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Research Article

A Combined Treatment of Chemotherapy and HAMLET Orally to a Patient with a Condition of Hodgkin's Lymphoma, Induced a Higher Functional UP Genes (Organelle Biogenesis, Immune System, Serotonin Hormone Signaling) than DOWN Regulated Genes (Cytokine and Signaling in Cancer, Hemostasis): A Report Case 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, it has been proposed the use of HAMLET (alpha-lactal albumin lethal to kill tumor cells) as palliative agent for cancer patients before and after chemotherapy and/or radiotherapy. In the present report the results of a classic Hodgkin's lymphoma (cHL) case in an adolescent female patient who was given Hamlet orally 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.

Keywords: Cancer, Hodgkin's Lymphoma, Human Maternal Milk, HAMLET, Chemotherapy, Radiotherapy, Immunotherapy

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 potential of the use of HAMLET as palliative agent for cancer patients before and after chemotherapy and/or radiotherapy [34,35]. In the present report the results of a cHL case in an adolescent female patient who was given Hamlet orally as 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/m^2sc/hr. Units: ml/kg/hr (milliliters per kilogram per hour) and ml/m^2sc/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

- Discharge home independently.
- Schedule an appointment if you experience any warning signs (fever, severe, unbearable pain, general malaise).
- 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: Gencitabine on August 7 and 14, 2024, Pegfilgrastimel 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 Cosió" 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 orally and the 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 under chemotherapy treatment was instructed to take 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 oral administration of HAMLET. 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

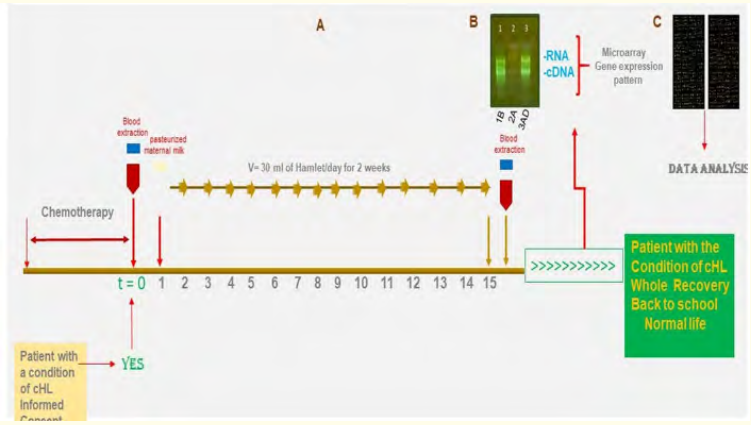

Figure 1: Scheme protocol follwed fo the analysis of the effect of HAMLET (human lactalbumin lethal to kill cells) orally after chemotherapy for two weeks to an individual with diagnosed with a condition of classical Hodgkin's lymphoma (cHL).The study was carried out using whole blood and DNA microarrays. After the written consent informed of the participant (s) (health and sick individual) (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 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).

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 diary orally, it was necessary to hybridize the cDNA of a healthy young children, and the cDNA of the young children with cHL 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 orally (at time two weeks after, with a hamlet orally and given diary for two weeks) (Figure 1A-C). 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

Differential DOWN and UP regulation of genes from a patient with a condition of classical Hodgkin's lymphoma (cHL) and combined treatment (chemotherapy/HAMLET orally).

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-C). 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 daily oral ingestion of HAMLET, the number of genes down regulated was decreased by few (n = 300), but the number of genes Up regulated increased (n = 140) (Table 1).

Table 1. Higher number of Up regulated genes after chemotherapy and dialy oral ingestión 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. Statiscal 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 Hodkins lymphoma (cHL) after chemotherapy. 1B vs 2A, patient with cHL, after chemotherapy and and after dialy oral ingestión of HAMLET for two weeks.

DOWN and UP regulation genes in the patient with a condition of classical Hodgkin's Lymphoma (cHL) after chemotherapy

Among the 25 most DOWN regulated genes with a Zscore 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. 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 (Table 2.A.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

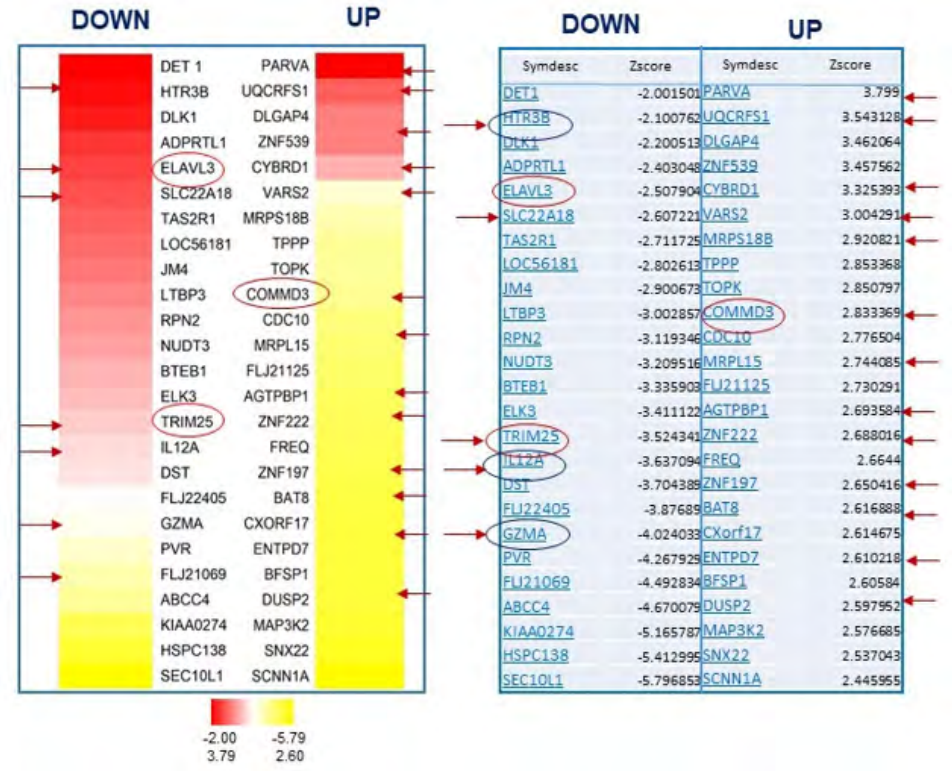

Figure 2A: Gene expression in a patient with a condition of classical Hodgkin's lymphoma after chemotherapy. Heat map (left panel) from the 25 most DOWN regulated genes with a Z score values from -2.0 to -5.79 that includes among others (right panel) to DET1, DET1 partner of COP1 E3 ubiquitin ligase 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. SLC67A1 solute carrier family 67 member 1. TRIM25 tripartite motif containing 25. 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. UP regulated genes (left panel) with Z values from 2.0 to 3.79 that includes a gene that is a member of the parvin family of actin-binding proteins (PARVA parvin alpha) (right panel). 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.

Table 2A.1 17 OF THE MOST DOWN REGULATED GENES AFTER CHEMOTHERAPY (Figure 2A, LEFT PANEL)

