Rapid selection of sotrovimab escape variants in SARS-CoV-2 Omicron infected immunocompromised patients

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Abstract

Background: Monoclonal antibodies (mAb) targeting SARS-CoV-2 are predominantly less effective against Omicron variants. Immunocompromised patients often experience prolonged viral shedding and are therefore at increased risk for viral escape mutations, when mAbs are used as monotherapy.

Methods: In an observational, prospective cohort, 57 patients infected with Omicron variants receiving sotrovimab alone or in combination with remdesivir were followed. The study endpoints were a decrease in SARS-CoV-2-RNA $<10^6$ copies/ml in nasopharyngeal swabs at day 21 and the emergence of resistance mutations at days 7, 14, and 21 after sotrovimab administration. All SARS-CoV-2 samples were analyzed by whole-genome sequencing, individual variants within the quasispecies were subsequently quantified and further characterized by a pseudovirus neutralization assay.

Results: 47/57 patients (82.5%) were infected with Omicron/BA.1 and 10/57 (17.5%) with Omicron/BA.2. The vast majority of patients (43/57, 75.4%) were immunodeficient, predominantly due to immunosuppression after organ transplantation or hematologic malignancies. 21 days after sotrovimab administration, 12/43 (27.9%) of immunodeficient patients had prolonged viral shedding compared to 1/14 (7.1%) immunocompetent patients (p=0.011). Longitudinal sequencing revealed that 14/43 (32.6%) immunodeficient patients had in part Omicron-specific viral spike protein mutations (e.g., P337S and/or E340D/V) that substantially reduced susceptibility to sotrovimab in a pseudovirus neutralization assay. Combination therapy with remdesivir significantly reduced the selection of escape variants.

Conclusions: Immunocompromised patients face a considerable risk of prolonged viral shedding and emergence of escape mutations after early therapy with sotrovimab. These findings underscore the importance of careful monitoring and the need to conduct dedicated clinical trials for this patient population.

Keywords: immunodeficiency, sotrovimab, SARS-CoV-2, Omicron, escape

Introduction

During the SARS-CoV-2 pandemic, numerous studies have shown that treatment options directly targeting SARS-CoV-2 itself are most successful in the early phase of COVID-19, whereas in the late phases of COVID-19 with pneumonia and hyperinflammation, immunomodulation is the main therapeutic principle. Several monoclonal antibodies (mAb) targeting SARS-COV-2, such as bamlanivimab/etesevimab or casirivimab/imdevimab, became available starting in late 2020 and were successfully used in the early phase of COVID-19 to prevent disease progression in high-risk patients [1]. With the emergence of the currently dominating variant of concern (VOC) Omicron in November 2021, a significant rise in infection rates was observed. This went along with a loss of *in vitro* activity of the mAb combination casirivimab/imdevimab, commonly used until then, because the target regions in the spike protein were altered through several mutations [2]. In January 2022, sotrovimab became available in Germany. It was one of the few mAbs found to be effective against the Omicron variant *in vitro*, and thus represented a promising treatment option for early SARS-CoV-2 infection [3-5].

Sotrovimab was approved for use in children over 12 and adults at high/moderate risk for developing severe infection [6]. Up until now, only two randomized controlled trials have evaluated the efficacy of sotrovimab in preventing hospitalization and disease progression but only the COMET-ICE trial showed a benefit [3, 4, 7]. However, these trials did not include severely immunodeficient patients such as solid organ transplant (SOT) recipients. Case series as well as two cohort studies evaluated the efficacy and safety of sotrovimab in SOT patients in the context of Omicron and reported a reduction in disease severity [8, 9]. However, it was suggested that therapy of SARS-CoV-2 infections with single mAbs might promote the emergence of escape mutations in the spike protein, especially in immunocompromised patients [10]. Recently, mutations have been reported after sotrovimab therapy in patients infected with the Omicron variant, but the risk factors for the occurrence

and the longitudinal development of resistance are still largely unclear [11, 12]. Therefore, we analyzed the outcome and risk factors for viral persistence after treatment with sotrovimab in our cohort of patients treated since January 2022, focusing specifically on the emergence of escape mutations.

