Universität zu Köln

A Phase 1/2a Trial of the Inhaled Administration of the SARS-CoV-2-Neutralizing Monoclonal Antibody DZIF-10c in SARS-CoV-2-Infected and -Uninfected Individuals

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EudraCT:	2020-004448-27
ClincialTrials.gov:	NCT04631705
Protocol code:	Uni-Koeln-4370

Version V04_0, 01-APR-2021

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Statement of Compliance

The clinical trial will be conducted in compliance with the protocol, with the International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP), as well as the applicable European and German law.

I. Signatures

[redacted]

On behalf of the Sponsor (University of Cologne) Signature Date

[redacted]

Coordinating Investigator Signature Date

[redacted]

Statistician Signature Date

[redacted]

II. Synopsis

Title	A Phase 1/2a Trial of the Inhaled Administration of the SARS-CoV-2-Neutralizing Monoclonal Antibody DZIF-10c in SARS-CoV-2-Infected and -uninfected Individuals
Identifiers	**EudraCT:** 2020-004448-27 **Sponsor ID:** Uni-Koeln-4370
Phase	Phase I/IIa
Indication	Healthy volunteers and SARS-CoV-2-infected individuals
Sponsor	University of Cologne Albertus-Magnus-Platz 50923 Cologne Germany Represented by: [redacted]
Coordinating Investigator	[redacted]
Study Design	This trial is a phase 1/2a clinical trial to evaluate the safety, pharmacokinetics, immunogenicity and antiviral activity of the monoclonal SARS-CoV-2-neutralizing antibody DZIF-10c administered by inhalation using a nebulizer. This trial includes both SARS-CoV-2-uninfected individuals (**Groups 1A-1C**) and SARS-CoV-2-infected individuals (**Groups 2A-2D**). Following an open-label single-administration dose-escalation phase (**Groups 1A-1C** and **Groups 2A-2C**), the highest tested and tolerated dose will be administered to an expansion cohort (**Group 2D**) in a randomized double-blind placebo-controlled manner. In addition to the inhaled administration of DZIF-10c or placebo, participants in this expansion cohort will receive an intravenous infusion of DZIF-10c or placebo. Enrollment into **Group 2D** and intravenous dose selection must be supported by the Safety Monitoring Committee based on data from the separate trial Uni-Koeln-4288 (EudraCT: 2020-003503-34) investigating the intravenous infusion of DZIF-10c.

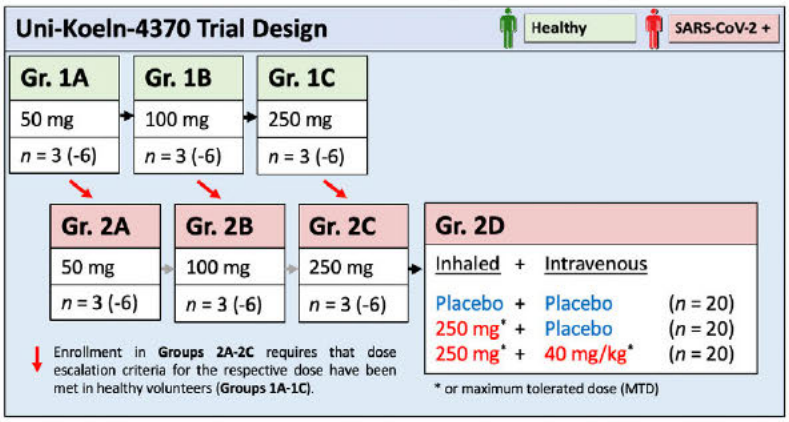

Figure 1. Study Design.

	Dose Escalation Phase During the dose escalation phase, SARS-CoV-2-uninfected (**Groups 1A-C**) and SARS-CoV-2-infected individuals (**Groups 2A-C**) will receive a single inhaled administration of DZIF-10c at the specified dose on day 0. **Groups 1A** and **2A**: 50 mg **Groups 1B** and **2B**: 100 mg **Groups 1C** and **2C**: 250 mg **Dose-Limiting Toxicities** A dose-limiting toxicity (DLT) for this trial will be defined as any grade 3 or higher adverse event during the dose escalation phase that the investigators determine to be related to DZIF-10c. **Dose Escalation Scheme** During the dose escalation phase of the study, only a single individual per dose may be enrolled per day. However, two individuals receiving different DZIF-10c doses may be enrolled on the same day. Initially, three individuals will be enrolled per group during the dose escalation phase. During the dose escalation phase, the following rules will apply: - If no DLT occurs up to the day 7 visit in the 3 enrolled participants within a group, dose escalation will proceed and no additional participants will be enrolled into the same group. - If a DLT occurs in one individual in a group up to the day 7 visit, three additional participants will be enrolled into the same group. If no additional DLTs occur up to the day 7 visit in these 3 additional participants, the trial will proceed to the next dose group. - If DLTs in a group occur in two or more participants, dosing will be halted, and the prior dose level will be declared as maximum tolerated dose (MTD). Study enrollment will start in **Group 1A**. Decisions on dose escalation and enrollment of SARS-CoV-2-infected individuals will be made by the Safety Monitoring Committee (SMC) who will review interim safety data after the day 7 visit of all participants within a group has occurred. Enrollment of SARS-CoV-2-infected individuals can start only after escalation criteria for the respective dose to be tested have been met in SARS-CoV-2-uninfected individuals, i.e.: Group to be Enrolled — Escalation Criteria must have been met in Group 2A — Group 1A Group 2B — Group 1B and Group 2A Group 2C — Group 1C and Group 2B
Number of Participants	A total of 18(-36) participants will be enrolled into the open-label dose escalation component of the trial. An additional 60 participants will be enrolled into the randomized expansion cohort.

Objectives	**Primary Objectives** - To evaluate the safety and tolerability of a single inhaled application of DZIF-10c in SARS-CoV-2-uninfected and SARS-CoV-2-infected individuals. - To evaluate the safety and tolerability of a single combined inhaled and intravenous application of DZIF-10c in SARS-CoV-2-infected individuals. **Secondary Objectives** All to be determined after a single inhaled application of DZIF-10c or a single combined inhaled and intravenous application of DZIF-10c: - To determine the systemic DZIF-10c exposure ($AUC_{0\text{-}672h}$ (i.e., from the day 0 to the day 28 visit)). - To determine the development of antibodies targeting DZIF-10c (anti-drug antibodies, ADA). - To determine the effect of DZIF-10c on SARS-CoV-2 shedding in nasopharyngeal swabs by qRT-PCR. **Exploratory Objectives** All to be determined after a single inhaled application of DZIF-10c or a single combined inhaled and intravenous application of DZIF-10c: - To evaluate the effect of DZIF-10c on viral shedding in nasopharyngeal swabs determined by the presence of infectious virions in SARS-CoV-2-infected individuals. - To evaluate the effect of DZIF-10c on viral shedding in oropharyngeal swabs determined by qRT-PCR in SARS-CoV-2-infected individuals. - To evaluate the effect of DZIF-10c on viral shedding in oropharyngeal swabs determined by the presence of infectious virions in SARS-CoV-2-infected individuals. - To evaluate the frequency of COVID-19-related hospitalization or medical contacts after DZIF-10c application in SARS-CoV-2-infected individuals. - To evaluate the effect of DZIF-10c administration on symptom resolution in SARS-CoV-2-infected individuals symptomatic at baseline. - To evaluate the effects of DZIF-10c administration on the viral sequence and the development of viral escape mutation that might arise. - To evaluate SARS-CoV-2-specific immune responses following DZIF-10c application. - To evaluate the effect of DZIF-10c application on viral replication in respiratory samples as determined by subgenomic mRNA levels in SARS-CoV-2-infected individuals. - To determine the further pharmacokinetic profile of DZIF-10c.
Endpoints	**Primary Endpoints** - The rate of Adverse Events after DZIF-10c inhalation. - The rate of Adverse Events after the combined inhalation and intravenous infusion of DZIF-10c. **Secondary Endpoints** To be determined after a single inhaled application of DZIF-10c or a single combined inhaled and intravenous application of DZIF-10c: - The Area under the Curve (AUC) for DZIF-10c levels from the day 0 to the day 28 visit ($AUC_{0\text{-}672h}$) - The frequency and magnitude of anti-drug antibodies targeting DZIF-10c. - SARS-CoV-2 RNA shedding in nasopharyngeal swabs determined by qRT-PCR in SARS-CoV-2-infected individuals.

	Exploratory Endpoints To be determined after a single inhaled application of DZIF-10c or a single combined inhaled and intravenous application of DZIF-10c: - Successful virus isolation from nasopharyngeal swabs in SARS-CoV-2-infected individuals. - SARS-CoV-2 RNA shedding in orpharyngeal swabs determined by qRT-PCR in SARS-CoV-2-infected individuals. - Successful virus isolation from oropharyngeal swabs in SARS-CoV-2-infected individuals. - Frequency of COVID-19-related hospitalization and medically-attended health care contacts in SARS-CoV-2-infected individuals not previously hospitalized. - Time to resolution of self-assessed SARS-CoV-2-related symptoms. - Differences of viral spike gene sequences obtained before and after study drug administration and the investigation of the effect of observed mutations on viral sensitivity to DZIF-10 in neutralization assays. - The activity and frequency of SARS-CoV-2-reactive immune cells following DZIF-10c application. - Subgenomic SARS-CoV-2 mRNA levels in respiratory samples in SARS-CoV-2-infected individuals. - The pharmacokinetic profile of DZIF-10c, including elimination half-life, peak serum concentration, area under the curve, clearance, and volume of distribution.

Inclusion and Exclusion Criteria	**Inclusion Criteria**
	
	<u>Groups 1A-1C</u>
	- Age 18 to 65.
	- SARS-CoV-2-RNA negative naso- or oropharyngeal swab obtained within 3 calendar days before study drug administration by NAAT (e.g., qRT-PCR).
	- Non-reactivity of serum antibodies (IgG; and IgA and/or IgM when tested) against SARS-CoV-2 by serological assay at screening.
	
	<u>Groups 2A-2D</u>
	- Age 18 to 70.
	- SARS-CoV-2-RNA positive naso- or oropharyngeal swab obtained within 3 calendar days before study drug administration by NAAT (e.g., qRT-PCR).
	- Onset of COVID-19 symptoms (e.g., sore throat, cough, fever, chills, fatigue, dys- or anosmia, dys- or angeusia, headache, muscle pain, gastrointestinal symptoms) within 7 days prior to study drug administration
	or
	Non-reactivity of serum or plasma antibodies (IgG; and IgA and/or IgM when tested) against SARS-CoV-2 by serological assay at screening.
	- Disease severity score 1-4 as defined by the WHO Clinical Progression Scale (WHO, Lancet Inf Dis 2020).
	
	Exclusion Criteria
	
	- Known hypersensitivity to any constituent of the investigational medicinal product.
	- Hepatitis B infection indicated by detectable HBsAg (Hepatitis B surface antigen) in blood.
	- Detectable antibodies against hepatitis C virus in blood unless active hepatitis C is ruled out by negative HCV-RNA.
	- HIV infection indicated by detectable HIV antigen and/or HIV antibodies in blood.
	- Blood laboratory parameter abnormalities as listed below
	  - Neutrophil count ≤1,000 cells/µl
	  - Hemoglobin ≤10 g/dl

	- Platelet count ≤100,000 cells/µl - ALT ≥2.0 x ULN - AST ≥2.0 x ULN - Total bilirubin ≥1.5 ULN - eGFR <60 ml/min/1.73m^2 - Pregnancy or lactation. - Any vaccination within 14 days prior to DZIF-10c administration. - Receipt of any SARS-CoV-2 vaccine or SARS-CoV-2 monoclonal antibody in the past. - Diagnosis of bronchial asthma or history of bronchial hyperresponsiveness, COPD, pulmonary fibrosis, or other chronic lung diseases. - Any chronic or clinically significant medical condition that in the opinion of investigator would jeopardize the safety or rights of the volunteer. - History of systemic corticosteroids, immunosuppressive anti-cancer, or other medications considered significant by the trial physician within the last 6 months (a single administration of systemic corticosteroids within ≤6 months and ≥4 weeks of enrollment is acceptable). - Participation in another clinical trial of an investigational medicinal product within the past 12 weeks or expected participation during this study. - Dependency on the principal investigator or study staff; or site personnel directly affiliated with this trial. - Legally incapacitated individuals. - Individuals held in an institution by legal or official order. - If engaging in sexual activity that could result in pregnancy, inability or unwillingness to comply with the requirements for highly effective contraception.
Study Product and Placebo	DZIF-10c is a recombinantly produced fully human monoclonal antibody of the IgG1k isotype that targets the spike protein (S) of SARS-CoV-2. When tested *in vitro*, DZIF-10c potently neutralizes SARS-CoV-2 and prevents viral infection. Solvent for dilution (formulation buffer) of DZIF-10c will be used as placebo for inhalation. Sterile normal saline (0.9% NaCl) will be used as placebo for the intravenous administration. <u>Dose</u> A volume of 5 mL containing metered doses of 50 mg, 100 mg, or 250 mg of DZIF-10c (or placebo) will be inhaled. For the intravenous infusion of DZIF-10c, the targeted dose of 40 mg/kg requires SMC confirmation and may be reduced if a separate trial on the intravenous infusion of DZIF-10c (Uni-Koeln-4288, EudraCT: 2020-003503-34) defined a lower maximum tolerated dose. <u>Route of Administration</u> For the inhalation, DZIF-10c or placebo will be administered through a mouthpiece following aerosol generation using a mesh nebulizer. For the infusion, DZIF-10c or placebo will be administered by intravenous infusion after dilution in formulation buffer as necessary using a 0.2 µm in-line filter.
Treatment Duration	DZIF-10c will be administered by a single inhalation or by a single inhalation followed by a single intravenous infusion. Participants will be followed for 90 days after the administration of DZIF-10c or placebo.

Tentative Time Plan	First patient first visit (FPFV): Q4/2020 Last patient first visit (LPFV): Q2/2021 Last patient last visit (LPLV): Q3/2021 End of trial: Q3/2021 Final study report: Q2/2022
Statistical Methodology	Quantitative data will be summarized by mean ± standard deviation and percentiles (0, 25, 50, 75, 100), qualitative data by absolute and relative frequency. Listings of the data will be given where appropriate. Data up to the day 7 visit after study treatment and during follow up after the day 7 visit will be evaluated separately, and in total where sensible. Results are described by group (dose and SARS-CoV-2-infection group in the dose escalation phase, treatment group in the randomized phase) and in total. Analysis cohorts include the safety population (as treated), including all trial participants who received any amount of the study drug, as well as the modified intention-to-treat (mITT) population in the expansion phase, including all randomized trial participants receiving study drug who have at least a baseline (day 0) value and a second measurement until (including) the day 7 visit after trial drug administration. Statistical analyses are descriptive by nature. The frequency of (serious) adverse events ((S)AEs) and adverse events of special interest (AESIs) occurring up to the day 7 visit after study drug application, during study follow up after the day 7 visit and in total will be given. Safety events of the primary endpoints will be listed. Main analysis is in the safety population. Secondary and exploratory endpoints will be summarized, figures may show development over time where appropriate. Additionally, explorative multivariable methods or confidence intervals may be calculated where sensible. Endpoints will be evaluated in the mITT population, and additional analysis populations can be defined. Viral RNA shedding determined by qRT-PCR will be evaluated as time weighted average change from baseline. A full description of the statistical methodology will be provided in the Statistical Analysis Plan.
Funding	Funding for this trial is provided by Boehringer Ingelheim.

