Lynch syndrome in the 21st century: clinical perspectives

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Summary

Lynch syndrome (LS) is the most common of all inherited cancer syndromes, associated with substantially elevated risks for colonic and extracolonic malignancies, earlier onset and high rates of multiple primary cancers. At the genetic level, it is caused by a defective mismatch repair (MMR) system due to presence of germline defects in at least one of the MMR genes—MLH1, MSH2, MSH6, PMS2 or EPCAM. An impaired MMR function during replication introduces infidelity in DNA sequence and leads to ubiquitous mutations at simple repetitive sequences (microsatellites), causing microsatellite instability (MSI). Although previously, clinicopathological criteria such as Amsterdam I/II and Revised Bethesda Guidelines were commonly used to identify suspected LS mutation carriers, there has been a recent push towards universally testing, especially in case of colorectal cancers (CRCs), through immunohistochemistry for expression of MMR proteins or through molecular tests (polymerase chain reaction, PCR) for MSI, in order to identify LS mutation carriers and subject them to genetic testing to ascertain the specific gene implicated. In this review, we have discussed the latest diagnostic strategies and the current screening and treatment guidelines for colonic and extracolonic cancers in clinically affected and at-risk individuals for LS.

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the USA, predicted to be responsible for about 132,700 new cases and 49,700 deaths in 2015.¹ CRC is a multifactorial disease, with variable interplay between genetic and environmental factors. It is estimated that about 70–80% cases of CRC are sporadic whereas the remaining 20–30% have an inherited component.² However, an inherited syndrome can be defined only in a small fraction of such cases due to genetic heterogeneity, variable penetrance and presentation, and a plethora of pathways through which CRC could evolve.

In the 20th century, before discovery of cancer-related genetic defects, the inherited cancer syndromes were identified by the familial predisposition to certain aggregates of cancers. One of such familial aggregates of CRC with stomach and endometrial cancers was reported by Lynch and colleagues in 1966 in two extended pedigrees and was designated cancer family syndrome.³ Later on, this condition was called hereditary non-polyposis colorectal cancer (HNPCC), to differentiate it from another inherited form of CRC, familial adenomatous polyposis (FAP). However, ‘nonpolyposis’ was indeed a misnomer because adenomatous polyps are much more prevalent and undergo malignant transformation more aggressively in patients with HNPCC than in the general population. In 1984, the term ‘Lynch syndrome’ (LS) was proposed to describe this condition and has been most commonly used since then.⁴ LS accounts for about 3% of newly diagnosed cases of CRC and exhibits characteristic features of cancer predisposition syndromes, including substantially elevated risks for specific cancers, earlier age of onset and high rates of multiple primary cancers.⁵
Genetic basis of LS

In the 1990s, mutation of genes in the DNA mismatch repair (MMR) pathway was implicated as the cause of LS. MMR genes function to maintain the fidelity of the DNA during replication by correction of nucleotide base mis-pairs and erroneous small insertions or deletions generated by slippage of DNA polymerase. A compromised MMR system leads to accelerated accumulation of somatic mutations, often resulting in carcinogenesis.

Presence of a germline defect (the inherited component) is a prerequisite for the diagnosis of LS. However, acquired loss of the corresponding normal allele (wild-type) in somatic tissue through genetic or epigenetic mechanisms is required to compromise the function of the entire MMR complex in order for LS to manifest phenotypically. LS, therefore, is recessive at cellular level, but inherited as a Mendelian dominant with variable expressivity at the phenotype level, causing predisposition to colorectal and extracolonic cancers.6,7

MLH1, MSH2, MSH6 and PMS2 are the genes that produce MMR proteins. An estimated 80–90% of LS is attributable to deleterious mutations in MLH1 and MSH2, with the remaining 10–20% due to mutations in MSH6 and PMS2. Up to 3% of LS is due to mutations in the EPCAM gene, which is directly upstream of MSH2. Deletions of the 3’-end of EPCAM result in epigenetic hypermethylation of the MSH2 promoter, producing a phenotype very similar to LS.8

