Pediatric Insights

BEST CHILDREN'S HOSPITALS U.S.News & WORLD REPORT NEONATOLOGY 2019-20

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An Update From the UPMC Newborn Medicine Program

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Affiliated with the University of Pittsburgh School of Medicine and ranked among the nation's best children's hospitals by U.S. News & World Report.

UPMC CHILDREN'S HOSPITAL OF PITTSBURGH

40 Years of ECMO Success at UPMC Children's

Extracorporeal membrane oxygenation (ECMO) has been performed at UPMC Children's Hospital of Pittsburgh for almost 40 years, beginning with its first neonatal patient in 1979. Historically speaking, in 1977 Robert L. Hardesty, MD, and Bartley P. Griffith, MD, from the University of Pittsburgh, visited Dr. Robert Bartlett's ECMO research lab at the University of California, Irvine, with the original intention to begin using ECMO as a modality for postoperative cardiac patients.

After nearly a year of testing and development at the University of Pittsburgh Cardiac Research lab, ECMO support was made available for clinical use at UPMC Children's Hospital. UPMC Children's was the second institution in the United States to offer this bypass therapy to neonates, and it also was the second center to join the Extracorporeal Life Support Organization (ELSO) in 1989, an alliance of accredited ECMO centers.

Since its inception, the ECMO Program at UPMC Children's has treated almost 800 neonates with cardiorespiratory failure. The first neonatal patient managed with ECMO support at UPMC Children's, a case of congenital diaphragmatic hernia, survived after three days of ECMO bypass.

UPMC Children's has been an active member of the ELSO registry database, voluntarily reporting on all patients requiring ECMO therapy. The UPMC Children's ECMO Program has been the recipient of a Gold Level ELSO Award for Excellence in Life Support since 2010 and continues to be recognized as an ELSO Center of Excellence, receiving the designation in reporting years 2010, 2013, 2016, and again in 2019.

The Neonatal ECMO Program

In 2009, the Newborn Medicine Program took over ECMO management of newborns and established a certified Neonatal ECMO Program at UPMC Children's. **Burhan Mahmood, MD**, was appointed as medical director of the Neonatal ECMO Program and tasked with leading this important transition. Previously, ECMO support for both pediatric and neonatal noncardiac patients was managed by the pediatric intensive care unit (PICU). The UPMC Newborn Medicine Program established guidelines, policies, and procedures for the management of neonatal ECMO patients, and ECMO equipment was updated as necessary to ensure patient safety and outcomes.

The Neonatal Intensive Care Unit (NICU) at UPMC Children's provides in-house coverage, with ECMO-trained staff and an American Board of Pediatrics-certified ECMO Credentialed Neonatologist available 24 hours a day, seven days a week. As the Neonatal ECMO Program director, Dr. Mahmood spearheads a Neonatal ECMO Core Team that supervises daily care of the ECMO patients and provides oversight of

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Interview with Neonatal Researcher Liza Konnikova, MD, PhD

Liza Konnikova, MD, PhD, is an attending neonatologist and an assistant professor and early stage investigator in the departments of Pediatrics, Developmental Biology, and Immunology at the University of Pittsburgh School of Medicine. Dr. Konnikova's research focus is on the development of neonatal immunity at mucosal surfaces and its role in the pathogenesis of diverse diseases such as sepsis, preterm labor, necrotizing enterocolitis and inflammatory bowel disease.

What is your laboratory currently studying?

Currently our lab is focused on studying what the normal development of neonatal immunity at mucosal surfaces looks like rather than studying a specific disorder. However, the disorder that I've always wanted to study is necrotizing enterocolitis (NEC), a severe inflammatory disorder of the GI tract that mainly affects very premature infants and has high rates of morbidity and mortality.

The problem with studying NEC is that we don't understand what normal immunity development is in the fetus and infant, so it is very difficult to come to conclusions or develop theories about what's wrong with these infants. Our aim right now is to describe what normal immunity is, and then start studying diseases like necrotizing enterocolitis, or preterm labor after we really understand what constitutes normal immunological development.

