Broccoli seed tea

Candidate	Biology	Safety
BRANDED NAME(S)	DRUG CLASS	SAFETY RECORD/TOXICOLOGY STUDIES
Not regulatory approved	Liquid formulation of broccoli seed	Preliminary pilot study data with PD volunteers indicates that the solution is safe and well tolerated
OTHER EXAMPLES	EFFICACY IN MODELS	MODE OF DELIVERY
None	Not for this formulation	Oral solution
OWNER/OTHER POSSIBLE SUPPORT	TESTED IN HUMANS?	SIDE EFFECTS
No IP associated	Yes	Unclear. To be determined. Limited based on previous pilot study
IP HISTORY	TARGET MOLECULAR PATHWAY?	SELECTIVITY
No IP associated	NRF2 pathway	To be determined
LICENCE STATUS	POSSIBLE OFF-TARGET EFFECTS	MAJOR CONTRAINDICATIONS
Investigational	To be determined	To be determined
TESTED/USED FOR WHICH CONDITIONS?	POTENTIALLY PROTECTIVE OR RESTORATIVE?	SPECIFIC MONITORING REQUIREMENTS?
Parkinson's	Protective; partially restorative	Cardiac monitoring
BLINDING POSSIBLE?	PK AND PD AND ADME	ANY PREVIOUS USE IN ANY OTHER NEUROLOGICAL CONDITIONS?
Yes	Limited PK/PD data available	None
ORIGIN OF DOSSIER?	CNS PENETRATION	APPROVED DOSAGES
Patient initiated study	Yes (for sulforaphane)	Not approved

(based on the literature); CP proposed drafting a dossier		
iLCT HISTORY	**BIOAVAILABILITY & HALF-LIFE**	**OTHER TRIALS USING THIS THERAPEUTIC?**
Never previously presented	Limited information available	There are no clinical trials registered for this agent on the ClinicalTrials.gov website

Rationale for conducting a PD trial using Broccoli seed tea within the iLCT Initiative

HYPOTHESIS: Long-term increase in NRF2 activity (via sulforaphane activation) will improve non-motor features of Parkinson's.

The aliphatic isothiocyanate sulforaphane is a potent inducer of the endogenous anti-oxidant transcription factor nuclear factor (erythroid-derived 2)-like 2 (NRF2), which can effectively counteract oxidative stress damage and mitochondrial dysfunction by up-regulating the Antioxidant Response Element (ARE) regions of genes encoding detoxification and antioxidant enzymes. Sulforaphane has demonstrated neuroprotective properties in a variety of models of Parkinson's but has not been clinically tested.

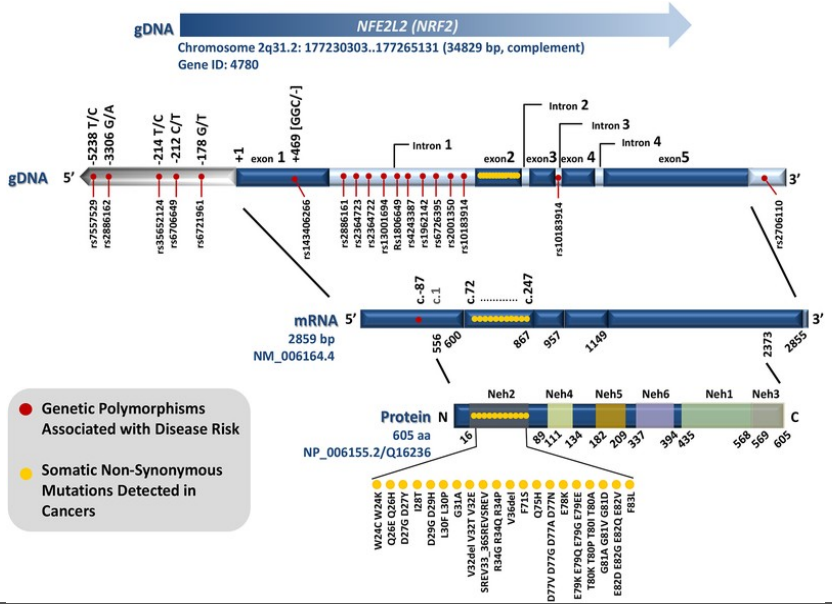
A patient researcher – Dr Albert F Wright (ARIC, PhD) – has devised a broccoli seed tea (from *B. oleracea,* var. *italica*), designed to deliver a therapeutic amount of sulforaphane based upon specific dose calculations. Preliminary pilot N=1 studies have provided encouraging results - particularly in terms of non-motor symptoms - which need to be examined in a larger, controlled study.

Cure Parkinson's is presenting this dossier to the iLCT committee with the goal of conducting a Phase IIa safety and biomarker study in a cohort of individuals with idiopathic Parkinson's (possibly conducted in under-represented geographical regions). Dr Wright helped with the production of this dossier.

