

Final report on the DZIF project

TTU 05.915 - Exploring the potential of circular epsilon RNA decoys for HBV treatment

Coordinating institution:

Ruprecht-Karls-Universität Heidelberg

Participating institution(s):

Universitätsklinikum Freiburg

Executive Summary/Abstract

Chronic HBV infection is associated with a high risk of developing potentially fatal liver cirrhosis and hepatocellular carcinoma, accounting for almost 1 mio deaths annually. Current HBV therapies are inefficient in achieving cure highlighting the need for novel therapeutic strategies. HBV replicates its DNA genome by reverse transcription of an RNA intermediate (pregenomic (pg) RNA) within nucleocapsids. Reverse transcription requires co-packaging of pgRNA together with the viral polymerase (P protein) which is critically dependent on binding of P to the pgRNA element epsilon. Hence, interference with epsilon-P interaction should have a profound dual impact on replication due to prevention of both, pgRNA packaging and DNA synthesis, rendering the epsilon-P complex an attractive novel antiviral target. We aimed to achieve this goal by a decoy strategy using artificial epsilon RNA aptamers to compete with authentic, pgRNA resident epsilon elements for P binding. In this project we have engineered highly stable, circular („non-linear“) epsilon RNA decoy (NERD) aptamers. In preliminary experiments we could detect efficient aptamer specific inhibition of HBV replication by transfection of plasmids capable of intracellular production of NERDs into HBV replicating cells. To translate this novel approach into HBV therapy we aimed to develop a NERD based RNA drug. Recently powerful RNA therapeutics based on mRNA and siRNA have emerged. Instrumental for the success were breakthroughs in RNA delivery by lipidnanoparticles (LNPs). In this 12 month flexfund project we employed established LNP technology to produce NERD-LNPs for cellular delivery into HBV replicating cells. Antiviral activity of NERD-LNPs was tested in stably HBV transfected, Tet-off regulated hepatoma cells and in HBV infected NTCP-HepG2 cells. In summary, our data demonstrate proof-of-principle of epsilon aptamer mediated inhibition of HBV replication and provide a basis for future drug development.

Chapter 1: Short report

a) Original task and scientific/technical status built on

Hepatitis B Virus (HBV) is one of the most important human pathogens. Globally about 250 million people are chronically infected with HBV and live with a high risk of developing potentially fatal liver fibrosis, cirrhosis and hepatocellular carcinoma, which accounts for more than 800,000 deaths annually. Current treatment regimens rarely lead to functional cure highlighting the need for novel therapeutic strategies. A yet unexplored anti-HBV target is the RNA-protein complex of the HBV pregenomic (pg) RNA element epsilon and the HBV P-protein. Functional epsilon-P interaction is critical for two essential steps in HBV replication, namely pgRNA packaging into nucleocapsids and subsequent reverse transcription of pgRNA into mature viral DNA (Fig. 1A). These critical roles in replication render epsilon an attractive new antiviral target with likely very low escape potential. From a therapeutic perspective interference with epsilon-P interaction should inhibit virion production by prevention of pgRNA packaging and DNA synthesis.

To achieve this goal we aimed to employ synthetic epsilon RNA aptamers as decoys to compete with authentic pgRNA epsilon for P binding. If epsilon aptamers are in excess over pgRNA, P should preferentially bind to the aptamers and less efficiently to pgRNA. This should lower the yield of packaged pgRNA, since pgRNA packaging is strictly dependent on P protein binding (Fig. 1B).

