Athens

5-7 October 2023

Intra-Articular PRP injection: Mechanism of Action and Their Degree of Efficacy

Isabel Andia Ph.D.

Biobizkaia Health Research Institute, Bizkaia, Spain

Regenerative Therapies: osteoarticular pathologies, tendinopathies

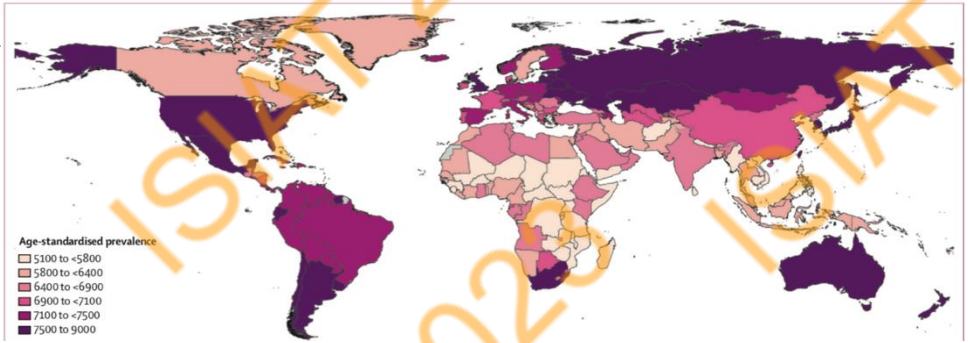
Athens

5-7 October 2023

DISCLOSURE

Scientific Advisory Board**, SPRY BIO, INC**

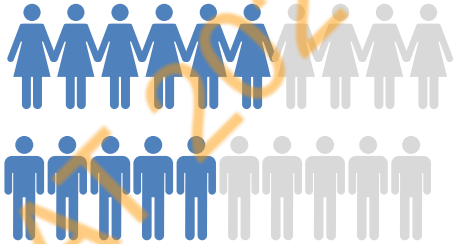
2021-2030, decade of healthy ageing (WHO)



The age standardized prevalence greatest in high income countries

Knee OA is the major contributor

Increase in case number
from 2020 to 2050
74.9% (59.4-89.9) for knee OA



Global burden of disease (2020):
654.1 (95%CI 565.6-745.6) millions (aged>40 yr) (Lancet 2023)

TKR BURDEN 2012-2050 in USA
expected rise 855%
Projected TKR health care costs (AUS) growth by 273% by 2030, $AUD5.32

Is PRP treatment just a form of modern art??

Something each individual is free to interpret as they wish?

Polarized opinions

Shirim Neshat (Iran)
"our house is on fire"

Polarized opinions

A single « product » that is going to change complex health outcomes??

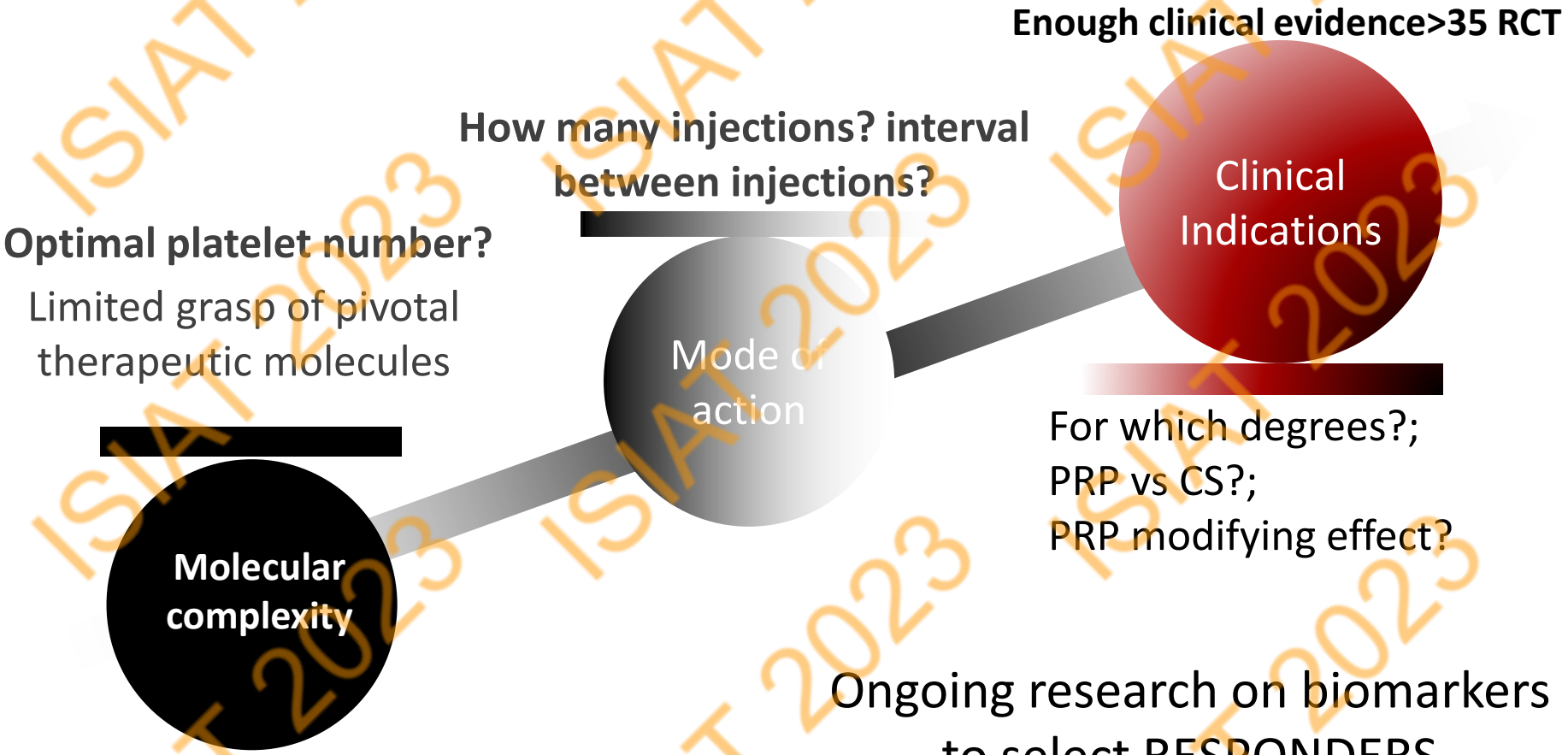
« SKEPTICS »:

- ✓ No standardization,
- ✓ Lack of robust clinical data,
- ✓ Highly lucrative for those who offer it

- **« BELIEVERS »:**

Recognize complexity of medical discovery
Causality is not black&white
The focus remains on advancing
in PRP refinement

PRP Investigation



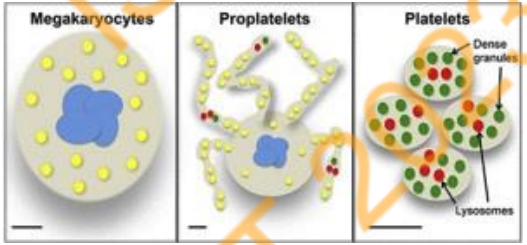
Ongoing research on biomarkers to select RESPONDERS

PRP Molecular complexity

Platelet secretome

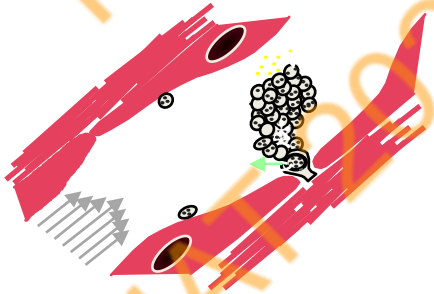
Megakaryocyte synthesis

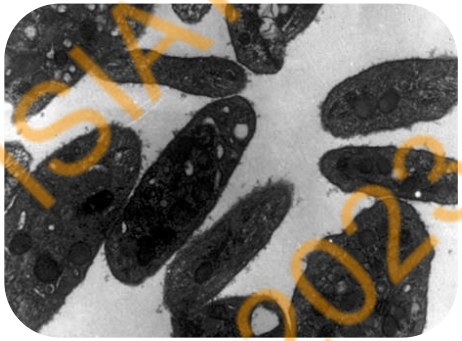
Platelets are cytoplasmic fragments released from the megakaryocyte in the bone marrow

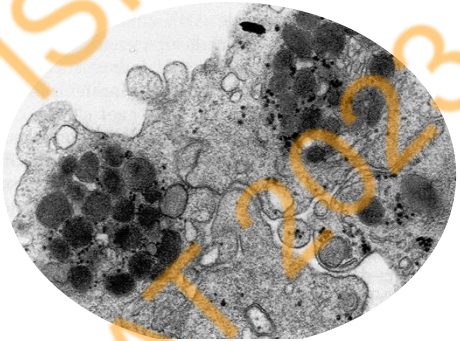


Captured by endocytosis in the blood stream

Platelets circulate for 8-10 days, during this time they capture proteins from the plasma



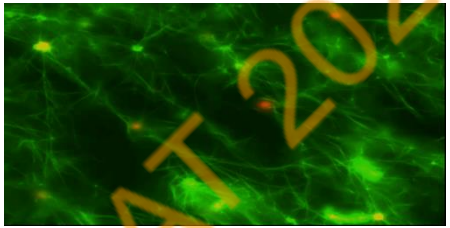




CORE elements in PRP secretome

Growth factors*, *cytokines and chemokines: PDGF-A, B & C, TGFβ-1, EGF, IGF-1, VEGF A & C, bFGF, HGF, PF4, β -TG, endostatins, BMP2, 4 & 6, RANTES, IL-8, MIP1, growth-regulated oncogene- β, ENA-8, MCP-3, angiopoietin…***Fibrinolytic proteins***:Plasminogen, PAI-I, osteonectin, β2-antiplasmin
TAFI ***Adhesive proteins***: VWF, Fg, Fn, Vn, TSP-1, laminin-8 ***Coagulation proteins***: FV/Va + multimerin, FXI, protein S, HMWK, antithrombin, Gas6

.



