Symposium

RECENT ADVANCES IN THE MOLECULAR DIAGNOSIS OF COLORECTAL CANCER MAKE PERSONALISED TREATMENT POSSIBLE EVEN IN RESOURCE-LIMITED SETTINGS

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Abstract

Colorectal cancer is the fourth most common cancer in Nigeria with a significant proportion presenting at advanced stage partly due to non-availability of organized screening programs. The oncological challenge posed by colorectal cancer (CRC) is equally shared by rich and poor countries alike. Studies on colorectal cancer molecular heterogeneity have used genome-wide gene expression-based data to group patients into four Consensus Molecular Subtypes (CMS), through which patients can now benefit from personalized immunotherapy. The recent report of complete remission in patients with locally advanced mismatch repair deficient rectal cancer treated with immunotherapy (PDL-1 inhibitor) is a game changer for the treatment of the disease. However, the exorbitant cost and sophistication of genetic analysis has precluded poor countries from benefitting from this new knowledge. Recently however, it was shown that immunohistochemistry-based CMS classification and patient stratification is feasible and was used to sub-classify a cohort of CRC patients at the Lagos University Teaching Hospital. This cost-effective method is now available for use in other resource-limited settings as ours.

Keywords: Colorectal Cancer, Immunohistochemistry, CMS Subtyping, Immunotherapy

INTRODUCTION

Colorectal cancer is the 4th most common cancer accounting for 10% of all cancers worldwide and the 2nd most common cause of cancer death (9.4%). (GLOBOCAN, 2020).[1] The incidence is almost ten times higher in Western countries compared to developing countries. In Nigeria, colorectal cancer (CRC) is the 4th most common cancer accounting for 6% of all cancer cases, and coming after breast, prostate, and cervical cancer respectively. [1]

The incidence of CRC rose from 1.3million new cases in 2012 to 1.9million worldwide in 2020. [1] It was projected that global incidence will increase by 80% in 2035 with significant rise in the young and underdeveloped countries. [2] Increasing incidence have been reported in Nigeria especially in the young, and most patient present at advanced stage of the disease. Irabor et al, in 2009 reported a three-fold increase in incidence from the Ibadan cancer registry data to an average of 70 cases per annum between 2002 and 2006. [3] We reported, in a systematic review of 2497 cases reported in Nigerian literature over 53yrs (1954 to 2007), increasing number of colorectal cancer cases from 18.2 cases/annum in the early 50s to 86.8/annum in the later years (1991-2007) [4].

In the systematic review of the Nigerian literature, and similar to most studies from within and outside Africa, CRC was more common in males than female with a male to female ratio of 1.3 to 1. The peak age is in the 5th decade in Nigeria, with the mean ages in most studies ranging from 39 to 50.7years (average of 46.2years), and about a third of patients are less than 40years of age[4]. Most studies showed that the mean age of occurrence of CRC in Nigeria and most other parts of Africa is about a decade or more compared to what obtains in developed countries[4].

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CRC is the most common cancer of the gastrointestinal system. Of a total of 713 malignant tumours of the gastrointestinal system reported from Lagos and Sagamu in Southwest Nigeria, CRC was the most common, accounting for 56% of all cases. [5] A significant proportion (23%) was found in patients below 40 years. Patients below 40years had more tumours located in the right colon and there were more mucinous and signet ring carcinomas in them compared to those above 40years[5].

Risk factors for CRC

CRC is a multifactorial disease with multiple aetiology/risk factors including environmental and dietary factors, host inflammatory GI disorders and genetic factors. Some factors have been reported to be protective against CRC such as the use of NSAIDs (COX 2 inhibitors), intake of vegetables and physical activity. Inflammatory bowel diseases (IBD) such as ulcerative colitis and Crohn's disease have been associated with CRC.

