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ORIGINAL ARTICLE

Malignancy among adult heart transplant recipients following patient-tailored dosing of anti-thymocyte globulin: a retrospective, nested case-control study of individualized dosing

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SUMMARY

Post-transplant malignancy is diagnosed in approximately 18% of heart transplant patients and is a leading cause of death post-transplant. One modifiable risk factor is the type and amount of immunosuppression received. Contemporary rabbit anti-thymocyte globulin (rATG) dosing strategy using T-cell-guided dosing, and its effect on malignancy in heart transplant patients is unclear. This was a single-center, retrospective chart review of heart transplant recipients receiving rATG for induction. Patients diagnosed with malignancy post-transplant were matched 1:2 to controls using a nested case-control design. The primary endpoint was to determine the relative risk of rATG exposure with the actual incidence of malignancy post-transplant. The secondary endpoint was the impact of maintenance immunosuppression on malignancy risk. Of the 126 patients included in the study, 25 developed malignancy and were matched to 50 control patients. The median cumulative rATG dose in milligrams (mg) between groups was 365 mg in malignancy cases and 480 mg in controls (OR 0.90, 95% CI 0.75–1.08, $P = 0.28$). In both the univariate and multivariable analysis, there was no statistically significant difference in malignancy risk found with any maintenance immunosuppressant. The results of this study showed that patient-tailored rATG dosing strategies may not be associated with malignancy development as previously thought.

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Key words
anti-thymocyte globulin, heart transplantation, immunosuppression, malignancy

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Introduction

Malignancy is a leading cause of death among heart transplant recipients, with increasing incidence each year following transplant [1,2]. Nonmodifiable risk factors affecting malignancy development include male gender and older age [2–4]. A modifiable risk factor related to post-transplant malignancy is a patient's overall immunosuppression exposure.

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Heart transplant recipients commonly maintain higher levels of immunosuppression in comparison with abdominal transplant patients and are twice as likely to acquire a malignancy post-transplant compared with kidney transplant recipients [1,5,6]. The increased cancer frequency has been partially attributed to the depletion of immune cells to prevent organ rejection; by broadly reducing immune cells, diseases regulated by protective immune cells, such as malignancies, are able to progress via altered immune surveillance, disrupted DNA repair and molecular signaling, and direct promotion of tumor growth by the immunosuppressive agents [1,5,7].

Induction immunosuppression in particular can have a pronounced and prolonged effect on the immune system, thereby contributing to malignancy. Rabbit antithymocyte globulin (rATG) is an induction immunosuppressant that causes depletion of various types of immune cells, with major effects on T- and B-lymphocytes [8]. Via profound immune modulation, rATG suppresses the immune system, and accommodates allograft acceptance [9,10]. Dosing strategies for rATG have varied, aimed at decreasing adverse events, including malignancy, without increasing recipient immunogenicity [9,11]. Previous studies on the relationship between rATG exposure and malignancy delivered mixed data and conclusions, and the association between contemporary dosing of rATG in heart transplantation and its impact on subsequent malignancy remains unclear [5,12,13]. Utilization of strategies such as T-cell-guided rATG dosing in heart transplant is limited [14–16]. The primary endpoint of this study was to explore the relationship between patient-tailored, T-cell-guided rATG exposure with the development of malignancy in heart transplant recipients. The secondary endpoint was to evaluate the impact of maintenance immunosuppression on the risk of malignancy in this patient population.

Materials and methods

Subjects

This was a single-center, retrospective, matched, nested case-control study. It was approved by the Institutional Review Board (IRB) and requirement of informed consent was waived by the IRB because of the retrospective nature and minimal risk of the study. This study was performed in accordance with the ethical standards set by the 2000 Declaration of Helsinki and the Declaration of Istanbul 2008. Heart transplant patients ≥18 years of age, who received rATG induction immunosuppression for a heart, heart-kidney, or heart-liver transplant between October 1st, 2008, when rATG was first used at this institution, and December 31, 2015 were included. Patients were excluded if they: received a heart transplant at another institution, did not receive rATG induction, received an unknown quantity of rATG, refused research participation consent, were considered a vulnerable population, or were a heart lung-transplant patient as these patients adhere to a different immunosuppression protocol.

