

KRAS-dependent and independent mechanisms of progressive disease in colorectal cancer patients with liver metastases while monitoring on circulating cell free DNA



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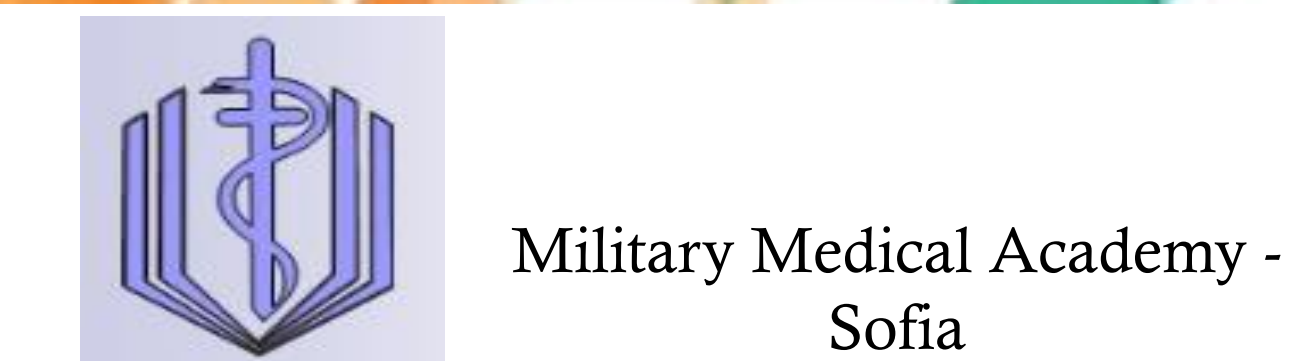
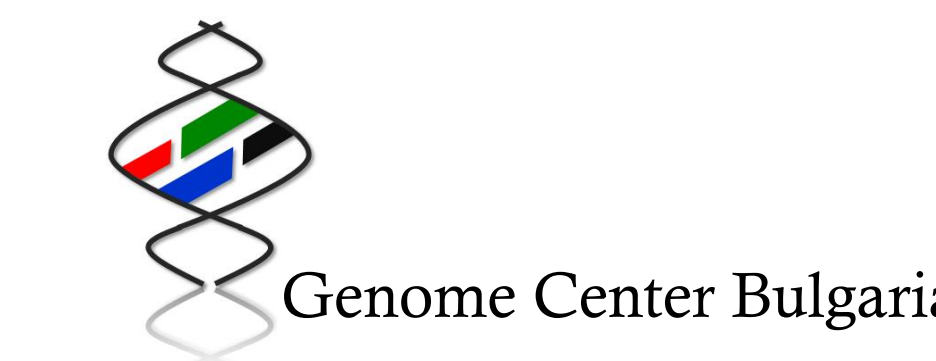
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BACKGROUND

KRAS mutational analysis in plasma ctDNA is an alternative to tissue analysis with concordance rate from 30 to 90%. The emergence of KRAS mutation during the course of anti-EGFR therapy is responsible for acquired resistance. We aimed to evaluate the RAS concordance rate between tissue and ctDNA in mCRC patients, and to monitor changes in RAS mutation status.

