Transient Receptor Potential Vanilloid 1 (TRPV1): a “Nobel” Future for Osteoarthritis Knee Pain

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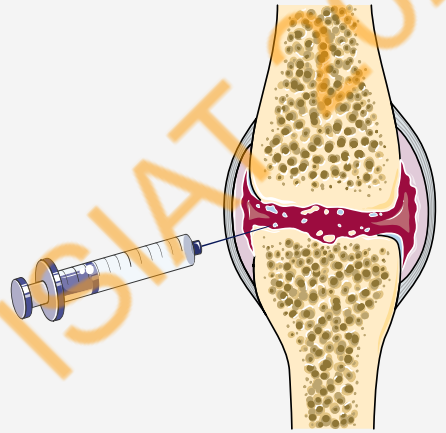
The Future is Intra-Articular
All New Osteoarthritis Drugs will be Intra-Articular

- TissueGene-C (Cell and Gene therapy using a combination of allogeneic chondrocytes and GP-293 cells) – Kolon TissueGene
- Fibroblast growth factor 18 (FGF-18) - Sprifermin – Merck KGaA
- Lorecivivint (SM04690) Samumed / BioSplice
- Zilretta (synthetic corticosteroid triamcinolone acetonide) - Flexion Therapeutics / Pacira Biosciences
- UBX0101 (anti-senolytic drug) – Unity Biotechnology
- Emerging intra-articular toxins

The Majority of Future OA Treatments will be Intra-Articular

- However, we need better biomarkers to assess the efficacy of these treatments
- Intra-articular treatment is ideally suited to intra-articular phenotypes of OA
- We must exploit opportunities to develop novel products for intra-articular injection
- “The earliest application is always the best” Alberto Migliore
- Synovial biopsies and biomarkers can be very useful for guiding rheumatology practice, monitoring disease progression and response to therapy
- Synovial fluid and biopsies may be needed for biomarker studies and future drug development
- However, we need to develop methods to non-invasively assess synovial inflammation



Osteoarthritic Joint

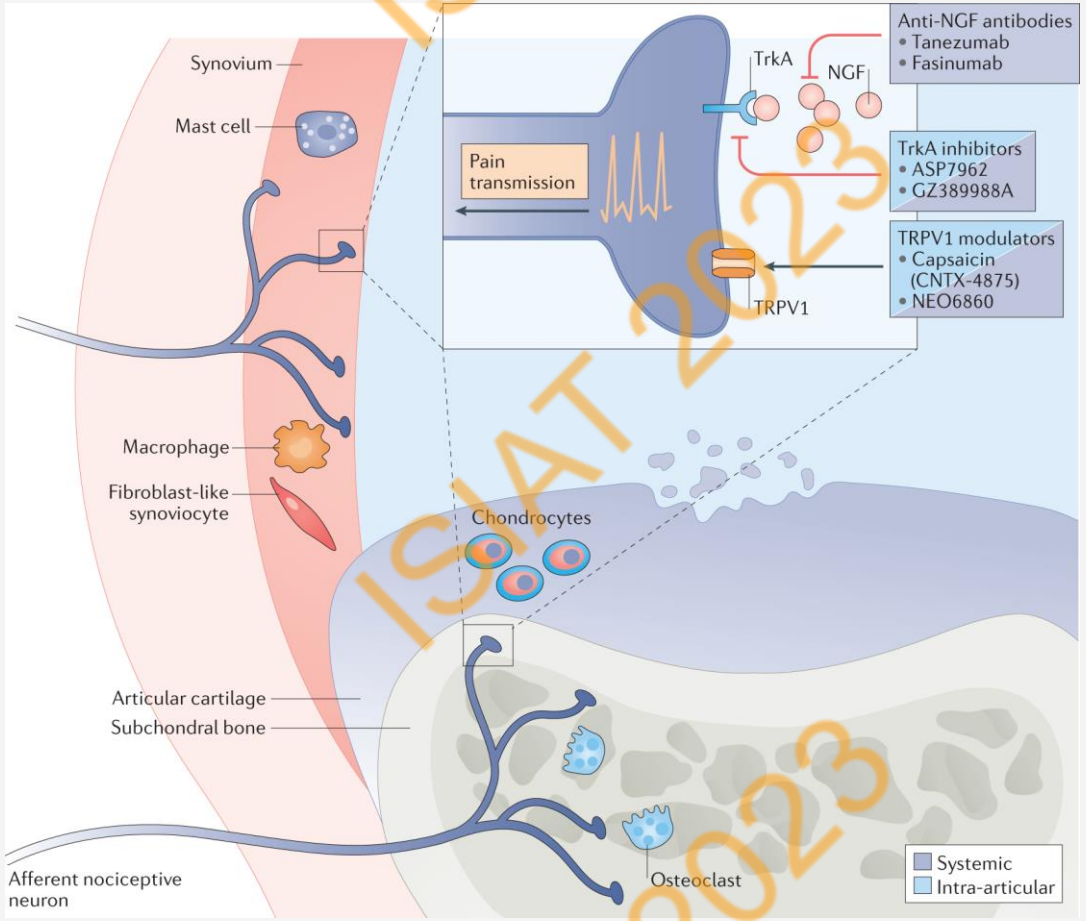
Multiple OA Phenotypes and Intra-Articular Injections

The role of metabolism in the pathogenesis of osteoarthritis

Ali Mobasheri[1,2], Margaret P. Rayman[3], Oreste Gualillo[4], Jérémie Sellam[5,6], Peter van der Kraan[7] and Ursula Fearon[8]

Slide courtesy of Professor Alberto Migliore Rome, Italy

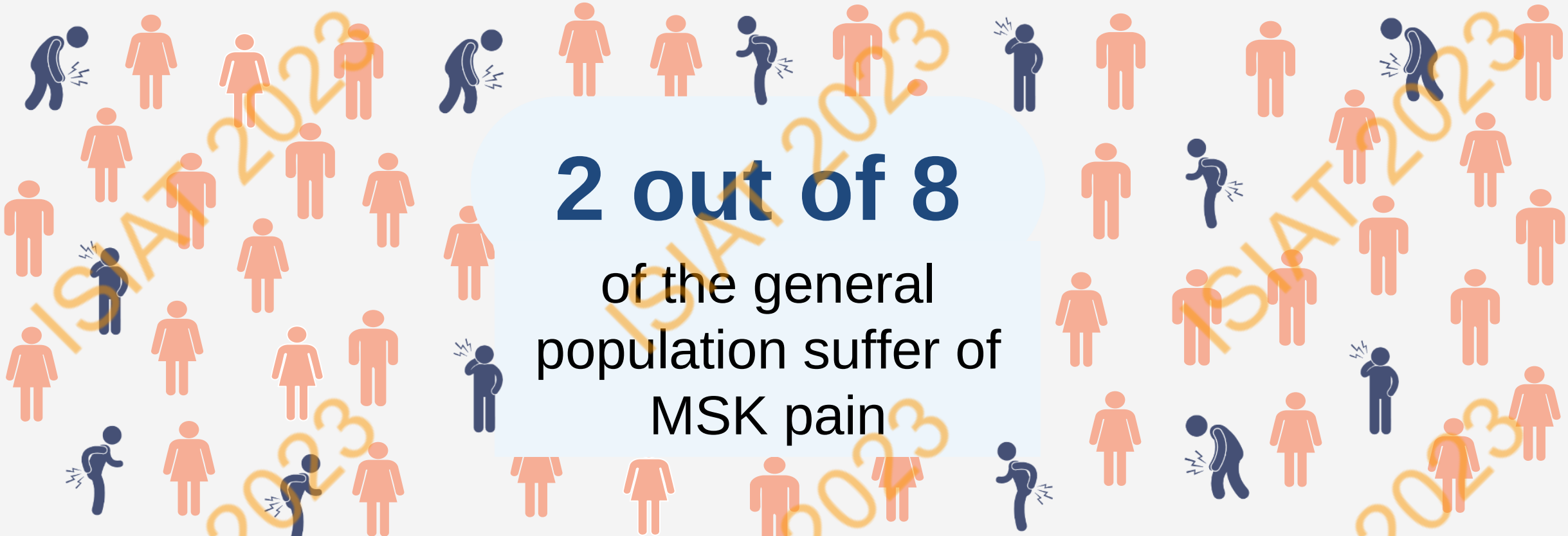
Drugs Targeting Pain in Osteoarthritis – Anti-NGF Antibodies



Latourte, A., Kloppenburg, M. & Richette, P. Emerging pharmaceutical therapies for osteoarthritis. *Nat Rev Rheumatol* **16,** 673–688 (2020). https://doi.org/10.1038/s41584-020-00518-6

The Prevalence of Musculoskeletal Conditions

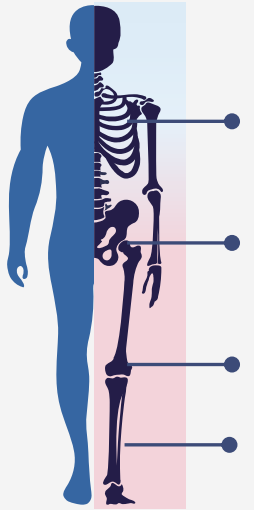
- Approximately 1.71 billion people have MSK conditions worldwide.



Musculoskeletal Pain

- IASP (2020) defines pain as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage.[1]
- Musculoskeletal (MSK) pain can affect **bones, joints, muscles, ligaments, or tendons**. MSK includes joint pain, a discomfort that arises from any joint (articular pain).[2]
- It is most often the consequence of cumulative trauma injury (jerking movements, falls, sprains), repetitive strain, or overuse.
  - Although, pain can also develop as a consequence of neuropathy, tendinitis, tendinosis (chronic scarring), myalgia, and even stress fractures.[2]
- The pain can be **acute or chronic**, diffuse or focal (even multifocal), in musculoskeletal or associated neural tissues.[2]

Symptoms



- Local or widespread pain
- Fatigue
- Burning sensation in the muscles
- Stiffness or aching

Symptoms progressively increase with greater tissue injury and inflammation in affected sites[1]

1. Srinivasa NR, et al. 2020 (IASP 2020). 2. IASP, 2017.

Pathophysiology of Musculoskeletal Pain

- Although the pathophysiology of MSK pain is not completely clear, the following mechanisms have been implicated.