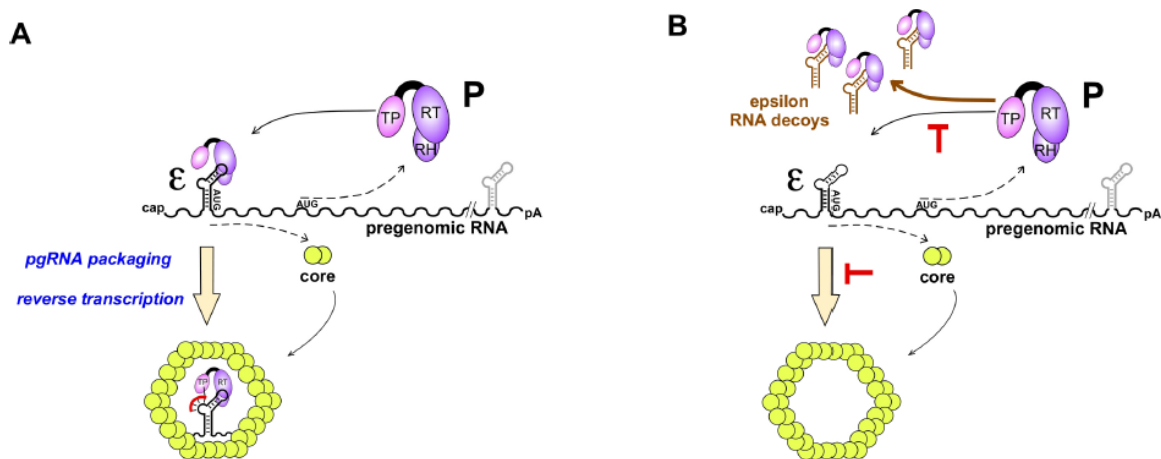


Fig. 1. (A) Roles of the pgRNA element epsilon in HBV replication. Epsilon is essential for packaging of pgRNA into capsids and contains the replication origin and is therefore indispensable for reverse transcription of the pgRNA into DNA. Both functions are strictly dependent on interaction with the HBV polymerase (P protein). **(B) Principle of the epsilon decoy strategy.** Short artificial epsilon RNA decoys compete with authentic pgRNA epsilon for P protein binding and impair pgRNA packaging and reverse transcription.

A major challenge in the decoy approach is generation of sufficient intracellular levels of RNA aptamers. Many intracellular RNAs are relatively short-lived and RNA steady-state levels are limited by RNA degradation. Circularization of RNA can dramatically improve the RNA half-life by orders of magnitude. Expression systems for generation of artificial circular RNAs have recently been developed. Of those the TORNADO (Twister-optimized RNA for durable over expression) system is particularly suited for expression of small structured aptamer RNAs like epsilon (*Nat Biotechnol* (2019) 37, 667–675). In these constructs the RNA sequence of interest is flanked by two cis-acting ribozymes. Ribozyme self-cleavage events cut out the RNA aptamer and the ubiquitous cellular tRNA ligase RtcB ligates the termini to form highly stable circular RNA aptamers.

In an unpublished pilot study we have already shown that circular epsilon RNA decoys (termed „NERD“ [non-linear epsilon RNA decoy]), if produced intracellularly from transfected TORNADO

plasmids, are potent inhibitors of HBV replication in human hepatoma cells. These data provided the scientific basis for the current flexfund project.

The ultimate/long-term goal of this project is to develop a NERD-based anti-HBV RNA drug. Recent breakthroughs in in vivo delivery of RNA, resulted in powerful, and meanwhile approved, RNA therapeutics based on siRNA („ONPATPRO“ (Alnylam)) and mRNA (Biontech/Pfizer and Moderna SARS-CoV2 vaccines). In these drugs the therapeutic RNA is encapsulated into multifunctional lipidnanoparticles (LNPs), which protect the RNA from RNase attack, promote endocytotic uptake and facilitate endosomal cargo release into the cytosol. LNPs are produced by rapid mixing of an organic lipid solution with an acidic aqueous RNA solution. The negatively charged RNA binds to the ionizable lipid, which is positively charged at low pH, and becomes entrapped in the LNPs. The PEGylated surface prevents LNP aggregation. The size of the LNPs can be adjusted by the fraction of the PEG-lipid in the lipid mix. This is critical for hepatocyte targeting since LNPs have to pass the fenestrae in the liver sinusoidal membrane. In the development of the siRNA drug ONPATPRO LNPs have already been optimized for siRNA delivery into hepatocytes (*Accounts Chem Res* (2019) 52(9):2435-2444). Upon intravenous administration those LNPs accumulate rapidly and efficiently in the liver and allow efficient RNA delivery into hepatocytes. The ONPATPRO LNP formulation and manufacturing protocols have been published and all required lipids are commercially available. In this flexfund project we applied state-of-the-art LNP technology to produce NERD-LNPs and investigated the antiviral effects in vitro in two different HBV cell culture models.