Research Questions

1st

2nd

3rd

4rd

***Mechanism of Action*:**

Platelet-cell interactions?

Or PRP secretome?

Exogenous activation of PRP?

Pivotal molecules:

Molecular drivers of clinical benefits?

Optimal platelet range?

***PRP indication*:**

Enough evidence?

For which KL degrees?

PRP vs CS?

***Pain*:**

Does PRP interfere with pain mechanisms?

Unwanted proteins in PRP?

Research Questions

***Mechanism of Action*:**

Platelet-cell interactions?
Or PRP secretome?
Exogenous activation of PRP?

Pivotal molecules:

Molecular drivers of clinical benefits?

Optimal platelet range?

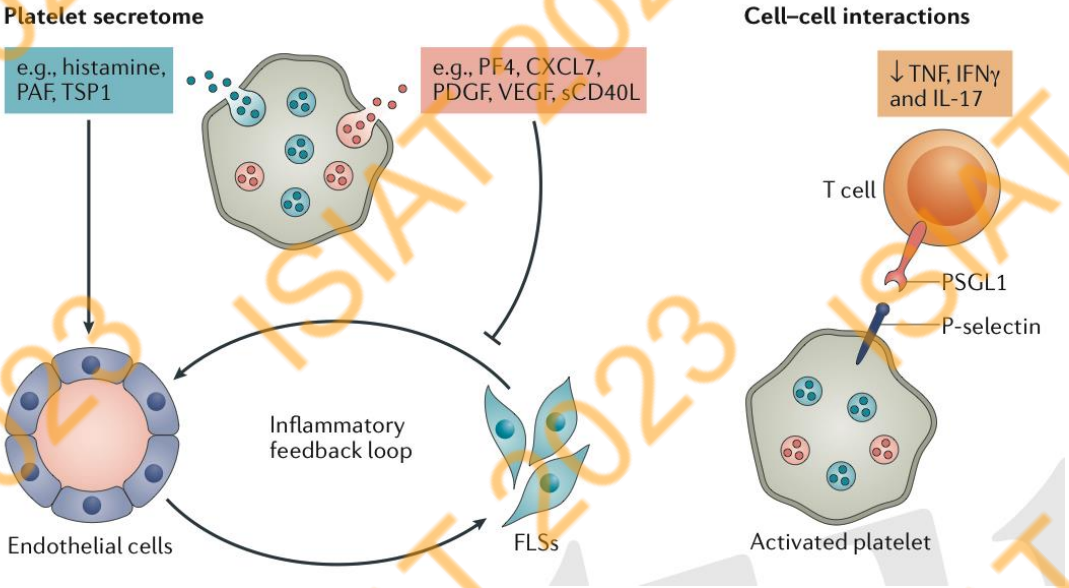
Experimental investigations are crucial for understanding the mode of action and can help answer questions like the optimal platelet dose and injection intervals.

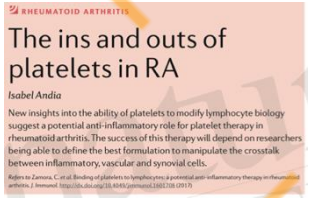
It's a two-way process that informs and improves clinical practice.

Two mechanisms of action

1. Direct platelet-cell interactions

2. Interaction of platelet secretome with local cells




RHEUMATOID ARTHRITIS

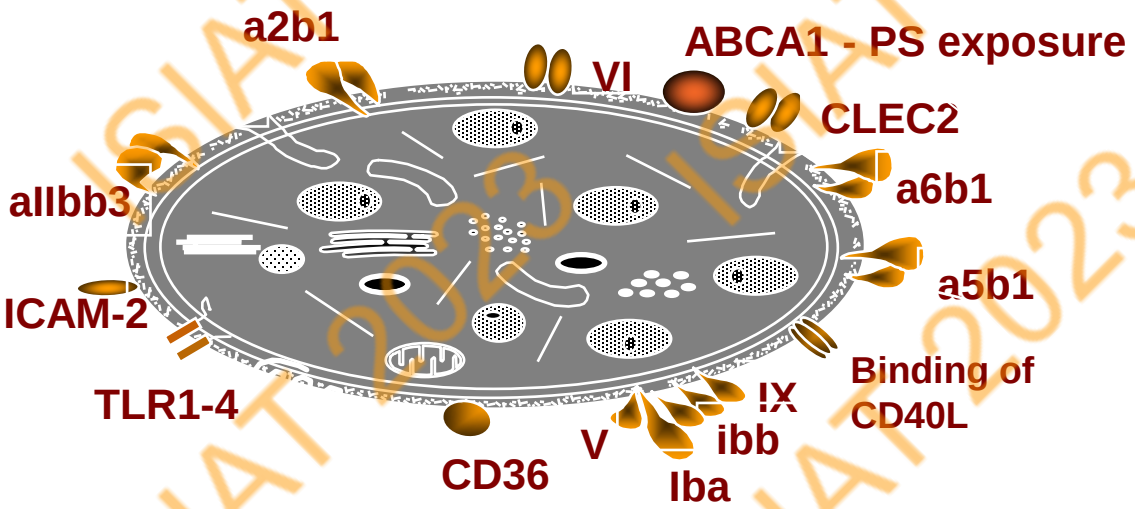
The ins and outs of platelets in RA

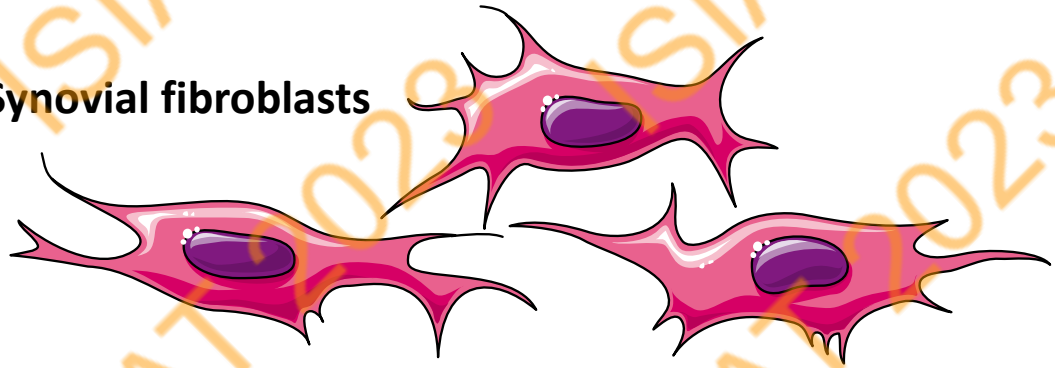
Isabel Andia

New insights into the ability of platelets to modify lymphocyte biology suggest a potential anti-inflammatory role for platelet therapy in rheumatoid arthritis. The success of this therapy will depend on researchers being able to define the best formulation to manipulate the crosstalk between inflammatory, vascular and synovial cells.

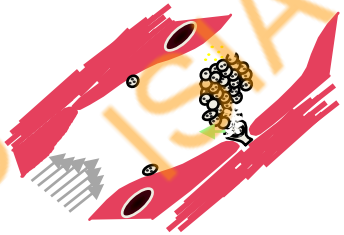
Refers to Zamora, C. *et al.* Binding of platelets to lymphocytes: a potential anti-inflammatory therapy in rheumatoid arthritis. *J. Immunol.* http://dx.doi.org/10.4049/jimmunol.1601728 (2017)

