Bone Cancer Pain & CB2 Cannabinoids

10th Annual NIH Pain Consortium

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THE UNIVERSITY OF ARIZONA HEALTH NETWORK

Breast Cancer

Breast cancer is the most frequent malignant tumor in women worldwide

(American Cancer Society (2011) Global Facts and Figures 2nd Edition)

http://epcnt.info/?p=31

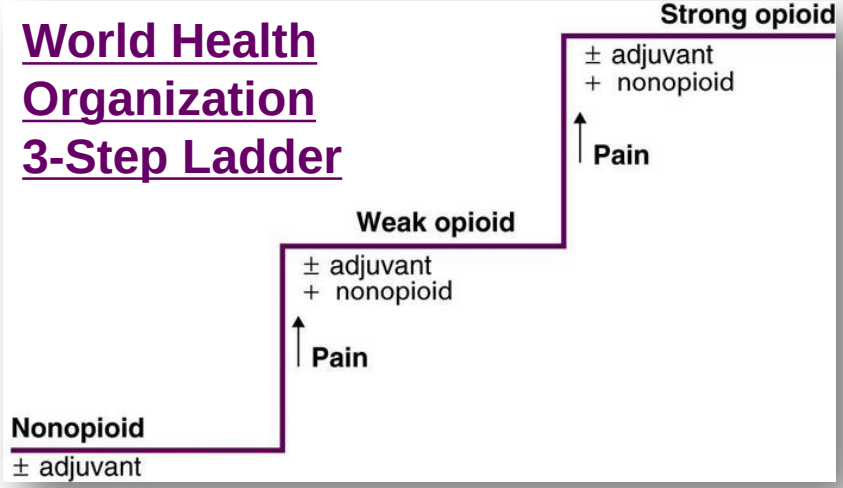
Breast Cancer and Bone Metastasis

- Breast cancer contributes the greatest number of bone metastases of all cancer types & detected in 70% of all advanced breast cancer patients (Coleman (2006) *Clinical Cancer Research* ***12***: 6243s-6249s)

Stage IV breast Cancer, Pet scan done due to the uncontrollable pain,

Radiology/Oncologist is looking for sites to perform radiation to help reduce pain

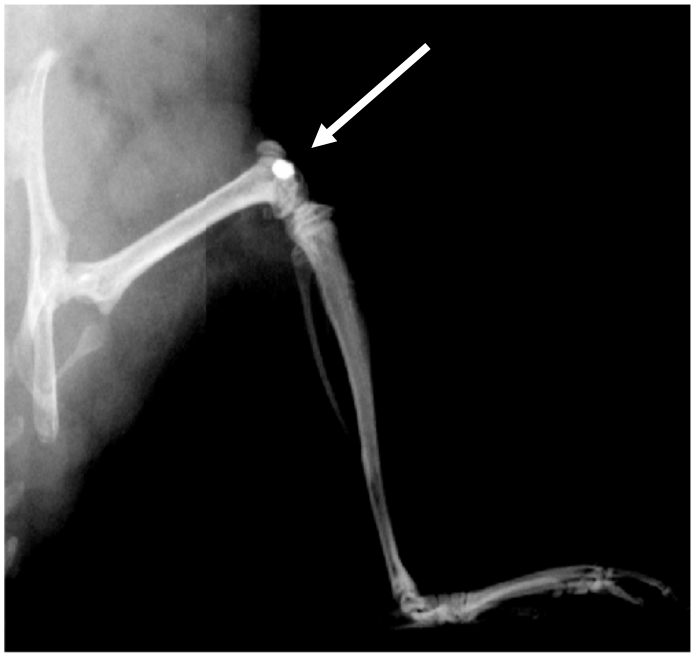
Breast Cancer and Bone Pain



- Radiation
- Opiates
  - Side effects
  - Tolerance
  - Accelerates bone loss and bone pain
    - King T, …., Vanderah TW and Porreca F (2007). Morphine treatment accelerates sarcoma-induced bone pain, bone loss and spontaneous fracture in a murine model of bone cancer. *Pain* November; 132(1-2): 154–168
- Bisphosphonates and Denosumab
  - Decrease bone loss
- Failed pain treatment with tumor progression

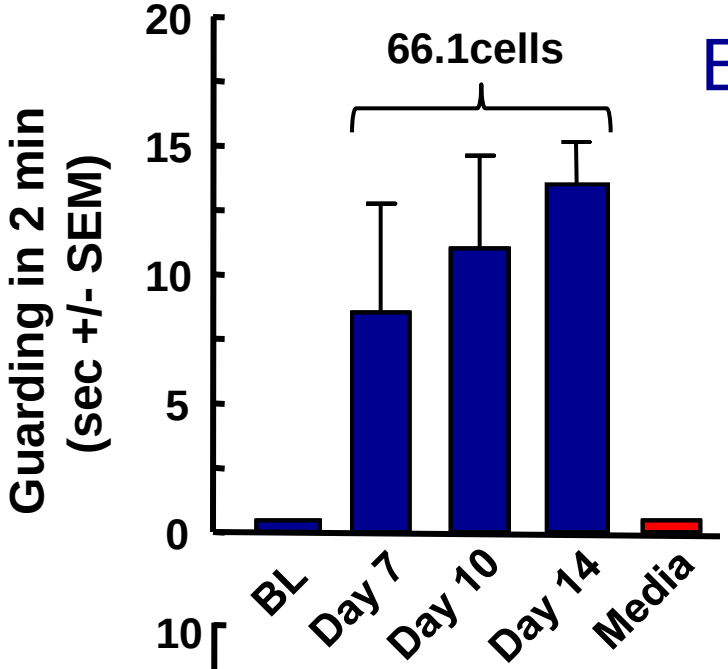
<u>Great need for novel therapy!</u>

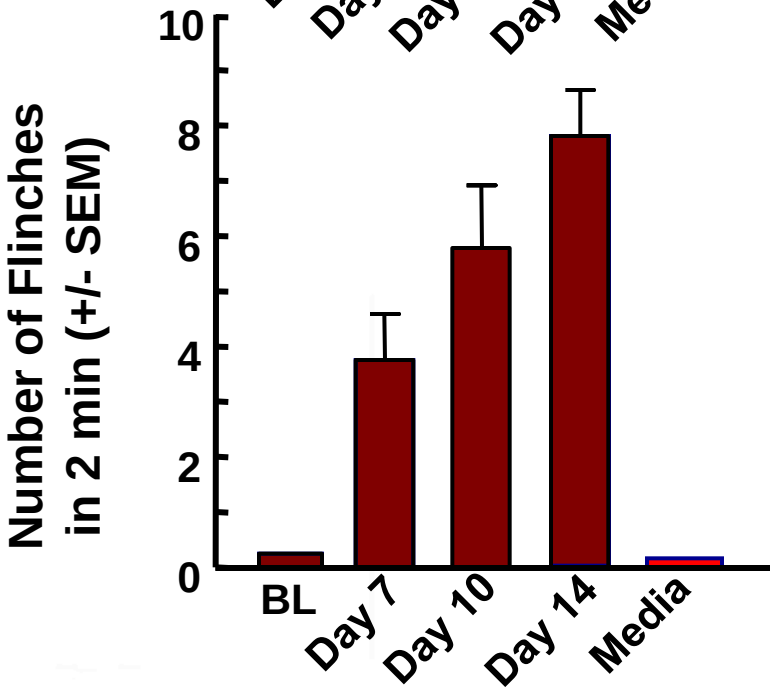
Animal Model Using Breast Cancer

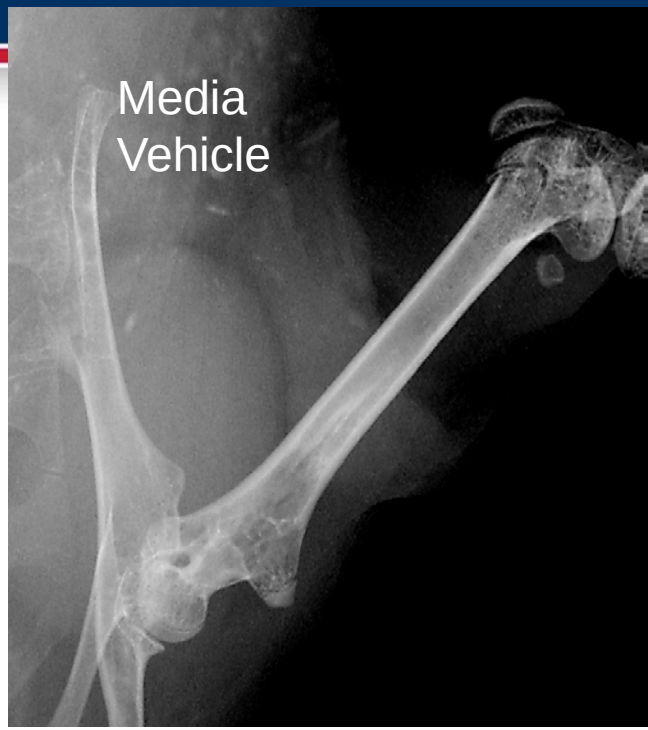


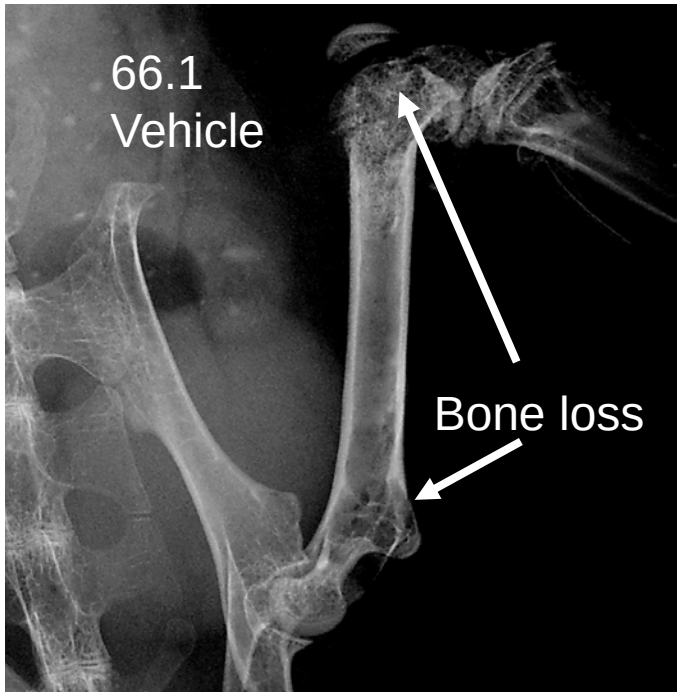
- Needle placement into the intramedullary space.
- spontaneously occurring mammary adenocarcinoma (66.1 cells, 10^5 in 5 µl) in a Non-immunocompromised BALB/cfC3H mouse.
- Dental amalgam/bone cement is used to seal the cells inside the bone.

