Clinical and molecular response of acute myeloid leukemia harboring non-canonical *FLT3* N676K driver mutations to contemporary FLT3 inhibitors

Treatment of acute myeloid leukemia (AML) has been enhanced by the development and regulatory approval of several novel agents, including midostaurin and gilteritinib (FLT3 inhibitors), venetoclax (BCL2 inhibitor), ivosidenib (IDH1 inhibitor), and enasidenib (IDH2 inhibitor).[1] A challenge during the era of molecular therapies, however, is determining the efficacy of these agents for patients with AML harboring atypical driver mutations. These atypical drivers were underrepresented in seminal clinical trials that led to the approval of targeted AML therapies, thereby limiting availability of data for clinical decision making.[2] The non-canonical *FLT3* N676K variant was initially described as an acquired resistance mechanism in patients with *FLT3* internal tandem duplication (ITD) mutations treated with midostaurin.[3] *In vivo* studies demonstrated *FLT3* N676K-mutated AML is sensitive to midostaurin and quizartinib, but suggested that co-operating ITD mutations confer resistance to both agents.[4] Clinical reports of *FLT3* N676K-mutated AML are limited to those of two individuals, both of whom developed *FLT3* N676K mutations at relapse.[4,5] Treatment outcomes for *de novo* disease with *FLT3* N676K mutations are lacking, and limited data exist regarding the utility of FLT3 inhibitors for *FLT3* N676K-mutated AML patients. The aim of this study was to use clinical and genomic data to investigate the efficacy of FLT3 inhibitors, both as monotherapy and in combination with other agents, for *FLT3* N676K-mutated AML.

We performed a retrospective analysis of patients with AML receiving care at the University of Chicago. The study was approved by the Institutional Review Board and conducted according to the Declaration of Helsinki. Our practice utilizes a validated 1,213 gene next-generation sequencing (NGS) panel that has been previously described.[6-8] NGS is employed at presentation and at subsequent time points to assess response or disease status. In cases of morphologic remission, NGS is not performed due to the anticipated lack of detectable tumor DNA. We identified nine patients with AML and *FLT3* N676K mutations. N676K was the only *FLT3* mutation detected in seven patients, whereas two patients had co-incident ITD or tyrosine kinase domain (TKD) mutations at some point during their clinical course. Two patients in the study were referred from outside of our institution and had an unknown *FLT3* mutation status at first presentation. The remaining seven patients were assessed by upfront NGS, and all patients had molecular reassessment longitudinally, including at relapse, by our in-house assay. The median age at AML diagnosis was 41 years (range, 17-79), with a mean presenting white blood cell (WBC) count of 53,300/μL. Four of seven (57%) patients with available cytogenetic data had normal cytogenetics. Laboratory and clinical data can be found in Table 1.

In order to infer antileukemic activity of FLT3 inhibitors for *FLT3* N676K-mutated AML and to characterize relapse dynamics, we analyzed *FLT3* N676K variant allele frequency (VAF) kinetics in patients for whom longitudinal NGS data were available. Regardless of disease status at the time of FLT3 inhibitor use (newly diagnosed *vs.* relapse), patients receiving FLT3 inhibitors had declines in *FLT3* N676K VAF. For individuals in whom *FLT3* N676K was the only *FLT3* mutation (patients 1, 2, 9), a mean treatment time of 95 days led to undetectable *FLT3* N676K. Suppression of *FLT3* N676K VAF generally paralleled clinical response and likelihood of survival at the time of our analysis (Figure 1A). One exception was patient 9, who achieved morphologic and molecular remission with gilteritinib salvage therapy but who unfortunately died of post-transplant veno-occlusive disease after exposure to gemtuzumab ozogamicin and subsequent allogeneic transplant.

One older patient pursued comfort care immediately after diagnosis. Six patients were treated with “7+3” induction therapy, with three of six patients having FLT3 inhibitors added to induction chemotherapy (midostaurin, n=2; sorafenib, n=1). Patient 1 had a complete morphologic and molecular remission. They proceeded to allogeneic transplantation after induction 7+3 therapy with sorafenib (discontinued for gastrointestinal toxicity) and subsequent midostaurin during pretransplant consolidation. Day 30 post-transplant bone marrow studies demonstrated a complete morphologic and molecular remission on midostaurin maintenance therapy.

