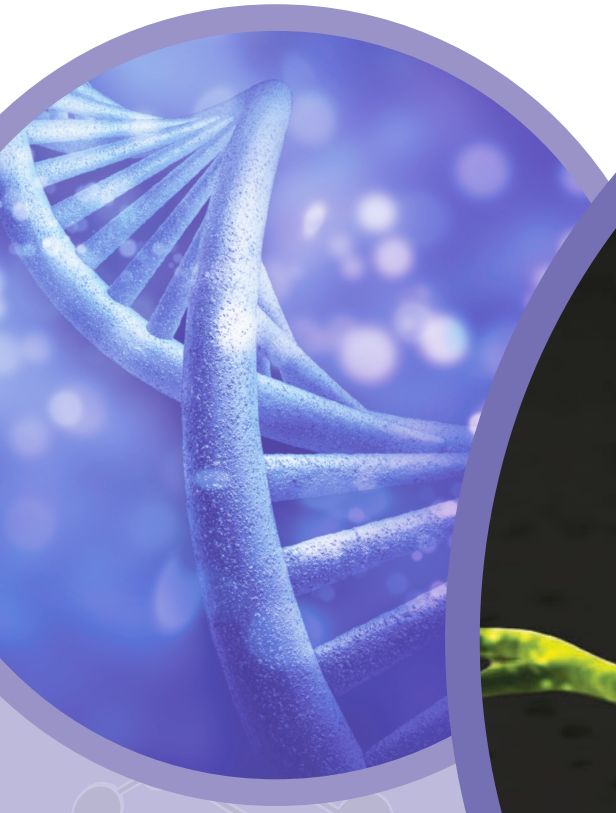
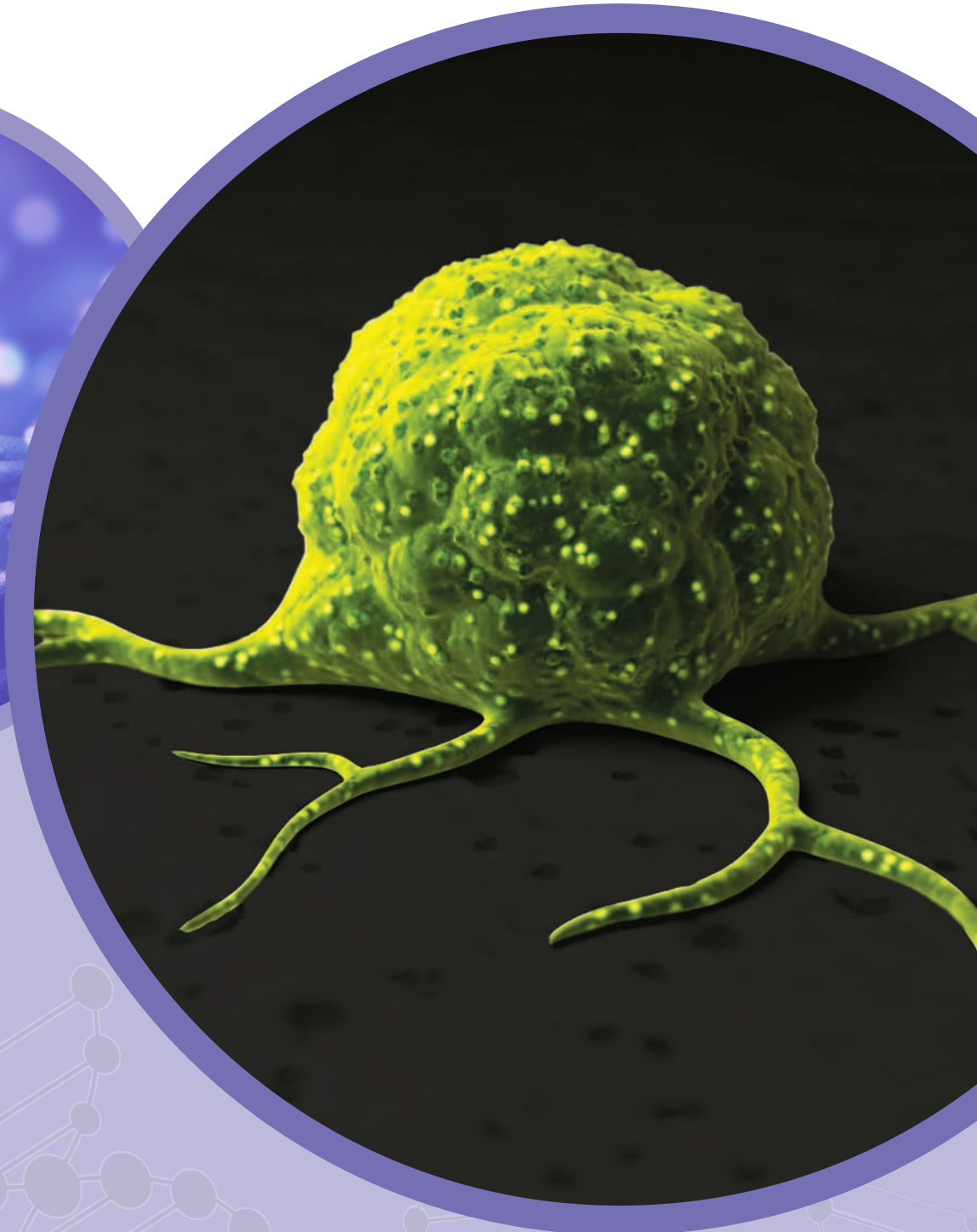
RANDOX
MOLECULAR

