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Hodgkin Lymphoma: A Complex Metabolic Ecosystem with Glycolytic Reprogramming of the Tumor Microenvironment

Lekha Mikkilineni^{1,*}, Diana Whitaker-Menezes^{2,*}, Marina Domingo-Vidal², John Sprandio³, Paola Avena⁴, Paolo Cotzia⁵, Alina Dulau-Florea⁶, Jerald Gong⁷, Guldeep Uppal⁷, Tingting Zhan⁸, Benjamin Leiby⁸, Zhao Lin², Barbara Pro⁹, Federica Sotgia¹⁰, Michael P. Lisanti¹⁰, and Ubaldo Martinez-Outschoorn^{2,+}

¹Department of Medical Oncology National Cancer Institute

²Department of Medical Oncology Thomas Jefferson University

³Department of Medical Oncology Chester County Memorial Hospital

⁴Department of Pharmacy and Health and Nutritional Sciences University of Calabria

⁵Department of Pathology Memorial Sloan Kettering Cancer Center

⁶Department of Pathology National Institutes of Health

⁷Department of Pathology Thomas Jefferson University

⁸Department of Pharmacology and Experimental Therapeutics, Thomas Jefferson University

⁹Division of Medical Oncology Northwestern University

¹⁰School of Environment and Life Sciences University of Salford

Abstract

Background—Twenty percent of patients with classical Hodgkin Lymphoma (cHL) have aggressive disease defined as relapsed or refractory disease to initial therapy. At present we cannot identify these patients pre-treatment. The microenvironment is very important in cHL since non-cancer cells constitute the majority of the cells in these tumors. Non-cancer intra-tumoral cells such as tumor-associated macrophages (TAMs) have been shown to promote tumor growth in cHL via crosstalk with the cancer cells. Metabolic heterogeneity is defined as high mitochondrial metabolism in some tumor cells and glycolysis in others. We hypothesized that there are metabolic differences between cancer cells and non-cancer tumor cells such as TAMs and tumor-infiltrating lymphocytes in cHL and that greater metabolic differences between cancer cells and TAMs are associated with poor outcomes.

*Corresponding Author: Ubaldo Martinez Outschoorn, MD, Department of Medical Oncology Sidney Kimmel Cancer Center, 233 S. 10th Street Suite 909 Philadelphia PA 19107, Phone: 2159558874, ubaldo.martinez-outschoorn@jefferson.edu.

+Co-first authors

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Methods—A case-control study was conducted with 22 tissue samples of cHL at diagnosis from a single institution. The case samples were from 11 patients with aggressive cHL who had relapsed after standard treatment with adriamycin bleomycin vinblastine and dacarbazine (ABVD) or were refractory to this treatment. The control samples were from 11 patients with cHL who achieved a remission and never relapsed after ABVD. Reactive non-cancerous lymph nodes from 4 subjects served as additional controls. Samples were stained by immunohistochemistry for three metabolic markers: translocase of the outer mitochondrial membrane 20 (TOMM20), monocarboxylate transporter 1 (MCT1) and monocarboxylate transporter 4 (MCT4). TOMM20 is a marker of mitochondrial oxidative phosphorylation (OXPHOS) metabolism. Monocarboxylate transporter 1 (MCT1) is the main importer of lactate into cells and is a marker of OXPHOS. Monocarboxylate transporter 4 (MCT4) is the main lactate exporter out of cells and is a marker of glycolysis. The immunoreactivity for TOMM20, MCT1 and MCT4 was scored based on staining intensity and percentage of positive cells, as follows: 0 for no detectable staining in > 50% of cells; 1+ for faint to moderate staining in > 50% of cells, and 2+ for high or strong staining in >50% of cells.

Results—TOMM20, MCT1 and MCT4 expression was significantly different in Hodgkin and Reed Sternberg (HRS) cells, which are the cancerous cells in cHL compared to tumor associated macrophages (TAMs) and tumor-associated lymphocytes. HRS have high expression of TOMM20 and MCT1 while TAMs have absent expression of TOMM20 and MCT1 in all but 2 cases. Tumor-infiltrating lymphocytes have low TOMM20 expression and absent MCT1 expression. Conversely, high MCT4 expression was found in TAMs, but absent in HRS cells in all but 1 case. Tumor-infiltrating lymphocytes had absent MCT4 expression. Reactive lymph nodes in contrast to cHL tumors had low TOMM20, MCT1 and MCT4 expression in lymphocytes and macrophages. High TOMM20 and MCT1 expression in cancer cells with high MCT4 expression in TAMs is a signature of high metabolic heterogeneity between cancer cells and the tumor microenvironment (TME). A high metabolic heterogeneity signature was associated with relapsed or refractory cHL with a hazard ratio of 5.87 [1.16–29.71] (two sided $p < 0.05$) compared to the low metabolic heterogeneity signature.

Conclusion—Aggressive cHL exhibits features of metabolic heterogeneity with high mitochondrial metabolism in cancer cells and high glycolysis in TAMs, which is not seen in reactive lymph nodes. Future studies will need to confirm the value of these markers as prognostic and predictive biomarkers in clinical practice. Treatment intensity may be tailored in the future to the metabolic profile of the tumor microenvironment and drugs that target metabolic heterogeneity may be valuable in this disease.

