**Supplementary Table S1. Baseline patients’ characteristics**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **HP** n (%) = 39 | **P** n (%) = 39 | **SD** n (%) = 42 | **R** n (%) = 32 | **TOTAL**n (%) = 152 |
| AGE  < 70years  ≥70 years | 29 (74.4)  10 (25.6) | 28 (71.8)  11 (28.2) | 28 (66.7)  14 (33.3) | 22 (68.7)  10 (31.3) | 104 (68.4)  48 (31.6) |
| SEX  M  F | 22 (56.4)  17 (43.6) | 21(53.8)  18 (46.2) | 28 (66.7)  14 (33.3) | 18 (56.3)  14 (43.7) | 91(59.9)  61(40.1) |
| ECOG PS  0-1  ≥2 | 34(87.2)  5 (12.8) | 36 (92.3)  3 (7.7) | 39 (92.9)  3 (7.1) | 32 (100.0)  0 (0.0) | 140 (92.1)  12 (7.9) |
| HISTOLOGY  squamous  non- squamous | 13(33.3)  26 (66.7) | 4 (10.2)  35 (89.8) | 11(26.1)  31 (73.9) | 13(40.6)  19(59.4) | 36 (23.7)  116 (76.3) |
| SMOKING  Current  Never  Former  unknown | 9 (23.1)  7 (18.0)  23 (58.9)  0 (0.0) | 8 (20.6)  5 (12.8)  24 (61.5)  2 (5.1) | 12 (28.6)  3 (7.1)  25 (59.5)  2 (4.8) | 11(34.4)  5 (15.6)  16 (50.0)  0 (0.0) | 40 (26.3)  20 (13.2)  88 (57.9)  4 (2.6) |
| BIOMOLECULAR ASSESSEMENT  EGFR +  ALK +  KRAS +  EGFR/ALK WT  unknown | 2 (5.1)  1 (2.6)  2 (5.1)  26 (66.7)  8 (20.5) | 4 (10.2)  0 (0.0)  3 (7.7)  30 (77.0)  2 (5.1) | 1(2.4)  0 (0.0)  4 (9.5)  27 (64.3)  10 (23.8) | 0 (0.0)  0 (0.0)  6 (18.8)  21 (65.6)  5 (15.6) | 7 (4.6)  1 (0.7)  15 (9.9)  104 (68.4)  25 (16.4) |
| PD-L1 STATUS  positive  negative  unknown | 10 (25.6)  12 (30.8)  17 (43.6) | 7 (18.0)  15 (38.4)  17 (43.6) | 11(26.1)  13 (31.0)  18 (42.9) | 17(53.1)  5(15.6)  10 (31.3) | 45(29.6)  45 (29.6)  62 (40.8) |
| METASTATIC SITES  ≤ 2  >2 | 15 (38.5)  24 (61.5) | 16 (41.1)  23 (58.9) | 20 (47.6)  22 (52.4) | 15 (46.9)  17 (53.1) | 66 (43.4)  86 (56.6) |
| BASELINE  LIVER METASTASES  yes  no | 13 (33.3)  26 (66.7) | 7 (18.0)  32 (82.0) | 4 (9.5)  38 (90.5) | 5 (15.6)  27 (84.4) | 29 (19.1)  123 (80.9) |
| LIVER  PROGRESSION  AT FIRST ASSESSMENT  yes  no  uk | 16 (41.0)  20 (51.3)  3 (7.7) | 8 (20.5)  27 (69.2)  4 (10.3) | 0 (0.0)  42 (100.0)  0 (0.0) | 0 (0.0)  32 (100.0)  0 (0.0) | 24 (15.8)  121 (79.6)  7 (4.6) |
| ICI TREATMENT  Anti PD-1  Anti PD-L1  Anti PD-1 + Anti CTLA4  Anti PD-L1 + Anti CTLA4  Anti CTLA4 | 28 (71.8)  6 (15.4)  1 (2.6)  4 (10.2)  0 (0.0) | 25 (64.1)  12 (30.8)  0 (0.0)  2 (5.1)  0 (0.0) | 29 (69.0)  13 (31.0)  0 (0.0)  0 (0.0)  0 (0.0) | 17 (53.1)  14 (43.7)  0 (0.0)  0 (0.0)  1 (3.2) | 99 (65.1)  45 (29.6)  1 (0.7)  6 (3.9)  1(0.7) |
| ICI TREATMENT  I line  II line  >II line | 8 (20.5)  14 (35.9)  17 (43.6) | 2 (5.1)  20 (51.3)  17 (43.6) | 9 (21.5)  19 (45.2)  14 (33.3) | 3(9.4)  11(34.4)  18 (56.2) | 22 (14.5)  64 (42.1)  66 (43.4) |

