Colorectal cancers with high density of tumor-infiltrating lymphocytes (TIL), especially of CD8+ T cells, are associated with a better prognosis (1–4), suggesting that a cytotoxic antitumor immune response could control colorectal cancer progression. Cytotoxic immune response is part of a complex immune reaction that includes different cell types, one of them being regulatory T cells (Tregs). Tregs are commonly characterized by the expression of the transcription factor FOXP3 and can suppress cytotoxic TIL (CTL) activities.

Colorectal cancers with microsatellite instability (MSI) represent around 15% of colorectal cancers, including Lynch syndrome, the most frequent hereditary form of colorectal cancer. MSI colorectal cancers are due to a defect of the DNA mismatch repair (MMR) system, leading to accumulation of mutations in microsatellite unstable (MSS) colorectal cancers (9–13), suggesting that MSI colorectal cancers are particularly prone to a local cytotoxic immune response. The link between stronger immunogenicity of MSI colorectal cancers and MMR deficiency is commonly explained by the accumulation of frameshift mutations within coding sequences and the synthesis of neoantigens (14). Degradation of such neoantigens can release immunogenic neopeptides, presented by human leukocyte antigen class I (HLA-I) molecules, on the tumor cell surface, and targeted by a specific CD8+ cellular immune response. In vitro, TILs from MSI colorectal cancer patients have already been activated against some immortalized cell lines (5–8) and a more dense infiltration of intraepithelial activated CD8+ TILs than microsatellite stable (MSS) colorectal cancers (9–13), suggesting that MSI colorectal cancers are more prone to a local cytotoxic cellular immune response. The link between stronger immunogenicity of MSI colorectal cancers and MMR deficiency is commonly explained by the accumulation of frameshift mutations within coding sequences and the synthesis of neoantigens (14).
frameshift mutation-derived peptides (FSP), underlining that such neopeptides could indeed be immunogenic (15).

We have previously shown, in 52 MSI colorectal cancers, that frameshift mutation number and spectrum correlated with total (CD3\(^{\text{+}}\)) TIL density (16). However, to our knowledge, the direct link between frameshift mutations in MSI tumor cells and TIL subpopulation densities has never been shown.

A total of 103 MSI tumors, from two independent series, were analyzed for the correlation between frameshift mutations within 19 selected MSI target genes and CD3\(^{\text{+}}\), CD8\(^{\text{+}}\), and FOXP3\(^{\text{+}}\) TIL densities. We found that CD8\(^{\text{+}}\) TIL density correlated with the number and spectrum of frameshift mutations. Furthermore, we provided evidence that specific CD8 cytotoxic antitumor T-cell responses could be mounted in vitro against frameshift mutations present in patients’ tumors, paving the way for developing new personalized immunotherapy strategies.

### Materials and Methods

#### Tumor samples

For the main series, colorectal tissues were collected from 106 MSI colorectal cancer surgical specimens, at Rouen University Hospital, Rouen, France, between 2003 and 2009. This series included 26 Lynch patients (7 with MLH1 mutation, 15 with MSH2 mutation, 3 with MSH6 mutation, and 1 without MMR gene mutation detected). In agreement with French regulation, all patients gave a written consent for the MMR gene germline mutation detected. In agreement with French regulation, all experiments were approved by ethical review boards. All experiments were performed according to the Helsinki guidelines.

#### Microsatellite instability assessment and determination of tumor cell proportion within the tumor samples

MSI was defined as an instability affecting at least two, among the five, consensus mononucleotide repeats (BAT25, BAT26, NR21, NR22, and NR24; ref. 17) within tumor DNA, compared with normal colon DNA. PCR products were separated on an ABI Prism 3100 DNA sequencer (Applied Biosystems).

As previously described (16), to evaluate the proportion of tumor cells within each tumor sample, the profile corresponding to the most unstable microsatellite was selected. The proportion of tumor cells within a tumor sample was given by the area of the unstable part of this microsatellite profile, divided by the total area of this microsatellite profile (Fig. 1A).

