

# The 7 Factors of Wellness

and the art and science  
of how to disable our immune system  
so we cannot stay well  
or regain health when we already lost it

Dietrich Klinghardt MD, PhD

Seattle, March 2019

# The 7 Factors of Wellness and Chronic Illness

## The Physical Body

- 1. Structural imbalances** (poor occlusion, spinal subluxations, fascial adhesions, poorly healed injuries)
- 2. Biochemical needs/deficiencies** (hormones, vitamins, minerals, enzymes and co-factors, water, oxygen, hydrogen)
- 3. Toxins** (microbial endo-and exotoxins, man-made chemicals, heavy metals, metabolic waste, senescent cells)

## The Energy Body

- 4. Biophysical stress** (microwave from cell-phone broadcasting or smart meter, household currents and fields, ground current, light pollution from computers, cell-phones, CFLs and fluorescent lights, magnetic fields, geopathic stress from mother earth)
- 5. Food, environmental and emotional “allergies”** (with immediate change in exclusion zone water, brain wave and autonomic nervous system activity followed by secondary level 1 phenomena such as cytokine and mast cell activation - and tertiary adrenal hormone secretion)
- 6. Energetic perturbances** (active scars with frictional electric discharges, metal implants in jaw or joints, charge build-up in spinal membranes and fascia, misfiring of autonomic and spinal ganglia, dissimilar metals in dental restorations, tattoos and piercings)

## The Higher Bodies

### 7. Psycho-spiritual issues:

Level 3: unresolved emotional and mental traumata and conflicts (from the personal biography)

Level 4: unresolved ancestral traumata and conflicts, curses and thought-field influences, carry-over of traumata and conflicts from past lives and pre-life realities

Level 5: guiding and correcting influences from the source (that may be perceived as illness, accident o

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The relevance of treating retroviral activity in chronic illness was recognized by this author with the use of the muscle-tension biofeedback method ART (developed by D.Klinghardt)

[This study shows that ART is both reliable and valid in allergy testing](#)

Altern Ther Health Med. 2018 Jan 15. pii: AT5703. [Epub ahead of print]

Autonomic Response Testing Compared With Immunoglobulin E Allergy Panel Test Results: Preliminary Report.

[Frandsen A](#), [McClure M](#), [Chung MK](#), [LaRiccia PJ](#).

### Abstract

*Chronically ill patients who have failed standard medical assessment and therapies are often assessed by integrative medical providers for atypical manifestations of allergies as the possible source or contributing factor(s) to their condition. Skin testing and immunoglobulin E (IgE) allergy panels increase the cost of care in these patients. Objective • The objective of this study was to determine the accuracy of autonomic response testing (ART) as compared with IgE allergy panel blood tests.*

*Design • This study was a retrospective chart review of patients who had ART and blood drawn for an IgE allergy panel at the same office visit. Outcome Measures • Sensitivity, specificity, positive predictive value, negative predictive value, overall accuracy, phi coefficient, and Cohen's kappa were calculated.*

*Results • A total of 14 charts were reviewed. All measures of accuracy were of either useful or excellent strength. The **strength of association measures** of the phi coefficient and Cohen's kappa **were strong**. Conclusion • This first and preliminary evaluation of the allergy assessment utility of ART is very promising and reveals the need for more vigorous follow-up studies.*

Frandsen, A., McClure, M., Chung, M. K., & LaRiccia, P. J. (2018). Autonomic Response Testing Compared With Immunoglobulin E Allergy Panel Test Results: Preliminary Report. *Alternative therapies in health and medicine*. 2018 - ncbi.nlm.nih.gov

**In this clinical study we could show, that when ART finds dysfunction in an organ or tissue, ultrasound can confirm the dysfunction in all cases. ART is a reliable tool to detect tissue dysfunction, establish an accurate diagnosis and effective treatment protocols**

The Ruggiero-Klinghardt (RK) Protocol for the Diagnosis and Treatment of Chronic Conditions

with Particular Focus on Lyme Disease

American Journal of Immunology, March 2017

Dietrich Klinghardt and Marco Ruggiero

**Abstract:** Here we describe the Ruggiero-Klinghardt (RK) Protocol that is based on **integration of Autonomic Response Testing (ART) with diagnostic ultrasonography** and on application of therapeutic ultrasounds; the latter are used as a provocation tool and as an instrument to optimize drug uptake and utilization in specific areas of the body. This protocol consists of a precise sequence of diagnostic and therapeutic procedures with the ultimate goal of improving sensitivity and specificity of diagnosis at the same time evaluating and optimizing efficacy of treatments in chronic conditions including, but not limited to, persistent Lyme disease. The RK Protocol represents **a paradigm shift in diagnostics and therapeutics: Thus, compartmentalized microbes, transformed cells, toxins and metabolites could be detected using a safe and non-invasive method.** In addition, the RK Protocol allows optimization of efficacy of drugs and other therapeutic interventions. Although the RK Protocol was initially developed for persistent Lyme disease, it shows significant potential in conditions ranging from cancer to neurodegenerative diseases and autism. In oncology, the RK Protocol may serve to facilitate early diagnosis and to increase sensitivity of cancer cells to the killing effects of a variety of remedies ranging from conventional radio- and chemotherapy to more recent forms of immunotherapy. Thus, the 1st goal of the RK Protocol is diagnostic: That is, to make pathogens, toxins, transformed cells and cells infected by viruses that are inaccessible to conventional diagnostic and therapeutic tools, “visible” to the therapist who can detect them with laboratory methods and deal with them with appropriate interventions; and also to make them “visible” to the immune system that can fight them in a physiological manner. The 2nd goal is to optimize drug uptake and utilization in the organs and tissues studied and targeted with these procedures.

**Keywords:** Lyme, Ultrasound, Autonomic Response Testing, Immune System, Imaging, Brain

25 year old woman with POTS and severe fatigue and brain fog. She tested negative with the Western Blot and appropriate IgG/IgM tests, Immunofluorescence - but positive for all 3 pathogens with ART

<b>DNA CONNEXIONS</b>			
Telephone: 888-843-5832 TIN: 47-2642690		Fax: 719-548-8220	
Lab Director: Christopher W. Shade, PhD, NRCC-EAC		Lab Manager: Leslie Douglas, PhD	
Patient: <b>Douglas, Diandra W</b>		Lyme Panel	
Doctor: <b>Dr. Kingham</b>		Test ID: <b>10441</b>	
<u>Sample Collected</u>	<u>Sample Received</u>	<u>Sample Tested</u>	<u>Test Reported</u>
02/24/2017	03/01/2017	04/04/2017	04/07/2017
Sample type: <b>Urine</b>		Test performed by: L. Douglas	
<p>This test utilizes the polymerase chain reaction (PCR) technology to detect the presence of targeted microbial DNA for the causative agent of Lyme disease and common tick-transmitted co-infections. Sensitivity of the test is 1 to 10 microbes with a specificity exceeding <math>5 \times 10^{18}</math>.</p> <p style="text-align: center;"><b>The <span style="background-color: yellow;">✓</span>highlighted microbes were detected in the submitted sample:</b></p> <ul style="list-style-type: none"><li>Borrelia burgdorferi F7</li><li>B. burgdorferi Osp A</li><li>B. burgdorferi Osp B</li><li><span style="background-color: yellow;">✓</span>B. burgdorferi Osp C-NSA</li><li>Babesia microti</li><li><span style="background-color: yellow;">✓</span>Babesia divergens-NSA</li><li>Babesia duncani</li><li>Bartonella bacilliformis</li><li><span style="background-color: yellow;">✓</span>Bartonella henselae-NSA</li><li>Bartonella quintana</li><li>Borrelia miyamotoi</li><li>Borrelia recurrentis</li><li>Ehrlichia chaffeensis</li><li>Anaplasma phagocytophilum</li><li>NONE</li></ul> <p><b>NSA:</b> Species specific <u>target microbial DNA was detected</u> but amplification product was not of expected size. More commonly detected in individuals with long-term infections. Product size differential possibly due to: degraded DNA, mutation of species, unspecified subspecies, other.</p> <p><b>Interpretation of Results Disclaimer:</b> DNA Connexions is not a clinical diagnostic laboratory and cannot provide a diagnosis for disease and/or subsequent treatment. These results are from DNA PCR testing, and indicate the presence of disease-causing agents known to be transferred by ticks. A positive result indicates the presence of DNA from B. burgdorferi and/or other tick-transmitted organisms. A negative result only indicates the absence of detectable targeted organismal DNA in the submitted specimen. The information is supplied as a courtesy to health care providers to aid in an overall assessment. This information alone should not be used to diagnose and/or treat a health problem or disease. All reported results are intended for research purposes only and consultation with a qualified health care provider is required.</p>			

# Introduction: Chronic illness treatment basics (KlinghardtInstitute.com)

## **Clean up your home:**

- Mitigate home electromagnetic radiation (Stetzer filters, absolutely no WiFi, appropriate shielding, no CFLs, intelligent handling of the cellphone).
- Measuring and mitigating home mold and internal mold
- Decreasing noise and light pollution Evaluation of food and environmental allergies – avoidance and treatment

## **Decrease your toxic load:**

- detoxing aluminium (silica, ionic footbath, alumina C 30, ecklonia cava from BioPure), lead (CaEDTA, Vit C), Mercury (OSR).
- Remove glyphosate: Restore, Matrix Minerals from BioPure (Shehata, Awad A., et al. "Distribution of glyphosate in chicken organs and its reduction by humic acid supplementation." *The Journal of Poultry Science* 51.3 (2014): 333-337)
- Treat pathogens that modulate your immune system (so you become a comfortable host): retroviruses, DNA viruses, Lyme and Co, Mold and parasites

## **Repair**

- the gut barrier (food allergies, Bravo suppositories, Megaspore/Thrive)
- and blood brain barrier (progesterone, AUT)

## **Psychotherapy**

- Clean up you personal and family biography with appropriate ongoing therapy

Immunome Res 2013, 9:1 <http://dx.doi.org/10.4172/1745-7580.1000069>

## Aluminum's Role in CNS-immune System Interactions leading to Neurological Disorders

Shaw CA<sup>1,2,3\*</sup>, Kette SD<sup>4</sup>, Davidson RM<sup>5</sup> and Seneff S<sup>6</sup>

Neural Dynamics Research Group, Department of Ophthalmology and

### Abstract

Multisystem interactions are well established in neurological disorders, in spite of conventional views that only the central nervous system (CNS) is impacted. We review evidence for **mutual interactions between the immune and nervous systems** and show how these seem to be **implicated in the origin and progression of nervous system disorders**. Well-established immune system triggers leading to autoimmune reactions are considered. Of these, aluminum, **a known neurotoxicant, may be of particular importance**. We have demonstrated elsewhere that aluminum has the potential to induce damage at a range of levels in the CNS leading to neuronal death, circuit malfunction and ultimately, system failure. Aluminum is widely used as an adjuvant in various vaccine formulations and has been implicated in a multisystem disorder termed “autoimmune/inflammatory syndrome induced by adjuvants” (ASIA). The implications of aluminum-induced ASIA in some disorders of the CNS are considered. We propose a unified theory capturing a progression from a local response to a systemic response initiated by disruption of water-based interfaces of exposed cells.

Mold, Matthew, et al. "Aluminium in brain tissue in autism." *Journal of Trace Elements in Medicine and Biology* 46 (2018): 76-82.

## 1. Introduction

[Autism spectrum disorder](#) (ASD) is a group of [neurodevelopmental conditions](#) of unknown cause. It is highly likely that both genetic [\[1\]](#) and environmental [\[2\]](#) factors are associated with the onset and progress of ASD while the mechanisms underlying its [aetiology](#) are expected to be multifactorial [\[3\]](#), [\[4\]](#), [\[5\]](#), [\[6\]](#). Human exposure to aluminium has been implicated in ASD with conclusions being equivocal [\[7\]](#), [\[8\]](#), [\[9\]](#), [\[10\]](#). To-date the majority of studies have used hair as their indicator of human exposure to aluminium while aluminium in blood and urine have also been used to a much more limited extent. [Paediatric vaccines](#) that include an aluminium [adjuvant](#) are an indirect measure of infant exposure to aluminium and their burgeoning use has been directly correlated with increasing prevalence of ASD [\[11\]](#). Animal models of ASD continue to support a connection with aluminium and to aluminium adjuvants used in human [vaccinations](#) in particular [\[12\]](#). Hitherto there are no previous reports of aluminium in brain tissue from donors who died with a diagnosis of ASD. We have measured aluminium in brain tissue in [autism](#) and identified the location of aluminium in these tissues.

## 5. Conclusions

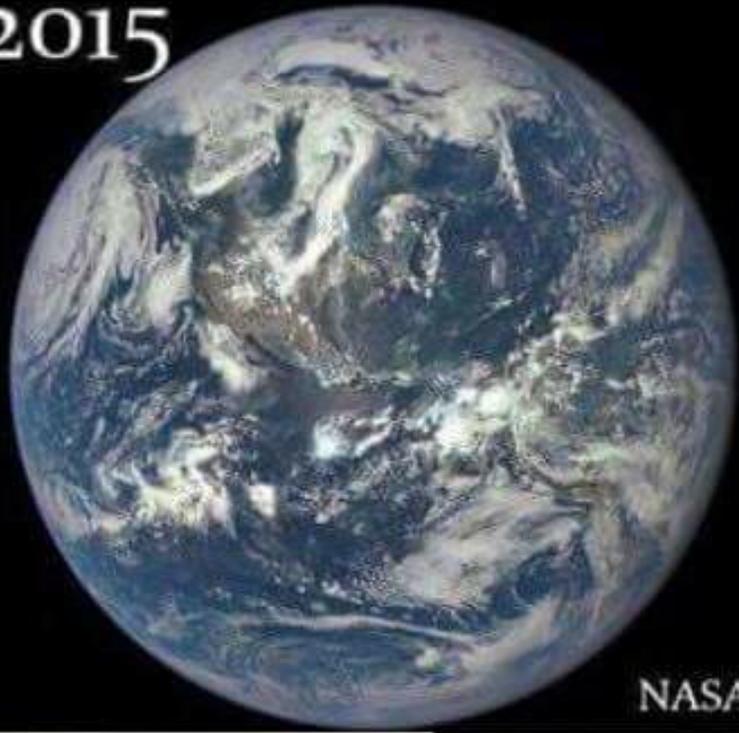
We have made the first measurements of **aluminium in brain tissue in ASD and we have shown that the brain aluminium content is extraordinarily high**. We have identified aluminium in brain tissue as both extracellular and intracellular with the latter involving both neurones and [non-neuronal cells](#). The presence of aluminium in [inflammatory cells](#) in the [meninges](#), vasculature, [grey and white matter](#) is a standout observation and could implicate aluminium in the [aetiology](#) of ASD.

# Aluminum Sky – where does it go?

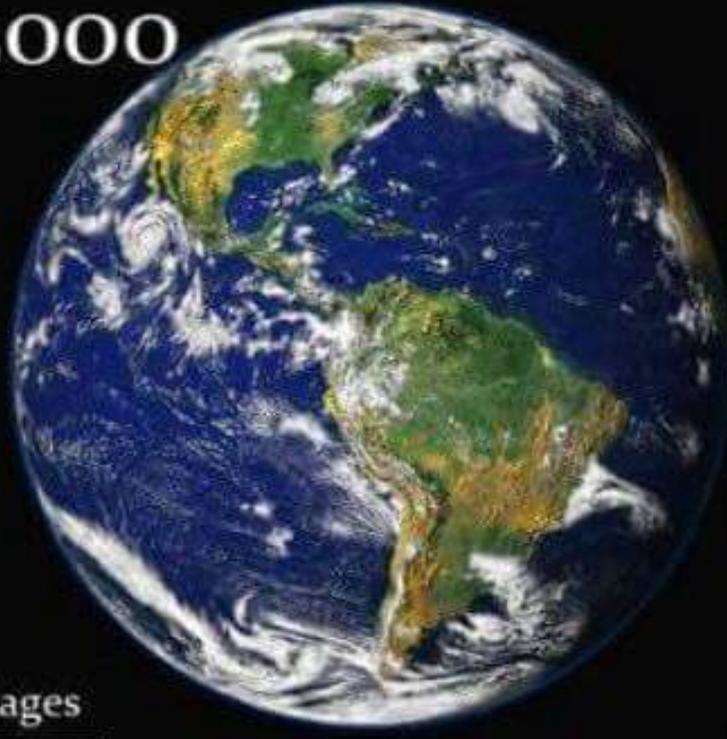


source: flickr.com, Pandoozy Photos, South Downs, Woodingdean

2015



2000



NASA images



## Exposing all of us, especially the foetus, to destructive radiation 24/7

“Brain proteome response following whole body exposure of mice to mobile phone or wireless DECT base radiation”

Electromagnetic Biology and Medicine; Posted online on January 20, 2012.

(doi:10.3109/15368378.2011.631068 (1–25) Adamantia F. Fragopoulou, Athina Samara, Marianna H. Antonelou, Anta Xanthopoulou, Aggeliki Papadopoulou, Konstantinos Vougas, Eugenia Koutsogiannopoulou, Ema Anastasiadou, Dimitrios J. Stravopodis, George Th. Tsangaris, Lukas H. Margaritis Department of Cell Biology and Biophysics, Athens University

### Abstract:

The objective of this study was to investigate the effects of two sources of electromagnetic fields (EMFs) on the proteome of cerebellum, hippocampus, and frontal lobe in Balb/c mice following long-term whole body irradiation. Three equally divided groups of animals (6 animals/group) were used; the **first** group was exposed to a **typical mobile phone**, at a SAR level range of 0.17–0.37 W/kg for 3 h daily for 8 months, the **second** group was exposed to a **wireless DECT base** (Digital Enhanced Cordless Telecommunications/Telephone) at a SAR level range of 0.012–0.028 W/kg for 8 h/day also for 8 months and the **third** group comprised the **sham**-exposed animals. Comparative proteomics analysis revealed that long-term irradiation from **both EMF sources altered significantly ( $p < 0.05$ ) the expression of 143 proteins** in total (as low as 0.003 fold downregulation **up to 114 fold overexpression**). Several neural function related proteins (i.e., Glial Fibrillary Acidic Protein (GFAP), Alpha synuclein, Glia Maturation Factor beta (GMF), and apolipoprotein E (apoE)), heat shock proteins, and cytoskeletal proteins (i.e., Neurofilaments and tropomodulin) are included in this list as well as proteins of the brain metabolism (i.e., Aspartate aminotransferase, Glutamate dehydrogenase) to nearly all brain regions studied. Western blot analysis on selected proteins confirmed the proteomics data. The observed **protein expression changes may be related to brain plasticity** alterations, indicative of **oxidative stress in the nervous system** or involved in **apoptosis** and might potentially explain human health hazards reported so far, such as **headaches, sleep disturbance, fatigue, memory deficits, and brain tumor long-term induction** under similar exposure conditions.

