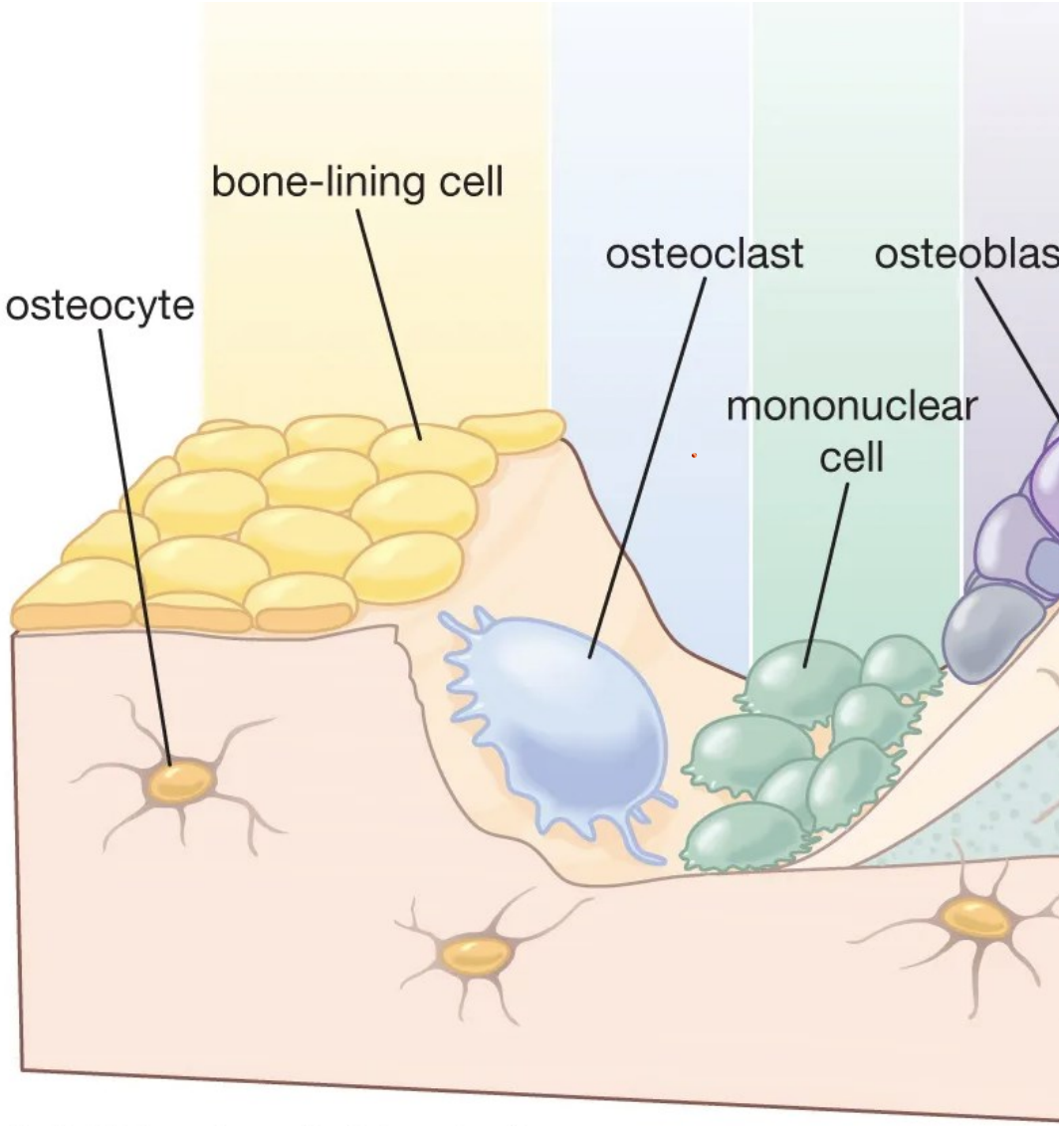
Vloga nekodirajočih RNA pri anabolnem zdravljenju s teriparatidom – primer farmako(epi)genomskega pristopa *in vitro*

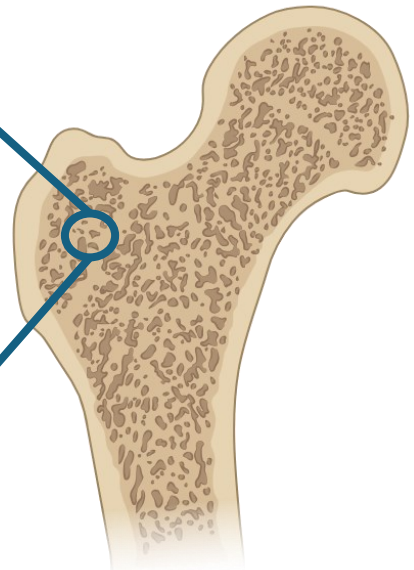
Asist. dr. Lucija Ana Vrščaj, mag. lab. biomed.
Prof. dr. Janja Marc, mag. farm.
Izr. prof. dr. Barbara Ostanek, mag. farm.

