·论 著·

基于免疫微环境特征的曲妥珠单抗与免疫治疗联合应用预测模型

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［**摘要**］**背景与目的：**人表皮生长因子受体2（human epidermal growth factor receptor 2，HER2）阳性乳腺癌患者的肿瘤免疫微环境（tumor immune microenvironment，TIME）与曲妥珠单抗治疗效果显著相关，提示免疫检查点疗法联合曲妥珠单抗治疗的临床潜力。本研究旨在探索HER2阳性乳腺癌联合治疗的预测因子，筛选联合治疗的潜在获益人群。**方法：**纳入高通量基因表达（Gene Expression Omnibus，GEO）数据库中509例接受曲妥珠单抗治疗的HER2阳性乳腺癌患者和癌症基因组图谱（The Cancer Genome Atlas，TCGA）数据库中67例HER2阳性乳腺癌患者的转录组与基因组数据，筛选曲妥珠单抗耐药组的差异表达基因进行功能富集分析、蛋白质互作网络构建。结合临床信息通过对数秩检验和多因素COX比例风险回归模型构建预测模型。并利用CIBERSORT反卷积法分析TIME特征，通过肿瘤免疫功能障碍和排斥（tumor immune dysfunction and exclusion，TIDE）评分预测免疫治疗获益。**结果：**通过分析曲妥珠单抗缓解组和曲妥珠单抗耐药组之间的免疫微环境与基因表达特征，构建了由4个核心基因（*GATA6*、*TRPV6*、*AMACR*、*ZHX2*）组成的曲妥珠单抗相关基因预测指数（trastuzumab related genetic prognostic index，TRGPI）。低TRGPI评分的患者的TIME含有更高比例的$CD8^+$ T淋巴细胞和激活的自然杀伤细胞，同时程序性死亡［蛋白］-1（programmed death-1，PD-1）的表达更高，更倾向于从曲妥珠单抗联合免疫治疗中获益。**结论：**本研究基于TIME重新定义了HER2阳性乳腺癌曲妥珠单抗联合免疫治疗的获益人群，并为临床应用提供了可选的治疗策略。

［**关键词**］肿瘤免疫微环境；曲妥珠单抗；免疫治疗；人表皮生长因子受体2阳性乳腺癌；预测模型

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Characterization of immune microenvironment identifies prognostic and immunotherapy benefit for trastuzumab-based therapy

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［**Abstract**］**Background and Purpose:** The tumor immune microenvironment (TIME) of breast cancer with positive human epidermal growth factor receptor 2 (HER2) is significantly related to the efficacy of trastuzumab, indicating the clinical potential of immunocheckpoint therapy combined with trastuzumab. This study aimed to explore the predictors of HER2-positive breast cancer combination therapy and screen the potential beneficiaries of combination therapy. **Methods:** Transcriptome and genome data of 509 HER2-positive breast cancer samples of patients receiving trastuzumab treatment from Gene Expression Omnibus (GEO) database and 67 HER2-positive breast cancer samples from The Cancer Genome Atlas (TCGA) databases were collected. Trastuzumab-

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resistant group's differentially expressed genes were identified and analyzed for functional enrichment and protein-protein interaction. The log-rank test and multivariate COX proportional hazards regression were used with clinical data to create the prediction model. The TIME landscape was characterized using the CIBERSORT. The immunotherapy benefit was valued by the tumor immune dysfunction and exclusion (TIDE) score. **Results:** The trastuzumab related genetic prognostic index (TRGPI) consisting of four hub genes (*GATA*6, *TRPV*6, *AMACR*, *ZHX*2) was constructed by analyzing the immune microenvironment and gene expression characteristics between trastuzumab-remission group and trastuzumab-resistance group. Importantly, the results revealed that patients with lower TRPGI were trastuzumab-sensitive and more likely to benefit from immunotherapy because of the increased percentages of $CD8^+$ T cells, active natural killer cells and programmed death-1 (PD-1) expression. **Conclusion:** This study redefined the benefit population through TIME and provided a selectable strategy of trastuzumab plus immunotherapy for HER2-positive breast cancer.

［**Key words**］ Tumor immune microenvironment; Trastuzumab; Immunotherapy; Human epidermal growth factor receptor 2-positive breast cancer; Prediction model

乳腺癌是一种异质性的疾病，临床上按照雌激素受体（estrogen receptor，ER）、孕激素受体（progesterone receptor，PR）、人表皮生长因子受体2（human epidermal growth factor receptor 2，HER2）的表达情况及Ki-67增殖指数将乳腺癌分为Luminal A型、Luminal B型、HER2阳性型及三阴性乳腺癌（triple-negative breast cancer，TNBC），其中HER2阳性乳腺癌具有分化水平较差、临床病理学分期较高和死亡风险增加的特点[1-2]。曲妥珠单抗是一种靶向HER2的单克隆抗体，可显著提高HER2阳性乳腺癌患者的无病生存率和总生存率[3]。尽管如此，仍有近一半的HER2阳性患者出现曲妥珠单抗耐药[4]。研究[5-9]表明，曲妥珠单抗耐药的机制主要包括表皮生长因子受体（epidermal growth factor receptor，EGFR）家族配体的表达、HER2表位不可及、*PTEN*基因的丢失、胰岛素样生长因子通路的激活和免疫逃逸。针对以上耐药机制，大量临床前和临床研究[10-18]提出了曲妥珠单抗的联合治疗策略，包括联合多种化疗药物、HER2靶向药物、免疫检查点抑制剂（immune checkpoint inhibitor，ICI）的治疗方案，并在过去的20年内很大程度改善了HER2阳性乳腺癌患者的预后状况。但目前证据[19]表明，许多HER2阳性乳腺癌患者面临着推荐方案下的过度医疗，而部分患者仍会经历转移性复发。因此需要更为有效的HER2阳性乳腺癌的复发风险预测生物标志物，以制订适合不同患者的个体化治疗方案。

免疫逃逸是曲妥珠单抗耐药的机制之一，表现为肿瘤免疫微环境（tumor immune microenvironment，TIME）中免疫细胞的数量减少或功能失调导致抗肿瘤作用的减弱[20-22]。曲妥珠单抗的细胞毒性依赖于免疫反应的激活，主要包括抗体依赖的细胞介导的细胞毒性作用（antibody-dependent cellular cytotoxicity，ADCC）[23-24]。在这个过程中，激活的自然杀伤（natural killer，NK）细胞通过其Fcγ受体与曲妥珠单抗的Fc结构域结合，识别癌细胞并释放颗粒酶和颗粒溶素引起癌细胞裂解[25-26]。此外，肿瘤浸润淋巴细胞（tumor-infiltrating lymphocytes，TILs）也被认为是曲妥珠单抗获益的预后预测生物标志物[27-30]。高表达程序性死亡［蛋白］-1（programmed death-1，PD-1）、程序性死亡［蛋白］配体-1（programmed death ligand-1，PD-L1）和其他免疫检查点分子的TILs提示联合免疫治疗的潜在获益[31]。PANACEA临床试验[10]结果证实，派姆单抗联合曲妥珠单抗治疗可以提高曲妥珠单抗耐药的HER2阳性乳腺癌患者的临床获益，但令人失望的是，仅有15%的PD-L1阳性患者获得客观缓解。因此，了解TIME如何影响曲妥珠单抗的治疗效果，并确定哪些患者会从联合治疗中获益显得尤为重要。本研究旨在描绘曲妥珠单抗治疗患者的肿瘤免疫浸润模式，构建曲妥珠单抗疗效相关的预测模型，拟为鉴别曲妥珠单抗联合免疫治疗的潜在获益人群提供依据。

1 资料和方法

1.1 患者与数据集

本研究从高通量基因表达（Gene Expression Omnibus，GEO）数据库中收集了509例接

受曲妥珠单抗新辅助治疗（GSE66305、GSE37946、GSE50948、GSE62327）和辅助治疗（GSE58984、GSE55348、GSE65095、GSE44272）的HER2阳性乳腺癌患者的RNA测序数据和临床病理学数据。此外，本研究还通过癌症基因组图谱（The Cancer Genome Atlas，TCGA）数据库获取了810例乳腺癌样本的RNA-seq数据和临床信息，其中包括67例HER2阳性乳腺癌样本。使用R软件的“limma”包去除RNA测序数据因批次效应导致的系统变异，并使用“FactoMineR”和“factoextra”进行主成分分析（principal component analysis，PCA）揭示数据的主要特征成分[32]。

1.2 差异基因筛选与富集分析

使用R软件的“limma”包，基于*P*-adjust＜0.05，|log2 fold change (FC)|＞0.585的阈值筛选出与曲妥珠单抗耐药相关的差异表达基因（differentially expressed gene，DEG）。设定*P*-adjust＜0.05为临界值，使用R软件的“clusterProfiler”包进行基因本体论（Gene Ontology，GO）、京都基因与基因组百科全书（Kyoto Encyclopedia of Genes and Genomes，KEGG）和基因集富集分析（gene set enrichment analysis，GSEA），揭示DEG的生物学功能和参与调控的信号转导通路。通过R软件的“VennDiagram”包筛选新辅助治疗队列和辅助治疗队列共有的DEG中52个核心基因。

