

Azimiplacel Modulates Synoviocyte and Chondrocyte Inflammation *in vitro*

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INTRODUCTION: Knee osteoarthritis (OA) is one of the most prevalent musculoskeletal disorders, currently affecting approximately 30 million adults in the United States [1-2]. The global burden of OA continues to rise, with prevalence projected to increase substantially in the coming decades [3]. Although surgical interventions are available, younger patients face a significant lifetime risk of revision [4-5], highlighting the need for nonsurgical therapies. A placental-derived injectable therapy, azimiplacel, is under development with the FDA as a biologic for the treatment of knee OA. This therapy contains a cocktail of anti-inflammatory and regenerative factors [6-7] and has shown promise in both preclinical and clinical settings [8-12]. To elucidate the potential mechanisms of action, two *in vitro* inflammatory models were developed using synoviocytes and chondrocytes, both of which are key contributors to the pathophysiology of OA, to evaluate the effects of azimiplacel on inflammatory signaling.

METHODS: Azimiplacel is an amniotic suspension allograft containing micronized amniotic membrane combined with cells derived from amniotic fluid and is stored at -80°C in a cryopreservation solution. *In vitro* synoviocyte and chondrocyte inflammation models were developed to evaluate the attenuation of inflammation by azimiplacel. Human synovial sarcoma cells and primary knee articular chondrocytes were cultured under cell-specific culture conditions. Prior to assaying, chondrocytes were differentiated for 7-8 days. Both synoviocytes and chondrocytes were treated with assay media (AM) to produce a basal environment or stimulated with inflammatory cytokines (TNF- α and IL-1 β ; INF). For the treatment phase, cells were then cultured with AM alone, INF alone, or INF plus azimiplacel conditioned media. Cell monolayers were collected to assess changes in gene expression and signaling following azimiplacel treatment.

RESULTS: In the *in vitro* synoviocyte inflammation model, INF alone (compared to AM) resulted in a significant upregulation in the gene expression of IL-1 β , IL-6, MMP-3, and TNF- α relative to the basal environment ($p < 0.001$ for all targets). The chondrocyte inflammation model similarly showed significant upregulation of ADAMTS4, COL10A1, and MMP-13 ($p < 0.001$ for all targets). For both synoviocytes and chondrocytes, treatment with azimiplacel led to significant downregulation of these inflammation-stimulated gene targets. Profiler arrays revealed that key pathways affected in both models were related to phospho-kinase and NF- κ B signaling. In synoviocytes and chondrocytes, 75% and 65% of phospho-kinase targets, respectively, were downregulated at least 0.15-fold relative to inflammatory conditions following azimiplacel treatment. Implicated signaling pathways included PI3K/Akt, JAK/STAT, NF- κ B, MAPK, and Wnt/ β -catenin. Of the targets identified, 45% were conserved between the two cell-based inflammation models. Western blots confirmed attenuation of both NF- κ B and GSK-3 β in both inflammation models. Additionally, AKT and MAPK signaling were significantly downregulated in synoviocytes, while STAT1 was significantly downregulated in chondrocytes.

DISCUSSION: Inflammation plays a critical role in knee OA, with multiple signaling pathways implicated and correlated to disease progression. To build on the current evidence supporting azimiplacel as a potential nonsurgical treatment option for OA, two *in vitro* inflammatory models were developed to investigate the potential mechanisms of action. In both models, azimiplacel treatment resulted in reduced expression of inflammatory and catabolic genes. As a complex product containing hundreds of proteins, azimiplacel attenuated key OA-related signals in both synoviocytes and chondrocytes, notably phospho-kinases and NF- κ B pathways.

SIGNIFICANCE/CLINICAL RELEVANCE: Crosstalk between synoviocytes and chondrocytes plays a key role in the regulation of inflammation. In OA, this positive feedback loop is formed by inflammatory and tissue breakdown products resulting in paracrine and autocrine signaling creating a positive feedback loop. The conserved downregulation of inflammation by azimiplacel was demonstrated in two primary cell types involved in knee OA, with consistency among inflammatory signals impacted. These studies further support the utility of azimiplacel as a promising nonsurgical treatment option of OA.

REFERENCES:[1] Zhao, X, 2019. [2] Dell'Isola, A, 2025. [3] Hootman, JM, 2006. [4] Price, AJ, 2018. [5] Parvizi, J, 2014. [6] Goldring, MB, 2000. [7] McQuilling, JP, 2017. [8] Gomoll, AH, 2021. [9] Willett, NJ, 2014. [10] Kimmerling, KA, 2019. [11] Kimmerling, KA, 2022, [12] Harmon, KA, 2024.

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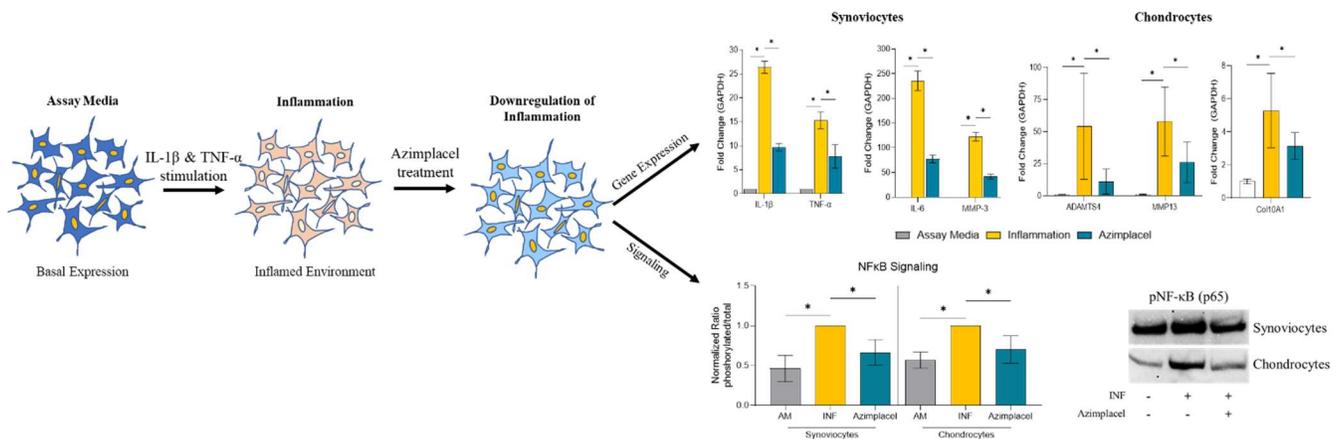


Figure 1. Synoviocyte and chondrocyte inflammatory models result in the downregulation of key-OA gene expression targets and NF- κ B signaling.