HP: Hyperprogressors; P: Progressors; SD: Stable disease; R: Responders; n: Number; M: Male; F: Female; ECOG: Eastern Cooperative Oncology Group; PS: Performance Status; ICI: Immune Checkpoints Inhibitors

**Supplementary Table S2.** **Criteria adherence in patients with hyperprogressive or progressive disease**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **PATIENTS WITH HYPERPROGRESSIVE DISEASE** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Patient ID Criteria** | 1 | 3 | 4 | 5 | 6 | 9 | 18 | 20 | 21 | 30 | 31 | 34 | 35 | 36 | 46 | 49 | 60 | 65 | 76 | 77 | 82 | 85 | 86 | 88 | 92 | 94 | 96 | 97 | 107 | 109 | 110 | 113 | 116 | 118 | 120 | 136 | 137 | 140 | 148 |
| TTF < 2 mts | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| STL ≥ 50% |  | x | x | x |  | x |  | x | x | x | x | x | x | x | x |  | x | x | x | x | x | x |  |  | x | x |  | x |  |  |  | x | x | x | x | x | x | x | x |
| NLSO ≥ 2 |  |  |  | x | x |  | x | x |  | x |  | x |  |  | x |  |  |  |  |  |  | x |  | x |  | x | x |  | x |  | x |  |  | x | x |  | x | x | x |
| NLDO ≥ 1 | x | x | x | x | x | x |  |  | x |  | x | x | x |  |  | x | x | x | x |  | x |  | x |  | x | x | x | x |  | x |  |  |  |  | x | x |  |  | x |
| WPS ≥ 2 | x |  |  | x | x | x | x |  |  | x | x | x |  | x | x | x | x | x | x | x | x | x | x | x | x |  |  | x | x | x | x | x | x | x | x |  | x | x | x |
| **PATIENTS WITH PROGRESSIVE DISEASE** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Patient ID Criteria** | 7 | 10 | 17 | 23 | 25 | 32 | 41 | 52 | 53 | 56 | 57 | 58 | 62 | 77 | 78 | 80 | 81 | 83 | 84 | 89 | 93 | 95 | 98 | 102 | 103 | 104 | 106 | 123 | 125 | 126 | 127 | 129 | 130 | 131 | 141 | 143 | 146 | 149 | 151 |
| TTF < 2 mts |  |  |  |  |  | x | x | x |  |  |  |  |  |  |  |  | x |  |  | x |  |  | x |  |  |  |  |  | x |  |  |  |  |  | x |  | x | x | x |
| STL ≥ 50% | x |  |  |  |  |  |  |  | x |  |  |  |  |  |  |  |  | x |  |  | x |  |  |  |  |  |  |  |  |  |  | x |  |  |  |  |  |  |  |
| NLSO ≥ 2 |  |  |  |  | x |  |  |  |  |  |  |  |  |  |  |  |  | x |  |  |  |  |  |  |  |  |  | x |  |  |  |  |  |  |  |  |  |  |  |
| NLDO ≥ 1 |  |  | x | x | x |  |  |  |  |  |  |  |  |  |  | x |  |  |  |  | x | x |  |  |  |  | x | x |  | x |  |  | x |  |  |  | x |  |  |
| WPS ≥ 2 |  |  |  |  |  | x |  |  |  |  |  |  |  |  |  |  | x |  |  | x |  |  | x |  |  | x |  |  | x |  |  |  |  |  | x |  |  | x | x |

Numbers represent patient ID.

TTF= Time-to-Treatment Failure (Time-to-treatment failure < 2 months, mts= months);

STL= Sum of Target Lesions (Increase of ≥ 50% in the sum of target lesions major diameters between baseline and first radiological evaluation);

NLSO= New Lesions in the Same Organ (Appearance of at least two new lesions in an organ already involved between baseline and first radiological evaluation);

NLDO= New Lesion in a Different Organ (Spread of the disease to a new organ between baseline and first radiological evaluation);

WPS= Worsening of Performance Status (Clinical deterioration with decrease in ECOG performance status ≥ 2 during the first 2 months of treatment);