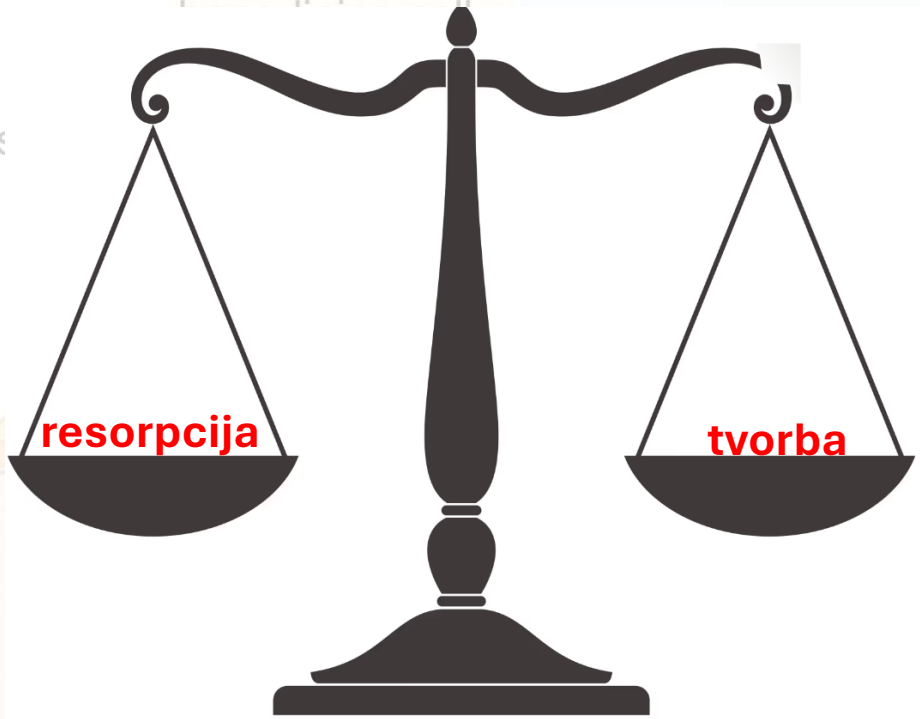
Kostna prenova

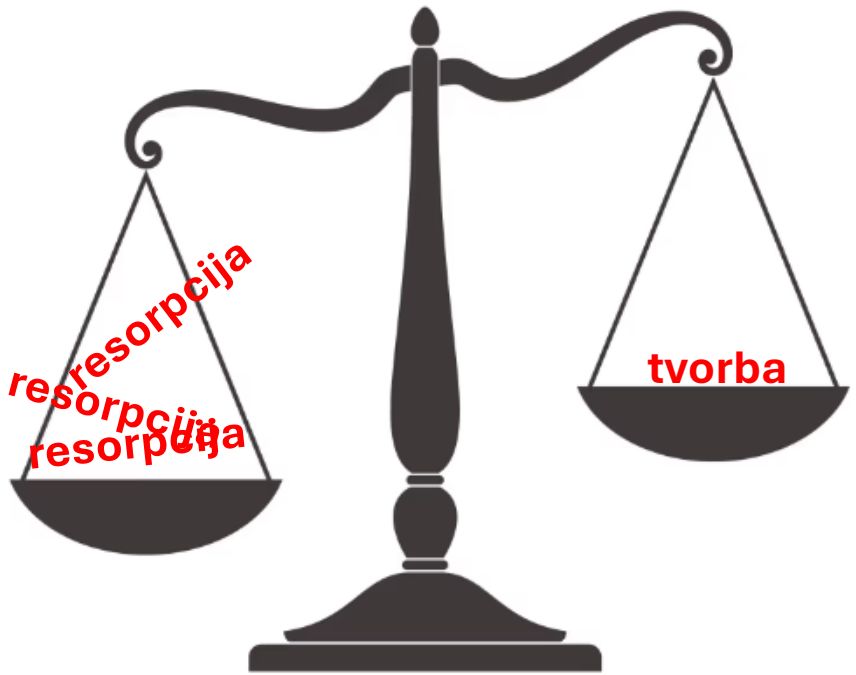
resorpcija

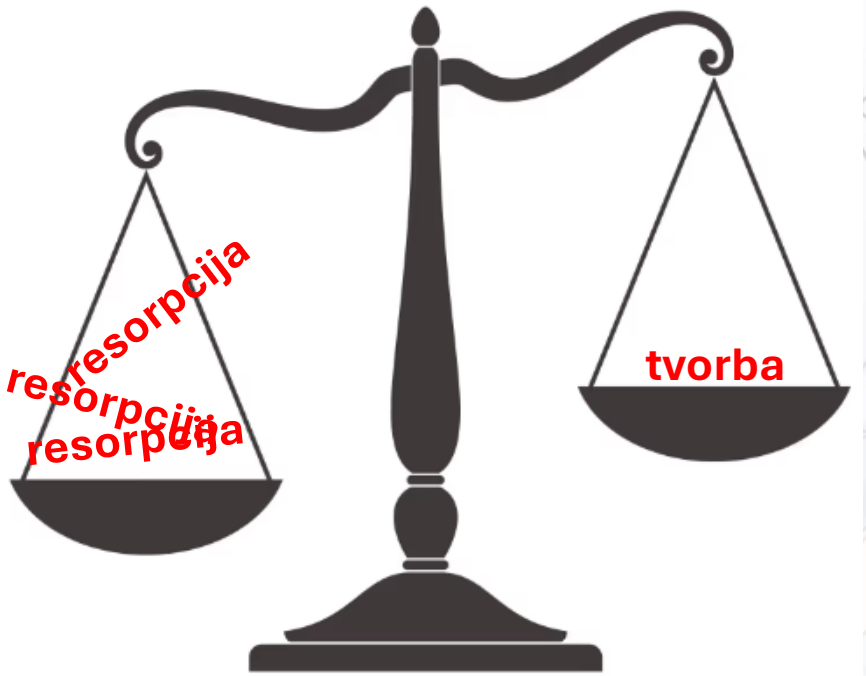
tvorba





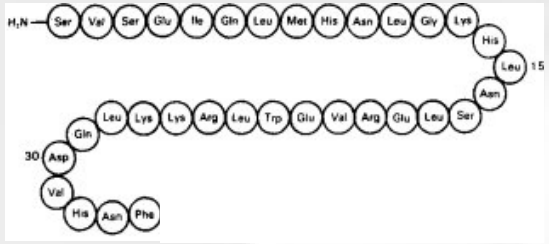






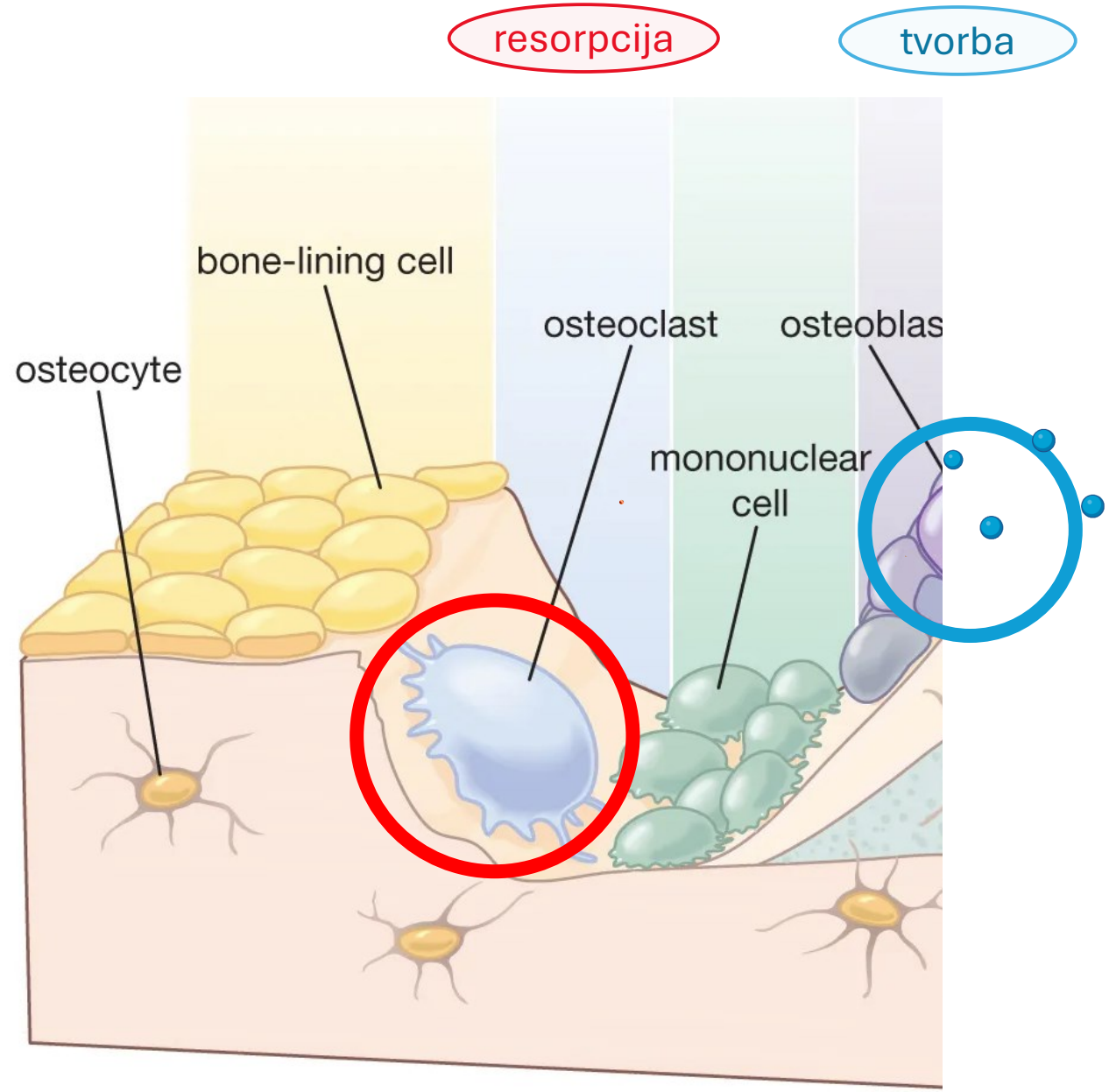
Osteoporoza

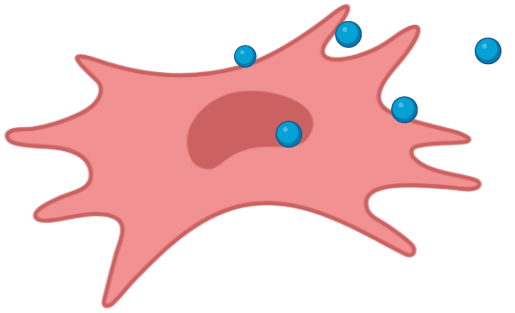
Zdravljenje



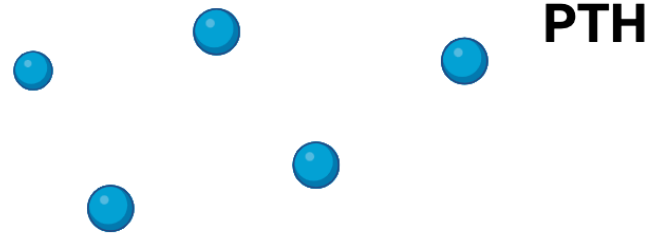
Antiresorptivi	Osteoanaboliki
• Pretežno v uporabi • Bisfosfonati in denosumab • Poleg resorpcije, zavirajo tudi kostno tvorbo • Neželeni učinki	• Pospešujejo kostno tvorbo • Teriparatid (TPTD) – PTH • Variabilnost učinkovitosti zdravljenja

Kostna prenova



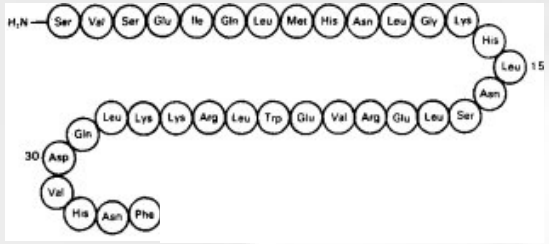


Mezenhimska stromalna matična celica



Intermitentno odmerjanje

Zdravljenje



Antiresorptivi

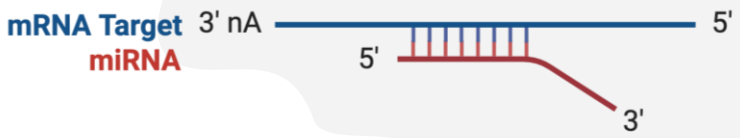
- Pretežno v uporabi
