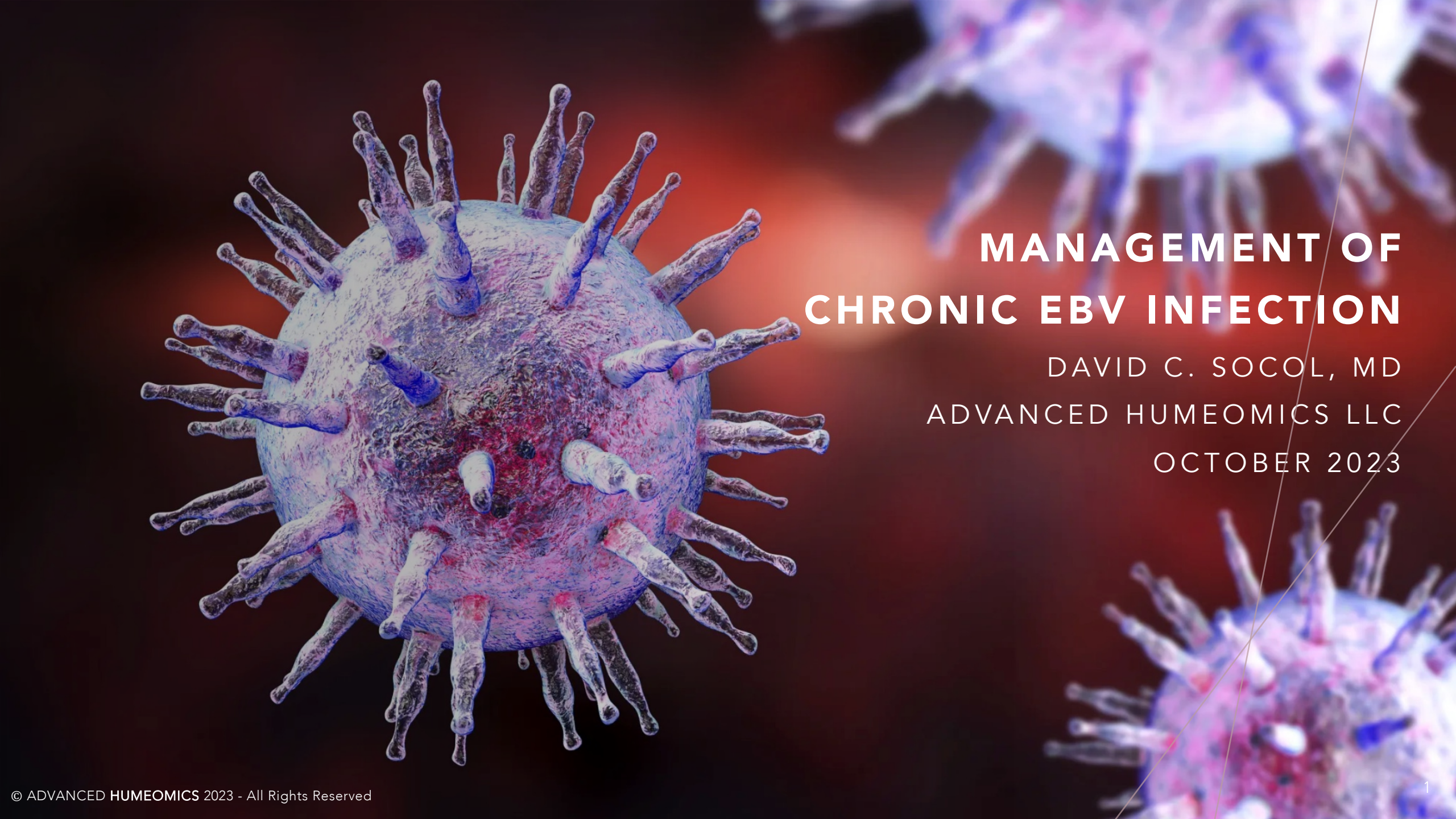


MANAGEMENT OF CHRONIC EBV INFECTION

DAVID C. SOCOL, MD
ADVANCED HUMEOMICS LLC

OCTOBER 2023

INTENDED USE

This presentation is intended for **healthcare professionals only**. It is not intended for general distribution.

SUPPLEMENTAL INFORMATION

WITH PERMISSION FROM THE AUTHOR, 2023.1022, AMMG, HOUSTON, TX

To learn more about the pathophysiology of EBV and other interventional options, return to the Provider's Corner of our website to upload:

Clinical Considerations & Protocols for Epstein Barr, Cytomegalo and Human Herpesvirus-6, by Christopher Bump, DC-MS, IFMCP

ACKNOWLEDGEMENTS

Our progress would not be possible without the following individuals:

Anne Tillman, formerly of Researched Nutritionals, who prompted the use of Transfer Factors in the approach to EBV management.

Dr. Aunna Herbst, who introduced Andrographis EP80 and shared information about her successes with the combination of Andrographis EP80 and Humavir. Aunna also encouraged our continued efforts when outcomes were not matching expectations.

Dr. Eddie Maristany, who introduced InfectoLab, and **Felix Scholz, Ph.D**., CEO of InfectoLab, who was generous with his time as I sorted through the nuances of using the T-cell compartment in lieu of antibodies as a marker of infectious disease activity.

Dr. Cynthia Keller, a faculty member at SSRP, for her coaching and guidance on the value of peptides in the management of infectious disease.

The **IntellxxDNA Community,** who provided some finishing details that made this slide deck more comprehensive than it would have otherwise been.

Our patients, whose patience made these outcomes possible.

RISKS BEYOND FATIGUE

COMPLICATIONS OF EBV INFECTION

- Nasopharyngeal carcinoma
- Multiple sclerosis
- Hodgkin lymphoma
- Burkitt lymphoma
- Lupus
- Rheumatoid arthritis
- Inflammatory bowel disease
- Type 1 diabetes
- Juvenile idiopathic arthritis
- Celiac disease
- Hashimoto's thyroiditis

Article | Published: 12 April 2023

Chromosomal fragile site breakage by EBV-encoded EBNA1 at clustered repeats

Julia Su Zhou Li, Ammal Abbasi, Dong Hyun Kim, Scott M. Lippman, Ludmil B. Alexandrov & Don W. Cleveland

Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM

Epstein–Barr virus is an agent of genomic instability

A protein from Epstein–Barr virus called EBNA1 has been shown to bind to and break human chromosome 11, producing instability in the genome that might cause a predisposition to cancer.

CLINICAL TOUCHPOINTS

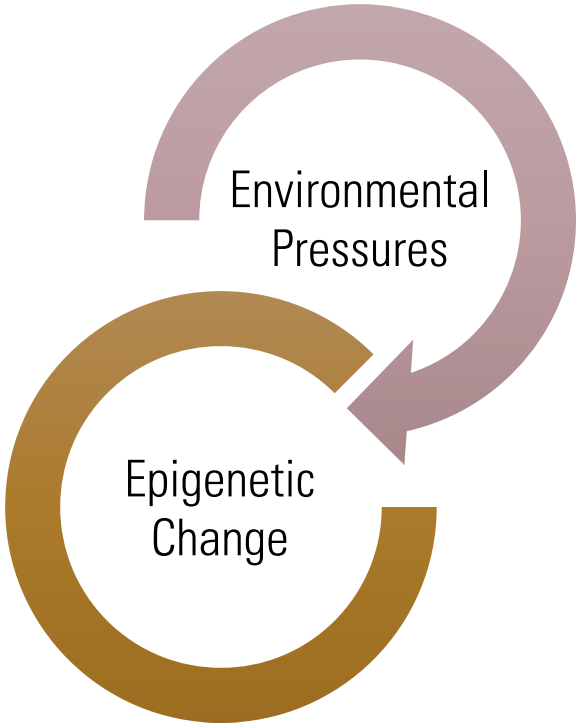
MANAGEMENT

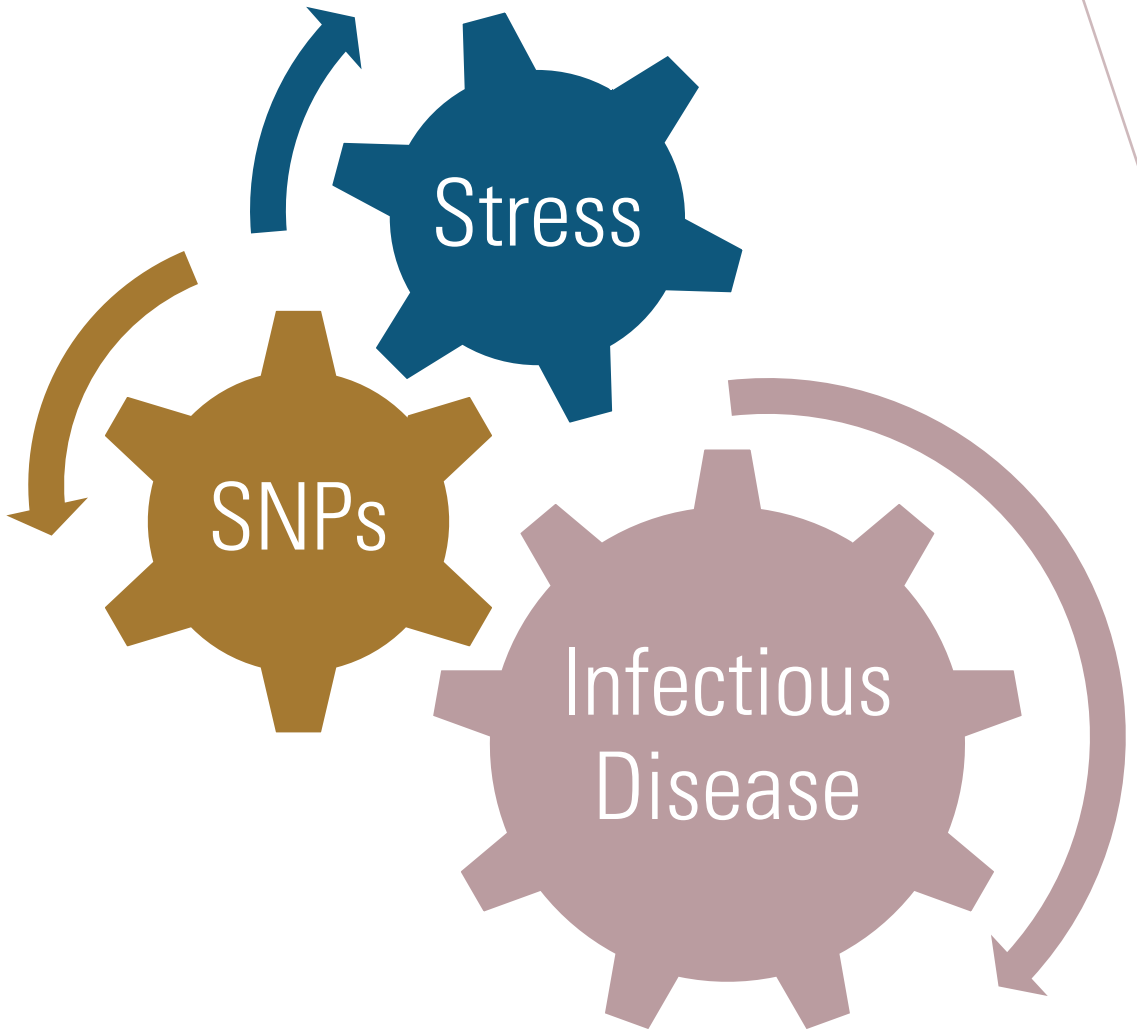
Mitochondrial

Immune System

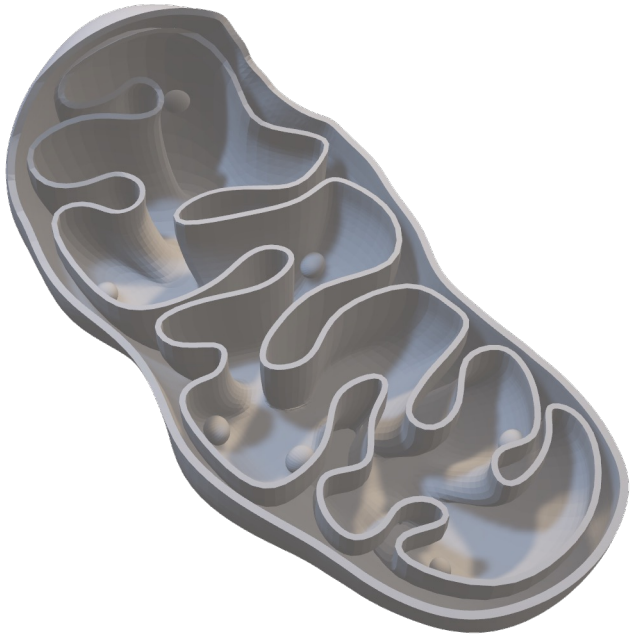
Antiviral Support

BACKGROUND PRESSURES





MITOCHONDRIA
ESSENTIAL FUNCTIONS



- Energy metabolism
- Innate immune response
- Production of ROS

DRIVERS OF MITOCHONDRIAL DYSFUNCTION

01
GENOMIC

02
ENVIRONMENTAL
- Micronutrient insufficiencies
- Pollution

03
HORMONAL
- Testosterone
- Estrogen

04
BODY COMPOSITION
- BMI > 25 kg/mg
- IL-6

05
INFECTIOUS DISEASE
- SARS-CoV-2
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)

GENOMIC DRIVERS

01

DIRECT

- TOMM40
- CBS
- GLRX
- MTHFD1

02

INDIRECT

- PPARGC1A
- CDH13
- SGSM2
- CACNA1C

"METABOLIC-OXIDATIVE STRESS INFLAMMATORY SPIRAL"

Thompson, E. A., et al. (2021). Metabolic programs define dysfunctional immune responses in severe COVID-19 patients. Cell reports, 34(11), 108863.

Epstein Barr Virus

- Induces ACE2 expression
- Drives mitochondrial dysfunction
- Infects T cells and B cells

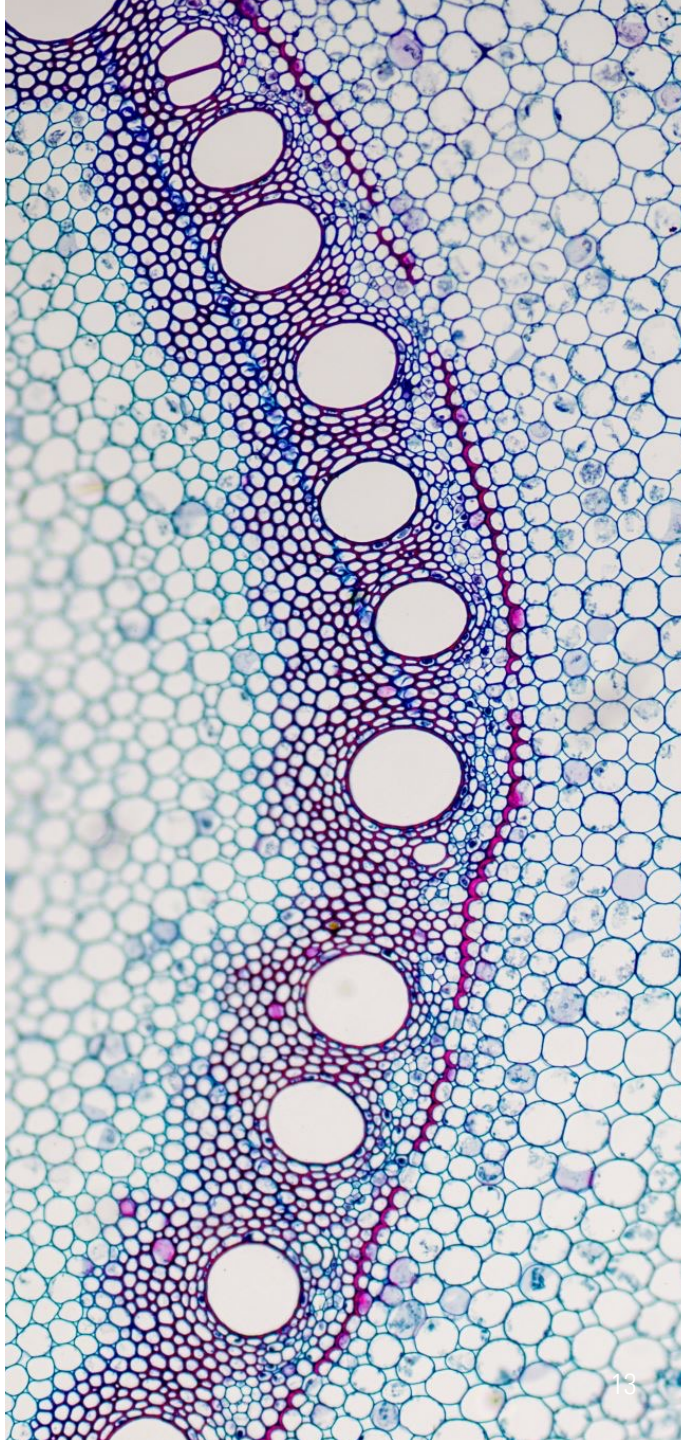
- Mitochondiral homeostatis
  - Essential for sustained killing by cytotoxic T cells

XIE, L., SHI, F., LI, Y., LI, W., YU, X., ZHAO, L., ZHOU, M., HU, J., LUO, X., TANG, M., FAN, J., ZHOU, J., GAO, Q., WU, W., ZHANG, X., LIAO, W., BODE, A. M., & CAO, Y. (2020).

