Schlussbericht zum DZIF-Vorhaben

TTU 09.914 - Corallopyronin A development through to phase 1 trial (first-into-man)

Förderkennzeichen: 8016809914 Uni BN bzw. 8004809914 HZI

Koordinierende Einrichtung:

Rheinische Friedrich-Wilhelms-Universität Bonn (UKB)

Beteiligte Einrichtung(en):

Rheinische Friedrich-Wilhelms-Universität Bonn (UKB)

Helmholtz-Zentrum für Infektionsforschung GmbH (HZI)

Executive Summary/Abstract

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See summary HZI

The results of this DZIF were instrumental in enabliing a cooperation with Eisia Co., Ltd, Japan to apply to the Global Health Inovative Technology (GHIT) Fund, Japan for funding to complete GLP toxicology in dogs and IND enabling safety studies, define the API, produce tech. and engineering batches of drug substance, and write a phase 1 study protocol and IND application. The GHIT funds were approved in Q2/2024.

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We are developing Corallopyronin A (CorA) as a treatment for human filarial nematode infections. CorA is a bacterial RNA polymerase inhibitor that sterically blocks the switch region. We have shown in vitro and in vivo CorA activity against Gram-negative Wolbachia endosymbionts, targets for controlling filarial infections causing lymphatic filariasis and onchocerciasis. Antiwolbachial therapy results in worm sterility and slow killing of worms. Importantly, our development activities have shown CorA efficacy against Rickettsia spp., Chlamydia trachomatis, Neisseria gonorrhoeae and multi-resistant Staphylococcus aureus. The latter three are United Nations Sustainable Development Goals and World Health Organization Priority Pathogen List targets requiring new antibiotics. Thus, we are developing CorA for the primary indication: new therapy for human filarial infections, and are exploring the secondary indications: C. trachomatis, N. gonorrhoeae and MRSA infections. With this, we fulfill candidate compound requirements for the Drugs for Neglected Diseases initiative (DNDi, www.dndi.org) and Global Antibiotic Research & Development Partnership (GARDP, www.gardp.org) to develop new drugs for Global Health.

Our PK/PD, ADME and safety results, including Cyp450 induction, hERG, AMES, micronucleus and off target receptor tests, support further development. CorA is better than the comparator rifampicin, having activity against multi-resistant S. aureus with no induction of drug-drug interactions that could negatively affect CorA administration with other treatments, e.g. HIV drugs. Due to the different MoA, it will also not drive rifampicin-resistant tuberculosis. It is lipophilic and stable in liver microsomes from dogs and humans .

We have overcome a crucial hurdle frequently encountered with natural products, cost-efficient production, by developing CorA heterologous production in Myxococcus xanthus and a streamlined downstream process without preparative HPLC, that is suitable for material from 500L scale pilot fermentations. These improvements reproducibly yield CorA with 90-95% purity, as confirmed by our validated quantitative NMR and HPLC analytical methods. The final production protocol will provide CorA at >90% purity for non-GLP pre-clinical studies, genotoxicity and non-GLP toxicity studies, and drug product formulation. We are engaged with the competent German regulatory authority (German Federal Institute for Drugs and Medical Devices, BfArM) and have received a first formal scientific advice regarding the CorA drug substance. BfArM has formally agreed that: i) heterologous production of CorA is not a barrier to developing CorA; ii) 90-95% CorA purity is acceptable for the pre-clinical toxicology and safety activities and the non-CorA contaminants, which are mainly CorA family compounds, do not need to be individually assessed for toxicity; and iii) the preliminary specification for the phase 1 API is acceptable. We identified and contracted two CMOs (BBEPP and Alphora) for the performance of a feasibility study. The tech transfer, and shipment of the producer strain and CorA standards were successful. BBEPP reproduced the fermentation at 100 L scale and were able to upscale the manufacturing process up to 15 m3. The SOPs established during that process will build the basis for the next step, the GMP manufacturing of CorA drug substance and product.

Kapitel 1: Kurzbericht (wird veröffentlicht)

a) Ursprüngliche Aufgabenstellung und wissenschaftlicher/technischer Stand, an den angeknüpft wurde

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See report HZI

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The main objective of the project is the development of CorA through formal preclinical studies that will culminate in submission of an IMPD and a phase 1 first-into-man clinical trial application for approval by the German Federal Institute for Drugs and Medical Devices (BfArM). The project objectives and work program were carried out as planned in the project plan, as parts of the DZIF basic project (TTU 09.822) and the flex funds project (TTU 09.914).

The greatest difficulty we have had in the past in developing CorA for a first-into-man study was the production of high quality CorA at the quantities needed for development at a reasonable cost. We could overcome this problem within the last funding period. Optimization of the producer strain and the down stream processing (DSP) has reduced the costs of production of technical grade CorA by at least 3-fold.

To achieve cost-efficient industrial-scale production of CorA, the HZI was involved in the WP 1 (Drug substance (DS) and DS characterization) with the subtasks:

DS I: Optimize heterologous CorA producer strain and initial scale-up (MINS and MWIS)
DS II: pre-GMP grade material and product specification (MWIS)

b) Ablauf des Vorhabens

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The work package for UKB was intended to engage a contract manufacturer (CMO) to produce the antibiotic Corallopyronin A. To be able to carry out this complex process, we first put out a feasibility study out to tender according to EU procurement law. Here, the CMO should first prove that they are technically capable of producing the substance to a defined purity. The CMO should then be able to carry out the further steps, the optimization of the fermentation and purification steps. The search for a CMO and the tendering process were time consuming and we were not able to identify a CMO who can conduct the fermentation as well as the downstream process (DSP). Therefore, we had to split the production process into the fermentation part with first DSP steps at one CMO and the final DSP part at another CMO facility. For the fermentation and beginning steps of DSP, we contracted the Belgium CMO Bio Base Europe Pilot Plant (BBEPP) in November 2020 for the feasibility study in 150 L and 1,500 L scale. The CMO Alphora, Canada, was contracted in January 2021 for the remaining DSP steps.

The Corona pandemic had an impact on the project, as contract manufacturers also had Corona-related problems and closures. This has made the negotiations more difficult and led to delays. Additionally, we were dependent on free time slots at the CMO and received a cost-neutral prolongation of the project duration. Due to the failure of the CMO Alphora, we shifted parts of the UKB budget in 2022 to the HZI, who were able to take over the DSP of the raw material produced by BBEPP.

In July 2023 we contracted the German/Canadian company Phyton Biotech, Ahrensberg, a GMP-CMO that can perform both the upstream and downstream processes to produce CorA to conduct

a feasibility study and calculate cost of goods based on the study results. This work is ongoing and funded in TTU 09.922.

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The corona pandemic led to delays in the project. Especially finding a CMO delayed the start of CMO activities (see report partner UK Bonn). In the project, HZI and UK Bonn received a cost-neutral prolongation of the project duration by 16 months. The new project end date was therefore June 30, 2023. In a second cost-neutral prolongation in 2023, only project partner UK Bonn received an additional prolongation of six months (new project end for UK Bonn: 31.12.2023). Due to failure of the contracted CMO Alphora in 2021 to produce pre-GMP grade material, the HZI (MWIS) took over the chromatographic purification of Cor A in industiral scale. According to the changed VHB Corallopyronin A was purified inhouse to overcome project delays. To proceed like this, the HZI received budget from UK Bonn in 2022, that was originally dedicated for the CMO. In addition to that, maximum tolerable dose (MTD) studies in rats and dogs were performed within the BMBF funded CORALS project, which defined the MTD as 1000 mg/kg of CorA. As such a low toxicity was not foreseeable, larger amounts of CorA must be produced for the 7-day-repeated dose studies.

Necessary adjustments were reproted to the DZIF, the VHB and AZK were updated accordingly. The work at HZI was performed according to plan with reference to adjusted milestone/delivery dates.

c) Wesentliche Ergebnisse und ggf. Zusammenarbeit mit anderen Forschungseinrichtungen

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We identified and contracted Bio Base Europe Pilot Plan (BBEPP), a CMO in Gent, Belgium to upscale the fermentation process from 150L to 15,000 L and to purify the drug substance into the stable intermediate product. This product was sent to Alphora (a Eurofins company), a CMO in Canada, for the final downstream process and analysis of the produced product. The tech-transfer to both companies was successful. BBEPP has received the heterologous producer strain and the CorA standard, and has started with the first test-fermentation at lab scale. In March 2022 the first 15,000 L fermentation run was successfully conducted, and was repeated in June and September 2022, followed by 3x further 15,000L runs in 2023 (the last one was covered by TTU 09.922). With these manufacturing runs, we received sufficient amounts of CorA for the performance of the non-GLP toxicity studies in rats and dogs. Additionally we used the drug substance to perfom *in vivo* efficacy studies at our mouse and jird infection model.