Procedures

Patients were evaluated from the date of transplant through December 2017 to allow a minimum of 2 years follow-up for observation of malignancy manifestation. ICD-9 or -10 codes for cancers were used to identify patients acquiring malignancy since transplant, which were then confirmed via chart review. Baseline demographics, laboratory values, malignancy details (type, date of diagnosis, time since transplant), and medication details (doses, duration, troughs as applicable) were collected. Cumulative dose of rATG included that used for induction, rejection, and a subsequent transplant meeting inclusion criteria.

Definitions and criteria

Patients receiving a heart transplant at this institution receive rATG 1.5 mg/kg intraoperatively, then 1.5 mg/kg post-operatively for 3–5 doses. The duration of rATG is determined by the provider based on the recipient's clinical parameters, namely renal function, to delay initiation of calcineurin inhibitors. Additionally, guiding dosing for clinicians are cluster of differentiation (CD) 4 and CD8 counts in place of previously utilized cumulative CD3 counts [14–16]. Via this T-cell guided approach, if either CD4 or CD8 surpasses >15 cells/mcL, redosing of rATG is considered [15]. T-cell subsets, analyzed by flow cytometry, are collected daily until tacrolimus is initiated. In select patients, for example those with leukopenia or thrombocytopenia, a dose reduction to 0.75 mg/kg may be utilized. Basiliximab may be given in place of rATG for a profound rabbit allergy, thrombocytopenia, or leukopenia; however, basiliximab patients were excluded from this study. Initial maintenance immunosuppression for all patients is composed of mycophenolate mofetil 1000 mg twice daily, tacrolimus adjusted to attain trough levels of 9–12 ng/ml, and a prolonged prednisone taper over 4–2 months. The tacrolimus trough goal is reduced to 5–10 ng/ml when greater than

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6 months post-transplant if appropriate based on the rejection risk for the individual patient. Patients can be transitioned from tacrolimus to sirolimus after 4 months if they have completed their prednisone taper and subsequently have one negative biopsy. If the patient is <1 year post-transplant, the trough goal for sirolimus is 10–14 ng/ml with consideration given to 8–12 ng/ml if greater than one year post-transplant and low risk for rejection. If the patient remains on tacrolimus, the sirolimus trough is 8–12 ng/ml. If a patient develops a malignancy, the general approach is conversion to sirolimus if possible or reduction in the tacrolimus trough goals. Mycophenolate dosing can be adjusted based on patient weight and tolerance during treatment, especially related to leukopenia when sirolimus is used. All patients included in this study, including heart/liver and heart/kidney, followed our institutional protocol for heart transplant and were managed by the heart transplant team.

Both the total cumulative dose of rATG and the duration of induction dosing, termed "exposure" were examined in this study. Exposure for rATG induction was started on day 1 of rATG and concluded the day after that last dose of rATG, thus representing time with recurring T cell depletion. For maintenance immunosuppression in this study, exposure was weighted as illustrated in Fig. 1 to allow for quantification of duration and magnitude of immunosuppression. This equation was developed by the study team in order to more accurately reflect a patient's cumulative exposure to maintenance immunosuppression.

