**A Phase II trial of Ipilimumab with Carboplatin/Paclitaxel in Unresectable Stage III or Stage IV Melanoma reveals peripheral and local immune signature**

Jamal et al.

Supplemental Tables

Supplemental Tables

Table S1. Demographic and Baseline Characteristics of the Patients Seperated by Arm (Number (percent))

|  |  |  |  |
| --- | --- | --- | --- |
| *Patient Characteristics* |  | *Arm A* | *Arm B* |
| **Total no. of patients** |  | **10 (33)** | **20 (67)** |
| **Median age, years (range)** |  | **55 (43-67)** | **52.5 (26-74)** |
|  |  |  |  |
| **Sex** |  |  |  |
| Female |  | **2 (20)** | **6 (30)** |
| Male |  | **8 (80)** | **14 (70)** |
|  |  |  |  |
| **Metastatic stage (n)** |  |  |  |
| M0 |  | **0** | **1 (5)** |
| M1a |  | **2 (20)** | **4 (20)** |
| M1b |  | **5 (50)** | **1 (5)** |
| M1c |  | **3 (30)** | **14 (70)** |
|  |  |  |  |
| **Lactate dehydrogenase** |  |  |  |
| ≤ ULN\* |  | **7 (70)** | **13 (65)** |
| > ULN |  | **3 (30)** | **7 (35)** |
|  |  |  |  |
| **ECOG** |  |  |  |
| 0 |  | **6 (60)** | **13 (65)** |
| 1 |  | **4 (40)** | **7 (35)** |
|  |  |  |  |
| **Primary site** |  |  |  |
| Cutaneous |  | **10 (100)** | **14 (70)** |
| Mucosal |  | **0** | **2 (10)** |
| Ocular  |  | **0** | **3 (15)** |
| Unknown primary |  | **0** | **1 (5)** |
|  |  |  |  |
| **BRAF status** |  |  |  |
|  BRAF mutated (V600E) |  | **3 (30)** | **6 (30)** |
|  BRAF wild type |  | **7 (70)** | **14 (70)** |
|  |  |  |  |
| **Prior therapies\*\*** |  |  |  |
|  Prior adjuvant therapy |  | **4 (40)** | **1 (5)** |
|  Prior therapy with a BRAF inhibitor |  | **3 (30)** | **3 (15)** |
|  |  |  |  |
| **Brain metastases** |  |  |  |
|  Patients without brain metastases |  | **7 (70)** | **19 (95)** |
|  Patients with brain metastases |  | **3 (30)** | **1 (5)** |
|  |  |  |  |
| \* ULN denotes upper limit of the normal |  |  |  |
| Table S2. Adverse Events |  |

