Review

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Hodgkin lymphoma cell lines: to separate the wheat from the chaff

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Abstract: Characteristic components of Hodgkin lymphoma (HL) tissue are the mono- or multinucleated Hodgkin-Reed-Sternberg (HRS) cells. Given the challenges of isolating these rare malignant cells and the difficulty in culturing cells from patients, many investigators have tried to establish cell lines in efforts to develop cellular tools for *in vitro* studies. A limited number of HL cell lines exist and have provided valuable insights into HL pathobiology. A literature survey indicated that 35 cell lines derived from HL patients have been published. To determine whether all these alleged HL cell lines hold up to scrutiny, we examined the available data and also put some of these cell lines to the test of hierarchical clustering, providing additional information regarding assignment to cell line type and tissue derivation. Hierarchical clustering separated the *bona fide* (classical) HL cell lines completely from cell lines derived from other lymphoma categories and proved conclusively that HL cell lines represent a distinct entity, irrespective of the cellular origin of the HRS cells. We conclude by pointing out the need for an intensified search for new cell culture avenues in order to develop a new generation of informative HL cell lines covering more widely the spectrum of HL stages and subtypes.

Keywords: cell lines; Hodgkin; lymphoma.

Introduction

Malignant lymphomas are systematized according to the World Health Organization (WHO) classification; the latest edition of 2016 distinguishes more than 100 entities defined according to their morphology, immunophenotype and genetic/molecular details (Swerdlow et al., 2016). The umbrella category Hodgkin lymphoma (HL) includes two major entities: on the basis of distinct biological, histological and clinical features nodular lymphocyte predominant HL is thought to represent a distinct entity and is classified separately from the other subtypes which are collectively known as classical HL (cHL). The WHO classification system recognizes four subtypes of cHL: nodular sclerosis, lymphocyte-rich, mixed cellularity and lymphocyte-depleted (Stein et al., 2008; Swerdlow et al., 2016).

In cHL most of the cells in the affected lymphoid tissue were shown to be non-malignant bystander cells. The atypical large mononuclear and multinuclear cells, termed Hodgkin and Reed-Sternberg (HRS) cells, respectively, commonly represent only 0.1–1% of the cells in any specimen (Küppers and Rajewsky, 1998). This abundance of normal bystander cells and the scarcity of the malignant HRS cells rendered any deeper and modern analysis of HRS cells rather difficult (Stein and Diehl, 2014). A pure population of HRS cells may help alleviate this problem and would allow for more reliable studies.

Hence, many investigators turned to the *in vitro* culture of HRS cells, either as short-term cultures with the cultured cells surviving for only weeks or months or preferably as immortalized cell lines. However, the development of a *bona fide* HRS-derived ‘HL cell line’ has proven to be very difficult: the vast majority of all such attempts have resulted in ‘normal’ Epstein-Barr virus-transformed B-lymphoblastoid cell lines (EBV+ B-LCL) derived from bystander B-lymphocytes, presumably normal B-cells. Few investigators succeeded in establishing permanently growing EBV-negative HL cell lines (Drexler, 1993).

Fortunately, research on HL was not impeded by the lack of adequate model systems and thus a handful of time-honored warhorses contributed a significant body of important data over several decades. However, there is a foreseeable need for new and more diverse canonical and available HL cell lines as the lack thereof

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could become a significant barrier to progress in the field. Here we review the value of the currently available true HL cell lines and distinguish the genuine and authentic cell lines from the wannabees. A number of reviews are available on the earlier HL cell lines literature (Diehl et al., 1982; von Kalle and Diehl, 1992; Drexler, 1993).

Cell line integrity is of critical importance for the suitability of any given cell line to serve as a faithful *in vitro* model for a certain disease. Cell line integrity obviously depends on the correct clinical diagnosis of the patient (fidelity of diagnosis); cell line-inherent parameters are derived from the index patient (authentication and exclusion of cross-contamination); and derivation from the malignant tissue and not from residual, non-malignant bystander cells (correct tissue of origin identity).

Some alleged HL cell lines have been found to contain cells other than HRS cells and are therefore clearly non-representative populations. Hence, studies based on such 'false' cell lines should be largely uninterpretable. In the interest of clarity, we distinguish in this review: (1) *bona fide* cHL cell lines; (2) malignant cell lines initially thought to be derived from cHL but in fact established from other malignancies than HL; (3) normal EBV+ B-LCL derived from HL patients; (4) the rest of the alleged HL cell lines: the cross-contaminated, the uncharacterized, the unavailable. Regarding the last point, there is a background of literature on the problem of cell line cross-contamination and its unforeseen dimensions (MacLeod et al., 1999; Drexler et al., 2003, 2017; Drexler, 2010).

For the validation and assessment of the panel of published HL cell lines we take advantage of the evidence published in the literature and of our own data (Drexler, 1993, 2010). In addition, we used the tool of hierarchical clustering of gene expression data which has been shown to represent a more systematic approach based on the simultaneous expression monitoring of genes using DNA microarrays (Golub et al., 1999). It has been suggested that this method is a precise and objective strategy for a uniform and comprehensive molecular classification of human cancers (Ramaswamy et al., 2001). Hierarchical clustering used as 'class prediction' allows for an accurate assignment to already-recognized classes (or subtypes) (Golub et al., 1999). We used the hierarchical tree structure here to supplement rather than replace existing assignment of cell lines to classes.

