ARTICLE

Characterization of Casirivimab Plus Imdevimab, Sotrovimab, and Bamlanivimab Plus Etesevimab-Derived Interference in Serum Protein Electrophoresis and Immunofixation Electrophoresis

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Background: Therapeutic monoclonal antibodies can be a source of assay interference in clinical serum protein electrophoresis (SPEP) and immunofixation electrophoresis (IFE), producing monoclonal bands that can be misinterpreted as a monoclonal gammopathy related to a B-cell or plasma cell neoplasm. The extent to which new anti-COVID-19 monoclonal antibodies produce this interference is unknown.

Methods: Casirivimab plus imdevimab, sotrovimab, and bamlanivimab plus etesevimab were spiked into patient serum samples to evaluate for SPEP/IFE interference, to characterize the position of therapy-derived bands relative to a reference band (either combined beta band or beta 1 band, depending on instrument platform), and to confirm heavy and light chain utilization of each medication. Serum samples from patients who had recently received casirivimab plus imdevimab or sotrovimab were also evaluated for comparison.

Results: When spiked into serum samples, all tested anti-COVID-19 monoclonal antibodies generated interference in SPEP/IFE. Importantly, the patterns of interference differed between spiked serum samples and serum from patients who had recently received casirivimab plus imdevimab or sotrovimab.

Conclusions: Imdevimab can be added to the growing list of therapeutic monoclonal antibodies that produce sustained interference in SPEP/IFE. Although casirivimab and sotrovimab also produce assay interference in vitro, these antibodies are not reliably detected in serum from recently infused patients. The value of relative band position in recognizing bands that may represent therapeutic monoclonal antibodies is also emphasized. Clinicians and laboratorians should consider therapeutic monoclonal antibody interference in diagnostic SPEP/IFE and review a patient's medication list when new or transient monoclonal bands are identified.

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IMPACT STATEMENT

The potential for anti-COVID-19 monoclonal antibody therapies to generate assay interference in clinical serum protein electrophoresis (SPEP) and immunofixation electrophoresis (IFE) is unexplored. Herein we demonstrate that imdevimab produces sustained assay interference (for at least 6 weeks) that could be misinterpreted as an IgG lambda monoclonal gammopathy. However, other tested monoclonal antibodies, including casirivimab and sotrovimab, fail to produce assay interference after medication administration. This knowledge should help laboratorians and clinicians better interpret new or transient bands identified by electrophoresis in the setting of recent anti-COVID-19 monoclonal antibody administration.

INTRODUCTION

Therapeutic monoclonal antibody-mediated interference in diagnostic serum protein electrophoresis (SPEP) and immunofixation electrophoresis (IFE) is well described, particularly in the setting of plasma cell myeloma treated with daratumumab and elotuzumab (1). These sources of assay interference can be suspected based on consistent patterns of band migration in electrophoretic gels or capillary tubes. Recently described methods for normalizing abnormal band position relative to the beta band can help to differentiate therapy-derived interference from endogenous monoclonal gammopathies (2), particularly when the position of a patient's monoclonal gammopathy has been historically established. Assay interference is suspected but not well-characterized for many other therapeutic monoclonal antibodies, including medications that have recently been approved for use in patients with COVID-19.

Starting in November of 2020, the Food and Drug Administration granted emergency authorization for 4 monoclonal antibody therapies for the treatment of mild to moderate cases of COVID-19 in patients who are at high risk of severe disease resulting in hospitalization or death: casirivimab plus imdevimab (3), bamlanivimab plus etesevimab (4), sotrovimab (5), and bebtelovimab (6). These therapies target epitopes of the SARS-CoV-2 virus spike protein, and have shown to reduce COVID-19 disease progression, hospitalization, and risk of death (7–10). An additional long-acting antibody cocktail, tixagevimab plus cilgavimab, also recently received authorization for use as COVID-19 pre-exposure prophylaxis (11). The FDA subsequently issued a revised authorization statement for casirivimab plus imdevimab and bamlanivimab plus etesevimab, which recommended limiting the use of these medications at present due to limited efficacy against the Omicron variant (12). Prior to this revised statement, Duke Health administered casirivimab plus imdevimab to >3000 patients. Currently, both Duke Health and UPMC are utilizing sotrovimab for patients with COVID-19 who meet clinical criteria (precise patient counts are not available at this time). Thus, the potential impact of these therapies on the interpretation of protein electrophoresis became a growing area of concern.

