Paired Tumor/Germline Testing for Lynch Syndrome – A Comprehensive Testing Approach

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Background: Appropriate candidates for Lynch syndrome genetic testing are often identified by abnormal microsatellite instability (MSI) and/or mismatch repair (MMR) protein immunohistochemistry (IHC) on colorectal and endometrial tumors. In the absence of a *BRAF* mutation, *MLH1* promoter hypermethylation, or an identifiable germline mutation, individuals with abnormal MSI and/or IHC have been managed as though they have Lynch syndrome. Recent data have shown that analyses of tumor DNA may exclude Lynch syndrome in many of these patients. This study aimed to describe the results of paired tumor/germline testing for Lynch syndrome at one laboratory.

Methods: A retrospective analysis was performed on data from colorectal and endometrial cancer patients with abnormal IHC who had paired tumor/germline MMR gene analysis between 12/05/16-07/09/17. Results of sequencing and deletion/duplication analyses of the MMR genes and *EPCAM* (del/dup only), *MLH1* promoter hypermethylation, and microsatellite instability (MSI) were assessed.

Results: Forty-one of 54 (76%) patients had either a germline MMR mutation, consistent with Lynch syndrome, or somatic changes to explain IHC results, significantly reducing the likelihood of Lynch syndrome in the majority of cases (Table 1). In 13 cases (24%), testing was uninformative (Table 2).

Conclusions: In this cohort, 47% of cases would have remained unexplained without the addition of somatic MMR gene sequencing and deletion/duplication analyses. Adding tumor MMR gene analyses to the testing algorithm allows for potential exclusion of Lynch syndrome and reduces the likelihood of discordant results. These data may provide clinicians with additional information to further tailor treatment and surveillance for each patient.

Table 1: *Informative Cases*

Molecular DNA Results	**Concordant with IHC?**	**Diagnosis**	**n (%)**
Germline mutation	Yes	Lynch Syndrome	7 (13%)
MLH1 promoter hypermethylation	Yes	IHC likely due to somatic changes	9 (17%)
		Explained by traditional testing algorithm	***16 (30%)***
Double somatic mutations*	Yes	IHC likely due to somatic changes	21 (39%)
Double somatic mutations in *MLH1*/presence of *MLH1* promoter hypermethylation w/ loss of PMS2 on IHC	No	IHC likely due to somatic changes	3 (6%)
Double somatic mutation + germline VUS**	Yes	IHC likely due to somatic changes	1 (2%)
		Explained by tumor MMR analyses	***25 (47%)***
		Total informative cases	**41 (76%)**

* One case was an apparent hyper/ultramutator phenotype

** Lynch syndrome could not be fully ruled out

Table 2: *Uninformative Cases*

Molecular DNA Results	**Concordant with IHC?**	**n (%)**
Single somatic mutation identified	Yes	6 (11%)
Single mutation in another gene identified	No	2 (4%)
No alterations identified	No	5 (9%)
	Total uninformative cases	**13 (24%)**