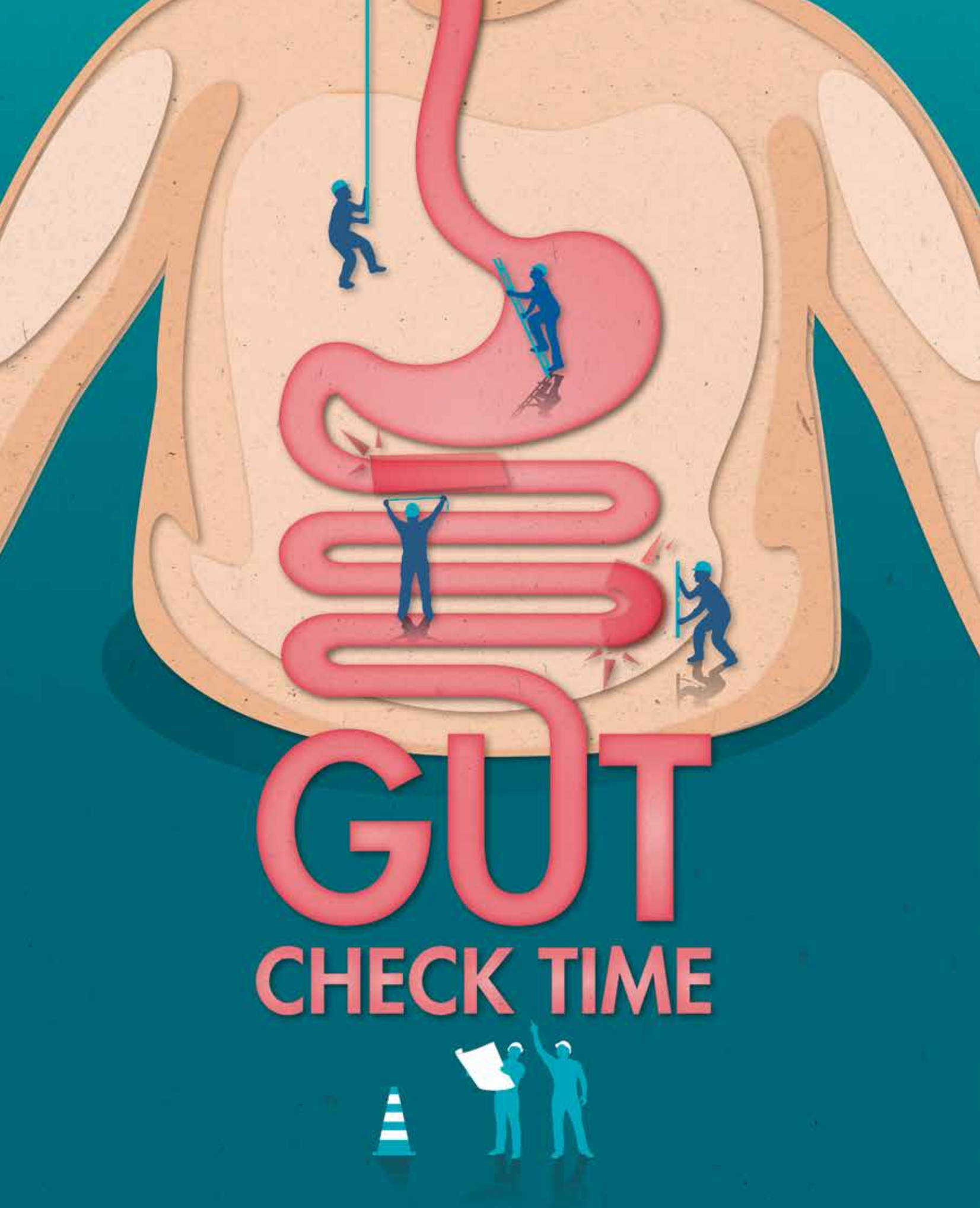
GUT
CHECK TIME

Investigators at The Saban Research Institute take a full-circle approach to a life-threatening intestinal disease that targets newborns.

Henri Ford, MD, MHA (center)

As a young surgical fellow at the University of Pittsburgh in the late 1980s, Henri Ford, MD, MHA, vowed he would try to unlock the mysteries of a terrible disease he saw all too often in the operating room—necrotizing enterocolitis, or NEC, which damages and even destroys the intestinal tissue of newborns.

"Too many babies were dying. We just didn't have enough answers," recalls Ford, vice president and surgeon in chief at Children's Hospital Los Angeles.

Now, thanks to a 360-degree offensive by multiple investigators at The Saban Research Institute of Children's Hospital Los Angeles, more clues are surfacing about some likely culprits for NEC, along with possible future intervention and prevention strategies. In addition to Ford, two of the leading investigators are also surgeons; like him, they have been inspired to look beyond their surgical training to biology. They are joined by basic researchers who are equally determined to defeat the most common and serious gastrointestinal disorder among newborns.

This impressive brain trust is good news for families dealing with NEC, which most often targets preemies, whose organs are still developing at birth. About 30 percent of babies who develop NEC will die. Survivors face life-changing consequences such as removal of part of their intestine—resulting in long-term dependence on intravenous nutrition and possible liver damage—or a bowel transplant. Either spells an uncertain future.

"It's imperative we come up with a means of preventing this disease," says Ford, who is also vice dean of medical education and professor and vice chair for clinical affairs in the Department of Surgery at the Keck School of Medicine of the University of Southern California (USC). "If we can understand what pathogen or injury triggers the cascade of events that lead to the inflammation characterized by NEC, perhaps we can prevent it."

