Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN SCIENZE BIOMEDICHE
Ciclo 27

Settore Concorsuale di afferenza: 06/D3
Settore Scientifico disciplinare: MED/15

HISTONE DEACETYLASE INHIBITORS IN ADULT PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA RELAPSED/REFRACTORY: A PHASE II STUDY WITH PANOBINOSTAT

Presentata da:

Dott.ssa Cinzia Pellegrini

Coordinatore Dottorato:
Chiar.mo Prof.

Lucio Cocco

Relatore:
Chiar.mo Prof.

Pier Luigi Zinzani

Esame finale anno 2015

INDEX

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) constitutes 25-30% of adult non-Hodgkin lymphomas in western countries. The incidence of DLBCL has largely increased in these last decades, particularly in older populations; it affects more frequently males and the median age at presentation is in the 7^{th} decade. R-CHOP (Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone) is at present the standard therapy for DLBCL, yielding to complete response rate of nearly 70-80% and 5 years progression free survival of 50 to 90% according to initial prognostic risk. The prognosis of patients with DLBCL relapsed and/or refractory to CHOP-R first line therapy is dismal. High dose therapy and autologous stem cell transplant (ASCT) is an active rescue therapy in nearly 50% of patients with chemo-sensitive disease. Therapy of patients who relapse after ASCT or result non eligible for ASCT because of refractory disease, age or co-morbidities, represents an unmet medical need.

Histone deacetylases (DACs) are involved in chromatin structure regulation and function. Treatment with DACs inhibitors leads to the activation or repression of genes regulating apoptosis, proliferation, differentiation, angiogenesis, immune responses. These agents resulted to be active for the treatment of T and B-cell lymphoma and other haematological malignancies. Previous in vitro studies underlined the possible pathophysiological role of DACs in diffuse large B-cell lymphoma (DLBCL)(1-6). Panobinostat is a potent pan-DACs inhibitor belonging to cinnamic hydroxamic acid class of compounds. It is highly potent class I/II pan-DAC inhibitor that has shown anti-tumor activity in pre-clinical model and in cancer patients.

In this regard, the ‵Fondazione Italiana Linfomi″ designed a phase II clinical trial aiming to assess the efficacy and the safety of the HDACi Panobinostat in DLBCL patients refractory to conventional therapies (Study ID: IIL _ PanAL10). The present research project will be performed within this clinical trial.

1. OVERVIEW OF DIFFUSE LARGE B CELL LYMPHOMA

1.1. Definition and incidence

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid malignancy in adults. The annual incidence of Non-Hodgkin's Lymphomas (NHL) is estimated to be 15–20 cases/100,000 (7) Based on the data of the International NHL study group (Non-Hodgkin's Lymphoma classification Project 1997) (8) and the WHO Classification of Tumour of Haematopoietic and Lymphoid Tissues (9), DLBCL accounts for approximately 31% of all NHL in Western Countries and 37% of B-cell tumours worldwide. The median age of DLBCL falls between the sixth and seventh decade, although other types of aggressive NHL present at a lower median age, as for instance Burkitt lymphoma and primary mediastinal lymphoma. DLBCL corresponds to a group of lymphoid malignancies composed of large cells with vesicular nuclei, prominent nucleoli, basophilic cytoplasm and a usually high proliferation rate. As acknowledged in the 2008 WHO Classification (9) morphological, biological and clinical studies have allowed the subdivision of DLBCLs into morphological variants, molecular and immunophenotypic subgroups and distinct disease entities. However, a large number of cases still remain biologically heterogeneous, for which there are no clear and accepted criteria for subclassification: these are collectively termed DLBCL, not otherwise specified (NOS). Importantly, the principles used for the present organization of DLBCLs are the ones established in 1994 by the Revised European American Lymphoma (REAL) Classification (10) and subsequently adopted in the third and fourth editions of the WHO Classification (9) in which each lymphoma entity is defined by the amalgamation of clinical data, morphology, phenotype, cytogenetics, and molecular characteristics as well as by the identification of a normal counterpart.

1.2. Classification

DLBCLs are defined as a heterogeneous group of malignancies composed of large cells with nuclei at least twice the size of a small lymphocyte and usually larger than those of tissue macrophages. They more often occur de novo but can also represent the progression or transformation of a less aggressive B-cell neoplasm, such as chronic lymphocytic leukaemia, lymphoplasmacytic lymphoma, marginal zone lymphoma,

follicular lymphoma and even lymphocyte predominant Hodgkin lymphoma. DLBCL can develop at either nodal or extra-nodal sites. In the fourth edition of the WHO Classification of Haematopoietic and Lymphoid Tumours (9), they have been subdivided into four categories, some of which not quoted in previous schemes: (a) DLBCL – not otherwise specified (DLBCL, NOS), (b) DLBCL with predominant extranodal location, (c) large cell lymphoma of terminally differentiated B-cells, and (d) borderline cases. Each category further includes morphologic and/or clinico-pathologic variants that make the organization of these neoplasms quite complex. In the following, we focus on the most frequent subgroup: DLBCL, NOS.

1.3. DLBCL, NOS

1.3.1 Morphology

DLBCL NOS is the most common form of NHL. It usually consists of a mixture of centroblasts and immunoblasts that grow diffusely, partly or completely effacing the normal structure of the involved organ(s). Centroblasts are defined as large cells with a central oval-round nucleus, finely dispersed chromatin, multiple small nucleoli at times adjacent to the nuclar membrane, and a moderate rim of basophilic cytoplasm. Immunoblasts have instead a slightly eccentric nucleus, one or more prominent nucleoli (sometimes with an inclusion-like appearance), and a rather large rim of basophilic cytoplasm with a more or less pronounced Golgi area (11). Features of intrasinusoidal spread are occasionally observed. The number of mitotic figures is globally high, the amount of reactive T-lymphocytes and/or histiocytes variable. In any case, the latter do not overwhelm the neoplastic population as instead observed in the so-called histiocyte/T-cell-rich B-cell lymphoma (HTCRBCL). In 10–15% of cases, one cytotype predominates over the other, representing more than 90% of the examined population: these cases are respectively referred to as “centroblastic” or “immunoblastic”. The latter may show aspects of plasmacellular differentiation, making the differential diagnosis with extra-medullary involvement by plasmablastic lymphoma or poorly differentiated plasma cell myeloma possibly difficult. A recent study has suggested that pure immunoblastic morphology does represent a negative prognostic indicator more relevant than the various immunohistochemical algorithms proposed during the last few years (12) Other morphologic variants are the multilobated and anaplastic large cell ones. The former may be observed in tumours arising at extranodal sites, especially the

bone, while the latter resembles T-cell anaplastic large cell lymphoma with Reed–Sternberg-like elements, intrasinusoidal diffusion and cohesive growth pattern but differs from it because of the phenotype, cytogenetics and, last but not least, definitely worse clinical course.

1.3.2 Phenotype

On immunohistochemistry, the cells of DLBCL, NOS are SIg+/−, CIg−/+ and express B-cell-associated antigens (CD19, CD20, CD22, and CD79a) (11). In particular, CD20 is relevant not only on diagnostic grounds but also for therapeutical purposes, being the target of the Rituximab humanized monoclonal antibody that has dramatically improved the prognosis of these neoplasms. DLBCL are usually CD45+ and PAX5+: the search for such molecules can, however, be negative in case of pronounced plasmacellular differentiation, producing strong positivity for BLIMP-1, CD138 and CIg. CD10, BCL6, IRF4, LMO2, GCET1, and FOXP1 are variably expressed: their combination has represented the base for the construction of algorithms aiming to surrogate gene expression profiling (GEP) results (13), (14) and (15). Cases with anaplastic morphology typically express CD30, while those possibly derived from marginal zone or monocytoid B-cells more often turn IRTA-1 positive. Recently, attention has been paid to CD5 positivity (16). This occurs in the absence of cyclin D1 over-expression, a fact that allows the easy differentiation of $CD5^{+}$ DLBCLs from polymorphic MCLs (17). Finally, additional useful markers are BCL-2, p53 and Ki-67 as well as the NF-kB components (RELA, RELB, REL, p50 and p52) and EBER in situ hybridization (ISH) (18). BCL-2 is expressed by about 50% of DLBCL of either the ABC type (see below) or carrying *t*(14;18) as shown by fluorescent ISH (FISH). The latter condition is not invariably due to FL transformation (see below). BCL-2 protein expression – that is down-regulated in normal GC where apoptosis plays a critical role in negative B-cell selection – is associated with an inferior outcome in DLBCL irrespective of the mechanism causing it (18) and (19). P53 positivity may be found in the setting of DLBCL derived from a less aggressive B-cell neoplasm (e.g. Richter's syndrome and related conditions in patients with chronic lymphocytic leukaemia) (12). Ki-67 marking is representative of the proliferation rate: in case it exceeds the 90% value, FISH analysis may be worthy to identify tumours carrying*MYC* complex karyotype (see below). On the same line, EBER ISH allows the recognition of EBV-infected DLBCL, which are not infrequently encountered in immunocompromised individuals (e.g. due to

AIDS or immunosuppressive therapies such as the ones following transplantation) (9). Last but not least, the search for the NF-kB components is indicative of the status of the canonical or alternative pathway, a fact that is provided with potential therapeutic implications (20;21).

Besides the above mentioned markers that refer to attributes of the neoplastic cells, other molecules may be prognostically relevant that correspond to different microenvironmental components (e.g.: CD68, FOXP1, CD21 and CD31).

1.3.3. Genetics

Genetic alteration may relate to**:**

- Antigen receptors genes: clonal reengagement of Ig encoding genes is generally recorded. By adopting the highly reliable BIOMED-2 approach, combined application of IGH (VH–JH and DH–JH) and IGK tubes is recommended in order to detect virtually all rearranged cases, in the light of the high levels of somatic mutations characteristic of DLBCL (12);
- Aberrant somatic hypermutations: these are detected in more than 50% DLBCL and affect multiple genes, including *PIM-1*, *MYC*, *Rho*/*TTF* and*PAX5*. They are thought to contribute to the oncogenesis of the tumour (12);
- Genetic aberrations: chromosomal translocations of band 3q27 are detected in 30–40% of cases and cause the rearrangement of *BCL-6*, a transcriptional repressor selectively expressed by germinal centre (GC) B-cells and controlling GC formation (22,23,24) . The translocation inhibits the down-regulation of *BCL-6* that is required for further differentiation of GC B-cells, and creates a DNA error prone GC microenvironment by functionally inactivating p53 (25,26). Translocation of the *BCL2* gene, i.e. *t*(14;18), a hallmark of FL, occurs in approximately 20% of DLBCL arising de novo and in most cases transformed from a prior follicular phase (27). A *MYC*rearrangement is observed in up to 10% of an unselected series of cases (28) and is usually associated with a complex pattern of genetic alterations (29). The *MYC* break partner is an *Ig*-gene in 60% and a non-*Ig*-gene in 40% of cases (29) Approximately 20% of cases with a *MYC* break have a concurrent *IGH-BCL2* translocation and/or *BCL6* break or both (29) and (30). These cases usually have a high proliferation (>90% Ki67+) and may be better categorized as "B-cell lymphomas, unclassified with features intermediate between diffuse large B-cell

lymphoma and Burkitt lymphoma" (31). Other rare molecular lesions occasionally involved in DLBCL pathogenesis include translocations of *BCL-8* and *MUC-1*, amplification of *REL*, and inactivation of the *p53*tumour suppressor gene (32). A recent study has identified monoallelic deletions and mutations inactivating *CREBBP* and *EP300* in nearly 39% of GCB-DLBCL and less frequently in ABC–DLBCL (17% of samples) (33). *CREBBP* and *EP300* are acetyltransferases that act as transcriptional co-activators in multiple signalling pathways. As a consequence of the mutations, *CREBBP*/*EP300* lose their ability to acetylate*BCL-6* and *p53,* a post-translational modification that inactivates *BCL-6* by disrupting the recruitment of histone deacetylases (HDACs) and thus hindering its capacity to repress transcription, while representing an essential requirement for *p53* activation (34) and (35). Thus, *CREBBP* and *EP300* mutations may contribute to lymphomagenesis by favouring the decreased activity of the tumour suppressor and constitutive activation of the oncogene (33). Interestingly, these mutations are also found in about 40% of FL, suggesting that DLBCL and FL share common pathogenetic events (33). Chromosomal translocations juxtaposing the IRF4 oncogene next to one of the immunoglobulin loci were recently identified as a novel recurrent aberration in mature B-cell lymphoma, predominantly GCB-type DLBCL and FL (36). These lymphomas shared strong expression of IRF4/MUM1 and BCL6, and lacked PRDM1/BLIMP1 expression and *t*(14;18)/BCL2 breaks. The GEP of IG/IRF4-positive lymphomas differs from other subtypes of DLBCL, and IG/IRF4 positivity is associated with young age and a favourable outcome. IRF4 translocations may be primary alterations in a subset of GCB-derived lymphomas. The probability for this subtype of lymphoma significantly decreases with age, suggesting that diversity in tumour biology might contribute to the age-dependent differences in prognosis of lymphoma.

1.4. Staging

A diagnosis of DLBCL cannot be established without the examination of tissue obtained at biopsy. Excisional biopsy is mandatory for DLBCL diagnosis; while core needle biopsy should be performed only in the case other surgical approaches are possible. On the basis of the lymphoma presentation, the biopsy can be performed on

enlarged superficial lymph node or on lymphoid tissue in Waldeyer's ring or on mediastinal adenopathy by core needle biopsy, mediastinoscopy or mediastinotomy (37)] or on retroperitoneal adenopathy by ultrasound-guided core needle biopsy, laparoscopy or laparotomy (38). Once the diagnosis has been established the first critical step is the pre-treatment evaluation and staging.

The standard staging system used for DLBCL was proposed at the Ann Arbor Conference in 1971 (39) and the Cotswlds modification (40). This staging system reflects the number of sites of involvement and their relation to the diaphragm, the existence of B symptoms (fevers >38 °C for at least three consecutive days, night sweats, body weight loss >10% during the 6 months prior to diagnosis) and the presence of extranodal disease. A careful history and physical examination are the most important factors in the patient's evaluation. Physical examination includes evaluation of all lymph node enlargement, recording site and size of all abnormal lymph nodes, inspection of Waldeyer's ring, evaluation of the presence or absence of hepatosplenomegaly, inspection of the skin, and detection of palpable masses. The presence or absence of B symptoms should be noted, and other symptoms may show specific sites of involvement. An assessment of performance status according to the ECOG scale is important in all patients, and especially for those entering into clinical research trials.

Laboratory studies that should be routinely performed in NHL patients include a complete blood count to assess bone marrow reserves and a white blood cell differential with careful examination of the peripheral blood to look for the presence of circulating lymphoma cells. Serum chemistry should include an assessment of hepatic and renal function. Lactic dehydrogenase (LDH) is also an important indicator of tumour activity and is included in the International Prognostic Index (41). The uric acid level may predict patients at increased risk for urate nephropathy. A test for a complete assessment of HIV, HBV, and HCV should be performed in all patients.

A bone marrow aspirate and biopsy should be performed in all patients. Bilateral bone marrow biopsies have been recommended because they increase sensitivity of detection of NHL involvement by 10–20%. However, an adequate (>2 cm) unilateral bone marrow specimen is generally sufficient. Additional testing in DLBCL may include lumbar puncture to assess liquor cytology identifying subclinical meningeal involvement and brain MRI in patients with high risk of central nervous system (CNS) progression.

