REGULAR ARTICLE

blood advances

Presentation of BK polyomavirus–associated hemorrhagic cystitis after allogeneic hematopoietic cell transplantation

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Key Points

- BKPyV-HC causes macroscopic hematuria and cystitis that last a median of 2.4 and 3.4 weeks, respectively.
- High-grade viremia and immune deficits are associated with prolonged and more severe disease.

BK polyomavirus (BKPyV) has been associated with hemorrhagic cystitis (HC) after allogeneic hematopoietic cell transplantation (HCT), but the natural history of HC and factors associated with the clinical course are incompletely understood. We retrospectively analyzed allogeneic HCT patients transplanted from 2007-2017 who presented after platelet engraftment or after day 28 post-HCT with BKPyV-associated HC (BKPyV-HC), which was defined as a positive urine BKPyV PCR, ≥1 plasma BKPyV viral load result, and macroscopic hematuria (Bedi grade ≥2). Factors associated with resolution of macroscopic hematuria and resolution of all cystitis symptoms within 90 days after HC diagnosis were investigated in multivariable models. In 128 patients with BKPyV-HC, the median times from diagnosis to resolution of all symptoms, macroscopic hematuria, and urinary clots (present in 55% [71/128]) were 24 days (15-44), 17 days (10-30), and 14 days (5-26), respectively. Ninety percent of patients had BKPyV viremia at the onset of HC with a median viral load of 1850 copies/mL (interquartile range, 240-8550). In multivariable models, high plasma viral load (≥10 000 copies/mL) and cytopenias at the beginning of BKPyV-HC were significantly associated with longer macroscopic hematuria and cystitis symptoms. Use of cidofovir was not associated with shorter duration of illness. In conclusion, BKPyV-HC after allogeneic HCT is characterized by prolonged and severe symptoms and requires improved management strategies. High-grade viremia and cytopenias were associated with a longer duration of BKPyV-associated HC. Accurate descriptions of disease and factors associated with prolonged recovery will inform end points of future clinical trials.

Introduction

BK polyomavirus (BKPyV) infects ≥90% of the general population, typically during early childhood, and persists in the renourinary tract. Asymptomatic urinary shedding of BKPyV occurs in ~10% of immunocompetent healthy blood donors,[1] but in patients with impaired immune control, the rate and magnitude of BKPyV viruria increases.[2,3] BKPyV has been associated with BKPyV-associated nephropathy, best described in kidney transplant recipients, and BKPyV-associated hemorrhagic cystitis (BKPyV-HC).[3,4] BKPyV-HC is estimated to complicate 5% to 25% of allogeneic hematopoietic cell transplantations (HCTs)[5] and typically occurs after engraftment.[6-10]

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The full-text version of this article contains a data supplement.

BKPyV replication is frequently detected in urine and plasma following HCT.[11-14] The presence of BKPyV viruria is not diagnostic for presence of symptomatic disease, but high-level viruria and/or the presence of viremia have been associated with diagnosis of BKPyV-HC.[5,12,15-19] Other reported risk factors for BKPyV-HC include acute graft-versus-host disease (GVHD), myeloablative conditioning, cord blood transplant, male gender, and cytomegalovirus (CMV) viremia.[2,5,20-24] Previous studies have noted associations between BKPyV viruria/viremia and renal impairment or mortality,[13,15,21,25-27] but the relationship between viremia and the duration and severity of BKPyV-HC is less well described.[28] One proposed mechanism for the pathogenesis of BKPyV-HC describes a multistep process[4,29-31]: (1) conditioning regimens damage urothelial mucosa; (2) BKPyV reactivates and replicates during host immune suppression; and (3) donor immune cells attack viral antigens, which perpetuates bladder and mucosal damage. This proposal has been discussed by several working groups.[5,12] Another explanation is that BKPyV causes cytopathic effects in bladder cells following chemotherapy-induced injury without an explicit role of immune-mediated mechanisms.[16]

The clinical manifestations and consequences of BKPyV-HC can be severe and prolonged and include urinary frequency, dysuria, gross hematuria, urinary blood clots, and urinary obstruction requiring continuous bladder irrigation or renal/bladder procedures, but exact data on the natural history are lacking. Patients with BKPyV-HC have high rates of hospitalization and blood/platelet transfusions.[2,18,28] It is important to understand the natural history of this illness for clinical management and to select clinically relevant end points and estimate effect sizes for the design of clinical trials.

The purpose of this study was to describe the natural history of BKPyV-HC and investigate risk factors for more severe or prolonged courses, with specific emphasis on the relationship between BKPyV viral load in urine and blood and symptoms.

Methods

Patients

We retrospectively identified all patients of any age who underwent allogeneic HCT and were followed by the Seattle Cancer Care Alliance from 1 January 2007 to 31 December 2017, met viral criteria for BKPyV-HC (defined in "Definitions"), and developed postengraftment macroscopic hematuria. A subset (n = 32) of patients had retrospective testing for BKPyV using quantitative polymerase chain reaction (PCR) on weekly plasma samples through 100 days post-HCT as part of a previous study.[13,14] We used these additional samples to perform analyses among this patient subset (see "Statistical Analysis") but used clinically obtained results alone for our primary analyses.

Patient demographics, viral loads, blood products, and transplant-specific data were obtained from a prospectively managed transplant database. BKPyV load testing is described in the supplemental Methods. Chart review was performed to assess the start and end of urinary tract symptoms, use of pain and other symptom-modifying medications, presence of coinfections, antiviral treatment, and need for bladder irrigation or surgical procedures. This study was approved by the Fred Hutchinson Institutional Review Board.

Definitions

Patients were included if they had a positive urine BKPyV PCR and a plasma BKPyV PCR performed within 7 days of their urine test. To avoid including cases of HC related to peritransplant chemotherapy, patients were only included if macroscopic hematuria occurred after platelet engraftment or day 28, whichever came first. The beginning of BKPyV-HC was defined as the later of urine BKPyV positivity or the start of macroscopic hematuria; the end of BKPyV-HC was the end of macroscopic hematuria. The degree of hematuria was graded according to Bedi et al[32] (grade 1, microscopic hematuria; grade 2, macroscopic hematuria; grade 3, macroscopic hematuria with clots; and grade 4, macroscopic hematuria requiring instrumentation). We defined symptoms of cystitis to include macroscopic hematuria, dysuria, frequency, urgency, urinary clots, abdominal pain, and flank pain; these signs and symptoms were described before and after the defined start date of BKPyV-HC.

