



Hamlet Oral Treatment Post Chemotherapy in a Patient with a Condition of Classical Hodgkin's Lymphoma Induced a Down and Up Regulation of a Set of Four Genes Involved in the Immune System, Nucleic Acid Binding and Sodium Transport: A Case Report Study

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Abstract

Hodgkin's lymphoma was first described in 1832. The etiology of this lymphoma, however, remained enigmatic for a long time. Only within the past 10 years has the B-cell nature of the pathognomonic Hodgkin and Reed-Sternberg (HRS) cells been revealed, along with several recurrent genetic lesions. It has been suggested that the Hodgkin's lymphoma microenvironment is dominated by an extensive mixed, potentially inflammatory cellular infiltrate. Understanding the contribution of all of these changes to the pathogenesis of this disease is essential for the development of novel immunotherapies. On the other hand, the anti-tumorigenic activity therapeutic effects of α -lactal albumin complexed with C18:1 fatty acid (oleic acid) or HAMLET (human α -lactal albumin made lethal to tumor cells) have been demonstrated in human skin papilloma's and bladder cancers. HAMLET limits the progression of human glioblastomas, with no evidence of toxicity for normal brain or bladder tissue. In a previous work, we reported the gene expression pattern induced by HAMLET oral treatment before chemotherapy in a patient with a condition of thyroid cancer. Herein, it is reported, that there is a set of four genes (TRIMM25, ELAVL3, ZNF and COMMD3) with a higher down and up regulation in an individual with condition of classic Hodgkin's lymphoma (cHL) after chemotherapy and Hamlet oral treatment. Altogether the data suggest that this could serve as a palliative measure to reduce the side effects of the chemotherapy treatment. The gene expression at systemic level is a potential tool that can allow to identify genetic changes that could be attributed to the consumption of the compound.

Keywords: Classical Hodgkin's Lymphoma; Human Maternal Milk; Alpha-Lactalbumin HAMLET; Chemotherapy; Radiotherapy

Introduction

Cancer is a disease in which some cells in the body multiply uncontrollably and spread to other parts of the body. Cancer can start anywhere in the human body. Under normal conditions, human cells should form and multiply to create new cells as the body needs them. When cells grow old or become damaged, they die and are replaced by new cells. In the case of cancer abnormal or damaged cells form and multiply spontaneously. These cells may form tumors, which are lumps of tissue, classified as malignant or benign [1-4].

On specifically, the Hodgkin's lymphoma, a tissue lymphatic cancer, used to be called Hodgkin disease, affecting primarily the lymphatic system (organs, glands, lymph nodes, vessels) and disseminated to the immune system, major body's germinal fighting immune system) (thymus, bone marrow). Lymphoma is characterized by an abnormal, and increased proliferation of white blood cells (lymphocytes) and the distinctive large Reed-Sternberg cells surrounded by bands of fibrotic collagen. Hodgkin lymphomas are classified into two main subtypes: classical Hodgkin lymphoma and nodular lymphocyte-predominant Hodgkin lymphoma. Classical Hodgkin lymphoma accounts for 95% of all Hodgkin lymphomas. This subtype is divided into four subtypes, which, according to the World Health Organization (WHO), are: a) Nodular sclerosis type Hodgkin lymphoma, which constitutes 55% of cases and is more common in adolescents and young adults; b) Mixed cellularity Hodgkin lymphoma represents 15%, more frequently in young adults, c) Lymphocyte-rich Hodgkin lymphoma, which has a similar frequency to the previous one, occurs mostly in elderly adults with a favorable prognosis, and d) Lymphocyte-depleted Hodgkin lymphoma, which is the least frequent subtype, represents 1% of cases and generally has the worst prognosis because it is associated with immunocompromised patients. Therefore, classification is of utmost importance in determining treatment [1-4]. Nodular sclerosis Hodgkin lymphoma is the most common subtype of classical Hodgkin lymphoma (cHL), as mentioned earlier. According to several studies the cause of Hodgkin lymphoma is unknown. However, it has been proposed that past infections, such as, with the Epstein-Barr virus (EBV) is thought to contribute to some cases. It has been shown for example, that people with HIV infection are at higher risk than the general population [1-4]. The immediate clinical symptoms may include any of the following: Feeling tired all the time. Intermittent fever and chills. Unexplained

itching all over the body. Loss of appetite. Profuse night sweats. Painless swelling of the lymph nodes in the neck, armpits, or groin (swollen lymph nodes). Unexplained weight loss. The current test to diagnose Hodgkin's lymphoma includes, Blood chemistry tests, for example, liver function tests, kidney function tests, and lactate dehydrogenase (LDH). Erythrocyte sedimentation rate (ESR). Bone marrow biopsy. Computed tomography (CT) scan of the chest, abdomen, and pelvis. Complete blood count (CBC) and white blood cell differential to check for anemia and low white blood cell count and Positron emission tomography (PET) [1-5]. Interestingly, this is one of the types of blood cancer that can be curable at early and late stages of the disease. Except in cases with comorbidities. Usually after the positive diagnostic positive for HL, the most recommendable treatment will depend of some factors, the type of the HL or localization of the tumor, if the biopsy reveals malignant or benign, the stage of the dissemination, the age and other clinical and medical conditions, or the comorbidities. However, nowadays, this type of cancer can be treated with chemotherapy, radiotherapy, immunotherapy or the combination of chemo-immunotherapy (chemo immunotherapy), chemo-radiotherapy (chemo radiation therapy) [6-15].

Of what depends the success of the cancer treatments? [15-20]. It is possible that the immunogenicity of the tumor play a role, since as an antigen can induce anti-tumor immune responses. Indeed, the recognition of the interplay of cancer and immunity has led to the deep investigation in how to harness from this interaction and from the immunologically active tumor cells [5,12,13,15]. The innate and mostly the adaptive immune response represented by the B and T cells play a key role in the protection of the body against myriad of pathogens and other non-infectious disease as cancer. The T cells can recognize specifically pathogens or other antigens through the histocompatibility complex (MHACI, MHAC-II), are enable with the capacity to kill pathogens or eliminated cancerous cells for example, through the coordination of the innate and adaptive immune responses. The traditional treatments against cancer has been characterized in the last decades for the use of chemical compounds or drugs, or secondary metabolites with anti-tumoral properties. Chemotherapy remains as the backbone for the treatment against cancerous tumors. Other classical therapies or cancer treatments includes in addition to chemotherapy, surgery, radiotherapy, and more recently, targeted therapy i.e., cancer vaccines (consist in the use of tumor antigens, to destroy cancer

cells. It can be preventive (HPV) or therapeutic (to manipulate tumor growth or tumor regression through genetically engineered, tumor whole-cell, dendritic cell, and protein-peptide vaccines) [19-21]. Monoclonal antibodies (might be of two types, Antibody-drug conjugates (ADCs) use the antigen-specificity of monoclonal antibodies to target and deliver cytotoxic drugs to tumor cells or designed to bind either two distinct antigens or two separate epitopes on the same antigen) CART cells,) and immunotherapy or immuno-oncology [7-9,15,16]. On referring specifically to the immunotherapies, or immuno-oncology, to control and kill tumor cells promoting or favoring the restoration of the immune system in most of the cases, or stimulating the T cell-based immune response protecting thus against cancerous tumor. It is very well known that tumor cells use the immune check points as a strategy for immune surveillance, and as evasion mechanism [7-9,15,16]. These checkpoints are being described as immune protector's factors that impede T cell over activity resulting as a consequence an autoimmune response and therefore damage tissue. Two of the best known immune checkpoints are the Cytotoxic T lymphocyte associated protein 4 (CTLA-4), programmed cell death-ligand 1 (PDL-1). Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) is a negative costimulatory molecule expressed on the surface of activated and regulatory T cells leading to an effectively attenuation of the T cell activation via competing with the costimulatory molecule CD28 to bind with ligands CD80 (B7.1) and CD86 (B7.2) on antigen-presenting cells (APCs) [15-17]. While PD-1 (programmed cell death-1) receptor is expressed on the surface of activated T cells and bind to its ligand, PD1--L1 and PD1---L2 expressed on the surface of the antigen presenting cells, dendritic cells or macrophages to regulate and limit T cell responses [20-24].

It has been developed immune checkpoint inhibitors (ICIs), intended to disrupt inhibitory signaling pathways and restoring thus, and T cell immune surveillance and kills tumor cells. These ICIs has been useful in several types of cancer, as non-small-cell lung cancer (NSCLC), metastatic melanoma, and renal cancers. A recent study for example have reported that in triple negative breast cancer has been found that there is an increase of infiltrated lymphocytes, expression of programmed cell death-ligand 1 (PDL-1) and tumor mutational load (TMB) [22,23,25]. Therefore, the use of the immune checkpoints inhibitors of the Cytotoxic T lymphocyte associated protein 4 (CTLA-4), and programmed cell death-ligand 1 (PDL-1), such as ipilimumab, nivolumab, and

atezolizumab is to overcome cancer's ability to resist the immune responses and thereby allow body's own immune system to remain awake to trigger defense against cancer [22,23,25]. Despite enormous improvement in the immunotherapies, still there are some alternatives that are being offered to enhance their success to approach personalized immunotherapies by immune modulation of the host anticancer response. One of this are the biomarkers determination which can be greatly important in diagnosis, prognosis and progression of the disease.

By other hand, in recent years, HAMLET (alpha-lactalbumin lethal to kill tumor cells) has been described as one of the best anti-cancer treatments, effectively eradicating cancer cells and reducing the volume of certain tumors. Based on several *in vitro* and *in vivo* studies, the functionalities of this complex have been gradually determined, with particular emphasis on its cytotoxic capacity. HAMLET, is a tumoricidal complex consisting of proteins and fatty acids found in fractions of human breast milk. It can be synthesized from pure components using a modified chromatographic process where pre-applied oleic acid binds to alpha-lactalbumin in the stationary phase. Native alpha-lactalbumin does not trigger cell death; it is only when combined with oleic acid that HAMLET exhibits cytotoxicity. Early *in vitro* experiments showed that HAMLET has broad antitumor activity with a high degree of tumor selectivity [26-29]. More recently, HAMLET's broad antitumor activity has been explained by specific effects on oncogenic transformation and by targeting metabolic machinery in tumor cells [30]. The tumoricidal activity of HAMLET maintains its relative selectivity for tumor tissue *in vivo*, as demonstrated in several human studies and various animal models. HAMLET treatment delayed the progression of human glioblastoma xenografts in nude rats and increased survival, triggering apoptotic changes in the tumor without evidence of cell death in healthy brain tissue [31]. Thus, in a placebo-controlled clinical trial, topical administration of HAMLET to cutaneous papillomas was eliminated without side effects [32]. In patients with bladder cancer, local instillations of HAMLET killed tumor cells but not healthy cells in the surrounding tissues. Furthermore, HAMLET triggered the rapid elimination of tumor cells in the urine and caused a reduction in tumor size in patients with bladder cancer [33]. In addition to this, it has been shown to have tumoricidal effectiveness against more than 40 different tumor cell lines of various origins. Moreover, the HAMLET bound directly to isolated 20S proteasomes *in vitro* and

in significant tumor cells. This interaction was confirmed by co-immunoprecipitation of HAMLET-treated tumor cell extracts. After brief activation, HAMLET inhibited proteasome activity *in vitro* and, in parallel, induced proteasome modification. Furthermore, in colon cancer cells with APC mutations, HAMLET altered the integrity and localization of β -catenin through an ion channel-dependent pathway, defining a new mechanism for controlling β -catenin signaling that is important or plays a role in cell-cell adhesion. In a previous work we have shown the promising palliative effect of HAMLET oral treatment in individual with a condition of Thyroid cancer before radiotherapy/surgery treatment [34,35]. Herein, it is reported the gene expression pattern of four genes (TRIMM25, ELAVI3, ZNF and COMMD3) most Down or Up regulated post chemotherapy and HAMLET oral treatment. Altogether the data suggest that Hamlet oral treatment might be a palliative measure to reduce the side effects of the treatment, as well as to identify genetic changes that could be attributed to the consumption of the compound.