KRAS, BRAF, PIK3CA Array

Targeted mutation profiling





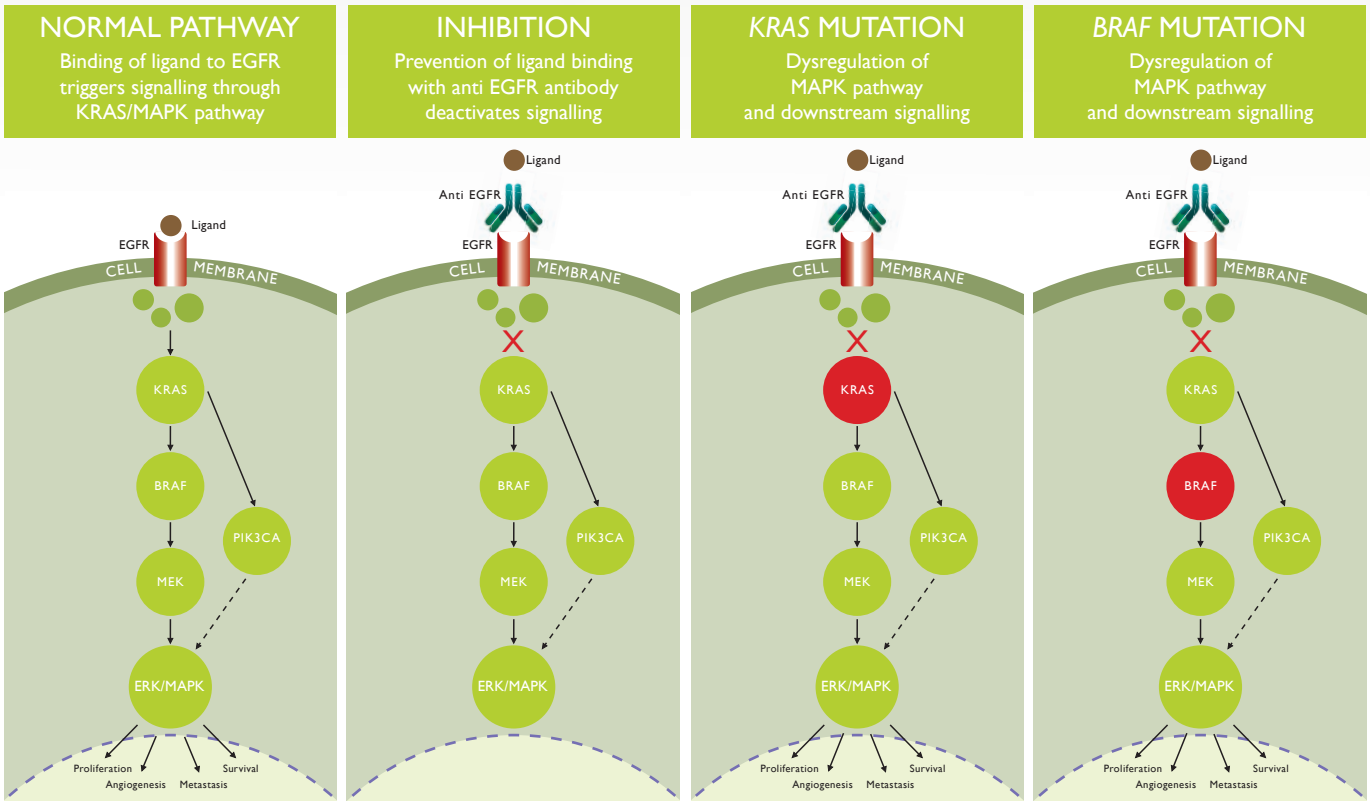
KRAS, BRAF, PIK3CA* Array

Rapid profiling of point mutations in the KRAS, BRAF and PIK3CA genes

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide.1 Metastatic disease accounts for 40-50% of newly diagnosed patients and is associated with high morbidity.1,2 Despite recent therapeutic advances, the prognosis for patients with metastatic CRC (mCRC) remains poor.3 In recent years monoclonal antibodies (moAbs), like cetuximab and panitumumab which target the epidermal growth factor receptor (EGFR), have proven to be effective in combination with chemotherapy or as single agents for the treatment of mCRC.4,5 These moAbs block the signal from EGFR inhibiting downstream signalling including KRAS, BRAF and PIK3CA mediated events (see diagram below). However, when KRAS, BRAF and PIK3CA* are mutated they are permanently 'turned on' permitting downstream events irrespective of anti-EGFR therapy. The Randox KRAS, BRAF, PIK3CA Array allows the clinician to detect important mutations in the KRAS, BRAF and PIK3CA genes, enabling the appropriate selection of patients for therapy.

EGFR pathway and its inhibition by EGFR targeted monoclonal antibodies



Targets detectable by the KRAS, BRAF, PIK3CA* Array

The KRAS, BRAF, PIK3CA* Array is designed for the rapid qualitative detection of point mutations within the genes KRAS, BRAF and PIK3CA from fresh/frozen and formalin fixed paraffin embedded (FFPE) tissue DNA (refer to below table for targets detectable).

KRAS						*BRAF*	*PIK3CA**		
codon 12		codon 13	codon 61		codon 146	codon 600	codon 542	codon 545	codon 1047
G12A	G12C	G13D	Q61K	Q61H(1)	A146T	V600E	E542K	E545K	H1047R
G12R	G12S	G13C	Q61L	Q61H(2)	A146P				
G12D	G12V	G13R	Q61R						

*PIK3CA for research use only

Why test the KRAS, BRAF, and PIK3CA genes?

