**Supplementary Table** **A1**

Associations between biomarkers and time to first change in photographic breast appearance in FAST trial patients.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Biomarker** | | **Events /Total (%)** | **Kaplan-Meier 5-year estimate of cumulative incidence event-free (95%CI), %** | **HR (95%CI)** | ***P*-value1** |
| **Primary biomarkers of interest** | | | | | |
| **Lymphocyte apoptosis (%)** | | 21/98 (21.4) | - | 1.00 (0.96-1.05) | 0.8753 |
| **Lymphocyte apoptosis (%)** | |  |  |  |  |
| <16 | | 8/36 (22.2) | 80.0 (62.6-89.9) | 1 | 0.8242 |
| 16-24 | | 8/38 (21.0) | 74.3 (54.2-86.6) | 0.92 (0.34-2.47) | 0.821 |
| >24 | | 5/24 (20.8) | 87.5 (66.1-95.8) | 0.88 (0.29-2.71) | 0.833 |
| **Lymphocyte apoptosis (%)** | |  |  |  |  |
| <15.63 | T1 | 8/33 (24.2) | 78.8 (60.6-89.3) | 1 | 0.6382 |
| 15.63-21.68 | T2 | 7/33 (21.2) | 73.8 (52.2-86.8) | 0.88 (0.32-2.44) | 0.729 |
| >21.68 | T3 | 6/32 (18.7) | 87.5 (70.0-95.1) | 0.78 (0.27-2.24) | 0.665 |
|  |  |  |  |  |  |
| **Total aberrations/cell** | | 21/101 (20.8) | - | 0.75 (0.40-1.41) | 0.3773 |
| **Total aberrations/cell** | |  |  |  |  |
| <6.16 | T1 | 9/35 (25.7) | 78.0 (58.4-89.1) | 1 | 0.4532 |
| 6.16-6.60 | T2 | 7/35 (20.0) | 78.9 (60.5-89.4) | 0.79 (0.29-2.13) | 0.622 |
| >6.60 | T3 | 5/31 (16.1) | 81.5 (55.8-93.0) | 0.66 (0.22-1.99) | 0.505 |
|  |  |  |  |  |  |
| **Foci/cell 4Gy at 24h** | | 19/96 (19.8) | - | 0.55 (0.32-0.95) | 0.0323 |
| **Foci/cell 4Gy at 24h** | |  |  |  |  |
| <9.3 | T1 | 6/33 (18.2) | 84.4 (66.5-93.2) | 1 | 0.3542 |
| 9.3-10.0 | T2 | 10/32 (31.2) | 71.2 (49.2-85.0) | 1.59 (0.57-4.38) | 0.388 |
| >10.0 | T3 | 3/31 (9.7) | 90.3 (72.9-96.8) | 0.48 (0.12-1.92) | 0.323 |
| **Secondary biomarkers of interest** | | | | | |
| **Dicentrics/cell** | | 21/101 (20.8) | - | 0.72 (0.27-1.90) | 0.5053 |
| **Dicentrics/cell** | |  |  |  |  |
| <3.04 | T1 | 10/35 (28.6) | 75.3 (55.9-87.1) | 1 | 0.9922 |
| 3.04-3.42 | T2 | 2/33 (6.1) | 93.7 (77.2-98.4) | 0.22 (0.05-1.00) | 0.028 |
| >3.42 | T3 | 9/33 (27.3) | 69.1 (45.8-84.0) | 1.04 (0.42-2.57) | 0.910 |
|  |  |  |  |  |  |
| **Foci/ cell 0.5Gy at 30min** | | 17/92 (18.5) | - | 0.66 (0.33-1.33) | 0.2463 |
| **Foci/ cell 0.5Gy at 30min** | |  |  |  |  |
| <8.2 | T1 | 6/32 (18.7) | 87.0 (69.2-94.9) | 1 | 0.4342 |
| 8.2-8.7 | T2 | 9/34 (26.5) | 73.0 (52.1-85.9) | 1.56 (0.55-4.41) | 0.384 |
| >8.7 | T3 | 2/26 (7.7) | 92.3 (72.6-98.0) | 0.43 (0.09-2.15) | 0.312 |
|  |  |  |  |  |  |
| **Ratio of 4Gy/0.5Gy**  **(per 0.1 unit change)3** | | 17/89 (19.1) | - | 0.90 (0.63-1.29) | 0.5783 |
| **Ratio of 4Gy/0.5Gy** | |  |  |  |  |
| <1.132 | T1 | 6/30 (20.0) | 77.3 (55.4-89.4) | 1 | 0.2472 |
| 1.132-1.208 | T2 | 8/30 (26.7) | 76.4 (52.6-89.3) | 1.23 (0.43-3.55) | 0.720 |
| >1.208 | T3 | 3/29 (10.3) | 93.1 (75.1-98.2) | 0.43 (0.11-1.71) | 0.243 |
|  |  |  |  |  |  |
| **Micronuclei no cut-off**  **(per 0.1 unit change)3** | | 20/97 (20.6) | - | 1.02 (0.94-1.10) | 0.6503 |
| **Micronuclei no cut-off** | |  |  |  |  |
| <0.320 | T1 | 7/32 (21.9) | 83.3 (64.2-92.8) | 1 | 0.2242 |
| 0.320-0.962 | T2 | 4/32 (12.5) | 87.5 (70.0-95.1) | 0.71 (0.21-2.44) | 0.537 |
| >0.962 | T3 | 9/33 (27.3) | 68.3 (42.7-84.2) | 1.87 (0.68-5.19) | 0.169 |
|  |  |  |  |  |  |
| **Micronuclei >4 cut-off**  **(per 0.1 unit change)3** | | 20/97 (20.6) | - | 0.99 (0.74-1.31) | 0.9203 |
| **Micronuclei >4 cut-off** | |  |  |  |  |
| <0.235 | T1 | 9/31 (29.0) | 76.4 (56.5-88.1) | 1 | 0.6382 |
| 0.235-0.432 | T2 | 5/34 (14.7) | 82.3 (60.8-92.7) | 0.58 (0.19-1.73) | 0.310 |
| >0.432 | T3 | 6/32 (18.7) | 82.6 (62.6-92.5) | 0.81 (0.28-2.31) | 0.727 |
|  |  |  |  |  |  |
| **Nuclear division index (per 0.1 unit change)3** | | 20/96 (20.8) | - | 1.33 (0.70-2.51) | 0.3863 |
| **Nuclear division index** | |  |  |  |  |
| <1.13 | T1 | 8/38 (21.0) | 78.9 (59.4-89.8) | 1 | 0.9092 |
| 1.13 – 1.18 | T2 | 5/30 (16.7) | 82.9 (58.2-93.7) | 0.74 (0.24-2.27) | 0.601 |
| >1.18 | T3 | 7/28 (25.0) | 77.3 (55.9-89.2) | 0.95 (0.34-2.65) | 0.948 |