Keywords

Hodgkin lymphoma; mitochondria; oxidative phosphorylation; glycolysis; lactate; ketone bodies

Introduction

It is estimated that over 9000 new cases of classical Hodgkin lymphoma (cHL) have been diagnosed in the United States in 2016 with over 1100 deaths, the majority of whom were adolescents and young adults who had relapsed or refractory disease¹. The most commonly used treatment for cHL in the United States is combination therapy with adriamycin,

bleomycin, vinblastine and dacarbazine (ABVD) with or without subsequent radiation therapy and approximately 80% of patients are cured with this approach². However, 20% of patients have aggressive cHL, which is defined as relapsed disease or disease which is refractory to initial therapy². In addition, the toxicity of treatment is significant with high rates of heart and lung disease, secondary malignancies and compromised fertility, which not only impact quality of life but increase mortality³. In fact, patients with cHL who are cured of their lymphoma will die most commonly from complications of treatment⁴.

Tools at diagnosis to accurately predict who will relapse or have refractory cHL are imprecise and are not used widely in clinical practice to reduce therapy or use alternative treatments⁵. Also, the majority of patients with high risk features are cured with their initial therapy⁶. In sum, cHL is an aggressive cancer where we need better predictive biomarkers for relapsed or refractory disease. This should allow us to design rational clinical trials in cHL, which provide less intensive curative treatment for the majority of subjects reducing toxicity, and provide alternative treatments for those patients with high-risk disease to improve long-term outcomes.

The Hodgkin Reed Sternberg (HRS) cell is the cancer cell in cHL and its origin is a germinal center or post-germinal center B cell^{7,8}. Specifically, this large cancer cell is called the Reed Sternberg cell if binucleated or Hodgkin cell if mononucleated. Interestingly, non-cancerous cells far outnumber cancer cells in cHL tumors and less than 1% of the tumor cells are HRS on average^{9,10}. The importance of non-cancer cells in these tumors is highlighted by the fact that the classification of cHL is based on the tumor microenvironment (TME) or non-cancerous cells within the tumor⁸. The four morphologic subtypes of cHL are nodular sclerosis (NS), mixed cellularity (MC), lymphocyte rich (LR) and lymphocyte depleted (LD)⁷. The TME in cHL consists of many different cell types such as tumor-associated macrophages (TAMs), reactive lymphocytes (RLs), fibroblasts, eosinophils, mast cells and plasma cells. HRS cells recruit non-cancer cells to the tumor and there is substantial cross-talk between these cells to favor the growth of the cancer cells allowing proliferation and resistance to cell death^{9,11}. The mediators of the crosstalk between TME cells and HRS include cytokines, chemokines, immune checkpoint receptors and the extracellular matrix^{9,11,12}. Markers of crosstalk between the TME and HRS have been studied in cHL and the number of macrophages in the tumor is a predictive biomarker of response to therapy⁹. Predictive biomarkers, attempting to reflect the underlying biology of cHL also have been studied. These include circulating cytokines, chemokines and soluble receptors such as CD30^{13,14,15,16,17}. Analyses of the tumor itself by protein expression or gene expression profiling also have been performed^{18,19,20}. However, none of the above markers is sufficiently reliable to predict outcomes for patients with cHL^{22,21}. We set out to investigate if metabolic markers in cHL can serve to predict outcomes.

Mitochondrial metabolism was studied here by measuring expression of translocase of the outer mitochondrial membrane subunit 20 (TOMM20). TOMM20 is a translocase found in the outer mitochondrial membrane, which recognizes and transports into mitochondria cytosolic proteins that are subunits of the oxidative phosphorylation (OXPHOS) machinery²². The majority of mitochondrial OXPHOS subunits are nuclearly encoded and TOMM20 provides these subunits to the mitochondria²³. TOMM20 expression is directly

related to OXPHOS as measured by oxygen consumption rates and its expression has been shown to be associated with complex IV activity²⁴²⁵²⁶²⁷²⁸. Cytochrome C Oxidase (COX) is the terminal enzyme in the mitochondrial electron transport chain required for OXPHOS to generate ATP²⁹. Low COX activity is a marker of mitochondrial dysfunction and is the gold standard to diagnose human myopathies and neurologic diseases due to mitochondrial dysfunction^{3031,32}.

Monocarboxylate transporters are a family of membrane proteins (MCT) that demonstrate proton-linked passive symport of lactate and pyruvate into and out of cells³³. MCT1 is expressed in many cell types and cancer cell lines and is associated with lactate uptake³³³⁴. MCT1 is upregulated and expressed most prominently in cells with increased mitochondrial OXPHOS, such as heart and red muscle, suggesting an important role in lactic acid and ketone body oxidation³⁴³⁵. MCT4 is the main transporter of lactate out of cells with some ability to also export ketone bodies out of cells; it acts as a marker of oxidative stress³⁴³⁶³⁷³⁸. The expression of MCT4 is upregulated by HIF-1 α which is the main glycolytic transcription factor induced by oxidative stress and hypoxia³⁸. In sum, MCT1 and MCT4 are markers of metabolic crosstalk.