Methods

Study design

We performed a prospective, observational cohort study in patients diagnosed with SARS-CoV-2 infection who received sotrovimab therapy between the 20th of January and the 25th of February 2022. Patients were either hospitalized or presented at the outpatient clinic at the University Hospital Düsseldorf. Inclusion criteria were: (I) polymerase chain reaction (PCR)-confirmed SARS-CoV-2 infection, (II) age over 12 years, (III) weight over 40 kg and (IV) risk factors for developing a severe course of COVID-19. All patients provided informed consent. Patients were pseudonymized with number IDs. A single dose of 500 mg of sotrovimab was administered intravenously over a one-hour period as part of routine clinical practice.

Baseline was defined as the day of sotrovimab administration. Nasopharyngeal swabs and clinical parameters were collected at baseline and during the follow-up period: every 7 days (+/-2 days) until viral clearance was achieved. The main endpoints of the study were percentage of patients with a decrease in SARS-CoV-2 RNA $<10^6$ copies/ml in nasopharyngeal swabs 21 days after sotrovimab administration and the characterization of the viral variants including the screening for escape mutations during the observation period of 28 days. Patients who did not attend their follow-up appointments and patients, for whom viral genome sequencing was unsuccessful at any time during the study, were excluded from the statistical analysis.

Definition of prolonged viral shedding

Prolonged viral shedding was defined as a persistent SARS-CoV-2 RNA concentration above 10^6 copies/ml 21 days after sotrovimab administration. The threshold of 10^6 SARS-CoV-2 RNA copies/ml or a Ct-value >25 is considered a measure of infectivity based on *in vitro* cell culture data showing a correlation between viral load and viral cultivability and the associated probability of transmission [13]. This cutoff value as correlate of contagiousness was also chosen following the German recommendations of the Robert Koch Institute for the isolation of SARS-CoV-2 infected hospitalized patients.

Laboratory SARS-CoV-2 analyses

All detailed information on SARS-CoV-2 detection and quantification, SARS-CoV-2 whole genome sequencing and resistance analysis, pseudovirus cloning, production and neutralization assays is provided in the Supplementary Appendix.

Statistical analysis

Detailed information on the statistical programs used and the statistical tests performed are documented in the Supplementary Appendix.

Results

Patients' characteristics

A total of 57 patients (female=21; male=36) were enrolled into this study, of which 47 patients (82.5%) were infected with Omicron variant BA.1 and 10 patients (17.5%) with Omicron variant BA.2 (Table 1). No symptoms were present in 21 of 57 (36.8%) patients, while the rest had symptoms consistent with early COVID-19. The median time from onset of symptoms to administration of sotrovimab was 3 days (interquartile range (IQR) 1-3.3). All participants were in the early phase of COVID-19 when sotrovimab was administered, two of

them required low levels of oxygen supplementation for reasons unrelated to COVID-19. 42 of 57 patients (73.7%) received at least three doses of SARS-CoV-2 vaccine in accordance with the recommendations of the Standing Committee on Vaccination (Supplementary Table 1). The median timespan since the last vaccination was 3 months (range 1-5). Two patients died from causes unrelated to COVID-19: one from stage IV malignant melanoma, the other from complications of acute lymphoblastic leukemia. In total, five patients could not be monitored because they either died (malignant melanoma) or did not present to follow-up (n=4).

Patients were grouped into immunocompetent (n=14) and immunodeficient (n=43). Immunodeficiency mostly comprised solid organ transplantation (SOT), stem cell transplantation (SCT), active hematologic malignancies and autoimmune diseases. The full spectrum of diseases is presented in Table 1. Immunosuppressive medication was given to 39 out of 43 patients (90%) classified as immunodeficient (Supplementary Table 1).

