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# *In vivo* proof-of-concept for two experimental antiviral drugs, both directed to cellular targets, using a murine cytomegalovirus model

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ABSTRACT

Infections with the human cytomegalovirus (HCMV) cause serious medical problems including organ rejection and congenital infection. Treatment of HCMV infections with currently available medication targeting viral enzymes is often accompanied with severe side effects and the occurrence of drug-resistant viruses. This demands novel therapeutical approaches like targeting genetically stable host cell proteins that are crucial for virus replication. Although numerous experimental drugs with promising *in vitro* efficacy have been identified, the lack of available data in animal models limits their potential for further clinical development. Recently, we described the very strong *in vitro* antiherpesviral activity of the NF-κB inhibitor TF27 and the CDK7 inhibitor LDC4297 at low nanomolar concentrations. In the present study, we present first data for the *in vivo* efficacy of both experimental drugs using an established cytomegalovirus animal model (murine CMV replication in immunodefective Rag −/− mice). The main findings of this study are (i) a strong inhibitory potency against beta- and gamma-herpesviruses of both compounds *in vitro*, (ii) even more important, a pronounced anticytomegaloviral activity also exerted *in vivo*, that resulted from (iii) a restriction of viral replication to the site of infection, thus preventing organ dissemination, (iv) in the absence of major compound-associated adverse events. Thus, we provide evidence for a strong antiviral potency *in vivo* and proof-of-concept for both drugs, which may encourage their further drug development, possibly including pharmacologically optimized derivatives, for a potential use in future antiherpesviral treatment.

## 1. Introduction

Human cytomegalovirus (HCMV, family Herpesviridae) is an important human pathogen that establishes life-long latency in the host. HCMV infection has a high prevalence worldwide with seropositivity rates that range between 40% and 95% depending on socioeconomic factors. Primary infection of immunocompetent individuals is either asymptomatic or associated with mild, mononucleosis-like symptoms. In contrast, life-threatening disease can occur in patients with a compromised, suppressed or underdeveloped immune system (Mocarski et al., 2013). Moreover, congenital abnormalities as well as developmental delay may result from HCMV infection during pregnancy, which

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has an incidence of approx. 1–2% in industrialized countries. About 10% of newborns with congenital infection are immediately symptomatic at birth (Hamilton et al., 2015; Rawlinson et al., 2017) and late developmental defects may additionally occur at a similar frequency. Not only primary infection but also reactivation represents a challenging medical issue, particularly in the field of antitumor chemotherapy and transplantation medicine. No vaccine has been licensed so far and treatment options with currently available drugs are limited. Antiviral therapy is currently mainly based on inhibitors of viral DNA synthesis such as ganciclovir (GCV), valganciclovir (VGCV), foscarnet (FOS) and cidofovir (CDV), which all have in common that they can induce drug-resistant HCMV variants thus impairing a long-term efficiency of therapy (Dropulic and Cohen, 2010). Importantly, letermovir (LMV, Prevymis®) has been approved by the U.S. Food and Drug Administration (FDA) very recently, followed by drug approval in a number of other countries, for the prophylaxis of HCMV infection in HCMV-seropositive recipients of allogeneic hematopoietic stem cell transplant (Bowman et al., 2017; Chemaly et al., 2014; Kropeit et al., 2018; Lischka et al., 2016). LMV exclusively targets the viral protein terminase and shows antiviral selectivity for HCMV infection. Thus, based on the fact that drug resistance may also arise during LMV therapy (Chou, 2015; Goldner et al., 2014) and driven by the necessity to extend the repertoire of antiherpesviral drugs, particularly including those exerting a broader range of inhibitory activity, novel compounds are investigated in various scientific settings worldwide. Since HCMV replication is a complex process regulated by a precisely coordinated interplay between numerous viral and cellular proteins, the exploitation of both, virus and host factors, could be a suitable strategy to improve the general problem of selecting drug-resistant viruses. The specific targeting of approved antiviral drugs to cellular proteins and cell-based regulatory pathways has not been achieved to date. Here, we describe two promising compounds both showing pronounced efficacy of *in vitro* antiviral activity and acting through cell-based mechanisms. The modes of antiviral action of LDC4297, a selective cyclin-dependent kinase (CDK) 7 inhibitor, and TF27, a trimeric derivative of the NF-κB-interfering drug artesunate, have been partly characterized by previous studies, mostly achieved by cell culture- and protein biochemistry-based experimentation (Efferth et al., 2008; Hahn et al., 2018 and references therein; Hutterer et al., 2015a and references therein; Hutterer et al., 2015b). In the present study, we present first data for the *in vivo* efficacy of both experimental drugs analyzed in an established cytomegalovirus animal model (murine CMV replication in the immunodefective mouse strain Rag −/−). We provide evidence for a strong antiviral potency, as considering parameters of viral replication efficiency and organ dissemination, and suggest an *in vivo* proof-of-concept for the further development of these or related compounds for their potential use in future antiherpesviral treatment.