LS and sporadic CRCs

Defect in one of the MMR genes leads to ubiquitous mutations at simple repetitive sequences (microsatellites) due to malfunctioning MMR mechanism, causing microsatellite instability (MSI). The defect in MMR genes could be due to presence of a germline mutation (LS) or secondary to hypermethylation of MLH1 promoter (somatic change/sporadic CRC). MSI is characterized by abnormal expansion or contraction of these microsatellite repeats, present mostly in intron sequences. Approximately 90% of CRCs in LS have MSI, compared with <10% of sporadic CRC cases. Most CRCs in LS are MSI-high (>30% markers unstable). Significance of MSI-low status is unclear, but it is most often seen due to somatic inactivation of MSH3 gene, which is quite common but not inherited.10

Although LS implies presence of a germline mutation in one of the MMR genes, immunohistochemistry (IHC) of the tumor specimen often identifies concurrent loss or partial production of two MMR gene proteins, and cannot distinguish between the loss of protein expression due to germline mutation vs. somatic hypermethylation. For example, MSH2 and MSH6 proteins are often lost concurrently and indicate MSH2 gene mutation. Similarly, MLH1 and PMS2 proteins are also mostly lost together, generally indicating loss of MLH1 function due to germline mutation or somatic silencing of MLH1 gene. Isolated loss of MSH2, MSH6 or PMS2 proteins represents presence of germline mutation in these genes, and is very specific for LS, except in a very small number of cases where loss of MSH2 protein expression could be due to mutation in the EPCAM gene (leading to MSH2 gene promoter methylation and hence loss of function) rather than MSH2 gene. MLH1 protein is also lost or underexpressed in about 12% of sporadic CRCs due to somatic methylation of MLH1 gene promoter.11 These cancers evolve through a CpG island methylator phenotype (seen in 15% of sporadic CRCs) caused due to somatic mutation of BRAF gene mostly at codon 600. Therefore, presence of a mutated BRAF in an MSI-high CRCs usually argues against LS.12

Rarely, there are LS-like cases involving germline MLH1 gene hypermethylation (epimutation), without any MLH1 sequence variation or rearrangements (mutations). This epimutation is mosaic (involving different tissues to a variable extent) and reversible so that the offspring are typically unaffected.13 By definition, therefore, such cases cannot be classified as LS.

Clinical spectrum of LS

LS is an autosomal dominant disorder with variable expressivity with CRC as the major clinical consequence.14 In addition to CRC, LS patients have a significantly increased risk for a wide variety of extracolonic malignancies. Cancer risks vary substantially depending upon the genes involved as well as within families with the same inherited germline mutation due to epigenomic changes secondary to gene–environment interactions.

In its early days, when genetics of LS was poorly understood, the clinical criteria for diagnosis were based upon identifying the familial clustering of CRC and associated cancers, after exclusion of FAP. In 1990, the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer established Amsterdam I criteria (3-2-1 mnemonic) for HNPCC. Later on, they were revised to Amsterdam II criteria, to include some extracolonic tumors as qualifying criteria for LS. Both Amsterdam and revised Amsterdam criteria were developed as research tools to identify high-risk families, with a sensitivity and specificity of around 60 and 70%, but neither of the two is optimal to identify LS patients. For example, families meeting Amsterdam II criteria have 22% sensitivity and 98% specificity for diagnosis of LS.15 Similarly, more than a third of families that meet the Amsterdam I criteria do not have LS.16 More recently, a third set of clinic criteria, The Revised Bethesda Guidelines, were developed to include evaluation of MSI and/or IHC in order to better identify individuals who deserve genetic testing to investigate for LS. The sensitivity and specificity for LS in those meeting any one of the guidelines is 82 and 77%, respectively.17 These clinicopathological criteria are summarized in Table 1.

Although the term HNPCC is often used interchangeably with LS, it is important to remember that HNPCC is a clinical diagnosis (for patients and/or families that meet Amsterdam I or II criteria) whereas diagnosis of LS requires presence of a genetically confirmed mutation in one of the MMR genes known to be implicated in LS (MLH1, MSH2, MSH6 and PMS2 and EPCAM). There are several variants of LS, some sharing genotypic changes and some with similar phenotype.

