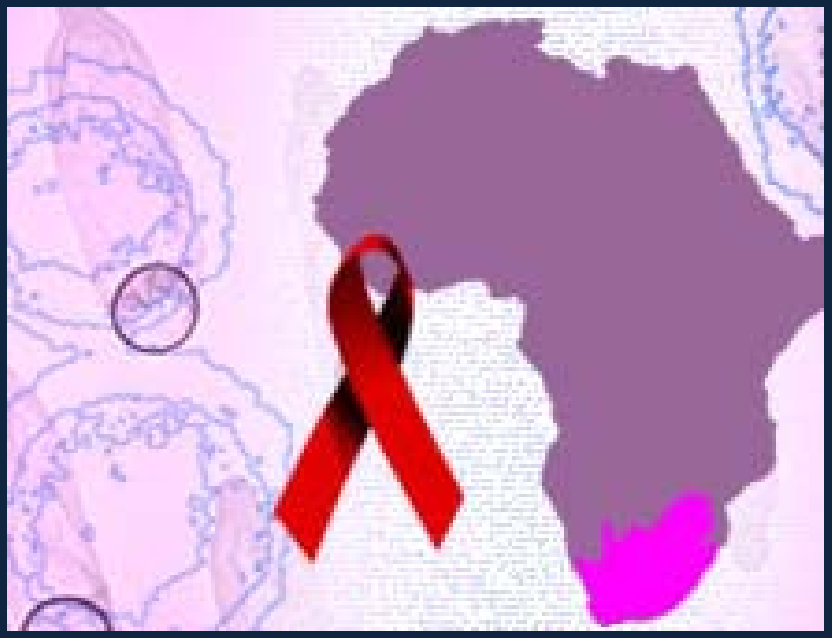
Neuropathogenesis of Subtype C HIV-1:
Research and Capacity Building in Zambia



Victor Mudenda

&

Charles Wood

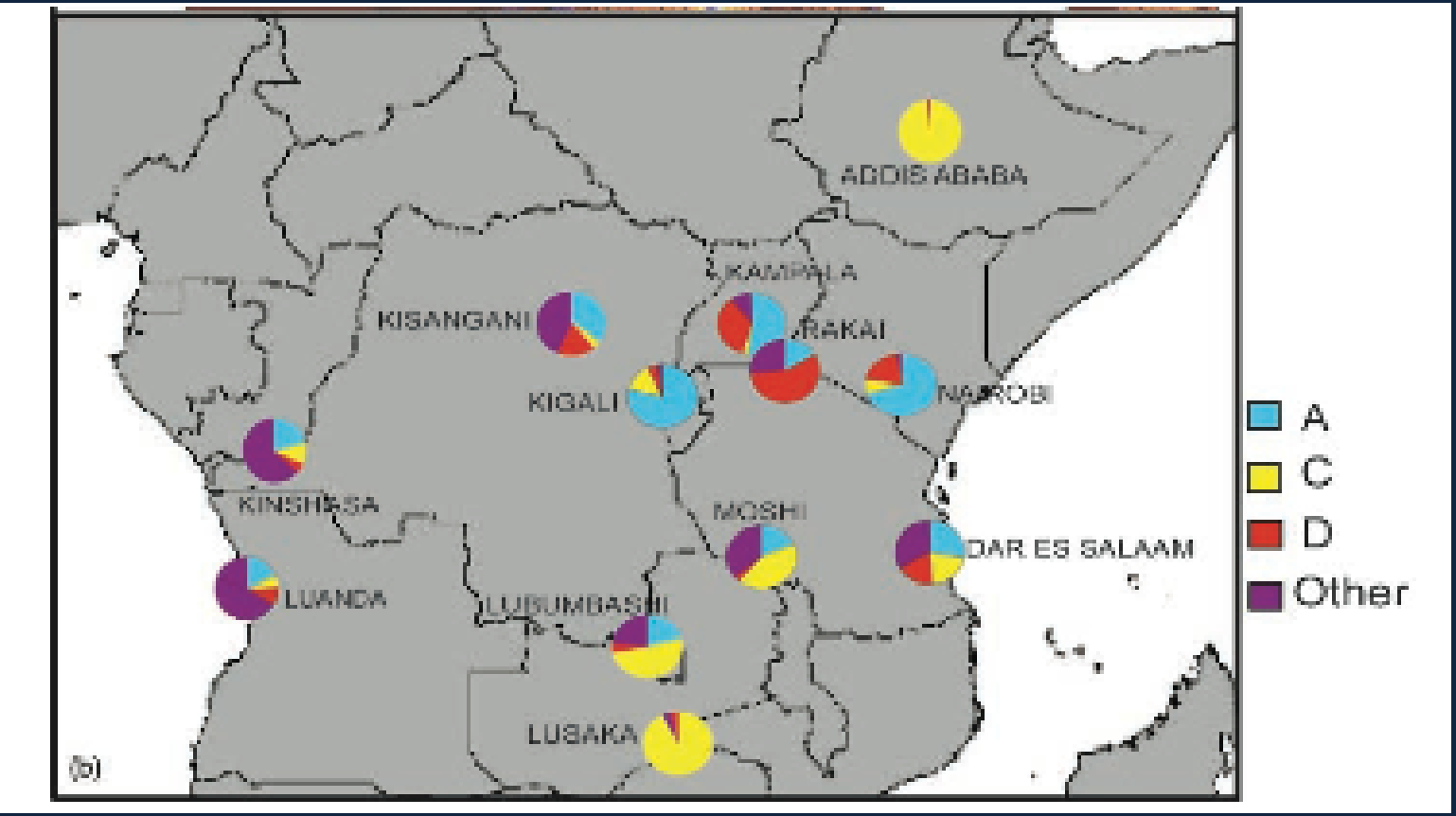
Zambia

- One of the most urbanized countries in Africa with population of about 12 million
- Lusaka has an estimated population of over 2 million
- Mining and agriculture are the major contributors to the GDP
- Poverty is rampant with 64% of the population below poverty level
- Life expectancy is 45 years, primarily attributed to the HIV epidemic

HIV Burden in Zambia

- HIV prevalence is 14%
- HIV mortality
- Neurological manifestation and neuropathological characterization of HIV-1 remains unknown
- Poor infrastructure
- Lack research capacity to understand HIV-1 neuropathology

HIV Subgroup Distributions



Greater than 95 percent of the viral isolates obtained from Zambia are subtype C

Gray, et. al., AIDS 2009

Objectives:

To determine the impact and extent of subtype C HIV-associated neuropathology in Zambia

- Neuropsychological assessments
- Neuropathological analysis
- Virological analysis

Neuropsychological Evaluation

- **Prospective study**
- **140 participants:**

  -15 - 45 years old

  - HIV seronegatives and seropositives
- **Site:**
  - University Teaching Hospital
  - Our Lady Hospice

- **Health assessment:**

  - Physical
  - Neurological
  - Psychological
  - Immunological: CD4 count
  - Virological: viral load
- **Neuropsychological assessment**

Table 1. Sociodemographics data

Variables	HIV- mean (sem)	percent	[Range]	HIV+ mean (sem)	percent	[Range]
Sex (M/F) – number	57 (30/27)			83 (32/51)		
Age-years	28 (1.2)		[18 – 56]	34 (0.8)		[18 – 57]
Education-years	9 (0.3)		[2 – 14]	9 (0.2)		[2 – 16]
Dependents	4 (0.4)		[0 – 12]	3 (0.3)		[0 – 9]
Marital status (number)						
Single	25	(44%)		17	(21%)	
Married	26	(46%)		37	(45%)	
Separated	0	(0%)		2	(2%)	
Divorced	2	(3%)		3	(4%)	
Widowed	0	(0%)		12	(14%)	
Unknown	4	(7%)		12	(14%)	
Type of Occupation						
Formal	10	(18%)		33	(40%)	
Informal	39	(68%)		38	(46%)	
Unknown	8	(14%)		12	(14%)	
Preferred Language						
English	35	(61%)		64	(77%)	
Nyanja	17	(30%)		19	(23%)	
Bemba	5	(9%)		0	(0%)	
Income Range (in kwacha)						
< 2,000,000 (poverty line)	48	(84%)		78	(94%)	
2,000,000 – K5,000,000	4	(7%)		4	(5%)	
>5,000,000	0	(0%)		1	(1%)	
Missing	5	(9%)				
Religious Affiliation						
Christian	55	(96%)		56	(67%)	
Muslim	0	(0%)		0	(0%)	
Traditional	0	(0%)		0	(0%)	
Other	0	(0%)		0	(0%)	
Unknown	2	(04%)		27	(33%)	

Table 2. MANCOVA results of HIV status groups and covariates

	IHDS Total	Motor Speed	Psychomotor Speed	Memory Recall	CTT 1	CTT 2	GPTdom	GPTnondom	TGT
HIV status									
P value	p =.33	p =.06	p =.36	p =.21	p =.02*	p =.000**	p =.000**	p =.001**	p =.002**
HIV+, mean (SE)	9.8 (.20)	3.1 (.10)	3.1 (.09)	3.5 (.07)	61.7 (2.76)	102.8 (4.29)	72.5 (1.81)	87.1 (1.79)	12.3 (0.26)
ARV, mean (SE)	10.1 (.22)	3.4 (.09)	3.3 (.13)	3.4 (.20)	71.0 (0.07)	140.3 (9.26)	91.6 (4.27)	108.7 (6.83)	10.8 (0.59)
HIV-, mean (SE)	10.1 (.32)	3.5 (.12)	3.1 (.14)	3.4 (.11)	71.9 (3.64)	149.5 (6.47)	82.9 (2.41)	97.5 (3.07)	12.3 (0.21)
Covariates									
Age	p =.08	p =.61	p =.25	p =.13	p =.50	p =.06	p =.06	p =.78	p =.27
Sex	p =.15	p =.19	p =.23	p =.46	p =.01**	p =.16	p =.04*	p =.01**	p =.003**
Education	p =.07	p =.28	p =.21	p =.25	p =.18	p =.59	p =.49	p =.14	p =.55
Clinical Symptoms	p =.10	p =.25	p =.59	p =.09	p =.78	p =.65	p =.51	p =.37	p =.63

Significant difference at $p \leq .05$* or $p \leq .01$ **. HIV status = HIV-, HIV+, ARV. IHDS = International HIV Dementia Scale; CTT 1 or 2 = Color Trials Test 1 or 2; TGT = Timed Gait Test; GPT dom or nondom = Grooved Pegboard Test dominant or nondominant hand.

