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Azithromycin Use and Increased Cancer Risk among Patients with Bronchiolitis Obliterans after Hematopoietic Cell Transplantation

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A B S T R A C T

Azithromycin exposure during the early phase of allogeneic hematopoietic cell transplantation (HCT) has been associated with an increased incidence of hematologic relapse. We assessed the impact of azithromycin exposure on the occurrence of relapse or new subsequent neoplasm (SN) in patients with bronchiolitis obliterans syndrome (BOS) after HCT who are commonly treated with azithromycin alone or in combination with other agents. In a retrospective study of patients with BOS from 2 large allograft centers, the effect of azithromycin exposure on the risk of relapse or SN was estimated from a Cox model with a time-dependent variable for treatment initiation. The Cox model was adjusted on time-fixed covariates measured at cohort entry, selected for their potential prognostic value. Similar models were used to assess the exposure effect on the cause-specific hazard of relapse, SN, and death free of those events. Sensitivity analyses were performed using propensity score matching. Among 316 patients, 227 (71.8%) were exposed to azithromycin after BOS diagnosis. The corresponding adjusted hazard ratio (HR) in patients exposed to azithromycin versus unexposed was 1.51 (95% confidence interval [CI], 0.90 to 2.55) for relapse or SN, 0.82 (95% CI, 0.37 to 1.83) for relapse, and 2.00 (95% CI, 1.01 to 3.99) for SN. Patients exposed to azithromycin had a significantly lower cause-specific hazard of death free of neoplasm and relapse (adjusted HR, 0.54; 95% CI, 0.34 to 0.89). In conclusion, exposure to azithromycin after BOS after HCT was associated with an increased risk of SN but not relapse.

INTRODUCTION

Recently, the European Medicines Agency and the US Food and Drug Administration issued warnings against the long-term use of azithromycin in the setting of early allogeneic hematopoietic cell transplant (HCT) for hematologic malignancies [1,2]. These warnings followed the results of ALLOZITHRO, a French randomized trial testing azithromycin as prophylaxis against lung chronic graft-versus-host disease (GVHD) (i.e., bronchiolitis obliterans syndrome [BOS] in recipients of allogeneic HCT) [3]. The rationale for testing azithromycin in this population derives from the effect of azithromycin prophylaxis in reducing the incidence of BOS in lung transplant recipients [4]. ALLOZITHRO was terminated early due to an unanticipated reduction in survival attributed to increased rates of hematologic relapse in patients who received azithromycin [3]. The mechanisms for relapse observed in the ALLOZITHRO trial are under investigation. It is hypothesized that azithromycin interferes with antitumor immune surveillance.

In light of these unexpected findings, the long-term use of azithromycin for the treatment of BOS after HCT has been called into question. Azithromycin is frequently used alone or as part of fluticasone, azithromycin, and montelukast treatment for established BOS [5,6], In the setting of HCT, BOS is

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usually diagnosed within the first 2 years after transplant [7] at a time when the risk for hematologic relapse decreases and the risk of subsequent neoplasms (SNs) increases [8,9].

The aim of this study was to determine if azithromycin treatment for BOS after allogeneic HCT is associated with an increased risk of cancer, including relapse of the original malignancy and SN.

METHODS

Study Cohort

Patients with BOS, aged 18 years and older, who survived at least 6 months post-transplant, from Fred Hutchinson Cancer Research Center (FHCRC)/Seattle Cancer Care Alliance in Seattle, Washington, and at the Hôpital St. Louis (SLS) in Paris, France, were included. Both sites have expertise in managing postallogeneic HCT lung complications. Any patient who received an allogeneic HCT between 2000 and 2016 (FHCRC) or who was referred for clinical care between 2000 and 2017 (SLS) and met criteria for BOS diagnosis were included. BOS was defined by the following 2014 National Institutes of Health spirometric criteria: forced expiratory volume in 1 second (FEV1) $<75\%$, FEV1/vital capacity (VC) <0.7, and $\geq 10\%$ absolute FEV1 decline compared to pretransplant baseline [10]. Absence of a bronchodilator response was not required for BOS diagnosis as this parameter was not uniformly available. Similarly, chest imaging was not used to determine BOS diagnosis as high-resolution studies were not available for many subjects. Chart review confirmed the absence of infectious diagnosis at the time of meeting spirometric criteria for BOS. Additionally, in the SLS cohort, as previously reported, BOS was also diagnosed in patients with a new-onset obstructive impairment characterized by a decrease in both FEV1 and VC, a normal FEV1/VC ratio, a normal total lung capacity, and elevated residual volume in the absence of alternative explanations for ventilatory impairment [11,12]. Before undergoing HCT, all subjects from both sites signed informed consent allowing the use of their clinical data for research. This study was approved by the appropriate institutional review boards for FHCRC and SLS.

Clinical Variables

Modified disease risk index (DRI) was used to assess disease risk at the time of transplant [13]. Acute GVHD was graded from 1 to 4, according to consensus criteria [14,15]. Chronic GVHD was graded according to the 2014 National Institutes of Health consensus guidelines [10] if the clinical data were available by chart review. Primary cause of death was determined by consensus when multiple morbidities were reported (G.-S.C. and A.B.). Causes of death were classified as respiratory (respiratory failure and respiratory infection), death related to relapse or SN, transplant-related mortality, others, and unknown.

