

# Intratumoural immune signature to identify patients with primary colorectal cancer who do not require follow-up after resection: an observational study

John N Primrose,<sup>1\*</sup> Siân A Pugh,<sup>1,2</sup> Gareth Thomas,<sup>1</sup> Matthew Ellis,<sup>1</sup> Karwan Moutasim<sup>1,3</sup> and David Mant<sup>4</sup>

<sup>1</sup>Cancer Sciences Division, University of Southampton, Southampton, UK

<sup>2</sup>Medical Oncology, Addenbrookes Hospital, Cambridge, UK

<sup>3</sup>Cellular Pathology, University Hospital Southampton NHS Foundation Trust, Southampton, UK

<sup>4</sup>Department of Primary Care, University of Oxford, Oxford, UK

\*Corresponding author [j.n.primrose@soton.ac.uk](mailto:j.n.primrose@soton.ac.uk)

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## Scientific summary

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# Scientific summary

## Background

Following primary surgical and adjuvant treatment for colorectal cancer, patients are routinely followed up for  $\geq 5$  years. This involves blood testing for carcinoembryonic antigens and a variable number of computerised tomography scans. The Follow-up After Colorectal Surgery (FACS) trial showed that this follow-up is effective in detecting recurrences that are treatable with curative intent. However, as only 20% of patients relapse, many patients are followed up (resulting in the spend of attendant costs and inducing patient anxiety) but never develop recurrence. It is known that the immunological response to cancer manifested by the presence of intratumoural lymphocytes, especially T cells [cluster of differentiation (CD)3+] and memory T cells (CD45RO+), correlates strongly with outcome. We surmised that it may be possible to use the data from immunophenotyping of the primary tumours to identify a cohort of patients in whom relapse is unlikely and follow-up is not needed. The FACS trial is an ideal opportunity to investigate this possibility as patients have a defined follow-up strategy after standard of care treatment and good follow-up data.

## Aim and objectives

The aim was to determine whether or not the density of CD45RO+ and CD3+ lymphocytes in the primary tumour from patients in the FACS trial can be used to predict the possibility of relapse. The data from the analysis of these initial markers were intended to provide guidance on what other markers may be of value in supporting the primary hypothesis.

## Methods

### *Pathology blocks*

Tissue was obtained from the primary tumours and from the FACS trial, a 2 × 2 pragmatic, randomised, factorial controlled trial comparing minimum post-surgery follow-up of colorectal cancer patients for 5 years with 3- to 6-monthly blood tests for carcinoembryonic antigen and 6- to 12-monthly computerised tomography imaging. As the overall survival was similar in all arms of the trial, we included all of the patients from the trial in the tissue collection. Tissue was stored in the Southampton Tissue Bank until it was used.

### *Tissue analysis*

A full-face section was taken from each block and stained with standard haematoxylin and eosin. The blocks were marked to allow sampling of cores to create tissue microarrays. Three cores were taken from the centre of the tumour and three were taken from the invasive margin. The tissue microarrays were sectioned and stained for CD45RO+ and CD3+ T lymphocytes as well as being stained with standard haematoxylin and eosin in the first instance.

### *Cell counting*

Initially, the intention was to manually count lymphocytes in the tissue microarrays. This was attempted but was abandoned because the time needed to undertake the work was not feasible for the individuals concerned. Counting was then attempted using an automated cell counter. A Zeiss Axio Scan.Z1 (ZEISS, Oberkochen, Germany) whole-slide scanner was utilised at a resolution equivalent to  $\times 20$  magnification. The initial image analysis was carried out using QuPath (developed at the University of Edinburgh; originally created at the Centre for Cancer Research & Cell Biology at Queen's University Belfast as

part of research projects funded by Invest Northern Ireland and Cancer Research UK). Various technical issues were encountered, including misregistration of the tissue microarray cores by the software (requiring manual relocation, which was a prolonged exercise). Some preliminary data were obtained on 287 patients prior to the scientist running the scanner relocating. These results represent only part of the cohort and, as such, are unsuitable for publication. However, they give an indication that a signal is present which warrants pursuing the project further.

### **Relevance and implications**

This study was not feasible as originally conceived because manual counting of this sample size would be possible within a 'citizen science'-type programme only, because of the time-consuming nature of the task. It has been demonstrated that machine counting may be possible if there is suitably functioning hardware and software combined with available technical expertise. It seems likely that the use of machine counting of tissue microarrays of colorectal tumours will give prognostic information, and preliminary data suggest that the survival benefit associated with a high infiltrate of CD3- and CD45RO-positive immune cells is limited to those with left-sided tumours. Further work is required to verify the results and to include a much larger cohort to analyse additional immune markers to confirm these observations. It is not clear whether or not the immune signature as assessed by these methods will be able to identify a subset of patients in whom follow-up is not required.

### **Trial registration**

This trial is registered as ISRCTN41458548.

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