Spring/Summer 2022

Pediatrics NATIONWIDE

Advancing the Conversation on Child Health

Optimizing the Body's Natural Cancer Killers

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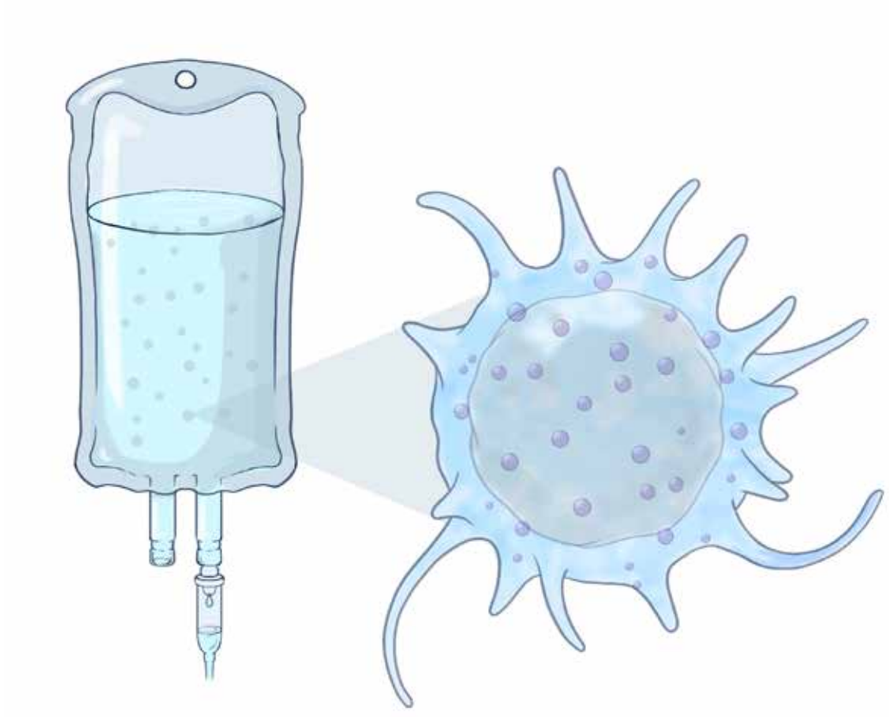

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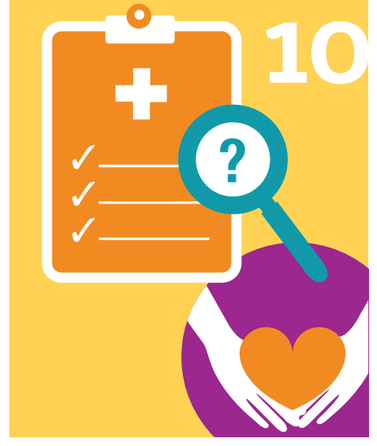


DEPARTMENTS

"We have to think about people, process and environment. We're doing this work now because we want to continue to live our mission. We want to better live our mission. If we want to improve the health of all children, we have to make sure all children are represented in our research."

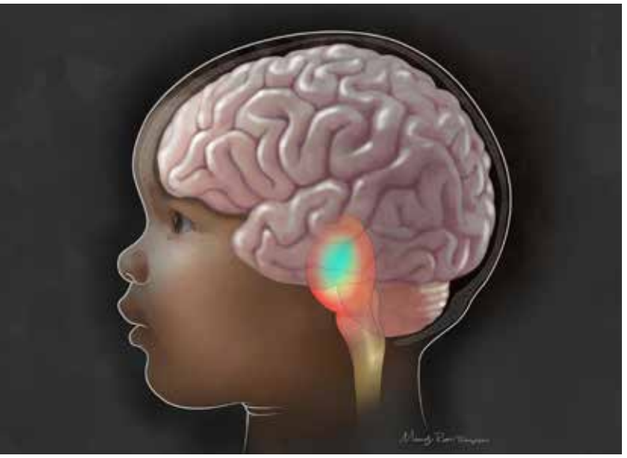
– Katherine J. Deans, MD, chief clinical research officer at Nationwide Children's Hospital

"The ability to begin enrolling patients so quickly is really a huge testament to the strengths of Nationwide Children's. All the committees that needed to sign off after the FDA cleared the IND were willing to work at lightning speeds to avoid delaying the start of a trial."

– Kathrin Meyer, PhD, principal investigator in the Center for Gene Therapy at Nationwide Children's Hospital

Exploring the RNA Cargo of Extracellular Vesicles in Malignant Pediatric Brain Tumors

Study provides novel insights into the potential roles of miRNAs in DIPG pathogenesis.



In the first study of its kind, Setty Magaña, MD, PhD, co-director of the Neuroimmunology Program at Nationwide Children's Hospital, and her research team isolated and characterized extracelluar vesicles (EVs) in medulloblastoma (MB) and diffuse infiltrative pontine glioma (DIPG). Dr. Magaña notes that their findings, recently published in the *Journal of Neuro-Oncology*, indicate that EVs are novel mediators of intracellular crosstalk and biomarkers in pediatric brain tumors.

The research team first cultured surgically resected tissue of each tumor type from pediatric patients to derive seven cell lines (4 MB cell lines, 3 DIPG cell lines). All but two cell lines were acquired in collaboration with David Daniels, MD, PhD, a pediatric neurosurgeon at the Mayo Clinic. Michelle Monje, MD, PhD, a pediatric neuro-oncologist at Stanford Medicine, provided the research team with autopsy samples for the remaining two DIPG cell lines.

Next, the team isolated and sequenced the RNA within the EVs and their parent cells, with a focus on microRNAs (miRNAs), a subtype of small noncoding RNAs (ncRNAs) that have garnered much interest as diagnostic and prognostic biomarkers in a variety of diseases. Several analyses were performed to compare miRNA between the EVs of MB and DIPG, as well as between the EVs and their parent cells.

Not unexpectedly, the composition of EV-associated miRNAs differed between MB and DIPG due to their distinct pathobiology. However, one of the most informative aspects of this study was the shared miRNAs between MB and DIPG.

"Much more is known about miRNAs in MB than DIPG. Therefore, by comparing the less well-characterized tumor (DIPG) to the more well-characterized tumor (MB), our study provides novel insights into the potential role of miRNAs in DIPG pathogenesis," says Dr. Magaña.

Notably, the miRNAs also differed between the parental cell of origin and the secreted EVs, indicating targeted, tumor-specific cargo loading of the EVs.

"Tumor cells use a selective process to determine which miRNAs get packed into the EVs," Dr. Magaña explains. "This allows them to metastasize and evade the immune system."

After comparing the miRNA cargo within the EVs, the research team predicted the gene targets of the miRNA and what cancer-related pathways those genes might be involved in. Some of the targets were involved in cancer pathways related to cell proliferation and cell-to-cell communication. Interestingly, the EVs and their parent cells differed in their gene targets, again supporting targeted loading of miRNAs into tumor EVs.

The EVs also contained Y RNA, another class of ncRNA. Dr. Magaña notes that their study was the first to implicate Y RNAs in pediatric brain tumor pathogenesis, further expanding the treatment and genetic landscape of these tumors and providing insight into brain tumor pathogenesis. Y RNAs, she explains, can help identify new potential therapeutic targets.

Magaña SM, Peterson TE, Evans JE, Decker PA, Simon V, Eckel-Passow JE, Daniels DJ, Parney IF. Pediatric brain tumor cell lines exhibit miRNA-depleted, Y RNA-enriched extracellular vesicles. *Journal of Neuro-Oncology*. 2022 Jan;156(2):269-279.

— JoAnna Pendergrass, DVM

How Does Allergic Disease Protect Against Developing Asthma After Viral Infection?

Neutrophils and IL-4 are critical in preventing post-viral airway disease in mice with pre-existing allergic disease.

In a new study, researchers from Nationwide Children's Hospital show that in a mouse model simulating human respiratory viral infection, pre-existing allergic disease prevents the development of asthma following viral infection. Further experiments revealed that this protection against post-viral asthma depends on neutrophils and the cytokine interleukin 4 (IL-4).

Although rates of asthma and other allergic disease have increased in much of the world, the mechanisms driving this increased prevalence are unclear. One area under investigation is the role respiratory viruses play in the development of asthma. For instance, infection with respiratory syncytial virus (RSV) in infancy is associated with an increased risk of asthma, primarily in those without any allergic disease at the time of infection.

To investigate how allergic disease could be protective against post-viral asthma, Mitchell Grayson, MD, chief of the Division of Allergy and Immunology at Nationwide Children's, and his team used a mouse model that mimics human infection with RSV. When these mice are infected with Sendai virus, they go on to develop post-viral airway disease. For the new study, Dr. Grayson and colleagues induced allergic disease in the mice before viral infection using house dust mite allergen.

"When we make the mice allergic, they do not go on to develop post-viral airway disease," says Dr. Grayson. "It suggests that making them allergic is actually protecting them from allergic disease following viral infection."

The researchers found that this protection depended upon neutrophils, as depletion of neutrophils at the time of infection restored the susceptibility of allergic mice to post-viral airway disease. Neutrophils appeared to take up viral particles through an IL-4-dependent process, leading to protection from post-viral airway disease in mice with pre-existing allergic disease.

In addition, Dr. Grayson and his team demonstrated that human neutrophils from donors with allergic disease were able to reduce RSV infection of human airway epithelial cells *in vitro*, suggesting these findings could be relevant in human patients. However, the researchers say that further studies are needed to firmly establish the protective link between pre-existing allergic disease and post-viral airway disease in humans.

Dr. Grayson, who is also professor of pediatrics at The Ohio State University College of Medicine, says that overall, the findings illustrate the significant overlap between allergic disease and antiviral immune responses.

