irradiated transfusions were associated with reduced short-term mortality compared with patients treated with conventional ABO identical, leukoreduced, irradiated transfusions. Long-term mortality in recipients of washed transfusions (20–40%) was half to two-thirds of that in the comparable historical comparison group and the current literature (60%) (Supporting Information Table 5). A limitation of these data, in addition to the lack of randomization, is that we did not collect detailed information on treatment regimens (e.g., choice and dose of anthracycline in AML). The striking differences we observed in long-term survival are unlikely solely due to progress in treatment regimens or supportive care. Identical differences were observed when we restricted the comparison to the years 2003–2005 and 2006–2008. For lower risk patients (favorable or intermediate cytogenetics; <46 years of age or younger) in New York State treated between 2006 and 2011 long-term mortality rate was 2.5-fold higher (50% versus 20%) in conventionally treated patients compared with recipients of washed transfusions.

This approach has the potential to substantially improve outcomes for many patients with AML. This may be limited, at present, to younger patients with favorable or intermediate cytogenetics. Larger randomized trials will be required to determine whether our promising results are generalizable and reproducible, and whether they might be applicable to older patients (who often receive less intensive therapy), and to patients with other hematologic malignancies or solid tumors.

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Authors Contribution

NB, KH, and DG had full access to the data. NB assumes full responsibility for the manuscript, and all authors contributed to the drafting, editing and finalizing of the manuscript.

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Additional Supporting Information may be found in the online version of this article.

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References

1. Blumberg N, Heal JM, Murphy P, et.al. Association between transfusion of whole blood and recurrence of cancer. Br Med J 1986;293:530–533.
2. Blumberg N, Heal JM, Rowe JM. A randomized trial of washed red blood cell and platelet transfusions in adult acute leukemia [ISRCTN76536440]. BMC Blood Disord 2004;4:6.
3. Roback J, Combs MR, Grossman B, Hillyer C. editors. AABB Technical Manual, 16th ed. Bethesda, MD: AABB; 2008.
4. Kalmin ND, Brown DJ. Platelet washing with a blood cell processor. Transfusion 1982;22: 125–127.

Treatment of central venous catheter-associated deep venous thrombosis in cancer patients with rivaroxaban

To the Editor: Central venous catheters (CVC) are an important tool in ongoing cancer therapy. However, central venous catheter-related, upper extremity deep venous thrombosis (CVC-UEDVT) is a common complication in patients with cancer [1]. These thromboses often lead to loss of the CVC, which presents an obstacle to ongoing treatment [2]. There is little prospective data and limited clinical trials on appropriate anticoagulation management of CVC thrombosis. However, low molecular weight heparin (LMWH) has been recommended and routine removal of the CVC has not been recommended [1–5]. There are no data available yet about the use of rivaroxaban for CVC-UEDVT.

TABLE I. Baseline Characteristics and Outcomes Of Patients With Central Venous Catheter-Associated Thrombosis Treated With Rivaroxaban

A. Baseline characteristics	
Average age	62 years
Sex: Male/Female	32/51
CVC-UEDVT, line type:	
Port	77
PICC	5
Leukapheresis catheter	1
CVC-UEDVT, anatomy:	
Internal jugular vein	37
Superior vena cava	16
Subclavian vein	10
Other	20
CVC-UEDVT, line type:	
Port	77
PICC	5
Leukapheresis catheter	1
Presentation:	
Symptoms of thrombosis	40
Line dysfunction	2
Incidental during routine scan	41
Initiation of rivaroxaban	
Time since event:	
Riva started within the first 7 days of diagnosis	60
Rivaroxaban start > 7 days after diagnosis	23
B. Outcomes	*N*
Completed 90 days	53
Line Removed <90 days	
Line removal for dysfunction	3
Line removal for other reasons. (completion of therapy, infection, thrombocytopenia,)	9
Major bleed	2
CRNMB leading to discontinuation of rivaroxaban	1
Death	6
New thrombosis in other blood vessel	3
Rivaroxaban discontinuation for medical reason other than endpoint	4
Transfer of care to other hospital	2
TOTAL	*83*

Since January 1, 2014, all rivaroxaban use at Memorial Sloan Kettering Cancer Center (MSKCC) is being monitored under an Internal Review Board (IRB) approved Quality Assessment Initiative. For this analysis, we have identified all patients with active cancer and a CVC-UEDVT from January 1, 2014 through February 24, 2016, treated with rivaroxaban. A CVC-UEDVT was identified by imaging study (CT, MRI, and/or ultrasound) and related clinical notes.

This was a retrospective analysis. The primary endpoint was preservation of line function through 90 days. Secondary endpoints included removal of central line for other medical reason, major bleeding (MB), clinically relevant non-major bleeding leading to discontinuation of rivaroxaban, death, and development of other venous thromboembolic event. Patients were censored if they reached an endpoint, or discontinued rivaroxaban prior to completion of 90 days. All clinical notes were reviewed by a combination of automated text search with predefined terms, followed by review by a study physician.