DRP1-DEPENDENT REMODELING OF MITOCHONDRIAL MORPHOLOGY TRIGGERED BY EBV-LMP1 INCREASES CISPLATIN RESISTANCE.

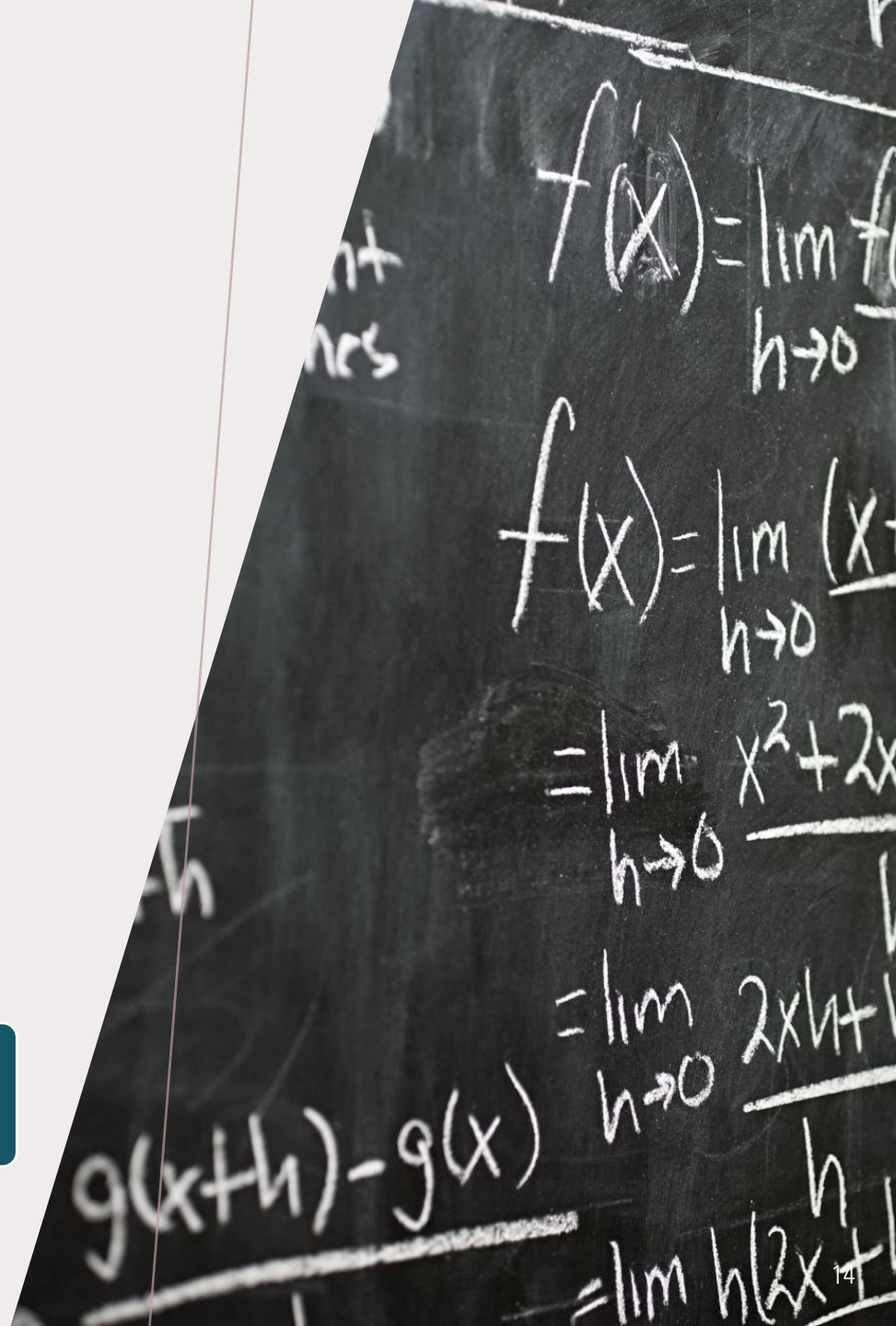
SIGNAL TRANSDUCTION AND TARGETED THERAPY, 5(1), 56.

- Alters mitochondrial dynamics & DNA replication
- Enhances glycolysis via latent membrane protein 1 (LMP1) and dynamin related protein 1 (Drp1)
- Decreases mitochondrial biogenesis and autophagy through metabolic reprogramming of monocytes, which ultimately increases apoptosis and compromises immune surveillance
- Modulates c-Myc activity and glutaminolysis and thus aerobic glycolysis
- Enhances the activity of glutaminase-1 (GLS-1) isoforms in mitochondria, which ultimately drives oncogenesis



CLINICAL PROCESS

- QUESTIONNAIRE
- LABORATORY
- SELECT INTERVENTIONS
- REPEAT QUESTIONNAIRE & LABORATORY
- ADJUST



CLINICAL PROCESS

- QUESTIONNAIRE
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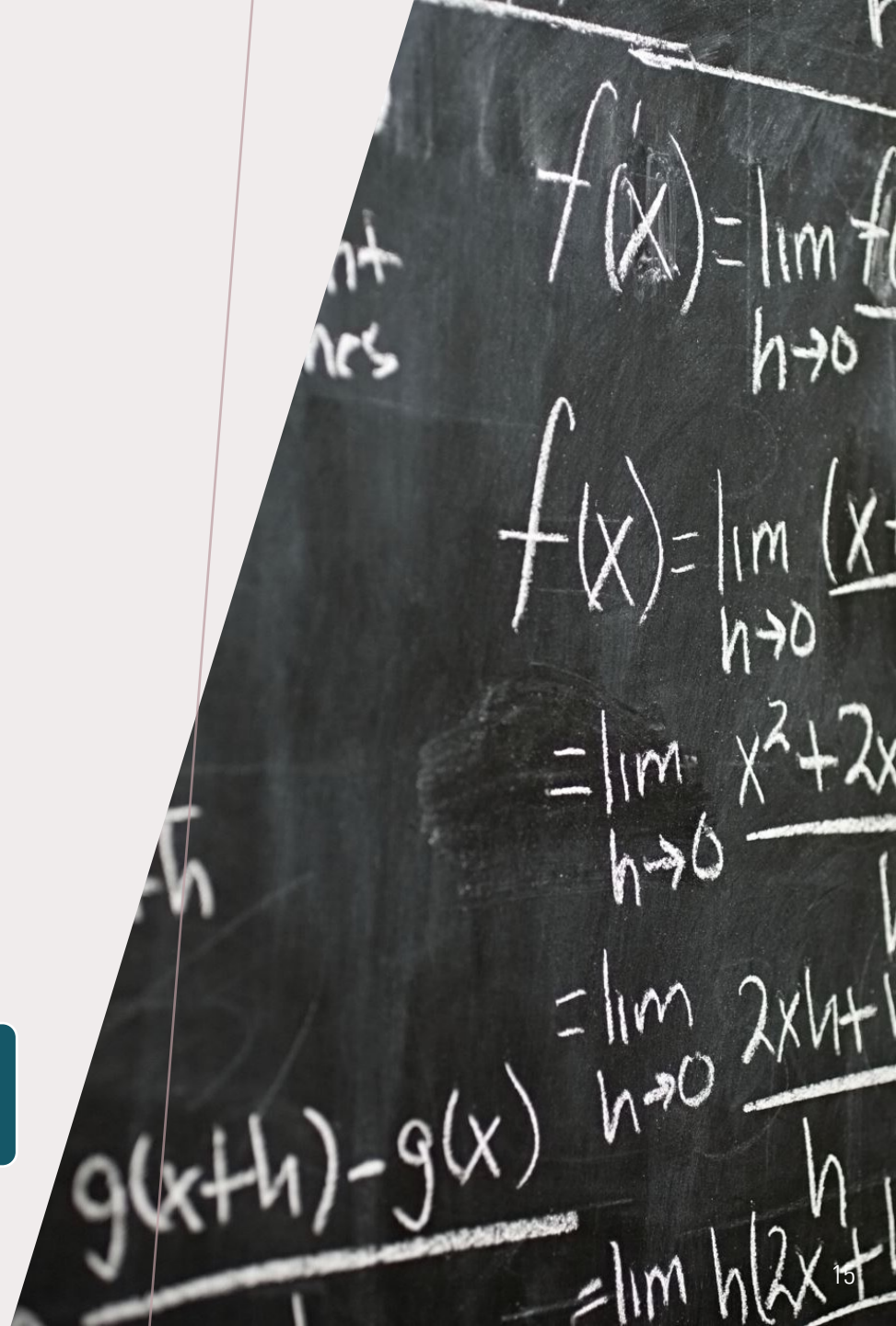
- SELECT INTERVENTIONS
- REPEAT QUESTIONNAIRE & LABORATORY
- ADJUST



QUESTIONNAIRE

Goal

- To standardize clinical insight and support monitoring

Limitations

- No validated EBV questionnaire

Rationale

- Relationship between EBV and multiple sclerosis

I have been less alert

I have had difficulty paying attention for long periods of time

I have been unable to think clearly

I have been clumsy and uncoordinated

I have been forgetful

I have had to pace myself in physical activities

I have been less motivated to do anything that requires physical effort

I have been less motivated to participate in social activities

I have been limited in my ability to do things away from home

I have trouble maintaining physical effort for long periods

I have had difficulty making decisions

MODIFIED FATIGUE IMPACT SCALE (MFIS)

Embedded in a larger questionnaire to audit multiple sclerosis symptoms

Minimum interval: q4 weeks, 21 questions

Results divided into several subsections

Physical	Cognitive	Psychosocial	Total Score

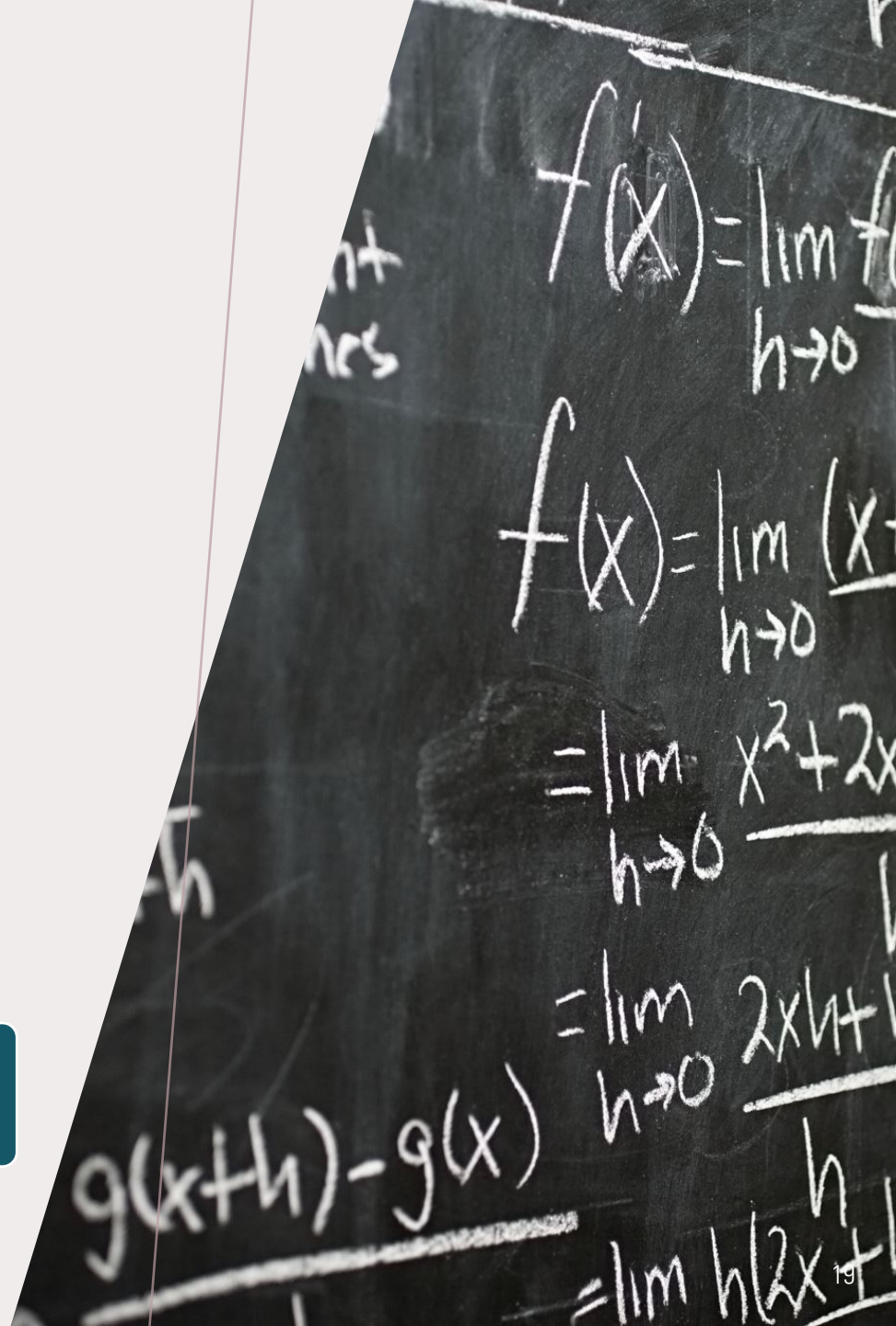
CLINICAL PEARL

QUESTIONNAIRE

Our use of a questionnaire has proven to be useful prior to, during and after treatment, for a minimum of 6 months.