Evidence of HAND in Subtype C HIV Predominant Regions

- 22% neuropsychological impairment based on IHDS

- Reports of other subtype C HIV predominant African countries
  - 38%, Botswana, Lawler K., 2010, J Int AIDS Soc
  - 24%, South Africa, Joska JA., 2010, AIDS and Behavior

- Comparative results are emerging

- Neurological assessments
- Neuropathological analysis
- Virological analysis

HIV-associated Neuropathology

- Autopsies of HIV infected individuals
  -18 to 45 years old
- Case note review
- Histology of brain specimens
  -Hematoxylin & Eosin (H&E)
  -Immunohistochemistry
- Viral sequence analysis to determine compartmentalization

Specimens

- 241 cases have been collected

- Eight different tissues were obtained from each case:

  frontal lobe, parietal lobe, temporal lobe, occipital lobe, hippocampus, cerebellum, basal ganglia, lymph node

Pathology

Opportunist Infections:

* *Cryptococcus neoformans*
* *Toxoplasma gondii*
* *Mycobacterium tuberculosis*

CNS Histology:

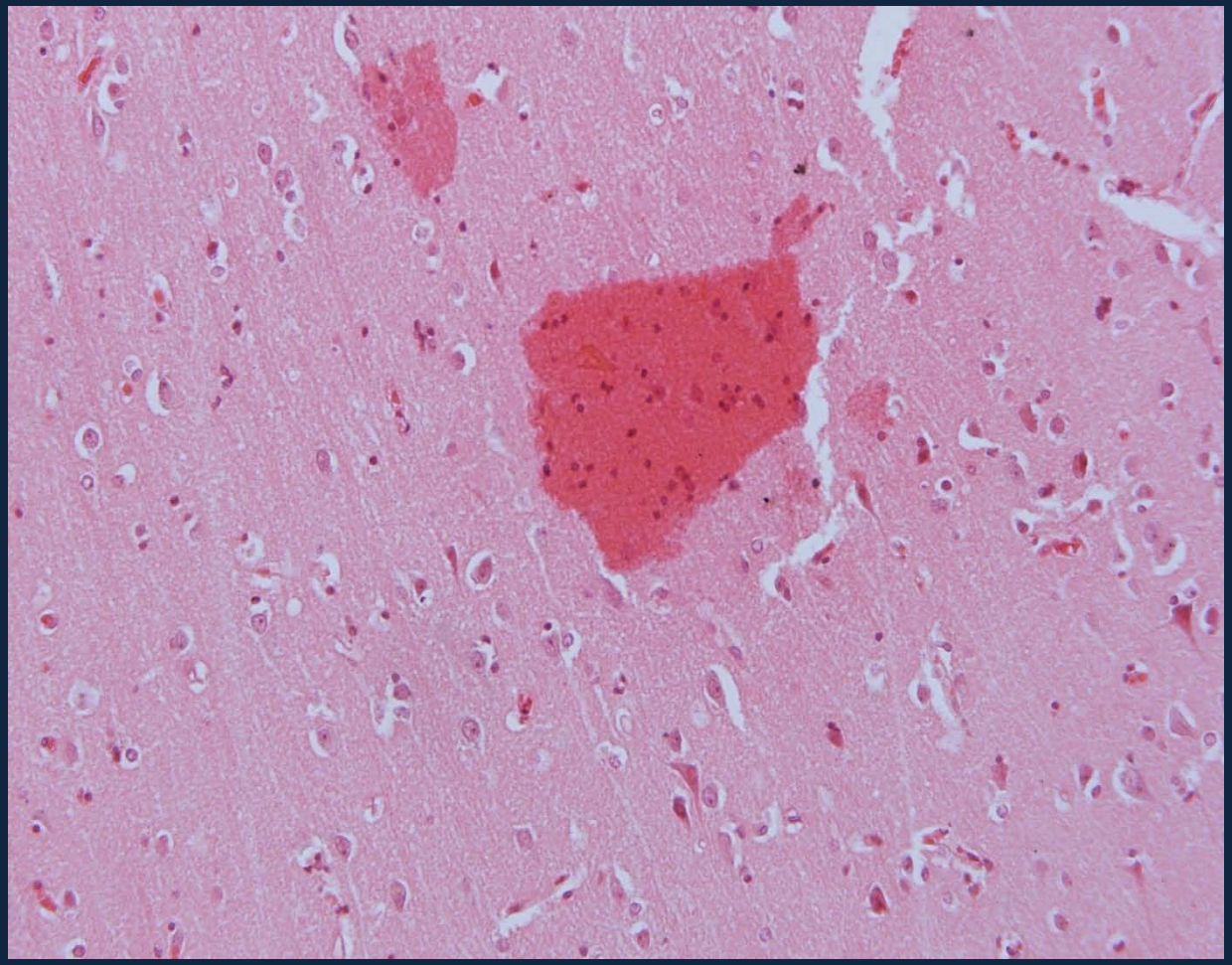
* CNS cells: neurons, microglia, astrocytes, oligodendrocytes
* Immune cells: CD8+, CD4+, neutrophils, NK cells, dendritic cells, macrophages, monocytes
* Pathologic lesions: necrosis, haemorrhage, inflammation,granulomata

Patients Information

Case #	Sex	Age	HIV	Cause of Death (COD) and Date of Death	HAART
35	M	29	+	RVD with PTB to R/O PCP (6/4/09)	-
38	M	33	+	RVD with Hepatic Encephalopathy(6/21/09)	+
39	M	29	+	Hepatic Encephalopathy (HAART or drug induced) (7/7/09)	+

* Sequences have been obtained from each of these cases

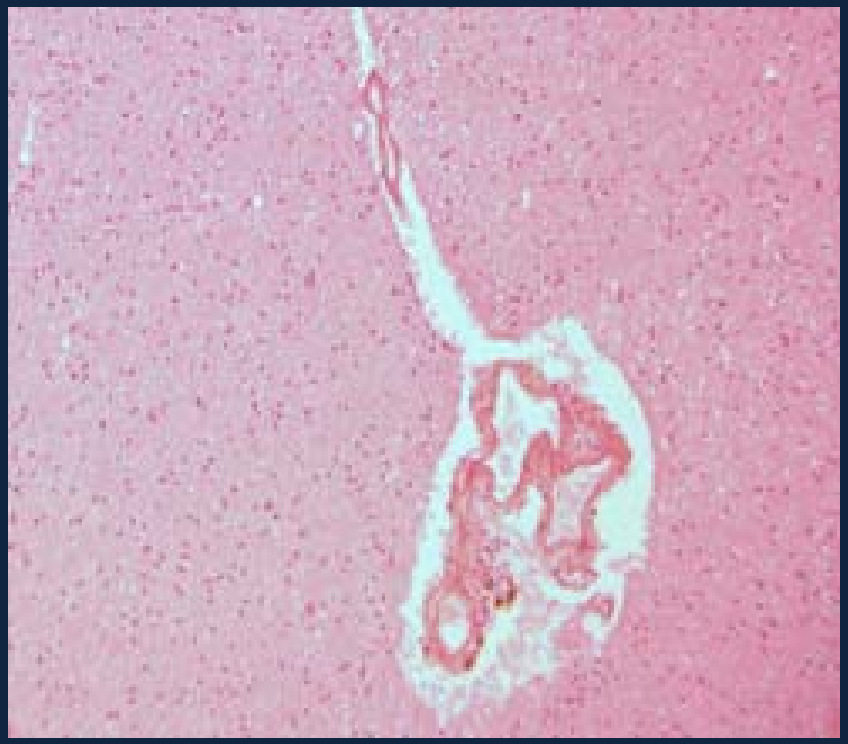
H&E Staining (C-35)