# Part 1: We are losing generations of children to ill health and neurological illness. The Elephant in the Living Room: Autism, chronic illness and Retroviral activity

- Torres, Anthony R., Alma Maciulis, and Dennis Odell. "The association of **MHC genes** with **autism**." Front Biosci 6 (2001): D936-43.
- Giles, T., Washington SJ Boyer-Lehnert, and Ohio P. Bassin. "Is **A Retrovirus The Root Cause Of The Autism Epidemic?**." (2011).
- Balestrieri, E., Arpino, C., Matteucci, C., Sorrentino, R., Pica, F., Alessandrelli, R., Coniglio, A., Curatolo, P., Rezza, G., Macciardi, F. and Garaci, E., 2012. HERVs expression in autism spectrum disorders. PloS one, 7(11), p.e48831. From the discussion: "***To the best of our knowledge, this is the first evidence linking retrotransposon activity and ASD***"
- Balestrieri, E., Cipriani, C., Matteucci, C., Capodicasa, N., Pilika, A., Korca, I., ... & Coniglio, A. (2016). Transcriptional activity of human **endogenous retrovirus** in Albanian children with **autism spectrum disorders**. The new microbiologica, 39(3), 228.
- Cipriani, Chiara, Laura Ricceri, Claudia Matteucci, Alessia De Felice, Anna Maria Tartaglione, Ayele Argaw-Denboba, Francesca Pica et al. "***High expression of Endogenous Retroviruses from intrauterine life to adulthood in two mouse models of Autism Spectrum Disorders.***" Scientific reports 8, no. 1 (2018): 629.

- What are retroviruses? The more familiar DNA viruses such as those from the “herpes family” - and many others - work their way from the DNA over to the RNA and from there to the manufacture of viral proteins. Retroviruses work their way backwards – from the RNA to the DNA – and then forward again from there. The most well-known and published retrovirus is HIV. However, there are countless others, grouped together in various “classes”. The generally accepted key contributors to chronic illness are inflammation, oxidative stress and microbial infection. All of these are known triggers for retroviral activity, and in turn also known causes of retroviral activity.
- Both **human and animal retroviruses can infect the CNS**. These are associated with many diseases of the CNS causing neurological disease directly through infection of immune cells which traffic to the brain and indirectly through increases in proinflammatory cytokines and chemokines or in the absence of detectable brain inflammation indirect effects known as bystander effects-caused chronic retroviral replication of immune cells.

**A retrovirus works via the enzyme “reverse transcriptase”.** Once inside the cell, it uses the enzyme to force the cell to create viral DNA. This viral DNA becomes integrated into the host-cell DNA. A retrovirus integrated into our genome may be passed from mother to child during pregnancy (Sakuma et al.,2012). **Only 2 % of our DNA is protein-coding, but 6-8% of our DNA is retroviral DNA** – passed down to us from our ancestors as battle-scars from our constant encounter with an often hostile microbial and virus-rich environment (Stoyle.,2006, Mayer et al.,2011; Li et al.,2001). These viruses are referred to as **Human Endogenous Retroviruses or HERVs**. An unintended source of retroviruses are some vaccines (Frontiers in Microbiology, January 2011).

The retroviruses are subdivided in different-lettered classes: Beta Retroviruses: HERV -K. Gamma Retroviruses: HERV -H and HERV-W.

Human endogenous retroviruses (HERVs) make up part of our genome (4-8%) and represent footprints of previous retroviral infection (length of HERV-DNA in a single patient: 150 000 times round the earth. What does it take to silence it?)

The HERV-K “superfamily” represents one of the most active HERVs and is capable of reproducing retroviral particles, i.e symptoms

Some HERVs may be of benefit to the host (p53, placenta; “Beneficial role of human endogenous retroviruses: facts and hypotheses”. *Scan J Immunol* 1998; 48:329-38), but can also be harmful, involved in cancer, autoimmune disease, fatigue and many other debilitating symptoms

HERVs possess a similar genomic organisation to present-day exogenous retroviruses (from tick and insect bites, vaccines, etc.).

Retroviruses can also be exogenous -acquired – and possess a similar genomic organisation as the endogenous retroviruses/HERV. Today they are present in the saliva of most biting insects and can be transferred to the host in the company of bacteria (*Borrelia*, *Bartonella*) or viruses: flaviviruses (FSME, zika, some flus, dengue, etc.), EBV, HSV-1/2. Also vaccines have tested positive for retroviruses. The baby or infant does not have a competent blood brain barrier or immune system to contain the retroviruses.

*Sources:*

- *“The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences”*; *Proc Natl Acad Sci USA* 1996; 93: 5177-84
- *“Demystified: Human endogenous retroviruses”*; *Mol Pathol* 2003 Feb; 56(1): 11-18; PN Nelson et al

## The Vaccine Issue:

we find that Lyme is far more symptomatic in the younger, more “completely” vaccinated population than in older people who had only a moderate number of vaccinations. Is this the cause of the severity of some Lyme cases? Is the fetal tissue found since over 20 years in many vaccines the cause of retroviral infection and autoimmunity?

*Retroviruses in vaccines?* Böni, Jürg, et al. "Detection of reverse transcriptase activity in live attenuated virus vaccines." *Clinical and diagnostic virology* 5.1 (1996): 43-53. From the paper: **Conclusions:** The data indicate the **systematic presence** of partially particle-associated **retroviral reverse transcriptase in attenuated live virus vaccines** that are produced in chicken-derived cells. The identification and further characterization of these particles, as well as the elucidation of possible interactions with the human organism are imperative goals despite the fact that these vaccines have been safely used for many years.

*“Pilot comparative study on the health of vaccinated and unvaccinated 6- to 12-year-old U.S. children”*

Anthony R Mawson, Brian D Ray, Azad R Bhuiyan and Binu Jacob

J Transl Sci, 2017 Volume 3(3): 1-12 (full text on the KI website – can strangely not be found anymore on Google search engine)

Mawson, Anthony R., et al. *"Preterm birth, vaccination and neurodevelopmental disorders: a cross-sectional study of 6-to 12-year-old vaccinated and unvaccinated children."*

J Transl Sci 3 (2017).

Both studies indicate a **14-fold increase in allergic, autoimmune and neurological problems** in the vaccinated group. What about the children, that do not develop frank autism? Is symptomatic Lyme a possible late outcome?

Address correspondence to: Theresa Deisher, tdeisher@soundchoice.org, 206-906-9922, 1749 Dexter Ave N, Seattle, WA 98109. 48: Issues in Law & Medicine, Volume 30, Number 1, 2015

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## **Epidemiologic and Molecular Relationship Between Vaccine Manufacture and Autism Spectrum Disorder Prevalence**

Theresa A. Deisher, Ph.D.;\* Ngoc V. Doan, B.S.;\*\*  
Kumiko Koyama, B.S.;\*\*\* Sarah Bwabye, B.S.\*\*\*\*

### **ABSTRACT**

**Objectives:** To assess the public health consequences of fetal cell line manufactured vaccines that contain residual human fetal DNA fragments utilizing laboratory and ecological approaches including statistics, molecular biology and genomics.

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\* President and Principal Scientist. Dr. Deisher conceptualized and designed the study, supervised all data collection and results, drafted the initial manuscript, revised and approved the final manuscript as submitted. Affiliations: <sup>1</sup>Sound Choice Pharmaceutical Institute, 1749 Dexter Ave N, Seattle, WA 98109. Address correspondence to: Theresa Deisher, tdeisher@soundchoice.org, 206-906-9922, 1749 Dexter Ave N, Seattle, WA 98109.

Ethics Statement: All data used in this manuscript was from public data files and therefore is exempt from IRB approval according to guidelines from The National Human Subjects Protection Advisory Committee (NHRPAC) recommendations on Public Use Data Files approved at the January 28-29, 2002 Committee meeting. (<http://www.hhs.gov/ohrp/archive/nhrpac/documents/data1r.pdf>): "Responsibility of Users of Public Use Data Files: Users of public use data files do not need to obtain IRB approval to use such files or seek a determination that the use of the public data files meets the criteria for being exempt from IRB review."

Transparency declaration: The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

All authors, external and internal, had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis

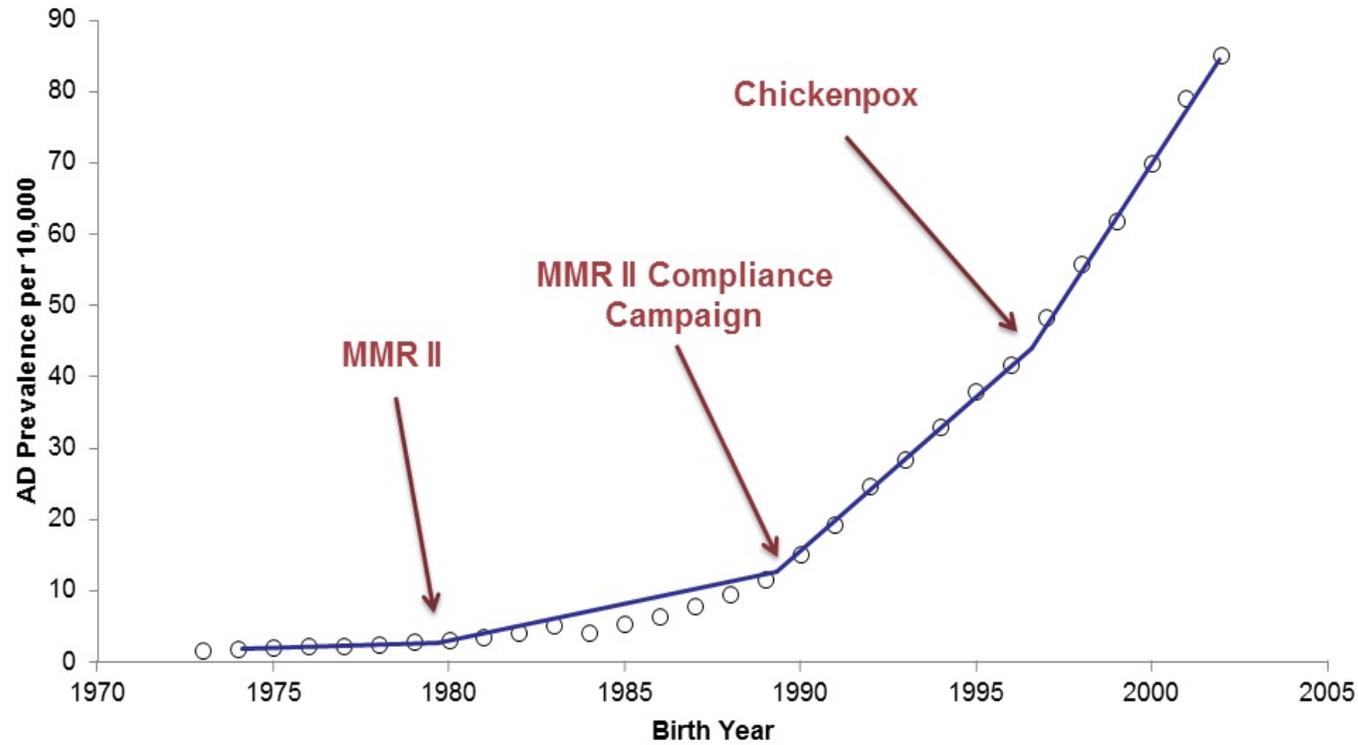
Funding sources: the study was funded by M.J. Murdock Charitable Trust and by private donations.

\*\* Research Associate. Ms. Doan collected and analyzed the data; operated data analysis instruments, reviewed the manuscript and approved the final manuscript as submitted.

\*\*\* Research Associate. Ms. Koyama coordinated and designed experiment, reviewed the manuscript and approved the final manuscript as submitted.

\*\*\*\* Research Associate. Ms. Bwabye assisted in collecting and revising the data and approved the final manuscript as submitted.

### Human Fetal DNA and Retrovirus Contaminants in Vaccines Coincide with Autism Changepoints



Vaccines today contain retroviral sequences with predictable adverse consequences

[Issues Law Med.](#) 2016 Fall;31(2):221-234.

**Insertional mutagenesis and autoimmunity induced disease caused by human fetal and retroviral residual toxins in vaccines.**

[Jarzyna P](#)<sup>1</sup>, [Doan NV](#)<sup>1</sup>, [Deisher TA](#)<sup>1</sup>.

## **Article excerpt**

### Introduction

Major concern of vaccination regarding childhood diseases in terms of Insertional Mutagenesis and Autoimmunity

The potential consequences of injecting our children with human fetal DNA contaminants include two well-established pathologies:

- 1) **Insertional mutagenesis** in which fetal DNA incorporates into the child's DNA **causing mutations**.
- 2) **Autoimmune disease triggered by the human fetal DNA in vaccines** leading a child's immune system to attack his or her own body.

Vaccines: The United States manufacturing process and vaccine history of using human fetal cell lines: In January 1979, the rubella manufacturing switch from animal based to the human fetal cell line WI-38 was approved by the FDA

Google and vaccines:

Sorry, no information is available for the URL <https://www.corvelva.it/speciali-corvelva/analisi/vaccingate-initial-results-on-infanrix-hexa-chemical-composition.html>

Sorry, no information is available for the URL <https://www.nature.com/articles/d41586-018-07464-0>

Infanrix Hexa: no antigens against the illnesses found, but strange proteins

A few less known references:

- 1. J Chromatogr B Analyt Technol Biomed Life Sci. 2017 Jun 1;1054:80-92 - The combined use of analytical tools for exploring tetanus toxin and tetanus toxoid structures.
- 2. Vaccine. 2007 Mar 8;25(12):2213-27. - Investigation of the detoxification mechanism of formaldehyde-treated tetanus toxin.

<https://pubchem.ncbi.nlm.nih.gov/search/>

<https://toxnet.nlm.nih.gov/>

- 5. Int J Med Microbiol. 2018 Aug;308(6):738-750. - The quest for bacterial allergens.
- 6. Front Microbiol. 2017 Oct 9;8:1938 - Morbid Sequences Suggest Molecular Mimicry between Microbial Peptides and Self-Antigens: A Possibility of Inciting Autoimmunity.  
Assoziierte Artikel\_
- Vaccingate: 5 of 7 vaccines analyzed are not compliant

# MMR contains human fetal DNA and Retroviruses

Deisher, Theresa A., Ngoc V. Doan, Kumiko Koyama, and Sarah Bwabye. "Epidemiologic and molecular relationship between vaccine manufacture and autism spectrum disorder prevalence." [Issues in law & Medicine](#) [01 Jan 2015, 30(1):47-70] (PMID:26103708)

## Abstract

**OBJECTIVES:** To assess the public health consequences of fetal cell line manufactured vaccines that contain residual human fetal DNA fragments utilizing laboratory and ecological approaches including statistics, molecular biology and genomics. **METHOD:** MMR coverage and autism disorder or autism spectrum disorder prevalence data for Norway, Sweden and the UK were obtained from public and government websites as well as peer reviewed published articles. Biologically, the size and quantity of the contaminating fetal DNA in Meruvax II and Havrix as well as the propensity of various cell lines for cellular and nuclear uptake of primitive human DNA fragments were measured and quantified using gel electrophoresis, fluorescence microscopy and fluorometry. Lastly, genomic analysis identified the specific sites where fetal DNA fragment integration into a child's genome is most likely to occur. **RESULTS:** The average MMR coverage for the three countries fell below 90% after Dr. Wakefield's infamous 1998 publication but started to recover slowly after 2001 until reaching over 90% coverage again by 2004. During the same time period, the average autism spectrum disorder prevalence in the United Kingdom, Norway and Sweden dropped substantially after birth year 1998 and gradually increased again after birth year 2000. Average single stranded DNA and double stranded DNA in Meruvax II were 142.05 ng/vial and 35.00 ng/vial, respectively, and 276.00 ng/vial and 35.74 ng/vial in Havrix respectively. The size of the fetal DNA fragments in Meruvax II was approximately 215 base pairs. There was spontaneous cellular and nuclear DNA uptake in HFF1 and NCCIT cells. Genes that have been linked to autism (autism associated genes; AAGs) have a more concentrated susceptibility for insults to genomic stability in comparison to the group of all genes contained within the human genome. Of the X chromosome AAGs, 15 of 19 have double strand break motifs less than 100 kilobases away from the center of a meiotic recombination hotspot located within an exon.

**CONCLUSION: Vaccines manufactured in human fetal cell lines contain unacceptably high levels of fetal DNA fragment contaminants.** The human genome naturally contains regions that are susceptible to double strand break formation and DNA insertional mutagenesis. The "Wakefield Scare" created a natural experiment that may demonstrate a **causal relationship between fetal cell-line manufactured vaccines and ASD prevalence.**

The overlooked co-infection in Lyme: the saliva of biting insects contains retroviruses

[Transmission of retroviruses by arthropods](#)

LD Foil, CJ Issel - Annual Review of Entomology, 1991 - annualreviews.org

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Song, S. U., M. Kurkulos, J. D. Boeke, and V. G. Corces. 1997. **Infection of the germ line by retroviral particles produced in the follicle cells**: a possible mechanism for the mobilization of the gypsy retroelement of *Drosophila*. Development 124:2789-2798.

