Supplementary description of methods regarding analysis of *KRAS, NRAS, BRAF, PIK3CA*

DNA isolation

Manual microdissection was performed before DNA extraction from primary and metastatic CRC tissue: A sufficient amount of neoplastic tissue was microscopically identified on hematoxylin- and eosin stained slides. This same area was then re-identified on the unstained 10 µm dewaxed, rehydrated and air-dried tissue section and separately isolated with a cannula, predominantly without adherent non-neoplastic tissue. Separately embedded resection margins without evidence of tumor were used as normal tissue for CRC patients. DNA isolation from paraffin embedded tissue was performed with the High Pure PCR Template Preparation Kit (Roche Molecular Biochemicals, Mannheim, Germany) and the innuPrep DNA Minikit (Analytik Jena, Jena, Germany). Quality of DNA was assessed by agarose gel electrophoresis.

Sequencing analysis

Sequencing analysis of *Kras* Exon 2 was done for part of samples as a means of quality control with primers F 5'AGGCCTGCTGAAAATGACTGAATA and R 5' CTGTATCAAAGAATGGTCCTGCAC. PCR amplification was performed in 12.5 µl sample volumes with 1-2 ng of genomic tumour or nonneoplastic DNA as template in 15 mM Tris/HCl, 50 mM KCl, with 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.1 nM primers, and 1 Unit HotStart Taq Polymerase (Applied Biosystems). An initial denaturation and activation step of 8 min at 95°C was followed by 30-35 cycles of 1 min at 95°C, 1 min at 55°C and 2 min at 72°C, and a 30 min final elongation step at 72°C. Sequencing was always carried out in both directions with the BigDye sequencing kit according to the manufacturer's instructions (Applied Biosystems). Each mutation was verified by a second sequencing reaction of an independent amplification product.

Assessment of Kras mutations with Kras strip assay

For assessment of mutation status of *Kras* positions 12, 13 and 61, we used the KRAS 12/13/61 StripAssay® (ViennaLab, Vienna, Austria) according to the manufacturer's instructions. It detects the mutations c.35G>C, c.34G>C, c.35G>A, c.34G>T, c.[34G>A;35G>T], c.[34G>C;35G>T], c.34G>A, c.35G>T, c.38G>A, c.37G>T, c.182A>G, c.183A>T and c.182A>T and includes positive and negative amplification and

hybridization controls. As quality control, the mutation status was verified by a second reaction directly by ViennaLab in 54 randomly chosen samples with no divergent results.

SNP analysis

Twenty-six recurrent cancer pathway mutations were included in this study as summarized in table S1 (primer sequences partly from Dias-Santagata [S1], and partly self-designed with primer 3 [S2]). They were combined to two SNaPshot assays with the additional safeguard against artefacts that neighbouring mutations were never amplified in one assay together. For both SNP analyses, multiplex PCR was done in a volume of 12.5 µl in the GeneAmp® PCR system 9700 (Applied Biosystems) with 0.5-2 ng of DNA as template in 15 mM Tris/HCl, 50 mM KCl, with 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.1 µM each primer (primer sequences see table S1) and 1.5 Units AmpliTaq Gold Polymerase (Applied Biosystems). PCR conditions were: Initial denaturation and activation step of 8 min at 95°C, 30 cycles of 1 min at 94°C, 1 min at 52°C and 2 min at 72°C, and a 45 min final elongation step at 60°C. SNaPshot analyses were performed with the SNaPshot Multiplex kit (Applied Biosystems) (primer sequences displayed in table S1) in accordance with the manufacturer's instructions and evaluated on an ABI310 Genetic Analyzer. Electrophoresis results were analysed using the GeneMapper® ID Software v3.2 with self-designed panels and bins sets.

Supplementary references

S1 Dias-Santagata D, Akhavanfard S, David SS, *et al*. Rapid targeted mutational analysis of human tumours: a clinical platform to guide personalized cancer medicine. *EMBO Mol Med* 2010;**2**:146-158.

S2 Primer 3: http://bioinfo.ut.ee/primer3-0.4.0/