materials a few days ago and it looked like it may have been on their nominating list, but I think the key question is not if they nominated it, I think the key question is we only saw that off-target when we looked in cells that carry that variant. So, unless that test was done, I think that would be uncertainty and based on our results, I think it would be extremely likely that in cells carrying that variant, there would be cleavage at that site.

Dr. Komor: Okay. Yeah, I guess they're in the briefing. It does mention that the additional off-targets they saw they did test them in patient samples that had the variant, but it's unclear which one. But then, I just wanted to ask about the edit distribution that third bullet point that you covered. The on-target end-all profile, I'm assuming, in theory, like the sequence surrounding the double stranded break site should be the same for all of these patients. And so, do you typically see for this guide RNA, those indel sequences are quite reproducible across different patients, or do we have to worry about human genetic variation on that point as well?

Dr. Bauer: There's no common genetic variants that that would disrupt the binding of that guide RNA. But I think it would be a vanishingly rare event where someone carried a variant that would impact that. But it's a possibility for any sequence-specific therapeutic and any gene editing. But yeah, like any guide RNA, the edit profile is quite reproducible and characteristic, and there's a certain set of indels that are seen at a given frequency. Now, it's

not the same, actually, in the cell product and in engrafting cells in that other clinical trial and in the experiments we've done in animal models. Likely due to differences in the editing in true and grafting hematopoietic stem cells versus progenitor cells in that edited cell product population. But certainly, those can be measured over time and would indicate clonal dynamics and clonal diversity.

Dr. Komor: But you would expect that to be similar across various patients. Or is that reasonable?

Dr. Bauer: I think that's reasonable. We have never seen patient differences in that, but it depends on the clonality of engraftment. If many cells engraft and it's highly polyclonal, then you might expect patients would have similar distribution. As it becomes more oligoclonal, there could be stochastic differences or other differences in terms of which cells engraft and which cells give rise to that. And it's known that hematopoiesis, the clonal contributions of different hematopoietic stem cells can vary over time with different factors influencing that. And so, I don't know that we can totally predict what would happen in patients.

Dr. Komor: Got it. Thank you.

Dr. Ahsan: Great. Thank you. And Dr. Komor, maybe your question about what the sponsor had identified might be a question for the FDA after the FDA presentation as well.

I think at this point, there are no more raised hands. Anyone else have any questions that they would like to ask of our two guest speakers, and I'd like to remind you that Dr. Bauer will not be available in the afternoon, so now is the opportunity. Dr. Tisdale if you could turn on your camera and go off mute.

Dr. Tisdale: Yeah, thank you. Dan, get this question in before you go. And I think the one thing that I'm interested in contextualizing is your view on the risk of off-target effects and how they thus should be monitored. Given that this tool is following Watson Crick base

pairing, can you say anything about the overall risk and how you see it, and thus, how it should be monitored?

Dr. Bauer: Yeah, like you said the tool nominates off-targets taking as input genetic variants, and it can use any external annotation. So, any off-target prediction tool could be implemented along with the tool. And as those prediction methods improve, our ability to predict variant associated off-targets will improve. I think the risk is modest that there's no biological significance that we know of it of editing at this off-target site. I think the indels in the intronic non-coding sequences are unlikely to be functional, but we don't know that for sure. I think the pericentric inversions that we saw something like one in 600 allele frequency are a little more notable, but still may not be of biological significance. I think there's really no methods that I would say that are reliable to predict the function of off-targets. I think the main benefit of doing gene editing in the blood system is that it's easy to follow blood samples over time and that a broad characterization of on-target edits should be able to find the pericentric inversion as a rearrangement between the on-target and off-target site, and it could easily be followed over time. And if it's nonfunctional, it could provide reassuring data on this point, but I don't think there's any preclinical analysis that could be convincing to say a given off-target effect is certainly safe. So, I think that's a main benefit of editing in blood disorders is that we can follow patients.

Dr. Tisdale: Fyodor, I see you on the edge of your chair. Does that mean you'd like to add something?

Dr. Urnov: I think any professional genome editor like Dan or myself or Alexis who looks the entire world in the eye and says, Look, we're completely certain that our nucleus is 100% of the time behave pristinely. We have a 30-year history of our field. And John, you wrote much of the textbook of it where things can happen clinically that we couldn't predict the one thing I will stand by is the thing I closed with, which is as I look at the trajectory of where we

were when genome editing entered the clinic, 2008, 2009, and where we are today, our ability to do deep analysis at the sequence level at the functional outcomes level is really in a different part of we're in a different dimension of how deeply we can look. And so, to me, as I said, again, I'm just going to stand by what I close my talk on, my take, technologically speaking, is the technology is, in fact, ready for prime time. And by that, we're reaching asymptotic places in terms of how we can de risk it non-clinically. I don't know what else to do at this point in terms of understanding the benefit risk, which again I'm grateful for the opportunity to be part of this today.

Dr. Tisdale: Thank you, Bill.

Dr. Ahsan: Great. So, it looks like we have addressed the questions from the committee. I do want to thank the two guest speakers today. Those were very thorough and informative presentations. I think that led to some good question and discussion. And I think for the rest of the day, it sets the stage to think about two things, which is when is enough theoretical data sufficient to support a patient-specific risk assessment? And also, to your point, Dr. Urnov, which is where are we in that curve of risk mitigation? And have we actually started getting to that asymptote? Or is there more work to be done? Those are two very important questions. And while we will start the discussion today, I think that this will continue to evolve over time. But thank you so much for setting the stage for the conversation today.

I look forward to the rest of the day. At this point, we have time for a 10-minute break, and we will reconvene at a little less than ten minutes at 10:35 A.M. So, thank you all and see you then.

Dr. Ahsan: Welcome back. At this point in the day, we're going to hear from the sponsor. And so, with that, I would ask that there's going to be a series of speakers from the sponsor that each speaker introduced the subsequent speaker, and I will introduce the first speaker.

So, first to speak will be Dr. Stephanie Krogmeier who is the vice president of Global Regulatory Affairs at Vertex Pharmaceuticals. Dr. Krogmeier if you would like to start.

Applicant Presentations: Exa-cel for the Treatment of Sickle Cell Disease: Introduction – Dr. Stephanie Krogmeier

Dr. Krogmeier: Good morning. My name is Stephanie Krogmeier, and I'm the head of Global Regulatory Affairs for Cell and Genetic Therapies at Vertex Pharmaceuticals. We are excited to be here today and would like to thank the FDA, the panelists, and the patients in our clinical trials, as well as their families, for making this meeting possible. Exa-cel was developed for the treatment of Sickle Cell Disease in patients 12 years and older with recurrent vaso-occlusive crises. In other words, Exa-cel was developed for severe Sickle Cell Disease. In parallel, Vertex is evaluating the same drug, Exa-cel, for the treatment of transfusion dependent theta thalassemia. This BLA is also under review by the FDA but is not being discussed today.

I'll begin by discussing Sickle Cell Disease. Severe Sickle Cell Disease is a serious, rare, debilitating, and life shortening genetic disorder affecting hemoglobin function. Approximately 20,000 people in the US have severe Sickle Cell Disease defined as two or more vaso-occlusive crises per year in each of the two previous years and who are candidates for transplant therapy. Of those patients, approximately 90% of people with Sickle Cell Disease in the US are of African descent. The clinical hallmark of Sickle Cell Disease is recurrent, painful VOCs. These events not only require care at a hospital, outpatient clinic, or ER, but culminate in acute and chronic organ complications, leading to significant morbidity and mortality. The current treatments for Sickle Cell Disease are only partially effective and do not eliminate VOCs. Allogeneic stem cell transplantation is the only curative option but has substantial limitations. Thus, there is a high unmet need for transformative therapy, and that is why we are here today. Exa-cel is a non-viral one-time autologous CRISPR-edited

cellular therapy. The development of Exa-cel is grounded in human genetics, showing that fetal globin can substitute for sickle globin in erythrocytes and eliminate VOCs. Specifically, the permanent, irreversible, and precise edit made by Exa-cel results in the reduction of BC11A gene transcription, which upon erythroid differentiation leads to the increase in HBF I just described. Consistent with this mechanism and site of action, comprehensive non-clinical studies demonstrate no off-target editing, which will be discussed in detail later in the presentation.

Turning now to the Sickle Cell Disease Development Program. The Exa-cel Development Program consists of Study 121, a pivotal Phase 123 study, and Study 131, a long-term safety and efficacy follow up study. Given this is a rare disease, with an intended population of only 20,000 people, combined with the expected treatment effect, we designed the study in collaboration with the FDA to be approximately 45 patients. Study 121 has completed enrollment and dosing of all patients, 46 in total, including 12 adolescents. The patient journey for Study 121 is shown here, and there are three things I will point out. Sickle Cell Disease patients undergo CD34 mobilization and cell collection utilizing single agent Palixafor. The editing process is non-viral and occurs ex vivo, via an electroporation of Cas9 and the highly specific guide RNA. And finally, the patient is prepped for transplant by undergoing myeloablative conditioning with Busulfan, to ablate their existing bone marrow prior to Exa-cel infusion.

In the presentation today, you will hear from Dr. Hobbs on the efficacy. The data were highly positive and met both the primary and key secondary endpoints. You will also hear from Dr. Altshuler on the comprehensive non-clinical safety package with a specific focus on the off-target assessment, which did not identify any evidence of off-target editing by Exa-cel. Finally, Dr. Simard will describe the safety profile of Exa-cel, which was generally safe and well-tolerated.

In summary, the results from the Exa-cel program in severe Sickle Cell Disease are unprecedented. Exa-cel has demonstrated transformative efficacy, a strong safety profile, and a highly positive benefit-risk for patients with severe Sickle Cell Disease. With that background, here is the agenda for the remainder of the presentation. Unmet need will be presented by Dr. Thompson, Chief of Hematology at the Children's Hospital of Philadelphia and Pediatric Hematologist who has cared for patients with Sickle Cell Disease for the past 30 years.

Next, efficacy will be reviewed by Dr. Hobbs, who is the Head of Hematology Clinical Development at Vertex and has spent his career treating people living with Sickle Cell Disease. Then, non-clinical safety will be discussed by Dr. Altshuler, Chief Scientific Officer at Vertex. Prior to Vertex, he was a founding member of the Broad Institute at Harvard and MIT with a deep background in population and human genetics.

Later, clinical safety of Exa-cel will be shared by Dr. Simard, Head of Clinical Safety for Cell and Gene Therapies at Vertex, who has been with the program since the first patient was dosed.

Lastly, clinical perspective will be presented by Dr. Frangroul, Director of the Pediatric Stem Cell Transplant Program at the Sarah Cannon Research Institute at Tri-State Centennial Children's Hospital in Nashville, Tennessee, and the lead investigator in the Sickle Cell Disease Exa-cel clinical trials. Dr. Thompson and Dr. Frangoul are presenting on behalf of Vertex and have been compensated for their time. We have we also have additional experts from Vertex here today who are available during the Q&A session. Thank you, and I will now turn the lectern over to Dr. Thompson.

Exa-cel for the Treatment of Sickle Cell Disease: Unmet Need – Dr. Alexis Thompson

Dr. Thompson: Thank you. I'm Alexis Thompson, and I'm the Division Chief of Hematology at the Children's Hospital of Philadelphia. For the past 30 years, I've cared for

patients with Sickle Cell and have regularly witnessed the debilitating consequences of this life-threatening disease. I'm pleased to be here today to discuss the current treatment landscape and why I believe that patients with Sickle Cell greatly need a cure to treatment. Let me share some background on the disease.

Sickle Cell is considered a rare condition in the United States affecting approximately 100,000 Americans. Among these, about 20,000 have what would be considered severe disease defined by recurrent VOCs and are therefore candidates for transplant therapy. Sickle Cell Disease occurs at disproportionately high rates among individuals of African ancestry and also at lower rates among individuals of Middle Eastern, Mediterranean, Indian, or Asian descent. People with Sickle Cell often live in low-income areas and communities with high unmet medical need, further adding to substantial healthcare disparities. Sickle Cell is caused by mutation, the beta-globin gene, which encodes a key component of hemoglobin. This mutation leads to production of an abnormal form of hemoglobin called sickle hemoglobin. In the deoxygenated state, sickle hemoglobin polymerizes and produces deformed or sickle-shaped red blood cells that are prone to hemolysis, leading to chronic anemia. Individuals with sickle cell disease commonly experience episodes of severe, acute pain, known as vaso-occlusive episodes or crises that can last a few hours to sometimes many days. Over time, with repeated sickling events, Sickle Cell results in progressive injury, potentially impacting multiple organs in the body, which can progress to organ failure and a shortened lifespan. Frequent painful episodes and chronic pain significantly diminish the quality of life, not only for the patients, but also for their caregivers and their families. In addition, Sickle Cell has profound psychosocial consequences for the patients, with high rates of anxiety, depression, and absenteeism from work and school.

In addition to high morbidity, VOCs are the most common cause of hospitalizations for individuals with Sickle Cell Disease, resulting in approximately 100,000 admissions per

year. VOCs that require hospitalizations are associated with increased risk of mortality. While the overall lifespan for patients with Sickle Cell has certainly improved over time, it is still reduced by 20 to 30 years compared to the general population with a median life expectancy of only 45 years in recent reports. Unfortunately, there is no broadly available treatment option that will eliminate VOCs. Allogeneic stem cell transplant, the only potentially curative option, is only available to approximately 18% of patients who will have a suitable donor. Allotransplants are associated with significant risk, including transplant-related mortality, graft failure, graft versus host disease, and other significant complications.

Turning to fetal hemoglobin, fetal hemoglobin is an established powerful modulator of clinical and hematologic features of Sickle Cell disease and has been robustly studied. Elevated levels of hemoglobin F result in improved morbidity and mortality in Sickle Cell Disease. And this is demonstrated by two examples from natural history.

The first are neonates or infants with Sickle Cell who by and large are asymptomatic when they produce primarily hemoglobin F, which is non-sickling. And Sickle Cell Disease patients who have co-inherited hereditary persistence of fetal hemoglobin. Fetal hemoglobin levels of 20% or greater have become the clinical target for patients with sickle cell to protect against disease complications. So, a durable therapy that consistently raises fetal hemoglobin higher than 20% would provide an important treatment option.

In summary, Sickle Cell Disease is a rare, debilitating, and life-shortening disease. Patients will suffer painful vaso-occlusive events and other recurrent issues that cause chronic complications across multiple organs and significantly impact their lives and lifespan. Allogeneic hematopoietic stem cell transplants are potentially curative, but they are not widely available for the majority of patients. In the current landscape of disease-modifying therapies, none of the approved agents are curative, nor will they fully eliminate Vaso-occlusive episodes. Hemoglobin F is an established and highly relevant clinical marker in

Sickle Cell, so a new treatment that raises fetal hemoglobin in a durable or sustained manner would provide an important therapeutic benefit. The bottom line is patients and families need curative medicines for this devastating disease. Thank you. I'll now turn the presentation over to Dr. Hobbs.

Exa-cel for the Treatment of Sickle Cell Disease: Efficacy – Dr. William Hobbs

Dr. Hobbs: Thank you, Dr. Thompson. I'm William Hobbs, head of hematology clinical development at Vertex. I'm a hematologist and have spent over 20 years working with people living with Sickle Cell Disease, including in patient care, and for the last 10 years in developing new treatment options for the severe progressive disease. And it's an honor and a privilege to be here today to share the clinical data showing the transformational and durable clinical benefit of Exa-cel in adolescents and adults with Sickle Cell Disease.

Exa-cel resulted in transformational clinical benefit, and I'll provide an overview of the efficacy data, which showed that the study met its primary and key secondary endpoint. The primary endpoint being the proportion of patients with no VOCs for at least 12 consecutive months, which is referred to as VF12. The key secondary endpoint was the proportion of patients with no in patient hospitalizations for VOCs. For at least 12 consecutive months, which is referred to as HF12. The efficacy of Exa-cel was consistent across the patient population, including both adolescents and adults. And the clinical benefit of Exa-cel was durable, including for approximately four years of follow-up.

Key characteristics of patients in the study were representative of patients with severe Sickle Cell Disease expected to be treated with Exa-cel. The primary efficacy set, or PES, includes all patients with at least 16 months of follow up who are analyzed for the primary and key secondary endpoints. The full analysis set or FAS, includes all patients who received Exa-cel. Adolescents represented a significant proportion of the study population, making up approximately 30% of the dose patients and 20% of patients evaluated for the primary and

key secondary endpoints. Patients experienced a mean of approximately four VOCs per year in each of the two years prior to Exa-cel, with a mean of almost three inpatient hospitalizations per year, resulting in approximately two to three weeks in the hospital per year. The study met the VF12 primary endpoint, demonstrating remarkable clinical benefit. 29 of 30 patients, nearly 97% achieved at least 12 consecutive months without a VOC, with a mean VOC free duration of over 22 months, almost two years, and ranging up to 46 months, or almost four years.

To further illustrate the treatment effect in more granular detail. This figure shows each of the 44 patients who received Exa-cel. Each black diamond indicates a VOC event, and you can see the remarkable absence of VOC events after Exa-cel. The light gray bars to the left indicate the two-year baseline period prior to Exa-cel, demonstrating the high frequency of VOC events before Exa-cel treatment. The purple bars to the right show the duration VOC free after Exa-cel. This evaluation period for VOC events began after a 60-day washout of transfused red blood cells which are given for post-transplant support and identified as the red and dark gray bars for each patient. There were only two patients in the PES who had VOCs after the endpoint evaluation period. One patient had a single event and is the patient towards the top of the figure. This patient achieved both VF12 and HF12. And then had the single event after approximately 20 months VOC free. And I'd like to highlight a few features of this event because it illustrates the protective benefit of Exa-cel. This event occurred in the setting of a documented parvovirus infection.

Parvoviral infections are known to cause severe and potentially life-threatening events in patients with Sickle Cell Disease. Due to parvoviral-induced acute severe anemia that typically requires hospitalization, often in an intensive care unit, and almost universally requires transfusion support. In contrast, this patient recovered quickly after an uncomplicated short hospital stay without any red blood cell transfusions. This case

highlights the protective effect of Exa-cel in preventing severe complications. even from known acute precipitants of what could otherwise be potentially life-threatening events. There was only one patient who did not achieve VF12, and this is the patient on the figure who had several VOC events after Exa-cel. None of these events required hospitalization, and the patient achieved HF12, which I will show you in a moment.

At the bottom of the figure are the patients who are not yet evaluable for the primary endpoint because they had not yet been followed for 16 months as of the data cut. One of these patients has had several VOC events and will not achieve VF12 but does remain eligible to achieve HF12. All of the other patients remain eligible to achieve both VF12 and HF12.

I want to focus for a minute on adolescent patients. This is the same data that I just showed you, but now focusing on the adolescent patients who are grouped together at the bottom of the figure. Exa-cel demonstrated consistent clinical benefit between adults and adolescents. And this was as expected given the same disease pathophysiology, the same mechanism of action of Exa-cel, and the same protective effect of HBF. The 12 adolescent patients who received Exa-cel, representing approximately 30% of all patients, have VOC-free treatment effects similar to adults, and all of the adolescent patients in the PES, or 100% of them, achieved VF12.

Turning here to the key secondary endpoint of avoiding hospitalization. All 30 patients, 100% of them, achieve the key secondary efficacy endpoint, HF12, which is defined as patients free from in-patient hospitalization for VOCs for at least 12 consecutive months. This endpoint is clinically important because it informs the absence of the subset of VOCs that are associated with higher acute mortality risk. These data are presented here in the same format that I just showed you for VOC data, but now each black diamond represents a hospitalization for a VOC. The gray bars to the left show the frequent hospitalizations for

VOCs the patients had over the two years prior to Exa-cel and the purple bars to the right show hospitalization events after Exa-cel, the clinical benefit of Exa-cel is clear. There was only one patient in the PES who had a hospitalization for AVOC after Exa-cel. This is the same patient event I previously described associated with the parvovirus infection for the patients. Not yet in the PES at the bottom of the figure. There were two other patients who experienced a hospitalization early after Exa-cel, with both maintaining the potential to achieve HF12. Exa-cel resulted in rapid, robust, and durable reactivation of fetal hemoglobin. As shown on the left, fetal hemoglobin levels increased to over 20%. And were maintained at approximately 40% over time. As Dr. Thompson described, increasing fetal hemoglobin to over 20% protects against disease complications, including eliminating VOCs. And this was clearly achieved. As shown on the right, adolescents increased fetal hemoglobin levels similar to adults. With all adolescents achieving fetal hemoglobin levels over 20%, which were also maintained at approximately 40% over time. Again, illustrating the similar treatment response of adolescents and adults.

To further demonstrate the durability of Exa-cel, shown here is patient's allelic editing in bone marrow at the top and peripheral blood on the bottom, which remains stable and durable throughout follow-up in every patient. This demonstrates the stable engraftment of edited long-term hematopoietic stem cells, with editing remaining durable through follow-up, including beyond two years.

In summary, Exa-cel demonstrated transformational clinical benefit in patients with Sickle Cell Disease. 97% of patients achieved the primary endpoint to VF12, and 100% achieved the key secondary endpoint of being free from inpatient hospitalizations for VOCs. This efficacy was consistent across all endpoints and all subgroups. And in particular, adolescent patients had similar efficacy responses as adults. Again, this is as expected given the same disease pathophysiology, the same mechanism of action of Exa-cel, and the same

protective effect of fetal hemoglobin. Efficacy was durable. Patients were VOC-free for an average of over 22 months, including up to almost four years. High protective levels of fetal hemoglobin were rapidly achieved and were durable over time. Allelic editing was stable for up to approximately four years of follow-up. In totality, the data support the remarkable clinical benefit of Exa-cel in patients with Sickle Cell Disease.

I'll now invite Dr. Altshuler to present the non-clinical safety.

Exa-cel for the Treatment of Sickle Cell Disease: Non Clinical Safety – Dr. David Altshuler

Dr. Altshuler: My name is David Altshuler and I'm the Chief Scientific Officer at Vertex. I will be discussing non-clinical safety with a focus on the strategies used to minimize the potential for off-target editing by Exa-cel. We designed and executed a comprehensive non-clinical safety package in support of the Exa-cel program. The package included analysis of on-target editing, of chromosomal integrity, potential for off-target editing, And studies of tumorgenicity, engraftment, persistence, and biodistribution. The non-clinical studies did not identify any Exa-cel specific risk. I will focus this presentation on the potential for off-target editing.

Ten years after the discovery of CRISPR gene editing. We now understand that the specificity of CRISPR is determined by the uniqueness of the on-target site and of the guide RNA. In cells exposed to CRISPR, the guide RNA guides the CRISPR enzyme to specific genomic locations based on sequence homology, that is, where the guide RNA matches the DNA of the host genome. For CRISPR, to edit a specific site, the DNA sequence must match both the guide RNA and a short adjacent sequence known as the Protospacer Adjacent Motif or PAM. If the on-target site is unique in the genome, as depicted on the left with a yellow dot, and if the guide is highly specific, then editing will occur only at the on-target site. However, if one were to choose an on-target DNA sequence that is present at multiple

genomic locations as depicted on the right, in red, and one designed to guide that binds promiscuously at many places in the genome, then off-target editing can occur. Based on this understanding, three strategies to minimize the risk of off-target editing are, first, to limit exposure to CRISPR. Second, to select an on-target site that is unique in the genome. And third, to optimize the guide RNA, not only for efficacy, but also for specificity. From the start of the Exa-cel program eight years ago, we were focused on minimizing and assessing the risk of off-target editing.