DET1 DET1 partner of COP1 E3 ubiquitin ligase [*Homo sapiens* (human)]. 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. Part of Cul4A-RING E3 ubiquitin ligase complex.
HTR3B 5-hydroxytryptamine receptor 3B [*Homo sapiens* (human)]. 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. It is not functional as a homomeric complex, but a pentaheteromeric complex with subunit A (HTR3A) displays the full functional features of this receptor.
DLK1 delta like non-canonical Notch ligand 1 [*Homo sapiens* (human)]. 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. This gene is located in a region of chromosome 14 frequently showing unparental disomy, and is imprinted and expressed from the paternal allele. A single nucleotide variant in this gene is associated with child and adolescent obesity and shows polar overdominance, where heterozygotes carrying an active paternal allele express the phenotype, while mutant homozygotes are normal.
PARP4 poly(ADP-ribose) polymerase family member 4 [*Homo sapiens* (human)]. This gene encodes poly(ADP-ribosyl)transferase-like 1 protein, which is capable of catalyzing a poly(ADP-ribosyl)ation reaction. This protein has a catalytic domain which is homologous to that of poly (ADP-ribosyl) transferase, but lacks an N-terminal DNA binding domain which activates the C-terminal catalytic domain of poly (ADP-ribosyl) transferase. Since this protein is not capable of binding DNA directly, its transferase activity may be activated by other factors such as protein-protein interaction mediated by the extensive carboxyl terminus.
ELAVL3 ELAV like RNA binding protein 3 [*Homo sapiens* (human)]. 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 (neuronal-specific RNA-binding proteins) recognized by the anti-Hu serum antibody present in sera from patients with paraneoplastic encephalomyelitis and sensory neuronopathy (PEM/PSN) suggests it has a role in neurogenesis. Two alternatively spliced transcript variants encoding distinct isoforms have been found for this gene.
SLC67A1 solute carrier family 67 member 1 [*Homo sapiens* (human)]. 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 gene is imprinted, with preferential expression from the maternal allele. Mutations in this gene have been found in Wilms' tumor and lung 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. Several alternatively spliced transcript variants encoding different isoforms have been described.
TAS2R1 taste 2 receptor member 1 [*Homo sapiens* (human)]. 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 intronless taste receptor gene encodes a 7-transmembrane receptor protein, functioning as a bitter taste receptor. 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.
LOC56181/MTFR1L mitochondrial fission regulator 1 like [*Homo sapiens* (human)]. Predicted to be involved in aerobic respiration and mitochondrial fission. Located in mitochondrion.
JAM4/PRAF2 PRA1 domain family member 2 [*Homo sapiens* (human)]. 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.
LTBP3 latent transforming growth factor beta binding protein 3 [*Homo sapiens* (human)]. 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. Three transcript variants encoding different isoforms have been found for this gene.
ELK3 ETS transcription factor ELK3 [*Homo sapiens* (human)]. 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. This protein is activated by signal-induced phosphorylation; studies in rodents suggest that it is a transcriptional inhibitor in the absence of Ras, but activates transcription when Ras is present. Alternate splicing results in multiple transcript variants.
TRIM25 tripartite motif containing 25 [*Homo sapiens* (human)]. 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.
IL12A interleukin 12A [*Homo sapiens* (human)]. This gene encodes a subunit of a cytokine that acts on T and natural killer cells, and has a broad array of biological activities. The cytokine is a disulfide-linked heterodimer composed of the 35-kD subunit encoded by this gene, and a 40-kD subunit that is a member of the cytokine receptor family. 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/iNOS2) is found to be required for the signaling process of this cytokine in innate immunity.
DST dystonin [*Homo sapiens* (human)]. This gene encodes a member of the plakin protein family of adhesion junction plaque proteins. Multiple alternatively spliced transcript variants encoding distinct isoforms have been found for this gene, but the full-length nature of some variants has not been defined. 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. Consistent with the expression, mice defective for this gene show skin blistering and neurodegeneration.
FLJ22405/MTMR14 myotubularin related protein 14 [*Homo sapiens* (human)]. This gene encodes a myotubularin-related protein. The encoded protein is a phosphoinositide phosphatase that specifically dephosphorylates phosphatidylinositol 3,5-biphosphate and phosphatidylinositol 3-phosphate. Mutations in this gene are correlated with autosomal dominant centronuclear myopathy. Alternate splicing results in multiple transcript variants. A pseudogene of this gene is found on chromosome 18
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. They are thought to protect their host by lysing cells bearing on their surface 'nonself' antigens, usually peptides or proteins resulting from infection by intracellular pathogens. 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.
SEC10L1/EXOC5 exocyst complex component 5 [*Homo sapiens* (human)]. The protein encoded by this gene is a component of the exocyst complex, a multiple protein complex essential for targeting exocytic vesicles to specific docking sites on the plasma membrane. Though best characterized in yeast, the component proteins and functions of exocyst complex have been demonstrated to be highly conserved in higher eukaryotes. 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

are regulatory proteins characterized by nucleic acid-binding zinc finger domains (Table 2.A.2).

Table 2A.2. 17 OF THE MOST UP REGULATED GENES AFTER CHEMOTHERAPY (Figure 2A, RIGHT PANEL)

PARVA parvin alpha [*Homo sapiens* (human)]. 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.
UQCRFS1 ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1 [*Homo sapiens* (human)]. 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.
DLGAP4 DLG associated protein 4 [*Homo sapiens* (human)]. 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. The encoded protein is one of at least four similar proteins that have been found. Alternatively spliced transcript variants encoding different isoforms have been found for this gene.
ZNF539/ZNF254 zinc finger protein 254 [*Homo sapiens* (human)]. 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
CYBRD1 cytochrome b reductase 1 [*Homo sapiens* (human)]. 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.
VARS2 valyl-tRNA synthetase 2, mitochondrial [*Homo sapiens* (human)]. 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. Alternative splicing of this gene results in multiple transcript variants.
MRPS18B mitochondrial ribosomal protein S18B [*Homo sapiens* (human)]. 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. They have an estimated 75% protein to rRNA composition compared to prokaryotic ribosomes, where this ratio is reversed. Another difference between mammalian mitoribosomes and prokaryotic ribosomes is that the latter contain a 5S rRNA. Among different species, the proteins comprising the mitoribosome differ greatly in sequence, and sometimes in biochemical properties, which prevents easy recognition by sequence homology. This gene encodes a 28S subunit protein that belongs to the ribosomal protein S18P family. The encoded protein is one of three that has significant sequence similarity to bacterial S18 proteins. The primary sequences of the three human mitochondrial S18 proteins are no more closely related to each other than they are to the prokaryotic S18 proteins. Pseudogenes corresponding to this gene are found on chromosomes 1q and 2q.
TPPP tubulin polymerization promoting protein [*Homo sapiens* (human)]. 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.
Topk/PBK PDZ binding kinase [*Homo sapiens* (human)]. This gene encodes a serine/threonine protein kinase related to the dual specific mitogen-activated protein kinase kinase (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. Alternative splicing results in multiple transcript variants.
SEPTIN7 septin 7 [*Homo sapiens* (human)]. This gene encodes a protein that is highly similar to the CDC10 protein of Saccharomyces cerevisiae. The protein also shares similarity with Diff 6 of Drosophila and with H5 of mouse. Each of these similar proteins, including the yeast CDC10, contains a GTP-binding motif. The yeast CDC10 protein is a structural component of the 10 nm filament which lies inside the cytoplasmic membrane and is essential for cytokinesis. 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. Alternative splicing results in multiple transcript variants.
MRPL15 mitochondrial ribosomal protein L15 [*Homo sapiens* (human)]. 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. They have an estimated 75% protein to rRNA composition compared to prokaryotic ribosomes, where this ratio is reversed. Another difference between mammalian mitoribosomes and prokaryotic ribosomes is that the latter contain a 5S rRNA. Among different species, the proteins comprising the mitoribosome differ greatly in sequence, and sometimes in biochemical properties, which prevents easy recognition by sequence homology. This gene encodes a 39S subunit protein that belongs to the EcoL15 ribosomal protein family. A pseudogene corresponding to this gene is found on chromosome 15q.
ZNF222 zinc finger protein 222 [*Homo sapiens* (human)]. Predicted to enable DNA-binding transcription factor activity. Predicted to be involved in regulation of DNA-templated transcription. Predicted to be located in nucleus.
ZNF197 zinc finger protein 197 [*Homo sapiens* (human)]. This gene product belongs to the zinc finger protein superfamily, members of which are regulatory proteins characterized by nucleic acid-binding zinc finger domains. The encoded protein contains 20 tandemly arrayed C2H2-type zinc fingers, a Kruppel-associated box (KRAB) domain, and a SCAN box. This transcript turns over rapidly and contains 3' UTR AUUUA motifs, which are often a hallmark of rapid turnover. It is overexpressed in some thyroid papillary carcinomas. This gene is located in a cluster of zinc finger genes at 3p21. Naturally-occurring read-through transcription is observed between this gene and the upstream zinc finger protein 660 gene and is represented by GeneID:110354863
BAT8/EHMT2 euchromatic histone lysine methyltransferase 2 [*Homo sapiens* (human)]. 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. This gene was initially thought to be two different genes, NG36 and G9a, adjacent to each other in the HLA locus. Alternative splicing results in multiple transcript variants.
CXorf17/FAM120C family with sequence similarity 120 member C [*Homo sapiens* (human)]. This gene encodes a potential transmembrane protein and lies in a region where mutations and deletions have been associated with intellectual disability and autism. Alternative splicing results in multiple transcript variants.
SCNN1A sodium channel epithelial 1 subunit alpha [*Homo sapiens* (human)]. Nonvoltage-gated, amiloride-sensitive, sodium channels control fluid and electrolyte transport across epithelia in many organs. These channels are heteromeric complexes consisting of 3 subunits: alpha, beta, and gamma. 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 mineralocorticoids. Alternatively spliced transcript variants encoding different isoforms have been described for this gene.

DOWN and UP regulated genes from the patient with a condition of classical Hodgkin's Lymphoma (cHL) under a combined treatment of chemotherapy and HAMLET orally

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 megaryocyte 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 metallopeptidase 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 aberrently 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 (Table 2.B.1.).

UP regulated genes from a Zscore (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 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. 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 with type II

Table 2B.1 17 OF THE MOST DOWN REGULATED GENES AFTER CHEMOTHERAPY AND HAMLET orally in a patient with a condition of cHL.

