A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Medicine in Internal Medicine.

Investigator: Dr Oswald HABYARIMANA

Supervisors: Dr. Mary Chamberlin

Dr Vincent DUSABEJAMBO

Dr Eric RUTAGANDA

Co-supervisors: Dr. Steve Bensen, Dr Tim Walker, Dr Belson Rugwizangoga,

Co-Investigators: Francine B. DeAbreu, Dr. Arief Surinawata, Dr.Greg Tsongalis.

Kigali, August 2017
DECLARATION

I declare that this Dissertation contains my own work except where specifically acknowledged. It is being submitted for the degree of Master of Medicine in Internal Medicine at University of Rwanda and has not been submitted before for any degree or examination in any university.

Dr Oswald HABYARIMANA………………………………………………

Dr. Mary Chamberlin …………………………………………………

Dr Vincent DUSABEJAMBO…………………………………………

Dr Eric RUTAGANDA …………………………………………………

August 2017
DEDICATION

I dedicate this work:

To the Only Almighty, Our God

To my family.
ACKNOWLEDGEMENTS

My gratitude goes to my supervisors

My profound gratitude goes to my lovely wife Iradukunda Laetitia and my son Rwema Ian

I wish to express my sincere appreciation to the entire staff of Internal Medicine CHUK for their tireless commitment and support throughout my studies.
ABSTRACT
Background: Gastric cancer is associated with high morbidity and mortality due to the late onset of symptoms. It is the third leading cause of mortality around the world. The aim of this study was to determine molecular profiles of Rwandan samples for mutations that could potentially identify new targets for treatment.

Methods: From 12th August 2015 to 30th November 2016, we conducted an analytical cross-sectional study by enrolling 76 patients. The participants were patients coming for EGD endoscopy visualized endoscopically to have gastric tumor lesions. 76 pair biopsies were sent for pathological evaluation at CHUK then 97 specimens sent to Dartmouth Hitchcock Medical Center for genetic analysis where DNA was extracted from fixed tissues using the QIAamp DNA FFPE Tissue Kit and then processed.

Results: Among 76 patients with gastric tumor lesions, males were 55.3% and females were 44.7%. There was a preponderance increase of males. 46% of our participants were from Eastern Province; roughly 15% were coming from North, South and Kigali City respectively. 76.3% were from rural area. 85.5% of patients had low level of education. 46% (35/74) have smoked. 78.9% histopathology concordance: gastric malignancy vs benign. Malignancy 89.5%: Intestinal adenocarcinoma 47%, Diffuse adenocarcinoma 21%, Mixed 10.5%, Lymphoma 10.5%. There was a significant association between types of gastric cancer and age. P<0.038. Relatively, there was an increase in diffuse type cancer in younger age and Intestinal type in old age. Higher total LCN2 expression in samples + gastric cancer, H pylori + compared to H pylori neg, Not statistically significant P<0.744, probably due to small sample size (n=19). Twelve mutations in 9 biopsies 41% (9/22) were identified among the sequenced specimens. TP53 was the most common mutation 50%. The only targetable mutation was PTEN 25%.

Conclusion: This study is a very big achievement in terms of gastric cancer exploration in Rwanda, a short overview from the demographic distribution pattern, risk factors, clinical presentation and histopathology types to the genetic profile level of gastric cancer. This study showed the feasibility of Next Generation Sequencing on gastric specimen and a paucity of mutations in gastric cancer. PTEN mutation was a potential for targeted therapy.
LIST OF SYMBOLS AND ACRONYMS

CagA: Cytotoxin-associated gene A

CMHS: College of Medicine and Health Sciences

CHUK: University Teaching Hospital, Kigali

CHUB: University Teaching Hospital, Butare

DNA: Deoxyribonucleic Acid

DHMC: Dartmouth Hitchcock Medical Center

DSMAC: Data Safety Monitoring and Accrual Committee.

EGD: Esophago-Gastro-Duodenal endoscopy

EGFR: Epidermal Growth Factor Receptor

EPIYA: Glutamate-Proline-Isoleucine-Tyrosine-Alanine

FFPE: Formalin-Fixed Paraffin-embedded

GMD: Geisel School of Medicine at Dartmouth

HER2: Human Epidermal Growth Factor Receptor

HIPAA: Health Insurance Portability and Accountability Act of 1996

H. Pylori: Helicobacter Pylori

IHC: Immunohistochemistry

IRB: Institutional Review Board

GIT: Gastro-Intestinal-Tract

LCN2: Lipocalin2

NCCC: Norris Cotton Cancer Center
NGS: Next Generation Sequencing

PHI: Protected Health Information

PTEN: Phosphatase and Tensin homolog

RNA: Ribonucleic acid

UR: University of Rwanda

VacA: Vacuolating cytotoxin

WHO: World Health Organization
# TABLE OF CONTENT

DECLARATION ................................................................................................................. i
DEDICATION .................................................................................................................... ii
ACKNOWLEDGEMENTS ................................................................................................... iii
ABSTRACT ........................................................................................................................ iv
LIST OF SYMBOLS AND ACRONYMS ........................................................................... v
TABLE OF CONTENT ...................................................................................................... vii
LIST OF TABLES ............................................................................................................... vii
LIST OF FIGURES ........................................................................................................... ix

Chapter 1. INTRODUCTION ........................................................................................... 1
  1.1. Background to the study ....................................................................................... 2
  1.2. Literature Review ............................................................................................... 5

Chapter 2. METHODOLOGY .......................................................................................... 12
  2.1. Study design ......................................................................................................... 12
  2.2. Study population ................................................................................................. 12
  2.3. Sampling strategy and Sample size ..................................................................... 12
  2.4. Evaluable Patients .............................................................................................. 13
  2.5. Study period ......................................................................................................... 13
  2.6. Statistical considerations .................................................................................... 13
  2.7. Endoscopic techniques for sampling the gastric biopsies .................................... 13
  2.8. Tissue biopsy preparation for Histopathology studies ......................................... 13
  2.9. Analysis of DNA ................................................................................................. 14
  2.10. Ethical consideration ......................................................................................... 14
  2.11. Data collection, handling and record keeping ................................................... 15
  2.12. Study Monitoring, Auditing and Inspecting .................................................... 16

Chapter 3. PRESENTATION OF THE RESULTS .............................................................. 17
  3.1. The recruitment and findings flow of study population ...................................... 17
  3.2. Demographic characteristics of study population ............................................ 19
3.3. Clinical characteristics of study population .......................................................... 20
3.4. Laboratory findings on gastric tumor specimens ..................................................... 22
3.5. Targetable mutations in gastric cancer ................................................................. 26
3.6. Comparative data quality and quantity between the two groups (pre group vs. post group)
 ............................................................................................................................................. 28
Chapter 4. DISCUSSION OF THE FINDINGS .................................................................. 30
Chapter 5. CONCLUSION AND RECOMMENDATION ................................................. 32
  5.1. Study limitations .................................................................................................. 32
  5.2. Conclusion ......................................................................................................... 32
  5.3. Recommendation ............................................................................................. 32
REFERENCE .................................................................................................................. 34
APPENDIX ..................................................................................................................... 39
**LIST OF TABLES**

Table 1. Tafe et al represents an example of molecular profiles of other types of cancers .......... 11

Table 2. Demographic characteristics of study population .......................................................... 19

Table 3. Risk Factors for Gastric cancer in our study population .................................................. 20

Table 4. Physical findings of patients presenting for endoscopy ................................................... 20

Table 5. Sign and symptoms of patients presenting to endoscopy ................................................ 21

Table 6. Findings on endoscopy ..................................................................................................... 21

Table 7. Prevalence of H.pylori, Lnc2 and UR vs GMD comparative histopathology of gastric cancer ......................................................................................................................... 22

Table 8. Association between Age and type of gastric cancer ......................................................... 23

Table 9. Gender and Type of Gastric cancer .................................................................................... 24

Table 10. Association of H pylori and gastric cancer type. .............................................................. 24

Table 11. LCN2 and gastric tumor types. ......................................................................................... 25

Table 12. Association of LCN2 expression, H. pylori IHC and presence of a gastric cancer ....... 25
LIST OF FIGURES

Figure 1: Flowchart of patients presenting for endoscopy and their findings.......................... 17

Figure 2: Flow chart of samples received and processed at DHMC .......................................... 18

Figure 3: Pie chart of gastric cancers types in our study population according to Lauren classification................................................................. 23

Figure 4: Flowchart of molecular profiling by next generation sequencing of 57 gastric tumor specimens. ............................................................................................................................................ 26

Figure 5: Types of mutations found in 9 biopsies of gastric cancer by NGS .............................. 27

Figure 6: Chart of quality control findings in Pre Group ................................................................. 28

Figure 7: Quantification Data in pre & post group ......................................................................... 29

Figure 8: Quality of DNA specimens in the 2 groups .................................................................... 29
Chapter 1. INTRODUCTION

This work is research to present at University of Rwanda, School of Medicine, College of Medicine and Health Sciences. It is the final work, to be submitted at University of Rwanda as partial fulfillment of the requirements for the award of Masters of Medicine in Internal medicine. This study was completed in March 2017.