By informing the optimal choice of a model system it is hoped that this summary will provide a valuable framework for HL studies using cell lines, separating clearly the canonical HL cell lines from the imposters.

Bona fide classical Hodgkin lymphoma cell lines

The five cell lines HDLM-2, KM-H2, L-428, L-540 and SUP-HD1 which were established in the 1970s and 1980s have been used extensively for many years as model systems and fully deserve the title of 'classical HL cell lines' (Table 1). The following decade saw the arrival of HL cell lines HD-70 and L-1236. The last two additions to the available panel of HL cell lines are U-HO1 and very recently AM-HLH. In comparison with other types of leukemia-lymphoma cell lines (Drexler, 2010), the overall number of HL cell lines is rather low and has not increased significantly over these four decades.

Hierarchical clustering showed that the gene expression profiles of these cHL cell lines were more similar to each other than to any other group of malignant hematopoietic cell lines and clustered as a major branch on their own in the dendrograms (irrespective of their B- or T-cell derivation), implying that the HL cell lines represent a distinct entity (Küppers et al., 2003) (Figure 1).

It is worth mentioning that nearly all cases of cHL are derived from B-cells, but that a few cases diagnosed as cHL have a T-cell origin (Küppers, 2002). This is relevant because two of the accepted cHL cell lines are of T-cell origin (HDLM-2 and L-540). As a support for the idea that these are HL cell lines and not cell lines derived from a misdiagnosed T-cell lymphoma, already an earlier study, comparing the gene expression of HL cell lines and cell lines of the closely related T-cell lymphoma anaplastic large cell lymphoma (ALCL), revealed that HDLM-2 clusters together with B-cell-derived HL cell lines and not with ALCL cell lines (Willenbrock et al., 2006).

Nine of the 10 HL cell lines listed in Table 1 are EBV-negative. As cell line L-591 is EBV-positive, one might suspect its origin to be from non-neoplastic residual normal bystander lymphocytes (see below the paragraph 'B-lymphoblastoid cell lines'). However, L-591 retained several characteristics of HRS cells (Vockerodt et al., 2002). Two further aspects are worth mentioning: (1) L-591 expresses an EBV latency program 3 which is untypical for EBV+ HRS cells which show latency 2 (i.e. no EBNA2 expression); (2) L-591 presumably carries crippled IgV genes, a hallmark of HRS cells (Vockerodt et al., 2002). Finally, L-591 clusters clearly with the true cHL cell lines (Figure 1); therefore the assignment to the HL cluster is independent from the EBV status. The

Table 1: *Bona fide* classical Hodgkin lymphoma cell lines.

Cell line	Patient/ Sample[a]	Original diagnosis/Disease status[a]	Authentication[b]	Clustering[c]	Reference
AM-HLH	65 M/PE/2013	HL, nodular sclerosis/terminal	Yes (+)	cHL	Hayashida et al., 2017
HD-70	69 M/PB/1989	HL, nodular sclerosis/refractory terminal	No (+)	cHL	Kanzaki et al., 1992
HDLM-2[d]	74 M/PE/1982	HL, nodular sclerosis/stage IV	No (+)	cHL	Drexler et al., 1986
KM-H2	37 M/PE/1974	HL, mixed cellularity→lymphocyte depletion/stage IV, at relapse	No (+)	cHL	Kamesaki et al., 1986
L-428	37 F/PE/1978	HL, nodular sclerosis/stage IVB, refractory terminal	No (+)	cHL	Schaadt et al., 1979, 1980
L-540[e]	20 F/BM/1981[f]	HL, nodular sclerosis/stage IVB, prefinal	No (+)	cHL	Diehl et al., 1981
L-591	31 F/PE/1982[f]	HL, nodular sclerosis /stage IVB, refractory terminal	No (+)	cHL	Diehl et al., 1982
L-1236	34 M/PB /1994	HL, mixed cellularity/stage IV, at 3rd relapse, refractory terminal	Yes (+)	cHL	Kanzler et al., 1996; Wolf et al., 1996
SUP-HD1	37 M/PE/1987	HL, nodular sclerosis→lymphocyte depletion/stage IIISA/IV, refractory terminal	Yes (+)	cHL	Naumovski et al., 1989
U-HO1	23 M/PE/2005	HL, nodular sclerosis/at 2nd relapse, refractory terminal	No (+)	cHL	Mader et al., 2007

