EX VIVO EXPLORATION OF PCRX-201 IN HUMAN KNEE OA TISSUE

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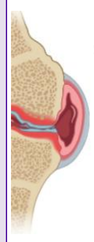
INTRODUCTION



Osteoarthritis (OA) is a degenerative joint disease affecting 595 million people worldwide characterised by progressive **cartilage breakdown, subchondral bone remodelling, and synovial inflammation**, leading to **pain and disability.**

A key driver of **OA pathogenesis** is an imbalance between anabolic and catabolic processes mediated by **interleukin-1 beta (IL-1β)**.

PCRX-201, a high-capacity adenoviral vector encoding **interleukin-1 receptor antagonist (IL-1Ra),** designed to inhibit IL-1 driven cellular pathogenesis (*Figure 1*).

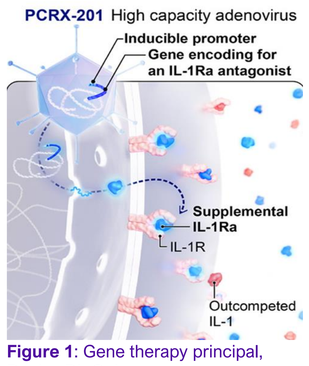


Figure 1: Gene therapy principal, Pacira BioSciences, Inc

This study utilised a physiologically relevant ***ex vivo* human OA knee model to evaluate PCRX-201's** dose response, and molecular effects within the human OA knee model.

EXPERIMENTAL DESIGN

Ex vivo co-culture model was established using human low-grade osteochondral (LGOC), high-grade osteochondral (HGOC) and synovium (S) explants. The explant model represents a ~350-fold reduction in size compared to the human knee joint; therefore, dosing within the *ex vivo* system was scaled to reflect clinically relevant concentrations *(Figure 2*).

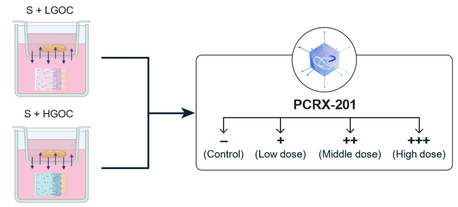


Figure 2: Experimental design

A pre-culture period of 7 days was followed by the induction of gene therapy (PCRX-201) on day 0, samples were cultured for a further 14 days, therefore for 21 days in total prior to assessment of outcome measures (*Figure 3*).

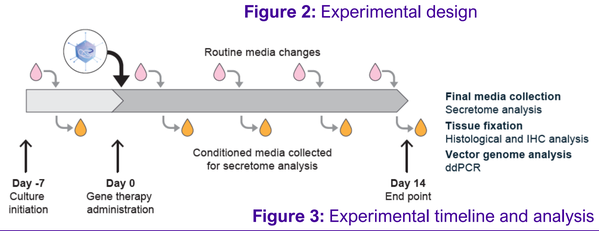


Figure 3: Experimental timeline and analysis

RESULTS

1) PCRX-201 transduction did not compromise cell viability or induce cytotoxicity

Lactate dehydrogenase (LDH) assay showed that PCRX-201 transduction did not compromise cell viability or induce cytotoxicity in the cultured tissues over 21 days of culture period (*Figure 4*).

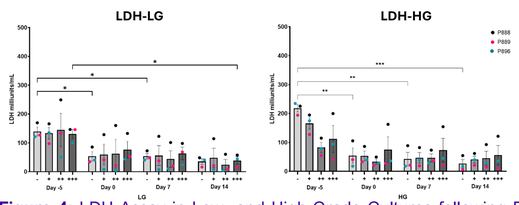


Figure 4: LDH Assay in Low- and High-Grade Cultures following PCRX-201 induction. N=3 patients: P888, P889, P896. *P<0.05, **P<0.01.

Semi-quantification of Caspase-3 staining (*Figure 5*) indicated that cell viability remained intact post-PCRX-201 transduction.

