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.

Bryostatin

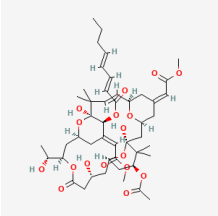
Evidence Summary

Bryostatin modulates PKC, a protein family involved in numerous cell activities. Repeated clinical trials of bryostatin in cancer and in AD have shown no clear efficacy.

Neuroprotective Benefit: Bryostatin has shown neuroprotective potential in preclinical models but has not shown efficacy in any of the multiple Phase 2 trials.

Aging and related health concerns: Bryostatin has not been studied clinically for age-related indications other than cancer. Bryostatin appears to have minimal to no efficacy as a monotherapy but may potentially have benefit in combination therapy.

Safety: Bryostatin has been studied in dozens of early-phase studies. At appropriate doses, bryostatin appears to be well-tolerated, with side effects such as headache, myalgia, and GI symptoms.

Availability: in clinical development	**Dose**: Efficacious dose still unknown; a dose of 20 µg biweekly, i.v., has been tested in AD patients	**Chemical formula:** $C_{47}H_{68}O_{17}$  **MW**: 905.0 g/mol Source: PubChem
Half-life: ~28-32 hours after a single dose	**BBB**: Penetrant	
Clinical trials: Largest clinical trial of AD patients included 147 participants	**Observational studies**: There have been no observational studies of bryostatin.	

What is it?

Bryostatins are a family of molecules that were isolated from a colony forming marine invertebrate *Bugula neritina*, part of the bryozoan phylum. This report will focus on bryostatin 1, the most studied family member (Trindade-Silva et al., 2010).

Bryostatins are modulators of protein kinase C (PKC), a family of several different enzymes known as isozymes: enzymes that perform the same function but have different structures. PKC activity is highest in the brain, particularly at the synapse. PKC exists as a pro-enzyme until it is activated. Typically, PKC is activated when a receptor on the membrane is activated by an extracellular signal and cleaves a particular membrane phospholipid to form diacylglycerol. Most PKC isozymes are activated by diacylglycerol alone or diacylglycerol and calcium. After activated, PKC can phosphorylate or interact with a number of different targets in a number of signaling cascades. PKC therefore plays a role in a wide-ranging variety of cellular activities, including cell-cycle regulation, apoptosis, cytoskeletal dynamics, and cellular differentiation (Nelson & Alkon, 2007; Callender & Newton, 2017). PKC is also involved in synaptic plasticity; thus, PKC is also involved with learning and memory. Once PKC is activated, it is also targeted for degradation (Callender & Newton, 2017).

Bryostatin is an activator of PKC. Binding of bryostatin to PKC results in the typical initial activation and then subsequent degradation. The extent and timing of this activation and degradation, as well as the downstream consequences, depend on the PKC isozyme that bryostatin binds to and the dosing concentration and frequency of bryostatin, among other factors (Nelson & Alkon, 2007).

Bryostatin has bene most thoroughly studied as a potential cancer therapeutic, as PKC plays a key role balancing between cell survival and cell death. More recently, bryostatin has been investigated for clinical use in AD and HIV. Preclinical work has also touched on other potential uses of bryostatin, including in diabetes, Crohn's disease, multiple sclerosis, and stroke (Raghuvanshi & Bharate, 2020).

While bryostatins have primarily been studied in the context of binding to PKC, bryostatins also bind to and activate other proteins such as the synaptic protein Munc-13, which is crucial for neurotransmitter release. Bryostatin may therefore act through different pathways with clinical effects that we do not yet recognize or understand (Blanco et al., 2019).

Neuroprotective Benefit: Bryostatin has shown neuroprotective potential in preclinical models but has not shown efficacy in any of the multiple Phase 2 trials.

Types of evidence:

- 3 clinical trials
- 6 reviews
- Numerous laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

Bryostatin has not been tested as a prevention strategy; it has only been administered to patients with dementia.