*1 p-value from log-tank test comparing T2, T3 versus T1 unless otherwise indicated*

*2 p-value from log-tank test for trend across categories*

*3 p-value from Wald test*

*4 Biomarkers re-scaled (multiplied by 10) so Hazard Ratio represents change in 0.1 units of the biomarker.*

**Supplementary Table A2**

Associations between biomarkers and time to first occurrence of breast shrinkage, induration on central axis, telangiectasia, and breast oedema of at least grade 2 in FAST trial patients.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Biomarker** | | **Events /Total (%)** | **KM 5yr estimate of cumulative incidence event-free5 (95%CI), %** | **HR (95%CI)** | ***P*-value1** |
| **Primary biomarkers of interest** | | | | | |
| **Lymphocyte apoptosis (%)** | | 60/100 (60.0) | - | 0.96 (0.93-1.00) | 0.0273 |
| **Lymphocyte apoptosis (%)** | |  |  |  |  |
| <16 | | 26/37 (70.3) | 43.2 (27.2-58.3) | 1 | 0.0172 |
| 16-24 | | 25/39 (64.1) | 51.3 (34.8-65.5) | 1.16 (0.65-2.06) | 0.596 |
| >24 | | 9/24 (37.5) | 79.2 (57.0-90.7) | 0.50 (0.25-1.00) | 0.012 |
| **Lymphocyte apoptosis (%)** | |  |  |  |  |
| <15.63 | T1 | 23/34 (67.6) | 47.1 (29.8-62.5) | 1 | 0.0412 |
| 15.63-21.68 | T2 | 23/33 (69.7) | 45.4 (28.2-61.2) | 1.10 (0.62-1.97) | 0.776 |
| >21.68 | T3 | 14/33 (42.4) | 72.6 (53.9-84.7) | 0.50 (0.25-0.96) | 0.038 |
|  |  |  |  |  |  |
| **Total aberrations/cell** | | 62/103 (60.2) | - | 1.17 (0.82-1.68) | 0.3793 |
| **Total aberrations/cell** | |  |  |  |  |
| <6.16 | T1 | 22/35 (62.9) | 57.1 (39.3-71.5) | 1 | 0.4522 |
| 6.16-6.60 | T2 | 18/36 (50.0) | 58.3 (40.7-72.4) | 0.79 (0.43-1.48) | 0.494 |
| >6.60 | T3 | 22/32 (68.7) | 46.5 (28.7-62.5) | 1.27 (0.70-2.30) | 0.414 |
|  |  |  |  |  |  |
| **Foci/cell 4Gy at 24h** | | 60/98 (61.2) | - | 1.08 (0.81-1.44) | 0.6033 |
| **Foci/cell 4Gy at 24h** | |  |  |  |  |
| <9.3 | T1 | 17/34 (50.0) | 61.5 (43.1-75.5) | 1 | 0.3642 |
| 9.3-10.0 | T2 | 24/33 (72.7) | 45.4 (28.2-61.2) | 1.81 (0.97-3.37) | 0.063 |
| >10.0 | T3 | 19/31 (61.3) | 54.8 (36.0-70.3) | 1.35 (0.70-2.60) | 0.400 |
| **Secondary biomarkers of interest** | | | | | |
| **Dicentrics/cell** | | 62/103 (60.2) | - | 1.16 (0.66-2.04) | 0.6043 |
| **Dicentrics/cell** | |  |  |  |  |
| <3.04 | T1 | 24/36 (66.7) | 52.8 (35.5-67.4) | 1 | 0.9412 |
| 3.04-3.42 | T2 | 17/34 (50.0) | 55.7 (37.6-70.5) | 0.77 (0.41-1.43) | 0.419 |
| >3.42 | T3 | 21/33 (63.6) | 54.5 (36.3-69.6) | 1..03 (0.58-1.86) | 0.918 |
|  |  |  |  |  |  |