1. Inflammation

- ↑ Pro-inflammatory cytokines and inflammatory mediators

2. Tissue degradation

- ↑ Collagen breakdown products
- ↑ Strain

3. Fibrosis

- ↑ Enzymes degrading extracellular matrix (metalloproteinases, aggrecanases)
- ↓Tissue load tolerance

4. Neurosensory disturbances

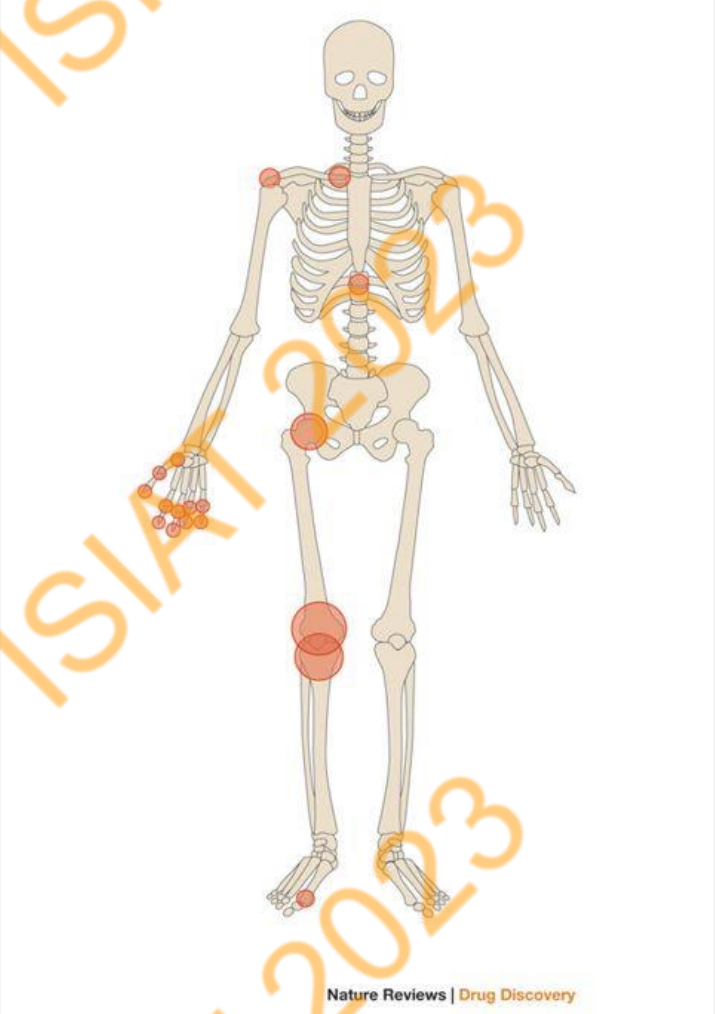
- ↑ Levels of neurotransmitters
- ↑ Peripheral nociceptor sensitisation or central amplification of pain

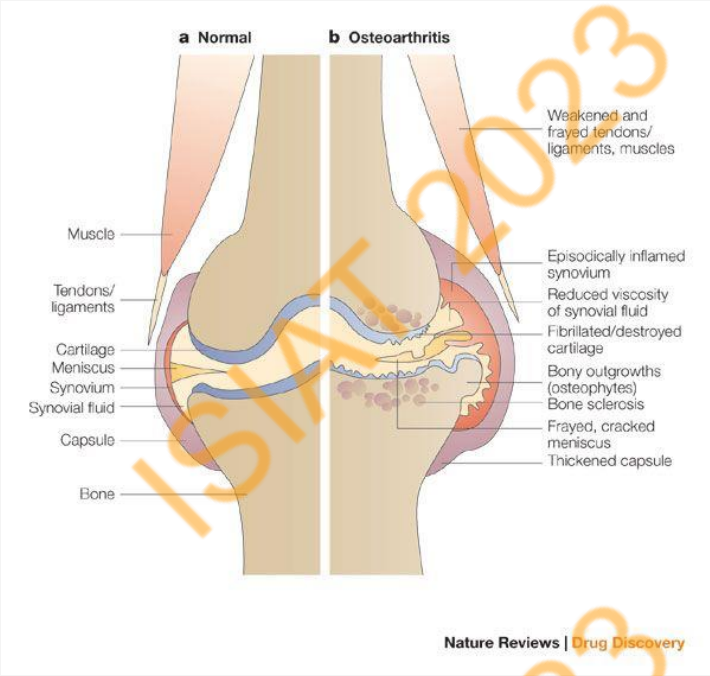
Osteoarthritis

The most common form of arthritis affecting millions of people worldwide

Increasing global burden

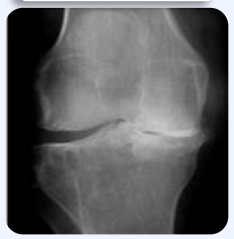
Lack of effective disease modifying drugs and safe pain treatments represent a significant unmet medical need





Current Osteoarthritis Treatment

Kellgren & Lawrence Grade[1]

Grade 1	Grade 2	Grade 3	Grade 4
			
Initial joint space narrowing	Increased joint space narrowing	Progressive joint space narrowing	Bone-on-Bone

Patient Ratio

X %	Y %	Z %

Duration

X years	Y years	Z years

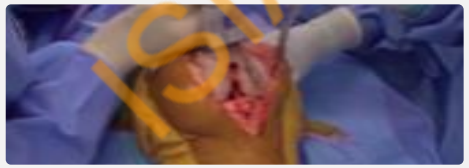
Current Treatment Paradigm


Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, hyaluronic acid (HA), COX2 inhibitors, SYSADOAS

- Temporary pain relief (need for frequent repeat administrations)
- Inability to treat the underlying cause (Degeneration continues)
- Side-effects (gastrointestinal, cardiovascular, renal)