Prolonged viral shedding in immunodeficient COVID-19 patients infected with an Omicron variant and treated with sotrovimab

We analyzed the kinetics of viral clearance after the first positive SARS-CoV-2 PCR test and after sotrovimab administration in immunocompetent and immunodeficient patients (Figure 1). All but one of the immunocompetent patients had a viral load (VL) below 10^6 copies/ml at day 14, while 21/43 (48.8%) of immunodeficient patients had prolonged viral shedding at this time (p=0.011). Moreover, even on day 21, 12/43 (27.9%) of the patients with immunodeficiency had not achieved a VL $<10^6$ copies/ml. The only immunocompetent patient who still had a VL $>10^6$ copies/mL on day 14 was lost to follow-up and therefore considered for analysis as having a VL $>10^6$ copies/mL on day 21 (patient 37, Supplementary Table 2). A higher proportion of patients presenting without COVID-related symptoms had prolonged viral shedding after days 14 and 21 (Supplementary Table 4).

Of note, 6 of 43 (13.9%) immunodeficient patients were infected with the BA.2 Omicron-variant, characterized by higher levels of *in vitro* resistance of sotrovimab compared to BA.1. 29 out of 43 (67.4%) immunodeficient patients received additional therapy with remdesivir at baseline (Supplementary Table 2). However, in the subgroup analysis, no significant association was found regarding the occurrence of prolonged viral shedding and following factors: Omicron variant, remdesivir administration, number of vaccinations and months since last vaccination or time between symptom onset and sotrovimab infusion (Table 2). The only risk factor identified for prolonged viral shedding was immunodeficiency (r=0.329; p=0.016).

Initial non-responders defined as patients whose symptoms either worsened despite sotrovimab administration and required hospitalization (#30, 34), or who experienced a viral rebound during the observation period (days 14-21: #8, 9, 10, 16; >21 days: #3, 26, 54) received further antiviral therapy. All patients who showed a slow but steady decline in SARS-CoV-2 VL did not receive further antiviral therapy, and five patients were lost to follow up. In all 10 patients retreated with further antiviral drugs, the virus was subsequently eliminated (Details in Supplementary Table 2).

Taken together, these results show that immunocompromised patients have a substantial rate of prolonged viral shedding, even after administration of sotrovimab, which was the standard therapy for patients infected with SARS-CoV-2 at high risk for disease progression at the time of enrollment.

Emergence and characterization of resistance mutations in Omicron VOC after the use of sotrovimab

Noting the prolonged viral shedding in immunocompromised patients after sotrovimab administration, we next performed whole-genome nanopore sequencing of all available viral samples with VL $>10^6$ copies/ml. Samples with detected resistance mutations were further analyzed with quantitative Illumina sequencing with spike amino acid coverage averaging 98.5% (range 91.6-100%) (Sample overview table, online data repository server). This

analysis revealed that mutations at spike protein residues associated with resistance to sotrovimab occurred in 14 of 57 patients (24.6%). No selection of escape mutations was observed in the immunocompetent patients, but only in immunodeficient patients (14/43, 32.6 %), most of which had prolonged viral shedding. This group comprised 6 patients with SOT, 2 allogeneic SCT recipients, 2 patients with active hematologic malignancy receiving chemotherapy as well as 1 patient each with cryoglobulinemic vasculitis, systemic lupus erythematosus, and liver cirrhosis (Child-Pugh class A), each of whom received additional immunomodulatory therapies, and finally 1 patient with common variable immunodeficiency (Supplementary Table 2).

While no variants with reduced susceptibility to sotrovimab were detected at baseline confirmed by Illumina sequence analysis, five patients had sotrovimab-resistant variants already at day 7, whereas most escape mutations occurred between day 7 and day 14. Details of the quantitative analysis of sotrovimab resistance mutations performed by Illumina sequencing on SARS-CoV-2 samples with evidence of immune escape in nanopore sequencing are shown in Figure 2. The first-appearing resistance mutations were detected exclusively at positions 337 or 340 in the spike protein, predominantly featuring the mutations P337S (n=8), E340K (n=9), and E340D (n=5). In addition, amino acid substitutions P337H/L/R and E340A/V were found during our observation period of up to 28 days (Supplementary Table 3). During the observation period, not only an increase in escape variants (e.g., patients 2, 31 and 53), but also a change of the frequency of mutated variants was observed, e.g., patient 10: E340D (d21) to E340K (d28) and patient 53: E340V (d7) to E340D (d14) (Figure 2, Supplementary Table 3).