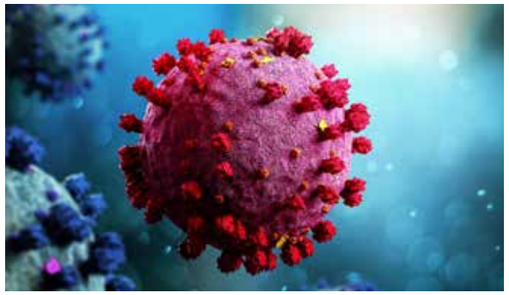
"A viral infection can drive the development of allergic disease, but the flip side is that allergic disease can prevent the development of post-viral airway disease," he says. "The study shows that allergic disease is not always bad. In fact, it suggests allergic disease might have evolved in part as a protective effect to respiratory viral infection."

Hussain SA, Rohlfing M, Resiliac J, Santoro J, Peeples ME, Garcin D, Grayson MH. Atopic neutrophils prevent postviral airway disease. *Journal of Immunology*. 2021;207(10):2589-2597.

— Mary Bates, PhD

Neurologic Impacts of COVID-19 in Kids

The pediatric arm of the GCS-NeuroCOVID study offers insights into varying neurologic effects of SARS-CoV-2 and MIS-C.



Several pathophysiologic mechanisms have been proposed to explain the impact of acute and post-infectious SARS-CoV-2 on the brain, including: direct viral invasion, vasoconstriction/occlusion of the macro and/or microvasculature, effects of treatment and immune system dysregulation. Neurologic manifestations have been frequently reported in the adult literature and have included headache, loss of smell and/or taste, seizure, weakness, meningitis, coma and even stroke. In fact, the adult Global Consortium Study of Neurologic Dysfunction in COVID-19 (GCS-NeuroCOVID) study noted an 82% incidence of neurologic manifestations. Strikingly, the presence of neurologic manifestations was associated with an increased risk of in-hospital death.

The recently published pediatric arm of the GCS-NeuroCOVID study provides insight into the neurologic manifestations of acute SARS-CoV-2 and the multisystem inflammatory syndrome (MIS-C) in hospitalized children. Forty-four percent of the 1,493 enrolled children at 30 sites presented with at least one neurologic sign or symptom. The most frequent manifestations included headache (20%), encephalopathy (16%) and seizure (8%). Children with acute SARS-CoV-2 had a slightly different pattern of neurologic involvement compared to children who developed MIS-C after clearing an acute infection. Children with acute infection had headache (16%), encephalopathy (15%) and seizures (8%) while children with MIS-C most frequently had headache (47%), encephalopathy (22%) and dizziness (12%). Throughout the entire cohort, the rates of severe complications such as meningitis/encephalitis (1.3%) and stroke (0.9%) were low. Although the overall mortality rate was low, 15 children died.

Children with MIS-C were more likely to have neurologic manifestations compared to those with acute SARS-CoV-2. Older children and those with pre-existing neurologic conditions had a higher rate of neurologic manifestations. Children often experienced neurologic symptoms before hospitalization. Importantly, children that presented with neurologic manifestations were more likely to require ICU-level care and had a longer hospital length of stay.

The incidence of neurologic manifestations reported in the GCS-NeuroCOVID study is higher than previously reported pediatric data. Taken together, the limited pediatric literature thus far reports severe, life-threatening neurologic complications in children. Though children have a lower rate of stroke, they have a higher rate of seizure than the adult population.

While we continue to learn about the acute effects of SARS-CoV-2 in children, we lack data on the long-term consequences on child health, including neurodevelopmental deficits, new comorbidities and health-related quality of life. Prior studies have shown that some children are being discharged home with new neurologic deficits, but it is unclear as to whether these deficits will progress or improve over time. Consortiums like the GCS-Neuro COVID group may help answer these questions as they follow a cohort of affected children after hospital discharge. With longitudinal data, researchers can begin to assess the true impact of SARS-CoV-2 on child health and help our community move past this pandemic.

Schober ME, Pavia AT, Bohnsack JF. Neurologic manifestations of COVID-19 in children: Emerging pathophysiologic insights. *Pediatric Critical Care Medicine*. 2021;22(7):655-661

Chou SH, Beghi E, Helbok R, et al. Global incidence of neurological manifestations among patients hospitalized with COVID-19-A report for the GCS-NeuroCOVID Consortium and the ENERGY Consortium. *JAMA Netwwork Open.* 2021;4(5):e2112131.

Fink EL, Robertson CL, Wainwright MS, et al. risk factors of neurologic manifestations in hospitalized children diagnosed with Acute SARS-CoV-2 or MIS-C. *Pediatric Neurology.* 2022;128:33-44.

LaRovere KL, Riggs BJ, Poussaint TY, et al. Neurologic involvement in children and adolescents hospitalized in the United States for COVID-19 or multisystem inflammatory syndrome. *JAMA Neurology* 2021;78(5):536-547.

— Marlina Lovett, MD

Biofilm Bacteria Protected by a Form of DNA Resistant to Current Treatments

A structural component of the bacterial biofilm matrix identified in a novel discovery has strong implications as to how biofilms resist current treatments.

Biofilms are communities of bacteria that are protected by an extracellular matrix that is rich in and requires DNA. When present in the body, biofilms act as the fortresses that shield bacteria from antibiotics and immune responses. While enzymes that digest DNA — for example, DNase and Pulmozyme® — can prevent biofilm formation, these same enzymes fail to disrupt biofilms that are mature despite the fact that extracellular DNA (eDNA) continues to accumulate within the biofilm over time.

This conundrum has baffled researchers in the biofilm field and clinicians who treat biofilm-mediated diseases such as the chronic, and ultimately fatal, pulmonary infections associated with cystic fibrosis. In these cases, treating with DNase has minimal efficacy.

ExeDNA is a key component of biofilm matrix structure. In a new study published in *Cell*, researchers from Nationwide Children's Center for Microbial Pathogenesis in the Abigail Wexner Research Institute present evidence that this abundant eDNA is not the typical well-known B-form of DNA, but is, instead, the rare Z-form of DNA.

Z-DNA forms a left-handed double helix, unlike the more common B-DNA, which forms a right-handed helix. Also, unlike B-DNA, a biological role for Z-DNA was not obvious.

Hallmarks of the Z-DNA structure include its left-handed double helix, zig-zag appearance, increased stiffness compared to B-DNA and its resistance to all known enzymes (nucleases) that degrade B-DNA. While Z-DNA is associated with gene regulation, innate immune sensing and inflammation, its appearance is uncommon and typically fleeting.

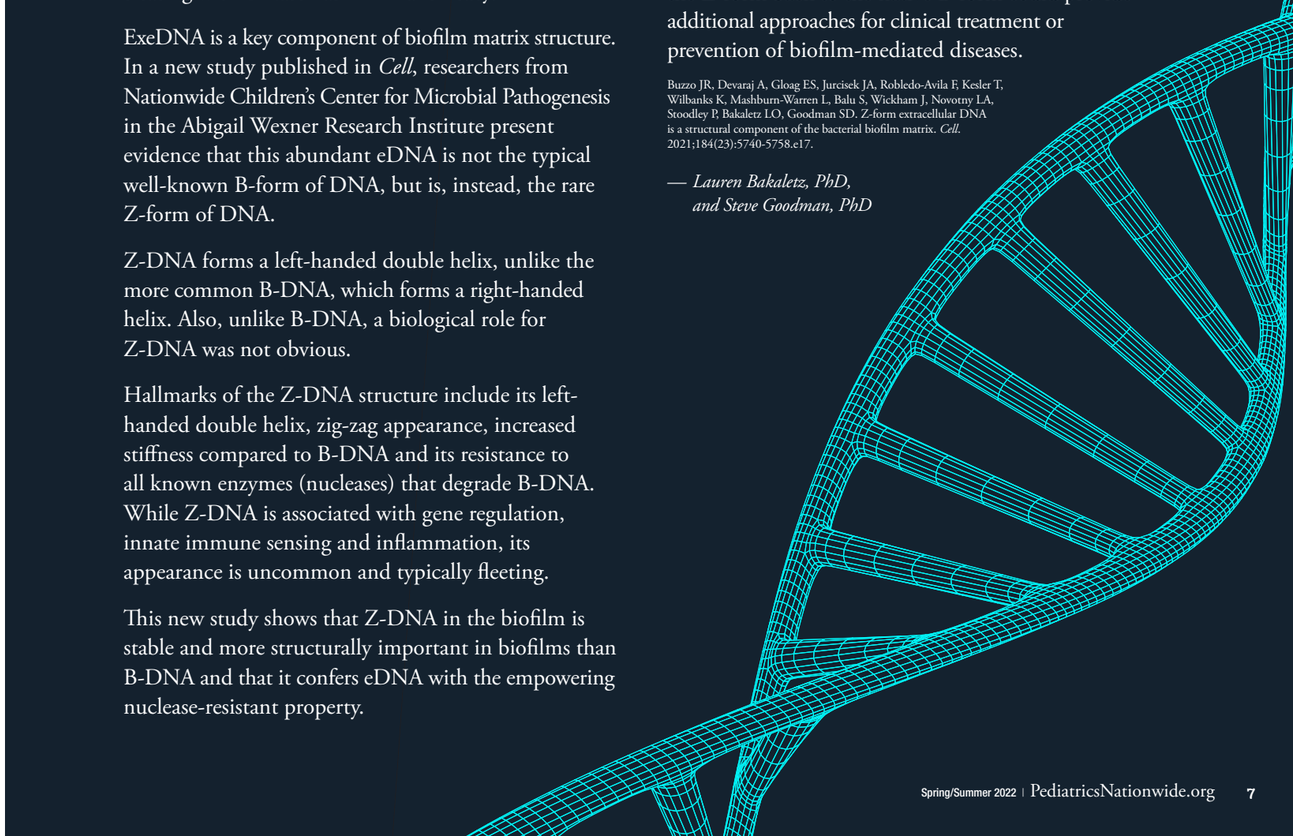
This new study shows that Z-DNA in the biofilm is stable and more structurally important in biofilms than B-DNA and that it confers eDNA with the empowering nuclease-resistant property.