Cystitis symptoms and the presence of macroscopic hematuria were obtained from nursing and provider clinical documentation. If the end of a specific symptom was not explicitly stated, then the last day of that symptom was noted as the last day on which that symptom was reported.

Platelet engraftment was defined as the first day in a 7-day period in which patients had a platelet count of $\geq 20 \times 10^9$/L and did not receive platelet transfusion support.[8,33] Acute GVHD was graded by a committee responsible for managing a transplant database and counted as peak grade of GVHD starting on the day of diagnosis of GVHD.[34]

Clinical diagnosis and management of BKPyV-HC

Urine and plasma samples were tested for BKPyV based on provider suspicion of BKPyV-HC. Institutional guidelines for the diagnosis of BKPyV recommend urine BKPyV PCR testing for investigation of hematuria and plasma BKPyV testing if urine PCR is positive. Most patients had a plasma BKPyV viral load assessed at the beginning of their HC episode. For patients who did not, we retrospectively tested an available plasma samples for BKPyV using real-time quantitative PCR as has been described previously.[35-37] BKPyV-HC management, including use of intravenous cidofovir, was at the discretion of the attending physician.

Statistical analysis

Patients were followed for 90 days from the diagnosis date of BKPyV-HC to measure the following clinical outcomes: resolution of macroscopic hematuria, resolution of all cystitis symptoms, need for continuous bladder irrigation or urinary-specific surgical procedure, number of blood and platelet transfusions, and development of blood clots during their HC episode. Univariable and multivariable logistic (probability of clots), linear (number of blood and platelet transfusions required), and Cox proportional hazard models (time to hematuria resolution, time to cystitis resolution, and time to continuous bladder irrigation or renal/bladder-specific surgical procedure) were used to assess potential risk factors for clinical outcomes. Death with ongoing symptoms or hematuria was rare in this population, so patients were censored at time of death for all time-to-event outcomes. Variables evaluated as potential risk factors are defined in the supplemental Methods.

Covariates with $P < .2$ in univariable analyses were candidates for inclusion in the multivariable models, and baseline plasma BKPyV $\geq$10 000 was included a priori in each model irrespective of P value.

A candidate variable was retained in the multivariable model if $P \leq .1$ was in the full model or its exclusion did not change the estimate of BKPyV-related variables by >10%. Two-sided $P < .05$ was considered statistically significant. SAS Version 9.4 TS1M3 (SAS Institute, Cary, NC) was used for statistical analyses and figures were created in R (R Core Team, 2017).

A subset of patients had additional plasma BKPyV viral loads analyzed at regular intervals through a previous study[13,14] and had results available at times they were asymptomatic, reported cystitis symptoms without macroscopic hematuria, reported cystitis symptoms with macroscopic hematuria, and reported cystitis symptoms with macroscopic hematuria and clots (where applicable). Univariable and multivariable generalized estimating equation (GEE) models were used to evaluate the association between grade of HC and $\log_{10}$ plasma BKPyV viral load. Potential covariates were selected into the multivariable model using the strategy described above.

Sensitivity analysis

We performed sensitivity analyses to explore the significance of non–hematuria cystitis symptoms, different thresholds of plasma BKPyV load at diagnosis, and presence of copathogens; details are included in supplemental Methods.

Results

Study cohort

We included 128 adult and pediatric allogeneic HCT recipients out of 2558 total allogeneic HCT recipients from 2007 to 2017 (selection diagram in supplemental Figure 1). Demographics of the study cohort are shown in Table 1.

Clinical characteristics of BKPyV-HC course

Aggregate symptom information for all patients in the cohort are shown in Figure 1A-H, which represents the number of patients with each symptom on each study day, including days with symptoms prior to the study-defined beginning date. Patients presented a median of 58.5 days after HCT (IQR, 46-77.75). The median duration of overall symptoms was 24 days (IQR, 15-44), and median duration of macroscopic hematuria was 17 days (IQR, 10-30). Fifty-five percent (71/128) of patients had clots during their course, and the most frequently reported cystitis symptom was dysuria (105/128 [82%]). Clinical characteristics of BKPyV-HC course, copathogens identified, and interventions needed are shown in Figure 2A-C. Initial urine and plasma viral loads are shown in supplemental Figure 2.

In our cohort, 26 out of 128 patients (20%) died within the 90 days following diagnosis of BKPyV-HC; however, only 8% of patients (10/128) died prior to resolution of BKPyV-HC (cumulative incidence of resolution and death shown in Figure 3). Notably, a small proportion of patients had symptoms (9/128 [7%]) or hematuria (4/128 [3%]) that lasted >90 days. No death was directly attributed to BKPyV-HC. Of the 7 patients requiring renal/bladder procedures during their course, 1 patient required ureteral stent placement, and the remainder needed diagnostic procedures, including cystoscopy (1/7), renal biopsy (4/7), or bladder (1/7) biopsy to evaluate potential renal obstruction or nephropathy. Neither BKPyV-HC nor BKPyV-associated nephropathy was definitively diagnosed by renal biopsy.

Intravenous cidofovir is the most commonly used antiviral agent for BKPyV-HC at our center but was given to few patients; intravesical cidofovir and leflunomide were not used, and fluoroquinolones were only used for BKPyV-HC in 1 patient. Of the 19 patients given intravenous cidofovir at the start of illness, 11 out of 19 (58%) received 0.25 to 0.5 mg/kg per week and 8 out of 19 (42%) received ≥1 mg/kg per week for a median duration of 28 days (IQR, 23.5-50 days) during the study period. Probenecid was given to patients receiving doses ≥5 mg/kg.

