Session 4 - Current research landscape in onchocerciasis
Clinical research in onchocerciasis

Onchocerciasis Research Network and DNDi stakeholder event
October 3 - 4, 2018
Kampala, Uganda

Ute Klarmann-Schulz
Institute for Med. Microbiology, Immunology and Parasitology (IMMIP)
German Center for Infection Research (DZIF), partner-site Bonn-Cologne
University Hospital of Bonn
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Current treatment of onchocerciasis

- Ivermectin (IVM)
ISRCTN50035143

Death to Onchocerciasis and Lymphatic Filariasis: Comparison of Ivermectin alone with Albendazole (ALB) plus Ivermectin (IVM) in their efficacy against Onchocerciasis

In total 272 Mf-positive participants, with at least one palpable onchocercoma were treated with either:

Annual treatment (0, 12, 24 months):
1) IVM 200µg/kg annually
2) IVM 200µg/kg plus ALB 800mg annually

Semi-annual treatment (0, 6, 12, 18, 24 months):
3) IVM 200µg/kg semi-annually
4) IVM 200µg/kg plus ALB 800mg semi-annually.
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Palpable onchocercomata
Surgical extirpation of onchocercomata (Nodulectomies)
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(Nodulectomies)
Hematoxylin and eosin (HE) staining

**Normal embryogenesis**  
**Degenerated embryogenesis**

**Evaluations**
- **Nodules**: nodule size; number & position of worms
- **Worms**: size, sex, age, morphology (hypodermis, cuticle), live / dead, sperms
- **Female worms**: embryogenesis, microfilaria, uterus (embryonic stages)
Perl's Prussian blue (FE III, iron stain)

Young worm (weak FE III)  Old worm (strong FE III)

**Evaluations**
- **Worms:** age
Lysosomal Aspartic Protease (APR) staining

Live worm (APR positive, left)
Dead worm (APR negative, right)

**Evaluations**
- **Worms**: live / dead
  - Dead worms: calcification, resorption
  - Live worms: morphology, sperms, uterus possible (embryonic stages)
With a range of 55 - 59% the proportion of dead worms did not differ between the 4 groups.

Number of live and dead female worms per treatment group. 4-group comparison $p = 0.9198$ (Proc Genmod, SAS®)
The addition of albendazole to ivermectin did not reduce female worm fertility

Number of live female worms with normal or degenerated embryogenesis, oocytes only or empty uterus per treatment group. 4-group comparison p = 0.1229 (Proc Genmod, SAS®)
Semiannual drug administration resulted in a sustained increase of MF negative individuals compared to annual treatment.
Semiannual drug administration resulted in a sustained increase of MF negative individuals compared to annual treatment.

Number of Mf-positive/ Mf-negative participants grouped for annual or semiannual treatment without taking the addition of ALB into account

\[ p = 0.024 \text{ (Fisher`\textquotesingle}s exact test) \]
Alternative treatment strategies (ATS) are needed where community directed treatment with ivermectin (CDTI) is not sufficient because of:
- low treatment coverage
- areas co-endemic for loiasis
- suboptimal response

(WHO ATS-report 2015)
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- low treatment coverage
- areas co-endemic for loiasis
- suboptimal response

→ drugs with adulticidal activity

- Re-purposing of registered drugs
- Optimization of drug candidates
- Identification of novel drugs that target *Wolbachia* or have another mode of action

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The endosymbiont *Wolbachia* as drug target

From: Hoerauf et al., Lancet 2000

*O. volvulus* female from an untreated patient

*O. volvulus* female after 6 weeks of 100 mg/kg doxycycline

From: Hoerauf et al., Lancet 2000
Targeting *Wolbachia* using doxycycline

- *Wolbachia* depletion
- female worm sterility after 6-12 months
- growth retardation of the worms
- macrofilaricidal effects
Publications from clinical trials in onchocerciasis targeting Wolbachia

Endosymbiotic bacteria in worms as targets for a novel chemotherapy in filariasis

Achim Hoerauf, Lars Volkmann, Christoph Hamelmann, Ohene Adjei, Ingo Autenrieth, Bernhard Fleischer, Dietrich W Böttner

THE LANCET • Vol 355 • April 8, 2000

Depletion of wolbachia endobacteria in Onchocerca volvulus by doxycycline and microfilaridermia after ivermectin treatment

Achim Hoerauf, Sabine Mand, Ohene Adjei, Bernhard Fleischer, Dietrich W Böttner

THE LANCET • Vol 357 • May 5, 2001

Newly acquired Onchocerca volvulus filariae after doxycycline treatment

Sabine Specht, Achim Hoerauf, Ohene Adjei, Alexander Debragh, Dietrich W. Böttner

