Lynch Syndrome - Cancer Pathways, Heterogeneity and Immune Escape

Lynch Syndrome – Cancer Pathways, Heterogeneity and Immune Escape

Sidhant Seth[1], Ann Ager[2], Mark Arends[3], Ian M Frayling[4]

1 University of Edinburgh Medical School, Edinburgh EH16 4SB

2 Division of Infection and Immunity, School of Medicine, and Systems Immunity Research Institute, Cardiff University, Cardiff CF14 4XN

3 Division of Pathology, Cancer Research UK Edinburgh Centre, Institute of Genetics & Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XR

4 Institute of Medical Genetics, University Hospital of Wales, Cardiff CF14 4XW, and Institute of Cancer & Genetics, Cardiff University, Cardiff CF14 4XN

FraylingIM@cardiff.ac.uk

ABSTRACT:

Recent work has provided evidence for genetic and molecular heterogeneity in colorectal cancers (CRCs) arising in patients with Lynch syndrome (LS), dividing these into two groups: G1 and G2. In terms of mutation and gene expression profile, G1 CRCs bear resemblance to sporadic CRCs with microsatellite-instability (MSI), whereas G2 CRCs are more similar to microsatellite-stable CRCs. Here we review the current state of knowledge on pathways of precursor progression to CRC in LS and how these might tie in with the new findings. Immunotherapy is an active field of research for MSI cancers and their potential use for cancer therapy for both sporadic and LS MSI cancers is discussed.

Lynch syndrome

Lynch syndrome (LS) is probably the most common hereditary cause of cancer in humans: a genetic condition with a prevalence of at least 1 in 200, that predisposes to various cancers, most frequently colorectal (CRC) and endometrial adenocarcinoma [1]. It is caused by constitutional ("germline") mutations in one of four DNA mismatch-repair (MMR) genes: *MLH1*, *MSH2*, *MSH6* and *PMS2*. Such heritable mutations, when combined with a sporadic somatic mutation of the normal allele, lead to a deficiency of DNA mismatch repair (dMMR) that results in the accumulation of genetic length changing mutations at microsatellites – so-called 'microsatellite instability' (MSI). This is accompanied by an incidental 100x – 1000x fold increase in mutation rate, but more importantly a reduced susceptibility to apoptosis induced by DNA damage recognised by the MMR pathway, conferring a strongly selectable Darwinian advantage to such cells. A subset (~14%) of sporadic colon cancers also display dMMR and MSI, predominantly related to promoter hypermethylation of the MMR gene *MLH1*, which arise from right-sided serrated lesions [2]. In contrast, sporadic rectal cancers with dMMR and MSI are rare: most rectal cancers with dMMR are due to Lynch syndrome [3,4]. LS accounts for 3.3% of bowel cancers in the UK, so similar to figures for Denmark (2.8%) and the USA (3.1%) [5–7].

The number of mutations reported in MMR genes varies [8]. This is a consequence of mutations in *MLH1* and *MSH2* conferring the highest risks, *MSH6* mutations less so, and *PMS2* mutations least of all, so cases of LS identified on the basis of family history are mostly found to have mutations in *MSH2* or *MLH1*. However, this may change as case finding by systematic testing of incident cancers becomes the norm [e.g. 3]. So far gene, gender, age, and previous cancer have all been identified as variables in LS cancer risk [10], as well as various environmental and lifestyle factors [1].

