Encapsulation of Gli-inhibitors blocks tumor invasion into the bone.

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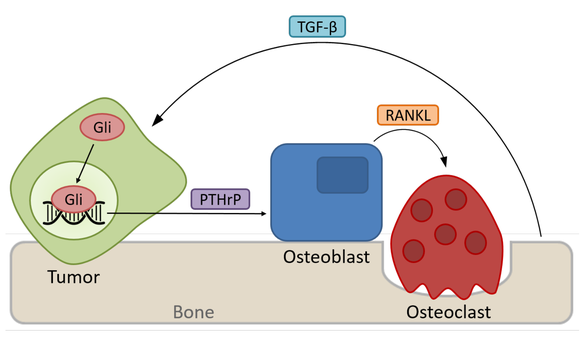
BACKGROUND

- Tumors including breast, prostate, lung, and oral cancer can establish in bone and disrupt normal bone remodeling.
- Our group and others have shown that Gli2, a Hedgehog signaling transcription factor regulates parathyroid hormone related protein expression and bone destruction.
- Genetic inhibition of Gli2 dramatically reduces tumor induced bone disease.
- Hedgehog inhibitors have not been successful in most tumor in bone, since they often do not express Hedgehog receptors.
- The available Gli inhibitors (including GANT58) are hydrophobic and challenging to deliver in vivo.



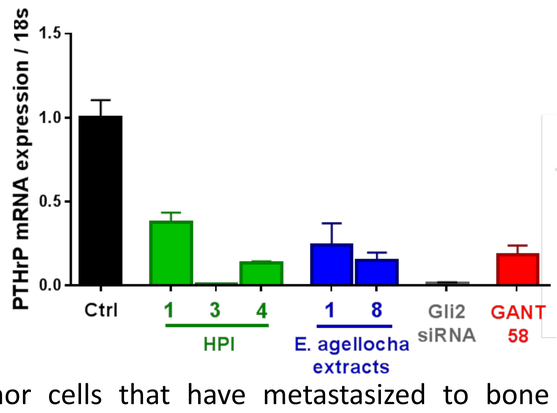


Figure 1: Pathologic role of Gli2 in TIBD. Tumor cells that have metastasized to bone overexpress Gli2, a transcriptional activator of Hedgehog (Hh) signaling. Gli2 stimulates the production of PTHrP which promotes osteoclast-mediated bone resorption. The subsequent release of bone-derived growth factors like TGF-β stimulates tumor growth and Gli2-induced expression of PTHrP, thus perpetuating the "vicious cycle". Gli inhibitors reduce PTHrP expression and subsequent tumor-induced bone disease.

HYPOTHESIS

Encapsulation of Gli inhibitors will facilitate delivery of these agents to tumors in bone will help reduce tumor induced bone disease.

MATERIALS & METHODS

MATERIALS

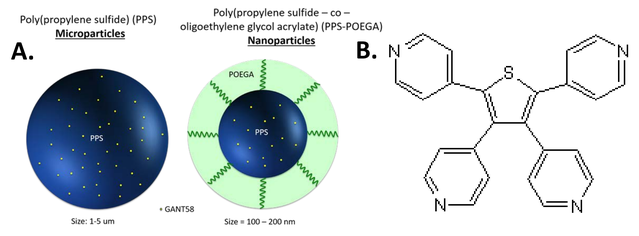


Figure 2: A. Schematic of GANT-58 loaded microparticles and nanoparticles. **B.** Structure of GANT58

PARTICLE FABRICATION

- **Microparticles:** GANT58 microparticles (GANT58-MPs) were fabricated using an oil-in-water single emulsion technique. GANT58-MPs averaged 4.2 µm in diameter and exhibited ROS-dependent release characteristics *in vitro*.

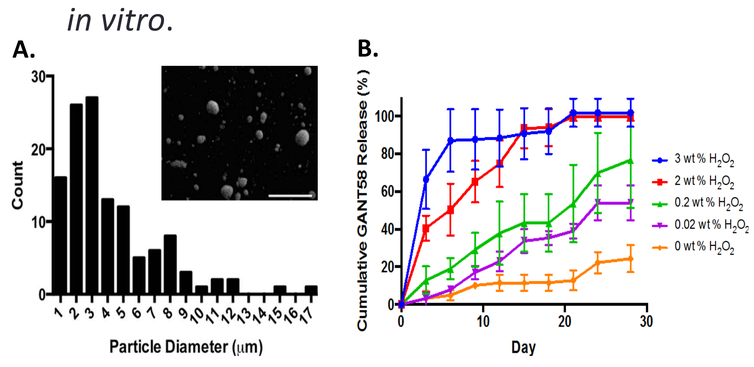


Figure 3: Microparticle characterization. A. GANT58-MP size characterization measured by SEM. Scale bar of inset SEM image is 50 µm. **B.** ROS-dependent temporal release characteristics of GANT58-MPs *in vitro*

- **Nanoparticles:** The GANT58 nanoparticles (GANT58-NPs) were made using an oil-in-water solvent evaporation method. Reactive oxygen species (ROS) trigger particle disassembly and drug release.