Breast-induced Bone Cancer Pain









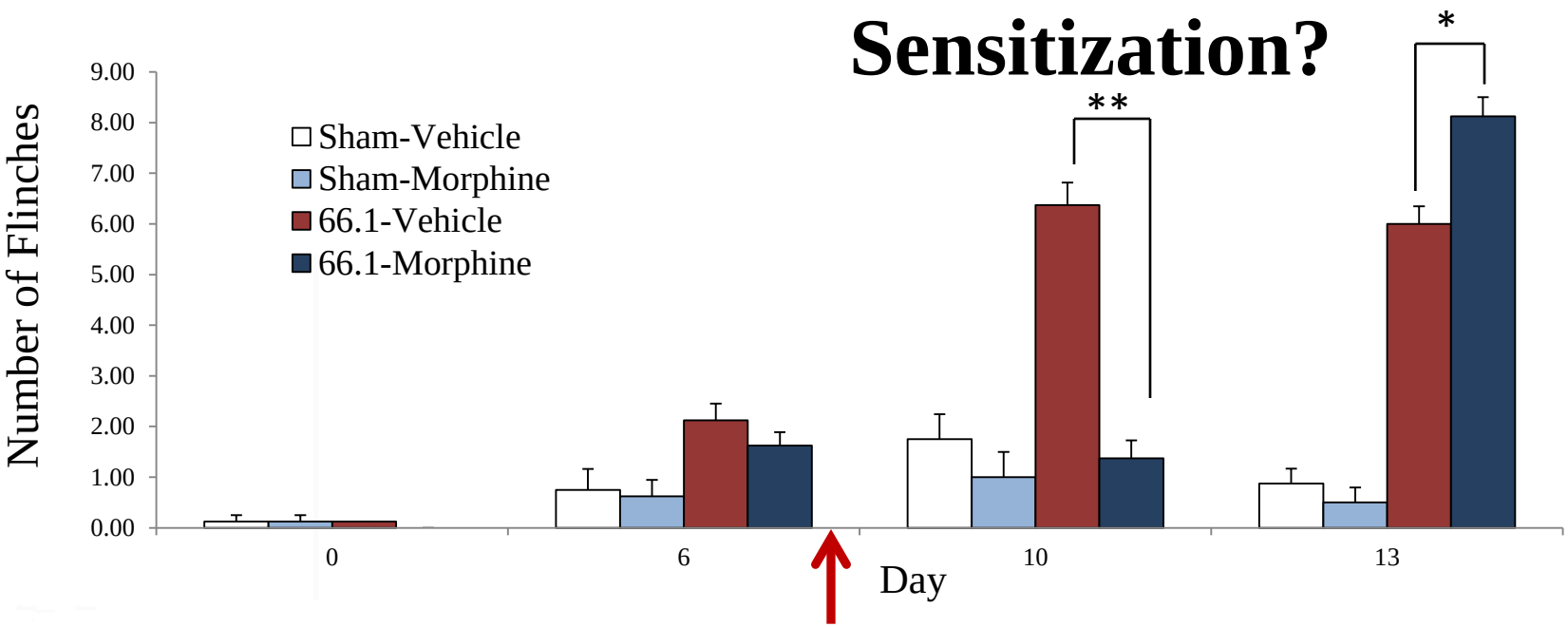
First line therapy for metastatic bone pain is insufficient and may be linked to bone degeneration

- Chronic morphine administration increases serum markers of osteolysis in rodents (Boshra 2011), and accelerates sarcoma-induced pain, bone-loss and fracture in mice. (King et al. 2007)
- Chronic opioid use increases the likelihood of spontaneous fracture in patient populations (Vestergaard et al. 2006).
- Clinical opioid use is a recognized risk factor for the development of osteoporosis (Mattia et al., 2012).
- Opioids accelerate loss of BMD in HIV+ women but not uninfected women (Sharma et al. 2011).

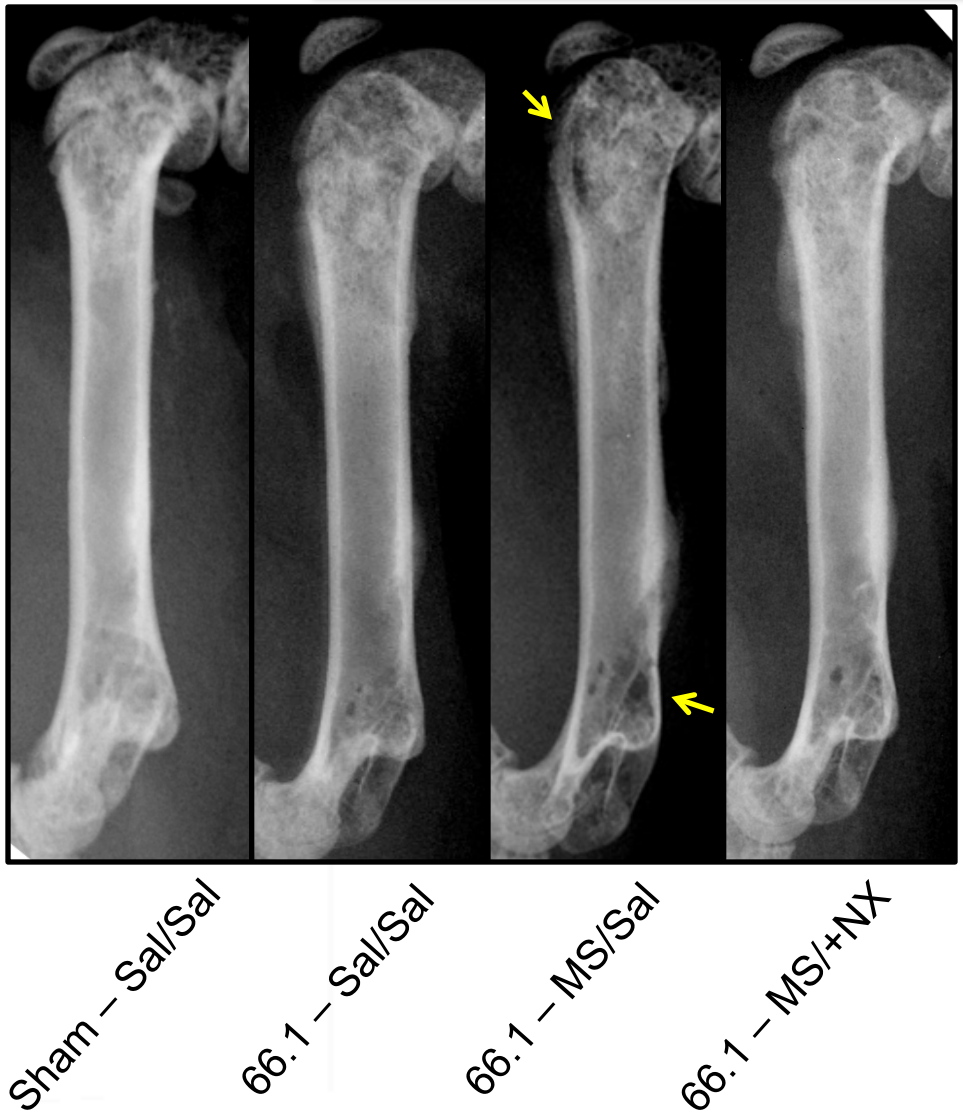
Chronic morphine administration in the 66.1-BALB/c model

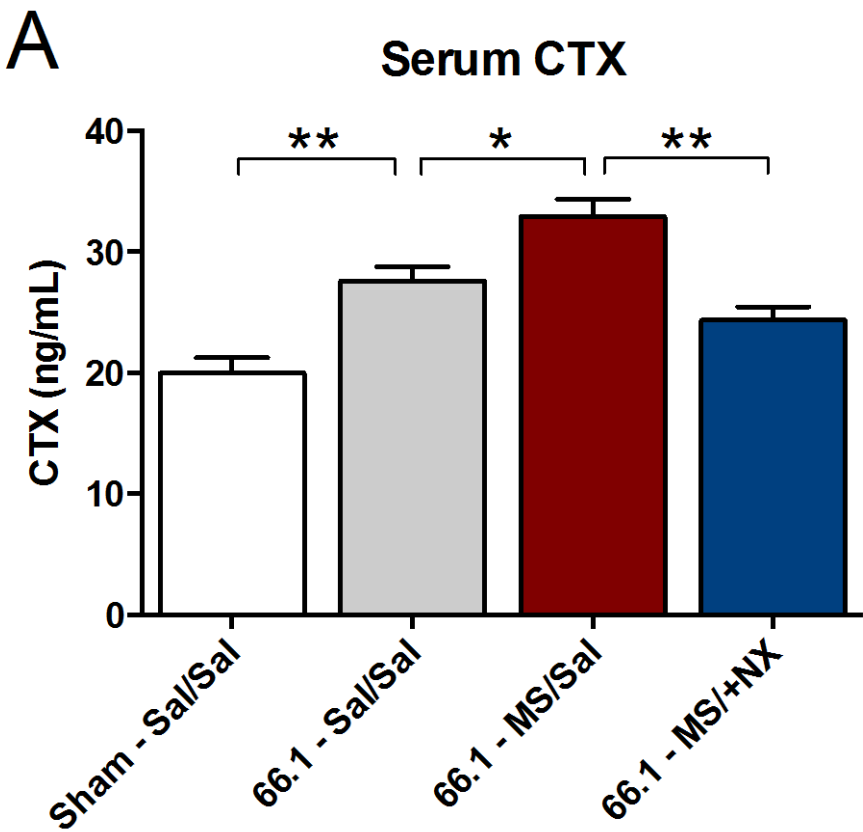
- Osmotic minipumps containing saline or morphine implanted 7 dpi (red arrow).
- 20 mg/kg/day in accordance with King et al. 2007
- n=8 animals/group, **p<0.01, *p<0.05

Tolerance?
Sensitization?



