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ARTICLE

# First-in-human, randomized, double-blind, placebo-controlled, dose escalation trial of the anti-herpes simplex virus monoclonal antibody HDIT101 in healthy volunteers

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## Abstract

HDIT101 is a first-in-class humanized monoclonal antibody recognizing a conserved epitope in glycoprotein B, a target present on the surface of herpes simplex virus 1 (HSV-1) and HSV-2 particles as well as on virus-infected cells. This was a first-in-human, single-center, double-blind, placebo-controlled trial in 24 healthy volunteers, randomized 3:1 (placebo:active) in each of the six dose levels with escalating doses up to 12,150 mg HDIT101. HDIT101 was administered intravenously, to study safety, pharmacokinetics (PKs), and immunogenicity. HDIT101 was well-tolerated in all recipients and no serious or severe adverse events, no infusion-related reactions, and no events suggestive of dose limiting off-target toxicity occurred. The mean serum exposure (area under the curve from zero to infinity [$AUC_{0-\infty}$]) of HDIT101 showed a linear increase from 4340 h*µg/ml at a dose of 50 mg to 1,122,247 h*µg/ml at a dose of 12,150 mg. No immunogenic effects following HDIT101 exposure were observed at any of the applied doses. HDIT101 demonstrated the expected PK properties of a monoclonal antibody was well-tolerated, and could be safely administered even at excessively high doses that may be required for treatment of patients with septical HSV spread.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Herpes simplex virus (HSV-1) and HSV-2 are a global disease burden with significant morbidity and serious consequences for patients mainly in

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immunocompromised settings. There is a need for new drugs that reduce symptoms, recurrences, and effectively suppress viral shedding.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

How safe is the administration of HDIT101, a first-in-class humanized monoclonal antibody recognizing the exclusively viral target glycoprotein B?

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**

HDIT101 is the first clinically tested monoclonal antibody against HSV. It was well tolerated by healthy volunteers and could be safely administered up to very high doses.

**HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?**

Our findings pave the way for efficacy trials in patients to evaluate the promising first-in-class therapeutic antibody for patients suffering from HSV-related diseases.

## INTRODUCTION

Herpes simplex viruses (HSVs) are highly infectious pathogens impacting the global population. Particularly newborns, elderly, and immunocompromised patients (e.g., human immunodeficiency virus-infected patients, organ and stem cell transplant recipients, and patients with cancer) may face serious consequences of HSV infections, such as birth defects (herpes neonatorum), blindness (herpes keratitis), and encephalitis, respectively.[1,2]

According to the World Health Organization, in 2020, an estimated 3.7 billion people under the age of 50, or 67% of the population, suffered from infection with HSV type 1 (HSV-1), which is transmitted mainly by oral-to-oral contact and causes herpes labialis (cold sores). In addition 491 million people aged 15–49 (13%) worldwide were living with HSV type 2 (HSV-2) infection, which is transmitted almost exclusively sexually and causes genital herpes (herpes genitalis).[3] Up to now, no cure or vaccine is available and drug-resistance against approved treatment options is increasing.

Among all symptomatic HSV infections, herpes labialis (cold sores primarily on the lips) and herpes genitalis (lesions in the genital area) are the most prevalent.[4,5] Herpes genitalis is the most common sexually transmitted viral disease and was primarily caused by HSV-2 in the past. However, genital HSV-1 infections have become more common in Western countries and estimates suggest that 140 (range 67–212) million people had prevalent genital HSV-1 infections, most of which occurring in the Americas, Europe, and Western Pacific.[5]

HSV infections are characterized by life-long persistence of the virus in infected tissue and corresponding nerve ganglia at the primary infection site. From this site, the virus may become re-activated and may cause recurrent local infections by retrograde transport to the respective associated mucocutaneous tissue.[6,7]

Mucosal sores are the common sign of an active infection, but HSV can also produce cutaneous lesions. Based on its affinity for neurons and epithelial cells, HSV is also capable of attacking the brain, resulting in encephalitis or meningitis.[8]

The entry of HSV into mammalian cells represents one of the most complex viral entry mechanisms studied so far. Among the 12 glycoproteins of the HSV envelope, glycoprotein B (gB), gD, and the gH/gL heterodimer display essential functions for both, entry of extracellular virions and cell-to-cell spread, a key mechanism by which HSV may escape humoral immune surveillance.[9]

Available treatment options for HSV-2-mediated anogenital infections, such as valaciclovir, aciclovir, and famciclovir have shown some efficacy in reducing clinical symptoms but have not been able to completely suppress viral shedding in affected mucocutaneous tissue. Subclinical shedding of the virus bears a high risk of sexual transmission even within symptom-free episodes. In a subset of patients, repeated annual recurrences are observed which – secondary to physical symptoms – may also cause severe psychosocial distress in those afflicted.[10]

HDIT101 is a humanized monoclonal antibody with a novel mechanism of action intended for the treatment of HSV-1 and HSV-2 infections. HDIT101, or its murine origin MAb2c, specifically recognizes viral gB, a glycoprotein present on the surface of HSV-1 and HSV-2 virions and on the surface of virus-infected cells,[11,12] whereas it does not bind to uninfected human cells or cells in which HSV is inactive.[9]

By blocking gB on HSV virions, HDIT101 is capable of neutralizing their infectivity with high efficacy. By binding to gB on HSV-infected cells, HDIT101 also inhibits cell-to-cell spread, a key mechanism by which HSV may escape humoral immune surveillance.[9,13]

The HDIT101 epitope on gB is highly conserved between clinical HSV-1 and HSV-2 isolates and efficacy in

neutralization of isolates resistant to standard-of-care medication has been demonstrated (data on file and in Ref. 13). Preclinical characterization revealed high efficacy of HDIT101 for treating HSV-1 and HSV-2 infected mice in both immunocompetent and immunocompromised settings, respectively. The favorable safety profile for HDIT101 observed in these animal models were further confirmed by the absence of adverse human tissue-cross reactivity and proinflammatory cytokine release in a whole blood assay.[14] In addition, biochemical and biophysical analyses revealed excellent stability as well as favorable large scale good manufacturing practice (GMP) manufacturing characteristics.[15] These features collectively qualified HDIT101 for further clinical evaluation as a first-in-class compound for the treatment of primary and chronic recurrent HSV infections. For the translation of HDIT101 into clinical development, we conducted a first-in-human (FIH) single-dose, dose-escalation trial in healthy volunteers to study pharmacokinetics (PKs), safety, and immunogenicity of HDIT101 as a basis for subsequent phase II clinical trials.