Markers of metabolism may be valuable to predict relapse in cHL. This is due to the fact that most cHL tumors have high glucose uptake on the basis of ¹⁸F-2-deoxy-glucose positron emission tomography (FDG-PET) scans and that loss of FDG-PET avidity in interim FDG-PET scans has been shown to be associated with a low risk of relapse or refractory cHL⁶. Hence, metabolic characteristics at diagnosis may be valuable as predictive biomarkers of response to therapy. Despite the fact that cHL tumors are very metabolically active with high glucose uptake and that non-cancer cells are the majority of cells within a tumor, a rigorous metabolic characterization of these tumor cell populations has not been undertaken. It is widely recognized that tumors generate lactate, which is the end product of glycolysis, yet it is unknown if all cells in a tumor or only some produce lactate. It has been argued that high FDG-PET avidity in cHL indicates that the cancer cells in these tumors are glycolytic yet since they are a minority of the cells that make up the tumor this is not a foregone conclusion. Also, it is important to highlight that FDG-PET avidity only indicates that glucose has been taken up by cells in a tumor and it does not provide information on whether the fate of glucose is glycolysis with lactate generation or mitochondrial OXPHOS with carbon dioxide and water generation³⁹. In this study we sought to metabolically characterize different cell populations in cHL and determine if there is an association between metabolic profiles and outcomes.

Methods/Materials

Three biomarkers based on immunohistochemistry were employed to assess the metabolic compartments of classical Hodgkin lymphoma (cHL). Translocase of outer mitochondrial membrane 20 (TOMM20), monocarboxylate transporter 1 (MCT1) and monocarboxylate transporter 4 (MCT4) protein expression was assessed.

We selected 22 cases of cHL treated at Thomas Jefferson University Hospital from 2009 to 2013. Eleven subjects achieved a complete remission and have not relapsed to date. Eleven

subjects had relapsed disease after adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) or had progressive refractory disease leading to death. The diagnosis of classical Hodgkin lymphoma (cHL) was confirmed independently by three hematopathologists who were blinded to outcomes and was based on morphology and immunohistochemistry (IHC). We identified tissues that showed the binucleated Reed-Sternberg cells and/or mononuclear Hodgkin cells surrounded by an inflammatory background of small lymphocytes and scattered histiocytes. Immunohistochemical stains were performed on 5-micron thick, formalin-fixed, paraffin-embedded tissue sections of 22 cHL tumors, using the horseradish peroxidase method. The primary antibodies used were: CD30, CD45, CD15, TOMM20, MCT1 and MCT4. Immunohistochemistry as expected revealed CD30 expressing HRS cells with variable CD15 expression, absence of CD45 and CD3 and dim or negative CD20 expression in all the cases. CD3 staining was present in the majority of reactive small lymphocytes while CD15 stained scattered histiocytes. We evaluated each case with low magnification fields (10X) and 5 higher magnification fields (40X).

The immunoreactivity for TOMM20 MCT1 and MCT4 was evaluated based on staining intensity and percentage of positive cells, as follows: 0 for no detectable staining in > 50% of cells; 1+ for faint to moderate stain in > 50% of cells, and 2+ for strong stain in >50% of cells. Small lymphocytes were mostly CD3-positive T-cells. 3 pathologists obtained final scoring after consensus review. Chi-square was performed for categorical variables and Student's t-test for variables on an interval scale. Kaplan Meier curves were plotted for progression free survival comparing tumor-staining patterns and a Cox proportional Hazards ratio was carried out.

Results

Our total patient group consisted of 22 patients who were diagnosed with classical Hodgkin lymphoma (cHL) from 2009 to 2013 at Thomas Jefferson University Hospital. The baseline characteristics of these patients are described in Table 1. The average age of the patients was 37 with the majority of patients (65%) being less than 40 years old at diagnosis. Thirteen of our 22 patients were men. Only 1 patient was infected with HIV. Of the 22 patients studied, 34% suffered a relapse and one patient had refractory disease (Table 1).

The majority of the samples had high TOMM20 and MCT1 expression in Hodgkin/Reed Sternberg cells (HRS), which are the cancer cells in cHL (Table 2 and Figures 1–3). The highest TOMM20 expression out of all cells analyzed was found in the HRS cells in 18 of the 22 samples. The non-cancer cell compartment in proximity to cancer cells had low functional mitochondrial mass and low monocarboxylate utilization as evidenced by low TOMM20 expression and low MCT1 expression (Table 2). TOMM20 expression in TAMs and tumor infiltrating lymphocytes was low and in TAMs was scored as 0 in 20 of the 22 samples and in tumor infiltrating lymphocytes was scored as 1 in 20 of 22 samples (Table 2).

The mean intensity score for TOMM20 in HRS was 1.8 and the median and mode intensity score was 2, while as for TAMs the mean intensity score was 0.1 and the median and mode intensity score was 0. The mean intensity score for TOMM20 in tumor infiltrating lymphocytes was 0.9 and the median and mode intensity score was 1. The mean intensity for

MCT1 in HRS was 1.6 and the median and mode intensity score was 2, while as for TAMs it was 0. MCT1 intensity was 0 in tumor-infiltrating lymphocytes. There was a statistically significant difference in intensity scores for TOMM20 and MCT1 comparing HRS to TAMs ($p<0.05$). In sum, the expression patterns for TOMM20 and MCT1 in non-cancer cells were opposite to what was observed in HRS cancer cells with high expression in cancer cells and low expression in non-cancer cells (Table 2).

MCT4, which is a marker of glycolysis was highly expressed in TAMs but there was low expression in HRS cells and tumor-infiltrating lymphocytes in cHL tumors (Table 2 and Figure 1–3). The mean intensity for MCT4 in TAMs was 1.8 and the median and mode intensity score was 2, while as for HRS the mean intensity score was 0.05 and the median and mode intensity score was 0. Tumor-infiltrating lymphocytes intensity scores for MCT4 were 0. There was a statistically significant difference in intensity scores for MCT4 comparing HRS to TAMs ($p<0.05$). Note that the MCT4 expression of HRS was 0 in 21 of 22 samples and the scoring in the other sample was 1.