^{18}Fluorodeoxyglucose Positron Emission Tomography (PET) is now a standard procedure both for staging and response assessment. Many studies showed that PET at the end of treatment is highly predictive of PFS and OS in aggressive lymphomas with or without residual masses detected with CT scan. PET scan is able to distinguish between lymphoma and necrosis or fibrosis in residual masses. The combination of International Workshop Criteria (IWC) and PET was evaluated in a retrospective analysis of 54 patients with NHL. On the basis of this study, the International Harmonization Project has provided new recommendations for response criteria for aggressive malignant lymphomas, incorporating PET into the definition of response at the end of treatment (42;44). PET scan should not be used in follow-up setting, mostly due to a high rate of false positives and the fact that its increased sensitivity does not translates into clinical benefit. Further studies are warranted to investigate the cost effectiveness and the benefit of using PET during the follow up phase.

1.5. Prognosis

Each DLBCL category and their morphologic and/or clinico-pathologic variants exhibit varied clinical presentation, behaviour and treatment sensitivity. Regarding DLBCL–NOS, it occurs in adult patients, with a median age in the seventh decade, but the age range is broad, and it may also occur in children. Clinical presentation, behaviour and prognosis of DLBCL–NOS are variable, depending mainly of the extranodal site when they arise. Patients most often have a rapidly enlarging, often asymptomatic, mass at a single nodal or extranodal site; up to 40% of presentations regard an extranodal organ. These malignancies present in localized manner in approximately 20% of patients. Disseminated extranodal disease is less frequent, and one third of patients have systemic symptoms. Overall, DLBCLs are aggressive but potentially curable malignancies. Cure rate is particularly high in patients with limited disease with a 5-year progression free survival (PFS) ranging from 80% to 85%. Patients with advanced disease or symptomatic disease have a 5-year PFS ≈ 50%. Prognostic factors in DLBCL can be divided into those related primarily to the patient (e.g. age and performance status), those related to the tumour itself (e.g. stage, tumour burden, proliferating fraction, extranodal involvement), those related to aggressiveness indicators (e.g. LDH serum level, β2-microglobulin levels, proliferating fraction), and those related to the therapeutic strategy. Some extranodal sites, like brain (45) or testis (46), require special treatment strategies, and DLBCLs arisen in these organs constitute particularly entities

with poor prognosis. Response rate after primary treatment is highly predictive of outcome.

The *International Prognostic Index* (*IPI*) and *age-adjusted International Prognostic Index* (*aaIPI*) have been developed as models for predicting outcomes based on clinical factors from more than 4000 patients (Table 1) (41). The models proved to be more accurate than the Ann Arbor classification in predicting survival. The aaIPI, which includes stage, LDH and performance status, is the most commonly used in clinical practice and it is helpful in stratifying patients below or over 60 years of age. A revised version has been developed in the post-rituximab era (R-IPI) (47). The IPI has been documented to be robust in different series of DLBCL and still represents the only benchmark of DLBCL prognosis, even in the rituximab era (Fig. 1) (48).

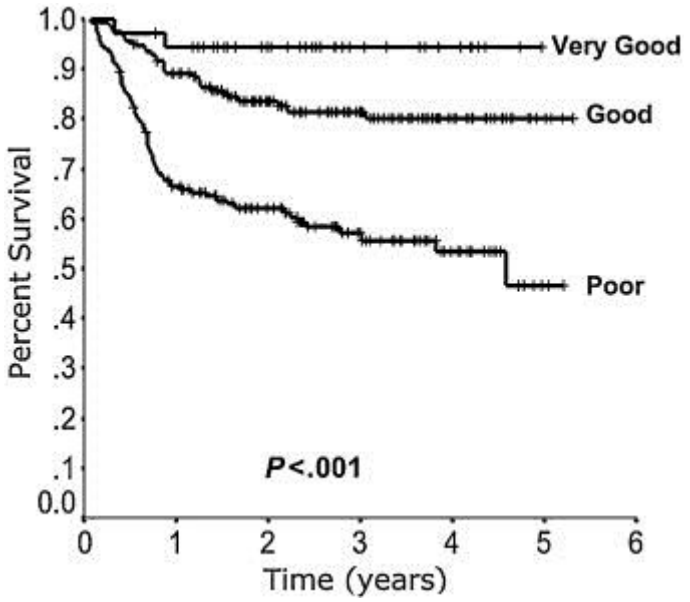
Table 1.

IPI		aa-IPI	
Risk group	IPI factors	Risk group	IPI factors
Low	0 or 1	Low	0
Low intermediate	2	Low intermediate	1
High intermediate	3	High intermediate	2
High	4 or 5	High	3
IPI Factors			
Older than 60 years of age (not used for aa-IPI)			
Disease stage III/IV			
Lactate dehydrogenase level elevated			
ECOG performance score ≥ 2			
Extranodal disease > 1 site (not used for aa-IPI)			

International Prognostic Index (IPI) for DLBCL.

aa-IPI, age-adjusted International Prognostic Index; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; R-IPI, revised International Prognostic Index.

Fig. 1.



R-IPI: Risk groups in DLBCL in the rituximab era.

Many biological parameters directly related to the tumour but also eventually to the host, have been identified as prognostic factors in DLBCL. This represents an exciting field of understanding of the disease and for designing of new therapeutics options, but the use of these factors for treatment decisions remains investigational. GEP of the malignant cells and their microenvironment is the most interesting procedure to reveal biological heterogeneity in DLBCL. Distinct subsets, including germinal-centre-like (GC) and activated B-cell-like (ABC) DLBCL, have been identified as having different genetic markers, activation pathways, and clinical outcomes (49;51). CD10, BCL-6, and MUM1 immunohistochemistry have been found to be potential surrogates for the gene expression profiles, having similar predictive capabilities (52). Currently, further investigation is needed before GEP or immunohistochemistry is applicable to clinical practice. However, some preliminary studies suggest that certain drugs are more active in one of the GEP subgroups of recurrent DLBCL and not in the other (53). A recently reported retrospective study showed that salvage therapy with lenalidomide is associated with a significantly higher response rate in ABC–DLBC with respect to GCB-cell-DLBCL (54). More solid confirmation on the prognostic value of these markers in large prospective trials is attended

1.6. Treatment

The choice of the first-line treatment for patients with DLBCL is based on the individual IPI score and age. Therefore, three major subgroups of DLBCL patients should be considered: elderly patients (>60 years, aaIPI = 0–3); young patients with low risk (≤60 years, aaIPI = 0–1); young patients with high risk (≤60 years, aaIPI = 2–3).

1.6.1. Treatment of elderly patients

The addition of the anti-CD20 monoclonal antibody rituximab to CHOP chemotherapy, administered every 14 or 21 days, is the standard treatment for elderly patients with DLBCL on a type 1 basis. A phase III study of Groupe d' Etude des Lymphomes de l'Adulte (GELA) randomized 399 previously untreated patients with DLBCL, 60–80 years old, to receive either 8 cycles of CHOP every 3 weeks (CHOP21) or 8 cycles of CHOP21 plus rituximab (55). Complete response (CR) rates, 2-year OS and Event-Free Survival (EFS) were significantly better in the R-CHOP arm without increase of adverse events. A recently updated follow-up analysis confirmed that the superiority of R-CHOP was maintained over time and the benefit was for all IPI subgroups (56,57). These figures are similar to those reported in a large retrospective study of the British Columbia University (58).

The real impact on survival of a dose-dense regimen in elderly patients with DLBCL remains a matter of debate. The German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL) have shown a survival improvement with a dose-dense programme as CHOP every 2 weeks (CHOP14) compared to CHOP21 in the NHL-B2 trial. This prospective randomized trial compared 6 cycles of CHOP-21, CHOP-14, CHOEP-21 and CHOEP-14 (bi-weekly) in a 2 × 2 factorial study design in patients older than 60 years showing that dose dense CHOP-14 significantly improved PFS and OS over the others (59). The following study conducted by the DSHNHL (RICOVER 60 trial) randomized 1222 patients to receive six or eight courses of CHOP14 with or without rituximab plus radiotherapy (RT) to sites of initial bulky disease (60). Six cycles of R-CHOP14 with 8 doses of rituximab significantly improved EFS, PFS, and OS over 6 cycles of CHOP-14 treatment (3-year OS: 78.1% vs. 67.7%). Moreover, no advantages were reported with the addition of 2 further courses of chemotherapy suggesting that response-adapted addition of chemotherapy beyond six cycles, though widely practiced, is not justified.

The optimal administration schedule of rituximab remains to be defined. Rituximab serum levels build up slowly after infusion and it may be that also dose dense immunotherapy may improve the efficacy of the treatment. Thus, the phase II "Dense-R-CHOP-14" trial explored in elderly patients, with supplemented dose intense rituximab during the first 2 cycles of R-CHOP14, maintaining a single dose in the subsequent cycles for a total of 12 doses of rituximab delivered in 6 courses of chemotherapy. One hundred patients were recruited, the results historically compared with those achieved in RICOVER 60 study, showed a markedly increase in rituximab serum level and suggested a higher efficacy in High-risk IPI patients (1-year EFS 74% vs. 65%) but an increased incidence of infection, mainly interstitial pneumonia, was also reported (61). This strategy is currently being compared with 8 doses over 2 week application (both in R-CHOP14 × 6 regimen) in a controlled randomized DSHNHL study.

A superiority of R-CHOP14 over R-CHOP21 has not been yet shown in randomized trials. Trials comparing R-CHOP21 with R-CHOP14 have been completed by the GELA in elderly patients (62), and by the British National Lymphoma Investigation (BNLI) in all age and IPI risk patients with DLBCL (63). The preliminary results of both trials did not show statistically significant differences for PFS and EFS between R-CHOP21 × 8 and R-CHOP14 × 6. However, in the French study many patients did not receive adequate supportive measures, such as prephase treatment and G-CSF administration, which are necessary to maintain an adequate dose-intensity when giving R-CHOP14 to elderly patients. In the British trial, the inclusion criteria were extended also to low-risk young patients with a potential risk of reducing the statistical power of the trial for high risk IPI and elderly patients. Hopefully, the final results of these and other randomized trials will clarify this issue.

1.6.2. Treatment of young patients with low-risk IPI

Following the outcome improvement observed in elderly patients, R-CHOP has been investigated also in younger patients. The retrospective study at the British Columbia University confirmed a significant improvement of OS and PFS also in patients of age < 60 years when rituximab was combined with CHOP compared to historical controls treated with CHOP alone (58) The benefit of rituximab in low-risk young patients has been also confirmed in a phase III multicentric study conducted by the Mabthera International Trial Group (the Mabthera International Trial – MInT).

Rituximab was combined with CHOP 21 or CHOP-like regimens (R-Chemo) and compared to the same chemotherapy regimens but without rituximab (chemo). Patients receiving R-chemo had a significantly better CR rate, and longer 3-year EFS and OS (64). No difference was demonstrated between R-CHOP21 and more intensive R-CHOP-like regimens (R-CHOEP-21, R-MACOP-B). This trial was recently updated, confirming significant better 6-year EFS and OS of R-Chemo over Chemo (64). The results of this trial support the use of six cycles of R-CHOP21 as standard therapy in younger patients with low IPI risk DLBCL on type 1 basis. The MInT trial has also identified two distinct prognostic sub-groups of patients with low IPI risk defined as favourable (i.e. IPI = 0 without bulky disease) or less-favourable (i.e. IPI = 1 or bulky disease, or both). The favourable group showed a significant better 6-year PFS compared to less-favourable group (89.5% vs. 76.7%, respectively) (64). In attempt to improve lymphoma-free survival in the less-favourable group, the GELA has conducted a randomized phase III trial that compared a dose-dense R-ACVBP to a conventional R-CHOP21. The preliminary results showed that the dose-dense R-ACVBP was associated with significant improvement in 3-year EFS, PFS and DFS, but increased haematological toxicity and mucositis were also reported. These results should be confirmed after a longer follow-up and compared to the updated 6-year results of R-CHOP21 of the MInT trial. In fact, recently updated MInt follow-up suggests that radiotherapy of bulky lesions could play a central role in the management of young patients with IPI = 1 DLBCL (65). Thus, R-ACVBP without radiotherapy or 6 courses of R-CHOP21 with radiotherapy to bulky disease could be proposed to these patients. A randomized phase III trial of the DSHNHL group (the UNFOLDER trial) who compare R-CHOP 14 to R-CHOP21 is ongoing and until those results will be available, R-CHOP21 will remain the standard regimen for the less favourable group. In the favourable group, considering the optimal survival rate obtained with six cycles of R-CHOP21, a reduction of the chemotherapy regimen is under investigation in a DSHNHL randomized phase III study (the FLYER trial).

1.6.3. Treatment of young patients with high-risk IPI

These patients have a poor outcome and represent probably the greatest current challenge in treating DLBCL. Around 30% of these patients develop refractory disease (progressing during first-line therapy or relapsing within one year). In the pre-rituximab era, consolidation with high-dose chemotherapy supported by autologous stem cell

transplant (HDC/ASCT) was employed in attempt to decrease relapse rate, but reported results were controversial in several randomized trial (68-72). Two meta-analyses on up to 11 randomized trials showed a similar OS in patients receiving first-line HDC/ASCT or standard chemotherapy. On the basis of observations, HDC/ASCT is not recommended as intensification/consolidative therapy for patients with newly diagnosed high-risk DLBCL outside a clinical trial. The routine use of HDC/ASCT is actually recommended as standard treatment only in young patients (<65 years) with DLBCL who did not achieve CR after first-line chemotherapy or in patients with chemosensitive DLBCL at relapse on type 1 basis (73).

Several non-randomized phase II studies incorporating rituximab into dose-dense or dose-intense regimens as R-CHOP14 or R-CHOP14-like (R-ACVBP, R-CHOEP) showed that such approaches are feasible and effective in high-risk young DLBCL patients. An Italian trial showed the feasibility of R-CHOP14 with pegfilgrastim support in 50 patients with newly diagnosed aggressive lymphoma with a relative dose intensity of 95% and a low incidence of febrile neutropenia (74). The estimation of the outcome of young high-risk patients treated with dose-dense or dose-intense schemes plus rituximab and without ASCT is difficult, it reports a 2–5 year PFS that did not exceeded 45–61%. These results indicate that there is a place for an HDC/ASCT strategy combined with dose-dense or dose-intense schemes in those high-risk groups of patients that are unlikely to be cured by standard R-CHOP (74;75).

The combination of rituximab with dose-dense chemotherapy and HDC/ASCT has been explored in 94 untreated poor risk young DLBCL (aa-IPI score 2–3) in a prospective phase II Italian trial. In this group of patients a CR was obtained in 82% of patients with a 4-year FFS and OS of 73% and 80% respectively (76). Similar results were presented in a case controlled study comparing a dose dense rituximab-ACVBP followed by ASCT (77). A summary of the most recent studies in poor-prognosis DLBCL treated with (rituximab) dose-dense chemotherapy with or without HDC/ASCT. These studies suggest that rituximab-dose-dense chemotherapy supplemented with HDC/ASCT may be effective in young DLBCL patients with a poor prognosis. However, the issue if rituximab-HDC/ASCT may be more effective compared with rituximab-dose-dense chemotherapy alone in these patients will be addressed only by randomized phase III trials that are currently ongoing into the major cooperative groups. The preliminary results of two of these randomized trials were recently reported. In the randomized phase II trial of the DSHNHL, which compares a dose-dense R-CHOEP14 to a dose-

escalated R-CHOEP plus HDC/ASCT, there were no statistical differences in PFS and OS but a significant better EFS with a lower haematological toxicity in the conventional arm (78). Also in a French cooperative randomized trial, the arm with intensified dose-dense regimen plus R-HDC/ASCT failed to demonstrate a significant benefit of lymphoma-free survival over a standard R-CHOP14 (79). The SWOG and the Italian Lymphoma Intergroup recently completed two randomized trials assessing the role of R-HDC and ASCT in younger high-risk (aaIPI = 2–3) DLBCL patients. The SWOG S9704 trial has investigated the role of HDC/ASCT as part of first-line treatment in patients with stage II–IV aggressive lymphoma, bulky disease and high-intermediate/high IPI in first remission after 5 cycles of CHOP ± R (80). Three hundred ninety-seven patients up to age 65 have been enrolled between 9/97 and 12/07 and induced with 5 cycles of CHOP (n = 215) or CHOP-R (n = 182); patients with complete or partial response (n = 253) have been randomly allocated to an additional course of CHOP ± R followed by autotransplant using TBI- or BCNU-based regimens (n = 125 – experimental arm) or to additional three courses of CHOP ± R (n = 128 – control arm). Experimental arm has been associated with a significantly better 2-year PFS (69% vs. 56%;p = 0.005), with no benefit in OS (2-year: 74% vs. 71%; p = 0.32). Only patients with high IPI achieved OS benefit after HDC/ASCT, with a 2-year OS of 82% and 64% respectively for experimental-arm and control-arm subgroups (80). Although the SWOG S9704 trial seems to support a role for upfront HDC/ASCT in patients with advanced DLBCL and high IPI grade, it is important to underline that accrual was long lasting (≈10 years), 54% of patients did not receive rituximab and several patients with histotypes other than DLBCL have been enrolled, with the potential introduction of relevant interpretation biases. In the Italian study (the DLCL04 trial) a full course of rituximab-dose-dense chemotherapy (R-CHOP14 or R-MegaCHOP14) alone was compared with the same rituximab-dose-dense chemotherapy followed by R-HDC/ASCT. Preliminary results of DLCL04 trial showed that upfront HDC/ASCT significantly reduces relapse rate in comparison to R-CHOP14 in young patients with high-risk DLBCL, but this PFS advantage did not translate into OS improvement (81). A longer follow-up will further clarify the role of first-line HDC/ASCT in these poor-prognosis patients. Considering preliminary results of the four randomized trials performed in the rituximab era (79;81), HDC/ASCT should not be used as upfront treatment for young high-risk patients outside prospective clinical trials.

