Radium-223 dichloride (Ra-223, Xofigo®), a targeted alpha therapy, is currently used for the treatment of patients with bone metastases (Corey et al., 2009; Parker et al., 2014). Ra-223 is a calcium-mimetic that selectively binds to hydroxyapatite in bone and targets areas of increased bone turnover. Its uptake into the bone matrix is mediated by osteoblasts (Suominen et al., 2011, 2015).

Ra-223 inhibits disease progression by reducing tumor growth and tumor-induced pathological bone reaction in breast and prostate cancer mouse models (Suominen et al., 2015, 2017).

The aim of this study was to investigate the effects of Ra-223, bortezomib and their combination on myeloma cell proliferation in vitro and on a myeloma bone disease model in mice.

### Materials and Methods

<table>
<thead>
<tr>
<th>Objective</th>
<th>Methods</th>
</tr>
</thead>
</table>
| Ra-223 and bortezomib inhibited proliferation of MM cells in vitro | Cell line proliferation assays were performed with human plasma cell leukemia (JJN-3, L-363), human MM (LP-1, MOLP-8, RPMI-8226 and OPM-2), and mouse multiple myeloma (MPM-1, MP-11, C13.MP) cell lines obtained from the American Type Culture Collection (ATCC), and cultured in RPMI-1640 (RPMI-8226 and OPM-2) or Iscove’s Modified Dulbecco’s Medium (IMDM, RPMI-8226, OPM-2, RPMI-8226 and OPM-2) containing 10% fetal bovine serum (FBS). Cells were treated with Ra-223 (300 Ci/mmol, 24 hours) and/or bortezomib (1 nM). BrdU incorporation was measured using the Cell Proliferation ELISA (BrdU) (Roche), and an increase of mitotic area was observed in the combination group. (Figures 2A, 2B, 2C, 2D)
| Combination of Ra-223 and bortezomib resulted in higherRa-223 incorporation to bone in vivo and combination therapy based on total activity measurements | Representative X-ray images (at sacrifice) of hind limbs of mice treated with vehicle (EtOH), bortezomib (1 nM), Ra-223 (300 Ci/mmol), vehicle + Ra-223, and bortezomib + Ra-223. The percentage of Ra-223 incorporation to bone was determined by scintigraphy and histology. (Figure 1A, 1B, 1C, 1D)

### Results

<table>
<thead>
<tr>
<th>Objective</th>
<th>Summary of the inhibitory effects of bortezomib, Ra-223, and Ra-223 in combination with bortezomib on myeloma cell proliferation.</th>
<th>Table 1</th>
<th>Table 2</th>
</tr>
</thead>
</table>
| Ra-223 and bortezomib inhibited proliferation of MM cells in vitro | The number of apoptotic cells was increased in the Ra-223 group, and an increase of necrotic area was observed in the combination group. (Figure 3A, 3C, 3D) | Combination of Ra-223 and bortezomib resulted in higherRa-223 incorporation to bone | The incorporation of Ra-223 to bone was measured using scintigraphy and histology. (Figure 5)

### Conclusions

The combination of Ra-223 with bortezomib virturally eradicates tumor-associated osteoclasts.

The incorporation of Ra-223 to bone matrix is improved, possibly via induction of osteoblast activity by bortezomib.

These data provide a strong rationale for combination of Ra-223 and bortezomib as possible therapy in MM. This is currently investigated in a Phase Ib/Ii trial in patients with early relapsed MM (NCT02928029).

### Acknowledgments

Aurexel Life Sciences Ltd (www.aurexel.com) is thanked for financial assistance in the preparation of this poster, funded by Bayer AG.