



Research

Development

Clinical

Commercial

Identification of mRNA genes that define Cetuximab sensitivity in either wild type or mutant KRAS colorectal primary tumors using formalin fixed paraffin embedded samples

Steven M. Walker Ph.D.

Team Leader Biomarker and Target Identification



Research

Development

Clinical

Commercial

Disclosures

- I am an employee of Almac Group LTD
- I will not discuss off label or investigational use of cetuximab.



Research

Development

Clinical

Commercial

Background

- Cetuximab improves overall and progression-free survival in a subset of advanced colorectal cancer patients
- DNA microarray analysis of freshly frozen liver metastases identified markers of Cetuximab sensitivity
- Standard clinical pathology practice is formalin fixation and paraffin embedding (FFPE)
- Routine biopsy of liver metastases is impractical in clinical practice

Research

Development

Clinical

Commercial

Study Goals

Aim : Analyse gene expression in FFPE colorectal primary samples from Cetuximab/Irinotecan-treated cohort

- 1) Identify differentially expressed genes (DEG) between responding and non responding groups for:
 - All tumors irrespective of KRAS status
 - KRAS wild type tumors
 - KRAS mutant tumors
- 2) Identify molecular pathways associated with KRAS mutation

Research

Development

Clinical

Commercial

Colorectal Cancer DSA™: Generation Overview

- Transcriptome of colorectal cancer identified by:
 - cDNA sequencing of many different colorectal tumors representing all stages and grades including normal.
 - Mining of public EST databases and publications.
 - Gene expression analysis using Affymetrix HG-U133 plus 2.
- Probesets designed to true 3' region of each transcript.
- Content put on Affymetrix GMP manufactured platform.