To evaluate whether COVID-19 monoclonal antibody therapies cause assay interference, we initially spiked serum samples with casirivimab plus imdevimab, sotrovimab, or bamlanivimab plus etesevimab to evaluate for therapy-derived bands. In vitro, each medication produced reproducible bands in the gamma region on SPEP, with anticipated immunoglobulin heavy and light chain patterns as determined by IFE. SPEP and IFE were also performed on serum samples from patients who had recently

received casirivimab plus imdevimab or sotrovimab; these studies from recently infused patients were informative, as the pattern of casirivimab plus imdevimab interference differed between spiked serum and patient samples, and definitive sotrovimab-mediated interference could not be demonstrated in serum samples from recently infused patients. Due to limited usage at both Duke Health and UPMC, patterns of interference seen in patients who have recently received bamlanivimab plus etesevimab, bebtelovimab, or tixagevimab plus cilgavimab are not characterized here.

Given widespread use of some of these therapies and the potential for therapy-derived interference to be misinterpreted as a monoclonal gammopathy, precise characterization of drug-induced bands, both in vitro and in patients, is critical for accurate clinical SPEP/IFE interpretation and for preventing unnecessary testing in these patients. We also emphasize that there may be potential discrepancies between the results of medication spiking studies and results from recently infused patients, necessitating a comprehensive strategy when characterizing interference due to new monoclonal antibody therapies.

MATERIALS AND METHODS

Evaluated Medications

A list of medications evaluated in this study, their reported antibody isotypes, dosing, maximum and day 29 serum concentrations, and method of evaluation, is included in Table 1. Dosage recommendations and concentration data are obtained from each medication's respective FDA Emergency Use Authorization Fact Sheet (13–15).

Medication Spiking Studies

This study was approved by the Institutional Review Boards of Duke University and the University of Pittsburgh. To evaluate for therapy-mediated interference, excess medication remaining after patient infusion (or unused medications approaching their expiration date) were spiked directly into waste serum samples that lacked a detectable monoclonal gammopathy. For enhanced band clarity on SPEP and IFE, supratherapeutic doses of these therapies were used for spiking (for casirivimab plus imdevimab, bamlanivimab, and etesevimab, 200 µg/mL; for sotrovimab, 2 mg/mL).

Table 1. Properties of COVID-19 monoclonal antibody therapies.

Medication	Isotype	Dosing	Cmax µg/mL (%CV)	CD29 µg/mL (%CV)	Method of Evaluation
Casirivimab	IgG1-kappa	Single 600 mg IV infusion	192 (42.1)	46.2 (48.3)	Spiking study and patient samples
Imdevimab	IgG1-lambda	Single 600 mg IV infusion	198 (42.8)	38.5 (51.2)	Spiking study and patient samples
Sotrovimab	IgG1-kappa	Single 500 mg IV infusion	143 (34.5)	40.7 (40.3)	Spiking study and patient samples
Bamlanivimab	IgG1-kappa	Single 700 mg IV infusion	187 (41.7)	25.7 (42.9)	Spiking study
Etesevimab	IgG1-kappa	Single 1400 mg IV infusion	422 (41.2)	116 (38.1)	Spiking study

Data obtained from each medication's respective FDA Emergency Use Authorization Fact Sheet (13–15). Medications in the same shade are coadministered. Abbreviations: Cmax = maximum serum concentration, CD29 = serum concentration on day 29, CV = coefficient of variation.

Identification of Recently Treated Patients

Using readily available electronic medical record search functions, patients who had received casirivimab plus imdevimab or sotrovimab infusion and subsequently underwent SPEP and IFE testing were identified. Alternatively, patients who had received one of these infusions and had leftover serum or plasma samples available in Duke's Central Automated Laboratory (DCAL) or UPMC's Automated Testing Laboratory (ATL) were identified via daily electronic medical record queries, with subsequent electrophoresis being performed.