III. Table of Contents

IV. Abbreviations

ACE2	Angiotensin-converting enzyme 2
ADE	Antibody-dependent enhancement
AE	Adverse Event
AESI	Adverse Event of Special Interest
CA	Competent authority
CI	Confidence interval
COVID-19	Coronavirus disease 2019
CRF	Case Report Form
Ct	Cycle threshold
CTCC	Clinical Trials Center Cologne
DLT	Dose-limiting toxicity
DSUR	Development Safety Update Report
EBOV	Ebola virus
EC	Ethics Committee
EMA	European Medicines Agency
Fc	Fragment crystallizable
FDA	Food and Drug Administration
HIV-1	Human immunodeficiency virus type 1
IC_{50}	50% inhibitory concentration
ISF	Investigator Site File
IMSB	Institute of Medical Statistics and Computational Biology
IMP	Investigational Medicinal Product
mAb	Monoclonal antibody
mITT	Modified Intention-To-Treat
NAAT	Nucleic acid amplification test
PI	Principal Investigator
PEI	Paul-Ehrlich-Institute
PP	Per Protocol
qRT-PCR	Quantitative real-time reverse transcription polymerase chain reaction
RBD	Receptor-binding domain
RSV	Respiratory syncytial virus
RNA	Ribonucleic acid
S	SARS-CoV-2 spike protein
SAE	Serious Adverse Event
SAP	Statistical analysis plan
SARS	Severe acute respiratory syndrome
SARS-CoV	Severe acute respiratory syndrome coronavirus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SMC	Safety monitoring committee
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File

1. Introduction

1.1. Background

After emerging at the end of 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread across the globe. By October 2020, over 40 million confirmed cases of SARS-COV-2 infection and more than 1,000,000 casualties of the associated coronavirus disease 2019 (COVID-19) have been reported[1]. The development of safe and effective options for treatment and prevention of SARS-CoV-2 infection is therefore of urgent concern for global public health.

SARS-CoV-2 is an enveloped positive-sense single-stranded RNA Betacoronavirus of the subgenus Sarbecovirus. It is related to SARS-CoV, the causative agent of the 2002/2003 SARS outbreak, and to coronaviruses circulating in bat populations. Although a definitive animal reservoir has not yet been identified, SARS-CoV-2 is likely of zoonotic origin[2].

Human-to-human transmission of SARS-CoV-2 occurs primarily through respiratory droplets and aerosols[3]. It is notable for the occurrence of superspreading events, in which a limited number of index cases results in the infection of a large number of contacts. Importantly, asymptomatic and presymptomatic individuals shedding the virus at high concentrations create a challenge to identify origins of transmission chains[4]. In addition to the respiratory route, transmission of SARS-CoV-2 by fomites has been suggested as plausible[5].

Following exposure, symptoms typically manifest after a median of 5-6 days, although this period can range from 1 to more than 14 days. In addition, a sizeable fraction of infected individuals appears not to develop clinically apparent signs. A majority of infections (approximately 80%) results in mild to moderately severe courses of disease that are characterized by symptoms of upper respiratory tract infections (e.g., cough, fever, rhinorrhea, coarse throat, or disturbances of smell or taste). More severe cases of COVID-19 primarily demonstrate signs of lower respiratory tract infection (e.g., dyspnea, pneumonia) and can result in respiratory failure with a need for mechanical ventilation or extracorporeal oxygenation of the blood. Beyond respiratory symptoms, an increasing number of additional manifestations have been associated with SARS-CoV-2 infection, including thromboembolic events, cardiac or systemic inflammation, and renal failure[6].

A number of investigational and approved drugs that were developed for the use in other indications are under evaluation for their potential use in the treatment of SARS-CoV-2 infection. For example, remdesivir is a nucleoside analogue prodrug that acts as an inhibitor of viral RNA polymerase following intracellular biotransformation. Based on its demonstrated *in vitro* activity against human coronaviruses, including SARS-CoV-2, clinical investigation of remdesivir for SARS-CoV-2 infection was rapidly initiated. In hospitalized adults with COVID-

19, administration of remdesivir resulted in a significantly reduced time to recovery compared to placebo[7]. Based on these observations, remdesivir has received full or conditional approval (e.g., by the FDA and EMA) for use in SARS-CoV-2 infected individuals requiring supplemental oxygen therapy. However, remdesivir requires a 5-to-10-day intravenous treatment course and its efficacy in prevention of SARS-CoV-2 infection has not been demonstrated yet. In addition to antiviral compounds, agents aimed at modulating immune-mediated organ injury are under investigation for their efficacy in COVID-19. Importantly, in hospitalized patients with COVID-19 requiring oxygen therapy, the anti-inflammatory corticosteroid dexamethasone has resulted in reduced 28-day mortality[8]. However, no effects in mortality were noted in individuals not receiving respiratory support [8].

SARS-CoV-2 infection of target cells occurs through the interaction of the receptor-binding domain (RBD) of the homotrimeric spike (S) protein on the viral surface with its host target cell receptor ACE2[9,10]. Moreover, SARS-CoV-2-infected cells can be identified through the expression on the SARS-CoV-2 S protein on the cellular surface[11]. Thus, strategies that target the S protein are of potential interest both for the treatment and prevention of SARS-CoV-2 infection.

1.2. Monoclonal Antibodies Targeting Infectious Pathogens

Human immunity is composed of an intricate network of different mechanisms aimed at preventing and resolving infection. Although the immune response can vary between different pathogens and individuals, infection as well as vaccination typically result in the induction of neutralizing and non-neutralizing pathogen-specific antibodies. Both types of antibodies can promote clearance or processing of targeted pathogens by engaging other components of the immune system. These functions are mediated through the antibodies' Fc domains (Fc, fragment crystallizable) that interact with Fc receptors expressed on the surface of effector cells (e.g., NK cells, macrophages, or dendritic cells) or with components of the complement system[12]. In addition, neutralizing antibodies (nAbs) are capable of rendering pathogens non-infectious by targeting critical surface epitopes[13]. For example, virus-specific neutralizing antibodies can prevent infection by targeting viral surface proteins that mediate attachment to target cells or the subsequent fusion of viral and cellular membranes[13,14]. Induction of neutralizing antibodies is therefore a key objective of many vaccination strategies and neutralizing antibodies are of particular relevance for antibody-mediated strategies against pathogens[15].

Administration of antibodies for treatment and prevention of infectious disease is a clinically well-established principle since its origins in the late 19th century when serum antitoxins were used against tetanus and diptheria[16]. For example, purified polyclonal

immunoglobulins enriched from human donors with high titers of pathogen-specific antibodies are routinely used for postexposure prophylaxis of infections including hepatitis B, rabies, or tetanus[17]. In addition, antibody-containing convalescent plasma donated by survivors of infection can provide a readily and rapidly available medication with clinical efficacy against newly emerging infectious pathogens. For example, the administration of convalescent plasma has been associated with reduced mortality after infection with SARS-CoV, for which no specific antiviral medication exists[18]. However, preparations of polyclonal immunoglobulins show donor-dependent variations resulting in difficulties for standardization and are not enriched for neutralizing antibodies with particularly high potency.

In contrast, individual monoclonal antibodies (mAbs) have well-defined specifities, and advances in antibody production technologies have facilitated the pharmaceutical development of monoclonal antibodies. Such antibodies have gained widespread medical use with >70 approved mAbs and derived constructs, mostly for malignant and autoimmune diseases. In addition, monoclonal antibodies have been approved for the treatment of drug-resistant HIV-1 (human immunodeficiency virus type 1), the prevention of recurrence *Clostridium difficile* infection, as well as for the prophylaxis of infection with RSV (respiratory syncytial virus)[14,19].

Monoclonal antibodies for clinical use have initially been obtained from immunized animals. However, recent developments in B cell cloning and antibody isolation methods have enabled the identification and recombinant production of fully human monoclonal antibodies derived from human samples[14]. Using these approaches, highly potent neutralizing antibodies targeting different viruses, including influenza virus, Ebola virus (EBOV), and HIV-1, could be identified[14]. When administered to heathy and infected individuals, potent neutralizing human monoclonal antibodies were generally safe and well tolerated[20-25]. Importantly, high *in vitro* neutralizing activity can be translated to clinical antiviral efficacy. For example, administration of potent human monoclonal antibodies significantly reduced mortality from Ebola virus compared to the antiviral agent remdesivir and a combination of antibodies obtained from immunized mice[25]. Moreover, a combination of potent human broadly neutralizing antibodies maintained suppression of HIV-1 without development of antibody resistance in individuals infected with sensitive strains[20,24]. Finally, human antibody-derived monoclonal antibodies targeting influenza and RSV resulted in significantly reduced viral titers compared to placebo in an influenza challenge study[26] and reduced hospitalizations during RSV season, respectively[27].

1.3. Neutralizing Antibodies Targeting SARS-CoV-2

SARS-CoV-2 infection induces a humoral immune response of varying magnitude[28]. Total and neutralizing antibody titers in serum depend on several factors, including disease severity[29]. Whether the development of neutralizing antibodies contributes to protection from re-infection and whether neutralizing antibodies are a correlate of protection in vaccinated individuals are subjects of intensive research efforts that include investigations on the longevity of the immune response[30].

SARS-CoV-2-neutralizing monoclonal antibodies have been isolated from phage display libraries[31], immunized animals[32,33], survivors of infection with the related betacoronavirus SARS-CoV[34,35], as well as from SARS-CoV-2-infected individuals[33,36-48]. Most of the identified neutralizing antibodies interfere with viral infection by targeting the RBD of the spike protein that interacts with the host cell receptor ACE2. However, neutralizing antibodies targeting other epitopes on the SARS-CoV-2 S protein have also been characterized[40,44,45]. When tested *in vitro* against authentic replication-competent SARS-CoV-2 or replication-deficient viruses pseudotyped with the SARS-CoV-2 spike protein, neutralizing antibodies can efficiently block or reduce viral infection, although the neutralizing potency between different neutralizing antibodies varies considerably[33,35,36,39-41,43,44,46,48]. In addition, the development of antibody escape mutations during viral passage *in vitro* suggests that combinations of antibodies targeting different epitopes may be beneficial[49].

Indicating their potential for use in prevention of SARS-CoV-2 infection, administration of neutralizing monoclonal antibodies prior to SARS-CoV-2 challenge resulted in reduced clinical disease, reduced viral concentrations, and/or reduced pulmonary pathological findings across diverse animal models[36,42,43,46,47]. For example, a serum concentration of 22 µg/ml of the human neutralizing antibody CC12.1 at the time of intranasal viral challenge resulted in protection from disease in a Syrian hamster model of SARS CoV-2 infection[46]. Taking the neutralizing potency of the administered human antibody CC12.1 against SARS-CoV-2 into account, a serum antibody concentration of 1.160-fold the pseudovirus IC_{50} was considered fully protective in this study[46]. Similarly, administration of human SARS-CoV-2-neutralizing antibodies prior to respiratory SARS-CoV-2 challenge resulted in reduced viral replication and reduced pathological findings in lung biopsies in non-human primate models of SARS-CoV-2 infection[42,43].

When administered in a therapeutic setting several hours after viral challenge, human SARS-CoV-2-neutralizing monoclonal antibodies resulted in reduced viral titers in transgenic mouse models expressing human ACE2[36,47]. In addition, antibody treatment 24 h and 72 h after viral challenge was associated with an expedited decline of viral RNA in respiratory swabs and reduced pathological signs of pulmonary inflammation in rhesus macaques[42].

These positive pre-clinical findings of potent human SARS-CoV-2-neutralizing antibodies have prompted the initiation of phase I to phase III clinical trials of different antibody candidates. In addition to establishing the safety and tolerability of neutralizing antibodies, these studies aim to evaluate antibody efficacy both in the therapeutic and preventive settings. For example, the SARS-CoV-2-neutralizing antibody LY-CoV555 has been investigated in recently SARS-CoV-2-infected outpatients with mild-to-moderate infection[50]. A single intravenous infusion of a dose of 700 to 7,000 mg was overall well tolerated[50]. While the effects on the decline of viral shedding were limited, a lower fraction of antibody-recipients reported COVID-19-related hospitalizations or emergency room visits compared to placebo recipients[50]. Interim results of trials investigating combinations of SARS-CoV-2 neutralizing antibodies to SARS-CoV-2-infected ambulatory trial participants have also suggested beneficial antibody-mediated effects, including reduced viral shedding and impoved clinical outcomes (e.g., reduced symptom duration, reduced rate of COVID-19-associated medical contacts)[51,52]. Based on these results, emergency use authorizations for the first monoclonal antibodies were recently granted by the FDA in SARS-CoV2-infected outpatients at high risk of disesase progression or hospitalization.

1.4. Application of Antibodies and Antibody-Derived Fragments by Inhalation

Intravenous antibody application results in limited antibody concentrations in the epithelial lining fluid (ELF) of the lung[53]. In addition, achieving peak antibody ELF concentrations can be considerably delayed (up to multiple days) after intraven ous infusion of an antibody[53].

To provide an immediate onset of action with high local concentrations, application of antibodies and antibody-derived fragments has been investigated for different indications in clinical trials. For example, inhalation of the humanized monoclonal antibody omalizumab (E25) was investigated in a total of 22 trial participants with mild allergic asthma[54]. In this trial, participants self- administered a solution containing 1 mg or 10 mg of omalizumab twice daily over a total treatment period of 8 weeks, using a PARI IS-2 nebulizer. Although no therapeutic effects as indicated by an attenuation of early phase responses to inhaled allergens were observed, the inhaled administration of omalizumab at both dose levels was considered to be well tolerated. Inhalation of full-length antibodies was also studied in a case series of three immunodeficient infants (2, 4, and 5 years) suffering from frequent upper respiratory tract infections (URTI). In an attempt to reduce the number of URTIs, a 4 mL dose of a 5% human polyclonal immunoglobulin solution (i.e., a 200 mg human IgG dose) was administered twice daily by inhalation for a period of >9 months[55]. To this end, an aerosol was generated using the eFLOW nebulizer (PARI GmbH) and applied using a children’s facemask. During the

treatment period, a reduced number of URTIs was observed. Importantly, no adverse events related to the inhalation of aerosolized full-length IgGs were reported despite a daily administration of 400 mg IgG a day for multiple months[55].

Moreover, inhalation of antibody-derived fragments was also investigated for viral respiratory infection. In an ascending-dose healthy volunteer trial of the trivalent respiratory syncytial virus-targeting nanobody ALX-0171, doses of aerosolized ALX-0171 ranging from 2.1 mg to 210 mg were well tolerated, including a twice-daily dose of 105 mg administered for 5 days[56]. In a phase 2b trial of ALX-0171 in children hospitalized with lower respiratory tract RSV infection, nebulized ALX-0171 at doses up to 9 mg/kg was not considered to be related to any serious adverse event[57]. Thus, inhaled administration of antibody-derived molecules appeared generally safe in the presence of a lower respiratory tract viral infection.

1.5. DZIF-10c

DZIF-10c is a potent human SARS-CoV-2-neutralizing antibody that was identified from a blood sample of a COVID-19-convalescent patient obtained 35 days after diagnosis of infection[48]. To isolate SARS-CoV-2-neutralizing antibodies from this and 11 additional patients, single memory B cells reactive to the SARS-CoV-2 spike protein were sorted using flow cytometry. Antibody heavy and light chain sequences were subsequently amplified from individually lysed cells using specific reverse transcription polymerase chain reaction (RT-PCR) and sequenced, facilitating the recombinant production of exact antibody replicates[58]. Using this approach, the human monoclonal antibody HbnC3t1p1_F4 was identified and later modified by eliminating the heavy chain C-terminal lysine to reduce potential charge heterogeneity, resulting in antibody DZIF-10c. DZIF-10c is a monoclonal antibody of the IgG1 kappa isotype with low somatic hypermutation compared to its germline sequence (% germline nucleotide identity of 93.2% and 96.1% for the heavy chain and light chain variable genes, respectively)[48].

DZIF-10c targets the receptor binding domain (RBD) of the SARS-CoV-2 spike protein with high affinity. When tested *in vitro*, DZIF-10c demonstrates potent neutralizing activity both against authentic SARS-CoV-2 as well as SARS-CoV-2 spike-typed pseudoviruses[48,59]. For example, infection of Vero E6 cells with the SARS-CoV-2 variant BavPat1/2020 was completely blocked in the presence of DZIF-10c at concentrations as low as 0.05 µg/ml as indicated by the absence of detectable cytopathic effects. Moreover, DZIF-10c demonstrated high activity against a panel of six SARS-CoV-2 pseudovirus variants with an average 50% inhibitory concentration (IC_{50}) of 0.007 µg/ml in a neutralization assay using ACE2-expressing human 293T cells.

DZIF-10c has also been studied in animal models of SARS-CoV-2-infection. Mice expressing human ACE2 after transtracheal transduction by a recombinant adenovirus encoding for ACE2 [redacted] can be challenged with SARS-CoV-2, resulting in viral replication in pulmonary tissues. To investigate its therapeutic activity, DZIF-10c was administered [redacted] after SARS-CoV-2 challenge either by intraperitoneal injection or intranasal instillation. While high titers of infectious virus could be detected in lung tissues of animals treated with isotype control antibodies, [redacted] undectable levels of infectious SARS-CoV-2 (ACE2-transduced mice) were observed after DZIF-10c treatment.