# METHODS

## Study design and participants

We conducted an FIH single-center, randomized, double-blind, placebo-controlled, single-dose, dose escalation trial with HDIT101 in participants of both sexes at the Early Clinical Trial Unit (KliPS) of the Department of Clinical Pharmacology and Pharmacoepidemiology, which is certified according to the DIN EN ISO 9001 standard. The trial followed the guideline of Good Clinical Practice, the ethical principles expressed in the Declaration of Helsinki, and all legal requirements for conducting clinical trials in Germany. The trial was approved by the Ethics Committee of the Medical Faculty of Heidelberg University (AFmo-702/2017) and the competent national authority (PEI, Langen, Germany, EudraCT: 2017-004452-37; DRKS00014678). Prior to participation in any trial-related procedures, each participant provided written informed consent. To qualify for participation in the trial, volunteers had to pass a satisfactory medical assessment with no clinically relevant abnormalities. There were no important changes to any methods or the trial outcome after the trial commenced. Further details of the trial protocol, including eligibility criteria, and blinding are described in the Supplemental Material. The trial was conducted between May 9, 2018, and April 1, 2019 and ended according to planning.

A modified 3 + 1 dose-escalation scheme was implemented (Figure 1), where six successive cohorts of four participants at each predefined dose level were randomized (3 to HDIT101 and 1 to placebo in each cohort). Single intravenous doses of placebo or 50, 150, 450, 1350, 4050, or 12,150 mg HDIT101 were administered as an infusion over 1 h. Participants within a given dose cohort were treated sequentially with a ≥48-h-observation period (first 24 h in-house) between participants. An end-of-trial visit was conducted 29 days after administration of HDIT101 or placebo. A safety follow-up call was carried out about 6 months after the end-of-trial visit. Dose levels for the respective cohorts were escalated after consultation of an independent data safety monitoring board that had access to all safety data of the previous cohorts.

## Primary and secondary end points of the trial

The primary end point of the trial was the number of participants experiencing a dose-limiting toxicity (DLT) during the 48-h-observation period after HDIT101 administration. Secondary end points were (i) incidence rate and severity of adverse events (AEs) and their relatedness to HDIT101, (ii) the noncompartmental PKs of HDIT101, and (iii) the occurrence of circulating anti-drug antibodies (ADAs) and their neutralizing potential.

## Preclinical findings and dose justification

Because mice infected with human HSV develop the same course of disease as humans and HDIT101 binds highly specific to an exclusive viral target, evaluation of preclinical toxicology and efficacy studies in only this single species was considered appropriate by the regulatory authority. For tolerability studies, naïve mice were treated with doses of HDIT101 ranging from 15 to 50 mg/kg and no signs of intolerance were observed.[14] For efficacy studies, HSV-1 or HSV-2 infected mice were treated intravenously (i.v.) or intraperitoneally with HDIT101 at doses ranging from 2.5 to 30 mg/kg and no sign of symptoms unrelated to the HSV-1/2 infection were observed. Indeed, symptoms and clinical scores were reduced in HDIT101 treated HSV-2 infected immunocompetent Balb/c mice as compared to untreated control mice within 10 days after infection.[14] Therapeutic effects with the minimal HDIT101 dose of 2.5 mg/kg were observed on suppression of viral shedding in the genital mucosa of non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice after HSV-1 infection, correlating with prolonged survival. This was considered the minimum anticipated biological effect level (MABEL).