Serum Protein Electrophoresis/ Immunofixation

Spiked serum samples or serum/plasma samples from recently infused patients were submitted for diagnostic SPEP and IFE testing similarly to routine clinical samples. At Duke Health, serum protein capillary electrophoresis and gel-based IFE were performed on Capillarys 2 and Hydrasys 2 platforms (Sebia Inc.). Protein electrophoresis tracings were then reviewed and annotated on Sebia's PHORESIS software, v.9.3.0. Images of corresponding immunofixation gels were captured with a Nikon D750 DSLR digital camera with a Nikon Micro-Nikkor 105 mm f2.8 lens on a Bessler Dual Mode Slide Duplicator Model 4102. At UPMC, gel-based SPEP and IFE were performed on the SPIFE 3000 platform (Helena Laboratories). Protein electrophoresis tracings were then reviewed and annotated on Helena's QuickScan Touch Plus software. Images of SPEP and IFE gels were captured on an Epson Perfection V800 scanner.

For spiking studies, relative position of therapy-derived bands was assessed as described previously (2), with reporting of the mean and standard deviation for band *x* axis value and the band/beta 1 ratio (on PHORESIS) or band/combined beta ratio (on QuickSan Touch Plus) after performing 5 repeats. *x* axis values were obtained directly from PHORESIS software (instrument-provided arbitrary units) and were derived manually on printouts from QuickScan Touch Plus software (measured in cm). In samples from patients, the imdevimab band *x* axis value and band/beta 1 ratio were assessed and compared to values obtained from spiking studies.

RESULTS

Casirivimab plus Imdevimab Produces Two Distinct Monoclonal Bands via Spiking Studies, but Only a Single Band in Infused Patients

Casirivimab (IgG kappa) plus imdevimab (IgG lambda) consists of 2 monoclonal antibodies that are currently coformulated in a single vial for administration. Serum samples lacking a detectable monoclonal gammopathy were used as a negative control (Fig. 1, A and B). Spiking studies using these medications produced 2 corresponding bands on SPEP (Fig. 1C), with anticipated immunoglobulin heavy and light chain patterns on IFE (Fig. 1D). Based on these spiking studies, casirivimab appears to migrate to the early to intermediate portion of the gamma region, while imdevimab runs toward the end of the gamma region. As has been demonstrated for daratumumab and elotuzumab (2), the band/beta 1 ratio proved to be a highly reproducible method for expressing relative band position after 5 repeats (Fig. 1C).

Three patients who had received casirivimab plus imdevimab within the preceding 6 weeks with subsequent SPEP/IFE were identified at Duke Health (window between administration and electrophoresis testing: <1 day, 4 weeks, and 6 weeks). All 3 patients demonstrated a similar pattern of medication-induced interference on SPEP/IFE, with only a single band corresponding to imdevimab. A representative case is illustrated in Fig. 2. This patient had a prior history of monoclonal gammopathy, with SPEP/IFE runs before