Thus, DZIF-10c is a highly potent human SARS-CoV-2-neutralizing monoclonal antibody with promising potential for use in the treatment and prevention of SARS-CoV-2 infection.

2. Objectives of the Clinical Trial

2.1. Rationale for the Clinical Trial

This clinical trial is the first-in-human study for the inhaled application of the SARS-CoV-2-neutralizing human monoclonal antibody DZIF-10c. It is intended to investigate the safety and tolerability of DZIF-10c by inhalation, including by combined administration by inhalation and intravenous infusion, in humans and to support the further development of DZIF-10c for treatment and prevention of SARS-CoV-2 infection.

2.2. Primary Objective

- To evaluate the safety and tolerability of a single inhaled application of DZIF-10c in SARS-CoV-2-uninfected and SARS-CoV-2-infected individuals.
- To evaluate the safety and tolerability of a single combined inhaled and intravenous application of DZIF-10c in SARS-CoV-2-infected individuals.

2.3. Secondary Objectives

All to be determined after a single inhaled application of DZIF-10c or a single combined inhaled and intravous application of DZIF-10c:

- To determine the systemic DZIF-10c exposure ($AUC_{0\text{-}672h}$ (i.e., from the day 0 to the day 28 visit)).
- To determine the development of antibodies targeting DZIF-10c (anti-drug antibodies, ADA).
- To determine the effect of DZIF-10c on SARS-CoV-2 shedding in nasopharyngeal swabs by qRT-PCR.

2.4. Exploratory Objectives

All to be determined after a single inhaled application of DZIF-10c or a single combined inhaled and intravous application of DZIF-10c:

- To evaluate the effect of DZIF-10c on viral shedding in nasopharyngeal swabs determined by the presence of infectious virions in SARS-CoV-2-infected individuals.
- To evaluate the effect of DZIF-10c on viral shedding in oropharyngeal swabs determined by qRT-PCR in SARS-CoV-2-infected individuals.
- To evaluate the effect of DZIF-10c on viral shedding in oropharyngeal swabs determined by the presence of infectious virions in SARS-CoV-2-infected individuals.

- To evaluate the frequency of COVID-19-related hospitalization or medical contacts after DZIF-10c application in SARS-CoV-2-infected individuals.
- To evaluate the effect of DZIF-10c administration on symptom resolution in SARS-CoV-2-infected individuals symptomatic at baseline.
- To evaluate the effects of DZIF-10c administration on the viral sequence and the development of viral escape mutation that might arise.
- To evaluate SARS-CoV-2-specific immune responses following DZIF-10c application.
- To evaluate the effect of DZIF-10c application on viral replication in respiratory samples as determined by subgenomic mRNA levels in SARS-CoV-2-infected individuals.
- To determine the further pharmacokinetic profile of DZIF-10c.

3. Organizational and Administrative Aspects of the Trial

3.1. Sponsor

University of Cologne

Albertus-Magnus-Platz

50923 Cologne

Germany

Represented by

[redacted]

3.2. Coordinating Investigator

[redacted]

3.3. Statistician

[redacted]

3.4. Safety Monitoring Committee

A Safety Monitoring Committee (SMC) will be established to provide assessments of the participants' safety during the conduct of the clinical trial and to provide recommendations on dose escalation. This SMC will also oversee the safety of participants in a parallel clinical trial on the intravenous infusion of DZIF-10c (Uni-Koeln-4288, EudraCT: 2020-003503-34). The SMC will review data from both trials as they progess to ensure consistent and complete overview of DIF-10c safety data independent of the route of administration. Details will be provided in the SMC charter.

The SMC will be composed of three independent individuals with no connection to the Sponsor, who are not involved in the conduct of the clinical trial beyond their role in the SMC, and who do not have any direct responsibility for the clinical care of the study participants outside of the clinical trial. In addition, a Pharmacovigilance designee of Boehringer Ingelheim may be assigned as standing member of the SMC without voting right. Principal Investigators (PIs), designees of the Sponsor or Boehringer Ingelheim, and/or designees of the Monitor may be invited by the SMC for participation in SMC meetings to provide information on the conduct of the clinical trial and to answer questions by the SMC.

Meetings of the SMC will be conducted through video conference, by phone, or in-person as deemed necessary by the SMC. If scheduling problems prevent a timely meeting, review and comment may alternatively conducted by E-Mail. One member will be appointed chair of the SMC and be responsible for summarizing SMC meetings and informing the Coordinating Investigator and Sponsor about meetings and recommendations in writing. The SMC chair may select another SMC member as designee to perform these tasks. A quorum for SMC meetings will require the participation of at least two SMC members with voting rights.

Ad-hoc SMC meetings will be conducted prior to enrolment into **Groups 1B**, **1C**, **2B, 2C**, and **2D** to provide recommendations on proceeding with the dose escalation scheme. A review of the safety data from the intravenous doses administered in the Uni-Koeln-4288 trial will be conducted prior to inclusion of the intravenous dose in **Group 2D** to decide on whether to include intravenous infusion of DZIF-10c and at which dose. A lower dose than the targeted 40 mg/kg dose will be used in case that a lower maximum tolerated dose for the intravenous infusion of DZIF-10c has been determined in the trial Uni-Koeln-4288.

Additional SMC meetings may be scheduled on an as-needed-basis if deemed necessary by the members of the SMC, the Sponsor, or the Principal Investigators.

On an interim basis, the SMC will also be asked to review the following data:

- Any grade 3 or higher Adverse Events (AEs) that are deemed to be related to DZIF-10c by the Investigators.
    - These events will also be assessed as potential late-occurring dose-limiting toxicities (i.e., after dose-escalation has proceeded and/or been completed) to evaluate whether a change to study dosing is warranted.
    - In case of grade 3 or higher AEs deemed related to DZIF-10c occurring in two or more participants, administration of DZIF-10c will be halted until a SMC review has been completed to provide recommendations regarding subsequent study enrolment.

- Serious Adverse Events (SAEs) that are deemed related to DZIF-10c by the Principal Investigator or designee. Such events need to be reported to the SMC within one business day of the Sponsor becoming aware of the event.
    - No additional administration of DZIF-10c will occur after an SAE deemed related to DZIF-10c until an SMC review has been completed to provide recommendations regarding subsequent study enrolment.
    - In case of life-threatening, fatal, or permanently disabling SAEs deemed related to DZIF-10c, no further administration of DZIF-10c will occur until a consensus plan has been approved by the SMC, Investigators, the Sponsor, the principal ethics committee, and the PEI.

3.5. Central Organization Unit

Administrative trial project management, data management, monitoring, and safety management for this trial are delegated to the Clinical Trials Center Cologne.

Clinical Trials Center Cologne (CTCC, ZKS Köln)
Gleueler Str. 269
50935 Cologne
Germany
Tel.: [redacted]
Fax: [redacted]
E-Mail: [redacted]

3.6. Principal Investigators and Trial Sites

This trial will be conducted as a multicenter trial at up to seven sites in Germany. Additional qualified trials sites may be recruited, if necessary, for timely recruitment.

3.7. Financing

This trial is funded by Boehringer Ingelheim.

DZIF-10c is produced and provided free of charge by Boehringer Ingelheim.

4. Trial Conduct

4.1. Trial Design

This trial is the first-in-human phase I/IIa clinical trial of DZIF-10c administered by inhalation to SARS-CoV-2-uninfected and SARS-CoV-2-infected individuals. Following a single inhalation open-label dose-escalation phase (**Groups 1A-1C** and **Groups 2A-2C**), DZIF-10c will be administered in a randomized placebo-controlled double-blind manner as a single combined inhalation and infusion to SARS-CoV-2-infected individuals in an exploratory expansion cohort (**Group 2D**). In this expansion cohort, DZIF-10c inhalation will be combined with DZIF-10c or placebo for infusion, and compared to placebo administered by both inhalation and infusion (see also **Figure 2**).

Table 1: Tentative Time Plan of the Trial

First Patient First Visit (FPFV):	Q4 2020
Last Patient First Visit (LPFV):	Q2 2021
Last Patient Last Visit (LPLV):	Q3 2021
End of Trial	Q3 2021
Final Study Report:	Q2 2022

End of the Clinical Trial

The end of this clinical trial is defined as the last visit of the last trial participant.

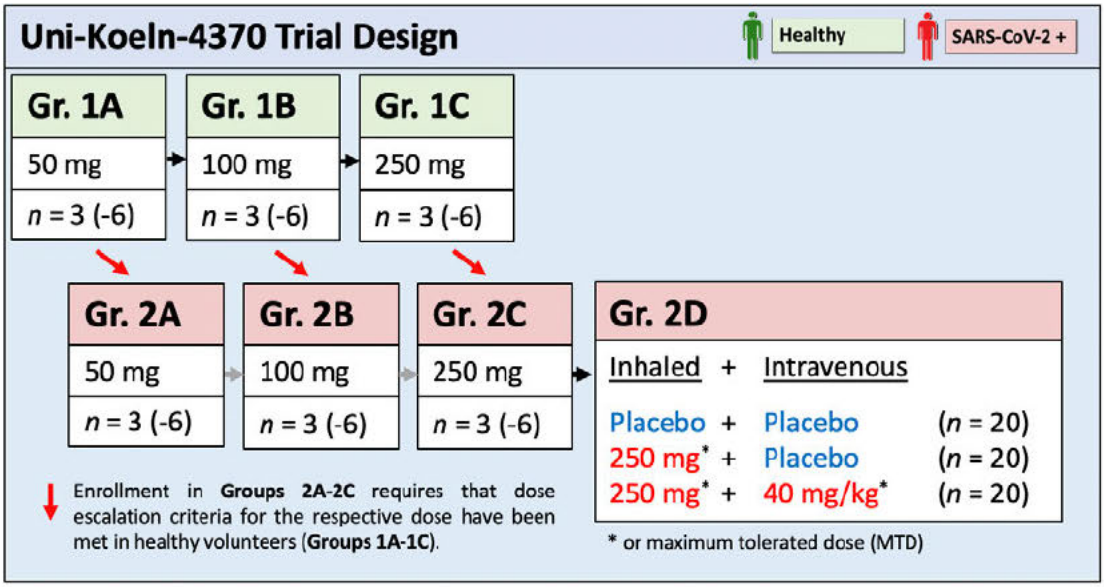

Figure 2. Trial Design.

This study includes a total of 7 trial groups.

Dose Escalation Phase

The Dose Escalation scheme is detailed in Section 4.1.1.

SARS-CoV-2-uninfected participants will receive an open-label single metered inhaled DZIF-10c dose of (*n*=3-6 per group):

Group 1A: 50 mg
Group 1B: 100 mg
Group 1C: 250 mg

SARS-CoV-2-infected participants will receive an open-label single metered inhaled DZIF-10c dose of (*n*=3-6 per group):

Group 2A: 50 mg
Group 2B: 100 mg
Group 2C: 250 mg

Randomized Placebo-Controlled Double-Blind Exploratory Expansion Phase

Group 2D – SARS-CoV-2-infected participants will be randomized (1:1:1) to receive

	by inhalation		**by i.v. infusion**
	Placebo	+	Placebo
or	DZIF-10c (250 mg)	+	Placebo
or	DZIF-10c (250 mg)	+	DZIF-10c (40 mg/kg)

Should a maximum tolerated dose (MTD) for DZIF-10c by inhalation have been defined during the dose escalation phase, or should an MTD have been defined for the intravenous administration of DZIF-10c in a parallel trial (Uni-Koeln-4288, EudraCT: 2020-003503-34), the respective MTDs will be applied in **Group 2D** after consultation with the SMC.

4.1.1. Dose Escalation Scheme

During the dose escalation phase, SARS-CoV-2-uninfected (**Groups 1A-1C**) and SARS-CoV-2-infected individuals (**Groups 2A-2C**) will receive a single inhalation of DZIF-10c at the specified dose on day 0.

Group 1A and **Group 2A**: 50 mg
Group 1B and **Group 2B**: 100 mg
Group 1C and **Group 2C**: 250 mg

During the dose escalation phase of the trial, only a single individual per dose may be enrolled per day. However, two individuals receiving different DZIF-10c doses may be enrolled on the same day.

Dose-Limiting Toxicities

A dose-limiting toxicity (DLT) for this trial will be defined as any grade 3 or higher adverse event during the dose escalation phase that the investigators determine to be related to DZIF-10c.

Dose Escalation Rules

Initially, three individuals will be enrolled per group during the dose escalation phase.

During the dose escalation phase, the following rules will apply:

- If no DLT occurs up to the day 7 visit in the 3 enrolled participants within a group, dose escalation will proceed and no additional participants will be enrolled into the same group.
- If a DLT occurs in one individual in a group up to the day 7 visit, three additional participants will be enrolled into the same group. If no additional DLTs occur up to the day 7 visit in these 3 additional participants, the trial will proceed to the next dose group.
- If DLTs in a group occur in two or more participants, dosing will be halted, and the prior dose level will be declared as maximum tolerated dose (MTD).

Scheme

Study enrollment will start in **Group 1A**. Decisions on dose escalation and enrollment of SARS-CoV-2-infected individuals will be made by the Safety Monitoring Committee (SMC) who will review interim safety data after the day 7 visit of all participants within a group has occurred. Enrollment of SARS-CoV-2-infected individuals can start only after escalation criteria for the respective dose to be tested have been met in SARS-CoV-2-uninfected individuals, i.e.:

Group to be Enrolled	Escalation Criteria must have been met in
Group 2A	Group 1A
Group 2B	Group 1B and Group 2A
Group 2C	Group 1C and Group 2B

Enrollment into **Group 2D** may begin based on the dose escalation criteria after the day 7 visit of all **Group 2C** participants and upon a positive vote by the SMC (or at a lower MTD upon a positive vote by the SMC).

4.1.2. Discussion of Trial Design

During the open-label dose escalation phase of the study, a standard “3+3” phase I design will be used, with stopping rules based on the occurrence of dose-limiting toxicities (DLTs). Dose escalation will be initiated in healthy volunteers (i.e., in the absence of the antibody target) to provide early indicators on the safety and tolerability of the inhalation of DZF-10c. Based on the pre-specified safety criteria and upon review by the SMC, dose escalation will be performed as defined above.

Enrollment of SARS-CoV-2-infected individuals (i.e., individuals with antigen present) will commence after the dose escalation critieria for the starting dose in SARS-CoV-2-uninfected individuals (**Group 1A**) have been met. Dose escalation in SARS-CoV-2-infected can occur only after dose escalation criteria for the dose to be tested have been met in SARS-CoV-2-uninfected individuals (see above).

Following the open label lead-in phase in SARS-CoV-2-infected individuals (**Groups 2A-2C**), an exploratory expansion cohort of SARS-CoV-2-infected individuals will be recruited (**Group 2D**). In this double-blind group, SARS-CoV-2-infected individuals will be randomized 1:1:1 to receive a.) placebo by inhalation and infusion, or b.) DZIF-10c at the highest tested tolerated dose by inhalation and placebo by infusion, or c.) DZIF-10c at the highest tested tolerated dose by inhalation and DZIF-10c by intravenous infusion. Enrollment into **Group 2D** will commence following a positive SMC vote based on the safety data of the clinical trial Uni-Koeln-4288 (EudraCT: 2020-003503-34) on the intravenous administration of DZIF-10c running in parallel (see section 3.4). Should this trial have defined an MTD of DZIF-10c for the intravenous infusion, this MTD will also be applied in **Group 2D** after confirmation by the SMC.

In addition to the analyses of safety and tolerability, pharmacokinetic parameters and the development of anti-drug antibodies, this trial aims to facilitate an exploratory assessment of

the antiviral activity of DZIF-10c. This assessment will be primarily based on virological endpoints in respiratory tract samples. SARS-CoV-2-infected participants enrolled in this trial will be individuals with mild-to-moderate SARS-CoV-2 infection not requiring supplemental oxygen at baseline (see Inclusion and Exclusion Criteria). In this cohort, current guidelines do not recommend medical intervention with specific antiviral medication. Therefore, formulation buffer (solvent for dilution of DZIF-10c) will be used as placebo for the inhalation and sterile normal saline (NaCl 0.9%) will be used as placebo for the infusion in **Group 2D**.