1.6.4 Treatment of relapsed or refractory disease

Patients with DLBCL who experience relapse or fail to achieve CR after first-line therapy have a poor outcome. The wide use of front-line rituximab-containing chemoimmunotherapy regimens has been associated with a substantial reduction in relapse rates, which remain 10–20% among patients with low IPI risk and 30–50% in patients with IPI score >2. Less than 10% of failed patients achieve a long-term DFS with salvage conventional-dose chemotherapy, and it is well established that salvage treatment in a patients with chemosensitive relapse should, whenever possible, include HDC/ASCT (83). A large number of phase-II trials have addressed HDC/ASCT in patients with relapsed/refractory DLBCL (84-88), reporting a median 3-year PFS of 35% and, virtually, all these studies have demonstrated the prognostic importance of chemosensitivity before transplantation. The use of HDC/ASCT for relapsed DLBCL has been validated after the results of the International PARMA prospective randomized trial (82). Two sub-analysis of the PARMA trial, have demonstrated that an IPI score >1 at relapse and a short time to relapse, defined as TTR < 12 months, correlated with a poor survival (89;90). All the available evidence suggests that HDC/ASCT should be considered the standard therapy for DLBCL patients with chemotherapy-sensitive relapse on a type 1 basis.

Advances in salvage therapy are needed mainly to overcome resistance to chemotherapy, enabling more patients to achieve CR in order to proceed and optimize transplantation procedures. Various chemotherapy regimens have been employed for DLBCL in the salvage setting. The effectiveness of these regimens has been evaluated mainly in non-randomized phase II studies and their outcome is generally expressed in terms of ORR and possibility of collecting an adequate number of stem cells for transplantation. The combination of rituximab with salvage chemotherapy regimens has significantly improved the CR rate also in patients with relapsed/refractory disease. This assumption has been demonstrated in a series of 36 patients where the CR rate was significantly higher (53% vs. 27% $p = 0.01$) in the group treated with rituximab and ICE (R-ICE) compared with an historical controls of patients treated with ICE alone (91). The more robust demonstration of the potential benefits of the addition of rituximab to platinum-based salvage regimens has been reported by the HOVON group in a prospective randomized trial in 239 patients with relapsed/refractory DLBCL who received a salvage regimen consisting of DHAP–VIM–DHAP with or without

rituximab followed by HDC/ASCT. A marked difference in favour of the rituximab-containing regimen in terms of CRR (75% vs. 54%) and 2-year EFS (50% vs. 24%) has been observed. However, in this study the prior rituximab use in each group of patients was not well described (92). While the benefits of rituximab combined to salvage chemotherapy is clearly established, the optimal chemotherapy regimen still needs to be determined. Recently, the results of 396 relapsed/refractory DLBCL patients after first-line therapy enrolled in the CORAL international randomized trial, which compare R-DHAP to R-ICE followed by HDC/ASCT, have been reported. The ORR was 63%, with 38% of CR. There was no difference in terms of ORR between R-ICE (63.5%) and R-DHAP (62.8%), in mobilization-adjusted response rate (52% vs. 54%), and in the 3-year EFS (26% vs. 35%$p = 0.6$). Fewer serious adverse events were reported in the R-ICE regimen compared to R-DHAP (93). Of note, the 3-year EFS was significantly affected by early relapse (20% vs. 45%, $p < 0.0001$), by a high IPI risk at relapse (18% vs. 40%, $p < 0.0001$) and by prior first-line treatment with rituximab (21% vs. 47%,$p < 0.0001$). Early relapse, IPI at relapse and prior treatment with rituximab have been significantly associated with ORR, OS, EFS, and PFS (94). A recent analysis performed on the CORAL database showed that R-DHAP was superior to R-ICE in patients with ABC-type DLBCL (95). In this context, PET-positive result prior to ASCT is associated with a short duration of response and a higher risk of relapse (96;97). Thus, the response prior to transplant is highly predictive of the outcome and PET scan should be incorporated in the definition of quality of the response.

The use of allogeneic SCT for relapsed/refractory DLBCL is still limited since the reasonable good outcome and low toxicity of HDC/ASCT.

1.6.5. Salvage treatment strategies without transplant

Because of age or comorbidities not all patients are eligible for a transplant. We can assume that about 40–60% of elderly patients with DLBCL treated with R-CHOP will be refractory or experience relapse during their clinical course. Effective and less toxic chemotherapy approaches are still therefore needed. New chemotherapy drugs or biological agents both alone or in combination are also worth considering for this specific and broad group of patients.

More recently, gemcitabine and oxaliplatin have been shown activity in relapsed or refractory DLBCL. In a single arm study involving 46 patients with relapsed DLBCL who received rituximab, gemcitabine and oxaliplatin in combination (R-Gem-OX), 38 patients (83%) responded and 23 (50%) achieved CR by the end of treatment. The regimen has been well tolerated but long-term results in patients previously treated with rituximab are not satisfactory (98). New biological drugs with different mechanisms of action should be encouraged in this unfavourable and frail group of elderly patients.

1.6.6. Novel approaches to DLBCL

Several novel agents are undergoing evaluation in DLBCL, as both single agents in the relapsed setting or in combination with standard chemotherapy R-CHOP. These agents have different activity degrees and some of their mechanisms are incompletely understood. These new approaches include immunomodulating agents (IMiDs) such as lenalidomide, m-TOR inhibitors as temsirolimus and everolimus, proteasome inhibitors as bortezomib, histone deacetylase inhibitors as vorinostat, and anti-angiogenetic agents (anti-VEGF) as bevacizumab.

IMIDs inhibit angiogenesis and tumour necrosis factor (TNF)-alpha, stimulate immune responses, alter cytokines and inhibit interleukin-12, affect stromal cells, induce apoptosis and inhibit pro-survival factors (Akt). Lenalidomide has been evaluated as single agent in a series of 73 relapsed/refractory DLBCL patients with an ORR of 29%. Toxicity was mild and mainly haematological (99). In another series of 49 heavily pre-treated patients with relapse/refractory DLBCL treated with lenalidomide 25 mg daily an objective response was observed in 35% of cases, with a 12% CRR (100). On this basis, the Fondazione Italiana Linfomi is running a prospective multicenter dose finding phase II pilot trial to evaluate efficacy and safety of treatment with lenalidomide plus R-CHOP21 (LR-CHOP21) for elderly patients with untreated Intermediate-High/high-risk IPI, with a MTD of lenalidomide in this combination of 15 mg over 14 days.

A variety of intracellular oncogenic pathways are potential targets for DLBCL therapy. Small molecules can selectively inhibit specific signalling molecules of pathways critical for survival of lymphoma cells. The targeting of PI3K/Akt/mTOR pathway seems to be a relevant strategy for the treatment of some lymphomas. The exact mechanisms of mTOR inhibitors remain unclear, but likely include induction of autophagy, anti-angiogenesis, immunoregulation and translation inhibition of cell survival proteins (101,102). First-generation mTOR inhibitors were soluble rapamycin

derivatives (rapalogs), temsirolimus and everolimus being the better known agents. Everolimus has promising single-agent activity in various lymphomas, with an ORR of 30% in DLBCL (103). This drug is being addressed as maintenance therapy in DLBCL in CR after RCHOP in a phase III trial. The strategy of targeting molecules upstream of mTOR, such as protein kinase 3 (AKT) and PI3K, is more potent than the use of mTOR inhibitors in in vitro studies. Enzastaurin is a selective inhibitor of the PKC and AKT pathways known to promote tumour angiogenesis, as well as tumour-cell survival and proliferation (104). A phase II trial with oral enzastaurin plus GEMOX is ongoing, while preliminary results of a small randomized phase II study suggest a better outcome with the combination of R-CHOP-enzastaurin vs. R-CHOP (104). Oral enzastaurin is also being tested in DLBCL patients as maintenance after the end of first-line chemotherapy in a large international phase III randomized trial.

Bortezomib is a proteasome inhibitor initially approved for use in multiple myeloma and currently under continued investigation in lymphomas, with reasonable response rates in mantle cell lymphoma. One postulated mechanism of action in lymphomas is the ability of bortezomib to ameliorate molecular dysregulation in NF-κB activation and regain cell cycle control. Bortezomib has been safely administered in combination with R-CHOP21 to patients with newly diagnosed DLBCL (106). This combination has been associated with an ORR of 100% and 86% CRR, with a 2-year PFS of 64% and 2-year OS of 70%. Unlike in DLBCL treated with R-CHOP alone, germinal-centre B-cell-like and nongerminal-centre B-cell DLBCL subtypes had similar outcomes (106) Moreover, some activity in combination with salvage regimens have been reported (107).

New generation of monoclonal antibodies are currently being investigated to assess their activity in DLBCL. Inotuzumab ozogamicin (CMC-544), a humanized anti-CD22 antibody conjugated to calicheamicin, a potent antitumor antibiotic, demonstrated an improved single-agent activity, with 15% ORR in DLBCLs (108). Recently, a new humanized anti-CD20 mAb, with high affinity for FcRγIII, named GA101, was developed. GA101 demonstrated antibody-dependent complement-mediated cytotoxicity and strong caspase-independent apoptosis activity upon CD20 binding (109). An European randomized phase-III trial finalized to compare rituximab vs. GA101 in association with standard chemotherapy CHOP in DLBCL is ongoing. Preliminary results of ongoing phase I and II clinical trials suggest a relevant activity with blinatumomab, a bispecific T-cell engager targeting CD19 and CD3 antigens, in relapsed or refractory follicular lymphoma and mantle cell lymphoma suggesting this

antibody should be assessed also in DLBCL. One advantage of blinatumomab is the use of activated CD3+ T cells to kill the malignant CD19+ B cells bypassing specialized effectors (110).

Histone deacetylases (HDACs) inhibitors play a role in regulating cell-cycle progression, survival, angiogenesis and immunity and constitutes a new group of drugs with demonstrated activity in some lymphoma categories, mostly cutaneous T-cell lymphoma and Hodgkin lymphoma. Expression of HAT1 and HDAC1, molecules that play a critical role in lymphomagenesis, is high in DLBCL, and it seems to be correlated with poor survival (111). These observations seem to suggest that HDACs inhibitors may play a therapeutic role also in DLBCL and constitute the background of ongoing prospective trials. Since HDACs modulate a variety of survival factors, the most probable use of their inhibitors will be in combination with other biological agents.

In the next future, the combination of these new biological drugs with conventional chemo-immunotherapy should be incorporated in the first-line management of DLBCL patients in attempt to increase the response rate and lymphoma-free survival.

2. OVERVIEW OF HYSTONE DEACETYLASE INHIBITORS

2.1. Overview of Panobinostat (LBH589)

Histone deacetylases (DACs) are enzymes that regulate chromatin structure and function through the removal of acetyl groups from lysine residues of core histones. In particular DACs target lysine groups on chromatin, transcription factors and various non-histone proteins such as p53, tubulin, HSP90 and Rb. Hystone deacetylase inhibitors (DACi) are a group of molecules able to induce rapid histone hyperacetylation and chromatin remodelling yielding to the activation or repression of genes regulating apoptosis, proliferation, differentiation, angiogenesis, immune responses. Panobinostat is a DACi belonging to cinnamic hydroxamic acid class of compounds. It is a highly potent class I/II pan-DAC inhibitor that has shown anti-tumor activity in pre-clinical models and in cancer patients. Panobinostat is formulated as an oral capsule and a solution for intravenous (IV) injection. Both the oral and IV formulations are currently being investigated in ongoing phase I and phase II studies in advanced solid tumors and haematological malignancies.

2.2. Proposed mechanism of anticancer activity of DAC inhibitors

Alterations in chromosome structure play critical roles in the control of gene transcription. These epigenetic alterations include modification of histones and others proteins by acetylation and/or phosphorylation. Normally, these modifications are balanced finely by maintenance of levels of deacetylase enzymes in check. The levels of these enzymes are altered and may be elevated in tumor cells, thereby causing an imbalance and thus leading to a closed and tightly bound chromatin structure. This leads to an inability of the transcription initiation factors to bind to the promoter regions on the DNA resulting in the modulation of the expression of a subset of genes, including the tumor suppressor genes, in a coordinated fashion. Several tumors suppressor genes associated with the malignant phenotype are repressed by epigenetic mechanisms in sporadic cancers. Thus, therapy with DAC inhibitors, which leads to an opening of the chromatin structure thereby allowing re-initiation of the transcription mechanisms, may alter tumor phenotype and inhibit growth in such tumors. Tumor growth inhibition and

apoptosis in response to DACi treatment may also be mediated by changes in acetylation of non-histone proteins (e.g., HSP90, p53, HIF-1α, α-tubulin). For example, the chaperone protein HSP90 has been shown to be acetylated in cells treated with DACi (112-114). Acetylation of HSP90 inhibits its ability to bind newly synthesized client proteins, thus preventing proper client protein folding and function. In the absence of HSP90 function, misfolded proteins are targeted for degradation in the proteasome. Many proteins that require HSP90 association are critical to cancer cell growth, including ErbB1, ErbB2, AKT, Raf, KDR, and BCR-ABL. Acetylation of HSP90 in cells treated with DACi inhibits the chaperone function of HSP90, leading to degradation of the client proteins and eventually cell death. The potential clinical utility of the use of DACi in cancer therapy was first suggested by the activity of sodium phenylbutyrate against acute promyelocytic leukemia (115). Vorinostat (Zolinza™), an orally administered, structurally-related DACi has been reported to have single-agent activity in cutaneous T-cell lymphoma (CTCL), diffuse large cell lymphoma, and head and neck cancer (116;117). Vorinostat was approved by the FDA for the treatment of cutaneous manifestations of CTCL in patients with progressive, persistent or recurrent disease (118). Similar activity has been reported in clinical studies with other DACi, including IV romidepsin (119), which was approved for the treatment of patients who have received at least one prior systemic therapy in CTCL in November 5, 2009.

