be cautious in extrapolating findings from murine studies, we did suggest the need for further studies on combining the targeting of MDSCs with anti-PD1/PD1 ligand (PD-L1) therapies. Nevertheless, we agree completely with Drs Kodach and Peppelenbosch that such studies should take into account the diversity of gastric cancer, because it is unlikely that there will be a uniform response.

The current predictors of response to immuno-oncology therapy include the mutational burden, the level of PD-L1 expression, and possibly the presence of infiltrating $CD8^+$ T cells.[3] In our studies, we used primarily a mouse model with a documented high mutational load, and other murine models (particularly genetically engineered mouse models) may not respond as well. In addition, the mouse model studied had a higher level of PD-L1 expression in our initial screen. However, the authors are correct that the mutational load and PD-L1 expression is not uniform in all types of gastric cancers. The current TCGA classification, developed by Adam Bass (now here at Columbia University) and colleagues described 4 subtypes[4] and the evidence to date does suggest that the microsatellite instability subtype, which is usually mismatch repair deficient, is the most responsive to anti-PD1/PD-L1 therapy, because of their increased mutational load and higher PD-L1 expression.[5] Pembrolizumab has been approved for any type of solid tumor with microsatellite instability-high.[6] The Epstein-Barr virus–positive subtype is also predicted to be more responsive because of increased PD-L1/2 expression and marked immune cell infiltration,[5] confirmed in the reference cited, but this subtype is more rare, and although it is enriched in responders, the overall responsiveness of Epstein-Barr virus–positive tumors to anti-PD1/PD-L1 therapy remains to be clarified. Nevertheless, although these 2 subgroups show significant responses to immuno-oncology therapy, the vast majority of PD-L1–positive tumors fail to respond. Furthermore, some PD-L1–negative tumors respond, and gastroesophageal junctional tumors are now approved by the US Food and Drug Administration for pembrolizumab combined with 5-fluoracil as first line, regardless of PD-L1 expression.

Although we would agree that PD-L1 expression tends to be low in the other 2 subtypes, the chromosomal instability (CIN) subtype and the genomically stable subtype, these 2 categories constitute the bulk of gastric tumors. Most CIN tumors do have an intestinal-type histology, as seen in most mouse models. The CIN subtype has been predicted to benefit the most from adjuvant chemotherapy,[7] but the tumors themselves express lower levels of PD-L1, and the majority of CIN tumors exhibit T-cell exclusion and infiltrating macrophages.[8] In contrast, one-half of genomically stable tumors have tertiary lymphoid structures, pointing to the potential for tumor immunity.[8] However, an important point of our study is that MDSCs suppress $CD8^+$ T-cell immunity and often express PD-L1, and thus targeting MDSCs can improve the response to immune checkpoint drugs, potentially in distinct tumor classes. In any case, we are now in the era of precision oncology, and we agree that therapies should be given to the patients most likely to benefit from them.

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References

1. Kodach LL, et al. Gastroenterology 2021;161:727.
2. Kim W, et al. Gastroenterology 2021;160:781–796.
3. Cristescu R, et al. Science 2018:362.
4. Cancer Genome Atlas Research Network. Nature 2014; 513:202–209.
5. Kim ST, et al. Nat Med 2018;24:1449–1458.
6. Marcus L, et al. Clin Cancer Res 2019;25:3753–3758.
7. Sohn BH, et al. Clin Cancer Res 2017; Jul 26 [Epub ahead of print].
8. Derks S, et al. Ann Oncol 2020;31:1011–1020.