4.2. Risk-Benefit Assessment

This is the first-in-human trial of the inhaled administration of the human monoclonal antibody DZIF-10c in both SARS-CoV-2-uninfected and SARS-CoV-2-infected individuals. It will also include the first combined administration of DZIF-10c by inhalation and intravenous infusion. Determining the safety and tolerability of DZIF-10c is the primary objective of this trial.

DZIF-10c is a human monoclonal antibody of the IgG1k isotype that was isolated by single cell sorting of SARS-CoV-2 spike protein-reactive memory B cells and antibody cloning[48]. It was identified in a blood sample obtained 35 days after diagnosis from an individual who had an asymptomatic course of SARS-CoV-2 infection[48]. Importantly, in this individual, the development of B cells encoding for DZIF-10c was not associated with clinically apparent adverse events.

4.2.1. Potential Risks and Strategies for Risk Mitigation

<u>Antibody-dependent enhancement</u>

Antibody-dependent enhancement (ADE) of disease or infection is a potential concern of all antibody-mediated strategies, including vaccination, against infectious pathogens. It is best, albeit rarely, documented for severe courses of disease after dengue virus (re)-infection in the presence of low neutralizing antibody titers[60]. However, dengue virus replicates in Fc receptor-expressing cells (e.g., monocytes and dendritic cells). Thus, Fc-mediated cellular uptake of antibody-bound virus may result in increased infection, viral propagation, and enhanced disease for dengue virus infection[61]. Antibody-mediated worsening of clinical symptoms can in principle also result from an overstimulation of the immune response, e.g., by immune complex-mediated activation of the complement system. However, monoclonal antibodies targeting the respiratory viral pathogens RSV and influenza virus have been administered for treatment and prevention of disease without signs of increased disease[27,62]. Importantly, the administration of reconvalescent plasma with varying levels of neutralizing and non-

neutralizing antibodies to tens of thousands of individuals has not been reported to be associated with an increased severity of disease[63,64]. Similarly, antibody-dependent enhancement of disease was not reported in a phase II clinical trial of a SARS-CoV-2-neutralizing monoclonal antibody administered to recently infected outpatients[50]. In addition, neither neutralizing nor sub-neutralizing concentrations of DZIF-10c were associated with increased infection or replication in CD14-derived human macrophages co-cultured with authentic SARS-CoV-2- *in vitro*.

Risk mitigation strategy: Infusion of DZIF-10c to SARS-CoV-2-infected individuals will be inititated open-label and staggered by at least one day during the dose escalation phase of the trial (i.e., a maximum of one participant to be enrolled per dose group oer day, **Groups 2A-2C**). Participants will be closely followed for clinical signs of disease enhancement (e.g., respiratory distress, reduced SpO2).

Allergic/Anaphylactic reactions

Administration of non-human, chimeric, or humanized antibodies can result in severe allergic and anaphylactic reactions because the non-human derived components can be recognized as foreign by the recipient's immune system. In contrast, administration of human immunoglobulins (e.g., IVIG, hepatitis B immunoglobulin) is a routinely performed and safe clinical procedure for the treatment of autoimmune diseases or immune deficiency or to prevent viral infections such as hepatitis B. Similarly, monoclonal antibodies of human origin have been shown to be generally safe and well tolerated[20-25]. DZIF-10c is derived from a human antibody that naturally developed in response to SARS-CoV-2 infection[48] and was recombinantly produced in a mammalian cell line without use of animal-derived media components. DZIF-10c has not been administed to humans prior to this study and thus, no clincal data on the tolerability of DZIF-10c is available.

Risk mitigation strategy: DZIF-10c by inhalation will be administered slowly over a period of approximately 15-20 minutes under supervision. DZIF-10c by infusion will administered slowly over an approximately 60-minute infusion. During the dose escalation phase, participants will be hospitalized for the inhalation for immediate access to emergency therapy. Antiinflammatory medication, bronchodilators, emergency kits and an intensive-care unit are available on-site. Infusion- and inhalation-related reactions will be solicited as Adverse Events of Special Interest (AESIs) until the trial visit scheduled for day 7 after study drug administration.

Irritation of Airways

Administration of DZIF-10c and excipients may result in airway irritation that could manifest as cough, wheezing, bronchial narrowing, or bronchospams resulting in respiratory distress.

Risk mitigation strategy: Inhalators and/or nebulizers for the application of bronchodilators will be available at the site of the trial drug administration. Inhalation-related reactions will additionally be solicited as Adverse Events of Special Interest (AESIs) until the trial visit scheduled for day 7 after study drug administration.

Off-target toxicity

DZIF-10c specifically targets the spike protein of SARS-CoV-2 that is expressed on the surface of the virus and infected cells. [redacted] As DZIF-10c does not bind to human proteins, it is not expected to have pharmacological activity in healthy humans. Based on these results and the limited value of animal toxicity studies in the evaluation of human monoclonal antibodies, animal *in vivo* toxicology studies and animal tissue cross-reactivity studies are not considered to provide relevant additional information. In the normal human immune system, B cell maturation and antibody development are tightly regulated to limit the risk for the development of self-reactivity. Indeed, autoreactive B cells are generally selected against and depleted from the memory B cell pool[65]. As DZIF-10c was identified from a human memory B cell in a SARS-CoV-2-convalescent individual and based on the results of off-target profiling, the risk of off-target-mediated toxicity of DZIF-10c can be considered as low.

Risk mitigation strategy: Participants will be closely followed for unspecific toxicities.

Viral resistance

Antibody-mediated selection pressure may result in the development of viral escape mutations conferring antibody resistance. To date, it is not known whether the selection of antibody escape variants of SARS-CoV-2 withing an antibody-treated host may be associated with changes in infectivity and/or pathogenicity.

Biosampling

Over the course of the trial, blood samples will repeatedly be obtained by venipuncture that can result in bleeding, infection, or nerve damage. Upper respiratory tract samples will be obtained by naso- and oropharyngeal swabs that may cause discomfort, tissue irritation or damage, or bleeding.

Risk mitigation strategy: Biosamples will be obtained by trained trial staff.

Visits to Trial Sites during SARS-CoV-2 Pandemic

Trial participants will be asked for visits to trial sites that are embedded into hospital infrastructure.

Risk mitigation strategy: Routine hygiene measures applicable for SARS-CoV-2 will be put in place at all trial sites.

Blunting of Vaccine Response

Systemic levels of DZIF-10c may blunt the response to a SARS-CoV-2 vaccine, should one become available to a participant. Additional or delayed vaccination may be required.

Risk mitigation strategy: Trial participants will be advised about the potential need to follow-up on anti-SARS-CoV-2 antibody levels following vaccination.

4.2.2. Potential Benefits

DZIF-10c has not been previously administered to humans and it is not known whether DZIF-10c inhalation results in beneficial clinical effects.

It is possible that healthy volunteers are protected from SARS-CoV-2-infection or from severe disease after DZIF-10c inhalation during the period of DZIF-10c exposure. In SARS-CoV-2-infected individuals, administration of DZIF-10c may reduce the risk of severe disease and contribute to accelerated viral clearance.

By participating in this clinicial trial, volunteers may contribute to the development of a novel option for the treatment and prevention of SARS-CoV-2 infection.

4.2.3. Overall Risk-/Benefit-Assessment

Taking into account the implemented procedures to mitigate risks, the potential risks to participants in this first-in-human trial are justified by the significant benefits that may be afforded to SARS-CoV-2-infected individuals and the community-at-large.

4.3. Selection of Trial Population

This trial is the first-in-human trial of the inhaled application as well as of the combined inhaled and intravenous application of DZIF-10c. DZIF-10c has potential use both in healthy individuals (prevention of SARS-CoV-2-infection by passive immunization) and SARS-CoV-2-infected individuals (treatment). Adults of both potential target groups will be included in this trial.

Participation in the cohort of SARS-CoV-2-infected individuals for this first-in-human trial will be restricted to adults aged 18-70 with mild-to-moderate infection not requiring supplemental oxygen at baseline (based on the inclusion criterion of the WHO Clinical Progression Scale, see **Appendix I**) as well as probable early infection (based on the duration of symptoms and/or absence of antibodies against SARS-CoV-2). In these individuals, current guidelines do not recommend the use of approved antiviral drugs. In addition, the exclusion of elderly individuals (>70 years) or individuals with significant co-morbidities will result in the enrolment of SARS-CoV-2-infected individuals at a relatively low risk of clinical progression into this first-in-human study that will focus the on safety and tolerability of DZIF-10c.

Gender Distribution

We expect the gender distribution in this clinical trial to reflect the population-at-large. Enrollment of SARS-CoV-2-infected individuals will primarily be driven by local epidemiological characteristics, but all efforts will be made to ensure equal trial access to all genders.

4.3.1. Inclusion Criteria

Groups 1A-1C

- Age 18-65.
- SARS-CoV-2-RNA negative in naso- or oropharyngeal swab obtained within 3 calendar days before study drug administration by NAAT (e.g., qRT-PCR).
- Non-reactivity of serum antibodies (IgG; and IgA and/or IgM when tested) against SARS-CoV-2 by serological assay.

Groups 2A-2D

- Age 18-70.
- SARS-CoV-2-RNA positive in naso- or oropharyngeal swab obtained within 3 calendar days before study drug administration by NAAT (e.g., qRT-PCR).
- Onset of COVID-19 symptoms (e.g., sore throat, cough, fever, chills, fatigue, dys- or anosmia, dys- or angeusia, headache, muscle pain, gastrointestinal symptoms) within 7 days prior to study drug administration

  or

  Non-reactivity of serum or plasma antibodies (IgG; and IgA and/or IgM when tested) against SARS-CoV-2 by serological assay at screening.
- Disease severity 1-4 as defined by the WHO Clinical Progression Scale (WHO, Lancet Inf Dis 2020).

4.3.2. Exclusion Criteria

- Known hypersensitivity to any constituent of the investigational medicinal product.
- Hepatitis B infection indicated by detectable HBsAg (Hepatitis B surface antigen) in blood.
- Detectable antibodies against hepatitis C virus in blood unless active hepatitis C is ruled out by negative HCV-RNA.
- HIV infection indicated by detectable HIV antigen and/or HIV antibodies in blood.
- Blood laboratory parameter abnormalities as listed below
  - Neutrophil count ≤1,000 cells/µl
  - Hemoglobin ≤10 g/dl
  - Platelet count ≤100,000 cells/µl
  - ALT ≥2.0 x ULN
  - AST ≥2.0 x ULN
  - Total bilirubin ≥1.5 ULN
  - eGFR <60 ml/min/1.73m^2
- Pregnancy or lactation.
- Any vaccination within 14 days prior to DZIF-10c administration.
- Receipt of any SARS-CoV-2 vaccine or SARS-CoV-2 monoclonal antibody in the past.
- Diagnosis of bronchial asthma or history of bronchial hyperresponsiveness, COPD, pulmonary fibrosis, or other chronic lung diseases.
- Any chronic or clinically significant medical condition that in the opinion of investigator would jeopardize the safety or rights of the volunteer.

- History of systemic corticosteroids, immunosuppressive anti-cancer, or other medications considered significant by the trial physician within the last 6 months (a single administration of systemic corticosteroids within ≤6 months and ≥4 weeks of enrollment is acceptable).
- Participation in another clinical trial of an investigational medicinal product within the past 12 weeks or expected participation during this study.
- Dependency on the principal investigator or study staff; or site personnel directly affiliated with this trial.
- Legally incapacitated individuals.
- Individuals held in an institution by legal or official order.
- If engaging in sexual activity that could result in pregnancy, inability or unwilligness to comply with the requirements for highly effective contraception (see section 4.3.2.1).

4.3.2.1. Requirements for Effective Contraception

Female participants

For the purpose of this trial, a woman is considered of childbearing potential following menarche and until becoming post-menopausal unless permanently sterile (e.g., by hysterectomy, bilateral salpingectomy, or bilateral oophorectomy). A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

Women of childbearing potential are eligible to participate if they are not pregnant or breastfeeding and agree to use a highly effective contraceptive method during the trial period and for at least 3 months after the administration of the study drug.

Highly effective contraceptive methods are methods with a failure rate of <1% per year when used consistently and correctly. Acceptable highly effective contraceptive methods for the purpose of this trial are:

- Combined (estrogen- and progestogen-containing) hormonal contraception methods associated with inhibition of ovulation (oral, intravaginal, or transdermal application)
- Progestogen-only hormonal contraception that prevent ovulation (oral, injectable, or implantable application)
    - Note that progestogen-only hormonal contraception where inihibition is ovulation is not the primary mode of action is not considered as highly effective
- Intrauterine devices (IUDs)
- Intrauterine hormone-releasing systems (IUSs)

- Bilateral tubal occlusion
- Vasectomized partner (if partner is sole sexual partner and has received medical assessment of surgical success)
- Abstinence from heterosexual intercourse if this is the preferred and usual lifestyle of the subject

Male participants

Male participants who engage in heterosexual intercourse with women of childbearing potential must be willing to use barrier contraception (i.e., condom) from the start of study drug administration until 3 months after trial drug administration, and ensure their partner is using an additional highly effective method of contraception (listed above) until at least 3 months after study drug administration. Men with pregnant partners should use condoms during the pregnancy of the partner.

5. Methods and Procedures

5.1. Trial Visit Overview

A summary overview of study visits and corresponding procedures is provided in the Visit Schedule (**Appendix II**).

5.2. Assessments and Sample Collection

5.2.1. Safety Assessments

Adverse Events

Adverse Events will be assessed by open questions or upon spontaneous reporting by the trial participants. All adverse events will be followed to resolution or until completion for the trial of the subject concerned. Serious Adverse Events (SAEs) will be collected during the entire study period for each individual trial subject until completion of the trial of the subject concerned (see also section 9.3). In the interest of participants' safety, follow-up for up to 30 days after the individual participants's study termination is required for SAEs that are not sufficiently resolved at the participant's final trial visit. The CTCAE (Common Terminology Criteria for Adverse Events) grading table (v5.0) will be used by trial investigators to report and grade Adverse Events. The development of Adverse Events will be followed until the day 90 visit (i.e., until the end of the trial).

Adverse Events of Special Interest (AESI)

Infusion-related reactions presenting as hypersensitivity/allergic reaction, anaphylaxis, or cytokine release syndrome as well as inhalation-related reactions presenting as allergic reaction (e.g., bronchospasm that may manifest with wheezing and/or respiratory distress) are designated as Adverse Events of Special Interest (AESIs).

Infusion of biological products including monoclonal antibodies may be associated with the development of infusion-related reactions that can occur during and/or within hours of the infusion. Symptoms of infusion-related reactions may be related to immediate hypersensivity (allergic reaction, anaphylaxis) or cytokine release syndrome. Symptoms of cytokine release syndrome may include, but are not limited to, fever, tachypnea, headache, tachycardia, hypotension, all types of rashes including flushing, and/or hypoxia.

Inhalation of pharmaceutical agents may result in airway irritation and/or hypersensitivity that can result in bronchospasm manifesting with wheezing, prolonged expiration and respiratory distress.

The presence or absence of infusion-related reactions presenting as hypersensitivity/allergic reaction, anaphylaxis, or cytokine release syndrome, as well the presence or absence of inhalated-related reactions presenting as allergic reaction (e.g., bronchospasm) will be solicited and documented until the study visit seven days after study drug administration.

Adverse Events of Special Interest should be communicated to the Sponsor in an expedited manner (see section 9.3.1 for requirements).

Dose-Limiting Toxicities

During the dose-escalation phase of the trial (**Groups 1A-1C**, **Groups 2A-2C**), grade 3 or higher Adverse Events that are deemed to be related to DZIF-10c by the Investigator are considered as Dose-Limiting Toxicities (DLTs). DLTs should be reported to the Sponsor in an expedited manner (see section 9.3.1 for requirements).

Routine Laboratory Parameters

Routine laboratory parameters from blood and urine samples will be assessed as indicated in the Visit Schedule (see **Appendix II**). Laboratory parameters will include a complete blood count with white blood cell differential, clinical chemistry (Na, K, Cl, Ca, AST, ALT, AP, total bilirubin, creatinine including eGFR, CRP, lipase), coagulation parameters (INR, PTT), urinalysis, and pregnancy testing in women of childbearing potential.

In female participants of childbearing age, the negative result of a serum beta-HCG test or a negative urine dipstick pregnancy test from a sample obtained on the screening day must be available prior to administration of trial medication.

In the event of abnormal laboratory results, participants may be asked to have additional samples collected at the discretion of the investigator or designee.