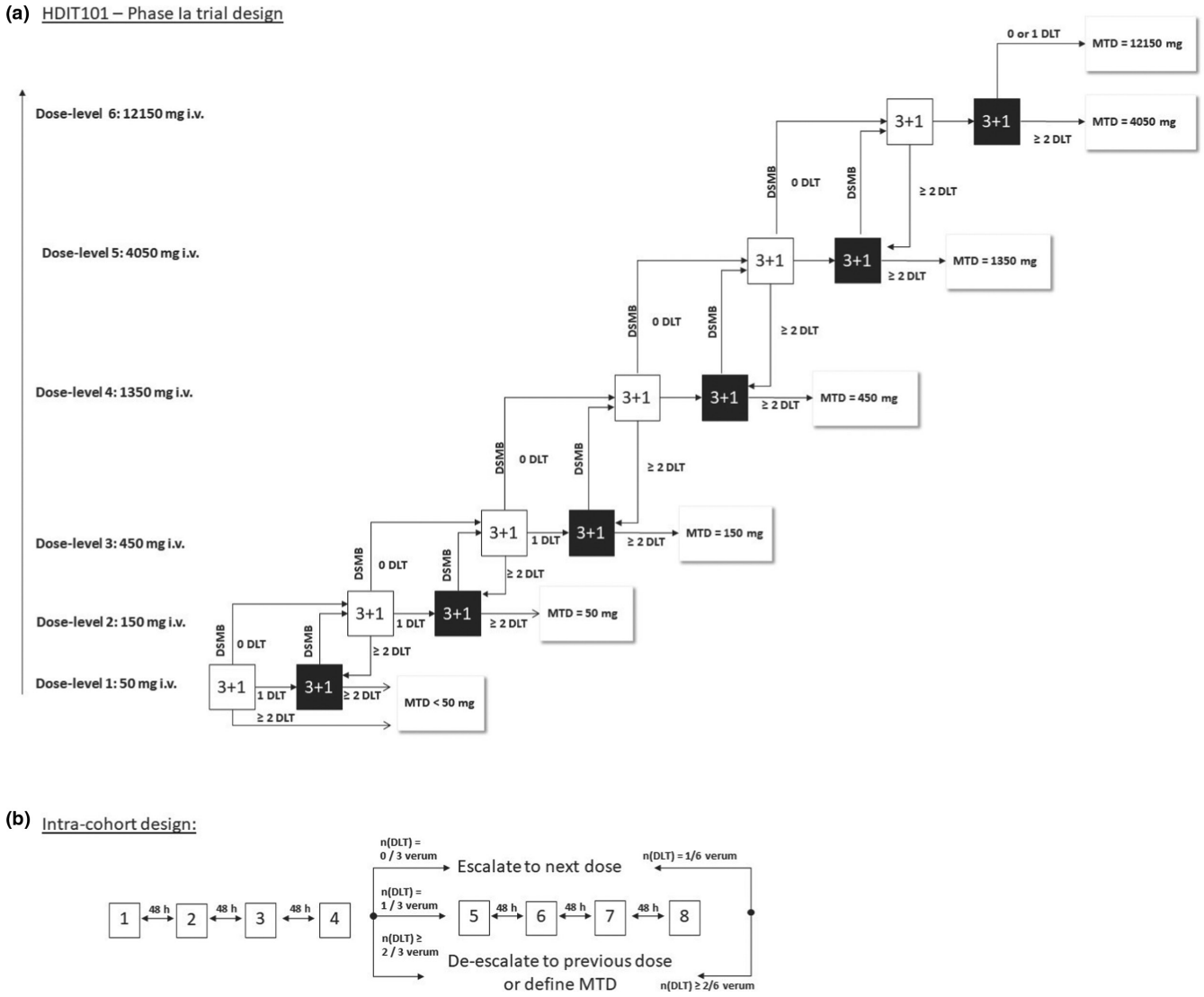


**FIGURE 1** (a) trial design and (b) intra-cohort design. DSMB, Data and Safety Monitoring Board; DLT, dose limiting toxicity; i.v. intravenous; MTD, maximum tolerated dose.

Based on preclinical in vitro and in vivo data for HDIT101 and available safety profiles of clinically established therapeutic antibodies targeting pathogen-specific antigens, a flat dose of 50 mg (corresponding to ~0.75 mg/kg) was estimated to be a safe starting dose of HDIT101 in healthy adults.[14] The starting dose was conservatively chosen about three-fold lower than the MABEL dose for safety reasons. This was because studies using nonactivated whole blood immune cells showed a weak increase of interleukin (IL)-1β, monocyte chemoattractant protein 1 (MCP-1), IL-8 and macrophage inflammatory protein (MIP)-1β in the presence of HDIT101.[14,16] The 50 mg dose was ~20-fold lower than the administered effective and safe dose (15 mg/kg) administered in mouse pharmacodynamic models, about three-fold lower than the lowest dose (2.5 mg/kg) of HDIT101 that showed a minimal biologic effect in mouse models, and ~20 to 100-fold lower than the therapeutic dose of other therapeutic IgG antibodies (e.g., rituximab, cetuximab, motavizumab, or palivizumab) or hyperimmune sera (e.g., to treat cytomegalovirus, respiratory syncytial virus, rabies, and hepatitis A and B; data on file and in Ref. 13). Because the target recognized by HDIT101 (HSV gB) is a viral discontinuous epitope that is not expressed on healthy human tissue, a dose escalation schedule with a factor of three was therefore considered safe. The proposed maximal dose of 12,150 mg was considered justified in discussions with the Paul-Ehrlich Institute during a scientific advice meeting in view of additional indications with excessive virus load and high medical need, such as disseminated septical HSV spread in severely immunocompromised patients.

## Blood sampling and pharmacokinetic assessments

Venous blood samples for the quantification of HDIT101 in serum were collected before administration, and at 0.5, 1 (end of infusion), 1.25, 1.5, 2, 2.5, 3, 4, 5, 7, 9, 11, and 13 h after the start of the 1-h infusion, as well as on days 2, 3, 4, 8, 15, and 29 from an antecubital vein of the arm not used for drug administration.

HDIT101 was quantified using an electrochemiluminescence assay based on the Mesoscale Discovery platform. Standards, controls, and test samples were incubated with the anti-idiotype monoclonal antibody (AIA#13-1-1) labeled with biotin. Biotinylated AIA#13-1-1 was immobilized on a Meso Scale Discovery (MSD) Streptavidin plate. Following addition of the samples, unbound material was washed away and HDIT101 was detected using a specific monoclonal AIA (AIA#54-22-1) labeled with Sulfo-TAG. The electrochemiluminescence signal from the Sulfo-TAG labels was measured using a MESO Sector S600 plate reader (Meso Scale Discovery; Rockville, MD) in the presence of a read buffer containing tripropylamine. The assay was validated according to the pertinent European Medicines Agency (EMA) and US Food and Drug Administration (FDA) guidelines.[17,18] The calibration range in the validation was 1.3–4000 ng/ml, the lower limit of quantification and upper limit of quantification of the assay were 3.3 and 2000 ng/ml, respectively.

