Systems biology as a compass to understand cancer-immune interactions in humans

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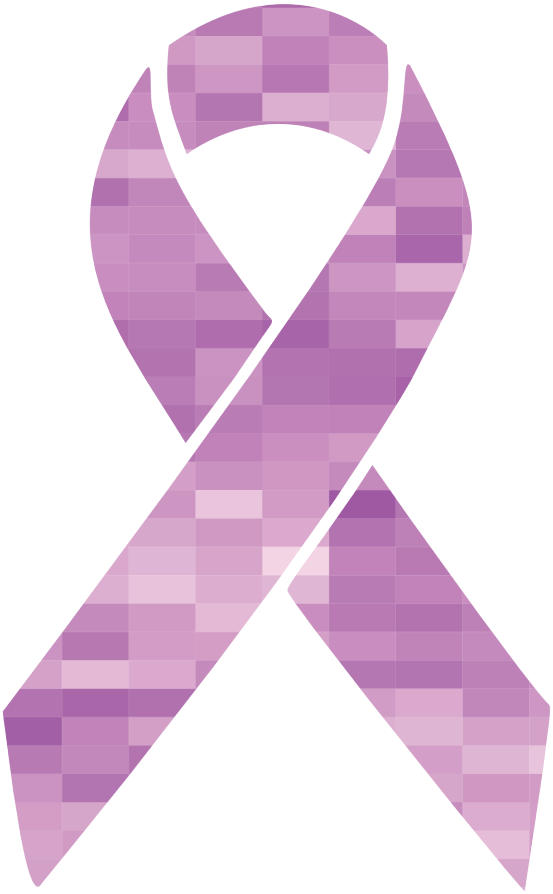

Chapter 5

Ancestry-associated transcriptomic profiles of breast cancer in patients of African, Arab and European ancestry

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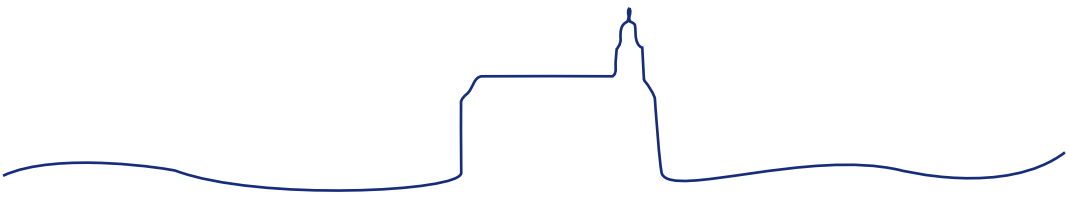


Abstract

Breast cancer largely dominates the global cancer burden statistics, however, there are striking disparities in mortality rates across countries. While socioeconomic factors contribute to population-based differences in mortality, they do not fully explain disparity among women of African (AA) and Arab (ArA) ancestry compared to women of European ancestry (EA). In this study, we sought to identify molecular differences that could provide insight into the biology of ancestry-associated disparities in clinical outcome. We applied a unique approach that combines the use of curated survival data from the TCGA Pan-Cancer clinical data resource, improved SNP-based inferred ancestry assignment and a novel breast cancer subtype classification to interrogate the TCGA and a local Arab breast cancer dataset. We observed an enrichment of BasalMyo tumors in AA patients (38% vs 16.5% in EA, p=1.30E-10), associated with a significant worse overall (HR=2.39, p=0.02) and disease specific survival (HR=2.57, p=0.03). Gene set enrichment analysis of BasalMyo AA and EA samples revealed differences in the abundance of T regulatory and T helper type 2 cells, and enrichment of cancer-related pathways with prognostic implications (AA: PI3K-Akt-mTOR and ErbB signaling; EA: EGF, estrogen dependent and DNA repair signaling). Strikingly, AMPK signaling was associated with opposing prognostic connotation (AA: 10yr-HR=2.79, EA: 10yr-HR=0.34). Analysis of ArA patients suggests enrichment of BasalMyo tumors with a trend for differential enrichment of T regulatory cells and AMPK signaling. Together, our findings suggest that the disparity in clinical outcome of AA breast cancer patients is likely related to differences in cancer-related and microenvironmental features.

Introduction

As we enter an era of personalized medicine in oncology, large-scale studies have been instrumental in deciphering the pathogenesis and evolution of tumors. Public data repositories such as The Cancer Genome Atlas (TCGA) have enabled researchers to define the genomic landscape of different types of cancers, including breast cancer. The public availability of large-scale datasets has led to a surge in candidate drug targets and novel prognostic and/or predictive gene signatures. However, it is important to note that the majority of patients in public datasets are of European ancestry and hence, the knowledge gained from such studies might not be applicable to patients of a different ancestry[1]. Given the global disparities in clinical behavior of breast cancer, it has become imperative to investigate ancestry-associated differences in tumor biology.

Breast cancer in women of African ancestry (AA) presents at a younger age, and is associated with more advanced disease and higher mortality rates as compared to breast cancer in age-matched patients of European (EA) or Asian (AsA) ancestry[2–10]. Several reports have demonstrated an increased frequency of the more aggressive triple negative breast cancer (TNBC) subtype and of the PAM50-molecular basal subtype in AA women[7–16]. Moreover, African American women with early stage TNBCs have been shown to exhibit a lower pathological complete response to neoadjuvant chemotherapy[17]. Interestingly, this discrepancy in clinical outcome remains after correcting for socioeconomic factors, suggesting the presence of molecular differences by ancestry[18,19]. The African American breast cancer epidemiology and risk consortium identified few rare germline single nucleotide polymorphisms (SNPs) that are associated with an increased risk of hormone receptor negative breast cancer and/or TNBC in African-American women[20,21]. Analysis of genotypic traits revealed that most somatic mutations and copy number variations are subtype specific rather than ancestrally determined[22,23]. Very few mutations showed dissimilar frequencies across African, African American or European American patient subgroups when considering a specific breast cancer subtype. Likewise, numerous differentially expressed genes have been identified between breast tumors of patients of African and European ancestry[24–28], however, there is little to no evidence linking these findings to differences in breast cancer survival or subtype-specific survival in relation to ancestry. Therefore, differential expression of genes involved in biological processes such as differentiation, cell cycle, DNA repair, invasion, metastasis, and angiogenesis could be related to the higher proportion of triple negative breast tumors in the African-American population. To address this, several studies investigated molecular differences within TNBC tumors of African American and European American patients. TNBC tumors of African American women were shown to display enrichment of gene sets related to a high proliferative rate, high genomic grade index, *BRCA1* deficiency, increased activation of insulin-like growth factor 1 receptor (IGF1R) and increased angiogenesis, closely resembling the basal like-1 TNBC subtype gene signature as described by Lehmann et al[23,28–33]. In addition, it has been suggested that an abundance of cancer stem cells might in part contribute to the worse survival of African American women with TNBC tumors[34–38].

Given the importance of immune cell infiltration in determining the prognosis and treatment response of breast cancer, and especially in TNBC, it is important to investigate whether differences in anti-tumor immunity may contribute to the divergent clinical behavior of breast cancer across populations[39–42]. To date, this phenotypic aspect of breast cancer is largely unexplored in the context of ancestry. Interestingly, systemic levels of pro-inflammatory cytokines such as IFN-γ and IL-6 have been found to be elevated in both healthy African American women and those affected with breast cancer as compared to European American women, suggesting ancestry-inferred differences in the immune response that might affect anti-tumor immunity and ultimately breast cancer clinical outcome[43,44]. In contrast, only subtle differences in immune gene

signatures related to immune cell infiltration were found in TNBC tumors of women of African ancestry[22,45].

In this study, we applied a unique approach to explore ancestry-associated heterogeneity of breast cancer outcome. First, we used improved and curated survival information from the TCGA Pan-Cancer clinical data resource (TCGA-CDR)[46]. Second, we applied SNP-based inference of ancestry[47,48] to improve ancestry assignment, enabling us to include a substantial number of additional patients from the TCGA dataset in our analysis thereby increasing the power of our study. Third, we performed a comprehensive transcriptomic analysis of both immunological and cancer-cell intrinsic parameters within breast cancer subtypes as defined by a novel PAM50 classification. This refined classifier utilizes a combination of Topological Data Analysis (TDA) signatures of normal mammary cell types (basal epithelial cells, luminal epithelial cells, myoepithelial cells, and Her2-related expression) to subgroup breast tumors into 7 distinct molecular subtypes with prognostic value[49]. Using this combined novel approach, we interrogated the TCGA breast cancer dataset, comprising of patients of African (n=184), European (n=811) and Asian (n=56) ancestry; and a local Arab/Asian breast cancer dataset from Qatar (n=24) for ancestry-specific molecular differences in breast cancer.

Methods

Patient cohorts

Two different breast cancer cohorts were included in this study; the public available TCGA breast cancer dataset and a local cohort from Qatar.