Azithromycin Exposure

Exposure to azithromycin was defined as the use of azithromycin at any time post-transplant, including for intentional treatment of BOS or other reasons, for any duration and at any dose, with the date of BOS diagnosis representing the entry into the cohort. Periods of exposure were defined by courses of azithromycin intake with a defined start and end date with at least 1 day free of intake between courses. Time-dependent dynamics of azithromycin exposure are displayed with a random sample of 50 patients in Figure 1 and demonstrate the following issues: (1) azithromycin may have been given before BOS diagnosis. (2) Azithromycin may have been given at BOS diagnosis. (3) In some instances, there is a time interval between BOS diagnosis and azithromycin intake. Each observation ends with patient death or last follow-up. Clinical practice for BOS treatment evolved during the study period for both cohorts; the use of azithromycin was more common in the latter part of the study period.

Outcomes

The primary outcome was cumulative incidence of all cancers subsequent to BOS, including relapse of the original hematologic disease and new SN. Secondary outcomes included cumulative incidence of relapse, of SN, and of death free of relapse and SN, as well as event-free survival (event being either relapse or SN). Data for clinical outcomes were locked on November 30, 2017.

Statistical Methods

To account for the time-dependent dynamics of the data, we used a multistate model with following states: BOS diagnosis, azithromycin treatment, and death (see Supplementary Figure S1). The effect of cumulative treatment exposure on the risk of relapse and/or SN after BOS diagnosis was estimated from a Cox model with time-dependent variable for treatment initiation. This provides unbiased estimates of the hazard ratio (HR), allowing for control of survival bias while avoiding selection bias [16,17]. The Cox model was adjusted on time-fixed covariates measured at the time of transplant that were selected as potentially of prognostic value: age, sex, tobacco use, total body irradiation, DRI, prior autologous HCT, and past exposure to azithromycin after HCT but before BOS. We also introduced chronic GVHD after BOS as a time-dependent covariate. Models were finally stratified on the site to handle potentially different baseline hazards.

Similar modeling strategies were used to assess the exposure effect on the cause-specific hazard of relapse, of SN, and of death free of relapse and SN. On each endpoint, we further tested the interaction of exposure effect according to the site using the Gail and Simon test [18].

To account for the use of azithromycin as an intermediate event and death as a potential competing event on the effect of azithromycin on the outcomes, we displayed cumulative hazards [19].

To account for changes in clinical practices in prescribing azithromycin, sensitivity analyses were performed using propensity score matching. The propensity score of azithromycin administration was estimated using a multivariable logistic model, including 9 potential confounders for relapse or SN (namely, age, sex, DRI, prior graft, acute leukemia, myeloablative conditioning, antithymocyte globulin, chronic GVHD, and time from HCT to BOS). Estimates of propensity scores were pooled from 30 imputed data sets, obtained by multiple imputations with chained equations. Quality of the score was measured on standardized mean difference of confounders and c-index of the model [20]. Then, 1:1 matching on the pooled propensity score was individually performed using the nearest neighbor method within a caliper of 0.20 standard deviations of the logit of the propensity score, with and without replacement [21]. Estimates of azithromycin exposure used generalized linear models to handle the matching, with inverse-probability weighting and design-based standard errors.

Summary statistics—namely, median (interquartile range [IQR]) and percentage—are reported. All tests are 2-sided, with P values less than .05 considered significant. Analyses were performed on R 3.5.1 software (R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

RESULTS

Description of the Cohort

A total of 316 patients with BOS were included in the study: 185 patients from FHCRC and 131 from SLS. Baseline characteristics of the cohort are reported in Table 1.

The median time to BOS diagnosis after HCT was 16.8 months (IQR, 10.8 to 30.6). The description of the patients according to the transplant center (FHCRC versus SLS) is reported in the online supplement (Supplementary Table S1, Supplementary Figure S2, and Supplementary Figure S3).

Azithromycin Exposure

Overall, 237 patients (75%) received azithromycin during their follow-up for 1 to 3 courses. Characteristics of both azithromycin-exposed and unexposed cohorts are summarized in Table 1. The description of azithromycin exposure any time after HCT is summarized in Table 2. The median length of exposure to azithromycin after BOS diagnosis was 16 months (IQR, 7 to 36).

Cancer Outcomes

The median time of follow-up after BOS was 41 months (IQR, 17 to 95). There were 53 (16.8%) patients who relapsed; 30 relapses were documented after BOS (Table 3).

