Robo4 expression in primary breast tumors is associated with worse prognosis in relapse-free survival.

![Figure 1. High expression of Robo4 was associated with bone metastasis-free survival across pH+ patients. Kaplan-Meier curves for rates of relapse-free survival in 254 patients with breast cancer, according to Robo4 status.](image1)

Robo4 silencing reduced experimental primary tumor growth but did not affect long-term bone colonization.

![Figure 2. Robo4 silencing decreased primary tumor development but not bone tumor burden.](image2)

Robo4 in breast cancer cells promoted early bone colonization and anchorage in the bone marrow.

![Figure 3. Robo4 silencing impaired tumor cells colonization and engraftment](image3)

Anti-Robo4 antibody treatment reduced breast cancer cell invasion and osteoblastic adhesion in vitro and anchorage in vivo.

![Figure 4. Targeting Robo4 with specific antibody decreased invasion in vitro and tumor cell development inside bone marrow in vivo](image4)

Targeting Slt2, the Robo ligand produced by the bone marrow microenvironment, disturbed osteoblast/tumor cell interaction.

![Figure 5. Robo ligand ShH2 production by osteoblast is promoted by tumor cells in the bone microenvironment and involved in the interaction between both cell types](image5)

Conclusion & Perspectives

Depletion of Robo by shRNA decreased the colonization as well as early engraftment of tumor cells in bone marrow in vivo. Interestingly, Robo4 silencing by an antibody strategy in B02 breast cancer cells decreased invasion in vivo but also engraftment of tumor cells in the bone marrow in vivo. Moreover, antibody targeting of Slt2 or Robo4 disturbed the interaction of B02 cells with osteoblasts during co-culture experiments. We postulate that the Slt2/Robo4 axis mediates the arrest of circulating metastatic cancer cells in the bone marrow.

These results provide strong evidence that the axon guidance receptor Robo4 is involved in bone metastasis formation and that the use of an antibody directed against Robo4 could lead to the development of innovative therapies to prevent metastasis formation in the bone marrow.