A Phase 1 study of the novel gamma-secretase inhibitor PF-03084014 in patients with T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma

Blood Cancer Journal (2015) 5, e350; doi:10.1038/bcj.2015.80; published online 25 September 2015

T-cell acute lymphoblastic leukemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL) are aggressive malignancies derived from neoplastic transformation of precursor T cells. T-ALL accounts for 10–15% of pediatric and 25% of adult ALL cases. Although current treatment with intensive chemotherapy regimens may achieve a cure rate of 80% in children with T-ALL, treatment of adult patients leads to a lower response rate.

Results from a number of studies have demonstrated a key role for the deregulation of NOTCH signaling pathways in the oncogenic transformation leading to the development of T-ALL and T-LBL, providing a rationale for the development of gamma-secretase inhibitors as a novel targeted therapy for these hematologic malignancies. PF-03084014 is a noncompetitive, reversible, targeted agent that selectively inhibits gamma secretase in multiple tumor types including leukemia and lymphoma. Treatment with PF-03084014 has demonstrated significant antitumor activity in preclinical models of lymphoid malignancies and solid tumors.

In study A8641014 (approved by the ethics and regulatory committees of the participating institutions, with signed patient informed consent, and following the Declaration of Helsinki and good clinical practice guidelines), eight patients with T-ALL or T-LBL received treatment with PF-03084014 150 mg twice daily in continuous cycles, and all were evaluable for safety and treatment response as well as pharmacokinetic and pharmacodynamic analyses. Mean patient age was 31 (range 18–43) years. Six patients were male and two were female; the majority (75%) was white (Table 1). Five patients had a primary diagnosis of T-LBL with a mean duration of 2.4 years and three had a primary diagnosis of T-ALL with a mean duration of 4.8 years. All eight patients had received prior systemic therapy; most patients (n = 7, 87.5%) had been treated with two or three prior regimens and three (37.5%) had received prior radiation therapy.

Treatment with PF-03084014 was associated with a complete response (CR) in a patient with T-ALL that lasted for ~3 months, with evidence of full hematologic recovery. This patient had been heavily pretreated, achieving a CR following induction therapy with cyclophosphamide, idarubicin, methotrexate, vincristine and dexamethasone, but not after salvage treatment with nelarabine, vincristine and cyclophosphamide; the patient also relapsed after a cord blood stem cell transplant performed 8 months before study entry.

NOTCH1 sequence analysis by conventional Sanger sequencing did not reveal any NOTCH1 mutation in the peripheral blood samples from five patients, including the T-ALL patient with a CR. Evaluation by the more sensitive deep-sequencing method revealed a known activating mutation, V1677D in bone marrow mononuclear cells from a non-responding patient with T-LBL. This suggests that mutation status does not consistently predict response to PF-03084014, in line with prior clinical trials.

The most common adverse events following treatment with PF-03084014 in patients with T-ALL/T-LBL were nausea and vomiting. Diarrhea was not a treatment-limiting toxicity in patients with evidence of full hematologic recovery.

Table 1. Patient baseline demographics and clinical characteristics

| Parameter | Patients (N = 8) |
|-----------|----------------|
| Age, years, mean (range) | 30.8 (18–43) |
| Male:female | 6:2 |
| Race, n (%) | |
| White | 6 (75.0) |
| Black | 1 (12.5) |
| Asian | 0 |
| Other | 1 (12.5) |
| ECOG PS, n (%) | |
| 0 | 3 (37.5) |
| 1 | 4 (50.0) |
| 2 (at baseline), 1 (at screening) | 1 (12.5) |
| Primary diagnosis, n (%) | |
| T-LBL | 5 (62.5) |
| T-ALL | 3 (37.5) |
| Disease duration since diagnosis, years, mean (range) | |
| T-LBL | 2.4 (0.4–7.4) |
| T-ALL | 4.8 (0.6–12.3) |
| Prior radiation therapy, n (%) | |
| Yes | 3 (37.5) |
| No | 5 (62.5) |
| Prior systemic therapies, n (%) | |
| Yes | 8 (100) |
| Systemic regimens, n (%) | |
| 1 | 1 (12.5) |
| 2 | 3 (37.5) |
| 3 | 4 (50.0) |