Clinic Case Report Description

On August 5, 2024, a 12-year-3-month-old female adolescent, born September 5, 2011, originally from and residing in Tacoaleche, Guadalupe, Zacatecas, was admitted to the Zacatecas General Hospital. Her anthropometric indicators were: weight-for-age 72%, height-for-age 86%, and weight-for-height 91%. She was diagnosed with classical Hodgkin lymphoma (cHL) with nodular sclerosis, stage IIIVB, in the surveillance phase, with probable tumor activity. The initial diagnosis was made at the Durango General Hospital on June 10, 2024, with an excisional biopsy of the right axillary lymph node.

There was no significant personal or family history relevant to the current condition: LHc E-IVB. The B indicates the presence of symptoms from the onset of the illness. These symptoms typically include painless swelling of the lymph nodes in the neck, as in most cases it is located in the mediastinum. Additionally, general symptoms such as fever, night sweats, and weight loss are present.

CI diagnoses: D479 tumors of uncertain or unknown behavior of lymphatic tissue, hematopoietic organs and related tissues, unspecified classical Hodgkin lymphoma with nodular sclerosis stage IV, E-IVB.

Hospital discharge due to clinical improvement on: July 12, 2023 with lymph node imprint report: Large, mononuclear cells, prominent nucleolus with clear cytoplasm, corresponding to Hodgkin cells, Reed-Sternberg cells, binucleated with eosinophilic nucleoli separated by a space of thickened nuclear membrane. With second cycle of remission.

Echocardiogram: Structurally healthy heart, moderate pericardial effusion, pulmonary artery systolic pressure (PASP): 19 mm Hg, left ventricular ejection fraction (LVEF) 78%.

Histopathology: Classical Hodgkin lymphoma with nodular sclerosis. Bone marrow aspirate with Reed-Sternberg cells. Lugano staging (CIL) stage IV-B.

Start protocol: With OPPA-COPDAC this protocol is a sequence of two different chemotherapy regimens: OPPA: Oncovin (Vincristine sulfate); Procarbazine; Prednisone; Adriamycin (Doxorubicin hydrochloride) and COPDAC: Cyclophosphamide; Oncovin (Vincristine sulfate); Prednisone (a steroid); Dacarbazine.

Administered: OPPA protocol: 1st cycle on July 28, 2023 without incident. 2nd cycle on August 18 without incident and with significant reduction in tumor size. COPDAC administration; Received 1st cycle on September 18, 2023; 2nd cycle on October 14; 3rd cycle on November 13. No data on adverse effects or associated toxicity; 4th cycle on December 12, 2023, without incident.

External consolidation radiotherapy with a dose of 36 Gy/20 sessions using mantle field radiotherapy from January 17 to February 21, 2024, under controlled monitoring. A PET-CT scan on June 14, 2024, revealed cervical and mediastinal lymphadenopathy and conglomerates associated with metabolism and related to lymphoproliferative neoplastic activity, corresponding to a Deauville score of 4.

CT scan (Computed tomography without contrast) May 16, with lymph node conglomerates in neck and thoracic chains; related to activity of the known primary tumor. Foci and Nodes (F and N): Not applicable.

Note: Pediatric Oncology discharge.

Until hospital discharge: Date of entry: 05/08/24 and hospital exit: 08/08/24.

Currently, the patient is in good general condition, tolerating oral intake, afebrile, and does not require supplemental oxygen to maintain optimal saturations. Vital signs (VS): HR bpm, RR rpm, BP mmHg, SpO₂%, temp °C, fluid balance, urine output ml/kg/hr, ml/m²sc/hr. Units: ml/kg/hr (milliliters per kilogram per hour) and ml/m²sc/hr (milliliters per square meter of body surface area per hour).

On physical examination, the patient was awake, active, and responsive, with neurological integrity, a normocephalic skull, symmetrical and normoreflexic eyes and palpebral reflexes, hydrated mucous membranes, palpable lymphadenopathy in the neck, symmetrical and well-ventilated hemithoraces, and regular precordial rhythm without added sounds, a soft, depressible, and non-tender abdomen, intact extremities, and capillary refill of 2 seconds. Discharge is planned due to the patient's good general condition, with readmission scheduled for the next chemotherapy session.

Laboratories: 08/05/24: Hb 13.7, Hct 39.5, platelets 318, Leuc 4.41, Neut 2.75, Linf 1.08, M 0.43, Gluc 69, BUN 9, Urea 19.6, Creatinine 0.6, BT 0.11, BD 0.09, BI 0.02, AST 22, FA 384, ALT 12.10, DHL 232, Alb 4.4, Na 141, K 3.9, Cl 107, Ca 9.5, P 4.2, Mg 1.85.

Medical recommendations and prognosis

Discharge instructions:

1. Discharge home independently.
2. Schedule an appointment if you experience any warning signs (fever, severe, unbearable pain, general malaise).
3. Attend chemotherapy on August 14, 2024.

Readmission: Current illness (CI): She was admitted from outpatient consultation to determine the appropriate treatment due to probable tumor activity. During this hospitalization, a second cycle of remission was initiated with the following regimen: Gemcitabine on August 7 and 14, 2024; Pegfilgrastim on August 15, 2024 (one dose); and Brentuximab Vedotin (a single dose) on August 7, 2024, administered on the corresponding dates with monitoring for tolerance and potential side effects.

Last chemotherapy and last hospitalization from 11-15/12/2024: 4 days with diagnosis of: Eutrophic female schoolchild (PE 72%; TE 86%; P/T 107%; BMI 17.58 Percentile 25-50 + LHc with nodular sclerosis E-IVB + Chemotherapy in 4th cycle of the COPDAC protocol. Hospital discharged on: 08/08/24. No report of relapses.

Methodology for the study of the effect of the combined treatment (Chemotherapy and oral ingestion of HAMLET) to a patient with a condition of classical Hodgkin's lymphoma (cHL)

The study and all medical research procedures with human subjects, including research with identifiable human material and data, were approved by the Research Ethics Committee of Zacatecas, "Luz González Cosío" General Hospital CONBIOETICA-32-CEI-001-20231023. Since the young child with a condition of cHL was under the chemotherapy treatment, the ethical procedure was firstly to talk with the parents and explained them about the effects of the HAMLET oral treatment did not interfere with the chemotherapy nor have any secondary effect. On the contrary, could be exerting a palliative effect with the secondary effects of the chemotherapy. The parents gave signed written consent (Figure 1A) Untreated healthy young children (3AD), a young children with a condition of Classical Hodgkin's lymphoma (cHL) under chemotherapy treatment(1B) and/ or under a combined treatment of chemotherapy and HAMLET orally (2A) gave signed written consent. The patient with condition of cHL after chemotherapy treatment was instructed for an ingestion of 30 milliliter of HAMLET early in the morning every day for two weeks. (Figure 1A). At time zero blood and after the last dose of HAMLET blood was collected in tempus tube for further RNA extraction. Individual were advised not to eat food before HAMLET oral treatment. Similarly, were instructed not to drink any probiotics during the study period.

Gene expression pattern determination: RNA extraction from whole blood was performed using a KIT and following the manufacturer's instructions (AMBION, Life technology). The integrity of the RNA was recorded in an agarose gel 1% in TBE 0.5 X and stained with Green loading buffer. The preparation of the microarrays was carried out according to the standardized method in the Microarray Unit of the Institute of Cellular Physiology of the UNAM. Mexico City. To carried out the analysis of the genes that are being up or down regulated at time zero before and after two weeks

of HAMLET oral treatment. cDNA of a healthy young children, and the cDNA of the young children with cHL, hybridized with the human DNA chip (n = 10,000 genes) (with only chemotherapy treatment). In the same chip of DNA, both cDNA were hybridized and from this, it is possible to determine which genes versus the cDNA of the healthy young children are up or down regulated and which genes in fact. In another DNA chip it was hybridized the cDNA of the patient (young children) with a condition of cHL, and chemotherapy treated at time zero, and the cDNA of this patient with the combined treatment of chemotherapy and HAMLET oral treatment (at time two weeks after) (Figure 1A-1C). The results obtained from image quantification were analyzed with Gena rise. The lists of regulated UP and/or DOWN genes are reported for Z-Score cuts (± 2.0 SD). Statistical analyzes were performed using Graph Pad Prism 6.0 (CA, USA) using nonparametric analysis of variance (ANOVA). A $p \leq 0.05$ was considered significant.

Results

TRIM 25, ELAVL3, COMMD3, and ZNF571 are the genes that are down and up regulated in a patient with a condition of classical Hodgkin's lymphoma with Hamlet oral treatment postchemotherapy

The analysis of the effect of the oral ingestion of HAMLET in a patient with a condition of classical Hodgkin's lymphoma (cHL) under chemotherapy treatment, and after chemotherapy and oral ingestion of HAMLET, was determined using DNA microarray and expressed a heat maps (Figure 1A-1C). The first analysis of how many genes are down or up regulated under these settings showed that cDNA from the patient condition (1B) hybridized to the human DNA and most of the genes are downregulated (n = 319) versus Up regulated genes (n = 87) (Table 1). However, after two weeks of HAMLET oral treatment, the number of genes down regulated was decreased by few (n = 300), but the number of genes Up regulated increased (n = 140) (Table 1).

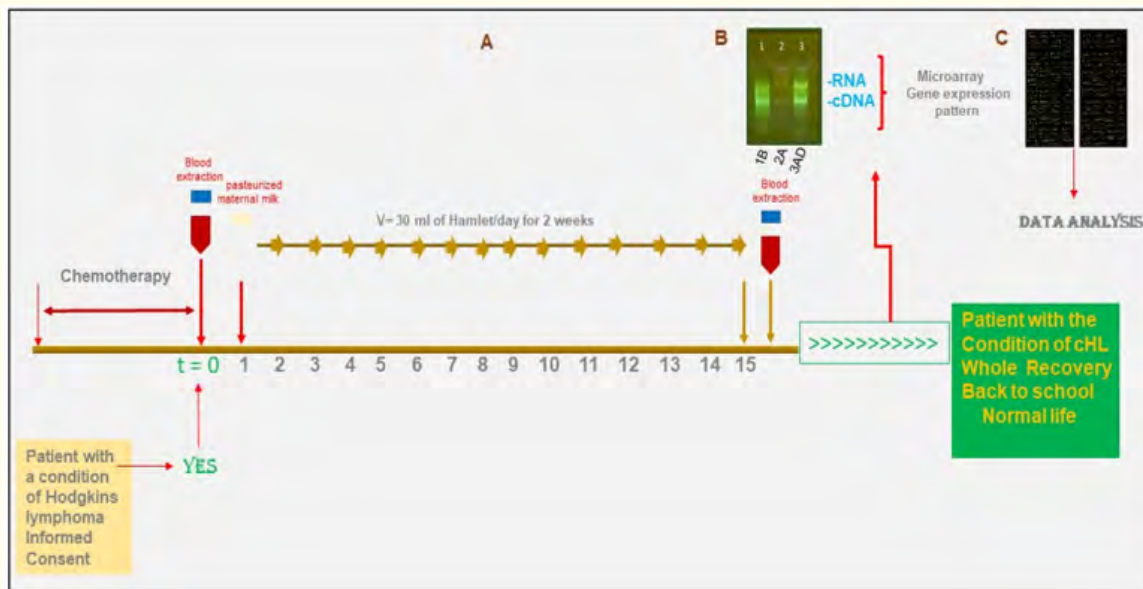


Figure 1: Scheme protocol followed for the analysis of the effect of HAMLET (human lactalbumin lethal to kill cells) oral treatment after chemotherapy for two weeks to an individual diagnosed with a condition of classical Hodgkin's lymphoma (cHL). The study was carried out using whole blood and DNA microarrays. After the written consent is obtained, the participant (s) (health and sick individuals) (A). RNA was prepared from whole blood extracted and analyzed in agarose gel 1% in TBE 0.5X and stained with Green loading buffer. (B) cDNA was prepared following the manufacturer's instructions. Gene expression pattern represented as heat maps of the crude extracted data of DNA-cDNA microarrays (C) (Unit of Microarray of the Institute of Cellular Physiology, UNAM, City of Mexico).