- Bisfosfonati in denosumab
- Poleg resorpcije, zavirajo tudi kostno tvorbo
- Neželeni učinki

Osteoanaboliki

- Pospešujejo kostno tvorbo
- Teriparatid (TPTD) – PTH
- Variabilnost učinkovitosti zdravljenja

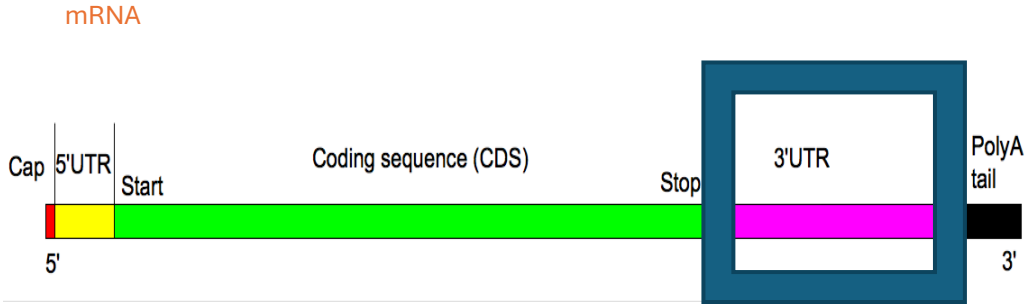
Točen mehanizem delovanja še ni poznan

miRNA



miRNA

- Majhne nekodirajoče molekule (približno 20 nukleotidov)
- Uravnavajo izražanje genov po transkripciji!
- Vežejo se na 3' UTR regijo mRNA
- Utišanje genov
  - Zaviranje translacije
  - Pospeševanje razgradnje mRNA



KROŽIJO V KRVI

- *So stabilne – dobri kandidati za biomarkerje*
- *Farmakoepigenomske študije o miRNA pri OP so redke, nobena ni tako celovita*

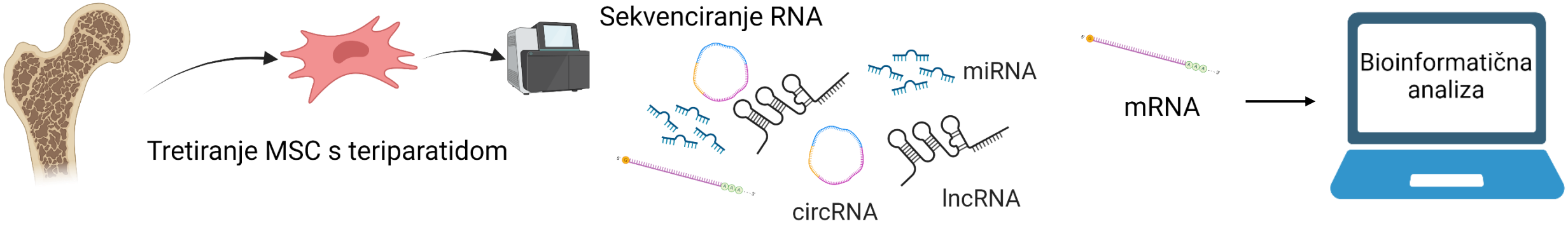
Namen študije

Pridobiti vpogled v osteoanabolni učinek teriparatida prek njegove regulacije miRNA v osteoblastih