Median time from first azithromycin exposure after BOS to relapse was 15.6 months (IQR, 8.5 to 36.3); from transplant to relapse was 41.8 months (IQR, 23.1 to 60.0). Excluding basal cell carcinomas, 43 (13.6%) patients developed a SN after BOS, including 10 patients who did not receive azithromycin before the diagnosis of SN and 33 in patients previously exposed to azithromycin. Among these 43 patients with SN after BOS, 18 developed more than 1 subsequent malignancy, including only 2 of 10 free of any azithromycin exposure and 16 of 33 after azithromycin exposure ($P = .15$). The median time of developing a SN after azithromycin exposure was 43.5 months (IQR, 8.5 to 36.3). Median time from transplant to SN was 81 months (IQR, 44.7 to 117.5). Twenty-four (56%) SNs were of squamous cell histology (Table 3). The type of hematologic relapse and SN

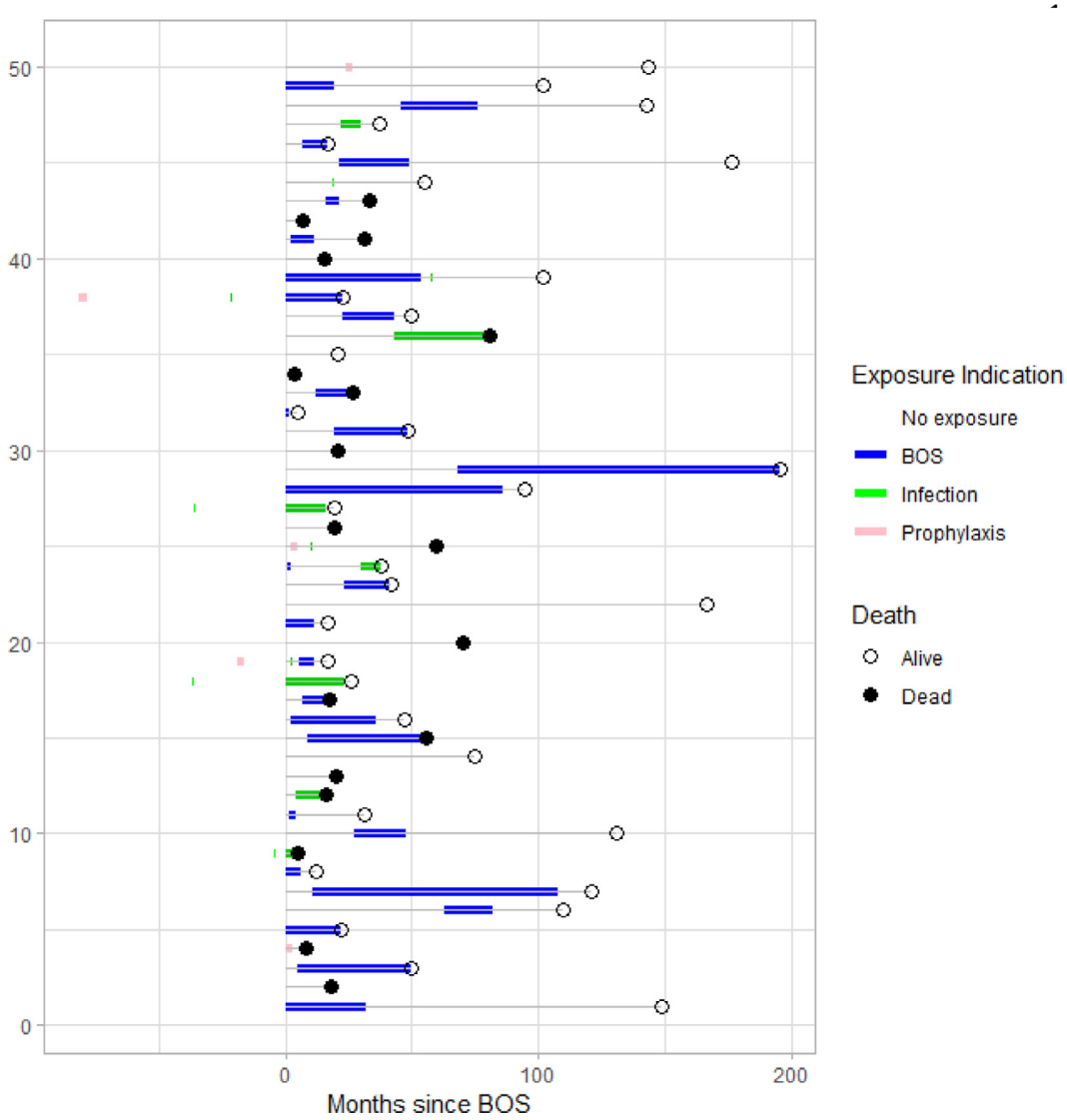

Figure 1. Graphical time-dependent display of 50 randomly selected patients. Each line shows 1 observed patient. To display the time dependency of azithromycin treatment, time free of any azithromycin exposure is marked in gray and a time of azithromycin exposure is marked in color according to the indication. The corresponding survival event is marked with a filled circle (death) or a transparent circle (alive). For instance, patient 2 (second line at the bottom) died early free of any exposure to azithromycin. At the top (first line), patient 50 only received a short course of azithromycin for prophylaxis.

according to sites is reported in Supplementary Table S2, as well as details on the duration of follow-up in each group of patients (Supplementary Table S3). There was no evidence of any difference in relapses/SN according to the association with fluticasone and montelukast ($P = .42$ by the exact Fisher test).

The cumulative hazards for relapse or SN are displayed in Figure 2.