The research has been conducted at CHUK (Centre Hospitalier-Universtaire de Kigali) one the two main public health institutions in Rwanda, referral and university teaching hospital, and the Geisel School of Medicine at Dartmouth in Hanover, NH, USA. CHUK is a 429 bed-based hospital in Kigali. It receives referred patients from almost all district hospitals (46 hospitals). Other referral hospitals may refer patients to CHUK for different reasons such as financial, special services like CT-Scan, neuro-surgery or physiological conditions in mentally disabled patients. The patients with premium health insurance, prisoners, and those in need of emergency services access immediately CHUK services without passing through the national referral system. In the Endoscopic unit of Internal Medicine, diagnostic endoscopies are performed 5 days per week and interventional endoscopies; three to four days per week.
1.1. Background to the study

Gastric cancer is associated with high morbidity and mortality due to the late onset of symptoms. It is the third leading cause of mortality around the world where approximately 700 000 patients die each year (Wroblewski et al. 2010). In Japan and China, gastric cancer is the most common type of cancer with an annual incidence of 12 to 15 per 100 000 (Shiota et al. 2013).

From 2007 to 2011 retrospectively 3294 cancer cases were registered in Rwanda and gastric cancer was the most prevalent cancer (9.6%). Age-standardized incidence rates (ASIR’s) per 100,000 and age-standardized death rates (ASDRs) per 100,000 for both sexes in 2013 was higher in developing countries vs developed countries for stomach cancer (Global Burden of Disease Cancer 2015).

The most common risk factor for gastric cancer is Helicobacter Pylori especially for non-cardia gastric cancers. Shiota (2) estimated 20-fold increase risk of gastric cancer development in HP infected patients.

In Rwanda, at Kigali university teaching hospital 2.31% of patients in endoscopic unity had gastric tumors (over a period of one year starting in April 2008); at Butare University teaching hospital, 4.45% of endoscopically scanned patients in a period of 1 year (2011-2012) had gastric tumors, overall prevalence of H. pylori in this study was 75.3% (622/825) Walker, T.D. et al., 2014. In all cases, to date, there is no Rwandan data concerning molecular profiles analyzing Rwandan samples for mutations that could potentially identify new targets for treatment, thereafter referred to as “targetable genetic changes.”

In addition to standard cytotoxic regimens, targeted therapies, which are small molecules or antibodies designed to disrupt the activity of specific oncogenic signaling pathways, have recently emerged as a promising therapeutic strategy (Deng et al. 2012). In the ToGA trial (Bang et al. 2010), trastuzumab, and anti-HER2/ ERBB2 targeting antibody, improved the overall survival of patients with HER2-positive tumors when combined with chemotherapy. However, because only 7-17% of gastric patients in Western countries, are HER2 positive (either gene amplification or overexpression) and thus suitable candidates for anti-HER2 therapy (Gravalos and Jimeno 2008; Hofmann et al. 2008; Tanner et al. 2005), further research is warranted to
increase the population of gastric cancer patients for which targeted treatments are clinical options. For resource poor countries, the incidence of HER2 positive gastric cancers is unknown but if substantially increased, could be a more effective target for curative or palliative treatments than standard chemotherapy. Some HER2 targeted drugs are oral agents which would be easier to administer and easier to transport to remote locations.

1.1.2. Statement of the problem

In Rwanda, epidemiological studies have been conducted on the prevalence of gastric cancer. The study at Butare University Teaching Hospital by Walker et al (Timothy D Walker et al. 2014) showed a high prevalence (4.45%) compared to the standards of the world. At Kigali University Teaching Hospital, a 3-month period study done (not published) in 2010 by Dusabem Emmanuel did not report on the incidence of H.Pylori. In this study the prevalence of gastric tumor lesions was also higher (5.33%).

Kidd et al (1999) conducted a literature review on different 24 studies done in 12 sub-saharan african countries, they demonstrated that the overall prevalence of gastric cancers was 3.4 %. In a subset presenting with vomiting and weight loss or GI bleeding the prevalence would be higher and that 4.45 % or 5.33% reported in Rwanda is reflective of all patients coming for upper endoscopy. In the region (sub-saharan Africa), the prevalence of gastric tumor lesions was low either due to paucity of literature on gastric cancers or the quality of studies which are reporting the incidence from the symptomatic patients coming for upper GI endoscopy(Kidd, Louw, and Marks 1999).

Additionally, although exposure to indoor cooking smoke is common, known risk factors for gastric cancer such as tobacco smoking and diets high in salted or smoked foods are rare practices in Rwanda. Despite this there is a high prevalence of gastric cancer, and there is no data on the molecular profiling which could help to point other etiologies as well as novel treatments to improve this problem.

The Helicobacter pylori is the most evidenced risk factor for non cardia gastric cancer. Up to 95% gastric cancers are attributable to Helicobacter Pylori (Malfertheiner et al. 2012).
Furthermore, H. pylori infection may be the inciting event for oncogenic genetic changes and if strong associations with targetable mutations are identified, it could be used as a surrogate marker for more treatable gastric cancers in resource-poor parts of the world.

Since last two decades, a family of proteins called Lipocalin2 has been discovered and emerging as a good biomarker for any tissue insult or injury. Then lipocalin2 expression in gastric cancer is a promising area of investigation for the potential future diagnostic and prognostic biomarker of gastric cancers.

1.1.3. OBJECTIVES

1.1.3.1. General Objective

To type targetable genetic changes associated with gastric tumors among patients attending the Esophago-Gastro-Duodenal (EGD) endoscopy. See Table 1 for an example of the type of genetic changes/data that has been expected to be collected in this study.

1.1.3.2. Specific objectives

1. To assess the clinico-demographic characteristics of patients with gastric tumors

2. To determine the histopathology of gastric tumor lesions.

3. To determine the prevalence of H. pylori in gastric tumor patients

4. To determine the level of lipocalin2 expression in gastric tumors.

5. To determine the feasibility of creating molecular profiles including genetic changes targetable by drugs known to treat other cancers with similar mutational changes (see Table 1 for an example of molecular profiles of other types of cancers)
1.1.3.3. Research questions

Considering the high causative effect of H. pylori in gastric cancers and lack of data on genetic mutations among gastric cancer patients to determine if they are targetable by known medications, we conduct this study to answer the following questions:

1. What are the socio-demographic characteristics of patients with gastric tumors attending EGD endoscopy?

2. What are the types of gastric tumors encountered in patients attending EGD endoscopy?

3. What is the prevalence of H. pylori among the gastric tumor patients attending EGD endoscopy?

4. What is the level of lipocalin2 expression in gastric tumor patients attending EGD endoscopy?

5. Do genetic alterations (mutations, deletions, rearrangements) in gastric tumor patients correlate with targetable therapies?