Molecular heterogeneity of Lynch syndrome tumours

Whilst research in sporadic CRC has focussed on delineating molecular heterogeneity, little work has been done on such heterogeneity in LS-CRC. Previously, it was assumed that CRCs occurring in LS patients were all part of the same disease process. However, recent studies have identified at least two precursors of LS-CRC. Firstly, via adenomatous polyp formation in which *APC* mutations (and other genetic or epigenetic changes) which occur early, and probably sporadically, and drive conventional polypoid adenoma formation. Subsequent loss of MMR in LS patients leads to invasive adenocarcinoma via more genetic mutations. Secondly, a separate pathway in which MMR deficiency occurs within single non-dysplastic crypt foci, that subsequently acquire more genetic changes to become flat intra-mucosal neoplastic lesions, that are more difficult to diagnose endoscopically [11]. Intriguingly, evidence is coming forward of a third pathway, related to the first two, comprising a subset of lesions that initially proceed along the second pathway, but which on acquisition of somatic *APC* mutations, secondary to the MMR deficiency, become dysplastic and polypoid [12]. It is thought that approximately half of the CRCs in LS may evolve from these flat, non-polypoid, neoplasms to form sub-mucosal "immediately invasive" cancers. These are often associated with *CTNNB1* mutational activation, instead of *APC* inactivation, although mutations of ASTE1*/HT001, AIM2* and *BAX* may also contribute to the progression of MMR-deficient precursors into MMR-deficient cancers. Moreover, these must arise at a slow rate, given the 10,000 or so dMMR crypts which have inactivated the second MMR allele in a LS large bowel, and yet LS patients develop only zero, one or perhaps two CRCs in a lifetime [13,14]. This is in contradistinction to the original school of thought that LS-CRCs must derive from very rapidly growing adenomas – an understandable logical consequence of assuming all CRCs derive from adenomas.

In turn, this starts to explain recent findings that surveillance colonoscopy in LS patients only reduces CRC-related mortality in LS by half, mostly by downstaging, whilst making no discernible

impact on the rate at which LS CRCs occur – in complete contrast to the situation in the general population undergoing screening, where polypectomy very significantly impacts on future CRC incidence [15,16]. Significantly, patients who have inherited mutations in both copies of the DNA repair genes *MSH2, MLH1, MSH6, PMS2, MSH3* and *MUTYH* all develop multiple colorectal adenomas, but patients with a mutation in only one copy of these genes do not.

Further new evidence now supports the contention that, in addition to different precursor lesions, distinct molecular and cellular subtypes of LS-CRC exist. Writing in this edition of *The Journal of Pathology*, Binder and colleagues utilise CRC resection specimens from LS patients to demonstrate at least two distinct genetic subtypes of LS-CRC, termed G1 and G2 [17]. The DNA sequencing data suggests that G1 tumours show a different mutation spectrum to G2 tumours, with higher mutation numbers and greater microsatellite instability. Interestingly, G1 cancers share these characteristics with MSI sporadic CRC, whereas the mutational profile of G2 cancers tends more to resemble that of MSS sporadic CRC. Additionally, a higher proportion of G1 cancers arise on a background of germline *MLH1* mutation compared with G2 cancers. These findings suggest that, even within LS, varying degrees of mutation frequency and MSI exist, consistent with multiple pathways to carcinogenesis in LS.

The authors also perform gene expression analysis on these two LS tumour groups. The G1 reference mucosa has upregulated transcription of genes associated with inflammatory pathways, whereas G1 tumours have strong upregulation of cell cycle and proliferation-associated genes. In contrast, G2 reference mucosa and tumours show a more heterogeneous expression signature with either a stromal or mucosal transcriptional programme. The inflammatory signature in G1 reference mucosa is characterised by overexpression of various T- and B-lymphocyte markers (e.g. *CD3*, *CD4*, *CD8* and *CD19*), and this is backed up by strong CD4+ staining demonstrated by immunohistochemistry. Moreover, G1 reference mucosa shows higher expression of genes related

to MHC antigen presentation and of chemokine receptor-ligand pairs compared with G1 tumours. These data suggests that G1 tumours undergo transcriptional reprogramming to dysregulate the immune response and hence evade immune destruction. The differences between G1 and G2 LS CRCs are summarised and compared to sporadic CRC in Table 1.

