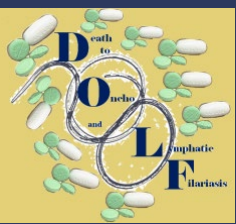


# DOLF RESEARCH ON ONCHOCERCIASIS

PETER U. FISCHER, PHD, DIVISION OF INFECTIOUS DISEASES



12 yrs, 18 kg

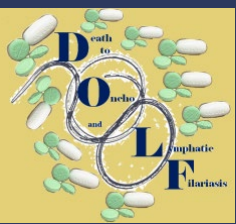


# Death to Onchocerciasis & Lymphatic Filariasis Project

(<https://dolf.wustl.edu/>)

- Established in November **2009** (PI Gary Weil)
- **Bill & Melinda Gates** Foundation funded project, 11 disease endemic countries
- Working with WHO, country MoH and many academic institutions
- **Optimization of Mass Drug Administration** (MDA) with existing drugs for lymphatic filariasis (LF) and onchocerciasis (Peter Fischer)
- **Clinical trials** to test the impact of new drug combinations and treatment schedules for LF and onchocerciasis using currently used drugs (Chris King, CWRU)
- Effect of MDA for LF/oncho on **soil-transmitted helminth** infections
- Community studies of **ivermectin+DEC+albendazole** (IDA) vs. DEC+albendazole (DA) for LF
- Pilot studies of I/IDA for onchocerciasis (**new grant until 2021**)

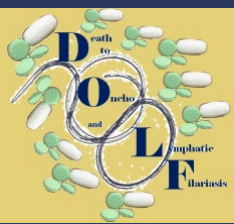




# Projects

- Community trials on annual vs semianual IVM/Alb for **lymphatic filariasis and onchocerciasis**
  - Liberia Foya district
  - Liberia Harper district
  - Cote d'Ivoire, Akoupe/Abengouru
- RCT on multiple doses of **IVM/Alb for onchocerciasis**
  - West Ghana (Kumasi, Alex Debra/Achim Hoerauf)
  - East Ghana (Hohoe, Nick Opoku)
- Pilot RCTs on **I/IDA for onchocerciasis**
  - East Ghana (Hohoe, Nick Opoku), ophthalmology
- Side projects: Diagnostics for onchocerciasis
  - Development of qPCR assays
  - Evaluation of Ov16 tests
  - Biomarker discovery for adult female *O. volvulus* worms
  - ◦ Histological assessment of adult *O. volvulus* in nodules





# DOLF Onchocerciasis Study Sites



1. ComT: Foya, Liberia, Partner National Public Health Institute (NPHI/LIBR, F. Bolay)
2. ComT: Harper, Liberia (NPHI)
3. ComT: Akoupe/Abengouru, Côte d'Ivoire, Partner MoH (A. Meite), CSRS
4. RCT: Ashanti region, Partner KNUST/KCCR (A. Debrah), Uni Bonn (A. Hoerauf)
5. RCT: Volta region, Partner UHAS/OCRC (N. Opoku)



# Annual vs semianual IVM/Alb for LF and oncho: Liberia

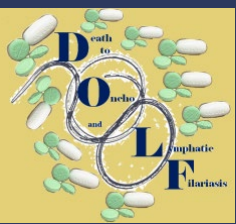
## Foya

Group	Year	Lymphatic Filariasis						Onchocerciasis			
		N	Adjusted Mf prevalence (%)	ICT (%)	FTS (%)	Gm Wb Mf/ml	CMFL Mf/ml	N	Ov Skin snip Mf positive (%)	Gm Ov Mf/mg	CMFL Mf/mg
(2x MDA)	2012	1169	1.7	13.6	ND	59.5	3.7	1142	<b>23.6</b>	2.2	0.34
	2014	1174	0.2	10.1	12.2*	16.7	0.2	367**	<b>27.8</b>	2.7	0.48
	2016	1132	0.0	ND	3.3	0.0	0.0	322**	<b>18.6</b>	1.2	0.18
	2017	1209	0.0	3.8	4.2	0.0	0.0	198**	<b>4.5*</b>	1.7	0.05
(1X MDA)	2012	997	1.6	12.5	ND	59.3	3.8	1008	<b>14.4</b>	2.2	0.20
	2014	639	0.0	6.1	3.6*	0.0	0.0	525	<b>9.1</b>	2.0	0.11
	2016	898	0.0	ND	1.8	0.0	0.0	494	<b>5.5</b>	1.4	0.05
	2017	1066	0.0	1.3	1.2	0.0	0.0	705	<b>3.7*</b>	1.0	0.03

## Harper

Group	Year	Lymphatic Filariasis						Onchocerciasis			
		N	Adjusted Mf prevalence (%)	ICT (%)	FTS (%)	Gm Wb Mf/ml	CMFL Mf/ml	N	Ov Skin snip Mf positive (%)	Gm Ov Mf/mg	CMFL Mf/mg
Coastal (2x MDA)	2013	1000	19.8	36.4	ND	65.3	5.7	-	<b>ND</b>	ND	ND
	2014	635	6.0	24.5*	22.8	158.3	14.8	-	<b>ND</b>	ND	ND
	2015	594	2.0	ND	24.2	170.8	1.0	349	<b>29.8</b>	3.6	0.63
	2016	677	0.8	13.4	17.4	97.7	0.3	534	<b>10.9*</b>	10.1	0.31
Forest (1X MDA)	2013	1125	9.0	19.3	ND	99.1	2.8	-	<b>ND</b>	ND	ND
	2014	798	2.7	11.9*	13.4	168.3	11.0	615	<b>31.1</b>	6.6	0.93
	2015	759	2.9	ND	15.8	147.8	1.0	356	<b>23.9</b>	2.6	0.40
	2016	650	0.0	5.1	5.4	0.0	0.0	568	<b>16.7*</b>	7.3	0.45