2.3. Preclinical studies

2.3.1. In vitro study

Preclinical activity of panobinostat was tested against a broad array of tumor cell lines, including the Hodgkin's lymphoma cell lines RPMI6666, HD-MY-Z, and L428. These cells were incubated in culture with different concentrations of panobinostat to determine the effect of the compound on the proliferation and viability of the cells. Consistent with the proposed mechanism of action for panobinostat, tumor cells exposed to panobinostat for 2 hours or more demonstrated increased levels of histone-H3 and -H4 acetylation lasting up to 72 hours. The HL cell lines exhibited extreme sensitivity to panobinostat with IC50 values ranging from 200 picomolar to 50 nanomolar; these concentrations can easily be achieved in the plasma of patients with doses that have been safely administered. Panobinostat induced cell death in transformed cells while, in similar conditions, induced growth arrest in normal cells.

A recent study (120) highlighted that the inhibition of histone deacetylase overcomes rapamycin-mediated resistance in diffuse large B cell lymphoma (DLBCL) by inhibiting Akt signaling through mTORC2. In this study, increasing doses of panobinostat (5-100 nM) suppressed the survival of each cell line in a dose-dependent manner. The anti neoplastic effect of *ex vivo* panobinostat was recently investigated in cells from biopsies of patients with various B-NHL, including 5 DLBCL. Fifteen out of 16 patient samples analyzed showed moderate to high sensitivity to panobinostat; Western blots of lymphocytes treated with panobinostat showed increased acetylation of histone H4 compared to untreated cells (121).

2.3.2 In vivo studies

The activity of panobinostat was assessed in several *in vivo* tumor models including BT474 (breast cancer) and CWR22Rv1 (prostate adenocarcinoma) mouse models. Panobinostat treated tumors regressed during the time of treatment whereas the vehicle treated tumors grew. Additional data and experiments, including those for hematological malignancy cell lines and xenograft models, are described in the panobinostat IB.

2.4. Clinical studies

2.4.1 Pharmacokinetics

Panobinostat is rapidly absorbed with a median T_{max} reached within 1 hour after oral administration. The absolute oral bioavailability of panobinostat is approximately 30%. The compound can be administer regardless of food intake as the variability and overall systemic exposure remained unchanged in patients taking panobinostat with or without food (122). AUC increased linearly and proportionally with doses up to 50 mg. *In vitro* plasma protein binding of panobinostat (mainly to albumin) is moderate (89.6% at 37°C) and independent of concentration. Elimination half-lives averaged 15 hours. Steady state is achieved by the third dose following days 1, 3, and 5 (TIW) weekly (QW) dosing. The metabolism of panobinostat is extensive and several metabolic pathways are involved including reduction, hydrolysis, oxidation, and glucuronidation processes. It is a substrate of cytochrome P450 3A4 (CYP3A4) with minor involvement of CYP2D6 and 2C19 as well as an inhibitor of CYP2D6. Panobinostat and its

metabolites (~at least 40 metabolites in circulating plasma) were nearly equally excreted in urine and bile/feces of patients.

2.4.2 Safety

Pooled safety data from patients with advanced hematological malignancies or solid tumors or lymphomas, enrolled in three Phase I and six Phase II studies of single-agent oral panobinostat have recently been reviewed. Data cut-off date was April 8, 2009 for 7 studies and Sept 24, 2009 and Oct 3, 2009 for two other studies, respectively. The overall population was divided in patients with solid tumors or lymphomas (N = 266) and patients with all other hematological malignancies (N = 293). Furthermore, data was analyzed separately for patients who received panobinostat three times a week every week (N = 226) and three times a week every other week (N = 40)

For the every week dosing schedule:

• Thrombocytopenia is the most common adverse event reported; it is noted at all dose levels with increasing frequency and severity with an increase in dose. In the clinical trials, thrombocytopenia has been successfully managed with dose interruption, dose reduction and platelet transfusion. Considering this common occurrence of thrombocytopenia, a guidance has been provided regarding exclusion of patients from the study entry if the baseline platelet count is below 100 $x10^9$/L and dose delay or reductions be instituted for low counts during the study.

• Gastrointestinal events such as diarrhea and nausea are also commonly reported but are primarily of grade 1 or 2 severity.

• Anemia and neutropenia are noted primarily at the 40 mg dose and since this dose has been studied mostly in patients with Hodgkin's lymphoma, the underlying disease may have a role in the occurrence of this event.

• Fatigue of grade 1 and 2 severity is reported across all doses but it does not seem to have an augmented incidence with the increasing in dosage. The underlying reason for fatigue is currently unknown.

Most common adverse events reported when panobinostat is administered three times every week (N=226)

	20 mg 3x week q wk N=190		30 mg 3x week q wk N=13		40 mg 3x week q wk N=86		60 mg 3x week q wk N=5	
Preferred term	**All grades**	**Grade 3/4**	**All grades**	**Grade 3/4**	**All grades**	**Grade 3/4**	**All grades**	**Grade 3/4**
Thrombocytopenia	81 (42.6)	34 (17.9)	8 (61.5)	6 (46.2)	73 (84.9)	70 (81.4)	5 (100)	5 (100)
Diarrhea	104 (54.7)	8 (4.2)	10 (76.9)	1 (7.7)	56 (65.1)	3 (3.5)	1 (20.0)	0 (0.0)
Nausea	88 (46.3)	2 (1.1)	6 (46.2)	0 (0.0)	53 (61.6)	2 (2.3)	3 (60.0)	0 (0.0)
Fatigue	110 (57.9)	12 (6.3)	7 (53.8)	0 (0.0)	52 (60.5)	14 (16.3)	3 (60.0)	0 (0.0)
Anemia	28 (14.7)	6 (3.2)	6 (46.2)	2 (15.4)	35 (40.7)	20 (23.3)	1 (20.0)	1 (20.0)
Hypokalemia	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	30 (34.9)	3 (3.5)	1 (20.0)	0 (0.0)
Vomiting	36 (18.9)	2 (1.1)	4 (30.8)	0 (0.0)	29 (33.7)	3 (3.5)	1 (20.0)	0 (0.0)
Pyrexia	31 (16.3)	3 (1.6)	4 (30.8)	0 (0.0)	24 (27.9)	3 (3.5)	2 (40.0)	0 (0.0)
Neutropenia	30 (15.8)	21 (11.1)	1 (7.7)	1 (7.7)	21 (24.4)	15 (17.4)	2 (40.0)	2 (40.0)
Cough	21 (11.1)	0 (0.0)	3 (23.1)	1 (7.7)	19 (22.1)	2 (2.3)	2 (40.0)	0 (0.0)
Anorexia	64 (33.7)	1 (0.5)	7 (53.8)	0 (0.0)	18 (20.9)	4 (4.7)	3 (60.0)	0 (0.0)
Constipation	26 (13.7)	0 (0.0)	1 (7.7)	0 (0.0)	17 (19.8)	0 (0.0)	0 (0.0)	0 (0.0)
* Table sorted in decreasing order based on All Grade events for 40 mg dose								

For the <u>every other week</u> dosing schedule, the number of patients treated is small and so direct comparisons cannot be made between the 2 schedules. The following findings can still be noted:

• In this small number of patients, fatigue is the most common adverse event, although the events are primarily of mild severity.

• While thrombocytopenia is commonly seen, the frequency of grade 3 or 4 events is much lower than that observed with the every week schedule

• Other adverse events are all of grade 1 and 2 severity.

Since thrombocytopenia is the most common adverse event observed with panobinostat, additional analysis have been conduced that suggest:

• The frequency of grade 3/4 thrombocytopenia incidence was generally higher with panobinostat weekly dosing, compared to dosing every other week

• Thrombocytopenia was more frequently observed in patients with hematologic malignancies

• For patients with solid tumors or lymphomas, new or worsening grade 3 or 4 thrombocytopenia onset often occurred within the first 2 weeks for ≥40 mg/dose every week schedule

• Median time to thrombocytopenia was generally shorter in the weekly schedules than that in every other week schedules, especially for patients with solid tumors and lymphomas

• The median duration of thrombocytopenia was relatively short (~8 days) and similar across both schedules

• In general, as supported by the preclinical findings, every other week dosing appears to be associated with lower hazard rate of new or worsening grade 3 or 4 thrombocytopenia compared to the weekly dosing schedule.

• The frequency of dose adjustments, interruptions, and discontinuation due to thrombocytopenia was generally higher with panobinostat weekly dosing, compared to every other week dosing at comparable dose levels, which suggests better tolerability of every other week dosing with oral panobinostat.

Most common adverse events reported when panobinostat is administered three times every other week (N=40)

	30 mg 3x week q other wk N=21		45 mg 3x week q other wk N=7		60 mg 3x week q other wk N=12	
Preferred term	**All grades**	**Grade 3/4**	**All grades**	**Grade 3/4**	**All grades**	**Grade 3/4**
Fatigue	17 (81.0)	1 (4.8)	7 (100)	0 (0.0)	9 (75.0)	3 (25.0)
Thrombocytopenia	8 (38.1)	6 (28.6)	5 (71.4)	4 (57.1)	9 (75.0)	9 (75.0)
Diarrhea	11 (52.4)	1 (4.8)	5 (71.4)	1 (14.3)	10 (83.3)	3 (25.0)
Nausea	12 (57.1)	0 (0.0)	4 (57.1)	0 (0.0)	9 (75.0)	0 (0.0)
Anorexia	15 (71.4)	0 (0.0)	4 (57.1)	0 (0.0)	7 (58.3)	0 (0.0)
Vomiting	9 (42.9)	0 (0.0)	3 (42.9)	0 (0.0)	7 (58.3)	0 (0.0)
Anemia	5 (23.8)	3 (14.3)	3 (42.9)	0 (0.0)	5 (41.7)	1 (8.3)
Pyrexia	9 (42.9)	1 (4.8)	3 (42.9)	0 (0.0)	4 (33.3)	0 (0.0)
Edema peripheral	5 (23.8)	0 (0.0)	3 (42.9)	0 (0.0)	2 (16.7)	0 (0.0)
Weight decreased	8 (38.1)	0 (0.0)	3 (42.9)	0 (0.0)	1 (8.3)	0 (0.0)
* Table sorted in descending order of occurrence based on All Grade events for 45 mg dose. If 2 or more events have same frequency at the 45 mg dose then further sorting based on 60 mg All Grade events						

2.5. Clinical activity in hematological malignancies

The safety and tolerability of panobinostat has been explored in several hematological malignancies.

Giles et al. described 15 patients with acute myeloid leukemia, acute lymphocytic leukemia or myelodisplastic syndrome who were treated with increasing doses of panobinostat (4,8 – 14 mg/m^2). Four dose-limiting toxicities (grade 3 QTcF prolongation) were observed; other toxicities included nausea (40%), diarrhea (33%), vomiting (33%), hypokalemia (27%), loss of appetite (13%), and thrombocytopenia (13%). In 8 of 11 patients with peripheral blasts, transient blast cell reductions occurred with a rebound following the treatment period (123).

Another large study was performed to evaluate panobinostat in patients with advanced hematologic malignancies including Hodgkin lymphoma, non-Hodgkin lymphoma, or multiple myeloma, acute myeloid leukemia, myelodysplastic syndrome, chronic idiopathic myelofibrosis, acute lymphoblastic leukemia, chronic myelomonocytic leukemia, chronic myeloid leukemia and chronic lymphocytic leukemia. 128 pts were enrolled: the most common grade 3/4 AEs (≥10%) were thrombocytopenia (43%),

neutropenia (22%), febrile neutropenia (20%), fatigue (20%), and anemia (11%). Anti-tumor activity was observed in a group of 13 response-evaluable patients with relapsed/refractory Hodgkin lymphoma, in 26 patients with acute myeloid leukemia and in patients with other hematologic malignancies (124).

Another study investigated safety and toxicity of panobinostat in resistant-refractory patients with mycosis fungoides and Sezary syndrome. Panobinostat was administered at the dose of 20 mg on days 1, 3, and 5 weekly until disease progression or intolerance. Diarrhea, thrombocytopenia, fatigue, asthenia, hyper-triglyceridaemia, nausea and pruritus were the most common toxicities, while no QTc prolongation >500 ms were evidenced. Three patients achieved a partial response and 4 patients maintained a stable disease (125).

Another study tested panobinostat in the same setting of patients: 10 patients with cutaneous T-cell lymphoma received 20 mg of panobinostat on days 1, 3 and 5 of each week on a 28-day cycle. Patients attained a CR (2), a PR (4), achieved stable disease with ongoing improvement (1), or progressed on treatment (2). The treatment was globally well tolerated, and the microarray data showed that the majority of genes involved in this neoplasm was repressed after treatment (126). Panobinostat use was investigated in myelofibrosis too. Thirteen patients have been treated, including 10 patients with idiopathic myelofibrosis and 3 pts with post-polycythemic disease. Among 12 patients evaluable for response, one has demonstrated a partial response, including an 85% reduction in spleen size; three patients demonstrated clinical benefit lasting ≥8 weeks: reduction in spleen size, transfusion independence and improvement in other disease-related symptoms were observed. Four patients had stable disease (127).

In another study, panobinostat was implied in 12 patients with primary myelofibrosis and post-polycythemia/essential thrombocythemia myelofibrosis: two patients had a greater than 50% reduction in spleen size. One of these patients has also had a significant reduction in RBC transfusion requirements and has complete resolution of splenomegaly at 7 months. Two patients experienced clinical improvement and 4 patients had stable disease. Thrombocytopenia was the only DLT observed; the most common non-hematologic AEs noted were grade 1 nausea, fatigue, diarrhea and musculoskeletal pain (128).

Two studies evaluated the possibility to imply panobinostat in chronic myeloid leukemia (CML); the first study investigated the efficacy and safety of panobinostat in patients with chronic phase CML who had received at least 2 prior BCR-ABL tyrosine

kinase inhibitors. 29 patients were enrolled, and no major cytogenetic response (MCyR), but 1 complete hematologic response (CHR) with eradication of the T315i mutation were observed (129). The second study used panobinostat in patients in accelerated phase or blastic crisis stage of a Ph positive CML. A total of 27 patients were enrolled, but no MCyR or CHR were observed (130). A more recent report, analyzed safety and tolerability of panobinostat given in combination with imatinib in CML patients. Five patients were enrolled: DLT (grade 3 thrombocytopenia) was observed in one patient. Toxicities included thrombocytopenia (Grade 3 [n=2]; grade 1-2 [n=4]), hypophosphatemia (grade 3 [n=1]; grade 2 [n=2]), fatigue (grade 1 [n=3]), hypocalcemia (grade 1 [n=2] and grade 1-2 GI symptoms (diarrhea [n=2]; nausea [n=3]; anorexia [n=2]; vomiting [n=3]; constipation [n=1]), notably, no significant QTc prolongation was observed on intensive. Q-PCR analyses showed reduction in BCR-ABL levels in 2 patients, with a patient that reached undetectable BCR-ABL after 3 months of treatment (131).

Panobinostat was largely tested also in multiple myeloma. In a first study, 38 patients with advanced refractory multiple myeloma were treated with 20 mg of this drug. Overall, panobinostat was well tolerated; mild or moderate level of nausea, as well as fatigue/asthenia, occurred in half of the patients. One clinical durable response (very good partial response – VGPR) and 3 stable disease observations longer than 3 months occurred in 3 patients (132). Another study explored maximum-tolerated dose (MTD), safety, tolerability, pharmacokinetic/pharmacodynamic profiles, and preliminary efficacy of the combination therapy including panobinostat and bortezomib in patients with advanced refractory multiple myeloma. A total of 29 patients have been enrolled into four dosing cohorts: encouraging clinical efficacy was observed in all four cohorts, with 14 responders (PR or better) in 28 evaluable patients (50%), including 4 with immunofixation negative CR. Four additional patients achieved minor responses, resulting in 64% overall response rate. Responses were also seen in the subset of patients refractory to prior bortezomib, suggesting a strong clinical correlation for synergism of the panobinostat plus bortezomib combination. Overall, the combination of panobinostat and bortezomib was safe and tolerated; hematologic adverse events (AEs) have been frequent, including grade 3/4 thrombocytopenia (25), neutropenia (18), and anemia (6): non-hematologic AEs included: diarrhea (18), fever (15), nausea (14), fatigue (14), and asthenia (11) (133). A recent study investigated safety, tolerability, PK/PD, and preliminary efficacy of combination treatment of panobinostat,

lenalidomide and dexamethasone. Twenty-two patients with relapsed or relapsed refractory MM were treated in three dose levels; the only study drug related SAE was fever in two patients. Safety and early efficacy have not been reported yet (134).