Morphine exacerbates demineralization in a model of bone cancer

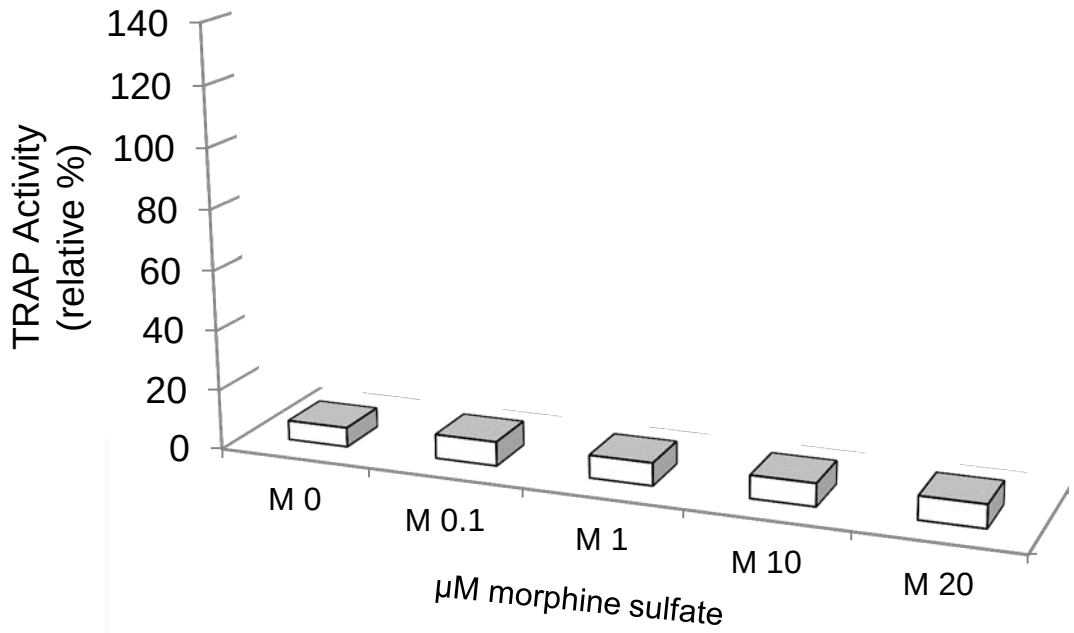




Morphine enhances osteoclastogenesis *in vitro*

RAW264.7 progenitor cells

Osteoclastogenesis Assay



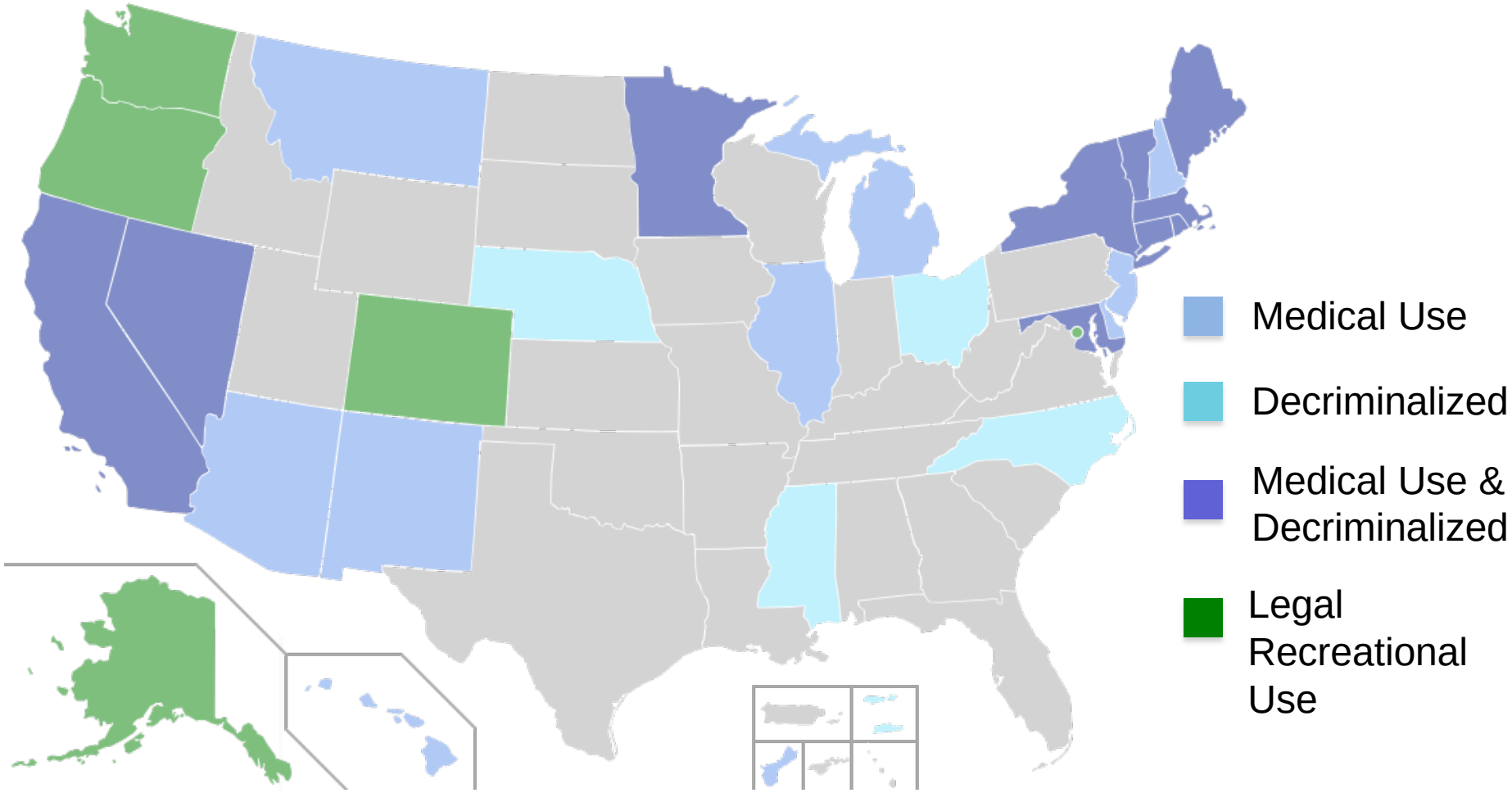
** indicates significance from control at $p<0.01$
n=5

The Need for New Pain Meds

- Sustained morphine infusion produces increased pain and bone loss in CIBP by favoring osteolytic processes.

Cannabinoids & Bone Cancer Pain

Medical use of Cannabinoids



13 Pending Legislation in 2015 (medical use)

Florida, Georgia, Indiana, Kansas, Kentucky, Missouri, Nebraska, North Carolina, Pennsylvania, South Carolina, Tennessee, Utah, West Virginia

CB_2 Receptors

Struttura dei recettori CB1 e CB2

- CB_2 receptors are found in the spleen, tonsils, immune cells, glia & osteoclasts

- CB_2 receptor activation attenuates inflammatory & neuropathic pain

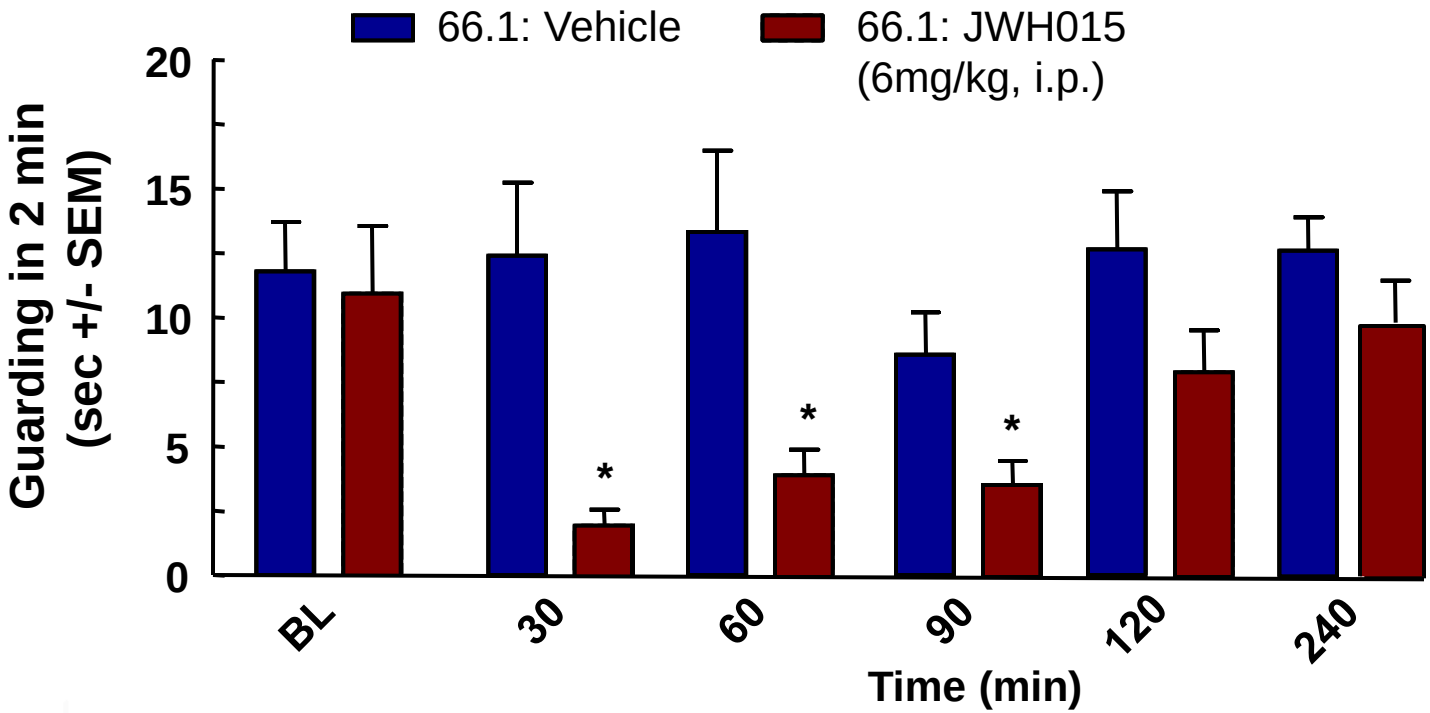
Ibrahim et al., PNAS; 2003 & 2005 *Quartilho et al., Anesthesiology; 2003*

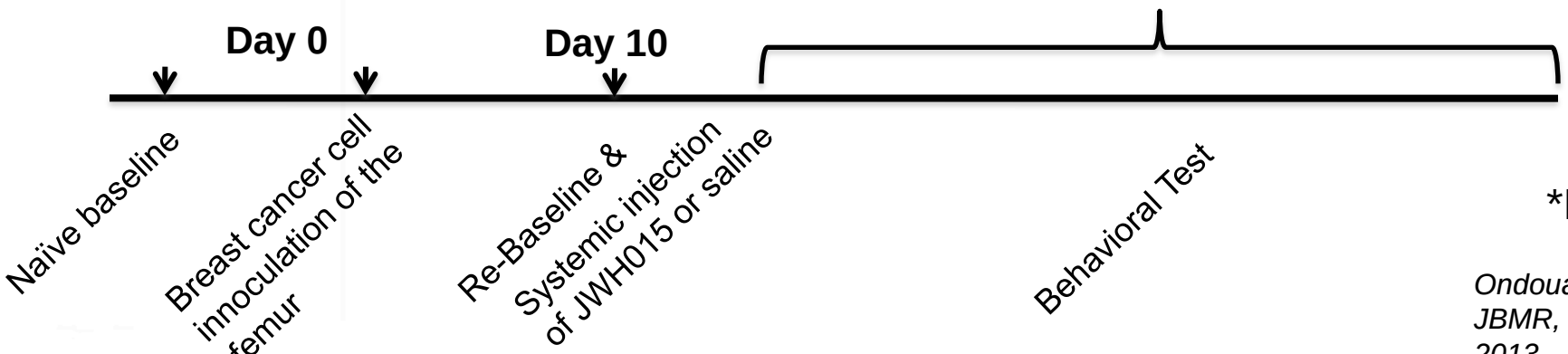
- CB_2 receptor activation decreases osteoclasts & maintains bone integrity

Ofek et al., PNAS 103; 2006

- Unlike CB_1, **CB_2 receptors do not produce psychotropic effects & are NOT rewarding**