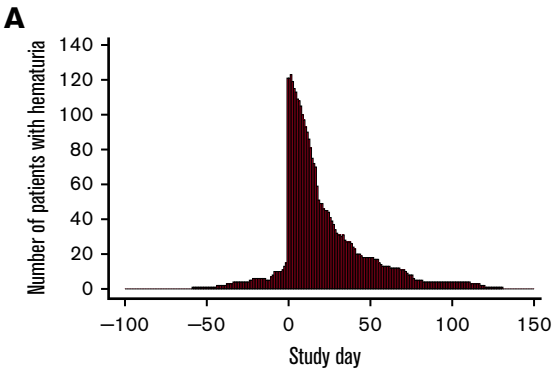
Table 1. Baseline demographics of the cohort

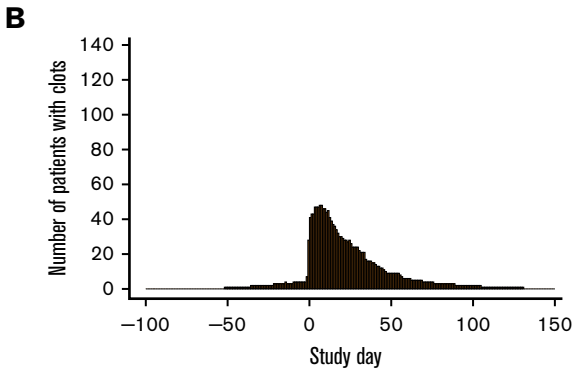
Characteristics	Number of patients (N = 128)
Age at transplant, median (IQR), y	47.2 (26.2-57.4)
Male sex	83 (64.8)
Primary disease requiring transplant	
Acute leukemia/MDS	65 (50.8)
Chronic leukemia	9 (7)
Other*	54 (42.2)
Number of transplants	
1	98 (76.6)
2	27 (21.1)
3	3 (2.3)
CMV serostatus	
D+R−	13 (10.2)
D-R+	52 (40.6)
D+R+	28 (21.9)
D-R−	35 (27.3)
Type of transplant	
Matched related	20 (15.6)
Matched unrelated	44 (34.4)
Mismatched	64 (50)
Source of transplant	
BM	23 (18)
Cord	29 (22.6)
PBSCT	76 (59.4)
Myeloablative conditioning	38 (29.7)
Cyclophosphamide conditioning	75 (58.6)
Posttransplant cyclophosphamide use	21 (16.4)
Any cyclophosphamide use	86 (67.2)
High-dose TBI conditioning	23 (18)
Busulfan conditioning	48 (37.5)

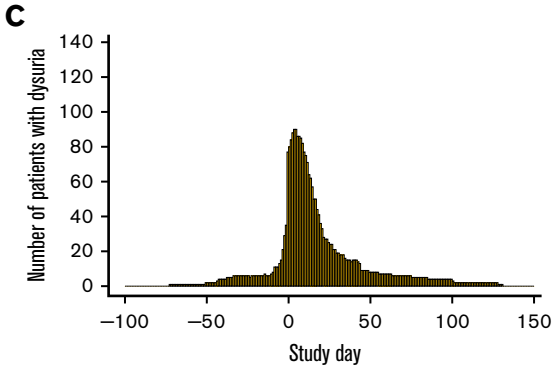
Values are presented as n (%) of patients unless otherwise indicated.
BM, bone marrow; D, donor; IQR, interquartile range; MDS, myelodysplastic syndrome; PBSCT, peripheral blood stem cell transplant; R, recipient; TBI, total body irradiation.
*Other includes aplastic anemia (n = 5), immunodeficiency disorders (n = 1), Hodgkin disease (n = 3), lymphoma (n = 14), multiple myeloma (n = 4), myelofibrosis (n = 10), other leukemia not fitting into above groups (n = 2), refractory anemia with or without blasts (n = 11), sarcoma (n = 1), sickle cell disease (n = 2), and systemic sclerosis (n = 1).

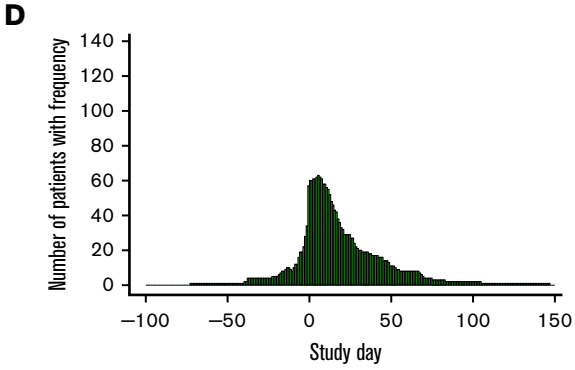
Analysis of factors associated with longer/more severe courses of BKPyV-HC

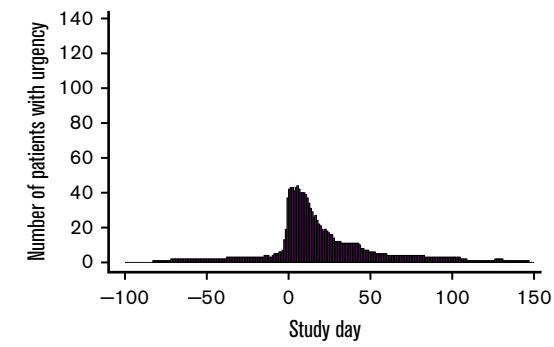
In univariable analyses, each increase in $\log_{10}$ plasma BKPyV load was associated with lower likelihood of, and therefore longer time to, resolution of all symptoms and resolution of macroscopic

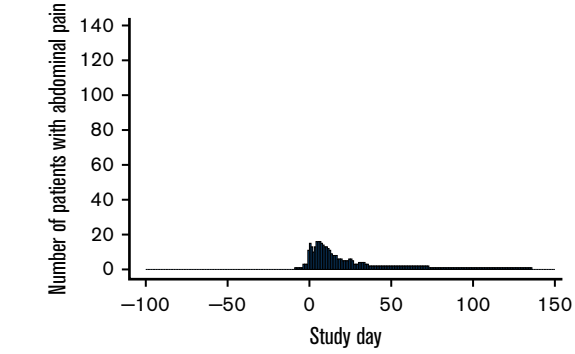


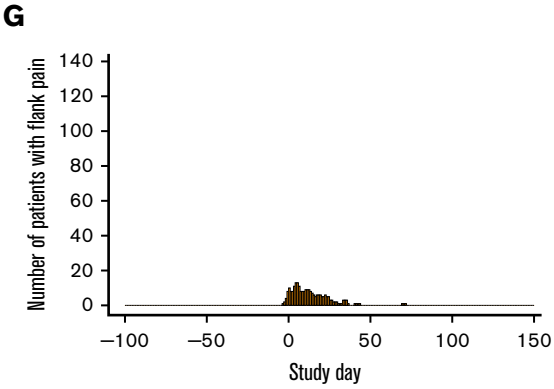












H

Symptom	N (%)	Mean (days)	Median days, (IQR)
Hematuria	128 (100)	26.14	17 (10 – 30)
Clots	71 (55)	22.3	14 (5 – 26)
Dysuria	105 (82)	22.96	14 (9 – 26)
Frequency	78 (61)	28.95	18 (10.5 – 36.5)
Urgency	57 (44)	27.04	14 (8 – 22)
Abdominal pain	24 (19)	17.16	7 (3 – 17)
Flank Pain	20 (16)	8.83	3 (2 – 17)
Use of phenazopyridin/ oxybutynin	82 (64)	27.54	17 (7 – 32)
Any cystitis symptom	128 (100)	38.5	24 (15 – 44)