Role of the Immune System in dMMR Immunosurveillance and Tumour Immune Escape

The link between the immune system and cancer was probably first recognised in the nineteenth century, when Rudolf Virchow noticed an infiltrate of leucocytes within tumours [18]. However, the exact role the immune system plays is complex and our understanding of this is continually evolving. Somewhat paradoxically, both 'avoiding immune destruction' and 'tumour-promoting inflammation' are now regarded as hallmarks of cancer [19], suggesting that the immune system has dual roles in protecting from and promoting carcinogenesis. The mechanisms by which cancer cells avoid immune destruction can be conceptualised as a continual process of 'immunoediting' and split into three phases: elimination, equilibrium and escape [20].

MSI cancers with high mutation rates producing many mutant proteins tend to be particularly immunogenic and are typically associated with a strong lymphocytic infiltrate [21]. Moreover, they tend to show overexpression of immune-checkpoint proteins (e.g. PD-1 and CTLA-4) [22], and early-stage clinical trials show promising results for anti-tumour efficacy of immune-checkpoint inhibitors in the treatment of advanced MSI CRC [23,24]. It is hypothesised that this immunogenicity is due to the generation of a multitude of neo-antigens that are recognised as 'foreign' and trigger a strong immune response [25]. With deficiency of MMR, cells develop a characteristic mutator phenotype, and accumulate indel mutations within microsatellites and other repetitive sequences. If this occurs within coding DNA, it can lead to a frameshift within the translational reading frame and hence synthesis of novel frameshift peptides (FSPs) that can act as

tumour-specific antigens. This hypothesis is backed up by previous work that shows tumour-infiltrating T-lymphocytes exhibiting reactivity against predicted FSPs are present in sporadic MSI CRCs but not MSS CRCs [26]. Interestingly, peripheral FSP-reactive T-cells are also identified in LS patients without CRC, but not individuals without LS, or CRC patients with cancers that do not have dMMR. This shows that patients with LS are 'auto-immunised' against FSP neo-antigens prior to cancer formation, and may explain the better survival [27] and reduced rate of metastasis [21] seen in LS CRCs compared to sporadic CRCs.

In the work by Binder et al. [17], G1 reference mucosa is shown to be more immunogenic than that of G2 with a greater CD4+ infiltrate and greater expression of immune response-associated genes. Furthermore, even within the G1 group, expression of MHC class II genes and the predicted number of FSP neo-antigens (from sequencing data) rises as a function of mutational load. This supports a conceptual model whereby dMMR leads to hypermutation within coding repetitive sequences, which in turn leads to generation of FSPs and consequently a strong anti-tumour immune response. Such a strong immune response was not seen in G2 reference mucosa, supporting the contention that there is heterogeneity concerning the mutation frequencies and degree of MSI in LS.

G1 tumours, in contrast with matched reference mucosa, show reduced expression of MHC class I and II genes as well as reduced infiltration with CD4+ cells [17], suggesting that they have undergone immune escape through reduced presentation of FSP neo-antigens. Such immune escape in MSI CRC has previously been linked to mutation in genes that regulate MHC class I (e.g. *B2M*) and class II (e.g. *CIITA, RFX5*) function [28,29]. Additionally, in this work, the authors describe recurrent mutations in *AIM2* (an upstream regulator of MHC class II function [30]) as a mechanism for reduced FSP presentation. Interestingly, G1 tumours show reduced expression of the T-cell immune checkpoint proteins, PD-1 and CTLA-4, compared to reference mucosa, consistent with a

reduced infiltration with lymphocytes. Conversely, sporadic MSI CRCs tend to have a strong lymphocytic infiltrate and show increased expression of immune-checkpoints [21,22]. Overall, these findings are consistent with marked immunoediting of G1 CRCs that arise in LS. In the early stages of carcinogenesis, the immune system is able to keep small foci of neoplastic cells in check (elimination phase). However, the inevitable accumulation of somatic mutations in dMMR cells leads to mechanisms to avoid immune destruction and the progression to CRC (equilibrium and escape phases) [see Figure 1].