RNA sequencing data from the TCGA breast cancer cohort (n=1082 patients) was downloaded using R (v3.5.1) and TCGA Assembler (v2.0.3,[50]). Sample data were extracted ensuring a single primary tumor sample per patient using the TCGA Assembler "ExtractTissueSpecificSamples" function. Clinical data for all patients were obtained from the TCGA-CDR[46]. Patient ancestry was obtained using SNP-based inferred ancestry data, focusing on the European, Asian, and African clusters[47,48]. To visualize major ancestry clusters within the TCGA BRCA cohort PCA results of Jian Carrot-Zhang were used to plot PC1 versus PC2 using ggplot [48]. Using these data, we were able to include 108 patients that previously had no reported ancestry. As SNP-based ancestry had a very high concordance with reported ancestry (99.1%), we decided to also include 63 patients for which only self-reported ancestry was available. We excluded 31 patients from our ancestry-based analyses. Firstly, 16 patients with American inferred ancestry as the number of samples in this cluster is limited as well as one patient who self-identified as not Hispanic or Latino. Secondly, six patients without self-reported or inferred ancestry and thirdly exceptional cases of discordance between self-reported and SNP-based ancestry (n=8; 0.9%) were excluded. The final TCGA breast cancer cohort used for analysis comprised of 1051 patients (811 of European, 184 of African and 56 of Asian ancestry). The tumor non-silent mutation rate, predicted neoantigen load, and aneuploidy score were obtained from Thorsson *et al.*[51], and predicted versus expected neoantigen values were extracted from Rooney *et al.*[52].

The RA-QA patient cohort constitutes a breast cancer cohort from Qatar (n=24 of which 16 of Arab ancestry) with patients that were newly diagnosed with breast cancer between 2004-2010 at the National Centre for Cancer Care and Research in Doha. Clinical information and self-reported ancestry were extracted from the medical records. The study was approved by the local ethical committees of the Hamad Medical Corporation (study approval number #14027/14), the Qatar Biomedical Research Institute (study approval number #2016-002), and Sidra Medicine (study approval number #1711015664), and was performed in accordance with the ethical

standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol was granted waiver of informed consent under the condition of anonymization and no additional intervention for the participants.

Total RNA sequencing

RNA was isolated from four 20 μm sections of Formalin Fixed Paraffin Embedded (FFPE) tumor samples of the RA-QA cohort using the AllPrep DNA/RNA FFPE kit (Qiagen, Germany), followed by a quality control for purity and integrity by the Agilent Bioanalyzer system. Total RNA was depleted from ribosomal RNA and random primed for cDNA synthesis using the TruSeq stranded total RNA kit (Illumina, USA). RNA sequencing was performed on the Illumina HiSeq2500 platform (Illumina) with Paired End 25X coverage (PE100-125). The FASTQ files were trimmed to remove adaptor sequences using flexbar (v3.0.3,[53]) and aligned to GRCh37/hg19 reference genome using hisat2 (v2.0.5,[54]), resulting in an average 10-15M aligned reads. Reads were counted to genomic features using subreads (v1.5.5,[55]). For both the TCGA and RA-QA cohort, RNA-seq data was corrected for GC content and normalized within and between lanes using the R package EDASeq (v2.12.0,[56]), and quantile normalized using the preprocessCore (v1.36.0,[57]).

Intrinsic molecular subtype classification

The intrinsic molecular subtype of each tumor sample was defined by the differential expression of a set of 50 genes (PAM50) using two distinct algorithms. First, the R package bioclassifier_R was used to predict sample subtype according to the Parker et al subtype predictor[58]. Second, a more recent classification model was applied using a robust classifier that integrates the PAM50 gene signature with Topological Data Analysis, resulting in 7 subgroups with well-defined gene expression patterns[49]. The TDA classifier is based on the observed expression of five gene groups, basal (a), myo1 (b), myo2 (c), luminal (d), and Her2 (e) **(Figure 1A**). The nomenclature of the identified TDA classes directly reflects the observed gene groups, e.g., BasalHer2 samples are characterized by increased expression of the basal (a) and the Her2 (e) gene groups, and LumBasal samples by basal (a) and luminal (d) gene expression etc. An explanatory summary of the characteristics of the different TDA classes is included in **Figure 1A**. Sample clustering according to both classification methods was visualized in a PAM50-based heatmap using the R package ComplexHeatmap (v1.20.0,[59]). Circos plots using the R package circlize (v0.4.6, [60]) depicted TDA reclassification of samples in comparison to PAM50 subtyping. The distribution of TDA subtypes within ancestries was assessed using stacked barplots and chi square tests.

Immunologic Constant of Rejection consensus clustering

Consensus clustering of samples according to the expression values of 20 ICR genes was performed using the ConsensusClusterPlus (v1.42.0,[61]) R package with the following parameters: 5.000 repeats, and agglomerative hierarchical clustering with ward criterion (Ward.D2) inner and complete outer linkage as previously described[62,63]. The optimal number of clusters for best segregation of samples was determined using the Calinski-Harabasz criterion with samples in intermediate clusters defined as “ICR Medium”. Samples of the TCGA dataset were clustered into 3 groups; ICR low (cluster 1), ICR medium (clusters 2 and 3) and ICR high (cluster 4). Due to the small number of samples, the RA-QA cohort was divided into 2 groups; ICR Low (cluster 1, 2, 3) and ICR High (cluster 4).

Single sample gene set enrichment analysis

Enrichment of specific gene sets, reflecting either abundance of immune cell populations or expression of tumor related pathways, was defined by single sample gene set enrichment analysis (ssGSEA) using R package GSVA (v.1.30.0, [64])[65]. Gene set signatures of 24 distinct immune cell types or leukocyte subgroup enrichment scores were used to deconvolute immune cell abundance[66]. Gene sets comprising numerous tumor-related pathways were obtained from multiple sources, including the Molecular Signatures Hallmark[67] and Ingenuity Pathway Analysis (IPA) gene set collections and several signatures that have been associated with tumor immune escape[68–71]. Gene signature enrichment scores were compared based on ancestry using the 2-tailed unpaired t-test.

XGBoost model

We utilized an optimized version of the white-box, non-linear, ensemble gradient boosting machine called XGBoost to build our cox-regression model for survival analysis[72,73]. Gradient Boosting is a machine learning technique based on a constructive strategy by which the learning procedure will additively fit new models, typically decision trees[74] and repetitively leverage the patterns in residuals to provide a more accurate estimate of the response variable or time to event i.e. death in case of survival analysis. The patients who are alive are considered as right censored and since the XGBoost model takes only one label for the response variable as input, the censored survival information is converted to negative labels while performing the cox proportional hazards modelling[75]. XGBoost is a scalable machine learning technique for tree boosting, a learning technique to improve the regression performance of weak regressors by repeatedly adding new decision trees to the ensembles, which enhances performance in comparison to other boosting algorithms[72]. The main components of XGBoost algorithm are the objective function and its iterative solution. The objective function is initialized to describe the model's performance. Given the training dataset, $D = \{x^i, y^i\}_{i=1}^{N}$ where $x^i \in R^d$, d = 54, $y^i \in R$, *N* denotes the total number of training samples, *R* depicts the set of real numbers and *D* represents the training set. The predicted output $\hat{y}^t$ obtained from the ensemble model can be represented as: $\hat{y}^t = \sum_{t=1}^{T} H_t(x^i)$, where $H_t(x^i)$ represents the prediction score of the tth decision tree for the ith patient in the training dataset. If the decision trees are allowed to grow unregulated, then the resulting model is bound to overfit[72]. Hence, the following objective has to be minimized:

$$J(H) = \sum_{i=1}^{N} L\left(y^i, \hat{y}^t\right) + \sum_{t=1}^{T} \Omega(H_t) \qquad \text{Eq. 1}$$

where *L* is the loss function and $\Omega()$ is the penalty that is used to prevent overfitting and is defined as $\Omega(H_t) = \gamma A + \frac{1}{2}\lambda \sum_{j=1}^{A} w_j^2$, where γ and λ are the parameters that control the penalty for number of leaf nodes (*A*) and leaf weights (*w*) respectively in the decision tree H_t.
The objective function can be re-written as $J(H) = \sum_{i=1}^{N} L\left(y^i, \hat{y}^t{}_{t-1} + H_t(x^i)\right) + \sum_{t=1}^{T} \Omega(H_t)$. After applying a Taylor expansion [76] and expanding $\Omega(H_t)$, we obtain:

$$J(H_t) = \sum_{i=1}^{N} \left[g_i H_t(x^i) + \frac{1}{2} h_i^2 H_t(x^i)\right] + \gamma A + \frac{1}{2}\lambda \sum_{j=1}^{A} w_j^2 \qquad \text{Eq. 2}$$

where $g_i = \partial_{y_{t-1}}\left(L\left(y^i, \hat{y}^t{}_{t-1}\right)\right)$ and $h_i = \partial^2_{y_{t-1}}\left(L\left(y^i, \hat{y}^t{}_{t-1}\right)\right)$ are the first and second order gradient statistics for the loss function *L*. For a fixed tree structure *H(x)*, where $I_j = \{i\}, \forall H(x^i) = j$ is an instance of leaf node *j*, the optimal weight w_j^o for leaf node *j* is given by:

$$w_j^o = \frac{-\sum_{i \in I_j} g_i}{\sum_{i \in I_j} h_i + \lambda}$$

The corresponding optimal objective function becomes:

$$J(H_t) = \frac{-1}{2} \sum_{j=1}^{A} \frac{\left(\sum_{i \in I_j} g_i\right)^2}{\left(\sum_{i \in I_j} h_i + \lambda\right)} + \gamma A \qquad \text{Eq. 3}$$

Equation 3 can be used as a scoring function to measure the quality of a tree structure H_t during iteration t. This score is equivalent to the impurity score used for evaluating decision trees in random forests[77]. We build our XGBoost model using the fast, greedy and iterative algorithm by Chen et al to identify the optimal tree structures[72].