With a focus on the study of normal immunological development, much of what you do likely involves the gut, where mucosal immunity is very important. Are there other organs or tissue sites that you focus on in terms of normal development?

Our laboratory actually studies two major organ systems that are both barrier sites. One, of course, is the GI tract as noted previously, and the other is the placenta.

A lot is known about the maternal part of the fetal-maternal interface, but very little is actually known about the fetal-maternal interactions. One of the projects in our group, led by Jessica Toothaker, is to figure out how, throughout gestation, the fetus communicates with the mother and then what goes awry to cause such things as preterm labor.

How have technological or clinical influences in the neonatal ICU impacted your research? For example, total parenteral nutrition (TPN) dependence early in life for premature infant populations. How do these kinds of environmental influences affect immunologic fetal and neonatal immunity?

It has been amazing to see that babies as early as 23 to 24 weeks have a good chance at survival. I think the next era in advancement will be to see improvements in survival without morbidities. One of these will certainly entail the immunological development.

The other thing to consider is that a lot of what we have to do to these infants is not normal. They are in incubators. They are in a hospital or in an ICU setting and they might not necessarily receive normal nutrition. How those things contribute to immune development is tricky to study because you have to obtain blood from those babies to study it, and babies have very little amounts of blood we can access for studies.

One of the fellows in my laboratory, Bunmi Olaloye, is working on a project taking tiny amounts of blood, essentially two drops, and trying to do deep immunophenotyping, examining what cells are present at weekly or bi-weekly intervals to better understand how environmental factors may be affecting development. It's an area we currently know little about.

Also, some of my previous work and that of many other groups, has shown that we ought to be feeding our babies. TPN is likely very bad for their guts. Because NEC is such a frightful condition, previous thinking was that not feeding babies would probably be a best practice. However, over the past 10 to 15 years, we have come to realize not feeding is the worst thing for them. We should start feeding them as early as possible. I think the new question to ask and study is: what do we feed them?

It's clear that being fed through a tube is much better than being fed through an IV, because when you're fed through the tube, the gut does not atrophy. Those cells that lie in the gut don't die. When you feed through an IV, the cells that are lining the gut do die.

What kind of technologies do you employ in your research, and how may the ever-growing improvements in these technologies directly influence the kinds of studies you can undertake and what you may learn?

As I said earlier, normal development has yet to be fully described and understood. One of the reasons is because it is so hard to obtain the necessary tissues for study. The other reason is simply that it is a very difficult area to study. The tissues are very valuable, and you get very little of them. People have studied one specific cell type at a time, be it T cells, B cells, macrophages, etc. It's a very slow process.

Recent advances in technology have allowed us to look at all the cells at the same time. The technology I use is called CyTOF, or mass cytometry by time-of-flight spectrometry. Because in this technology the antibodies are linked to heavy metals and not fluorophores, you can combine many together in single studies. We routinely combine 40 antibodies at once, which allows us to look at 40-plus different cell types at the same time. Doing so allows for not only the description of what cells are present, but perhaps more important how they interact with each other. Instead of focusing on one particular cell, we focus on the entire tissue. This approach has driven the advancement in the field such that we now see there exists much more cellular diversity and intracellular interactions than we had appreciated.

What are some of the changes in our understanding of mucosal immunity in infants possibly related to the development of NEC?

We used to think that T cells and B cells didn't play a huge role in infants because, at least in mouse models, the adaptive immune system is really not developed. However, our recent research has upended that thinking and shown that both T cells and B cells are actually quite developed and occupy a large proportion of the gut even in preterm infants as early as 16 weeks gestation.

Looking at samples from patients that have developed NEC, they are fully lacking the adaptive immune system which coincides with the previous hypothesis that perhaps the immune system wasn't developed. Now we know that it was developed. We think that there is a large contribution of the adaptive immune system that plays a role in developing NEC. That is how we will attempt to study it.

How much do we know about how the immunity develops in utero, and what influence does the maternal-fetal interface play in ultimately providing protection or predisposition to disease, either in infants born prematurely or at normal gestational age?

