

To the editor:

A phase 2 trial of HQK-1001 in HbE- β thalassemia demonstrates HbF induction and reduced anemia

β -thalassemia syndromes comprise a global health burden. Hemoglobin E (HbE)- β thalassemia represents 60% of regional populations, with an increasing prevalence in the coastal United States.^{1,2} Serious complications related to hemolysis and anemia in β -thalassemia intermedia (BTI, or nontransfusion-dependent thalassaemia [NTDT]) are common, with frequent progression to transfusion dependency and alloimmunization.³ There is no effective therapeutic to improve globin balance and reduce anemia for treatment of BTI. Renewed or persistent γ -globin expression is established to compensate for deficient β -globin; affected infants become anemic only after γ -globin suppression and patients with higher fetal hemoglobin (HbF) levels are less anemic than counterparts within genotypes with lower HbF levels.⁴⁻⁸

An oral, nonmutagenic, noncytotoxic short-chain fatty acid derivative, sodium 2,2 dimethylbutyrate (HQB-1001; HemaQuest Pharmaceuticals) induces the γ -globin gene promoter in predictive models, and prolongs erythroid cell survival through enhanced expression of Bcl-xL.⁹ A dose-ranging trial of HQK-1001 at 10, 20, 30, and 40 mg/kg daily for 8 weeks in 21 BTI subjects with HbE- β^0 and β^+ β^0 thalassemia mutations increased HbF by a mean of 6.6% above baseline at 20 mg/kg, the most effective dose.¹⁰ This suggested that investigating HQK-1001 treatment of longer periods was warranted.

Accordingly, an open-label trial (NCT01609595), approved by the Ethics Committee of the Department of Medical Science, Ministry of Public Health, Nonthaburi, Thailand, was performed in

10 NTDT patients with HbE- β^0 thalassemia, (7 female), ages 20 to 39 years. Subjects received HQK-1001 20 mg/kg once daily for 24 weeks, with 4 weeks' follow-up observation. Baseline complete blood counts (CBCs) and HbF were obtained twice before dosing and averaged; total Hb ranged from 6 to 8.7 g/dL. CBCs, HbF levels, and safety assessments (including examinations and chemistry panels) were performed monthly, urinalyses bimonthly, and electrocardiograms every 3 months. End points included change from baseline in HbF and total hemoglobin (Hb) as well as safety.

HQK-1001 was well tolerated. Adverse events consisted of mild nausea in 2 subjects, transient elevations in bilirubin, aspartate aminotransferase, and alanine aminotransferase, and 1 case each of tonsillitis and poststreptococcal nephritis.

HbF increased in all subjects from a mean of 36.4% to 47.3%; the mean increase in percentage of HbF was 10.9%, (range, 5%-21%; $P < .001$). Mean absolute HbF increased by 0.96 g/dL, from 2.9 to 3.9 g/dL ($P < .002$). Total Hb increased in 7 of 10 subjects; the mean change was 0.93 g/dL (range, 0.5-2.2 g/dL; $P < .05$), as shown in Figure 1. Rises in total Hb began often at week 20, suggesting higher responses might be achieved with longer treatment, particularly in more severely anemic patients, with baseline Hb < 8.0 g/dL. Two subjects who did not achieve a rise in total Hb had infections associated with declines in HbF.

These findings confirm that HQK-1001 increases HbF and total Hb levels in some β -thalassemia syndromes. Longer and alternate regimens, such as intermittent pulsed dosing,⁵ warrant evaluation to determine its hematologic potential. These findings showing higher responses with longer treatment than in prior trials suggest HQK-1001 merits further investigation in well-characterized β -thalassemia subjects, to determine whether select subsets, and younger subjects with greater hematologic reserve, will respond optimally.

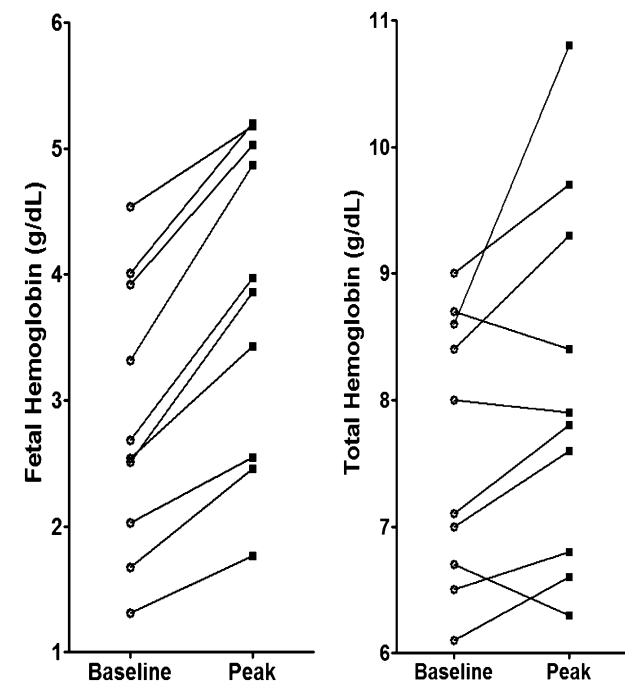


Figure 1. Baseline and peak HbF and total Hb in HbE- β^0 thalassemia subjects treated with HQK-1001.