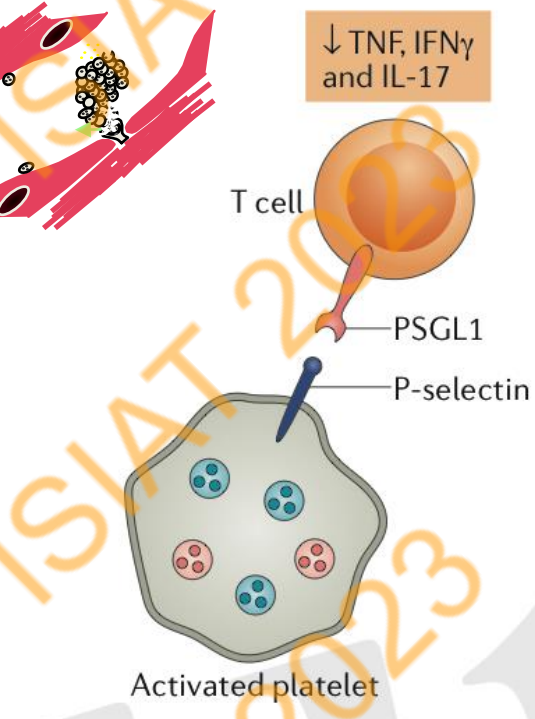
Platelet-CELL interactions



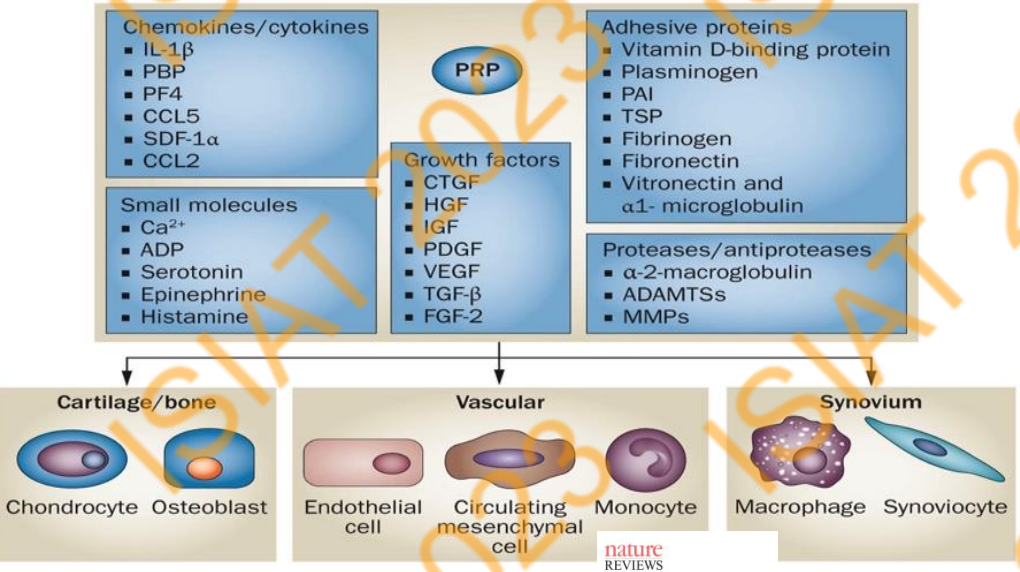


Platelet-leukocyte





Modification of the molecular milieu and cross-talk between cell phenotypes



Andia&Maffulli. Nat Rheum Rev 2013

Exogenously activated PRP more effective than non activated PRP

Systematic Review and Metaanalysis (N=14 RCT)

Clinical Rheumatology (2023) 42:1397–1408
https://doi.org/10.1007/s10067-022-06463-x

ORIGINAL ARTICLE

Check for updates

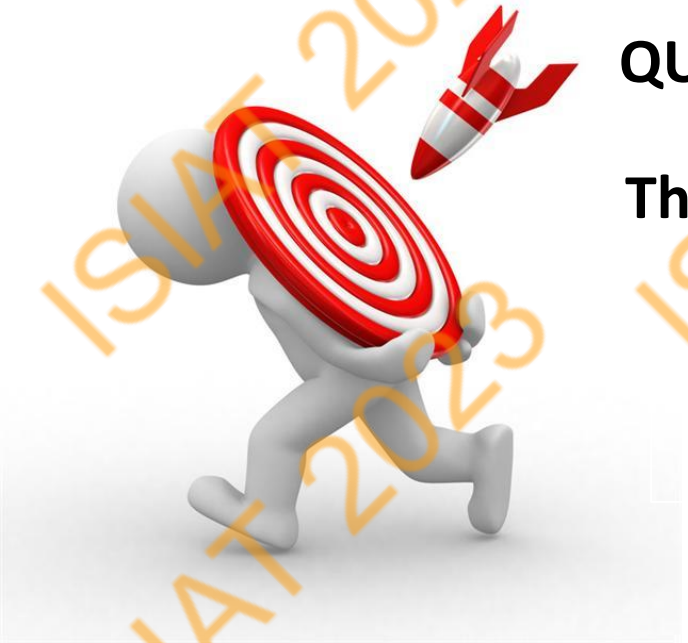
Comparison of the clinical effectiveness of activated and non-activated platelet-rich plasma in the treatment of knee osteoarthritis: a systematic review and meta-analysis

Mario Simental-Mendía[1] · Daniela Ortega-Mata[1] · Yadira Tamez-Mata[1] · Carlos A. Acosta Olivo[1] · Félix Vilchez-Cavazos[1]

To answer optimal platelet range we have to identify molecular drivers of PRP clinical effects

The medical literature tells that the most prominent Growth Factors in PRP include **PDGF, TGF-beta, EGF, VEGF, IGF and FGF**

Are we overlooking the functions of other groups of molecules?



QUESTIONING THIS FOCUS IS IMPORTANT

The cells we target with the PRP secretome

- ✓Local stromal and **stem cell niche**
- ✓Vascular and lymphatic
- ✓**Immune infiltrating compartment:**
  T-cells, mast cells, monocyte/macrophages

Are we overlooking the functions of other groups of molecules?

Bansal et al. Sci. Rep. 2021

The growth factors secreted by the platelets *stimulate the proliferation of chondrocytes and mesenchymal stem cells thereby assisting in synthesis of type II collagen.*

Tan et al. Arthroscopy 2021

"PRP provides concentrated ***growth factors*** *for use as n intraarticular injection"*

Singh et al. Am J Sports Med 2021

"PRP, defined as an autologous formulations derived from Whole blood that is centrifuged to extract a solution with A platelet concentration 3- to 5- fold greater in multiple ***growth factors*** *compared with normal plasma"*

Kon et al. Exp Opin Biol Therapy 2020

"PRP consists of a volume of autologous plasma with a concentration of platelets above the baseline containing a ***high levels of several growth factors*** *including IGF-I, TGF-b, FGF, PDGF, VEGF, EGF, which*

Are we barking up the wrong tree??

Optimal platelet range?
Pivotal cytokines?

Interleukins

IL-9

IL-10

IL-21

IL-29

Chemokines

PF-4 (CXCL4)

NAP2 (CXCL7)

CXCL16

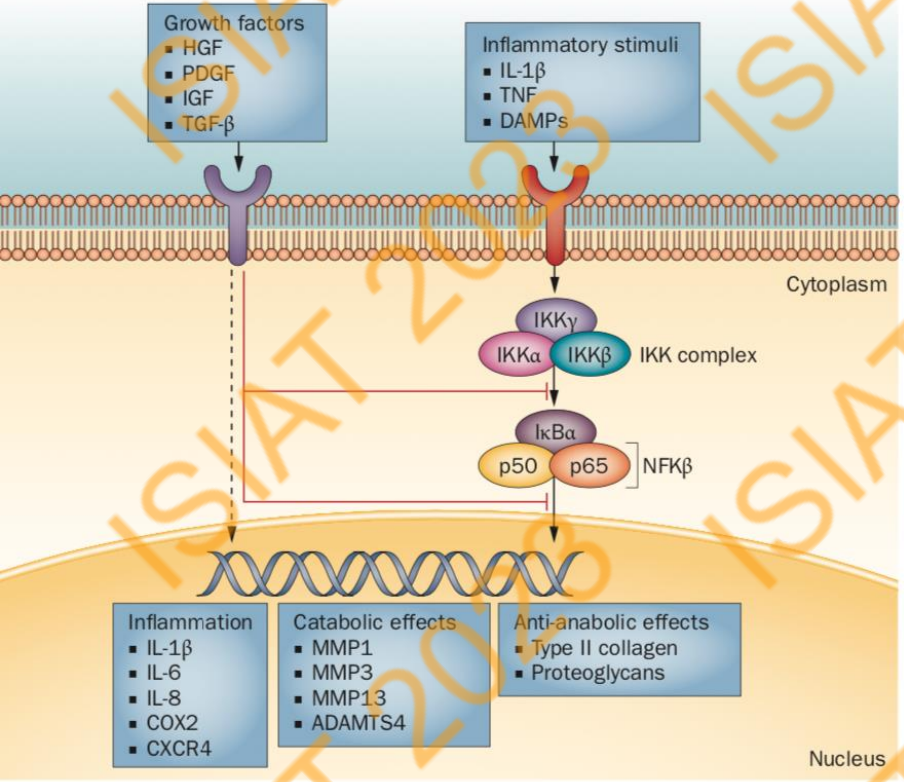
Multifunctional immune regulation

Chemotactic neutrophils& monocytes

Macrophage polarisation

Looking into a different direction

Shall we interrogate specific signaling pathways?

