CLINICIAN INFORMATION SHEET

Study Title	Prognostic value of ploidy and digital tumour-stromal morphometric analyses for guiding chemotherapy treatment for Stage II / IIIa Colon Cancer Patients (ONCOPROG_AI)
Chief Investigator	Prof David Kerr
Contact Number	01865 784743
Protocol Number	V.16.1
Sponsor Name	Oxford Cancer Biomarkers Limited (OCB)
IRAS ID	330060
Name of Research Ethics Committee	East of England – Cambridge South

Title of Study

OncoProg®: Prognostic value of ploidy and digital tumour-stromal morphometric analyses for guiding chemotherapy treatment for stage II / IIIa Colon Cancer Patients

Summary

OncoProg® is a CE and UKCA marked digital pathology software as a medical device, for assessing the risk of cancer recurrence after surgical resection. Based on digital images and clinical data on over 2,500 patients with stage II-III CRC, OncoProg had a prognostic value with a hazard ratio (HR) for the intermediate - versus low-risk group [HR 1.9 (95% CI: 1.4–2.6)] and for the for the high- versus low-risk group, [HR (2.9 (95%CI:2.0–4.3)].

Unmet Medical Need in Early-Stage Colorectal Cancer (CRC)

CRC is frequently cited as the third most common cancer after breast and lung in both men and women. In 2020 there were 1.93 million new CRC diagnoses globally accounting for 10.7% of all new cancers, and CRC accounted for 9.2% of all cancer related deaths only second only to lung cancer [(1)].

The prevalence of CRC carries an economic burden to healthcare systems resulting in approximately 10% of total cancer related costs, with annual costs in the EU of €13 billion. In the UK alone 41,804 new cases were diagnosed in 2015 accounting for 12% of the total cancer diagnoses. Of these CRC diagnoses around 10% of patients were diagnosed at stage I, 35% were diagnosed at stage II, 35% at stage III and 20% at stage IV. Whilst treatment options for stage III and IV patients are generally accepted, with clinical guideline information to assist clinicians in making treatment choices for their patients, the situation for stage II cancer is more unclear [(2-4)].

In stage II patients, 5-year overall survival (OS) after surgical resection alone is approximately 80%. A proportion (around 20%) of stage II cancers carry the risk of micrometastatic disease and the main purpose of adjuvant chemotherapy after surgery is to destroy these micrometastases before they develop further [(1,4)]. This suggests that patients with stage II cancer are most likely comprised of a heterogeneous population of patients that consist of those curable by surgery alone (80%), those with micrometastatic disease that may

not be susceptible to adjuvant chemotherapy (16%) and those with micrometastatic disease that would be eradicated by adjuvant chemotherapy (4%) [5].

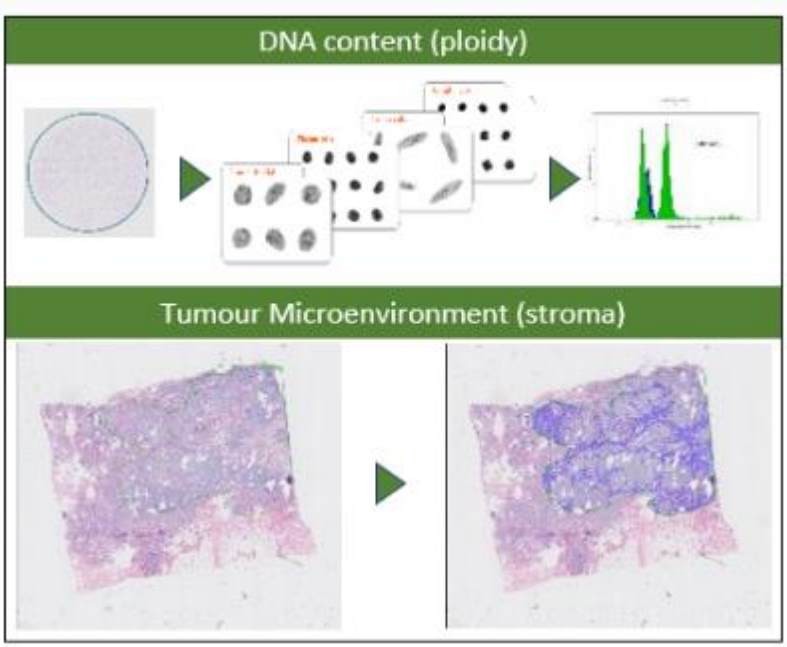
Due to this small margin of benefit, determining which patients would benefit most from chemotherapy (i.e. determining those at highest risk of mortality or disease recurrence) is challenging, and no clear guidelines exist to aid clinicians[6].