Specific objectives of the project were:

- (i) Synthesis of circular NERD RNAs by in vitro transcription, gel purification and enzymatic ligation (WP1).
- (ii) Encapsulation of NERD RNAs into lipidnanoparticles (NERD-LNPs) by rapid mixing technology (WP2).
- (iii) Biophysical and biochemical characterization of NERD-LNPs (WP2).
- (iv) Monitoring of LNP-mediated RNA delivery to cells (WP3.1).
- (v) Investigation of antiviral effects in the tetracycline regulated inducible HBV cell line HepG2.117 (WP3.1).
- (vi) Investigation of antiviral effects in HBV-infected HepG2-NTCP cells (WP3.2).

b) Course of the project

For this 12 month flexfund project personnel was recruited in time at the planned project start in March 2023 and the project could be immediately implemented without delay. All work packages have been executed according to the time line in the work program and there were no significant adjustments of the work program.

c) Main results and cooperation with other research institutions, if applicable

The major goal of this project was to demonstrate proof-of-principle of in vitro synthesis, cellular delivery and antiviral activity of NERD-LNPs. In this project we successfully devised a multistep-protocol for large scale synthesis of circular epsilon aptamers (NERDs). We managed to efficiently encapsulate these NERD RNAs into lipidnanoparticles (LNPs) of desired size and demonstrated cellular RNA uptake. Antiviral activity of NERD-LNPs was investigated in a stably HBV-transfected cell-line and in an in vitro infection system (the latter in cooperation with Stephan Urban, University of

Heidelberg). In both systems we could observe substantial inhibition of HBV replication by NERD-LNPs. Inhibition was strongly dependent on P protein binding ability of NERD suggesting that NERDs act by the intended decoy mechanism. The developed tools and the generated data provide a basis for further development of epsilon RNA aptamers into an anti-HBV antiviral drug.

Chapter 2: Detailed description

a) Detailed presentation of the results achieved

WP 1

In vitro synthesis of circular epsilon RNAs (NERDs)

For generation of NERDs (Milestone 1) we first had to adapt the Tornado expression system to in vitro synthesis of circular NERD RNAs. In initial experiments we established the RNA synthesis protocol consisting of the following steps: (i) PCR-mediated generation from synthetic oligonucleotides of DNA templates consisting of epsilon sequences (wt and variants) flanked by two self-cleaving ribozymes (ii) production of ribozyme-epsilon RNA precursors by in vitro transcription of the DNA templates, (iii) processing of the RNA precursors by ribozyme cleavage in cis at both termini, (iii) denaturing polyacrylamide gel electrophoresis (PAGE) purification of the processed epsilon RNA fragments, (iv) circularization of the epsilon RNA fragments using RtcB ligase, (v) PAGE purification of the circular ligation product.

Initially we have established the protocol in small scale pilot experiments and demonstrated feasibility of circular NERD synthesis. Preparative RNA synthesis by in vitro transcription was performed in 200 µl scale using the T7 RNA Pol Ampliscribe kit (Lucigen). Ribozyme cleavage was about 90% efficient for epsilon wt and the P protein binding deficient variant "delta bulge", which was used as specificity control. Due to the high demand of RtcB ligase for large scale NERD production we cloned, expressed and purified E. coli RtcB ligase. Our home made enzyme prep is highly active and achieved circularization yields of $\geq 90\%$ even in large scale ligations of 100 µg RNA. Efficacy of circularization was analysed by denaturing PAGE, which separates the linear from the circular RNA conformation. The quality of the final purified circular NERD RNAs was checked by denaturing PAGE and consisted of a single band. Circularity was judged by exonuclease treatment. RNA concentrations were measured photometrically. The final yields of purified circular NERD RNAs were usually ≥ 150 µg from 200 µl in vitro transcription reaction.

In summary, quality and quantity of purified NERD RNAs were sufficient for subsequent NERD-LNP production (Milestone 1). Due to the high demand of NERD-RNAs in our subsequent experiments the circular RNAs had to be resynthesized 2 times later on.

