Supplementary Information for

**Collapse of the Plasmacytoid Dendritic Cells compartment in advanced cutaneous melanomas by components of the tumor cell secretome**

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**Running title:** Plasmacytoid dendritic cells and melanoma

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**Supplementary Tables**

Supplementary Table S1 details on the Clinical data of the PCM cohort

Supplementary Table S2 details on the List of the antibodies

Supplementary Table S3 details on the Clinical data of the MM cohort

Supplementary Table S4 illustrates the Molecular profile of Melanoma Cells Lines

Supplementary Table S5 details on the Correlation of PDC and LK density and prognostic features of PCM

Supplementary Table S6 illustrates the Genomic landscape of 50 selected PCM

Supplementary Table S7 details on the Clinical data of SNL

Supplementary Table S8 details on Clinical data of the melanoma metastasis cohort.

Supplementary Table S9 illustrates the Differential expression of Gene signatures and prognosis in different sites of disease

Supplementary Table S10 details on the CK-R expression in MM patients

Supplementary Table S11 details on the CK-R expression in HD

Supplementary Table S12 illustrates the Correlation between absolute number of immune cells and stages in MM patients

**Supplementary Table S1. Clinical data of the PCM (superficial spreading melanomas) CH1 cohort.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient** | **Gender** | **Age** | **PDC/mm²** | **LK/mm²** | **PDC fraction** | **Breslow (mm)** | **Mitosis (n/mm² )** | **Regression** | **Ulceration** | ***Stage*** | **D/A** | **Follow up** | **Relapse** | **Time to relapse** | **Anatomical sites** |
| #1 | M | 55 | 169,90 | 2414,22 | 7,04 | 1,00 | 5 | Present | Absent | IB | Alive | 93,00 | 0 |  | Trunk |
| #2 | F | 71 | 8,75 | 3026,55 | 0,29 | 0,15 | 0 | Present | Absent | IA | Alive | 60,00 | 0 |  | Trunk |
| #3 | M | 31 | 6,00 | 1875,62 | 0,32 | 1,00 | 0 | Present | Absent | IA | Alive | 107,00 | 0 |  | Trunk |
| #4 | M | 23 | 90,60 | 2189,38 | 4,14 | 0,60 | 0 | Present | Absent | IA | Alive | 95,00 | 0 |  | Arm |
| #5 | M | 61 | 128,00 | 2244,63 | 5,70 | 1,10 | 0 | Present | Absent | IB | Alive | 81,00 | 0 |  | Trunk |
| #6 | F | 67 | 11,05 | 743,47 | 1,49 | 1,00 | 3 | Absent | Absent | IB | Alive | 59,00 | 0 |  | Leg |
| #7 | F | 74 | 7,53 | 946,86 | 0,80 | 1,40 | 1 | Absent | Absent | IB | Alive | 72,00 | 0 |  | Arm |
| #8 | M | 70 | 0,51 | 1064,34 | 0,05 | 1,75 | 2 | Absent | Absent | IB | Alive | 74,00 | 0 |  | Leg |
| #9 | F | 73 | 1,38 | 436,81 | 0,32 | 1,10 | 6 | Absent | Absent | IB | Alive | 72,00 | 1 | NA | Leg |
| #10 | F | 56 | 0,00 | 13,47 | 0,00 | 1,90 | 2 | Absent | Absent | IB | Alive | 71,00 | 0 |  | Leg |
| #11 | M | 69 | 11,12 | 2061,82 | 0,54 | 0,40 | 0 | Absent | Absent | IV | Dead | 22,00 | 1 | 3,00 | Leg |
| #12 | F | 76 | 6,43 | 3751,44 | 0,17 | 1,70 | 3 | Absent | Absent | IB | Alive | 7,00 | 0 |  | Arm |
| #13 | M | 59 | 11,25 | 628,88 | 1,79 | 1,20 | 0 | Present | Absent | IB | Alive | 60,00 | 0 |  | Trunk |
| #14 | F | 43 | 92,81 | 2489,09 | 3,73 | 0,90 | 3 | Absent | Absent | IB | Alive | 59,00 | 0 |  | Trunk |
| #15 | F | 55 | 10,67 | 1276,25 | 0,84 | 0,90 | 1 | Absent | Absent | IB | Alive | 43,00 | 0 |  | Leg |
| #16 | M | 46 | 118,99 | 4078,50 | 2,92 | 1,69 | 4 | Absent | Present | IIA | Alive | 69,00 | 0 |  | Trunk |
| #17 | F | 22 | 3,01 | NV | NV | 1,40 | 9 | Absent | Absent | IB | Alive | 65,00 | 0 |  | Arm |
| #18 | M | 63 | 0,00 | 1690,48 | 0,00 | 0,90 | 0 | Present | Absent | IA | Alive | 52,00 | 0 |  | Trunk |
| #19 | F | 61 | 80,31 | 4504,18 | 1,78 | 1,00 | 1 | Present | Absent | IB | Alive | 56,00 | 0 |  | Leg |
| #20 | M | 65 | 89,96 | 685,22 | 13,13 | 1,45 | 4 | Absent | Absent | IB | Alive | 2,00 | 0 |  | Trunk |
| #21 | F | 42 | 21,56 | 665,43 | 3,24 | 1,20 | 2 | Absent | Absent | IB | Alive | 54,00 | 0 |  | Leg |
| #22 | F | 51 | 6,16 | 909,40 | 0,68 | 1,20 | 10 | Absent | Absent | IB | Alive | 59,00 | 0 |  | Leg |
| #23 | M | 45 | 34,66 | 2769,47 | 1,25 | 1,90 | 2 | Absent | Absent | IB | Alive | 44,00 | 0 |  | Leg |
| #24 | M | 38 | 366,79 | 2382,50 | 15,40 | 0,80 | 1 | Absent | Absent | IB | Alive | 56,00 | 0 |  | Trunk |
| #25 | F | 69 | 18,26 | 245,04 | 7,45 | 1,50 | 3 | Absent | Absent | IB | Alive | 48,00 | 0 |  | Leg |
| #26 | M | 32 | 5,19 | 1559,31 | 0,33 | 0,40 | 0 | Absent | Absent | IA | Alive | 51,00 | 0 |  | Trunk |
| #27 | F | 65 | 1,41 | 290,47 | 0,48 | 0,60 | 0 | Absent | Absent | IA | Alive | 37,00 | 0 |  | Leg |
| #28 | F | 51 | 40,92 | 1141,07 | 3,58 | 1,00 | 2 | Absent | Absent | IB | Alive | 35,00 | 0 |  | Trunk |
| #29 | F | 63 | 256,34 | 3163,71 | 8,12 | 0,70 | 0 | Absent | Absent | IA | Alive | 45,00 | 0 |  | Leg |
| #30 | M | 69 | 20,62 | 778,42 | 2,65 | 1,35 | 1 | Absent | Absent | IB | Alive | 15,00 | 0 |  | Leg |
| #31 | M | 58 | 160,46 | 2811,74 | 5,71 | 1,35 | 2 | Absent | Absent | IB | Alive | 41,00 | 1 | 1,00 | Trunk |
| #32 | F | 80 | 57,74 | 1619,69 | 3,56 | 1,10 | 7 | Present | Absent | IB | Alive | 10,00 | 0 |  | Leg |
| #33 | M | 63 | 361,90 | 6471,49 | 5,60 | 2,00 | 5 | Present | Present | IIA | Dead | 17,00 | 1 | 5,00 | Trunk |
| #34 | M | 25 | 62,35 | 1429,46 | 4,35 | 0,95 | 0 | Absent | Absent | IA | Alive | 42,00 | 0 |  | Trunk |
| #35 | F | 48 | 3,04 | 3087,39 | 0,10 | 0,60 | 2 | Absent | Absent | IB | Alive | 15,00 | 0 |  | Leg |
| #36 | F | 34 | 27,37 | 512,80 | 5,33 | 0,76 | 2 | Absent | Absent | IB | Alive | 23,00 | 0 |  | Leg |
| #37 | F | 48 | 13,69 | 1763,49 | 0,78 | 0,60 | 1 | Present | Absent | IB | Alive | 30,00 | 0 |  | Trunk |
| #38 | M | 69 | 11,30 | 3005,86 | 0,38 | 1,00 | 3 | Absent | Absent | IB | Alive | 26,00 | 0 |  | Trunk |
| #39 | M | 36 | 104,31 | 2891,58 | 3,61 | 1,20 | 1 | Absent | Absent | IB | Alive | 30,00 | 0 |  | Trunk |
| #40 | M | 41 | 286,11 | 3746,38 | 7,64 | 1,60 | 1 | Present | Absent | IB | NA | 29,00 | 1 |  | Arm |
| #41 | F | 46 | 6,03 | 1300,27 | 0,46 | 1,20 | 0 | Absent | Absent | IB | Alive | 36,00 | 0 |  | Leg |
| #42 | M | 71 | 16,28 | 1568,92 | 1,04 | 0,90 | 2 | Absent | Absent | IB | Alive | 23,00 | 0 |  | Arm |
| #43 | F | 22 | 44,28 | 5712,35 | 0,78 | 0,80 | 1 | Absent | Absent | IB | Alive | 18,00 | 0 |  | Leg |