Abbreviations: ECOG PS, Eastern Cooperative Oncology performance status; T-ALL, T-cell lymphoblastic leukemia; T-LBL, T-cell lymphoblastic lymphoma.
with T-ALL/T-LBL, as previously observed with other investiga
tional gamma-secretase inhibitors\(^1,3,4\) and it was mostly low grade (grades 1 and 2). In contrast with the results obtained in patients with solid tumors,\(^5\) no rash and hypophosphatemia were reported in patients with T-ALL/T-LBL treated with PF-03084014, although the duration of treatment was shorter and the number of treated patients was substantially lower in this population. The causality of the dose limiting toxicity reported in this study, elevations in liver enzymes, remains unclear as it was observed in a patient who was receiving concomitant treatment with hepatotoxic drugs, and had chronic graft-versus-host disease and a suspected hepatic infection (candidiasis). Furthermore, no hepatic enzyme abnormalities (AST and ALT) and bilirubin elevations were noted when PF-03084014 administration was restarted at the reduced dose of 130 mg twice daily and continued for at least 2 months.

Pharmacokinetic analysis of PF-03084014 following single-dose and multiple-dose administration to patients with T-ALL or T-LBL demonstrated a favorable pharmacokinetic profile. Steady state was achieved by day 8 of treatment and the mean terminal half-life was 18 h (s.d., 3.6), after repeated daily dosing of PF-03084014. Treatment with PF-03084014 induced inhibition of HES4 gene expression levels at days 8, 15 and 21 of cycle 1 in the peripheral blood (as surrogate tumor tissue with no leukemic blast separation) of the majority of patients with T-ALL and T-LBL, thus providing a biomarker for measuring in vivo modulation of NOTCH pathway-related targets. Of note, HES4 gene expression levels were inhibited throughout cycle 1 in the responding patient with T-ALL, with an increase above baseline levels at disease relapse (end-of-treatment sample).

In conclusion, the anti-T-ALL activity demonstrated by PF-03084014 in this study, as well as the antitumor activity observed in patients with solid tumors,\(^5\) supports further evaluation of PF-03084014 in patients with T-ALL or T-LBL in an earlier therapeutic setting to limit the confounding factor represented by the poor prognosis associated with relapsed or refractory disease.

**CONFLICT OF INTEREST**

CP, DJD, WS, FK, GG-M and GM have no relevant disclosures. JCA is a consultant for Cell Signaling Technology and CytomX, Inc. BH, NMS, RC, DJD, WS, FK, GG-M and GM were full-time employees of Pfizer during the conduct of this study. The remaining authors declare no conflict of interest.

**ACKNOWLEDGEMENTS**

This study (registered at ClinicalTrials.gov, NCT00878189) was sponsored by Pfizer and in part by an award received by Giovanni Martinelli from ELN, AIL, AIRC, PRIN, progetto Regione-Università 2010-12 (L Bolondi, FP7 NGS-PTL project). The authors thank Deborah A. Thomas, MD, PhD, of Engage Scientific Solutions and was funded by Pfizer.

**AUTHOR CONTRIBUTIONS**

All authors (1) conceived and/or designed the work that led to the submission, acquired data and/or had an important role in interpreting the results; (2) drafted or revised the manuscript; and (3) approved the final version.

C Papayannidis\(^1\), DJ DeAngelo\(^2\), W Stock\(^3\), B Huang\(^4,5\), MN Shaik\(^5\), R Cesari\(^6\), X Zheng\(^7\), JM Reynolds\(^5\), PA English\(^5\), M Ozeck\(^5\), JC Aster\(^8\), F Kuo\(^7\), D Huang\(^5\), PD Lira\(^5\), KR McLachlan\(^7\), KA Kern\(^5\), G Garcia-Manero\(^8\) and G Martinelli\(^1\)

1Department of Experimental, Diagnostic, and Specialty Medicine, University of Bologna, Bologna, Italy; 2Adult Leukemia Institute, Dana-Farber Cancer Institute, Boston, MA, USA; 3Department of Medicine, Hematology/Oncology Section, University of Chicago, Chicago, IL, USA; 4Pfizer Oncology, Groton, CT, USA; 5Pfizer Oncology, San Diego, CA, USA; 6Pfizer Oncology, Milan, Italy; 7Department of Pathology, Dana Farber Cancer Institute, Boston, MA, USA and 8Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