#### Detection of frameshift mutations in target genes: fluorescent multiplex PCRs

As previously described (16), two fluorescent multiplex PCRs were performed on paired normal and tumor DNA samples. In brief, 22 short coding repeat in 19 genes were amplified with a dicistronic vector encoding a puromycin-resistance element and one of the following frameshift peptides: RSLSVCPVA, GMCVKVSS1, and VLRTEGPE1, called FSP02 (22), FSP27, and FSP30 (23). The high affinity of these peptides for the from tumor and paired nonmalignant colorectal tissue DNAs were superimposed. Shifts in the lengths of PCR products corresponded to nucleotidic deletions or insertions (i.e., frame-shift mutations).

We evaluated the proportion of cells harboring each mutation within the tumor sample: for each mutation, on the PCR profile obtained from tumor DNA sample, the area corresponding to mutated DNA was divided by the total area, corresponding to mutated and not mutated DNA. If this percentage was similar to the percentage of malignant cells in the sample, we concluded that this frameshift mutation was present in all tumor cells, given that chromosomal instability is extremely rare in MSI colorectal cancers (18, 19; Fig. 1B). PCRs were performed when both tumor and normal DNA could be obtained (for 87 patients from the main series and the 35 patients from the validation series). Percentage of mutations within a tumor was given by the number of mutated genes divided by the number of analyzed genes.

#### Tissue microarray, immunohistochemistry, and TIL quantification

Tissue microarrays (TMA) were constructed for 86 of the 106 patients from the main series (for some patients, no tumor tissue was available). Tissue cores of 0.6-mm diameter were inserted in recipient paraffin blocks. Four cores were taken at the tumor center, four at the invasion margin, and four from distant histologically normal colonic mucosa (Tissue Arrayer). Four-micrometer-thick sections from these TMA blocks were used for immunohistochemistry staining of CD3\(^{\text{+}}\), CD8\(^{\text{+}}\), and FOXP3\(^{\text{+}}\) cells, as previously described (20), with mouse monoclonal primary anti-CD3, anti-CD8 (Dako), and anti-FOXP3 (AbCam) antibodies. Ventana Bench Mark XT IHC/ISH Staining Module (Ventana Medical Systems) was used according to the manufacturer’s recommended protocol. Each slide, scanned with Mirax Scan (Zeiss Systems), was analyzed as already described (20), with the Chips’N Cheap TMA analysis program, using Aphelion 3.2 software (ADCIS), further optimized for this purpose. On each chip, the number of cells per tissue surface area unit.

#### Construction of artificial antigen presenting cells expressing FSPs

As previously described (21), NIH3T3 fibroblasts were sequentially transfected with five replication-defective gammaretroviral vectors encoding HLA-A*0201 (A*0201 heavy chain and human β2-microglobulin), and three human costimulatory molecules ICAM-1 (CD54), LFA-3 (CD58), and B7.1 (CD80). These artificial antigen presenting cells (AAPC) were then transduced with a dicistronic vector encoding a puromycin-resistance element and one of the following frameshift peptides: RSLSVCPVA, GMCVKVSS1, and VLRTEGPE1, called FSP02 (22), FSP27, and FSP30 (23). The high affinity of these peptides for the
Figure 1. Fluorescent multiplex PCR frameshift mutation analysis. A, MSI status was determined with five consensus mononucleotide repeats (BAT25, BAT26, NR21, NR22, and NR24). For this tumor, all five markers were unstable. To evaluate the percentage of tumor cells within the tumor sample, on the most unstable microsatellite, the area corresponding to mutated DNA was divided by the total area of this microsatellite tumor profile. B, frameshift mutations were characterized based on multiplex PCR profiles. To detect frameshift mutations, mononucleotide repeats of 19 genes were simultaneously amplified in two multiplex PCRs. Tumor and nontumor DNA sample profiles were superimposed and shifts between profiles were identified. For this tumor, one of the two multiplex PCRs revealed that ACVR2, IGFR2, SEC63, TGFBR2, OGT, MSH6, and TCF7L2 were mutated. To evaluate the percentage of cells harboring a given mutation [here a (−1) mutation in ACVR2] within a tumor sample, the area corresponding to mutated DNA was divided by the total area of this gene tumor profile. The percentage of cells harboring the given mutation divided by the percentage of tumor cells in the tumor sample gives the proportion of tumor cells harboring this mutation.
HLA-A*0201 molecule was validated using the SYFPEITHI algorithm (24).