Terzian, C., A. Péliison, and A. Bucheton. 10 August 2001, posting date. Evolution and phylogeny of **insect endogenous retroviruses**. BMC Evol. Biol. 1:3. [Online.] <http://www.biomedcentral.com>.

Teyssset, L., J. C. Burns, H. Shike, B. L. Sullivan, A. Bucheton, and C. Terzian. 1998. A Moloney murine **leukemia virus-based retroviral vector** pseudotyped by the insect retroviral *gypsy* envelope can infect *Drosophila* cells. J. Virol. 72:853-856.

Dual Infection: most Lyme patients are co-infected with retroviruses

“Viral Association with the Elusive Rickettsia of Viper Plague from Ghana, West Africa” From the abstract: *A type D retrovirus was observed in vacuoles in all infected cells. **The virus and rickettsia infection was associated*** with transfer of cytopathic effect

Annals of the New York Academy of Sciences. 15 December 2008

<https://doi.org/10.1196/annals.1428.092>

Most biting insects harbour **Insect retroviruses**. This includes ticks, stinging flies, fleas and spiders

- Pearson, Margot N., and George F. Rohrmann. "Transfer, incorporation, and substitution of envelope fusion proteins among members of the Baculoviridae, Orthomyxoviridae, and Metaviridae (**insect retrovirus**) families." *Journal of virology* 76.11 (2002): 5301-5304.
- Recent research suggests that members of the *Baculoviridae* family can be divided into two groups on the basis of their envelope fusion proteins. One group utilizes proteins related to GP64. Homologs of GP64 are also used by the thogotoviruses, a genus of the *Orthomyxoviridae* family. Members of the other group of baculoviruses utilize envelope fusion proteins related to a protein called LD130. LD130 has been shown to be related to the envelope protein of insect **retroviruses** in the genus *Errantivirus* (family *Metaviridae*).
- In this review, the evidence for these data is outlined and possible pathways of transfer, incorporation, and substitution are discussed.

# Cat fleas not only give you Bartonella but also a multitude of retroviruses with life-long consequences

Vobis, M., J. D'haese, H. Mehlhorn, and N. Mencke. "The feline leukemia virus (FeLV) and the cat flea (*Ctenocephalides felis*)." *Parasitology research* 90, no. 3 (2003): S132-S134.

## Abstract

The feline leukemia virus (FeLV) is naturally occurring and widespread retrovirus among domestic cats. The virus is mainly transmitted horizontally through saliva, blood and other body fluids by close contact between cats. Other vectors than cats, e.g. blood sucking parasites, have not been reported. This study tested the vectors potential of the cat flea (*C.felis*) for FeLV. In a first feeding, fleas were fed for 24 hours with blood from a FeLV-infected cats with persistent viremia. FeLV could be detected in the fleas , as well as in their faeces. Fleas were then divided in two populations and fed in a second feeding for 5 and 24 hours, respectively, with uninfected non-viremic blood. **The FeLV was again detected in the fleas and their faeces.** In addition, the two resulting blood samples of the second feeding were subsequently tested for FeLV, and both samples were positive for FeLV-RNA. The cat flea transmitted the feline leukemia virus from one blood sample to another. In an third feeding, the same populations of fleas were fed again with uninfected blood for 5 and 24 hours, respectively. This time, neither in the fleas, nor in the faeces or blood samples FeLV was detectable. Results show, that cat fleas are potential vectors for the feline leukemia virus RNA *in-vitro* and probably also *in-vivo*.

# What chronic illnesses are caused or perpetuated by retroviruses?

## ADHD and retroviruses

Balestrieri, Emanuela, Mariabernarda Pitzianti, Claudia Matteucci, Elisa D'Agati, Roberta Sorrentino, Antonia Baratta, Rosa Caterina et al. "Human endogenous retroviruses and ADHD." *The World Journal of Biological Psychiatry* 15, no. 6 (2014): 499-504. *Conclusions.* Since the ADHD aetiology is due to a complex interaction of environmental, biological and genetic factors, **HERVs may represent a link among these factors and clinical phenotype of ADHD.**

## Schizophrenia and Retroviruses

O'Reilly, Richard L., and Shiva M. Singh. "Retroviruses and schizophrenia revisited." *American Journal of Medical Genetics Part A* 67, no. 1 (1996): 19-24. From the abstract: **"the involvement of an endogenous retrovirus would be compatible with some of the puzzling epidemiological findings in schizophrenia."**

## Inflammatory Brain diseases

Johnston, James B., et al. "Monocyte activation and differentiation augment **human endogenous retrovirus expression: implications for inflammatory brain diseases.**" *Annals of neurology* 50.4 (2001): 434-442.: **three- to ninefold increases in HERV-W, HERV-K, and HERV-H RNA levels.** In addition, **elevated reverse transcriptase activity**

## **Multiple Sclerosis** caused by retroviruses:

- HERV-W - also referred to as MSR/V - is involved in MS (also HERV -H). The activity and presence of MSR/V in MS is well-published: in active plaques in every MS brain examined to date on both macrophages and microglia, also in astrocytes in MS lesions of the brain, as well as in the inner lining of the venous system. The highly neurotoxic MSR/V envelope protein was found in the serum of 73% of MS patients and not in controls [Perron et al.,2012].
- “Expression of sequence variants of endogenous retrovirus RGH in particle form in multiple sclerosis”. Lancet 1998; 352: 1033. T Christensen et al
- “Expression of endogenous retrovirus in blood mononuclear cells in brain tissue from multiple sclerosis patients”. Acta Neural Scand 1995; 169: 38-44. HB Rasmussen et al.

## **Breast cancer** caused by retroviruses:

- “Human endogenous retrovirus expression and reverse transcriptase activity in the T47D mammary carcinoma cell line”. J Virol 1996; 70:2654-7. C Patience et al

## **Auto-Immunity** caused by retroviruses

- “Immunity and retroviral superantigens in humans” Trends Immunol 2002: 23; 57-8. DL Woodland
- “The potential role of endogenous retroviruses in autoimmunity”. Immunol Rev 1996; 152:194-236

**Diabetes Type 1:** is Ritchie Shoemaker’s “dreaded mold type” really a patient with activated retroviruses?

- “Retroviral superantigens and type 1 diabetes mellitus”. Cell 1998; 95:9-11
- “A human endogenous retroviral superantigen as candidate autoimmune gene in type 1 diabetes”. Cell 1997; 90: 303-13
- “Endogenous retroviral long terminal repeats of the HLA-DQ region are associated with susceptibility to insulin-dependent diabetes mellitus. Hum Immunol 1996; 50: 103-10. K Badenhoop et al

## **CCSVI** and other vascular problems caused by retroviruses

- “Cytokine regulation of env gene expression of human endogenous retrovirus-R in human vascular endothelial cells”. Clin Immunol 1999; 93: 75-80

## **Rheumatoid Arthritis** caused by retroviruses

- A positive correlation between HERV K levels and objective markers of disease activity in Rheumatoid arthritis (Raynier et al.,2009) and **Sjogren** Syndrome (Ohyama et al.,1998)
- “Retroviruses in rheumatic diseases”. Ann Rheum Dis 1995; 55:441-2
- “Retrovirus-associated rheumatic syndromes”. Current Opin Rheumatol 1998; 10:347-54
- “An endogenous retroviral long terminal repeat at the HLA-DQB1 gene locus confers susceptibility to rheumatoid arthritis”. Hum Immunol 1999; 60: 63-8. C Seidl et al

## **Sjogren’s** Syndrome caused by retroviruses

- “Detection of serum antibodies to retroviral proteins in patients with primary Sjogren’s syndrome (autoimmune exocrinopathy). Arthritis Rheum 1990; 33:774-81

## **Lupus** and retroviruses:

- There is a robust body of evidence implicating HERV-caused DNA hypomethylation in the etiology of SLE (Zhou et al.,2008; Talaber et al.,2014).

# Alzheimer's Disease and Retroviral Activity

- Küry, Patrick, Avindra Nath, Alain Créange, Antonina Dolei, Patrice Marche, Julian Gold, Gavin Giovannoni, Hans-Peter Hartung, and Hervé Perron. "Human endogenous retroviruses in neurological diseases." *Trends in molecular medicine* (2018).
- Rasmussen, H. B., H. Perron, and J. Clausen. "Do endogenous retroviruses have etiological implications in inflammatory and degenerative nervous system diseases?." *Acta neurologica scandinavica* 88.3 (1993): 190-198.
- Manuelidis, Laura, Geoffrey Murdoch, and Elias E. Manuelidis. "Potential involvement of retroviral elements in human dementias." *Novel infectious agents and the central nervous system, Ciba Foundation Symposium*. Vol. 135. 1988.
- Akowitz, A., E. E. Manuelidis, and L. Manuelidis. "Protected endogenous retroviral sequences copurify with infectivity in experimental Creutzfeldt-Jakob disease." *Archives of virology* 130.3-4 (1993): 301-316.
- Sharpe, Arlene H., et al. "Role of abortive retroviral infection of neurons in spongiform CNS degeneration." *Nature* 346.6280 (1990): 181.

- **ME/CFIDS:** the connection of ME/CFIDS with retroviruses has been firmly established by the work of F.Ruscetti and J.Mikovits (*Mikovits. Plague: One Scientist's Intrepid Search for the Truth about Human Retroviruses and Chronic Fatigue Syndrome (ME/CFS), Autism, and Other Diseases. Skyhorse Publishing Inc., 2017*)
- **Non-Hodgkin Lymphoma:** the likelihood of a CFS sufferer to contract Non-Hodgkin lymphoma was deemed to be 250 times higher than a typical healthy person.
- **Cancer:** the relationship of retroviral activity and cancer is well established, but inconvenient. The association of the XMRVs (xenotropic murine retrovirus) with prostate cancer was found in 23% of patients with the cancer, but those who tested positive for XMRV were the ones with the aggressive form of it (Schalberg et al.,2009). The researching team on this discovery, led by Dr. Ila Singh of the University of Utah, reported, "The presence of virus in malignant cells invokes classical pathways for retroviral pathogenesis, i.e. inactivation of a tumor suppressor or activation of an oncogene by retroviral integration, as possible mechanisms of tumorigenesis."
- **Schizophrenia:** a number of studies have found increased levels of reverse transcriptase activity in the cerebrospinal fluid of patients with the diagnosis (Yolken et al.,2004).
- **Autism:** that real time PCR revealed that the copy number of HERV-H was indeed higher in children with autism

- But not all **embedded retroviral DNA** is bad. Some sections have become a functional part of our genome because they have given us an evolutionary advantage such as the **formation of the p53 gene regulatory network** (Shin et al.,2013; Barbusecu et al.,2001). The behaviour of endogenous retroviruses as part of an intrinsic anti- viral defense-network is also well documented (Bieniasz et al.,2014). Other retroviruses have to be silenced throughout life – mainly through DNA methylation and acetylation. And here is one of the focal points of retroviral research: **the transcription of the non-serving retroviral DNA makes the infected person susceptible to numerous de-novo genetic mutations, including MTHFR, DNMT** and other genes which control methylation. Many other illness-producing effects are known: there is evidence implicating HERV- K in the pathogenesis of neuroinflammatory and autoimmune illnesses. For a patient to get well today it is rarely enough to just interpret the DNA SNPs and to substitute the nutrients of the methylation cycle such as SAME, Dimethylglycine, methylated folate or B12, etc. Another approach has been needed for a long time. Our health span has dramatically decreased in the last 20 years due to the rise in chronic illness.
- In animal studies on Gamma retroviruses, the virus titre correlates with disease progression, which is a typical finding in other retrovirus infections (Denner and Young.,2013). If the hypothesis of this author (and several well-respected researchers) is correct, that the driving force of chronic illness is the activation of HERVs, which in turn opens the door for numerous other pathogens to enter the system, we have to look at - and understand - the forces in our environment that have disabled our mechanisms to silence the HERVs and other more recent retroviral infections.

## **How do we become infected? Why do HERVs become activated and pathogenic**

Retroviruses can be introduced into a system as an aerosol (inhaled), injected inadvertently in vaccines (review Miyazawa et al.,2010) or via blood based products (i.v.IG, etc.), acquired via sex (HIV) and many other avenues. The HERV (Human Endogenous Retro Viruses) are already in our system since conception.

This author has good evidence from his clinical experience, that the current permanent **2.4 Gigahertz microwave irradiation** of our system (cell-phone radiation) is the main driver of chronic inflammation, a precondition for the explosion of the virus within and is probably silencing our methylation and acetylation enzymes. Several toxins present everywhere in our world are suspected as cofactors: **glyphosate** (from food), **nanonized aluminium** and lead (from polluted air) and mercury (from fish and dental amalgam fillings).

HERVS can also become activated by a number of other influences such as a **viral infection, chronic inflammation**, involving elevated cytokine production with up-regulation of NF-kappaB and STAT-3 (Manghera and Douville,2013). The ubiquitous **Epstein Barr** virus induces expression of the HERV-K envelope gene and the transactivation of the Multiple Sclerosis retrovirus (Mameli et al.,2007; Sutkowski et al.,2001). **Herpes simplex type 2** activates members of the HERV-W family. These, and other, mechanisms are likely responsible for the transactivation of HERVs seen in RA, SLE , Sjorgens disease, Schizophrenia, Autism, MS and cancer.

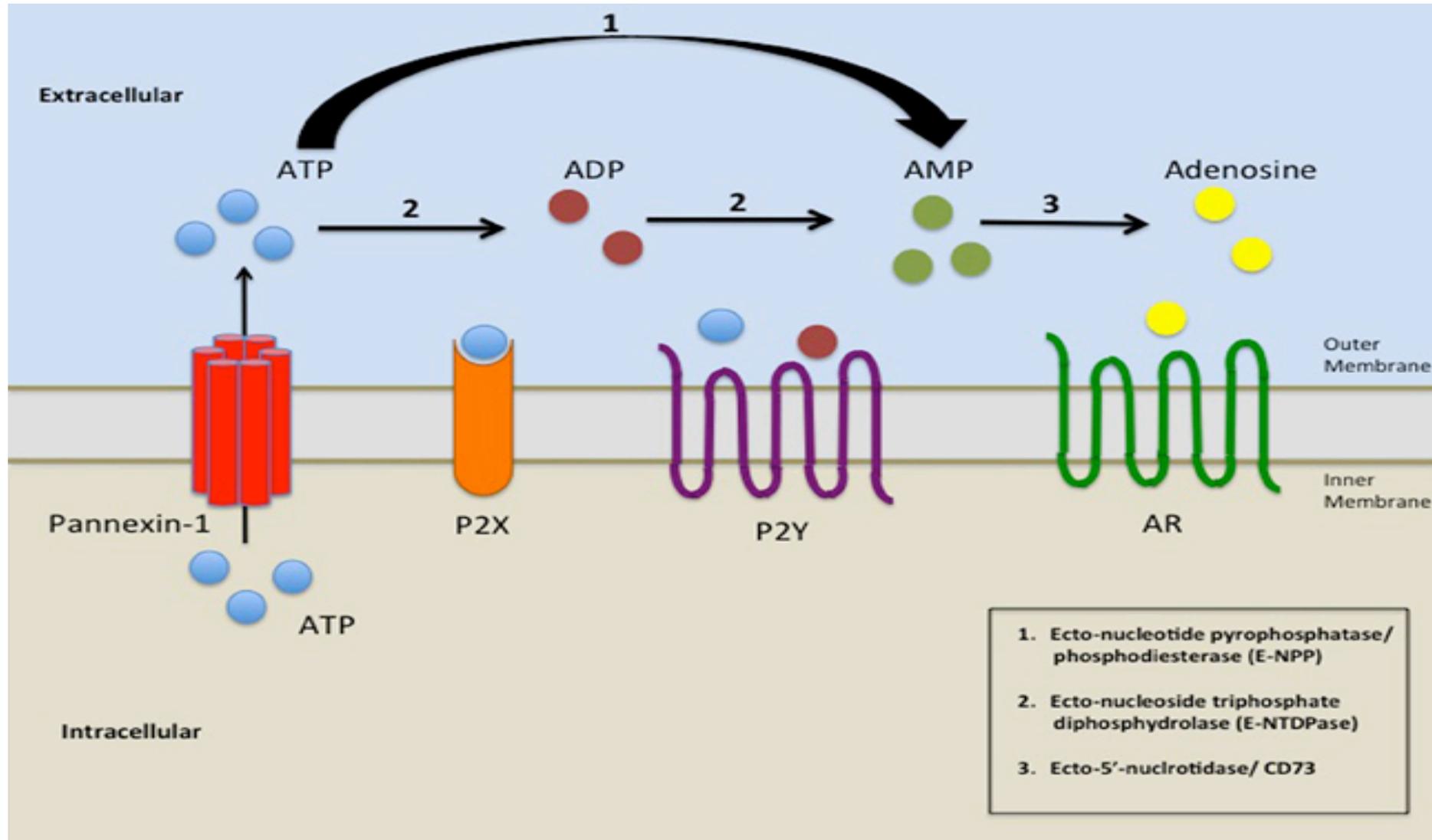
## Retroviruses cause brain inflammation and elicit the “cell danger response”

Johnston, James B., et al. "Monocyte activation and differentiation augment human endogenous retrovirus expression: implications for inflammatory brain diseases." *Annals of neurology* 50.4 (2001): 434-442.