Two additional patients (patients 5 and 6) harbored *de novo* disease with co-occurring *FLT3* mutations, one with a *FLT3* ITD mutation (patient 5) and the other a *FLT3* TKD mutation (patient 6). Each had midostaurin added to 7+3 induction. By days +71 and +32 after midostaurin treatment, respectively, both demonstrated remission with no detectable *FLT3* VAF (Figure 1B).

Patient 5 had *de novo* disease with co-occurring *FLT3* mutations (*FLT3* ITD F612_G613ins25 and N676K). She received 7+3+midostaurin induction and had a morphologic

and molecular complete remission 71 days after initiating midostaurin. Induction was complicated by fungal pneumonia and repeated episodes of acute kidney injury. She was not a candidate for cytotoxic consolidation therapy and started gilteritinib. She continued to experience multiple episodes of acute kidney injury unrelated to gilteritinib. Gilteritinib was held during these episodes, and she eventually presented with 25% circulating blasts after

Table 1. Clinical and laboratory characteristics of patients with *FLT3* N676-mutated acute myeloid leukemia.

	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9
Sex	F	F	M	M	F	F	F	M	M
Age at diagnosis in years	46	68	41	79	51	38	38	27	17
Presenting WBC, $x10^9$/L	70	1.5	87.8	123	93	25	15.4	2.8	60.2
Presenting BM blasts, %	56	37	65	na	na	74	95	80	78
Presenting PB blasts, %	5	0	43	92	76	68	90	0	80
Cytogenetics	t(8;16)	46, XX	46, XY	NA	+8	46, XX	NA	del(13q)	46, XY
FLT3 mutation at diagnosis	N676K	None	None	N676K	ITD N676K	D835V N676K	NA	NA	N676K
Induction backbone	7+3	Enasidenib	7+3	None	7+3	7+3	7+3	7+3	7+3+ Etoposide
Frontline FLT3i	Sorafenib	None	None	None	Midostaurin	Midostaurin	None	None	None
Induction response	CR MFC MRD-	PD	CR	None	CR *NPM1* MRD-	CR *NPM1* MRD-	CR MFC MRD-	CR MFC MRD-	PD
FLT3 mutation at relapse	None	N676K	N676K	None	ITD	N676K	N676K	N676K	N676K
Subsequent FLT3i?	Midostaurin	Gilteritinib monotherapy followed by azacitidine, venetoclax, and gilteritinib combination therapy	Midostaurin	None	Gilteritinib	None	None	None	Gilteritinib
Setting for subsequent FLT3i	Maintenance	Salvage & Post-HCT maintenance	Post-HCT maintenance	None	Maintenance	None	None	None	Salvage
Best response on FLT3i monotherapy/maintenance	NA	CR (0% blasts)	CR *NPM1* MRD-	NA	CR (0% blasts)	NA	NA	NA	PD
Best response on FLT3i combination therapy	CR MFC MRD-	CR (0% blasts)	NA	NA	CR *NPM1* MRD-	CR *NPM1* MRD-	NA	NA	PD
HCT	Yes	Yes	Yes	No	No	Yes	No	Yes	Yes
Alive	Yes	Yes	Yes	No	No	No	No	No	No
Survival in days*	NR (217)	NR (1,407)	NR (2,864)	4	445	516	239	577	1,363

Pt: patient; F: female; M: male; WBC: white blood count; BM: bone marrow; PB: peripheral blood; FLT3i: FLT3 inhibitor; HCT: hematopoietic cell transplant; NA: not available/not applicable; CR: complete remission; PD: progressive disease; NR: not reached; MFC: multiparameter flow cytometry; MRD: measurable residual disease. *For living patients, survival calculated as of manuscript submission and included in parentheses.

approximately 100 days of intermittent gilteritinib administration. NGS at relapse showed ascendancy of the same *FLT3* ITD clone (VAF 43%) that was present at diagnosis, but an absence of the *FLT3* N676K mutation. The patient chose comfort measures.

