Supplementary material

Integration of Serum Metabolomics into Clinical Assessment to Improve Outcome Prediction of Metastatic Soft Tissue Sarcoma Patients Treated with Trabectedin

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Table S1. Serum levels of 53 amino acids, 15 bile acids and urea, determined by metabolomics profiling in patients with metastatic soft tissue sarcoma.

| Metabolite | Mean (SD), M | Median, M | Range |
|------------|--------------|-----------|-------|
| 3-Methylhistidine | 3.98 (1.33) | 4.01 | 1.81–7.59 |
| 2-Aminoacidic acid | NA | – | – |
| 2-Aminobutyric acid | NA | – | – |
| 3-Aminoisobutyric acid | NA | – | – |
| 1-Methylhistidine | 4.84 (5.72) | 2.70 | 0.24–22.29 |
| 5-Hydroxylysine | 0.20 (0.11) | 0.18 | 0.10–0.62 |
| Asymmetric dimethyl-arginine (ADMA) | 0.53 (0.08) | 0.52 | 0.33–0.71 |
| Agmatine | 6.88 (6.62) | 5.02 | 3.18–35.09 |
| Alanine | 358.37 (114.16) | 355.01 | 147.11–559.64 |
| Anserine | NA | – | – |
| Arginine | 59.37 (16.19) | 59.22 | 34.46–97.09 |
| Argininosuccinic acid | NA | – | – |
| Asparagine | 44.71 (9.52) | 44.11 | 22.45–63.56 |
| Aspartic acid | 7.39 (2.65) | 6.78 | 3.34–14.77 |
| Beta-alanine | NA | – | – |
| Carnosine | NA | – | – |
| Citrulline | 33.64 (11.07) | 34.79 | 12.22–58.95 |
| Creatinine | 85.35 (22.01) | 86.34 | 42.85–120.70 |
| Cystathionine | 0.50 (0.29) | 0.44 | 0.16–1.27 |
| Cysteine | NA | – | – |
| Cystine | 13.06 (13.11) | 9.59 | 1.33–47.06 |
| Ethanolamine | 6.66 (1.47) | 6.97 | 3.75–9.26 |
| Gamma-aminobutyric acid | NA | – | – |
| Glutamic acid | 95.24 (69.28) | 78.65 | 30.46–376.71 |
| Glutamine | 585.21 (118.95) | 595.57 | 199.51–764.53 |
| Glycine | 215.91 (62.36) | 213.13 | 129.34–429.64 |
| Histidine | 73.41 (12.19) | 76.46 | 46.67–92.08 |
| Homocitrulline | 2.48 (1.81) | 2.25 | 0.83–8.81 |
| Homocystine | NA | – | – |
| Isoleucine | 59.04 (13.08) | 59.59 | 35.71–86.48 |
| Kynurenine | 1.55 (0.62) | 1.35 | 0.75–2.91 |
| Leucine | 146.39 (28.47) | 151.36 | 80.66–187.99 |
| Lysine | 200.71 (35.44) | 200.69 | 132.85–272.86 |
| Methionine | 27.06 (7.27) | 28.60 | 14.83–45.21 |
| Methionine sulfoxide | 0.20 (0.11) | 0.17 | 0.07–0.64 |
| N-Acetylputrescine | NA | – | – |
| O-Phosphorylethanolamine | NA | – | – |
| O-Phosphoserine | NA | – | – |
| Substance                                | Mean (SD) | Minimum | Maximum |
|------------------------------------------|-----------|---------|---------|
| Ornithine                                | 75.69 (16.92) | 75.93 | 39.53–102.09 |
| Phenylalanine                            | 70.76 (11.40) | 69.57 | 54.80–93.64 |
| Phenylethylamine                         | 0.48 (0.13) | 0.46 | 0.13–0.71 |
| Proline                                  | 234.76 (71.76) | 256.47 | 91.43–339–48 |
| Sarcosine                                | NA        | –       | –       |
| Symmetric dimethyl-arginine (SDMA)       | 0.42 (0.09) | 0.42 | 0.27–0.60 |
| Serine                                   | 103.34 (21.61) | 102.28 | 63.84–145.72 |
| Serotonin                                | 0.04 (0.06) | 0.02 | 0.00–0.27 |
| Taurine                                  | 12.26 (2.97) | 11.53 | 6.90–19.23 |
| Threonine                                | 141.43 (30.29) | 136.14 | 86.84–198.10 |
| Trans-4-hydroxyproline                   | 1.31 (0.69) | 1.18 | 0.51–3.42 |
| Tryptophan                               | 46.13 (10.88) | 46.12 | 16.17–62.91 |
| Tyrosine                                 | 62.52 (15.45) | 60.15 | 38.75–97.69 |
| Urea                                     | 6.4 10^(1.6 10^3) | 5947.18 | 4.0 10^3–1.1 10^3 |
| Valine                                   | 197.87 (29.68) | 193.44 | 145.42–265.92 |
| **Bile acid**                            |           |         |         |
| Cholic acid (CA)                         | 0.20 (0.31) | 0.06 | 0.01–1.13 |
| Chenodeoxycholic acid (CDCA)             | 0.29 (0.44) | 0.18 | 0.011–1.87 |
| Deoxycholic acid (DCA)                   | 0.45 (0.44) | 0.31 | 0.02–1.70 |
| Glycocholic acid (GCA)                   | 0.37 (0.39) | 0.28 | 0.00–1.41 |
| Glycochenodeoxycholic acid (GCDCA)       | 1.21 (0.94) | 1.09 | 0.06–3.39 |
| Glycodeoxycholic acid (GDCA)             | 0.49 (0.48) | 0.32 | 0.01–1.72 |
| Glycolithocholic acid (GLCA)             | 0.02 (0.03) | 0.01 | 0.00–0.12 |
| Glycoursodeoxycholic acid (GUDCA)        | 0.12 (0.19) | 0.06 | 0.00–0.86 |
| Lithocholic acid (LCA)                   | 0.01 (0.01) | 0.01 | 0.00–0.03 |
| Taurocholic acid (TCA)                   | 0.10 (0.18) | 0.04 | 0.00–0.68 |
| Taurochenodeoxycholic acid (TCDCA)       | 0.28 (0.34) | 0.17 | 0.01–1.56 |
| Taurodeoxycholic acid (TDCA)             | 0.09 (0.15) | 0.06 | 0.01–0.74 |
| Taurolithocholic acid (TLCA)             | 0.01 (0.004) | 0.003 | 0.00–0.02 |
| Taursodeoxycholic acid (TUDCA)           | 0.01 (0.01) | 0.00 | 0.00–0.06 |
| Ursodeoxycholic acid (UDCA)              | 0.07 (0.06) | 0.04 | 0.01–0.24 |

