**Supplementary Table S1.Clinical pathological parameters of enrolled patients**

|  |  |  |
| --- | --- | --- |
| **Clinicopathologic factor** | **training cohort** | **validation cohort** |
| **Age (year)** |  |  |
| Median | 52 | 51 |
| **Gender n (%)** |  |  |
| Male | 13 (41.9) | 35 (41.2) |
| Female | 18 (58.0) | 50 (58.8) |
| **Ulceration n (%)** |  |  |
| Yes | 9 (29.0) | 25 (29.4) |
| No | 8 (25.8) | 22 (25.9) |
| NA | 16 (51.6) | 38 (44.7) |
| **Thickness (mm)** |  |  |
| Median | 4.8 | 5 |
| **TNM Stages n (%)** |  |  |
| III | 2 (6.5) | 8 (9.4) |
| IV | 29 (93.5) | 77 (90.6) |
| **M Stages n (%)** |  |  |
| M0 | 2 (6.5) | 8 (9.4) |
| M1 | 29 (93.5) | 77 (90.6) |
| **Subtype n (%)** |  |  |
| Acral subtype | 17 (54.8) | 32 (37.6) |
| Mucosal subtype | 0 (0) | 13 (15.3) |
| CSD subtype | 7 (22.6) | 17 (20.0) |
| Non-CSD subtype | 4 (12.9) | 10 (11.7) |
| Unknown primary subtype | 3 (9.7) | 13 (15.3) |
| **LDH n (%)** |  |  |
| LDH normal | 21 (67.7) | 55 (64.7) |
| LDH>ULN | 10 (32.3) | 30 (35.3) |
| **clinical benefit (%)** |  |  |
| CR | 1 (3.2) | 0 (0) |
| PR | 4 (12.9) | 17 (20) |
| SD | 15 (48.4) | 27 (31.8) |
| PD | 11 (35.5) | 41 (48.2) |

CSD: chronic sun-induced damage; Non-CSD: non chronic sun-induced damage; Upper limit of normal (ULN) for LDH serum level is 250 U/L.