The sotrovimab-specific escape mutations (P337S, E340D/K/V) already detected on day 7 were characterized in the BA.1 and BA.2 omicron background using a pseudovirus neutralization assay (Figure 3). While in the B.1 background (a common lineage early in 2020[14]), only E340K und E340D were associated with reduced neutralization by sotrovimab (IC_{50}: >100 µg/ml and IC_{50}: 0.162 µg/ml, respectively), all other detected

mutations completely abrogated neutralization by sotrovimab in both the BA.1 and BA.2 backgrounds (IC_{50}: >100 µg/ml).

To characterize the risk factors for the selection of escape mutations, correlation analyses were performed (Table 2). This analysis revealed that two factors correlated with the emergence of resistance mutations: immunodeficiency (r=0.305, p=0.021) and days until VL below 10^6 SARS-CoV-2 RNA copies/ml was achieved after sotrovimab administration (r=0.322, p=0.019). In detail, patients with emergence of mutations had significantly delayed time to viral clearance (mean 28.2, SD 16.2 days) compared to those without mutations (12.9, SD 9.9 days); odds ratio 5.04 (95%CI, 1.29-18.3). In addition, for patients with tacrolimus therapy, higher tacrolimus levels at baseline positively correlated with the emergence of escape mutations (r=0.523, p=0.015). In immunodeficient patients, administration of remdesivir in combination with the corresponding duration correlated negatively with the occurrence of resistance mutations against sotrovimab (r=-0.392, p=0.009). Most patients with selection of sotrovimab-specific escape mutations (13/14; 92.8%) were infected with the BA.1 variant; however, only 6/43 immunodeficient patients were infected with BA.2.

Together, these findings suggest that sotrovimab monotherapy in immunocompromised patients is associated with the risk of de-novo development of specific mutations leading to immune escape.

Discussion

To our knowledge, this is one of the few studies reporting the frequent emergence of escape mutations after sotrovimab treatment in a predominantly immunodeficient cohort of patients infected with Omicron variants.

Previous publications showed a decreased severity of SARS-CoV-2 disease with the Omicron variant [15]. Consistent with this, all patients in our high-risk cohort had uncomplicated disease throughout the follow-up period and there was no SARS-CoV-2-

related mortality. Due to the observational nature of our study, it remains unclear whether the clinical course might have been less favorable in some patients without early antiviral therapy. When comparing the BA.1 and BA.2 Omicron variants in terms of prolonged viral shedding after sotrovimab administration, there was no significant difference found in our cohort. At this point, however, it must be emphasized that BA.2 was underrepresented in our study cohort compared to BA.1 (17.5% vs. 82.5%, respectively). In our pseudovirus neutralization assays (Figure 3), as well as in other studies, a reduced neutralization activity of sotrovimab against BA.2 was described [16, 17]. These data have led the Food and Drug Administration (FDA) to revoke the approval of sotrovimab for patients infected with BA.2 in April 2022 [18].

A unique feature of our cohort is the high number of immunodeficient patients, almost half of whom were patients with SOT, resulting in a higher risk of prolonged viral shedding, therefore potentially promoting the emergence of highly mutated viruses [19-21]. In this context, a higher baseline tacrolimus serum level was associated with the selection of escape mutations in our study, which highlights the importance of considering treatment adjustments of immunosuppressive medication during SARS-CoV-2 infection.

In our cohort, all but one of the immunocompetent patients (13/14, 92.9%) were below the defined viral threshold of 10^6 SARS-CoV-2 RNA copies/ml at day 14 and no selection of resistant variants to sotrovimab was detected. In contrast, sotrovimab escape mutations were detected in 32.6% of immunodeficient patients, who predominantly experienced prolonged periods of viral replication. Similarly, treatment with other mAbs or antiviral agents (such as remdesivir) is also reported to promote the selection of viral mutations particularly in immunosuppressed patients [10, 22-24].