\*12 mo after the last MDA •



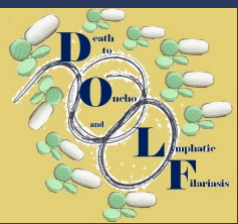
# Annual vs semianual IVM/Alb for LF and oncho: Cote d'Ivoire

Group	Year	Lymphatic Filariasis					Onchocerciasis			
		N	Adjusted Mf prevalence* (%)	FTS (%)	Gm Wb Mf/ml	CMFL Mf/ml	N	Ov Skin snip Mf positive (%)	Gm Ov Mf/mg	CMFL Mf/mg
Akoupe (2x MDA)	2014	1973	7.6 (6.7-8.6)	25.6	230.7	4.33	1993	<b>23.2</b>	2.9	0.43
	2015	2029	6.7 (5.7-7.7)	21.0	174.9	2.67	-	<b>ND</b>	ND	ND
	2016	2009	3.6 (2.8-4.4)	14.8	183.2	1.33	-	<b>ND</b>	ND	ND
	2017	1743	2.8(2.1-3.6)	11.6	134.9	1.00	1713	<b>7.7*</b>	2.5	0.12
Abengourou (1X MDA)	2014	1924	9.5 (8.4-10.7)	24.2	200.6	4.33	1926	<b>23.0</b>	4.5	0.55
	2015	1708	5.5 (4.5-6.6)	20.3	134.9	2.00	-	<b>ND</b>	ND	ND
	2016	1635	3.3 (2.4-4.1)	12.2	231.6	1.33	-	<b>ND</b>	ND	ND
	2017	1300	3.5 (2.5-4.5)	12.6	83.1	0.83	1273	<b>9.6*</b>	0.8	0.06

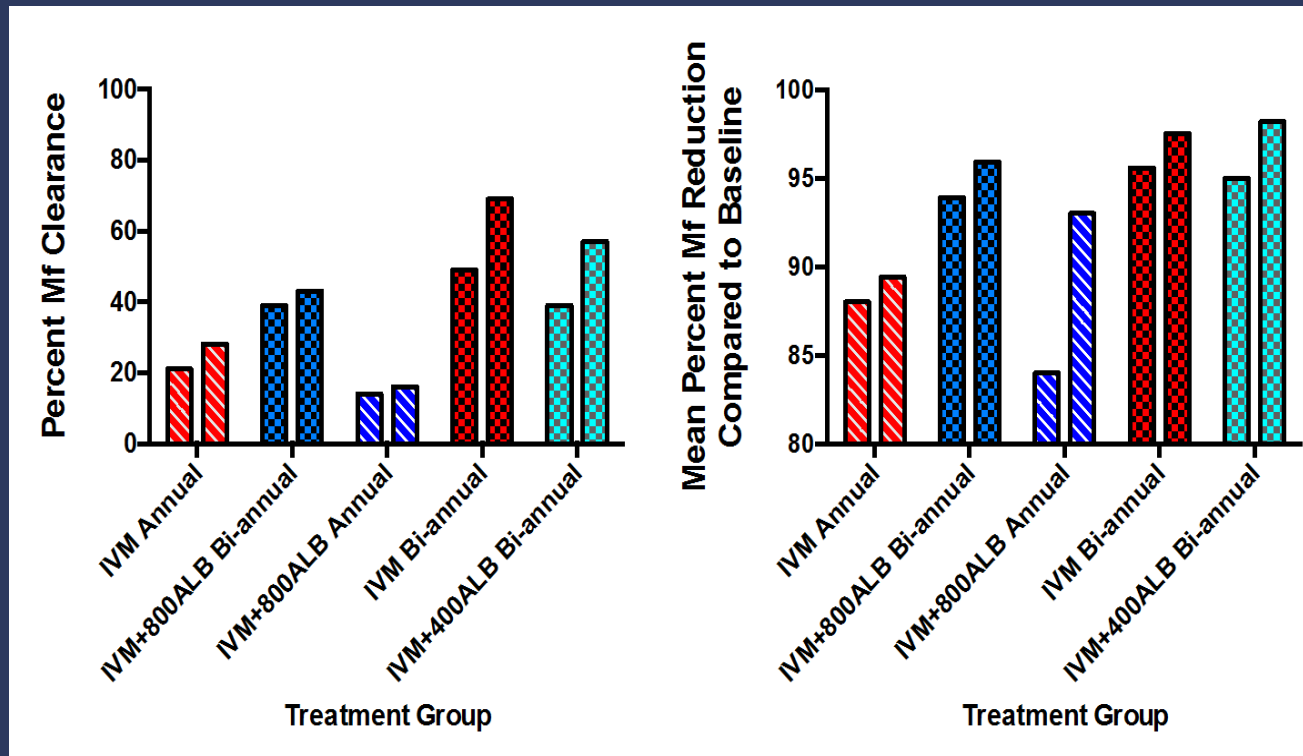
\*12 mo after the last MDA

## Conclusions:

- Detailed surveys still discover areas hypo – or low meso- endemic for onchocerciasis in some areas (ie Harper)
- Despite at least 3 or 5 rounds of high compliance MDA, 12 months after the last MDA *O. volvulus* Mf rates can be as high as 17%
- Twice yearly MDA may not be cost-effective for LF, but may be for oncho



