Backgrounder on Anifrolumab and Type I Interferons

The positive results for the investigational drug anifrolumab in the Phase III TULIP 2 trial, announced today in the *New England Journal of Medicine*, shine a spotlight on type I interferons, the immune system molecules that anifrolumab disrupts. Type I interferons are one of the main drivers of lupus as demonstrated by numerous scientific studies [1].

The Lupus Research Alliance (including its legacy organizations) have sponsored more than 20 studies on type I interferons, including work that identified a unique combination of genes that had been switched on in the blood of lupus patients and galvanized research into the molecules' role in the disease. Here is more information about type I interferons and how scientists, including many funded by the Lupus Research Alliance, discovered their connection to lupus.

What are type I interferons and what is their function?
Type I interferons are a family of 17 similar molecules that help protect us from infections [1]. Most of the time, type I interferons are beneficial because they trigger changes in our cells that help fight off viruses and other disease-causing microbes. The main source of type I interferons is a type of immune cell known as a plasmacytoid dendritic cell, but other types of cells can also release them [2]. Plasmacytoid dendritic cells serve as guards, releasing type I interferons when they detect potentially harmful bacteria and viruses.

How important are type I interferons in lupus?
In lupus the immune system attacks patients' own tissues. Although the mechanisms of the disease are very complex, researchers consider type I interferons one of the main factors that leads to tissue damage and disease symptoms. "I've been convinced for many years that type I interferons contribute to lupus pathogenesis," says Dr. Mary Crow, Physician-in-Chief and Chair of the Department of Medicine at Hospital for Special Surgery in New York City and Co-chair of the Lupus Research Alliance's Scientific Advisory Board. Many patients with lupus have chronically high levels of type I interferons [1]. In contrast, during an infection in healthy people, the body ramps up production of the molecules for just a few days before returning to a normal level.

How do type I interferons affect the immune system in lupus?
Over the long term, high levels of type I interferons may disrupt the immune system and lead to lupus symptoms. But as Dr. Crow notes, "there's probably not one mechanism by which type I interferons cause the immune problems we see in lupus, there are many mechanisms." One example involves the immune cells called B cells, which in lupus release proteins called antibodies that spur tissue damage. Type I interferons bind to an interferon receptor that juts out from the surface of cells and allows them to respond to type I interferon molecules. When these molecules bind to receptors on B cells and T cells, they stimulate the cells to mature and promote

production of a more destructive variety of antibodies [1]. Dr. Virginia Pascual, Director of the Drukier Institute for Children's Health and Ronay Menschel Professor of Pediatrics at Weil Cornell Medicine, discovered that high levels of interferon influenced the maturation of immune cells known as dendritic cells which play a central role in activating B and T cells [3]. Scientists have also found that type I interferons can make skin cells more sensitive to UV radiation which may explain the photosensitivity experienced by many lupus patients [4].

Is there a treatment that blocks the type I interferons?
When scientists realized the important role type I interferons played in promoting lupus symptoms, they worked on creating therapies to block their effect on cells. Initially, they tried blocking the type I interferon molecule itself, but it wasn't very effective in reducing lupus activity in clinical trials [5].

Scientists then created a monoclonal antibody, anifrolumab, that instead recognizes and binds to the type I interferon receptor [6]. By blocking the receptor, anifrolumab prevents all type I interferons from affecting these cells.

Who discovered the first evidence that type I interferons might be involved in lupus?
In the late 1960s, Alfred Steinberg and colleagues at the National Institutes of Health (NIH) tested mice that have an autoimmune disease similar to lupus. Giving the mice a compound that stimulates cells to produce more type I interferons made the disease worse [7]. Studies by other scientists in the 1970s then revealed high levels of type I interferons in the blood of patients with lupus [8, 9].

