Supplementary Materials: Anti-tumor Activity and Epigenetic Impact of the Polyphenol Oleacein in Multiple Myeloma

Giada Juli, Manuela Oliverio, Dina Bellizzi, Maria Eugenia Gallo Cantafio, Katia Grillone, Giuseppe Passarino, Carmela Colica, Monica Nardi, Marco Rossi, Antonio Procopio, Piersandro Tagliaferri, Pierfrancesco Tassone and Nicola Amodio

Table S1. Characteristics of MM cell lines.

<table>
<thead>
<tr>
<th>MM Cell Line</th>
<th>Cytogenetic</th>
<th>TP53 Status*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMO-1</td>
<td>t(12;14)</td>
<td>WT</td>
</tr>
<tr>
<td>AMO-bzb</td>
<td>t(12;14)</td>
<td>WT</td>
</tr>
<tr>
<td>OPM2</td>
<td>t(4;14)</td>
<td>HD</td>
</tr>
<tr>
<td>MM1s</td>
<td>t(14;16)</td>
<td>WT</td>
</tr>
<tr>
<td>NCI-H929</td>
<td>t(4;14)</td>
<td>HD</td>
</tr>
<tr>
<td>RPMI-8226</td>
<td>t(14;16)</td>
<td>HD</td>
</tr>
<tr>
<td>JJN3</td>
<td>t(14;16)</td>
<td>HD</td>
</tr>
<tr>
<td>U266</td>
<td>t(11;14)</td>
<td>HD</td>
</tr>
</tbody>
</table>

*WT indicates wild type; HD indicates homozygous mutation or homozygous deletion; MM indicates multiple myeloma.

Figure S1. Western Blot (WB) of pro-caspase 7, cleaved caspase 7, pro-caspase 9 and cleaved caspase 9 in NCI-H929 cells after 24 hours of oleacein treatment.

Figure S2. Histone deacetylase (HDAC) activity was determined in JJN3 cells treated with oleacein, as reported in materials and methods; Trichostatin A (TSA) was used as positive control. Results are expressed as % of HDAC activity as compared to DMSO-treated cells. * p < 0.05 as compared to vehicle.
Figure S3. miR-29b and miR-22 expression levels were determined by qRT-PCR in JJN3 cells treated for 24 hours with oleacein; miRNA expression was normalized on RNU44. *p < 0.05 as compared to vehicle.

Figure S4. Cell Titer Glo (CTG) assay was performed on NCI-H929 cells treated with oleacein (2.5, 5.0 or 10.0 µM) and bortezomib (1.0, 2.0 and 5.0 nM). Results are expressed as the percentage of the viability of vehicle-treated cells. The right panel reports values of fraction affected (Fa) and combination indexes (CI) for each drug combination, as calculated by the CalcuSyn software, in a triplicate experiment.
Figure S5. Whole blots for Figure 2.
Figure S6. Whole blots for Figure 3.
Cancers 2019, 11, 990

---

**Figure Legend:**

The figure shows Western blot analysis of HDACs in cells treated with OLE at different concentrations (2.6, 6, 10 μM) compared to vehicle control. The molecular weight markers for proteins are indicated at the left side of each lane: 250, 150, 100, 75, 50, 37, 25, 20, 15, 10, 7, and 5 kDa. The proteins analyzed include HDAC6, HDAC4, HDAC1, HDAC2, HDAC3, and GAPDH.

**Key Points:**
- **HDAC6**: Shows a decrease in expression with increasing OLE concentration.
- **HDAC4**: Exhibits a similar trend to HDAC6.
- **HDAC1**: Appears to be less affected by OLE treatment compared to HDAC6 and HDAC4.
- **HDAC2**: No significant change in expression across the OLE concentrations.
- **HDAC3**: Similar expression levels as HDAC2.
- **GAPDH**: Used as a loading control and remains constant across all conditions.

**Conclusion:** The results suggest that OLE may have a differential effect on the expression of HDACs, with HDAC6 and HDAC4 being more sensitive to OLE treatment compared to HDAC1, HDAC2, and HDAC3.
Figure S7. Whole blots for Figure 4.
Figure S8. Whole blots for Figure 5.
Figure S9. Whole blots for Figure S1.

© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).