**Supplementary Table S2. The sample information of QuantiGenePlex RNA assay**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample code** | **Sample type** | **Age of tissue** | **Prep technique** | **Location of biopsy** |
| T-1 | tumor biopsies | 51 | formalin soak | right foot subcutaneous metastase |
| T-2 | tumor biopsies | 65 | formalin soak | right calf subcutaneous metastase |
| T-3 | tumor biopsies | 59 | formalin soak | left foot subcutaneous metastase |
| T-4 | tumor biopsies | 36 | formalin soak | scalp subcutaneous metastase |
| T-5 | tumor biopsies | 51 | formalin soak | right foot subcutaneous metastase |
| T-6 | tumor biopsies | 39 | formalin soak | right calf subcutaneous metastase |
| T-7 | tumor biopsies | 45 | formalin soak | left foot subcutaneous metastase |
| T-8 | tumor biopsies | 49 | formalin soak | right inguinal lymph node metastasis |
| T-9 | tumor biopsies | 53 | formalin soak | vaginal recurrence |
| T-10 | tumor biopsies | 36 | formalin soak | left calf subcutaneous metastase |
| T-11 | tumor biopsies | 60 | formalin soak | left inguinal lymph node metastasis |
| T-12 | tumor biopsies | 52 | formalin soak | right axillary lymph node metastasis |
| T-13 | tumor biopsies | 45 | formalin soak | right foot subcutaneous metastase |
| T-14 | tumor biopsies | 55 | formalin soak | right foot subcutaneous metastase |
| T-15 | tumor biopsies | 53 | formalin soak | left ear recurrence |
| T-16 | tumor biopsies | 52 | formalin soak | left chest wall recurrence |
| T-17 | tumor biopsies | 42 | formalin soak | right foot subcutaneous metastase |
| T-18 | tumor biopsies | 40 | formalin soak | left inguinal lymph node metastasis |
| T-19 | tumor biopsies | 67 | formalin soak | left foot subcutaneous metastase |
| T-20 | tumor biopsies | 48 | formalin soak | right inguinal lymph node metastasis |
| T-21 | tumor biopsies | 63 | formalin soak | right back subcutaneous metastase |
| T-22 | tumor biopsies | 53 | formalin soak | right knee subcutaneous metastase |
| T-23 | tumor biopsies | 52 | formalin soak | left foot subcutaneous metastase |
| T-24 | tumor biopsies | 53 | formalin soak | right inguinal lymph node metastasis |
| T-25 | tumor biopsies | 54 | formalin soak | left foot subcutaneous metastase |
| T-26 | tumor biopsies | 51 | formalin soak | right inguinal lymph node metastasis |
| T-27 | tumor biopsies | 47 | formalin soak | left foot subcutaneous metastase |
| T-28 | tumor biopsies | 60 | formalin soak | right upper arm subcutaneous metastase |
| T-29 | tumor biopsies | 58 | formalin soak | left waist subcutaneous metastase |
| T-30 | tumor biopsies | 31 | formalin soak | left eyebrow |
| T-31 | tumor biopsies | 20 | formalin soak | right ankle |
| T-32 | tumor biopsies | 28 | formalin soak | left hand subcutaneous metastase |
| T-33 | tumor biopsies | 57 | formalin soak | left nasal cavity |
| T-34 | tumor biopsies | 45 | formalin soak | left hand subcutaneous metastase |
| F-1 | FFPE tumor tissues | 49 | RNAlater soak | left foot |
| F-2 | FFPE tumor tissues | 30 | RNAlater soak | right inguinal lymph node metastasis |
| F-3 | FFPE tumor tissues | 53 | RNAlater soak | nasal cavity |
| F-4 | FFPE tumor tissues | 48 | RNAlater soak | scalp |
| F-5 | FFPE tumor tissues | 60 | RNAlater soak | left foot |
| F-6 | FFPE tumor tissues | 69 | RNAlater soak | right calf |
| F-7 | FFPE tumor tissues | 59 | RNAlater soak | left foot |
| F-8 | FFPE tumor tissues | 57 | RNAlater soak | right calf |
| F-9 | FFPE tumor tissues | 51 | RNAlater soak | right foot |
| F-10 | FFPE tumor tissues | 59 | RNAlater soak | right nasal cavity |
| F-11 | FFPE tumor tissues | 42 | RNAlater soak | scalp |
| F-12 | FFPE tumor tissues | 67 | RNAlater soak | left forearm |
| F-13 | FFPE tumor tissues | 38 | RNAlater soak | meninges |
| F-14 | FFPE tumor tissues | 55 | RNAlater soak | left inguinal lymph node metastasis |
| F-15 | FFPE tumor tissues | 55 | RNAlater soak | left foot |
| F-16 | FFPE tumor tissues | 48 | RNAlater soak | left inguinal lymph node metastasis |
| F-17 | FFPE tumor tissues | 57 | RNAlater soak | scalp |
| F-18 | FFPE tumor tissues | 63 | RNAlater soak | left inguinal lymph node metastasis |
| F-19 | FFPE tumor tissues | 42 | RNAlater soak | right inguinal lymph node metastasis |
| F-20 | FFPE tumor tissues | 53 | RNAlater soak | right inguinal lymph node metastasis |
| F-21 | FFPE tumor tissues | 51 | RNAlater soak | left inguinal lymph node metastasis |
| F-22 | FFPE tumor tissues | 48 | RNAlater soak | anal |
| F-23 | FFPE tumor tissues | 54 | RNAlater soak | right foot |
| F-24 | FFPE tumor tissues | 46 | RNAlater soak | left hip |
| F-25 | FFPE tumor tissues | 49 | RNAlater soak | gum |
| F-26 | FFPE tumor tissues | 68 | RNAlater soak | left hand |
| F-27 | FFPE tumor tissues | 48 | RNAlater soak | right hip |
| F-28 | FFPE tumor tissues | 53 | RNAlater soak | rectum |
| F-29 | FFPE tumor tissues | 27 | RNAlater soak | left foot |
| F-30 | FFPE tumor tissues | 41 | RNAlater soak | left foot |
| F-31 | FFPE tumor tissues | 52 | RNAlater soak | vaginal |
| F-32 | FFPE tumor tissues | 52 | RNAlater soak | left foot |
| F-33 | FFPE tumor tissues | 51 | RNAlater soak | right inguinal lymph node metastasis |
| F-34 | FFPE tumor tissues | 64 | RNAlater soak | right foot |
| F-35 | FFPE tumor tissues | 65 | RNAlater soak | right foot |
| F-36 | FFPE tumor tissues | 45 | RNAlater soak | left axillary lymph node metastasis |
| F-37 | FFPE tumor tissues | 32 | RNAlater soak | vulva |
| F-38 | FFPE tumor tissues | 52 | RNAlater soak | left foot |
| F-39 | FFPE tumor tissues | 63 | RNAlater soak | chest |
| F-40 | FFPE tumor tissues | 62 | RNAlater soak | left foot |
| F-41 | FFPE tumor tissues | 45 | RNAlater soak | left waist |
| F-42 | FFPE tumor tissues | 43 | RNAlater soak | back |