1.3 预测模型的构建和验证

选取GSE58984（n=91）作为训练集构建曲妥珠单抗相关基因预测指数（trastuzumab related genetic prognostic index，TRGPI），GSE55348（n=53）作为测试集进行独立验证。TRGPI的构建流程如下：① 以每个核心基因的中位表达值作为截断值，通过对数秩检验筛选出12个与复发风险显著相关的核心基因（P＜0.05）；② 在12个核心基因中，采用单因素和多因素COX比例风险回归模型筛选出与复发风险独立相关的4个基因；③ 用COX模型中各基因的表达值乘以各基因的系数的总和作为每例患者的TRGPI。并根据TRPGI的中位数，将患者分为TRGPI高组（高于中位数）和TRGPI低组（低于中位数）两个亚组。采用多因素COX比例风险回归模型评估TRGPI的独立预后价值。采用R的“timeROC”包绘制表达分析和受试者工作特征（receiver operating characteristic，ROC）曲线，并计算C指数和ROC曲线的曲线下面积（area under curve，AUC），评价TRGPI的预后价值。

1.4 基因组特征分析

将TCGA队列的原始数值转化为每千碱基的转录数（transcripts per kilobase million，TPM）以保证不同来源数据之间的可比性。使用R软件的“maftools”包分析基因突变及其相关性。每个样本的肿瘤突变负荷（tumor mutation burden，TMB）根据每百万碱基（megabase，Mb）的突变总数进行计算[33]。

1.5 免疫微环境特征分析

使用R软件的“CIBERSORT”包量化每例患者TIME中的22种免疫细胞浸润情况[34]。应用单因素COX回归分析探讨免疫细胞的预后价值，并通过R软件的“GSVA”包和“Hmisc”包进行单样本GSEA，计算28种免疫细胞与52个核心基因的相关性。免疫亚型分析用来描述不同TRGPI亚组的TIME特征[35]。

1.6 免疫治疗效果预测

通过基因集（*HLA-A*、*HLA-C*、*TAP*2、*NLRC*5、*TAP*1、*PSMB*9、*PSMB*8、*HLA-B*和*B2M*）的表达量计算主要组织相容性复合体（major histocompatibility complex，MHC）评分用以评估T细胞的抗原加工呈递能力[36]。采用肿瘤免疫功能障碍和排斥（tumor immune dysfunction and exclusion，TIDE）评分计算T细胞排斥和功能障碍分数，用于评估T细胞功能和预测ICI疗效[37]。

1.7 细胞培养

本研究中所使用的HMEC、MCF-10A、MCF-7、ZR-75-1、BT-474、AU565、HCC1954、MDA-MB-453、SK-BR-3、BT-549、HCC937和MDA-MB-231细胞系均购自中国科学院典型培养物保藏委员会细胞库和美国典型培养物保藏中心（American Type Culture Collection，ATCC）。以上细胞均在37 ℃、CO_2体积分数为5%的培养箱中培养，每2 d更换1次新鲜培养液。

1.8 RNA含量测定

使用美国Invitrogen公司的TRIzol从细胞中提取总RNA，并使用HiScript® Ⅲ 1st Strand cDNA Synthesis Kit进行反转录生成cDNA。引物使用SnapGene软件设计，实时荧光定量聚合酶链反应（real-time fluorescence quantitative polymerase chain reaction，RTFQ-PCR）采用ChamQTM Universal SYBR®qPCR Master Mix进行。以β-actin作为内标，使用$2^{-\Delta\Delta Ct}$法定量每种RNA的相对表达量。

1.9 统计学处理

所有统计分析均采用R软件（4.0.3版本）进行。两两比较采用Wilcoxon检验，两组以上采用Kruskal-Wallis检验。采用对数秩检验进行Kaplan-Meier生存分析，采用COX比例风险回归模型进行单因素和多因素生存分析。Pearson相关性分析用来计算免疫细胞与52个中枢基因的相关系数。$P<0.05$为差异有统计学意义。

2 结　果

2.1 预测模型的构建流程

本研究纳入了509例接受曲妥珠单抗新辅助治疗和辅助治疗的HER2阳性乳腺癌患者，通过整合其转录组、基因组数据和临床病理学信息，综合分析了通路水平、免疫微环境、基因组特征等对曲妥珠单抗疗效的影响，并构建了曲妥珠单抗疗效的预测模型TRGPI，同时该模型也显示出对免疫治疗效果的预测作用（图1）。

2.2 曲妥珠单抗耐药患者的免疫反应抑制

509例接受曲妥珠单抗治疗患者的转录组数据的PCA提示新辅助治疗和辅助治疗队列患者的转录组水平呈现明显差异（图2），因此将新辅助治疗和辅助治疗队列分别进行分析并各分为两组：① 曲妥珠单抗缓解组（$n=307$），定义为新辅助治疗中病理学完全缓解（pathological

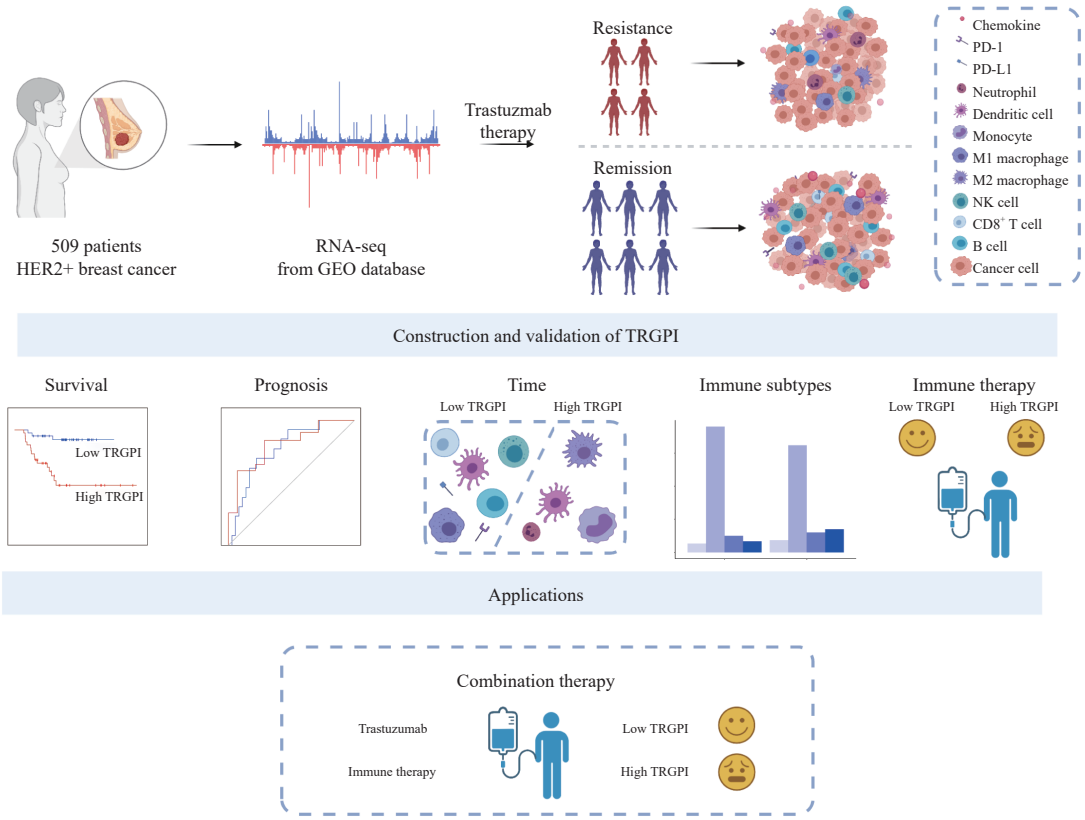

图1 研究流程图

Fig. 1 Graphical flowchart

complete remission，pCR）或辅助治疗队列中无远处转移的患者；② 曲妥珠单抗耐药组（n=202），新辅助治疗队列中无pCR或辅助治疗队列中有远处转移的患者。根据差异表达分析，新辅助队列共获得697个DEG，包括321个下调基因和376个上调基因（图3A）；辅助队列共获得905个DEG，包括350个下调基因和555个上调基因（图3B）。