Patient 6 had *de novo* disease with co-occurring *FLT3* TKD (D835V) and N676K mutations. She underwent induction with 7+3+midostaurin, which led to morphologic and molecular remission 32 days after initiating midostaurin. She proceeded to hematopoietic cell transplantation in first remission. Despite MRD negativity at transplant, she relapsed after 6 months. She did not receive post-transplant FLT3 inhibitor therapy. NGS performed at relapse demonstrated expansion of the *FLT3* N676K population (VAF 33%) and absence of the original *FLT3* TKD clone. Salvage measures with donor lymphocyte infusion and high dose cytarabine/mitoxantrone were unsuccessful. She died of complications from central nervous system leukemic infiltration.

We also analyzed the spectrum of other pathogenic mutations co-existing with *FLT3* N676K in our cohort (*Online Supplementary Figure S1*). Co-mutational clusters were most notable for *FLT3* N676K and either *FLT3* ITD or *FLT3* TKD mutations (Figure 1C). In order to understand the structural properties of therapeutic inhibition of FLT3 in the presence of the N676K mutation, we utilized PyMOL (Schrödinger), an open source molecular graphics tool that is commonly used for visualization of macromolecules, to study the *FLT3* TKD harboring N676K in the presence and absence gilteritinib.[9] Upon activation, three residues, Asp-Phe-Gly (DFG), shift inward (DFG-in) from the inactive state (DFG-out). Mutations at D835 within the TKD favor the active DFG-in state and promote resistance to type II FLT3 inhibitors.[10] Gilteritinib and other type I FLT3 inhibitors bind directly to the ATP-binding site, maintaining their activity regardless of DFG conformation.[11] As shown in Figure 2, the N676K mutation does not prohibit the transition from DFG-out to DFG-in or the interaction of gilteritinib with the ATP-binding site.

A recent analysis of the mutational landscape of patients with *FLT3*-mutated AML treated on CALGB 10603 (RATIFY) showed 26 of 275 (5.5%) patients harbored non-canonical *FLT3* mutations.[2] Ten of these 26 patients (38%) had *FLT3* N676K-mutated AML.[2] Growing clinical application of NGS will increase the identification of atypical driver mutations. Robust clinical series focused on *FLT3* N676K-mutated AML patients are lacking, and the benefit of FLT3 inhibitor therapies in this population was previously unknown.