We also intended to include epsilon variants with enhanced P protein affinity which have been screened in our lab in an unrelated unpublished study. However, for unknown reason those constructs displayed very poor ribozyme cleavage and the yields of circular RNA were too low for NERD-LNP production. Nevertheless we included these epsilon RNA variants in their linear conformation in our study and purchased the corresponding synthetic RNAs from commercial vendors.

WP 2

Production of NERD containing Lipidnanoparticles (NERD-LNPs)

In this work package we produced LNPs containing the RNAs synthesized in WP1. Recent data proved that LNPs are efficient vehicles for in vitro and in vivo RNA delivery. Furthermore, LNPs have been optimized for targeting hepatocytes. Thus production of NERD-LNPs (Milestone 2) appeared to be straightforward and was approached by using established and published protocols for the approved hepatocyte targeted siRNA therapeutic ONPATRO. LNPs consisting of 4 different lipids (Cholesterol, DSPC, DLin-MC3-DMA, DMG-PEG 2000) in optimised proportions plus various cargo RNAs were produced in collaboration with lipid biochemist Dr. Gerhard Pütz (Dept. of Clinical Chemistry, Universitätsklinikum Freiburg). NERD-LNPs were generated in a microfluidic mixing chamber by simultaneous injection of a lipid solution in organic solvent and an acidic aqueous solution containing 100 µg of the synthesized NERD RNAs. Rapid mixing in the Y-shaped herringbone capillary results in LNP assembly. RNA becomes entrapped in the LNPs by ionic interaction with the ionizable lipid DLin-MC3-DMA, positively charged at acidic pH. Upon exchange of the acidic buffer against PBS by ultrafiltration the LNP preparations were ready for use. LNPs were stored at 4 C and appeared to be stable for at least several weeks. Before use in WP3, LNPs were quality controlled. Nanoparticle formation was demonstrated by Taylor dispersion analysis (TDA) using FIDA technology (Fidabio) recently established in the lab of Dr. Pütz. LNP preparations appeared to consist of monodisperse particles with hydrodynamic diameters of 92 to 105 nm, which is in agreement with the intended size of 80-100 nm. RNA packaging into LNPs was monitored by resistance to RNase digestion. Typically more than 90% of the input RNA was protected from digestion, demonstrating efficient RNA packaging into LNPs. In addition to circularized RNAs, linear epsilon wt NERD precursor RNA was encapsulated to examine the role of RNA circularity for replication inhibition. Furthermore, we also generated LNPs containing a validated anti-HBV siRNA as positive control for LNP-mediated RNA delivery into HBV replicating cells. As a corresponding negative control we produced LNPs containing siRNA against Green Fluorescent Protein (GFP) which is absent from our assays. Finally we prepared LNPs containing high affinity eps variants as described above in WP1. Due to the high demand of LNPs in our subsequent experiments each LNPs had to be resynthesized 2 times. In summary, all aims of Milestone 2 were reached.

WP 3

Functional testing of NERD-LNPs in HBV cell culture models

WP 3.1

Antiviral activity of NERD-LNPs in stably HBV transfected cells

Before functional testing of NERD-LNPs in HBV cell culture models we first monitored LNP-mediated RNA delivery into cells (Milestone 3). We used two independent approaches. In the first approach we packaged a commercially available, fluorescently labelled small RNA targeted to the nucleus (siGLO, Horizon Biology) into LNPs, which allows direct microscopic visualisation of internalization of LNP-delivered RNA. Upon addition of siGLO-LNPs to HepG2 cells the majority of cells became fluorescently labeled demonstrating efficient cellular RNA delivery. Unpackaged free siGLO RNA did not stain the cells indicating LNP-mediated delivery. In the second approach we transfected a

chimeric luciferase-HBV reporter plasmid containing the target site for our anti-HBV siRNA. Treatment of the transfected cells with anti-HBV siRNA-LNPs dropped the level of luciferase expression by >20-fold, whereas application of the free siRNA did not affect luciferase activity. Hence, both methods consistently demonstrate efficient LNP-mediated delivery of functional RNAs into HepG2 cells (Milestone 3). The methods we used are superior to the methods originally suggested in the project description (qRT-PCR or Northern blotting), because they unambiguously demonstrate cellular uptake of functional RNA into cytoplasm and nucleus. In contrast, qRT-PCR and Northern blotting require isolation of RNA and are not able to discriminate between RNA released into the cytoplasm and non-functional RNA trapped in LNPs bound to cells.