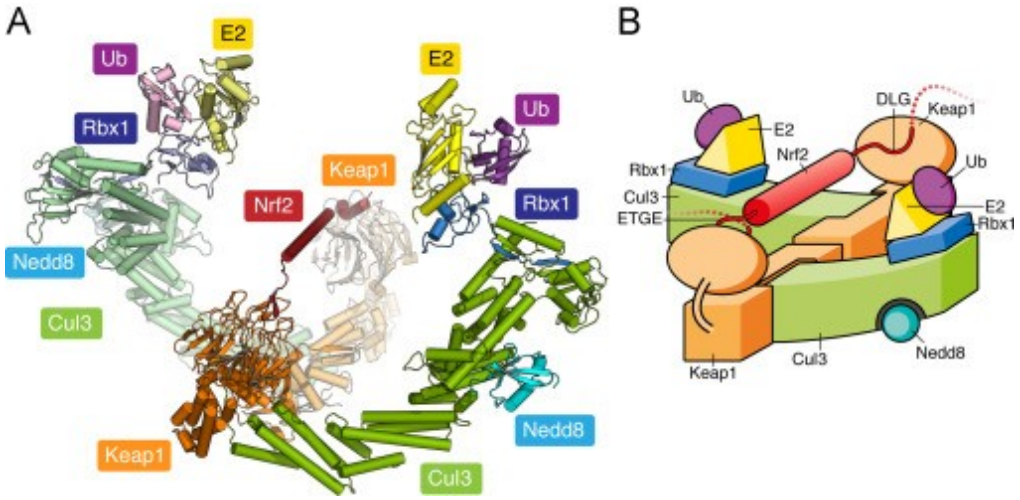
Scientific background

The NRF2 pathway

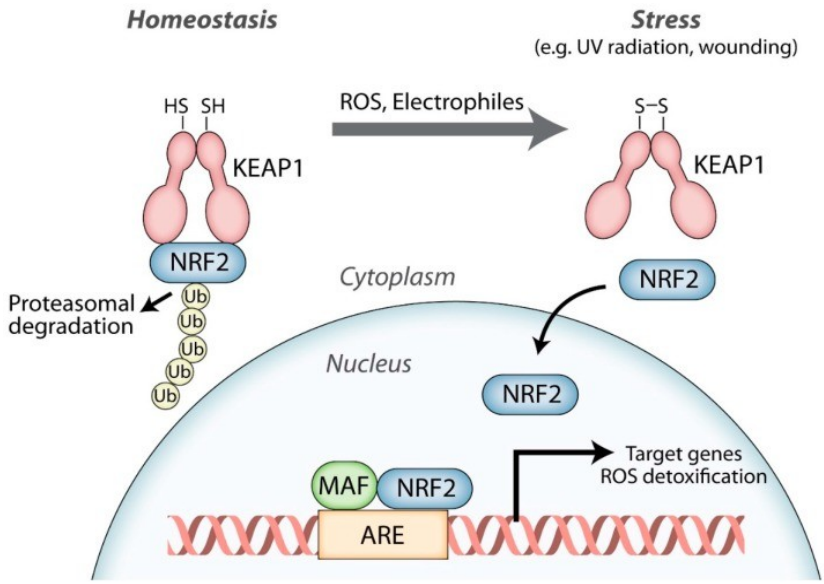
Nuclear factor (erythroid-derived 2)-like 2 (NRF2) is a basic leucine zipper protein member of the cap'n'collar family of transcription factors. It functions as a major regulator of antioxidant and cellular protective genes and is activated in response to oxidative stress. Human NRF2 is located on chromosome 2, spanning approximately 34.8 kb (gene ID: 4780). NRF2 mRNA is 2,859 base pairs long, encoding a 605 amino acid protein:



NRF2 is a short-lived protein, whose homeostasis is regulated by Kelch-like erythroid-derived Cap'n'Collar Homology (ECH)-associated protein 1 (KEAP1), a cytoplasmic NRF2 suppressor. Under homeostatic conditions, KEAP1 functions as (i) a substrate adaptor for a Cullin 3 (CUL3)-based E3 ubiquitin ligase which polyubiquinates NRF2 for proteasomal degradation, and (ii) a cysteine-based sensor for a myriad of physiological and pharmacological NRF2 activators.:



However, electrophilic and oxidative insults are known to modify thiol residues in KEAP1, which alter binding interactions between KEAP1, CUL3, and NRF2, and permit newly synthesized NRF2 to bypass KEAP1 inhibition. This "activated" NRF2 heterodimerizes with JUN and small musculoaponeurotic fibrosarcoma (sMAF) proteins, translocates to the nucleus and binds to the antioxidant response element (ARE) or the electrophile-response element (EpRE) in the promoter region of NRF2 target genes.



This results in the coordinated expression and activation of antioxidant, antiapoptotic, metabolic, and detoxification proteins. Among the proteins with antioxidant activity regulated by NRF2 are superoxide dismutase (SOD), catalase (CAT), heme-oxygenase 1 (HO-1), glutathione peroxidase 1 (GPx-1) and NAD(P)H: quinone oxidoreductase 1.

As a result of this action, molecules that help to activate NRF2 are of therapeutic interest:

https://pubmed.ncbi.nlm.nih.gov/29261130/
Nrf2, the Master Regulator of Anti-Oxidative Responses.
Vomund S, Schäfer A, Parnham MJ, Brüne B, von Knethen A.
Int J Mol Sci. 2017 Dec 20;18(12):2772.

https://pubmed.ncbi.nlm.nih.gov/26281945/
Nrf2--a therapeutic target for the treatment of neurodegenerative diseases.
Johnson DA, Johnson JA.
Free Radic Biol Med. 2015 Nov;88(Pt B):253-267.

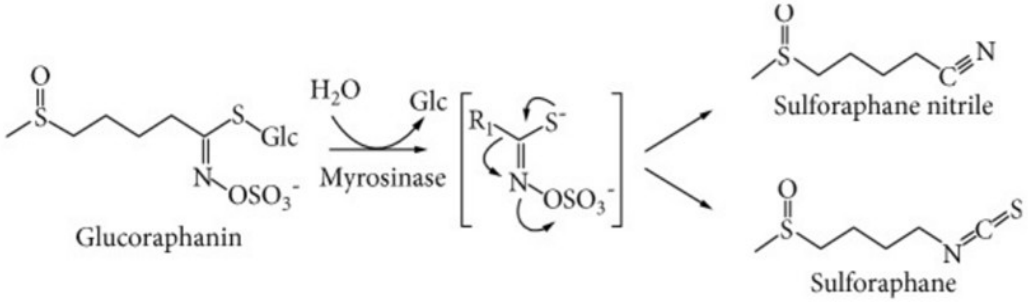
In addition to its antioxidant action, a large volume of research indicates that NRF2 plays a significant role in the maintenance of mitochondrial health, including the mitochondrial membrane potential, respiration, mitophagy and mitochondrial biogenesis.

https://pubmed.ncbi.nlm.nih.gov/26812787/
Nrf2 activation in the treatment of neurodegenerative diseases: a focus on its role in mitochondrial bioenergetics and function.
Esteras N, Dinkova-Kostova AT, Abramov AY.
Biol Chem. 2016 May;397(5):383-400.

https://pubmed.ncbi.nlm.nih.gov/32681666/
Activation of transcription factor Nrf2 to counteract mitochondrial dysfunction in Parkinson's disease.
Bento-Pereira C, Dinkova-Kostova AT.
Med Res Rev. 2021 Mar;41(2):785-802.

