Advancing rare disease drug discovery using consistent, defined human cells

How To Cure a Rose used ioGABAergic Neurons to progress towards a potential ASO therapy for an ultra-rare neurodevelopmental disorder.

bit.bio case study
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TO CURE A ROSE FOUNDATION

Overview

In 2016, rockstar frontman Casey McPherson and his wife welcomed their second daughter (Rose) into the world with the hope and wonder that washes over new parents. That same year, scientists would describe for the first time a rare neurodevelopmental condition stemming from mutations in the HNRNPH2 gene[1]. To date, little more than 50 individuals have been diagnosed with this ultra-rare condition since its discovery[2]. In 2019, Rose McPherson would become one of those unlucky few.

According to the World Health Organisation, a rare disease is defined as one that affects fewer than 1 in 2000 people[3]. Though each of the 7,000+ described conditions in this category are individually rare, they are, as a group, common, affecting more than 300 million people worldwide[3]. Despite their prevalence, few if any therapeutic options are available to patients, due in part to the significant economic and regulatory hurdles that slow drug development efforts in this space.

Faced with the dire reality of Rose's situation, the McPhersons set out on a journey to find a cure, one that would ultimately lead them to form the non-profit organisation To Cure A Rose. The organisation took the unusual step of building a comprehensive laboratory, one that is designed to enable rapid pre-clinical development of therapeutics for rare diseases. Beginning with a focus on disease characterisation, the team searches for genetic drivers of each patient's pathology and uses this information to build patient-inspired models for multi-modal therapeutic screening. Just three years after its formation, To Cure A Rose is nearing clinical trials with two promising therapeutic candidates, each targeting the defective HNRNPH2 gene that's responsible for Rose's condition.

The laboratory's success thus far has inspired hope, not only for Rose's family but for many others affected by rare diseases. But to be successful, the team has had to overcome many challenges, one of which is the need for reliable model systems that can be used in drug screening. For this, they turned to bit.bio.

Rodney Bowling Jr, Ph.D,

Founder and Chief Scientific Officer

To Cure A Rose

The challenges of rare disease drug development

"The scientific enterprise that surrounds drug development, both the basic disease research and the pipeline itself, wasn't built for rare disease patients" explains Rodney A. Bowling Jr, Founder and Chief Scientific Officer at To Cure A Rose. "The system isn't built for 'N=1' studies".

The typical drug development pathway is designed to maximise confidence in therapeutic candidates before they reach the market. With the prospect of treating thousands to millions of patients, and the potential costs of failure (both financial and medical), drug sponsors must invest in extensive evidence gathering during preclinical stages to evaluate the risk-reward of moving forward with any one candidate. Such a process requires considerable time and money, neither of which are common in the rare-disease space[4], [5], [6].

Instead, drug development in rare diseases is often fueled by limited funding raised by the families of affected individuals, many of whom are racing against the clock to help their loved ones.

According to Bowling Jr, "these families are operating with a 'Time Is Life' urgency. They don't need to spend time making sure this drug works for everyone, they just need it to work for their child."

Rather than building confidence in a therapeutic's market potential, preclinical studies for rare diseases like Rose's can be a more focused affair, one that simply needs to inspire reasonable confidence that the therapeutic will work in a single patient. In theory, this should free developers up to streamline their studies and accelerate progress towards the clinic, culminating in an 'N=1' trial to treat the affected patient[7].

However, the success of this approach hinges on the research team's ability to model key aspects of each patient's disease in vitro, and to do so at the scale needed for rapid, multimodal drug screening. It is then critical to tailor preclinical studies to the patient.

"Every disease is different," explained Bowling Jr. "When you are studying a rare disease, you really have to break it down and explore it from the gene level back up to the cell line and then extrapolate that to the patient."

