for one third of the cases and hMLH1 hypermethylation for the remaining part [4,5]. Typically, MSI cancers beside exhibiting peculiar pathological features such as medullary histology [6], have a lower pathological stage at diagnosis, and thus a better prognosis [5]. MSI prevalence in gastric, uterine and ovarian cancers approaches that of colon cancer [7,8]. Considering the ominous prognosis of pancreatic cancer [9,10], it would be relevant whether MSI testing could identify PDAC patients with better survival [11,12]. However, the prevalence of MSI remains undefined in pancreatic cancer.

Studies based upon the review of family history, found a risk of pancreatic cancer 7–8 times higher in LS families than in the general population [13,14]. On the other hand, a few studies assessed the prevalence of MSI in PDAC specimens. Goggins first reported 3 (3.7%) MSI cases in a North American series comprising 82 PDAC [15], while European studies from Poland

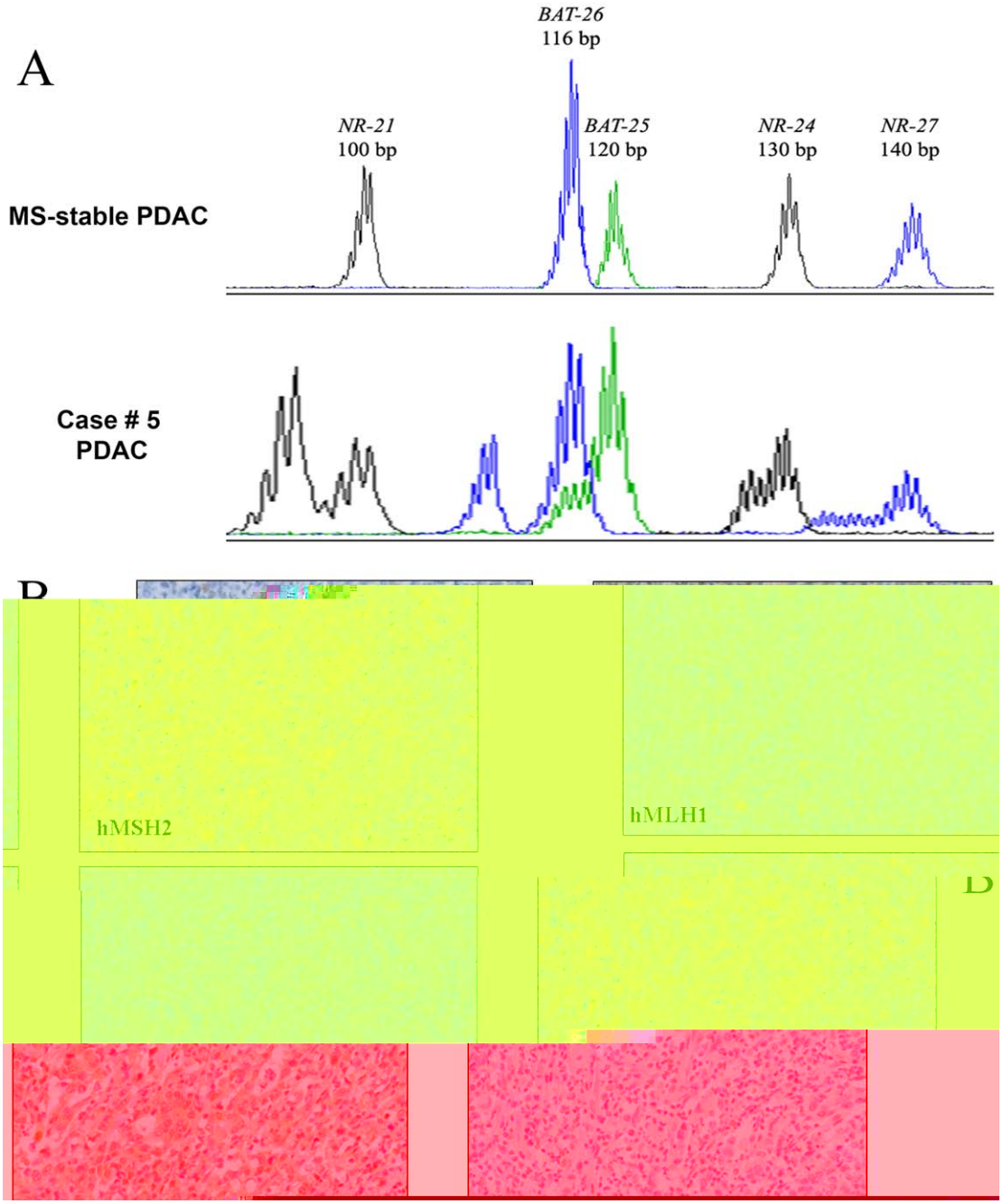

Figure 1. Electropherograms of the analysis of mononucleotide microsatellite markers *BAT26, BAT25, NR-21, Nr-24* and *NR-27* in pancreatic cancers. From top to bottom, an example of MS-Stable PDAC, and the only MSI PDAC (Case #5) (Panel A). Immunohistochemical analysis of MMR protein expression in the medullary hMLH1-deficient PDAC (Case #5), retaining hMSH2 and hMSH6 expression. Note the loss of PMS2, due to protein degradation in the absence of the cognate partner hMLH1 (Objective magnification ×10) (Panel B).
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was diagnostic for PDAC, but no tissue was available for MS-status testing. The other case, treated by resection of the pancreatic head, was an ampullary cancer, with histological features of pancreato-biliary poorly-differentiated (G3) adenocarcinoma, showing MSI and hMSH2 deficiency.

Discussion

MSI was an extremely rare event in the largest and only consecutive series of PDAC ever studied. The implications of our results involve assessment methods, pathological features, and clinical behavior of MSI PDAC as reported so far.