| #44 | M | 69 | 0,95 | 236,32 | 0,40 | 0,50 | 0 | Absent | Absent | IA | Alive | 36,00 | 0 |  | Trunk |
| #45 | M | 40 | 0,00 | 2225,35 | 0,00 | 0,90 | 2 | Present | Absent | IB | Alive | 27,00 | 0 |  | Arm |
| #46 | F | 48 | 18,35 | 676,80 | 2,71 | 1,10 | 2 | Absent | Absent | IB | Alive | 31,00 | 0 |  | Leg |
| #47 | F | 68 | 12,11 | 1828,57 | 0,66 | 1,80 | 4 | Absent | Absent | IB | Alive | 28,00 | 0 |  | Trunk |
| #48 | M | 37 | 47,86 | 1387,42 | 3,45 | 1,20 | 0 | Absent | Absent | IB | Alive | 18,00 | 0 |  | Arm |
| #49 | F | 45 | 9,13 | 1698,17 | 0,54 | 0,45 | 1 | Present | Absent | IB | Alive | 27,00 | 0 |  | Leg |
| #50 | F | 50 | 79,11 | 621,08 | 12,75 | 1,04 | 1 | Absent | Absent | IB | Alive | 23,00 | 0 |  | Leg |
| #51 | M | 74 | 32,82 | 2458,86 | 1,33 | 0,80 | 0 | Present | Absent | IA | Dead | 39,00 | 1 | 31,00 | Trunk |
| #52 | F | 66 | 0,84 | 105,34 | 0,80 | 1,00 | 1 | Absent | Absent | IIIB | NA | NA | NA | NA | NA |
| #53 | M | 68 | 312,21 | 2939,94 | 10,62 | 1,90 | 2 | Absent | Absent | IIIA | Alive | 92,00 | 0 |  | Arm |
| #54 | F | 28 | 76,43 | 2345,92 | 3,25 | 1,00 | 0 | Present | Absent | IIIA | Alive | 93,00 | 0 |  | Trunk |
| #55 | F | 79 | 6,84 | 461,85 | 1,48 | 0,97 | 1 | Absent | Absent | IIIB | Dead | 58,00 | 1 | 3,00 | Trunk |
| #56 | F | 27 | 37,88 | 289,41 | 13,09 | 1,40 | 0 | Absent | Absent | IV | Dead | 88,00 | 1 | 76,00 | Head |
| #57 | M | 21 | 40,79 | 2803,23 | 1,46 | 1,30 | 3 | Absent | Absent | IIIA | Alive | 101,00 | 0 |  | Trunk |
| #58 | F | 69 | 0,27 | 558,45 | 0,05 | 1,00 | 0 | Absent | Absent | IIIA | NA | NA | NA | NA | NA |
| #59 | F | 45 | 289,71 | 6248,27 | 4,64 | 0,87 | 0 | Absent | Absent | IIIA | Alive | 78,00 | 0 |  | Trunk |
| #60 | F | 47 | 134,13 | 4741,65 | 2,83 | 1,30 | 0 | Absent | Absent | IIIA | Alive | 78,00 | 0 |  | Trunk |
| #61 | M | 47 | 37,33 | 1643,88 | 2,27 | 1,40 | 8 | Absent | Absent | IIIA | Alive | 67,00 | 0 |  | Leg |
| #62 | F | 30 | 51,88 | 1402,80 | 3,70 | 1,20 | 5 | Absent | Present | IIIB | Alive | 50,00 | 0 |  | Leg |
| #63 | F | 33 | 1,07 | 1033,24 | 0,10 | 0,80 | 0 | Absent | Absent | IIIA | Alive | 48,00 | 0 |  | Arm |
| #64 | F | 66 | 88,33 | 2122,36 | 4,16 | 0,70 | 0 | Present | Absent | IIIA | Alive | 29,00 | 0 |  | Trunk |
| #65 | F | 42 | 124,13 | 997,82 | 12,43 | 1,10 | 0 | Absent | Absent | IIIA | Alive | 25,00 | 0 |  | Trunk |
| #66 | F | 48 | 106,73 | 2320,25 | 4,60 | 1,10 | 2 | Absent | Absent | IIIA | Alive | 28,00 | 0 |  | Trunk |
| #67 | M | 47 | 5,21 | 483,44 | 1,08 | 0,55 | 2 | Absent | Absent | IIIB | Alive | 22,00 | 0 |  | Trunk |
| #68 | F | 40 | 98,80 | 2926,36 | 3,38 | 1,80 | 14 | Absent | Present | IIIB | Alive | 27,00 | 0 |  | Arm |
| #69 | M | 27 | 15,59 | 1821,56 | 0,86 | 1,80 | 2 | Present | Absent | IIIA | Alive | 30,00 | 0 |  | Arm |
| #70 | F | 42 | 225,27 | 1671,17 | 13,48 | 0,80 | 1 | Absent | Absent | IIIB | Alive | 16,00 | 0 |  | Leg |
| #71 | F | 59 | 6,54 | 393,47 | 1,66 | 0,90 | 0 | Absent | Absent | IIIA | Dead | 85,00 | 1 | 9,00 | Trunk |
| #72 | F | 74 | 14,59 | 501,91 | 2,91 | 2,2 | 8 | Absent | Absent | IIA | Alive | 14,00 | 0 |  | Leg |
| #73 | F | 52 | 41,74 | 1971,52 | 2,12 | 13,5 | 11 | Absent | Present | IV | NA | 20,00 | 0 | 19,00 | Arm |
| #74 | F | 34 | 152,35 | 1677,52 | 9,08 | 2,1 | 2 | Absent | Absent | IIA | Alive | 17,00 | 0 |  | Trunk |
| #75 | F | 52 | 35,85 | 671,40 | 5,34 | 3,4 | NV | Absent | Absent | IIA | Alive | 32,00 | 0 |  | Arm |
| #76 | F | 87 | 10,75 | 1901,03 | 0,57 | 5 | 6 | Absent | Present | IIC | Alive | 47,00 | 0 |  | Leg |
| #77 | M | 73 | 0,25 | 67,36 | 0,38 | 2,9 | 4 | Absent | Absent | IIIA | Dead | 18,00 | 1 | 2,00 | Arm |
| #78 | F | 83 | 6,29 | 2209,99 | 0,28 | 12 | 6 | Present | Absent | IV | Dead | 21,00 | 1 | 4,00 | Arm |
| #79 | M | 30 | 8,93 | 271,28 | 3,29 | 5,4 | 10 | Absent | Absent | IV | Dead | 9,00 | 1 | 0,00 | Trunk |
| #80 | F | 69 | 4,62 | 228,40 | 2,02 | 5 | 9 | Absent | Present | IIIB | Alive | 79,00 | 0 | 69.00 | Leg |
| #81 | M | 61 | 124,15 | 2668,05 | 4,65 | 2,1 | 2 | Absent | Absent | IIA | Alive | 19,00 | 0 |  | Arm |
| #82 | F | 74 | 0,19 | 2318,38 | 0,01 | 2,3 | 6 | Present | Present | IIB | Alive | 8,00 | 0 |  | Leg |
| #83 | F | 63 | 35,81 | 2114,64 | 1,69 | 2,3 | 10 | Absent | Absent | IV | Dead | 28,00 | 1 | 5,00 | Arm |
| #84 | F | 49 | 39,14 | 2468,15 | 1,59 | 2,3 | 5 | Absent | Absent | IIA | Alive | 22,00 | 0 |  | Leg |
| #85 | M | 69 | 67,21 | 837,42 | 8,03 | 2,2 | 5 | Absent | Absent | IV | NA | 41,00 | 0 | 24,00 | Arm |
| #86 | F | 69 | 3,89 | 530,86 | 0,73 | 2,2 | 5 | Absent | Absent | IIIA | Alive | 12,00 | 0 |  | Leg |
| #87 | M | 74 | 9,84 | 1300,01 | 0,76 | 2,1 | 1 | Absent | Absent | IIA | Alive | 26,00 | 0 |  | Leg |
| #88 | M | 74 | 1,62 | 38,77 | 4,17 | 2,1 | 3 | Absent | Absent | IIA | Dead | 21,00 | 1 |  | Leg |
| #89 | M | 26 | 22,90 | 437,75 | 5,23 | 3,4 | 12 | Absent | Present | IV | Dead | 4,00 | 1 | 0,00 | Arm |
| #90 | F | 59 | 19,10 | 644,95 | 2,96 | 2,2 | 10 | Present | Absent | IIA | Alive | 11,00 | 0 |  | Trunk |
| #91 | F | 83 | 0,31 | 126,88 | 0,24 | 2,4 | 10 | Absent | Present | IV | Alive | 42,00 | 0 | 5,00 | Leg |
| #92 | M | 43 | 11,67 | 1170,31 | 1,00 | 2,6 | 3 | Absent | Absent | IIA | Alive | 26,00 | 0 |  | Arm |
| #93 | M | 57 | 0,05 | 5095,48 | 0,00 | 2,9 | 6 | Absent | Absent | IV | NA | 34,00 | 0 | 18,00 | Leg |
| #94 | F | 77 | 0,00 | 492,41 | 0,00 | 2,1 | 1 | Present | Absent | IIA | Alive | 25,00 | 0 |  | Leg |
| #95 | F | 34 | 28,22 | 309,21 | 9,12 | 2,1 | 1 | Absent | Absent | IIIA | Alive | 39,00 | 0 |  | Leg |
| #96 | M | 20 | 10,91 | 766,02 | 1,42 | 2,6 | 3 | Present | Absent | IIA | Alive | 21,00 | 0 |  | Trunk |
| #97 | F | 32 | 19,57 | 3953,18 | 0,50 | 3,3 | 2 | Present | Absent | IIA | Alive | 25,00 | 1 |  | Leg |
| #98 | M | 45 | 14,67 | 2126,06 | 0,69 | 2,4 | 10 | Absent | Absent | IV | Alive | 57,00 | 0 | 27,00 | Trunk |
| #99 | F | 54 | 35,26 | 1195,20 | 2,95 | 2,1 | 6 | Absent | Absent | IIIA | Alive | 53,00 | 0 |  | Trunk |
| #100 | M | 75 | 39,49 | 1738,72 | 2,27 | 2,2 | 7 | Present | Present | IIB | Alive | 30,00 | 0 |  | Trunk |
| #101 | M | 40 | 16,07 | 1624,64 | 0,99 | 2,5 | 10 | Absent | Present | IV | Dead | 30,00 | 0 | 9,00 | Trunk |