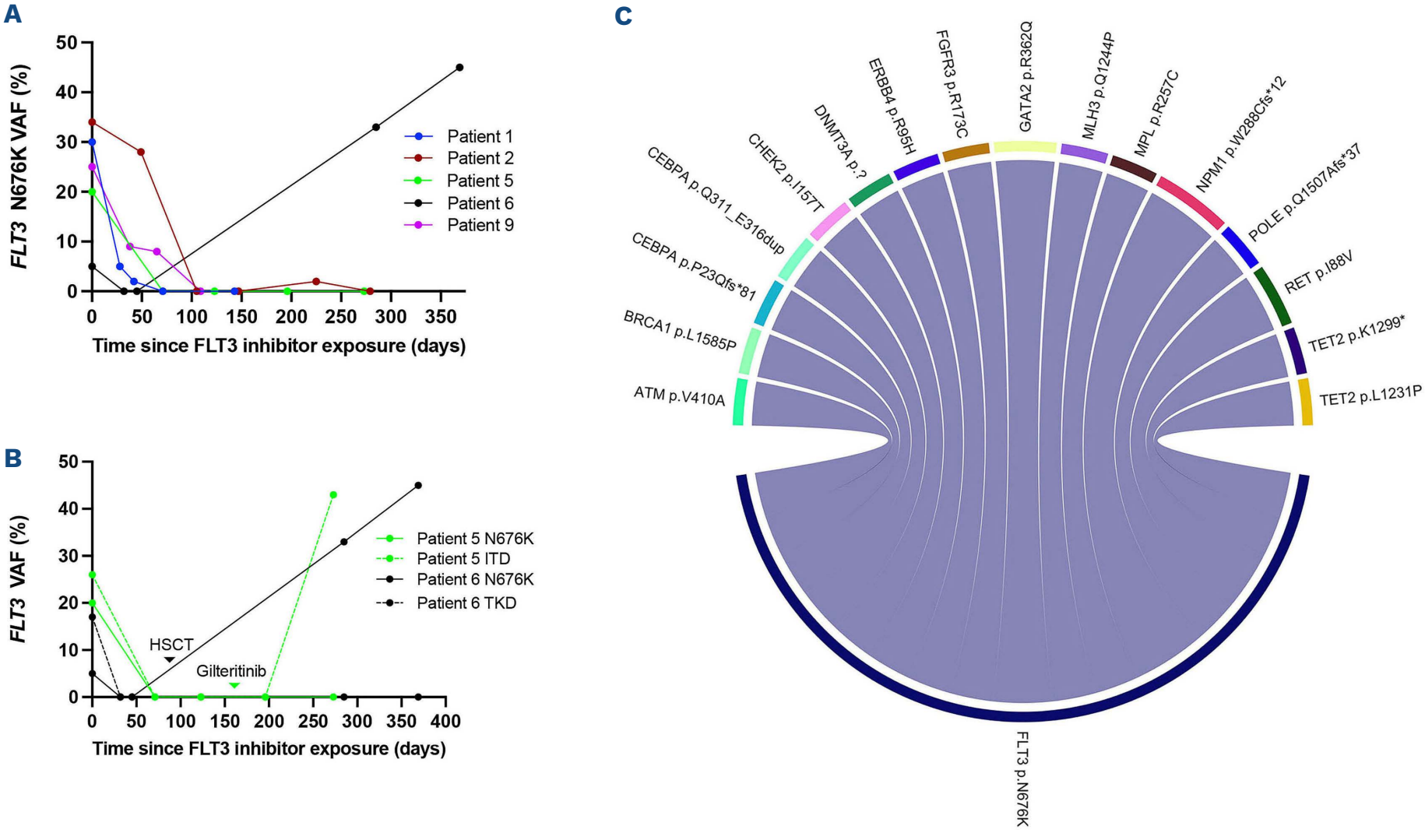

Figure 1. Clinical and molecular characterization of acute myeloid leukemia harboring atypical *FLT3* N676K mutations. (A) Longitudinal detection of *FLT3* N676K by next-generation sequencing from the time of FLT3 inhibitor initiation. (B) Clinical course and relapse dynamics of 2 patients with *FLT3* N676K-mutated acute myeloid leukemia (AML) and coincident internal tandem duplication or tyrosine kinase domain mutations. (C) Co-mutational networks of *FLT3* N676K-mutated AML seen in 2 or more patients within the cohort. Chord thickness reflects the number of co-occurrences between 2 genes.