A debated issue has been the technical reproducibility and accuracy of the methods to test MS-status [4]. Already the first proposed microsatellite panel was aimed to standardize MS-status assessment [21], although the di-nucleotide markers initially employed generate false positives [4,22], while mononucleotides are specific [4,23] and can detect MSI [24] without matched normal tissue [4,22]. Noteworthy, the 5 employed markers were fully concordant in all MS-stable (or -unstable) cases. Accordingly,

testing multiple mononucleotide repeats does not increase sensitivity and specificity in a cancer type in which MSI phenotype is so rarely encountered. At any event, our study is the only one performed by using a standard panel of 5 mononucleotide repeats [4,23,25] on the largest series ever investigated. Accordingly, our method reflects the true prevalence of MSI cancers. MSI prevalence$\cong$20% such as reported by Nakata et al. using di-nucleotide markers only and not supported by immuno-histo-

their privacy, the approval for the use of pathology specimens with a waiver of consent was granted by the Review Board of the Humanitas Clinical and Research Center and of the Chìarite Campus Virchow, and by the Ethics Committees of the University Hospital Trust of Verona. A coded data-base was prepared by clinical researchers unaware of molecular data, and deidentified samples under code were obtained from the pathology archives for molecular analysis.

MS-status Assessment and Analysis of MMR Defects

DNA was extracted from 5 micron thick, paraffin-embedded specimens, and cancer tissue was micro-dissected if tumor cells did not account for at least 50% of the sample. MSI assignment was based on the analysis of mononucleotide repeats. After DNA extraction by proteinase-K digestion and phenol-chloroform purification, amplification of the mononucleotide microsatellites BAT25, BAT26, NR-21, NR-24 and NR-27 with fluorescent dye-labeled primers was followed by capillary-gel electrophoresis (ABI PRISM 310 DNA Sequencer, Perkin-Elmer, Foster City, CA, USA) [4,5,25,37,38].

hMLH1 and hMSH2 MMR protein defects were tested by immunohistochemistry in the Verona series, as well as in MSI

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