Table 1. Higher number of Up regulated genes after chemotherapy and dialy oral ingestion of HAMLET in a patient with a condition of classical Hodgkin lymphoma (cHL).

Sample	UP	DOWN
1B vs 3AD	87	319
1B vs 2A	140	300

*Genarise program to analyze the gene expression pattern reported for two Z score cuts (2 SD) of the up and down regulated. Statistical analyses were performed using Graph Pad Prism (Ca, USA). A p< 0.05 was considered significant. 1B and 3AD, patient with a condition of classic Hodgkins lymphoma (cHL) after chemotherapy. 1B vs 2A, patient with cHL, after chemotherapy and and after dialy oral ingestion of HAMLET for two weeks.

Table 1: Higher number of Up regulated genes after chemotherapy and hamlet oral treatment to a patient with a condition of classical Hodgkin lymphoma (cHL).

Down and Up regulation genes in the patient with a condition of classical Hodgkin's Lymphoma (cHL) with chemotherapy treatment

Among the 25 most down regulated genes with a Z-score values from -2.0 to -5.79 (Figure 2A) that includes among others, to DET1, DET1 partner of COP1 E3 ubiquitin ligase that is part of the Cul4A-RING E3 ubiquitin ligase complex; involved in the positive regulation of proteasome ubiquitin-dependent protein catabolic process. DLK1 delta like non-canonical Notch ligand 1, that contains multiple epidermal growth factor repeats that functions as a regulator of cell growth. ELAVL3 ELAV like RNA binding protein 3. A member of the ELAVL protein family, ELAV-like 3 is a neural-specific RNA-binding protein which contains three RNP-type RNA recognition motifs. It plays a role in neurogenesis. SLC67A1 solute carrier family 67 member 1. This gene is one of several tumor-suppressing sub transferable fragments located in the imprinted gene domain of 11p15.5, an important tumor-suppressor gene region. Genes involved in the immune system, such as TRIM25 tripartite motif containing 25. The protein is an RNA binding protein, functions as an ubiquitin E3 ligase and is involved in multiple cellular processes, including regulation of antiviral innate immunity. IL-12, this gene encodes a subunit of a cytokine that acts on T and natural killer cells, and has a broad array of biological activities. GZM, granzyme A. Cytolytic T lymphocytes (CTL) and natural killer (NK) cells share

the remarkable ability to recognize, bind, and lyse specific target cells (Figure 2A and table 1A.1).

While the most UP regulated genes with Z values from 2.0 to 3.79 (Figure 2A) PARVA parvin alpha gene, which encodes a member of the parvin family of actin-binding proteins. Parvins are associated with focal contacts and contain calponin homology domains that bind to actin filaments. The encoded protein is part of the integrin-linked kinase signaling complex and plays a role in cell adhesion, motility and survival. ZNF539/ZNF254 zinc finger protein 254. Zinc finger proteins have been shown to interact with nucleic acids and to have diverse functions. CYBRD1 cytochrome b reductase 1. This gene is a member of the cytochrome b (561) family that encodes an iron-regulated protein. It has ferric reductase activity and is believed to play a physiological role in dietary iron absorption. MRPS18B mitochondrial ribosomal protein S18B, and MRPL15 mitochondrial ribosomal protein L15. Mammalian mitochondrial ribosomal proteins are encoded by nuclear genes and help in protein synthesis within the mitochondrion. ZNF222 zinc finger protein 222. ZNF197 zinc finger protein 197. Predicted to enable DNA-binding transcription factor activity. Predicted to be involved in regulation of DNA-templated transcription. Predicted to be located in nucleus. This gene product belongs to the zinc finger protein superfamily, members of which are regulatory proteins characterized by nucleic acid-binding zinc finger domains (Figure 2A and table 1A.2).

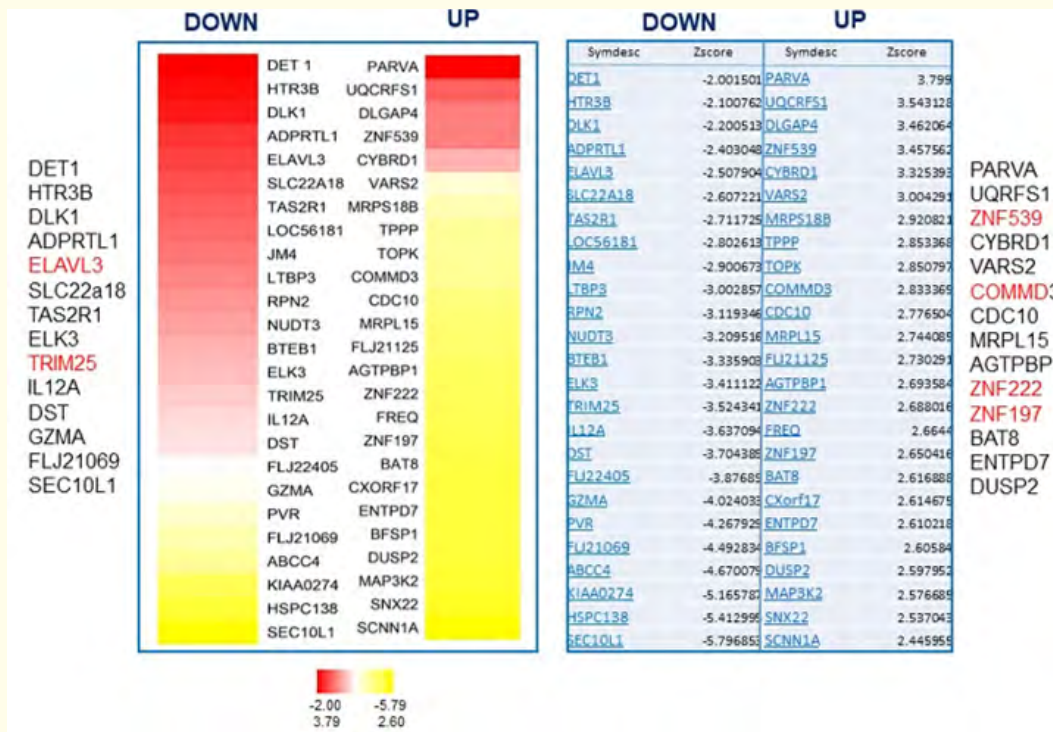


Figure 2A: Gene expression in a patient with a condition of classical Hodgkin's lymphoma after chemotherapy. Heat map (left panel) from the 25 most DOWN-regulated genes with a Z score value from -2.0 to -5.79, which includes, among others (right panel) to ELAVL3, ELAV-like RNA binding protein 3. A member of the ELAVL protein family, ELAV-like 3 is a neural-specific RNA-binding protein that contains three RNP-type RNA recognition motifs. TRIM25 tripartite motif containing 25. Functions as an ubiquitin E3 ligase and is involved in multiple cellular processes, including the regulation of antiviral innate immunity. IL-12, this gene encodes a subunit of a cytokine that acts on T and natural killer cells, and has a broad array of biological activities. GZM, granzyme A. Cytolytic T lymphocytes (CTL) and natural killer (NK) cells share the remarkable ability to recognize, bind, and lyse specific target cells. UP-regulated genes (left panel) with Z values from 2.0 to 3.79, that includes PARVA gene, which plays a role in regulating a variety of cellular processes in multicellular organisms, including motility, shape change, survival, proliferation, and differentiation. UQCRFS1 complex III assembly of the cytochrome c (cytochrome bc1) reductase (Complex III in the mitochondrial inner membrane, mitochondrial matrix. ZNF539/ZNF254 zinc finger protein 254. Zinc finger proteins have been shown to interact with nucleic acids and to have diverse functions. COMMD3 COMM domain containing 3. Predicted to be involved in sodium ion transport. Predicted to be located in the extracellular region and ficolin-1-rich granule lumen. VARS2.

Table 1A.1. MOST DOWN REGULATED GENES in a patient with a condition of classical Hodgkins lymphoma after chemotherapy.