We next compared staining patterns in cHL tumors and reactive non-cancerous lymph nodes. We focused on comparing HRS cells to lymphocytes in non-cancerous lymph nodes since these are their normal counterpart. We also compared TAMs in cHL to macrophages in non-cancerous reactive lymph nodes since these are their normal counterparts. The reactive lymph nodes had absent TOMM20, MCT1 and MCT4 staining globally and specifically in lymphocytes and macrophages. TOMM20 and MCT1 expression was high in HRS but low in lymphocytes in reactive lymph nodes (Table 2). There was a statistically significant difference in intensity scores for TOMM20 and MCT1 comparing HRS to lymphocytes in reactive lymph nodes ($p<0.05$). MCT4 expression was high in TAM but was low in macrophages in reactive LNs (Table 2). There was a statistically significant difference in intensity scores for MCT4 comparing TAMs to macrophages in reactive lymph nodes ($p<0.05$).

Two metabolic compartments exist: a mitochondrial metabolism compartment with high TOMM20 and MCT1 expression and a glycolytic and a lactate exporting tumor associate macrophage compartment (Table 2 and Figures 1–3). However, there was variability in the expression of these markers in the different cHL tumors (Table 2). We grouped the tumors as having high metabolic heterogeneity if the tumors had high expression of TOMM20 and MCT1 in HRS cancer cells (2+) and high expression of MCT4 in tumor associated macrophages (TAMs) (2+). Tumors without this previously described expression pattern were considered tumors with low metabolic heterogeneity (TOMM20 and MCT1 in HRS not 2+ and/or MCT4 in TAMs not 2+).

All reactive lymph nodes also displayed a TOMM20, MCT1 and MCT4 staining pattern of low metabolic heterogeneity (Table 3). A majority of relapsed and refractory cases had high metabolic heterogeneity (9 of 11 cases) (Table 3). Also, three patients had experienced fatal recurrence. Their tumor analysis showed strong metabolic compartmentalization. The majority of tumors from patients who did not relapse or have refractory disease had low metabolic heterogeneity (10 of 11 subjects) (Table 3). Progression free survival was plotted for patients with high metabolic heterogeneity and low metabolic heterogeneity in their

tumors. High metabolic heterogeneity was associated with shorter progression free survival (Figure 4). The Cox proportional Hazards Ratio for progression comparing high metabolic heterogeneity versus low metabolic heterogeneity was 5.87 (1.16–29.71) with a $p < 0.05$. A very low relapse rate was observed in subjects with low metabolic heterogeneity. These patients continue to be followed and 80% have not had a relapse despite 40% having stage three or four disease.

Discussion

The current study demonstrates the existence of two metabolic compartments in classical Hodgkin lymphoma (cHL) tumors. The sparsely strewn HRS cells have high expression of TOMM20, a marker for mitochondrial mass, and stand in stark contrast to the more abundant population of stromal cells which include tumor-associated macrophages that highly express MCT4, a marker of lactate export. Although Otto Warburg, a pioneer of cancer metabolism, hypothesized that glycolysis was the metabolic pathway utilized exclusively by cancer cells in tumors, there is growing evidence that the stroma also utilizes glucose at high rates in tumors⁴⁰. Cancer cells may thrive when they are able to reprogram their neighboring cells into catabolic machinery to support tumor growth. The current study suggests that cancer cells in Hodgkin lymphoma transform neighboring cells to induce aggressive tumors.

Specifically, cancer cells transform their neighboring stromal cells into a catabolic state by inducing autophagy and mitophagy, which results in a shift to aerobic glycolysis^{41,35}. By doing so, the malignant cell creates an environment where the tumor stroma becomes a generator of high-energy mitochondrial substrates such as lactate, ketone bodies and glutamine for cancer cell consumption, enabling the malignant cells to generate ATP efficiently by mitochondrial oxidative phosphorylation (OXPHOS)⁴². This shift to catabolism and aerobic glycolysis by tumor stromal cells can be measured by evaluating the expression of monocarboxylate transporter 4 (MCT4), which is the main exporter of intracellular lactate. The expression of MCT4 correlates with glycolytic activity⁴³. In turn, the lactate secreted via MCT4 is taken up by cancer cells via monocarboxylate transporter 1 (MCT1), leading to the generation of ATP via OXPHOS⁴⁴. The metabolic relationships between cancer and non-cancer cells within a tumor has been studied in several human malignancies including breast, ovarian, prostate, colon, head and neck, thyroid, sarcoma and lymphoma and has been termed two compartment metabolism or metabolic symbiosis^{35,43}. The current study demonstrates that metabolic heterogeneity exists in cHL.

The microenvironment is very important in cHL since non-cancer cells constitute the majority of the cells in these tumors. cHL has been described as a paradigm of how cancer cells transform the tumor microenvironment to promote the expansion of malignant cells^{7,911124546474849}. Aberrant chemokine and cytokine production stimulates the growth and migration of CD4+ memory cells, regulatory T cells, macrophages, fibroblasts, eosinophils and mast cells¹¹⁵⁰.

Targeting the interactions between lymphoma cells and their microenvironment is already being utilized clinically in cHL. T cells allow HRS cells to evade the adaptive immune

system⁵¹⁵⁰. The PD-1 inhibitors, nivolumab and pembrolizumab, are effective treatments for refractory cHL⁵¹⁵². PD ligands found on HRS cells and macrophages, PDL-1 and PDL-2, engage their PD-1 receptor counterpart on T cells and induce a state of “exhaustion” where the T cell is subsequently inactivated and its proliferation is inhibited⁵¹. By blocking PD ligand-receptor interactions with drugs such as nivolumab and pembrolizumab, the adaptive immune system can kill the cancer cells. Studies to understand the cancer-microenvironment metabolic relationship hold promise to develop drugs that target tumor metabolism in cHL. Treatment intensity may also be tailored in the future to the metabolic profile of the tumor microenvironment.