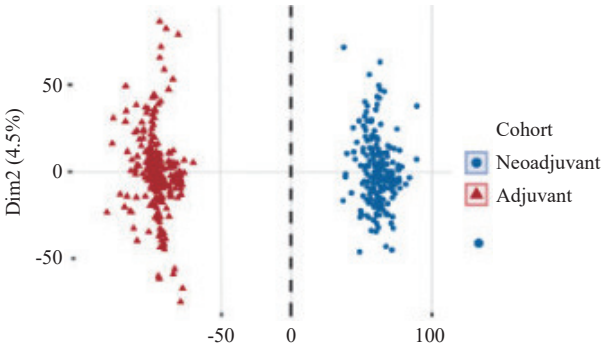

图2　新辅助治疗与辅助治疗队列的PCA

Fig. 2　PCA of neoadjuvant and adjuvant cohorts

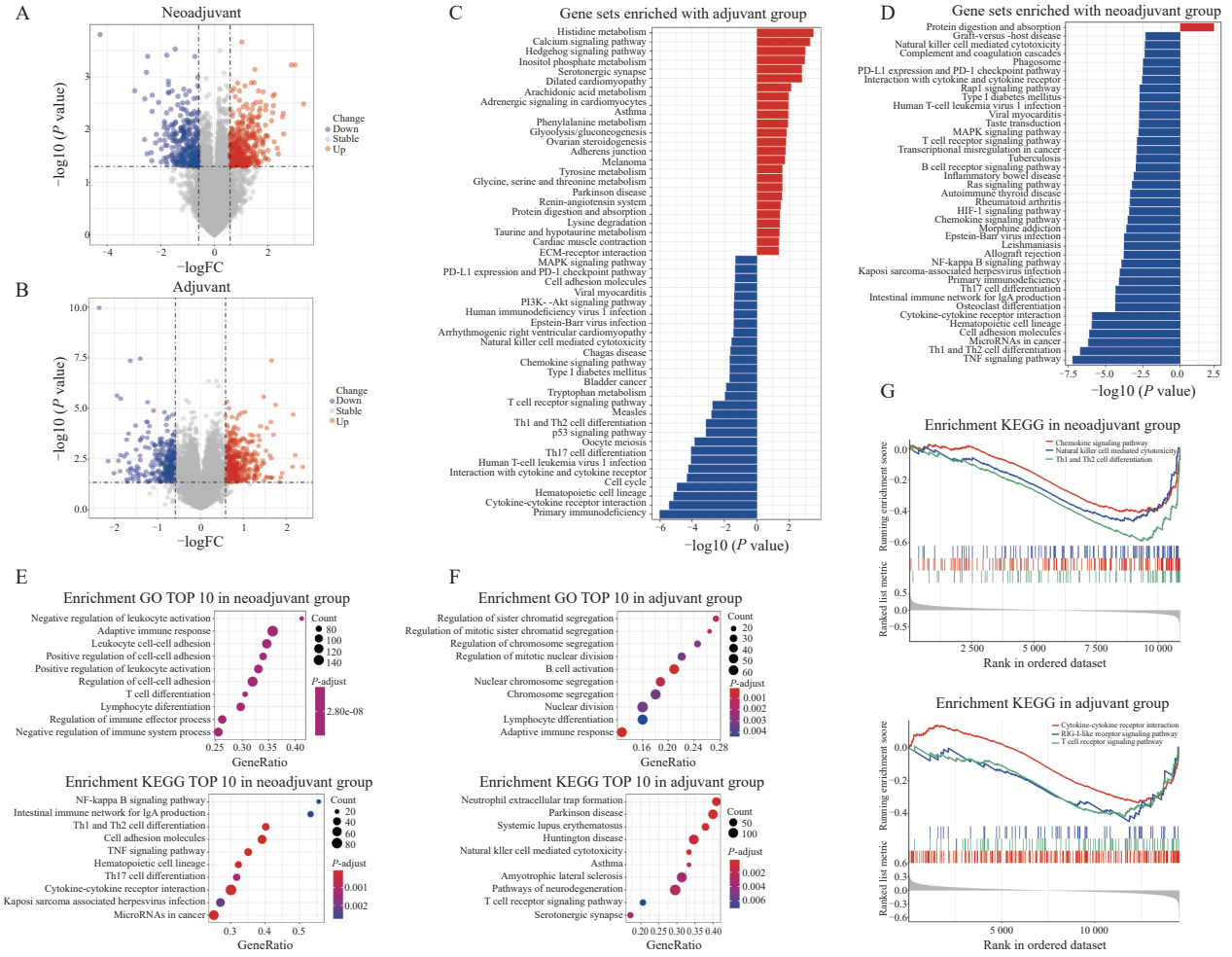

图3　曲妥珠单抗耐药患者的免疫反应抑制

Fig. 3　Immune deficiency in trastuzumab-resistant patients

A, B: Volcano plot of RNA-seq gene expression changes in the trastuzumab-resistant neoadjuvant and adjuvant cohorts. C, D: KEGG pathway analysis in the trastuzumab-resistant neoadjuvant and adjuvant cohorts. E, F: Top 10 candidate GO terms and pathways in the functional annotation of GSEA of the trastuzumab-resistant neoadjuvant and adjuvant cohorts. G: Representative pathways of GSEA in the trastuzumab-resistant group (P-adjust<0.05) of neoadjuvant and adjuvant cohorts..

功能富集分析提示，无论是新辅助治疗还是辅助治疗队列，其DEG均与免疫通路显著相关（图3C~3G）。KEGG分析提示有19条通路共同富集于新辅助治疗和辅助治疗队列，其中8条为免疫相关通路，且均在曲妥珠单抗耐药组中下调，包括趋化因子信号通路、细胞因子-细胞因子与抑制的免疫反应相关受体相互作用、T细胞受体信号通路、Th1和Th2细胞分化、Th17细胞分化、NK细胞介导的细胞毒性、原发性免疫缺陷、肿瘤中PD-L1表达和PD-1检查点通路（图3C、3D）。此外，GSEA提示曲妥珠单抗耐药组抗肿瘤免疫反应的抑制，主要表现为NK细胞的杀伤作用、T细胞受体信号通路等通路发生下调（图3G）。免疫细胞组成分析显示，曲妥珠单抗获益患者的TIME中含有更高比例的$CD8^+$ T淋巴细胞和活化NK细胞（图4）。上述结果均提示曲妥珠单抗耐药与免疫抑制相关。

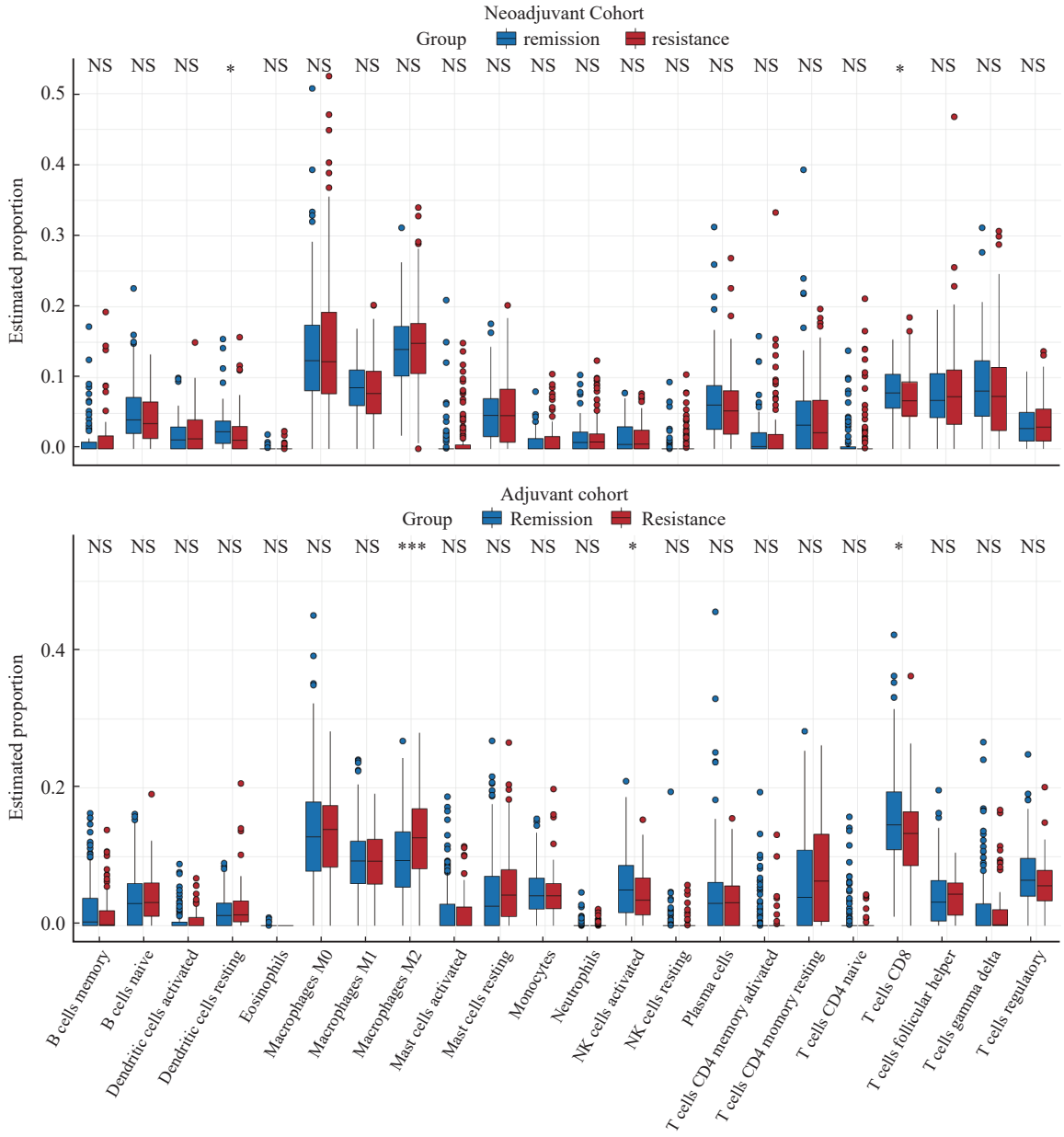

图4　新辅助治疗与辅助治疗队列的免疫细胞组成分析

Fig. 4　TIME analysis of neoadjuvant and adjuvant cohorts

Significant differences between the two subgroups were assessed using the Wilcoxon test (NS: Not significant; *: P<0.05; ***: P<0.001).

