**Supplementary Tables**

**Table S1.** **Antibodies used in flow cytometry experiments.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibody** | **Fluorochrome** | **Clone** | **Dilution** | **Source** |
| Fixable viability dye | eFluor™ 450 |  | 1:500 | Thermo |
|  | eFluor™ 506 |  | 1:500 | Thermo |
| Anti-mouse CD11b | eFluor™ 450 | M1/70 | 1:400 | Thermo |
| Anti-mouse CD8α | APC | 53-6.7 | 1:400 | Thermo |
|  | APC/Cy7 | 53-6.7 | 1:400 | Thermo |
|  | Alexa FluorTM 488 | 53-6.7 | 1:100 | Thermo |
| Anti-mouse CD69 | APC | H1.2F3 | 1:200 | Thermo |
| Anti-mouse IFNγ | PE/Cy7 | XMG1.2 | 1:100 | Thermo |
| Anti-mouse CD62L | APC | MEL-14 | 1:200 | Thermo |
| Anti-mouse TCR Vβ5.1/5.2 | APC | MR9-4 | 1:200 | Thermo |
| Anti-mouse CCR7 | PE/Cy7 | 4B12 | 1:50 | Thermo |
| Anti-mouse CD183 (CXCR3) | FITC | CXCR3-173 | 1:100 | Thermo |
| Anti-mouse CD366 (TIM3) | PE/Cy7 | RMT3-23 | 1:200 | Thermo |
| Anti-mouse CD62L | Brilliant VioletTM 711 | MEL-14 | 1:200 | Biolegend |
| Anti-mouse CD45 | APC/Cy7 | 30-F11 | 1:200 | Biolegend |
|  | PerCP | 30-F11 | 1:200 | Biolegend |
| Anti-mouse CD4 | PerCP/Cy5.5 | RM4-5 | 1:400 | Biolegend |
|  | APC/Cy7 | RM4-5 | 1:400 | Biolegend |
| Anti-human/mouse Granzyme B | Alexa FluorTM 647 | GB11 | 1:100 | Biolegend |
| Anti-mouse CD44 | Brilliant Violet TM 510 | IM7 | 1:500 | Biolegend |
|  | PerCP/Cy5.5 | IM7 | 1:300 | Biolegend |
| Anti-mouse TCR Vα2 | PerCP/Cy5.5 | B20.1 | 1:200 | Biolegend |
| Anti-mouse CD279 (PD-1) | PE | 29F.1A12 | 1:300 | Biolegend |
| Anti-mouse TCRβ | Brilliant Violet TM 421 | H57-597 | 1:300 | BD Biosciences |

**Table S2.** **Probes used for quantitative RT-PCR analyses.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Gene** | **Exon location** | **Assay ID** |
| Mouse | *Hprt* | Exon 2-3 | Mm.PT.58.32092191  |
| Mouse | *Plxna4* | Exon 2-3 | Mm.PT.58.8104978 |
|  |  | Exon 19-20 | Mm.PT.58.42494501 |
| Mouse | *Sema6a* | Exon 7-8 | Mm.PT.58.8787256  |
| Mouse | *Sema6b* | Exon 7-9 | Mm.PT.58.9406966  |
| Mouse | *Sema3a* | Exon 17-18 | Mm.PT.58.11314988  |
| Human | *TBP* | Exon 1-2 | Hs.PT.58v.39858774 |
| Human | *PLXNA1* | Exon 20-21 | Hs.PT.58.4352083 |
| Human | *PLXNA2* | Exon 22-23 | Hs.PT.58.39698368 |
| Human | *PLXNA3* | Exon 32-33 | Hs.PT.58.3100864 |
| Human | *PLXNA4* | Exon 29-30 | Hs.PT.58.4195119 |