Acute CB_2 Agonists Attenuate Bone Cancer-induced Spontaneous Pain

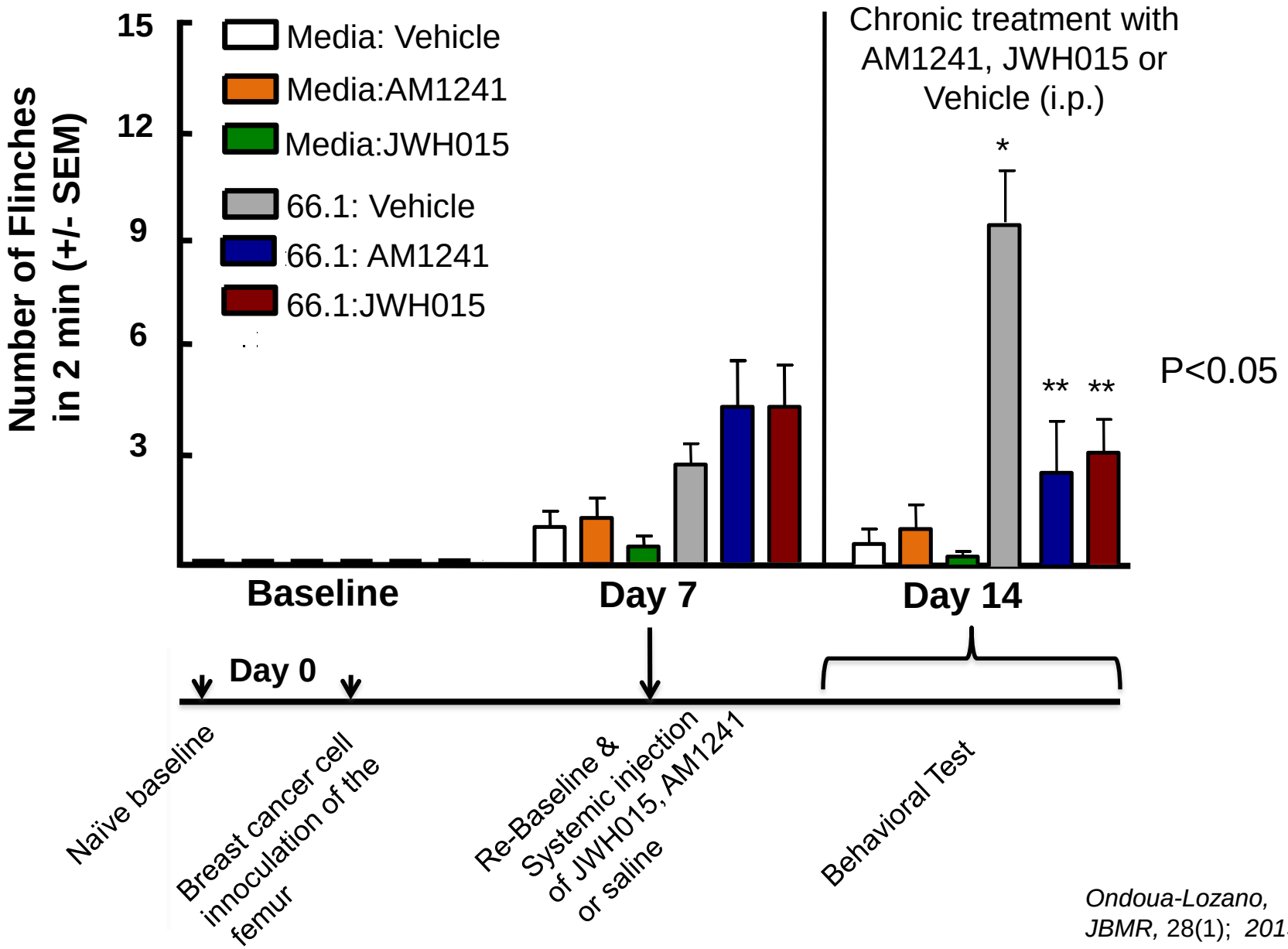




*P<0.05

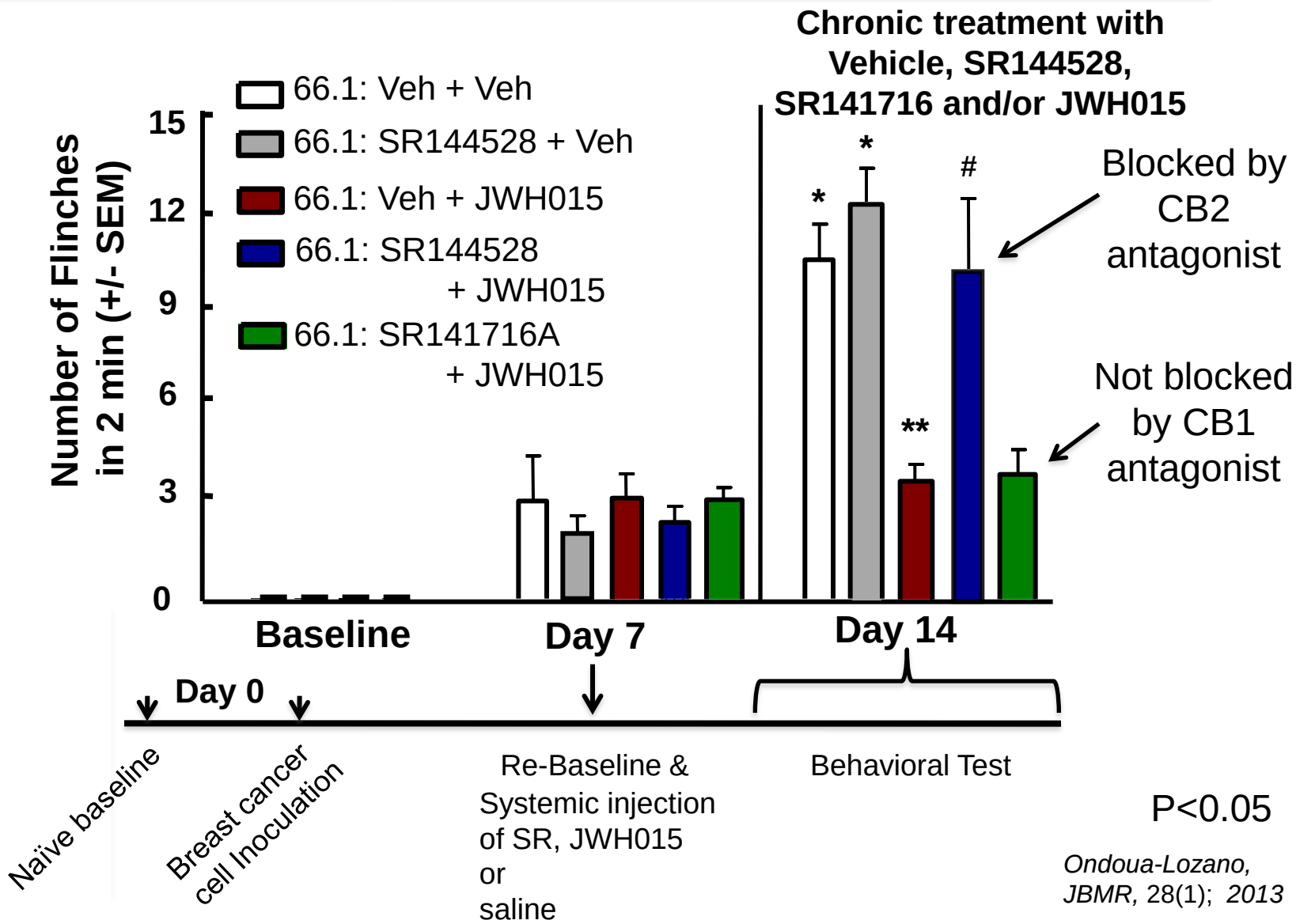
Ondoua-Lozano, JBMR, 28(1); 2013

Chronic CB_2 Agonists Attenuate BC Spontaneous Pain



Ondoua-Lozano, JBMR, 28(1); 2013

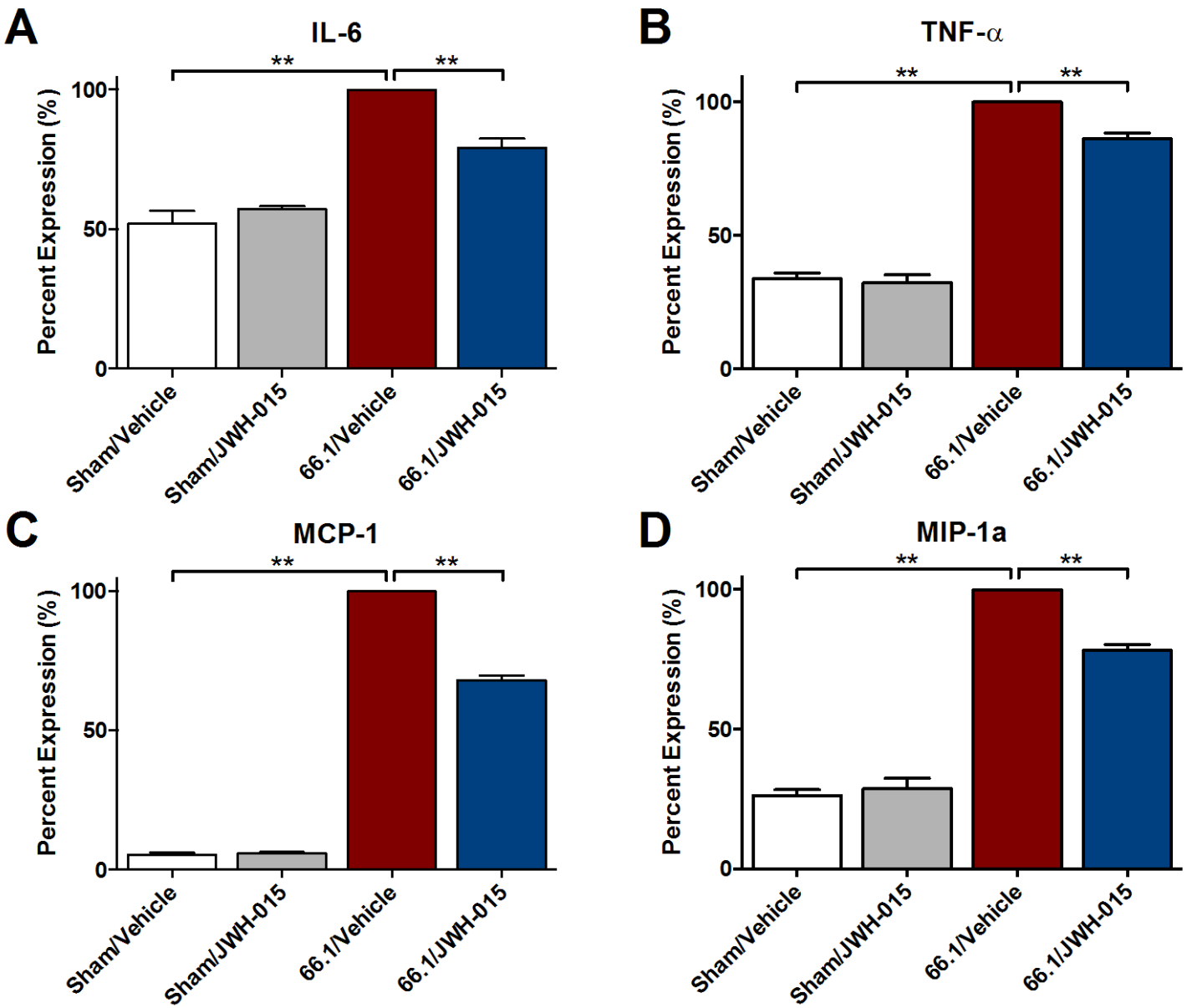
Chronic CB_2 Agonist Attenuation of Cancer-induced Spontaneous; Blocked by CB_2 Antagonist



