Supplementary Materials

Methods

Antibodies used and immunohistochemistry

The following primary antibodies were used as following: anti-B7-H4 (clone HPA054200, 1:400; Sigma–Aldrich, Ronkonkoma, NY, USA), anti-LAG3 (clone D2G40, 1:100; Cell Signaling Technology, CST, Beverly, Massachusetts), anti-PD-L1 (clone E1L3N, 1:200 dilution; CST), anti-B7-H3 (clone D9M2 L, 1:300; CST), anti-IDO1 (clone D5J4E; 1:400; CST), anti-VISTA (clone D1L2G, 1:500; CST), anti-TIM-3 (clone D5D5R, 1:400; CST), anti-ICOS (clone D1K2T, 1:1600; CST), and anti-OX40 (ab119904, 1:1600; Abcam, Cambridge, UK).

Digital image analysis

The immune checkpoint PD-L1 is expressed by both tumor cells and tumor-associated immune cells and were assessed in combination with both compartments. In contrast, B7-H3 is expressed only by tumor cells whereas IDO1, VISTA, TIM-3, ICOS, and OX40 are expressed by tumor-associated immune cells and were assessed only in the tumor cells or tumor-associated immune cells compartment. B7-H4 and LAG3 were all negative in the tissue samples. All cell nuclei in the tumour bulk were identified in haematoxylin channel images after stain deconvolution [1] using a Mask-RCNN-based instance segmentation model. [2] The positive nuclei were further recognized according to the intensities in the DAB channel image, and the density of the positive nuclei in the tumor bulk was calculated. Based

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on a computational pathology analysis, the expression of the immune checkpoints was digitally defined and quantified as the number of positive expression in combined areas (per mm^2), including tumor cells and/or tumor-associated immune cells.

Random forest for ICS construction

Random forest is a complex nonlinear model and built using the R package "randomForestSRC 3.1.0. As the number of trees increased, the error rate gradually stabilized between 0.42 and 0.44 (**Supplementary Figure S2A**). We used an ensemble of 177 estimator trees and Gini entropy to evaluate the quality of a split at each code of a tree. The variable performance measures were generated based on the Gini index of the random forest model (**Supplementary Figure S2B**). Eventually, the top four immune checkpoints, OX40, B7-H3, ICOS, and TIM-3, were selected for the model (**Supplementary Figure S2B**). The out-of-bag (OOB) Brier and OOB continuous rank probability score (CRPS) were consistently used to evaluate the prediction accuracy of the model. The OOB Brier and OOB CRPS gradually increased as the OS time increased (**Supplementary Figure S2C and S2D**). **Supplementary Figure S2E** shows a decision tree in the random forest. A nonlinear relationship existed between the four selected genes and mortality (**Supplementary Figure S2F, S2G, S2H and S2I**). The model was constructed using the discovery set of our FFPE cases (n = 212), and then, we grouped the NB patients into low- and high-risk groups based on the ICS scores using a minimum *P* value.

The model was further validated in the GSE85047 dataset. We used the "predict.rfsrc" function of the R package "randomForestSRC" to confirm the prediction value of the ICS in the validation set.

References

1 Ruifrok AC, Katz RL, Johnston DA. Comparison of quantification of histochemical staining by hue-saturation-intensity (HSI) transformation and color-deconvolution. Appl Immunohistochem Mol Morphol 2003; 11: 85-91.

2 Jung H, Lodhi B, Kang J. An automatic nuclei segmentation method based on deep convolutional neural networks for histopathology images. BMC Biomed Eng 2019; 1: 24.