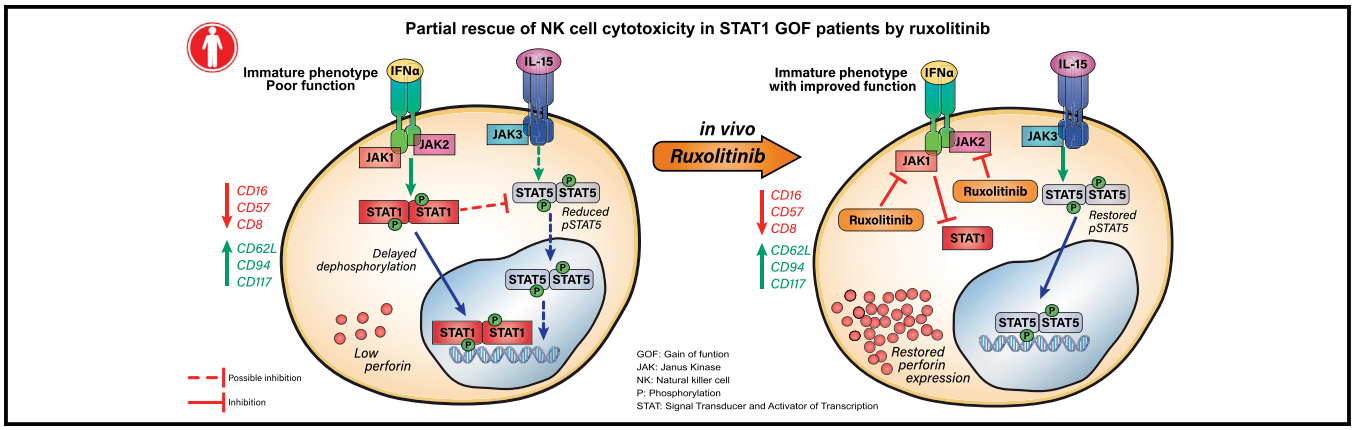
Ruxolitinib partially reverses functional natural killer cell deficiency in patients with signal transducer and activator of transcription 1 *(STAT1)* gain-of-function mutations

Alexander Vargas-Hernández, PhD,[a,b] Emily M. Mace, PhD,[a,b] Ofer Zimmerman, MD,[c] Christa S. Zerbe, MD,[c,d] Alexandra F. Freeman, MD,[c,d] Sergio Rosenzweig, MD, PhD,[c,d] Jennifer W. Leiding, MD,[e] Troy Torgerson, MD, PhD,[f] Matthew C. Altman, MD,[f] Edith Schussler, MD,[g,h] Charlotte Cunningham-Rundles, MD, PhD,[g,h] Ivan K. Chinn, MD,[a,b] Alexandre F. Carisey, PhD,[a,b] Imelda C. Hanson, MD,[a] Nicholas L. Rider, DO,[a,b] Steven M. Holland, MD,[c,d] Jordan S. Orange, MD, PhD,[a,b] and Lisa R. Forbes, MD[a,b] *Houston, Tex, Bethesda, Md, St Petersburg, Fla, Seattle, Wash, and New York, NY*

GRAPHICAL ABSTRACT



Background: Natural killer (NK) cells are critical innate effector cells whose development is dependent on the Janus kinase–signal transducer and activator of transcription (STAT) pathway. NK cell deficiency can result in severe or refractory viral infections. Patients with *STAT1* gain-of-function (GOF) mutations have increased viral susceptibility.

Objective: We sought to investigate NK cell function in patients with *STAT1* GOF mutations.

Methods: NK cell phenotype and function were determined in 16 patients with *STAT1* GOF mutations. NK cell lines expressing patients' mutations were generated with clustered regularly interspaced short palindromic repeats (CRISPR-Cas9)–mediated gene editing. NK cells from patients with *STAT1* GOF mutations were treated *in vitro* with ruxolitinib.

Results: Peripheral blood NK cells from patients with *STAT1* GOF mutations had impaired terminal maturation. Specifically,

From [a]the Department of Pediatrics, Baylor College of Medicine, Houston; [b]Texas Children's Hospital, Center for Human Immunobiology, Department of Allergy, Immunology and Rheumatology, Houston; [c]the National Institute of Allergy and Infectious Diseases and [d]Clinical Center, National Institutes of Health, Bethesda; [e]the Division of Allergy and Immunology, Department of Pediatrics, University of South Florida at Johns Hopkins–All Children's Hospital, St Petersburg; [f]the Center for Allergy and Inflammation, University of Washington, Seattle; and [g]the Division of Allergy and Immunology and [h]the Department of Medicine and Pediatrics, Immunology Institute, Icahn School of Medicine at Mount Sinai, New York.

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Corresponding author: Lisa R. Forbes, MD, Department of Pediatrics, Immunology, Allergy, and Rheumatology, Center for Human Immunobiology, 1102 Bates, Suite 330, Houston, TX 77030. E-mail: lisa.forbes@bcm.edu.

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patients with *STAT1* GOF mutations have immature CD56^{dim} NK cells with decreased expression of CD16, perforin, CD57, and impaired cytolytic function. STAT1 phosphorylation was increased, but STAT5 was aberrantly phosphorylated in response to IL-2 stimulation. Upstream inhibition of STAT1 signaling with the small-molecule Janus kinase 1/2 inhibitor ruxolitinib *in vitro* and *in vivo* restored perforin expression in CD56^{dim} NK cells and partially restored NK cell cytotoxic function.