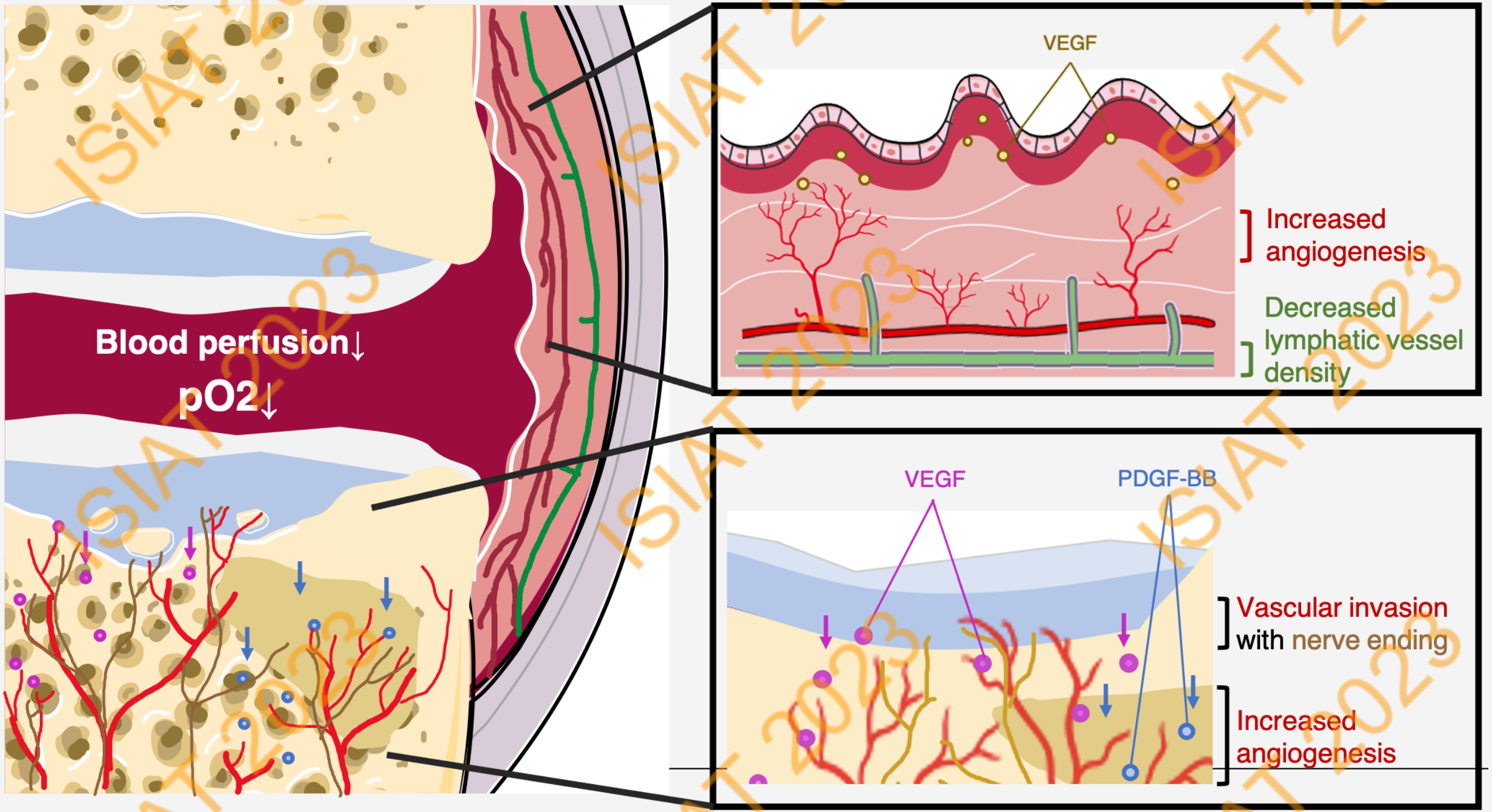
Treatment Gap

Unmet Medical Need

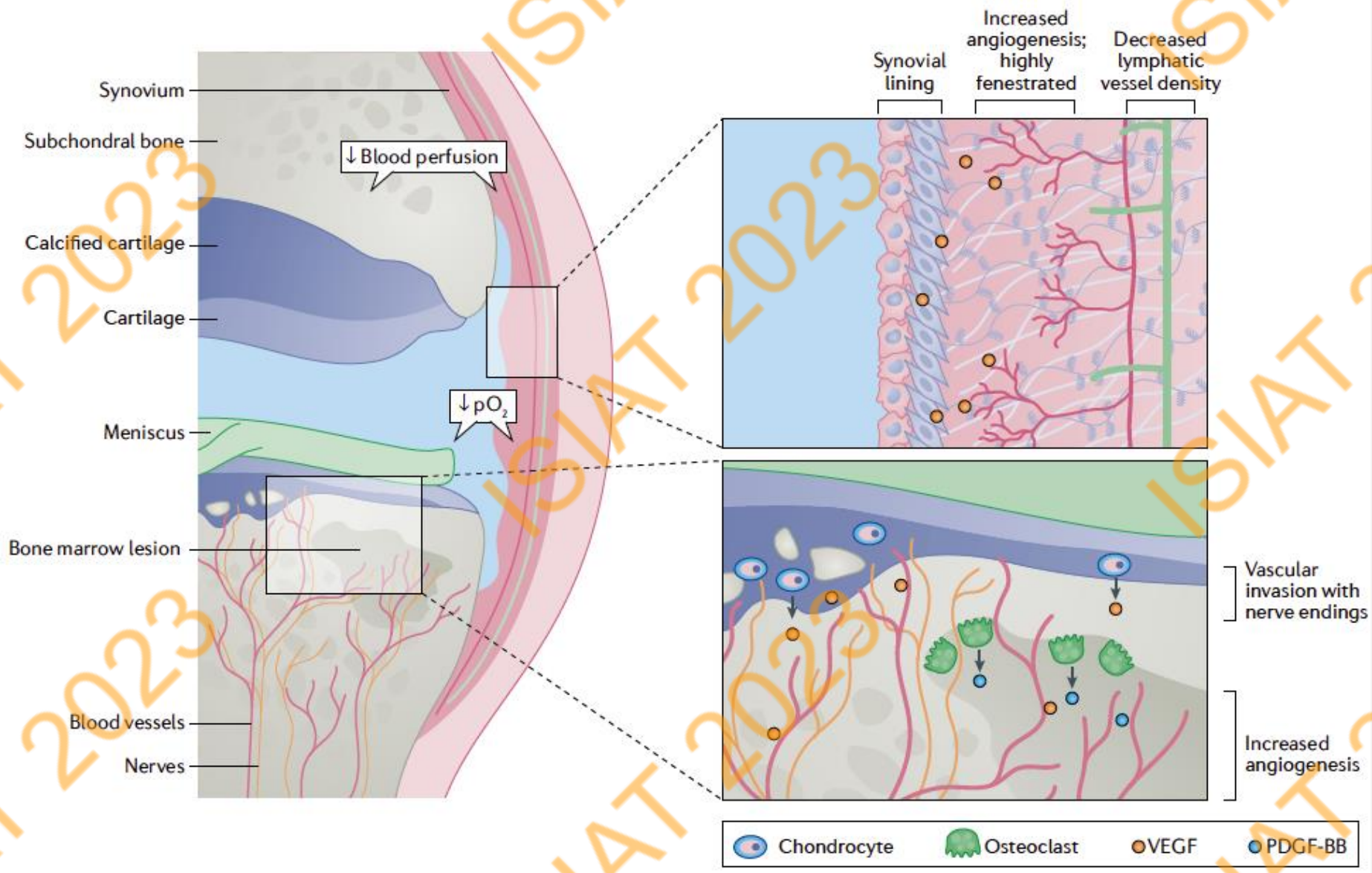


Joint arthroplasty

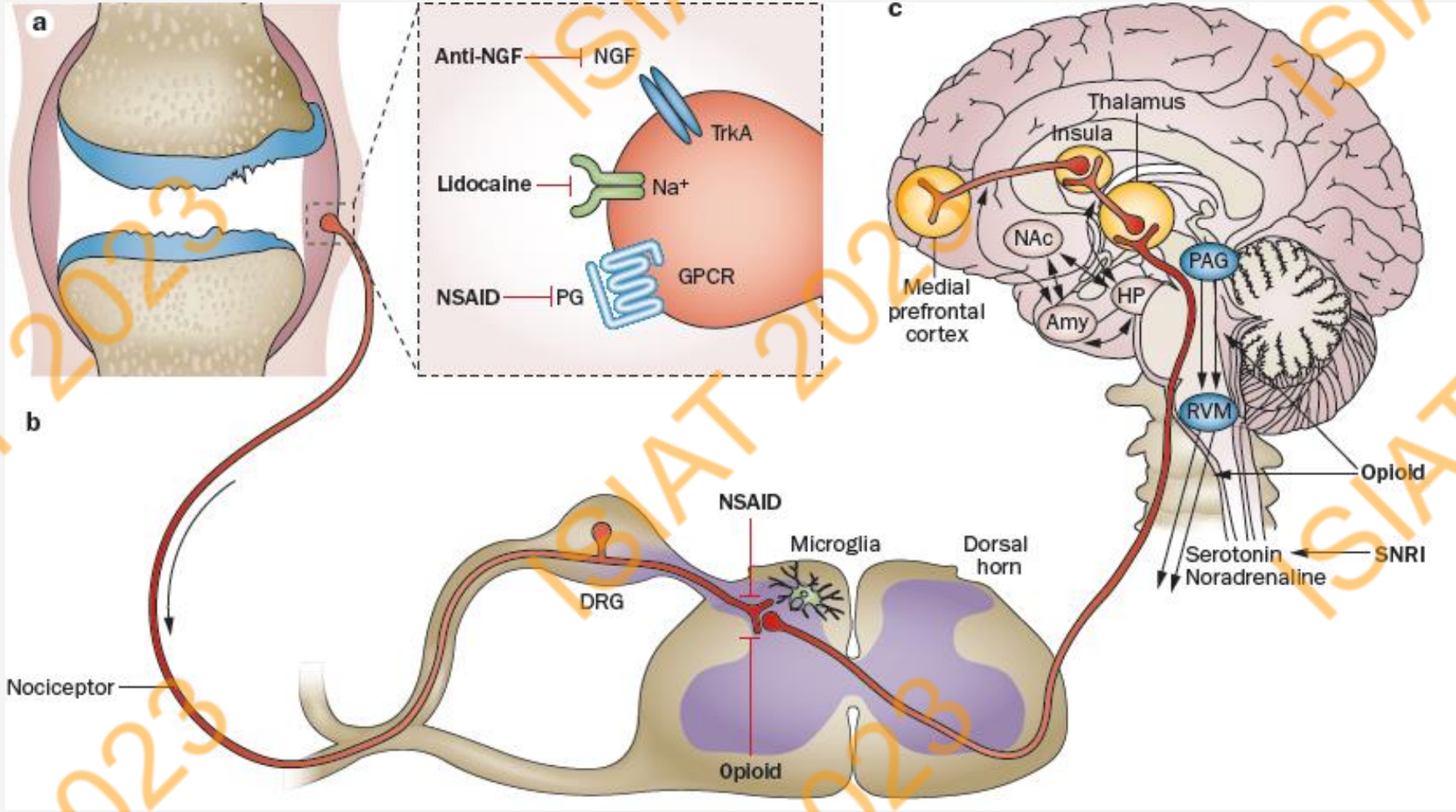
- Last resort for OA patients with end-stage disease
- High surgical and rehabilitation costs
- Prolonged joint pain and rehabilitation period
- Potential need for a second surgery
- Death in surgery for elderly and vulnerable patients


Blood perfusion↓
pO2↓
VEGF
Increased angiogenesis
Decreased lymphatic vessel density
VEGF
PDGF-BB
Vascular invasion with nerve ending
Increased angiogenesis

Vascular Changes and Neo-Innervation in OA



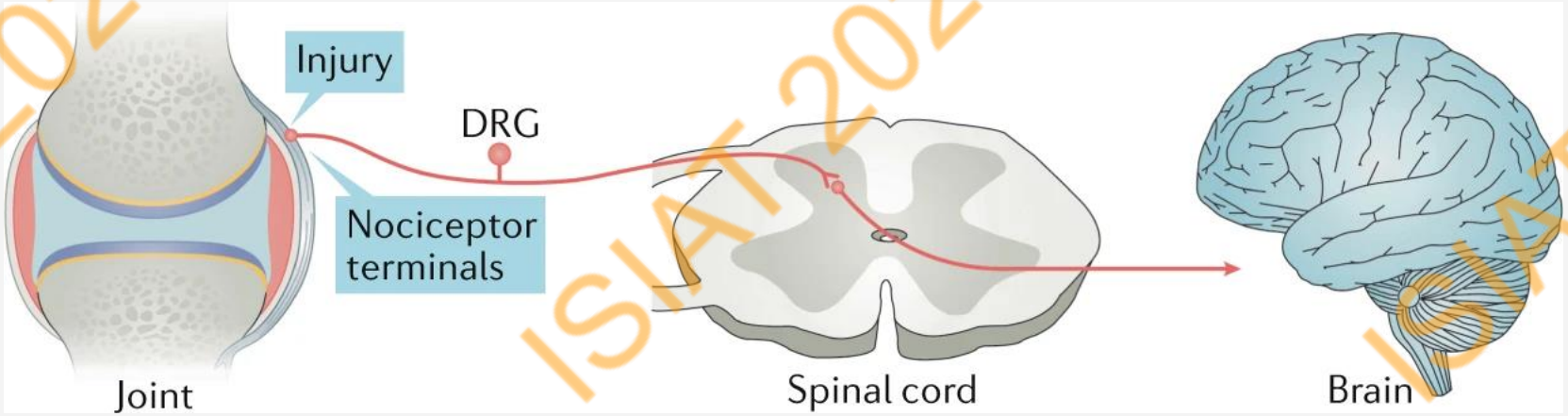
“MSK Pain” Pathways



Slide kindly provided by Professor Philip Conaghan (University of Leeds)