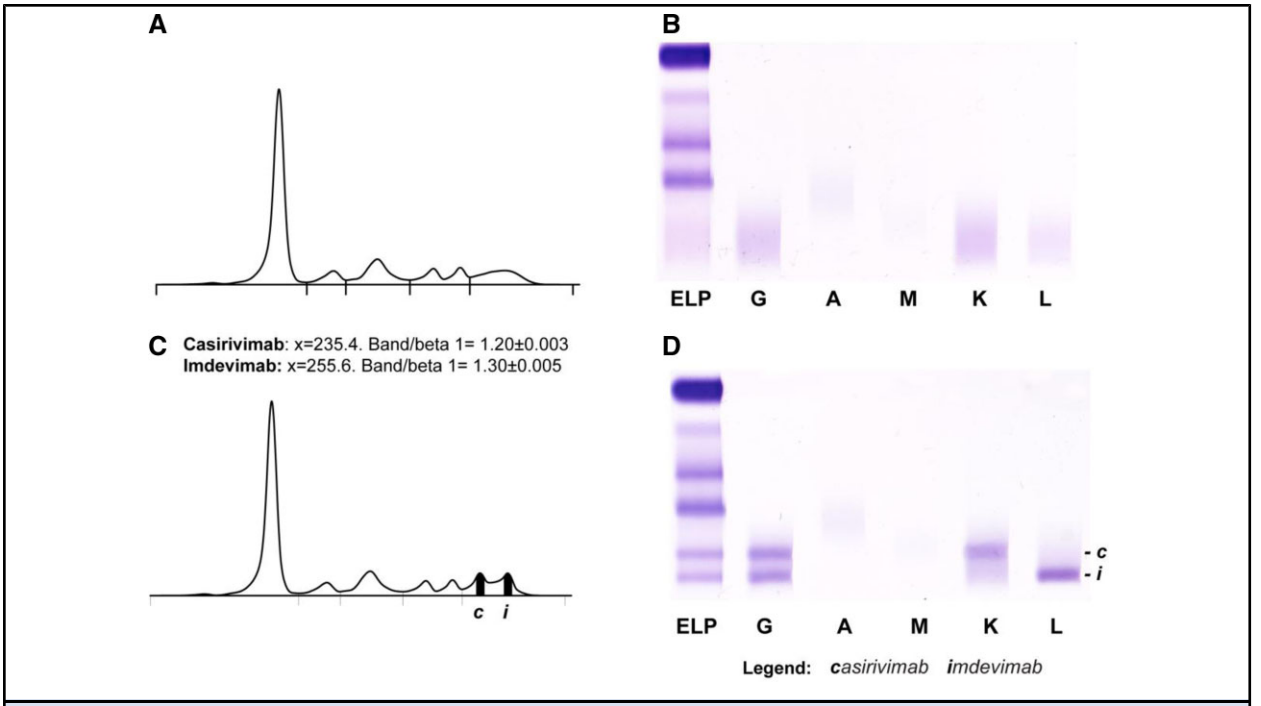

Fig. 1. Casirivimab plus imdevimab spiking studies. (A), SPEP tracing in a normal serum sample; (B), IFE on the same sample as Fig. 1A; (C), SPEP on serum spiked with casirivimab (*c*) plus imdevimab (*i*), 200 µg/mL. Mean band *x* axis value and mean band/beta 1 ratio ± 1 SD are reported; (D), IFE corresponding to Fig. 1C, with casirivimab (*c*, IgG kappa) and imdevimab (*i*, IgG lambda).

and after casirivimab plus imdevimab administration, as well as a subsequent run several months later, available for comparison. The patient had a stable low-level IgG lambda monoclonal gammopathy, which is usually undetectable by SPEP but is reliably captured by IFE near the beta 2 region. Six weeks after receiving casirivimab plus imdevimab, SPEP/IFE identified a new IgG lambda band with a relative band position (band/beta 1 ratio = 1.32) very close to the predicted migration site for imdevimab based on spiking studies (Fig. 2, A and B). Repeat testing 3 months later showed the new band did not persist (not shown). In all 3 evaluated patients, including a patient who had a sample drawn for SPEP/IFE within hours of receiving casirivimab plus imdevimab, an IgG kappa component corresponding to casirivimab was not detected.

Sotrovimab Produces a Distinct Monoclonal Band via Spiking Studies, But No Definitive Interference Is Detected in Infused Patients

Sotrovimab (IgG kappa) is administered as a single monoclonal antibody infusion. Spiking studies using this medication produced a monoclonal band on SPEP that runs toward the end of the gamma region (Fig. 3A); in serum samples spiked with both sotrovimab and daratumumab (which has a well-described band position in the distal gamma region on SPEP), the bands essentially co-migrate (not shown). As expected, IFE identified an IgG kappa band (Fig. 3B).

Using daily electronic medical record queries, 2 samples (one serum, one plasma) located in DCAL and UPMC's ATL were identified from patients who had received sotrovimab one to 2 days before sample collection; these samples