The design of Exa-cel was shaped by three strategies to minimize off-target risk. First, we use an ex vivo approach and transiently express CRISPR only in cells of the hematopoietic lineage. Second, we selected the on-target site in an intron of BCL11A that has a unique sequence with no other match in the human genome. Third, we screened hundreds of candidate guide RNAs to select an optimal guide RNA that has no other match elsewhere in the human genome. Now, having designed Exa-cel to minimize potential for off-target editing, we then systematically evaluated the risk of off-target editing using multiple orthogonal methods to detect potential off-target edits, including sites nominated based on human genetic diversity, and performing risk assessments as appropriate. And the conclusion is that the design of Exa-cel minimized potential for off-target risk and multiple systematic evaluations did not identify evidence of off-target editing by Exa-cel. I'll start by describing the framework used for off-target evaluation of Exa-cel. As depicted in the box on the left, our approach involved three steps.

First, we nominated candidate off-target sites using two orthogonal methods, Computational Homology Search, and a laboratory method known as GUIDE-seq. As will be discussed in the next section, the nominating process included analysis of human genetic diversity relevant to the Exa-cel patient population. Both nomination methods are known to be sensitive in their ability to detect sites at which off-target editing may occur, but both

methods have high rates of false positives. And for this reason, to determine if any off-target editing occurs at any nominated site, the second step was to compare the DNA sequences of edited as compared to unedited cells using high coverage, hybrid capture, next generation sequencing. The third step was to perform a risk assessment in two settings. First, if any sites were confirmed as having an off-target edit, and second, for any site nominated based on a rare genetic variant that was not directly evaluated in our hybrid capture experiments. I'll now review in a bit more detail each step in this process.

One nomination method was a systematic computational homology search of the human genome sequence. In the box in the right you can see the DNA sequence of the Exa-cel guide on the target site and the NGG PAM. Below that is sequence of a potential off-target site that has three mismatches and an alternative PAM sequence highlighted in red. In the first study, we searched the genome and nominated 5007 candidate sites based on criteria of up to five mismatches or a bulge or an alternative PAM sequence. While sites with a bulge or alternative PAM are very unlikely to cut, we included them for completeness.

In the second study, we narrowed the mismatch criteria to include only those sites with up to three mismatches because this enabled sequencing more deeply at the candidate sites with the highest likelihood of having any off-target editing.

The third study added 50 additional candidate sites nominated based on human genome sequence diversity. Now on the next slide, to help quantitate the risk of off-target editing, I will review literature and how the number of mismatches between a guide RNA and the cell's genome sequence can impact the likelihood of off-target editing. This table includes data from a paper by Haeussler et al. that measured the likelihood that any given site would be subject to off-target editing by CRISPR Cas9 as a function of the number of mismatches between the guide RNA and that genomic sequence. This paper analyzed many different guides and many different off-target sites. They found that sites with one or two mismatches

to a guide RNA have a reasonably high chance of detectable off-target editing. By the time there are three mismatches, less than 2% of such sites with three mismatches have any detectable off-target editing. And by the time there are five mismatches, only one in 20,000 such sites had off-target editing.

Now, how does this data apply to Exa-cel? In the human genome sequence, there are no sites with zero mismatches or one mismatch or two mismatches as compared to the Exa-cel guide RNA. In fact, there are only six sites in the entire human genome with three mismatches to the guide RNA. So, all the other sites that we nominated and tested have more than three mismatches and/or contain a bulge or an alternative PAM. And sites with these features have an even lower likelihood of off-target editing.

Now, the last two slides were about computational homology search. We also nominated candidate sites using a second, orthogonal, laboratory-based method known as GUIDE-seq. GUIDE-seq is a well-established, empirical nomination method that is performed directly in living cells. And we performed GUIDE-seq in CD34 cells from both healthy volunteers. And from patients with Sickle Cell Disease and transfusion dependent thalassemia using the process used by Exa-cel. Now GUIDE-seq has high sensitivity, but to validate this in our experiments, in each experiment we use the on-target site as an internal positive control to document that editing occurred and could be detected. But GUIDE-seq also has a high rate of false positives. And this is because normal cells have double strand breaks even in the absence of genome editing. Given that both Computational Homology Search and GUIDE-seq have a high rate of false positives, it was necessary to perform a second, independent test to determine if off-target editing actually occurs at any of the nominated sites.

To test each candidate site for off-target editing, we used a sensitive and accurate method known as high-coverage hybrid capture sequencing. Specifically, we compared the

genomes of edited and unedited cells at each of the candidate off-target sites. We used very high sequencing coverage depth, ranging from 2,500-fold in the first study to 19,000-fold in the third study. We used such high sequencing coverage depth to enable detection of off-target edits in as few as two in a thousand DNA copies in edited as compared to unedited cells. Finally, in each hybrid capture experiment, we again used the on-target BCL11A site as an internal positive control, confirming that editing occurred, and that hybrid capture sequencing could detect it.

The third step in our framework was to perform risk assessment. The reason to perform risk assessment is that the presence of an off-target edit, if one were to be found, does not, in and of itself, create risk to the patient. The risk of a potential off-target edit would be if it increased risk of malignancy or impacted the function of a gene known to play a role in cells edited by Exa-cel. For this reason, we performed risk assessments on sites meeting either of two criteria.

First, if hybrid capture sequencing had found any confirmed off-target edit, we would have performed a risk assessment.

Second, some of our candidate sites were nominated based on genetic diversity, and if a specific variant allele was not present in any of the samples tested with hybrid capture, we would perform a risk assessment. The pre-specified questions considered our risk assessment were, does the off-target site overlap a gene known to play a role in hematologic malignancy? Does the off-target site overlap an exon? And does the off-target site overlap a gene known to play a functional role and be expressed in blood cells? In a few minutes, I'll discuss the results of these studies. But first, I next discuss the approach used to include genetic diversity in the off-target analysis. Because the intended patient population for Exa-cel is diverse, our off-target analysis includes genetic diversity. We nominated candidate sites based on a variant aware homology search. This identified additional sites that met the criteria for

potential off-target site only in the presence of a genetic variant. Specifically, we identified all variant sites in the 1000 Genomes Project database with a frequency greater than 1% in samples from populations living in each of five continents. That is, donors residing in, or with ancestry from, Africa, East Asia, South Asia, Europe, and the Americas. And in the 1000 Genomes Project, there are more than 21 million genetic variant sites with a frequency greater than 1% in one or more of the populations. We included these 21 million variant sites in our off-target variant aware homology search, and this led to the nomination of 50 additional candidate sites.

As a second approach to include genetic diversity, the 14 donors, in whose cells we performed hybrid capture sequencing, had self-reported ancestry that was diverse, and this included four donors with African American ancestry, three of whom had Sickle Cell Disease. To evaluate the adequacy of this approach to incorporating genetic diversity, it's helpful to review two aspects of our current understanding of the human genome sequence and how it varies across populations.

First, any two copies in the human genome sequence are 99.9% identical. That is, they differ on average at only one of a thousand DNA letters. Of the one in a thousand or so DNA letters that vary in any individual, the overwhelming majority are due to genetic variants that turn out to be common and shared across populations. The reason for this is that all eight billion people living on the planet today are descended from a small founder population that lived in Africa tens of thousands of years ago. Our shared ancestry means that we are very similar to one another at the level of DNA sequence. Because most human genetic variation is both common and shared across populations, it's possible to build a comprehensive database of common human genetic variation.

The second aspect of the human genome that I want to mention is that only 1% spans protein-coding exons. This means that of the millions of genetic variants in each of us, only a

tiny fraction overlaps functional sequences. To survey human genetic variation, we used the 1000 Genomes Project, an NIH-funded, gold standard, global reference database of human genetic variation. And we used the 1000 Genomes Project database because all samples were consented, for public data release, including community consultation. The sample set is large and diverse, including 2504 individuals from 26 different global populations and of the 2504 samples, 661 resided in and/or have recent ancestry from West Africa, East Africa, African American, or Afro-Caribbean populations.

This slide briefly compares the 1000 Genomes Project database to another well-known database called the Human Genome Diversity Project that is discussed in the FDA briefing book. In addition to the 1000 Genomes Project having informed consent for public data release, the 1000 Genomes Project contains more individuals than the HGDP, 2504 as compared to 929, more samples residing in or with recent African ancestry, 661 as compared to 104, and contains 61 samples from individuals with African ancestry residing in the United States. These data document that the 1000 Genomes Project database is an appropriate resource for studies of human genome variation relevant to the Exa-cel target population.

We also performed calculations to evaluate the power to detect variants with a frequency 1% or higher in the 661 donors of the 1000 Genomes Project from individuals residing in or with recent ancestry from Sub-Saharan Africa. The answer is that 661 individuals provides greater than 99% power to discover variants with a frequency greater than 1% in these population samples. Moreover, both internal and external analyses have independently confirmed the completeness of the 1000 Genomes Project database for variants of frequency 1% and above in these samples. Now, I want to again emphasize that because most genetic variation of each individual is shared across populations, and because patients potentially treated with Exa-cel will have ancestry from many parts of the globe, all of the

samples from the 1000 Genomes Project contribute information relevant to our off-target assessment.

Having described the framework for evaluating off-target editing and inclusion of genetic diversity, I will now review the results of the off-target analysis. In summary, three off-target studies were performed, and these studies did not identify any evidence of off-target editing by Exa-cel. The first assessment was performed in four healthy donors and more than 5000 candidate sites, nominated by both Homology Search and GUIDE-seq. The coverage depth was 2500-fold, median depth, and no off-target editing was detected at any site in any individual.

The second study was performed in four additional samples from healthy donors, and this study focused on 171 sites with three or fewer mismatches. Or a bulge or an alternative PAM and also included additional sites nominated from GUIDE-seq in two additional healthy donors. The sequence depth was increased to a median of 15000-fold depth and no off-target editing was detected in any individual at any site.

The third study was performed in six patient samples, three each with Sickle Cell Disease and transfusion dependent thalassemia. As discussed, we performed variant aware homology search, nominating 50 additional sites based on genetic diversity. We included these sites, we included sites also nominated by GUIDE-seq in each of the patient samples. The sequence depth was a median of 19,000-fold depth. No off-target editing was detected at any site in any individual. To the best of our knowledge, this is the most comprehensive evaluation of off-target potential performed to date.

This slide provides an alternative visualization of the hybrid capture data for all nominated sites tested in one patient with Sickle Cell Disease. The X-axis is chromosomal position with chromosome one on the left and the X and Y chromosomes on the right. The Y-axis is the rate of editing at each in edited CD34 cells using our manufacturing process as

compared to unedited CD34 cells using the same process. You can see the purple dot showing the high rate of editing at the on-target site. You can also see that no other site was edited and detected above the threshold of detection. This slide provides the hybrid capture results for the eight healthy donors from the study one and two, and each case you can see very consistent results. High rates of editing at the on-target site and no evidence of off-target editing. And this slide shows a very similar result for the six patient samples, three with Sickle Cell Disease, three with transfusion dependent thalassemia. Again, the results are consistent, high rates of editing at the on-target site, and no evidence of off-target editing at the nominated sites.

We genotyped each of the samples tested for the genetic variants that led to the nomination of the 50 additional sites, that is, we genotyped each sample tested with hybrid capture for the sites, the 50 additional sites nominated based on genetic diversity. Of those 50 sites, nine were nominated based on a common genetic variant with a global frequency greater than 10%. And at each of these nine sites, one or more individuals characterized by hybrid capture carried the genetic variant. In the individual donors who carried the variant allele, no off-target editing was detected. For the remaining 41 of the 50 sites, the genetic variant that led to nomination had a frequency less than 10% globally. And at three of these 41 sites, the variant allele was observed in one or more patient samples studied by hybrid capture, and no evidence of off-target editing was detected. But because some of the variant alleles were not seen in our hybrid capture samples, we performed a risk assessment of each as if editing had been seen. This is what we would have done if editing had been observed in hybrid capture experiments. We found that none of the sites overlapped with the gene involved in hemologic malignancy. And none of the sites overlapped with a protein-coding exon. We also evaluated the candidate genomic site described earlier this year in a paper by Cancellieri et al.

Cancellieri et al. developed a computational algorithm, as you heard from Dr. Bauer, for identifying candidate off-target sites based on genetic diversity. And they used BCL11A as a test case and highlighted the particular variant site as having the potential risk of off-target editing. Our initial homology search actually nominated this candidate based not on genetic diversity but based on the presence of an alternative PAM sequence.

So, we tested this locus in all 14 hybrid capture off-target assessments, and no off-target editing was observed. But we genotyped each of the 14 donors to see if any carried the low frequency site. Discussed by Cancellieri et al. and none of the 14 donors carried that allele. Now, this is unsurprising given the variant has a frequency of 5% in both 1000 Genomes Project and the Human Genome Diversity Project in samples from Africa. Because none of our donor samples contain the variant, we perform the risk assessment to determine if potential off-target editing of this site would be expected to create risk for patients in whom it might occur. The site occurs in a non-coding intron of a gene called CPS1. CPS1has no known or hypothesized role in malignancy. CPS1 encodes a metabolic enzyme that is expressed specifically in the liver and small intestine and is not expressed in any blood cells. Thus, the risk assessment did not highlight any specific risk attributable to potential gene editing at the Cancellieri site.

In summary, we designed Exa-cel to minimize off-target risk by choosing an ex vivo editing procedure with transient expression of CRISPR Cas9, selected an on-target site with a sequence that is unique in the human genome, and carefully screened guides to select one that is highly precise and specific for the on-target site. We empirically assessed off-target editing using hybrid capture, high coverage sequencing in cells edited with our protocol, including sites nominated based on sequence diversity. And we performed a risk assessment on each potential site that was nominated based on genetic variation, including and in addition for the site highlighted by Cancellari et al. And none of the candidate sites nominated based on

genetic variation overlapped with the gene involved in hematologic malignancy, nor a coding exon of any gene. In summary, a comprehensive non-clinical data package did not identify any evidence for off-target editing by Exa-cel.

And I will now turn to Dr. Simard, who will discuss clinical safety.

Exa-cel for the Treatment of Sickle Cell Disease: Safety– Dr. Christopher Simard

Dr. Simard: Thank you, Dr. Altshuler. Good morning. I'm Christopher Simard, Vice President of Global Patient Safety at Vertex. This morning, I'll be sharing a summary of the clinical safety data for Exa-cel, which supports a favorable benefit risk in adults and adolescents with severe Sickle Cell Disease. By way of an overview, adverse events, and serious adverse events after Exa-cel were consistent with that of myoblative conditioning with Busulfan and hematopoietic stem cell transplant. No patients experienced graft rejection or graft failure, and all patients successfully achieved both neutrophil and platelet engraftment. Safety has also been similar across subgroups, including adults and adolescents. No new or unique safety events have emerged during long-term follow-up, including no malignancies. And we'll lastly review key elements of our proposed post-approval pharmacovigilance plan, including product labeling and long-term follow-up.

Beginning with the safety database for Exa-cel consists of 44 patients with Sickle Cell Disease, which included 32 adults and 12 adolescents. Patients have been followed for an average of 20.1 months with 73 and a half patient years cumulative follow-up. 30 patients or 68% have been followed for 18 months or longer with a maximum follow-up of approximately four years.

Now let's look at the adverse event data. The safety profile following Exa-cel can best be summarized as being consistent with that myeloablative conditioning with Busulfan and hematopoietic stem cell transplant. All patients in Study 121 experienced at least one adverse event. 30% had adverse events considered related to Exa-cel. All of these were non-serious.

One patient died. This was from COVID-19 infection, which led to respiratory failure nine months after treatment. The event was attributed to COVID-19 infection and possibly related to Busulfan.

On this slide, we summarize the adverse event rates per patient months over time. Not unexpectedly, following myeloablation, most adverse events, grade three and higher adverse events, and serious adverse events occurred in the first three months. And all decreased over time.

Here we see the most common adverse events, including those grade three and higher. These two were all consistent with the known safety of busulfan myeloablation in NHST. And while we're just showing the most common adverse events here, additional details on adverse events, serious adverse events, as well as safety in adolescents and adults, which was similar has been included in the briefing materials. Turning to engraftment, all patients who received Exa-cel successfully achieved both neutrophil and platelet engraftment. Platelet engraftment time was somewhat longer than reported in the AlloHECT literature. However, overall platelet recovery was robust and patients with longer times to platelet engraftment had similar efficacy. And safety outcomes as other patients in the study.

Before we conclude, I'd like to briefly summarize our post-approval pharmacovigilance plan. Within product labeling, we propose to include the risk of delayed platelet engraftment, as well as risks associated with Busulfan myeloablative conditioning. used as part of the Exa-cel regimen. We also plan to monitor the safety of Exa-cel over the long-term, including clinical trial patients and patients treated post-approval in a registry for 15 years. We have multiple surveillance mechanisms in place to closely monitor patients for long-term safety post-approval. Beginning on the far left, we will follow all clinical trial patients for 15 years. Including the safety and efficacy data shown. In addition, we are fortunate that data from over 90% of patients who undergo bone marrow transplant in the US

is collected and available through the CIBMTR transplant registry. Importantly, all centers in the US where Exa-cel will be used participate in CIBMTR and will provide data on Exa-cel treated patients to the registry and we will have access to this data. We are further also planning a 250 patient Vertex registry-based study which will leverage CIBMTR, and European Transplant Registries, where patients will be followed for 15 years. The study will collect all the data which the registries collect, as well as all SAEs and malignancies, which will be reported to us immediately or within 24 hours.

I would like to point out that for patients in the pivotal studies and the long term follow up Study 131, we collect and store bone marrow and blood samples before Exa-cel treatment and at periodic intervals following Exa-cel treatment.

Finally, in addition to what is summarized on the slide, I'd like to further highlight that as part of our manufacturing process, we collect and store samples of CD34 positive cells before and after editing in all clinical trial patients, and we plan to do the same for all patients who will receive Exa-cel in the post-approval setting. And all of these samples would be available for DNA testing should the need arise. Through these extensive surveillance activities, we will closely monitor patients for potential safety signals over the long-term.

In conclusion, the safety data demonstrate that Exa-cel has a favorable safety profile in patients with severe Sickle Cell Disease. The clinical safety profile of Exa-cel has been consistent with that of Busulfan myeloablation and HSCT with delayed platelet engraftment, the only Exa-cel specific risk. All patients that were able to successfully achieve and maintain both neutrophil and platelet engraftment after Exa-cel. The data also demonstrate the safety profile of Exa-cel was similar in adults and adolescents. To date, we've seen no long-term safety findings, including no malignancies in the entire Exa-cel program and long-term monitoring will continue post-approval. In totality, these data demonstrate that Exa-cel has a

favorable safety profile and support a positive benefit-risk in adults and adolescents with severe Sickle Cell Disease.

Thank you, and I will now turn the lecture to Dr. Frangoul to share his clinical perspective.

Exa-cel for the Treatment of Sickle Cell Disease: Clinical Perspective – Dr. Haydar Frangoul

Dr. Frangoul: Thank you. I'm Haydar Frangoul. I'm the Medical Director of Pediatric Hematology Oncology and Cellular Therapy at the Sarah Cannon Research Institute in Nashville, Tennessee. I'm a hematologist and a stem cell transplant physician. So, I see patients with Sickle Cell Disease and their families from all over the region who are referred to our center to discuss transplant options. I'm also the lead investigator in the study presented today, and I have seen firsthand the impact Exa-cel has on my patients with Sickle Cell Disease. It has been such a rewarding experience to take part in this program, and I'm excited to be here today to provide my clinical perspective and experience using Exa-cel.

As you've heard from Dr. Thompson, Sickle Cell Disease is debilitating and shortens a patient's lifespan. Patients who experience severe recurrent vaso-occlusive crisis live with debilitating pain and chronic progressive complications across multiple organs. I see this diminish the quality of life for my patients and their families. So, it is clear that patients need a curative therapy. I have been performing allogeneic transplant for Sickle Cell Disease for more than 20 years. And I have seen the impact of a cure on patients and their families, it's truly life changing. But we must remember that 80% to 85% of patients with Sickle Cell Disease do not have an HLA identical related donor. And there are many risks involved with transplants using alternative donor transplant that a patient must consider. Unrelated and haploidentical transplants have been associated with the risk of graft rejection, transplant

related mortality and high rates of acute and chronic graft versus host disease, especially in the unrelated setting. I would like to share some patient stories to illustrate this experience.

The first patient we consented was a 33-year-old mother of four children and had been in and out of the hospital roughly seven times over two years. She was suffering with severe and painful Sickle Cell crises, where at times she couldn't walk or even hold up a spoon to feed herself. She described the pain as lightning striking her chest. And because of this, she couldn't keep a job and was struggling to care and enjoy time with her four active children. The patient was initially referred to us for a haploidentical bone marrow transplant, but she was worried about the risk of graft versus host disease and the need for prolonged immune suppression and decided to enroll on the Exa-cel trial. Following Exa-cel, she has remained VOC-free and is now spending time with her family and working full time, something she was not able to do prior to receiving Exa-cel.

The second patient is a 13-year-old girl who was diagnosed with Sickle Cell Disease on newborn screening. She had her first hospital admission at six months of age, and despite hydroxyurea therapy, she was hospitalized many times per year, including an episode of severe acute chest syndrome. She could not attend school regularly because of her pain crisis. Following Exa-cel treatment, she had not experienced any VOC, she has not been hospitalized once, and she's attending school and enjoying her teenage years.

The highlighted stories are not unique to those patients. I see the same effect on the patients with Sickle Cell Disease I have treated with Exa-cel. And many of the adult patients wish they were given the opportunity to be treated at a younger age, so they could have their lives and live it to the fullest. My patients who participated in the trial went on to benefit in the same way as my post-allogeneic transplant patients do over time. They live their lives as patients who do not have the disease, without the severe, painful vaso-occlusive crisis and hospitalizations. They go back to school or work, they participate in their normal activities,

and they enjoy time with families and friends, all things that were previously challenging because of their Sickle Cell Disease.

Because Exa-cel is an autologous product, it avoids the major limitation of allogeneic transplant because every patient is their own donor. Therefore, there is no risk of graft versus host disease or graft rejection and no need for long term immune suppression. And Exa-cel eliminates those risks while providing transformational clinical benefit and a potential functional cure for Sickle Cell Disease. I am from the camp that says to treat at a younger age if possible. Over time, Sickle Cell Disease can cause lasting organ impairment, such as kidney disease, stroke, or bone damage, because some of the damage that occurs prior to transplant is irreversible. I explain it to my patient this way, Sickle Cell Disease is like a hammer hitting a wall. If you hit the wall with a hammer, it leaves damage. With transplant, I can take away the hammer, but we cannot reverse the irreversible damage. We cannot fix the wall. So, if someone comes in with a joint that has been completely destroyed by Sickle Cell Disease, a transplant will stop another joint from being destroyed, but it will not repair the original joint. That is why intervening early is better. I want to take away the hammer before it permanently damages the organs. For Sickle Cell Disease, we have no way to tell what an individual patient trajectory will be. But we consistently see the disease will get worse as children and adolescents approach adulthood. That's why some hematologists perform HLA typing on patients and their siblings early in childhood to identify potential match siblings, even when no signs and symptoms of the disease are yet present.