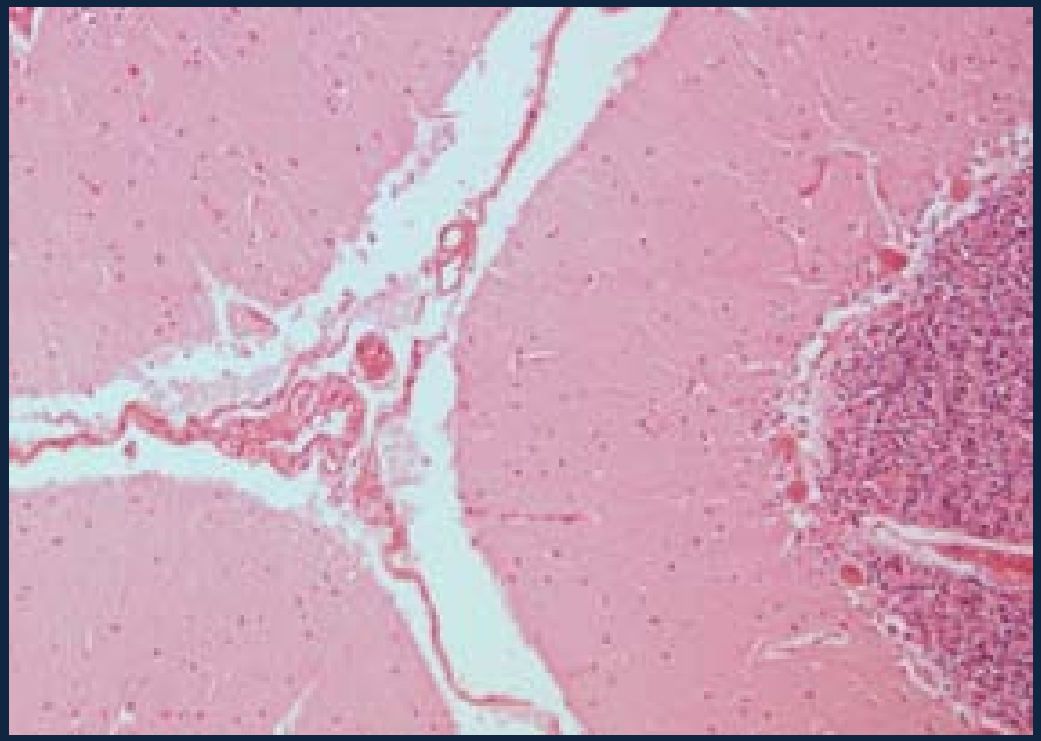
C35 RTL 20X

* Micro-hemorrhage

H&E Staining (C-38)

