PainWeek®

The Potential Role of the Type 3 Adenosine Receptor Modulators (A3RMs) in the Management of Pain

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Consulting Fee (e.g., Advisory Board): BDSI, Vertex, Teva, Amgen, Lilly, Neumentum, Collegium, Lundbeck, Gruenenthal, Redhill Pharma

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Speakers' Bureau: Abbvie, Amgen, Lilly, Teva, Lundbeck, Biohaven, Red Hill Pharma, Gruenenthal

Royalty: Elsevier

Paul J. Christo, MD

- Consulting Fee (e.g., Advisory Board):
- Y-mAbs, Eli Lilly, GSK Consumer Healthcare, Exicure

Learning Objectives

- Describe the prevalence of acute pain in the US
- Explain the progression of acute pain to chronic pain
- List risks and benefits of NSAIDs and non-NSAID analgesics
- Outline the adenosine receptor system
- Define the potential mechanism of action related to analgesia
- Review existing data on a novel Adenosine Receptor 3 Modulator, NTM-006

Charles Argoff, MD

Part 1: Overview of Acute and Chronic Pain; Risks and Benefits of Current Non-Opioid Analgesics

The Need for New Approaches to Managing Pain is Real

- The prevalence and burden of pain is high
  - Chronic and acute pain
- There is an opioid crisis
  - Prescription opioids are often misused and overprescribed
  - Opioid overdoses and death
- Many nonpharmacological pain management modalities are inadequately reimbursed/accesible
  - Eg, physical therapy, occupational therapy, acupuncture
- Pain management is inadequately addressed in most medical training curricula

IOM (Institute of Medicine). 2011. *Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research*. Washington, DC: The National Academies Press; University of Wisconsin–Madison. Pain & Policy Studies Group. www.painpolicy.wisc.edu. Accessed January 17, 2018; Nahin RL. *J Pain*. 2015;16(8):769-780; Kroenke K, Cheville A. *JAMA*. 2017;317(23):2365-2366.

The Prevalence of Acute Pain in the US is High

- 100 million surgical procedures annually in the US
- 40 million injury-related visits to the ER annually
  - 80% of surgical procedures and trauma result in acute pain – approximately 100 million individuals
  - Most acute pain will resolve, but can sometimes evolve into chronic pain
- Severe acute postoperative pain is a predictive factor for persistent postoperative pain
  - Clinical discomfort lasting >2 months postsurgery without other causes such as infection or pain from a condition preceding the surgery

www.cdc.gov/nchs/faststats. Apfelbaum JL, et al. *Anesth Analg* 2003 Aug;97(2):534-40. Richebe P, et al. *Anesthesiology*.2018;129(3):590-607

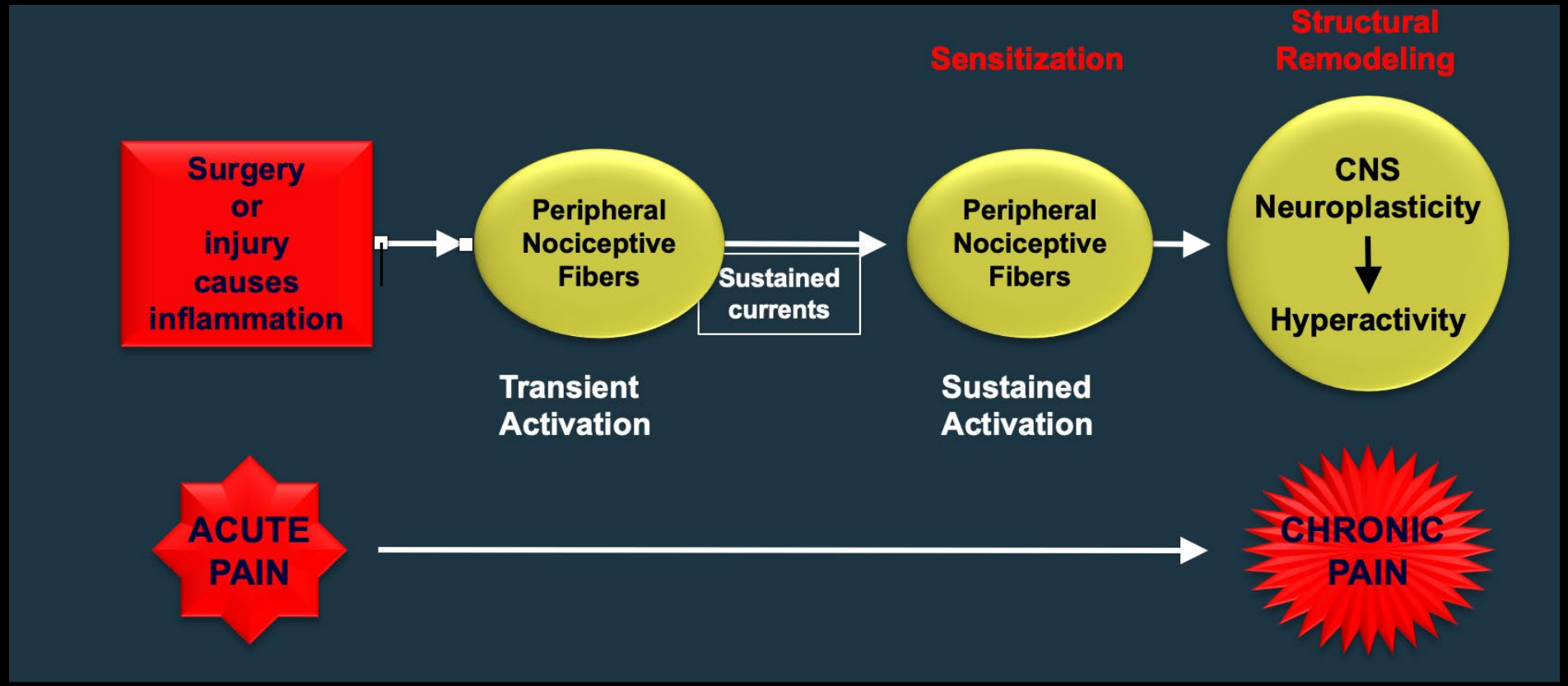
The Prevalence of Chronic Pain in the US is High

- Chronic pain is defined as pain that persists or recurs for > 3 months
- The prevalence and burden of chronic pain is high
  - 100 million Americans experience chronic pain (33%)
  - 25.3 million US adults report daily (chronic) pain; 23.4 million report a lot of pain
  - Numerous studies indicate undertreated pain: eg, cancer, older adults, children, minorities
  - Low back pain, neck pain, and osteoarthritis are among the 9 leading causes of disability
  - Low back pain is the leading cause of years lived with disability in the United States and accounts for one-third of all work loss

IOM (Institute of Medicine). 2011. *Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research*. Washington, DC: The National Academies Press; University of Wisconsin–Madison. Pain & Policy Studies Group. www.painpolicy.wisc.edu. Accessed January 17, 2018; Nahin RL. *J Pain*. 2015;16(8):769-780; Kroenke K, Cheville A. *JAMA*. 2017;317(23):2365-2366.

Long-Term Consequences of Acute Pain: Potential for Progression to Chronic Pain

Hypothesized Mechanism of Progression from Acute to Chronic Pain



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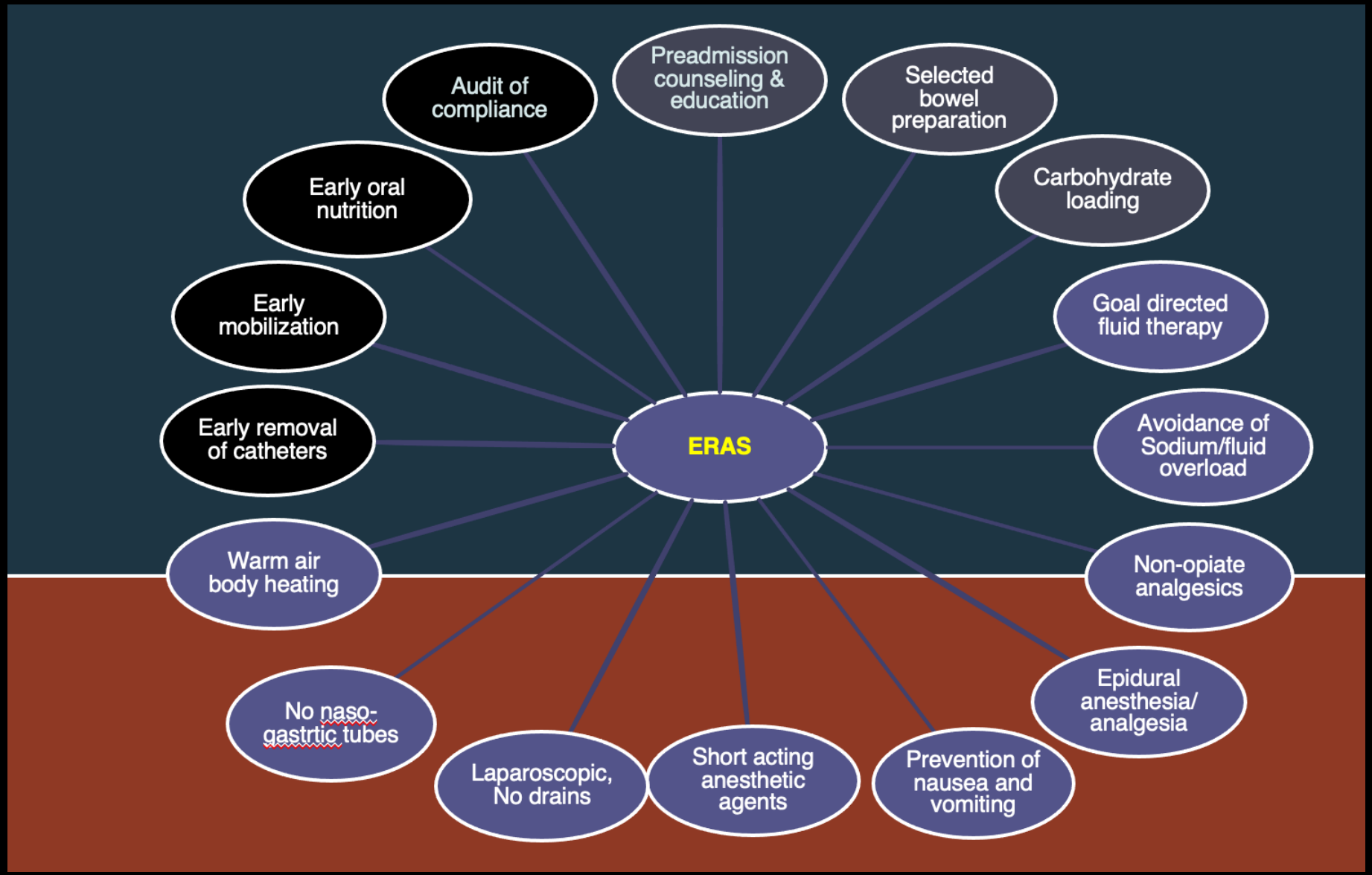
Source: Woolf. Ann Intern Med. 2004;140:441; Petersen-Felix. Swiss Med Weekly. 2002;132:273-278; Woolf. Nature.1983;306:686-688;
Source: Woolf et al. Nature. 1992;355:75-8.



