

## **Supplementary Appendix**

### **Improved prognosis and evidence of enhanced immunogenicity in tumor and circulation of high-risk melanoma patients with unknown primary**

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Table S1. Five-year relapse free survival (RFS) and overall survival (OS) rates (%) and 95% confidence intervals by primary tumor status and stage group among all study patients (S1A), patients treated with ipilimumab (S1B) and patients treated with high dose interferon- $\alpha$  (S1C).

| Table S1A<br>(All patients) |                | Known primary | Unknown primary | Combined    |
|-----------------------------|----------------|---------------|-----------------|-------------|
| <b>RFS</b>                  | <b>IIIB</b>    | 50 (46, 54)   | 57 (40, 72)     | 51 (48, 55) |
|                             | <b>IIIC</b>    | 36 (32, 40)   | 49 (36, 60)     | 38 (34, 42) |
|                             | <b>M1a/M1b</b> | 39 (28, 51)   | 59 (41, 73)     | 46 (37, 55) |
|                             | <b>Total</b>   | 44 (41, 47)   | 56 (48, 64)     | 46 (43, 48) |
| <b>OS</b>                   | <b>IIIB</b>    | 72 (68, 75)   | 82 (70, 88)     | 74 (71, 77) |
|                             | <b>IIIC</b>    | 64 (59, 68)   | 69 (57, 79)     | 64 (60, 68) |
|                             | <b>M1a/M1b</b> | 69 (56, 78)   | 83 (67, 92)     | 73 (64, 80) |
|                             | <b>Total</b>   | 69 (66, 77)   | 76 (69, 82)     | 70 (67, 72) |
| Table S1B<br>(Ipilimumab)   |                | Known primary | Unknown primary | Combined    |
| <b>RFS</b>                  | <b>IIIB</b>    | 51 (46, 56)   | 70 (54, 81)     | 53 (48, 57) |
|                             | <b>IIIC</b>    | 41 (36, 46)   | 51 (35, 66)     | 42 (37, 47) |
|                             | <b>M1a/M1b</b> | 35 (22, 49)   | 53 (30, 72)     | 41 (29, 52) |
|                             | <b>Total</b>   | 46 (43, 50)   | 57 (45, 67)     | 48 (44, 51) |
| <b>OS</b>                   | <b>IIIB</b>    | 72 (69, 77)   | 85 (70, 93)     | 73 (69, 77) |
|                             | <b>IIIC</b>    | 67 (62, 72)   | 72 (55, 84)     | 68 (63, 72) |
|                             | <b>M1a/M1b</b> | 67 (62, 72)   | 76 (52, 89)     | 73 (61, 82) |
|                             | <b>Total</b>   | 70 (67, 73)   | 81 (73, 87)     | 70 (68, 74) |
| Table S1C                   |                | Known primary | Unknown primary | Combined    |

| (Interferon- $\alpha$ ) |                |             |             |             |
|-------------------------|----------------|-------------|-------------|-------------|
| <b>RFS</b>              | <b>IIIB</b>    | 48 (41, 55) | 57 (35, 73) | 49 (43, 55) |
|                         | <b>IIIC</b>    | 28 (21, 35) | 45 (26, 62) | 30 (24, 37) |
|                         | <b>M1a/M1b</b> | 48 (28, 66) | 67 (38, 85) | 49 (43, 55) |
|                         | <b>Total</b>   | 39 (34, 44) | 55 (42, 64) | 42 (37, 46) |
| <b>OS</b>               | <b>IIIB</b>    | 72 (65, 77) | 75 (55, 87) | 72 (66, 77) |
|                         | <b>IIIC</b>    | 56 (49, 64) | 65 (45, 79) | 58 (51, 65) |
|                         | <b>M1a/M1b</b> | 70 (47, 85) | 99 (61, 99) | 79 (63, 89) |
|                         | <b>Total</b>   | 66 (61, 70) | 75 (63, 84) | 67 (63, 71) |

**Table S2.** Tumor infiltrating lymphocytes cell types estimated by CIBERSORT signatures found to be significantly enriched in unknown primary melanomas as compared to known primary as computed by gene set enrichment analysis (GSEA) (NES: Normalized enrichment score).

| <b>CIBERSORT CELL TYPES</b>  | <b>NES</b> | <b>NOM p-val</b> | <b>FDR q-val</b> |
|------------------------------|------------|------------------|------------------|
| MACROPHAGES M1               | 2.48       | 0.0000           | 0.0000           |
| B CELLS MEMORY               | 2.35       | 0.0000           | 0.0000           |
| T CELLS GAMMA DELTA          | 2.34       | 0.0000           | 0.0000           |
| B CELLS NAIVE                | 2.26       | 0.0000           | 0.0000           |
| PLASMA CELLS                 | 2.20       | 0.0000           | 0.0000           |
| T CELLS CD8                  | 2.19       | 0.0000           | 0.0000           |
| T CELLS CD4 MEMORY RESTING   | 2.12       | 0.0000           | 0.0000           |
| NK CELLS RESTING             | 2.08       | 0.0000           | 0.0000           |
| NK CELLS ACTIVATED           | 2.06       | 0.0000           | 0.0000           |
| T CELLS CD4 MEMORY ACTIVATED | 2.04       | 0.0000           | 0.0000           |
| T CELLS REGULATORY (TREGS)   | 1.96       | 0.0000           | 0.0002           |
| T CELLS FOLLICULAR HELPER    | 1.93       | 0.0000           | 0.0002           |
| DENDRITIC CELLS ACTIVATED    | 1.90       | 0.0000           | 0.0004           |

|                         |      |        |        |
|-------------------------|------|--------|--------|
| MACROPHAGES M2          | 1.72 | 0.0016 | 0.0060 |
| MHC-I                   | 1.68 | 0.0066 | 0.0081 |
| MONOCYTES               | 1.67 | 0.0000 | 0.0089 |
| DENDRITIC CELLS RESTING | 1.65 | 0.0017 | 0.0110 |
| MACROPHAGES M0          | 1.52 | 0.0287 | 0.0362 |

## SUPPLEMENTAL FIGURE LEGEND

**Figure S1.** E1609 Consolidated Standards of Reporting Trials diagram (adult patient population). <sup>a</sup>E1609 included a pediatric component (ages 12–17 years) consisting of three separate cohorts randomized to the three treatment regimens and analyzed separately for safety per study protocol. Total pediatric accrual was three subjects; <sup>b</sup>these overlap with but are not limited to treatment-related grade 5 events previously reported; <sup>c</sup>concurrently randomized cases. HDI, high-dose interferon alpha-2b; lpi3, ipilimumab 3 mg/kg; lpi10, ipilimumab 10 mg/kg; ITT, Intent to Treat.