The Z-DNA matrix protects the bacterial biofilm and acts offensively to inactivate the part of the immune system that restricts biofilm growth. This two-pronged effect has strong implications for treating biofilm infections and other autoimmune diseases such as systemic lupus erythematosus (Lupus), where the counter-effective immune response drives Z-DNA formation and thus biofilm growth.

Now, with an understanding of the role of Z-DNA in the eDNA matrix, diseases that were untreatable can be examined with new eyes. Z-DNA is not just part of the biofilm, it impinges on the immune system in a way that begins to tie a lot of loose ends together. Developing therapeutic agents to drive the biofilm eDNA from the Z-form back to the native B-form could provide additional approaches for clinical treatment or prevention of biofilm-mediated diseases.

Buzzo JR, Devaraj A, Gloag ES, Jurcisek JA, Robledo-Avila F, Kesler T, Wilbanks K, Mashburn-Warren L, Balu S, Wickham J, Novotny LA, Stoodley P, Bakaletz LO, Goodman SD. Z-form extracellular DNA is a structural component of the bacterial biofilm matrix. *Cell*. 2021;184(23):5740-5758.e17.

— Lauren Bakaletz, PhD, and Steve Goodman, PhD



Engineered Viruses Help the Immune System Target Cancer Cells

The seemingly unlikely "partnership" leads to strong antitumor responses in pediatric tumor models.

A recent study from researchers at Nationwide Children's Hospital, published in the *Journal for ImmunoTherapy of Cancer*, describes how a type of viroimmunotherapy activates the immune system to preferentially target tumor cells.

"Pediatric tumors typically lack neoantigens — new proteins that form due to mutations in the cancer cells' DNA because they have not undergone years of environmental damage like adult cells. They can, however, express shared antigens that help them grow," says senior author of the study Kevin Cassady, MD, a principal investigator for the Center for Childhood Cancer and Blood Diseases at the Abigail Wexner Research Institute at Nationwide Children's.

"We hypothesized that we could help the immune system 'see' tumor cells by creating a virus that expresses a shared antigen prevalent on tumor cells. With introduction of the antigen-expressing virus, we break the immune system's self-tolerance, and the shared antigen is now recognized as foreign by the immune system rather than as a self-antigen, allowing immune cells to develop a response against it," added Dr. Cassady.

To test this hypothesis, Dr. Cassady and his team used an oncolytic herpes simplex virus (oHSV), which is one of many virus-based platforms that has been safely used in clinical trials for a wide range of cancer types.

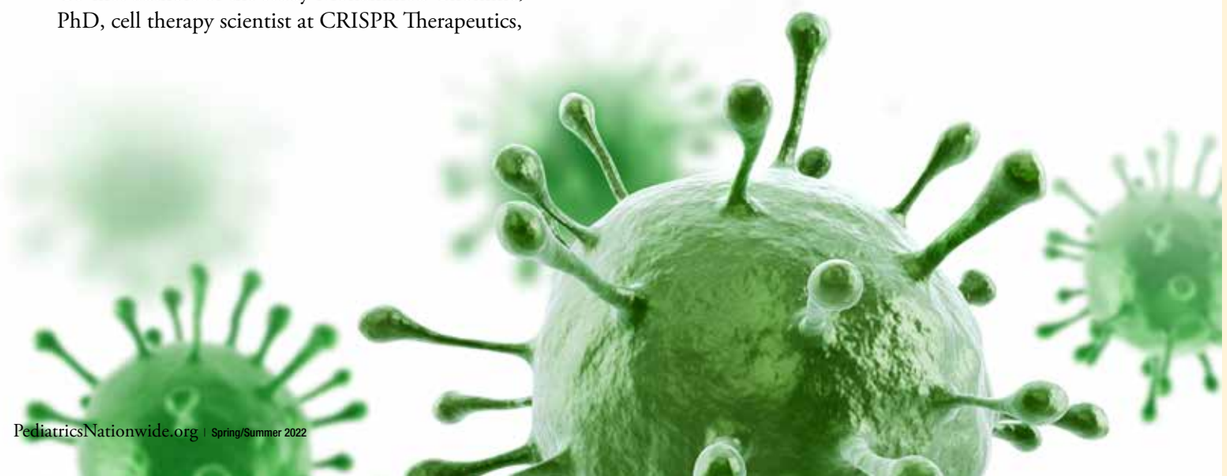
Co-first authors of the study Mohammed Ghonime, PhD, cell therapy scientist at CRISPR Therapeutics, and Uksha Saini, PhD, a research scientist in Dr. Cassady's lab, conducted experiments in two murine tumor models that express high levels of the antigen EphA2. They generated an oHSV encoding the EphA2 antigen and evaluated the immune-mediated antitumor response after mice with malignant brain tumors were treated with the engineered virus.

The team found that the EphA2-expressing oHSV induced antitumor activity and significantly improved survival of the mice relative to those treated with a control virus (no EphA2). Furthermore, when survivor mice given the EphA2-expressing oHSV were rechallenged with injection of tumor cells to their flanks, they displayed a memory response, resisting tumor growth. Dr. Saini conducted detailed experiments demonstrating that the viral treatment predominantly induced CD8 T cells that recognized the EphA2 antigen and eliminated the tumor cells.

"The immune system was highly activated with a strong antitumor response from a single dose of the virus," says Dr. Saini. "In addition to the brain tumors, we confirmed the findings in a nerve sheath tumor model, meaning this system could potentially be applied to other tumor types as well."

Ghonime MG, Saini U, Kelly MC, et al. Eliciting an immune-mediated antitumor response through oncolytic herpes simplex virus-based shared antigen expression in tumors resistant to viroimmunotherapy. *Journal for ImmunoTherapy of Cancer*. 2021;9(10):e002939.

— Lauren Dembeck, PhD



SARS-CoV-2 RNA in the Blood is Associated With Worse Outcomes in Kids

Researchers have identified a risk factor for more severe illness in children with COVID-19: detectable levels of SARS-CoV-2 RNA circulating in the blood.

Children with detectable SARS-CoV-2 RNA in the blood, called RNAemia, were more likely to require oxygen and be admitted to the intensive care unit than children with COVID-19 who did not have detectable RNAemia. This finding, based on a study of 103 children who tested positive for COVID-19, may help identify a subset of children at risk for severe disease.

"Detection of viral RNA in the blood is not common with other respiratory viruses, and when you find it — especially in patients with abnormal immune systems — it is associated with severe outcomes," says Asuncion Mejias, MD, PhD, principal investigator in the Center for Vaccines and Immunity at the Abigail Wexner Research Institute and an infectious disease clinician at Nationwide Children's Hospital.

"Extrapolating what we knew from other viruses and emerging findings in adults, we decided to assess how common SARS-CoV-2 RNAemia was in children hospitalized with COVID-19 and whether it was associated with worse clinical outcomes," says Octavio Ramilo, MD, chief of the Division of Infectious Diseases at Nationwide Children's, who co-led the study with Dr. Mejias.

Drs. Mejias, Ramilo and colleagues conducted the study using blood samples from a convenience sample of patients ages 20 and younger admitted to Nationwide Children's who tested positive for SARS-CoV-2 from March 2020 to May 2021.

SARS-CoV-2 RNAemia was detected in 26 patients who had symptomatic COVID-19, representing 33% of the symptomatic COVID-19 cohort (n=80); it was also detected in one child that presented with a ruptured appendix (representing 4% of the 23 patients identified by screening only). Patients with RNAemia had a shorter duration of symptoms at the time of study enrollment and were more likely to have higher viral loads in respiratory samples, fever and lymphopenia. They were also more likely to receive anti-COVID-19 therapies, oxygen and admission to the ICU.

Patients with RNAemia had slightly longer hospital stays and were younger (median age of 1.6 years vs 11.1 years) than non-RNAemia patients, but these differences did not reach statistical significance. Underlying diseases, race, ethnicity and sex were not associated with RNAemia or clinical outcomes.

"COVID can be so unpredictable in hospitalized children in the hospital," Dr. Mejias says. "This could be a tool to help us assess and classify our patients and the severity of their disease, with the opportunity to maybe implement earlier interventions."

The study is the largest examining the relationship between blood RNAemia and COVID-19 severity in pediatric patients as of press time. Further work is in progress with a much larger sample size to confirm the link and explore the potential mechanisms behind RNAemia and host response to SARS-CoV-2 infection.

Mertz C, Glowinski R, Cohen SH, Mertz S, Ye F, Hall MW, Peeples ME, King T, Wang H, Leber AL, Sanchez PJ, Ramilo O, Mejias A. SARS-CoV-2 RNAemia and clinical outcomes in children with COVID-19. *The Journal of infectious Diseases*. 2022;225(2):208-213.

— Katie Brind'Amour, PhD

When Every Week Matters:

ADVANCING A TREATMENT TO THE CLINIC

Researchers and regulatory experts bring a potential new therapy for a deadly neurodegenerative disease from IND application to clinical trial enrollment in a matter of weeks, setting a new standard in translating therapies from bench to bedside.

Written by Natalie Wilson

In October 2021, the Office of Research Regulatory Affairs (ORRA) at Nationwide Children's Hospital submitted an Investigational New Drug (IND) application to the U.S. Food and Drug Administration (FDA) for gene therapy AAV9.IGHMBP2.

Within the same month, the ORRA received official notice that a clinical trial could begin. The first patient was enrolled by November.

"The ability to begin enrolling patients so quickly is really a huge testament to the strengths of Nationwide Children's," says Kathrin Meyer, PhD, a principal investigator in the Center for Gene Therapy in the Abigail Wexner Research Institute (AWRI) at Nationwide Children's who led the preclinical work to develop the AAV9 gene therapy. "All the committees that needed to sign off after the FDA cleared the IND were willing to work at lightning speeds to avoid delaying the start of a trial."

This Phase 1 clinical trial, which is currently underway, is the first to study a treatment for disorders caused by mutations in the IGHMBP2 gene, marking another significant milestone in the history of translational research at Nationwide Children's.

