

A Phase I/II first-line study of R-CHOP plus B-cell receptor/NF- κ B-double-targeting to molecularly assess therapy response

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The ImbruVerCHOP trial is an investigator-initiated, multicenter, single-arm, open label Phase I/II study for patients 61–80 years of age with newly diagnosed CD20⁺ diffuse large B-cell lymphoma and a higher risk profile (International Prognostic Index ≥ 2). Patients receive standard chemotherapy (CHOP) plus immunotherapy (Rituximab), a biological agent (the proteasome inhibitor Bortezomib) and a signaling inhibitor (the Bruton's Tyrosine Kinase-targeting therapeutic Ibrutinib). Using an all-comers approach, but subjecting patients to another lymphoma biopsy acutely under first-cycle immune-chemo drug exposure, ImbruVerCHOP seeks to identify an unbiased molecular responder signature that marks diffuse large B-cell lymphoma patients at risk and likely to benefit from this regimen as a double, proximal and distal B-cell receptor/NF- κ B-co-targeting extension of the current R-CHOP standard of care.

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Lay abstract: The study investigates a new therapeutic concept for elderly patients newly diagnosed with a particularly aggressive B-cell lymphoma type that combines classical chemotherapy and a therapeutic antibody (together reflecting the current standard) with two modern agents, directed against a critical signaling cascade in this cancer type. Beyond feasibility and efficacy, it is particularly important in this study to collect tumor samples not only prior to but also immediately during first drug exposure. Molecular profiling of the tumor co-interpreted with patient outcome is expected to predict which patients are likely to benefit from such an extension of the standard regimen.

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Introduction to the trial

About 30–40% of the patients diagnosed with diffuse large B-cell lymphoma (DLBCL) will not be cured by standard R-CHOP induction [1,2] and, unfortunately, the majority of those will eventually succumb to their disease despite next-line treatment options that include salvage and high-dose chemotherapy with autologous stem cell support, allogeneic stem cell transplantation and CAR-T cell therapies. Given the limited curative potential those treatment strategies come with, it is generally consented that higher complete remission rates must be achieved by the induction regimen to reduce the risk of death in this entity. Hence, how to improve the R-CHOP backbone

for patients at particular risk to fail, and how to select those likely to benefit from such an extended treatment, are pivotal questions in DLBCL care and conceptually go well beyond this specific type of lymphoma.

The ImbruvERCHOP Trial consists of combination treatment with Ibrutinib (Imbruvica®), Bortezomib (Velcade®), Rituximab and CHOP (i.e., Cyclophosphamide, Doxorubicin, Vincristin and Prednisone) for elderly patients (age 61–80 years) with CD20⁺ DLBCL who are at higher risk (International Prognostic Index [IPI] ≥ 2). The trial is an investigator-initiated, multicenter, single-arm, open label Phase I/II study (supported by Janssen-Cilag [High Wycomb, UK]) that offers a rational, preclinically and clinically supported treatment concept, based on the hypothesis that the B-cell receptor (BCR)/NF- κ B pathway operates as the central cellular survival pathway in DLBCL.

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Background & rationale

Standard first-line treatment in DLBCL

DLBCL accounts for about 30% of all Non-Hodgkin's lymphoma (NHL) and 80% of aggressive NHL and is the most frequent type of malignant lymphoma. The median age at diagnosis is in the mid-60s, and most patients present with advanced-stage disease [3]. The CHOP regimen has been the standard of care for DLBCL for over 4 decades, producing cures in about half of the patients [4]. By additional application of the anti-CD20 antibody Rituximab, overall survival (OS) can be improved by another 10–15% [1,2]. Moreover, dose densification by reducing the 3-week interval (R-CHOP-21) to an R-CHOP-14 rhythm produced no consistently superior results, thus confirming R-CHOP-21 as the standard with R-CHOP-14 remaining a possible alternative in the elderly patient population [5–7]. However, nearly 40% of all patients are refractory to or relapse after initial therapy.

Predicting outcome

Long-term outcome can be predicted by clinical factors, for example, by applying the IPI, which was established more than 25 years ago [3], or by biological characteristics, among them genome-wide, transcriptome-based gene expression profiles (GEP) [8–10]. More recently, much deeper molecular analyses utilizing next-generation DNA and RNA sequencing plus comparative genomic hybridization to cover copy number alterations [11–13] linked to the clinical responsiveness and long-term outcome of the patients whose DLBCL samples were molecularly profiled. GEP of DLBCL actually pioneered the 'omics'-driven dissection of a histopathologically indistinguishable cancer entity, thereby unveiling at least two distinct subgroups that were characterized by different outcomes in response to standard therapy prior to and in the Rituximab (R) era [3,14–16]. Specifically, reflecting a postulated 'cell-of-origin (COO)' signature, GEP revealed a germinal center B-cell-like (GCB) DLBCL subtype and an activated B-cell-like (ABC) subtype [14]. Importantly, in a GEP-profiled cohort of 233 DLBCL patients undergoing R-CHOP-like first-line therapy, those diagnosed with an ABC DLBCL presented with a significantly inferior progression-free survival (PFS) and OS when compared with an equally treated group of GCB-type DLBCL patients [15]. Notably, although intense efforts have been made to identify surrogate markers that recapitulate the GEP findings, especially immunohistochemistry (IHC)-based markers applicable to formalin-fixed and paraffin-embedded archive material (the so-called 'Hans' classifier), it takes genome-wide GEP or, more recently, a Nanostring®-based assay that quantifies the expression levels of 20 transcripts, termed Lymph2Cx, to faithfully assign individual DLBCL cases via a linear predictor score to either GCB, an undetermined group or the ABC cohort and predict clinical outcome [17–24]. Moreover, only genome-wide profiling, but not surrogate markers or limited gene panels, enable unbiased discoveries such as the link between stromal signatures and long-term outcome in R-CHOP-treated DLBCL [6].