2.3 筛选曲妥珠单抗疗效相关的核心基因

通过韦恩分析，我们得到了52个在新辅助治疗和辅助治疗队列的耐药组中表达均上调或下调的核心基因。相关性热图提示这52个核心基因的表达与免疫细胞的丰度显著相关（图5A）。其中*POU2AF1*、*CD69*、*GZMB*为免疫激活相关基因，与免疫细胞浸润正相关，且在曲妥珠单抗耐药组中下调。通过Kaplan-Meier分析，筛选出12个与无远处转移生存期（distant disease-free survival，DDFS）显著相关的基因，并通过单因素与多因素COX回归分析进一步筛选出4个曲妥珠单抗疗效的独立预后基因：*ZHX2*、*GATA6*、*AMACR*和*TRPV6*（图5B、5C）。其中*ZHX2*、*GATA6*在曲妥珠单抗耐药组中表达上调，提示较少的曲妥珠单抗获益，而AMACR、TRPV6在曲妥珠单抗耐药组中表达下调且与曲妥珠单抗获益相关。进一步在乳腺癌细胞系与乳腺癌组织中对以上4个预后相关基因的表达量进行验证。RTFQ-PCR结果提示，与HER2阴性乳腺癌细胞（MCF-7、ZR-75-1、BT549、HCC1937、MDA-MB-231）和正常乳腺细胞（HMEC、MCF-10A）相比，这4个核心基因在HER2阳性乳腺癌细胞系（AU565、HCC1954、SK-BR-3、MDA-MB-453）中表达水平相对较高（图5D）。同时发现与曲妥珠单抗耐药相关的*ZHX2*、*GATA6*基因分别在Luminal B型和TNBC中也具有较高的表达水平。在TCGA队列中，进一步分析了以上4个核心基因在不同乳腺癌分子亚型中的表达量，与细胞PCR结果较为一致（图5E）。上述结果提示*ZHX2*、*GATA6*、*AMACR*和*TRPV6*是影响曲妥珠单抗治疗获益的关键基因，因此纳入以上基因进行后续预测模型的构建。

2.4 构建预测模型

选取GSE58984作为训练集，纳入以上4个预后相关的核心基因构建多因素COX比例风险回归模型，并计算每个样本的TRGPI：$TRGPI=\sum_{1}^{4}(\text{prognostic gene}\times\text{coefficients})$，并以GSE55348作为测试集进行独立验证。多因素COX比例风险回归提示TRGPI是曲妥珠单抗治疗患者的独立预后因素［训练集：HR=4.13（1.33～12.82），*P*=0.013 4；验证集：HR=8.26（2.54～26.85），*P*=0.000 4，图6A、6B］。通过C指数进一步评估TRGPI的预测性能（训练集C指数为0.78，测试集C指数为0.73）。以TRGPI中位数为临界值将患者分为低TRGPI组和高TRGPI组，Kaplan-Meier法分析提示低TRGPI组患者的DDFS优于高TRGPI组（训练集：*P*=0.006；测试集：*P*=0.00018，图6C）。在时间依赖的ROC曲线分析中，TRGPI表现出对DDFS较强的预测性能，训练集18和36个月的平均AUC分别为0.82和0.84，测试集18和36个月的AUC值分别为0.77和0.80（图6D）。TRGPI升高提示较高的复发风险和较短的DDFS（图6E）。

2.5 TRGPI亚组的基因组特征

为了比较不同TRGPI亚组的体细胞突变特征，研究收集了来自TCGA数据库的67例HER2阳性乳腺癌样本。基因组分析结果显示，最常见的体细胞突变类型是错义突变，其次是无义突变和移码突变（图7A）。既往研究[38]提出TP53基因突变与PD-L1高表达及ICI反应有关，而其在TRGPI低评分组（79%）中也呈现出高于TRGPI高评分组（62%）的突变频率。同时，在TRGPI高评分组中，*PIK3CA*具有最高的变异等位基因频率（variant allele frequency，VAF），此基因也被认为是曲妥珠单抗治疗生存率显著较差的生物标志物（图7B）[39]。两个TRGPI亚群之间的突变共现关系和互斥关系也存在较为明显的差异（图7C），而两组的TMB没有显著差异（图7D）。上述研究揭示了TRGPI亚组之间不同的基因组突变特征，提示TRGPI与曲妥珠单抗和ICI在基因组水平上的潜在相关性。

2.6 TRGPI亚组的TIME特征

通过CIBERSORT反卷积算法对不同TRGPI评分亚组的TIME特征进行描绘。无论在训练集还是测试集中，TRGPI低评分组的TIME均富集了更高比例的$CD8^+$ T淋巴细胞、活化的树突状细胞和NK细胞；TRGPI高评分组的TIME则浸润了更高比例的γδ T细胞和$CD4^+$静息记忆T细胞（图8A）。结合临床病理学数据进行分析显示，不同TRGPI亚组的TIME与年龄、ER状态和PR状态无显著相关性（图8B）。

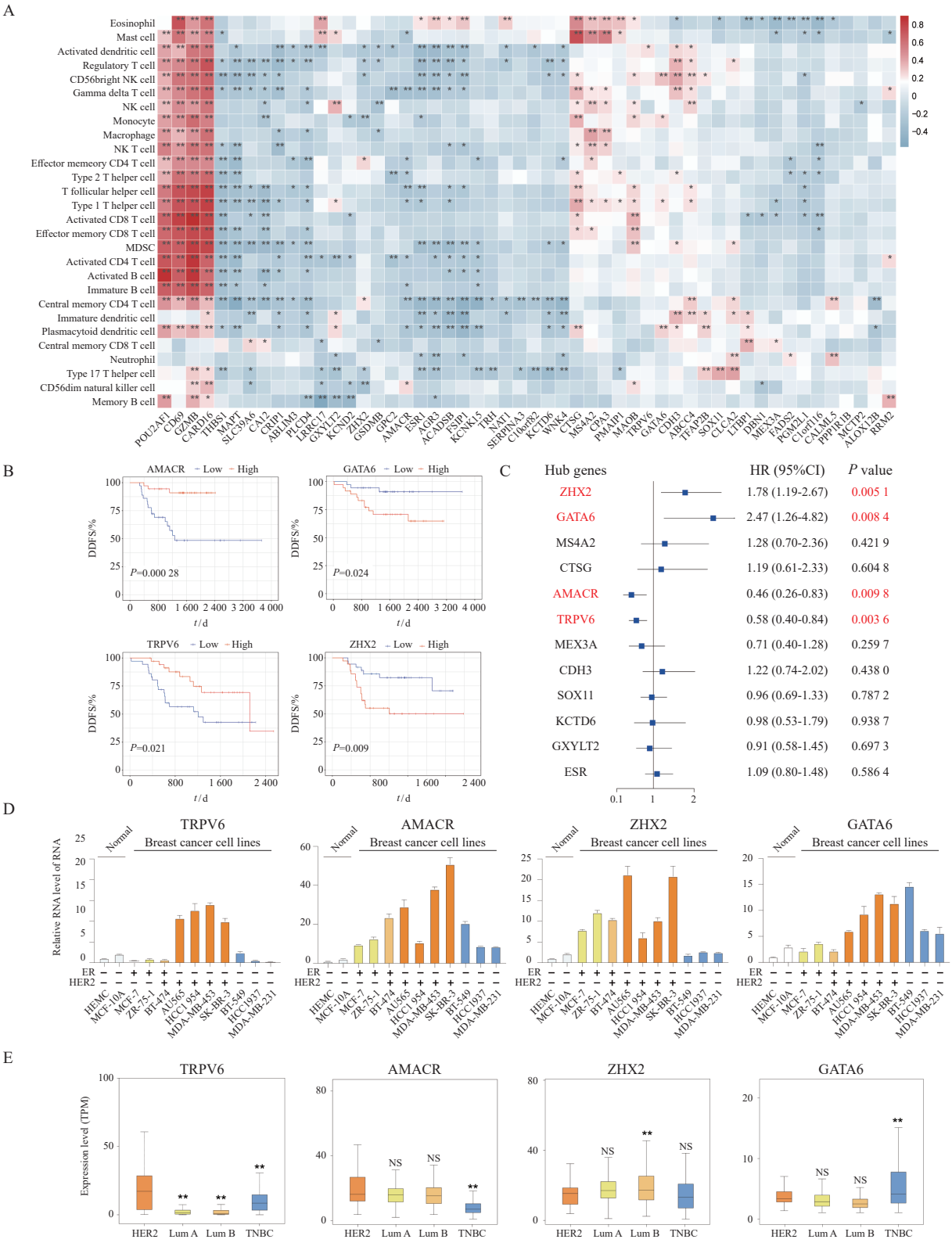

图5 曲妥珠单抗耐药相关核心基因

Fig. 5 Trastuzumab-response related hub genes

A: Correlation coefficient heatmap to demonstrate the immune characteristics of the hub genes. B: Kaplan-Meier analysis of the DDFS curves for the four genes (of the 12 hub genes) with significant prognostic value (log-rank test, $P<0.05$). C: Univariate COX regression analysis of the 12 hub genes. D: RTFQ-PCR validation of the four prognostic genes in breast cancer cell lines. E. Expression analysis of the four prognostic genes in TCGA cohort. Significant differences between the two subgroups were assessed using the Wilcoxon test (NS: Not significant; *: $P<0.05$; **: $P<0.01$).