During the study period, we identified a key cohort of 83 patients with active cancer and a CVC-UEDVT, in whom the central line was present and functional at initiation of rivaroxaban. Patients whose CVC had been removed prior to initiation of anticoagulation were not included in this cohort.

Table I summarizes the baseline characteristics of the 83 patients. Forty one of the CVC-UEDVT events were identified incidentally on routine imaging studies and 42 were identified following symptoms ($N = 40$) or line dysfunction ($N = 2$). Most of the thromboses were found in an internal jugular vein ($N = 37$), the superior vena cava ($N = 16$), and subclavian vein ($N = 10$). An indwelling port was the predominant central access device.

In 55 patients rivaroxaban was the sole anticoagulant and 5 additional patients received less than 7 days of LMWH prior to transition to rivaroxaban. The remaining

patients transitioned to rivaroxaban after initial anticoagulation with LMWH. In three patients, the CVC-UEDVT developed in patients already on another anticoagulant for either atrial fibrillation or a previous thromboembolic event, and the patients were transitioned to rivaroxaban when the new CVC-UEDVT was identified. The majority of patients were in an advanced cancer stage; 60% stage IV and 13% stage III.

Our analysis focused on the 90-day period after the thrombosis. Within the 90-day period, in only three patients was the CVC line removed due to development of line dysfunction. These three patients developed inability to aspirate from a Port type central line on day 15, 20, and 36 of rivaroxaban anticoagulation. Fifty-three patients (64%) completed a follow-up time of 90 days without the removal of their central line, or reaching another endpoint (Table I). In addition, nine other patients had their CVC lines removed within the 90-day period, but not due to line failure. These were for end of cancer treatment ($N = 6$), infection ($N = 1$), thrombocytopenia ($N = 1$), and patient preference ($N = 1$). Other primary endpoints of note are listed in Table I(B), including six deaths, three new VTE at other sites, two major bleeds, and one clinically relevant non-major bleeding leading to discontinuation of rivaroxaban.

In this single institutional experience, rivaroxaban appears to be a good choice for treatment of a CVC-UEDVT. The failure rate at three months of treatment with rivaroxaban in this cohort is low, with only 3 patients out of 83 (3.6%) requiring CVC line removal due to development of line dysfunction. The overall rate of CVC line removal for any cause in our rivaroxaban cohort was 12 of 83 (14%). Our cohort study does not lend itself to direct comparisons with previous reports. With that limitation in mind, in the previously published Catheter Study of LMWH followed by warfarin, the overall rate of CVC line removal was 43% [3].

The safety profile of rivaroxaban use for CVC-UEDVT was encouraging. Major bleeding events occurred in two patients treated with rivaroxaban, with an estimate of 2.4%. In The Catheter Study and the upper-Extremity DVT arm of the RIETE trial, major bleeding was reported in 10.9% and 2.1%, respectively [3,6].

Overall the safety and efficacy of rivaroxaban use in patients with active cancer for treatment of central venous catheters associated upper extremity deep venous thrombosis is very favorable in this single institutional cohort. Nevertheless, randomized controlled trials are needed to confirm these results.

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References

1. Debourdeau P, Farge D, Beckers M, et al. International clinical practice guidelines for the treatment and prophylaxis of thrombosis associated with central venous catheters in patients with cancer. J Thromb Haemost 2013;11:71–80.
2. Verso M, Agnelli G. Venous thromboembolism associated with long-term use of central venous catheters in cancer patients. J Clin Oncol 2003;21:3665–3675.
3. Kovacs MJ, Kahn SR, Rodger M, et al. A pilot study of central venous catheter survival in cancer patients using low-molecular-weight heparin (dalteparin) and warfarin without catheter removal for the treatment of upper extremity deep vein thrombosis (The Catheter Study). J Thromb Haemost 2007;5:1650–1653.
4. Kucher N. Clinical practice. Deep-vein thrombosis of the upper extremities. N Engl J Med 2011;364:861–869.
5. Lee AY, Levine MN, Baker RI, et al. Low-molecular-weight heparin versus a coumarin for the prevention of recurrent venous thromboembolism in patients with cancer. N Engl J Med 2003;349:146–153.
6. Munoz FJ, Mismetti P, Poggio R, et al. Clinical outcome of patients with upper-extremity deep vein thrombosis: Results from the RIETE Registry. Chest 2008;133:143–148.