| **Foci/ cell 0.5Gy at 30min** | | 56/93 (60.2) | - | 0.78 (0.54-1.12) | 0.1763 |
| **Foci/ cell 0.5Gy at 30min** | |  |  |  |  |
| <8.2 | T1 | 23/33 (69.7) | 51.5 (33.5-66.8) | 1 | 0.2102 |
| 8.2-8.7 | T2 | 20/34 (58.8) | 46.9 (29.6-62.4) | 0.97 (0.53-1.77) | 0.925 |
| >8.7 | T3 | 13/26 (50.0) | 65.4 (44.0-80.2) | 0.64 (0.32-1.26) | 0.188 |
|  |  |  |  |  |  |
| **Ratio of 4Gy/0.5Gy**  **(per 0.1 unit change)4** | | 55/90 (61.1) | - | 1.09 (0.91-1.32) | 0.3453 |
| **Ratio of 4Gy/0.5Gy** | |  |  |  |  |
| <1.132 | T1 | 19/30 (63.3) | 46.2 (27.9-62.7) | 1 | 0.6482 |
| 1.132-1.208 | T2 | 17/30 (56.7) | 56.7 (37.3-72.1) | 0.76 (0.39-1.46) | 0.427 |
| >1.208 | T3 | 19/30 (63.3) | 56.7 (37.3-72.1) | 0.86 (0.45-1.62) | 0.605 |
|  |  |  |  |  |  |
| **Micronuclei no cut-off**  **(per 0.1 unit change)4** | | 60/99 (60.6) | - | 1.00 (0.96-1.04) | 0.9793 |
| **Micronuclei no cut-off** | |  |  |  |  |
| <0.320 | T1 | 20/33 (60.6) | 57.6 (39.1-72.3) | 1 | 0.3082 |
| 0.320-0.962 | T2 | 18/33 (54.5) | 57.6 (39.1-72.3) | 0.91 (0.48-1.73) | 0.757 |
| >0.962 | T3 | 22/33 (66.7) | 45.2 (27.9-61.1) | 1.38 (0.75-2.52) | 0.291 |
|  |  |  |  |  |  |
| **Micronuclei >4 cut-off**  **(per 0.1 unit change)4** | | 60/99 (60.6) | - | 1.02 (0.88-1.19) | 0.8003 |
| **Micronuclei >4 cut-off** | |  |  |  |  |
| <0.235 | T1 | 20/33 (60.6) | 57.6 (39.1-72.3) | 1 | 0.7932 |
| 0.235-0.432 | T2 | 22/34 (64.7) | 47.1 (29.8-62.5) | 1.20 (0.65-2.20) | 0.546 |
| >0.432 | T3 | 18/32 (56.2) | 56.2 (37.6-71.3) | 1.08 (0.57-2.04) | 0.809 |
|  |  |  |  |  |  |
| **Nuclear division index (per 0.1 unit change)4** | | 59/98 (60.2) | - | 0.96 (0.65-1.41) | 0.8243 |
| **Nuclear division index** | |  |  |  |  |
| <1.13 | T1 | 24/39 (61.5) | 43.6 (27.9-58.3) | 1 | 0.2932 |
| 1.13 – 1.18 | T2 | 21/30 (70.0) | 50.0 (31.3-66.1) | 1.18 (0.65-2.12) | 0.658 |
| >1.18 | T3 | 14/29 (48.3) | 68.8 (48.6-82.4) | 0.68 (0.35-0.31) | 0.278 |

*1p-value from log-tank test comparing T2, T3 versus T1 unless otherwise indicated*

*2p-value from log-tank test for trend across categories*

*3p-value from Wald test*

*4Biomarkers re-scaled (multiplied by 10) so Hazard Ratio represents change in 0.1 units of the biomarker.*

*5Kaplan-Meier estimates of cumulative incidence event-free calculated at 5 years 3 months, to allow for follow-up assessments occurring after due date*

**Supplementary Table A3**

Distribution of endpoint events and biomarker descriptive statistics by fractionation for FAST patients used in the analysis.