In vivo studies indicated that panobinostat is well tolerated and induces clinical responses in several hematological malignancies including HL and CTCL. On these grounds several new clinical studies are now ongoing to test the clinical activity of panobinostat in different clinical setting. Some *in vitro* studies suggest the possible therapeutic activity also in patients with aggressive B cell lymphoma.

3. A PHASE II STUDY OF ORAL PANOBINOSTAT IN ADULT PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA RELAPSED/REFRACTORY AFTER HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSFUSION (ASCT) OR NOT ELEGIBLE FOR ASCT.

3.1. Purpose/Rationale

Treatment of patients with DLBCL who relapse after ASCT or result non eligible for ASCT because of refractory disease, age or co-morbidities represents an unmet medical need. The use of DACis is approved in cutaneous T-cell lymphoma, but the efficacy in other lymphomas, particularly B-cell Non-Hodgkin's lymphoma, remains to be established. Even if the therapeutic activity of panobinostat in DLBCL is not yet defined, some preclinical data suggests the possible of this agent for these diseases. The rationale of this study is to investigate the therapeutic activity of panobinostat in patients with DLBCL in order to consider the possible future use of this agent either in combination with standard therapy or as maintenance therapy.

3.2. Objectives

Primary objective

1. To explore the anti-tumor activity of panobinostat in term of overall response (OR) at the end of the induction phase (i.e. month 6 from the beginning of panobinostat)

Secondary objectives

1. To explore the anti-tumor activity of panobinostat in terms of Complete Response (CR)
2. To assess the time to response (TTR)
3. To evaluate Progression Free Survival (PFS)
4. To assess the safety and tolerability of panobinostat
5. To evaluate the Overall Survival (OS)

Exploratory objectives

1. To study the impact of pharmacogenetics in predicting the response to panobinostat
2. To study the impact of immunohistochemical patterns and patient's specific gene expression and response to panobinostat
3. To assess the correlation between "telomeric asset" and response to panobinostat

Endpoints

- CT scan evaluation to assess the response rate and for monitoring progression according to the Cheson 1999 response criteria; the reason to adopt the Cheson 1999 criteria is because of the high number of radiological evaluation indicated in the study, particularly during the induction phase (every two months). Overall response is defined as CR or PR
- Safety: Common Terminology Criteria for Adverse Events (CTCAE Version 4.0)
- SNPs genotypic variant (pharmacogenetics)
- Immunophenotypic analysis on tissue-microarray (to assess whether the distinction of DLBCL subtypes according to the supposed histogenesis GBC-type vs. ABC-type vs. NC performed by immunohistochemistry has prognostic value in patients treated with DACi)
- Gene expression analysis (to identify molecular signatures related to treatment response/resistance; to test previously identified molecular profiles associated to resistance to conventional chemotherapy in patients receiving DACi)
- Assessment of telomere-deregulation by assessing the following parameters: whole telomere lenght by southern blot and STELA (single telomere length analysis), 3' overhang size by G-tail telomere hybridization protection assay (Gt-telomere HPA), and expression of telomere-related proteins.
- The Progression Free Survival (PFS) is defined as the time from enrolment to disease progression or death from any cause
- The time to response is defined as the time from enrolment to OR
- The Overall Survival (OS) is defined as the time from enrolment to death from any case

3.3. Study design

This will be a multi-center, italian, open-label, phase II study. All participating centers are members of FIL (Italian Lymphoma Foundation) with specific expertise in the management of patients with lymphoma.

The trial is conducted according to the optimal two-stage design of Simon with alpha 0.05 and beta 0.10, considering the following two hypotheses: first a response rate (RR) less than 10% is of no further interest; and second, an RR 30% is clinically meaningful. In the initial stage, 18 patients have to enter onto the study. If less than 3 responses (≤2 in 18) will be observed, the trial would be terminated. Otherwise, accrual will continue to a total of a maximum of 35 patients. At the end of the trial, if 6 or fewer responses will occur among the 35 patients (≤6 in 35), it will be concluded that the regimen is not worthy of further investigations for that group of patients.

The treatment is divided in three phases: induction phase (course 1 to 6), consolidation phase (courses 7 to 12), maintenance phase (from course 13 until the end of therapy for any reason). This choice is made in order to have a well defined initial period of response evaluation (induction phase) and to understand the possible impact of subsequent continuous administration of panobinostat in terms of improvement of response (consolidation phase) and response duration (maintenance phase). This study is expected to start in January 2011. The last patient is expected to be enrolled at the end of December 2011. Considering a possible treatment duration of 24 months, this trial is due to be completed by December 2013. Approximately 15 centers are involved.

3.4. Inclusion/Exclusion criteria

3.4.1 Inclusion criteria:

1. Patient age is ≥ 18 years
2. Patient has an Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 2
3. Patient has a history of DLBCL according to the WHO classification
4. Patient has progressive disease after receiving at least CHOP-R or CHOP-R like first line regimen, standard second line therapy (DHAP, ESHAP, ICE or similar salvage regimens) inclusive ASCT

5. Patient has progressive disease after receiving at least CHOP-R or CHOP-R like first line regimen and is not considered eligible for intensive salvage therapy including ASCT because of age, co-morbidities, impossibility to perform ASCT

6. Patient undergoes at baseline new lymph node or other pathologic tissue biopsy for confirmation of diagnosis and biologic studies; bone marrow biopsy is not adequate for this purpose and should be performed only for staging. Patients with primary refractoriness, not eligible for intensive salvage therapy including ASCT, who performed a previous biopsy with stored frozen material 6 months or less before enrolment into the study do not have to repeat a new biopsy

7. Patient has at least one site of measurable nodal disease at baseline ≥ 2.0 cm in the longest transverse diameter as determined by CT scan (MRI is allowed only if CT scan can not be performed). Note: Patients with bone marrow involvement are eligible, but this criteria alone should not be used for disease measurement

8. Patient has the following laboratory values (labs may be repeated, if needed, to obtain acceptable values before screen fail):

- Absolute neutrophil count (ANC) ≥ 1.5 x 109/L [SI units 1.5 x 109/L]
- Platelet count ≥ 100 x 109/L
- Serum potassium, magnesium, phosphorus, sodium, total calcium (corrected for serum albumin) or ionized calcium within normal limits (WNL) for the institution
- Serum creatinine ≤ 1.5 x ULN
- Serum bilirubin ≤ 1.5 x ULN (or ≤ 3.0 x ULN, if patient has Gilbert syndrome)
- AST/SGOT and/or ALT/SGPT ≤ 2.5 x upper limit of normal (ULN) or ≤ 5.0 x ULN if the transaminase elevation is due to disease involvement

9. Clinically euthyroid. Note: Patients are permitted to receive thyroid hormone supplements to treat underlying hypothyroidism

10. Written informed consent was obtained from the patient prior to any study-specific screening procedures

11. Patient has the ability to swallow capsules or tablets

12. Practice acceptable birth control

3.4.2 Exclusion criteria

1. Patient has a history of prior treatment with a DAC inhibitors including panobinostat

2. Patient will need valproic acid for any medical condition during the study or within 5 days prior to the first panobinostat treatment

3. Patient has been treated with monoclonal antibody therapy (e.g., rituximab or anti CD-30 antibody, etc.) within 4 weeks of start of study treatment

4. Patient has been treated with any other anti lymphoma therapy within 3 weeks of start of study treatment

5. Patient is using any anti-cancer therapy concomitantly

6. Patient has been treated with > 5 prior systemic lines of treatment

7. Patient has received prior radiation therapy ≤ 4 weeks or limited field radiotherapy ≤ 2 weeks prior to start of study treatment

8. Patient treated with allogeneic hematopoietic stem cell transplant with active progressive cGVHD; patient has received DLI ≤ 6 weeks prior to start of study treatment; patient is planned to receive DLI

9. Patient has a history of another malignancy ≤ 3 years before study entry, with the exception of non-melanoma skin cancer and carcinoma in situ of uterine cervix

10. Patient has a history of CNS involvement with lymphoma

11. Patient has impaired cardiac function including any of the following:

• Complete left bundle branch block or use of a permanent cardiac pacemaker, congenital long QT syndrome, history or presence of ventricular tachyarrhythmias, clinically significant resting bradycardia (<50 beats per minute), QTcF > 450 msec on screening ECG, or right bundle branch block + left anterior hemiblock (bifascicular block)

• Presence of unstable atrial fibrillation (ventricular heart rate >100 bpm). Patients with stable atrial fibrillation are allowed in the study provided they do not meet the other cardiac exclusion criteria

• Previous history angina pectoris or acute MI within 6 months

• Congestive heart failure (New York Heart Association functional classification III-IV)

12. Patient has any other clinically significant heart disease (e.g., uncontrolled hypertension)

13. Patient has an impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of panobinostat (e.g., ulcerative disease, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, obstruction, or stomach and/or small bowel resection)

14. Patient has unresolved diarrhoea ≥ grade 2

15. Patient has any other concurrent severe and/or uncontrolled medical condition(s) (e.g., uncontrolled diabetes mellitus, active or uncontrolled infection, chronic

obstructive or chronic restrictive pulmonary disease including dyspnoea at rest from any cause) that could cause unacceptable safety risks or compromise compliance with the protocol

16. Patient has a known history of HIV seropositivity

17. Patient has active HBV hepatitis. The following categories of patients HBV positive but with non evidence of active hepatitis may be considered for the study and treated with panobinostat (see also Section 8.12):

- patient is HBsAg + with HBV DNA < 2000 UI/ml (inactive carriers); HBV DNA > 2000 UI/ml is criteria of exclusion
- patient is HBsAg – HBsAb +
- patient is HBsAg – but HBcAb +

18. Patients with HCV active hepatitis are excluded from the study. Patient with no evidence of active hepatitis and/or advanced chronic liver disease according to liver biopsy or fibro-scan evaluation may be included into the study (see also Section 8.13)

19. Patient is using medications that have a relative risk of prolonging the QT interval or of inducing Torsade de Pointes, where such treatment cannot be discontinued or switched to a different medication prior to starting study drug

20. Women who are pregnant or breast feeding or women of childbearing potential (WOCBP) not willing to use a double method of contraception during the study and 3 months after the end of treatment. One of these methods of contraception must be a barrier method. WOCBP are defined as sexually mature women who have not undergone a hysterectomy or who have not been naturally postmenopausal for at least 12 consecutive months or had menses any time in the preceding 12 consecutive months. WOCBP must have a negative serum pregnancy test at baseline

21. Male patient whose sexual partner(s) are WOCBP who are not willing to use a double method of contraception, one of which includes a condom, during the study and for 3 months after the end of treatment

22. Patient does not have before entering into the study a new lymph node or other pathologic tissue biopsy for confirmation of diagnosis and biologic studies; bone marrow biopsy is not adequate for this purpose and should be performed only for staging.

3.5. Dose, regimen, treatment cycle

The duration of a treatment course will be 28 days. The first dose of panobinostat in course 1 defines day 1 of the treatment cycle, and each cycle thereafter will begin 28 days later.

Induction phase (course 1 to 6)

Patients will receive panobinostat for 6 courses (1 course = 28 days). Response assessment will be performed every two courses until the end of the induction phase. Patients with responsive (complete or partial response) or stable disease during each assessment will complete the induction phase.

Consolidation phase (courses 7 to 12)

Consolidation phase includes courses from 7 to 12 (1 course = 28 days). Response assessment will be performed every three courses until course 12. Patients with responsive (complete or partial response) or stable disease after the consolidation phase will continue therapy according to the maintenance phase.

Maintenance phase (from course 13 to the end of therapy)

Patients will continue therapy with panobinostat until disease progression, intolerability, withdrawal of consent and/or if the investigator determines that further therapy is not in the patient's best interest (e.g., due to non-compliance, toxicity etc.). Response assessment will be performed every three courses until course 36. For patients still in therapy with panobinostat after course 36, the subsequents response assessments will be performed according to each institutional policy.

Treatment

Panobinostat should be taken p.o. at the dose of 40 mg/day three-times every week (QW) (e.g., on Monday, Wednesday, and Friday or Tuesday, Thursday, and Saturday), as part of a 4 week (28 days) treatment cycle.

Oral panobinostat capsules should be administered as follows:

• Patients should be instructed to take their three times a week oral dose of panobinostat at the same time on each dosing day (e.g. day 1, 3, and 5). Doses should be separated by a minimum of 30 hours

• Patients should be instructed to take the daily dose of oral panobinostat, after a minimum 2-hour fast and should continue to fast for 2 hours post dose. Each dose of panobinostat should be taken with a glass (approximately 240 mL) of non-carbonated water. Patients should be instructed to swallow the capsules whole and not chew them

• If vomiting occurs during the course of treatment, then no re-dosing of the patient is allowed before the next scheduled dose

• Patients must avoid grapefruits, grapefruit juice, Seville (sour) oranges and Seville orange juice during the entire study period.

Duration of treatment with panobinostat

Patients may continue treatment with panobinostat until the patient experiences unacceptable toxicity that precludes any further treatment, and/or the investigator determines that further therapy is not in the patient's best interest (e.g., due to non-compliance, toxicity etc.).

Patients who discontinue treatment with panobinostat (non-compliance, toxicity or start of new anti cancer therapy) remain into the study and are evaluated for follow up.

Patients who experience disease progression must be follow up for vital status until death or at least 36 months.

Patients who withdraw consent must be permanently discontinued from the study.

3.6. Study drug discontinuation

Patients who discontinue study drug for any reason should be scheduled for a visit as soon as possible and all of the assessments listed for the final visit should be performed. All patients must have evaluations for 28 days after the last dose of study treatment, including the monitoring of adverse events and concomitant medications (including anti-neoplastic therapy). If applicable, any adverse event marked as 'continuing' as of the last dose of study medication must be followed at least once per week for at least 4 weeks until resolution of the event, return to baseline status, or clinical stability is reached. At a minimum, all patients who discontinue study treatment, including those who refuse to return for a final visit, will be contacted for safety evaluations during the 28 days following the last dose of study drug. Patients lost to follow up should be recorded as such on the CRF. Patients who discontinue study treatment for reasons other than progression should continue to have tumor assessments according to the protocol until disease progression. If patients refuse to return for these visits or are unable to do so, the patient should be considered off-study, and a Study Evaluation Completion form should be completed. If possible, survival information must be registered at the end of the study.

3.7. End of treatment

Patients may voluntarily withdraw from the study or be dropped from it at the discretion of the Investigator at any time. If such withdrawal occurs, or if the patient fails to return for visits, the Investigator must determine the primary reason for a patient's premature withdrawal from the study and record this information on the End of Treatment CRF. Therapy may be interrupted for one of the following reasons:

· Adverse event(s)

· Abnormal laboratory value(s)

· Abnormal test procedure result(s)

· Protocol deviation

· Subject withdrew consent

· Lost to follow-up

· Administrative problems

· Death

· Initiation of new cancer therapy

· Disease progression

· The investigator determines that further therapy is not in the patient's best interest (e.g., due to non-compliance, toxicity etc.)