Poramin Patthamalai
Mahidol University,
Bangkok, Thailand

Suthat Fuchareon
Mahidol University,
Bangkok, Thailand

Nattawara Chaneiam
Mahidol University,
Bangkok, Thailand

Richard G. Ghalié
HemaQuest Pharmaceuticals,
San Diego, CA

David H. K. Chui
Boston University School of Medicine,
Boston, MA

Michael S. Boosalis
Boston University School of Medicine,
Boston, MA

Susan P. Perrine
Boston University School of Medicine,
Boston, MA

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Contribution: S.P.P. and R.G.G. designed the study and wrote the letter; S.F., M.S.B., and D.H.K.C. analyzed laboratory data; and S.F., N.C., and P.P. treated patients and collected data.

Conflict-of-interest disclosure: R.G.G. is an employee of HemaQuest Pharmaceuticals; S.P.P. is an inventor on patents licensed to, and has equity interest in, HemaQuest Pharmaceuticals; and S.F. has received research support from HemaQuest Pharmaceuticals. The remaining authors declare no competing financial interests.

Correspondence: Susan P. Perrine, Boston University School of Medicine, 72 E Concord St, L-908, Boston, MA 02118; e-mail: sperrine@bu.edu.

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To the editor:

Ibrutinib antagonizes rituximab-dependent NK cell-mediated cytotoxicity

Ibrutinib is an irreversible inhibitor of Bruton's tyrosine kinase (BTK) with promising activity in CD20⁺ B-cell malignancies including recent US Food and Drug Administration approval in mantle cell lymphoma.¹ Given the homology between BTK and interleukin-2 inducible tyrosine kinase (ITK), we recently confirmed that ibrutinib irreversibly binds ITK.² ITK expression in Fc receptor (FcR)-stimulated natural killer (NK) cells leads to increased calcium mobilization, granule release, and cytotoxicity.³ As rituximab is a backbone of lymphoma therapy, with mechanisms of action including antibody-dependent cell-mediated cytotoxicity (ADCC), as well as direct induction of apoptosis and complement-dependent cytotoxicity⁴ and FcR stimulation is requisite for ADCC, we investigated if ibrutinib influenced rituximab's anti-lymphoma activity in vitro by assessing NK cell interferon- γ secretion, degranulation by CD107a mobilization, and cytotoxicity by chromium release using CD20⁺ cell lines and autologous patient samples with chronic lymphocytic leukemia (CLL), as well as in xenotransplant lymphoma athymic, ν/ν mouse models, as previously described.⁵ Trastuzumab and human epidermal growth factor receptor 2⁺ (HER2⁺) breast cancer cell lines provided an ADCC control, and CGI-1746, lacking ITK inhibition, represented a BTK selective control.⁶

We found that FcR-stimulated NK cells following exposure to rituximab-coated lymphoma cells express high and moderate levels of ITK and BTK, respectively. Ibrutinib inhibited both rituximab- and trastuzumab-induced NK cell cytokine secretion in a dose-dependent manner at 0.1 and 1 μ M of ibrutinib in vitro (Figure 1A; $*P = .009$, $**P = .001$, $***P < .001$). Similarly, ibrutinib prevented FcR-stimulated NK cell degranulation by $\sim 60\%$ and $\sim 90\%$ at 0.1 and 1 μ M, respectively (Figure 1B; $*P = .013$, $**P = .017$, $***P = .002$, $****P = .001$). Despite direct in vitro cytotoxicity due to ibrutinib independent of NK cells, NK cell-mediated cytotoxicity of both rituximab-coated, chromium-labeled lymphoma cells and trastuzumab-coated, chromium-labeled breast cancer cells was

inhibited in an ibrutinib dose-dependent manner (Figure 1C; $*P < .001$, $**P = .045$, $***P = .036$, $****P = .010$). We hypothesize that a dose effect is seen in Figure 1C with trastuzumab and not with ibrutinib as a result of increasing apoptosis, which is a direct dose-dependent effect of ibrutinib monotherapy. Therefore, in vitro, as higher doses of ibrutinib are combined with rituximab, the direct effect of BTK inhibition outweighs the inhibition of NK cell's ability to perform ADCC. In contrast, in vitro, CGI-1746 had no antagonistic effect on ADCC against rituximab-coated lymphoma cell lines or autologous CLL cells (Figure 1D; $*P < .001$). Abrogation of trastuzumab-dependent NK cell-mediated cytotoxicity was confirmed in vivo with concurrent ibrutinib daily dosing for 2 weeks during trastuzumab treatment (4 doses), as measured by tumor growth and survival (Figure 1E, $*P < .001$; Figure 1F, $*P = .18$). Concurrent ibrutinib daily dosing for 2 weeks during 4 doses of rituximab therapy similarly antagonized rituximab's efficacy, with anti-lymphoma activity of the combination equivalent to ibrutinib monotherapy (Figure 1G, $*P = .049$, $**P = .032$; Figure 1H, $*P = .29$). Sequential ibrutinib for 1 week followed by 2 doses of rituximab or sequential rituximab (2 doses) followed by ibrutinib for 1 week resulted in restored anti-lymphoma activity superior to concurrent combination therapy of ibrutinib for 2 weeks and 4 doses of rituximab (Figure 1I, $*P < .001$; Figure 1J, $*P < .001$).

The combination of a promising novel agent and current standard of care for the treatment of B-cell lymphomas is currently being explored in multiple phase 2 and 3 trials. Surprisingly, our preclinical investigation of the combination of ibrutinib and rituximab results in antagonistic effects. We demonstrate that the abrogation of both rituximab's and trastuzumab's antitumor efficacy is a result of ibrutinib's inhibition of FcR-stimulated NK cell function, specifically ADCC. Selective BTK inhibitors or alternative ibrutinib dosing schedules, sequential vs concurrent, may preserve the anti-lymphoma efficacy of both agents.