Abstract
Purpose of Review While solid organ transplant (SOT) recipients are at the highest risk for severe complications and increased mortality from COVID19 disease, their vaccination against SARS-CoV-2 remains challenging due to fear of immune-mediated adverse events and suboptimal immune response. Our current review is aimed to summarize current knowledge about the safety and efficacy of SARS-CoV-2 vaccines, describe factors that are correlated with immune response, and discuss strategies to improve vaccine immunogenicity in SOT recipients.

Recent Findings SARS-CoV-2 vaccines are safe in SOT recipients and not related to rejection or other major adverse events. The immune response to two doses of vaccine is suboptimal and correlated to age and magnitude of immunosuppression. Administration of a third vaccine dose brings to significant amplification of immune response.

Summary This review strengthens the existing recommendation of vaccination by three doses of vaccine in all SOT recipients and completion of vaccination before transplantation if possible.

Keywords COVID-19 in transplant recipients · SARS-CoV-2 vaccine · Immunosuppression and vaccine · Immune response to the vaccine in transplant recipients

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the resulting disease, coronavirus disease 2019 (COVID-19), have affected millions of persons worldwide. COVID-19 in solid organ transplant (SOT) recipients is associated with increased morbidity and mortality due to comorbidities and immunosuppression state [1–4].

Nair et al. [5] showed a 30% increased risk of ventilation or death due to COVID-19 in SOT recipients compared with matched controls, and although symptoms of COVID-19 disease are generally similar to those in non-transplant patients [6, 7], rates of COVID-19-related hospitalizations in SOT recipients are higher than the general population [6–8].

In addition, immunocompromised patients who do not mount a strong immune response against SARS-CoV-2 are likely to shed the virus for a longer time and be a source of continued unintended exposure to others [9–11], as well as a potential source of new variants [9].

Vaccines to prevent SARS-CoV-2 infection are considered the most promising approach for controlling the pandemic and are being vigorously pursued. Based on data from other vaccinations, the immune response of solid organ recipients to vaccination may be blunted [12–14], and an adequate vaccine response cannot be assumed.

All transplant recipients are eligible for vaccination unless contraindicated from other reasons. Although the immunogenicity and efficacy of COVID-19 vaccines are uncertain in solid organ transplant recipients [15], the potential for benefit from vaccination likely outweighs this uncertainty.

In this review, we aimed to expand the knowledge and understanding and present an overview of the safety and immunogenicity of the SARS-CoV-2 vaccine in solid organ transplant recipients.
Safety

Although there are clear evidence of immune activation caused by SARS-CoV-2, based on large observational cohort studies of transplant patients with COVID-19, there has not yet been detected any correlation of SARS-CoV-2 infection with subsequent rejection [15]. Vaccines could induce allostimmunity by triggering an immune response that is cross-reactive with the allograft, by stimulating previously alloreactive immune cells, or through the non-specific stimulatory effects of adjuvants that could lead to de novo allostimmunity.

While there is a theoretical concern that vaccination may trigger organ rejection [16–19], or production of de novo anti-donor-specific antibody (DSA) [19–21], also reported for SARS-CoV-2 vaccine [22, 23], numerous studies of some common vaccines have shown no casual association between the two [24–29].

Similar to that, mRNA SARS-CoV-2 vaccines in SOT recipients were not significantly related to rejection events in most studies (Table 1).

In one study [30], the authors quote a sixfold higher incidence of rejection in third-dose versus second-dose recipients, based on only one case of rejection per group and the putative mechanism is unclear.

Taking into consideration high SARS-CoV-2 vaccine effectiveness and severe clinical spectrum of COVID-19 disease, these rare cases of rejection did not provide any basis to withholding vaccination in SOT recipients.

In immunocompetent individuals, mRNA SARS-CoV-2 vaccines, similar to other common vaccines, are noted to cause mild-to-moderate side effects, including local pain, headaches, and fatigue [31], with the only rare occurrence of major adverse events [32, 33]. Among transplant recipients, SARS-CoV-2 vaccines were not associated with any unexpected short-term local and systemic side effects (Table 1).

Immune Response and Efficacy of Two Doses of Vaccine

Data regarding immune response to SARS-CoV-2 vaccines have been exponentially accumulating over the last several months.

Some of the recent publications on this topic are summarized in Table 1.