Abnormal laboratory parameters determined to be clinically significant by the investigator or designee will be graded according to the CTCAE (Common Terminology Criteria for Adverse Events) grading table (v5.0) and reported as Adverse Events.

Physical Examination and Vital Signs

A general physical examination including weight, height, and examination of skin, respiratory, cardiovascular, nervous, and gastrointestinal systems will be performed at screening. Symptom-directed physical examinations will be performed at later visits as indicated and include vital signs and any further examination indicated by history or observation.

Vital signs to be determined are systolic and diastolic blood pressure, heart rate, respiratory rate, body temperature, and peripheral oxygen saturation.

Pulmonary function testing

Pulmonary function testing will be performed by spirometry using a hand-held spirometer at screening, prior to the inhalation, and at pre-specified timepoints after the inhalation (see **Appendix II**). FEV1 (forced expiratory pressure in one second), FVC (forced vital capacity), FEV1/FVC ratio, VC (vital capacity), IVC (inspiratory vital capacity), PEF (peak expiratory flow), and PIF (peak inspiratory flow) will be recorded.

ECG

A 12-lead ECG will be performed at screening and assessed by the Investigators.

5.2.2. Pharmacokinetic Assessments

Non-compartmental analysis methods will be used to calculate pharmacokinetic parameters, and descriptive results will be presented for each dose group. Parameters to be assessed will include

- $AUC_{0\text{-}672}$ (the area under the concentration-time curve of DZIF-10c in serum over the time interval from 0 to 672 hours (Day 28)) (secondary endpoint)
- $AUC_{0\text{-}504}$ (the area under the concentration-time curve of DZIF-10c in serum over the time interval from 0 to 504 hours) in healthy volunteers
- $AUC_{0\text{-}inf}$ (the area under the concentration-time curve of DZIF-10c in serum over the time interval from 0 extrapolated to infinity)
- C_{max} (maximum measured concentration of DZIF-10c in serum)
- t_{max} (time from dosing to C_{max} of DZIF-10c in serum)
- $t_{1/2}$ (the terminal elimination half-life of DZIF-10c in serum)

- CL/F (apparent clearance after extravascular administration) for individuals receiving DZIF-10c by inhalation only
- Vz/F (apparent volume of distribution during the terminal phase after extravascular administration) for individuals receiving DZIF-10c by inhalation only

Further PK parameters may be calculated, if considered reasonable.

Data may be used to develop exploratory pharmacokinetic/pharmacodynamic models using non-linear mixed effects techniques, if feasible. Modeling activities will be planned and documented separately. Serum levels of DZIF-10c will be determined using a validated immunoassay [redacted].

Exploratory assays for PK analyses under development include serum/plasma neutralization assays against pseudoviruses and authentic SARS-CoV-2. These assays will be performed at the Institutes of Virology at the University Hospital Cologne and/or at the University of Marburg.

5.2.3. Assessment of Viral Shedding

SARS-CoV-2 RNA assessment

SARS-CoV-2 RNA levels will be determined from frozen (-80°C) transport medium using a clinically validated quantitative reverse-transcripase real-time PCR assay (qRT-PCR) assay. Results will be provided as Ct (Cycle threshold) values as well as quantitatively (copies/ml) based on a reference standard. These assays will be performed at the Institute of Virology at the University Hospital Cologne.

At the screening visit, a single naso- and/or oropharyngeal swab is sufficient to document the absence or presence of SARS-CoV-2 infection by qRT-PCR. This assay is performed at the trial site's local laboratory. In any case, in SARS-CoV-2-infected individuals, both nasopharyngeal and oropharyngeal swabs must be obtained for the central laboratory prior to study drug administration.

To provide participants with early results about the presence or absence of SARS-CoV-2 in swab samples prior to the determination of results at the central laboratory, an additional oropharyngeal swab may be obtained and sent for NAAT (e.g., qRT-PCR) at the local routine laboratory. This optional additional swab is to be obtained after the oropharyngeal swab for central laboratory evaluation.

Assessment of the presence of infectious SARS-CoV-2

Viral transport medium will be incubated on VeroE6 cells to determine the presence of infectious virions. Cell culture supernatants will be assessed for virus production by qRT-PCR and/or cells will be visually inspected for the development of cytopathic effects. These assays will be performed at the Institutes of Virology at the University Hospital Cologne and/or the University of Marburg.

Assessment of viral replication by subgenomic RNA

Levels of subgenomic mRNA that is transcribed in infected cells but not packaged into virions will be determined from RNA extracted from swab samples by qRT-PCR. These assays will be performed at the Institutes of Virology at the University Hospital Cologne and/or the University of Marburg.

5.2.4. Assessment of Anti-Drug Antibodies

Serum samples will be analyzed for reactivity against DZIF-10c by a validated immunoassay. [redacted]

5.2.5. Assessment of the Development of Viral Mutations and Antibody Resistance

Viral RNA will be extracted from transport medium and cDNA will be generated by reverse transcription. Spike sequences will be amplified from cDNA using polymerase chain reaction (PCR) and purified PCR products will be sequenced by Sanger sequencing or next-generation sequencing. Sequences from samples obtained prior to trial drug administration will be compared to sequences obtained after trial drug administration.

Selected recurrent or individual mutations observed in the SARS-CoV-2 *S* gene will be introduced into plasmids encoding the SARS-CoV-2 *S* gene by site-directed mutagenesis. Modified plasmids will be used to generate replication-deficient SARS-CoV-2 pseudoviruses and the DZIF-10c sensitivity of unmodified and modified variants will be compared to evaluate the effect of individual spike mutations on antibody sensitivity.

These assays will be performed at the Institutes of Virology at the University Hospital Cologne and/or the University of Marburg.

5.2.6. Assessment of COVID-19-related Hospitalizations and Medically-Attended Health Care Contacts

Any hospitalization and medically-attended health care contact (i.e., health care contacts that were not routine/scheduled visits; including, but not limited to, emergency room visits, unscheduled visits to primary care physicians) reported by participants during the trial duration will be assessed by the investigators based on the available medical information on its relatedness to COVID-19 and conditions associated with SARS-CoV-2-infection.

5.2.7. Assessment of Time to Symptom Resolution

Participants will be asked to self-record selected SARS-CoV-2 symptoms (cough, feverishness, body ache, headache, sore throat, shortness of breath, changes in/loss of taste of taste and smell) from the day of study drug administration through the day 28 visit and grade them as absent, mild, moderate, or severe using a participant diary (**Groups 2A-2D**).

5.2.8. Assessment of Immunological Parameters

SARS-CoV-2-reactive T cell responses will be evaluated in T cells purified from peripheral blood mononuclear cells by multiparametric cytokine flow cytometry and the expression of immune activation and exhaustion markers. These assays will be performed at the Institute of Virology at the University Hospital Cologne and/or collaborating Institutes.

5.2.9. Sample Collection, Storage, and Shipment

Blood samples will be obtained by venipuncture.

Nasopharyngeal swabs will be obtained by inserting a swab in one nostril along the nasal septum to the nasopharynx until resistance is felt, reaching a depth that is proximately the length from the outer nostril opening to the ear. Once resistance is felt, the swab will be left in place for a few seconds to allow for absorption of secretions and is slowly removed while rotating.

Oropharyngeal swabs will be obtained by swabbing the posterior pharynx and tonsillar areas. Care should be taken to avoid accidental swabbing of the gums, tongue, or teeth.

All biospecimens will be handled according to Standard Operating Procedures (SOPs) defined in a Lab Manual that will be provided to all trial sites. Blood (serum, plasma, PBMCs) and respiratory swab samples will be processed at the trial site, aliquoted as needed, and stored

as indicated in the lab manual. Long-term storage of samples will be located at the Institute of Virology at the University Hospital Cologne.

5.3. Recruitment

SARS-CoV-2-uninfected participants will be recruited through advertisements and the CTCC volunteer database. SARS-CoV-2-infected individuals will additionally be recruited by referrals of primary care physicians or following a patient contact by the diagnosing health care institution or health care authorities to determine potential interest in trial participation.

5.4. Screening Procedures

Pre-Screening

Healthy volunteers and SARS-CoV-2-infected individuals may undergo a pre-screening procedure conducted by phone or video call to inform them about the aims and nature of the trial, potential risks, and the trial schedule. In addition, potential participants will be provided with the opportunity to discuss all questions and concerns they may have about the trial. Moreover, study recruiters will preliminarily assess study eligibility based on the previous medical history as well as the trial inclusion and exclusion criteria.

Screening Visit

At the screening visit, the Principal Investigator or subinvestigator will explain and discuss the aims and nature of the trial, potential risks, and the trial schedule with potential participants who will be provided the opportunity to address all questions and concerns they may have with study staff, relatives, and friends. Prior to the initiation of any study-related procedures, written informed consent will obtained from each participant.

If the potential participant provides informed consent to participate in this trial, study staff will

- Obtain a complete medical history including the use of concomitant medication (including medication within the last 4 weeks prior to screening)
- Perform a general physical exam, including height, weight, vital signs (heart rate, blood pressure, body temperature, respiratory rate, and pulse oximetry), and examination of the skin and respiratory, cardiovascular, abdominal, and neurological systems
- Provide pregnancy counseling
- Determine the clinical score based on the WHO Clinical Progression Scale (**Groups 2A-2D**)

- Perform a pulmonary function test (**Groups 1A-1C**)
- Collect blood, urine, and swab samples for all tests indicated in the Visit Schedule (**Appendix II**)
    - At the screening visit, a single nasopharyngeal and/or oropharyngeal swab may be obtained to document absence or presence of SARS-CoV-2 infection. This swab is to be analyzed at the trial site's routine laboratory. An available SARS-CoV-2-RNA NAAT result documented in writing from a sample obtained within three days prior to trial drug administration is considered as an acceptable alternative. In all cases, nasopharyngeal and oropharyngeal swabs must be obtained at baseline prior to study drug administration in SARS-CoV-2-infected individuals.

Screening and Trial Drug Administration Visit Procedures may occur on the same day during the expansion phase of the trial (**Group 2D**).

Screening Failures

Participants who have provided informed consent to participate in the clinical trial but do not receive any study drug administration are defined as Screening Failures. Information on demography, details on the cause for the screen failure and inclusion/exclusion criteria, as well as any Adverse Events should be recorded to facilitate transparent reporting. Individuals with a Screening Failure may not be rescreened unless for Screening Failures due to scheduling issues in healthy volunteers (**Groups 1A-1C**). In case of Screening Failures due to abnormalities in blood laboratory parameters (hematology and/or clinical chemistry), the deviating parameter may be retested once during the Screening window.

5.5. Trial Drug Adminstration Visit

Prior to the study drug administration, trial staff will

- Review the informed consent form
- Discuss the planned procedure and any potential questions with the participant
- Review the interim medical history including concomitant medication
- Review screening laboratory results including safety laboratory parameters
- Review eligibility criteria
- Perform a symptom-directed physical exam
- Perform procedures and obtain samples as outlined in the Visit Schedule (**Appendix II**)
- Obtain a baseline PK serum sample
- Perform baseline assessments of vital signs
- Perform the randomization procedure (**Group 2D** only)

- Perform a pulmonary function test (**Groups 1A-1C** only)

If the screening visit of healthy volunteers (**Groups 1A-1C**) occurred >3 calendar days prior to the start of the antibody administration, the negative NAAT result of an additional naso- or oropharyngeal swab obtained within (inclusive) 3 calendar days of the antibody inhalation must be available prior to the start of the drug administration (e.g., rapid PCR from swab obtained on the day of inhalation).

DZIF-10c or placebo will be prepared by the local pharmacy and administered as described in Section 5.9.

Sequence of Trial Drug Administration in Group 2D

In **Group 2D** participants will first receive the trial drug by inhalation, followed by the infusion after a waiting period of at least 15 min after the end of the inhalation.

Participants in **Groups 1A-1C** and **2A-2C** will be admitted as inpatients on the day of DZIF-10c inhalation and will be hospitalized overnight to allow for immediate access to medical interventions in case of unexpected emergencies. For participants in **Group 2D**, the study drug may be administered on an inpatient or outpatient basis at the discretion of the investigator.

Observation Requirements

Dose Escalation Phase **Groups 1A-1C** and **2A-2C** *- Phase I Component -*	**Expansion Cohort** **Group 2D** *- Phase IIa Component -*
• Inpatient admission (overnight) on Intermediate Care Unit (or ward with equivalent monitoring and observation capacity, e.g., phase I unit)	• Outpatient ward or inpatient admission
• Immediate observation by trial staff during and until at least 15 min after trial drug administration; nursing staff on the ward at all times; dedicated physician available (24/7 availability on premises or ward)	• Immediate observation by trial staff for at least 10 min after the start of the inhalation and infusion; continuous presence by trial staff on the ward during the treatment and observation period; physician on the ward during treatment and available on premises during observation period

• Immediate access to emergency medication (e.g., antihistamines, glucocorticoids, epinephrine, bronchodilators) and emergency ventilation equipment	
• Resuscitation ICU team availability at all times	
• Intensive monitoring with continuous ECG, automated blood pressure assessments (at least every 30 minutes), and pulse oximetry during and for at least 4 hours after the end of the infusion. Continuous ECG monitoring and pulse oximetry will be continued for at least 12 hours after the end of the infusion and until at least 6 am. Continuous monitoring may be intermittently interrupted for mobilization on the ward.	• ECG, blood pressure cuffs, pulse oximetry readily available on demand.
• Assessment of vital signs by trial staff at the end of the inhalation, and 1 h (+/- 10 min) and 4 h (+/- 15 min) after the end of the inhalation	• Assessment of vital signs by trial staff at the end of the inhalation, 15 min (+/- 5 min) after infusion start, at the end of the infusion, and 1 h (+/- 10 min) and 2 h (+/- 10 min) after the end of the infusion
• Discharge assessment by trial physician after day 1 visit procedures	• Assessment of discharge qualification by trial physician at the end of the observation period (at least 2 h after the end of the infusion)

Should inhalation-related adverse events (e.g., bronchospasm) or infusion-related adverse events (e.g., hypersensitivity, anaphylaxis, or cytokine release syndrome) occur, it would typically be expected that such events develop within the first hour of the antibody administration as a consequence of antibody or excipient exposure and/or high serum antibody concentrations.

To enable intensive monitoring in the lead-in phase of the trial, participants in the dose escalation cohort (**Groups 1A-1C** and **Group 2A-2C**) will be admitted to an Intermediate Care Unit or ward with equivalent monitoring and observational capacity (e.g., phase I unit, ICU). Participants will be intensively monitored by continuous ECG, automated blood pressure cuffs, and pulse oximetry during the inhalation and for at least 4 hours after the end of the inhalation, followed by at least ECG monitoring and pulse oximetry for at least 12 hours after the end of

the inhalation and overnight (until at least 6 am). Prior to discharge, participants will be assessed by a trial physician.

Participants in the expansion cohort **Group 2D** will be clinically observed for at least two hours after the end of the trial drug administration and may be discharged only after a final assessment by a trial physician.

The presence or absence of infusion-related reactions and inhalation-related reactions will be assessed and documented for all participants 1 h (+/- 10 min) after the end of the inhalation (**Groups 1A-1C** and **2A-2C**) or infusion (**Group 2D**).

Samples for PK analysis will be obtained prior to trial drug administration.

In addition, in **Groups 1A-1C** and **2A-2C**, an additional sample will be obtained 4 h (+/- 15 min) after the end of the inhalation.

In participants in **Group 2D**, an additional sample will be obtained 1 h (+/- 10 min) after the end of the infusion.

5.6. Follow-up Visits

Participants will be followed for a total of 90 days (+/- 7 days) after trial drug administration.

At these follow-up visits, trial staff will

- Review the interim medical history including concomitant medication
- Review safety laboratory results
- Perform symptom-directed physical exams
- Perform procedures and obtain samples as outlined in the Visit Schedule (**Appendix II**)

For SARS-CoV-2-infected indivduals, trial visits may be conducted at home during the period of quarantine. Trial visits on days 5 and days 10 are considered optional for SARS-CoV-2-infected individuals in quarantine.

In healthy volunteers (**Groups 1A-1C**), an optional visit at day 5 may be included to facilitate denser PK sampling.

Should trial participants become hospitalized during the follow-up phase, the visit schedule should be followed as possible.