The discovery that metabolic heterogeneity exists in cHL may lead to a better understanding of which intratumoral compartment is being visualized with ¹⁸F-2-deoxy-glucose (FDG) positron emission tomography (PET) imaging, more refined prognostication and novel drug development. Cancer’s distinct metabolic profile allows the use of FDG-PET imaging. FDG-PET is routinely used to evaluate stage and response to therapy in Hodgkin lymphoma since FDG uptake is very high in lymphoma tumors and rapidly decreases in tumors that are responding appropriately to therapy⁵³⁵⁴⁶. FDG-PET avidity in cHL is likely measuring glycolytic activity of the tumor stroma since they are the major component and novel PET methods using lactate as a probe may be valuable in this disease.

To further risk stratify patients with cHL, clinicians might utilize TOMM20, MCT1 and MCT4 immunohistochemical staining in the future. Metabolic characterization of patient samples could focus on cancer cells and tumor associated macrophages to make analysis easier since these two cell types display the greatest metabolic differences and there is interpatient variation in cHL. Cancer cells that have robust mitochondrial function as indicated by strong TOMM20 expression may have a higher tolerance to chemotherapy drugs that disrupt OXPHOS function such as adriamycin. Similarly, stroma that has been glycolytically transformed with high MCT4 expression may be more resistant to drugs that alter the cellular redox state. Targeted therapy aimed at reducing OXPHOS with drugs such as arsenic or metformin in combination with other biologic agents such as PD1 inhibitors may be synergistic³⁵⁵⁵. Also, agents that inhibit the transport of lactate into HRS cells would cut the nutrient supply source to lymphoma cells, essentially starving the cancer cells. Therapy success may be dependent on affecting preferentially HRS and transformed stromal cells without affecting normal cells.

This study provides the first evidence of metabolic heterogeneity in cHL. Also, metabolic heterogeneity in cHL is associated with poor outcomes with higher rates of relapsed and refractory disease. Hodgkin lymphoma provides a unique opportunity to explore the relationship between cancer cells and its environment because the majority of tumor cells are non-cancerous. Future studies will need to confirm if metabolic heterogeneity with high cancer cell expression of TOMM20 and MCT1 with high TAM expression of MCT4 is a prognostic and predictive biomarker in cHL that can be used in clinical practice. Studies will also need to assess if targeting metabolic heterogeneity between cancer and non-cancer cells in cHL tumors is an effective anticancer strategy.

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Bibliography

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016; 66:7–30. [PubMed: 26742998]
2. Johnson P, McKenzie H. How I treat advanced classical Hodgkin lymphoma. *Blood*. 2015; 125:1717–23. [PubMed: 25564404]
3. Bartlett NL. Fine-Tuning the Treatment of Hodgkin's Lymphoma. *N Engl J Med*. 2016; 374:2490–2. [PubMed: 27332908]
4. Matasar MJ, Ford JS, Riedel ER, Salz T, Oeffinger KC, Straus DJ. Late morbidity and mortality in patients with Hodgkin's lymphoma treated during adulthood. *J Natl Cancer Inst*. 2015:107.
5. Connors JM. Risk assessment in the management of newly diagnosed classical Hodgkin lymphoma. *Blood*. 2015; 125:1693–702. [PubMed: 25575542]
6. Johnson P, Trotman J, Federico M. Interim PET-CT Scan in Advanced Hodgkin's Lymphoma. *N Engl J Med*. 2016; 375:999–1000.
7. Kuppers R, Engert A, Hansmann ML. Hodgkin lymphoma. *J Clin Invest*. 2012; 122:3439–47. [PubMed: 23023715]
8. Mathas S, Hartmann S, Kuppers R. Hodgkin lymphoma: Pathology and biology. *Semin Hematol*. 2016; 53:139–47. [PubMed: 27496304]
9. Steidl C, Lee T, Shah SP, et al. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. *N Engl J Med*. 2010; 362:875–85. [PubMed: 20220182]
10. Pileri SA, Ascani S, Leoncini L, et al. Hodgkin's lymphoma: the pathologist's viewpoint. *J Clin Pathol*. 2002; 55:162–76. [PubMed: 11896065]
11. Aldinucci D, Gloghini A, Pinto A, De Filippi R, Carbone A. The classical Hodgkin's lymphoma microenvironment and its role in promoting tumour growth and immune escape. *J Pathol*. 2010; 221:248–63. [PubMed: 20527019]
12. Steidl C, Connors JM, Gascoyne RD. Molecular pathogenesis of Hodgkin's lymphoma: increasing evidence of the importance of the microenvironment. *J Clin Oncol*. 2011; 29:1812–26. [PubMed: 21483001]
13. Casasnovas RO, Mounier N, Brice P, et al. Plasma cytokine and soluble receptor signature predicts outcome of patients with classical Hodgkin's lymphoma: a study from the Groupe d'Etude des Lymphomes de l'Adulte. *J Clin Oncol*. 2007; 25:1732–40. [PubMed: 17389336]
14. Visco C, Nadali G, Vassilakopoulos TP, et al. Very high levels of soluble CD30 recognize the patients with classical Hodgkin's lymphoma retaining a very poor prognosis. *Eur J Haematol*. 2006; 77:387–94. [PubMed: 16879607]
15. Sarris AH, Kliche KO, Pethambaram P, et al. Interleukin-10 levels are often elevated in serum of adults with Hodgkin's disease and are associated with inferior failure-free survival. *Ann Oncol*. 1999; 10:433–40. [PubMed: 10370786]
16. Vassilakopoulos TP, Nadali G, Angelopoulou MK, et al. Serum interleukin-10 levels are an independent prognostic factor for patients with Hodgkin's lymphoma. *Haematologica*. 2001; 86:274–81. [PubMed: 11255274]
17. Younes A, Gopal AK, Smith SE, et al. Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. *J Clin Oncol*. 2014; 30:2183–9.
18. Scott DW, Chan FC, Hong F, et al. Gene expression-based model using formalin-fixed paraffin-embedded biopsies predicts overall survival in advanced-stage classical Hodgkin lymphoma. *J Clin Oncol*. 2013; 31:692–700. [PubMed: 23182984]
19. Steidl C, Telenius A, Shah SP, et al. Genome-wide copy number analysis of Hodgkin Reed-Sternberg cells identifies recurrent imbalances with correlations to treatment outcome. *Blood*. 2010; 116:418–27. [PubMed: 20339089]