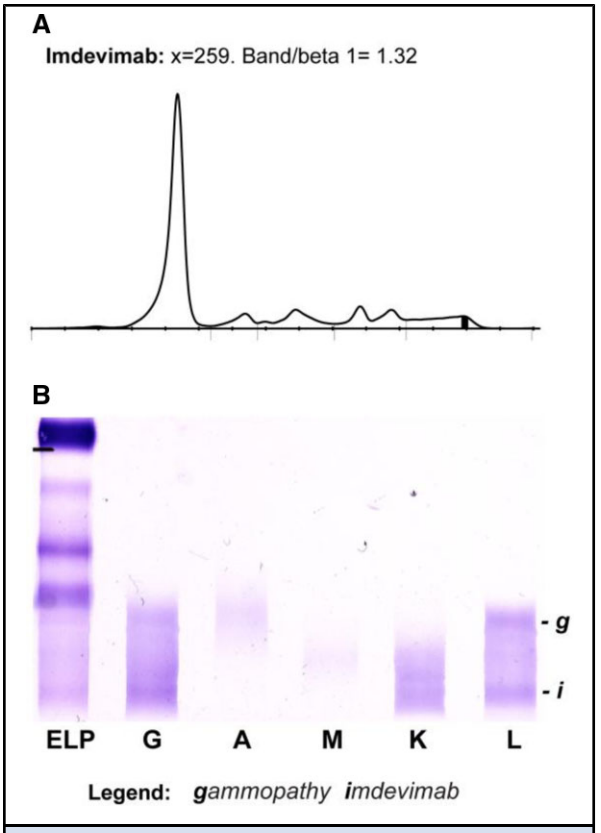

Fig. 2. Casirivimab plus imdevimab interference in a recently infused patient. (A), SPEP tracing in a patient with IgG lambda monoclonal gammopathy, 6 weeks after receiving casirivimab plus imdevimab. The patient's gammopathy partially overlaps with beta 2 and is not usually detectable by SPEP. The irregularity in the distal gamma region represents imdevimab; (B), IFE in the same patient, with endogenous gammopathy (*g*) and imdevimab (*i*).

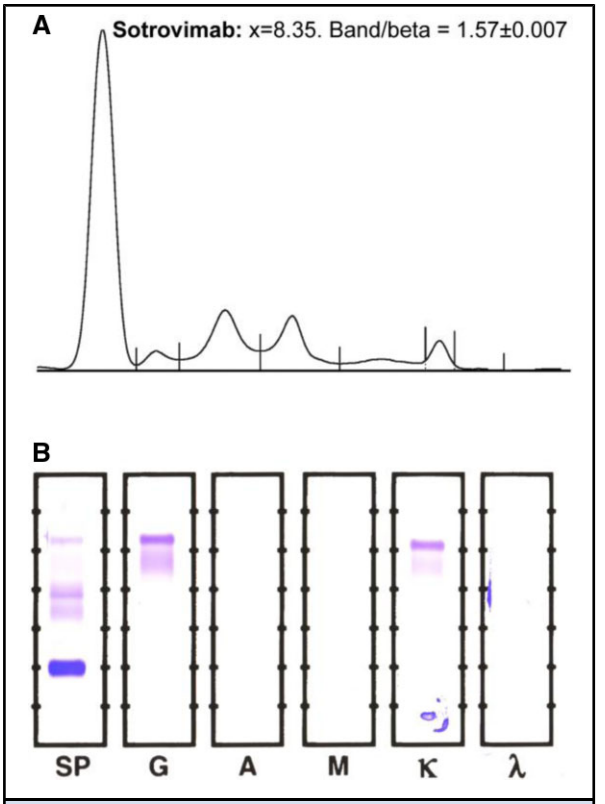

Fig. 3. Sotrovimab spiking studies. (A), SPEP in serum spiked with sotrovimab, 2 mg/mL. Mean band *x* axis value and mean band/beta ratio ± 1 SD are reported (*x* axis values in cm obtained manually from Helena QuickScan Touch Plus software); (B), IFE corresponding to Fig. 3A with IgG kappa band representing sotrovimab.

were submitted for electrophoresis. Neither sample showed a discrete monoclonal band on SPEP (Fig. 4A). The sample obtained at Duke was also submitted for IFE, with no evidence of a monoclonal band (Fig. 4B). Interestingly, 4 additional patients with an established history of monoclonal gammopathy that had received sotrovimab and subsequently underwent SPEP/IFE were identified at Duke Health (window between administration and electrophoresis testing ranging from 2 to 4 weeks). A new band corresponding to sotrovimab was not identified in any of these patients (not shown).