<p>DETI partner of COP1 E3 ubiquitin ligase. Enables ubiquitin protein ligase binding activity and ubiquitin-like ligase-substrate adaptor activity. Involved in positive regulation of proteasomal ubiquitin-dependent protein catabolic process; protein ubiquitination, and protein-containing complex assembly.</p>
<p>HR23B 5-hydroxytryptamine receptor 3B. The product of this gene belongs to the ligand-gated ion channel receptor superfamily. This gene encodes subunit B of the type 3 receptor for 5-hydroxytryptamine (serotonin), a biogenic hormone that functions as a neurotransmitter, a hormone, and a mitogen. This receptor causes fast, depolarizing responses in neurons after activation.</p>
<p>DLK1 delta like non-canonical Notch ligand 1. This gene encodes a transmembrane protein that contains multiple epidermal growth factor repeats that functions as a regulator of cell growth. The encoded protein is involved in the differentiation of several cell types including adipocytes.</p>
<p>PARP4 RP4 [ADP-ribosyl transferase family member 4]. This gene encodes poly(ADP-ribose) polymerase family member 4. This protein, which is capable of catalyzing a poly(ADP-ribose)ylation reaction. This protein has a catalytic domain which is homologous to that of poly(ADP-ribose) transferase, but lacks an N-terminal DNA binding domain which activates the C-terminal catalytic domain of poly(ADP-ribose) transferase.</p>
<p>ELAVL3 ELAV like RNA binding protein 3. ELAV-like 3 is a neural-specific RNA-binding protein which contains three RNP-type RNA recognition motifs. The observation that ELAVL3 is one of several Hu antigens (neural-specific RNA-binding proteins) recognized by the anti-Hu serum antibody present in sera from patients with paraneoplastic encephalomyelitis and sensory neuropathy (PEM/PSN) suggests it has a role in neurogenesis.</p>
<p>SLC67A1 solute carrier family 6 member 1. This gene is one of several tumor-suppressing subtransferable fragments located in the imprinted gene domain of 11p15.5, an important tumor-suppressor gene region. Alterations in this region have been associated with the Beckwith-Wiedemann syndrome, Wilms tumor, rhabdomyosarcoma, adrenocortical carcinoma, and lung, ovarian, and breast cancer. This protein may act as a transporter of organic cations, and have a role in the transport of chloroquine and quinidine-related compounds in kidney.</p>
<p>TAS2R1 taste 2 receptor member 1. This gene encodes a member of a family of candidate taste receptors that are members of the G protein-coupled receptor superfamily and that are specifically expressed by taste receptor cells of the tongue and palate epithelia. This gene is mapped to chromosome 5p15, the location of a genetic locus (PROP) that controls the detection of the bitter compound 6-n-propyl-2-thiouracil.</p>
<p>CO5G18 MITF1 mitochondrial fission regulator 1 like Predicted to be involved in aerobic respiration and mitochondrial fission. Located in mitochondrion.</p>
<p>JNM1/PRAF2 PRA1 domain family member 2. Predicted to be involved in L-glutamate transmembrane transport. Predicted to be located in endosome membrane. Predicted to be active in several cellular components, including GABA-ergic synapse; glutamatergic synapse; and postsynapse.</p>
<p>LTBP3 latent transforming growth factor beta binding protein 3. The protein encoded by this gene forms a complex with transforming growth factor beta (TGF-beta) proteins and may be involved in their subcellular localization. Activation of this complex requires removal of the encoded binding protein. This protein also may play a structural role in the extracellular matrix.</p>
<p>RPN2 ribophorn II. This gene encodes a type I integral membrane protein found only in the rough endoplasmic reticulum. The encoded protein is part of an N-oligosaccharyl transferase complex that links high mannose oligosaccharides to asparagine residues found in the Asn-X-Ser/Thr consensus motif of nascent polypeptide chains.</p>
<p>NUDT8 nudix hydrolase 8. NUDT8 belongs to the Nudix protein family. Nudix proteins act as homeostatic checkpoints at important stages in nucleoside phosphate metabolic pathways, guarding against elevated levels of potentially dangerous intermediates, like 8-oxo-dGTP, which promotes AT-to-CG transversions.</p>
<p>BTBD1 KLF9 KLF transcription factor 9. The protein encoded by this gene is a transcription factor that binds to GC box elements located in the promoter. Binding of the encoded protein to a single GC box inhibits mRNA expression while binding to tandemly repeated GC box elements activates transcription.</p>
<p>ELK3 ETS transcription factor ELK3. This gene encodes a member of the ETS-domain transcription factor family and the ternary complex factor (TCF) subfamily. Proteins in this subfamily regulate transcription when recruited by serum response factor to bind to serum response elements.</p>
<p>TRIM25 tripartite motif containing 25. The protein encoded by this gene is a member of the tripartite motif (TRIM) family. The TRIM motif includes three zinc-binding domains, a RING, a B-box type 1 and a B-box type 2, and a coiled-coil region. The protein is an RNA binding protein, functions as a ubiquitin E3 ligase and is involved in multiple cellular processes, including regulation of antiviral innate immunity.</p>
<p>IL12A interleukin 12A. This gene encodes a subunit of a cytokine that acts on T and natural killer cells, and has a broad array of biological activities. This cytokine is required for the T-cell-independent induction of interferon (IFN)-gamma, and is important for the differentiation of both Th1 and Th2 cells. The responses of lymphocytes to this cytokine are mediated by the activator of transcription protein STAT4. Nitric oxide synthase 2A (NOS2A/NOS2) is found to be required for the signaling process of this cytokine in innate immunity.</p>
<p>DST dystonin. This gene encodes a member of the plakin protein family of adhesion junction plaque proteins. It has been reported that some isoforms are expressed in neural and muscle tissue, anchoring neural intermediate filaments to the actin cytoskeleton, and some isoforms are expressed in epithelial tissue, anchoring keratin-containing intermediate filaments to hemidesmosomes.</p>
<p>PLUZ2/05/MTMR14 myotubularin related protein 14. This gene encodes a myotubularin-related protein. The encoded protein is a phosphoinositide phosphatase that specifically dephosphorylates phosphatidylinositol 3,5-bisphosphate and phosphatidylinositol 3-phosphate. Mutations in this gene are correlated with autosomal dominant centronuclear myopathy.</p>
<p>GZMA granzyme A [Homo sapiens (human)]. Cytolytic T lymphocytes (CTL) and natural killer (NK) cells share the remarkable ability to recognize, bind, and lyse specific target cells. The protein described here is a T cell- and natural killer cell-specific serine protease that may function as a common component necessary for lysis of target cells by cytotoxic T lymphocytes and natural killer cells.</p>
<p>PVR PVR cell adhesion molecule. The protein encoded by this gene is a transmembrane glycoprotein belonging to the immunoglobulin superfamily. The external domain mediates cell attachment to the extracellular matrix molecule vitronectin, while its intracellular domain interacts with the dynein light chain Tctex-1/DYLNLT1.</p>
<p>CLIP4 CAP-GLY domain containing linker protein family member 4. Predicted to be involved in cytoplasmic microtubule plus-end binding activity. Predicted to be involved in intracellular membrane-bounded organelle.</p>
<p>ABCC4 ATP binding cassette subfamily C member 4 [PE blood group]. The protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. This protein is a member of the MRP subfamily which is involved in multi-drug resistance. This family member plays a role in cellular detoxification as a pump for its substrate, organic anions. It may also function in prostaglandin-mediated cAMP signaling in cilogenesis.</p>
<p>KIAA0274 FGA FGA phosphoinositide 5-phosphatase. The protein encoded by this gene belongs to the SAC domain-containing protein gene family. The SAC domain, approximately 400 amino acids in length and consisting of seven conserved motifs, has been shown to possess phosphoinositide phosphatase activity. Membrane-bound phosphoinositides function as signaling molecules and play a key role in vesicle trafficking in eukaryotic cells.</p>
<p>HSPC136 HKESH heat shock protein nuclear import factor hspc136. This gene encodes an evolutionarily conserved nuclear transport receptor that mediates heat-shock-induced nuclear import of 70 kDa heat-shock proteins (Hsp70s) through interactions with P23-nucleoporins. The protein mediates transport of the ATP form but not the ADP form of Hsp70 proteins under conditions of heat shock stress.</p>
<p>SEC10L1/EXOC5 exocyst complex component 5. This gene encodes a component of the exocyst complex, a multiple protein complex essential for targeting exocytic vesicles to specific docking sites on the plasma membrane. At least eight components of the exocyst complex, including this protein, are found to interact with the actin cytoskeletal remodeling and vesicle transport machinery. The complex is also essential for the biogenesis of epithelial cell surface polarity.</p>

Table 1A.2. MOST UP REGULATED GENES in a patient with a condition of classical Hodgkins lymphoma after chemotherapy.

<p>PARVA parvin alpha. This gene encodes a member of the parvin family of actin-binding proteins. Parvins are associated with focal contacts and contain calponin homology domains that bind to actin filaments. The encoded protein is part of the integrin-linked kinase signaling complex and plays a role in cell adhesion, motility and survival.</p>
<p>UQCRCF51 ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1. Predicted to enable oxidoreductase activity. Involved in mitochondrial respiratory chain complex III assembly and respiratory electron transport chain. Located in mitochondrion. Part of respiratory chain complex III. Implicated in mitochondrial complex III deficiency.</p>
<p>DLGAP4 DLG associated protein 4. The product of this gene is a membrane-associated guanylate kinase found at the postsynaptic density in neuronal cells. It is a signaling molecule that can interact with potassium channels and receptors, as well as other signaling molecules. The protein encoded by this gene can interact with PSD-95 through its guanylate kinase domain and may be involved in clustering PSD-95 in the postsynaptic density region.</p>
<p>ZNF539/ZNF254 zinc finger protein 254. Zinc finger proteins have been shown to interact with nucleic acids and to have diverse functions. The zinc finger domain is a conserved amino acid sequence motif containing 2 specifically positioned cysteines and 2 histidines that are involved in coordinating zinc. Kruppel-related proteins form 1 family of zinc finger proteins</p>
<p>CYBRD1 cytochrome b reductase 1. This gene is a member of the cytochrome b(561) family that encodes an iron-regulated protein. It highly expressed in the duodenal brush border membrane. It has ferric reductase activity and is believed to play a physiological role in dietary iron absorption.</p>
<p>VARS2 valyl-tRNA synthetase 2, mitochondrial. This gene encodes a mitochondrial aminoacyl-tRNA synthetase, which catalyzes the attachment of valine to tRNA(Val) for mitochondrial translation. Mutations in this gene cause combined oxidative phosphorylation deficiency-20, and are also associated with early-onset mitochondrial encephalopathies.</p>
<p>MIRPS18B mitochondrial ribosomal protein S18B. Mammalian mitochondrial ribosomal proteins are encoded by nuclear genes and help in protein synthesis within the mitochondrion. Mitochondrial ribosomes (mitoribosomes) consist of a small 28S subunit and a large 39S subunit. MRPL15 mitochondrial ribosomal protein L15.</p>
<p>TPPP tubulin polymerization promoting protein. Enables several functions, including magnesium ion binding activity; microtubule nucleator activity; and protein homodimerization activity. Involved in several processes, including microtubule cytoskeleton organization; negative regulation of tubulin deacetylation; and positive regulation of protein polymerization. Located in several cellular components, including cytoskeleton; mitochondrion; and perinuclear region of cytoplasm. Is active in Golgi apparatus.</p>
<p>Topk/PBK PDZ binding kinase. This gene encodes a serine/threonine protein kinase related to the dual specific mitogen-activated protein kinase family (MAPKK) family. Evidence suggests that mitotic phosphorylation is required for its catalytic activity. The encoded protein may be involved in the activation of lymphoid cells and support testicular functions, with a suggested role in the process of spermatogenesis. Overexpression of this gene has been implicated in tumorigenesis.</p>
<p>COMMD3 COMMD domain containing 3. Predicted to be involved in sodium ion transport. Predicted to be located in extracellular region and ficolin-1-rich granule lumen.</p>
<p>SEPTIN7 septin 7. This gene encodes a protein that is highly similar to the CDC10 protein of <i>Saccharomyces cerevisiae</i>. This human protein functions in gliomagenesis and in the suppression of glioma cell growth, and it is required for the association of centromere-associated protein E with the kinetochore.</p>
<p>MRPL15. Mammalian mitochondrial ribosomal proteins are encoded by nuclear genes and help in protein synthesis within the mitochondrion. This gene encodes a 39S subunit protein that belongs to the Ecol.15 ribosomal protein family.</p>
<p>FLJ12125. RTL10 retrotransposon Gag like 10. Involved in mitochondrial outer membrane permeabilization and regulation of mitochondrial membrane potential. Located in mitochondrion.</p>
<p>AGTBP1 ATP/GTP binding carboxypeptidase 1. NNA1 is a zinc carboxypeptidase that contains nuclear localization signals and an ATP/GTP-binding motif.</p>
<p>ZNF222 zinc finger protein 222. This gene product belongs to the zinc finger protein superfamily, members of which are regulatory proteins characterized by nucleic acid-binding zinc finger domains. Predicted to enable DNA-binding transcription factor activity... Predicted to be located in nucleus.</p>
<p>FREQ1/NCST1 neuronal calcium sensor 1. This gene is a member of the neuronal calcium sensor gene family, which encode calcium-binding proteins expressed predominantly in neurons. The protein encoded by this gene regulates G protein-coupled receptor phosphorylation in a calcium-dependent manner and can substitute for calmodulin. The protein is associated with secretory granules and modulates synaptic transmission and synaptic plasticity.</p>
<p>Znf197 Zinc finger protein 197. The encoded protein contains 20 tandemly arrayed C2H2-type zinc fingers, a Kruppel-associated box (KRAB) domain, and a SCAN Box. It is overexpressed in some thyroid papillary carcinomas.</p>
<p>BAT8/TEHMT2 euchromatic histone lysine methyltransferase 2. This gene encodes a methyltransferase that methylates lysine residues of histone H3. Methylation of H3 at lysine 9 by this protein results in recruitment of additional epigenetic regulators and repression of transcription.</p>
<p>CXorf17/FAM120C family with sequence similarity 120 member C. This gene encodes a potential transmembrane protein and lies in a region where mutations and deletions have been associated with intellectual disability and autism.</p>
<p>ENTPD7 ectonucleoside triphosphate diphosphohydrolase 7. This gene encodes a purine-converting ectoenzyme which belongs to the ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) family. The encoded protein hydrolyzes extracellular nucleoside triphosphates (UTP, GTP, and CTP) to nucleoside monophosphates as part of a purinergic signaling pathway. This gene affects oxidative stress as well as DNA damage and is a mediator of senescence</p>
<p>BFSP1, beaded filament structural protein 1. This gene encodes a lens-specific intermediate filament-like protein named filensin. The encoded protein is expressed in lens fiber cells after differentiation has begun. This protein functions as a component of the beaded filament which is a cytoskeletal structure found in lens fiber cells</p>
<p>DUSP2 dual specificity phosphatase 2. The encoded gene protein is a member of the dual specificity protein phosphatase subfamily. These phosphatases inactivate their target kinases by dephosphorylating both the phosphoserine/threonine and phosphotyrosine residues. They negatively regulate members of the mitogen-activated protein (MAP) kinase superfamily (MAPK/ERK, SAPK/JNK, p38), which are associated with cellular proliferation and differentiation. This gene product inactivates ERK1 and ERK2, is predominantly expressed in hematopoietic tissues, and is localized in the nucleus</p>
<p>MAP3K2, mitogen-activated protein kinase kinase 2. The protein encoded by this gene is a member of serine/threonine protein kinase family. This kinase preferentially activates other kinases involved in the MAP kinase signaling pathway. This kinase has been shown to directly phosphorylate and activate I kappa B kinases, and thus plays a role in NF-kappa B signaling pathway. This kinase has also been found to bind and activate protein kinase C-related kinase 2, which suggests its involvement in a regulated signaling process.</p>
<p>SNX22 sorting nexin 22. The protein encoded by this gene is a sorting nexin that is found in the cytoplasm, where it interacts with membrane-bound phosphatidylinositol 3-phosphate. The encoded protein may play a role in intracellular trafficking.</p>
<p>SCNN1A sodium channel epithelial 1 subunit alpha. Nonvoltage-gated, amiloride-sensitive, sodium channels control fluid and electrolyte transport across epithelia in many organs. This gene encodes the alpha subunit, and mutations in this gene have been associated with pseudohypoaldosteronism type 1 (PHA1), a rare salt wasting disease resulting from target organ unresponsiveness to mineral corticoids.</p>