### Abstract

Human endogenous retroviruses (HERVs) have been implicated as causative agents in diseases characterized by inflammation and macrophage activation, such as multiple sclerosis. Because monocyte activation and differentiation influence retroviral transcription and replication, we investigated the contribution of these processes to the expression of four HERV families (HERV-W, HERV-K, HERV-E, and HERV-H) in human monocytes and autopsied brain tissue from patients with brain diseases associated with increased macrophage activity. Reverse transcriptase-polymerase chain reaction analysis of primary macrophages and U937 monocytoid cells stimulated with phorbol-12-myristate-13-acetate or lipopolysaccharide revealed **three- to ninefold increases in HERV-W, HERV-K, and HERV-H RNA levels**. In addition, **elevated reverse transcriptase activity** and HERV RNA were detectable in supernatants from PMA-stimulated U937 cultures, properties that could be attenuated with the inhibitor of monocyte differentiation threonine-lysine-proline. In contrast, stimulation of monocytes decreased or had no effect on HERV-E expression. Compared with controls, HERV-W and HERV-K expression was increased in brain tissue from patients with multiple sclerosis or human immunodeficiency virus infection or AIDS, with concomitant elevated tumor necrosis factor- $\alpha$  levels. Similarly, elevated HERV-W levels were detected in patients with Alzheimer's dementia only when tumor necrosis factor- $\alpha$  expression was also evident (2 of 6 cases). The **detection of several HERVs in inflammatory brain diseases** and the capacity to augment HERV expression in monocytes with compounds that influence cellular activity suggest that increased expression of these viruses is a consequence of increased immune activity.

ATP from damaged cells as extracellular messenger signals the cell danger response. It is degraded to adenosine, which is anti-inflammatory or immunosuppressive. Degradation of ATP by the ectonucleotidases (CD 39 and CD 73). The Purine receptor P2Y becomes dysregulated.



# Brain inflammation from HERV-activation elicits the “cell danger response” and “purinergic signalling”

Pathophysiology of astroglial purinergic signalling. *Purinergic signalling*, 8(3), 629-657

Franke, H., Verkhratsky, A., Burnstock, G., & Illes, P. (2012).

Abstract

Astrocytes are fundamental for central nervous system (CNS) physiology and are the fulcrum of neurological diseases. **Astroglial cells control development of the nervous system, regulate synaptogenesis, maturation, maintenance and plasticity of synapses and are central for nervous system homeostasis.** Astroglial reactions determine progression and outcome of many neuropathologies and are critical for regeneration and remodelling of neural circuits following trauma, stroke, ischaemia or neurodegenerative disorders. They secrete multiple neurotransmitters and neurohormones to communicate with neurones, microglia and the vascular walls of capillaries. **Signalling through release of ATP is the most widespread mean of communication between astrocytes and other types of neural cells.** ATP serves as a fast excitatory neurotransmitter and has pronounced long-term (trophic) roles in cell proliferation, growth, and development. **During pathology, ATP is released from damaged cells and acts both as a cytotoxic factor and a proinflammatory mediator, being a universal “danger” signal.** In this review, we summarise contemporary knowledge on the role of purinergic receptors (P2Rs) in a variety of diseases in relation to changes of astrocytic functions and nucleotide signalling. We have focussed on the role of the ionotropic P2X and metabotropic P2YRs working alone or in concert to modify the release of neurotransmitters, to activate signalling cascades and to change the expression levels of ion channels and protein kinases. All these effects are of great importance for the initiation, progression and maintenance of astrogliosis—the conserved and ubiquitous glial defensive reaction to CNS pathologies. We highlighted specific aspects of reactive astrogliosis, especially with respect to the involvement of the P2X<sub>7</sub> and P2Y<sub>1</sub>R subtypes. Reactive astrogliosis exerts both beneficial and detrimental effects in a context-specific manner determined by distinct molecular signalling cascades. **Understanding the role of purinergic signalling in astrocytes is critical to identifying new therapeutic principles to treat acute and chronic neurological diseases.**

Burnstock, Geoffrey. "Purinergetic signalling and neurological diseases: an update." *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)* 16.3 (2017): 257-265.

Purinergetic signalling, i.e. ATP as an extracellular signalling molecule and co-transmitter in both peripheral and central neurons, is involved in the physiology of neurotransmission and neuromodulation.

Receptors for purines have been cloned and characterised, including 4 subtypes of the P1(adenosine) receptor family, 7 subtypes of the P2X ion channel nucleotide receptor family and 8 subtypes of the P2Y G protein-coupled nucleotide receptor family. The roles of purinergetic signalling in diseases of the central nervous system and the potential use of purinergetic compounds for their treatment are attracting increasing attention. In this review, the focus is on the findings reported in recent papers and reviews to update knowledge in this field about the **involvement of purinergetic signalling in Alzheimer's, Parkinson's and Huntington's diseases, multiple sclerosis, amyotrophic lateral sclerosis, degeneration and regeneration after brain injury, stroke, ischaemia, inflammation, migraine, epilepsy, psychiatric disorders, schizophrenia, bipolar disorder, autism, addiction, sleep disorders and brain tumours.** The use in particular of P2X7 receptor antagonists for the treatment of neurodegenerative diseases, cancer, depression, stroke and ischaemia, A2A receptor antagonists for Parkinson's disease and agonists for brain injury and depression and P2X3 receptor antagonists for migraine and seizures has been recommended. **P2Y receptors have also been claimed to be involved in some central nervous disorders.**

Ulrich H, Abbracchio MP, Burnstock G (Sep 2012). Stem Cell Reviews. 8 (3): 755–67.  
[doi:10.1007/s12015-012-9372-9](https://doi.org/10.1007/s12015-012-9372-9). [PMID 22544361](https://pubmed.ncbi.nlm.nih.gov/22544361/). "Extrinsic purinergic regulation of neural stem/progenitor cells: implications for CNS development and repair".

**Purinergic receptors**, also known as **purinoceptors**, are a family of plasma membrane molecules that are found in almost all mammalian tissues. Within the field of purinergic signalling, these receptors have been **implicated in learning and memory, locomotor and feeding behaviour, and sleep**. More specifically, they are involved in several cellular functions, including proliferation and migration of neural stem cells, vascular reactivity, apoptosis and cytokine secretion. These functions have not been well characterized and the effect of the extracellular microenvironment on their function is also poorly understood.

*Wikipedia: **Molecular mechanisms:** Generally speaking, all cells have the ability to release [nucleotides](#). In neuronal and neuroendocrinal cells, this mostly occurs via regulated [exocytosis](#). Released [nucleotides](#) can be hydrolyzed extracellularly by a variety of cell surface-located enzymes referred to as [ectonucleotidases](#). The purinergic signalling system consists of transporters, enzymes and receptors responsible for the synthesis, release, action, and extracellular inactivation of (primarily) [ATP](#) and its extracellular breakdown product [adenosine](#). The signalling effects of [uridine triphosphate](#) (UTP) and [uridine diphosphate](#) (UDP) are generally comparable to those of ATP.*

**Ectonucleotidases:** levels and activity are directly related to the cell danger signal and can be measured

and consist of families of [nucleotide metabolizing enzymes](#) that are expressed on the [plasma membrane](#) and have externally oriented active sites. These enzymes metabolize [nucleotides](#) to [nucleosides](#). The contribution of ectonucleotidases in the modulation of [purinergic signaling](#) depends on the availability and preference of substrates and on cell and tissue distribution.

Adenosine generation: The first step in the production of adenosine involves the conversion of ATP/ADP to AMP. It is carried out by [ENTPD1](#), also known as **CD39**. The second step involves the conversion of AMP to adenosine. It is carried out by [NT5E](#), also known as **CD73**.

Classification: Subfamilies of ectonucleotidases include: [CD39](#)/NTPDases (ecto-nucleotide triphosphate diphosphohydrolases), Nucleotide pyrophosphatase/phosphodiesterase (NPP)-type ecto-phosphodiesterases, alkaline phosphatases and ecto-5'-nucleotidases/[CD73](#)

Function: Ectonucleotidases produce key molecules for purine salvage and consequent replenishment of ATP stores within multiple cell types. Dephosphorylated nucleoside derivatives interact with membrane transporters to enable intracellular uptake.

Ectonucleotidases modulate [P2](#) purinergic signaling. In addition, ectonucleotidases generate extracellular adenosine, which abrogates nucleotide-mediated effects and activates adenosine receptors, often with opposing (patho-) physiological effects.

Miller, Catherine M., et al. "The role of the P2X7 receptor in infectious diseases." *PLoS pathogens* 7.11 (2011): e1002212.

## Abstract

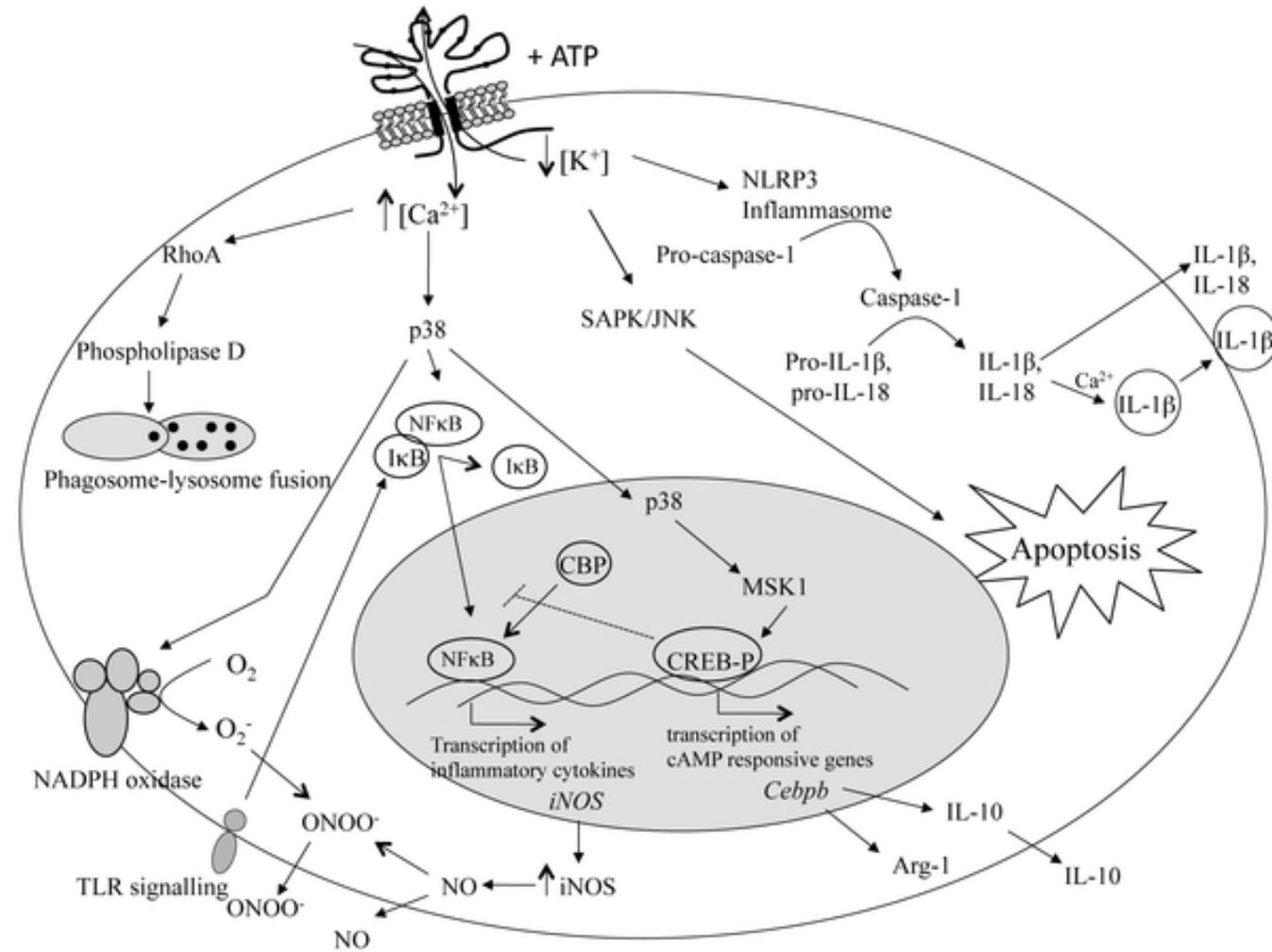
**ATP is an extracellular signal for the immune system**, particularly during an inflammatory response. It is sensed by the P2X<sub>7</sub> receptor, the expression of which is upregulated by pro-inflammatory cytokines. **Activation of the P2X<sub>7</sub> receptor** opens a cation-specific channel that alters the ionic environment of the cell, activating several pathways, including (i) **the inflammasome**, leading to production of IL-1 $\beta$  and IL-18; (ii) the stress-activated protein kinase pathway, resulting in **apoptosis**; (iii) the mitogen-activated protein kinase pathway, leading to generation of **reactive oxygen and nitrogen intermediates**; and (iv) phospholipase D, stimulating phagosome-lysosome fusion. The P2X<sub>7</sub> receptor can initiate host mechanisms to remove pathogens, most particularly those that parasitise macrophages. At the same time, **the P2X<sub>7</sub> receptor may be subverted by pathogens to modulate host responses**. Moreover, recent genetic studies have demonstrated significant associations between susceptibility or resistance to parasites and bacteria, and loss-of-function or gain-of-function polymorphisms in the P2X<sub>7</sub> receptor, underscoring its importance in infectious disease. The P2X<sub>7</sub> receptor is highly expressed by cells of the haemopoietic lineage and can mediate cell death, killing of infectious organisms, and regulation of the inflammatory response. The involvement of the P2X<sub>7</sub> receptor in these pathways suggests that it functions as a major regulator of inflammation.

Naviaux RK. Metabolic features of the cell danger response. *Mitochondrion* 2014;**16**:7–17

## Abstract

The cell danger response (CDR) is the evolutionarily conserved metabolic response that protects cells and hosts from harm. It is triggered by encounters with chemical, physical, or biological threats that exceed the cellular capacity for homeostasis. The resulting metabolic mismatch between available resources and functional capacity produces a cascade of changes in cellular electron flow, oxygen consumption, redox, membrane fluidity, lipid dynamics, bioenergetics, carbon and sulfur resource allocation, protein folding and aggregation, vitamin availability, metal homeostasis, indole, pterin, 1-carbon and polyamine metabolism, and polymer formation. **The first wave of danger signals consists of the release of metabolic intermediates like ATP and ADP, Krebs cycle intermediates, oxygen, and reactive oxygen species (ROS), and is sustained by purinergic signaling.** After the danger has been eliminated or neutralized, a choreographed sequence of anti-inflammatory and regenerative pathways is activated to reverse the CDR and to heal. When the CDR persists abnormally, whole body metabolism and the gut microbiome are disturbed, the collective performance of multiple organ systems is impaired, behavior is changed, and chronic disease results. Metabolic memory of past stress encounters is stored in the form of altered mitochondrial and cellular macromolecule content, resulting in an increase in functional reserve capacity through a process known as mitocellular hormesis. The systemic form of the CDR, and **its magnified form, the purinergic life-threat response (PLTR)**, are under direct control by ancient pathways in the brain that are ultimately coordinated by centers in the brainstem. Chemosensory integration of whole body metabolism occurs in the brainstem and is a prerequisite for normal brain, motor, vestibular, sensory, social, and speech development. **An understanding of the CDR permits us to reframe old concepts of pathogenesis for a broad array of chronic, developmental, autoimmune, and degenerative disorders. These disorders include autism spectrum disorders (ASD), attention deficit hyperactivity disorder (ADHD), asthma, atopy, gluten and many other food and chemical sensitivity syndromes, emphysema, Tourette's syndrome, bipolar disorder, schizophrenia, post-traumatic stress disorder (PTSD), chronic traumatic encephalopathy (CTE), traumatic brain injury (TBI), epilepsy, suicidal ideation, organ transplant biology, diabetes, kidney, liver, and heart disease, cancer, Alzheimer and Parkinson disease, and autoimmune disorders like lupus, rheumatoid arthritis, multiple sclerosis, and primary sclerosing cholangitis.**

Figure 1. Intracellular pathways in immune cells stimulated by P2X7 receptor activation.



Miller CM, Boulter NR, Fuller SJ, Zakrzewski AM, Lees MP, et al. (2011) The Role of the P2X7 Receptor in Infectious Diseases. *PLOS Pathogens* 7(11): e1002212. <https://doi.org/10.1371/journal.ppat.1002212>  
<http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1002212>

## Suramin/antipurinergic agents inhibit HIV/retrovirus replication

Séror, C., Melki, M. T., Subra, F., Raza, S. Q., Bras, M., Saïdi, H., ... & Martins, I. (2011). Extracellular ATP acts on P2Y2 purinergic receptors to facilitate HIV-1 infection. *Journal of Experimental Medicine*, 208(9), 1823-1834.

### Abstract

**Extracellular adenosine triphosphate (ATP) can activate purinergic receptors of the plasma membrane and modulate multiple cellular functions.** We report that ATP is released from HIV-1 target cells through pannexin-1 channels upon interaction between the HIV-1 envelope protein and specific target cell receptors. Extracellular ATP then acts on purinergic receptors, including P2Y2, to activate proline-rich tyrosine kinase 2 (Pyk2) kinase and transient plasma membrane depolarization, which in turn stimulate fusion between Env-expressing membranes and membranes containing CD4 plus appropriate chemokine co-receptors. **Inhibition of any of the constituents of this cascade (pannexin-1, ATP, P2Y2, and Pyk2) impairs the replication of HIV-1 mutant viruses that are resistant to conventional antiretroviral agents.** Altogether, our results reveal a novel signaling pathway involved in the early steps of HIV-1 infection that may be targeted with new therapeutic approaches.

Naviaux, J. C., et al. "Reversal of autism-like behaviors and metabolism in adult mice with single-dose antipurinergic therapy." *Translational psychiatry* 4.6 (2014): e400.