FLJ23342/MSANTD2 Myb/SANT DNA binding domain containing 2 [*Homo sapiens* (human)]. Located in nucleoplasm, and in nuclear body.
TRIM25 tripartite motif containing 25 [*Homo sapiens* (human)]. 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 [*Homo sapiens* (human)]. 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 [*Homo sapiens* (human)]. Enables protein-N-terminal amino-acid acetyltransferase activity. Involved in protein stabilization. Located in cytosol and nucleus. Part of NatC complex.
EREG epiregulin [*Homo sapiens* (human)]. 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. Alternative splicing results in multiple transcript variants encoding distinct protein isoforms. Additional transcript variants of this gene have been described, but their full-length nature is not known
HBD hemoglobin subunit delta [*Homo sapiens* (human)]. 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. Two alpha chains plus two delta chains constitute HbA-2, which with HbF comprises the remaining 3% of adult hemoglobin. Five beta-like globin genes are found within a 45 kb cluster on chromosome 11 in the following order: 5'-epsilon--Ggamma--Agamma--delta--beta-3'. Mutations in the delta-globin gene are associated with beta-thalassemia. Involved in the megaryocyte and platelet development
PFN2 profilin 2 [*Homo sapiens* (human)]. 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. There are two alternatively spliced transcript variants encoding different isoforms described for this gene.
LMNB1 lamin B1 [*Homo sapiens* (human)]. 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 [*Homo sapiens* (human)]. 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 [*Homo sapiens* (human)]. 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. The genes of the alpha and beta subunits of the mitochondrial trifunctional protein are located adjacent to each other in the human genome in a head-to-head orientation.
GRM3 glutamate metabotropic receptor 3 [*Homo sapiens* (human)]. 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. The metabotropic glutamate receptors are a family of G protein-coupled receptors, that have been divided into 3 groups on the basis of sequence homology, putative signal transduction mechanisms, and pharmacologic properties. Group I includes GRM1 and GRM5 and these receptors have been shown to activate phospholipase C. Group II includes GRM2 and GRM3 while Group III includes GRM4, GRM6, GRM7 and GRM8. Group II and III receptors are linked to the inhibition of the cyclic AMP cascade but differ in their agonist selectivities.
CDSN corneodesmosin [*Homo sapiens* (human)]. This gene encodes a protein found in corneodesmosomes, 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 metallopeptidase domain 12 [*Homo sapiens* (human)]. 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. Expression of this gene has been used as a maternal serum marker for pre-natal development. Alternative splicing results in multiple transcript variants encoding different isoforms. Shorter isoforms are secreted, while longer isoforms are membrane-bound form
ELAVL3 ELAV like RNA binding protein 3 [*Homo sapiens* (human)]. 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 (neuronal-specific RNA-binding proteins) recognized by the anti-Hu serum antibody present in sera from patients with paraneoplastic encephalomyelitis and sensory neuronopathy (PEM/PSN) suggests it has a role in neurogenesis. Two alternatively spliced transcript variants encoding distinct isoforms have been found for this gene.
MYB MYB proto-oncogene, transcription factor [*Homo sapiens* (human)]. 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 aberrently expressed or rearranged or undergo translocation in leukemias and lymphomas, and is considered to be an oncogene. Alternative splicing results in multiple transcript variants.
SLC25A4 solute carrier family 25 member 4 [*Homo sapiens* (human)]. This gene is a member of the mitochondrial carrier subfamily of solute carrier protein genes. 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 mitochondria membrane. Mutations in this gene have been shown to result in autosomal dominant progressive external opthalmoplegia and familial hypertrophic cardiomyopathy
CLCN5 chloride voltage-gated channel 5 [*Homo sapiens* (human)]. This gene encodes a member of the CIC 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. Alternatively spliced transcript variants have been found for this gene.

diabetes and susceptibility to insulin resistance. BFAR 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) (Table 2.B.2).

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

The analysis of the functionality of the up and down regulated genes under treatment of chemotherapy only (1B) (Mat and Methods), and after HAMLET orally (2A)(Mat and Methods), using REACTOME base data, highlight the pathways of the human biology in which UP and DOWN regulated genes participate. This is calculated based on the probability score, which is corrected for false discovery rate using the Benjamin-Hochberg method [36,37]. The lower p values means a higher probability that at least these genes hit or influence one of the pathways (Tables 3A-C). Thus, after Reactome analysis, we found that the 25 most Up or Down regulated genes (identifiers), in a patient with a condition of cHL, and under chemotherapy treatment, 19 identifiers participate in 231 pathways, and 18 of 25 up regulated genes participate in 186 pathways (table 3A). By other hand, in a patient with a condition

Table 2B.2. 17 OF THE MOST UP REGULATED GENES AFTER CHEMOTHERAPY AND HAMLET orally in a patient with a condition of cHL.

COMMD3 COMM domain containing 3 [*Homo sapiens* (human)]. 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 [*Homo sapiens* (human)]. 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.
NFRKB nuclear factor related to kappaB binding protein [*Homo sapiens* (human)]. 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 nucleoplasm.
EDIL3 EGF like and discoidin domains 3 [*Homo sapiens* (human)]. 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.
RNASE6 ribonuclease A family member 6 [*Homo sapiens* (human)]. 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.
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. 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. Four transcript variants encoding two different isoforms have been found for this gene. This gene is highly similar to the Y chromosome TBL1Y gene.
IRS1 insulin receptor substrate 1 [*Homo sapiens* (human)]. 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.
MAK male germ cell associated kinase [*Homo sapiens* (human)]. The product of this gene is a serine/threonine protein kinase related to kinases involved in cell cycle regulation. Studies of the mouse and rat homologs have localized the kinase to the chromosomes during meiosis in spermatogenesis, specifically to the synaptonemal complex that exists while homologous chromosomes are paired. Mutations in this gene have been associated with ciliary defects resulting in retinitis pigmentosa 62. Alternative splicing results in multiple transcript variants.
BFAR bifunctional apoptosis regulator [*Homo sapiens* (human)]. 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. Located in endoplasmic reticulum and membrane
SLC2A9 solute carrier family 2 member 9 [*Homo sapiens* (human)]. 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. Two transcript variants encoding distinct isoforms have been identified for this gene.
ZNF571 zinc finger protein 571 [*Homo sapiens* (human)]. Predicted to enable DNA-binding transcription factor activity, RNA polymerase II-specific and 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.
HTR1A 5-hydroxytryptamine receptor 1A [*Homo sapiens* (human)]. This gene encodes a G protein-coupled receptor for 5-hydroxytyptamine (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
SMCR7/MIEF2 mitochondrial elongation factor 2 [*Homo sapiens* (human)]. 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. Alternative splicing results in multiple transcript variants encoding different isoforms.
ZMYND10 zinc finger MYND-type containing 10 [*Homo sapiens* (human)]. 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. Alternative splicing results in multiple transcript variants.
ZNF304 zinc finger protein 304 [*Homo sapiens* (human)]. 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.
HLA-DOB major histocompatibility complex, class II, DO beta [*Homo sapiens* (human)]. 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). The beta chain is approximately 26–28 kDa and its gene contains 6 exons. Exon one encodes the leader peptide, exons 2 and 3 encode the two extracellular domains, exon 4 encodes the transmembrane domain and exon 5 encodes the cytoplasmic tail.
ACTR1B actin related protein 1B [*Homo sapiens* (human)]. This gene encodes a 42.3 kD subunit of dynactin, a macromolecular complex consisting of 10 subunits ranging in size from 22 to 150 kD. Dynactin 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. These two proteins, which are of equal length and share 90% amino acid identity, are present in a constant ratio of approximately 1:15 in the dynactin complex

of cHL, under chemotherapy and HAMLET orally, 18 identifiers participate in 292 pathways, and from 25 most up regulated genes, 17 identifiers participate only in 68 pathways (Table 3A).

The analysis of the functionality of the Up and Down regulated genes (Tables 3A, 3B, right and left panel is divided in two part, Part I is described below and Part II supplementary description of the Up and down regulated genes functionality using the Reactome base data is in the link, https://drive.google.com/file/d/1md6Pc9CzrZ4Ox8Ad8EvJ116ZkpRAu8z/view?usp = sharing.

Table 3A. A higher amount of pathways are hitted by **UP** regulated genes than down regulated genes after combined treatment of chemotherapy and HAMLET orally.

Sample	UP	DOWN
1B vs 3AD	68(17/25)	292(18/25)
1B vs 2A	186(18/25)	231(19/25)

- According to the p values from the reactome data analysis, low values of p (< values <<<), a higher amount of pathways are hitted by **UP (right) regulated genes than DOWN (left) regulated genes. For example, proportion of identifiers (x/x) found in the Reactome where i.e. a number of pathways (X) were hit** by at least one of them (Fabregat et al., 2016; 2017). Reactome pathways are enriched in the submitted data (n= 25 genes). My list contain more proteins **(x/x)** for pathway **X** than would be expected by chance. **3AD**, healthy individual; **1B**, patient with a condition of classic Hodkins lymphoma (cHL) treated with chemotherapy, and before HAMLET orally at time zero; 1B vs 2A, patient with cHL, before, and after chemotherapy and HAMLET orally for two weeks.

