A NEW FAST AND HIGHLY SENSITIVE ASSAY FOR THE DETECTION OF KRAS MUTATIONS

F. Molinari1, A. Riva1, C. Nielsen2, M.H. Kyneb3, T. Wolff3, L. Mazzucchelli1, U.B. Christensen2, M. Frattini1

1Laboratory of Molecular Pathology, Institute of Pathology, Locarno, SWITZERLAND
2Pentabase Aps, Pentabase Aps, Odense C, DENMARK
3Life Science Department, Danish Technological Institute, Aarhus C, DENMARK

Aim: The demonstrated efficacy of anti-EGFR drugs (mAbs) in KRAS wild-type (wt) patients affected by colorectal cancer (CRC) resulted in increasing demand for mutation analyses. Mutation testing techniques have therefore become an urgent concern as a powerful mean to select wt patients. However, it is still unclear which is the most sensitive, specific and efficient protocol to detect KRAS mutations in clinical samples. The aim of this study was to compare the new Pentabase kit with direct sequencing (DS) (gold standard) and mutant-enriched PCR (ME-PCR) (highly sensitive method) with regard to sensitivity, specificity and efficiency of testing.

Methods: Eight tumour cell lines, 7 specific for each KRAS mutation and 1 with the KRAS wt sequence, were used to perform a sensitivity assay. Seven different percentages of mutated DNA in fixed amounts of wt DNA were tested by all the methods (10%, 1%, 0.5%, 0.1%, 0.05%, 0.01% and 0%). KRAS mutations in exons 2 (codons 12 and 13) were evaluated by DS, ME-PCR and the Pentabase kit in a sensitivity assay and in 100 consecutive CRC patients. The Pentabase kit is based on real-time PCR using SuPrimers™ (DNA primers with increased specificity), BaseBlockers™ (oligos suppressing amplification of wt genes) and HydrolEasy probes (hydrolysis probe with increased signal-to-noise ratio and sensitivity).

Results: In the cell line experiments, DS had a sensitivity of 10%, ME-PCR of up to 0.1% and the Pentabase kit of <0.1%. In CRC cases, we found 29% KRAS mutated patients by DS, 40% by ME-PCR and 42% by the Pentabase kit. In particular, ME-PCR detected the same mutations as DS and 11 additional cases. The Pentabase kit found the same mutations detected by ME-PCR and 2 additional cases (G12C and G13D).

Conclusions: Overall, through the application of highly sensitive KRAS analysis methods, we detected additional KRAS alterations in up to 13/71 (18%) and 2/60 (3%) patients after DS and ME-PCR had shown them to be KRAS wt, respectively. The new kit allows the KRAS mutational typing in only 2 hours, compared to DS and ME-PCR, which require at least 2 days. Therefore the Pentabase kit represents a new, faster, more sensitive and more reliable method for the analysis of KRAS mutations in CRC and can be proposed for the identification of patients resistant to anti-EGFR mAbs.

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