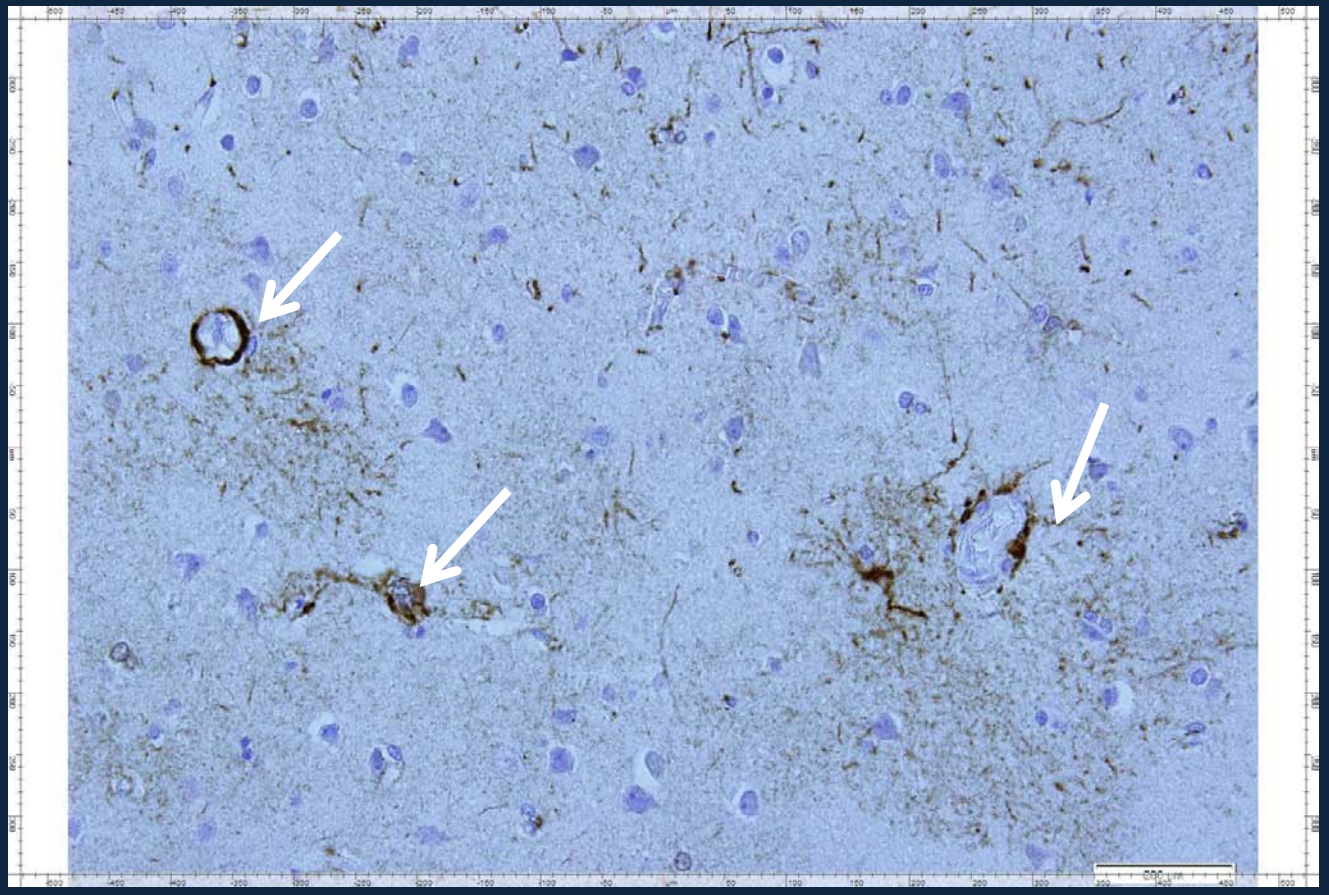


C38 RPL 10X white matter



C38 Cereb 10X

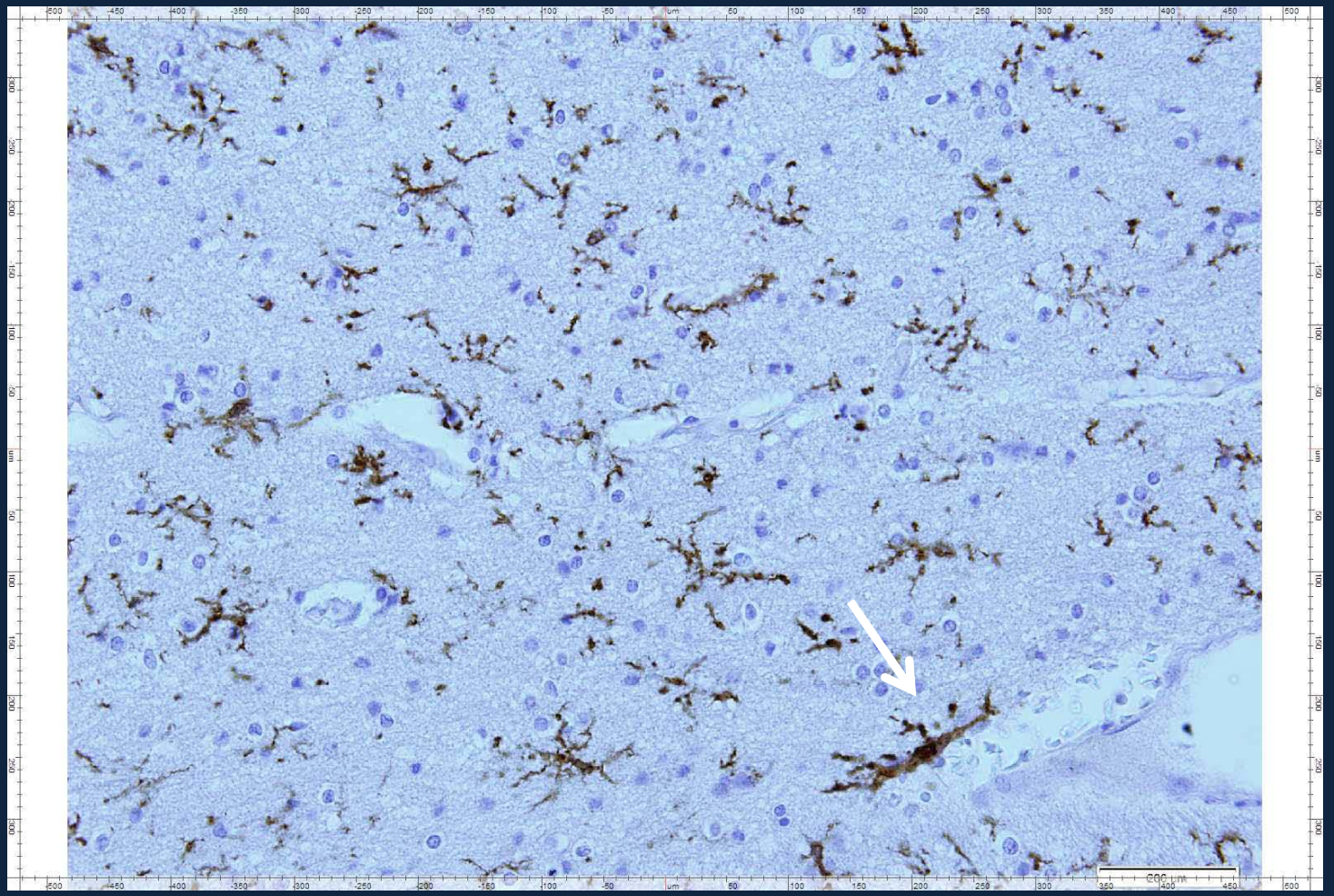
GFAP Immunostain



C38 RTL GFAP 40X

* Astrocytes are activated on all sections
* Astrocytes wall off lesions

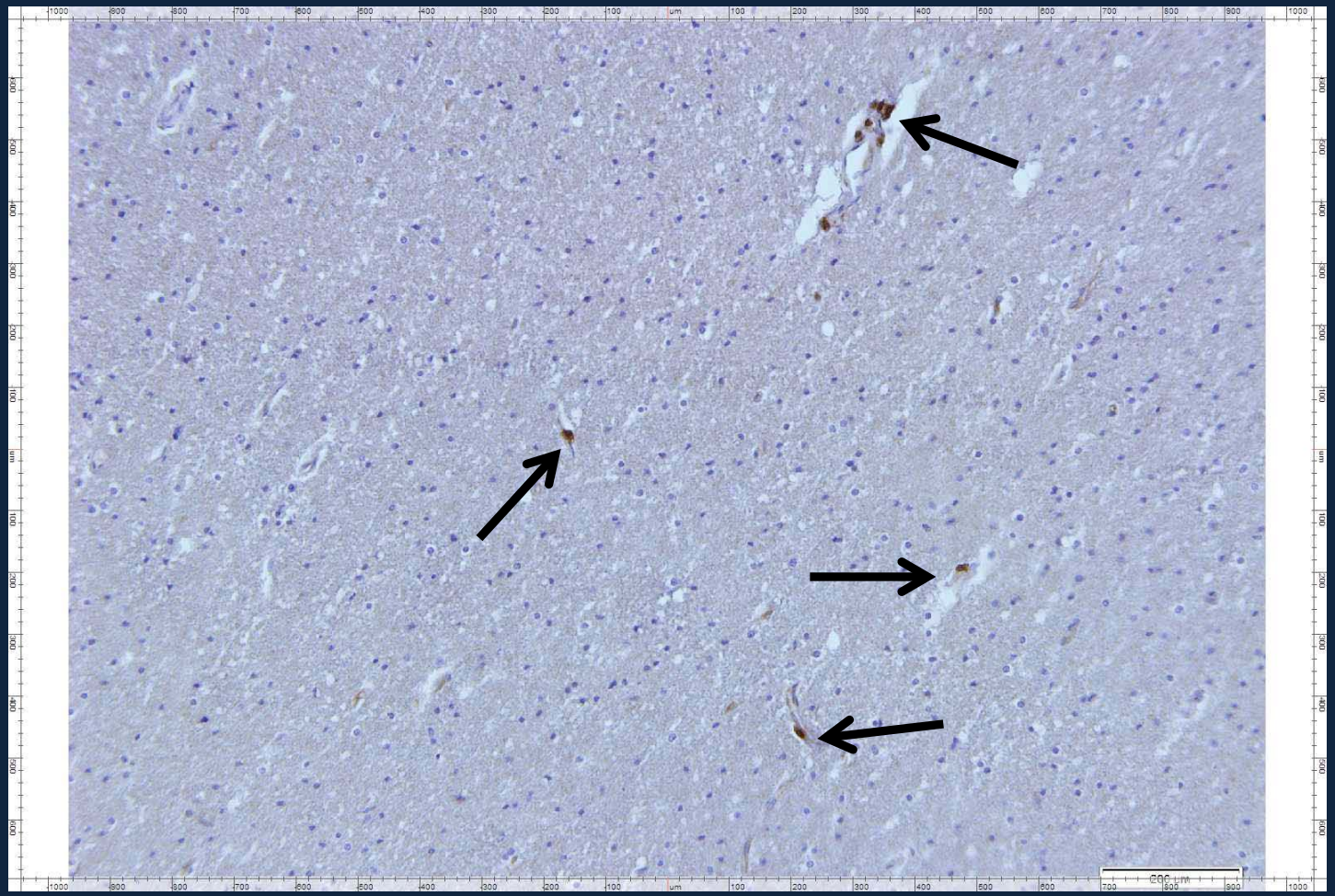
Iba-1 Immunostain



C38 RFL Iba-1 40X

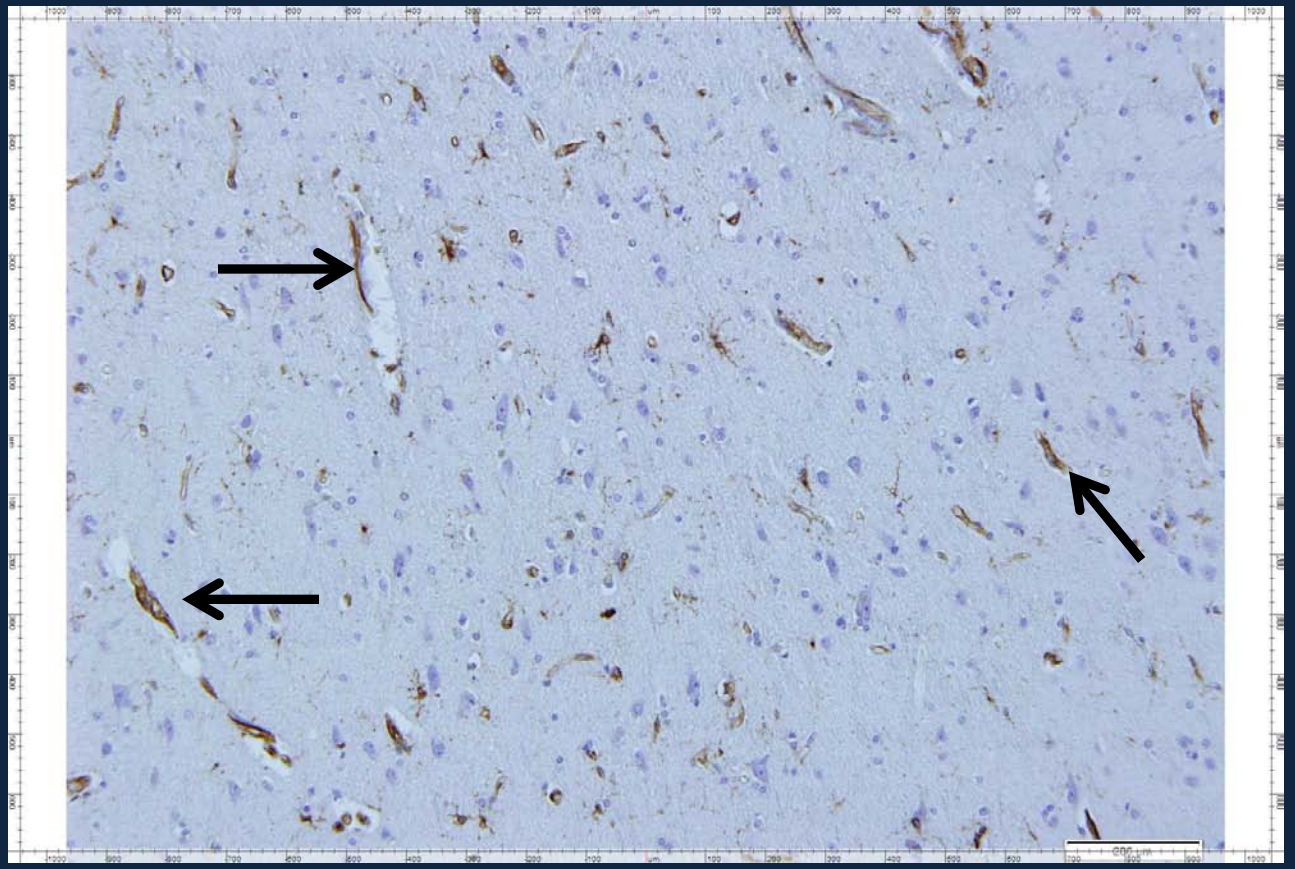
** Antibody against ionized calcium binding adaptor molecule 1 expressed by microglia and macrophages.*