Figure 1. Clinical presentation of BKPyV-HC on each study day by reported symptom. (A) Macroscopic hematuria. (B) Urinary clots. (C) Dysuria. (D) Frequency. (E) Urgency. (F) Abdominal pain. (G) Flank pain. Day 0 represents the diagnosis of BKPyV-HC, defined as the later of a positive urine BKPyV sample or the start of macroscopic hematuria. (H) A summary of proportion of patients reporting each symptom and median duration. If a patient died, then the symptom was only counted for the days they were alive.

A

	n=128 (%)
Plasma BKPyV viral load, median (IQR)	1850 copies/mL (242 – 8550)
Urine BKPyV viral load, median (IQR)	$8.9*10^8$ copies/mL ($2.1*10^8$ – $4.6*10^9$)
Plasma CMV viral load, median (IQR)	0 IU/mL (0 – 50.5)
ALC[a]	
<100	17 (13)
≥100 and <300	42 (33)
≥300	69 (54)
AMC[a]	
<100	65 (51)
≥100 and <300	36 (28)
≥300	24 (19)
ANC[a]	
<500	36 (28)
≥500 and <1000	8 (6)
≥1000	74 (58)
Acute GVHD diagnosis[b]	
grade 0-1	38 (30)
grade 2	58 (45)
grade 3-4	32 (25)
Chronic GVHD diagnosis[c]	15 (12)
Steroid dose (mg/kg of prednisone equivalents)	
<1	85 (66)
≥1 and <2	33 (26)
≥2	10 (8)
Cidofovir use[b]	19 (15)
Timing of BKPyV-HC diagnosis after transplant	
<60 days	66 (52)
≥60 days	62 (48)
Bedi grade at presentation of BKPyV-HC[d]	
2	90 (70)
3	38 (30)

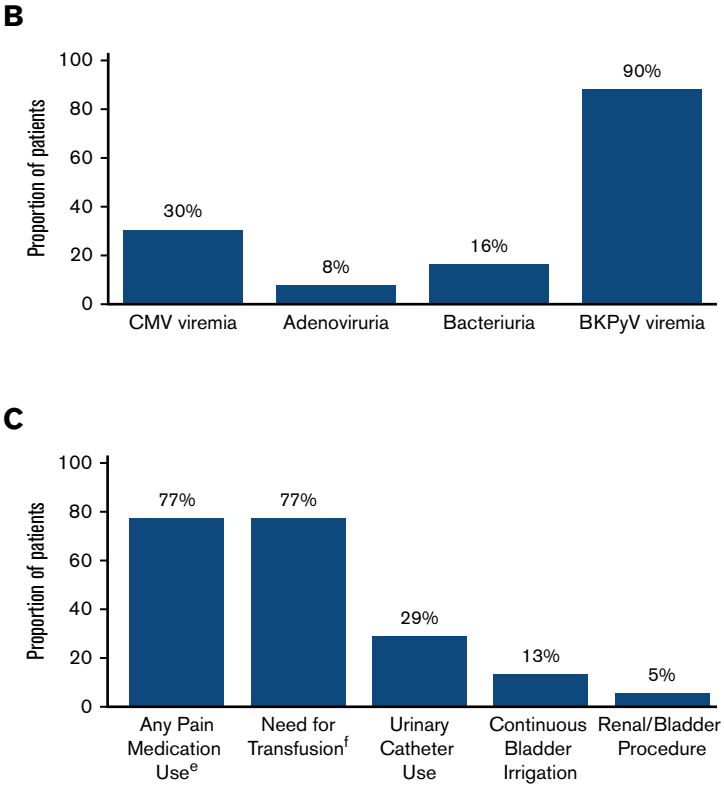


Figure 2. Characteristics of patients at time of diagnosis of BKPyV-HC. (A) Laboratory data, comorbidities, and medication use. (B) Presence of copathogens detected at time of diagnosis. (C) Specific clinical outcomes observed during their course. [a]Cell counts are from the closest test within 14 days before or after beginning of BKPyV-HC and are measured in cells/μL. [b]Including diagnoses up to 90 days before or 7 days after beginning of BKPyV-HC. Grade is the peak grade of the episode. [c]Includes cidofovir use up to 7 days before or after baseline date. Among the 19 patients on cidofovir, 4 (21%) had a concomitant diagnosis of adenovirus. [d]Bedi score 2 indicates macroscopic hematuria, and 3 indicates presence of clots.32 [e]Pain medication use includes opiates (n = 74), oxybutynin (n = 46), or phenazopyridine (n = 72). [f]Transfusion includes red blood cell (n = 92) or platelet (n = 79) transfusions. ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count.

hematuria but was not associated with the number of platelet transfusions received; plasma BKPyV expressed as the presence or absence of viremia was not associated with the number platelet transfusions received, time to resolution of symptoms, or time to resolution of macroscopic hematuria (supplemental Table 1A-C). Baseline urine BKPyV viral load (expressed in $\log_{10}$ copies/mL as a continuous variable) was associated with an increased time to hematuria resolution but no other end points, and a time-varying urine BKPyV viral load (expressed as the presence of viruria $\geq 10^9$ copies/mL) was not significant in any of the time-dependent Cox models. To focus our analyses on the contribution of high-grade BKPyV viremia, we included baseline BKPyV viral load ≥10 000 copies/mL in our multivariable models; this cutoff was close to the upper quartile of baseline viral loads and has been previously associated with BKPyV disease.[25,27,38] BKPyV viremia ≥10 000 copies/mL at the start of BKPyV-HC was significantly associated with longer time to resolution of all symptoms and macroscopic hematuria as well as the number of platelet transfusions required.