SHAP model

One of the disadvantages of the feature importance scores obtained from the XGBoost model is that the directionality is not apparent. For instance, when a particular pathway attains a high enrichment score it is not clear whether this corresponds to a higher or lower risk of death. Moreover, at the test phase, it is a challenge for traditional white-box, tree-based, machine-learning techniques to provide information about the top 5 features driving the prediction to better or poorer survival prognosis. Recently, several techniques have been proposed to overcome aforementioned limitations, including LIME (Local Interpretable Model-agnostic Explanations)[78] and SHAP (SHapley Additive exPlanations)[79]. These methods have the ability to interpret feature importance scores from complex training models and provide interpretable predictions for a test sample based on the top k features for that particular test instance. In our work, we used the SHAP method as it has been shown to outperform the LIME method and to be better aligned with human intuition[79]. The SHAP method is an additive feature attribution method where a test instance prediction is defined as a linear function of features that satisfies 3 critical properties: local accuracy, missingness and consistency.

The explicit SHAP regression values are derived from a game-theory framework[80,81] and can be computed as:

$$\phi_i = \sum_{S \subseteq Q - \{i\}} \frac{|S|!\,(|Q| - |S| - 1)!}{|Q|!} \left[H_{S \cup \{i\}}\left(x_{S \cup \{i\}}\right) - H_S(x_S)\right]$$

where Q represents the set of all d features, S represents the subsets obtained from Q after removing the ith feature and ϕ_i is an estimate of the importance of feature i in the model. In order to refrain from undergoing $2^{|Q|}$ differences to estimate ϕ_i, the SHAP method approximates the Shapley value by either performing Shapley sampling[82] or Quantitative Input Influence[83]. A detailed description of model interpretation using the SHAP method has been outlined by Samek W et al[79]. In our work, SHAP values associated with a particular pathway in the XGBoost model provide information on the change in log (risk of death) for each feature of the Cox proportional hazards model.

Survival analysis

Kaplan-Meier curves were generated using the ggsurvplot function from R package "survminer" (v0.4.8) to compare overall survival and disease specific survival between ancestries, ICR clusters, and AMPK subgroups. Univariate Cox proportional hazards regression analysis was performed with the R package "survival". AJCC pathologic tumor stage as described in the TCGA-CDR was used for stratified analysis within the BasalMyo class. Forest plots were generated using the R package forestplot (v1.7.2).

Results

Ancestry of patient populations

To date, studies investigating molecular differences between ancestries have been solely based on self-identified ancestry. In our study, we applied a novel approach combining self-reported ancestry and SNP-based inference of ancestry[47,48]. Ancestries were assigned using principal component analysis (PCA) of SNP array genotyping calls following the method as described by Carrot-Zhang et al[48] (**Supplementary Figure 1**)**.** As such, we included 1051 patients from the TCGA breast cancer dataset in our analysis of which 811 EA, 184 AA, and 56 AsA patients (**Table 1**). Ancestry of patients in the local Retrospective Arab cohort from Qatar (RA-QA) was solely based on self-reported ancestry, subgrouping 16 patients as Arab ancestry (ArA), five as AsA, two as EA and one as Persian (**Table 2**).

Table 1. Cohort demographics TCGA breast cancer cohort.

TCGA BRCA cohort (n=1082)		
Median FU (yrs)	2.37	
Events		
OS	151	
DSS	83	
Age (yrs)		
median	58	
range	26-90	
	n	%
Ancestry[1]		
European	811	75
African	184	17
Asian	56	5.2
Undefined	31	2.9
AJCC stage		
I	179	16.8
II	613	56.6
III	247	22.7
IV	19	1.8
NA	24	2.2
PAM50 subtype		
Basal	233	22
Her2-enriched	160	14
Luminal A	337	31
Luminal B	241	22
Normal-like	111	10
TDA subtype		
BasalHer2	82	8
BasalMyo	219	20
BasalLumHer2	90	8
Lum	283	26
LumBasal	209	19
MyoLumA	102	9

MyoLumB	35	3
MyoLumHer2	62	6

[1]SNP-based ancestry

Table 2. Cohort demographics RA-QA breast cancer cohort.

RA-QA cohort (n=24)		
Median FU (yrs)	8.02	
Events		
OS	7	
Age (yrs)		
median	48.5	
range	28-63	
	n	%
Ancestry[1]		
Arab	16	66.7
Asian	5	20.8
Caucasian	2	8.4
Persian	1	4.2
AJCC stage		
I	4	16.7
II	10	41.7
III	4	16.7
IV	0	0
NA	6	25
PAM50 subtype		
Basal	9	37.5
Her2-enriched	3	12.5
Luminal A	7	29.2
Luminal B	2	8.3
Normal-like	3	12.5
TDA subtype		
BasalHer2	2	8.3
BasalMyo	7	29.2
BasalLumHer2	2	8.3
Lum	6	25
LumBasal	2	8.3
MyoLumA	1	4.2
MyoLumB	1	4.2
MyoLumHer2	3	12.5

[1]Self-reported ancestry

Distribution of molecular breast cancer subtypes

Numerous studies have demonstrated a higher prevalence of TNBC and of tumors of the molecular basal subtype among AA women and have linked the increased frequency of these aggressive breast tumors to ancestry-associated disparity in breast cancer clinical outcome. Using our novel combined approach, we interrogated the TCGA and RA-QA datasets to subgroup patients according to TDA-defined molecular subtype and ancestry[49]. Heatmaps of TCGA and RA-QA samples based on TDA gene signatures (basal, myo1, myo2, luminal, and Her2) show a clear segregation of samples in 7 molecular subtypes, each defined by a unique combination of expression of five distinct gene groups, demonstrating the accuracy and robustness of the novel classifier (**Figure 1A**). As can be seen in the circos plots in **Figure 1B**, and in accordance with the METABRIC analysis by Mathews *et al.*[49], we found that Luminal A tumors are mainly reclassified into Lum and MyoLum subgroups, while luminal B tumors are mainly subgrouped into LumBasal and Lum tumors. In addition, tumors of the normal-like PAM50-subtype are mainly reclassified into the Myo classes. Her2 enriched tumors are predominantly subdivided into BasalHer2, BasalLumHer2 and LumBasal tumors. Further, the vast majority of basal tumors are reclassified as BasalMyo (88%). **Figure 1C** clearly demonstrates differences in molecular subtype frequency across ancestries, with a strong enrichment in AA patients of BasalMyo (38.0% vs 16.5% in EA, X-squared = 41.3, p=1.30E-10) and a reduced proportion of MyoLumA (2.7% vs 11% in EA, X-squared = 11.7, p=0.0006) and Lum (17% vs 29% in EA, X-squared = 10.9, p=0.001) tumors, and in AsA patients an enrichment of BasalHer2 tumors (21.7% vs 6.4% in EA, X-squared = 19.0, p=1.33E-05). While several studies reported an increase in basal tumors with worse outcome in AA patients[7,9,11,12,29,84], we were able to fine-tune this observation to a strong increase of BasalMyo tumors, accounting for the majority of basal tumors. Furthermore, we observed an increase in the proportion of BasalMyo tumors in ArA patients (25.0% vs 16.5% in EA, X-squared = 1.0E-4, ns), although this did not reach statistical significance as a likely result of the small cohort size.