|  | | **Event/Total (%)** | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **50Gy/25f** | | **30Gy/5f** | | **28.5Gy/5f** | |
| **Mild/marked change in photographic breast appearance** | |  | |  | |  | |
|  | | 3/29 (10) | | 7/35 (80) | | 11/37 (30) | |
| **Any moderate/marked adverse event (breast shrinkage, induration on central axis, telangiectasia or breast oedema)** | | 16/29 (55) | | 24/36 (67) | | 22/37 (60) | |
|  | |  | |  | |  | |
| **Lymphocyte apoptosis**  Median (IQR)  Range | | 20.2 (14.6-25.5)  7.9-50.3 | | 17.3 (11.6-21.7)  6.4-25.3 | | 18.3 (13.2-25.5)  8.0-42.5 | |
| **Total aberrations / cell** | |  | |  | |  | |
| Median (IQR) | | 6.4 (5.9-6.7) | | 6.4 (5.9-6.8) | | 6.4 (5.9-6.8) | |
| Range | | 4.8-7.4 | | 4.8-7.9 | | 4.8-7.6 | |
| **Foci / cell 4Gy at 24h** | |  | |  | |  | |
| Median (IQR) | | 9.9 (9.3-10.8) | | 9.5 (8.9-9.9) | | 9.7 (9.0-10.3) | |
| Range | | 8.7-12.3 | | 7.2-11.5 | | 7.9-11.5 | |
|  | **Event/Total (%)** | | | | | |
| **50Gy/25f** | | **30Gy/5f** | | **28.5Gy/5f** | |
| **Mild/marked change in photographic breast appearance** |  | |  | |  | |
|  | 3/29 (10) | | 7/35 (80) | | 11/37 (30) | |
| **Any moderate/marked adverse event (breast shrinkage, induration on central axis, telangiectasia or breast oedema)** | 16/29 (55) | | 24/36 (67) | | 22/37 (60) | |
|  |  | |  | |  | |
| **Lymphocyte apoptosis**  Median (IQR)  Range | 20.2 (14.6-25.5)  7.9-50.3 | | 17.3 (11.6-21.7)  6.4-25.3 | | 18.3 (13.2-25.5)  8.0-42.5 | |
| **Total aberrations / cell** |  | |  | |  | |
| Median (IQR) | 6.4 (5.9-6.7) | | 6.4 (5.9-6.8) | | 6.4 (5.9-6.8) | |
| Range | 4.8-7.4 | | 4.8-7.9 | | 4.8-7.6 | |
| **Foci / cell 4Gy at 24h** |  | |  | |  | |
| Median (IQR) | 9.9 (9.3-10.8) | | 9.5 (8.9-9.9) | | 9.7 (9.0-10.3) | |
| Range | 8.7-12.3 | | 7.2-11.5 | | 7.9-11.5 | |

