

Point-of-Care Diagnosis of Kaposi Sarcoma in Sub-Saharan Africa Using KS-Detect



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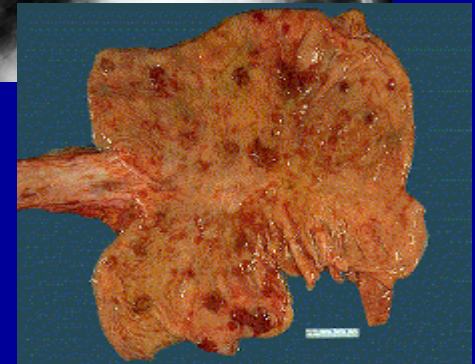
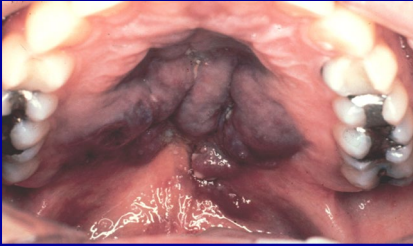
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Sibley School of Mechanical and Aerospace Engineering, Cornell University Ithaca, New York.

Kaposi Sarcoma

- Cancer of endothelial cell origin, almost always affecting skin and mucous membranes, often extending to internally (lungs, GI tract)



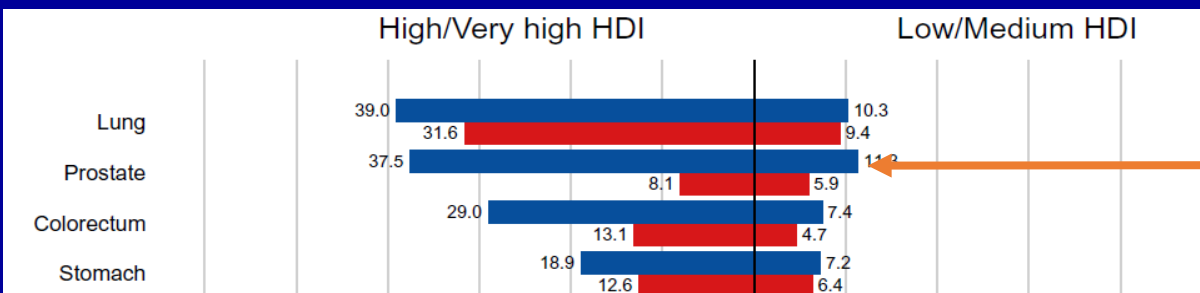
- **Caused by a virus**
 - Kaposi's sarcoma-associated herpesvirus (KSHV; also known as human herpesvirus 8) is a necessary (albeit not sufficient) cause of KS
- **Had been rare (e.g., in U.S.) or uncommon (e.g., Africa) but in early 1980's became scarlet letter of HIV epidemic**

Incidence of KS with Normalized Immune Status

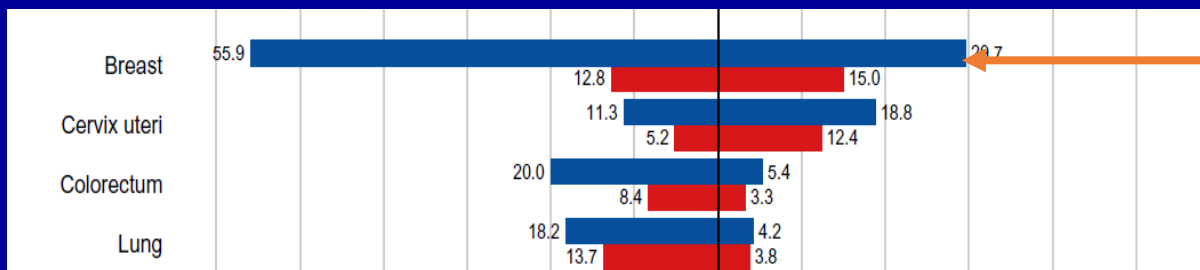
Group	All Patients			Non-ART Users			ART Users			New ART Users		
	Female n = 94,334	Male n = 46,218	Overall n = 140,552	Female n = 84,552	Male n = 43,039	Overall n = 127,591	Female n = 66,470	Male n = 31,440	Overall n = 97,910	Female n = 51,283	Male n = 23,839	Overall n = 75,122
CD4+ T cell, cells/mm ³ 6												
>350	66 (39,112)	80 (36,179)	70 (45,109)	141 (76,263)	161 (60,428)	146 (87,247)	29 (11,76)	40 (10,161)	32 (14,70)	40 (13,125)	43 (6,0,302)	41 (15,109)
>500	60 (29,126)	121 (46,323)	74 (41,133)	146 (66,325)	158 (39,630)	149 (74,298)	13 (1,9,95)	99 (25,394)	31 (10,98)	0 ² (0,98)	118 (17,834)	22 (3,1,154)