Table 1: Familial Syndromes in CRC

Condition	Abnormal Genes	Lifetime cancer risks	Features
Hereditary non-polyposis coli (HNPCC) syndrome	DNA mismatch repair gene MLH1, MLSH2, MSH6, PMS2	50-80%	No polyps, microsatellite instability
Familial adenomatous polyposis (FAP) syndrome	APC gene mutation in on chromosome 5q21.	100% by age 40yrs	Multiple adenomatous polyps throughout the GIT (50-500 polyps)

IBD-induced CRC represents only about 1.2% of all cases of CRC but the mortality rate in such patients is higher compared to sporadic CRC. The risk increases with duration of the IBD and the anatomic extent of the disease. [6] Familial syndromes that are known to increase the risk of CRC are varied and some of these are shown in Table 1.

Environmental and dietary factors that have been associated with CRC are:

- High content of red meat and animal fat
- Low content of un-absorbable fiber in diet
- Low overall fruits and vegetable intake
- Low intake protective micronutrients such as vitamins C, D, E
- Alcohol and tobacco consumption
- Obesity/overweight
- Sedentary lifestyle

Classification of CRC

CRC is a heterogeneous disease consisting of diverse subtypes that have specific clinical, morphological, and molecular characteristics. It has been classified variously using different parameters, such as the location of the tumour, associated genetic and epigenetic abnormalities and whether it is hereditary or sporadic. Hereditary CRC accounts for 20-30%, examples are Lynch syndrome and Familial Polyposis syndrome. Sporadic CRC accounts for 70-80% of all cases, occurring without any identifiable underlying disease. A small proportion of cases results from the complication of Inflammatory bowel syndrome. Based on location of the tumour, CRC is classified as right-sided or left-sided colon cancer.

Table 2: Differences between Right and Left Sided CRC (Baran et al 2018) [8]

Right Sided CRC	Left sided CRC
Mucinous adenocarcinomas, sessile serrated adenomas	Tubular, villous adenocarcinomas
Flat like morphology	Polypoid like morphology
MSI-high and mismatch repair deficient	Chromosomal instability tumours
Highly immunogenic, high T cell infiltration	Low immunogenic
Metastasis in peritoneal region	Liver and lung metastases
Occur in older ages	Occur in younger ages
Predominantly occur in females	Predominantly occurs in males
Better prognosis at early stages (stage I & II)	Better prognosis at late stages (stage II & IV)
Respond well to immunotherapy	Respond well to adjuvant chemotherapies including standard chemo and targeted therapies

Right-sided CRC are those located in the caecum, ascending colon, and transverse colon, while left-sided CRC are cancers located in the splenic flexure colon, descending colon, sigmoid colon, and rectum and these exhibit different clinicopathologic characteristics [7].

CRC exhibits different clinical, histological, and molecular characteristics based the anatomical location of the tumor. [7-9] Left-sided cancer tends to occur more in males while right-sided cancer is commoner in females. Right-sided colon cancer tends to have more advanced tumour stage, a higher risk of peritoneal metastasis, and a poorer outcome than LCRC in a study of 1503 patients [7]. Microsatellite instability (MSI)-high, CpG island methylator phenotype (CIMP)-high and BRAF mutation are more often seen in right - sided colon cancer while chromosomal instability is observed in left-sided tumour. Adenomatous polyposis, KRAS mutation and p53 are also more often associated with left-sided cancer. Other differences are detailed in Table 2. Gender-specific disparities have also been suggested, as women tend to have a higher risk of

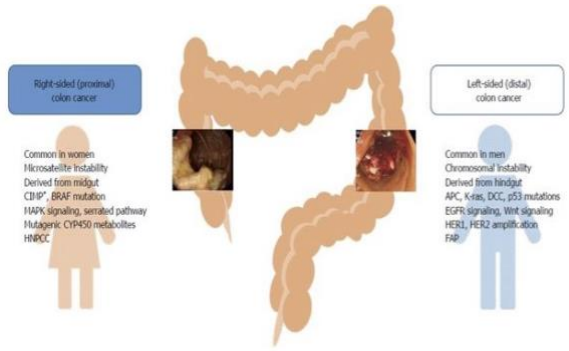

Figure 1: Differences between Right- and Left Sided CRC, (Kim SE, et al, 2015)[10]