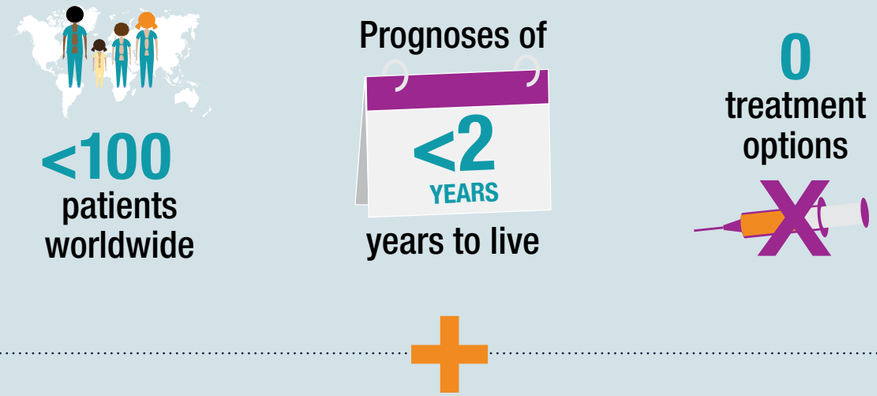
"We have a wonderful multifaceted team here that allows us to transition new experimental therapies to patients as safely and efficiently as possible," says Megan Waldrop, MD, a pediatric neurologist at Nationwide Children's and the principal investigator of the current trial.

The Difference Innovation Makes

The journey of a new therapeutic — from a discovery or concept to an FDA-approved, commercially-available treatment — is winding and fraught with challenges. The time between when an IND is submitted, when an institution receives notice from the FDA that it may proceed and when actual treatment of patients begins can stretch over months.

"These kinds of things are very complex. There are so many pieces that need to fall in place to keep the program moving forward," says Dr. Meyer.

> "We have a wonderful multifaceted team here that allows us to transition new experimental therapies to patients as safely and efficiently as possible."
>
> – Megan Waldrop, MD, pediatric neurologist at Nationwide Children's Hospital



Yet for a child with a neurodegenerative disease, waiting weeks to months might mean losing the ability to walk, stand, sit — or even breathe.

In the last seven years, the FDA has greenlit clinical trials for five investigational drugs that grew out of the research programs of Dr. Meyer and Brian Kaspar, PhD, who was a faculty member at Nationwide Children's for over 13 years, founded gene therapy startups during his tenure with Nationwide Children's, and made the fundamental discovery that an adeno-associated virus vector could cross the blood brain barrier when injected into the vascular system to deliver genes directly to motor neurons. None of these five trials were delayed by the review process.

"This shows how well-oiled our translational machinery is at Nationwide Children's," says Dr. Meyer. "These teams, and their commitment to quality, collaboration

> This IND was notably complicated and marks the largest and most thorough regulatory submission Nationwide Children's has sent to the FDA to date. I'm still impressed by the number and quality of preclinical studies performed to support it, the careful and detailed documentation of clinical protocols, and the rigor of this review."
>
> – Kevin Bosse, PhD, RAC, Director of Regulatory Affairs at Nationwide Children's Hospital

and constant improvements — and most of all to patients — are what kept things moving forward at this pace. People here understand there are patients behind the process, and it matters to those patients whether committees meet this week or next week."

To prepare to submit an IND application for AAV9.IGHMBP2, teams across Nationwide Children's, including researchers, regulatory staff, sponsored project officers, the Institutional Review Board (IRB) and Institutional Biosafety Committee (IBC), cores and shared resources, licensing team and legal services, as well as numerous external collaborators, were involved in a thorough and constant review process.

"This IND was notably complicated and marks the largest and most thorough regulatory submission Nationwide Children's has sent to the FDA to date," says Kevin Bosse, PhD, RAC, who leads the ORRA. "I'm still impressed by the number and quality of preclinical studies performed to support it, the careful and detailed documentation of clinical protocols, and the rigor of this review."

The Start of the Road

The road to commercial viability for AAV9.IGHMBP2 began in Italy, when Dr. Meyer's mentor, Dr. Kaspar, and the Corti Lab at Associazione Centro Dino Ferrari headed up a collaborative effort to develop an initial version of a gene therapy vector for treating IGHMBP2-related disease phenotypes — including spinal muscular atrophy with respiratory distress type 1 (SMARD1), a severe disease associated with deterioration of motor neurons of the spinal cord, and Charcot-Marie-Tooth disease type 2S (CMT2S), a milder, but still rare, disease that causes progressive weakness and sensory loss but is less often characterized by significant respiratory compromise early in life.

In 2018, inspired by Dr. Meyer's track record of translational neurodegenerative disease research and moving therapeutic programs towards clinical trials, a patient foundation, smashSMARD, approached her to pick the project back up.

"When smashSMARD visited to ask me to lead the preclinical development of a gene therapy to treat SMARD1, they told me stories of patients and showed me photos," says Dr. Meyer. "It was incredibly powerful; I obviously had to help."

Dr. Meyer and her team examined the initial gene therapy vector that had been developed and modified it to make it more efficient.

Same Gene, Different Disease: SMARD1 vs. CMT2S

As defined by the Orphan Drug Act of 1983, a rare disease is any condition that affects fewer than 200,000 people in the United States. While SMARD1 is the second most common form of spinal muscular atrophy (SMA) and the second most common motor neuron disease of infancy, it affects fewer than 100 patients worldwide. Its uncommonness, however, is of little comfort to patients and families impacted by its traumatizing and devastating pathology.

Within the first six months of life, infants diagnosed with the severe disease may be smaller, move less than expected and begin to show signs of breathing issues. If breathing stops, it can cause or be confused with sudden infant death syndrome (SIDS). Ultimately, infants diagnosed with this severe type of SMA may be permanently paralyzed and unable to survive without a ventilator. Most die before their second birthday if they do not have a feeding tube or breathing tube placed.

"Sadly, when a child gets a SMARD1 diagnosis, families are told by doctors who have never encountered anyone with the disease before, 'Go home and love your child, because there are literally no options out there,'" says Dr. Meyer. "When no disease-modifying treatment options exist, the impact a single gene therapy could have on these families is profound."

With an estimated prevalence of one in 2,500, Charcot-Marie-Tooth (CMT) disease is the most common inherited degenerative nerve disease. The milder phenotype caused by IGHMBP2 mutations represents only a small subset of CMT patients. These patients may have typical early development and may not be diagnosed until later in life when gait abnormalities or progressive weakness first appear.

When patients have mutations of the same gene, it can be unclear whether or why one will have milder disease than another. Some children with more severe disease have deletion mutations or mutations that more strongly reduce or even destroy protein function, but the correlation isn't always perfect. Even patients who are related can present with different clinical phenotypes. The team, and the FDA, were interested in learning whether a new gene therapy treatment would work across all disease severities.

By collaborating with Gregory Cox, PhD, associate professor at The Jackson Laboratory, who cloned the gene for neuromuscular degeneration in a mouse model for SMARD1, and with funding support from smashSMARD and smashSMARD Germany, the teams tested two versions of the vectors in three mouse models with varying disease progression — and they identified a clear lead. The gene therapy worked not only for SMARD1 but also for the milder disease, CMT2S.

All Hands on Deck From Bench to Bedside

Moving towards a trial required a coordinated effort across the Abigail Wexner Research Institute.

The ORRA team, including director Dr. Bosse and regulatory specialist Rachel Manthe-Gross, PhD, oversaw all pre-IND interaction with the FDA. Before the IND was submitted, the ORRA team and Robyn Cunningham, ASQ, CQA, CHRC, director of the Office of Research Compliance and Integrity at Nationwide Children's, performed an official review of the study reports to ensure all the necessary data and documentation were included in the IND in the correct format.

Early licensing and agreements support during preclinical research was essential. Jocelyn Eidahl, PhD, a senior licensing associate in the Office of Technology Commercialization, and Tabatha Simmons, PhD, director of the Gene Therapy Clinical Research Unit (GT-CRU) at Nationwide Children's, facilitated discussions and contract negotiations with both smashSMARD and Alcyone Therapeutics Inc., a gene therapy biotech startup that provided funding and non-monetary support of preclinical development including chemistry, manufacturing and control expertise and that is now funding the clinical trial. Dr. Simmons also oversaw budgeting, the manufacture of the clinical vector — the first one produced by Andelyn Biosciences, an affiliate of Nationwide Children's — and other aspects of clinical trial start up necessary to begin enrollment.

"This process involved essentially every component of the integrated system of bench-to-bedside research and regulatory support that has been developed at Nationwide Children's over many years," says Dennis Durbin, MD, MSCE, president of AWRI.

"These teams are incredibly dedicated and passionate about moving research forward into the clinic to help patients," says Dr. Meyer. "Together, as One Team, we have achieved an incredible milestone to tackle another disease with no other treatment options to date. We can change the lives of many children and families who are suffering."

> This process involved essentially every component of the integrated system of bench-to-bedside research and regulatory support that has been developed at Nationwide Children's over many years."
>
> – Dennis Durbin, MD, MSCE, President of the Abigail Wexner Research Institute at Nationwide Children's Hospital

Moving Emergency Medicine Research Forward

Utilizing exception from informed consent studies and a national network of emergency medicine experts, clinician-scientists are advancing research to uncover best practices and improve care.

Written by Abbie Roth

Exception from informed consent (EFIC) is a special rule set utilized by the U.S. Food and Drug Administration (FDA) to support research in emergency situations.

The word exception is particularly important when talking about EFIC studies.

"Often, people mistakenly say exempted from informed consent," says Manish Shah, MD, MS, chief of Academic Development and Strategy in the Division of Pediatric Emergency Medicine at Texas Children's Hospital. "But that is incorrect. While the treatment may be administered before informed consent can be obtained due to the critical needs of the patient, informed consent must still be obtained to use the data collected from the patient."