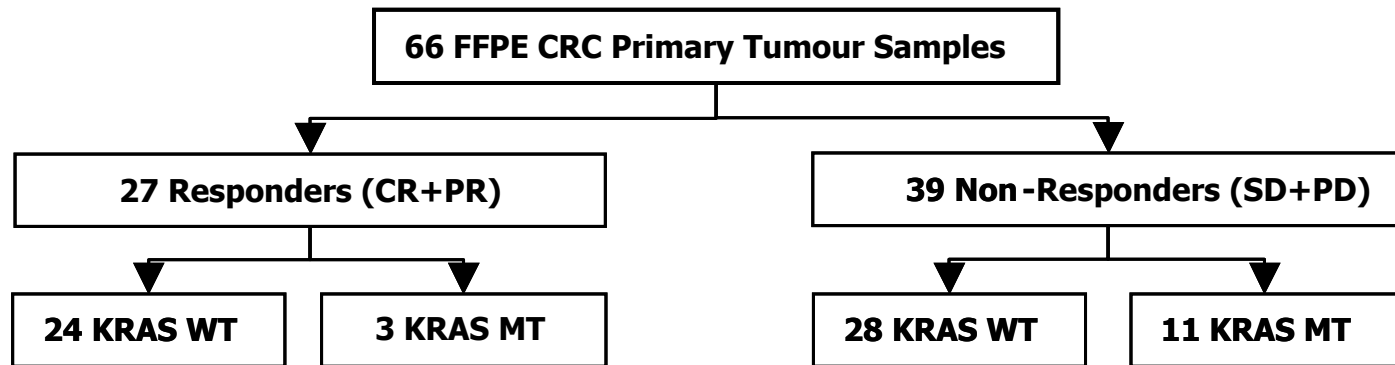
Research

Development

Clinical

Commercial

Study Design

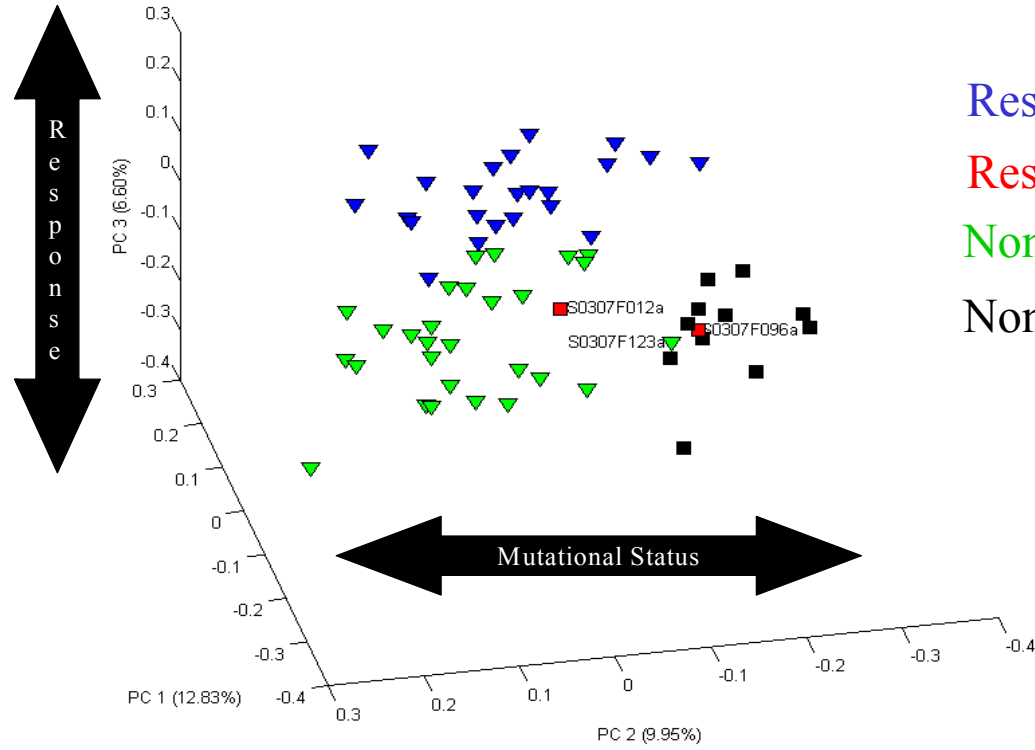


RNA extracted from one 10 μ m slice of FFPE tissue

Amplified and hybridised to Colorectal Cancer DSA™ research tools



Principle Component Analysis Demonstrating Separation of Samples by KRAS Mutational Status and Response



Responder KRAS wt
Responder KRAS mt
Non-Responder KRAS wt
Non-Responder KRAS mt

Research

Development

Clinical

Commercial

Study Goals

Aim : Analyse gene expression in FFPE colorectal primary samples from Cetuximab/Irinotecan-treated cohort

- 1) Identify differentially expressed genes (DEG) between responding and non responding groups for:
 - All tumors irrespective of KRAS status
 - KRAS wild type tumors
 - KRAS mutant tumors
- 2) Identify molecular pathways associated with KRAS mutation

Research

Development

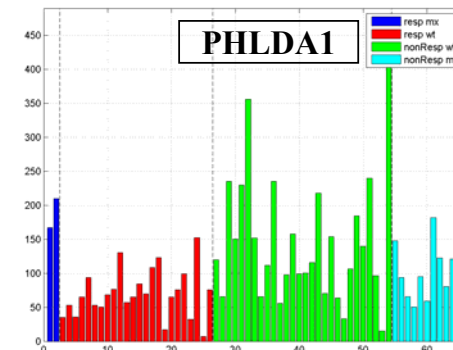
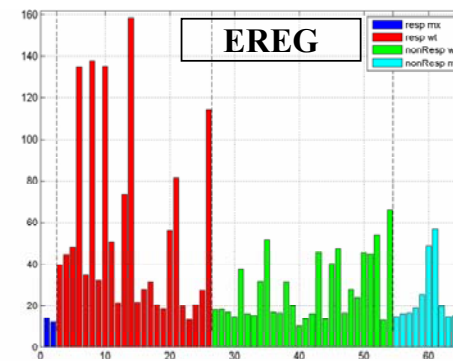
Clinical

Commercial

Responder vs. Non-Responder Analysis

- 243 DEGs identified between responders and non-responders irrespective of KRAS status.
- Included the vast majority of those identified previously by other investigators from fresh frozen material:
 - EREG,
 - DUSP4,
 - LYZ,
 - PHLDA1,
 - KIAA0040,
 - TBXAS1.