$P<0.05$

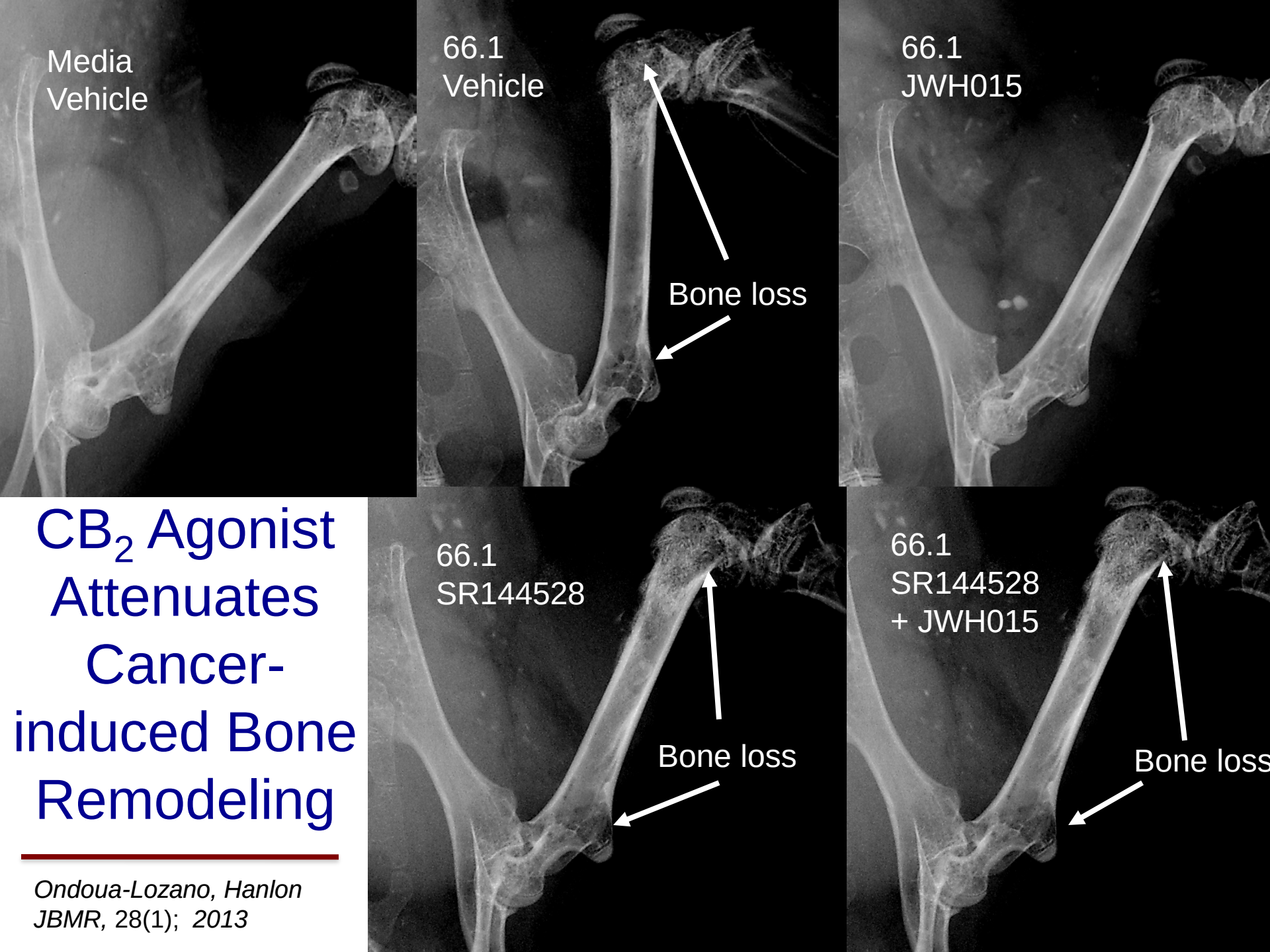
Ondoua-Lozano, JBMR, 28(1); 2013

CB_2 Agonist Attenuates Tumor-Associated Inflammation



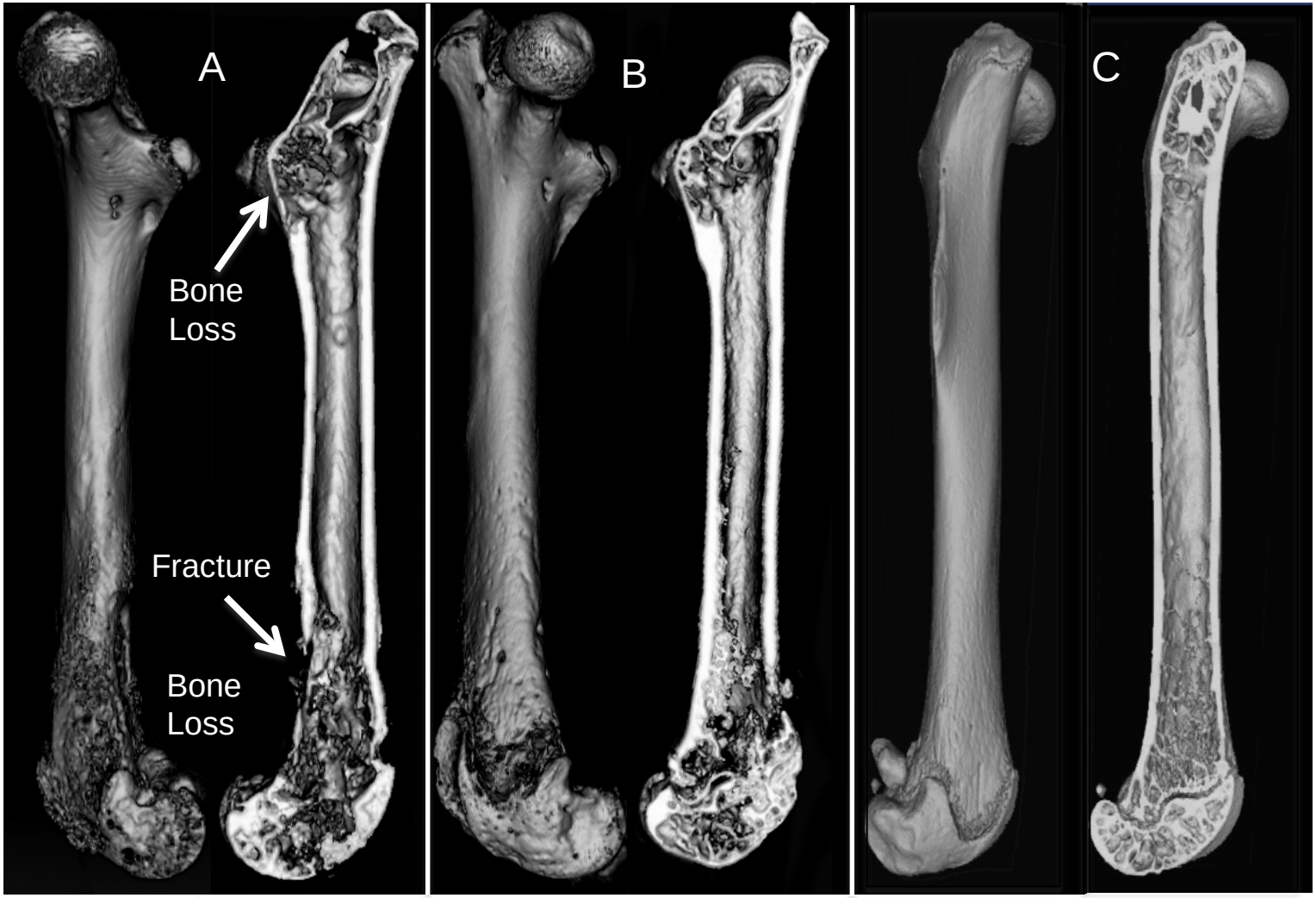
(JWH 015 6mg/kg, i.p. 1x/day for 7 days)

CB_2 Agonist Attenuates Cancer-induced Bone Remodeling



Ondoua-Lozano, Hanlon
JBMR, 28(1); *2013*

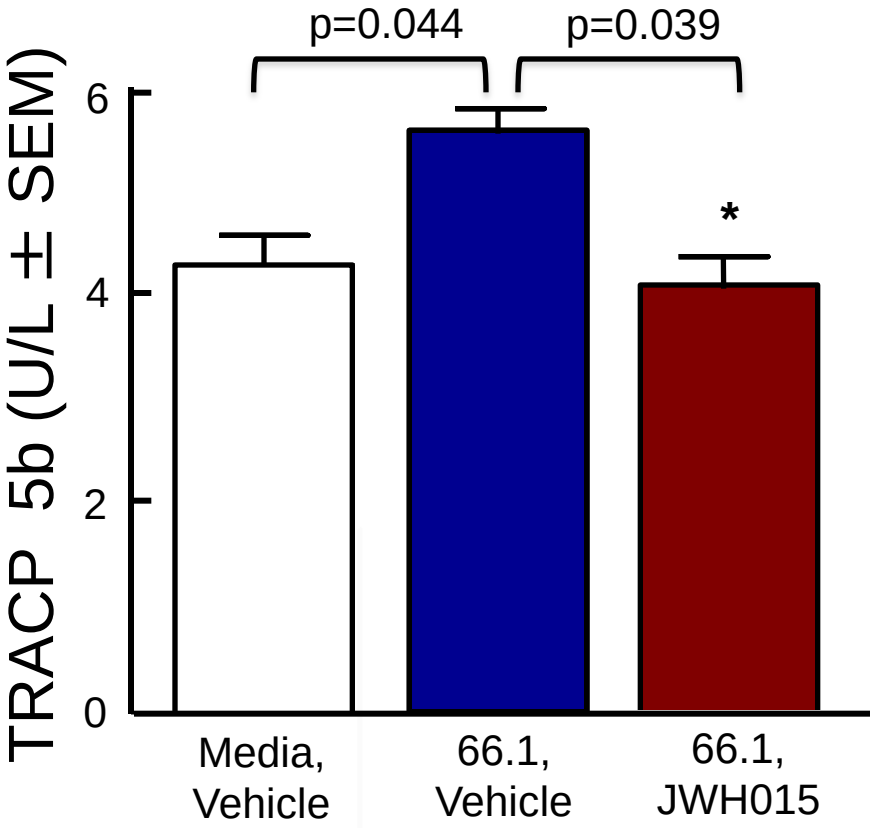
CB_2 Agonist Attenuates Cancer-induced Bone Remodeling

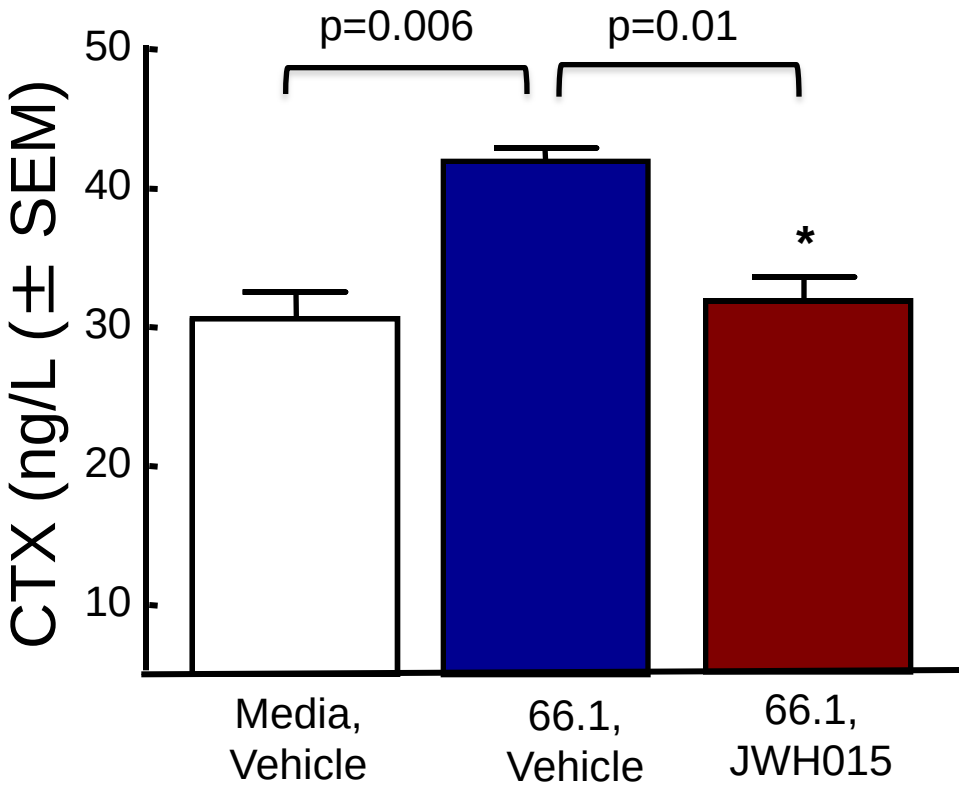


Ondoua-Lozano, JBMR, 28(1); *2013*

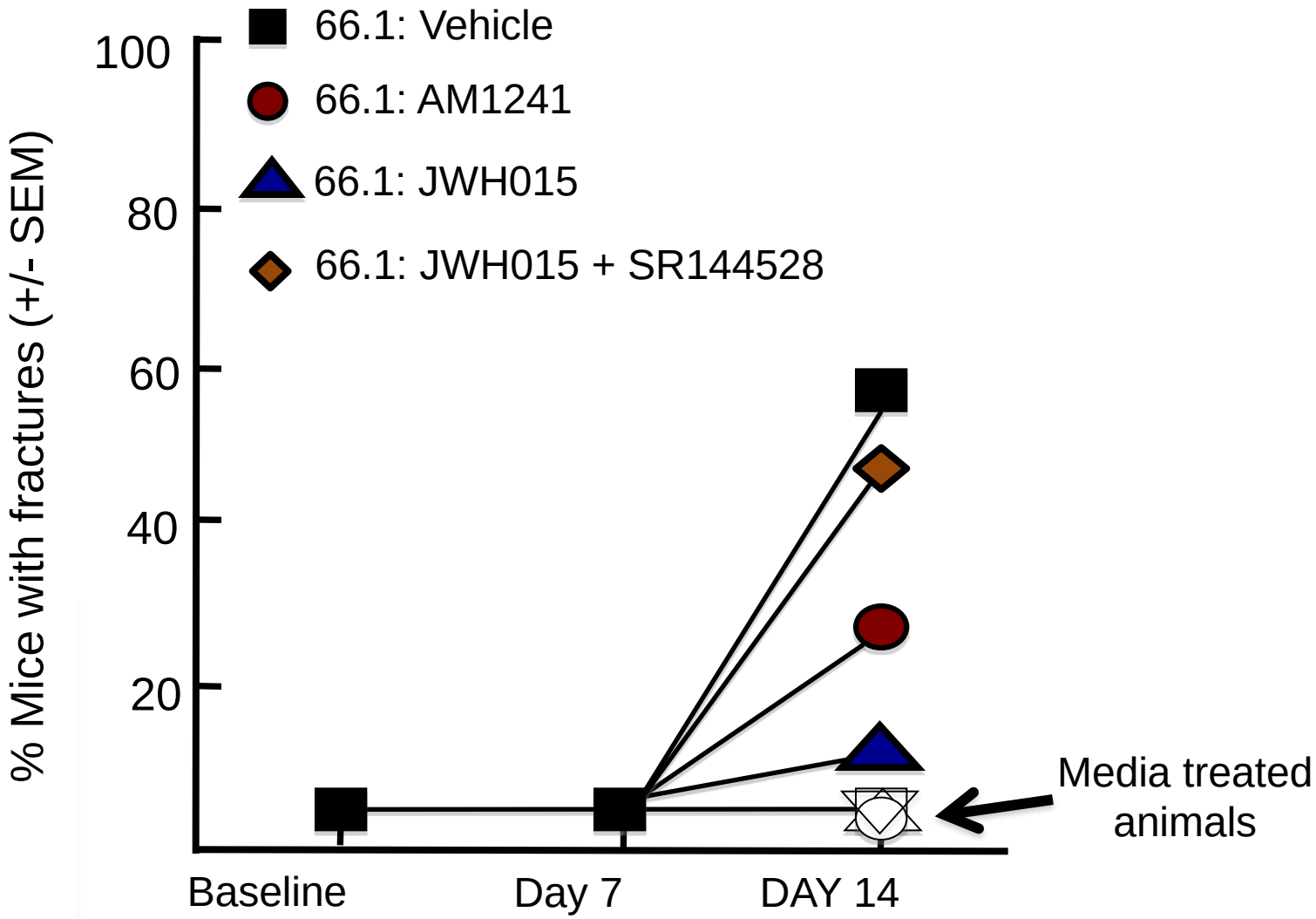
CB_2 Agonist Attenuates Cancer-induced Bone Remodeling