Antiviral effects of NERD-LNPs were tested in the stably HBV transfected hepatoma cell line HepG2.117 (Milestones 4+5). In this cell line the full-length wt HBV genome is integrated into the cellular genome. HBV replication is tightly regulated on the level of pgRNA transcription by a tetracycline responsive promoter and can be induced by withdrawal of tetracycline (TetOFF system). To test antiviral activity of LNPs we first induced HBV replication in HepG2.117 cells, and then applied LNPs twice per week. After 2 weeks cells were lysed, the HBV capsids in the cytoplasmic fraction containing replicated DNA were resolved by native agarose electrophoresis (NAGE) and blotted in parallel onto nylon and nitrocellulose membranes. HBV capsid protein was detected on nitrocellulose membranes by Western blotting. Replicating HBV DNA in capsids immobilized on nylon membranes was detected by Southern blotting using a radiolabeled anti-HBV probe. To measure virion production HBV-DNA was extracted from cell culture supernatants and analysed by qPCR. Production of the viral antigens HBs and HBe was monitored using commercial ELISA kits. In initial experiments we titrated the amount of LNPs which were applied to the cells and measured the antiviral effects and cytotoxic activities (CellTiter Blue Cell Viability Assay). We identified an optimal LNP dose, characterized by no significant cytotoxicity and high antiviral activity, which was routinely used in all experiments. Generally the toxicity of the LNPs was low. Even a very high dose of NERD-LNPs (containing 10 µg of packaged RNA) when applied on a well of a 12-well plate of HepG2.117 cells was only moderately cytotoxic. Importantly, we could detect sequence-specific inhibition of HBV replication by NERD-LNPs (Milestone 4), which was the main goal of the project. Treatment with LNPs containing circular wt epsilon RNA suppressed production of intracellular capsid DNA and extracellular virion DNA up to 5-fold (Milestone 5). In contrast, the P-protein binding deficient epsilon variant „delta bulge“ did not significantly inhibit HBV replication, corroborating the intended decoy mechanism of replication inhibition mediated by direct interaction between epsilon and P. In agreement with targeting of P we found that NERD-LNPs did not affect HBV antigen expression. The amounts of capsid protein, Hbs and HBe were not significantly affected. Surprisingly, we could not detect any significant difference in replication inhibition between circular and linear epsilon RNAs questioning the proposed benefit of circular RNAs. However, this outcome may be related to the specific design of the experiment, where we repeatedly administered LNPs in short time intervals which may lead to saturating intracellular concentrations of epsilon.

We also employed epsilon variants screened for high P-protein affinity in vitro (linear conformation, packaged in LNPs). However, none of the tested epsilon variants was superior in antiviral activity to wt epsilon suggesting that P affinity of epsilon may be different in vitro and in cells, or that other factors are rate-limiting in the cellular environment. The anti-HBV siRNA-LNPs, originally implemented as positive control for LNP-mediated delivery, inhibited HBV replication extremely potently. Replicating HBV DNA was undetectable in highly sensitive radioactive Southern blots, suggesting that RNA delivery by our LNPs is highly efficient. To further evaluate the efficacy of LNP-mediated RNA delivery we performed siRNA and NERD-RNA transfections using commercial RNA

transfection reagents (Horizon Dsiccovery). While NERD-RNAs inhibited HBV replication to a similar extend with both delivery systems, anti-HBV siRNA was more efficient when delivered by LNPs suggesting that our LNP-based RNA delivery system is at least as efficient as state of the art transfection reagents. We also used the method of RNA transfection to test the antiviral activity of epsilon variants which could only be produced in low amounts in circular form (insufficient quantities for LNP production). We found that none of the tested variants inhibited replication significantly better than circular wt epsilon.