[a]Age/sex of patient, source of material, year of establishment and disease (sub)type/status of disease of the patient as indicated in the original publication quoted.
[b]Indicates whether the original investigators have proven that the cell line was derived from the patient described, thus excluding cross-contamination and overgrowth by an already established cell line; (+) indicates indirect authentication, meaning that DNA fingerprinting/profiling at DSMZ excluded cross-contamination and confirmed identity of cell line as far as possible using the DSMZ DNA Fingerprint Database comprising more than 800 human leukemia-lymphoma cell lines.
[c]Cell line clusters with this group in the dendrograms showing the hierarchical clustering based on gene expression data (see Figure 1).
[d]HDLM-1/-2/-3 are simultaneous sister cell lines with similar or identical features.
[e]L-538 is a sister cell line of L-540 which is, however, not used any longer and might have been lost.
[f]The exact year of establishment is not known, the year when the paper was published is indicated.
ALCL, anaplastic large cell lymphoma; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ASC, ascites; B-NHL, B-cell non-Hodgkin lymphoma; BM, bone marrow; cHL, classical Hodgkin lymphoma; CTCL, cutaneous T-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; F, female; HL, Hodgkin lymphoma; HRS, Hodgkin-Reed-Sternberg (cell); LCL, lymphoblastoid cell line; LN, lymph node; M, male; n.a., not analyzed; PB, peripheral blood; PC, pericardial effusion; PE, pleural effusion; PMBL, primary mediastinal B-cell lymphoma; SP, spleen; T-ALL, T-cell acute lymphoblastic leukemia; T-LL, T-cell lymphoblastic lymphoma; TU, tumor.
Details on these cell lines are listed in ref. (Drexler, 2010) which is available upon request from the first author at <hdr@dsmz.de>. All cHL cell lines (except for cell line AM-HLH) are available from the non-profit cell lines repository bank DSMZ (see www.dsmz.de).

morphology of L-591 cells in culture as assessed under the inverted microscope is comparable with, but still distinct from the EBV+ B-LCLs (Figure 2).

It can also be mentioned that for most of the reliable cHL cell lines both cytogenetic studies and gene mutation studies further support their HRS cell origin as HRS cell-typical chromosomal aberrations and gene mutations were found in these cell lines (Drexler, 2010).

Cell lines from other hematopoietic malignancies

The cell lines summarized in Table 2 were originally described as being derived from HL. However, old and new data have cast serious doubts on this assumption – and clearly these cell lines do not qualify as cHL cell lines. In addition to available immunological, cytogenetic, molecular genetic and other data, again gene expression profiling was used here for definitive classification. All four cell lines showed gene expression patterns separate from cHL cell lines and in the hierarchical trees clustered with other well-defined cell line categories.

Cell line DEV was first published as being established from a patient with HL nodular sclerosis (Poppema et al., 1985). Later the original authors presented the 'real' diagnosis as HL lymphocyte predominant (which does not belong to the category of cHL) (Maggio et al., 2002). As expected as the tumor cells of these subtypes (lymphocyte predominant versus classical) are well known for their phenotypic differences (Küppers et al., 2003), cell line DEV does not cluster

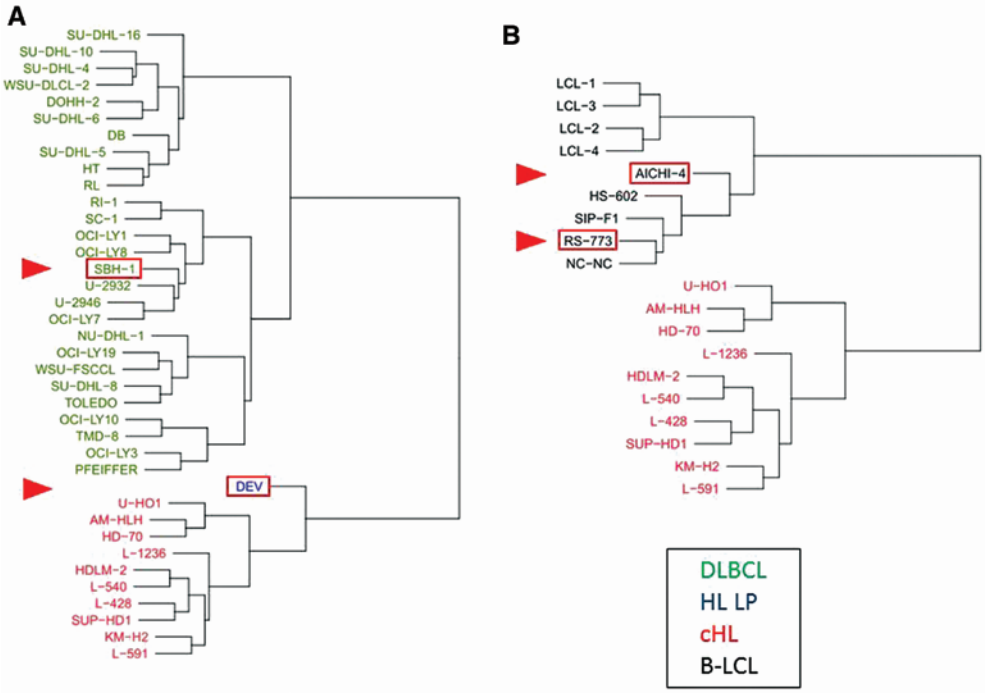

Figure 1: Dendrograms showing hierarchical clustering of gene expression data generated from cell lines derived from diffuse large B-cell lymphoma (DLBCL), lymphocyte predominant HL (HL LP), classical HL (cHL) and EBV-transformed B-lymphoblastoid cell lines (B-LCL). (A) Note: this analysis shows that the cHL cell lines, the DLBCL cell lines, and cell line DEV form separate branches; hence DEV is clearly neither a cHL nor a DLBCL cell line and forms its own cluster (red arrowhead); alleged cHL cell line SBH-1 clusters with 26 known DLBCL cell lines (red arrowhead). (B) Note: the cHL cell lines and EBV+ B-LCLs form distinct clusters; alleged cHL cell lines AICHI-4 and RS-773 (red arrowheads) cluster with proven B-LCL. Cell lines are color-coded according to their cellular origin: DLBCL, green; HL LP, blue; cHL, red; B-LCL, black. Biostatistical analysis: To build the dendrograms the gene expression of a subset of genes (30%) were considered which varied the most among the cell lines studied and are hence most informative. The hierarchical clustering algorithm employed to generate the dendrograms is Ward's method and calculated on the Euclidean distance matrix. The authors performed themselves the gene chip studies for all the cell lines included, hence clusterings are based on own previously unpublished data.