Lynch-like syndrome (LLS) describes cases where molecular testing demonstrates the presence of MSI and/or abnormal expression of MMR proteins on IHC testing of tumors, without presence of characteristic germline mutations seen in LS. About half of LLS patients have biallelic somatic mutations of MLH1 or MSH2 genes to explain the MMR-deficient tumors without having causal germline or promoter mutations.21

Muir-Torre syndrome is a rare phenotypic variant of LS characterized by high incidence of skin sebaceous gland or hair follicle neoplasms (sebaceous adenomas/carcinomas and keratoacanthomas) in LS patients.22 These cases have characteristic germline changes seen in LS patients, most commonly MSH2 gene mutations.23

Turcot’s syndrome is a phenotypic syndrome that refers to patients and/or families with CRC and brain tumors. However, this is not an independent entity because it represents either LS families (with glioblastomas) or FAP families (with medulloblastomas).24

Table 1

Evaluation of MSI and IHC for LS

<table>
<thead>
<tr>
<th>Clinicopathological Criteria</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
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<tbody>
<tr>
<td>Amsterdam I</td>
<td>22</td>
<td>98</td>
</tr>
<tr>
<td>Amsterdam II</td>
<td>82</td>
<td>77</td>
</tr>
<tr>
<td>Revised Bethesda Guidelines</td>
<td>82</td>
<td>77</td>
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Note: Sensitivity and specificity values are approximate and may vary depending on the population and the diagnostic tool used.
Table 1. Clinicopathological criteria to identify HNPCC/LS

Amsterdam I criteria (3-2-1)
1. Three or more relatives with histologically confirmed CRC, with one being a first-degree relative of the other two, provided that FAP has been excluded
2. Two or more generations with CRC
3. One or more CRC cases diagnosed before the age of 50 years

Amsterdam II criteria
1. Three or more relatives with histologically confirmed CRC or other LS-associated tumors, with one being a first-degree relative of the other two, provided that FAP has been excluded
2. Two or more generations involved
3. One or more cancers diagnosed before the age of 50 years

Revised Bethesda Guidelines
1. CRC diagnosed before the age of 50 years
2. Presence of synchronous or metachronous CRC or other LS-associated tumors
3. CRC with MSI-high pathologic features (Crohn’s like lymphocytic reaction, medullary growth pattern, mucinous/signet cell differentiation) diagnosed after age 60 years
4. Patient with CRC and CRC or LS-associated tumor diagnosed in at least one first-degree relative younger than 50 years of age
5. Patient with CRC and CRC or LS-associated tumor at any age in two first-degree or second-degree relatives

Constitutional MMR deficiency syndrome refers to patients/families with biallelic mutations of MMR gene, and is characterized by café-au-lait spots and high incidence of LS-associated cancers in childhood as well as small intestinal polyps, brain tumors, and hematologic malignancies.

Familial colorectal cancer type X refers to cases meeting Amsterdam I criteria but lacking MSI characteristics of LS. CRC in these cases is diagnosed at a slightly older age compared with LS, and risk of extracolonic cancers is not more than that of the average-risk population.

Diagnostic strategies for LS

LS is a genetic diagnosis, which requires identification of a germline mutation in one of the MMR genes. Because CRC is by far the most common of LS-associated cancers, a growing body of evidence supports universal genetic testing for at least all newly diagnosed CRCs because it can reduce morbidity and mortality of relatives of patients with LS. Supporting this practice is the finding that one of every 35 of all CRC patients has LS. However, there are serious concerns regarding the cost-effectiveness and pragmatic feasibility of universal genetic testing of all new CRCs. Therefore, efforts have been made to develop strategies to identify cases with highest pre-test probability of LS diagnosis and subject only those cases to genetic testing. This is the fundamental principle of the diagnostic strategies for LS (as summarized in Figure 1).

The traditional testing strategy depends upon clinicopathological criteria (such as Amsterdam I/II criteria and Revised Bethesda guidelines), CRC risk assessment tool and computational models (such as MMRpredict, MMRpro and the PREMM models). However, none of these models have been consistently successful in identifying LS cases. For example, molecular studies of the CRCs reveal that up to a quarter of LS cases will be missed even with the most liberal of clinical criteria- the Revised Bethesda guidelines. Similarly, computational models also fall short of expectations and could not be reliably used clinically.