CLINICAL PROCESS

- QUESTIONNAIRE
- LABORATORY TESTING
- SELECT INTERVENTIONS
- REPEAT QUESTIONNAIRE & LABORATORY
- ADJUST

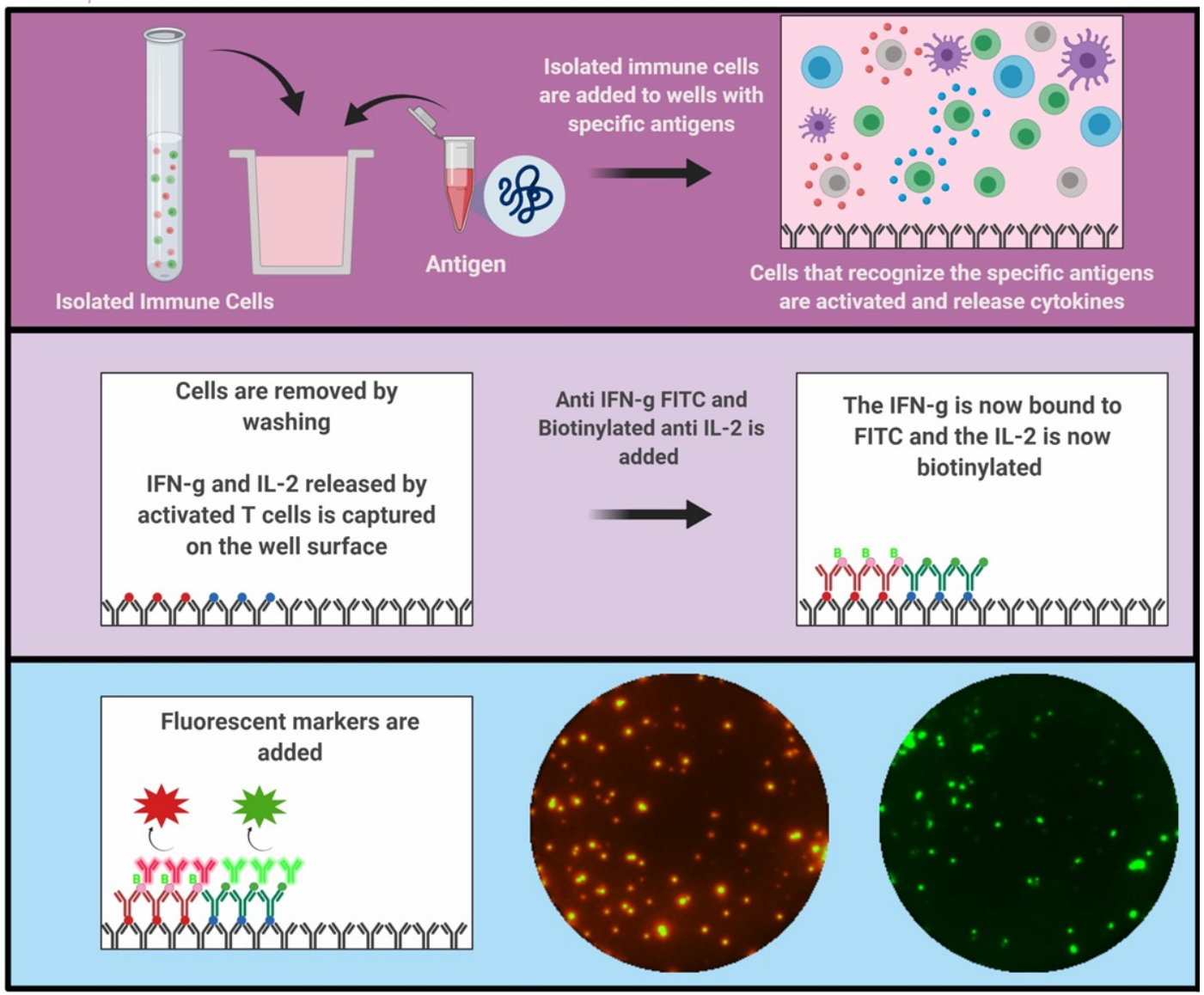


LABORATORY

Goals

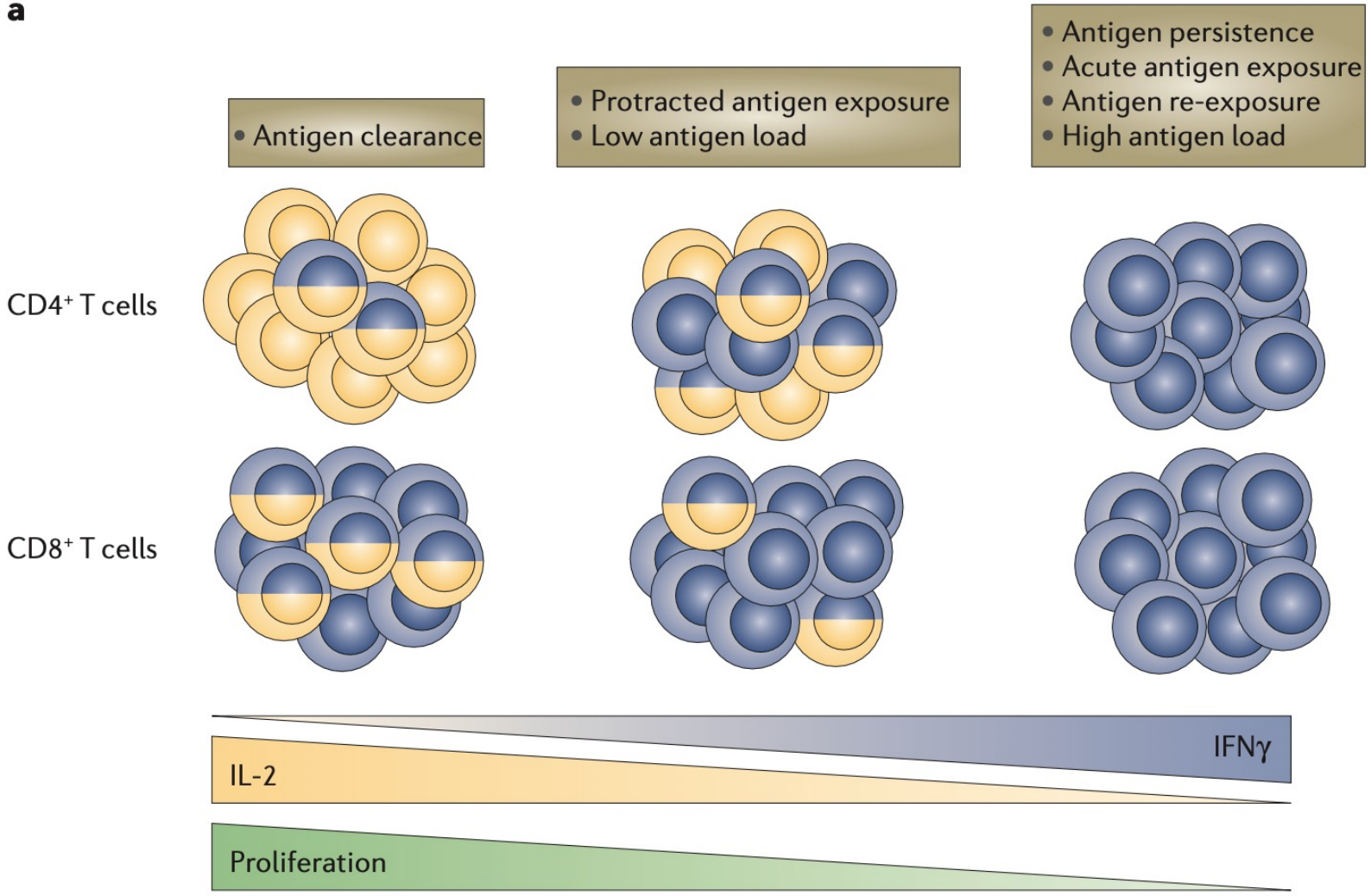
- Calibrate viral load and **lytic vs. latent** status
- Calibrate immune status - CBC
- Optional
  - Adrenocortical competency - cortisol
  - Mitochondrial function
  - Micronutrient testing

HOW TO MONITOR SPECIFIC T-CELL SUBSETS



- PBMCs (white blood cells) are isolated
- Plated on a pre-coated plate
- Specific antigens are used for re-stimulation
- After over night incubation cells are washed away
- Released cytokines are visualized
- Each spot represents one antigen specific T-cell of the patient

PANTALEO, G., & HARARI, A. (2006). FUNCTIONAL SIGNATURES IN ANTIVIRAL T-CELL IMMUNITY FOR MONITORING VIRUS-ASSOCIATED DISEASES. NATURE REVIEWS. IMMUNOLOGY, 6(5), 417–423.

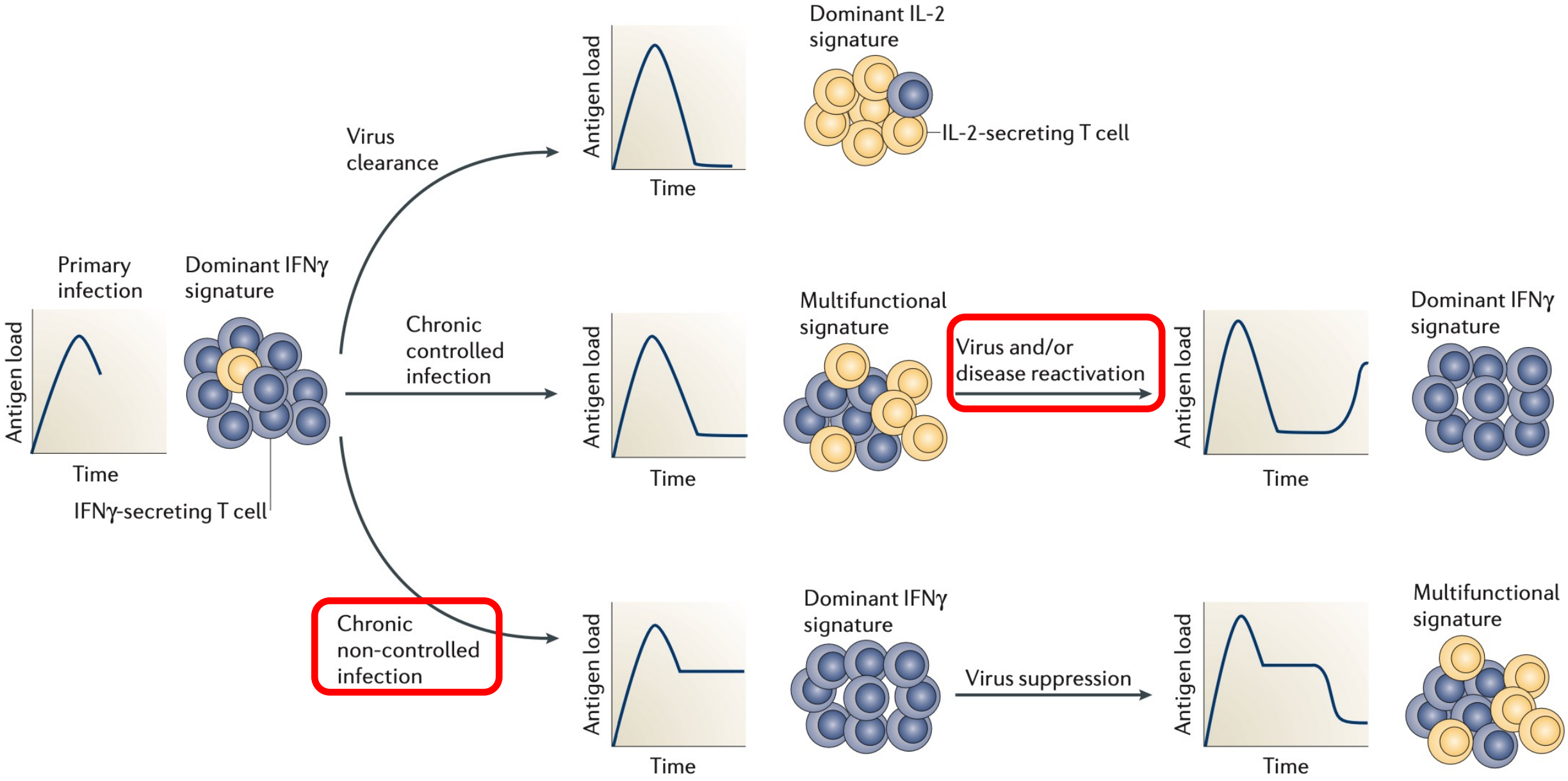


CLINICAL PEARL

INFECTOLAB TESTING

InfectoLab testing may be repeated every 12-16 weeks based on the lifespan of T cells. Our practice typically tests at 16-week intervals, but we may also test at 8 weeks if we're looking to validate prior clinical decision making.

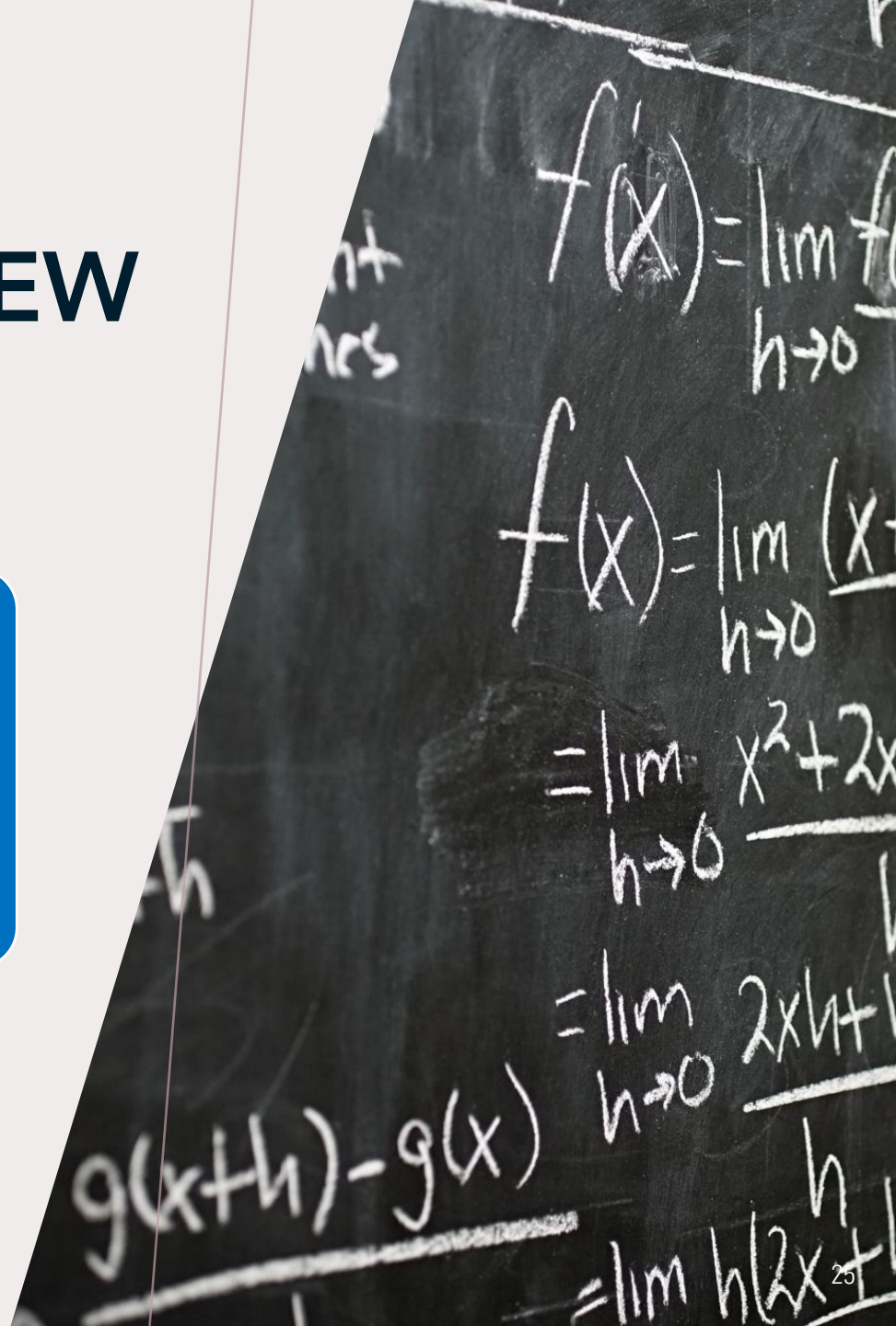
Aside: Immunologists use an IgG that is 5x greater than normal as an indication of EBV reactivation.