Bamlanivimab plus Etesevimab Produces Two Closely Migrating Bands via Spiking Studies

Bamlanivimab (IgG kappa) plus etesevimab (IgG kappa) consists of 2 monoclonal antibodies that are packaged separately in most formulations but are coadministered. To enhance band clarity, in vitro studies for these medications were conducted separately. Spiking studies using these monoclonal antibodies produced 2 corresponding IgG kappa bands

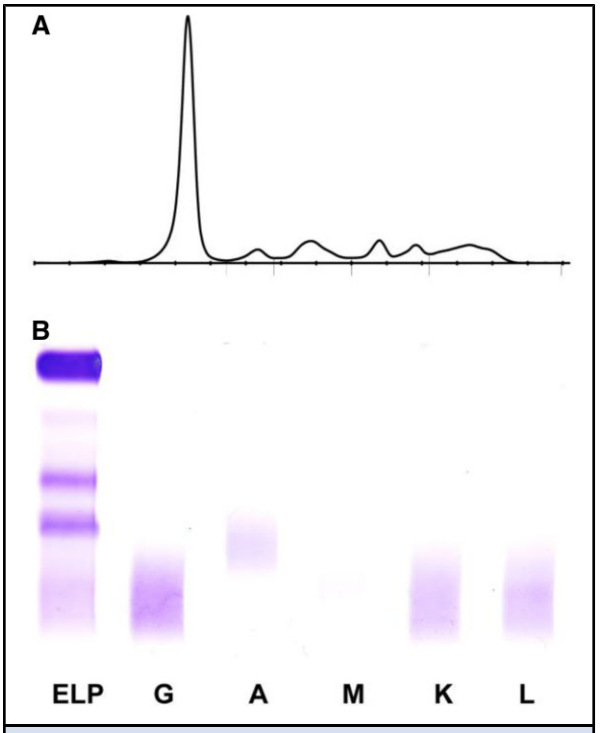

Fig. 4. No definitive sotrovimab interference in a recently infused patient. (A), SPEP from a patient who received sotrovimab 2 days before serum collection. A discrete band is not present; (B), IFE corresponding to Fig. 4A, with no evidence of a monoclonal band.

on SPEP and IFE (Fig. 5, A–D). Both antibodies run toward the end of the gamma region and may at least partially overlap if coadministered. Due to prescribing practices at Duke Health and UPMC, samples from patients who had received these medications were not available for evaluation.

DISCUSSION

The advent of monoclonal antibody therapy has revolutionized large sectors of medicine, including oncology (16), neurology (17, 18), rheumatology (19), and, with the introduction of anti-COVID-19 therapies, infectious disease. However, structural similarities between these medications and endogenous monoclonal gammopathies can result in clinical SPEP/IFE assay interference, potentially leading to test misinterpretation and unnecessary evaluation in patients (ranging from relatively innocuous blood work to invasive bone marrow biopsy to exclude a B-cell or plasma cell neoplasm). After these patterns of assay interference were characterized, we notified our oncology physicians that this is a potential source of misinterpretation in our assays. Laboratorian–clinician teams are encouraged to exclude monoclonal antibody-derived interference whenever possible and repeat SPEP/IFE if there is any doubt as to the source of monoclonal bands, particularly before invasive evaluations (such as bone marrow biopsy) are considered.

As monoclonal antibody therapies grow in number and enjoy an expansion in their approved clinical indications, laboratories may need to develop creative ways to characterize the electrophoretic properties of these medications and recognize interference. This study also provides some important insights about the role of spiking studies in evaluating assay interference due to new medications. While spiking studies are certainly helpful for characterizing the relative position and heavy and light chain features of therapy-derived bands, these studies do not obviate the need to evaluate samples from recently infused patients.

The fact that casirivimab plus imdevimab only produces an IgG lambda band in serum from infused patients, while essentially all other monoclonal antibody therapies on the market utilize an IgG kappa band, may make this particular medication even more difficult to recognize on a routine electrophoresis service. An explanation for why casirivimab and sotrovimab are not readily detected in patients after administration, despite having dosages and anticipated serum concentrations that are comparable to imdevimab, is elusive. Differences in medication stability may at least partially contribute; we noted in our spiking studies for sotrovimab that >200 μg/mL was required to detect discrete bands. It is also possible that casirivimab and sotrovimab are more readily cleared after binding SARS-CoV-2 virus.

