immediately. However, in centers that have an easier access to CAR-T cell therapy, the sequencing of BCMA-targeting agents may be the opposite.

There are indeed some preliminary experiences with BsA after prior BCMA-targeting agents including CAR-T cells. Results with the BsA teclistamab and erlanatamab are already available. In patients previously exposed to ADC or CAR-T, teclistamab yielded a 52.5% response rate including complete responses with no difference between ADC and CAR-T. The safety profile was similar to that observed in BCMA-naive patients.[5] In the Magnetis MM-3 phase 2 trial of erlanatamab in patients with RRMM, cohort B was specifically focused on prior BCMA-directed ADC or CAR- T. Results from this study are not yet available.[6]

Another possibility for patients previously exposed to BCMA-targeting agents is to use a different target. In the MonumenTAL1 phase 1 trial of the GPRC5D targeting BsA talquetamab, almost 30% of RRMM had received BCMA-targeted therapy.[7] Similarly, cevostamab targeting the Fc receptor homolog 5 showed encouraging preliminary results with responses in 7 of 10 patients with prior BCMA-targeted therapies (ADC or CAR-T).[8]

In conclusion, BCMA-directed therapies represent a major breakthrough in the treatment of RRMM. In addition to a better understanding of the mechanisms of resistance, a crucial question is the optimal sequencing of the different BCMA-targeted therapies. Repeated treatments with these agents appear to be possible, but soon, enhanced benefit and improved outcome may be optimized by their use in better sequencing or at earlier stages.[9]

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Kumar et al, page 238

TORing the impact of sirolimus on immune health

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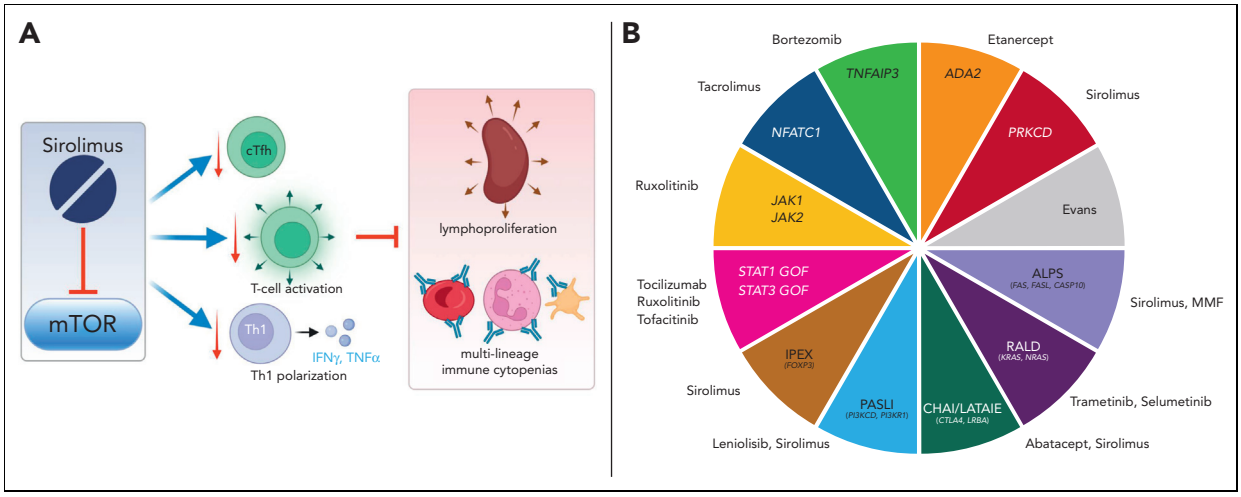
In this issue of *Blood*, Kumar et al[1] investigate the impact of sirolimus on immune health in a cohort of children treated with sirolimus for multilineage immune cytopenias (m-ICs). Sirolimus, also known as rapamycin, is an antifungal compound first identified in a soil sample obtained from Easter Island (Rapa Nui) as part of a 1960s drug discovery program.[2] Shortly after its isolation, sirolimus was found to be a potent immunosuppressive agent, forming a complex with FK-binding protein-12 that blocks activation of the protein kinase, mammalian target of rapamycin (mTOR), arresting the cell cycle and inducing autophagy.[2] In 1999, sirolimus was approved by the US Food and Drug Administration to prevent solid organ transplant rejection, and over the past 20 years, it has been found to be a well-tolerated drug with an excellent safety profile.[2]