Right now, we are not really sure about those questions. At present we've only examined the neonatal GI tract to significant detail. What we have shown is that as early as 16 weeks gestation, which is very early in the second trimester, all of the major immune populations that are supposed to be there are present in the fetus. Not only are they present but they are functional. The fetal GI immune cells are producing the cytokines that they should be, similar to cytokine production in adult tissues. They're able to react to stimulation in similar ways to how adult cells react. There are also specific T cells that have retained some immune memory. We have seen the classical markers that are known to be indicative of memory in T cells, and those markers are expressed on the cells in the fetus as early as 16 weeks.

A majority of the pre-setup of what the immune system should be like actually happens in utero. And then once the fetuses are born and are exposed to the microbiome, then there are changes in that immune environment. But the initial environment, or ecosystem between the immune cells, the epithelial cells, and all the other cells that are there is actually quite developed before birth and very early on in development. How that happens, though, we do not yet understand.

This is really the next question that we hope to answer. My thought is that because fetuses swallow amniotic fluid continuously starting at 14 weeks gestation, they are getting antigens from the mother that cross the placenta into the amniotic fluid and are then ingested. We have shown that development of the immune system occurs much earlier in the small intestine than the large intestine. Consistent with that as the fetus swallows the fluid, it is first going to travel through the small intestine and that system will develop first. Eventually it will reach the large intestine, and then that system will develop. At least that is the hypothesis right now.

Do you have any thoughts, relative to epigenetics circumstances, that what is happening to or in the mother is clearly happening to the child? At these critical stages of immune system development, is that part of the conversation?

That's a great question. It's a very difficult thing to study unfortunately. I think because we're just starting to investigate what normal immunity is like, it is a big unknown. Once we have the normal development well-described then we can start examining these kinds of questions. The maternal part of this is much trickier to study ethically because these are fetal tissues we are dealing with and so we don't have any information whatsoever on the mother. However, I think as we develop a more detailed understanding of normal development, we can then begin to study all sorts of diseases downstream.

New Research by Liza Konnikova, MD, PhD, Expands Knowledge on Gut Immunity Development Before Birth

Most biology textbooks explain that the fetal immune system is largely undeveloped and that it learns after being exposed to the world at birth. New research from the University of Pittsburgh School of Medicine and UPMC Children's Hospital of Pittsburgh challenges that paradigm, and provides the first comprehensive look at the immune system of the developing gut.

The findings, published in October in the journal *Developmental Cell,* show that the fetal gut has far more well-developed immune capabilities than previously thought.

"Understanding intestinal immune development is crucial as it may have major impacts on the risk of developing autoimmune and autoinflammatory conditions like inflammatory bowel disease later on in life," says co-senior author Dr Konnikova. "It also opens the door to developing new maternal vaccines that may offer lifelong protections against major infectious diseases even before birth."

Dr. Konnikova and her colleagues applied advanced cellular and genomic analyses to study the makeup of the immune system in gut tissues from 14- to 23-week-old fetuses and infants undergoing surgery to correct gut defects.

"We were surprised to find that almost complete immune capacity in the gut had developed as early as 14 weeks, and it remained mostly stable through infancy," says Dr. Konnikova.

The fetal gut had cells from both the innate and adaptive immune systems. The innate immune system is always present in the body and is not specific, but refers to barriers, such as skin, and immune cells that respond quickly to invaders. The adaptive immune system is created in response to a foreign substance, making it specific to that invader, but needs to be primed to recognize the pathogen before it can work, which can be achieved with immunization or prior infection.