Sulforaphane

Sulforaphane (Molar mass: 177.29 g/mol) is naturally derived from certain species of the Brassica vegetable family, most notably broccoli. Classified as cruciferous vegetables, they are known for their disease-preventive effects. When ingested, the bioactivity of crucifers is dependent on the dual presence of a precursor molecule, Glucoraphanin, and an enzyme, myrosinase, which hydrolyses the precursor; the product is an isothiocyanate:



Glucoraphanin occurs in all tissues of broccoli plants, though it is most abundant in the seeds and young sprouts. Sulforaphane has been demonstrated to have an absolute bioavailability of between 70-80%, and to peak in the bloodstream at approximately 1 hour following ingestion:

https://pubmed.ncbi.nlm.nih.gov/21372038/
Bioavailability of Sulforaphane from two broccoli sprout beverages: results of a short-term, cross-over clinical trial in Qidong, China.
Egner PA, Chen JG, Wang JB, et al, Talalay P, Groopman JD, Kensler TW.
Cancer Prev Res (Phila). 2011 Mar;4(3):384-395.

https://pubmed.ncbi.nlm.nih.gov/17347138/
Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast.
Cornblatt BS, Ye L, Dinkova-Kostova AT, et al, Talalay P, Kensler TW, Visvanathan K.
Carcinogenesis. 2007 Jul;28(7):1485-1490.

https://pubmed.ncbi.nlm.nih.gov/11750273/
Quantitative determination of dithiocarbamates in human plasma, serum, erythrocytes and urine: pharmacokinetics of broccoli sprout isothiocyanates in humans.
Ye L, Dinkova-Kostova AT, Wade KL, Zhang Y, Shapiro TA, Talalay P.
Clin Chim Acta. 2002 Feb;316(1-2):43-53.

In vivo experiments have demonstrated an increased NRF2 expression and nuclear localization after sulforaphane treatment, as well as augmented transcriptional activity:

https://pubmed.ncbi.nlm.nih.gov/23353773/
Prevention by sulforaphane of diabetic cardiomyopathy is associated with up-regulation of Nrf2 expression and transcription activation.
Bai Y, Cui W, Xin Y, Miao X, Barati MT, Zhang C, Chen Q, Tan Y, Cui T, Zheng Y, Cai L.
J Mol Cell Cardiol. 2013 Apr;57:82-95.

https://pubmed.ncbi.nlm.nih.gov/29684505/
Protective Effects of Sulforaphane on Cognitive Impairments and AD-like Lesions in Diabetic Mice are Associated with the Upregulation of Nrf2 Transcription Activity.
Pu D, Zhao Y, Chen J, Sun Y, Lv A, Zhu S, Luo C, Zhao K, Xiao Q.
Neuroscience. 2018 Jun 15;381:35-45.

https://pubmed.ncbi.nlm.nih.gov/27006750/
Sulforaphane Attenuates Contrast-Induced Nephropathy in Rats via Nrf2/HO-1 Pathway.
Zhao Z, Liao G, Zhou Q, Lv D, Holthfer H, Zou H.
Oxid Med Cell Longev. 2016; 9825623.

Sulforaphane binds to the regulatory cys151 sensor in the Keap1 dimer, thus stopping NRF2 degradation and increasing ARE gene expression:

https://pubmed.ncbi.nlm.nih.gov/32574549/
KEAP1, a cysteine-based sensor and a drug target for the prevention and treatment of chronic disease.
Dayalan Naidu S, Dinkova-Kostova AT.
Open Biol. 2020 Jun;10(6):200105.

https://pubmed.ncbi.nlm.nih.gov/21391649/
Modification of keap1 cysteine residues by sulforaphane.
Hu C, Eggler AL, Mesecar AD, van Breemen RB.

Chem Res Toxicol. 2011 Apr 18;24(4):515-21.

The NRF2-dependent antioxidant response diminishes with age:

https://pubmed.ncbi.nlm.nih.gov/34012501/
The Keap1-Nrf2 System: A Mediator between Oxidative Stress and Aging.
Yu C, Xiao JH.
Oxid Med Cell Longev. 2021 Apr 19;2021:6635460.

https://pubmed.ncbi.nlm.nih.gov/30654017/
Redox regulation by NRF2 in aging and disease.
Schmidlin CJ, Dodson MB, Madhavan L, Zhang DD. Free Radic Biol Med. 2019 Apr;134:702-707.

https://pubmed.ncbi.nlm.nih.gov/28863281/
Aging-related decline in the induction of Nrf2-regulated antioxidant genes in human bronchial epithelial cells.
Zhou L, Zhang H, Davies KJA, Forman HJ.
Redox Biol. 2018 Apr;14:35-40.

Sulforaphane treatment has been shown to increase NRF2 transcription, activation, nuclear translocation, DNA-binding, and antioxidant gene expression in epithelial cells isolated from old rats and elderly humans:

https://pubmed.ncbi.nlm.nih.gov/29074861/
Sulforaphane reactivates cellular antioxidant defense by inducing Nrf2/ARE/Prdx6 activity during aging and oxidative stress.
Kubo E, Chhunchha B, Singh P, Sasaki H, Singh DP.
Sci Rep. 2017 Oct 26;7(1):14130.

Therefore, sulforaphane could be an important inducer of the antioxidant and protective response during aging.