There is a growing body of evidence that systemic application of testing for LS in newly diagnosed CRC at <70 years of age could provide substantial clinical benefits at affordable cost and an overall sensitivity of 83% and specificity of 89%. Of MMR gene proteins, loss of MLH1 protein is usually due to somatic events (acquired mutation or hypermethylation) causing inactivation of MLH1 gene, whereas loss of MSH2 protein is more likely due to a germline mutation of MSH2 gene. Because loss of MLH1 protein expression could also be due to hypermethylation of MLH1 gene promoter in sporadic CRCs (with increasing incidence with age), it is imperative to check for presence of BRAF gene mutation (responsible for MLH1 gene promoter hypermethylation) whenever MLH1 protein is lost or underexpressed in CRCs in order to distinguish between sporadic CRC (mutated BRAF gene) from LS (mutated MLH1 gene). On the other hand, if IHC demonstrates normal expression of MLH1 protein but underexpression or loss of other MMR proteins, targeted germline testing for that particular MMR gene is high yield. More recently, there have been reports suggesting that universal tumor IHC testing regardless of age at diagnosis has greater sensitivity for identification of LS compared with any other strategy.

Another less commonly applied strategy is molecular (polymerase chain reaction, PCR) testing of CRCs to check for presence of MSI. This is based upon the knowledge that over 90% of CRCs in LS are MSI-high. Hence, lack of MSI has an excellent negative predictive value for LS. The sensitivity for diagnosing LS using this molecular technique is 85% with about 90% specificity.

Genetic testing for LS

Diagnosis of LS requires evidence of presence of germline mutation in one of the MMR genes - MLH1, MSH2, PMS2 or EPCAM. Traditional testing strategy to determine the need for genetic testing depends upon individuals meeting clinicopathological criteria (Amsterdam I/II criteria, Revised Bethesda Guidelines), having known MMR gene mutation carriers in family, >5% chance of harboring MMR genes mutation by prediction models or personal history of uterine cancer before age of 50 years.
Universal testing strategy, on the other hand, determines the eligibility for genetic testing based upon the loss of MMR proteins expression on IHC of tumor specimen. There is general consensus in favor of universal tumor testing for all CRCs diagnosed under the age of 70 years. Moreover, many institutions have opted to perform tumor testing on all CRCs regardless of age of diagnosis to further enhance detection of LS. In CRC cases diagnosed after age 70 years where tumor testing is not performed, it is important to assess for risk of LS by using traditional testing strategy as mentioned earlier and recommend genetic testing if the criteria are met.

The algorithm for genetic testing depends upon two factors: (i) whether the individual is clinically affected; (ii) whether germline family mutation is known. If family mutation is known, both clinically affected and at-risk individuals should be genetically tested for that particular MMR gene mutation. If they do not harbor that mutation, LS can be safely excluded and they should only get age-appropriate average-risk cancer screening and surveillance based upon individual risk factors. In absence of genetic testing, clinically affected as well as at-risk family members (when family mutation is known) should be assumed to be carriers of LS mutation and aggressive surveillance should be carried for LS-associated cancers per guidelines.

However, when the family mutation is not known, the testing should start with the family member with highest pre-test probability of harboring a mutation- that is clinically affected individual. MSI or IHC testing of the tumor tissue from an affected individual (even if deceased, because the federal laws mandate keeping the CRC tissue for at least 7 years after procurement) can be the very first step which can help rule out LS altogether and hence abrogate the need for further testing, including that in unaffected family members. When tumor tissue is not available, genetic testing should first be offered to clinically affected family member. If an MMR gene mutation is detected in the clinically affected member, the unaffected at-risk family members could be genetically tested for that particular mutation to determine their LS mutation carrier status. If genetic testing could not be performed in a clinically affected member (deceased or unwilling), at-risk family member should be considered for the same. A detailed algorithm for genetic testing in clinical affected and at-risk family members is described in a recently published consensus statement on genetic evaluation and management of LS by the US Multi-Society Task Force on Colorectal Cancer.