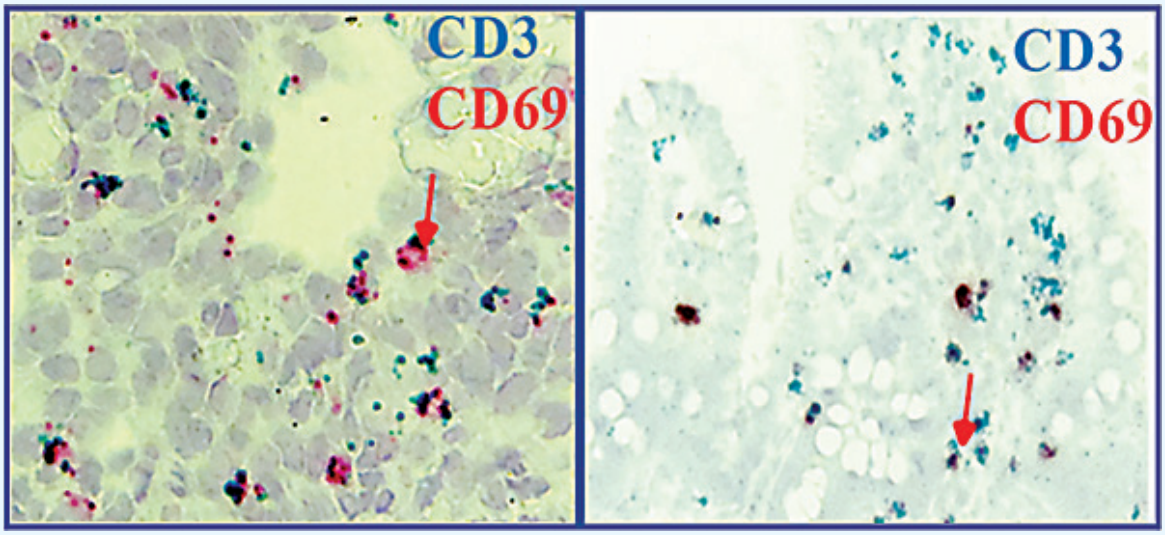
In the innate immune system, the researchers found a large variety of antigen-presenting cells, which are crucial to priming and activating the adaptive immune system, and natural killer cells, which attack virus-infected cells and tumor cells. Other innate immune cells called neutrophils, which are recruited only to sites of inflammation, were found in infants after birth, but not in the fetuses.

Dr. Konnikova and her colleagues also found abundant amounts of B cells and T cells in the fetal gut, which are part of the adaptive immune system.

Surprisingly, most of the T cells were of the "memory" type that help the body remember past invaders in order to respond faster to repeat attacks in the future.

"Finding memory T cells was completely unexpected because these cells need to be exposed to a pathogen to form, and you would think that the placenta would prevent most pathogens from entering the womb," says Dr. Konnikova. She speculated that the fetuses could be exposed to molecular byproducts of pathogens from the amniotic fluid that they float in, which they begin swallowing as early as 12 weeks.

The study authors suggest that the large numbers of memory T cells could help provide some initial protection at birth, when the baby is exposed to a sudden onslaught of microbes, and also prevent it from being overactivated.



Microscopic image showing the presence of memory T cells in the fetal small intestine (left) and large intestine (right).

Dr. Konnikova notes that the study has a few caveats: The immune system in the gut may not reflect that in other tissues; the fetal tissue was limited to the second trimester; and the neonatal tissue was not obtained from completely healthy infants.

In the future, the researchers plan to study exactly what the fetal immune system is exposed to in the womb, and whether it can be manipulated to benefit the growing fetus. The hope, according to Konnikova, is that the immune system can be monitored or manipulated before birth, potentially leading to diagnoses of disease at an extremely early stage or developing beneficial maternal vaccines.

The full text of the new paper can be found at the following reference: Stras SF, Werner L, Toothaker JM, Olaloye OO, Oldham AL, McCourt CC, Lee YN, Rechavi E, Shouval DS, Konnikova L. Maturation of the Human Intestinal Immune System Occurs Early in Fetal Development. *Developmental Cell.* 2019; 51: 1-17. Epub ahead of print.

Additional authors on this study include Stephanie Stras, Jessica Toothaker, Collin McCourt, Austin Oldham, and Oluwabunmi Olaloye of the University of Pittsburgh; and Lael Warner, PhD, Yu Nee Lee, PhD, Erez Rechavi, MD, and Dror S. Shouval, MD, all of Tel Aviv University in Israel.

The study was funded by the University of Pittsburgh.