Next, we explored ancestry-related differences in clinical outcome using curated survival data from the TCGA-CDR[46]. Clinical outcome of breast cancer patients, irrespective of molecular subtype, was not different between EA and AA patients **(Figure 1D)**. Among all seven TDA subtypes, BasalMyo tumors were the only tumors that were associated with significant different 10-year overall survival (OS, p= 0.020) and disease specific survival (DSS, p=0.033) rates for AA versus EA patients **(Figure 1D and Supplementary Figure 2)**. The 5-year OS rates for BasalMyo tumors were 85.5% for EA and 70.1% for AA patients (p=0.07), and the 5-year DSS rates were 90.1% for EA and 73.6% for AA patients (p=0.05). Interestingly, compared to TNBC and basal tumors, we observed a larger disparity in 10-year OS (HR=2.39, p=0.020) and 10-year DSS (HR=2.57, p=0.033) by ancestry in BasalMyo tumors **(Figure 1D)**. To exclude that this survival difference results from a higher frequency of more advanced stage BasalMyo tumors in AA patients, we compared the AJCC pathological stage between EA and AA patients and found no significant difference in stage distribution by ancestry (X-squared=2.83, p=0.092) **(Supplementary Figure 3)**. Additionally, we performed survival analysis stratified by early (Stage I and II) and advanced (Stage III and IV) stage and found rather large hazard ratios, although not significant, indicating worse overall survival of AA patients within strata **(Supplementary Figure 3).** Adjustment for tumor stage and/or age in multivariate analysis showed similar results with African ancestry being associated with worse survival (**Supplementary Figure 3**), albeit with borderline significance, implying that additional factors beyond pathological stage contribute to the divergent clinical outcome of AA patients with BasalMyo tumors compared to EA patients.

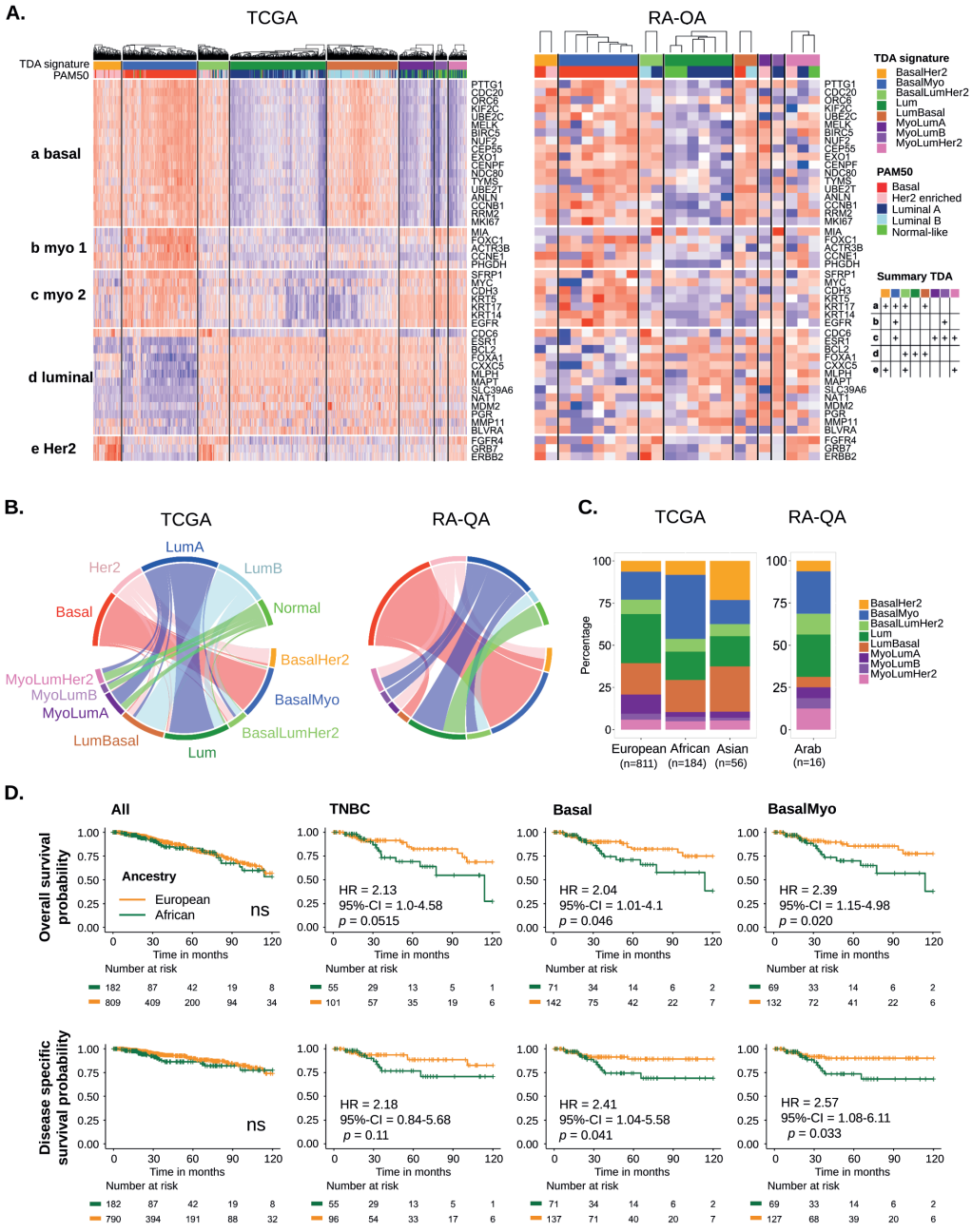


Figure 1. Distribution of breast cancer molecular subtypes defined by topological data analysis (TDA) signatures across ancestries. A. Heatmap of expression of PAM50 genes organized by TDA signature classes in TCGA breast cancer and RA-QA cohort. Samples are annotated by TDA signature

class (*upper annotation bar*) and classical PAM50 intrinsic molecular subtype (*lower annotation bar*). The combination patterns of upregulated expression of 5 distinct gene groups defining each TDA class are summarized in a table on the right (*Summary TDA*). **B.** Re-classification of breast cancer samples from classical PAM50 intrinsic molecular subtypes (*upper part of circos*) to TDA signature classes (*lower part of circos*) in TCGA and RA-QA breast cancer cohorts. **C.** Stacked barchart of distribution of TDA classes by ancestry. **D.** Kaplan Meier plots showing overall survival (*upper panels*) and disease specific survival (*lower panels*) by ancestry. Difference between survival of patients with European and African ancestry is shown for the complete TCGA breast cancer cohort (*left*), patients with TNBC according to hormone receptor status (*middle left*), patients with PAM50-defined basal breast cancer (*middle right*), and patients with tumors classified as BasalMyo by TDA classification (*right*). Censor points are indicated by vertical lines.

Ancestry-associated differences in immunological parameters

In an effort to elucidate potential ancestry-inferred differences in tumor biology, we compared the immune microenvironment of tumors from patients with different ancestry. More specifically, we assessed tumor immune disposition using the prognostic Immunologic Constant of Rejection (ICR) immune gene signature[62,85] and deconvoluted immune cell abundance using leukocyte subgroup enrichment scores (LES)[66]. The ICR 20-gene signature consists of genes encoding CXCR3/CCR5 chemokine ligands (*CXCL9, CXCL10*, and *CCL5*), genes encoding molecules involved in Th1 signaling (*IFNG, TXB21, CD8B, CD8A, IL12B, STAT1*, and *IRF1*) and effector immune functions (*GNLY, PRF1, GZMA, GZMB*, and *GZMH*), as well as counter regulatory molecules (IDO1, PDCD1/PD-1, CD274/PD-L1, CTLA4, *FOXP3*). Using the ICR gene signature, we previously classified breast cancer samples into four classes with the highest activation of the anti-tumor immune response in the ICR4 class[62]. In a follow-up study of more than 8000 non-metastatic breast cancer cases, we demonstrated that the ICR signature was the strongest independent prognostic predictor for metastatic relapse, in particular for patients with Her2+-enriched and triple negative breast tumors[86]. Since we didn't consider ancestry in our previous findings, the present study aimed to investigate whether the prognostic value of ICR holds true across ancestries or whether there could be immune-related dysregulations that in part explain the disparity in clinical outcome of AA breast cancer patients. First, we used the ESTIMATEscore, ImmuneScore and StromalScore to compare tumor cellularity, proportion of the stromal component and level of infiltration of immune cells of all TDA subtypes in EA versus AA patients[87]. We did not observe significant differences within subtypes by ancestry, indicating that any potential changes in immune-related gene expression in AA versus EA patients is not caused by differences in stromal and immune cell composition (**Supplementary Figure 4**).