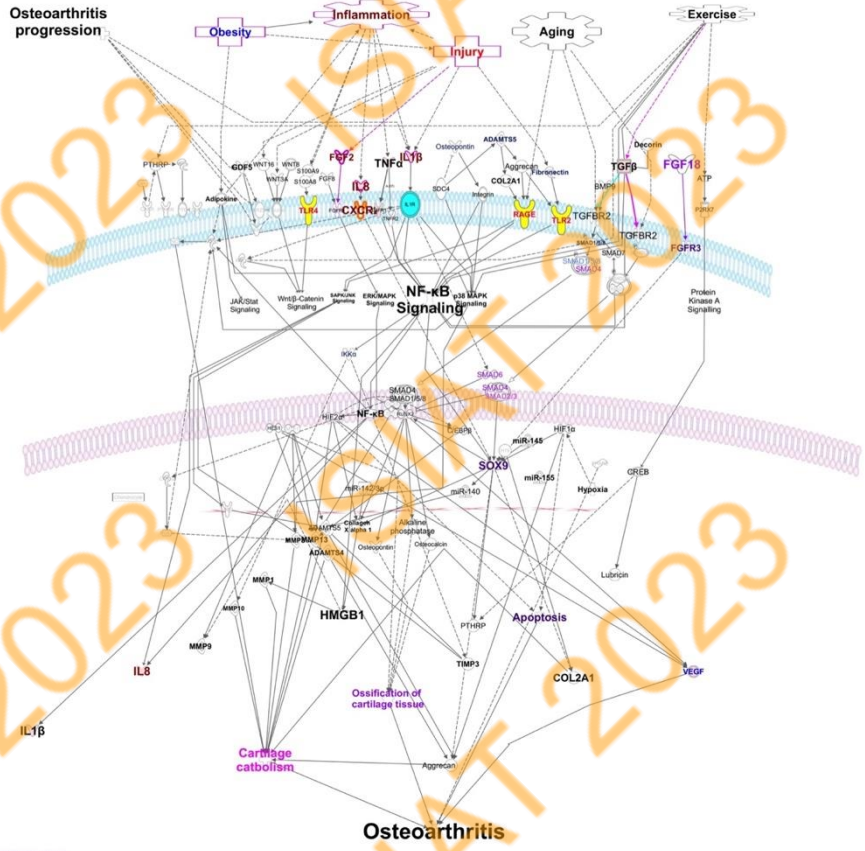


Journal of Clinical Medicine
MDPI

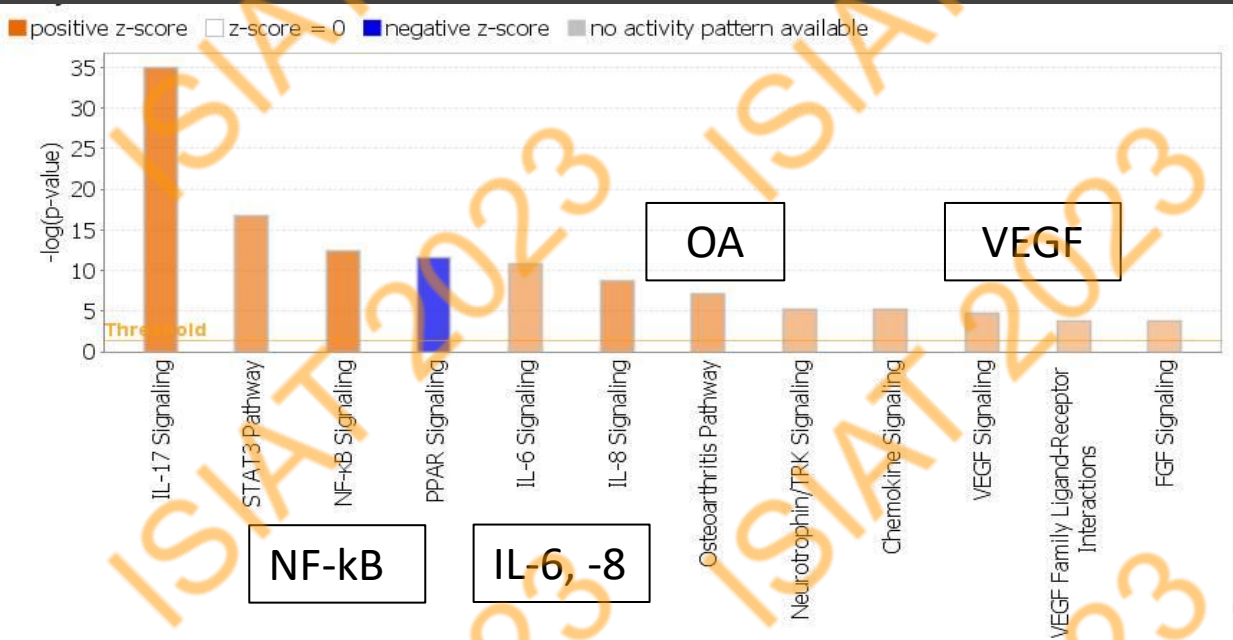
Article

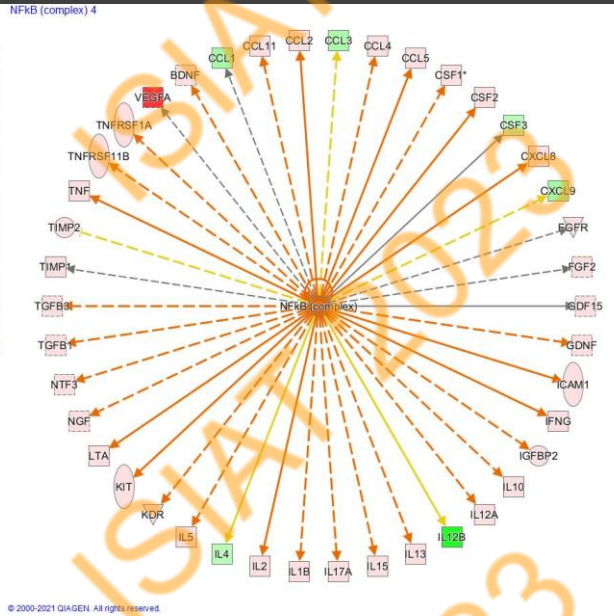
Unraveling the Signaling Secretome of Platelet-Rich Plasma: Towards a Better Understanding of Its Therapeutic Potential in Knee Osteoarthritis

Cristina Del Amo [1], Arantza Perez-Valle [1], Leire Atilano [1,2] and Isabel Andia [1,*]



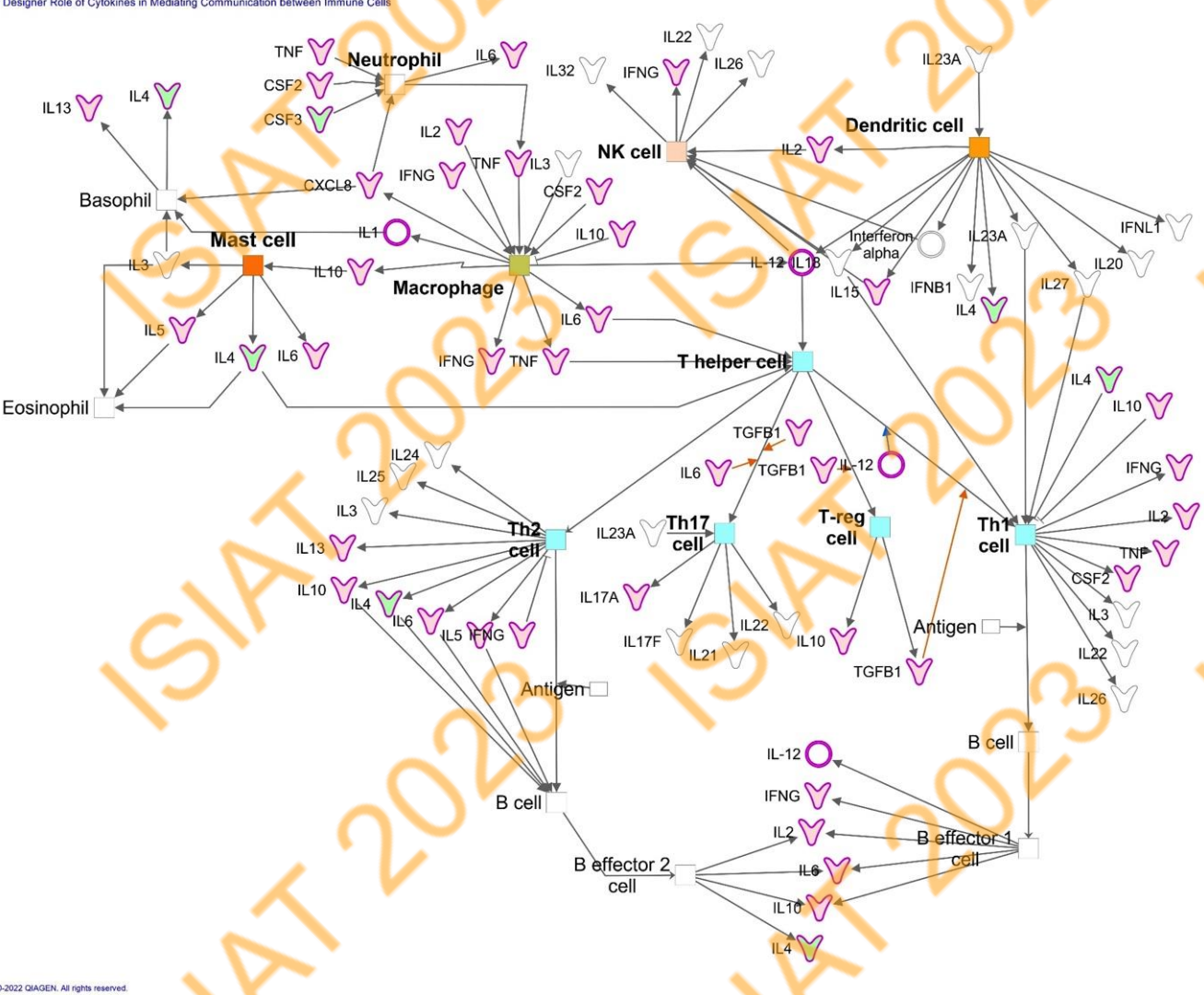
Identify biological mechanisms that are enriched
More than would be expected by chance