Each peptide coding sequence was cloned downstream of the human CD8α leader sequence, for the peptide to be addressed into the endoplasmic reticulum. AAPCs expressing these peptides were selected with puromycin (Sigma-Aldrich) at 8 μg/mL for 1 week.

Peripheral TL purification and stimulation of antigen-specific CTLs
Peripheral blood mononuclear cells (PBMC) from HLA-A*02+ colorectal cancer patients and healthy donors (HLA phenotype assessed in the laboratory) were used upon informed consent and agreement of the local ethic committee. PBMCs were collected by density centrifugation on a lymphocyte separation medium (Eurobio). The next day, nonactivated TLs were negatively sorted using Dynabeads untouched human T cell kit (Invitrogen) according to the manufacturer’s instructions. Irradiated AAPCs (25 Gy) were plated (10^6 per well) in a 24-well plate the day before as previously described (25). T cells were added (1 × 10^6 per well) to the AAPCs and cultured for 21 days. A second stimulation was performed for 14 days: irradiated AAPCs were plated as for the first stimulation and 3 × 10^5 T cells were added per well. IL-2 (Proleukin Chiron) was added at 20 IU/mL to the cocultures every second day from the seventh day of coculture.

Cytotoxicity assays
As previously described (25), standard 51Cr release assays were performed using HLA-A*0201+ T2 cells (ATCC) loaded with the different peptides (irrelevant, FSP02, FSP27, and FSP30, synthesized by Rouen University Proteomic Platform, IRIB, Inserm U982, France), at 10 μmol/L for 1 hour at room temperature, or using HLA-A*0201+ HCT116 and Colo205 colorectal cancer–derived cell lines (ATCC) incubated 24 hours with IFNγ (Imukin) at 200 IU/mL. T2, HCT116, and Colo205 target cells were labeled with 51Cr (for 1 hour at 37°C). A total of 5 × 10^3 target cells were used per well in 96-well U-bottomed plates at different effector to target (E:T) ratios and incubated at 37°C for 4 hours for the T2 cells, and 18 hours for the colorectal cancer cell lines.

Statistical analyses
Associations between dichotomous characteristics (e.g., presence or absence of frameshift mutations) and continuous variables (e.g., cell densities) were assessed using the Mann–Whitney nonparametric test. Correlations between quantitative variables (e.g., TIL densities and number of frameshift mutations) were assessed using Spearman’s rank correlation coefficient. The Kruskal–Wallis post hoc test was used for pairwise comparisons among three groups.
To select the frameshift mutations associated with tumor infiltration, among 19 studied genes, while accounting for multiple testing, the following procedure was used: a mutation was deemed correlated with TIL density if it was associated with an increased infiltration in at least two among four independent tumor compartments (i.e., epithelium in tumor center, epithelium in invasion front, stroma in tumor center, stroma in invasion front, normal tissue).
front) at the 0.05 level using the Mann–Whitney test. This way, the probability to find, only by chance, a mutation associated with TIL density was lower than 5%.