with the cHL cell lines but forms a separate branch in the dendrograms (Figure 1), thus also corroborating the correction of diagnosis. In a prior study (Schumacher et al., 2010) gene expression profiling of DEV in comparison to primary lymphoma cells of cHL and of HL lymphocyte predominant showed that DEV is much more similar to the latter cells than to HRS cells of cHL, confirming that the reclassification of DEV as a cell line model for HL lymphocyte predominant is correct. Interestingly, DEV despite its B-cell derivation does not cluster with cell lines derived from Burkitt lymphoma, primary mediastinal B-cell lymphoma, pleural effusion lymphoma, mantle cell lymphoma or diffuse large B-cell lymphoma (DLBCL), but always forms its own branch in the expression profiles (Figure 1; Supplementary Figure 1).

Cell line HD-MAR which was published as being established from a patient with HL mixed cellularity displays immunophenotypical and cytogenetic features of a T-cell malignancy-derived cell line (Drexler, 2010). Furthermore, HD-MAR cells show T-leukemia-type morphology and cluster with T-cell malignancies (Figure 3; Supplementary Figure 2).

Cell line HD-MY-Z with myelomonocytic features is another controversial case. Genetically this cell line is neither of B- nor T-cell in origin as it lacks rearrangements of the B-cell and T-cell receptor genes (Drexler, 2010). The comparative hierarchical clustering showed that this cell line retains a genetic signature which readily separates it from cHL cell lines and places it among myelo-monocytic leukemia cell lines (Figure 3; Supplementary Figure 2). Hence, all available evidence suggests this cell line contains myelomonocytic cells.

The diagnosis of the patient from whom the cell line SBH-1 was derived was not clearly established (DeCoteau et al., 1995). The SBH-1 cells have a B-cell immunoprofile and carry the balanced translocations t(8;14) and t(14;18) which are typically seen in B-NHL (Drexler et al., 2016). Finally, SBH-1 clusters with a large panel of B-NHL cell lines (Figure 1).

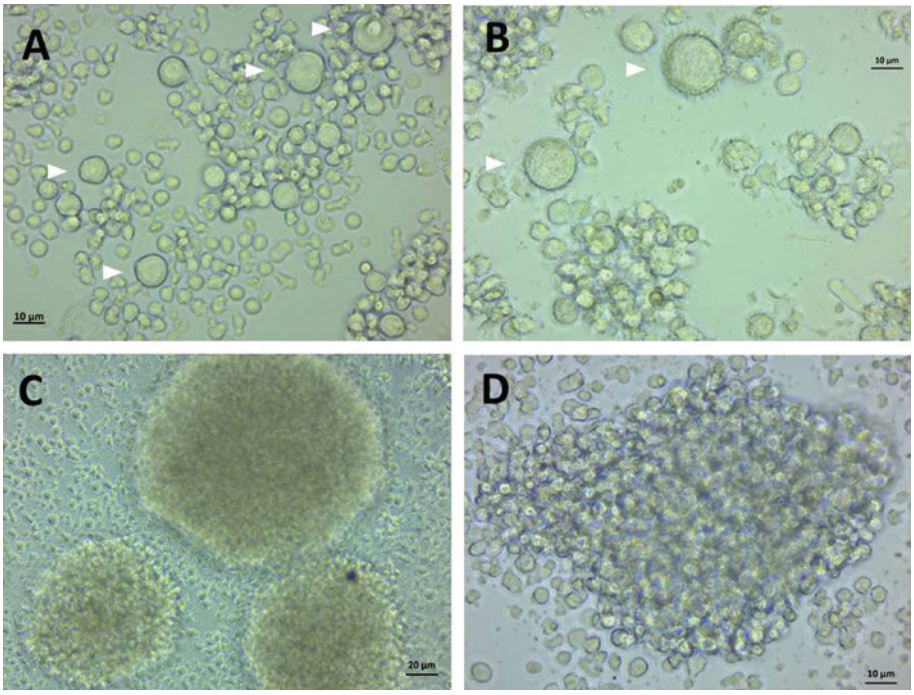

Figure 2: Cell lines in culture under the inverted microscope.
(A) and (B) cHL cell lines HDLM-2 and L-1236 grow as single cells or in small loose clumps. Note: some of the giant polynucleated cells are indicated by white arrowheads; up to 20% of the HDLM-2 population consists of polynucleated cells (Drexler et al., 1989), the majority of the cells are mononuclear. (C) EBV+ B-LCL HS-602 grows in very dense, tightly packed, large clumps (note the lower magnification) which is the typical growth pattern for B-LCLs. B-LCL cells show a strong tendency to form clumps with the uropods (slender pseudopodia) pointing away from the center as described in detail previously (Nilsson and Pontén, 1975). (D) cHL cell line L-591 also grows in clumps which are, however, smaller and less packed.