Sotrovimab-specific resistance mutations were first described in an Australian cohort of patients infected with the Delta variant [25]. Genome sequencing of samples from the COMET-ICE trial detected 20 patients with sotrovimab escape mutations, out of which P337L, E340A and E340K showed reduced susceptibility to sotrovimab in pseudotyped viral-like particles (>100-fold change in EC_{50} value) [3, 18]. In the study published by Rockett *et*

al., eight out of 100 included subjects developed one of the following mutations, E340A/K/V or P337L combined with the E340 mutation occurring 6 to 13 days after sotrovimab administration [25]. While in the Delta background mainly the P337L and the E340A were selected, in the Omicron background other amino acids were selected at the same positions, predominantly the P337S/R and the E340D/K, as also reported by other recent studies [11, 12].

In our longitudinal study, after detection of the sotrovimab-specific escape mutations P337S/L/R and E340A/D/K/V at day 7, additional variants were detected during our observation period (P337H). Moreover, changes in frequency of different escape variants over time were observed, as already described in infections with the Delta variant, presumably indicating ongoing viral evolution [25].

In the *in vitro* analyses carried out in our study, the pseudovirus neutralization assays confirmed that both, the sotrovimab mutations E340K and E340V, which were also selected in Delta, and the mutations P337S and E340D newly described in the Omicron context completely abrogate the neutralization activity of sotrovimab. In the B.1 background, on the other hand, a strongly reduced neutralization activity could only be observed for E340K, whereas the E340D mutation reduced the neutralization activity of sotrovimab to a much lesser extent. These data clearly show that not only the escape mutation itself, but also the broader genetic background of the spike protein influences the impact of a specific escape mutation on mAbs efficacy, as has already been observed in several efficacy studies for mAbs [17, 26].

In a previous small cohort study conducted before the Omicron era, we had found that the E484K mutation occurred upon bamlanivimab monotherapy in 83% of patients and in a major portion of the viral population in the respective patients [10]. In contrast, in our study, the frequency of sotrovimab-resistant viral variants was lower in most patients and showed a very heterogeneous mutation spectrum [27, 28].

Our study has limitations that should be considered in further studies: (I) the relatively small cohort, making subgroup analysis difficult (II) quantitative analysis with Illumina

sequencing was performed only in patients in whom spike protein mutations were detected in nanopore sequencing, and therefore the diversity of viral quasispecies cannot be compared to patients without detection of mutations in nanopore sequencing. However, failure to account for possible minor spike protein mutations in this group seems unlikely, as these were not detected in the patients with emerging sotrovimab resistance mutations.

There is growing evidence to support the hypothesis that new SARS-CoV-2 variants preferentially occur in immunocompromised patients with persistent SARS-CoV-2 infection. Since some of these variants may be more transmissible or may have better immune escape, this has potentially significant implications for individual medical care and public health. In immunocompromised patients, prolonged viral shedding must therefore be considered with respect to infection control. Given the available data, administration of a single mAb or single antiviral drug should be avoided in immunocompromised patients because of the risk of emergent mutations. In our study, we could demonstrate that presence and length of remdesivir therapy at baseline was associated with a reduced emergence of escape mutations. In addition, a second remdesivir administration over a longer period of 10 days and combination antiviral therapy resulted in a sustained decrease in viral load in the vast majority of patients with persistently high nasopharyngeal VL and successfully terminated viral shedding in them.

In summary, combination therapies with at least two mAbs or other antivirals such as remdesivir, molnupiravir, and nirmatrelvir/ritonavir should be considered when treating immunodeficient patients with SARS-CoV-2 infection. These results also highlight the importance of particularly careful monitoring and the need to conduct dedicated clinical trials to establish the optimal treatment strategy for this patient population. This is especially true in this stage of the pandemic since, with the availability of vaccines that prevent severe disease courses for most patients, immunodeficient patients represent one of the most vulnerable and severely affected patient groups.

Notes

Author contributions

SG, NL, AK, TL and BJ were responsible for conceptualization and supervised the study. SG, NL, AK, HG, AW, ATD, CL, CF, SK, JV, TS, AZ, TF, JB, H-MO, FK, JT, TL and BJ contributed to investigation and data curation. SG, NL, AK, BJ, HG and JT conducted the formal analysis. SG, BJ, TL, NL, AK, JT, HG and FK were responsible for methodology, data validation and visualization. SG, NL, AK, HG, JT, TL and BJ contributed to the original draft. All authors critically revised the manuscript and approved the final version.