These accumulating data consistently demonstrate a suboptimal humoral immune response among transplant recipients following two doses of mRNA SARS-CoV-2. Among kidney and lungs transplant recipients, the majority of patients did not develop an appreciable humoral immune response (seropositivity rates are between 8.2 to 66% and 10 to 47.4% for kidney and lung transplant recipients, respectively), while liver and heart recipients had a better response (seropositivity rates between 37.5 to 80% and 18.2 to 62% for liver and heart transplant recipients, respectively) (Table 1).

In addition, impairments in T-cell response were also described, with a high rate but reduced magnitude of spike-specific T helper cell response, as well as limited effector cytokine production in SOT recipients [34•, 35, 36•, 37••], and diminished generation of plasmablasts and memory B cells in response to mRNA vaccine [38]. Although cellular immunity may offset the absence of post-vaccination neutralizing antibodies in SOTRs, among 148 kidney transplant recipients, 35% developed neither humoral nor cellular responses following vaccination [39•].

The clinical importance of this laboratory finding is supported by growing evidence, suggesting that elapsed time after the vaccination has a major role in breakthrough infections due to waning immunity and declining of antibody titers [40]. Bergwerk et al. [41] described breakthrough infections with SARS-CoV-2 in a cohort of healthcare workers and a correlation of these infections with the declining of neutralizing antibody titers in the peri-infection period.

Growing bulk of evidence confirm that this observation is true also for immunocompromised patients. Wadei et al. [42] reported on seven SOTRs with undetectable or low titer anti-spike antibodies who developed COVID-19 infection after receiving one or two doses of the SARS-CoV-2 mRNA vaccine. The clinical presentation and course of these patients were comparable to those of SOT recipients who had COVID-19 infection and have not been vaccinated. Qin et al. [43••] analyzed breakthrough SARS-CoV-2 infections in 18,215 fully vaccinated SOTRs at 17 transplant centers and demonstrated an 82-fold higher risk of breakthrough infection and 485-fold higher risks of breakthrough infection associated with hospitalization and death, compared to the general population. Furthermore, while breakthrough COVID-19 is predominantly mild, recent studies show severe and fatal breakthrough infection in transplant recipients with suboptimal humoral responses [43••, 44–49].