The corresponding unadjusted HRs (azithromycin exposed versus unexposed) are 1.49 (95% confidence interval [CI], 0.91 to 2.44) for relapse or SN, 0.90 (95% CI, 0.42 to 1.93) for relapse, and 1.88 (95% CI, 1.00 to 3.51) for SN. The effect of azithromycin exposure on hazard of malignancy was confirmed in adjusted multivariate models (HR, 2.00; 95% CI, 1.01 to 3.99; $P = .048$; Table 4), adjusting for factors associated with the occurrence of relapse or SN or both after BOS on univariable analyses (see Supplementary Table S4). Further adjusting on year of allograft, indication for the graft (distinguishing acute myelogenous leukemia from other diagnoses), total body irradiation dose, the use of a myeloablative regimen, and antithymocyte globulin did not modify these findings (see Supplementary Table S5).

Sensitivity analysis considering only extensive chronic GVHD rather than overall chronic GVHD and adding time from HCT to BOS diagnosis in the adjusted multivariate models confirmed the azithromycin exposure effect on the occurrence of SN (HR, 2.03; 95% CI, 1.01 to 4.08; $P = .047$) (see Supplementary Table S6). There was no effect of cumulative months of exposure to azithromycin before the occurrence of relapse or SN; restriction of azithromycin exposure to greater than 7 days or greater than 28 days (adjusted HR, 2.05; 95% CI, 1.01 to 4.19; $P = .047$) did not modify the hazard of malignancy in exposed patients compared with unexposed, likely due to the duration of exposure of at least 7 months in 75% of the exposed patients.

There was no evidence of any azithromycin exposure by site interaction (FHCRC or SLS) on adjusted estimates, with P values of Gail and Simon interaction tests ($P = .38$ for relapse or malignancy; $P = .18$ for relapse; $P = .78$ for subsequent malignancy) (see Supplementary Figure S4 and Figure S5). That is, the effect of azithromycin appeared to be the same for patients treated at FHCRC and those treated at SLS.

Table 1
Characteristics of the study cohort at baseline and at BOS diagnosis according to azithromycin exposure after BOS diagnosis

Characteristic	Total (N = 316)	Azithromycin Exposure	
		No (n = 89)	Yes (n = 227)
Baseline characteristics at HCT			
Women, n (%)	129 (40.8)	31 (34.8)	98 (43.1)
Age, median (IQR), yr	48.6 (33.6-58.6)	47.9 (36-59)	48.7 (33-57.6)
Diagnosis, n (%)			
Acute leukemia	140 (44.3)	35 (39.3)	105 (33.5)
Chronic myeloid leukemia	32 (10.1)	12 (13.5)	20 (8.8)
Other myeloproliferative disorders	8 (2.5)	3 (3.4)	5 (2.2)
Myelodysplastic disorders	52 (16.4)	20 (22.5)	32 (36.6)
Lymphoid malignancies	73 (23.1)	16 (18.0)	57 (25.1)
Others	11 (3.5)	3 (3.4)	8 (3.5)
Disease risk index at HCT, n (%)			
Low	47 (14.9)	18 (21.7)	29 (13.3)
Intermediate	176 (55.7)	43 (51.8)	133 (61.0)
High	68 (21.5)	19 (22.9)	49 (22.5)
NA	25 (7.9)	9 (10.1)	16 (7.0)
History of smoking, n (%)	122 (38.6)	45 (51.1)	77 (33.9)
History of solid cancer before HCT, n (%)	31 (9.8)	4 (4.5)	27 (11.9)
Prior autologous HCT, n (%)	96 (30.4)	19 (21.3)	77 (33.9)
Donor type, n (%)			
Related	138 (43.7)	42 (47.2)	96 (42.3)
Haploidentical	5 (1.6)	2 (2.2)	3 (1.3)
Unrelated HLA-match*	130 (41.1)	32 (36.0)	98 (43.0)
Unrelated HLA-mismatch†	36 (11.4)	9 (10.1)	27 (11.9)
Donor/recipient sex, n, (%)‡			
Male/male	91 (29.5)	28 (32.9)	63 (28.3)
Male/female	57 (18.5)	16 (18.8)	41 (18.4)
Female/male	92 (29.9)	27 (31.8)	65 (29.1)
Female/female	68 (22.1)	14 (16.5)	54 (24.2)
Source of stem cells graft, n (%)			
Peripheral blood	273 (86.4)	74 (83.1)	199 (87.7)
Bone marrow	36 (11.4)	11 (12.4)	25 (11)
Cord blood	7 (2.2)	4 (4.5)	3 (1.3)
Conditioning regimen, n (%)			
Myeloablative	180 (57.0)	47 (52.8)	133 (58.6)
Nonmyeloablative	136 (43.0)	42 (47.2)	94 (41.4)
TBI	159 (50.3)	33 (37.1)	126 (55.5)
Dose, median (IQR), Grays	2 (2-12)	2 (2-12)	2 (2-12)
Antithymocyte globulin§	39 (12.3)	14 (15.7)	25 (11.0)
Lung function			
FEV1 (% predicted), median (IQR)	92.8 (82.8-100.2)	91.1 (83.1-97.9)	92.9 (82.7-100.7)
FVC (% predicted), median (IQR)	95.1 (85.9-105.6)	92.0 (83.9-103.7)	96.2 (86.5-106.0)
Characteristics at BOS diagnosis			
Months from HCT, median (IQR)	16.8 (10.8-30.6)	13.9 (10.7-24.5)	18.1 (11-33.5)
Chronic GVHD			
Before BOS	273 (86.4)	76 (85.4)	197 (86.8)
At or after BOS, n (%)	33 (10.0)	10 (11.0)	23 (10.0)
Grade max			
Mild	25 (7.9)	13 (14.8)	12 (5.4)
Moderate	117 (37.0)	33 (37.5)	84 (37.8)
Severe	161 (50.9)	39 (44.3)	122 (54.9)
Death free of chronic GVHD	5 (1.6)	2 (2.2)	3 (1.3)
Absence of chronic GVHD	5 (1.6)	1 (1.1)	4 (1.8)
Lung function			
FEV1 (% predicted), median (IQR)	55.9 (42.9-65.5)	60.4 (51.1-68.8)	53.2 (40.4-63.7)
FVC (% predicted), median (IQR)	71.9 (64.0-83.5)	72.9 (65.5-83.6)	71.3 (63-83.5)
FEV1/FVC, median (IQR)	0.63 (0.51-0.69)	0.67 (0.60-0.73)	0.61 (0.48-0.67)