Table 3B. DOWN and UP genes with high probability to be involved in different pathways of the human biology in a patient with a condition of cHL and under chemotherapy treatment

Pathway name	Entities found	Entities ratio	Entities p-value	Entities FDR*	Reactions found	Reactions ratio
Cell junction organization	5 / 298	0.018	2.44e-04	0.044	8 / 162	0.01
Cell-Cell communication	5 / 336	0.021	4.22e-04	0.044	8 / 185	0.012
Interleukin-35 Signalling	2 / 16	9.91e-04	4.48e-04	0.044	24 / 26	0.002
Adherens junctions interactions	4 / 238	0.015	0.001	0.08	5 / 141	0.009
Cell-cell junction organization	4 / 271	0.017	0.002	0.103	5 / 146	0.009
Molecules associated with elastic fibres	2 / 37	0.002	0.002	0.115	2 / 10	6.30e-04
Elastic fibre formation	2 / 45	0.003	0.003	0.142	2 / 17	0.001
Defective SLC22A18 causes lung cancer (LNCR) and embryonal rhabdomyosarcoma 1 (RMS1)	1 / 2	1.24e-04	0.004	0.142	1 / 1	6.30e-05
TGF-beta receptor signaling activates SMADs	2 / 51	0.003	0.004	0.143	2 / 44	0.003
Regulation of CDH1 Expression and Function	3 / 192	0.012	0.006	0.143	4 / 92	0.006
Regulation of Expression and Function of Type I Classical Cadherins	3 / 192	0.012	0.006	0.143	4 / 92	0.006
Regulation of Homotypic Cell-Cell Adhesion	3 / 215	0.013	0.008	0.143	4 / 125	0.008
Translation of Structural Proteins	2 / 85	0.005	0.012	0.143	2 / 46	0.003
Interleukin-10 signaling	2 / 86	0.005	0.012	0.143	1 / 15	9.45e-04
Nectin/Necl trans heterodimerization	1 / 7	4.34e-04	0.013	0.143	1 / 8	5.04e-04
Regulation of PD-L1(CD274) Post-translational modification	2 / 94	0.006	0.014	0.143	4 / 25	0.002
Interleukin-12 family signaling	2 / 96	0.006	0.015	0.143	36 / 114	0.007
Late SARS-CoV-2 Infection Events	2 / 102	0.006	0.016	0.143	2 / 69	0.004
Signaling by TGF-beta Receptor Complex	2 / 108	0.007	0.018	0.143	2 / 100	0.006
ABC-family proteins mediated transport	2 / 111	0.007	0.019	0.143	2 / 27	0.002
Type I hemidesmosome assembly	1 / 11	6.81e-04	0.021	0.143	3 / 6	3.78e-04
Positive Regulation of CDH1 Gene Transcription	1 / 13	8.05e-04	0.025	0.143	2 / 14	8.82e-04
NF-kB activation through FADD/RIP-1 pathway mediated by caspase-8 and -10	1 / 14	8.67e-04	0.027	0.143	5 / 5	3.15e-04
Extracellular matrix organization	3 / 351	0.022	0.029	0.143	3 / 330	0.021
Maturation of nucleoprotein	1 / 17	0.001	0.032	0.143	1 / 9	5.67e-04
* False Discovery Rate						

Pathway name	Entities found	Entities ratio	Entities p-value	Entities FDR*	Reactions found	Reactions ratio
Mitochondrial translation initiation	3 / 88	0.007	9.91e-04	0.02	3 / 4	2.55e-04
Mitochondrial translation elongation	3 / 88	0.007	9.91e-04	0.02	5 / 8	5.11e-04
Mitochondrial ribosome-associated quality control	3 / 90	0.007	0.001	0.02	5 / 5	3.19e-04
Translation	5 / 370	0.031	0.001	0.02	22 / 126	0.008
Mitochondrial translation termination	3 / 102	0.008	0.002	0.02	7 / 7	4.47e-04
Mitochondrial translation	3 / 113	0.009	0.002	0.022	20 / 24	0.002
tRNA Aminoacylation	2 / 42	0.003	0.004	0.036	2 / 42	0.003
Localization of the PINCH-ILK-PARVIN complex to focal adhesions	1 / 4	3.31e-04	0.009	0.071	1 / 2	1.28e-04
Sensory perception of salty taste	1 / 6	4.97e-04	0.013	0.093	1 / 2	1.28e-04
Regulation of cytoskeletal remodeling and cell spreading by IPP complex components	1 / 8	6.62e-04	0.018	0.106	2 / 5	3.19e-04
Phosphate bond hydrolysis by NTPDase proteins	1 / 8	6.62e-04	0.018	0.106	1 / 12	7.66e-04
Cell-extracellular matrix interactions	1 / 18	0.001	0.039	0.164	3 / 10	6.38e-04
Regulation of TP53 Activity through Methylation	1 / 19	0.002	0.042	0.164	1 / 12	7.66e-04
Mitochondrial tRNA aminoacylation	1 / 21	0.002	0.046	0.164	1 / 21	0.001
RAF-independent MAPK1/3 activation	1 / 24	0.002	0.052	0.164	1 / 12	7.66e-04
Cytosolic tRNA aminoacylation	1 / 24	0.002	0.052	0.164	1 / 21	0.001
Complex III assembly	1 / 26	0.002	0.057	0.164	5 / 10	6.38e-04
Transcriptional Regulation by E2F6	1 / 34	0.003	0.073	0.164	4 / 33	0.002
Nucleotide catabolism	1 / 38	0.003	0.082	0.164	1 / 63	0.004
Regulation of endogenous retroelements by the Human Silencing Hub (HUSH) complex	1 / 39	0.003	0.084	0.164	1 / 8	5.11e-04
Transcriptional Regulation by VENTX	1 / 39	0.003	0.084	0.164	1 / 13	8.30e-04
EIF2S3 (CSB) and EIF4T2 (G9a) positively regulate rRNA expression	1 / 44	0.004	0.094	0.164	2 / 4	2.55e-04
Negative regulation of MAPK pathway	1 / 44	0.004	0.094	0.164	1 / 17	0.001

1825
Identifiers were found in the Reactome were 292 were hit by at least one of them

17/25
Identifiers were found in the Reactome were 68 were hit by at least one of them

Note: Data from DOWN(LEFT) and UP (RIGHT) genes from a patient with a condition of classcial Hodgkin's Lymphoma (cHL) AND CHEMOTHERAPY. AT low values of p (< values <<<), A higher probability that Down (LEFT) or Up (right) genes affecte or influence a certain pathway.

Table 3C. Down and Up genes from a patient with a condition of cHLwith high probability to be involved in the different pathways of the human biology in a patient with a condition of cHL, under a combined treatment of chemotherapy and HAMLET orally

Pathway name	Entities found	Entities ratio	Entities p-value	Entities FDR*	Reactions found	Reactions ratio
Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation	4 / 73	0.005	1.19e-05	0.003	2 / 36	0.002
Interleukin-12 signaling	4 / 84	0.005	2.06e-05	0.003	2 / 56	0.004
Interleukin-12 family signaling	4 / 96	0.006	3.46e-05	0.003	2 / 114	0.007
Factors involved in megakaryocyte development and platelet production	4 / 194	0.012	5.06e-04	0.032	3 / 43	0.003
Protein localization	3 / 171	0.011	0.004	0.128	7 / 53	0.003
Cytokine Signaling in Immune system	7 / 1,106	0.069	0.004	0.128	14 / 806	0.051
Breakdown of the nuclear lamina	1 / 3	1.86e-04	0.006	0.128	1 / 3	1.89e-04
Signaling by EGFR	2 / 64	0.004	0.007	0.128	41 / 50	0.003
Signaling by Interleukins	5 / 646	0.04	0.007	0.128	3 / 505	0.032
Peroxisomal protein import	2 / 67	0.004	0.007	0.128	3 / 26	0.002
Vpr-mediated induction of apoptosis by mitochondrial outer membrane permeabilization	1 / 4	2.48e-04	0.008	0.128	1 / 2	1.26e-04
Invadopodia formation	1 / 5	3.10e-04	0.01	0.128	1 / 1	6.30e-05
ESR-mediated signaling	3 / 257	0.016	0.013	0.128	8 / 114	0.007
RUNX1 regulates transcription of genes involved in differentiation of HSCs	2 / 92	0.006	0.014	0.128	2 / 15	9.45e-04
Signaling by Overexpressed Wild-Type EGFR in Cancer	1 / 8	4.96e-04	0.015	0.128	1 / 2	1.26e-04
Inhibition of Signaling by Overexpressed EGFR	1 / 8	4.96e-04	0.015	0.128	1 / 2	1.26e-04
Hemostasis	5 / 805	0.05	0.018	0.128	4 / 342	0.022
EGFR interacts with phospholipase C-gamma	1 / 11	6.81e-04	0.021	0.128	3 / 3	1.89e-04
RHOT2 GTPase cycle	1 / 11	6.81e-04	0.021	0.128	1 / 3	1.89e-04
Miro GTPase Cycle	1 / 12	7.43e-04	0.023	0.128	1 / 6	3.78e-04
NF-kB activation through FADD/RIP-1 pathway mediated by caspase-8 and -10	1 / 14	8.67e-04	0.027	0.128	5 / 5	3.15e-04
PI3K events in ERBB4 signaling	1 / 15	9.29e-04	0.028	0.128	2 / 2	1.26e-04
Plasmalogen biosynthesis	1 / 15	9.29e-04	0.028	0.128	2 / 4	2.52e-04
Beta oxidation of myristoyl-CoA to lauroyl-CoA	1 / 16	9.91e-04	0.03	0.128	3 / 4	2.52e-04
Beta oxidation of palmitoyl-CoA to myristoyl-CoA	1 / 16	9.91e-04	0.03	0.128	3 / 4	2.52e-04
* False Discovery Rate						