Relevance to Parkinson's:

Oxidative stress and mitochondrial dysfunction are believed to play an important role in the parthenogenesis of Parkinson's:

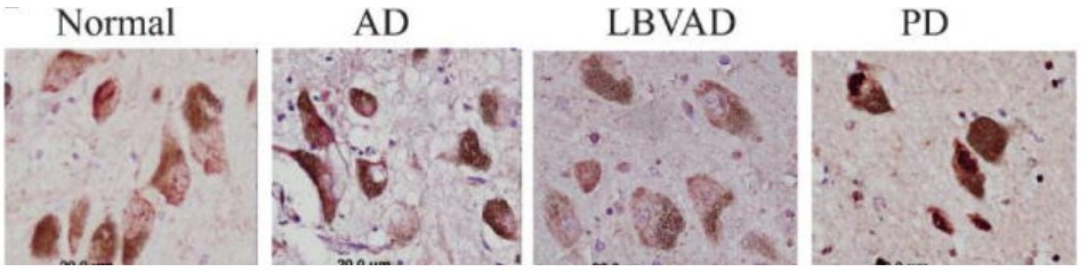
https://pubmed.ncbi.nlm.nih.gov/24252804/
The role of oxidative stress in Parkinson's disease.
Dias V, Junn E, Mouradian MM. J
Parkinsons Dis. 2013;3(4):461-491.

https://pubmed.ncbi.nlm.nih.gov/22735187/
Mitochondrial dysfunction in Parkinson's disease: molecular mechanisms and pathophysiological consequences.
Exner N, Lutz AK, Haass C, Winklhofer KF.
EMBO J. 2012 Jun 26;31(14):3038-3062.

NRF2 has been explored in the context of Parkinson's:

https://pubmed.ncbi.nlm.nih.gov/27145762/
Nrf2: a modulator of Parkinson's disease?
Todorovic M, Wood SA, Mellick GD.
J Neural Transm (Vienna). 2016 Jun;123(6):611-619.

NRF2 has been reported to predominantly localise to the cytosol in nigral dopamine neurons from postmortem control cases, whereas in age-matched PD patients (early Braak staging 1–2), NRF2 is found within in the cell nucleus – suggesting a possible attempt (in the PD cells) to reduce oxidative stress:



https://pubmed.ncbi.nlm.nih.gov/17204939/
Expression of Nrf2 in neurodegenerative diseases.
Ramsey CP, Glass CA, Montgomery MB, et al, Hamilton RL, Chu CT, Jordan-Sciutto KL.
J Neuropathol Exp Neurol. 2007 Jan;66(1):75-85.

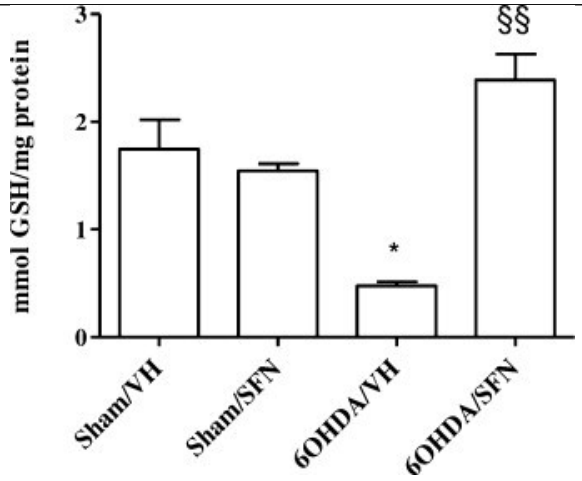
Administration of sulforaphane provides neuroprotection for a variety of PD models:

https://pubmed.ncbi.nlm.nih.gov/31132231/
Dietary intake of glucoraphanin prevents the reduction of dopamine transporter in the mouse striatum after repeated administration of MPTP.
Pu Y, Qu Y, Chang L, Wang SM, Zhang K, Ushida Y, Suganuma H, Hashimoto K.
Neuropsychopharmacol Rep. 2019 Sep;39(3):247-251.

https://pubmed.ncbi.nlm.nih.gov/27553905/
Sulforaphane protects against rotenone-induced neurotoxicity in vivo: Involvement of the mTOR, Nrf2, and autophagy pathways.
Zhou Q, Chen B, Wang X, et al, Ye J, Shen J, Cao P.
Sci Rep. 2016 Aug 24;6:32206.

https://pubmed.ncbi.nlm.nih.gov/23518299/
Neuroprotective effect of sulforaphane in 6-OHDA-lesioned mouse model of PD.
Morroni F, Tarozzi A, Sita G, Bolondi C, Zolezzi Moraga JM, Cantelli-Forti G, Hrelia P.
Neurotoxicology. 2013 May;36:63-71.

Twice weekly administration of sulforaphane (5mg/kg) for four weeks reduced motor complications, reduced dopaminergic neurodegeneration, and rescued glutathione levels:



https://pubmed.ncbi.nlm.nih.gov/33711331/
Sulforaphane inhibits NLRP3 inflammasome activation in microglia through Nrf2-mediated miRNA alteration.
Tufekci KU, Ercan I, Isci KB, Olcum M, Tastan B, Gonul CP, Genc K, Genc S.
Immunol Lett. 2021 May;233:20-30.

https://pubmed.ncbi.nlm.nih.gov/21254817/
Pharmacological targeting of the transcription factor Nrf2 at the basal ganglia provides disease modifying therapy for experimental parkinsonism.
Jazwa A, Rojo AI, Innamorato NG, Hesse M, Fernández-Ruiz J, Cuadrado A.
Antioxid Redox Signal. 2011 Jun 15;14(12):2347-2360.

Summary of this intervention

Diagnosed with Parkinson's in May 2018, Dr Albert Wright has been investigating the impact of naturally-sourced isothiocyanate activators of NRF2 on his own condition. After decades of experience as a research scientist at the Institut Laue–Langevin in Grenoble, Dr Wright decided to apply his knowledge to identifying a better treatment for Parkinson's.

At diagnosis, Dr Wright experienced non-motor features of sudden acute fatigue, neck, arm and leg pain, poor balance, confusion, quiet voice, urinary urgency, poor sleep, vivid dreams, apathy, and mood changes. His primary motor symptoms were bradykinesia, dystonia (foot), left hand stiffness and pain, general stiffness, stoop, gait change, and left-hand weakness.

