Supplemental Methods

Clinical data collection and histopathological assessment

Clinicopathological data were collected by reviewing electronic medical records and microscopic examination of hematoxylin and eosin (H&E)-stained tissue slides by experienced pathologists (J.H.K., J.A.L., and H.J.O.). The data included age, sex, tumor location, gross tumor type, tumor size, depth of tumor invasion (pT category), lymph node metastasis (pN category), distant metastasis (pM or cM category), early recurrence (defined as clinically or pathologically confirmed tumor recurrence within two years after curative surgical removal), lymphatic invasion, venous invasion, perineural invasion, tumor differentiation grade, mucinous histology, medullary histology, signet ring cell histology, tumor budding, and poorly differentiated clusters. Histological assessment of tumor budding and poorly differentiated clusters was performed based on the International Tumor Budding Consensus Conference 2016 recommendation and Ueno criteria, respectively.[1 2]

PD-L1 expression scoring

PD-L1 expression was separately evaluated in tumor and immune cells using IHC and the H-score.[3] To calculate the H-score, staining intensity was semi-quantitatively evaluated by assigning it to one of four grades, as follows: 0, negative expression; 1+, weak expression; 2+, moderate expression; 3+, strong expression. The results were then applied to the following formula: H-score = [1 × (% area showing staining intensity 1+)] + [2 × (% area showing staining intensity 2+)] + [3 × (% area showing staining intensity 3+)].[3] The total PD-L1 expression score in each tumor was calculated as follows: total PD-L1 score = H-score of PD-L1 expression in tumor cells (PD-L1 tumor cell score) + H-score of PD-L1 expression in immune cells (PD-L1 immune cell score). The PD-L1 tumor cell score or PD-L1 immune cell score can range from 0–300, and the total PD-L1 score can range from 0–600.

Histomorphometric assessment of TLS activity

Assessment of the activity of TLS in the peritumoral area was performed using a histomorphometric method.[4 5] The unique formation of multiple TLSs below the invasive front of CRCs has also been referred to as the Crohn-like lymphoid reaction (CLR).[4] Among multiple assessment strategies for TLS or CLR in CRCs, we employed Ueno's assessment criteria for CLR activity as a validated strong prognosticator.[4 5] For each of the 73 MSI-H and 411 MSS CRCs (selected cases with multiple H&E tumor sections available), the largest TLS was measured on H&E-stained digitally scanned slides by three pathologists (J.H.K., J.A.L., and H.J.O.) and analyzed using digital pathology software (Aperio ImageScope version 12.4, Leica Biosystems). Cases were classified as TLS-active if the largest TLS was ≥1,000 μm; otherwise, cases were classified as TLS-inactive (online supplemental figure 3).[4 5]

Analyses of *KRAS*/*BRAF* mutations and CpG island methylator phenotype

Oncogenic hotspot mutations in *KRAS* and *BRAF* were assessed using peptide nucleic acid (PNA) clamping-mediated real-time polymerase chain reaction (PCR) and Sanger sequencing. Initially, the 73 MSI-H CRCs were subjected to PNA clamping-mediated real-time PCR for *KRAS* codons 12, 13, 59, 61, 117, and 146 and *BRAF* codon 600 using the PNAClamp Mutation Detection Kit (Panagene, Daejeon, South Korea). To confirm the *KRAS*/*BRAF* mutation results, Sanger sequencing for *KRAS* exons 2, 3, and 4 and *BRAF* exon 15 was additionally performed in all the 73 MSI-H CRCs, as previously described.[6] The CpG island methylator phenotype (CIMP) status of the 73 MSI-H CRCs was analyzed using the methylation-specific quantitative real-time PCR method (MethyLight assay), as previously described.[6]

Exome data analysis

To detect somatic mutations, raw data (FASTQ) were aligned to the reference genome (GRCh38) using BWA-MEM.[7] PCR duplicates were marked using Picard, and base recalibration and recalibrating base qualities were performed using the Genome Analysis Toolkit (GATK, 4.0.11.0) according to GATK best practice.[8] We used Strelka2[9] to detect somatic single-nucleotide variants (SNVs) and insertions and deletions (indels), and output was annotated using vcf2maf with VEP.[10] Only nonsynonymous coding

variants were selected. To remove common germline variants, we further filtered dbSNP variants or variants that are present in gnomAD with AF ≥ 0.005, while keeping the following known pathogenic variants: (1) listed as pathogenic in ClinVar; (2) reported in COSMIC; or (3) located in hotspots. Functional impacts of variants were assessed using CADD (score > 20), Polyphen2, SIFT, CONDEL, and Fathmm-MKL coding prediction to define deleterious or damaging mutations. TMB was calculated as the number of nonsynonymous somatic mutations per megabase in the exome target region (50 Mb). For mutational process analysis, mutational signatures were determined using maftools with nonsynonymous variants per sample with COSMIC version 3 (https://cancer.sanger.ac.uk/signatures/ sbs). To identify somatic copy number variations (CNVs), the allele-specific copy number was called using Sequenza[11] and GISTIC[12] to analyze recurring amplification and deletion. CNV load was calculated as the number of CNV events (amplification and loss) at the gene level.

Transcriptome data analysis

RNA-Seq reads were aligned to the reference genome GRCh38 with Gencode v29 using STAR v2.6.0c with two-pass mapping.[13] The count value per sample was generated using the STAR with --quantMode GeneCounts option and normalized using the variance stabilizing transformation (VST) provided in the DESeq2 package.[14] DEGs were tested using the Mann-Whitney U test (for two groups) or the Kruskal–Wallis test (for ≥3 groups). To reveal the informative gene features for immune groups, the random forest recursive feature elimination algorithm with 5-fold cross validation was used and repeated 100 times. For the inputted genes in modeling, the most variable gene set (over standard deviation 0.9, N = 1241) was used. Fusion transcripts were identified using JAFFA[15] and FusionCatcher.[16] The scheme of fusion transcripts was visualized using Arriba (https://github.com/suhrig/arriba), and potential functional consequences of the fused protein were assessed using AGFusion. FusionHub was used as an annotation database for known somatic fusions. The novel *SFPQ-NTRK1* fusion discovered by RNA-Seq was verified using the Genome Rearrangement IDentification Software Suite (GRIDSS)[17] and GeneFuse[18] with whole-exome sequencing data.

Prediction of neoantigen load

Human leukocyte antigen (HLA) typing was performed using OptiType (v1.3.2) with tumor DNA reads from sequencing.[19] Neoantigen prediction was performed using NeoPredPipe.[20] Binding affinity prediction of nine amino acid peptide sequences containing mutated sites was performed using NetMHCpan4.0[21] together with HLA types per sample determined by using OptiType. Neoantigen load was calculated with a strong binding peptide according to the rank of the predicted affinity, which was compared to a set of random natural peptides (% rank < 0.5). We also calculated the neoantigen peptide load based on the unique peptides.

Exploration of immuno-oncological targets

To identify novel and alternative immuno-oncological targets for subtype-specific groups, we used a list of immuno-oncological targets in the CRI iAtlas database.[22]

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