(*continued*)

Table 1 (*Continued*)

Characteristic	Total (N = 316)	Azithromycin Exposure	
		No (n = 89)	Yes (n = 227)
Residual volume (% predicted), median (IQR)	131.7 (103.0-161.3)	134.8 (113.9-161.1)	130.8 (100.8-161.3)
Treatments for BOS,‖ n (%)			
Azithromycin	197 (62.0)	0 (0)	197 (87.0)‡
Systemic steroids	105 (33.0)	16 (18.0)	89 (39.0)
ICS/LABA	153 (48.0)	44 (49.0)	109 (48.0)
ICS without LABA	154 (49.0)	18 (20.0)	98 (43.0)
Montelukast	146 (46.0)	7 (8.0)	139 (62.0)

IQR indicates interquartile range; NA, not available; TBI, total body irradiation; FVC, forced vital capacity; ICS, inhaled corticosteroids; LABA, long-acting beta-agonist.

* Seventy-four patients minimal 8/8 HLA-match and 56 patients 10/10 HLA-match.

† Thirty-four patients 7/8 HLA-match and 2 patients 9/10 HLA-match.

‡ Excluding cord blood recipients and 1 missing value for a recipient of a peripheral blood stem cell graft.

‖ Treatments administered for BOS at the time of diagnosis or thereafter; of the 227 patients who received azithromycin after the diagnosis of BOS, 197 received it specifically for the treatment of BOS and 30 for another indication (antimicrobial prophylaxis or treatment of an infection).

§ No patients had HCT with ex vivo depletion of donor T cells.A total of 95 patients (30%) received the azithromycin in the fluticasone, azithromycin, and montelukast regimen.

Table 2
Description of Azithromycin Exposure Any Time after HCT for 237 Patients*

Characteristic	Value
Number of azithromycin exposures per patient†	
1	165 (52.2)
2	60 (19.0)
3	12 (3.8)
Characteristics of first exposure	
Duration, median (IQR), d	230 (7-847)
Before BOS	66 (20.9)
At or after BOS	167 (52.9)
Both before and after BOS	4 (1.3)
Characteristics of second exposure	
Duration, median (IQR), d	244.5 (90-884.8)
Before BOS	7 (2.2)
At or after BOS	64 (20.2)
Both before and after BOS	1 (0.32)
Characteristics of third exposure	
Duration, median (IQR), d	191.5 (69.2-783.2)
Before BOS	0 (0)
At or after BOS	12 (3.8)
Both before and after BOS	0 (0)

Values are presented as number (%) unless otherwise indicated.

* Ten patients received azithromycin only before BOS for a median time of 7 days among the 237 patients who received azithromycin during the study period.

† A single exposure of azithromycin was defined by azithromycin intake with a start and end date; the minimum duration of the first exposure after BOS was 7 days. A subsequent azithromycin exposure was considered if there was at least 1 day free of intake between 2 courses of azithromycin.

Table 3
Type of Hematologic Relapses and Subsequent Neoplasms after BOS Diagnosis According to Prior Exposure to Azithromycin

Characteristic	Azithromycin Exposure	
	No (n = 89)	Yes (n = 227)
Hematologic relapses type after BOS, n (%)	10 (11.0)	20 (9.0)
Acute leukemia	3	5
Chronic myeloid leukemia	1	2
Lymphoid malignancies	4	8
Myelodysplastic disorders	2	5
Others	0	0
Subsequent neoplasms,* n (%)	8 (9.0)	35 (15.0)
		33 after azithromycin†
Types, n (%)		
Squamous cell carcinoma	3 (37.5)	21 (60.0)
Adenocarcinoma	2 (25.0)	8 (22.9)
Verrucous carcinoma	1 (12.5)	0
Carcinoma NOS	0 (0)	2 (5.7)
Bowen disease	0 (0)	2 (5.7)
Malignant melanoma	0 (0)	2 (5.7)
Lymphoma	1 (12.5)	0
Mast cell leukemia	1 (12.5)	0
Anatomic site, n (%)		
Breast	0 (0)	3 (10.7)
Gut	1 (16.7)	3 (10.7)
Skin	2 (33.3)	14 (50.0)†
Oral cavity	1 (16.7)	7 (20.0)
Pancreas	1 (16.7)	0 (0)
Penis	0 (0)	1 (3.6)†
Prostate	1 (16.7)	3 (10.7)
Uterus	0 (0)	2 (5.7)
Blood	1 (12.5)	0 (0)
Lung	1 (16.7)	1 (3.6)
Brain	0 (0)	1 (3.6)

NOS indicates not other specified.