Booster

Observation of the waning immunity demonstrated by decreased antibody levels after two doses of vaccine in the general population [41] as well as breakthrough infections among vaccinated SOT recipients, including severe COVID19 cases [43••, 44–49], together with a suggested correlation between breakthrough infections and the time that has passed since the second vaccine dose, has led experts including the FDA to recommend the administration
| Table 1  | Humoral response to two doses of SARS-CoV-2 vaccine in solid organ transplant recipients |
|--------------------|---------------------------------------------|
| Organ transplanted, number of patients | Type of vaccine | % Seropositive humoral response | Associations with seronegative response | Associations with seropositive response | Adverse Events (AE) | Time from second dose of vaccine |
| **Bertrand et al. [35]** | Kidney, 45 | BNT162b2 (Pfizer-BioNTech) | 17.8% | Duration of kidney transplantation and a cyclosporin-based immunosuppressive regimen |  | 1 month |
| **Boyarsky et al. [77]** | Total = 436 | - BNT162b2 (Pfizer-BioNTech) | 17.4% | Regimen includes antimetabolite (OR = 0.22 (0.1–0.3)) | mRNA-1273 (OR = 2.1 (1.3–3.5)) compared to BNT162b2 | 20 days post first dose |
| | Kidney, 219 | - mRNA-1273 (Moderna) | 18.7% | Age (OR = 0.83 (0.7–0.9)) |  |  |
| | Liver, 78 | | 47.4% |  |  |  |
| | Heart, 66 | | 18.2% |  |  |  |
| | Lung, 50 | | 10% |  |  |  |
| **Boyarsky et al. [78••]** | Total = 658 | - BNT162b2 (Pfizer-BioNTech) | 54% | Younger age | Low-dose mycophenolic acid Cyclosporine-based regimen | 28–31 days |
| | Kidney, 322 | - mRNA-1273 (Moderna) | 48% | Type of vaccine (BNT162b2 > BNT162b2) | No serious AE |  |
| | Liver, 129 | | 80% | More years since transplantation |  |  |
| | Heart, 97 | | 57% | Organ transplanted |  |  |
| | Lung, 71 | | 39% | Maintenance includes antimetabolite |  |  |
| | Pancreas, 5 | | 20% |  |  |  |
| **Bruminhent et al. [79]** | Kidney, 35 | Inactivated whole-virus SARS-CoV-2 vaccine | 9% |  | No serious AE | 2 weeks |
| **Crespo et al. [80]** | Kidney, 90 | mRNA-1273 (Moderna) | 63% | > 6 months after transplantation (OR 0.29 (0.08–1.0)) | No serious AE | 28 days |
| **Cucchiari et al. [39•]** | Kidney, 117 | mRNA-1273 (Moderna) | 30% | Combination Tacrolimus + mTOR inhibitors (OR 0.3 (0.09–0.8)) | No serious AE | 2 weeks |
| **Grupper et al. [74]** | Kidney, 136 | BNT162b2 (Pfizer-BioNTech) | 37.5% | Older age (1.7 (1.2–2.7)) High-dose corticosteroids in the last 12 months (1.3 (1.09–1.8)), Maintenance with triple immunosuppression (1.4 (1.06–2.1)) Regimen that includes mycophenolate (1.5 (1.3–2.3)) | No serious AE | 14–21 days |
| Table 1 (continued) | Organ transplanted, number of patients | Type of vaccine | % Seropositive humoral response | Associations with seronegative response | Associations with seropositive response | Adverse Events (AE) | Time from second dose of vaccine |
|---------------------|--------------------------------------|-----------------|-------------------------------|--------------------------------------|--------------------------------------|---------------------|----------------------------------|
| Grupper et al. [63••] | Kidney, 128 | BNT162b2 (Pfizer-BioNTech) | 52% | Vaccination post-transplantation (22.4 (3.6–36)) Age (1.04 (1.01–1.1)) Time on dialysis ((1.02 (1.01–1.04)) | Higher lymphocyte count ((0.52 (0.3–0.9)) | No serious AE | 95 days after second dose |
| Guarino et al. [81] | Liver, 365 | BNT162b2 (Pfizer-BioNTech) | 75% | Older age (> 65 years) Higher BMI Shorter time from transplantation Immunosuppressive regimens (anti-metabolite, mTORS, 2 <Immunosuppressive medications) | | No serious AE | 4 weeks |
| Hallett et al. [82] | Heart, 134 Lung, 103 | • BNT162b2 (Pfizer-BioNTech) • mRNA-1273 (Moderna) | 62% 36% | Heart vs. lung transplant (1.55 (1.2–2.0)) Anti-metabolite maintenance immunosuppression (0.7 (0.5–0.8)) Younger age ((0.61 (0.4–0.9)) > 6 years out from transplantation (1.22 (1.1–1.3)) | | No serious AE | 28 days |
| Itzhaki Ben Zadok et al. [83] | Heart, 37 | BNT162b2 (Pfizer-BioNTech) | 49% | Older age Treatment with anti-metabolite-based protocols | | No serious AE | 35–40 days post first dose |
| Marion et al. [84•] | Total = 367 Kidney, 271 Liver, 58 Thoracic organs, 33 | • BNT162b2 (Pfizer-BioNTech) • mRNA-1273 (Moderna) | 34% 33% 50% 12% | Anti-metabolite drugs (5.7 (3.0–11.5)) or steroids (3.6 (2.1–6.6)) Age (1.03 (1.01–1.06)) Impaired allograft function (0.98 (0.96–0.99)) Transplant < 4 years (2.9 (1.70–5.1)) | Most mild 1 acute cellular rejection in kidney recipient | | 28 days |
| Masset et al. [85•] | Kidney, 456 | BNT162b2 (Pfizer-BioNTech) | 50% | | | | 1 month |
| Table 1 (continued) | Organ transplanted, number of patients | Type of vaccine | % Seropositive humoral response | Associations with seronegative response | Associations with seropositive response | Adverse Events (AE) | Time from second dose of vaccine |
|----------------------|----------------------------------------|----------------|-------------------------------|---------------------------------------|----------------------------------------|---------------------|----------------------------------|
| Mazzola et al. [86]  | Total = 133 Kidney, 59 Liver, 58 Heart, 26 | BNT162b2 (Pfizer-BioNTech) | 28% 16.6% 37.5% 34.8% | Transplantation < 2 years (2.8 (1.06–7.7)) Diabetes (3.4 (1.3–8.5)) Kidney transplantation (4.0 (1.5–10.2)) | No serious AE | 28 days |
| Peled et al. [87]    | Heart, 77 | BNT162b2 (Pfizer-BioNTech) | 18% | Mycophenolate use (0.12 (0.01–0.8)) | No serious AE | 28 days |
| Przedecki et al.     | Kidney, 920 | ChAdOx1 (Oxford University–AstraZeneca) BNT162b2 (Pfizer-BioNTech) | Pfizer 66% Astra zeneca 44% <1 year after transplant (0.2–0.5) Diabetes (0.6 (0.4–0.9)) Vaccination with BNT162b2 (2.4 (1.7–3.4)) Tacrolimus monotherapy (5.2 (3.6–7.6)) | No serious AE | 31 days |
| Rabinowich et al.    | Liver, 80 | BNT162b2 (Pfizer-BioNTech) | 47% | Age (1.3 (1.1–1.9)) Lower eGFR (0.8 (0.4–0.9)) High-dose prednisone in the past 12 months (1.8 (1.5–4.6)) Triple therapy immunosuppression (1.7 (1.2–2.5)) Low-dose steroids (1.5 (0.9–4.1)) MMF (1.8 (1.1–3.5)) | No serious AE | 10–20 days |
| Rashidi-Alavijeh et al. [90] | Liver, 43 | BNT162b2 (Pfizer-BioNTech) | 79% | MMF treatment | No serious AE | 15 days |
| Rozen-Zvi et al. [91] | Kidney, 308 | BNT162b2 (Pfizer-BioNTech) | 36% | eGFR (1.02 (1.01–1.04)) Lower mycophenolic acid dose (2.3 (1.8–3.1)) Younger age (1.03 (1.01–1.05)) Lower calcineurin inhibitor blood level (1.9 (1.1–3.4)) | No serious AE | 2–4 weeks |
| Sattler et al. [34•] | Kidney, 39 | BNT162b2 (Pfizer-BioNTech) | 8.3% | No significant associations | No serious AE | 24 days |
| Shostak et al. [92]  | Lung, 168 | BNT162b2 (Pfizer-BioNTech) | 18% | Age (0.9 (0.92–0.98)) Use of antimetabolites (0.2 (0.08–0.8)) Use of mTOR (0.1 (0.02–0.99)) | No serious AE | 14–21 days |
### Table 1 (continued)