Malfait & Schnitzer 2013. Nat Rev Rheum

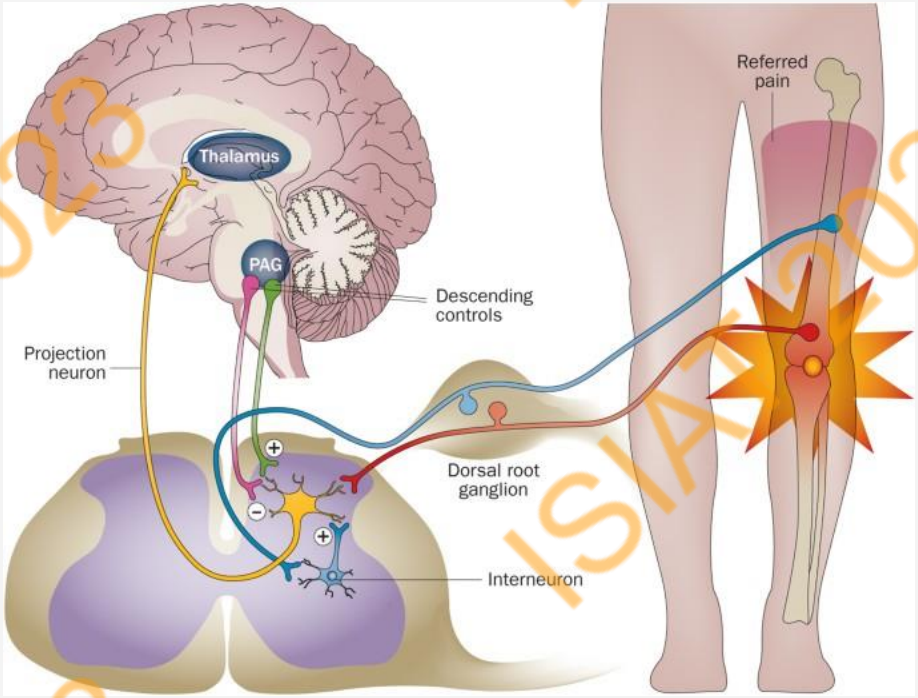
The joint–spine–brain connection in osteoarthritis nociception: anatomy of the basic pain pathway from the periphery to the brain



Conaghan, P.G., Cook, A.D., Hamilton, J.A. *et al.* Therapeutic options for targeting inflammatory osteoarthritis pain. *Nat Rev Rheumatol* **15**, 355–363 (2019). https://doi.org/10.1038/s41584-019-0221-y

Nociceptive Pain

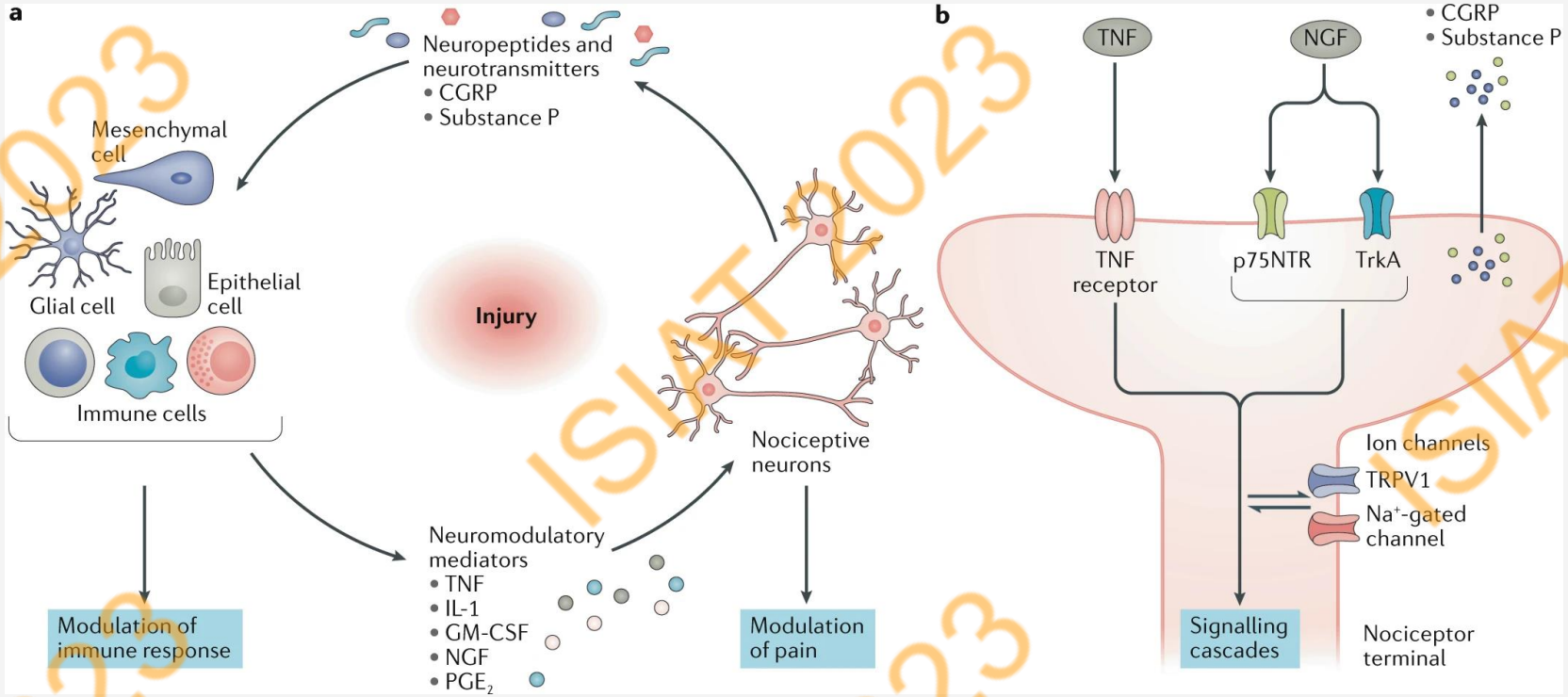
- This is the most common type of OA pain and is typically associated with the degeneration of joint tissues such as cartilage and bone. It's often described as a dull, aching pain that worsens with activity and improves with rest.



Neuropathic Pain

Neuropathic pain occurs when there is damage or dysfunction in the nerves that transmit pain signals. Some individuals with OA may experience tingling, burning, or shooting pain, which is characteristic of neuropathic pain.

In some cases, neuropathic OA pain may have an inflammatory component, characterized by swelling, redness, and warmth in the affected joint. This type of pain is often more constant and may be responsive to anti-inflammatory medications.



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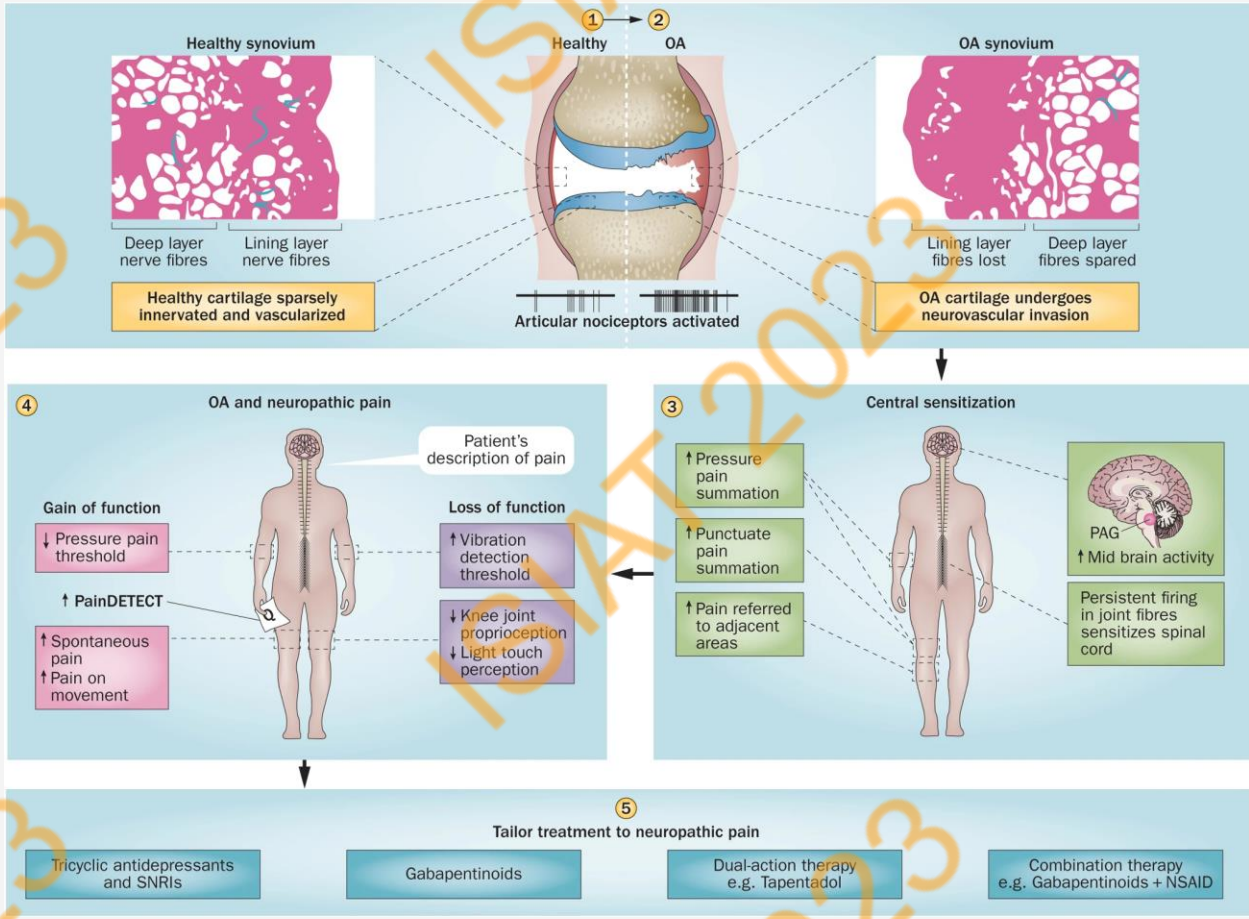
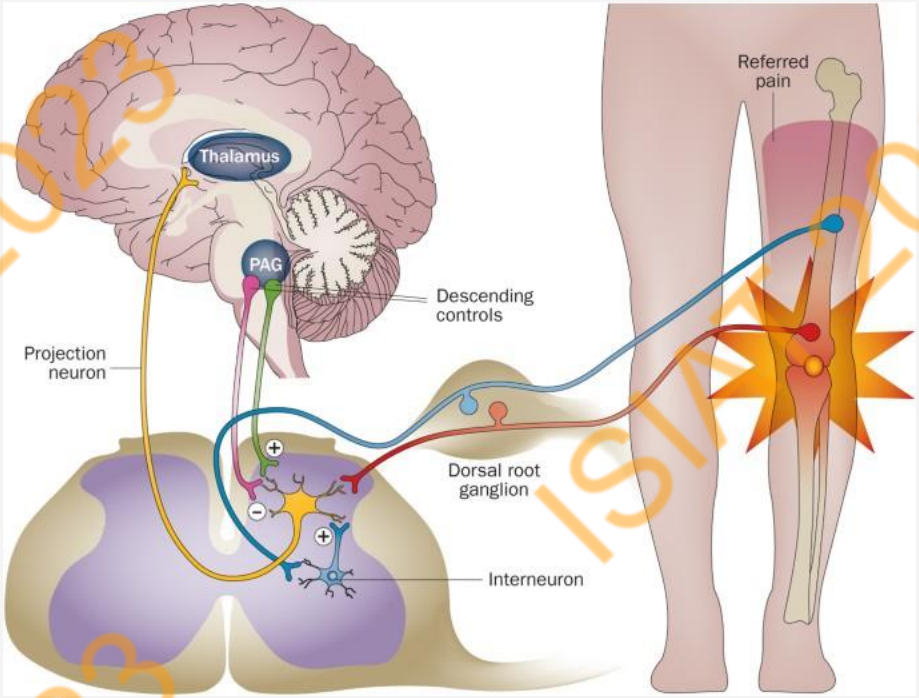
Development of Neuropathic Pain in Osteoarthritis