# RCT: Multiple doses of IVM/Alb for *O. volvulus* (East Ghana)



- 36 mo follow-up completed in early 2018
- Nodulectomies performed in February and June 2018
- ~ 1,000 nodules obtained from ~ 260 subjects
- ~ 600 nodules stained (H&E, Gmori iron, APR immunostain)
- ~ 500 nodules read by the 1<sup>st</sup> reader

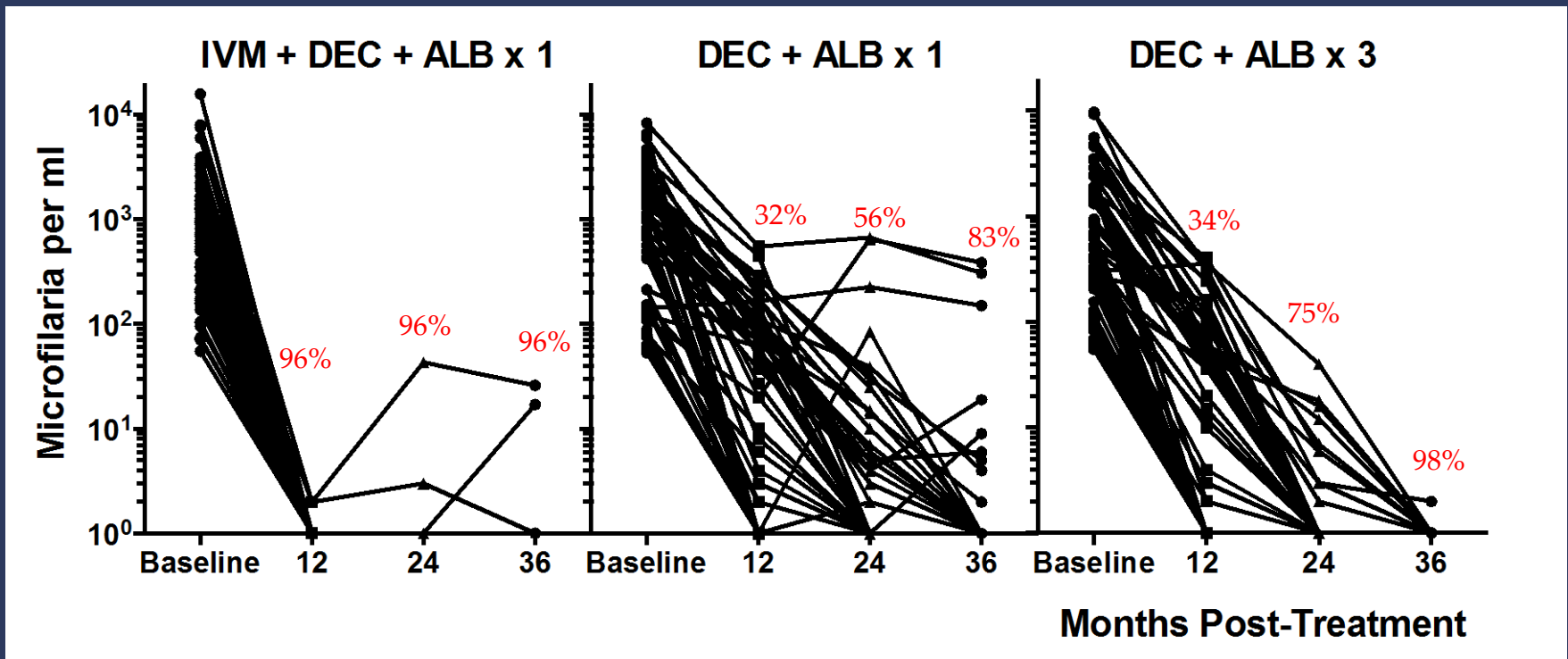


# IDA – Combination Therapy using Established Drugs

- IDA uses **three well established drugs** together. **PK studies** have shown no significant interactions
- All three drugs have an impressive **safety record** in uninfected persons and in persons with LF
- DOLF: **IDA clears Mf** of *W. bancrofti* and *B. timori* more efficiently than DA or IA
- DOLF: **IDA inactivates *W. bancrofti* adult worm** nests more efficiently than IA
- DOLF: **IDA is safe for MDA outside** of Africa (>14,000 subjects studied in 5 countries)
- WHO recommends **IDA MDA for LF elimination** in countries that currently use DA (including those in Africa)
- Merck: **Expanded their ivermectin donation** (additional 100 million doses per year through 2025)



# A Single Dose of Triple Drug is Superior to Two Drugs and Equivalent to DEC/ALB given Annually x3 for clearing *W. bancrofti* Mf