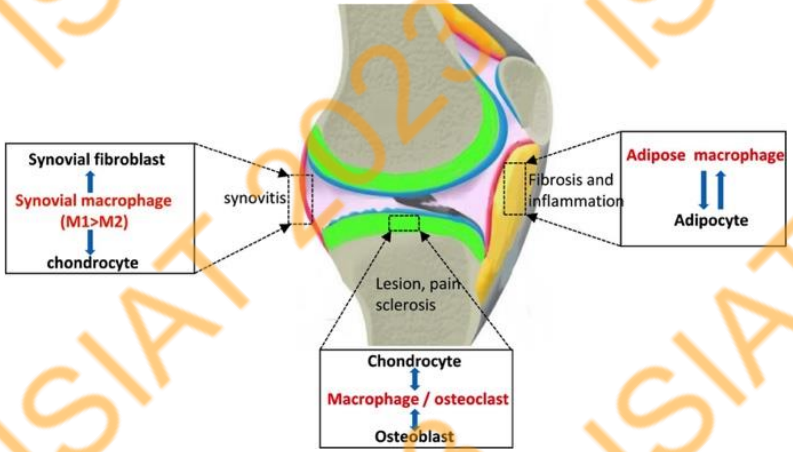
Activation z-score, infer likely activation states of biological functions

(based on comparisons with a model that assigns randon regulation directions)

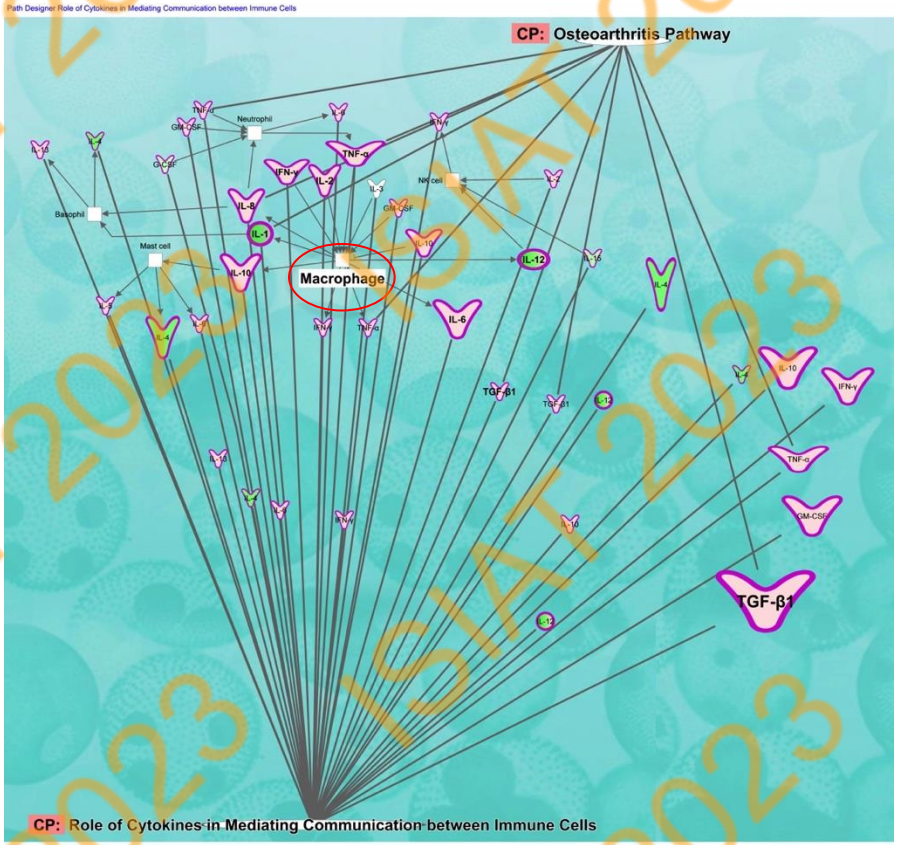


- PRP mediates immune cell recruitment
- PRP cytokines mediate the communication between immune cells

Resident macrophages maintain organ homeostasis



Xie J et al. Clinical implications of macrophage dysfunction. Cytokine and Growth Factor Reviews 46 (2019)



Research Article

For reprint orders, please contact: reprints@futuremedicine.com

Regenerative Medicine

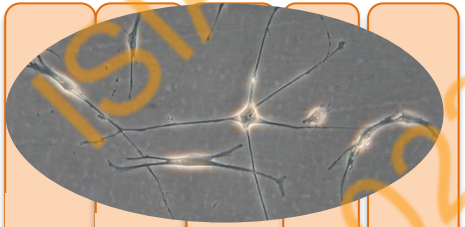
Pure platelet-rich plasma and supernatant of calcium-activated P-PRP induce different phenotypes of human macrophages

Gisselle Escobar[1], Alejandro Escobar[2], Gabriel Ascui[1], Fabián I Tempio[1,4], María C Ortiz[2], Claudio A Pérez*[,3,4] & Mercedes N López**[,1,4]

Regulation of macrophage polarization

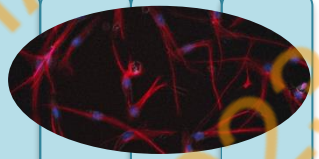
Preclinical research tools

2D Monolayer/cocultures



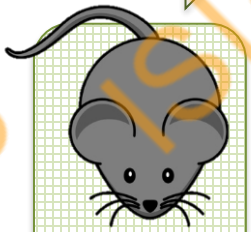
- Cell proliferation and migration
- RNA expression (microarrays)
- Protein secretion (CM)
- RNA-seq
- RNA silencing

3D Explants/Scaffold systems



- Cell encapsulation (bioprinting)
- Cell survival and adhesion
- Tubulogenesis (angiogenesis)
- organoids
- Arrays/bioinformatics
- Pathway inference

In vivo



- In vivo, animal models

BART

- Molecular arrays and bioinformatics

In silico

- Mechanistic models of how to control cell phenotype

Research Questions

***PRP indication*:**

Enough evidence?
For which KL degrees?
PRP vs CS?

***Pain*:**

Does PRP interfere with pain mechanisms?
Unwanted proteins in PRP?

GRIIP, Expert consensus
Modified Delphi Methodology

Knee Surgery, Sports Traumatology, Arthroscopy (2021) 29:3195–3210
https://doi.org/10.1007/s00167-020-06102-5

KNEE

Intra-articular injections of platelet-rich plasma in symptomatic knee osteoarthritis: a consensus statement from French-speaking experts

Florent Eymard[1] · Paul Ornetti[2] · Jérémy Maillet[3] · Éric Noel[4] · Philippe Adam[5] · Virginie Legré-Boyer[6] · Thierry Boyer[7] · Fadoua Allali[8] · Vincent Gremeaux[9] · Jean-François Kaux[10] · Karine Louati[11] · Martin Lamontagne[12] · Fabrice Michel[13] · Pascal Richette[14] · Hervé Bard[15] on behalf of the GRIP (Groupe de Recherche sur les Injections de PRP, PRP Injection Research Group)

GRIIP, PRP Injection Research Group
French speaking experts

PRP treatment should be offered as a second line treatment (1A, best evidence)

Better results in young patients with mild-moderate OA

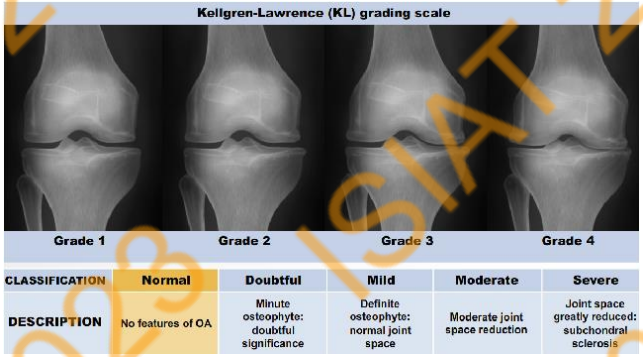
ESSKA/ICRS CONSENSUS
Delphi Methodology

ESSKA **ORBIT Consensus**
Use of injectable orthobiologics for the treatment of knee osteoarthritis

Part 1: blood-derived products (alias PRP)

Chairpersons: Laura de Girolamo, Lior Laver

ORthoBIologicsIniTiative



CLASSIFICATION	Normal	Doubtful	Mild	Moderate	Severe
DESCRIPTION	No features of OA	Minute osteophyte: doubtful significance	Definite osteophyte: normal joint space	Moderate joint space reduction	Joint space greatly reduced: subchondral sclerosis

Age
Tibiofemoral
Patellofemoral
Effussion

ESSKA/ICRS, ORBIT CONSENSUS

Delphi methodology (28 questions)

Laura de Girolamo
Chairperson

Lior Laver
Chairperson

ESSKA **ORBIT Consensus**
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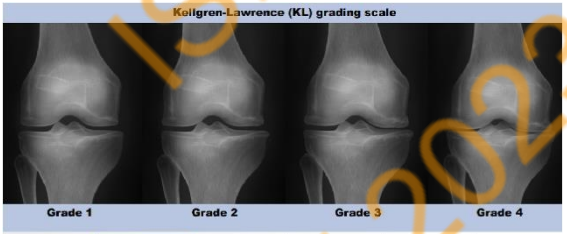