Dr. Hobbs showed us earlier that the Exa-cel data in adolescents is consistent with the adult data. As we would expect, given that the mechanism of disease and mechanism of Exa-cel are the same, regardless of age. And adolescent patients often tolerate the myeloablative conditioning and transplantation procedure better than adults, further supporting the benefit

of treating early. Therefore, the extrapolation of adult data to adolescent is very appropriate, and I would be happy to have this therapy available for my adolescent patients.

In conclusion, Exa-cel data have demonstrated transformational and durable clinical benefit for patients with Sickle Cell Disease. And I have seen this clearly in the patients I have treated in the study. All study patients received substantial clinical benefit, and these results were demonstrated consistently across adolescent and adult patients. Regarding safety, Exa-cel was generally safe and well tolerated, consistent with that of Busulfan myeloablation and hematopoietic stem cell transplant. As we did in the trials, experienced medical staff who regularly care for patients receiving transplant will be able to monitor appropriately for safe use of this therapy. It has been an honor to participate in this trial and see Exa-cel change my patients' lives. I hope to soon have it available as an approved treatment option for patients suffering with Sickle Cell Disease. Thank you, and I'll now turn the presentation back to the sponsor to take your questions.

Dr. Ahsan: Great, thank you very much for those presentations. That was very informative. We'll now take questions from the committee for the sponsor. And I just wanted to remind people that there will be an FDA presentation after lunch and so, opportunity for questions there as well for the FDA and then, of course, discussion in the afternoon. So, Dr. Tisdale, can you go on camera and unmute yourself, please?

Q & A

Dr. Tisdale: Yeah, thank you for the presentation. This is, of course, quite interesting. I have a number of questions. I'm just going to start off with one or two and then see how the questions go. One is that you've shown really robust and stable percent levels of edits and hemoglobin F. So, these percentages stay stable over time, but you didn't show hemoglobin levels or markers of hemolysis. Were they similarly stable? And can you comment on whether they normalized?

Dr. Krogmeier: I will ask Dr. Hobbs to address your question. Bill Hobbs, Clinical Development at Vertex. Your question is two-fold. One is about hemoglobin and one is about hemolysis.

First, I'll start with hemolysis. And we looked at hemolysis in a couple of different ways. We focused on measures of intravascular hemolysis because these measures, like LDH and haptoglobin and LDH in particular, are associated with increased effects on mortality as well as other vascular complications. And what we observed was a decrease in LDH and an improvement in haptoglobin. Shown here is LDH levels, which normalized after nine months in patients and remain normal. For haptoglobin, we saw an increase in haptoglobin as would be expected with a resolution of homolysis or an improvement in homolysis with levels becoming detectable in patients and remaining detectable in patients over time.

The second part of your question is regarding total hemoglobin partly because of the anemia of the disease and not unexpected for Sickle Cell Disease. We saw increases for the effect of fetal hemoglobin. We saw increases in total hemoglobin as a function of that fetal hemoglobin with levels achieving normal or near normal levels in almost all patients at approximately 12 grams per deciliter, in large part due to that pancellular distribution of fetal hemoglobin, which is shown on the right. And so, I think across the data, which is also in the briefing book for additional review was an improvement in anemia and improvement in homolysis across all study patients.

Dr. Tisdale: You also look at reticulocytes in total bilirubin.

Dr. Hobbs: We did look at additional measures of hemolysis, which included reticulocytes. And reticulocyte counts also improved over time in patients which is shown here with a decrease from baseline, although remaining still perhaps somewhat elevated compared to normal.

Dr. Tisdale: Great. Thank you. I also have a question about the hybrid capture method that you used. So, one thing that puzzled me about the method was that you only had 60% on-target editing in your donor samples. And that doesn't seem to be reflective of the graphs you put into participants because they had much higher even in vivo levels of editing. If the editing rate is higher in the participant samples, I would think that the off-target rates might also be similarly higher. Why was it only 60% on-target editing in these in these samples used for the hybrid capturing?

Dr. Krogmeier: I'll ask Dr. Altschuler to address your question.

Dr. Altshuler: The hybrid capture experiments were done using the same manufacturing process and the same cells and the distribution of on-target editing was the same in the distribution of all patients treated and the hybrid capture experiments.

Dr. Tisdale: So, your participant samples products were 60% edited and then gave 90% neutrophil editing during follow-up?

Dr. Altshuler: The 60% of that you're referring to might be the one slide, but there's a variety of different on-target edits and the samples used. Obviously, it's not the samples from the clinical trial, it's the set of samples we used non-clinically, but it went through the same process, sampled in the same way, and had the same results.

Dr. Krogmeier: I can ask Dr. Moore to address your manufacturing question.

Dr. Moore: Kim Moore, CMC. I just want to add that each patient may receive more than one lot. And so, the specific lot used in the hybrid capture may have contributed to part of the dose, but more than one lot can be used.

Dr. Ahsan: Great. Thank you. Dr. Ott, can you go on camera and unmute yourself, please?

Dr. Ott: Yes, thank you very much. I have a question about the off-target effect of the on-target editing. In other words. I would like to learn more about the lack of BCL2A expression in erythrocytes or precursors and other lineages coming out of HSCs. I understand

that the editing is done in a very specific enhancer, but nothing is always complete, and I would like to know whether the lack of BCL2A has any effects in other lineages. For example, is the delayed platelet engraftment caused by this. And also, do you anticipate any other effects there other than the effect on the hemoglobin F gene?

Dr. Krogmeier: Dr. Altshuler?

Dr. Altshuler: I think the simple answer is we do not expect any other effects but let me explain a little bit more detail. So, I'd like to present here our analysis of on-target editing. So just to contextualize for everybody, the on-target site is in an intron of the gene, as you can see here, depicted between exon two and exon three and just give you a sense of how large and non-coding region this is, the nearest exon on the right is 26,000 base pairs away, and the nearest on the left is 50,000 base pairs away. And you can see the distribution of indels of the different genetic perturbations or edits from Exa-cel in the graph below where the on-target site is right in the middle and you can't really see, the bullet shows you, but in fact, if you quantify it, 88% of all indels are less than 30 base pairs in length. But there are some that are larger. But they're all as you can see here modest in size. If the question then is, what would happen outside of erythrocytes? I actually think there are two ways of answering that question.

One is experimentally, where we actually transplanted the cells into not, and I'm answering non-clinically. We transplanted the cells into animals and looked at the distribution of edits across different cell types, and it was unchanged for the different edited cells. And the other was obviously the clinical data, which could be described. But I think that there's another piece of data that's very informative, which is others, not Vertex, have done extensive characterization of this region. In fact, Dr. Bauer published some very beautiful papers where both in human cells and in animals, in mice, they actually created systematic modification of this non-coding region, including edits much larger than the ones

we see with Exa-cel. And they then looked in the animals and also in the cells and saw no effect of editing this non-coding region and this erythroid specific enhancer in any other setting. And I guess the last point is the genetic variant we're recreating, which is this whole program was motivated by a genetic variant discovered in a genome-wide association study that increased hemoglobin F and decreased the risk of the severity of symptoms with both the thalassemia and Sickle Cell Disease, and then that variant has been studied in millions of human beings. It's a common variant to look for other phenotypic consequences of modulating the site, and none were observed.

Dr. Ott: Thank you.

Dr. Ahsan: Thank you. Dr. Wolfe, will you go on camera and unmute yourself, please?

Dr. Wolfe: Yeah. So, I had a follow up question on the off-target event. It was identified in the Cancellieri paper by the Bauer lab and others. What's the plan to follow up with regards to treated patients to look at editing events at this off-target site in the context of the therapy?

Dr. Krogmeier: I will ask Dr. Hobbs to address your question.

Dr. Hobbs: Thank you. Bill Hobbs, clinical development. This is a really important question that we've thought really long and hard about, and not only in relationship to the Cancellieri variant that was identified, but really to any potential off-target risk. And our approach to this, if you boil it down, is really that to do close clinical monitoring and follow-up, which we recommend and do for all patients in the clinical study, irrespective of the variant or not. And the rationale for the approach is that we know we have established a strongly positive benefit-risk for this in a patient population with severe unmet need who not only have a disease that impacts quality of life, but also shortens their life as the disease relentlessly progresses.

The approach that Dr. Altshuler described for the non-clinical package, which didn't identify any specific off-target risks, and did a risk assessment of any additional variant that could potentially occur in a patient, concluded that there was a low risk of a functional consequence to a patient, and therefore we had neither an off-target to follow, nor a specific variant of concern for a clinical outcome. And we concluded from that, that the appropriate approach for all patients in the clinical study was close and careful clinical monitoring, which is independent of whether they have the variant or not, and assumes that any particular patient could have an off-target effect, which we could then pick up.

In that process, we also collected laboratory samples from both before and after treatment. That would allow us to go and then subsequently investigate, should the need arise. And so, this is also reflected in our pharmacovigilance plan that Dr. Simard described. And so, our approach has been to do careful, close clinical monitoring, which was also referred to in the session this morning as the appropriate approach for all patients who receive a genetic therapy like Exa-cel.

Dr. Wolfe: So, there's no plan for molecular follow-up to look at editing at this off-target site? It seems like there is quite a bit that could be learned with regards to off-target editing rates in your treated patient population by looking retrospectively at editing at this site now that you've treated more than 45 patients.

Dr. Krogmeier: I will ask Dr. Altshuler to address your question.

Dr. Altshuler: It's an important question, and we've thought deeply about it. And the way we think about it is first taking into consideration, not only the off-target assessment we've done, but all the other assessments and the package of data. And as Dr. Hobbs described, we don't believe from the totality of data that we've collected that additional, non-clinical studies are going to be informative, and then we believe for clinical studies, the important thing is to

follow all the patients, see if any events occur, and then we'll have the samples and the data to try and understand those events, and that's our approach.

Dr. Ahsan: Thank you. Dr. Shapiro, can you turn on your camera and come off mute, please?

Dr. Shapiro: Yes, thank you. I have some clinical questions. I think specifically for Dr. Thompson. Can you comment on fertility preservation protocols and what standard of care and issues specifically related to that in individuals of childbearing age or pre-puberty for individuals who might undergo this therapy?

Dr. Krogmeier: I'm going to take that in two parts. First, I'm going to ask Dr. Hobbs to comment on the clinical perspective and then Dr. Thompson to comment on the patient perspective.

Dr. Hobbs: Bill Hobbs, clinical development. And thank you for the question because this is, I think, a really important one for patients and families as they think about going through a treatment such as Exa-cel. In the clinical studies, for all patients, we offered fertility preservation. And that's largely because the reason for that is the Busulfan myeloablative conditioning that patients get, which has a high frequency of potential infertility afterwards, not related to Exa-cel itself. But we did offer that for all patients in the clinical study. And I'll turn it over to Dr. Thompson to discuss the additional clinical perspective on that for patients.

Dr. Thompson: Thank you, Dr. Shapiro, for asking the question. I think this is a critical issue that we need to deal with in terms of advocacy. I think it's safe to say prior to programs like this current program, this was not the standard of care, although one could have made the argument some time ago, given that myeloablative therapy even used in allogeneic stem cell transplants has been associated with infertility. It's been very reassuring that recent programs in this space have included that as part of the studies, including the payment for it. I do believe that many of us have an opportunity to advocate with insurers to be sure that they also

consider this in the totality of costs for transplantation. It is absolutely tragic for families having to choose between a possible cure and their children having future children. And so, we would strongly, as a community, support any and all efforts, including those by Be The Match, which will now help to support, in a limited way, fertility preservation for individuals with Sickle Cell Disease who are undergoing chemotherapy related conditioning that may impact their fertility.

Dr. Ahsan: Great, thank you—

Dr. Shapiro: Actually, I have a follow up question. Is that okay? Or do I need to read—

Dr. Ahsan: Yes. If we can keep it brief, we have a few people with questions. Yes.

Dr. Shapiro: Okay. In this regard, would you if both allogeneic stem cell transplant were available as well as Exa-cel, would you prefer Exa-cel over allogeneic treatment for individuals with Sickle Cell?

Dr. Krogmeier: I will ask Dr. Frangoul to address your question.

Dr. Frangoul: Thank you. This is Haydar Frangoul from Sarah Cannon. I think the decision to go with Exa-cel versus an allogeneic transplant, even when there is an HLA identical sibling identified, is a decision that should be made by the physician as well as the family. There are so many things to consider, including recovery time, the need for immune suppression, the collection of cells from the donor, which can put the donors at risk to donate bone marrow. So, there are multiple variables, but the results we are seeing are equivalent to what we see with the HLA identical sibling transplant. And I think that discussion should take place between the physician and the families.

Dr. Ahsan: Great. Thank you. So actually, I'm going to insert myself here to ask a question myself, which is the efficacy seems to be very impressive in terms of data and its durability. Is there any reason to believe a change in off-target effects? With repeat treatment. We're hoping it seems like there's a propensity to try to treat earlier with adolescents, but this

is a lifelong disease. If you were to do a repeat treatment, or if the patient were to receive another genetic editing therapy later in life, do we expect a difference in off-target effects?

Dr. Krogmeier: I will ask Dr. Hobbs to address your question.

Dr. Hobbs: Thank you, Bill Hobbs, Clinical Development at Vertex. Exa-cel was developed and is intended to be a one-time treatment, and we do not envision any need or approach that would include re-treatment.

Dr. Ahsan: Great and could you speak a little to the off-target effects if they were to receive a different genetic editing therapy subsequently in life?

Dr. Hobbs: Yep thank you for the question. I'll turn that over to Dr. Altshuler to continue that answer.

Dr. Altshuler: I'd like to just go back and answer your question to the transient nature of CRISPR editing with excess cells. So, the cells obviously are harvested and then in the manufacturing process, they are briefly exposed to the CRISPR Cas9 enzyme using ribonucleotide RNA protein particle and are in a protein complex and then that is a shortly short Duration of editing and then it's gone. So, I guess if your question again is Dr. Hobbs said we do not imagine the need for nor intend there to be another treatment. But just as your hypothetical, the exposure to CRISPR Cas9 is extremely brief and not in the body of the person who has the cells because it all takes place in the manufacturing process and is then gone and none of it residually is there and makes it into the patient

Dr. Ahsan: Great, thank you. You expect no residual effects in the cells once they've been edited. Dr. Breuer if you could go on camera and take yourself off mute.

Dr. Breuer: Thank you for your presentations and congratulations on your promising clinical trial results. My question pertains to the labeling. Was any consideration given to adding possible off-target effects to the label? While I recognize that your preclinical studies did not show evidence of that. I think given the nature of this meeting and the emerging field

and the difficulty of potentially trying to identify these things ahead of time, might that be something to consider?

Dr. Krogmeier: Yeah, we are still in discussions with the agency on the label. We, those discussions will certainly include the safety of Exa-cel.

Dr. Breuer: Thank you.

Dr. Ahsan: Great, thank you. Dr. Tisdale.

Dr. Tisdale: Thank you again. So, I have a question about clone diversity. There's a lot of talk about off-target effects and the effects that an off-target could have on subsequent hematopoiesis, but I think one buffer against a clone getting out of control is to have a diverse set of hematopoietic stem cells that have this edit. So, I'm wondering if you have ways to estimate HSC number contributing over time. I know it's more difficult than, for example, an integrating vector where you can use integration sites to do that. But perhaps, the diversity of edits could somehow give you a sense of how many corrected cells you're putting back in and whether that's a high number and a number high enough to hopefully prevent clonal events later.

Dr. Krogmeier: Dr. Altshuler.

Dr. Altshuler: It's a great question. And I'll think during the break if there's a quantitative answer to your question, but I can tell you that just in terms of you're trying to estimate the number, but I will tell you a couple of things that are relevant to your question. One is in the New England Journal paper in 2021, Frangoul et al. we actually published a plot of the distribution of indels, which are not each, of course, clonal because you got the same indel occur multiple times. We see a very broad distribution of different indels. And that figure in the New England Journal paper shows three different lots, and then they were each transplanted into mice, and there were many mice for each. And we both followed the number of clones and also the distribution of those clones across the animals. And it was,

there were many different indels in each animal and in the cell line and across many different animals that diversity was maintained and similar. And then the other thing we've done is as part of process quantification, we have characterized the 19 different donor lots just for the distribution of indels and again see a very broad distribution of indels. So, I'll think about your specific question if I can quantify it, but I think the answer is that there is a broad distribution of cells that get engrafted and there's a broad distribution of different indels to the nature of what you were asking. I think that is the case, but I'll think about your very specific question. See if I can come up with a more quantitative answer for you.

Dr. Ahsan: Great, thank you. We are going over time, but I think this is an important discussion. So, we'll continue a little bit more before we go to lunch. Dr. London.

Dr. London: Yes, thank you. I'm wondering about the samples from only 14 donors of patients that were tested for off-target editing. How was the sample size chosen? It seems small for detecting the kind of rare event that we're concerned about.

Dr. Krogmeier: Dr. Altschuler.

Dr. Altschuler: Thank you. If I could have the slide from the core presentation on genetic diversity. The way that that we think about this in terms of the assessment of genetic diversity really comes back to and actually, if I could have the slide on the two different types of genetic diversity, please, the 1000 Genomes Project and the sample of Zoners. So, the way we think about this is, first, that as described in the both our presentation and the previous presentations, we do understand that off-target events are directly related to homology. Between the guide and between the host genome, we know the sequence of the host genome, and we also know the sequence using 1000 Genomes project of 2504 people, including more than 21 million genetic variants. We did the analysis, so I would think of it as the nomination of sites is not about 14 people. It's actually about 2500 people from around the world, including 661 people within Sub-Saharan Africa. Having used all those genetic variants to

nominate the sites, then we went and looked at were any of those sites, either from the reference genome or the genetic diversity, did they have off-target editing?

And we did look at those in 14 individuals of diverse ancestry.

But the 14 individuals are not the limit of detection for the variation in people, because we know the variation from the 1000 Genomes Project of 2500 people and we looked at all those sites and all of the sites in the reference genome, of course, we examined all the sites with a frequency greater than 10% in the human population from the 1000 genomes were directly evaluated and there were sites that were low frequency, like 1% in a group from one continent or another, and we didn't see them all.

We acknowledge that. So, we perform the risk assessment that we would have performed had we seen off-target editing. And that risk assessment did not identify any genes overlapping with a gene involved in hemologic malignancy using the myelocyte panel or an exon of any gene. So, we believe that the assessment is not an assessment of 14 people, it's an assessment of the genetic variation across 2500 people that there was queried in appropriate samples.

Dr. Ahsan: Great, thank you. Dr. Komor.

Dr. Komor: Hi, I have a quick question. In the brief, it said that no chromosomal abnormalities were detected, but I was just wondering what was the assay for looking at those and if that would have picked up any larger insertions or inversions or translocations or truncations?

Dr. Krogmeier: I will ask Dr. Altshuler to address your question.

Dr. Altshuler: We evaluated chromosomal abnormalities using two different orthogonal methods. One was karyotyping edited cells, which is a standard approach. And the other was we used a combination of long-range PCR and split read analysis to evaluate both the indel patterns at the site, because as one of the talks mentioned, I think it was Dr. Bauer, you can't

simply use PCR to look at large indels because there's an amplification bias against large sites. So, we used a thing called split read analysis and got actually very similar results for those. So, we saw no chromosomal abnormalities in these studies. And I would just note a few other points just in how we think about it, which is that to the best of our understanding, creation of a chromosomal abnormality involves cutting at two sites. And one would be the on-target site, and as we said, the systematic evaluation we described did not identify any on-target, off-target editing by Exa-cel that would be the substrate. And then just two other points that we at least think about are, one, that cells contain that DNA repair system that Dr. Urnov described, which exists to identify DNA damage, and then either arrest the cells, and either repair the DNA damage or induce apoptosis. And so even if such sites are created, it doesn't necessarily mean they'll survive.

And the last point, just because a lot of discussion in the field is about laboratory experiments that are transient, rather than transplant experiments, in order for such cells, if they did have any damage, and they did actually survive the DNA repair response, they'd also have to survive, the engraftment process and make it to the patient. So, those are just additional considerations.

Dr. Komor: Thanks for the clarification.

Dr. Ahsan: Great. Thank you. We are getting pressed for time. Dr. Wu, if you could keep it brief, that would be great.

Dr. Wu: Yes. A very quick question. You may have covered this already, but what is the number of cells of the hematopoietic cells that you infuse back to the patient and do you have a sense of, what percent of these cells have been successfully edited from patient to patient? And then you also showed a patient that have recurrence at the VOC. Is it because the number edited cells was lower compared to the other batches compared to other patients?

Dr. Krogmeier: I will ask Dr. Hobbs to address your questions.

Dr. Hobbs: Bill Hobbs Clinical Development. The first part of your question is about the number of cells infused in patients. And the protocol specified a minimum of three times 10 to the sixth per kilogram and a maximum of 20 times 10 to the sixth per kilogram. And in the clinical study, that range was infused into patients.

r. Ahsan: Great, thank you very much. I think we've addressed all the questions for now. There will be opportunity if we need to, if the committee feels like they have questions directly for the sponsor, we can arrange for that. But I think for now, we're set and we'll be taking a break for lunch and we will reconvene at 12:35. So, in 30 minutes. And so, enjoy your break and I'll see you all then.

Open Public Hearing

Dr. Ahsan: Welcome back to the meeting from the lunch break. We're now going to move forward with an open public hearing, and I have an announcement to read. Welcome to the open public hearing session. Please note that both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the open public hearing session of the Advisory Committee Meeting, FDA believes that it is important to understand the context of an individual's presentation. For this reason, FDA encourages you, the open public hearing speaker, at the beginning of your written or oral statement, to advise the committee of any financial relationship that you may have with the sponsor, its product, and if known, its direct competitors. For example, this financial information may include the sponsor's payment of expenses in connection with your participation in this meeting. Likewise, FDA encourages you, at the beginning of your statement, to advise the committee if you do not have any financial relationships. If you choose not to address this issue of financial relationships at the beginning of your statement, it will not preclude you from speaking. So with that, we'll move forward, and I hand this over to Cicely Reese who will be handling the open public hearing.

Dr. Reese: Thank you, Dr. Ahsan. This is Cicely Reese speaking. Before I begin calling the registered speakers, I would like to add the following guidance. FDA encourages participation from all public stakeholders in its decision-making processes. Every advisory committee meeting includes an open public hearing session during which interested persons may present relevant information or views. Participants during the open public hearing session are not FDA employees or members of this advisory committee. FDA recognizes that the speakers may present a range of viewpoints. The statements made during the open public hearing session reflect the viewpoints of the individual speakers or their organizations, and are not meant to indicate agency agreement with the statements made. In fairness to all open public hearing speakers here today, since this is a one-hour session, we ask that you please remain within your four-minute time frame. To assist speakers in adhering to four minutes each, we are placing a timer in the lower left of the screen for each presentation. We greatly appreciate your cooperation. When I call your name, please unmute your microphone and open your camera if you would like. And start your presentation. If you are not available at that time, we will come back to you after the other speakers have spoken. We will now begin with open public hearing speaker number one.