The ICR gene signature clearly clusters breast tumors of the TCGA dataset into three immune phenotypes with varying degrees of immune activation (ICR low, ICR medium and ICR high), while tumors of the RA-QA cohort were subdivided into two immune phenotypes (ICR low and ICR high) (**Figure 2A**). In accordance with our previous work, tumors with an ICR low immune phenotype were associated with a worse survival in EA patients (p=0.028) (**Figure 2B**). Likewise, we observed a large, although not significant, difference in survival between ICR low and ICR high patients within the AA and ArA groups. In line with these findings, the prognostic value of gene signatures that reflect abundance of individual immune cell populations was overall similar across ancestries with leukocyte subpopulations classically associated with better prognosis such as CD8+ T cells and cytotoxic cells having the same trends in EA and AA patients (**Supplementary Figure 5**). Next, we investigated whether the immune disposition, inferred from the ICR enrichment score, varies within TDA subtypes by ancestry (**Figure 2C**). Comparison of the continuous ICR enrichment score demonstrated modest variation between TDA subtypes with overall higher scores in non-luminal tumors (BasalHer2 and BasalMyo), which was not affected by ancestry. For instance, no significant difference in ICR enrichment score was found in

BasalMyo tumors by ancestry, suggesting a similar overall immune disposition across ancestries. In accordance, we did not find any significant differences in expression of individual ICR genes based on ancestry (data not shown). Further analysis of BasalMyo tumors, however, revealed differences within ICR clusters whereby ICR low and ICR medium patients were grouped into one subgroup due to limited sample size of each cluster within BasalMyo tumors. Although BasalMyo tumors of AA patients were overall associated with worse overall survival, this was more pronounced in ICR medium+low tumors (10y OS, p=0.03; 5y OS, p=0.07) (**Figure 2D**). In multivariate analysis, African ancestry remained significantly associated with worse survival when adjusted for tumor stage, and reached borderline significance when adjusted for tumor stage and age (**Supplementary Figure 3**).

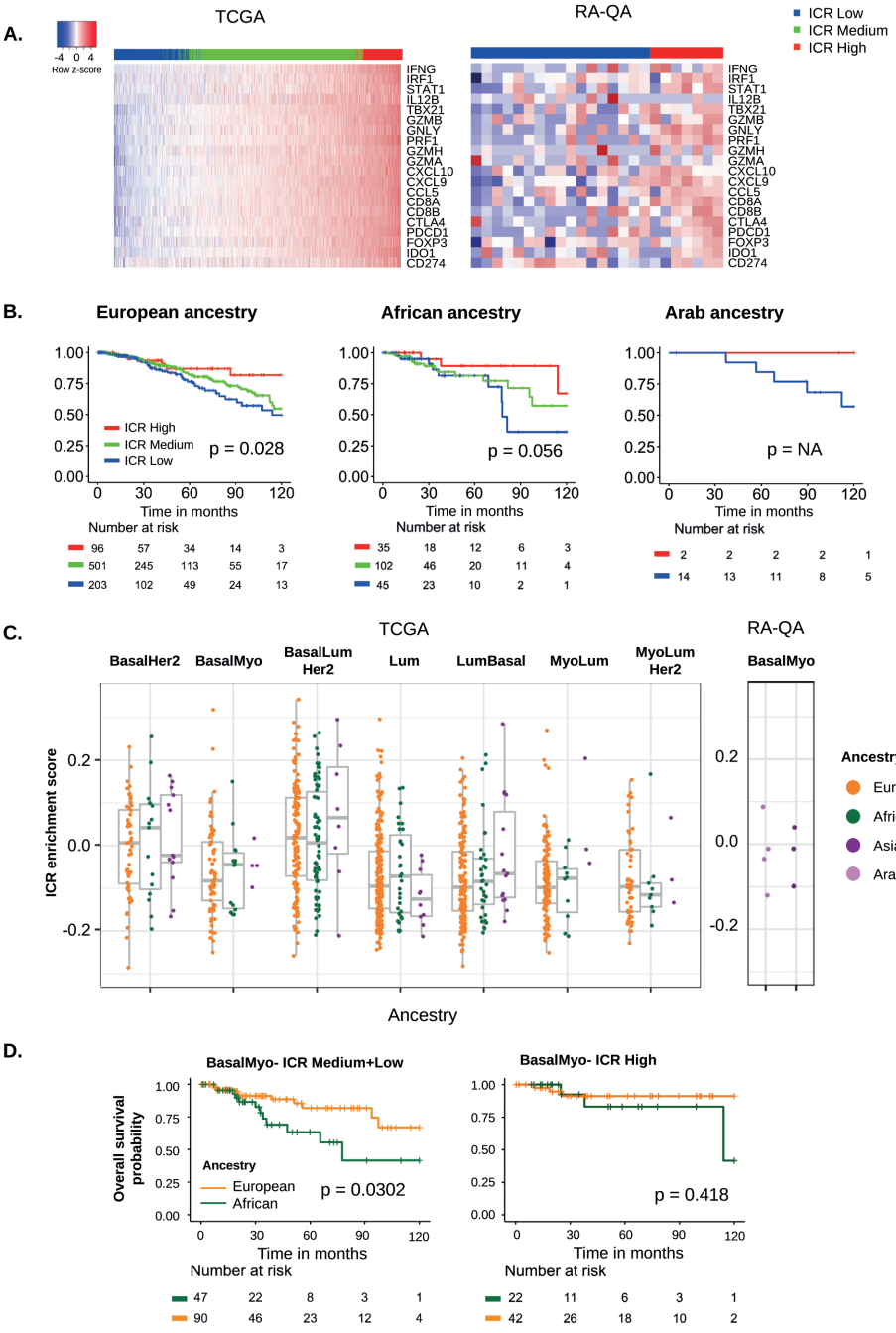

A.
TCGA
RA-QA
Row z-score
ICR Low
ICR Medium
ICR High
IFNG
IRF1
STAT1
IL12B
TBX21
GZMB
GNLY
PRF1
GZMH
GZMA
CXCL10
CXCL9
CCL5
CD8A
CD8B
CTLA4
PDCD1
FOXP3
IDO1
CD274
B.
European ancestry
African ancestry
Arab ancestry
ICR High
ICR Medium
ICR Low
p = 0.028
p = 0.056
p = NA
Time in months
Number at risk
96 57 34 14 3
501 245 113 55 17
203 102 49 24 13
35 18 12 6 3
102 46 20 11 4
45 23 10 2 1
2 2 2 2 1
14 13 11 8 5
C.
TCGA
RA-QA
BasalHer2
BasalMyo
BasalLum Her2
Lum
LumBasal
MyoLum
MyoLum Her2
BasalMyo
ICR enrichment score
Ancestry
European
African
Asian
Arab
D.
BasalMyo- ICR Medium+Low
BasalMyo- ICR High
Overall survival probability
Ancestry
European
African
p = 0.0302
p = 0.418
Time in months
Number at risk
47 22 8 3 1
90 46 23 12 4
22 11 6 3 1
42 26 18 10 2

Figure 2. Tumor immune phenotypes and clinical outcome by ancestry. A. Heatmap of ICR gene expression in TCGA and RA-QA breast cancer cohorts. Classification of samples by ICR consensus clustering segregates TCGA samples in ICR Low, ICR Medium, and ICR High groups. Samples of RA-QA cohort were classified as ICR High or ICR Low. **B.** Kaplan Meier plots showing overall survival across ICR groups in breast cancer TCGA patients of EA (*left*), TCGA patients of AA (*middle*), and RA-QA patients of ArA (*right*). **C.** ICR enrichment scores across ancestries within TDA signature classes. Box plots indicate medians and interquartile range, whiskers represent 10th and 90th percentile. All data points are plotted individually. **D.** Overall survival of EA and AA patients in TCGA BasalMyo samples classified as ICR Medium+Low (*left*), and ICR High (*right*). Censor points are indicated by vertical lines.

This finding raised the question whether the worse outcome of AA patients with BasalMyo tumors is linked to molecular differences in ICR medium+low tumors also known as cold tumors. For this purpose, we determined the LES of 24 distinct immune cell types (**Figure 3A**). Focused analysis of BasalMyo cold (ICR medium+low) tumors revealed a significant decrease in T regulatory cell (Tregs) and T helper 2 cell (Th2) enrichment scores (p=0.036; p=3.36E-4, respectively), and a small increase in B cell enrichment score (p=0.039) in AA versus EA patients, whereas dendritic cell enrichment scores were reduced in ICR hot (ICR high) tumors (p=0.009).