Autism spectrum disorders (ASDs) now affect 1–2% of the children born in the United States. Hundreds of genetic, metabolic and environmental factors are known to increase the risk of ASD. Similar factors are known to influence the risk of schizophrenia and bipolar disorder; however, a unifying mechanistic explanation has remained elusive. Here we used the maternal immune activation (MIA) mouse model of neurodevelopmental and neuropsychiatric disorders to study the effects of a single dose of the antipurinergic drug suramin on the behavior and metabolism of adult animals. **We found that disturbances in social behavior, novelty preference and metabolism are not permanent but are treatable with antipurinergic therapy (APT) in this model of ASD and schizophrenia. A single dose of suramin (20 mg kg<sup>-1</sup> intraperitoneally (i.p.)) given to 6-month-old adults restored normal social behavior, novelty preference and metabolism.** Comprehensive metabolomic analysis identified purine metabolism as the key regulatory pathway. Correction of purine metabolism normalized 17 of 18 metabolic pathways that were disturbed in the MIA model. Two days after treatment, the suramin concentration in the plasma and brainstem was 7.64  $\mu\text{M}$   $\text{pmol } \mu\text{l}^{-1}$  ( $\pm 0.50$ ) and 5.15  $\text{pmol mg}^{-1}$  ( $\pm 0.49$ ), respectively. These data show good uptake of suramin into the central nervous system at the level of the brainstem. Most of the improvements associated with APT were lost after 5 weeks of drug washout, consistent with the 1-week plasma half-life of suramin in mice. **Our results show that purine metabolism is a master regulator of behavior and metabolism in the MIA model, and that single-dose APT with suramin acutely reverses these abnormalities, even in adults.**

Naviaux, Robert K., et al. "Low-dose suramin in autism spectrum disorder: a small, phase I/II, randomized clinical trial." *Annals of clinical and translational neurology* 4.7 (2017): 491-505.

**Abstract:** No drug is yet approved to treat the core symptoms of autism spectrum disorder (ASD). Low-dose suramin was effective in the maternal immune activation and Fragile X mouse models of ASD. The Suramin Autism Treatment-1 (SAT-1) trial was a double-blind, placebo-controlled, translational pilot study to examine the safety and activity of low-dose suramin in children with ASD.

**Methods:** Ten male subjects with ASD, ages 5–14 years, were matched by age, IQ, and autism severity into five pairs, then randomized to receive a single, **intravenous infusion of suramin (20 mg/kg)** or saline. The primary outcomes were ADOS-2 comparison scores and Expressive One-Word Picture Vocabulary Test (EOWPVT). Secondary outcomes were the aberrant behavior checklist, autism treatment evaluation checklist, repetitive behavior questionnaire, and clinical global impression questionnaire.

**Results:** Blood levels of suramin were  $12 \pm 1.5 \mu\text{mol/L}$  (mean  $\pm$  SD) at 2 days and  $1.5 \pm 0.5 \mu\text{mol/L}$  after 6 weeks. The terminal half-life was  $14.7 \pm 0.7$  days. A self-limited, asymptomatic rash was seen, but there were no serious adverse events. **ADOS-2 comparison scores improved** by  $-1.6 \pm 0.55$  points ( $n = 5$ ; 95% CI =  $-2.3$  to  $-0.9$ ; Cohen's  $d = 2.9$ ;  $P = 0.0028$ ) in the suramin group and did not change in the placebo group. Secondary outcomes also showed **improvements in language, social interaction, and decreased restricted or repetitive behaviors.**

**Interpretation:** The safety and activity of low-dose suramin showed promise as a novel approach to treatment of ASD in this small study. We hypothesized that **there is a conserved cellular response to metabolic perturbation or danger that is shared by all children with ASD. This is called the cell danger hypothesis.**

Pacheco, Paulo AF, et al. "Putative roles of purinergic signaling in human immunodeficiency virus-1 infection." *Biology direct* 9.1 (2014): 21.

## Abstract

Nucleotides and nucleosides act as potent extracellular messengers via the activation of the family of cell-surface receptors termed purinergic receptors. These receptors are categorized into P1 and P2 receptors (P2Rs). P2Rs are further classified into two distinct families, P2X receptors (P2XRs) and P2Y receptors (P2YRs). These receptors display broad tissue distribution throughout the body and are involved in several biological events. Immune cells express various P2Rs, and **purinergic signaling mechanisms have been shown to play key roles in the regulation of many aspects of immune responses**. Researchers have elucidated the involvement of these receptors in the host response to infections. The evidences indicate a dual function of these receptors, depending on the microorganism and the cellular model involved. Three recent reports have examined the relationship between the level of extracellular ATP, the mechanisms underlying purinergic receptors participating in the infection mechanism of HIV-1 in the cell. Although preliminary, these results indicate that purinergic receptors are putative pharmacological targets that should be further explored in future studies.

Croon JJ, Wolff HL: The **inhibition of yellow fever virus multiplication by suramin**: a preliminary note. *Acta Leiden*. 1982, 48: 5-8. [PubMedGoogle Scholar](#)

Xu K, Ren H, Zhu J, Yang Y, Liao F: **Suramin inhibits** the in vitro expression of **encephalitis B virus proteins NS3 and E**. *J Huazhong Univ Sci Technolog Med Sci*. 2003, 23 (4): 375-379.

## Cannabis as anti-retroviral medicine

Milloy, M-J., et al. "High-intensity cannabis use associated with lower plasma human immunodeficiency virus-1 RNA viral load among recently infected people who use injection drugs." *Drug and alcohol review* 34.2 (2015): 135-140.

**Introduction and Aims:** Cannabis use is common among people who are living with human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS). While there is growing pre-clinical evidence of the immunomodulatory and anti-viral effects of cannabinoids, their possible effects on HIV disease parameters in humans are largely unknown. Thus, we sought to investigate the possible effects of cannabis use on plasma HIV-1 RNA viral loads (pVLs) among recently seroconverted illicit drug users.

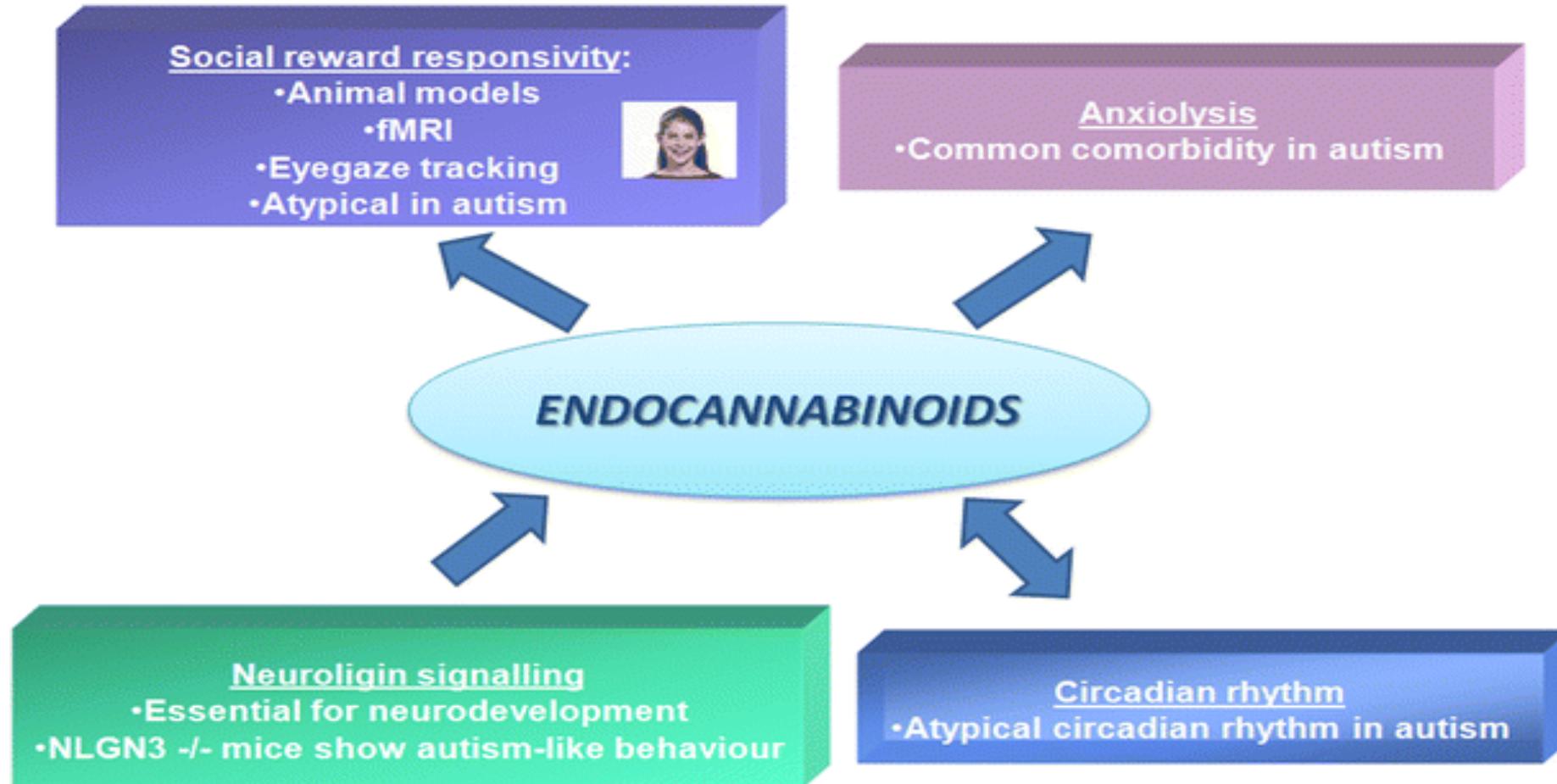
**Results:** Between May 1996 and March 2012, 88 individuals seroconverted after recruitment and were included in these analyses. Median pVL in the first 365 days among all seroconverters was 4.66  $\log_{10}$  c mL<sup>-1</sup>. In a multivariable model, at least daily cannabis use was associated with 0.51  $\log_{10}$  c mL<sup>-1</sup> lower pVL ( $\beta = -0.51$ , standard error = 0.170,  $P$  value = 0.003).

**Discussion and Conclusions:** Consistent with the findings from recent *in vitro* and *in vivo* studies, including one conducted among lentiviral-infected primates, we observed a strong association between cannabis use and lower pVL following seroconversion among illicit drug-using participants. Our findings support the further investigation of the immunomodulatory or antiviral effects of cannabinoids among individuals living with HIV/AIDS. [Milloy M-J, Marshall B, Kerr T, Richardson L, Hogg R, Guillemi S, Montaner JSG, Wood E. High-intensity **cannabis use associated with lower plasma human immunodeficiency virus-1 RNA viral load** among recently infected people who use injection drugs. *Drug Alcohol Rev* 2015;34:135–40]

## Cannabis extracts to improve outcomes in ASD

- Földy, Csaba, Robert C. Malenka, and Thomas C. Südhof. "Autism-associated neuroligin-3 mutations commonly disrupt tonic endocannabinoid signaling." *Neuron* 78.3 (2013): 498-509.
- Schultz, Stephen T. "Can autism be triggered by acetaminophen activation of the endocannabinoid system?." *Acta neurobiologiae experimentalis* 70.2 (2010): 227-231.
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- Wei, Don, Drake Dinh, DaYeon Lee, Dandan Li, Allison Anguren, Guillermo Moreno-Sanz, Christine M. Gall, and Daniele Piomelli. "Enhancement of anandamide-mediated endocannabinoid signaling **corrects autism-related social impairment.**" *Cannabis and cannabinoid research* 1, no. 1 (2016): 81-89.

Chakrabarti, Bhisimadev, et al. "Endocannabinoid signaling in autism." *Neurotherapeutics* 12.4 (2015): 837-847.



## Part 2: Towards Diagnosing Retroviral Activity

Varga, Tímea, et al. "Higher serum DPP-4 enzyme activity and decreased lymphocyte CD26 expression in type 1 diabetes." *Pathology & Oncology Research* 17.4 (2011): 925.

**Abstract: Dipeptidyl peptidase-4 (DPP-4) is involved in the metabolism of peptide hormones, T-cell activation and proliferation.** In type 1 diabetes mellitus (T1DM)  $\beta$ -cell destruction involves a number of dysregulated T-cells. Our aim was to assess the serum DPP-4 activity and the lymphocyte membrane bound CD26 expression in patients with type 1 diabetes and healthy controls. Ninety-eight (T1DM: 48, F/M = 20/28, mean age: 34.4y; control: 50, F/M = 39/11 mean age: 32,4y) individuals were included. Fasting serum DPP-4 enzymatic activity, plasma glucose (FPG), CD26 expression on CD3+, CD4+ and CD8+ lymphocytes, HbA1c and body mass index (BMI) were assessed. ICA and GAD antibodies were assessed in the T1DM group. DPP-4 enzymatic activity was determined by kinetic enzyme assay, ICA and GAD were assessed by ELISA. Determination of the CD26 expression on CD3+, CD4+ and CD8+ lymphocytes was performed by flow-cytometric analysis. We found higher serum DPP-4 activity (Mean: T1DM: 30.069 U/L, control: 22.62 U/L,  $p < 0.0001$ ) and decreased CD26 lymphocyte expression on all lymphocyte subpopulations in T1DM. Fasting serum DPP-4 activity was independent from the ICA or GAD status of patients with T1DM. Here we first present that the serum DPP-4 activity is increased and the lymphocyte membrane bound **CD26 expression is decreased in type 1 diabetes.** Decreased lymphocyte membrane bound CD26 expression in T1DM might be a novel part of the T-lymphocyte regulatory dysfunction observed in type 1 diabetes mellitus. These results might provide some basis for the clinical implication of DPP-4 inhibition in patients with T1DM.

Maina, Ayub K., et al. "The Potential for DPP-4/CD26 usage as a surrogate marker for Antiretroviral Therapy Efficacy in HIV Infected populations." *African Journal of Pharmacology and Therapeutics* 5.4 (2017).

**Background:** Human Immunodeficiency Virus (HIV) viral load and CD4<sup>+</sup> cell counts are the most commonly used markers for monitoring efficacy of anti-retroviral therapy (ART) in HIV infected individuals. The high cost of viral load monitoring limits its usage in resource limited countries, often leaving the use of CD4<sup>+</sup> T cell counts as the only alternative. Though cheaper and more readily available, CD4<sup>+</sup> cell counts as a measure of detecting treatment failure, is an unreliable predictor of disease progression. Hence, there is a need for more sensitive alternative, but less costly techniques for detecting treatment failure which can be used in resource limited settings.

**Objective:** To evaluate the feasibility of using plasma CD26/Dipeptidyl peptidase IV (DPPIV) as a novel marker for clinical evaluation of treatment efficacy in HIV infected children.

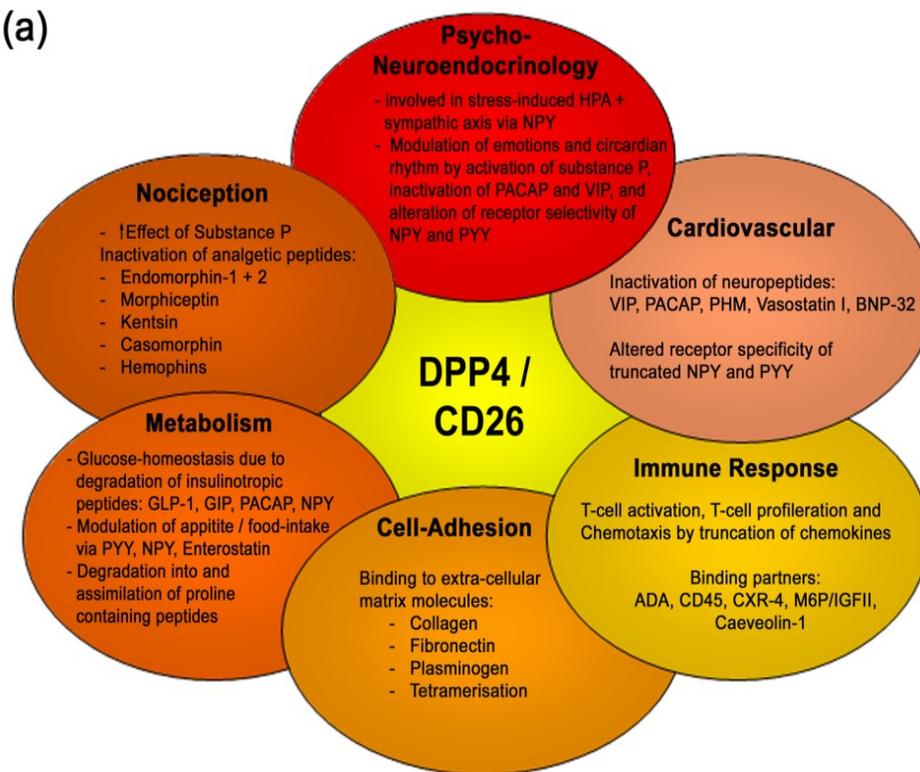
**Method:** Blood samples collected from HIV<sup>+</sup> children (n=76) before and after initiation on ART, were assessed for HIV RNA (viral load), CD4<sup>+</sup> T-cell count and DPPIV/CD26 levels. Viral load levels were analyzed using Roche Amplicor HIV-1 Monitor Test kit; CD4<sup>+</sup> T-Cell Counts were analyzed using BD FACS Calibur flow cytometer while DPPIV/CD 26 levels were analyzed using Human DPPIV/CD26 Quantikine ELISA kit (R&D Systems, Minneapolis MN).

**Results:** The plasma DPPIV/CD26 levels increased significantly in children after ART initiation ( $p = 0.017$ ), while the viral load levels declined after ART initiation with subsequent CD4<sup>+</sup> cell counts increase. **The DPPIV/CD 26 increase positively correlated with viral load decrease** while negatively correlating to the CD4<sup>+</sup> cell count increase.