Additionally, the members of the *Sotrovimab study group* had the following contribution: SK, JVP, TPS and AZ were instrumental in patient management, as well as the coordination of outpatient care for the study participants. AT and MD coordinated the Illumina sequencing and performed the bioinformatic analysis of the sequencing data.

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Data availability

Raw data are generated at the University Hospital Düsseldorf and in cooperation at the University Hospital Cologne. Derived or additional data supporting the findings of this study could be provided by the corresponding authors upon reasonable request. All Nanopore and

Illumina sequence data can be found on the Open Science Framework (OSF) server https://osf.io/q7js6/?view_only=59884b79343e449ea9f7103f99c118a9.

Ethical approval

The investigations were performed in accordance with the Declaration of Helsinki, our study on COVID-19-associated risk factors, clinical course, and viral genomes was approved via the ethics vote of the local ethics committee of the medical faculty of Heinrich-Heine-University (study number 5350). All patients gave written informed consent.

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Conflict of interest

NL received honoraria for presentations from Gilead, MSD, Abbvie, ViiV (outside the submitted work) and served on advisory boards for ViiV and Theratechnologies (outside the submitted work), including consulting fees for ViiV and Theratechnologies. BJ received honoraria for presentations from Gilead (remdesivir) and GSK (sotrovimab) as well as Falk, Janssen-Cilag, ViiV, Gilead, Fresenius Medical Care (outside the submitted work), received travel support from Gilead and served on advisory boards for ViiV, Gilead, Theratechnologies (outside the submitted work); consulting fees from Gilead, ViiV, and Theratechnologies; and leadership or fiduciary role in other board, society, committee or advocacy group for Development of National Recommendations on COVID-19 treatment

(COVRIIN) (unpaid participation). TF was PI for a Gilead clinical trial (remdesivir) and served on Gilead advisory boards (outside the submitted work) (PI and participation in SIMPLE trials on Remdesivir for moderate and severe COVID-19, no personal fees; publication on the treatment of Remdesivir, authorships amongst others including Gilead team members, no personal fees). TF reports a leadership or fiduciary role with Robert Koch Institute on the development of national recommendations on COVID-19 treatment. TL received honoraria for lectures from Abbvie, BMS, Gilead and travel support von Gilead und Abbvie and served on advisory boards for Gilead. TL was involved in the development of the national recommendation on COVID-19 treatment and received honoraria for presentations for Abbvie, BMS and Gilead and received travel support from Gilead and Abbvie. HG and FK are listed as inventors on patent applications on SARS-CoV-2 neutralizing antibodies filed by the University of Cologne. AK received lecture fees from Gilead and participated on Advisory Boards for Gilead. AK was supported for attending meetings from Abbvie. All other authors declare no competing interest regarding this work.

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Tables

Table 1. Baseline characteristics of patients grouped by immunodeficiency

Variable	n
Total	57
Gender	
• Male	36
• Female	21
Groups	
• **Immunocompetent**	**14**
• **Immunodeficient**	**43**
Solid organ transplantation (SOT)	
Kidney	18
Heart	2
Heart+ Kidney	1
Heart+ Lung	1
Kidney + Pancreas	1
Stem cell transplantation (SCT)	
Allogeneic	5
Autologous	2
Leukemia	
Acute lymphoblastic leukemia (ALL)	2
Acute myeloblastic leukemia (AML) *	2
AML+ chronic myelomonocitic leukemia (CMML)	1
CMML	1
Lymphoma	
Diffuse large B cell lymphoma (DLBCL)	1
T-cell lymphoma*	1
AL amyloidosis/ smoldering multiple myeloma *	1
Other malignancies	
Stage IV Malignant melanoma and Stage IV NSCLC**	1
Common variable immune deficiency (CVID)	1
Autoimmune diseases	
Cryoglobulinemic vasculitis	1
p-ANCA vasculitis	1
Rheumatoid arthritis	1
Systemic lupus erythematosus (SLE)	1
Ulcerative colitis	1
Liver cirrhosis Child A ***	1
Liver fibrosis with portal hypertension**	1

*: patients with previous allogeneic (2) and autologous (1) SCT and malignancy relapse

