SUPPLEMENTARY METHODS

p53 and HMGCR immunohistochemistry

All immunohistochemistry (IHC) was performed in the Northern Ireland Molecular Pathology Laboratory, a research laboratory that has UK Clinical Pathology Accreditation. Sections for IHC were cut at 4µm on a rotary microtome and dried at 37°C overnight. IHC was performed on the fully automated Leica BOND-MAX (Leica Microsystems (UK) Ltd, Milton Keynes, UK) platform using a polymer based antibody detection system. Following initial internal validations of both technical and expression parameters, p53 IHC was performed on the TMAs with a DO-7 antibody clone to p53 (Dako UK Ltd, Ely, UK - catalogue number M7001) with 30 minutes of Epitope Retrieval Solution 1 (Leica Microsystems) as optimal pre-treatment and 15 minutes incubation in a 1:100 dilution of the primary antibody. Similarly, HMGCR expression was evaluated on the TMAs with a rabbit polyclonal antibody to HMGCR (Atlas Antibodies AB, Stockholm, Sweden - catalogue number HPA008338) with 20 minutes of Epitope Retrieval Solution 2 (Leica Microsystems) as optimal pre-treatment and 60 minutes incubation in a 1:50 dilution of the primary antibody.

Immunohistochemical assessment and scoring

Scanned images of immunohistochemically stained TMA sections were hosted remotely by PathXL (Belfast, Northern Ireland). *QuPath* (Queen's University Belfast, Northern Ireland) image analysis software facilitated digital immunoscoring using QuPath's custom integration with PathXL's application

programming interface. Briefly, scanned images of immunohistochemically stained TMA sections were dearrayed and computational color deconvolution[1] was applied to separate the haematoxylin and 3,3'-diaminobenzidine (DAB) stains. A novel detection algorithm was used to differentiate tumour and non-tumour cells. After calibration of immunopositivity thresholds modified H-scores were calculated based on the extent and intensity of cytoplasmic or nuclear staining where appropriate (H-score = 3 x % of strongly staining cytoplasm + 2 x % of moderately staining cytoplasm + 1 x % of weakly staining cytoplasm, giving a range of 0 to 300).[2]

Following review of these expression patterns and visual comparison of images with their corresponding H-scores, the H-score thresholds for the extreme negative, non-extreme, and extreme positive categories of staining patterns were set as ≤10, >10 - ≤150, and >150 respectively. To assess the validity of these thresholds an expert gastrointestinal histopathologist independently assessed p53 expression patterns in a random sample (n=89) of the triplicate TMA cores. There was strong concordance between H-score and histopathologist-based p53 expression grades (agreement=95.5%, weighted κ=0.88).

DNA extraction

Tumour-rich areas of the representative blocks from each case were annotated for macrodissection. DNA was extracted according to the manufacturer's instructions from five 5μm sections using the Maxwell 16 Instrument (Promega, Southampton, UK) and Promega DNA extraction kit.

Quantification of DNA was performed by an absorbance method using the NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, USA).

KRAS mutation and microsatellite instability status

A mass spectrometry platform incorporating the pre-validated ColoCarta Panel (Agena Bioscience, Hamburg, Germany) was used to assess for the presence of KRAS mutations. The ColoCarta Panel includes assays that capture 98% of the known KRAS mutations in colorectal cancer.[3] Samples were processed at the Genomics Core Technology Unit (Queen's University Belfast, Northern Ireland) and the Assays by Agena Custom Services Laboratory (Hamburg, Germany) using previously described methods.[4] Detection of somatic mutations at a frequency of greater than 10% for any of 11 alleles (A59T, G12A, G12C, G12D, G12F, G12R, G12S, G12V, G13D, G61H, Q61L) was considered evidence of a mutation in the KRAS gene. A failed reaction at a single position resulted in missing data for KRAS status only if the reactions at other positions were wild-type.[3]

The Northern Ireland Molecular Pathology Laboratory assessed for microsatellite instability (MSI) according to the manufacturer's instructions using the MSI Analysis System, version 1.2 kit (Promega, Southampton, UK) for five mononucleotide repeat markers (BAT-25, BAT-26, NR-21, NR-24 and MONO-27). PCR products were separated by capillary electrophoresis using an ABI 3500 Genetic Analyzer (Fisher Scientific – UK Ltd, Loughborough, UK). The output data was analysed using GeneMapper® v4.1 (Fisher Scientific – UK Ltd, Loughborough, UK) to determine MSI-high status (MSI-high versus non MSI-high).

REFERENCES

1. Ruifrok AC, Johnston DA. Quantification of histochemical staining by color deconvolution. *Anal Quant Cytol Histol*. 2001;23(4):291-299.

2. McCarty KS, Szabo E, Flowers JL, et al. Use of a monoclonal anti-estrogen receptor antibody in the immunohistochemical evaluation of human tumors. *Cancer Res*. 1986;46(8 Suppl):4244s-4248s.

3. Gavin PG, Colangelo LH, Fumagalli D, et al. Mutation profiling and microsatellite instability in stage II and III colon cancer: an assessment of their prognostic and oxaliplatin predictive value. *Clin Cancer Res*. 2012;18(23):6531-6541.

4. Fumagalli D, Gavin PG, Taniyama Y, et al. A rapid, sensitive, reproducible and cost-effective method for mutation profiling of colon cancer and metastatic lymph nodes. *BMC Cancer*. 2010;10:101. doi:10.1186/1471-2407-10-101.