Ms. Gray: Good afternoon. I do not have any ties to get paid financially to be at this meeting. My name is Victoria Gray. I'm a 38-year-old mother and wife. I'm the first sickle cell patient to be treated with CRISPR gene therapy. Before this treatment, my entire childhood and most of my adult life was plagued with severe pain, fatigue, numerous hospital stays, and the fear of dying. The pain would come on so suddenly, it felt like I was being hit by a truck and struck by lightning at the same time. In order to manage my pain, I had to take three different opioids, oxycodone, dilaudid, and fentanyl. Even with this combination, I was still in a lot of pain. I received regular blood transfusions in hopes to increase my blood counts and improve my symptoms of pain and fatigue. But it was only a temporary solution.

One hospital stay in particular has been permanently imprinted into my mind. It was in October 2010 that I had one of the worst sickle cell crises of my life. It ended my college pursuit of being a nurse. With this crisis, I was awake for three days straight. I couldn't use my legs or my arms. I was in so much pain that I couldn't even lift my hips enough to sit on a bedpan. I couldn't lift a fork to feed myself or use my hands to wash my face. I depended on a physical therapy team to help me regain the control of my body. This was all a result of a severe pain episode from sickle cell disease. I didn't get released from this hospital stay until January 2011. I missed Thanksgiving, Christmas, and all four of my children's birthdays. I became so weak from being beat down by this disease, I had to have someone come into my home to help me with my normal day to day routines. It wasn't until my son's teacher called me to say that his behavior had changed, excuse me, because he thought that I was going to die. I knew I had to fight for my kids.

When I met Dr. Fringle in Nashville, he presented the opportunity for me to join gene therapy trial. I said yes without hesitation, knowing that I would be the first person. But this was my opportunity to fight. After receiving this treatment, I no longer have pain, so I no longer have to take opioids. I no longer have hospital stays or receive blood transfusions. I get to participate with my kids and join them in their activities when they play sports, cheer them on at their dance events, and just be here, and just to play with them and, knowing that I no longer have to leave them to go to the hospital. I now work full time and I contribute to my household and my community. I believe if you say yes to this treatment, that it's going to change the lives positively of many people who are suffering from diseases and disorders who now feel hopeless. But once it comes, they can feel hope again, just like I did. Thank you.

Dr. Reese: Thank you so much for sharing your personal story. We'll now have open public hearing speaker number two.

Mr. Abrams: Yes, good afternoon. Can you hear me okay?

Dr. Reese: Yes.

Mr. Abrams: Thank you. Good afternoon, everyone. Michael Abrams here, from Public Citizen's Health Research Group. We have no financial conflicts of interest on this matter. The exocell gene editing therapy to reduce the frequency of vaso-occlusive crises in patients with sickle cell has demonstrated apparent efficacy in at least 29 of 30 subjects who have received this therapy thus far. This therapy, as we've heard, involves stem cell extraction from patients, CRISPR editing, aimed at reigniting the expression of fetal hemoglobin, and autologous re-infusion of the re-engineered stem cells back into the patient. Chemotherapy, of course, is required and used to prepare patients for this autotransplant. The FDA scientific review of exocell has concluded that these results, although limited to small single arm studies, are overall strongly positive. This review also notes that, if the therapy is approved, a 15 year follow up study, yet pending in design, has been proposed to fully evaluate safety outcomes, including the possibility that barren (phonetic) gene editing may lead to plausible adverse effects such as malignant cancers, blood diseases, organ damage, transplantation-related illness, and even the possibility of early death. The focus of this meeting is accordingly, not so much on the efficacy of exocell, but on its safety. Specifically, there is considerable uncertainty about off target gene editing, that is unintended editing of other genes besides those which turn on the expression of fetal hemoglobin. Per the FDA's review, the sponsor has thus far assessed the probability of off target gene editing in two ways. First, by using algorithmic or silico reviews of existing genome databases, and second, by using more direct cellular assays, looking at cells, how they've been modified with the exocell therapy.

Unfortunately, at present, both of those evaluations have insufficient scope. The algorithmic analysis relies on a limited amount of sequencing data, that may not capture all of

the variants that are vulnerable to off target editing. For example, the review notes specifically that only 61 whole genome maps of individuals of African descent from the southwest U.S. were actually used to consider whether tens of millions of genetic variants may be at risk for off target editing. Moreover, the review notes that one recent silico study published in Nature Genetics, which we heard about this morning, did not identify the same variant of concern that were identified by the sponsor study described today. A discrepant finding, that may underscore sampling concerns. Finally, the cellular assay data was limited to just nine subjects. Three healthy, three with thalassemia, three with sickle cell disease. As stated by the FDA in their packet quote, it is unclear whether this limited sample size will provide for an adequate understanding of the potential risk of off target editing. Sickle cell disease, for example, is known to alter chromatin structure and stem cell function. Such alterations could plausibly affect the risk of off target editing. Accordingly, Public Citizen's Health Research Group presently strongly believes that more study is needed to determine if off target gene editing is a concern for patients receiving the therapy. We thus encourage this advisory committee and the FDA to require additional comprehensive studies to be completed before exocell is approved for wider spread use. Thank you very much.

Dr. Reese: Thank you. We greatly appreciate your comments. We would like to have open public hearing speaker number three.

Mr. Onehare: Hi, my name is Jimmy Onehare (phonetic). Hi, my name is Jimmy over here. I participated in exocell about 36 months ago, and I've got nothing to disclose. For most of my adult existence, my life has revolved around one thing, sickle cell disease. It dominated every facet of my life. Hospital admissions were so regular that they even had a bed reserved for me. It was a circus. Bouncing from specialist to specialist, and constantly desecrating my body with endless amounts of prescription pills, all in the hopes of finding a sliver of what it feels like to be truly alive. So when the opportunity came to participate in a gene editing

clinical trial, I leaped at that chance with no concern of any future consequences. Now, instead of gloomy hospital rooms, I'm out here living life to the fullest. No more days wasted under the fluorescent lights of the ER. No more pain, and subsequently, no more pain meds. No more endless forms, no insurance battles, and no waiting room that seemed designed to test your patients. I can breathe easier, both literally and figuratively.

Prior to the therapy, I had focused on the short term. Life was in a state of touch and go. Long-term planning meant planning for a world without me being able to support my family. Now those long-term plans include me. My family can do more and achieve more, because we're all able to work towards the same goals. My quality of life has soared to new heights, allowing me to achieve things I once thought were impossible. Gene therapy has given me the ability to take full control of my life. I can chase the proverbial sunset, write novels, and even dance in the rain without a care in the world. Most importantly, gene therapy has given me the ability to be a present father, and not encumber my children with the burden of caretaking. In a world where the deck was stacked against me, gene therapy has been a winning hand. While I recognize gene editing won't be the solution for everyone, I strongly recommend embattled warriors to consider this one-time therapy, as it has the potential not only to change the individual's life, but also impact generations to come. Thank you.

Dr. Reese: Thank you, open public hearing number three. We really appreciate you sharing your personal story. We'll have open public hearing speaker number four. Excuse me, we'll have open public hearing speaker number five. We'll try to come back to number four.

Ms. Ashley: Hello, my name is Brianna Ashley. I've lived my entire life with sickle cell. I had constant crises. I had a crisis every two weeks. I mean, twice a week. Constant hospitalizations. And I was approached with the gene therapy where they take my cells, altered my fetal hemoglobin, he gave me my own cells back. And after the process, I haven't had any crises, any hospitalizations. Sickle cell. I had a little brother that was two years

younger than me, and he passed away from sickle cell because of organ failure. And I wish that this gene edit editing was around longer, and I want others to have it as well, and have the opportunity to it. So that way, everyone else can experience it, just like I wish my brother could have. My life has changed drastically. I have more energy. Like I said, I don't have any crises. I'm not in the hospital. I haven't been in the hospital in six months. I'm at my six-month period and I haven't had any problems with sickle cell. So, I ask that this is offered to others. Thank you.

Dr. Reese: Thank you, for your moving comments. We appreciate it. We'll now move on to open public hearing speaker number six.

Ms. Howard: Hello. Can you hear me?

Dr. Reese: Yes, we can hear you.

Ms. Howard: Okay. My name is Darday (phonetic) Kelly Howard, and I have sickle cell disease. Before the age of one, I was hospitalized over 13 times. Last year, I was hospitalized 100 times. Over the years, I have experienced stigma surrounding my disease. I have been doubted, dismissed and judged for having pain. Throughout my life, all I could think was, I wish there was a cure. I wish I didn't have to go through all this pain. I wish I didn't have to be in the hospital. Well, today, I'm here to tell you that I am three months post op of having a BMT. Although it's still early, this transplant has improved my quality of life tremendously. It has relieved me of so much pain. It's freed me from continuous hospital stays and has given me some quality of life back. This process, it's liberating me from a disease that I have been fighting all 33 years of my life. I am so grateful because I don't know where I would be without the transplant.

However, BMT is not accessible to all SCD warriors, because they do not have a stem cell donor. Gene therapy is an additional option that can cure SCD as well. It's more accessible. It doesn't require full body radiation, and has a shorter recovery time. I'm asking

the advisory committee to prioritize research and development of both methods. These are life altering treatments that are desperately needed. Awareness and access are extremely important to improve and save the lives of people battling SCD. This is my plea. I pray you take note, and action. Thank you, from a surviving sickle cell warrior.

Dr. Reese: Thank you for your comments. We greatly appreciate your comments. We'll now have open public hearing speaker number seven.

Mr. Sandhurst: Hello, everyone. My name is Evan Sandhurst (phonetic) and I have no financial conflict of interest in this matter. I'm here today on behalf of my incredible wife, Elodie Antala (phonetic), who is a sickle cell warrior, and also on behalf of the roughly 100,000 sickle cell warriors living in the United States who are battling sickle cell disease every day. I am here to voice my support for gene therapy as a curative therapy for sickle cell disease. Elodie and I met in 2018, and I quickly fell in love with her infectious laugh, her wisdom, and her immense enthusiasm for adventure and everyday life. I also learned quickly about the very real challenges and obstacles that Elodie faced as someone living with sickle cell. When we met, Elodie was recovering from a severe stroke and was receiving eight units of blood every six weeks via an exchange blood transfusion. These transfusions served as a treatment and helped her sickle cell stay at bay. However, by 2019, to keep up with the progression of the disease, she was having to receive a blood exchange every four weeks. As you can imagine, this was a huge challenge for her and her family.

Even though our life had many hurdles because of sickle cell, we were still able to have many moments of joy and celebration. And by 2020, we decided to get married. By 2021, we were exploring our options to become parents. It was then that we found out through a brain MRI that Elodie had small vessel disease in her brain. We were told that she was likely to have another stroke. This incredibly difficult news served as the catalyst for us beginning to research available curative therapy options to cure Elodie of sickle cell.

In our search, we met with multiple doctors and hospitals in Wisconsin, Virginia, and Ohio. We learned about the two curative therapy options, which were gene therapy and bone marrow transplant. Elodie's first choice was gene therapy as she felt it was less scary, less risky, and has a better chance of success. However, due to her history with stroke, gene therapy was not an option for her. We decided to pursue the bone marrow transplant as a possible cure for Elodie. Luckily, we found out rather quickly that Elodie's father was a bone marrow match and would be able to be her donor. In September 2021, Elodie successfully received her bone marrow transplant and was cured of her sickle cell. Elodie, her family and friends, and I recently celebrated her two-year anniversary of her successful transplant, and it marked a truly life changing milestone in her battle as a sickle cell warrior.

I just want to share a quick photo if I can. This is... Oh, I don't think I'm able to. Okay. Today Elodie is living her best life. She is able to have a full-time job. She is able to swim and exercise regularly. No longer has to battle regular pain crises. She doesn't have to receive monthly blood transfusions or live with the worry of having another stroke. I am here today sharing Elodie's story to highlight the immense importance for all sickle cell warriors to have access to life changing curative therapies like bone marrow transplant and gene therapy. Thank you all for your time.

Dr. Reese: Thank you so much for your moving story. We really appreciate that. We'll now have open public hearing speaker number eight.

Ms. Shapiro: I have no disclosures. I'm Adrienne Shapiro, and I'm here representing five generations of mothers in my family to have a child born with sickle cell disease. I think of these mothers often. I think of their pain, I think of their children dying, I think of their reality of their lifetime. In 1865, there was the Emancipation Proclamation. In 1890, boom, the Wounded Knee Massacre. In 1815, the beginning of World War I. In 1940, World War II. In 1965, the Civil Rights Movement was going on. And in my daughter's generation, the

1990s, was the beginning of the Genome Project. As a young child, I told my mother that someday I was going to speak to the FDA. I can't remember why, but she always said to me, God is good, but science is going to fix this. She was the first generation of mothers to understand the cause of the disease, and I was the first to benefit from trait testing. No trait, science said, I was good.

We were all taught to look after my brother. As my mother learned, we all learned. We learned about colds, viruses, weather, sleep, hydration, visits to the doctor with just the three of us. We learned about life, division of parenting duties, and the isolation that comes from having a warrior. You're in your family. Everybody told my mom that she should send my brother away. He had a stroke at three and it left him mentally and physically disabled. She kept him with us, when everybody said he was going to die. She kept him living. So when my daughter was diagnosed with sickle cell I learned two things. Cheap science is bad science, and sickle cell disease was not just a disease for black Americans. Everybody said anyone in the family could meet this challenge. It was me. I was trained by the best. So now we have two more generations living through this well documented lens of sickle cell disease trauma.

Nothing was ever going to be normal. Education, employment, enjoyment, nothing. I was determined she was going to remember, staying alive, and she was going to be healthy. Well, she was alive but not healthy. For generations she spent months in either the ER, the ED, somewhere. And until we got treatments that made her have a better life. I know that I may not be the last mother with a child with sickle cell disease in my family. But with these treatments, I will be the last mother to watch my child suffer and die without hope. Science is fixing this, and science is only going to get better. Please support this. Thank you.

Dr. Reese: Thank you very much for your very moving personal story. We'll now have open public hearing speaker number nine.

Speaker # 9: Thank you for having me today. I don't have any ties or disclosures to speaking today. I'm gonna start off by saying I am a 42-year-old male who lives with sickle cell disease. I was diagnosed with sickle cell at the age of two. I was the only one out of four kids to have sickle cell. So as you can imagine, life for me was different. I was in and out of the hospital with pain crisis because of my complications from sickle cell. I had pneumonia as a kid. I had my gallbladder removed as a kid. And I dealt with excruciating pain crises that would have me in and out of hospital for days to weeks at a time, because the pain was so excruciating. It felt like I was being hit with a hammer or someone had a vice grip around my arms and was just squeezing and I couldn't get rid of the pain on my own. Even with the prescribed medications I had at home, like Percocet. So I had to go to the hospital and rely on the hospital. And that took a heavy financial burden on my family.

My mother, she had to take off of work to care for me because I couldn't be in the hospital alone as a child. So she would miss days to weeks at a time at her work. And when her work didn't understand that she had a child with sickle cell, and didn't know what sickle cell was, she would be relieved of her duties at times. That also put the pressure on my dad because while I was in the hospital and my mother was in the hospital with me, he would have to take care and provide and run the household for the other three children. And his job also didn't understand. So, at times, because of the financial struggle we had, because of my health, we would go without things or have to borrow money so that we can have food on the table. Simple necessities like tissue. And so, over a lifetime, that financial burden doesn't go away. It continues. Within a year, over 10,000 is spent on medical costs, medical care. And as I got older, I would still be in the hospital with the same excruciating pain, pain that, if it was in my legs I couldn't walk from here to the bathroom, or pour a glass of water from a pitcher.

So it's very important that we support gene therapy because it's a lot of people like me who want to be relieved of this pain, and the stigma of going to the hospital. The biases of my

disease left me with inadequate care because I was a man. I didn't get the right and proper care. So it's important that we address the issues and concerns for sickle cell because our entire life we came into this world fighting. We fight with hospital systems. We fight for our health. We fight with insurance companies for coverage. We fought with pharmaceutical companies to come up with medications that would help us. So that we wouldn't have to go through this pain, because 100,000 people live with this pain. And just the fact that we only had four medications. And now that we have the pharmaceutical companies on our side, and they see the importance, and they're taking action, and they understand how much this affects the community. I think it's important that we support gene therapy, so those hundred thousand people can live a normal healthy life. Can work, and can have jobs and be providers for their family. So I thank you for this time and I ask that you support this gene therapy.

Dr. Reese: Thank you so much. We appreciate hearing from you.

Speaker # 9: Thank you.

Dr. Reese: We'll now have open public hearing speaker number 10.

Ms. Ebbs: Hello, my name is Trinity Ebbs (phonetic), and I do not have any financial ties on this matter. I was born with sickle cell hemoglobin SS disease. At the age of 16, I received the CRISPR stem cell transplant, and since then, my life has been so much better than I imagined it could be. All my life, I suffered from chronic and severe pain crises, along with other complications that came with sickle cell disease. Many times, the pain would put me in the hospital to receive IV fluids, strong pain medications, and blood transfusions. When I was not in the hospital, I had to take pain medicine just about every day of my life. When I was in elementary school, getting up in the morning was hard for me. I was tardy just about every day, with some of the time having to use a wheelchair to assist me around the school, because it was too painful for me to walk. Frequently, after having so many consecutive missed days of school, I would have to be put on homebound schooling. Physical activity, or a change in

the weather, could also bring the onset of pain crisis. I could always tell when the rain or first cold front of the season was on its way, three or four days before, and even times with a prediction not even being made by the meteorologists.

By the time I got to middle school, my condition became worse. With pain crisis episodes, with me still having many hospital visits, making it extremely hard for me to attend school. Eventually, when I became old enough, I became dependent on hydroxyurea, which minimized some of my hospital visits. But, not long after, I had to have surgery to have my spleen removed, which is common for patients with sickle cell. Shortly thereafter, my mom made the decision to remove me from public school and enrolled me in a self-paced online private school, because I fell too far behind in my classes and was not learning anything from missing so many days of school.

It's been two years now since my transplant, and I have not have had to been hospitalized due to any sickle cell pain. I have minimal pain, so taking pain medication has been reduced. I have no longer of pain when the weather changes. I can be physically active, walking a mile without having a pain crisis. I can swim staying in the water for long periods of time without needing a wet suit to keep me from getting cold. I am now currently finishing up my last two years of high school, attending in person learning for the first time since middle school. with the ability to focus and learn with almost perfect attendance. Some of my classes consist of dual credit courses, and I plan to attend college after I graduate. My overall health has improved 95 percent, and I'm able to spend time with my family and friends without having to miss out on special events all the time. I'm so glad I enrolled in the CRISPR study, and would like other patients to have this opportunity to receive this treatment. The best part of the transplant is that you are able to choose your own cells, especially when you have no one else as a match. Thank you for this opportunity to speak.

Dr. Reese: Thank you so much for sharing your story. We truly appreciate it. We'll now have open public hearing speaker number 11.

Dr. Hsu: Good afternoon. I'm Lewis Hsu, and my colleague, Dr. Donnell Ivy and I would like to represent Sickle Cell Disease Association of America. We volunteer as Chief Medical Officer and Vice Chief Medical Officer, respectively. I declare no financial ties in this matter. Next, please. Sickle Cell Disease Association of America has a mission to advance the search for universal cure, and that's what this gene therapy is about. Sickle cell disease is a rare disease, but if you count up the families impacted, it is probably half a million or more affected by a disease with a lot of suffering and day to day insults, as you've heard, as well as high cost and high utilization of the emergency department and of the hospital. Next, please.

And you've already heard what the community feels about gene therapy, enthusiasm, and seeing the potential benefits for individuals living with sickle cell disease and their caregivers. And Sickle Cell Disease Association of America likewise says yes for gene therapy. And it's a "yes, but". Next, please. So there are issues to deal with as we seek that there could be approval of this gene therapy approach. That this would be something where you do pay attention to coverage for fertility preservation. That there can be, addressing lack of insurance coverage in many states for fertility preservation. There can be also attention not just to pain and to cancer risk, but also to behavioral and mental health. And that the services provided for people with sickle cell disease, who don't get gene therapy, don't get tossed to the side as we pursue gene therapy. Plus, for those who have the gene therapy that there can be ongoing care, ongoing attention during the planned 15 years of follow up, to look for additional problems. Whether there could be something beyond secondary cancers, organ damage or other kinds of effect. I'm going to turn the rest of the time to my colleague, Dr. Donnell Ivy. Next slide.

Dr. Donnell Ivy: Hello. I do not have any financial relationships to disclose. Thank you for this opportunity to provide testimony on behalf of the Sickle Cell Disease Association of America, and on behalf of individuals with sickle cell disease. My name is Edward Ivy, and in addition to serving as the vice chief medical officer for the Sickle Cell Disease Association of America, I also am an individual living with sickle cell disease. As we have heard this morning from experts, the potential of gene editing treatments for sickle cell disease would be of tremendous benefit to individuals suffering from this painful condition. As with many other therapies to treat disease, a potential risk-benefit analysis for gene therapy must continue to be evaluated, and strategies to adequately inform patients and their families of this risk benefit must be provided to the population in language that is easy to understand and helps individuals to make informed decisions. As pointed out by several speakers this morning, sickle cell disease is a very serious disease, so the absence of therapy can also be present as a risk factor for individuals, and this should be accounted for in any risk-benefit analysis that is made.

However, in addition to the risk benefit from the gene editing therapies, we must also consider the societal factors that can affect the therapies for this population. It is estimated that over 100,000 Americans suffer from sickle cell disease. The majority of individuals with sickle cell disease are lower income and depend on government-sponsored health insurance for their care. Given the expected high cost of this one-time treatment, the risk of many patients who cannot afford this therapy will be left out of this potentially life-altering benefit must be considered. Although the role of the FDA to ensure access to the therapies from the cost perspective is limited, it is important that the FDA remains in conversation as the risk benefit analysis is continued to be evaluated. This is particularly important on how the FDA develops language around the analysis of the risk benefit, so the determination of who receives the therapy remains between the sickle cell expert provider and the individuals

receiving therapy. I see that my time is out. So thank you so much. And I encourage you to consider the risk-benefit analysis for this patient, particularly around cost as this therapy moves forward. Thank you.

Dr. Reese: Thank you for sharing your comments. We'll have open public hearing speaker number 12.

Dr. Bailey: Greetings. I am Dr. Lakeya (phonetic) Bailey. I have no personal financial disclosures to make at this time. I am a sickle cell disease patient warrior, research scientist, and disease expert, as well as community leader as the executive director of the Sickle Cell Community Consortium. I have experienced it all, from stroke, multiple acute chest syndromes, bilateral hip replacement, the most recent of which was two months ago, and the hip still has not healed.

And I have tried it all, trials, every kind of experimental treatment, even bone marrow transplant, where I have failed to find a consistent donor. But yet, by the grace of God, I have made it to age 45. And at 45, I have made it to this transition of the second transition in sickle cell. From a young adult living with sickle cell to an older adult. For a very long time I was the oldest person that I knew living with sickle cell disease. I did not realize how that affected me until I began to meet those much older than me. This past July, at our Annual Warriors Convention, where we brought together hundreds of sickle cell warriors, we recognized for the first time something that we named Golden Warriors. And as those Golden Warriors shared with us their age, all over 55, some all the way into their 70s, as they shared with us their life, I realized that they represent hope. A hope that I had needed to see and hadn't seen. Despite all of my activity and work in this space, I needed to see that hope, and that is what this gene therapy represents. These warriors represent hope, these golden warriors. And so does the option of genetic therapy, these curative therapies. This idea that I could be my own

donor and that through some of these trials, I could potentially see a day where I do not wake up in excruciating pain.