CLINICAL DEVELOPMENT

INFECTOLAB TESTING - NEW

We have started using the Functional Immune Assessment in our more challenging cases. Outcome data is pending.



CLINICAL PEARL

INTERVENTIONS

In our experience, no single intervention solves the problem of chronic EBV infection. Scaffolding within a category (e.g. multiple antivirals) or across categories (e.g. immune and antiviral support) is a pre-requisite for success as is a good understanding of patient needs and an iterative approach to care.

CLINICAL PROCESS

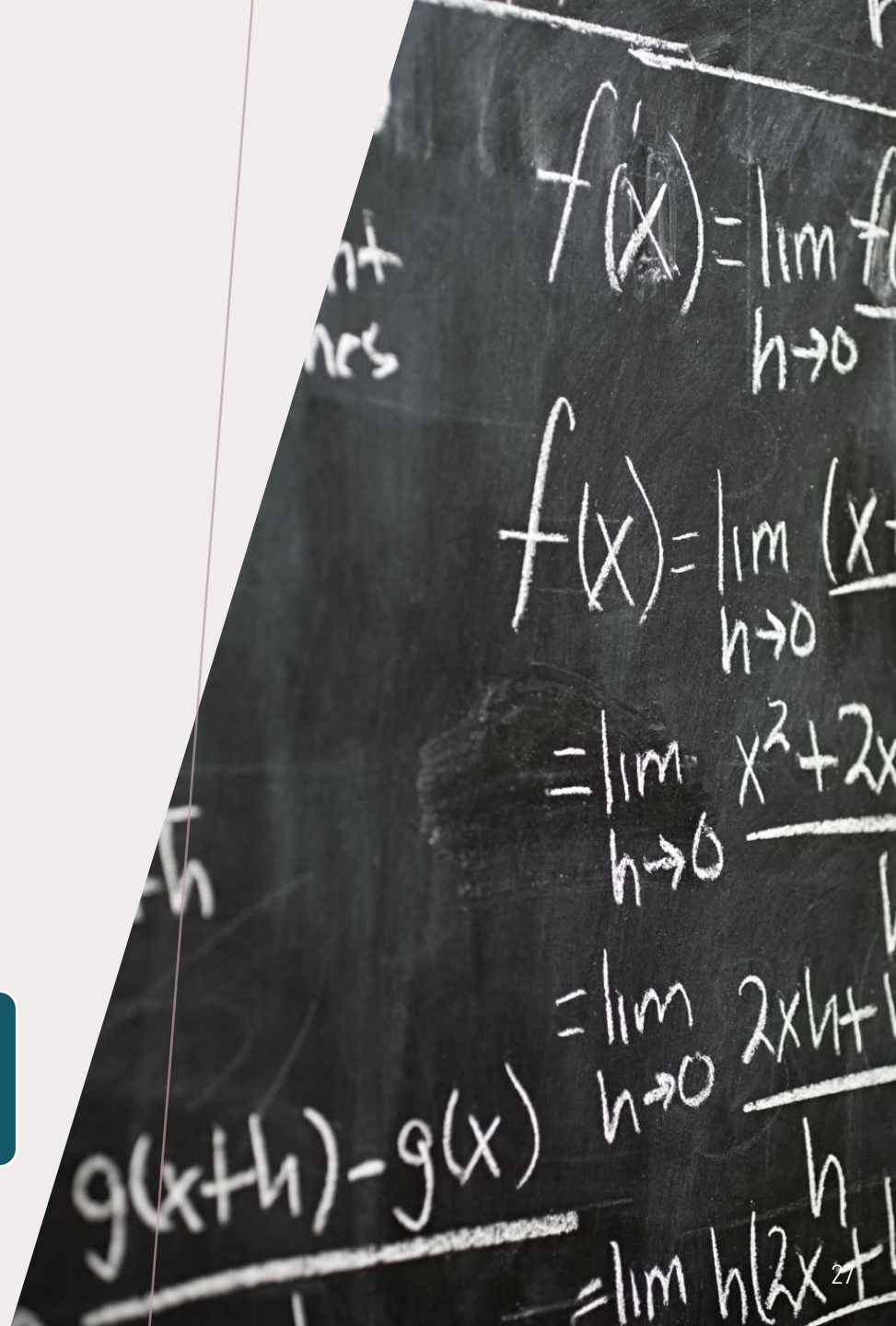
QUESTIONNAIRE & LABORATORY

SELECT INTERVENTIONS

- Antiviral, Immune Support, and/or Mitochondrial

REPEAT QUESTIONNAIRE & LABORATORY

ADJUST



NIH IN VITRO DATA – HUMIC ACID & EBV

EFFECTIVE INHIBITORY CONCENTRATION AT 50% (IC_{50}) AND 90% (IC_{90}) OF HUMIC ACID (HA) vs. REFERENCE COMPOUND WITH SELECT HERPESVIRIDAE

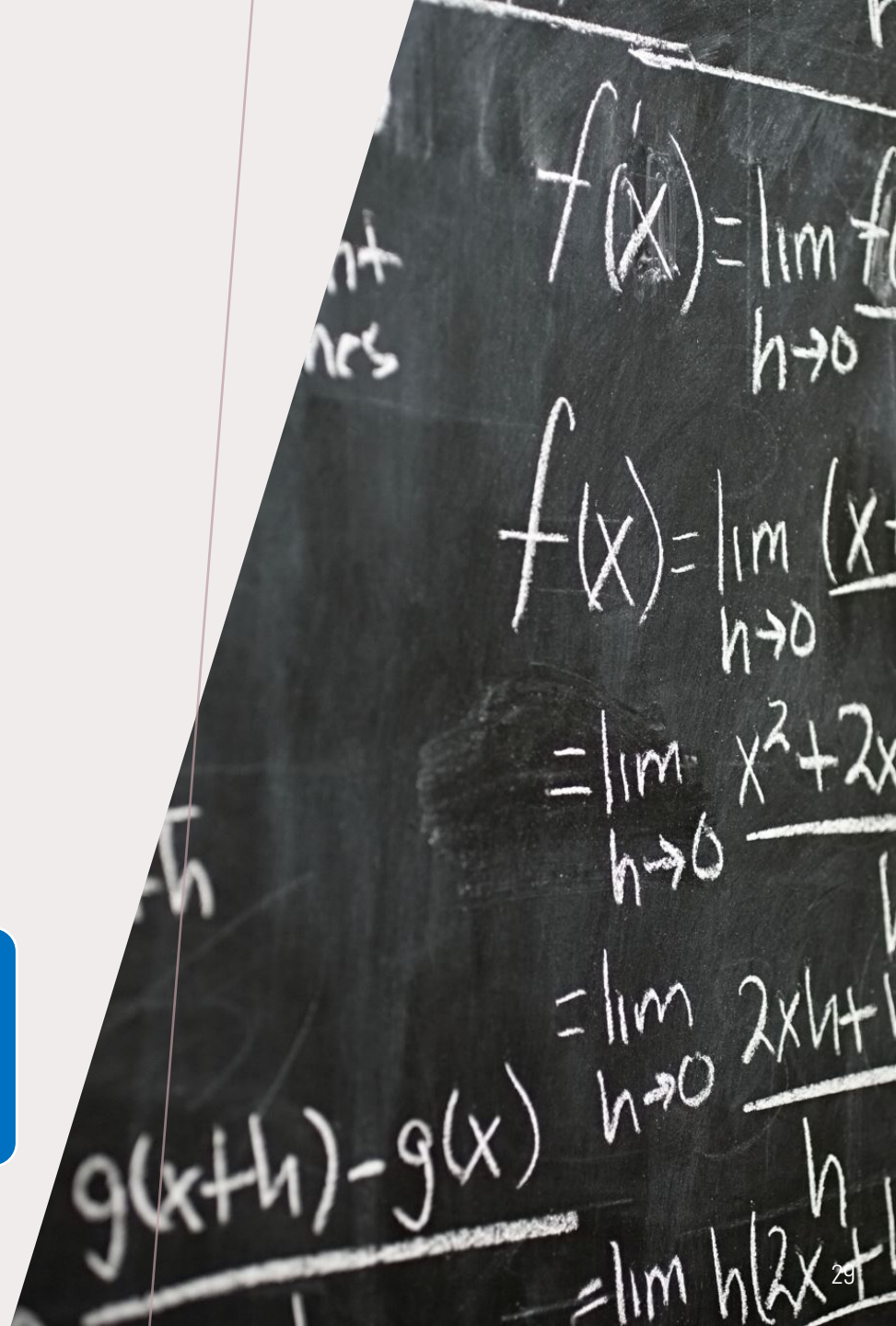
	IC_{50} mcg/mL		IC_{90} mcg/mL	
	HA	Acyclovir	HA	Acyclovir
HSV2 - Herpes Simplex Virus Type 2 [a]	2.5	1.1 – 1.3	6.7	9.5
EBV - Epstein Barr Virus [b]	>50	1.8 – 2.4	>50	16.3
	HA	Gancyclovir	HA	Gancyclovir
hCMV – Human Cytomegalovirus [a]	32.3	0.3 – 0.76	47	0.6 – 1.3
	[a] Human foreskin fibroblast cells; [b] Daudi cells			

CLINICAL PEARL
HUMIC ACID

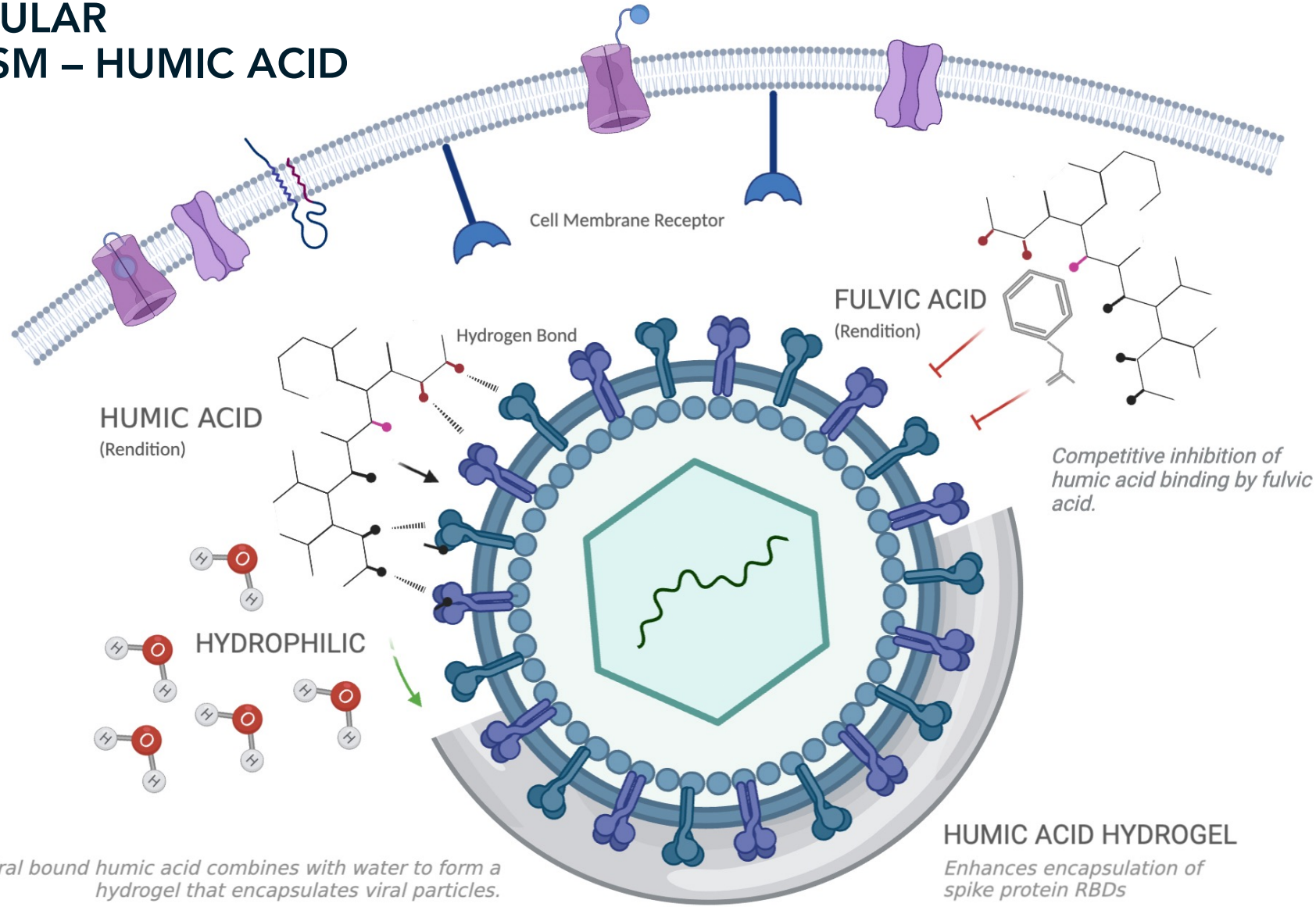
Baseline IC_{50} and IC_{90} EBV & Varicella data was the least promising of all *in vitro* testing performed for humic acid; however, Humavir's enhancements unexpectedly made it clinically relevant in the management of EBV, but ONLY **in tandem** with other products. VZV has not been tested.

Dr. Herbst was the first to use Humavir in combination with Andrographis EP80 to improve clinical outcomes.

In our experience and others, none of the products cited in this presentation stand on their own vs. EBV, but they work well together.



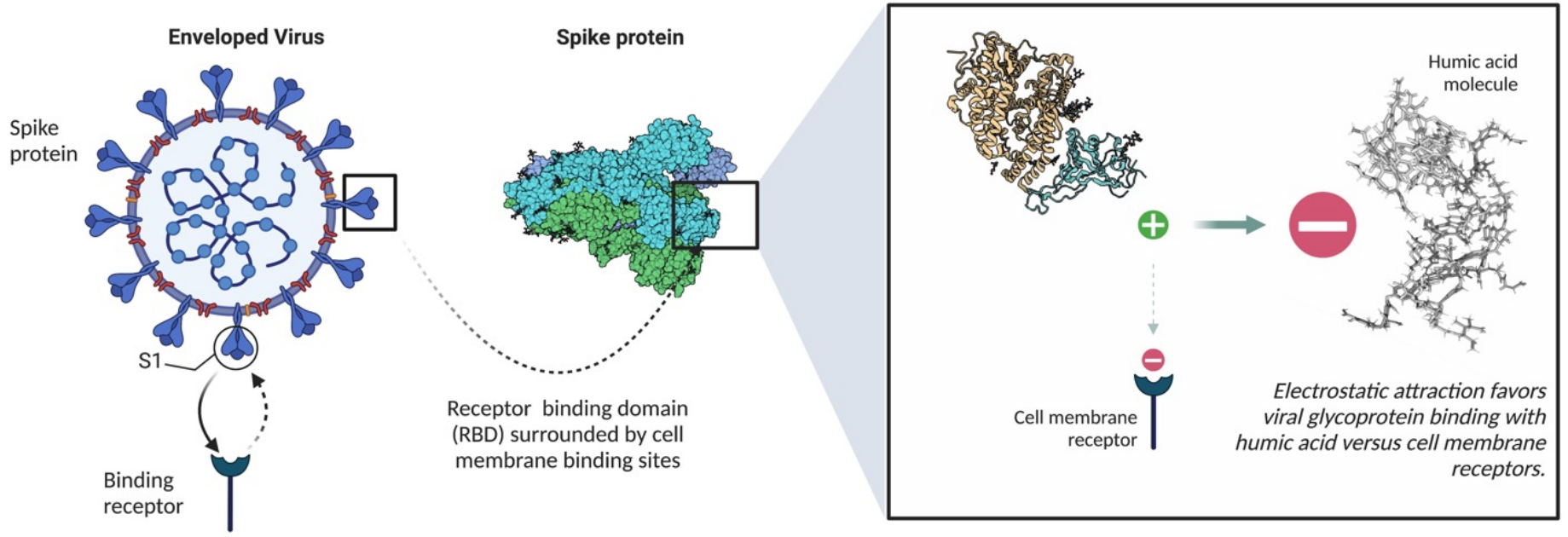
EXTRACELULAR MECHANISM – HUMIC ACID



Socol, D. C. (2023). Clinical review of humic acid as an antiviral: Leadup to translational applications in clinical humeomics. *Frontiers in Pharmacology*, *13*, 1018904.