5.7. Withdrawal of Trial Participants

Participants may be withdrawn from the trial for any of the following reasons:

- Any participant may withdraw from the trial at any time and for any reason if he or she wishes to do so
- At the discretion of an investigator or designee following an Adverse Event
- At the discretion of an investigator or designee if the participant is judged to be at risk of failing to comply with the study protocol which may result in harm or interfere with the validity of the study results
- At the discretion of the primary care provider if he or she thinks that further participation is no longer in the best interest of the participant

5.7.1. Follow-Up after Withdrawal

In case of an early termination of a trial participant, the date and reason for early termination should be collected and reported to the Principal Investigator who will provide this information to the Sponsor and to the SMC. At the time of withdrawal and if the participant agrees, all procedures specified in the Final Study Visit according to the corresponding Visit Schedule (**Appendix II**) will be performed.

Any Adverse Event resulting in withdrawal of a participant will be followed up until resolution or until the adverse event is judged by the principal investigator or designee to have stabilized where possible.

Should a study participant become pregnant during the conduct of the clinical trial, separate informed consent for follow-up until delivery and for assessment of the newborn's health status or a report by the responsible pediatrician will be sought.

Participants who are withdrawn from the trial after DZIF-10c administration will not be replaced, except for participants during the dose-escalation phase of the trial that are not followed until at least the day 7 visit after inhalation for reasons not related to safety events.

5.8. Closure of Trial Sites/Premature Termination of the Clinical Trial

5.8.1. Closure of Trial Sites

At any time, the study can be terminated at an individual trial site if:

- The trial site cannot comply with the requirements of the protocol.
- The trial site cannot comply with GCP standards.

- The trial sites first patient is not recruited within 6 weeks after initiation of the trial site.

Should the study be prematurely terminated, all study materials (IMPs, etc.) must be returned to the Sponsor. After consultation with the Sponsor, IMP may be also destroyed at the site according to the local procedures.

5.8.2. Premature Termination of the Clinical Trial

The sponsor has the right to terminate the trial prematurely if there are any relevant medical or ethical concerns, or if completing the trial is no longer practicable. If such action is taken, the reasons for terminating the trial must be documented in detail. All trial subjects still under treatment at the time of termination shall undergo a final examination which must be documented.

Premature termination of the trial will be considered if:

- The risk-benefit balance for the trial subject changes markedly
- It is no longer ethical to continue treatment with the IMP
- The sponsor considers that the trial must be discontinued for safety reasons (e.g., on the advice of the SMC)
- It is no longer practicable to complete the trial

A decision on premature termination by the Sponsor will be made in consultation with the Principal Coordinating Investigator, the trial statistician, and the SMC.

5.9. Treatment

5.9.1. Administration

Inhalation

DZIF-10c or placebo will be administered as a single inhalation through a mouthpiece (Aerogen Ultra) using a mesh nebulizer (Aerogen Solo) to generate an aerosol. The inhalation procedure will require approximately 15-20 minutes.

Infusion

DZIF-10c or placebo for intravenous infusion will be administered using a peripherally inserted catheter, preferably into an upper extremity vein, using a 0.2 µm in-line filter and polyurethane-based infusion system. In individuals receiving a DZIF-10c dose ≤5 g, a volume of 100 ml will be administered within 60 min (+/- 10 min). In individuals receiving a higher dose, the infusion volume will be increased as required to administer the calculated dose of DZIF-10c without further dilution; in these cases, the duration of the infusion will be extended accordingly to maintain a flow rate of 1.67 ml/min. Polypropylene-coated infusion bags containing the appropriate quantitify of diluted or undiluted DZIF-10c or placebo will be provided by the local study pharmacy after preparation according to the handling instructions. At the end of the infusion, the infusion system will be flushed using at least 25 ml of sterile normal saline at the infusion flow rate. The dose of DZIF-10c or the amount of placebo will be body weight-adjusted.

Sequence and timing of trial drug administration in individuals receiving study drug both by infusion as well as by inhalation (**Group 2D**) are described in Section **5.5**.

5.9.2. Description of the Investigational Medicinal Product and Placebo

DZIF-10c is a human monoclonal antibody of the IgG1 kappa isotype (see section 1.4). [redacted]

The solvent for dilution of DZIF-10c will also be used a placebo for the inhaled application. Sterile normal saline (NaCl 0.9%) procured from a commercial source and approved for clinical application will be used as placebo for the intravenous infusion.

5.9.2.1. Manufacture of the Investigational Medicinal Product

[redacted]

[redacted]

5.9.2.2. Labelling of Investigational Medicinal Product

DZIF-10c stock vials and vials with solvent for dilution (diluent) will be labelled by Boehringer Ingelheim. For potential shelf-life extensions after shipment of DZIF-10c and diluent to the trial sites, labels for re-labelling at the trial sites will be provided by Boehringer Ingelheim.

Bags of DZIF-10c or normal sterile saline prepared for infusion as well as inhalation containers used for inhalation will be labelled by the local pharmacies according to national requirements.

5.9.2.3. Shipment and Storage of Investigational Medicinal Product

DZIF-10c stock vials and diluent will be transported to the trial sites in temperature-controlled conditions using a temperature logger following a qualified order by the sponsor. Study pharmacies will acknowledge receipt of DZIF-10c and diluent, and document integrity of the shipment and the logged temperature in writing to be stored in the Pharmacy Trial File. DZIF-10c stock vials and diluent will be stored at the local pharmacies at the recommended storage temperature of 5±3°C. In case of temperature excursions during shipment or storage, the product shall not be administered, and the sponsor is to be informed about the temperature deviation without undue delay.

5.9.3. Dispensing and Return of Investigational Medicinal Product

During the open-label dose escalation phase, DZIF-10c for administration will be ordered based on the individual participant's group assignment using local standard procedures.

DZIF-10c or placebo for inhalation will be prepared by the unblinded local study pharmacists in syringes for transfer into the nebulizer container at the site of administration, or directly applied to the nebulizer container. Based on the participant's groups assignment, the appropriate dose of DZIF-10c and/or dilutent will be combined to provide a final volume of 5 mL for inhalation in all dose groups. In the blinded study phase (**Group 2D**), DZIF-10c or placebo will be provided to the blinded clinical team in masked syringes for transfer to masked nebulizer containers or in masked nebulizer containers.

DZIF-10c or placebo for infusion will also be prepared by the unblinded local study pharmacist. For participants assigned to receive DZIF-10c, the appropriate volume of DZIF-10c stock solution based on the individual participant's body weight will be diluted in diluent to or provided undiluted, as appropriate. For participants assigned to receive placebo, an infusion bag containing normal sterile saline at the nominal volume that would be provided for weight-adjusted DZIF-10c will be prepared. DZIF-10c or placebo will be provided to the blinded study team by the local study pharmacy in infusion bags of identical visual appearance.

Following completion of the inhalation and/or infusion, the used nebulizer containers and/or infusion bags will be discarded as per local standard procedures.

Product Accountability and Product Dispensions Forms will be maintained by the local study pharmacy and stored in the Pharmacy Trial File. Following completion or termination of the clinical trial, unused material of DZIF-10c will be destroyed as per local guidelines after consultation with the Sponsor. Destruction of study material will be documented in writing.

5.9.4. Assignment of Trial Participants to Treatment Groups

In the expansion cohort (**Group 2D**) participants are randomized 1:1:1 (placebo only:DZIF-10c inhalation and placebo i.v.:DZIF-10c inhalation and DZIF-10c i.v.) into the trial groups without stratification. Randomization will be implemented by a 24/7-Internet service (ALEA 17.1, FormsVision BV, Abcoude, NL) and prepared centrally by the Institute of Medical Statistics and Computational Biology (IMSB) at the University of Cologne.

5.9.5. Selection of Dosage of DZIF-10c

Inhalation

Repeated exposure to inhaled antibodies and antibody-derived fragments has been determind to be overall safe and well tolerated (see section 1.4). For example, inhalation of the antibody-derived fragment ALX-0171 at doses up to 9 mg/kg in children <24 months hospitalized with acute severe lower respiratory tract RSV infection was not associated with treatment-emerging (serious) adverse events that were considered to be related to the study drug[57]. In addition to antibody doses previously administered by inhalation, the target dose of 250 mg is also based on technical considerations [redacted], results of preclinical animal studies [redacted], and viral dynamical models. Moreover, the target dose of 250 mg was selected to take potential differences in lower respiratory tract deposition between individiuals (e.g., due to different inhalation techniques) into account. Finally, average IgG concentrations in epithelial lining fluid

in humans have been reported to range from 1-2 mg/mL[66]. Assuming an overall ELF volume of 20-40 mL[67] and taking limited deposition of inhaled antibody in the lower respiratory drug into account, the starting dose of 50 mg is expected to result in an ELF concentration that is lower than the total IgG concentration in the epithelial lining fluid.

Infusion

Combining a single inhaled dose with a single intravenous dose of DZIF-10C could immediately provide high levels of antibody, which may be superior when there is need for rapid anvitial efficacy while maintaining systemic and long-term acitivity. The target antibody concentration for the intravenous administration of DZIF-10c is based on viral dynamic modelling calculations suggesting that a 40 mg/kg dose provides adequate coverage for SARS-CoV-2 neutralization. Moreover, similar doses of human monoclonal antibodies have previously been shown to be well-tolerated and effective against other viral pathogens in clinical trials, including HIV-1 and Ebola virus, as well against SARS-CoV-2 (see sections 1.2 and 1.3). The final dose selection will be informed by a separate clinical trial on the intravenous infusion of DZIF-10c (Uni-Koeln-4288). A recommendation on the intravenous dose of DZIF-10c for the combined inhalation and infusion will be made by the Safety Monitoring Committee.

5.9.6. Blinding

During the open-label dose-escalation phase of this clinical trial (**Groups 1A-1C** and **2A-2C**), study participants as well as the study team will be aware of group and dose assignments.

During the expansion cohort of SARS-CoV-2-infected individuals (**Group 2D**), participants and study investigators will be blinded to the treatment assignment (DZIF-10c or placebo). Infusions/Inhalations will be prepared by the unblinded local pharmacist who will provide the infusion bags in a blinded manner (i.e., labeled in a way that does not reveal the content of the infusion), and the nebulizer solution in masked nebulizer containers or masked syringes for transfer to masked nebulizer containers. Study pharmacists will be informed about the participants' group assignments through the randomization software. Blinding for participants will be maintained until the end of the trial, while the sponsor may be unblinded after the visit scheduled for day 7 after the final study drug administration of the trial.

5.9.6.1. Unblinding

Emergency unblinding of the treatment assignment for a patient may be necessary due to a medical emergency, a serious adverse event (SAE) that is unexpected and for which a causal

relationship to IMP cannot be ruled out, or any other significant medical event (e.g., pregnancy).

If unblinding is required for a medical emergency:

- Only the investigator will make the decision to unblind the treatment assignment.
- Only the affected patient will be unblinded.
- An emergency envelope will provide the treatment assignment to the investigator.
  The investigator will notify the sponsor and/or designee immediately that the patient has been unblinded.

Emergency unblinding will be done by sealed unblinding envelopes. The envelopes will be prepared centrally by IMSB and sealed by the study pharmacists. Study pharmacists receive a request for a specific patient ID and randomise the patient using his or her patient ID via ALEA 17.1. The study pharmacists then write the patient ID on a prepared letter that contains the randomized treatment for this patient and seal the letter so that the patient ID can be seen in the window of the envelope.

Data will be unblinded after the last participant has completed the visit planned for day 7 after trial drug administration for a first data analysis of the expansion cohort (**Group 2D**). Unblinding is done by the IMSB. Results are not communicated to participants during the trial and are used to inform the further development of DZIF-10c.

5.9.7. Previous and Concomitant Medication

Following trial drug administration, the standard of care may be provided as required by the medical condition and deemed appropriate by health care providers.

5.9.8. Rescue Therapy for Emergencies

All trial sites are embedded into a maximum-care hospital facility capable of providing immediate emergency therapy including specialized medical intensive care.

No specific rescue therapy for DZIF-10c is available. In case of an acute infusion reaction, the infusion will be interrupted and not be re-initiated. For infusion reactions requiring medical intervention, rescue medication including, but not limited to, acetaminophen/paracetamol, antihistamines, glucocorticoids, and IV fluids must be available on-site. In case of bronchial hyperresponsiveness (e.g., bronchospasm), bronchodilators and antiinflammatory medication may be provided by inhalation or intravenously as deemed clinically appropriate.

5.9.9. Continuation of Treatment after the End of the Clinical Trial

Study participants will receive no additional administrations of DZIF-10c beyond the planned administration as per group assignment.

6. Data Quality Assurance

6.1. Monitoring and Audits

6.1.1. Monitoring

Regularly scheduled monitoring will be performed by the Clinical Trials Center Cologne (CTCC) Monitoring team at all trial sites. This monitoring process aims to ensure the study participants' safety, that data is collected accurately and completely, that the clinical trial is conducted according to the trial protocol, and that all principles of GCP and applicable guidance are followed.

A monitoring plan will define the specific extent of the monitoring procedures for all sites. All Principal Investigators will agree to regularly performed monitoring and provide appropriate support to the delegate of the CTCC Monitoring team to fully conduct the monitoring procedures and ensure access to all relevant trial-related documentation including source documents. Monitoring procedures will additionally include drug accountability checks at the local study pharmacies to ensure accurate documentation and handling of study drug, as well as monitoring of local study research laboratories to ensure proper sample handling and storage.

Each monitoring visit will be followed by a written visit report summarizing the visit and any potential findings.

6.1.2. Audits / Inspections

For purposes of quality assurance, the sponsor or a delegate assigned by the sponsor will have the right to audit trial sites and other institutions involved in this clinical trial. The objectives of such audits include the ensurance of trial participants' safety, and the verification of data accuracy, validity, and completeness. To perform these tasks, auditors will receive access to all relevant trial documentation including source documentation, drug accountability documentation, and any trial-related correspondence.

Inspections may be performed by competent authorities as indicated by applicable law and guidelines.

The sponsor and all trial sites will commit to support auditors and inspectors of competent authorities at all times, and to provide all necessary original documentation.

6.2. Documentation

Principal Investigators will oversee and coordinate data collection, entry, and protection. Trial-specific data will be collected by the clinical trial staff using designated source documents. Standard GCP practices will be followed to ensure accurate, reliable and consistent data collection. Any correction to source documentation needs to ensure a valid correction trail, including leaving original entries legible and signed or initialed dated corrections.

All trial data must be verifiable to the source documentation. All source documents will be kept in a locked facility at the clinical site. Source documents include but are not limited to

- Informed Consent Forms
- Reported laboratory results
- Lists of adverse events
- Lists of concomitant medication
- Documentations of existing conditions.

Medical records will be archived by the Medical Record Department of the hospital.
All data relevant to the trial are documented by the Principal Investigators (PI) or designees in corresponding electronic case report forms (eCRFs). All eCRFs and laboratory reports will be reviewed by the clinical team to ensure that they are accurate and complete.
The Principal Investigators may authorize trial staff members to sign the eCRFs to confirm accuracy of the data. At the completion of the follow-up visits, final sign-off must be performed by the Principal Investigators.

6.2.1. Data Management

The IT infrastructure and dedicated staff will be provided by the Clinical Trials Center Cologne (CTCC, ZKS Köln). A trial-specific database will be developed and validated prior to data entry based on standard operating procedures established at the CTCC. The data management system is based on commercial, validated trial software.
All changes to data stored within the trial database will be documented in an audit trail. The trial software has a user and role concept that can be adjusted on a trial-specific basis. Access to the trial database is restricted, and the database is embedded into an IT infrastructure with a dedicated safety concept including a firewall and backup systems. Data within the trial database are backed up daily.

Data will be entered online at the local trial sites via the internet. During data entry, automated plausibility checks will be performed, thereby detecting many discrepancies immediately. CTCC staff will conduct additional checks for data completeness and plausibility and clarify any questions with the local trial staff using an electronic query system of the trial software.

These electronic queries have to be answered by the trial site without unreasonable delay. Further details will be specified in the data management plan.

eCRF Completion Requirements will be provided to the trial sites for detailed descriptions of the respective procedures.

After completion or termination of the clinical trial and data cleaning, the database will be locked and the data exported for statistical analysis.

6.2.2. Archiving

All essential trial documents including investigator and sponsor parts of the TMF will be archived for 15 years according to ICH-GCP (Chapter 8).

The trial master file, the eCRFs, the code envelopes and other material supplied for the performance will be archived according to applicable regulations and laws.

