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Ultra-low doses of microARNs restore a strong immune response in patients with Chronic Active Epstein-Barr Virus infection (CAEBV)

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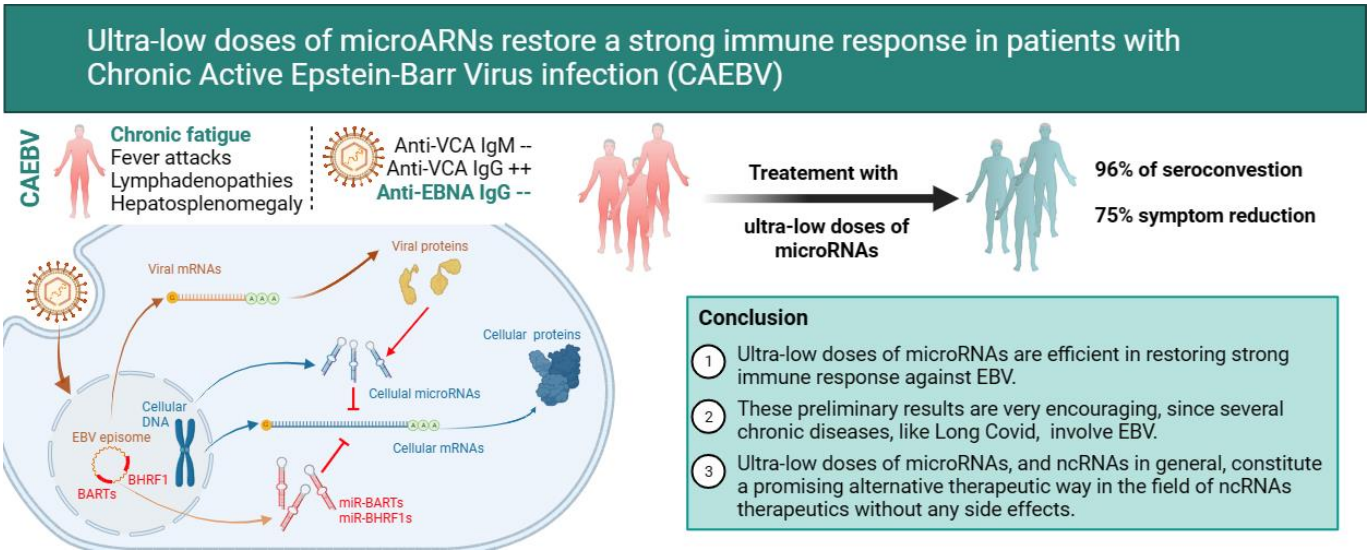
Abstract

Chronic active Epstein-Barr Virus infection (CAEBV) is an abnormal response of the immune system to EBV infection, characterized by the absence of anti-EBNA (EBV Nuclear Antigens) IgG and associated with chronic fatigue and several immune related diseases. We present here an innovative therapeutic approach of CAEBV based on the use of ultra-low doses of non-coding RNAs (ncRNAs) involved in the host-virus interaction. We performed a retrospective study on 26 patients treated with our remedy. All the patients expect one developed positive EBNA IgG within eighteen months of starting treatment. Among the 25 cases that seroconverted, 75% report a significant improvement of their symptoms, especial of chronic fatigue. This study gives the proof of the efficiency of ultra-low doses of ncRNAs in restoring a strong immune response against EBV. More generally, this therapeutic approach can potentially be used in almost all pathologies.



Keywords: Epstein-Barr Virus (EBV); Chronic fatigue; Non-coding RNA (ncRNA); MicroRNA (mRNA); Ultra-low doses; BioImmun(G)ene Medicine (BI(G)MED)

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1 Introduction

Everyone knows about the Epstein-BarrVirus ... or at least thinks they know about it, because this double-stranded DNA human virus belonging to the *Herpesviridae* family is present worldwide and about 95% of the adult population has antibodies against EBV (1). Its transmission through saliva makes it highly contagious. Due to its high prevalence, first exposition occurs in childhood where it is usually asymptomatic (2). When it occurs later in life, 30% to 50% cases manifest clinically as infectious mononucleosis (IM), characterized by fever, lymphadenopathy and pharyngitis (3) that spontaneously heals in few weeks. As many other herpes viruses, EBV is associated with several pathologies like autoimmune diseases among which SLE, multiple sclerosis, Sjögren's syndrome, and systemic sclerosis (4) and various malignancies such as nasopharyngeal carcinoma, gastric carcinoma and Burkitt's lymphoma (5). But many years of clinical practice have enabled us to identify many other symptoms and syndromes that can be linked to this virus.