For patients who are lost to follow-up, the investigator should show "due diligence" by documenting in the source documents steps taken to contact the patient, e.g., dates of telephone calls, registered letters, etc. Patients must be followed for 28-days post-last treatment of study drug for toxicity. All patients who have been discontinued from study drug treatment for any reason other than progression should continue to have tumor assessments until the end of the study or PD. Once a patient discontinues study drug administration and no additional study assessments will be performed other than survival information, a Study Evaluation Completion form should then be completed. The reasons for study evaluation completion may include the following:

· Protocol deviation

· Subject withdrew consent

· Lost to follow-up

· Administrative problems

· Death

· New cancer therapy

· Disease progression

3.8. Histopatology and gene expression profiling studies

3.8.1. Background

Diffuse large B cell lymphoma (DLBCL) is currently regarded in the WHO classification as a neoplasm of large B lymphoid cells with nuclear size equal to or exceeding normal macrophage nuclei or more than twice the size of a normal lymphocyte, that has a diffuse growth pattern. Morphological, biological and clinical studies have subdivided DLBCL into morphological variants, molecular and immunophenotypic subgroups and distinct disease entities. However, a large number of cases remain that may be biologically heterogeneous, but for which there are no clear and accepted criteria for subdivision. These are classified as DLBCL, not otherwise specified (NOS). Noteworthy, recent gene expression profile (GEP) studies demonstrated the existence of at least two main molecular subgroups of DLBCL, namely germinal centre B-type (GCB-type) and activated B-cell type (ABC-type) provided with significantly different prognosis (135-138). In particular, such tumours are classified according to the expression of genes reflecting their cell of origin (germinal centre vs. late germinal centre/plasmablast (139)), proliferation rate and host immune response to the tumour. Of note, the molecular classification has been demonstrated to predict the clinical outcome for patients treated with CHOP or R-CHOP (and similar) regimes, and, intriguingly, target therapies specific for each subtypes have been recently proposed, such as NFKB inhibition in ABC-cases (140). On the other hand, a discrete fraction of cases, still remain unclassifiable basing on GEP, not belonging to either one subgroup (termed type 3 or unclassified).

In the clinical practice, due to the frequent unavailability of fresh/frozen pathological material and the relatively high costs necessary for GEP analysis, surrogate markers for DLBCL classification have been required. In particular, in the last few years, several attempts have been made to design diagnostic algorithms based on immunohistochemical studies (141-143). However, it was clear that although such systems provided useful prognostic information, they were not able to definitely recapitulate the molecular classification. Thus, a better definition of the immunohistochemical diagnostic markers is warranted.

3.8.2. Aims of the study

1) To define the molecular subgroups of DLBCL
    a. Basing on GEPs
    b. Basing on novel immunohistochemical patterns
2) To identify possible molecular signatures associated with panobinostat resistance/sensitivity
    a. Reflecting the current molecular classification (GCB vs. ABC vs. type 3)
    b. Reflecting different molecular features

3.8.3. Samples and Methods

Sample collection. Formalin-fixed paraffin embedded (FFPE) tissue blocks will be centralized at the Unit of Hematopathology, Molecular Pathology Laboratory, Department of Haematology and Oncology "L. and A. Seràgnoli" (To the Attention of Prof. Stefano A. Pileri/Dr. Pier Paolo Piccaluga). When available, frozen tissue will be collected as well. FFPE tissues will be shipped at room temperature; when available, fresh tissue will be first cryopreserved and then shipped in dry ice (and delivered within 24 hours).

Tissue microarray analysis. Tissue microarrays (TMAs) will be constructed as previously described (31). Three different cores for each case will be considered. Immunohistochemistry will be carried out in order to 1) confirm the diagnosis of DLBCL; 2) to investigate the ability of a newly developed immunohistochemical pattern to discriminate GCB, ABC and type 3 DLBCLs; 3) to investigate whether the immunophenotypic profile correlates with clinical outcome in patients receiving HDACi. The immunohistochemical analysis will include the antibodies directed against the following antigens: CD10; CD20; CD79a; CD30; BCL2; BCL6; IRF4; IRTA1; CD138; LMO2; JAW1; PAG; GCET; BLIMP1; VpreB3; FOXP1; HGAL.

In addition, ISH and FISH/CISH will be performed on TMAs in order to assess the prevalence and the clinical impact of EBV integration and translocations involving BCL2, BCL6 and MYC, respectively.

Gene expression profile analysis. Gene expression profile (GEP) analysis will be performed on pathologic specimens by using the DASL technology and Illumina DNA-microarrays. Total RNA will be extracted by using the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Applied Biosystems/Ambion, Austin, TX USA) and converted to cDNA according to a standard protocol (Illumina, USA). Finally, after incubation with the whole genome DASL assay pool (DAP), and enzymatic extension

and ligation steps, the nucleic acids will be hybridized to whole genome gene expression BeadChips and then scanned on a BeadArray^{TM} Reader using BeadScan software. Analysis of GEPs will be then carried on by using GenSpring 10.3/11 as well Partek suite. Supervised, unsupervised analyses as well as principal component analysis (PCA), GSEA and cell type classification will be performed as previously described (144). Non parametric rather then parametric tests will be applied, if indicated, according to the number of cases included in each subgroup.

3.9. Statistical methods and data analysis

3.9.1 Sample size

The sample size is established according to evaluation of the primary endpoint. The trial is conducted according to the optimal two-stage design of Simon with alpha 0.5 and beta 0.10, considering the following two hypotheses: first a response rate (RR) less than 10% is of no further interest; and second, an RR 30% is clinically meaningful. In the initial stage, 18 patients have to entered onto the study. If no more than 2 responses (≤ 2 in 18) will be observed, the trial would be terminated. Otherwise, accrual will continue to a total of a maximum of 35 patients. At the end of the trial, if 6 or fewer responses will be occurred among the 35 patients, it will be concluded that the regimen is not worthy of further investigations for that group of patients. The decision to proceed to stage 2 will be based on Investigator assessment of tumor response by clinical evaluation and CT scan.

Since the biologic studies have only an explorative purpose the sample size is not calculated according to these analysis.

3.9.2. Efficacy

Primary efficacy endpoint is the objective response rate as determined by evaluation of CT scans/MRI for response by clinical site investigator. The response criteria according to Cheson 1999 will be adopted.

On these grounds:

- **Complete response (CR)** requires the following:

1. Complete disappearance of all detectable clinical and radiographic evidence of disease and disappearance of all disease-related symptoms if present before therapy, and

normalization of those biochemical abnormalities (eg, lactate dehydrogenase definitely assignable to NHL

2. All lymph nodes and nodal masses must have regressed to normal size (1.5 cm in their greatest transverse diameter for nodes > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their greatest transverse diameter before treatment must have decreased to 1 cm in their greatest transverse diameter after treatment, or by more than 75% in the sum of the products of the greatest diameters (SPD)

3. The spleen, if considered to be enlarged before therapy on the basis of a CT scan, must have regressed in size and must not be palpable on physical examination. However, no normal size can be specified because of the difficulties in accurately evaluating splenic size. For instance, spleens thought to be of normal size may contain lymphoma, whereas an enlarged spleen may not necessarily reflect the presence of lymphoma but variations in anatomy, blood volume, the use of hematopoietic growth factors, or other causes. The determination of splenic volume or splenic index by CT scan are cumbersome and not widely used. Any macroscopic nodules in any organs detectable on imaging techniques should no longer be present. Similarly, other organs considered to be enlarged before therapy due to involvement by lymphoma, such as liver and kidneys, must have decreased in size

4. If the bone marrow was involved by lymphoma before treatment, the infiltrate must be cleared on repeat bone marrow aspirate and biopsy of the same site. The sample on which this determination is made must be adequate (20 mm biopsy core). Flow cytometric, molecular, or cytogenetic studies are not considered part of routine assessment to document persistent disease at the present time

- **CR/unconfirmed (CRu)** includes those patients who fulfill criteria 1 and 3 above, but with one or more of the following features:

1. A residual lymph node mass greater than 1.5 cm in greatest transverse diameter that has regressed by more than 75% in the SPD. Individual nodes that were previously confluent must have regressed by more than 75% in their SPD compared with the size of the original mass

2. Indeterminate bone marrow (increased number or size of aggregates without cytologic or architectural atypia)

- **Partial response (PR)** requires the following:

1. 50% decrease in SPD of the six largest dominant nodes or nodal masses. These nodes or masses should be selected according to the following features: (a) they should be clearly measurable in at least two perpendicular dimensions, (b) they should be from as disparate regions of the body as possible, and (c) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved

2. No increase in the size of the other nodes, liver, or spleen

3. Splenic and hepatic nodules must regress by at least 50% in the SPD

4. With the exception of splenic and hepatic nodules, involvement of other organs is considered assessable and not measurable disease

5. Bone marrow assessment is irrelevant for determination of a PR because it is assessable and not measurable disease; however, if positive, the cell type should be specified in the report, eg, large-cell lymphoma or low-grade lymphoma (ie, small, lymphocytic small cleaved, or mixed small and large cells)

6. No new sites of disease

- **Stable disease** is defined as less than a PR (see above) but is not progressive disease (see below).
- **Relapsed disease** (CR, CRu) requires the following:

1. Appearance of any new lesion or increase by 50% in the size of previously involved sites

2. 50% increase in greatest diameter of any previously identified node greater than 1 cm in its short axis or in the SPD of more than one node

- **Progressive disease** (PR, non-responders) requires the following:

1. 50% increase from nadir in the SPD of any previously identified abnormal node for PRs or non responders

2. Appearance of any new lesion during or at the end of therapy

Table 2. Response Criteria for Non-Hodgkin's Lymphoma

Response Category	Physical Examination	Lymph Nodes	Lymph Node Masses	Bone Marrow
CR	Normal	Normal	Normal	Normal
CRu	Normal	Normal	Normal	Indeterminate
	Normal	Normal	> 75% decrease	Normal or indeterminate
PR	Normal	Normal	Normal	Positive
	Normal	≥ 50% decrease	≥ 50% decrease	Irrelevant
	Decrease in liver/spleen	≥ 50% decrease	≥ 50% decrease	Irrelevant
Relapse/progression	Enlarging liver/spleen; new sites	New or increased	New or increased	Reappearance

NOTE. See text for definitions of "normal" and "indeterminate."

3.9.3. Response Assessment

Response is currently assessed on the basis of clinical, radiological, and pathologic (ie, bone marrow) criteria.

1. CT scans remain the standard for evaluation of nodal disease. Thoracic, abdominal, and pelvic CT scans are recommended even if those areas were not initially involved because of the unpredictable pattern of recurrence
2. A bone marrow aspirate and biopsy should only be performed to confirm a CR if they were initially positive or if it is clinically indicated by new abnormalities in the peripheral blood counts or blood smear
3. A PET or a CT-PET scan is requested in patients attaining CR or PR every 6 months

3.9.4. Statistical analysis

Descriptive statistics and their 95% confidence intervals will be used to summarize the activity and the safety endpoints. Time to event variables will be analysed using the Kaplan-Meier method. A subgroup analysis for patients with DLBCL relapsed and/or refractory and those not eligible for ASCT will be performed, if the number of patients in each group will be sufficient. Subgroup analysis for ancillary studies (pharmacogenetic studies and histopatology and gene expression profiling studies) will be analysed with explorative purpose.

3.9.5. Safety assessment

The severity of adverse events will be graded on a scale of 1 to 5 according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events version 3.0 (NCI CTCAE).

3.10. Preliminary results

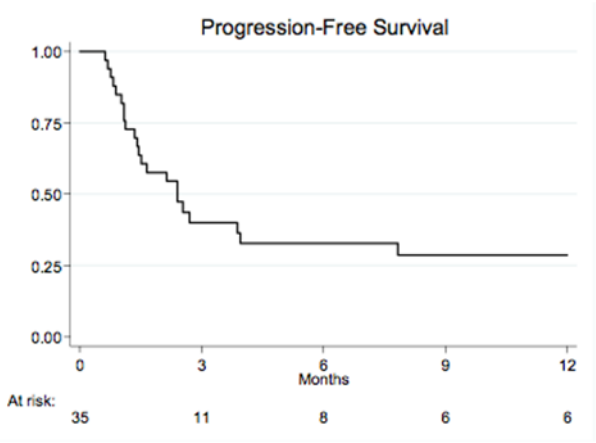
Thirty-five patients, 21 males (60%), were enrolled between June 2011 and March 2014. Clinical characteristics were: median age 73 (range 65-75), stage IV in 18 (55%), B-symptoms in 9 (28%), increased LDH in 24 (69%), high-intermediate or high International Prognostic Index (IPI) in 18 (51%). Patients received a median of 2 prior lines of therapy (range 1-4).

At the end of induction phase, 7 responses (20%) were observed, including 4 CR (11%), while 28 patients (80%) discontinued treatment due to progressive disease (PD) in 21

(60%) or adverse events in 7 (20%). Median TTR in 9 responders was 2.6 months (range 1.8-12). With a median follow up of 6 months (range 1-34), the estimated 12 months PFS and OS were 27% and 30.5%, respectively (Fig.2 and 3)

In univariate analysis, favourable IPI score and cutaneous involvement at enrollment showed a trend toward a higher ORR (p=0.007 and 0.061, respectively); pharmacogenetics, immunohistochemical and gene expression profile studies are still ongoing.

No toxic deaths were reported; 18 patients died, 17 due to lymphoma progression and one for allogeneic transplant related complications, performed after PD. Grade 3-4 thrombocytopenia and neutropenia were the most common toxicities (in 29 (83%) and 12 (34%) patients, respectively), while grade 3-4 extra-hematological toxicity included diarrhoea in 4 (12%), infectious complications in 1 (3%) and supraventricular arrhythmia in 2 patients (6%).



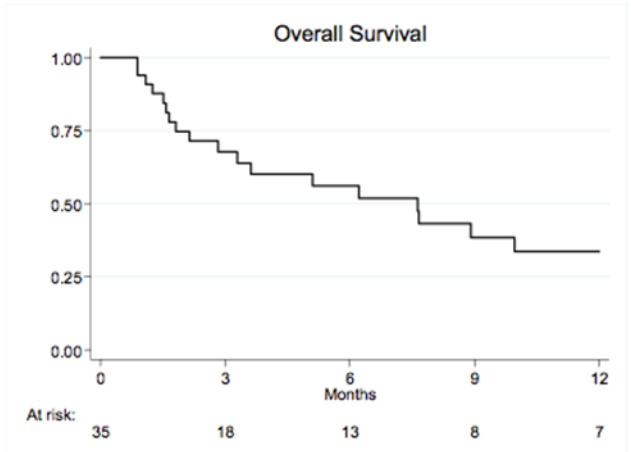


Fig. 2 and 3: the estimated 12 months PFS and OS were 27% and 30.5% respectively.

4. CONCLUSION

The prognosis of patients with DLBCL relapsed and/or refractory to CHOP-R first line therapy is dismal. High dose therapy and autologous stem cell transplant (ASCT) is an active rescue therapy in nearly 50% of patients with chemo-sensitive disease. Therapy of patients who relapse after ASCT or result non eligible for ASCT because of refractory disease, age or co-morbidities, represents an unmet medical need. Novel treatment strategies are needed in such patients with refractory or recurrent lymphoma. There are multiple new agents that are currently being evaluated for the treatment of patients with lymphoma (146,147). However, when these compounds are tested in unselected patients with relapsed lymphoma, they typically produce low response rates with short response duration.

Histone deacetylases (DACs) are involved in chromatin structure regulation and function. Treatment with DACs inhibitors leads to the activation or repression of genes regulating apoptosis, proliferation, differentiation, angiogenesis, immune responses. These agents resulted to be active for the treatment of T and B-cell lymphoma and other haematological malignancies. Previous in vitro studies underlined the possible pathophysiological role of DACs in diffuse large B-cell lymphoma (DLBCL).

FIL-PanAL10 (NCT01523834) is a phase II, prospective multicenter trial of the Fondazione Italiana Linfomi (FIL). In this study we evaluate the therapeutic activity and safety of Panobinostat, a potent pan-DACs inhibitor, in patients with relapsed or refractory (R/R) DLBCL. Exploratory objectives evaluate the predictive role of pharmacogenetics, immunohistochemical and specific gene expression in relation to the response to Panobinostat are still ongoing; for this aim a new lymph node or other pathologic tissue biopsy is requested before starting treatment. and to evaluate a possible relationships between response and any biological features.