1.2. Literature Review

1.2.1. Gastric cancer

1.2.1.1. Definition

Cancer is a medical term to mean an abnormal cell division and growth in malignant manner. All cancers are caused by alterations in oncogenes, tumor-suppressor genes, and microRNA genes (Croce 2008). These alterations are physically the results from an insult of carcinogens (viruses and bacteria, irradiations, chemicals) on susceptible host (cells). In gastric cancer, there is an alteration of micro RNA gene (miR21) that inhibits the expression of tumor suppressor gene PTEN. The PTEN is dilated, silenced or mutated in advanced gastric cancer. PTEN mutations in the ATP-binding domain are also implicated in an inheritable condition called Cowden Syndrome which pre-disposes individuals to an increased risk of many malignancies (Croce 2008).
1.2.1.2. Epidemiology

In 2011, the WHO reported that there were approximately 25 million people suffering from cancer. An epidemiological report showed that cancer is the second leading cause of death worldwide where 13 to 17 million deaths occur every year. GLOBOCAN project in Asombang’s paper classified gastric cancer as the second most common type of cancer globally and the ninth cause of cancer mortality in Africa. Holocombe introduced the idea of “African enigma”; the increased number of H.pylori infection unproportionally to the diseases associated with it in Africa. However, registries used in reports from african countries were not comprehensive to mean that, the epidemiology of gastric cancer is underreported thus the “African enigma” may be a speculative notion (Asombang and Kelly 2012).

Among 3924 registered cancers in Rwanda (from 2007 to 2013), tissue biopsy confirmed cancers were 58%; the gastric cancer was among the first five prevalent cancers at a percentage of 9.6 (Rwanda cancer registry).

1.2.1.3. Etiopathology

When a tumor lesion in stomach is biopsied, it should be sent for histopathological studies. Gastric tumors range from MALT (Mucosal associated lymphoid tissue) lymphoma to gastric carcinoma as well as benign tumors. Non cardia tumors are mainly adenocarcinomas and are mainly associated with H. pylori.

Extensive research studies have been conducted among eastern and western countries to assess the link between the gastric cancers and H. pylori. The latter has been confirmed as a type I carcinogen in gastric cancer by International Agency for Research on Cancer and WHO. Moreover, other risk factors also have been mentioned in other studies like salted and smoked food, tobacco and alcohol use (Feng et al. 2004).

The virulence of the H. pylori strain plays a big role by invading and damaging the gastric mucosa. For example in the intestinal type of gastric cancers, the patho-physiology is a cascade of consecutive process from chronic gastritis to gastric mucosa atrophy followed by intestinal metaplasia then dysplasia into carcinoma (Asombang and Kelly 2012; Wang et al. 2007).
1.2.1.4. Clinical features

Gastric cancer often presents in the advanced stage. Symptoms of early gastric cancers are very non-specific. In other countries with a high incidence of gastric cancer, like Japan, routine screening by endoscopy is performed. This results in earlier diagnosis and improves outcomes. The symptoms of advanced gastric cancer include severe unintentional weight loss, early satiety, epigastric pain, anorexia and vomiting. Anemia, melena, hematemesis, meat intolerance are also significant clinical features of bleeding gastric tumor.

1.2.1.5. Diagnosis

The diagnosis of cancer is based on a tissue biopsy studies. Based on physical examination and endoscopic inspection a gastric cancer may be highly suspected but a benign-looking tumor on endoscopy can be found to be malignant. It is a good practice to biopsy every gastric tumor or ulcer scoped during endoscopy to have a clear diagnosis. The barium swallow has a low specificity but it can detect the aggressive type of diffuse gastric cancer known as linitis plastica which endoscopy and biopsy studies can miss.

1.2.1.6. Treatment

Early diagnosis of gastric cancer is a prognostic factor in management of gastric cancer where, the surgical removal remains a cornerstone. There is no clear data on whether a primary prevention of gastric cancer may be achieved. Eradication of H. pylori will help by halting the progression of atrophic gastritis. To date, there have been no large scale trials examining the effectiveness of reversing the process towards gastric cancer by treating H. pylori infection (Mccoll 2010).

Due to the late clinical presentation, the management of gastric cancer consists of improving the quality of life. Surgical removals of the tumors, bypassing the obstructing tumor or implanting ostomies or feeding tubes to facilitate feeding are the options in palliative management of gastric cancers. Morbidity of gastrectomy is high, and unfortunately metastatic recurrence is common within 6-12 months and prognosis is poor. More effective therapies prior to surgery may help significantly improve at least the quality of life, if not overall survival. However this is not the case for MALT lymphoma, this low-grade lymphoma can often respond to H. pylori eradication.
treatment. In advanced grades, the treatment of MALT lymphoma consists of adding chemotherapy and ablative radiotherapy to \textit{H. pylori} eradication (Malfertheiner et al. 2012).

\textbf{1.2.2. Helicobacter pylori}

\textbf{1.2.2.1. Definition}

Helicobacter \textit{pylori} is spiral shaped, microaerophilic a gram-negative bacterium that found on the gastric mucosa, first isolated by Robin Warren and Barry Marshall in 1983, they got Nobel Prize award of Medicine in 2005 (Karlsson et al. 2012; Kusters et al. 2006). Chronically it induces inflammation of the gastric mucosa. Humans are the most likely reservoir of the bacterium, its transmission thought to be oral-fecal, oral-oral or iatrogenic as result of occupational exposure to gastric content where nurses and gastro-enterologists were documented to be more exposed. Normally, \textit{H. pylori} is contracted during the first years of life and is more prevalent in lower socioeconomic communities (Crowe, Feldman, and Grover 2014).

Unless eradicated, it persists for life in the stomach where it survives the acidic environment by its high urease activity by converting the urea of gastric juice to alkaline ammonia and carbon dioxide. \textit{H. pylori} is a factor in developing chronic gastritis, peptic ulcers (duodenal or gastric ulcers, gastric cancer and gastric mucosa associated lymphoid-tissue lymphoma (Mccoll 2010).

\textbf{1.2.2.2. Pathogenicity}

\textit{H. pylori} damages the host cell by its toxins especially cytotoxin-associated gene A (CagA) and vacuolating cytotoxin (VacA). The genes and alleles encoding for CagA and VacA have been widely studied and their association with gastric cancer have been established (Garcia et al. 2010). CagA was more likely to be associated with a high risk of gastric cancer (Jang et al. 2010).

The CagA has EPIYA (glutamate-proline-isoleucine-tyrosinealanine) motifs, which are located within the carboxyl-terminus of CagA. Depending on the amino acid sequences surrounding the EPIYA motifs, there exist EPIYA-A, -B, -C and -D motifs. These help us to differentiate other strains from the western countries strains (Europe, North America and Australia). They typical contains themselves the EPIYA-C (Karlsson et al. 2012). Inside the cell, the CagA EPIYA motifs are phospharylared; this results in targeting and interacting with numerous intracellular effectors to lower the threshold for carcinogenesis (1). VacA strains damage the host cell by vacuolating it,
the more the cell is vacuolated, the more likely to become malignant (1). VacA protein has a structure where different alleles have been identified. The signal (s) and mid region (m) where each has 2 different alleles (s1/s2, m1/m2) are the main strain’s characteristics playing a role in virulence. The gastric cancer was more associated with the variant s1/m1 (Uchida et al. 2009).

1.2.2.3. Epidemiology

Estimates infer H. pylori infection to 50 percent of world’s population. H. pylori is the most common chronic bacterial infection in human beings (Crowe et al. 2014). The infection is acquired in first few years of life in developing countries compare to developed countries. Around 20% of infected population clinically manifest H. pylori associated diseases whereas the remaining 80% they are chronically colonized by the bacteria without an obvious clinical features (Bridge and Merrell 2013).

1.2.3. Carcinoma and Targetable mutation therapies

The nomenclature of cancers reflects on the histology of affected tissue. According to the International Classification of Diseases for Oncology third edition (ICD-O-3), “carcinoma and adenocarcinoma are malignant neoplasms of epithelial tissue and glands confined to this tissue. Carcinoma is by far the most type of cancer accounting around 90% of all cancer cases”. Adenocarcinoma is the most common type of cancer found in the stomach where two types of this cancer are documented, well differentiated or intestinal type adenocarcinoma and undifferentiated or diffuse type adenocarcinoma(Parkin, Pisani, and Ferlay 1999)

The targetable molecule for therapy is HER-2, first tried in breast cancers with promising results. Due to its amplification in other carcinoma especially more than 10% in gastric cancer, it is a potential way in new medical management of such cancers(Marx et al. 2009).