Results
Frameshift mutation number is highly variable but associated with age and some histopathological features

We studied the mutational status of coding repeat sequences within 19 selected genes by performing comparative multiplex PCRs on tumor and normal colon tissues of 122 MSI tumors from two independent series (Fig. 1). Most of the frameshift mutations were single nucleotide deletions (>95%) within the studied mononucleotidic repeat sequences. As previously reported (16), ACVR2, TAF1B, ASTE1, and TGFBR2 were the most frequently mutated genes (in more than 75% of MSI colorectal cancers). Moreover, in 30% or more of the tumors harboring mutations in ACVR2, TGFBR2, and ASTE1 genes, these given genes were mutated in all malignant cells, whereas TAF1B almost never was (1/99; Supplementary Table S1).

Number of frameshift mutations was correlated with age and some histopathological characteristics, notably the VELIPI (Vascular emboli, lymphatic invasion and perinervous invasion) criteria (Supplementary Table S2), and was highly variable from a tumor to another, ranging from 0 to 18 mutation(s) among the 22 analyzed repeat sequences. No association was found between mutation number and MMR gene status (data not shown).

The median number of frameshift mutations per tumor was 9 for the main series and 11 for the validation one.

TIL density is highly variable but associated with frameshift mutation number

In 86 tumors from the main series (for some patients, no tumor tissue was available) and 35 tumors for the validation series, CD3+, CD8+, and FOXP3+ TILs were quantified in the tumor center, the invasion front, and in nonmalignant distant tissue, using TMAs (Fig. 2). For the 52 patients included in both our previous study (16) and this one, CD3+ T cell density found on TMAs was correlated (P = 0.0001) with the ones previously found on representative fields of whole slides, confirming the reliability of this TMA-based analysis (Supplementary Fig. S1). For the 35 tumors from the validation series, CD3+, CD8+, and FOXP3+ TILs were quantified in the tumor center and the invasion front, using another TMA-based analysis system (as mentioned in Material and Methods), which reliability had also been previously veriﬁed on 230 colorectal tumors (P < 0.0001, data not shown).

TIL densities highly differed between tumors, ranging from 0.16% to 16.5% for CD3, 0.06% to 13% for CD8, and 0.03% to 1.18% for FOXP3 in the main series (percentages of antibody-labeled surfaces), and from 44 to 1,320 cells/mm² for CD3, 10 to 1,020 cells/mm² for CD8, and 0 to 127 cells/mm² for FOXP3 in the validation series.

Looking for correlations between TIL densities in whole tumor tissues and increasing frameshift mutation percentages, we found that, in both series, only CD8+ TIL density signiﬁcantly increased with the percentage of frameshift mutations (Fig. 3 and Supplementary Fig. S2A). There was a tendency for CD3+ and CD8+ TIL density to increase with this percentage, whereas FOXP3+ TIL density was not higher in tumors containing more mutations.

Figure 3.
Associations between frameshift mutation percentages and CD3+ TIL density (% of total stained areas). In the main series, we looked for unbiased robust associations between frameshift mutations in each of the 19 selected genes and CD3+, CD8+, and FOXP3+ TIL densities. For 68 patients, the x-axis gives the percentage of mutations found within the 22 studied coding repeat sequences and the y-axis gives the percentage of total stained areas calculated from TMA immunostained cores (Spearman tests).

ASTE1, HNF1A, and TCF7L2 frameshift mutations are associated with higher CD8+ TIL densities