Human research to suggest benefits to patients with dementia:

Nelson et al., 2017, reported on the results of a small randomized double-blinded placebo-controlled Phase 2a study of bryostatin 1 in 9 patients with AD. The authors also included information from a three patient expanded access / compassionate use portion of the study. In the randomized trial, patients received single doses of either 25 $\mu g/m^2$ bryostatin 1 or placebo via IV. The primary outcome was safety and tolerability; the secondary outcomes were pharmacokinetics, pharmacodynamics, and effects on cognition. The authors report that there was a statistically significant increase in MMSE scores 3 hours after dosing in the group treated with bryostatin 1 ($p = 0.041$). It is worth noting that taking the same cognitive test in rapid succession can potentially result in better scores through practice effects rather than true cognitive improvement.

	Baseline	3 Hours	Change from Baseline
Placebo	22.00±1.53	21.00±2.00	–1.00±1.53
Bryostatin	21.33±1.50	23.16±1.89	+1.83±0.70

In the expanded access / compassionate use portion of the study, three patients with severe dementia were given multiple doses of bryostatin 1. One patient was given 4 doses, one was given 11 doses, and a third was given 26. Dosing was 25 µg/m^2 weekly for 3 weeks, followed by successively lower doses given at longer intervals. Doses were again given intravenously. The authors report qualitatively significant behavioral improvements in the three patients; for instance, cessation of 'constant' hallucinations, improved verbal communication in previously non-verbal patients, and improved motor function and ability to perform self-care activities. These effects were reported to occur soon after drug administration (within 3 hours) and then persisted over weeks. Given the small size of the group and the severity of their dementia, cognitive testing was not possible. As this portion of the study was an open-label design, placebo effects cannot be ruled out.

The study also reported on levels of bryostatin and activated PKCε, one of the PKC isozymes, in peripheral blood and PBMCs, respectively (AlzForum). They report a significant increase in PBMC PKCε at 1 hour ($p = 0.0185$, repeated measures ANOVA) followed by a downregulation, which is an expected behavior of activated PKC. They observed a trend, though not a significant relationship, between higher PKCε and improved cognitive function.

Farlow et al., 2019, from the same group as the 2017 paper, reported on the results from a randomized double-blinded Phase 2 trial of bryostatin 1 in patients with moderately severe to severe AD. This study enrolled 150 patients and tested IV-administered doses of 20 µg and 40 µg of bryostatin and compared to placebo treatment. Unlike the first study, all patients received the same dose, regardless of body weight or volume. Patients received loading doses of 24 or 48 µg of bryostatin if they were in the 20 µg or 40 µg group, respectively, or matching placebo, with the doses spaced 1 week apart. Patients then received their assigned study drug every other week for 10 weeks, thus receiving a total of 7 doses.

The primary outcomes were safety, tolerability, and effects on cognitive function as measured by the Severe Impairment Battery (SIB). Secondary outcomes included other measures of cognitive function, including the ADCS-ADL, Severe Impairment Version (ADCS-ADL-SIV), the Clinical Global Impression of Improvement (CGI-I), and Neuropsychiatric Inventory (NPI). They performed their assessments both of the intent-to-treat groups (defined as patients who were randomized and received at least one dose of study drug) as well as the completer group (defined as patients who completed the dosing regimen). They assessed results at 5, 9, and 13 weeks after start of dosing. They do not appear to have corrected for multiple comparisons.

The authors prespecified use of one-sided t-tests for the statistical analysis of their primary and secondary outcomes and said that they will not interpret their p-values as having statistical significance. Therefore, any differences are framed as trends towards benefit. When comparing the change from baseline SIB scores of the 20 µg group to the placebo group, there were trends towards benefits at 5 weeks in the intent-to-treat group, and at 5 and 13 weeks in the completer group (see table below).

Comparing Change from Baseline SIB Scores by One Sided T-Test, 20 µg vs. Placebo			
	Week 5	Week 9	Week 13
Intent to Treat	Trend towards benefit	No difference	No difference
Completer Group	Trend towards benefit	No difference	Trend towards benefit

The authors also report trends towards improvement in patients receiving 20 µg bryostatin as compared to placebo in the completer group at 13 weeks as measured by the ADCS-ADL-SIV. They observed no trend towards any difference as measured by the NPI; they did not report any outcome as measured by CGI.