Among ART-treated with “normalized” their CD4 count (>500 cells/mm₃), KS incidence = 31/100,000 person-yrs

Semeere et al. Cancer Medicine 2016



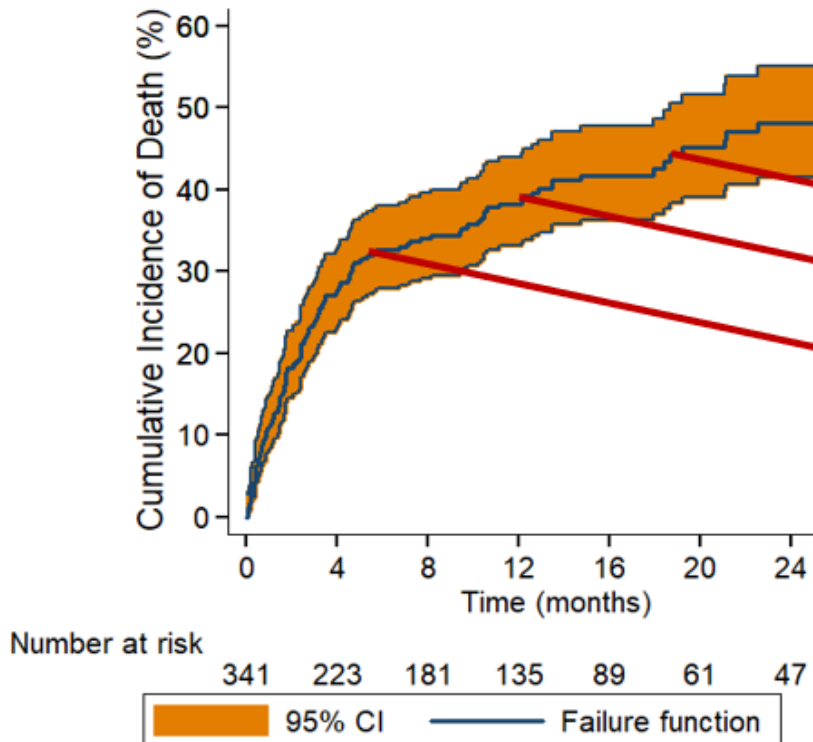
KS Incidence of 31 per 100,000 PYs



KS Incidence of 31 per 100,000 PYs

Sung et al. CA Cancer J Clin 2021

Survival following KS Diagnosis



Number of deaths among 367 with newly-diagnosed KS =141

Month: Cum. Incidence (95% CI)

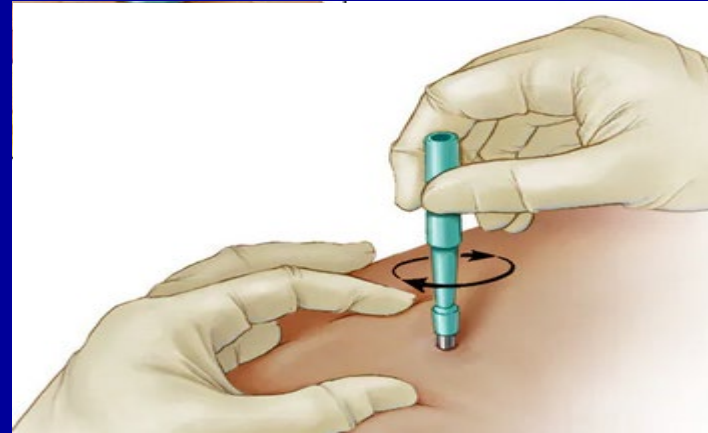
18 mo: 43% (37% to 49%)

12 mo: 38% (33% to 44%)

6 mo: 33% (28% to 38%)