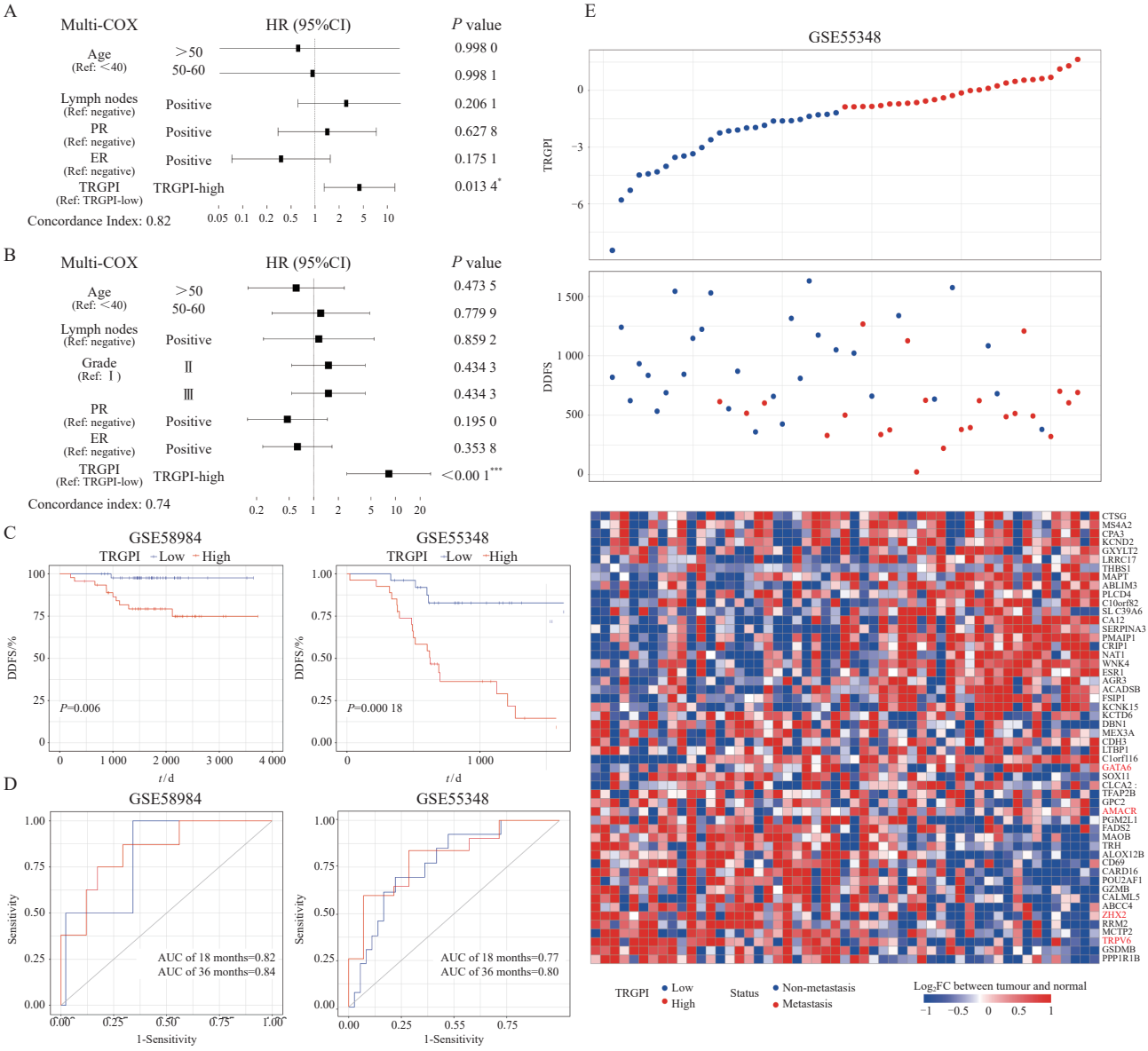

图6 曲妥珠单抗耐药预测模型构建与评价

Fig. 6 TRGPI construction and evaluation

Multivariate COX regression analysis of clinicopathological factors and TRGPI in the training set (A) and test set (B). C: Kaplan-Meier survival analysis of the TRGPI subgroups in the training set (left) and test set (right) (log-rank test, $P<0.05$). D: ROC curve analysis of the prognostic value of TRGPI for DDFS at 18 months and 36 months in the training set (left) and the test set (right). E: The TRGPI score curve showing the distribution of patients under trastuzumab therapy in the test set. Significant differences between the two subgroups were assessed using the Wilcoxon test (*: $P<0.05$; ***: $P<0.001$).

对于低TRGPI评分患者来说，不论接受新辅助治疗还是辅助治疗，其TIME均富集了较高比例的$CD8^+$ T淋巴细胞、记忆B细胞、活化的肥大细胞和NK细胞（图9A），且白细胞介素（interleukin，IL）-2和IL-21表达较高（图9B），提示低TRGPI评分组肿瘤免疫增强。同时，低TRGPI评分组的PD-1表达较高（$P<0.001$）。既往研究[35]表明，C2免疫亚型［γ干扰素（interferon-γ，IFN-γ）主导型］含有最高比例的TILs，TCR多样性最大。本研究发现，低TRGPI评分组相比于高TRGPI评分组含有更高比例的C2免疫亚型。上述结果均提示曲妥珠单抗获益与TIME密切相关（图9C）。

2.7 TRGPI评分与ICI获益

免疫逃逸被认为是临床ICI疗效欠佳的主要原因，包括适应性免疫抵抗和抗原呈递功能障

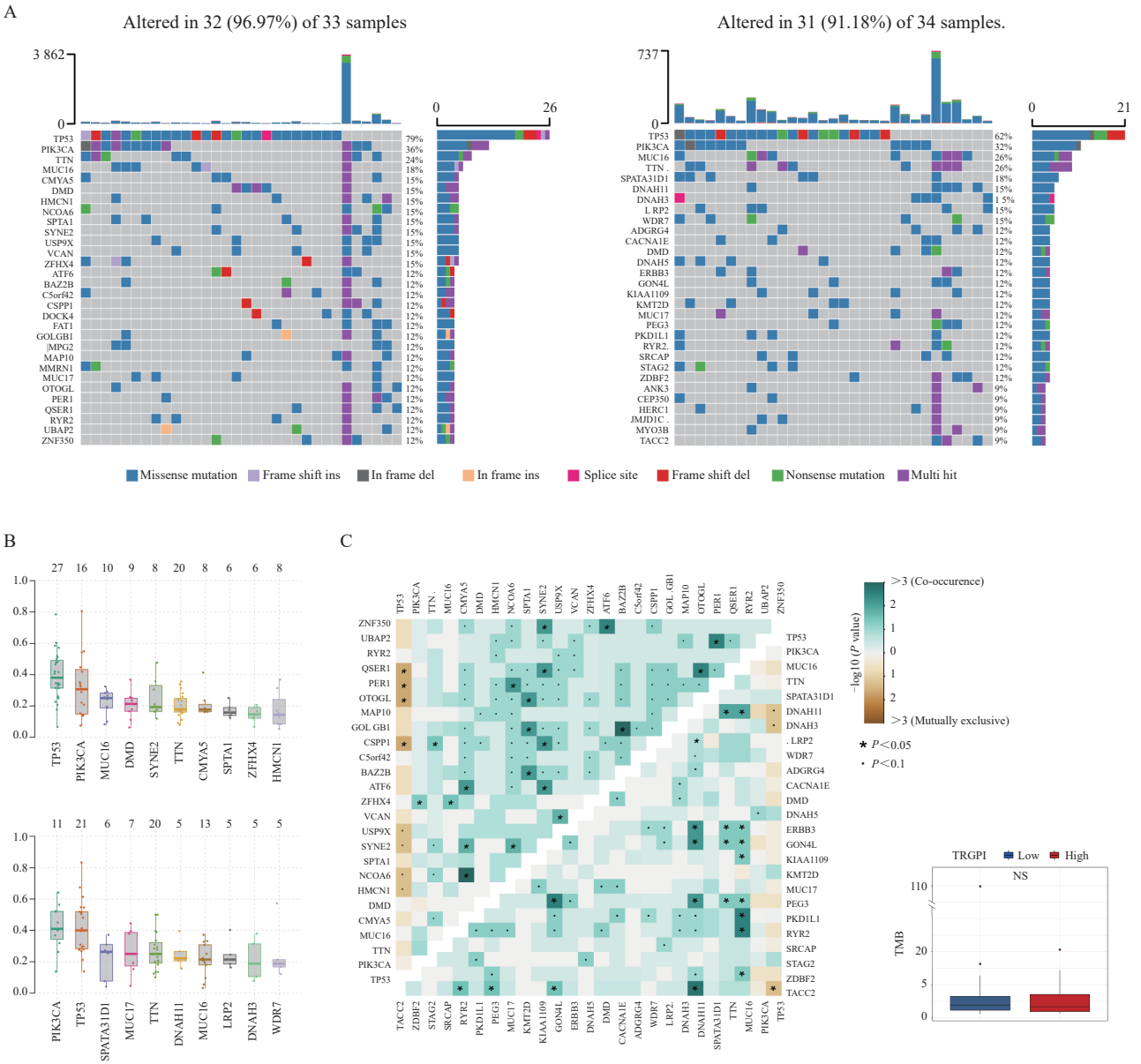

图7 不同TRGPI亚组的体细胞突变特征

Fig. 7 Genomic features in TRGPI subgroups

A: Waterfall plots of 30 highly variant mutant genes demonstrated the mutation landscape in the TRGPI-low group (left) and TRGPI-high group (right). B: Distribution of the top 10 somatic mutations VAF in the TRGPI-low group (upper) and TRGPI-high group (lower). C: The mutation co-occurrence and exclusion analyses in TRGPI-low group (upper left) and TRGPI-high group (lower right). NS: No significance. D: Differences in TMB among different TRGPI subgroups. Wilcoxon test was used to compare the statistical difference (NS: Not significant).

