Myeloma bone marrow serum primes healthy donor (HD) bone marrow cells to support the survival and growth of myeloma plasma cells (MMPC) in co-culture. Following interaction with the MMPC, the phenotype of the HD bone marrow stroma changes – expression of immune regulatory factors is increased while expression of genes associated with osteoblastogenesis and WNT signaling is suppressed. The aim of this study was to determine whether exosomes in the myeloma bone marrow serum have a role in priming the microenvironment.

### METHODS

- Serum was separated from clotted bone marrow aspirates from myeloma patients obtained during scheduled clinic visits.
- Exosomes were prepared from 1-2 ml serum using Invitrogen’s Total Exosome Isolation kit according to manufacturer’s instruction. Exosomes were 30-130 nm in diameter as determined by Dynamic Light Scatter on a Brookhaven Instruments Corp. ZetaPlus Particle Sizing Software or NanoSight NS300, and visualized by scanning electron microscopy.
- Small RNA (≤200 nucleotides) was isolated using Qiagen’s miRNeasy kits, quality and quantity assessed with Qubit 2. 96 miRNAs were analyzed by qRT-PCR on Fluidigm’s BioMark platform using 96x96 multiplex chips and normalized to spiked in C. elegans miR mimetic.
- Primary CD138-expressing cells were isolated from EDTA-anticoagulated bone marrow aspirates using RoboSep.
- Luciferase-expressing stroma-dependent myeloma cells were described previously (Bam, BMC Cancer 2015;864). Healthy donor mesenchymal stem cells (MSC) were prepared from bone particles obtained during orthopedic surgery, and cultured in α-MEM supplemented with 10% FBS.
- Primary myeloma cells or luciferase-expressing stroma dependent myeloma cells were added to wells containing monolayers of healthy donor (HD) MSC in α-MEM supplemented with 10% FBS in 96 well culture plates. For each experiment 2 conditions were used: a control group and a group to which exosomes isolated from a patient's bone marrow serum aliquot were added. The number of primary MMPC dictated that these experiments were carried out in duplicates, while the stroma-dependent cells were plated in 5 replicates. Viable primary cells counts or luciferase bioluminescence were evaluated after 4 days of co-culture.

### RESULTS

- Exosomes (Figure 1) supported survival and growth of primary MMPC. Exosomes from 5 of the 7 patients supported growth of stroma-dependent myeloma cells co-cultured with HD MSC.
- In a representative experiment, stroma-dependent myeloma cells bioluminescence was 44% higher (p=0.02) when exosomes were used (Table 1).
- The levels of 10 miRs were at least 2.5 fold higher while 4 were at least 2.5 fold lower in MM than in HD exosomes.
- The effect was reproduced when primary cells were used: in a representative experiment, the number of primary MPC recovered from the co-cultures supplemented with exosomes was 28% higher than in controls (Table 2). Primary MMPC did not survive in the absence of MSCs even when supplemented with bone marrow serum or exosomes.

### CONCLUSIONS

- Exosome from bone marrow serum of myeloma patients prime the bone marrow microenvironment to support survival and growth of primary MMPC.
- Bone marrow serum more effective, indicating that other factors in the serum are also important.
- The differences in microRNA contents between healthy donor and myeloma exosomes may provide an insight of the regulatory mechanisms molding the myeloma bone marrow microenvironment.