**Supplementary Table S3. Comparison of immunophenotype between HP and non-HP (P+SD+R) patients (Univariate analysis)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variables** | **HP**  ***median* (*IQR)*** | **Non HP**  ***median* (*IQR)*** | **p-Value** |
| CD8 Intratumoral immune cells  - within the stroma  - within the tumor  CD8 Peritumoral immune cells | 10.0 ( 5.0-15.0)  7.0 ( 3.0- 9.0)  2.0 ( 1.0- 3.0)  20.0 (15.0-20.0) | 10.0 ( 5.0-15.0)  5.3 ( 4.0-12.8)  1.8 ( 0.8- 6.0)  12.5 ( 5.0-20.0) | 0.9295  0.7601  0.5492  0.1105 |
| CD4 Intratumoral immune cells  - within the stroma  - within the tumor  CD4 Peritumoral immune cells | 15.0 (10.0-15.0)  9.0 ( 8.0-13.5)  3.0 ( 1.5- 6.0)  20.0 (15.0-30.0) | 10.0 ( 5.0-15.0)  7.0 ( 4.0-11.3)  2.0 ( 0.5- 5.3)  17.5 ( 7.5-22.5) | 0.2985  0.2498  0.5429  0.2387 |
| CD3 Intratumoral immune cells  - within the stroma  - within the tumor  CD3 Peritumoral immune cells | 20.0 (15.0-30.0)  16.0 (12.0-18.0)  6.0 ( 3.0- 8.0)  30.0 (20.0-35.0) | 15.0 (10.0-30.0)  12.0 ( 8.0-20.5)  4.5 ( 2.5- 9.0)  25.0 (15.0-30.0) | 0.6156  0.3932  0.8296  0.5339 |
| FOXp3 Intratumoral immune cells  - within the stroma  - within the tumor  FOXp3 Peritumoral immune cells | 1.0 ( 1.0-10.0)  1.0 ( 0.9- 7.0)  0.2 ( 0.0- 1.0)  5.0 ( 1.0-10.0) | 5.0 ( 1.0- 5.0)  3.5 ( 1.0- 4.5)  0.5 ( 0.1- 1.0)  3.0 ( 1.0-10.0) | 0.8827  0.8773  0.9432  0.5690 |
| PD-1 Intratumoral immune cells  - within the stroma  - within the tumor  PD-1 Peritumoral immune cells | 5.0 ( 1.0-10.0)  4.0 ( 1.0- 6.0)  1.0 ( 0.2- 3.5)  10.0 ( 5.0-15.0) | 5.0 ( 1.0-10.0)  2.8 ( 0.9- 6.0)  1.2 ( 0.2- 3.3)  5.0 ( 5.0-10.0) | 0.9221  0.8925  0.9921  0.3409 |
| PDL-1 Tumor Cells  PDL-1 Intratumoral immune cells  PDL-1 Peritumoral immune cells | 1.0 ( 0.0-10.0)  5.0 ( 5.0- 5.0)  10.0 ( 5.0-15.0) | 5.0 ( 1.0-55.0)  5.0 ( 1.0-10.0)  5.0 ( 5.0-10.0) | **0.0457**  0.8928  0.3144 |
| Arginase Tumor Cells  Arginase Intratumoral immune cells  - within the stroma  - within the tumor  Arginase Peritumoral immune cells | 0.0 ( 0.0- 0.0)  20.0 (10.0-35.0)  9.0 ( 5.0-17.5)  8.0 ( 2.0-17.5)  25.0 (20.0-35.0) | 3.0 ( 0.0-20.0)  10.0 ( 5.0-17.5)  7.8 ( 4.0-11.3)  4.3 ( 1.5- 6.5)  15.0 ( 5.0-25.0) | 0.0927  0.2681  0.6246  0.0893  0.0666 |
| CD33 Intratumoral immune cells  - within the stroma  - within the tumor  CD33 Peritumoral immune cells | 15.0 ( 5.0-20.0)  7.5 ( 3.5-12.0)  7.5 ( 2.0-12.0)  20.0 (10.0-35.0) | 10.0 (10.0-17.5)  7.5 ( 6.0-12.0)  3.5 ( 2.5- 6.8)  15.0 ( 5.0-22.5) | 0.8723  0.6335  0.3677  0.1660 |
| CD163 Intratumoral immune cells  - within the stroma  - within the tumor  CD163 Peritumoral immune cells | 20.0 (15.0-25.0)  12.0 ( 9.0-14.0)  9.0 ( 6.0-10.0)  30.0 (25.0-30.0) | 20.0 (10.0-25.0)  10.5 ( 7.5-14.5)  6.0 ( 3.3-12.0)  20.0 (15.0-30.0) | 0.5561  0.8590  0.2635  0.0618 |
| MPO Intratumoral immune cells  - within the stroma  - within the tumor  MPO Peritumoral immune cells | 5.0 ( 1.0-10.0)  1.0 ( 0.5- 4.0)  3.5 ( 0.1- 6.0)  1.0 ( 1.0- 5.0) | 1.0 ( 1.0- 5.0)  0.8 ( 0.3- 1.5)  0.4 ( 0.0- 3.0)  1.0 ( 0.5- 1.0) | 0.0737  0.3436  **0.0497**  0.1732 |
| CD123 Intratumoral immune cells  - within the stroma  - within the tumor  CD123 Peritumoral immune cells | 1.0 ( 1.0- 5.0)  0.9 ( 0.8- 4.0)  0.4 ( 0.1- 1.0)  5.0 ( 1.0- 5.0) | 1.0 ( 1.0- 3.0)  0.9 ( 0.6- 2.0)  0.2 ( 0.0- 0.7)  1.0 ( 1.0- 5.0) | 0.5963  0.4074  0.7915  0.1592 |
| CD138 Intratumoral immune cells  - within the stroma  - within the tumor  CD138 Peritumoral immune cells | 5.0 ( 5.0- 5.0)  4.0 ( 2.0- 4.5)  1.0 ( 0.5- 2.0)  5.0 ( 1.0-10.0) | 3.0 ( 1.0- 5.0)  2.0 ( 0.8- 3.8)  0.6 ( 0.2- 1.5)  1.0 ( 1.0- 5.0) | 0.1884  0.2214  0.2699  0.3710 |