(6mg/kg, i.p. 1x/day for 7 days)

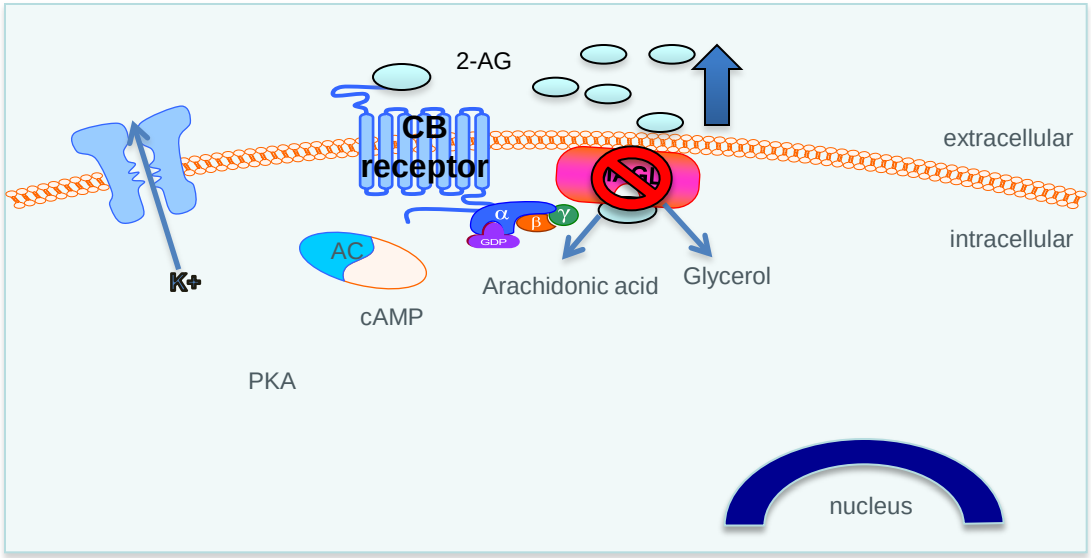


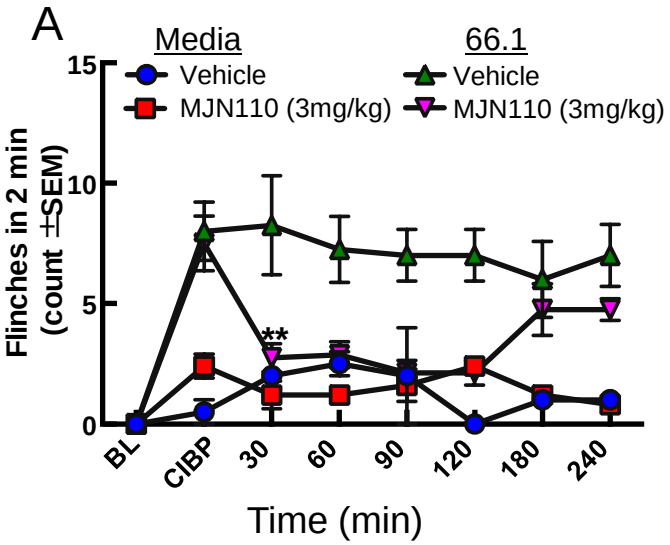


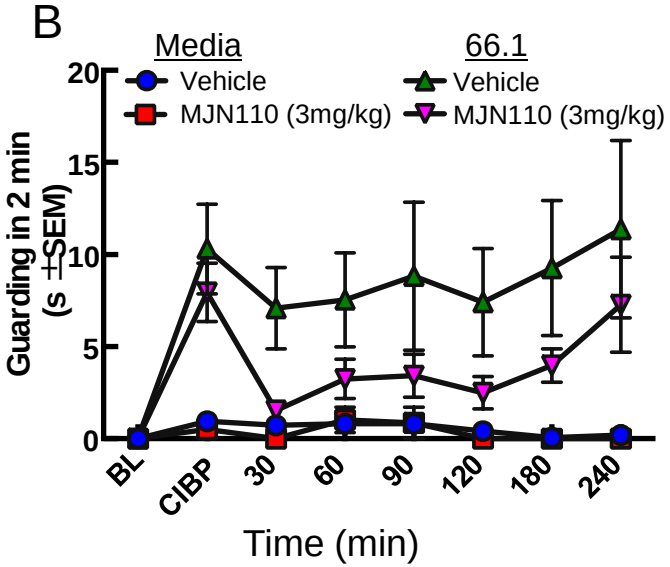
CB_2 Agonist Attenuates Cancer-induced Bone Remodeling



Acute Inhibition of MAGL With MJN110 Attenuates Spontaneous Pain Behavior on D7

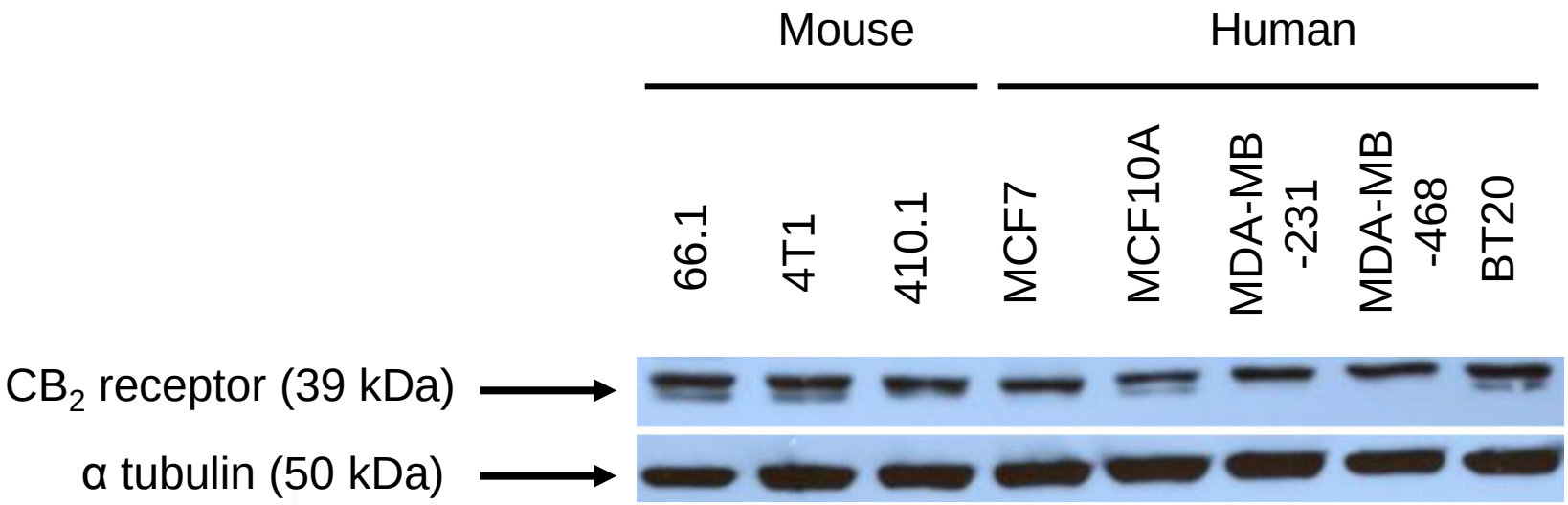




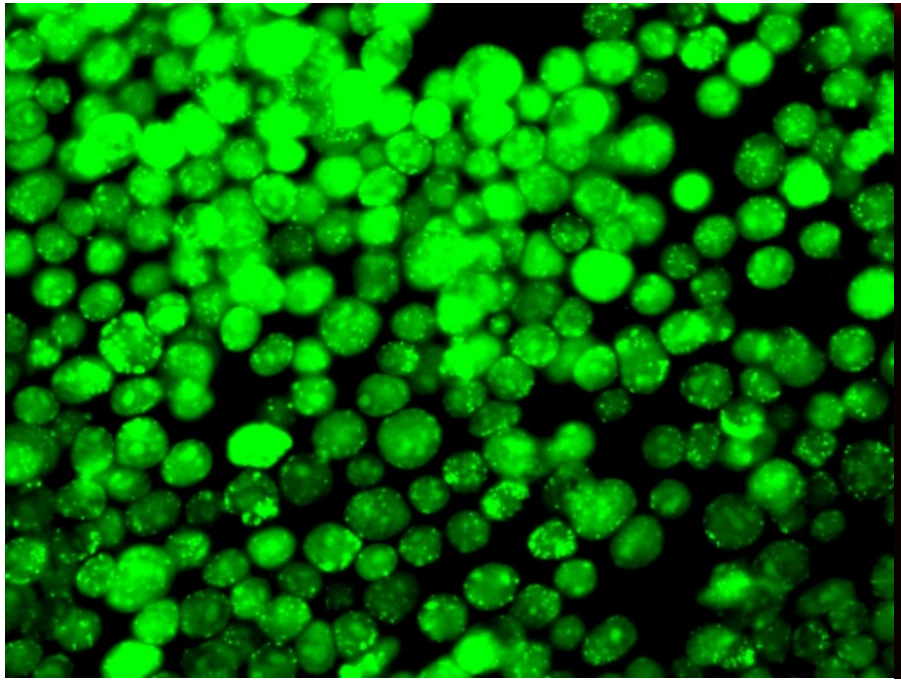


What Effects does CB_2 agonist have on tumor burden?