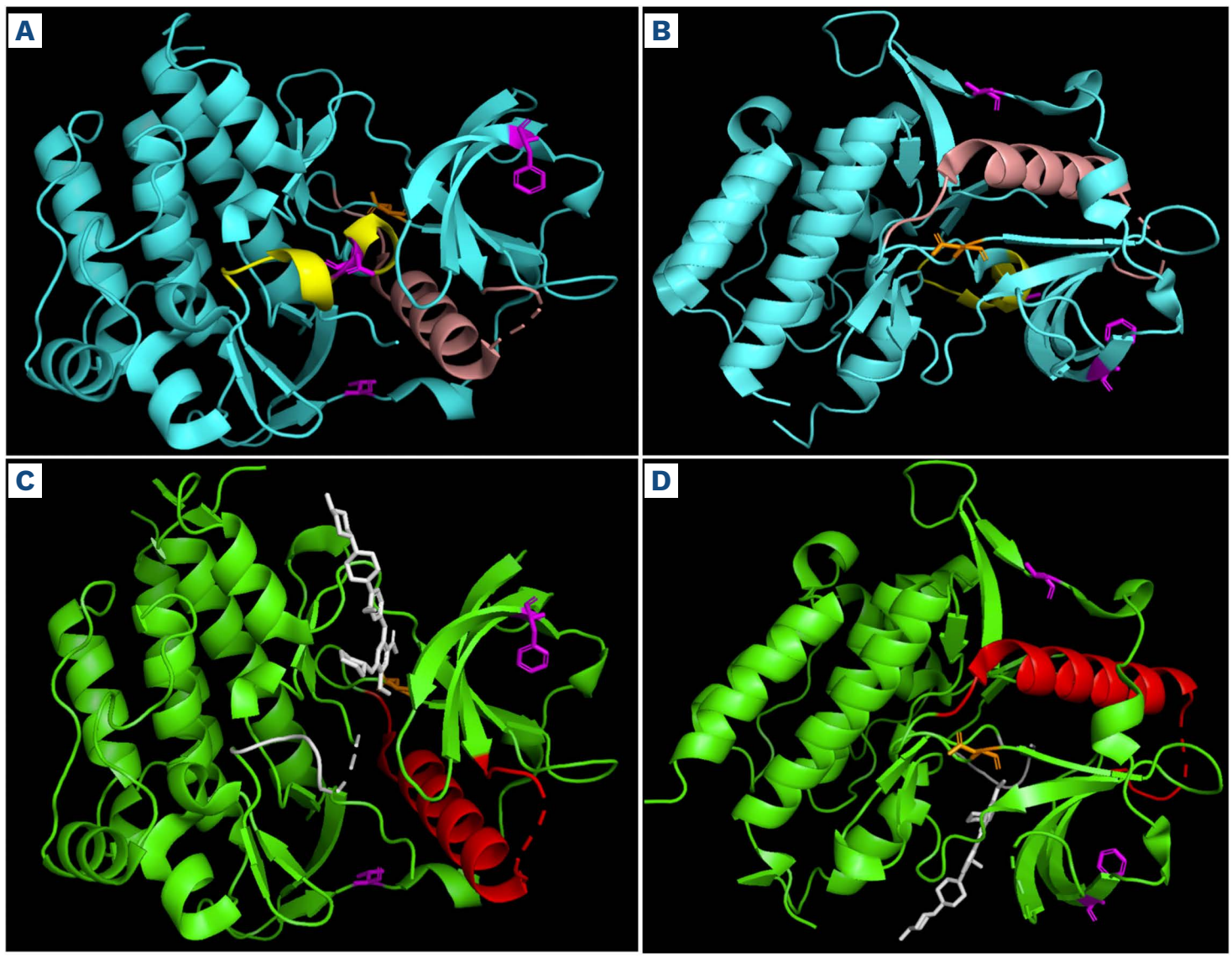

Figure 2. Macromolecular modeling of the FLT3 tyrosine kinase domain. (A) PyMol visualization models of the uninhibited FLT3 tyrosine kinase domain with N676K (residue shown in orange) and activation loop (yellow). Residues F612 and D835 (corresponding to internal tandem duplication and tyrosine kinase domain mutations, respectively) are shown in magenta. (B) Upon activation, the 18-fluoro-deoxyglucose (FDG)-containing α helix (salmon) translocates inward. Inhibition by gilteritinib at the ATP-binding site is depicted in the inactive, FDG-out (C) and activated, FDG-in (D) state with the FDG-containing α helix shown in red.

Here, we used clinical and genomic data to assess the utility of FLT3 inhibitors in the largest series of *FLT3* N676K AML patients described to date. Although previously described to be enriched in populations of core binding factor AML,[4] none of the seven patients in our cohort with available metaphase cytogenetic data available had core-binding factor AML. Seven of nine (78%) individuals received intensive induction chemotherapy. FLT3 inhibitors were utilized in three patients during frontline induction in combination with cytotoxic chemotherapy, and in five patients during subsequent lines of therapy. We observed reduction, and in some instances, complete molecular suppression of detectable *FLT3* N676K VAF on NGS, underscoring the activity of FLT3 inhibitors in this population, regardless of the line of therapy. All patients with *FLT3* N676K mutations who were treated with FLT3 inhibitors had a best response of MRD negativity via flow cytometry or NGS at some point during their care.