The Sickle Cell Consortium started at an FDA meeting, the FDA patient focused drug development meeting back in 2013 or 14, and it has now come full circle back to this meeting at the FDA, where once again, hope is on the horizon, and we are looking towards this hope for a change of the lives that we are living of excruciating pain. We are often faced with a population growing older and yet significant unmet needs. These unmet needs have left us feeling, in many cases, hopeless. But at the convention, where there were dozens and dozens of young people there, that hope was renewed. We had many, many discussions about gene therapy, and the sickle cell community is excited and ready to walk into these curative therapies. We often find ourselves going and facing mistreatment, and finding ourselves having to choose between what sounds ridiculous to say, but death and dignity. Do I choose my life or my dignity? Poor treatment, unmet needs. Many of us do not choose the way that you would instantly think that we should. We are now here to ask for support, for not only dignity, but hope. Hope that we can have a better life and a better future. I'm grateful to have made 45 and I look forward to another 20 or 30 years to provide hope to the next warrior. Thank you.

Dr. Reese: Thank you so much for sharing your, your personal story. We greatly appreciate it. We'll now have open public hearing speaker number 13.

Ms. Scott: Good afternoon. I do not have any financial disclosures. My name is Mariah Jacqueline Scott. I'm a 32-year-old sickle cell warrior from New Jersey. First and foremost, I would like to express my gratitude for allowing me to speak to the FDA Advisory Committee today. This is a special day with a community voice, our journeys, and how we need to see the advancement of sickle cell therapies in our health care system. As I speak to you today, I woke up in pain as I have chronic pain every minute and every day, and yet I keep hope for

what is about to be approved, gene therapy. This gene therapy, exocell, has future implications that a warrior like myself has been looking for forever since we became aware of what sickle cell disease can do. This would be the first gene therapy approved after it was first discovered over a century ago. In addition, not many are aware of the depression anxiety sickle cell creates for our families and ourselves. Alleviating the fear and worry of pain and suffering after gene therapy can prevent some of these mental anxieties.

I was diagnosed with sickle cell disease at six months old, in 1991, with parents that were unaware they carry the trait. Living with this disease was challenging for myself and my family. I came into this world wondering if I will live, after being resuscitated from an emergency C-section. After my first crisis resulting in splenectomy, the doctors told my parents, I may not live past five. Living beyond those predictions was the first milestone in battling this disease. I always had a fervent drive for education and learning more about the disease at a molecular level, how immunity can cause infections, and vasoclusive (phonetic) crises, and how patient-reported outcomes are valuable measures of what is important to the patient. I'm applying these skills to my community as a research coordinator with sickle cell.

However, these accomplishments did not come easy. In 2016, I received my first shoulder replacement. In 2018, I needed my right hip replaced. This continued to 2020, when my left hip required a joint replacement. Yet, what is ingrained in my mind is that in December 2016, I went to an annual OBGYN appointment and came out crying because the physician directed me to be on birth control because I should not have children. In quotes, your risk of dying is too high. My mental health declined. I became depressed so much that I couldn't work as a professor, and my physical health declined. Despite the many times sickle cell made me feel alone and won't have any chance of having a fruitful life, I had my beautiful daughter with my fiancé in 2021. And yet that came with a price. My veins are incredibly hard to access due to being in the hospital. I was stuck for IV access four times

before the anesthesiologist decided to put a central line in my neck. In addition, a year after my daughter, I was chronically in the hospital requiring my fourth joint replacement, just after two months giving birth. Imagine a new mom unable to have skin to skin contact, because my shoulder collapsed after rocking her to sleep. I provided my postpartum hospital data between March 2022 and March 2023. According to this graph, I had eight hospital stays, where my average length of stay was 10 days. That was 10 days I had to FaceTime my baby. 10 days when I couldn't see her take her first steps, and 10 days where I couldn't burp her after a bottle and put her to sleep with Winnie the Pooh. To this committee, I want to highlight what sickle cell can take away. But you can give hope after this approval for exocell for that future mother, father, and anyone who feels sickle cell hinders their future, for the devastating medical and mental implications this disease can cause. This gene therapy is another chance for this community to live free from pain, hurt, and for dreams to come true. Thank you once again for this opportunity to speak.

Dr. Reese: Thank you so much for your comments. We greatly appreciate it. So, thank you so much. So, we were going to give speaker number four the opportunity to speak, but speaker four has not had the ability to log in. So, with that, we are grateful to each of you for sharing your thoughtful remarks today with this committee and with the agency, and for taking the time to be with us today. We invite you to watch the rest of the day's proceedings on the YouTube link provided earlier, and also on the committee's webpage. Thank you so much, and we will now proceed to the next portion of our meeting, and I'll hand it back over to Dr. Ahsan.

Dr. Ahsan: Thank you, Cicely, and thank you so much for all of the folks that took the time out of their day for the open public hearing, that their viewpoint is very much appreciated and really an important component of the day in terms of how we look at the discussion points. So, thank you very much for everyone's comments and sharing of their

experiences. So, at this point, we move on, and close off the open public hearing. We have a break now and we will start at 1:45 PM. So we have a little bit of extra time. And then we will see everyone there for the FDA presentation and then the subsequent discussion.

FDA Presentation: BLA 125787 Exagamglogene Autotemcel (Exa-cel) – Dr. Karl Kasamon

Dr. Ahsan: Welcome back, and now, we're going to move forward with the FDA presentation. There'll be two speakers, and I will present; I will introduce first Dr. Karl Kasamon, who's a reviewer in the office of clinical evaluation, evaluation division of hematology, benign hematology branch, OTP, CBIR, FDA. So, if Dr. Kasamon and if you could move, go on camera, and unmute yourself, that would be great.

Dr. Ahsan: Oh, Dr. Kasamon, we cannot hear you.

Dr. Ahsan: Oh, I think it's working now. Oh, nope.

Dr. Karl Kasamon: How about now?

Dr. Ahsan: Yes.

Dr. Karl Kasamon: Okay. I'm so sorry. I don't know why that did that. Okay.

Dr. Ahsan: No worries.

Dr. Karl Kasamon: Thank you. So, welcome back to this part of the Cellular Tissue and Gene Therapies Advisory Committee meeting regarding biologics license application number 125787 on exogambular gene autotemcel, which is CRISPR Cas9 modified autologous CT34 positive hematopoietic stem and progenitor cell cellular therapy, which seeks the indication for the treatment of sickle cell disease and patients 12 years and older with recurrent vasoclusive crises. Next slide. Please.

My name is Karl Kasamon, as was mentioned, and I'm a hematologist and a reviewer at the office of therapeutic products within the FDA Center for biologics evaluation and research. Next slide.

The goal of my presentation is to briefly review the clinical aspects of this BLA and set the stage for Dr Singh, a bioinformatics expert, to lead you through a crucial discussion of potential off-target editing by exa-cel and strategies to manage this issue.

I'd like to start by introducing sickle cell disease and its current therapy, then talk about exa-cel, including the mechanism of action and how it is manufactured. After which, we will review the studies providing evidence to support efficacy and safety. Next slide.

Sickle cell disease is a group of hemoglobinopathies that include sickle cell anemia, sickle beta plus and sickle beta zero thalassemia, and sickle SC disease. Sickle SC disease will not be further included in this presentation, as it was not studied in the clinical trials that will be discussed. Sickle cell disease largely affects persons of African, Southeast Asian, and Mediterranean ancestry, including about 80,000 patients in the US.

As shown in this graphic, sickle hemoglobin differs from adult hemoglobin by a point mutation that substitutes avaline for aglutamine at the beta-globin gene when de-oxygenated sickle hemoglobin polymerizes, creating rigid fibers that deform red blood cells, making them sticky and leading to occlusion of blood vessels and hemolysis. Next slide.

As shown in this slide, sickle cell disease causes a number of debilitating manifestations, which include recurring, severely painful episodes called vaso-occlusive crises, in addition to anemia, retinopathy, strokes, pulmonary hypertension, and chronic ischemic damage to various organs such as brain, kidney, liver, and bone. And to date, sickle cell disease continues to shorten survival substantially, especially for adults. Next slide.

Sickle cell management consists of supportive care, including analgesics and red blood cell transfusions during vaso-occlusive crises, and in children, penicillin prophylaxis, and transcranial Doppler monitoring. Approved drugs include hydroxyurea, L-glutamine, voxelotor, and crizanlizumab. While these have modestly improved the outcomes of many

patients with sickle cell disease, none of these is curative, and they require lifelong adherence.

Furthermore, not all patients can tolerate these. The only available curative therapy is allogeneic hematopoietic stem cell transplant. However, fewer than 20% of patients with sickle cell disease have an appropriately matched donor. Consequently, treatment for severe sickle cell disease remains an unmet medical need. Next slide.

At this time, we'll go over the mechanism of action of exa-cel and look at how it is manufactured. Next slide.

To help explain exa-cel's mechanism of action, it's useful to review the role of BCL11a and the control of hemoglobin expression around the time of birth.

Hemoglobin is an oxygen-carrying protein within red cells, and, as shown in this figure, it's a tetramer that is made up of two copies of two distinct peptides. Fetal hemoglobin consists of two alpha and two gamma globin chains, and adult hemoglobin consists of two alpha and two beta globin chains.

BCL11A, which is shown here in orange, is a zinc finger protein that's responsible for the transition from gamma globin to beta globin. The line graph at the bottom shows that starting late in fetal development, gamma globin expression becomes repressed by BCL11A, leading to a coordinated transition from fetal hemoglobin to adult hemoglobin. Next slide.

Exa-cel is a cell-based gene therapy product that is composed of autologous CD34-positive hematopoietic stem cells edited by means of a SPY101 guide RNA and a CRISPR endonuclease at the erythroid lineage-specific enhancer region of the BCL11A gene. This diagram shows the mechanism of action of exa-cel. The exa-cel works by disrupting GATA1, binding, and downregulating BCL11a expression. Therefore, it uninhibits gamma globin expression and upregulates fetal hemoglobin production within RET cells.

It's important to consider why increasing fetal hemoglobin and decreasing sickle hemoglobin would be expected to be clinically desirable. It has been observed that fetal hemoglobin is therapeutic in individuals who have a coinheritance of sickle hemoglobin and hereditary persistence of fetal hemoglobin.

Therefore, upregulation of fetal hemoglobin by the action of exa-cel is predicted to lessen symptoms of sickle cell disease. Next slide.

The manufacture of exa-cel, as shown in this diagram, starts with the collection of autologous hematopoietic stem cells using apheresis. Then, CD34-positive cells are isolated, purified, and genome-edited with a CRISPR endonuclease and the SPY101 guide RNA. Following editing, the cells are incubated in a culture medium, washed, and cryopreserved.

After completion of lot release testing and manufacture of the appropriate dose, exa-cel lots are shipped to qualified clinical centers for infusion. Next slide.

The next section will focus on the clinical data. Next slide.

The clinical data come from a single study, 121, and the long-term rollover safety follow-up study, 131. Study 121 was launched in 2018 as a phase 1 study with a planned population of 17 subjects and evolved over time to become a phase 1, 2, and 3 study that is still ongoing as a multinational single-arm trial with a planned population of approximately 45 of whom 12 are adolescents under age 18. Following participation in study 121, all subjects who have been dosed with exa-cel are eligible and encouraged to participate in study 131, where they will continue follow-up for 15 years more following exa-cel infusion. Next slide.

The primary efficacy endpoint was defined as a proportion of subjects achieving VF12, which is freedom from severe VOCs for a period of at least 12 months at any point on study 121 after exa-cel infusion. Evaluation of VF12 started only 60 days or more after any

last red blood cell transfusion was given for post-transplant support or sickle cell disease management.

Notable secondary efficacy endpoints included a proportion of subjects achieving freedom from hospitalization for severe VOCs for a period of at least 12 months after exa-cel, which was called HF12, as well as several other endpoints that assess the durability of effect, expression of fetal hemoglobin above 20%, or reduction in the rates of VOCs, hospitalizations, and transfusion requirements compared with the baseline.

Finally, safety endpoints assess neutrophil and platelet engraftment reported on adverse events, abnormal laboratory values, and mortality. Next slide.

Study 121 enrolled adolescents and adults 12 to 35 years of age with a genotype that is shown here, who had severe sickle cell disease. Phenotype severity was demonstrated by having had at least two documented clinical sequelae that are quite specific for sickle cell disease, such as acute chest syndrome, splenic sequestration, or prolonged priapism that would require a visit to a health care facility, or having had at least two severe vaso-occlusive painful crises in each of the two years preceding screening. To be considered a severe VOC required that a subject had an evaluation for a pain event at a healthcare facility and received either red blood cell transfusion, intravenous NSAIDs, or opioids.

Key exclusion criteria included having a matched donor for an allogeneic stem cell transplant, having had a history of a prior stem cell transplant, a baseline fetal hemoglobin above 15%, or several clinical features that could make the autologous transplant process unsafe. Next slide.

I'd like to use this diagram to explain the schema of Study 121.

Starting on the left and going across, you'll note that in Stage 1, screening, eligible subjects were advised of the option of fertility preservation and began red blood cell transfusions for a minimum of eight weeks before mobilization, with the goal to lower their

sickle hemoglobin to less than 30% while keeping total hemoglobin no greater than 11 grams per deciliter.

In stage 2, mobilization, each subject is injected with Plerixafor in order to mobilize the stem cells and allow them to be collected from the peripheral blood with apheresis, which would then permit the manufacture of exa-cel. In stage three, which was myeloablative conditioning and exa-cel infusion, first, Busulfan was administered intravenously either daily or every six hours for four consecutive days, and after a washout of Busulfan, exa-cel was given IV.

Finally, in stage four, subjects remained in the hospital until neutrophil engraftment was observed and then were followed in the study for up to two years after exa-cel. Next slide.

Forty-four subjects have received exa-cel as of the time of data lock, and of these subjects, those 30 with at least 16 months of follow-up after exa-cel are considered evaluable for efficacy. This population is defined as a primary efficacy set, or PES, which will be the focus of the remainder of the efficacy discussion. At baseline, evaluable subjects had a median annualized rate of severe VOCs of 3.3 and spent a median of 12 days in the hospital for severe VOCs. The subjects required transfusion of a median of 3.3 annualized units of red cells for sickle cell disease. Next slide.

I'd like to reiterate that the primary efficacy endpoint was VF12, which again was the absence of severe VOCs for a period of at least 12 months on study 121 following exa-cel. This was achieved by 29 out of the 30 subjects who were followed for at least 16 months and thus were eligible for efficacy analysis. All 30, 100%, of the valuable subjects, reached the key secondary efficacy end point HF12 and thus avoided hospitalization for severe VOCs for a period of at least 12 months while on study after exa-cel.

All 30 evaluable subjects had a sustained fetal hemoglobin level of 20% or more for a period of at least 12 consecutive months starting 60 days after any last red blood cell transfusion. While all six treated adolescent subjects followed for at least 16 months did achieve VF12, a seventh adolescent subject with 14.3 months of follow-up experienced recurrent VOCs between month 11 and month 14 and, therefore, cannot meet the definition of VF12 responder regardless of additional follow-up. Next slide.

I'd like to walk you through this rather busy slide to illustrate some important efficacy endpoints. On the left, you'll notice the gray timeline of baseline severe VOCs, and on the right is a follow-up after exa-cel among the 44 subjects who received exa-cel. Adolescents are in purple, and adults are shown in green.

The dark blue diamonds are the severe VOCs. The 30 subjects who are shown above the orange line are those with at least 16 months of follow-up.

The safety profile of exa-cel recipients in study 121 was largely consistent with the toxicities typically seen with autologous transplants. Next slide.

In conclusion, exa-cel administration to patients with severe sickle cell disease led to the achievement of primary and secondary efficacy endpoints by a large majority of patients. The long-term outcome following CRISPR-based gene therapy in humans remains unknown, and questions still remain regarding off-target unintended genome editing. This important topic will be further discussed at this time by Dr. Komudi Singh of bioinformatics, and therefore, I'd like to turn it over to her. Thank you.

FDA Presentation: BLA 125787 Exagamglogene Autotemcel (Exa-cel) – Dr. Komudi Singh

Dr. Komudi Singh: Thank you, Dr. Kasamon. Good afternoon, everybody. My name is Komudi Singh. I'm a bioinformatics reviewer at the office of therapeutics product at CBER.

In this presentation, I will provide an overview of the applicants' off-target safety assessment of exa-cel using the bioinformatics method. Next slide, please.

I will first introduce the CRISPR Cas9 technology, which will be a recap of the presentation provided by Professor Urnov earlier this morning: the risk associated with off-target editing and methods of off-target analysis. I will then present the applicant's off-target safety analysis of exa-cel, summarize potential issues, leading to the discussion topic today. Next slide, please.

The CRISPR Cas9 systems are naturally occurring microbial defense systems that have been engineered to introduce DNA breaks in animal and human cells. A double-strand DNA break caused by Cas9 endonuclease occurs upon base pairing between the guide RNA and the target sequence in the genome in the presence of a short protospacer adjacent motif sequence, or PAM sequence for short, that is present on the non-complementary strand of the genomic DNA. Precise editing by Cas9 endonuclease at an intended genomic location can be achieved by designing the guide RNA to align with the region of the genome targeted for editing in the presence of a PAM sequence.

The PAM sequence motif serves as a binding signal for Cas9 and is strictly required for a Cas9-mediated double-strand break. Shown on the right side of the slide is a CRISPR Cas9 ribonucleoprotein complex that shows a perfect base pairing between the guide RNA and the target genomic sequence that would result in an on-target double-strand break or an on-target edit. However, a growing body of evidence has shown that Cas9-mediated edits can also occur when there is an imperfect base pairing between the guide RNA and the genomic DNA in other locations, giving rise to unintended off-target editing, as shown in the figure on the lower right portion of the slide. Next slide, please.

If an unintended genome editing occurs at the region of the genome known to have regulatory elements, then a double-strand break in such locations can disrupt regulatory

function. Similarly, off-target editing at the gene coding region can lead to gene inactivation. If the gene happens to play an essential role in cell function, then such unintended edits can be deleterious. These edits can also increase the risk of cancer. Therefore, an adequate off-target analysis is needed to allow for safety assessments of genome editing products intended for therapeutic purposes. To provide context to the information I will be presenting today, my talk is going to revolve around the off-target safety assessment of exa-cel that the applicant conducted and the adequacy of these approaches.

I will spend some time to introduce the off-target editing methods that have been developed for the safety assessment of CRISPR Cas9 genome editing products before presenting the applicant's off-target safety assessment of exa-cel. Next slide, please.

The CRISPR Cas9-based genome editing technology is rapidly expanding, and so are the bioinformatic tools that are being developed to assess off-targets. These bioinformatic tools use sequencing information alone or with next-generation sequencing data to perform off-target analyses. These methods are broadly divided into three categories. Next slide, please.

First, the in-silico off-target analysis methods use computational algorithms that require user-provided guide RNA sequence information and user-provided mismatch criteria while scanning the human genome reference sequence to nominate potential off-target loci. The cartoon on the middle-left section of the slide shows an example case of a perfect base pairing between a guide RNA and the target genomic sequence. The in-silico algorithm will nominate additional loci across the reference genome by identifying regions with imperfect base pairing occurring due to a mismatch, as shown in the cartoon depicted in the center of the slide, or when an imperfect base pairing occurs because of a gap between a guide RNA and genomic sequence, shown on the right section of the slide.

These methods are straightforward to implement. However, the findings of this type of analysis are biased by user-provided mismatch criteria. Additionally, these methods do not account for cell type specificity arising from the unique chromatin landscape within a cell. Next slide, please.

The cellular methods of off-target analysis use the genomic sequence information of CRISPR Cas9 genome-edited cells. The cells, in this case, are edited in the presence of an oligonucleotide tag that marks the loci where double-strand breaks have occurred. The genomic material from these cells is isolated and subjected to high-throughput sequencing and analysis.

These methods can provide high confidence in target candidates. However, determining the right experimental parameters needs careful consideration. Additionally, this method can be hard to implement due to toxicity associated with oligonucleotide tags in certain cell types. A third method for off-target analysis includes biochemical methods that use genomic material from the cells that are edited and assessed for off-targets. Since the applicant did not use this method, we will not be discussing this in the presentation today. For the remainder of my talk, I will present the applicant's off-target safety assessment of exa-cel, leading to the discussion question for today's Advisory Committee meeting.Next slide, please.

The applicant used two orthogonal methods to perform off-target safety assessments of exa-cel. In one of the approaches, they used in-silico methods to nominate off-targets based on homology to the human genome reference sequence. We would like to note that the Cas9 endonuclease recognizes a native or cognate PAM sequence, NGG, shown in the bold font on this slide, where n can be any nucleotide base. Additionally, Cas9 has also been shown to recognize different variations of the PAM sequence but exhibit lower activity at these alternate PAM sequences, and I will refer to them as suboptimal PAM sequences.

The applicant used three in-silico analysis tools for this analysis, and they included both cognate or native PAM sequences, as well as suboptimal PAM sequence patterns, in their search.

In the second method, the applicant performed a cellular GUIDE-Seq off-target analysis on healthy donor and sickle cell disease donor cells. These cells were edited with Cas9 SPY 101 guide RNA, and the genomic material was extracted for high throughput sequencing and analyzed. I will now present the findings of the off-target safety assessment of exa-cel and discuss potential issues surrounding this analysis. Next slide, please.

As mentioned in the previous slide, the applicant used three different in-silico off-target analysis tools. They used two mismatch limits of three and five when scanning the human genome reference sequence and nominated off-targets for SPY 101 guide RNA. The mismatch criteria were inclusive of many mismatches and permissive of one gap.

The applicant used a more linear mismatch criteria of five when including cognate or native PAM sequence patterns in their search, and suboptimal PAM sequence patterns were tested with lower mismatch criteria. Increasing the mismatch criterion would result in the inclusion of more loci as potential off-targets.

Consistent with this, the applicant identified 171 loci when the homology-based search was implemented with three mismatches, and they identified 5007 loci when the search was implemented with five mismatches. The data presented by the applicant shows that the number of mismatches implemented can impact the number of off-target loci nominated.

We would like to note that several of these in-silico nominated sites are sequences in the genome that can base pair with the guide RNA, withstanding the applicant-provided mismatched criterion, and harbors any of the PAM sequence patterns that the applicant used in their search.

For such nominated loci, confirmatory testing should be performed, ideally using more than one sample to allow for testing editing potentials at these sites in the presence of all potential PAM patterns used in the in-silico nomination process. We will discuss this issue when presenting the applicant's confirmatory testing in the later part of the talk. Next slide, please.

One of the issues with the in-silico off-target analysis method is that these tools while scanning the reference genome sequence, does account for individual genetic variations that may result in off-target editing at a new locus harboring the variation. Shown below is a cartoon representation of several genomes harboring nucleotide variations across individuals, contributing to heterogeneity.

These individual nucleotide variations could be of concern if they contribute to decreasing the mismatch between guide RNA and genomic DNA, as shown in the cartoon below, or if it contributes to the generation of a PAM site. Next slide, please.