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Important note: The work packages, milestones, and deliverables of the BMBF/EU project as well as the planned follow-up project, are not subject of the present final report.

WP 1 Drug substance (DS) and DS characterization

DS I: Optimize heterologous CorA producer strain and initial scale-up

Our work aims to improve current heterologous CorA production titers based on the publication of Pogorevc et al. 2019 (Pogorevc D, Panter F, Schillinger C, Jansen R, Wenzel SC, Müller R.: Production optimization and biosynthesis revision of corallopyronin A, a potent anti-filarial antibiotic. Metab Eng. 2019, 55:201-211), which reports heterologous expression of the corallopyronin A (CorA) biosynthesis genes in Myxococcus xanthus with yields of ~100 mg/L of CorA and additional ~160 mg/L of preCorallopyronin A (preCorA).

During the project, we could scale up the production up to 300 L and enhance the exclusive

production of preCorA in a range of ~300-350 mg/L. However, despite all efforts (e.g. deletion of the corO gene (encoding cytochrome P450) and the generation of mutant strains for overexpression of the CorA biosynthetic gene cluster) production yields of 300 mg/L CorA could not be reached. We could conclude, that M. xanthus is not the optimal heterologous system for high-yield CorA production. In order to overcome the problem of self-resistance during high-yield production of antibacterials, we started a project for alternative strategies involving non-bacterial production systems (see TTU 09.920) in Q4 2022.

DS II: pre-GMP grade material and product specification
An analytical certificate proving the CorA quality was delivered by the Alphora company in 2021. In addition the chromatographic purification was scaled up further to multi gramm scale. Each batch 70 g CorA raw extract were sucessfully purified. Thereby almost 500 g CorA high quality research grade material (HQ-RGM) were produced in the HZI in 2022. Although the HQ-RGM has a purity of 90-95%, it is not equal to the pre-GMP grade material that could have been produced by a certified CRO. However, for the purposes within this project the quality was sufficient. The majority of the product was produces in Q4 of the project to serve preclinical trials in dogs. Having done that project goals could be kept.

Kapitel 2: Eingehende Darstellung (wird veröffentlicht)

a) Ausführliche Darstellung der erzielten Ergebnisse im Einzelnen

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We have successfully applied for additional funding outside of DZIF to finalize the preclinical development of CorA and enter into phase 1.

DS II (IMMIP): pre-GMP grade material and product specification

With the help of our external consultant Dr. Koenig (Koenig & Funk Biotech GmbH) we contacted several CMOs for a CorA manufacturing feasibility study. The first contacted CMO Huvepharma did not provide a quote for the feasibility study. The second contacted CMO Olon, Italy, was interested in our project but due to the corona crisis, this company decided not to facilitate a project in this early stage of development. We identified and contracted Bio Base Europe Pilot Plan, a CMO in Gent, Belgium to upscale the fermentation process (150 L and 15,000 L fermentation) and to purify the drug substance into the stable intermediate product. This product should then be sent to Alphora (a Eurofins company), a CMO in Canada, for the final downstream process and analysis of the produced product. The tech-transfer to both companies was successful. In 2020, BBEPP has received the heterologous producer strain and the CorA standard, and has started with the first test-fermentation at lab scale.

In spring 2021 BBEPP performed two 150 L runs, to learn the production process, which was followed by upscaling to 1,500 L. The direct upscale to 15,000L was thought to be too risky, without the experience the company received with the 1,500 L fermentation, which was successful. The intermediate product was sent to HZI for analysis and aliquots of the product were sent to Alphora. Alphora analyzed a sample of the CorA toxicity batch and established a certificate of analysis, which was needed for the toxicity studies. Furthermore, Alphora tested different columns and cartridges for the detection of the best purification method. Although we had many telephone conferences and HZI transferred the SOP of the DSP in detail, Alphora failed to reproduce the SOPs from HZI and were not able to perform the DSP to our satisfaction. We decided that HZI (MWIS) will take over

the purification process and to terminate the contract with Alphora in February 2022. With this choice we are able to produce CorA at HQ-RGM at HZI and meanwhile were looking for a CMO for the production under GMP conditions.

In March 2022 the first 15,000 L fermentation run at the BBEPP facility was successfully conducted, and was repeated in June and September 2022. With these three manufacturing runs, we received sufficient amounts of CorA to start the non-GLP toxicity studies in rats and dogs. Since we had to use very high CorA doses, we need much drug substance. Therefore, three further 15,000L fermentation runs were performed at BBEPP in 2023 (the last run was covered by TTU 09.922), which yielded enough CorA for the 7 day toxicity studies in dog using three different CorA doses. The intermediate and DSP products of each run were sent to HZI for analysis and to produce HQ-RGM. Because BBEPP has no GMP licence we looked for further CMOs for the production under GMP conditions. We had discussions with three different companies under CDA to present the SOPs of the production process in more detail. At the time of the EU-tendering process, three different companies were intersted in submitting a bid. But at the end of December 2022, we received only one offer, from the CMO Phyton Biotech, Ahrensberg, which was contracted in July 2023 to conduct a feasibilty study (with DZIF FF budget granted in 2023, TTU 09.922).
The tech transfer, including standards and CorA raw material to establish the DSP, was completed in 2023.

In preparation for the GMP production of CorA, we contracted the CRO Naicons, Italy, for characterization of the heterologous producer strain, preparation of the Master Cell Bank (MCB) and long-term stability investigation of the MCB. The characterization of the producer strain was finalized and in November 2021 150 vials of the MCB were released by the qualified Person Dr. Thumann. In December 2021 we had a virtual audit at Naicons led by our qualified person. The audit outcome supported the commissioning of Naicons for the creation and qualification of the MCB. Premises, equipment and processes were found GMP compliant. The long-term stability investigations are performed since February 2022 and will be repeated annually until Jan 2026.

In December 2021 we contracted the company Biokryo, Saarbrücken, to store the MCB for 5 years under GMP conditions. The MCB stored at Biokryo in Saarbrücken was used in 2022 and 2023 for stability tests at Naicons and also for a run at the Belgium production site, BBEPP, to confirm the productivity and genetic stability of the strain after the fermentation.

WP4 Drug efficacy testing of CorA produced as DS and DP:

Using the HQ-RGM produced by HZI, we performed *in vivo* efficacy studies, including PK studies to determine the minimal effective dose. For a prophylactic treatment in mice 18 mg/kg QD CorA for a treatment time of 14 days were used and for a therapeutic treatment in infected jirds 30 mg/kg CorA BID for a period of 14 days are necessary. In 2021 the dose for a prophylactic treatment in mice could be reduced to 9 mg/kg QD CorA for a treatment time of 14 days. Using these data, we predicted a human equivalent dose according to FDA guidelines of 0.5-4 mg/kg, which will help us as to define a starting point for the therapeutic dose range and a treatment schedule for human.

We also investigated if the new oral formulations povidone and coppovidone are active *in vivo*. In the efficacy model in mice, we demonstrated that 24 mg/kg lead to a 1-log reduction of *Wolbachia* load. The application with 12 mg/kg BID or 8 mg/kg TID were sufficient to yield a 3-log reduction. Thus, the BID or TID administration is better than QD.

The synergy of CorA and albendazole was investigated by treating infected mice with combinations of both substances. The treatment time as well as the dosage could be reduced to achieve the same

depletion of *Wolbachia* and killing of adult worms. In further studies we investigated to which amount the CorA can be reduced to get the macrofilaricidal effect.

The CorA content of the plasma samples of each *in vivo* study was monitored by LC/DAD-MS at the Department of Pharmaceutical Technology and Biopharmacy, to determine the plasma level in comparison to PK studies that gives data concerning the AUC, Cmax, CL, tmax and $t_{1/2}$. The bioavailability of the new developed oral formulation was investigated in mouse and jirds and a patent application was filled out, which is still pending. Funded by NIAID a first thigh model was performed by PDS, Taiwan using ICR mice that were infected in the muscle with S. aureus. The used CorA concentrations were too low to achieve muscle levels that were high enough to kill the bacteria. To investigate how much CorA is necessary to reach levels above the MIC for 5 hours in the thigh, we performed PK studies in mice and determined the CorA level in plasma and different organs (in cooperation with HZI). Using this results a study plan for the next thigh model was established.