The exception to the informed consent is in the timing, not in the requirement of having informed consent. Researchers still have an obligation to notify research participants that they have been enrolled in a study at the earliest feasible opportunity, and they must still give them choices about ongoing participation once that notification has been made.

"We have clinical research coordinator coverage seven days a week for just this reason," says Julie Leonard, MD, MPH, prinicipal investigator in the Abigail Wexner Research Institute at Nationwide Children's Hospital and professor of Pediatrics at The Ohio State University College of Medicine. "We make sure our teams discuss the trial with the caregivers as soon as possible. If they decide to withdraw, we don't use their data. But usually, families are happy to be part of the trials."

Before an EFIC study, researchers must provide information about the study to the community and gather community feedback. This helps to increase awareness about the study before families even need emergency care. In some cases, investigators may offer an opt-out option for individuals who know ahead of time that they may likely need services and also know that they do not want to be part of the study. For most studies however, the decision to withdraw from ongoing participation is offered after care has been initiated.

Power in Numbers: Connecting Researchers Through Networks

Nationwide Children's currently has two emergency medicine EFIC studies open and enrolling. Both are multicenter studies running through the Pediatric Emergency Care Applied Research Network (PECARN).

PECARN is a federally funded multi-institutional network for research in pediatric emergency medicine. It operates through seven geographic nodes with sites in each node that represent academic, community, urban, general and children's hospitals, as well as emergency medical service (EMS).

"The tools, resources and opportunities for collaboration in PECARN have been instrumental in driving these large EFIC studies," says Dr. Leonard. "Nationwide Children's Hospital is the lead site for the Great Lakes EMS for Children Research Node of PECARN and has been a key collaborator in studies that have changed the emergency management of children with serious injuries and illnesses."

PediDOSE

An upcoming multicenter study takes EFIC out of the emergency department and into the field. The Pediatric Dose Optimization for Seizures in EMS (PediDOSE) trial is investigating the administration of medication by EMS to stop seizures.

Exception from informed consent (EFIC) is a special rule set utilized by the U.S. Food and Drug Administration (FDA) to support research in emergency situations.

EFIC CAN ONLY BE USED IF

- The person's life is at risk

  AND

- Available treatments are unproven or unknown and collection of valid scientific evidence is necessary to determine the safety and effectiveness of a therapy

  AND

- The person could benefit from the study

  AND

- It is not possible to get permission from the person because of the condition, or from the parent/guardian because of the short amount of time to treat the medical problem

  AND

- There is no reasonable way to identify prospectively individuals likely to become eligible for participation

  AND

- The clinical investigation could not practicably be carried out without the exception

> We know children are getting underdosed in these emergency situations. We want to find out if the protocol can change that."
>
> – Julie Leonard, MD, MPH, prinicipal investigator in the Abigail Wexner Research Institute at Nationwide Children's Hospital

"Far too often, a child is still seizing when they arrive at the emergency department. As many as 1 in 3 children come to the emergency department for seizures are still seizing when they arrive," says Dr. Shah, who is the leader of the national study.

How does this happen? Well, it all comes down to math. When EMS arrives at the scene, they first assess the child, check their airway and pulse, then administer medication to treat the seizure.

"We typically don't have another paramedic on the scene with us and are also supervising the basic emergency technician on the scene while they are checking vital signs. In addition, parents are very stressed if the seizure is a new onset," says Joseph Zarbaugh, paramedic and firefighter for the Columbus Division of Fire. "As the paramedic, I'm working to get the medication ready to administer."

The medication given by a paramedic is typically a benzodiazepine — often midazolam. According to emergency medicine physicians, midazolam administered via nasal spray or injection is preferred, but paramedics frequently give it intravenously. Additionally, different bottles may have different concentrations.

In order to find the correct dose, the EMS personnel must measure the seizing child with a tape to estimate weight, then use that weight to calculate the dose in milligrams, and then convert that dose to milliliters.

"Doing math in a high-stress situation is a challenge for caring for children having seizures at the scene," says Zarbaugh. "There are a lot of moving parts on the scene, and emergencies involving children are even more stressful."

Paramedics like Zarbaugh do these calculations in high-pressure and time-sensitive situations: while the child is having a seizure and the family members are present and upset and every second counts. In these circumstances, it's no wonder that some children receive incorrect doses. And if you're erring on the side of caution, you probably are going to underdose the benzodiazepine. Overdosing puts the heart and lungs at risk. Underdosing prolongs the risk of damage to the brain. It's a hard line to walk.

The idea behind the PediDOSE trial is to optimize the timing, the amount, and the method of giving the medication: It does this by emphasizing the use of a nasal spray or intramuscular injection only — no intravenous administration. The patient care protocol also lowers the priority of getting a blood glucose test until after the first dose of medication is administered. And finally, it eliminates the need for complex math. Children are given one of four age-based doses instead of a calculated weight-based dose.

"We know children are getting underdosed in these emergency situations," says Dr. Leonard, principal investigator for the Nationwide Children's study site. "We want to find out if the protocol can change that."

Notably, the age-based doses in the protocol are safe doses that are already used in various medical settings.

"Our team thought very carefully about the dose ranges," says Dr. Shah. "We are confident they are optimal based on standardized growth charts for children."

The PediDOSE trial will be launched in 20 cities across the United States, including Columbus, Ohio, where it will be rolled out as a collaboration between Nationwide Children's and Columbus Division of Fire. In each city, each participating EMS agency will first follow their current protocol, but will then switch to the new protocol at some point over the next years.

Researchers will then compare the data from all sites to see if more children arrive at the ED with their seizures stopped when treated under the new protocol versus the EMS agencies' current protocols.

Zarbaugh is optimistic that the trial will lead to improvements in emergency care for children.

"Anything that reduces resources and complexity in a high-stress environment is going to afford a better outcome on emergency calls," he says. "Caring for children in general is especially stressful for EMS. Anything that can simplify and streamline their care will be a positive outcome, and a welcome improvement for both the EMS and the children we treat."

PROMPT Bolus Study

Each year, 100,000 children arrive at emergency departments with severe sepsis. More than 2,500 of them die, 25 times more than the number of children who die from the flu each year.

Previous research has shown that quickly hydrating children who come to the ED with sepsis is critical to improving their outcomes. The two main choices for hydration are normal saline and lactated ringers.

Normal saline is just what it sounds like — a saltwater solution. Lactated ringers is similar to normal saline, but is considered a balanced crystalloid solution because it contains other electrolytes that more closely approximate those found in blood and tissues.

In recent years, adult studies have shown a slight but significant benefit of lactated ringers over normal saline, but to date, no evidence exists to show if this might be true for children.

"Both normal saline and lactated ringers are effective for hydrating pediatric patients with sepsis," says Julia Lloyd, MD, attending emergency medicine physician and site principal investigator at Natiownide Children's for the PROMPT Bolus study. "But is one safer? We don't know. Adult studies show clinical evidence for lactated ringers in adults, with a small but meaningful reduction in major adverse kidney events and hospital mortality. However, no trial has compared the effectiveness of different crystalloid fluid types for resuscitation in children with sepsis, and the two largest observational pediatric studies reported conflicting results."

The PROMPT Bolus (PRagMatic Pediatric Trial of Balanced vs nOrmaL saline flUid in Sepsis) study is designed as a randomized trial to compare the two options. The protocol, first developed as a pilot at Children's Hospital of Philadelphia and later published in the *Academy of Emergency Medicine*, has now been extended to a national study through PECARN.

Using EFIC, the pilot study had 85% enrollment of eligible patients; of those, 98% completed the study.

PROMPT Bolus will enroll children (age 6 months to 18 years) who come into the ED with suspected sepsis. These children will be randomly assigned to two groups: normal saline or lactated ringers. All other tests, medicines and care will be given according to the normal routines of the hospital.

"If a patient is coming in with sepsis, they're going to receive one of these two fluids anyway," says Dr. Lloyd. "The difference with the study is that an envelope will tell us which to give and enable us to collect the data needed to find out if one is better than the other or if they really are equally effective."

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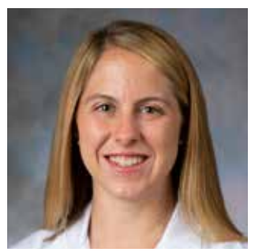


Optimizing the Body's

NATURAL CANCER KILLERS

Recent advances in the expansion and production of natural killer cells offers pediatric patients new hope for remission after high-risk cancer diagnoses.

Written by Katie Brind'Amour, PhD

Natural killer (NK) cells are the innate immune system's first line of defense for viral infections. Although these white blood cells don't have the antigen-specific "memory" that characterizes T cells or the antibody-producing capacity characteristic of B cells, they act faster than T or B cells because they do not require priming — prior activation or exposure to a pathogen or tumor cell — in order to recognize and attack problematic cells.

Instead, NK cells operate on constant patrol, bumping into other cells and checking for signs of cancer, infection or metabolic stress via receptors on their surface. Certain signals and molecules from potentially dangerous cells activate NK cell receptors and trigger a full-scale assault: the NK cell releases toxic granules that infiltrate the target cell and cause cell death.

In healthy individuals, NK cells — and the army of immune cells they recruit for assistance — keep most infections and potential tumors at bay. But there are important limitations.

Sometimes viral replication is too fast for the NK cells to manage alone, and an infection takes root until the rest of the system kicks into gear, or a tumor may develop mechanisms of hiding from immune cells. While medicine can help with some of these circumstances, in others, we have few treatment options at our disposal to achieve what our bodies cannot.