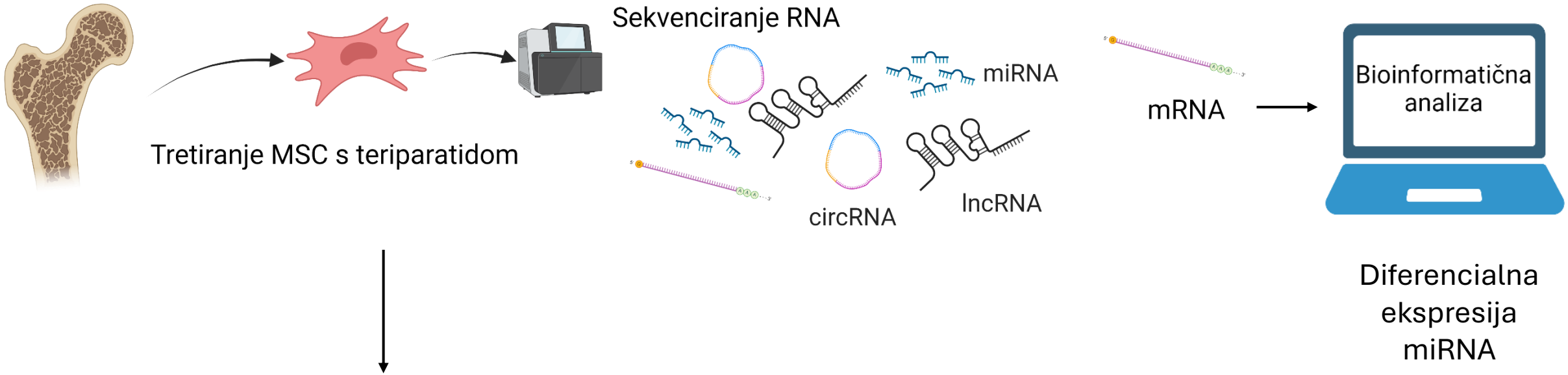
Analiza ravni miRNA v serumu pri bolnikih, ki prejemajo teriparatid

Metode

1


Tretiranje MSC s teriparatidom
Sekvenciranje RNA
miRNA
circRNA
lncRNA
mRNA
Bioinformatična analiza

Sekvenciranje malih RNA

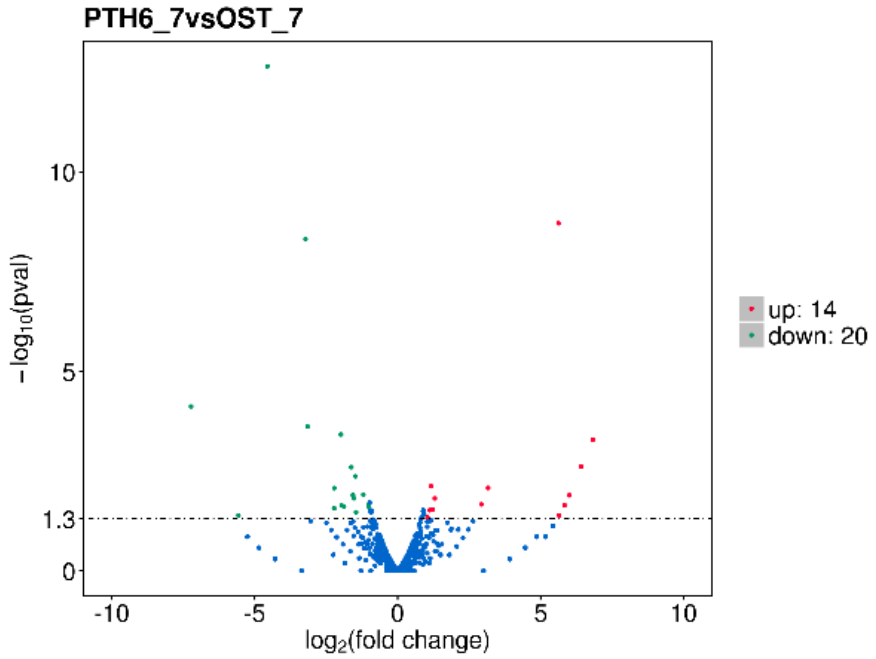


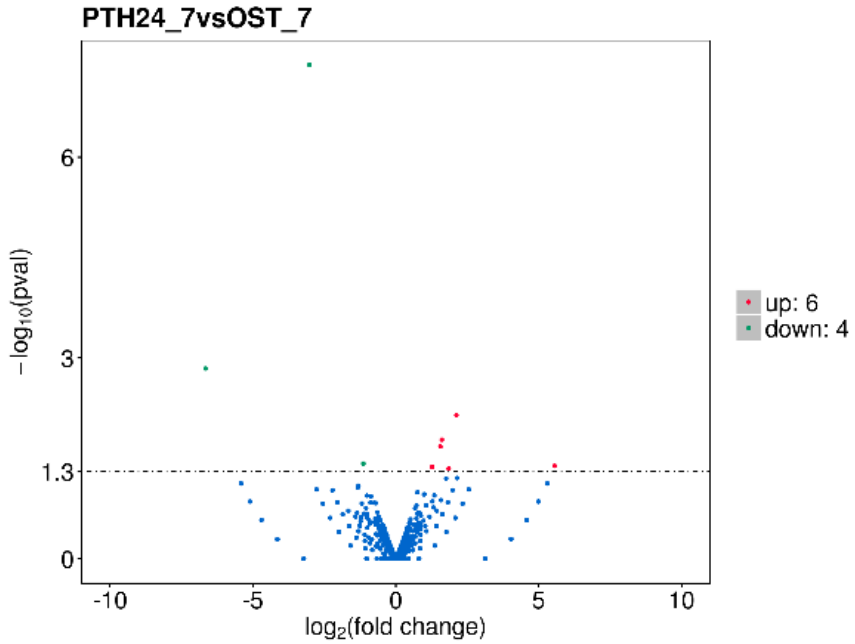
Diferencialna ekspresija miRNA

Intermitentno odmerjanje → PTH za 6 ur na 2-3 dni.

Kontinuirano odmerjanje → PTH 24/7, sveže dodan na 2-3 dni.