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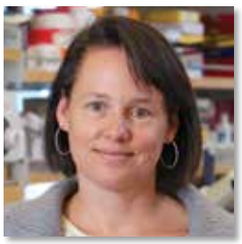
Anatoly Grishin, PhD

Christopher Gayer, MD, PhD

Mark Frey, PhD



Tracy Grikscheit, MD

Zeroing in on bacteria

Bacterial colonization starts immediately after birth, but so-called "good" bacteria don't emerge until weeks 2 to 4, giving time for NEC to enter the equation. Normally, the intestinal epithelium, or lining, is tight, so no bacteria can cross over. As NEC begins its attack, the intestine becomes inflamed and openings can appear in the lining, allowing viruses and bacteria to enter.

In Ford's lab at The Saban Research Institute, research led by Anatoly Grishin, PhD, focuses on a bacterial species called Cronobacter sakazakii, associated with NEC and neonatal meningitis. Researchers believe that Cronobacter sakazakii is just one example of a broad group of opportunistic pathogens in babies with weakened immune systems. "If we can identify which bacteria are the harmful ones, and which protective, we hope to also identify babies at high risk for NEC and intervene with targeted therapies aimed at specific pathogens," says Grishin.

Also important, he adds, is "finding the defining event that causes the gut barrier to break down the first time." CHLA researchers were the first to discover that abnormally high levels of nitric oxide, a molecule that aids in cellular signaling, induces cell and tissue death in the intestinal lining. They also found that it is not nitric oxide itself, but rather short-lived compounds resulting from its reaction with oxygen, that damage cellular proteins and kill cells.

An investigational anti-inflammatory drug called semapimod appears to block nitric oxide synthesis, a finding that led the Ford team to apply for a patent on its use in NEC.

Finding a biomarker

Christopher Gayer, MD, PhD, a surgeon at CHLA, is curious about another player in our digestive tract, bile acids, which facilitate the movement and absorption of fats and cholesterol. As they enter the small intestine, bile acids are "changed into about 100-plus different forms, depending on the bacteria there," says Gayer, an assistant professor of Surgery at the Keck School of Medicine of USC, whose postgraduate work focused on intestinal wound healing.

His hypothesis: Certain bacteria interacting with bile acids will inhibit the ability of the intestine to heal and may be one mechanism behind NEC—something Ford calls "an exciting new avenue of investigation."

In addition to studying the molecular signals that govern bile acid migration, Gayer is measuring bile acids and bacteria in stool specimens of babies with NEC to determine which bacteria are in highest concentrations. Ultimately, he suspects he won't find a single type of bacteria behind NEC but a combination of them, all sharing the ability to create toxic bile acids.

He'd like to identify a biomarker—a biological predictor of disease—that could indicate whether a newborn is likely to develop NEC even before symptoms manifest. "We often can't diagnose NEC until it's very advanced and the baby is already sick," Gayer explains.

Protective factors

Mark Frey, PhD, is principal investigator on a study at The Saban Research Institute that recently showed that a protein called neuregulin-4 (NRG4)—present in breast milk but missing in infant formula—may help guard against intestinal damage caused by NEC.

During NEC's inflammatory assault, specialized intestinal cells called Paneth cells, which protect the intestine from microbial damage, are lost. NRG4 binds with a receptor found in the intestine, ErbB4, to block that damage. When mice were given a chemical that damages Paneth cells, NRG4 protected the animals from developing NEC. NRG4 also provided protection when newborn, formula-fed rats and cultured intestinal cells were given bacteria related to strains that may induce NEC in humans. Ford was among the contributors to Frey's study, published in the American Journal of Pathology.

"Given that NEC is a significant clinical problem without an effective treatment, we plan to evaluate NRG4 for its therapeutic potential," says Frey, who is also an assistant professor of Pediatrics and Biochemistry and Molecular Biology at the Keck School of Medicine of USC.

Tissue engineering

Tracy Grikscheit, MD, a researcher and surgeon at CHLA, wants to help babies who have surgery to remove diseased or dead intestinal tissue and subsequently develop short-bowel syndrome, for which there is no cure except transplant.

In January, Grikscheit, who is also an assistant professor of Surgery at the Keck School of Medicine of USC, received her second five-year, $3.1 million grant from the California Institute for Regenerative Medicine to continue her efforts to engineer intestinal tissue from stem cells. So far, her team has succeeded in growing pieces of large and small intestine in the lab, using stem cells from intestinal tissue discarded during surgery.

The good news: All the necessary cell types are present in the engineered tissue and, adds Grikscheit, "as the cells grow, the new intestine is able to perform all the functions of a healthy organ—absorbing nutrients and providing a barrier against infection." Along the way, she and her team have found that their stem cell-growing techniques seem to support tissue engineering of other organs as well.

Now they're focused on another task—finalizing a device that can help standardize the process of tissue engineering. The machine under development will cryopreserve the stem cells, maintaining their viability, and then thaw them without damage to the cells. Grikscheit envisions a day when such a device may be used in an operating room, enabling parents to save their baby's stem cells for later use if, for example, a tissue-engineered small intestine is needed. As for what drives her and other investigators, she says, "I get to take care of these babies and families, and I want to be able to tell them that we have more options."

With so many dedicated investigators in one place, CHLA is "poised to emerge as the leading center in the U.S., if not the world, studying this disease."

– Henri Ford, MD, MHA

In a powerful synchronicity, the inquiries of these NEC-focused surgeon-scientists and researchers complement other current CHLA studies of intestinal conditions such as inflammatory bowel disease. With so many dedicated investigators in one place, says Ford, Children's Hospital Los Angeles is "poised to emerge as the leading center in the U.S., if not the world, studying this disease." ■