# Assay result variability during determination of mismatch repair deficiency status using immunohistochemistry – a transatlantic comparative study

UNIVERSITY OF LEEDS

**G Hutchins[1]**, K Handley[2], L Magill[2], F Baehner[3], M Lopatin[4], H Yaziji[5], M Lee[4], M Seymour[6], D Kerr[7], R Gray[2] and P Quirke[1] on behalf of the QUASAR-1 trial collaborators

[1]Leeds Institute of Molecular Medicine, University of Leeds, UK. [2]Birmingham Clinical Trials Unit, University of Birmingham, UK. [3]University of California, San Fransisco, CA. [4]Genomic Health, Redwood City, CA. [5]Vitro Molecular Laboratories, Miami, FL. [6]CRUK Cancer Centre, University of Leeds, UK. [7]Sidra Medical and Research Centre, Doha, Qatar

Pathological Society Understanding Disease | yorkshire cancer research | Leeds Institute of Molecular Medicine | The Birmingham Clinical Trials Unit | QUASAR

## Purpose

Colorectal cancer (CRC) patients with deficient mismatch repair (dMMR) have a significantly reduced risk of tumour recurrence and may respond less well to chemotherapy. Determination of MMR status is therefore advocated to identify patients in whom adjuvant therapy is not indicated because of their low recurrence risk.

Despite substantial evidence to support the use of immunohistochemistry (IHC) to determine MMR status, little is known regarding the variability of assay results – how reproducible is IHC in the determination of MMR status? We aimed to define MMR IHC assay reproducibility using formalin-fixed, paraffin-embedded (FFPE) material from the QUASAR randomised control trial (ISRCTN82375386) contained within heterogeneity-prone tissue microarray (TMA) material. Single observer reproducibility (intra-observer agreement) was also assessed.

## Methods

Resected pathological material was obtained from 3239 patients (91% stage II) entered into the QUASAR randomised control trial of 5-fluorouracil (5-FU) / folinic acid (FA) chemotherapy versus observation alone (ISRCTN82375386). Material from 2007 patients was suitable for TMA construction. Tissue sections derived from identical TMAs were distributed to Leeds Institute of Molecular Medicine (LIMM) in the United Kingdom (UK) and Vitro Molecular Laboratories (VML) in the United States (US) for MMR testing using IHC techniques.

Expression of MMR proteins MLH1 and MSH2 was independently evaluated by LIMM and VML using IHC. Exact IHC methodologies employed by each laboratory were variable and blinded to the other unit. For ***inter-laboratory agreement*** analyses (table 1 a,b,c.), following case exclusions or losses, the MMR status for 1224 stage II colon cancer patients was determined independently in both laboratories and compared; outcomes assessed included anatomical distribution of discordant cases, % agreement of scores and kappa coefficients. For ***intra-observer agreement*** analyses (table 2 a,b,c.), following losses, MMR status was comparable in 1826 stage II / III CRC patients. ***Intra-observer*** agreement was determined by a single pathologist assessment of MMR-stained slides from both VML and LIMM. Again outcomes assessed included the anatomical distribution of discordant cases, % agreement and kappa coefficients.

## Results – Inter-laboratory / intra-observer agreement

### INTER-LABORATORY AGREEMENT

| MMR | VML MMR STATUS (n, %) | | | |
|---|---|---|---|---|
| LIMM MMR STATUS (n, %) | | dMMR | pMMR | TOTALS |
| | dMMR | 140 (12.2) | 39 (3.2) | 179 |
| | pMMR | 20 (1.6) | 1025 (83.7) | 1045 |
| | TOTAL | 160 | 1064 | 1224 |

**Table 1a – Inter-laboratory MMR scoring agreement**
**95.1% agreement**
**κ=0.798 (0.748 – 0.848), p<0.0001**

| MLH1 | VML MLH1 STATUS (n, %) | | | |
|---|---|---|---|---|
| LIMM MLH1 STATUS (n, %) | | dMMR | pMMR | TOTALS |
| | dMMR | 115 (9.4) | 36 (2.9) | 151 |
| | pMMR | 20 (1.6) | 1053 (86) | 1073 |
| | TOTAL | 135 | 1089 | 1224 |

**Table 1b – Inter-laboratory MLH1 scoring agreement**
**95.3% agreement**
**κ=0.778 (0.722 – 0.835), p<0.0001**

| MLH2 | VML MSH2 STATUS (n, %) | | | |
|---|---|---|---|---|
| LIMM MSH2 STATUS (n, %) | | dMMR | pMMR | TOTALS |
| | dMMR | 24 (2) | 7 (0.6) | 31 |
| | pMMR | 3 (0.2) | 1189 (97.2) | 1192 |
| | TOTAL | 27 | 1196 | 1223 |

**Table 1c – Inter-laboratory MSH2 scoring agreement**
**99.1% agreement**
**κ=0.823 (0.714 – 0.932), p<0.0001**

### INTRA-OBSERVER AGREEMENT

| MMR | VML SLIDE MMR STATUS (n, %) | | | |
|---|---|---|---|---|
| LIMM SLIDE MMR STATUS (n, %) | | dMMR | pMMR | TOTALS |
| | dMMR | 175 (9.6) | 18 (1) | 193 |
| | pMMR | 5 (0.3) | 1628 (89.2) | 1633 |
| | TOTAL | 180 | 1646 | 1826 |