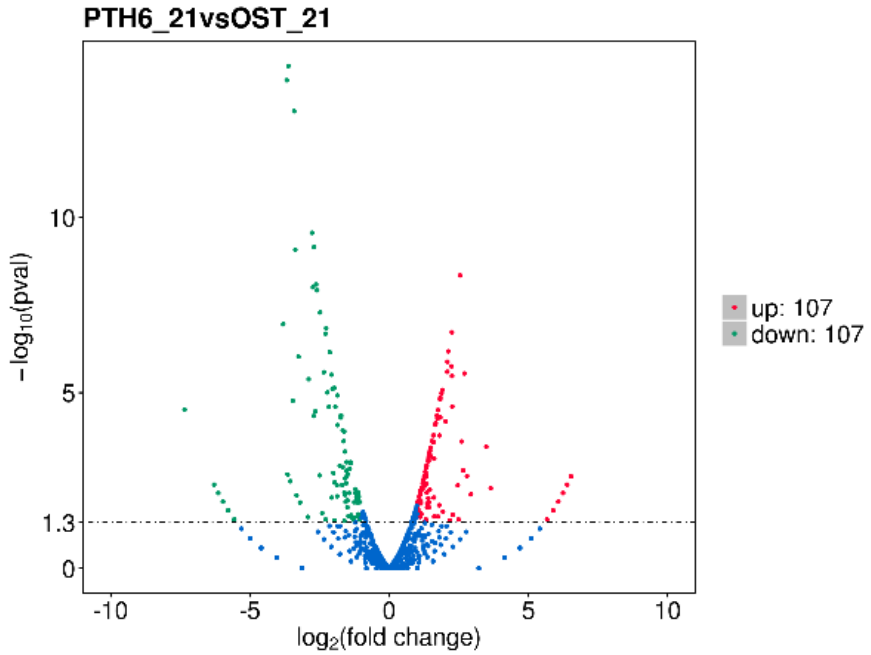
V osteogenem mediju, 21 dni.

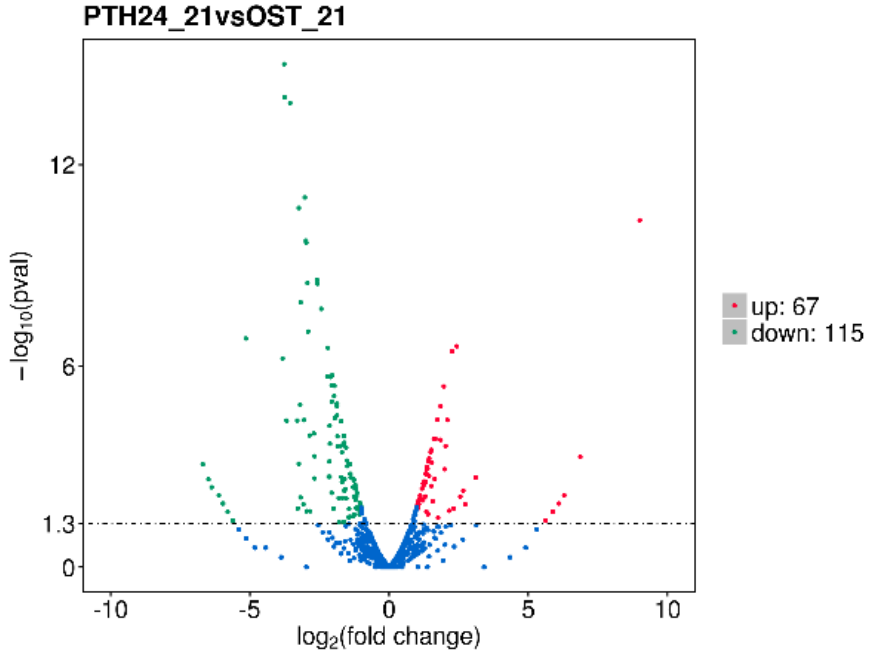




Rezultati sekvenciranja **miRNA**

Diferencialno izražene miRNA intermitentno tretiranih celic MSC proti netretiranim celicam MSC (7. dan)			
povečano izražanje	p vrednost	zmanjšano izražanje	p vrednost
hsa-miR-375-3p	1.93E-09	***hsa-miR-363-3p***	2.22E-13
hsa-miR-1277-3p	0.000517	hsa-miR-4454	4.79E-09
hsa-miR-4786-5p	0.002435	***hsa-miR-20b-5p***	7.55E-05
Diferencialno izražene miRNA kontinuirano tretiranih celic MSC proti netretiranim celicam MSC (7. dan)			
povečano izražanje	p vrednost	zmanjšano izražanje	p vrednost
hsa-miR-629-3p	0.007168	hsa-miR-4454	4.17E-08
hsa-miR-548bc	0.016655	hsa-miR-1245a	0.001437
hsa-miR-548f-5p	0.021096	hsa-miR-499a-5p	0.03769





Rezultati sekvenciranja **miRNA**

Diferencialno izražene miRNA intermitentno tretiranih celic MSC proti netretiranim celicam MSC (21. dan)			
povečano izražanje	p vrednost	zmanjšano izražanje	p vrednost
hsa-miR-543	4.56E-09	hsa-miR-451a	4.93E-15
hsa-let-7a-3p	1.90E-07	hsa-miR-887-3p	1.25E-14
hsa-miR-148b-3p	6.64E-07	***hsa-miR-31-3p***	9.58E-14
Diferencialno izražene miRNA kontinuirano tretiranih celic MSC proti netretiranim celicam MSC (21. dan)			
povečano izražanje	p vrednost	zmanjšano izražanje	p vrednost
hsa-miR-1298-5p	4.50E-11	***hsa-miR-31-3p***	9.90E-16
hsa-miR-1271-5p	2.58E-07	hsa-miR-887-3p	9.63E-15
hsa-miR-122-5p	3.67E-07	hsa-miR-451a	1.44E-14

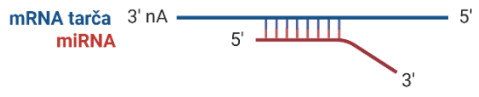
+ hsa-miR-133a-3p iz literature – naši rezultati sicer tudi