The results of this study indicate that Panobinostat might be remarkably active in some patients with R/R DLBCL, showing durable CR. Feasibility was impaired by relevant hematological toxicity, mainly frequent and dose limiting grade 3-4 thrombocytopenia. Data that will be obtained from biological exploratory studies could hopefully be useful to better address the use of Panobinostat in peculiar subsets of patients.

REFERENCES

1. Kelly WK, et al. Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. J Clin Oncol. 2005 Jun 10;23(17):3923-31. Epub 2005 May 16.

2. Mann BS et al. Oncologist. FDA approval summary: vorinostat for treatment of advanced primary cutaneous T-cell lymphoma. 2007 Oct;12(10):1247-52.

3. Gupta M, et al. Blood. Inhibition of histone deacetylase overcomes rapamycin-mediated resistance in diffuse large B-cell lymphoma by inhibiting Akt signaling through mTORC2. 2009 Oct 1;114(14):2926-35.

4. Nielsen T, et al. Histone deacetylase inhibitor treatment in B-cell non-Hodgkin's lymphoma - effect of *ex vivo* treatment on primary patient samples. Proceeding AACR 101st Annual Meeting 2010 abst. 5449.

5. Giles F, et al. A phase I study of intravenous LBH589, a novel cinnamic hydroxamic acid analogue histone deacetylase inhibitor, in patients with refractory hematologic malignancies. Clin Cancer Res. 2006 Aug 1;12(15):4628-35.

6. Ellis L, et al. Histone deacetylase inhibitor panobinostat induces clinical responses with associated alterations in gene expression profiles in cutaneous Tcell lymphoma. Clin Cancer Res 2008, 14:4500-4510.

7. S.G. Fisher, et al. The epidemiology of non-Hodgkin's lymphoma Oncogene, 23 (38) (2004), pp. 6524–6534.
8. Clinical evaluation of the International Lymphoma Study Group classification of non Hodgkin's lymphoma. The non-Hodgkin's lymphoma classification project. Blood, 89 (1997), pp. 3909–3918
9. S.H. Swerdlow, et al. WHO classification of tumors of haematopoietic and lymphoid tissues. (4th ed.) Lyon: IARC Press; (2008).
10. N.L. Harris, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood, 84 (5) (1994), pp. 1361–1392.
11. H. Stein, et al. Diffuse Large B-cell Lymphoma, not otherwise specified. In S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein (Eds.), et al., WHO classification of tumors of haematopoietic and lymphoid tissues (4th ed.), IARC Press, Lyon (2008), pp. 233–237.
12. G. Ott, et al. Immunoblastic morphology but not the immunohistochemical GCB/nonGCB classifier predicts outcome in diffuse large B-cell lymphoma in the RICOVER-60 trial of the DSHNHL Blood, 116 (23) (2010), pp. 4916–4925.
13. P.N. Meyer, et al. Immunohistochemical methods for predicting cell of origin and survival in patient with diffuse large B-cell lymphoma treated with rituximab. Journal of Clinical Oncology, 29 (2) (2011), pp. 200–207.
14. W.W. Choi, D.D, et al. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clinical Cancer Research, 15 (17) (2009), pp. 5494–5502.

15. C.P. Hans, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood, 103 (1) (2004), pp. 275–282.
16. M. Yamaguchi, et al. De novo CD5+ diffuse large B-cell lymphoma: a clinicopathologic study of 109 patients. Blood, 99 (3) (2002), pp. 815–821.
17. L. Colomo, A. et al.Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. Blood, 101 (1) (2003), pp. 78–84.
18. M.H. Kramer, J. Hermans, E. Wijburg, K. Philippo, E. Geelen, J.H. van Krieken, et al. Clinical relevance of BCL2, BCL6, and MYC rearrangements in diffuse large B-cell lymphoma. Blood, 92 (9) (1998), pp. 3152–3162.
19. R.D. Gascoyne, et al. Prognostic significance of Bcl-2 protein expression and Bcl-2 gene rearrangement in diffuse aggressive non-Hodgkin's lymphoma. Blood, 90 (1) (1997), pp. 244–251.
20. R.E. Davis, et al. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. The Journal of Experimental Medicine, 194 (12) (2001), pp. 1861–1874
21. P.J. Jost, J. Ruland. Aberrant NF-kappaB signaling in lymphoma: mechanisms, consequences, and therapeutic implications. Blood, 109 (7) (2007), pp. 2700–2707.
22. K. Offit, F. et al. Rearrangement of the bcl-6 gene as a prognostic marker in diffuse large-cell lymphoma. New England Journal of Medicine, 331 (2) (1994), pp. 74–80.|
23. B.H. Ye, et al. The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation. Nature Genetics, 16 (2) (1997), pp. 161–170.
24. B.H. Ye, F. Lista, F. Lo Coco, D.M. Knowles, K. Offit, R.S. Chaganti, et al. Alterations of a zinc finger-encoding gene, BCL-6, in diffuse large-cell lymphoma. Science (New York, NY), 262 (5134) (1993), pp. 747–750.|
25. B.H. Ye, et al. Chromosomal translocations cause deregulated BCL6 expression by promoter substitution in B cell lymphoma. EMBO Journal, 14 (24) (1995), pp. 6209–6217.
26. R.T. Phan, R. Dalla-Favera. The BCL6 proto-oncogene suppresses p53 expression in germinal-centre B cells. Nature, 432 (7017) (2004), pp. 635–639.
27. G. Volpe, et al. Molecular heterogeneity of B-lineage diffuse large cell lymphoma. Genes, Chromosomes and Cancer, 16 (1) (1996), pp. 21–30.
28. J.J. Yunis, et al. Bcl-2 and other genomic alterations in the prognosis of large-cell lymphoma. New England Journal of Medicine, 320 (16) (1989), pp. 1047–1054.
29. M. Hummel, et al. A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. New England Journal of Medicine, 354 (23) (2006), pp. 2419–2430.
30. C. Uedaet al. Coexistent rearrangements of c-MYC, BCL2, and BCL6 genes in a diffuse large B-cell lymphoma. International Journal of Hematology, 79 (1) (2004), pp. 52–54.
31. P. Kluin, N.L. Harris, H. Stein, L. Leoncini, M. Raphael, E. Campo, et al. B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. In S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein (Eds.), et al., WHO classification of tumors of haematopoietic and lymphoid tissues (4th ed.), IARC Press, Lyon (2008), pp. 265–266.
32. V.G. Dyomin, et al. BCL8, a novel gene involved in translocations affecting band 15q11–13 in diffuse large-cell lymphoma. Proceedings of the National Academy of Sciences of the United States of America, 94 (11) (1997), pp. 5728–5732

33. L. Pasqualucci, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. Nature, 471 (7337) (2011), pp. 189–195.
34. O.R. Bereshchenko, W. Gu, R. Dalla-Favera. Acetylation inactivates the transcriptional repressor BCL6. Nature Genetics, 32 (4) (2002), pp. 606–613.
35. W. Gu, R.G. Roeder. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell, 90 (4) (1997), pp. 595–606.
36. I. Salaverria, et al. Translocations activating IRF4 identify a subtype of germinal center-derived B-cell lymphoma affecting predominantly children and young adults. Blood, 118 (1) (2011), pp. 139–147.
37. T. Andersson, P.G. Lindgren, A. Elvin. Ultrasound guided tumour biopsy in the anterior mediastinum. An alternative to thoracotomy and mediastinoscopy. Acta Radiologica, 33 (5) (1992), pp. 423–426.
38. V.I. Pappa, et al. Role of image-guided core-needle biopsy in the management of patients with lymphoma. Journal of Clinical Oncology, 14 (9) (1996), pp. 2427–2430.
39. P.P. Carbone, H.S. Kaplan, K. Musshoff, D.W. Smithers, M. Tubiana. Report of the committee Hodgkin lymphoma staging classification. Cancer Research, 31 (1971), pp. 1860–186.
40. T.A. Lister, et al. Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: cotswolds meeting. Journal of Clinical Oncology, 7 (11) (1989), pp. 1630–1636.
41. A predictive model for aggressive non-Hodgkin's lymphoma. The international non-Hodgkin's lymphoma prognostic factors project. N Engl J Med, 329 (14) (1993), pp. 987–994
42. P. Seam, M.E. Juweid, B.D. Cheson. The role of FDG-PET scans in patients with lymphoma. Blood, 110 (10) (2007), pp. 3507–3516
43. B. Cheson. The case against heavy PETing. Journal of Clinical Oncology, 27 (11) (2009), pp. 1742–1742-3.
44. M.E. Juweid et al. Use of positron emission tomography for response assessment of lymphoma: consensus of the Imaging Subcommittee of International Harmonization Project in Lymphoma. Journal of Clinical Oncology, 25 (5) (2007), pp. 571–578.
45. A.J. Ferreri. How I treat primary CNS lymphoma. Blood, 118 (3) (2011), pp. 510–52.
46. E. Zucca, et al. Patterns of outcome and prognostic factors in primary large-cell lymphoma of the testis in a survey by the International Extranodal Lymphoma Study Group. Journal of Clinical Oncology, 21 (1) (2003), pp. 20–27
47. L.H. Sehn, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood, 109 (5) (2007), pp. 1857–1861
48. M. Ziepert, D. et al. Standard International prognostic index remains a valid predictor of outcome for patients with aggressive CD20+ B-cell lymphoma in the rituximab era. Journal of Clinical Oncology, 28 (May (14)) (2010), pp. 2373–2380 [Erratum in Journal of Clinical Oncology 2011;29(6):779]
49. G. Lenz, et al. Stromal gene signatures in large-B-cell lymphomas. New England Journal of Medicine, 359 (22) (2008), pp. 2313–2323.
50. A.A. Alizadehet al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature, 403 (6769) (2000), pp. 503–511.
51. M.A. Shipp, et al. Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. Nature Medicine, 8 (1) (2002), pp. 68–74.

52. C.P. Hans, D.D. et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood, 103 (1) (2004), pp. 275–282.
53. K. Dunleavy et al. Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. Blood, 113 (24) (2009), pp. 6069–6076.
54. F.J. Hernandez-Ilizaliturri, et al. Higher response to lenalidomide in relapsed/refractory diffuse large B-cell lymphoma in nongerminal center B-cell-like than in germinal center B-cell-like phenotype. Cancer, 117 (22) (2011), pp. 5058–5066.
55. B. Coiffier, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. New England Journal of Medicine, 346 (4) (2002), pp. 235–242.
56. P. Feugier, A. et al. Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte. Journal of Clinical Oncology, 23 (18) (2005), pp. 4117–4126.
57. B. Coiffier, et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. Blood, 116 (12) (2010), pp. 2040–204.
58. L.H. Sehn, et al. Introduction of combined CHOP plus rituximab therapy dramatically improved outcome of diffuse large B-cell lymphoma in British Columbia. Journal of Clinical Oncology, 23 (22) (2005), pp. 5027–5033.
59. M. Pfreundschuh, et al. Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of elderly patients with aggressive lymphomas: results of the NHL-B2 trial of the DSHNHL. Blood, 104 (3) (2004), pp. 634–641;
60. M. Pfreundschuh, et al. Prognostic significance of maximum tumour (bulk) diameter in young patients with good-prognosis diffuse large-B-cell lymphoma treated with CHOP-like chemotherapy with or without rituximab: an exploratory analysis of the MabThera International Trial Group (MInT) study. Lancet Oncology, 9 (5) (2008), pp. 435–444;
61. M. Pfreundschuh, et al. Dose-escalated CHOEP, for the treatment of young patients with aggressive non-Hodgkin's lymphoma: II. Results of the randomized high-CHOEP trial of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL). Annals of Oncology, 19 (3) (2008), pp. 545–552;
62. R. Delarue, H. Tilly, G. Salles, et al. R-CHOP14 compared to R-CHOP21 in elderly patients with diffuse large B cell lymphoma: results of the interim analysis of the LNH03-6B GELA study. Blood, 114 (2009), p. 169 [abstract 406].
63. D. Cunningham, et al. CHOP14 versus R-CHOP21: result of a randomized phase III trial for the treatment of patients with newly diagnosed diffuse large B-cell non-Hodgkin lymphoma. Journal of Clinical Oncology, 29 (2011) [suppl; abstract 8000].
64. M. Pfreundschuh, et al. CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. Lancet Oncology, 7 (5) (2006), pp. 379–391.
65. M. Pfreundschuh, et al. CHOP-like chemotherapy with or without rituximab in young patients with good-prognosis diffuse large-B-cell lymphoma: 6-year results of an open-label randomised study of the MabThera International Trial (MInT) Group. Lancet Oncology, 12 (11) (2011), pp. 1013–1022.

66. C. Haioun, et al. Incidence and risk factors of central nervous system relapse in histologically aggressive non-Hodgkin's lymphoma uniformly treated and receiving intrathecal central nervous system prophylaxis: a GELA study on 974 patients, Groupe d'Etudes des Lymphomes de l'Adulte. Annals of Oncology, 11 (6) (2000), pp. 685–690.
67. A.M. Gianni, et al. High-dose chemotherapy and autologous bone marrow transplantation compared with MACOP-B in aggressive B-cell lymphoma. New England Journal of Medicine, 336 (18) (1997), pp. 1290–1297
68. N. Milpied, et al. Initial treatment of aggressive lymphoma with high-dose chemotherapy and autologous stem-cell support. New England Journal of Medicine, 350 (13) (2004), pp. 1287–1295
69. M. Martelli, et al. Early autologous stem-cell transplantation versus conventional chemotherapy as front-line therapy in high-risk, aggressive non-Hodgkin's lymphoma: an Italian multicenter randomized trial. Journal of Clinical Oncology, 21 (7) (2003), pp. 1255–1262
70. U. Vitolo, et al. High dose sequential chemotherapy with autologous transplantation versus dose-dense hemotherapy MegaCEOP as first line treatment in poor-prognosis diffuse large cell lymphoma: an "Intergruppo Italiano Linfomi" randomized trial. Haematologica, 90 (6) (2005), pp. 793–801.
71. A. Greb, J. et al. Gisselbrecht. High-dose chemotherapy with autologous stem cell support in first-line treatment of aggressive non-Hodgkin lymphoma – results of a comprehensive meta-analysis. Cancer Treatment Reviews, 33 (4) (2007), pp. 338–346.
72. J. Strehl, U. et al. High-dose chemotherapy followed by autologous stem cell transplantation as first-line therapy in aggressive non-Hodgkin's lymphoma: a meta-analysis. Haematologica, 88 (11) (2003), pp. 1304–1315.
73. G. Barosi, et al. Management of nodal indolent (non marginal-zone) non-Hodgkin's lymphomas: practice guidelines from the Italian Society of Hematology, Italian Society of Experimental Hematology and Italian Group for Bone Marrow Transplantation. Haematologica, 90 (9) (2005), pp. 1236–1257.
74. E. Brusamolino, et al. Dose-dense R-CHOP-14 supported by pegfilgrastim in patients with diffuse large B-cell lymphoma: a phase II study of feasibility and toxicity. Haematologica, 91 (4) (2006), pp. 496–502.
75. B. Glass, et al. Dose-escalated CHOP plus etoposide (MegaCHOEP) followed by repeated stem cell transplantation for primary treatment of aggressive high-risk non-Hodgkin lymphoma. Blood, 107 (8) (2006), pp. 3058–3064.
76. U. Vitolo, et al. Dose-dense and high-dose chemotherapy plus rituximab with autologous stem cell transplantation for primary treatment of diffuse large B-cell lymphoma with a poor prognosis: a phase II multicenter study. Haematologica, 94 (9) (2009), pp. 1250–1258
77. C. Gisselbrecht. Use of rituximab in diffuse large B-cell lymphoma in the salvage setting. British Journal of Haematology, 143 (5) (2008), pp. 607–621
78. N. Schmitz, et al. Conventional chemotherapy (CHOEP-14) with rituximab or high-dose chemotherapy (MegaCHOEP) with rituximab for young, high-risk patients with aggressive B-cell lymphoma: an open-label, randomised, phase 3 trial (DSHNHL 2002-1). Lancet Oncology, 13 (12) (2012), pp. 1250–1259
79. P. Milpied, et al. IL-17-producing invariant NKT cells in lymphoid organs are recent thymic emigrants identified by neuropilin-1 expression. Blood, 118 (11) (2011), pp. 2993–3002