Cellular rejection was defined as grade 2R or greater on the 2005 Revision of the International Society for Heart and Lung Transplant (ISHLT)-1990 criteria, and antibody-mediated rejection (AMR) based on immunopathologic features, clinical descriptors, and histological evidence, if present, based on the 2011 ISHLT pathology consensus guidelines for AMR diagnosis [17]. Per protocol at this institution, a biopsy is considered to be performed at specific times post-transplant, after any significant alteration in maintenance immunosuppression, and when there is significant clinical concern for cardiac rejection. All biopsy specimens must be pathologically adequate for interpretation (three or more pieces and evaluated for AMR, including staining for C4d and CD68). Grade 2R rejection at this institution is treated with methylprednisolone 10 mg/kg IV daily for 3 days if <3 months post-transplant. Grade 3R and 4R rejection is treated with methylprednisolone 10 mg/kg IV daily for 3 days or providers may consider using thymoglobulin 1.5 mg/kg daily up to 5 days in the case of steroid refractory rejection or based on immunopathologic features, clinical descriptors, and histological evidence. The use of additional thymoglobulin for rejection was captured in the cumulative thymoglobulin dose for patients in this study. Cytomegalovirus (CMV) and Epstein–Barr virus (EBV) viremia were defined as detectable viral loads in the blood, noting tissue-invasion and biopsy evidence if available. Resolution was considered achieved when viral blood levels were undetectable on two occasions at least one week apart.

Statistics

A nested, case-control study design was chosen for its beneficial use in small populations when observing rare outcomes. Patients who developed cancer were matched to those that had not developed cancer by that post-transplant time point in a 1:2 method based on age, sex, EBV donor and recipient status, and year of transplant to eliminate the confounding nonmodifiable risk factors for malignancy, using sampling with replacement. Year of transplant was necessary to control for the era effect of rATG dosing observed in this population. Immunosuppression was not controlled for as it was the endpoint of this study. With this design, the follow-up time for controls was only as long as their match who developed cancer, so if a patient developed cancer 120 days after transplant, their matches would only be followed for the first 120 days after transplant.

Descriptive statistics are reported as means and standard deviations (SD) or medians and interquartile ranges (IQR) for continuous variables, and frequencies and percentages for categorical variables. Prior to matching, the incidence of malignancy post-transplant was calculated using the Kaplan–Meier method. Conditional logistic regression models were used to measure the association of cumulative rATG and maintenance immunosuppression exposure with the occurrence of malignancy post-transplant. Odds ratios (OR) and 95% confidence intervals (CI) were used to summarize the

$$\left[\frac{\text{Number of days on certain dose}}{\text{Total number of days}}\left(\begin{matrix}\text{average trough}\\ \text{during that time}\end{matrix}\right)+\frac{\text{Number of days on otherdose}}{\text{Total number of days}}\left(\begin{matrix}\text{average trough}\\ \text{during that time}\end{matrix}\right)\right]\times \text{Total number of days}$$

Figure 1 Equation used to calculate exposure to maintenance immunosuppression.

associations. The expected dosing range of rATG for this timeframe was 1.5–15 mg/kg, which yielded a predicted standard deviation of 3.4. Approximately 25 malignancy cases were anticipated based on the projected 18% malignancy rate in heart transplant patients found in the analysis of ISHLT transplant registry at the time of study development [18]. Based on an rATG standard deviation of 3.4, a 2:1 matched study provided 80% power to detect an odds ratios of 1.22 using a two-sided, alpha = 0.05 test.

Results

Population demographics

The study began with 186 heart transplant patients identified. Sixty patients were excluded, the most common causes being non-rATG induction immunosuppression or unknown rATG dose. The remaining 126 patients were included (Fig. 2). No patients received a subsequent transplant during the study.