EBV infection associated immune response results in the production of antibodies among which EBV-Capsid Antigen (VCA) IgG and IgM, and EBV Nuclear Antigens (EBNA) IgG are routinely detected in patient's sera. Classically, recent infection is characterized by positive VCA IgM and IgG, more or less positive EA-IgG and negative EBNA IgG, whereas the presence of VCA IgG and EBNA IgG without VCA IgM is typical of past infection (table 1).

However, some atypical serological patterns may occur with negative EBNA IgG despite positive VCA IgG and negative VCA IgM. Those cases are considered as a chronic active Epstein-Barr Virus (CAEBV) infection syndrome (6). This pathology, also known as chronic mononucleosis, is characterized by chronic fatigue, recurrent fever attacks, lymphadenopathies, and/or hepatosplenomegaly (7). Here again, our long experience as clinicians has enabled us to associate multiple clinical situations with this incomplete EBV immunization. And let's not forget that sometimes there can be no symptoms at all, just silence on the part of the organism... until the day when something changes and a pathology takes hold that can be difficult to control.

Table 1 Different serological profiles following EBV infection.

	VCA IgM	**VCA IgG**	**EBNA IgG**
Recent infection	+	+	-
Past infection	-	+	+
Chronic infection	-	+	-

"+" means positive serology test, "-" means negative serology test.

EBV primary infects B-cells in the oropharynx and starts the lytic phase of infection that involves virus replication (8). During this phase, EBV expresses more than 100 genes (9). After acute infection, EBV establishes lifelong latency in memory B-cells and escapes immune response by silencing almost all viral genes (10). Epigenetic factors play an important role in the interaction between EBV and the host immune system. In a recent review, Shareena and Kumar highlighted the role of both virus and host DNA methylation in the regulation of lytic and latent genes as well as in the activation of oncogenic host cell's phenotype (11). EBV is the first human virus that has been identified to produce microRNAs (miRNAs). EBV-encoded miRNAs interact with viral genes as well as with host genome. They enable infected cells to escape immune recognition by inhibiting the expression of viral antigens (12). EBV miRNAs also interfere with host cell's messenger RNA (mRNA) to modulate immune cell activation, and are also involved in cell proliferation and apoptosis (13).

Non coding RNAs (ncRNAs) constitute an innovative new therapeutic approach for almost all acute and chronic diseases (14–17). However, ncRNA therapeutics face several challenges like delivery method, nuclease-mediated degradation of nucleic acids and inoculation dose that are not fully elucidated (18). Ultra-low doses of ncRNAs constitute a promising alternative therapeutic way that has been already demonstrated in EBV associated pathologies (19,20) as well as other disorders like auto-immune diseases, allergies or viral infections (21–26).

In this original paper, we aim to show how ultra-low doses of non-coding ARNs can restore a strong immune response against EBV, by acting in the epigenetic level to support B-cells producing anti-EBNA antibodies. For this goal, we present the results of a retrospective study on 26 patients presenting CAEBV and treated with a cocktail of ncRNAs at ultra-low doses.

2 Material and methods

2.1 Identification of the ncRNAs implicated in the host-EBV interaction

Regulatory non coding RNAs are a class of functional RNA that are not translated into protein. They can be classified into microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs), small interfering RNAs (siRNAs), and long non-coding RNAs (lncRNAs) (see table 2).

All EBV ncRNAs described to date are expressed during latency III as well as in infected B-cells. But a majority of all these EBV ncRNAs are also expressed in the other latency stages, thus demonstrating the importance of their contribution to maintaining the virus' latent state. Sequencing studies have identified 40 mature micro-RNAs within these EBV ncRNAs, which target different EBV and cellular transcripts. They are encoded in two genomic regions: BART and BHRF1 (figure 1).