20. Chetaille B, Bertucci F, Finetti P, et al. Molecular profiling of classical Hodgkin lymphoma tissues uncovers variations in the tumor microenvironment and correlations with EBV infection and outcome. *Blood*. 2009; 113:2765–3775. [PubMed: 19096012]
21. Moccia AA, Donaldson J, Chhanabhai M, et al. International Prognostic Score in advanced-stage Hodgkin's lymphoma: altered utility in the modern era. *J Clin Oncol*. 2012; 30:3383–8. [PubMed: 22869887]
22. Yano M, Kanazawa M, Terada K, Takeya M, Hoogenraad N, Mori M. Functional analysis of human mitochondrial receptor Tom20 for protein import into mitochondria. *J Biol Chem*. 1998; 273:26844–51. [PubMed: 9756929]
23. Yano M, Terada K, Mori M. Mitochondrial import receptors Tom20 and Tom22 have chaperone-like activity. *J Biol Chem*. 2004; 279:10808–13. [PubMed: 14699115]
24. Wurm CA, Neumann D, Lauterbach MA, et al. Nanoscale distribution of mitochondrial import receptor Tom20 is adjusted to cellular conditions and exhibits an inner-cellular gradient. *Proc Natl Acad Sci U S A*. 2011; 108:13546–51. [PubMed: 21799113]
25. Whitaker-Menezes D, Martinez-Outschoorn UE, Flomenberg N, et al. Hyperactivation of oxidative mitochondrial metabolism in epithelial cancer cells in situ: visualizing the therapeutic effects of metformin in tumor tissue. *Cell Cycle*. 2011; 10:4047–64. [PubMed: 22134189]
26. Curry JM, Tuluc M, Whitaker-Menezes D, et al. Cancer metabolism, stemness and tumor recurrence: MCT1 and MCT4 are functional biomarkers of metabolic symbiosis in head and neck cancer. *Cell Cycle*. 2013; 12:1371–84. [PubMed: 23574725]
27. Gehrke S, Wu Z, Klinkenberg M, et al. PINK1 and Parkin control localized translation of respiratory chain component mRNAs on mitochondria outer membrane. *Cell Metab*. 2015; 21:95–108. [PubMed: 25565208]
28. Ko YH, Domingo-Vidal M, Roche M, et al. TIGAR Metabolically Reprograms Carcinoma and Stromal Cells in Breast Cancer. *J Biol Chem*. 2016
29. Fontanesi F, Soto IC, Horn D, Barrientos A. Assembly of mitochondrial cytochrome c-oxidase, a complicated and highly regulated cellular process. *Am J Physiol Cell Physiol*. 2006; 291:C1129–47. [PubMed: 16760263]
30. Papadopoulou LC, Sue CM, Davidson MM, et al. Fatal infantile cardioencephalomyopathy with COX deficiency and mutations in SCO2, a COX assembly gene. *Nat Genet*. 1999; 23:333–7. [PubMed: 10545952]
31. Borthwick GM, Johnson MA, Ince PG, Shaw PJ, Turnbull DM. Mitochondrial enzyme activity in amyotrophic lateral sclerosis: implications for the role of mitochondria in neuronal cell death. *Ann Neurol*. 1999; 46:787–90. [PubMed: 10553999]
32. Ross JM. Visualization of mitochondrial respiratory function using cytochrome c oxidase/succinate dehydrogenase (COX/SDH) double-labeling histochemistry. *J Vis Exp*. 2011:e3266. [PubMed: 22143245]
33. Sonveaux P, Vegran F, Schroeder T, et al. Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. *J Clin Invest*. 2008; 118:3930–42. [PubMed: 19033663]
34. Bonen A. The expression of lactate transporters (MCT1 and MCT4) in heart and muscle. *Eur J Appl Physiol*. 2001; 86:6–11. [PubMed: 11820324]
35. Martinez-Outschoorn UE, Peiris-Pages M, Pestell RG, Sotgia F, Lisanti MP. Cancer metabolism: a therapeutic perspective. *Nat Rev Clin Oncol*. 2016
36. Dimmer KS, Friedrich B, Lang F, Deitmer JW, Broer S. The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. *Biochem J*. 2000; 350(Pt 1):219–27. [PubMed: 10926847]
37. Brooks GA. Cell-cell and intracellular lactate shuttles. *J Physiol*. 2009; 587:5591–600. [PubMed: 19805739]
38. Ullah MS, Davies AJ, Halestrap AP. The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1 α -dependent mechanism. *J Biol Chem*. 2006; 281:9030–7. [PubMed: 16452478]
39. Erez A, DeBerardinis RJ. Metabolic dysregulation in monogenic disorders and cancer - finding method in madness. *Nat Rev Cancer*. 2015; 15:440–8. [PubMed: 26084394]

