Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Apilimod

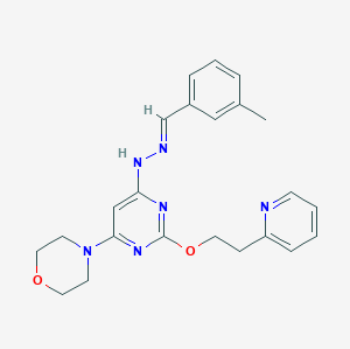
Evidence Summary

Apilimod failed to improve Crohn's disease or rheumatoid arthritis. A clinical trial is ongoing for non-Hodgkin's lymphoma. Systemic PIKfyve inhibition may impact immune responses and insulin sensitivity.

Neuroprotective Benefit: A study of a frontotemporal dementia mouse model suggests that aplimod, when injected directly into the hippocampus, increases lysosomes and decreases excitotoxicity. Neuroprotective potential is not known if used systemically.

Aging and related health concerns: Clinical trials for Crohn's disease and rheumatoid arthritis have failed. A clinical trial for non-Hodgkin's lymphoma is ongoing. The apilimod dose necessary for anti-proliferative effects may also inhibit PIKfyve activity in healthy immune cells, leading to an inhibition of IL-12/23 signaling.

Safety: Clinical trials in Crohn's disease and rheumatoid arthritis have reported mild to moderate adverse events including nausea and headache. Preclinical studies suggest that systemic PIKfyve inhibition could alter immune responses and insulin sensitivity.

Availability: Not available; in clinical trials.	**Dose**: Not established. Clinical trials in rheumatoid arthritis and Crohn's disease have used doses ranging from 50 to 100 mg daily.	**Chemical formula:** $C_{23}H_{26}N_6O_2$ **MW**: 418.5  Source: PubChem
Half life: Not documented.	**BBB**: Not documented.	
Clinical trials: The largest clinical trial has been in 220 patients with Crohn's disease.	**Observational studies**: None available.	

What is it? Apilimod was initially identified as an inhibitor of interleukins IL-12 and IL-23 and was developed for the treatment of autoimmune conditions including Crohn's disease and rheumatoid arthritis. However, clinical trial results failed to show efficacy for these indications. Apilimod was later found to also inhibit the PIKfyve kinase with nanomolar specificity (Cai et al., 2013). PIKfyve is a class-III phosphatidylinositol-5-kinase (PI5K) that synthesizes PI(3,5)P2 from PI3P (Staats et al., bioRxiv). Cellular functions of PIKfyve include the regulation of various aspects of both degradative and recycling endosomal trafficking, cytoskeletal rearrangement, and autophagy (Sbrissa et al., 2018). PIKfyve inhibition promotes endosomal maturation by increasing PI3P levels, and PI3P is also critical for autophagosome formation and engulfment of proteins designated for degradation. Apilimod has since been studied for indications including non-Hodgkin's lymphoma and viral diseases (e.g., Ebola). It is under clinical development by AI Therapeutics (LAM-002; AI Therapeutics website).

Neuroprotective Benefit: A study of a frontotemporal dementia mouse model suggests that aplimod, when injected directly into the hippocampus, increases lysosomes and decreases excitotoxicity. Neuroprotective potential is not known if used systemically.

Types of evidence:

- 1 laboratory study in C9orf72 +/- and -/- mice, models of frontotemporal dementia

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?
None available.

Human research to suggest benefits to patients with dementia:
None available.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:
One study evaluated the effects of apilimod in a model of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), the C9orf72 +/- and -/- mice. The C9orf72 repeat expansion causes neurodegeneration through loss- and gain-of-function mechanisms (Staats et al., bioRxiv). Reduced C9orf72 levels alter endosomal trafficking, autophagy, and lysosomes *in vitro*, and increase glutamate receptors on neurons. C9orf72 gain-of-function mechanisms include neurotoxic dipeptide repeat proteins. The authors performed a phenotypic screen to identify small molecules that could rescue the C9orf72 deletion-induced death of motor neurons and identified apilimod and confirmed PIKfyve as the therapeutic target.

In the C90rf72 +/- and -/- mouse model of FTD and ALS, infusion of apilimod (0.3 µL of 0.5, 3, or 20 µM in PBS) directly into the hippocampus increased the number of endosomes and lysosomes (LAMP1+) in neurons and astrocytes while decreasing the NMDA-induced excitotoxicity caused by the increase in glutamate receptors (Staats et al., bioRxiv). Apilimod also alleviated the gain-of-function pathology induced by the C9orf72 hexanucleotide repeat expansion (HRE) by decreasing levels of dipeptide repeat proteins derived from both sense and antisense C9orf72 transcripts in hippocampal neurons. While these findings are encouraging, it is not known what the therapeutic potential is for apilimod when used systemically.

APOE4 interactions: Unknown.

Aging and related health concerns: Clinical trials for Crohn's disease and rheumatoid arthritis have failed. A clinical trial for non-Hodgkin's lymphoma is ongoing. The apilimod dose necessary for anti-proliferative effects may also inhibit PIKfyve activity in healthy immune cells, leading to an inhibition of IL-12/23 signaling.