In December 2019, he was able to demonstrate to his own satisfaction that a brassica-seed tea (*Brassica rapa* var. *cymosa* in particular) could be prepared to deliver a good yield of the small, but volatile isothiocyanate, 3-butenyl isothiocyanate.

https://pubmed.ncbi.nlm.nih.gov/14759128/
Screening crucifer seeds as sources of specific intact glucosinolates using ion-pair high-performance liquid chromatography negative ion electrospray mass spectrometry.
Bennett RN, Mellon FA, Kroon PA.
J Agric Food Chem. 2004 Feb 11;52(3):428-438.

https://pubmed.ncbi.nlm.nih.gov/31261930/
Bioavailability of Sulforaphane Following Ingestion of Glucoraphanin-Rich Broccoli Sprout and Seed Extracts with Active Myrosinase: A Pilot Study of the Effects of Proton Pump Inhibitor Administration.
Fahey JW, Wade KL, Stephenson KK, et al, Fuchs E, Holtzclaw WD, Cheskin LJ.
Nutrients. 2019 Jun 29;11(7):1489.

Self-experimentation in early 2020 resulted in a profound effect on certain symptoms of his Parkinson's. He experienced a rapid and robust alleviation of his fatigue, pain, urinary problems, and apathy, whilst at the same time slightly increasing his tremor. This experiment was later duplicated using a broccoli seed tea (B. oleracea, var. italica), designed to deliver a therapeutic dose of sulforaphane based upon dose calculations:

https://pubmed.ncbi.nlm.nih.gov/31590459/
Broccoli or Sulforaphane: Is It the Source or Dose That Matters?
Yagishita Y, Fahey JW, Dinkova-Kostova AT, Kensler TW.
Molecules. 2019 Oct 6;24(19):3593.

A wash-out period (March 2020) resulted in a gradual deterioration of improvements and a return to baseline symptoms. Re-administering the broccoli seed tea (April 2020), led to the general improvement in fatigue, pain and urinary problems again. Improved vocal abilities were also noted. There were occasional digestive problems (diarrhea and nausea). A slight reduction in dosing (from 1.5g/day to 1g/day) led to continued improvements in all non-motor symptoms, except olfaction. Reduced bradykinesia, dystonia and stiffness were also noted in terms of motor symptoms. In addition, there was a reduction in dyskinesias.

After 8 months of self-experimentation, the results were then shared with a community of interested individuals living with Parkinsonians, a number of whom expressed their wish to do their own self-experimentation. The results of their efforts were recorded (see clinical history section below).

By November 2020, the dose had been further reduced to 0.8g/day and Dr Wright was no longer taking any painkillers. He also observed an improvement in left/right sequencing (the left hand knowing precisely what the right hand was doing). In May 2021, a further adaptation of the tea processing protocol resolved the gastrointestinal issues and improved internal tremor. Dr Wright noted continued improvement in all non-motor symptoms, except olfaction.

Preparation of the broccoli-seed tea solution:

Glucoraphanin is hydrolysed by the enzyme myrosinase, which is also present in broccoli seeds, but only when crushed in the presence of water. The reaction can take several paths of which only one leads to the formation of sulforaphane. Broccoli seeds also contain a protein called "epithiospecifier" protein (ESP) which acts as a co-factor to myrosinase and directs the hydrolysis reaction to produce an inactive by-product. Unlike glucoraphanin, both ESP and myrosinase are temperature sensitive. ESP is inactivated at temperatures above 55°C whereas myrosinase is destroyed at temperatures above 65-70°C,

which leaves a rather narrow temperature window in which to optimise the yield of sulforaphane:

https://pubmed.ncbi.nlm.nih.gov/15184012/
Heating decreases epithiospecifier protein activity and increases sulforaphane formation in broccoli.
Matusheski NV, Juvik JA, Jeffery EH.
Phytochemistry. 2004 May;65(9):1273-1281.

To ensure inactivation of ESP, the protocol included a step to heat-treat dry broccoli seeds at 60°C followed by adding a few % of untreated white mustard seeds (*Sinapis alba*) as a source of fresh myrosinase. Mustard seeds are not a significant source of ESP. This seed mixture was then reduced to a coarse powder in a coffee grinder and served as the stock source material for making the tea.

The tea was made by adding a defined quantity of the seed powder to water at 60°C. Under these conditions, hydrolysis is complete in a few minutes and the resulting suspension can be filtered through a fine-mesh tea strainer to remove particulate material. An alternative and possibly better method is to extract the glucoraphanin into boiling water so as to ensure complete elimination of the ESP protein and add a few % of white mustard seed once the solution has cooled to below 60°C.

Assessment of target engagement

Sulforaphane and metabolites can readily be assayed in blood and urine to measure the internal dose received:

https://pubmed.ncbi.nlm.nih.gov/33996874/
The Challenges of Designing and Implementing Clinical Trials With Broccoli Sprouts… and Turning Evidence Into Public Health Action.
Fahey JW, Kensler TW.
Front Nutr. 2021 Apr 29;8:648788.

https://pubmed.ncbi.nlm.nih.gov/32070059/
Measuring Sulforaphane and Its Metabolites in Human Plasma: A High Throughput Method.
Langston-Cox A, Anderson D, Creek DJ, Palmer K, Wallace EM, Marshall SA.
Molecules. 2020 Feb 13;25(4):829.

NRF2 and its target genes can be readily assayed from PBMC:

https://pubmed.ncbi.nlm.nih.gov/33396641/
Peripheral Blood NRF2 Expression as a Biomarker in Human Health and Disease.
Neilson LE, Quinn JF, Gray NE.
Antioxidants (Basel). 2020 Dec 30;10(1):28.

https://pubmed.ncbi.nlm.nih.gov/29888232/
Sulforaphane Augments Glutathione and Influences Brain Metabolites in Human Subjects: A Clinical Pilot Study.
Sedlak TW, Nucifora LG, Koga M, et al, Barker PB, Fahey JW, Sawa A.