|  |  |  |
| --- | --- | --- |
|   | **ARM A (n=10)** | **ARM B (n=20)** |
|   | **Total** | **Grade3/4/5** | **Total** | **Grade3/4/5** |
| **All adverse events** |  |  |  |  |
| Any event | 10 (100) | 6 (60) | 20 (100) | 15 (75) |
| Hepatotoxicity |  |  |  |  |
|  high ALT | 2 (20) | 1 (10) | 2 (10) | 1 (5) |
| Gastrointestinal Disorders |  |  |  |  |
|  nausea | 4 (40) | 0 | 17 (85) | 1 (5) |
|  vomiting | 3 (30) | 0 | 11 (55) | 0 |
|  diarrhea | 7 (70) | 0 | 16 (80) | 3 (15) |
|  *C.difficile* colitis | 1 (10) | 1 (10) | 0 | 0 |
| Electrolyte |  |  |  |  |
|  hypophosphatemia | 1 (10) | 0 | 3 (15) | 2 (10) |
|  hypokalemia | 2 (20) | 0 | 4 (20) | 1 (5) |
|  hypomagnesemia | 2 (20) | 0 | 6 (80) | 1 (5) |
| Hematological |  |  |  |  |
|  anemia | 2 (20) | 0 | 2 (10) | 2 (10) |
|  febrile neutropenia | 0 | 0 | 2 (10) | 2 (10) |
|  neutropenia | 3 (30) | 2 (20) | 5 (25) | 3 (15) |
|  thrombocytopenia | 2 (20) | 1 (10) | 4 (20) | 2 (10) |
| Nervous System |  |  |  |  |
|  seizure | 0 | 0 | 2 (10) | 1 (5) |
|  vasovagal reaction | 0 | 0 | 1 (5) | 1 (5) |
| Infection |  |  |  |  |
|  pneumonia | 1 (10) | 1 (10) | 0 | 0 |
| Constitutional |  |  |  |  |
|  fatigue | 6 (60) | 1 (10) | 11 (55) | 1 (5) |
| Vascular Disorder |  |  |  |  |
|  pulmonary embolism | 0 | 0 | 1 (5) | 1 (5) |
|  |  |  |  |  |
| **Immune-related adverse events** |  |  |  |  |
| Any event | 8 (80) | 1 (10) | 18 (90) | 3 (15) |
| Gastrointestinal Disorders |  |  |  |  |
|  diarrhea | 3 (30) | 0 | 8 (40) | 2 (10) |
|  vomiting | 2 (10) | 0 | 4 (20) | 0 |
|  autoimmune colitis | 0 | 0 | 1 (5) | 1 (5) |
| Endocrine |  |  |  |  |
|  hypothyroidism | 1 (10) | 0 | 0 | 0 |
|  pituitary disorder | 0 | 0 | 1 (5) | 0 |
| Skin Disorders |  |  |  |  |
|  rash | 2 (20) | 0 | 5 (25) | 0 |
|  pruritus  | 3 (30) | 0 | 6 (30) | 0 |
|  urticaria | 0 | 0 | 1 (5) | 0 |
|  vitiligo | 0 | 0 | 1 (5) | 0 |
|  erythroderma | 1 (10) | 0 | 0 | 0 |
| Constitutional |  |  |  |  |
|  fatigue | 3 (30) | 1 (10) | 8 (40) | 0 |
|  |  |  |  |  |

Table S3. Tumor Response (by irRC, mWHO and Arm)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|   | All patients | Arm A | Arm B |   |
|   | n=30 | n=10 | n=20 | A *vs.* B |
| Response | No. of pts (%) | No. of pts (%) | No. of pts (%) | P-value |
|   |   |   |   |   |
| **irBOR** |   |   |   |   |
|  irCR | 1 | 0 | 1 |   |
|  irPR | 7 | 2 | 5 |   |
|  irSD | 9 | 5 | 4 |   |
|  irPD | 13 (43) | 3 (30) | 10 (50) | 0.18 |
|  irDCR  | 17 (57) |  7(70) | 10 (50) | 0.43 |
|  irCBR | 13 (43) | 6 (60) | 7 (35) | 0.23 |
|  irBORR  |  8 (27) | 2 (20) | 6 (30) | 0.29 |
|   |   |   |   |   |
| **mWHO-BOR** |   |   |   |   |
|  mWHO-CR | 0 | 0 | 0 |   |
|  mWHO-PR | 4 | 2 | 2 |   |
|  mWHO-SD | 7 | 4 | 3 |  |
|  mWHO-PD | 19 (63) | 4 (40) | 15 (75) | 0.11 |
|  mWHO-DCR  | 11 (37) | 6 (60) | 5 (25) | 0.09 |
|  mWHO-CBR  | 8 (27) | 6 (60) | 2 (10) | 0.01 |
|  mWHO-BORR | 4 (13) | 2 (20) | 2 (10) | 0.15 |
|   |   |   |   |   |
| *Abbreviations: BOR, best overall response; ; ir, immune-related; mWHO, modified WHO; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; BORR, best overall response rate; DCR, disease control rate (CR+PR+SD); CBR, clinical benefit rate (CR+PR+SD≥24 weeks)* |