DOI 10.1007/s00436-009-1624-5

Efficacy of 5-week doxycycline treatment on adult Onchocerca volvulus

Achim Hoerauf, Sabine Specht, Yeoobah Marfo-Debrekyei, Marcelle Böttner, Alexander Yaw Debragh, Sabine Mand, Linda Batsa, Norbert Brattig, Peter Konadu, Claudia Bandi, Rolf Fimmers, Ohene Adjei, Dietrich W. Böttner

DOI 10.1007/s00436-008-1217-8

Doxycycline Leads to Sterility and Enhanced Killing of Female Onchocerca volvulus Worms in an Area With Persistent Microfilaridermia After Repeated Ivermectin Treatment: A Randomized, Placebo-Controlled, Double-Blind Trial


CID 2015:61 (15 August)

Comparison of Doxycycline, Minocycline, Doxycycline plus Albendazole and Albendazole Alone in Their Efficacy against Onchocerciasis in a Randomized, Open-Label, Pilot Trial


2003

2017

Institute for Med. Microbiology, Immunology and Parasitology (IMMIP)
Current recommendations for doxycycline treatment

Lymphatic filariasis
- DOX 200 mg/d for 6 weeks if benefit to disease is wanted in addition to macrofilaricidal effect
- DOX 200 mg/d for 4 weeks if focus is on the macrofilaricidal effect

Onchocerciasis
- DOX 200 mg/d for 6 weeks if macrofilaricidal effect is wanted
- DOX 200 mg/d for 4 weeks or 100 mg/d for 5 weeks if only worm sterility is wanted

Hoerauf, Curr Opin Infect Dis 2008,
Taylor-Hoerauf-Bockarie Lancet 2010
Comparison of doxycycline alone vs doxycycline plus rifampicin in their efficacy against onchocerciasis

Randomised, placebo-controlled, double-blind trial
508 patients
5 treatment arms (Doxycycline 200mg/d and 100mg/d for 6 weeks)
Nodulectomies after 6 and 20 months
Doxycycline 100mg for 6 weeks is as good as doxycycline 200mg for 6 weeks to achieve sterility of live female worms.

DOX 200 and DOX 100 for 6 weeks:
- Superiority to DOX + RIF for 3 weeks,
- Superiority RIF for 6 weeks
- Superiority to Placebo*

DOX + RIF for 3 weeks:
- Superiority to RIF for 6 weeks
- Superiority to Placebo*

*alternating logistic regression taking the dependency of several nodules in one patient into account (Proc Genmod, SAS®) at an alpha-level of 0.0083 one-sided
**Wolbachia** Surface Protein (WSP) staining

**Evaluations**
- **Worms:** number of *Wolbachia* in worm and uterus, uterus possible (embryonic stages)
Both doxycycline regimens showed complete absence of *Wolbachia* by immunohistology in > 96% of the live female worms, corresponding to the anti-parasitic effect.

**DOX 200 and DOX 100 for 6 weeks:**
- Superiority to DOX + RIF for 3 weeks
- Superiority to RIF for 6 weeks
- Superiority to Placebo.*

**DOX + RIF for 3 weeks:**
- Superiority to RIF for 6 weeks*

*alternating logistic regression taking the dependency of several worms in one patient into account (Proc Genmod, SAS®) at an alpha-level of 0.0083 one-sided
Histological analysis of *Wolbachia* was confirmed by nodule PCR.
Doxycycline 100mg for 6 weeks is as good as doxycycline 200mg for 6 weeks to achieve sustained absence of microfilariae.

DOX 200 and DOX 100 for 6 weeks:
- Superiority to DOX + RIF for 3 weeks,
- Superiority to RIF for 6 weeks
- Superiority to Placebo.*

DOX + RIF for 3 weeks
- Superiority to RIF for 6 weeks*

*Fisher’s exact test
A-WOL Mino

156 patients
5 treatment arms (Doxycycline 200mg/d for 4 weeks)
Nodulectomies after 6 months
These results confirm earlier studies that DOX 4w is sufficient for *Wolbachia* depletion and the desired parasitological effects.

The data further suggest that there is an additive/synergistic effect of ALB 3d on top of that of DOX 3w alone, and that MIN 3w has a stronger potency than DOX 3w. These latter two results are preliminary and need confirmation in a full randomized controlled phase 2 trial.