NA, data not available, concentrations below the low limit of quantification.
Figure S1. Kaplan-Meier curves of overall survival in patients with metastatic soft tissue sarcoma. (a) L-sarcomas (leiomyosarcomas and liposarcomas, n = 9, blue) vs. other histotypes (n = 15). (b) Performance status score of 0 (n = 13, blue) vs. 1 (n = 11). (c) Tumor grade G3 (n = 16, blue) vs. G2 (n = 8). (d) Age < 65 (n = 15, blue) vs. ≥ 65 years (n = 9). (e) Absolute neutrophil count < 7.5 10⁹ cells/L (n = 21, blue) vs. ≥ 7.5 10⁹/L (n = 3). (f) Hemoglobin < 12 g/dL (n = 9, blue) vs. ≥ 12 g/dL (n = 15). (g) Albumin < 3.5 g/dL (n = 7, blue) vs. ≥ 3.5 g/dL (n = 17). (h) Lactate dehydrogenase < 320 U/L (n = 12, blue) vs. ≥ 320 U/L (n = 12).
Figure S2. Correlation heat map of serum metabolites based on Pearson’s correlation coefficient. Positive correlations are shown in red, and negative correlations are shown in blue. The circle marks a cluster of bile acids whose levels correlate. Abbreviations are defined in Table S1.
Figure S3. Quartile stratification of STS patients according to the risk score from the Cox regression model. In the box-and-whisker plot, the central box represents the values from the lower to upper quartile and the middle line the median. Patients with a risk score > or ≤ the upper quartile value (-6.19) were included in the H-Risk (orange) and LM-Risk groups (blue), respectively.

Figure S4. Diagnostic discriminatory power of serum citrulline and hemoglobin, by receiving operator characteristic curve analysis. For citrulline, the area under the curve was 0.926, with a sensitivity of 100% and a specificity of 77.8%. The cut-off value was 33.7 µM. For hemoglobin, the area under the curve was 0.870, with a sensitivity of 100% and a specificity of 72.2%. The cut-off value was of 12.4 g/dL.