**Supplementary Table S3. Correlation of CDK4 pathway aberrations withPD-L1 protein expression in melanoma patients.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **PD-L1 expression** | | |
|  | **PD-L1 positive** | **PD-L1 negative** | ***P***a |
| **Clinical benefit** |  |  |  |
| CB | 11 | 19 | 0.024 |
| NCB | 6 | 37 |  |
| ***CDK4* Aberration** |  |  |  |
| Gain | 10 | 38 | 0.492 |
| Normal | 7 | 18 |  |
| ***CCND1* Aberration** |  |  |  |
| Gain | 9 | 30 | 0.964 |
| Normal | 3 | 12 |  |
| loss | 5 | 14 |  |
| ***CDKN2A* Aberration** |  |  |  |
| loss | 10 | 34 | 0.889 |
| Normal | 7 | 22 |  |

*P*a for the X2 test or Fisher exact test was used for evaluating the clinical benefit and CDK4 pathway aberrations. CB: clinical benefit; NCB: no clinical benefit

**Supplementary Figure S1. The flow chart describing the total population of the study.**

**Supplementary Figure S2. The PD-1 staining of C57BL/6-hPD-1 mice.** Mouse PD-1 staining with APC anti mouse CD279(PD-1). Human PD-1 staining with JS001(10 µg/mL) and mouse anti human IgG4 Fc PE.

**Supplementary Figure S3. CDK4/6 inhibitor treatment positively regulates PD-L1 protein expression and influences immune gene expression.**

(A) Palbociclib treatment increases PD-L1 protein levels in the SK-MEL-5 cell line, AMC-3 cell line, C57BL/6-hPD-1 and HIS PDX murine models. (B) GSEA was performed on RNA-seq from palbociclib treatment melanoma samples in SK-MEL-5 cell line, AMC-3 cell line and C57BL/6-hPD-1 murine model. Enrichment plots show differential expression of IL6/JAK/STAT3 signaling pathway gene set.

1. **Bioinformatics analysis of WES results**

The raw sequencing data were subjected to data quality control firstly, removing reads containing an adaptor or poly-N, and low-quality reads. Clean reads in FASTQ format were aligned to human reference genome (hg19) by Burrows-Wheeler Aligner (BWA, v0.7.13). Samtools (v1.3) and Picard (v2.2.4) were used to filter duplicates for the aligned BAM files. GATK were used to do local realignment and base quality recalibration to generate final BAM files for computation of the sequencing coverage and depth. Somatic variants were detected by MuTect2. After filtering, the locus with a minimum of 329.7M reads covering in the tumor and minimum of 78723 variant allele reads, as well as 102M reads in the corresponding normal sample and maximum of 82080 variant allele reads were regarded as variants.

Variants were annotated the variants call format (VCF) using ANNOVAR software against multiple databases including HGVS variant description, population frequency（1000G, ExAC, dbSNP）, disease or phenotype(OMIM, COSMIC, ClinVar) and variant functional prediction(PolyPhen-2, SIFT). Somatic CNVs was identified by Control-FREEC. The GISTIC was used to infer recurrently amplified or deleted genomic regions. HLA typing was performed using HLA-VBSeq.

1. **Data analysis for gene expression based on RNA-Seq**

Raw sequencing reads were filtered with seqtk before mapping to the human GRCh38 reference genome using Hisat2 (v2.0.4). Differentially expressed genes between tumor and normal tissues were identified using edgeR. The P-value significance threshold in multiple tests was set based on the false discovery rate (FDR). Differentially expressed genes were selected using the following filter criteria: FDR ≤0.05 and fold-change ≥2.