Cancer incidence

Twenty-five of the 126 included patients experienced at least one post-transplant malignancy, with an incidence rate of 23.8% (95% CI 14.9–31.7) at 5 years post-transplant. Fourteen patients had more than one malignancy, providing 52 total malignancy episodes. The median time from transplant to diagnosis of malignancy was 407 days (range 124–1455 days, IQR 467). Cutaneous malignancies made up 82.7% of episodes whereas noncutaneous malignancies comprised 17.3%. Squamous cell carcinoma of the skin accounted for 46.2% of all cases, and 34.6% were basal cell carcinoma. Five episodes of post-transplant-lymphoproliferative disorder (PTLD) occurred, of which four episodes were in patients with an EBV donor (D) recipient (R) D+/R− match status. There was one episode each of melanoma, pancreatic, breast, prostate, and hepatocellular carcinoma. The smaller number of episodes of each individual cancer did not allow for further division into subgroups based on malignancy. Three of the 25 cases had a history of cancer, two with Non-Hodgkin Lymphoma and one with hepatocellular carcinoma. Only the hepatocellular carcinoma case was a recurrence of the former cancer. There was only one control patient with a history of malignancy in the form of Hodgkin Lymphoma.

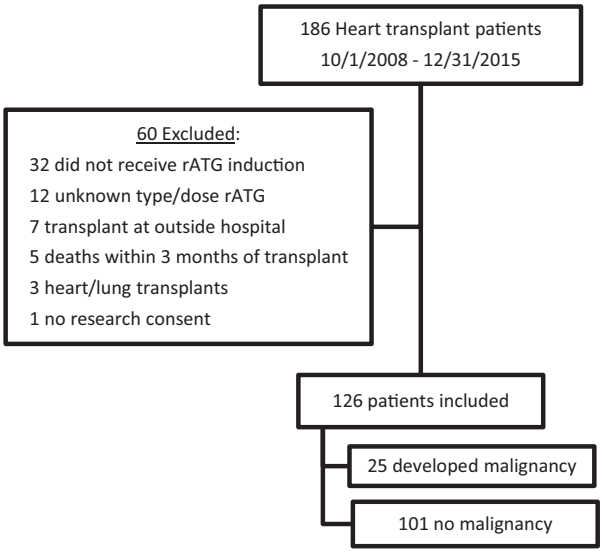

Figure 2 Study cohort (rATG, rabbit anti-thymocyte globulin).

From the 25 patients that demonstrated malignancy, 50 controlled pairings were made based on age, gender, EBV match status, and year of transplant. Patients were matched within 1 year of transplant and within 2.5 years of age with two exceptions to maintain match with EBV status of D+/R−, a well-described contributor to PTLD yet rare in our study population (8 of 126 patients, 6.3%). One D+/R− patient was matched within 5 years of transplant, and a second D+/R− patient was matched to a patient of the opposite sex and within 9 years of age.

The demographics between the cases and controls were comparable. A solitary heart transplant was the most common (73.3%), with 16% and 10.7% receiving heart/kidney and heart/liver transplants, respectively. A greater percentage of patients with CMV IgG positivity, that is, R+, were present in the malignancy group compared with the control group at the time of transplant. Episodes of EBV viremia, CMV viremia, and treated rejection episodes were similar between groups (Table 1).

Primary endpoint—relation of cumulative thymoglobulin dose to cancer incidence

Median rATG cumulative dose in milligrams (mg) was 365 mg in cases and 480 mg in controls (per 100 mg: OR 0.90, 95% CI 0.75–1.08, $P = 0.28$). This equated to a median cumulative rATG dose of 4.7 mg/kg in cases vs. 5.8 mg/kg in controls (per 1 mg/kg: OR 0.92, 95% CI 0.78–1.09, $P = 0.34$). There was no statistical difference in the individual doses of rATG given, the number of days of administration, or the median days of rATG induction exposure between the groups that did and

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Table 1. Baseline demographics.