Comparison to ALB 3d:
- DOX 4w: p < 0.0001 (OR 145)
- DOX 3w + ALB 3d: p < 0.0001 (OR 8.2)
- MIN 3w: p = 0.0016 (OR 5.8)
- DOX 3w: p = 0.0084 (OR 4.2)

Comparison to DOX 4w:
- DOX 3w + ALB 3d: p = 0.0052 (OR 18)
- MIN 3w: p = 0.0022 (OR 26)
- DOX 3w: p = 0.0007 (OR 36)

No difference between the 3 experimental regimens
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**MF PCR (mouse model)**

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<td>-99.91 vs. VEH</td>
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**Graph:**
- **L. sigmodontis microfilaria** (mf/10µl blood)
- **weeks post treatment [wpt]**
- **treatment**
  - VEH bid 14d PO
  - DOX 40 mpk bid 14d PO
  - Experimental treatment
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MF PCR (mouse model)

### Graphs:

**Graph 1:**
- **L. sigmodontis microfilaremia** (mf+1/10µl blood)
- **treatment**
- **VEH bid 14d PO**
- **DOX 40 mpk bid 14d PO**
- **Experimental treatment**

**Graph 2:**
- **weeks post treatment [wpt]**
- **Wolbachia FtsZ per microfilaria**
- **Experimental treatment**
- **DOX 40 mpk bid 14d PO**
- **VEH bid 14d PO**

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**Wolbachia** depletion in microfilariae is an indicator for **Wolbachia** depletion in adult worms.
MF PCR (human samples)

*Mann-Whitney-U-test (Kruskal-Wallis-test (over all 4 groups): p = 0.003)

-validated method to detect *Wolbachia* FtsZ/ MF
-reproducible on different days by using the same or a different master-mix
-currently limited to MF-counts > 5MF/sample
TPP: Target product profile (min.)

- Death or permanent sterilization of the adult onchocerciasis worms
- After 1 course, ~70% (model-based) of treated individuals achieve death or permanent sterilization of all adult onchocerciasis worms
- All adults and children age ≥5 who are infected, excluding pregnant women
- Oral dose, once daily, up to 7 days (DNDi: 14 days) or single, intra-muscular injection.
- One dosage for adults, and height-based dosing for children
Rifapentine plus Moxifloxacin

**Mouse Litomosoides Screening**

**L. sigmodontis adult worm**

**BALB/c WT mouse model:**

- Mf counts 7wpt
  - untreated
  - vehicle
  - DOX 17d
  - DOX 21d
  - DOX 28d
  - RPT MOX 2x100mg/kg 7d
  - RPT MOX 2x100mg/kg 14d
  - RPT MOX 200mg/kg 14d

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The efficacy of Rifapentine 900mg/d plus Moxifloxacin 400mg/d given for 14 or 7 days against Onchocerciasis – a randomized, controlled, parallel-group, open-label, phase II pilot trial.

In collaboration with:

Interventions*:
Treatment A (experimental): Rifapentine + Moxifloxacin for 14 days
Treatment B (experimental): Rifapentine + Moxifloxacin for 7 days
Treatment C (control): Doxycycline 200mg for 4 weeks
Treatment D (control): Nodulectomy only

*All participants will be treated with ivermectin (IVM) at the standard MDA dosage of 150 μg/kg following the nodulectomies 6 months after study onset.

Skin snipping (for Mf-counts/ Mf-PCR): pre-treatment, 3.5 months and 6 months after treatment onset

Nodulectomies: 6 months after treatment onset

Primary outcome: Absence of Wolbachia endobacteria in adult female worms assessed by immunohistology 6 months after treatment onset.
“The efficacy of Rifapentine 900mg/d plus Moxifloxacin 400mg/d given for 14 or 7 days against Onchocerciasis – a randomized, controlled, parallel-group, open-label, phase II pilot trial.”

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Recruitment of patients for this trial starts this week
Acknowledgements

Achim Hoerauf
Marc Huebner
Kenneth Pfarr
Janina Kuehlwein
Sabine Mand
Andrea Schiefer
Anna Albers
Bettina Dubben
Arcangelo Ricchiuto
Barbara Gruetzmacher
Christine Laemmer
and the whole team

Matthias Schmid
Rolf Fimmers
Jennifer Nadal

External Collaborators:
Peter Konadu
Mike Osei-Atweneboana

DOLF: Drugs for Neglected Diseases initiative

Sabine Specht
Ivan Scandale
Rob Don
Frederic Monnot

CWRU:
Christopher L. King
James Kazura

WUSTL:
Gary Weil
Kerstin Fischer

LSTM
Mark Taylor
Steve Ward

Samuel Wanji

DZIF

Institute for Med. Microbiology, Immunology and Parasitology (IMMIP)
Acknowledgements

Thanks to all study participants and thanks for your attention!
### Trial in onchocerciasis of lymphatic filariasis?

**Main outcome parameter:**

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