Background
- Bioactive compounds from marine organisms offer a source of anti-tumor drugs.
- In the FFP project special (Sponge Enzymes and Cells for Innovative Applications) we screened marine sponge extracts and identified two acetylenic compounds (AA1 and AA2) from Cribrochalina vasculum with anti-tumor potential (Zovko, Viktorsson et al., Mol Cancer Ther 2014). (Fig.1A-B)
- Both compounds triggered cell death and blocked proliferation signal in non-small cell lung cancer (NSCLC) and other tumor cells but not in normal cells e.g. PMBC and lung fibroblasts (Zovko, Viktorsson et al., Mol Cancer Ther 2014). (Fig.1B-C)
- The compounds also inhibited insulin like growth factor receptor (IGF-1R) β phosphorylation and cellular thermal shift assay (CESTA) confirmed binding of AA1 to IGF-1R (Zovko, Novak et al., Oncotarget 2016). (Fig.1C)
- Importantly, AA1 also caused specific degradation of IGF-1R β in tumor cells. (Fig.1C)

Aim
- To investigate if the derived marine compound AA1 / AA2 could induce cytotoxicity in NSCLC cells derived from pleural effusion.
- To analyze AA1 – induced anti-tumor efficacy in IGF-1R driven multiple myeloma (MM) tumors grafted into SCID-mice.

Methods
Cell culture and cytotoxicity assessment: NSCLC U-1810, primary NSCLC cells obtained from pleural effusion, multiple myeloma cells (RPMI-8226)(Table 1) were profiled for AA1 or AA2 cytotoxicity at 72h post drug treatment using MTT cell viability assay. Viability is given as % of DMSO-treated controls.

Table 1. Tumor cell lines and primary cells used in the current study

<table>
<thead>
<tr>
<th>Tumor cell line</th>
<th>Primary cells used in the current study</th>
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<tbody>
<tr>
<td>U-1810</td>
<td>NSCLC, large cell</td>
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<tr>
<td>H1975</td>
<td>NSCLC, adeno</td>
</tr>
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<td>PE002</td>
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<tr>
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<tr>
<td>PE011</td>
<td>NSCLC, adeno</td>
</tr>
<tr>
<td>U1810</td>
<td>Multiple Myeloma ND</td>
</tr>
</tbody>
</table>

IGF-1R expression analysis: Basal IGF-1R expression in PE cells and in RPMI-8226 MM cells before and after treatment with AA1 was analyzed using flow cytometry with total-α and p-IGF-1R antibody, by Western blotting or by immunofluorescence using Atto-488 nM-conjugated IGF-1R affibody.

In vivo tumor efficacy in RPMI-8226 xenograft tumors: SCID mice were injected s.c. with 2*10^6 RPMI-8226 MM cells. Tumors appeared approximately one month (25 days) after injection and 8-10 mm developed tumors. When the tumor volume reached 167 mm^3 (v=1/6 * l * w^2 * 2), the animals were treated i.p. biweekly for 3 weeks with:
- AA1 - 12.5 mg/kg (n=2)
- Bortezomib (VEGCADE) - 1mg/kg (n=2)
- Sham - DMSO 1:2 (n=2, vehicle used for AA1) (n=2)

Figure 3. AA1 has anti-tumor activity in multiple myeloma cells in vitro. (A) Cell morphology (B) 2D cultures of RPMI-8226 cells after 72h treatment with 1 µM AA1.

Figure 4. AA1 induced cytotoxicity and survival in MM SCID-mouse xenografts.

Figure 5. AA1 reduced proliferation and survival in MM SCID-mouse xenografts.

Results

Conclusions
- We demonstrate that acetylenic compounds from marine sponge hold anti-tumor activity in both NSCLC and MM.
- Our results illustrate that these compounds may offer a way to treat IGF-1R driven tumors and suggest IGF-1R β phosphorylation to be a marker of responsiveness.

References

Funding agencies

Marine sponge compounds offer a novel way to combat IGF-1R-driven tumors
Adam Sierakowiak, Petra Hägg, Ravi Saini, Veslilaci Arapi, Ana Zovko, Metka Novak, Theresa Holmlund, Therese Backe, Dima Kovalerchick, Andrea Alimonti, Katarina Farnegard, Yvonne I. Ivan, Shnuel Carmeli, Kristina Viktorsson, Rolf Lewensohn

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We are currently developing a library of different compounds for cancer treatment based on our findings in the marine sponges.