Microsatellite Instability of *BAT-26* and *NR-24* among Breast Cancer Patients in Senegal

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Abstract: *Microsatellite instability high (MSI-H) tumours are known to have better prognosis than microsatellite stable (MSS) tumours and diagnostic characterization of these tumours reveal differences in clinical, pathological and molecular characteristics.Differentpopulations have variable results for MSI tumours due to varied environmental factors that influencediverse outcomes. Therefore, with the successes in the exploration of immunotherapy in cancers and currently in breast cancer, this study aims to evaluate the microsatellite phenotypes of breast cancer patients in Senegal to better understand the pattern of tumour progression and design therapies for improved treatment outcome. Sixty-five breast tumours were genotyped for microsatellite instability of BAT-26 and NR-24 markers.The results showed MSI in more than 50% of the tumours with variant alleles of 92.5% and 81.4% in BAT-26 and NR-24 respectively. Statistical significance (p-value = 0.041) was observed for SBR grade between MSS and MSI tumours.This study shows the MSI phenotype of BAT-26 and NR-24 markers among breast cancer patients in Senegal with a positive correlation between MSI and SBR grade in NR-24 marker. We propose thatNR-24 whichoften shows little or no variation among healthy individuals in different populations may be a marker of interest in the determination of MSI status as a prognostic factor among breast cancer patients in Senegal.*

I.   Introduction

Globally, female breast cancer (FBC) ranks second (11.6%) and fifth (6.6%) in terms of incidence and death respectively. Additionally, FBC along with lung and colorectal cancersare responsible for one third of the burden of cancer incidence and death (Bray, 2018).In Africa,it ranks fifth in incidence and five-year prevalence, as well asthird in mortality.Likewise, in Senegal, FBC is second to cervical cancer in number of new cases (18.97%) and deaths (12.46%) (Ferlay *et al.*, 2018).

Being a multifactorial disease that results from the association between various genetic, environmental, hormonal and lifestyle factors, breast cancer development involves a multistep process that is associated with various genetic alterations(Mehrgou and Akouchekian, 2016).Only about 5– 10 percent of breast cancer have clearly defined genetic predispositions while about 90 – 95 percent of the disease have no defined germline mutation butmay develop through epigenetic inactivation or acquired mutations that may arise from a defective mismatch repair (dMMR) system (Walsh *et* al., 2006; Chacon and Costanzo, 2010; Guler*et al*., 2011; Prat and Perou, 2011).

The mismatch repair (MMR) system maintains the integrity of the genome by correcting accumulated mutations that occur as a result of DNA polymerase slippage errors during DNA replication (Levinson and Gutman, 1987; Ellegren, 2000; Scarpa *et al.*, 2016).The MMR system in humans is regulated by MutSα(a heterodimer complex of *hMSH2*and*hMSH6*that binds to base-base mismatches and the loops of one or several nucleotides) and MutSβ(a MutS-related complex composed of the heterodimers *hMSH2*and *hMSH3*that repairs the heteroduplexes with two or several extrahelical bases (Acharya *et al.,* 1996; Genschel*et al.,* 1998).Together, MutSα and MutSβ then binds to MutLα (a heterodimer of MutL-homologues *hMLH1* and *hPMS2*), excising the DNA strand with the mismatch (Buermeyer*et al.,* 1999; Kolodner and Marsischky, 1999; Jiricny, 2000).The inactivation of any of these genes due to mutation results in dMMR leading to an increased rate of mutation especially in the microsatellite regionsand this is referred to as microsatellite instability (MSI) (Modrich and Lahue, 1996; Geurts-Giele*et* al., 2014; Joly *et* al., 2015; Richman, 2015).