*f: fraction*

**Supplementary Table A4**

Associations between biomarkers and time to first grade ≥2 RTOG bladder or bowel toxicity in CHHiP trial patients.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Biomarker** | | **Events /Total (%)** | **KM 5yr estimate of cumulative incidence event-free5 (95%CI), %** | **HR (95%CI)** | ***P*-value1** |
| **Primary biomarkers of interest** | | | | | |
| **Lymphocyte apoptosis (%)** | | 57/296 (19.3) | - | 0.97 (0.94-1.01) | 0.1103 |
| **Lymphocyte apoptosis (%)** | |  |  |  |  |
| <16 | | 27/115 (23.5) | 71.3 (56.9-81.6) | 1 | 0.1482 |
| 16-24 | | 17/97 (17.5) | 83.4 (74.3-89.5) | 0.71 (0.39-1.31) | 0.276 |
| >24 | | 13/84 (15.5) | 77.9 (56.5-89.7) | 0.63 (0.33-1.23) | 0.159 |
| **Lymphocyte apoptosis (%)** | |  |  |  |  |
| <15.14 | T1 | 21/99 (21.2) | 72.9 (56.9-83.8) | 1 | 0.1702 |
| 15.14-22.79 | T2 | 23/99 (23.2) | 77.6 (68.1-84.7) | 1.13 (0.62-2.04) | 0.684 |
| >22.79 | T3 | 13/98 (13.3) | 81.5 (63.5-91.2) | 0.60 (0.30-1.20) | 0.139 |
|  |  |  |  |  |  |
| **Total aberrations/cell** | | 57/297 (19.2) | - | 0.79 (0.54-1.17) | 0.2383 |
| **Total aberrations/cell** | |  |  |  |  |
| <5.58 | T1 | 21/100 (21.0) | 79.0 (69.6-85.7) | 1 | 0.1172 |
| 5.58-6.14 | T2 | 24/98 (24.5) | 71.3 (57.5-81.3) | 1.17 (0.65-2.10) | 0.628 |
| >6.14 | T3 | 12/99 (12.1) | 81.7 (58.3-92.7) | 0.55 (0.27-1.12) | 0.100 |
|  |  |  |  |  |  |
| **Foci/cell 4Gy at 24h** | | 55/275 (20.0) | - | 1.05 (0.80-1.38) | 0.7253 |
| **Foci/cell 4Gy at 24h** | |  |  |  |  |
| <9.6 | T1 | 19/95 (20.0) | 72.2 (51.5-85.2) | 1 | 0.8982 |
| 9.6-10.4 | T2 | 18/91 (19.8) | 80.2 (70.4-87.0) | 1.01 (0.53-1.92) | 0.958 |
| >10.4 | T3 | 18/89 (20.2) | 80.9 (71.1-87.6) | 1.04 (0.55-1.99) | 0.907 |
| **Secondary biomarkers of interest** | | | | | |
| **Dicentrics/cell** | | 57/297 (19.2) | - | 0.64 (0.36-1.15) | 0.1363 |
| **Dicentrics/cell** | |  |  |  |  |
| <2.66 | T1 | 22/101 (21.8) | 78.2 (68.8-85.1) | 1 | 0.0582 |
| 2.66-3.08 | T2 | 24/97 (24.7) | 71.3 (55.2-82.5) | 1.16 (0.65-2.08) | 0.609 |
| >3.08 | T3 | 11/99 (11.1) | 83.1 (65.3-92.3) | 0.48 (0.23-0.99) | 0.038 |
|  |  |  |  |  |  |
| **Foci/ cell 0.5Gy at 30min** | | 51/258 (19.8) | - | 0.75 (0.52-1.10) | 0.1413 |
| **Foci/ cell 0.5Gy at 30min** | |  |  |  |  |
| <8.2 | T1 | 26/99 (26.3) | 73.0 (62.8-80.8) | 1 | 0.0892 |
| 8.2-8.6 | T2 | 11/73 (15.1) | 86.3 (76.0-92.4) | 0.55 (0.27-1.12) | 0.086 |
| >8.6 | T3 | 14/86 (16.3) | 73.3 (50.9-86.7) | 0.59 (0.31-1.13) | 0.117 |
|  |  |  |  |  |  |
| **Ratio of 4Gy/0.5Gy**  **(per 0.1 unit change)4** | | 50/253 (19.8) | - | 1.20 (0.97-1.48) | 0.0963 |
| **Ratio of 4Gy/0.5Gy** | |  |  |  |  |
| <1.155 | T1 | 12/85 (14.1) | 79.0 (57.0-90.6) | 1 | 0.0512 |
| 1.155-1.260 | T2 | 16/84 (19.0) | 80.9 (70.8-87.9) | 1.41 (0.67-2.98) | 0.354 |
| >1.260 | T3 | 22/84 (26.2) | 74.1 (62.9-82.4) | 1.98 (0.98-4.01) | 0.053 |
|  |  |  |  |  |  |
| **Micronuclei no cut-off**  **(per 0.1 unit change)4** | | 57/293 (19.4) | - | 0.93 (0.85-1.02) | 0.1133 |
| **Micronuclei no cut-off** | |  |  |  |  |
| <0.230 | T1 | 26/98 (26.5) | 74.4 (64.5-81.9) | 1 | 0.0212 |
| 0.230-0.469 | T2 | 18/98 (18.4) | 76.7 (60.3-87.0) | 0.67 (0.36-1.22) | 0.186 |
| >0.469 | T3 | 13/97 (13.4) | 80.5 (62.6-90.5) | 0.47 (0.24-0.91) | 0.023 |
|  |  |  |  |  |  |
| **Micronuclei >4 cut-off**  **(per 0.1 unit change)4** | | 57/293 (19.4) | - | 0.77 (0.59-1.00) | 0.0513 |
| **Micronuclei >4 cut-off** | |  |  |  |  |
| <0.201 | T1 | 25/98 (25.5) | 75.4 (65.6-82.8) | 1 | 0.0362 |
| 0.201-0.281 | T2 | 19/98 (19.4) | 76.2 (60.7-86.2) | 0.75 (0.41-1.36) | 0.348 |
| >0.281 | T3 | 13/97 (13.4) | 79.5 (59.2-90.4) | 0.49 (0.25-0.97) | 0.036 |
|  |  |  |  |  |  |
| **Nuclear division index (per 0.1 unit change)4** | | 55/285 (19.3) | - | 1.24 (0.89-1.73) | 0.2063 |
| **Nuclear division index** | |  |  |  |  |
| <1.14 | T1 | 19/103 (18.4) | 71.2 (51.5-84.1) | 1 | 0.0952 |
| 1.14–1.19 | T2 | 13/103 (12.6) | 88.3 (80.4-93.2) | 0.68 (0.33-1.37) | 0.269 |
| >1.19 | T3 | 23/79 (29.1) | 70.9 (59.5-79.6) | 1.69 (0.92-3.11) | 0.081 |

*1p-value from log-tank test comparing T2, T3 versus T1 unless otherwise indicated*

*2p-value from log-tank test for trend across categories*

*3p-value from Wald test*

*4Biomarkers re-scaled (multiplied by 10) so Hazard Ratio represents change in 0.1 units of the biomarker*

*5Kaplan-Meier estimates of cumulative incidence event-free calculated at 5 years 3 months, to allow for follow-up assessments occurring after due date*

**Supplementary Table A5**

Distribution of primary endpoint events and biomarker descriptive statistics by fractionation for CHHiP patients used in the analysis.