Table 2 Main non-coding RNAs with their known functions

ncRNAs	abbreviation	Length (nt)	Function
Long non coding RNAs	lncRNA	> 200	Heterogeneous class. Involved in epigenetic modification and post-transcriptional processing
Small interfering RNAs	siRNA	20-25	Form complexes with Argonaute proteins. Operate through RNA interference (RNAi) pathway. Promote mRNA degradation
Micro RNAs	miRNA	21-24	Post-transcriptional regulation of gene expression. RNA silencing. May have an extracellular location
PIWI-interacting RNAs	piRNA	36-31	Epigenetic and post-transcripitonal gene silencing of retrotransposons in germ line cells

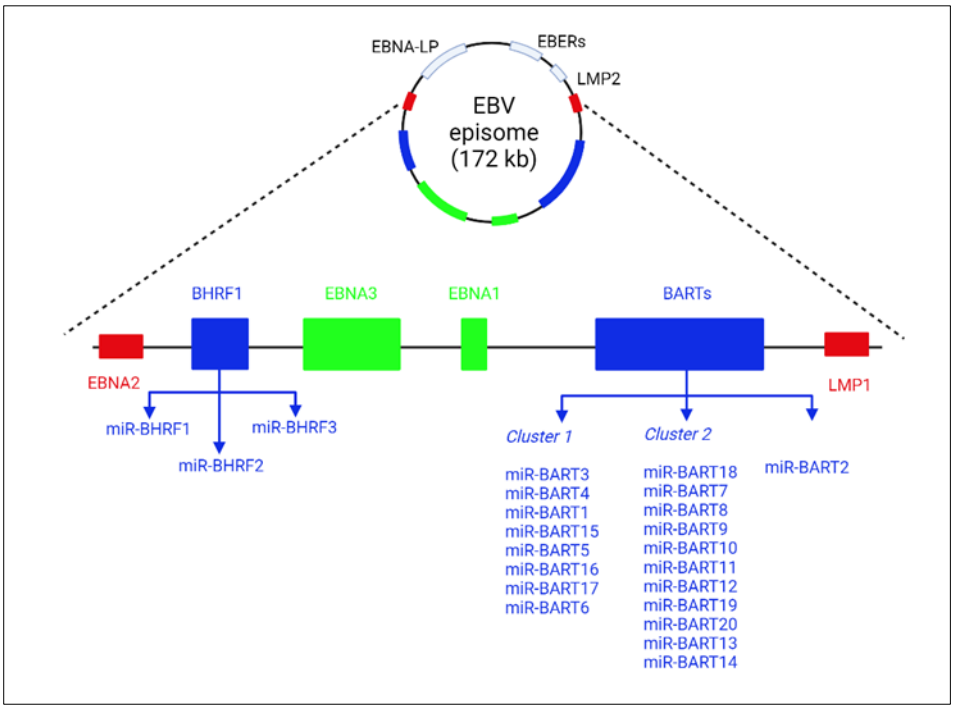

Figure 1 EBV episome and BamH1 restriction map containing the BHFR1 and BARTs loci coding for the micro RNAs expressed by EBV.

Interestingly, EBV miRNAs can be transferred by secreted exosomes from infected cells. Thus, these miRNAs are potential factors for genome regulation of both infected and uninfected cells.

Target identification studies have shown that multiple biological processes and cell signalling pathways can be regulated by viral miRNAs, most of which are also intrinsically regulated by cellular miRNAs (figure 2). Other functions include EBV-driven B-cell immortalization in vitro, and potentially, the development of cancer in vivo.