CD8 Immunostain



C38 LOL CD8+ 20X

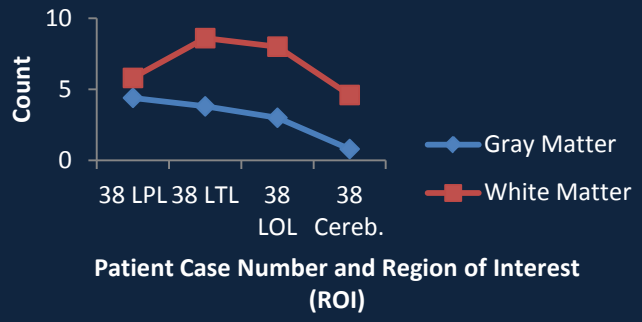
HLA-DR Immunostain



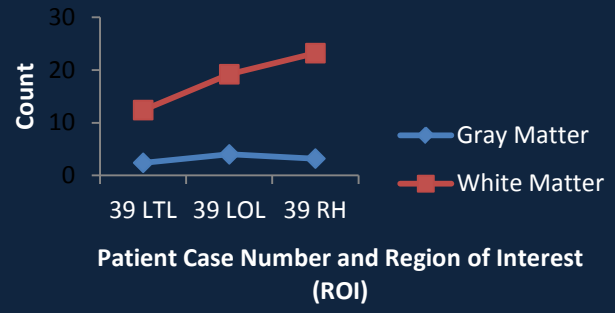
C38 LPL HLA-DR 20X

CD8+ Infiltrations

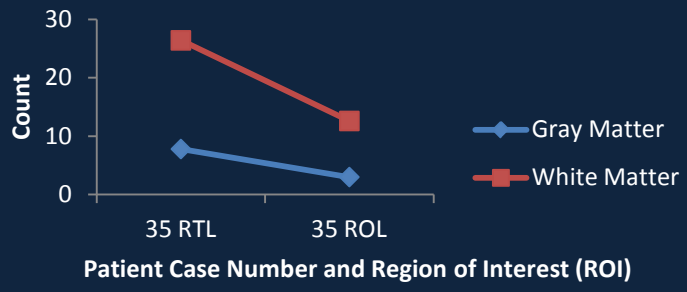
CD8+ cells/microscope frame view (20X; Patient #38)



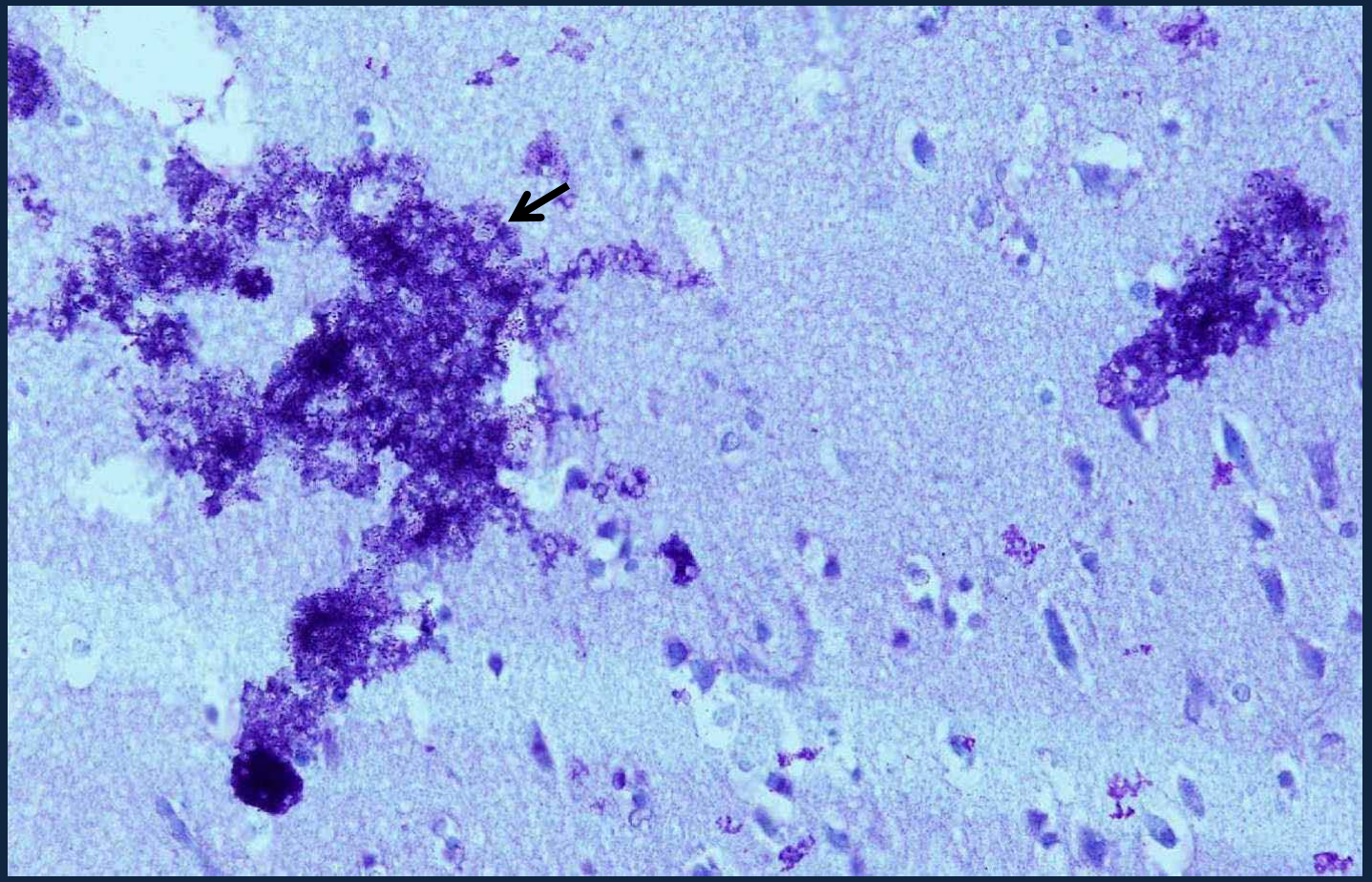
CD8+ cells/microscope frame view (20X; Patient #39)



CD8+ cells/microscope frame view (20X; Patient #35)