| Organ transplanted, number of patients | Type of vaccine | % Seropositive humoral response | Associations with seronegative response | Associations with seropositive response | Adverse Events (AE) | Time from second dose of vaccine |
|---------------------------------------|----------------|-------------------------------|----------------------------------------|----------------------------------------|---------------------|----------------------------------|
| Strauss et al. [93] Liver, 161         | - BNT162b2 (Pfizer-BioNTech)  
- mRNA-1273 (Moderna) | 81%                           | Antimetabolites (0.6 (0.5–0.8))        | mRNA-1273 (1.25 (1.09–1.4))           | Most mild, 1.6% hospitalization due to side effects | 30 days |
| Stumpf et al. [36] Kidney, 368         | - BNT162b2 (Pfizer-BioNTech)  
- mRNA-1273 (Moderna) | 42%                           | Age (1.02 (1.008–1.04)) Time since transplantation (0.9 (0.91–0.98)) Number of immunosuppressive drugs (2.0 (1.3–3.1)) mRNA-1273 v (0.35 (0.2–0.6)) compared to BNT162b2 | | 8 weeks post first dose |
| Thuluvath et al. [94] Liver, 62        | - Johnson & Johnson  
- BNT162b2 (Pfizer-BioNTech)  
- mRNA-1273 (Moderna) | 39%                           | Use of 2–3 immunosuppressive medications (14.4 (5–40.7)) Vaccination with a single dose of Johnson & Johnson Vaccine (0.02 (0.01–0.1) compared to Moderna 0.06 (0.02–0.24 compared to Pfizer) | No serious AE | 28 days |
of a booster (third) vaccine dose to immunocompromised individuals, including SOT recipients [50–52].

The CDC recommends that patients who are moderately to severely immunocompromised (including SOT recipients) should receive an additional dose of mRNA COVID-19 vaccine at least 28 days after the second dose [53]. Several countries have made similar recommendations [54–57].