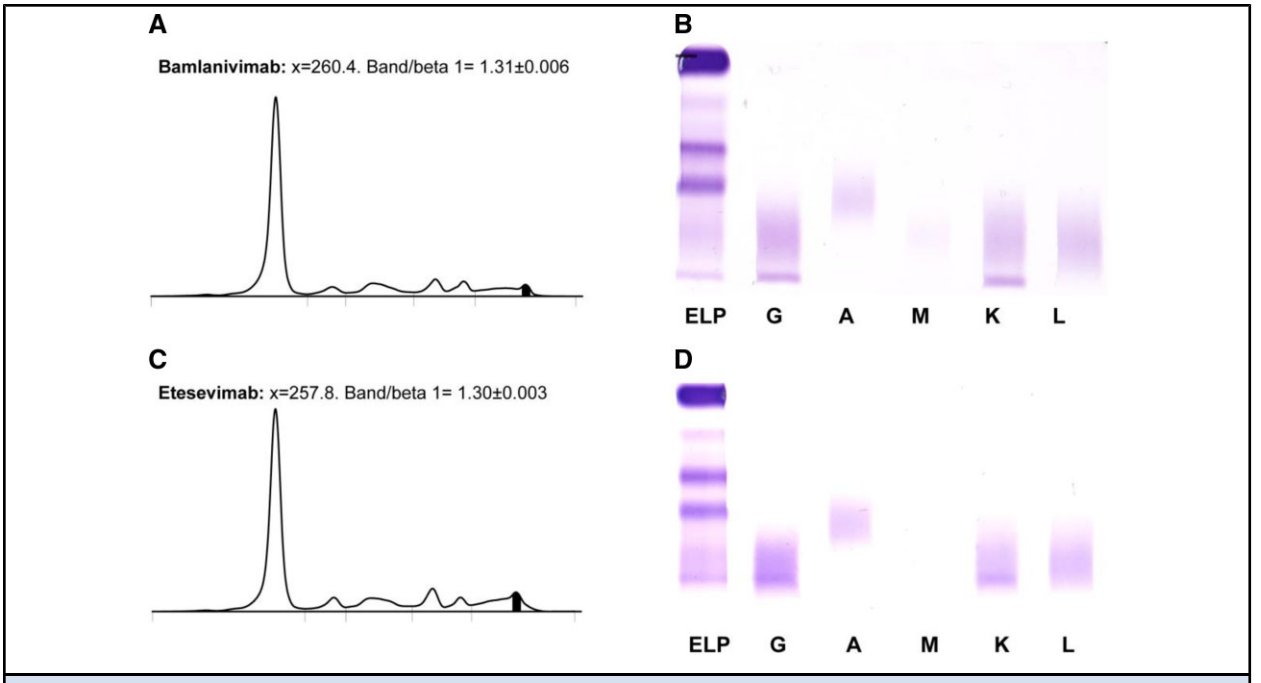

Fig. 5. Bamlanivimab plus etesevimab spiking studies. (A), SPEP in serum spiked with bamlanivimab, 200 µg/mL. Mean band *x* axis value and mean band/beta 1 ratio ± 1 SD are reported; (B), IFE corresponding to Fig. 5A; (C), SPEP in serum spiked with etesevimab, 200 µg/mL. Labels are similar to Fig. 5A; (D), IFE corresponding to Fig. 5C.

Alternatively, imdevimab may bind virus with less avidity and thus remain at a higher serum concentration after administration.

Detection of antibody-mediated interference (or a lack thereof) is not equivalent to well-controlled pharmacokinetic studies for these therapies, and SPEP/IFE evaluation is not intended to serve as a monitoring tool for appropriate dosing or efficacy. These observations do, however, emphasize the need for laboratorians to remain vigilant and informed as technologies and clinical circumstances change (often at a considerably faster rate than our routine clinical assays). It remains to be seen whether some of these therapies will be used for prolonged periods as the COVID-19 pandemic continues. Alternatively, some of these medications may find new, unexpected applications in the future; considering sotrovimab is derived from a patient who was exposed to SARS-CoV-1 during the initial coronavirus outbreak nearly 20 years ago (20), this is certainly a possibility.

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