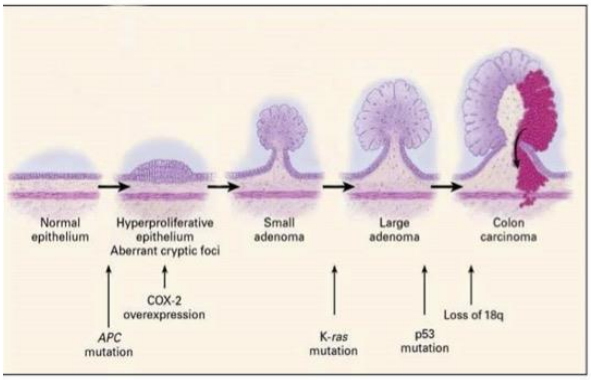

Figure 2: Adenoma-Carcinoma Progression Sequence Table

developing right-sided (proximal) colon cancer than men, which is associated with poorer prognosis [10]. These authors assert that understanding the sex- and gender-related biological and socio-cultural disparities in colorectal cancer risk, specific strategies can be produced for screening, treatment and prevention in order to reduce the mortality and improve the quality of life[8,9] (Figure 1).

Pathogenetic Pathways and Molecular Subtyping of CRC

Colorectal cancer is not a single disease but a collection of multiple diseases. It is a highly heterogenous and dynamic disease with multiple genetic and epigenetic alterations underlying its pathogenesis. The different subtypes have distinct clinical, morphological, and molecular characteristics which explain the differences in disease outcomes and response to therapy. This heterogeneity in tumour biology, response to therapy and prognosis, hindered a clinically relevant classification but has motivated efforts towards the search for a molecular classification that best categorizes these tumours into clinically relevant and prognostically significant subtypes.

In CRC, the transformation of colonic mucosa into invasive cancer occurs through an accumulated somatic or inherited changes within the genome and epigenome, so-called multi-hit, and multi-step phenomenon in which several mutations occur in multiple genes following exposure to multiple hits of environmental and dietary risk factors. This is aptly depicted by the 'adenoma-carcinoma sequence' in which there is progressive evolving of invasive carcinoma, over 10-15years, from the earliest lesion of micro-adenoma as a result of genetic and epigenetic changes (figure 2). [11,12] There are three recognised pathogenetic pathways of CRC; Chromosomal instability (CIN), Hypermutated (microsatellite instability MSI) and CpG island methylated (CIMP) or serrated pathway (Table 3) [13,14].

Differences between the Three Pathogenetic Pathways in CRC (Table 3)

Chromosomal instability means an accelerated rate of gains or losses of whole or large portions of chromosomes whole or large portions of chromosomes resulting in variable karyotypes within the cells. The CIN tumours are those that are associated with oncogene activation (KRAS, PIK3CA) and tumour suppressor gene inactivation (e.g., APC, SMAD4, TP53, WNT). They tend to be left-sided, have well differentiated histology, insignificant lymphocyte infiltration and worse prognosis (after adjustment for stage). [13,14]

MSI tumours have defective DNA mismatch repair system (MMR) with widespread microsatellite Instability, tend to be right sided, have poorly differentiated histology, significant lymphocytic infiltration and have better prognosis (after adjustment for stage). Microsatellites are short repetitive DNA sequences scattered throughout the genome and are prone to frequent mutations and mismatch. [13,14]

The MMR system consists of six enzymes (MLH1, MLH3, MSH2, MSH3, MSH6, PMS1, and PMS2) that detect and repair DNA., thus defect in the MMR system causes inadequate DNA repair, with attendant high degree of DNA replication errors, shortening or lengthening of these microsatellites.

Table 3. Differences between the Three Pathogenetic Pathways in CRC

Chromosomal instability (CIN)	Hypermutated (microsatellite instability (MSI)	Serrated pathways Tumours
Oncogene activation (KRAS, PIK3CA) Tumour suppressor gene inactivation (e.g., APC, SMAD4, p53) Early abnormalities of WNT pathway	Defective DNA mismatch repair (MMR). MSH2 or MLH1, MSH6, PMS2 CIMP, BRAF	Overlapping with CIN and MSI Mutation in KRAS or BRAF→hyperactivation of MAP kinase pathway
Sporadic-80% Hereditary- FAP-<1%	Sporadic-15% Hereditary-Lynch syndrome 3-4%	10-20%
Adenoma→Carcinoma sequence Classical adenocarcinoma Distal Bowel	Poorly differentiated tumour Tumour infiltrating lymphocytes Proximal bowel Better prognosis for early cancer, resistance to chemotherapy	Premalignant lesions- Traditional serrated adenomas (TSA) and Sessile serrated adenomas/polyps Poor prognosis and therapy Resistance