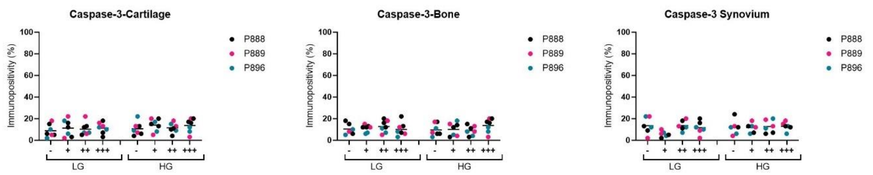


Figure 5: Semi-quantification Caspase-3 immunopositivity in Low- and High-Grade Cultures following PCRX-201 transduction.

2) IL-1Ra expression confirms tissue uptake and retention of PCRX-201 within the model

Immunohistochemical staining of IL-1Ra (*Figure 6*) showed increased positivity within synovium, cartilage and bone tissue with escalating gene therapy dosage, confirmed by semi-quantitative analysis, demonstrating a dose-dependent increase in IL-1Ra expression in low grade synovium and significant increase in higher doses in high-grade cartilage.

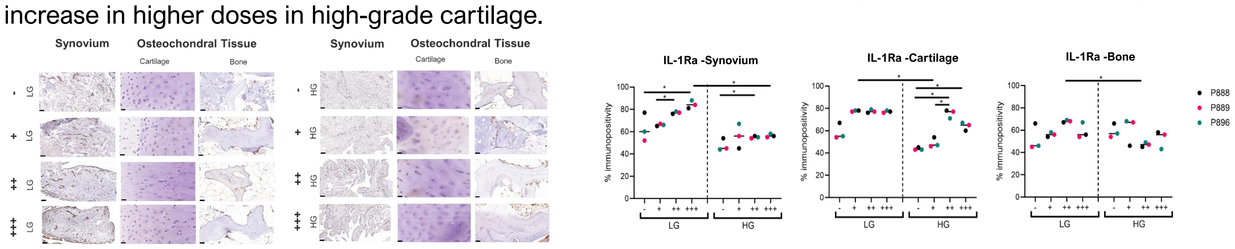


Figure 6: IHC staining of IL-1Ra and semi-quantification of immunopositivity in Low- and High-Grade Cultures following PCRX-201 transduction. *P<0.05.

Cumulative ELISAs highlight a clear dose-dependent increase in cumulative IL-1Ra concentration within media over the 21-day culture period across all treatment groups compared to the control *(Figure 7).*

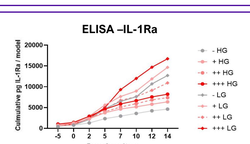


Figure 7: Cumulative IL-1Ra in culture media from Low- and High-Grade cultures.

3) PCRX-201 supressed inflammatory and catabolic markers while enhancing expression of anabolic markers

In low- and high-grade co-cultures, PCRX-201 reduced inflammatory and catabolic cytokine IL-1β in a dose-dependent manner in both cartilage and synovium *(Figure 8).*

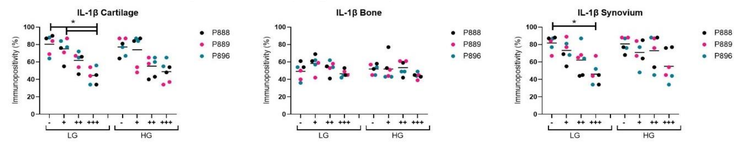


Figure 8: Semi-quantification IL-1β immunopositivity in Low- and High-Grade Cultures following PCRX-201 transduction. *P<0.05

PCRX-201 transduction resulted in reduced immunopositivity of catabolic factors IL-6 and MMP-13. IL-6 decreased in high-grade cartilage at the highest dose and in low-grade synovium at mid and high doses. MMP-13 was reduced in low-grade cartilage and bone at high dose, and in high-grade synovium across all doses. Conversely, immunopositivity of the matrix-associated protein collagen type II increased, with a significant rise observed in high-grade cartilage at the highest dose *(Figure 9).*