Metode

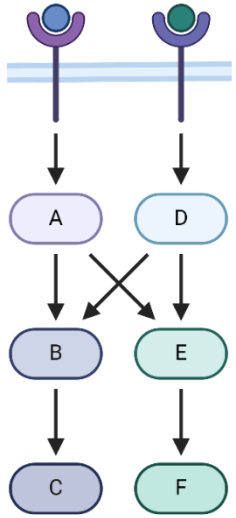
2

Bioinformatična analiza

Iskanje potencialnih tarč miRNA

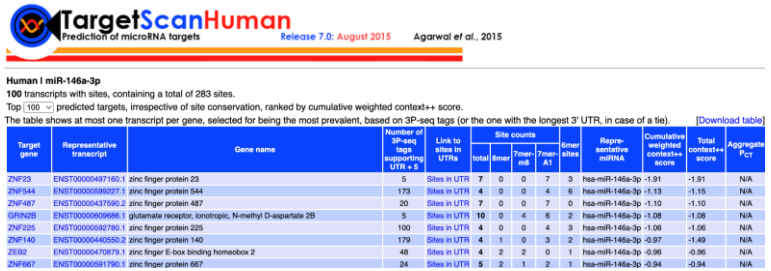


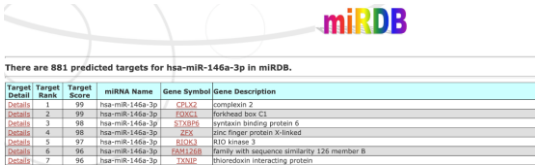
Obogatitvena analiza

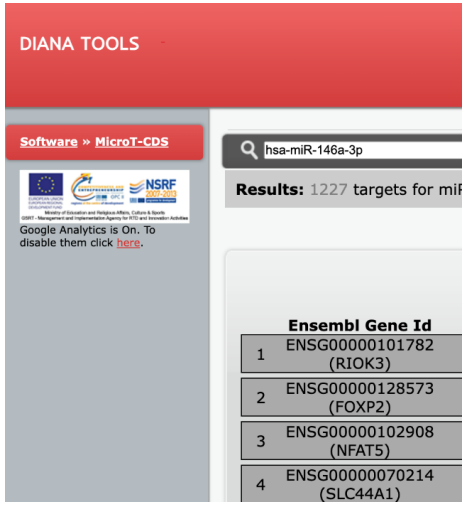


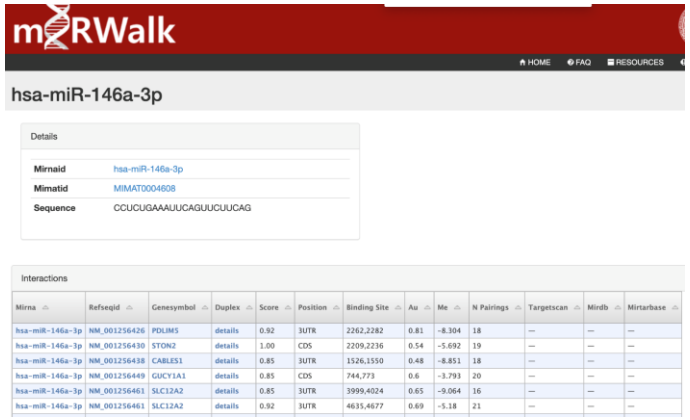
Serumski vzorci

Pacienti







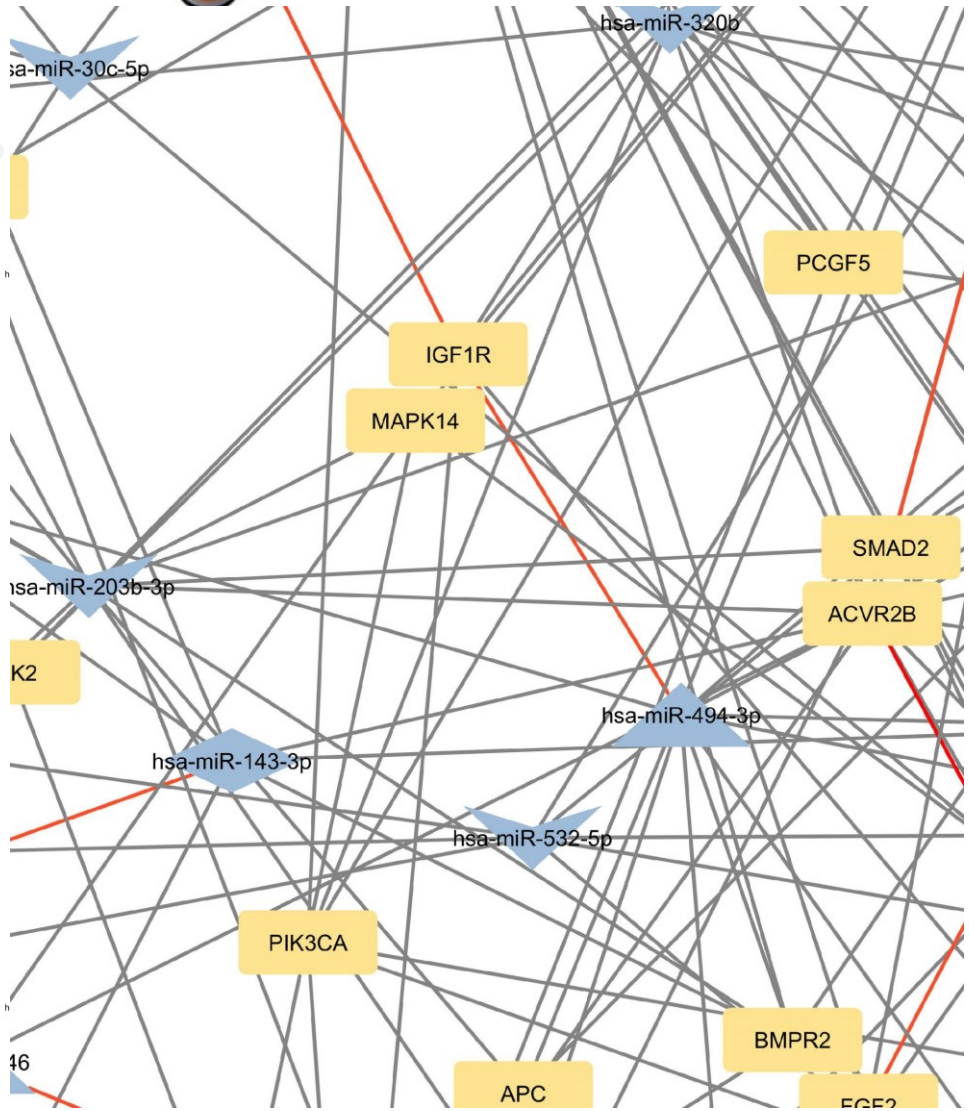


Predikcije vezave na tarčne gene – bioinformatična orodja

- DIANA
- miRWalk
- miRDB
- TargetScan

Interaktom

- Cytoscape
- miRTarBase
  - Validirane interakcije



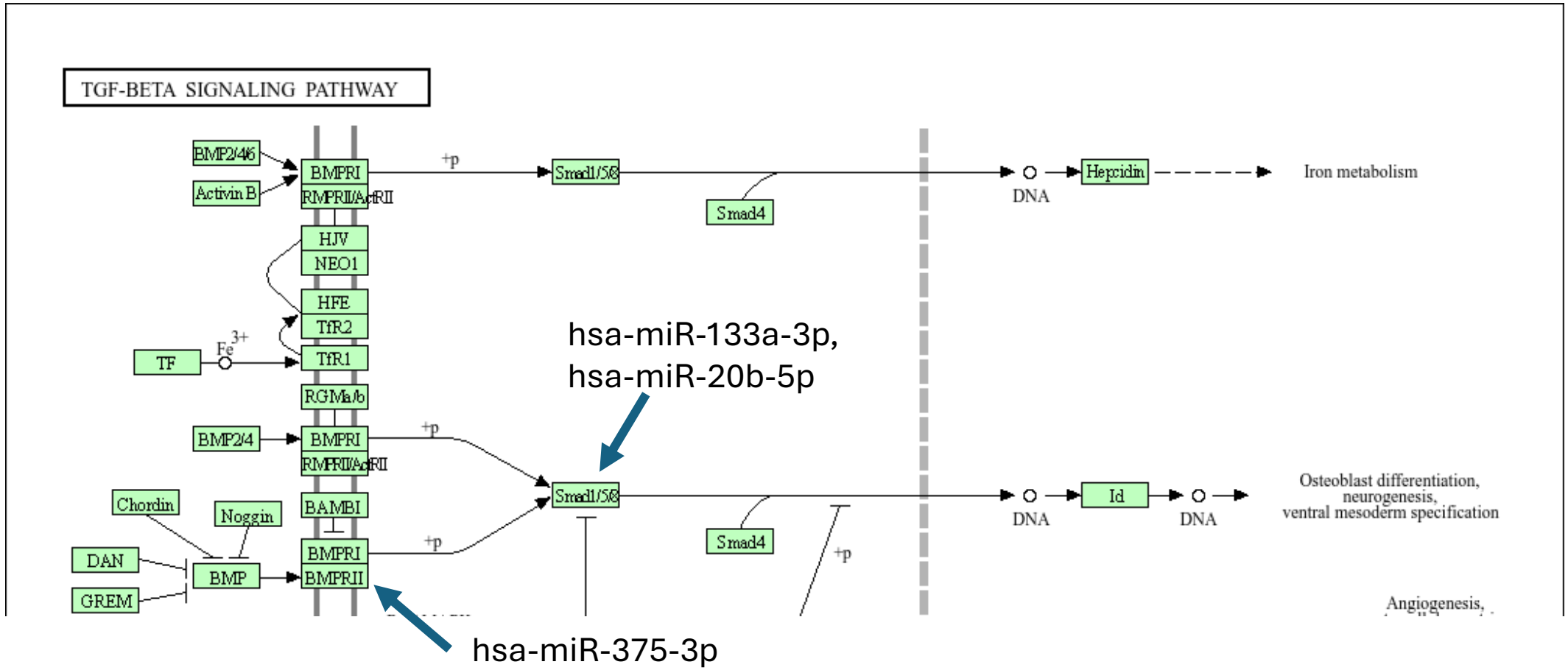
Obogatitvena analiza

- DIANA TOOLS - mirPath

KEGG pathway	p-value	#genes	#miRNAs
Renal cell carcinoma (hsa05211)	1.25924E-09	48	24
Proteoglycans in cancer (hsa05205)	2.0867E-08	108	24
Signaling pathways regulating pluripotency of stem cells (hsa04550)	6.09134E-08	84	21