The epidermal growth factor receptor family consists of four members: EGFR (HER1), HER2, HER3, and HER4. Activation of EGFR and HER2 expression in several types of tumour can lead to uncontrolled proliferation of tumour cells. Because EGFR and HER2 are overexpressed in a subset of oesophageal, gastric, and colorectal cancer, these receptors are strong candidates for targeted therapy(Fujita 2013).
The targeted therapy includes for instance Cetuximab, which is a human murine chimeric anti-EGFR monoclonal antibody and panitumumab, a human monoclonal antibody specific to human EGFR. Trastuzumab, an anti-HER2 antibody, is effective for treatment of HER2-positive gastric and gastro-oesophageal junction cancer, with increased benefits reported in patients with high levels of HER2 expression (Fujita 2013). Trastuzumab showed its potentiality when is associated to adjunctive cytotoxic chemotherapy (5-FU or capecitabine plus cisplatin) among gastric cancer patients with an improved survival rate (Bouché and Penault-Llorca 2010; Gunturu et al. 2013).
Table 1. Tafe et al represents an example of molecular profiles of other types of cancers

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Diagnosis</th>
<th>Prior therapy</th>
<th>Amino acid change(s) (p.)</th>
<th>DNA mutation(s) (c.)</th>
<th>SITG interpretation and recommendation</th>
<th>Next therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rhabdomyosarcoma</td>
<td>M</td>
<td>3</td>
<td>R</td>
<td>Recommend treatment with standard therapy; referral genetic counseling to rule out germine mutation.</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>NSCLC, adeno.</td>
<td>M</td>
<td>0</td>
<td>A (3 y)</td>
<td>EGFR mutation may result in decreased activation.</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>NSCLC, adeno.</td>
<td>M</td>
<td>2</td>
<td>R</td>
<td>BRAF mutation may confer resistance to EGFR inhibitor and sensitivity to BRAF inhibitor.</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>NSCLC, adeno.</td>
<td>M</td>
<td>1</td>
<td>R</td>
<td>KRAS mutation confers resistance to EGFR-targeted therapy. Recommend treatment with standard second-line therapy, followed by clinical trial with Hedgehog or CDK4/6 inhibitor.</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>NSCLC, adeno</td>
<td>M</td>
<td>1</td>
<td>R</td>
<td>KIT mutation; poor performance status; trial not recommended.</td>
<td>NA</td>
</tr>
</tbody>
</table>

(ND- none detected; NR- not reported; NA- not applicable; pt- patient; PS- performance status; SOC- standard of care; RT- radiation therapy; NSCLC- non-small cell lung cancer; adeno.- adenocarcinoma; ER- estrogen receptor alpha status; HER2- HER2 proto-oncogene status.)
Chapter.2. METHODOLOGY

2.1. Study design
This was an analytical cross-sectional study using quantitative strategy of data collection.

2.2. Study population
The study population was made by patients referred for EGD endoscopy who presented with clinical features of gastric tumor or gastric outlet obstruction. Those features included one or more of the following symptoms: constitutional symptoms, visible or palpable epigastric mass other than hepatomegaly, sister Mary Joseph’s nodes, Virchow node, immediate post prandial vomiting, left to right visible peristaltism, succession splash, upper or lower GIT bleedings.

2.3. Sampling strategy and Sample size
We enrolled every single patient coming for EGD endoscopy with clinical features of gastric tumor and retained only those visualized endoscopically to have gastric tumor lesions. This has been a non-random purposeful sampling strategy.

Over the year 2013, In CHUK endoscopic unit records, 75 among 1975 patients were diagnosed to have gastric tumor lesions. This gives a prevalence of 3.8% over a period of 12 months. To increase the power of the findings, we used the prevalence reported by Walker et al (2014) which was 4.45% among patients attended upper EGD at CHUB.

Thus, we are aiming to reach a sample size (N) of

\[ N = \frac{Z^2 \times P \times Q}{D^2} \]

N= Sample size

Prevalence (P) of gastric tumor =4.45%.

\[ Z=1.96 \]

\[ Q=1-P \]

\[ D = \text{precision (0.05) with 95\%CI} \]

\[ N = 66 \text{ patients} \]
2.4. Evaluable Patients
All subjects who consented to and completed the tissue biopsies were evaluable. Subjects not able to tolerate the biopsy, they were recorded as screen failures and replaced.

2.5. Study period
The study period is complete after tissue biopsies are obtained. Data collection started 12th August 2015 to 30th November 2016. Subjects proceeded to treatment at the discretion of their treating oncologist.

2.6. Statistical considerations
PRIMARY ENDPOINT – The primary endpoint of this trial is to determine the frequency (%) of gastric tumor samples with mutations or genetic changes for which there are targeted therapies available.
SECONDARY ENDPOINT—To determine the relative rates of mutations compared to relative rates of H Pylori positivity.

2.7. Endoscopic techniques for sampling the gastric biopsies
In patients suspected of having a gastric malignancy the following sets biopsies has been obtained using standard endoscopic biopsy technique with a large or jumbo endoscopic biopsy forceps. Ideally, Six to eight endoscopic biopsies in different locations of the suspected lesion were supposed to be obtained to assure adequate sampling. Two additional biopsies were obtained from the stomach in a location that appears normal. Both sets of biopsies were sent for pathological evaluation at CHUK as well as stored for transfer to Dartmouth Hitchcock Medical Center for genetic analysis.

2.8. Tissue biopsy preparation for Histopathology studies
If fixed in formalin, the ideal was that tissues must be removed from formalin and processed within 24 hours (fixation time of 6 hours is preferable).

Formalin-fixed cores/biopsies were transported to Pathology and fixed in formalin for paraffin-embedding, routine evaluation of histology and cellularity, and HER2 status.
After confirmation of tumor cellularity in frozen/FFPE biopsy specimens, Pathology generated 4 unmounted sections (“ribbons”) ≥10 microns thick of each tissue block. Unmounted tissue sections were placed into a 1.5-mL tube, and used for DNA analysis by the Genomics Shared Resource.

2.9. Analysis of DNA

DNA was extracted from fixed tissues using the QIAamp DNA FFPE Tissue Kit. Library preparation was performed using 50 ng gDNA for each sample. Samples were normalized using Qubit, pooled and sequenced on the v3 cartridge on the Illumina’s MiSeq® system using the Pillar NGS SLIMamp™ Lung Hot Spot Panel. For data analysis, FASTq files were uploaded to Pillar Biosciences, where sequence alignment, annotation, and variant classification were performed. Two hundred nanograms of RNA were used for cDNA and quality check using FusionPlex SolidTumor panel.

2.10. Ethical consideration

University of Rwanda ethical and research committee (CMHS IRB) accepted this study. The permission to conduct this study was obtained from CHUK Ethic Committee and approval from Hospital administration as well. The verbal consent was obtained from each participant at the time of seeking a rendez-vous of upper GI endoscopy prior to biopsy, at this time of pre-screening session a counseling to make participants’ disposition free from fearing the study and thorough physical examination was done by a senior medical resident or a physician and at the end all information was treated confidentially. Patients were consented using a written free consent form prior to endoscopy and counseled that if a tumor is found during the evaluation, research biopsies will be obtained from the tumor and surrounding normal tissue. If no tumor was seen, only standard biopsies were obtained. A material transfer agreement form was completed between CHUK and DHMC. The study was presented at IRB/Dartmouth and approved for molecular profiling of biopsies.
2.11. Data collection, handling and record keeping

2.11.1. Confidentiality

Information about study subjects is kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Participants signed an authorization that includes the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization.

Loss of patient confidentiality is a risk of participation. Study participant identities are kept confidential except as required by law. Subjects’ samples are identified by code only (i.e., linked, but de-identified). Patient samples are de-identified at the time and site of collection. Electronic case report forms, participant, and study information are kept in the Velos eResearch password-protected database. Additionally, documents containing participant identifiers, such as those from the medical record to confirm eligibility, are filed in binders and kept in a locked, secure location in the Office of Clinical Research at the Norris Cotton Cancer Center.

2.11.2. Data Retention

Following closure of the study, the investigator maintained study records in a safe and secure location. The records are maintained to allow easy and timely retrieval when needed (e.g., audit or inspection) and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Upon completion of study analysis, research information is stored in Dartmouth College Records Management off-site storage located at 6218 Etna Road, Hanover, NH 03755. Documents are shredded on site after 50 years of storage.
2.12. Study Monitoring, Auditing and Inspecting

2.12.1. Safety and Data Monitoring

This study has been monitored by the Data Safety Monitoring and Accrual Committee (DSMAC) of the Norris Cotton Cancer Center.