In the early 2000s, sirolimus was studied as a treatment for autoimmune lymphoproliferative syndrome (ALPS), a disorder driven by defects in Fas-mediated apoptosis that causes inappropriate proliferation of $CD4^-$ and $CD8^-$ T lymphocytes, termed "double-negative" T cells (DNTs), resulting in symptoms including lymphadenopathy, splenomegaly, and m-ICs.[3,4] Multiple studies have since confirmed that sirolimus is a highly effective therapy for ALPS, inducing apoptosis of DNTs while promoting the developing of regulatory T cells, leading to the resolution of m-ICs without causing significant immune compromise when used as monotherapy.[5,6] Interestingly, sirolimus has subsequently been shown to be effective in the treatment of m-ICs in patients without ALPS.[7] In many of these patients, sirolimus does not significantly change absolute lymphocyte counts or immunoglobulin levels, and its mechanism of activity remains unclear. However, prior studies of the impact of sirolimus on immune health are not robust.[8]



(A) Sirolimus, an mTOR inhibitor, is effective in children with m-IC syndromes, improving and/or eliminating abnormal lymphocyte populations that drive immune dysregulation, including cTfh and decreasing markers of abnormal T-cell activation, senescence, and exhaustion on $CD4^+$ and $CD8^+$ effector memory T cells, along with a reduction of Th1 polarization. Ultimately, sirolimus improved disease manifestations without leading to immune deficiency. Panel B depicts targeted therapies currently available to treat children and adults with different monogenic causes of m-IC. Similar studies evaluating the mechanism of action and short- and long-term impacts on immune health are needed. ADA2, adenosine deaminase deficiency 2; ALPS, autoimmune lymphoproliferative syndrome; CHAI, CTLA4 haploinsufficiency with autoimmune infiltration; Evans, syndrome characterized by m-ICs without known cause; GOF, gain of function; IFN, interferon; IPEX, immune dysregulation polyendocrinopathy, enteropathy, X-linked; LATAIE, LRBA deficiency with autoantibodies, regulatory T (Treg) cell defects, autoimmune infiltration, and enteropathy; MMF, mycophenolate mofetil; PASLI, p110d-activation with senescent T cells, lymphadenopathy, and immunodeficiency; PRKCD, protein kinase C delta deficiency; RALD, ras-associated leukoproliferative disease; TNF, tumor necrosis factor; TNFAIP3, tumor necrosis factor alpha infused protein 3.

There is increasing recognition that m-ICs in children are commonly a manifestation of disorders of immune dysregulation, which may or may not be monogenic, and are often amenable to therapy with immune modulatory medications. To determine how sirolimus affects immune dysfunction in pediatric m-IC, Kumar et al performed robust longitudinal quantitative and qualitative immune profiling of 12 patients with m-ICs before and after initiation of sirolimus, with a median follow-up time of 17 months, compared with 21 healthy pediatric controls. Although 4 patients were found to have pathogenic gene variants in *LRBA*, *PI3KCD*, and *FAS*, an underlying genetic etiology was not identified in 8 patients. All patients responded to sirolimus, with 2 experiencing partial remission of their cytopenias and 10 achieving complete remission. The majority also experienced improvement in lymphoproliferation (lymphadenopathy and/or splenomegaly).

Sirolimus treatment was consistently associated with normalization of T-cell distribution and characteristics, without changes in absolute numbers of $CD4^+$ and $CD8^+$ T cells or immunoglobulin levels (see figure panel A). All patients had a significant reduction in circulating T follicular helper cells (cTfh), a cell type often elevated in the setting of autoimmunity and immune dysregulation.[9] Sirolimus also led to a decrease in markers of cell activation, senescence, and exhaustion on $CD4^+$ and $CD8^+$ effector memory T cells, along with a reduction of Th1 polarization. Of note, the authors did not report whether they observed changes in DNT counts following initiation of mTOR inhibition. Sirolimus use was associated with a decrease in CXCL9 and CXCL10 levels, consistent with flow cytometry results demonstrating decreased Th1 polarization. Strikingly, similar trends were observed in m-IC patients regardless of etiology or whether they were found to have an underlying pathogenic genetic variant.