## Safety and tolerability assessment

Safety was assessed by monitoring AEs throughout the 29-day trial and by repeated physical examinations, assessment of vital signs, electrocardiogram recordings, routine laboratory tests (hematology, biochemistry, coagulation, and urinalysis), and additional laboratory tests in case of infusion-related reactions (IL-6 and IL-8). AEs were classified according to the Common Terminology Criteria for Adverse Events (CTCAE, version 4.03; US Department of Health and Human Services, Bethesda, MD) and coded using the Medical Dictionary for Regulatory Activities (version 18.0; MedDRA MSSO, McLean, VA). AEs were tabulated in a descriptive way.

## Immunogenicity assessments (ADA)

Blood sampling for ADAs occurred on day 1 predose, day 8, day 15, and on the last study day, on day 29. For ADA assays, HDIT101 was labeled with biotin as the capture reagent and with Sulfo-TAG as the detection reagent. ADA samples were mixed with the labeled compounds. If present, ADAs form a bridge between the biotin-labeled drug and the Sulfo-TAG-labeled drug. The complex was captured onto a high binding avidin-coated MSD platform plate. The electrochemiluminescence signal was measured using a MESO Sector S600 plate reader in the presence of a read buffer containing tripropylamine and was normalized to the median negative control signal in each plate. ADA detection was based on a multitiered approach to screen, confirm, and titer ADAs. The samples were screened for the presence or absence of anti-HDIT101 antibodies. Samples with ratios to negative controls above or equal to the screening cutoff point were defined as screened positive and were tested in the confirmatory assay. The specificity of the screened positive samples was confirmed by competition using unlabeled HDIT101. The immunogenicity assay had a drug tolerance (DT) of 20, 250, and ≥800 µg/ml HDIT101 in serum for tested ADA concentrations of 7 ng/ml (low positive control [LPC]), 100 ng/ml (medium positive control [MPC]), and 1000 ng/ml (high positive control [HPC]), respectively. The assay was validated according to the pertinent EMA and FDA guidelines.[17,18]

## Statistical analysis and sample size

This was an exploratory phase 1 FIH trial and no formal power calculations to determine sample size were performed. Cohort sizes were chosen to balance the need for adequate exposure to a meaningful dose range and minimizing exposure of humans with a new biological entity. Six cohorts of four evaluable participants (randomized 3:1, verum:placebo) per dose level were planned to be enrolled into the trial. Dose groups could be expanded if DLTs occurred and dropouts could be replaced leading to a minimum of 24 and a maximum of 64 participants to be enrolled. Further details for blinding and statistical methods are described in Supplemental Material.

## Pharmacokinetic analysis

A noncompartmental PK analysis was carried out by using SAS version 9.4. HDIT101 peak concentrations ($C_{max}$) and the time to reach $C_{max}$ ($T_{max}$) were obtained directly from the concentration–time profiles. The area under the concentration–time curve from start of the infusion until a given sampling time ($AUC_{[0-t]}$) was calculated using the linear trapezoidal rule. The terminal phase elimination rate constant $\lambda_z$ was calculated by least squares linear regression of the log(ln)-linear terminal elimination phase

of the concentration–time curve. The adjusted $r^2$ had to be at least 0.85 for the estimate to be considered sufficiently reliable. Systemic clearance ($CL_{sys}$) was calculated as dose/$AUC_{0-\infty}$, where $AUC_{0-\infty}$ (AUC extrapolated to infinity) was calculated as $AUC_{(0\text{-tlast})} + C_{last}/\lambda_z$. For the last quantifiable concentration ($C_{last}$) actually measured was used rather than an estimated value. The extrapolation was considered sufficiently reliable only if the extrapolated part of the $AUC_{0-\infty}$ accounted for <20%. Plasma terminal half-life ($t_{1/2}$) was calculated using ($\ln^2/\lambda_z$) and volume of distribution ($V_d$) was calculated using dose/($AUC_{0-\infty}$*$\lambda_z$). PK parameters were analyzed with descriptive methods and dose proportionality was assessed by ANOVA with dose-normalized AUC and $C_{max}$. The diagrams were created with GraphPad Prism 9.2.0 (GraphPad Software Inc., La Jolla, CA).

# RESULTS

## Participants

Thirty-six participants were screened for eligibility and 24 (12 women, 92% White) were enrolled and randomized, of whom 18 were treated with HDIT101 and six received placebo. The median age of all enrolled participants was 39 years (range: 21–56 years), their mean weight was 73.4 kg (SD 12.89), and the mean body mass index was 24.4 (SD 2.87); the values of the placebo and verum group were comparable and demographics for all groups are displayed in Table S1. All participants completed the trial as planned and each cohort included four participants. One participant in the highest dose group experienced extravasation 39 min after the start of infusion. The PK data of this volunteer were censored for the aggregated evaluation. The volunteer received about 65% of the planned HDIT101 dose, reached $C_{max}$ at day 7 and showed an overall $AUC_{0-\infty}$ of about 50% as compared to the full i.v. dose of 12,150 mg HDIT101.

## Primary end point and safety

Single intravenous doses of HDIT101 were well tolerated up to the highest dose of 12,150 mg. In total, 13 (70.8%) participants receiving HDIT101 and four receiving placebo (66.7%) reported 38 AEs of any grade, and half of them experienced AEs (three CTCAE grade 2 AEs and all others grade 1) for which at least a causal relationship with the study treatment was possible (Table 1). The most common AEs after HDIT101 were asymptomatic laboratory changes with elevations of serum lipase ($N = 3$), C-reactive protein ($N = 2$), and leukopenia and lymphopenia ($N = 2$), and two participants each reported respiratory symptoms (nasal congestion) and signs of a common cold. Increased serum lipase was also reported in the placebo group ($N = 2$) and one placebo participant experienced an oral herpes infection. Table 2 gives an overview on AEs based on preferred terms.