40. DeNicola GM, Cantley LC. Cancer's Fuel Choice: New Flavors for a Picky Eater. *Mol Cell*. 2015; 60:514–23. [PubMed: 26590711]
41. Wallace DC. Mitochondria and cancer. *Nat Rev Cancer*. 2012; 12:685–98. [PubMed: 23001348]
42. Martinez-Outschoorn UE, Lisanti MP, Sotgia F. Catabolic cancer-associated fibroblasts transfer energy and biomass to anabolic cancer cells, fueling tumor growth. *Semin Cancer Biol*. 2014; 25:47–60. [PubMed: 24486645]
43. Bovenzi CD, Hamilton J, Tassone P, et al. Prognostic Indications of Elevated MCT4 and CD147 across Cancer Types: A Meta-Analysis. *Biomed Res Int*. 2015; 2015:242437. [PubMed: 26779534]
44. Martinez-Outschoorn UE, Sotgia F, Lisanti MP. Power surge: supporting cells “fuel” cancer cell mitochondria. *Cell Metab*. 2012; 15:4–5. [PubMed: 22225869]
45. Kuppers R. The biology of Hodgkin's lymphoma. *Nat Rev Cancer*. 2009; 9:15–27. [PubMed: 19078975]
46. Banerjee D. Recent Advances in the Pathobiology of Hodgkin's Lymphoma: Potential Impact on Diagnostic, Predictive, and Therapeutic Strategies. *Adv Hematol*. 2011; 2011:439456. [PubMed: 21318045]
47. Kamper P, Bendix K, Hamilton-Dutoit S, Honore B, Nyengaard JR, d'Amore F. Tumor-infiltrating macrophages correlate with adverse prognosis and Epstein-Barr virus status in classical Hodgkin's lymphoma. *Haematologica*. 2011; 96:269–76. [PubMed: 21071500]
48. Tan KL, Scott DW, Hong F, et al. Tumor-associated macrophages predict inferior outcomes in classic Hodgkin lymphoma: a correlative study from the E2496 Intergroup trial. *Blood*. 2012; 120:3280–7. [PubMed: 22948049]
49. Montanari F, Diefenbach CS. Hodgkin lymphoma: targeting the tumor microenvironment as a therapeutic strategy. *Clin Adv Hematol Oncol*. 2015; 13:518–24. [PubMed: 26351815]
50. Liu Y, Sattarzadeh A, Diepstra A, Visser L, van den Berg A. The microenvironment in classical Hodgkin lymphoma: an actively shaped and essential tumor component. *Semin Cancer Biol*. 2014; 24:15–22. [PubMed: 23867303]
51. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med*. 2015; 372:311–9. [PubMed: 25482239]
52. Armand P, Shipp MA, Ribrag V, et al. Programmed Death-1 Blockade With Pembrolizumab in Patients With Classical Hodgkin Lymphoma After Brentuximab Vedotin Failure. *J Clin Oncol*. 2016
53. Hutchings M, Loft A, Hansen M, et al. FDG-PET after two cycles of chemotherapy predicts treatment failure and progression-free survival in Hodgkin lymphoma. *Blood*. 2006; 107:52–9. [PubMed: 16150944]
54. Tirelli U, Spina M. PET-adapted salvage therapy in Hodgkin's lymphoma. *Lancet Oncol*. 2015
55. Scharping NE, Menk AV, Whetstone RD, Zeng X, Delgoffe GM. Efficacy of PD-1 Blockade Is Potentiated by Metformin-Induced Reduction of Tumor Hypoxia. *Cancer Immunol Res*. 2017; 5:9–16. [PubMed: 27941003]

Fig. 1. TOMM20, MCT1 and MCT4 staining in classical Hodgkin lymphoma (cHL)

Samples were stained and the intensity was assessed in cancer cells (HRS) and non-cancer cells within tumors. Note that the neoplastic cells in cHL have high expression of TOMM20 and MCT1 with low expression of MCT4. The stromal cells have an opposite pattern with low expression of TOMM20 and MCT1 with high expression of MCT4.

Fig. 2. MCT1 and CD68 double labeling and MCT4 and CD68 double labeling in cHL

A negative control is shown in addition to the MCT1 and MCT4 double labeling with the macrophage marker CD68 by immunohistochemistry. Note that cancer cells express MCT1 (brown) while as the macrophages that express CD68 (red) do not express MCT1. Also, note that macrophages expressing CD68 (red) express MCT4 (brown) while as the cancer cells do not express MCT4.

Fig. 3. CD68 and MCT1 staining by immunofluorescence in cHL

Immunofluorescence was performed on cHL samples staining for CD68 in green, MCT1 in red and DAPI blue counterstain to highlight nuclei. Note that CD68 macrophages do not stain for MCT1 since the red and green stains are not found on the same cells.