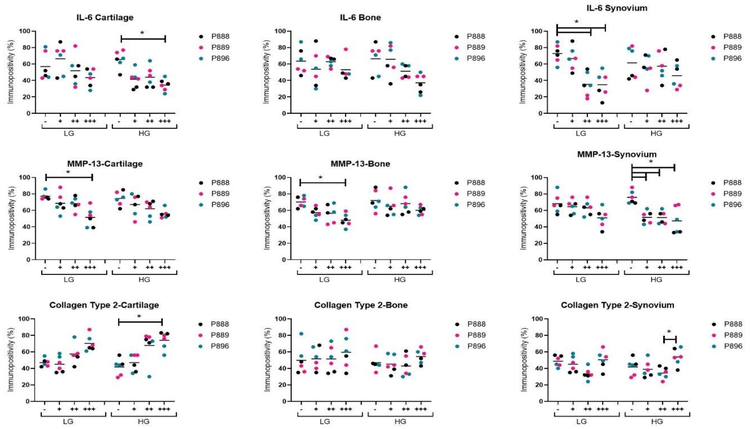


Figure 9: Semi-quantification IL-16, MMP-13 and Collagen type II immunopositivity in Low- and High-Grade Cultures following PCRX-201 transduction. *P<0.05.

4) PCRX-201 showed minimal effect on secretome post-transduction

Following 14 days post-transduction with PCRX-201, limited differential expression of secreted factors was observed using multivariate statistical analysis across 42 proteins *(Figure 10).*

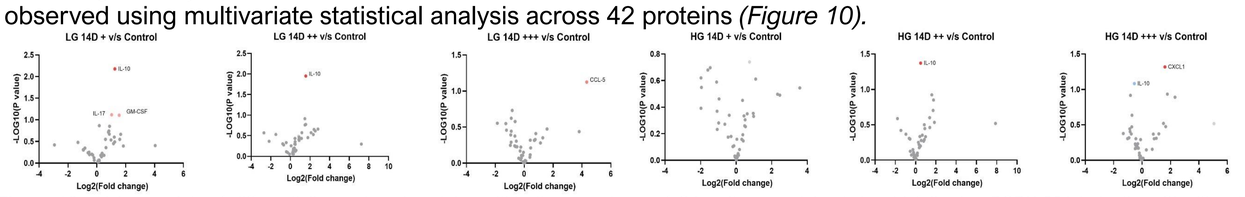


Figure 10 : Volcano plots of differentially produced proteins within the secretome of OA culture models at day 14 following PCRX-201 induction. Significant factors named and coloured (Red = increase; Blue = decrease) Adjusted P value <0.05.

CONCLUSIONS

- Using a **physiologically relevant *ex vivo* human knee OA model**, PCRX-201 gene therapy demonstrated potential to modulate IL-1-driven pathways linked to OA progression.
- Immunohistochemical analysis demonstrated **clear induction of IL-1Ra expression**, decreased immunopositivity for catabolic factors and increased anabolic matrix proteins following transduction with PCRX-201.
- **Secretome analysis demonstrates a highly complex secretome** within OA models with donor specific secretome profiles, and limited significant secretome changes following 14 days post transduction, this was influenced by the large secretomic panel investigated and n=3 donor samples. To further assess changes at the cellular level, intracellular protein expression will be evaluated using proteins lysates.
- *Ex vivo* co-culture system represents **a robust tool to deepen mechanistic understanding of OA pathophysiology, accelerate therapeutic development, and evaluate clinically translatable interventions.**
- Future work involves the vector genome titer of ex vivo knee OA tissues, to determine the transduction efficiency of PCRX-201, longer term studies, and increased donor samples.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST STATEMENT

This work was funded by Pacira Biosciences Inc. DJ and MK are employees of Pacira Biosciences Inc.