Down and Up regulated genes from the patient with a condition of classical Hodgkin's Lymphoma (cHL) after chemotherapy and HAMLET oral treatment

The most down regulated genes regulated genes with a Z score values from -2.0 to -5.01, (Figure 2B) which includes among others, TRIM25 tripartite motif containing 25. The protein encoded by this gene is a member of the tripartite motif (TRIM) family. The protein is an RNA binding protein, functions as an ubiquitin E3 ligase and is involved in multiple cellular processes, including regulation of antiviral innate immunity. SIAT7F/ST6GALNAC5 ST6 N-acetylgalactosaminide alpha-2, 6-sialyltransferase 5. ST6GALNAC6 belongs to a family of sialyltransferases that modify proteins and ceramides on the cell surface to alter cell-cell or cell-extracellular matrix interactions. MAK3/NAA30 N-alpha-acetyltransferase 30, NatC catalytic subunit. Enables protein-N-terminal amino-acid acetyltransferase activity. Involved in protein stabilization. EREG epiregulin. The protein encoded by this gene forms a tetrameric cation channel that is permeable to calcium, sodium, and potassium and is regulated by free intracellular ADP-ribose. The encoded protein is activated by oxidative stress and confers susceptibility to cell death. HBD hemoglobin subunit delta. Involved in the megakaryocyte and platelet development. The delta (HBD) and beta (HBB) genes are normally expressed in the adult: two alpha chains plus two beta chains constitute HbA, which in normal adult life comprises about 97% of the total hemoglobin. Mutations in the delta-globin gene are associated with beta-thalassemia. PFN2 profilin 2. The protein encoded by this gene is a ubiquitous actin monomer-binding protein belonging to the profilin family. It is thought to regulate actin polymerization in response to extracellular signals. LMNB1 lamin B1. This gene encodes one of the two B-type lamin proteins and is a component of the nuclear lamina. FLJ1901/FASTKD1 FAST kinase domains 1. Enables RNA binding activity. Involved in mitochondrial RNA metabolic process and regulation of mitochondrial mRNA stability. HADHA hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha. This gene encodes the alpha subunit of the mitochondrial trifunctional protein, which catalyzes the last three steps of mitochondrial beta-oxidation of long chain fatty acids. CDSN corneodesmosin. This gene encodes a protein found in corneodesmosomes. The encoded protein undergoes a series of cleavages during corneocyte maturation. This gene is highly polymorphic in human populations, and variation has been

associated with skin diseases such as psoriasis, hypotrichosis and peeling skin syndrome. ADAM12 ADAM metalloproteinase domain 12. This gene encodes a member of a family of proteins that are structurally related to snake venom disintegrins and have been implicated in a variety of biological processes involving cell-cell and cell-matrix interactions, including fertilization, muscle development, and neurogenesis. MYB MYB proto-oncogene, transcription factor. This gene encodes a protein with three HTH DNA-binding domains that functions as a transcription regulator. This protein plays an essential role in the regulation of hematopoiesis. This gene may be aberrantly expressed or rearranged or undergo translocation in leukemia's and lymphomas, and is considered to be an oncogene. SLC25A4 solute carrier family 25 member 4. This gene is a member of the mitochondrial carrier subfamily of solute carrier protein genes (Figure 2B and table 1B.1).

Up regulated genes from a Z-score (2.63 to 4.20)

Among them, includes to COMMD3 COMM domain containing 3. Predicted to be involved in sodium ion transport. Predicted to be located in extracellular region and ficolin-1-rich granule lumen. SEZ6L seizure related 6 homolog like. Predicted to be involved in synapse maturation. Predicted to act upstream of or within activation of protein kinase C activity. NFRKB nuclear factor related to kappa-β binding protein. Enables protease binding activity. Involved in several processes, including chromatin remodeling; regulation of chromosome organization; and regulation of nucleobase-containing compound metabolic process. EDIL3 EGF like and discoidin domains 3. The protein encoded by this gene is an integrin ligand. It plays an important role in mediating angiogenesis and may be important in vessel wall remodeling and development. TBLIX transducin beta like 1 X-linked. The protein encoded by this gene has sequence similarity with members of the WD40 repeat-containing protein family. The WD40 group is a large family of proteins, which appear to have a regulatory function. It is believed that the WD40 repeats mediate protein-protein interactions and members of the family are involved in signal transduction, RNA processing, gene regulation, vesicular trafficking, cytoskeletal assembly and may play a role in the control of cytotypic differentiation. IRS1 insulin receptor substrate 1. This gene encodes a protein which is phosphorylated by insulin receptor tyrosine kinase. Mutations in this gene are associated

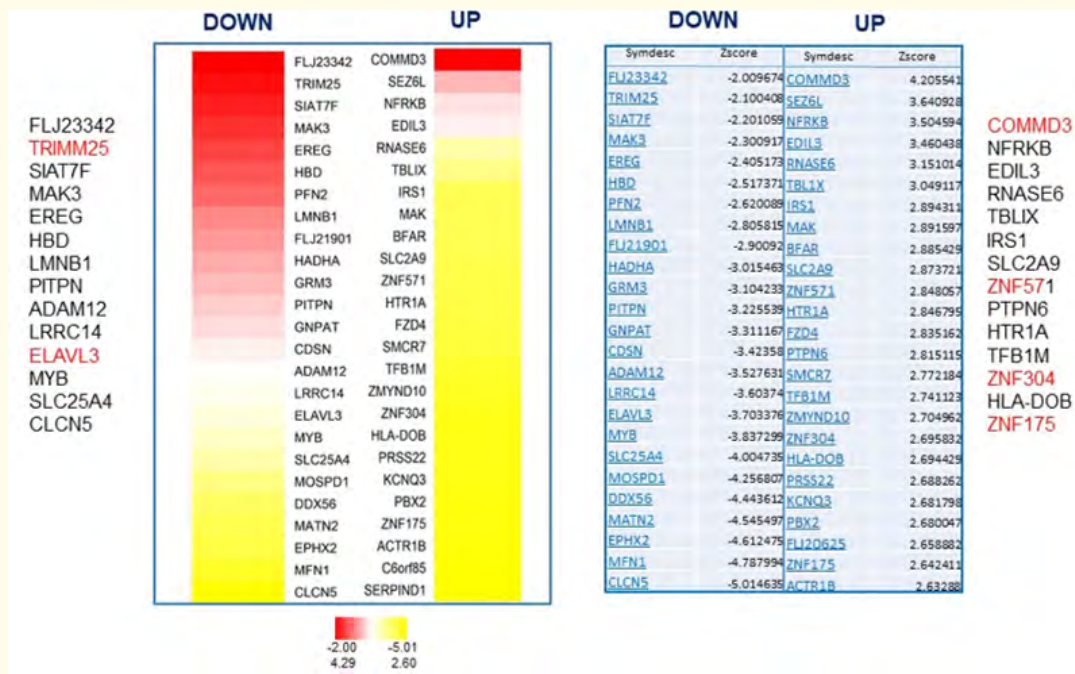


Figure 2B: Gene expression in a patient with a condition of classical Hodgkin's lymphoma after chemotherapy and HAMLET oral treatment. Heat map (left panel) from the DOWN-regulated genes with a Z score from -2.0 to -5.01 that includes, among other genes, TRIM25, tripartite motif containing 25. The protein encoded by this gene is a member of the tripartite motif (TRIM) family, it is an RNA-binding protein, functions as a ubiquitin E3 ligase, and is involved in multiple cellular processes, including regulation of antiviral innate immunity. ELAVL3 ELAV-like RNA-binding protein 3. ELAV-like 3 is a neural-specific RNA-binding protein that contains three RNP-type RNA recognition motifs. It is involved in neurogenesis. EREG epiregulin. The protein encoded by this gene forms a tetrameric cation channel permeable to calcium, sodium, and potassium. The encoded protein is activated by oxidative stress and confers susceptibility to cell death. HBD hemoglobin subunit delta. Involved in the megakaryocyte and platelet development. UP-regulated genes from a Z-score (2.63 to 4.20). COMMD3, COMM domain containing 3. Predicted to be involved in sodium ion transport. Predicted to be located in the extracellular region and the ficolin-1-rich granule lumen. HTR1A 5-hydroxytryptamine receptor 1A. This gene encodes a G protein-coupled receptor for 5-hydroxytryptamine (serotonin). Serotonin has been implicated in a number of physiologic processes and pathologic conditions. HLA-DOB major histocompatibility complex, class II, DO beta. Class II molecules are expressed in antigen-presenting cells (APC: B lymphocytes, dendritic cells, macrophages).

Table 1B.1. MOST DOWN REGULATED GENES in a patient with a condition of classical Hodgkins lymphoma after chemotherapy and HAMLET oral treatment