Some treatments for high-risk pediatric cancers, such as acute myeloid leukemia (AML), cause damage to the innate immune system; these include chemotherapy and radiation, which kill cells of the innate immune

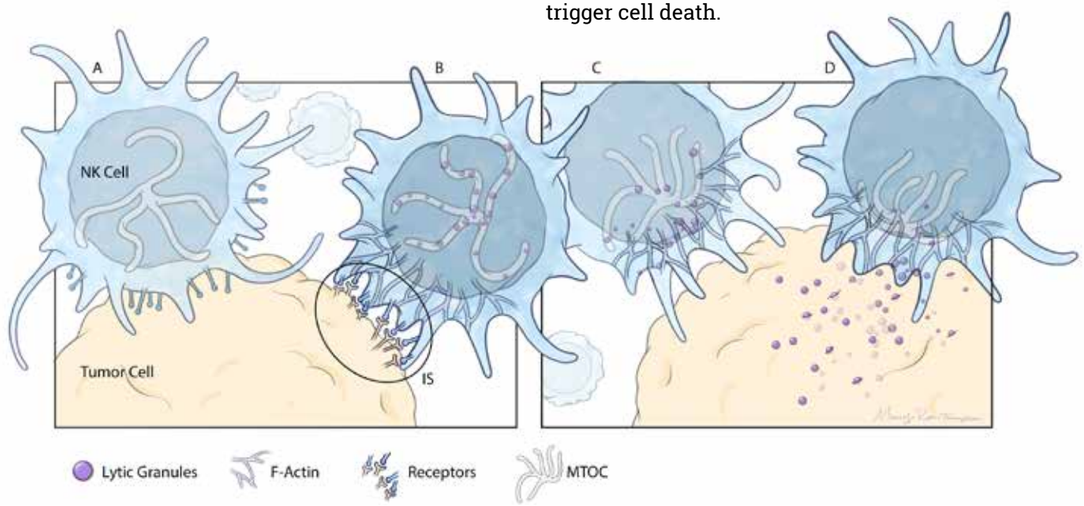
HOW THE NK CELLS WORK

A: NK cells are in constant circulation in the bloodstream, bumping into other cells in an attempt to detect invaders, such as viral or tumor cells.

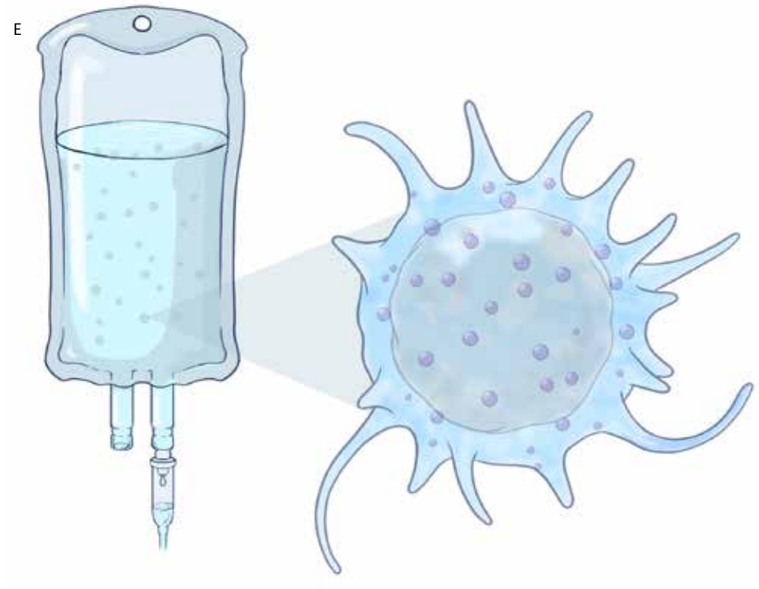
B: When they collide with invaders, receptors on the NK cell surface identify dangerous proteins or antigens produced by the foreign cells.

C: These antigens trigger an attack from the NK cell. It activates F-actin , a group of bundled, polarized polymers that stabilize the NK cell's attachment to the foreign cell's membrane.

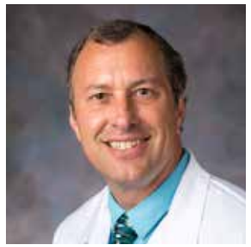
D: The NK cell releases lytic granules from its microtubule-organizing center (MTOC). These killer granules pass into the foreign cell and trigger cell death.



E: Natural killer cells can be extracted from donors, expanded in the lab, and infused directly into patients.



> We found a combination that worked very well to consistently grow cells from any donor, as well as from monkeys, dogs, cord blood, stem cells and in many other settings. And 14 years later, it's still the best way we know of to grow NK cells."
>
> – Dean A. Lee, MD, PhD, director of the Cellular Therapy and Cancer Immunology Program at Nationwide Children's Hospital Hospital



system and temporarily stop NK cells from continuing their valuable work. Furthermore, a potentially curative treatment for leukemia patients, hematopoietic stem cell transplant (HSCT), requires immune suppression for several months.

Outcomes for these already high-risk patients take a nose-dive when post-transplant infections hit before the immune system has recovered (most often in the first 6 months after the procedure). Without a functional stock of NK cells circulating to fight off invaders, many patients fall prey to common illnesses. Worse still, the lack of NK cells results in an immune system unable to detect early reemergence of leukemia cells, resulting in a cancer relapse.

To address the immune suppression caused by these cancer therapies, many scientists have been methodically cultivating the concept of lab-made or cultured immune cells. From NK cells to CAR T cells (chimeric antigen receptor T cells, which are genetically altered to fight specific cancer antigens), innovations in immune cell research are poised to revolutionize the field of immunotherapy.

MAKING MAGIC FROM DONOR NK CELLS

Unfortunately, NK cells have proven difficult to select from blood donations, and the relatively small quantities obtained naturally may not be enough for effective therapies. Furthermore, there appears to be natural variation in the quality of our NK cells, and they may not last long enough for a single infusion to do much good. Multiple doses may be required to maximize protective potential after chemotherapy or stem cell transplant. Traditionally, to make this work, a donor would have to repeatedly give blood or undergo a longer apheresis procedure to collect enough starting material.

"I have been studying the biology of NK cells in the lab and their clinical use as immunotherapy for well over a decade and have been really amazed by the sophisticated power of these immune cells," says Monica Thakar, MD, director of Bone Marrow Transplantation Inpatient Services at Seattle Children's Hospital and an associate professor of pediatrics at Fred Hutchinson Cancer Research Center and University of Washington.

Her two initial NK cell clinical trials in children with high-risk cancers infused haploidentical, or "half-matched," NK cells from family members to prevent post-HSCT cancer relapse. NK cells were isolated from the donors' peripheral blood obtained via apheresis, a lengthy and technically complicated procedure. The purified NK cells were then directly infused into patients the same day of collection, as a fresh cell therapy product.

"I realized that maybe we needed a lot more NK cells to go in, or that we needed to do something to the cells so that they persisted or were more activated than just our single fresh infusion," says Dr. Thakar. "We were making an impact, but we kept hitting limitations. That's where Dean and I connected."

Dean Lee, MD, PhD, a bone marrow transplant specialist and director of the Cellular Therapy and Cancer Immunology Program at Nationwide Children's Hospital, joined the world of NK research by chance. Early in his career, while working on methods to expand T cells using genetically engineered feeder cells, he observed that about 10-20% of the time, dramatic NK cell overgrowth occurred instead. Encouraged by his advisor at the time, he investigated whether he could reliably direct the growth of NK cells from donor blood using the feeder cells and various combinations of cytokines and cell stimulants.

> Right now, we don't have a lot of therapies to help prevent relapse in AML, and even with all of the best treatments, only 60% of children remain well. In Dean's adult AML NK cell data, relapse rates fell from around 40% down to 4%. That's a big drop. We know NK cell infusions are safe and nontoxic, so we really want to see if we can get the same improvement in AML recurrence in kids."
>
> – Hemalatha Rangarajan, MD, a clinician-scientist on the Hematology, Oncology & Blood and Marrow Transplant team at Nationwide Children's Hospital

"We found a combination that worked very well to consistently grow cells from any donor, as well as from monkeys, dogs, cord blood, stem cells and in many other settings," says Dr. Lee, who joined Nationwide Children's in 2016 and is the DiMarco Family Endowed Chair in Cell Based Therapy as well as a professor of pediatrics at The Ohio State University College of Medicine. "And 14 years later, it's still the best way we know of to grow NK cells."

The method, which encourages cell expansion using membrane-bound interleukin-21, produced cell quantities many times greater than other approaches. Dr. Lee's approach enabled comparatively rapid production of large batches of NK cells from a single donation — enough for about six NK cell-abundant infusions. This improvement, detailed in *PLoS One* in 2012, catapulted NK cells into the realm of feasible clinical development.

A handful of companies began to generate NK cells as commercial products using the method, and while there is ongoing scientific debate as to the ideal type or quality of NK cell, no other method grows more. The change in production capacity also made it possible for clinical trials to incorporate NK cell infusion regimens, and the first adult trials using Dr. Lee's methods launched in 2013.

LIGHTING A FIRE UNDER NK CELL IMMUNOTHERAPY

NK infusions are of particular interest in AML because the cancer cells express proteins that attract NK cells and activate their receptors to promote killing. There are also studies showing that patients with the largest number of circulating NK cells post-HSCT have the best outcomes, so that's where Dr. Lee started. He and his team initiated their AML NK cell studies in adults in Brazil and in Texas, where Dr. Lee previously served as section chief of Pediatric Cellular Therapy at the University of Texas M.D. Anderson Cancer Center.

"Right now, we don't have a lot of therapies to help prevent relapse in AML, and even with all of the best treatments, only 60% of children remain well," says Hemalatha Rangarajan, MD, a clinician-scientist on the Hematology, Oncology & Blood and Marrow Transplant team at Nationwide Children's. "In Dean's adult AML NK cell data, relapse rates fell from around 40% down to 4%. That's a big drop. We know NK cell infusions are safe and nontoxic, so we really want to see if we can get the same improvement in AML recurrence in kids."

