K-RAS mutation detection in colorectal cancer- A combined approach using TaqMan® Mutation Detection assays (TMDA) and digital PCR.

TRINITY COLLEGE DUBLIN

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Introduction

The discovery of pivotal genetic alterations and the understanding of their potential role in cancer is leading to remarkable successes in translational medicine.

Activating RAS mutations occur in 30% of human cancers and specific RAS genes are mutated in different cancers, including colorectal cancer (CRC), non-small cell lung cancer, pancreatic cancer and others. KRAS mutations typically occur in codons 12 or 13 (exon 2) or in codon 61 (exon 3) of the *KRAS gene.*

KRAS is a protein located on the cytoplasmic side of the cell membrane and functions as a signal transducer in the EGFR pathway that promotes cell growth. Mutations in the KRAS gene cause it to lose GTPase activity, resulting in a constitutively active protein that promotes unregulated, proliferative cell growth.

EGFR is the primary therapeutic target in the management of colorectal cancer (CRC). KRAS mutations, found in approximately 40% of cases, have been widely demonstrated as major predictive markers of resistance to cetuximab or panitumumab.

The development of new technologies and diagnostics with increased specificity and sensitivity of KRAS mutation detection may therefore pave the way to individualized treatment for patients with CRC.

Objectives

The aim of this study was to investigate the feasibility of TaqMan® Mutation Detection Assays in conjunction with Digital PCR analysis as test for assessment of KRAS mutation in FFPE/blood samples.

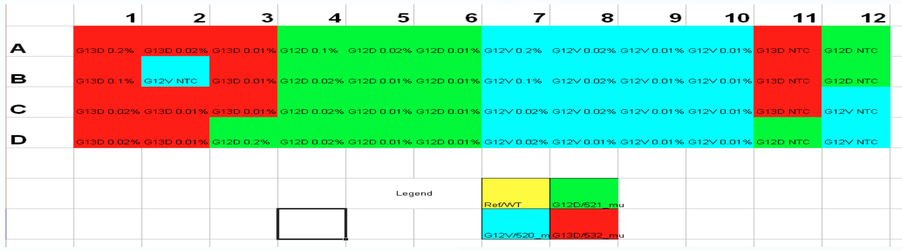
Methods

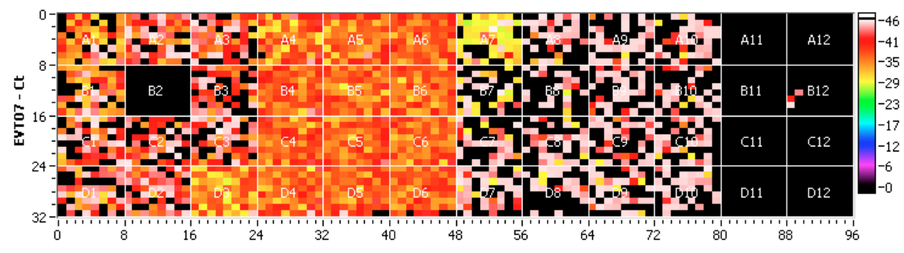
- Evaluate the efficacy of a panel of novel TaqMan® Mutation Detection assays (TMDA) based on competitive allele specific TaqMan PCR to detect KRAS mutations.
- 96 archival FFPE specimens were examined and analysed for presence of KRAS mutations using (a) pyrosequencing, (b) Custom conventional TaqMan assays and (c) TMDA.
- Use TMDA assays in conjunction with the OpenArray system to digitally detect the 3 most common KRAS mutations – G13D, G12D and G12V.
- Establish proof of principle in a cell line model by performing serial dilutions of each mutated DNA in a background of wild type/normal DNA (Nthy-ori), followed by TMDA/OpenArray analysis on n=25 clinical formalin-fixed paraffin-embedded (FFPE) samples.