PAINWEEK

Ljungqvist O, Scott M, Fearon KC. Enhanced Recovery After Surgery: A Review. JAMA Surgery 2017 March 1;152(3): 292-298.

Pharmacological Alternatives

- Implement multi-modal therapy
  - Synchronous administration of two or more pharmacological agents or approaches, each with a distinct mechanism of action
- Rationale:
  - Targeting of different receptors and pain pathways
  - Synergism of multiple agents
  - Allow for dose reduction of individual agents, leading to less risk of adverse effects
- The choice of medication, dose, route, and duration of therapy should be individualized.

Acetaminophen: Mode of Action (MOA

- MOA evidence now suggests that the analgesic effect of APAP is partly due to the indirect activation of cannabinoid CB(1) receptors.
- Thus, acetaminophen my have multiple MOA, one of which ultimately acts as a pro-drug, the active one being a cannabinoid.
  - Dual effect may be both a direct analgesic effect and modulation effect

Bertolini A et al. CNS Drug Rev. 2006;12:250-75
Graham CC et al. Inflammopharmacology. 2013;21:201-232.
Anderson BJ. Paediatr Anaesth. 2008;18:915-21

Acetaminophen in LBP

- Acute or subacute low back pain
  - ACP: Low-quality evidence showed no difference between acetaminophen and placebo for pain intensity or function through 4 weeks
- Chronic low back pain
  - ACP: no indication
  - APS-ACP: first line option
    - Acetaminophen is a slightly weaker analgesic than NSAIDs (10 points on a 100-point visual analogue pain scale)

Qaseem A, et al. *Ann Intern Med* 2017 doi:10.7326/M16-2367
Chou R, et al. *Ann Intern Med.* 2007;147:478-491

Acetaminophen in OA

- OARSI recommends acetaminophen for patients without co-morbidities
- ACR conditionally recommends it too
- The AAOS was unable to recommend for or against its use
- A recent systematic review and network meta-analysis of 137 studies comprising 33 243 participants concluded that all active interventions were significantly better than acetaminophen

McAlindon TE, et al. *Osteoarthritis Cartilage* 2014;22:363-88 Kolasinski SL et al. *Arthritis Rheumatol* 2020;Jan 6
Jevsevar DS, et al. *J Bone Joint Surg Am*. 2013;95:1885-6
Bannuru RR, et al. *Ann Intern Med.* 2015;162:46-54. doi:10.7326/M14-1231

Acetaminophen Adverse Effects

- Thrombocytopenia, agranulocytosis, pancytopenia, hemolytic anemia
- Methemoglobinemia
- Hypoglycemia
- Hypothermia
- Pancreatitis
- Nephrotoxicity
- Hepatotoxicity (with overdose; > 4 g/d), hepatic necrosis
- Pneumonitis
- Rash, and hypersensitivity

Qaseem A, et al. *Ann Intern Med* 2017 doi:10.7326/M16-2367

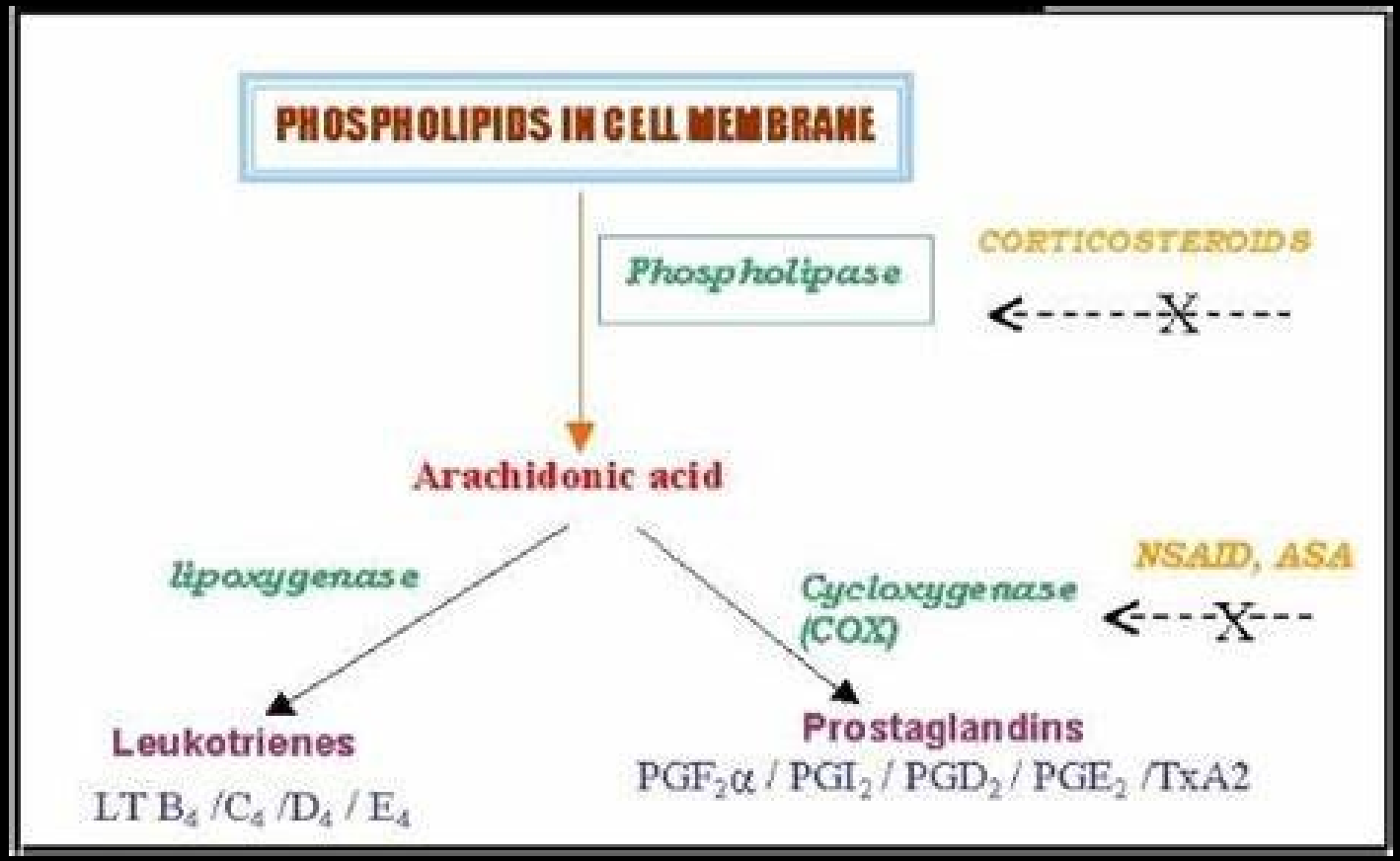
Acetaminophen

- Routes of administration and formulations – tablets, capsules, liquid, IV
- Initial dosing, dose titration and tapering –
  - start at 350 mg Q 4-6 h
  - titrate to 500 mg Q 6 h
  - maximum daily dose 4 g/d
  - maximum daily dose 3 g/day age 65 and older
  - < 2 g/d in patients with a history alcoholism
- Contraindications – liver dysfunction

Graham GG et al. Am J Ther. 2005 Jan-Feb;12(1):46-55. www.rxlist.com

Non-Steroidal Anti-Inflammatory Agents (NSAIDs)

- Mechanism of action – inhibition of cyclo-oxygenase (COX), the enzyme responsible for biosynthesis of prostaglandins and thromboxane; nonselective NSAIDs inhibit both COX-1 and COX-2; COX-2 selective NSAIDs only inhibit COX-2



Non-Steroidal Anti-Inflammatory Agents (NSAIDs)

- Contraindications – avoid in individuals with known coronary artery disease, angina, history of stroke, and those at a higher than average risk for these conditions; avoid in individuals with kidney disease, heart failure, cirrhosis; not recommended in third trimester of pregnancy
- Adverse events – FDA warning of risk for heart attack and stroke as early as the first weeks of use; GI toxicity (selective NSAIDs have less potential to cause ulcers or GI bleeding); liver toxicity; kidney toxicity; hypertension; congestive heart failure
- Drug interactions – caution in patients on diuretics; increased risk of bleeding in patients on low dose aspirin, any anticoagulant; phenytoin; cyclosporine

Bacchi S et al. *Antiinflamm Antiallergy Agents Med Chem*. 2012;11(1):52-64; Solomon DH et al. *UpToDate* 2020

NSAIDs in Low Back Pain (LBP)

- Moderate-quality evidence showed that NSAIDs were associated with small to moderate pain improvement compared with placebo
- Low-quality evidence showed that NSAIDs were associated with no to small improvement in function
- Moderate quality evidence showed that most head-to-head trials of one NSAID versus another showed no differences in pain relief in patients with chronic low back pain
- Regardless, NSAIDs were positioned as first-line therapy
- There were no data on COX-2–selective NSAIDs.

Qaseem A, et al. *Ann Intern Med* 2017

NSAID in Osteoarthritis (OA)

- The OARSI recommmends oral nonselective NSAIDS for treatment of all OA subphenotypes
- COX-2 selective oral NSAIDs were deemed appropriate for individuals without comorbidities and mutiple-joint OA with moderate co-morbidity risk.
- Proton-pump inhibitor (PPI) co-prescription with oral NSAIDs is not recommended for those with no co-morbidity risk
- Use of oral NSAIDs is strongly NOT recommended for individuals with high co-morbidity risk

McAlindon TE, et al. *Osteoarthritis Cartilage* 2014;22:363-88

NSAIDs in OA (cont'd)

- The 2019 ACR guideline strongly recommends oral NSAIDs for knee, hip and/or hand OA

- A 2013 clinical practice guideline from the American Academy of Orthopaedic Surgeons (AAOS) recommends the use of oral NSAIDs for symptomatic osteoarthritis of the knee