There is increasing evidence that improved stratification of stage II patients into groups that reflect the risk of micrometastases should improve patient outcomes. It follows that there is an unmet clinical need for predictive markers to risk-stratify patients within this stage II subset so that there is a better understanding of who is at the greatest risk of recurrence and therefore who would most benefit from adjuvant chemotherapy.

The Solution – OncoProg®

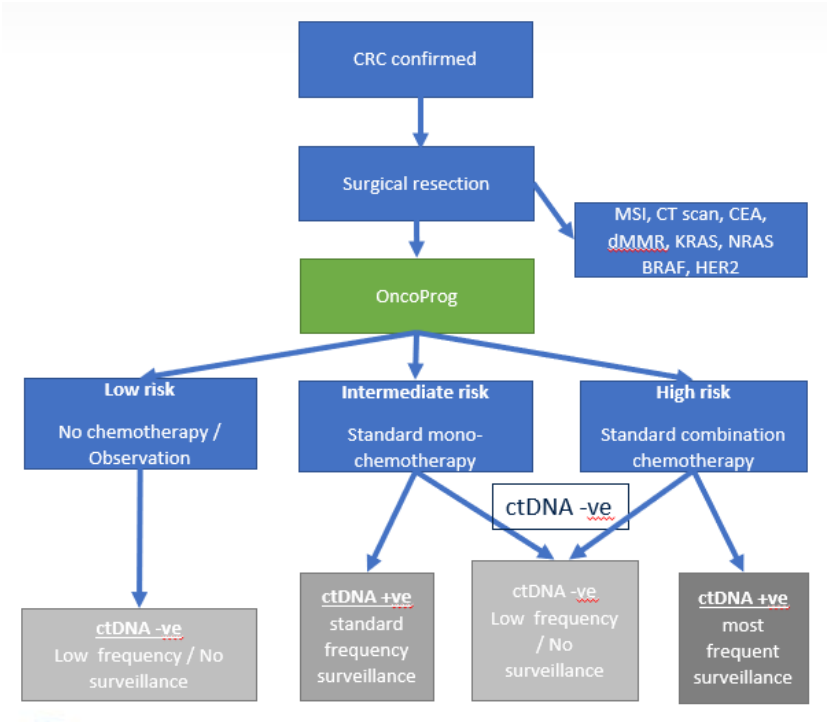
There are several pathological features that are associated with poor prognosis in stage II CRC, and these should be considered when adjuvant chemotherapy is considered. Based on these observations, Oxford Cancer Biomarkers Ltd (OCB) have developed a digital pathology-based product, OncoProg®.

OncoProg® is a CE and UKCA-marked tool that uses image processing software algorithms to analyse whole slide images of resected tumour tissue. The system combines analyses of tumour cell DNA content as a measure of genomic instability (DNA ploidy) and an evaluation of the impact of the tumour microenvironment (tumour stroma content) to stratify samples into categories indicative of the risk of disease relapse ("low", "intermediate" or "high" risk). This information is then used by the clinicians as a decision-making tool to identify those people who would benefit from adjuvant chemotherapy.



Adoption of OncoProg® into clinical pathways may lead to better identification of patients who should be offered standard single agent chemotherapy ("intermediate risk") or combination treatment ("high risk") whilst also identifying "low risk" patients who may be amenable to observation alone and therefore spared unnecessary chemotherapy and risk of side effects. A reduction in the proportion of patients treated with chemotherapy may also lead to reduced overall health care costs. The key benefit of OncoProg® is to provide a clinical decision support in predicting whether a patient with CRC (stage II) would benefit from chemotherapy following surgical resection.

