Supplementary Online Content

Cohen R, Hain E, Buhard O, et al. Association of primary resistance to immune checkpoint inhibitors in metastatic colorectal cancer with misdiagnosis of microsatellite instability or mismatch repair deficiency status. *JAMA Oncol.* Published online November 15, 2018. doi:10.1001/jamaoncol.2018.4942

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix. Methods

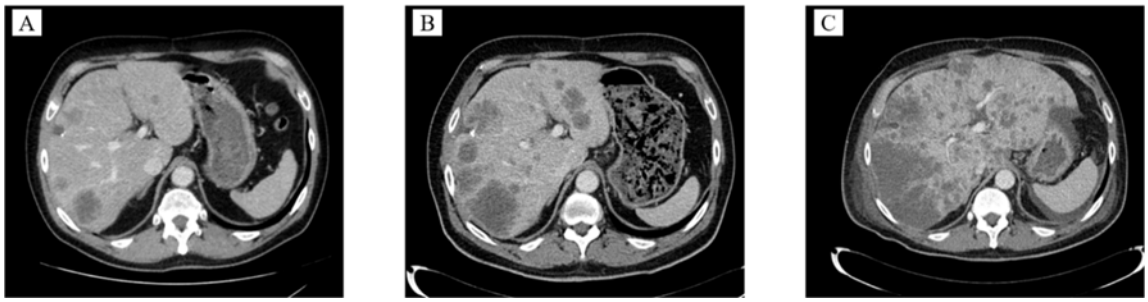
Immunohistochemistry

Formalin-fixed, paraffin-embedded (FFPE) tumor specimens were stained with anti-MLH1, -PMS2, -MSH2 and -MSH6 antibodies. Protein expression was considered negative when nuclear staining was absent in the tumor cells but present in adjacent non-tumor tissue used as an internal positive control. dMMR was defined as loss of expression of MLH1 and/or PMS2, MSH2 and/or MSH6, PMS2 alone, or MSH6 alone. Proficient MMR (pMMR) was defined as unequivocal expression of all 4 MMR proteins in tumor cells.

Microsatellite instability testing

Tumor DNA was extracted from paraffin sections using the DNeasy Blood and Tissue DNA isolation kit (Qiagen, Hilden, Germany). MSI status was determined using the pentaplex mononucleotide repeat panel (BAT-25, BAT-26, NR-21, NR-24 and NR-27). All pentaplex markers were analyzed for size on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). MSI was defined by alterations in the size of at least two of the five markers.

eFigure. CT Scan Evolution of a False-Positive Patient Treated with Immune Checkpoint Inhibitor (Patient #181)



Tumor evolution using CT-scan at the last evaluation 9 weeks before immunotherapy initiation (A), at baseline (B) and at the first evaluation after 6 weeks (C); the patient was diagnosed with a metastatic colorectal cancer harboring mismatch repair deficiency as determined at the originating center (loss of MLH1 expression) and was included in an immunotherapy trial after a 9-week wash-out period; the CT-scan showed disease progression at the first evaluation; re-analysis of the sample in our experienced molecular diagnostic center revealed a microsatellite stable and mismatch repair-proficient tumor.

eTable. Detection Rate and Sensitivity of Immunohistochemistry, Pentaplex and *HSP110* T17 PCR

	Immunohistochemistry	**Pentaplex PCR**	***HSP110* HT17 PCR**
Test results (N=119)*			
Inconclusive results (N)	0	22	2
True positives (N)	114	93	105
False negatives (N)	5	4	12
Detection rate (95% CI)	100% (97.0-1.00)	81.5% (73.4-88.0)	98.3% (94.1-99.8)
Sensitivity (95% CI)	95.8% (90.5-98.6)	78.2% (69.7-85.2)	88.2% (81.1-93.4)

*: samples misdiagnosed by originating laboratories were excluded of this analysis (N=12)
The detection rate of the diagnostic methods is defined as the probability of obtaining a conclusive result (either positive or negative) amongst all tested samples. The sensitivity is defined as the proportion of true positives amongst dMMR and/or MSI samples.