Kolasinski SL et al. *Arthritis Rheumatol* 2020;Jan 6

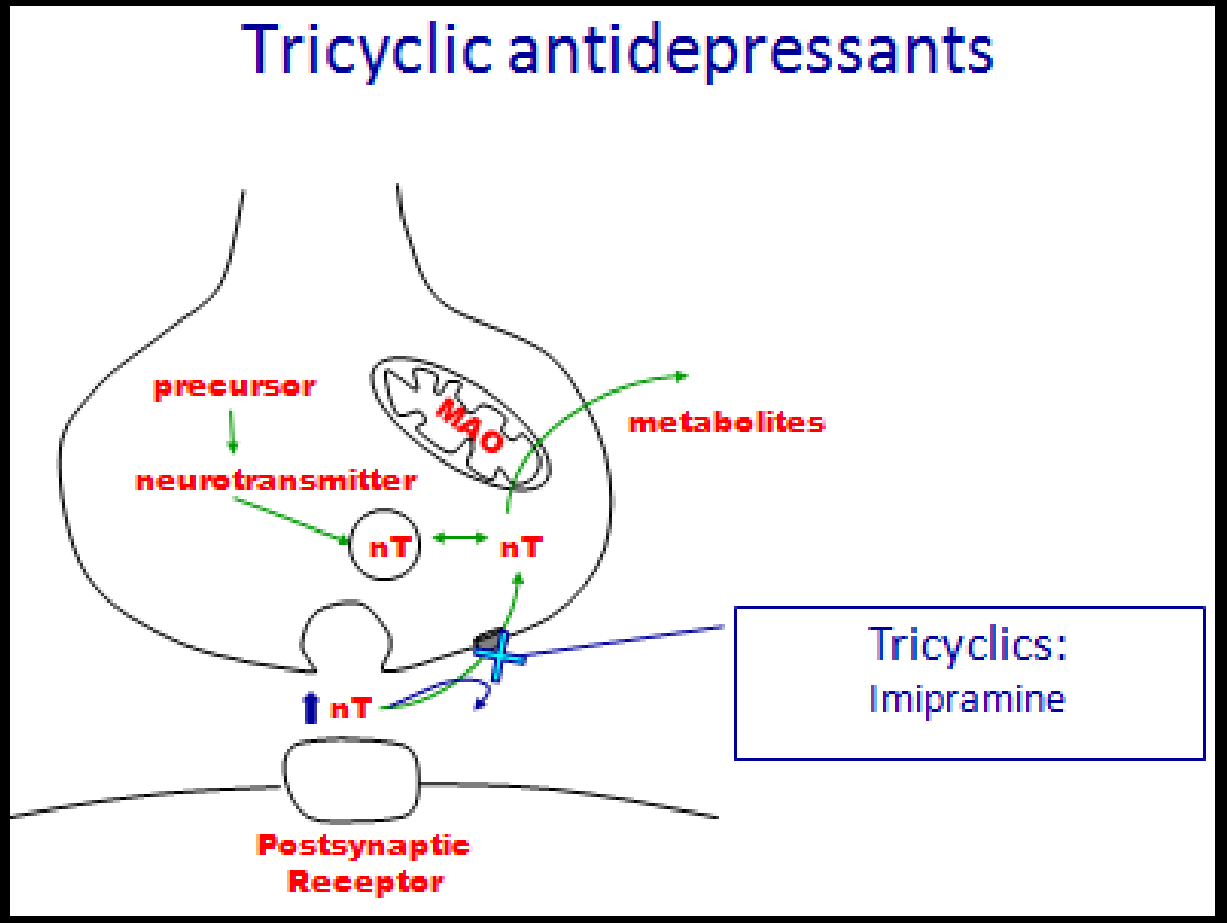
NSAID TOPICAL Agents (T-NSAIDs) in OA

- 2019 ACR guideline position on topical NSAIDs:
  - Strongly recommended for knee OA
  - Conditionally recommended for hand OA
- OARSI recommends:
  - Uncertain for multiple-joint OA and in patients with co-morbidities
  - Topical NSAIDs for knee-only OA
- A 2013 clinical practice guideline from the American Academy of Orthopaedic Surgeons (AAOS) recommends topical NSAIDs

Kolasinski SL et al. *Arthritis Rheumatol* 2020;Jan 6 McAlindon TE, et al. *Osteoarthritis Cartilage* 2014;22:363-88
Jevsevar DS, et al. *J Bone Joint Surg Am*. 2013;95:1885-6

Non-Opioid Pharmacologic Analgesic Therapy – Tricyclic Antidepressants (TCAs)

- Mechanism of action – inhibition of reuptake of both serotonin and norepinephrine



Non-Opioid Pharmacologic Analgesic Therapy – Tricyclic Antidepressants (TCAs)

- Indications and uses for pain management – even though well studied, none of the tricyclics carry an indication for pain management; first-line treatment for neuropathic pain
- Routes of administration and formulations – pills for oral administration
  - Nortriptyline is the only TCA available in a liquid form

Rosenquist EWK, et al. *UpToDate* 2019

Non-Opioid Pharmacologic Analgesic Therapy – Tricyclic Antidepressants (TCAs)

- Initial dosing, dose titration and tapering – doses used for pain are weeks (eg, 10 mg/d to a maximum dose of 100 mg/d)
- Contraindications – MAOIs, avoid in patients with severe cardiac disease (pretreatment ECG may be needed) and in patients with severe GI dysfunction; black box warning for suicidal thinking and behavior in children, adolescents, and young adults
- Drug interactions – P-gp, CYP3A4 and CYP2D6 substrate
  - Lowers seizure threshold
  - Serotonergic effect – strong
  - Hyponatremia

Rosenquist EWK, et al. *UpToDate* 2019

Tricyclic Antidepressants - Adverse Events

- Commonly reported AEs (generally anticholinergic):
  - Blurred vision
  - Cognitive changes
  - Constipation
  - Dry mouth
  - Orthostatic hypotension
  - Sedation
  - Sexual dysfunction
  - Tachycardia
  - Urinary retention

Desipramine

Nortriptyline

Imipramine

Doxepin

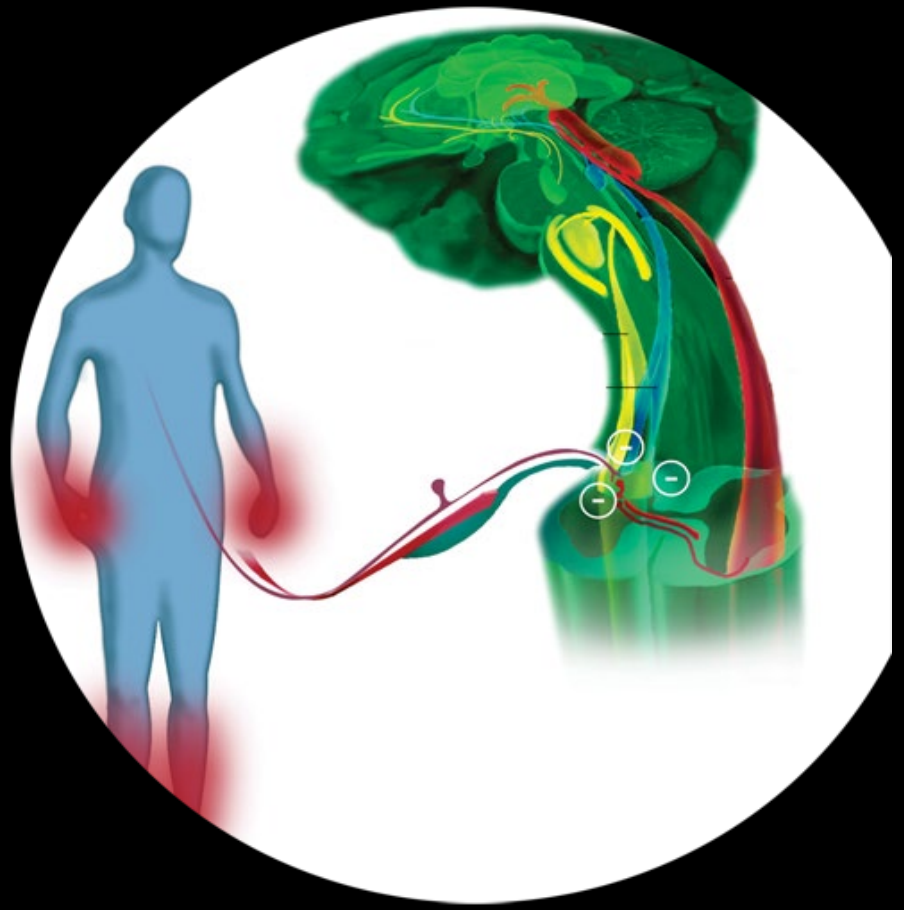
Amitriptyline

AEs = adverse events

Beers MH. *Arch Intern Med*. 1997;157:1531-1536; Mackin GA. *J Hand Ther*. 1997;10:96-109; McCue RE. *Clin Geriatr Med*. 1992;8:323-334.

Non-Opioid Pharmacologic Analgesic Therapy – Serotonin Norepinephrine Reuptake Inhibitors

- Mechanism of action – inhibition of reuptake of both serotonin and norepinephrine



Non-Opioid Pharmacologic Analgesic Therapy – Serotonin Norepinephrine Reuptake Inhibitors

- Indications and uses for pain management –
  - Venlafaxine – effective in acute and chronic neuropathic pain and in diabetic neuropathy (indicated for depression and anxiety)
  - Duloxetine
    - Indicated for chronic musculoskeletal pain: low back pain and OA
    - Fibromyalgia
    - Painful diabetic neuropathy
  - Milnacipran – indicated for fibromyalgia
- Routes of administration and formulations – pills, capsules

Rosenquist EWK, et al. *UpToDate* 2019

Non-Opioid Pharmacologic Analgesic Therapy – Serotonin Norepinephrine Reuptake Inhibitors

- Initial dosing, dose titration and tapering – eg,
  - Duloxetine starting doses: 30 mg/d for chronic musculoskeletal pain and fibromyalgia; 60 mg/d for DPNP; maximum dose 120 mg/d
- Contraindications – do not use with MAOIs
- Adverse events
  - Cardiac conduction abnormalities, nausea, dry mouth, insomnia, drowsiness, constipation, fatigue
  - Black box warning for suicidal thinking and behavior in children, adolescents, and young adults
  - Monitor liver function during its use
- Drug interactions – MAOIs; potent inhibitors of CYP1A2 and 2D6

Rosenquist EWK, et al. *UpToDate* 2019

Serotonin Norepinephrine Reuptake Inhibitors for LBP

- Moderate-quality evidence showed no difference in pain between tricyclic antidepressants (TCAs) or selective serotonin reuptake inhibitors (SSRIs) versus placebo
- Low-quality evidence showed no differences in function for antidepressants
- Moderate-quality evidence showed that duloxetine was associated with a small improvement in pain intensity and function compared with placebo
- Duloxetine was recommended as second-line therapy

Qaseem A, et al. *Ann Intern Med* 2017

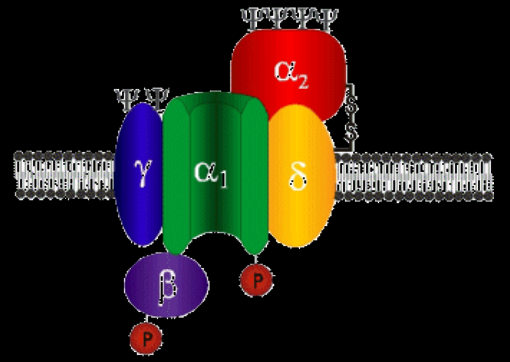
Serotonin Norepinephrine Reuptake Inhibitors for OA

- Duloxetine is recommended for most subphenotypes by OARSI
- However, associated adverse events and availability of more targeted therapies predicated uncertain appropriateness for individuals with knee-only OA and co-morbidities
- The ACR has no recommendations regarding the use of duloxetine

McAlindon TE, et al. *Osteoarthritis Cartilage* 2014;22:363-88
Hochberg MC, et al. *Arthritis Care Res* 2012;64:465-74

Non-Opioid Pharmacologic Analgesic Therapy – Anticonvulsants

- Mechanism of action – Drug dependent
  - Voltage gated calcium channel modulation: gabapentinoids, valproic acid
  - Voltage gated sodium channel modulation: carbamazepine, oxcarbazepine, lamotrigen, topiramate, valproic acid
  - Inhibition of glutamate release: Carbamazepine, oxcarbazepine, lamotrigen, gabapentin
  - Increase GABA inhibition: Valproic acid, topiramate