## 2. Materials and methods

### 2.1. Antiviral compounds

Antiviral drugs were obtained from the following sources: TF27 (Vichem Chemie Research Ltd., Budapest, Hungary) (Reiter et al., 2015; Hutterer et al., 2015a,b), valganciclovir (VGCV; Sigma Aldrich) and LDC4297 (Lead Discovery Center, GmbH, Dortmund, Germany). The chemical structures of TF27 and LDC4297 are shown in Fig. 1A. Stock aliquots were prepared in DMSO, 5% Transcutol P (Gattefossé, Saint-Priest, France) dissolved in 0.9% NaCl solution (vehicle) or 30% 2-Hydroxypropyl-β-cyclodextrin (cyclodextrin) and stored at −20 °C.

### 2.2. Cultured cells and viruses

Human foreskin fibroblasts (HFFs) were grown in minimal essential medium (MEM, Gibco) supplemented with 10% (vol/vol) fetal bovine serum (FCS, Sigma-Aldrich), 10 µg/ml gentamicin, and 350 µg/ml glutamine. Murine embryonic fibroblasts (MEFs) cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM, Gibco), 10% FCS, 10 µg/ml gentamicin and 350 µg/ml glutamine. HCMV strain AD169-GFP (Marschall et al., 2000) or MCMV Smith-GFP (Mathys et al., 2003) were propagated in HFFs or MEFs and used for GFP-based replication assays (Marschall et al., 2000). $EC_{50}$ values are mean values derived from quadruplicates. *In vivo* experiments were performed using the luciferase-tagged MCMV Smith strain (MCMV-del157luc) (Klenovsek et al., 2007).

### 2.3. CMV GFP-based replication assay and cytotoxicity assay

Cytomegalovirus GFP-based replication assays were performed as described previously (Hutterer et al., 2015a; Marschall et al., 2000). In brief, 200.000 HFFs or MEFs were cultivated in 12-well plates, infected with HCMV AD169-GFP or MCMV Smith-GFP at a MOI of 0.25 (i.e. 25% GFP-forming dose of a multi-round infection measured at 7 d) and treated with antiviral drugs by onetime addition of the drug immediately after virus infection. At 7 days post-infection (d p.i.), the cells were lysed, and the lysates were subjected to automated GFP quantitation using a Victor 1420 multilabel counter (Perkin-Elmer, Germany). All infections were performed in duplicate; GFP quantifications were performed in quadruplicate. Similarly, this assay system was also applied to other GFP-expressing recombinant viruses. Cytotoxicity was measured by neutral red uptake assay (Repetto et al., 2008). HFFs were treated with TF27 at increasing concentrations for 3 d or 7 d. Cells were incubated with 40 µg/ml neutral red (Sigma Aldrich) solution for 3 h at 37 °C. Cells were destained with 1% acetic acid in 50% ethanol solution and neutral red amounts quantitated by fluorometry (excitation/emission at 560/630 nm) in a Victor X4 microplate reader (Perkin Elmer).

### 2.4. Marek's disease virus replication assay

The virulent Marek's disease virus (MDV) strain RB1B was propagated in chicken embryo cells (CEC). Passaged CECs were maintained in MEM (PAN-Biotech, Germany) supplemented with 10% FCS (PAN-Biotech, Germany), 100U/ml penicillin and 100 µg/ml streptomycin (Applichem, Germany) at 37 °C and 5% $CO_2$ in the presence or absence of the respective antiviral compound. MDV replication was assessed by qPCR. In brief, $10^6$ CECs were infected with 100 plaque forming units (pfu) and analyzed at 5 d p.i.. MDV genome copies were determined using specific primers and a probe for the viral gene MDV084 (ICP4). ICP4 copy numbers were normalized against genome copies of the chicken inducible nitric oxide synthase (iNOS) as described previously (Greco et al., 2014).

### 2.5. Quantitative in vitro luciferase assay and quantitative Droplet Digital™ PCR

For performing quantitative *in vitro* luciferase assays, animals were sacrificed, spleen and lung tissues were prepared and directly homogenized in 1 ml Glo Lysis Buffer (Promega) using a Precellys 24 homogenizer (Bertin Technologies, France). Homogenates were centrifuged at 4 °C for 10 min at 14.000 rpm, and protein concentrations were determined using Pierce™ BCA Protein Assay Kit. Determination of luciferase signals were performed in triplicates using 30 µg of each protein lysate and 1 mM D-luciferin. Detection of chemiluminescence was performed with an Orion II microplate luminometer (Berthold Technologies, Germany).

Viral genomes were determined by using Droplet Digital™ PCR (ddPCR) technology (Bio-Rad). Homogenates of dissected organs were used as templates and ddPCR was performed according to the manufacturer's manual. MCMV genome copies were determined using 5′-MCMV (TGCCATACTGCCAGCTGAGA) and 3′-MCMV (GGCTTCATGAT CCACCCT GTT) and the probe 5′-Fam/3′-BHQ1 (CTGGCATCCAGGAA AGGCTTGGTG) for the viral gene immediate early 1 (IE1).