2.12.2. On-Site Monitoring

Clinical research monitoring for regulatory compliance and data integrity was conducted according to the NCI-approved NCCC Data and Safety Monitoring Plan. Internal monitoring was conducted by appropriately trained staff of the NCCC Office of Clinical Research and Dartmouth-Hitchcock Medical Center Clinical Trials Office who are not involved in the study.

2.12.3. Adverse event reporting

Patients were instructed to report the occurrence of any adverse event. An adverse event is any undesirable event occurring with the use of this procedure. Adverse events were graded according to the NCI Common Toxicity Criteria Version 4.0. A copy of the CTC version 4.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov). All appropriate treatment areas should have access to a copy of the CTC version 4.0. This trial was independently monitored by the Norris Cotton Cancer Center Data Safety and Accrual Monitoring Committee.

The following definitions are used to assess causality:

No: The clinical adverse event is definitely unrelated to the study procedure (e.g., does not follow a reasonable temporal sequence from study procedure, present prior to procedure, etc.)

Unlikely: The study procedures do not have any reasonable association with the observed experience; however, relationship cannot be definitely excluded.

Possibly: The connection with study procedure appears feasible, but cannot be excluded with certainty.

Probably: The clinical experience appears related to the study procedures with a high degree of certainty.
Chapter 3. PRESENTATION OF THE RESULTS

3.1. The recruitment and findings flow of study population

Figure 1 represents the recruitment and findings flow of patients who presented for Endoscopy and had clinical suspicion of gastric cancer. Within 16 months from Aug 2015 to Nov 2016, 1938 patients were scoped in CHUK, endoscopy unity and 76 were enrolled in the study (3.9%).

Figure 1: Flowchart of patients presenting for endoscopy and their findings.

PRE GROUP: biopsies taken before first analysis

POST GROUP: biopsies taken and sent for analysis with suggesting technique changes after first data analysis in October 2016

23 patients excluded from the study, did not meet criteria to be sent for molecular profiling

1938 patients scoped in endoscopy unity at CHUK

76 pts enrolled (3.9%)
Confirmed gastric tumor on EGD

PRE GROUP: 34 pair biopsy specimens (1 path & 1 non-path)
Molecular profiling by NGS

POST GROUP: 19 pair biopsy specimens (1 path & 1 non-path)
- Comparative USA vs UR H&E pathology
- Helicobacter Pylori & LCN2
Figure 2: Flow chart of samples received and processed at DHMC

Total: 105 samples

1st batch: 57 samples
"Pre group"
- 5 Cancelled (not enough slides)
- Total: 52 samples
  - DNA & RNA extraction
  - NGS

2nd batch: 10 samples
"Pre group"

3rd batch: 38 samples
"Post group"
- 8 not tumor
- Total: 30 samples
  - Histology, HP&LCN2 IHC
### 3.2. Demographic characteristics of study population

Table 2. Demographic characteristics of study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td><strong>Age group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-40</td>
<td>12</td>
<td>15.8</td>
</tr>
<tr>
<td>41-60</td>
<td>34</td>
<td>44.7</td>
</tr>
<tr>
<td>61-80</td>
<td>30</td>
<td>39.5</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>34</td>
<td>44.7</td>
</tr>
<tr>
<td>Male</td>
<td>42</td>
<td>55.3</td>
</tr>
<tr>
<td><strong>Residence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>58</td>
<td>76.3</td>
</tr>
<tr>
<td>Urban</td>
<td>18</td>
<td>23.7</td>
</tr>
<tr>
<td><strong>Province</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>East</td>
<td>35</td>
<td>46</td>
</tr>
<tr>
<td>North</td>
<td>12</td>
<td>15.8</td>
</tr>
<tr>
<td>MVK</td>
<td>11</td>
<td>14.5</td>
</tr>
<tr>
<td>South</td>
<td>12</td>
<td>15.8</td>
</tr>
<tr>
<td>West</td>
<td>6</td>
<td>7.9</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>Married</td>
<td>56</td>
<td>73.7</td>
</tr>
<tr>
<td>Widow</td>
<td>15</td>
<td>19.7</td>
</tr>
<tr>
<td>Divorced</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>Medical insurance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Community based insurance</td>
<td>73</td>
<td>96.1</td>
</tr>
<tr>
<td>Premiums</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>No insurance</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>Level of study</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nil</td>
<td>31</td>
<td>40.8</td>
</tr>
<tr>
<td>Primary</td>
<td>34</td>
<td>44.7</td>
</tr>
<tr>
<td>Secondary</td>
<td>7</td>
<td>9.2</td>
</tr>
<tr>
<td>More than secondary</td>
<td>3</td>
<td>3.9</td>
</tr>
</tbody>
</table>
Among patients with gastric tumor, males were 55.3% and females were 44.7%. There was a preponderance increase of males. 60.5% were below 60 years, 39.5% > 60. The youngest was 25, the oldest was 76, and mean age was 55.6 years.

46% of our patients were from Eastern Province; roughly 15% were coming from North, South and Kigali City respectively. Only 8% came from Western Province. 76.3% was from rural area. 73.4% (56/75) were married; few were single or divorced 5.2% (4/75) and 19.7% were widow. 85.5% of patients had low level of education. 40.8% have not been at school, 44.7% have done only primary. 96.1% (73/75) were using community based health insurance.

### 3.3. Clinical characteristics of study population

#### Table 3. Risk Factors for Gastric cancer in our study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoked food</td>
<td>12</td>
<td>15.8</td>
</tr>
<tr>
<td>Family history</td>
<td>12</td>
<td>15.8</td>
</tr>
<tr>
<td>Tobacco smoke</td>
<td>35</td>
<td>46.1</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>53</td>
<td>69.7</td>
</tr>
<tr>
<td>Salted food</td>
<td>13</td>
<td>17.1</td>
</tr>
</tbody>
</table>

69.7% (53/74) have been drinking 46% (35/74) have smoked. Family history, Smoked and salted food had low frequency in our population 15.8% (12/74), 15.8% (12/74) & 17.1% (13/74) respectively.

#### Table 4. Physical findings of patients presenting for endoscopy

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle wasting</td>
<td>56</td>
<td>73.7</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>13</td>
<td>17.1</td>
</tr>
<tr>
<td>Pallor</td>
<td>23</td>
<td>30.3</td>
</tr>
<tr>
<td>Lymphadenopathies</td>
<td>3</td>
<td>3.9</td>
</tr>
<tr>
<td>Epigastric mass</td>
<td>22</td>
<td>28.9</td>
</tr>
<tr>
<td>Succussion splash</td>
<td>8</td>
<td>10.5</td>
</tr>
<tr>
<td>Melena/Hematochezia</td>
<td>6</td>
<td>7.9</td>
</tr>
</tbody>
</table>

The commonest physical findings were muscle wasting, epigastric mass and pallor with 73.7%, 30.3%, 28.9% respectively.
Table 5. Sign and symptoms of patients presenting to endoscopy

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heartburn</td>
<td>38</td>
<td>50.0</td>
</tr>
<tr>
<td>Regurgitation</td>
<td>29</td>
<td>38.2</td>
</tr>
<tr>
<td>Early postprandial epigastric pain</td>
<td>47</td>
<td>61.8</td>
</tr>
<tr>
<td>Late postprandial epigastric pain</td>
<td>19</td>
<td>25.0</td>
</tr>
<tr>
<td>Epigastric tumor</td>
<td>25</td>
<td>32.9</td>
</tr>
<tr>
<td>Bloating</td>
<td>25</td>
<td>32.9</td>
</tr>
<tr>
<td>Bloody vomitus</td>
<td>28</td>
<td>36.8</td>
</tr>
<tr>
<td>Black stool</td>
<td>15</td>
<td>19.7</td>
</tr>
<tr>
<td>Weight loss</td>
<td>68</td>
<td>89.5</td>
</tr>
<tr>
<td>Symptoms of anemia</td>
<td>30</td>
<td>39.5</td>
</tr>
<tr>
<td>Early satiety</td>
<td>20</td>
<td>26.3</td>
</tr>
<tr>
<td>Eructation</td>
<td>24</td>
<td>31.6</td>
</tr>
<tr>
<td>Vomiting</td>
<td>60</td>
<td>78.9</td>
</tr>
<tr>
<td>Hiccups</td>
<td>29</td>
<td>38.2</td>
</tr>
</tbody>
</table>

The three most common clinical presentations were weight loss 89.5%, vomiting 78.9%, and epigastric pain 61.8%.