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WP 1 Drug substance (DS) and DS characterization

DS I: Optimize heterologous CorA producer strain and initial scale-up (MINS, MWIS)

Optimize heterologous CorA producer strain (MINS): Our work aims to improve current heterologous CorA production titers based on the publication of Pogorevc et al. 2019 (Pogorevc D, Panter F, Schillinger C, Jansen R, Wenzel SC, Müller R.: Production optimization and biosynthesis revision of corallopyronin A, a potent anti-filarial antibiotic. Metab Eng. 2019, 55:201-211), which reports heterologous expression of the corallopyronin A (CorA) biosynthesis genes in Myxococcus xanthus with yields of ~100 mg/L of CorA and additional ~160 mg/L of preCorallopyronin A (preCorA). Furthermore, deletion of the corO gene (encoding cytochrome P450) from the expression construct and its expression in M. xanthus led to exclusive production of preCorA in a range of ~300-350 mg/L.

One main strategy to potentially increase production titers in the heterologous M. xanthus host was pursued in 2021 based on the limited self-resistance of the host organism M. xanthus DK1622. To circumvent this bottleneck, spontaneous resistance development of the host strain has previously been tried by continuous sub-cultivation on gradually increasing CorA concentrations. The method led to an increase of MIC values from 4 µg/mL CorA in the wild type strain to >64 µg/mL CorA in the obtained resistant mutants. The obtained mutant strains were used for overexpression of the CorA biosynthetic gene cluster, including the introduction of several copies of the BGC in these mutant strains. Unfortunately, no further significant increase of CorA production was monitored during these studies.

As an alternative to spontaneous development of resistance, co-expression of additional native copies of RNAP subunits (RpoB and RpoC) that interact with CorA as targets was performed in the heterologous host organism M. xanthus DK1622. However, the experiment did not result in a positive development of self-resistance in the strain. Therefore, a more advanced approach was implemented by coexpressing additional resistant copies of RNAP subunits. CorA resistance-conferring mutations have already been identified in the RpoB and RpoC subunits of several bacterial strains and described in the literature (Mukhopadhyay 2008, Mariner 2011). The corresponding resistance-promoting mutations were artificially introduced into the RNAP subunit-

encoding genes of M. xanthus before co-expression in the heterologous producer strain. Unfortunately, this approach also did not result in increased self-resistance of the strain and had no or negative effects on corallopyronin production.

However, despite all efforts, the production yields of 300 mg/L CorA could not be reached. Therefore, MS1 could not been reached. The drug substance strains with the best CorA production titers and the according protocols for optimized CorA production (small-to-medium scale) were transferred to MWIS (Del1 fulfilled). While the current yields of CorA will most likely be sufficient to take the last hurdles of preclinical development, there remains the task of implementing the expensive GMP-conform production that is bound to follow. Indeed, a robust and efficient method for large-scale production of a drug candidate molecule (with yields preferably in the range of (multi-)gram per liter fermentation broth) is highly advantageous for process transfer to GMP manufacturing. In the case of CorA, the DSP procedure is already rather efficient, also owing to the fact that almost no interfering side products are observed. M. xanthus turned out to be not the optimal heterologous system for high-yield CorA production. In order to overcome the problem of self-resistance during high-yield production of antibacterials, we propose to test alternative strategies involving non-bacterial production systems. One pragmatic approach is the use of a heterologous eukaryotic platform for production of CorA and other antibacterial natural products (see TTU 09.920). As we could not optimize producer strains > 300 mg/L CorA production, the working cell bank (100 cryo vials) could not been generated and characterized.
Initial scale-up of production (MWIS): It was finally possible to scale up production to 300 L, resulting in similar yields as in the shake flask experiments, but unfortunately the titers could eventually not be much further increased, neither by fed-batch fermentation nor extended modification of the bacterial producer including the insertion of multiple copies of the biosynthetic gene cluster and the overexpression of self-resistance factors (Pogorevc et al., 2019).

DS II: pre-GMP grade material and product specification (MWIS)
An analytical certicicate proving the CorA quality was delivered by the Alphora company in 2021. The transfer of the finalized producer strain, the fermentation and DSP processes will be performed by scientists from MWIS and supported by the external consultancy firm Koenig & Funk Biotech GmbH, www.kfbiotech.de. Therefore the BBEPP company was supported to perform HZI procedures in their company, scale the process up and adjust procedures to industrial scale. Alphora was not able to reproduce the MWIS SOPs to produce CorA at the defined quality, therefore the contract was cancelled in 2021. BBEPP up-scaled the fermentation to 15,000 L and provided the resulting material to MWIS, where the compound was purified in the required quality (high quality research grade material; HQ-RGM) and quantity to perform the preclinical experiments and improvement of the formulation. Alltogether BBEPP performed 3 x 15,000 L fermentation runs. Therefore the chromatographic purification at MWIS was scaled up further to multi gramm scale. Each batch 70 g CorA raw extract were sucessfully purified. Thereby almost 500 g CorA were produced in the HZI in 2022 (MS10 and Del9 fulfilled). The majority of the product was produces in Q4 of the project to serve preclinical trials in dogs. Having done that project goals could be kept. Until 30.06.2023 in total 35 g CorA were delivered to UK Bonn and in total 150 g were produced for the following trails. In addition, efforts to identify drug substance impurities in further detail were started.

During 01.01.2021 and 30.06.2023, six publications (5 scientific reports and 1 review article) were made on CorA research (including DZIF projects TTU 09.822 and 09.920 as well as the BMBF funded project CORALS) with collaboration of HZI and UK Bonn. For details see chapter 2 g.

b) Meilensteine und Liefergegenstände

Meilensteine (UKB)

Nr.	Titel	Arbeitspaket	Einrichtung	Datum lt. Vorhabenbeschreibung	Korrigiertes Datum	Status	Kommentar
2	**Drug substance** Transfer of the production process to a CMO	1	Rheinische Friedrich-Wilhelms-Universität Bonn	31.12.2022	30.06.2023	completed	Delays in finding a CMO and the Corona pandemic delayed start of CMO activities. Nevertheless, BBEPP was contracted for the feasibility study and scaled the fermentation process up to 15,000 L scale. Alphora failed to replicate the HZI protocols, therefore we had to search for an alternative CMO. The EU tendering process was finalized 2022 and CMO Phyton was contracted in 2023 for the production of GMP grade material.
3	**Drug substance** Validated production process of pre-GMP grade material	1	Rheinische Friedrich-Wilhelms-Universität Bonn	31.12.2022	30.06.2023	Not achievable	The Master Cell Bank for production of the pre-GMP and GMP grade material is finalized and released. The contract with Phyton achieved in Q2/2023 and the tech transfer and the feasibility study at the new CMO started in Q3/2023. So the basis for the pre-GMP production is build, this MS is not achievable in TTU09.914. It will be fullfilled during the follow up project.
7	Drug efficacy Finalization of pre-clinical development (drug efficacy)	4	Rheinische Friedrich-Wilhelms-Universität Bonn	30.06.2022		completed	Minimal effective dose *in vivo* was used to calculate the HED (4 mg/kg)

Liefergegenstände (UKB)

Nr.	Titel	Arbeitspaket	Einrichtung	Datum lt. Vorhabenbeschreibung	Korrigiertes Datum	Status	Kommentar
2	Drug substance pre-GMP grade material	1	Rheinische Friedrich-Wilhelms-Universität Bonn	31.03.2023	30.06.2023	Not achievable	With the Corona pandemic and the new production strategy, the delivery of pre-GMP material was pushed back. The new CMO Phyton started their work in Q3/2023, so this Del is not achievable in the time of TTU 09.914, but during the next funding period. See also MS 3
6	Drug efficacy Minimal effective dose and definition of the starting dose for first in human	4	Rheinische Friedrich-Wilhelms-Universität Bonn	31.07.2022		completed	For the first in human also the results of the GLP toxicity will be taken into account.

Meilensteine (HZI)

Nr.	Titel	Arbeitspaket	Einrichtung	Datum lt. Vorhabenbe-schreibung	Korrigiertes Datum	Status	Kommentar
1	Drug substance Heterologous producer strain with CorA production titers of about 300 mg/L generated	1	Helmholtz-Zentrum für Infektionsforschung GmbH	31.03.2020	30.06.2021	Nicht erreichbar	Despite all efforts, the titer could not be improved over 100 mg/L for CorA and 160 mg/L for preCorA. Alternatively, heterologous expression in yeast will be attempted.