The microsatellites region consists of tandem nucleotide repeat motifs of 1 to 6 bp and are interspersed throughout the eukaryotic genome in the coding and non-coding regions of the DNA (Subramanian *et* al., 2003; Cuadrado *et al.,* 2008; Richman, 2015). They are considered to be mutational hot-spots because ofthe high level of polymorphisms observedamong individuals and populations and therefore are widely used as molecular markers in evolutionary studies, genetic mapping, bone marrow engraftment monitoring and

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DNAfingerprinting (Lee *et* al., 2001; Schlotterer and Har, 2001; De Bustos *et al.*, 2016). Although, microsatellites are stable in each individual, their mutation rates are significantly elevated not only in thedMMR system, but also in tumour suppressor genes resulting in instability of the microsatellite repeats (Imai and Yamamoto, 2008; Shia, 2015).MSIhas been identified in about 10% - 15% ofcommon human tumours such as colorectal, gastric, stomach and endometrium (Boland *et* al., 1998; Hamelin *et al.*, 2008; Zhu *et al.*, 2015; Ratti*et al.*, 2018).The slippage errors that occur during DNA replicationoften lead to frameshift mutationsin target genes andencourage the growth of tumour cells. These drivethe process of carcinogenesis in microsatellite instability high (MSI-H) tumoursand are more likely to occur in long runs of G:C than A:Trepeats (Sagher *et al., 1999*). The evaluation of MSI in tumours has proven to be an efficient tool for the prognosis of various cancers and they are more sensitive in mononucleotide repeats than dinucleotide repeats (Imai and Yamamoto, 2008). Thus, there isthe need to utilize poly-T or poly-Amononucleotides microsatellite markers for the analysis of MSI phenotype in tumour cells (Ionov*et al.*, 1993; Boland *et al.*, 1998).

The evaluation of MSI is standardized in some cancers especially in hereditary non-polypopsis colorectal cancers (HNPCC)in which MSI-H tumours are known to have better prognosis than microsatellite stable (MSS) tumours (Popat*et al., 2005*; Shokal and Sharma, 2012). These tumours exhibit specific pattern of gene expression in their phenotype while their diagnostic characterization reveal differences in clinical, pathological and molecular characteristics(Abida*et al.*, 2018). Therefore, it is necessary toanalyze different tumours in diverse populations with the vast demographic differences from varied geographical locations. MSI has been reported in various frequencies of between 0 – 33% in patients with breast canceralthough the differences observed may be due to the non-uniform selection of microsatellite markers, samples, and the use of different criteria for analysis (De Marchis *et* al., 1997; Arzimanoglou*et al.*, 1998; Anbazhagan*et al., 1999*; Dudley *et al., 2016*).

After its discovery in I993, several highly variable markers were initially used for the evaluation of MSI but to have a consensus and facilitate comparison between studies, the NCI in 1997 proposed a panel of two mononucleotides (*BAT-25* and *BAT-26*) and three dinucleotides (*D5S346, D2S123* and *D17S250*) repeats (Ionov*et al.*, 1993; Boland *et al.*, 1998; Akiyama *et* al., 1997; Perucho, 1999). However, apart from the highly polymorphic nature of dinucleotide repeats requiring the use of corresponding germline DNA (which can be expensive and time consuming), there have also been evidence of misclassification of MSI-H tumours when using dinucleotide repeats or the Bethesda panel (Hoang *et al.*, 1997; Loukola*et al.*, 2001; Suraweera*et al., 2002*).Hence, to circumvent these challenges, an optimized pentaplex panel of five quasimonomorphic mononucleotide repeats has been opined to be more sensitive and specific for the detection of MSI-H tumours (Suraweera*et al.*, 2002; Bacher*et al., 2004*; Umar *et al., 2004*).*BAT-25* and *BAT-26*which are common to both panelsare the mostcommonly used mononucleotide markers and it has been suggested that testing them alone would be sufficient to establish the MSI status of a tumour without the need for the analysis of corresponding normal tissues (Hatch *et* al., 2005; Malesci*et* al., 2007; Xicola*et al., 2007*). Also, the analysis of *BAT-26* alone has been suggested to be sufficient for the detection of the MSI-H phenotype in most cases (Hoang *et al., 1997*). Furthermore, polymorphic variations of these markers (*BAT-25* and *BAT-26*) have been observed in several populations depending on ethinicity in sub-Saharan Africa including Senegal, showing variant alleles corresponding to 5% and more (Samowitzand Slattery,1999; Pyatt*et al.*, 1999; Suraweera*et al., 2002*; Buhard *et al., 2006*). Besides, the use of *BAT-26* alone might possibly lead to the determination of false positive MSI-H ormayallow for underestimation ofthe true levels of MSI due to its failure to recognize rare cases with biallelic *MSH2* deletions since it lies in the intragenic region of *MSH2*.Hence, the use of at least one other mononucleotide marker (e.g., *BAT-25* or *NR-24*) has been suggested (Laghi*et al.*, 2008). Since diverse populations of the world have variable results for MSI tumours due to various environmental factors ensuing dissimilar outcomes (Shokal and Sharma, 2012), and considering the current success in the exploration of immunotherapy in breast cancer (Hu and McArthur, 2018), it becomes imperative to evaluate the microsatellite phenotypes of *BAT-26* and *NR-24* among breast cancer patientsin Senegal for a better understanding of the disease progression to tailor diagnosis and design of therapiesfor a better treatment outcome.