Pathway name	Entities found	Entities ratio	Entities p-value	Entities FDR*	Reactions found	Reactions ratio
Transcriptional activation of mitochondrial biogenesis	3 / 89	0.006	5.54e-04	0.047	4 / 32	0.002
Regulation of MITF-M-dependent genes involved in extracellular matrix, focal adhesion and epithelial-to-mesenchymal transition	2 / 23	0.001	8.07e-04	0.047	2 / 22	0.001
Growth hormone receptor signaling	2 / 29	0.002	0.001	0.047	3 / 28	0.002
Signaling by ALK fusions and activated point mutants	3 / 119	0.007	0.001	0.047	4 / 63	0.004
Signaling by ALK in cancer	3 / 119	0.007	0.001	0.047	4 / 71	0.005
Mitochondrial biogenesis	3 / 131	0.008	0.002	0.051	4 / 36	0.002
Interleukin-37 signaling	2 / 36	0.002	0.002	0.051	1 / 14	8.93e-04
Diseases of signal transduction by growth factor receptors and second messengers	5 / 536	0.033	0.002	0.057	7 / 516	0.033
Signaling by ALK	2 / 46	0.003	0.003	0.063	3 / 42	0.003
Nuclear events stimulated by ALK signaling in cancer	2 / 56	0.003	0.005	0.083	2 / 32	0.002
Defective SLC2A9 causes hyperuricemia renal 2 (RHUC2)	1 / 4	2.48e-04	0.007	0.122	1 / 1	6.38e-05
IRS activation	1 / 7	4.35e-04	0.013	0.155	3 / 3	1.91e-04
Co-inhibition by BTLA	1 / 7	4.35e-04	0.013	0.155	1 / 4	2.55e-04
Loss of MECP2 binding ability to the NCoR/SMRT complex	1 / 8	4.97e-04	0.014	0.155	1 / 1	6.38e-05
Signaling by RNF43 mutants	1 / 8	4.97e-04	0.014	0.155	1 / 1	6.38e-05
SOS-mediated signalling	1 / 10	6.21e-04	0.018	0.155	2 / 2	1.28e-04
Activated NTRK3 signals through PI3K	1 / 10	6.21e-04	0.018	0.155	2 / 5	3.19e-04
Organelle biogenesis and maintenance	3 / 340	0.021	0.023	0.155	4 / 86	0.005
PI3K/AKT activation	1 / 13	8.07e-04	0.023	0.155	4 / 5	3.19e-04
Serotonin receptors	1 / 13	8.07e-04	0.023	0.155	3 / 6	3.83e-04
Signal attenuation	1 / 13	8.07e-04	0.023	0.155	3 / 7	4.47e-04
Signaling by Leptin	1 / 13	8.07e-04	0.023	0.155	2 / 19	0.001
PECAM1 interactions	1 / 14	8.69e-04	0.025	0.155	1 / 7	4.47e-04
MHC class II antigen presentation	2 / 137	0.009	0.025	0.155	3 / 26	0.002

19/25
Identifiers were found in the Reactome were 231 were hit by at least one of them

18/25
Identifiers were found in the Reactome were 186 were hit by at least one of them

Note: Data from DOWN(LEFT) and UP (RIGHT) genes from a patient with a condition of classical Hodgkin Lymphoma (cHL), CHEMOTHERAPY AND DIALY ORAL INGESTION OF HAMLET. At low values of p (< values <<<), A higher probability that Down (LEFT) or Up (right) genes affecte or influence a certain pathway.

UP regulated genes from a patient with a condition of classical Hodgkin's lymphoma (cHL), and under chemotherapy only (1B) (Table 3B, right panel)

Part I

- The pathways affected or influenced by the Up regulated genes: Mitochondrial translation initiation, mitochondrial translation elongation, mitochondrial ribosome-associated quality control, Translation, Mitochondrial translation termination mitochondrial translation (MRPL15, MRPS18B genes). tRNA Aminoacylation, Mitochondrial tRNA aminoacylation (VARS2 gene). tRNA synthetases catalyze the ligation of tRNAs to their cognate amino acids in an ATP-dependent manner. A single synthetase mediates the charging of all of the tRNA species specific for any one amino acid but, with three exceptions, glycine, lysine, and glutamine, the synthetase that catalyzes aminoacylation of mitochondrial tRNAs is encoded by a different gene than the one that acts on mitochondrial tRNAs. Both mitochondrial and cytosolic tRNA synthetase enzymes are encoded by genes in the nuclear genome. The role of these enzymes in human development and disease.

- Localization of the PINCH-ILK-PARVIN complex to focal adhesions (PARVA gene). The interactions among ILK, PINCH, and parvins are necessary but not sufficient for localization of ILK to cell-ECM adhesions. Additional proteins that interact with PINCH-ILK parvin complex components likely participate in mediating its localization. Sensory perception of salty taste (SCNN1A gene). The identity of salt-tasting cells remains a subject of current research. The ability to taste low concentrations of salt is at least partially due to an amiloride-sensitive sodium channel believed to be an SCNN channel (ENAC cannel) (SCNN1A). Regulation of cytoskeletal remodeling and cell spreading by IPP complex components (PARVA gene). The PINCH-ILK-Parvin complexes function in transducing diverse signals from ECM to intracellular effectors. Interacting partners for components of these complexes have been identified, a number of which regulate and/or mediate its functions in cytoskeletal remodeling and cell spreading.

- Phosphate bond hydrolysis by NTPDase proteins (ENTPD 7 gene). The ectonucleoside triphosphate diphosphatase (E-NTPDase family) of ectonucleotidases includes 8 enzymes. Different family members show different specificity for particular nucleotides. NTPDases are involved in various biological processes, such as hemostasis, immune response and development of the nervous system.

- Cell-extracellular matrix interactions (ECM) (PARVA gene). Interactions play a critical role in regulating a variety of cellular processes in multicellular organisms including motility, shape change, survival, proliferation and differentiation.

- RAF-independent MAPK1/3 activation (DUS2 gene). Cellular compartments: cytosol, nucleoplasm. Depending upon the stimulus and cell type mitogen-activated protein kinases (MAPK) signaling pathway can transmit signals to regulate many different biological processes by virtue of their ability to target multiple effector proteins. In particular, the extracellular signal-regulated kinases MAPK3(ERK1) and MAPK1 (ERK2) are involved in diverse cellular processes such as proliferation, differentiation, regulation of inflammatory responses, cytoskeletal remodeling, cell motility and invasion through the increase of matrix metalloproteinase production. The canonical RAF: MAP2K:MAPK1/3 cascade is stimulated by various extracellular stimuli including hormones, cytokines, growth factors, heat shock and UV irradiation triggering the GEF-mediated activation of RAS at the plasma membrane and leading to the activation of the RAF MAP3 kinases. However, many physiological and pathological stimuli have been found to activate MAPK1/3 independently of RAF and RAS. Tumor progression locus 2 (TPL2), also known also known as MAP3K8 and COT is another MAP3 kinase which promotes MAPK1/3 (ERK)-regulated immune responses downstream of toll-like receptors (TLR), TNF receptor and IL-1β signaling pathways.

- Nucleotide catabolism (ENTPD7 gene). The purine bases guanine and hypoxanthine (derived from adenine by events in the purine salvage pathways) are converted to xanthine and then to uric acid, which is excreted from the body. The end-point of this pathway in humans and hominoid primates is unusual. Most other mammals metabolize uric acid further to yield more soluble end products, and much speculation has centered on possible roles for high uric acid levels in normal human physiology.

- Transcriptional Regulation by VENTX (BAT8 gene). The VENTX (also known as VENT homeobox or VENTX2) gene is a member of the homeobox family of transcription factors. VENTX is expressed in human blood cells and appears to play an important role in hematopoiesis. VENTX promotes cell cycle arrest and

differentiation of hematopoietic stem cells and/or progenitor cells. VENTX induces transcription of cell cycle inhibitors TP53 (p53) and p16INK4A and activates tumor suppressor pathways regulated by TP53 and p16INK4A, as well as macrophage colony stimulating factor receptor (CSF1R) and inhibits transcription of cyclin D1 (CCND1) and Interleukin-6 (IL6). While VENTX expression may suppress lymphocytic leukemia, high levels of VENTX have been reported in acute myeloid leukemia cells, with a positive effect on their proliferation. Another homeobox transcription factor that regulates differentiation of hematopoietic stem cells is DLX4. Studies on colon cancer showed that VENT X regulates tumor associated macrophages and reverts immune suppression in tumor microenvironment. VENTX induces TP53-independent apoptosis in cancer cells.

Functionality of the DOWN regulated genes in a patient with a condition of classical Hodgkin's lymphoma (cHL), and under chemotherapy (Table 3B left panel)

Part I

The pathways in which down regulated genes are involved and participate are:

- Cell junction organization (BTEB1, PVR, DST1, and RPN2 genes). Cell junction organization in Reactome currently covers aspects of cell-cell junction organization, cell-extracellular matrix interactions, and Type I hemidesmosome assembly.