In order to identify which LES may harbor prognostic value, we focused on BasalMyo tumors irrespective of ICR class due to sample size limitations and adopted a machine-learning strategy which has empirically been shown to work efficiently on small size datasets[88–90], despite a slight tendency for overfitting (EA, n=134; AA, n=70). First, we performed a sensitivity model analysis that enabled us to identify the XGboost models that have an optimal set of hyper-parameters (Harrell's C index EA=0. 58, AA =0.63) with relatively small variance (data not shown). Next, we used XGBoost modeling for nonlinear multivariate cox-regression survival analysis followed by the SHapley Additive exPlanations (SHAP) method for the AA and EA subgroups separately (**Supplementary Figure 6**). This approach provided information on which features or gene signatures are the most important and their range of effects over the dataset, including the breadth (SHAP value) and the direction of the effect (positive or negative). Both the Treg and Th2 signature were classified as features with more importance for predicting outcome in AA patients as compared to outcome in EA patients, with reduced enrichment scores being associated with increased risk of death. In accordance, we found that AA, but not EA, patients could be stratified into different risk groups based on the expression of the Treg and Th2 cell signatures with borderline statistically different clinical outcome (**Figure 3B**). More specifically, stratification by Treg LES subgrouped AA patients with BasalMyo tumors in a low-risk group with higher expression and 5-year OS rate of 77%, and a high-risk group with low expression and 5-year OS rate of 59% (10y-HR=2.99, 95%-CI=1.02-8.77). Th2 LES-based stratification grouped AA patients with BasalMyo tumors into a low-risk/high expression group with 5-year survival rate of 84% and a high-risk/low expression group with 5-year survival rate of 55% (10y-HR=3.13, 95%-CI= 0.98-10.00). No differences in survival were noted for DC and B cell LES (data not shown), which supports their lower rank of importance in the SHAP plot of AA patients (**Supplementary Figure 6**).

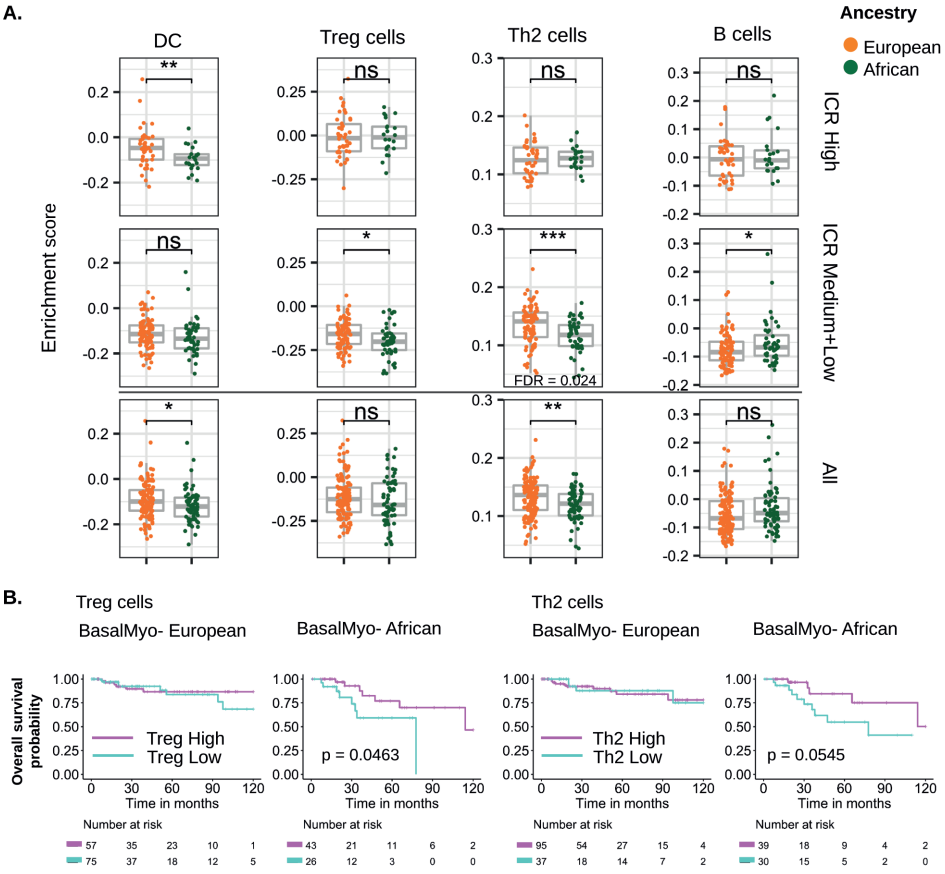


Figure 3. Enrichment of immune cell subpopulations in AA and EA patients with BasalMyo breast tumors. A. Enrichment scores of signatures reflecting abundance of dendritic cells (DC), T-regulatory cells (TReg), T-helper 2 (Th2), and B cells in BasalMyo tumor samples of EA and AA patients. Boxplots are facetted by ICR groups, ICR High (*upper panels*), ICR Medium+Low (*middle panels*), and across all samples (*lower panels*). Box plots indicate medians and interquartile range, whiskers represent 10th and 90th percentile. All data points are plotted individually. T-test (two-sided): *p < 0.05, ** p < 0.01, *** p < 0.001 and ns; not significant. Adjusted p-value (FDR) by Benjamini & Hochberg method. **B.** Kaplan Meier plots of overall survival in EA and AA patients with BasalMyo breast cancer dichotomized by enrichment scores of TReg (*left panels*) and Th2 cell signatures (*right panels*). Cutoff for dichotomization in "High" and "Low" categories is based on optimal enrichment cutoff determined by XGBoost model used for survival analysis. Censor points are indicated by vertical lines.

Ancestry-associated differences in cancer-cell intrinsic features

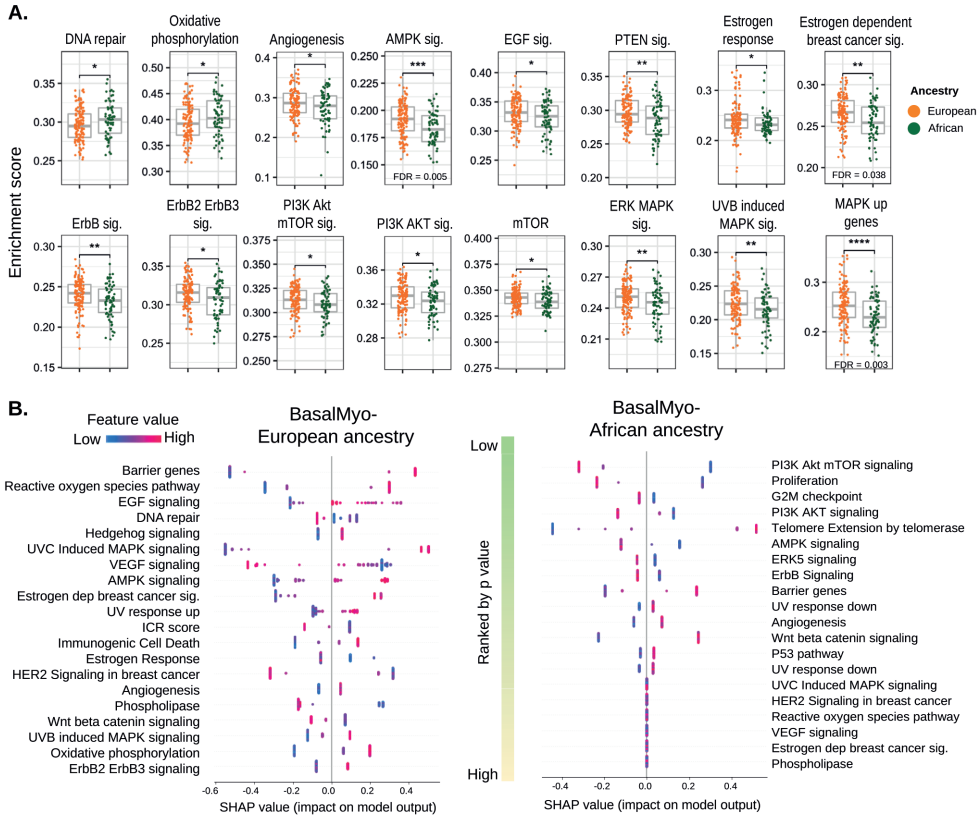
Next, we investigated whether specific cancer-cell intrinsic features might contribute to the worse survival of AA patients with BasalMyo tumors. First, we examined potential changes in common cancer-associated genomic aberrations, including mutational load, neoantigen load and tumor aneuploidy. Remarkbly, non-silent mutation rate was significantly lower in AA patients compared to EA (p=0.025), while the number of predicted single nucleotide variant (SNV)

neoantigens was similar between both patient populations (**Supplementary Figure 7**). Therefore, we speculated that AA BasalMyo tumors undergo less immunoediting and immune-mediated elimination of neoantigens compared to EA BasalMyo tumors. To address this hypothesis, we used an "immunoediting score", defined as the observed ratio (number of point mutations predicted to generate neo-epitopes divided by the total count of non-silent point mutations) compared to the expected ratio (expected numbers based on silent mutation rate) [52]. Indeed, the ratio of the observed/expected neoantigens was increased in AA patients (p=0.033), suggesting reduced immunoediting in AA samples (**Supplementary Figure 7**). However, we did not observe any survival difference between tumors with a high observed/expected neoantigen ratio compared to tumors with a low ratio (HR=1.1, 95%-CI= 0.43-2.79, p=0.842), suggesting that this tumor attribute does not explain the observed survival differences between AA and EA BasalMyo tumors. Similarly, while we observed a significantly increased tumor aneuploidy score in samples of AA patients (p=0.008, **Supplementary Figure 7**), this tumor characteristic was not associated with a difference in survival (HR=0.691, 95%-CI=0.32-1.48, p=0.34).