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PRP-Rationale/indication

Enough evidence to recommend PRP? **Grade A,** high scientific level

For which degree?, KL<= 3 **Grade A,** high scientific level

PRP-Protocol, Preparation/characterization

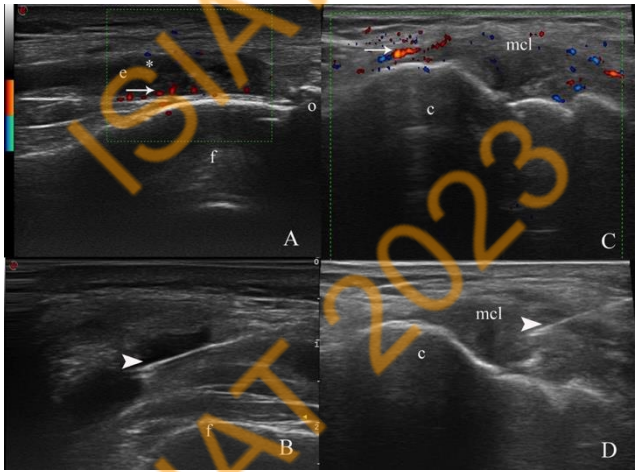
Both L-PRP and P-PRP valid options **Grade B,** scientific presumption

Number of injections **2-4** **Grade B,** scientific presumption

Interval between injections 1-3 wks **Grade B,** scientific presumption

Platelet number, concentration **Grade C,** low scientific level

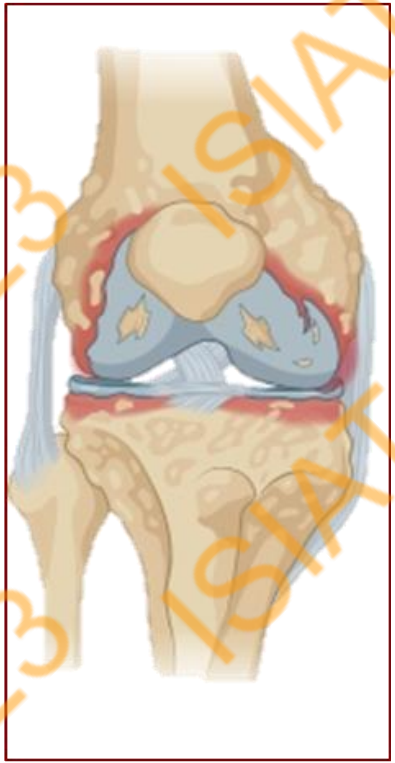
Personalize therapies by refining phenotypes/endotypes



Prominent synovitis
Hoffa involvement
Ligament alterations
Meniscal extrusions

Cartilage/meniscus driven phenotype

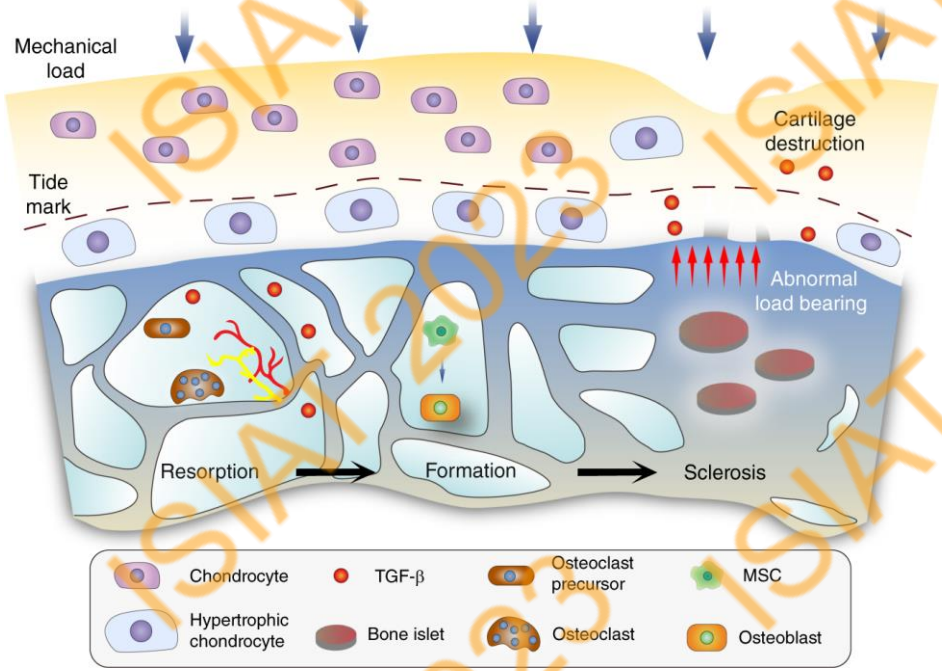
Subchondral bone phenotype

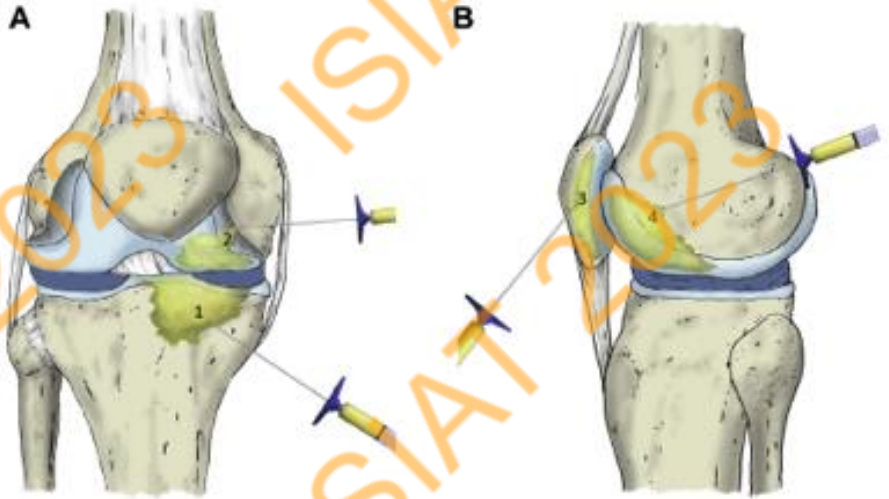


Synovium driven inflammatory phenotype

Need for studies combining biochemical markers with US-based markers

PRP therapies should consider both **procedure** and **product**





Combination of intraosseous and intraarticular injections

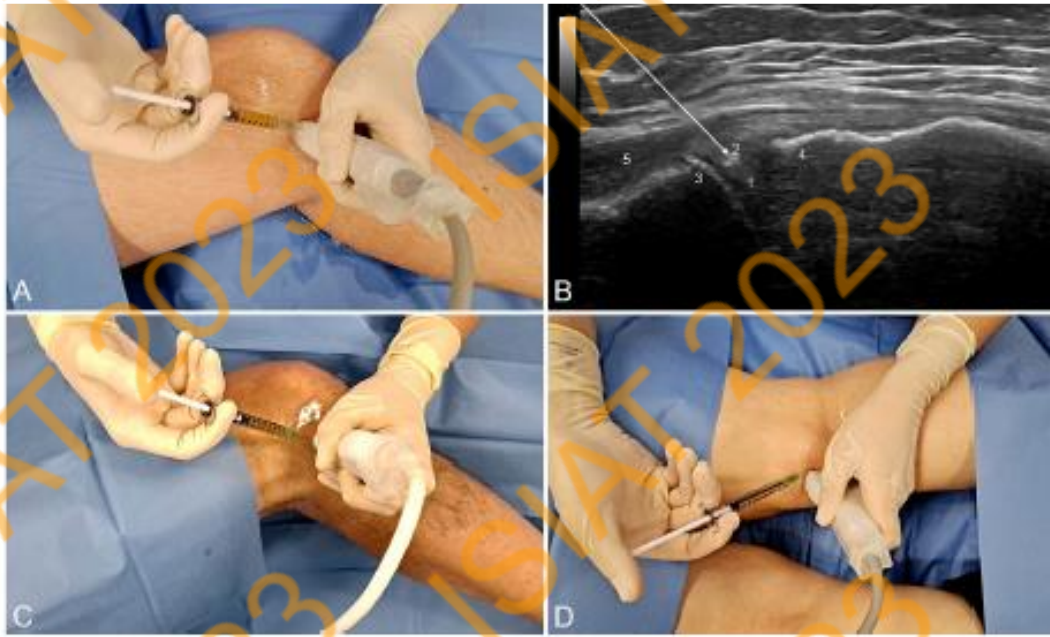
PRP therapies should consider both **procedure** and **product**

Knee Surgery, Sports Traumatology, Arthroscopy (2023) 31:4246–4256
https://doi.org/10.1007/s00167-023-07470-4

KNEE

High survival rate after the combination of intrameniscal and intraarticular infiltrations of platelet-rich plasma as conservative treatment for meniscal lesions

Mikel Sánchez[1,2] · Cristina Jorquera[2] · Ane Miren Bilbao[1] · Saínza García[2] · Maider Beitia[2] · João Espregueira-Mendes[3,4,5,6,7] · Sergio González[1] · Jaime Oraa[1] · Jorge Guadilla[1] · Diego Delgado[2]