Non-Opioid Pharmacologic Analgesic Therapy – Anticonvulsants (cont'd)

- Indications and uses for pain management –
  - Gabapentin – indicated for postherpetic neuralgia; effective in painful diabetic neuropathy
  - Pregabalin – indicated for painful diabetic neuropathy, postherpetic neuralgia, fibromyalgia, neuropathic pain associated with spinal cord injury
  - Carbamazepine – indicated for trigeminal neuralgia
  - Other anticonvulsants have been studied in pain conditions; may be considered as second line if other options have failed
- Routes of administration and formulations – pills, capsules, solution, chewable tablets (carbamazepine)
- Drug interactions – co-administration of gabapentiods and opioids increase the risk of respiratory depression

Rosenquist EWK, et al. *UpToDate* 2019; Gomes T, et al. PLOS Medicine

Non-Opioid Pharmacologic Analgesic Therapy – Anticonvulsants in LBP

- Evidence was insufficient to determine the effect of anticonvulsant medications on acute or subacute low back pain
- Evidence was insufficient to determine the effect of anticonvulsant medications on chronic low back pain
- There was insufficient evidence to determine the effect of anticonvulsant medications on radicular low back pain

Qaseem A, et al. *Ann Intern Med* 2017

Non-Opioid Pharmacologic Analgesic Therapy – Anticonvulsants

- OA
  - No recommendations by ACR, AORSI, or AAOS

Hochberg MC, et al. *Arthritis Care Res* 2012;64:465-74
McAlindon TE, et al. *Osteoarthritis Cartilage* 2014;22:363-88
Jevsevar DS, et al. *J Bone Joint Surg Am*. 2013;95:1885-6

Examples of New/Novel Analgesic Targets

- Opioid: peripherally restricted kappa selective agents, mu-agonist/delta anatagonist, mu agonist/CB1 antagonist, Biased ligands
- Cannabinoid Receptors
- Angiotensis type-2 receptor
- Alpha 2 adrenergic receptors
- Chemokine receptors
- TRPV1 channels
- Sodium channels : Nav 1.7, 1.8, 1.9
- Calcium channels
- Potassium channels Kv7, K2P
- Type 3 Adenosine Receptor Modulators

Yekkirala AJ, etal. Breaker barriers to novel analgesic development. NAT REV DRUG DISCOV. 2019 AUGUST; 16(8):545-564

Conclusions

- The effective management of acute and chronic pain for all people remains elusive
- A multimodal approach to pain management is optimal
- Novel pharmacological targets are being investigated

Paul J. Christo, MD, MBA

Part 2: Adenosine and the CNS

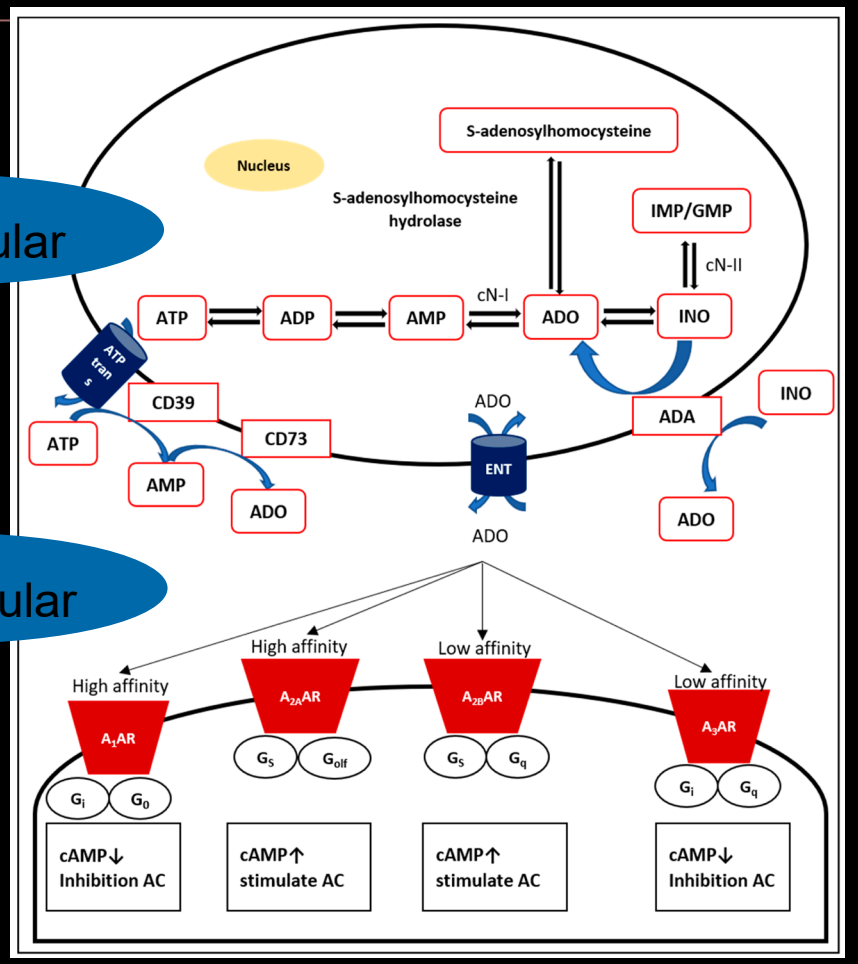
Adenosine and the CNS

- Acts as a neuromodulator for neurotransmitter systems involving glutamate, GABA, Ach, and dopamine
  - Limits neuroexcitability and regulates neuroplasticity
- Pharmacological blockade of adenosine kinase phosphorylation (inactivates adenosine) causes adenosine mediated inhibition of spinal nociceptive transmission
- Integral in CNS pain processing by regulating excitatory neurotransmission, neuronal signaling, and regulation of glial activation/proliferation
- Clinically, intrathecal adenosine produced relief of chronic neuropathic pain for hours or even months

Janes J, Symons-Liguori AM, Jacobson KA et al. Identification of A3 adenosine receptor agonists as novel non-narcotic analgesics. British Journal of Pharmacology (2016) 173 1253-1267

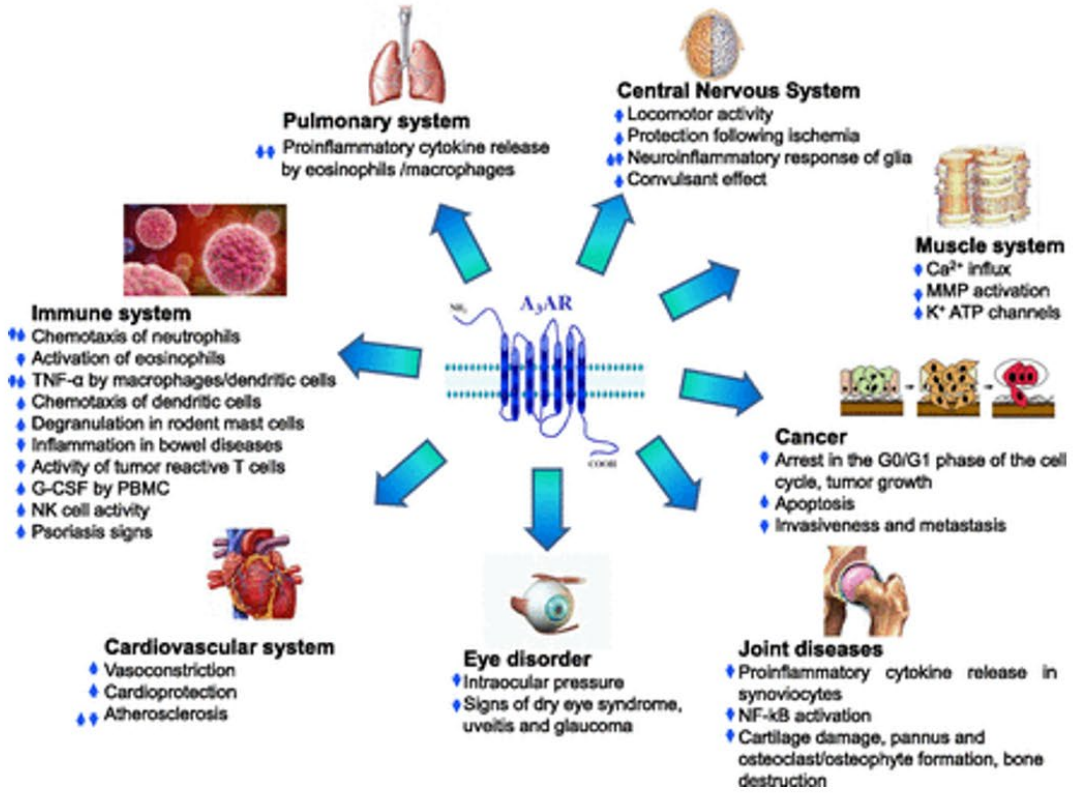
Adenosine Receptors

- Carry out different biological effects depending on location on specific cells
- Four receptor subtypes
  - A1 and A3 inhibit adenylate cyclase
  - A2A and A2B stimulate adenylate cyclase
- Part of G protein coupled family of receptors
- Distribution
  - A1: distributed in CNS, spinal cord, kidney, testis
  - A2A: distributed in liver, heart, lung, immune system
  - A2B: distributed in bowel, bladder, lung
  - A3: distributed in testis, CNS, lung, heart, kidney
- Immune Cells
  - Trigger proinflammatory and anti-inflammatory effects on mast cells, neutrophils, macrophages, lymphocytes, dendritic cells



Effendi WI, Nagano T, Kobayashi K, et al. Focusing on Adenosine Receptors as a Potential Targeted Therapy in Human Diseases. Cells 2020, 9, 785

Functions of the A3 Receptor



Borea Pa, Varani K, Vincenzi F, et al. The A3 Adenosine Receptor: History and Perspectives. Pharmacological Reviews (2015) 67:74-102

Adenosine Receptors and Pain

- A1 Receptor
  - Highly expressed on neurons in the CNS, glia in the brain, superficial laminae of spinal dorsal horn
  - Data suggest receptor is responsive to neuroinflammatory and nociceptive pathology
    - May involve differential expression of the receptor post injury (increased or decreased)
  - Beneficial outcomes with agonists in preclinical studies of acute/chronic pain, peri-operative pain, inflammatory pain, central pain after spinal cord injury, painful diabetic neuropathy
    - Several clinical trials of agonists occurred, but stopped due to limited efficacy
  - Presence in the AV node leads to high grade AV block by agonists
    - Hurdle to therapeutic use of the receptor
- A2A Receptor
  - Mostly in the striatium of the brain, and some areas of the hippocampus, cerebral cortex, glia
  - Preclinical studies produce mixed results
  - Clinical trial of an agonist for diabetic neuropathy was abandoned.