|  | **Event/Total (%)** | | |
| --- | --- | --- | --- |
| **74/37f** | **60Gy/20f** | **57/19f** |
| **Bladder or bowel grade ≥2 toxicity** |  |  |  |
|  | 17/103 (17) | 19/96 (20) | 21/98 (21) |
| **Lymphocyte apoptosis**  Median (IQR)  Range | 18.2 (13.5-24.4)  5.2-61.9 | 17.8 (13.5-24.7)  4.6-52.4 | 19.2 (14.0-25.4)  6.3-46.4 |
| **Total aberrations / cell** |  |  |  |
| Median (IQR) | 5.9 (5.2-6.4) | 5.9 (5.6-6.3) | 5.9 (5.3-6.2) |
| Range | 4.1-8.2 | 4.7-8.4 | 4.3-7.1 |
| **Foci / cell 4Gy at 24h** |  |  |  |
| Median (IQR) | 9.9 (9.2-10.6) | 10.1 (9.3-10.7) | 10.0 (9.3-10.6) |
| Range | 7.6-12.8 | 7.8-12.0 | 7.6-11.7 |

*f: fraction*

**Supplementary Table A6**

Associations between biomarkers and time to first grade ≥2 RTOG bladder toxicity in CHHiP trial patients.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Biomarker** | | **Events /Total (%)** | **KM 5yr estimate of cumulative incidence event-free5 (95%CI), %** | **HR (95%CI)** | ***P*-value1** |
| **Primary biomarkers of interest** | | | | | |
| **Lymphocyte apoptosis (%)** | | 146/296 (49.3) | - | 1.00 (0.98-1.02) | 0.8433 |
| **Lymphocyte apoptosis (%)** | |  |  |  |  |
| <16 | | 56/115 (48.7) | 50.5 (40.8-59.4) | 1 | 0.7672 |
| 16-24 | | 47/97 (48.4) | 51.2 (40.8-60.7) | 0.97 (0.66-1.43) | 0.887 |
| >24 | | 43/84 (51.2) | 46.5 (34.8-57.5) | 1.07 (0.72-1.59) | 0.742 |
| **Lymphocyte apoptosis (%)** | |  |  |  |  |
| <15.14 | T1 | 46/99 (46.5) | 52.4 (41.8-62.0) | 1 | 0.8152 |
| 15.14-22.79 | T2 | 53/99 (53.5) | 46.2 (36.1-55.7) | 1.21 (0.81-1.79) | 0.342 |
| >22.79 | T3 | 47/98 (48.0) | 50.1 (39.2-60.1) | 1.05 (0.70-1.58) | 0.817 |
|  |  |  |  |  |  |
| **Total aberrations/cell** | | 146/297 (49.2) | - | 0.83 (0.66-1.05) | 0.1233 |
| **Total aberrations/cell** | |  |  |  |  |
| <5.58 | T1 | 55/100 (55.0) | 43.6 (33.4-53.4) | 1 | 0.1282 |
| 5.58-6.14 | T2 | 49/98 (50.0) | 48.5 (37.8-58.4) | 0.88 (0.60-1.29) | 0.499 |
| >6.14 | T3 | 42/99 (42.4) | 57.5 (47.2-66.6) | 0.73 (0.49-1.09) | 0.129 |
|  |  |  |  |  |  |
| **Foci/cell 4Gy at 24h** | | 135/275 (49.1) | - | 0.96 (0.81-1.14) | 0.6623 |
| **Foci/cell 4Gy at 24h** | |  |  |  |  |
| <9.6 | T1 | 48/95 (50.5) | 47.3 (36.3-57.5) | 1 | 0.8002 |
| 9.6-10.4 | T2 | 41/91 (45.0) | 54.9 (44.1-64.4) | 0.91 (0.60-1.38) | 0.680 |
| >10.4 | T3 | 46/89 (51.7) | 47.9 (37.2-57.9) | 1.06 (0.70-1.58) | 0.789 |