Demographics	Cases (*N* = 25)	Controls (*N* = 50)	Total (*N* = 75)
Age (years), mean (SD)	60.2 (7.6)	59.7 (9.1)	59.9 (8.6)
Sex (*n*, %)			
Male	19 (76%)	39 (78%)	58 (77.3%)
Female	6 (24%)	11 (22%)	17 (22.7%)
Race			
Non-Hispanic/Caucasian	24 (96%)	46 (92%)	70 (93.3%)
Hispanic	1 (4%)	1 (2%)	2 (2.7%)
Black or African American	0 (0%)	3 (6%)	3 (4.0%)
Body Mass Index, mean (SD)	26.9 (4.6)	28 (4)	27.7 (4.2)
Organs transplanted			
Heart only	18 (72%)	37 (74%)	55 (73.3%)
Heart/kidney	5 (20%)	7 (14%)	12 (16%)
Heart/liver	2 (8%)	6 (12%)	8 (10.7%)
CMV status			
D+/R+	10 (40%)	13 (26%)	23 (30.7%)
D+/R−	5 (20%)	20 (40%)	25 (33.3%)
D−/R−	5 (20%)	14 (28%)	19 (25.3%)
D−/R+	5 (20%)	3 (6%)	8 (10.7%)
EBV status			
D+/R+	19 (76%)	40 (80%)	59 (78.7%)
D+/R−	4 (16%)	8 (16%)	12 (16%)
D−/R−	0 (0%)	0 (0%)	0 (0%)
D−/R+	2 (8%)	2 (4%)	4 (5.3%)
Rejection episodes	10 (40%)	19 (38%)	20 (38.7%)
EBV viremia	3 (12%)	2 (4%)	5 (6.7%)
CMV viremia	6 (24%)	18 (36%)	24 (32%)
History of smoking	8 (32%)	21 (42%)	29 (38.6%)
History of malignancy	3 (12%)	1 (2%)	4 (5.3%)

CMV, cytomegalovirus; D, donor IgG; EBV, Epstein–Barr virus; IQR, interquartile range; R, recipient IgG; SD, standard deviation.

did not develop malignancy (Table 2). When comparing the difference in rATG dosing from year-to-year, the cumulative rATG dose (mg/kg) decreased by an average of 1.57 mg/kg per year ($P < 0.001$, $R^2 = 0.55$; Fig. 3). Assessing patients from the first of half of the timeline from 2008 to 2011 versus the second half from 2012 to 2015, the difference in cumulative rATG dose (mg/kg) was statistically significantly different at a median of 7.9 mg/kg for 2008–2011 and 4.5 mg/kg for 2012–2015 ($P < 0.001$). Despite this difference, which was potentially because of heightened awareness to longer term side effects of induction immunosuppression yet not

Table 2. Primary endpoint – rATG exposure and malignancy risk.

Induction	Cases (*N* = 25)	Controls (*N* = 50)	Odds ratio (95% CI)	*P*-value
rATG cumulative dose (mg), median (IQR)	365 (270, 560)	480 (300, 630)	0.90 (0.75–1.08)*	0.28
rATG cumulative dose (mg/kg), median (IQR)	4.7 (3, 6.7)	5.8 (3.7, 7.3)	0.92 (0.78–1.09)†	0.34
Number of rATG doses, median (IQR)	4.0 (4, 7)	5.0 (4, 8)	0.97 (0.87–1.09)	0.63
rATG days of exposure, median (IQR)	5 (5, 8)	6 (5, 9)	0.96 (0.86–1.08)	0.53
Additional ATGAM used (yes)	2 (8.0%)	1 (2.0%)	4.00 (0.36–44.11)	0.26
Cumulative ATGAM dose (mg/kg), mean (SD)	15.0 (0.1)	14.6	—	—

CI, confidence interval; IQR, interquartile range; rATG, rabbit anti-thymocyte globulin; SD, standard deviation.

*Per 100 mg.

†Per 1 mg/kg.

