Immune Age Acceleration as a Biomarker for Immune-Related Adverse Events in Early Triple-Negative Breast Cancer Treated with Perioperative Pembrolizumab

Ayelet Alpert[1], Margarita Makarov[1], Mor Sabary-Ben Simon[1], Dvir Aran[2], Ayelet Shai[1,2]

[1]Oncology Department, Rambam Health Care Campus; [2]Faculty of Biology, Technion Israel Institute of Technology

Introduction

Immunotherapy is a cornerstone in the management of early-stage, high-risk triple-negative breast cancer (TNBC). However, a considerable number of patients develop immune-related adverse events (irAEs), which can be severe and even life-threatening. Currently, no validated biomarkers exist to predict the risk of high-grade irAEs in this population, limiting early identification and monitoring efforts.

Recent advances in immunological profiling enabled the quantification of immune system function by leveraging insights from the physiological decline in immune competence that accompanies aging—a process characterized by increased low-grade inflammation. The variability in immune aging rates among individuals has led to the development of *immune age metrics*, which use omics data to estimate the biological age of the immune system and predict age-related morbidity and all-cause mortality more accurately than chronological age.

Aim

In this work, we aim to unravel study the association between immune age at baseline and development of irAE in high-risk, triple-negative breast cancer patients undergoing immunotherapy.

Methods

Serum samples were collected from 136 patients with stage I–III breast cancer before treatment. Serum levels of 92 inflammatory proteins were quantified using the Olink® Inflammation panel. Twenty-four samples collected following corticosteroid administration were excluded, leaving 112 samples for immune-age analysis.

To estimate immune age, an elastic net linear regression model with cross-validation was trained on the principal component analysis (PCA) space derived from inflammatory protein profiles of post-surgical, treatment-naïve patients (n = 56), thereby minimizing tumor-related immune perturbations. The trained model was then applied to the full cohort to infer immune age. Immune age acceleration was defined as the difference between the predicted immune age and the expected immune age based on chronological age, and this value was standardized as a z-score. The trajectories of inflammatory marker expression along the immune age continuum were also examined.

Among 25 TNBC patients treated with perioperative pembrolizumab and chemotherapy, baseline immune age acceleration was compared between individuals who did and did not experience high-grade (grade 3–4) immune-related adverse events (irAEs). Additionally, the incidence of high-grade irAEs was compared between patients with positive versus negative immune age acceleration.

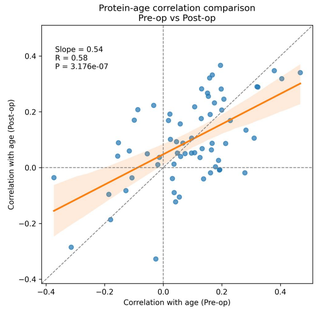
Results

Of the 136 patients, 35 patients (25.7%) had TN disease, 73 patients (53.7%) had ER-positive, HER2-negative disease, and 28 patients (20.6%) had HER2-positive breast cancer. The mean age was 51.05 years, range 23-75.5 years. No significant difference was observed in age distribution across the different breast cancer subtypes (ANOVA p = 0.36). Among the 35 TN patients, 25 received perioperative pembrolizumab. Of these, 10 patients (40%) developed grade 3-4 irAEs, while 15 patients (60%) either did not develop irAEs or experienced low-grade adverse events (see Table).

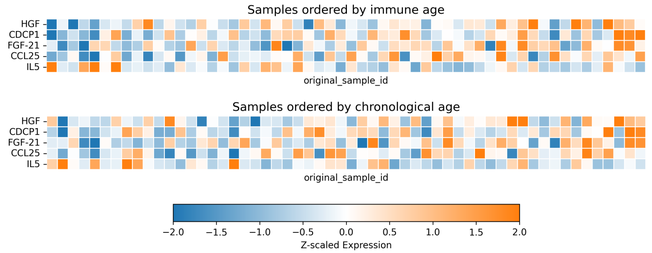
Table 1. irAEs of the patients' cohort

	Adverse events
Grade 1	Diarrhea (n=1); Arthritis (n=1); Rash (n=1)
Grade 2	Hypothyroidism (n=2); Arthritis (n=3); Pneumonitis (n=1); Hepatitis (n=1)
Grade 3	Arthritis (n=3); Rash (n=5); Gastritis/Duodenitis (n=2)
Grade 4	Neutropenic fever (n=1); Rash (n=1)

