Title: Azimplacel, intended to treat pain associated with knee osteoarthritis, retains anti-inflammatory properties of amniotic tissues.

Author Names: Miranda Burnette PhD, Kelly Kimmerling PhD, MaryRose Kammer MS, and Katie Mowry PhD

Background: Amniotic tissues have a long history of use in tissue-based therapies due to their unique properties. Azimplacel is an amniotic suspension allograft (ASA) comprised of cells derived from amniotic fluid and milled amniotic membrane from the same donor and stored at -80°C in a cryopreservation solution. After more than a decade on the market regulated by FDA as a human cell, tissue, or cellular and tissue-based product (HCT/P) under Section 361 of the Public Health Service Act (PHS Act), azimplacel is now under development as an Investigational New Drug (IND) for the treatment of pain associated with knee osteoarthritis in preparation for submission of a Biologics License Application (BLA). Azimplacel is hypothesized to function by downregulating inflammation through the protein contributed from the allogenic amniotic cells and tissue.

Hypothesis: The native anti-inflammatory properties of amniotic tissues are maintained in azimplacel, enabling it to potently downregulate inflammation in the osteoarthritic knee.

Methods: The potency of azimplacel in downregulating inflammation was assessed using various analytic, *in vitro*, and pre-clinical models relevant to knee osteoarthritis. Specifically, the anti-inflammatory content of azimplacel was measured using a proteomic array and confirmed via ELISA. A synovial model of inflammation was used to assay the impact of azimplacel on the inflamed environment using gene expression and protein content assays. Finally, three animal models ranging in osteoarthritis severity, including one chemical and two mechanical models, were used to assess the effects of azimplacel on pain, function, and synovial fluid content.

Results: Azimplacel was demonstrated to retain a variety of anti-inflammatory proteins that confer high potency in downregulating inflammatory-associated phenotypes in multiple models. Specifically, azimplacel contains high levels of IL-1Ra, an anti-inflammatory protein that competitively inhibits inflammatory IL-1β signaling, and TIMPs, which inhibit tissue degradation and inflammation caused by metalloproteinases. The functional activity of these proteins was supported by the synoviocyte model, where azimplacel treatment led to diminished gene expression and protein levels of inflammatory proteins such as TNF-α, IL-1β, and MMPs. Finally, in animal models, azimplacel treatment resulted in decreased pain, increased function, and increased levels of anti-inflammatory IL-10 in synovial fluid samples.

Conclusions: Together, these results are supportive of the proposed mechanism of action of azimplacel in downregulating inflammation to treat pain associated with knee osteoarthritis.

Azimplacel, intended to treat pain associated with knee osteoarthritis, retains anti-inflammatory properties of amniotic tissues

Miranda Burnette PhD[1], Kelly Kimmerling PhD[1], **MaryRose Kammer MS**[1], and Katie Mowry PhD[1]
[1]Organogenesis Discovery Center, Birmingham, AL

BACKGROUND

Amniotic tissues have a long history of use in tissue-based therapies due to their unique properties. Azimplacel is an amniotic suspension allograft (ASA) comprised of cells derived from amniotic fluid and milled amniotic membrane from the same donor and stored at -80°C in a cryopreservation solution. After more than a decade on the market regulated by FDA as a human cell, tissue, or cellular and tissue-based product (HCT/P) under Section 361 of the Public Health Service Act (PHS Act), azimplacel is now under development as an Investigational New Drug (IND) for the treatment of pain associated with knee osteoarthritis in preparation for submission of a Biologics License Application (BLA). Azimplacel is hypothesized to function by downregulating inflammation through the protein contributed from the allogenic amniotic cells and tissue.

METHODS

- Azimplacel is manufactured from micronized amniotic membrane and amniotic fluid cells.
- Anti-inflammatory content of azimplacel, and the contribution of both the amniotic membrane and fluid components were quantified using single-target ELISAs.
- A synovial model of inflammation was used to assess the impact of azimplacel on the inflamed environment using gene expression (PCR) and protein content (ELISA) assays.
- The effects of azimplacel on pain, function, and synovial fluid content were evaluated in three animal models of osteoarthritis: monosodium iodoacetate (MIA), medial meniscal tear (MMT), and medial meniscal destabilization (DMM).
- Clinical impact of azimplacel was evaluated in a randomized controlled trial.

AZIMPLACEL MODULATES INFLAMMATION *IN VITRO*

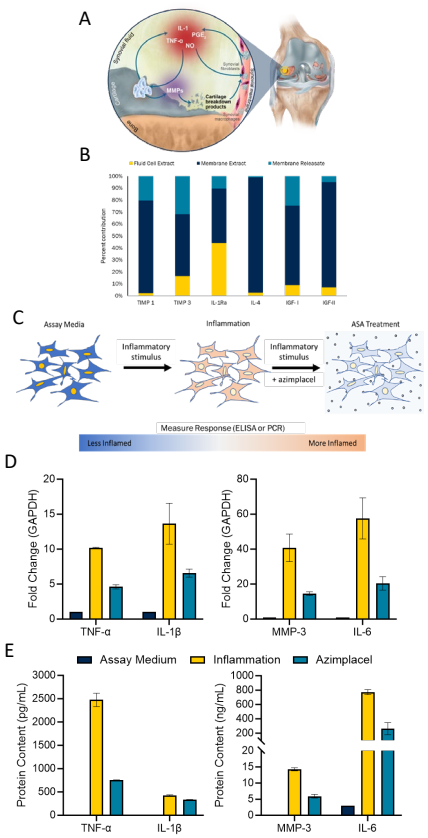

Figure 2: Overview of the role of inflammation in knee osteoarthritis (OA) (A). Both amniotic membrane and amniotic fluid cells contribute the to anti-inflammatory, anti-catabolic protein load (B). Overview of synovial inflammation model of OA (C). Down-regulation in gene expression (D) and protein content (E) of inflammatory and catabolic markers relevant to OA *in vitro* in response to treatment with azimplacel.