LS-associated cancers
Colorectal cancers
Patients with LS are at much higher risk of CRC than average risk population, with certain distinctive characteristics such as right side predominance, younger age at diagnosis and rapid adenoma to cancer conversion. Therefore, early screening and annual colonoscopic surveillance for CRC in those with known MMR gene mutation or the first-degree relatives of affected patient (unless MMR gene mutation ruled out) is associated with significant decrease in mortality (~72%) from CRC. It is necessary to have good visualization of the cecum where one-third of the CRCs occur. In general, risk of CRC is much higher with MLH1 and MSH2 gene mutations compared with MSH6 or PMS2, so colonoscopic screening and annual surveillance can begin at later age, at 30 and 35 years for MSH6 and PMS2 mutation carriers, respectively.

Standard treatment for patients with colon cancer or endoscopically unresectable polyp is colectomy with ileorectal...
anastomosis and surveillance for rectal cancer through flexible sigmoidoscopy every 1–2 years. In older patients, hemicolectomy could be considered to improve post-op recovery and quality of life. Rectal cancer occurs in up to 20% of LS patients and typically requires neoadjuvant chemotherapy, proctocolectomy and ileal pouch-anal anastomosis.36

In terms of chemoprevention, there are reasonably compelling data supporting that aspirin is protective against CRC and some extracolonic tumors.38 Although current guidelines do not routinely recommend its use, aspirin at low dosage (75–81 mg daily) is usually prescribed unless there are contraindications to its use. It should be noted that in regard to LS, long-term use of aspirin has been found to be necessary to reduce the incidence of CRC.39

Gynecological (endometrial and ovarian) cancers
Endometrial cancer is the second most common cancer in LS patients, with 20–60% lifetime risk depending upon specific gene mutation. Fortunately, majority of symptomatic patients with endometrial cancer have stage 1 disease with 5-year survival rate in excess of 85%. Therefore, it is unlikely that screening for endometrial cancer will offer any significant survival benefit. However, consensus guidelines currently recommend annual pelvic exam and endometrial sampling starting at age 30–35 years.40

The lifetime risk of ovarian cancer in women with LS ranges from 0.3 to 20%. Although screening for ovarian cancer through transvaginal ultrasound and CA-125 has not been shown to be effective in any study (including patients with hereditary breast cancer from mutation of BRCA1 or BRCA2), consensus opinion is in favor of transvaginal ultrasound starting at age 30–35 years in LS patients.36

Based upon cost-effectiveness analysis, current guidelines recommend prophylactic hysterectomy and bilateral salpingo-oophorectomy at age 40 or after having finished childbearing.41 This option should be given to women in large part because of the ineffectiveness of screening for gynecologic cancers, especially ovarian cancer.42

Gastric cancer
The lifetime risk of gastric cancer in LS patients is estimated to be 5–10% without any familial clustering.33 Most of the gastric cancers in LS patients are intestinal type, and hence amenable to endoscopic surveillance. Currently, all society guidelines recommend screening with upper endoscopy (esophagogastroduodenoscopy, EGD) with gastric antrum biopsy at age 30–35 years to identify Helicobacter pylori infection with subsequent treatment if detected. Surveillance EGD every 2–3 years can be considered based upon individual risk factors such as detection of intestinal metaplasia on prior EGD.36

Small bowel cancer
The lifetime risk of small bowel cancer in LS patients is 0.4–12% without any familial clustering, and the majority of these cancers occur in the duodenum or ileum which can at least partly be examined with EGD and colonoscopy (ileal intubation).44 Currently, the consensus opinion is against routine screening and surveillance for small bowel cancer, due to lack of data regarding its effectiveness as well as relatively high cost. However, the National Comprehensive Cancer Network (NCCN) does suggest capsule endoscopy screening every 2–3 years starting at age 30–35.35,45

Urinary tract cancers
The lifetime risk of urinary tract cancers (transitional cell carcinoma of bladder, renal pelvis and ureter) ranges from 0.2–25% in LS patients and appears to be more prevalent with MSH2 mutations.46 Urine cytology and urinalysis for microscopic hematuria has not been shown to be sensitive or specific enough and benefit of ultrasound screening is unclear. However, urinalysis is inexpensive and usually a part of routine physical examination. Therefore, consensus opinion is in favor of annual urinalysis starting at age 30–35 years in individuals at risk for or affected with LS.47

Other LS-associated extracolonic cancers
There are conflicting data regarding the lifetime risk of pancreatic, prostate and breast cancers in LS patients, with some studies showing slightly increased risk whereas others show no difference compared with average-risk populations. Regardless, the general consensus is that screening guidelines for pancreatic, prostate and breast cancers in LS patients should be no different than what is applicable to general population.48

On the other hand, the lifetime risk of CNS tumors (glioblastomas) and sebaceous neoplasms in LS mutation carriers is 1–4% and 1–9%, respectively, higher than the general population (<1%). However, no specific screening strategy is recommended for clinically affected or at-risk patients above and beyond annual physical examination including skin examination. Table 2 summarizes the current screening guidelines for clinically affected or at-risk persons for LS.