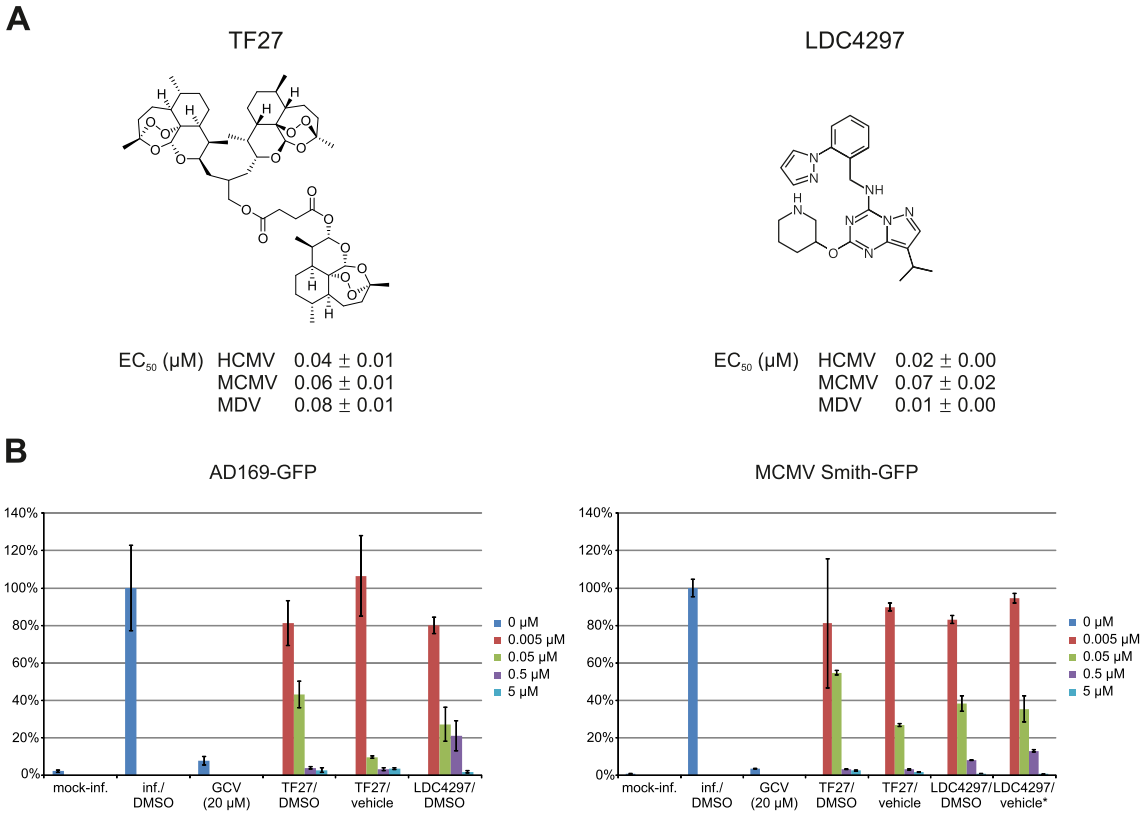


**Fig. 1. Antiviral activity of TF27 and LDC4297**. (A) Chemical structures of artesunate trimer TF27 and the CDK7-specific inhibitor LDC4297. $EC_{50}$ values of respective compounds for different herpesviruses are displayed in μM (Hutterer et al., 2015a). (B) To quantitate antiviral activity, TF27 and LDC4297 were assayed for their antiviral activity in a GFP-based replication assay using HCMV AD169-GFP for the infection of HFFs or MCMV Smith-GFP for infection of MEFs (MOI of 0.01). Antiviral compounds were added immediately p.i. at the concentrations indicated. Cells were lysed 7 d p.i. to perform quantitative GFP fluorometry. Mean values were calculated from determinations in quadruplicate. Standard deviations (SD) are given in black bars. *, LDC4297 was dissolved in 30% cyclodextrin.

### 2.6. Animal models

B6.129S7-*Rag1*$^{tm1Mom}$/J mice (Rag −/−, 6 weeks old) were purchased from the Jackson Laboratories, maintained under specific pathogen free conditions and utilized between 6 and 8 weeks of age. Caging was performed in groups of 5 mice and body weight was monitored on days 0, 5 and 7 post-infection (p.i.). Animals were infected with MCMV at $5.0 \times 10^5$ PFU intraperitoneal (i.p.) in a final volume of 100 μl PBS or remained mock-infected. Antiviral compounds were administered daily (d 1 to d 6) via the intraperitoneal route (i.p.) using 5% Transcutol in 0.9% NaCl as solvent and also as vehicle control. For VGCV, we used 30 mg/kg/d as it was described previously (Marschall et al., 2013). With regard to TF27 and LDC4297, both drugs were analyzed for solubility in 5% Transcutol and concentrations were designated to 5 mg/kg/d (TF27) and 20 mg/kg/d (LDC4297). Mice were utilized for *in vivo* imaging (5 d p.i.) and blood sampling (6 d p.i.). At 7 d p.i., mice were sacrificed, spleen and lung were dissected and stored at −80 °C. Experimental protocols were reviewed and approved by the Regierung von Mittelfranken, Würzburg, Germany (permit 55.2-2532-2-416; Jun 06, 2017). All parameters of *in vivo* experiments are summarized in Table S2.