A total of 12 participants (50.0%) experienced AEs of any grade, suspected possibly or probably related to the study treatment (9 HDIT101 participants, 50.0%; 3 placebo participants, 50.0%). The most common AEs of any grade at least possibly related to the study medication that occurred in subjects treated with HDIT101 were increased lipase and amylase, (2 participants, 11.1%, and 1 participant, 5.6%, respectively) as well as leukopenia and lymphopenia (each in 2 participants, 11.1%). In the placebo treatment group, two cases of increased serum lipase (33.3%) were reported, one subject presenting also with increased amylase levels (16.7%), and one additional participant suffered from lower abdominal pain (16.7%).

There was no relationship of AEs to dose. The only grade 2 AEs were observed in the 450-mg and in the 12,150-mg dose group: the participant in the 450-mg group had an episode of grade 2 presyncope, unlikely related to the study treatment, and in the 12,150-mg group, one participant experienced grade 2 leukopenia and one grade 2 infusion-site edema due to extravasation in the 12,150 mg dose group, both were considered possibly related to HDIT101.

**TABLE 1** Overview of adverse events and their evaluation after intravenous administration of the humanized monoclonal antibody HDIT101 or placebo

| | All participants $N = 24$ | HDIT101 – all dose groups $N = 18$ | Placebo $N = 6$ |
|---|---|---|---|
| All AEs, *N* | 38 | 32 | 6 |
| Participants with AEs, *N* (%) | 17 (70.8) | 13 (72) | 4 (66.7) |
| Serious AEs, *N* | 0 | 0 | 0 |
| Dose-limiting toxicity, *N* | 0 | 0 | 0 |
| Related/unrelated AEs[a], *N*/*N* | 28/10 | 23/9 | 5/1 |

Abbreviation: AEs, adverse events.

[a]Events evaluated as being not related or unlikely related to the trial medication were considered unrelated, events evaluated as being possibly, probably or definitely related to the trial medication were considered related.

**TABLE 2** All adverse events (preferred term) by treatment group (relation to trial medication a not related, b unlikely, c possible, d probable, e definite)

| | 50 mg HDIT101 (*N* = 3) | 150 mg HDIT101 (*N* = 3) | 450 mg HDIT101 (*N* = 3) | 1350 mg HDIT101 (*N* = 3) | 4050 mg HDIT101 (*N* = 3) | 12,150 mg HDIT101 (*N* = 3) | Placebo (*N* = 6) | Total (*N* = 24) |
|---|---|---|---|---|---|---|---|---|
| Subjects with at least one AE | 3 (100%) | 2 (66.7%) | 2 (66.7%) | 2 (66.7%) | 1 (33.3%) | 3 (100%) | 4 (66.7%) | 17 (70.8%) |
| Abdominal pain | – | – | – | – | – | 1 (33.3%) | – | 1 (4.2%) |
| Relationship to trial medication | | | | | | a | | |
| Abdominal pain lower | – | – | – | – | – | – | 1 (16.7%) | 1 (4.2%) |
| Relationship to trial medication | | | | | | | c | |
| Amylase increased | – | – | 1 (33.3%) | – | – | – | 1 (16.7%) | 2 (8.3%) |
| Relationship to trial medication | | | c | | | | c | |
| Alanine aminotransferase increased | 1 (33.3%) | – | – | – | – | – | – | 1 (4.2%) |
| Relationship to trial medication | c | | | | | | | |
| Aspartate aminotransferase increased | 1 (33.3%) | – | – | – | – | – | – | 1 (4.2%) |
| Relationship to trial medication | c | | | | | | | |
| Cough | – | 1 (33.3%) | – | – | – | – | – | 1 (4.2%) |
| Relationship to trial medication | | b | | | | | | |
| C-reactive protein increased | – | – | – | – | 1 (33.3%) | 1 (33.3%) | – | 2 (8.3%) |
| Relationship to trial medication | | | | | c | a | | |
| Diarrhea | – | – | – | – | – | 1 (33.3%) | – | 1 (4.2%) |
| Relationship to trial medication | | | | | | a | | |
| Dysuria | – | – | 1 (33.3%) | – | – | – | – | 1 (4.2%) |
| Relationship to trial medication | | | b | | | | | |
| Hematoma | – | – | – | – | – | 1 (33.3%) | – | 1 (4.2%) |
| Relationship to trial medication | | | | | | b | | |
| Headache | – | 1 (33.3%) | – | – | – | – | – | 1 (4.2%) |
| Relationship to trial medication | | a | | | | | | |
| Hyperuricemia | – | – | – | – | – | – | 1 (16.7%) | 1 (4.2%) |
| Relationship to trial medication | | | | | | | b | |
| Infusion-site edema | – | – | – | – | – | 1 (33.3%) | – | 1 (4.2%) |
| Relationship to trial medication | | | | | | c | | |
| Lipase increased | 1 (33.3%) | – | 1 (33.3%) | 1 (33.3%) | – | – | 2 (33.3%) | 5 (20.8%) |
| Relationship to trial medication | b | | c | d | | | c, c | |
| Leukopenia | – | – | – | – | 1 (33.3%) | 1 (33.3%) | – | 2 (8.3%) |
| Relationship to trial medication | | | | | c | c | | |
| Lymphopenia | – | – | – | – | 1 (33.3%) | 1 (33.3%) | – | 2 (8.3%) |
| Relationship to trial medication | | | | | c | c | | |
| Medical device site erythema | 1 (33.3%) | – | – | – | – | – | – | 1 (4.2%) |
| Relationship to trial medication | a | | | | | | | |
| Nasal congestion | – | – | – | 1 (33.3%) | 1 (33.3%) | – | – | 2 (8.3%) |
| Relationship to trial medication | | | | b | a | | | |
| Nasopharyngitis | – | – | – | – | – | 2 (66.7%) | – | 2 (8.3%) |
| Relationship to trial medication | | | | | | a, a | | |
| Nausea | – | 1 (33.3%) | – | – | – | – | – | 1 (4.2%) |
| Relationship to trial medication | | d | | | | | | |