II. Materials And Methods

Sample collection
      All consecutive breast cancer patients who visited the Institut Joliot-Curie Cancer Centre, of the Aristide le Dantec Hospital Dakar, Senegal between 2014 and 2015 were approached for the study. Majority of them agreed to participate in the study with a refusal rate of 4%. Aristide le Dantec Hospital is the major and only referral hospital for cancer patients in the country serving an immediate population of about 2.45 million people. Sixty-five participants between ages 15 and 80 years old were recruited for the study. Cancerous tissue samples were collected by the surgical oncologists through biopsy or later by mastectomy from each patient after informed consent and a standardized questionnaire was used to collect the patients' data. All tissue samples were histologically confirmed to be tumour samples before they were taken in for molecular analysis.

Ethical approval

This study was conducted in compliance with the ethical approval and standards of the Universite Cheikh Anta Diop de Dakar Research Ethics Committee (Reference number: Protocole 0269/2015/CER/UCAD). The recommendations guidelines of the Declaration of Helsinki for biomedical research was followed and informed consent was obtained from all participants after detailed explanation of the objectives, benefits,seeming inconveniences (in terms of sample collection) and contribution of the study.

Choice of markers used in the study and Laboratory Analysis

The markers used in this study are as outlined, location and primer sequences are as outlined in in Table 1.DNA was extracted from the sixty tumour tissues using the standard protocol for the Qiagen kit and thereafter, they were coded and stored at -20°C for PCR analysis. The amplifications were carried out in a monoplexreaction volume of 50 μlfor each repeat and the PCR reaction was made up of 2.5 μl of the forward and reverse primers, 2 μl of each dNTP, 2 μl of $MgCl_2$ and 0.1 μl of *Taq*polymerase. The PCR was performed in an Eppendorf-type thermal cycler and the conditions were as follows: Initial denaturation at 94^0C for 5 minutes, 35 cycles of denaturation at 94^0C for 30 seconds, annealing at 55^0C for 30 seconds, extension at 72^0C for 30 seconds and final extension at 72^0C for 7 minutes. Thereafter, the PCR products were run on gel electrophoresis using 1.5% agarose. For the sequencing,30μl of each amplicon was placed in a 96-well microtiter microplates along with each primer (15μl of 10Mm) for each sample and sent to Macrogen, South Korea for Sanger sequencing (Mbaye*et al.,* 2015).