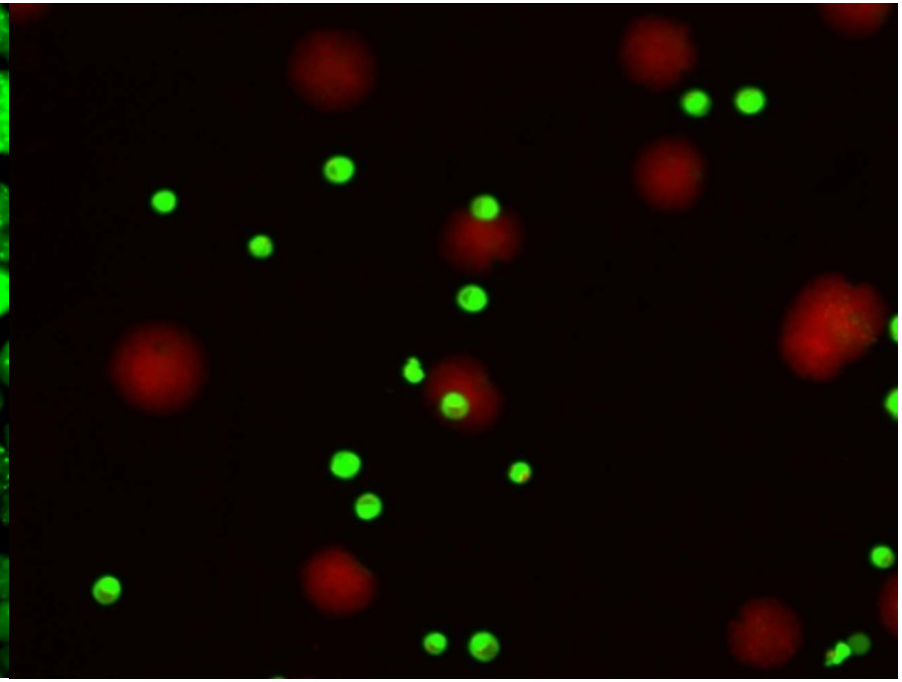
CB_2 Receptors on Breast Cancer Cells



CB_2 induced Apoptosis of Breast Cancer Cells

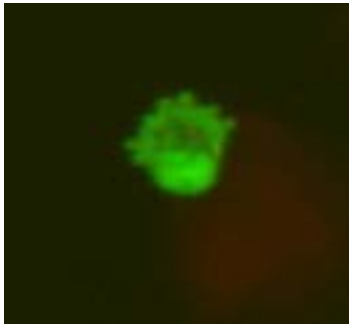


Vehicle (.01% DMSO) 66.1 Cells

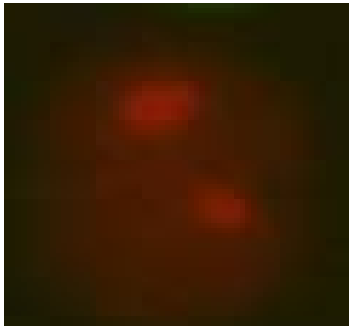


Treated (1 uM AM1241) 66.1 Cells

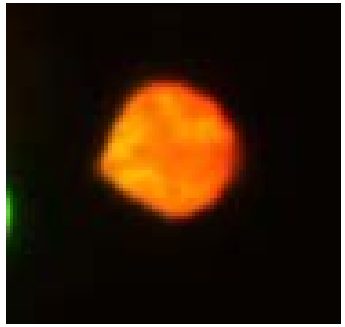
Normal cell



Pre-Apoptotic cell

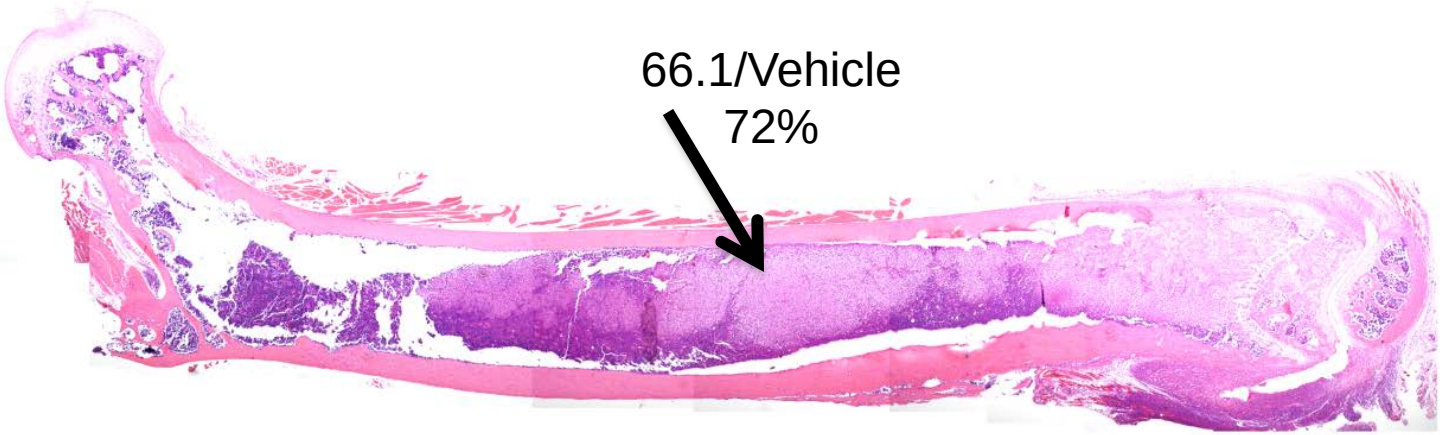


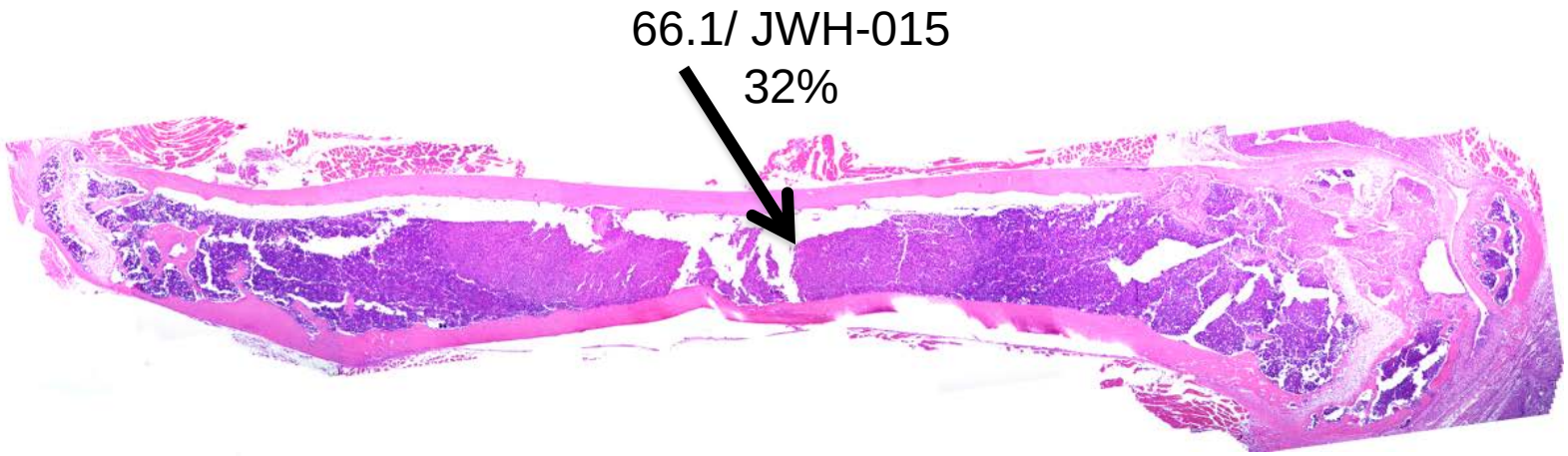
Apoptotic cell



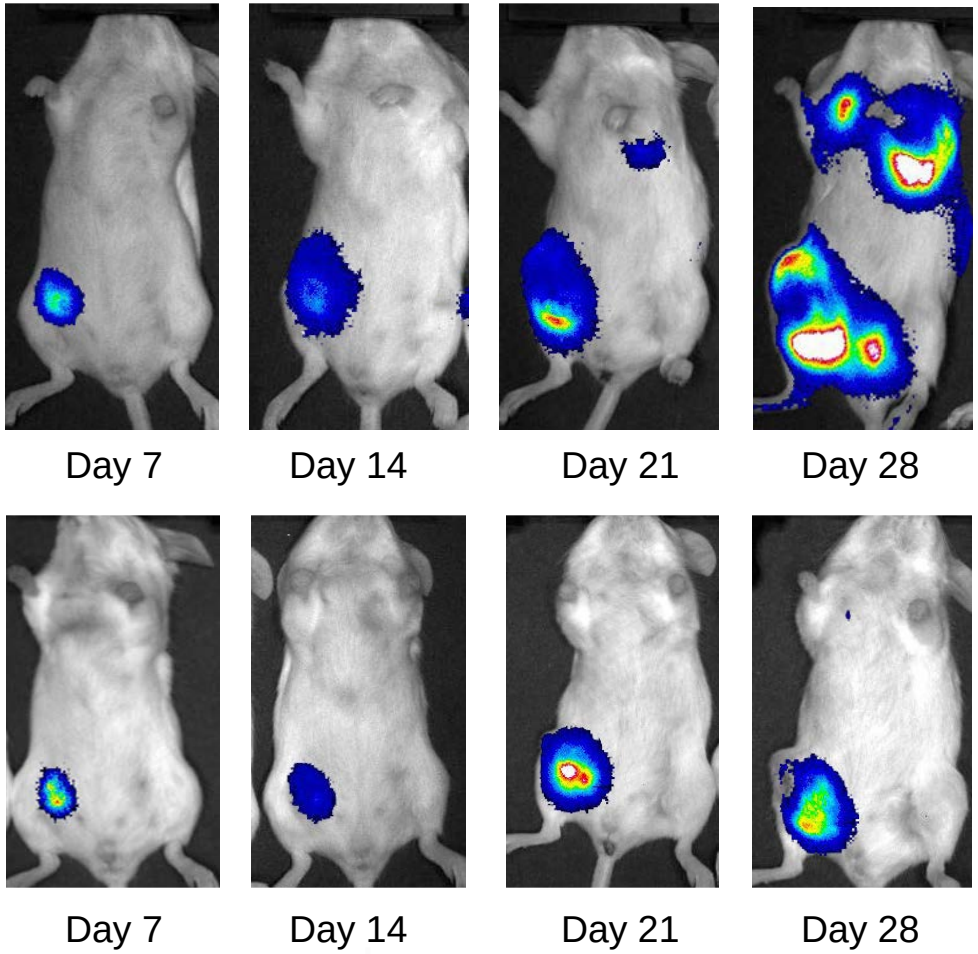
Necrotic cell

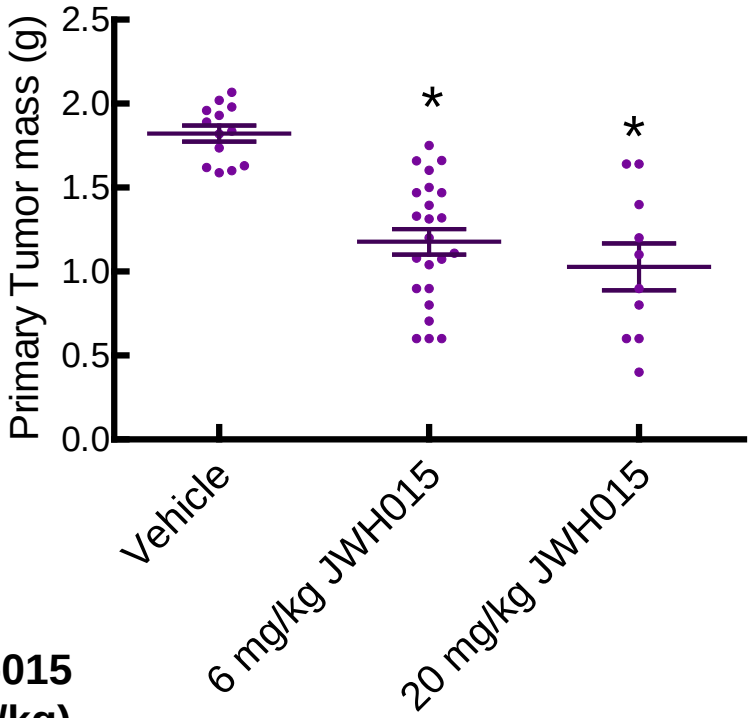
H & E Staining of Breast Cancer Cells in Animal Model





CB_2 Agonist Attenuates Primary Tumor Burden & Metastasis





Summary & Conclusions

- CB_2 cannabinoids inhibit pain and bone wasting by significantly decreasing several cytokines and chemokines as well as significantly decreasing osteoclast number and activity.

- CB_2 agonists significantly attenuates breast cancer proliferation in vitro and in vivo.

Future Studies

- Clinical Trials using the CB_2 agonists, **LY2828360**
- Analgesic Synergy studies on Opioid and CB2 agonists
- Mechanism of CB2-antiproliferative mechanism

Acknowledgements

Vanderah Lab

- Dr. William Staatz
- Dr. Ashley Liguori (former)
- Alex Sandweiss
- Shaness Grenald
- Lynn Bui (former)
- Warren Wang
- Neemah BassiriRad (former)
- Brittany Forte
- Ryan Vanderah
- Jackie Hu
- Madison Young
- Nicole Sayers
- Mary McIntosh
- Josh Stark

Collaborators

- Dr. Tally Largent-Milnes
- Dr. Patrick Mantyh
- Dr. Mohab Ibrahim
- Dr. Frank Porreca
- Dr. Daniela Salvemini (St. Louis Univ)
- Dr. Katherine Hanlon (UNE)
- Dr. Edward Bilsky (UNE)

Company Support

- Lilly Inc.
- Takeda Inc.
- Neurex Inc.
- Virobay Inc.