Khambata-Ford et al 2007



Research

Development

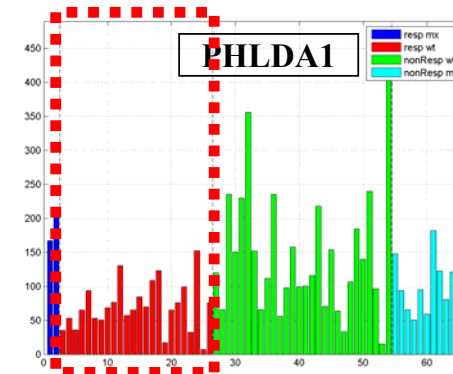
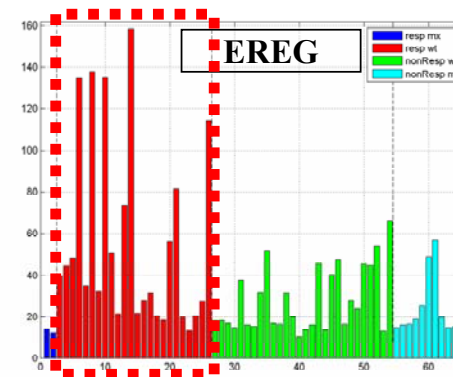
Clinical

Commercial

Responder vs. Non-Responder Analysis

- 243 DEGs identified between responders and non-responders irrespective of KRAS status.
- Included the vast majority of those identified previously by other investigators from fresh frozen material:
 - EREG,
 - DUSP4,
 - LYZ,
 - PHLDA1,
 - KIAA0040,
 - TBXAS1.

Khambata-Ford et al 2007



Research

Development

Clinical

Commercial

Study Goals

Aim : Analyse gene expression in FFPE colorectal primary samples from Cetuximab/Irinotecan-treated cohort

- 1) Identify differentially expressed genes (DEG) between responding and non responding groups for:
 - All tumors irrespective of KRAS status
 - KRAS wild type tumors
 - KRAS mutant tumors
- 2) Identify molecular pathways associated with KRAS mutation

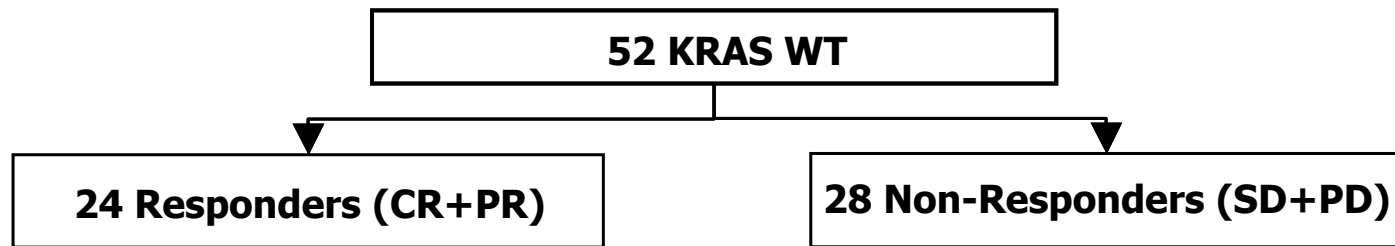
Research

Development

Clinical

Commercial

KRAS WT Responders versus Non-Responders



- 46% of KRAS wild type patients responded to Cetuximab
- 689 DEGs identified between KRAS wild type responder and non-responders.
- Supports the feasibility of developing a gene signature predictive of response to Cetuximab within KRAS WT patients.
- A number of these DEGs are unique to the Colorectal Cancer DSA™ research tools

Research

Development

Clinical

Commercial

Study Goals

Aim : Analyse gene expression in FFPE colorectal primary samples from Cetuximab/Irinotecan-treated cohort