Table 4 Clinicopathologic Parameters and CMS status of Colorectal Cancer Cases*[24]

Clinicopathologic parameter	No. Cases (n, %)	CMS1	CMS2	CMS3	CMS4	p-value (Fisher's exact)
Age (years)						**0.0001**
<40	27(38%)	1(7.1%)	6(27.3%)	1(7.7%)	19(86.4%)	
≥40	44(62%)	13(92.9%)	16(72.7%)	12(92.3%)	3(13.6%)	
Sex						0.210
Male	45(60%)	7(43.8%)	17(70.8%)	6(46.2%)	15(68.2%)	
Female	30(40%)	9(56.3%)	7(29.2%)	7(53.9%)	7(31.8%)	
Tumor Site						**0.0001**
RCC	17(23%)	12(80.0%)	2(8.3%)	0(0)	3(13.6%)	
LCC	57(77%)	3(20.0%)	22(91.7%)	13(100%)	19(86.4%)	
Histologic Diagnosis						0.608
Well differentiated	45(60%)	11(68.8%)	13(54.2%)	7(53.9%)	14(63.6%)	
Moderately differentiated	17(22.7%)	3(18.8%)	6(25.0%)	3(23.1%)	5(22.7%)	
Poorly differentiated	6(8%)	1(6.2%)	4(16.7%)	0(0)	1(4.6%)	
Undifferentiated	2(2.7%)	0(0)	0(0)	2(15.4%)	0(0)	
Mucinous carcinoma	3(4%)	0(0)	1(4.2%)	1(7.7%)	1(4.6%)	
Signet ring cell carcinoma	2(2.7%)	1(6.2%)	0(0)	0(0)	1(4.6%)	

*Totals for analyses are inconsistent due to missing data

The third pathway is CpG island methylated or serrated pathway which exhibits gene silencing due to hypermethylation of CpG islands, and has overlapping features of CIN and MSI. It is characterized by widespread CpG islands methylation (CIMP) in promotor regions, resulting in inactivation of tumour suppressor genes such as MLH1. It is associated with premalignant lesions such traditional serrated adenomas (TSA) and sessile serrated adenomas/polyps. The tumor tends to have poor prognosis. [13,14]

The Consensus Molecular Subtyping of CRC

The Tumour Cancer Genome Atlas network (TCGA) proposed a molecular classification using array- based and sequencing technologies utilizing

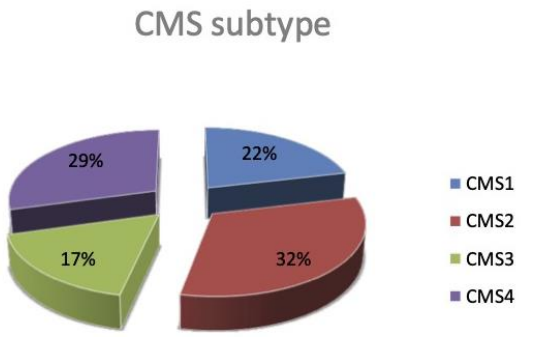

Figure 3-Frequencies of Consensus Molecular Subtypes of Colorectal Cancer. [24]

genomic and transcriptomic characterization in 2012. [15]. TCGA defined three subtypes: hypermutated (13%), ultra mutated (3%) and CIN (84%). There were several other classification systems that were proposed by different groups. Application of this gene expression-based subtype classification approach was associated with inconsistencies; thus, in 2015, an international consortium of experts was formed which produced four consensus molecular subtypes, reported to be more robust and has better biological interpretation and clinical relevance[16]. This classification is based on gene expression profiling of CRCs after coalescing information from six CRC gene expression datasets. The four CMS subtypes are biologically distinct, have different clinical courses, and prognostic significance for patient management.