GSEA (Gene set enrichment analysis, v3.0) was performed to identify differential expressed pathways among different groups. Hallmark gene sets (h.all.v6.2.symbols.gmt) downloaded from Molecular Signatures Database (MsigDB, http://software.broadinstitute.org/gsea/index.jsp) were chosen for grouping. Both significant and non-significant genes were taken into account for enrichment analysis. Gene markers were ranked using Signal2Noise. P-values and false discovery rates (FDR) were calculated and gene sets with p-value < 0.05 and FDR< 0.25 were considered as significantly regulated.

1. **QuantiGenePlex RNA assay (panel)**

Tissue homogenates were prepared according to the procedure described in the user manual of QuantiGene Sample Processing Kit for FFPE Tissues (Panomics). Briefly, 5-8 pieces of deparaffinized sections (4-10 μm each) were incubated with 150 μl homogenizing solution supplemented with 1.5 μl of proteinase K (50 μg/μl) at 65°C for 6 h. The tissue homogenate was separated from debris by brief centrifugation and then transferred to a new tube.

Briefly, the sample (40 μl) of each assay well was denatured in each well of the hybridization plate containing working bead mix. The hybridization plate was sealed and incubated at 54°C ± 1°C in a shaking incubator at 600 rpm for 18- 22 h. The next day, the unbound material was removed using the bio-plex pro II wash station (Bio-Rad), and the beads were sequentially hybridized with pre-amplifier, amplifier, label probe and SAPE. Fluorescence intensities were measured by the bio-plex 100 system (Bio-Rad).

For each sample, the average signal (MFI) was determined for all genes. The background values were subtracted from each probe set signal, and values of test genes were normalized to the geometric means of *Gapdh and ACTB*.

1. **DNA preparation and TaqMan copy number assays**

Genomic DNA was extracted from FFPE sections using a QIAamp DNA FFPE Tissue Kit (Qiagen). Quantitative real-time PCR was performed using the ABI 7500 FAST real-time PCR system (Applied Biosystems). Copy numbers were then quantified with CopyCaller v2.0 software (Applied Biosystems) using the comparative Ct (DDCt) method. A gain in copy number for CDK4 or CCND1 was determined when the relative copy number was greater than 3.0; a loss in copy number for CDKN2A was considered to have occurred when the relative copy number was less than 2.0.

1. **Immunofluorescence (IF)**

IF staining was performed using humanized immune system (HIS) patient-derived xenograft (PDX) models. Briefly, mouse tumor samples were prepared as FFPE sections. Tissue sections were mounted on slides with DAPI-containing Vectashield mounting medium (Vector Laboratories) and examined and photographed under an inverted three-color fluorescence microscope system (Nikon Ti-U). Images were analyzed and quantified using ImageJ software (NIH).

1. **NanoString-based gene expression profiling**

Total RNA was isolated from FFPE tumor samples using the RNeasy FFPE Kit (73504, Qiagen) according to the manufacturer’s protocol. The RNA concentration and purity were estimated using a NanoDrop 2000 spectrophotometer (Thermo Scientific). NanoString Technologies-based gene expression profiling was performed using 300 ng of total RNA from each sample according to the manufacturer’s instructions. The mRNA hybridization, detection and scanning were performed following the nanoString protocol. Normalization of raw data was performed using nSolver software (NanoString Technologies) based on the 10 most relevant housekeeping genes.

1. **Cell lines and primary cell culture**

A2058 (catalog no. CRL-11147), SK-MEL-5 (catalog no. HTB-70), WM-266-4 (catalog no. CRL-1676), B16-OVA cell lines were a gift from Shanghai Junshi Biosciences. Above cell lines were cultured at 37°C under 5% CO2 in Dulbecco’s Modified Eagle Medium (DMEM, Invitrogen) supplemented with 10% FBS, 100 UI/ml penicillin, and 100 μg/ml streptomycin.

AMC-3 (acral primary melanoma cell) cell lines were derived from a patient-derived xenograft (PDX) model. The tumor tissue was minced into 1-mm3 fragments and resuspended in 30 ml of DMEM containing 50× collagenase IV (Invitrogen) and 1× DNase (Takara, Kusatsu, Japan). After a 2-h incubation at 37°C, the suspensions were collected and slowly transferred onto 15 ml of Histopaque (Sigma, St. Louis, MO). The interface cell fraction was collected after centrifugation, and the cells were maintained in serum-free stem cell medium supplemented with growth factors at 37°C in 5% CO2.