### 2.7. In vivo imaging of luciferase-based bioluminescence

Mice were anesthetized with isoflurane and placed on a heated bed at 37 °C of an *in vivo* optical imaging system (IVIS Spectrum, Perkin Elmer). The bioluminescence signal in mice was acquired 10 min after intraperitoneal administration of luciferin (150 mg/kg body weight) using 1 min of exposure and binning set to 8 with a field of view of 13.2 cm. In the respective regions of interest, the total flux (in photon per second) was acquired.

### 2.8. Determination of compound plasma levels by mass spectrometry and NMR

Blood samples were collected from MCMV-infected or mock-infected mice at 6 d p.i., 30 min after the administration of inhibitors. The plasma concentrations of TF27 from *in vivo* studies were determined by NMR. Samples were extracted with 1 ml dichloromethane. 40 μl of the extract were diluted with a dichloromethane: acetonitrile (1:1) mixture, which was also used for the background measurement, and analyzed via APPI method (Instrument: maXis, 288882.20183). For NMR analysis, samples were collected and extracted with dichloromethane ($3 \times 1.5$ ml). The combined organic phases were dried over $MgSO_4$ and the solvent was evaporated. The resulting residue (0.1 mg) was dried and dissolved in $CDCl_3$ for the measurement (Bruker, 600 MHz).

The plasma concentrations of LDC4297 and TF27 from *in vivo* studies were determined by LC-MS/MS analysis. For this purpose, plasma proteins were precipitated by addition of one volume of 100% acetonitrile containing an internal LC-MS/MS standard and samples were filtered. A calibration curve was obtained from blank plasma samples spiked with a titrated concentration of LDC4297. The probes were measured using a Shimadzu UPLC system connected to a QTrap 5500 hybrid triple quadrupole/linear ion trap mass spectrometer (AB Sciex). The regression equation of the calibration curve was used to calculate plasma concentration of LDC4297 in each probe.