Figure 2: Binding Mechanism of Humic Acid with Spike Proteins

Net negatively charged humic acid binds to positively charged viral glycoproteins to inhibit viral binding to target cell membrane receptors.



INTRACELULAR MECHANISM – HUMIC ACID & ANDROGRAPHOLIDE

OPEN

Humic acid inhibits HBV-induced autophagosome formation and induces apoptosis in HBV-transfected Hep G2 cells

Received: 11 July 2016
Accepted: 14 September 2016
Published: 06 October 2016

Kishor Pant[1], Ajay K. Yadav[1], Parul Gupta[1], Abhishek Singh Rathore[1], Baibaswata Nayak[2] & Senthil K. Venugopal[1]

Article

Andrographolide Inhibits Epstein–Barr Virus Lytic Reactivation in EBV-Positive Cancer Cell Lines through the Modulation of Epigenetic-Related Proteins

Praphatson Malat [1,2], Tipaya Ekalaksananan [1,2], Chukkris Heawchaiyaphum [3], Supawadee Suebsasana [4], Sittiruk Roytrakul [5], Yodying Yingchutrakul [5] and Chamsai Pientong [1,2,*]

CLINICAL PEARL

ANDROGRAHPIS

EuroMedica's Andrographis EP80 is the most potent Andrographis in the market. It is marked exclusively by EuroMedica.

Some providers have successfully managed EBV by combining Andrographis EP80 with Humavir where the former did not provide adequate improvement on its own

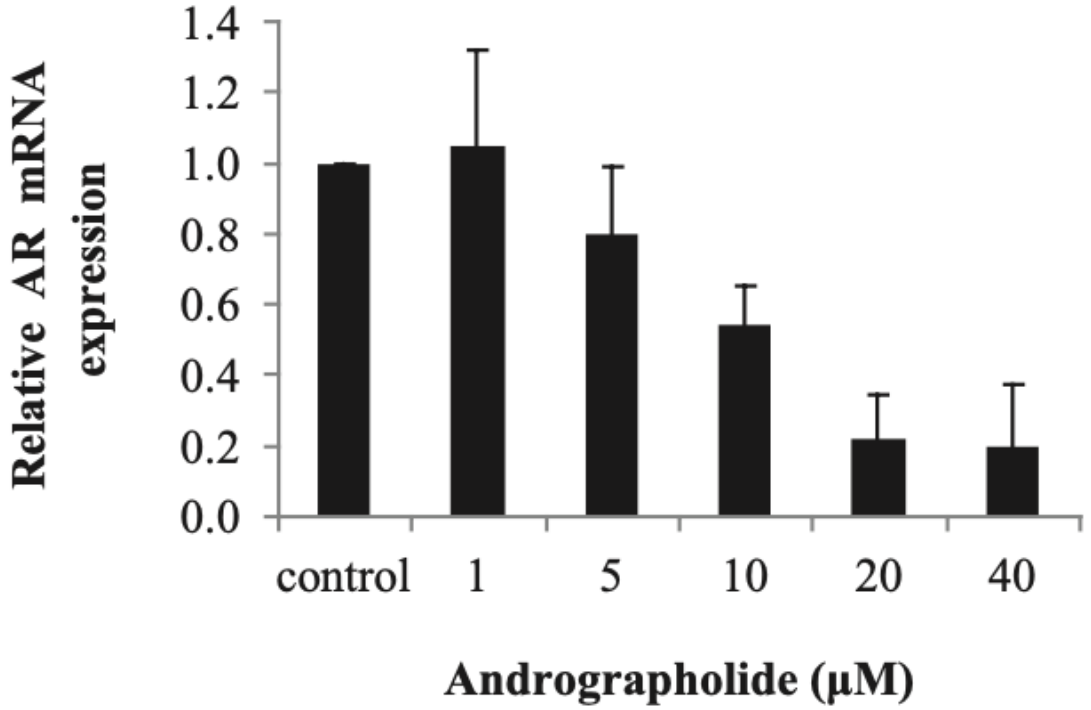
LIU, C., NADIMINTY, N., TUMMALA, R., CHUN, J. Y., LOU, W., ZHU, Y., SUN, M., EVANS, C. P., ZHOU, Q., & GAO, A. C. (2011).

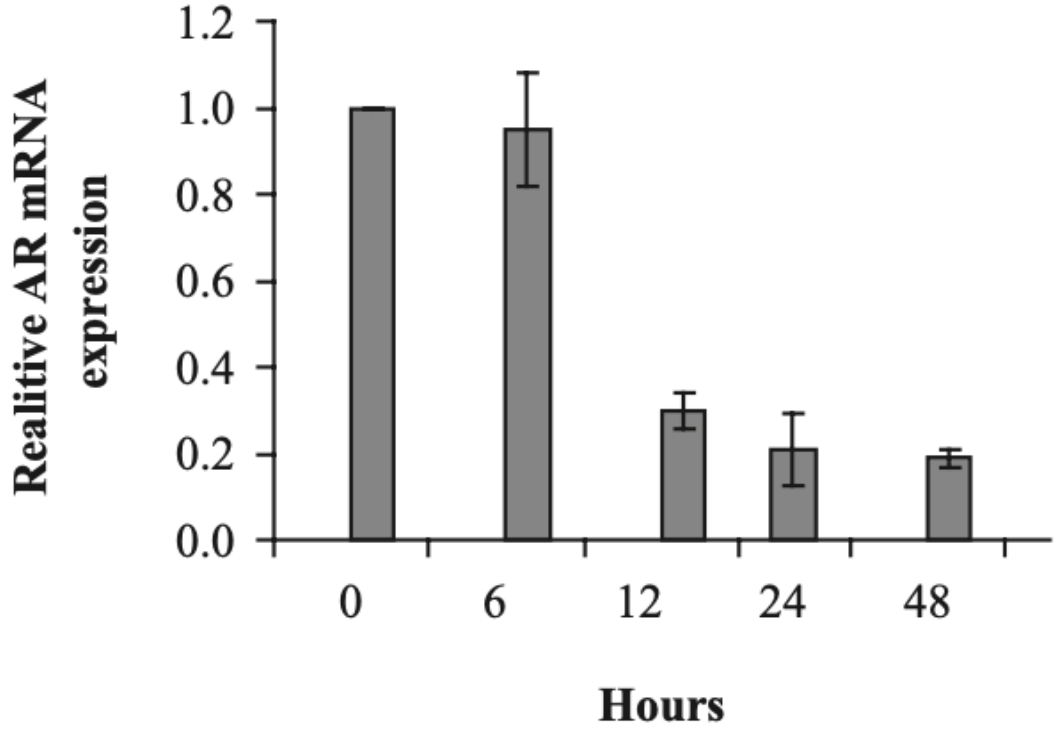
ANDROGRAPHOLIDE TARGETS ANDROGEN RECEPTOR PATHWAY IN CASTRATION-RESISTANT PROSTATE CANCER.

GENES & CANCER, 2(2), 151–159. HTTPS://DOI.ORG/10.1177/1947601911409744

Andrographolide down-regulates androgen receptor expression at both mRNA and protein levels, prevents its nuclear translocation, and inhibits transactivation of its target genes.

LIU, C., NADIMINTY, N., TUMMALA, R., CHUN, J. Y., LOU, W., ZHU, Y., SUN, M., EVANS, C. P., ZHOU, Q., & GAO, A. C. (2011). **ANDROGRAPHOLIDE TARGETS ANDROGEN RECEPTOR PATHWAY IN CASTRATION-RESISTANT PROSTATE CANCER.** GENES & CANCER, 2(2), 151–159. HTTPS://DOI.ORG/10.1177/1947601911409744





SELECT INTERVENTIONS

- Immune Support and Mitochondrial

Immune Support

Transfer Factor PlasMyc
Th1 vs. Th2 Support

Thymosin Alpha-1

Fludrocortisone

Glutathione

Mitochondrial Support

Alpha Lipoic Acid

Acetyl-l-Carnitine

CoQ10, PQQ, B Vitamins, phosphatidylcholine, …

CLINICAL REFRESHER

TRANSFER FACTORS

Transfer factors are immune proteins produced by lymphoid cells that can be cultivated from lymphocytes, egg yolks or colostrum.

CLINICAL REFRESHER

Th1 vs. Th2 PATHWAYS

Th1 cells activate the cellular immunity in response to an intracellular infection with bacteria or viruses. The cytokine footprint of this response includes IFN-gamma, IL-2 and TNF-a proliferation, which stimulate macrophage to kill pathogens. Th1 cells are also responsible for driving the emergence of cytotoxic T cells, which kill infected cells directly.

The hallmark of the Th2 pathway is an antibody-mediated immune response and includes the recruitment of B cells to make antibodies and eosinophilic activation. While the Th1 response is associated with viral and bacterial infections, the Th2 response is associated with parasitic and helminth infections. Associated cytokines include IL-21, IL-22, and TGF-beta.

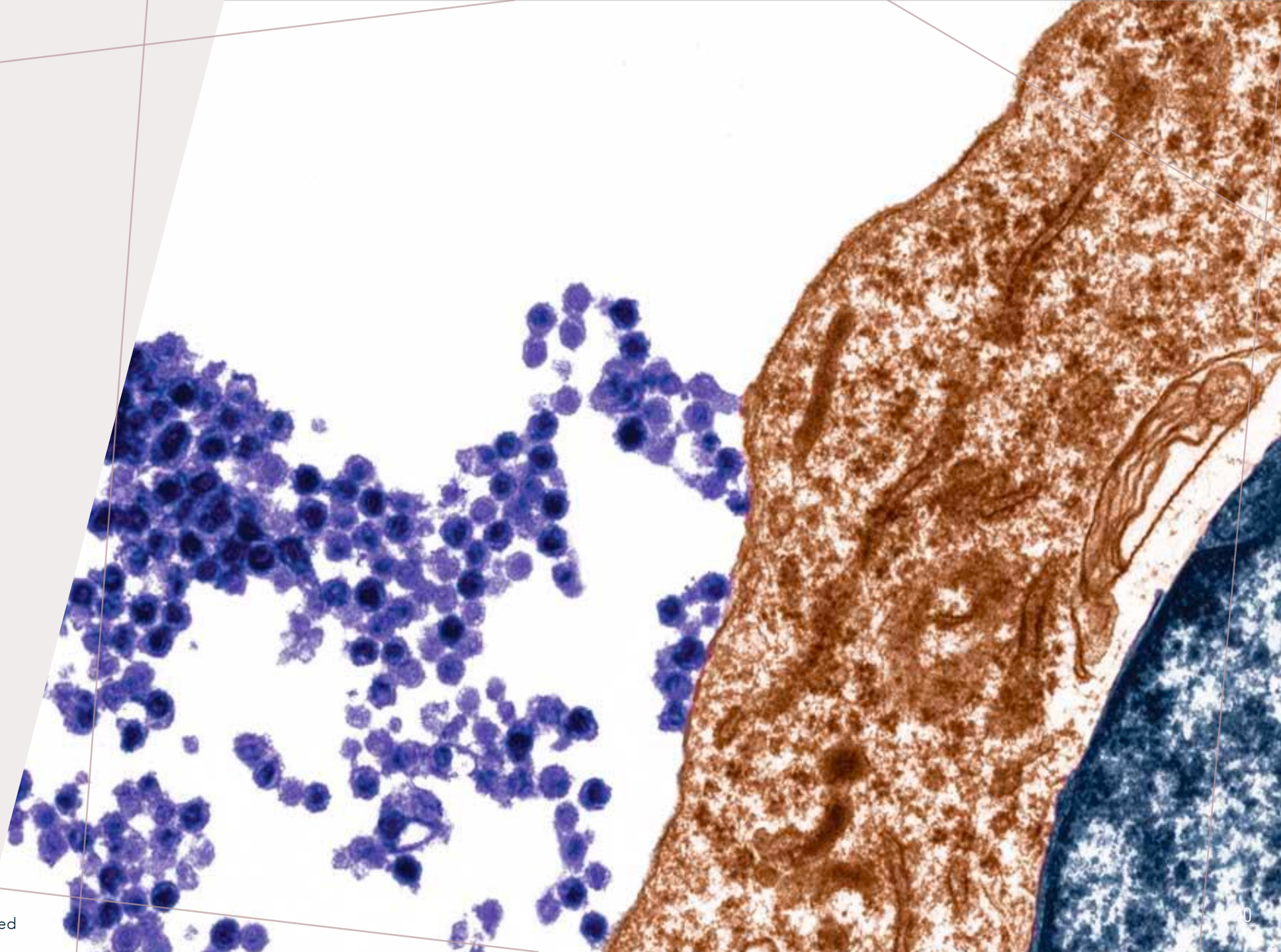
CLINICAL PEARL

GLUTATHIONE

The immune system requires "intermediate levels" of reduced glutathione to function properly. For patients with a glutathione insufficiency, use of a liposomal glutathione is a temporary interventional option but not per se best practice in the opinion of this provider. Use of liposomal glutathione replacement, regardless of whether a person carries a GCLC and/or NFE2L2 SNP and/or others, disrupts downstream activity of the Antioxidant Response Element (ARE) while creating a larger challenge in the ROS/oxidative stress domain. A presentation scheduled for February 2024 on updating the paradigm for managing oxidative stress will offer more detail on this point plus relevant workarounds. In the interim, consider IF2000™ as a substitute product to promotes glutathione production by using the **precursor method** without imposing the negative feedback on the broader system.