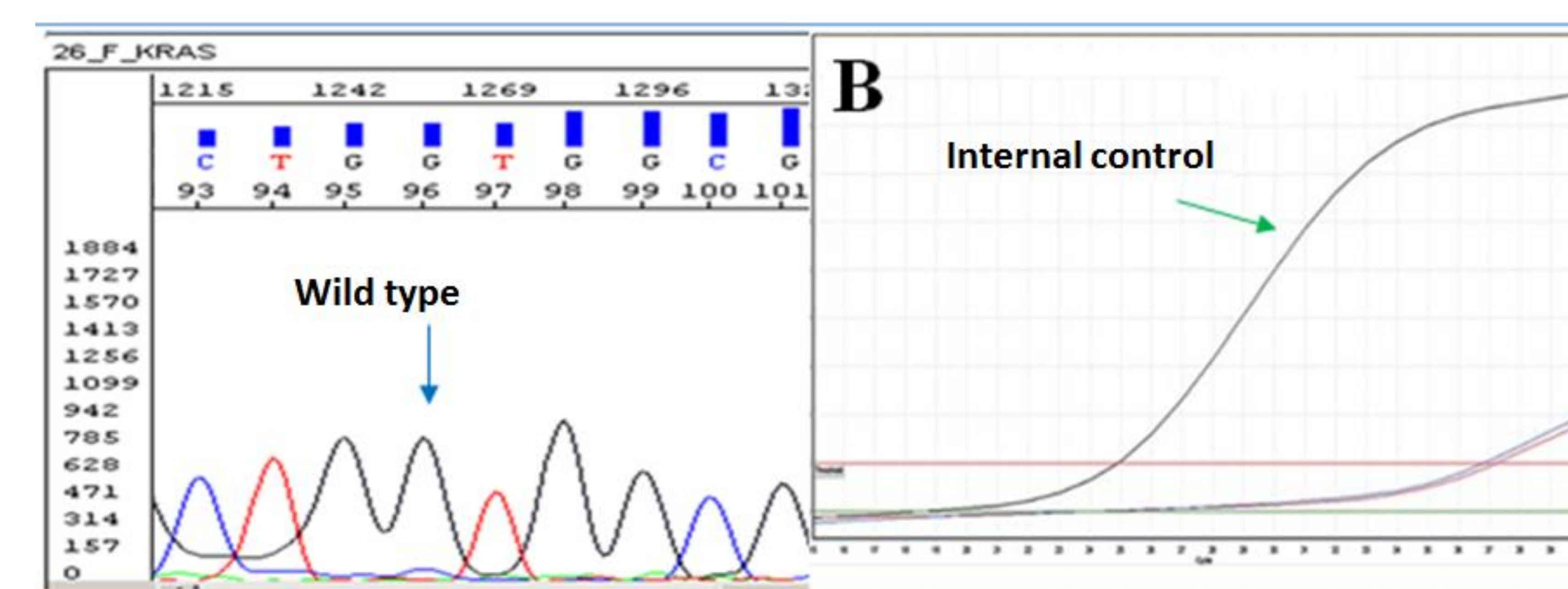
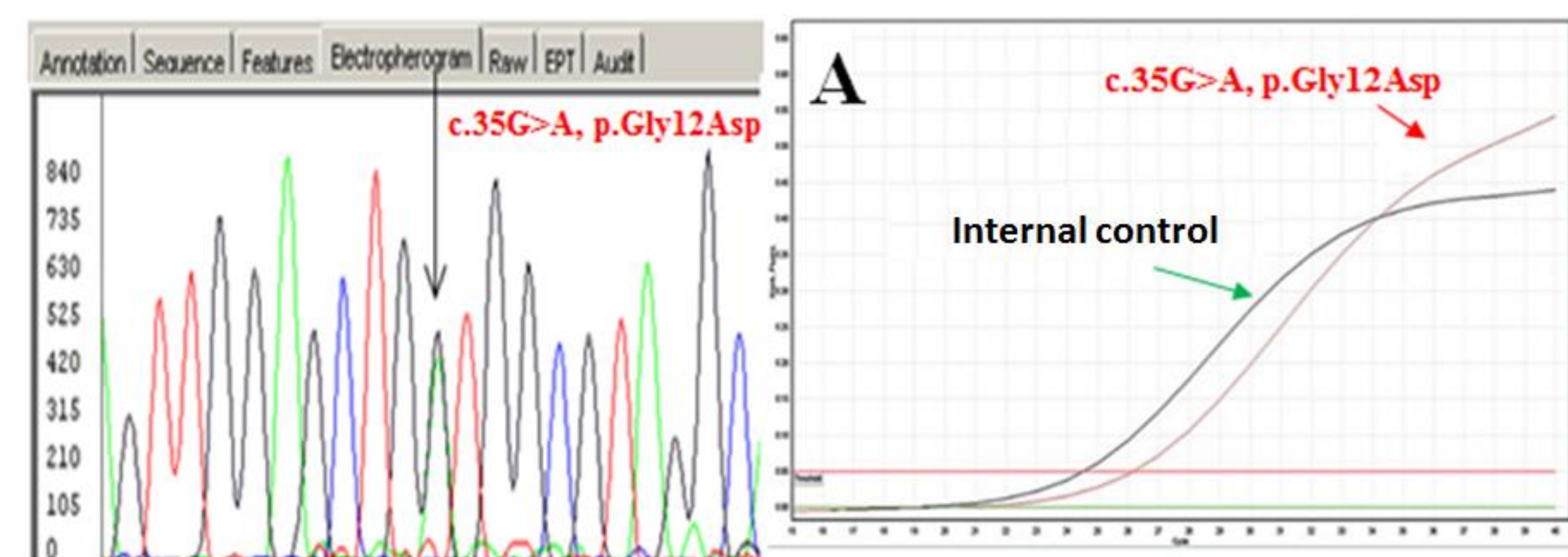
METHODS