**Supplementary Table S2. List of the antibodies used for IHC and FC**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reagent | Clone | Diluition | Source | | Catalog number |
| Primary antibodies used in IHC |  |  |  | |  |
| CD303 | 124B3.13 | 1:75 | Dendritics, Lyon, France | | DDX0043 |
| CD45 Rp | X16/19 | 1:200 | Leica Biosystem, Nussloch, Germany | | NCL-L-LCA |
| Melan-A/ MART-1 | A103 | 1:50 | Dako, Glostrip, Denmark | | M7196 |
| CD123 | 7G3 | 1:50 | BD Pharmingen, New Jersey, USA | | 554527 |
| CD2AP | B-4 | 1:1000 | Santa Cruz, Dallas, Texas | | sc-25272 |
|  |  |  |  | |  |
| MITF | D5 | 1:50 | Dako, Glostrip, Denmark | | M3621 |
| BRAF V600E | VE-1 | 1:30 | Courtesy of Prof.Andreas von Deimling | |  |
| Caspase 3  FOXP3  E2.2/ TCF4 | Polyclonal rabbit  FJK-16s  6A | 1:1800  1:200  1:500 | R&D Systems, Minneapolis, USA  Thermo Fisher Scientific, Waltham, USA  AbCam, Cambridge, UK | | AF835  4-5773-80  ab217668 |
| Reagent | **Clone** | **Conjugation** | **Source** | | **Catalog number** |
| Panel #1 Identification of mature DC and CKR by flow cytometry  PRIMARY ANTIBODIES |  |  |  | |  |
| CD303 | AC144 | FITC or APC | | Miltenyi Biotec, Bergisch Gladbach, Germany | 130-090-510 or 130-113-190 |
| CD123 | AC145 | VioBlue | | Miltenyi Biotec, Bergisch Gladbach, Germany | 130-113-329 |
| CD16 | 3G8 | PE | | Becton Dickson, New Jersey, USA | 555407 |
| CD8 | HIT8a | PerCP Cy5.5 | | Becton Dickson, New Jersey, USA | 555636 |
| CD3 | UCHT1 | PE Cy7.7 | | Becton Dickson, New Jersey, USA | 563423 |
| CD45RA | 2D1 | APC H7 | | Becton Dickson, New Jersey, USA | 557833 |
| CD4 | RPA-T4 | V450 | | Becton Dickson, New Jersey, USA | 560345 |
| CD1c | F10/21A3 | PE | | Becton Dickson, New Jersey, USA | 564900 |
| CD19 | HIB19 | FITC | | Becton Dickson, New Jersey, USA | 555412 |
| CD14 | M5E2 | FITC | | Becton Dickson, New Jersey, USA | 555397 |
| CCR5  CCR6  CCR7  CXCR3  CXCR4  Panel #2 Identification of DC progenitors by flow cytometry  PRIMARY ANTIBODIES | J418F1  REA190  REA546  REA232  12G5 | APC Cy7.7  PE  APC  PE Vio-770  PerCP Cy5.5 | | BioLegend, SanDiego, CA, USA  Miltenyi Biotec, Bergisch Gladbach, Germany  Miltenyi Biotec, Bergisch Gladbach, Germany  Miltenyi Biotec, Bergisch Gladbach, Germany  BioLegend, SanDiego, CA, USA | 359110  130-100-375  130-108-286  130-101-381  306516 |
| CD135 | 4G8 | Brilliant Violet 451 | | Becton Dickson, New Jersey, USA | 566256 |
| CD117 | 104D2 | Brilliant Violet 510 | | BioLegend, SanDiego, CA, USA | 313220 |
| CD1c | AD5-E7 | FITC | | Miltenyi Biotec, Bergisch Gladbach, Germany | 130-113-301 |
| CD141 | AD5-14H12 | FITC | | Miltenyi Biotec, Bergisch Gladbach, Germany | 130-113-317 |
| CD66b | REA306 | FITC | | Miltenyi Biotec, Bergisch Gladbach, Germany | 130-104-395 |
| CD335 | 9E2 | Vio-Bright | | Miltenyi Biotec, Bergisch Gladbach, Germany | 130-104-518 |
| CD303 | AC144 | FITC | | Miltenyi Biotec, Bergisch Gladbach, Germany | 130-090-510 |
| CD34 | 8G12 | PE | | Becton Dickson, New Jersey, USA | 348057 |
| CD123 | 7G3 | PerCP Cy5.5 | | Becton Dickson, New Jersey, USA | 560904 |
| CD115 | 9-4D2-1E4 | PE Cy7 | | BioLegend, SanDiego, CA, USA | 347307 |
| CD116 | REA211 | APC | | Miltenyi Biotec, Bergisch Gladbach, Germany | 130-100-984 |
| CD45RA | 2D1 | APC H7 | | Becton Dickson, New Jersey, USA | 557833 |