Giemsa Stain

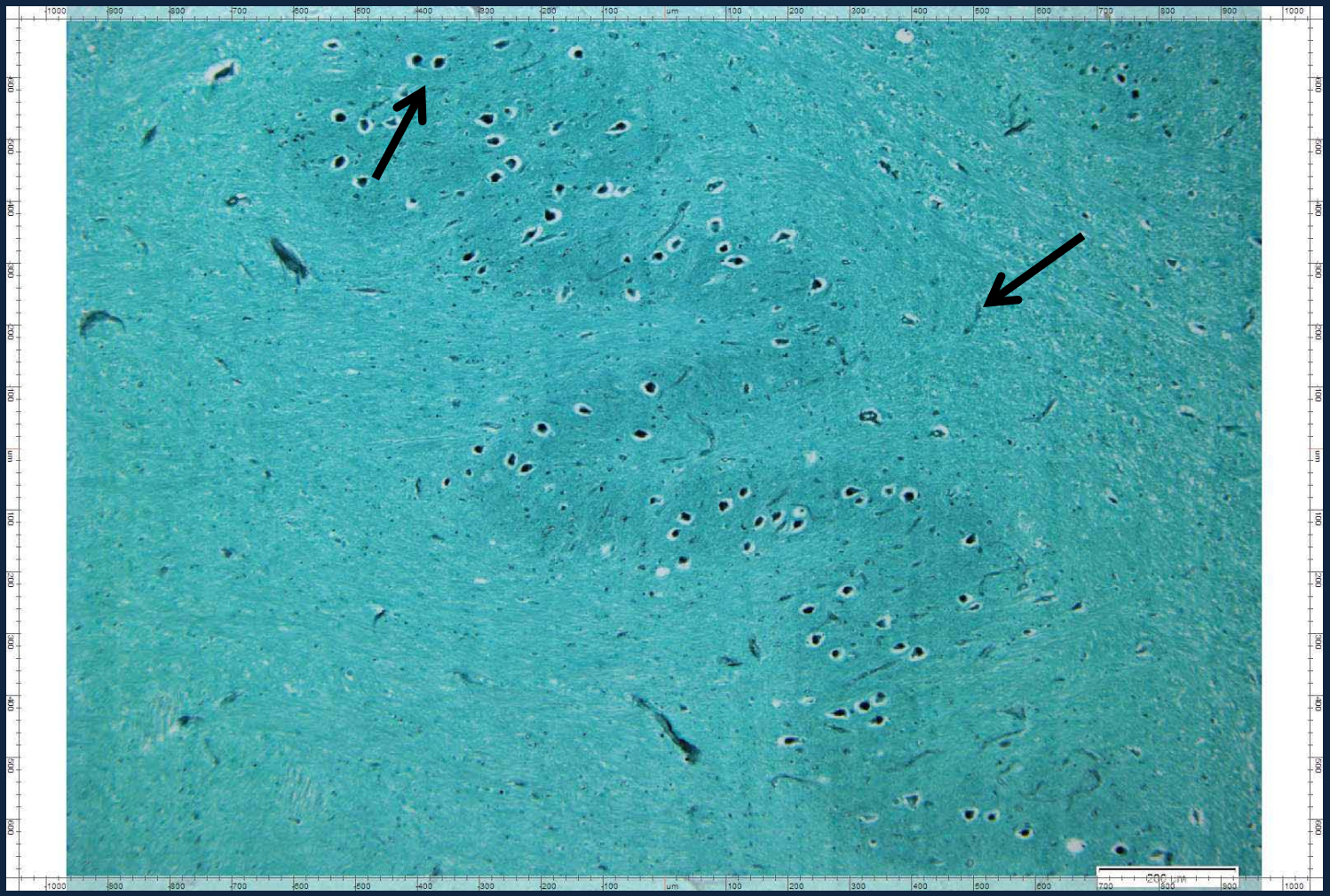


C38 RH Giemsa 40X

Toxoplasma gondii

G.M.S. Stain

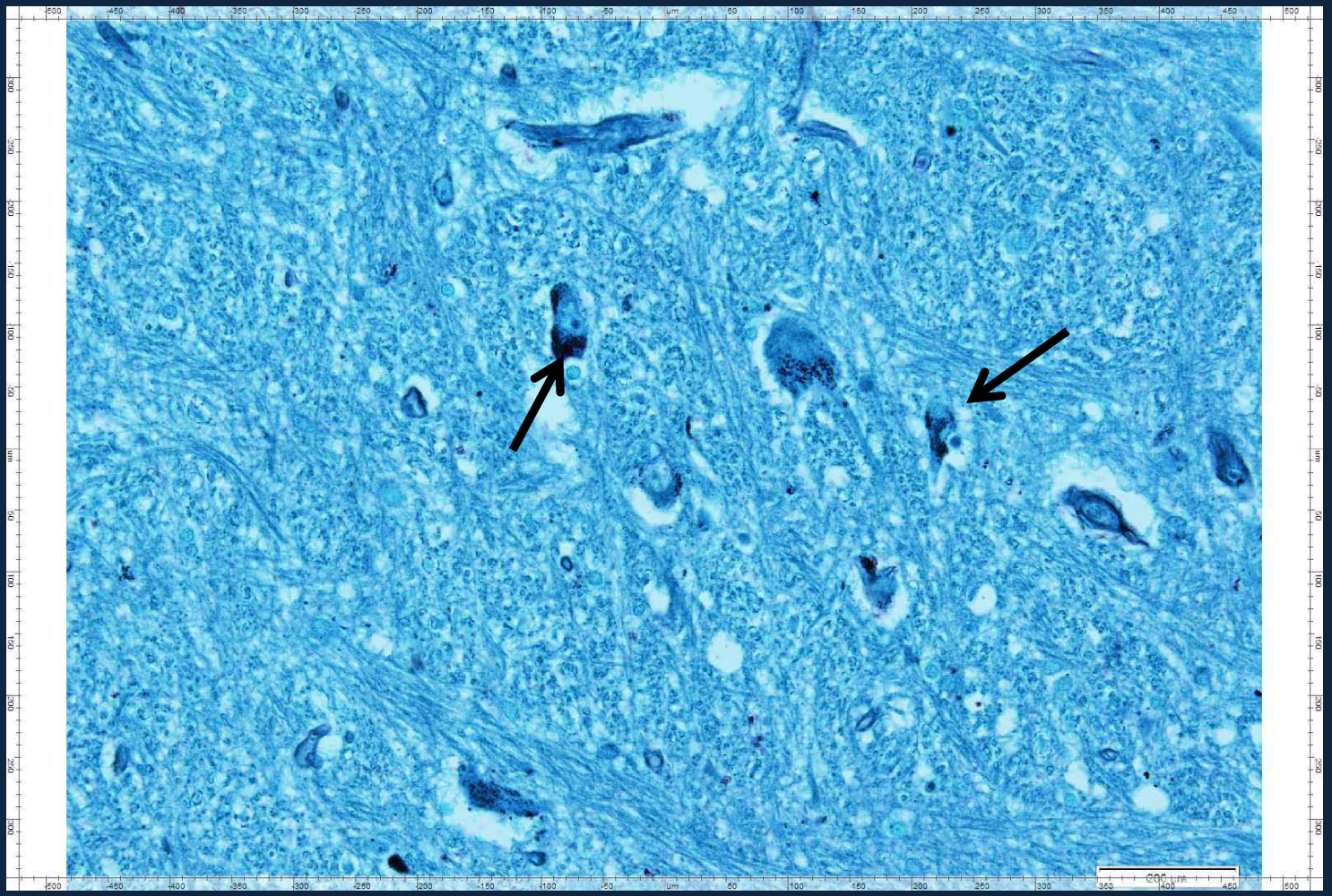
** Stains for fungi (gray to black)*



* Rods are aspergillus
* Black dots are cryptococci

C38 BG2 GMS 10X

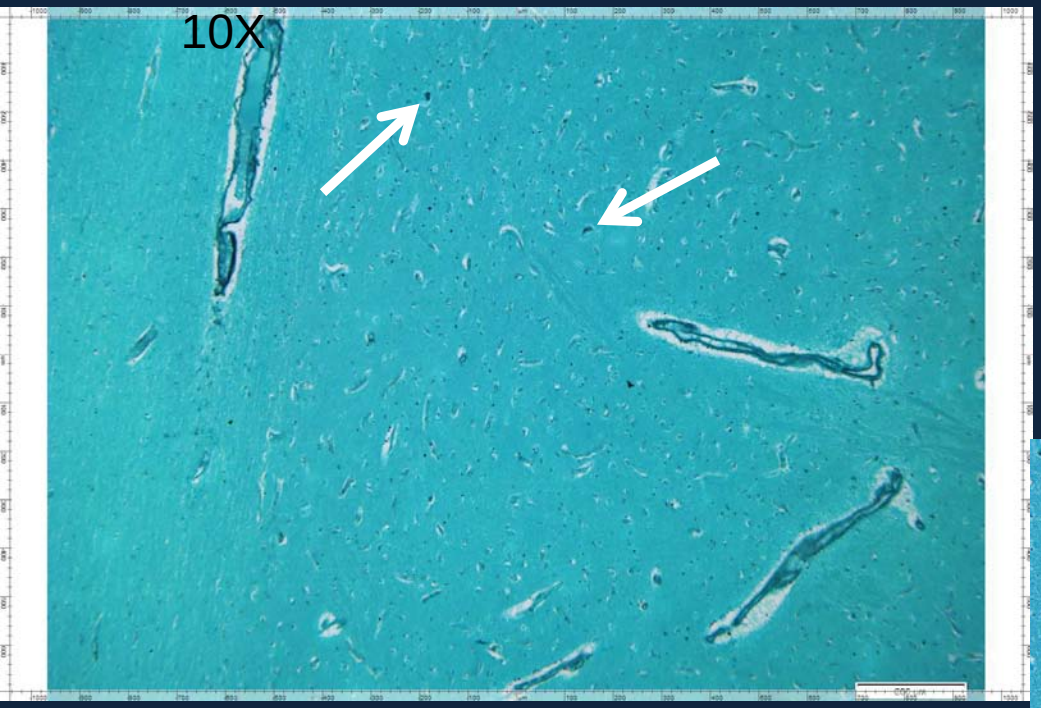
G.M.S. Staining



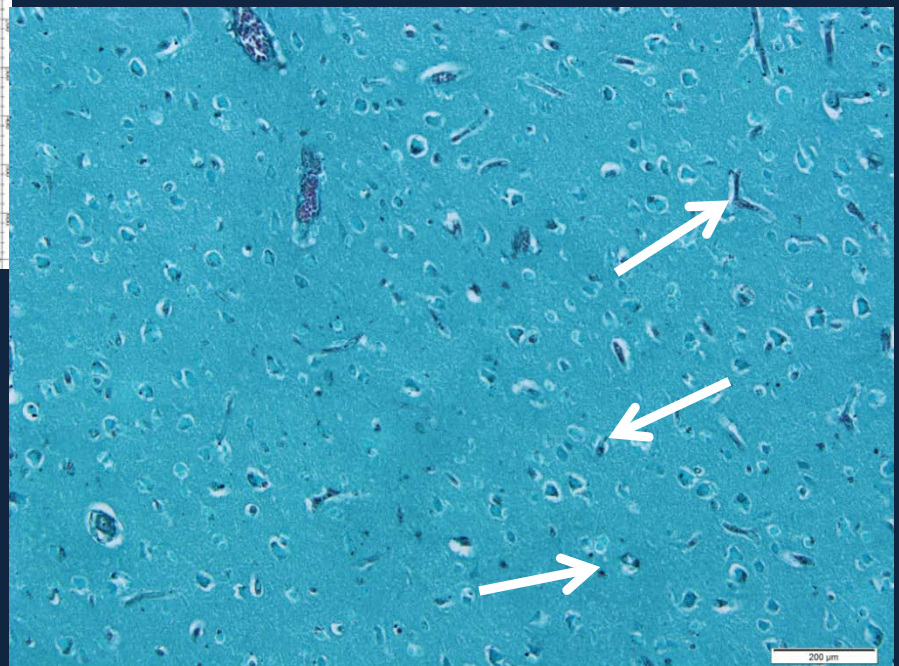
C38 BG GMS 40X.