- Cell-Cell communication (BTEB1, PVR, DST1, and RPN2 genes). Cell-to-Cell communication is crucial for multicellular organisms because it allows organisms to coordinate the activity of their cells. Some cell-to-cell communication requires direct cell-cell contacts mediated by receptors on their cell surfaces. Members of the immunoglobulin superfamily (IgSF) proteins are some of the cell surface receptors involved in cell-cell recognition, communication and many aspects of the axon guidance and synapse formation-the crucial processes during embryonal development. Processes annotated here as aspects of cell junction organization mediate the formation and maintenance of adherens junctions, Tight junctions, and gap junctions, as well as aspects of cellular interactions with extracellular matrix and hemidesmosome assembly. Interactions among members of the signal regulatory protein family are important for the regulation of migration and phagocytosis by myeloid cells.

- Interleukin-35 Signalling (IL12A gene). Interleukin 35 (IL35) is an IL12 family cytokine produced by regulatory but not effector T-cells. It is a dimeric protein composed of IL-12RB2 and IL27RA chains. IL35 suppresses inflammatory responses of immune cells.

- Defective SLC22A18 causes lung cancer (LNCR) and embryonal rhabdomyosarcoma 1 (RMSE1) (SLC22A18 gene). Diseases: lung cancer, rhabdomyosarcoma.The human gene SLC22A18 (aka TSSC5) encodes organic cation transporter-like protein 2 (ORCTL2). It is expressed at high levels in kidney, liver and colon and at lower levels in heart, brain and lung. ORCTL2 can transport organic cations such as chloroquine and quinidine with the antiport of protons. The human chromosome region 11p15.5 is linked with Beckwith-Wiedemann syndrome (associated with susceptibility to Wilms' tumor, rhabdomyosarcoma and hepatoblastoma). SLC22A18 is located in this region. Mutations and/or reduced expression of SLC22A18 have been found in certain tumors such as lung cancer (LNCR, MIM: 211980) and embryonal rhabdomyosarcoma 1 (RMSE1, MIM: 268210).

- Regulation of CDH1 Expression and Function CDH1 (BTEB1, RPN2 genes). Also known as E-cadherin, epithelial cadherin, Cadherin-1, CADH1, or uvomorulin), is single-membrane-spanning protein with a conserved cytoplasmic domain and five extracellular cadherin domains separated by interdomain Ca2+ binding sites. CDH1 is connected to the cytoskeleton via its interactions with catenins. Loss-of-function missense mutations in CDH1 are an underlying cause of about 30% of cases of hereditary diffuse gastric cancer (HDGC), and they affect various points in CDH1 posttranslational processing, trafficking, and interaction with protein partners, and a polymorphism in CDH1 gene promoter has also been associated with increased gastric cancer risk. CDH1 is frequently downregulated in tumors of epithelial origin and is considered to be a tumor suppressor gene. Loss of CDH1 expression promotes epithelial-to-mesenchymal transition

(EMT), implicated in tumor invasiveness. The early stage of EMT is thought to involve removal of CDH1 from the plasma membrane and proteolytic degradation, while the later stage/established EMT is thought to involve repression of CDH1 gene transcription.

- IL-10 signaling (IL12A gene). Interleukin-10 (IL10) was originally described as a factor named cytokine synthesis inhibitory factor that inhibited T-helper (Th) 1 activation and Th1 cytokine production. It was found to be expressed by a variety of cell types including macrophages, dendritic cell subsets, B cells, several T-cell subpopulations including Th2 and T-regulatory cells (Tregs) and Natural Killer (NK) cells. IL10 inhibits a broad spectrum of activated macrophage/monocyte functions including monokine synthesis, NO production, and expression of class II MHC and costimulatory molecules such as IL12 and CD80/CD86. IL-10 antagonizes the expression of MHC class II and the co-stimulatory molecules CD80/CD86 as well as the pro-inflammatory cytokines IL1β, IL6, IL8, TNF-α and especially IL-12. The biological role of IL10 is not limited to inactivation of APCs, it also enhances B cell, granulocyte, mast cell, and keratinocyte growth/differentiation, as well as NK-cell and CD8+ cytotoxic T-cell activation, stimulating proliferation of activated NK and T cells and induces production of interferon gamma (IFN-γ) by these cells, NF-kβ activation through FADD/RIP-1 pathway mediated by caspase-8 and 10.

- Regulation of PD-L1 (CD274) Post-translational modification (RPN2 gene). Post-translational modification (PTM) of CD274 (PD-L1) is a crucial regulatory mechanism that significantly impacts its stability, surface presentation and interaction with its receptor PD-1, thus modulating normal immune function and immune response in cancers by influencing tumor immune evasion. PTMs of PD-L1 includes glycosylation, ubiquitination/de-ubiquitination, and phosphorylation. These post-translational modifications collectively modulate the effectiveness of PD-L1 in immune checkpoint path ways and have significant implications for the development of immune therapies targeting PD-L1 in various diseases, including cancer. These processes reveal potential therapeutic targets for enhancing the efficacy of PD-L1 inhibitors by manipulating its post-translational regulation (RPN2) (Table 3B, left panel).

- Interleukin-12 family signaling (IL-12A gene). Interleukin-12 (IL-12) is a heterodimer of interleukin-12 subunit alpha (IL12A, IL-12p35) and interleukin-12 subunit beta (IL12B, IL-12p40). It is a potent immuno regulatory cytokine involved in the generation of cell mediated immunity to intracellular pathogens. It is produced by antigen presenting cells, including dendritic cells, macrophages/monocytes, neutrophils and some B cells. It enhances the cytotoxic activity of natural Killer (NK) Cells and Cytotoxic T cells, stimulating proliferation Of activated NK and T cells and induces production of interferon gamma (IFN gamma) by these cells. IL-12 also plays an important role in immunomodulation by promoting cell mediated immunity through induction of a class 1 T Helper cell (Th1) Immune response. IL-12 may contribute to immunopathological conditions such as rheumatoid arthritis.

- Signaling by TGF-beta Receptor Complex (LTBP3 gene). The TGF-beta/BMP pathway incorporates several signaling pathways that share most, but not all, components of a central signal transduction engine. The general signaling scheme is rather simple: upon binding of a ligand, an activated plasma membrane receptor complex is formed, which passes on the signal towards the nucleus through a phosphorylated receptor SMAD (R-SMAD). The R-SMAD:Co-SMAD complex can interact with a great number of transcriptional co-activators/co-repressors to regulate positively or negatively effector genes, so that the interpretation of a signal depends on the cell type and cross talk with other signaling pathways such as Notch, MAPK and Wnt. The pathway plays a number of different biological roles in the control of embryonic and adult cell proliferation and differentiation, and it is implicated in a great number of human diseases.

- NF-kB activation through FADD/RIP-1 pathway mediated by caspase-8 and 10 (TRIM25 gene). Cellular compartments: mitochondrial outer membrane. Fas-Associated DeathDomain (FADD) and receptor interacting protein 1 (RIP1) are death domain containing molecules that interact with the C-terminal portion of MAVS (IPS-1) and induce NF-kB through interaction and activation of initiator caspases (caspase-8 and -10). Caspases are usually involved in apoptosis and inflammation. But they also exhibit nonapoptotic functions. These

nonapoptotic caspase functions involve prodomain-mediated activation of NF-kB. Processed caspases (caspase-8/10) encoding the DED (death effector domain) strongly activate NF-kB. The exact mechanism by which caspases Mediate NF-kB Activation is unclear, but the prodomains of caspase-8/10 may act as a scaffolding and allow the recruitment Of the IKK complex in association with other signaling molecules.

UP regulated genes from a patient with a condition of classical Hodgkin's lymphoma (cHL), chemotherapy and HAMLET orally influence the following pathways (Table 3C, right panel).