* Basal cell carcinomas excluded.

† Two malignancies occurred after BOS but before azithromycin onset, including 1 malignant melanoma of the upper extremity and 1 squamous cell carcinoma of the penis.

Survival Outcomes

Because death is a competing event for SN or relapse, the effect of azithromycin exposure after BOS on deaths free of relapse and/or SN was examined. In total, 120 (38%) subjects had died as of last follow-up, including 32 patients who died of relapse and 7 of cancer (Table 5).

Besides relapse, respiratory causes (n = 54, 45%) were the most common primary causes of death. Regarding the effect of azithromycin exposure, the hazard of death free of malignancy and relapse was not modified, either unadjusted or adjusted for prognostic variables, compared with unexposed patients (HR, 0.69; 95% CI, 0.42 to 1.12; P = .13 and HR, 0.71; 95% CI, 0.43 to 1.19; P = .19, respectively). Patients exposed to azithromycin had a significantly decreased cause-specific hazard of death free of malignancy (adjusted HR, 0.54%; 95% CI, 0.34 to 0.89; P = .014; Figure 2d), whereas their cause-specific

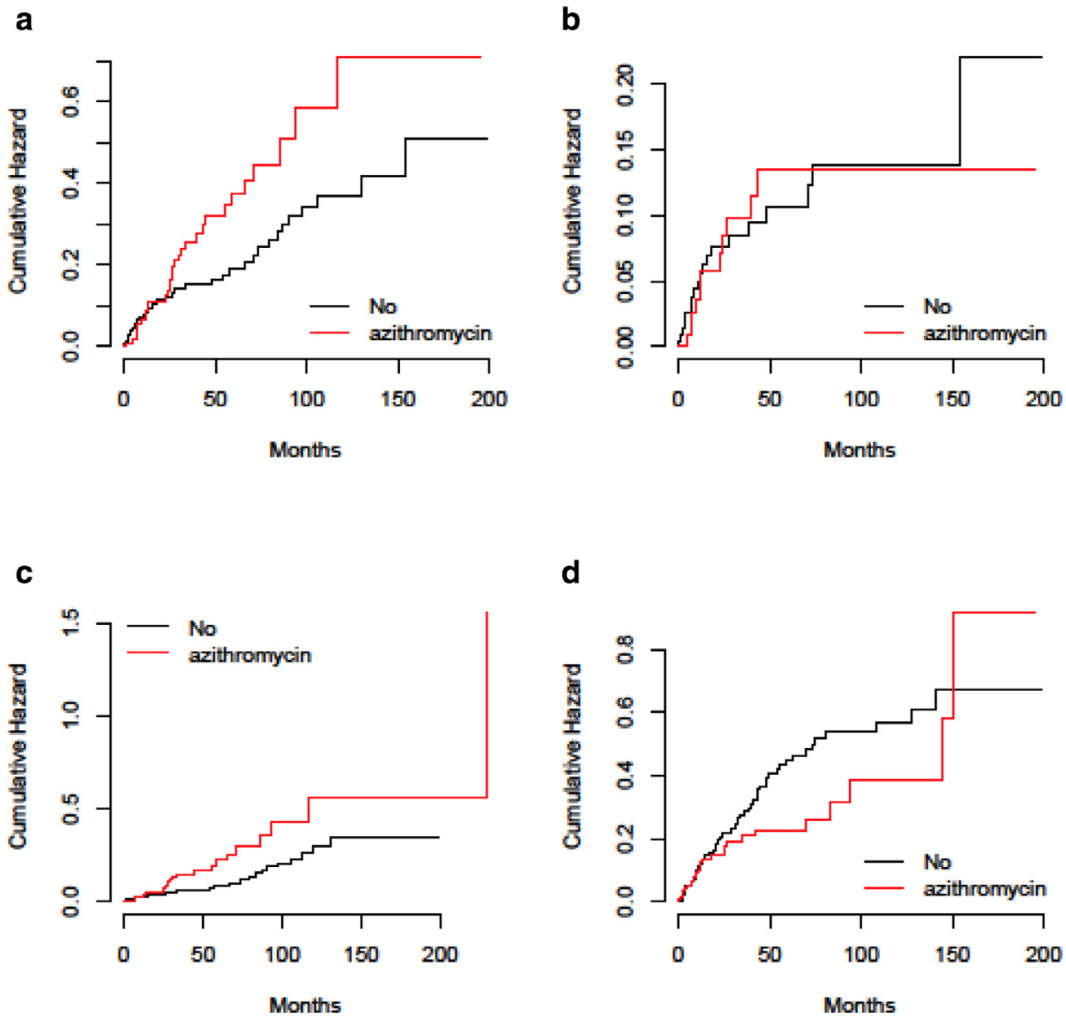

Figure 2. Cumulative hazard of relapse and/or subsequent neoplasm, either overall (a) or for relapse (b) and subsequent neoplasm (c), separately, and death free of relapse and subsequent neoplasm (d). Cumulative hazard can be interpreted as the probability of failure at time t given survival until time t. Note that when the cumulative hazard function is a straight line (such as in panel a for the nonexposed group), the underlying hazard function is constant over time.