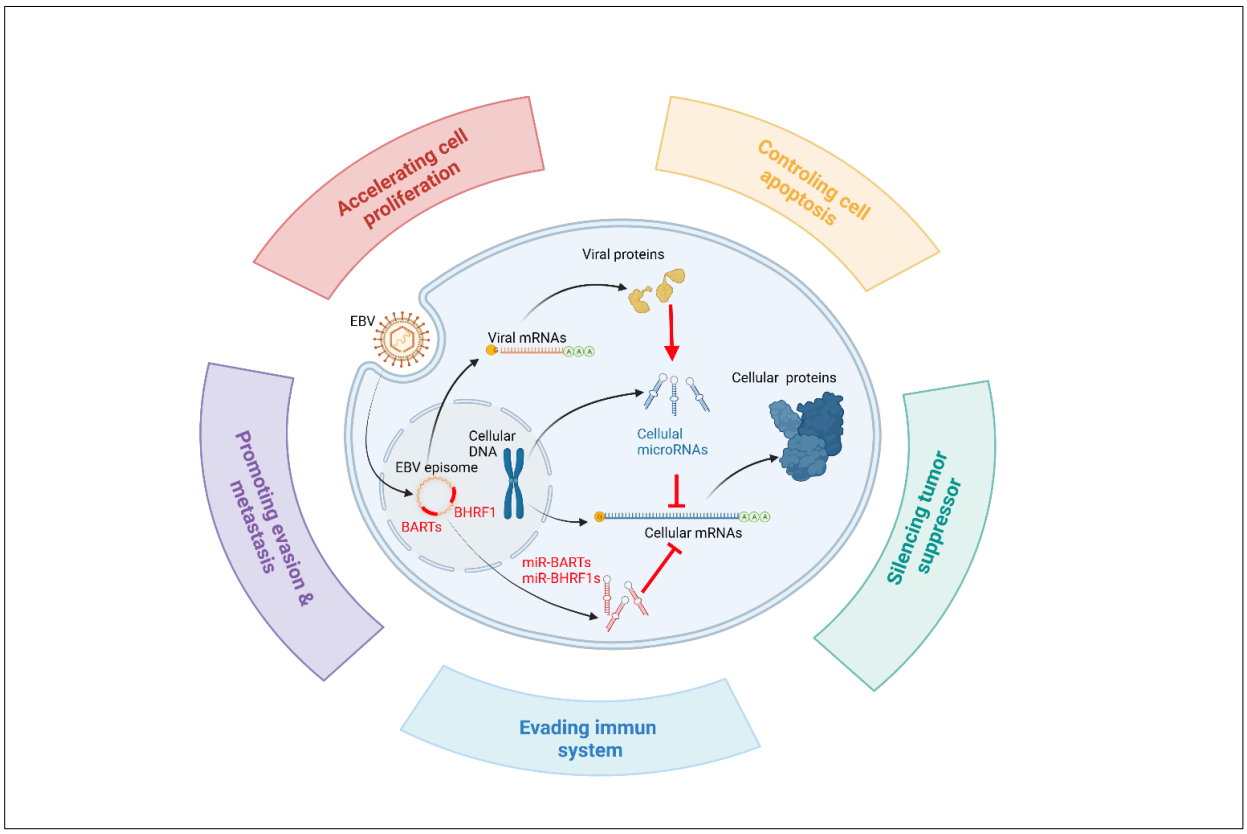

Figure 2 After EBV infection, viral genome enters cell nucleus and circularizes in the latent phase. Viral miR-BARTs and miR-BHRF1 interfere with cellular messenger RNAs. From another side, viral proteins modulate cellular micro RNAs production. Therefore, infected cells protein synthesis is affected by viral microRNAs, inducing several dysfunctions.

Another EBV strategy involves the activation of certain of its proteins to induce the expression of specific cellular microRNAs that may be players in the EBV life cycle.

2.2 Formulation of the remedy according to the Bio Immune(G)ene MEDicine (BI(G)MED) method

BI(G)MED formulations use molecular components in ultra-low doses to achieve a biomimetic action. Here, the dilutions used range from nanograms and picograms to femtograms and beyond. Indeed, it's now well known that many cellular receptor and signalling systems can be engaged by very low doses of biochemical and biological component at concentration ranging from 10^{-18} to 10^{-24} M (27).

These molecules, prepared at such high dilutions, then undergo a process of shaking to activate them. This succussion step allows the formation of Exclusion-Zones that could contain surface-specific information (28) in accordance with the principles of the ab initio molecular dynamics (29, 30).

For each component of the remedy, the level of dilution is adapted to the desired effect according to the Hormesis concept. Applied at the molecular level, this concept has led to the formulation of the so-called law of inversion of action, according to which very low doses of a substance exert an opposing action to high doses, with all possible intermediates (31-34).

By this way, it becomes entirely possible to regulate all the disturbances affecting a cell's molecular behaviour, whether at genomic, epigenomic or proteomic level. This is the most effective way of restoring cellular homeostasis while constantly aiming for a biomimetic effect.