Fig. 4. TOMM20, MCT1 and MCT4 staining patterns in cHL and outcomes

Progression free survival was graphed according to whether the tumor sample had high or low metabolic heterogeneity. High metabolic heterogeneity was defined as having high TOMM20 and MCT1 expression in cancer cells (HRS) and high MCT4 in tumor associated macrophages (TAMs). Specifically, high metabolic heterogeneity was defined as 2+ TOMM20 and 2+MCT1 in HRS and 2+MCT4 in TAMs. Any other expression pattern was defined as low metabolic heterogeneity. Note that high metabolic heterogeneity in cHL tumors is associated with shorter progression free survival ($p < 0.05$).

Fig. 5. Model of Cancer-Macrophage Metabolic Heterogeneity in cHL

A model of two-compartment tumor metabolism with neoplastic-macrophage metabolic heterogeneity is shown. The tumor-associated macrophages (TAMs) have high MCT4 expression, which is consistent with high generation and release of monocarboxylates and low mitochondrial metabolism. The neoplastic cells in cHL or Hodgkin/Reed Sternberg cells (HRS) have high MCT1 expression, which is consistent with high utilization of monocarboxylates. HRS also have high mitochondrial metabolism with high TOMM20 expression.

Table 1

Baseline characteristics

Demographics	Number of Patients (Percentage, N=22)
Male	13 (59%)
Female	9 (41%)
Average Age	
Patients less than 40 years old	14 (64%)
Patients greater than 40 years old	8 (36%)
Patients who relapsed	9(41%)
Patients with refractory disease	2 (9%)
Patients with B symptoms [*] on presentation	9(41%)
HIV + patients	1 (5%)
Histological Subtype	
Nodular Sclerosis (NS)	15 (68%)
Mixed Cellularity (MC)	4 (18%)
Not Otherwise Specified (NOS)	3 (14%)

^{*} B-symptoms on initial presentation including fever > 100.4, drenching night sweats and greater than 10 pound weight loss.

Table 2

Staining characteristics of samples from cases and controls.

Patient	Subtype	Rel/Refr	TOMM20			MCT1			MCT4		
			HRS	L	TAM	HRS	L	TAM	HRS	L	TAM
RRcHL-1	NOS	Y	2	1	0	2	0	0	1	0	2
RRcHL-2	NS	Y	2	1	0	2	0	0	0	0	2
RRcHL-3	NS	Y	2	1	0	2	0	0	0	0	2
RRcHL-4	MC	Y	2	1	0	2	0	0	0	0	2
RRcHL-5	NOS	Y	2	1	0	2	0	0	0	0	2
RRcHL-6	NS	Y	2	1	0	2	0	0	0	0	2
RRcHL-7	NOS	Y	2	1	0	2	0	0	0	0	2
RRcHL-8	NS	Y	2	1	1	2	0	0	0	0	2
RRcHL-9	NS	Y	2	1	0	2	0	0	0	0	2
RRcHL-10	NS	Y	2	0	0	1	0	0	0	0	2
RRcHL-11	NS	Y	2	1	0	1	0	0	0	0	1
NRcHL-2	MC	N	2	1	0	2	0	0	0	0	2
NRcHL-6	NS	N	2	1	0	2	0	0	0	0	1
NRcHL-9	NS	N	2	1	0	2	0	0	0	0	1
NRcHL-8	NS	N	2	1	0	1	0	0	0	0	1
NRcHL-11	MC	N	2	1	0	1	0	0	0	0	2
NRcHL-10	NS	N	2	1	0	1	0	0	0	0	2
NRcHL-7	NS	N	2	1	1	1	0	0	0	0	2
NRcHL-4	NS	N	1	1	0	2	0	0	0	0	2
NRcHL-5	NS	N	1	1	0	2	0	0	0	0	2
NRcHL-1	NS	N	1	0	0	2	0	0	0	0	2
NRcHL-3	MC	N	1	1	0	0	0	0	0	0	1
RL-1	React LN	N/A	N/A	0	0	N/A	0	0	N/A	0	0
RL-2	React LN	N/A	N/A	0	0	N/A	0	0	N/A	0	0
RL-3	React LN	N/A	N/A	0	0	N/A	0	0	N/A	0	0
RL-4	React LN	N/A	N/A	0	0	N/A	0	0	N/A	0	0

RRcHL-Relapsed or refractory classical Hodgkin lymphoma tumor. NRcHL-Non-relapsed or refractory classical Hodgkin lymphoma. React LN-Reactive non-cancerous lymphadenopathy. HRS-Hodgkin Reed Sternberg Cell, TAM-Tumor Associated Macrophage, L-reactive lymphocyte, NS- Nodular sclerosis subtype of cHL, NOS- Not Otherwise Specified, MC-mixed cellularity, N/A-not applicable.

Table 3

Metabolic Heterogeneity staining patterns in cases and controls.

	Cases-RRcHL	Controls-NRcHL	Controls-Reactive LN	Total
High Metabolic Heterogeneity	9	1	0	10
Low Metabolic Heterogeneity	2	10	4	16
Total	11	11	4	26

Relapsed refractory classical Hodgkin lymphoma (RRcHL), Non-relapsed refractory classical Hodgkin lymphoma (NRcHL), Non-cancerous reactive lymphadenopathy (Reactive LN). Definitions of High and Low Metabolic Heterogeneity are as follows: High Metabolic Heterogeneity is 2+TOMM20 in HRS, 2+ MCT1 in HRS and 2+ MCT4 in TAM. Low Metabolic Heterogeneity is any pattern excluding that of High Metabolic Heterogeneity.