Controlled baricitinib release from hydrogels through reversible thioimidate bonds to treat autoimmune skin diseases

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Statement of Purpose: Janus kinase (JAK) inhibitors have tremendous potential to treat autoimmune skin diseases but are met by significant systemic toxicities including malignancy, infections, and cardiovascular events.[1] To overcome these limitations, we developed an injectable hydrogel system for local and sustained release of baricitinib, an FDA-approved JAK1/2 inhibitor. Baricitinib forms a reversible thioimidate adduct between an aliphatic nitrile and thiolated hyaluronic acid, leading to conjugation. Thiols then form disulfides, which crosslinks the polymer into a hydrogel. Towards clinical translation, we tested baricitinib hydrogels in three models of autoimmune skin disease that converse on JAK signaling: psoriasis, alopecia areata, and hidradenitis suppurativa. Compared to controls, intradermally injected baricitinib hydrogels led to improvements in mouse models of both psoriasis and alopecia areata and reduced secretion of inflammatory cytokines in cultured *ex vivo* human hidradenitis skin.

Methods: *Materials:* Thiolated HA was synthesized through an amidation between HA and cysteamine and commercially available for purchase.[2] *Thioimidate formation:* Reactions between cysteamine and baricitinib or thiolated HA and baricitinib were quantified through 1H NMR, 13C NMR, and spectroscopy. *In vitro release and bioactivity:* Hydrogels were formed and releasates were collected and replaced. Absorbance at 300 nm was used to quantify baricitinib release. Releasates were added recombinant interferon-stimulated HEK293 cells with the luciferase gene stably integrated control of the interferon-stimulated response element. Luminescence was used to measure JAK activity. *In vivo psoriasis model:* Hydrogels were injected intradermally into C57BL/6 mice followed by five days of topical imiquimod. Immunohistochemistry and immunofluorescence were performed at seven days. *In vivo alopecia areata model:* Hydrogels were injected intradermally into C3H/HeJ mice at the time of grafting; ventral hair loss was followed by photography for three months. *Ex vivo hidradenitis model:* Explanted human hidradenitis skin from the dermatology clinic was injected and cultured in a transwell; cytokines were analyzed after two weeks using the Luminex 18-plex kit.[2]

Results:
Baricitinib formed thioimidates with cysteamine, leading to a distinct peak at 170 ppm on ^{13}C NMR and downfield shifts on ^{1}H NMR. Following conjugation of cysteamine to hyaluronic acid, baricitinib also consumed thiols on thiolated HA in a dose-dependent fashion (not shown).[1] Hydrogels led to tunable release of baricitinib with ~60-80% released at two weeks. When added to interferon-stimulated HEK293 cells, releasates led to significant attenuation of JAK activity as measured by luminescence, which could be observed as early as two days and as late as six weeks. In the C57BL/6 imiquimod mouse model of psoriasis, intradermal injections led to marked reductions in epidermal thickness and pSTAT3 expression (downstream of JAK), with reductions in scale and erythema by one week. In our C3H/HeJ mouse model of alopecia areata, alopecia was established through skin grafting. Following grafting, baricitinib hydrogels were injected intradermally. Controls receiving hydrogel or baricitinib alone exhibited rapid hair loss over three months, while baricitinib hydrogels had significantly attenuated hair loss.

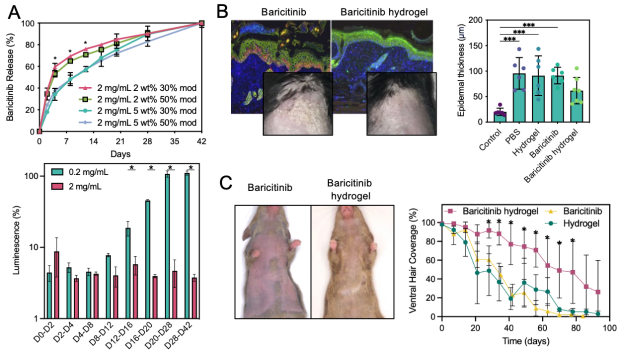

Figure 1. A) Tunable release of baricitinib (0.2 or 2 mg/mL) from hydrogels (2 wt%) measured by absorbance at 300 nm followed by addition of releasates (5 μL) to IFN-stimulated HEK293 cells, leading to decreased luminescence at all timepoints tested. B) Psoriasis model following imiquimod; intradermal injections (0.2 mg/mL, 4 x 25 μl) regulate epdermal thickness (green) and pSTAT3 expression (red), and scaling and erythema grossly (inset) at seven days. ***p<0.001. C) Alopecia areata model wherein grafting induces progressive alopecia; intradermal injections (2 mg/mL, 4 x 25 μl) control ventral hair loss progressing over three months. *p<0.05.

To validate our approach in humans, we sought to test our hydrogel in an inflammatory skin disease, hidradenitis suppurativa. Following clinical deroofing procedures, human hidradenitis skin was injected with baricitinib hydrogel followed by *ex vivo* culture for two weeks. Baricitinib hydrogels led to significant reductions in IL-2 and IL-23 over the time periods measured, with notable decreases of IL-17, TNF-α. These inflammatory biomarkers may reflect therapeutic potential clinically.

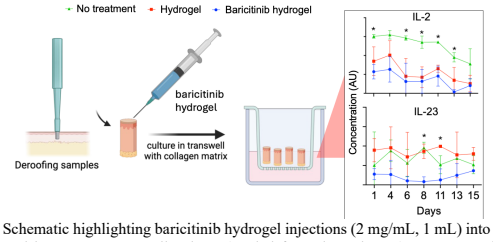

Figure 2. Schematic highlighting baricitinib hydrogel injections (2 mg/mL, 1 mL) into human HS skin followed by *ex vivo* transwell culture (pooled from three donors). Serum cytokines were measured for two weeks by Luminex® cytokine assay. Studies were performed under an approved IRB protocol; *p<0.05.

Conclusions: We have developed an injectable baricitinib hydrogel to treat autoimmune skin diseases, leveraging a novel reversible thioimidate chemistry. This controlled release approach leads to significant attenuation of psoriasis and alopecia areata in mice, while regulating secreted cytokines in human hidradenitis. With completion of preclinical studies including toxicology in pigs as well as a pre-IND meeting, we are pursuing an IND following the 505(b)(2) pathway.

References:
1. Wang et al. *Advanced Healthcare Materials*, 2024;13(12):e2303256.
2. Goliwas et al. *Inflammation*, 2022; 45(3):1388-1401.