The fundamental mechanism of BI(G)MED's mode of action thus becomes clearly apparent: the molecules activated as described above will provide the cell with regulatory information, enabling it gradually to modulate all the molecular signalling pathways disrupted in the context of a given pathology, and thus progressively to restore an appropriate cellular functioning. This places our method in the new scientific field of an informational approach to life and health (35, 36).

2.3 Studied population

All the patients came for consultation between 01/02/2020 and 30/06/2022 to the office of one of the seven BI(G)MED physicians involved in the study. Informed consent was obtained from all individual participants included in the study. They have been diagnosed with CAEBV by an immunofluorescence serology characterized by the absence of EBNA IgG. Patients undergoing antiviral, immunosuppressive or psychotropic treatment were excluded. All were offered treatment with the BI(G)MED formula during eighteen months or until the apparition of EBNA IgG. They have been monitored with a clinical interview and a serology every six months. In total, we have 26 cases.

3 Results and discussion

The studied population is composed of 7 males and 19 females. The average age was 52 years old. 16 cases presented chronic fatigue, 1 had an auto-immune disease. 9 cases were diagnosed as CAEBV during routine laboratory tests without specific symptoms.

Among the 26 cases, only on case did not develop EBNA IgG. In other terms, 96% of the patients with CAEBV developed EBNA IgG after a treatment with our formula.

For each case that seroconverted, we have evaluated the time between the beginning of the treatment and the first serology presenting positive EBNA IgG. The mean duration is 7,5 months with a minimum of 4 months and a maximum of 17 months. The median is 6,5 months. 42,3% of the cases seroconverted in the first 6 months of the treatment and 80,7% in the first year (figure 3).

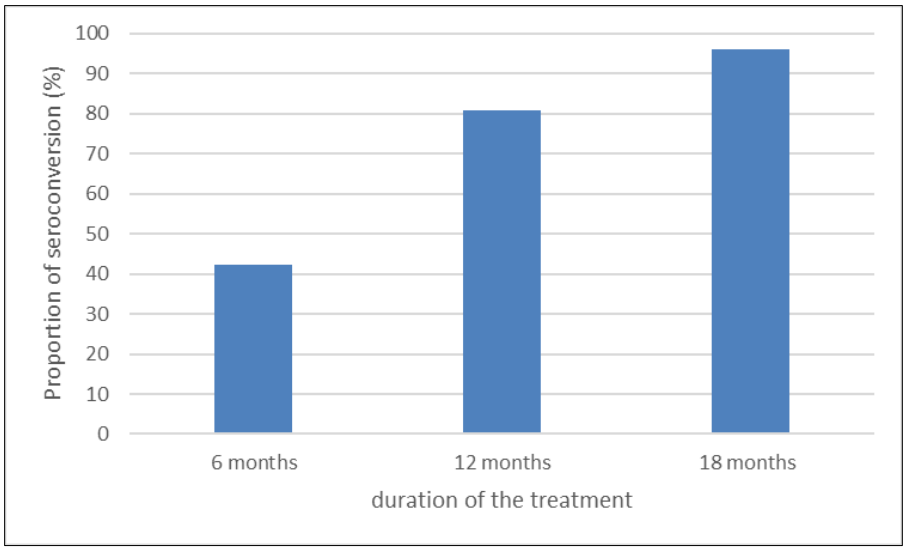

Figure 3 Proportion of seroconversion after 6, 12 and 18 months (in %)

Among the 16 patients with chronic fatigue, 12 cases (75%) report a disappearance of their symptoms. The case with auto-immune disease reports a clear improvement.

Finally, we haven't reported any side effect of the treatment among the 26 cases, which is the general rule for this type of treatment.

4 Conclusion

Non coding RNAs constitute a highly promising therapeutic approach for almost all kind of diseases. Significant advances have been made in identifying the associations of ncRNAs with cancers, viral infections, immune diseases, cardiovascular diseases, biological development and other areas of medicine. However, to date, not more than five drugs have been FDA-approved despite the far great number of potential molecules already developed. This is mainly due to the non-specificity of microRNAs, each one being involved in the silencing of several hundred genes. There are a number of hurdles to overcome before lncRNA-based therapies can be routinely administered to the human population. Among which, we can mention vector delivery, off-target effects, toxicity mediation, immunological activation and dosage determination.