G.M.S. Stain

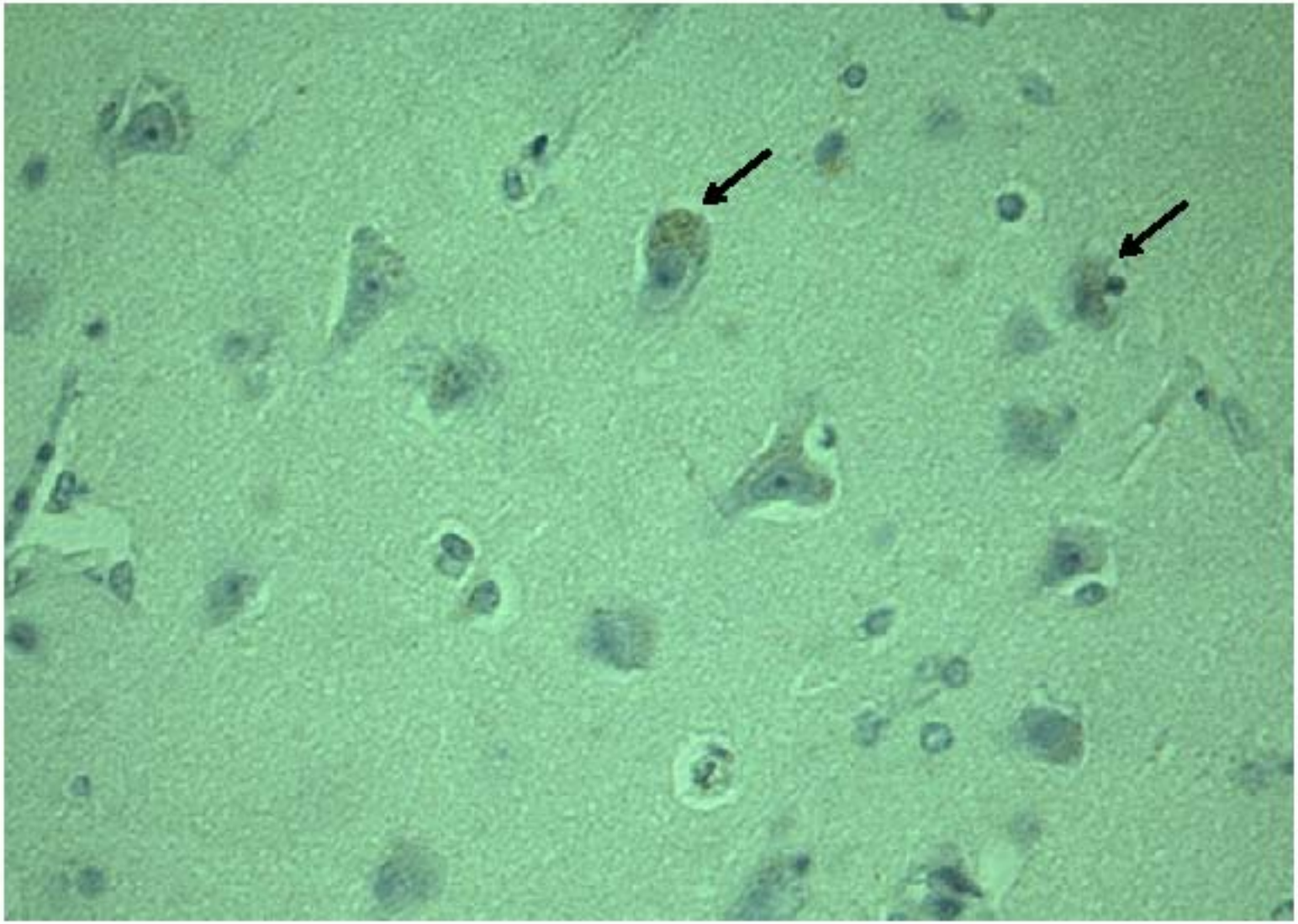
C39 BG GMS
10X



C44 LFL GMS 20X



HIV p24 Immunostain



C38 RTL p24 40X

Summary

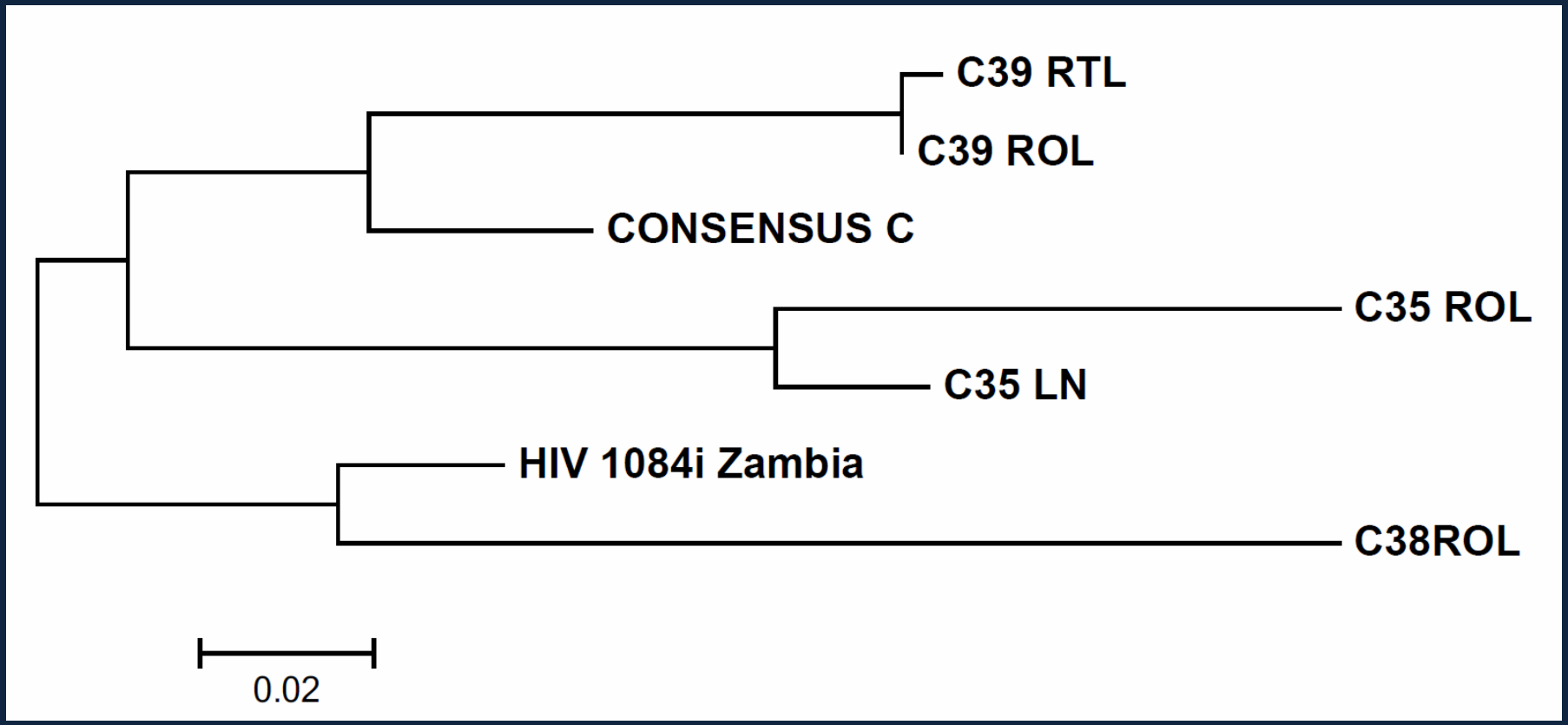
- HAND is common in subtype C HIV infected individuals in Zambia
- Co-infections and meningitis are found in HIV infected brains
- High Frequency of meningitis and lymphocyte infiltration in HIV infected brains