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https://doi.org/10.1053/j.gastro.2021.04.027

Tacrolimus Use and COVID-19 Infection in Patients After Solid Organ Transplantation

To the Editors:

We read with great interest the study by Belli et al[1] in which the authors have retrospectively analyzed the effect of comorbidities, immunosuppression, and ageing on overall mortality in liver transplant patients with coronavirus disease 2019 (COVID-19). In this multicenter cohort study, multivariable Cox regression analysis showed that tacrolimus use had a positive effect on patient survival (hazard ratio, 0.55; 95% confidence interval [CI], 0.31–0.99).[1] However, this association was not that solid owing to defects in the study design. In this study, 39 patients (16%) received homecare and 204 (84%) needed hospitalization. However, patients receiving homecare had a survival rate of 100% and 82.05% received tacrolimus, whereas those patients in hospital only had a survival rate of 76.0% and 63.7% received tacrolimus. Additionally, for inpatients, 7.8% stopped calcineurin inhibitors (CNI) and 17.6% decreased CNI compared with 0% stopping CNI and 5.13% decreasing CNI in outpatients. This point means that it was more likely for patients with a good prognosis (those receiving homecare) to use tacrolimus, whereas those needing hospitalized or intensive care unit admission with worse prognosis tended to stop tacrolimus after diagnosis of COVID-19. Hence, preexisting selection bias in the study contributed to the favorable association between tacrolimus use and a better prognosis. Although multivariate Cox analyses were conducted, disease severity was not

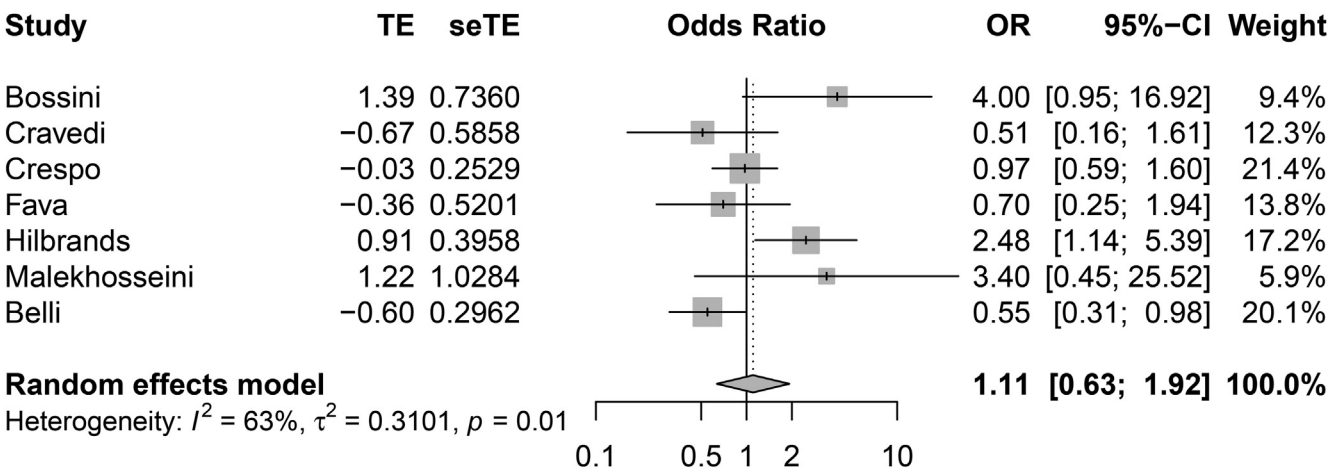

Figure 1. Forest plot of studies investigating the association between tacrolimus use and mortality in solid organ transplant recipients with COVID-19.

adjusted. It may be more reasonable to do patient stratification or enroll in-hospital patients alone to explore the impact of tacrolimus on prognosis.

In addition, in the study by Colmenero et al[2] of 111 hospitalized liver transplant recipients, more patients with nonsevere COVID-19 received tacrolimus initially (64.5% vs 48.6%), and tacrolimus use was not associated with severe COVID-19 (relative risk, 0.54; 95% CI, 0.29–1.07; $P = .08$).[2] Of interest, mycophenolate use was an independent predictor of severe COVID-19 (relative risk, 3.94; 95% CI. 1.59–9.74; $P = .003$). In the prospective cohort study involving 414 kidney transplant recipients with COVID-19,[3] tacrolimus use was not associated with mortality (hazard ratio, 0.974; 95% CI, 0.593–1.598; $P = .918$). Bossini et al[4] even reported that tacrolimus use was associated with an increased risk of death in a retrospective cohort of 53 kidney transplant recipients (odds ratio [OR], 4.0; 95% CI, 1.1–19.7; $P = .05$).[4]

