Title: Case Study in Nested Knowledge: Researching Biological Mechanisms of Neurodegeneration

Sub-title text: *This case study shows a full Systematic Literature Review project completed in the Nested Knowledge software on the role of FLAP Inhibition and leukotriene signaling in neuroinflammation and neurodegeneration.*

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Neurodegenerative Diseases: patient burden and current state

Neurodegenerative diseases are among the most prevalent and intractable disorders facing both patients and the healthcare system. Researchers estimate that over 50 million people worldwide suffer from a neurodegenerative disease, and this is expected to double in the next 30 years as the population ages. Furthermore, many of these disease states–notably Alzheimer's and ALS–have few therapeutic options, likely due to an incomplete understanding of the pathophysiology of neurodegeneration.

Recent research has shown that neuroinflammation is essential to the pathogenesis of many neurodegenerative diseases, including Alzheimer's. However, despite many theories and billions spent (including over $42 billion on Alzheimer's alone), the specific biological mechanisms underlying this neuroinflammation and resultant neurodegeneration remain opaque. In effect, neurodegeneration remains one of the most pressing areas for expanding both understanding and therapeutic options.

Leukotriene biosynthesis: neuroinflammatory pathways

Bioscience Pharma is a company dedicated to both understanding and treating neurodegeneration. Notably, founder and Chief Scientific Officer Dr. Kim Heidenreich (a neuroscientist with over 30 years of research experience) has led research into leukotrienes, a key inflammatory mediator, as well as methods to prevent and respond to neuroinflammation. Dr. Heidenreich's discovery that FLAP Inhibitors may be efficacious in blocking leukotriene production has shown promise in several disease areas, and Dr. Heidenreich's existing research has demonstrated that novel FLAP Inhibitors block the synthesis of pro-inflammatory

leukotrienes in the brain, as well as reducing levels of activated microglia and reactive astrocytes.

To put all the pieces together, the neuroinflammatory pathway is mediated by leukotrienes, and inhibition of leukotriene production reduced inflammation as shown by reduction in microglia and astrocytes. FLAP Inhibitors, several of which have been demonstrated to be safe in studies of asthma, may therefore present an opportunity for treatment of neurodegeneration, if they can demonstrate both effective blocking of leukotriene production, and if this inhibition can reduce subsequent neuroinflammation.

Case Study: Reviewing Neuroinflammatory pathways and FLAP Inhibition

While further research on novel FLAP Inhibitors is underway, the existing literature on these well-researched drugs contains a great deal of information that can assist with the understanding of both the pathways leading to neurodegeneration–especially inflammatory pathways–and the current state of the art on FLAP Inhibitors.

Nested Knowledge, a leading software company for AI-assisted systematic literature review, whose software has been widely adopted for clinical, epidemiological, and economic studies, engaged to address the demands of this research use case—an expansion into a uniquely challenging discipline—collating evidence on biological mechanisms. A dedicated research team set out to collate all relevant evidence on both neuroinflammation and FLAP Inhibitors in two related reviews. In this Case Study, we present both the performance and outputs of these Nested Knowledge reviews of the biological mechanisms underlying neurodegeneration.