Table 1:Primer Sequences for the Microsatellite Instability Assay (Suraweera*et al.,* 2002)

Name	Gene	Genbank number	Repeat	Primer Sequences	Amplicon size (bp)
BAT-26	hMSH2	U41210	26(A) Intron 5	F: TGACTACTTTTGACTTCAGCC R:AACCATTCAACATTTTAACCCC	120
NR-24	Zinc finger 2 (ZNF-2)	X60152	24T 3′UTR	F: CCATTGCTGAATTTTACCTC R: ATTGTGCCATTGCATTCCAA	132

Sequence variant nomenclature and classification

Antonarakis and the Nomenclature Working Group (1998) suggested a nomenclature system for the description of mutations and polymorphisms observed in DNA and protein sequences. This nomenclature is known as the Human Genome Variation Society (HGVS) recommendations/ nomenclature (den Dunnen and Antonarakis, 2000). The sequence variant nomenclature constitutes the changes observed in a specific sequence when compared to a reference sequence.This gives a stable, accurate, meaningful, unequivocal, unambiguous but flexible description (den Dunnen*et al.,* 2016).

Molecular and Statistical Analysis

Data obtained after capillary electrophoresis were analyzed withBio-Edit Sequence Alignment Editor version 7.1.9 (Thompson *et al.,* 1994; Hall, 1999). To test the association between the association of breast cancer and instability of the studied markers, the sequences were cleaned, corrected and aligned to the reference sequence from the GenBank (Table 1) using the Clustal algorithm (Thompson *et al.,* 1994; Hall, 1999). Since normal DNA was not examined in this study, every sample with a polymorphic variation in any of the mononucleotide repeats (*BAT-26*and *NR-24*)in terms of size and pattern of variations (deletion, insertion, transition or transversion) was scored positive for instability (Wong *et al.,*2006).The Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS, Inc. Chicago IL, USA) was used to evaluate the frequencies of different variables as well as the chi-square values to determine the statistical differences or otherwise among test variables. The statistically significant value was placed at p-value < 0.05. Tumours were classified as MSI-H tumours if they were unstable in the two markers, MSI-low (MSI-L) if only one marker unstable and MS-stable (MSS) if none of the markers was unstable.

III. Results

Frequency of MSI and their haplotypes

Of the 65 tumours genotyped for microsatellite instability status among breast cancer patients in Senegal, only 40 of the sequences for *BAT-26* and 43 of the sequences for *NR-24* were clean after alignment and confirmation of each sequence with their chromatogram.Based on the classification of MSI (as earlier stated),7 (10.8%) were MSS, 45 (69.2%) were MSI-L, and 13 (20%) were MSI-H tumours were identified from the analyzed sequence. The composition of the allele sizes and variants for each marker are presented in Table 2 for *BAT-26* and Table 3 for *NR-24.*The percentage of variant alleles observed for each marker was 92.5% and 81.4% for *BAT-26* and *NR-24* respectively.

For *BAT-26*with 26A repeats, shortened alleles of 1 to 3 bp was observed as well as INDELS (hereafter denoted as 'delins') 'delins' of up to 3 bp with deletion of the A and insertion of G. Three haplotype groups had substitution of A with G (represented as > for substitution). The highest haplotype with frequency of 10 had a deletion of A at the 25th position and A>G at the 26th position of the microsatellite repeat.Eight tumours (18.6%) were stable for *NR-21* having the wildtype allele of 24T repeat. The variants alleles observed included deletions, substitutions, deletion-insertion and duplication. The highest number of deletions was 4 bp and the haplotype with the highest frequency of 10 had substitution of T>G.