碍[40-41]。为进一步评估TRGPI与ICI疗效的关系，本研究计算了不同TRGPI亚组的MHC评分用以比较两组的抗原呈递能力，并使用TIDE评分计算T细胞排斥和功能障碍分数，用于评估T细胞功能和预测ICI疗效。结果显示，低TRGPI评分组的MHC评分较高，提示较强的T细胞抗原加工和递呈能力（图10A）。同时，低TRGPI评分组具有更低的TIDE评分，提示ICI的潜在临床获益（图10B）。上述结果均提示TRGPI对ICI疗效具有一定的预测价值。

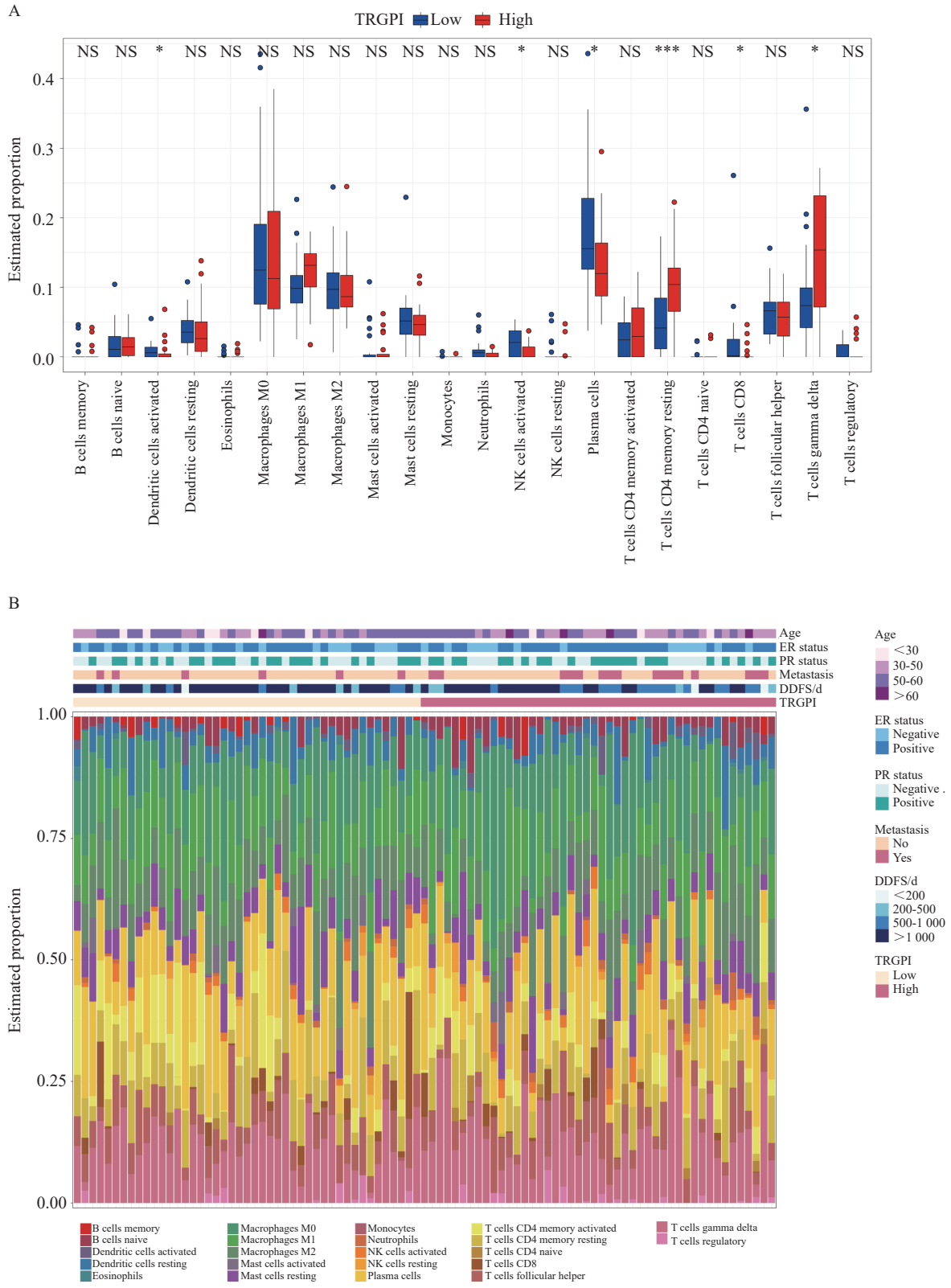

图8 不同TRGPI亚组的TIME特征

Fig. 8 TIME characteristics in different TRGPI subgroups of the training set

A: The proportions of TIME cells in different TRGPI subgroups. Significant differences between the two subgroups were assessed using the Wilcoxon test. B: The TRGPI grouping and proportions of TIME cells for 91 patients. Age, ER status, PR status, metastasis, DDFS, and TRGPI were used as patient annotations. NS: Not significant; *: $P<0.05$; ***: $P<0.001$.

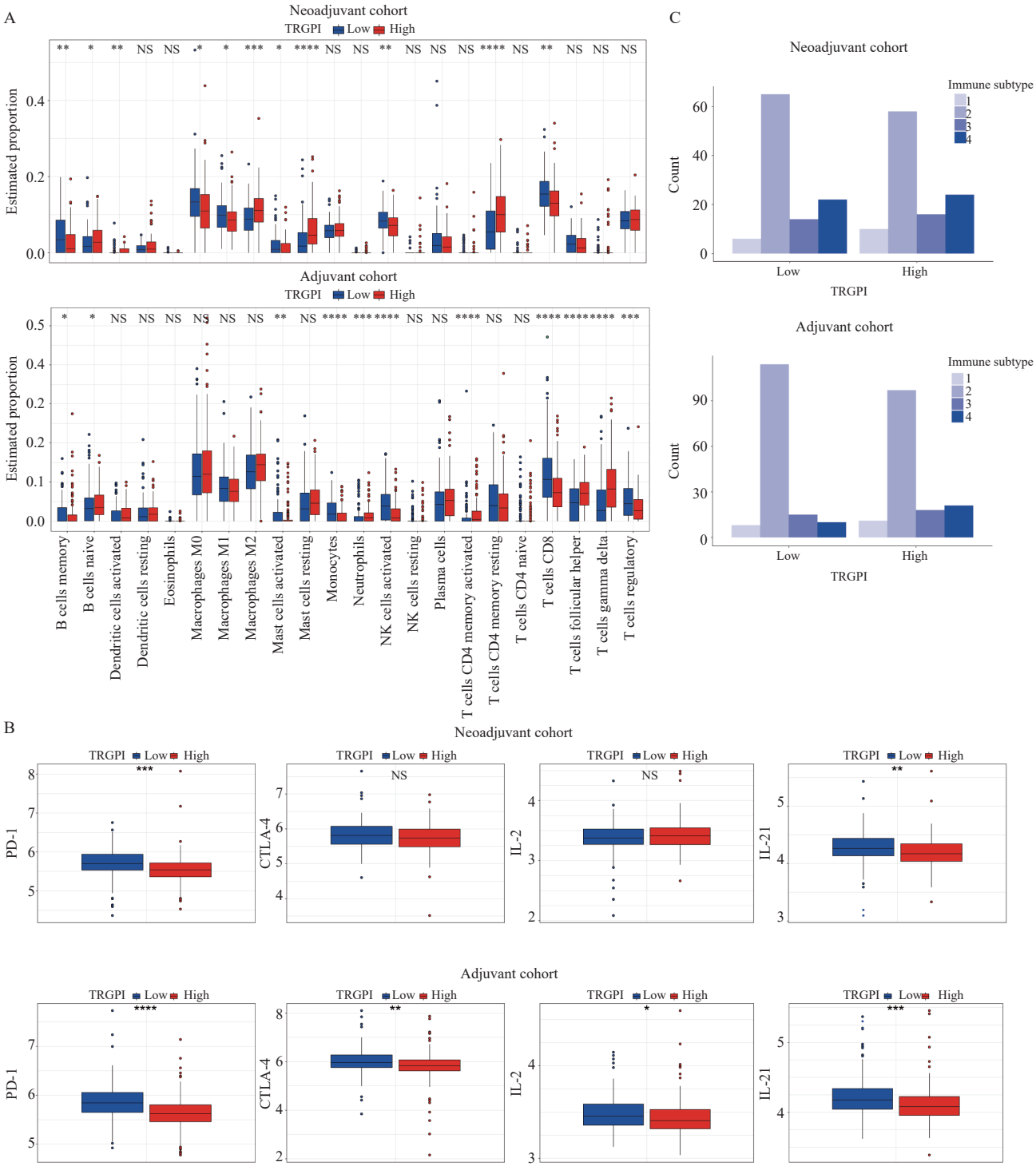

图9 新辅助与辅助治疗队列不同TRGPI亚组的TIME特征

Fig. 9 TIME characteristics of TRGPI subgroups in neoadjuvant and adjuvant cohorts

A: The percentage of immune cells; B:The expression levels of PD-1, CTLA-4, IL-2, and IL-21 from different TRGPI subgroups were compared in the neoadjuvant and adjuvant cohorts. Significant differences between the two subgroups were assessed using the Wilcoxon test. C: Immune subtype analysis of TRGPI subgroups in the neoadjuvant and adjuvant cohorts. NS: Not significant; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ****: $P<0.000\ 1$..