Mol Neuropsychiatry. 2018 May;3(4):214-222.

In addition to NRF2 levels (compared to baseline measures), elevation in mRNA levels for target genes NAD(P)H:quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HO-1), glutamate-cysteine ligase catalytic subunit (GCLC), catalase (CAT), superoxide dismutase (SOD) could also be evaluated.

This approach has already been explored in PD cohorts:

https://pubmed.ncbi.nlm.nih.gov/31682033/
Systemic activation of Nrf2 pathway in Parkinson's disease.
Petrillo S, Schirinzi T, Di Lazzaro G, et al, Mercuri NB, Piemonte F, Pisani A.
Mov Disord. 2020 Jan;35(1):180-184.

Inflammatory markers could also be considered, such as serum CRP, COX-2, TNF-α, IL-6 and IL-1β:

https://pubmed.ncbi.nlm.nih.gov/32242086/
Biomarker Exploration in Human Peripheral Blood Mononuclear Cells for Monitoring Sulforaphane Treatment Responses in Autism Spectrum Disorder.
Liu H, Zimmerman AW, Singh K, et al, Stephenson KK, Dinkova-Kostova AT, Fahey JW.
Sci Rep. 2020 Apr 2;10(1):5822.

https://pubmed.ncbi.nlm.nih.gov/32784785/
Current Landscape of NRF2 Biomarkers in Clinical Trials.
Yagishita Y, Gatbonton-Schwager TN, McCallum ML, Kensler TW.
Antioxidants (Basel). 2020 Aug 7;9(8):716.

Preclinical data

The broccoli seed tea protocol described in this dossier has not been preclinically tested.

Clinical history

A set of N=1 studies have been conducted with the broccoli seed tea protocol. Eight participants were involved in this pilot work. They were requested to keep all other medications and supplements unchanged during a 6 week analysis and to record their assessment of 30 common symptoms of Parkinson's prior to consuming the tea and each week thereafter for a period of 6 weeks.

The analysed symptoms were sorted into 2 groups, representing 15 motor symptoms and 11 non-motor symptoms. The remaining 4 symptoms in the original list had very low or zero incidence for the participants involved and were excluded. A scale with 4 degrees of symptom severity was adopted with the following scores:

0: insignificant or absent

Broccoli seed extract to reduce oxidative stress in Parkinson's disease

Symptom Record

Identity code: dd/mm/yy			Starting date: 10/30/20				
Symptom	**Baseline**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
Daily product quantity (g)	0	0,5	0,5	0,5	0,5	0,6	0,6
Number of doses per week	7	7	7	7	7	7	7
Daytime urinary urgency	1	1	1	1	1	1	1
Nocturnal urinary frequency	1	1	1	1	1	1	1
Constipation	1	1	1	1	0	0	0
Mood changes	1	1	0	0	0	0	0
Brain fog	1	1	0	0	0	0	0
Lack of motivation	1	0	0	0	0	0	0
General fatigue	1	0	1	0	0	0	0
Sleep quality	1	1	1	1	1	0	0
Dreams, Rem sleep	0	0	0	0	0	0	0
Memory	1	1	1	1	1	0	0
Sense of smell	1	1	1	1	1	1	1
Total non-motor	**10**	**8**	**7**	**6**	**5**	**3**	**3**
Dyskinesia,	0	0	0	0	0	0	0
Dystonia	0	0	0	0	0	0	0
Slowness	1	1	1	1	1	1	1
Rigidity	1	1	1	1	1	1	1
Hand pain	1	1	1	1	1	1	1
Leg pain	0	0	0	0	0	0	0
Balance	1	1	1	1	1	1	1
Speech difficulty	0	0	0	0	0	0	0
Soft voice	0	0	0	0	0	0	0
Eye fatigue	0	0	0	0	0	0	0
Swallowing	0	0	0	0	0	0	0
Coordination	1	1	1	1	1	1	1
Typing ability	1	1	1	1	1	1	1
Walking	0	0	0	0	0	0	0
Tremor	1	1	1	1	1	1	1
Total motor	**7**	**7**	**7**	**7**	**7**	**7**	**7**
Total symptom score	**17**	**15**	**14**	**13**	**12**	**10**	**10**
Overall evaluation							
Notes	a		b				
Events		1		2			

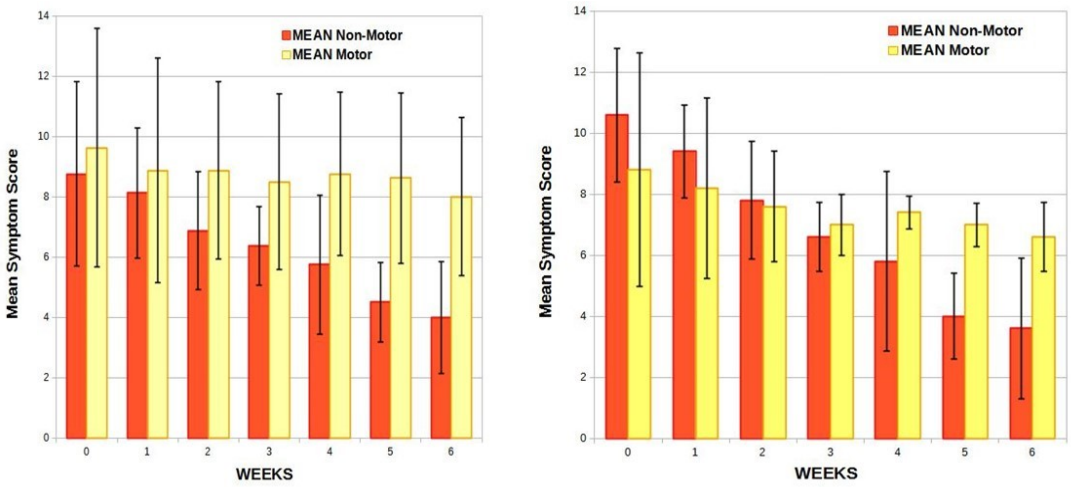
1: moderate but manageable
2: severe or difficult to manage
3: major handicap or disabling

An example of the data sheet for a participant after exclusion of the low incidence symptom records is provided below:
A recommended starting dose of 0.5g of ground seed powder used in the daily dose of tea was applied. If no improvement in symptoms was observed and no adverse effects were experienced, participants were free to modify the quantity of seed powder by 0.1 or 0.2g each week. In the event that a symptom improvement was observed, the dose was to be maintained at that level. In case of adverse effects (the most common being digestive problems), the recommendation was to reduce the dose and/or pause it for a few days.