Thakur, M., Dickenson, A. & Baron, R. Osteoarthritis pain: nociceptive or neuropathic?. *Nat Rev Rheumatol* **10**, 374–380 (2014). https://doi.org/10.1038/nrrheum.2014.47

Central Sensitization

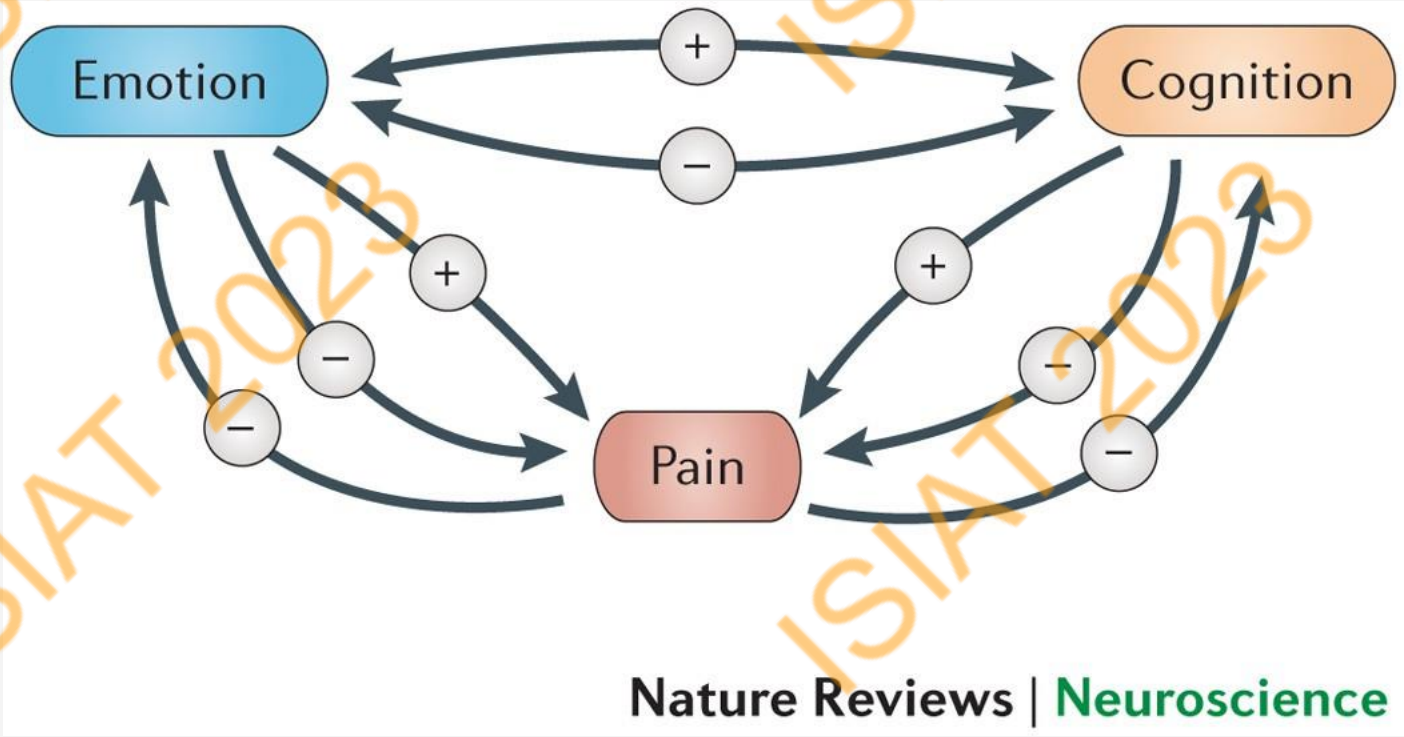
This is a phenomenon where the central nervous system becomes hypersensitive to pain signals, leading to amplified pain perception. Some people with OA may develop central sensitization, resulting in more severe and widespread pain



Thakur, M., Dickenson, A. & Baron, R. Osteoarthritis pain: nociceptive or neuropathic?. *Nat Rev Rheumatol* **10**, 374–380 (2014). https://doi.org/10.1038/nrrheum.2014.47

Existence of Mixed Phenotypes

- Many individuals with OA experience a combination of these different pain phenotypes. For example, they may have both nociceptive and neuropathic pain components.
- In addition, there is some degree of plasticity involved in the sensation of pain in responses to different emotional and environmental settings.

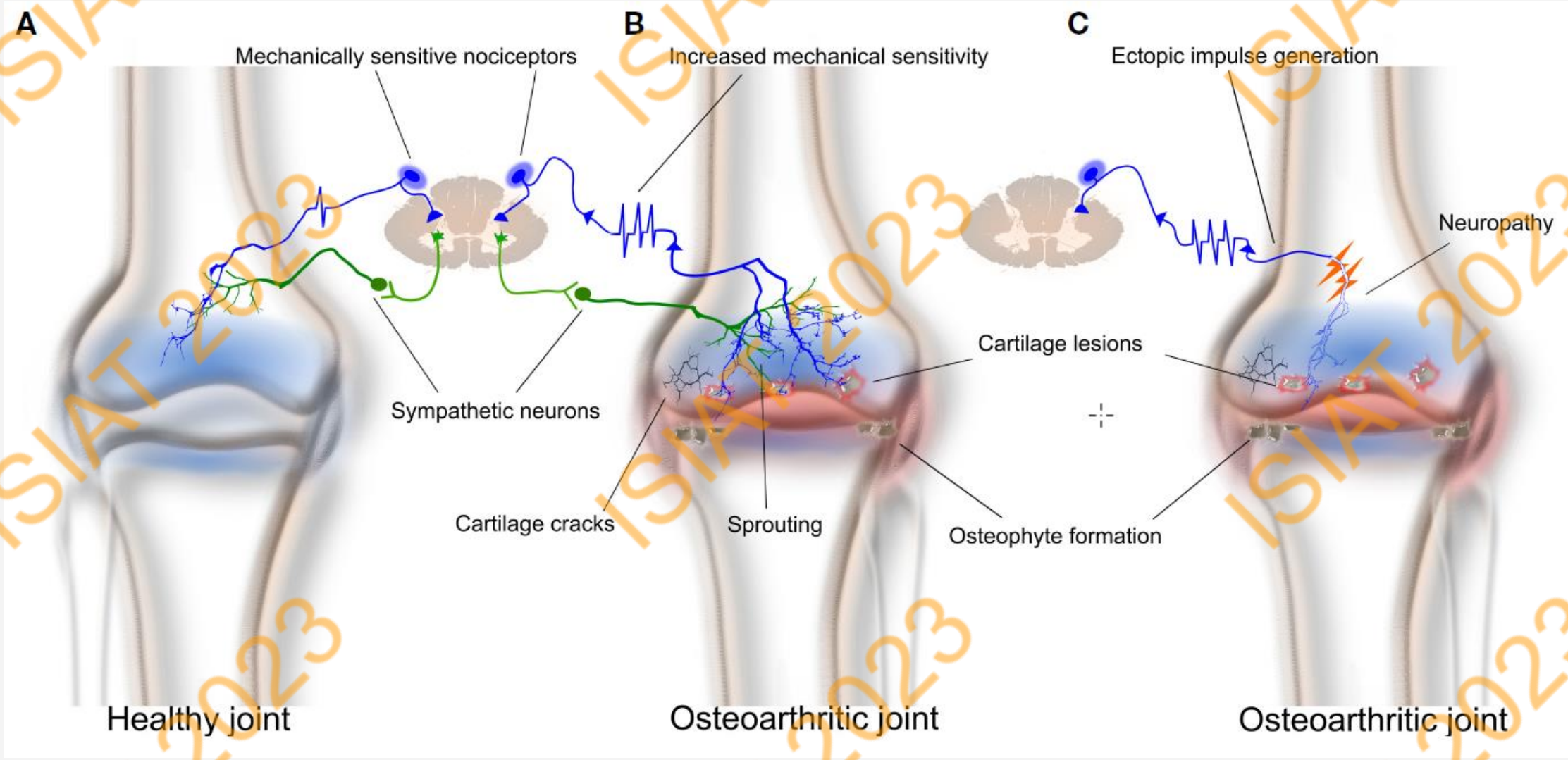


Bushnell, M., Čeko, M. & Low, L. Cognitive and emotional control of pain and its disruption in chronic pain. *Nat Rev Neurosci* **14**, 502–511 (2013). https://doi.org/10.1038/nrn3516