Formulation	Diameter (nm)	Drug Loading (%)	Encapsulation Efficiency (%)
PPS-PEG	170	13	44

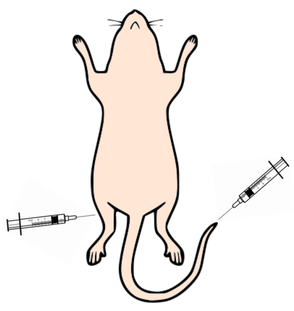
Table 1: Summary of GANT58-NP characterization. Poly(propylene sulfide)-poly(ethylene glycol) (PPS-PEG). The average size of the loaded particles is reported.

RESULTS

NANOPARTICLE SYSTEMIC DELIVERY OF GANT58

Experimental Design:

Intratibial injection of 2.5 x 10^5 MDA-MB-231 GFP cells or PBS.



≤10 mg/kg of GANT58-NPs in 100 µl administered intravenously.

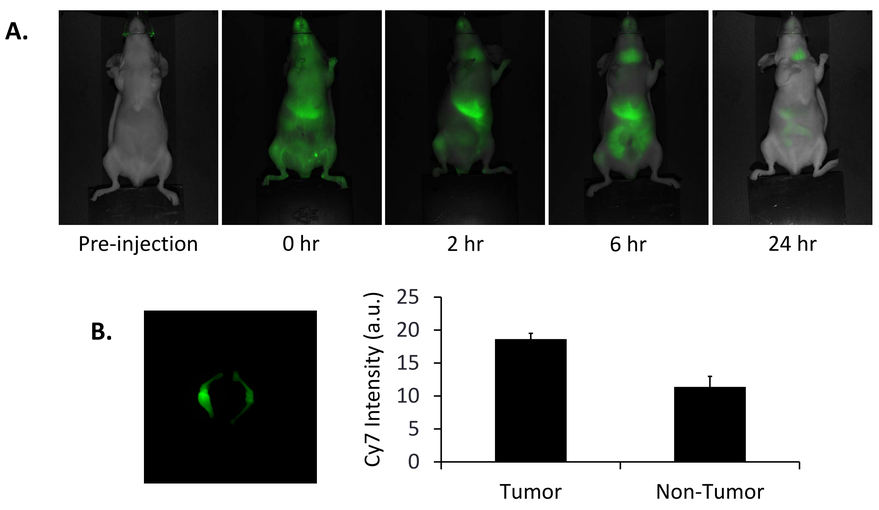


Figure 4: GANT58-NP biodistribution. A. In vivo imaging shows the GANT58-NPs in circulation after injection and its gradual clearance through the liver and kidneys. **B.** Ex vivo imaging shows a preferential localization of the NPs at the tumor site in bone (left) compared to non-tumor bone (right). Images were taken with the Pearl® Impulse system and are representative.

MICROPARTICLE LOCAL DELIVERY OF GANT58

Experimental Design: 1x10^6 cells were injected into the right and left masseter muscle (parallel to the mandible) of 4-6 week old athymic male mice from Harlan Laboratories. Tumor control mice received PBS injections. Drug treatments began once tumors were palpable (~10 days) which consisted of 50-75 µl injections of ~5mg/kg GANT58 loaded microparticles or empty-PPS microparticles as controls.