The group had a pre-specified exploratory analysis of their bryostatin groups to assess whether there were differences in cognitive performance based on whether or not the patients were also receiving memantine therapy. The analysis was pre-specified because PKC is thought to regulate NMDA receptors, which memantine blockades. For this analysis, they looked at the change from baseline performance at weeks 5, 9, 13 and an average of week 13 and 15, using two-sided t-tests.

The authors found that there was no difference between patients receiving placebo and patients receiving 20 µg bryostatin + memantine. But, they did find that patients who received 20 µg bryostatin and were not on memantine had significantly better SIB scores at week 13 as compared to their own baseline (difference=5.6 points; 95% CI 0.4 – 10.9; p = 0.035). They also looked at an average of the week 13 and week 15 scores and also compared to baseline; the difference between those who took bryostatin without memantine as compared to those who took placebo was greater than at 13 weeks (difference=6.1 points; 95% CI 1.5 -10.7; p = 0.012). The authors posit that this difference in the no-memantine group is a hint towards efficacy of bryostatin.

No trend towards or statistically significant benefit was seen in the 40 µg group compared to either the lower bryostatin dose or the placebo group in any analysis.

A second double-blinded placebo-controlled Phase 2 trial was then performed, this time enrolling 108 patients with moderately severe or severe AD who were not taking concomitant memantine therapy (NCT03560245). In this trial, the authors stratified patients based on their MMSE scores (one group for patients with scores from 4-9, one group for patients with scores from 10-15) and then randomized to either placebo or 20 µg bryostatin, taken intravenously. The dosing schedule was the same as in the

study published by Farlow and colleagues: patients received two loading doses of 24 µg bryostatin or matching placebo 1 week apart, and then received 20 µg doses of bryostatin or matching placebo every other week. Patients received a total of 7 doses over the course of 12 weeks. Dosing was also the same between patients again, regardless of body weight or volume. Their primary outcomes were safety and cognitive function as assessed by the SIB from baseline to week 13. Their secondary outcomes were other measures of cognitive function such as the MMSE and also other analyses of the SIB, including the change in SIB at different timepoints during the trial, or the change in cognitive function in the patients with more or less severe dementia symptoms.

This second Phase 2 trial failed to meet its endpoints. However, the group found that there was a chance imbalance of SIB scores at baseline; the placebo group had higher SIB scores than the group given bryostatin, and the SIB score at baseline was statistically associated with both the score at 13 weeks and also the change in score. They found that this imbalance was larger when comparing between placebo and bryostatin groups when looking at the patients with more severe illness; patients with more moderate illness were less unbalanced between the two groups.

The authors therefore performed a post-hoc analysis of the data from both Phase 2 trials, focusing on the moderate severity stratum. These analyses were published in Thompson et al., 2022, and completed using data from both trials individually and also pooled together.

Again using one-sided t-tests, but this time using the phrasing 'statistically significant' in comparison to their 2019 paper, they report that bryostatin treated patients with MMSE scores between 10 and 14 who did not take memantine had improvements in SIB scores in both Phase 2 trials when analyzed individually ($p=0.005$, 0.016) and when combined ($p<0.001$) as compared to placebo patients. They state bryostatin showed 'complete safety' but they do not report on the safety results from the second trial.

A third double-blinded placebo-controlled Phase 2 trial was then conducted (NCT04538066). This trial enrolled 100 patients with moderate AD. Patients were randomized to either placebo or 20 µg bryostatin. The dosing schedule was similar to the prior study (two loading doses of 24 µg or matching placebo one week apart, followed by a 20 µg dose every other week for 10 weeks, giving patients a total of seven doses over 12 weeks). However, in this study, patients then took a 30-day break and repeated the same dosing schedule again. The outcomes are safety and cognitive function as assessed by SIB. This study was completed in late 2022; results have not yet been published.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

Through activation of PKC, bryostatin is thought to be potentially neuroprotective in a variety of ways. Some studies have found PKC levels to be lower in the brains of AD patients as compared to controls (Khan et al., 2015). Activated PKC activates α-secretase, and thus can reduce Aβ levels through shunting APP towards production of soluble APP rather than towards Aβ formation (Hongpaisan et al., 2011,

Schrott et al., 2020). PKC is also involved in phosphorylation of tau (Alkon et al., 2007; Callender & Newton, 2017). Some preclinical work reports that administration of bryostatin increases BDNF levels in wildtype mice. Bryostatin may also mitigate oxidative stress (Sen et al., 2018; Liu et al., 2023).