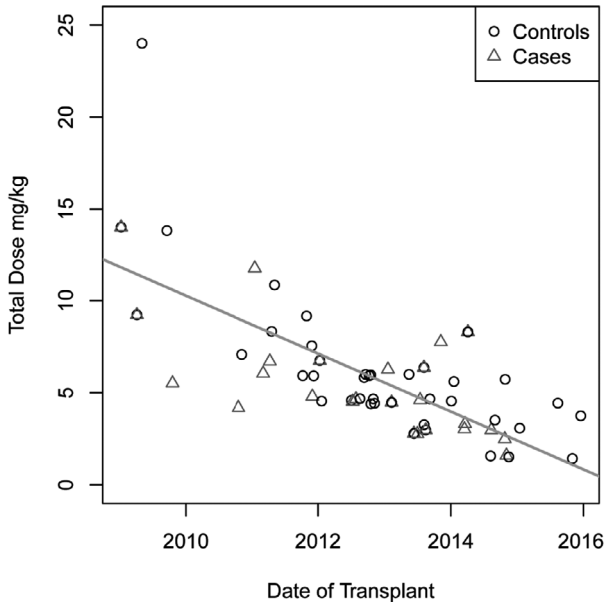

Figure 3 Date of transplant vs. dose of rATG (mg/kg) between cases and controls.

fully understood, the incidence of cancer between these two time periods was not statistically significantly different (HR = 1.19, 95% CI: 0.51–2.77, $P = 0.69$).

Secondary endpoint—relation of maintenance immunosuppression to cancer incidence

A univariate analysis for individual maintenance immunosuppressive agents was performed for tacrolimus, sirolimus, and mycophenolate mofetil. No patients in the malignancy cohort received azathioprine and too few received cyclosporine to complete the analyses. None of the maintenance immunosuppression agents showed a statistically significant association with the odds of developing cancer based on use of the drug, average trough/dose, days of therapy, or the calculation for overall exposure (Table 3). Though not statistically significant, potential trends toward decreased malignancy development in the sirolimus group were observed (OR 0.97, 95% CI 0.94–1.01, $P = 0.10$). A multivariable analysis was also conducted with various combinations of the maintenance immunosuppressants, but there was no statistically significant difference in malignancy found with any combination.

Discussion

Anti-thymocyte globulin is a commonly utilized medication in solid organ transplant, yet its place as induction in heart transplant remains controversial. When used, dosing strategy is variable and weighing of the risk of over- or under-immunosuppressing of patients is tenuous. Herein we analyzed the relationship between rATG exposure using an individualized dosing strategy and the risk of malignancy post-transplant in heart transplant patients. At our institution, CD 4/8 biomarkers are used daily at the time of transplant to guide rATG dosing based on presence of T-cells in the blood. By creating a biological threshold of CD 4 or CD 8 counts being >15 cells/mcL, dosing is tailored to the immune system of the patient and the ability to potentiate T cell rebound. This tailored dosing approach is

Table 3. Secondary endpoints—maintenance immunosuppression and malignancy risk, univariate analysis.

Univariate analysis	Cases ($n = 25$)	Controls ($n = 50$)	Odds Ratio (95% CI)	*P*-value
Sirolimus total exposure			0.97 (0.94–1.01)*	0.10
Use (yes vs. no)	$n = 16$	$n = 34$	0.69 (0.16–2.94)	0.62
Days of use, median (IQR)	210.5 (139.5, 458)	216.0 (114, 588)	0.75 (0.52–1.09)*	0.13
Mean (SD) trough value (mg)	8.9 (3.1)	8.5 (2.6)	1.18 (0.08–16.58)*	0.90
Tacrolimus total exposure			1.01 (0.98–1.04)*	0.58
Use (yes vs. no)	$n = 21$	$n = 40$	1.32 (0.36–4.80)	0.67
Days of use, median (IQR)	259 (228, 407)	274 (220.5, 449)	1.08 (0.80–1.45)*	0.63
Mean (SD) trough value (mg)	9.9 (1.3)	9.9 (1.5)	0.97 (0.68–1.40)*	0.88
Mycophenolate mofetil total exposure			1.00 (0.999–1.001)†	0.58
Use (yes vs. no)	$n = 25$	$n = 46$	—‡	—‡
Days of use, median (IQR)	393 (197, 714)	264.5 (115, 671)	1.13 (0.93–1.39)*	0.22
Mean (SD) daily dose (mg)	1642.6 (579.6)	1792.9 (542.7)	0.94 (0.84–1.04)*	0.24

CI, confidence interval; IQR, interquartile range; SD, standard deviation.