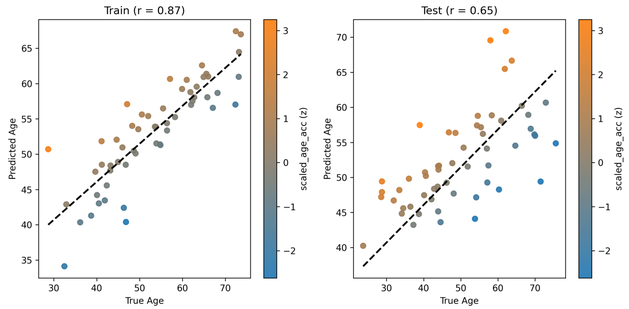
To evaluate whether age-related effects on inflammatory proteomics persist in early breast cancer, we focused on post-operative patients, in whom tumor-associated immune perturbations are minimal. For each protein, we computed its correlation with age in both pre- and post-operative conditions. Although several proteins exhibited reduced correlations after surgery, the overall age-associated inflammatory signature remained largely preserved. These findings suggest that immune age metrics derived from inflammatory profiles can be reliably applied to cancer samples.



To evaluate the relationship between the immune age metric and proteomic profiles, we ranked the top age-associated proteins according to both immune age and chronological age. The expression patterns of these proteins aligned more strongly with the immune age axis than with chronological age, indicating that immune age captures proteomic variability more effectively and provides a more biologically informative measure of aging.

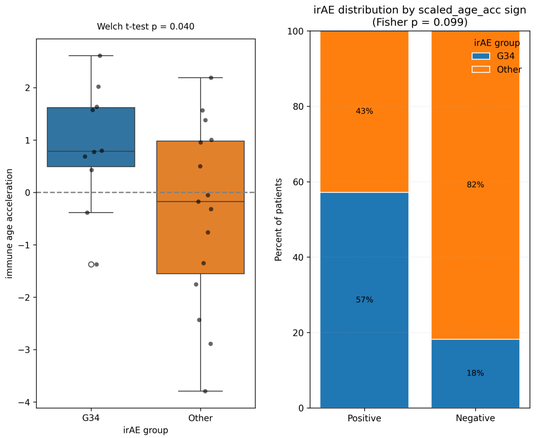


The regression of model predictions against true chronological age reveals a strong correlation between the two (Pearson r = 0.87 for the training set and 0.65 for the test set), emphasizing the close relationship between immune age and chronological age in both the training (n = 56) and test (n = 56) sets. However, we observed notable deviations from the regression line, which were attributed to immune-age acceleration of individual patients. These deviations were more pronounced in the test set compared to the training set. The deviations were z-scaled and represented with a color gradient ranging from blue (low immune age acceleration) to orange (high immune age acceleration).



Immune age acceleration measured in baseline samples of TN patients who subsequently developed high-grade irAE (n=10) was significantly higher (t test P = 0.04) compared to patients who did not develop high grade irAE (n=15). This reflects the higher baseline inflammatory state of these patients.

Among the 14 patients with positive immune age acceleration, 57% experienced high-grade immune-related adverse events (irAEs). In contrast, of the 11 patients with negative immune age acceleration, only 18% developed high-grade irAEs. These findings suggest a potential correlation between immune age acceleration and the likelihood of developing severe irAEs.



Conclusions

- Age strongly influences the proteomic landscape in early breast cancer patients, enabling development of an immune-age model.
- The immune-age model outperforms chronological age in explaining proteomic variation.
- Baseline immune-age acceleration correlates with the subsequent occurrence of immune-related adverse events.
- Further research should be performed to validate these observations on larger cohorts.

We are happy to collaborate: a_alpert@rambam.health.gov.il

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