Effects of hydroxyurea on F-cells in sickle cell disease and potential impact of a second fetal globin inducer

To the Editor: Biochemical, epidemiologic, clinical, and genetic research over several decades has shown that any increment in fetal hemoglobin (HbF) reduces the clinical severity of sickle cell anemia, with significantly improved survival in US patients with HbF levels above the 75% percentile (8.6%) or with an absolute HbF $\geq$ 0.5 g/dL with hydroxyurea (HU) treatment [1,2]. While having 100% F-cells results in a benign condition in compound heterozygotes for HbS and hereditary persistence of HbF (HPFH), a level of 70–75% F-cells has been observed in the milder haplotypes, such as the Arabian-Indian haplotype [3–5]. Perhaps the most important protector of the sickle erythrocyte from deoxy HbS polymer-induced injury is the concentration of HbF/F-cell. A recent analysis of a population of African patients found low concentrations of HbF/F-cells in sickle cell patients in Tanzania, supporting the importance of this parameter [6]. The amount of HbF/F-cell required to entirely prevent HbS polymerization was recently proposed as a therapeutic target [1].

To investigate the impact of HU on HbF expression parameters other than total HbF in adult patients, we analyzed F-cells and HbF/F-cell in 56 adult sickle cell disease patients attending a sickle cell clinic for routine care, of whom 33 (60%) were taking HU at modest stable doses of 1,000–1,500 mg/day. Subjects were 20–65 years of age, with median age 31 years; 45% were females. Patients with an acute illness or transfusion within 4 weeks were not included. Proportions of F-cells and mean fluorescent intensity (MFI) of F-cells were analyzed from heparinized peripheral blood by flow cytometry. Cells were stained with a specific HbF antibody (Becton–Dickinson). F-cells and mean fluorescence intensity of positive cells was determined using Cell Quest software and used as an estimate of HbF/F-cell. HbF was analyzed by HPLC (Variant).

The mean HbF in HU-treated subjects was 8.8% compared to 5.0% in untreated subjects (Fig. 1A), a level nearly identical to that observed in the Multi-Center Study of Hydroxyurea that led to its FDA approval. Mean % F-cells was 34% in HU-treated subjects compared to 22.9% in untreated subjects ($P = 0.01$, t-test), shown in Fig. 1B. Fourteen of the 33 (42%) of HU-treated subjects demonstrated F-cell proportions $\geq$ 40%. Mean fluorescent intensity of F-cells in untreated patients compared to HU-treated patients was 37 vs. 48 fluorescence units, respectively, shown in Fig. 1B ($P = 0.01$). Several recently identified targeted HbF therapeutic inducing agents which act through differing mechanisms to increase fetal globin mRNA, HbF, and F-cells in vitro and in vivo, including sodium 2,2 dimethylbutyrate (ST20), benserazide (BEN), and the LSD-1 inhibitor RN-1 were evaluated for effects on HbF expression in erythroid progenitor cells cultured from at least 10 sickle cell patients [3]. All therapeutic candidates significantly induced fetal globin mRNA levels by 2.5- to 10-fold above untreated control cells from the same patients (Fig. 1C); mean increases above control were 2.5- to 2.8-fold with HU, RN-1, or ST20 (all, $P < 0.01$); 5.8-fold with BEN ($P < 0.001$); and 7-fold with combined treatment with BEN and HU ($P = 0.01$), analyzed by a nonparametric test.

Therapeutic targets for amelioration of clinical severity of sickle cell disease have been proposed as 20–30% HbF, 70–75% F-cells, and 10 pg HbF/cell, twice the threshold of 4–6 pg/cell which is the minimum previously detectable in flow cytometry assays [1]. F-cells undergo selective survival and have longer lifespans than non-F cells [3–5]. We used a pathway analysis to deconstruct the total effects of HU as either direct (HbF) or indirect (mediated by F-cell percentage). Pathway analysis tests a hypothetical pathway from predictors to responses against observed data using multiple regression equations. Standardized regression coefficients are computed for each relationship, adjusted for the other relationships, and shown next to each line connecting predictors to responses, and is shown for the patient data in Fig. 1D. This analysis indicates that HU contributes first to higher proportions of F-cells ($r = 0.47$, $P < 0.001$), and secondly to the amount of HbF ($r = 0.85$, $P < 0.01$), whereas, in contrast, a direct effect of HU to HbF was not statistically significant ($r = 0.09$, $P = 0.3$). In this analysis, 82% of the total effect of HU on HbF is an indirect effect mediated by F-cells. These data suggest that addition of a second, or perhaps multiple, HbF inducers may produce higher concentrations of HbF content in erythroid cells which differentiate with, or are primed by, HU. The findings here particularly suggest that addition of benserazide as a second therapeutic with HU may induce HbF expression closer to therapeutic targets proposed. As individual patients have highly variable baseline HbF expression patterns, monitoring these parameters may guide treatments to ameliorate clinical severity and indicate when multiple therapies are warranted.

Author Contributions

Y. Dai, J. Sangerman, A. D. Faller, D. Maharaj and X. Niu performed assays; A. Rock, O. Owoyemi, and P. Oneal obtained clinical correlations; S. Perrine, M. Nouraie, M. Steinberg, analyzed results and wrote the paper. M. Nouraie performed statistical analyses. S. Nekhai, S. Cui, and R. Taylor reviewed the manuscript.

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