*Per 100 units.

†Per 1000 units.

‡All cases used mycophenolate mofetil therefore an odds ratio using conditional logistic regression was not possible.

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used in an effort to reduce infectious and cancer-associated complications by decreasing overall exposure to the agent, while still maintaining T-cell efficacy by following each patient's unique response daily [14–16].

Previous studies have found rATG use to be associated with post-transplant malignancy development when flat dose or standardized weight-based dose strategies were implemented [5,12]. One hundred percent of patients included in this study received rATG per our tailored dosing method. With this individualized dosing approach, cancer incidence in our cohort did not differ significantly with post-transplant rates reported in the general heart transplant population that is comprised of both rATG-treated and non-rATG-treated patients. This contrasts the limited literature that has shown an increase in cancer with the use of rATG in heart transplant patients [5,12]. Data from a Spanish Post-Heart-Transplant Tumor Registry study reported increased incidences of skin cancers and lymphomas with thymoglobulin; however this data combined equine and rabbit thymoglobulin and did not report specific doses received other than a range of 1–20 days of therapy [12]. A study by Rinaldi and colleagues explored the incidence of malignancy between OKT3, antilymphocyte globulin (ALG), and rATG. Cancer rates were higher with OKT3 and ALG compared with rATG, but present-day clinical application of this study is limited by the 1985–1998 timeframe; no knowledge of actual rATG doses received; and concomitant cyclosporine and azathioprine as maintenance immunosuppression, which are no longer standards of therapy [5]. Our study's findings align with results from one study done by El-Hamamsy and colleagues who administered flat-dose rATG 125 mg/day for 3 days to all heart-transplant patients and did not find an association with cancer incidence; though power to detect an association because of a smaller sample size and unreported rATG dosage received limit its interpretability [13].

Our results indicate that the use of lower cumulative rATG doses and judicious dose tailoring per patient may elicit less malignancy risk when compared with previous studies utilizing cumulative doses as high as 12.5 and 20 mg/kg [9]. Cancer incidence (23.8%) was similar to the approximate 18% post-transplant malignancy rate reported in the heart transplant population at the time of study development despite 100% of these patients having received rATG [18]. The malignancy types were comparable to those previously described, at approximately 80% cutaneous malignancies and 20% noncutaneous malignancies [1,13,19].

Potential benefits of individualizing rATG regimens for patients are several. Heart transplant recipients entering into transplant with underlying hematologic conditions or sensitivities may receive limited exposure to the rATG polyclonal depleting antibody; cost savings are incurred when doses are given under guidance of T cell markers rather than daily; and risks of post-transplant infectious or oncologic processes associated with rATG are normalized to that of the general heart transplant population.

More patients may benefit from polyclonal, depleting induction via this patient-specific approach. The innovations of ventricular assist devices and intra-aortic balloon pumps have provided prolonging options of temporary bridges to transplant. While increasing the time for organ availability, longer wait times prior to transplant incur the ongoing risk of bleed necessitating blood transfusions and furthering sensitization. In addition to these highly sensitized patients, patients in need of a renal-sparing post-transplant regimen may further benefit from a conservative, tailored, extended approach of rATG dosing, and calcineurin inhibitor avoidance. If tailored rATG dosing strategies are not associated with an increased incidence of cancer, consideration of additional rATG doses to delay calcineurin inhibitor initiation and provide depleting induction may be reasonable.