To further explore tumor intrinsic features that could contribute to the divergent survival outcomes, we explored the differential enrichment of 54 cancer-associated pathways (**Figure 4A**). A total of 16 pathways were found to be differentially enriched between BasalMyo tumors of AA versus EA patients. Of note, only 2 out of 16 pathways, DNA repair and oxidative phosphorylation, were associated with an increased enrichment in AA patients. A number of enriched pathways were identified multiple times as they were included in more than one database, including estrogen response and estrogen-dependent breast cancer signaling, ErbB signaling and ErbB2/ErbB3 signaling, PI3K Akt mTOR signaling and PI3K AKT signaling or mTOR signaling, and ERK MAPK signaling, UVB-induced MAPK signaling and MAPK up genes. Furthermore, the pathways defined as angiogenesis, AMPK signaling, EGF signaling and PTEN signaling were significantly less enriched in BasalMyo tumors of AA versus EA patients. Using the same approach we applied to explore the prognostic value of immune gene signatures, we used XGBoost modeling and the SHAP method to identify which cancer-associated pathways are the most powerful indicators of poor survival in AA versus EA patients with BasalMyo tumors (**Figure 4B-C**). Based on the summary SHAP plots, we observed that among the top 10 pathways affecting survival in EA patients, the majority displayed an inverse correlation of enrichment with survival including barrier genes, reactive oxygen species pathway, EGF signaling, hedgehog signaling, UVC-induced MAPK signaling, AMPK signaling, estrogen-dependent breast cancer signaling and UV response up genes (**Figure 4B**). In contrast, increased enrichment of DNA repair and VEGF signaling pathways were associated with better survival in EA patients. In AA patients, the majority of the top 10 pathways determining survival exhibited better survival with increased enrichment including PI3K Akt mTOR signaling, proliferation, G2M checkpoint, PI3K AKT signaling, AMPK signaling, ERK5 signaling, and ErbB signaling (**Figure 4B**). On the other hand, we found that pathway enrichment for telomere extension by telomerase, barrier genes and UV response down corresponded to worse survival.

In analogy with our analysis of the prognostic value of enriched immune gene signatures, we performed a combined analysis of differentially enriched pathways and the top 10 pathways with importance for prediction of survival (**Figure 4C**). Using this approach we identified three differentially enriched pathways with prognostic value in EA patients with higher enrichment of EGF signaling (p=0.02, optimal enrichment cutoff=0.334) and estrogen-dependent breast cancer signaling (p=0.076, optimal enrichment cutoff=0.268) being associated with worse prognosis, while a better survival was observed for enrichment of DNA repair (p=0.03, optimal enrichment cutoff=0.304). Focusing on AA patients, we found three differentially enriched pathways with prognostic connotation whereby enrichment of PI3K-Akt-mTOR signaling (p=9.00E-04, optimal enrichment cutoff=0.307), PI3K-Akt signaling (p=0.006, optimal enrichment cutoff=0.328), and

ErbB signaling (p=0.053, optimal enrichment cutoff=0.232) was associated with better outcome (**Figure 4B-C**). Interestingly, we found AMPK signaling to be the sole pathway to be differentially enriched between BasalMyo tumors of AA and EA patients with prognostic value in patients of both ancestries. Further analyses revealed an inverse correlation of AMPK enrichment with overall survival in AA versus EA patients. While in EA patients, pathway enrichment was associated with worse survival, it bestowed a survival advantage for AA patients (**Figure 4D**). The 5-year OS rate of EA patients with BasalMyo tumors enriched for AMPK signaling was reduced by 12% from 91% to 79% (10y-HR=0.343, 95% CI=0.11-1.10), while the opposite was observed in AA patients where the 5-year OS rate was increased by 21% from 57% to 78% (10y-HR= 3.598, 95% CI=1.18-10.94).



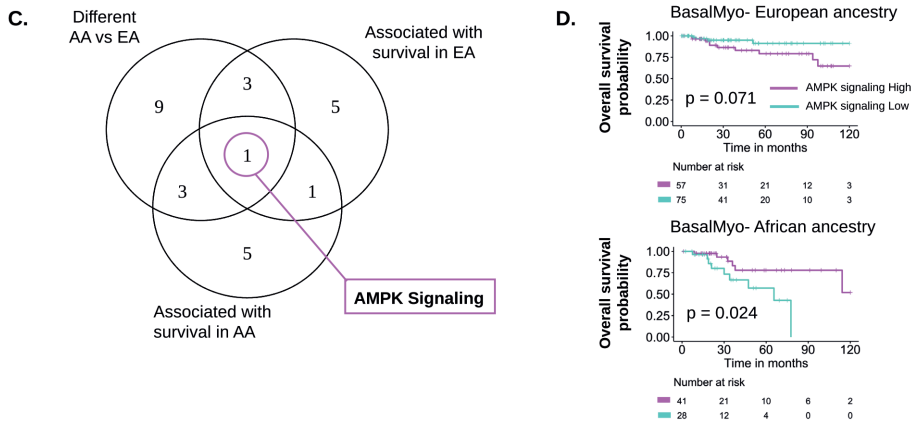


Figure 4. Differentially enriched oncogenic pathways with prognostic connotation in EA and AA patients with BasalMyo breast tumors. A. Enrichment scores of signatures of tumor-associated pathways that are differentially regulated between AA and EA patients with BasalMyo tumors. Box plots indicate medians and interquartile range, whiskers represent 10th and 90th percentile. All data points are plotted individually. T-test (two-sided): *p < 0.05, ** p < 0.01, *** p < 0.001 and ns; not significant. Adjusted p-value (FDR) by Benjamini & Hochberg method. **B.** SHAP plots of tumor-associated pathways that are associated with overall survival in EA (*left*) and AA (*right*) patients with BasalMyo breast tumors. Pathways are ranked by p-value to reflect the importance of each feature in the survival model. Each dot represents a single sample and is colored by relative enrichment score. Corresponding impact on model output (SHAP value) ranges from -1 (indicating absence of an event) to +1 (indicating occurrence of an event, in this case death). **C.** Intersection of differentially enriched tumor-associated pathways with ten most important pathways in AA and EA patients with BasalMyo breast tumors. AMPK signaling is differentially regulated in AA versus EA and is of importance in survival models of both AA and EA patients. **D.** Kaplan Meier curves visualizing the prognostic value of AMPK signaling in EA (*upper*) and AA (*lower*) BasalMyo patients. Dichotomization of samples by AMPK signaling is based on optimal enrichment score cutoff as determined by XGBoost model. Censor points are indicated by vertical lines.

Molecular alterations in Arab breast cancer patients

Given the similarity in TDA subtype distribution of ArA and AA patients (**Figure 1C**), we investigated whether the increased frequency of BasalMyo tumors in ArA patients was associated with differential enrichment of LES and cancer-associated pathways. Specifically, we focused our analyses on Treg, Th2 and AMPK signaling signatures that showed differentially enrichment with prognostic value in AA patients. Due to limited cohort size, we assessed enrichment patterns in all Arab patients without subgrouping by TDA subtype. Compared to AsA patients, ArA patients showed a trend towards lower enrichment scores of the Treg and AMPK signature (**Figure 5A**). In order to compare patterns of enrichment between ancestries of both cohorts, we performed a similar analysis across TCGA ancestries (EA, AA, AsA) without TDA subgrouping (**Figure 5B**). Out of the three signatures, only the differential enrichment of AMPK signaling holds true when comparing the overall AA versus EA patient population. Since BasalMyo tumors constitute a large

proportion of breast tumors in the AA patients (38%) and are associated with a strong reduction in AMPK signaling (p=1.78E-04), we cautiously speculate that the overall reduced enrichment of AMPK signaling in AA patients might be related to our findings in BasalMyo tumors. Similarly, it could be plausible that our findings in Arab patients might be related to differential enrichment signatures in BasalMyo tumors, supporting the need for larger Arab patient cohorts to enable statistically powered subanalysis of TDA subgroups.