OncoProg® could complement existing and future precision medicine clinical decision tools.

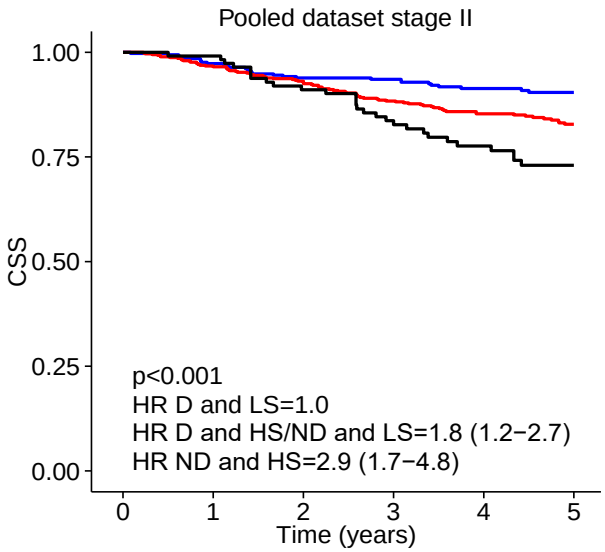


Clinical Evidence

The prognostic value of these two parameters were combined and evaluated in a study by Danielsen *et al*, using tissue samples collected from participants of 3 large studies (one prospectively recruiting multi-centre randomised controlled-trial [the QUASAR trial], and 2 large cohort studies) representing a total of 2,624 tumour samples from patients with early-stage CRC including over 1,000 patients with stage II disease.

The combination of DNA ploidy and tumour stroma fraction allowed stratification of stage II patients into three clinically useful groups with a 5-year cancer-specific survival (CSS) of 90% vs 83% vs 73% (HR=1·77 [95% CI 1·13-2·77] and HR=2·95 [95% CI 1·73-5.03], p<0·001 respectively)[7]. Overall, OncoProg® categorised stage II patients into :

- "low risk" (diploid and low stroma) - 33.5%
- "intermediate risk" (non-diploid and low stroma OR diploid and high stroma) - 55%
- "high risk" (non-diploid and high stroma) - 11.5%



The combination of ploidy and stroma has also been clinically validated in an initial study of 188 stage II colon cancer patients in China where the OncoProg® test predicted disease-free survival (DFS) with patients stratified into high and low risk groups with HR = 4.036 [1.556-10.47] (p=0.011)[8]. This initial study was then expanded into a multi-centre study (9 clinical centres, 1227 stage II CRC patients) where ploidy plus stroma was shown to be an independent prognostic factor of DFS independent of age, gender, tumour location and lymph node infiltration with stratification of risk recurrence at a level consistent with the initial clinical validation study (HR = 3.15 [95% CI 1.246-7.963] p = 0.015)[9]. This study demonstrated the prognostic value of the OncoProg® test with clear stratification of patients into "low" and "high-risk" categories.

See **Appendix C** for references.

OncoProg® Clinical Workflow

The OncoProg® test integrates into hospital pathology workflows and is deployed at the same time as standard tests are ordered after initial surgery to remove the tumour. FFPE tissue blocks are the main input

for the OncoProg® test and the samples are processed, and risk report issued within the two-week timeframe i.e., from initial surgery and the consultation between the patient and their oncologist to assist in determining the most beneficial course of treatment.

Aims of the study

This study is being sponsored by Oxford Cancer Biomarkers Limited and funded by the Department of Health and Social Care through and AI in Health and Care Award. For further information please see https://www.england.nhs.uk/aac/what-we-do/how-can-the-aac-help-me/ai-award/

The primary objectives of the study are to:

1. Assess the differences, if any, in the recommendation of adjuvant chemotherapy with stand-of-care data alone then with the addition of OncoProg® results

2. Demonstrate the health economic benefit of adopting OncoProg® as a tool for guiding chemotherapy treatment for CRC Stage II and Stage IIIA.

What is my role as an Oncologist in the study?