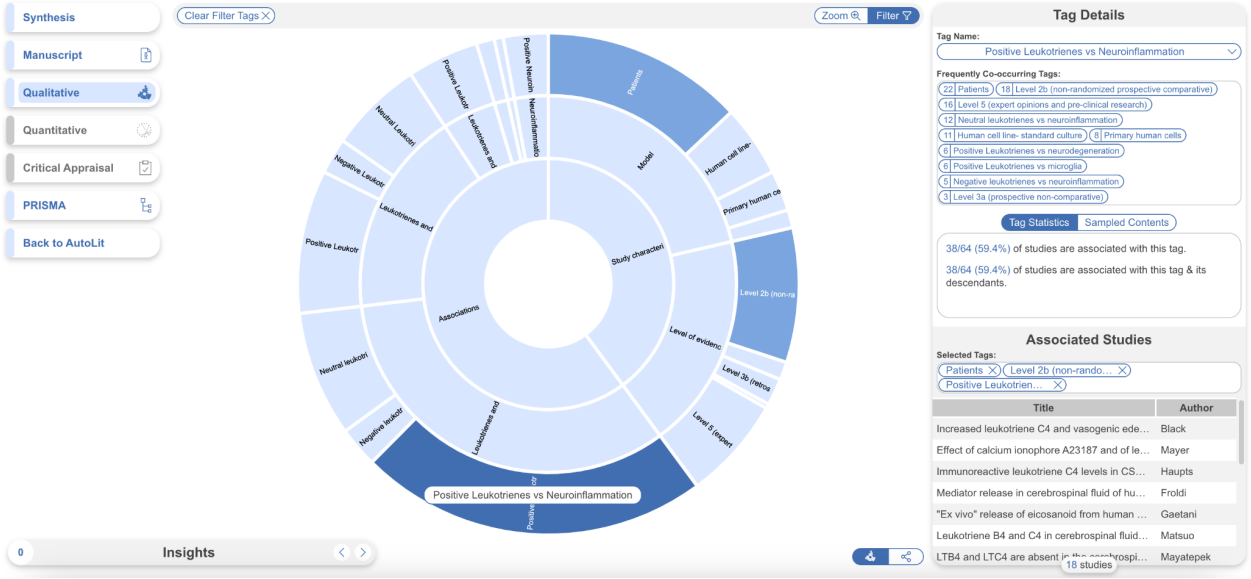
Methods of Literature Review

In brief, an updatable (living) search strategy was created, focusing on (1) **Leukotrienes and Neuroinflammation:** clinical and pre-clinical studies reporting leukotriene signaling and microglia, neuroinflammation, or neurodegeneration, and (2) **FLAP Inhibitors:** preclinical and clinical studies investigating the therapeutic efficacy of 5-lipoxygenase activating protein (FLAP) inhibitors in the treatment of neurodegenerative diseases.

All relevant records were screened and extracted in the Nested Knowledge AutoLit platform, and study characteristics, patient characteristics (clinical studies), model used (pre-clinical studies), and key outcomes and findings were extracted and visualized.

Leukotrienes: Upstream of microglia, astrocytes, and inflammatory markers

Of 1,314 search results, 64 studies were found that addressed associations between leukotrienes, microglia, astrocytes, and neuroinflammation (or neurodegeneration): 23 preclinical studies (cell lines or cadaver), one case series, six retrospective, and 34 prospective studies (all but four of which were comparative). To summarize, the association between leukotrienes and downstream markers of neuroinflammation were coded as ‘positive’, ‘negative’, or ‘neutral’; summary of these associations were automatically visualized in the Qualitative Synthesis (available here; see figure below).



The associations are also summarized here:

Association	Positive (studies)	Neutral (studies)	Negative (studies)	Mixed positive and negative (studies)
Leukotriene signaling with microglia	**6** Citations	0	0	0
Leukotriene signaling with neuroinflammation	**33** Citations	4 Citations	1 Citations	5 Citations
Leukotriene signaling with neurodegeneration	**21** Citations	3 Citations	3 Citations	1 Citations
Microglia with neuroinflammation	**2** Citations	0	0	0
Microglia with neurodegeneration	**2** Citations	0	0	0

Neuroinflammation with neurodegeneration	**4** Citations	0	0	0

The trends were clear at the high level, and when details were examined, Leukotriene signaling was found to correlate with microglial activation or infiltration in Alzheimer's, brain tumors, and a cell culture model of microglia and neuronal cells. While some negative associations were found with neuroinflammation, the vast majority of evidence supported a link between Leukotriene signaling and neuroinflammation, and the 'mixed' studies posited that feedback loops and the presence of high drug concentrations or other immune modulators may impact findings.

Leukotriene signaling was also strongly associated with neurodegeneration, though the studies that differed from this main finding indicate that leukotriene signaling may function differently in different neurodegenerative diseases, such as familial ALS. Neuroinflammation was also found to be directly associated with neurodegeneration, not only through microglia but also inflammatory cytokines, other innate immune cells, B cells, and T cells.