In, summary the presented data demonstrate that we have completed milestones 3, 4 and 5.

WP 3.2

Antiviral activity of NERD-LNPs in HBV infected cells

In addition to the stably transfected HepG2.117 cell line, antiviral effects of NERD-LNPs were investigated in an in vitro infection system in collaboration with Stephan Urban's lab (University of Heidelberg). Na⁺-taurocholate cotransporting polypeptide (NTCP) is an essential receptor for HBV infection. Due to strong down-regulation of NTCP the hepatoma cell line HepG2 is not infectable by HBV. However, this block can be overcome by overexpression of NTCP. The Urban lab has generated infectable HepG2 cells by lentiviral transduction of NTCP. These HepG2-NTCP cells have been used in this work package to investigate the effects of NERD-LNPs on HBV replication in HBV infected cells. HB Virus stocks subsequently used for infection of HepG2-NTCP cells were produced by large scale propagation of HepG2.117 and subsequent concentration of HBV virions from collected supernatants by PEG precipitation. Virus stocks were analysed for content of viral genome equivalents by NAGE and southern blotting with radiolabeled probes. Virus stocks were further tested for their ability to infect HepG2-NTCP cells. Infection yielded robust amounts of secreted HBs and HBe (both detected by ELISA) and of intracellular HBc (in situ fluorescence hybridization). Viral antigen expression diminished to base-line in cells treated with the entry inhibitor Myrcludex B. Thus, detected antigens were derived from infected cells and not from the virus inoculum demonstrating NTCP-dependent viral infection. LNP-mediated RNA delivery into HepG2-NTCP was observed by fluorescence microscopy using siGLO-LNPs.

To test antiviral activity of NERD-LNPs HepG2-NTCP cells were first infected with HBV, and then LNPs were applied twice per week. After 2-3 weeks cells were lysed and DNA was extracted from lysates (for analysis of replicative HBV intermediates) and from supernatants (for analysis of HBV virion DNA) using commercial DNA extraction kits. Production of the viral antigens HBs and HBe was monitored by ELISA. LNP-mediated delivery of anti-HBV siRNA dropped HBs and HBe levels to near background indicating efficient RNA delivery into infected cells. As expected from their mode of action, NERD-LNPs did not significantly affect HBs and HBe levels. Due to low amounts of replicating DNA in infected cells we initially intended to switch the method of HBV DNA detection and quantification from capsid NAGE-Southern blot (as performed in WP3.1) to highly sensitive qPCR. Our qPCR data revealed about 10-fold reduction of HBV DNA in supernatants of cells treated with anti-HBV siRNA-LNP, and 2-3 fold reduction by wt epsilon NERD-LNP (normalized to the epsilon specificity control variant „delta bulge“) demonstrating specific inhibition of virion production by NERD-LNPs in an HBV infection system (Milestone 6). The values suggest that replication inhibition may be less efficient in the infection system than initially observed in HepG2.117 (WP3.1). However, this difference may be due to an artificial qPCR background derived from residual viral DNA fragments of the virus inoculum used for infection. This issue appeared to be even more pronounced in DNA extracted from cellular

lysates, where we could not reproducibly detect significant differences in qPCR signals between the various tested LNPs suggesting that a considerable amount of input HBV DNA may remain associated with cells and may obscure the RNA treatment-induced differences in levels of replicating HBV DNA. To address this issue we additionally analysed the DNA samples extracted from lysates by Southern blotting. In line with our hypothesis, we now observed profound differences in signal strength in agreement with data from WP3.1. Quantification of the rc-DNA band (representing mature HBV DNA) revealed strong replication inhibition. We quantified about 30-fold reduction of rc-DNA by the positive control anti-HBV siRNA-LNP and about 8-fold reduction by circular wt epsilon-LNP, normalized to the P binding deficient negative control epsilon RNA variant „delta bulge“ (Milestone 6). We did not find a significant difference in antiviral activity between the linear and circular conformation of wt epsilon-LNPs. All of the tested epsilon sequence variants (tested only in linear conformation) appeared to be less efficient than wt.