Table 6. Findings on endoscopy

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of the tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardia</td>
<td>9</td>
<td>11.8</td>
</tr>
<tr>
<td>Fundus</td>
<td>4</td>
<td>5.3</td>
</tr>
<tr>
<td>Body</td>
<td>11</td>
<td>14.5</td>
</tr>
<tr>
<td>Antrum</td>
<td>32</td>
<td>42.1</td>
</tr>
<tr>
<td>Pyloric</td>
<td>4</td>
<td>5.3</td>
</tr>
<tr>
<td>whole stomach</td>
<td>11</td>
<td>14.5</td>
</tr>
<tr>
<td>Bleeding during gastroscopy</td>
<td>27</td>
<td>35.5</td>
</tr>
</tbody>
</table>

47.4% localized in the distal stomach (antrum and pylorus) and 18.3% in the proximal stomach. The whole stomach was invaded by cancer in 14.5%. 35.5% (27/76) were bleeding at the time of endoscopy.
### 3.4. Laboratory findings on gastric tumor specimens

Table 7. Prevalence of H.pylori, Lnc2 and UR vs GMD comparative histopathology of gastric cancer.

<table>
<thead>
<tr>
<th>Number</th>
<th>UR path</th>
<th>GMD path</th>
<th>H Pylori IHC</th>
<th>LCN2 IHC</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Inconclusive</td>
<td>Diffuse</td>
<td>NEG 3+</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Diffuse</td>
<td>Diffuse</td>
<td>POS 7+</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>Intestinal</td>
<td>Mixed</td>
<td>POS 5+</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Intestinal, well diff</td>
<td>Intestinal, Mod diff</td>
<td>NEG 6+</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Intestinal, well diff</td>
<td>Intestinal, Poorly diff</td>
<td>NEG 2+</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>Intestinal</td>
<td>Intestinal, poorly diff</td>
<td>NEG 5+</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>Gastritis</td>
<td>No tumor</td>
<td>NEG 0</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>Gastritis</td>
<td>Diffuse</td>
<td>POS 4+</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>No tumor</td>
<td>No tumor</td>
<td>NEG 3+</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>Gastritis</td>
<td>No tumor</td>
<td>POS 3+</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>Adenoca, NOS</td>
<td>Intestinal, Mod diff</td>
<td>NEG 4+</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>12</td>
<td>Intestinal, NOS</td>
<td>Intestinal, poorly diff</td>
<td>POS 6+</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>13</td>
<td>Adenoca, NOS</td>
<td>Intestinal, Poorly diff</td>
<td>NEG 5+</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>Adenoca</td>
<td>Intestinal, mod diff</td>
<td>NEG 1+</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>Adenoca, NOS</td>
<td>Mixed</td>
<td>NEG 4+</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>16</td>
<td>Diffuse</td>
<td>Diffuse</td>
<td>NEG 4+</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>17</td>
<td>Diffuse</td>
<td>Lymphoma</td>
<td>NEG 2+</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>18</td>
<td>Gastritis</td>
<td>Intestinal, poorly diff</td>
<td>NEG 3+</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>19</td>
<td>Intestinal, NOS</td>
<td>Intestinal, poorly diff</td>
<td>NEG 5+</td>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

78.9% pathology concordance: gastric malignancy vs benign
3.4.1. Histopathology, LCN2 and H Pylori findings in gastric cancer specimens

Malignancy 89.5%: Intestinal adenocarcinoma 47% Diffuse adenocarcinoma 21%, Mixed 10.5%, Lymphoma 10.5%

Figure 3: Pie chart of gastric cancers types in our study population according to Lauren classification

Table 8. Association between Age and type of gastric cancer

<table>
<thead>
<tr>
<th>Age group</th>
<th>Lauren classification</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No tumor</td>
<td>Intestinal</td>
</tr>
<tr>
<td>20-40 years</td>
<td>0 (0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>41-60 years</td>
<td>2(25%)</td>
<td>3(37.5%)</td>
</tr>
<tr>
<td>61-80 years</td>
<td>0(0%)</td>
<td>6(85.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>2(10.5%)</td>
<td>9(47.4%)</td>
</tr>
</tbody>
</table>

There was a statistically significant association between types of gastric cancer and age group. P<0.038

Relatively, there was an increase in diffuse type cancer in younger age and intestinal type in old age.
Table 9. Gender and Type of Gastric cancer

<table>
<thead>
<tr>
<th>Gender</th>
<th>Lauren classification</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No tumor</td>
<td>Intestinal</td>
</tr>
<tr>
<td>Male</td>
<td>1(11.1%)</td>
<td>5(55.6%)</td>
</tr>
<tr>
<td>Female</td>
<td>1(10%)</td>
<td>4(40%)</td>
</tr>
<tr>
<td>Total</td>
<td>2(10.5%)</td>
<td>9(47.4%)</td>
</tr>
</tbody>
</table>

Diffuse type were common in female 4/4, 40/%) and intestinal type are common in male (55.6%, 5/9) P=0.194

Table 10. Association of H pylori and gastric cancer type.

<table>
<thead>
<tr>
<th>Lauren classification</th>
<th>H. pylori pos</th>
<th>H. pylori neg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No tumor</td>
<td>1(20.0%)</td>
<td>1(7.1%)</td>
<td>2(10.5%)</td>
</tr>
<tr>
<td>Intestinal</td>
<td>2(40.0%)</td>
<td>7(50.0%)</td>
<td>9(47.4%)</td>
</tr>
<tr>
<td>Diffuse</td>
<td>1(20%)</td>
<td>3(21.4%)</td>
<td>4(21.1%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>1(20%)</td>
<td>1(7.1%)</td>
<td>2(10.5%)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>0(0%)</td>
<td>2(14.3%)</td>
<td>2(10.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>5(100%)</td>
<td>14(100%)</td>
<td>19(100.0%)</td>
</tr>
</tbody>
</table>

26.3 % (4/19) of gastric cancer were H pylori positive. There was no statistical significant association between H Pylori and types of gastric cancer.  P= 0.744
### Table 11. LCN2 and gastric tumor types.

<table>
<thead>
<tr>
<th>Lauren classification</th>
<th>No tumor</th>
<th>Intestinal</th>
<th>Diffuse</th>
<th>Mixed</th>
<th>Lymphoma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1+</td>
<td>1(50.0%)</td>
<td>1(50.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>2(100.0%)</td>
</tr>
<tr>
<td>2+</td>
<td>0(0.0%)</td>
<td>1(50.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>1(50.0)</td>
<td>2(100.0%)</td>
</tr>
<tr>
<td>3+ and above</td>
<td>1(6.7%)</td>
<td>7(46.7%)</td>
<td>4(26.6%)</td>
<td>2(13.3%)</td>
<td>1(6.7%)</td>
<td>15(100.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>2(10.5%)</td>
<td>9(47.4%)</td>
<td>4(21.1%)</td>
<td>2(10.5%)</td>
<td>2(10.5%)</td>
<td>19(100.0%)</td>
</tr>
</tbody>
</table>

There was a higher (3+above) LCN2 expression in gastric cancer types, even though not statistically significant \( P < 0.398 \).

### Table 12. Association of LCN2 expression, H. pylori IHC and presence of a gastric cancer.

<table>
<thead>
<tr>
<th>Lauren classification</th>
<th>No tumor</th>
<th>Tumor (cancer)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H. pylori +ve</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>0-1+</td>
<td>H. pylori –ve</td>
<td>1(50.0%)</td>
<td>1(50.0%)</td>
</tr>
<tr>
<td>2+</td>
<td>H. pylori +ve</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td></td>
<td>H. pylori –ve</td>
<td>0(0.0%)</td>
<td>2(100.0%)</td>
</tr>
<tr>
<td>3+ and above</td>
<td>H. pylori +ve</td>
<td>1(20.0%)</td>
<td>4(80.0%)</td>
</tr>
<tr>
<td></td>
<td>H. pylori –ve</td>
<td>0(0.0%)</td>
<td>10(100.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>2(10.5%)</td>
<td>17(89.5%)</td>
<td></td>
</tr>
</tbody>
</table>

Higher total LCN2 expression in samples + gastric cancer, H pylori + compared to H pylori neg. Not statistically significant. \( P < 0.744 \). Probably due to small sample size (n=19).
3.5. Targetable mutations in gastric cancer

Figure 4 shows the findings of 57 biopsies from which next generation sequencing was done for genetic alterations.