About the UPMC Newborn Medicine Program

The UPMC Newborn Medicine Program, led by division chief **Thomas Diacovo, MD**, sees and treats infants of all gestational ages with life-threatening medical emergencies and congenital malformations. Each of our NICUs features a full complement of specially trained physicians, neonatal nurse practitioners, nurses, respiratory therapists, and support staff. Neonatologists provide and supervise around-the-clock care at all units. In addition to neonatologists, experts in pediatric medicine and pediatric surgery are available at any time. Our physicians are experts in managing state-of-the-art therapies for critically ill infants, including the use of inhaled Nitric Oxide (iNO), Extracorporeal Membrane Oxygenation (ECMO), and induced Whole Body Hypothermia.

The UPMC Newborn Medicine Program has dedicated plans for consensus management of many neonatal conditions, including gastroschisis, congenital diaphragmatic hernia, Pierre Robin sequence, myelomeningocele, NAS, and others. These plans include:

- NICUs at four hospitals
- UPMC Children's is western Pennsylvania's only AAP-designated Level IV NICU
- Immediate access to every pediatric medical and surgical specialty
- 24/7 neonatal transport via ground or air
- Extracorporeal membrane oxygenation (ECMO) patient transports
- Extracorporeal Life Support Organization (ELSO) Center of Excellence
- Quality Improvement Leader in the Children's Hospitals Neonatal Consortium

The UPMC Newborn Medicine program is one of the nation's fastest-growing pediatric research programs with current projects that include:

- Developing and testing of novel therapies to prevent and treat blood clots in neonates
- Protective strategies for necrotizing enterocolitis
- Mechanisms of bilirubin-induced neurotoxicity
- Identifying premature infants at risk for necrotizing enterocolitis
- The use of bubble CPAP for ventilatory support
- Rapid whole-genome sequencing for diagnosing rare disorders

UPMC Physician Resources

For the latest news, events, videos, and free CME courses presented by UPMC clinicians and researchers, visit **UPMCPhysicianResources.com.**

Video Rounds

Video Rounds is a series of short, informative, and educational videos created for physicians and covering a variety of medical and surgical disciplines. Current topics in neonatology include:

- **Congenital Diaphragmatic Hernia Part 1** with Burhan Mahmood, MD
- **Congenital Diaphragmatic Hernia Part 2** with Kalyani Vats, MD

40 Years of ECMO Success at UPMC Children's *Continued from Page 1*

their management. To ensure optimal patient care, members of the Neonatal ECMO Core Team also provide an ECMO back-up call service for newly credentialed faculty. Currently, the Neonatal ECMO Program at UPMC Children's provides the most advanced forms of life support available to neonates experiencing acute cardiorespiratory failure, and it is the only center in western Pennsylvania to provide extracorporeal support to sick neonates, with ECMO referrals made from all of western Pennsylvania, western West Virginia, and northeastern Ohio.

Since 2009, the Neonatal ECMO Program has provided extracorporeal support to 115 critically ill babies with an overall survival rate of 81 percent coming off ECMO and 68 percent at discharge. A total of 21,375 ECMO patient hours, or around 890 ECMO patient days, have accrued since the UPMC Children's Neonatal ECMO Program was established.

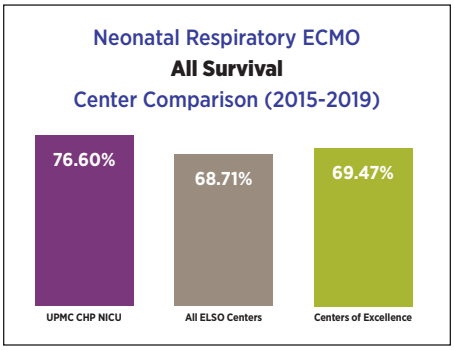
With the ongoing refinement of ECMO equipment and management strategies during the last five years, neonates are now on the bypass for a shorter duration than before, with fewer complications, while overall survival has improved significantly to 96 percent coming off ECMO and close to 80 percent at discharge. These neonatal respiratory ECMO outcomes are better than the outcomes reported by other ELSO centers of excellence.