Dröge, W., & Breitkreutz, R. (2000). Glutathione and immune function. The Proceedings of the Nutrition Society, 59(4), 595–600. https://doi.org/10.1017/s0029665100000847

CLINICAL CASES



28 Y/O FEMALE, PROFOUND CHRONIC FATIGUE

The biggest thing is that I want to have more capacity athletic wise. Before the whole EBV stuff, I was doing something physical every day. It helps my mental health so much. (4.26.2022)

Engagement Benchmarks:

- Exercise tolerance : None
- Employment: Self employed, not applicable
- Children: Deferred pregnancy given EBV burden

PROOF OF CONCEPT: DRIVING LYTIC EBV INFECTION TO LATENT

28 Y/O FEMALE, PROFOUND CHRONIC FATIGUE

QUEST DIAGNOSTICS DOS: 1.28.2022
STARTED CARE: 3.1.2022

BIOMARKER	RESULT (U/mL)	INTERPRETATION
CMV Ab (IGG/IGM)	< 0.60	Negative
CMV Ab (IGM)	< 30	Negative
EBV Early Antigen D Ab (IGG)	> 150	Positive Negative: < 9
EBV Viral Capsid Ag Ab (IGM)	< 36	Negative
EBV Nuclear Ag Ab (IGG)	< 18	Negative

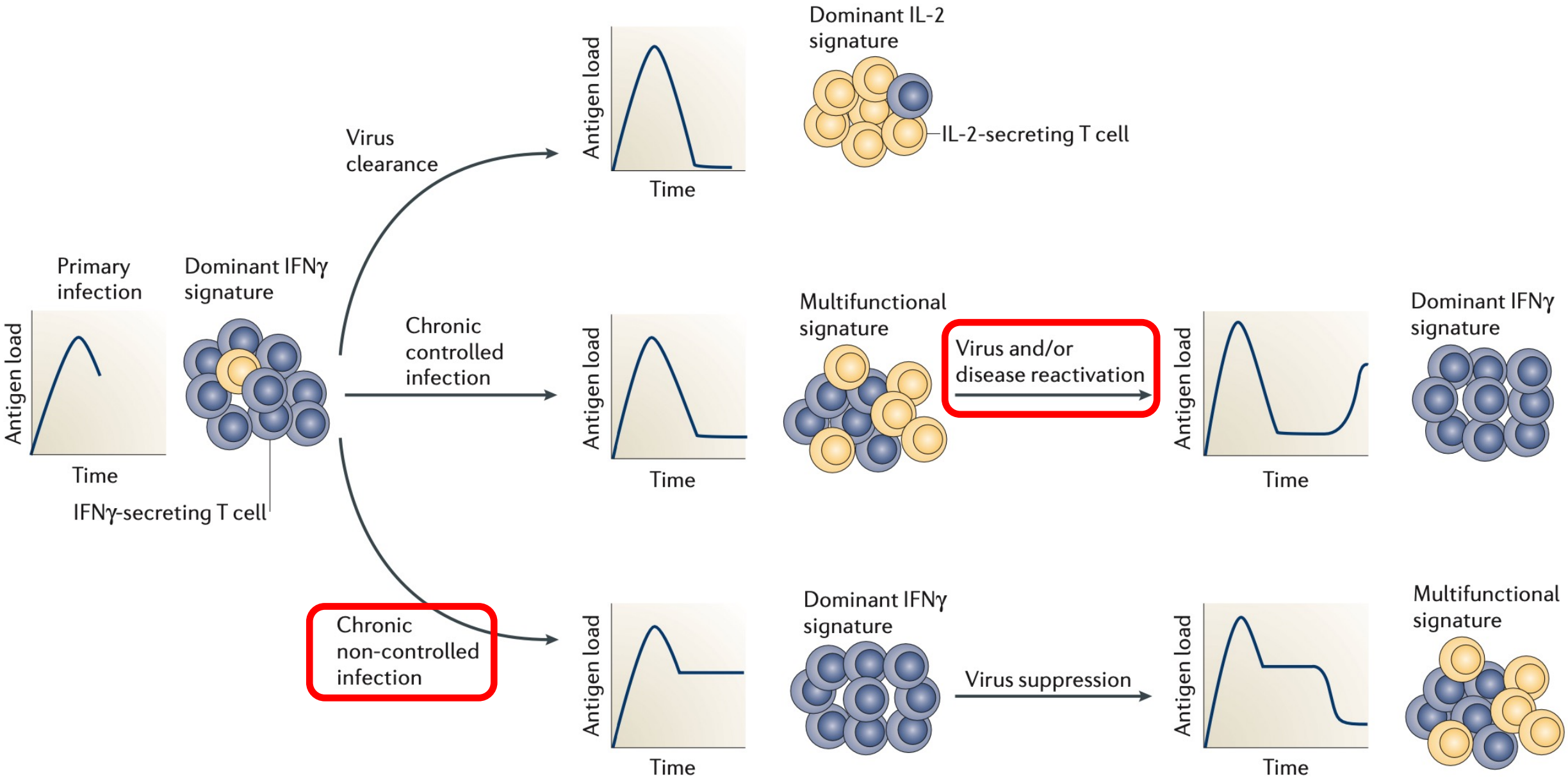
LYTIC TO "LATENT" EBV INFECTION

28 Y/O FEMALE, CHRONIC FATIGUE. INFECTOLAB DOS: 6.7.2022

EBV EARLY ANTIGEN D AB (IGG) — **129.00 H** U/mL

U/mL	Interpretation
<9.00	Negative
9.00-10.99	Equivocal
>10.99	Positive

Epstein Barr Virus Latent Interferon gamma (IFN-γ)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00
Epstein Barr Virus Latent Interleukin-2 (IL-2)*	0.67	SI	**≤2.00**	2.01-3.99	≥4.00
Epstein Barr Virus Lytic Interferon gamma (IFN-γ)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00
Epstein Barr Virus Lytic Interleukin-2 (IL-2)*	0.33	SI	**≤2.00**	2.01-3.99	≥4.00



LYTIC TO "LATENT" EBV INFECTION

28 Y/O FEMALE, CHRONIC FATIGUE. INFECTOLAB DOS: 7.12.2023

Analysis	Result	Units	Reference Range		
			Negative	Borderline	Positive
3129 Long Haul Viral					
Cytomegalovirus IE1 Interferon gamma (IFN-γ)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00
Cytomegalovirus IE1 Interleukin-2 (IL-2)*	1.00	SI	**≤2.00**	2.01-3.99	≥4.00
Cytomegalovirus pp65 Interferon gamma (IFN-γ)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00
Cytomegalovirus pp65 Interleukin-2 (IL-2)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00
Epstein Barr Virus Latent Interferon gamma (IFN-γ)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00
Epstein Barr Virus Latent Interleukin-2 (IL-2)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00
Epstein Barr Virus Lytic Interferon gamma (IFN-γ)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00
Epstein Barr Virus Lytic Interleukin-2 (IL-2)*	0.50	SI	**≤2.00**	2.01-3.99	≥4.00

EASY (PROOF OF CONCEPT)

CLINICAL PROCESS

QUESTIONNAIRE & LABORATORY

- Quest Diagnostics and InfectoLab

SELECT INTERVENTIONS

- Antiviral: Humavir 1 bid
- Immune Support: TF PlasMyc, 1 qd x 1week and then 2 qD
- Mitochondrial Support: Omega-3, Primal Multi (Baseline)

REPEAT QUESTIONNAIRE & LABORATORY

- MFIS – q2 months
- InfectoLab – 4 month window

CLINICAL PEARL

THERAPEUTIC ENDPOINT

After cessation of Transfer Factor and Humavir, patient symptoms rebounded. She was restarted on her regimen and symptoms resolved. No laboratory testing was performed to assess the patient's status objectively.

As of October 2023, patient is using Humavir 1x qD and no TF PlasMyc with success.

TIMELINE

1/23/2022
Quest Diagnostics

3/1/2022
Rx Humavir & TF PlasMyc

6/7/2022
Quest & InfectoLab

3/17/2023
Symptomatic Improvement first noted

7/12/2023
InfectoLab

3.17.23,, no edits

My depression is very much a symptom of my EBV. All I can feel is a general fatigue. The fact that I can see that through the winter, that I was no longer feeling good, it didn't dawn on my that it was my depression or the EBV.

The level of clarity this past week while being back on the PlasMyc with the Humavir is remarkable. It's like my brain can think clearly. And I can see that my depression is intrinsically linked to this.

4/26.23, no edits

Since starting the Humavir & PlasMyc my energy has been far greater than it was the 6 months prior, such that I think that when I got my first flu shot, every time that I thought my energy was back, it dissipated. I now realize, oh wow, I really was fatigued for the past 2 years.

COMMUNITY PROVIDER

UNSOLICITED TESTIMONIAL

I started going through premature ovarian failure at 35 and went through menopausal symptoms by 38. I was trying to not start hormone replacement therapy and find root cause. My sixth physician I found, an Internal Medicine/Integrative Medicine MD, had me tested for EBV and after testing positive, start your supplements. I have ovulated every month since. I have had three ovulation cycles in a row, and this has not happened for me for almost four years. Your supplement has completely changed my life.

CHRONIC EBV INFECTION

38 Y/O MALE, CHRONIC FATIGUE

- Active-duty Special Operator
- Traumatic Brain Injury (blast & blunt trauma)
- Partial Androgen Deficiency
- "Weak immune system"
- Chronic musculoskeletal pain
- Chronic fatigue
- Relapsing RUQ pain

- Highly organized
- Trains in austere environments routinely
- No respite
- Subject to deployment

CLINICAL PEARL

CONFOUNDERS?

TESTOSTERONE & NALTREXONE THERAPY

This patient was started on TESTOSTERONE therapy in April 2022, and then on NALTREXONE 4.5mg daily to assist in management of TBI, chronic fatigue and chronic pain. Testosterone positively influences immune function.

For details on the potential value of naltrexone in the context of "chronic fatigue and myalgic encephalomyelitis" caused by EBV infection, see the reference below.

https://casereports.bmj.com/content/13/1/e232502

CLINICAL PROCESS

QUESTIONNAIRE

- Baseline MFIS**:** **72/84**, 10.6.2022

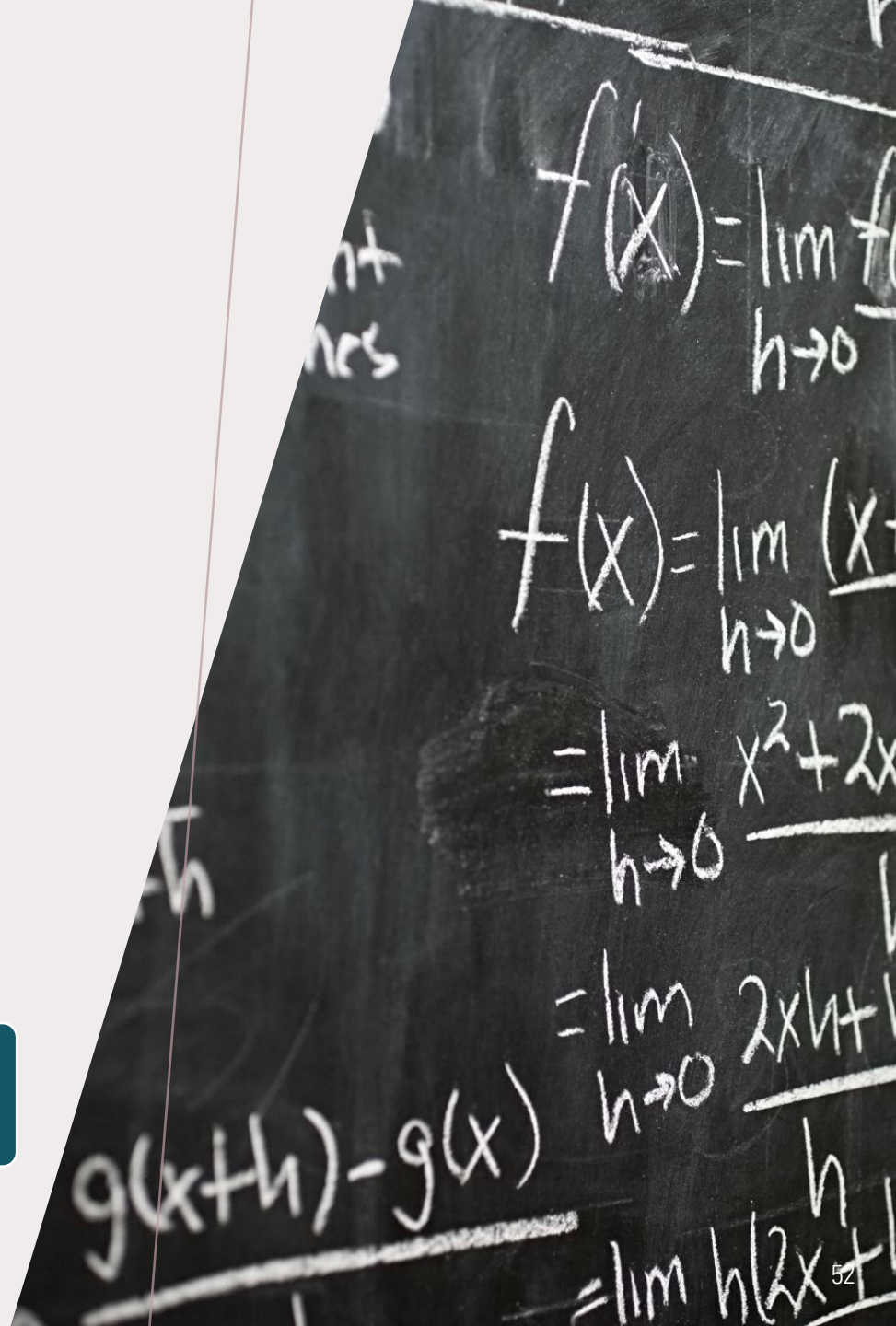
LABORATORY TESTING

- CBC 6.16.2022; InfectoLab, 9.13.2022

SELECT INTERVENTIONS

REPEAT QUESTIONNAIRE & LABORATORY

ADJUST



"LYTIC" EBV INFECTION

38 Y/O MALE

CBC DOS: 6.16.2022; INFECTOLAB DOS: ***9.13.2022***

WHITE BLOOD CELL	3.1 L	3.9 - 11.4	K/ul	

Analysis	Result	Units	Reference Range		
3081 Chronic Fatigue Lytic & Latent			**Negative**	**Borderline**	**Positive**
Cytomegalovirus IE1 Interferon gamma (IFN-γ)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00
Cytomegalovirus IE1 Interleukin-2 (IL-2)*	0.76	SI	**≤2.00**	2.01-3.99	≥4.00
Cytomegalovirus pp65 Interferon gamma (IFN-γ)*	0.33	SI	**≤2.00**	2.01-3.99	≥4.00
Cytomegalovirus pp65 Interleukin-2 (IL-2)*	0.62	SI	**≤2.00**	2.01-3.99	≥4.00
Epstein Barr Virus Latent Interferon gamma (IFN-γ)*	14.33	SI	≤2.00	2.01-3.99	**≥4.00**
Test indicates positive Interferon gamma (IFN-γ) active T-Lymphocytes.					
Epstein Barr Virus Latent Interleukin-2 (IL-2)*	0.66	SI	**≤2.00**	2.01-3.99	≥4.00
Epstein Barr Virus Lytic Interferon gamma (IFN-γ)*	34.67	SI	≤2.00	2.01-3.99	**≥4.00**
Test indicates positive Interferon gamma (IFN-γ) active T-Lymphocytes.					
Epstein Barr Virus Lytic Interleukin-2 (IL-2)*	0.66	SI	**≤2.00**	2.01-3.99	≥4.00

MOST CHALLENGING CASE

CLINICAL PROCESS

QUESTIONNAIRE & LABORATORY

- Quest Diagnostics and InfectoLab

SELECT INTERVENTIONS (9.30.2022)

- Antiviral: Humavir 1 bid
- Immune Support: TF PlasMyc, 1 qd x 1week and then 2 qD
- Mitochondrial Support: Custom Formula

REPEAT QUESTIONNAIRE & LABORATORY

- MFIS – 8 weeks
- InfectoLab – 16 weeks

HARD, STEP #1

CLINICAL PROCESS

QUESTIONNAIRE & LABORATORY

- Quest Diagnostics and InfectoLab

INTERVENTIONS (9.30.2022)

- Antiviral: Humavir 1 bid
- Immune Support: TF PlasMyc, 1 qd x 1week and then 2 qD
- Mitochondrial Support: Custom Formula

REPEAT QUESTIONNAIRE (11.29.2022)

- MFIS – **51/84** (Baseline: 72)

REACTIVATED "LYTIC" EBV INFECTION

38 Y/O MALE; COVID 19: 6.23.2022; DOS: 2.9.2023

WHITE BLOOD CELL	3.3 L	3.9 - 11.4	K/ul

FINAL REPORT

Analysis	Result	Units	Reference Range		
			Negative	Borderline	Positive
3116 EBV Lytic and Chronic Spot					
Epstein Barr Virus Latent Interferon gamma (IFN-γ)*	4.50	SI	≤2.00	2.01-3.99	**≥4.00**
Test indicates positive Interferon gamma (IFN-γ) active T-Lymphocytes.					
Epstein Barr Virus Latent Interleukin-2 (IL-2)*	1.00	SI	**≤2.00**	2.01-3.99	≥4.00
Epstein Barr Virus Lytic Interferon gamma (IFN-γ)*	46.00	SI	≤2.00	2.01-3.99	**≥4.00**
Test indicates positive Interferon gamma (IFN-γ) active T-Lymphocytes.					
Epstein Barr Virus Lytic Interleukin-2 (IL-2)*	1.00	SI	**≤2.00**	2.01-3.99	≥4.00

HARD, STEP #2

CLINICAL PROCESS

LABORATORY

- Last InfectoLab: 9.13.2022, 2.9.2023
- Last CBC: 6.16.2022, 2.9.2023

UPDATE INTERVENTIONS (2.14.23)

- Antiviral: Humavir 2 BID
- Immune Support: TF PlasMyc 2 BID to 3 BID
- Mitochondrial Support: Custom Formula

QUESTIONNAIRE (3.23.23)

- MFIS: 56/84 (Prior: 51/84, 11.29.2022)

HARD, STEP #3

CLINICAL PROCESS

Pressure of forthcoming deployment and schedule for laboratory redraws prompted reconsideration of approach. Started ANDROGRAPHIS EP80.