Targeting Pain – A Strategic Priority in Osteoarthritis

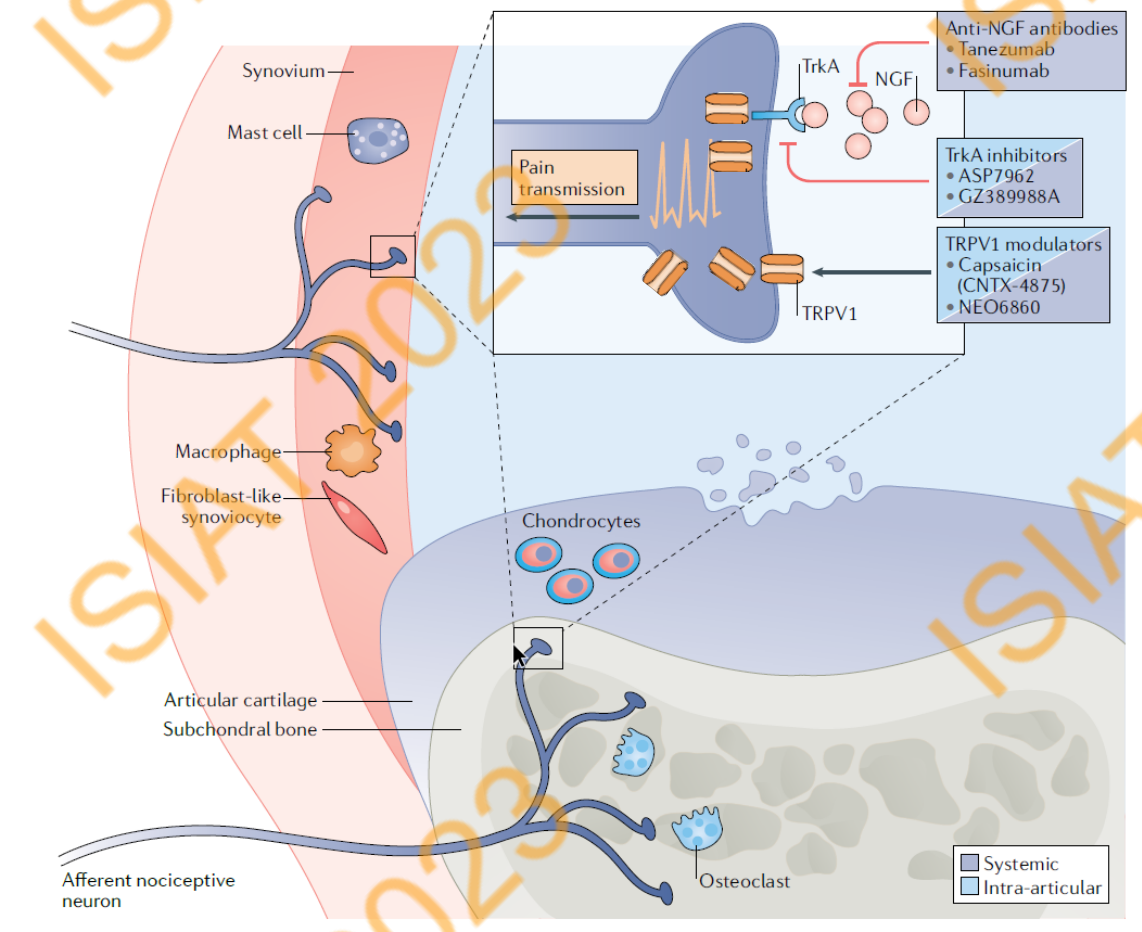
- Pain is a predominant symptom in knee OA
- **Many patients continue to suffer despite the best efforts of current therapies**
- The knee joint is a complex structure and is richly innervated (both normal & pathological states)
- **Subchondral bone, synovium, ligaments, fat pads, even menisci house c- and a-delta nociceptive fibres**
- As OA develops these nerves can become more abundant in certain structures and/or infiltrate previously denervated structures/territories, such as articular cartilage
- These nerves express many receptors, ion channels and other pharmacological targets, that all, to some extent contribute to pain signalling

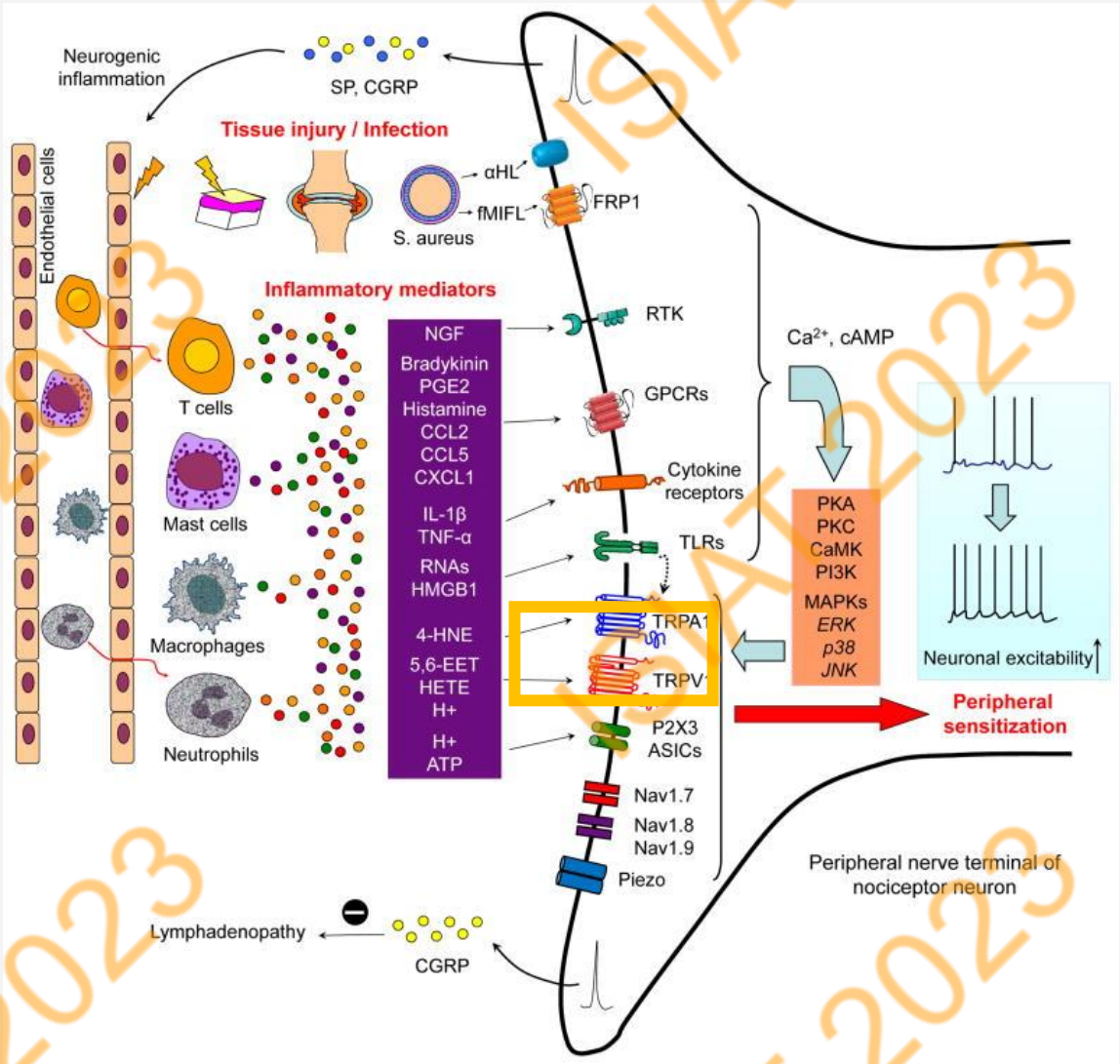
Pain Fibres in Knee Joint Osteoarthritis