- Neurological assessments
- Neuropathological analysis
- Virological analysis

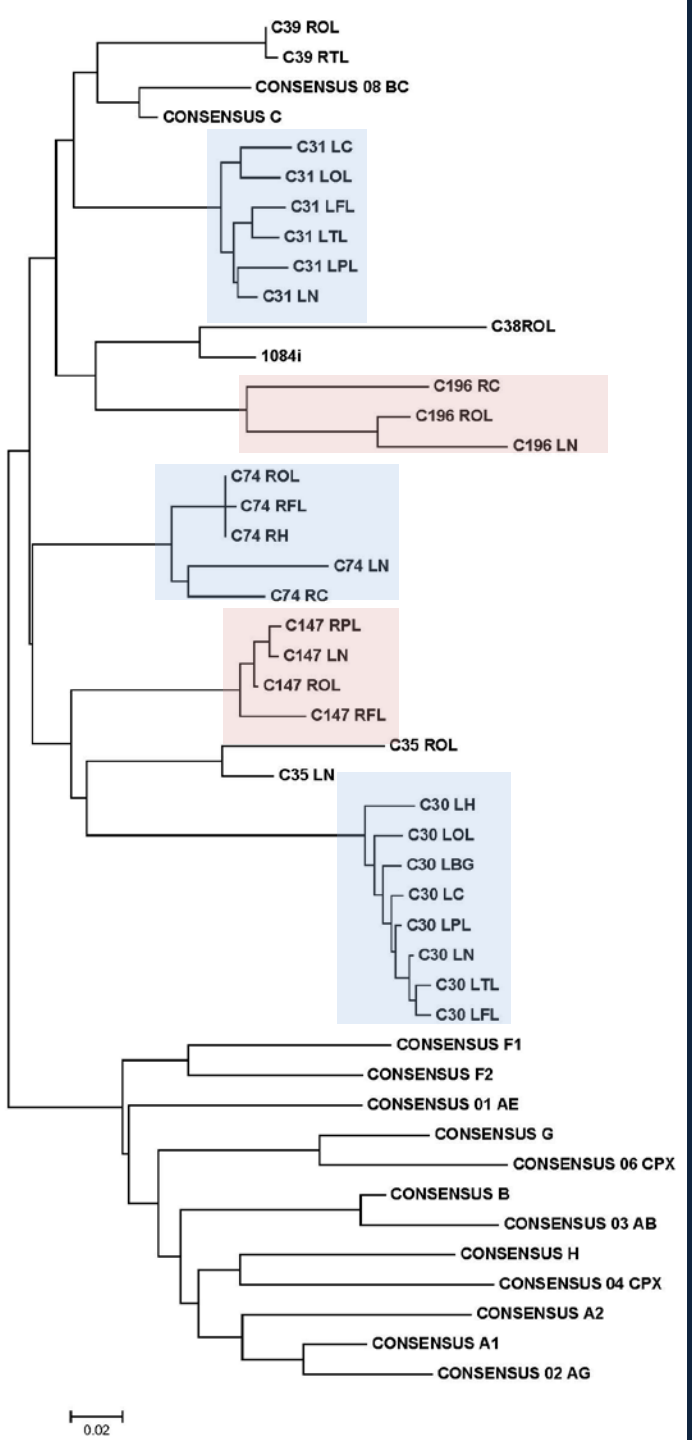
HIV Detection

Case	ARV	CD4	frontal lobe	parietal lobe	temporal lobe	occipital lobe	hippocampus	cerebellum	basal ganglia	lymph node
C30	naïve	NA	+	+	+	+	+	+	+	+
C31	naïve	351	+	+	+	+	+	+	neg	+
C35	naïve	NA	neg	neg	+	+	+	neg	neg	+
C38	treated	NA	+	+	+	+	neg	+	neg	ND
C39	treated	NA	neg	neg	+	+	+	+	neg	ND
C74	treated	4	+	+	+	+	+	+	+	+
C147	treated	46	+	+	+	+	+	neg	+	+
C196	naive	424	+	+	neg	+	+	+	+	+

Env C1-C5 Phylogenetic Analysis



Phylogenetic Analysis of Env C1-C5



Summary

- HAND is common in subtype C HIV infected individuals in Zambia
- Co-infection and meningitis are found in HIV infected brains
- High Frequency of meningitis and lymphocyte infiltration in HIV infected brains
- Viral compartmentalization and quasispecies are observed in HIV infected brains

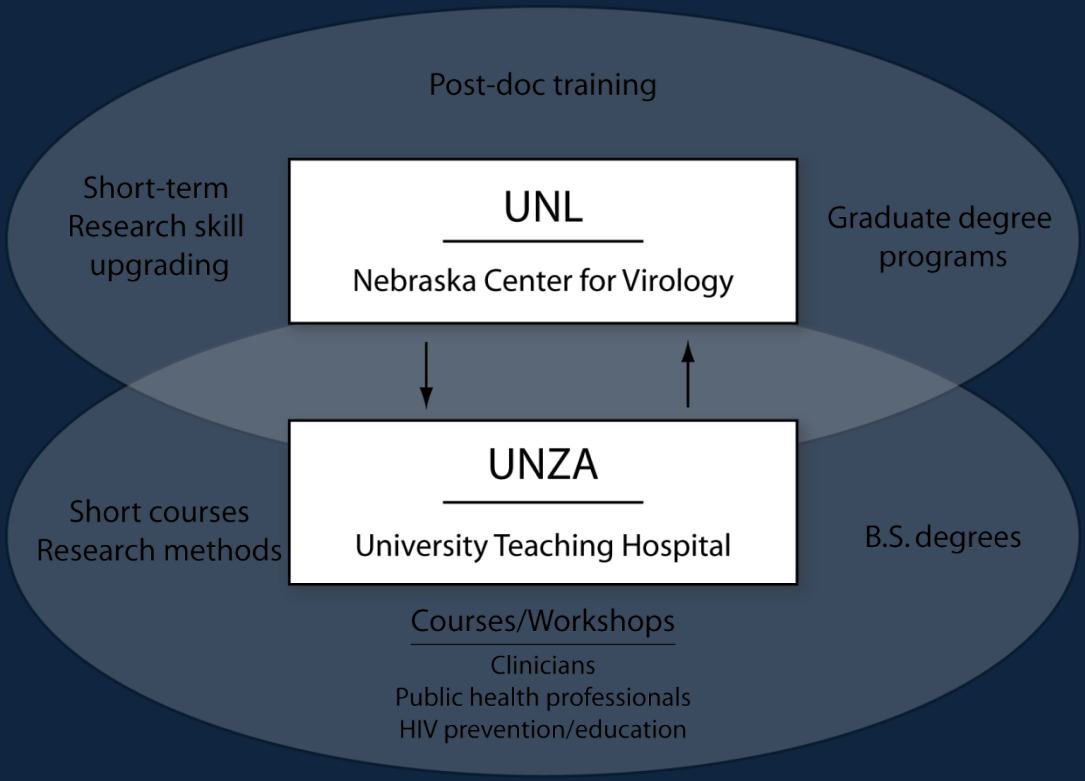
Future Direction

- Continue to collect autopsy cases
- Correlate opportunistic infections with neuropathology
- Analyze clinical history to determine if associations exist between ART and clinical symptoms with neuropathology
- Further analysis viral compartmentalization, including ART resistance genotypes

Challenges in Zambia

- Brain drain: trained people leaving Zambia
- Research infrastructure
- A great need to develop capacity
  - Training of people
  - Facilities

Fogarty International Training Program



- ***Results:*** 52 fellows trained in the US, many more health professionals trained in workshops in Lusaka

Developing Facilities and Infrastructure

- Infrastructure to support research of returning trainees
- Built clinic in the hospital in 2002
- Staffed by UNL/UTH research personnel – nurses, data managers, medical personnel, many of them trained through the collaborative program

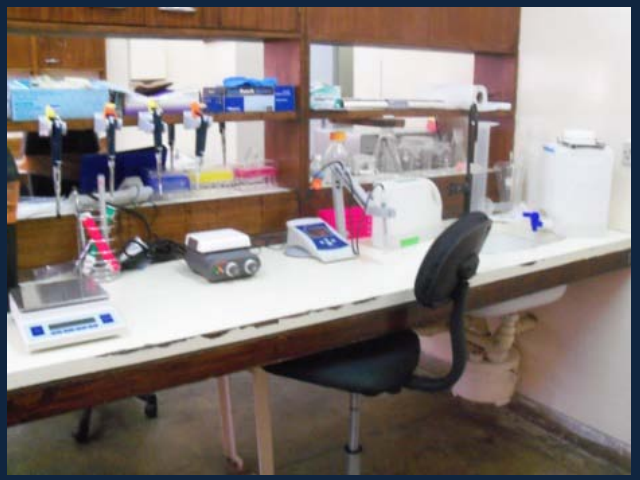
Research Lab Expansion

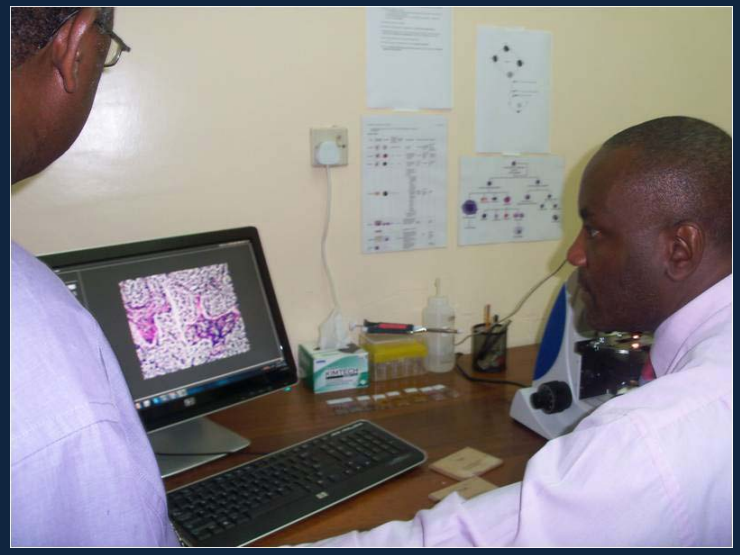
- In 2005, dedicated expanded and newly equipped research laboratory
- Headed and staffed by Fogarty trainees
- Partnership with the U.S. CDC – will use lab as a Center of Excellence in Pediatric & Family HIV Care

Lab dedication 2005

Neuropathology Laboratory

- Immunohistochemistry
- Training of personnel

Dr. Mudenda

Dr. Shibemba

Acknowledgment

University of Nebraska

Adelina Holguin
Dawn Eggert
Sandra Gonzales

University Teaching Hospital

Victor Mudenda – In-country collaborator
Aaron Shibemba - Pathologist
Jonus Kalwaji - Clinical officer
Samson Banda - Mortuary attendant
Isaac Mweemba Histopathology technician

www.nimh.nih.gov
NIMH

National Institute of
Neurological Disorders and Stroke
National Institutes of Health
Reducing the burden of neurological disease...

Study Site

UTH, Lusaka

Study Clinic

Study Lab

The Nebraska Center for Virology

'Multidisciplinary research in virology producing a new generation of innovative researchers'

University of Nebraska–Lincoln
University of Nebraska Medical Center
Creighton University

NCRR