7. Ethical and Regulatory Aspects

7.1. Ethics Committee

A favorable opinion by the competent ethics committee will be obtained before initiating any trial procedures.

7.2. Ethical and Legal considerations

This trial will be conducted in agreement with the principles of the Declaration of Helsinki as well as the Good Clinical Practice guidelines by the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH-GCP), and all applicable law.

7.3. Registration and Notification of Authorities

This clinical trial has been registered at the EU Clinical Trials Register (EudraCT: 2020-004448-27) and at ClinicalTrials.gov (NCT04631705).

Prior to study initiation, approval will be obtained from the federal competent authority (Paul-Ehrlich-Institute), and state and local authorities will be notified as per applicable regulations.

7.4. Obtaining Informed Consent from Trial Participants

Prior to the initiation of any clinical trial-related procedures, potential trial participants will be provided with a copy of the most recent informed consent form approved by the local ethics committee to read. In addition, the Principal Investigator or a Subinvestigator will discuss the specifics of the clinical trial with the potential participants verbally. This discussion will include but not be limited to the purpose for conducting the clinical trial, the procedures to be conducted, the time commitment required by the participant, the drug and placebo to be administered, potential benefits and risks of participation, alternative options for treatment, patient confidentiality, and potential access to data by representatives of the sponsor (e.g., monitors or auditors) and competent authorities. As part of this discussion, potential participants will be informed that participation in the clinical trial is completely voluntary, and that withdrawal of consent is possible at any time without the need to provide reasons and will not jeopardize the participant’s future medical treatment. The informed consent discussion will be led in a comprehensible manner using language that is readily understandable by the potential participant.

Potential participants will be given the opportunity to ask questions to the investigators and express potential concerns, and to discuss potential participation in the clinical trial with family, friends, and/or other health care providers.

Originals of the signed informed consent forms will be stored in the investigator site file, and trial participants will be provided with a copy of the participant information sheet and the signed informed consent form. Receipt of this copy will be confirmed by the participants by signature on the informed consent form.

7.5. Insurance of Trial Subjects

All study participants will be provided insurance in accordance with § 40 AMG. The insurer's name, contact details, and policy number will be provided in the participant's information sheet.

7.6. Data Protection

All investigational materials and data will be pseudonymized using unique alphanumerical codes for each trial participant, and all applicable provision of the data protection legislation will be followed. As part of the informed consent process, potential trial participants will be informed about the requirement to agree to handling of pseudonymized data in accordance with applicable legislation to be eligible to participate in the trial.

The sponsor (University of Cologne) is responsible for data processing (University of Cologne, Albertus-Magnus-Platz, 50923 Cologne, Data Protection Officer: Phone: [redacted], Fax: [redacted], E-Mail: [redacted]). In this trial, the sponsor is represented by [redacted]. Data processing will be carried out by the Clinical Trials Centre Cologne at the University of Cologne (CTCC, Gleueler Str. 269, 50935 Cologne).

8. Statistical Methods and Sample Size Considerations

8.1. Statistical and Analytical Plan

For each study phase (dose escalation and randomized) a first analysis is planned after the data from up to the day 7 visit after administration of the study drug is collected and data is cleaned. In the expansion cohort (**Group 2D**), data will be unblinded for this analysis. The final analysis will take place after the follow up for all subjects of both phases is completed. The final CTR will include all study phases.

In group 2D, no adjustments for dropouts are planned.

8.1.1. Analysis Populations

Dose-Escalation Phase (**Groups 1A-1C** and **Groups 2A-2C**)

Analyses are conducted “as treated”, which includes all participants who received any amount of the study drug.

Randomized Placebo-Controlled Phase (**Group 2D**)

The primary dataset for analysis is the safety population (as treated). This population includes all trial subjects who received any amount of the study drug.

The secondary dataset for analysis is a modified intention-to-treat (mITT) population. This dataset includes all randomized trial participants receiving study drug inhalation and infusion and who have at least a baseline (day 0) value and a second measurement of viral RNA shedding in nasopharyngeal swabs by qRT-PCR up to the day 7 visit. The evaluation is carried out strictly in accordance with the allocation by randomization.

Additional analysis populations can be defined in the SAP.

8.1.2. Description of Trial Participants Groups

Quantitative data will be summarized by mean ± standard deviation and percentiles (0, 25, 50, 75, 100). Qualitative data will be summarized by absolute and relative frequency. Confidence intervals can support interpretation, if sensible. Listings of the data will be given where appropriate. Data up to the day 7 visit after administration of the study drug and during follow up will be evaluated separately and in aggregate as appropriate.

Dose-Escalation Phase (**Groups 1A-1C** and **Group 2A-2C**)

Descriptions will be provided by DZIF-10c dose and infection status, as well as in aggregate. Listings of the data by participant will be provided for all safety data.

Randomized Placebo-Controlled Phase (**Group 2D**)

Descriptions will be provided by treatment group and in aggregate. Listings of the data by participant will be provided for all safety data.

Screening failures

Screening failures will be listed with available information on demography, the cause for the screen failure and Adverse Events.

8.1.3. Primary Outcome and Target Variables

Primary target variables are Adverse Events (including Serious Adverse Events and Adverse Events of Special Interest), as defined in chapter 5.2.1 and chapter 9. Evaluation will be performed in the safety population. Safety events will be listed by participant. Safety events are reported in total, by seriousness, relatedness, and severity.

Analysis is descriptive. Safety events will be evaluated as rate and frequency of participants who experienced the event and also as number of events.

Safety events will be evaluated in the time period until the day 7 visit after trial drug administration, after the day 7 visit until the end of study, and in aggregate. An exception are AESIs, which will be documented as AESIs until the day 7 visit only.

8.1.4. Secondary and Exploratory Outcomes and Target Variables

Analysis is descriptive in nature. Secondary and exploratory endpoints will be evaluated by dose or treatment group and visit. Endpoints will be evaluated mainly in the safety population (dose escalation phase) or in the mITT population (**Group 2D**). Figures may show development over time where sensible. Endpoints can additionally be analysed by suitable multivariable models, including covariates. Substitution of missing values is not planned; the main efficacy analysis of time weighted average change of viral RNA shedding is possible in the mITT without substitutions. Complete methods will be specified in the SAP.

Outcomes and Target Variables

Laboratory Parameters, Vital Signs, Symptoms

Changes in safety laboratory parameters, vital signs and symptoms will be summarized descriptively. Changes in laboratory parameters and vital signs will additionally be calculated relative to the results obtained at baseline (day 0 prior to study drug administration).

Pharmacokinetic Parameters

Pharmacokinetic parameters of DZIF-10c in antibody recipients will be calculated using standard non-compartmental analysis. The complete methodology will be defined in the Statistical Analysis Plan.

Anti-Drug Antibodies

The frequency of participants with antibodies and magnitude (i.e., titer) of antibodies targeting DZIF-10c will be calculated and described in tables.

Viral Shedding Determined by qRT-PCR

Viral RNA shedding in nasopharygeal and oropharyngeal swabs will be measured by qRT-pCR as Ct values and copies/ml. Viral RNA shedding will be evaluated as original values and difference to baseline by visit, and as time weighted average change between baseline and the day 7 visit, as well as between baseline and the day 14 visit (**Groups 2A-2D**). In addition, the proportion of SARS-CoV-2-RNA positive participants per group and swab type will be determined (**Groups 2A-2D**).

Viral Shedding Determined by the Isolation of Infectious Virus

Frequency of viral shedding as determined by successful isolation of infectious virus in virus isolation assays will be analysed by visit (**Groups 2A-2D**).

Viral Replication Determined by Subgenomic SARS-CoV-2 mRNA

Levels of subgenomic SARS-CoV-2 mRNA will be determined in swab samples by qRT-PCR and summarized (**Groups 2A-2D**).

Frequency of COVID-19-related hospitalizations and medically-attended contacts

The frequency of unplanned hospitalizations and medically-attended contacts deemed to be related to COVID-19 by the Investigator will be described (**Groups 2A-2D**).

Time to symptom resolution

The duration of COVID-19-related symptoms will be described based on participants's self-assessment documented on patient diaries (**Groups 2A-2D**).

Differences in Viral Sequences and Change in Neutralization Sensitivity

Sequencing results of SARS-CoV-2 spike genes will be described. The impact of individual mutations (e.g., within the antibody epitope or recurrent mutations) on DZIF-10c sensitivity will be determined by neutralization assays and 50% inhibitory concentrations (IC_{50}s) and/or 100% inhibitory concentrations (IC_{100}s) will be determined (**Groups 2A-2D**).

Activity and Frequency of SARS-CoV-2-reactive immune responses

The frequency and response magnitude of SARS-CoV-2-reactive lymphocytes or lymphocyte subset will be evaluated and summarized per participant (**Groups 2A-2D**).

8.1.5. Subgroup Analyses

All subgroup analyses will be descriptive only. A subgroup analyses for sex will be performed for the primary endpoint.

Additional subgroup analyses are planned in the expansion cohort (**Group 2D**) for age, sex, BMI, viral RNA shedding at baseline, and trial center. The definition of Subgroup categories and minimum number of participants for a subgroup analysis will be specified in the SAP.

8.1.6. Interim Analysis

No formal interim analysis is planned.

8.2. Sample Size Considerations

During the dose escalation phase of the trial, a routine 3+3 trial design will be employed.

Since this is a hypothesis-generating trial, no formal sample size calculation is done for the randomized expansion study part. Selection of the group size included considerations of feasibility for an early phase first-in-human trial in quarantined individuals while facilitating outcome measures that can inform later stage trials. 60 participants will be included in the

expansion cohort with 20 participants per arm. Assuming a similar rate of participants experiencing Adverse Events as observed in a previous clinical trial of a SARS-CoV-2-neutralizing antibody in ambulatory SARS-CoV-2-infected participants[50], the following confidence intervals for the rate of Adverse Events in the expansion cohort can be calculated per group of 20 participants:

Rate of AEs (Participants)	**90% confidence interval** (Participants with AEs)
20%	[5% ; 35%]
25%	[9% ; 41%]
30%	[13% ; 47%]

While this exploratory trial is not powered to determine statistically significant differences in the change of the viral load between the treatment arms in the expansion cohort, simulations based on effects observed for previously reported SARS-CoV-2 neutralizing antibody responses[68,69] indicate that an AUC ratio >1.45 between a treatment arm of with a similar effects and controls can be observed with >80% probability at a group size of 20.

9. Safety

9.1. Definitions of Adverse Events and Adverse Drug Reactions

9.1.1. Adverse Event

An adverse event (AE) is any untoward medical occurrence in a clinical trial participant administered a study drug (i.e., IMP or placebo), even if the event is not considered to be caused by study drug. The adverse event may be, but is not restricted, to any new illness, worsening of a sign or symptom of COVID-19, or deterioration of a pre-existing medical condition. Abnormal laboratory results and results of physical examinations after the screening examination constitute Adverse Events to be reported and graded according to the CTCAE Grading table (v 5.0) if they are considered as clinically significant and/or require treatment.

Pregnancy

For reasons of drug safety, the occurrence of a pregnancy during the conduct of this trial is to be regarded as an AE. For details of special reporting requirements for pregnancy, see section 9.3.2.

Adverse Events of Special Interest

Infusion-related reactions presenting as hypersensitivity/allergic reaction, anaphylaxis, or cytokine release syndrome; as well as inhalation-related reactions presenting as allergic reaction (e.g., bronchospasm that may manifest with wheezing and/or respiratory distress) are designated as Adverse Events of Special Interest. For details of reporting requirements, see section 9.3.1.

9.1.2. Adverse Drug Reaction

All adverse events judged by either the investigator or the sponsor as having a reasonable suspected causal relationship to the study drug product qualify as adverse drug reactions (ADR).

9.1.3. Serious Adverse Events and Serious Adverse Reactions

A serious adverse event (SAE) or serious adverse drug reaction (SAR) is any adverse event or adverse drug reaction that

- results in death, and/or
- is life-threatening at the time of the event, and/or
- requires inpatient hospitalization or prolongation of existing hospitalization, and/or
- results in persistent or significant disability/incapacity, and/or
- is a congenital anomaly/birth defect, and/or
- is any other medically important event in the opinion of the investigator (e.g., an event that may jeopardize the trial subject or may require intervention to prevent one of the outcomes listed above)

Inpatient hospitalization is defined as any stay in a hospital that includes at least one night (midnight to 6 am).

Admission to hospital that was planned before the first study drug administration will not considered as SAE but must be documented in the trial subject's medical records and CRF.

Inpatient hospitalization for the purpose of IMP administration is not considered as SAE, but must be documented in a proper manner in the trial subject's medical record and CRF. Prolongation of hospitalization must be documented and reported as SAE.

9.1.4. Unexpected Adverse Drug Reaction

An unexpected adverse drug reaction is an ADR that is not consistent with the potential ADRs listed in the Investigator's Brochure in severity, nature and/or outcome.

9.1.5. Suspected Unexpected Serious Adverse Reactions

A suspected unexpected serious adverse reaction (SUSAR) is a serious adverse event (SAE) the nature or severity of which is not consistent with the potential ADRs listed in the Investigator's Brochure, and that has at least a possible causal relationship to the study drug.

9.1.6. Dose-Limiting Toxicities

A dose-limiting toxicity (DLT) will be defined as any grade 3 or higher adverse event during the dose escalation phase of the trial (**Groups 1A-1C, 2A-2C**) that is assessed by the trial investigators to be related to DZIF-10c. For details on reporting requirements see section 9.3.1.

9.2. Documentation and Follow-Up of Adverse Events

9.2.1. Documentation of Adverse Events and Adverse Drug Reactions

The occurrence of (S)AEs and ADRs will be documented starting with the provision of informed consent and until the termination of the clinical trial for the individual participant using the applicable case report form (CRF).

All adverse events, regardless of whether a causal relationship between the AE and study drug is suspected, will be followed-up until the associated symptoms have subsided, abnormal laboratory results have returned to pre-event levels, or the study has been completed or terminated for the individual participant.

Persistent AEs that do no resolve between individual visits will be recorded once. The initial severity will be documented at the time the event is first reported, and the highest severity will be documented should the persistent AE become more severe.

Should an AE become serious within the reporting period, it should be reported to the sponsor immediately (i.e., no more than 24 hours after learning that the event became serious) by using the trial-specific SAE form. Additionally, as soon as a documented Adverse Event becomes serious, the SAE information should be amended on the AE eCRF page by providing the date that the event became serious as onset date of the SAE and completing all data fields related to serious adverse event.

Documentation of AEs includes

- Description of AE
- Date and time of onset and resolution
- Grading/Severity
- Relationship to study drug
- Seriousness
- Effect on the trial drug administration schedule
- Need for treatment and action taken
- Outcome
- Written summary / narrative (for SAEs, AESIs, and DLTs)

Pre-existing diseases (i.e., medical conditions starting before study drug administration) are not documented as AEs but as medical history. However, worsening of pre-existing diseases will be documented as AEs.

9.2.2. Severity of Adverse Events

The CTCAE (Common Terminology Criteria for Adverse Events) grading table (v5.0) will be used by trial investigators to classify adverse events. It comprises the following categories:

- Grade 1: Mild.
  Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate.
  Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living* (ADL).
- Grade 3: Severe or medically significant but not immediately life-threatening.
  Hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
- Grade 4: Life-threatening consequences.
  Urgent intervention indicated.
- Grade 5: Death related to AE.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

9.2.3. Causality Assessment of Adverse Events

Every AE will be assessed by a trial investigator for potential causal relationship to the study drug. This assessment will include consideration of the nature and type of reaction, the temporal relationship with the study drug, the clinical status of the trial participant, concomitant medication and other relevant clinical factors. If the event is considered to be a consequence of lack of study drug efficacy or to be a symptom or sign of the underlying disorder, no causal relationship to the study drug will be assumed.

AEs/SAEs will be assessed according to the causality determinations of CIOMS VI-Group (Council for International Organizations of Medical Sciences) as follows:

- Related: There is a reasonable possibility that the AE is related to the IMP.
- Not related: There is not a reasonable possibility that the AE is related to the IMP.

Events for which no causal relationship to the IMP is given or which are classified as “not assessable” will be regarded as related until enough information is provided to allow a proper assessment of the causal relationship.

The causality assessment given by the investigator should not be downgraded by the Sponsor. If the Sponsor disagrees with the Investigator’s causality assessment, the more critical assessment is to be used for further processing of the case and the opinion of both, the Investigator and the Sponsor, will be provided the report narrative.