10	Drug substance Purification of the CorA-intermediat product	1	Helmholtz-Zentrum für Infektionsforschung GmbH	31.12.2022		Erfüllt	Upscale of the DSP established for the purification of 500 g CorA intermediate product. Purification of 500 g CorA- intermediate product to 90-95% pure CorA-API.

Liefergegenstände

Nr.	Titel	Arbeitspaket	Einrichtung	Datum lt. Vorhabenbe-schreibung	Korrigiertes Datum	Status	Kommentar
1	Drug substance Strains and protocols for optimized CorA production (small-to-medium scale) transferred to MWIS and CMO	1	Helmholtz-Zentrum für Infektionsforschung GmbH	30.09.2020		Erfüllt	
9	Drug substance HQ-RGM CorA	1	Helmholtz-Zentrum für Infektionsforschung GmbH	30.06.2023		Erfüllt	

c) Wichtigste Positionen des zahlenmäßigen Nachweises

Personalmittel

Rheinische Friedrich-Wilhelms-Universität Bonn
Einsatz: Post-doc, Project manager, female 12 months 65%
Sachverhalt:
In 2019 – 2020 no personal budget was spent

The personnel budget in this project for 2021 was used for the Project manager (Post-Doc, 65%).

In 2022 no personnel budget was spent, according to the VHB.

Helmholtz-Zentrum für Infektionsforschung GmbH
Einsatz: MINS:
Technical assistant, male, 01.01.2020 - 31.12.2020, 12 months, 57%
Technical assistant, male, 01.01.2021 - 28.02.2021, 2 months, 82,05%
Technical assistant, male, 01.03.2021 - 31.12.2021, 10 months, 100%

MWIS:
Technical assistant, female (01.08.2022 - 30.06.2023), 11 months, 100%
Technical assistant, female (01.07.2022 - 31.10.2022), 4 months, 100%
Technical assistant, female (07.11.2022 - 30.06.2023), 8 months, 51%
PD, female (01.10.2022 - 31.03.2023), 6 months, 100%
PD, female (01.04.2023 - 31.05.2023), 2 months, 65%
PD, female (01.06.2023 - 30.06.2023), 1 month, 92%
Sachverhalt: In 2022, budget was transferred from UK Bonn to HZI to perform the required work in WP1 DS II (total: 229,921.95 €; personell: 186,907.50 €, consumables: 43,014.45 €). The budget was spent as planned and was used as originally proposed.

Sachmittel (auch Aufträge)

Rheinische Friedrich-Wilhelms-Universität Bonn
Einsatz:
The consumables budget was spent for animal costs, HPLC reagents and columns, plastic ware for cell culture or PCR and PCR reagents.

Sachverhalt:
- Subcontractors for feasibility study for production of CorA have been hired and payments have been made for the fermentation (BBEPP) processes, which included 3 runs at 15,000 L scale in 2022 and 2 x 15,000 L runs in 2023.
-After the Alphora contract was canceled, we transferred parts of the budget (229.921,95 €) to HZI for the performance of the DSP.
-Naicons was contracted for preparation of the master cell bank and for stability testing of the master cell bank samples.
-Additionally, we ordered the storage of the MCB under GMP conditions at Biokryo.
- External consulting was contracted to A) the toxicologist Dr. Ingo Stammberger, who guided us through the non-GLP toxicity studies and was necessary for the planning of the GLP-toxicity and safety pharmacology studies. B) the law office Dageförde, the lawyer Mr. Bretzel

helped the team with the difficult tendering processes and C) the patent application concerning the oral formulations, which was tested in WP 4.

Helmholtz-Zentrum für Infektionsforschung GmbH
Einsatz: The budget was spent for kits and enzymes for DNA work/cloning, media and supplements for fermentation, solvents, plasticware, and chemicals.

Sachverhalt: In 2022, budget was transferred from UK Bonn to HZI to perform the required work in WP1 DS II (total: 229,921.95 €; personell: 186,907.50 €, consumables: 43,014.45 €). The budget was spent as planned and was used as originally proposed.

Investitionsmittel

Rheinische Friedrich-Wilhelms-Universität Bonn
Einsatz: N.A.
Sachverhalt: N.A.

Helmholtz-Zentrum für Infektionsforschung GmbH
Einsatz: N.A.
Sachverhalt: N.A.

Reisekosten

Rheinische Friedrich-Wilhelms-Universität Bonn
Einsatz:
The travel budget was spent as planned.

Sachverhalt:
The travel budget was used to attend meetings at which the CorA activities and results were presented.

Helmholtz-Zentrum für Infektionsforschung GmbH
Einsatz: N.A.
Sachverhalt: N.A.

d) Notwendigkeit und Angemessenheit der geleisteten Arbeiten

Rheinische Friedrich-Wilhelms-Universität Bonn
The personnel and material resources (consumables) were used achieve the milestones and deliverables reported herein.

Helmholtz-Zentrum für Infektionsforschung GmbH
The personnel and material resources (consumables) were used in the budgeted amount and were necessary and appropriate for the work in the project.

e) Voraussichtlicher Nutzen des Vorhabens, insb. die Verwertbarkeit des Ergebnisses im Sinne des fortgeschriebenen Verwertungsplans

Rheinische Friedrich-Wilhelms-Universität Bonn

The results of the current project will be used to finalize the preclinical development of CorA that will culminate in a phase I trial. The planning and conduct of a phase I trial will be a significant scientific success for the publicly funded institutes (UKB and HZI). The feasibility study at BBEPP showed that the production process can be upscaled to industrial scale and the SOPs established by them will be used as basis for the pre-GMP and GMP manufacturing protocol. The patent application for the oral formulation, developed by AG Wagner, was submitted in 2020. This patent will make the existing patents more attractive for licensing. The final oral formulation will be transferred to the GMP-certified CMO for production of material for a phase I clinical trial (planned start in Q1/2025).

Economic chances of success are unchanged regarding envisaged clinical trials and market entry in mid-to-long-term perspective.

The translation of CorA into an effective compound that can be used for antibiotic therapies is important for the treatment of infected human as well as for the veterinary field. During the last DZIF project, we showed the activity of CorA against the following pathogens:

1) Filarial nematode infections are our primary indication for CorA. There are circa 200 million human infections found in Africa, India, South East Asia, and Latin America. The nematodes cause high morbidity in the endemic countries and are a major hindrance to economic development. Over 1 billion people are at risk of contracting these infections. Additionally, companion animal infections of *Dirofilaria immitis* and *D. repens* are found in North and South America, Europe, Japan and Australia. An estimated 1.5 million dogs are infected in the USA, where circa 1 billion USD/year are paid for this disease, so the market potential for "Heart-Worm-Disease" is big. Our experiments have demonstrated that filarial infections can be treated with CorA (Schiefer et al. 2012 J Infect Dis, Schiefer et al. 2020 Plos Negl Trop Dis).

Currently human filarial infections are treated using diethylcarbamazine (DEC) and ivermectin (IVM). Both mainly only kill the microfilariae, not the long-lived (5-15 years) adult worms, and therefore require long treatment times. Increasing reports of suboptimal responses against standard IVM and DEC therapy have arisen, demonstrating a need for new antifilarial drugs or treatment options. The use of antibiotics fulfills this need, as shown by depletion studies with doxycycline or rifampicin. Because doxycycline has contra-indications and rifampicin cannot be used in areas co-endemic with TB, CorA is an alternative new therapeutic agent. Like the other antibiotics it is macrofilaricidal and slowly kills the adult worms, avoiding adverse events seen with compounds that rapidly kill the worms. It has a unique mode of action from rifampicin, binding of the bacterial DNA-dependent RNAP rather than the active site. Since Mycobacteria are insensitive to CorA it can be used in areas co-endemic with TB without driving resistance to rifampicin. Compared to the competitor doxycycline, CorA treatment time can be shortened to 14 days, instead of the 3-5 weeks treatment needed for doxycycline.

