Epidermal Growth Factor Receptor Immunohistochemistry Image Analysis in Advanced Colorectal Cancer.

Ryan Hutchinson¹*, Jacqueline James¹, Richard Wilson¹, Richard A. Adams², Bharat Jasani², Manuel Salto-Tellez¹ and Peter W. Hamilton¹

1 Centre for Cancer Research and Cell Biology, Queen’s University Belfast, Belfast, United Kingdom
2 Institute of Cancer and Genetics, Section of Oncology and Palliative Medicine, Cardiff School of Medicine, Cardiff, United Kingdom

rhutchinson09@qub.ac.uk
Image Analysis: Linking Drug Development and Biomarker Development

Drug Development

- Target Discovery
- Lead Optimisation
- Preclinical/animal Studies
- Clinical Development I
- Clinical Development II
- Clinical Development III
- Approval
- Market

Image Analysis

Quantitative automated assessment of tissue biomarkers (IHC, ISH)

Companion Algorithms

Quantitative assays to support patient stratification and therapeutic selection

Biomarker Development

- Biomarker Discovery
- Biomarker Validation
- Assay Development
- Clinical Utility Testing
- Approval
- Market
The Era Of Personalised Medicine

The current dilemmas in tissue biomarker quantification include:

- Tumour heterogeneity.
- Increased sensitivity of new technologies and how to interpret the clinical significance of the quantified biomarker.
- Qualitative or Quantitative method.

Some therapy decisions are still reliant and based on immunohistochemistry.
Manual IHC

- Subjective, time consuming.
- Inherent intra-observer variability.
- Semi-quantitative data.
- Difficult to quantitate.

Image Analysis IHC

- **Objective quantification** of IHC staining.
- Reproducible data.
- Continuous output.
- Ability to batch process, time efficient.
- An additional tool for the pathologist.
Colorectal Cancer

- Second most common cause of cancer related death in the United Kingdom.

- Most colorectal cancers develop as a result of a stepwise progression from normal mucosa to adenoma to invasive carcinoma.

- Progression is controlled by the accumulation of alterations or mutations in a number of growth regulating genes.
EGFR in Colorectal Cancer

- Epidermal Growth Factor Receptor (EGFR) is a cell surface receptor.

- Successful binding of the EGFR is known to initiate signals along pathways.

- EGFR has been found to be overexpressed in 80% of colorectal cancers (Goldstein et al.).

- Monoclonal antibodies such as Cetuximab can bind to the EGFR and prevent intra-cellular signals being transduced.
The COIN Trial: A Background

• The COIN Trial: a major multi-national study sponsored by the Medical Research Council (MRC), with the recruitment of 2445 patients.

• Investigated the potential of EGFR IHC as a predictive biomarker.

• Examined the comparison of three chemotherapy combinations as a first line therapy in previously untreated advanced colorectal cancer patients.

1. How well the combination of chemotherapy and Cetuximab worked for KRAS wildtype (WT) patients with advanced colorectal cancer.

2. Whether EGFR IHC is predictive of response to Cetuximab.

Lancet 2011; 377; 2103-14
Lancet Oncol 2011; 12: 642-53
• Conventional EGFR IHC scoring did not indicate any predictive value for treatment with Cetuximab and chemotherapy in first line therapy.

• Visual EGFR IHC assessment did not demonstrate any evidence for use as a predictive biomarker in KRAS wildtype advanced colorectal tumours.

Lancet 2011; 377; 2103-14
Lancet Oncol 2011; 12: 642-53

Can IA-based EGFR IHC provide a more reliable predictive biomarker for patient stratification in advanced colorectal cancer?
The application of Image Analysis to assess EGFR IHC

1. To quantify EGFR IHC expression in COIN trial sampled using state of the art image analysis software.

2. To compare EGFR IA-derived H score with original Manual EGFR H-score

3. To evaluate the predictive potential of IA-based EGFR IHC in each of the treatment arms of the COIN Trial.

\[ H \text{ Score} = (1 \times \text{Amount of cells weakly stained cells}) + (2 \times \text{Amount of cells with medium staining intensity}) + (3 \times \text{Amount of cells with high staining intensity}). \]
COIN Trial: IHC Methods

• EGFR IHC expression on primary tumour samples stained using the DakoCytomation PharmDx kit® were assessed at a central reference laboratory.

• Freshly cut samples were used for the generation of the tissue microarrays (TMAs).

• Tumour sample TMAs for all available COIN patients were scored by three blinded expert pathologists using Mirax digital scanning and imaging software.

• Positive EGFR staining was identified using the Dako® recommendations.

www.dako.com : Guidelines for interpreting EGFR pharmDx™
Methods: Sample set

- 2445 patients.
- 6264 arrayed 0.6 mm cores (up to 3 per patient).
- Varying intensities of EGFR positivity used in training set.
- Image Acquisition Mirax Scanner @ 20x.
Methods: Image Analysis

• The digitised slides were imported into Definiens Tissue Studio (TMA) for both automated and quantitative image analysis.

• All digitised TMA cores were successfully dearrayed using Tissue Studio.

• A user defined region recognition module was applied to define histological regions of interest.

• Subsets of identified EGFR positive tumour regions were used to create a digital solution for the quantification of positive membrane staining across the EGFR IHC images.
Methods: TMA Dearray
Methods: EGFR+ Region Recognition
Methods: EGFR+ Region Recognition
Methods: Nuclei and Cell Detection
Methods: Membrane Quantification
Methods: Membrane Detection and Quantification
Methods: Membrane Detection and Quantification
Methods: Mark-Ups Used for Training
<table>
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Results: Algorithm Validation

Spearman Rank Correlation

R = 0.96
P = <0.0001

Concordance correlation coefficient
0.98

95% CI
0.9588763->0.9919103
Results

• The defined algorithm was capable of accurately segmenting and measuring positive cell membrane EGFR expression across the range of colorectal cancer samples.

• This allowed for the successful selection of epithelial tumour cells and exclusion of positivity in stromal/non tumour regions.

• A direct comparison between visual IHC scores and computerised image analysis derived IHC scores showed a strong correlation ($r=0.96$, $P<0.0001$).

• Some results suggested that visually marked up cores with a lower histological score were scored higher in some instances using image analysis.
Future Work

• Although visual and automated IHC evaluation showed strong concordance in this sample set, the clear advantage of image analysis is reproducibility and consistency across large numbers of samples.

• Our EGFR algorithm can now be applied across the entire patient cohort.

• Examine the role of EGFR image analysis in predicting response to Cetuximab in the complete COIN trial patient cohort.

• Comparison of Image Analysis derived EGFR Histological Score (H-Scores) with corresponding clinicopathological data.

• Investigation of the predictive role of EGFR IHC in both KRAS mutant and wildtype tumours.
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• COIN Trial Patients