Janes J, Symons-Liguori AM, Jacobson KA et al. Identification of A3 adenosine receptor agonists as novel non-narcotic analgesics. British Journal of Pharmacology (2016) 173 1253-1267

Adenosine Receptors and Pain

- A3 Receptor
  - Found in high levels in glial cells, (immune cells), and in peripheral and central neurons in the brain and spinal cord
  - Functionally relevant in pain at the level of the peripheral afferent, spinal cord, and rostral ventromedial medulla (RVM)
    - Based on studies examining agonists introduced at each of these sites with subsequent reduction of neuropathic pain behaviors
      - The receptor is functionally expressed at multiple levels of pain processing

Little JW, Ford A, Symons-Liguori AM, et al. Endogenous adenosine A3 receptor activations selectively alleviates persistent pain states. Brain (2015) 138:28-35

Janes J, Symons-Liguori AM, Jacobson KA et al. Identification of A3 adenosine receptor agonists as novel non-narcotic analgesics. British Journal of Pharmacology (2016) 173 1253-1267

Adenosine Receptor Drugs

- Many clinical trials for cardiovascular diseases, metabolic diseases, and CNS diseases
- May be full agonists, antagonists, partial agonists
- All four receptors targeted by drugs in cardiovascular and metabolic conditions
- All but A2B targeted in CNS diseases
- Examples of diseases studied:
  - Heart failure, Arrythmia, Hypertension, Angina
  - Diabetes mellitus, Atherosclerosis, Glaucoma
  - Pain, Stroke, Sleep, Schizophrenia, Epilepsy, Drug Addiction

Effendi WI, Nagano T, Kobayashi K, et al. Focusing on Adenosine Receptors as a Potential Targeted Therapy in Human Diseases. Cells 2020, 9, 785

A3 Receptor Role in Pain States

- Nociceptive Effect
  - Produces a nociceptive and proinflammatory response leading to edema, and acts on sensory nerve terminals
    - Mediated by serotonin and histamine release (mast cells)
- Antinociceptive Effects
  - Mice studies show mediation of heat hyperalgesia and decreased sensitivity to certain stimuli
  - Rat studies show an intrathecal ligand reduced inflammatory component of the formalin test
  - Agonists blocked mechanically and chemotherapy-induced neuropathic pain in rats, and augmented analgesic effect of analgesics used in the study
  - Other animal models demonstrate that agonists inhibit oxaliplatin and paclitaxel induced peripheral neuropathic pain
  - Laboratory studies suggest no impact of A3 agents on normal protective nociception (pain), just pathological pain states
- Overall: A3 Receptor influences sensory nerve fibers, mediates hyperalgesia and inflammatory pain, and can suppress neuropathic pain (chemotherapy induced)

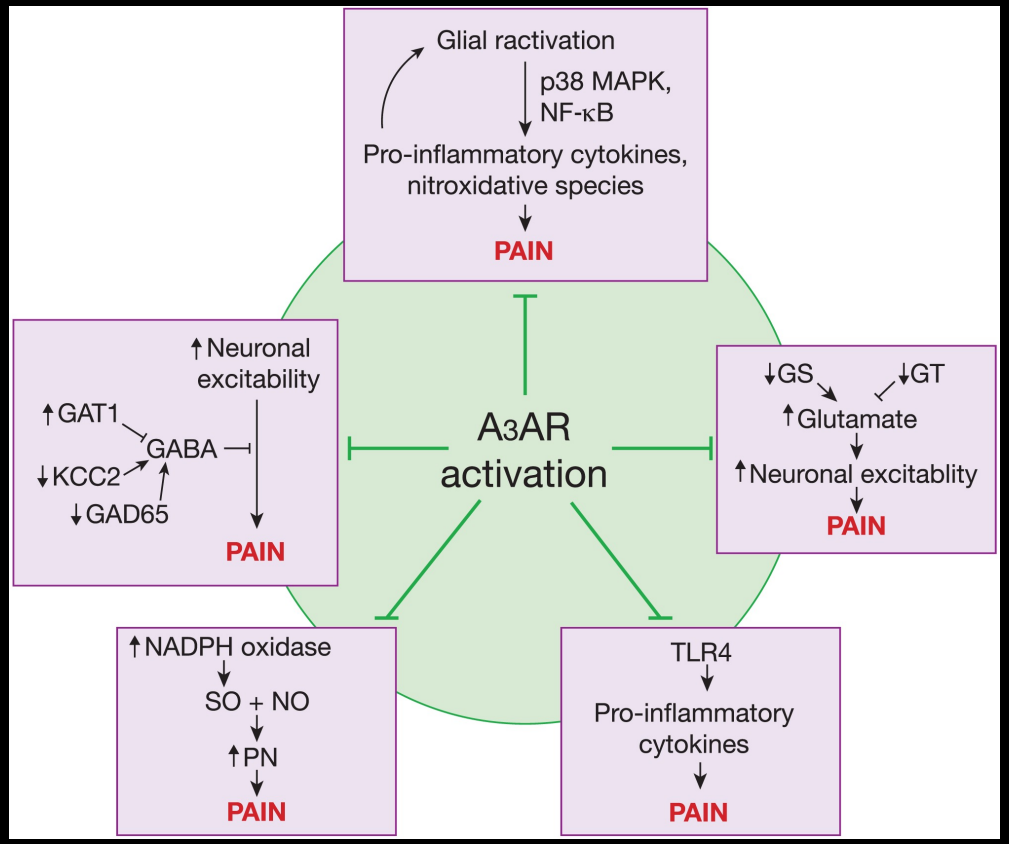
Borea Pa, Varani K, Vincenzi F, et al. The A3 Adenosine Receptor: History and Perspectives. Pharmacological Reviews (2015) 67:74-102

A3 Receptor and Abuse Potential

- Antinociceptive effects of agonists not incorporate endogenous opioid or endocannabinoid pathways
- Preclinical studies show that A3 agonists don't produce tolerance
- Preclinical studies suggest A3 receptor activation reduces pain behavior without producing a reward
- Hypothesized that A3 receptor agonists circumvent tolerance and abuse potential seen in opioid therapy

A3 Receptor Proposed Mechanism of Action

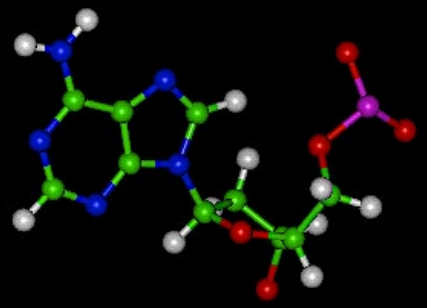
- Modulates pathways involved in producing pain states
  - Impaired GABAergic neurotransmission
  - Increased glial hyperactivation and toll like receptor (TLR4) signaling (enhancing neuroinflammation)
  - Increased glutamatergic signaling
  - Heightened production of nitroxidative species
- Mechanism of Antinociception
  - GABA – restores this inhibitory system and
  - reverses neuropathic pain
  - Glia – inhibits the p38 MAPK, NF pathway
  - Nitrooxidative products – inhibits NADPH oxidase
  - Glutamate – inactivates glutamate transporter and glutamate synthase
  - TLR4 (immune receptor on glial cells)-decreases release of pro-inflammatory mediators like TNF and increases levels of IL-10 (anti-inflammatory)



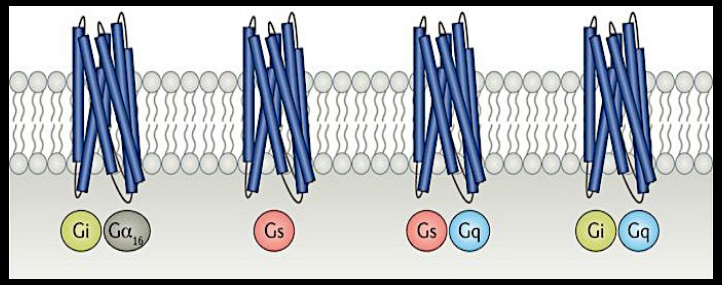
Proposed chronic pain pathways

Janes J, Symons-Liguorm AM, Jacobson KA et al. Identification of A3 adenosine receptor agonists as novel non-narcotic analgesics. British Journal of Pharmacology (2016) 173 1253-1267

Adenosine A_3 Receptors are Associated with Analgesia Without Cardiovascular AEs



A_1 A_{2a} A_{2b} A_3



Cronstein & Sitkovsky (2017) Nature Revs Rheumatol **13:**41–51.
Borea *et al.* (2018

Subtype	Analgesia	CV AEs
A_1	Yes	Yes
A_{2a}	Yes	Yes
A_{2b}	No	–
A_3	Yes	No

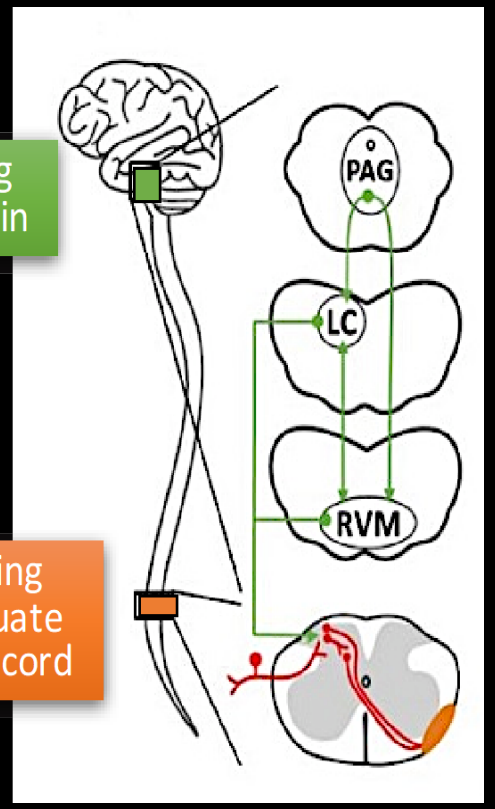
- Robust anti-nociception
- Variety of pain types
- Does not alter protective nociception
- Does not produce inherent reward

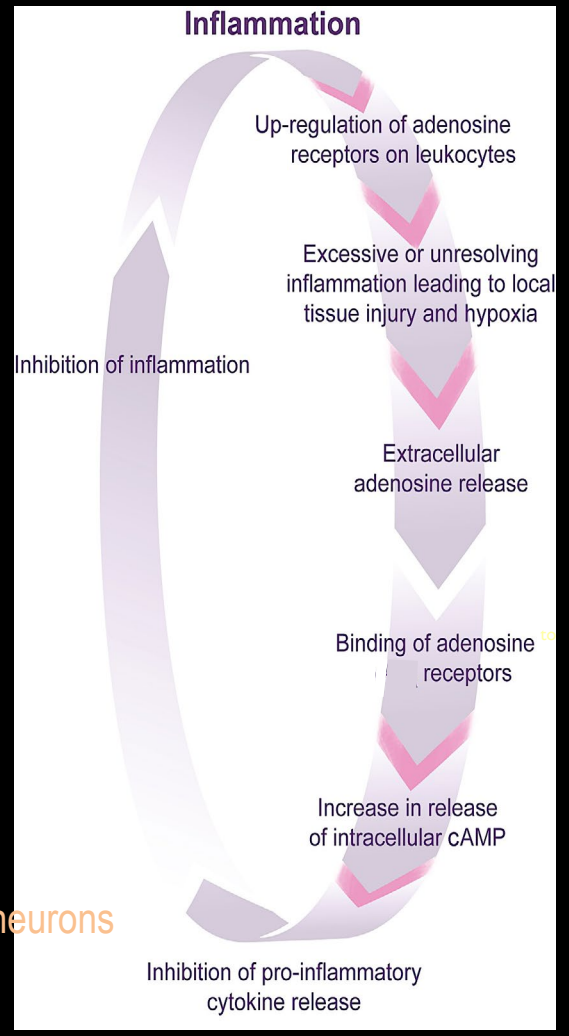
A_3R is an Established Analgesic and Anti-Inflammatory Mechanism – Potential for Unique Analgesic Activity