Results

	Pyrosequencing	TMDA assays	Custom TaqMan SNP assay
G12C	2	2	2
G12S	0	0	0
G12R	0	0	0
G12V	5	6	25
G12D	17	14	1
G12A	3	2	2
G13S	0	0	-
G13R	0	0	-
G13D	4	3	4
Q61R	0	1	-
Q61L	1	2	-
Q61H	0	0	-
Total	32/105	30/96	34/113

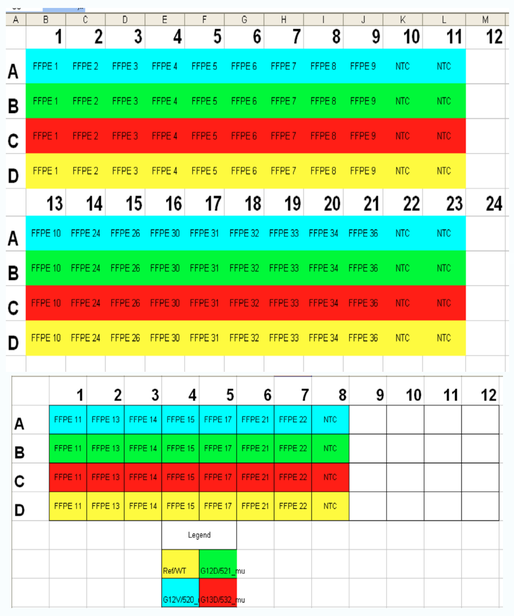
Results

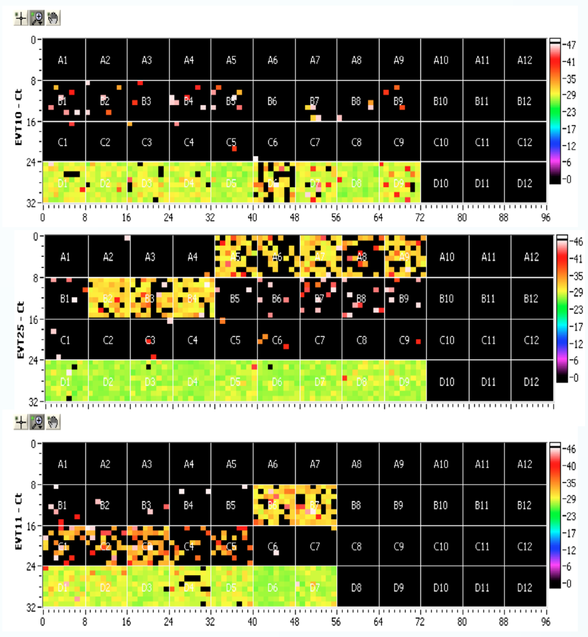




Serial Dilution results:

- *In general the lowest Dilution (0.01%) too concentrated for accurate digital analysis.*
- *Likely to be successful for low copy detection from clinical samples*





•A clear distinction can be made between the differentially mutated sample cohorts with minimal observed cross-talk between assays.

Discussion & Conclusions

- >96% concordance across 3 testing methods.
- TMDA PCR as accurate as pyrosequencing for KRAS mutant detection in heterogenous CRC samples
- TMDA PCR and TaqMan SNP assays detected 2 mutations not detected by conventional sequencing.
  - Possibly due to sampling
- TaqMan SNP assays susceptible to "cross-talk" (mutant detected but not clear cut).
- TMDA PCR eliminates these specificity issues.

- We have shown that TMDA assays can be used in conjunction with OpenArray for low copy mutation detection. A clear distinction can be made between the differentially mutated sample cohorts with minimal cross-talk between assays.
- The combined approach represents a feasible option for assessment of KRAS mutation in FFPE/blood samples.
- The increased sensitivity and specificity can facilitate accurate mutation detection of heterogeneous CRC samples. Its sensitivity renders it a potential option for the future detection of circulating tumour cells or tumoural DNA in peripheral blood samples.