Implications for cancer therapy and clinical practice

Clinically, dMMR cancers tend to be immunogenic and therefore have the potential to be targeted with specific immunotherapy. MSS CRCs, on the other hand, are not as immunogenic and would be less likely to respond to immunotherapy. Although LS CRCs have dMMR, their response to immunotherapy may be more difficult to predict. G2 CRCs are less immunogenic, bearing resemblance to sporadic MSS CRCs, and therefore might be less likely to respond. G1 CRCs arise in a highly immunogenic environment, suggesting that they would be good candidates for treatment with immunotherapy. However these tumours have undergone immune escape with reduced antigen presentation and reduced lymphocytic infiltrate, meaning that some immunotherapies may not be as effective as compared with sporadic MSI CRCs.

Clinical trials are already underway for the use of immune-checkpoint inhibitors such as pembrolizumab and nivolumab for MSI CRC [23,24]. Another approach, based on the idea of 'auto-immunisation' in patients with LS, is to develop a vaccine against common FSP neo-antigens expressed by MSI CRCs. Such a vaccine has completed phase I/IIa clinical trials with long-term results awaited [31]. Different approaches for vaccine delivery have also been tried, including peptide loading of patient's monocyte-derived dendritic cells *ex vivo* and administration to the patient as a

cellular vaccine [32]. In addition, as adoptive transfer of 'CAR T-cells' (cytotoxic T-lymphocytes engineered to express a chimeric antigen receptor recognising a particular antigen [33]) has recently gained FDA approval for the management of various haematological malignancies [34,35], then such an approach has the potential to be used in MSI CRC by creating engineered T-cells, either with an engineered T-cell receptor (TCR) or CAR, against FSP neo-antigens. Adoptive transfer of cytotoxic T-cells, with an engineered TCR against a common MSI FSP, has demonstrated efficacy in a xenograft mouse model [36], but a trial in humans is yet to be conducted. Although not yet experimentally tested, CAR T-cells against membrane-associated FSP neo-antigens could represent an ideal way to target G1 LS CRCs and sporadic MSI CRCs that have undergone immune escape, as there is no requirement for FSP neo-antigens to be presented by MHC class I (since the immunoglobulin domain of a CAR can directly target FSPs).

The implications of these findings of LS tumour heterogeneity include the need for greater use of MMR analysis in all LS and sporadic MSI cancers, both biopsies and resections, including CRCs, endometrial cancers and other LS-related cancers. This should include combined MMR immunohistochemistry and tumour MSI testing for LS screening, prognosis, prediction of treatment responsiveness (both conventional chemotherapy and immunotherapies), and pre-operative planning of appropriate surgical operations. Binder et al's work [17] also argues for further work to establish whether this should also now involve further tumour testing for G1 versus G2 MSI subtypes, along with quantitative analysis and characterisation of tumour infiltrating lymphocytes for more sophisticated prediction of responsiveness to the emerging range of immunotherapies. Moving forward into the era of digital pathology, more advanced image-analysis techniques will facilitate such a quantitative description of tumour infiltrating lymphocytes, and prognostic parameters such as the 'Immunoscore' [37] are likely to become an integral part of the pathology report in CRC.

References:

1 Syndromes - InSiGHT. [Accessed April 27, 2018] Available from: https://www.insight-group.org/syndromes/.

2 Noffsinger AE. Serrated Polyps and Colorectal Cancer: New Pathway to Malignancy. *Annu Rev Pathol Mech Dis* 2009; **4**: 343-364

3 Nilbert M, Planck M, Fernebro E, *et al.* Microsatellite instability is rare in rectal carcinomas and signifies hereditary cancer. *Eur J Cancer* 1999; **35**: 942-945

4 de Rosa N, Rodriguez-Bigas MA, Chang GJ, *et al.* DNA Mismatch Repair Deficiency in Rectal Cancer: Benchmarking Its Impact on Prognosis, Neoadjuvant Response Prediction, and Clinical Cancer Genetics. *J Clin Oncol* 2016; **34**: 3039-3046

