Pre-clinical development of Corallopyronin A – a natural product active against helminths, STIs and Staphylococci

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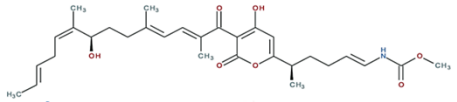
Abstract

Corallopyronin A (CorA) inhibits bacterial DNA-dependent RNA polymerase and has a different binding site to rifampicin. Thus, it is effective against rifampicin-resistant *Staphylococcus aureus*. We have shown that CorA kills Gram-negative *Wolbachia* endobacteria of filarial nematodes, causative agents of onchocerciasis (river blindness) and lymphatic filariasis (elephantiasis). Depleting the essential endosymbionts results in worm sterility and slow adult worm killing. Within DZIF we demonstrated CorA activity against *Neisseria gonorrhoeae* and multi-resistant *S. aureus*. At 4x MIC no spontaneous CorA resistance in *N. gonorrhoeae* could be detected, predicting a frequency of mutation of $\leq 10^{-10}$. We have also shown CorA activity against established *S. aureus* biofilms and their formation. Furthermore, CorA has excellent biodistribution into bone. We have received funding to investigate CorA as a new antibiotic class for treating osteomyelitis and *S. aureus* biofilms. In support of CorA as a novel solution to several targets of the WHO Priority Pathogen List for which new antibiotics are needed, we have conducted standard non-GLP ADMET studies. *In vitro* toxicity tests (off-target, AMES, micronucleus, hERG, phototoxicity) demonstrated that CorA is non-toxic and pharmacologically safe; supported by non-GLP *in vivo* toxicity studies in rats and dogs in which the maximal tolerated dose (MTD) in both species was 1000 mg/kg CorA, causing mild symptoms. Results of the 7-day repeated dose studies in rats and dogs that will form the basis for the design of the regulatory-conform GLP toxicity and safety pharmacology studies will be presented. CorA drug substance is heterologously produced using genetically modified *Myxococcus xanthus*. In preparation for pre-GMP and GMP manufacturing, the first up-scale into industrial scale (15 m3) was achieved in 2022 at Bio Base Europe Pilot Plant (BBEPP, Ghent, Belgium). The Helmholtz Centre for Infection research, Braunschweig performed the DSP of this large amount of material, achieving 90-95% pure HQ-RGM material. Using amorphous solid dispersion (ASD) formulation principles, two solid oral formulations were developed with increased stability (>3 months at 30 °C, >6 months at 5 °C) and oral bioavailability (mouse >59%, rat 100%, dog >53%) compared to neat CorA. After finalization of the pre-clinical work, we plan to enter the clinical phase I in 2024/2025.



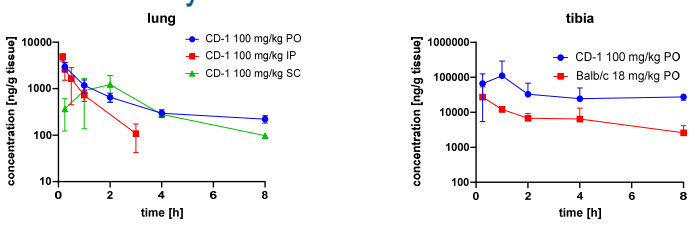
Non-GLP safety and toxicity

In vitro ADME		Conclusion
Plasma protein binding	6 species	98 % - 99 %; equivalent to Ibuprofen, Warfarin
Plasma stability	6 species	> 4 hours
Stability in intestinal fluids		Stable in FeSSIF (pH 5.8) $t_{1/2}$: > 139 min
Liver microsome metabolic stability Human, Dog, Mouse, Monkey, Rat, Mini-pig		Human and dog: few metabolites after 45 min (mainly M1, M2)
UGT phenotyping UGT 1A4, UGT 1A1, UGT 2B7, UGT 2B10, UGT 1A9, UGT 1A6		CorA is not a UGT substrate: % turnover <8.6 (FDA = >25% turnover)
CaCo cells:		Predicted high intestinal absorption P_{app} A to B = 2.0E-05 cm/s
Drug substance stability 30°C, 65% RH, 3 months		Incorporation of CorA with polymers increased stability: neat CorA 25%, Povidone 96%, Copovidone 97%

In vitro and *in vivo* safety data	Conclusion
Off target profiling	A3, PPARγ, COX1; EC_{50} = 170-850X higher than CorA EC_{50} = 0.016 µM of against *Wolbachia in vitro*
Cyp inhibition 1A2, 2B6, 2D6, 3A4, 2C8, 2C19, 3A4	No inhibition of six recombinant human CYPs; inhibition of 2CP
CYP 3A4 induction via PXR	Minimal inducer: 12 µM CorA vs 1.5 µM, DDI unexpected
Non-GLP Micronucleus	No induction of chromosomal damage, no genotoxicity
Non-GLP AMES (5 strains)	No evidence of genotoxicity
Phototoxicity	No phototoxicity up to limit of solubility (38 µM)
Liver toxicity	No toxicity in hepatocytes from rats or humans (200µM)
Non-GLP hERG	Predicted IC_{50} = > 10 µM
MTD rat	1000 mg/kg; mild clinical symptoms
MTD dog	1000 mg/kg; moderate, transient symptoms
7d repeated-dose rat Vehicle, 250, 1000 mg/kg/d	250 mg/kg/d, no effects seen
7d repeated-dose dog Vehicle, 150, 450, 750 mg/kg/d	**NOAEL**: 150 mg/kg bw/d; Predicted **HED** = 4 mg/kg. No effect: ECG, blood pressure, hematology, coagulation, necropsy, organ weights; histology to be done