Table 2: Composition of allele sizes and variants for *BAT-26*

Haplotypes	Allele Sizes	Allele variants	Senegal : No.
Hap 1	**26A (Wild type)**	AAAAAAAAAAAAAAAAAAAAAAAAAA	**3**
Hap 2	26del**A**	AAAAAAAAAAAAAAAAAAAAAAAAAA –	8
Hap 3	24_26delins**G**	AAAAAAAAAAAAAAAAAAAAAAAGGG	5
Hap 4	25del**A**; 26A>**G**	AAAAAAAAAAAAAAAAAAAAAAAAA–G	10
Hap 5	24_26del**A**	AAAAAAAAAAAAAAAAAAAAAAAA — –	2
Hap 6	25_26>**G**	AAAAAAAAAAAAAAAAAAAAAAAAGG	6
Hap 7	24del**A**; 25>**G**; 26del**A**	AAAAAAAAAAAAAAAAAAAAAAA– G –	1
Hap 8	25del**A**	AAAAAAAAAAAAAAAAAAAAAAAAA –A	1
Hap 9	24_25del**A**	AAAAAAAAAAAAAAAAAAAAAAAA—A	1
Hap 10	26>**G**	AAAAAAAAAAAAAAAAAAAAAAAAAA	3
		Total	40

(del = deletion, **>** = substitution,delins = deletion-insertion)

Table 3: Composition of allele sizes and variants for *NR-24*

Haplotypes	Allele Sizes	Allele variants	Senegal : No.
Hap 1	**24T (Wild type)**	TTTTTTTTTTTTTTTTTTTTTTTT	**8**
Hap 2	24dup**T**	TTTTTTTTTTTTTTTTTTTTTTTTT	1
Hap 3	24**T**>**G**	TTTTTTTTTTTTTTTTTTTTTTTG	10
Hap 4	22del**T**	TTTTTTTTTTTTTTTTTTTTT–TT	1
Hap 5	22del**T**; 24**T**>**G**	TTTTTTTTTTTTTTTTTTTTT–TG	3
Hap 6	1del**T**; 22delins**G**; 24dup**T**	–TTTTTTTTTTTTTTTTTTTTGTTT	1
Hap 7	21del**T**; 22delins**G**; 24dup**T**	TTTTTTTTTTTTTTTTTTTT–GTTT	2
Hap 8	20delins**G**	TTTTTTTTTTTTTTTTTTTGTTTT	1
Hap 9	20delins**G**; 24**T**>**G**	TTTTTTTTTTTTTTTTTTTGTTTG	2
Hap 10	20delins**G**; 22_24del**T**	TTTTTTTTTTTTTTTTTTTGT– – –	1
Hap 11	20del**T**; 24**T**>**G**	TTTTTTTTTTTTTTTTTTT–TTTG	5
Hap 12	20del**T**; 23del**T** ;24**T**>**G**	TTTTTTTTTTTTTTTTTTT–TT–G	1
Hap 13	20_21del**T**; 24**T**>**G**	TTTTTTTTTTTTTTTTTT– – TTG	1
Hap 14	20_21del**T** ;22delins**G**	TTTTTTTTTTTTTTTTTT– – GTT	2
Hap 15	1del**T**; 24**T**>**G**	-TTTTTTTTTTTTTTTTTTTTTTG	3
Hap 16	17**T**>**G**; 20delins**G**; 21_24del**T**	TTTTTTTTTTTTTTTGTTG– – – –	1
		Total	43

(del = deletion, dup = duplication, **>** = substitution,delins = deletion-insertion)

Correlation between studied markers and Age at diagnosis, SBR grade and tumour stage of patients.

Statistical analysis of some clinic-pathological characteristics (age at diagnosis, SBR grade and TNM stage) showed no significant difference between MSS and MSI tumours for the two markers except in the SBR grade for *NR-24* (p-value = 0.041).As presented in Table 4, analysis of *BAT-26* showed that cases with MSI were more frequent in the age group that are 50 years old (64%) than in those above who were 50 years and above (28%), 73.3% had grade 2 tumour (SBR grading) and 75.7% were either at stage 3 or stage 4 (TNM staging). Likewise, for *NR-24*, majority of the cases with MSI tumours were of younger age (86.2% < 50). They were more among those who presented either at stage 3 or 4 (62.2%) and had tumourgrade two (56%) (Table 5).

