Supplementary Material

# Supplementary Figures and Tables

## Supplementary Figures

SUPPLEMENTARY FIGURE 1

 (A) The size of the tumor pre-treatment in each group. NONE, surgery alone; (B) Changes in size pre and post-treatment in the NCT group and NICT group; (C) The ratio of clinical staging raise between NCT group and NICT group; (D) Clinical cases. The maximum thickness of tumors in cross-sectional CT images. *\*p* < 0.05, *\*\*p* <0.01, *\*\*\*p* < 0.001.

SUPPLEMENTARY FIGURE 2

(A) Comparison of pathway enrichment analysis between the NONE and NICT groups; (B) Comparison of pathway enrichment analysis between the NONE and NCT groups; (C) Comparison of function cluster of the differential genes between the NONE and NCT groups; (D) Comparison of cycle net plot between the NONE and NCT groups; (E) The network of regulating immune function suggested that the increasing expressions of genes in the NICT group promoted adaptive immune response; (F) The network of regulating immune function shows the increasing expressions of genes in the NICT group can activate and regulate NK cells; (G) Expression of CD3G,NKG7, and FCGR3A in T cells and NK cells analyze by single-cell analysis; (H) The proportion of various types of immune cells in esophageal cancer and normal tissue; (I) The proportion of T cells and NK cells in tumor and normal tissue.

SUPPLEMENTARY FIGURE 3

 (A) Heatmap shows the production of protein phosphorylation and regulation of kinase activity in clinical cases among the NONE, NCT, and NICT groups; (B) Compare the expression of cytokines in the NONE group, NCT group, and NICT group; (C) Expres-sion levels of PRF1 in immune cells and tumors based on the Human Protein Atlas(HPA) database; (D) Expression levels of GZMA and GZMM in immune cells and tumors based on HPA database; (E) The correlation between CD8A and CD8B+T cells and the expression of EOMES,IFN-γ,TCIRG1,PRF1,GZMA and GZMM based on HPA database.

SUPPLEMENTARY FIGURE 4

 (A) HPA database showed the expression level of PDCD1 in tumors; (B) HPA database showed the expression level of PDCD1 in immune cells; (C) Expression status of PDCD1 in ESCA from GEPIA database; (D) IHC showed the expression status of CT8+T cells among NONE, NCT and NICT groups,and performed cell count analysis; (E) Expression status of TCIRG1 in ESCA from GEPIA database; (F) IHC showed the expression status of TCIRG1 among NONE, NCT and NICT groups; (G) HPA database showed the expression level of IFN-γ in immune cells and tumors. *\*p* < 0.05, *\*\*p* <0.01, *\*\*\*p* < 0.001.

SUPPLEMENTARY FIGURE 5

(A) Heatmap shows the expression status of NK cell characteristic antigen in each group of cases; (B) IHC showed the expression status of CD16+NK cells among NONE, NCT and NICT groups; (C) HAP database showed the types of T cells and NK cells expressing IFN-γ in ESCA; (D) Heatmap showed the main targets of ICBs in each group of clinical cases; (E) HAP database showed the expression level of CD276 in many tumors; (F) HAP database showed the expression level of CD276 in immune cells; (G) Expression status of CD276 in ESCA from GEPIA database; (H) Expression status of CD276 in ESCA and the normal tissues from UALCAN database; (I) IHC showed the expression status of CD276 cells in each group; (J) Multiplex immunofluorescence showed the expression status and quantity changes in CD8+ cells and CD276+ cells among the NICT and NCT groups in pre-NT; (K) Multiplex immunofluorescence showed the expression status and quantity changes in CD8+ cells and CD276+ cells among the NICT and NCT groups in post-NT.

1.2 Supplementary Tables

SUPPLEMENTARY TABLE S1 Baseline characteristics of patients in three treatment groups.

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristics  | NONE(%)a(n=120) | NCT(%)b(n=64)  | NICT(%)c(n=95) |
| Sex:MaleFemale | 111 （92.5）9 (7.5) | 62（96.9）2（3.1） | 94（98.9）1（1.1） |
| Age(year):<60≥60 | 46（38.3）74（61.7） | 27（42.2）37（57.8） | 49（51.6）46（48.4） |
| History of smoking No Yes | 54（44.8）66（55.2） | 19（29.2）45（70.8） | 39（40.8）56（59.2） |
| History of alcohol No Yes | 55（45.9）65（54.1） | 23（35.4）41（64.6） | 42（40.8）61（59.2） |
| Comorbidityd No Yes | 86（71.3）34（28.7） | 45（69.2）19（30.8） | 76（79.6）19（20.4） |
| Family historyNoYes | 105（87.5）15（12.5） | 59（92.2）5（7.8） | 81（85.3）14（14.7） |
| ECOGe01 | 5（4.2）115（95.8） | 2（3.1）62（96.9） | 2（2.1）93（97.9） |
| Tumor locationfUpperMiddleLower | 5（4.2）32（26.7）83（69.1） | 7（10.9）24（37.5）33（51.6） | 6（6.3）24（25.3）65（68.4） |
| PathologySquamous-cell carcinomaAdenocarcinomaOther | 99（86.7）11（7.7）10（5.5） | 54（84.6）5（7.7）5（7.7） | 91（96.1）1（1.0）3（2.9） |
| DifferentiationWellModeratelyPoorlyUnknown | 9（7.5）60（50）44（36.7）7（5.8） | 5（7.8）26（40.6）27（42.2）6（9.2） | 7（7.4）36（37.9）24（25.3）28（29.4） |
| Clinical T stageT1T2T3T4aCould not be determined | 16（13.3）40（33.3）63（52.6）1（0.8）0（0） | 8（12.5）11（17.2）36（56.2）9（14.1）0（0） | 2（2.1）23（24.2）64（67.4）6（6.3）0（0） |
| Clinical N stageN0N1N2N3Could not be determined | 51（42.5）51（42.5）14（11.7）0（0）4（3.3） | 22（34.4）27（42.1）14（21.9）1（1.6）0（0） | 18（18.9）52（54.7）21（22.2）2（2.1）2（2.1） |
| Clinical stagegIIIIIIIVACould not be determined | 15（12.5）52（43.3）48（40.0）1（0.8）4（3.3） | 8（12.5）18（28.1）26（40.6）12（18.8）0（0） | 2（2.1）28（29.5）58（61.1）7（7.4）0（0） |