The main finding of this review was that the majority of evidence links Leukotriene signaling not only with microglia and neuroinflammation, but also directly with neurodegeneration, though with different responses in certain neurodegenerative diseases. This should be interpreted with caution– not only were several negative studies found (implicating other immune and drug responses as well as potential feedback loops), but the wide-ranging topics of the review also mean that topical studies may not have been found in all cases. Furthermore, the qualitative nature of the extraction and range of study methods meant that no statistical analysis could be performed. Nevertheless, the available evidence shows that Leukotriene signaling may be an upstream inflammatory mediator and viable target to reduce neuroinflammation and resultant neurodegenerative diseases.

FLAP Inhibitors: Preclinical and Clinical Evidence

Of 1,316 search results, 17 were found that tested FLAP Inhibitors (one randomized controlled trial and 16 comparative animal studies). Of these animal studies, 15 were murine and one was in guinea pigs; while the plurality of studies addressed traumatic brain injury, one addressed multiple sclerosis, one addressed Parkinson's disease, and four addressed Alzheimer's disease.

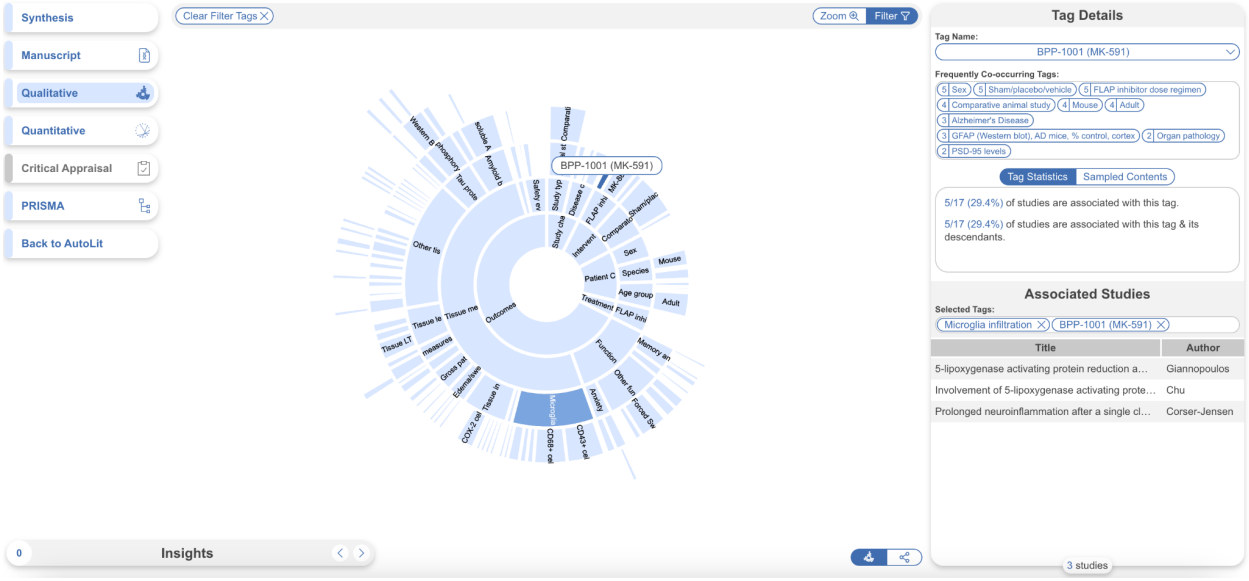
The randomized controlled trial performed did not address neurodegenerative diseases and was thus included solely for safety data.

To summarize efficacy, the results from animal studies were coded as ‘positive’, ‘negative’, or ‘neutral’, across studies of memory and motor function as well as tissue and blood studies, as shown here:

Metric	Positive (studies)	No effect (studies)	Negative (studies)	Mixed positive and negative (studies)
Memory	**2**	0	0	0
Motor	**2**	1	0	0
Other functional tests	1	0	1	0
Tissue	13	6	0	0
Blood	0	3	0	0

Notably, **all memory tests**– water mazes, fear conditioning, field excitatory postsynaptic potentials, and radial water mazes– except paired-pulse ratios were positive (paired-pulse ratios had no significant results). Related functional tests were positive only with sustained doses of FLAP Inhibitors, while one study found negative results in old mice (but no significant results in adult mice). Interestingly, one forced-swim-away found increased climbing behaviors in the FLAP Inhibitor group, which is similar to what has been found for antidepressants.