Working with the Research Nurse:

- Complete a pre-study questionnaire to record your opinions on the use of the OncoProg® test in clinical practice
- Introduce patients with suspected CRC stage II and IIIA to the study verbally and providing the patient a Patient Information Sheet
- Consent the patient to the study using the Patient Consent Form
- Document standard-of-care test results in the eCRF
- Document adjuvant treatment recommendation based on standard of care data in the eCRF
- Document adjuvant treatment recommendation based on standard of care data plus OncoProg® report in the eCRF
- Document any change in your decision in the eCRF
- Document the treatment agreed with the patient in the eCRF
- Complete an acceptability questionnaire to record your opinions on the use of OncoProg in your treatment decision making

What are the risk in participating in this study?

- Any decisions about treatment including the decision whether to prescribe chemotherapy are a joint decision with the patient.
- Consent forms are signed and carefully prepared information on the side effects of chemotherapy are given to each patient so the process of consent for the study and subsequent chemotherapy are highly formal.
- Clinical liability insurance is provided by the sponsor. Fur further information please contact guy.mozolowski@oxfordbio.com.

How will we use information about you?

We will need to use information from your medical records for this research project.

This information will include your

- Name
- Employer and Job Title

- GMC registration number

People will use this information to do the research or to check your records to make sure that the research is being done properly.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a participant identification number (PIN) instead.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

What will happen to my data?

- Data protection regulation requires that we state the legal basis for processing information about you. In the case of research, this is 'a task in the public interest.' Oxford Cancer Biomarkers Limited is the sponsor for this study. It is the data controller and is responsible for looking after your information and using it properly.

- We will be using information from you, your hospital and NHS England in order to undertake this study and will use the minimum personally-identifiable information possible.

- Data protection regulation provides you with control over your personal data and how it is used. When you agree to your information being used in research, however, some of those rights may be limited in order for the research to be reliable and accurate.
- You can find out more about how we use your information on our website www.oxfordbio.com/privacy-policy by contacting the study team.

Who do I contact if I have comments or issues or technical questions about the study or technology?

For questions concerning the study protocol or eCRF please contact : Guy Mozolowski @ guy.mozolowski@oxfordbio.com

For questions concerning the technical aspects of OncoProg including slide preparation please contact : Susan Fotheringham @ susan.fotheringham@oxfordbio.com

For questions concerning the technical aspects of OncoProg including scanning and use of the OncoProg software please contact: Kevin Xu @ kevin.xu@oxfordbio.com

Appendix A: OncoProg® Clinical Report

OncoProg® Clinical Report (Page 1)

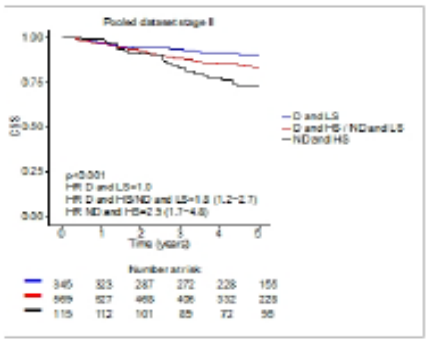
ONCOPROG CRC PLOIDY/STROMA ANALYSIS

Report Form

PATIENT INFORMATION	SAMPLE INFORMATION
LAST NAME: <Created>	DATE SPECIMEN COLLECTED: 01 Jan 0001
FIRST NAME:	DATE TESTED: 21 May 2021
NHS/PATIENT No.:	ORDERING HOSPITAL:
DOB: 01 Jan 0001	ORDERING ONCOLOGIST:
ONCOPROG SAMPLE REF: C18000013	ADDITIONAL REPORT RECEIPT:

ONCOPROG CRC ASSAY DESCRIPTION

OncoProg CRC assay uses digital pathology to determine the DNA content (ploidy) and tumour microenvironment (stroma content) of formalin-fixed paraffin embedded (FFPE) tissue from patients with Stage II colorectal cancer. These biomarkers are combined to stratify patients into categories informing on risk of recurrence. Patients with diploid (normal) DNA content and low stroma (<50%) have been shown to be at low risk of recurrence and therefore have a favourable prognosis. Patients with diploid DNA content and high stroma (≥50%) and patients with non-diploid (abnormal) DNA content and low stroma (<50%) have been shown to be at an intermediate risk of recurrence. Patients with non-diploid (abnormal) DNA content and high stroma (≥50%) have been shown to be at high risk of recurrence and therefore have an unfavourable prognosis. The test results will indicate Low Risk, Intermediate Risk or High Risk with the results for both ploidy (DNA histogram) and stroma (tumour tissue image) provided.