In summary, we obtained quantitative data on replication inhibition by NERD-LNPs in infected cells and completed milestone 6.

b) Milestones and Deliverables

Milestones

No	Title	Work package	Institution	Date as per project description	Corrected date	Status	Comment
1	Provision of NERD RNAs	1	UK FR – 805	01.05.2023		completed	
2	Provision of NERD LNPs	2	UK FR – 805	01.07.2023		completed	
3	Proof-of-principle of NERD-LNP transfection	3.1	UK FR – 805	01.09.2023		completed	
4	Proof-of-principle of antiviral activity of NERD-LNPs	3.1	UK FR – 805	01.11.2023		completed	
5	Quantitative evaluation of antiviral activity of NERD LNPs in stably HBV transfected cells	3.1	UK FR – 805	01.02.2024		completed	
6	Quantitative evaluation of antiviral activity of NERD LNPs in primary HBV infection	3.2	Uni HD -601	01.02.2024		completed	

Deliverables

Nr	Titel	Work package	Institution	Date as per project description	Corrected date	Status	Comment
1	Report on cellular delivery and antiviral activity of NERD-LNPs	3.1	UK FR – 805	29.02.2024		completed	
2	Report on antiviral activity of preselected NERD-LNPs	3.2	Uni HD - 601	29.02.2024		completed	

c) Most important items of the financial report

Personnel expenses

Universitätsklinikum Freiburg

Use:

1 postdoc (TVL-E13, 100%) was employed for 10 month (05/2023-02/2024) with the help of the current DZIF fund TTU05.915 for working on the work packages 1, 2 and 3.

Circumstances:

Ruprecht-Karls-Universität Heidelberg

Use:

No funding by TTU05.915

Circumstances:

Personnel expenses were covered by the infrastructure project TTU05.709

Consumables (also contracts)

Universitätsklinikum Freiburg

Use:

RNA synthesis kits, enzymes, chemicals, buffers, Mol Biol reagents, DNA- and RNA-oligonucleotides, lipids, mixing chambers, syringes, siRNAs, cell culture reagents, transfection reagents, Western/Southern blotting reagents, blotting membranes, ELISA kits, antibodies, radiochemicals, PCR reagents, nucleic acid extraction kits

Circumstances:

Ruprecht-Karls-Universität Heidelberg

Use:

cell culture reagents

Circumstances:

Investment funds

Universitätsklinikum Freiburg

Use:

Not applicable

Circumstances:

Ruprecht-Karls-Universität Heidelberg

Use:

Not applicable

Circumstances:

Travel expenses

Universitätsklinikum Freiburg

Use:

Travel funds were re-purposed to purchase consumables.

Circumstances:

Meetings were arranged and attended online.

Ruprecht-Karls-Universität Heidelberg

Use:

No travel expenses

Circumstances:

Only online meetings

d) Necessity and adequacy of the work done

Universitätsklinikum Freiburg

DZIF funding was absolutely essential to execute the project. There were no additional funds available neither for personnel nor for consumables. Due to the complexity of the topic, the ambitious work plan and the rather short 12-month funding period a postdoc in full-time position was essential for the success of the project. The allocated budget for consumables was necessary to purchase all items required for the work.

Ruprecht-Karls-Universität Heidelberg

In vitro HBV infection experiments (WP3.2) were crucial to evaluate the antiviral potency of the NERD-LNPs. Due to the strong expertise of the Urban lab in in vitro infection these experiments were performed at UNI-HD and could be finished successfully.

e) Prospective benefit of the project, in particular the usability of the results according to the exploitation plan

Universitätsklinikum Freiburg

In this project we demonstrate proof-of-principle of synthesis and cellular delivery of HBV epsilon decoys, and of their antiviral activity in HBV cell culture models. The developed tools and the generated data provide a basis for future in vivo studies in HBV animal models.

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see UK FR

f) Progress/Advances by third parties during implementation of the project

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The funding recipient is not aware of any currently performed third party studies on the topic of epsilon RNA decoys.

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see UK FR

g) Successful or planned publication of the results according to No. 5 of the “Nebenbestimmungen für Zuwendungen” (NABF/NKBF 2017)

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Results have not been published yet. Future publication in a scientific journal is intended.

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see UKF