EVIDENCE OF ENHANCED IMMUNE ACTIVATION WITHIN THE TUMOR MICROENVIRONMENT AND THE CIRCULATION OF FEMALE PATIENTS WITH HIGH-RISK MELANOMA COMPARED TO MALES

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Background Sex differences in tumor immunity and response to immunotherapy were shown in murine models and descriptive analyses from recent clinical trials. We recently reported that female gender is a favorable prognostic marker for survival benefit following ipilimumab and high dose interferon- α (HDI) adjuvant therapy of high-risk melanoma in the ECOG-ACRIN E1609 trial (N=1670). Therefore, we investigated differences in candidate immune biomarkers in the circulation and tumor microenvironment (TME) of female and male patients.

Methods Gene expression profiling (GEP) was performed on the tumor biopsies of 718 (454 male, 264 female) patients. The primary endpoint was mRNA expression profiling using U133A 2.0 Affymetrix gene chips. Raw microarray data sets were normalized by using the Robust Multi-array Average (RMA) method using Affymetrix Power Tools (APT) as previously published. Multiple probe sets representing the same genes were collapsed by using the probe with maximum gene expression. Gene set enrichment analysis (GSEA) was performed by comparing the female and male tumor samples, and gene sets with FDR q-value <0.1 were deemed as significant. Similarly, peripheral blood (serum and PBMC) samples were tested for soluble (Luminex) and cellular (multicolor flow cytometry) prognostic biomarkers in a subset of patients (N=321; 109 female and 212 male). All patients provided an IRB-approved written informed consent.

Results Among the subset of patients tested for circulating biomarkers, females were significantly younger than males (P=0.03). Testing PBMCs, the percentages of CD3+ T cells (P=0.04) and CD3+CD4+ helper T cells (P=0.0005) were significantly higher in female patients compared to males. Also, there were trends toward higher levels of proinflammatory cytokines IL1 β (P=0.07) and IL6 (P=0.06) in females. On the other hand, males had significantly higher percentages of monocytes (P=0.03). Further, there were trends toward higher percentages of CD3+/CD4+/CD25^{hi}/Foxp3+ (P=0.1) and CD3+/CD4+/CD25+/CD127^{low}+ (P=0.1) T-reg in male patients compared to females. Among the cohort of patients (N=718) with tumor GEP data, females were significantly younger than males (P=0.0009). GEP identified pathways and genes related to immune cell infiltration and activation that were significantly enriched in the tumors of females compared to males (table 1).

Abstract 88 Table 1 Immune pathways significantly enriched in tumors of females

Pathways	P-value	FDR q-value
CIBERSORT_T_CELLS_GAMMA_DELTA	0.005199	0.11409
CIBERSORT_NK_CELLS_ACTIVATED	0.010187	0.060707
CIBERSORT_NK_CELLS_RESTING	0.011824	0.055186
CIBERSORT_DENDRITIC_CELLS_ACTIVATED	0.010638	0.046483
CIBERSORT_T_CELLS_REGULATORY_(TREGS)	0.008547	0.050286
CIBERSORT_DENDRITIC_CELLS_RESTING	0.013986	0.063591
CIBERSORT_T_CELLS_CD8	0.027027	0.082961
CIBERSORT_MACROPHAGES_M1	0.016393	0.082415
CIBERSORT_T_CELLS_CD4_NAIVE	0.029412	0.093233

Conclusions Female gender was associated with adjuvant immunotherapeutic benefits and female patients were more likely to have evidence of immune activation within the TME and the circulation, supporting a potentially important role for female related factors in the immune response against melanoma, and these require further investigation.

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Ethics Approval The E1609 study protocol was approved by the institutional review board of each participating institution and conducted in accordance with Good Clinical Practice guidelines as defined by the International Conference on Harmonization. All patients provided an IRB-approved written informed consent.

Consent Not applicable.

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