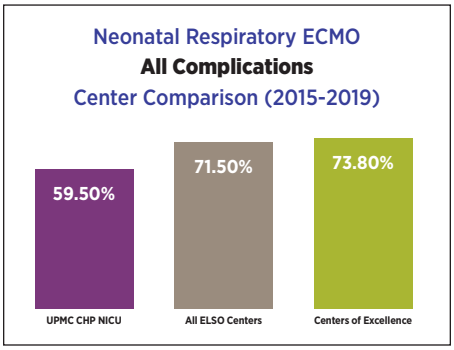
Neonatal ECMO Competency

To ensure that all clinicians and advanced practice providers (APPs) become ECMO certified, UPMC Children's established well-defined in-house training programs and credentialing measures in collaboration with the UPMC Children's Perfusion Services Department. These measures include clinical case reviews and troubleshooting scenarios with intensive, hands-on ECMO wet lab sessions and ECMO simulation to better understand the nuances of neonatal ECMO management. The wet lab training and ECMO simulation provide a detailed overview of the ECMO circuit, including control and safety mechanisms of the system in a nonclinical setting.

Incoming neonatology fellows, new faculty members, and APPs for the UPMC Newborn Medicine Program receive a basic ECMO course prior to starting clinical service. The course curriculum covers a review of the ECMO equipment, physiology, and patient management. This initial ECMO certification course is mandatory for new faculty, fellows, APPs, and nurse practitioners before they can start caring for ECMO patients. Completion of ECMO educational, simulation, and training sessions — especially involving the equipment — are required for faculty, fellows, and advanced practice providers for ECMO certification.

Other activities that promote patient safety and enhance continued education of extracorporeal life support (ECLS) topics include daily ECMO rounds in the NICU, weekly ECMO educational rounds, monthly neonatal ECMO patient reviews, morbidity and mortality conferences, ECMO journal clubs, multidisciplinary ECMO care meetings, and the annual UPMC Neonatal and Pediatric ECMO Conference. Under the oversight of Dr. Mahmood, these endeavors are conducted regularly and ensure ongoing ECMO competency of all clinical practitioners who provide neonatal ECMO support at UPMC Children's.





Dr. Mahmood at 2019 ELSO Conference with Dr. Robert Bartlett.

