

To address the potential impact of clinicopathological variables in our results, we first examined the association of all TCR β variables linked to response with clinicopathological variables such as age (using the Pearson test), sex, and tumor type (using Mann-Whitney/T-Student tests). All tumor-infiltrating variables associated with response were statistically significantly correlated with age (evenness: $R = -0.70$, $p = 0.011$; *TRBV6.5*: $R = -0.60$, $p = 0.040$; *TRBV11.3*: $R = -0.66$, $p = 0.019$; *TRBJ2.1*: $R = -0.68$, $p = 0.015$), but not with sex or tumor type. In contrast, no circulating variable was associated with any clinicopathological variable, although some showed a tendency with age. Consequently, we focused on the role of age as an influencing factor in the signature related to immunotherapy response prediction.

Next, we evaluated the predictive capacity of each variable in the signature through a logistic regression (fit.logit). It is important to note that due to the limited cohort size, the fit.logit model lacks sufficient statistical power (as indicated by the warning from the test) when all variables are included, resulting in p-values of 1. Therefore, we assessed the predictive capacity of each variable individually. While age was not predictive ($p = 0.998$), all TCR β variables (except for circulating *TRBJ2.6*: $p = 0.998$) were close to statistical significance both in tissue (evenness: $p = 0.0913$; *TRBV6.5*: $p = 0.0735$; *TRBV11.3*: $p = 0.109$; *TRBJ2.1*: $p = 0.0688$) and blood (*TRBV5.3*: $p = 0.153$; *TRBV27*: $p = 0.0999$; *TRBV28*: $p = 0.103$; *TRBJ2.1*: $p = 0.189$)

Finally, we applied a Random Forest analysis to assess the contribution of each variable to the signature's performance, along with ROC curve analyses of the signatures with and without age. For the tissue signature, age appeared to be the variable with the highest contribution. However, the signature including age (AUC = 0.80) demonstrated lower predictive capacity than the one with only TCR variables (AUC = 0.83). This is understandable, as all TCR β variables are associated with age, but they still explain more than age. For the blood signature, similar results were found, again showing that including age does not enhance the signature's predictive capacity.

These findings support that while TCR β variables may be influenced by age, they still possess individual predictive capacity. Indeed, in the case of tissue (where TCR variables are associated with age), the accuracy of the signature in predicting response is higher when age is excluded. However, the observed increase in TCR clonality with age, may limit its predictive effectiveness in older patients.