Given the disputes on the immunosuppression in solid organ transplant (SOT) recipients with COVID-19, we have registered a systematic review and meta-analysis in PROSPERO aimed to explore the risk factors of mortality in SOT patients (CRD42020215987). PubMed, Embase, and Cochrane library were searched, and the last search was conducted on December 15, 2020. Disease severity defined in the original study was adopted in this meta-analysis. The quality of observational studies was assessed by using the Newcastle-Ottawa Scale.[5] A meta-analysis was performed using R statistical software (version 4.0.0), with the package "meta." A random effects analysis was used for all meta-analyses, owing to the clinical heterogeneity inherent in the data and the different sample sizes of included studies. The ORs and 95% CIs were pooled by the inverse variance method.[5]

Finally, 11 cohort studies were included.[1–4,6–12] Among them, 7 studies involving 1348 SOT patients explored the association between tacrolimus use and mortality and other 4 involving 229 SOTs explored the association between tacrolimus use and severe COVID-19 (Supplementary Table 1). Four studies only included hospitalized patients, and 7 included both in- and out-patients. Seven studies included kidney transplant recipients, 2 included liver transplant recipients, and 2 included SOTs. Study population size ranged from 25 to 414 patients. COVID-19 was diagnosed based on real-time polymerase chain reaction (RT-PCR) in 6 studies, 4 studies included both PCR and specific chest image confirmed COVID-19 patients, and 1 did not report the COVID-19 diagnosis method. The median time from SOT to COVID-19 diagnosis ranged from 0 to 168 months. Based

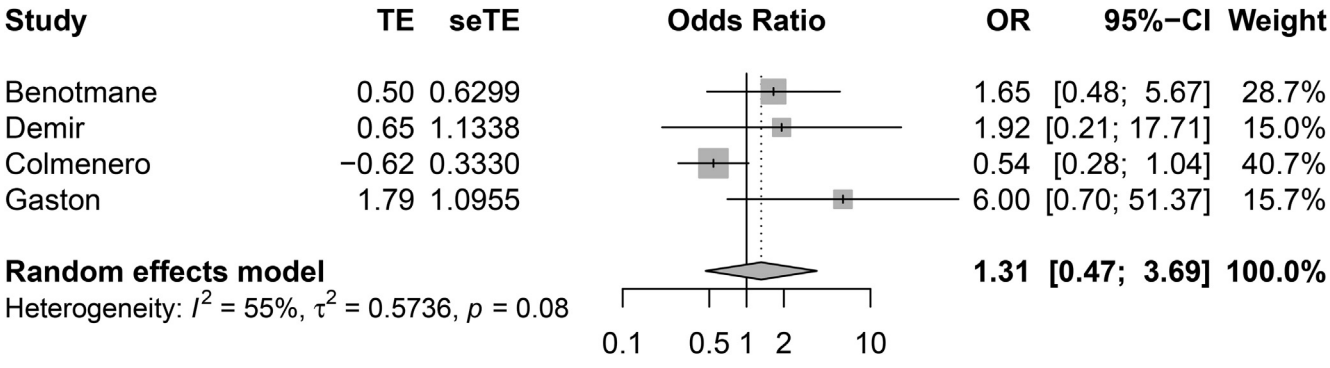

Figure 2. Forest plot of studies investigating the association between tacrolimus use and severe COVID-19 in solid organ transplant recipients.

on the Newcastle-Ottawa Scale, 2 studies were of high quality, 6 of moderate, and 2 of low quality (Supplementary Tables 2 and 3).