King, et al. in press



# Why test IDA for onchocerciasis?

1. If IDA could be safely used in **oncho areas**, it would be more widely used for LF elimination in Africa
2. IDA may **sterilize** female *O. volvulus*
3. IDA may have **macrofilaricidal** activity vs *O. volvulus*
4. **Short time required** for IDA testing/deployment
5. An independent bioethics review concluded that it would be **unethical to not test IDA for onchocerciasis** because of its potential to accelerate oncho elimination

# Potential Drivers of Adverse Events After **DEC** or **IVM** in Oncho

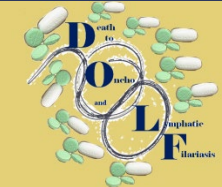


- Mazzotti: **Mf** density in the **skin** (most studies treated moderate to high intensity infections (>50 mf/snip))
- Duration of treatment: Most DEC studies provided 8 days or longer courses of treatment
- **Ocular AEs after a course of DEC:** Uveitis, retinal damage, optic neuritis. Risk related to **Mf** density in the anterior chamber. Very low risk if Mf are absent in the AC.
- After **DEC** Mf are killed/degraded *in situ* (skin)
- After **IVM** Mf are usually killed/degraded in draining lymph nodes



# Hypotheses & Assessment of ocular AEs

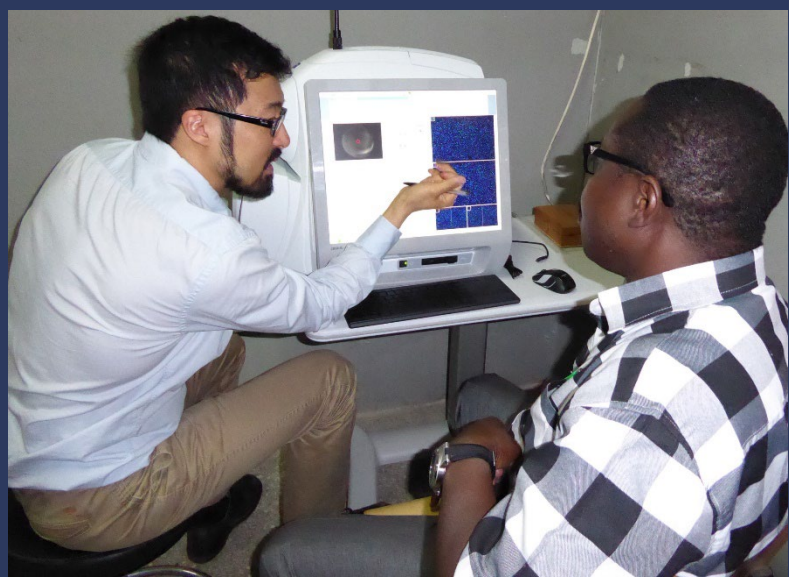
- ❖ IVM/DEC/ALB (following ivermectin pretreatment) is more effective for killing or sterilizing adult female *O. volvulus* worms than IVM/ALB
- ❖ IVM/DEC/ALB (following ivermectin pretreatment) is as safe as IVM/ALB alone in persons with onchocerciasis
  - Visual acuity, fields, pupillary reflex testing
  - Applanation tonometry (intraocular pressure).
  - Slit lamp examination (corneal changes)
  - Indirect ophthalmoscopy (evaluate the fundus of the eye posterior segment) and retinal photography
  - Slit-lamp exam for Mf.
  - Optical coherence tomography (OCT)



# Ophthalmology Training



Slit lamp



Optical Coherence Tomography



Retinal Camera



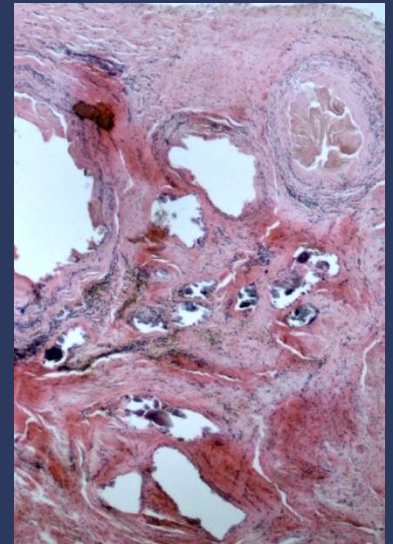
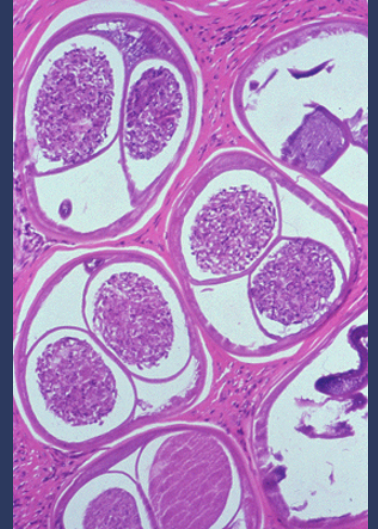
# Enrollments & Experiences so Far