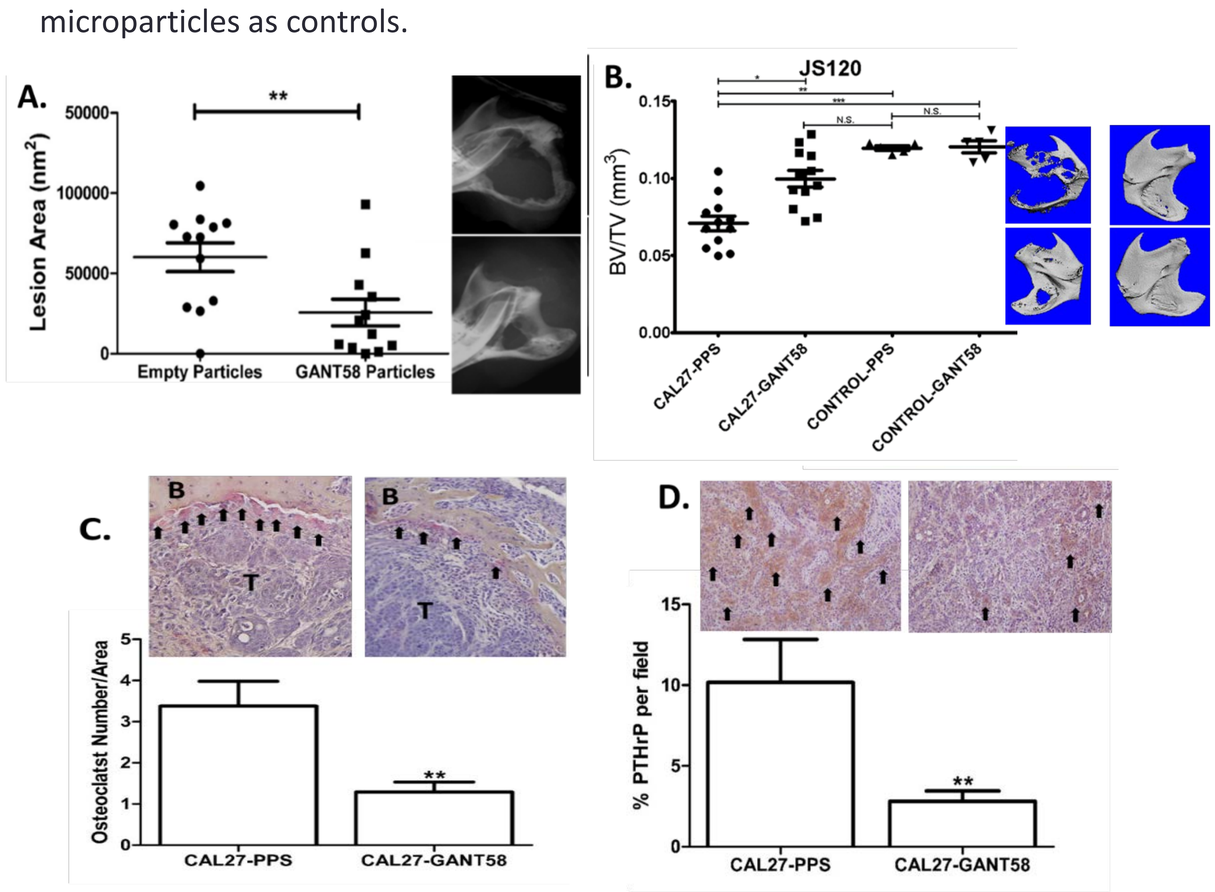


Figure 5: Male athymic mice treated with GANT58 loaded microparticles show a significant decrease in **A.** lesion area by high resolution x-rays and **B.** have significantly more bone volume as compared to the control mice. Importantly, non-tumor bearing mice treated with GANT58 microparticles have bone volumes similar to that of mice treated with control particles. Mice treated with GANT58 microparticles also show decreased levels of **C.** osteoclasts, as indicated by TRAP positive multi-nucleated cells (black arrows) as well decreased levels of **D.** PTHrP positive staining.

CONCLUSIONS

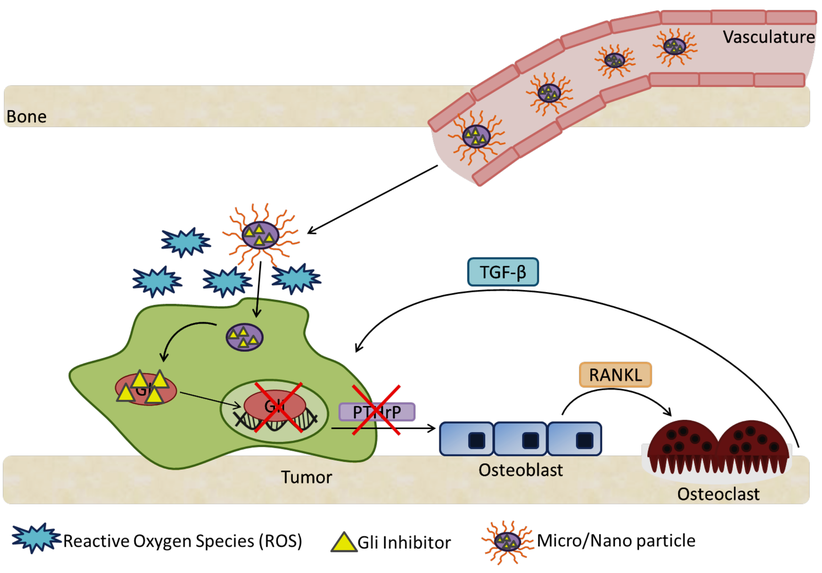


Figure 6: Encapsulated GANT58 travels to the bone through the vasculature or by local injection, where the particles are dissolved by Reactive Oxygen Species (ROS) present in the tumor microenvironment. Free GANT58 is then taken up by the cell where it inhibits Gli2, decreasing PTHrP expression and preventing subsequent bone destruction

FUNDING

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