Part I

- Growth hormone receptor signaling (IRS1, PTPN6). Growth hormone (Somatotropin or GH) is a key factor in determining lean body mass, stimulating the growth and metabolism of muscle, bone and cartilage cells, while reducing body fat. It has many other roles, it acts to regulate cell growth, differentiation, apoptosis, and reorganization of the cytoskeleton, affecting diverse processes such as cardiac function, immune function, brain function, and aging. GH also has insulin-like effects such as stimulating amino acid transport, protein synthesis, glucose transport, and lipogenesis. The growth hormone receptor (GHR) is a member of the cytokine receptor family. Phosphorylation of key tyrosine residues in its cytoplasmic domains and activation of associated tyrosine kinase JAK2, leads to recruitment of signaling molecules such as STAT5 and Src family kinases such as Lyn leading to ERK activation.
- Signaling by ALK fusions and activated point mutants, signaling by ALK (IRS1, PTPN6). The anaplastic lymphoma kinase (ALK) is a transmembrane receptor tyrosine kinase that, along with related receptor LTK (leukocyte tyrosine kinase receptor) is a member of the insulin receptor superfamily. ALK was discovered as an oncogene in anaplastic large cell lymphomas (ALCLs), but also plays an oncogenic role in other cancer types, such as non-small-cell lung cancer (NSCLC), inflammatory myofibroblastic tumours (IMT), melanoma, neuroblastoma, and glioblastoma. ALK is activated in a range of cancers as a result of amplification or overexpression, fusion event or activating point mutations, resulting, in general, in constitutive activation of intracellular signaling.
- Signaling by ALK in cancer (IRS1, PTPN6). Diseases: cancer. Anaplastic lymphoma kinase (ALK) was first identified in the context of an oncogenic fusion with nucleophosmin (NPM) in anaplastic large cell lymphoma (ALCL). In addition to translocation events that lead to fusion proteins, the ALK gene also contributes to oncogenesis as a result of gene amplification and overexpression events, as well as being subject to activating missense mutations. Oncogenic ALK activity can be targeted with tyrosine kinase inhibitors, although resistance often arises due to secondary mutations or activation of bypass pathways.
- Interleukin-37 signaling (PTPN6). Interleukins (IL) are immunomodulatory proteins that elicit a wide array of responses in cells and tissues. Interleukin 37 (IL37), also known as IL 1F7, is a member of the IL 1 family. Isoform b of IL37 (referred just as IL37) is synthesized as a precursor that requires processing (primarily by caspase1) to attain full receptor agonist or antagonist function. Processed IL37 can bind with mothers against decapentaplegic homolog 3 (SMAD3) in the cytosol and then translocate to the nucleus, where it facilitates transcription of Tyrosine protein phosphatase non receptors (PTPNs). These events ultimately lead to suppression of cytokine production in several types of immune cells resulting in reduced inflammation.
- Diseases of signal transduction by growth factor receptors and second messengers (FZD4, PTPN6, IRS1, and TBLIX). Signaling processes are central to human physiology, and their disruption by either germ-line or somatic mutation can lead to serious disease. Here, the molecular consequences of mutations affecting visual signal transduction and signaling by diverse growth factors are annotated.
- Nuclear events stimulated by ALK signaling in cancer (PTPN6 gene). Diseases: cancer. Signaling through oncogenic forms of ALK activate nuclear events that drive cellular survival, escape from apoptosis and transformation. Changes to gene expression are effected both by epigenetic mechanisms and by inducing expression of key transcription factors and cell cycle regulators, among other critical targets. Many of these gene expression events are dependent on activation of STAT3 and to a lesser extent, MAP kinase signaling downstream of ALK. Unique among fusion proteins identified to date, the well-studied NPM-ALK fusion appears to be partially localized

to the nucleus by virtue of oligomerization with endogenous full-length NPM.

- Co-inhibition by BTLA (PTNP6 gene). BTLA (B and T Lymphocyte Attenuator) is a co-inhibitory receptor that plays a crucial role in regulating immune responses, maintaining immune homeostasis, and preventing, autoimmunity. BTLA Interacts with its ligand, HVEM (Herpesvirus Entry Mediator), a member of the tumor necrosis. BTLA is expressed on various immune cells, including T cells, B cells, and dendritic cells, and it functions similarly to other immune checkpoint molecules like CTLA-4 and PD-1, but it has unique structural and functional properties.
- Signaling by RNF43 mutants (FZD4 gene) Cellular compartments: plasma membrane. Diseases: cancer. RNF43 and related protein ZNRF3 are E3 ubiquitin ligases that negatively regulate WNT signaling by downregulating FZD receptors at the cell surface. Frameshift loss-of-function mutations in RNF43 that enhance WNT signaling have been identified in pancreatic and colorectal cancers, the proliferation of these cells is dependent on the presence of secreted WNT as their growth is abrogated by treatment of cells with the Porcupine inhibitor LGK974.
- PI3K/AKT activation (IRS1 gene). PI3K/AKT signaling is a major regulator of neuron survival. It blocks cell death by both impinging on the cytoplasmic cell death machinery and by regulating the expression of genes involved in cell death and survival. In addition, it may also use metabolic pathways to regulate cell survival. The PI3K/AKT pathway also affects axon diameter and branching, and regulates small G proteins like RhoA, which control the behavior of the F-actin cytoskeleton. Moreover, through its connection with the TOR pathway, it promotes translation of a subset of mRNAs.
- Serotonin receptors (HTR1A gene). Serotonin (5-HT) is a monoamine neurotransmitter that plays an important role as a modulator of anger, aggression, body temperature, mood, sleep, sexuality, appetite, metabolism, as well as stimulating vomiting. Several classes of drugs target the 5-HT system including some antidepressants, antipsychotics, anxiolytics, antiemetics and antimigraine drugs. The activity of 5-HT is modulated by 5-HT receptors, made up of seven families (5-HT1-7). All but 5-HT3 (ligand-gated ion channel) are GPCRs and these receptors bind different G proteins resulting in differing outcomes.
- Signaling by Leptin (IRS1 gene). Leptin (LEP, OB, OBS), a circulating adipokine, and its receptor LEPR (DB, OBR) control food intake and energy balance and are implicated in obesity-related diseases, cancer, inflammation and angiogenesis. Leptin binding to LEPR induces canonical (JAK2/STATs, MAPK/ERK 1/2, PI-3K/AKT) and non-canonical signaling pathways (PKC, JNK, p38 MAPK and AMPK) in diverse cell types. The long isoform (LEPRb, OBRb) is expressed in the hypothalamus and all types of immune cells.
- PECAM1 interactions (PTPN6 gene). PECAM-1/CD31 is a member of the immunoglobulin superfamily (IgSF) and has been implicated to mediate the adhesion and trans-endothelial migration of T-lymphocytes into the vascular wall, T cell activation and angiogenesis. Under conditions of platelet activation, PECAM-1 is phosphorylated by Src kinase members.
- MHC class II antigen presentation (ACTR1B, HLA-DOB genes). Antigen presenting cells (APCs) such as B cells, dendritic cells (DCs) and monocytes/macrophages express major histocompatibility complex class II molecules (MHC II) at their surface and present exogenous antigenic peptides to CD4+ T helper cells. CD4+ T cells play a central role in immune protection. On their activation they stimulate differentiation of B cells into antibody-producing B cell blasts and initiate adaptive immune responses. Exogenous antigens are internalized by the APC by receptor mediated endocytosis, phagocytosis or pinocytosis into Antigenic peptides are then loaded into the class II ligand-binding groove. Then move to the cell surface, where they are scanned by CD4+ T cells for specific recognition.

DOWN regulated genes in a patient with a condition of classical Hodgkin's lymphoma (cHL), chemotherapy and HAMLET orally influence the following pathways (Table 3C, left panel)

Part I

- Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation. Interleukin-12 signaling. Interleukin-12 family signaling (LMNB1, PITPN genes). IL-12RB2 is considered to play the key role in IL12 function, in part because its expression on activated T cells is stimulated

by cytokines that promote Th1 cell development and inhibited by those that promote Th2 cells development. Receptor dimerization leads to juxtaposition of the cytosolic domains and subsequent tyrosine phosphorylation and activation of JAK2 and TYK2. These Activated kinases, in turn, tyrosine phosphorylate and activate several members of the signal transducer and activator of transcription (STAT) family. The STATs translocate to the nucleus to activate transcription of several genes, including IFN gamma. The production of IFN gamma has a pleiotropic effect in the cell, stimulating production of molecules important to cell mediated immunity.

- Factors involved in megakaryocyte development and platelet production (HBD, MFN1, and MYB genes). Megakaryocytes (MKs) give rise to circulating platelets (thrombocytes) through terminal differentiation of MKs which release cytoplasmic. Fragments as circulating platelets. The processes of megakaryocytopoiesis and platelet production occur within a complex microenvironment where chemokines, cytokines and adhesive interactions play major roles. Megakaryocytopoiesis is regulated at several levels including proliferation, differentiation and platelet release. Thrombopoietin (TPO/c-Mpl ligand) is the most potent cytokine stimulating proliferation and maturation of MK progenitors but many other growth factors are involved. MK development is controlled by the action of multiple transcription factors. Nuclear factor erythroid 2 (NF-E2), which has an MK-erythroid specific 45-kDa subunit, controls terminal MK maturation, proplatelet formation and platelet release.
- Cytokine Signaling in Immune system (LMNB1, PITPN, LRRC14, TRIM25). Cytokines are small proteins that regulate and mediate immunity, inflammation, and hematopoiesis. They are secreted in response to immune stimuli, and usually act briefly, locally, at very low concentrations. Cytokines bind to specific membrane receptors, which then signal the cell via second messengers, to regulate cellular activity.
- Signaling by EGFR (ADAM12, EREG genes). The epidermal growth factor receptor (EGFR) is one member of the ERBB family of transmembrane glycoprotein tyrosine receptor kinases (RTK). Binding of EGFR to its ligands induces conformational change that unmasks the dimerization interface in the extracellular domain of EGFR, leading to receptor homo- or heterodimerization at the cell surface. Ligand activated EGFR dimers trans-autophosphorylate on tyrosine residues in the cytoplasmic tail of the receptor. Phosphorylated tyrosines serve as binding Sites for the recruitment of signal transducers and activators of intracellular substrates, which then stimulate intracellular signal transduction cascades that are involved in regulating cellular proliferation, differentiation, and survival.
- Signaling by Interleukins (LMNB1, PITPN genes). Cellular compartments: plasma membrane. Interleukins are low molecular weight proteins that bind to cell surface receptors and act in an autocrine and/or paracrine fashion. They were first identified as factors produced by leukocytes but are now known to be produced by many other cells throughout the body. They have pleiotropic effects on cells which bind them, impacting processes such as tissue growth and repair, hematopoietic homeostasis, and multiple levels of the host defense against pathogens where they are an essential part of the immune system.
- Vpr-mediated induction of apoptosis by mitochondrial outer membrane permeabilization (SLC25A4, PI2235 genes). Diseases: Human immunodeficiency virus infectious disease. In one model of Vpr mediated induction of apoptosis, Vpr acts directly on the mitochondrial permeability transition pore complex through its interaction with adenine nucleotide translocator (ANT). This interaction promotes the permeabilization of the mitochondrial membranes resulting in the release of cytochrome c and apoptosis-inducing factors.
- ESR-mediated signaling (EREG, MYB genes). Estrogens are a class of hormones that play a role in physiological processes such as development, reproduction, metabolism of liver, fat and bone, and neuronal and cardiovascular function. Estrogens bind estrogen receptors, members of the nuclear receptor superfamily. Ligand-bound estrogen receptors act as nuclear transcription factors to regulate expression of genes that control cellular proliferation and differentiation, among other processes, but also play a non-genomic role in rapid signaling from the plasma membrane.
- RUNX1 regulates transcription of genes involved in differentiation of HSCs (MYB gene). The RUNX1: CBFB complex regulates transcription of the SPI1 (PU.1) gene, involved in differentiation of Hematopoietic stem cells