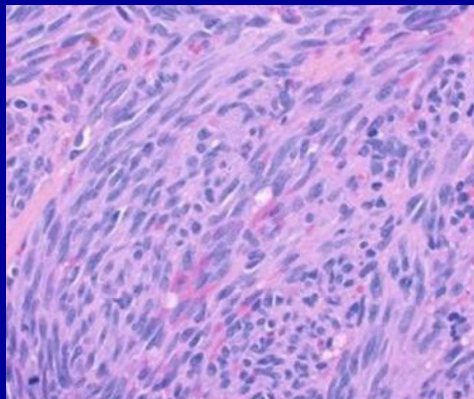
KS Diagnosis in Resource-Rich Settings

- Following clinical suspicion and biopsy

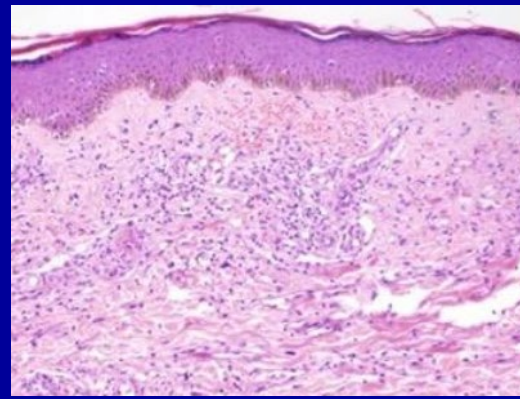


- KS is a pathologic diagnosis

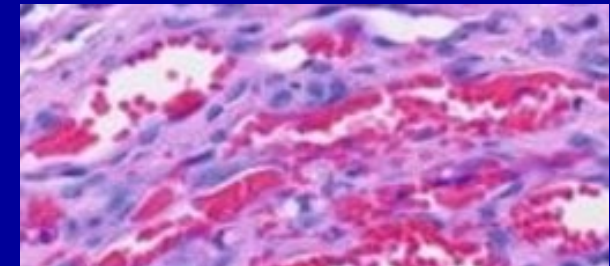
- Triad:



spindle cells



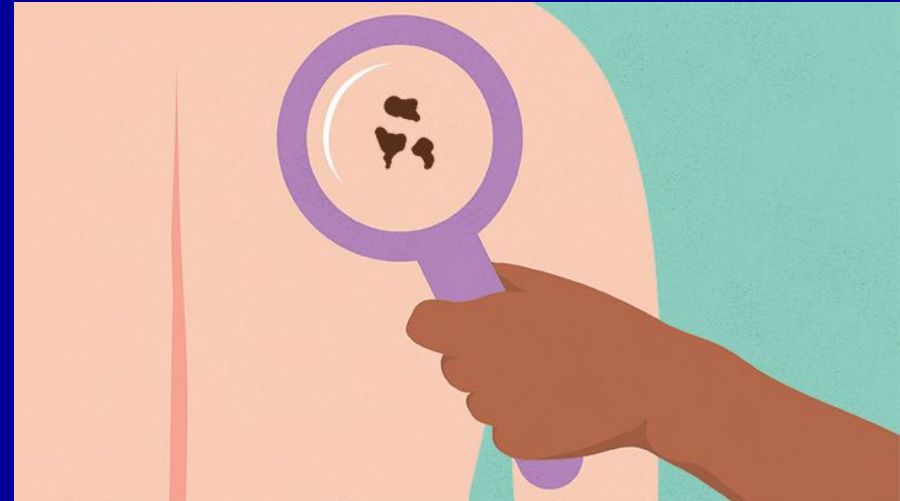
inflammatory infiltrate



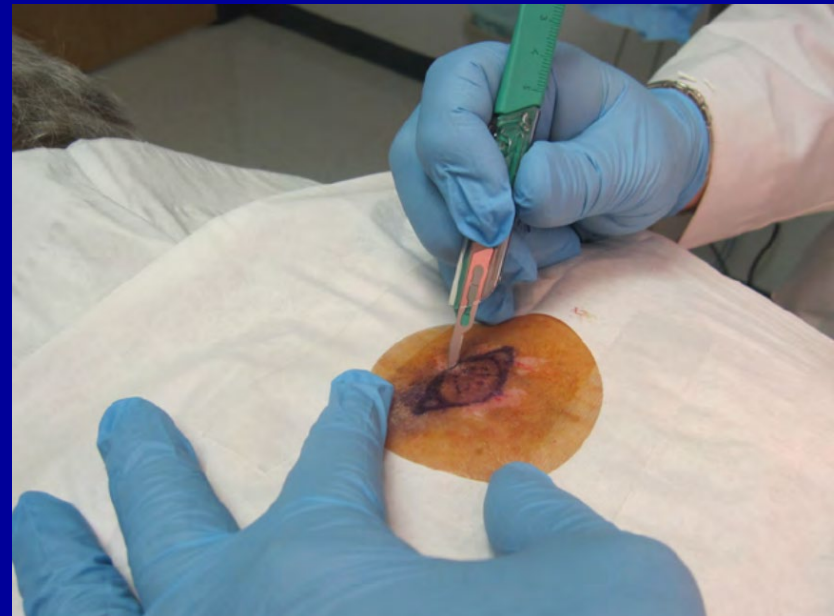
abnormal
vasculature

KS Diagnosis in Sub-Saharan Africa

- As of mid-2000's, clinical (i.e., macroscopic visual) diagnosis of KS was common



- Where biopsy was being performed, it was by resource-intensive excisional means via surgeons