Strength of this study is in the comprehensive approach used to assess cumulative drug exposure by including both induction rATG doses and any rATG doses used for rejection treatment to capture a patient's lifetime exposure of rATG paired with patient-tailored dosing strategy using T-cell-guided dosing, which builds upon previous findings by Aliabadi *et al.* [20] For induction, T-cell counts are used to determine the frequency of rATG, allowing for individualization of the rATG dosage, interval, and duration of treatment. By utilizing this patient-specific approach, a lesser incidence of malignancy may develop than that previously reported with rATG usage in heart transplant. Additionally, the impact of maintenance immunosuppression on cancer risk was explored in a more granular approach than previous studies that utilize a dichotomous "yes" or "no" approach to drug exposure, or listing goal doses and troughs without collecting the true troughs attained or doses received.

The maintenance immunosuppression agents evaluated in this study did not have a statistically significant impact on malignancy development in the univariate or multivariable analyses. This may imply there is not one immunosuppression combination that has the greatest impact on cancer risk. Previous studies have shown mixed results regarding maintenance immunosuppression and

cancer risk. Generally, mammalian target of rapamycin (mTOR) inhibitors have demonstrated a decreased risk [21,22], tacrolimus has not had a significant impact on malignancy [23–25], and mycophenolate mofetil has shown both decreased and negligible effects [18,21,22]. Azathioprine has previously been associated with an increased risk [3,21,26,27], and cyclosporine has shown comparable risk to that of tacrolimus [24,25,28]. In our study, sirolimus had trends toward anti-oncotic effects, though it was not statistically significant. In the interest of hypothesis generation, we share that sirolimus showed a decreased cancer risk in patients with more days of sirolimus use compared with those with less, and those with higher cumulative exposure to sirolimus compared with patients with less exposure. These findings are supported by previous studies, though there are minimal studies in heart transplant patients alone [18,26–28]. Overall, the trends found with this study support that the chronic use of sirolimus may be beneficial in lowering the risk of post-transplant malignancy in heart transplant recipients.

Limitations

The low incidence of malignancy in heart transplant patients gives a relatively small study population with a variety of cancer types represented. The use of the nested, case-control design was selected to mitigate some of these limitations. By matching patients on the year of transplant, the impact of era effect, that is, earlier patients receiving more rATG, was minimized. This allowed comparison of cases and controls in patients receiving similar rATG based on similar dosing strategies. There is also a varied timeline for malignancy diagnosis post-transplant. While most were diagnosed within the first 1.5 years in this study, the chance of missing the manifestation of later malignancies cannot be fully eliminated. Additionally, accurately representing maintenance immunosuppression over the post-transplant period can be challenging because of the dynamic nature of the regimens. Using a novel formula for maintenance immunosuppression exposure allowed quantification of the amount and duration of immunosuppression in a more comprehensive way than previously reported though is still subject to limitations.

Conclusion

In conclusion, the findings of this study are hypothesis generating in that judicious strategies of rATG dosing may infer less cancer risk when compared with previously reported literature. This study did not find a statistically significant correlation between malignancy development and cumulative rATG exposure. There was no difference in malignancy risk when assessing the maintenance immunosuppression agents or regimens; although a potential trend of decreasing malignancy was seen with sirolimus. Additional studies and immunosuppressive biomarkers are needed to wholly assess a safe amount of rATG that could be used; however, thoughtful individualization and dose-tailoring of rATG may bring about new approaches to its implementation in the care of heart transplant recipients.

Authorship

RJB: designed research/study, performed research/study, collected and analyzed data, wrote manuscript. RAD: designed research/study, analyzed data, and performed statistical analysis. KCM: designed research/study, analyzed data, and performed statistical analysis. RCD: designed research/study, analyzed data, wrote manuscript. SSK: designed research/study, analyzed data, wrote manuscript. ALC: designed research/study, analyzed data, wrote manuscript. SAB: designed research/study, performed research/study, collected and analyzed data, wrote manuscript.

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Conflict of interest

The authors have no conflicts of interest to disclose.

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