**TABLE 2** (Continued)

| | 50 mg HDIT101 (*N* = 3) | 150 mg HDIT101 (*N* = 3) | 450 mg HDIT101 (*N* = 3) | 1350 mg HDIT101 (*N* = 3) | 4050 mg HDIT101 (*N* = 3) | 12,150 mg HDIT101 (*N* = 3) | Placebo (*N* = 6) | Total (*N* = 24) |
|---|---|---|---|---|---|---|---|---|
| Neutrophil count decreased | – | – | – | – | – | 1 (33.3%) | – | 1 (4.2%) |
| Relationship to trial medication | | | | | | c | | |
| Oral herpes | – | – | – | – | – | – | 1 (16.7%) | 1 (4.2%) |
| Relationship to trial medication | | | | | | | a | |
| Oropharyngeal pain | – | 1 (33.3%) | – | – | – | – | – | 1 (4.2%) |
| Relationship to trial medication | | b | | | | | | |
| Presyncope | – | – | 1 (33.3%) | – | – | – | – | 1 (4.2%) |
| Relationship to trial medication | | | b | | | | | |
| Rash | – | – | – | – | – | 1 (33.3%) | – | 1 (4.2%) |
| Relationship to trial medication | | | | | | a | | |
| Sunburn | 1 (33.3%) | – | – | – | – | – | – | 1 (4.2%) |
| Relationship to trial medication | c | | | | | | | |
| Vomiting | – | 1 (33.3%) | – | – | – | – | – | 1 (4.2%) |
| Relationship to trial medication | | d | | | | | | |
| Wheezing | – | 1 (33.3%) | – | – | – | – | – | 1 (4.2%) |
| Relationship to trial medication | | d | | | | | | |
| Total number of AEs | 5 | 6 | 4 | 2 | 4 | 11 | 6 | 38 |

Abbreviation: AE, adverse event.

**TABLE 3** Noncompartmental PK parameters of the humanized monoclonal antibody HDIT101 after a 1-h intravenous infusion in six different doses in 17 healthy volunteers

| Administered dose of HDIT101 | Number of participants | $AUC_{0-\infty}$ (μg/ml*h) | $C_{max}$ (μg/ml) | $T_{max}$ (h after start of infusion) | $t_{½}$ (h) |
|---|---|---|---|---|---|
| 50 mg | 3 | 4300 [2860; 6460] | 18.6 [15.7; 21.9] | 3.64 [2.43, 5.46] | 260 [179; 376] |
| 150 mg | 3 | 15,500 [9800; 24,400] | 63.3 [41.8; 95.9] | 2.17 [0.51; 9.26] | 302 [194; 470] |
| 450 mg | 3 | 49,400 [18,700; 131,000] | 174 [82.7; 366] | 1.86 [0.78; 4.44] | 319 [225; 451] |
| 1350 mg | 3 | 126,000 [104,000; 152,000] | 458 [354; 593] | 1.45 [0.87; 2.42] | 291 [239; 355] |
| 4050 mg | 3 | 415,000 [304,000; 566,000] | 1600 [1230; 2100] | 3.37 [1.38; 8.21] | 281 [190; 414] |
| 12,150 mg | 2[a] | 1,100,000 [105,000; 1,560,000] | 4360 [1560; 12,100] | 2.71 [0.88; 8.40] | 264 [226; 307] |

*Note*: Data are reported as geometric mean [95% confidence interval].

Abbreviations: $AUC_{0-\infty}$, area under the concentration-time curve extrapolated to infinity; $C_{max}$, serum peak concentration; PK, pharmacokinetic; $T_{max}$, time to reach $C_{max}$; $t_{½}$, elimination half-life.

[a]One participant was not considered in the aggregated PK analysis because of extravasation.

In addition, AE edema by extravasation was evaluated as being related to the HDIT101 application, but most likely not related to the pharmacological properties of HDIT101 but rather to local irritation after extravasation. All but three AEs resolved by the end of trial participation.

## HDIT101 pharmacokinetics

The PKs of HDIT101 are shown in Table 3, Figure 2, and Figure S1. With a 243-fold increase in dose (from 50 to 12,150 mg), there was a linear increase in $C_{max}$ (235-fold, $r^2 = 0.99$) and in mean systemic exposure ($AUC_{0-\infty}$; $r^2 = 0.96$, Table 2 and Figure S1). After an initial distribution phase, there was a dose-independent decline with a $t_{1/2}$ ranging from 260 h (50 mg) to 319 h (450 mg).

One participant in the highest dose group experienced extravasation 39 min after the start of the infusion. At that time, administration was stopped. The PK data of this volunteer were censored for the aggregated evaluation but are included in Figure 2. The volunteer received ~65% of the planned HDIT101 dose, reached $C_{max}$ on day 7, and showed