- Combined activity at brain, spinal cord, and periphery likely adds to therapeutic effects
- But likely results in less AEs at any one site

Potential Clinical Relevance:

- Multimodal (multisite) mechanism
- Suggestive of efficacy in chronic Inflammatory, Nociplastic and Neuropathic pain





Brain: inhibits N-type Ca^{2+} channels and excess cell excitation
Spinal cord: inhibits excess microglia and anomalous convergence of neurons

Function and Therapeutic Applications of the A3 Receptor: Chronic Pain

- Rheumatoid Arthritis
  - A3 receptor upregulated in RA, and overexpressed in untreated RA
    - Found that A3 can regulate inflammation due to an increase in receptor density/functionality
  - A3 density inversely correlates with Disease Activity Score in the joints
    - Endogenous activation of these receptors may have direct role in the control of RA inflammation
- Studies
  - A3 agonists prevent cartilage damage, osteoclast/osteophyte formation, bone destruction in rat models
  - CF101 clinical trial showed improvement in signs and symptoms, and proved safe and well tolerated

Borea Pa, Varani K, Vincenzi F, et al. The A3 Adenosine Receptor: History and Perspectives. Pharmacological Reviews (2015) 67:74-102

Function and Therapeutic Applications of the A3 Receptor in Chronic Pain

- Osteoarthritis
  - P38 MAPKs second messenger and signaling pathway stimulated by A3 activation, and leads to proinflammatory cytokine release in OA
- Studies
  - CF101 causes inflammatory cell apoptosis, and protects cartilage in a rat model
  - CF101 found to be safe and effective in a clinical study in patients with knee OA
    - May be suitable for OA treatment

Borea Pa, Varani K, Vincenzi F, et al. The A3 Adenosine Receptor: History and Perspectives. Pharmacological Reviews (2015) 67:74-102

NTM-006 A3 Receptor Modulator Drug

- New Chemical Entity: oral, non-opioid, non-NSAID
- Novel Mechanism of Action – Putative Adenosine A3 Modulator
- Clinical trial in development
-
- Planning to target Chronic Inflammatory and Neuropathic Pain

NTM-006: Extensive Preclinical Research

Initial Target Product Profile

Acute-pain
→ OTC market
Non-opioid, non-NSAID
Vs. Acetaminophen

- **Focus was Acute Pain Followed by OTC replacement for Tylenol**
- Extensive Pre-clinical Data – 10 in-vivo studies
- Positive Phase 2a Dental Pain study showed unique efficacy on single dose
- Binding assays showed high selectivity for Adenosine A3 receptors

New Target Profile

Identified as an A_3RM (MoA)
→ chronic / inflammatory pain

Path for Chronic Nociplastic Pain

- Pharmacological profile differs greatly from APAP
- Presumptive MoA = Adenosine A_3 Receptor Modulator
- Animal model supports Chronic-pain effect
- MOA & Anti-inflammatory effects of product point to chronic pain with neuropathic and/or inflammatory component
- Potential once/day dose

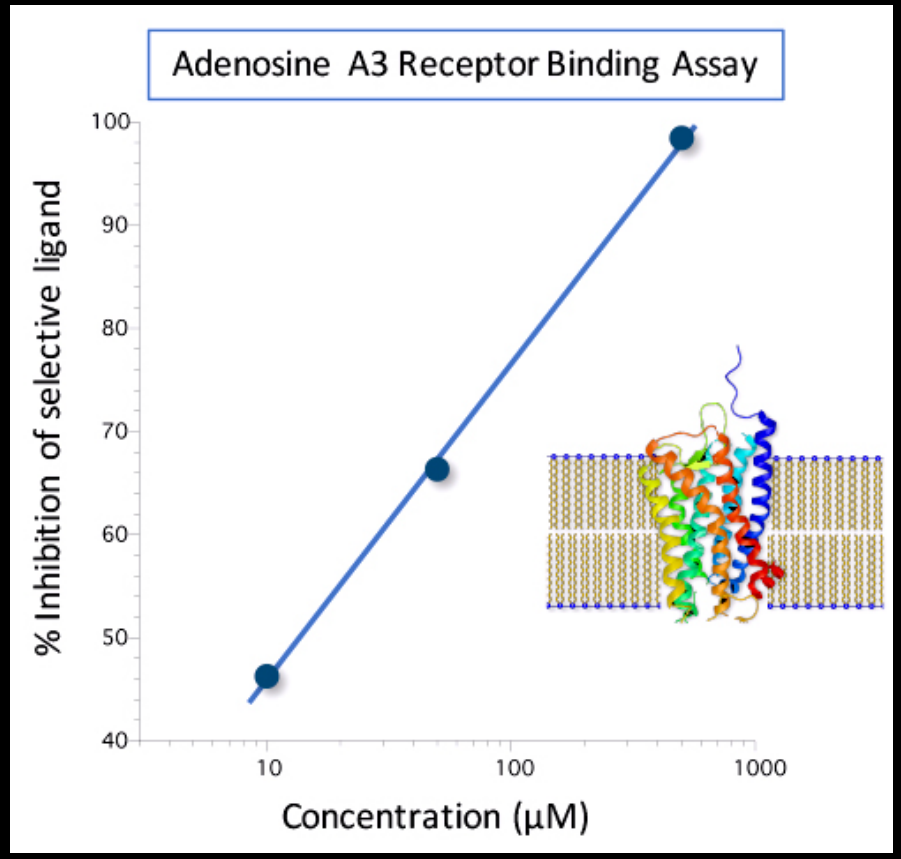
A_3Receptor Modulator is the Presumed Mechanism of Action for NTM-006

NTM-006 Demonstrated No Other Activity Except in A_3 Receptor Binding Assays

The effect is concentration-dependent

It occurs at concentrations present in brain at doses that produce pain relief in animal models

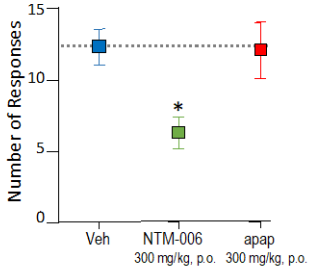
- Non-opioid (MOR, DOR or KOR)
- Non-NSAID
- Inactive at other receptors
- Inactive at uptake transporters
- Inactive at ion channels
- Orally active
- Phase-2 metabolism
- No ECG effects



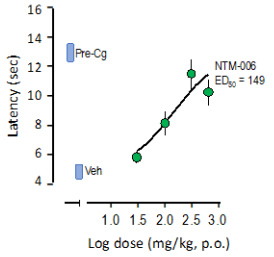
NTM-006 is Active in Multiple Animal Models of Pain and Fever

- Abdominal-irritant (nociceptive)
- Carrageenan (hyperalgesia)
- Complete Freunds Adjuvant (antihyperalgesic)
- Long time-course
- Yeast-induced pyresis (antipyretic)

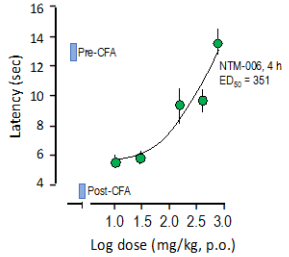
RESULTS



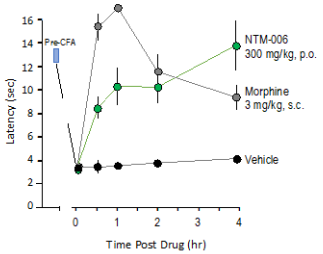
Antinociceptive effect in phenylquinone-induced abdominal irritant model. * P < 0.05. Mice



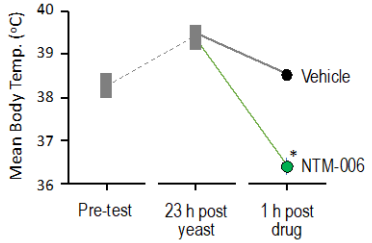
Antihyperalgesic effect in carrageenan(Cg)-induced thermal hyperalgesia. Rats.



Antihyperalgesic effect (4 hr) in model of thermal hyperalgesia induced by i.pl. injection of complete Freund's adjuvant (CFA). Rats.



Time-course of antihyperalgesic effect in model of complete Freund's adjuvant-induced thermal hyperalgesia. Rats.

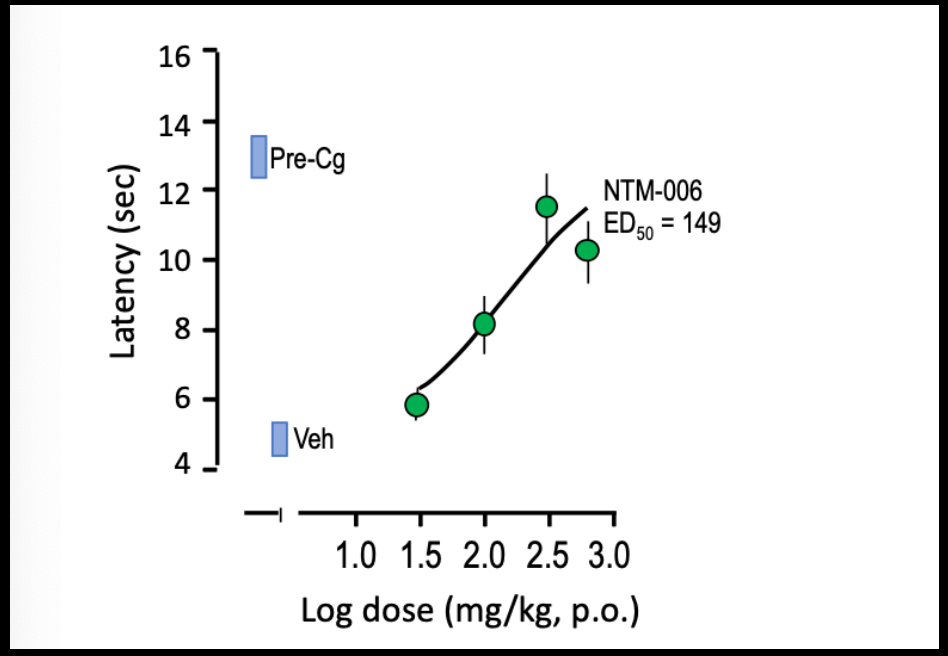


Antipyretic evaluation at 600 mg/kg, p.o. in a yeast-induced pyresis model. Rats.