Unlike single-lineage autoimmune cytopenias, m-ICs in children are often chronic, regardless of whether they are found to be secondary to a monogenic disorder or are classified as idiopathic (eg, Evans syndrome). Although some of these children, especially those with certain monogenic disorders, may benefit from hematopoietic stem cell transplant (HSCT), HSCT is often avoided because graft rejection is high in patients with aberrant immune activation, and HSCT is fraught with significant morbidity and mortality.[10] Accordingly, during formative stages of growth and development, many of these children require long-term immunomodulation/suppression, and it is critical that we understand the short- and long-term complications of these therapies, particularly their effect on immune health. The authors' data suggesting that mTOR inhibition ameliorates underlying immune dysregulation in patients with m-ICs irrespective of underlying genetic etiology without suppressing the immune response are highly encouraging and support long-term use of monotherapeutic sirolimus in pediatric m-ICs.

Although the total number of patients studied was small, the consistency of results is compelling, especially in light of the genetic heterogeneity in the cohort. Nevertheless, larger studies focused on individual cohorts of patients with longer follow-up are needed to confirm these findings. For example, sirolimus and abatacept have been anecdotally used successfully for patients with *LRBA*- and *CTLA4*-mutant disease, and multicenter studies are needed to assess the short- and long-term impacts of immunomodulatory medications including sirolimus in each of these diseases.[7] We have entered an exciting time in the management of treatment of children with disorders of immune dysregulation, as multiple targeted precision therapies are now

available (see figure panel B). For example, patients with inherited mutations in Jak/Stat pathway genes may benefit from ruxolitinib, patients with gain of function mutations in *STAT3* may benefit from tocilizumab, and patients with Ras-associated leukoproliferative disorder may benefit from MAPK inhibitors.[7] It is critical that we continue to study the impact of these medicines on immune health in these populations to determine whether novel targeted approaches are acting through expected mechanisms and to confirm whether they are safe for long-term use.

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Charlebois et al, page 271

Non–transferrin-bound iron takes the driver's seat

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In this issue of *Blood*, Charlebois et al[1] report that non–transferrin-bound iron (NTBI) is the primary driver of bone morphogenetic protein 6 (BMP6) expression in liver sinusoidal endothelial cells (LSECs) during iron overload. This finding is important because LSEC-derived BMP6 prompts the liver to produce hepcidin, the chief iron-regulatory hormone that regulates body iron balance.

Otto von Bismarck, the "Iron Chancellor" of the German Empire in the late 19th century, argued that great questions of national policy are settled by iron and blood. One can make a similar argument for present-day research in iron biology, in which a great and unresolved question will be settled by iron. In essence, the question is how the body "senses" iron status so that it can adapt to absorb more iron when needed but avoid accumulating too much of the metal, which can be toxic in excess. Such regulation is essential for body iron balance because humans cannot excrete excess iron. An important advance in recent years has been the identification of LSECs as the site of iron sensing.[2] LSECs respond to iron loading by increasing the expression and secretion of BMP6,[3,4] which activates in neighboring hepatocytes a signaling pathway that induces the expression of hepcidin,[5] the hormone that controls how much iron the intestine absorbs. However, the form of iron taken up by LSECs that triggers *Bmp6* expression in vivo and the molecular mechanisms involved have not been well defined.

The 2 most plausible candidates for conveying the iron signal to LSECs are transferrin-bound iron and NTBI. In normal blood plasma, iron circulates nearly exclusively as transferrin-bound iron (ie, holotransferrin), which cells take up via transferrin receptor 1 (TFR1)-mediated endocytosis. In iron overload conditions, plasma iron increases to levels that exceed the iron-carrying capacity of transferrin, giving rise to NTBI, a poorly defined, heterogenous, and variable mixture that includes ferric citrate and high-mass iron aggregates.[6,7] Usually undetectable in normal healthy individuals, plasma NTBI becomes measurable when transferrin saturations surpass 70%, such as in the iron overload disorders hereditary hemochromatosis and thalassemia major. Cells take up NTBI via divalent metal-ion transporters such as ZIP14, ZIP8, and DMT1.[6] Although previous studies have shown that either holo-transferrin or NTBI (as ferric ammonium citrate) can load primary mouse liver endothelial cell cultures with iron and induce *Bmp6* expression,[3,4] how these iron sources contribute to LSEC BMP6 production in vivo requires clarification. Using mouse models and single-cell transcriptomics, Charlebois et al conclude that NTBI is the main regulator of LSEC BMP production during iron overload.

To define the role of LSEC TFR1 in the iron-dependent regulation of *Bmp6* expression, the authors generated mice with endothelial-specific inactivation of the TFR1-encoding *Tfrc* gene. They found that mice lacking endothelial TFR1 display no alterations in systemic or tissue iron levels and express normal amounts of BMP6 and hepcidin, indicating that endothelial TFR1 does not play a major