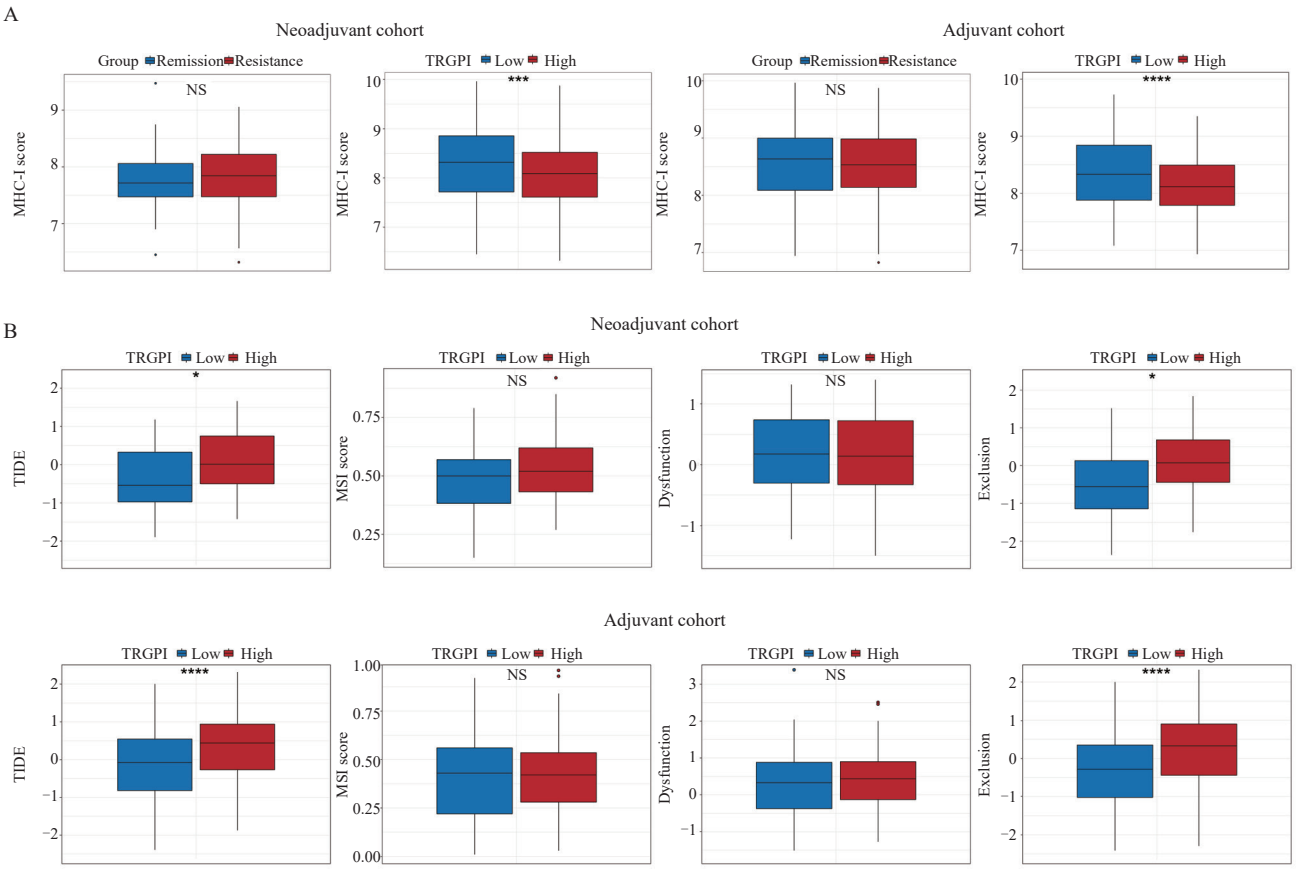

图10 TRGPI评分预测ICI获益

Fig. 10 The TRGPI-low subgroups benefit more from ICI

A: MHC-I scores; B: TIDE, MSI, T cell exclusion and dysfunction scores between the two TRGPI subgroups were compared using the Wilcoxon test. NS: Not significant. *: $P<0.05$; ***: $P<0.001$; ****: $P<0.000\ 1$.

3 讨 论

长期以来，乳腺癌被认为是一种非免疫性疾病，直到越来越多的证据[42-43]表明，免疫反应在乳腺癌，特别是在HER2阳性乳腺癌中发挥关键作用。近年来，曲妥珠单抗被广泛应用于HER2阳性乳腺癌的一线治疗，但仍有超过一半的患者在曲妥珠单抗治疗期间出现复发或疾病进展[2, 44]。目前已有大量研究对曲妥珠单抗耐药机制进行了探索，同时提出了以曲妥珠单抗为基础联合其他药物的治疗策略，其中就包括联合ICI帕博利珠单抗。然而在PANACEA临床研究[10]中，仅有15%的PD-1阳性患者观察到临床获益，因此寻找有效的疗效预测生物标志物，筛选联合治疗获益人群尤为重要。

本研究对接受曲妥珠单抗治疗的HER2阳性乳腺癌患者的TIME进行分析，曲妥珠单抗耐药组相比于曲妥珠单抗缓解组患者在免疫细胞浸润、免疫因子和免疫相关途径等方面均表现出显著差异。例如，$CD8^+$ T淋巴细胞作为促进肿瘤免疫的正向调控因子与HER2阳性乳腺癌的预后正相关，本研究结果也表明，曲妥珠单抗耐药组的TIME浸润了较低比例的$CD8^+$ T淋巴细胞。与之前的研究结果[45-46]一致，曲妥珠单抗耐药组表现出NK细胞介导的细胞毒性、T细胞受体信号通路和T细胞激活的下调。此外，本研究构建了曲妥珠单抗的预测模型TRGPI，其被证明是DDFS的独立预后因素。TRGPI同时也显示出较好的预测性能，特别是用于预测36个月的生存率，AUC在训练集中达到0.84，在测试集中达到0.80。同时，基因组分析提示，TRGPI高评分组与低评分组具有不同的基因突变特征，主要表现为*PIK3CA*、*TP53*突变频率不同以及突变共现关系

和互斥关系的差异，这也在基因水平上对TRGPI预测ICI和曲妥珠单抗治疗效果进行了解释。多项研究[47-48]表明，TIME的细胞和分子特征与抗肿瘤免疫具有明显相关性，因此为进一步从生物学角度了解TRGPI亚群的免疫学特征，本研究分析了TRGPI亚组的TIME特征。在曲妥珠单抗反应较差的TRGPI高评分组中，肿瘤浸润激活的NK细胞和$CD8^+$ T淋巴细胞水平较低，同时IL-2和IL-21表达较低，而PD-1和CTLA-4表达降低。通过分析不同TRGPI亚组免疫治疗TIDE评分，发现TRGPI低评分组更倾向于从曲妥珠单抗与ICI的联合治疗中获益。与以往仅关注TILs的研究相比，本研究更全面地分析了曲妥珠单抗治疗患者的TIME，探索了曲妥珠单抗获益与ICI的潜在相关性，并首次提出曲妥珠单抗获益的患者也更倾向于从ICI治疗中获益。然而本研究仍存在一定的局限性：首先，TRGPI依赖于公共数据库，需要在更多的体内和体外实验中进一步验证；其次，本研究缺乏曲妥珠单抗联合ICI治疗队列的临床验证。

本研究进一步阐明了TIME与曲妥珠单抗耐药的关系，并构建了预测曲妥珠单抗联合ICI疗效的TRGPI评分，可为临床筛选曲妥珠单抗联合免疫治疗的获益人群提供参考。

利益冲突声明：所有作者均声明不存在利益冲突。

［参　考　文　献］

［1］ SLAMON D J, CLARK G M, WONG S G, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene［J］. Science, 1987, 235(4785): 177-182.

［2］ SWAIN S M, SHASTRY M, HAMILTON E. Targeting HER2-positive breast cancer: advances and future directions［J］. Nat Rev Drug Discov, 2023, 22(2): 101-126.

［3］ SLAMON D J, LEYLAND-JONES B, SHAK S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2［J］. N Engl J Med, 2001, 344(11): 783-792.

［4］ VOGEL C L, COBLEIGH M A, TRIPATHY D, et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer［J］. J Clin Oncol, 2002, 20(3): 719-726.

［5］ GENNARI R, MENARD S, FAGNONI F, et al. Pilot study of the mechanism of action of preoperative trastuzumab in patients with primary operable breast tumors overexpressing HER2［J］. Clin Cancer Res, 2004, 10(17): 5650-5655.

［6］ BUSSOLATI G, MONTEMURRO F, RIGHI L, et al. A modified trastuzumab antibody for the immunohistochemical detection of HER-2 overexpression in breast cancer［J］. Br J Cancer, 2005, 92(7): 1261-1267.

［7］ DIERMEIER S, HORVÁTH G, KNUECHEL-CLARKE R, et al. Epidermal growth factor receptor coexpression modulates susceptibility to herceptin in HER2/neu overexpressing breast cancer cells via specific erbB-receptor interaction and activation［J］. Exp Cell Res, 2005, 304(2): 604-619.