| **Secondary biomarkers of interest** | | | | | |
| **Dicentrics/cell** | | 146/297 (49.2) | - | 0.75 (0.53-1.07) | 0.1183 |
| **Dicentrics/cell** | |  |  |  |  |
| <2.66 | T1 | 54/101 (53.5) | 45.4 (35.1-55.1) | 1 | 0.0642 |
| 2.66-3.08 | T2 | 55/97 (56.7) | 43.2 (33.2-52.8) | 1.17 (0.81-1.71) | 0.394 |
| >3.08 | T3 | 37/99 (37.4) | 60.3 (49.0-69.9) | 0.66 (0.44-1.01) | 0.051 |
|  |  |  |  |  |  |
| **Foci/ cell 0.5Gy at 30min** | | 125/258 (48.4) | - | 0.72 (0.57-0.92) | **0.007**3 |
| **Foci/ cell 0.5Gy at 30min** | |  |  |  |  |
| <8.2 | T1 | 55/99 (55.6) | 43.5 (33.3-53.2) | 1 | 0.0192 |
| 8.2-8.6 | T2 | 36/73 (49.3) | 50.7 (38.7-61.4) | 0.85 (0.56-1.30) | 0.459 |
| >8.6 | T3 | 34/86 (39.5) | 57.9 (45.8-68.3) | 0.60 (0.39-0.92) | 0.017 |
|  |  |  |  |  |  |
| **Ratio of 4Gy/0.5Gy**  **(per 0.1 unit change)4** | | 123/253 (48.6) | - | 1.13 (0.98-1.29) | 0.0803 |
| **Ratio of 4Gy/0.5Gy** | |  |  |  |  |
| <1.155 | T1 | 34/85 (40.0) | 57.7 (45.6-68.0) | 1 | 0.0302 |
| 1.155-1.260 | T2 | 42/84 (50.0) | 49.9 (38.8-60.0) | 1.40 (0.89-2.20) | 0.141 |
| >1.260 | T3 | 47/84 (55.9) | 43.2 (32.2-53.7) | 1.63 (1.05-2.53) | 0.029 |
|  |  |  |  |  |  |
| **Micronuclei no cut-off**  **(per 0.1 unit change)4** | | 144/293 (49.1) | - | 0.97 (0.93-1.02) | 0.2933 |
| **Micronuclei no cut-off** | |  |  |  |  |
| <0.230 | T1 | 49/98 (50.0) | 48.9 (38.2-58.7) | 1 | 0.4162 |
| 0.230-0.469 | T2 | 52/98 (53.1) | 45.7 (35.2-55.5) | 1.03 (0.70-1.53) | 0.861 |
| >0.469 | T3 | 43/97 (44.3) | 54.7 (43.9-64.2) | 0.84 (0.56-1.27) | 0.416 |
|  |  |  |  |  |  |
| **Micronuclei >4 cut-off**  **(per 0.1 unit change)4** | | 144/293 (49.1) | - | 0.87 (0.74-1.02) | 0.0973 |
| **Micronuclei >4 cut-off** | |  |  |  |  |
| <0.201 | T1 | 53/98 (54.1) | 45.9 (35.8-55.4) | 1 | 0.1632 |
| 0.201-0.281 | T2 | 48/98 (49.0) | 49.0 (38.0-59.0) | 0.85 (0.57-1.25) | 0.402 |
| >0.281 | T3 | 43/97 (44.3) | 54.4 (43.5-64.0) | 0.75 (0.50-1.12) | 0.165 |
|  |  |  |  |  |  |
| **Nuclear division index (per 0.1 unit change)4** | | 141/285 (49.5) | - | 1.04 (0.83-1.31) | 0.7283 |
| **Nuclear division index** | |  |  |  |  |
| <1.14 | T1 | 51/103 (49.5) | 49.6 (39.3-59.0) | 1 | 0.8112 |
| 1.14–1.19 | T2 | 50/103 (48.5) | 49.1 (38.3-59.1) | 0.96 (0.65-1.41) | 0.822 |
| >1.19 | T3 | 40/79 (50.6) | 49.1 (37.6-59.6) | 1.06 (0.70-1.60) | 0.782 |