Results:

Each weekly data point for a given symptom group is the mean score per patient of either the sum of 11 non-motor symptoms or the sum of 15 motor symptoms. The mean grouped non-motor symptom score for all 8 participants (left) showed a rapid and regular decline by 54.3% over the 6-week period, whereas motor symptom score were much less affected (-14.8%). Further examination of the data revealed that 5 patients exhibited a strong and rapid attenuation of their total non-motor symptoms, whereas those of the 3

remaining patients were practically unchanged. A closer examination showed that all the non-responders had declared very much lower total scores for non-motor symptoms at baseline, equal to about half of those for the 5 responders. Their potential for improvement of their non-motor scores was therefore extremely low.



Data from the 5 strong responders alone (right) shows an even stronger, 66% decline in the non-motor score and a modest decline in the motor score of 25%

Individual symptom responses:

The symptoms with the greatest reduction over the course of the experiment were: a) fatigue and b) lack of motivation. Every participant reported an improvement in fatigue and the overall score for fatigue was reduced by 90%. These are symptoms known to be related to compromised energy production by mitochondria. Urinary urgency and frequency, symptoms which have a strong impact on quality of life also responded well. No difference in sense of smell was reported.

Additional clinical trials have been conducted on sulforaphane, including:

https://pubmed.ncbi.nlm.nih.gov/16965241/
Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase I study.
Shapiro TA, Fahey JW, Dinkova-Kostova AT, et al, Wade KL, Ye L, Talalay P.
Nutr Cancer. 2006;55(1):53-62.

https://pubmed.ncbi.nlm.nih.gov/17347138/
Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast.
Cornblatt BS, Ye L, Dinkova-Kostova AT, et al, Talalay P, Kensler TW, Visvanathan K.
Carcinogenesis. 2007 Jul;28(7):1485-1490.

https://pubmed.ncbi.nlm.nih.gov/25431127/
A phase II study of sulforaphane-rich broccoli sprout extracts in men with recurrent prostate cancer.
Alumkal JJ, Slottke R, Schwartzman J, et al, Tucker E, Kleinschmidt R, Mori M.
Invest New Drugs. 2015 Apr;33(2):480-489.

https://pubmed.ncbi.nlm.nih.gov/29507103/
Transcription Factor NRF2 as a Therapeutic Target for Chronic Diseases: A Systems Medicine Approach.
Cuadrado A, Manda G, Hassan A, et al, Valverde AM, Guney E, Schmidt HHHW.
Pharmacol Rev. 2018 Apr;70(2):348-383.

Dosing

A 4-week randomized controlled clinical trial investigating the effect of 5 grams (yielding 112.5 µmol sulforaphane) and 10 grams (yielding 225 µmol sulforaphane) daily of broccoli sprout powder on 81 individuals with Type 2 diabetes reported that sulforaphane could reduce lipid peroxidation, especially significant at the higher dose (P=0.001 for treatment effect). No SAEs occurred, and only mild gastrointestinal events, including flatulence (n=5) and increased defecation (n=1) were reported.

https://pubmed.ncbi.nlm.nih.gov/21559038/
Broccoli sprouts reduce oxidative stress in type 2 diabetes: a randomized double-blind clinical trial.
Bahadoran Z, Mirmiran P, Hosseinpanah F, Hedayati M, Hosseinpour-Niazi S, Azizi F.
Eur J Clin Nutr. 2011 Aug;65(8):972-7.

Delivery

Sulforaphane is chemically unstable unless highly purified. It is however readily available in a stable form as the precursor molecule glucoraphanin and the enzyme myrosinase in broccoli sprouts or seeds. Ingesting these two components does not however guarantee the delivery of the molar equivalent quantity of sulforaphane to that of the ingested glucoraphanin. Conversion rates in humans can be highly variable.

To avoid this variability, Dr Wright developed a method for the preparation of a broccoli seed tea designed to optimize the conversion of glucoraphanin to sulforaphane prior to ingestion. This method was used in his own self-experimentation and the 8 x n =1 experiments reported above.

https://pubmed.ncbi.nlm.nih.gov/33996874/
The Challenges of Designing and Implementing Clinical Trials With Broccoli Sprouts… and Turning Evidence Into Public Health Action.
Fahey JW, Kensler TW.
Front Nutr. 2021 Apr 29;8:648788.

Safety/Tolerability

Sulforaphane from cruciferous vegetables is considered to be safe and nontoxic.

Based on the results from toxicity studies, the median toxic dose (TD_{50}) and the median lethal dose (LD_{50}) of sulforaphane were 191.58 mg/kg and 212.67 mg/kg, respectively.

https://pubmed.ncbi.nlm.nih.gov/33023225/
Discovery of Sulforaphane as a Potent BACE1 Inhibitor Based on Kinetics and Computational Studies.
Youn K, Yoon JH, Lee N, Lim G, Lee J, Sang S, Ho CT, Jun M. Nutrients. 2020 Oct 2;12(10):3026.

Previous clinical studies of some NRF2 activators have reported increased rate of cardiovascular events:

https://pubmed.ncbi.nlm.nih.gov/24206459/
Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease.
de Zeeuw D, Akizawa T, Audhya P, et al, Wittes J, Wrolstad D, Chertow GM; BEACON Trial Investigators.
N Engl J Med. 2013 Dec 26;369(26):2492-503.

https://pubmed.ncbi.nlm.nih.gov/32733266/
The Dark Side of Nrf2 in the Heart.
Zang H, Mathew RO, Cui T.
Front Physiol. 2020 Jul 9;11:722.