Mucin type O-Glycan	2.05291E-07	18	13
Hippo signaling pathway (hsa04390)	4.18173E-07	81	21
	4.64518E-07	15	14
Adherens junction (hsa04520)	1.05553E-05	47	19
	1.27573E-05	73	22
TGF-beta signaling pathway (hsa04350)	5.91744E-05	48	19
pathway (hsa04015)	9.40696E-05	111	22
PI3K-Akt signaling pathway (hsa04151)	9.40696E-05	168	25
Circadian rhythm (hsa04710)	0.000107691	24	19

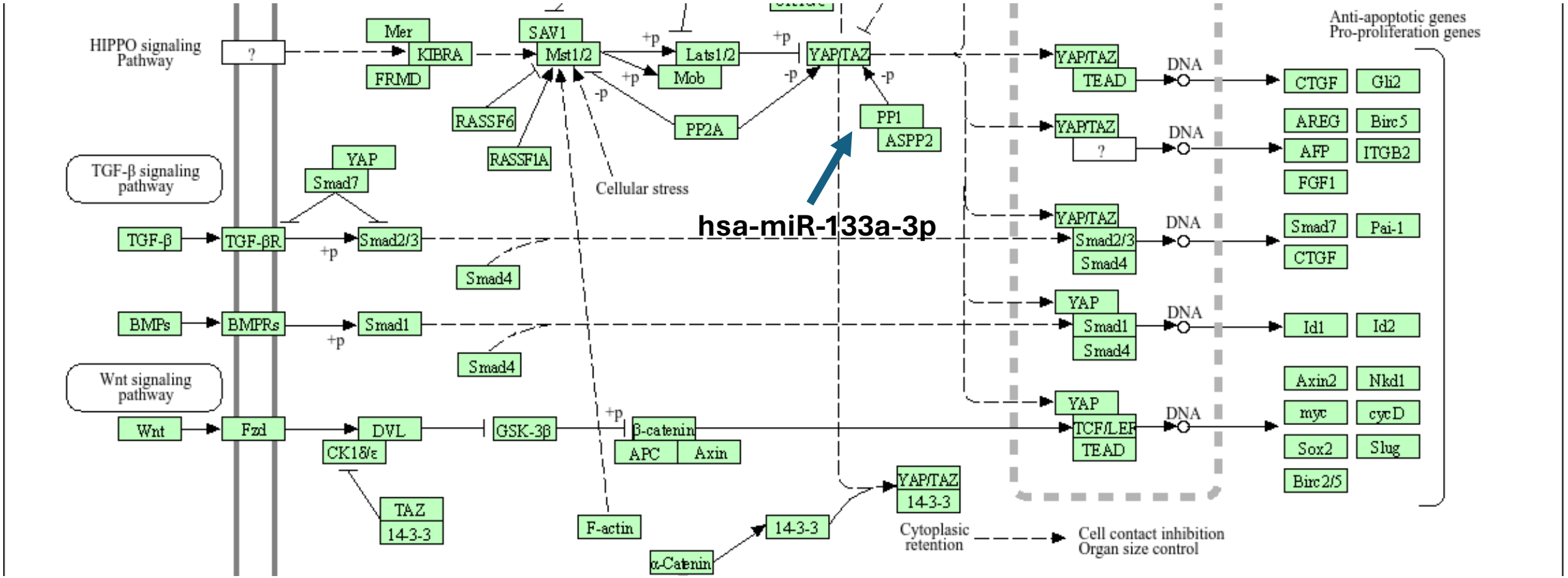
Signalne poti

hsa-miR-375-3p
hsa-miR-20b-5p
hsa-miR-133a-3p
hsa-miR-31-3p



Signalne poti

hsa-miR-375-3p
hsa-miR-20b-5p
hsa-miR-133a-3p
hsa-miR-31-3p



Vzorci pacientov

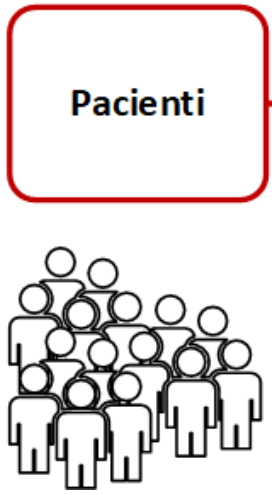
Serumski vzorci

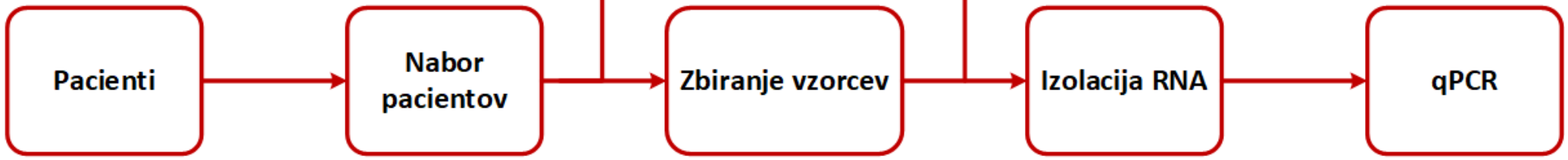
UKC Ljubljana - Klinični oddelek za endokrinologijo, diabetes in presnovne bolezni