1) Identify differentially expressed genes (DEG) between responding and non responding groups for:

- All tumors irrespective of KRAS status
- KRAS wild type tumors
- KRAS mutant tumors

2) Identify molecular pathways associated with KRAS mutation

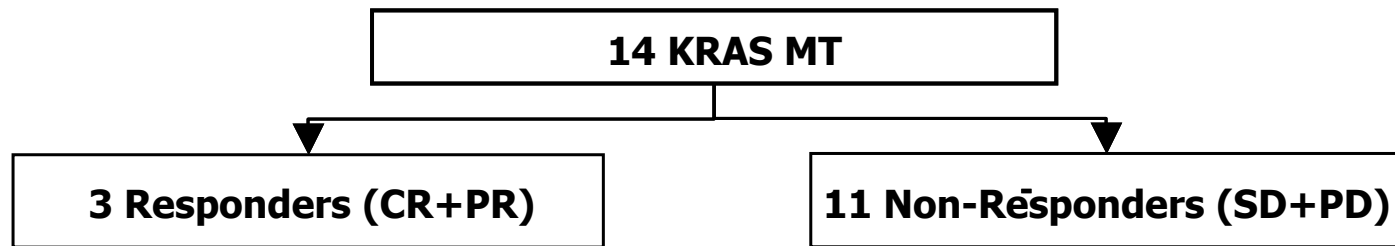
Research

Development

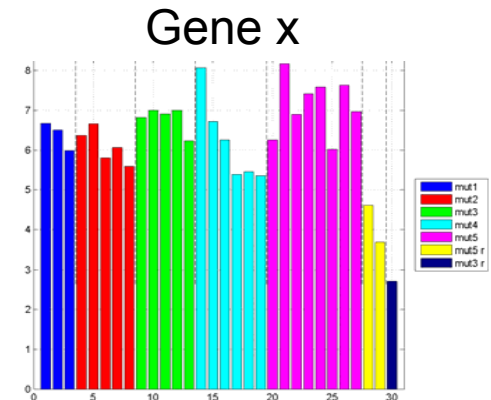
Clinical

Commercial

KRAS MT Responders versus Non-Responders



- 3/14 of KRAS mutant patients responded to Cetuximab, indicating a novel group of KRAS mutant tumours
- 95 DEGs identified between KRAS mutant responder and non-responders, suggesting the possibility of a biomarker of response.



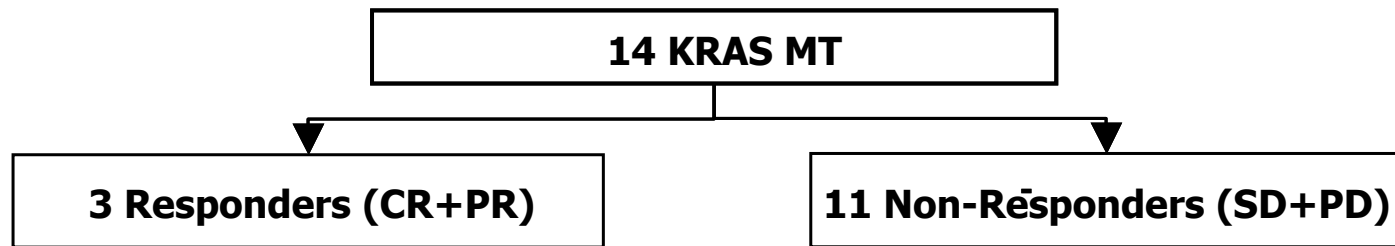
Research

Development

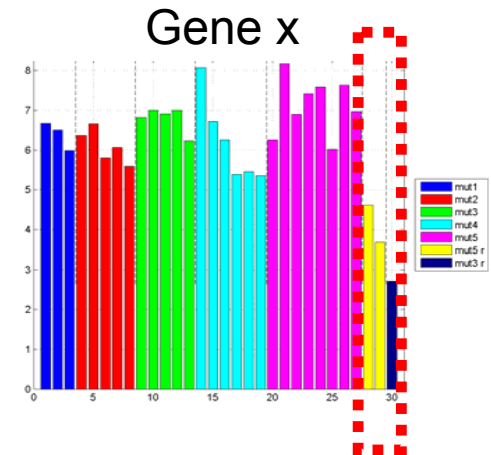
Clinical

Commercial

KRAS MT Responders versus Non-Responders



- 3/14 of KRAS mutant patients responded to Cetuximab, indicating a novel group of KRAS mutant tumours
- 95 DEGs identified between KRAS mutant responder and non-responders, suggesting the possibility of a biomarker of response.