AZIMPLACEL IS AN AMNIOTIC SUSPENSION ALLOGRAFT

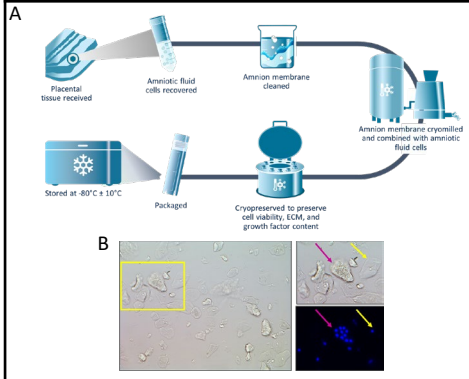

Figure 1: Overview of azimplacel manufacturing process (A). Azimplacel under the microscope (B). Pink arrows identify amniotic membrane and yellow arrows identify amniotic fluid cells. DAPI staining of nuclei (bottom right).

ASA IMPROVES PAIN AND FUNCTION IN RAT MODELS OF OA[1,2]

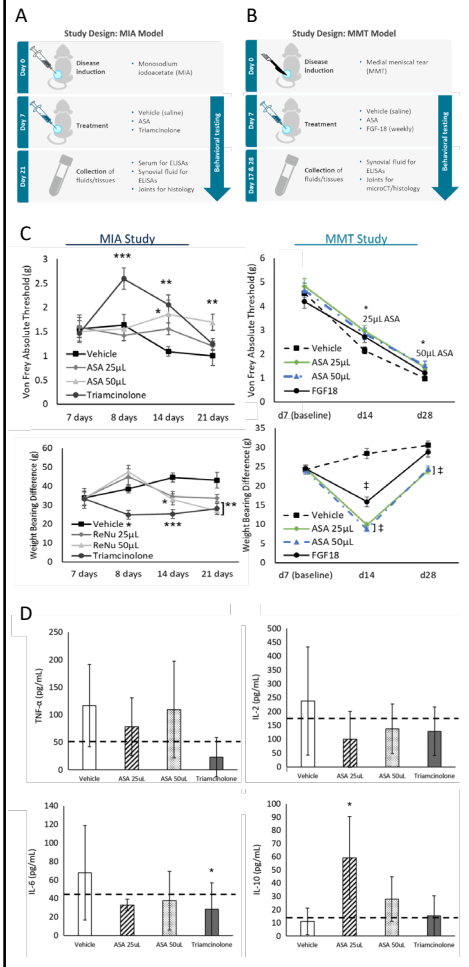

Figure 3: Overview of evaluation of azimplacel in monosodium iodoacetate (MIA, A) and medial meniscal tear (MMT, B) rat models of OA. Azimplacel resulted in decreased pain and increased function in rat models of OA (C). Azimplacel resulted in significant increases in anti-inflammatory IL-10 and decreases in IL-6 (not significant) in synovial fluid (D).

MULTIPLE INJECTIONS OF ASA IMPROVE PAIN AND FUNCITON IN A RAT MODEL OF OA[3]

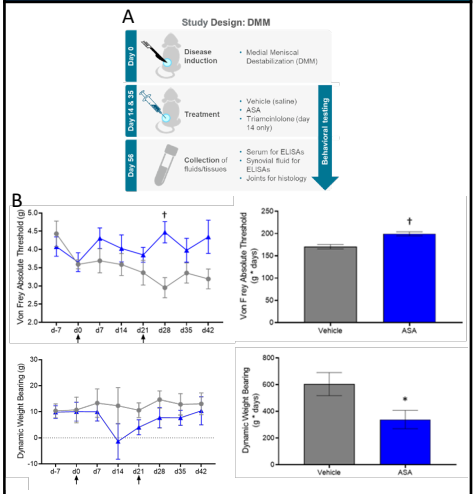

Figure 4: Overview of multiple injection study in a rat medial meniscal destabilization model of OA (A). Multiple injections of azimplacel result in improved pain and function (B).

AZIMPLACEL IMPROVES OUTCOME SCORES CLINICALLY[4]

Figure 5: Overview of clinical study performed with ASA during prior commercial experience (A). ASA improved patient reported outcome scores for 12 months as evaluated by KOOS pain (B) and KOOS ADL (C). Top line results of reduction in pain and maintenance of function in phase 3 clinical trial (D).

CONCLUSIONS

- Azimplacel contains key regulatory proteins, including anti-inflammatory IL-1Ra, which is contributed by both the amniotic membrane and amniotic fluid components.
- In an *in vitro* synoviocyte inflammation model of OA azimplacel reduced inflammatory and catabolic gene expression and protein production.
- In animal models of OA, azimplacel treatment resulted in decreased pain, increased function, and increased levels of anti-inflammatory proteins in synovial fluid.
- Prior clinical experience with ASA resulted in improved pain and functional scores durable out to 12 months.
- Phase 3 topline data showed statistically significant improvements in pain and maintenance of function[5].
- Collectively these results support the proposed mechanism of action of azimplacel downregulating the inflammatory cascade associated with knee OA.

ACKNOWLEDGEMENTS

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