(HSCs). SPI1 transactivation represses self-renewal and proliferation of HSCs and is needed for commitment of HSCs to specific hematopoietic lineages. As a component of the TAL1 transcription factor complex, involved in acute T cell lymphoblastic leukemia (T-ALL), RUNX1 can promote growth and inhibit apoptosis of hematopoietic stem cells by stimulating transcription of the MYB gene and possibly the TRIB2 gene.

- Signaling by Overexpressed Wild-Type EGFR in Cancer (EREG gene). Diseases: cancer. Signaling by EGFR is frequently activated in cancer through genomic amplification of the EGFR locus, resulting in over-expression of the wild-type protein.
- Hemostasis (HBD, MYB, MFN1, and PFN2). Hemostasis is a physiological response that culminates in the arrest of bleeding from an injured vessel. Under normal conditions the vascular endothelium supports vasodilation, inhibits platelet adhesion and activation, suppresses coagulation, enhances fibrin cleavage and is anti-inflammatory in character. Under acute vascular trauma, vasoconstrictor mechanisms predominate and the endothelium becomes prothrombotic, procoagulatory and proinflammatory in nature. This is achieved by a reduction of endothelial dilating agents: adenosine, NO and prostacyclin, and by the direct action of ADP, serotonin and thromboxane on vascular smooth muscle cells to elicit their contraction. The vessel constricts, reducing the loss of blood. Platelets adhere to the site of injury, become activated and aggregate with fibrinogen into a soft plug that limits blood loss, a process termed primary hemostasis.
- NF-kB activation through FADD/RIP-1 pathway mediated by caspase-8 and 10 (TRIM 25 gene). Cellular compartments: Mitochondrial outer membrane. Fas-Associated Death Domain (FADD) and receptor interacting protein 1 (RIP1) are death domain containing molecules that interact with the C-terminal portion of MAVS (IPS-1) and induce NF-kB through interaction and activation of initiator caspases (caspase-8 and -10). Caspases are usually involved in apoptosis and inflammation but they also exhibit nonapoptotic functions. These nonapoptotic caspase functions involve prodomain-mediated activation of NF-kB. Processed caspases (caspase-8/10) encoding the DED (death effector domain) strongly activate NF-kB. The exact mechanism by which caspases mediate NF-kB activation is unclear, but the prodomains of caspase-8/10 may act as a scaffolding and allow the recruitment of the IKK complex in association with other signaling molecules.

Discussion

The objective of the present study was to evaluate the effect of the combined treatment chemotherapy and Hamlet orally in patient with a condition of classical Hodgkin's lymphoma. Herein, we are presenting the results of the gene expression represented as heat maps of the UP and DOWN regulated genes (values of Z score). In addition, the functionality of the 17 most UP or DOWN regulated genes (values of p <<<) in different pathways of the human biology and under the clinical settings of the case. Hodgkin's lymphoma (HL) a lymphatic cancer tissue/lymphatic system). 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 [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. It is curable even at late stages, however, it depends of several factors, one of them, the age and the type of HL.

In a previous work we have reported that hamlet present in the maternal milk exerted a palliative prophylactic effect in a patient with a condition of Thyroid cancer [34,35]. The effect was measured in the feces and in whole blood. The HAMLET orally administered each four days for two weeks. At each time point feces were recollected and the number of forming colonies (CFUs) measured. Whole blood was recollected at time zero and at the end of the HAMLET orally. Under these settings, it was found that HAMLET reduced the CFUs by at least 0.5 logs with respect to the healthy individual. It induced a higher number of down-regulated genes (around 70 genes), and no difference in the up-regulated

genes versus healthy individuals. The number of pathways affected by both up (n = 109), and down-regulated genes (n = 41), are chromatin organization, developmental biology, circadian clock, and metabolism of proteins, immune system, DNA repair, organelle biogenesis and maintenance, cell cycle, gene expression (transcription) [36,37].

In the present study, following a different protocol, such as daily HAMLET orally for two weeks (Figure 1A), after chemotherapy doses (after the second dose of chemotherapy). Whole blood was recollected at time before HAMLET orally and two weeks after. From the data (Table 1), it was found that before HAMLET orally, there is high number of Down regulated genes (N = 319) than Up regulated genes (n = 87). While after HAMLET orally and chemotherapy the number of genes Up regulated genes increased significantly (n = 140). Down regulated genes was reduced by a very small number of genes (n = 300). In general the pattern of genes DOWN regulated in the patient with the condition of cHL and chemotherapy are: HTR3B, ELAVL3 ELAV like RNA binding protein, SLC67A1, MTFR1L, and TRIM25, UP regulated genes ZNF539/ZNF254, 179, 330, 222 and 197 and SCNN1A (Figure 2A). Of note is that the functionality of the genes using REACTOME base data (Table 2A.1, 2A.2) the pathways in which these genes participate are (Table 3B), cell junction organization, cell-cell communication, Interleukin 35 signaling, TFG-bet signaling, IL-12 signaling, adherents-junction interactions (Table 2B, left panel). UP regulated genes in Metabolism of proteins, sensory perception, gene transcription, and metabolism and cell-cell communication (Table 2B, right panel).

Furthermore, the pattern of genes after HAMLET orally and chemotherapy (Table 3C). UP regulated such as HTR1A, MIEF2, SLC2A9 while maintaining the UP regulation of zinc finger DNA binding proteins such as ZNF 571, 304. In addition, other important genes that participate in the immune system, are up regulated, NFRκβ, HLA-DOB (major histocompatibility complex, class II, DO beta. HLA-DM. Class II molecules are expressed in antigen presenting cells (APC: B lymphocytes, dendritic cells, macrophages). Of note is the up regulation of the gene 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. DOWN regulated genes, include TRIM25, ELAVL3 ELAV like RNA binding protein, SLC25A4 solute carrier family 25 member 4(member of the mitochondrial carrier subfamily of solute carrier protein genes), CLCN5 (voltage gated channel) and of great relevance the DOWN regulation of the MYB oncogene (Figure 2B). Interestingtly it was observed that several genes downregulated form the patient with a condition of cHL,and chemotherapy down, were really downregulated after HAMLET orally and many of them have a role in the mitochondria function. This is important since it has been reported that in the mechanism of action of HAMLET is at the level of the mitochondria, and in the NF-κβ translocation to the nucleus, for the induction of a pro-inflammatory response, in the proteasome regulation, in actin tubulin reorganization, in the binding to the DNA for DNA fragmentation [38]. Indeed, after HAMLET orally there is a DOWN regulation of the genes that participate in the immune system, in the transport of small molecules, in disease, in cell-cell communication, in signal transduction (Table 2A.2, Table 3C, left panel). While the genes UP regulated after HAMLET orally and chemotherapy, that participate in addition to the above mentioned pathways, are functional in hemostasis, protein localization, cell cycle, extracellular matrix organization, in programmed cell death, in Gene transcription, and in cellular response to stimuli (Table 3C. right panel). Of relevance is that VENTX promotes cell cycle arrest and differentiation of hematopoietic stem cells and/or progenitor cells. VENTX induces transcription of cell cycle inhibitors TP53 (p53) and p16INK4A and activates tumor suppressor pathways regulated by TP53 (Table 3C) [39]. The causes of the Hodgkin's lymphoma are unknown, 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]. In patients with HIV, the HL is common. Despite that until now the treatment for HL it has been successful, still it greatly depends of the condition of the individual, the type and the tissue affected as well as social factors. Anyway, the genetic data of the effect of the combined treatment, chemotherapy and HAMLET orally showed that it is potential alternative as palliative and therapeutic since it promotes the expression of genes that function in pathways that play a role in the return to normal condition since the patient with the condition of cHL, the tumor in the neck decreased significantly, and now is back to normal life and to the school.

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Declarations Statement

The authors declare no conflict of interest.

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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