Early studies conducted on mainly heavily pre-treated chemotherapy-refractory patients and also chemotherapy-naive patients with mCRC indicated that only 10-20% of patients clinically benefited from anti-EGFR moAbs.5,6 Consequently oncogenic activation of EGFR downstream effectors was investigated with respect to clinical outcome to moAb therapy. Analysis confirmed that patients with mCRC carrying activating KRAS gene mutations do not benefit from anti-EGFR moAb therapy.7,8 KRAS mutations have since emerged as the major negative predictor of efficacy in patients receiving cetuximab or panitumumab.3 The occurrence of KRAS mutations however only accounts for approximately 35-45% of nonresponsive patients.3 The identification of additional genetic determinants of primary resistance to EGFR-targeted therapies in colorectal cancers is therefore important. Recent studies have focused on the molecular analysis of the molecules involved in downstream EGFR signalling with mutations in BRAF9 and PIK3CA10 genes being reported to affect patient response to EGFR-targeted moAbs.

KRAS, BRAF, PIK3CA* Array Protocol

Mutations on this array are also implicated in other cancers such as KRAS in lung cancer and PIK3CA in breast cancer

Step 1 Extraction
Genomic DNA is extracted from fresh/ frozen or FFPE tissue samples

Step 2 Amplificaton
Single tube multiplex PCR reaction

Step 4 Detection
Imaging and result processing by Evidence Investigator analyser

Multiple Mutation Profiling
The *KRAS, BRAF, PIK3CA* Array detects 20 mutations in these three genes, which are implicated in mCRC therapy within 3 hours

Step 3 Hybridisation
Amplicon hybridisation/ conjugation to biochip array

Benefits of the KRAS, BRAF, PIK3CA Array

To the patient

- KRAS, BRAF, PIK3CA* Array is a rapid simple method for mutation detection
- Determining mutational status informs selection of appropriate therapy

To the laboratory

- Detection of 1% mutant in a background of wildtype genomic DNA
- Compatible with a broad range of genomic DNA input and type:
  - Formalin fixed paraffin embedded (FFPE) tissue
  - Fresh/frozen tissue
- Single DNA sample required
- Turnaround time of 3 hours
- Single reaction multiplex PCR coupled to a biochip provides greater mutation coverage of the three most important genes (*KRAS*, *BRAF* and *PIK3CA*) implicated in metastatic colorectal cancer therapy response
- Streamlined workflow – protocol and reagents are optimised for the molecular laboratory

Evidence Investigator

Multiplexing...proven, perfected, evolved

The Evidence Investigator is a semi-automated, benchtop biochip analyser which offers complete patient profiling.

Save time and costs
Multiplexing reduces time, labour and reagents associated with multiple individual tests

Increase throughput
For greater laboratory efficiency

Consolidation
Of immunoassays and molecular diagnostics, improving laboratory efficiency

Result traceability
Chain of custody features and bar coded reagents

No hidden costs
Package includes imaging module, PC and imaging software, thermoshaker, biochip carrier handling tray and barcode scanner

Ease of operation
Straightforward testing procedure, ready-to-use biochips and minimal sample handling

Extensive QC
Internal quality controls ensure all key assay steps have been performed correctly i.e. amplification

Retrospective reporting
Enabling additional analysis of previously captured sample data

Ordering Details

Description	Size	Cat. No.
KRAS, *BRAF*, *PIK3CA* Array	54 Biochips	EV3799A & EV3799B
Evidence Investigator Analyser		EV3602

*Note: Extraction reagents are not included
PIK3CA* for research use only

References

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7. Lièvre, A., Bachet, J.B., Le Corre, D. et al. (2006) KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Research 66, 3992-3995.
8. Benvenuti, S., Sartore-Bianchi, A., Di Nicolantonio, F. et al. (2007) Oncogenic activation of the RAS/RAF signalling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Research 67, 2643-2648.
9. Di Nicolantonio, F., Martini, M., Molinari, F. et al. (2008) BRAF wildtype is required for response to panitumumab or cetuximab in metastatic colorectal cancer. Journal of Clinical Oncology 26, 5705-5712.
10. De Roock, W., Claes, B., Bernasconi, D. et al. (2010) Effects of KRAS, BRAF, NRAS and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy –refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncology 11, 753-762.

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