Consistent with previous evidence,[3,4] concurrent canonical ITD or TKD *FLT3* mutations were associated with loss of treatment response. *In silico* modeling of FLT3 in the presence of gilteritinib suggests that the mechanism of N676K-mediated resistance is not due to disruption of FLT3 inhibitor binding at the ATP-binding site but is likely influenced by other allosteric forces on the protein structure. Individuals with *FLT3* N676K-mutated AML in our cohort whose treatment included FLT3 inhibitors had longer median survival (940 days) than those who did not (408 days, excluding patient 4 who immediately pursued comfort measures). This difference was not significant, likely because of the small size of this study (P=0.2). The three patients who remained in an ongoing remission at the time of manuscript submission, however, were all treated with FLT3 inhibitors. With emerging evidence supporting the role of post-transplant FLT3 inhibitor maintenance therapy for suppression of *FLT3* ITD-mutated AML,[12] further studies evaluating the durability of FLT3 inhibitor maintenance for patients with non-canonical driver mutations in both transplant and non-transplant settings is warranted.

In conclusion, this is the largest study to date demonstrating that the atypical *FLT3* N676K driver mutation is sensitive to contemporary FLT3 inhibitors, such as midostaurin and gilteritinib. This mutation has been infre-

quently detected in seminal studies of FLT3 inhibitors. However, our data demonstrate FLT3 inhibitors should be included both in upfront induction setting and relapsed/refractory settings for patients harboring the atypical *FLT3* N676K mutation.

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Contributions

MWD conceived the study. MWD and GWR developed the concept and design of the study. MWD, GWR, FW and AS collected and analyzed the data. MWD, GWR, ASA, SK, WS, OO, RAL, HL, LAG, MJT, AAP, CKD, ASD, MTN and ED cared for the patients described. GDW, SG, CF, JPS, PW provided pathological support. GWR and MWD drafted the manuscript. All authors contributed to editing the manuscript.

Data-sharing statement

Data from the current work are available on request.

References

1. Estey EH. Acute myeloid leukemia: 2021 update on risk-stratification and management. Am J Hematol. 2020;95(11):1368-1398.
2. Jahn N, Jahn E, Saadati M, et al. Genomic landscape of patients with FLT3-mutated acute myeloid leukemia (AML) treated within the CALGB 10603/RATIFY trial. Leukemia. 2022;36(9):2218-2227.
3. Heidel F, Solem FK, Breitenbuecher F, et al. Clinical resistance to the kinase inhibitor PKC412 in acute myeloid leukemia by mutation of Asn-676 in the FLT3 tyrosine kinase domain. Blood. 2006;107(1):293-300.
4. Opatz S, Polzer H, Herold T, et al. Exome sequencing identifies recurring FLT3 N676K mutations in core-binding factor leukemia. Blood. 2013;122(10):1761-1769.
5. Daver N, Price A, Benton CB, et al. First report of sorafenib in patients with acute myeloid leukemia harboring non-canonical FLT3 mutations. Front Oncol. 2020;10:1538.
6. Kadri S, Long BC, Mujacic I, et al. Clinical validation of a next-

generation sequencing genomic oncology panel via cross-platform benchmarking against established amplicon sequencing assays. J Mol Diagn. 2017;19(1):43-56.
7. Patel AA, Rojek AE, Drazer MW, et al. Therapy-related myeloid neoplasms in 109 patients after radiation monotherapy. Blood Adv. 2021;5(20):4140-4148.
8. Cahill KE, Karimi YH, Karrison TG, et al. A phase 1 study of azacitidine with high-dose cytarabine and mitoxantrone in high-risk acute myeloid leukemia. Blood Adv. 2020;4(4):599-606.
9. Kawase T, Nakazawa T, Eguchi T, et al. Effect of Fms-like tyrosine kinase 3 (FLT3) ligand (FL) on antitumor activity of gilteritinib, a FLT3 inhibitor, in mice xenografted with FL-overexpressing cells. Oncotarget. 2019;10(58):6111-6123.
10. Smith CC, Lin K, Stecula A, Sali A, Shah NP. FLT3 D835 mutations confer differential resistance to type II FLT3 inhibitors. Leukemia. 2015;29(12):2390-2392.
11. Larrosa-Garcia M, Baer MR. FLT3 inhibitors in acute myeloid leukemia: current status and future directions. Mol Cancer Ther. 2017;16(6):991-1001.
12. Xuan L, Wang Y, Huang F, et al. Sorafenib maintenance in patients with FLT3-ITD acute myeloid leukaemia undergoing allogeneic haematopoietic stem-cell transplantation: an open-label, multicentre, randomised phase 3 trial. Lancet Oncol. 2020;21(9):1201-1212.