5 Snowsill T, Huxley N, Hoyle M, *et al.* A systematic review and economic evaluation of diagnostic strategies for Lynch syndrome. *Health Technol Assess (Rockv)* 2014; **18**: 1-406

6 Jensen LH, Bojesen A, Byriel L, *et al.* Implementing population-based screening for Lynch syndrome. *J Clin Oncol* 2013; **31**: 6600

7 Yurgelun MB, Kulke MH, Fuchs CS, *et al.* Cancer Susceptibility Gene Mutations in Individuals With Colorectal Cancer. *J Clin Oncol* 2017; **35**: 1086-1095

8 International Society for Gastrointestinal Hereditary Tumours Database. [Accessed April 27, 2018] Available from: https://www.insight-database.org/genes.

9 Molecular testing strategies for Lynch syndrome in people with colorectal cancer | Guidance and guidelines | NICE. [Accessed April 27, 2018] Available from: https://www.nice.org.uk/guidance/dg27.

10 Nakken S, Hovig E, Møller P. Prospective Lynch Syndrome Database (PLSD) - cumulative risk

for cancer by age, genetic variant, and gender. [Accessed April 28, 2018] Available from: www.PLSD.eu.

11 Ahadova A, von Knebel Doeberitz M, Bläker H, *et al.* CTNNB1-mutant colorectal carcinomas with immediate invasive growth: a model of interval cancers in Lynch syndrome. *Fam Cancer* 2016; **15**: 579-586

12 Ahadova A, Gallon R, Gebert J, *et al.* Three molecular pathways model colorectal carcinogenesis in Lynch syndrome. *Int J Cancer* 2018; **143**: 139-150

13 Kloor M, Huth C, Voigt AY, *et al.* Prevalence of mismatch repair-deficient crypt foci in Lynch syndrome: a pathological study. *Lancet Oncol* 2012; **13**: 598-606

14 Staffa L, Echterdiek F, Nelius N, *et al.* Mismatch Repair-Deficient Crypt Foci in Lynch Syndrome – Molecular Alterations and Association with Clinical Parameters. Castresana JS, ed. *PLoS One* 2015; **10**: e0121980

15 Møller P, Seppälä T, Bernstein I, *et al.* Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. *Gut* 2017; **66**: 464-472

16 Seppälä T, Pylvänäinen K, Evans DG, *et al.* Colorectal cancer incidence in path_MLH1 carriers subjected to different follow-up protocols: a Prospective Lynch Syndrome Database report. *Hered Cancer Clin Pract* 2017; **15**: 18

17 Binder H, Hopp L, Schweiger MR, *et al.* Genomic and transcriptomic heterogeneity of colorectal tumours arising in Lynch syndrome. *J Pathol* 2017; **243**: 242-254

18 Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539-545

19 Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. *Cell* 2011; **144**: 646-674

20 Dunn GP, Old LJ, Schreiber RD. The Three Es of Cancer Immunoediting. *Annu Rev Immunol* 2004; **22**: 329-360

21 Buckowitz A, Knaebel H-P, Benner A, *et al.* Microsatellite instability in colorectal cancer is associated with local lymphocyte infiltration and low frequency of distant metastases. *Br J Cancer* 2005; **92**: 1746-1753

22 Llosa NJ, Cruise M, Tam A, *et al.* The Vigorous Immune Microenvironment of Microsatellite Instable Colon Cancer Is Balanced by Multiple Counter-Inhibitory Checkpoints. *Cancer Discov* 2015; **5**: 43-51

23 Diaz LA, Le DT, Yoshino T, *et al.* KEYNOTE-177: Randomized phase III study of pembrolizumab versus investigator-choice chemotherapy for mismatch repair-deficient or microsatellite instability-high metastatic colorectal carcinoma. *J Clin Oncol* 2017; **35**: TPS815

24 Overman MJ, McDermott R, Leach JL, *et al.* Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol* 2017; **18**: 1182-1191

25 Kloor M, von Knebel Doeberitz M. The Immune Biology of Microsatellite-Unstable Cancer. *Trends in cancer* 2016; **2**: 121-133