Why were early studies of interferon gene signatures important?
These studies provided strong evidence that type I interferons were promoting lupus, not a response to it. Although research in the 1970s had found high levels of type I interferons in patients with lupus, the patients also had many other changes in their immune systems that could be responsible for the disease. In 2003, four groups of researchers, including Dr. Pascual's team, detected interferon gene signatures in the blood cells of patients with lupus [10-13]. Two of the studies were funded by the Lupus Research Alliance: those by Dr. Crow and by Dr. Timothy Behrens. The finding that "this molecular pathway was turned up in a coordinated way made you think it could be very important in lupus," says Dr. Crow.

"Longitudinal studies sponsored by the Lupus Research Alliance also permitted us to follow children with lupus longitudinally. These studies highlighted the value of the interferon gene signature as a biomarker of disease activity", says Dr. Pascual. (Chaussabel et al., Immunity. 2008 Jul 18;29(1):150-64. doi: 10.1016/j.immuni.2008.05.012)

What is an interferon gene signature?
Type I interferons work by stimulating cells to turn on several hundred genes. When researchers measure which genes are switched on in blood cells of lupus patients, as

first done in 2003 and discussed above, they can detect a specific pattern of interferon-activated genes called an interferon gene signature. Multiple studies have subsequently confirmed this finding [15,16]. Pivotal work by Dr. Virginia Pascual showed that up to 84% of pediatric patients with lupus exhibit the signature [17]. For this and other important research contributions to lupus, she was awarded the 2017 Lupus Insight Prize by the Lupus Research Alliance.

What other findings support type I interferons' role in lupus?
A variety of additional data from human and animal studies points to a connection between type I interferons and lupus. Researchers have found that genetically altering mice to lack the type I interferon receptor prevents some lupus symptoms, for example [18]. Studies of DNA from large numbers of patients with lupus, including work by the International Consortium on the Genetics of Systemic Lupus Erythematosus (SLEGEN), funded by the Lupus Research Alliance, have identified links between changes in DNA and the type I interferon pathway [19]. These alterations may affect how much type I interferons patients release or how their cells respond to it. In work sponsored by the LRA, scientists found that several gene variants associated with increased risk of lupus involve the type I interferon pathway [20]. Increased type I interferon signatures have also been shown to be associated with both active nephritis [21] and pregnancy [22] in patients with lupus.

What causes cells to overproduce type I interferons in lupus?
Researchers are still investigating that question. However, one mechanism likely involves cells responding abnormally to RNA, a chemical cousin of DNA with vital function in cells. But because some viruses contain RNA, it can also be a sign of a viral infection. Cells have proteins, including one called toll-like receptor 7 (TLR7), to detect viral RNA and stimulate the immune system to fight back, including by producing type I interferons [5]. Scientists now think that in patients with lupus, the virus detection system mistakes normal cellular RNA for viral RNA and turns on the immune system.

The Lupus Research Alliance funded research that helped build the case that this mistaken identity promotes lupus. Dr. Franck Barrat of Dynavax Technologies and colleagues designed molecules that block TLR7 and showed that they reduce the amount of one type I interferon released by plasmacytoid dendritic cells that have been exposed to virus RNA [23]. A team led by Dr. Lars Ronnblom of Uppsala University in Sweden found that RNA from dead blood cells prompted plasmacytoid dendritic cells to release type I interferons [24].

Does DNA also trigger release of type I interferons in lupus?
Some viruses carry DNA, and as in the case of RNA, cells have toll-like receptors for viral DNA that may be inappropriately stimulated by normal cellular DNA in lupus. A recent study demonstrated that in lupus patients, immune cells known as neutrophils released DNA that could induce a type I interferon response [25].

The discovery of type I interferons and the subsequent development of anifrolumab is a long story filled with twists and turns, breakthroughs and setbacks that have led to the satisfying conclusion of a successful clinical trial. The last pages are still being written, but the outlook is promising for a happy ending with positive late-stage data and a submission for FDA approval in the not-so-distant offing. The LRA looks forward to the sequel.

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