With the Bio Immune(G)ene Medicine, we have developed a method to take advantage of the therapeutic potential of ncRNAs without adverse effects. Indeed, the use of ultra-low doses makes us part of the biomimetic medicine. The various molecules are used by cells at physiological or even sub-physiological doses. Our remedies help restoring the abnormal signaling pathways underlying the disease, thus promoting cellular self-regulation and enhancing homeostasis.

In this paper, we have presented the results of a retrospective study on 26 cases of Chronic Active EBV infection (CAEBV) treated with a cocktail of ncRNAs at ultra-low doses. We have shown that only one person didn't seroconvert in the 18 months of treatment and that seroconversion was associated with an improvement of health condition in 75% of the cases.

This study opens interesting perspectives in the treatment of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). CFS/ME prevalence is about 1%, with women approximately 1.5 to 2 folds higher than men , and is highly increasing since SARS-CoV 2 emergence. Indeed, it has been proven that EBV is strongly associated with Long Covid. The next step of this study would be to conduce a clinical study on patients suffering Long Covid. The small number of cases studied does not allow us to make similar observations concerning other chronic pathologies.

Compliance with ethical standards

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Gilbert Glady, is the president of the European Bio Immune(G)ene Medicine Association.

Narges Bahi-Jaber is the scientific coordinator of the European Bio Immune(G)ene Medicine Association.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Kerr JR. Epstein-Barr virus (EBV) reactivation and therapeutic inhibitors. J Clin Pathol. 2019, 72(10):651–8.

[2] Naughton P, Healy M, Enright F, Lucey B. Infectious Mononucleosis: diagnosis and clinical interpretation. Br J Biomed Sci. 2021, 78(3):107–16.

[3] Sylvester JE, Buchanan BK, Silva TW. Infectious Mononucleosis: Rapid Evidence Review. Am Fam Physician. 2023, 107(1):71–8.

[4] Hedström AK. Risk factors for multiple sclerosis in the context of Epstein-Barr virus infection. Front Immunol. 2023, 14:1212676.

[5] Fugl A, Andersen CL. Epstein-Barr virus and its association with disease - A review of relevance to general practice. BMC Family Practice. 2019, 20:62-71.

[6] Savoldo B, Huls MH, Liu Z, Okamura T, Volk HD, Reinke P, et al. Autologous Epstein-Barr virus (EBV)-specific cytotoxic T cells for the treatment of persistent active EBV infection. Blood. 2002, 100(12):4059–66.

[7] Straus SE. The chronic mononucleosis syndrome. J Infect Dis. 1988, 157(3):405–12.

[8] Smatti MK, Al-Sadeq DW, Ali NH, Pintus G, Abou-Saleh H, Nasrallah GK. Epstein-barr virus epidemiology, serology, and genetic variability of LMP-1 oncogene among healthy population: An update. Frontiers in Oncology. 2018, 8:211

[9] Cohen J. Epstein Barr virus infections. N Engl J Med. 2000, 343(7):481–92.

[10] Cai J, Zhang B, Li Y, Zhu W, Akihisa T, Li W, et al. Prophylactic and therapeutic ebv vaccines: Major scientific obstacles, historical progress, and future direction. Vaccines. 2021, 9:1290

[11] Shareena G, Kumar D. Epigenetics of Epstein Barr virus - A review. Biochim Biophys acta Mol basis Dis. 2023, 1869(8):166838.

[12] Li W, He C, Wu J, Yang D, Yi W. Epstein Barr virus encodes miRNAs to assist host immune escape. Journal of Cancer, 11:2091–100.

[13] Wang M, Yu F, Wu W, Wang Y, Ding H, Qian L. Epstein-Barr virus-encoded microRNAs as regulators in host immune responses. Int. J. Biol. Sci. 2018 *14:*565–76.

[14] Natarelli N, Boby A, Aflatooni S, Tran JT, Diaz MJ, Taneja K, et al. Regulatory miRNAs and lncRNAs in Skin Cancer: A Narrative Review. Life. 2023, 13(8):1696.

[15] Jiang Y, Zhao Y, Li Z yi, Chen S, Fang F, Cai J hui. Potential roles of microRNAs and long noncoding RNAs as diagnostic, prognostic and therapeutic biomarkers in coronary artery disease. Inter. J. Cardio. 2023, 384: 90-99

[16] Hao Q, Wang Z, Wang Q, Xia W, Cao H, Lu Z, et al. Differential expression profile of long noncoding RNAs in chronic HBV infection: New insights into pathogenesis. J Med Virol. 2020 , 92(12):3390–402.