The results of multivariable models are shown in Figure 4. Baseline BKPyV viral load ≥10 000 copies/mL was significantly associated with a longer time to resolution of hematuria and longer time to resolution of all cystitis symptoms; it showed a trend toward being associated with the number of platelet transfusions but was not significantly associated with the number of blood transfusions needed (difference in mean number of transfusions, 2.7 [95% confidence interval (CI), −4.1, 9.5], $P = .44$) or the odds of developing clots (odds ratio, 1.31 [95% CI, 0.5, 3.46], $P = .58$). A time-varying covariate of BKPyV viral load ≥10 000 copies/mL was not associated with the hazard of needing bladder irrigation or renal/bladder procedures (hazard ratio [HR], 1.15 [95% CI, 0.42, 3.14], $P = .78$).

To examine the potential contribution of immune reconstitution to prolonged symptoms or hematuria, we examined the slope of the absolute lymphocyte count between the beginning and end of BKPyV-HC; this was not significantly associated with outcomes in any of our models. Cytopenias (defined as absolute lymphocyte

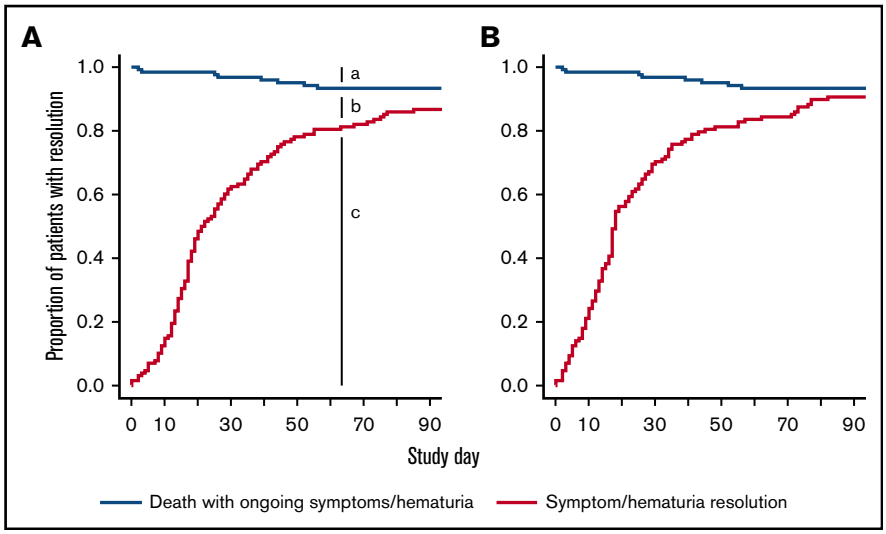


Figure 3. Incidence of disease resolution and mortality over the study period. Cumulative incidence (red) of symptom resolution (A) and hematuria resolution (B) plotted with the proportion of the population who died with ongoing symptoms or hematuria (blue). Among patients who died with ongoing BKPyV-HC, causes of death in this cohort included bleeding events (n = 2), hepatic failure due to vanishing bile duct syndrome (n = 1), *Clostridioides difficile* colitis (n = 1), GVHD (n = 1), pneumonia (n = 3), acute heart failure (n = 1), and sepsis related to cholecystitis (n = 1). No autopsies were done among this group of patients. At any time point, the height over the blue line represents the proportion of patients who have died during their BKPyV-HC episode (a), the height between blue and red lines represents the proportion of patients who are alive with ongoing symptoms/hematuria (b), and the height under the red line represents the proportion of patients whose symptoms/hematuria have resolved (c).

count <100 cells/μL, absolute monocyte count <100 cells/μL, and absolute neutrophil count <500 cells/μL) at the beginning of BKPyV-HC were associated with the number of platelet transfusions, prolonged symptoms, and macroscopic hematuria and were included in multivariable models.

In adjusted models, baseline cytopenias were also associated with longer or more severe courses of BKPyV-HC (Figure 4). Baseline neutrophil count <500 cells/μL was significantly associated with a longer duration of hematuria, baseline monocyte count <300 cells/μL was significantly associated with longer durations of cystitis, baseline monocyte count <300 cells/μL showed a trend to for higher risk of bladder irrigation or renal/bladder procedures (HR, 5.84 [95% CI, 0.78, 43.7], $P = .09$), and baseline lymphocyte counts <100 cells/μL were significantly associated with higher platelet and red blood cell transfusions (difference in mean number of transfusions, 10.5 [95% CI, 2.8, 18.3], $P = .008$) (supplemental Table 2).

Association between cidofovir use and outcomes

Due to the small number of patients who received cidofovir, we did not analyze dosing strategies. Intravenous cidofovir use of any dose was not associated with development of clots or number of platelet or red blood cell transfusions in univariable models. In univariable models, intravenous cidofovir use was associated with a trend toward higher hazard for bladder irrigation or renal/bladder surgical procedure (6/19 patients[32%] with cidofovir use vs 17/109 patients [16%] without; HR, 2.52 [95% CI, 0.91, 7.03], $P = .08$) and a trend toward longer duration of BKPyV-HC (median 23 days for patients with cidofovir use vs 17 days without; HR for resolution, 0.70 [95% CI, 0.43, 1.12], $P = .14$) and was significantly associated with longer duration of all cystitis symptoms (median 30 days for patients with cidofovir use vs 19 days without; HR for resolution, 0.58 [95% CI, 0.35, 0.97], $P = .038$) (factors with HR <1 are associated with lower likelihood of symptoms or HC and therefore longer duration of symptoms or HC). Finding an association between cidofovir and longer or more severe courses of BKPyV-HC is at least in part because of a substantial selection bias in patients who received cidofovir; cidofovir was more likely to be given in patients with higher baseline Bedi scores ($P = .02$) and higher baseline plasma viral loads ($P = .04$). As a result, in our primary model, we reported multivariable models without adjustment for cidofovir use.

However, to evaluate whether cidofovir use was confounding our estimates, we examined additional models that included cidofovir use when it met inclusion criteria and remained in the final model (time to symptom resolution and time to hematuria resolution). Cidofovir use was not significantly associated with either outcome (HR, 0.51 [95% CI, 0.91, 1.40], $P = .19$ for time to hematuria resolution; HR, 0.49 [95% CI, 0.18, 1.35], $P = .17$ for time to cystitis resolution), and estimates for other covariates did not qualitatively or quantitatively change as a result of this addition.