CMS1 (MSI-immune; 14%), is the first among these subtypes. These are hypermutated tumours with microsatellite instability and strong immune activation (PD1 activation, NK cell, Th1 cell and cytotoxic T cell infiltration signatures). In addition, they frequently show BRAF mutations and have low single copy number alterations (SCNAs). The high immune response makes CMS1 to better respond to immune checkpoint inhibitors. A response rate of 40% was demonstrated in a phase 2 clinical trial using pembrolizumab (PD-1 inhibitor) in metastatic MMR deficient CRC, and no response in MMR proficient tumours. MMR deficient (MSI-H) CRC patients have poor prognosis and are less responsive to conventional chemotherapy[14-17]

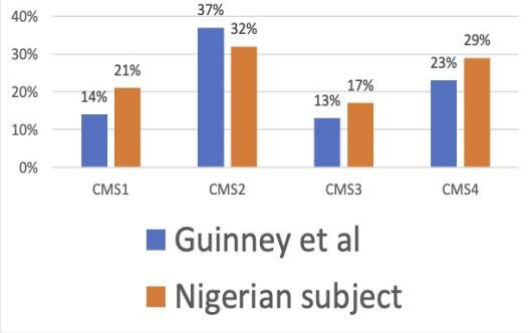

Figure 4. Colorectal Cancer Molecular Subtypes by Immunohistochemistry in a Patient-Cohort of seventy-five cases from Nigeria (compared to Guiney et al[16]).[24]

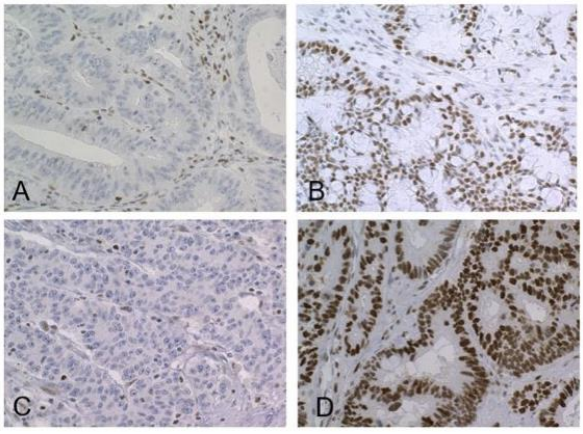

Figure 5. Immunohistochemical staining of colorectal cancer with MMR markers. Magnification x20. A. Negative immunohistochemical staining of colorectal cancer with MLH1. Lymphocytes serves as internal positive control. B. Positive staining with MLH1. C. Negative staining with MSH2. D. Positive staining with MSH2.[24]

CMS2 (Canonical, 37%), consists of tumours that exhibit epithelial signatures with increased WNT and MYC signalling activation. They harbor loss of tumour suppressor genes and show mutations in oncogenes and associated with better survival after relapse. Patients with CMS2 tumours do not harbor *BRAF* or *RAS* mutations and are thus more likely to benefit from anti-EGFR therapies [14-17].

CMS3 subtype (metabolic, 13%), are epithelial tumours with evidence of metabolic dysregulation. They frequently harbor RAS, *PIK3CA*, and *PTEN* mutations, which confer resistance to anti-EGFR therapy. They may however benefit from other agents due to increased activity in several metabolic pathways such as glycolysis, glycogen synthase kinase, and amino acid metabolic pathways [14-17].

CMS4 (Mesenchymal, 23%), tumours show increased expression of epithelial-mesenchymal transition (EMT) genes and prominent transforming growth factor-beta activation, angiogenesis, and stromal invasion. Patient may therefore benefit from agents that inhibit the TGF-β signalling pathway and inhibitors of angiogenesis. CMS4 tumours tend to display worse overall and relapse free survival rates. [14-17]The consensus molecular subtyping of CRC currently represents the best attempt at a clinically relevant molecular classification of colorectal cancers as it captures the highly heterogeneous nature of this group of neoplasms. The classification has over the past few years become a validated prognostic tool in the diagnosis and management of CRC. [18] Despite the novelty of this method, it has been difficult to adopt it in routine pathology practice because the original classification is based on gene expression profiling, requires a cumbersome genetic testing procedure, and prohibitive cost of reagents and materials. [18] These challenges make the translation of the classification for routine patient's management impracticable especially in resource limited countries such as Nigeria. Efforts towards ameliorating these challenges have been fruitful as recent advances in molecular cancer diagnostics have led to the development of a protocol that combines MSI testing; by PCR or immunohistochemistry with an immunohistochemical assay for four other markers and an online tool that is capable of accurately classifying CRC patients into the four major CMS groups. [18,10]