**Table S3. Clinicopathologic features of melanoma cancer patients and cancer-free controls. Related to Figure 6.**

|  |  |  |
| --- | --- | --- |
| **Clinicopathologic features** | **Cases****n (%)** | **Controls****n (%)** |
| **Demographics** |  |  |
| Number of cases | 31 (100) | 16 (100) |
| Number of males | 15 (48) | 10 (62) |
| Number of females | 16 (52) | 6 (38) |
| Age (years), mean ± SD | 65,8 ± 11,8 | 53,4 ± 8,3 |
| **Tumor subtype** |  |  |
| Nodular | 11 (36) |  |
| Superficial spreading | 12 (39) |  |
| Desmoplastic | 1 (3) |  |
| Acral lentigenous | 1 (3) |  |
| Spitzoid | 1 (3) |  |
| Unknown | 5 (16) |  |
| **Tumor stage** |  |  |
| IIIb | 3 (10) |  |
| IIIc | 10 (32) |  |
| IIId | 1 (3) |  |
| IV | 17 (55) |  |
| **BRAF mutation status** |  |  |
| WT | 9 (29) |  |
| V600E | 13 (42) |  |
| non-V600E | 3 (10) |  |
| multiple | 2 (6) |  |
| N/A | 4 (13) |  |
| **NRAS mutation status** |  |  |
| WT  | 16 (52) |  |
| MT | 9 (29) |  |
| N/A | 6 (19) |  |
| **NF1 mutation status** |  |  |
| WT | 23 (74) |  |
| MT | 3(10) |  |
| N/A | 5 (16) |  |
| **Paired blood samples (before and after 1 treatment cycle)** |  |  |
| Number of cases | 20 (100) |  |
| **Treatment** |  |  |
| Nivolumab | 4 (20) |  |
| Pembrolizumab | 11 (55) |  |
| Nivolumab + Ipilimumab | 5 (25) |  |
| **Treatment setting** |  |  |
| Palliative (unresectable disease) | 14 (70) |  |
| Curative (resectable disease) | 6 (30) |  |
| **Response evaluation** |  |  |
| Responders | 7 (35) |  |
| Non-responders | 11 (55) |  |
| Unknown | 2 (10) |  |

**Table S4. Inclusion and exclusion criteria for the study of metastatic melanoma patients. Related to Figure S6.**

|  |  |
| --- | --- |
| **Inclusion criteria**  | **Exclusion criteria** |
| Signed informed consent and age >18 years at the time of signing informed consent form. | Pregnancy, breast feeding or intention to become pregnant during the study. Women of childbearing potential must have a negative serum pregnancy test result within 7 days prior to initiation of the study. |
| Active Belgian health insurance covering in-patient and out-patient treatment. | History of known intolerance to anti-PD-1/anti-PD-L1 blocking agents. |
| Histologically confirmed stage IV metastatic or stage III unresectable and resectable malignant melanoma according to AJCC classification 8th edition. | Inability to comply with the study protocol or study related procedures. |
| Treatment naïve to anti-PD-1 or anti-PD-L1 ICIs used in the setting of metastatic malignant melanoma. Adjuvant treatment with anti-PD-1 or anti-PD-L1 ICIs is permitted if patients relapse >6 months after the last dose of ICI. | Patient must not have received previous anti-PD-1 or anti-PD-L1 therapy for metastatic or unresectable melanoma. Patients who had received prior anti-PD-1 therapy in the adjuvant setting are eligible if remained recurrence free for 1 year after stopping adjuvant treatment. |
| Measurable disease according to RECIST v1.1.  | Patients with ocular melanoma. |
| ECOG performance status 0, 1 or 2. | Patients with an active infection requiring antibiotic, antimycotic or antiviral therapy within 4 weeks prior to starting the study. |
| Patients must provide a blood sample at baseline and on treatment. Blood samples during treatment will be taken 3 weeks after the first infusion, the pre-treatment sample will be taken during the screening period. Blood samples will not be taken in the event of withdrawn consent, termination of the study by the sponsor, the patient becoming ineligible for the study or if the patient deceases. | Presence of absolute contraindications for the application of anti-PD-1/anti-PD-L1 blocking agents including but not restricted to autoimmune disease which are likely to become aggravated during therapy or where aggravation would pose a significant health risk. Previous immune-related AE’s due to anti-CTLA-4 antibodies do not pose an exclusion unless aggravation or relapse of the immune-related AE’s would outweigh the potential therapeutic benefit of the anti-PD-1/anti-PD-L1 blocking agent.  |
| Patient has an hematological status as defined by :* Hemoglobin >8g/dL (transfusion allowed)
* Neutrophil count >1000/ml
* Platelet count >75.000 x 109/L
 | Patients may not have lab abnormalities with regard to kidney, bone marrow, liver function as well as electrolyte abnormalities and endocrine abnormalities that would pose a contraindication to apply anti-PD-1/anti-PD-L1 therapy. Results of routine lab tests should be in line with common clinical practice applied for patients receiving immune checkpoint inhibitors. |
| Patient has an adequate liver and kidney function allowing participation in the study and safe administration of an ICI according to the judgment of the treating physician. | Treatment with systemic immunosuppressive agents including but not limited to prednisone, cyclophosphamide, azathioprine, methotrexate, thalidomide and anti-TNF alpha agents within 2 weeks prior to study start. Patients receiving mineralocorticoids, corticosteroids for COPD or low doses of corticosteroids (<10mg of methylprednisone or equivalent) are allowed to participate. |
| Patient has no medical or psychiatric condition that would form a contra-indication to take a blood sample and to treat the patient with an ICI according to the judgment of the treating physician. | Patients having received a live vaccine within 30 days of planned study entry. |
| Patients, both males and females, of childbearing/reproductive potential must agree to use adequate contraception while participating in the study. | Patients with a history of severe bleeding or a condition that would pose a risk factor for bleeding |
|  | Patients who are positive for HIV |
|  | Other cancers than malignant melanoma within the past 5 years before study entry except cancers known to pose a low risk of metastasis including but not limited to adequately treated squamous cell carcinoma of the skin, basocellular carcinoma, cervical cancer, low risk prostate cancer. |
|  | Patients receiving concomitant anti-cancer therapy. Exceptions are patients who receive irradiation of tumor lesions for palliation of symptoms. Lesions subjected to irradiation during the study, or which have been irradiated before entering the study may not be chosen as target lesions unless documented disease progression occurs in a formerly irradiated area.  |