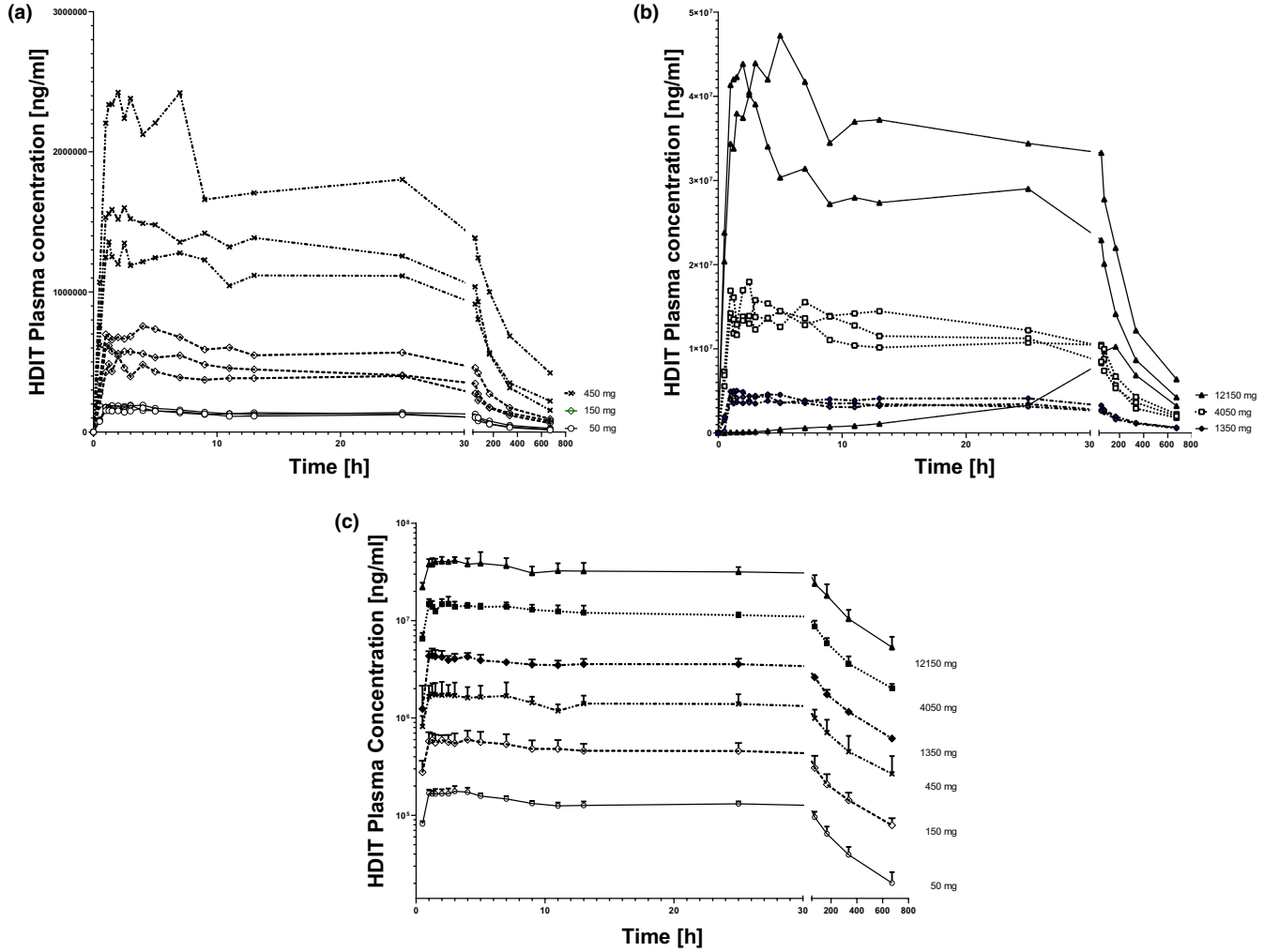


**FIGURE 2** Serum concentration–time profiles of HDIT101 after intravenous administration of six ascending doses to three healthy volunteers in each dose group. (a,b) Individual concentration–time curves including a volunteer with extravasation in the 12,150 mg cohort separated in cohorts with lower (a) and higher doses (b). (c) Semilogarithmic plot of geometric cohort means and error bars showing standard deviations of all evaluable volunteers (only $N = 2$ in the 12,150 mg cohort).

an overall $AUC_{0-\infty}$ of ~50% compared to participants who received the entire i.v. dose of 12,150 mg HDIT101.

## Immunogenicity

For ADA evaluation, HDIT101 concentrations did not exceed DT at LPC, or MPC ADA levels at any study timepoint in the 50 mg dose group. Participants of the 150 mg HDIT101 group reached DT on day 8 or day 15 after infusion at LPC ADA level, whereas at the MPC ADA level, the HDIT101 concentration was below DT at all timepoints. HDIT101 concentrations were below DT at LPC ADA level on day 29 for one participant of the 450 mg HDIT101 group, whereas the two other individuals did not reach DT at LPC ADA level. For all participants of dose groups 50–4050 mg, HDIT101 serum concentrations were below DT at MPC ADA level on day 29, whereas none of the two individuals of the 12,150 mg HDIT101 dose group reached DT at LPC or MPC ADA level within the study period. HDIT101 concentrations were below the highest evaluated DT at HPC ADA level during assay validation (800 µg/ml) for all participants across different dose groups on day 29.

One out of 18 participants had a confirmed predose ADA titer of 1 (dilution excluding minimal required dilution of 1/68) but no ADA levels at HPC level (≥1000 ng/ml) were detected on day 29 in this patient. Evaluation of lower ADA titers in this patient was hampered within the study time by the DT limitation of the assay. The detailed analysis of DT levels per dose group can be found in (Table S2).