Dr. Lee and Dr. Rangarajan brought the idea to the Pediatric Transplant and Cellular Therapy Consortium (PTCTC) to find partner sites for initiating the trial, and quickly heard from a dozen institutions interested in and capable of conducting such a trial. Study Vice-Chair Dr. Rangarajan and Study Chair Dr. Thakar wrote the protocol in partnership with the regulatory team at the Children's Hospital of Los Angeles and the PTCTC. This first-in-world pediatric-focused protocol infusing Dr. Lee's expanded NK cells — manufactured on site at Nationwide Children's in the Good Manufacturing Practices (GMP) Cell-Based Therapy Core — after allogenic transplantation, dosed the first patient in late 2021.

This Phase 2 Expanded NK Cells study, called the EXCEL Trial (NCT04836390), expects to enroll about 30 patients with AML across 13 institutions. Each child will receive multiple NK cell infusions post-HSCT in an attempt to stave off relapse, graft-vs-host disease and post-transplant infections.

"This trial is very exciting because of the possibility

> By infusing them at the time of immunosuppression, we're hoping the NK cells will be able to bridge the gap between engraftment and development of a more functional immune system in the patient."
>
> – Challice Bonifant, MD, PhD, a pediatric oncologist on the blood and marrow transplantation team at the Sidney Kimmel Comprehensive Cancer Center

to track the NK cells and see how long they might persist," says Challice Bonifant, MD, PhD, a pediatric oncologist on the blood and marrow transplantation team at the Sidney Kimmel Comprehensive Cancer Center and an assistant professor of Pediatric Oncology at the Johns Hopkins University School of Medicine. Dr. Bonifant uses Dr. Lee's NK cell expansion method for her own work studying modified NK cells; her latest efforts use CAR-NK cells to prevent SARS-CoV-2 from binding to its target cells, published in *Frontiers in Immunology*.

"By infusing them at the time of immunosuppression," Dr. Bonifant says, "we're hoping the NK cells will be able to bridge the gap between engraftment and development of a more functional immune system in the patient."

Using the rapid expansion method, post-transplant infusions can provide large doses of NK cells, and re-infusion can be done quickly and easily if the child continues to show no native NK cell production.

One mechanism of evasion that AML uses post-HSCT transplant is downregulation of HLA (human leukocyte antigen) molecules, which are necessary for T-cells to bind and kill leukemic blasts. This downregulation hurts T-cell response but actually lowers NK cell inhibition toward attacking potentially dangerous cells, possibly making them even more potent against invaders than they normally would be. AML cells themselves also express NK cell-activating ligands.

"This disease is a great target for NK cells in their natural state," says Dr. Bonifant.

While the multi-institutional research team is enthused about the potential this current trial holds, they're already looking forward to another possibility that could transform care to a much greater degree: off-the-shelf NK cell products available for infusion at any time using optimized, universal donor cells.

THE NEXT PHASE IN NK CELL RESEARCH

"The one drawback with the current trial is that Dr. Lee is manufacturing these infusions from donor blood, so it still takes a couple of weeks to expand the cells," says Dr. Thakar, who is developing her own NK cell program at Fred Hutchinson Cancer Research Center. "It would be so much easier to have an 'off-the-shelf' product that is ready to thaw and infuse on demand."

Dr. Lee agrees. He learned from his AML studies in adults that waiting for family donors and patient-by-patient cell expansion can take too long, allowing some patients to fall ill by the time the cells are ready to infuse.

That's why he's been working to tease out what it is about some donors' cells that makes for a highly expandable, potent, efficient response to cancer and infection, so that he can identify "super donors" from cell banks and generate large quantities of NK cells using their donations. These cells can then be cryopreserved, banked and kept available for patients whenever needed. This could make or break treatment success in very ill patients who require an urgent immune boost. It also enables the use of NK cells for post-chemotherapy infusions and post-transplant infusions without any added burden on a family donor.

"For patients with AML or other rapidly proliferating diseases, one challenge in immune cell therapies is that, because they require autologous products, there is a lag in time while we manage cell collection and then cell expansion *ex vivo*," says Dr. Bonifant. "The opportunity to have cells immediately available from either the transplant or other allogeneic donor would decrease costs. More importantly, it would decrease the time between identifying a patient in need and getting them the cell therapy product. It's like a dream for this population."

> In a perfect world, we could think about injecting them directly into solid tumors to get past the physical barriers impeding their infiltration, or maybe combining NK cells with different classes of drugs to overcome immunologic barriers of the tumor micro-environment. We have lots of work to do to see what's possible, and we will be working hard to establish safety and explore other avenues for pediatric cancer, including treating active relapse."
>
> – Monica Thakar, MD, director of Bone Marrow Transplantation inpatient Services at Seattle Children's Hospital

Dr. Lee's work toward this aim is almost ready for prime time. His team has already identified a handful of optimal donors that have the "best" anti-cancer NK phenotype from a national registry and begun the process of expanding cells; multiple pediatric post-chemotherapy infusion trials using the universal NK cell donor product will launch this year.

AML IS JUST THE BEGINNING

AML was an excellent first target for pediatric NK cell-focused research, but the utility of NK cells may extend far beyond AML.

"There is potential for NK cell therapy in every kind of cancer," says Dr. Lee. "We haven't found any cancer yet that is uniformly resistant to NK cells — some patients may have resistant tumors, but there's promise in any cancer type. Moving forward, we can test whether giving large numbers of high-functioning NK cells will do something to whatever cancer you want to try."

Dr. Lee's team has already done studies in brain tumors and neuroblastoma, and together with The Ohio State University, they are set to open new studies for sarcomas, melanoma, breast cancer, T-cell lymphomas and skin lymphoma, and additional studies for AML, neuroblastoma and brain tumors. And the EXCEL study investigators feel this is just the beginning.

"In a perfect world, we could think about injecting them directly into solid tumors to get past the physical barriers impeding their infiltration, or maybe combining NK cells with different classes of drugs to overcome immunologic barriers of the tumor microenvironment," says Dr. Thakar. NK cell therapies developed using other donor and expansion modalities have already been tried in high-risk solid tumors with modest success. "We have lots of work to do to see what's possible, and we will be working hard to establish safety and explore other avenues for pediatric cancer, including treating active relapse."

These experts are not the only ones to recognize the potential of this technology. NK cell therapies utilizing Dr. Lee's expansion method are currently under study

> We're really excited about continuing our work with NK modification to see what can help children the most. We hope to have the first CAR-NK cell in clinical trials by the end of this year, with many more to come."
>
> – Margaret Lamb, MD, pediatric oncologist and principal investigator at Nationwide Children's Hospital

by a handful of pharmaceutical companies in adults with leukemias, neuroblastomas and more. In addition, investigators around the world are working to generate NK cells from induced pluripotent stem cells, exploring alternative ways to expand and infuse them or, like Drs. Lee, Thakar, Bonifant and their colleagues, exploring ways to alter NK cells to target specific cancers by creating CAR-NK cells.

"Can we overcome suppressive factors in the tumor microenvironment and redirect NK cells to identify specific tumors?" Dr. Lee asks. "Can we combine NK cells with antibodies or other immune-modulating drugs to improve survival and function? That's what is coming in the next NK innovation wave."

To that end, the CRISPR/Gene Editing Core staff at Nationwide Children's and The Ohio State University, led by Meisam Naeimi Kararoudi, DVM, PhD, has been working on CAR-NK cells for the past five years. Their work with Margaret Lamb, MD, a pediatric oncologist and HSCT specialist at Nationwide Children's and lead investigator for the upcoming universal NK cell AML trial, has allowed the team to develop new CAR-NK cell study protocols as well.

"We're really excited about continuing our work with NK modification to see what can help children the most," says Dr. Lamb.

The NK team at Nationwide Children's has plans for growth for the cell production capacities both of donor-specific NK and CAR-NK cell lines and universal cell batches. The GMP facility will be able to support numerous multi-center trials simultaneously, especially if the universal donor concept proves successful.

"We hope to have the first CAR-NK cell clinical trials by the end of this year," Dr. Lamb says, "with many more to come."

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Making (Lots of) NK Cells

When Dr. Lee joined Nationwide Children's and The Ohio State University Comprehensive Cancer Center in 2016, the institutions gave him leadership of a joint program: the Cellular Therapy and Cancer Immunology Program (CTCI), which serves to develop research efforts into clinical care for both pediatric and adult patients.

The CTCI team has worked hard to develop techniques to generate universal NK cell-based therapies and the ability to manufacture a wide range of other immunotherapies. They currently produce the NK cells used in their multi-site AML study and will also supply a host of other NK cell studies slated to open in nearly a dozen indications once enough cells are processed for their one-of-a-kind bank.

Manufacturing was initially established at The Ohio State University to support clinical trials at both institutions. Since the development of a dedicated Good Manufacturing Practice (GMP) facility for cellular therapy at Nationwide Children's, known as the Cell-Based Therapy (CBT) Core, the group has begun transitioning manufacturing for most of the patient-specific and universal-donor NK cell pediatric studies to the children's hospital.

"Having the ability to make NK cell products right here gives us rapid access for clinical studies and ensures we have oversight of the innovative processes we have underway," says Dr. Lamb, who has written multiple universal-donor NK cell protocols that are FDA-approved and pending launch. The team expects to open these studies at multiple sites and ship the product to participating institutions as patients enroll. "The biggest thing is the time it saves for patients who need treatment right away — making these ourselves means we can get cells straight to patients without waiting for the donation and expansion process."

The CRISPR/Gene Editing Core at Nationwide Children's, also in collaboration with The Ohio State University, is led by Dr. Naeimi Kararoudi, who works with Dr. Lee and his team to drive forward multiple NK cell gene modification (knock-out and/or CAR-NK) efforts. In addition to NK cells, other cell products are also currently under study.

4
national cancer consortia funding CTCI NK cell research

4-6
weeks faster treatment using off-the-shelf NK cells

20×
as many cells as old NK cell expansion methods

50-100
patients treated per universal-donor collection

8+
cancer protocols awaiting universal-donor NK cell supply

12+
patents filed for the team's NK cell techniques

4+
pharma companies using the team's NK cell technology

Improving Racial Diversity and Equity in Clinical Trials

There is now broad consensus across medicine that clinical trials must be more representative of minority populations. How can that be achieved?