**Supplementary Table S3. Clinical data of the MM CH3 cohort used for the peripheral blood analysis of PDC and MDC.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Patient*** | ***Age at the diagnosis of MM*** | ***AJCC (7th Edition) Staging*** | ***NRAS*** | ***BRAF*** |
| 1 | 63 | M1c | Q61R | wt |
| 2 | 53 | M1c | wt | wt |
| 3 | 45 | M1c | wt | V600E |
| 4 | 50 | M1b | wt | V600E |
| 5 | 43 | M1b | wt | V600E |
| 6 | 63 | M1a | wt | V600K |
| 7 | 49 | M1c | wt | V600E |
| 8 | 67 | M1c | Q61R | wt |
| 9 | 63 | M1b | wt | V600E |
| 10 | 76 | M1c | Q61K | wt |
| 11 | 64 | M1c | wt | wt |
| 12 | 32 | M1c | wt | V600E |
| 13 | 58 | M1c | Q61K | wt |
| 14 | 50 | M1b | Q61R | wt |
| 15 | 60 | M1a | wt | wt |
| 16 | 67 | M1c | wt | wt |
| 17 | 54 | M1a | Q61R | wt |
| 18 | 48 | M1c | Q61R | wt |
| 19 | 62 | M1c | wt | V600K |
| 20 | 79 | M1b | wt | wt |
| 21 | 60 | M1b | wt | V600E |
| 22 | 66 | M1b | wt | V600K |
| 23 | 57 | M1c | wt | V600E |
| 24 | 23 | M1c | wt | V600E |
| 25 | 43 | M1a | wt | V600E |
| 26 | 61 | M1b | wt | V600E |
| 27 | 61 | M1b | wt | wt |
| 28 | 77 | M1a | wt | V600E |
| 29 | 69 | M1c | wt | V600K |

**Supplementary Table S4. Molecular profile of Melanoma Cells Lines**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Mutation** | **Mel 146** | **Mel 252** | **Mel 327** | **Mel 336** | **Mel 346** |
| **APHA 10** | wt | wt | wt | wt | wt |
| **AKT3** | wt | wt | wt | wt | wt |
| **ARID 2** | wt | wt | wt | P38L | P941T |
| **BRAF** | V600E | wt | wt | V600K | wt |
| **CDK4** | wt | wt | wt | wt | wt |
| **CXCR4** | wt | wt | wt | wt | wt |
| **CTNNBB1** | wt | wt | wt | wt | wt |
| **EPHB6** | wt | G404S | wt | wt | G404S |
| **ERBB4** | wt | wt | wt | wt | wt |
| **GNA11** | wt | wt | wt | wt | wt |
| **GNAQ** | wt | wt | wt | wt | wt |
| **GRIN2A** | N193K | wt | Y423H | wt | S912F |
| **KIT** | wt | wt | wt | wt | wt |
| **KRAS** | wt | wt | wt | wt | wt |
| **MAP2K1** | wt | wt | wt | wt | wt |
| **MET** | wt | wt | wt | wt | wt |
| **NEK10** | wt | wt | wt | wt | wt |
| **NF1** | wt | wt | wt | wt | DelALLV548A |
| **NRAS** | wt | Q61R | Q61K | wt | G13D |
| **PIK3CA** | wt | wt | wt | wt | wt |
| **PREX2** | wt | wt | wt | wt | wt |
| **PTEN** | wt | wt | wt | wt | wt |
| **TP53** | wt | G279W | wt | wt | M133T |

The mutations that result benign (search in the COSMIC and ICGC database as well as analyzed by PolyPhen and SIFT predictor) were not considered. Pathogenic mutations (by the above predictors) that were not found in COSMIC and ICGC databases are reported in red.

**Supplementary Table S5. Correlation of PDC and LK density and prognostic features of PCM.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Features** | **Case no.** | **PDC/mm²** | | **p-value** | **LK/mm²** | | **p-value** | **PDC Fraction** | | **p-value** |
|  |  | Low or no (n,%)a | High  (n,%)b |  | Low or no (n,%)a | High (n,%)b |  | Low or no (n,%)a | High  (n,%)b |  |
| *Age (years)* |  |  |  | 0,33 |  |  | 0,2542 |  |  | 0,0875 |
| ≤ 60 | 59 | 42 (55,26) | 17 (68) |  | 42 (56) | 16 (64) |  | 42 (56) | 16 (64) |  |
| ≥ 60 | 42 | 34 (44,74) | 8 (32) |  | 33 (44) | 9 (36) |  | 33 (44) | 9 (36) |  |
| *Gender* |  |  |  | 0,15 |  |  | 0,3149 |  |  | 0,7992 |
| Female | 58 | 43 (58,11) | 13 (52) |  | 45 (60) | 12 (48) |  | 45 (60) | 12 (48) |  |
| Male | 43 | 31 (41,89) | 12 (48) |  | 30 (40) | 13 (52) |  | 30 (40) | 13 (52) |  |
| *Breslow* |  |  |  | 0,0227\* |  |  | 0,0728 |  |  | 0,2665 |
| <2 | 71 | 48 (63,16) | 22 (88) |  | 49 (65,33) | 21 (84) |  | 52 (69,33) | 18 (72) |  |
| >2 | 30 | 28 (36,84) | 3 (12) |  | 26 (34,67) | 4 (16) |  | 23 (30,67) | 7 (28) |  |
| *Ulceration* |  |  |  | 0,6337 |  |  | 0,3585 |  |  | 0,3809 |
| Absent | 89 | 67 (88,16) | 22 (88) |  | 66 (88) | 22 (88) |  | 65 (86,67) | 23 (92) |  |
| Present | 12 | 9 (11,84) | 3 (12) |  | 9 (12) | 3 (12) |  | 10 (13,33) | 2 (8) |  |
| *Mitosisc* |  |  |  | 0,1853 |  |  | 0,677 |  |  | 0,2557 |
| <1 | 41 | 27 (36) | 14 (56) |  | 32 (42,66) | 9 (36) |  | 30 (40) | 11 (44) |  |
| >1 | 60 | 48 (64) | 11 (44) |  | 43 (57,34) | 17 (64) |  | 45 (60) | 14 (56) |  |
| *Staging* |  |  |  | 0,2518 |  |  | 0,1981 |  |  | 0,8211 |
| I-II-IIIa | 82 | 59 (77,63) | 23 (92) |  | 58 (77,33) | 23 (92) |  | 60 (80) | 21 (84) |  |
| IIIb/c-IV | 19 | 17 (22,37) | 2 (8) |  | 17 (22,67) | 2 (8) |  | 15 (20) | 4 (16) |  |
| *Regression* |  |  |  | 0,5808 |  |  | 0,0281\* |  |  | 0,1646 |
| Absent | 76 | 59 (77,63) | 17 (68) |  | 57 (76) | 18 (72) |  | 54 (72) | 21 (84) |  |
| Present | 25 | 17 (22,37) | 8 (32) |  | 18 (24) | 7 (28) |  | 21 (28) | 4 (16) |  |
| *Associated Naevus* |  |  |  | 0,593 |  |  | 0,9716 |  |  | 0,8661 |
| Absent | 63 | 43 (58,11) | 18 (72) |  | 45 (60) | 18 (72) |  | 47 (62,67) | 16 (64) |  |
| Present | 38 | 31 (41,89) | 7 (28) |  | 30 (40) | 7 (28) |  | 28 (37,33) | 9 (36) |  |
| *Overall Survival* |  |  |  | 0,086 |  |  | 0,2182 |  |  | 0,9307 |
| Median (95% CI) months |  | 31,5 | 41 |  | 30 | 40 |  | 31 | 35,5 |  |

