87 ENHANCED IMMUNOGENICITY WITHIN THE TUMOR MICROENVIRONMENT AND THE CIRCULATION OF HIGH-RISK MELANOMA PATIENTS WITH UNKNOWN PRIMARY COMPARED TO THOSE WHOSE PRIMARY MELANOMA IS KNOWN

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Background We recently reported data supporting the unknown primary status as a potentially distinct prognostic group among high-risk melanoma patients treated with ipilimumab and high dose interferon-alfa (HDI) in the ECOG-ACRIN E1609 trial (N=1670) with improved RFS and OS outcomes compared to known primary. Therefore, we investigated differences in candidate immune biomarkers in the circulation and tumor microenvironment (TME) of patients with unknown compared to those with known primary melanoma enrolled in this trial that tested adjuvant ipilimumab at 3 and 10 mg/kg versus HDI.

Methods Gene expression profiling (GEP) was performed on the tumor biopsies of 718 (102 unknown, 616 known primary) melanoma 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 unknown and known primary 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) immune biomarkers in a subset of patients (N=321; 66 unknown and 255 known primary). All patients provided an IRB-approved written informed consent.

Results Unknown primary melanoma cases represented 12.8% of the total E1609 study population (N=1670) including 11.7% on the ipilimumab arms and 14.7% on the HDI arm. Stratifying by stage, relapse free survival (RFS) (P=0.001) and overall survival (OS) (P=0.009) were significantly better for patients with unknown primary tumor compared to known primary. Including only ipilimumab-treated patients, RFS (P=0.005) and OS (P=0.023) were significantly better in favor of the unknown primary status. Among the cohort of patients with tumor GEP data (N=718), GEP identified pathways and genes related to autoimmunity, inflammation, immune cell infiltration and immune activation that were significantly enriched in the unknown primary tumors compared to known primaries (table 1). Among the subset of patients tested for circulating biomarkers, patients with unknown primary melanoma had significantly higher circulating levels of IL-2R than those with known primary (P=0.04).

Abstract 87 Table 1 Immune pathways enriched in unknown primary melanoma

Pathways	P-value	FDR q-value	Pathways	P-value	FDR q-value
CIBERSORT_MACROPHAGES_M1	0	0	KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	0	0
CIBERSORT_B_CELLS_MEMORY	0	0	KEGG_AUTOIMMUNE_THYROID_DISEASE	0	0
CIBERSORT_T_CELLS_GAMMA_DELTA	0	0	KEGG_ALLOGRAFT_REJECTION	0	0
CIBERSORT_B_CELLS_NAIVE	0	0	KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	0	0
CIBERSORT_PLASMA_CELLS	0	0	KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	0	1.62E-04
CIBERSORT_T_CELLS_CD8	0	0	KEGG_GRAFT_VERSUS_HOST_DISEASE	0	1.35E-04
CIBERSORT_T_CELLS_CD4_MEMORY_RESTING	0	0	KEGG_LEISHMANIA_INFECTION	0	7.42E-04
CIBERSORT_NK_CELLS_RESTING	0	0	KEGG_PRIMARY_IMMUNODEFICIENCY	0	0.001084
CIBERSORT_NK_CELLS_ACTIVATED	0	0	KEGG_TYPE_I_DIABETES_MELLITUS	0	0.00115
CIBERSORT_T_CELLS_CD4_MEMORY_ACTIVATED	0	0	KEGG_DNA_REPLICATION	0.00974	0.038615
CIBERSORT_T_CELLS_REGULATORY_(TREGS)	0	2.39E-04	KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	0	0.036395
HISTONES	0	2.19E-04	KEGG_VIRAL_MYOCARDITIS	0.003067	0.034207
CIBERSORT_T_CELLS_FOLLICULAR_HELPER	0	2.02E-04	KEGG_PANTOTHENATE_AND_COA_BIOSYNTHESIS	0.020305	0.035828
CIBERSORT_DENDRITIC_CELLS_ACTIVATED	0	3.56E-04	KEGG_PRION_DISEASES	0.008432	0.05644
CIBERSORT_MACROPHAGES_M2	0.001623	0.006049	KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	0.002963	0.053087
CIBERSORT_MHCI	0.006579	0.008082	KEGG_PROTEIN_EXPORT	0.018771	0.05286
CIBERSORT_MONOCYTES	0	0.008912	KEGG_ASTHMA	0.022337	0.06327
CIBERSORT_DENDRITIC_CELLS_RESTING	0.001681	0.010999	KEGG_STARCH_AND_SUCROSE_METABOLISM	0.016129	0.077571
CIBERSORT_MACROPHAGES_M0	0.028716	0.03623	KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	0.014151	0.077391
			KEGG_CELL_ADHESION_MOLECULES_CAMS	0.035294	0.126863

Conclusions Unknown primary high-risk melanoma patients had significantly better prognosis and evidence of significantly enhanced immune activation within the TME and the circulation, supporting the designation of unknown primary melanoma as a distinct prognostic marker in patients with high-risk melanoma.

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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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