Pooled results showed that tacrolimus use was associated with neither higher risk of severe COVID-19 (OR, 1.31; 95% CI, 0.47–3.69) or increased mortality (OR, 1.11; 95% CI, 0.63–1.92) in SOT patients with COVID-19 infection (Figures 1 and 2). For mortality, similar results were indicated in subgroup analyses of hospitalized SOT patients (OR, 0.61; 95% CI, 0.28–1.30), kidney transplants (OR, 1.22; 95% CI, 0.65–2.30), a sample size of >100 patients (OR, 0.89; 95% CI, 0.52–1.53), and PCR-confirmed cases (3 studies, OR, 0.97; 95% CI, 0.36–2.61). For severe COVID-19, similar results were also observed in hospitalized SOT patients (OR, 3.46; 95% CI, 0.74–16.21), kidney transplant recipients (OR, 1.71; 95% CI, 0.58–5.03), and PCR-confirmed cases (OR, 1.39; 95% CI, 0.30–6.41).

In conclusion, our study found that tacrolimus use is not a risk factor for mortality and severity in SOT patients with COVID-19. Well-designed prospective study is encouraged to verify these findings in the future.

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References

1. Belli LS, et al. Gastroenterology 2020;160:1151–1163.
2. Colmenero J, et al. J Hepatol 2021;74:148–155.
3. Crespo M, et al. Transplantation 2020;104:2225–2233.
4. Bossini N, et al. Am J Transplant 2020;20:3019–3029.
5. Sethia R, et al. Gastroenterology 2020;116:600–608.
6. Benotmane I, et al. Transplantation 2021;105:158–169.
7. Cravedi P, et al. Am J Transplant 2020;20:3140–3148.
8. Demir E, et al. Transpl Infect Dis 2020;22:e13371.
9. Favà A, et al. Am J Transplant 2020;20:3030–3041.
10. Hilbrands LB, et al. Nephrol Dial Transplant 2020;35:1973–1983.
11. Ali Malekhosseini S, et al. Transplantation 2021;105:90–99.
12. Gaston DC, et al. Am J Transplant 2021;21:1304–1311.

Data availability: After publication, data and R code are available as supplementary materials.

Funding
Supported by grants from 1.3.5 Project for Disciplines of Excellence Clinical Research Incubation Project, West China Hospital, Sichuan University (ZY2016104).
Saifu Yin and Xianding Wang contributed equally to this work and shared the co-first authorship.

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https://doi.org/10.1053/j.gastro.2021.01.223

Beneficial Effect of Tacrolimus… Cyclosporin A, Still up for Discussion!

To the Editors:

The management of immunosuppression in liver transplant recipients with coronavirus disease 2019 (COVID-19) is a matter of concern in scientific communities. Belli et al[1] published the first multicenter study that demonstrate a beneficial effect of tacrolimus. They described in a large multicenter study that included 243 adult symptomatic cases from 36 centers and 9 countries that the use of tacrolimus was associated with a better survival in liver transplant recipients. Interestingly, they found no beneficial effect of the cyclosporin A (CsA), another calcineurin inhibitor.

An important point should be discussed; tacrolimus and CsA have similar intracellular mechanisms—an indirect immunomodulator activity and a direct antiviral activity, 2 related but independent mechanisms. Briefly, calcineurin is a calcium-calmodulin-activated serine/threonine-specific phosphatase that is a key player in T-cell activation.[2,3] Its phosphatase activity will allow the nuclear factor of activated T cells to be dephosphorylated, allowing nuclear translocation of its substrate, and consequently the expression of immune genes like IL-2, IL-4, and IL-6, the so-called immune response.[4] CsA enters into the cells and forms a binary complex with its intracellular partners, the cyclophilins. In turn, these binaries sequester the calcineurin into a ternary complex and thus inhibit calcineurin activity. In this manner, CsA suppresses the immune response secondary to activation of cytotoxic and helper T cells.[2–4] Tacrolimus is functionally but not structurally related to CsA. The immunosuppressive properties of tacrolimus depend on the formation of binary complex with FKBP proteins, that constitute the immunophilin superfamily together with cyclophilins. These binaries sequester the calcineurin into a ternary complex and thus inhibit calcineurin activity.[2] Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication relies on a variety of host factors, and expresses several structural proteins and many nonstructural proteins.[5] Nonstructural protein 1 interacts with different cellular partners (CypA, CypB, CypH, CypG, FKBP1A, FKBP1B), which in turn increases signaling through the nuclear factor of activated T-cell pathway and enhances the induction of IL-2, IL-4, and IL-6.[3,6] CsA and tacrolimus have an antiviral effect by binding to the cyclophilins and FKBP proteins with subsequent inhibition of their peptidyl-prolyl isomerase activity, whose enzymatic activities are supposed to promote coronavirus replication.[3,6] The exact mechanism by which CsA and tacrolimus interact in coronavirus replication are unknown. Based on this information, both drugs should have similar mechanism and in theory they might have the same beneficial effect in SARS-CoV-2 infection.