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To Cure A Rose's rare disease therapeutic development workflow

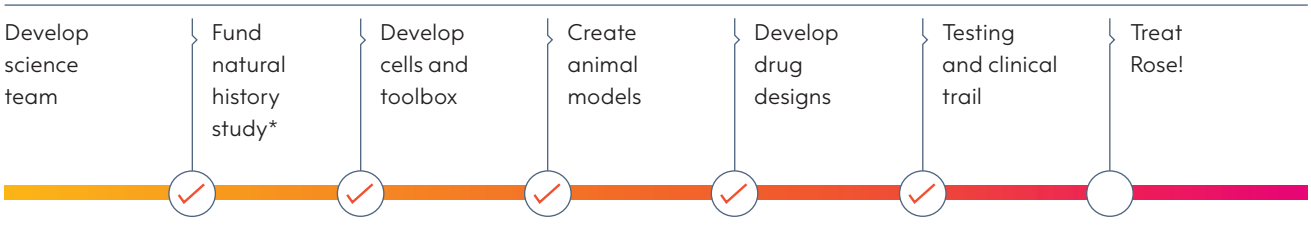
Though the exact workflow may vary on a patient-by-patient basis, the basic process at To Cure A Rose aims to rapidly characterise the child's disease, arm the families with resources to help them fundraise, and quickly identify potential therapeutic options.

1. Patient cell collection & genetic analysis
   Collect a cell line from the affected child. If the causal mutation is unknown, perform genomic and transcriptomic sequencing
2. iPSC generation
   If possible, reprogram the patient's cells into induced pluripotent stem cells (iPSCs) to supplement disease modeling
3. Assay development
   Design a functional assay that reveals the disease-related dysfunction
4. Therapeutic strategy & amenability study
   Explore different therapeutic modalities—such as ASOs, gene editing, or small molecules—to identify potential ways to correct the dysfunction. Compile this into an "amenability study": a document and presentation that families can use to rally funding and support
5. Parallel therapeutic development
   Launch therapeutic development across multiple modalities simultaneously, operating with a "time is life" mindset. For example, initiate both a small-molecule repurposing screen and an ASO development track for the same pathway to maximise shots on goal
6. Mouse model development & controls
   Order a custom mouse model early, as it can take 18+ months to develop. In parallel, generate isogenic CRISPR-corrected control cell lines for more rigorous in vitro testing
7. Iterative testing & optimisation
   Use multimodal testing to identify the most promising intervention, whether an approved drug for short-term relief or a bespoke therapy for long-term correction
8. Advance into toxicology studies
   Advance promising candidates into relevant animal models to assess toxicological profile
9. Assemble IND and move for a clinical trial
   Present cumulative evidence to regulators and apply for the initiation of clinical trial(s)

For Rose, however, step 2, 'iPSC generation' within the standard workflow had to be adapted. Generating patient-derived cell lines proved challenging, so the team turned to commercially available iPSC-derived cells from another donor.

TCAR Foundation's pathway to developing genetic treatments quickly and sustainably

TCAR PHASE 1



Modelling a Rose

Next-generation sequencing had shown that Rose's condition was driven by a missense mutation in the HNRNPH2 gene. Among its many roles, the protein product of this gene is responsible for binding to RNA transcripts in the nucleus, where it can guide trafficking and splicing. Evidence suggests this protein is critically involved in neuronal differentiation, proliferation, and apoptosis. Rose's mutation disrupts the normal function of HNRNPH2, a nuclear RNA-binding protein that plays a critical role in pre-mRNA splicing.

In healthy cells, HNRNPH2 associates with specific RNA transcripts and facilitates their recruitment to the spliceosome, the macromolecular complex responsible for removing introns and generating mature mRNA. However, Rose's mutation alters HNRNPH2's ability to deliver its bound RNAs to the spliceosome, preventing proper intron removal. As a result, transcripts that depend on HNRNPH2 remain incompletely spliced, leading to widespread mRNA processing defects and likely contributing to the neuronal dysfunction observed in Rose's condition. Fortunately, previous studies have demonstrated that the homologous HNRNPH1 (H1) protein can compensate for the loss of HNRNPH2 (H2) expression[2]. Notably, this compensation does not occur when mutant forms of H2 are expressed. The team saw a potential therapeutic angle in this: With small molecules, ASOs, or gene therapies, they could prevent mutant H2 expression in Rose's cells, enabling her natural compensation mechanisms to kick in and begin overexpressing H1.

However, testing this theory proved tricky, largely because Rose's condition is hard to model in vitro.

"Rose's cells didn't want to grow," recalled Bowling Jr. "They wouldn't differentiate well, which in retrospect is not surprising: She has a neurodevelopmental condition, and so we had trouble making neurons from her cells."