Analysis of association between viral load and grade of HC

Because our study used only samples obtained in the context of signs/symptoms of HC, BKPyV viral loads were not obtained in asymptomatic individuals. Thus, we could not draw conclusions about the relationship between viral load and the presence/lack of symptoms. However, 32 of our patients had regularly obtained plasma BKPyV viral loads obtained in the first 100 days posttransplant as part of a separate prospective study.[13,14] Demographics and clinical characteristics of this subset are in supplemental Tables 3 and 4. In these 32 patients, we performed univariable and multivariable GEE models to examine the impact of plasma viral load on the evolution of asymptomatic to symptomatic disease among patients who eventually developed HC. We found that the plasma

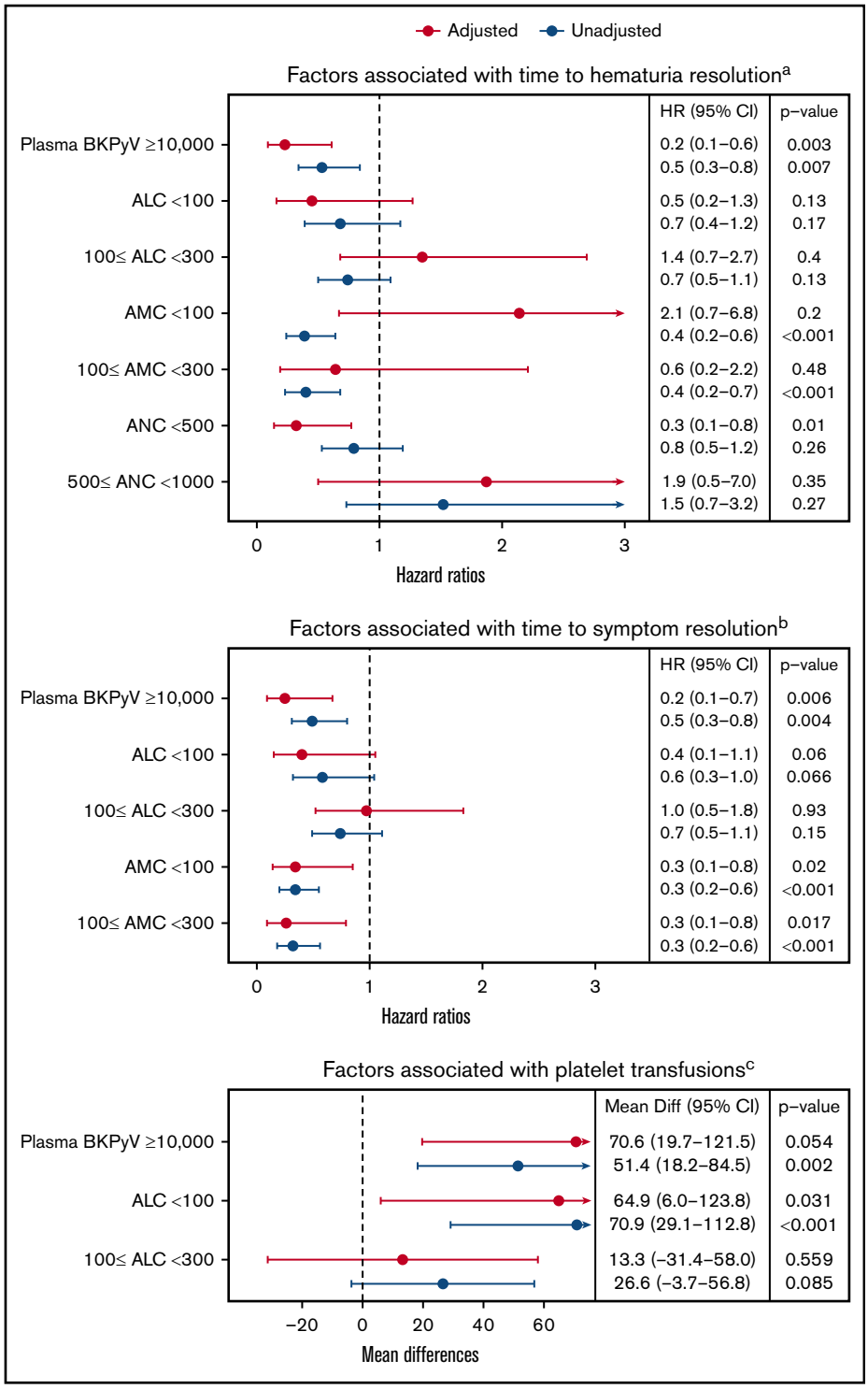
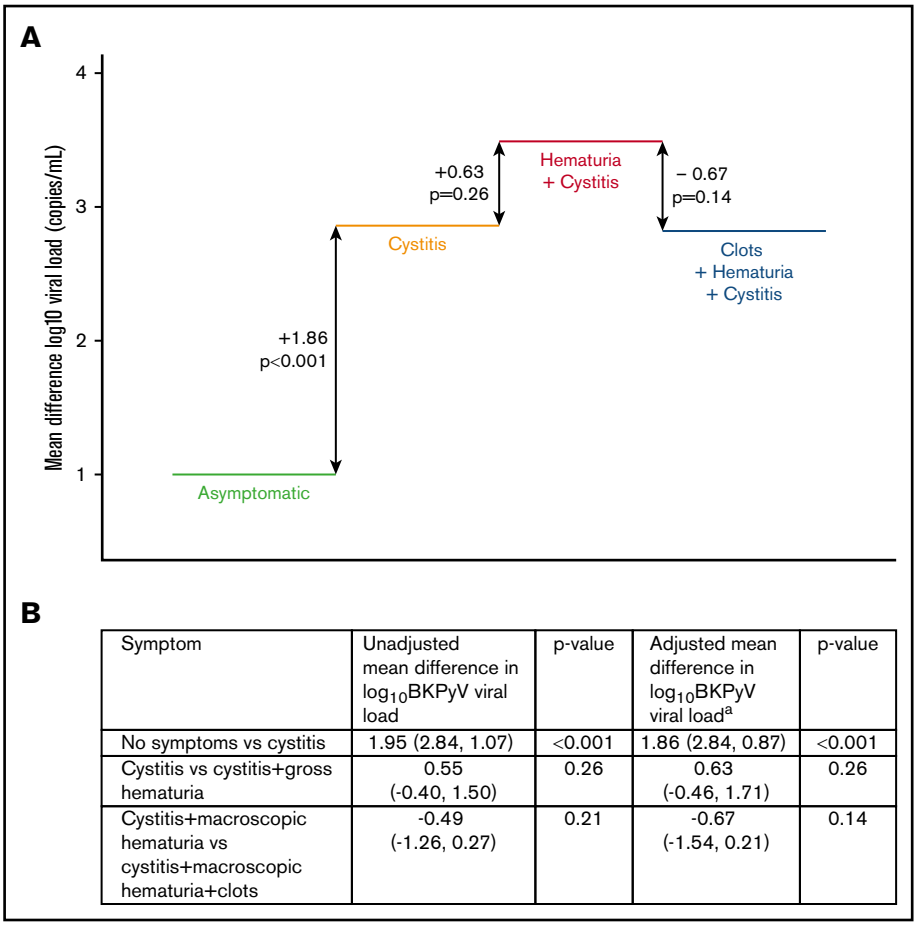