FLJ23842	MSANTD2	Myo/SANT DNA-binding domain containing 2 . Ubiquitous expression in thyroid (RPKM 1.6), endometrium (RPKM 1.2) and 25 other tissues
TRIM25		tripartite motif containing 25. The protein encoded by this gene is a member of the tripartite motif (TRIM) family. The TRIM motif includes three zinc-binding domains, a RING, a B-box type 1 and a B-box type 2, and a coiled-coil region. The protein is an RNA-binding protein. Functions as a ubiquitin E3 ligase, and is involved in multiple cellular processes, including regulation of antiviral innate immunity.
SIAT7F	ST6GALNAC5	ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 5 . ST6GALNAC5 belongs to a family of sialyltransferases that modify proteins and ceramides on the cell surface to alter cell-cell or cell-extracellular matrix interactions.
MAK3	NAA30N	alpha-acetyltransferase 30, NatC catalytic subunit . Enables protein-N-terminal amino-acid acetyltransferase activity. Involved in protein stabilization. Located in cytosol and nucleus.
EREG		epiregulin. The protein encoded by this gene forms a tetrameric cation channel that is permeable to calcium, sodium, and potassium and is regulated by free intracellular ADP-ribose. The encoded protein is activated by oxidative stress and confers susceptibility to cell death.
HBD		hemoglobin subunit delta. The delta (HBD) and beta (HBB) genes are normally expressed in the adult; two alpha chains plus two beta chains constitute HbA, which in normal adult life comprises about 97% of the total hemoglobin. Mutations in the delta-globin gene are associated with beta-thalassemia. Involved in the megaryocyte and platelet development
PFM2		profilin 2 . The protein encoded by this gene is a ubiquitous actin monomer-binding protein belonging to the profilin family. It is thought to regulate actin polymerization in response to extracellular signals.
LMNB1		lamin B1 . This gene encodes one of the two B-type lamin proteins and is a component of the nuclear lamina. A duplication of this gene is associated with autosomal dominant adult-onset leukodystrophy (ADLD). Alternative splicing results in multiple transcript variants.
FLJ1901	FASTKD1	FAST kinase domains 1 . Enables RNA binding activity. Involved in mitochondrial RNA metabolic process and regulation of mitochondrial mRNA stability. Located in mitochondrion and nucleoplasm.
HADHA		hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha . This gene encodes the alpha subunit of the mitochondrial trifunctional protein, which catalyzes the last three steps of mitochondrial beta-oxidation of long chain fatty acids. The mitochondrial membrane-bound heterocomplex is composed of four alpha and four beta subunits, with the alpha subunit catalyzing the 3-hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase activities. Mutations in this gene result in trifunctional protein deficiency or LCHAD deficiency.
GRM3		glutamate metabotropic receptor 3 . L-glutamate is the major excitatory neurotransmitter in the central nervous system and activates both ionotropic and metabotropic glutamate receptors. Glutamatergic neurotransmission is involved in most aspects of normal brain function and can be perturbed in many neuropathologic conditions.
PIP5PA		phosphatidylinositol transfer protein alpha . This gene encodes a member of a family of lipid-binding proteins that transfer molecules of phosphatidylinositol or phosphatidylcholine between membrane surfaces. The protein is implicated in phospholipase C signaling and in the production of phosphatidylinositol 3,4,5-trisphosphate (PIP3) by phosphoinositide-3-kinase
GNPAT		glycerophosphatase o-acyl transferase . This gene encodes an enzyme located in the peroxisomal membrane which is essential to the synthesis of ether phospholipids. Mutations in this gene are associated with rhizomelic chondrodysplasia punctata.
CDSN		corneodesmosin . This gene encodes a protein found in corneo desmosomes, which localize to human epidermis and other cornified squamous epithelia. The encoded protein undergoes a series of cleavages during corneocyte maturation. This gene is highly polymorphic in human populations, and variation has been associated with skin diseases such as psoriasis, hypotrichosis and peeling skin syndrome
ADAM12		ADAM metalloproteinase domain 12 . This gene encodes a member of a family of proteins that are structurally related to snake venom disintegrins and have been implicated in a variety of biological processes involving cell-cell and cell-matrix interactions, including fertilization, muscle development, and neurogenesis.
LRRC14		leucine rich repeat containing 14 . This gene encodes a leucine-rich repeat-containing protein. Alternate splicing results in multiple transcript variants.
ELAVL3		ELAV like RNA binding protein 3 . A member of the ELAVL protein family, ELAV-like 3 is a neural-specific RNA-binding protein which contains three RNP-type RNA recognition motifs. The observation that ELAVL3 is one of several Hu antigens (neural-specific RNA-binding proteins) recognized by the anti-Hu serum antibody present in sera from patients with paraneoplastic encephalomyelitis and sensory neuropathy (PEM/PSN) suggests it has a role in neurogenesis.
MYB		MYB proto-oncogene, transcription factor . This gene encodes a protein with three HTH DNA-binding domains that functions as a transcription regulator. This protein plays an essential role in the regulation of hematopoiesis. This gene may be aberrantly expressed or rearranged or undergo translocation in leukemias and lymphomas, and is considered to be an oncogene
SLC25A4		solute carrier family 25 member 4 . This gene is a member of the mitochondrial carrier subfamily of solute carrier proteins. The product of this gene functions as a gated pore that translocates ADP from the cytoplasm into the mitochondrial matrix and ATP from the mitochondrial matrix into the cytoplasm. The protein forms a homodimer embedded in the inner mitochondrial membrane. Mutations in this gene have been shown to result in autosomal dominant progressive external ophthalmoplegia and familial hypertrophic cardiomyopathy
MOSPD1		male sperm domain containing 1 . Predicted to be involved in negative regulation of transcription by RNA polymerase II and positive regulation of transcription by RNA polymerase II. Predicted to be located in nucleus and perinuclear region of cytoplasm. Predicted to be active in cytoplasm.
DOK65		DEAD-box helicase 56 . This gene encodes a member of the DEAD box protein family. DEAD box proteins, characterized by the conserved motif Asp-Glu-Ala-Asp (DEAD), are putative RNA helicases. They are implicated in a number of cellular processes involving alteration of RNA secondary structure such as translation initiation, nuclear and mitochondrial splicing, and ribosome and spliceosome assembly. The protein encoded by this gene shows ATPase activity in the presence of polynucleotides and associates with nucleoplasmic 65S preribosomal particles. This gene may be involved in ribosome synthesis, most likely during assembly of the large 60S ribosomal subunit
MATN2		matrin 2 . This gene encodes a member of the von Willebrand factor A domain containing protein family. This family of proteins is thought to be involved in the formation of filamentous networks in the extracellular matrices of various tissues. This protein contains five von Willebrand factor A domains. The specific function of this gene has not yet been determined.
EPH4C		epoxide hydrolase 2 . This gene encodes a member of the epoxide hydrolase family. The protein, found in both the cytosol and peroxisomes, binds to specific epoxides and converts them to the corresponding dihydrodiols. Mutations in this gene have been associated with familial hypercholesterolemia.
MFN1		mitofusin 1 . The protein encoded by this gene is a mediator of mitochondrial fusion. This protein and mitofusin 2 are homologs of the Drosophila protein fuzzy onion (Fzo). They are mitochondrial membrane proteins that interact with each other to facilitate mitochondrial targeting
CLCN5		chloride voltage-gated channel 5 . This gene encodes a member of the ClC family of chloride ion channels and ion transporters. The encoded protein is primarily localized to endosomal membranes and may function to facilitate albumin uptake by the renal proximal tubule. Mutations in this gene have been found in Dent disease and renal tubular disorders complicated by nephrolithiasis.

Table 1B.2. MOST UP REGULATED GENES in a patient with a condition of classical Hodgkins lymphoma after chemotherapy and HAMLET oral treatment

<p>COMMD3 domain containing 3. Predicted to be involved in sodium ion transport. Predicted to be located in extracellular region and ficolin-1-rich granule lumen.</p> <p>SEZ6L seizure related 6 homolog like. Predicted to be involved in synapse maturation. Predicted to act upstream of or within activation of protein kinase C activity; adult locomotory behavior; and cerebellar Purkinje cell layer development. Predicted to be located in endoplasmic reticulum membrane. Predicted to be active in several cellular components, including glutamatergic synapse; neuronal cell body; and postsynaptic membrane.</p> <p>NFRKB nuclear factor related to kappaB binding protein. Enables protease binding activity. Involved in several processes, including chromatin remodeling; regulation of chromosome organization; and regulation of nucleobase-containing compound metabolic process. Located in Ino80 complex and nucleolus.</p> <p>EDIL3 EGF like and discoidin domains 3. The protein encoded by this gene is an integrin ligand. It plays an important role in mediating angiogenesis and may be important in vessel wall remodeling and development. It also influences endothelial cell behavior.</p> <p>RNA5E6 ribonuclease A family member 6. The protein encoded by this gene is a member of the ribonuclease A superfamily and functions in the urinary tract. The protein has broad-spectrum antimicrobial activity against pathogenic bacteria.</p> <p>TBUX1 tubulin beta like 1 X-linked. The protein encoded by this gene has sequence similarity with members of the WD40 repeat-containing protein family, which appear to have a regulatory function. It is believed that the WD40 repeats mediate protein-protein interactions and members of the family are involved in signal transduction, RNA processing, gene regulation, vesicular trafficking, cytoskeletal assembly and may play a role in the control of cytoplasmic differentiation. This encoded protein is found as a subunit in corepressor SMRT (silencing mediator for retinoid and thyroid receptors) complex along with histone deacetylase 3 protein. This gene is located adjacent to the ocular albinism gene and it is thought to be involved in the pathogenesis of the ocular albinism with late-onset sensorineural deafness phenotype.</p> <p>IRS1 insulin receptor substrate 1. This gene encodes a protein which is phosphorylated by insulin receptor tyrosine kinase. Mutations in this gene are associated with type II diabetes and susceptibility to insulin resistance.</p> <p>MAK male germ cell associated kinase. The product of this gene is a serine/threonine protein kinase related to kinases involved in cell cycle regulation. Mutations in this gene have been associated with ciliary defects resulting in retinitis pigmentosa 62.</p> <p>BFA1 bifunctional apoptosis regulator. Enables caspase binding activity; protein-macromolecule adaptor activity; and ubiquitin mediated unfolded protein response; proteasome-mediated ubiquitin-dependent protein catabolic process; and protein ubiquitination. Acts upstream of or within negative regulation of apoptotic process.</p> <p>SLC2A9 solute carrier family 2 member 9. This gene encodes a member of the SLC2A facilitative glucose transporter family. Members of this family play a significant role in maintaining glucose homeostasis. The encoded protein may play a role in the development and survival of chondrocytes in cartilage matrices.</p> <p>ZNF571 zinc finger protein 571. Predicted to enable DNA-binding transcription factor activity. RNA polymerase II cis-regulatory region sequence-specific DNA binding activity. Predicted to be involved in regulation of transcription by RNA polymerase II. Located in nucleus.</p> <p>HTR1A 5-hydroxytryptamine receptor 1. This gene encodes a G protein-coupled receptor for 5-hydroxytryptamine (serotonin), and belongs to the 5-hydroxytryptamine receptor subfamily. Serotonin has been implicated in a number of physiologic processes and pathologic conditions. Inactivation of this gene in mice results in behavior consistent with an increased anxiety and stress response. Mutation in the promoter of this gene has been associated with menstrual cycle-dependent periodic fevers.</p> <p>FZD4 frizzled class receptor 4. This gene is a member of the frizzled gene family. Members of this family encode seven-transmembrane domain proteins that are receptors for the Wingless type MMTV integration site family of signaling proteins. Most frizzled receptors are coupled to the beta-catenin canonical signaling pathway. This protein may play a role as a positive regulator of the Wingless-type MMTV integration site signaling pathway.</p> <p>PTPN6 protein tyrosine phosphatase non-receptor type 6. The protein encoded by this gene is a member of the protein tyrosine phosphatase (PTP) family. PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. This PTP is expressed primarily in hematopoietic cells, and functions as an important regulator of multiple signaling pathways in hematopoietic cells. This PTP has been shown to interact with, and dephosphorylate a wide spectrum of phospho-proteins involved in hematopoietic cell signaling.</p> <p>SMCR7/MIIEF2 mitochondrial elongation factor 2. This gene encodes an outer mitochondrial membrane protein that functions in the regulation of mitochondrial morphology. It can directly recruit the fission mediator dynamin-related protein 1 (Drp1) to the mitochondrial surface. The gene is located within the Smith-Magenis syndrome region on chromosome 17.</p> <p>TFB1M transcription factor B1, mitochondrial. The protein encoded by this gene is a dimethyltransferase that methylates the conserved stem loop of mitochondrial 12S rRNA. The encoded protein also is part of the basal mitochondrial transcription complex and is necessary for mitochondrial gene expression. The methylation and transcriptional activities of this protein are independent of one another.</p> <p>ZMYND10 Zinc Finger MYND-type containing 10. This gene encodes a protein containing a MYND-type zinc finger domain that likely functions in assembly of the dynein motor. Mutations in this gene can cause primary ciliary dyskinesia. This gene is also considered a tumor suppressor gene and is often mutated, deleted, or hypermethylated and silenced in cancer cells.</p> <p>ZNF304 zinc finger protein 304. This gene encodes a member of the Krueppel C2H2-type zinc-finger family of proteins. The encoded protein functions as a transcriptional repressor that recruits a corepressor complex to stimulate promoter hypermethylation and transcriptional silencing of target genes. Expression of this gene is upregulated in colorectal, ovarian and breast cancer, and this gene may promote cancer cell survival, growth and invasion.</p> <p>HLA-DQB major histocompatibility complex, class II, DO beta. HLA-DQB belongs to the HLA class II beta chain paralogs. This class II molecule is a heterodimer consisting of an alpha (DOA) and a beta chain (DOB), both anchored in the membrane. It is located in intracellular vesicles. DO suppresses peptide loading of MHC class II molecules by inhibiting HLA-DM. Class II molecules are expressed in antigen presenting cells (APC: B lymphocytes, dendritic cells, macrophages).</p> <p>PRSS22 serine protease 22. This gene encodes a member of the trypsin family of serine proteases. The enzyme is expressed in the airways in a developmentally regulated manner. The gene is part of a cluster of serine protease genes on chromosome 16.</p> <p>KCNQB potassium voltage-gated channel subfamily Q member 3. This gene encodes a protein that functions in the regulation of neuronal excitability. The encoded protein forms an M-channel by associating with the products of the related KCNQ2 or KCNQ5 genes, which both encode integral membrane proteins. M-channel currents are inhibited by M1 muscarinic acetylcholine receptors and are activated by retigabine, a novel anti-convulsant drug.</p> <p>PBX2 PBX homeobox 2. This gene encodes a ubiquitously expressed member of the TALE/PBX homeobox family. It was identified by its similarity to a homeobox gene which is involved in t(1;19) translocation in acute pre-B-cell leukemias. This protein is a transcriptional activator which binds to the TLX1 promoter. The gene is located within the major histocompatibility complex (MHC) on chromosome 6.</p> <p>LAMTOR1 late endosomal/lysosomal adaptor, MAPK AND MTOR activator 1. Enables GTPase binding activity and protein-membrane adaptor activity. Contributes to guanyl-nucleotide exchange factor activity and molecular adaptor activity. Involved in several processes, including cholesterol homeostasis; positive regulation of TORC1 signaling; and regulation of cholesterol transport. Located in lysosome. Part of FNP-folliculin RagC/D GAP. Is active in Ragulator complex and lysosomal membrane.</p> <p>ZNF175 zinc finger protein 175. Enables DNA-binding transcription activator activity. RNA polymerase II-specific; DNA-binding transcription repressor activity. Involved in negative regulation of transcription by RNA polymerase II and positive regulation of transcription by RNA polymerase II. Located in cytosol and nucleus.</p> <p>ACTR1B actin related protein 18. This gene encodes a 42.3 kD subunit of dynactin, a macromolecular complex which binds to both microtubules and cytoplasmic dynein and is involved in a diverse array of cellular functions, including ER-to-Golgi transport, the centripetal movement of lysosomes and endosomes, spindle formation, chromosome movement, nuclear positioning, and axonogenesis. This subunit, like ACTR1A, is an actin-related protein.</p>
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with type II diabetes and susceptibility to insulin resistance. BFA1 is a bifunctional apoptosis regulator. Enables caspase binding activity; protein-macromolecule adaptor activity; and ubiquitin protein ligase activity. Involved in negative regulation of IRE1-mediated unfolded protein response; proteasome-mediated ubiquitin-dependent protein catabolic process; and protein ubiquitination. Acts upstream of or within negative regulation of apoptotic process. HTR1A 5-hydroxytryptamine receptor 1A. This gene encodes a G protein-coupled receptor for 5-hydroxytryptamine (serotonin), and belongs to the 5-hydroxytryptamine receptor subfamily. Serotonin has been implicated in a number of physiologic processes and pathologic conditions. HLA-DOB major histocompatibility complex, class II, DO beta. HLA-DOB belongs to the HLA class II beta chain paralogues. This class II molecule is a heterodimer consisting of an alpha (DOA) and a beta chain (DOB), both anchored in the membrane. It is located in intracellular vesicles. DO suppresses peptide loading of MHC class II molecules by inhibiting HLA-DM. Class II molecules are expressed in antigen presenting cells (APC: B lymphocytes, dendritic cells, macrophages) (Figure 2B and table 1B.2).