Several Animal Models Support an Effect of NTM-006 in Chronic Pain with Inflammatory Component (1 of 3)

Chronic inflammatory arthritis model in the rat[1,2]

- NTM-006 produced an orally-active dose-related antinociceptive (analgesic) effect in the rat model of **carrageenan-induced thermal hyperalgesia**
- The Carrageenan Test is a well-known model used to predict potential efficacy for OA pain

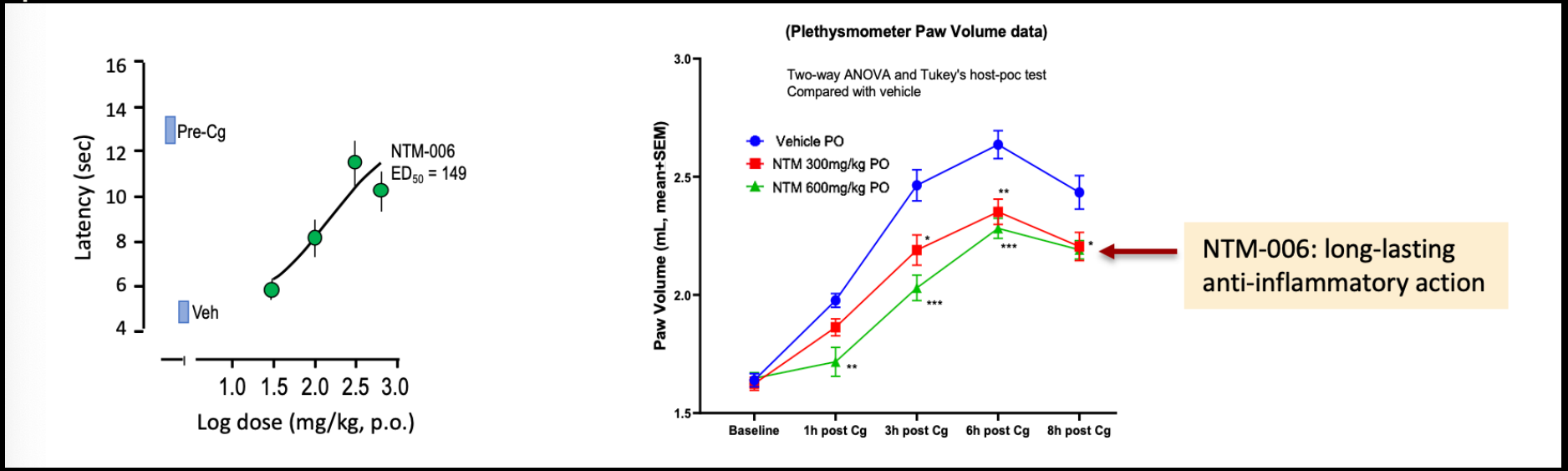


[1] Fehrenbacher et al. (2012) Models of inflammation: Carrageenan- or complete Freund's adjuvant (CFA)–Induced edema and hypersensitivity in the rat. Curr Protoc Pharmacol Chapter 5:Unit5.4

Several Animal Models Support an Effect of NTM-006 in Chronic Pain with Inflammatory Component (2 of 3)

Chronic inflammatory arthritis model in the rat[1,2]

- NTM-006 produced **orally-active**, **dose-related** antinociceptive (analgesic) effect *and* **orally-active**, **dose-related**, and **long-duration** inhibition of paw-volume (anti-inflammatory) effect
- The Carrageenan Test is a well-known model used to predict potential efficacy for OA pain

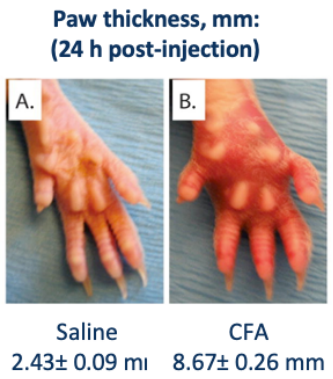


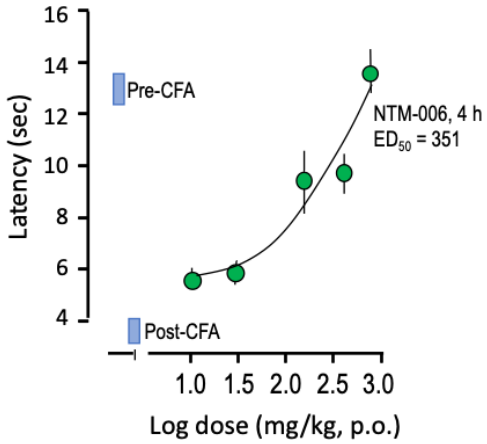
[1] Fehrenbacher et al. (2012) Models of inflammation: Carrageenan- or complete Freund's adjuvant (CFA)–Induced edema and hypersensitivity in the rat. Curr Protoc Pharmacol Chapter 5:Unit5.4

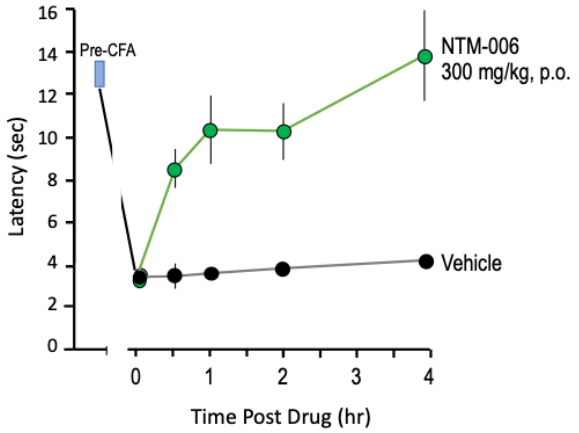
Several Animal Models Support an Effect of NTM-006 in Chronic Pain with Inflammatory Component (3 of 3)

Chronic inflammatory arthritis and chronic-pain model in the rat[1,2]

- NTM-006 produced an orally-active positive dose-related antinociceptive (analgesic) effect in the rat CFA (Complete Freund's Adjuvant) test
- The Carrageenan Test is a well-known model used to predict potential efficacy for OA pain





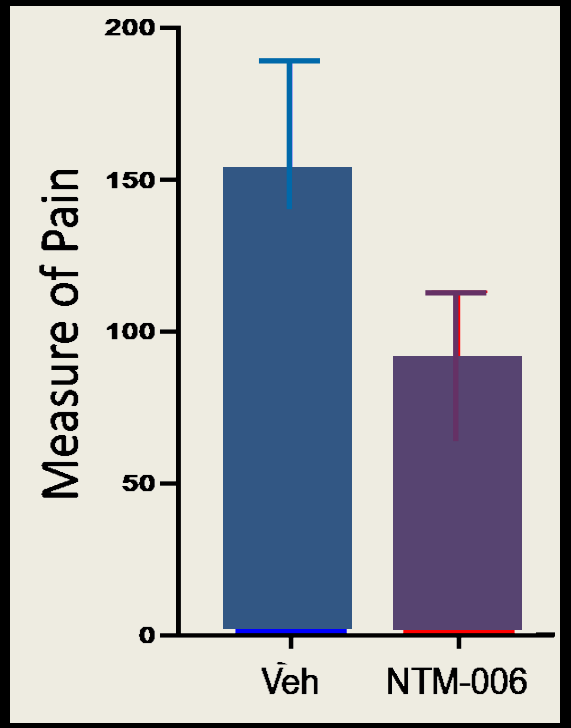


[1] Snekhalatha et al. (2013) Evaluation of complete Freund's adjuvant-induced arthritis in a Wistar rat model. Comparison of thermography and histopathology. Z Rheumatol 72:375-82

Animal Model Supports Potential Application for NTM-006 in Chronic Pain

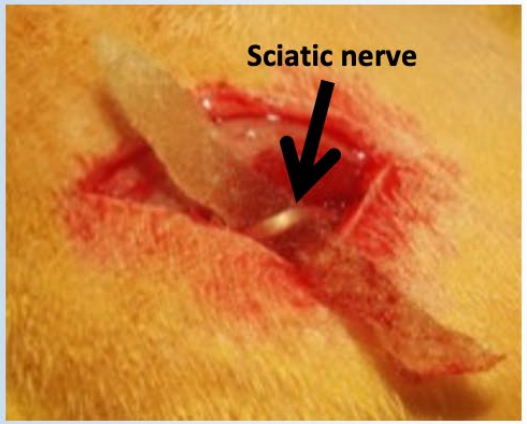
Formalin model of chronic pain in the rat[1,2]

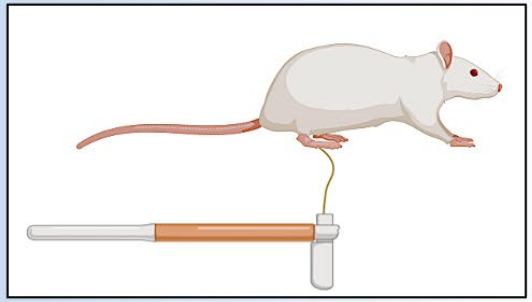
- NTM-006 reduced pain response in rat model of formalin-induced moderate continuous pain
- Consistent with efficacy against conditions with chronic-inflammation component
- Consistent with A3R-related mechanism of action
- The Formalin Test is a well-known predictive model for chronic pain [1,2]