Thus, it would be prudent to include cardiac monitoring in the proposed clinical trial.

Pharmacokinetics/pharmacodynamics

Due to sulforaphane's small molecular weight and its relatively high lipophilicity, it is rapidly absorbed in the jejunum across the enteric cells after oral administration. It reaches the highest concentrations in plasma 3 hours after consumption (approximately 0.9 µmol/L), and slowly decreases after the second hour, having an approximate half-life of 2.2 hour. Total elimination is reached in the 12th hour post-ingestion. NRF2 activation leads to the expression of antioxidants and antioxidant enzymes over a longer period with expression of NQO1 and HO-1 peaking at about 12 hours and remaining elevated for more than 48 hours.

https://pubmed.ncbi.nlm.nih.gov/17347138/
Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast.
Cornblatt BS, Ye L, Dinkova-Kostova AT, et al, Talalay P, Kensler TW, Visvanathan K.
Carcinogenesis. 2007 Jul;28(7):1485-90.

https://pubmed.ncbi.nlm.nih.gov/18566435/
The Transcription Factor Nrf2 Is a Therapeutic Target against Brain Inflammation
Innamorato NG, Rojo AI, García-Yagüe AJ, Yamamoto M, de Ceballos ML, Cuadrado A.
J Immunol July 1, 2008, 181 (1) 680-689.

https://pubmed.ncbi.nlm.nih.gov/21240766/
Sulforaphane absorption and excretion following ingestion of a semi-purified broccoli powder rich in glucoraphanin and broccoli sprouts in healthy men.
Cramer JM, Jeffery EH.
Nutr Cancer. 2011;63(2):196-201.

The primary sulforaphane metabolism sites are the intestinal walls, the liver (where it is conjugated with glutathione), the kidney (where it is conjugated with N-acetyl cysteine), and the bladder, and it accumulates mainly in those same organs, and in lower concentrations in plasma, skin, and lung tissues:

https://pubmed.ncbi.nlm.nih.gov/19035553/
Glucosinolates in Brassica vegetables: the influence of the food supply chain on intake, bioavailability and human health.
Verkerk R, Schreiner M, Krumbein A, et al, Gerhäuser C, Mithen R, Dekker M.
Mol Nutr Food Res. 2009 Sep;53 Suppl 2:S219.

https://pubmed.ncbi.nlm.nih.gov/24975513/
Isothiocyanate metabolism, distribution, and interconversion in mice following consumption of thermally processed broccoli sprouts or purified sulforaphane.
Bricker GV, Riedl KM, Ralston RA, Tober KL, Oberyszyn TM, Schwartz SJ.
Mol Nutr Food Res. 2014 Oct;58(10):1991-2000.

Brain penetrance

Both preclinical and clinical research indicates that CNS penetrance of sulforaphane.

In healthy human volunteers, a consistent increase in brain glutathione levels (as determined by MRS) in response to seven days of sulforaphane treatment was been reported, indicating brain penetrance:

https://pubmed.ncbi.nlm.nih.gov/29888232/
Sulforaphane Augments Glutathione and Influences Brain Metabolites in Human Subjects: A Clinical Pilot Study.
Sedlak TW, Nucifora LG, Koga M, et al, Barker PB, Fahey JW, Sawa A.
Mol Neuropsychiatry. 2018 May;3(4):214-222.

In addition, gavage administration of sulforaphane penetrated the blood brain barrier in its intact structure and accumulated in brain tissues with a maximum increase and disappearance after 15 min and 2 h, respectively in mice:

https://pubmed.ncbi.nlm.nih.gov/21254817/
Pharmacological targeting of the transcription factor Nrf2 at the basal ganglia provides disease modifying therapy for experimental parkinsonism.
Jazwa A, Rojo AI, Innamorato NG, Hesse M, Fernández-Ruiz J, Cuadrado A.
Antioxid Redox Signal. 2011 Jun 15;14(12):2347-2360.

https://pubmed.ncbi.nlm.nih.gov/21681606/
Metabolism and tissue distribution of sulforaphane in Nrf2 knockout & wild-type mice.
Clarke JD, Hsu A, Williams DE, Dashwood RH, Stevens JF, Yamamoto M, Ho E. Pharm Res.
2011 Dec;28(12):3171-3179.

Summary

Sulforaphane is recognized as a potent inducer of the endogenous antioxidant transcription factor NRF2. It has also been shown to have beneficial effects in models of Parkinson's. Patient researcher Dr Albert Wright – a chemist by training - devised a broccoli seed tea solution for the delivery of a therapeutic amount of sulforaphane and improved the protocol via self-experimentation.

Preliminary pilot N=1 studies by other volunteers have provided encouraging results - particularly in terms of non-motor symptoms. A wash out period experienced by Dr Wright suggests that the beneficial effects are not due to a placebo response.

Dr Wright now has experience of self-administration of the broccoli seed tea for a total of 20 months and has continued to benefit from decreasing frequency and intensity of all non-motor symptoms (including complete remission from acute pain, acute fatigue and urinary urgency). Over the last eight months he has also noted a modest reduction of the intensity of motor symptoms including tremor. A further observation is the reduced need for levodopa (from 500 to 300 mg/day) and more recently, reducing the frequency of the broccoli seed tea (now once every 3 days) is giving better well-being.

The initial results of this patient-initiated study may be symptomatic, but in order to determine whether the broccoli seed tea has any disease modifying potential, a larger, longer controlled study is required. Cure Parkinson's is presenting this dossier to the iLCT committee with the goal of conducting a Phase IIa safety and biomarker study in a cohort of individuals with idiopathic Parkinson's.

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More information about Albert's research can be found at:
https://www.researchgate.net/publication/351099763_A_pilot_study_of_a_broccoli-seed_tea_by_eight_Parkinson's_disease_patients