2) One secondary indication that we are developing CorA for is Methicillin resistant *Staphylococcus aureus* (MRSA), which is of clinical importance due to its high incidence within healthcare settings. In 2009, MRSA caused 171,200 (44%) healthcare-associated infections in Europe. Worldwide, MRSA accounts for at least 25-50% of *S. aureus* infections in hospitals. In the USA, there is increasing incidence of so-called community associated MRSA, making it

more difficult to control its spread. MRSA is often resistant to other classes of antibiotics, requiring newer, more expensive antibiotics, many of which are less effective than β-lactams in susceptible strains. The restricted antibiotic arsenal available for MRSA is detrimental because MRSA can cause life-threatening diseases, such as necrotizing fasciitis or necrotizing pneumonia, thus MRSA is associated with a greater negative prognosis and higher mortality. Drug-drug interactions as with rifampicin are not expected with CorA, so combinations with other antibiotics should be easier to use, which gives CorA the potential to become a reserve antibiotic for patients in intensive care.
We have shown CorA efficacy against *S. aureus in vitro* and *in vivo*, and that the frequency of mutation is much lower than that for rifamicin, the comparator (Balansky et al. 2022 Antibiotics, Rox et al. 2022 Pharmaceutics).

3) Sexually Transmitted Infections (STIs) are also secondary indications, which include the WHO high priority listed gonorrhoea and chlamydial infections. *Neisseria gonorrhoeae* infections are responsible for 78 million STIs worldwide, causing infertility in both genders and neonatal conjunctivitis. Antimicrobial resistance development in gonococci is increasing, making untreatable gonorrhoea a universal threat. The most common STI is *Chlamydia trachomatis* infection with 92 million genital tract infections, causing infertility in women. Our data show activity of CorA against both pathogens, making it attractive to combat these WHO priority pathogens (Edwards et al. 2022 mSphere, Shima et al. 2018 Int J Antimicrob Agents, Loeper et al. 2019 Front Microbiol). Importantly, we have shown that the development of resistance to CorA in *N. gonorrhoeae* is extremely low (Balthazar et al. 2024 Microbiol Spectr).

The basis of this project is formed by the patents from the University Bonn (US 9168244 B2, EP 2704708 B1, US 9687470 B2) and the HZI (WO 2014/181000 A1), and the patent application EP 20172409.3 -1109, which are supervised and commercialized by PROvendis, the patent agency of the University Bonn. The commercialization of the project results will occur by licensing the EP and US patents. Licensing will be interesting for the pharmaceutical industry (e.g. Eisai Co., Ltd.) to develop CorA as treatment option for filarial, MRSA or STIs. The development of CorA will also be valuable for veterinary companies (e.g. Merial, Vétoquinol or Virbac) for combating Heartworm disease.

The ongoing results of the CorA project were presented by PROvendis at the digital Bio Europe Meeting in November 2020. The French company Virbac was very interested in our substance and a CDA for discussions on confidential results is in preparation. CorA has the potential to be developed by pharmaceutical company under the rubric of the FDA's Priority Voucher System, further increasing its worth to pharma.

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The translation of CorA into an effective compound that can be used for antibiotic therapies is important for the treatment of differently infected human as well as for the veterinary field. CorA has activity against filarial nematodes (treatment for filarial infections). In additon, CorA has good activities against Methicillin resistant Staphylococcus aureus (MRSA), N. gonorrhoea and C. trachomatis. In combination with its preliminary low toxicity in dogs, CorA has the potential to be developed by pharmaceutical company under the rubric of the FDA´s priority voucher system. Further increasing its worth to pharma. The results of the current project will be used scientifically for the finalization of the preclinical development of CorA that will culminate in a phase I trial. The planning and conduction of a phase I trial will be a significant scientific success for the publicly funded institutes (Uni BN and HZI).

The commercialization of the project results will occur by licensing the already existing EP and US patents. Licensing will be interesting for the pharmaceutical industry (e.g. Johnson & Johnson, Sanofi or Pfizer) to develop CorA as treatment option for filarial, MRSA or STIs. The development of CorA will also be valuable for veterinary companies (e.g. Merial, Vétoquinol or Virbac) for combating Heartworm disease. The onging results of the CorA project are presented by PROvendis (the patent agency of the University Bonn) amongst others at the BioVaria to different companies (e.g. Ceva Santé Animale). Thus, upcoming results of the project that strengthen CorA clinical application will increase the attractiveness of the patents and simplify the commercialization by licensing.

f) Während der Durchführung des Vorhabens dem Zuwendungsempfänger bekannt gewordener Fortschritt auf dem Gebiet des Vorhabens bei anderen Stellen

Rheinische Friedrich-Wilhelms-Universität Bonn

To our knowledge, there has been no relevant work or publications by other groups.

Helmholtz-Zentrum für Infektionsforschung GmbH

To our knowledge, there has been no relevant work or publications by other groups.

g) Erfolgte oder geplante Veröffentlichung der Ergebnisse nach Nr. 5 der Nebenbestimmungen für Zuwendungen (NABF/NKBF 2017)

Rheinische Friedrich-Wilhelms-Universität Bonn

Manuscripts

1. Balansky J, Pfarr K, Szekat C, Kehraus S, Aden T, Grosse M, et al. The RNA polymerase inhibitor corallopyronin A has a lower frequency of resistance than rifampicin in *Staphylococcus aureus*. Antibiotics. 2022;11 7:920; doi: ARTN 92010.3390/antibiotics11070920. https://www.mdpi.com/2079-6382/11/7/920.
2. Becker T, Krome AK, Vahdati S, Schiefer A, Pfarr K, Ehrens A, et al. In *vitro-in vivo* relationship in mini-scale-enabling formulations of corallopyronin A. Pharmaceutics. 2022;14 8:1657; doi: 10.3390/pharmaceutics14081657. https://www.mdpi.com/1999-4923/14/8/1657.
3. Edwards JL, Balthazar JT, Esposito DLA, Ayala JC, Schiefer A, Pfarr K, et al. Potent *in vitro* and *ex vivo* anti-gonococcal activity of the RpoB inhibitor corallopyronin A. mSphere. 2022;7 5:e0036222; doi: 10.1128/msphere.00362-22. https://www.ncbi.nlm.nih.gov/pubmed/36094073.
4. Ehrens A, Schiefer A, Krome AK, Becker T, Rox K, Neufeld H, et al. Pharmacology and early ADMET data of corallopyronin A, a natural product with macrofilaricidal anti-wolbachial activity in filarial nematodes. Front Trop Dis. 2022;3: 983107.; doi: 10.3389/fitd.2022.983107.
5. Krome AK, Becker T, Kehraus S, Schiefer A, Gutschow M, Chaverra-Munoz L, et al. Corallopyronin A: antimicrobial discovery to preclinical development. Nat Prod Rep. 2022;39 9:1705-20; doi: 10.1039/d2np00012a. https://www.ncbi.nlm.nih.gov/pubmed/35730490.
6. Rox K, Becker T, Schiefer A, Grosse M, Ehrens A, Jansen R, et al. Pharmacokinetics and Pharmacodynamics (PK/PD) of Corallopyronin A against Methicillin-Resistant Staphylococcus aureus. Pharmaceutics. 2022;15 1; doi: 10.3390/pharmaceutics15010131. https://www.ncbi.nlm.nih.gov/pubmed/36678760.

Balthazar JT, Golparian D, Unemo M, Read TD, Grosse M, Stadler M, et al. A laboratory-based predictive pathway for the development of *Neisseria gonorrhoeae* high-level resistance to

corallopyronin A, an inhibitor of bacterial RNA polymerase. Microbiol Spectr. 2024:e0056024; doi: 10.1128/spectrum.00560-24. https://www.ncbi.nlm.nih.gov/pubmed/38647280.

7. Becker T, Heitkötter J, Krome AK, Schiefer A, Pfarr K, Ehrens A, et al. Mesoporous silica as an alternative vehicle to overcome solubility limitations. Pharmaceutics. 2024;16 3; doi: 10.3390/pharmaceutics16030386.

Conferences and symposia

- A. Schiefer, K. Pfarr, A. Krome, S. Kehraus, S. Hüttel, R. Jansen, G. König, C. Keller, J. Rupp, M. Hübner, R. Müller, K. Wagner, T. Hesterkamp, M. Stadler, A.Hoerauf . Pre-clinical development of Corallopyronin A - an antibiotic active against helminths, STIs and Staphylococci. 12th Berlin Conference on Life Sciences, Novel Antimicrobials and AMR Diagnostics 14–15 March 2019, Berlin

- K. Pfarr, A. Schiefer, A. Krome, S. Kehraus, S. Hüttel, R. Jansen, L. Chaverra-Munoz, D. Pogorevc, G. König, C. Keller, J. Rupp, M. P. Hübner, R. Müller, K. Wagner, T. Hesterkamp, M. Stadler, A. Hoerauf. Pre-clinical development of Corallopyronin A, an antibiotic with activity against filarial nematodes, STIs and Staphylococci. Conference on Tropical Medicine and Global Health, April 4 -6, 2019, Munich.