*1 p-value from log-tank test comparing T2, T3 versus T1 unless otherwise indicated*

*2 p-value from log-tank test for trend across categories*

*3 p-value from Wald test*

*4 Biomarkers re-scaled (multiplied by 10) so Hazard Ratio represents change in 0.1 units of the biomarker*

*5 Kaplan-Meier estimates of cumulative incidence event-free calculated at 5 years 3 months, to allow for follow-up assessments occurring after due date*

**Supplementary Table A7**

Associations between biomarkers and time to first grade ≥2 RTOG bowel toxicity in CHHiP trial patients.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Biomarker** | | **Events /Total (%)** | **KM 5yr estimate of cumulative incidence event-free5 (95%CI), %** | **HR (95%CI)** | ***P*-value1** |
| **Primary biomarkers of interest** | | | | | |
| **Lymphocyte apoptosis (%)** | | 102/296 (34.5) | - | 0.98 (0.96-1.00) | 0.0943 |
| **Lymphocyte apoptosis (%)** | |  |  |  |  |
| <16 | | 45/115 (39.1) | 60.3 (50.6-68.6) | 1 | 0.0672 |
| 16-24 | | 35/97 (36.1) | 62.1 (51.0-71.5) | 0.93 (0.60-1.44) | 0.743 |
| >24 | | 22/84 (26.2) | 70.5 (57.2-80.4) | 0.61 (0.37-1.02) | 0.055 |
| **Lymphocyte apoptosis (%)** | |  |  |  |  |
| <15.14 | T1 | 37/99 (37.4) | 61.8 (51.3-70.7) | 1 | 0.0982 |
| 15.14-22.79 | T2 | 40/99 (40.4) | 57.9 (47.0-67.4) | 1.18 (0.75-1.84) | 0.469 |
| >22.79 | T3 | 25/98 (25.5) | 71.9 (60.1-80.7) | 0.64 (0.38-1.06) | 0.077 |
|  |  |  |  |  |  |
| **Total aberrations/cell** | | 102/297 (34.3) | - | 0.96 (0.73-1.27) | 0.7913 |
| **Total aberrations/cell** | |  |  |  |  |
| <5.58 | T1 | 35/100 (35.0) | 62.1 (50.7-71.6) | 1 | 0.6962 |
| 5.58-6.14 | T2 | 34/98 (34.7) | 65.3 (55.0-73.8) | 0.97 (0.60-1.55) | 0.871 |
| >6.14 | T3 | 33/99 (33.3) | 64.4 (53.0-73.7) | 0.91 (0.56-1.46) | 0.697 |
|  |  |  |  |  |  |
| **Foci/cell 4Gy at 24h** | | 96/275 (34.9) | - | 1.03 (0.84-1.27) | 0.7573 |
| **Foci/cell 4Gy at 24h** | |  |  |  |  |
| <9.6 | T1 | 34/95 (35.8) | 64.2 (53.7-72.9) | 1 | 0.9292 |
| 9.6-10.4 | T2 | 30/91 (33.0) | 65.6 (54.3-74.7) | 0.91 (0.56-1.49) | 0.698 |
| >10.4 | T3 | 32/89 (36.0) | 60.4 (48.0-70.7) | 1.02 (0.63-1.66) | 0.911 |
| **Secondary biomarkers of interest** | | | | | |
| **Dicentrics/cell** | | 102/297 (34.3) | - | 0.86 (0.56-1.32) | 0.4933 |
| **Dicentrics/cell** | |  |  |  |  |
| <2.66 | T1 | 36/101 (35.6) | 61.9 (50.8-71.3) | 1 | 0.3932 |
| 2.66-3.08 | T2 | 36/97 (37.1) | 60.3 (48.6-70.1) | 1.01 (0.64-1.61) | 0.938 |
| >3.08 | T3 | 30/99 (30.3) | 69.6 (59.5-77.7) | 0.81 (0.50-1.31) | 0.377 |
|  |  |  |  |  |  |
| **Foci/ cell 0.5Gy at 30min** | | 91/258 (35.3) | - | 0.88 (0.66-1.17) | 0.3723 |
| **Foci/ cell 0.5Gy at 30min** | |  |  |  |  |
| <8.2 | T1 | 38/99 (38.4) | 59.6 (48.3-69.1) | 1 | 0.2812 |
| 8.2-8.6 | T2 | 27/73 (37.0) | 60.3 (46.9-71.3) | 0.94 (0.58-1.54) | 0.812 |
| >8.6 | T3 | 26/86 (30.2) | 69.2 (58.1-77.9) | 0.76 (0.46-1.25) | 0.277 |
|  |  |  |  |  |  |
| **Ratio of 4Gy/0.5Gy**  **(per 0.1 unit change)4** | | 87/253 (34.4) | - | 1.09 (0.93-1.29) | 0.2763 |
| **Ratio of 4Gy/0.5Gy** | |  |  |  |  |
| <1.155 | T1 | 27/85 (31.8) | 68.1 (57.0-76.9) | 1 | 0.4042 |
| 1.155-1.260 | T2 | 28/84 (33.3) | 66.4 (55.2-75.5) | 1.08 (0.63-1.83) | 0.791 |
| >1.260 | T3 | 32/84 (38.1) | 56.4 (43.0-67.8) | 1.24 (0.74-2.07) | 0.400 |
|  |  |  |  |  |  |
| **Micronuclei no cut-off**  **(per 0.1 unit change)4** | | 102/293 (34.8) | - | 0.90 (0.84-0.97) | **0.0073** |
| **Micronuclei no cut-off** | |  |  |  |  |
| <0.230 | T1 | 39/98 (39.8) | 57.6 (46.3-67.4) | 1 | 0.0202 |
| 0.230-0.469 | T2 | 40/98 (40.8) | 57.4 (46.3-67.1) | 1.02 (0.65-1.58) | 0.931 |
| >0.469 | T3 | 23/97 (23.7) | 76.2 (66.4-83.5) | 0.54 (0.32-0.90) | 0.016 |
|  |  |  |  |  |  |
| **Micronuclei >4 cut-off**  **(per 0.1 unit change)4** | | 102/293 (34.8) | - | 0.82 (0.68-1.00) | 0.0473 |
| **Micronuclei >4 cut-off** | |  |  |  |  |
| <0.201 | T1 | 35/98 (35.7) | 62.5 (51.4-71.7) | 1 | 0.0322 |
| 0.201-0.281 | T2 | 47/98 (48.0) | 49.5 (38.3-59.7) | 1.42 (0.91-2.20) | 0.112 |
| >0.281 | T3 | 20/97 (20.6) | 79.3 (69.8-86.1) | 0.52 (0.30-0.91) | 0.021 |
|  |  |  |  |  |  |
| **Nuclear division index (per 0.1 unit change)4** | | 98/285 (34.4) | - | 1.13 (0.88-1.47) | 0.3433 |
| **Nuclear division index** | |  |  |  |  |
| <1.14 | T1 | 33/103 (32.0) | 65.4 (54.2-74.6) | 1 | 0.2932 |
| 1.14–1.19 | T2 | 34/103 (33.0) | 65.0 (54.1-73.9) | 1.05 (0.65-1.69) | 0.847 |
| >1.19 | T3 | 31/79 (39.2) | 60.6 (49.0-70.4) | 1.31 (0.80-2.14) | 0.278 |

*1 p-value from log-tank test comparing T2, T3 versus T1 unless otherwise indicated*

*2p-value from log-tank test for trend across categories*

*3p-value from Wald test*

*4Biomarkers re-scaled (multiplied by 10) so Hazard Ratio represents change in 0.1 units of the biomarker*

*5Kaplan-Meier estimates of cumulative incidence event-free calculated at 5 years 3 months, to allow for follow-up assessments occurring after due date*