80. P.J. Stiff, J.M. et al. Randomized phase III U.S./Canadian intergroup trial (SWOG S9704) comparing CHOP ± R for eight cycles to CHOP ± R for six cycles followed by autotransplant for patients with high-intermediate (H-Int) or high IPI grade diffuse aggressive non-Hodgkin lymphoma (NHL). Journal of Clinical Oncology, 29 (Suppl.) (2011) [abstract 8001].
81. A. Chiappella, et al. A randomized multicentre phase III study for first line treatment of young patients with high risk (aaIPI 2-3) diffuse large b-cell lymphoma (DLBCL): rituximab (R) plus dose-dense chemotherapy CHOP14/megaCHOP14 with or without intensified high-dose chemotherapy (HDC) and autologous stem cell transplantation (ASCT). Results of DLCL04 trial of Italian Lymphoma Foundation (FIL). Annals of Oncology, 22 (Suppl. 4) (2011) [abstract #072].
82. C. Bonnet, et al. CHOP alone compared with CHOP plus radiotherapy for localized aggressive lymphoma in elderly patients: a study by the Groupe d'Etude des Lymphomes de l'Adulte. Journal of Clinical Oncology, 25 (7) (2007), pp. 787–792
83. T. Philip et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. New England Journal of Medicine, 333 (23) (1995), pp. 1540–1545.
84. T. Philip, et al. High-dose therapy and autologous bone marrow transplantation after failure of conventional chemotherapy in adults with intermediate-grade or high-grade non-Hodgkin's lymphoma. New England Journal of Medicine, 316 (24) (1987), pp. 1493–1498.
85. G.L. Phillips, et al. The treatment of progressive non-Hodgkin's lymphoma with intensive chemoradiotherapy and autologous marrow transplantation. Blood, 75 (4) (1990), pp. 831–838.
86. F.B. Petersen, et al. Autologous marrow transplantation for malignant lymphoma: a report of 101 cases from Seattle. Journal of Clinical Oncology, 8 (4) (1990), pp. 638–647.
87. J.M. Vose. Autologous transplantation for aggressive non-Hodgkin's lymphoma: results of a randomized trial evaluating graft source and minimal residual disease. Journal of Clinical Oncology, 20 (9) (2002), pp. 2344–2352.
88. A. Bosly, et al. Bone marrow transplantation prolongs survival after relapse in aggressive-lymphoma patients treated with the LNH-84 regimen. Journal of Clinical Oncology, 10 (10) (1992), pp. 1615–1623.
89. J. Blay, et al. The International Prognostic Index correlates to survival in patients with aggressive lymphoma in relapse: analysis of the PARMA trial. Parma Group. Blood, 92 (10) (1998), pp. 3562–3568.
90. C. Guglielmi, et al. Time to relapse has prognostic value in patients with aggressive lymphoma enrolled onto the Parma trial. Journal of Clinical Oncology, 16 (10) (1998), pp. 3264–3269
91. T. Kewalramani, et al. Rituximab and ICE as second-line therapy before autologous stem cell transplantation for relapsed or primary refractory diffuse large B-cell lymphoma. Blood, 103 (10) (2004), pp. 3684–3688 [Epub 2004 January 22].
92. E. Vellenga, et al. Rituximab improves the treatment results of DHAP–VIM–DHAP and ASCT in relapsed/progressive aggressive CD20+ NHL: a prospective randomized HOVON trial. Blood, 111 (2) (2008), pp. 537–543
93. C. Gisselbrecht, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. Journal of Clinical Oncology, 28 (27) (2010), pp. 4184–4190

94. C. Gisselbrecht, et al. Maintenance with rituximab after autologous stem cell transplantation in relapsed patients with CD20 diffuse large B-cell lymphoma (DLBCL): CORAL final analysis. Journal of Clinical Oncology, 29 (Suppl.) (2011) [abstract 8004]
95. C. Thieblemont, et al. Prognostic impact of germinal center (GC)/activated B-cell (ABC) classification analysed by immunochemistry, FISH analysis and GEP. In Relapsed/refractory diffuse large B-cell lymphoma (DLBCL): the bio-CORAL study blood (ASH), 116 (2010) [abstract 993]
96. K. Spaepen, et al. Prognostic value of positron emission tomography (PET) with fluorine-18 fluorodeoxyglucose ([18F]FDG) after first-line chemotherapy in non-Hodgkin's lymphoma: is [18F]FDG-PET a valid alternative to conventional diagnostic methods? Journal of Clinical Oncology, 19 (2) (2001), pp. 414–419.
97. J.E. Filmont, et al. The impact of pre- and post-transplantation positron emission tomography using 18-fluorodeoxyglucose on poor-prognosis lymphoma patients undergoing autologous stem cell transplantation. Cancer, 110 (6) (2007), pp. 1361–1369.
98. T. El Gnaoui et al. Rituximab, gemcitabine and oxaliplatin: an effective salvage regimen for patients with relapsed or refractory B-cell lymphoma not candidates for high-dose therapy. Annals of Oncology, 18 (8) (2007), pp. 1363–1368.
99. M.S. Czuczman, et al. Confirmation of the efficacy and safety of lenalidomide oral monotherapy in patients with relapsed or refractory diffuse large-B-cell lymphoma: results of an international study (NHL-003). Blood (ASH), 112 (2008) [abstract 268]
100. P.H. Wiernik, et al. Lenalidomide monotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma. Journal of Clinical Oncology, 26 (30) (2008), pp. 4952–4957.|
101. A.W. Thomson, H.R. Turnquist, G. Raimondi. Immunoregulatory functions of mTOR inhibition. Nature Reviews Immunology, 9 (5) (2009), pp. 324–337
102. A. Younes. Therapeutic activity of mTOR inhibitors in mantle cell lymphoma: clues but no clear answers. Autophagy, 4 (5) (2008) pp. 707–709
103. T.E. Witzig, et al. A phase II trial of the oral mTOR inhibitor everolimus in relapsed aggressive lymphoma. Leukemia, 25 (2) (2011), pp. 341–347
104. J. Vose, S. et al.Phase II study of enzastaurin, a protein kinase C beta inhibitor, in patients with relapsed or refractory diffuse large B-cell lymphoma. Journal of Clinical Oncology, 25 (13) (2007), pp. 1741–1746
105. J.D. Hainsworth, et al. Randomized phase II study of R-CHOP plus enzastaurin versus R-CHOP in the first-line treatment of patients with intermediate- and high-risk diffuse large B-cell lymphoma (DLBCL): preliminary analysis. Journal of Clinical Oncology, 29 (Suppl.) (2011) [abstract 8016]
106. J. Ruan, et al. Bortezomib plus CHOP-rituximab for previously untreated diffuse large B-cell lymphoma and mantle cell lymphoma. Journal of Clinical Oncology, 29 (6) (2011), pp. 690–697R.L. Elstrom, B. Andemariam, P. Martin, J. Ruan, T.B. Shore, M. Coleman, et al.
107. Bortezomib in combination with rituximab, dexamethasone, ifosfamide, cisplatin and etoposide chemoimmunotherapy in patients with relapsed and primary refractory diffuse large B-cell lymphoma. Leukemia and Lymphoma, 53 (8) (2012), pp. 1469–1473
108. B.Y. Wong, N.H. Dang. Inotuzumab ozogamicin as novel therapy in lymphomas. Expert Opinion on Biological Therapy, 10 (8) (2010), pp. 1251–1258

109. W. Alduaij, A. Ivanov, J. Honeychurch, E.J. Cheadle, S. Potluri, S.H. Lim, et al. Novel type II anti-CD20 monoclonal antibody (GA101) evokes homotypic adhesion and actin-dependent, lysosome-mediated cell death in B-cell malignancies. Blood, 117 (17) (2011), pp. 4519–4529
110. D. Nagorsen, P.A. Baeuerle. Immunomodulatory therapy of cancer with T cell-engaging BiTE antibody blinatumomab. Experimental Cell Research, 317 (9) (2011), pp. 1255–1260
111. S.K. Min, Y.H. Koh, Y. Park, H.J. Kim, J. Seo, H.R. Park, et al. Expression of HAT1 and HDAC1–3 in diffuse large B-cell lymphomas, peripheral T-cell lymphomas, and NK/T-cell lymphomas. Korean Journal of Pathology, 46 (2) (2012), pp. 142–15
112. Yu X, et al. Modulation of p53, ErbB1, ErbB2, and Raf-1 expression in lung cancer cells by depsipeptide FR901228. J Natl Cancer Inst. 2002 Apr 3;94(7):504-13.
113. Fuino L, et al. Histone deacetylase inhibitor LAQ824 down-regulates Her-2 and sensitizes human breast cancer cells to trastuzumab, taxotere, gemcitabine, and epothilone B. Mol Cancer Ther. 2003 Oct;2(10):971-84.
114. Nimmanapalli R, et al. Histone deacetylase inhibitor LAQ824 both lowers expression and promotes proteasomal degradation of Bcr-Abl and induces apoptosis of imatinib mesylate-sensitive or -refractory chronic myelogenous leukemia-blast crisis cells. Cancer Res. 2003 Aug 15;63(16):5126-35.
115. Warrell RP Jr, et al. Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase. J Natl Cancer Inst. 1998 Nov 4;90(21):1621-5.
116. Heaney 2003
117. Kelly WK, et al. Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. J Clin Oncol. 2005 Jun 10;23(17):3923-31. Epub 2005 May 16.
118. Mann BS et al. Oncologist. FDA approval summary: vorinostat for treatment of advanced primary cutaneous T-cell lymphoma. 2007 Oct;12(10):1247-52.
119. Kim YH, et al. Romidepsin (depsipeptide) Induces Clinically Significant Responses in Treatment-Refractory CTCL: An International, Multicenter Study. Blood (ASH Annual Meeting Abstracts), Nov 2007; 110: 123.
120. Gupta M, et al. Blood. Inhibition of histone deacetylase overcomes rapamycin-mediated resistance in diffuse large B-cell lymphoma by inhibiting Akt signaling through mTORC2. 2009 Oct 1;114(14):2926-35.
121. Nielsen T, et al. Histone deacetylase inhibitor treatment in B-cell non-Hodgkin's lymphoma - effect of ex vivo treatment on primary patient samples. Proceeding AACR 101st Annual Meeting 2010 abst. 5449.
122. Lewis et al, 2009.
123. Giles F, et al. A phase I study of intravenous LBH589, a novel cinnamic hydroxamic acid analogue histone deacetylase inhibitor, in patients with refractory hematologic malignancies. Clin Cancer Res. 2006 Aug 1;12(15):4628-35.
124. Ottmann OG, et al. Phase IA/II study of oral panobinostat (LBH589), a novel pan-deacetylase inhibitor (DACi) demonstrating efficacy in patients with advanced hematologic malignancies. ASH Annual Meeting Abstracts 2008;112:958.
125. Duvic M, et al. Phase II study of oral panobinostat (LBH589), a potent pandeacetylase inhibitor, in patients with refractory Cutaneous T-cell Lymphoma (CTCL). J Clin Oncol 2008, 26:abstr 8555.

126. Ellis L, et al. Histone deacetylase inhibitor panobinostat induces clinical responses with associated alterations in gene expression profiles in cutaneous Tcell lymphoma. Clin Cancer Res 2008, 14:4500-4510.
127. De Angelo DJ, et al. Activity of Oral Panobinostat (LBH589) in Patients with Myelofibrosis. Blood (ASH Annual Meeting Abstracts), Nov 2009; 114: 2898.
128. Mascarenhas J, et al. A Phase I Study of LBH589, a Novel Histone Deacetylase Inhibitor in Patients with Primary Myelofibrosis (PMF) and Post-Polycythemia/Essential Thrombocythemia Myelofibrosis (Post-PV/ET MF). Blood (ASH Annual Meeting Abstracts), Nov 2009; 114: 308.
129. Zaritskey A, et al. A Phase II Study of Oral Panobinostat (LBH589) for Chronic Phase Chronic Myeloid Leukemia (CML) with Resistance to 2 BCR-ABL Tyrosine Kinase Inhibitors. Blood (ASH Annual Meeting Abstracts), Nov 2008; 112: 4254.
130. Guilhot F, et al. A Phase II Study of Oral Panobinostat (LBH589) in Accelerated Phase (AP) or Blast Crisis (BC) Chronic Myeloid Leukemia (CML) Patients Resistant to 2 BCR-ABL Tyrosine Kinase Inhibitors Blood (ASH Annual Meeting Abstracts), Nov 2008; 112: 4263.
131. Bhatia R, et al. A Phase I Study of the HDAC Inhibitor LBH589 in Combination with Imatinib for Patients with CML in Cytogenetic Remission with Residual Disease Detectable by Q-PCR. Blood (ASH Annual Meeting Abstracts), Nov 2009; 114: 2194.
132. Wolf JL, et al. A Phase II Study of Oral Panobinostat (LBH589) in Adult Patients with Advanced Refractory Multiple Myeloma Blood (ASH Annual Meeting Abstracts), Nov 2008; 112: 2774.
133. San-Miguel JF, et al. A Phase IB, Multi-Center, Open-Label Dose-Escalation Study of Oral Panobinostat (LBH589) and I.V. Bortezomib in Patients with Relapsed Multiple Myeloma. Blood (ASH Annual Meeting Abstracts), Nov 2009; 114: 3852.
134. Spencer A, et al. Panobinostat plus lenalidomide and dexamethasone phase I trial in multiple myeloma (MM). J Clin Oncol 27:15s, 2009 (suppl; abstr 8542).
135. Alizadeh AA, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000 Feb 3;403(6769):503-11.
136. Rosenwald A, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med. 2002 Jun 20;346(25):1937-47.
137. Wright G, et al. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. Proc Natl Acad Sci U S A. 2003 Aug 19;100(17):9991-6.
138. Lenz G, et al. Stromal gene signatures in large-B-cell lymphomas. N Engl J Med. 2008 Nov 27;359(22):2313-23.
139. Lenz G, et al. Aggressive lymphomas. N Engl J Med. 2010 Apr 15;362(15):1417-29.
140. Davis RE, et al. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. J Exp Med. 2001 Dec 17;194(12):1861-74.
141. Hans CP, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004 Jan 1;103(1):275-82

142. Zinzani PL, et al. Identification of outcome predictors in diffuse large B-cell lymphoma. Immunohistochemical profiling of homogeneously treated de novo tumors with nodal presentation on tissue micro-arrays. Haematologica. 2005 Mar;90(3):341-7.
143. De Jong D, et al. Immunohistochemical prognostic markers in diffuse large B-cell lymphoma: validation of tissue microarray as a prerequisite for broad clinical applications--a study from the Lunenburg Lymphoma Biomarker Consortium. J Clin Oncol. 2007 Mar 1;25(7):805-12.
144. Piccaluga PP, et al. Gene expression analysis of peripheral T cell lymphoma, unspecified, reveals distinct profiles and new potential therapeutic targets.J Clin Invest. 2007 Mar;117(3):823-34.
145. Cartron G, et al. J Clin Oncol. 2003.
146. Younes A. Beyond chemotherapy: new agents for targeted treatment of lymphoma. Nat Rev Clin Oncol 2011; 8:85-96;
147. Younes A. Berry DA. From drug discovery to biomarker-driven clinical trials in lymphoma. Nat Rev Clin Oncol 2012;9: 646-653.