- 54 Participants enrolled, treated with ivermectin and completed Day 7 follow-up
- First IDA treated cohort estimated to be around Mar 2019
- First nodulectomies Sept 2020
- 14 (25.9%) had ocular Mf at screening
- 6/14 participants (42.9%) oMf negative at day 7
- 5/40 participants (12.5%) were oMf positive at day 7 who were negative at screening
- Few, mild AEs observed - mainly itching, rash, and limb swelling (14 participants)
- 2 participants with mild itching eyes
- 2 participants with mild painful eyes



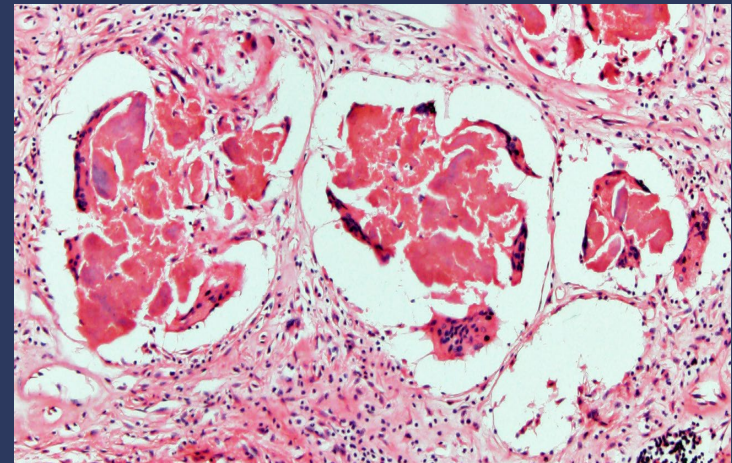
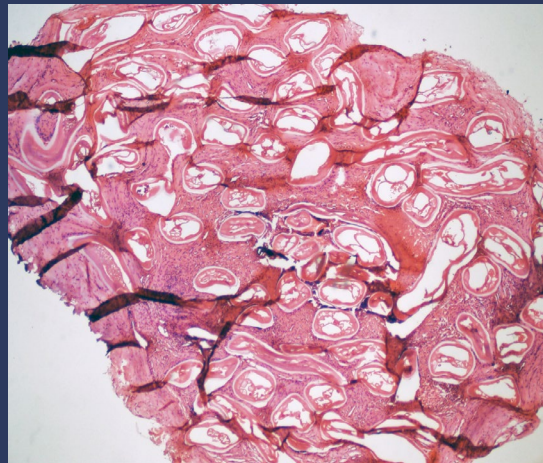
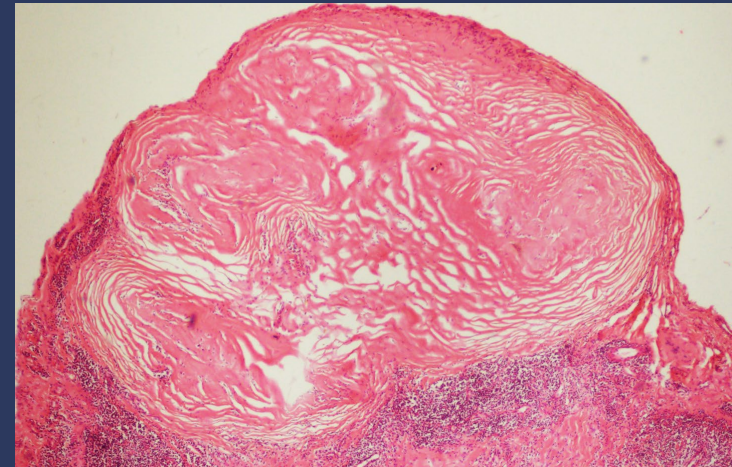
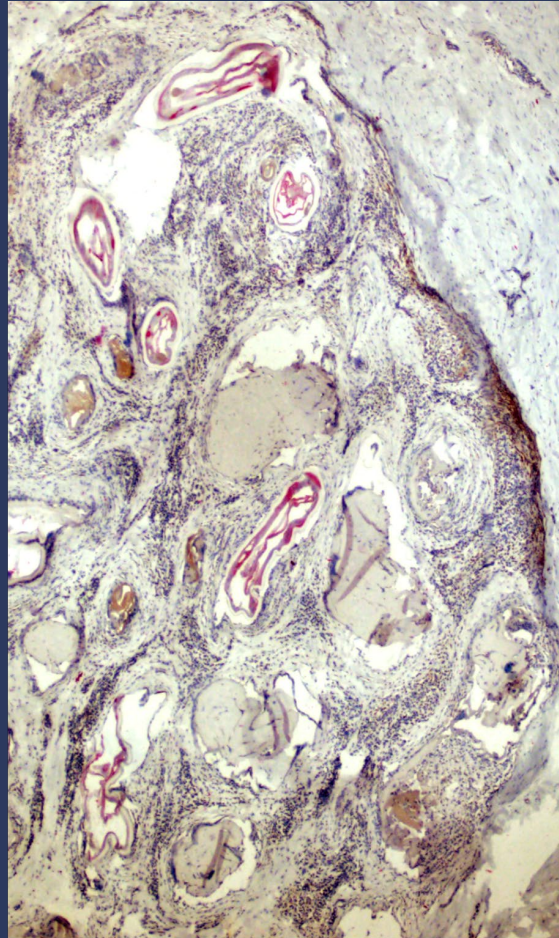
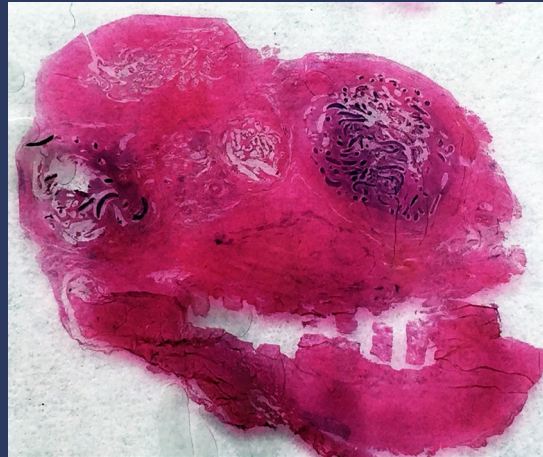
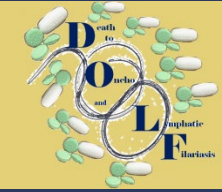
# Diagnosis of Onchocercias