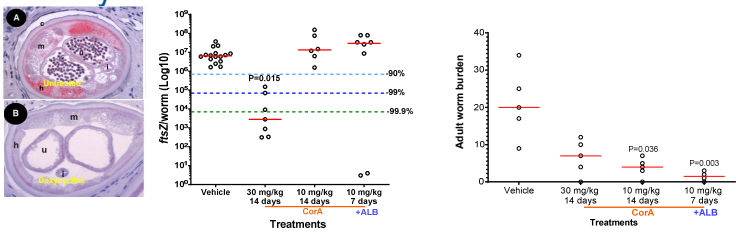
CorA has no prohibitive safety issues

Bioavailability



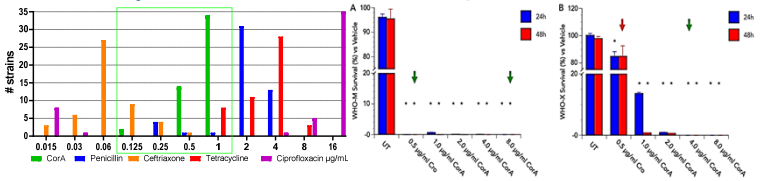
- Excellent oral PK biodistribution allows pursuing efficacy in S. *aureus* lung infection and osteomyelitis models
- DZIF grant to generate data for treatment of biofilm-associated intracellular infections and osteomyelitis

Primary indication: helminths – anti-wolbachial



- Filarial nematodes require *Wolbachia* endobacteria for development, fecundity and survival
- CorA depletes *Wolbachia* from all life stages
- Better efficacy than the comparator substances in *Litomosoides sigmodontis* model (doxycycline)
- Combination with albendazole lowers dose and treatment time (as with doxycycline and albendazole in human trials)
  - Fits Target Product Profile of >90% reduction

Secondary indication: *Neisseria gonorrhoeae*



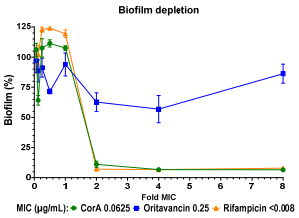
- MIC = 0.5-1 µg/mL vs 50 CDC + 14 WHO MDR/XDR strains
- **No spontaneous resistance selected at 4X MIC**
  - Predicted frequency of mutation $\leq 10^{-10}$ (clinical strains)
- Three-step resistance selection needed for MIC of 32
- MIC ≤2 µg/mL in infected primary cervical cells
  - Time to kill ≤48 hours
- **Efficacy in established biofilms; prevents biofilm formation**

Collaborating with leaders in gonorrhoeae research:
- Prof. Dr. William Shafer: Emory Antibiotic Resistance Center, Emory School of Medicine
- Prof. Dr. Jennifer Edwards: Center for Microbial Pathogenesis, Nationwide Children´s Hospital
- Prof. Dr. Magnus Unemo: WHO Collaborating Centre for Gonorrhoea and Other STIs, Sweden

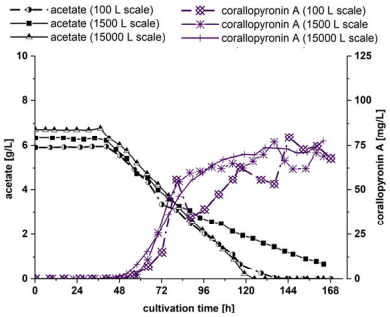
Secondary indication: *Staphylococcus aureus*

CorA rate of mutation is lower than rifampicin
- CorA: $1.67x10^{-8}$
  - 3 RpoB and 2 RpoC mutation sites
- Rifampicin: $1.03x10^{-7}$
  - 11 RpoB mutation sites
- No cross resistance

Efficacy against *S. aureus* in established biofilms and prevents biofilm formation



Drug substance and drug product



Upscaling to industrial scale
- USP successfully scaled up to 15,000 L
  - 3 successful runs
  - Titers equivalent to those observed at HZI

Formulation - amorphous dispersion
- Improved oral bioavailability in mouse, rat and dog
  - F from 5% to 35%-100%
- Improved stability
  - stable >3 months at 30 °C, >6 months at 25 °C
- Formulation Patent EP 20 172 409.3

Current and future work

- Feasibility study at cGMP CMO; production of GMP trial material
- GLP safety and toxicity study in rats (Q4 2023) and dogs (Q2 2024)
- 3rd BfArM Scientific Advice
- Prepare phase 1 clinical trial

Publications

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Funding

DZIF
Bundesministerium für Bildung und Forschung
EFRE.NRW Investitionen in Wachstum und Beschäftigung
HORIZON 2020

Patents

- US Patent: US 9168244 B2, granted 2015 (treatment of filariasis)
- US Patent: US 9687470 B2, granted 2017 (prevention of filariasis)
- EU Patent: EP 2704708 B1, granted 2017 and validated in: DE, GB, NL, CH, IT, ES, FR and HR
- WO 2014/181000 A1 Heterologous production of myxopyronin and its derivatives, granted 2018
- PCT/EP2021/061310 CorA formulations, filed on 29.4.2021, claiming priority of EP 20 172 409.3