9.3. Reporting of Serious Adverse Events, Adverse Events of Special Interest, Dose-Limiting Toxicities, Pregnancy and Changes in Risk-Benefit Assessment

Every SAE that occurs from the provision of infomed consent to participate in the trial until the trial participant’s last study visit must be documented on the appropriate CRF and in addition be reported on a SAE form to the sponsor. In the interest of participants’ safety, follow-up for up to 30 days after the individual participants’s study termination is required for SAEs that are not sufficiently resolved at the participant’s final trial visit.

Adverse Events of Special Interest (AESIs) and Dose-Limiting Toxicites (DLTs) should be reported to the sponsor within 24 h of the site becoming aware of the event.

Pregnancies must be documented on a separate pregnancy form and reported to the sponsor within the defined periods (see Section 9.3.2.).

An Investigator Handout providing detailed information of reporting procedures will be provided to the trial sites.

9.3.1. SAE, AESI and DLT Reporting

All serious adverse events (SAEs) occurring between the provision of informed consent to participant in the trial and the participant’s last trial visit must be reported by the local trial investigator to the sponsor without delay and at the latest within 24 hours of becoming aware of the SAE.

To this end, the provided SAE report forms must be completed and submitted by fax or E-Mail to the Clinical Trials Center Cologne (CTCC, ZKS Köln) to which the sponsor has delegated the SAE management procedures:

SAE Dedicated Facsimile: [redacted]

SAE Reporting E-Mail: [redacted]

Similarly, AESIs should be reported on AESI report forms and must be completed and submitted by fax or E-Mail to the Clinical Trials Center Cologne (CTCC, ZKS Köln) within 24 h of the site becoming aware of the event.

Finally, DLTs occurring during the dose-escalation phase of the trial (**Groups 1A-1C**, **Groups 2A-2C**) must be reported by submitting the AE/DLT report form by fax or E-Mail to the Clinical Trials Center Cologne (CTCC, ZKS Köln) within 24 h of the site becoming aware of the event.

9.3.2. Pregnancy Reporting

The trial investigator will inform the sponsor of any pregnancy in a female trial participant reported during the conduct of the trial within 24 hours of the investigator becoming aware of the pregnancy.

To this end, the provided pregnancy report forms must be completed and submitted by fax or E-Mail to the Clinical Trials Center Cologne (CTCC, ZKS Köln) to which the sponsor has delegated the safety management procedures:

Pregnancy Dedicated Facsimile: [redacted]

Pregnancy Reporting E-Mail: [redacted]

Pregnant trial participants will be asked to provide separate informed consent for pregnancy follow up, and the parents will be asked to provide separate informed consent for follow-up of the child until 4 weeks after life birth.

9.3.3. SAE Assessment by the Sponsor

All cases of suspected SAEs will additionally be assessed by the Coordinating Investigator or its delegate (Sponsor assessors) with regard to seriousness, causality, and expectedness, regardless of the investigator's assessments.

9.3.4. Unblinding for SUSAR when Treatment is Blinded

The trial treatment is unblinded by the Sponsor in the individual trial subject to verify causality before reporting the event to the responsible ethics committee, the competent authority and SMC members.

The Sponsor will notify the Investigator (blinded SUSAR) of relevant information about SUSARs that could adversely affect the safety of subjects in a timely fashion. Follow-up information may be submitted if necessary.

Details on emergency unblinding are described in section 5.9.6.1.

9.3.5. Notification of Competent Authority and Ethics Committee

Every SUSAR (suspected unexpected serious adverse reaction) will be reported by the sponsor to the competent authority and the responsible ethics committee.

- Fatal and life-threatening SUSARs

  The competent authority and responsible ethics committee must be informed by the sponsor without delay, and at the latest within 7 calendar days of becoming aware of the minimum criteria for reporting. In all cases, attempts will be made to obtain further relevant information which will be supplied within an additional 8 days.

- SUSARs that are not fatal or life-threatening

  The competent authority and the responsible ethics committee must be informed without delay by the sponsor, at the latest within 15 calendar days of becoming aware of the minimum criteria for reporting. Additional relevant details will be passed on as soon as possible.

If the information provided at the time of reporting is incomplete for a final assessment, additional information will be requested from the investigator or other available sources.

9.3.6. Reporting of Potential Changes in the Risk-Benefit Ratio

The sponsor will inform the competent authority and the responsible ethics committee of any occurrences that may change the risk-benefit ratio of the IMP. This will be done without delay, and at the latest within 15 calendar days of becoming aware of such an occurrence. Such occurrences include, but are not restricted to:

- Reports of expected serious adverse drug reactions (SARs) with an unexpected outcome
- An unexpected high frequency of expected SARs

- SUSARs in trial participants occurring following trial completion (end-of-trial visit)
- Events in connection with the conduct of the study or the development of the IMP that may affect the safety of the trial subjects.

In case of any new event or safety issues relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the trial participants, the Sponsor and the Investigator must take appropriate urgent safety measures to protect the participants against any immediate hazard. The Sponsor has to inform the competent authority, ethics committee and SMC of those new events and the measures taken.

9.3.7. Informing the Investigators

All site investigators will be informed by the sponsor on all SUSARs in blinded form including all relevant further information within the periods defined by the competent authority.

In addition, the sponsor will communicate any changes resulting in the risk-benefit-assessment to all site investigators.

9.4. Annual Safety Report (DSUR)

An annual report (Development Safety Update Report, DSUR) compiled according to ICH guideline E2F and comprising all available and relevant information concerning trial participants' safety will be provided by the sponsor to the competent authority and ethics committee.

This report will be submitted annually within 60 calendar days of the DSUR data lock point, defined as one year from the first authorization to conduct the clinical trial by the sponsor.

10. Reporting of Trial Results

10.1. Reports

10.1.1. Interim Reports

No formal interim reports are planned.

10.1.2. Final Report

Within 90 days of the end of the trial, the competent authority and ethics committee will be informed about the end the trial.

Within one year of the end of the trial, a final study report will be provided to the competent authority and a summary will be provided to the ethics committee.

11. Amendments to the Trial Protocol

Amendments to the trial protocol may only be implemented in joint agreement between the sponsor, the sponsor's representative, the Coordinating Investigator, and the trial statistician.

Substantial amendments will be implemented only after approval by the competent authority and after receiving a favourable opinion of the ethics committee. Amendments necessary to avoid immediate danger to the trial participants are exempt from this requirement.

12. Appendices

Appendix I WHO Clinical Progression Scale

Patient State	Descriptor	Score
Uninfected	Uninfected; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic; viral RNA detected	1
	Symptomatic; independent	2
	Symptomatic; assistance needed	3
Hospitalised: moderate disease	Hospitalised; no oxygen therapy*	4
	Hospitalised; oxygen by mask or nasal prongs	5
Hospitalised: severe diseases	Hospitalised; oxygen by NIV or high flow	6
	Intubation and mechanical ventilation, pO_2/FiO_2 ≥150 or SpO_2/FiO_2 ≥200	7
	Mechanical ventilation pO_2/FiO_2 <150 (SpO_2/FiO_2 <200) or vasopressors	8
	Mechanical ventilation pO_2/FiO_2 <150 and vasopressors, dialysis, or ECMO	9
Dead	Dead	10

*If hospitalized for isolation only, record status as for ambulatory patient.

Appendix II Visit Schedule

Appendix II A - Visit Schedule - Groups 1A-1C - **HEALTHY PARTICIPANTS** **Version: V04_0 01-APR-2021**

Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Study Week	**Screen**	**Wk 0**				**Wk 1**	**Wk 2**	**Wk 3**	**Wk 4**	**Wk 8**	**Wk 13**
Study Day	**D-7 to D-1**	**D 0**	**D 1**	**D 3**	**D 5[1]**	**D 7**	**D 14**	**D 21**	**D 28**	**D 56**	**D 90**
Visit Window (+/- days)	**-**	**-**	**-**	**1**	**1**	**1**	**2**	**3**	**3**	**4**	**7**
Eligibility and History											
Informed Consent	x										
Inclusion/Exclusion criteria	x										
Demographics	x										
Medical History	x	x	x	x	x	x	x	x	x	x	x
Concomitant Medication	x	x	x	x	x	x	x	x	x	x	x
Study Intervention											
In-Patient Admission		x									
DZIF-10c Inhalation		x									
Study Procedure											
ECG	x										
AESI / Inhalation-related reactions		x	x	x	x	x					
Adverse Events		x	x	x	x	x	x	x	x	x	x
General Physical	x										
Directed Physical[2]		x	x	x	x	x	x	x	x	x	x
Vital Signs[3]	x	x[4]	x	x	x	x	x	x	x	x	x
Weight	x								x		x
Height	x										
Pregnancy counseling	x										
Pregnancy testing in women of childbearing potential	x	x[5]					x		x	x	x
Pulmonary function testing	x	x[6]	x			x			x		
Biosamples											
Virology screening labs[7] (Local lab)	x										
Safety lab[8] (Local lab)	x	x	x	x	x	x	x		x	x	x
Coagulation parameters (INR, PTT) (Local lab)	x										
Urinalysis[9]		x				x			x		x
SARS-CoV-2 antibody serology[10] (Local lab)	x	x									
PK samples (Central lab)		x[11]	x	x	x	x	x	x	x	x	x
Naso- or Oropharyngeal SARS-CoV-2 swab (Local lab)[12]	x	x[13]				x					
Anti-DZIF-10c antibody (ADA) assay sample (Central lab)		x					x		x	x	x
Research lab (PBMCs) (Central lab)		x							x		x

1. Optional visit.
2. Symptom-oriented examination.
3. Vital signs: Heart rate, systolic/diastolic blood pressure, temperature, respiratory rate, SpO2 (pulse oximetry).
4. Vital signs determined prior to inhalation, at the end of the inhalation, 1 h (+/- 10 min) post end of inhalation, and 4 h (+/- 15 min) post end of inhalation.
5. Negative serum or dipstick urine b-HCG from sample obtained on day of infusion must be available prior to the start of the administration.
6. Pulmonary function testing to be performed prior to inhalation, 30 min (+/- 5 min) post end of inhalation, and 4 h (+/- 15 min post end of inhalation). Parameters: FEV1, FVC, FEV1/FVC, VC, IVC, PEF, PIF.
7. Virology screening labs: HIV-1/2 antigen/antibody test, HBsAg, anti-HCV antibodies (HCV-RNA if anti-HCV positive).
8. Safety labs: *Hematology:* Complete blood count with white cell differential;
   Chemistry: Sodium, Potassium, Chloride, Calcium, AST, ALT, AP, total bilirubin, creatinine incl. eGFR, CRP, lipase.
9. Urinalysis: Protein, White blood cells/Leukocytes, Red blood cells/Erythrocytes.
10. Serological assay that includes at least IgG antibodies against SARS-CoV-2.
11. PK samples drawn prior to inhalation and 4 h (+/- 15 min) post end of inhalation.
12. A single naso- or oropharyngeal swab is acceptable. Analysis is to be performed at the routine diagnostic laboratory for SARS-CoV-2 NAAT of the trial site.
13. If swab for NAAT at screening was obtained >3 calendar days prior to inhalation, the day 0 NAAT result must be available and negative prior to starting the inhalation.

Appendix II B - Visit Schedule - Groups 2A-2D - **SARS-CoV-2-INFECTED PARTICIPANTS** **Version: V04_0 01-APR-2021**

Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Study Week	Screen[1]	Wk 0				Wk 1		Wk 2	Wk 4	Wk 8	Wk 13
Study Day	D-3 to D 0	D 0	D 1	D 3	D5[2]	D 7	D10[2]	D 14	D 28	D 56	D 90
Visit Window (+/- days)	-	-	-	1	1	1	1	2	3	4	7
Eligibility and History											
Informed Consent	x										
Inclusion/Exclusion criteria	x										
Demographics/Medical History	x										
Medical History	x	x	x	x	x	x	x	x	x	x	x
Concomitant Medication	x	x	x	x	x	x	x	x	x	x	x
Study Intervention											
Randomization		(x)[3]									
In-Patient Admission		(x)[4]									
Study Drug Administration		x									
Study Procedure											
ECG	x										
Inhalation-related Events (+ Infusion-related in **Group 2D**)		x	x	x	x	x					
Adverse Events		x	x	x	x	x	x	x	x	x	x
General Physical	x										
Directed Physical[5]		x	x	x	x	x	x	x	x	x	x
Vital Signs[6]	x	x[7]	x	x	x	x	x	x	x	x	x
Weight	x								x		x
Height	x										
Pregnancy counseling	x										
Pregnancy testing in women of childbearing potential	x	x[8]						x	x	x	x
WHO R&D Clinical Progression Scale	x	x	x	x	x	x	x	x	x	x	x
Biosamples											
Virology screening labs[9] (Local lab)	x										
Safety lab[10] (Local lab)	x	x	x	x		x		x	x	x	x
Coagulation parameters (INR, PTT) (Local lab)	x										
SARS-CoV-2 antibody serology (Local lab)	x	x									
Urinalysis[11] (Local lab) (**Groups 2A-2C only**)		x				x			x		x
PK samples (Central lab)		x[12]	x	x	x	x	x	x	x	x	x
Nasopharyngeal SARS-CoV-2 swab (Central lab)		x	x	x	x	x	x	x	x	x	x
Oropharyngeal SARS-CoV-2 swab (Central lab)		x	x	x	x	x	x	x	x	x	x
Naso- or Oropharyngeal SARS-CoV-2 swab (Local lab)	x[13]										
Oropharyngeal SARS-CoV-2 swab (Local lab)		(x[14])	(x[14])	(x[14])	(x[14])	(x[14])	(x[14])	(x[14])	(x[14])	(x[14])	(x[14])
Anti-DZIF-10c antibody (ADA) assay sample (Central lab)		x						x	x	x	x
Research lab (PBMCs) (Central lab)		x							x		x

1. In **Group 2D**, screening and infusion procedure may be performed on the same day. SARS-CoV-2 swabs for central lab PCR are mandatory to be obtained prior to the infusion.
2. Trial visits on day 5 and day 10 are considered optional pending local capacity for quarantine visits.
3. Randomization in **Group 2D** only.
4. In-patient admission required in **Groups 2A-2C,** and at the discretion of the investigator in **Group 2D.**
5. Symptom-oriented examination.
6. Vital signs: Heart rate, systolic/diastolic blood pressure, temperature, respiratory rate, SpO2 (pulse oximetry).
7. **Groups 2A-2C:** Vital signs determined prior to inhalation, at the end of the inhalation, 1 h (+/- 10 min) post end of inhalation, and 4 h (+/- 15 min) post end of inhalation.
   Group 2D: Vital signs determined prior to inhalation, at the end of the inhalation, 15 min after infusion start, at the end of the infusion, 1 h (+/- 10 min) post end of infusion, and 2h (+/- 10 min) post end of infusion.
8. Negative serum or dipstick urine b-HCG from sample obtained on the day of the administration must be available prior to the start of the administration.
9. Virology screening labs: HIV-1/2 antigen/antibody test, HBsAg, anti-HCV antibodies (HCV-RNA if anti-HCV positive).
10. Safety labs: *Hematology:* Complete blood count with white cell differential;
    Chemistry: Sodium, Potassium, Chloride, Calcium, AST, ALT, AP, total bilirubin, creatinine incl. eGFR, CRP, Lipase.
11. Urinalysis: Protein, White blood cells/Leukocytes, Red blood cells/Erythrocytes (for **Groups 2A-2C** only).
12. **Groups 2A-2C:** PK samples drawn prior to inhalation and 4 h (+/- 15 min) post end of inhalation.
    Group 2D: PK samples drawn prior to inhalation and 1 h (+/- 10 min) post end of infusion.
13. A single naso- and/or oropharyngeal swab is acceptable. Analysis is to be performed at the routine diagnostic laboratory for SARS-CoV-2 NAAT of the trial site. If a documented SARS-CoV-2 swab NAAT with a sample-obtained-date within 3 days prior to infusion is available, an additional swab at screening is not mandatory, however, nasopharyngeal and oropharyngeal swabs for the central lab PCR must be obtained prior to the infusion.
14. Optional oropharyngeal swab to obtained after central lab samples, and for NAAT to be performed at routine diagnostic laboratory for SARS-CoV-2 NAAT at trial site to provide participants with early results.

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