HP: Hyperprogressors; P: Progressors; SD: Stable Disease; R: Responders; IQR: interquartile range; FOXp3: forkhead box P3; PD-1: Programmed Cell Death 1; PD-L1: Programmed death-ligand; MPO: Myeloperoxidase

**Supplementary Table S4. Macrophage markers and “complete phenotype” associate with HP patients**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Variables** | **TRUE +** | **FALSE +** | **LR+** | **95% CI** | **p-Value** |
| Epithelioid morphology | 11/11 | 9/24 | 2.67 | 1.59- 4.47 | 0.0006 |
| PD-L1 | 11/11 | 10/23 | 2.30 | 1.44- 3.66 | 0.0109 |
| CD163 | 11/11 | 13/24 | 1.85 | 1.28- 2.67 | 0.0075 |
| CD33 | 11/11 | 14/24 | 1.71 | 1.22- 2.40 | 0.0125 |
| Complete phenotype | 11/11 | 4/24 | 6.00 | 2.45-14.68 | < 0.0001 |

PD-L1: Programmed death-ligand 1; CI: Confidence Interval; LR: Likelihood ratio

**Supplementary Table S5. Overall distribution of macrophage markers among ICI-treated NSCLC patients**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| PATIENTS NUMBER | TREATMENT RESPONSE | EPITHELIOID MORPHOLOGY | PD-L1 | CD163 | CD33 | COMPLETE PHENOTYPE |
| 4 | HP | + | + | + | + | + |
| 5 | HP | + | + | + | + | + |
| 9 | HP | + | + | + | + | + |
| 20 | HP | + | + | + | + | + |
| 30 | HP | + | + | + | + | + |
| 34 | HP | + | + | + | + | + |
| 35 | HP | + | + | + | + | + |
| 46 | HP | + | + | + | + | + |
| 82 | HP | + | + | + | + | + |
| 116 | HP | + | + | + | + | + |
| 120 | HP | + | + | + | + | + |
| 32 | P | + | - | + | + | - |
| 53 | P | - | - | - | - | - |
| 75 | P | - | - | - | - | - |
| 78 | P | + | NE | - | + | - |
| 89 | P | + | + | + | + | + |
| 104 | P | - | + | + | + | - |
| 16 | SD | + | + | + | + | + |
| 24 | SD | - | - | - | - | - |
| 27 | SD | + | + | - | - | - |
| 51 | SD | - | - | - | - | - |
| 54 | SD | + | - | + | + | - |
| 55 | SD | + | - | + | + | - |
| 59 | SD | - | - | - | - | - |
| 100 | SD | - | - | + | + | - |
| 105 | SD | + | + | + | + | + |
| 121 | SD | - | + | + | + | - |
| 134 | SD | - | + | + | + | - |
| 15 | R | - | - | - | - | - |
| 19 | R | - | - | - | - | - |
| 64 | R | - | - | - | - | - |
| 67 | R | - | - | - | - | - |
| 111 | R | - | + | + | + | - |
| 115 | R | + | + | + | + | + |
| 135 | R | - | + | + | + | - |

Numbers represent patient ID.

ICI: Immune checkpoint inhibitors; NSCLC: Non Small Cell Lung Cancer; HP: Hyperprogressors; P: Progressors; SD: Stable disease; R: Responders; PD-L1: Programmed death-ligand 1; NE: not evaluable.