Abbreviations: RESIST, response evaluation criteria in solid tumors; ECOG, Eastern Cooperative Oncology Group; AE, adverse event; COPD, Chronic Obstructive Pulmonary Disease.

**Table S5. Clinicopathologic features of cancer patients and cancer-free controls for monocyte isolation. Related to Figure S6.**

|  |  |  |
| --- | --- | --- |
| **Clinicopathologic features** | **Cases****n (%)** | **Controls****n (%)** |
| **Demographics** |  |  |
| Number of cases | 24 (100) | 13 (100) |
| Number of males | 17 (71) | 7 (54) |
| Number of females | 7 (29) | 6 (46) |
| Age (years), mean ± SD |  65,7 ± 6,6 | 53,7 ± 7,8 |
| **Cancer type** |  |  |
| Colorectal  | 17 (71) |  |
| Colon  | 2 (9) |  |
| Esophageal | 1 (4) |  |
| Breast | 1 (4) |  |
| Head and neck | 1 (4) |  |
| Pancreatic | 1 (4) |  |
| Ovarian | 1 (4) |  |

**Table S6.** **Hematological parameters in WT and *Plxna4* KO mice. Related to Figure 1.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Cell Type** | **WT** | ***Plxna4* KO** | ***p*-value** |
| **WBC (k/µL)** | 3.63 ± 0.24 | 3.44 ± 0.25 | 0.59 |
| **Neu (k/µL)** | 0.21 ± 0.02 | 0.19 ± 0.02 | 0.64 |
| **Lym (k/µL)** | 3.23 ± 0.23 | 3.03 ± 0.24 | 0.54 |
| **Mon (×102/µL)** | 0.67 ± 0.10 | 0.75 ± 0.13 | 0.65 |
| **Eos (×102/µL)** | 0.81 ± 0.07 | 0.98 ± 0.10 | 0.17 |
| **Bas (×101/µL)** | 0.38 ± 0.13 | 0.15 ± 0.08 | 0.10 |
| **RBC (M/µL)** | 4.43 ± 0.05 | 4.46 ± 0.05 | 0.72 |

Data corresponds to 20 mice per condition. Abbreviations: WBC, white blood cells; Neu, neutrophils; Lym, lymphocytes; Mon, monocytes; Eos, eosinophils; Bas, basophils and RBC, red blood cells. Data show mean ± SEM.

**Table S7. Hematological parameters in WT🡪WT and *Plxna4* KO🡪WT mice. Related to Figure 1.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Cell Type** | **WT🡪WT**  | ***Plxna4* KO 🡪 WT** | ***p*-value** |
| **WBC (k/µL)** | 9.42 ± 0.65 | 9.65 ± 0.84 | 0.83 |
| **Neu (k/µL)** | 0.40 ± 0.03 | 0.41 ± 0.06 | 0.87 |
| **Lym (k/µL)** | 8.84 ± 0.60 | 8.73 ± 0.75 | 0.80 |
| **Mon (×102/µL)** | 0.11 ± 0.01 | 0.11 ± 0.02 | 0.98 |
| **Eos (×102/µL)** | 0.18 ± 0.02 | 0.22 ± 0.04 | 0.44 |
| **Bas (×101/µL)** | 0.39 ± 0.03 | 0.32 ± 0.04 | 0.23 |
| **RBC (M/µL)** | 8.21 ± 0.74 | 9.23 ± 0.15 | 0.20 |

Data corresponds to 12 mice per condition. Abbreviations: WBC, white blood cells; Neu, neutrophils; Lym, lymphocytes; Mon, monocytes; Eos, eosinophils; Bas, basophils and RBC, red blood cells. Data show mean ± SEM.