KS Diagnosis in Resource-Limited Settings

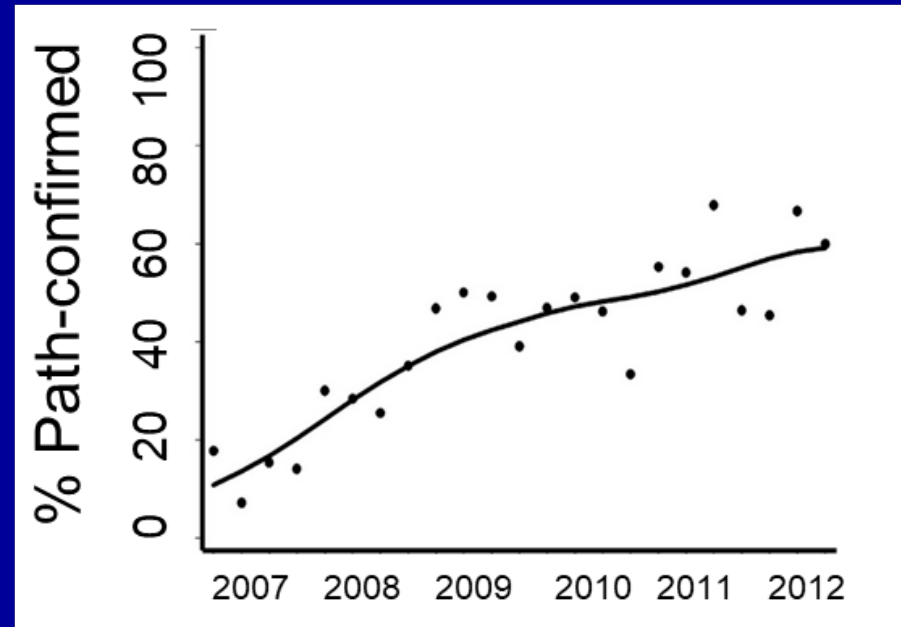
- In sub-Saharan Africa, lack of available pathology services has made clinical (i.e., macroscopic visual) diagnosis of KS common

- Just how common is clinical diagnosis?

- Zimbabwe (SIKO Study): 23% of 703 dx were path-confirmed

- Uganda/Kenya: 36% of 2439 dx were path-confirmed

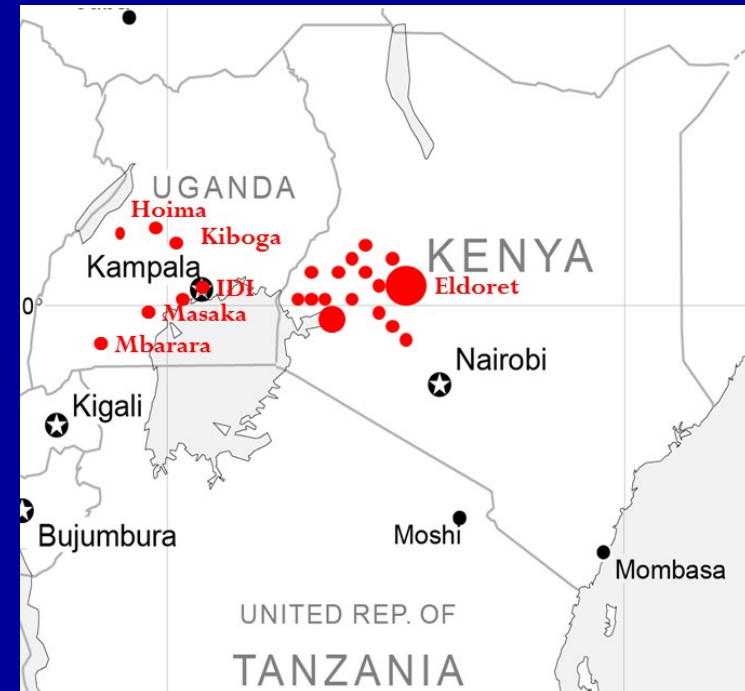
(Semeere et al. 2012)



- Tertiary centers require path before a patient is even seen?
 - Likely not seeing all KS in your community

Building a Service Provision Platform for an Alternative

- Establishment of free-of-charge skin punch services in Uganda and Kenya
- 5 mm cylindrical punch; Gelfoam® for hemostasis; performed by mid-level personnel (nurses and phlebotomists)