Analysis of the functionality of the genes from a patient with a condition of classical Hodgkin's lymphoma (cHL) using REACTOME analysis

Reactome's annotated data describe reactions possible if all annotated proteins and small molecules were present and active simultaneously in a cell. By overlaying an experimental dataset (Up and down regulated genes in a patient with a condition of cHL with chemotherapy and Hamlet oral treatment) on these annotations, a user can perform a pathway over-representation (enrichment) analysis. By overlaying quantitative expression data (Microarrays), a user can visualize the extent of change in affected pathways, and the reactions (binding, activation, translocation, degradation and classical biochemical events and its progression (i.e., HAMLET oral treatment). This is calculated based on the probability score (p-value) which is corrected for false discovery rate using for the multiple testing (Benjamini-Hochberg procedure) [36,37] that arises from evaluating the submitted list of identifiers (Up or down regulated genes) against every pathway. The lower p values means a higher probability that these genes hit or influence one of the pathways or reactions. Moreover, it determines whether certain Reactome pathways are over-represented (enriched) in the submitted data (Up or down regulated genes) (Figure 2A and 2B).

Functionality of the Up and down set of genes determined using microarrays from the patient with the condition of cHL and chemotherapy treatment

Based on this, it was found that 25 most DOWN regulated genes from a patient with a condition of (cHL) (Figure 3A), showed that 292 pathways are enriched from 18 submitted genes of 25, and hit by at least by one of these genes. Among the pathways enriched

by these aforementioned genes, from lower (higher probability to influence or affect the pathway or reaction) to higher p-values (lower probability to influence or affect the pathway or reaction) are: Cell junction organization (2.4e-04), Cell-Cell communication (4.2e-04), Interleukin-35 signaling (4.48e-04), Adherens junctions interactions (0.001), Cell-cell junction organization (0.002) >, Elastic fibre formation (0.003) >, Defective SLC22A18 causes lung cancer (LNCR) and embryonal rhabdomyosarcoma 1 (RMSE1) (0.004) >, TGF-beta receptor signaling activates SMADs Regulation of Expression (0.004) >, and Function of Type I Classical Cadherins (0.006) >. Of note is that in the Reactome pathways/reactions analyzed seven identifiers (ELAVL3, ELK3, FLJ21069, FLJ22405, GZMA, JM4, and LOC56181 genes) were not found neither mapper to any entity in Reactome curate data base. Moreover, the Genome-wide overview of this Reactome pathway analysis, arranged in a hierarchy order, Cell-Cell communication >, Immune system >, Disease, and Signal transduction >, Transport of Small Molecules >, Extracellular matrix organization >, Organelle biogenesis and maintenance (Figure 3A).

In marked contrast, the analysis of 25 most UP regulated genes from a patient with a condition with cHL showed that 68 pathways are enriched from 17 submitted genes of 25, and hit by at least one of the 17. Similarly as pinpointed above, from lower to higher p-values, Mitochondrial translation initiation (9.85e-04) >, Mitochondrial translation elongation (9.85e-04) >, Mitochondrial ribosome-associated quality control (0.001) >, Translation (0.001) >, Mitochondrial translation termination (0.002) >, Mitochondrial translation (0.002) >, tRNA Aminoacylation (0.004) >. Seven identifiers (FDDX56, ELAVL3, FLJ21901, FLJ23392, and MATN2), ELK3, FLJ21069, FLJ22405, GZMA, JM4, and LOC56181 genes) were not found neither mapper to any entity in Reactome curate data base (Figure 3A). Genome-wide overview of this Reactome pathway analysis, arranged in a hierarchy order, Metabolism of proteins >, Cell-Cell communication >, Sensory perception >, Metabolism >, Gene Transcription >, Signal transduction >, Chromatin organization (Figure 3A).

Functionality of the DOWN and UP regulated genes from a patient with a condition of cHL after chemotherapy and HAMLET oral treatment

25 most DOWN regulated genes from a patient with a condition of cHL, showed that 232 pathways are enriched from 20 submitted genes of 25, and hit by at least by one of these genes (n= 20 genes). Among the pathways enriched by these aforementioned genes, from lower (higher probability to influence or affect the pathway or reaction) to higher p-values (<higher probability to influence or affect the pathway or reaction), Gene and protein expression by JAKSTAT signaling after Interleukin-12 stimulation (1.19e-05) >, Interleukin-12 signaling (2.06e-05) >, Interleukin-12 family signaling (3.46e-05) >, Factors involved in megakaryocyte

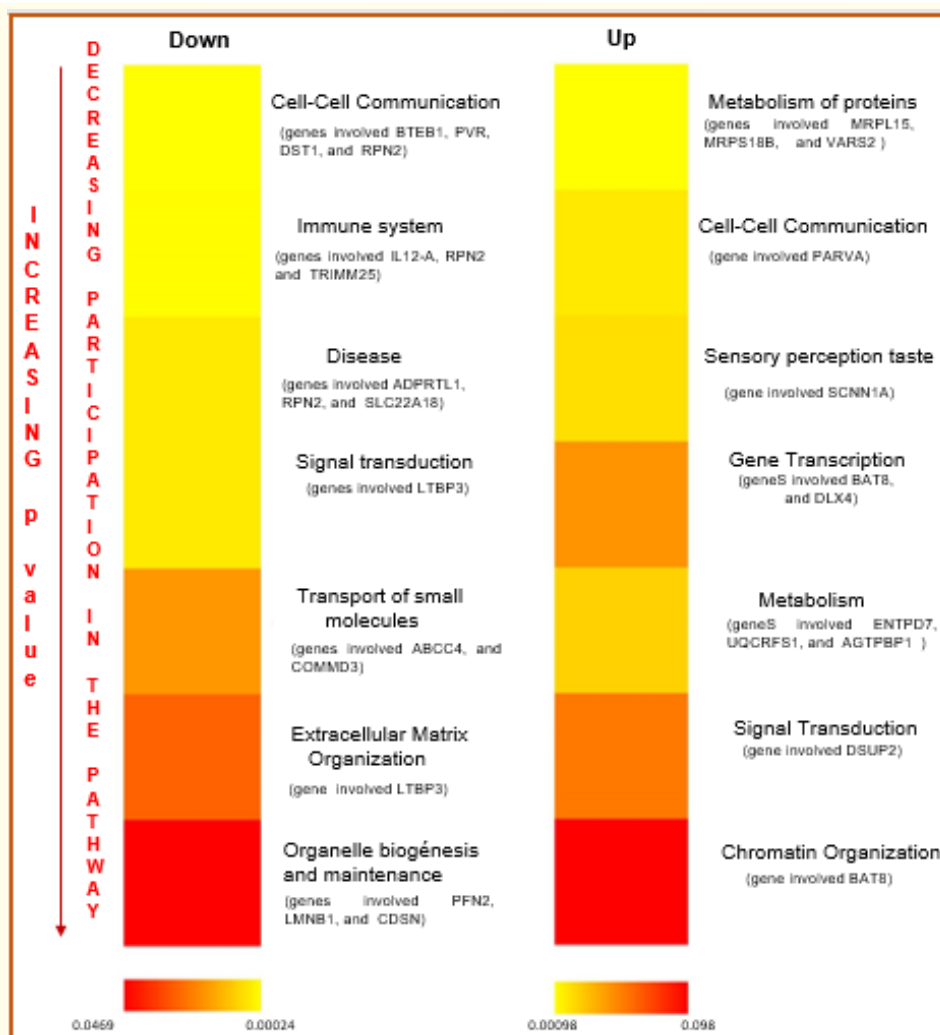


Figure 3A: Functionality of the Down and Up-regulated genes in pathways (Immune system, cell-cell communication, gene transcription) and/o reactions (binding, activation, phosphorylation), sorted by p-values (Reactome curated database), and expressed as heat maps from a patient with a condition of classical Hodgkin's lymphoma (cHL). After chemotherapy, down regulated genes involved from higher hierarchy (lower p-values) in Cell-Cell communication (BTEB1, PVR, DST1 and RPN2 genes) > Immune system (IL-12A, RPN2, TRIMM25 genes) > Disease (ADPRTL1, RPN2, and SLC22A18 genes) > signal transduction (LTBP3 gene). Up regulated genes participate in Metabolism of proteins (MRPL15, MRPS18B, and VARS2 genes) >, Cell-Cell communication (PARVA gene) >, Sensory perception (SCNN1A gene) >, Gene transcription (BAT8, and DLX4 genes) >, Metabolism (ENTPD7, UQCRCF1, and AGTPBP1) > Signal transduction (DUSP2).

development and platelet production ($5.06e-04$) >, Protein localization (0.004) >, Cytokine Signaling in Immune system (0.004)>. Five identifiers (DDX56, ELAVL3, FLJ21901, FLJ23342, and MATN2 genes) instead of seven were not found neither mapper to any entity in Reactome curate data base. While the genome-wide overview of this Reactome pathway analysis of the patient with a condition of cHL, the number of pathways were higher and different (7 versus 12), arranged in a hierarchy order, Immune system >, Hemostasis >, Protein Localization >, Signal

transduction >, Disease >, Extracellular matrix Organization >, Gene Transcription > Metabolism (Figure 3B).