To account for heterogeneity, the applicant used the 1000 Genomes project database and included variants present at greater than 1% frequency in this database, which includes greater than 1% frequency in every subcontinental group represented in this database. Specifically, they applied a 1% frequency cutoff, and I will present this analysis result in the next slide. Next slide, please.

The database that the applicant used had 83 million single nucleotide variations. Of these, 21 million variants were present at a frequency greater than 1%. The applicant implemented a variant-aware homology search that expanded the homology space to include sites that will either have a decreased mismatch or would include a PAM sequence in the presence of a variant. From this analysis, they identified 50 additional off-target loci that accounted for heterogeneity. Next slide, please.

Since all the loci that were reported were nominated using computational algorithms, the applicant performed confirmatory testing using hybrid capture sequencing. Briefly, this technique allows for the enrichment of DNA fragments using biogenerated RNA fragments that act as baits or probes. In this case, the probes were designed to enrich DNA fragments from the loci that were nominated by the in-silico of target analysis. To ensure optimal capture of target DNA, the baits were tiled around the off-target loci. The genomic material from control and CRISPR-edited cells was incubated; the captured DNA was sequenced and aligned; and after the removal of duplicated sequences, reeds carrying indels within three base pairs of potential cleavage sites were counted. Next slide, please.

The applicant used genomic material from four replicates of CRISPR or control-edited healthy cells. The target DNA sequences were captured for hybrid capture sequencing. Sequences with suboptimal coverage, high GC content, high background indels, and homopolymers were excluded from this analysis. As a result, 4,340 loci out of 5,007 were tested.The applicant performed confirmatory testing for these 4,340 loci in four samples, for which they did not provide any sample metadata information. They, however, provided sample metadata information for four independent samples that were used in confirmatory testing of 171 loci. They reported that one sample was from an individual of African-American ethnicity, and the remaining samples were from three individuals of Hispanic ethnicity. Next slide, please.

We would like to note that the applicant's off-target nomination strategy included scanning the genome with predefined mismatch criteria that were inclusive of different PAM sequence patterns that we had presented in slide 28. In this case, Confirmatory testing should be performed at all these loci in the vicinity of all PAM patterns included in the nomination process.

It is unclear if the four samples used in the hybrid capture sequencing allowed for testing of all PAM sequence patterns used in the nomination process. Based on this, we conclude that many of the off-target loci nominated were not experimentally tested. The applicant reported that no off-target editing was detected at any of the loci nominated in the in-silico analysis, as shown in the table on this slide. Next slide, please.

For the additional 50 off-target loci nominated by the variant-aware homology search, the applicant performed confirmatory hybrid capture sequencing using genomic material from one sickle cell disease donor sample and two transfusion-dependent thalassemia donor samples. The applicant reported that no off-target editing was detected at any of the 50 loci nominated from the variant aware search.

We want to point out that these 50 loci were nominated as potential off-targets because of the presence of variants at these sites. Therefore, the presence of variants in the sample is necessary for confirmatory testing. The applicant reported the presence of 13 variants in at least one of the samples that were used for confirmatory testing. Hence, the absence of editing shown by hybrid capture does not completely rule out off-target editing at the remaining 37 loci nominated from this analysis.

Additionally, we would like to note that out of the 50 loci, 20 nominated to the 18 genic locations. These genic locations were mostly intronic regions with one locus close to an intron exon border. Since intronic regions are known to have regulatory functions, adequate risk assessment of potential disruption of these sequences will be needed. Next slide, please.

Several factors need consideration when performing in an in-silico analysis accounting for heterogeneity. Implementation of off-target analysis accounting for heterogeneity requires using variant information from a sequencing database. A database used in this type of analysis would be adequate if it contains an adequate amount of samples from which the sequencing data is generated. The sample should be from individuals,

representative of the drug product target population, a good quality of sequencing data to ensure optimal variant identification, and a suitable allele frequency cutoff to subset variants for this analysis. All these factors would ensure adequate variant sampling that can be used to account for heterogeneity. As mentioned before, the applicant used the 1000 Genomes Project database that had sequencing data from 2,504 individuals across different continents. Of this, 661 sequencing data were from individuals representing the target population of exa-cel.

Among the 661, there's only data from 61 individuals in the United States. The limited amount of sequencing data may not adequately represent the drug product target population across the United States. As mentioned in the previous slide, the applicant reported 50 additional off-target loci from this analysis. Next slide, please.

We would like to refer back to the talk by Dr. Bauer earlier today, where he presented some data on the heterogeneity assessment of guide RNA that targets the same locus on the BBC11a gene as exa-cel. The Cancellieri study and the applicant's exa-cel analysis reported different numbers of variants contributing to potential off-target loci.

Before I go deeper into the Cancellieri study, I want to remind you that the Cas9 endonuclease recognizes the native PAM sequence NGG, shown in the bold font on the slide, where N can be any nucleotide base. It has also been shown to recognize different variations in PAM sequences, some of which are listed on this slide.

One of the variants reported in the Cancellieri study was a variant in the CPS1 intronic region that changed the TGA PAM sequence present on the reference sequence highlighted in the orange color box to a canonical TGG PAM sequence highlighted in the blue colored box on this slide. The Cancellieri study reported a higher off-target editing score at the TGG PAM locus compared to the TGC PAM locus present in the reference genome.

The CPS1 locus was nominated by applicants in their in-silico homology-based off-target assessment in which the applicant had included alternate PAM sequences in their search. However, the applicant performed confirmatory testing in samples that harbored TGC PAM sequence only. Hence, editing potential at this locus with TGG PAM was not empirically tested by the applicant.

A potential off-target editing at this locus cannot be ruled out until sufficient information is provided. This lack of empirical testing applies to other loci that were nominated by the applicant in their prior in-silico off-target analysis studies. Next slide, please.

We would like to note that while the applicant reported the CPS1 locus in their homology-based analysis, they did not report the variant in their heterogeneity analysis, even though this variant is present at a greater than 1% frequency in the 1000 Genomes Project Database.

An off-target locus that is potentially impacted by a variant is a critical finding that needs to be reported and fully assessed for editing potential using appropriate samples. With the available data, we cannot perform an adequate risk assessment at this locus in the presence of this variant.

The applicant, however, reported other variants in their heterogeneity analysis from other loci, and we show some of them in Table 1. The variant locus and the associated gene information are present in Table 6 of the briefing document.

These loci were likely reported in the applicants' in-silico analysis as they fulfilled the mismatch criteria, they applied in their prior silico study. We have provided the mismatch criteria that the applicant used in Table 2. Hence, it is not clear why the CPS1 variant was not reported in the applicant's heterogeneity study.

Because of the applicant's implemented criteria and curations to the database, it is unclear how many other variants were not reported in the applicant's heterogeneity analysis and how many potential variants may have overlapped with the Cancellieri study. Since the Cancellieri study included variants from different databases when compared to the database used by the applicant, some variants may be excluded due to different variants reported in specific databases. Additionally, different variant allele frequency cutoffs used in these two studies may also result in the exclusion of variants from the applicant's study. For instance, in Table 3, we present a variant that was reported in the study to contribute to an off-target locus. However, this variant would not meet the applicant's 1% allele frequency criteria they applied in their heterogeneity assessment. Next slide, please.

To summarize the two studies accounting for heterogeneity, the differences in the findings published in the Cancellieri study and those reported by the applicant may stem from different factors we have listed in this table. First, the applicant implemented a variant aware homology search, while the authors of the Cancellieri Study developed and implemented a tool to account for heterogeneity. The applicant used the 1000 Genomes Project database that included sequences in sequencing information from 2,504 individuals across continents.

The authors of the Cancellieri study used two different databases. The Human Genome Diversity Project Dataset comprising of sequencing data from 929 individuals, and the Genome Aggregation Database that has sequencing data from a much bigger sample. The applicant reported 50 potential off-target loci that were contributed by one or two variants, and the Cancellieri study mainly reported a detailed assessment of a variant that resulted in the creation of the PAM site and a potential off-target locus. Next slide, please.

To summarize the in-silico off-target safety assessment of exa-cel, we are concerned about the different numbers and subsets of nucleotide variants, variations that were identified in the two studies that contributed to off-target loci. These differences may arise potentially

because of the limited number of sequencing information present in the databases and the potential differences in the performance of in-silico algorithms used in these studies.

It is not clear if the small sample size of the database would allow for sufficient sampling of variants. Additionally, we would like to point out that the confirmatory testing of off-target loci requires that the cells or genomic material used in this test harbors a variant contributing to an off-target loci.

Since appropriate cell samples harboring variants were not used in the confirmatory testing, the majority of off-target loci arising from variants were not empirically tested. On the same lines, a subset of in-silico nominated off-target loci were also not empirically tested. The lack of clarity on these indicated aspects of off-target analysis accounting for heterogeneity and the lack of confirmatory testing using appropriate samples may support the need for additional studies to further assess the safety of exa-cel. Next slide, please.

I will now present the applicant's cellular method of off-target safety assessment of exa-cel. Specifically, they implemented GUIDE-Seq to identify off-targets in SPY101 guide RNA edited CD34+ HSPCs. In these experiments, the software edited with the Cas9 ribonucleoprotein complex in the presence of a double-strand oligonucleotide tag, or dsODN for short. The oligonucleotide tag will mark all the DNA breaks occurring during genome editing. The genomic DNA from these samples was sequenced by high-throughput sequencing and assessed using the GUIDE-Seq pipeline. The applicant performed this experiment using three healthy donors and three sickle cell disease donor cells. Next slide, please.

The GUIDE-Seq analysis of three healthy donor cells helps identify several off-target loci in each sample, as shown in the table. Also shown in this table are GUIDE-Seq data from the analysis of samples derived from three transfusion-dependent thalassemia donors. We would like to point out that two different dsODN concentrations were used, which could

interfere with the identification of a consistent subset of off-target loci. However, the sponsors stated that they were able to detect an adequate number of on-target reads shown in the fifth column of this table and hence consider these parameters to be optimal. The applicant then used hybrid capture sequencing on four independent, healthy donors and reported that no off-target editing was detected at these loci. Next slide, please.

In the next experiment, the applicant performed GUIDE-Seq on three sickle cell disease donor-derived cells. They reported optimal cell viability, as shown in column three of the table, high on-target editing frequency, shown in column four, and sufficient on a sufficient number of on-target reads in each sample, as shown in column five. From this analysis, they reported several off-target role loci in each sample tested, as shown in column six of the statement. Next slide, please.

They manually assessed a subset of the off-target loci identified in the GUIDE-Seq experiment. For confirmatory testing, they used hybrid capture sequencing on the same three samples but reported lower on-target editing rates in these samples prepared for hybrid capture, as shown in column four of the table on the left side of the slide. The applicant stated that the high sequencing depth would allow for the detection of editing at off-target loci. From this analysis, they reported that no editing was observed at the off-target loci identified in the GUIDE-Seq experiment.

They identified three loci with indels that mapped to a DNA break hotspot. Consistent with this observation, they provided a manual assessment of a subset of these loci that also reported a DNA break hotspot at the same location, and these DNA breaks were independent of CRISPR Cas9 editing. Next, they postulated that the off-target loci identified in these samples are likely false positives. To address this, they used false positive filtering and reported that all of the off-target loci identified in the experimental samples were removed. Next slide, please.

To summarize the cellular off-target safety assessment of exa-cell, the applicant performed two GUIDE-Seq experiments; one using three healthy donor-derived CD34 positive HSBCs and another using three sickle cell donor-derived cells.

From these experiments, they identified several potential off-target loci, but they reported that no off-target editing was observed in their confirmatory testing. None of the off-target loci identified in the Guide SEQ overlapped with the 171 in-silico nominated loci.

We would like to note that sickle cell disease has been shown to impact HSPC function and lineage and induce stress responses. These changes are likely to impact the cell's chromatin landscape, that is known to impact off-target editing. It is not clear if off-target analysis using healthy donor cells would adequately inform us of the off-target editing risk in exa-cel. We are also concerned about the adequacy of using a small number of samples in a cellular off-target analysis. Next slide, please.

In summary, the applicant performed an off-target safety assessment accounting for heterogeneity using the 1000 Genomes Project database. However, the small number of sequencing data present in the database and the lack of confirmatory testing of all off-target loci in samples harboring the variants is concerning.

We are also concerned about the adequacy of the small sample size in the cellular off-target analysis of exa-cel and if the use of a small number of healthy donor and sickle cell donor cells would adequately inform us of the potential off-target editing risk of exa-cel.

We would like the advisory committee members to weigh in on these issues and provide recommendations.

We would like to thank the advisory committee members for their time and for participating in this advisory committee meeting today.

This concludes the FDA's presentation on the clinical assessment of exa-cel and the applicant's off-target safety assessment of exa-cel in this DLA.

Thank you.

Q & A

Dr. Ahsan: Thank you very much, Drs. Kasamon and Singh, for thorough presentations that will help inform this conversation. So, we now have time for questions from the committee members directed toward the FDA speakers. We'll then follow that up with a committee discussion where there will be a discussion between the members of the committee.

If we feel at that time, which will start at around 3:00 PM, that we have pointed questions for the sponsor for clarification, we can do that then. But at this point, this Q&A is for the FDA speakers. So, if members want to raise their hands for those that have questions for the FDA speakers. Dr. Scot, please go on camera and take yourself off mute.

Dr. Wolfe: Dr. Singh. Thank you very much for the detailed overview of the applicants' off-target analysis. One comment you made about their computational assessments of off-targets and subsequent analysis was that you had a concern about their analysis of off-target sites with suboptimal PAMs. I was wondering if you could expand on that just a little bit to clarify what your concerns are there with regard to the Reference Genome.

Dr. Singh: Right. This Is Komudi Singh, Bioinformatics Reviewer from the FDA. Thank you for the question. The applicant had, in their description of their in-silico nomination process, they had used two different mismatch criteria and different variations of PAM sequences in their off-target in-silico nomination process. If that is the case, what the analysis would do would scan the genome to identify additional loci, which would have mismatches to the guide RNA, which is within the limits of the mismatched criteria applied. In that case, the applicant had performed searches across the genome with suboptimal PAM sequences with up to three mismatch criteria and a more lenient mismatch of five when they were using a cognate or native PAM sequence. Our concerns are not with the criteria they have used in their in-silico nomination process, but our concerns are with the confirmatory testing that they subsequently

followed this nomination process with, and those confirmatory tests were done on four samples, and it is not clear if they had used a certain number of PAM sequences in their searches, did the samples have these off-target loci with the indicated PAM sequences in their confirmatory testing?

Dr. Ahsan: Dr. Singh, so it sounds like you are unsure of the exact analysis that the sponsors did on the confirmatory aspect of the in-silico, correct?

Dr. Singh: Yes, so we are not sure about what variations of sequences were empirically tested in their confirmatory testing. Correct.

Dr. Ahsan: OK. So that is a question that we can ask them directly, not right now, but at the beginning of the discussion that we will hold.

Okay. Thank you very much. And Dr. Wolf, I apologize for calling you Dr. Scot, just quickly reading off the names here. Dr. London, if you could, uh, go on camera and take yourself off mute.

Dr. London: Yes. Yes. Thank you very much, Dr Singh. I am wondering. I appreciate your concern for the small sample size and just wondering if the FDA has guidance on the methodology that could be used to determine how many more samples should be analyzed. I mean, does the FDA have guidance about how many more patient samples would need to be studied in order to identify enough variance?

Dr. Singh: We are not prescriptive about the methodologies that the sponsors select in their analysis, and right now, we do not have any guidance to indicate the number of samples or recommend the number of samples to be tested. In fact, one of the issues that we would like the advisory members to weigh in on is if given the issue and given the lack of clarity on how many samples should be needed; we would like to hear advisory committee members discuss this issue and provide us with recommendations.

Dr. Ahsan: OK. Thank you. Dr. Singh. Dr. Wu, could you go on camera and take yourself off mute?

Dr. Wu: Yeah, so I think I asked this question in the very, very beginning to the applicant. So, do you get a sense of why it's so difficult to just take the patients that they have done the hematopoietic cell transplant and just analyze the samples that they have because they've had these samples for several years? Why is it so difficult? Why, why just show the data of three sickle cell patients instead of 30 or so patients that they've already done?

Dr. Singh: I would defer to the applicant to address that question.

Dr. Wu: OK. I mean, I'll ask again, but you know, I asked the same question, but I think they just didn't answer it. Yeah.

Dr. Ahsan: OK. So, if the sponsor who's listening can be prepared at the start of our discussion, I think there's two questions and, and Dr. Singh, correct me, Dr. Singh, and Dr. Wu, correct me if I did not capture it correctly. The second question was from Dr. Wu, which is, is there a reason why we cannot do a cellular-based analysis of samples from the patients that have already been treated? Right? And the first question that Dr Singh was asking which is the methodology for looking at the confirmatory studies of in-silico and whether the PAM variations were accounted for there. OK. So, we will get to that when we start our open discussion. Any other questions from the committee members for the FDA speakers?

Oh, Dr. Shapiro, if you could go off, go on camera and come off mute.

Dr. Shapiro: Well, perhaps this is what Dr. Wu was asking, but I was asking if any of the patients treated were positive for the RS114518452 variant. I think that's essentially what he's asking as well.

Dr. Ahsan: I don't know, Dr. Wu, would you like to come on camera and confirm whether that's the same question?

Dr. Wu: Yeah. So, that's one question. But that's based on in-silico modeling. Right? So, for any of any of these samples, besides the in-silico, you still want to do the hard-core experiment, taking the cells, analyzing them, and see what happens if there's any surprises outside of what the in-silica model predictions are. Yeah, I mean, this is a perfect opportunity to look into that, right? Instead of just focusing on three samples of SCB patients and that you see in-silica modeling.

Dr. Ahsan: Yeah. Right, so that's kind of a subset of your question, a more broader question, Dr. Wu.

Dr. Verdun: To Dr. Shapiro's question, I think that the applicant needs to address that as well. We don't have that data at FDA.

Dr. Ahsan: OK. Great. Good to know. So, both of those are questions for the applicants that we can have when we open up the discussion of a short period of questions for them.

Dr. Lee, I saw that you had raised your hand and then lowered it. Please raise it again. If you want to ask your question in the interim, Dr. Ott, could you go ahead and ask your question?

Dr. Ott: Yeah, I had the same question about the guidelines, you know, what the FDA is expecting, from applicants in terms of off-target, you know, effects that are that are there and, and then I also would like to just confirm that the in-silico prediction was not overlapping at all with the experimental of target data that were that were achieved. I just wanted to confirm this with Dr Singh.

Dr. Singh: The applicant's report had performed a comparison of off-target loci nominated from the in-silico analysis where they had up to three mismatches and reported 171 loci, and they had reported all the low size identified from GUIDE-Seq data, and they reported none of those low size overlapped. That is correct.

Dr. Ahsan: Thank you. Dr. Singh, maybe you could tell me, could you speak to why there may be results that are not overlapping like that? Could you could you bring that to the forefront for the committee and the public?

Dr. Singh: Yeah, it's a very good question. The in-silico nomination process is done using the applicant-decided preset mismatch criteria. The 171 off-target loci that were nominated and reported by the applicant where loci derived when they had searched the genome using up to three mismatch criteria, and those three mismatches were either all of them were three mismatches or a three mismatch inclusive of a gap. The GUIDE-Seq analysis default cutoff used in the GUIDE-Seq analysis is up to six mismatches. So, it is likely that the loci identified in the GUIDE-Seq experiments were off-target edited loci that were permissive of many more mismatches than was allowed in the in-silico nomination process. As a result, you would not have then identification of a common subset of off-target loci.

Dr. Ahsan: I see. So, the GUIDE-Seq experiments allowed for more variation than the in-silico experiments per the way the applicant had set up the in-silico experiments.

Dr. Singh: That can be one of the explanations. Yes.

Dr. Ahsan: Okay. Great. Dr. Ott, do you still have another question? Yeah, your hand is still raised. Maybe not.

Dr. Ott: I have a follow-up question. Sorry. Just after this explanation, would it not be more likely to find off-target effects with the more stringent criteria in the in-silico analysis, you know, three versus six mismatches? Could you just briefly comment on this, Dr. Singh, because it would be understandable if there would be additional mismatches, but exclusive mismatches with, you know, exclusive of off-target effects with more mismatches? I was just wondering whether you could comment on this.

Dr. Singh: With more number of mismatches, you're likely to nominate many, many more off-target loci. And while we are not very prescriptive to the sponsors about what is the

mismatch criteria, they should use in their in-silico nomination process, we do review the data that is presented, and as long as there is a reasonable mismatch criteria selected by the applicant, we accept that information.

The issue surrounding performing in-silico nomination with higher mismatches is that you would then get a prohibitively long list of off-target loci, and then confirmative testing of those loci would be difficult. So, one way to do it would be you can perform in-silico nomination using an increased number of mismatches, but only a subset of those that showed up in an orthogonal assay as a confirmative testing, but I defer to the applicant for them to provide their reasoning about the strategy that they used and provided us with the report. Thank you.

Dr. Ahsan: Great. Right. So, when we move from 3 to 6, we would actually expect that there would be thousands more off-target loci in the in-silico experiment, correct, Dr. Singh? And the GUIDE-Seq experiments give a more limited number, a more manageable number, but can I ask, is there a way to know and confirm that the ones that were identified in the GUIDE-Seq that were not identified in the in-silico experiments are actually ones with greater than 3 variables and less than six that they are in fact in that looser range of criteria.

Dr. Singh: The applicant in the report had pointed to that information, and I will defer to them to provide you with more information.

Dr. Ahsan: Okay. Great. Thank you. Dr. Komor.

Dr: Komor: Yeah. Well, this is mainly; I just want to make a comment about the GUIDE-Seq method. It's not like, there isn't, I don't know what the right word is, but it's not like, oh, only six possible mismatches or whatever. It's experimentally validating. And anytime you get a cut site, you could get incorporation of that oligo, and it might pop up as a potential off-target. But in reality, if you're actually doing genome editing, many of those double-stranded breaks would get perfectly repaired during experimental conditions.

And a lot of times in these GUIDE-seq experiments, we will see certain off-targets that have many more mismatches than we would expect, and that's kind of dependent on the sequence of the protospacer. If you have a higher GC content, for example, you might see an off-target pop-up. But so, it's not super uncommon to see the GUIDE-Seq analysis pop up a lot of off-targets that maybe weren't in the in-silico analysis if you're only looking at three potential mismatches there. It's just experimental conditions. In terms of everything that I've read, I'm not too surprised about that, but I would like to see the sequences of the additional off-targets that the sponsor did identify in the GUIDE-Seq. I'd be interested to see.

Dr. Ahsan: Great, that's great feedback. Maybe the sponsor can be prepared to provide that information, and that'll be very important, Dr. Komor, and maybe we can look to you during the discussion to see if this is just different data, as opposed to just data regarding a larger number of mismatches. Okay, Dr. Kwilas. If you could go on camera and take yourself off mute.

Dr. Kwilas: Hi, everyone. Thank you. Dr. Ahsan. So, I just wanted to address; there were a couple of comments asking about our guidelines, in particular regarding some of these studies. So, I just wanted to touch upon that a little bit. As Dr. Singh mentioned, we don't have finite guidelines for an exact number of different donor material or patient material that should be used in some of these studies. What we do say is that the material should be representative of the product of the indication and should have supportive data to support that the material that's being used is indicative of those two qualities. And then the number should be based on the analysis that they've done to date to determine the number of appropriate samples based on, you know, particularly when we're talking about the confirmatory testing, based on, say, for example, the number of sites that they have identified, based on their false positive screening and things of that nature.