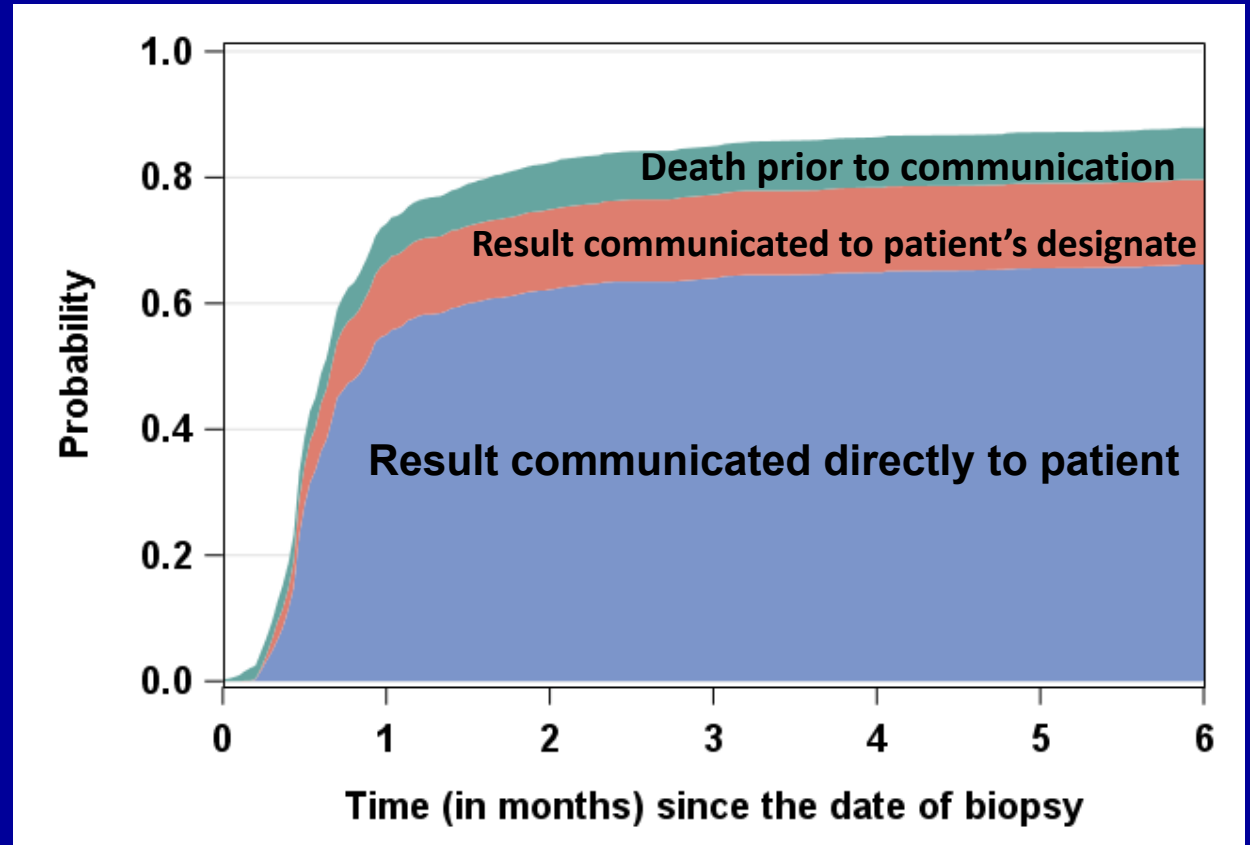
Even When Skin Punch & Pathology Are Available

- **Turn-around-time often unacceptable**

- N=958 biopsies in Uganda: at 1 month, 1 of 3 results NOT returned to patient or designate (Semeere et al. 2019)

- **Accuracy often unacceptable**

- Uganda/Kenya pathology compared to U.S. dermatopathologist gold standard (N=897) (Amerson et al. 2016)



Concordance	Sensitivity	Specificity
69% (66%-72%)	68% (65%-72%)	89% (83%-93%)

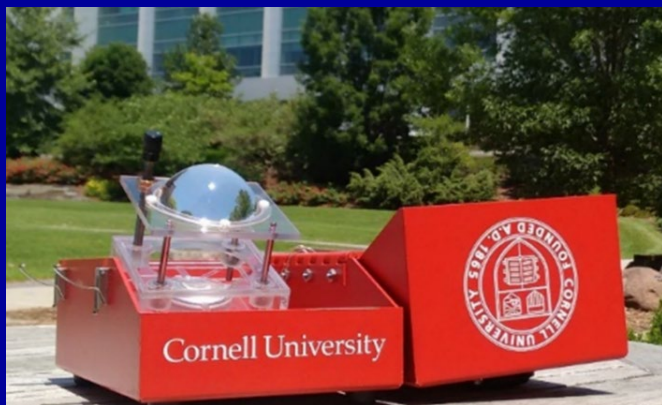
Is There a Better Way?