Figure 4. Results of multivariable models. For the models describing factors associated with time to macroscopic hematuria resolution or time to resolution of all cystitis symptoms, an HR <1 is associated with lower likelihood of resolution of disease at any given time point and thus a longer duration of disease. All cell counts reported in cells/μL. [a]Multivariable model is adjusted for CMV serostatus and duration of cystitis symptoms prior to beginning of BKPyV-HC. Although CMV serostatus was included in the final model, detection of CMV viremia was not significantly associated with resolution of macroscopic hematuria (supplemental Table 1A). [b]Multivariable model is adjusted for duration of cystitis symptoms prior to beginning of BKPyV-HC. [c]Multivariable model is adjusted for duration of cystitis symptoms prior to beginning of BKPyV-HC, HLA match, and diagnosis of chronic GVHD.

viral load (log_{10} scale) was significantly lower during asymptomatic periods compared with periods with symptoms of cystitis but without macroscopic hematuria (adjusted mean difference −1.86 [95% CI, −2.84, −0.87], $P < .001$). However, there were no statistically significant differences in viral load between times when patients had symptoms of cystitis without macroscopic hematuria vs symptoms of cystitis and macroscopic hematuria vs symptoms of cystitis, macroscopic hematuria, and urinary clots (Figure 5), and the effect sizes were smaller.

Sensitivity analyses

Results of sensitivity analyses, which examined a stricter definition of BKPyV-HC, varied thresholds for high-grade BKPyV viremia, and excluded patients with copathogens are included in supplemental



B

Symptom	Unadjusted mean difference in log_{10}BKPyV viral load	p-value	Adjusted mean difference in log_{10}BKPyV viral load[a]	p-value
No symptoms vs cystitis	1.95 (2.84, 1.07)	<0.001	1.86 (2.84, 0.87)	<0.001
Cystitis vs cystitis+gross hematuria	0.55 (-0.40, 1.50)	0.26	0.63 (-0.46, 1.71)	0.26
Cystitis+macroscopic hematuria vs cystitis+macroscopic hematuria+clots	-0.49 (-1.26, 0.27)	0.21	-0.67 (-1.54, 0.21)	0.14

Figure 5. Multivariable GEE model examining the relationship between evolution of symptoms and viral load in the subset of patients who had regular BKPyV viral load testing. (A) Multivariable GEE models examining difference in mean viral loads at different symptomatic stages of BKPyV-HC. (B) Multivariable and univariable GEE models examining differences in mean viral loads. [a]Multivariable models were adjusted for transplant cell source, underlying disease, baseline absolute lymphocyte count, baseline absolute monocyte count, race, and diagnosis of chronic GVHD.

Results, supplemental Tables 5-8, and supplemental Figure 3. Overall, the sensitivity analyses performed did not qualitatively change our overall findings.

Discussion

In a large cohort of HCT recipients with BKPyV, we found that a typical episode of HC with macroscopic hematuria lasted 2 to 4 weeks and that high-grade BKPyV viremia and cytopenias at the beginning of BKPyV-HC were significantly associated with longer durations of illness.

One key goal of our study was to characterize the clinical course and severity of BKPyV-HC in detail both to guide clinicians and to estimate expected disease duration and severity that would influence the end points and design of future interventional clinical trials. We found that median duration of hematuria was ~17 days, and median duration of all symptoms was 24 days; a small proportion of patients had prolonged symptoms (>90 days; 9/128 [7%]). Other than hematuria, the most commonly reported sign/symptom was dysuria, and over half of patients had urinary clots, which is consistent with previous reports.[2,28] The proportion of patients requiring pain medication, urinary catheterization, continuous bladder irrigation, and renal/bladder surgical procedures was high, underscoring the need for improved treatment of BKPyV-HC. Ultimately, an accurate description of symptomatology and duration of illness is required for measuring effectiveness of potential therapeutic strategies.

Another key objective was to examine the associations among viral, immune, and transplant factors and a prolonged or severe BKPyV-HC course. We found that high plasma BKPyV load at the beginning of BKPyV-HC was significantly associated with longer durations of hematuria and symptoms, but initial urine BKPyV load was not. Paired plasma and urine BKPyV loads were not correlated with each other, in contrast to the relationship between urine and plasma BKPyV loads that characterize BKPyV nephropathy in kidney transplant recipients.[39] This finding suggests that plasma viral load may have more prognostic utility than urine viral load, possibly related to the pathophysiology of BKPyV-HC. Alternatively, very high BKPyV loads ($>10^9$ copies/mL) may exceed the linear range of the assay, and thus urine viral loads may be subject to higher variability,[40,41] in addition to the interassay variability of both plasma and urine viral loads.[35]

By assessing the relationship of symptoms and plasma viral load among patients who were regularly tested for plasma BKPyV-HC, we could make notable inferences. We found a substantially higher plasma BKPyV load when patients had cystitis symptoms vs when they were asymptomatic but no differences in viral load when they had cystitis symptoms alone vs macroscopic hematuria vs hematuria with clots. Overall, our analyses highlighted the association of initial high-grade viremia with prolonged or severe courses of BKPyV-HC.