This method, called IHC-CMS classifier uses immunohistochemistry to detect antibodies against mismatch repair (MMR) proteins (MLH1 and MSH2) and cases with high-levels MSI considered unstable, are designated CMS1 (MSI). Other cases are assigned either as "epithelial" (CMS2/CMS3) or "mesenchymal" (CMS4) subtypes by staining for the protein products of four genes (i.e., CDX2, FRMD6, HTR2B, and ZEB1). CDX2 is a transcription factor in intestinal epithelial cells, which is expected to be highly expressed in epithelial-like tumours; HTR2B is a serotonin receptor with high expression in actin skeleton that is expressed in colon glandular cells and has a higher expression in mesenchymal-like mesenchymal like tumours; FRMD6 is an adaptor protein linking plasma membrane associated proteins to tumours, while ZEB1 is an indicator for epithelial-mesenchymal transition (EMT). KER is a pan cytokeratin marker that is used to normalize the other markers for tumour content. [16] A semi-quantitative pathologic scoring system is used, which records the percentage of cells stained and the intensity of the immunohistochemical stain. Except for the limitation of not being able to clearly discriminate between CMS2 and CMS3 tumours, this protocol can improve the clinical utilization of the CMS status. [19]

Table 5.-Frequencies MSI-positive Colorectal Cancers Detected by Different Techniques Reported in Different Studies in Nigeria and Ghana[24]

Author/year	Study site	Detection method	Sample Size	MSI Rate
Duduyemi et al, 2013	Ibadan	IHC-2 markers	26	**23%**
Adegoke et al, 2017	Ile-Ife	IHC, two markers	55	**34.5%**
Irabor et al 2017	Ibadan	Genetic testing, ten markers	35	**43%**
Aminu et al, 2017	Kano	IHC-2 markers	53	**53%**
Raskin et al, 2013	Ghana	Genetic testing, ten markers	70	**41%**
Abdulkareem et al, (2022	Lagos	IHC, four markers	75	**21%**

Alatise et al, utilized a combination of immunohistochemistry and genetic sequencing techniques to carry out a genetic/molecular profiling of colorectal cancers among a cohort of Nigerian patients [20]. However, that study was not aimed at subtyping CRCs according to the CMS classification.

IHC-Based Consensus Molecular Subtyping of CRC In a Cohort of Nigerian Cases

IHC-based CMS classifiers were recently developed to bring this molecular classification to bear on patient diagnosis, management, and prognostication of colorectal cancers. Good concordance has been reported between the transcriptome-based profiling of CRC and the IHC-based profiling for the purpose of CMS classification. Trinh et al reported a high concordance rate of 87%.[21] This is a good development as recent reports have shown that CMS subtype is an independent prognostic factor in individuals with metastatic CRC who receive first-line therapy; and it can guide in the selection of patients who may benefit from anti-VEGF and anti-EGFR therapy. [22,23] Due to the reported benefit of consensus subtyping of CRC in patient management, we sought to utilize immunohistochemistry in determining the CMS status of CRC diagnosed in Lagos.

We carried out a study to classify CRC into the four main CMS groups using immunohistochemistry on archival formalin-fixed paraffin embedded tissue blocks of seventy-five patients diagnosed with CRC[24]. Tissue microarrays were constructed from the tissue blocks of CRC, these were stained for mismatch repair (MMR) proteins (MLH1, MSH2, MSH6, and PMS2) and four other markers (CDX2, HTR2B, ZEB1, and Ki-6) by IHC. Semi-quantitative scoring was performed for the other four markers. A panel of CDX2, HTR2B, and ZEB1 was then used to distinguish between CMS4 and CMS2/CMS3 subtypes, whereas Ki-67 was used to separate CMS2 from CMS3 subtype. MMR status was used to identify CMS1 subtype (Figure 5). Associations between CMS categories and categorical demographic and tumour characteristics were analysed while HTR2B and Ki67 were compared between CMS group. *P- values* $\leq$ 0.05 were considered statistically significant [24].