We hypothesized:

- **A point-of-care diagnostic test for KS can be developed**
 - taking advantage of the central dogma of KS which is that KSHV is a necessary causal agent
- **An automated and objective “liquid biopsy” for KSHV DNA content could, in large part, replace solid-phase pathology**
- **Assaying for KSHV DNA should be highly sensitive for KS, but a possible problem with this approach is specificity**
 - 40 to 80% of adults in sub-Saharan Africa: KSHV-antibody-positive
 - 10 to 30% of KSHV-infected: detectable KSHV DNA in blood

User Driven Evolution of the POC KS-Detect System

V1 (2014)



V2 (2016)



V3 (2018)

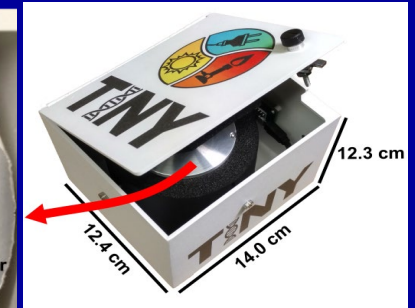
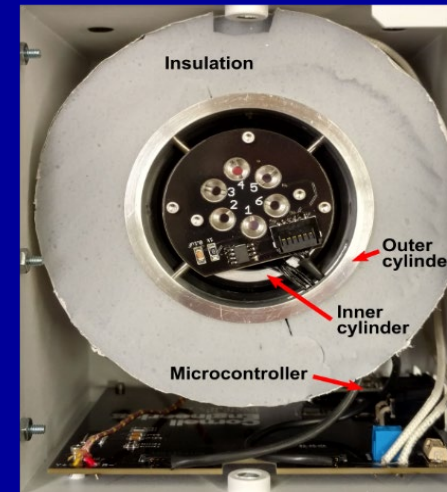


KS-Detect technological evolution driven by local user feedback and field experience



Inside the TINY (Tiny Isothermal Nucleic acid amplification sYstem)

- Portable, Inexpensive & Easy to Use
- Florescence based detection
- 6x multiplexing using 0.2mL PCR tube consumables
- Ability to operate off electric, battery, solar, thermal energy
- Phase change materials to maintain constant temperature



Electricity



Heat Source



Sunlight



**5 mm cylindrical punch
Gelfoam® for hemostasis**



Bisect

Histopathology

Uganda

- Anti-LANA stain available upon discretion of pathologist

U.S. (Cornell & UCSF):

- Examination of original slide
- New section and/or anti-LANA stain available upon discretion of pathologist
- Final interpretation: consensus ≥ 2 readers

**Quantification of KSHV DNA
under optimal conditions**

U.S. (Cornell):

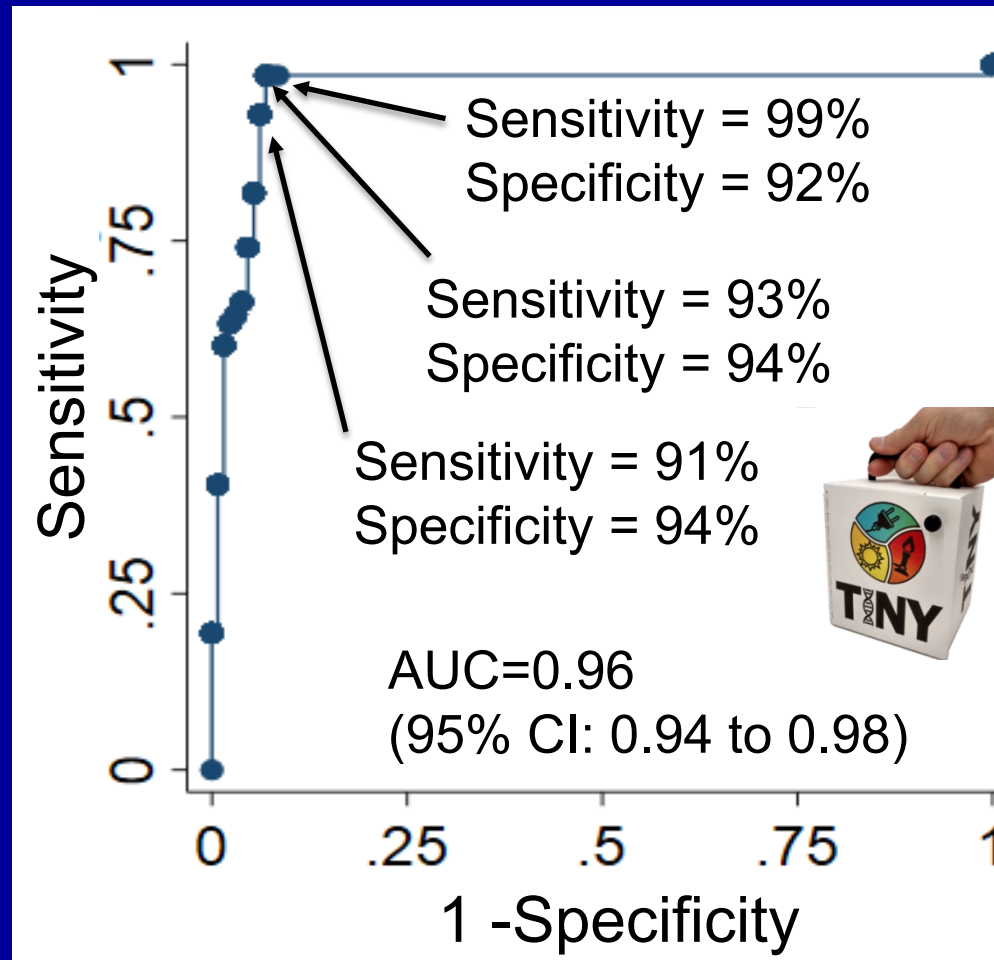
- DNA extraction by Qiagen kit
- Target : KSHV ORF 26 DNA
- Quantitative PCR &
- Loop-mediated isothermal amplification (LAMP) in a POC device: TINY