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Patient-derived cell lines are critical for rare disease studies, but they are also limited in significant ways. First, accessing a sufficient number of patient cells for disease modelling and drug screening may require invasive procedures followed by several rounds of proliferation in vitro, the latter becoming difficult as differentiated cells tend to be non-proliferative. Alternatively, fibroblasts can be collected from the patient and used to generate induced pluripotent stem cells (iPSCs). In theory, these patient-derived iPSCs can proliferate indefinitely and be differentiated into any desired cell type. In reality, however, methods for differentiating iPSCs are challenging in their complexity, time consuming, and result in batch-to-batch inconsistencies[8], [9].

Additionally, as was the case for Rose, generating the affected cell type, neurons, with patient-derived iPSCs may be impossible if the child's disease affects neurodevelopmental pathways of iPSC differentiation. To study Rose's condition, the team was in need of an alternative source of neurons that both expressed HNRNPH2 and could be reliably produced in large quantities for drug screening.

"We needed cells that gave us more control and scale, something that is absolutely uniform and consistent. We hoped we could get that by ordering iPSC-derived cell lines from one prominent vendor. Unfortunately, the cost was quite high, and they wouldn't collaborate with us to identify the right neuronal cell line [one that expresses H2]. In the end, we didn't get enough cells from them, customer service was very poor, and the cells we did get didn't express our protein. That's when we discovered bit.bio."

"Right away, we could tell a difference with bit.bio. They helped us identify the neuronal population expressing H2; they worked with us to ensure we grew these cells properly; and they helped customise our sample size to ensure we had enough to carry out screening. It was just incredible customer service."

Solution: ioGABAergic Neurons

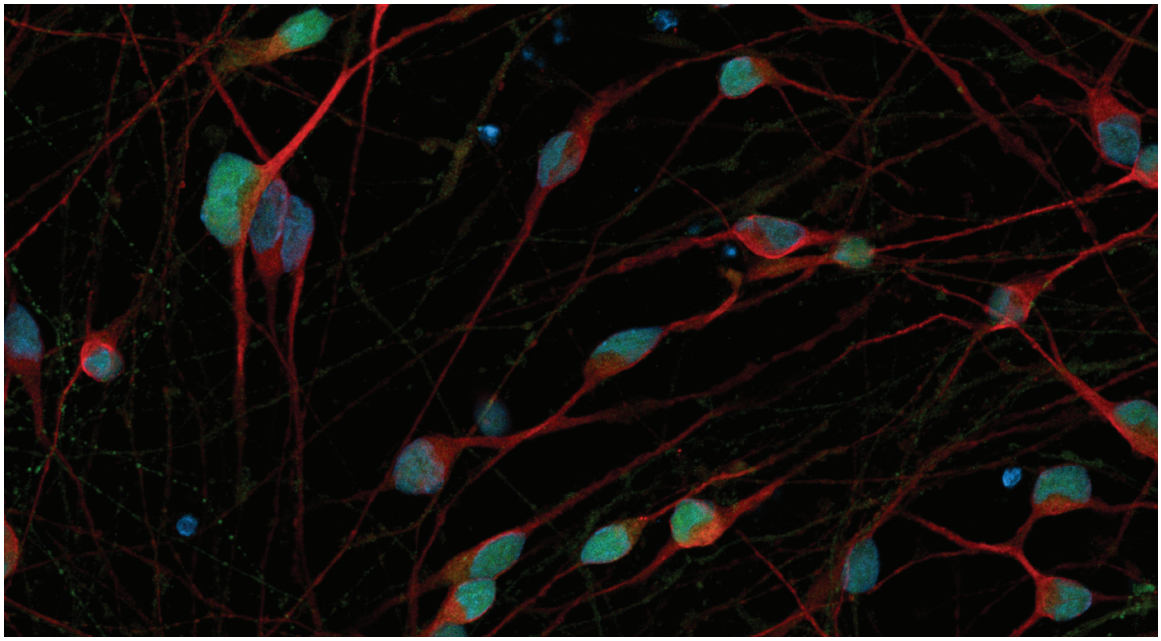
bit.bio is a synthetic biology company providing programmed human cells for research and drug discovery.
The company applies its opti-ox™ deterministic programming technology, a patented gene targeting strategy that leverages genomic safe harbours, to inducibly express genetic information in iPSCs. When induced, the iPSCs rapidly, precisely and consistently convert into highly defined somatic cells. In contrast to the high variability and heterogeneous output of commonly used iPSC differentiation approaches, bit.bio's technology produces consistently uniform populations of differentiated cells on a large-scale, and the technology can be applied to any cell type, including multiple neuronal cell types.