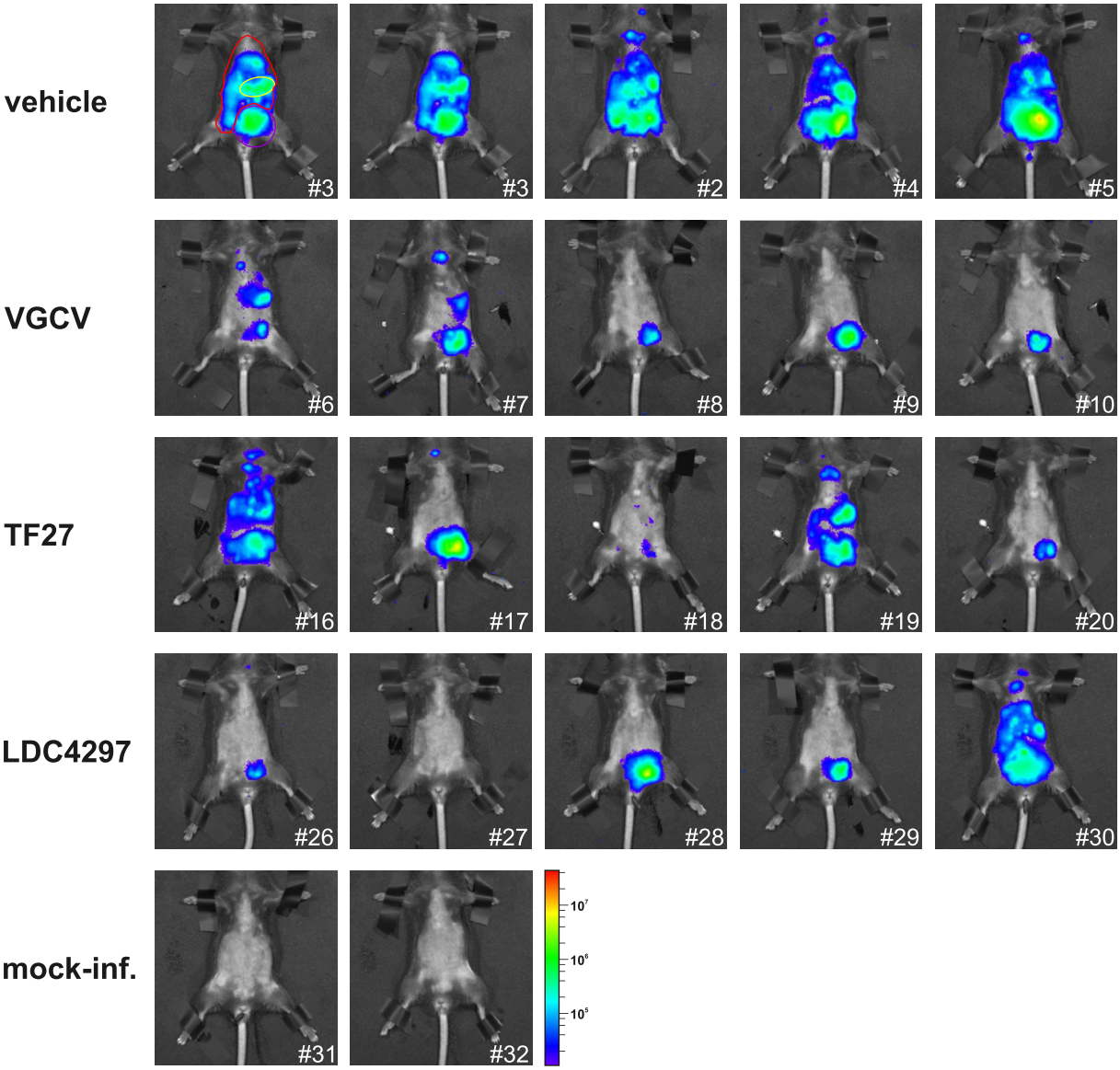


**Fig. 2. Impairment of viral *in vivo* replication and organ dissemination by TF27 and LDC4297 treatment**. Luciferase reporter system was used for *in vivo* imaging of MCMV-infected mice. Rag −/− mice were i.p. infected ($5 \times 10^5$ PFU) with MCMV-del157luc. Antiviral drug treatment was performed daily with 30 (VGCV), 5 (TF27) or 20 (LDC4297) mg/kg/d. At 5 d p.i., animals were anesthetized and analyzed for viral replication by *in vivo* luciferase imaging. Distinct areas used for quantification are highlighted in purple (site of infection, primary replication), red (virus dissemination, secondary replication) or yellow (spleen-specific replication).

## 3. Results and discussion

### *3.1. TF27 and LDC4297 exhibit a strong and broad inhibitory activity against herpesviruses*

The very promising anti-HCMV potential of TF27 (chemical class trioxanes/sesquiterpenes) had been confirmed by previous studies, as based on the proven interference with the NF-κB pathway (Efferth et al., 2008; Hahn et al., 2018; Hutterer et al., 2015b). LDC4297, a compound of the chemical class pyrazolotriazines, selectively inhibits CDK7 *in vitro* and shows a high antiviral potency against various DNA viruses (Hutterer et al., 2015a). Concerning HCMV infection, $EC_{50}$ and $CC_{50}$ values have been determined for LDC4297 in detail before, i.e. 0.02 ± 0.001 μM and 5.22 ± 0.50 μM at 7 d (Hutterer et al., 2015a). For TF27, an HCMV-specific $EC_{50}$ value of 0.04 ± 0.01 μM was published (Hutterer et al., 2015b), and novel data produced here show $CC_{50}$ values in primary human fibroblasts (HFF) of 42.6 ± 0.30 μM at 3 d and 24.3 ± 1.3 μM at 7 d (summarized in Table S1). Interestingly, very early assessments (24 h) did not indicate any cytotoxicity for TF27 at all, and a comparison of confluent HFF layers (standardly used) to subconfluent layers resulted in approx. 2-fold increased sensitivity. A pronounced *in vitro* anti-HCMV and anti-MCMV activity was demonstrated for both groups of compounds before (Hutterer et al., 2015a, 2015b; Hahn et al., 2018; Fröhlich et al., 2018), whereby a pretreatment strategy could substantially increase the efficacy of artesunate-related compounds, underlining their cell-targeted mode of action (Efferth et al., 2008; Chou et al., 2011). For LDC4297, similar pretreatment/time-of-addition experiments will have to be performed in a future study.

The present study extended the characterization and the antiviral potency of both compounds *in vitro* and further data were added for selected herpesviruses, i.e. HCMV, MCMV and Marek's Disease virus (MDV), with $EC_{50}$ values at nanomolar concentrations (Fig. 1A). All three herpesviruses are relevant for human or animal disease. MCMV is considered as a model of HCMV infection and thus was investigated here in a murine *in vivo* system, while MDV represents a model for

tumor-associated herpesvirus infection and will be further described elsewhere. Concerning MCMV infection, GFP-based replication assays displayed an $EC_{50}$ for TF27 of 0.06 ± 0.01 μM (Fig. 1). Moreover, these antiviral activities are compatible with solvents suitable for *in vivo* administration (Transcutol or cyclodextrin) and were capable of reducing CMV replication in a dose-dependent manner (Fig. 1B). With these results, we further illustrated the previous finding that both compounds are highly biologically active in a nanomolar range and display broad inhibitory activity against a selection of human and animal herpesviruses.