Table 2: Malignant cell lines not derived from classical Hodgkin lymphoma.

Cell line	Patient/ Sample[a]	Original diagnosis/Disease status[a]	Commentary	Clustering[b]	Reference
DEV	51 M/PE	HL, nodular sclerosis/stage II, at relapse	Diagnosis reclassified by original authors to HL, nodular lymphocyte predominance	Own cluster	Poppema et al., 1985; Maggio et al., 2002
HD-MAR	20 M/ PE/1977	HL, mixed cellularity/at relapse, advanced stage, terminal	Immunoprofile/karyotype typical for T-cell lymphoma	T-ALL/T-LL	Ben-Bassat et al., 1980
HD-MY-Z	29 M/ PE/1991	HL, nodular sclerosis/stage III→IV, refractory, terminal	Immunoprofile typical for myelomonocytic cells	AML	Bargou et al., 1993
SBH-1	78 F/PE	HL/at diagnosis	Originally diagnosis not clearly established; karyotype typical for B-NHL	B-NHL/DLBCL	DeCoteau et al., 1995

[a]Age/sex of patient, source of material, year of establishment and disease (sub)type/status of disease of the patient as indicated in the original publication quoted.
[b]Cell line clusters with this group in the dendrograms showing the hierarchical clustering based on gene expression data, but not with cHL (Figure 1; Supplementary Figures 1 and 2).
For Abbreviations see legend to Table 1.

B-lymphoblastoid cell lines from Hodgkin lymphoma patients

B-lymphoblastoid cell lines (B-LCL) are relatively easy to establish in long-term culture (Belpomme et al., 1972). In the majority of cases these cell lines represent *in vitro* transformation of non-malignant B-cells by EBV apparently, EBV has a strong facilitating effect on establishment *in vitro*.

EBV-carrying B-LCLs can be obtained from non-neoplastic cells from healthy as well as from diseased

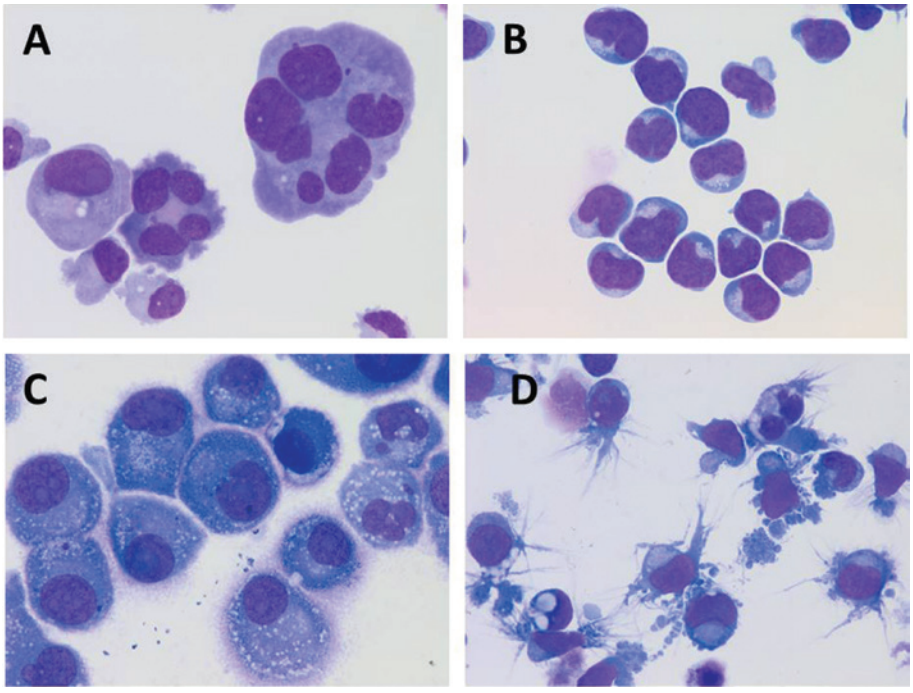

Figure 3: Morphology of HL cell lines at the single cell level (cells were cytospin-centrifuged on slides and stained using the May-Grünwald-Giemsa stain).
(A) cHL cell line HDLM-2; note: a giant polynucleated cells, two large polynucleated cells, two smaller mononucleated cells, highly pleomorphic morphology. (B) Cell line HD-MAR shows a morphology typical for T-cell leukemia-lymphoma cell lines; note: cells are small and homogenous, monomorphic morphology. (C) Cell line HD-MY-Z shows a macrophage-like morphology; note: single nuclei and abundant, vacuolated cytoplasm. (D) Cells of cell line RS-773 can be villous all over the surface and displays slender pseudopodia located at one end (the uropod) as described in detail previously (Nilsson and Pontén, 1975); very pleomorphic cell image.

individuals. In fact, as non-neoplastic cells are inevitably present as contaminants in any explant, malignant hematopoietic tissues (such as lymph nodes, spleen, etc.) frequently give rise to the B-LCL cell line type rather than to a cell line derived from the neoplastic cells (Nilsson and Pontén, 1975). Any leukemia-lymphoma cell line is difficult to establish; even when the neoplastic cells initially dominate the biopsy, the less exacting lymphoblastoid cells tend to outgrow the malignant cells (the latter usually dying off quickly) (Nilsson and Pontén, 1975). Hence, for every cell line the question arises whether the surviving and proliferating cells in the culture are indeed neoplastic or are they merely a progeny of normal cells. Taken together, it does not automatically follow that a cell line derived from a patient with a hematopoietic malignancy is of neoplastic origin (Nilsson and Pontén, 1975).