Qualitative findings were automatically visualized in Qualitative Synthesis (see figure below).



Tissue metrics were inconsistently reported (two studies reported tau protein metrics, two studies reported amyloid β metrics, and seven studies reported other protein expression metrics; five studies also reported tissue leukotriene metrics), but where found, generally indicated either positive or no effect of FLAP Inhibitors.

Summary of tissue findings: Both studies reporting tau metrics found that while treatment with a FLAP Inhibitor decreased tau phosphorylation, it had no significant effect on the level of insoluble tau. Both studies reporting amyloid β metrics found that treatment with a FLAP Inhibitor decreased all forms of amyloid β. Seven studies of GFAP (astrocyte marker), CD11b (microglia marker), IL-1β, TNF-α, IL-6, and over 20 other markers associated with neuroinflammation and/or neurodegeneration all found positive impact of FLAP Inhibitors.

With respect to astrocytes and microglia: Significant reduction in GFAP (a marker of astrocyte activity) levels were observed after treatment with FLAP inhibitors relative to disease state for the Alzheimer's model in one study, but no difference was found in the multiple sclerosis model. Treatment with a FLAP inhibitor was found to reduce microglia activation in two Alzheimer's studies, multiple sclerosis models, and TBI models.

In all measured cases, treatment with FLAP inhibitors was found to reduce leukotriene levels relative to the disease state, and in nearly all cases, treatment with a FLAP inhibitor significantly reduced the neuroinflammatory response in neurodegenerative disease models.

With respect to safety: No drug related side effects were reported for the animal studies and no significant differences in weight, feeding behavior, blood pressure, or heart rate were observed between treatment and vehicle groups. In the randomized controlled trial of FLAP inhibitor use in humans, numerous side effects were reported including lack of response, decreased hematocrit level, abdominal pain and/or diarrhea, malignant neoplasm of the brain, worsened ulcerative colitis, headache, abdominal pain, upper respiratory infection, nausea, rash, flatulence, dizziness, weight loss, vomiting, and sinusitis. However, of all reported safety events, only a dose-dependent rash was believed to be drug related.

Notably, the extremely diverse nature of these studies prevented quantitative analysis beyond basic summarization; the different methods, models, and markers or behavioral outcomes reported –not to mention the range of disease states being researched– is a major limitation of this review, and the results should be interpreted cautiously. Furthermore, the dearth of clinical evidence confirming the animal findings means that caution must be used in translating any pre-clinical finding to a clinical context.

In summary, FLAP Inhibitors were found to positively impact memory, motor, and function in the majority of murine models. While FLAP Inhibitors did not impact the level of insoluble tau, they reduced leukotriene levels in all measured cases, as well as astrocyte markers, microglia markers, and the majority of inflammatory markers. While a wide range of safety events impacted patients in the only randomized trial of FLAP Inhibitors, only rash was found to be drug-related, and no negative side effects were reported in animal studies. Therefore, FLAP Inhibitors were believed to have promise in both improving function and decreasing key inflammatory markers in the majority of preclinical studies; accordingly, early safety was demonstrated in a clinical study outside of neurodegeneration. Further study of FLAP Inhibition in a clinical context is recommended to confirm safety, efficacy, and the impact on key biomarkers.

Case Study Findings

In this use of the Nested Knowledge AutoLit software, evidence ranging from preclinical studies of cell lines to clinical studies of novel drugs were synthesized and interpreted, with the links along the chain of causes and mediators of neuroinflammation demonstrated across the available evidence. The findings from this effort are dual: First, the technology itself is flexible enough to take on complex biological questions and reduce complex mechanisms to the associations of each mediator in the pathway. Secondly, the reviews showed not only a consensus of studies linking leukotrienes to microglia and neuroinflammation, but also direct links between leukotrienes and certain neurodegenerative diseases, as well as the promise of FLAP Inhibitors in several neurodegenerative diseases, especially Alzheimer's. While these findings are preliminary and must be confirmed with clinical studies of sufficient power, they show the need for further examination of the leukotriene-neuroinflammation association and the safety and efficacy of FLAP Inhibitors for treating neurodegeneration.

Full reports available on request to:

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