**: dexamethasone therapy for cerebral metastases

***: patients with liver fibrosis/cirrhosis had a concurrent autoimmune disease

NSCLC: non-small cell lung cancer

Table 2. Bivariate correlation between clinical parameters, duration until viral load <10^6 copies/ml and resistance mutations

Parameter	VL <10^6 copies/ml since first pos. PCR test (days)	VL <10^6 copies/ml since Sotrovimab administration (days)	Mutations day 7 (0=none, 1=mutation)	Mutations day 14 (0=none, 1=mutation)	Mutations overall (0=none, 1=mutation)
	Correlation coefficient (r) *p-value*				
Remdesivir therapy at baseline (0= 0, 1= 3 and 2=5 days)	-0.355 0.234	-0.224 0.462	NA		
• Immunocompetent					
• Immunodeficient	0.057 0.726	-0.036 0.827	**-0.372** **0.015**	-0.261 0.099	**-0.392** **0.009**
Omicron variant (0=BA.1,1=BA.2)	0.068 0.824	0.207 0.498	NA		
• Immunocompetent					
• Immunodeficient	0.032 0.844	0.107 0.510	-0.150 0.343	-0.095 0.555	-0.137 0.383
Time since last vaccination (months)	0.073 0.822	0.080 0.805	N/A		
• Immunocompetent					
• Immunodeficient	0.298 0.109	0.241 0.199	0.011 0.953	0.179 0.345	0.217 0.240
Number of vaccinations	0.408 0.167	0.496 0.085	NA		
• Immunocompetent					
• Immunodeficient	0.041 0.804	0.046 0.780	0.117 0.467	-0.125 0.422	-0.104 0.512
Immunodeficiency (0= immunocompetent, 1=immunodeficient)	**0.329** **0.016**	0.208 0.135	**0.320** **0.015**	**0.275** **0.042**	**0.305** **0.021**
Viral clearance after sotrovimab administration (days)	NA		0.258 0.062	**0.401** **0.004**	**0.322** **0.019**
Tacrolimus levels at baseline (ng/ml)	0.349 0.132	0.275 0.240	0.161 0.486	**0.451** **0.046**	**0.523** **0.015**
Days since first symptoms (number of pairs= 35 *)	0.075 0.669	-0.251 0.146	0.090 0.600	-0.144 0.417	-0.10 0.955

Significant correlations are marked with bold. NA: not applicable. *: 1 patient was lost to follow-up and not included in this analysis

Figure Legends

Figure 1: Patients with persistent viral replication (≥ 10^6 copies/ml) after sotrovimab administration.

a: Prolonged viral shedding day 21 after first positive SARS-CoV-2 PCR test according to immunocompetence; **b**: Prolonged viral shedding day 21 after sotrovimab administration in immunocompetent patients and patients with immunodeficiency. Numbers at risk are patients with a viral load ≥ 10^6 copies/ml; censored are patients lost to follow-up (one patient was first lost to follow-up day 28 and was included in numbers at risk)

Figure 2. Prevalence and evolution of escape mutations in the spike protein of SARS-CoV-2 after sotrovimab treatment.

Detected amino acid exchanges in the spike protein at positions 337 and 340 on day 0, day 7 and day 14 after sotrovimab administration. The frequency of reads in % is indicated by the color scale. The determined patient-related SARS-CoV-2 variant is shown. Only patients with detected mutations after sotrovimab treatment are indicated. Patients selecting a spike protein mutation after day 14 are not included in this figure (patient #51).

Figure 3: Neutralization of SARS-CoV-2 spike mutants by sotrovimab.

SARS-CoV-2 variant specific pseudoviruses harboring mutations emerging after sotrovimab treatment were analyzed in sotrovimab neutralization assays (a). All samples were tested in duplicates. Symbols and bars indicate mean and standard deviation, respectively. The determined IC_{50} values are shown in (b).