NIH Funding

- NCI R01 CA142115
- NIDA P01-DA06284
- STTR R41-CA192455-01

Analgesic synergy observed in various models of pain

Relative A_{50} ±95% CI

Pain Model	Behavioral Assay	Morphine	JWH015	Morphine:JWH015	Theoretical Additive	Drug Interaction
Thermal	Tail flick	7.124 ± 53.0	2.447E4 ± 4.380E5	1:3 39.2 ± 43.75	28.47 ± 71.67	Additive
Inflammatory	1.5% Formalin flinch	0.4348 ± 0.3928	0.2404 ± 0.7315	2:1 0.0108 ± 0.0216*	0.3425 ± 0.1726	Synergistic
Post-operative incision	Infrared (I.R.)	1.535 ± 7.778	3.741 ± 23.13	1:1 0.1372 ± 0.3553*	2.177 ± 8.747	Synergistic
	Von frey	1.535 ± 7.778	4.515 ± 13.87	1:1 0.1068 ± 0.4132*	2.291 ± 8.846	Synergistic
Neuropathic Spared Nerve Injury (SNI)	Infrared (I.R.)	1.955 ± 1.324	1.65 ± 17.11	1:1 0.9533 ± 2.053	1.79 ± 10.07	Additive
	Von frey	7.693 ± 24.5	4.368 ± 4.178	1:1 0.8221 ± 1.517*	5.52 ± 7.272	Synergistic
Breast cancer-induced bone pain (CIBP)	Spontaneous flinching	3.959 ± 23.35	6.422 ± 3.157	1:10 4.483 ± 9.312	6.078 ± 11.42	Additive
	Spontaneous guarding	2.107 ± 6.027	6.854 ± 8.165	1:10 6.313 ± 27.69	5.689 ± 20.81	

*significant difference from the theoretical A_{50}, indicating a synergistic relationship

CB_2 Agonist & Clinical Trials

Dental Pain 3rd Molar Tooth Extraction GW842166 &
phase IIa (proof of concept) study will be conducted as a multi-centre study - randomised, single blind, placebo- controlled, parallel group design with a positive control arm to evaluate the analgesic efficacy of pre-emptive doses of **GW842166** following dental surgery (3rd molar tooth extraction). 112 enrolled

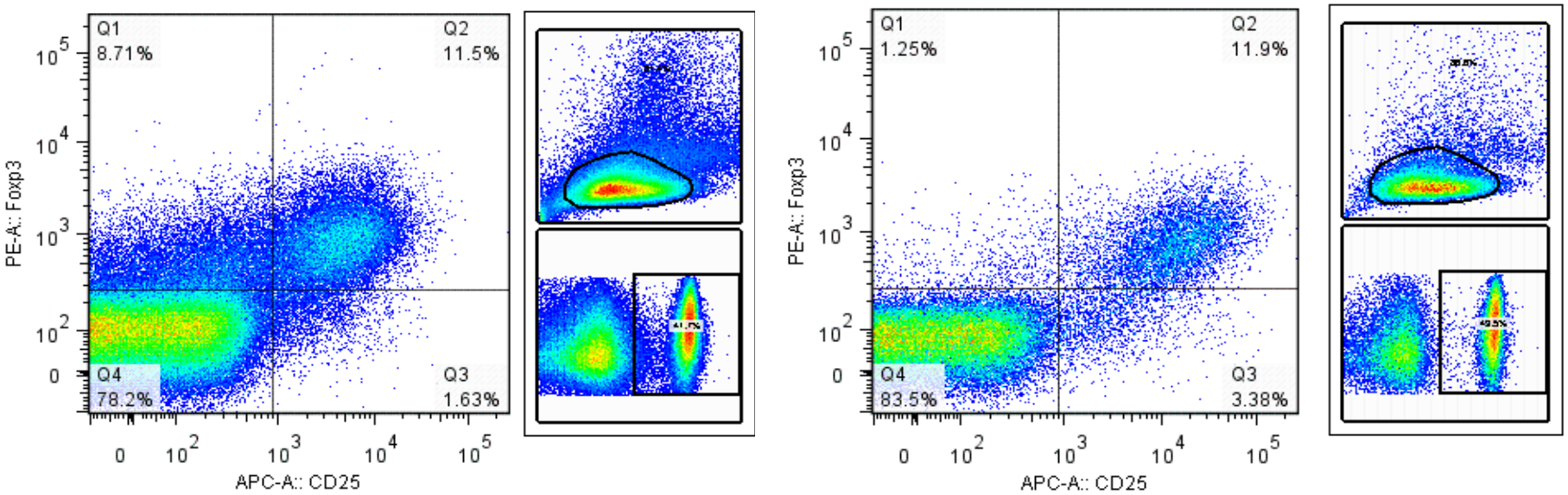
A Study of GW842166 in Adults With Osteoarthritis Pain
A Multicentre, Randomised, Double-Blind, Placebo and Naproxen (500mg) BID Controlled, Phase II Proof of Concept, Parallel Group Study to Assess the Efficacy and Safety of Oral **GW842166** at Two Dose Levels Administered for 4 Weeks in Adults With Osteoarthritis of the Knee, 308 enrolled

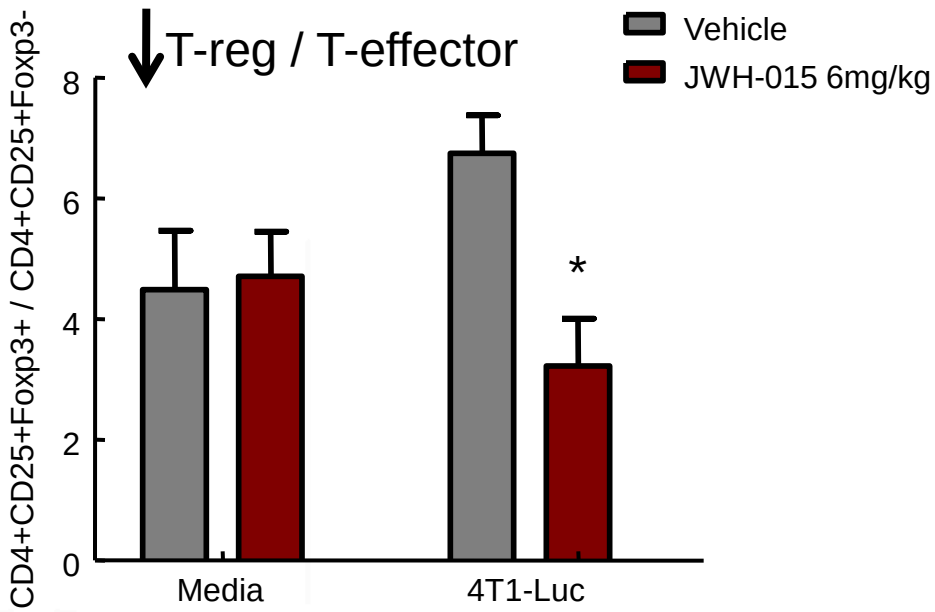
CB_2 Agonist & Clinical Trials

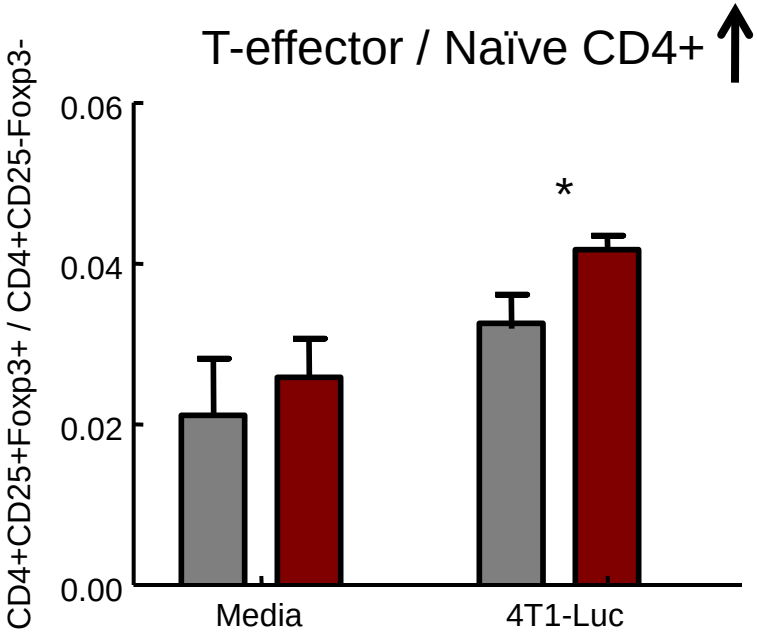
A Phase Ib/IIa, Double-Blind, Randomized Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of S-777469 in Subjects With Atopic Dermatitis
A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Efficacy and Safety Study of 2 Doses of **S-777469** (400 mg BID and 800 mg BID) in Patients With Atopic Dermatitis, 209 enrolled , Shionogi Inc.

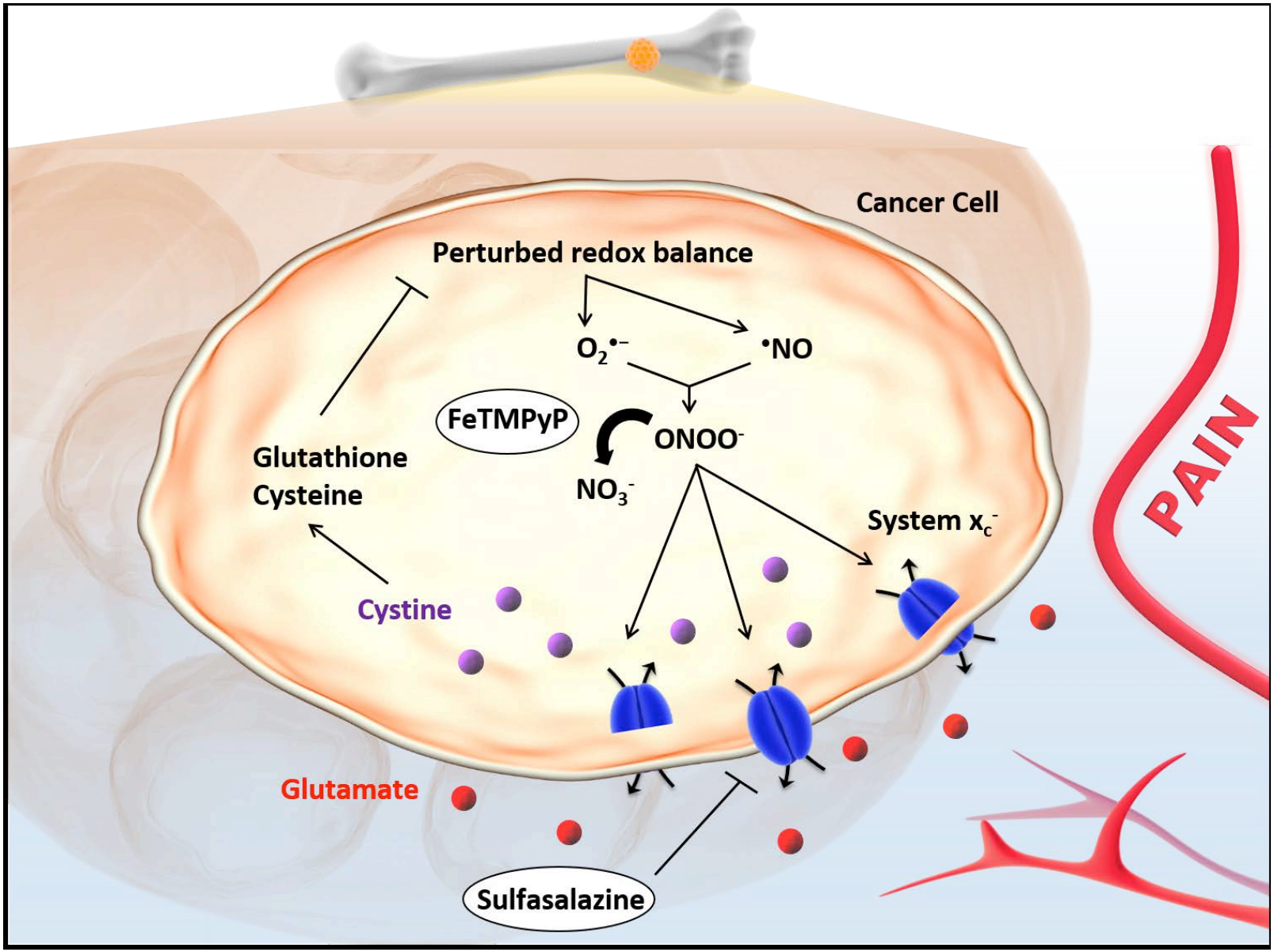
A Study of LY2828360 in Patients With Osteoarthritic Knee Pain
80 milligrams (mg) of LY2828360 daily by mouth for 4 weeks: placebo daily by mouth for 4 weeks. There is a washout period of 3 weeks between treatments. 39 enrolled

JWH-015 Attenuates Ratio of T-regulatory to T-effector Cells








Cancer Cell
Perturbed redox balance
$O_2^{\bullet-}$
$^{\bullet}NO$
FeTMPyP
$ONOO^-$
NO_3^-
Glutathione
Cysteine
System x_c^-
Cystine
Glutamate
Sulfasalazine
PAIN