aCut off point 75th quartile

b Cut off point 75th quartile

c Staining index, cut off point lower quartile

**Supplementary Table S6. Genomic Landscape of 50 selected PCM cases.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient** | **PDC/mm²** | **CD8/mm²** | **ARID2** | **BRAF** | **CDK4** | **EPHB6** | **GRIN2A** | **KIT** | **KRAS** | **MAP2K1** | **MET** | **NF1** | **NRAS** | **PIK3CA** | **PREX2** | **PTEN** | **TP53** |
| #1 | 0,00 | 196,38 |  |  |  |  | D1415N |  |  |  |  |  | Q61K |  |  |  |  |
| #2 | 0,00 | 47,71 |  | V600E |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #3 | 0,00 | 63,79 |  |  |  |  |  |  |  |  |  | R1362\* |  |  |  |  |  |
| #4 | 0,23 | 460,81 |  |  |  |  |  |  |  |  |  |  |  |  |  | P38L |  |
| #5 | 0,25 | 3,64 |  |  |  |  | M701L |  |  |  |  |  | Q61H |  |  |  |  |
| #6 | 1,38 | 181,66 |  | V600K |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #7 | 1,62 | 28,84 |  |  |  |  |  |  |  |  |  |  | Q61K |  |  |  |  |
| #8 | 2,32 | 362,98 | P1001L |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #9 | 2,37 | 45,06 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #10 | 3,01 | 41,27 |  |  |  |  |  |  |  |  |  |  | Q61K |  |  |  |  |
| #11 | 3,89 | 54,42 |  |  |  |  |  |  |  |  |  |  | Q61R |  |  |  |  |
| #12 | 4,62 | 40,81 |  | V600E |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #13 | 5,91 | 969,91 |  |  |  |  |  |  |  |  |  |  | Q61H |  |  |  |  |
| #14 | 6,00 | 1190,54 |  | V600E |  |  |  |  |  |  |  |  | Q61R | R88Q |  |  |  |
| #15 | 6,03 | 179,03 |  |  |  |  |  |  | Q61R |  |  |  |  |  |  |  |  |
| #16 | 6,16 | 112,35 | G125S | V600E |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #17 | 7,53 | 124,58 |  |  |  |  |  |  |  |  |  |  | Q61K |  |  |  |  |
| #18 | 8,93 | 185,95 |  | V600E |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #19 | 9,84 | 253,07 |  |  |  |  |  |  |  |  |  |  | Q61R |  |  |  |  |
| #20 | 15,59 | 114,48 |  | V600E |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #21 | 15,71 | 417,58 |  | V600E |  |  |  |  |  |  |  | H553R |  |  |  |  |  |
| #22 | 17,18 | 319,60 |  |  |  | G404S |  |  |  |  |  |  | Q61K |  |  |  |  |
| #23 | 18,26 | 24,85 |  | V600E |  |  | D1115N |  |  |  |  |  |  |  |  |  |  |
| #24 | 20,29 | 93,68 |  | V600E |  |  |  |  |  |  |  |  |  |  |  | K62I fs Ter35 |  |
| #25 | 22,11 | 318,88 |  | V600R |  |  | S511L |  |  |  |  |  |  |  |  | H93Y |  |
| #26 | 24,69 | 177,90 |  | V600E |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #27 | 38,40 | 170,74 |  |  |  |  |  |  |  | A372T |  |  | Q61R |  |  |  | G154S |
| #28 | 41,81 | 202,30 |  | V600E | R24C |  |  |  |  |  |  |  |  |  |  |  |  |
| #29 | 43,49 | 235,85 |  | V600R/  L597P |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #30 | 48,66 | 353,64 |  | V600E |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #31 | 49,70 | 292,60 | R274\* | V600K |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #32 | 51,30 | 1166,71 |  |  |  |  |  |  |  |  |  |  | Q61R |  |  |  |  |
| #33 | 53,02 | 112,94 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #34 | 55,16 | 512,76 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #35 | 60,70 | 26,13 |  | V600E |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #36 | 65,79 | 150,21 |  | V600E |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #37 | 67,51 | 756,72 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #38 | 74,41 | 215,33 |  | V600E |  |  |  |  |  |  | T992I |  |  |  |  |  |  |
| #39 | 80,72 | 445,90 |  | V600E |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #40 | 87,06 | 29,45 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #41 | 114,95 | 253,90 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #42 | 118,99 | 3810,91 |  | V600E |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #43 | 119,08 | 340,05 | Q1606\* | K601E |  |  |  |  |  |  |  |  |  |  | R263Q |  |  |
| #44 | 124,13 | 204,84 |  |  |  |  |  |  |  |  |  |  | Q61R |  | N212T |  |  |
| #45 | 124,15 | 1695,53 |  | V600K |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #46 | 128,00 | 357,69 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #47 | 134,13 | 861,09 |  | V600E |  |  |  |  |  |  |  |  |  |  |  |  |  |
| #48 | 169,90 | 805,30 |  | V600K |  |  | Q891\* | V559A |  |  |  |  |  |  |  |  |  |
| #49 | 183,24 | 377,58 | Q720\* | V600K |  |  |  |  |  |  |  |  |  |  | P1145H |  |  |
| #50 | 312,21 | 507,37 |  | V600E |  |  |  |  |  |  |  |  |  |  |  |  | S241F |

Note 1. The mutations that result benign by COSMIC, PolyPhen, ICGC and SIFT, were not considered. The gene mutations that were not found in COSMIC and ICGC databases are reported in red. The following genes were resulted wild type in all samples analyzed: 1) AKT3, CXCR4, CTNNB1, EPHA10, ERBB4, GNA11, GNAQ, MEK, NEK10, PDGFRA, PTK2B, ROR2 for hot spot mutations of Melacarta Panel; 2) RAC1 by NGS analysis.Note 2. Patient 1# to patient 37# were considered PDClow , whereas patient# 38 to patient#50 were considered PDClow

Note 3. Four groups of patients were identified by molecular profiling: the BRAF+ (n= 27), NRAS+ (n= 13), NF-1+ (n= 2), and the triple negative group (n= 11)

**Supplementary Table S7. Clinical data of the SLN CH1 cohort.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient** | **Gender** | | **Age** | | **Pathology** | | **PDC/mm²** | | **Metastasis** | | | **Localization** |
| #1 | M | | 47 | | Melanoma Sentinel LN | | 514,46 | | yes | | | Inguinal dx |
| #2 | F | | 28 | | Melanoma Sentinel LN | | 290,57 | | yes | | | Inguinal sx |
| #3 | F | | 63 | | Melanoma Sentinel LN | | 228,63 | | yes | | | Axilla sx |
| #4 | M | | 26 | | Melanoma Sentinel LN | | 130,16 | | yes | | | Axilla sx |
| #5 | F | | 27 | | Melanoma Sentinel LN | | 438,51 | | yes | | | Skin |
| #6 | M | | 41 | | Melanoma Sentinel LN | | 232,32 | | yes | | | Axilla sx |
| #7 | M | | 57 | | Melanoma Sentinel LN | | 24,14 | | yes | | | Inguinal sx |
| #8 | F | | 33 | | Melanoma Sentinel LN | | 206,53 | | yes | | | Axilla dx |
| #9 | M | | 30 | | Melanoma Sentinel LN | | 699,68 | | yes | | | Axilla Sx |
| #10 | M | | 27 | | Melanoma Sentinel LN | | 171,21 | | yes | | | Axilla |
| #11 | M | | 61 | | Melanoma Sentinel LN | | 329,75 | | yes | | | Inguinal sx |
| #12 | F | | 40 | | Melanoma Sentinel LN | | 504,07 | | yes | | | Axilla dx |
| #13 | F | | 48 | | Melanoma Sentinel LN | | 241,26 | | yes | | | Leg sx |
| #14 | F | | 48 | | Melanoma Sentinel LN | | 246,87 | | yes | | | Axilla sx |
| #15 | M | | 68 | | Melanoma Sentinel LN | | 677,29 | | yes | | | NA |
| #16 | M | | 61 | | Melanoma Sentinel LN | | 254,17 | | yes | | | Inguinal dx |
| #17 | F | | 34 | | Melanoma Sentinel LN | | 822,95 | | no | | | Axilla dx |
| #18 | M | | 46 | | Melanoma Sentinel LN | | 291,98 | | no | | | NA |
| #19 | M | | 68 | | Melanoma Sentinel LN | | 326,19 | | no | | | NA |
| #20 | M | | 63 | | Melanoma Sentinel LN | | 459,21 | | no | | | Inguinal dx |
| #21 | M | | 61 | | Melanoma Sentinel LN | | 161,03 | | no | | | Inguinal dx |
| #22 | M | | 58 | | Melanoma Sentinel LN | | 508,90 | | no | | | Axilla dx |
| #23 | F | | 43 | | Melanoma Sentinel LN | | 321,52 | | no | | | Inguinal sx |
| #24 | F | | 74 | | Melanoma Sentinel LN | | 178,23 | | no | | | Axilla sx |
| #25 | M | | 73 | | Melanoma Sentinel LN | | 73,31 | | no | | | NA |
| #26 | M | | 63 | | Melanoma Sentinel LN | | 285,83 | | no | | | Axilla dx |
| #27 | F | | 56 | | Melanoma Sentinel LN | | 103,21 | | no | | | Inguinal sx |
| #28 | M | | 73 | | Melanoma Sentinel LN | | 263,78 | | no | | | Axilla sx |
| #29 | M | | 69 | | Melanoma Sentinel LN | | 78,80 | | no | | | Inguinal sx |
| #30 | F | | 33 | | Melanoma Sentinel LN | | 249,36 | | no | | | Axilla dx |
| #31 | M | | 70 | | Melanoma Sentinel LN | | 379,98 | | no | | | Inguinal dx |
| #32 | F | | 34 | | Melanoma Sentinel LN | | 360,82 | | no | | | Inguinal |
| #33 | F | | 69 | | Melanoma Sentinel LN | | 246,39 | | no | | | Inguinal dx |
|  | |  | |  | |  |  |  | |  |