- Monitoring and evaluation of intervention
  - Development of qPCR assays
  - Evaluation of antibody tests (ie Ov16)
  - Development of antigen detection tests
- Assessment of viability of adult female *O. volvulus*
  - Biomarker discovery
  - Histological assessment of adult *O. volvulus* in nodules



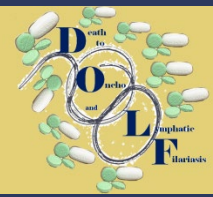


# Histological assessment of adult *O. volvulus* in nodules

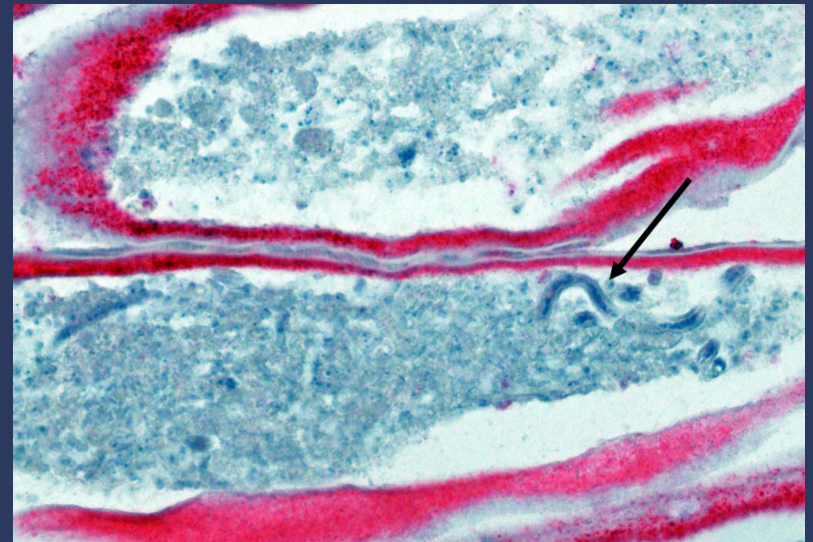
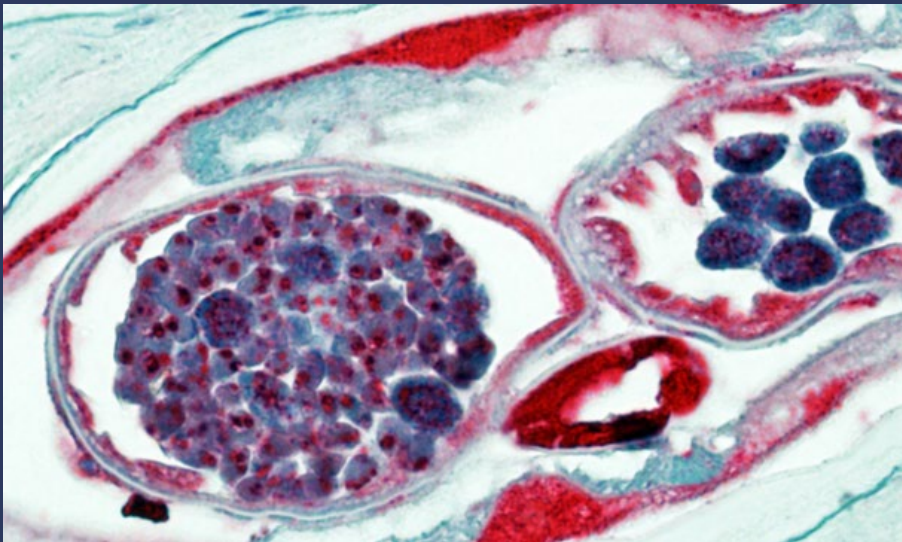
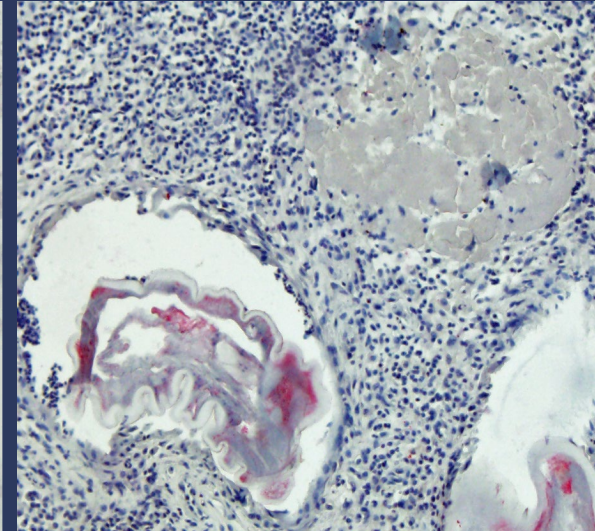
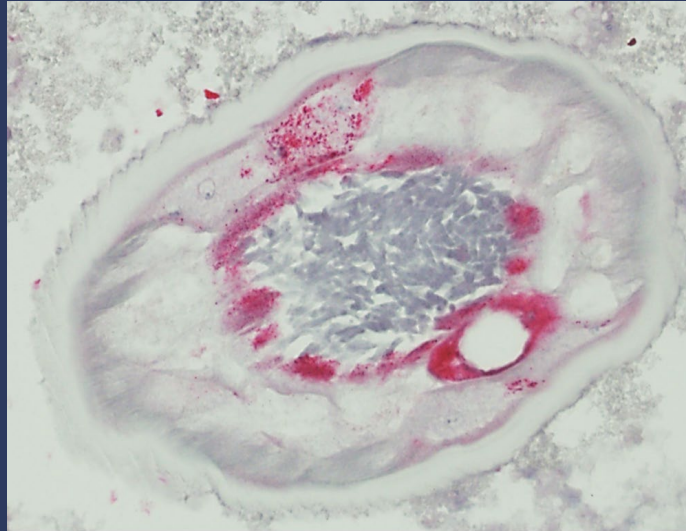