"Right away, we could tell a difference with bit.bio," Bowling Jr reflected. "They helped us identify the neuronal population expressing H2; they worked with us to ensure we grew these cells properly; and they helped customise our sample size to ensure we had enough to carry out screening. It was just incredible customer service."

"I called Neil at bit.bio (my account manager) to look through bit.bio's extensive catalogue of cells, specifically at neurons, I needed a neuron, as H2 is expressed highly throughout the brain." explained Bowling Jr.

Bowling Jr's team had identified ASOs as the modality of choice. Previous studies in animals had suggested that knockdown of H2 with an ASO would lead to increased, compensatory H1 expression. Now they needed to confirm this hypothesis in human neurons.

Starting with 55,000 ASO sequences and chemistry options, the team computationally honed it down to 57 distinct ASOs that had promise and could advance into wet lab screening[10]. Their goal was to assess the therapeutic ability of the ASOs to both knockdown H2 and prompt the increase in H1 expression. To do this, they used bit.bio's human iPSC-derived GABAergic neurons.



The value of transparency

Are you using the right cells for your experiment? It's a simple question, but one that can be frustratingly difficult to answer. Immortalised cell lines change over time and the quality of iPSC differentiation can vary from lot to lot. Such variability can be costly: Consider running a large-scale drug screen only to discover that your drug target is not expressed in your cell line.

To avoid this scenario, the To Cure A Rose team needed help. They knew Rose's condition was affecting her central nervous system and that H2 is highly expressed in the brain. But it is not ubiquitous among cell types. The team needed help identifying a reliable source of neurons that express H2. For this, they turned to bit.bio