Diagnostic Performance of Quantification of KSHV DNA for the Diagnosis of KS

LAMP for KSHV ORF 26 in TINY

TINY performed in U.S. laboratories

Evaluation of TINY in sub-Saharan African labs is underway



ROC curves for diagnosis of KS; gold-standard = US-based pathology (341 KS and 150 Non-KS).

Currently: Validating TINY in Real-world Clinical labs



We are here

Dissemination and
promotion of
real-world use
“Translation”

Validation in real-
world settings

Validation in
optimal setting

Development
in optimal setting
(Calibration/Training)
(e.g., choosing cutoffs)

Multi-site Network For Clinical Validation

Uganda

- Infectious Diseases Institute (IDI) – Kampala: Coordinating Center
- Mbarara Regional Referral Hospital
- Masaka Regional Referral Hospital
- Lacor Hospital Gulu

Rwanda

- Rwanda Military Hospital , Kigali

Kenya

- AMPATH-Moi University, Eldoret
- Chulaimbo District Hospital

Tanzania

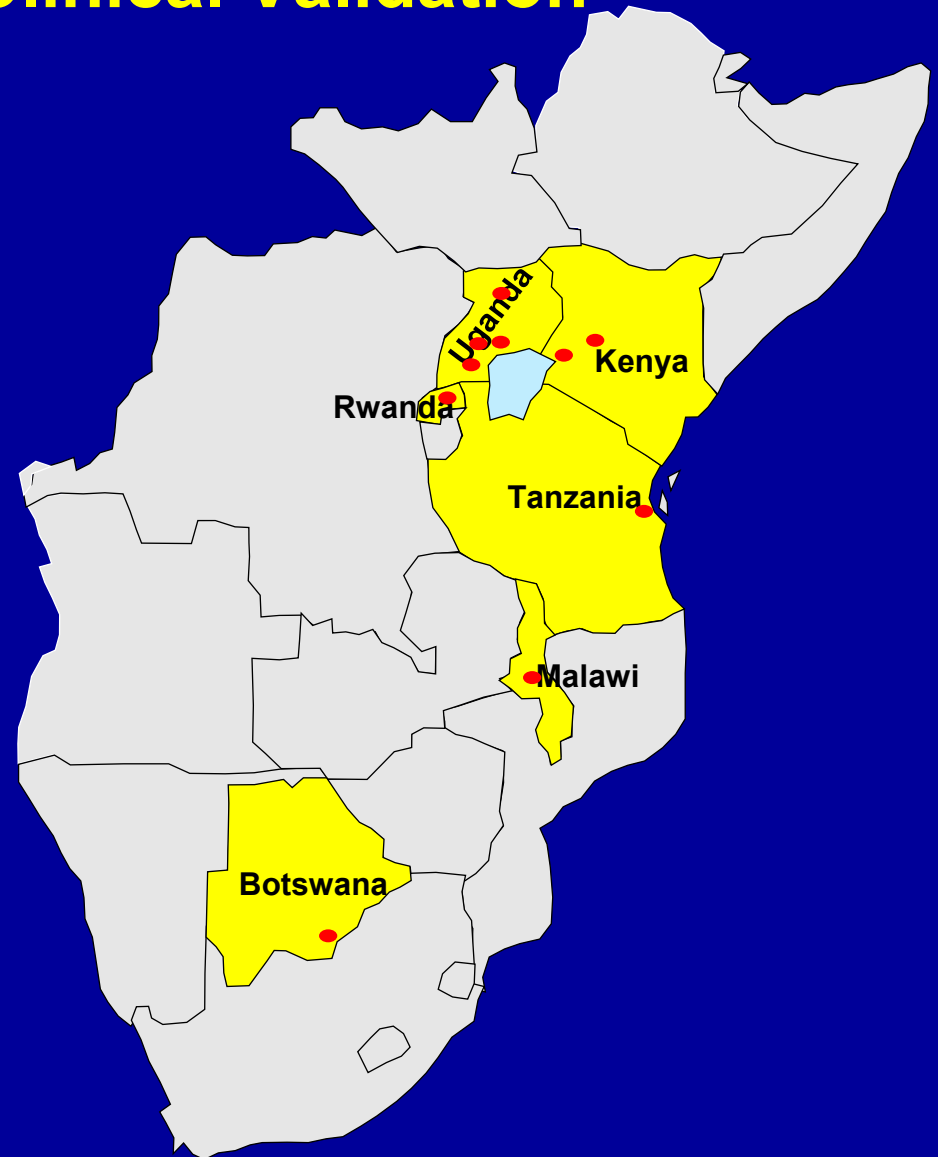
- Ocean Road Cancer Institute, Dar es Salaam