In the main series, we looked for unbiased robust associations between frameshift mutations in each of the 19 selected genes and CD3+, CD8+, and FOXP3+ TIL densities. We found that frameshift mutations of ASTE1, HNF1A (also known as TCF1), and TCF7L2 (also known as TCF4) genes were correlated with an increased CD8+ TIL density (P < 0.05 in at least two among four independent compartments, that is, epithelium in tumor center, epithelium in invasion front, stroma in tumor center, stroma in invasion front). ASTE1 frameshift mutation was also correlated with an increased CD8+ TIL density (P < 0.05 in at least two among four independent compartments). On the contrary, these mutations were associated neither with FOXP3+ infiltration in any compartment, nor with CD3+ or CD8+ infiltration in normal tissue compartments (Table 1).
The first HLA-A’02+ MSI colorectal cancer patient (P1) included in this functional study was a 27-year-old Lynch patient. In P1’s tumor, we detected (−) mutations in coding repeat sequences of TGFBR2, TAF1B, and ASTE1 genes (Fig. 5A), leading to the putative synthesis of the following neo-peptides of high affinity for HLA’A’0201: RLSSCVPVA (FSP02), GMCVKVSSI (FSP27), and VLRTEGEPL (FSP30). Therefore, we constructed A’0201-restricted AAPCs expressing these frame-shift peptides (AAPCA2.1/FSP02, AAPCA2.1/FSP27, and AAPCA2.1/FSP30, Fig. 5B). After two cocultures with AAPCs encoding FSP02, FSP27, or FSP30, P1’s peripheral CTLs could specifically lyse T2 cells pulsed with the corresponding peptide (Fig. 5C). Moreover, peripheral CTLs stimulated with AAPCA2.1/FSP02 or AAPCA2.1/FSP30 could specifically lyse the HLA-A’0201+ MSI colorectal cancer cell line HCT116, which harbors the same mutations as P1 in TGFBR2 and ASTE1 genes. On the contrary, peripheral CTLs stimulated with AAPCA2.1/FSP27 did not lyse HCT116 cells, which do not harbor the same mutation as the patient in TAF1B gene (Fig. 5D).

We performed similar experiments on another MSI HLA-A’02+ Lynch patient (P2, 39-year-old), whose tumor harbored the (−) TGFBR2 and (−) TAF1B frameshift mutations, but not the ASTE1 (−) mutation. After two stimulations, P2’s peripheral CTLs could specifically lyse T2 cells pulsed with FSP02 and FSP27, but not T2 cells pulsed with FSP30. Moreover, peripheral CTLs stimulated with AAPCA2.1/FSP02 could specifically lyse HCT116 cells (Supplementary Fig. S3A).

Functional assays were also performed on four HLA-A’02+ additional donors: an MSI colorectal cancer Lynch patient (P3, 49-year-old), an MSS colorectal cancer patient (49-year-old), and two healthy donors (26- and 47-year-old). In the tumors of both colorectal cancer patients, we did not detect mutations in TGFBR2, TAF1B, and ASTE1 genes. We cocultured their peripheral CTLs with AAPCA2.1/FSP02, AAPCA2.1/FSP27, AAPCA2.1/FSP30, and AAPCA2.1 encoding M1m (A0221/A021/M1m). M1m is a peptide derived from MART-1, a melanocyte auto-antigen. Here, AAPCA2.1/A021/M1m were used to ascertain TL functionality, because they can easily activate anti-M1m TLs in vitro (25). After two rounds of stimulation on the corresponding AAPCs, TLs from these four donors were cytotoxic against T2 cells pulsed with M1m, but not against T2 cells presenting FSP02, FSP27, or FSP30 (Supplementary Fig. S3B).

Discussion
In this study, we showed that ACRV2, TAF1B, TGFBR2, and ASTE1 genes harbored frameshift mutations in the majority of the MSI colorectal tumors (>75%), confirming results previously obtained on a smaller series (16). To determine whether frameshift mutations could lead to an increased density of different TL populations in MSI colorectal cancers, we studied CD3\(^+\), CD8\(^+\), and FOXP3\(^+\) TLs on 103 patients from two independent series, using TMA.

CD4\(^+\) TLs represent a major TL population, which includes both helper and regulatory T cells, but, unfortunately, they had to be kept out of the scope of this work, as it is often the case when large series of patients are studied by immunohistochemistry (1, 2, 12, 26). Indeed, as many groups and as previously discussed (20), we could not find any anti-CD4 antibody allowing a staining of good enough quality, exploitable with a digital image analysis software.