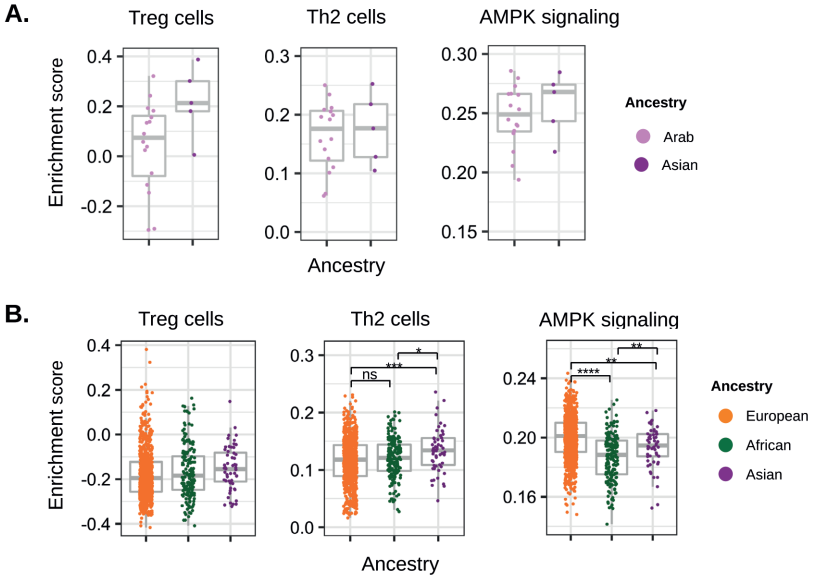


Figure 5. Enrichment of selected immune cell subpopulations and oncogenic pathways in Arab breast cancer patients. Enrichment scores for signatures for T regulatory cells (Tregs, *left*), T-helper 2 cells (Th2, *middle*), and AMPK signaling (*right*) in **A.** RA-QA cohort comparing ArA to AsA breast cancer patients, independent of molecular subtype. **B.** TCGA breast cancer cohort comparing AA, EA, and AsA breast cancer patients, independent of intrinsic molecular subtype. Box plots indicate medians and interquartile range, whiskers represent 10th and 90th percentile. All data points are plotted individually. T-test (two-sided): *p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, and ns; not significant.

Discussion

An increasing effort is expended to decipher the molecular differences that are associated with global disparities in breast cancer outcome. Several studies have investigated the presentation of breast tumors in patients of African ancestry in comparison to women of European origin. A consensus across studies is that women of African ancestry display a higher prevalence of the unfavorable triple negative breast cancer subtype and of the molecular PAM50-defined basal subtype[7–15]. We interrogated the TCGA breast cancer cohort using curated survival data, improved ancestry assignment and a refined classifier that reclassifies breast tumors into 7 subgroups using the PAM50 signature in combination with topological data analysis. Comparison of the classical PAM50 and the TDA classifier revealed that the large majority of basal tumors belong to the BasalMyo TDA subgroup, and that the reported enrichment of basal tumors in

patients of African ancestry is largely dominated by the BasalMyo subtype. Moreover, we were able to demonstrate that BasalMyo tumors are the only TDA subgroup that is associated with an ancestry-associated disparity in clinical outcome, underlining the clinical relevance of BasalMyo tumors in African patients.

In order to elucidate the underlying biological processes contributing to the worse survival of AA patients with BasalMyo tumors as compared to EA patients, we assessed transcriptomic differences in immunological parameters and cancer-cell intrinsic features. To date, only few population-based studies have considered ancestry-related changes in the immune response of breast cancer patients[22,43–45]. Overall, very few immunological differences in tumor tissues have been reported between patients of African and European ancestry[22,45]. Pitt et al reported subtle differences in tumor immune signatures when adjusting for PAM50-defined subtype[22]. They found an enrichment of the type I IFN signature in luminal A and luminal B tumors of patients of African ancestry, including African-American and Nigerian women, as compared to patients of European ancestry. A study by O'Meara *et al*. reported no significant differences in the expression of 14 immune metagenes in TNBC tumors of AA and EA patients, whereas the proportion of resting CD4+ memory cells, as determined by CIBERSORT, was significantly higher in TNBC tumors of EA patients[45]. Based on the notion that the CIBERSORT algorithm determines the relative abundance of immune cell subpopulations within a tumor rather than between tumors, we did not include CIBERSORT in our analyses. We explored ancestry-related differences in immune disposition using the ICR classifier of tumor immune phenotypes and leukocyte subgroup enrichment scores. As such, we found that the prognostic value of the ICR immune gene signature holds true across ancestries and that the lower enrichment of T regulatory and T helper 2 immune cells in patients of African ancestry negatively correlated with outcome. Although this seems a counterintuitive finding, it is important to note that the presence of immunosuppressive cells could be a result of prior immune activation. In line with this, we previously found that FoxP3 expression heavily correlates with T-cell infiltration as a counter regulatory signal and hence is an important marker of the ICR signature[85]. In addition, a number of studies have reported that increased expression of immunosuppressive gene signatures supports chemotherapy sensitivity and hence better clinical outcome in (triple negative) breast cancer[92–95].

Subsequently, we explored whether we could identify ancestry-specific enriched oncogenic pathways with prognostic relevance in BasalMyo tumors. In support of this concept, a recent transcriptome-wide association study of the Caroline Breast Cancer Study transcriptomic dataset, comprised of self-identified African American and European American women, demonstrated that ancestry-stratified predictive risk models did not perform across ancestries and/or subtype[96]. Through integrative analysis of differential enrichment and prognostic connotation we identified 7 differentially enriched signaling pathways with prognostic connotation in patients of European and/or African ancestry. Enrichment of EGF and estrogen-dependent signaling was associated with worse clinical outcome in patients of European ancestry, while enrichment of DNA repair genes correlated with better outcome. Conversely, enrichment of PI3K-Akt/PI3K-AKT-mTOR and ErbB signaling was associated with better prognosis in patients of African ancestry. Although this survival-favorable correlation appears contradictory in relation to mTOR and ErbB-mediated oncogenic signaling, recent studies have demonstrated enrichment of PI3K-AKT signaling in immunogenic TNBC tumors suggesting that hyperactivation of this signaling pathway might promote immunogenic activity and result in better prognosis[92,97,98]. This raises the question whether BasalMyo tumors enriched in PI3K and ErbB signaling could similarly infer an immune favorable tumor phenotype in a subset of AA patients. Furthermore, analysis of the individual molecules constituting the ErbB signaling pathway revealed a reduced enrichment of ErbB2, ErbB3 and ErbB4 and downstream signaling, irrespective of ancestry, in hormone receptor negative tumors and in particular BasalMyo tumors compared to hormone receptor positive

tumors (data not shown). On the other hand, hormone receptor negative tumors and BasalMyo tumors feature a higher enrichment of ErbB1/EGFR and its downstream molecules, which may be driving the overall increased enrichment of ErbB signaling in those tumors (data not shown). These findings highlight the importance of obtaining a more granular view of the changes in the ErbB pathway in BasalMyo tumors such as the relative effect of individual EGFR ligands on ErbB signaling enrichment. Notably, AMPK signaling was associated with opposing prognostic significance in EA and AA patients, with a positive connotation in the latter group. AMP-activated protein kinase or AMPK is a key regulator of cancer metabolism and oncogenic signaling, is frequently upregulated in TNBC versus non-TNBC tumors and is generally associated with poor clinicopathological factors and shorter survival[99,100]. Several lines of evidence however point towards a more complex role for AMPK in cancer whereby AMPK activation has been associated with both pro-tumorigenic and anti-tumorigenic effects depending on specific metabolic cues[101]. For example, activation of AMPK signaling has been shown to inhibit the PI3K-AKT-mTOR pathway, the expression of EGFR and cyclins, and the phosphorylation of Src, STAT3 and MAPK, culminating in reduced tumorigenic potential and better clinical outcome[102–104]. It remains to be determined if metabolic-mediated dysregulation of AMPK signaling could be regulated by ancestry-specific traits. Indeed, few studies have reported ancestral disparity in cancer metabolomics[105–107]. Our finding illustrates that metabolic pathways might be governed by different regulators depending on ancestry, and hence reiterates the need to account for ancestry in biomarker and cancer target research.

To conclude, the rapidly evolving technological landscape and refinement of cancer treatment towards precision cancer medicine has led to the recognition that breast cancer is not a single disease but should be studied and clinically managed as multiple distinct disease entities. It is now well appreciated that the complexity and heterogeneity of breast cancer arises from differences in cancer-cell intrinsic mechanisms as well as from dysregulation of the interplay with the stromal and immune microenvironment. Our findings support the notion of an additional level of complexity introduced by ancestry-associated traits and urge for more studies on underrepresented populations such as patients of Arab ancestry. Therefore, we advocate accounting for ancestry-specific molecular features in breast cancer research and in clinical decision making in order to guide precision cancer medicine.

Data availability

TCGA-BRCA cohort is available through GDC data portal (https://gdac.broadinstitute.org/) or by using TCGA-Assembler as detailed in the method section. TCGA-Assembler is open-source and freely available at http://www.compgenome.org/TCGA-Assembler/. The downloaded data product name is “illuminahiseq_rnaseqv2-RSEM_genes_normalized”.

Data pertaining to the RA-QA cohort can be downloaded via figshare: 10.6084/m9.figshare.12901928. Scripts used in this manuscript can be found on zenodoo/github : 10.5281/zenodo.3707660.

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