Research

Development

Clinical

Commercial

Study Goals

Aim : Analyse gene expression in FFPE colorectal primary samples from Cetuximab/Irinotecan-treated cohort

- 1) Identify differentially expressed genes (DEG) between responding and non responding groups for:
 - All tumors irrespective of KRAS status
 - KRAS wild type tumors
 - KRAS mutant tumors
- 2) Identify molecular pathways associated with KRAS mutation

Research

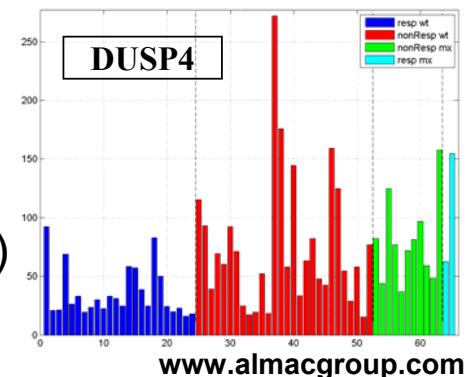
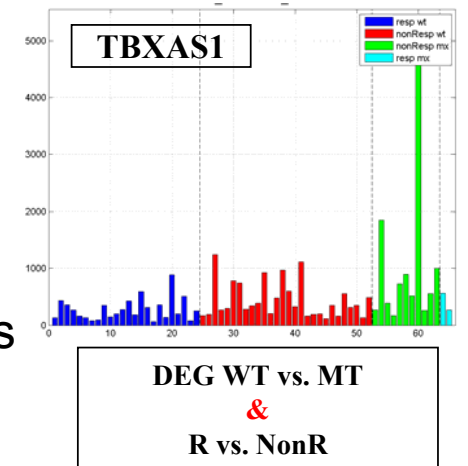
Development

Clinical

Commercial

KRAS WT vs. MT Functional Analysis

- 380 DEGs between KRAS wild type & mutant tumors
- Only two genes feature within KRAS mutant & non-response gene lists (TBXAS1, DUSP4)
- Functional analysis reveals several molecular pathways associated with KRAS mutation, including:
 - Mu-type opioid receptor regulation of proliferation (p=0.000631)
 - Transition & termination of DNA replication (p=0.00114)
 - Activation of PKC via G-Protein receptors (p=0.034)
- May represent targets for novel therapeutic agents



Research

Development

Clinical

Commercial

Conclusions

- Markers of Cetuximab sensitivity/response previously identified in a microarray analysis of frozen liver metastases were detected in FFPE sections from primary colorectal tumors.
- Many additional markers unique to the colorectal cancer DSA™ research tool.
- Groups of significantly differentially expressed genes identified:
 - 243 DEGs between Cetuximab responders vs non-responders irrespective of KRAS status.
 - 689 DEGs between KRAS wild type responders vs non-responders tumours.
 - 95 DEGs between KRAS mutant responders vs non-responders.
 - 380 DEGs between KRAS wild type vs mutants.
- Functional analysis identified relevant pathways (including kinase signalling pathways) for therapeutic targeting in KRAS mutant tumours.



ALMAC



Research

Development

Clinical

Commercial

Acknowledgements

KATHOLIEKE UNIVERSITEIT
LEUVEN



ALMAC
Diagnostics

- Sabine Tejpar
- Wendy De Roock

- Richard Kennedy
- Claire Wilson
- Paul Harkin
- Michael Sloan
- Vitali Proutski