UPDATE INTERVENTIONS (3.15.2023 & 4.27.2023)

- Antiviral: Humavir 2 BID & **ANDROGRAPHIS EP80 1 BID x2 weeks and then increased to 2 BID**
- Immune Support: TF PlasMyc 3 BID & Fludrocortisone 0.1mg BIW
- Mitochondrial Support: Custom Formula

REACTIVATED “LYTIC” EBV INFECTION

38 Y/O MALE

POST-COVID; DOS: CBC 3.29.23, INFECTOLAB DOS: ***PENDING***

WHITE BLOOD CELL	4.0	3.9 - 11.4	K/ul

5.8.2023

Interval laboratory, VA WBC = 5.0.

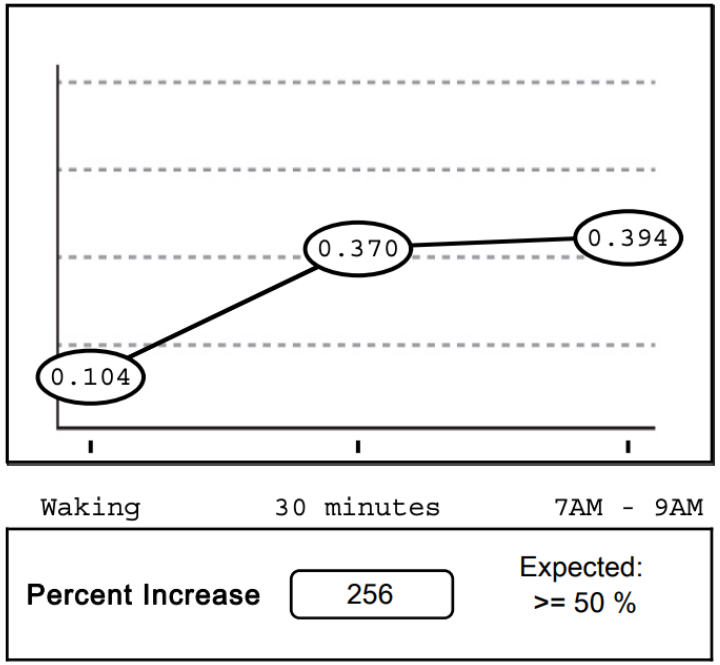
WBC is trending appropriately, which is atypical per the patient, but symptoms by MFIS are stagnant.

REACTIVATED "LYTIC" EBV INFECTION

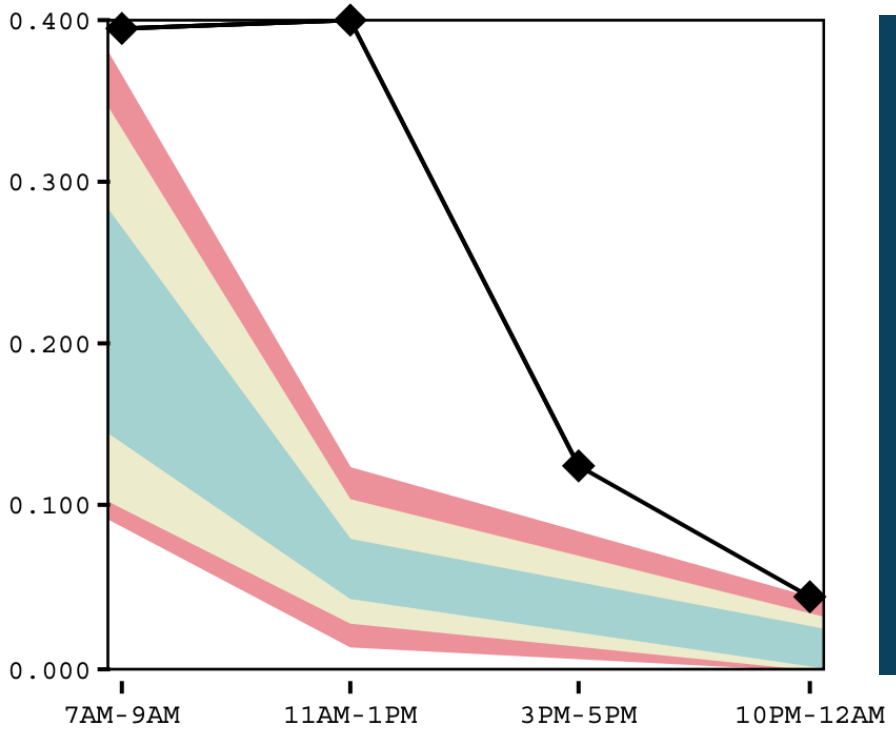
38 Y/O MALE

ADRENOCORTEX PROFILE W/ CORTISOL AWAKENING RESPONSE, DOS: ***5.30.2023***

Cortisol Awakening Response

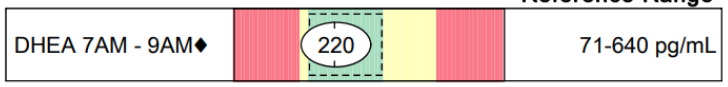


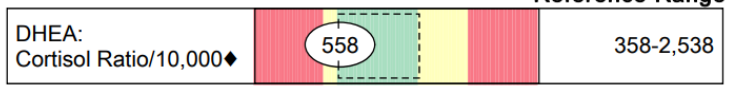
Salivary Cortisol



This is reflective of how I feel. And I have lived this way for years. Everything that I have been through professionally, and then add in family, kids, and life - this makes sense. I'm always training and always stressed

DHEA





HARD #4

CLINICAL PROCESS

LABORATORY

- Last InfectoLab: 9.13.2022, 2.9.2023
- Last CBC: 6.16.2022, 2.9.2023
- Adrenocortex Profile: 5.30.2023

UPDATE INTERVENTIONS (5.30.23)

- Unchanged: Humavir 2 BID + Andrographis EP80 2 BID + TF PlasMyc 3 BID + Mitochondrial Support
- Immune Support: **FLUDROCORTISONE 0.1mg BIW**

LABORATORY

- InfectoLab, scheduled in 2 months to validate approach; early relative to T-cell compartment turnover timeline

REACTIVATED "LYTIC" EBV INFECTION

38 Y/O MALE

POST-COVID; INFECTOLAB DOS: ***7.10.2023***

Analysis	Result	Units	Reference Range		
3116 EBV Lytic and Chronic Spot			**Negative**	**Borderline**	**Positive**
Epstein Barr Virus Latent Interferon gamma (IFN-γ)*	3.00	SI	≤2.00	**2.01-3.99**	≥4.00
Test indicates borderline Interferon gamma (IFN-γ) active T-Lymphocytes.					
Epstein Barr Virus Latent Interleukin-2 (IL-2)*	0.50	SI	**≤2.00**	2.01-3.99	≥4.00
Epstein Barr Virus Lytic Interferon gamma (IFN-γ)*	17.00	SI	≤2.00	2.01-3.99	**≥4.00**
Test indicates positive Interferon gamma (IFN-γ) active T-Lymphocytes.					
Epstein Barr Virus Lytic Interleukin-2 (IL-2)*	0.50	SI	**≤2.00**	2.01-3.99	≥4.00

EBV Lytic IFN-gamma:
% change from peak: -63%
% change from prior: -56.4%

HARD, STEP #5

CLINICAL PROCESS

LABORATORY

- Last InfectoLab: 9.13.2022, 2.9.2023, 7.10.2023
- Last CBC: 6.16.2022, 2.9.2023
- Adrenocortex Profile: 5.30.2023

UPDATE INTERVENTIONS (7.13.23)

- Antiviral: Humavir 2 BID & Andrographis EP80 1 BID
- Immune Support: TF PlasMyc 3 BID & Fludrocortisone 0.1mg BIW
- Mitochondrial Support: Custom Formula

QUESTIONNAIRE

- MFIS Pending. On base. States, "I'm feeling better (more energy), but not 100%.

LABORATORY

- InfectoLab scheduled 9.15.2023

PRE-DEPLOYMENT PREP

Prior to deployment, patient's spouse had been encouraging the use of Thymosin Alpha-1. Given demands of pre-deployment preparations, client started oral "Thymogen." Clinical value is not known. No other changes were made to patient regimen.

Lapse in MFIS Questionnaire completion was due to pre-deployment commitments.

REACTIVATED "LYTIC" EBV INFECTION

38 Y/O MALE

POST-COVID; INFECTOLAB DOS: ***9.5.2023, PRE-DEPLOYMENT***

FINAL REPORT

Analysis	Result	Units	Reference Range		
3116 EBV Lytic and Chronic Spot			**Negative**	**Borderline**	**Positive**
Epstein Barr Virus Latent Interferon gamma (IFN-γ)*	1.50	SI	**≤2.00**	2.01-3.99	≥4.00
Epstein Barr Virus Latent Interleukin-2 (IL-2)*	0.50	SI	**≤2.00**	2.01-3.99	≥4.00
Epstein Barr Virus Lytic Interferon gamma (IFN-γ)*	5.00	SI	≤2.00	2.01-3.99	**≥4.00**
Test indicates positive Interferon gamma (IFN-γ) active T-Lymphocytes.					
Epstein Barr Virus Lytic Interleukin-2 (IL-2)*	1.00	SI	**≤2.00**	2.01-3.99	≥4.00

EBV Lytic IFN-gamma

% change from peak: -89%

% change from prior: -70.6%

Treatment interrupted by deployment

PRE-DEPLOYMENT MODIFICATIONS

Decrease:	Humavir 1 BID
Continue	Fludrocortisone 0.1mg BIW TF PlasMyc 1 BID
Suspended	Thymogen (questionable value)
Reserve	Andrographis EP80

SUMMARY

TIMELINE, DOSE MAXIMUMS & LINKS

INITIAL TREATMENT TIMELINE

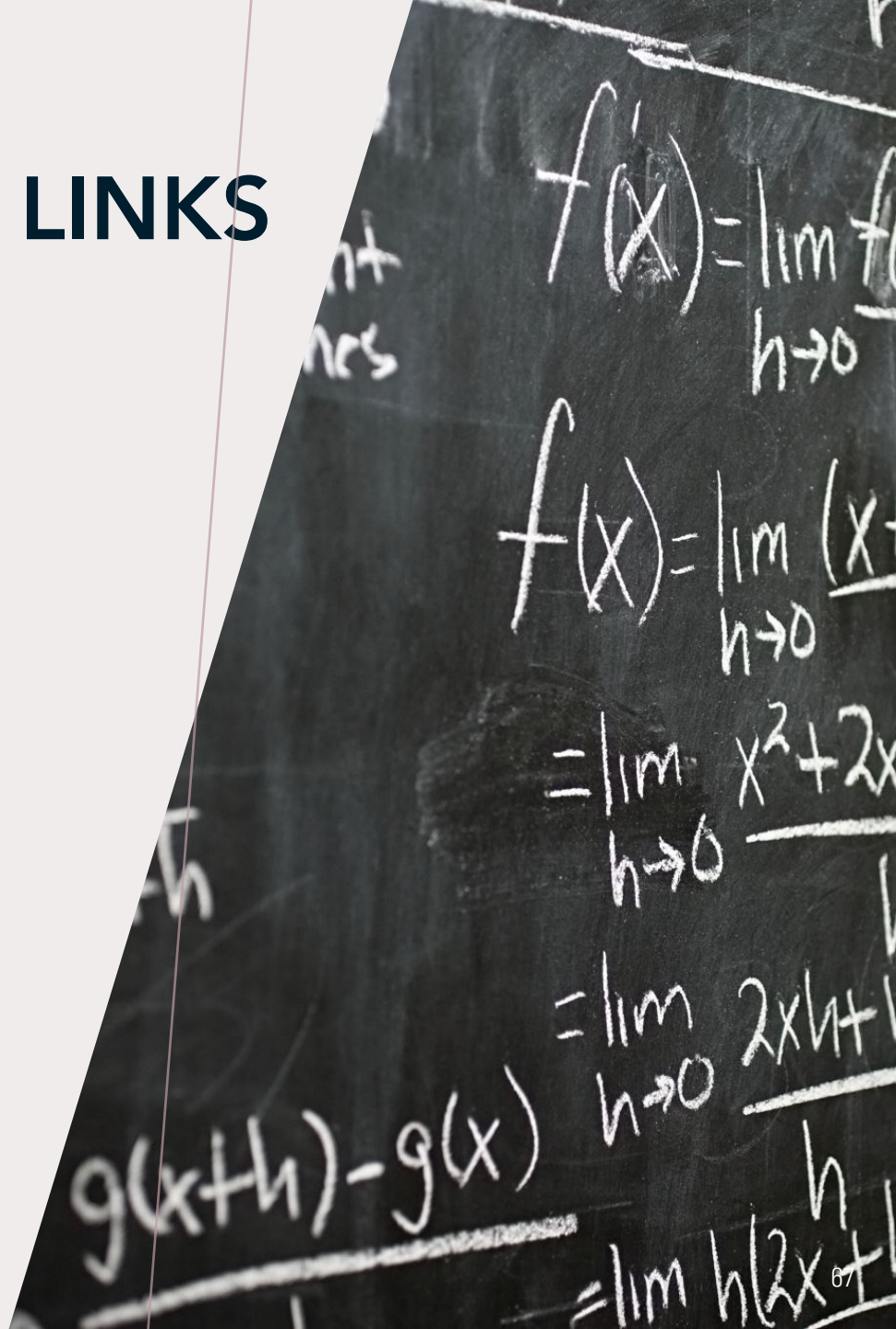
- Up to 12 months based on patient adherence and case complexity

DOSE MAXIMUMS

- Humavir: Up to 2 capsules 3x daily
- Andrographis EP80: Up to 2 capsules 2x daily
- TF PlasMyc: Up to 3 capsules 3x daily
- Fludrocortisone: Lowest effective dose
- Thymosin Alpha-1: Based on compounding source
- Th1 vs. Th2 Support: Per product

QUESTIONNAIRE

- Modified Fatigue Impact Scale, 21 questions
- Fatigue Impact Scale, 40 questions



ACKNOWLEDGEMENT
ENTEROIMMUNOLOGICAL INFLUENCES

Gut dysfunction imposes significant stress on the immune system. Though this presentation does not provide guidance on this thread, the diversity of the microbiome and status of the commensal microbiome is relevant to supporting immune function. Interventions include prebiotics, probiotics, and polyphenols, to name but a few.