- A. Hoerauf, K. Pfarr, A. Schiefer. Pre-clinical development of Corallopyronin A - an antibiotic active against helminths, STIs and Staphylococci. IRAADD Workshop, 15-16 October 2019, Saarbrücken

- A. Hoerauf, A. Schiefer, R. Jansen, S. Hüttel, A. Krome, M. Stadler, K. Wagner, K. Pfarr. räklinische Entwicklung von Corallopyronin A, einem Antibiotikum mit Wirkungen gegen Würmer, sexuell übertragbare Infektionen und Staphylokokken. Wirkstoffinitiative, 08.10.2019 in Berlin, BMBF

- K. Krome, T. Becker, A. Schiefer, T. Aden, M. P. Hübner, K. Pfarr, S. Kehraus, G. M. König, A. Hoerauf, K. G. Wagner. Preclinical Development of Corallopyronin A Formulations, Pharmacokinetics and Efficacy . TTU NAB Meeting, 14-15.10.2019, Saarbrücken

- K. Pfarr, A. Schiefer, A. Krome, S. Kehraus, S. Hüttel, R. Jansen, G. König, C. Keller, J. Rupp, M Hübner, R. Müller, K. Wagner, T. Hesterkamp, M. Stadler, A. Hoerauf. Pre-Clinical Development of Corallopyronin A against Helminths, STIs and Staphylococci. ASM/ESCMID Conference on Drug Development to Meet the Challenge of Antimicrobial Resistance, 3-6.09.2019, Boston

- A. Schiefer, M. Stadler, K. G. Wagner, R. Jansen, A. K. Krome, S. Kehraus, S. Hüttel, M. P. Hübner, D. Pogorevc, G. M. König, R. Müller, T. Hesterkamp, K. Pfarr, A. Hoerauf. Corallopyronin A: Non-GLP toxicity and safety pharmacology results support continued development to clinical trials. Joint Annual Meeting of DGI and DZIF in Bad Nauheim. 21-23.11.2019

- A. K. Krome, T. Becker, A. Schiefer, K. Pfarr, M. P. Hübner, S. Kehraus, G. M. König, M. Stadler, A. Hoerauf, K. G. Wagner. Evaluating preclinical and enabling formulations for Corallopyronin A. Joint Annual Meeting of DGI and DZIF in Bad Nauheim. 21-23.11.2019

- Achim Hörauf. Pre-clinical development of Corallopyronin A. Meeting at the Center for Global Health 02.03.20

- M. P. Hübner. Von der präklinischen Entwicklung zur klinischen Testung – der aktuelle Stand zu den neuen Medikamenten-Kandidaten für die Onchozerkose. PEG 01.09.2020

- A. Hoerauf. Pre-clinical development of Corallopyronin A – a novel natural product active against helminths, STIs and Staphylococci. Antimicrobial Chemotherapy Conference 2021, February 2021, virtual conference

- A. K. Krome, T. Becker, N. Ngo, S. Kehraus, A. Schiefer, K. Pfarr, M. Hübner, A. Hoerauf, G. König, K. Wagner. Insights from the preclinical development of corallopyronin A: Establishing analytical assays for the determination of corallopyronin A, corallopyronin isomers and degradation products of stability and bioanalytical samples. 12th World Meeting on Pharmaceutics, Biopharmaceutics, and Pharmaceutical Technology 2021, virtual conference.

- T. Becker, A. K. Krome, A. Schiefer, K. Pfarr, T. Aden, M. P. Hübner, S. Kehraus, G. M. König, A. Hoerauf, K. G. Wagner. Insights from the preclinical development of Corallopyronin A: Formulation evaluations by physiology-based pharmacokinetics and in vivo predictive in vitro tools. 12th World Meeting on Pharmaceutics, Biopharmaceutics, and Pharmaceutical Technology 2021, virtual conference

- A. Schiefer, M Stadler, K Wagner, R Jansen, A Krome, S Kehraus, S Hüttel, M P Hübner, D Pogorevc, S Alt, G M König, R Müller, T Hesterkamp, K Pfarr, A Hoerauf. Pre-clinical development of Corallopyronin A, an antibiotic active against filarial nematodes. 29th Annual Meeting of the German Society for Parasitology, 15-17.03.2021, virtual conference

- A. Schiefer, M Stadler, K Wagner, R Jansen, A Krome, T Becker, S Kehraus, M P Hübner, J Rupp, C Keller, W Shafer, S Alt, G M König, R Müller, T Hesterkamp, K Pfarr, A Hoerauf. Präklinische Entwicklung des neuen Antibiotikums Corallopyronin A. Kongress für Infektionskrankheiten und Tropenmedizin, 16-19.06.2021, virtuell

- K. G. Wagner**.** Formulation Development of Corallopyronin A. DZIF PDU: Preclinical Workshop on Small Molecules and Biologicals, 22.09.2021 (online)

- K. Pfarr, A Schiefer, A Krome, T Becker, S Kehraus, T Aden, H Neufeld, C Lämmer, R Jansen, G König, M Hübner, S Alt, R Müller, M Unemo, G Bierbaum, W Shafer, B Henrichfreise, K Wagner, T Hesterkamp, M Stadler, A Hoerauf. Pre-clinical development of the natural compound antibiotic Corallopyronin A. Antimicrobial Chemotherapy Conference 2021, February 2021, virtual conference

- A. Hörauf. Doxycycline and beyond – pros and cons of anti-*Wolbachia* drugs against onchocerciasis. American Society for Tropical Medicine and Hygiene 70th Annual Meeting Symposium: Clinical Development of New Treatments to Support the Global Elimination of Onchocerciasis 17.-21.11. 2021, virtual.

- T. Becker, A. K. Krome, A. Schiefer, K. Pfarr, T. Aden, M. P. Hübner, S. Kehraus, G. M. König, A. Hoerauf, K. G. Wagner. IVIVR in Mini Scale – Enabling Formulations for Corallopyronin A. World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology (28.-31.03.2022), Rotterdam, Netherlands.

- K. Pfarr, A. Schiefer, W. Shafer, J. Edwards, T. Becker, G. Bierbaum, S. Kehraus, M. Grosse, R. Jansen, S. Alt, G. König, A. Ehrens, T. Schneider, M. P. Hübner, R. Müller, K. Wagner, T. Hesterkamp, M. Stadler, A. Hoerauf. Pre-clinical development of Corallopyronin A – a novel natural product active against helminths, STIs and Staphylococci. American Society for Microbiology (ASM) ASM Microbe 07.06.2022, Washington, D.C., USA

- A. Schiefer, K. Rox, G. Bierbaum, A. Krome, T. Becker, M. Grosse, S. Alt, R. Jansen, S. Kehraus, M. P. Hübner, A. Ehrens, G. M. König, R. Müller, T. Hesterkamp, M. Stadler, K. Wagner, K. Pfarr, A. Hoerauf. Präklinische Entwicklung des Antibiotikums Corallopyronin A zur Behandlung von Filariosen und multiresistenter Staphylokokken. Konferenz der Deutschen Gesellschaft für Tropenmedizin, Reisemedizin und Globale Gesundheit e.V. (CTM), 23.-25.06.2022, Rostock

- K. Pfarr, A. Schiefer, M. Große, K. Rox, A. Krome, R. Jansen, T. Becker, S. Kehraus, A. Ehrens, G.M. König, R. Müller, S. Alt, T. Hesterkamp, M.P. Hübner, K. Wagner, M. Stadler, A. Hoerauf.

Pre-clinical development of Corallopyronin A – a novel natural product active against filarial nematodes. Parasitology (DGP) TransAlp Conference on Human and Animal Parasitic Diseases July 12th – 15th, 2022

- K. Pfarr, A. Schiefer, M. Große, K. Rox, A. Krome, R. Jansen, T. Becker, S. Kehraus, M P. Hübner, A. Ehrens, G.M. König, R. Müller, S. Alt, T. Hesterkamp, K. Wagner, M. Stadler, A. Hoerauf. Pre-clinical development of Corallopyronin A – a novel natural product active against filarial nematodes. 15th International Congress of Parasitology (ICOPA), 21.-26.08.2022 Copenhangen, Denmark.

