Inhibition of Vascular Endothelial Growth Factor Causes Low Bone Blood Flow, Bone Strength, and Bone Hydration with no Effect on Bone Mass

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Avastin™ Background

- Humanized monoclonal antibody to VEGFA (bevacizumab [BVZ]) that slows cancer growth by inhibiting blood vessel proliferation and normalizing blood vessel structure within solid tumors.

- First approved 15yrs ago as adjuvant chemotherapy for colorectal, lung (non–small cell), ovarian, cervical, and renal cancer.
Avastin™ Adverse Events

- Disrupts bone elongation

- Clinical adverse events include:
  - Hypertension (20–40%)
  - Delayed wound healing (>70%)
  - Heart attack
  - Stroke
  - Periodontitis/osteonecrosis of jaw (?)
Hypothesis

- Inhibition of VEGF in adult mice decreases bone blood flow in trabecular bone regions, with no effect on bone mass or bone strength.
Experimental Design

- 10 week old BALB/cJ male mice
- Two groups (N=12 each)
  - Vehicle (0.9% saline, SC)
  - Anti-VEGFA rodent antibody (B20–4.1.1; 5mg/kg 2X/wk SC; Genentech; South San Francisco, CA)
- Necropsy after 6 wks. Obtain:
  - LV6 and right femur wrapped in gauze at –20 °C
  - Right proximal humerus in 70% EtOH
Endpoints

- Bone Blood Flow (Distal Femur)
  - *In Vivo* $^{18}$F–PET/CT, a morphometric method; $K_1$ – rate of blood flow to bone

- Bone Strength (Lumbar Vertebrae Body 6)
  - Ultimate Load (Compression Test)

- Bone Mass (trabecular region of Proximal Humeral Metaphysis)
  - BMD (pQCT)

- Bone Hydration ($^1$H NMR) (Whole Femur)
  - Volume Fraction of Bound Water (%)
18F–NaF–PET/CT

- Position anesthetized mouse on scanning bed with fiducial markers
- Start PET Scan, then inject 18F IV. Scan continuously for 30min (0.8mm voxel)
- Do CT scan (0.15mm voxel)
- Reconstruct scans (PET Scan in selected timeframes w/r to 18F location); superimpose PET & CT scans
- Place VOIs in left ventricle at 0–1min and trabecular & cortical regions at 15–20min post–18F injxn
- Quantitate 18F in each VOI; calculate K1 (ml/cc/min), rate of 18F flow from blood to bone ECF
$^{18}$F–NaF–PET/CT
Blood Flow (K1)
Right Distal Femur

Mean ± SEM
(N=6 grp)

diff from VEH (p=0.009)
Ultimate Load (N)

Lumbar Vertebral Body 6

Mean±SEM
(N=12/grp)

VEH

anti-VEGF

diff from VEH (p=0.013)
Trabecular BMD (mg/cm²)

Proximal Humeral Metaphysis

Mean ± SEM
(N=11 grp)

VEH  anti-VEGF
Volume Fraction of Bound Water (%)

Whole Femur

Mean±SEM
(N=12/grp)

diff from VEH (p=0.003)
Data Summary

In trabecular bone regions, anti-VEGFA causes:

- 41% lower bone blood flow (distal femur)
- 23% lower ultimate load (LV6 body)
- no effect on BMD (proximal humeral metaphysis)
- 10% lower bone hydration (whole femur)
Ultimate Load vs. Volume Fraction of Bound Water

Vertebral Body

$r=0.70; P<0.0004; (N=21)$
Conclusion

- Anti-VEGFA reduces bone blood flow in trabecular bone of young adult mice.
- Anti-VEGFA reduces bone strength *without* affecting bone mass.
- Anti-VEGFA reduces bone hydration.
- Bone strength is well-correlated to volume fraction of bound water.
- Hypothesis not validated, but...!
Weaknesses

- Studied three *different* trabecular bone rich regions:
  - could have measured both blood flow and BMD in distal femur
  - could have measured both BMD and bone strength in LV6 vertebral body
- Should have used IgG antibody as VEH
- No histomorphometric analyses completed
- Expand analyses to cortical bone
- Recommend larger N
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Measuring Bone Blood Flow

- Past techniques include:
  - Necropsy day perfusion with India ink or MicroFil followed by morphometric evaluation of vessel area/volume
  - Necropsy day in vivo $^{95}$Nb or $^{103}$Ru–labeled microspheres
  - Laser Doppler flowmetry

- Technology for measuring bone blood flow has improved over the past decade ($^{18}$F–NaF–PET/CT)
Ultimate Load vs. Blood Flow

Vertebral Body

$r=0.68; \ P<.03; \ (N=10)$

LV6 Ultimate Load (N)

K1 (ml/cc/min)

VEH
anti-VEGF
Blood Flow vs. Volume Frxn of Bound Water

Vertebral Body

$r = -0.66; P < .04; (N=12)$