So, while we don't have a finite number, it is dependent on not only the indication, the type of product, but also the previous data that's been obtained. I just wanted to qualify that.

Dr. Ahsan: Great. Thank you very much for that input regarding the guidelines. And I suspect if there are hard guidelines, we wouldn't be having this meeting. So, let's see. I don't see any other members having questions for the FDA at this point.

Committee Discussion

Okay. Last chance. Nope. All right. So, at this point, we're a little bit ahead of schedule, but I think we can move on to the committee discussion. So, I think the best way for us to do this is to have a finite time where we have our specific questions that we have amassed for the sponsor. We can go through them one by one, but those should be very targeted answers by the sponsor. And please keep them brief and on point to the questions that the members are asking. Because what is very important is that we then have that session where we have a discussion among the members on this topic. And, so with that, perhaps Dr. Wu, you could ask your question of the sponsor now, and they can give, and they can give their answer to what you are asking about, the patient samples.

Dr. Wu: Yeah. So, it gets back to the patient sample. I think the study the sponsor there was on three SCD patients, and you've done many more patients since then; probably 30 or 40 patients. And you've had several years with these samples; why not just do the actual analysis rather than doing the in-silico modeling? Because in-silico is always in-silico. It just depends on how good the algorithm is. And also, with the actual experiment, you can find surprises that were not predicted by your in-silico. And you know, you mentioned about the incubation time with your enzyme that's very sensitive, you don't want to over incubate it. All of these have variabilities among different patients. So, I just, I just wonder why is it so difficult to do

it for all the patients that you've had so far, especially the ones that you follow for more than two to three years. Yeah.

Dr. Ahsan: The sponsor can come online.

Dr. Krogmeier: Thank you. Yes. Can you hear me?

Dr. Ahsan: I can. We can see you.

Dr. Krogmeier: Are we are we able to show our slides?

(Recording stopped. Please keep recording in progress).

Dr. Krogmeier: Great. And I will ask Dr. Altshuler to address your question.

Dr. Altschuler: So, the question is about, if I understand correctly, about testing of the patient samples from the clinical trial for off-target assessment. And the first point I would make just for clarity is we've tested 14 samples, of which three had sickle cell disease, three had TDT, and the other six were healthy volunteers, and there's no data to suggest that the result would be different for patients with sickle cell than the other possibilities. But if the question is whether we could do that, we do have the samples, and we have the method. So, it's possible, but we've thought a lot about whether to do this or not, and our view of this, and I'll just quickly pull up a slide, is to ask the question, what would we learn from doing such a study and the reason that I raised that is that we have this multi-step process and actually that's not the slide that I wanted. Actually, I wanted the slide that was up a second ago, if you could, the framework analysis of how we did the analysis. Thank you.

When we set out, you said we've been doing this for years; we set out a framework, which was to test with computational homology search and independently, as noted, check with GUIDE-Seq. And then the real way to know whether any of these are actual editing sites or not is to do a very sensitive experiment where you repeat the experiment and see if you see any editing and at both the computational homology sites and the GUIDE-Seq sites; we did not see any editing in the confirmatory testing, saying that none was seen. But there are, and

we absolutely acknowledge, there are rare variants that we did not see in our hybrid capture samples, and whether we did 14 or the 40 or 50, we wouldn't see every rare variant site because there are rare variant sites that are present at 1% frequency. So, the question is, what do you do then? And what we did in our pre-specified approach was to say we would then perform a risk assessment. And we perform the risk assessment if editing was seen because, of course, it's not the case that the presence of an off-target edit necessarily translates to biological meaning, let alone clinical prediction.

And so, what we did was for the sites that we didn't see since there were no sites that had confirmed on-target editing using our approach and our cells, we then said, well, let's treat all the samples at which there is a variant site nominated by sequencing of 2,504 people, and we tested all those sites.

And we asked if we don't see that variant site in one of the samples we queried, let's perform the risk assessment that we would have performed had editing been seen. And that risk assessment with pre-specified questions was, does the gene overlap anywhere in the entirety of the gene, not just the exons known to play a role in hematologic malignancy?

And for that, we use the MyeloSeq panel, which is a clinical test from Washington University in St. Louis, which has named those genes that have clinically interpretable results in terms of hematologic malignancy. We also looked at the entire genome. And as does the entire genome have any exon, a site where one can do functional annotation in a meaningful way, and then the answer to that question for all of the sites that we looked at with the sites identified by looking at 21 million different genetic variants was that for the common ones with a greater than 9% frequency. Uh. 10% frequency, the nine out of nine, we did see them, and there was no editing observed, but it's absolutely the case that there were three out of 41 that were seen and the rest were not. So, we performed that risk assessment and that risk assessment showed that there was no overlap with the gene known to play a role in

hematologic malignancy when it's mutated in the blood by the MyeloSeq panel, and there was no overlap with an exon that could be functionally annotated in a clear way and the one variant from the Cancellieri paper is in a gene called CPS1 that is a mitochondrial gene that is not expressed in the blood. It's only expressed in the liver and small intestine, and as what we noted and was noted previously, there's no clear functional, let alone clinical, interpretation of that site.

So, whether we were to do the testing, Dr. Wu, that you suggested or not, we'd end up in the same place, I believe, which is this is the risk assessment. And the key question then becomes, following patients over time, and you heard our plan for 15 years of follow up both of the clinical trial and the registry because that is what will tell us what actually happens to patients, and then we'll do the investigations with the clinical data and the samples that are mandated by what actually happens. So, that's our approach, at least.

Dr. Krogmeier: Dr. Ahsan, I believe you're on mute.

Dr. Ahsan: Oh, thank you. Sorry. Could you speak to what Dr Singh had raised as to a lack of clarity in your methodology about the PAM sites and the variation of the PAM sites? Could you speak to that, please?

Dr. Krogmeier: Yes, Dr. Altshuler?

Dr. Altschuler: Yes, no; thank you for the opportunity to clarify. So, as noted in the core presentation, I won't pull up the slides for time. We nominated sites based on mismatches. You know, not having a gap or not having an alternative PAM. And again, there were only six such sites that had three mismatches: zero with two mismatches, zero with one, and zero perfect matches. But we also, for completeness, also nominated sites that had a gap, which, or bulge, which means there's a base missing or added. Those are very unlikely to be cut based on the empirical literature. We also included alternative PAMs that weren't seen in the

human genome, but because we thought there's some evidence in the literature that even if the canonical PAM is not present at a given site, it's possible for the enzyme to cut.

So then, when we did the confirmatory testing, the PAM that is present in the human genome was the one that was present, and so we didn't see any cutting. I think if I interpreted the question, it was, did we have cells that contained the alternative PAM? And the answer was no because the alternative PAM is not present in the human genome.

So, it was really that we tested the PAMs that didn't match to see if they could possibly be cut. Not that we were looking to find an example of that PAM. With the exception, I should note, just to be complete, where there was a variant that created a different PAM that was like the conciliary variant; that's a relevant question. But in the case where there's no variation, and the PAM is a PAM that doesn't exist in the human genome, it was tested to see if CRISPR Cas9 would cut despite the wrong PAM. Not that we had cells or that we know of any cells that have that alternative PAM. I hope that clarifies.

Dr. Wu: Maybe, can I ask a follow-up question? I mean, let's say suppose your product goes to the market and let's say it's going to be used for 2,000 patients; 1,000 patients in the next few years; you will be comfortable just with getting three genome editing, I mean, our target data on three SCD patients. Which is what do you have so far?

And then the other readout that you have is looking in humans. Let's say, for example, cancer. Uh. But those usually pop up much later, right? So, I'm just curious why you're so confident that you can get all the data you need based on three SCD samples.

Dr. Krogmeier: Yeah, I'm going to actually take that in two parts. First up, Dr. Altshuler follow up on the nonclinical package, and then I'm gonna have Dr. Hobbs speak to you about the clinical side of the assessment.

Dr. Altschuler: Yes. So, if, I could have slide 40, please, from the core presentation? Our view of this is that the way that we – Oh, thank you. I forgot to push the button. Our view of

this is that the way we character – All the data we know of says that true off-target editing occurs at sites that have a partial mismatch to the guide. That's consistent with what Dr. Urnov said, Dr. Bauer said, and everything in the literature. There's no information we're aware of where a site with no homology to the guide actually has reproducible off-target cutting.

So, we're looking for sites that have homology to the guide. And in this case, the relevant number is not three, the number of sickle cell patients, or 14, the number of total samples. It's actually, we know a lot about human genetic variation because millions of human genomes have been sequenced. We know the patterns of human genetic diversity, and we have the 1000 Genomes Project, which, if you go to the next slide, or slide 42, actually has 2,504 individuals from 26 different populations.

I do want to make clear that while many people with sickle cell disease are African American, as noted by multiple of the presenters, it's also present the disease in samples from people of South Asian origin, of European Southern European origin, and other parts of the world. And also, the world is cosmopolitan. So, people have a self-reported ancestry may have ancestry from multiple populations, which is why we looked at the entire human genome diverse, the entire, I should say, 1000 Genomes Project. We looked at variants that had 1% or higher frequency in any one of the five continental groups, which are samples from Sub-Saharan Africa, from East Asia, from South Asia, from Europe, and from the Americas.

There are 21 million genetic variants. So, this is vastly more complete than whether we looked at ten people, three people, ten people, or 50 people. This is a sequencing of 2,500 people. And then those variants include samples, include 661 individuals. Populations from Nigeria, from Gambia, from Kenya, from Sierra Leone, from another population from

Nigeria, as well as African Caribbean, self-reported African Caribbean samples from Barbados and the 61 individuals residing in the United States.

Those 661 people, as well as the 1,943 other people in the database, all contribute variants. We have annotated the human genome with all of those variants, and looking at 21 billion variants; you identify 50, five zero, new sites. That gives you a sense of how few sites there are in the human genome that have any homology to our guide, such that having one of these 21 billion genetic variants, only 50 of 21 million actually nominated a new site.

So, then, when we tested the assessment of those sites, we also included the power calculation. I know I'm going on, so I'll stop, but on slide 44, if you could just pull up slide 44 for a second? If you question if you want to know whether or not the power is good in 661 samples, you can see the power calculation to find variance of 1% or higher in 661 people is 99.xx%.

So, all those, the genomes, have been annotated with all the sites from those people, and then we went and looked in our samples, did we query them? And the only ones that weren't directly queried were the ones that we previously discussed and those we performed a risk assessment. So, hopefully, that answers the question.

Dr. Ahsan: Thank you. Did you want the clinical…

Dr. Krogmeier: And yeah, in the interest of time, we can turn it back over to you.

Dr. Ahsan: Great. So, just to put one more point on it, and maybe I've misunderstood. But what you're saying is about the CPS1 variant; you did the risk assessment, and therefore, you did not do the hybrid, right?

Dr. Krogmeier: That's exactly right. We did the risk assessment as if there was an on-target.

Dr. Ahsan: OK. Great. And so that explains the discrepancy between your reporting it out and what the FDA was asking. Okay. So, unless there are pointed questions to the sponsor from the committee members, is there anyone else who has content questions for the sponsor?

Dr. Komor?

Dr. Komor: Yeah, I just wanted to follow up on the GUIDE-Seq off-targets and why there was no overlap with the in-silico and just very briefly if there's like an explanation of if there are additional mismatches or why there wasn't an overlap.

Dr. Krogmeier: Great. Dr. Altshuler.

Dr. Altshuler: Our interpretation is that cells that are alive without editing have double-strand breaks that can be detected by GUIDE-Seq that have nothing to do with genome editing. And, in fact, one of the reasons we say that as we perform the GUIDE-Seq in edited and unedited cells, and you see a similar number of false positives in both. So, it's clear that GUIDE-Seq is truly detecting, you know, sites that have a double-strand break in the cells you have to be, you happen to be characterizing, and that is the case in normal cells can have, and I think you said a moment ago, that happens all the time; DNA repair notes them, stops the cell, either corrects it or kills the cell, undergoes apoptosis But so we believe is going on is we're just detecting the background rate of double-strand breaks in cells and culture and the evidence for that, as I said, is that there's similar rates and edited and unedited cells, and they've no overlap with the things nominated by homology. And then, we test them in independent experiments. We don't see any editing, and I could give you an example, and I won't for the sake of time. But you know, there's one that's, I believe, a 17 run of 17 Ts in a row that's edited more frequently in the unedited than the edited cells. So, I mean, like, it's not the case that these are true gene editing inspired. They're just the background rate of a method that is very sensitive.

Dr. Komor: Okay. So, you didn't really see any homology at all to the guide RNA. Because a lot of times, I mean, you can get a very rare Cas9 cutting event, but then it just immediately, like 99.99% of the time, it's going to get repaired perfectly. But if you're seeing like no homology at all to the guide RNA, then yeah, I would consider that to be just background.

But if you did see some homology, maybe it's just a very, very rare event that, under your experimental conditions, is just perfectly getting repaired, and you don't have to worry about it.

Dr. Altshuler: That's exactly what we see. So, there's not homology. And I think the FDA presentation they noted that the method of GUIDE-Seq has something called a false positive filter, which is to filter out such things. We didn't apply it because we were trying to be as complete and comprehensive as possible. So, we left those in. But as the presentation from the FDA showed, if you actually apply the false positive filter in the publication, there are zero findings from any of our GUIDE-Seq experiments.

Dr. Komor: Got it. Thank you.

Dr. Ahsan: Thank you. Dr. Tisdale.

Dr. Tisdale: Yes. Thank you. I had a question about the predictability of in vitro assays in this space. You know that over the years, we've had a lot of trouble predicting what we get in an engrafted cell versus what we can measure in cells that have had some ex vivo manipulation. So, now that you've had some experience, I wonder if you can comment on the degree to which at least the editing types I know that, you know, with the off-target, it's going to be more difficult to compare this, but at least with just editing types, you know NHEJ versus MMEJ we see some discrepancy in engraftment, large animals. And when we try to do HDR, even further discrepancy between HDR rates in the cells, ex vivo, and in those that engraft. And those engrafting cells, you know, they may have a different set of requirements for engrafting that could even possibly eliminate some of those cells with edits that you don't want. So, I wonder if you can just comment in general on, now that you have clinical experience with looking at edits in vitro and in patients in vivo, how will they predict?

Dr. Krogmeier: Uh, can I just confirm that that is a question for the sponsor?

Dr. Tisdale: Yes, that's for the sponsor.

Dr. Krogmeier: Great, I will ask Dr. Altshuler; Dr. Ahsan, is that okay? With the FDA, yes? With the chair?

Dr. Ahsan: Yeah, let's keep it kind of limited because it's more of a commentary than a question about factual information from the BLA application.

Dr. Krogmeier: Understood. Dr. Altshuler?

Dr. Altshuler: Yes. No, thank you, Dr. Tisdale. And I'll be brief and also only refer to information that is in the BLA. If you could pull up, I think it's slide AA3; so just a quick bit of data. This figure on the left. Oh, I'm sorry. Gotta remember to push the button. That data on the left, which you can now see, is from our New England Journal paper. I believe it's supplemental figure one. And what that figure shows is three different samples from three different patients that were transplanted or three different people transplanted into mice; is the one on the left.

But what it shows is, for three different and then the different colors, and you can look at the New England Journal paper, it's obviously a lot of information there just to having a slide, but it shows the indel patterns that are seen. And you can see the indel patterns are similar across the cells and similar across many different animals that have engraftment. And then also in the manufacturing process qualification, looking at 19 lots and we assess the indel patterns. And the indel patterns are consistent with those seen in the non-clinical package.

So, the indel patterns are consistent, and we have this data from the animal studies that showed that they're consistent after engraftment. Thank you.

Dr. Tisdale: Just to put a finer point on it. My question was about how the in vitro predicts the in vivo observed not in xenografted mice but in patients. The experience that I was talking about was autologous transplantation in large animals. So, I think that's a model

where we get maybe a better view of what might happen in humans. But, my question was about how the in vitro predicts the in vivo in humans.

Dr. Ahsan: I think that might be a better question to leave for the committee members to discuss among ourselves. So, that's great. Dr. Tisdale. Maybe we can bring that up again in a few moments. So, I think at this point, we'll relieve the sponsor from answering any more questions. Uh. I think the committee has gotten the facts that they need from the sponsor and appreciates the sponsor coming back and returning to answer some questions as well as the presentations from the FDA.

Uh. And so now, if we can present the discussion point, that would be great. And I can read that off. Okay. So, today, our discussion question is: please discuss the applicants' off-target analysis, for example, in-silico and cellular methods, and provide recommendations for additional studies if needed to assess the risk of off-target editing for exa-cel.

And so, I think we have two discussions that we would like to start off our conversation, and then we will, of course, bring it up for all members. So, Dr. Wolfe, if you could please start to address this discussion question, that would be very helpful.

Dr. Wolfe: Sure. Happy to start things off. To start off with, thinking about the silico analysis that the applicant has used, I think that it's pretty detailed. They've used three different programs to search for near-cognate sequences to their guide RNA. And used criteria with regard to the number of mismatches that should capture the majority of potential sites that could be active. Their method for sequence capture seems reasonable and should avoid, at least, most bias for small indels. And, I think the only thing that could be improved potentially with regard to the analysis of their sites is the depth of sequencing. So, for the larger sample size that they did of 5,000 sites, they only look down to cut off with 1% editing, and then for the smaller subset of 200 sites, they look to about 0.2% editing, where you know, more in-depth analysis that's sort of done these days would be down to 0.1%. But

they're supplementing that with regards to the empirical analysis of GUIDE-Seq, which really is a gold standard right now for capturing off-target sites using double-strand DNA that is co-introduced with regards to the editing product.

So, overall, I think they've covered their bases with regard to the reference genome pretty well. I think that with regards to variant analysis, you know, the differences between the applicants' variant sites that they looked at and the sites that were identified by the Cancellieri authors. That's something that's of interest to think about, exploring in a little more detail, especially the off-targeted ending at CPS1. It would be really interesting, I think, to look at that in actual patient samples that have been, those that have been treated with exa-cel. There's enough patients that have been tested that, in principle, there will probably be multiple individuals that will have had the variant of interest, and it should be possible to look both in the input sample and in the engrafted material as a function of time and look at the persistence of edits at that off-target site if it's present, and also for the inversion that potentially could be taking place since both the off-target site and the on-target site are on the same chromosome. It would be, I think, something where we could learn quite a bit about, you know, the outcomes of genome editing with the patient population that the applicant now has. It's really exciting to see how many patients have been treated and how positive the results are.

I think the, you know, the other thing that I would mention with regards to off-target analysis is that, you know, we want to be careful to not let the perfect be the enemy of the good. And right now, I feel that you can do a lot of in-depth analysis with regards to cellular analysis and in-silico analysis, and, you know, samples that are treated prior to introduction into patients, and you want to do as good a job as you possibly can. But at some point, you have to just try things out in patients, and I think in this case that, you know, there's a huge unmet need for individuals with sickle cell disease. And, it's important we think about how

we can, you know, advance therapies that could potentially help them. And I certainly think that this is one of them. Dr. Ahsan: Thank you very much. If I could probe just a little bit in, in your initial analysis, which is, could you speak a bit to, in the in-silico studies, the number of genomes that were litigated, et cetera, in terms of getting to the data analysis that they performed.

Dr. Wolfe: Yeah, so they looked at both normal donors and sickle cell donor samples. Admittedly, the number of different donors that were analyzed was relatively modest, but I think that as Dr. Urnov and Dr. Bauer spoke to, typically, you know, with at least with regards to thinking about the reference genome, the editing outcomes that are observed in one sample reflect those that are observed in another. So, if you do three different donors and you look at off-target analysis across hundreds of different sites, generally, you're going to find that if they all have the reference sequence, that they're going to fall in line with regards to editing rates.

The only times you typically would see outliers for one individual would be if there is a sequence variant that overlaps the potential off-target site. So, hopefully, that answered your question. I honestly think that the number of samples that they've analyzed is reasonable. There's only so much you can learn from additional samples unless you're going to focus in on, in my mind, sequence variants and trying to find samples that would have sequence variants that would allow you to interrogate off-target sites that, you know, aren't common within the human population.

Dr. Ahsan: Great. I like to think about spanning the experimental space. And I think what I am hearing is doing more of the same type of samples, such as the healthy donors or the SCD ones, would only get you a repeated analysis of the same off-targets and not necessarily new information.

Dr. Wolfe: Yeah.

Dr. Ahsan: Great. OK. Thank you very much. Dr. Komor, could you provide us with some initial comments? And then, I will get to the questions from the committee. But if Dr. if you could provide some initial analysis, that would be helpful.

Dr. Komor: Yeah. I mean, I agree a lot with pretty much everything. Dr. Wolfe said. A couple of things I'll just point out. Their, yeah, their initial in-silico analysis was quite expansive. I think it was the thresholds that they used were quite lenient. And in fact, those thresholds of up to, you know, three or five mismatches and all these alternative PAMs actually would take into account a lot of the genetic variation, just because, oh, if a genetic variant pops up here to generate a potential off-target, well, that would have had, you know, three mismatches instead of two in their in-silico analysis.

And you saw that with the Cancellieri off-target that that everybody's been talking about. That did pop up in their initial in-silico analysis. And then, in terms of, I mean, each individual on the planet has several million genetic variants in their genome. And so, like, the perfect off-target analysis would be: sequence the patient, use that as a reference genome, and then individually validate every single off-target. And is that reasonable here? You know, especially, I love the quote from Dr. Wolfe. You know, expecting perfection at the expense of progress here. Like, do we have the technology to do that? To sequence every single patient and do an expansive individualized off-target analysis on each one? Probably, but is that reasonable to expect from them at this point? I don't know. And then. Additionally, for GUIDE-Seq, GUIDE-Seq is a very specialized technique. It's difficult to do in certain cell types. It nominates putative off-targets. Many of those nominated off-targets don't end up being bona fide off-targets just because they're very low levels, and the cell can repair those perfectly under genome editing conditions.

And so, again, would the ideal analysis be to perform GUIDE-Seq in the patient samples and then go in and individually validate each one? Yeah. But is that reasonable to

ask? I don't think so. I think, you know, what we see here, I think, especially given the benefits of this treatment or this cure and what we've these patients are dealing with without having this treatment, you know, I think I think the benefits far outweigh the risks here.

Dr. Ahsan: Great. Thank you very much. That's helpful commentary as we start to get to this. I mean, I think that that's a major point, right, which was brought up in the guest presentations early on in the morning, which is, at what levels is the theoretical analysis sufficient given that the safety is on a per patient or target population level? Dr. London.

Dr. London: Yes, thank you. You almost went so far as to say this, but I think the very next step is can anyone offer an opinion on what we would need to see in additional studies that would shift the risk such that we would think the risks outweigh the benefits. I mean, many people have said that they think doing other studies may not be reasonable. But even if they, if we did all the studies that we could, what would we need to see to make us think that the risk outweighed the benefits?