CLINICAL PEARL

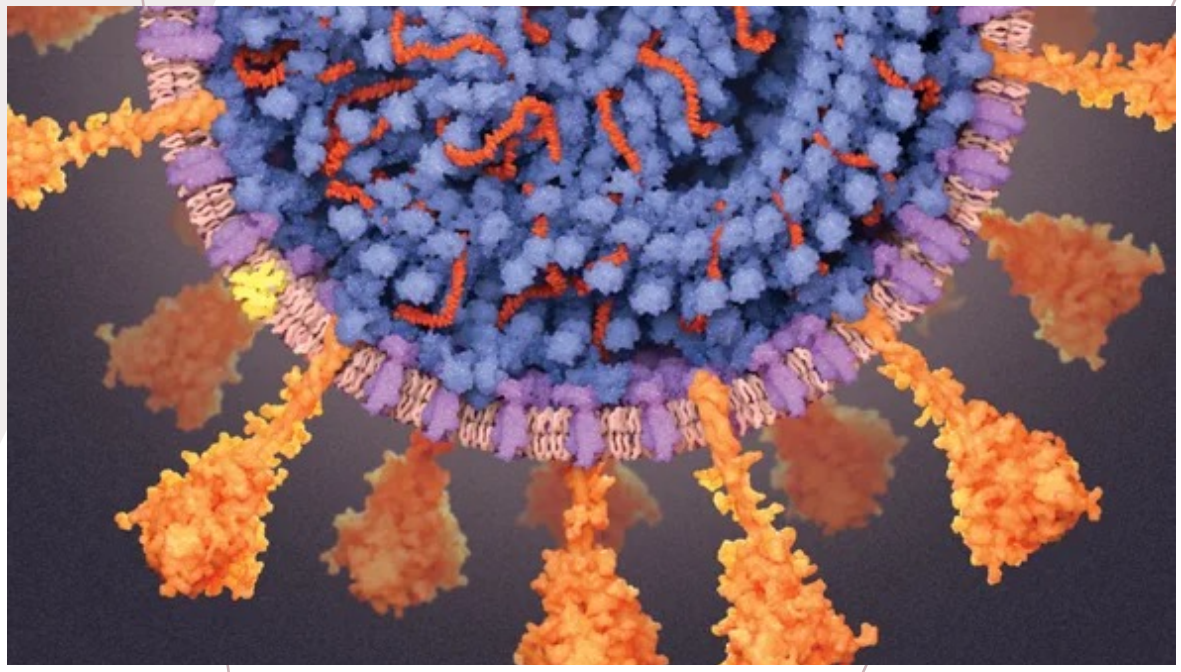
CONCLUDING REMARK

We found that the greatest limitation to successful management of Chronic EBV and related symptoms is clinical flexibility. Positive outcomes are dependent on an iterative approach to care.

Unfortunately, it also seems that to maintain resolution of the chronic EBV infection, some level of ongoing monitoring and therapy is required in many cases. We rely on the MFIS questionnaire to provide early subjective insight and release the questionnaire q30 days for at least 6 months.

SUPPLEMENTAL
SARS-COV-2

MITOCHONDRIAL DYSFUNCTION
EBV REACTIVATION



Veronica Falconieri Hays; Source: Lorenzo Casalino, Zied Gaieb and Rommie Amaro, U.C. San Diego (spike model with glycosylations)

DEFINITION

LONG COVID

- Persistent fatigue, brain fog, sleep difficulties, arthralgia, pharyngitis, myalgia, headaches, fever, gastrointestinal upset, and skin rashes with a variety of presentations three or more months after primary COVID infection that did not exist prior to an individual's COVID-19 infection (CDC)

SCAFFOLDING – COVID & EBV

- Dysfunctional mitochondria can be a source of oxidative stress, which in turn, further compromises mitochondrial recovery due to feedback and their role in inflammatory signaling….

  … Even if corona virus is cleared, mitochondrial imbalance can continue for some time; the second EBV whammy, whether coincident, or caused by reactivation due to reduced immune function, only makes this worse.

SARS-COV-2 CAUSES MITOCHONDRIAL DYSFUNCTION AND MITOPHAGY IMPAIRMENT

SHANG, C., LIU, Z., ZHU, Y., LU, J., GE, C., ZHANG, C., ... & LI, X. (2022). FRONTIERS IN MICROBIOLOGY, 12, 4159.

SARS-CoV-2 INFECTION

Membrane depolarization

Increased membrane permeability (TOM20)

Increased ROS production

DYSFUNCTION

↑ Pink1 and Parkin Protein

Pink1 mediated recruitment of p62

Mitophagy clock

MITOCHONDRIAL PRESERVATION

Viral mediated inhibition of p62 and LC3 binding

Inhibits emergence of autophagosomes

Zombification

"METABOLIC-OXIDATIVE STRESS INFLAMMATORY SPIRAL"

Thompson, E. A., et al. (2021). Metabolic programs define dysfunctional immune responses in severe COVID-19 patients. Cell reports, 34(11), 108863.

EBV

- Induces ACE2 expression & entry of SARS-CoV-2 binding to epithelial cells
- Drives mitochondrial dysfunction
- Infects T & B cells

SARS-CoV-2

- (+) Subset of T cells with an altered metabolic profile are linked to disease severity and upregulation of voltage dependent anion channel 1 (VDAC1) expression.
- VDAC1: Part of a complex that regulates the exchange of ATP and ADP between the mitochondria and cytosol, associated with mitochondrial cell death signaling and autoimmunity
- T cell changes → prone to mitochondrial apoptosis → secondary lymphopenia

- Mitochondiral homeostatis
  - Essential for sustained killing by cytotoxic T cells

PREVALENCE OF LONG HAUL COVID

- Household Pulse Survey
  - Phase 1: 19%
    - Phase 3.5, June 1, 2022
  - Phase 2: 7.5%
    - Phase 3.6, September 14, 2022
    - Symptomatic > 90 days
  - Cooperative effort: CDC, Census Bureau & National Center for Health Statistics

INVESTIGATION OF LONG COVID PREVALENCE AND ITS RELATIONSHIP TO EPSTEIN-BARR VIRUS REACTIVATION

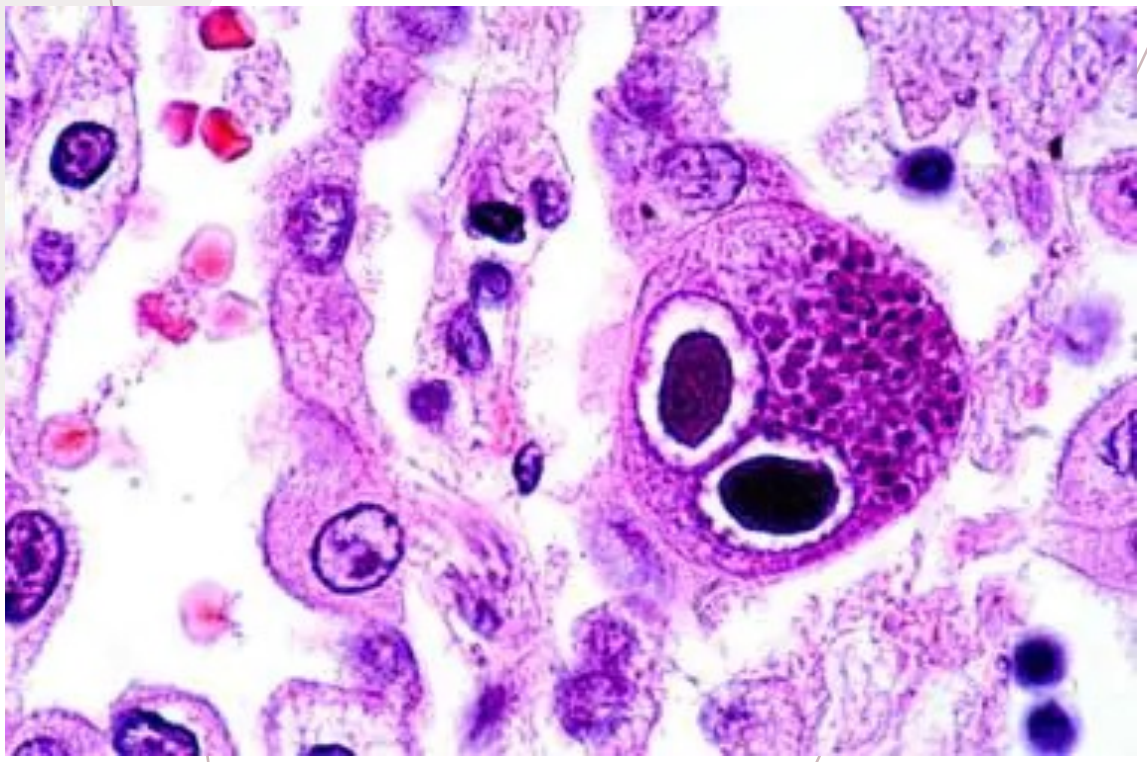
Gold, J. E., Okyay, R. A., Licht, W. E., & Hurley, D. J. (2021), Pathogens, 10(6), 763

- Prevalence, Long COVID
  - 185 candidates applied for study
  - (+) Historical COVID-19 infection verified
  - (+) None aware of intent to study long COVID
  - (+) Symptomatic at least **30 days** after initial infection
  - **30.3%** (56/185)

- Prevalence, Long-Term COVID
  - (+) Symptomatic at least **90 days** after diagnosis
  - Laboratory: EBV early antigen or EBV viral capsid antigen, $p < 0.001$, Fisher's exact test
  - **66.7%** (20/30) showed evidence of EBV reactivation

- Prevalence, Short-Term COVID
  - (+) Symptomatic between **21-90 days** after diagnosis
  - **66.7%** (6/9) showed evidence of EBV reactivation

SUPPLEMENTAL
CMV



CMV INFECTION - BASELINE

36 Y/O FEMALE, CHRONIC FATIGUE. INFECTOLAB DOS: 6.1.2022

Analysis	Result	Units	Reference Range		
			Negative	Borderline	Positive
3065 Chronic Viral Lytic and Latent					
Cytomegalovirus IE1 Interferon gamma (IFN-γ)*	9.00	SI	≤2.00	2.01-3.99	**≥4.00**
Test indicates positive Interferon gamma (IFN-γ) active T-Lymphocytes.					
Cytomegalovirus IE1 Interleukin-2 (IL-2)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00
Cytomegalovirus pp65 Interferon gamma (IFN-γ)*	28.50	SI	≤2.00	2.01-3.99	**≥4.00**
Test indicates positive Interferon gamma (IFN-γ) active T-Lymphocytes.					
Cytomegalovirus pp65 Interleukin-2 (IL-2)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00

EASY (PROOF OF CONCEPT)

CLINICAL PROCESS

QUESTIONNAIRE & LABORATORY

- Quest Diagnostics and InfectoLab

SELECT INTERVENTIONS

- Antiviral: Humavir 1 bid
- Immune Support: TF PlasMyc, 1 qd x 1week and then 2 qD
- Mitochondrial Support: Omega-3

REPEAT QUESTIONNAIRE

- MFIS – q2 months

CMV INFECTION

36 Y/O FEMALE, CHRONIC FATIGUE NEARLY RESOLVED. INFECTOLAB DOS: 10.12.22

TX: HUMAVIR & TRANSFER FACTOR

Analysis	Result	Units	Reference Range		
3117 CMV IE-1 and pp65 Spot			**Negative**	**Borderline**	**Positive**
Cytomegalovirus IE1 Interferon gamma (IFN-γ)*	0.92	SI	**≤2.00**	2.01-3.99	≥4.00
Cytomegalovirus IE1 Interleukin-2 (IL-2)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00
Cytomegalovirus pp65 Interferon gamma (IFN-γ)*	2.85	SI	≤2.00	**2.01-3.99**	≥4.00
Test indicates borderline Interferon gamma (IFN-γ) active T-Lymphocytes.					
Cytomegalovirus pp65 Interleukin-2 (IL-2)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00

(+) COVID DX: 10.19.2022

Coincidental lab draw on same date as COVID diagnosis via PTP

CMV INFECTION

36 Y/O FEMALE, RESURGENT CHRONIC FATIGUE, POST COVID.
INFECTOLAB DOS: 2.9.23

CMV Reactivation

Analysis	Result	Units	Reference Range		
3065 Chronic Viral Lytic and Latent			**Negative**	**Borderline**	**Positive**
Cytomegalovirus IE1 Interferon gamma (IFN-γ)*	14.50	SI	≤2.00	2.01-3.99	**≥4.00**
Test indicates positive Interferon gamma (IFN-γ) active T-Lymphocytes.					
Cytomegalovirus IE1 Interleukin-2 (IL-2)*	1.50	SI	**≤2.00**	2.01-3.99	≥4.00
Cytomegalovirus pp65 Interferon gamma (IFN-γ)*	23.50	SI	≤2.00	2.01-3.99	**≥4.00**
Test indicates positive Interferon gamma (IFN-γ) active T-Lymphocytes.					
Cytomegalovirus pp65 Interleukin-2 (IL-2)*	0.50	SI	**≤2.00**	2.01-3.99	≥4.00

CMV INFECTION: 36 Y/O FEMALE, RESURGENT CHRONIC FATIGUE, POST COVID

INFECTOLAB DOS: 5.3.23; TX: HUMAVIR & TRANSFER FACTOR

Analysis	Result	Units	Reference Range		
			Negative	Borderline	Positive
3065 Chronic Viral Lytic and Latent					
Cytomegalovirus IE1 Interferon gamma (IFN-γ)*	5.00	SI	≤2.00	2.01-3.99	**≥4.00**
Test indicates positive Interferon gamma (IFN-γ) active T-Lymphocytes.					
Cytomegalovirus IE1 Interleukin-2 (IL-2)*	0.00	SI	**≤2.00**	2.01-3.99	≥4.00
Cytomegalovirus pp65 Interferon gamma (IFN-γ)*	6.00	SI	≤2.00	2.01-3.99	**≥4.00**
Test indicates positive Interferon gamma (IFN-γ) active T-Lymphocytes.					
Cytomegalovirus pp65 Interleukin-2 (IL-2)*	0.50	SI	**≤2.00**	2.01-3.99	≥4.00

IMPROVED CMV SIGNATURE – but still symptomatic

36 Y/O FEMALE, RESURGENT CHRONIC FATIGUE; DOS: 5.3.23

COVID LONG HAULER PANEL

LH CYTOKINE 14 PANEL

IL-2	10.0	HIGH	pg/mL	1.6 - 7.0
IL-4	124.9	HIGH	pg/mL	2.3 - 6.2
IL-6	872.5	HIGH	pg/mL	1.4 - 3.0
IL-8	126.2	HIGH	pg/mL	5.4 - 21.0
IL-10	6.4	HIGH	pg/mL	0.7 - 1.2
IL-13	32.1	HIGH	pg/mL	1.5 - 6.1
GM-CSF	33.7	NORMAL	pg/mL	5.8 - 77.0
SCD40L	11286	HIGH	pg/mL	35.0 - 9236.0
CCL3 (MIP-1 ALPHA)	52.3	HIGH	pg/mL	3.5 - 33.0
CCL4 (MIP-1 BETA)	26.7	NORMAL	pg/mL	1.5 - 93.0
CCL5 (RANTES)	9475.1	NORMAL	pg/mL	7.2 - 11800.0
TNF-ALPHA	102.0	HIGH	pg/mL	3.7 - 11.0
IFN-GAMMA	138.7	HIGH	pg/mL	1.8 - 3.5
VEGF	137.4	HIGH	pg/mL	2.0 - 12.3
LONG HAULER INDEX	5.56	HIGH	INDEX	< 0.70

CLINICAL PEARLS

CYTOMEGALOVIRUS

CMV will always yield an IL-2 finding on T-cell compartment testing, but magnitude of IL-2 signature should improve with interventions and time.

Recall: CMV is more responsive to unenhanced humic acid than EBV based on NIH testing (Slide 28). Enhancements also make it more effective.

UNCITED REFERENCES

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