E-mail: martg@tin.it or giovanni.martinelli2@unibo.it

**REFERENCES**

1 Ferrando AA, Neuberg DS, Staunton J, Loh ML, Huard C, Raimondi SC et al. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell 2002; 1: 75–87.
2 Pai CH, Robinson LL, Look AT. Acute lymphoblastic leukaemia. Lancet 2008; 371: 1030–1043.
3 Aster JC, Backlow SC, Pear WS. Notch signalling in T cell acute lymphoblastic leukaemia/lymphoma and other hematological malignancies. J Pathol 2011; 223: 262–273.
4 Pear WS, Aster JC. T cell acute lymphoblastic leukaemia/lymphoma: a human cancer commonly associated with aberrant NOTCH1 signaling. Curr Opin Hematol 2004; 11: 426–433.
5 Palermo T, Barnes KC, Real PJ, Glade-Bender JL, Sulis ML, Murty VV et al. CUTL1, a novel human T-cell lymphoma cell line with t(7;9) rearrangement, aberrant NOTCH1 activation and high sensitivity to gamma secretase inhibitors. Leukemia 2006; 20: 1279–1287.
6 Shi L, Wang TL. Notch signalling, gamma-secretase inhibitors and cancer therapy. Cancer Res 2007; 67: 1879–1882.
7 Armstrong F, Brunet de la Grange P, Gerby B, Rouyez MC, Calvo J, Fonteny M et al. NOTCH is a key regulator of human T-cell acute leukaemia initiating cell activity. Blood 2009; 113: 1730–1740.
8 Wei P, Walls M, Qiu M, Ding R, Denlinger RH, Wong A et al. Evaluation of selective gamma-secretase inhibitor PF-03084014 for its antitumor efficacy and gastrointestinal safety to guide optimal clinical trial design. Mol Cancer Ther 2010; 9: 1618–1628.
9 Samon JB, Castillo-Martin M, Hadler M, Ambesi-Impriato A, Paietta E, Racevskis J et al. Preclinical analysis of the γ-secretase inhibitor PF-03084014 in combination with glucocorticoids in T-cell acute lymphoblastic leukemia. Mol Cancer Ther 2012; 11: 1565–1575.

**Table 2. NOTCH1 mutations detected by deep sequencing in PF-03084014-treated patients**

| Primary diagnosis | Sample type | Blast (%) | Sequencing method | NOTCH1 mutation detected | Domain |
|-------------------|-------------|-----------|------------------|--------------------------|--------|
| T-ALL             | Bone marrow | 75        | Ion torrent      | None detected            | Not applicable |
| T-LBL             | Bone marrow | 11        | Ion torrent      | V1677D                   | HD     |
| T-ALL             | Bone marrow | 11        | Ion torrent      | V2444F*35                | PEST   |
| T-LBL             | Bone marrow | Undetectable | Ion torrent | L1679P                   | HD     |
| T-ALL             | Bone marrow | Undetectable | Ion torrent | L1679P                   | HD     |
| T-LBL             | Bone marrow | Undetectable | Ion torrent | V1672I                   | HD (SNP) |

Abbreviations: HD, heterodimerization domain; PEST, proline-, glutamic acid-, serine-, and threonine-rich domain; SNP, single-nucleotide polymorphism; T-ALL, T-cell lymphoblastic leukemia; T-LBL, T-cell lymphoblastic lymphoma.
10 Zhang CC, Pavlicek A, Zhang Q, Lira ME, Painter CL, Yan Z et al. Biomarker and pharmacologic evaluation of the gamma-secretase inhibitor PF-03084014 in breast cancer models. Clin Cancer Res 2012; 18: 5008–5019.

11 Gutiérrez A, Look AT. NOTCH and PI3K-AKT pathways intertwined. Cancer Cell 2007; 12: 411–413.

12 Palomero T, Sulis ML, Cortina M, Real PJ, Barnes K, Ciofani M et al. Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. Nat Med 2007; 13: 1203–1210.

13 DeAngelo DJ, Stone RM, Silverman LB, Stock W, Attar EC, Fearen I et al. A phase I clinical trial of the notch inhibitor MK-0752 in patients with T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) and other leukemias. J Clin Oncol 2006; 24: 6585.

14 Paganin M, Ferrando A. Molecular pathogenesis and targeted therapies for NOTCH1-induced T-cell acute lymphoblastic leukemia. Blood Rev 2011; 25: 83–90.

15 Messersmith WA, Shapiro GI, Cleary JM, Jimeno A, Dasari A, Huang B et al. A Phase I, dose-finding study in patients with advanced solid malignancies of the oral γ-secretase inhibitor PF-03084014. Clin Cancer Res 2015; 21: 60–67.