One hypothesized mechanism of BKPyV-HC pathogenesis posits that postengraftment immune reconstitution exacerbates bladder

inflammation in response to BKPyV replication,[4,12,29-31,42] while another theorizes that tissue destruction is from viral cytopathic effect against urothelium alone.[16] To examine the contribution of immune reconstitution to prolonged/severe disease, we found that the slope of lymphocyte recovery/decline was not associated with longer/more severe disease in any model. Baseline cytopenias were significantly associated with multiple end points predicting longer/more severe courses, but the cell lineage associated with worse disease varied. However, we did not examine bladder or BKPyV-specific immunity. High grade BKPyV viremia at the beginning of illness may reflect viral replication occurring in renal tubular cells, spillover from denuded bladder tissue into the bloodstream, or a combination.[43,44] Whether HC is solely due to viral cytopathic effects or occurs in the context of prior urotoxic conditioning with or without immune-inflammatory reactions requires further characterization of the specific pathophysiologic mechanisms in renal and bladder tissue during BKPyV-HC.

The use of intravenous cidofovir to treat BKPyV-HC is controversial[6]; small observational data have suggested benefit (resolution or improvement of symptomatic disease or lower viral loads),[5,45-50] but randomized controlled trials are required to definitively evaluate efficacy. Since our data are similarly observational and cidofovir was used in more severe cases of BKPyV-HC, we cannot make firm conclusions about benefit. However, in univariable analysis or multivariable sensitivity analyses there was no signal for improved symptoms, and in some models a reverse association. A recent prospective examination of BKPyV-HC in children and adolescents found that cidofovir use did not improve clearance of BKPyV viremia.[27] Lastly, in a recent phase 3 trial of CMV prophylaxis, there appeared to be no protective benefit of CMX001, a cidofovir prodrug, on BK infection diagnosis.[51] Our data, with the caveat of being retrospective, do not support the routine use of cidofovir for BKPyV-HC, and the efficacy of cidofovir should be specifically studied. We were not able to examine differences in cidofovir dosing regimens due to the small number of patients who received the drug.

Our study had notable strengths. Our cohort of patients with BKPyV-HC is the largest published, and we used a symptom-driven definition of disease and patient-centered end points. We also analyzed our data in a way that attempted to address biases inherent to a retrospective study of an uncommon, poorly defined disease. Asymptomatic patients are infrequently tested for BKPyV, but >50% of allogeneic HCT recipients have BKPyV viremia, and an even higher proportion have viruria.[12-14] By performing all analyses within a group of patients with disease, we avoided biased results that would result from using a control group that was misclassified because of lack of symptom documentation or lack of testing. Lastly, a subgroup of our patients had regular testing of plasma BKPyV viral load, and we were uniquely able to evaluate the role of viral load in diagnosis of BKPyV-HC in this subset.

There were also several limitations to our study. Data were obtained retrospectively through chart review, so were susceptible to documentation error or bias related to more detailed documentation among patients with more medical visits. BKPyV testing, monitoring, and management was clinician dependent. Our case definition included only patients with macroscopic hematuria in order to describe a group of patients with more severe disease. We also only included postengraftment HC to increase the likelihood that disease was attributable to BKPyV rather than effects of chemotherapy or thrombocytopenia.[8] In these ways, our definition is stricter than previously published definitions of BKPyV-HC.[2,5,28] Unlike other definitions, we did not require a specific BKPyV load (in urine or plasma), require that patients report cystitis signs/symptoms other than macroscopic hematuria, or exclude patients with coinfections. However, the majority (≥90%) of patients presented with viruria $>10^7$ copies/mL, a subset analysis of patients with macroscopic hematuria and other cystitis symptoms showed results consistent with those of the whole cohort, and we included the contribution of coinfection to symptom/hematuria duration in our univariable and multivariable models to assess their effects on outcome (all components of the ECIL definition[5]). We additionally assessed subsets of patients without copathogens, and the association between high-grade baseline viremia and duration of symptoms or macroscopic hematuria was largely unchanged; however, the role of co-pathogen detection should be specifically studied. At our center, PCR testing for BKPyV viruria is recommended for clinical HC, but whether testing was done in all patients cannot be determined. To make conclusions about urine and plasma BKPyV testing, we required that testing was ordered in a specific way, and therefore, our incidence of BKPyV-HC likely underrepresents disease in an allogeneic HCT population. We used an arbitrary cutoff plasma BKPyV load of ≥10 000 to reflect what has been significant in the literature[25,38]; we explored different plasma BKPyV thresholds and found that using the upper quartile (8550 copies/mL) or 10 000 copies/mL gave similar results and were more associated with our end points relative to lower thresholds. Also, kidney injury attributable to BKPyV-associated nephropathy was not examined. Lastly, our analyses were exploratory and should be confirmed in future studies.

In this study, we found a prolonged BKPyV-HC occurs in a significant number of patients and that severe manifestations of BKPyV-HC occur frequently, and we showed that high-grade BKPyV viremia and cytopenias at baseline are associated with longer durations of illness. There is currently a lack of effective and evidence-based strategies to treat BKPyV-HC. Knowledge of the natural history and factors associated with severe disease is important for the day-to-day management of these patients and the rational design of clinical trials testing novel therapies.[3,52,53]

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Authorship

Contribution: H.I., L.E.K., P.S.P., A.P.L., and M.B. devised the project; H.I., L.E.K., and M.-L.H. defined the patient cohort and collected data; H.I., H.X., E.R.D., and W.M.L. planned and performed the analytic calculations; J.A.H., E.R.D., M.-L.H., A.P.L., S.A.P., K.R.J., F.M., W.G.N., and H.H.H. provided critical expertise and feedback regarding chosen methods and content; A.P.L. and M.B. supervised the project; and all authors discussed the results and contributed to the final manuscript.

Conflict-of-interest disclosure: J.A.H. has served as a consultant for Nohla Therapeutics and Amplyx and has received research support from Nohla Therapeutics, Karius, and Takeda (formerly Shire), all unrelated to this research. S.A.P. has received research funding from Global Technologies and has participated in clinical trials with Chimerix. A.P.L. reports support in the form of research funding and as site investigator and consultancy for Merck, site investigator for Astellas, a member of the data monitoring committee for Novartis, a consultant and site investigator for Gilead, site investigator for Roche Diagnostics, site investigator for Hologic, and site investigator for Oxford Immunotech, all unrelated to this research. W.G.N. is employed by Chimerix. P.S.P. is an employee and shareholder of Vir Biotechnology. M.B. has served as consultant and received research funding from Chimerix and Vir Biotechnology. The remaining authors declare no competing financial interests.

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