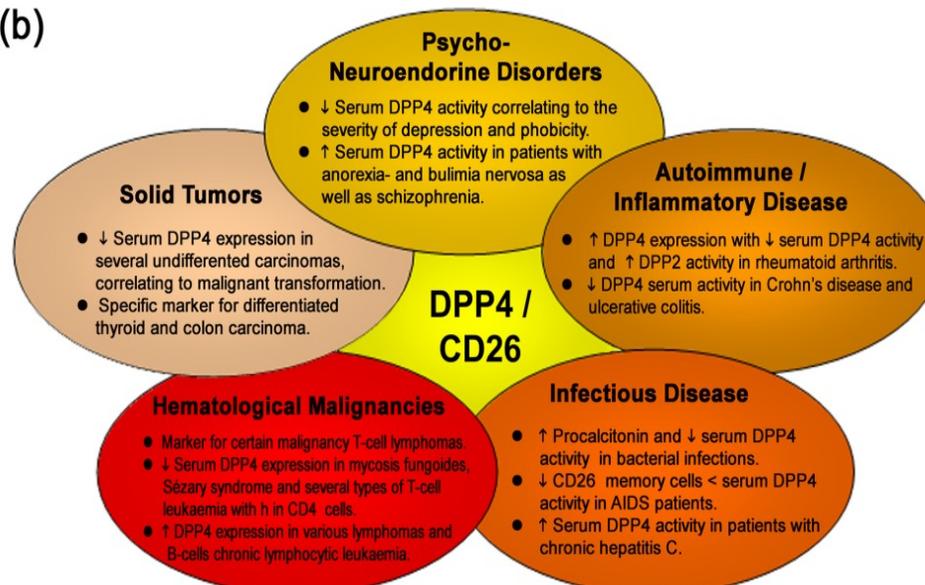
**Conclusion:** These findings demonstrate **an inverse relationship between DPPIV/CD26 levels and HIV viral load** and the direct proportionality of CD4<sup>+</sup> Cell counts and DPPIV/CD26 levels, suggesting potential for use of DPPIV/CD26 as a surrogate marker for evaluating HIV disease progression in children receiving anti-retroviral therapy.

**Key words:** CD26/Dipeptidyl peptidase IV (DPPIV), ELISA, Surrogate marker, Viral Load, CD4 Count, antiretroviral.

(a)



(b)



Bradstreet, James Jeffrey, Emar Vogelaar, and Lynda Thyer. "Initial observations of elevated alpha-N-acetylgalactosaminidase activity associated with autism and observed reductions from Gc protein--macrophage activating factor injections." *Autism Insights* 4 (2012): 31.

**Abstract Background:** Autism spectrum disorders (ASD) are developmental disorders affecting 1:88 children, and which appear to be associated with a variety of complex immune dysregulations including autoimmunity. The enzyme, alpha-N-acetylgalactosaminidase (Nagalase) deglycosylates serum Gc protein (vitamin D3 – binding protein) rendering it incapable of activating macrophage defenses. **Increased Nagalase activity has been associated with a variety of malignancies, immune disorders and viral infections.** Macrophage activating factor (GcMAF) has been repeatedly published as an intervention to lower serum Nagalase activity for a variety of cancer and HIV patients. GcMAF is a naturally occurring protein with well-established safety and therapeutic benefit(s) supported by numerous human studies. **Methods:** Initially, parents of 40 individuals with ASD sought testing for Nagalase serum activity as part of an evaluation of immune dysregulation. Nagalase enzyme activity measurement was performed by the European Laboratory of Nutrients (ELN), Bunnik, the Netherlands, using an end-point enzymatic assay of a chromogenic substrate. Some parents of patients with elevated Nagalase activity opted for weekly GcMAF injections provided by Immuno Biotech Ltd., Guernsey UK ([www.gcmf.eu](http://www.gcmf.eu)). GcMAF is purified from human serum obtained from the American Red Cross using 25-hydroxyvitamin D3-Sepharose high affinity chromatography. The protein is then further diluted to obtain therapeutically appropriate levels for patients based on their clinical presentations. **Results:** Individuals with ASD (32 males and 8 females, n = 40, ages: 1 year 4 months - 21 years 2 months) had initial and post treatment assessment of Nagalase activity. Dosing of GcMAF was recommended based on previously reported response curves adjusted by the treating clinician for age, weight, and Nagalase levels. The average pre-treatment Nagalase activity of the autism group was 1.93 nmol/min/mg of substrate. This was well above the laboratory reported normal range of ,0.95 nmol/min/mg. For the ASD group the average level at the time of second testing was 1.03 nmol/min/mg, reflecting an average reduction of 0.90 nmol/min/mg (P , 0.0001). Apart from the likely immunological benefits of lowering the Nagalase activity of these individuals, uncontrolled observations of GcMAF therapy indicated substantial improvements in language, socialization and cognition. No significant side-effects were reported during the course of injections.

**Conclusions:** In this first report of **Nagalase activity in patients with autism**, it appears that most individuals have **substantially higher levels** than the expected healthy ranges. Although Nagalase is a nonspecific marker of immune dysregulation, its observed levels in autism may have both etiological and therapeutic significance. Importantly, this is also the first report of reduction of Nagalase activity in an autism population with GcMAF injections.

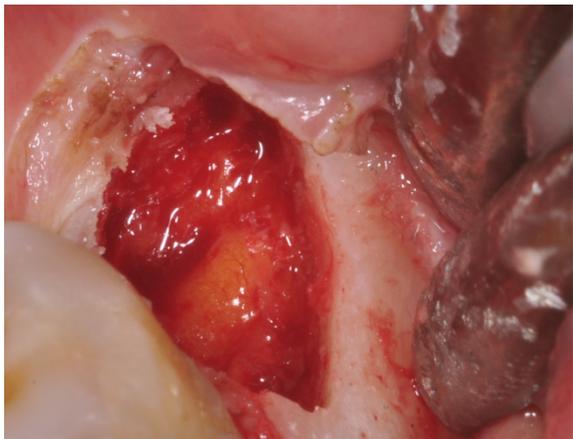
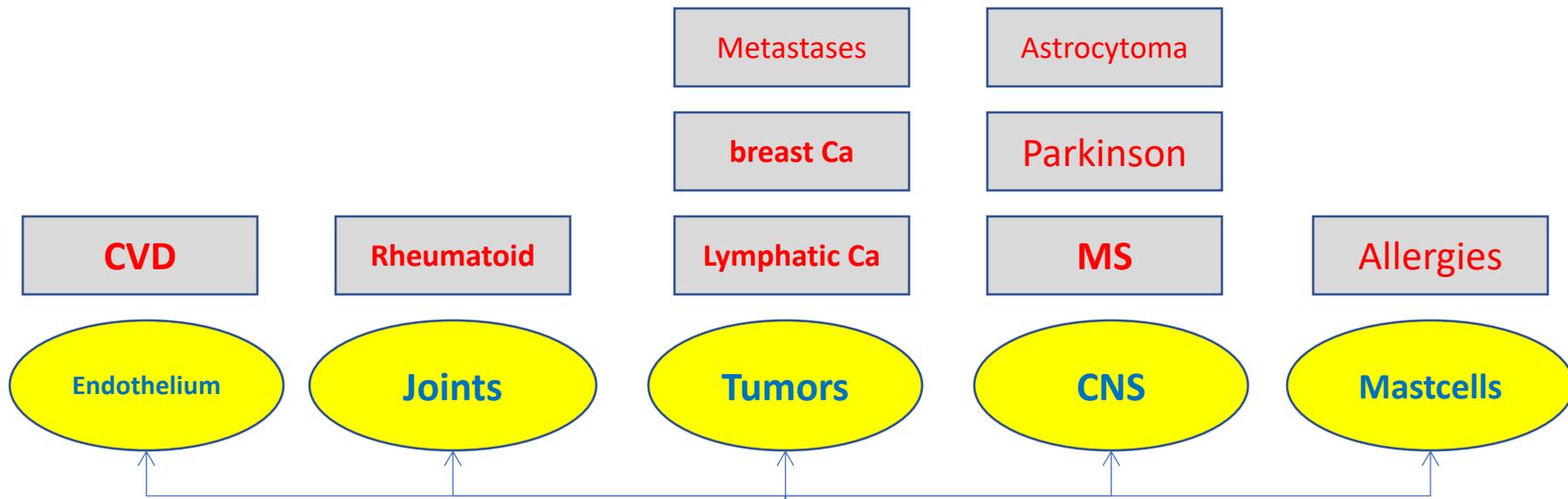
## RANTES is a marker for retroviral virulence and elevated in ASD

Gordon, Cynthia J., et al. "Enhancement of human immunodeficiency virus type 1 infection by the CC-chemokine RANTES is independent of the mechanism of virus-cell fusion." *Journal of virology* 73.1 (1999): 684-694.

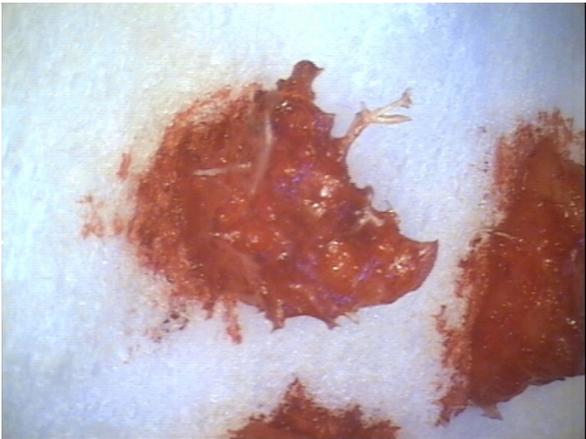
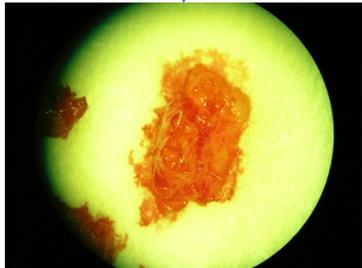
### ABSTRACT

We have studied the effects of CC-chemokines on human immunodeficiency virus type 1 (HIV-1) infection, focusing on the **infectivity enhancement caused by RANTES**. High RANTES concentrations increase the infectivity of HIV-1 isolates that use CXC-chemokine receptor 4 for entry. However, RANTES can have a similar enhancing effect on macrophagetropic viruses that enter via CC-chemokine receptor 5 (CCR5), despite binding to the same receptor as the virus. Furthermore, RANTES enhances the infectivity of HIV-1 pseudotyped with the envelope glycoprotein of murine leukemia virus or vesicular stomatitis virus, showing that the mechanism of enhancement is independent of the route of virus-cell fusion. The enhancing effects of RANTES are not mediated via CCR5 or other known chemokine receptors and are not mimicked by MIP-1 $\alpha$  or MIP-1 $\beta$ . The N-terminally modified derivative aminooxypentane RANTES (AOP-RANTES) efficiently inhibits HIV-1 infection via CCR5 but otherwise mimics RANTES by enhancing viral infectivity. There are two mechanisms of enhancement: one apparent when target cells are pretreated with RANTES (or AOP-RANTES) for several hours, and the other apparent when RANTES (or AOP-RANTES) is added during virus-cell absorption. We believe that the first mechanism is related to cellular activation by RANTES, whereas the second is an increase in virion attachment to target cells.

**Regulated upon activation, normal T-cell expressed, and secreted (RANTES)**



**NICO**



**In Vivo 25: 307-314 (2011)**

## **Xenotropic Murine Leukemia Virus-related Virus-associated Chronic Fatigue Syndrome Reveals a Distinct Inflammatory Signature**

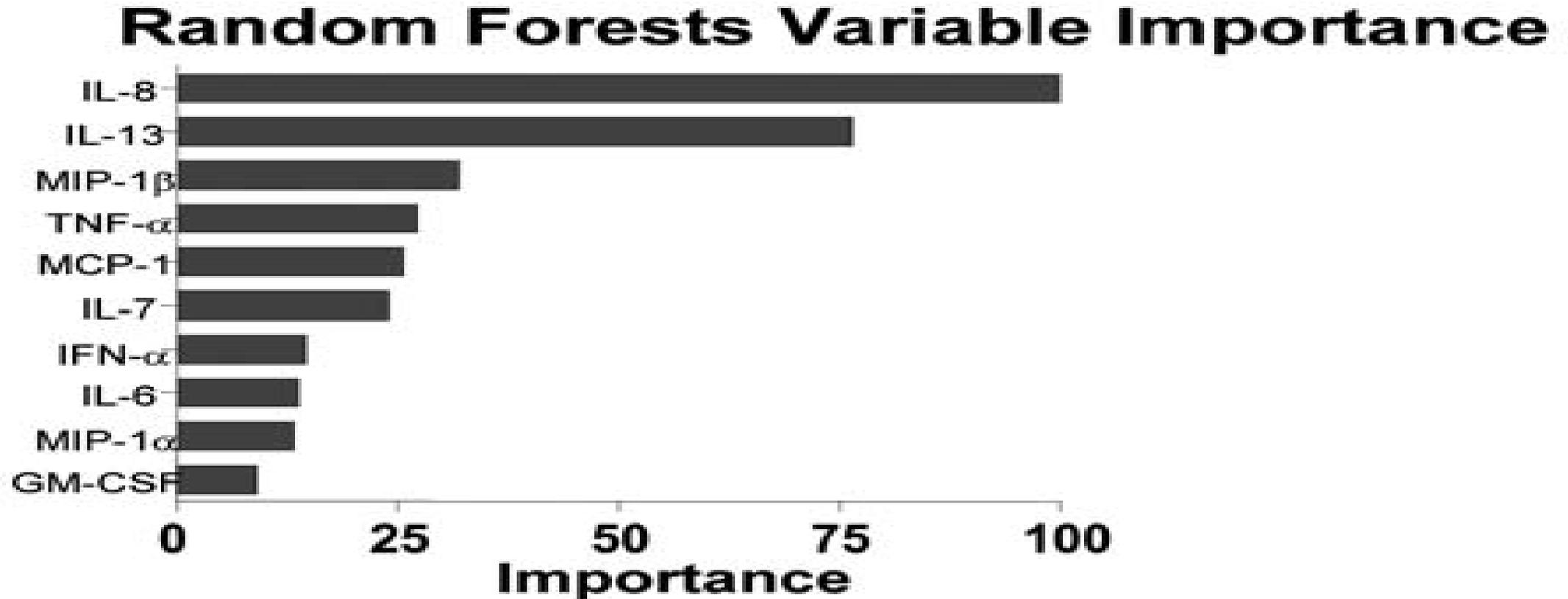
VINCENT C. LOMBARDI<sup>1</sup>, KATHRYN S. HAGEN<sup>1</sup>, KENNETH W. HUNTER<sup>4</sup>, JOHN W. DIAMOND<sup>2†</sup>, JULIE SMITH-GAGEN<sup>3</sup>, WEI YANG<sup>3</sup> and JUDY A. MIKOVITS<sup>1</sup>

**Abstract.** *Background: The recent identification of xenotropic murine leukemia virus-related virus (XMRV) in the blood of patients with **chronic fatigue syndrome (CFS)** establishes that **a retrovirus may play a role in the pathology in this disease**. Knowledge of the immune response might lead to a better understanding of the role XMRV plays in this syndrome. Our objective was to investigate the cytokine and chemokine response in XMRV-associated CFS. Materials and Methods: Using Luminex multi-analyte profiling technology, we measured cytokine and chemokine values in the plasma of XMRV-infected CFS patients and compared these data to those of healthy controls. Analysis was performed using the Gene Expression Pattern Analysis Suite and the Random Forest tree classification algorithm. Results: This study identifies a signature of 10 cytokines and chemokines which correctly identifies XMRV/CFS patients with 93% specificity and 96% sensitivity. Conclusion: These data show, for the first time, an immunological pattern associated with XMRV/CFS.*

	<b>CFS SFFV ENV+ vs. SFFV ENV- Control</b>
Total Cellularity	No Difference
CD45+ Leukocytes	No Difference
CD45+ Lymphocytes	Reduced in CFS
CD45+ CD3- Lymphocytes	Reduced in CFS
CD3+ CD56+ NKT cells	Increased in CFS
CD3- CD56+ NK cells	Reduced in CFS
CD56 <sup>DIM</sup> NK subpopulation	Reduced in CFS
CD56 <sup>DIM</sup> CD16+ NK subpopulation	Reduced in CFS
CD19+ B cells	Reduced in CFS
CD19+ CD20+ CD23+ B subpopulation	Increased in CFS
CD33+ CD14+ CD123- myeloid Population	Reduced in CFS
Activated APCs	Increased in CFS

Judy Mikovits , et al

In this study healthy volunteers were compared with CFIDS/ME patients with activated retroviruses. The larger the bar, the greater the difference between the 2 groups. IL-8 was overexpressed, IL-13 silenced



# The Treatment of retroviral activation/infection

## 1. The cautious use of glutathione – but do not use it until parasites, protozoa, fungi and bacteria are under control!

Sprietsma, J.E., 1999. Cysteine, glutathione (GSH) and zinc and copper ions together are effective, natural, intracellular inhibitors of (AIDS) viruses. *Medical hypotheses*, 52(6), pp.529-538. Abstract

Sufficient essential nutrients such as methionine, cysteine, copper, selenium, zinc and vitamins C and E are indispensable for the maintenance of optimal (immune) cell functions. Parasitic organisms such as protozoa, fungi, bacteria and viruses also depend on these essential nutrients for their multiplication and functioning. An evolutionarily developed optimal distribution of available nutrients between host (cells) and parasitic organisms normally prevents diseases, the nature of which will depend on genetic and environmental factors. The way in which the right amount of cysteine, glutathione (GSH), and copper and zinc ions made available in the right place at the right time and in the right form can prevent an unchecked multiplication of (AIDS) viruses in a more passive or active way forms the basis for the AIDS zinc-deficiency hypothesis (A–Z hypothesis) presented in this article.

Zinc and copper ions stimulate/inhibit/block in a concentration-dependent way the (intracellular) activation of essential protein-splitting enzymes such as HIV proteases. Zinc and copper ions as 'passive' virus inhibitors. Apart from this, zinc ions directly or indirectly regulate, via zinc finger protein molecular structures, the activities of virus-combating Th-1 cells such as cytotoxic T-cells (CTLs). Zinc ions as regulators of the active, virus-combating Th-1 cells. **Zinc and copper ions that remain available in sufficient amounts via cysteine/GSH are effective natural inhibitors/combaters of (AIDS) viruses** and thereby prevent the development of chronic virus diseases that can lead to AIDS, autoimmune diseases, (food) allergies and/or cancer.