Conclusions: Properly regulated STAT1 signaling is critical for NK cell maturation and function. Modulation of increased STAT1 phosphorylation with ruxolitinib is an important option for therapeutic intervention in patients with *STAT1* GOF mutations. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Signal transducer and activator of transcription 1, gain of function, natural killer cell deficiency, perforin, Janus kinase inhibition, ruxolitinib, natural killer cell maturation*

Abbreviations used
ADCC: Antibody-dependent cellular cytotoxicity
CCD: Coiled-coil domain
CMC: Chronic mucocutaneous candidiasis
CRISPR: Clustered regularly interspaced short palindromic repeats
DBD: DNA-binding domain
GOF: Gain of function
JAK: Janus kinase
NK: Natural killer
pSTAT: Phosphorylated STAT
SOCS: Suppressor of cytokine signaling
STAT: Signal transducer and activator of transcription
WT: Wild-type

Natural killer (NK) cells account for approximately 10% to 15% of all circulating lymphocytes[1] and are important early effectors in the innate immune response to a variety of viral infections.[2] Within peripheral blood, NK cells comprise 2 phenotypic and functional subsets[3]: CD56^{dim} NK cells are considered to be terminally mature and are the primary mediators of contact-dependent lysis of target cells,[1,3] and CD56^{bright} NK cells contain less mature NK cell subsets and are potent producers of cytokines.[4,5]

NK cells are derived from CD34^{+} precursors,[3,6] and their development can be stratified to 5 stages[7] characterized by specific surface receptors and proliferative and functional capacities.[8,9] CD56^{bright} NK cells are the minor subset (approximately 10%) of NK cells within peripheral blood.[5] They are characterized by low expression of perforin and high expression of CD94-NKG2A receptors,[7] and a subset of cells retain CD117 (c-Kit) expression.[7,10] NKG2D, NKp46, CD62 ligand, and detectable CD122 are expressed at high levels.[10-14] These cells are sources of cytokines reflected by high levels of IFN-γ and GM-CSF.[1,7-10] Functional and phenotypic intermediate populations between CD56^{bright} and CD56^{dim} cells have also been described in healthy donors.[9,11,15] NK cell terminal maturation is defined by upregulation of perforin, CD16, CD57, CD8, and NKp46 and downregulation of CD94.[7,11,13,14,16-19] NK cell development and homeostasis require IL-15,[20,21] and in both mouse and human systems, NK cells do not develop in the absence of IL-15.[22]

Classical natural killer deficiency is characterized by the absence of both NK cells and their cytotoxic function,[23-27] whereas functional NK cell deficiency is characterized by normal frequencies of NK cells in peripheral blood with decreased function.[28,29] There are several immune deficiency diseases that affect NK cell development, function, or both.[29] Patients with NK cell deficiency have increased susceptibility to viral infections, including herpesviruses, varicella zoster, herpes simplex, cytomegalovirus, and human papilloma virus.[23-27,30] Severe herpesvirus infection with decreased NK cell natural cytotoxicity has been reported in patients with loss-of-function mutations in signal transducer and activator of transcription (STAT) 1[31] and recently in 8 patients with *STAT1* gain-of-function (GOF) mutations.[32]

The STAT family includes 7 members: STAT1 to STAT4, STAT5a and STATb, and STAT6.[33] Activation through intracellular domains of cytokine receptors, including those for IFN-α/γ, IL-2, IL-4, IL-15, IL-21, and IL-6,[34-36] leads to association with Janus kinase (JAK) family members and recruitment and phosphorylation of STAT proteins.[33,37-40] Phosphorylated STAT (pSTAT) proteins form homodimers or heterodimers and translocate to the nucleus, where they bind to consensus sequences in the promoters of target genes.[33,41] In addition to roles in development and homeostasis, STAT proteins mediate viral defense in NK cells.[42] Upon IL-2 stimulation, pSTAT5 binds 2 enhancers located in the 5′ region of the perforin 1 gene *(PRF1)*, promoting its transcription[43]; upon IL-6 and IL-12 stimulation, this enhancer is bound by pSTAT1 and pSTAT4, respectively.[44,45] Stat5b knockout mice have significantly lower levels of perforin expression at baseline and greatly decreased NK cell cytolytic function.[46] In human subjects STAT5b deficiency is associated with abnormal NK cell development causing susceptibility to severe viral infections in these patients.[47] Heterozygous GOF mutations in *STAT1* lead to significantly higher levels of pSTAT1 and increased STAT1 response to type I and II interferons.[48] These mutations are mostly located in the coiled-coil domain (CCD) or DNA-binding domain (DBD) and lead to an excess of pSTAT1-driven target gene transcription.[48-50] Patients with these mutations can develop recurrent or persistent chronic mucocutaneous candidiasis (CMC) or other cutaneous mycosis,[48,49] staphylococcal infections, disseminated dimorphic fungal infections (*Coccidioides inmitis* and *Histoplasma capsulatum*), viral infections, and autoimmune disease.[51-54]

Our investigations of patients with unexplained significant viral susceptibility identified functional NK cell defects in patients with *STAT1* GOF mutations, suggesting that STAT1 is important for human NK cell differentiation and function. In this study we describe an immature and poorly functioning CD56^{dim} NK cell population with low perforin expression and impaired cytotoxic capacity in patients with *STAT1* GOF mutations. Administration of the specific JAK1/2 inhibitor ruxolitinib, both *in vitro* and *in vivo*, restored perforin expression in immature CD56^{dim} NK cells and partially restored NK cell cytotoxic function. Together, these data demonstrate the effects of ruxolitinib treatment and identify decreased perforin expression and impaired terminal maturation as contributing to functional NK cell defects in patients with *STAT1* GOF mutations.