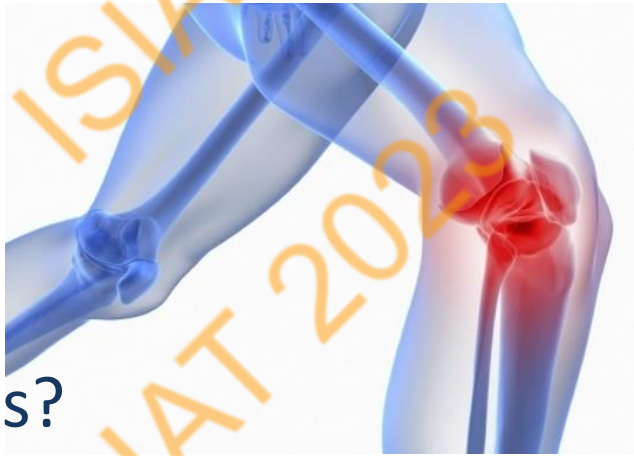
"The combination of **intrameniscal** and **intraarticular** PRP infiltrations is a valid conservative treatment for meniscal injuries avoiding the need for surgical intervention. Its efficacy is higher in **horizontal tears** and decreases when joint degeneration is present"

Combination of intrameniscal and intraarticular injections

PAIN (VEGF paradox)

4rd

OA, **clinical syndrome of joint PAIN** accompanied by varying degrees of **functional limitation**



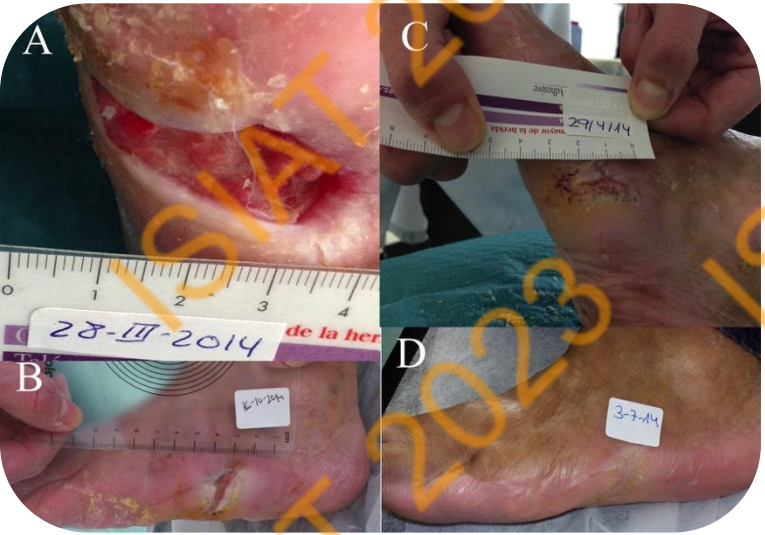
Does PRP interfere with pain mechanisms?

<u>Pain endotypes</u>

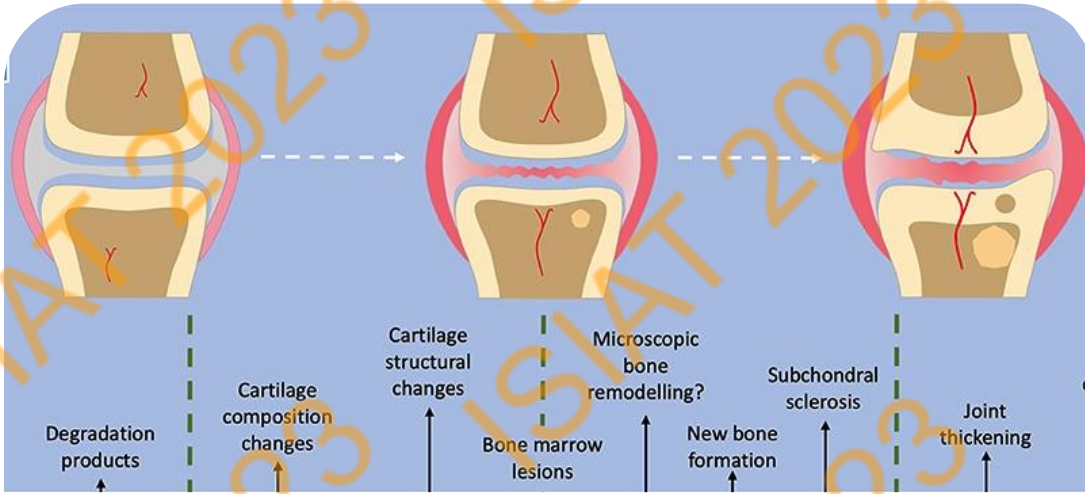
Nociceptive, inflammatory, neuropathic

VEGF paradox

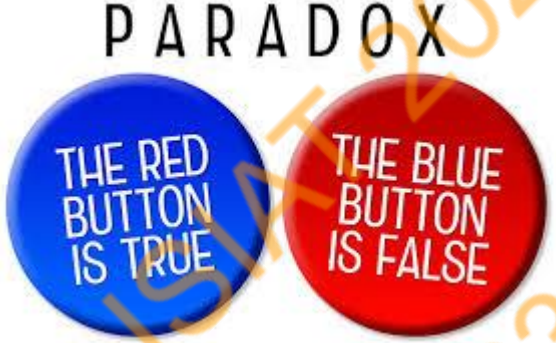
VEGF is a potent proangiogenic factor and a key mediator of neovascularization



Increased VEGF in SF of OA patients correlated with higher pain and OA progression



What accounts for the differences in PRP effects when applied to different conditions?



VEGF paradox

VEGF A is involved in cartilage degeneration and OA pain transmission

Pazopanib (VEGF inhibitor) reduces joint pain and cartilage degeneration

Potential PRP responders

Inflammed synovium

SYNOVITIS, Synovial hypertrophy, Effusion, Doppler

Int. J. Biol. Sci. 2023, Vol. 19 675

IVYSPRING INTERNATIONAL PUBLISHER

International Journal of Biological Sciences

2023; 19(2): 675-690. doi: 10.7150/ijbs.79125

Research Paper

Targeting Vascular Endothelial Growth Factor Receptors as a Therapeutic Strategy for Osteoarthritis and Associated Pain

Kaige Ma[1,2], Gurjit Singh[1], Jun Wang[1], InSug O-Sullivan[1], Gina Votta-Velis[3], Benjamin Bruce[4], Arivarasu Natarajan Anbazhagan[5], Andre J. van Wijnen[1,6], Hee-Jeong Im[1,4]

Received: 26 May 2022 | Revised: 5 May 2023 | Accepted: 26 May 2023

DOI: 10.1111/1756-185X.14781

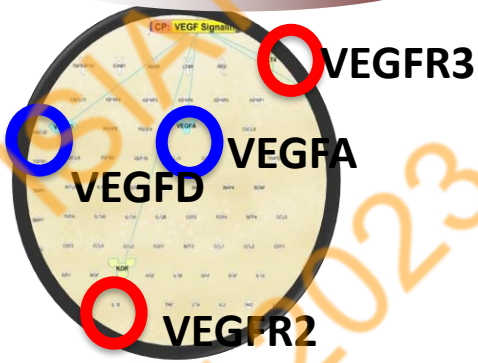
ORIGINAL ARTICLE

International Journal of Rheumatic Diseases WILEY

Inflammatory ultrasound features as prognostic factors of pain and functional outcomes following intra-articular platelet-rich plasma in knee osteoarthritis

Win Min Oo[1,2] | James Linklater[3] | Kim L. Bennell[4] | Shirley P. Yu[1] | Vicky Duong[1] | David J. Hunter[1]

VEGF signaling

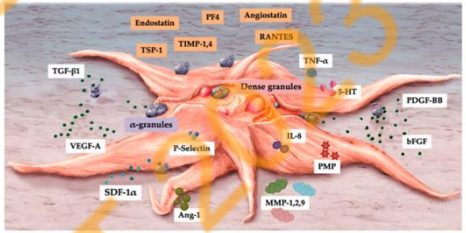


KDR and FLT4
Decoy receptors for VEGF

PRP is a SIGNALING SYSTEM

- **Molecules with opposite effects**
- **Molecular networks** (VEGF signaling, IL signaling)
- **Redundancy, synergisms**

Anti-angiogenic Proteins
Angiotensin, endostatin,
PF4, TSP1



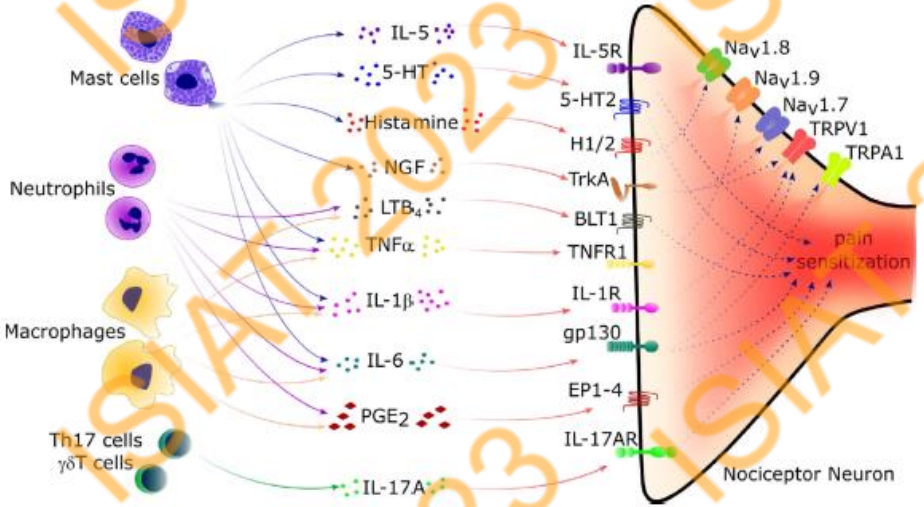
Published in final edited form as:
Biomater Sci. ; 10(9): 2172–2181. doi:10.1039/d1bm01873f.