➤ Nodules/worms are not always pretty!





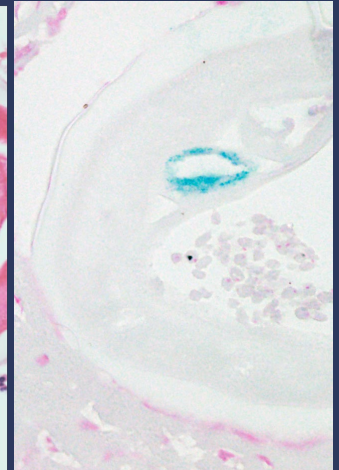
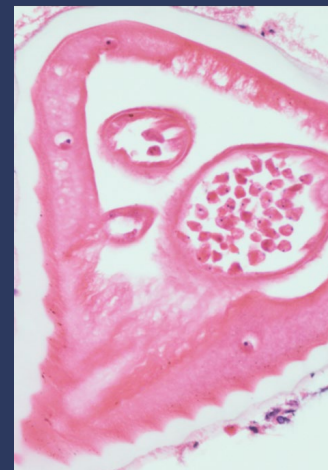
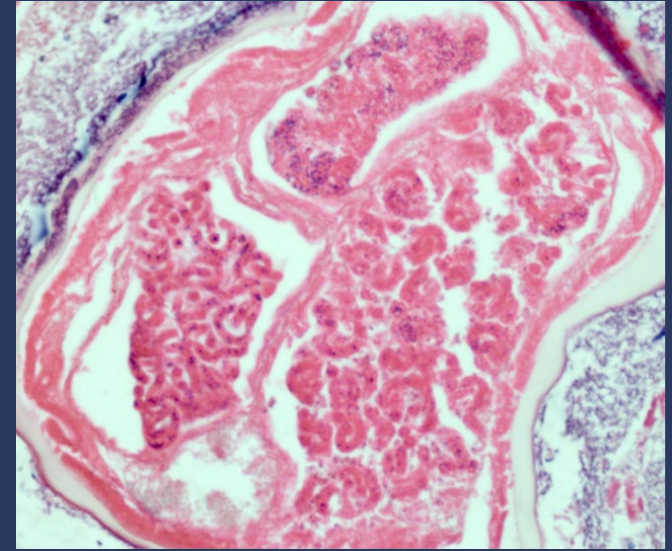
# APR-Immunostain detects living *O. volvulus*





# Assessment of *O. volvulus* after Drug Treatment

- Histology expertise vanishing
- Standardization of methods difficult (2 independent readers, double data entry)
- Ideally at least 3 (4) stains: H&E (overview), Gomori iron (age), APR (dead/alive), (Wsp for *Wolbachia*)
- Provides information beyond the presence of living adult worms
  - Adult worm population (no, age)
  - Embryogenesis
  - Intranodular Mf
  - Stages most affected by treatment





# Biomarker Discovery for Viable Female *O. volvulus*

- Approach:
  - Bioinformatic prioritization
  - Mass spec analysis of clinical samples
  - Discovery mode leads to targeted mode (increased sensitivity)
  - Enrichment methods (immunoprecipitation, removal of common proteins)
  - Identification of false positives before assay development
    - Large number of negative controls
    - Pool testing and subsequent individual testing
    - Samples from different endemic/non-endemic countries