- T. Becker, A. K. Krome, A. Schiefer, K. Pfarr, T. Aden, M. P. Hübner, S. Kehraus, G. M. König, A. Hoerauf, K. G. Wagner. Biorelevant Dissolution and PBPK Modelling to Forecast Oral Absorption – Enabling formulations for Corallopyronin A. American Association of Pharmaceutical Scientists (AAPS) PharmSci 360 (16.-19.10.2022), Boston, USA

- K. Pfarr, A. Schiefer, M. Große, A. Krome, T. Becker, S. Kehraus, A. Ehrens, R. Jansen, G. M. König, S. Alt, R. Müller, T. Hesterkamp, M.P. Hübner, M. Stadler, K. Wagner, A. Hoerauf. Pre-clinical development of Corallopyronin A – a novel antibiotic active against *Wolbachia* endosymbionts of filarial nematodes. American Society for Tropical Medicine and Hygiene 71th Annual Meeting 30.10-03.11.2022, Seattle, USA.

- K. Pfarr, A. Schiefer, W. Shafer, J. Edwards T. Becker, G. Bierbaum, S. Kehraus, M. Grosse, R. Jansen, S. Alt, G. König, A. Ehrens, T. Schneider, M. P. Hübner, R. Müller, K. Wagner, T. Hesterkamp, M. Stadler, A. Hoerauf. Pre-clinical development of Corallopyronin A – a natural product active against helminths, STIs and Staphylococci. 7th AMR Conference, 16.-17.03.2023, Basel, Switzerland.

- M. P. Hübner, K. Pfarr, A. Ehrens, A. Schiefer, F. Risch, S. Specht, A. Hoerauf. Oxfendazol und Corallopyronin A, zwei neue Kandidaten für Filariosen und weitere Indikationen. Frühjahrs-Tagung der PEG, 3.-4.04.2023 Bonn, Germany.

- A. Schiefer, K. Rox, G. Bierbaum, T. Becker, M. Grosse, S. Alt, R. Jansen, S. Kehraus, M. P. Hübner, A. Ehrens, T. Schneider, .G. M. König, R. Müller, T. Hesterkamp, M. Stadler, K. Wagner, K. Pfarr, A. Hoerauf. Präklinische Entwicklung des Antibiotikums Corallopyronin A zur Behandlung von Filariosen und multiresistenten Staphylokokken. 16. Kongress für Infektionskrankheiten und Tropenmedizin, 14.-17.06.2023, Köln, Deutschland.

- K. Pfarr. Developing the natural product Corallopyronin A to treat filariasis, STIs and Staphylococci. 2nd Onchocerciasis Associated Epilespy Workshop, 19.-20.09.2023, Antwerp, Netherlands.

- A. Schiefer, K. Wagner, T. Becker, M. Stadler, R. Jansen, S. Kehraus, M. P. Hübner, A. Ehrens, M. Große, G. M. König, S. Alt, R. Müller, T. Hesterkamp, K. Pfarr, A. Hoerauf. Corallopyronin A: Successful non-GLP in vivo toxicity tests in rat and dog support continued development to first in human trials. Annual Meeting of the German Center for Infection Research, 25.-27.09.2023, Hannover, Germany.

- K. Pfarr, A. Schiefer, W. Shafer, J. Edwards T. Becker, G. Bierbaum, S. Kehraus, M. Grosse, A. Ehrens, T. Schneider, K. Rox, M. P. Hübner, K. Wagner, T. Hesterkamp, M. Stadler, A. Hoerauf. Developing the natural product Corallopyronin A to treat filariasis, STIs and Staphylococci. ASTMH 71st Annual Meeting, 18.-22.10.2023, Chicago, USA.

- K. Pfarr. Developing the natural product Corallopyronin A to treat filariasis, Staphylococci and STIs. Emory University Medical School, invited speaker Graduate Student Symposium (23.10.2023), Atlanta, USA.

- M. P. Hübner. Identification of novel anthelmintics – preclinical. European Congress on Tropical Medicine and International Health (23.11.2023), Utrecht, Netherlands.

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During 01.01.2021 and 30.06.2023, six publications (5 scientific reports and 1 review article), six conference talks as well as four conference posters were published on CorA research (including DZIF projects TTU 09.822 and 09.920) with collaboration of HZI and UK Bonn. Peer-reviewed publications within the project:

- Peukert C, Vetter AC, Fuchs HLS, Harmrolfs K, Karge B, Stadler M, Brönstrup M. Siderophore conjugation with cleavable linkers boosts the potency of RNA polymerase inhibitors against multidrug-resistant E. coli. Chem Sci. 2023 Apr 26;14(20):5490-5502. doi: 10.1039/d2sc06850h
- Rox K, Becker T, Schiefer A, Grosse M, Ehrens A, Jansen R, Aden T, Kehraus S, König GM, Krome AK, Hübner MP, Wagner KG, Stadler M, Pfarr K, Hoerauf A. Pharmacokinetics and Pharmacodynamics (PK/PD) of Corallopyronin A against Methicillin-Resistant Staphylococcus aureus. Pharmaceutics. 2022 Dec 30;15(1):131. doi: 10.3390/pharmaceutics15010131
- Edwards JL, Balthazar JT, Esposito DLA, Ayala JC, Schiefer A, Pfarr K, Hoerauf A, Alt S, Hesterkamp T, Grosse M, Stadler M, Golparian D, Unemo M, Read TD, Shafer WM. Potent In Vitro and Ex Vivo Anti-Gonococcal Activity of the RpoB Inhibitor Corallopyronin A. mSphere. 2022 Oct 26;7(5):e0036222. doi: 10.1128/msphere.00362-22
- Krome AK, Becker T, Kehraus S, Schiefer A, Gütschow M, Chaverra-Muñoz L, Hüttel S, Jansen R, Stadler M, Ehrens A, Pogorevc D, Müller R, Hübner MP, Hesterkamp T, Pfarr K, Hoerauf A, Wagner KG, König GM. Corallopyronin A: antimicrobial discovery to preclinical development. Nat Prod Rep. 2022 Sep 21;39(9):1705-1720. doi: 10.1039/d2np00012a
- Becker T, Krome AK, Vahdati S, Schiefer A, Pfarr K, Ehrens A, Aden T, Grosse M, Jansen R, Alt S, Hesterkamp T, Stadler M, Hübner MP, Kehraus S, König GM, Hoerauf A, Wagner KG. In Vitro-In Vivo Relationship in Mini-Scale-Enabling Formulations of Corallopyronin A. Pharmaceutics. 2022 Aug 9;14(8):1657. doi: 10.3390/pharmaceutics14081657
- Balansky J, Pfarr K, Szekat C, Kehraus S, Aden T, Grosse M, Jansen R, Hesterkamp T, Schiefer A, König GM, Stadler M, Hoerauf A, Bierbaum G. The RNA Polymerase Inhibitor Corallopyronin A Has a Lower Frequency of Resistance Than Rifampicin in Staphylococcus aureus. Antibiotics (Basel). 2022 Jul 8;11(7):920. doi: 10.3390/antibiotics11070920

Conference talks and posters:

- 29th Annual Meeting of the German Society for Parasitology, 15.-17.03.2021, virtual conference; Pre-clinical development of Corallopyronin A, an antibiotic active against filarial nematodes. A Schiefer, M Stadler, K Wagner, R Jansen, A Krome, S Kehraus, S Hüttel, M P Hübner, D Pogorevc, S Alt, G M König, R Müller, T Hesterkamp, K Pfarr, A Hoerauf - Talk
- Antimicrobial Chemotherapy Conference 2021, February 2021, virtual conference; Pre-clinical development of the natural compound antibiotic Corallopyronin A. K Pfarr, A Schiefer, A Krome, T Becker, S Kehraus, T Aden, H Neufeld, C Lämmer, R Jansen, G König, M Hübner, S Alt, R Müller, M Unemo, G Bierbaum, W Shafer, B Henrichfreise, K Wagner, T Hesterkamp, M Stadler, A Hoerauf - Poster
- DZIF Jahrestagung 2022, 01.-03.06.2023 in Stuttgart; Steps towards Phase I – drug substance and drug product development of Corallopyronin A. M Große, K Wagner, T Becker, A Krome, R Jansen, D Pogorevc, A Schiefer, S Alt, R Müller, T Hesterkamp, K Pfarr, M Stadler, A Hoerauf - Poster