PKC is also thought to be instrumental in synaptic plasticity. PKC regulates a number of different neurotransmitter receptors and therefore influences long term potentiation / depression. PKC is also involved in cytoskeletal dynamics, which are necessary for synaptic formation and maintenance (Callender & Newton, 2017). Bryostatin may decrease dendritic spine density but increase synaptogenesis, which the authors postulate represented an increase of signal over noise (Ly et al., 2020). Bryostatin administration is reported to improve cognition in animal models of AD (Hongpaisan et al., 2011, Schrott et al., 2015) and also in an animal model of Fragile X (Cogram et al., 2020).

Preclinical work also suggests that bryostatin may be beneficial for other neurological conditions such as stroke, traumatic brain injury, and Fragile X (Sun et al., 2008; Lucke-Wold et al., 2015; Cogram et al., 2020).

There is also some conflicting evidence. At least one study has not replicated the increase in BDNF after bryostatin administration (Giarratana et al., 2020), and another study reported that PKC levels were increased in brains from AD patients compared to controls (Lorden et al., 2022). There are also some indications that activating PKC may be detrimental in neurodegeneration. For instance, mutations in one PKC isozyme cause spinocerebellar ataxia. Moreover, a genome-wide association study identified a gain-of-function variant of one PKC isozyme that co-segregated with AD patients (Callender & Newton, 2017). Transgenic animals with this variant had increased activity of PKC and showed enhanced synaptic depression in the presence of Aβ, reduced spine density, and cognitive impairments (Lorden et al., 2022). Whether these differences are due to the complexity of the PKC family and signaling pathways or to other factors is not yet known.

APOE4 interactions:

No clinical trials have reported on differential effects of bryostatin use in APOE4 carriers. Preclinical work hints that APOE may activate PKC, and that APOE3 may increase expression of or activate PKC more potently than APOE4. If these preclinical hints reflect a biological truth, it is possible that bryostatin could partly compensate for the reduced activation in APOE4 carriers. Whether this is the case, and whether this leads to any cellular or clinical benefit, is not yet known (Cedazo-Mínguez et al., 2001; Sen et al., 2017).

Aging and related health concerns: Bryostatin has not been studied clinically for age-related indications other than cancer. Bryostatin appears to have minimal to no efficacy as a monotherapy but may potentially have benefit in combination therapy.

Types of evidence:

- 2 reviews of cancer clinical trials

Cancer: LIKELY NO BENEFIT ALONE; POTENTIAL USE IN COMBINATION

Bryostatin has been studied for efficacy in cancer in more than 42 trials. Most of these studies have found that bryostatin is not an effective anti-cancer treatment on its own, though some report benefit in some patients. Overall, it seems bryostatin has more promise as a potential agent to use in combination therapy rather than as a monotherapy (reviewed by Kortmansky & Schwartz, 2003, Raghuvanshi & Bharate, 2020).

Safety: Bryostatin has been studied in dozens of early-phase studies. At appropriate doses, bryostatin appears to be well-tolerated, with side effects such as headache, myalgia, and GI symptoms.

Types of evidence:

- 2 clinical trials
- 2 reviews of cancer clinical trials

Bryostatin has been studied in dozens of Phase 1 and 2 clinical trials for cancer. The most common side effects of bryostatin reported from these studies are myalgia and GI symptoms such as nausea, vomiting, and diarrhea. At high doses, typically 40 µg and above, dose-limiting toxicity has been observed. The myalgia is thought to be due to effects of bryostatin on mitochondrial function and/or vasoconstrictive actions (reviewed by Kortmansky & Schwartz, 2003, Raghuvanshi & Bharate, 2020).