![Flowchart](image)

**Figure 4: Flowchart of molecular profiling by next generation sequencing of 57 gastric tumor specimens.**

It has been 12 mutations in 9 biopsies 41% (9/22) among the sequenced specimens, 15.7% (9/57) of the sample size.
Figure 5: Types of mutations found in 9 biopsies of gastric cancer by NGS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>4</td>
<td>50%</td>
</tr>
<tr>
<td>SAMD4</td>
<td>2</td>
<td>25%</td>
</tr>
<tr>
<td>ERBB4</td>
<td>2</td>
<td>25%</td>
</tr>
<tr>
<td>PTEN</td>
<td>2</td>
<td>25%</td>
</tr>
<tr>
<td>FBXW7</td>
<td>1</td>
<td>12.5%</td>
</tr>
<tr>
<td>KRAS</td>
<td>1</td>
<td>12.5%</td>
</tr>
</tbody>
</table>

TP53 was the commonest mutation (50%). The only targetable therapy mutation was PTEN 25%. 
3.6. Comparative data quality and quantity between the two groups (pre group vs. post group)

Figure 6: Chart of quality control findings in Pre Group

**Total: 57 samples**

- **22 samples**: passed qPCR. Sequenced and analyzed
  - **22 samples**: Archer Fusion Panel
    - **19 Failed quality check**
    - **3 Passed**

**Orange**: 57 samples

**Blue**: DNA Pillar Panel (only 22 were sequenced)

**Red**: RNA Archer Fusion Panel (the same 22 samples sequenced using DNA Pillar Panel). RNA concentration: 7.8-66.8 ng/µL. Quality check (PreSeq QC Kit from Archer): 19 samples failed. Two out of three samples were flagged (due to low quality), and only one sample showed good quality. None of them were sequenced
The majority of the samples were below 10 ng/μL. The red line shows that most of the samples below 50ng/μL were part of the “pre” group. However, most of the samples in the “post” group are below 10ng/μL.

Figure 8: Quality of DNA specimens in the 2 groups

1 Great quality DNA: genomic DNA band.
2 Low quality DNA: no genomic DNA band (wells 3-7): pre group
3 Low quality DNA: fragmented DNA (wells 8, 9 and 10): post group
Chapter 4. DISCUSSION OF THE FINDINGS

Gastric cancer is a disease of old age but it is not always the case. In this study, 15.8% (12/76) was less than 40 years the youngest was 25, the oldest was 76 years, and the mean age was 55.46 years which is not different from other areas with high prevalence. Pakistan is an example where 14.8% had age below 40 (Durrani et al. 2009), India, Kashmir 56% are between 50-60 years (Vol 2000). Gastric cancer tends to be in males than in females, males are 55.3% and females are 44.7% (Li et al. 1995). We find similarities of risk factors for gastric cancers in this study with other studies such as low social economic status, low level of education, tobacco, smoking, smoked & salted food and family history of gastric (Karpeh and Brennan 1998)(Chen et al. 2000).

Localization of gastric cancer proximal vs distal is paramount. Proximal cancers are less frequent and more aggressive. In this study, the majority of cancers were found in the distal stomach (antrum and pylorus) and few of them were in the proximal part of the stomach. This is consistent with results shown by Tajima et al. (2007) where 70.3% gastric cancers were localized in distal part of the stomach. The Brooklyn veterans study 1995–1999 (Vol 2000) 21% of gastric cancer were proximal, 29% mid-stomach, 25% distal stomach or extensive. We can assume that frequency of distal gastric cancer are related to fact that helicobacter pylori is commonly found in the same area (Timothy D. Walker et al. 2014) and have irreversibly damaged gastric mucosa leading to malignancy (Lechago and Ph 2000).

The selection of study participants was based on clinical and endoscopic suspicion of gastric cancer. Histology confirmed malignancy at 89.5%, similar to other studies. This study added the particularity to see the concordance of histology findings in two different laboratories 79.8%. In a study done in California pathologists agreed on the Lauren classification at 77% (Shibata, Longacre, and Puligandla 2001).

Intestinal adenocarcinoma (47%) is the most common followed by diffuse adenocarcinoma 21%. Other studies have found the similar pattern, even more wide difference in frequency 76%, 13% and others 11% respectively (Henson et al. 2004). The histopathology agreement was made only
on positive cancer biopsies as they were firstly confirmed by the CHUK pathologists. This might be a way to new study to evaluate the agreement between different laboratories where negative biopsies for cancer also should be considered.

Since 2 decades, lipocalin2 has become a surrogate marker of interest for clinical monitoring of tissue response to a variety of injuries or insults including cancers (Li and Chan 2011). Still it is a potential use for early detection, diagnosis and monitoring of disease progression (Bolignano et al. 2010). It has been shown to be highly expressed in malignancies, gastric cancer is an example (Wang et al. 2010). A Higher total LCN2 expression in samples of gastric cancer, H pylori positive compared with H pylori negative was found. This is identical to what has been seen in the quoted link.” Virchows Arch. 2009 Sep; 455(3):225-33: Neutrophil gelatinase-associated lipocalin (NGAL/Lcn2) is upregulated in gastric mucosa infected with Helicobacter pylori”.

Next generation sequencing done on 22 gastric cancer specimens found 12 mutations in 9 biopsies were found. Same findings in other studies were noted, with some mutations like TP53, KAS and so on (Tumour Biol. 2015 Sep; 36(10):7385-94). The most frequent type of mutations in this study is TP53 50% (Cancer 2015)(Cancer and Atlas 2014). The only mutation for targeted therapy found was PTEN 25%, currently no available PTEN targeted therapy in use for gastric cancer. Some molecules targeting PTEN mutations are under studies (Page 2016). PTEN inhibitors everolimus and temsirolimus have been shown in phase I trials to have xx response rates in some gastric cancers but numbers are small. Everolimus and temsirolimus are not approved by FDA for gastric cancer (Cancer 2015).

The quality and quantity of DNA in samples for sequencing has been low in 2 groups even though we tried to improve the technique in post group. However it has been possible to do Next Generation Sequencing. Some studies have showed a way to improve the quality and quantity of DNA and then success of Next Generation Sequencing (Goswami et al. 2016). A larger study to determine the incidence of PTEN mutations and a clinical trial on therapy of PTEN mutation in gastric cancer patients in Rwanda is warranted.
Chapter 5. CONCLUSION AND RECOMMENDATION

5.1. Study limitations
Small sample size for histology, LCN2 IHC & Pylori IHC studies. This has been done on 19 samples. Lack and poor quality of the equipment used to take biopsies and poor tissue preparation, causing insufficient tissue and DNA quality for next generation sequencing. This may be a source of sampling bias and other study with big sample may show other findings which are not shown here. However, this is a key to feasibility study between developing and developed countries.

This was a cross sectional study. It might have been limited by a recall bias due to lack of scrutinization of ideas from respondents especially in evaluation of risk factors for gastric cancer and clinical demographic characteristics of study population.

5.2. Conclusion
This study is a very big achievement in terms of gastric cancer exploration in Rwanda, a short overview from the demographic distribution pattern, risk factors, clinical presentation, and histopathology types to the genetic profile level of gastric cancer. This study showed the feasibility of Next Generation Sequencing on gastric specimen and a paucity of mutations in gastric cancer. PTEN mutation is a potential for targeted therapy.

5.3. Recommendation
Considering the results from this study our recommendation goes to what should be done next.

1. The requirements to meet the next generations sequencing testing success on gastric biopsies taken in Rwanda.

2. This study makes us curious to know what are the leading risk factors of gastric cancer in Rwanda.

3. There were no mutations in 59% (13/22) histologically confirmed gastric cancer. It is worthy to know which other types of molecular changes happened and caused cancer.
4. By doing fusion panel and amplification profiling may increase the yield of targetable molecular alterations.