Initially a B-LCL culture is considered to be polyclonal; however, with prolonged cultivation usually monoclonality prevails indicating a selection *in vitro*. In the early days of tumor cell culture it was thought that it would be possible to differentiate malignant cell lines from B-LCL on the basis of three parameters: karyotype, colony formation in agar and growth after heterotranspantation into mice (Belpomme et al., 1972). However, after prolonged cultivation *in vitro* B-LCL may show chromosomal alterations, form colonies in agar and grow also in mice, hence, these parameters are not reliable criteria for the malignancy of cell lines (Belpomme et al., 1972; Nilsson et al., 1977).

Furthermore, early on it was reckoned that by comparative morphology at the single cell level and in the culture vessel, B-LCL may be separated in most cases from other malignant cell lines (Nilsson and Pontén, 1975), but this discrimination requires expertise and experience and such microscopic examination is unfortunately not always as clearcut and as easy as hoped.

The literature abounds with 'cancer cell lines' derived from hematopoietic malignancies and other neoplasms which are in reality B-LCLs but are nevertheless used as *in vitro* models of tumors (Nilsson and Pontén, 1975). Unfortunately, such B-LCLs are occasionally advertised by cell line repositories (which clearly should know better) or in literature databases carelessly and even negligently as 'Hodgkin cell lines' or 'being derived' from HL patients (without further specification)

Table 3: EBV+ B-LCL from Hodgkin lymphoma patients.

Cell line	Patient/Sample[a]	Original diagnosis/Disease status[a]	Commentary	Clustering[b]	Reference
AICHI-4	50 M/LN/1968	HL, lymphocyte predominance	EBV+; no detailed characterization; B-LCL	B-LCL	Ito et al., 1968
HS-445	56 F/LN/1970	HL (stage 1)	EBV+; no detailed characterization; most likely B-LCL	n.a	Arnstein et al., 1974
RPMI-6666	29 M/PB	HL	EBV+; no detailed characterization; most likely B-LCL	n.a	Moore et al., 1966
RS-773		HL, mixed cellularity	EBV+; no detailed characterization; B-LCL	B-LCL	Zocchi et al., 2012

[a]Age/sex of patient, source of material, year of establishment and disease (sub)type/status of disease of the patient as indicated in the original publication quoted.
[b]Cell line clusters with this group in the dendrograms showing the hierarchical clustering based on gene expression data.
For Abbreviations see legend to Table 1.

and, hélas, some naïve user may not entertain any suspicion.

We identified the four cell lines: AICHI-4, HS-445, RPMI-6666 and RS-773 which were reported to be derived from patients with HL as B-LCLs (Table 3). These cell lines are EBV-positive and show the typical B-LCL morphology in culture (Figure 2) and on cytospin slides (Figure 3). Two of these cell lines were examined in the hierarchical clustering analysis; this exercise showed that AICHI-4 and RS-773 cluster with known and validated B-LCLs (Figure 1).

Again, vigilance with regard to cell line integrity (fidelity of diagnosis, authentication and exclusion of cross-contamination, tissue of origin identity) and extensive characterization are paramount. The technical advances for the indispensable work up of any cell line are available to fully meet these requirements. Until additional data are forthcoming (which is unlikely to happen), the cell lines listed in Table 4 do by no means qualify to be included in the well-defined entity of HL cell lines.

Alleged Hodgkin lymphoma cell lines: the cross-contaminated, uncharacterized, unavailable

During the 1970s and 1980s as many as one in three cell lines deposited in cell culture repositories were imposters, one cell line overtaking or masquerading another (O'Brien, 2001). In 1981 the discovery that four human HL cell lines were in reality monkey-derived cell lines became a scientific scandal and opened some eyes (Harris et al., 1981; Broad and Wade, 1983). Overall seven alleged HL cell lines have been found to be cross-contaminated and overgrown with other cell lines (Drexler et al., 2003; Drexler, 2010) (Table 4).

The fate of another 10 alleged HL cell lines is unknown (Table 4). Some of the data on these cell lines are of low quality: insufficient presentation of clinical data of the patient, poor characterization of cell line and no follow-up publication. One must assume that several 'established' cell lines were in reality not immortalized cell lines (and therefore stopped proliferating and may have never been cell lines) or have simply been lost.

Cell lines from malignancies secondary to Hodgkin lymphoma

Though modern therapy has greatly improved survival for HL, the relative risk of developing a secondary cancer, in particular aggressive hematopoietic malignancies such as secondary acute myeloid leukemia (AML), B-cell non-Hodgkin lymphoma (B-NHL) and others, is considerably higher for HL patients than for the general population (Schaapveld et al., 2015). The relatively long latency between HL treatment and disease onset in many cases is suggestive of a multi-step process of transformation, possibly involving the induction of genomic instability on the basis of an inherent susceptibility to toxic agents; microsatellite instability and concomitant DNA mismatch repair loss may play roles in this scenario, resulting in the cumulative acquisition of mutations and deletions in key genes and pathways.