Malawi

- UNC Project Lilongwe

Botswana

- University of Botswana



Multi-site Study to Validate TINY

Site	No. Screened	No. Enrolled	No. Biopsied	No. with Pathology Result Returned
Kenya				
<i>Moi</i>	22	19	19	17
<i>Chulaimbo</i>	9	8	8	7
Uganda				
<i>Mbarara</i>	113	95	95	88
<i>Masaka</i>	100	98	98	94
<i>IDI</i>	412	320	320	310
Malawi	101	98	98	97
Tanzania	35	35	35	24
Rwanda	7	7	7	5
Botswana	20	20	20	12
Total	819	700	700	654

Multidisciplinary Research

Funding



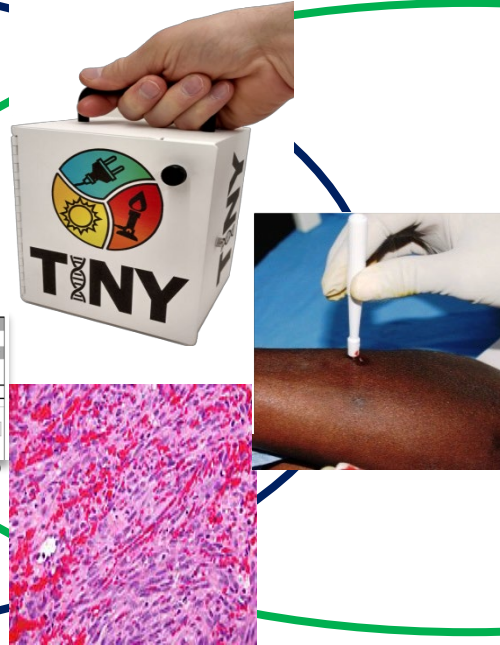
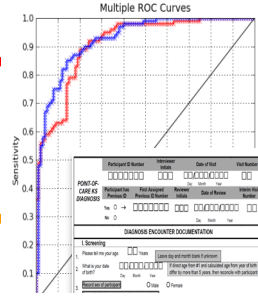
U54 CA190153
 UH3 CA202723
 K23 AI136579

Engineering

David Erickson, Ryan Snodgrass, Jens Duru,
 Varun Koppa & Duncan McCloskey
 (Cornell – Ithaca).

Clinical Epidemiology

- Africa - Aggrey Semeere, Miriam Laker-Oketta, Racheal Ayanga, (IDI-Makerere); Other clinical sites: ISS-Mbarara, UG Cares-Masaka, AMPATH Kenya, ORCI-Tanzania, RMH-Rwanda, UB-Botswana, UNC-Malawi.
- U.S. - Jeff Martin, Megan Wenger, & Melissa Assenzio (UCSF)



Dermatology

- Toby Maurer (UCSF)
- Esther Freeman (Harvard)

Pathology

- Africa - Robert Lukande (Makerere)
- U.S. - Ethel Cesarman, (Cornell-NYC); Phil Leboit, Tim McCalmont (UCSF)



RWANDA MILITARY HOSPITAL



University of California San Francisco



Summary

- In sub-Saharan Africa, clinical “visual” diagnosis of KS is sub-standard
- Where available, pathology suffers from slow turn-around and inaccuracy
- Quantitative detection of KSHV DNA content in skin lesions by LAMP (in TINY), performed under optimal laboratory conditions:
 - Has both high sensitivity and specificity for the diagnosis of KS
 - Awaits real-world testing to confirm robustness
 - May also be relevant for resource-rich settings
- **Easy to envision a future in which automated objective molecular diagnosis for KS is standard approach in any setting**