5. What may be the outcome of trial of available targeted therapy for PTEN mutations?
REFERENCE


Page, Cover. 2016. TO : FROM : SUBJECT : ECOG-ACRIN Clinical Research Associates and Investigators with Patients for EAY131 ECOG-ACRIN Operations Office This Addendum Has Been Reviewed and Approved by the Central IRB Which Is the Sole IRB of Record for This Study . Local IR.


APPENDIX

QUESTIONNAIRE FOR TARGETED THERAPY AMONG GASTRIC CANCER PATIENTS AT CHUK

1. Questionnaire number: …………………
2. CHUK ID: …………………………
3. Telephone Number: ………………………

SECTION A: SOCIO-DEMOGRAPHIC CHARACTERISTICS

Q1. Age …… Years
Q2. Sex:  F / M
Q3. Address: Sector…………………District………………Province…………………
Q4. Residence: 1 Rural 2 Urban
Q5. Transferred from (Health facility): …………………………………………………………………
Q6. Maritus State: 1 Single 2 Married 3 Widow 4 Divorced
Q7. Level of education: 1 Nil 2 Primary 3 Secondary 4 More than 2ndary
Q8. Health insurance: 1 Community based insurance 2 Premiums 3 none
Q9. Gastric cancer risk factors: 1 smoked food 2 salted food 3 Alcohol use 4 tobacco smoking 5 family history of gastric cancer.

SECTION B: CLINICAL MANIFESTATIONS:

Q10. Chief complaint (name it): ………………………………………………………………………

Q11. Other symptoms (circle the number if yes)

1. Heartburn
2. Regurgitation
3. Early post-prandial epigastric pain
4. Late post-prandial epigastric pain
5. Epigastric pain radiating to the back
6. Epigastric tumor
7. Bloating (uncomfortable distension)
8. Hematemesis
9. Melena
10. Loss of weight,
11. Early satiety,
12. Eructation,
13. Vomiting,
14. Hiccups
15. Symptoms of anemia

Q12. Clinical Findings (circle if yes)
  1. Muscle wasting
  2. Tachycardia
  3. Pallor
  4. Lymphadenopathies
  5. Epigastric mass
  6. Succussion splash
  7. Melena/Hematochezia

SECTION C: GASTRIC TUMOR PROFILE

Q13 Tumor localization (circle if yes)
  1. Extending in the whole stomach
  2. Cardia
  3. non cardia
     a. Fundus
     b. Body
c. Antrum

d. Pyloric

Q14. Bleeding

Yes:

No:

Q15. MRU for H.pylori (cercle the answer)

1. Positive

2. Negative

Q16. Histopathology study results

1. Adenocarcinoma

2. MALT

3. Benign tumor

4. Other (to be precised)………………………..

SECTION C: MOLECULAR ANALYSIS AND TARGET THERAPY

Q17. Molecular profiling of gastric tumor result : List the targetable mutations for therapy

1.

2.

3.

4.

Q18. Molecular profiling of normal tissue result: List the targetable mutations for therapy

1.

2.

3.
BIOPSY CONSENT FORM

I,.............................................................., am aware that the medical team has recommended a biopsy to aid in the diagnosis and treatment of my illness. I have been advised by Dr..................................... regarding the biopsy, as follows:

It has been explained that a biopsy is a small piece of tissue that is removed from my body and will be tested in a laboratory. This tissue may come from my skin, bone, blood, urine, lymph node, any solid organ, or any other body part or bodily fluid as deemed necessary by the medical team to assist with diagnosis and treatment of my illness. I have been advised that the biopsy results will help determine the correct treatment for my illness, and help better understand the course of my illness. I understand that the risks of taking the biopsy include infection and bleeding. I understand that the results of my biopsy will be communicated to me and treatment options discussed at that point. Furthermore, I understand that the biopsy sample may be sent outside of Rwanda if it is deemed necessary by the medical team or the pathology laboratory which receives the sample. The biopsy will only be used for diagnostic purposes to aid in the treatment of my illness, and it will not be used for research purposes under any circumstances. I am aware that the results of the biopsy will be recorded and stored in keeping with standards and regulations for routine medical records. Research on these, and other biopsy results, would only be conducted in concordance with Rwandan laws, with the express written permission of the appropriate regulatory bodies or human research in Rwanda and internationally, and using only data that cannot identify individual patients.

I…………………………………. understand that I am undergoing a procedure called endoscopy to explore the lining of my stomach for abnormalities.

I agree to biopsies as needed to diagnose my problem, and to additional samples to be taken of normal stomach and suspicious areas, for future research purposes.

I understand risks of biopsy include bleeding, infection, perforation, need for surgery or hospitalization.

I understand that research samples will be stored at CHUK without personal identifying information, and may be transported to the Geisel School of Medicine at Dartmouth in Hanover, NH in USA for additional testing.

For any needed information or explanation about this research, fill free to contact Dr Eric RUTAGANDA, one of researchers, on +250 (0)788685432 or Mr SUNDAY Francois Xavier, the secretary of IRB/CMHS on +250 (0)788563312
CONSENT FOR ADULT PATIENT

I give permission that Dr. .............................. may perform a biopsy on me, under the above conditions. I understand the benefits and risks of having a biopsy performed, and have had all my questions answered.

**Patient**

Name.......................................................... Signature..........................................................
Date (dd/mm/yyyy)......../.............../..................

**Doctor**

Name.......................................................... Signature..........................................................
Date (dd/mm/yyyy)......../.............../..................

**Witness**

Name.......................................................... Signature..........................................................
Date (dd/mm/yyyy)......../.............../..................
MATERIAL TRANSFER AGREEMENT

This is an agreement made in order to protect material obtained from: KIGALI UNIVERSITY TEACHING HOSPITAL
between the Recipient (hereafter "RECIPIENT"), represented by RECIPIENT
SCIENTISTS:
1. Mary Chamberlin
2. Steve Bensen

and the Kigali University Teaching Hospital (hereafter “PROVIDER”), represented by
PROVIDER SCIENTISTS:
1. Vincent Dusabjambo
2. Eric Rutaganda

“Original Material”:
“Gastric biopsies from Patients with gastric tumor lesions”: The Original Material as
described, as well as all unmodified or modified derivatives.

Both parties agree as follows:

1. The Original Material is the sole property of the PROVIDER and is made
available as a service to the research community. The RECIPIENT shall have no
right in the Original Material other than as provided in this agreement. Ownership
of modifications and direct/indirect derivates of material, and income arising from
commercializing the direct/indirect derivates of material shall be negotiated in
good faith by the parties hereto depending upon (a) their relative contribution to
the creation of said modifications and derivates, and (b) applicable laws and
regulations relating to the inventor ship.

2. The Material will be used for research purposes only and will not be used for
commercial purposes or sublicensed to any third party unless another license is
obtained from the PROVIDER.

3. The Recipient agrees to provide the PROVIDER with a copy of any publication,
which contains experimental results obtained from the use of the Material. The
RECIPIENT guarantees that the PROVIDER will be part of the publication team.
The RECIPIENT shall acknowledge the PROVIDER as the source of the material
in all publications containing any data or information about the Material, unless
the PROVIDER indicates otherwise.
Accepted by:

Provider Scientists
Signature: [Signature]
Printed Name: Eric Rutaganda

Recipient Scientists:
Signature: [Signature]
Printed Name: Steve Bensen

Provider Institution Approval
Signature: [Signature]
Date: [Date]

Recipient Institution Approval
Signature: [Signature]
Date: [Date]

Institution:

Institution:

Chairman, Gastroenterology
Outreach with Cork Medical
ADDENDUM TO MATERIAL TRANSFER AGREEMENT

This is to certify the above mentioned material agreement transfer between Kigali University teaching Hospital (provider) and DHMC (receiver) consist about 2 cassettes for every patient: one pathologic and non-pathologic. After being processed, the cassette containing pathologic tissue biopsy will be brought back to Kigali University teaching Hospital, Laboratory Department.

Coordinator of the transfer procedures Dr. Oswald HABYARIMANA

Head of Anathomopathology department, CHUK Dr. Belson Rugwizangoga.

Dartmouth Medical School and Medical Oncology Dr. Mary Chamberlin