# Biomarker Discovery Example: Verification Test Method

METHOD: PRM19\_4-24-18

- ❑ PEPTIDES SELECTED FROM DEPLETED SERUM(WEIL-061) & IC (WEIL-063) DISCOVERY (DDA) RUNS; 13 IN CURRENT INVENTORY; **LOW SCORES**
- ❑ 19 PEPTIDES IN MEHTOD: 4 OVOL PEPTIDES ID'D IN POSITIVE SAMPLES ONLY + 6 OVOL PEPTIDES IN A NEGATIVE SAMPLE + 4 IF + 5 REF
- ❑ 1 PEPTIDE PER RET TIME WINDOW

Protein Description	PEPTIDE-NO MODS	PEAKS SCORE	PRM-19_04-24-18	WEIL-61-SERUM-BOUND		WEIL-61-SERUM-FLOW THROUGH		WEIL-63-IC-DDA		WEIL-63-IC-PNGaseF-DDA	
				UGANDA-OVPOS	INDIA-OVNEG	UGANDA-OVPOS	INDIA-OVNEG	UGANDA-OVPOS	INDIA-OVNEG	UGANDA-OVPOS	INDIA-OVNEG
			<b>10</b>	<b>9</b>	<b>6</b>	<b>4</b>	<b>7</b>	<b>5</b>	<b>1</b>	<b>7</b>	<b>4</b>
OVOC7893	LILPGELAK	35.84	X	X	-	X	X	-	-	-	-
OVOC11703	NQFFIK	34.35	X	-	-	-	-	X	-	X	X
OVOC3336	RSTPGTLNSR	30.98	X	X	X	X	X	X	-	X	X
OVOC6919	LLIYESEAK	30.63	-	X	X	X	X	-	X	X	-
OVOC921	YIPTFGTNK	29.75	-	X	X	-	X	-	-	X	-
OVOC2895	LSVISQPK	27.97	X	X	X	-	X	X	-	X	X
OVOC8759	LLLYDGTR	26.64	-	X	X	X	X	-	-	-	-
OVOC659	LVDESASEEEVK	24.29	X	-	-	-	-	X	-	-	-
OVOC12054	IDQIVSWLEMPASKRPK	24.04	X	X	-	-	-	-	-	-	-
OVOC4989	NTLFEVQNLIK	23.62	X	X	X	-	X	-	-	X	-
OVOC1550	FPIYETVR	23.59	X	X	-	-	-	-	-	-	-
OVOC677	QIQFLQER	21.98	X	-	-	-	-	-	-	X	-
OVOC10597-IF	EKINQWK	21.6	X	-	-	-	-	X	-	-	X

**RESULT: POSSIBLY FALSE POSITIVES**



# Real-time qPCR for *O. volvulus* in skin snips

Acta Tropica 146 (2015) 114–118



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journal homepage: [www.elsevier.com/locate/actatropica](http://www.elsevier.com/locate/actatropica)

## Conventional parasitology and DNA-based diagnostic methods for onchocerciasis elimination programmes

Melanie M. Lloyd<sup>a,\*</sup>, Rebecca Gilbert<sup>a</sup>, Nathalie Tebao Taha<sup>b</sup>, Gary J. Weil<sup>a</sup>, Aboulaye Meite<sup>b</sup>, Ilunga M.M. Kouakou<sup>b</sup>, Peter U. Fischer<sup>a</sup>

<sup>a</sup> Infectious Diseases Division, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA

<sup>b</sup> Ministry of Health and Social Welfare of Côte d'Ivoire, Abidjan, Cote d'Ivoire

- Microscopy of 2 snips after 24 h in PBS
- Both snips (without PBS) were dried for 24h
- Samples were stored at RT with desiccants
- DNA extracted from both snips
- O-150 probe based qPCR

**Table 1**

Comparison of onchocerciasis test results obtained by qPCR, microscopic detection of microfilariae (MF) in skin snips, and nodule palpation for 369 individuals in the Akoupé district, Côte d'Ivoire.

Method	Positive	Negative	% Pos
qPCR	210	159	56.91
Skin snip MF	96	273	26.00*
Nodule palpation	140	229	37.94*
Skin snip MF or nodules	196	173	53.12
Any test positive	269	100	72.90*

\* Rates that are significantly different from the % positive by qPCR ( $P < 0.05$ , determined by a test of equal proportions).



# Parallel Comparison of Commercial Antibody Tests for Filariasis

Plasma from patients with proven	N	Loa SXP Rapid Test* N (%)	BLF (Bm SXP) Rapid Test (%)	Wb123 Oncho/LF IgG <sub>4</sub> bplex	<u>Ov16</u> <u>Oncho/LF</u> <u>IgG<sub>4</sub> bplex</u>	Bm14 (Bm SXP) ELISA
<i>W. bancrofti</i>	16	5 (31)	14 (88)	13 (81)	<b>0 (0)</b>	14 (88)
<i>B. timori</i>	11	3 (27)	10 (91)	2 (18)	<b>0 (0)</b>	11 (100)
<i>L. loa</i>	18	18 (100)	12 (67)	0 (0)	<b>1 (5)</b>	13 (72)
<i>O. volvulus</i>	19	8 (42)	16 (84)	0 (0)	<b>17 (89)</b>	15 (78)
<i>M. perstans</i>	14	5 (36)	11 (79)	0 (0)	<b>3 (21)</b>	8 (57)
no filarial infection	22	0 (0)	1 (5)	0 (0)	<b>0 (0)</b>	0 (0)

# Acknowledgements