Characteristics of cases according to CMS status and Clinical Implications

As shown in Table 4, of the total evaluable 75 CRC cases, 38% were <40 years old, 60% were males, with mean of 44.8 years (SD = 16.1). Fifty-nine patients (79%) had MSS, and the remaining 16 (21%) had MSI (i.e., CMS1). Thirty-seven (49%) were classified as CMS2 (n=24) or CMS3 (n=13) and 22 (29%) of the cases were classified as CMS 4 (Figure 3). The CMS4 subtype was significantly more likely to occur among young patients (p<0.001). CMS1 subtype was more in patients older than 40 years and 75% of right-sided cancers were CMS1 (p<0.001) [24]

The proportions of the various subtypes identified in our study (21% of CRC cases as CMS1, 32% as CMS2, 17% as CMS3 and 29% as CMS4, respectively) concur with data obtained using the transcriptome-based gene profiling technique except for CMS1, which according to the traditional classification accounted for about 14% of cases but our study found a proportion of 21% (Figure 4).

This further supports the findings in earlier studies that a significant proportion of CRC in Nigeria are associated with microsatellite instability (MSI) [20,25,26]. Studies have indicated that there is higher proportion of MSI tumours in the Black populations. Ashktorab et al recorded 43% proportion of MSI tumours in African Americans compared to <20% in the general population in 2005. [27] However, a meta-analysis of 22 studies within the USA about 10years after, reported the overall rate of MSI in all the studies analysed was 17%.[28] Several small IHC-based study cohorts from Nigeria showed rates ranging from 23% to 53% while the Ghanaian study which is based on genetic testing of 10 markers reported 43%. [26,29,30] A more recent study on molecular and phenotypic profiling of CRC in West Africa by Alatise et al reported 28.1% of the 64 Nigerian specimens that underwent MSK-IMPACT, to be MSI-high and 21.3% (20 of 94) by immunohistochemistry, compared to 7.2% (7 of 97) African American in the cases from MSKCC. [20] All of these clearly show that CMS 1 tumours constitute a significant proportion in Nigerian CRC (Table 5).

CMS1 subtype of colorectal cancers has a predilection for the proximal colon while CMS2 has a predilection for the distal colon or rectum according to observations in other studies. [31] Our study showed a significant association between tumour site and CMS status, with 71% (12/17) of all right sided tumours identified as CMS1, which corroborates the finding by Alatise et al in which 66.7% of all MSI- H cases in their cohort were located on the right [20]. CMS1 cases equates to the MSI-positive cases, which according to extant characterization are known to mostly have a proximal colon location and harbor significant tumours infiltrating lymphocytes and may benefit from immune checkpoint inhibitors. [14-17].

Recently, complete complete remission in mismatch repair deficient locally advanced rectal cancer was reported in 18 patients after using a newly approved drug, dostarlimab, an immune-check point inhibitor for six months. [32] With this, a new paradigm of treatment has been established and Nigerian patients should not be excluded.

Many cases of CRC involving the left side were CMS2, about 39% (22/57) and distal location equally predominates for CMS3 and CMS4. This closely mirrors the report of Alatise et al who reported a high frequency of MSS tumours in left-sided CRC cases. [20] Overall, left sided CRC are the most common and the present study does not differ on this finding as 77% of the cases investigated involved the distal colon.