Table 4: Comparison of Age, SBR grade and TNM stage between MSS and MSI *BAT-26* phenotype among breast cancer patients in Senegal

Characteristics		*BAT-26* (MSS)	*BAT-26* (MSI)	*p-value*
Age	< 50	2 (5%)	25 (64%)	0.680[a]
	≥ 50	1 (3%)	11 (28%)	
SBR Grade	1	-	3 (10%)	0.075[b]
	2	-	22 (73.3%)	
	3	1 (3.3%)	4 (13.3%)	
TNM Stage	1 & 2	1 (2.7%)	6 (16.2%)	0.477[b]
	3 & 4	2 (5.4%)	28 (75.7%)	

p-value[a] = fisher's exact test; [b] = χ**2**

Table 5: Comparison of Age, SBR grade and TNM stage between MSS and MSI *NR-24* phenotype among breast cancer patients in Senegal

Characteristics		*NR-24* (MSS)	*NR-24* (MSI)	p-value
Age	< 50	4 (13.8)	25 (86.2%)	0.202[a]
	≥ 50	4 (33.3%)	8 (66.7%)	
SBR Grade	1	2 (6%)	2 (6%)	0.041[b]
	2	2 (6%)	18 (56%)	
	3	4 (13%)	4 (13%)	
TNM Stage	1 & 2	1 (2.7%)	6 (16.2%)	1.000[b]
	3 & 4	7 (18.9%)	23 (62.2%)	

p-value[a] = fisher's exact test; [b] = $\chi 2$

IV. Discussion

The utilization of microsatellite markers as diagnostic tools to determine the prognosis of certain cancers in order to properly tailor treatment and predict patients' response is increasing rapidly for most solid tumours especially with the FDA approval of drugs for the treatment of patients with MSI-H or dMMRtumours (FDA, 2017).Although breast cancer was not known to be inherently immunogenic, current successes in clinical trials especially for triple negative breast cancer is fast making immunotherapy a possible therapeutic strategy asmonotherapy or in combination with the currently used conventional therapies (Williams *et* al., 2017; De LaCruz and Czerniecki, 2018).Nevertheless, the constant challenge has been to select patients who will most benefit from this treatment and therefore, the need for the evaluation of molecular markersespecially for the African population which is plagued with the menace of triple negative breast cancer.Although the use of *BAT-25* and *BAT-26* has already been proposed (de la Chapelle, 1999), polymorphism of these markers exist in individuals from Africa (Pyatt*et al*., 1999) and this may lead to misrepresentation of the MSI-H phenotype if the two markers are used together. Hence, the need for the evaluation of another mononucleotide marker which can be used alongside either of *BAT-25* and *BAT-26*.

In this study, we evaluated the microsatellite instability status of sixty-five breast tumours from Senegal using *BAT-26* and *NR-24*.20% of the tumours showed instability in the two markers while *BAT-26* and *NR-24* alone were instable in 92.5% and 81.4% of the tumours respectively. In a similar study carried out by Mbaye*et al*., (2015) using *BAT-25* and *BAT-26*, they observed that 70.6% of the tumours were unstable for the *BAT-26* phenotype. Also, the shortening of the *BAT-26* as observed in this study have been positively associated with MSI-H tumours.Consistent with this, *BAT-26* also showed variant alleles of up to 14 alleles out of its quasi-monomorphic variation range (QMVR) in a Brazilian population analysis of the pentaplex panel while no variant alleles were observed for *NR-24* out of its QMVR (Campanella *et al*., 2014). *NR-24*has been shown to be a remarkably stable marker with little or no variation among several studies that have evaluated mononucleotide repeats in human cancers (Buhard *et al*., 2006; Mamdouh *et al*., 2015).Results of microsatellite instability in breast cancer vary for different studies and in different populations with rates as low as 2.9% (Glebov*et* al., 1994; Patel *et* al., 1994; Yee *et* al., 1994; Bergthorsson*et* al., 1995; Paulso*et* al., 1996; Risinger*et al*., 1996; De Marchis *et al*., 1997;Sourvinos*et al*.,1997; Fujii*et* al., 1998; Walsh *et* al., 1998; Zhou *et* al., 1998; Siah*et al*., 1999; Tomita *et al*., 1999; Siah*et al*., 2000).It has also been suggested that the choice of markers used for the analysis, ranging from mononucleotides to tetranucleotide repeats and the sample sizes may have influenced these varying results (Siah*et al.,* 2000).