All blood samples were collected in cfDNA Preservative Tubes (Norgen Biotek Corp., Canada). ctDNA was extracted within 3 days after sampling, the extraction was performed by commercial kit (Plasma/Serum cfDNA Purification Mini Kit, Norgen Biotek). KRAS (ex. 2, codons 12 and 13) mutations on ctDNA and tumor tissue were detected by real-time PCR kits TheraScreen: K-RAS Mutation Kit. The other mutations were detected by Sanger sequencing (BigDye Terminator v3.1 Cycle Sequencing Kit - Thermo Fisher Scientific).

RESULTS



In the concordance rate evaluation were enrolled prospectively 63 mCRC patients. Only mutation analysis had 52,4% (33) patients, from whom - 27,3%(9) had primary resectable liver metastases. The median time from tissue biopsy (primary -97%) to plasma collection was 275, 7 days (range 26 - 1560). Tissue mutation analysis revealed 58,7% (37) mutant patients from whom 78,4% (29) had KRAS ex.2 mutations. cfDNA evaluation showed the distribution of mutation to wild - 81%/19% with KRAS concordance rate of 58,7%. To reduce time between tissue and plasma samples mutation analysis in the monitoring analysis were included only 31 patients who had primary unresectable liver metastases, with baseline plasma collection. In 5 patients with KRAS positive after converting treatment, liver metastases resection resulted in wild type on consecutive cfDNAs. In responding patients (10) with wild type disease on baseline ctDNA, there was no change in mutational analysis consequently. In non-responders with wild type the appearance of KRAS mutations was noted in 6 patients, while in the rest 3 the progressive disease was not correlated to KRAS mutation. In non-responders with KRAS mutation on baseline ctDNA (6 patients), monitoring of ctDNA revealed disappearance of KRAS mutations contemporary with mainly liver metastases progressive disease.



Sanger sequencing electropherogram vs real-time PCR results of the KRAS mutation (A) NM_033360.3(KRAS):c.35G>A (p.Gly12Asp) and wild type KRAS (B).

CONCLUSIONS:

The estimated concordance rate between primary tumor and ctDNA KRAS mutation analysis was 58%. The emergence of KRAS mutation in wild type patients revealed acquired resistance to anti-EGFR therapy. In patients with mutation KRAS on baseline ctDNA, liver resection in responders and liver metastases progressive disease in non-responders correlated with loss of KRAS mutation as a mechanism of acquired resistance to anti-angiogenesis treatment in the later.

Disclosure statement - All authors declare that they have no conflict of interest.