## DISCUSSION

This study provides the first data of HDIT101 administered in humans and confirms a good safety profile

without DLTs or serious AEs up to the very high dose of 12,150 mg. Concurrently and in the absence of the drug target (gB on shed HSV or infected cells), it revealed linear PKs of the humanized antibody. The administered doses between 50 and 12,150 mg cover a broad exposure range, well covering the expected therapeutic exposure required for HSV therapy. The starting dose of 50 mg was ~20-fold lower than the effective and safe dose (15 mg/kg) in immunocompetent Balb/c mice and approximately three-fold lower than the lowest dose (2.5 mg/kg) of HDIT101 that showed an effect in an immunocompromised NOD/SCID mouse model.[13] The maximum dose level of 12,150 mg was estimated to be about four times higher than the most effective dose (45 mg/kg) of HDIT101 that was administered systemically to NOD/SCID mice infected intravaginally with clinical isolates of HSV-2. Moreover, HDIT101 might subsequently also be developed for the treatment of severe herpes sepsis or HSV-mediated encephalitis. These patients carry a very high viral load and may need substantially higher HDIT101 doses for complete virus neutralization.[19]

HDIT101 was well-tolerated and no infusion-related reactions occurred. Only one participant experienced an AE during infusion, an edema caused by extravasation of infusion fluid. However, there was no consecutive local inflammation and no other symptoms suggestive of local toxicity by the extravasation. Approximately 70% of the participants treated with HDIT101 (72.2%) or placebo (66.7%) reported AEs, and half of these AEs were at least possibly related to the study treatment. Overall, the pattern and frequency of AEs were comparable between the participants treated with HDIT101 in increasing doses up to 12,150 mg and the participants in the placebo group. Similarly, also the mean changes in the safety assessments were similar in both groups, with the exception of sporadic reports of transient decreases of leukocyte, lymphocyte, and neutrophil counts associated with the treatment with HDIT101. Pancreas enzymes were elevated in a small number of volunteers on treatment and a similar number on placebo. Considering mean changes from baseline, no clear trends toward specific organ toxicities over time and no distinct dose dependencies were detected.

There was no indication that HDIT was immunogenic. One participant of the 12,150 mg dose group had confirmed anti-HDIT101 ADAs prior to HDIT101 administration, whereas all tests, where concentrations were below the DT, were negative in immunogenicity assessments during the trial. Because ADA levels in the participant with pre-existing ADAs did not increase following HDIT101 infusion, the initial result can be considered most likely false positive on the basis of inherent interference with the assay.

The PK characteristics of HDIT101 in serum are consistent with the general understanding of PKs and disposition of humanized monoclonal antibodies.[20,21] However, because, in our participants, the target HSV gB was most likely absent due to lack of an active HSV lesion in a healthy volunteer population, it remains open whether target-mediated drug disposition will occur in patients with active HSV eruptions, which could be expected in situations with excessive viral load, such as serious systemic HSV infections. A viral target may, however, not have the same strong impact on the PKs as a solid target has. In addition, the target will most likely only be present for part of the exposure, as it is hoped and expected that patients will be clearing the virus prior to the antibody being washed out.

As expected for an antibody, the estimated half-life was long (~11 days) but in the lower range of humanized monoclonal antibodies. The half-life may, however, be underestimated because the concentration decline appeared not strictly mono-exponential and at the end-of-trial visit, the antibody concentration was still measurable. The terminal slope of the kinetic profile therefore, bears some uncertainty which may result in underestimation.

The long half-life and the excellent tolerability of HDIT101 observed in this healthy volunteer trial, at even exceedingly high doses, provided the basis for currently exploring this antibody in two independent randomized clinical phase II trials in the orolabial HSV-1 infection (NCT04539483) or anogenital HSV-2 infection (NCT04165122) settings, respectively.

There are limitations to this study, that should be mentioned. This study has been conducted in healthy volunteers without active HSV infection (i.e., participants not expressing the target of HDIT101), and therefore the PK characteristics of antibodies in a disease situation, which may follow a target-mediated drug disposition, could not be evaluated.[21] Another limitation is that the follow-up period was only 4 weeks and therefore the $t_{½}$ may have been underestimated.

In conclusion, in an FIH trial in healthy volunteers, HDIT101, a humanized monoclonal antibody directed against gB of herpes simplex viruses, was safely administered and showed an excellent safety and tolerability profile up to very high doses and a dose-proportional PK. The results of this study form the basis for continuing clinical development with two phase II studies.

## AUTHOR CONTRIBUTIONS

A.B., N.H., G.M., F.E.S., M.A., T.S., R.L., J.K., and W.E.H. wrote the manuscript. A.B., N.H., G.M., M.A., D.T., B.S.-B., T.S., O.S.-K., J.K., A.M.-S., and W.E.H. designed the research. A.B., G.M., M.D., M.S.-S., E.E., and W.E.H.

performed the research. A.B., N.H., G.M., F.E.S., M.A., T.S., J.K., R.L., O.S.-K., and W.E.H., analyzed the data.

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## CONFLICT OF INTEREST

Antje Blank, received funding for this and other trials from Heidelberg ImmunoTherapeutics GmbH, the sponsor of this trial. Nicolas Hohmann, received funding another trial from Heidelberg ImmunoTherapeutics GmbH. Gerd Mikus, received funding for this trial from Heidelberg ImmunoTherapeutics GmbH. Daniel Thomas, was employed by Heidelberg ImmunoTherapeutics GmbH. Beate Schmitt-Bormann, carried out consulting activities for Heidelberg ImmunoTherapeutics GmbH during study conduct. Torsten Schaller was employed by Heidelberg ImmunoTherapeutics GmbH. Rico Laage was contracted by Heidelberg ImmunoTherapeutics GmbH. Oliver Schönborn-Kellenberger, Biostastician, Cogitars Gmbh, was consulting to Heidelberg ImmunoTherapeutics GmbH. Michaela Arndt, was employed by Heidelberg ImmunoTherapeutics GmbH. Walter E. Haefeli, received funding for this and other trials from Heidelberg ImmunoTherapeutics GmbH. Jürgen Krauss, holds shares in Heidelberg ImmunoTherapeutics GmbH. All other authors declared no competing interests for this work.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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