**Supplementary Table S8. Clinical data of the melanoma metastasis CH2 cohort.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Patient** | **Gender** | **Age** | **Pathology** | **PDC/mm²** | **LK/mm²** | **Localization** |
| #1 | M | 68 | Metastatic Melanoma | 29,06 | 936,49 | Skin |  |
| #2 | M | 40 | Metastatic Melanoma | 3,31 | 220,12 | Skin |  |
| #3 | F | 48 | Metastatic Melanoma | 35,69 | 888,57 | Skin |  |
| #4 | F | 63 | Metastatic Melanoma | 7,48 | 2582,52 | Skin |  |
| #5 | F | 48 | Metastatic Melanoma | 6,24 | 672,65 | Skin |  |
| #6 | F | 71 | Metastatic Melanoma | 8,56 | 603,68 | Skin |  |
| #7 | M | 40 | Metastatic Melanoma | 0,03 | 10,21 | Skin |  |
| #8 | F | 64 | Metastatic Melanoma | 0,00 | 723,86 | Skin |  |
| #9 | F | 69 | Metastatic Melanoma | 5,58 | 1951,08 | Skin |  |
| #10 | M | 69 | Metastatic Melanoma | 1,02 | 80,20 | Skin |  |
| #11 | M | 79 | Metastatic Melanoma | 3,74 | 209,86 | Skin |  |
| #12 | M | 75 | Metastatic Melanoma | 0,65 | 35,41 | Skin |  |
| #13 | F | 66 | Metastatic Melanoma | 1,26 | 9,05 | Skin |  |
| #14 | F | 60 | Metastatic Melanoma | 5,83 | 227,13 | Skin |  |
| #15 | F | 45 | Metastatic Melanoma | 1,98 | 10,80 | Skin |  |
| #16 | F | 85 | Metastatic Melanoma | 6,33 | 978,99 | Skin |  |
| #17 | M | 75 | Metastatic Melanoma | 1,93 | 1678,43 | Skin |  |
| #18 | F | 80 | Metastatic Melanoma | 14,35 | 471,33 | Skin |  |
| #19 | M | 79 | Metastatic Melanoma | 0,02 | 21,19 | Skin |  |
| #20 | F | 46 | Metastatic Melanoma | 15,25 | 369,41 | Skin |  |
| #21 | M | 15 | Metastatic Melanoma | 2,57 | 1163,76 | Skin |  |
| #22 | M | 46 | Metastatic Melanoma | 0,46 | 104,23 | Skin |  |
| #23 | F | 88 | Metastatic Melanoma | 2,93 | 40,14 | Skin |  |
| #24 | M | 66 | Metastatic Melanoma | 9,03 | 603,87 | Skin |  |
| #25 | M | 39 | Metastatic Melanoma | 0,01 | NA | Skin |  |
| #26 | M | 68 | Metastatic Melanoma | 46,67 | 1844,63 | Skin |  |
| #27 | F | 54 | Metastatic Melanoma | 16,15 | NA | Skin |  |
| #28 | M | 47 | Metastatic Melanoma | 11,15 | 15,70 | Skin |  |
| #29 | M | 57 | Metastatic Melanoma | 8,15 | 3952,56 | Lung |  |
| #30 | F | 57 | Metastatic Melanoma | 2,86 | 1779,64 | Lung |  |
| #31 | M | 55 | Metastatic Melanoma | 5,16 | 687,31 | Lung |  |
| #32 | F | 67 | Metastatic Melanoma | 0,68 | 341,51 | Lung |  |
| #33 | F | 61 | Metastatic Melanoma | 5,93 | 709,74 | Lung |  |
| #34 | M | 65 | Metastatic Melanoma | 1,35 | 198,29 | Lung |  |
| #35 | M | 51 | Metastatic Melanoma | 7,48 | 78,38 | Lung |  |
| #36 | F | 74 | Metastatic Melanoma | 7,11 | NA | Lung |  |
| #37 | F | 68 | Metastatic Melanoma | 0,21 | 92,22 | Lung |  |
| #38 | M | 41 | Metastatic Melanoma | 0,36 | 10,03 | Lung |  |
| #39 | F | 57 | Metastatic Melanoma | 0,00 | 365,79 | Lung |  |
| #40 | F | 52 | Metastatic Melanoma | 11,27 | 234,11 | Liver |  |
| #41 | NA | NA | Metastatic Melanoma | 3,03 | NA | Liver |  |
| #42 | F | 53 | Metastatic Melanoma | 2,12 | NA | Salivary glands |  |
| #43 | NA | NA | Metastatic Melanoma | 10,63 | NA | Salivary glands |  |
| #44 | M | 71 | Metastatic Melanoma | 0,01 | 238,16 | Brain |  |
| #45 | F | 31 | Metastatic Melanoma | 0,05 | 36,86 | Brain |  |
| **Patient** | **Gender** | **Age** | **Pathology** | **PDC/mm²** | **LK/mm²** | **Localization** |
| #46 | M | 68 | Metastatic Melanoma | 2,90 | NA | Brain |  |
| #47 | F | 66 | Metastatic Melanoma | 0,59 | 373,49 | Bones |  |
| #48 | F | 52 | Metastatic Melanoma | 26,95 | 263,74 | Bones |  |
| #49 | F | 73 | Metastatic Melanoma | 0,01 | 143,58 | Bones |  |
| #50 | M | 62 | Metastatic Melanoma | 31,72 | 1887,40 | Skeletal Muscles |  |
| #51 | F | 72 | Metastatic Melanoma | 6,19 | 142,49 | Gastroenteric Tract |  |
| #52 | NA | NA | Metastatic Melanoma | 0,54 | 125,14 | Gastroenteric Tract |  |
| #53 | NA | NA | Metastatic Melanoma | 54,55 | 977,26 | Gastroenteric Tract |  |
| #54 | NA | NA | Metastatic Melanoma | 0,02 | 8,42 | Gastroenteric Tract |  |
| #55 | NA | NA | Metastatic Melanoma | 15,75 | NA | Adrenal glands |  |
| #56 | NA | NA | Metastatic Melanoma | 3,62 | 514,64 | Adrenal Glands |  |
| #57 | NA | NA | Metastatic Melanoma | 0,20 | 292,86 | Adrenal Glands |  |
| #58 | NA | NA | Metastatic Melanoma | 15,20 | 469,69 | Adrenal Glands |  |
| #59 | NA | NA | Metastatic Melanoma | 1,11 | NA | Pancreas |  |
| #60 | NA | NA | Metastatic Melanoma | 4,58 | 1327,13 | Pancreas |  |