Dr. Ahsan: Yeah, I mean, that's, I think, a good point. One of the things that, one of the words I started thinking about early on during the day was, what should we know versus what can we know? Because when we do all of this theoretical analysis, at some point, it has diminishing returns and inhibits progress, as Dr. Wolfe suggested. Dr. Lee.

Dr. Lee: Thank you. I guess another way of framing this or what I've been thinking about today is and trying to get at is there seems to be a lot of uncertainty, a lot of unknowns about what these off-target changes might mean, and that was repeated over and over this morning. And my question is, you know, is the unknown given the theoretical possibilities, right? So, there is some limit to what the unknown is, but given the theoretical possibility, and given that we don't know them, is it more harmful? Are those unknowns more harmful than not allowing this to go forward, right? That's this risk thing we're constantly, this tension we're trying to cope with. And so, you know, if we anticipated, or there was some theoretical

possibility that if this found just the right off-target, somebody would drop dead. That's a very different kind of risk than, you know, I mean, even leukemia, depending on, you know, there are lots of different kinds, and they can be, you know, very, they vary with respect to lethality, et cetera. But given what people are dealing with right now, and given that the evidence for the efficacy of this treatment is overwhelming, I really wonder, you know, what would we, what would we not be able to tolerate with respect to the unknown? So, you know, even reading through all this stuff earlier this week, I just kept thinking, you know, what more could we know that would lead us to say, you know, the risk is too high relate relative to the harm of not doing anything.

Dr. Ahsan: Yeah, that's a great point. You know, we always think about the risk-to-benefit ratio. And the benefit seems to be not that equivocal as it might be in other situations. There seems to be a strong sense of benefit, and the risk is theoretical. And so that does lead the ratio towards one direction versus another. So, that's always something that we need to think about and something that it's almost, it's difficult in this scenario where we're not comparing... We're comparing theoretical versus real-life clinical outcomes. Dr. Ott.

Dr. Ott: Yes, thank you very much. I have just a more sort of clarifying question and comment. Weighing the two methods, the in-silico method versus the experimental method. Originally, I thought the in-silico method doesn't seem to predict anything that is actually happening in vivo, and why should we do it? But then we learned in the last, you know, the question from the sponsor that presumably the experimental method might be too sensitive and too many non-relevant sites might be coming up. So, I wanted just to hear a little bit also going into a recommendation from the experts here what they think about, you know, weighing these two methods and how, you know, clearly they're not totally overlapping currently, and how we can reconcile this and is there really too much sensitivity in one and maybe less sensitivity in the others?

Dr. Ahsan: Yeah, that would be great. I would ask if Dr. Komor could speak to that as she had given some opinion on this before if you can expound on that.

Dr. Komor: Sure. I think the overall thing to note is that both strategies are kind of like they identify potential off-targets, and so they give you a list, and usually, I think honestly, I will also note that a lot of people have looked at this guide RNA. There has been a ton of, like, not just Vertex, many academic labs, other companies, a lot of people have looked at this guide, and I think it is a very, very specific guide RNA. And so maybe this is not your typical situation when looking at off-targets, but, usually, the in-silico analysis, you know, we'll give you a list of potential off-targets and a subset of that might actually be off-targets. You usually then, if you do the GUIDE-Seq again, it's, it's very, it's very sensitive. But also it, it will pick up, you know, it won't pick up targets that either Cas9 won't bind that because of chromatin accessibility or because, you know, the binding just doesn't happen. So, it cuts those down, but then, since it is more sensitive, it picks up more than what you're going to see as an actual off-target. And you typically do see some overlap between the in-silico analysis and the GUIDE-Seq targets. I guess, in this case, when I, you know, ask them their question, a question about the GUIDE-Seq targets, they said they were all sort of false positives. And so that's why, in this case, there was no overlap because, basically, GUIDE-Seq didn't nominate any additional off-targets. But generally, with a typical guide RNA that is not this specific, you do see some overlap, and both strategies, I think, are quite useful. I don't know if Dr. Wolfe could add.

Dr. Wolfe: I thought that was an excellent explanation. Dr. Komor. I wholeheartedly agree. I think that they're very complimentary techniques, and in our experience, GUIDE-Seq usually finds off-targets for most guides, and those overlap with what you predict computationally as well. And so, by taking both approaches, I think the applicant is, you know, trying to both

take an empirical and computational approach and, thereby, you know, not being too biased with regards to their discovery of potential off-target sites.

Dr. Ahsan: Great. If I can ask a question of you all, and this is not my area of expertise, but it does seem like we're trying to triangulate to find those off-targets, and there was a third method, right? The naked DNA that the biochemical approach. Just to raise that question for completeness. The sponsor did not utilize that. Would that have been beneficial in any way to have conducted those experiments as well?

Dr. Wolfe: Certainly, that would be another approach one could take. The in vitro methods on purified genomic DNA tend to be, tend to give you a lot more potential off-target sites, there tend to be a lot more false positives that are associated with it, but it'll also give you a much larger list of sites that you can interrogate on treated samples to see if there's actual editing.

So, it's certainly a valid way to go. And Shengdar Tsai's lab has developed some really nice approaches for doing that with regard to genomic DNA. So, it's a valid way to go. I don't know if it's worth the effort at this point, given, you know, the analysis that they've already completed.

Dr. Ahsan: And can I ask you another question while I have you, Dr. Wolfe? Which is the reference databases that they used. Do you feel that those were appropriate?

Dr. Wolfe: Yes, so for the 1000 Genomes Project is a solid database to use with regards to looking at variation. You know, I'm really not an expert. Maybe Dr. Komor knows more about exploring sequence variance. It's not particularly my forte, but I would say that that was the primary database that the Cancellieri paper leaned on. So, they pulled out the CPS1 variant based on their analysis of the 1000 Genomes Project. So, I think it's a really good place to start. And as the applicant indicated, based on their power analysis and, like I said, I'm not capable of doing those calculations, it sounded to me like they felt that it would have, that the 1000 genomes project would have the majority of sequence variants that were, I

guess, greater than 1% frequency in the, in the patient, in the human population. So, that seems like a pretty good place to start.

Dr. Ahsan: OK. I do have a follow-up, but I see that Dr. Verdun has a comment to make. Dr. Verdun: Hi, thank you. I just wanted to make a just a clarifying statement. I appreciate the conversation. We just heard a comment, you know, considering too much risk or outweighing benefit, and that was sort of not the setting that we were talking about this. So, I just wanted to sort of make that clear. You know, we're not here discussing any concern with the benefit. What we were more wanting to have a conversation about is whether the committee recommends any additional studies, and just realizing that we also have certain regulatory authorities where those could be in the post-market setting. So, post-market requirements or commitments or otherwise, if needed. And, so I just wanted to, you know, make that comment as we're having the discussion. Thank you.

Dr. Ahsan: Great. Yeah, that's helpful. So. Another way to think about it. So, I think the one of the ways we can be helpful to the FDA is what would be some follow-up analysis that we might want to include as we as they move forward. Dr. Lee.

Dr. Lee: Yeah, just on that note, I guess I, I would love to hear folks' impressions of the plans they have for the post-market follow-up. I mean, they've got this 15-year plan to have a registry, et cetera, and continue post-market surveillance. And it seemed fairly strong to me and quite a commitment, and I just wondered what others might have thought about that plan.

Dr. Ahsan: Were there committee members that had a viewpoint on that? Dr. Wolfe.

Dr. Wolfe: Yeah, I agree that the 15-year follow-up seems really good. The one thing that I thought was missing that I'd love to see is, you know, a molecular analysis of on-target edits, the distribution of sequences. I think that's what Dr. Bauer was getting at in his presentation, that you can use those as sort of a fingerprint to look for clonal expansion potentially within the patient's hematopoietic cells. And it seems to me that that sort of analysis would be

relatively straightforward. In principle, the applicant is already generating this data because they're following the indel rates over time. So, they're actually sequencing peripheral blood to look at this. And, so it should be relatively straightforward to follow up with regards to the indel spectrum, and does it change over time? And with that, provide a surrogate and sort of an early warning sign of something going wrong with regards to the hematopoietic system.

Dr. Ahsan: Great. Dr. Tisdale.

Dr. Tisdale: Well, Scot just basically said what I was going to say. I mean, I really think it's worthwhile to follow these edits, you know, in real-time. And, they're getting these data, and then, you know, if anything happens, they can look backward. So, it would be really, really good to follow this. I also had another question, which I think would be interesting to know the answer to, and that is to what extent of the data they plan to share with the CIBMTR. Because I think this is also a very good plan, but there are two different ways to share there. It's limited or full, and I think I got the impression from the slide that the data were the full clinical data, at least, but it would be nice to see that.

Dr. Ahsan: Great. I do have a question. So, thinking about the presentation, the first presentation in the morning, talking about where are we on the risk mitigation curve. And I think Dr. Wolfe, you mentioned something about the value of the biochemical analysis or the DNA analysis that could be done. Is there something to think about there in terms of any emerging disruptive technology that would actually result in a step change in the evaluation of these off-targets? Something that we might want to ask them to do in a, for, in a monitoring way to help generate data.

Dr. Wolfe: Well, I think that the other thing that Dr. Bauer touched on that is potentially of interest would be long-range sequencing, so nanopore or some other sequencing method at the target site that would provide greater information with regards to large deletions or other, features like that. It would be one other way to look at the outcomes there. But, you know,

I'm honestly not sure what we'll learn. But that would be the one technology that I think is potentially of interest to apply in this setting.

Dr. Ahsan: Right. Any other questions or comments from the committee? Oh, Dr. Ott

Dr. Ott: Sorry. Just, just to come back to the CPS1 variant. What is sort of the consensus on this? Is it a risk variant for off-target effects? Or is it just a silly, cool, you know, prediction? Just wanted to hear what the experts thought about this. Thanks.

Dr. Ahsan: Dr. Wolfe, if you want to answer. I see your camera on.

Dr. Wolfe: Yeah, I mean, I think that it's clear from the study that's been published that there can be off-target editing there, so it seems that, it seems to me, it would be good to follow up in the patients that have been treated so far to look at whether or not off-target editing had occurred for individuals that have the variant. I mean, I agree with the applicant that ultimately, you know, you need to assess, the patients and is there a bad outcome. And that, you know, any given off-target doesn't necessarily, or off-target anything, does not necessarily mean that there's going to be a bad outcome. So, but it would; I do think it's worth taking a look at the patients they now have. If they have 45 patients that have been treated, that's 90 alleles. So, you know, with a 4.5% frequency in the African American population, you would expect that they'd have, you know, four or five alleles to look at, at this point.

Dr. Ahsan: Can I ask one question that I may not have understood correctly regarding that, which is, I thought I understood that they had identified that and they had vetted that for its biological relevance and found it not to be biologically meaningful? Did I misunderstand that?

Dr. Wolfe: No, I, that's what the applicant said, and they don't think that you know, cutting within this gene or indels within this gene would be a risk factor. So, you know, and I have no reason to not believe them in that.

Dr. Ahsan: Yeah, I mean, I guess the question that comes to me, which is if that one is of a particular interest, even though we feel that it may not be biologically meaningful versus all the other nominations. So, I don't know what a strategy might be about monitoring.

Dr. Wolfe: Uh, well, I mean, I don't know how much of a risk factor it is. But I think, though, the other thing that would be interesting just from a scientific point of view is, if you compared input for, versus, engrafted cells, and for instance, looked at the inversion that was detected by the Bauer lab is that only seen in input cells and does it, you know, not occur in grafted cells? Those kinds of questions would be really valuable to have answered. Dr. Komor, did you want to comment further?

Dr. Komor: Yeah, I mean, I'll just point out. So, in the publication, they identified the off-target. They found that it was a real off-target in their system. We don't know if, you know, the experimental conditions of that are significantly different enough from what Vertex did. Like if that would actually be a real off-target if a patient had that genetic variant in the system. But I mean, I certainly think – Yeah, as Dr. Wolfe said, Vertex has the sort of patient samples to get this data, you know, well genome sequencing is not prohibitively expensive anymore. And then also, I mean, I'm not an expert at this, so I'm wondering what other people's opinions of their risk assessment was. On all of the sort of putative off-targets where there wasn't, you know, one of these genetic variants in the samples that they tested, but then they did a risk assessment and said, oh, it wouldn't matter if there was an off-target, an off-target in any of the, yeah, an indel in any of these off-targets. I'm wondering what other people's opinions on the risk assessment are.

Dr. Ahsan: Yeah, that would be great. I mean, I don't know if others have expertise in that. But that was also a point of major consideration, which is how do you assess whether it's a biologically meaningful variation or not. And so, is there anyone who would have an opinion on that that they would want to share? Dr. Wu.

Dr. Wu: Yeah, so, I think we all, well, most of us, we all agree that, you know, the benefits outweigh the risk, right? So, these patients are quite sick, and this is a, this is a very good therapy. I think the question for us is the biology side. What is the frequency of these off-target effects? And I think unless you do whole genome sequencing, you wouldn't know. And I think, as mentioned earlier, it's very inexpensive. I mean, it costs less than a thousand dollars just to hold a genome sequencing sample before, hold a genome sequencing sample afterward, do it on, you know, 20 of their patients, and see what the data looks like. This is information that can be further fed into their AI machine learning in silica model to help improve the whole process; that's also going to help improve the whole field, right? And so, I just don't understand why the hesitation of not doing it.

Dr. Ahsan: Yeah. Dr. Tisdale.

Dr. Tisdale: Well, I have to say I'm mostly curious because I don't know that it's necessary given all that they've presented today, but it would be, I think it would be nice for the field to look to see if this snip that the Bauer group identified is present and any of their subjects, if there was off-target editing in any of their subjects, and then to look at the overall percentage of that edit over time in individuals to see if there was any change in the contribution to amount of [indiscernible] by cells with that edit based on the overall percentage of that edit being present. I mean, that, to me, is just a really interesting experiment to do. I mean, I'm not sure it's necessary, but it's pretty easy to do. And I think interesting for the field in general.

Dr. Ahsan: Yeah, that's a great point that both you, Dr. Wu, and others have raised, which is they could do it. And I think when you asked Dr. Wu, they said, well, we didn't do that, but we've done all these other aspects. One thing to think about is that it might be of interest, but one of the questions in the discussion is we should delineate what we would recommend if

there were studies that are needed, what we would recommend that they just do, and so we might want to distinguish those two categories. Dr. Wolfe.

Dr. Wolfe: Yeah, so I just wanted to follow up on the suggestion of whole genome sequencing. So, I think the challenge there is that editing rates at off-target sites may be quite low. And so whole genome sequencing is great for getting sort of the sequence of the most common genome that's present in an individual. But with regards to picking up low-frequency edits, I don't know that whole genome sequencing will be really effective for doing that. I think it might turn out to be challenging. I think that the error rate might start to get at the point where it would be, where it'd be challenging unless there's a high rate of editing. So, others may have more experience with whole gene genome sequencing than myself, but I'm not sure that it will give us the information that we'd like.

Dr. Ahsan: Dr. Komor, did you have a comment regarding that?

Dr. Komor: Yeah, I'm just going to say I agree. I think the whole genome sequencing would be able to get like a reference genome for that particular patient. But yeah, I don't know like the sequencing depth required to identify some of these low-efficiency off-target events potentially. I don't know if that would work, but for identifying additional putative off-targets and, especially, the one that we've been talking about all day, you could identify that.

Dr. Ahsan: Great. Dr. Shapiro.

Dr. Shapiro: I'm thinking about this from a clinical perspective. I'm wondering if, you know, you're going to do this 15-year follow up and have this registry to see how patients do. The issue is if you find a few patients who are having problems, wouldn't you want the whole genome sequencing to begin with? Because if there are specific polymorphisms or differences within the individuals who have problems, you'd want to know that. Otherwise, you're gonna have to go back and look at everybody afterward. I mean, this is a complicated issue. The patients are getting Busulfan, which can cause pulmonary problems. Patients with

sickle cell can have pulmonary hypertension, can have cardiac disease. This particular mutation, the CPS1 variant, can be associated with pulmonary hypertension, but and I think my understanding of that is it's only expressed in the liver and in specific parts of the GI tract, and perhaps it's related to its expression there that is associated with pulmonary hypertension, but it's hard to unravel all of this. So, it's more of a question. Would that help you with your registry?

Dr. Ahsan: Yeah, I don't know. An additional question. Is there, and maybe some of the experts on the panel, is there any value in doing a differential analysis between from samples from the patient that had had VOCs and had multiple of those versus the patients that did not? Because there was that one patient.

Dr. Wu: I think I asked them that question, right? So, I wasn't sure how much the transfection, the genome editing efficiency is, and whether the patient who didn't have a good benefit had a repeated VCO. Maybe the product they injected, they told us it's about three to 20 million cells. So, within that three to 20 million cells, is it 80% all edited? Is it 10% edited? Is it 40% edited? We don't know. I don't think he answered that question. And I think with regard to the genome editing, I mean with regard to the whole genome sequencing, you know, even with sometimes with the electroporation process, you could cause indels, insertion deletions, right? And so, they're doing electroporation with the vector. The in-silico is all predicting that the vector binds to that sequence specificity. But the whole transfection process itself, the whole electroporation process itself, can cause changes, stresses to the cell and could cause a whole bunch of other stuff, and maybe the stress opens up more possibility. So, again, I'm not questioning that this product is important for our patients. I'm just saying that we are at a point in which we, you know, this thing's gonna take off, and wouldn't it be nice to have more additional data, and they already have the samples? They could just analyze it before and after to show us what it is. I mean, we do that for IPS cells. I mean, we

generated IPS cells in 2,000 patients. We did the PBMC before and the IPS cells afterward. A similar idea, except this one's genome editing. Yeah.

Dr. Ahsan: So, I see that Vertex has raised their hand. Maybe you can tell me what you'd like to address before we get too deep into it.

Dr. Krogmeier: Yes. We would like to address the comment on the patient specifically who had a VOC. Provide an explanation of what exactly was received.

Dr. Ahsan: is it related? Maybe you can do it related to the off-target analysis.

Dr. Krogmeier: Yeah, you know, we have a very fast follow up from Dr. Hobbs to Dr. Wu's specific question.

Dr. Ahsan: OK. Great.

Dr. Hobbs: Hi Bill Hobbs. Clinical development. And I apologize. Dr. Wu, for not fully answering your question earlier, which was about the drug product editing in the patient who still had VOCs. And the figure that I'm, and if I could show a figure, which is, you know, a picture is always worth a thousand words, and being cognizant of time… In short, the answer is that the patient who had VOCs in the study, in the PES population, had similar drug product editing as all patients and, in fact, was at the higher end of drug product editing compared to all patients. The range of editing is approximately 65 to 90%. This patient was at the higher end, and so the reason for the VOCs is not due to an insufficiency of editing. I will also just point out quickly that this is a non-viral system, and there's no vector involved.

Dr. Ahsan: Great, thank you. All right, any other questions or comments from the committee members? OK. Dr. Verdun, was there any aspect that you would want to hear more on? Or should I move towards summarizing the discussion?

Dr. Verdun: No, I think we can move towards summarizing the discussion. This has been extremely helpful for us. You know, as you know, this is something that's not

straightforward. And, you know, it's new. And, you know, we're all learning here. So, I appreciate the conversation. And this has been very helpful for us. Thank you.

Dr. Ahsan: Great. Okay. So, let me try to summarize a lot of the comments that were made, starting at the highest level. One of the questions was, where are we on this risk mitigation curve? Are we at a point where we have the technology in order to really address these questions? It does lead us to this thought that we have this theoretical analysis that can be done against reference samples or specific cells, but the safety aspect is really related on a per-patient basis or a target population.

So, one of the questions becomes, when have we done enough theoretical analysis to allow us to move forward? And that's, I think, the major question that we want to look at. I think, overall, the sentiment was that the in-silico analysis was quite detailed. It used quite lenient thresholds so that the criteria were set to really be able to create a good list of off-targets. Maybe there is some room there for doing some deeper sequences.

There was also this GUIDE-Seq empirical analysis. That seems to be a growing standard in the field. It was appreciated that they were doing that, and it gave us different results from the in-silico, and there were reasons behind that that seemed very rational that were presented by the experts, and it just gives you more nominations to consider. At the end of the day, there needs to be some assessment as to whether these off-targets are biologically meaningful, and there might be some flexibility there in terms of how you evaluate that.

In terms of suggesting studies moving forward, there was quite a bit of discussion about the monitoring of the samples over the next 15 years. It would be nice to see some evaluation of monitoring the edits over real-time, looking at clonal expansion, but it's unsure of the technology that would be used to do that. Whether whole genome sequencing would actually have the detection levels to give us meaningful information there. But, thinking about new technologies related to long-range sequencing would be very good for potentially

monitoring the CPS1 variant. But again, I think what it comes down to was that there was a robust approach using multiple methods to try to identify these off-targets, and I think there's an opportunity to generate more data monitoring these patients moving forward. There seems to be a deep plan for deep monitoring over 15 years, and that can be very, very helpful in generating data and monitoring these patients. I think that that is the bulk of what we have to do. Was there any aspect that I failed to touch upon that one of the members may think should be reiterated at this point? Dr. Wolfe.

Dr. Wolfe: Yeah, I guess the only other thing that maybe we didn't touch on, and I apologize for not mentioning this, but whether there would be, you know, a plan for pre-screening for, you know, patients that have a variant of CPS1 in the future, and, how that would affect, you know, when they would receive treatment.

Dr. Ahsan: Great, great. Adding to the selection criteria of the patients.OK. So, I think we talked about a lot of different aspects. There was a kind of a robust conversation that hopefully will be informative to the FDA as they start to evaluate different paradigms for off-target analysis. This is likely just the first of many more discussions around this topic as products come for regulatory approval. OK. So, I think with that, I will pass it over.

Closing Remarks

Before I pass it over to Dr. Verdun, I do want to thank all the committee members for their efforts. I know it takes a lot of your time before the meeting, and then this is a long day to participate in, and everyone is quite busy, and I appreciate the time and the effort you've put into it. I do want to thank the FDA staff, who do an excellent job of making sure that this meeting goes off very smoothly and seamlessly. And all the AV support that goes into that as well. So, thank you, everyone, for your time and your efforts. And with that, I'll pass it on to Dr. Verdun for some closing remarks.

Dr. Verdun: Thank you. I would like to really thank the advisory committee for the thoughtful questions, discussion, and the recommendations. And thank you first to the FDA advisory committee staff, to the FDA review team, to Vertex, and to our very informative speakers this morning. I would also like to thank all of those who spoke during the open public hearing and shared their personal experiences and thoughts.

The FDA team will be taking all of the discussion and the recommendations and reviewing it in its entirety. In a rapidly evolving field like this, it's important to have these public discussions, and we are committed to doing the very important work of bringing advancements to sickle cell disease and then partnering with all of our stakeholders.

An important part of our mission is not just evaluating efficacy but safety, both short and long term, and doing what we can to evaluate both the known and unknown risks of therapy, including potential monitoring of any off-target effects of exa-cel therapy and discussing some of the limitations. So, thank you very much for playing a role in this process.

And I would like to turn it back over to Cicely Reese. Thank you.

Adjournment

Dr. Reese: Thank you, Dr. Verdun. I'd like to also say thank you to the committee members. I'd like to say thank you to CBER staff for working so hard alongside the FDA, the AV team, who also worked very hard in making this meeting a successful one. I now call this meeting officially adjourned at 4:01 PM Eastern Time. Have a wonderful evening.