**Table 2a – Intra-observer MMR scoring agreement**
**98.74% agreement**
**κ=0.931 (0.903 – 0.959), p<0.0001**

| MLH1 | VML SLIDE MLH1 STATUS (n, %) | | | |
|---|---|---|---|---|
| LIMM SLIDE MLH1 STATUS (n, %) | | dMMR | pMMR | TOTALS |
| | dMMR | 149 (8.2) | 16 (0.9) | 165 |
| | pMMR | 5 (0.3) | 1656 (90.7) | 1661 |
| | TOTAL | 154 | 1672 | 1826 |

**Table 2b – Intra-observer MLH1 scoring agreement**
**98.85% agreement**
**κ=0.928 (0.897 – 0.958), p<0.0001**

| MSH2 | VML SLIDE MSH2 STATUS (n, %) | | | |
|---|---|---|---|---|
| LIMM SLIDE MSH2 STATUS (n, %) | | dMMR | pMMR | TOTALS |
| | dMMR | 26 (1.4) | 6 (0.3) | 36 |
| | pMMR | 0 | 1793 (98.2) | 1793 |
| | TOTAL | 26 | 1799 | 1825 |

**Table 2c – Intra-observer MSH2 scoring agreement**
**99.67% agreement**
**κ=0.895 (0.811 – 0.978), p<0.0001**

## Results – discordant cases

| Discordant MMR cases (n, %) | | | |
|---|---|---|---|
| | **VML dMMR** | **LIMM dMMR** | **Totals** |
| **Left colon / rectum** | 11 (55) | 5 (12.8) | 16 |
| **Right colon** | 9 (45) | 33 (84.6) | 42 |
| **No data** | 0 (0) | 1 (2.5). | 1 |
| **TOTAL** | 20 | 39 | 59 |

**Table 3a – Inter-laboratory dMMR status discordance by anatomical distribution**

| Discordant MLH1 cases (n, %) | | | |
|---|---|---|---|
| | VML dMMR | LIMM dMMR | **Totals** |
| **Left colon / rectum** | 12 (60) | 4 (11.1) | 16 |
| **Right colon** | 8 (40) | 31 (86.1) | 39 |
| **No data** | 0 (0) | 1 (2.77). | 1 |
| **TOTAL** | 20 | 36 | 56 |

**Table 3b – Inter-laboratory MLH1 status discordance by anatomical distribution**

| Discordant MSH2 cases (n, %) | | | |
|---|---|---|---|
| | VML dMMR | LIMM dMMR | **Totals** |
| **Left colon / rectum** | 1 (33.3) | 2 (28.5) | 3 |
| **Right colon** | 2 (66.6) | 4 (57.1) | 6 |
| **No data** | 0 (0) | 1 (14.2). | 1 |
| **TOTAL** | 3 | 7 | 10 |

**Table 3c – Inter-laboratory MSH2 status discordance by anatomical distribution**

- ***Inter-laboratory*** and ***intra-observer*** agreement was good to excellent for all comparisons (tables 1 and 2). ***Inter-laboratory*** agreement varied from 95.1% (dMMR status overall) to 99.1% agreement (MSH2). ***Intra-observer*** agreement ranged from 98.7% for dMMR overall to 99.6% for MSH2.
- ***Inter-laboratory*** correlation coefficients for dMMR status, MLH1 status and MSH2 status were 0.798, 0.778 and 0.823 respectively (p<0.0001). Identical measures for ***intra-observer*** dMMR, MLH1 and MSH2 status were 0.93, 0.92 and 0.89 respectively (p<0.0001)
- 59 of 1224 cases(4.82%) in the ***inter-laboratory*** comparisons were discordant for dMMR status. The majority of dMMR discordance was a consequence of MLH1 discordance [56 of 1224 cases (4.6%)]. 12 of 20 (60%) MLH1 discordant cases reported by VML (pMMR by LIMM) originated in the left colon. The majority of discordant MLH1 cases (31 of 36, 86.1%) reported by LIMM were in the right colon. 10 of 1223 (0.8%) cases assessed for MSH2 status were discordant.
- The precise reasons for dMMR / MLH1 / MSH2 case discordance are currently under investigation.

## Conclusions

- Independent determination of MMR status by IHC on CRC TMA material is associated with good to excellent ***inter-laboratory*** and ***intra-observer*** agreement with the latter demonstrating excellent assay reproducibility (tables 1 / 2).
- The anatomical distribution of ***intra-laboratory*** discordant MMR cases ***may*** highlight possible false-negative cases as the dMMR phenotype is associated with the right colon.
- These data validate the routine use of IHC to determine MMR status, particularly as a result of whole tissue section IHC being less vulnerable to sampling heterogeneity when compared to TMAs.
- The precise reasons for MMR status discordance are currently under investigation.
- Inter-observer agreement is remains to be defined and is currently under investigation.