Types of evidence:

- 3 clinical trials, 2 in Crohn's disease and 1 in rheumatoid arthritis
- Numerous laboratory studies

Rheumatoid arthritis: NO BENEFIT.
In a phase 2a double blind randomized controlled proof-of-concept trial of 29 patients with active rheumatoid arthritis, treatment with apilimod (100 mg/day) showed a small reduction in the Disease Activity Score in 28 Joints (DAS28) on day 29 and day 57 compared with baseline, but these changes were not classified as a clinically meaningful response according to the European League Against Rheumatism (EULAR) response criteria (Krausz et al., 2012). American College of Rheumatology Criteria (ACR20) response was reached in only 6% of patients on day 29 and 25% of patients on day 57, similar to the percentage of responders in the placebo group. Increasing the dosage (100 mg twice daily) did not improve clinical efficacy. Apilimod also failed to show an effect on expression of synovial biomarkers. In apilimod-treated and placebo-treated patients, there was no significant difference in the number of CD68+ synovial macrophages on day 29 compared with baseline. Furthermore, no significant changes in the expression of other synovial markers were observed. There was no effect of apilimod on synovial IL-12 and IL-23 expression.

Crohn's disease: NO BENEFIT.
In a phase 2 double-blind randomized controlled trial for 220 patients with moderate-to-severe Crohn's disease, aplimod mesylate treatment (50 mg daily or 100 mg daily) failed to demonstrate efficacy over placebo based on an analysis by the Data Monitoring Committee and the trial stopped further enrollment (Sands et al., 2010). A clinical response was experienced by 18 patients (24.7%) in the 50-mg daily group (n = 73) and 19 patients (25.7%) in the 100 mg daily group (n = 74), as compared with 21 patients (28.8%) in the placebo group (n = 73) on day 29 (p=0.71 for each comparison).

Cancer: POTENTIAL BENEFIT BASED ON PRECLINICAL STUDIES WITH SOME CAVEATS.
High-throughput screen of clinical-stage drug library identified apilimod as a highly potent antiproliferative drug. Disruption of lysosomal homeostasis with apilimod represents a novel approach to treat non-Hodgkin's lymphoma (NHL) (Gayle et al. 2017). NHL B-cells display acute sensitivity to

apilimod *in vitro*. Apilimod displays antitumor activity in NHL B-cells *in vivo* in a lymphoma xenograft model. Oral dosing of 60 mg/kg apilimod dimesylate (~41 mg/kg apilimod free base; twice daily) resulted in a 48% inhibition of tumor growth in a mouse model. Apilimod combined with rituximab had an 83% inhibition to tumor growth. Disruption of lysosomal homeostasis is central to apilimod's mechanism of action.

Apilimod exerts a strong anti-proliferative effect in most (>75%) of the 48 tested non-Hodgkin's lymphoma (NHL) B-cell lines (Ikonomov et al., 2019). Selective toxicity, associated with large cytoplasmic vacuoles, is documented in NHL B-cells at doses not affecting the viability of normal B-lymphocytes. Based on the markedly higher apilimod sensitivity of NHL B-cell lines, the significantly and dose-dependently delayed growth of subcutaneous Daudi Burkitt lymphoma xenografts in mice by oral apilimod dimesylate treatment, and the evidence that apilimod is tolerated by other clinical populations, oral apilimod dimesylate is currently in a clinical trial of patients with B-cell malignancies (NCT02594384). Apilimod may also be tested for other cancers because of the reported sensitivity observed in ~75% of cell lines derived from kidney or colorectal cancers (Gayle et al. 2017).

While the preclinical data appear promising, a review of PIKfyve inhibitors for cancer therapeutics discusses unfavorable pharmacokinetics (e.g., unexpectedly low plasma levels even at maximum oral dosage). A single oral dose of apilimod dimesylate (15 mg; 102.7 mg free apilimod base) led to a peak plasma apilimod concentration of ~225 ng/ml after ~ 1 hour and then, after 6 hours, the apilimod concentration dropped below 50 ng/ml (Ikonomov et al., 2019). Importantly, a concentration of 200 nM (83.7 ng/ml free base) is determined to inhibit the proliferation of over 75% of 48 tested NHL B-cell lines (Gayle et al. 2017). Thus, 6 hours after single treatment the plasma concentration of apilimod was already below the desired effective concentration. The ongoing clinical trial is testing a higher dose of 25 or 50 mg, 2 or 3 times per day(NCT02594384).

Another issue worth noting is that apilimod appears to become inactivated in different cell culture systems (e.g., HEK293 cells, C2C12 myoblasts, mouse embryonic fibroblasts), which complicates the optimization of treatment for cancer. The authors argue that the potential widespread use of PIKfyve inhibitors as cancer therapeutics requires progress on multiple fronts: advances in methods for isolating relevant cancer cells from individual patients, delineation of the molecular mechanisms potentiating the vacuolation induced by PIKfyve inhibitors in sensitive cancer cells, design of PIKfyve inhibitors with favorable pharmacokinetics, and development of effective drug combinations (Ikonomov et al., 2019).