We have summarized a panel of 11 leukemia-lymphoma cell lines that were derived from patients with hematopoietic malignancies secondary to treatment for HL (Table 5). One cell line (SAML-1) that was allegedly derived from an AML patient subsequent to the diagnosis

Table 4: Cross-contaminated, uncharacterized and unavailable cell lines (reportedly from Hodgkin lymphoma).

Cell line	Patient/Sample[a]	Original diagnosis/Disease status[a]	Commentary	Reference
AG-F	BM	HL	Cross-contaminated (false cell line) = T-ALL cell line CCRF-CEM	Gazitt et al., 1993; Drexler et al., 2003
CO (COLE)	F/LN/1980	HL, nodular sclerosis	Cross-contaminated (false cell line) = T-ALL cell line CCRF-CEM	Jones et al., 1985; Drexler et al., 2003
EU-HE	15 F/LN	HL, mixed cellularity	No further publication/characterization, unknown availability	Miranda et al., 1999
FQ			Cross-contaminated (false cell line) = monkey cell line	Long et al., 1977; Harris et al., 1981
HKB-1	14 F/TU/1994	HL, nodular sclerosis	Cross-contaminated (false cell line) = Burkitt cell line BJAB	Wagner et al., 1998; Drexler, 2010
HO (HOLDEN)	F/LN	HL, nodular sclerosis/stage II	No further publication/characterization, unknown availability	Jones et al., 1989
HPL-HOD-1	57 ?/ PE/1972	HL	No further publication/characterization, unknown availability	Torii et al., 2001
HUT-11	6 F/LN	HL, mixed cellularity/stage IIA	No further publication/characterization, unknown availability	Roberts et al., 1978
MDA-E		HL	No further publication/characterization, unknown availability[b]	Goy et al., 2003
MDA-V		HL	No further publication/characterization, unknown availability[b]	Goy et al., 2003
L-439	39 M/PE/1979	HL, nodular sclerosis/stage IVB	Cell line stopped growing	Schaadt et al., 1980
RB			Cross-contaminated (false cell line) = monkey cell line	Long et al., 1977; Harris et al., 1981
RN[c]	14 M/LN	HL, mixed cellularity/stage IV	EBV+, possibly EBV+ B-LCL; no further publication/ characterization, unknown availability[b]	Friend et al., 1978
RY			Cross-contaminated (false cell line) = monkey cell line	Long et al., 1977; Harris et al., 1981
SPR			Cross-contaminated (false cell line) = monkey cell line	Long et al., 1977; Harris et al., 1981
SU-RH-HD-1	12 M/SP/1980	HL, nodular sclerosis/stage III, at diagnosis	No further publication/characterization, unknown availability[b]	Olsson et al., 1984
ZO	26 F/PC	HL, nodular sclerosis/stage II, at relapse	No further publication/characterization, unknown availability[b]	Poppema et al., 1989

[a]Age/sex of patient, source of material, year of establishment and disease (sub)type/status of disease of the patient as indicated in the original publication quoted.
[b]No response to request for cell line.
[c]Sister cell line RSP (also EBV+) was derived from the spleen of the same patient and is also possibly an EBV+ B-LCL.
For Abbreviations see legend to Table 1.

Table 5: Cell lines from secondary hematopoietic malignancies post-Hodgkin lymphoma.

Cell line	Patient/Sample[a]	Primary diagnosis/Disease status→Secondary disease[a]	Cell type of cell line	Reference
FE-PD	46 F/PB/1991	HL, nodular sclerosis, stage IIIAE→10 months later: Hodgkin-like ALCL	T-cell	Del Mistro et al., 1994
HEL	30 M/PB/1980	HL→AML M6	Myelo-erythroid	Martin and Papayannopoulou, 1982
L-660	38 M/PB/1981	HL, mixed cellularity, stage IIISA→4 years later: ALL	B-cell	Fonatsch et al., 1982
MAC-1[b]	45 M/PB/1985	HL, mixed cellularity, stage IIA→10 years later: CTCL	T-cell	Davis et al., 1992
MAC-2A[b]	47 M/TU/1987	HL, mixed cellularity, stage IIA→CTCL→2 years later: ALCL	T-cell	Davis et al., 1992
OCI-AML4	35 F/PB/1987	HL, nodular sclerosis, stage IIIA→2 years later: AML M4	Myelo-monocytic	Koistinen et al., 1991
OHN-GM	60 M/BM/1995	HL, mixed cellularity→5 years later: AML	Myeloid	Nagai et al., 1997
SAML-1	50 M/BM/1992	HL, nodular sclerosis, stage IVB→15 years later: AML; cell line is cross-contaminated (false cell line) = U-937	Myeloid	Knutsen et al., 2003
SAML-2	42 M/BM/1996	HL, mixed cellularity→5 years later: AML	Myeloid	Knutsen et al., 2003
U-2932	29 F/ASC/1996	HL, nodular sclerosis, stage IVB→14 years later: B-NHL (DLBCL ABC-type)	B-cell	Amini et al., 2002
U-2940	18 F/PE/1991	HL, nodular sclerosis, stage IIB→8 months later: PMBL	B-cell	Sambade et al., 2006