### 3.2. Demonstration of a potent anti-MCMV effect by TF27 and LDC4297 in vivo

In order to investigate the antiviral capacity of both compounds *in vivo*, an established animal model using Rag −/− mice was utilized (Marschall et al., 2013). For intraperitoneal (i.p.) infection, a recombinant MCMV strain harboring a luciferase expression module was used (Klenovsek et al., 2007). The inhibitors VGCV (30 mg/kg/d), TF27 (5 mg/kg/d), LDC4297 (20 mg/kg/d) or vehicle control (5% Transcutol) were administered daily via an i.p. route and caging was performed in groups of five. Individual animal parameters, such as body weight, were determined on days 0, 5 and 7 p.i. and behavioral characteristics were monitored daily (Table S2). All mice displayed no drug-related side effects or toxicity at the used concentrations. During the course of experimentation, weight gain or loss of the mice remained in a normal and acceptable range and did not show drug-related variation within the investigated cohort of animals. At 5 d p.i., animals were anesthetized and analyzed for viral replication by *in vivo* luciferase imaging. Notably, almost all of compound-treated mice showed a reduced replication in comparison to vehicle-treated group (Fig. 2, compare animals #6-30 with #2-5). Moreover, viral organ dissemination excluding salivary glands (#3, red circle) was impaired and replication was markedly restricted to sites of infection (purple circle; i.e. #18, 20 and 26) or completely abrogated (#27). It should be mentioned that in one of five investigated mice the treatment effect was poorly detectable (#16 and #30) which may be due to a technical limitation (possibly arising from suboptimal handling of mice at certain steps of drug administration). No luciferase signals were seen when mice were mock-infected (#31-32). Thus, TF27 and LDC4297 allow mild replication in primary sites after i.p. infection, however, systemic viral spread seems to be strikingly decreased.

To quantitate the observed bioluminescence signals, we focused on three distinct areas of the murine abdominal part, namely site of infection (primary replication, purple circle), virus dissemination (secondary replication, red circle) and specifically on spleen-specific replication (yellow circle, Fig. 2, #3). In line with our *in vivo* imaging results, all quantitated areas revealed diminished virus loads in the respective replication sites (at least 75%) (Fig. 3). Statistical evaluation by ordinary one-way ANOVA analysis indicated a significant reduction of viral replication in sites of infection by VGCV and LDC4297, whereas, TF27 was not significant (Fig. 3A). However, a highly significant effect was observed for all compounds in sites of secondary replication and for spleens when compared to the vehicle control (Fig. 3B and C). High standard deviations resulted from variable antiviral effects of administered compounds within the individual mice (compare panels in Fig. 2). Thus, the relatively low concentrations of 5 mg and 20 mg administered for TF27 and LDC4297, respectively, produced similar efficacies as a 30 mg concentration of the potent anti-HCMV reference drug VGCV. Furthermore, TF27 and LDC4297 did not exclusively inhibit primary viral replication but also prevented systemic dissemination of virus to the relevant organs.

Recent data provide evidence that spleen regulates viremia and serves as a transfer hub for viral systemic dissemination (Zhang et al., 2016). For quantitative evaluation of spleen-specific viral replication, animals were sacrificed seven days post-infection and spleen tissues