First reports on the administration of a third dose of the mRNA vaccine to SOT recipients have been shown to improve the immune response without causing any short-term, serious adverse events (Table 2). The third dose of the vaccine in SOT transplant recipients was effectively resulted in increased IgG anti-S levels, including in transplant recipients who were seronegative after two doses.

Hall et al. [58] studied 120 solid organ transplant recipients who had received two doses of an mRNA vaccine and were randomly assigned to either the third dose of mRNA vaccine or saline placebo. A serologic response was present in 55% of patients in the vaccine group compared with 18% in the placebo group.

Kamar et al. [59••] reported on SOT recipients (kidney, liver, lung, heart, pancreas) who received three doses of BNT162b2; the prevalence of anti-SARS-CoV-2 antibodies was 0%, 4%, and 40% before the first, second, and third doses, respectively, and 68% 4 weeks after the third dose. Unfortunately, approximately 50 to 70% of SOTRs who were seronegative after two doses remained seronegative. Adverse effects were similar to those reported after prior doses (Table 2).

Longitudinal follow-up and evaluation of the clinical implication of the third dose in this population are needed to more completely characterize the impact of additional vaccine doses.

Timing

The ideal timing of vaccination in the post-transplantation setting is not known. In all cases, the likelihood of contracting COVID-19 (based on community prevalence) against the optimal timing with the highest probability of developing a protective immune response to vaccination should be weighted.

ISHLT recommendations are to delay vaccination for at least one month from the time of transplantation and for at least three months after use of T-cell-depleting agents (e.g., anti-thymocyte globulin) or specific B cell-depletion agents (e.g., rituximab) [60].

According to NIH recommendation [61], transplant surgery should be delayed until ≥2 weeks after COVID-19 vaccination. If delayed surgery is not possible, it may be advisable to delay vaccination of transplant recipients for several weeks to months after surgery and the associated use of highly immunosuppressive medications.

The American Society of Transplantation (AST) also recommends vaccinating at least two weeks before transplantation but does not specify whether vaccination should be delayed when T or B cell-depleting agents are used [62].

Our group [63••] has demonstrated a significantly higher rate (90%) of positive humoral response to BNT162b2 SARS-CoV-2 vaccine in kidney transplant recipients vaccinated before transplantation, as compared to individuals vaccinated after transplantation (45%), with appreciable anti-spike antibody response sustained in pre-transplant vaccinated patients even after induction immunosuppression and high-dose steroids treatment. Mean antibody levels in seropositive recipients were similar to healthy controls, suggesting a better chance of responding to the vaccine when administered pre-transplantation.

Parameters Correlated with Immune Response to SARS-CoV-2 Vaccine

Age

Advanced age was found to be associated with low antibody response in immunocompetent patients after COVID-19 disease [64], as well as after mRNA SARS CoV-2 vaccination [65]. Older age was a consistent variable related to a poor immune response in recent studies (Table 1), demonstrating that even in immunosuppressed patients, age is an independent predictor of poor immunological response.

Immunosuppression

The seroresponse to vaccination is related to the net burden of immunosuppression: The greater the degree of immunosuppression, the less likely the patient will respond to immunization. These findings are not only clinically obvious but also supported by previous studies that found a strong correlation of poor seroprotection [21] and increased infection rates [66] with increasing intensity of immunosuppression regimen.

The influence of different immunosuppressive regimens on vaccination response was studied in previous years. A number of studies evaluated response rates to influenza and pneumococcal vaccination in patients receiving glucocorticoids. While the immune response was preserved, although mildly reduced, in most patients on a chronic low dose of glucocorticoids [67–69], the response was inadequate in patients on high dose [70].

Additional studies demonstrated a dose-related correlation of MMF to decreased influenza and cholera vaccine responsiveness in renal transplant subjects [71–73].