## Table S4. PD-L1 expression in the two Best Overall Response groups



No correlation between the percentage of PD-L1 positive melanoma cells and BOR (Pearson Chi-Square 1.257, 2-sided Fisher Exact Test 0.334)

## Table S5. Univariate Cox regression models

|  |  |  |
| --- | --- | --- |
| Variables | P value | HR (95% CI) |
| PD-L1 | 0.920 | 0.998 (0.954 – 1.044) |
| CCL3 | 0.035 | 14.144 (1.211 – 165.249) |
| CCL4 | 0.001 | 5.941 (2.062 – 17.120) |
| CXCL8 | 0.236 | 1.866 (0.665 – 5.235) |
| Bm2 | 0.007 | 0.113 (0.023 – 0.549) |
| eBm5+Bm5 | 0.027 | 10.268 (1.302 – 80.959) |
| Pre PD1+/CD8+ | 0.057 | 1.792 (0.983 – 3.268) |
| W10 PD1+/CD8+ | 0.002 | 1.444 (1.141 – 1.827) |
| W13 PD1+/CD8+ | 0.001 | 1.528 (1.178 – 1.982) |
| W24 PD1+/CD8+ | 0.007 | 2.518 (1.291 – 4.911) |
| Pre CD25+/CD4+ Teff | 0.581 | 1.045 (0.893 – 1.223) |
| W10 PD-1+/CD4+ | 0.743 | 1.029 (0.867 – 1.221) |
| Pre Treg/CD4+ | 0.363 | 1.267 (0.761 – 2.108) |
| W10 ICOS+/CD4+ | 0.214 | 1.070 (0.962 – 1.119) |
| W10 ICOS+/CD8+ | 0.438 | 1.048 (0.932 – 1.178) |

**Cox regression models: significance p<0.05 and hazard ratio (HR) are indicated. CI : Confidence Interval**

## Table S6. Antibodies for characterization of circulating immune cells

|  |  |  |  |
| --- | --- | --- | --- |
| Antibodies | Fluorochromes | Antibodies | Fluorochromes |
| CD3 | FITC | CD45RA | FITC |
| CD3 | Alexa Fluor 700 | CD56 | FITC |
| CD4 | FITC | CD62L | PE-CF594 |
| CD4 | V450 | CD66b | PE |
| CD8 | APC-H7 | CD69 | PerCPCy5.5 |
| CD11c | APC-H7 | CD80 | APC-H7 |
| CD11c | V450 | CD86 | Alexa Fluor 700 |
| CD14 | FITC | CD127 | PE-CF594 |
| CD14 | PerCPCy5.5 | CD152 | APC |
| CD16 | PE-CF594 | CD206 | FITC |
| CD19 | PE | CD274 | PE-Cy7 |
| CD19 | PerCPCy5.5 | CD278 | PE |
| CD20 | APC-H7 | CD279 | PE |
| CD23 | APC-H7 | CCR7 | PE-Cy7 |
| CD24 | PE-Cy7 | sIgD | PE-CF594 |
| CD25 | APC | HLA-DR | PE-Cy7 |
| CD25 | PerCPCy5.5 | FoxP3 | V450 |
| CD27 | Alexa Fluor 700 | IFN-γ | PE-CF594 |
| CD33 | APC | Granzym B | FITC |
| CD38 | PE | IL-4 | APC |
| CD43 | APC | IL-17A | PE |

## Table S7. Peripheral soluble cytokines/chemokines/soluble receptors studied by multiplex.

|  |  |  |
| --- | --- | --- |
| Cytokines | Chemokines | TNF Family |
| IL-1β IL-2 | CXCL8 | TNF-α |
| IL-4 IL-5 IL-6 | CXCL9 | TNF-RIs |
| IL-7 IL-10 IL-12p70 | CXCL10 | TNF-RIIs |
| IL-13 IL-17 | CCL3 |  |
| IFN-γ | CCL4 |  |
| GM-CSF | CCL5 |  |
|  | VEGF |  |