Kaplan-Meier plot illustrating cancer-specific survival (CSS) for patients with tumours that were diploid and low stroma (D and LS), diploid and high stroma or non-diploid and low stroma (D and HS/ND and LS), and non-diploid and high stroma (ND and HS) among patients with stage II tumours

The Risk is calculated using data from a study using the QUASAR2 clinical trial [1,2] and two other studies (total patient number = 2624) and allows Stage II CRC patients to be classified into low, intermediate and high risk groups [3].

RESULT

HIGH RISK	The test indicates for this individual there is a **HIGH RISK** of disease recurrence.

ADDITIONAL INFORMATION

Chief Medical Officer: David Kerr CBE FRCP FMedSci

This report is provided by Oxford Cancer Biomarkers Ltd, Oxford Science Park, Robert Robinson Avenue, Oxford, OX44GA, United Kingdom, Tel: +44 (0)1865 784743.
Analysis carried out by University Hospitals Coventry and Warwickshire

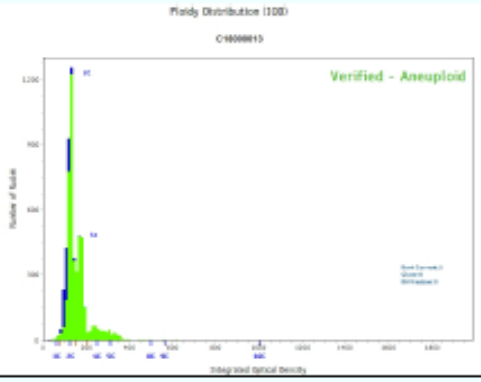
1. Kerr RS, et al. Adjuvant capecitabine plus bevacizumab versus capecitabine alone in patients with colorectal cancer (QUASAR 2): an open-label, randomised phase 3 trial. Lancet Oncol. 2016 Sep 19. pii: S1470-2045(16)30172-3.
2. Fotheringham S et al. A prognostic marker for colorectal cancer: combining analyses of ploidy and stroma. Annals of Oncology 27 (Supplement 2): ii118–ii128, 2016.
3. Danielsen HE et al. Prognostic markers for colorectal cancer; estimating ploidy and stroma. Annals of Oncology, mdx794, https://doi.org/10.1093/annonc/mdx794. Published online: 27 December 2017

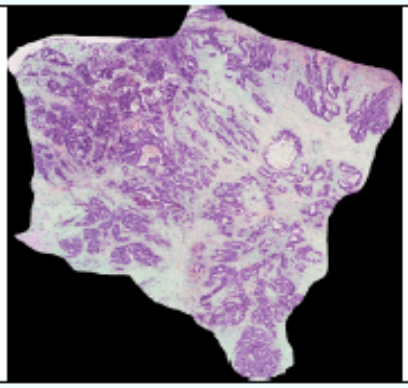
Ordering Information:
Report Queries:

ONCOPROG CRC PLOIDY/STROMA ANALYSIS

Report Form

PATIENT INFORMATION	SAMPLE INFORMATION
LAST NAME: <Created>	DATE SPECIMEN COLLECTED: 01 Jan 0001
FIRST NAME:	DATE TESTED: 21 May 2021
NHS/PATIENT No.:	ORDERING HOSPITAL:

PLOIDY RESULTS	PLOIDY CLASSIFICATION
	DNA content analysis: **NON-DIPLOID**

STROMA RESULTS	STROMA CLASSIFICATION
	Stroma fraction 54% : **HIGH**

ONCOPROG RISK DESIGNATION
HIGH RISK: Non-diploid DNA ploidy and High stroma fraction indicate unfavourable prognosis. Treatment with fluoropyrimidine combination therapy could be merited, in patients under 70 years of age.