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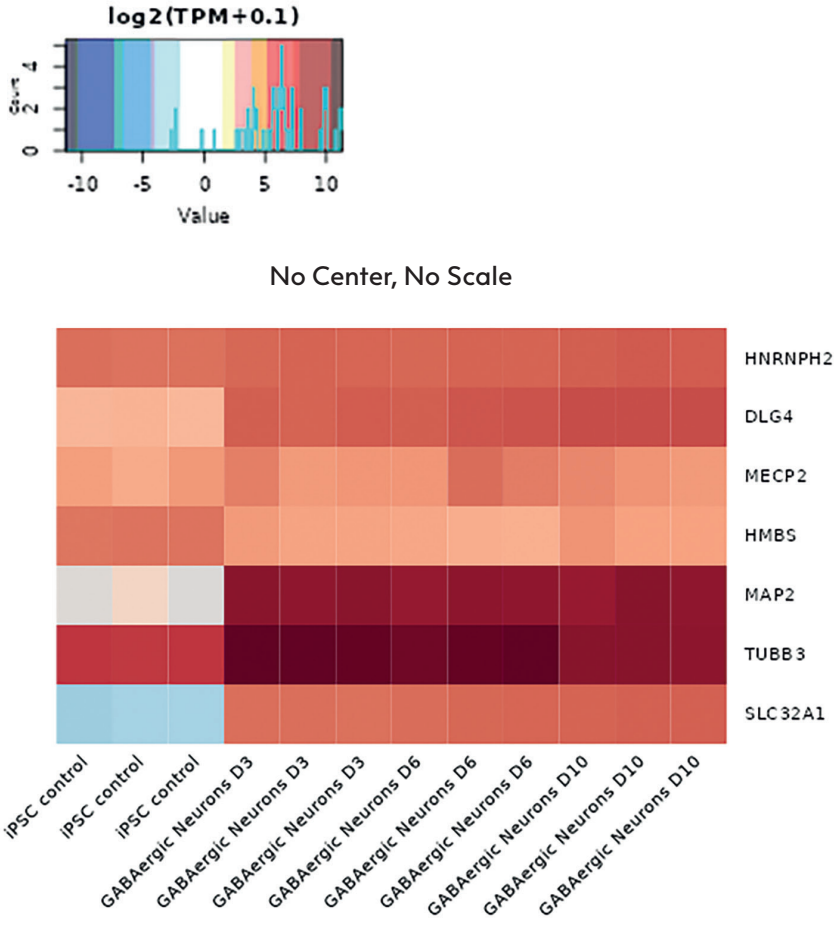
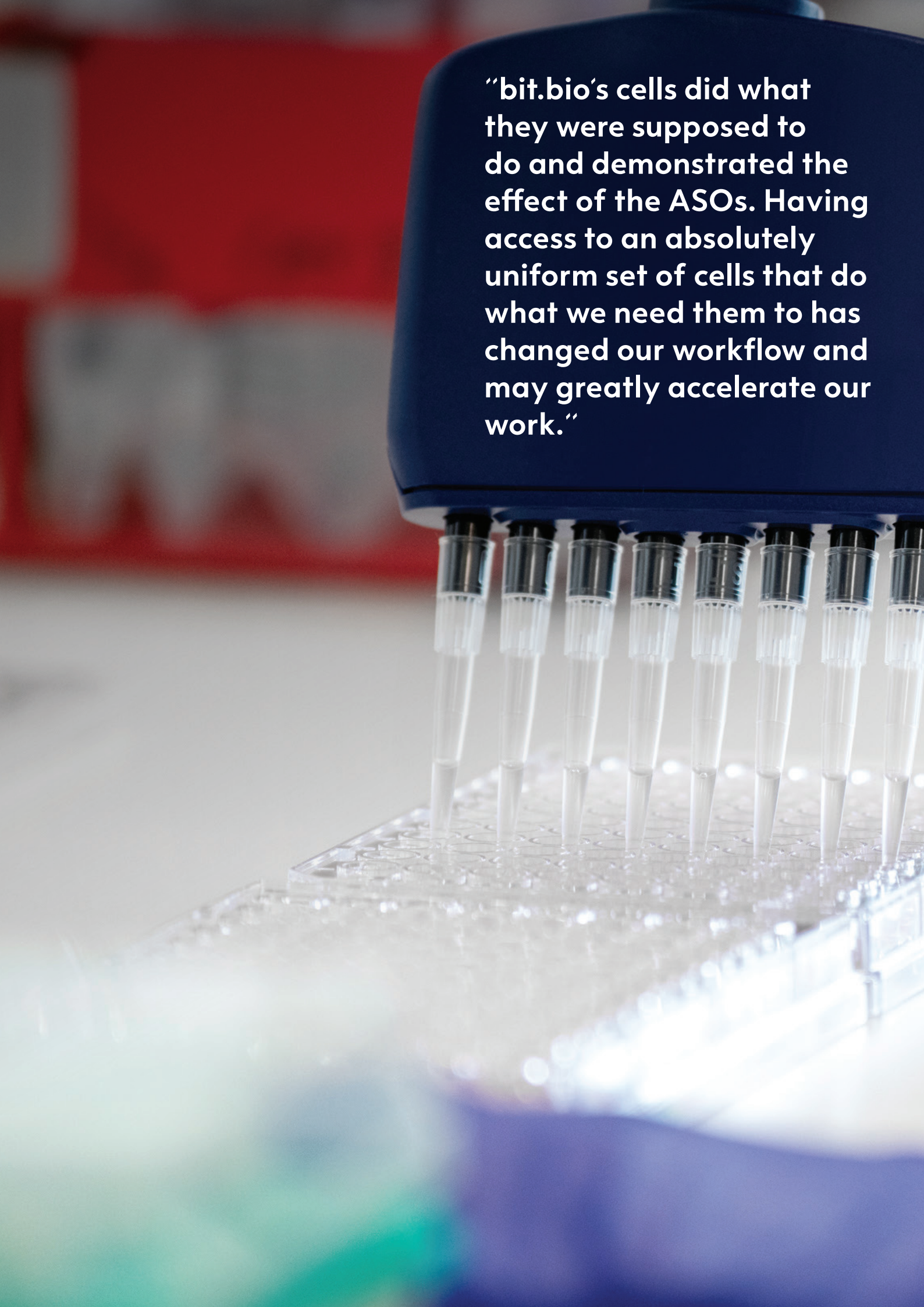
bit.bio routinely generates a comprehensive characterisation data package for its cell types that includes representative morphology images, protein expression for key markers, bulk RNA sequencing, among other datasets. From the bulk RNA sequencing data, researchers who request information on their specific genes of interest will receive an easy to read gene expression heatmap along with the associated transcripts per million (TPM) values. This data provides immediate insight, allowing scientists like Bowling Jr to confirm the expression of their target gene(s) and decide if this is the right cell to commit to.