Table 4
Estimation of Azithromycin Effect Using Multivariable Time-Dependent Cox Models

Characteristic	Relapse or Subsequent Neoplasm		Relapse		Subsequent Neoplasm	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
AZM exposure	1.51 (0.90-2.55)	.12	0.82 (0.37-1.83)	.63	2.00 (1.01-3.99)	.048
TBI	1.17 (0.62-2.23)	.62	0.55 (0.22-1.40)	.21	1.96 (0.90-4.27)	.091
DRI	1.64 (1.20-2.24)	.002	2.07 (1.35-3.16)	.0008	1.53 (1.02-2.29)	.038
cGVHD-t	0.86 (0.34-2.15)	.74	1.28 (0.37-4.41)	.69	0.65 (0.18-2.37)	.52
Age	1.00 (0.98-1.02)	.91	0.99 (0.96-1.02)	.40	1.02 (0.99-1.04)	.14
Sex/male	1.07 (0.64-1.79)	.79	1.06 (0.46-2.46)	.89	0.94 (0.50-1.77)	.86
History of smoking	1.28 (0.74-2.23)	.38	1.45 (0.64-3.33)	.38	1.08 (0.55-2.13)	.82
Prior autologous HCT	1.99 (1.03-3.87)	.041	4.24 (1.61-11.1)	.003	1.37 (0.68-2.77)	.38
AZM exposure before BOS	0.59 (0.30-1.17)	.13	0.85 (0.31-2.36)	.76	0.53 (0.23-1.22)	.14

AZM indicates azithromycin; cGVHD-t, time-dependent occurrence of chronic GVHD.

hazard of death free of relapse was not significantly decreased (adjusted HR, 0.62%; 95% CI, 0.38 to 1.03; P = .06). Cause-specific hazard of death from all respiratory causes was similar in both groups of patients (HR, 1.01%; 95% CI, 0.68 to 1.51; P = .96), as well as that of death from respiratory failure (HR, 1.13%; 95% CI, 0.68 to 1.86; P = .64) and from respiratory infection (HR, 0.84%; 95% CI, 0.43 to 1.64; P = .61).

Sensitivity Analyses

A propensity score for receiving azithromycin was estimated; this differed between exposed and unexposed patients as measured by the c-index of the model (at 0.66) and the standardized mean differences of the potential confounders across the treatment groups with an average value at 0.17. Of the 227 patients who received azithromycin, 76 (33%) could be

Table 5
Causes of Death

Primary Cause of Death (n = 120)	n (%)
Respiratory causes	54 (45.0)
Respiratory failure*	37 (30.8)
Respiratory infection	17 (14.2)
Relapse/SN causes	32 (26.7)
Relapse	25 (20.8)
SN	7 (5.8)
Transplant-related mortality	8 (6.7)
Others	12 (10)
Unknown cause	14 (11.7)

* Six patients died of multiple causes after lung transplantation.

matched without replacement, and up to 225 (99%) were matched when replacement was allowed. Balance was improved with an average standardized mean difference and c-index at 0.076 and 0.499, respectively, without replacement, and at 0.10 and 0.504, respectively, with replacement. Estimates of exposure effects confirmed previous results, with an increased risk of SN (although based on the sample of 76 treated and 76 untreated, this was not statistically significant) and no increased risk of relapse (Table 6).

DISCUSSION

In this analysis of a large multisite cohort of patients with BOS after HCT, exposure to azithromycin after BOS diagnosis was associated with an increased risk of developing a SN but not with risk for relapse of the original malignancy. These results were independent of chronic GVHD status and were further confirmed for each site independently. In the ALLOZITHRO trial, azithromycin was given early post-transplant when the risk of relapse was inherently high. In the current study, azithromycin was given many months to years after HCT for established BOS when the risk of relapse diminished and the risk of SN increased in association with chronic GVHD and prolonged immunosuppressive treatment [22]. In this context, an increase in relapse associated with azithromycin would have required a much larger cohort for analysis, but an increased risk of SN associated with azithromycin is consistent with the natural history of long-term survivors. Thus, these results constitute a second signal suggesting the potential association of azithromycin with cancer in allogeneic HCT.

Antibiotic use has previously been associated with various cancers [23]. Azithromycin may directly interfere with antitumor immune surveillance through an inhibitory effect on various cell types, including lymphocytes, dendritic cells, and natural killer cells in a dose-dependent way [24-26]. Long-term, low-dose azithromycin was shown to be associated with downregulation of genes regulating antigen presentation, interferon and T cell responses, and numerous inflammatory pathways in patients with neutrophilic chronic obstructive pulmonary disease [27]. There is growing evidence that antibiotics may alter immune functions that are important for surveillance and control of malignancy through the modification of gut microbiota [28], which have a composition that was shown to be associated with tumorigenesis [29,30]. In the specific setting of HCT, alterations in gut microbiota within the first month following HCT were associated with both incidence and severity of GVHD and hematologic relapse [31,32]. These data may explain the increase of relapse found in the ALLOZITHRO trial where patients received azithromycin before and during engraftment, which is an immunologically vulnerable period for the control of the hematologic malignancy [33,34]. Similar mechanisms may be involved in the development of SN associated with azithromycin exposure later in the course of survivorship.