Patients’ peripheral CD8\(^+\) TLs can be activated against neo-peptides derived from frameshift mutations present in their tumor

We then tested whether tumor-specific frameshift mutations could indeed be immunogenic in MSI colorectal cancers. Therefore, we developed a functional assay based on in vitro peripheral specific CTL activation with AAPCs, expressing the most frequent HLA class I molecule (A’0201), the main costimulatory molecules, ICAM-1, LFA-3, and B7.1 (21), and tumor-specific frameshift peptides.
Figure 4.
Associations between ASTE1, HNF1A, and TCF7L2 mutations and CD8$^+$ TIL densities. A, CD8$^+$ TIL density in tumors harboring no frameshift mutation or at least one frameshift mutation in ASTE1, HNF1A, or TCF7L2 gene. Mean values are shown with SEM. Mann–Whitney tests were performed. B, CD8$^+$ TIL density in the tumors harboring no mutation, at least one mutation in some tumor cells only, and at least one mutation within all tumor cells in ASTE1, HNF1A, or TCF7L2 gene. Bars, mean values; error bars, SEM. Pairwise comparisons were performed with a Kruskal–Wallis post hoc test. A and B, *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. 

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TMA reliability having often been questioned, because of the small tissue areas studied in very heterogeneous tumors (27), we first validated our TMA-based techniques by confirming the concordance of TIL density analysis results obtained after both TMA and whole slide staining.

In our main series, we found that total TIL density was significantly higher within the tumors harboring a higher number of frameshift mutations or within tumors harboring particular mutations. Noteworthy, TGFBR2 frameshift mutation was not associated with FOXP3+ (neither CD3+ nor CD8+) TIL density. This finding does not support the notion that the increased concentration of TGFβ in the tumor microenvironment, due to TGFβR2 mutation, could lead to the differentiation of

In vitro activation of antitumor FSP-specific T cells from an MSI colorectal cancer patient. A, percentage of tumor cells within patient's tumor sample was evaluated on the PCR profile of the most unstable MSI marker, NR22. Mutations in target genes were detected in two multiplex PCRs. Three frameshift mutations, potentially leading to the synthesis of FSPs, were present in more than a third of all tumor cells: TGFBR2 (-1), TAFIB (-1), and ASTE1 (-1). B, construction of AAPCs expressing FSP02, FSP27, or FSP30 (AAPC21903, AAPC219027, and AAPC219030). Nonreplicative dicistronic gamma retroviral vector encoding FSP02, FSP27, or FSP30 and a puromycin-resistance element (puroR, puromycin-N-acetyltransferase) in two coding sequences separated by an internal ribosome entry site was used. LTR, long terminal repeat; hCD8αL, human CD8α leader; SD, splice donor site; SA, splice acceptor site; ψ+, extended packaging signal. C, specific cytotoxic activity of patient's peripheral TLs stimulated with AAPC21903, AAPC219027, or AAPC219030 was assessed in standard 51Cr release assays on HLA-A*0201+ T2 cells pulsed with an irrelevant or a relevant (FSP02, FSP27, or FSP30) peptide at different effector-to-target (E:T) ratios. D, top, for both HLA-A*0201 colorectal cancer cell lines, HCT116 (MSI) and Colo205 (MSS), TGFBR2, TAFIB, and ASTE1, multiplex PCR profiles are shown. HCT116 cells harbored TGFBR2 (-V-1), TAFIB (-3/wt), and ASTE1 (-1/-1) mutations; bottom, specific cytotoxic activity of patient's peripheral TLs stimulated with AAPC21903, AAPC219027, or AAPC219030 was assessed in standard 51Cr release assays on HCT116 and Colo205 cell lines, at different effector-to-target ratios.
effect TILs into regulatory FOXP3+ TILs (28) and to a general increase in TIL density (29, 30). The fact that FOXP3+ TIL density was not correlated with frameshift mutations could be due to a lack of FSP-specific infiltrating Tregs able to inhibit FSP-specific CD8+ cytotoxic response, as suggested by Bauer and colleagues (31).