For Bowling Jr, this was essential. "[Within days,] Neil reported back to me that H2 was expressed in both ioGlutamatergic Neurons and ioGABAergic Neurons."

Learn more about our cell characterisation data here



Gene expression heatmap for select genes of interest. TPM normalisation quantifies transcript abundance by calculating how many of the specific transcript are detected out of 1 million RNA molecules. This bulk RNA-sequencing data was generated using cells from a continuous culture, without the cryopreservation and thawing steps. For simplicity, the culture days on the heatmap are labelled with the equivalent days in the cryopreserved ioGABAergic neurons. The iPSC control sample is from the same parental line as the ioGABAergic Neurons, but has undergone no doxycycline treatment. It is possible that minor variations will exist between this data of the continuous culture and RNA sequencing data from the cryopreserved culture. In addition to the genes of interest queried by the user, control genes are included for reference: HMBS (low-expression housekeeping gene), MAP2 (high-expression pan-neuronal marker), TUBB3 (high-expression pan-neuronal marker), and SLC32A1 (intermediate-expression GABAergic neuron-specific transporter).



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Results: An embarrassment of riches

Screening of the 57 ASOs in GABAergic neurons revealed 30 candidates with desirable activity. A second screen further narrowed this list down to just 7 ASOs, 4 of which were capable of reducing H2 expression by at least 90% and 3 of which also prompted increased H1 activity[10].

In subsequent animal studies, the team was able to reduce their focus to 2 of the ASO candidates. Both show strong activity and have thus far shown no signs of toxicity, despite being administered in concentrations 10-fold higher than would be given to human patients.

"Functionally, we have an embarrassment of riches now with two promising ASOs," emphasised Bowling Jr. The team is currently working through toxicology studies with both ASOs and hopes to enter a clinical phase trial in the coming year, one in which Rose can finally be treated for her condition.

Future Directions

Since its founding, To Cure A Rose has begun research on other neurological and muscular rare diseases. bit.bio's cells and supporting expertise have now proven to be a valuable tool that the To Cure A Rose team plans to integrate across projects. According to Bowling Jr, "physiologically, bit.bio's cells did what they were supposed to do and demonstrated the effect of the ASOs. Having access to an absolutely uniform set of cells that do what we need them to has changed our workflow and may greatly accelerate our work. Now, each project starts with me going to bit.bio and saying 'hey, I need a neuron that expresses X gene,' and seeing what cells they have that can help us blast through this quickly."

The ASOs that Bowling Jr and his team have thus far discovered inspire hope, for the McPhersons as well as others suffering from her condition.

"There are two other children with the same mutation as Rose that we know of, but there are children with similar mutations who, functionally, have the same disease as Rose. This agnostic H2 knockdown approach has the potential to help all of those children as well" hopes Bowling Jr.

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Is your gene of interest expressed?

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About bit.bio

bit.bio is a synthetic biology company focused on human cells, advancing medicine and enabling curative treatments. The company does this by industrialising the manufacture of human cells and making them more accessible.

bit.bio combines the concepts of cell programming and biology to provide human cells for research, drug discovery and cell therapy, enabling a new generation of medicines.

This is possible with our deterministic cell programming technology opti-ox* – a gene engineering approach that enables unlimited batches of any human cell to be manufactured consistently at scale.

More information:
www.bit.bio

About To Cure A Rose Foundation

To Cure A Rose is a patient-centred rare disease research initiative providing a preclinical proof-of-concept lab for families.
Their mission is to accelerate therapeutic development with urgency and scientific rigor, empowering patients with data, intellectual property, and personalised research programs.
The team takes a modality-agnostic approach, exploring antisense oligonucleotides (ASOs), gene therapies, and small molecule repurposing to maximise chances of success. By developing cell lines and disease models for each patient, they enable personalised medicine at the preclinical stage. Current projects are focused on a range of rare diseases affecting neurological and muscular systems.

More information:
www.tocurearose.org

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