26 Schwitalle Y, Kloor M, Eiermann S, *et al.* Immune Response Against Frameshift-Induced Neopeptides in HNPCC Patients and Healthy HNPCC Mutation Carriers. *Gastroenterology* 2008; **134**: 988-997

27 Stigliano V, Assisi D, Cosimelli M, *et al.* Survival of hereditary non-polyposis colorectal

cancer patients compared with sporadic colorectal cancer patients. *J Exp Clin Cancer Res* 2008; **27**: 39

28 Kloor M, Michel S, Buckowitz B, *et al.* Beta2-microglobulin mutations in microsatellite unstable colorectal tumors. *Int J Cancer* 2007; **121**: 454-458

29 Surmann E-M, Voigt AY, Michel S, *et al.* Association of high CD4-positive T cell infiltration with mutations in HLA class II-regulatory genes in microsatellite-unstable colorectal cancer. *Cancer Immunol Immunother* 2015; **64**: 357-366

30 Lee J, Li L, Gretz N, *et al.* Absent in Melanoma 2 (AIM2) is an important mediator of interferon-dependent and -independent HLA-DRA and HLA-DRB gene expression in colorectal cancers. *Oncogene* 2012; **31**: 1242-1253

31 Kloor M, Reuschenbach M, Karbach J, *et al.* Vaccination of MSI-H colorectal cancer patients with frameshift peptide antigens: A phase I/IIa clinical trial. *J Clin Oncol* 2015; **33**: 3020

32 Dendritic Cell Vaccination in Patients With Lynch Syndrome or Colorectal Cancer With MSI. [Accessed April 27, 2018] Available from: https://clinicaltrials.gov/ct2/show/NCT01885702.

33 Houot R, Schultz LM, Marabelle A, *et al.* T-cell-based Immunotherapy: Adoptive Cell Transfer and Checkpoint Inhibition. *Cancer Immunol Res* 2015; **3**: 1115-1122

34 U.S. Food and Drug Administration. Press Announcements - FDA approval brings first gene therapy to the United States. [Accessed December 2, 2017] Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm.

35 U.S. Food and Drug Administration. Press Announcements - FDA approves CAR-T cell therapy to treat adults with certain types of large B-cell lymphoma. [Accessed December 2, 2017] Available from:

https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm581216.htm.

36 Inderberg EM, Wälchli S, Myhre MR, *et al.* T cell therapy targeting a public neoantigen in microsatellite instable colon cancer reducesin vivotumor growth. *Oncoimmunology* 2017; **6**: e1302631

37 Galon J, Mlecnik B, Bindea G, *et al.* Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. *J Pathol* 2014; **232**: 199-209

Table 1: Comparison of properties of G1 and G2 CRCs in LS (as defined by Binder et al. [17]) with both MSI and MSS sporadic CRC. Data all from [17].

	Colorectal cancers			
	Lynch Syndrome		**Sporadic**	
	MSI (G1)	**MSI (G2)**	**MSI**	**MSS**
Relative Mutation Numbers	High	Low	High	Low
Microsatellite slippage	High	Low	High	Low
Gene expression profile	DOWN-regulation of immune system and inflammatory genes. UP-regulation of cell cycle and proliferation genes	UP-regulation of mucosal and stromal genes	UP-regulation of cell cycle and proliferation genes	UP-regulation of cell cycle and proliferation genes

Figure 1: Mechanisms of Immunoediting in dMMR CRC. Both G1 CRCs in Lynch syndrome and sporadic MSI CRCs elicit a strong immune response and undergo significant immunoediting. Cytotoxic T-lymphocytes (CTL) are able to recognise immunogenic frameshift peptides (FSPs) that are synthesised in dMMR cells as a result of MSI. However, tumour cells may evolve mechanisms to prevent immune destruction including inhibition of CTLs by upregulating expression of immune-checkpoints (e.g. PD-L1) or through various genetic mutations leading to loss FSP antigen presentation.