Morgan, et al., 2022. Frontiers in Endocrinology

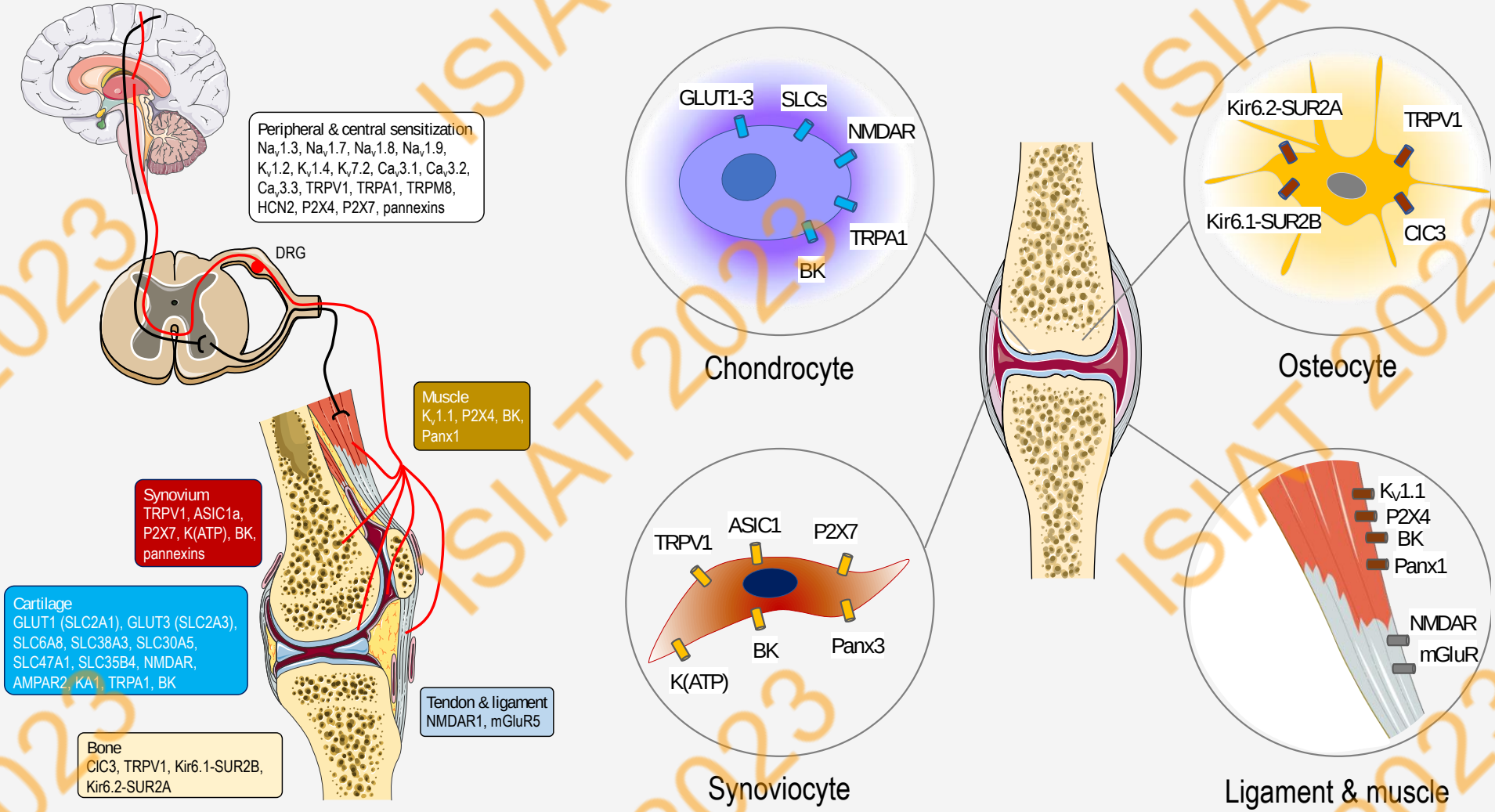
Pain fibres in osteoarthritis: receptors, channels and inflammatory mediators





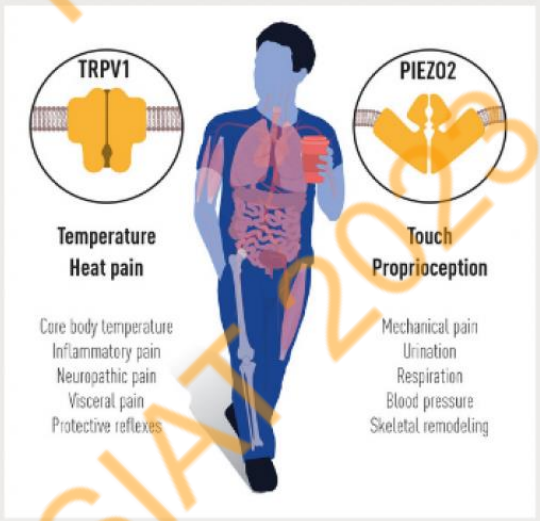
Latourte, A., et al., 2020 Nat Rev Rheumatol; Ji R-R, et al., 2014. Nature Review, Drug Discovery

Ion Channels, Inflammation and Pain in Osteoarthritis



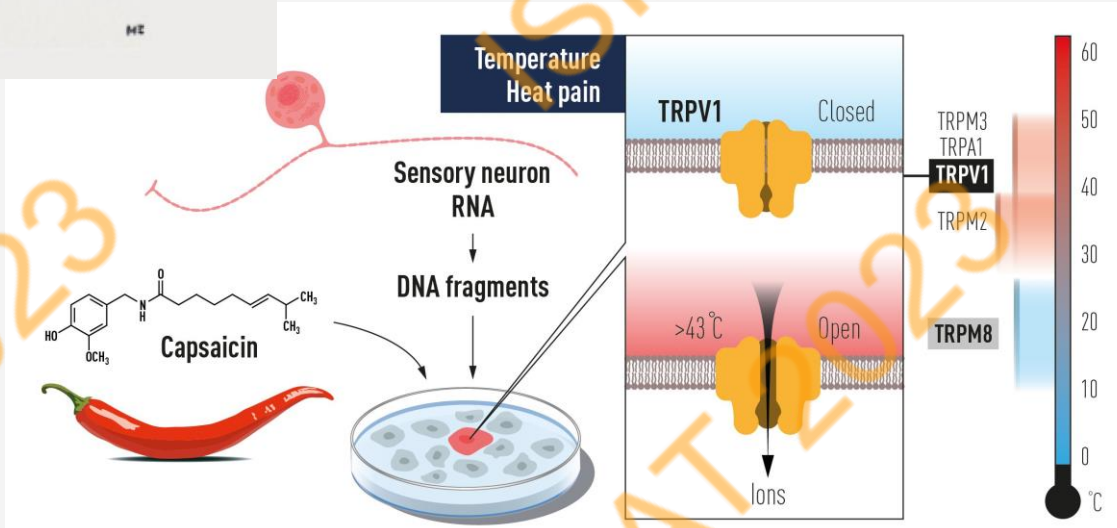
Matta C, Takács R, Ducza L, Ebeid RA, Choi H, **Mobasheri A**. Ion channels involved in inflammation and pain in osteoarthritis and related musculoskeletal disorders. Am J Physiol Cell Physiol. 2023 Jun 12. doi: 10.1152/ajpcell.00040.2023.

THE 2021 NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE:
DISCOVERING HOW WE FEEL HEAT AND TOUCH USING ANIMALS AND CELLS



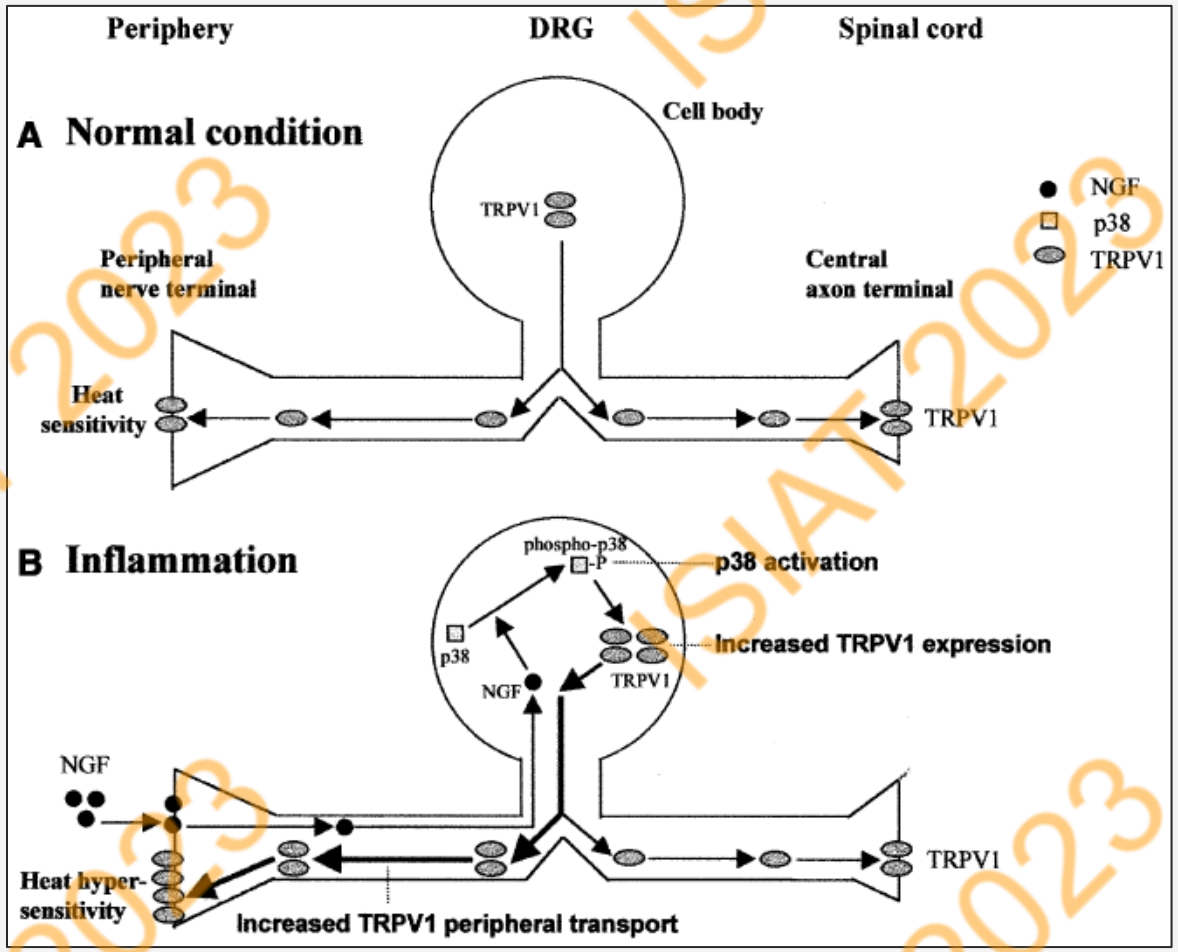
American scientists David Julius and Ardem Patapoutian won the 2021 **Nobel Prize** for Medicine for the discovery of receptors in the skin that sense temperature and touch.

The discovery of TRPV1 using a gain of function screen of genes expressed in sensory neurons for reconstitution of capsaicin responsiveness in a non-responsive cell line. This paved the way to the unravelling of additional temperature-sensing TRP receptors, which together code for temperature sensation.