［8］ LU Y, ZI X, ZHAO Y, et al. Insulin-like growth factor-Ⅰ receptor signaling and resistance to trastuzumab (Herceptin)［J］. J Natl Cancer Inst, 2001, 93(24): 1852-1857.

［9］ NAGATA Y, LAN K H, ZHOU X Y, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients［J］. Cancer Cell, 2004, 6(2): 117-127.

［10］ LOI S, GIOBBIE-HURDER A, GOMBOS A, et al. Pembrolizumab plus trastuzumab in trastuzumab-resistant, advanced, HER2-positive breast cancer (PANACEA): a single-arm, multicentre, phase 1b-2 trial［J］. Lancet Oncol, 2019, 20(3): 371-382.

［11］ STAGG J, LOI S, DIVISEKERA U, et al. Anti-ErbB-2 MAb therapy requires type Ⅰ and Ⅱ interferons and synergizes with anti-PD-1 or anti-CD137 MAb therapy［J］. Proc Natl Acad Sci U S A, 2011, 108(17): 7142-7147.

［12］ BASELGA J, BRADBURY I, EIDTMANN H, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial［J］. Lancet, 2012, 379(9816): 633-640.

［13］ CAREY L A, BERRY D A, CIRRINCIONE C T, et al. Molecular heterogeneity and response to neoadjuvant human epidermal growth factor receptor 2 targeting in CALGB 40601, a randomized phase Ⅲ trial of paclitaxel plus trastuzumab with or without lapatinib［J］. J Clin Oncol, 2016, 34(6): 542-549.

［14］ GIANNI L, PIENKOWSKI T, IM Y H, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial［J］. Lancet Oncol, 2012, 13(1): 25-32.

［15］ SCHNEEWEISS A, CHIA S, HICKISH T, et al. Pertuzumab plus trastuzumab in combination with standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer: a randomized phase Ⅱ cardiac safety study (TRYPHAENA)［J］. Ann Oncol, 2013, 24(9): 2278-2284.

［16］ GIORDANO S H, FRANZOI M A B, TEMIN S, et al. Systemic therapy for advanced human epidermal growth factor receptor 2-positive breast cancer: ASCO guideline update［J］. J Clin Oncol, 2022, 40(23): 2612-2635.

［17］ FU Z W, LI S J, HAN S F, et al. Antibody drug conjugate: the "biological missile" for targeted cancer therapy［J］. Signal Transduct Target Ther, 2022, 7(1): 93.

［18］ TORRES E T R, EMENS L A. Emerging combination immunotherapy strategies for breast cancer: dual immune checkpoint modulation, antibody-drug conjugates and bispecific antibodies［J］. Breast Cancer Res Treat, 2022, 191(2): 291-302.

［19］ FERNANDEZ-MARTINEZ A, PASCUAL T, SINGH B, et

al. Prognostic and predictive value of immune-related gene expression signatures *vs* tumor-infiltrating lymphocytes in early-stage ERBB2/HER2-positive breast cancer: a correlative analysis of the CALGB 40601 and PAMELA trials [J]. JAMA Oncol, 2023, 9(4): 490-499.

[20] SHARMA P, HU-LIESKOVAN S, WARGO J A, et al. Primary, adaptive, and acquired resistance to cancer immunotherapy [J]. Cell, 2017, 168(4): 707-723.

[21] KALAORA S, NAGLER A, WARGO J A, et al. Mechanisms of immune activation and regulation: lessons from melanoma [J]. Nat Rev Cancer, 2022, 22(4): 195-207.

[22] LABRIE M, BRUGGE J S, MILLS G B, et al. Therapy resistance: opportunities created by adaptive responses to targeted therapies in cancer [J]. Nat Rev Cancer, 2022, 22(6): 323-339.

[23] COOLEY S, BURNS L J, REPKA T, et al. Natural killer cell cytotoxicity of breast cancer targets is enhanced by two distinct mechanisms of antibody-dependent cellular cytotoxicity against LFA-3 and HER2/neu [J]. Exp Hematol, 1999, 27(10): 1533-1541.

[24] LEWIS G D, FIGARI I, FENDLY B, et al. Differential responses of human tumor cell lines to anti-p185HER2 monoclonal antibodies [J]. Cancer Immunol Immunother, 1993, 37(4): 255-263.

[25] KOHRT H E, HOUOT R, MARABELLE A, et al. Combination strategies to enhance antitumor ADCC [J]. Immunotherapy, 2012, 4(5): 511-527.

[26] CLYNES R A, TOWERS T L, PRESTA L G, et al. Inhibitory Fc receptors modulate *in vivo* cytotoxicity against tumor targets [J]. Nat Med, 2000, 6(4): 443-446.

[27] SALGADO R, DENKERT C, CAMPBELL C, et al. Tumor-infiltrating lymphocytes and associations with pathological complete response and event-free survival in HER2-positive early-stage breast cancer treated with lapatinib and trastuzumab: a secondary analysis of the NeoALTTO trial [J]. JAMA Oncol, 2015, 1(4): 448-454.

[28] CORTAZAR P, ZHANG L J, UNTCH M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis [J]. Lancet, 2014, 384(9938): 164-172.

[29] INGOLD HEPPNER B, UNTCH M, DENKERT C, et al. Tumor-infiltrating lymphocytes: a predictive and prognostic biomarker in neoadjuvant-treated HER2-positive breast cancer [J]. Clin Cancer Res, 2016, 22(23): 5747-5754.

[30] CHIC N, LUEN S J, NUCIFORO P, et al. Tumor cellularity and infiltrating lymphocytes as a survival surrogate in HER2-positive breast cancer [J]. J Natl Cancer Inst, 2022, 114(3): 467-470.

[31] DENKERT C, VON MINCKWITZ G, BRASE J C, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers [J]. J Clin Oncol, 2015, 33(9): 983-991.

[32] RITCHIE M E, PHIPSON B, WU D, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies [J]. Nucleic Acids Res, 2015, 43(7): e47.

[33] CHALMERS Z R, CONNELLY C F, FABRIZIO D, et al. Analysis of 100 000 human cancer genomes reveals the landscape of tumor mutational burden [J]. Genome Med, 2017, 9(1): 34.

[34] NEWMAN A M, LIU C L, GREEN M R, et al. Robust enumeration of cell subsets from tissue expression profiles [J]. Nat Methods, 2015, 12(5): 453-457.

[35] THORSSON V, GIBBS D L, BROWN S D, et al. The immune landscape of cancer [J]. Immunity, 2018, 48(4): 812-830. e14.

[36] LAUSS M, DONIA M, HARBST K, et al. Mutational and putative neoantigen load predict clinical benefit of adoptive T cell therapy in melanoma [J]. Nat Commun, 2017, 8(1): 1738.

[37] JIANG P, GU S Q, PAN D, et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response [J]. Nat Med, 2018, 24(10): 1550-1558.

[38] SCHOENFELD A J, RIZVI H, BANDLAMUDI C, et al. Clinical and molecular correlates of PD-L1 expression in patients with lung adenocarcinomas [J]. Ann Oncol, 2020, 31(5): 599-608.

[39] JENSEN J D, KNOOP A, LAENKHOLM A V, et al. PIK3CA mutations, PTEN, and pHER2 expression and impact on outcome in HER2-positive early-stage breast cancer patients treated with adjuvant chemotherapy and trastuzumab [J]. Ann Oncol, 2012, 23(8): 2034-2042.

[40] VIGANO S, ALATZOGLOU D, IRVING M, et al. Targeting adenosine in cancer immunotherapy to enhance T-cell function [J]. Front Immunol, 2019, 10: 925.

[41] YAMAMOTO K, VENIDA A, YANO J, et al. Autophagy promotes immune evasion of pancreatic cancer by degrading MHC-I [J]. Nature, 2020, 581(7806): 100-105.

[42] PRAT A, GUARNERI V, PASCUAL T, et al. Development and validation of the new HER2DX assay for predicting pathological response and survival outcome in early-stage HER2-positive breast cancer [J]. EBioMedicine, 2022, 75: 103801.

[43] FERNANDEZ-MARTINEZ A, KROP I E, HILLMAN D W, et al. Survival, pathologic response, and genomics in CALGB 40601 (alliance), a neoadjuvant phase Ⅲ trial of paclitaxel-trastuzumab with or without lapatinib in HER2-positive breast cancer [J]. J Clin Oncol, 2020, 38(35): 4184-4193.

[44] WONG H, LEUNG R, KWONG A, et al. Integrating molecular mechanisms and clinical evidence in the management of trastuzumab resistant or refractory HER-2+ metastatic breast cancer [J]. Oncologist, 2011, 16(11): 1535-1546.

[45] MITTAL D, CARAMIA F, MICHIELS S, et al. Improved treatment of breast cancer with anti-HER2 therapy requires interleukin-21 signaling in CD8+ T cells [J]. Cancer Res, 2016, 76(2): 264-274.

[46] KILLOCK D. Targeted therapy: leveraging ADCC to enhance anti-HER2 therapy [J]. Nat Rev Clin Oncol, 2017, 14(4): 200.

[47] ROSENBERG S A, YANG J C, SHERRY R M, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy [J]. Clin Cancer Res, 2011, 17(13): 4550-4557.

[48] VESELY M D, KERSHAW M H, SCHREIBER R D, et al. Natural innate and adaptive immunity to cancer [J]. Annu Rev Immunol, 2011, 29: 235-271.

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