Several studies on SOT recipients in mRNA SARS CoV-2 vaccination demonstrate concordant results with significant
| Table 2  | Humoral response to the third dose of SARS-CoV-2 vaccine in solid organ transplant recipients |
|----------|-----------------------------------------------------------------------------------------------|
| **Organ transplanted, number of patients** | **Type of vaccine** | **% Seropositive before third dose** | **% Seropositive after third dose** | **Associations with response** | **Adverse Events (AE)** | **Time from second dose of vaccine, time after injection** |
| Benotmane et al. [95] | Kidney, 159 | mRNA-1273 (Moderna) | 0% | 49% | Tacrolimus, mycophenolate and steroids were less likely to respond. Patients with a weak response after the second dose were more likely to respond. | No serious AE | Third dose 51 days after the second dose, Serology test was done 28 days after the third dose |
| Del Bello et al. [96•] | SOT, 396 | BNT162b2 (Pfizer-BioNTech) | 41% | 68% | Seroconversion was more likely in younger patients (OR = 0.95 (0.93–0.97)). Patients receiving mycophenolic acid (0.28 (0.1–0.5)), belatacept (0.14 (0.4–0.4)), patients that received at least a triple immunosuppression (0.4 (0.2–0.8)) presented lower seroconversion rates. | No serious AE | Third dose 59 days after the second dose, Serology test was done 4 weeks after the third dose |
| Hall VG et al. [97] | SOTR, 60 | mRNA-1273 vaccine (Moderna) | 12% | 55% |  | No serious AE | Third dose 2 months after second dose, Serology test was done 1 month after the third dose |
| Kamar N et al. [98] | SOT, 101 | BNT162b2 (Pfizer-BioNTech) | 40% | 68% | Seronegative patients were older, had a higher degree of immunosuppression, and had a lower eGFR. | No serious AE | Third dose 61 days after the second dose, Serology test was done 4 weeks after the third dose |
| Masset C et al. [85•] | Kidney, 136 | BNT162b2 (Pfizer-BioNTech) | 50% | 69% | Lymphocyte count < 1500/mm³ increased the likelihood of being a nonresponder (3.84 (1.6–19.9)). Impairment of allo-graft function (0.97 (0.94–0.99)). |  | Third dose 50 days after the second dose, Serology test was done 4 weeks after the third dose |
reduction of immune response with anti-metabolite medication, high-dose steroid treatment, or number of chronic immunosuppressive medications (Table 1).

For example, Grupper et al. [74] showed a decrease of 40% in humoral response for kidney transplant recipients on triple vs. double immunosuppressive medications with the significant negative influence of MMF and high-dose steroids.

How to Enhance the Immune Response to Vaccination

One possible approach to overcome the low response rate in SOTRs is to choose optimal timing for vaccination, with accumulating evidence of the advantage of pre-transplant vaccination of transplant candidates. After the transplantation, the decision of optimal vaccination timing is more challenging and should take into consideration epidemiological data of the danger of SARs-Cov-2 contraction versus pursuit to delay vaccination at least 1 to 3 months after transplantation considering a decreased immune response to the vaccine during this period (Table 1).

While multiple studies are concordant in the conclusions about the negative influence of immunosuppression (especially MMF and high-dose glucocorticoids) on the vaccination success, there are insufficient data to guide modifications of immunosuppression in anticipation of, or preparation for, vaccination, and society guidelines do not recommend routine modification of immunosuppression [75].

The introduction of a third (booster) dose is currently the best way to increase the immune response to the SARs-Cov-2 vaccine, and we have sufficient evidence to recommend it in SOT recipients. The longevity of this immune response should be evaluated and may guide us in the decision about the need for additional boosters in the future.

Promising results from the very recent study that used switching to a different vaccine platform in non-responders (e.g., viral vector-based vaccination after failure to achieve a response after mRNA vaccination) propose an additional useful strategy [76].

Conclusion

Accumulating data about SARA-CoV-2 vaccination in SOT recipients repeatedly confirm the high safety profile of mRNA vaccines. Reduced immunogenicity of the vaccine in SOT recipients is correlated mainly with high immunosuppression burden (and proximity to transplant surgery) and older age. While additional long-term studies are needed to evaluate the possible strategies to increase vaccine-induced protection in SOT recipients, this review strengthens the existing recommendation of vaccination by three doses of
vaccine in all SOT recipients and completion of vaccination before transplantation if possible. In the meantime, transplant recipients should be advised to continue other protective measures regardless of the number of vaccine doses received.

**Declarations**

**Ethics Approval and Consent to Participate** This article does not contain any studies with human or animal subjects performed by any of the authors.

**Conflict of Interest** The authors declare no competing interests.

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Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

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