**Supplementary Table S9. Differential expression of Gene signatures and prognosis** **in different sites of Melanoma disease**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Signature A** | | | | | | | | | | | | | | |
|  | **Metastasis Regional Skin vs Primary** | | | **Regional Lymph Node vs Metastasis Regional Skin** | | | **Regional Lymph Node vs Primary Melanoma** | | | **Survival**  **Primary Melanoma** | | | **Survival**  **Regional Lymph Node** | | |
| ***Gene*** | ***FC*** | ***p-value*** | ***FDR*** | ***FC*** | ***p-value*** | ***FDR*** | ***FC*** | ***p-value*** | ***FDR*** | ***HR*** | ***p-value*** | ***FDR*** | ***HR*** | ***p-value*** | ***FDR*** |
| ***CCL3*** | 1,10 | 0,67 | 0,84 | 1,37 | 0,04\* | 0,05\* | 1,51 | 0,04 | 0,05\* | 0,86 | 0,42 | 0.96 | 0,97 | 0,70 | 0,78 |
| ***CCL4*** | 1,19 | 0,50 | 0,83 | 2,04 | <0,01\* | <0,01\* | 2,43 | <0,01\* | <0,01\* | 0,70 | 0,07 | 0.96 | 0,86 | 0,01\* | 0,06 |
| ***CCL5*** | 0,91 | 0,74 | 0,86 | 2,30 | <0,01\* | <0,01\* | 2,09 | <0,01\* | <0,01\* | 0,79 | 0,16 | 0.96 | 0,88 | 0,01\* | 0,07 |
| ***CCL8*** | 0,98 | 0,93 | 0,93 | 1,76 | <0,01\* | <0,01\* | 1,72 | 0,02\* | 0,02\* | 1,05 | 0,84 | 0.96 | 0,83 | <0,01\* | 0,02\* |
| ***CCL19*** | 0,76 | 0,54 | 0,83 | 5,35 | <0,01\* | <0,01\* | 4,05 | <0,01\* | <0,01\* | 0,92 | 0,34 | 0,96 | 0,96 | 0,31 | 0,44 |
| ***CCL20*** | 0,78 | 0,43 | 0,83 | 1,12 | 0,61 | 0,61 | 0,87 | 0,64 | 0,64 | 1,05 | 0,58 | 0,96 | 0,89 | <0,01\* | 0,04\* |
| ***CCL21*** | 0,49 | 0,19 | 0,62 | 6,24 | <0,01\* | <0,01\* | 3,07 | 0,02\* | 0,03\* | 0,98 | 0,80 | 0,96 | 0,99 | 0,78 | 0,78 |
| ***CXCL9*** | 1,11 | 0,78 | 0,86 | 2,85 | <0,01\* | <0,01\* | 3,18 | <0,01\* | <0,01\* | 0,84 | 0,16 | 0,96 | 0,87 | <0,01\* | 0,02\* |
| ***CXCL10*** | 1,19 | 0,62 | 0,83 | 2,27 | <0,01\* | <0,01\* | 2,69 | <0,01\* | <0,01\* | 0,87 | 0,30 | 0,96 | 0,86 | <0,01\* | 0,03\* |
| ***CXCL11*** | 1,25 | 0,51 | 0,83 | 1,98 | <0,01\* | <0,01\* | 2,47 | <0,01\* | <0,01\* | 0,92 | 0,51 | 0,96 | 0,86 | <0,01\* | 0,03\* |
| ***CXCL12*** | 1,35 | 0,17 | 0,62 | 1,47 | <0,01\* | 0,01\* | 1,99 | <0,01\* | <0,01\* | 1,00 | 0,99 | 0,99 | 0,86 | 0,15 | 0,27 |
| ***RARRES2*** | 1,26 | 0,26 | 0,76 | 1,15 | 0,33 | 0,35 | 1,45 | 0,05\* | 0,07 | 0,93 | 0,71 | 0,96 | 0,84 | 0,04\* | 0,11 |
|  | **Signature B** | | | | | | | | | | | | | | |
|  | **Metastasis Regional Skin vs Primary** | | | **Regional Lymph Node vs Metastasis Regional Skin** | | | **Regional Lymph Node vs Primary Melanoma** | | | **Survival**  **Primary Melanoma** | | | **Survival**  **Regional Lymph Node** | | |
| ***Gene*** | ***FC*** | ***p-value*** | ***FDR*** | ***FC*** | ***p-value*** | ***FDR*** | ***FC*** | ***p-value*** | ***FDR*** | ***HR*** | ***p-value*** | ***FDR*** | ***HR*** | ***p-value*** | ***FDR*** |
| ***BCL11A*** | 0,57 | 0,06 | 0,29 | 2,40 | <0,01\* | <0,01\* | 1,37 | 0,24 | 0,26 | 0,99 | 0,96 | 0,99 | 0,98 | 0,71 | 0,78 |
| ***CD2AP*** | 1,06 | 0,62 | 0,83 | 1,12 | 0,15 | 0,18 | 0,19 | 0,10 | 0,12 | 0,96 | 0,87 | 0,96 | 1,05 | 0,75 | 0,78 |
| ***CLEC4C*** | 1,60 | 0,06 | 0,29 | 1,72 | <0,01\* | <0,01\* | 2,76 | <0,01\* | <0,01\* | 1,08 | 0,53 | 0,96 | 0,98 | 0,75 | 0,78 |
| ***IL3RA*** | 1,10 | 0,47 | 0,83 | 1,25 | 0,02\* | 0,02\* | 1,38 | 0,01\* | 0.02\* | 0,72 | 0,24 | 0,96 | 0,78 | 0,06 | 0,12 |
| ***TCF4*** | 1,72 | <0,01\* | 0,08 | 1,21 | 0,14 | 0,17 | 2,07 | <0,01\* | <0,01\* | 1,04 | 0,86 | 0,96 | 0,90 | 0,27 | 0,42 |
| ***TCL1A*** | 1,31 | 0,54 | 0,83 | 4,71 | <0,01\* | <0,01\* | 6,17 | <0,01\* | <0,01\* | 1,15 | 0,25 | 0,96 | 0,95 | 0,19 | 0,32 |
| ***TLR7*** | 1,96 | <0,01\* | 0,08 | 1,59 | <0,01\* | <0,01\* | 3,12 | <0,01\* | <0,01\* | 0,95 | 0,72 | 0,96 | 0,89 | 0,08 | 0,16 |
| ***TLR9*** | 1,03 | 0,87 | 0,91 | 1,14 | 0,34 | 0,35 | 1,17 | 0,35 | 0,36 | 1,06 | 0,72 | 0,96 | 0,94 | 0,34 | 0,45 |