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PRINT NAME	**DATE**

Ordering Information:
Report Queries:

2/2 ONCOPROG SAMPLE REF: C18000013

APPENDIX B: OncoProg® Technology

The first biomarker is based on the principle that the number of DNA copies in the epithelial cells in the tumour area is a good indicator of the tumour development for carcinomas. The ploidy analysis method, a type of image cytometry approach, was developed to measure the DNA content in the nuclei. This methodology uses formalin fixed paraffin embedded (FFPE) tissue samples resected from the patient tumour as the input. A 50µm sample is cut from the tissue and is then processed so that the cell nuclei can be isolated from the sample to produce a nuclear monolayer slide for analysis. An image of the prepared slide is then taken at 40x magnification. Based on a large quantity of image data, a digital imaging processing algorithm was developed to classify the cell nuclei in the tumour area using a rule-based classification network built upon the extracted nuclei morphological feature. This employs 13 geometric parameters including perimeter, shape factor, form factor, jaggedness, symmetry, concavity etc. A statistical data model was then developed around the integrated optical density (IOD) value of each nucleus that represents the nucleus' absorbance of the light as a surrogate for the amount of DNA in each nucleus. For each slide, sufficient numbers of epithelial cells (at least 2,000) and reference lymphocytes and fibroblasts (at least 1,000) are included in the calculation to allow the application of the statistical model for predicting if the majority of the epithelial cells are diploid or non-diploid.

The second biomarker used in OncoProg® was discovered in tumour microenvironment observations that a larger proportion of stroma in the tumour area leads to a poorer prognosis for the cancer patients. A digital image processing algorithm was developed to assess the percentage of stroma present in the resected tumour. The FFPE tumour sample is stained with H&E (haematoxylin and eosin) and the whole slide image of this stained tissue is use to calculate the fraction of the stroma component out of the tumour area. Technical challenges including the background noise interference, pink and purple colour overlapping in colour spaces were overcome to allow accurate quantitative measurement of the stroma fraction. Multiple large patient data sets were used to determine that the stroma fraction above the threshold of 50% for the CRC patients and 56% for the prostate cancer patients leads to a poor prognosis outcome.

Whilst both ploidy and stroma function as prognostic biomarkers individually, they have enhanced performance in combination. These two biomarkers are combined within the OncoProg® test to stratify patients into categories informing on risk of recurrence. Risk categorisation using OncoProg® suggests that stage II CRC patients with a "low risk" of recurrence (similar to stage I patients) should avoid adjuvant treatment, whilst patients with an "intermediate risk" might benefit from adjuvant fluoropyrimidine monotherapy and those with a "high risk" of recurrence might consider a combination therapy with oxaliplatin if patients are under 70 years of age. It has been concluded that OncoProg® is a useful tool for the clinicians to make more informed decisions on any adjuvant treatment strategy.

Recent technology improvements have allowed the OncoProg® system to move its ploidy analysis away from the initial microscope-based hardware system to enable OncoProg® to function as an entirely whole slide image (WSI) scanner-based system. The principal benefits of the new system are reduced hardware infrastructure cost, reduced laboratory space requirement, increased through-put, and decreased time to result whilst delivering equivalent performance to that of the previous system.

This combination biomarker technology is protected by a patent filed in 2016 (PCT/EP2016/055102). This patent has been granted in Europe (3271864), the USA (US 10,774,385 B2) and a number of other territories.

Appendix C: References

1. Bray F, *et al*. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov;68(6):394-424.
2. https://www.wcrf.org/int/cancer-facts-figures/data-specific-cancers/colorectal-cancer-statistics.
3. http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer.
4. Luengo-Fernandez R, *et al*. Economic burden of cancer across the European Union: a population-based cost analysis. Lancet Oncol. 2013 Nov;14(12):1165-74.
5. Quasar Collaborative Group, Gray R, *et al*. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. Lancet. 2007 Dec 15;370(9604):2020-9.
6. Fotheringham S, *et al*, Challenges and solutions in patient treatment strategies for stage II colon cancer, Gastroenterology Report, Volume 7, Issue 3, June 2019, Pages 151–161
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9. Cai S *et al*, Prognostic stratification in stage II colorectal cancer using a combination of DNA ploidy and interstitial tumour stroma ratio. Submitted. (Data summary available on request)