a NONE，surgery alone (non-neoadjuvant therapy); b NCT, neoadjuvant chemotherapy; c NICT, neoadjuvant immunochemotherapy; d Comorbidity, including hypertension, cardiovascular and cerebrovascular diseases, diabetes, hepatitis B and C; e ECOG, Eastern Cooperative Oncology Group; f Tumor location, the location of the tumor is mainly determined by gastroscopy,CT and esophagography; g Clinical stage, based on Union for International Cancer Control/American Joint Committee on Cancer 8th edition staging criteria (UICC/AJCC ).

SUPPLEMENTARY TABLE S2 Sequences of qRT-PCR primer

|  |  |
| --- | --- |
| Primer name | Sequences （5'→3'） |
| Q-GAPDH-F | ACAACTTTGGTATCGTGGAAGG |
| Q-GAPDH-R | GCCATCACGCCACAGTTTC |
| Q-TNFAIP3-F | TCCTCAGGCTTTGTATTTGAGC |
| Q-TNFAIP3-R | TGTGTATCGGTGCATGGTTTTA |
| Q-FCGR3A-F | CCTCCTGTCTAGTCGGTTTGG |
| Q-FCGR3A-R | TCGAGCACCCTGTACCATTGA |
| Q-IL17A-F | AGATTACTACAACCGATCCACCT |
| Q-IL17A-R | GGGGACAGAGTTCATGTGGTA |
| Q-IFI30-F | CCCCTCTGCAAGCGTTAGAC |
| Q-IFI30-R | CCCGCAGGTATAGATTGCCT |
| Q-BAX-F | CCCGAGAGGTCTTTTTCCGAG |
| Q-BAX-R | CCAGCCCATGATGGTTCTGAT |
| Q-BACH1-F | TCTGAGTGAGAACTCGGTTTTTG |
| Q-BACH1-R | CGCTGGTCATTAAGGCTGAGTAA |
| Q-CNN2-F | ACCGGCTCCTGTCCAAATATG |
| Q-CNN2-R | CCCGGCTGTAGCTTGTTCA |
| Q-CDK10-F | GCCTGCGTCATCCGAACAT |
| Q-CDK10-R | AGGGTGTTGGCATATTCTCCA |
| Q-TNK2-F | CTGTCCCACTTTGAGTACGTC |
| Q-TNK2-R | GACTTGCGTTTGCACAAGGC |
| Q-TYK2-F | GAACCGGCTGTGTACCGTT |
| Q-TYK2-R | ACGTCATTCACAAACTCATGCTT |
| Q-TYR-F | TGCACAGAGAGACGACTCTTG |
| Q-TYR-R | GAGCTGATGGTATGCTTTGCTAA |
| Q-PPP3CA-F | GCGCATCTTATGAAGGAGGGA |
| Q-PPP3CA-R | TGACTGGCGCATCAATATCCA |
| Q-TCIRG1-F | CATGGTCCTTGCGGAGAACC |
| Q-TCIRG1-R | GCCGGTGTAGATGGAGAACAG |
| Q-TBX21-F | GGTTGCGGAGACATGCTGA |
| Q-TBX21-R | GTAGGCGTAGGCTCCAAGG |
| Q-EOMES-F | GTGCCCACGTCTACCTGTG |
| Q-EOMES-R | CCTGCCCTGTTTCGTAATGAT |
| Q-IFN-γ-F | TCGGTAACTGACTTGAATGTCCA |
| Q-IFN-γ-R | TCGCTTCCCTGTTTTAGCTGC |
| Q-GZMM-F | ACACCCGCATGTGTAACAACA |
| Q-GZMM-R | GGAGGCTTGAAGATGTCAGTG |

SUPPLEMENTARY TABLE S3 Primary antibody and secondary antibody

|  |  |  |
| --- | --- | --- |
| Antibody name | Brand | Catalog number |
| IFN-γ | ProteinTech  | 15365-1-AP |
| TCIRG1 | ProteinTech  | 12649-1-AP |
| CD8 | ProteinTech | 66868-1-lg |
| CD16 | ProteinTech | 66779-1-lg |
| CD16 | ProteinTech | 16559-1-AP |
| CD276 | ProteinTech | 14453-1-AP |
| CD276 | ProteinTech | 66481-1-lg |
| Goat anti-Mouse IgG | ProteinTech | SA00001-1 |
| Goat anti-Rabbit IgG | ProteinTech | SA00001-2 |