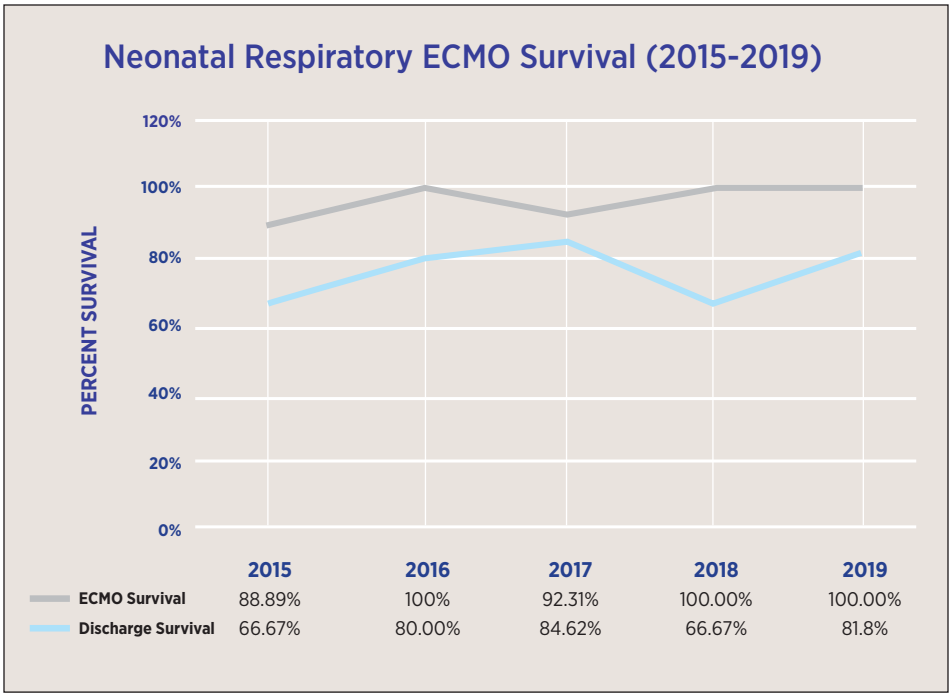
New Training Measures in Development

Moving forward, the quality of ECMO education and training is being enhanced by employing newer educational and training resources for the neonatal staff, physicians, and APPs. These measures include computer-based learning models, online content, and the development of more robust high-fidelity ECMO simulation models. The ECMO training program will collect performance data after caregivers complete teaching modules and complete online testing. This information will be utilized to assess how the program can be improved, and then required changes will be implemented. These initiatives will provide a quality learning experience and further enhance neonatal ECMO education and training and promote excellent patient outcomes.

Neonatal-Perinatal Fellowship Advanced ECMO Training

The UPMC Newborn Medicine Program has established an ECMO track within the UPMC Neonatal-Perinatal Medicine fellowship program to provide advanced training for fellows who are interested in enhancing their ECMO experience as a career pathway. Neonatology fellows have the option to participate in this advanced ECMO training to build this skill set, particularly if they have career plans to work at an ECMO center.

Completion of this advanced ECMO experience is noted as an additional mastered skill set at the time of the fellow's graduation from the program. This training occurs in all the critical care units of UPMC Children's and consists of bedside ECMO pump management with the perfusionist, attendance at cannulation/decannulation, priming procedures of the ECMO system, troubleshooting drills including emergency circuit change, and participation in the design of ECMO simulations. Completion of this program requires attendance at the UPMC Children's ECMO Specialist Training Course and successful completion of the Neonatal ECMO Training Exam to ensure ECMO certification at the time of graduation from the fellowship program.



"That's Pediatrics" Research Podcast Series

UPMC Children's Hospital of Pittsburgh medical podcast series for physicians, scientists, and other health care professionals features compelling interviews with the hospital's leading researchers and clinicians discussing innovative basic, translational, and clinical research. New episodes are released every two weeks.

"Going back to the polio vaccine, Pittsburgh has always been a hub of very innovative research, and in recent years has really become a nexus for some groundbreaking research in pediatric medicine," says **John Williams, MD**, chief of the Division of Pediatric Infectious Diseases at UPMC Children's and one of the podcast hosts. "There is a spirit of collaboration here in Pittsburgh that makes it somewhat unique nationally and we really want to explore the research that is happening here and how we have a real opportunity to change the way pediatric medicine is practiced around the world."

In addition to Dr. Williams, "That's Pediatrics" hosts are:

Carolyn Coyne, PhD, director of the Center for Microbial Pathogenesis, UPMC Children's Hospital

Stephanie Dewar, MD, director of Pediatric Residency Training Program, UPMC Children's Hospital

Brian Martin, DMD, vice president, Medical Affairs, UPMC Children's Hospital

That's PEDIATRICS
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Current episodes of "That's Pediatrics" from **neonatology faculty** and related topics include:

- *Researching Early Immune Development* with **Liza Konnikova, MD, PhD**
- *Neonatal Cardiovascular Research* with **Thomas Diacovo, MD**, Newborn Medicine Division Chief
- *The Unique Microbiome of Premature Infants* with **Michael Morowitz, MD**

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About UPMC Children's Hospital of Pittsburgh

Regionally, nationally, and globally, UPMC Children's Hospital of Pittsburgh is a leader in the treatment of childhood conditions and diseases, a pioneer in the development of new and improved therapies, and a top educator of the next generation of pediatricians and pediatric subspecialists. With generous community support, UPMC Children's Hospital has fulfilled this mission since its founding in 1890. UPMC Children's is recognized consistently for its clinical, research, educational, and advocacy-related accomplishments, including ranking 15th among children's hospitals and schools of medicine in funding for pediatric research provided by the National Institutes of Health (FY2018) and ranking on *U.S. News & World Report's* Honor Roll of America's Best Children's Hospitals (2019–20).

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