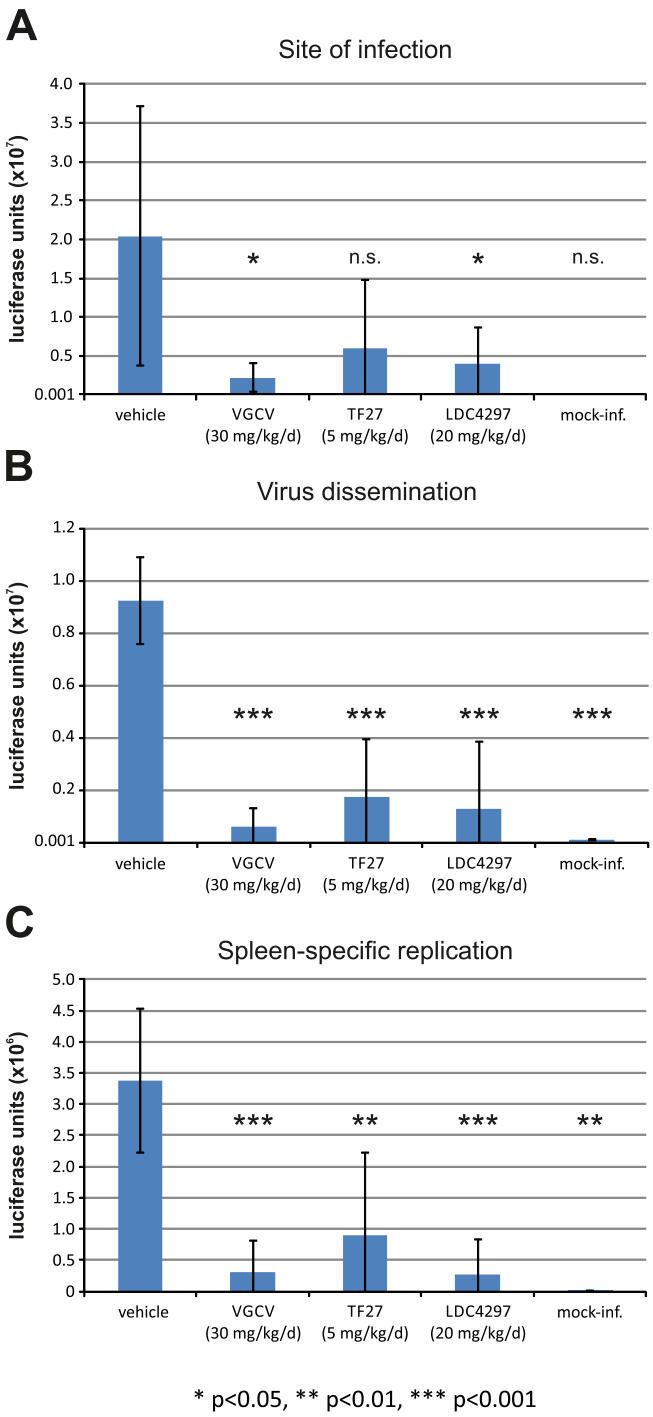


**Fig. 3. Quantitation of *in vivo* bioluminescence signals**. Defined areas of the murine abdominal part were used for quantitation of viral load. Evaluation of signals for (A) site of infection (primary replication), (B) virus dissemination (secondary replication) or (C) spleen-specific replication were performed using Living Image 4.5. The statistical significance was determined by ordinary one-way ANOVA analysis performed in GrahpPad Prism. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s., not significant.

were prepared to homogenates. Equivalent protein concentrations were adjusted and used for measurements of luciferase activity under standard buffer conditions (Klenovsek et al., 2007) (Fig. 4). In addition, homogenates were subjected to ddPCR in order to determine MCMV genome levels of infected spleens. In line with our *in vivo* imaging results, treated mice exhibited a markedly reduced virus load in individual spleens (Fig. 4A and C, #5-30). Of note, viral loads of mice

