# Supplementary Tables

**Supplementary Table S1: TP53 shRNA hairpin and oligos sequences**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **shRNA name** |  **Sigma Aldrich reference** | **Hairpin sequence (5‘ to 3’)** | **Forward oligo (5‘ to 3’)** | **Reverse oligo (5‘ to 3’)** |
| shTP53-1 | TRCN0000003755 | CCGGGTCCAGATGAAGCTCCCAGAACTCGAGTTCTGGGAGCTTCATCTGGACTTTTT | CCGGGTCCAGATGAAGCTCCCAGAACTCGAGTTCTGGGAGCTTCATCTGGACTTTTTG | AATTCAAAAAGTCCAGATGAAGCTCCCAGAACTCGAGTTCTGGGAGCTTCATCTGGAC |
| shTP53-2 | TRCN0000003756 | CCGGCACCATCCACTACAACTACATCTCGAGATGTAGTTGTAGTGGATGGTGTTTTT | CCGGCACCATCCACTACAACTACATCTCGAGATGTAGTTGTAGTGGATGGTGTTTTTG | AATTCAAAAACACCATCCACTACAACTACATCTCGAGATGTAGTTGTAGTGGATGGTG |

**Supplementary Table S2: *TP53* mutations of the GSCs studied**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **#GSC** | **LD50 *in vitro*** | ***TP53* mutation** | **LOH** | **Effect** | **FATHMM prediction** **(COSMIC database)** |
| 3 | 6.8 Gy | p.R175C | Yes | Missense | Pathogenic (score 0.97) |
| 7 | 6.5 Gy | p.A159V | Yes | Missense | Pathogenic (score 0.99) |
| 8 | 6.0 Gy | p.A159V | Yes | Missense | Pathogenic (score 0.99) |
| 9 | 6.4 Gy | p.P151T | Yes | Missense | Pathogenic (score 0.99)  |
| 12 | 3.4 Gy | p.G266R | Yes | Missense | Pathogenic (score 1.00) |
| 13 | 3.7 Gy | p.W146\*c.919+1G>T | No | NonsenseSplice Donor Variant | Neutral (score 0.17)n/a |

LOH: Loss of heterozygosity

**Supplementary Table S3: Clinical data overview**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **H3.1-K27M (n=22)** | **H3.3-K27M (n=51)** | **Overall (n=73)** |
|  | ***TP53WT* (n=19)** | ***TP53MUT* (n=3)** | ***TP53WT* (n=12)** | ***TP53MUT* (n=37)** | ***TP53NK* (n=2)** |  |
| **Age — years Median (range)** | 4.9 (3.3-8.6) | 5.2 (4.5-5.8) | 6.3 (3.9-15.2) | 9.2 (3.6-14.6) | 5.1 (4.4-5.7) | 6.6 (3.3-15.2) |
| **Gender — males n (%)** | 9 (47.3) | 1 (33.3) | 5 (41.6) | 18 (48.6) | 2 (100) | 35 (47.9) |
| **Clinical response n (%)** | **n=17** | **n=2** | **n=11** | **n=33** | **n=1** |  |
|  *worse* | 0 (0) | 0 (0) | 1 (9.0) | 4 (12.1) | 0 (0) | 5 (7.8) |
|  *stable* | 2 (11.7) | 2 (100) | 1 (9.0) | 13 (39.4) | 1 (100) | 19 (29.6) |
|  *improvement* | 15 (88.2) | 0 (0) | 9 (81.8) | 16 (48.5) | 0 (0) | 40 (62.5) |
| **Radiological response** **n (%)** | **n=15** | **n=2** | **n=9** | **n=32** | **n=1** |  |
|  *worse* | 0 (0) | 0 (0) | 0 (0) | 6 (18.7) | 0 (0) | 6 (10.1) |
|  *stable* | 1 (6.6) | 0 (0) | 0 (0) | 8 (25) | 0 (0) | 9 (15.2) |
|  *pseudo-progression* | 1 (6.6) | 2 (100) | 0 (0) | 5 (15,6) | 1 (100) | 9 (15.2) |
|  *improvement* | 13 (86.6) | 0 (0) | 9 (100) | 13 (40,6) | 0 (0) | 35 (59.3) |
| **TTP1 (months) Median (range)** | **n=19** 9.8 (3.3-17.6) | **n=3** 5.3 (4.9-18.5) | **n=11**  5.8 (2.7-17.7) | **n=34** 4.0 (1.4-19.3) | **n=2**  (4.0-7.9) | 5.8 (1.4-19.3) |
| **OS (months) Median (range)** | 15.1 (7.2-35.3) | 9.1 (9.1-26.4) | 12.1 (3.6-27.5) | 8.5 (2.4-25.6) | 10.5 (10.2-10.8) | 10.8 (2.4-35.3) |

WT: Wild-type, MUT: mutated, NK: Not know, TTP1: the time to first progression, OS: Overall survival

**Supplementary Table S4: Multivariable logistic regression (Firth’s approach) for predicting the clinical and radiological responses**

|  |  |  |
| --- | --- | --- |
|  | **Clinical Response** | **Radiological Response** |
| Characteristics | **Odds ratio** | **95% CI** | **p-value** | **Odds ratio** | **95% CI** | **p-value** |
| Age at diagnosis | 1.045 | 0.834 - 1.310 | 0.6995 | 0.895 | 0.694 - 1.154 | 0.3936 |
| H3 status  H3.1-K27M H3.3-K27M  | 11.168 | 0.198 - 6.894 | 0.8637 | 18.242 | 0.357 - 190.042 | 0.1877 |
| *TP53* status *TP53* wild-type *TP53* mutated | 10.137 | 0.028 - 0679 | 0.0150 | 10.023 | 0.001 - 0.458 | 0.0133 |

CI: Confidence interval