[a]Age/sex of patient, source of material, year of establishment and disease (sub)type/status of disease of the patient as indicated in the original publication quoted.
[b]MAC-1 is a serial sister cell line; MAC-2A and MAC-2B are simultaneous sister cell lines.
For abbreviations see legend to Table 1.

of HL was, however, found to be in reality the well-known and classical monocytic cell line U-937, hence, another case of cross-contamination at source. The other 10 cell lines have been authenticated directly or indirectly and have been established from the secondary diseases AML (n = 4), ALCL (n = 2), two types of B-NHL (n = 2), acute lymphoblastic leukemia (ALL, n = 1) and cutaneous T-cell lymphoma (CTCL, n = 1) between less than 1 year at the earliest and up to 15 years after treatment of the antecedent HL.

These cell lines represent unique models to dissect the distinct characteristics of the development of HL and the 'progression' to secondary malignancies and might also help to determine the pathophysiological aspects which may be rather therapy-related and those which may be rather patient-inherent.

Regarding the latter notion, there is the recent discovery that some apparently therapy-related myeloid neoplasms may evolve from somatically mutated hematopoietic clones which are already present prior to treatment and hence are not necessarily caused by genotoxic chemo-/radiotherapy; instead myelo-/immunosuppressive stress may lead to selection of a pre-existing clone, ultimately increasing the risk of development of a myeloid neoplasm (Bejar, 2017). In this scenario molecular comparison of a cell line (established from a malignancy secondary to HL) with the primary material of the index patient, both germ line normal cells and malignant cells, would be of great value.

Establishment of new Hodgkin lymphoma cell lines

In general human tumor cell lines are vital tools for gaining a detailed mechanistic understanding of the pathogenesis of the diseases from which they are derived and for identifying recurrent and actionable genetic alterations with the aim to translate these findings into effective anticancer therapeutic strategies.

In 1963 the first human lymphoma cell line was established (this cell line was named RAJI and was derived from a patient with Burkitt) (Drexler, 2010). Since then techniques for the isolation of cell lines from lymphoid tissues have improved but are still far from being perfect. This is probably due to the imperfection of the culture conditions (Nilsson, 1983). Nevertheless several 100 lymphoma cell lines have been established (Drexler, 2010).

Bona fide HL cell lines are particularly difficult to establish. The success rate for the establishment of authentic HRS cell lines is not known, but it can be guessed that it is in the one-digit percent range or even below. Standard tissue culture methodology is clearly insufficient for the growth of the majority of tumor cells, and hence it is not surprising that only a handful of cell lines have been established from HL. Further innovation is required to meet the '*in vitro* needs' of the cells so they can survive and multiply.

From our concise summary it is very obvious that any new HL cell line must be carefully characterized to ensure

that it is not an EBV-transformed B-LCL. The new tool of hierarchical clustering might be a useful addition to the armamentarium of validating techniques.

The present conventional culture techniques therefore seem not to be adequate in substituting the environment in which the malignant cell clone has evolved *in vivo* and that improvements of the composition of growth media (e.g. adjustments of basic nutrients and addition of specific factors) are badly needed (Nilsson, 1983). Clearly, future studies prioritizing the culture conditions or means of better manipulating the artificial environment would be beneficial for this cell lines cohort.

Conclusions

HL is one of the hematopoietic malignancies in which cell lines have been particularly informative. However, there has been significant confusion regarding the availability of true HL cell lines and the concern, of course, is for the reliability of the research data obtained using these types of cell lines, which prompted us to try to bring some light into the darkness and clear the fog. Hence, it was our aim to provide a reliable analysis of the *bona fide* HL-derived cell lines and of the wannabes, underscoring the significance of cell line integrity.

Given the general importance of cell lines as model systems for the study of many tumor types, the tool of hierarchical clustering should be performed systematically in order to solidify the proper lineage assignment of each cell line which certainly can inform the utility of a given cell line. At the same time we recognize that such data will reflect an evolving picture that can be refined by future studies.

The large body of scientific publications using HL cell lines reinforces the important value of these model systems for research on a multitude of aspects of HRS cell biology but it also underlines the currently unmet need for new and more diverse HL cell lines. Overall, there a really very few *bona fide* HL cell lines (n = 10), most of which are 'old cell lines' from the 1970s and 1980s (n = 7). Seven cell lines were derived from pleural effusion material at mostly stage IV of terminally ill HL patients, most of them having nodular sclerosis subtype (n = 8). This highlights the need for continuing to try to establish new cell lines from early disease stages, other subtypes and different tissues. The lack of standardized cell culture procedures need not be prohibitive. The establishment of a true HL cell line is a challenging task, but might be approachable with the right mix of evolving cell culture techniques and perseverance.

The study of HL cell lines remains a dynamic area of investigation. Newer techniques such as next generation sequencing may allow for a more nuanced understanding of the malignant processes associated with HL development (Liu et al., 2014; Hudnall et al., 2016). Therefore the availability of the most important and validated HL cell lines will be crucial resources to explore the genomic landscape in HL.

In sum, the present data produced the most comprehensive review of *bona fide* HL cell lines and imposter cell lines, providing a framework for the use of these valuable models in efforts to dissect the pathophysiological underpinnings of HL to move the field forward.

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