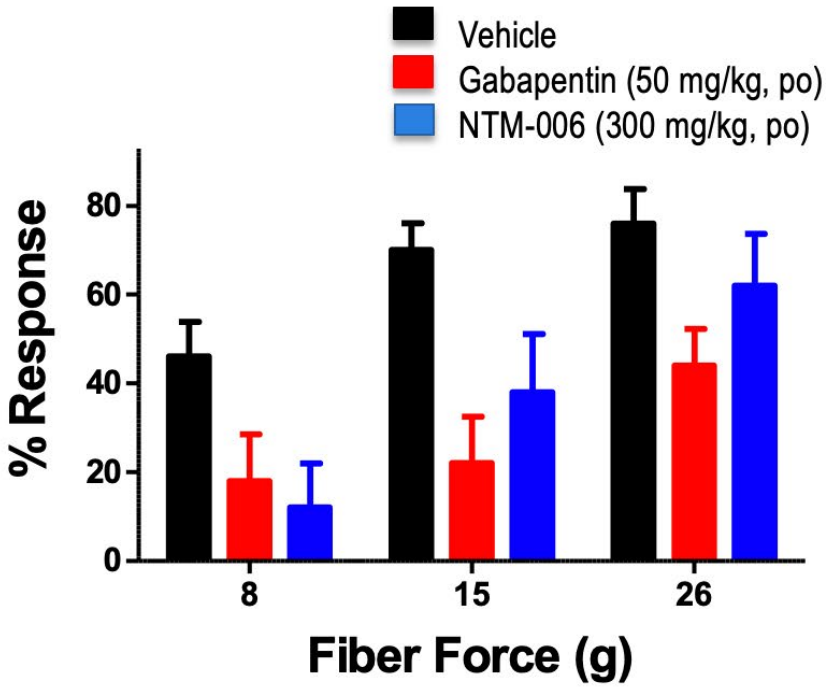


Active in Animal Model of Neuropathic Pain

- Carrageenan-induced Neuritis (rat)
- Model of tactile allodynia

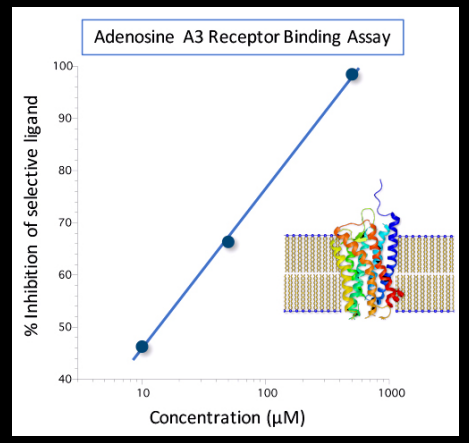


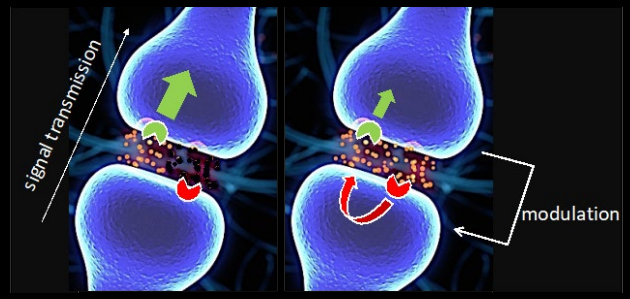


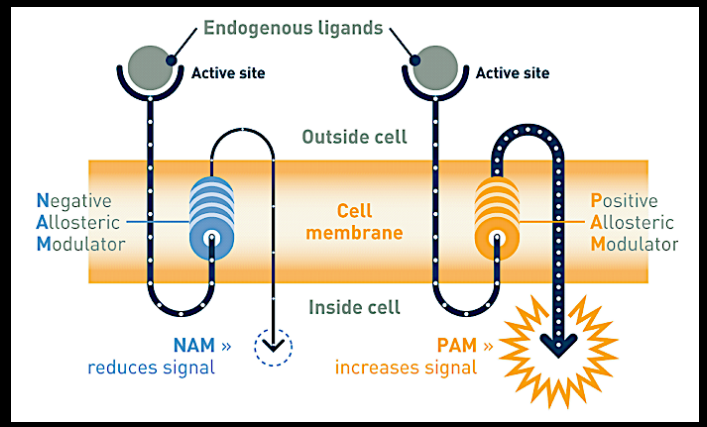


Summary of Preclinical Evidence for NTM-006

- NTM-006 inhibits selective radioligand at adenosine A_3 receptors
- Has no activity at other receptors, enzymes, ion channels, transporter
- Has no apparent direct agonist or antagonist action
- Thus, action appears to be indirect – by modulation
- *Should adjust adenosine back to homeostatic levels*
- *Should be site- and activity-related*
- Full efficacy and breadth of clinical applications still to be explored







Development of NTM-006 Through Ph2a

Pre-clinical Findings

- Non-opioid (MOR, DOR or KOR)
- Non-NSAID
- Inactive at other receptors
- Inactive at ion channels
- Inactive at uptake transporter
- Presumptive MoA = A_3RM
- Orally active
- Phase-2 metabolism
- No ECG effects
- Good toxicology profile

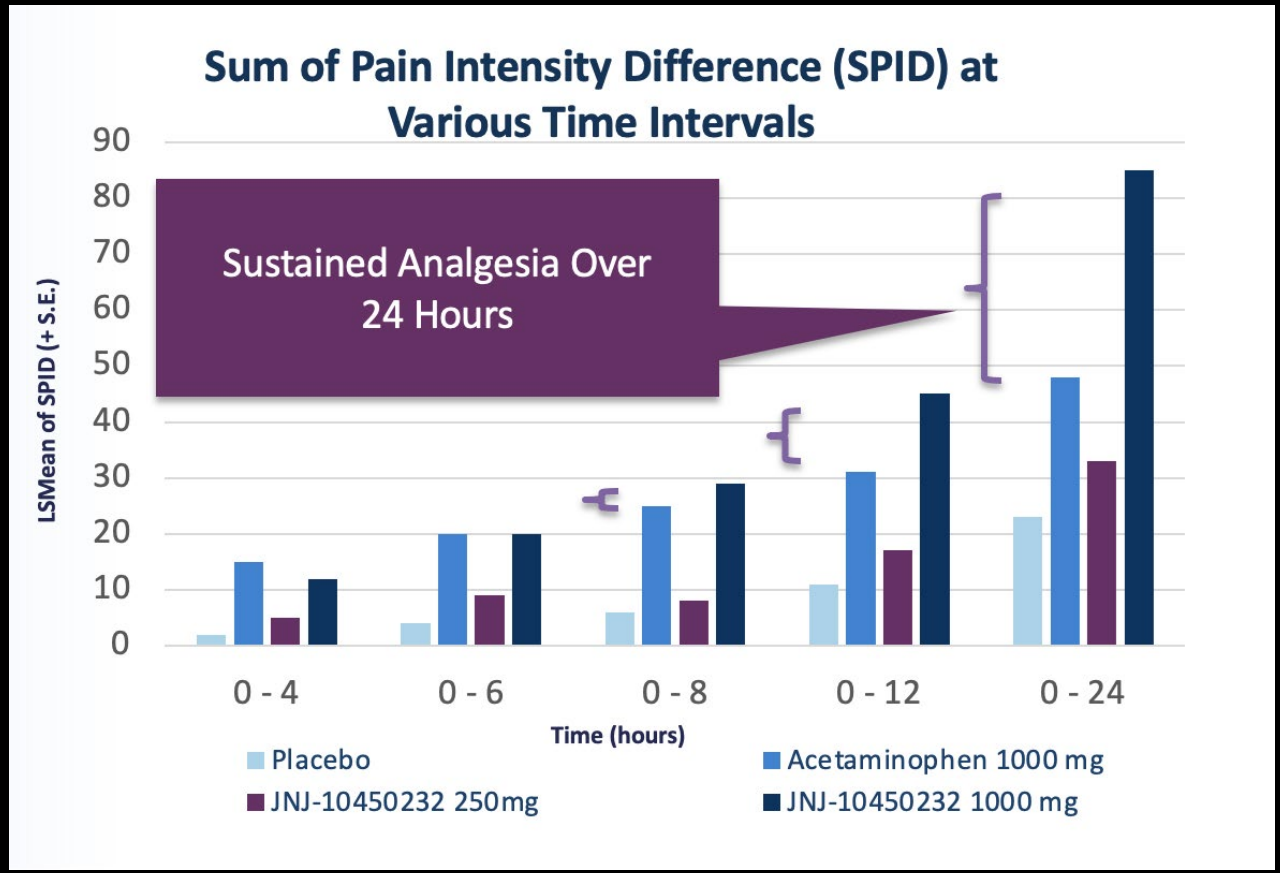
Clinical Findings

• Phase 1/FIH Single- and Multiple-Dose Study to Investigate Safety, Tolerability and Pharmacokinetics	Safe use up to 6000 mg SD/5000 mg MD
• An Open-Label, Multiple-Dose, Safety, and Pharmacokinetics Study	No significant AEs except a mild self-resolving skin rash
• Phase-2A Single-Dose Efficacy Study in Dental Pain Model	Efficacy of 1000 mg SD in moderate to severe pain for 24 hours

Ph2a Proof of Concept Study in Humans Showed Meaningful Analgesia with NTM-006 for Moderate to Severe Pain

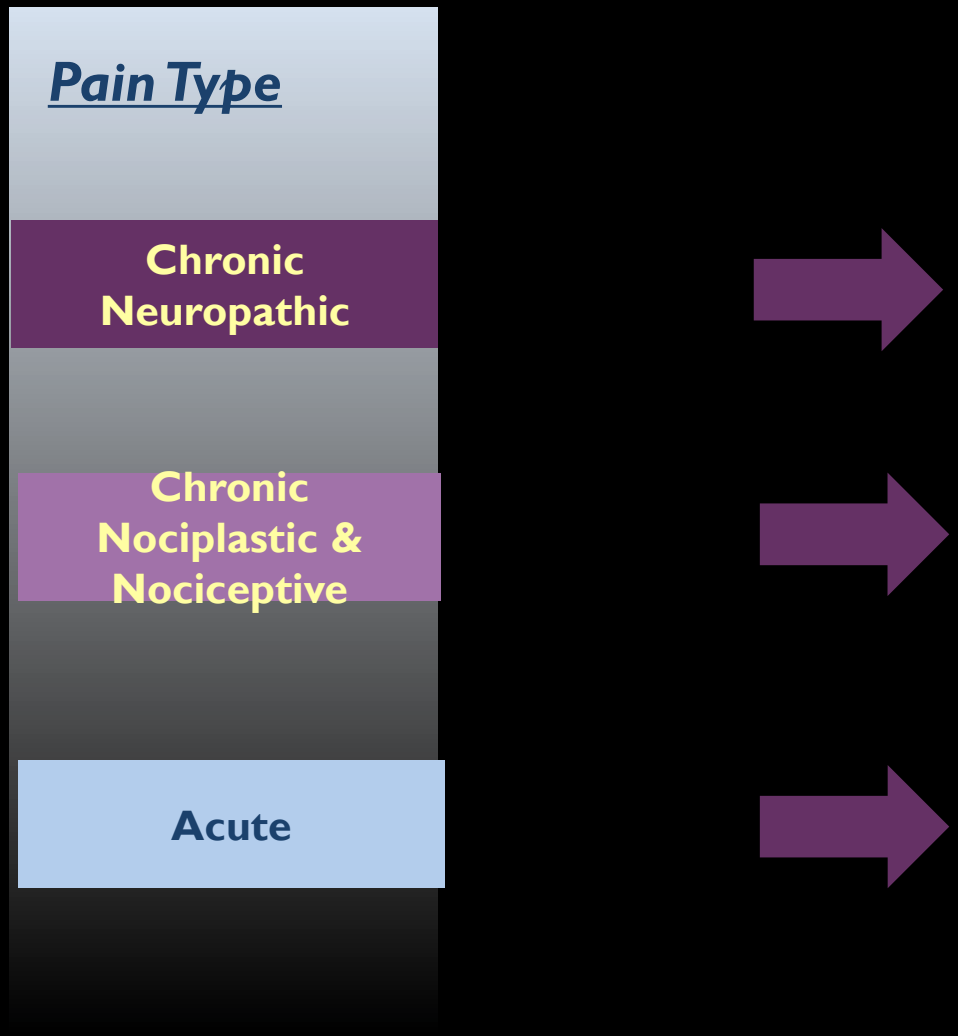
- Single-dose, randomized, double-blind, placebo- and active-controlled study – 269 patients
- Moderate to severe pain, following 3rd molar extraction
- Objectives - to evaluate overall efficacy (and safety /PK) vs placebo.
  - APAP included to validate model

Development program will include a variety of chronic pain models and formulation work to fully explore the product's full analgesic potential



Source: Data on file, Clinical Study Report for phase IIa dental pain study number CO-131230135611-CTPA

NTM-006 Has Potential in a Variety of Chronic/Inflammatory Pain Indications



Pain Indications

Neuropathic

- Peripheral Neuropathic Pain
- DPN/Sciatica/ small-fiber neuropathy

Nociceptive/Nociplastic

- Musculoskeletal
- Osteoarthritis
- Low back pain
- Fibromyalgia

- Postoperative/rehab
- Musculoskeletal

Conclusions

- Pharmacological studies related to AR ligands as new drugs have been widely developed
- The A3 receptor offers robust antinociceptive properties via agonists in a variety of pathological pain states
- Emerging evidence that targeting the endogenous A3 receptor pathway leads to effective pain relief without altering normal protective nociception, producing reward effects, or inducing cardiovascular adverse effects
- A novel A3 modulator may be effective in inflammatory, neuropathic, or chronic pain conditions