The Phase 2a study from Nelson et al., 2017 included 9 patients in the randomized placebo-controlled portion and 3 patients in the expanded access. All patients had AD. The 9 patients in the randomized portion received single doses of either 25 µg/m^2 bryostatin 1 or placebo via IV, whereas the 3 patients in the expanded access portion all received multiple doses of 25 µg/m^2 bryostatin. The authors report that there were no serious adverse events, deaths, or clinically significant changes in vital signs. They also report that all laboratory blood testing and functional tests like cardiac assessments were unremarkable after drug administration. Three patients experienced adverse events of headache, dizziness, and rash; the authors write that the headache was experienced by a patient in the bryostatin group and that it was not considered related to the drug; they report the patient experienced worsening of AD symptoms.

The Phase 2 study from Farlow et al., 2019 enrolled 150 patients with moderately severe to severe AD, and compared administration of 20 µg or 40 µg bryostatin to placebo. Patients were evenly distributed between groups. Patients received a total of 7 doses of the study drug. They report no apparent differences in ECG, vital signs, or laboratory values between the three groups. The authors report broadly similar adverse event data in the placebo and 20 µg group, but found the 40 µg to be not well-tolerated. While 12.5% of patients in the placebo group and 17.5% of the patients in the low dose bryostatin group withdrew from the trial, 38.8% of patients withdrew from the high dose bryostatin group. There were 28 treatment-emergent adverse events in the placebo group, 30 in the 20 µg group, and 39 in the 40 µg group. There were 3 serious adverse events in the placebo group, 1 in the 20 µg group, and 6 in the 40 µg group. There was one death in the high dose bryostatin group that was considered unrelated to study drug.

When comparing the adverse events seen in the placebo and the 20 µg group, two events were more common in the treatment group: GI symptoms such as diarrhea and infusion site reactions. The authors report that after training on aseptic technique, IV infusions, and universal precautions there were no further infusion site reactions; it is therefore possible that this adverse event was not study drug related. Other common side effects were headache, myalgia, and fatigue. Diarrhea, headache, fatigue, and myalgia were all more common in the highest dose group than the lower dose group or placebo. Weight loss was also observed in both the low dose (mean of -1.65 kg) and high dose (mean of -2.98 kg) bryostatin groups, whereas patients in the placebo group trended towards weight gain (mean of +0.44 kg).

Drug interactions:

The drug interactions of bryostatin-1 have not been fully elucidated or published. Clinical trials indicate that co-administration of memantine may interfere with the mechanism of bryostatin-1; bryostatin is thought to regulate the NMDA receptor, which memantine blockades. The trial did not explore or comment on whether the reverse is true, that bryostatin may interfere with the mechanism of memantine. Thus, bryostatin likely should not be prescribed to someone taking memantine as it appears bryostatin action is blocked (Farlow et al., 2019).

Research underway:

There are no currently ongoing studies investigating bryostatin.

One study of the effects of bryostatin in patients with moderately severe AD was recently completed in March 2023 and has not yet published results. This randomized, double-blinded, placebo-controlled Phase 2 study, NCT04538066, enrolled 117 patients. Bryostatin is administrated intravenously. Patients received two loading doses of 24 µg weekly, and then five doses of 20 µg every other week for 10

weeks. After a 30-day break, the patients received another round of the identical treatment. The placebo group underwent the same routine, receiving placebo instead of bryostatin. The primary outcomes were safety as measured by treatment-related adverse events and efficacy on cognitive function at 28 weeks as measured by the Severe Impairment Battery (SIB) total score. Secondary outcomes included cognitive function at other timepoints throughout the study, including at a week 42 follow up.

Bryostatin was also granted orphan drug designation for Fragile X, and Synaptogenix signaled that they intend to run a clinical trial in collaboration with Nemours AI DuPont Hospital for Children (Press Release).

Search terms:
Pubmed, Google: bryostatin, PKC

- Dementia, Alzheimer's disease, APOE4, aging, cancer

Websites visited for bryostatin:

- Clinicaltrials.gov
- PubChem
- DrugBank.ca