Viral infection: NO BENEFIT AGAINST EBOLA/LASSA VIRUSES BASED ON RODENT STUDIES. Apilimod is involved in the inhibition of traffic by Lassa and Ebola viruses (Hulseberg et al., 2019). Apilimod blocks Ebola virus trafficking to late endosomes, which serve as portals for both Ebola and Lassa viruses. However, apilimod did not protect mice from lethal Ebola virus challenge, likely due to its inhibition of interleukin 12/23 (IL-12/23) production.

Safety: Clinical trials in Crohn's disease and rheumatoid arthritis have reported mild to moderate adverse events including nausea and headache. Preclinical studies suggest that systemic PIKfyve inhibition could alter immune responses and insulin sensitivity.

Types of evidence:

- 3 clinical trials, 2 in Crohn's disease and 1 in rheumatoid arthritis
- 1 review on PIKfyve inhibitors as cancer therapeutics

The largest clinical trial to date was a phase 2 double-blind randomized controlled trial for 220 Crohn's disease patients and aplimod mesylate treatment (50 mg daily or 100 mg daily) did not result in significant adverse safety signals (Sands et al., 2010). Adverse events occurred at similar rates across the apilimod and placebo groups; 73% of patients receiving apilimod 50 mg daily, 80% of those taking 100 mg daily, and 81% of patients assigned to placebo. The majority of adverse events were mild or moderate in severity, with about 40% (n=59) of patients receiving apilimod (50 or 100 mg) experiencing at least 1 adverse event considered by the investigator to be related to study treatment. The adverse events most frequently reported by patients taking apilimod were nausea (20%), headache (14%), fatigue (11%), nasopharyngitis (11%), pyrexia (10%), and arthralgia (10%). Ten (7%) patients receiving apilimod (6 taking 50 mg daily and 4 taking 100 mg daily) and 8 (11%) patients receiving placebo had a serious adverse event during the study. A total of 27 patients withdrew from the study because of an adverse event (9 in placebo group, 10 in the 50 mg daily group, and 8 in the100 mg daily group).

In a phase 1/2a open-label dose escalation trial in 73 patients with active Crohn's disease, apilimod treatment (14 mg twice daily, 35 mg daily, 28 mg twice daily, or 70 mg daily) up to 4 weeks was well-tolerated (Burakoff et al., 2006). Reported adverse events were similar across dose cohorts and the most common (>15%) drug-related adverse events observed were dizziness, nausea, headache, and fatigue.

In a phase 2a double blind randomized controlled proof-of-concept trial of 29 patients with active rheumatoid arthritis, treatment with apilimod resulted in only mild adverse events (mainly gastrointestinal) for those taking the 100 mg dose daily for 4 weeks or 8 weeks, but all patients taking 100 mg twice daily for 8 weeks experienced headache and/or nausea (Krausz et al., 2012). One patient withdrew from the trial due to severe headache.

Based on a review of PIKfyve inhibitors as cancer therapeutics, preclinical studies suggest that systemic PIKfyve inhibition could alter patients' immune responses and insulin sensitivity (Ikonomov et al., 2019). The dose of apilimod necessary for anti-proliferative action in a large number of malignant cell lines also inhibits PIKfyve activity in "normal" immune cells, causing an inhibition of IL12/23 signaling (Cai et al., 2013), and defects in the presentation of the major histocompatibility complex in T-lymphocytes.

The review also discusses the unfavorable pharmacokinetics (e.g., unexpectedly low plasma levels) and apparent inactivation of apilimod in cell culture systems that complicate the optimization of treatment for cancer.

Drug interactions: Drug interactions have not been well studied.

Sources and dosing: Apilimod is being developed by AI Therapeutics for B-cell non-Hodgkin's lymphoma and amyotrophic lateral sclerosis. Clinical trials in rheumatoid arthritis and Crohn's disease have used doses ranging from 50 to 100 mg daily (Krausz et al., 2012; Sands et al., 2010). The ongoing trial in B-cell non-Hodgkin's lymphoma is testing apilimod at doses of 25 mg or 50 mg taken 2 or 3 times daily (NCT02594384).

Research underway: There is only one ongoing clinical trial testing apilimod based on ClinicalTrials.gov. A phase 1 dose escalation study of apilimod (25 mg or 50 mg capsules, 2 or 3 times daily) in repeated 28-day cycles will be evaluated in 62 patients with relapsed or refractory B-cell non-Hodgkin's lymphoma (NCT02594384). Apilimod will be administered alone or in combination with rituximab or atezolizumab (AI Therapeutics website; see LAM-002). This study is scheduled to be completed in December 2020.

Search terms:

Pubmed, Google: apilimod, LAM-002A, STA-5326

- + cognitive, + apolipoprotein, + Alzheimer, + cancer

Websites visited for apilimod:

- Clinicaltrials.gov (1)
- DrugAge (0)
- Geroprotectors (0)
- Drugs.com (0)
- WebMD.com (0)
- PubChem
- DrugBank.ca
- Cafepharma (0)
- Pharmapro.com (0)

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