Tumours with MSI-H phenotype have distinct clinical and pathological features from MSStumours. In this study, statisticalsignificance was observed for only SBR grade and *NR-24* markerbetween MSS and MSI tumours.However, younger patients are more likely to be unstable for themicrosatellite phenotype as recorded in our study where more than half (64%) of individuals below 50 years were unstable for both markers. This has been observed in studiesinvolving other tumours with instability in about 58% of tumours from younger individuals (Lamberti *et al*., 1999; Gryfe *et al*., 2000; Boland and Goel*.,* 2010). We found that MSI was more frequent in grade 2 tumours for both markers (73.3% for *BAT-26* and 56% for*NR-24*), with statistical significance (p = 0.041) for grade and *NR-24*. Similarly, Ward and colleagues (2001), observed that MSI-H tumours were associated with high grade tumours.The percentage of MSI varied with tumour stage (which was grouped into 2 due to the smallsample size and data). About 63% of the microsatellite instable tumours were for individuals whowere either at stage 3 or stage 4 of the disease with presence of lymph nodes status.This is in agreement with other studies (Soreide*et al.,* 2009; Eveno*et al.,* 2010; Belt *et al.,* 2012;) and mayindicate an association between MSI and aggressiveness of the disease leading to increase likelihood for metastasis as observed in this population where patients present at late stage with aggressive tumour.However, further studies need to be carried out to access if MSI leads toaggressive tumours or vice versa.Nevertheless, ourresult in contrast to Merok*et al.,* (2012) whoobserved high frequency of MSI in stage twotumours. It was suggested that

this isreflexiveof tumours that areless aggressive withlower metastatic tendencyas MSI may induce larger lymph nodes.

Two modes of microsatellite instability have been proposed to occur in human cancers: Type A alterations with length changes of ≤6 bp and Type B alterations with length changes of ≥8 bp (Oda *et al*., 2005).In our study, the Type A mode of MSI was observed as no haplotype had an alteration that was ≥8 bp. Being that Type A MSI is connected with a dMMR system, we can therefore propose that the MSI observes here is as a result of the inactivation and lose of function of the DNA MMR system. To establish this theory, analysis that identifies particular mutations in the MMR genes such as immunohistochemistry (IHC) must be carried out. Because of the missense or in-frame insertion/deletion mutations that occur in microsatellite instable tumours, which do not result in the truncation of a protein, assessment of theMSI status could help to ascertain the true functional consequences of these variant mutations especially in some DNA MMR genes like the *MLH1*and *MSH6* genes.This may not be true for the routine IHC analysis which may give a false negative result stemming from biological or technical reasons, therefore, evaluation of MSI allows for the elimination of confusion in case of inconsistent result. MSI analysis provides a functional analysis of the deficient MMR activity elucidating functionality of the MMR system (Zhang and Li., 2013).

V. Conclusion

Finally, the current success recorded in the use of immune checkpoint blockade molecules in the treatment of cancers that are resistant to chemotherapy such as TNBC requires the efficacious utilization of a molecular marker that will betterpredict immunotherapeutic response in patients.This study demonstrates microsatellite instability of *BAT-26* and *NR-24* markers among breast cancer patients in Senegal with a positive correlation between MSI and SBR grade in *NR-24*. Also, *NR-24* which shows little or no variation was shown to exhibit instability in our study and may be a better marker for the diagnosis of MSI status among breast cancer patients unlike the previously suggested *BAT-26* which is very polymorphic. Nevertheless, both markers could work effectively as a pair due to their varying polymorphic phenotype in different populations.

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