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A CORRELATION STUDY TO CHARACTERIZE TISSUE TYPE DEPENDENT STROMA IMMUNE COMPLEXITY IN SOLID TUMOR TMAS

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Background CD40 and 4-1BB have been established as relevant immunotherapy targets in cancer treatment, however, they have heterogeneous expression amongst immune cells within the tumor microenvironment. In the current work, we studied CD40 expression patterns in tumor associated stroma and its correlation with 4-1BB in three epithelial tumor microarrays (TMA) including non-small cell lung carcinoma (NSCLC), head and neck squamous cell carcinoma (HNSCC) and pancreatic ductal adenocarcinoma (PDAC). To further elucidate whether the correlation pattern was impacted by the number of myeloid or lymphoid populations in the tumor microenvironment, the prevalence of CD3 (+) lymphocytes and CD68/CD163 (+) myeloid cells were also evaluated.

Methods Sequential FFPE tissue sections from each TMA were immunohistochemistry stained with 41BB, CD3 and macrophage cocktail CD68/CD163 on Leica Bond autostainer at Genmab. CD40 staining was performed by CellCarta. Slides were then scanned in AT2 scanner using brightfield default settings. CD40 was scored using an overall stroma staining method as 0, 1+, 2+, 3+ based on staining intensity and number of % positive stroma cells. 4-1BB, CD3 and CD68/

CD163 were scored digitally and reported as cell density/mm². The average score of all studied biomarkers/case from one or more cores was then used to generate a protein expression & correlation heat map. Pearson's correlation coefficients are reported to show correlations between targets.

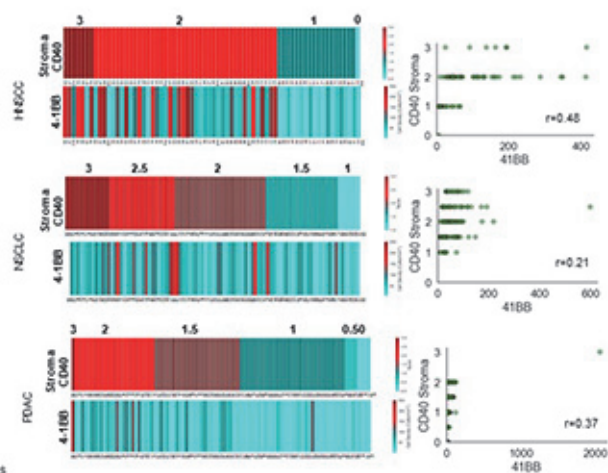
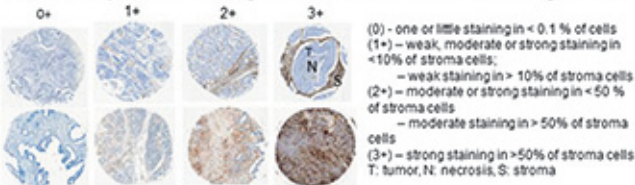
Results and Conclusions CD40 showed a broad range of positivity in tumor associated stroma compartment, presumably in myeloid/macrophages, in all studied TMAs. Fibroblast, endothelial cells, and B cell lineages including plasma cells also showed positive CD40 staining. The density of 4-1BB positive cells showed a varying degree of correlation with the density of CD3 positive cells, with the highest being in PDAC (r=.94) (figure 1). Different with 4-1BB, stroma CD40 showed different correlation patterns with CD3 and CD68/CD163 positive cells. Among all studied TMAs, the highest correlation between the overall score of stroma CD40 and the density of positive 4-1BB cells was observed in HNSCC (r=0.48) (figure 2), followed by PDAC (figure 3) and NSCLC (r = 0.37 and 0.21, respectively). In conclusion, this study indicates that 4-1BB expression and its correlation with stroma CD40 may be impacted by CD3+ lymphoid cells; however, stroma CD40 expression and its correlation with 4-1BB was impacted by different immune components among the three studied TMAs. Such correlation study among different immune components further elaborates the tissue specific immune complexity and it may add predictive value for disease indication selection for targeted immune therapy.

Epithelial TMA	No. cases/cores
LC1922b; Lung cancer (NSCLC includes squamous cell carcinoma and Adenocarcinoma)	96 cases/192 cores
HN804; Head and neck cancer (H&N squamous cell carcinoma, HNSCC)	80 cases/80 cores
PA1921a; Pancreas cancer (presumably ductal adenocarcinoma, PDAC)	96 cases/192 cores

Method and protein expression & correlation heatmap (right)