- American Society for Microbiology (ASM) ASM Microbe 07.06.2022; Pre-clinical development of Corallopyronin A – a novel natural product active against helminths, STIs and Staphylococci. K. Pfarr, A. Schiefer, W. Shafer, J. Edwards, T. Becker, G. Bierbaum, S. Kehraus, M. Grosse, R. Jansen, S. Alt, G. König, A. Ehrens, T. Schneider, M. P. Hübner, R. Müller, K. Wagner, T. Hesterkamp, M. Stadler, A. Hoerauf - Talk
- Konferenz der Deutschen Gesellschaft für Tropenmedizin, Reisemedizin und Globale Gesundheit e.V. (CTM), 23.-25.06.2022, Rostock; Präklinische Entwicklung des Antibiotikums Corallopyronin A zur Behandlung von Filariosen und multiresistenter Staphylokokken. A. Schiefer, K. Rox, G. Bierbaum, A. Krome, T. Becker, M. Grosse, S. Alt, R. Jansen, S. Kehraus, M. P. Hübner, A. Ehrens, G. M. König, R. Müller, T. Hesterkamp, M. Stadler, K. Wagner, K. Pfarr, A. Hoerauf - Talk
- 22nd Drug Design & Development Seminar (DDDS) of the German Society for Parasitology (DGP) TransAlp Conference on Human and Animal Parasitic Diseases July 12th – 15th, 2022; Pre-clinical development of Corallopyronin A – a novel natural product active against filarial nematodes. K. Pfarr, A. Schiefer, M. Große, K. Rox, A. Krome, R. Jansen, T. Becker, S. Kehraus, A. Ehrens, G.M. König, R. Müller, S. Alt, T. Hesterkamp, M.P. Hübner, K. Wagner, M. Stadler, A. Hoerauf - Talk
- ASTMH 71th Annual Meeting 30.10-03.11.2022; Pre-clinical development of Corallopyronin A – a novel antibiotic active against Wolbachia endosymbionts of filarial nematodes. K. Pfarr, A. Schiefer, M. Große, A. Krome, T. Becker, S. Kehraus, A. Ehrens, R. Jansen, G. M. König, S. Alt, R. Müller, T. Hesterkamp, M.P. Hübner, M. Stadler, K. Wagner, A.Hoerauf – Poster
- HIPS Symposium 04.05.2023 Saarbrücken; Development of a repeated batch process for the Corallopyronin A production. L Berger, S Bernecker, R Jansen, B Sandargo, A Schiefer, S Alt, R Müller, T Hesterkamp, K Pfarr, K Wagner, M Stadler, A Hoerauf, M Große - Talk
- DECHEMA Himmelfahrtstagung; 15.-17.5.2023 Weimar; Heading to phase I – Scale up of the production process of the antibiotic candidate Corallopyronin A into industrial scale.M Große, K Wagner, T Becker, A Krome, R Jansen, B Sandargo, A Schiefer, S Alt, R Müller, T Hesterkamp, K Pfarr, M Stadler, A Hoerauf – Poster
- 15th International Congress of Parasitology (ICOPA), 21.-26.08.2023 Copenhangen, Denmark; Pre-clinical development of Corallopyronin A – a novel natural product active against filarial nematodes. K. Pfarr, A. Schiefer, M.Große, K. Rox, A. Krome, R. Jansen, T. Becker, S. Kehraus, M P. Hübner, A. Ehrens, G.M. König, R.Müller, S. Alt, T. Hesterkamp, K. Wagner, M. Stadler, A. Hoerauf - Talk

Kurzbericht

DZIF-Vorhaben-Nr.:	***TTU 09.914***
Titel des Vorhabens:	**Corallopyronin A development through to phase 1 trial (first-into-man)**
Laufzeit des Vorhabens:	***01.06.2019-31.03.2024***

Zuwendungsletztempfänger (ZLE)

1. Rheinische Friedrich-Wilhelms-Universität Bonn (UKB)
2. Helmholtz-Zentrum für Infektionsforschung GmbH (HZI)

Angaben zum Vorhaben:

Corallopyronin A (CorA) ist ein Antibiotikum, das aktiv gegenüber Wolbachien ist, Endosymbionten von Filarien die Menschen infizieren. Bisher haben wir non-GLP ADME und *in vitro* toxikologische Studien durchgeführt: CorA ist oral verfügbar, nicht toxisch, induziert nicht CYP450 3A4 und hat eine gute Adsorption. Mit unserer entwickelten HPLC und NMR-Analyse Methode können wir die absolute Reinheit jedes CorA Batches, der Isoformen und Tautomeren bestimmen. Um die Produktion von klinischem Material zu ermöglichen, haben wir uns für die heterologe Expression des CorA Biosynthese Genklusters in *M. xanthus* durch das HZI entschieden. Ziel des DZIF Projektes war es, den heterologen Produzentenstamm weiter zu optimieren und anschließend den Produktionsprozess zu einer CMO zu transferieren, die die Produktion von pre-GMP und GMP CorA durchführt.

Wir haben *in vitro* und *in vivo* CorA-Aktivität gegen gramnegative Wolbachia-Endosymbionten gezeigt. Das Abtöten der Wolbachien führt zu Wurmsterilität und einer langsamen Abtötung der Würmer. Zusätzlich konnten wir die Wirksamkeit von CorA gegen Rickettsia spp., *Chlamydia trachomatis*, *Neisseria gonorrhoeae* und multiresistenten *Staphylococcus aureus* nachweisen. Die letzten drei Pathogene sind bei der WHO gelistet, als vorrangige Krankheitserreger für die neue Antibiotika erforderlich sind. Daher entwickeln wir CorA für die primäre Indikation: eine neue Therapie für menschliche Filarieninfektionen und untersuchen die sekundären Indikationen: *C. trachomatis-, N. gonorrhoeae-* und MRSA-Infektionen.

Durch die Entwicklung der heterologen CorA-Produktion in *Myxococcus xanthus* und eines optimierten Downstream-Prozesses ohne präparative HPLC, haben wir eine entscheidende Hürde überwunden, die bei Naturprodukten häufig auftritt: eine kosteneffiziente Produktion. Diese Verbesserungen ergeben reproduzierbar CorA mit einer Reinheit von 90–95 %, wie durch unsere validierten quantitativen NMR- und HPLC-Analysemethoden bestätigt. Das endgültige Produktionsprotokoll wird CorA mit einer Reinheit von >90 % für nicht-GLP-präklinische Studien, Genotoxizitäts- und Nicht-GLP-Toxizitätsstudien sowie die Arzneimittelformulierung bereitstellen. Wir sind mit der zuständigen deutschen Zulassungsbehörde (Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM) in Kontakt und haben eine erste formelle wissenschaftliche Stellungnahme zum Arzneimittelwirkstoff CorA erhalten. Das BfArM hat offiziell zugestimmt, dass: i) die heterologe Produktion von CorA kein Hindernis für die Entwicklung von CorA darstellt; ii) eine CorA-Reinheit von 90–95 % für die präklinischen Toxikologiestudien akzeptabel ist und die Nicht-CorA-Komponenten nicht

einzeln auf Toxizität bewertet werden müssen; und iii) die vorläufige Spezifikation für die Phase-1-API akzeptabel ist. Wir haben zwei CMOs (BBEPP und Alphora) identifiziert und mit der Durchführung einer Machbarkeitsstudie beauftragt. Der Technologietransfer und der Versand des Produzentenstamms und der CorA-Standards verliefen erfolgreich. BBEPP reproduzierte die Fermentation im 100-L-Maßstab und konnte den Herstellungsprozess auf 15 m^3 steigern. Die während dieses Prozesses festgelegten SOPs bilden die Grundlage für den nächsten Schritt, die GMP-Herstellung der CorA-Arzneimittelsubstanz und des CorA-Produkts.

Die Ergebnisse dieses DZIF Projektes waren ausschlaggebend für die Ermöglichung einer Zusammenarbeit mit Eisai Co., Ltd, Japan, um beim Global Health Innovative Technology (GHIT) Fund, Japan, eine Finanzierung für die weitere Entwicklung des Naturstoffes zu beantragen. Das GHIT-Projekt wurden im zweiten Quartal 2024 genehmigt.

Der Inhalt des Kurzberichts ist aus meiner/unserer Sicht veröffentlichungswürdig:
Nein

Gab es zu dem Projekt bereits Pressemitteilungen? Falls ja, bitte Datum und herausgebende Pressestelle angeben.

- 30.11.2022: Parasitologen lehren Würmer das Fürchten, Tagesspiegel Background

Gab es Ihrer Kenntnis nach Medienberichte zu dem Projekt? Falls ja, bitte Medium und Datum angeben.

- 08.08.2022: Antibiotikum gegen folgenschwere Wurminfektionen. Provendis YouTube Kanal und Website. https://www.youtube.com/watch?v=cOqIB-brlCE&t=20s

Koordinator des Vorhabens: Prof Dr med Achim Hörauf

Ort, Datum
Bonn, 09.01.2025