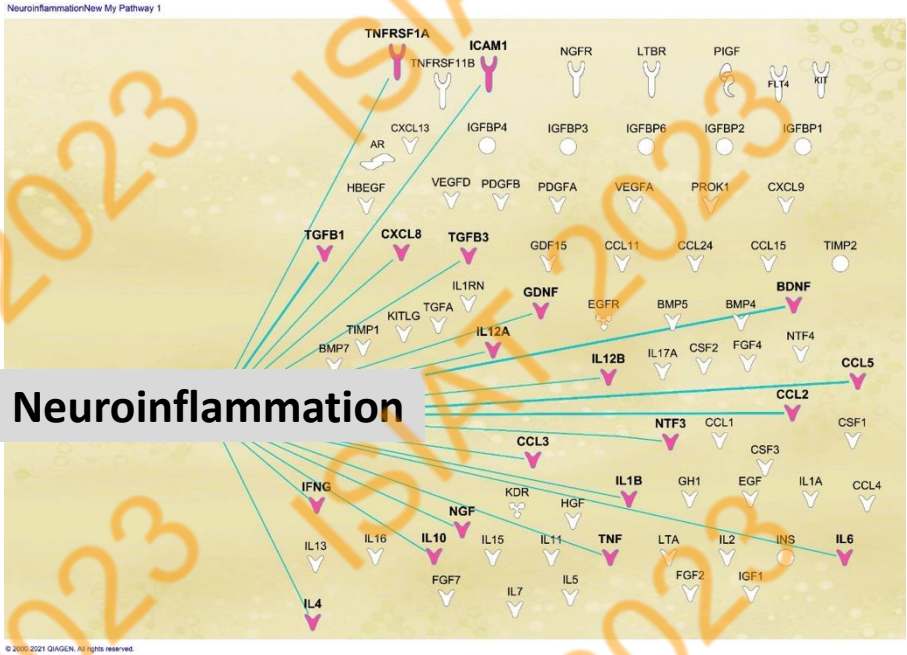
VEGF-attenuated platelet-rich plasma improves therapeutic effect on cartilage repair

Jae Sung Lee[1], Ping Guo[2], Katarina Klett[2], MacGregor Hall[3], Krishna Sinha[3], Sudheer Ravuri[2], Johnny Huard[2], William L. Murphy[1,4,5,*]

Active crosstalk between nociceptor neurons and immune system to regulate pain and inflammation

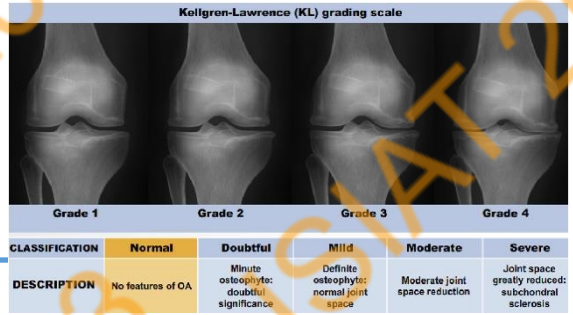
The joints are densely innervated by nociceptors

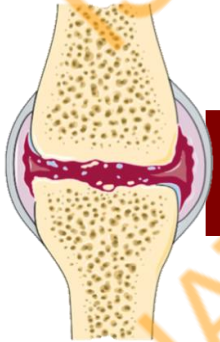




Imaging biomarkers readily available and cheap

Rx

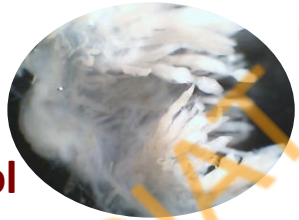


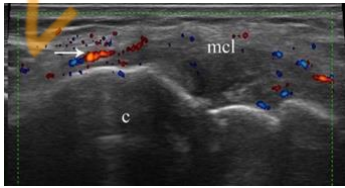


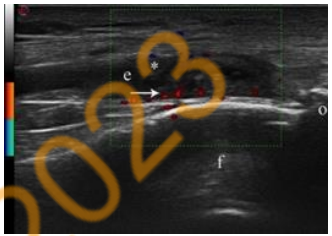
Imaging biomarkers

Synovium changes

OMERACT scanning protocol







Four separate US scans indicative of inflammation

- Synovitis (0-3)
- Hypertrophy (0-1)
- Effusion (0-1)
- Doppler (0-1)

Effect Of Intra-articular PRP Vs Placebo Injection On Pain And Medial Tibial Cartilage Volume

PRP **vs** **placebo**

RESTORE trial

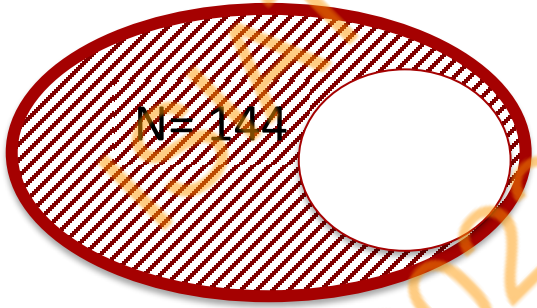
JAMA®
The Journal of the American Medical Association

Conclusions and Relevance

Among patients with symptomatic mild to moderate radiographic knee OA, intra-articular injection of PRP, compared with injection of saline placebo, **did not result in a significant difference in symptoms or joint structure at 12 months**.

These findings do not support use of PRP for the management of knee OA.

Subset of PRP treated patients from RESTORE trial (n= 44/144)



This exploratory cohort study showed that **ultrasound prognostic factors** representative of inflammation (especially **global synovitis**) were significantly **associated with improvements in some pain and functional outcomes** in people with mild to moderately severe knee OA undergoing a series of three weekly intra-articular PRP injections

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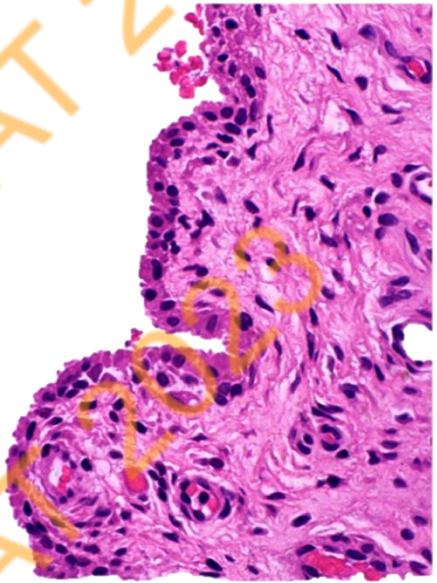
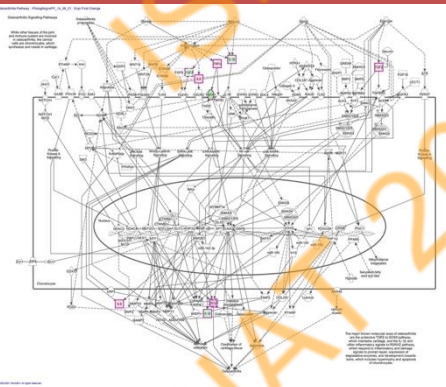


Where we are?

Updating PRPs as new information becomes available

Improving delivery procedures

Identification of “responders”



Knee Surgery, Sports Traumatology, Arthroscopy (2023) 31:4246–4256
https://doi.org/10.1007/s00167-023-07470-4

KNEE

High survival rate after the combination of intrameniscal and intraarticular infiltrations of platelet-rich plasma as conservative treatment for meniscal lesions

Mikel Sánchez[1,2] · Cristina Jorquera[2] · Ane Miren Bilbao[1] · Saínza García[2] · Maider Beitia[2] · João Espregueira-Mendes[3,4,5,6,7] · Sergio González[1] · Jaime Oraa[1] · Jorge Guadilla[1] · Diego Delgado[2]

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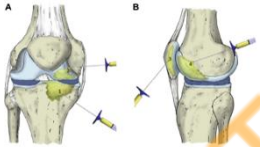

Osteoarthritis and Cartilage Open 4 (2022) 100318
Contents lists available at ScienceDirect
Osteoarthritis and Cartilage Open
journal homepage: www.elsevier.com/journals/osteoarthritis-and-cartilage-open/2665-9131

The role of Platelet-Rich Plasma (PRP) intraarticular injections in restoring articular cartilage of osteoarthritic knees. A systematic review and meta-analysis

Apostolos D. Prodromidis [a,*], Charalambos P. Charalambous [b,c], Emma Moran [a], Ram Venkatesh [a], Hemant Pandit [a,d]

TAKE HOME MESSAGES

US
biomarkers

Potential PRP responders (evaluate the synovium**)**
Inflammed synovium
Synovitis, Synovial hypertrophy, Effusion, Doppler

Tailor PRP intervention

Tailored intervention to joint pathology
Intraarticular PRP
+ intrameniscal
+ intraosseous

Mechanism of action

PRP IMPACTS the knee organ and modulates
The composition of the sinovial fluid
The crosstalk between immune cells
The crosstalk between nociceptors and immune cells

Simple questions... but.... complex answers!!

Q: Is swimming good for your figure?

A: If swimming good for figure, explain whale (balena) to me.