At present, it is well-known that the risk factors of poor outcome in COVID-19 infection include older age, male sex, and the presence of comorbidities.[5] The lack of beneficial

Supplementary Table 1. Baseline Characteristics of Included Studies (the Other 10 Studies)

Author	Location	Period	Organ	Total No. of Patients	Total No. of Hospitalized Patients	Test	Age (years)	Male sex (%)	Duration after Transplant	Initial Maintenance Therapy	Changed Maintenance Therapy after COVID-19	Treatment	Follow-up
Benotmane et al[6]	Europe	March 4 and April 7, 2020	Kidney	49	41	RT-PCR and/or typical lung lesions from chest CT	62.2 (52.3–67.8)	75.5	7.1 (2.9–14.4)	Tac (53.1%)/Cyc (32.7%) + MMF (77.6%) + mTOR (22.5%) + steroids (57.1%)	MMF/MPA withdrawal (100%) + calcineurin inhibitors withdrawal (41.7%) + delayed belatacept administration (50%) + mTORi withdrawal (41.7%)	Azithromycin (65%) + azole (2.5%) + lopinavir-ritonavir (12.2%) + hydroxychloroquine (36.6%) + tocilizumab (9.8%) + high-dose corticosteroids (34.2%)	Unknown
Bossini et al[4]	Europe	March 1 to April 16, 2020	Kidney	53	45	RT-PCR	Median 60 (IQR 50–67)	79	9.2 (IQR 4–16)	Tac (58%)/Cyc (32%) + MMF (60%) + mTORi (11%) + pred (57%)	Unknown	Lopinavir/ritonavir (34%) + darunavir plus ritonavir (26%) + hydroxychloroquine (79%)	Unknown
Cravedi et al[7]	North America	March 2 and May 15, 2020	Kidney	144	144	Unknown	60 (±12)	66	Unknown	Tac (91%)/Eve (7.6%) + MMF (77.1%) + pred (86.8%)	MMF withdrawal (68%) + calcineurin inhibitor withdrawal (23%)	Hydroxychloroquine (71%) + antibiotics (74%) + tocilizumab (13%) + and antivirals (14%)	Median 52 days (IQR, 16–66 days)
Crespo et al[3]	Europe	March 18 to May 16	Kidney	414	380	RT-PCR or bronchoalveolar lavage	Median 62 (IQR: 52–71)	64	Unknown	Tac (82.6%)/ mTORi (23%) + MMF (72.6%) + Pred (75.8%)	Unknown	Hydroxychloroquine (89.1%) + azithromycin (49.8%) + glucocorticoids (45%) + lopinavir/ ritonavir (33.8%) + tocilizumab (18.6%)	Mean, 44 days
Colmenero et al[2]	Europe	February 28, 2020 to April 7, 2020	Liver	111	96	RT-PCR	65.34 ± 10.96	68	105 (35–168)	Tac (66%)/Cyc (6%) + MMF (57%) + Eve (23%) + steroid (24%)	Unknown	Azithromycin (60%) + hydroxychloroquine (88%) + lopinavir/ ritonavir (40%) + remdesivir (1%) + interferon beta (3%) + tocilizumab (15%) + corticosteroids (12%)	Median follow-up of 23 days