Genetic counseling
Genetic counseling is a key to patient education and an impetus for germline mutation testing. Over one million patients will manifest CRC annually.49 Approximately 3.6% or over 36 000 cases will have LS; on average, each CRC affected will have three relatives with LS.50 Each case belongs to a family with clinical concerns that require genetic counseling. DNA testing for MMR gene mutations, and screening for CRC and extracolonic LS cancers. Burton-Chase et al. have shown the attitudes toward behavioral screening in concert with pre-test genetic counseling and post-test disclosure in LS families. Interestingly, mutation positive patients ’... reported increasingly positive attitudes toward CRC screening after receiving genetic test results, potentially reinforcing long-term colonoscopy adherence’.50 In conclusion, given limitations of physicians’ time and lack of sufficient remuneration for extended genetic counseling this role is being more and more assumed by professional genetic counselors, genetic counseling centers and medical geneticists.

Future perspective
LS represents a vexing clinical problem for both diagnosis and management. From a diagnostic perspective, LS is the most common cancer predisposing syndrome and vastly underdiagnosed since estimates suggest population prevalence of more than 1 in a 1000 yet LS is relatively rare in most clinician’s practices. This underscores the need for more vigilance in rigorously considering family history in all patients. Furthermore, given the myriad of clinical presentations, it supports the role for diagnostic evaluation on all CRC tissues for LS, regardless of demographics. From a management perspective, the goal is to personalize surveillance and therapy. This will be aided by the rapid scientific advances on not only genotype-phenotype correlates but modifier loci and also impact of gene–environment
Table 2. Current screening guidelines for clinically affected or at-risk persons for LS

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>Colonoscopy</td>
<td>- Every 1–2 years</td>
</tr>
<tr>
<td>- Attempt ileal intubation</td>
<td>- Starting at age 20–25 years or 2–5 years younger than youngest age at CRC diagnosis in family if diagnosed before age 25 years</td>
</tr>
<tr>
<td></td>
<td>- Start at age 30 years in MSH6, and 35 years in PMS2 mutation families</td>
</tr>
<tr>
<td>EGD with antrum biopsy</td>
<td>- Every 2–3 years</td>
</tr>
<tr>
<td>- Attempt enteroscopy</td>
<td>- Starting at age 30–35 years</td>
</tr>
<tr>
<td>Pelvic examination with endometrial sampling</td>
<td>- Every year</td>
</tr>
<tr>
<td></td>
<td>- Starting at age 30–35 years</td>
</tr>
<tr>
<td>Transvaginal ultrasound</td>
<td>- Every year</td>
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<tr>
<td></td>
<td>- Starting at age 30–35 years</td>
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<tr>
<td>Urinalysis</td>
<td>- Every year</td>
</tr>
<tr>
<td></td>
<td>- Starting at age 30–35 years</td>
</tr>
<tr>
<td>Video-capule study</td>
<td>- Every 2–3 years</td>
</tr>
<tr>
<td>- Recommended only by NCCN</td>
<td>- Starting at age 30–35 years</td>
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interactions (smoking, diet, obesity etc.). New biological insights into fields such as synthetic lethality may lend themselves to allow care of LS patients with cancer to enter the era of precision medicine.

**Funding**

This work was supported by revenue from Nebraska cigarette taxes awarded to Creighton University by the Nebraska Department of Health and Human Services. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the State of Nebraska or the Nebraska Department of Health and Human Services. Funding was also received from Kicks for a Cure. Dr Henry Lynch’s work is partially funded through the Charles F. and Mary C. Heider Chair in Cancer Research, which he holds at Creighton University.

**Conflict of interest:** None declared.

**References**