[17] Zhang Y, Yang M, Xie H, Hong F, Yang S. Role of miRNAs in Rheumatoid Arthritis Therapy. Cells. 2023, 12(13):1749.

[18] Rupaimoole R, Han H-D, Lopez-Berestein G, Sood AK. MicroRNA therapeutics: principles, expectations, and challenges. Chin J Cancer. 2011, 30(6):368–70.

[19] Glady G. Epstein-Barr virus Reactivation Associated with an Increased Thymidine Kinase and Normalized by an Immuno Modulatory Nano-Therapy: Three Case Reports. J Clin Exp Immunol. 2018, 3(1):1–5.

[20] Glady G. Epstein-Barr Virus Associated with Hashimoto's Thyroiditis and Treated with a Novel Nanobiological Therapy Based on Micro RNAs: A Case Report. J Clin Rev Case Rep. 2017, 2(3):1–4.

[21] Santilari CMP, Glady G. Bio Immune(G)ene Medicine and the Use of miRNAs to Regulate the Cell. Adv Bioeng Biomed Sci Res. 2018 1(2):1–6.

[22] Glady G. The Interplay between MiRNAs and LncRNAs in the Pathogenesis of Asthmatic Diseases. Arch Immunol Allergy. 2019, 2(2):1–9.

[23] Glady G. The Bio Immune(G)ene Medicine or How to Use a Maximum of Molecular Resources of the Cell for Therapeutic Purposes. Edelweiss Appli Sci Tech. 2019, 3:26–29.

[24] Cuvelier O, Glady G. Spondylitis Associated with Parvovirus B19 and Treated with a Novel Nanobiological Therapy Based on MicroRNAs : A Case Report. Clin Immunol Infect Dis. 2018, 2(1): 1-3.

[25] Glady G, Bahi-Jaber N. How Nanotherapy Fails SARS-CoV-2: The BI(G)MED Experience. Clin Immunol Res. 2021, 5(2):1–4.

[26] Glady G. Clinical efficacy of implementing Bio Immune(G)ene MEDicine in the treatment of chronic asthma with the objective of reducing or removing effectively corticosteroid therapy: A novel approach and promising results. Exp Ther Med. 2018, 15:5133–5140.

[27] Calabrese EJ, Giordano J. Ultra low doses and biological amplification: Approaching Avogadro's number. Pharmacol Res. 2021, 170: 105738.

[28] Upadhyay RP. The Possible Mechanism of Memory through Nanoparticles and Exclusion Zones. Water. 2017, 7:158–76.

[29] Manzhos S, Carrington T. Neural Network Potential Energy Surfaces for Small Molecules and Reactions. Chem Rev. 2021, 121(16):10187–217.

[30] Vollath D, Fischer FD, Holec D. Surface energy of nanoparticles - influence of particle size and structure. Beilstein J. Nano. 2018, 9: 2265-2276.

[31] Ullman D. Exploring Possible Mechanisms of Hormesis and Homeopathy in the Light of Nanopharmacology and Ultra-High Dilutions. Dose-Response. 2021, 19: 1-13.

[32] Agathokleous E, Kitao M, Calabrese EJ. Hormesis: Highly Generalizable and Beyond Laboratory. Trends Plant Sci. 2020, 25(11):1076–86.

[33] Li X, Yang T, Sun Z. Hormesis in Health and Chronic Diseases. Trends Endocrinol Metab. 2019, 30:944–58.

[34] Dei A, Bernardini S. Hormetic effects of extremely diluted solutions on gene expression. Homeopathy. 2015, 104(2):116–22.

[35] Walker SI, Callahan BJ, Arya G, Barry JD, Bhattacharya T, Grigoryev S, et al. Evolutionary dynamics and information hierarchies in biological systems. Ann N Y Acad Sci. 2013, 1305(1):1–17.

[36] Marijuán PC, Navarro J. From molecular recognition to the “vehicles” of evolutionary complexity: An informational approach. Int. J. Mol. Sci., 2021, 22: 11965 .