blood®
Regular Article

LYMPHOID NEOPLASIA

Genome-wide CRISPR-Cas9 screen identifies rationally designed combination therapies for *CRLF2*-rearranged Ph-like ALL

Kensuke Sasaki,[1,2] Takuji Yamauchi,[1,2] Yuichiro Semba,[1] Jumpei Nogami,[1] Hiroshi Imanaga,[1] Tatsuya Terasaki,[1] Fumihiko Nakao,[1] Koshi Akahane,[3] Takeshi Inukai,[3] Els Verhoeyen,[4,5] Koichi Akashi,[1] and Takahiro Maeda[2-6]

[1]Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan; [2]Center for Cellular and Molecular Medicine, Kyushu University Hospital, Fukuoka, Japan; [3]Department of Pediatrics, School of Medicine, University of Yamanashi, Yamanashi, Japan; [4]CIRI-International Center for Infectiology Research, INSERM, Unité 1111, Université Claude Bernard Lyon 1, Centre National de la Recherche Scientifique, Unité Mixte de Recherche (UMR) 5308, Ecole Normale Supérieure de Lyon, Université Lyon, Lyon, France; [5]Université Côte d'Azur, INSERM, Centre Méditerranéen de Médecine Moléculaire (C3M), Nice, France; and [6]Division of Precision Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan

KEY POINTS

- **STAT signaling is dispensable for survival of *IgH-CRLF2*-r Ph-like ALL cells.**
- **A precision medicine approach based on mutational status, namely of *RAS*, is key for treatment of *IgH-CRLF2*-r Ph-like ALL.**

Acute lymphoblastic leukemia (ALL) harboring the *IgH-CRLF2* rearrangement (*IgH-CRLF2*-r) exhibits poor clinical outcomes and is the most common subtype of Philadelphia chromosome-like acute lymphoblastic leukemia (Ph-like ALL). While multiple chemotherapeutic regimens, including ruxolitinib monotherapy and/or its combination with chemotherapy, are being tested, their efficacy is reportedly limited. To identify molecules/pathways relevant for *IgH-CRLF2*-r ALL pathogenesis, we performed genome-wide CRISPR-Cas9 dropout screens in the presence or absence of ruxolitinib using 2 *IgH-CRLF2*-r ALL lines that differ in *RAS* mutational status. To do so, we employed a baboon envelope pseudotyped lentiviral vector system, which enabled, for the first time, highly efficient transduction of human B cells. While single-guide RNAs (sgRNAs) targeting *CRLF2*, *IL7RA*, or *JAK1/2* significantly affected cell fitness in both lines, those targeting *STAT5A*, *STAT5B*, or *STAT3* did not, suggesting that STAT signaling is largely dispensable for *IgH-CRLF2*-r ALL cell survival. We show that regulators of RAS signaling are critical for cell fitness and ruxolitinib sensitivity and that *CRKL* depletion enhances ruxolitinib sensitivity in *RAS* wild-type (WT) cells. Gilteritinib, a pan-tyrosine kinase inhibitor that blocks CRKL phosphorylation, effectively killed *RAS* WT *IgH-CRLF2*-r ALL cells in vitro and in vivo, either alone or combined with ruxolitinib. We further show that combining gilteritinib with trametinib, a MEK1/2 inhibitor, is an effective means to target *IgH-CRLF2*-r ALL cells regardless of *RAS* mutational status. Our study delineates molecules/pathways relevant for *CRLF2*-r ALL pathogenesis and could suggest rationally designed combination therapies appropriate for disease subtypes.

Introduction

Philadelphia chromosome-like acute lymphoblastic leukemia (Ph-like ALL, also known as *BCR-ABL1*-like ALL) is a disease entity of B-cell ALL (B-ALL) that exhibits a gene expression profile similar to that of Philadelphia chromosome-positive ALL (Ph^+ ALL).[1-4] Ph-like ALL comprises 15% to 30% of childhood and adult B-ALL and generally exhibits poor clinical outcomes.[4-7] Ph-like ALL is categorized into 2 disease subtypes: ABL-class and CRLF2/JAK pathway types, both of which harbor gene alterations that constitutively activate cytokine/growth factor-related signals.[4,8] The ABL-class type harbors alterations in genes encoding tyrosine kinases, such as *ABL1*, *ABL2*, *CSF1R*, *PDGFRA*, and *PDGFRB*, while the CRLF2/JAK pathway type harbors rearrangements of genes such as *CRLF2*, *EPOR*, and *JAK2*.[4,8]

Ph-like ALL with *CRLF2* rearrangements (*IgH-CRLF2* and *P2RY8-CRLF2*) comprises more than half of Ph-like ALL cases,[5,6,8] and those cases exhibit worse prognosis than those without *CRLF2* rearrangements.[5,6,9] Notably, nearly 60% of *CRLF2*-rearranged (*CRLF2*-r) ALL cases harbor pathogenic mutation(s) in *JAK2* and/or Ras-related genes, including *NRAS*, *KRAS*, *PTPN11*, and *NF1*.[10,11] The frequency of *IgH-CRLF2*-r ALL is reportedly more than twice that of *P2RY8-CRLF2* and increases with age.[8]

Tyrosine kinase inhibitor (TKI)-based treatment regimens are effective in treating ABL-class type Ph-like ALL[12]; however, no standard regimen has been established for the CRLF2/JAK pathway type. While ruxolitinib, a JAK1/2 inhibitor, has been tested in preclinical models, and ruxolitinib monotherapy and/or its combination with chemotherapy are being tested in clinical trials,[8] their efficacy is reportedly limited.[13] Thus, novel