The analysis of 25 most UP regulated genes from a patient with a condition with cHL), showed that 186 pathways are enriched from the 18 submitted genes of 25, and hit by at least one of the 18. Similarly as pinpointed above, from lower to higher p-values, Transcriptional activation of mitochondrial biogenesis ($6.08e-04$)>, Regulation of MITF-M-dependent genes involved in

extracellular matrix, focal adhesion and epithelial-to-mesenchymal transition (8.60e-04) >, Growth hormone receptor signaling (0.001) >, Signaling by ALK fusions and activated point mutants (0.001) >, Signaling by ALK in cancer (0.001) >, Mitochondrial biogenesis (0.002) >, Interleukin-37 signaling (0.002) > (Figure 2). Seven identifiers (BFAR FLJ20625 MAK PRSS22 SEZ6L SMCR7 ZMYND10) were not found neither mapper to any entity in

Reactome curate data base (Figure 2). Interestingly, genome-wide overview of this Reactome pathway analysis, showed a differential number and types of pathways versus down regulated genes (12 versus 8), arranged in a hierarchy order, Organelle biogenesis and maintenance >, Developmental Biology >, Disease and Immune system >, Signal transduction >, Hemostasis >, Cell-Cell communication >, Metabolism of RNA > (Figure 3B).

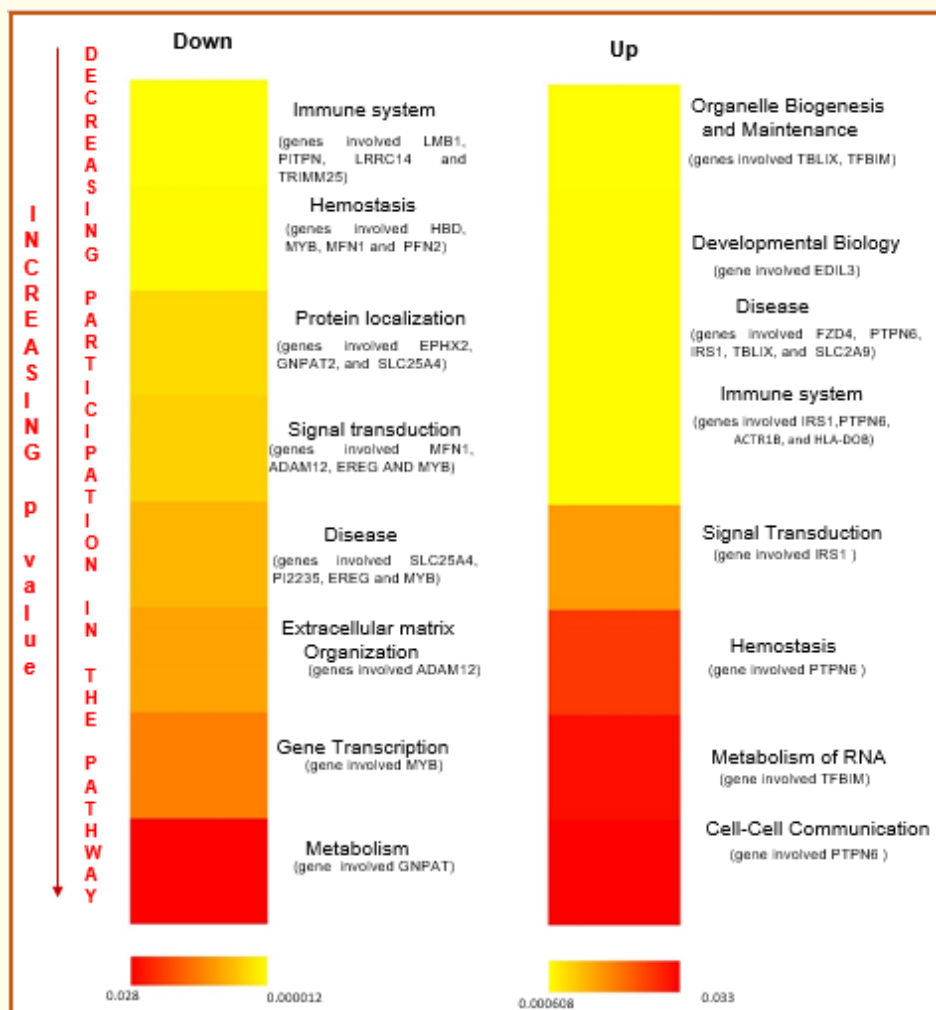


Figure 3B: Functionality of the Down and Up-regulated genes in pathways (Immune system, cell-cell communication, gene transcription) and/o reactions (binding, activation, phosphorylation), sorted by p-values (Reactome curated database), and expressed as heat maps from a patient with a condition of classical Hodgkin's lymphoma (cHL). After chemotherapy and HAMLET oral treatment (3B). Down regulated genes involved from higher hierarchy, lower p-values in immune system (LMB1, PITPN, LRRC14 and TRIMM25), > hemostasis (HBD, MYB, MFN1 and PFN2), > protein localization (EPHX2, GNPAT2, and SLC25A4), > Signal transduction (MFN1, ADAM12, EREG AND MYB) >, Disease (SLC25A4, PI2235, EREG and MYB) >, Extracellular matrix organization (ADAM12) >. Up regulated genes participate in from higher hierarchy (lower p-values) in organelle biogenesis and maintenance (involved TBLIX, TFBIM) >, Developmental Biology (EDIL3) >, Disease (FZD4, PTPN6, IRS1, TBLIX, and SLC2A9) >, Immune system (IRS1, PTPN6, ACTR1B, and HLA-DOB)>, Signal Transduction (IRS1) >, hemostasis (PTPN6) >.

Discussion and Conclusion

The objective of the present case report study was to evaluate HAMLET oral treatment as a palliative measure to reduce the side effects of the chemotherapy treatment, as well as to identify genetic changes that could be attributed to the consumption of the compound [36,37] in an individual with a condition of classic.

Gene expression pattern represented as heat maps expressing as values of Z-scores ± 2 SD of the Up and Down regulated genes from a patient with a condition of cHL and after chemotherapy (Figure 2A), or after chemotherapy and hamlet orally (Figure 2B). The comparison of the gene expression pattern after chemotherapy and after chemotherapy and hamlet orally, showed the up and down regulation of TRIMM 25, ELAVL3, COMMD3 and ZNF (539, 222, 197) proteins, that function in different pathways and reactions of the human biology (i.e., immune system, ion transport and gene transcription).

Lymphoma is characterized by an abnormal, and increased proliferation of white blood cells (lymphocytes) and the distinctive large Reed-Sternberg cells surrounded by bands of fibrotic collagen. Hodgkin lymphomas are classified into two main subtypes: classical Hodgkin lymphoma and nodular lymphocyte-predominant Hodgkin lymphoma. Classical Hodgkin lymphoma (cHL) accounts for 95% of all Hodgkin lymphomas [1-5,22,23]. According to several studies the cause of Hodgkin lymphoma is unknown. However, it has been proposed that past infections, such as, with the Epstein-Barr virus (EBV) is thought to contribute to some cases. It has been shown for example, that people with HIV infection are at higher risk than the general population. The treatment of the cancer is based on chemotherapy, radio immunotherapy. Hodgkin's lymphoma mechanism are unknown, However, it has been proposed that some virus could cause this, since virus enhanced significantly the proliferative rate of the line cell cultures (Epstein Bar) [1-5,22,23]. Hodgkin lymphoma is one of the most curable types of cancer. A cure is even more likely if it is diagnosed and treated in its early stages. Unlike many other types of cancer, Hodgkin lymphoma is also highly curable in its later stages. Symptoms may include any of the following: Feeling tired all the time. Intermittent fever and chills. Unexplained itching all over the body. Loss of appetite. Heavy night sweats. Painless swelling of the lymph nodes in the neck, armpits, or groin (swollen lymph nodes). Unexplained weight loss. After the diagnostic (presentation of the case report). The patient was under

chemotherapy with several secondary effects and the size of the tumor was prominent (data not shown). Treatment depends on the following: the type of Hodgkin lymphoma, the stage (how far the disease has spread), age and other medical conditions. Other factors, including whether you have weight loss, night sweats, and fever. They may have chemotherapy, radiation therapy, chemo immunotherapy, chemo radiation therapy, or a combination of all of these therapies. In the present study, written informed consent was obtained from the patient and following the Declaration of Helsinki, and approved by the Ethic Committee in Research of the Zacatecas, General Hospital "Luz Gonzalez Cosío" CONBIOETICA-32-CEI-001-20180807, a study was conducted with the patient, who was given Hamlet oral treatment as a palliative measure and to reduce the side effects of the chemotherapy treatment, as well as to identify genetic changes that could be attributed to the consumption of the compound (Figure 1A). Briefly, an increase in the number of Up regulated genes (n = 140 versus 87). Down regulated genes was reduced (n = 300 versus 319). Of note is that after HAMLET orally, TRIMM25 (NF- κ B activation through FADD/RIP-1 pathways mediated by caspase 8 and 10); was more Down regulated than before HAMLET orally (Z-score of -2.1 versus -3.5), ELAVL3 ELAV like RNA binding protein was less downregulated (Z-score of -2.507 versus -3.7). COMMD3 (participates in sodium iron transport) higher up regulation than before HAMLET orally (Z-score 2.83 versus 4.205); while ZNF575 (Zinc finger protein, DNA binding transcription factor) less Up regulated than before HAMLET orally (Z-score of 3.45 versus 2.84) (Figure 2A and 2B). The identity of each of the 25 most up or down regulated genes are showed in tables 1A.1. 1A.2, 1B.1 and 1B.2.

Furthermore, these Down or Up regulated genes functionality was analyzed using REACTOME curated database, sorted by p-values (lower p-values higher probability of participate in any of the pathways) and represented as heat maps (Figure 3A and 3B). The comparison of the functionality of these aforementioned genes, after chemotherapy or after chemotherapy and HAMLET oral treatment, suggest that after only chemotherapy, the pathways in which down and up regulated genes from figure 2A, are cell-cell communication and signal transduction (Figure 3A), while after chemotherapy and hamlet orally, the down and up regulated genes of figure 2B, are immune system, hemostasis, disease and signal transduction (Figure 3B). Moreover, the comparison between down regulated genes with chemotherapy and chemotherapy and

hamlet orally treatment the probability that the genes participate is after chemotherapy and hamlet orally in the immune system than in disease and extracellular matrix organization. In marked contrast, Up regulated genes probability to participate in a different pathways such as Organelle biogenesis and maintenance than in Cell-Cell communication (Figure 3B). There is no data regarding a same condition of the patient with cHL and hamlet orally, to compare and confirm the aforementioned pattern of genes that are being down or up regulated genes and can be used as genetic markers of the effects of HAMLET orally. This is the subject of further investigation of the mechanism of action of HAMLET which has been proposed to involve reactions and interactions with the machinery of the mitochondria (MRPL15, VARS2), NF- κ B translocation to the nucleus (TRIMM25) leading to the induction of a pro-inflammatory response, proteasome regulation, actin tubulin reorganization, and binding to the DNA for DNA fragmentation [38,39].

Supplementary Material

<https://docs.google.com/document/d/1cnM2o8RejoCHLGqhmLlTS0JtJnd5PE8r/edit?usp=sharing&ouid=113974552194826485902&rtpof=true&sd=true>

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Ethics Approval and Consent to Participate

The study and all the procedures for medical research involving human subjects, including research on identifiable human material and data were performed under the principles of the Declaration of Helsinki, and approved by the Ethic Committee in Research of the Zacatecas, General Hospital "Luz Gonzalez Cosío" CONBIOETICA-32-CEI-001-20180807.

Consent for Publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review.

Competing Interests

The authors declare no competing of interests.

Availability of Data and Material

Data will be shared following institutional guidelines. The review of the literature was based on search and data from PubMed database without limitation to 2024.

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Author's Contributions

G.G.G.M. and A.A.C.: Conceptualization. G.G.G.M.: Methodology, analysis, and writing. D.C.R.M. D.C.S. P.R.M.: Collaboration in patient's contact, clinic lab samples analysis, discussion. A.E.T.: Methodology. All authors have read and approved the manuscript.

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