Written by Jeb Phillips

Last year, a group of Nationwide Children's Hospital neonatologists published an unusually pointed critique of racial and ethnic representation in neonatal clinical trials in the *Journal of Perinatology*.

They wrote that the lack of urgency in addressing crises such as the inequities in health outcomes for Black birthing people and infants characterized "an insidious apathy among researchers and clinicians that spans centuries in this country." They called out how the "inability to acknowledge and account for how structural racism affects social determinants of health directly impedes study design, recruitment, analysis and interpretation."

"I am frustrated with my field," says lead author and neonatologist Valencia Walker, MD, who also serves as associate chief diversity and health equity officer at Nationwide Children's.

Dr. Walker is not the only one, and neonatology is not the only field. This wasn't even the only publication of its kind that came from Nationwide Children's authors over the last year. At least three other commentaries addressed increasing racial and ethnic diversity in pharmaceutical trials, critical care research and the human genome reference.

While there have been criticisms for decades about inequitable representation in clinical trials, the last two years have seen an explosion of them across medicine. They have come from academic health care organizations, research funders, policy makers and third-party analysts such as the Milken Institute's FasterCures initiative.

They are not just critiques, however. They are calls to action, and in many cases, they offer roadmaps to improvement.

A HISTORICAL, AND CURRENT, PROBLEM

The reasons for the lack of ethnic and racial diversity in United States' clinical trials are in some ways completely obvious to people who have a basic grasp of this country's history — and in others, so complex and nuanced that they have been difficult to fully explain.

But there are two core historical points that recent analyses make plain:

1. The American medical system has mistreated people of color for centuries.
2. As a result, many people of color have mistrusted the American medical system for centuries.

These two points come together most infamously in what was called the "Tuskegee Study of Untreated Syphilis in the Negro Male." This clinical trial was approved and led by the United States Public Health Service, which sent researchers to work at the Tuskegee Institute in Alabama (now Tuskegee University). Researchers "enticed" hundreds of Black men with syphilis to enroll in the study with incentives that would not normally be available to them, including regular health care, according to an account of the study from Tuskegee's National Center for Bioethics in Research and Health Care. They were told they would be treated for "bad blood."

The study began in 1932. Even though penicillin had become the standard treatment for syphilis by 1947, that treatment was withheld from the study participants. Dozens of the men died from their infections. Many of their family members also contracted syphilis, including babies born with congenital syphilis infections. The study only ended in 1972, when it was exposed in news articles, after so many were infected and suffered needlessly.

In short, a group of people already experiencing marginalization and racism were convinced to participate in medical research with lies, and the harm they experienced was one of the goals of the research. Given this and other medical-historical traumas "it would be illogical and irrational not to approach providers and health care entities with some level of skepticism," wrote researchers from Vanderbilt University in a widely-cited publication on equity and trust in medical research.

"That's all completely true," says Dr. Walker. It also doesn't tell the whole story.

"People always bring up Tuskegee," she says. "But there were people mistreated when they went to the hospital *today*. People don't want to confront that quite as much, because we believe we're good people now. What we need to do is divorce the often false individual moral and character accusations from the actions we need to make for structural and behavioral changes. This also requires us to look for our blinds spots when it comes to these issues."

There are many measures of just how skewed racial and ethnic representations still are in clinical trials; an FDA summary of drug trials from 2015 through 2019 showed that 76% of participants were White, for example, while approximately 58% of the overall U.S. population identifies as White.

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>
> – Valencia Walker, MD, neonatologist and associate chief diversity and health equity officer at Nationwide Children's Hospital

> We have to better empower families to take part in research. We have to think about people, process and environment. We're doing this work now because we want to continue to live our mission. We want to better live our mission. If we want to improve the health of all children, we have to make sure all children are represented in our research."
>
> – Katherine J. Deans, MD, chief clinical research officer at Nationwide Children's Hospital

Those statistics, though, are just the start, says Yasmeen Long, MA, director of the FasterCures center at the Milken Institute. She and her colleague Esther Krofah, MPP, executive director of FasterCures and the Center for Public Health, are authors of the report "Achieving Health Equity: An Action Plan to Address Diversity Across Clinical Trials and Biomedical Research," released in November 2021.

"Participants in clinical trials, regardless of their age, rarely represent the actual patients and populations bearing the burdens of the disease under study," she says.

That's always been the case, but rarely has it been clearer, or received more attention, than during the COVID-19 pandemic.

THE RECENT ATTENTION

Through the first year of the COVID-19 pandemic in the United States, Black people, Hispanic/Latinx people and American Indian and Alaska Native (AIAN) people had disproportionately high mortality rates compared to White people. The disparity in outcomes between minority populations and White people has decreased over time, but a February 2022 age-adjusted analysis from the Kaiser Family Foundation showed that Black, Hispanic and AIAN people were still about twice as likely to die from COVID-19 as White people.

The disparities were alarming and resulted in calls, including from Anthony Fauci, MD, director of the National Institute for Allergy and Infectious Diseases, for the pharmaceutical industry to overrepresent people of color in COVID-19 vaccine trials.

Pfizer and Moderna communicated about their efforts to recruit more people of color; Moderna actually slowed its trial in adults to do a better job of minority recruitment. While never coming close to Dr. Fauci's goal, the trials did better represent minority populations than many previous ones.

Both the comparatively poor COVID-19 outcomes among minority populations, and the clear lesson that focusing on current interactions and structures within the medical trials systems could improve representation, combined to draw greater attention to the issue.

"The COVID-19 pandemic brought health inequity into starker focus, and it's clearer than ever that when trials are appropriately diverse, they can improve the health of the broader population, including urban and rural populations and populations who have limited use of technology," says Lee.

The problems reach beyond history into 2022. The sometimes-under-resourced hospitals where people of color are more likely to receive care have small numbers of clinical trials. It can be difficult to travel, or get time off work, to reach to the relatively few academic medical centers that host clinical trials. Some research suggests that providers aren't as likely to discuss clinical trials with people of color. Clinical trial recruitment efforts may not account for health literacy, the need for translated materials or other cultural issues.

Many of the recent calls-to-action hope to address all of these issues and more.

THE WAY FORWARD

As nearly everyone will admit, it's not going to be easy.

"We're so guilty of wanting to come in with one sweeping change and say, 'we fixed it,'" says Dr. Walker. "We didn't get to this point in one year or three years or five years, but that's how we're trained to deal with things, because that's how our grant system works. It's how our political system works. If we are trying to change generations, centuries, of mistreatment, it doesn't happen in a year."

The FasterCures report proposes changes in community engagement, funding, data collection, workforce diversity and accountability across five huge stakeholder groups: federal and regulatory agencies, academic and research institutions, the biopharmaceutical industry, nonprofit and private research funders and clinical trials research sites.

Some of what FasterCures suggests is already happening, in fits and starts. For example, in February, a bipartisan group of members of Congress introduced the Diverse and Equitable Participation in Clinical Trials (DEPICT) Act. Among other provisions, it includes a requirement of "Diversity Action Plans" from Investigational New Drug and Investigational Device Exemption applicants that lay out engagement and outreach strategies, and it would provide grant funding to community health centers to increase their capacity to participate in clinical trials and research.

Pediatric academic medical centers have a particular responsibility to ensure equity in trials, and places such as Nationwide Children's already have many of the pieces in place to accelerate those efforts, says Katherine J. Deans, MD, chief clinical research officer at Nationwide Children's. The hospital's newest strategic plan is, in part, an effort to do just that.

"Integrating clinical research more deeply and broadly into clinical care is at the very heart of our new strategic plan. This is, in part, because we know that better integration will promote more inclusive access to research participation for families. By eliminating some of the barriers for underrepresented populations to participate in research, we are ensuring that the results of our research are more generalizable to these populations," Dr. Deans says. "Bringing research to the point of care allows families to hear about opportunities from the doctors and nurses that they trust. It also means that starting with our schedulers and our staff at registration desks, we are thinking through how to connect families with research that matters to them."

In addition, Dr. Deans says, if Nationwide Children's and other hospitals are serious about better including underrepresented people, research studies need to be planned around their concerns, their needs and their time constraints.

"We have to better empower families to take part in research — not just participating in it, but also having a voice in what we study and how we study it," Dr. Deans says. "We need to remember that we're doing this work now because we want to continue to live our mission. If we want to improve the health of all children, we have to make sure all children are represented in our research."

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First Multicenter, Prospective Study of Short-Term Outcomes in Patients Undergoing Total Pancreatectomy With Islet Autotransplantation (TPIAT)

A recent study, led by Jaimie Nathan, MD, FACS, chief of Pediatric Abdominal Transplant and Hepatopancreatobiliary Surgery at Nationwide Children's, is the first multicenter, prospective look at a novel approach to treating children with chronic pancreatitis. As a renowned expert in TPIAT, he and his team are currently building a TPIAT program at Nationwide Children's.

PediatricsNationwide.org/TPIAT

What is "Normal" Thyroid Functioning in Preterm Infants?

Currently in the United States, the thyroid function of all infants is evaluated within the first few days of life as a component of the state-mandated metabolic newborn screen. However, there is little data establishing reference interval values for preterm infants at various gestational ages. In a new study, researchers worked to establish reference intervals for thyroid-stimulating hormone and free thyroxine in preterm infants at specific postmenstrual ages.

PediatricsNationwide.org/Preterm-Thyroid-Range

The Success of EMR-Based, Health-Related Social Needs Screening in Pediatrics

Millie Dolce, PhD, MSW, a program evaluator and wellness initiatives analyst at Nationwide Children's Hospital, and her colleagues investigated the implementation of an electronic medical record-based social needs screening in a recent study published in *Pediatric Quality and Safety*. The study, done using quality improvement methodology, aimed to improve screening compliance in primary care settings, specialty clinics and urgent care centers from 0% to 70% within 12 months.

PediatricsNationwide.org/EMR-Based-HRSN

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