**Supplementary Table S10. CK-R expression in MM patients.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cases** | **% CCR5** | **% CCR6** | **% CCR7** | **% CXCR3** | **%**  **CXCR4** | **MFI CCR5** | **MFI CCR6** | **MFI CCR7** | **MFI CXCR3** | **MFI**  **CXCR4** |
|  |  |  |  |  |  |  |  |  |  |  |
| **#1** | 97,6 | 5,05 | 85,5 | 99,6 | 90 | 1120 | 919 | 671 | 1469 | 900 |
| **#2** | 24,4 | 5,65 | 97,5 | 100 | 54,5 | NA | NA | NA | NA | NA |
| **#3** | 55,8 | 9,64 | 95,2 | 97,2 | 45,4 | NA | NA | NA | NA | NA |
| **#4** | 47,8 | 6,68 | 96,3 | 99 | 31,8 | NA | NA | NA | NA | NA |
| **#5** | 98,2 | 11,3 | 95,2 | 100 | 86 | 1172 | 539 | 1212 | 4411 | 867 |
| **#6** | 97,2 | 18,3 | 94,5 | 98,9 | 52,9 | 1399 | 930 | 3731 | 2473 | 1378 |
| **#7** | 99,3 | 8,22 | 92,9 | 99,5 | 96,3 | 1261 | 1897 | 3834 | 1644 | 956 |
| **#8** | 95,5 | 9,44 | 79,4 | 96,1 | 21,7 | 1103 | 667 | 1127 | 1246 | 1370 |
| **#9** | 89,3 | 1,62 | 33,4 | 93,9 | 19,7 | 745 | 372 | 225 | 594 | 986 |
| **#10** | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| **#11** | 94,6 | 7,39 | 55,6 | 89,1 | NA | 826 | 216 | 169 | 433 | NA |
| **#12** | 96,9 | 1,04 | 30,6 | 97,3 | 87,6 | 765 | 261 | 273 | 507 | 740 |
| **#13** | 64,5 | 4,34 | 27,8 | 84,9 | 65,1 | 531 | 407 | 224 | 433 | 551 |
| **#14** | 75,2 | 5 | 25,6 | 86,9 | 75,1 | 517 | 281 | 194 | 448 | 609 |
| **#15** | 86 | 1,73 | 34,1 | 88 | 76,9 | 780 | 651 | 292 | 536 | 686 |
| **#16** | 49,9 | 5,07 | 18,8 | 47,8 | 20,6 | 1077 | 1246 | 280 | 1051 | 1473 |
| **#17** | 92,9 | 3,55 | 62,6 | 99,4 | 82,2 | 720 | 400 | 248 | 1013 | 628 |
| **#18** | 90,5 | 10,8 | 34,1 | 87 | 83,9 | 740 | 881 | 171 | 828 | 504 |
| **#19** | 91,1 | 3,51 | 16,4 | 93,6 | 80,6 | 813 | 311 | 251 | 570 | 538 |
| **#20** | 52,3 | 2,16 | 33,9 | 96,8 | 73 | 570 | 386 | 202 | 558 | 632 |
| **#21** | 89,3 | 0,71 | 65,5 | 99,2 | 71,3 | 705 | 361 | 321 | 609 | 529 |
| **#22** | 99,5 | 0,51 | 87,1 | 99 | 74,8 | 1382 | 291 | 649 | 690 | 509 |
| **#23** | 92,6 | 0,46 | 76 | 97 | 50,6 | 636 | 469 | 512 | 396 | 256 |
| **#24** | 88,7 | 0,7 | 64,4 | 54,9 | 28,3 | 1165 | 296 | 392 | 309 | 341 |
| **#25** | 95,1 | 5,19 | 34,2 | 96 | 50,5 | 1361 | 852 | 388 | 480 | 500 |
| **#26** | 94,8 | 2,99 | 83,4 | 91,1 | 73,6 | 881 | 638 | 638 | 473 | 488 |
| **#27** | 68,9 | 3,7 | 60,8 | 92,3 | 62,5 | 862 | 399 | 319 | 324 | 437 |
| **#28** | 37,5 | 0,7 | 90,8 | 73,6 | 93,4 | 696 | 379 | 761 | 222 | 217 |
| **#29** | 20,9 | 7,1 | 21,9 | 22,6 | 17,8 | NA | NA | NA | NA | NA |

**Supplementary Table S11. CK-R expression in HD.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cases** | **% CCR5** | **% CCR6** | **% CCR7** | **% CXCR3** | **%**  **CXCR4** | **MFI CCR5** | **MFI CCR6** | **MFI CCR7** | **MFI CXCR3** | **MFI**  **CXCR4** |
|  |  |  |  |  |  |  |  |  |  |  |
| **#1** | 11,2 | 7,15 | 93,1 | 99,8 | 43,1 | NA | NA | NA | NA | NA |
| **#2** | 62 | 5,73 | 97,7 | 100 | 38,6 | 1680 | 604 | 5874 | 4617 | 1013 |
| **#3** | 51,5 | 4,1 | 97,4 | 100 | 48,2 | 1382 | 659 | 3823 | 3364 | 908 |
| **#4** | 92,4 | 1,39 | 93,9 | 99,1 | 54,8 | 714 | 506 | 1137 | 1473 | 575 |
| **#5** | 47,6 | 5,76 | 95,4 | 99,2 | 4,64 | 390 | 709 | 9828 | 3478 | NA |
| **#6** | 93,2 | 7,73 | 96,7 | 99,8 | 84,8 | 1281 | 636 | 4125 | 4425 | 775 |
| **#7** | 90,5 | 12,8 | 99,2 | 99,9 | 77,1 | 881 | 900 | 16954 | 2413 | 1341 |
| **#8** | 71,5 | 5,88 | 97 | 99,7 | 82 | 600 | 1054 | 3457 | 3719 | 667 |
| **#9** | 99,8 | 4,41 | 95,2 | 99,9 | 5,42 | 1361 | 1289 | 2271 | 3521 | 329 |
| **#10** | 90 | 2,84 | 97,8 | 99,9 | 85,3 | 722 | 311 | 17214 | 2652 | 641 |
| **#11** | 91 | 3,34 | 73,3 | 82,3 | 62 | 729 | 231 | 305 | 347 | 523 |
| **#12** | 97 | 1,99 | 5,59 | 99,4 | 78,5 | 1032 | 313 | 284 | 1022 | 653 |
| **#13** | 95,1 | 1,38 | 35,6 | 96,2 | 74,4 | 787 | 287 | 251 | 497 | 544 |
| **#14** | 90,9 | 2,26 | 60,1 | 99,2 | 78,6 | 738 | 649 | 250 | 826 | 647 |
| **#15** | 79,3 | 2,68 | 25,7 | 98,3 | 88,5 | 645 | 333 | 164 | 682 | 659 |
| **#16** | 92,8 | 2,07 | 52,1 | 90 | 79,6 | 758 | 655 | 193 | 366 | 634 |
| **#17** | 97,5 | 3,51 | 50,5 | 97,7 | 92 | 998 | 445 | 214 | 651 | 758 |
| **#18** | 97,6 | 1,83 | 26,8 | 98,6 | 85,6 | 852 | 441 | 176 | 582 | 591 |
| **#19** | 96,1 | 1,67 | 28,5 | 97,8 | 86 | 846 | 371 | 205 | 653 | 643 |
| **#20** | 95,7 | 4,1 | 71,5 | 91,9 | 78,3 | 933 | 305 | 276 | 284 | 372 |
| **#21** | 97,8 | 3,49 | 88,8 | 96,1 | 58,7 | 1151 | 342 | 1151 | 472 | 409 |
| **#22** | 67,8 | 1,32 | 88 | 95,9 | 88,7 | 607 | 241 | 451 | 352 | 398 |
| **#23** | 98,3 | 0,53 | 93,8 | 99,1 | 75,5 | 1124 | 291 | 684 | 578 | 271 |
| **#24** | 99,2 | 2,01 | 94,6 | 99,6 | 78,5 | 1265 | 485 | 828 | 596 | 604 |
| **#25** | 96,8 | 1,58 | 81,9 | 90,3 | 63,7 | 1107 | 482 | 647 | 436 | 538 |

**Supplementary Table S12. Correlation between absolute numbera of immune cells and stages in MM patients.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **M1a+b** | | | **M1c** | | |  |
| **Immune cells population** | ***N*** | ***Median*** | ***IQR*** | ***N*** | ***Median*** | ***IQR*** | ***p-value*** |
| **PDC** | 14 | 349,5 | 237-503 | 15 | 168 | 43-304 | 0,0013\* |
| **MDC** | 14 | 476,5 | 300-820,8 | 15 | 249 | 158-300 | 0,0002\* |
| **CD3** | 14 | 114751 | 92413- 148525 | 15 | 98064 | 70406- 127534 | 0,1368 |
| **CD4** | 14 | 74521 | 55801- 81833 | 15 | 45671 | 30973- 65763 | 0,0056\* |

a A minimum of 2x105 PBMCs was acquired according to the forward light scatter versus side light scatter profile.