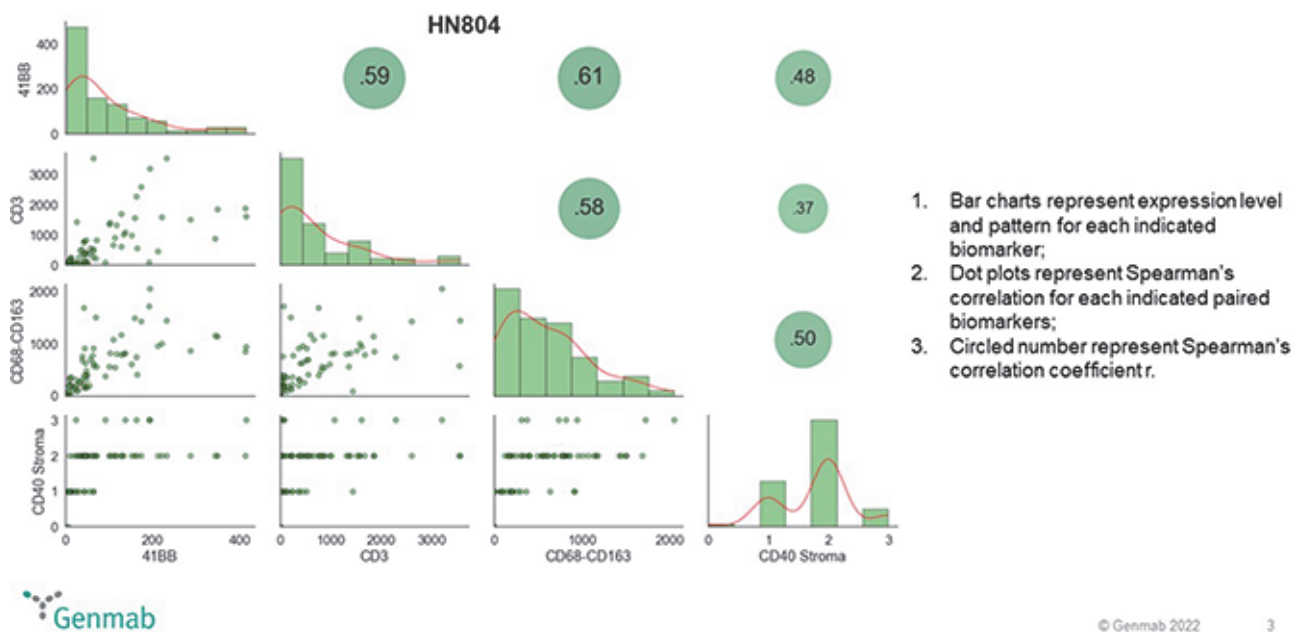
Sequential FFPE tissue sections from 3 epithelial tumor microarrays (TMA) as indicated were IHC stained with anti-CD40 and anti-4-1BB. Stroma CD40 was scored using overall stroma staining method as 0, 1+, 2+, 3+ based on staining intensity and number of % positive stroma cells. 4-1BB was scored digitally as cell density/mm². The average score of CD40 and 4-1BB /case from 1 or more cores were then used to generate the protein expression & correlation heatmap as shown in figure (right). Spearman's correlation coefficient r was analyzed.

CD40 IHC representative images to illustrate stroma CD40 scoring method



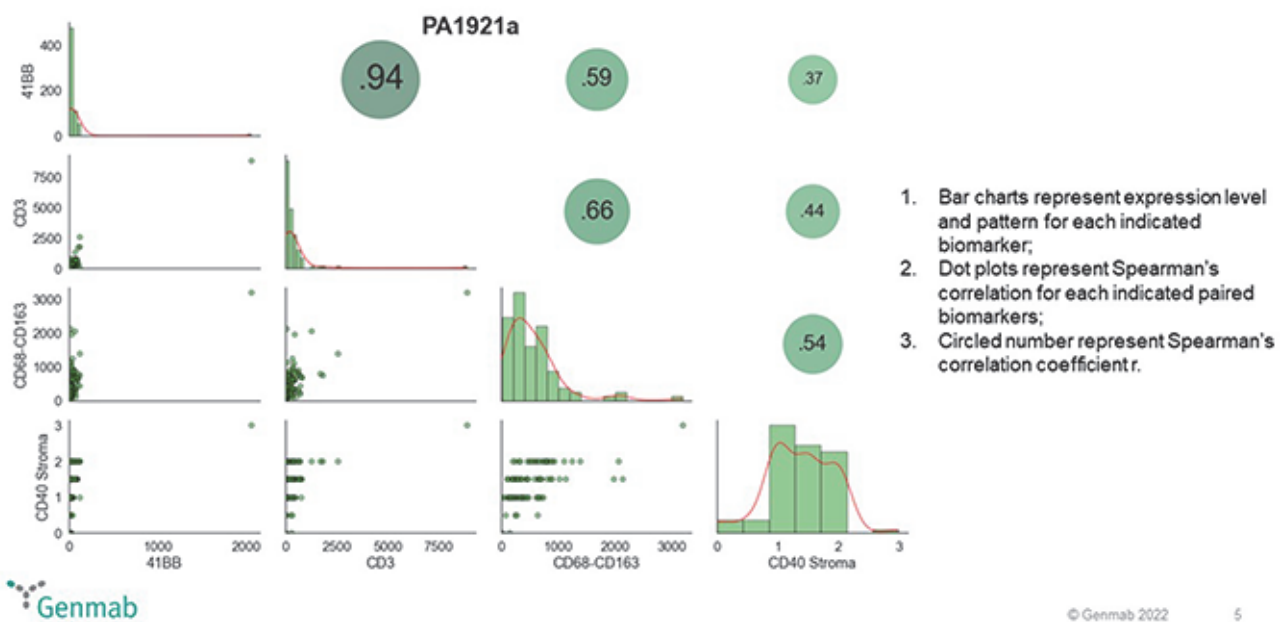
Abstract 953 Figure 1

Biomarker expression & correlation map in HNSCC



Abstract 953 Figure 2 Biomarker expression & correlation map in HNSCC. Bar charts represent expression level and pattern for each indicated biomarker; dot plots represent Spearman's correlation for each indicated paired biomarkers; circled number represent Spearman's correlation coefficient r .

Biomarker expression & correlation map in PDAC



Abstract 953 Figure 3 Biomarker expression & correlation map in PDAC. Bar charts represent expression level and pattern for each indicated biomarker; dot plots represent Spearman's correlation for each indicated paired biomarkers; circled number represent Spearman's correlation coefficient r .

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