The most important result of this study was certainly the correlation found between CD8+ TIL density and frameshift mutation number, in MSI colorectal cancer. Proteins derived from these mutations could be degraded into immunogenic peptides responsible for the increased CD8+ TIL density. In line with this, in vitro CD8+ TIL responses against FSPs have already been reported in MSI colorectal cancer patients, although no correlation could be found between these responses and the frameshift mutations present in the tumors (15, 32).

We observed that frameshift mutations in ASTE1, HNF1A, and TCF7L2 genes were robustly associated with an increased CD8+ TIL density. Mutated TCF7L2 mRNA expression, in MSI colorectal cancers, had already been found correlated with a stronger peritumoral lymphoid reaction (33) and with CD3+ infiltration (34), but we showed for the first time, to our knowledge, a correlation of these three mutated genes with an increased CD8+ tumoral infiltration. Moreover, CD8+ TIL densities were higher in tumors harboring ASTE1, HNF1A, or TCF7L2 mutation in all tumor cells. These correlations suggest that frameshift mutations in these genes can lead to the production of neoantigens fragmented into particularly immunogenic neopeptides, recognized by specific CD8+ TILs.

It could be argued that some FSPs could not be presented because frameshift mutations generally give rise to premature termination codon-containing mRNAs, which are prone to degradation by nonsense-mediated mRNA decay (NMD; ref. 35). Among the three mutations we found to be robustly correlated with CD8+ infiltration, ASTE1 and TCF7L2 mRNAs are not predicted to be degraded by NMD but HNF1A mRNA is. However, NMD is not a completely efficient mechanism in tumor cells (36, 37) and, moreover, it has been shown that a major source of antigenic peptides for the MHCI pathway is the pioneer round of mRNA translation that precedes putative NMD (38).

CD8+ TIL density was not correlated with prognosis in these independent series of patients with MSI colorectal cancer (20). Our series might be too small for robust survival statistical analysis, but there could also be a balance between beneficial effects of the immune response and deleterious effects of some studied mutations, many of the studied genes being tumor suppressor genes. Moreover, the correlations found between CD8+ cell density and frameshift mutation number and spectrum suggest that specific CD8+ TILs could be retained at the tumor site, but more detailed TIL in situ function studies, especially of activity markers such as granzymes, perforines, or cytokines, would be needed to better understand the relationships between these mutations and the immune cells.

After specific activation with AAPCs, MSI colorectal cancer patients’ peripheral TILs could recognize FSPs derived from frameshift mutations present in their tumor, especially the most correlated one with CD8+ TIL density, that is, ASTE1 (−1) mutation, and the most studied one in terms of immunogenicity, that is, TGFBR2 (−1) mutation (22). On the contrary, peripheral TILs from colorectal cancer patients whose tumors did not harbor these mutations or from healthy donors could not be activated with AAPCs encoding the same FSPs. This strongly suggests that, in vivo, the patient’s TILs had already encountered these FSPs, expressed by the patient’s tumor cells, allowing an in vitro memory specific recall response against the same antigen.

Altogether, this work establishes the link between frameshift mutations and CD8+ TIL tumor infiltration in MSI colorectal cancer patients, and emphasizes the interest, in MSI colorectal cancer patients and especially in young Lynch syndrome patients, of developing personalized cellular adoptive immunotherapy strategies based on in vitro stimulation of their own CTLs against tumor-specific immunogenic neopeptides derived from frameshift mutations found in their tumor.

Disclosure of Potential Conflicts of Interest

J. Galon is a cofounder and consultant at HalioDx. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: P. Maby, D. Tougeron, J.-B. Latouche


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Correlation between Density of CD8+ T-cell Infiltrate in Microsatellite Unstable Colorectal Cancers and Frameshift Mutations: A Rationale for Personalized Immunotherapy

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