We observed that greater than one third of the cases evaluated are younger than 40 years which concurs with the observations of increasing incidence of CRC among young patients[1]. Sixty three percent of the samples investigated were younger than 50 years old and this agrees with previous studies from Nigeria[3,4] We recorded 32.2% of 2497 cases in the systematic review of CRC in Nigeria to occur under 40years. [4] The mean ages in most studies ranged from 39 to 50.7years with average of 46.2years. [4] The same trend has been reported in developed countries which has brought about renewed efforts on investigating factors responsible for the rise in early onset CRC, with a view to lowering the age of screening initiation to 45 years. Several factors including changing diet, excess body weight and other lifestyle factors have been suggested to be responsible for this rise. [1]

Our study found that CMS subtype significantly differed in terms of age distribution. About 93% of CMS1 cases were aged 40 years and above and 86% of cases designated CMS4 were younger than 40 years. This finding agrees with the finding of Inamura and colleagues, who described CMS1 as adult- onset cancer[31] but contrasts with those of Willauer et al, who reported a higher proportion of CMS1 in patients younger than forty and observed that

CMS3 and CMS4 were less common below 40 years. [33]

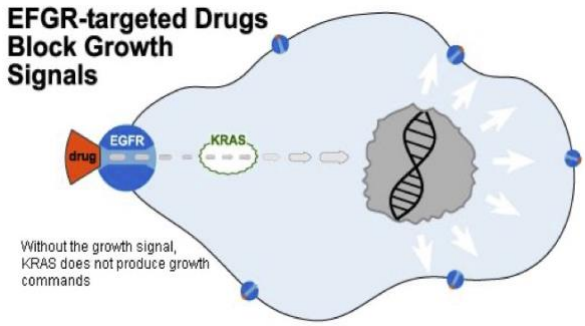

Figure 6: Role of KRAS in EGFR-targeted Therapy

It appears that CMS status has no significant association with gender, and histologic type of CRC in Nigeria. For instance, CMS1 tumours have been reported to be more prevalent in females, [31] however no significant difference was observed in our study. Similarly, Alatise et al reported no significance association with regards to age and gender of patients with MSI-H tumours in Nigeria. [20] Li et al in their study of 165 CRCs equally reported no significant relationship between CMS status and gender, as well as CMS status and histologic type or degree of tumour differentiation. [14] It therefore appears that no differences exist between the subtypes and extent of tumour differentiation. Larger series are required to further investigate this association.

Unlike the previous study by Trinh et al in 2017, [21] in our study, CMS2 and CMS3 were grouped as separate entities by utilizing Ki67 to discriminate between the two epithelial like subtypes. This is an improvement on the limitation of similar studies conducted by other authors in the past. Although CMS2 and CMS3 are known to have similar prognosis, it is important to separate the subtype because of differences that exist due to major metabolic dysregulation in CMS3.

CMS3 are known to have KRAS mutation. *KRAS* proto-oncogene codes for K-Ras G-protein which is located downstream of Epidermal Growth Factor Receptor-a transmembrane receptor (EGFR); an essential component of EGFR signalling cascade. This pathway is the basis of the use of anti-EGFR inhibitors (such as (cetuximab or panitumumab) for the treatment of CRC associated with EGFR over-expression (Figure 5). It is known that in the presence of a *KRAS* mutation particularly in exon 2, the inhibition of this pathway by EGFR inhibitors becomes ineffective. The result of our study in Nigerian colorectal patients showed KRAS mutation rate of 21% compared to higher rate of 41% in Caucasians ($p<0.0001$), while the Ghanaian study reported 32%.[34,35] The implication of lower rates of KRAS mutation implies that Nigerian patients are more likely to benefit from immunotherapy with anti-EGFR inhibitors.

CONCLUSION

Advancement in the diagnosis of colorectal cancer, now divided into four molecular subtypes has resulted in paradigm shift in the treatment modalities as patients now benefit from personalized immunotherapy. There is a dearth of studies on molecular profiling of CRC in Africa but a few studies available indicate that MSI cancer (CMS1) represent a significant proportion thus patients can benefit from PDL-1 inhibitor. KRAS mutation, which is commonly found in CMS3, has lower rate in Nigeria, implying that patients will benefit from the use of anti-EGFR inhibitor therapy such as cetuximab and panitumumab treatment. Also, EGFR is overexpressed in CMS2 cancers and are likely to respond to anti-EGFR therapy. Similar to studies from other parts of the world, our study has shown that molecular subtyping of CRC using immunohistochemistry, in our setting is feasible, cost-effective for prognostication and stratification of patients for the purpose of treatment.

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