A safe, relatively inexpensive and extensively tested medicine such as **N-acetylcysteine (NAC) can help in supplying extra cysteine**. The anti-HIV peptide T22, synthesized on the basis of two natural peptides from the *Tachypleus tridentatus* and *Limulus polyphemus* crabs, appears to be able to serve as supplier/carrier molecule of cysteine and zinc and/or to hinder the entry of HIVs into cells by way of the CD4 receptor.

Brundu, S., Palma, L., Picceri, G. G., Ligi, D., Orlandi, C., Galluzzi, L., ... & Mannello, F. (2016). Glutathione depletion is linked with Th2 polarization in mice with a retrovirus-induced immunodeficiency syndrome, MAIDS: role of pro-glutathione molecules as immunotherapeutics. *Journal of virology*, JVI-00603. **Glutathione GSH, NAC and OSR against Retroviruses. But beware of compounded "GSH".** The FDA has made sure that commercially available Glutathione is not GSH – this institution has ruled a few years ago that during the compounding of GSH the vial cannot be topped up with Nitrogen – causing the GSH to be oxidized, turning it into the GSH antidote rather than the active principle.

Masutani, H., S. Ueda, and J. Yodoi. "The thioredoxin system in retroviral infection and apoptosis." *Cell death and differentiation* 12.S1 (2005): 991.

**Glutathione and related Thio-Redoxins (OSR, NAC) are low in Retrovirally Infected Persons**

Breitkreutz, R., Pittack, N., Nebe, C., Schuster, D., Brust, J., Beichert, M., ... & Dröge, W. (2000). Improvement of immune functions in HIV infection by sulfur supplementation: two randomized trials. *Journal of Molecular Medicine*, 78(1), 55-62. **NAC is low in infected individuals**

Herzenberg, Leonore A., et al. "Glutathione deficiency is associated with impaired survival in HIV disease." *Proceedings of the National Academy of Sciences* 94.5 (1997): 1967-1972.

**NAC supplementation is helpful in HIV, probably also in other retroviral infections**

Roederer, Mario, et al. "Cytokine-stimulated human immunodeficiency virus replication is inhibited by N-acetyl-L-cysteine." *Proceedings of the National Academy of Sciences* 87.12 (1990): 4884-4888.

Fraternale, A., et al. "Erythrocytes as carriers of reduced glutathione (GSH) in the treatment of retroviral infections." *Journal of Antimicrobial Chemotherapy* 52.4 (2003): 551-554.

Dröge, W., & Breitkreutz, R. (2000). Glutathione and immune function. *Proceedings of the Nutrition Society*, 59(4), 595-600. **Give cysteine where cysteine is needed!**

Palamara, Anna T., et al. "Inhibition of murine AIDS by reduced glutathione." *AIDS research and human retroviruses* 12.14 (1996): 1373-1381.

Palamara, Anna Teresa, et al. "Glutathione inhibits HIV replication by acting at late stages of the virus life cycle." *AIDS research and human retroviruses* 12.16 (1996): 1537-1541.

Irminix (OSR, Emeramide): is currently only available via IRB certified institutions like all natural effective anti-retroviral agents made unavailable by the FDA to the general public. It is more potent and more specific than GSH, but similar. It is GSH squared.

Prof. Emeritus Boyd Haley PhD.: a 2010 IAOMT YouTube video explains how OSR may be the best anti-viral and anti-retroviral remedy. Here is how:

- It increases your own intracellular production of GSH by 10% in a few weeks
- GSH inserts into viral proteins and marks them for removal
- GSH molecules contain tight disulfide links and when the client has GSH that your cell has manufactured inside of itself, a sulphite exchange reaction occurs. Now GSH attaches to the virus inside the cell
- GSH coats that protein and prevents it from docking onto other cells and marks this viral protein for removal by the biliary transport system. It also shows the immune system through this tag that it can and should attack the enemy inside that cell. **GSH becomes best inhibitor of viral replication in culture cells – known to man**
- GSH or much better OSR, inhibits this viral replication without creating any toxicity.
- Prof. Haley suggests to give up to 6 gms NAC/day. But beware of its biofilm breaking effect!
- In the utube video many studies, backing this up
- Dr.K: best, if combined with R-Lipoic to shuttle toxic compound through the liver cells into the bile + repeated gallbladder flushes

## 2. Melatonin – a must in treating retroviral infections without drugs

Boga, Jose Antonio, et al. "Beneficial actions of melatonin in the management of viral infections: a new use for this “molecular handyman”?." *Reviews in medical virology* 22.5 (2012): 323-338.

Melatonin (N-acetyl-5-methoxytryptamine) is a multifunctional signaling molecule that has a variety of important functions. Numerous clinical trials have examined the therapeutic usefulness of melatonin in different fields of medicine. Clinical trials have shown that melatonin is efficient in preventing cell damage under acute (sepsis, asphyxia in newborns) and chronic states (metabolic and neurodegenerative diseases, cancer, inflammation, aging). The beneficial effects of melatonin can be explained by its properties as a potent antioxidant and antioxidant enzyme inducer, a regulator of apoptosis and a stimulator of immune functions. These effects support the use of melatonin in viral infections, which are often associated with inflammatory injury and increases in oxidative stress. In fact, melatonin has been used recently to treat several viral infections, which are summarized in this review. The role of melatonin in infections is also discussed herein.

*At SHI we may use transdermal melatonin in very high doses as a treatment for all infections, including retroviral activity*

Zhang, Z., et al. "Prevention of immune dysfunction and vitamin E loss by dehydroepiandrosterone and **melatonin supplementation** during murine retrovirus infection."

*Immunology* 96.2 (1999): 291.

## ABSTRACT

Female C57BL/6 mice infected with the LP-BM5 leukaemia retrovirus developed murine acquired immune-deficiency syndrome (AIDS). Dehydroepiandrosterone (DHEA) and melatonin (MLT) modify immune dysfunction and prevent lipid peroxidation. We investigated whether DHEA and MLT could prevent immune dysfunction, excessive lipid peroxidation, and tissue vitamin E loss induced by retrovirus infection. Retrovirus infection inhibited the release of T helper 1 (Th1) cytokines, stimulated secretion of Th2 cytokines, increased hepatic lipid peroxidation, and induced vitamin E deficiency. Treatment with DHEA or MLT alone, as well as together, largely prevented the reduction of B- and T-cell proliferation as well as of Th1 cytokine secretion caused by retrovirus infection. Supplementation also suppressed the elevated production of Th2 cytokines stimulated by retrovirus infection. DHEA and MLT simultaneously reduced hepatic lipid peroxidation and prevented vitamin E loss. The use of DHEA plus MLT was more effective in preventing retrovirus-induced immune dysfunction than either DHEA or MLT alone. **These results suggest that supplementation with DHEA and MLT may prevent cytokine dysregulation, lipid oxidation and tissue vitamin E loss induced by retrovirus infection.** Similarly, hormone supplementation also modified immune function and increased tissue vitamin E levels in uninfected mice.

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- Maria Helena Coelho Cruz, Claudia Lima Verde Leal, Jurandir Ferreira da Cruz, Dun-Xian Tan and Russel J. Reiter, **Role of melatonin on production and preservation of gametes and embryos: A brief review**, *Animal Reproduction Science*, 10.1016/j.anireprosci.2014.01.011, **145**, 3-4, (150-160), (2014).
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- Miroslav Pohanka, **Impact of melatonin on immunity: a review**, *Open Medicine*, 10.2478/s11536-013-0177-2, **8**, 4, (2013).
- Özge Kılınçel, Emel Çalışkan, İdris Şahin, Cihadiye Elif Öztürk, Nida Kılıç and Şükrü Öksüz, **The effect of melatonin on antifungal susceptibility in planktonic and biofilm forms of Candida strains** isolated from clinical samples, *Medical Mycology*, 10.1093/mmy/myx157, (2018).
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### 3: Proven biological Treatments

Anti-retroviral interventions we learned during the treatment of autistic children:

#### a. Broccoli sprouts (in capsules: [www.biopureUS.com](http://www.biopureUS.com))

- [Singh K, Zimmerman AW. Sulforaphane Treatment of Young Men with Autism Spectrum Disorder. CNS Neurol Disord Drug Targets. 2016;15\(5\):597-601.](#)
- Singh, K., Connors, S. L., Macklin, E. A., Smith, K. D., Fahey, J. W., Talalay, P., & Zimmerman, A. W. (2014). Sulforaphane treatment of autism spectrum disorder (ASD). *Proceedings of the National Academy of Sciences*, October 2014; *111*(43), 15550-15555. from the text: . “Sulforaphane, which showed negligible toxicity, was selected because it **upregulates genes that protect aerobic cells against oxidative stress, inflammation, and DNA-damage**, all of which are prominent and possibly mechanistic characteristics of ASD”
- Tester, Jodie, and Tessa Finney-Brown. "Sulforaphane treatment of autism spectrum disorder." *Australian Journal of Herbal Medicine* 27.1 (2015): 41.
- [Armah CN, Traka MH, Dainty JR, Defernez M, Janssens A, Leung W, Doleman JF, Potter JF, Mithen RF. A diet rich in high-glucoraphanin broccoli interacts with genotyped to reduce discordance in plasma metabolite profiles by modulating mitochondrial function. Am J Clin Nutr. 2013 Sep;98\(3\):712-22.](#)
- [Yang L, Palliyaguru DL, Kensler TW. Frugal chemoprevention: targeting Nrf2 with foods rich in sulforaphane. Semin Oncol. 2016 Feb;43\(1\):146-153.](#)
- [Heiss E, Herhaus C, Klimo K, Bartsch H, Gerhäuser C. Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. J Biol Chem. 2001 Aug 24;276\(34\):32008-15.](#)
- [Gan N, Wu YC, Brunet M, Garrido C, Chung FL, Dai C, Mi L. Sulforaphane activates heat shock response and enhances proteasome activity through up-regulation of Hsp27. J Biol Chem. 2010 Nov 12;285\(46\):35528-36.](#)

## b. CCSVI/TVAM treatment

- CCSVI-chronic spinal cerebro venous insufficiency
- Outcome of chronic infections affecting endothelium
- **TVAM (Transvascular Autonomic Modulation)** is an endovascular procedure in which a catheter is inserted via a small incision and threaded up into the jugular vein.
  - Treatment involves stretching the vein with small catheters, activating autonomic nerve fibers located within the outer tissues of the vein.
  - By stimulating the nerve fibers, we activate the venous distension reflex leading to increased sympathetic tone

Treatment: **Transdermal creams:**

“SophiaFlow” ([www.SophiaNutrition.com](http://www.SophiaNutrition.com)):  
Apply 2 times daily to front of neck ( bioactive molecules/often rapid decrease of retroviral markers). Alternate with Bee venom ointment ([www.BioPureUS.com](http://www.BioPureUS.com))



## c. **Cistus Incanus** as anti-retroviral remedy ([www.BioPureUS.com](http://www.BioPureUS.com))

Stephanie Rebensburg, Markus Helfer, Martha Schneider, Herwig Koppensteiner, Josef Eberle, Michael Schindler, Lutz Gürtler, Ruth Brack-Werner. Potent in vitro antiviral activity of **Cistus incanus** extract against HIV and Filoviruses targets viral envelope proteins. *Scientific Reports*, 2016; 6: 20394 DOI: [10.1038/srep20394](https://doi.org/10.1038/srep20394)

Scientists at the Helmholtz Zentrum München discover that extracts of the medicinal plant *Cistus incanus* (Ci) prevent human immunodeficiency viruses from infecting cells. Active antiviral ingredients in the extracts inhibit docking of viral proteins to cells. Antiviral activity of *Cistus* extracts also targets Ebola- and Marburg viruses.

### **HIV: broad activity, no resistance**

The Brack-Werner team found potent activity of Ci extracts acted against a broad spectrum of clinical HIV-1 and HIV-2 isolates. This also included a virus isolate resistant against most available drugs. "Antiviral ingredients of Ci extracts target viral envelope proteins on infectious particles and prevent them from contacting host cells," Brack-Werner explains. No resistant viruses were detected during long-term treatment (24 weeks) with Ci extract, indicating that Ci extract attacks viruses without causing resistance. Since antiviral activity of Ci extracts differs from all clinically approved drugs, Ci-derived products could be an important complementation to current established drug regimens,

## Cistus

Rauwald, H. W., et al. "On the **antispirochaetal activity** of manoyloxides and carvacrol from the oleoresin labdanum of *Cistus creticus* L." *Planta Medica* 79.13 (2013): PN53.

Bouamama, H., et al. "Antibacterial and **antifungal activities** of **Cistus incanus** and *C. monspeliensis* leaf extracts." *Therapie* 54.6 (1999): 731-733..

Cistus is also a very effective **biofilm breaker**, unmasking persistent infections:

Hannig, Christian, et al. "Effects of **Cistus-tea** on bacterial colonization and enzyme activities of the in situ pellicle." *Journal of dentistry* 36.7 (2008): 540-545.

Whole leaf Stevia has been shown in a study by Northwest University in the US to be as effective or more effective in the treatment of Lyme disease than triple antibiotic therapy, including the use of Daptomycin:

Effectiveness of **Stevia Rebaudiana Whole Leaf Extract** Against the Various Morphological Forms of **Borrelia Burgdorferi** in Vitro. *Eur J Microbiol Immunol (Bp)*. 2015 Dec ;5(4):268-80. Epub 2015 Nov 12; P A S Theophilus, M J Victoria, K M Socarras, K R Filush, K Gupta, D F Luecke, E Sapi

**SophiaFlow**: apply transdermal cream twice daily to the front of the neck (contains bioactive molecules; from [www.SophiaNutrition.com](http://www.SophiaNutrition.com))

**d. RetroV Powder anti-retroviral mix ([www.KiScience.com](http://www.KiScience.com)) or tincture (En-Vi from BiopureUS.com)**

**a. Baicalin extract from Scullcap root/Scutalaria**

- Li, B. Q., T. Fu, Y. D. Yan, N. W. Baylor, F. W. Ruscetti, and H. F. Kung. "Inhibition of HIV infection by baicalin--a flavonoid compound purified from Chinese herbal medicine." *Cellular & molecular biology research* 39, no. 2 (1993): 119-124.
- Zhao, Qing, Yang Zhang, Gang Wang, Lionel Hill, Jing-Ke Weng, Xiao-Ya Chen, Hongwei Xue, and Cathie Martin. "A specialized flavone biosynthetic pathway has evolved in the medicinal plant, *Scutellaria baicalensis*." *Science advances* 2, no. 4 (2016): e1501780.

**b. ST John's Wort**

- Meruelo, Daniel, Gad Lavie, and David Lavie. "Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin." *Proceedings of the National Academy of Sciences* 85.14 (1988): 5230-5234.
- [Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin.](#)
- D Meruelo et al., Proc Natl Acad Sci U S A

**c. Green Tea**

- Nakane, H., Hara, Y., & Ono, K. (1994). Tea polyphenols as a novel class of inhibitors for human immunodeficiency virus reverse transcriptase.

#### **d. Reishi Mushroom**

- Min, B. S., Nakamura, N., Miyashiro, H., BAE, K. W., & Hattori, M. (1998). Triterpenes from the spores of *Ganoderma lucidum* and their inhibitory activity against HIV-1 protease. *Chemical and Pharmaceutical Bulletin*, 46(10), 1607-1612.

#### **e. Stinging Nettle**

- Balzarini, J., Neyts, J., Schols, D., Hosoya, M., Van Damme, E., Peumans, W., & De Clercq, E. (1992). The mannose-specific plant lectins from *Cymbidium hybrid* and *Epipactis helleborine* and the (N-acetylglucosamine) n-specific plant lectin from *Urtica dioica* are potent and selective inhibitors of human immunodeficiency virus and cytomegalovirus replication in vitro. *Antiviral research*, 18(2), 191-207.

#### **f. Olive Leaf**

- Lee-Huang, S., Zhang, L., Huang, P. L., Chang, Y. T., & Huang, P. L. (2003). Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. *Biochemical and Biophysical Research Communications*, 307(4), 1029-1037.

#### **g. Bitter Melon**

- Trivedi, R. V., Wadher, K. J., Taksande, J. B., Mahore, J. G., & Umekar, M. J. (2011). *Momordica charantia*: A Natural and Safe Approach for the Treatment of HIV Infection. *Int J of Pharm Tech Research*, 3, 1660-1666.
- 4 Ng T B, Wong C M, Li W W, Yeung H W. [Isolation and characterization of a galactose binding lectin with insulinomimetic activities from the seeds of the bitter melon \*Momordica charantia\* \(Cucurbitaceae\)](#). *International Journal of Peptide and Protein Research*. 1986; 28 163-72

**e. Pantethine (B5)** activates gene and histone acetylation and slows replication of retroviral DNA  
Kristensson, Krister. "Microbes' roadmap to neurons." *Nature Reviews Neuroscience* 12.6 (2011): 345

Abstract: The nervous system is protected by barriers that restrict the invasion of pathogens. Nevertheless, mechanisms have evolved by which microbes can pass these barriers, enter and exit neurons and target various regions of the nervous system. In the brain, immune responses to pathogens are generally not robust, so microbes can hide and survive or, conversely, cause severe uncontrolled infections. Depending on their sites of entry and the regions that they target, microbes can cause diverse nervous system dysfunctions and even influence host behaviour to their own advantage. This Review discusses routes by which microbes can reach the nervous system and cause persistent or life-threatening infections.

From the text: ... dysfunctions in the neurovascular units. Administration of a low-molecular-weight thiol, **pantethine**, interrupts overproduction of microparticles and **prevents signs of cerebral malaria** in a mouse model 26 .