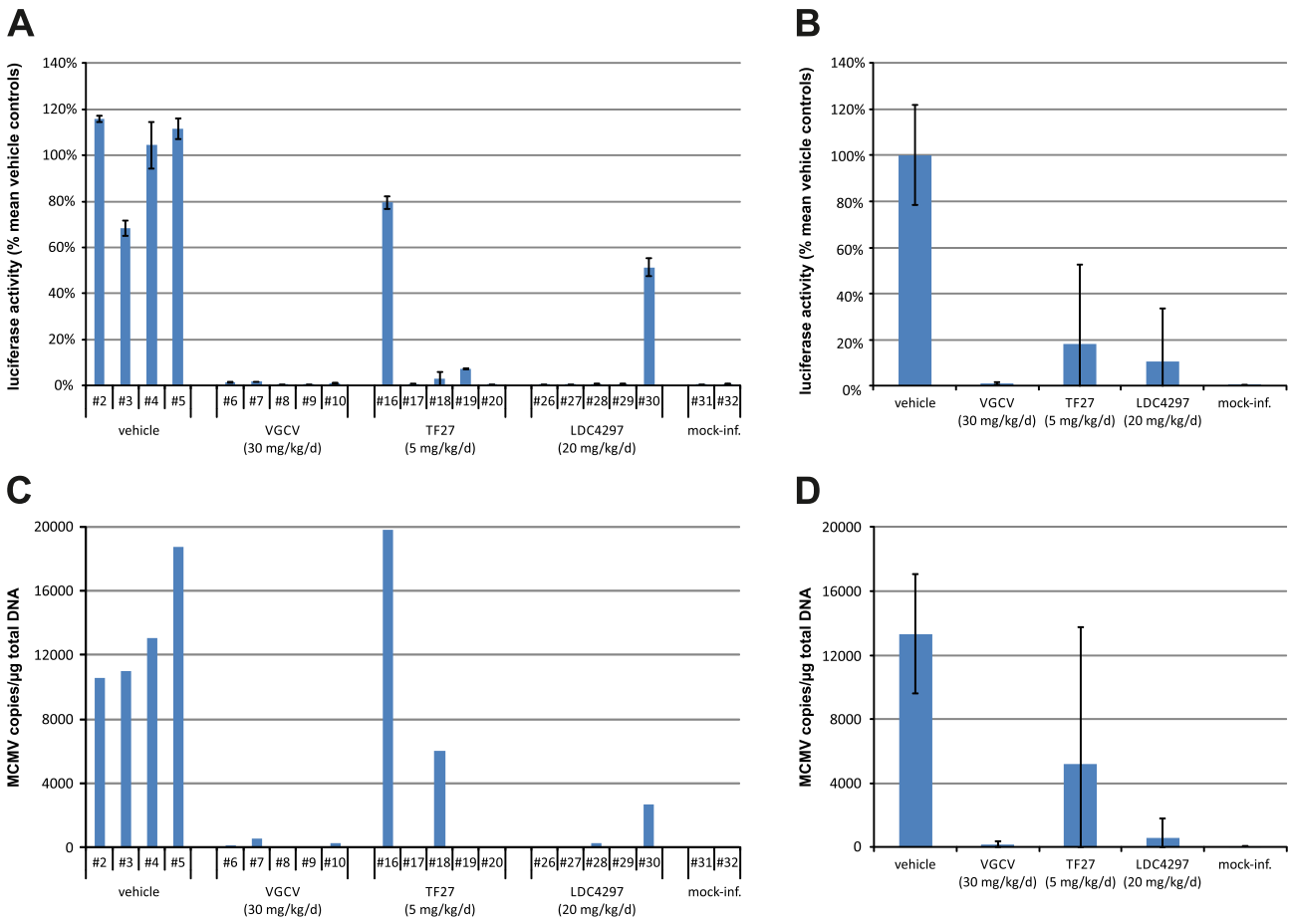


**Fig. 4. Determination of viral genomic load in spleen samples of infected mice**. Mice were sacrificed at 7 d p.i. and spleen samples were dissected and homogenized. For determination of luciferase signals, homogenates were subjected in triplicates to quantitative *in vitro* luciferase assay. Luciferase activity of individual mice (A, determination in triplicate) or groups of mice (B, mean values ± SD) are given in relation to the vehicle control group. Viral genomic load was determined by ddPCR. Homogenates of spleens were used for amplification of the HCMV IE1 region. Genome copy numbers of individual mice (C, single determinations) or groups of mice (D, mean values ± SD) are given.

#16 and #30 were substantially higher than signals of the remaining mice within the same groups, thereby confirming the less pronounced viral restriction as seen in Fig. 2. Consistent with our *in vivo* quantification, luciferase mean values of each group indicate a high reduction of viral loads below 20% in comparison to vehicle group (Fig. 4B). The reduced MCMV genomic load of each group suggests an inhibitory effect of both compounds not exclusively on protein production but additionally on the level of genomic replication (Fig. 4D).

### 3.3. Determination of plasma levels of the antiviral compounds

Finally, blood samples were taken 30 min after inhibitor application from MCMV-infected or mock-infected mice in order to determine the plasma levels of TF27 or LDC4297 by mass spectrometry (MS) analysis (Table S3). For 20 mg/kg LDC4297, mean plasma concentration was 560.14 ng/ml, as determined 0.5 h post-administration. Notably, experiments using 100 mg/kg/d showed a plasma concentration of 1297.6 ng/ml and a bioavailability of 97.7% for 8 h under same experimental conditions (Hutterer et al., 2015a). Here, we could demonstrate a high antiviral activity with considerable fewer amounts of LDC4297, whereas, mean plasma concentrations were approximately two times less. Hence, treatment with lower doses of LDC4297 might achieve a similar antiviral efficacy as high amounts. With regard to TF27, MS analysis and nuclear magnetic resonance (NMR) have been performed, however, TF27 amounts were below quantifiable levels (Table S3). Precise data regarding the stability of TF27 in biological systems are still lacking and could give insights in to half-life or conversion to metabolites. Initial experiments revealed that the activity remains stable for least seven days in cell culture-based experiments when TF27 is added to media and incubated under standard conditions. However, *in vivo* degradation or conversion into few or multiple active metabolites may occur at different rates. On the other hand, TF27 uptake from peritoneum to blood could proceed with strongly delayed kinetics resulting in negative measurements. Despite the missing detection of TF27 in plasma, an antiviral capacity was still demonstrated. Thus, TF27 might not be stable in its original, unmetabolized structure, but may, at least partly, be converted into primary (e.g. dihydroartemisinin) and secondary metabolites with so far unknown chemical identity.

## 4. Conclusions

Our data provide evidence that TF27 and LDC4297 exhibit a high antiherpesviral activity *in vivo*. In particular, viral dissemination was mainly restricted to sites of infection, which could be confirmed by various biochemical and imaging techniques. Strikingly, treatment with both compounds led to no compound-related adverse effects. With regard to LDC4297, plasma concentrations remained multiple times higher than the *in vitro* $EC_{50}$ value. Despite its high anti-MCMV activity, TF27 was not detectable in plasma samples. It is tempting to speculate that TF27 does not remain present in its unmetabolized structure, but may rather be converted into active metabolites, which may maintain

sufficient plasma levels for a longer time period and restrict viral spread *in vivo*. Further chemical analyses of TF27 might provide insights into the pharmacokinetic properties. Combined, the data provide first evidence that both TF27 and LDC4297 possess a high antiviral potential *in vitro* and *in vivo* and will be very helpful for further antiviral compound research. After an optimization of parameters like solubility, treatment conditions and dosing strategies, these experimental compounds might even be considered as candidates of future drug development.

## Competing interests

The authors have declared that no competing interests exist.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2018.11.008.

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