Axonal trafficking and phenotype switching

- Sensory neurons utilise an anterograde and retrograde axonal trafficking system[1]
  - For transporting trophic factors and cell surface receptors, including TRPV1
- Trafficking is subject to change in response to peripheral and central signals[1,2]



Schematic representation of p38 involvement in heat hyperalgesia after peripheral inflammation

Figure from Ji R-R, et al. Neuron 2002.[1]

(1) Ji R-R, et al. *Neuron.* 2002;36:57–68. (2) Mannion RJ, et al. *Proc Natl Acad Sci.* USA 1999;96:9385–90.

TRPV1 expression in the knee joint: MIA model of OA

FB dye back-labelling was used to identify sensory afferents from the knee joint at the L4 DRG in adult male Wistar rats after iodoacetate-induced OA.[1]

- IHC of those neurons labelled with FB was used to quantify expression levels of TRPV1 and CGRP[1]
- At day 28, there was an increase in joint-specific expression of TRPV1 and CGRP in the iodoacetate model compared with saline-injected controls[1]

Day 28 post-iodoacetate injection	Mean percentage (%) positively labelled cells (± SE)			
	Controls	p value	MIA model	p value
TRPV1 expression	54.3 (±9.8)	<0.0005	71.7 (±6.8)	<0.0005
CGRP expression	76.7 (±4.6)	<0.0005	86.4 (±8.4)	<0.0005

Shows potential role for CGRP and TRPV1 in the manifestation of pain behaviour associated with knee OA changes in the MIA model[1]

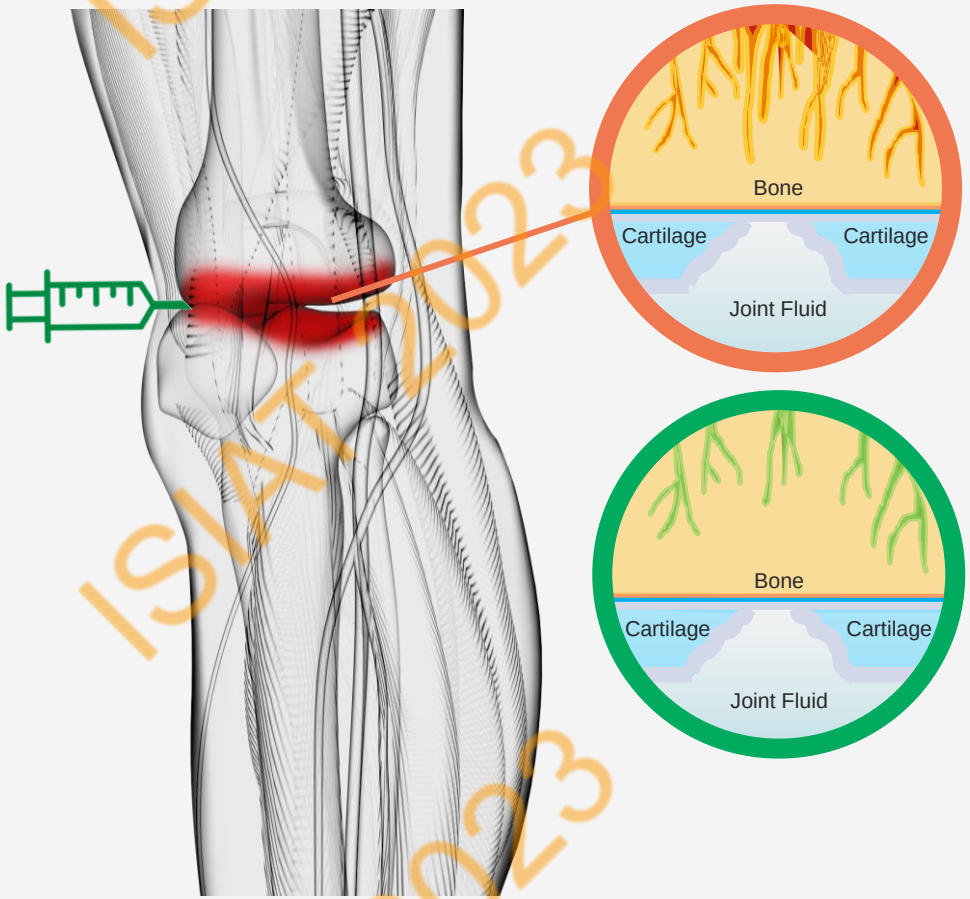
(1) Fernihough J, et al. *Neurosci Lett.* 2005;388:75–80.

CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglion; FB, fast blue; IHC, immunohistochemistry; L, lumbar; MIA, monosodium iodoacetate; OA, osteoarthritis; TPRV1, transient receptor potential vanilloid 1.

Resiniferatoxin

- Resiniferatoxin (RTX) is present in the latex of *Euphorbia resinifera*
  - The dried latex (Euphorbium), has been in use since in ancient medicine
  - Written records of the therapeutic potential of Euphorbium date back to the time of Roman Emperor Augustus
  - RTX was first isolated in 1975
- Experimental descriptions of its pharmacological activity date back to 1989 and indicate similarities to capsaicin
- Involved in hypothermia, neuroinflammation, acute pain, desensitization of capsaicin response

RTX-GRT7039: intra-articular injection comprising resiniferatoxin (RTX) – a potent TRPV1 agonist[1,2]



Activated pain fibres trigger pain[3]

- TRPV1 expression has been shown to play an important role in neuropathic and inflammatory pain
- TRPV1-expressing neurones are present in the synovium of the human OA knee joint

Pain fibres may desensitise after application of RTX[4]

- Administration of RTX can selectively activate TRPV1-expressing nociceptors, inducing an influx of calcium ions into the nerve endings
- This may result in long-lasting but reversible retraction of the peripheral terminals of Aδ- and C-nerve fibres and a subsequent prolonged analgesic effect
- The cell body and axon remain intact, and the nerve endings regenerate within a few months

The mechanism of action of RTX is well-understood
(i.e. similar to high concentration capsaicin)[1,4]

(1) Grünenthal. Grünenthal and NovaQuest enter agreement to advance the resiniferatoxin global Phase III program in osteoarthritis. https://www.grunenthal.com/en/press-room/press-releases/2022/gruenenthal-and-novaquest-enter-development-agreement-for-resiniferatoxin-rtx. Accessed February 2023. (2) Jerman JC, et al. *Br J Pharmacol*. 2000;130:916–22. (3) Fernihough J, et al. *Neurosci Lett*. 2005;388:75–80. (4) National Center for Biotechnology Information. PubChem Compound Summary for CID 5702546, Resiniferatoxin. https://pubchem.ncbi.nlm.nih.gov/compound/5702546. Accessed February 2023.

OA, osteoarthritis;
TRPV1, transient receptor potential vanilloid–1.

RTX and TRPV1

TRPV1 was previously known as capsaicin receptor and the vanilloid receptor 1 (VR1)[1]

- A study with rat dorsal root ganglion neurons demonstrated that TRPV1 can account for:
  - ✓ **RTX ligand binding[2]**
  - ✓ **Calcium ion uptake[2]**
    - ➢ **TRPV1 allows calcium ions into the neuron when the ligand is bound to it[2]**

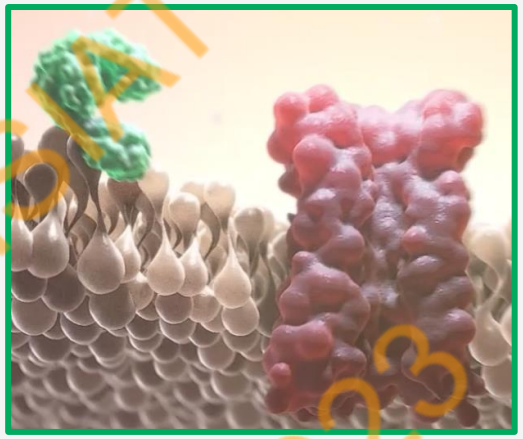
- Regarding binding characteristics, studies have demonstrated:
  - ✓ **Lower pH (5.5–7.0) does not affect RTX binding to TRPV1[3,4]**
  - ✓ **RTX binding to TRPV1 is temperature dependent[4]**
  - ✓ **RTX noncompetitively binds to TRPV1 (based on preliminary characterisation)[4]**

RTX is a potent TRPV1 agonist[3]

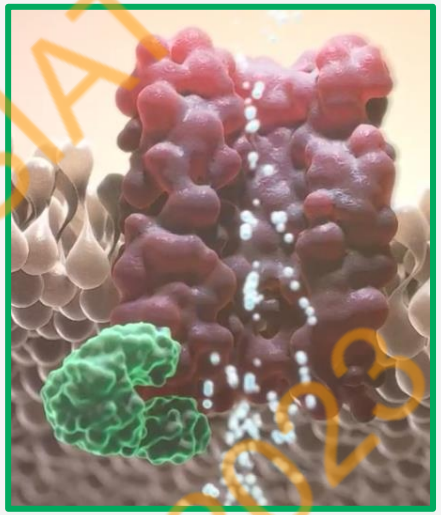
Ranking of TRPV1 agonist potency:
RTX > capsaicin > olvanil > PPAHV[3]

On the Scoville Scale (measure of spice 'heat'), RTX is around 1,000x higher than capsaicin[5]

RTX (green) not attached to TRPV1 (red), meaning TRPV1 is closed and Ca^{2+} ions are not moving through



RTX (green) attached to TRPV1 (red), meaning Ca^{2+} ions are moving through TRPV1



Images taken from Grünenthal's mechanism of action animation

(1) Shuba YM. *Front Cell Neurosci*. 2021;14:612480. (2) Szallasi A, et al. *Mol Pharmacol.* 1999;56:581–7. (3) Jerman JC, et al. *Br J Pharmacol*. 2000;130:916–22. (4) Szallasi A & Blumberg PM. *Naunyn Schmiedebergs Arch Pharmacol.* 1993;347:84–91. (5) alimentarium. The Scoville scale. Available at: https://www.alimentarium.org/en/magazine/infographics/scoville-scale. Accessed February 2023.

PPAHV, phorbol 12-phenylacetate 13-acetate 20-homovanillate; RTX, resiniferatoxin; TRPV1, transient receptor potential vanilloid–1.

Acknowledgements

GRÜNENTHAL