osteomiRs are circulating microRNAs that serve as novel biomarkers for bone quality and musculoskeletal diseases.

The **osteomiR™ test** is

- a novel fracture-risk assessment tool
- minimally invasive (200 µL serum required)
- fast and simple workflow
- software facilitating data analysis is included

**Highlights**

- Circulating microRNAs are novel biomarkers for **bone disorders**.
- We have identified **11 microRNAs in human serum** of osteoporotic patients, which are informative about fracture-risk in primary and secondary osteoporosis
- The osteomiR™ workflow is a **rapid and robust** solution to quantify osteomiRs in human serum samples.

microRNAs are small non-coding RNAs that regulate gene expression through RNA interference and therefore contribute to the regulation of correct cell and tissue function. MicroRNA secretion (contained in exosomes or microvesicles) has been observed in many different cell types, and is believed to serve as an endocrine pathway for cell-to-cell communication (see Figure 1). Therefore, the analysis of circulating microRNAs in serum, urine or saliva can be used to obtain valuable information about tissue physiology and pathophysiology. Circulating microRNAs are a novel class of relevant serum biomarkers.

The contribution of microRNAs to the regulation of bone tissue homeostasis has been extensively characterized (ref 1-6). Together with leading scientists we have investigated the profiles of up to 384 different circulating microRNA species in patients diagnosed with primary or secondary osteoporosis. From these data sets we have extracted a list of top microRNAs, which were repeatedly found to be associated with high-risk of fragility fractures.

Table 1 features this list of microRNAs, which is also covered by the osteomiR™ qPCR workflow. The key publications leading to the identification of these novel biomarker candidates are listed below. Figure 2 illustrates the expression of 11 osteomiRs in a range of different tissues. Some microRNAs are exhibited tissue-enriched expression (e.g. miR-155 in lymphoid cells)
Table 1: list of miRNAs

<table>
<thead>
<tr>
<th>microRNA</th>
<th>female postmenopausal OP</th>
<th>male/female idiopathic OP</th>
<th>female type 2 diabetes OP</th>
<th>bone formation</th>
<th>bone resorption</th>
<th>high tissue expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-let-7b-5p</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>ubiquitous</td>
</tr>
<tr>
<td>hsa-miR-127-3p</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>adrenal tissue</td>
</tr>
<tr>
<td>hsa-miR-155-5p</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>haematopoietic cells</td>
</tr>
<tr>
<td>hsa-miR-188-3p</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>muscle and PBMCs</td>
</tr>
<tr>
<td>hsa-miR-199b-5p</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ubiquitous</td>
</tr>
<tr>
<td>hsa-miR-203a-3p</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>alimentary tissue</td>
</tr>
<tr>
<td>hsa-miR-214-3p</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ubiquitous</td>
</tr>
<tr>
<td>hsa-miR-29b-3p</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>muscle tissue</td>
</tr>
<tr>
<td>hsa-miR-335-5p</td>
<td>✓</td>
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<td></td>
<td></td>
<td></td>
<td>alimentary tissue</td>
</tr>
<tr>
<td>hsa-miR-550a-5p</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mesenchymal tissue</td>
</tr>
</tbody>
</table>

**let-7b-5p (ref 4,5)**
Serum levels of let-7b-5p were found to elevated in primary postmenopausal osteoporosis as well as different forms of secondary osteoporosis. Let-7b-5p is highly abundant in serum and a validated regulator of bone formation.

**miR-127-3p (ref 4)**
Serum levels of miR-127-3p were found to be down-regulated in postmenopausal osteoporosis. miR-127-3p is highly expressed in adrenal tissue and known to positively affect bone resorption of bone marrow macrophages in vitro.
miR-155-5p (ref 4)
Serum levels of miR-155-5p were found to be down-regulated in postmenopausal osteoporosis (4). miR-155-5p is highly expressed in hematopoietic cells and an important regulator of monocyte and osteoclast development.

miR-188-3p (ref 4)
Serum levels of miR-188-3p are generally low. However, a significant decrease has been observed in post-menopausal osteoporosis. Overexpression of miR-188-3p in stem cells was observed to improve osteogenic differentiation.

miR-199b-5p (ref 4,5)
Serum levels of miR-199b-5p are increased in patients with fragility fractures. This microRNAs is induced during osteoblast development, however, its function in bone remodeling has not yet been investigated.

miR-203a-3p (ref 4)
Serum levels of miR-203a-3p were found to be increased in postmenopausal osteoporosis and decreased in diabetic osteopathy. This miRNA is known to regulate expression of RUNX2, however, it has not yet been tested for its effects on osteogenic differentiation.

miR-214-3p (ref 4,5)
Serum levels of miR-214-3p were found to be increased in postmenopausal osteoporosis. miR-214 is a potent inhibitor of bone formation in vitro and in vivo.

miR-29b-3p (ref 5)
Serum levels of miR-29b-3p were found to be down-regulated in patients with primary and secondary forms of osteoporosis and fragility fractures. This microRNA has validated effects on osteoblast and osteoclast formation and contributes to the regulation of collagen synthesis. It was found to be associated with bone turnover markers.

miR-31-5p (ref 2)
Serum levels of mir-31-5p are frequently changed in patients with postmenopausal as well as diabetic osteoporosis and fractures compared to control cohorts. The role of miR-31 for the regulation of bone remodeling has been deciphered on the basis of several in vitro and in vivo studies.

miR-335-3p (ref 5)
Serum levels of miR-335-3p are significantly higher in patients with primary and secondary osteoporosis compared to healthy controls. This microRNA is known to impact WNT signaling in pre-osteoblasts through regulation of Dickkopf-1 expression.

miR-550a-5p (ref 4,5)
Serum levels of miR-550a-5p are generally low, and few information on tissue expression levels are available. However, in our studies, this microRNA was frequently observed to be up-regulated in different form of osteoporosis. In addition, overexpression of miR-550a-5p inhibits proliferation and differentiation of mesenchymal stem cells.
Key Publications: