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.

GM-CSF

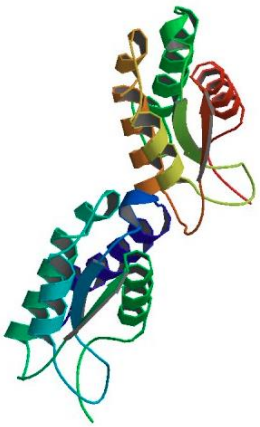
Evidence Summary

Preclinical studies suggest that activation of the immune system by GM-CSF may improve the ability of microglia to get rid of amyloid, but consistent increases in inflammation in preclinical studies make it unclear what effects it may have in Alzheimer's patients.

Neuroprotective Benefit: GM-CSF has beneficial effects in Alzheimer's animal models, though its immunostimulatory effects in Alzheimer's may be detrimental depending on the stage of the disease.

Aging and related health concerns: GM-CSF's effects on certain age-related diseases can be beneficial or detrimental, depending on the disease.

Safety: GM-CSF has some well-known side effects that can be mitigated, though the long-term side effects in elderly individuals is not clear.

Availability: Available with a prescription as sargramostim (Leukine) or molgramostim with IV or subcutaneous administration	**Dose:** 250µg/m² per day in Alzheimer's trial, 6µg/kg per day in Parkinson's trial	**Chemical formula:** $C_{639}H_{1006}N_{168}O_{196}S_8$ **MW:** 14434.5 Da **Source:** Drugbank 
Half life: 162 minutes (subcutaneous)	**BBB:** Penetrant (in animals)	
Clinical trials: 1 ongoing in 40 patients	**Observational studies:** 1 study in 95 patients	

What is it?

Granulocyte-macrophage colony stimulating factor (GM-CSF) is a secreted glycoprotein that promotes the proliferation and differentiation of hematopoietic stem cells to myeloid cells (macrophages and dendritic cells). It is one of four colony-stimulating factors (macrophage-CSF, granulocyte-CSF, and IL-3 are the other three). It is used to promote white blood cell recovery after bone marrow transplant. Other uses of GM-CSF include improvement of neutrophil recovery after chemotherapy in patients with acute myeloid leukemia and to improve survival in patients exposed to myelosuppressive doses of radiation (sargramostim label). Pharmaceutical-grade GM-CSF drugs include sargramostim (Leukine) and molgramostim (Leucomax).

The hypothesis for its use in Alzheimer's disease stems from the observation that patients with rheumatoid arthritis (RA) have a reduced risk for Alzheimer's disease. At first it was thought that the anti-inflammatory medications taken by individuals with RA may reduce the risk for Alzheimer's. However, after the failure of many NSAID trials, some investigators thought there might be something intrinsic in RA for reducing risk. GM-CSF is increased in joints in RA, and research begun to look at the link between GM-CSF and Alzheimer's.

Later research suggested that GM-CSF may also be a neurotrophic factor in the brain. GM-CSF and its receptor, GM-CSFR, are expressed in the brain, and GM-CSF may be involved in the proliferation and differentiation of neural stem cells, astrocytes, and microglia. GM-CSF signals through a heterodimeric receptor with an α-binding subunit (GM-CSFRα) and a β-signaling subunit (GM-CSFRβ) (Ridwan et al, 2012).

Neuroprotective Benefit: GM-CSF has beneficial effects in Alzheimer's animal models, though its immunostimulatory effects in Alzheimer's may be detrimental depending on the stage of the disease.

Types of evidence:

- One observational study in cancer patients
- One pilot study in Alzheimer's disease
- One pilot study in Parkinson's disease
- Multiple biomarker studies
- Multiple preclinical studies

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

In 95 cancer patients undergoing hematopoietic cell transplantation (a situation which can cause cognitive decline), patients concurrently taking recombinant GM-CSF plus granulocyte-colony stimulating factor (G-CSF) had greater cognitive improvement at 6 months (but not 12 months) than those taking only G-CSF. At 12 months, both groups had improved about equally (Jim et al, 2012).

Human research to suggest benefits to patients with dementia

Interim results from a small study of 20 patients with Alzheimer's disease suggested that sargramostim (250ug/m^2 per day for five days per week) over three weeks improved cognition and function compared to placebo. However, there were no improvements in follow up at 45 and 90 days (Potter et al, 2017) (from an abstract, details unavailable).

In a randomized controlled trial (RCT) of 20 Parkinson's patients, 56-day treatment with sargramostim (6ug/kg per day) increased the number of regulatory T cells, altered immune-linked plasma metabolites, and improved scores of motor function. Side effects included injection site reactions (100% of treated, 40% of non-treated), abnormal laboratory values (increased white blood cell count – 100% of treated, 30% non-treated), and abnormal pain at non-injection sites (e.g. bone extremities, chest-tightening – 70% of treated, 30% non-treated) (Gendelman et al, 2017).

Histopathology

GM-CSF and GM-CSFRα were expressed throughout the brain in Alzheimer's patients and non-Alzheimer's patients, primarily in neurons with some expression in astrocytes, ependymal cells, and cells in the choroid plexus. Although there was no change in GM-CSF expression, GM-CSFRα expression was reduced in the hippocampus of Alzheimer's patients and slightly reduced in the cortex (Ridwan et al, 2012).

Biomarkers

In a study examining multiple cerebral spinal fluid (CSF) inflammatory biomarkers, Taipa et al (2019) reported that GM-CSF CSF levels were not increased in patients with Alzheimer's disease or frontotemporal dementia. However, there was an inverse correlation with disease progression (suggesting that higher levels were more beneficial) . Llano et al (2012) also reported no difference in CSF levels of GM-CSF in Alzheimer's patients or controls. However, Tarkowski et al (2001) reported an increase in CSF and serum levels of GM-CSF in patients with Alzheimer's and vascular dementia. The differences in the results could be due to variability of the assays or the different characteristics of the patients. Each of the previous studies was small (~20 patients per group).

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

Labeled GM-CSF was reported to cross the blood brain barrier in rodents (McLay et al, 1997). However, the GM-CSF used in the study was murine, recombinant GM-CSF. Sargramostim has a leucine instead of a proline at position 23 and is produced in yeast, thus it has a different glycosylation pattern. Molgramostim is produced in *E. Coli* and is unglycosylated. It is not clear whether these differences would alter the blood brain barrier penetration in humans.

In an *ex vivo* study of brain explants from aged Alzheimer's mice, treatment with GM-CSF, but not other molecules (IL-10, TGF-B, IL-6, IL-12/p40), reduced the amyloid halo around plaques, leaving only a core amyloid plaque. This was accompanied by the proliferation and accumulation of microglia around amyloid plaques. Additionally, co-culturing microglia from young mice, as well as young GM-CSF knockout mice, also reduced the amyloid halo around plaques, suggesting that other young factors besides GM-CSF can increase microglia uptake of amyloid (Daria et al, 2017). In an Alzheimer's animal model, mouse recombinant GM-CSF (50ug/kg – once/day for ten days followed by two rounds of five injections) increased the number of regulatory T cells in the plasma. There were no effects on the number of amyloid plaques, though amyloid beta oligomers were decreased in the brain. GM-CSF also improved cognition and increased the number of microglia (i.e. Iba1 expression), increased synaptic

density, and increased neurogenesis (Kiyota et al, 2018). In another study in an Alzheimer's animal model, a single intrahippocampal injection of GM-CSF reduced amyloid burden one week later. Twenty daily subcutaneous injections reduced amyloid load, increased microglial markers (i.e. Iba1 expression), increased synaptic density, and improved cognition (Boyd et al, 2010).

On the other hand, Shang et al (2016) reported that GM-CSFRβ (but not GM-CSFRα) mRNA was increased in Alzheimer's patients' monocytes. In an *in vitro* model of the blood brain barrier, monocytes with high GM-CSFRβ had increased permeability through the barrier and reduced the expression of tight junction proteins. Intracerebral injection of a GM-CSF neutralizing antibody reduced the infiltration of peripheral monocytes into an Alzheimer's mouse brain. In addition, Manczak et al (2009) reported that five days after intracerebral injection of an GM-CSF neutralizing antibody in an Alzheimer's animal model there was a reduction in amyloid plaques, a non-significant reduction in Aβ42, a non-significant increase in Aβ40, and reductions in a number of inflammatory cytokines (IL-6, IL-1) and inflammation markers (CD40, CD11b).

The reason for these discrepant results is unclear. Both Kiyota et al (2018) and Manczak et al (2009) used older Alzheimer's animals while Boyd et al (2010) started treating animals at a younger age. The doses or types of administration could be responsible for some of the opposing effects.

Non-Alzheimer's animal models

Intracerebral ventricular injection of GM-CSF also increased neuroinflammation which was prevented by co-injection of a GM-CSF neutralizing antibody (Reddy et al, 2009). In addition, intrahippocampal injection of GM-CSF was used as a model of schizophrenia. It increased inflammation and behavioral deficits, results that were partially reduced by coadministration of minocycline (Zhu et al, 2014).

GM-CSF knockout mice were reported to have cognitive deficits. Although there were no gross structural changes in GM-CSF knockout mice hippocampi, there were microstructural alterations, including a reduction in dendrite length and spine density, though this did not result in a reduction of hippocampal long-term potentiation. AAV-viral reduction or enhancement of GM-CSFRα expression in the hippocampus slightly reduced or increased cognition, respectively (Krieger et al, 2012). Similarly, GM-CSF-enriched human umbilical cord plasma improved cognition in aged mice, though it did not alter levels of neurogenesis (Castellano et al, 2017).

Conclusion
The results from preclinical studies are conflicting. However, GM-CSF treatment appears to increase markers of inflammation in multiple animal models. There is an ongoing debate whether markers of inflammation are beneficial (they are a sign of microglial uptake of amyloid) or detrimental (a sign of the release of pro-inflammatory cytokines).

APOE4
None reported

Aging and related health concerns: GM-CSF's effects on certain age-related diseases can be beneficial or detrimental, depending on the disease.

Types of evidence:
- 1 clinical study in coronary artery disease
- Multiple preclinical studies of cardiovascular disease and stroke

Cardiovascular Disease: MIXED
The potential effects of GM-CSF on atherosclerosis are mixed with one study in hyperlipidemic rabbits suggesting a reduction in plaque size (45% vs. 74% for GM-CSF vs. placebo) (Shindo et al, 1999) and another study suggesting an increase in plaque size in ApoE-/- mice on a high fat diet (25% vs. 10% for GM-CSF vs. placebo) (Haghighat et al, 2007). Similarly, injection of GM-CSF increases the proliferation of cells in an atherosclerotic lesion in a mouse model of atherosclerosis while injection of an anti-GM-CSF antibody reduces proliferation (Zhu et al, 2009). Other preclinical cardiovascular models suggest that GM-CSF promotes myeloid and dendritic cell infiltration, proliferation, and pro-inflammatory cytokine release (Mindur and Swirski, 2019). A summary of the effects of GM-CSF (and the related IL-3) on difference inflammatory disease can be found in Borriello et al, 2019.

However, GM-CSF (and G-CSF) may be beneficial in certain cardiovascular complications (such as ischemia or myocardial infarction), as they can also promote arteriogenesis and neovascularization in preclinical studies (Kovacic et al, 2007). In addition, a small clinical study reported that local administration of GM-CSF over two weeks improved coronary collateral flow in patients with advanced coronary artery disease (Seiler et al, 2001).

In support of these studies, multiple preclinical studies of ischemic stroke have reported that administration of GM-CSF reduces infarct size, reduces cell death, and improves neurological

outcomes. CSF levels of GM-CSF are increased in humans after acute stroke, though levels were not correlated with improved neurological outcomes (Lanfranconi et al, 2011).

Safety: GM-CSF has some well-known side effects that can be mitigated, though the long-term side effects in elderly individuals is not clear.

Types of evidence:

- 1 meta-analysis of RCTs in Crohn's disease
- 1 RCT in Parkinson's disease

In a meta-analysis of randomized controlled trials (RCTs) for Crohn's disease, more patients in the sargramostim group experienced a serious adverse event than patients in the placebo group (2 studies, 251 participants; pooled RR = 2.17; 95%CI 0.82-5.70). Adverse events include injection site reactions (90% vs 12% in drug vs. placebo), bone pain (37% vs. 7%), musculoskeletal chest pain (36% vs. 5%), and dyspnea (13% vs. 0%) (Roth et al, 2012). Other reported adverse events include hypersensitivity reactions (anaphylactic reactions), risk of myelosuppression when administered within 24 hours of chemotherapy, edema and capillary leak syndrome, supraventricular arrhythmias (reversible after discontinuation), leukocytosis (an increase in white blood cell count), possibly myeloid malignancies, and induction of neutralizing anti-drug antibodies (sargramostim label).

Although long-term effects of sargramostim in Alzheimer's patients are not known, in a Parkinson's trial side effects included injection site reactions (100% of treated, 40% of non-treated), abnormal laboratory values (increased white blood cell count – 100% of treated, 30% non-treated), and abnormal pain at non-injection sites (e.g. bone extremities, chest-tightening – 70% of treated, 30% non-treated) (Gendelman et al, 2017).

Sargramostim should not be used in those who have a hypersensitivity to GM-CSF or yeast-derived products. A formulation containing benzyl alcohol is toxic to babies, and sargramostim has not been tested in pregnant women (sargramostim label).

Drug interactions:
Sargramostim is associated with many moderate drug interactions, most of which are cancer drugs (drugs.com).

Sources and dosing:
GM-CSF is available as sargramostim or margramostim. Doses used in clinical trials include 250µg/m² of sargramostim per day five days per week in an Alzheimer's trial, 6µg/kg of sargramostim per day in Parkinson's trial.

Research underway:
Sargramostim (Leukine) is currently in a phase 2 study for Alzheimer's Disease (NCT01409915). One small study is ongoing for cancer (NCT03790670). More than 350 studies are ongoing as a combination with other drugs for cancers.

Search terms:
granulocyte-macrophage colony stimulating factor +
Alzheimer, aging, atherosclerosis, lifespan, cardiovascular
sargramostim

Websites:
- Clinicaltrials.gov
- Pubmed
- Pubchem
- Drugbank
- DrugAge