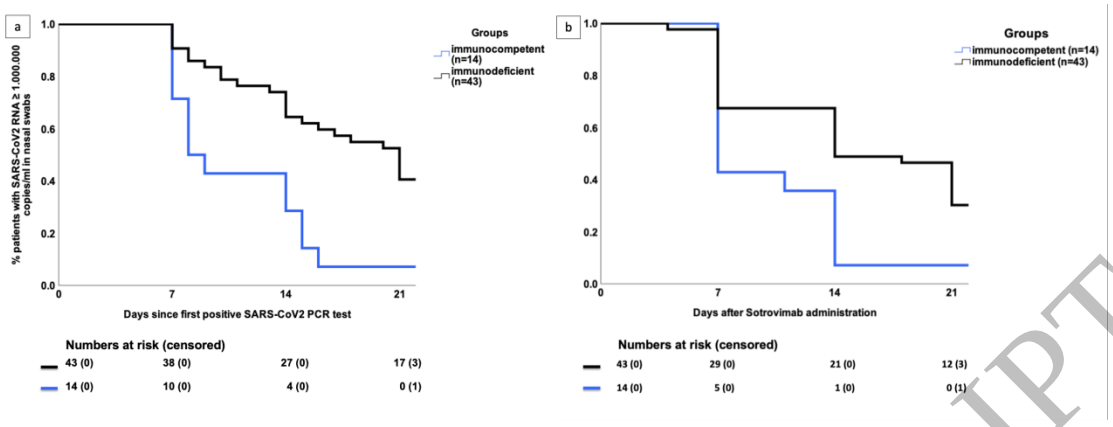

Figure 1
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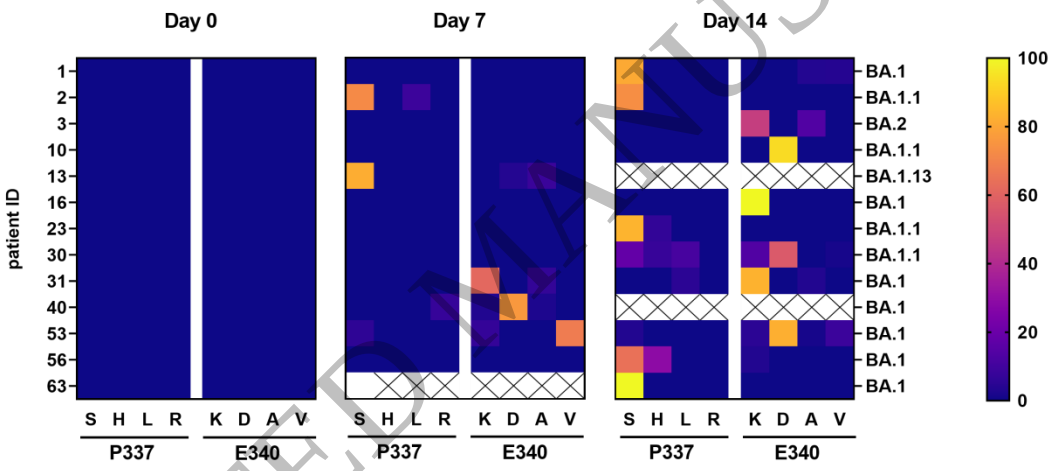

Figure 2
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Figure 3

Click here to access/download;Figure (.tif and .eps files only);Figure_3_R1.tif

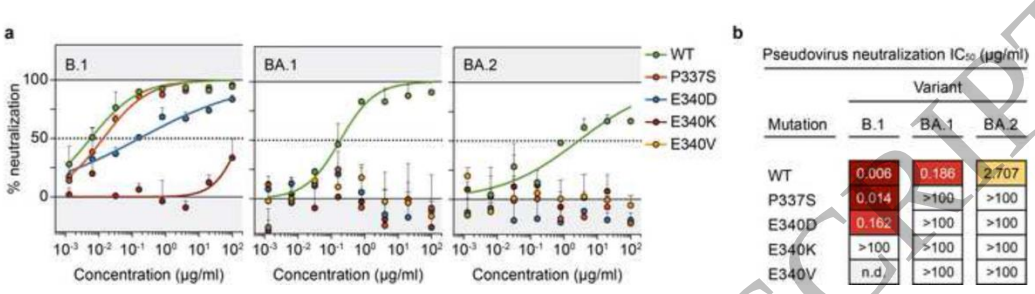

Figure 3

162x41 mm (x DPI)