Long-term immunosuppressive treatments are known to be associated with the development of SN. Unfortunately, because of the retrospective design of our study, which included a long period of patient follow-up, complete data on immunosuppressive therapy for GVHD were not available. However, when taking into account in the statistical model the severity of GVHD, a reflection of the intensity of immunosuppressive treatment, the effect of azithromycin on SN persists. We could not identify whether patients with cancer predisposition syndromes were included in our cohort of patients. Notably, however, the association of azithromycin with SN also persists when adjusted for DRI, which likely reflects a predisposition to SN.

Patients who received azithromycin developed more SN, but very few died of their SN. Most SN in azithromycin-exposed patients after BOS belonged to an intermediate prognostic group of malignancies [35]. We postulate that the duration of follow-up after cancer diagnosis may not have been long enough to assess the effect of the malignancy on mortality.

The paradoxical finding of more SN but decreased cause-specific hazard of death free of malignancy with azithromycin exposure may result from attenuating the progression of BOS or by reducing the number of respiratory exacerbations related to the underlying obstructive lung disease. A steroid-sparing effect of azithromycin [5] may reduce infections or other life-limiting complications related to chronic corticosteroid use. Similarly, a recent retrospective analysis demonstrated a survival benefit of extracorporeal photopheresis in patients with BOS after HCT in the absence of an impact on FEV1 [36]. Our retrospective data did not allow for a full exploration of these hypotheses, as some long-term survivors are managed outside of the transplant center for nonmalignancy-related concerns, and intercurrent infectious events are not comprehensively captured. Furthermore, the full effect of azithromycin on the respiratory function of patients with BOS was beyond the scope of our current analysis.

In addition to the limitations noted above, this study is limited by the retrospective design. However, a retrospective cohort analysis is the only way to address the serious concerns about azithromycin arising from the ALLOZITHRO trial and the European Medicines Agency and Food and Drug Administration warnings in a timely fashion. This study represents the largest cohort of patients with BOS analyzed to date, although the overall cohort remains relatively small due to the rarity of this complication. The study was designed to look specifically at azithromycin exposure after any designation of BOS. Despite

Table 6
Estimation of Azithromycin Effect Using Matched Samples on Propensity Score to Receive Azithromycin

AZM Exposure	Relapse or Subsequent Neoplasm		Relapse		Subsequent Neoplasm	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
With replacement	1.21 (0.77-1.91)	.42	0.78 (0.42-1.43)	.43	2.25 (1.19-4.25)	.013
Without replacement	2.12 (0.67-3.07)	.36	0.88 (0.30-2.58)	.81	2.21 (0.78-6.32)	.14

differences in clinical practice between FHCRC and SLS, the effect of azithromycin was similar at each site, which further adds to the strength of our analysis. The era of transplant experienced by the study subjects spanned 2 decades, during which time transplant practices and indications have changed, although we did standardize disease risk and GVHD grading as much as possible. Significantly, the results were not modified when the year of transplant was included in the models. Nevertheless, as with any predictive analysis from observational cohort data, residual unobserved confounders (for instance in the reasons for administering azithromycin) cannot be excluded in our study.

We also recognized the potential for immortal time bias in our cohort, in which the exposed group may have an inherent survival bias, when comparing the effect of azithromycin with varying exposures over time. To address this bias, we used a cohort design with time-dependent Cox models in which the estimated HR represents the adjusted incidence rate ratio. This approach compares the risk of an event between exposed and nonexposed patients at each event time and reevaluates to which risk group each person belonged based on whether there had been an exposure by that time, and it tends to result in estimates with lower bias and greater precision compared with a nested case-control design [16,17]. Given the potential confounding by indication due to observational data, causal inference methods based on propensity score matching were further used as sensitivity analyses. This confirmed an increased occurrence of SN in the azithromycin group; note that it was no longer statistically significant when replacement was not allowed, likely due to a lack of power given the limited sample size.

In the light of these results, a careful assessment of the potential risks and benefits should be performed for each patient with BOS to determine whether azithromycin treatment should be prescribed. The increased risk of SN must be weighed against the potential benefit of chronic exposure. Azithromycin has been generally considered by most practitioners to be safe and is in widespread use for respiratory infections and various chronic respiratory diseases. In the setting of HCT, although azithromycin has become standard of care for BOS treatment at many centers, robust data supporting its efficacy in ameliorating lung dysfunction are lacking. As in other chronic lung diseases, long-term use may reduce infectious morbidity, but this has never been demonstrated in BOS after HCT. In addition to known cardiovascular and hearing loss side effects, concerns regarding antibiotic resistance and changes in microbiome diversity related to chronic azithromycin use and its consequences have emerged [37]. Additional studies are needed both to determine whether azithromycin is beneficial for patients with BOS after HCT and to elucidate the underlying mechanisms of azithromycin-associated cancers.

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SUPPLEMENTARY MATERIALS

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