Odzivni/neodzivni

- BMD ≥ 3% po 12 mesecih na mestu ledvenih vretenc

652 pacientov





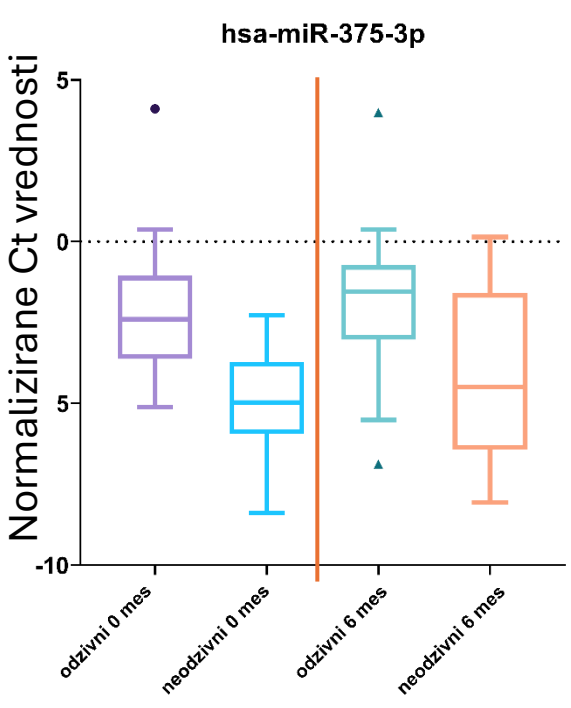
48 pacientov

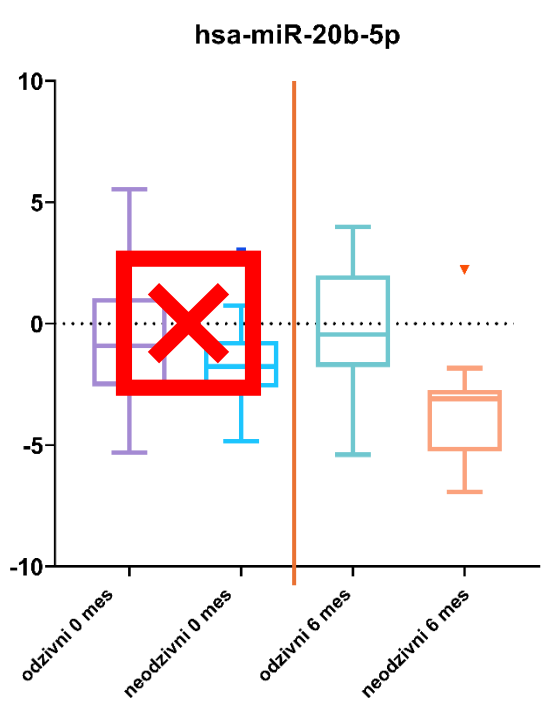
Pomenopavzna osteoporoza
Zdravljenje s TPTD

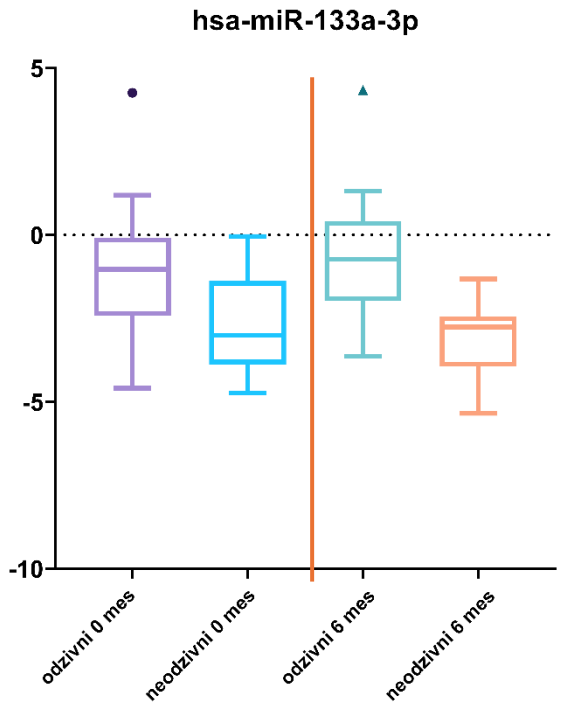
215 pacientov

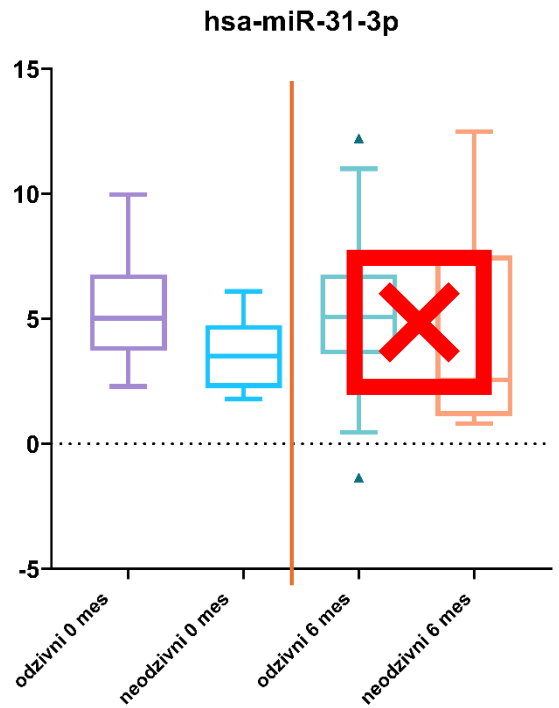
hsa-miR-375-3p
hsa-miR-20b-5p
hsa-miR-133a-3p
hsa-miR-31-3p

miRNA **375-3p, 20b-5p, 133a-3p in 31-3p** vplivajo na odzivnost zdravljenja s TPTD









Vrednost P: **0,0002** **0,0031**

Vrednost P: 0,3295 **0,0008**

Vrednost P: **0,0085** **<0,0001**

Vrednost P: **0,0175** 0,4929

Zaključki

- **Prva celogenomska farmakoepigenetska študija, ki združuje *in vitro* in *in vivo* pristop**
- Interaktom zagotavlja osnovo za boljše razumevanje osteoanabolnega delovanja PTH
- hsa-miR-375-3p, hsa-miR-20b-5p, hsa-miR-133a-3p, hsa-miR-31-3p lahko služijo kot potencialni biomarkerji za učinkovitost zdravljenja s teriparatidom ali kot nove terapevtske tarče

Katedra za klinično biokemijo – Fakulteta za farmacijo, Univerza v Ljubljani

V sodelovanju z

Oddelek za endokrinologijo, diabetes in presnovne bolezni – Univerzitetni klinični center, Ljubljana

Hvala za vašo pozornost