## f. Luteolin as potent anti-retroviral remedy (GliaLia from Italy or Mirica/OptiPEA-Netherlands)

Paterniti, Irene, et al. "Neuroprotection by association of palmitoylethanolamide with luteolin in experimental **Alzheimer's disease** models: the control of neuroinflammation." *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)* 13.9 (2014): 1530-1541.

Alzheimer's disease (AD) is the most common neurodegenerative disorder. Its neuropathological hallmarks include deposition of beta amyloid ( $A\beta$ ) fibrils in senile plaques. Numerous biochemical events, leading to  $A\beta$  neurotoxicity in AD, have been proposed and it seems that neuroinflammation plays a prominent role among these. Thus, since inflammatory processes and oxidative stress are considered to play an important role in neuroinflammatory disorders and in AD pathology, in the present work we decided to test a new composite, which is a formulation constituted of an anti-inflammatory compound such as palmitoylethanolamide (PEA) and the well recognized antioxidant flavonoid luteolin (Lut), subjected to an ultra-micronization process, here designated co-ultraPEALut. We investigated the effect of co-ultraPEALut in both an in vitro and ex vivo organotypic model of AD. For the in vitro model, we used human neuronal cells, obtained by differentiating SH-SY5Y neuroblastoma cells into sustainable neuronal morphology. These well-differentiated cells express features specific to mature neurons, such as synaptic structures and functional axonal vesicle transport, making this new concept for in vitro differentiation valuable for many neuroscientific research areas, including AD. Differentiated SH-SY5Y cells were pre-treated with co-ultraPEALut (reference concentrations: 27, 2.7 and 0.27  $\mu$ M PEA) for 2 h. AD features were induced by  $A\beta$ 1-42 stimulation (1  $\mu$ M). Twenty-four hours later cell vitality was evaluated by the colorimetric MTT assay, whereas the neuroinflammation underlying AD was observed by Western blot analysis for I $\kappa$ B $\alpha$  degradation and nuclear factor- $\kappa$ B translocation, as well as glial fibrillary acidic protein expression. For the organotypic model of AD, hippocampal slice cultures were prepared from mice at postnatal day 6 and after 21 days of culturing the slices were pre-treated with co-ultraPEALut (reference concentrations: 27, 2.7 and 0.27  $\mu$ M PEA) for 2 h and then incubated with  $A\beta$ 1-42 (1  $\mu$ g/ml) for 24 h. Pre-treatment with co-ultraPEALut significantly reduced inducible nitric oxide synthase and glial fibrillary acidic protein expression, restored neuronal nitric oxide synthase and brain-derived neurotrophic factor and reduced the apoptosis. **Taken together our results clearly showed that co-ultra PEALut is able to blunt  $A\beta$ -induced astrocyte activation and to exert a marked protective effect on glial cells. These findings suggest that the association of co-**

Ko, Yeon-Ju, et al. "Flavonoids as potential inhibitors of retroviral enzymes." *Journal of the Korean Society for Applied Biological Chemistry* 52.4 (2009): 321-326.

Mehla, Rajeev, Shalmali Bivalkar-Mehla, and Ashok Chauhan. "A flavonoid, **luteolin, cripples HIV-1** by abrogation of tat function." *PLoS One* 6.11 (2011): e27915.

**Palmitoyl-Ethanolamide to help regulate cannabinoid receptors and re-regulate microglia**  
Zeidán-Chuliá, Fares, et al. "The glial perspective of autism spectrum disorders." *Neuroscience & Biobehavioral Reviews* 38 (2014): 160-172.

Bertolino, Bartolomeo, et al. "Beneficial Effects of Co-ultramicrosomalized Palmitoylethanolamide/luteolin in a Mouse Model of Autism and in a Case Report of Autism." *CNS neuroscience & therapeutics* 23.1 (2017): 87-98.

### **PEA antidotes glia activation**

Sarnelli, Giovanni, et al. "HIV-1 Tat-induced diarrhea is improved by the PPAR-alpha agonist, palmitoylethanolamide, by suppressing the activation of enteric glia." *Journal of neuroinflammation* 15.1 (2018): 94.

## g. **Ecklonia Cava** (brown ocean algae; [www.biopureUS.com](http://www.biopureUS.com))

- Ahn, Mi-Jeong, et al. "Inhibition of HIV-1 reverse transcriptase and HIV-1 integrase and antiviral activity of Korean seaweed extracts." *Journal of Applied Phycology* 14.5 (2002): 325-329.

Abstract: Forty-seven species of marine macroalgae from the coast of Korea have been screened for the presence of inhibitory compounds against human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) and HIV-1 integrase (IN). One of 4 Chlorophyta, 8 of 17 Phaeophyta and 6 of 26 Rhodophyta showed inhibitory **activity against HIV-1 reverse transcriptase**. Five species (***Ecklonia cava***, *Ishige okamurae*, *Sargassum confusum*, *Sargassum hemiphyllum*, *Sargassum ringgoldianum*) belonging to Phaeophyta showed to inhibit the 3'-processing activity of HIV-1 integrase. In cell-based assays, the methanol extracts of *Bossiella* sp. and *Chondriocrassicaulis* inhibited cytopathogenicity of HIV-1 at a concentration below that cytotoxic for MT4 cells.

- Kim, Moon-Moo, Se-Kwon Kim, and Sang-Hoon Lee. "Phloroglucinol derivatives from *Ecklonia cava* having anti-HIV-1 inhibitory activity." U.S. Patent Application No. 13/130,335.
- Kim, Moon-Moo, Se-Kwon Kim, and Sang-Hoon Lee. "HIV-1 inhibiting pharmaceutical composition containing an *Ecklonia cava*-derived phloroglucinol polymer compound." U.S. Patent Application No. 13/964,687.
- Ahn, Mi-Jeong, et al. "Inhibition of HIV-1 reverse transcriptase and protease by phlorotannins from the brown alga *Ecklonia cava*." *Biological and Pharmaceutical Bulletin* 27.4 (2004): 544-547.

**h. Licorice** or intravenous glycyrrhizic acid against retroviral replication (compounding pharmacies)

**Amsar** Private Limited. Phytochemicals--Monoammonium glycyrrhizinate.  
<http://www.amsar.com/PhytoChemicals/monoammonium.htm>

**Cantelli-Forti** G, Maffei F, Hrelia R, Bugamelli F, Bernardi M D'Intino R, Maranesi M, and Raggi MA. Interaction of Licorice on Glycyrrhizin Pharmacokinetics. *Environmental Health Perspectives*. 1994 Nov;102 Suppl 9:65-8. Presented at the IV European ISSX Meeting on Toxicological Evaluation of Chemical Interactions: Relevance of Social, Environmental and Occupational Factors held 3-6 July 1992 in Bologna. <http://www.ncbi.nlm.nih.gov/pubmed/7698088>

**Fiore** C, Eisenhut M, Krausse R, Ragazzi E, Pellati D, Armanini D, Bielenberg J. Antiviral effects of Glycyrrhiza species. *Phytother Res*. 2008 Feb;22(2):141-8.  
<http://www.ncbi.nlm.nih.gov/pubmed/17886224>

**Ikegami** N., S. Kinoshita, T. Kanasaki, K. Uno, K. Akatani, T. Kishida. Glycyrrhiza glabra. Evaluation of long-term treatment with glycyrrhizin and of combination therapy with glycyrrhizin and AZT or DDI on HIV-1 carriers. *Antiviral Research*. Volume 30, Issue 1, pp. A33-A33, April, 1996.  
<http://www.ingentaconnect.com/content/els/01663542/1996/00000030/00000001/art80277>

**Ito** M, Sato A, Hirabayashi K, Tanabe F, Shigeta S, Baba M, De Clercq E, Nakashima H, Yamamoto N. Mechanism of inhibitory effect of glycyrrhizin on replication of human immunodeficiency virus (HIV). *Antiviral Res*. 1988 Dec 11;10(6):289-98.  
<http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=18974890>

- **Saidi H**, Melki M-T, and Gougeon M-L. HMGB1-Dependent Triggering of **HIV-1** Replication and Persistence in Dendritic Cells as a Consequence of NK-DC Cross-Talk *PLoS ONE*. 2008; 3(10): e3601. Published online 2008 October 31. doi: 10.1371/journal.pone.0003601.  
<http://www.ncbi.nlm.nih.gov/pubmed/18974890>
- **Melki MT**, Saidi H, Dufour A, Olivo-Marin JC, Gougeon ML.. Escape of **HIV-1**-infected dendritic cells from TRAIL-mediated NK cell cytotoxicity during NK-DC cross-talk--a pivotal role of HMGB1. *PLoS Pathog*. 2010 Apr 15;6(4):e1000862.  
<http://www.ncbi.nlm.nih.gov/pubmed/20419158>
- **Yao WH**, Zhao W, Wu YW, Zhao H, Wei HX, Cheng C, Zhu P, Chi Y. Effect of compound glycyrrhizin on peripheral T-lymphocyte subset in **AIDS** patients. *Zhonghua Nan Ke Xue*. 2006 Jul;12(7):598-601.  
<http://www.ncbi.nlm.nih.gov/pubmed/16894934>
- **Yuan H**, Ji WS, Wu KX, Jiao JX, Sun LH, Feng YT. Anti-inflammatory effect of Diammonium Glycyrrhizinate in a rat model of **ulcerative colitis**. *World J Gastroenterol*. 2006 Jul 28;12(28):4578-81.  
<http://www.ncbi.nlm.nih.gov/pubmed/16874877>

*At SHI we frequently and successfully use compounded glycyrrhizin intravenously*

## i. Selenium to silence retroviruses

- Bologna, Rosa, et al. "Selenium and immunity in HIV-1 infected pediatric patients." *Journal of Nutritional immunology* 3.1 (1994): 41-49.
- Baum, Marianna K., et al. "High risk of HIV-related mortality is associated with selenium deficiency." *JAIDS Journal of Acquired Immune Deficiency Syndromes* 15.5 (1997): 370-374.
- Campa, Adriana, et al. "Mortality risk in selenium-deficient HIV-positive children." *Journal of acquired immune deficiency syndromes and human retrovirology: official publication of the International Retrovirology Association* 20.5 (1999): 508-513.
- Cirelli, Augusto, et al. "Serum selenium concentration and disease progress in patients with HIV infection." *Clinical biochemistry* 24.2 (1991): 211-214.
- Dworkin, Brad M. "Selenium deficiency in HIV infection and the acquired immunodeficiency syndrome (AIDS)." *Chemico-biological interactions* 91.2-3 (1994): 181-186.
- Schrauzer, Gerhard N., and Juliane Sacher. "Selenium in the maintenance and therapy of HIV-infected patients." *Chemico-Biological Interactions* 91.2-3 (1994): 199-205.
- Baeten, Jared M., et al. "Selenium deficiency is associated with shedding of HIV-1--infected cells in the female genital tract." *Journal of acquired immune deficiency syndromes (1999)* 26.4 (2001): 360-364.

## j. Vagal nerve stimulation as anti inflammatory strategy

Trends in Molecular Medicine, September 2016, Vol. 22, No. 9

### MicroRNA Regulators of Anxiety and Metabolic Disorders

Chanan Meydan; Shani Shenhar-Tsarfaty and Hermona Soreq

Biomedical research views the ANS as fulfilling a more extended role than classically imagined, and ANS modulation of inflammation via cholinergic activities has become implicated in MetS [54]. The inflammatory reflex associated with cholinergic activity plays a central part in keeping inflammation under control [54]. The administration of nicotine, for example, has been known to ameliorate ulcerative colitis, with clinical and histologic improvement relative to placebo [55]. This outcome reflects a nicotinic anti-inflammatory effect, which may theoretically be mediated by miR-132 and modified AChE activity [28,51,56]. **Autonomic influence also ameliorates sepsis, as demonstrated in rats exposed to endotoxin and then to external stimulation of vagus nerves [57].** The anti-inflammatory activities of the ANS and endocrine system presumably limit the inflammatory response to the local level, avoiding a widespread systemic reaction. In parallel, the efferent reaction to inflammation is led by the parasympathetic arm of the ANS, and ACh acts to inhibit target cells in both the innate immune system (e.g., in macrophages) and in non-immune cells which participate in inflammation (such as vascular smooth muscle cells [58]). **At the cellular level, the parasympathetic system negates inflammation by inhibiting the recruitment, migration, and activation of innate immune cells [56].** This effect operates by reducing proinflammatory cytokines, such as tumor necrosis factor (TNF), IL-1, IL-6, and IL-18, and signaling molecules such as MCP-1 and HMGB-1, while also elevating anti-inflammatory cytokines [46,56,57,59]. This bidirectional process may involve miRNA-mediated control over the cholinergic surveillance of inflammation, contributing to the cholinergic-mediated bridge between anxiety and metabolic.....

### 3: Using pharmaceuticals

Dreyfus, David H. "Autoimmune disease: a role for new anti-viral therapies?." *Autoimmunity reviews* 11.2 (2011): 88-97.

Abstract: Many chronic human diseases may have an underlying autoimmune mechanism. In this review, the author presents **a case of autoimmune CIU (chronic idiopathic urticaria) in stable remission after therapy with a retroviral integrase inhibitor**, raltegravir (Isentress). Previous reports located using the search terms "autoimmunity" and "anti-viral" and related topics in the pubmed data-base are reviewed suggesting that novel anti-viral agents such as retroviral integrase inhibitors, gene silencing therapies and eventually vaccines may provide new options for anti-viral therapy of autoimmune diseases. Cited epidemiologic and experimental evidence suggests that increased replication of epigenomic viral pathogens such as Epstein–Barr Virus (EBV) in chronic human autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus Erythematosus (SLE), and multiple sclerosis (MS) may activate endogenous human retroviruses (HERV) as a pathologic mechanism. Memory B cells are the reservoir of infection of EBV and also express endogenous retroviruses, thus depletion of memory b-lymphocytes by monoclonal antibodies (Rituximab) may have therapeutic anti-viral effects in addition to effects on B-lymphocyte presentation of both EBV and HERV superantigens. Other novel anti-viral therapies of chronic autoimmune diseases, such as retroviral integrase inhibitors, could be effective, although not without risk.

*At SHI we good experience working with Truvada, starting with ¼ tablet (50/75 mg). Other anti retroviral approaches for chronic illness are now in the pipeline.*

The use of **Quinacrine** (a group known for their anti Malaria effects)

[Front Microbiol.](#) 2017 Oct 17;8:2007. doi: 10.3389/fmicb.2017.02007. eCollection 2017.

**Curaxin CBL0100 Blocks HIV-1 Replication and Reactivation through Inhibition of Viral Transcriptional Elongation.**

[Jean MJ](#)<sup>1</sup>, [Hayashi T](#)<sup>1</sup>, [Huang H](#)<sup>1</sup>, [Brennan J](#)<sup>1</sup>, [Simpson S](#)<sup>1</sup>, [Purmal A](#)<sup>2</sup>, [Gurova K](#)<sup>3</sup>, [Keefer MC](#)<sup>4</sup>, [Kobie JJ](#)<sup>4</sup>, [Santoso NG](#)<sup>1</sup>, [Zhu J](#)<sup>1,5</sup>.

Despite combination antiretroviral therapy (cART), acquired immunodeficiency syndrome (AIDS), predominantly caused by the human immunodeficiency virus type 1 (HIV-1), remains incurable. The barrier to a cure lies in the virus' ability to establish a latent infection in HIV/AIDS patients. Unsurprisingly, efforts for a sterilizing cure have focused on the "shock and kill" strategy using latency-reversing agents (LRAs) to complement cART in order to eliminate these latent reservoirs. However, this method faces numerous challenges. Recently, the "block and lock" strategy has been proposed. It aims to reinforce a deep state of latency and prevent sporadic reactivation ("blip") of HIV-1 using latency-promoting agents (LPAs) for a functional cure. Our studies of curaxin 100 (CBL0100), a small-molecule targeting the facilitates chromatin transcription (FACT) complex, show that it blocks both HIV-1 replication and reactivation in *in vitro* and *ex vivo* models of HIV-1. Mechanistic investigation elucidated that CBL0100 preferentially targets HIV-1 transcriptional elongation and decreases the occupancy of RNA Polymerase II (Pol II) and FACT at the HIV-1 promoter region. In conclusion, CBL0100 is a newly identified inhibitor of HIV-1 transcription that can be used as an LPA in the "block and lock" cure strategy

<https://www.ncbi.nlm.nih.gov/pubmed/29089933>

*We have used compounded Quinacrine in small doses successfully in adults with chronic illness. Loading dose: 200 mg every 15 minutes to a total of 800 mg. Followed by 100 mg t.i.d for 10 days. Premedicate with B vitamins and R-Lipoic acid to avoid side effects. Amazing results!*

## Suramin

- Ruprecht, R. M., Rossoni, L. D., Haseltine, W. A., & Broder, S. (1985). Suppression of retroviral propagation and disease by suramin in murine systems. *Proceedings of the National Academy of Sciences*, 82(22), 7733-7737.
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*In my Swiss practice I have successfully used suramin at the published dose of 20 mg/kg every 6 weeks. Surprising results, especially in children with autism*

# Contact Information

**For referrals/patients:** [www.SophiaHealthInstitute.com](http://www.SophiaHealthInstitute.com)

Location: Woodinville, WA - with satellite clinics in LA and Bay area

European patients: [www.SwissBioHealth.com](http://www.SwissBioHealth.com)

**For practitioner education:**

[www.klinghardtInstitute.com](http://www.klinghardtInstitute.com); [Info@klinghardtinstitute.com](mailto:Info@klinghardtinstitute.com)

[www.klinghardtAcademy.com](http://www.klinghardtAcademy.com)

Germany: [www.INK.com](http://www.INK.com) (Institut fuer Neurobiologie Klinghardt, Freiburg)

Switzerland: CINAK (Centre International Neurobiology alla Klinghardt, Geneva)

**For patient education/self development:** [www.SophiaEducation.com](http://www.SophiaEducation.com)