Role of BCR/NF- κ B-signaling

Importantly, the Dalla-Favera and Staudt laboratories showed that ABC DLBCL preferentially present with and depend on activated NF- κ B signaling, which is mainly driven by chronic active BCR signaling and NF- κ B-activating mutations in gene loci such as *CARD11*, *TNFAIP3/A20*, *CD79A/B* or *MYD88*, which are preferentially detectable in ABC DLBCL, although (a subset of) GCB lymphomas may also rely on BCR/NF- κ B signaling [25–29]. While ABC DLBCL present with particularly poor outcome, it is important to note that its merely signature-defined COO designation does not establish an actionable target principle *per se*. Given the still unsatisfying treatment results in DLBCL in general, there is urgent medical need to improve the current standard of care, in other words, R-CHOP-like immune-chemotherapy, either for the entire population of DLBCL patients, or a molecularly defined

subset of patients at particular risk that would selectively benefit from this novel therapeutic concept. Functional analyses in transgenic mouse lymphoma models unveiled differentially wired BCR/NF- κ B-related signaling network components as key determinants of treatment effector programs (such as apoptosis and cellular senescence) [30]. Cross-species interrogation of these signaling components in combined human transcriptome-plus-clinical outcome DLBCL datasets led to the identification of hitherto unknown and prognostically distinct DLBCL subgroups beyond their mere COO designation, and, thus, fueled the BCR/NF- κ B working hypothesis now underlying the ‘all-comers but in-depth molecularly profiling’ ImbruvERCHOP trial [30]. In essence, ImbruvERCHOP aims to demonstrate a safe and feasible way to enhance outcome in a certain subset of DLBCL patients marked by yet-to-be-determined genetic signatures or biomarkers of responsiveness to this novel regimen.

Extended treatment: addition of Bortezomib

Addition of Bortezomib (Velcade®), the clinically approved first-in-class inhibitor of the proteasome with significant activity in multiple myeloma, may have the potential to improve the DLBCL standard R-CHOP induction, since DLBCL cell lines display vulnerability to NF- κ B inhibition *in vitro* [31,32], with Bortezomib found to suppress NF- κ B signaling via blocked degradation of the NF- κ B inhibitor I κ B α [33]. Bortezomib is marketed but not approved for DLBCL. Subsequent Phase I trials have documented safety and activity alone or in combination with conventional chemotherapeutic compounds, including the CHOP backbone against B-cell and T-cell malignancies [34–36]. Important information regarding Bortezomib’s efficacy based on the COO-classified subtype came from a multicenter study investigating a total of 49 patients with relapsed or refractory (R/R) DLBCL; while Bortezomib failed to demonstrate activity as a single agent in this trial, it produced – as part of a dose-adjusted EPOCH regimen (Etoposide, Prednisone, Vincristine, Cyclophosphamide and Doxorubicin) – a significantly higher response rate and, for the first time, superior OS in ABC as compared with GCB DLBCL patients, while otherwise ABC and GCB subtypes have comparable outcomes upon R-CHOP relapse [37]. Encouraging data were subsequently reported from a small first-line DLBCL Phase I/II trial, where addition of Bortezomib to the R-CHOP backbone neutralized the historically inferior outcome of patients harboring ABC subtype lymphoma [38]. As a rational next step, the Bortezomib-R-CHOP combination was tested in previously untreated DLBCL patients in a randomized Phase III trial in the UK (‘REMoDL-B’; NCT01324596), where the lymphoma material was subjected to COO GEP analysis by Illumina (CA, USA) *DASL* gene expression arrays [39]. No significant differences were found in this trial between the ABC and GCB subtype, not even for R-CHOP alone, thereby prompting the question whether a representative proportion of higher-risk patients in need of more intense therapy was actually enrolled in REMoDL-B. In a more selective approach, considering only non-GCB DLBCL (by ‘IHC-Hans’ criteria) for randomized Bortezomib addition to R-CHOP induction in the Phase II PYRAMID trial (NCT00931918), patients did surprisingly well under R-CHOP alone, again indicative of a less failure-prone population and, again, with no significant improvement in PFS and OS in combination with Bortezomib [40].

Extended treatment: addition of Ibrutinib

Another novel agent of interest is Ibrutinib, an inhibitor of the Bruton’s Tyrosine Kinase (BTK), which relays upstream signals from the BCR and other mediators to its downstream NF- κ B cascade. Ibrutinib was co-developed by Pharmacyclics, Inc. (CA, USA) and Janssen Research & Development, LLC (NJ, USA) for the use in several B-cell malignancies and approved by the European Medicines Agency (Amsterdam, The Netherlands) and the US FDA (MD, USA) in autumn 2014 for the treatment of CLL patients who received at least one previous therapy and in first line for patients with a *del(17p)* or *TP53* mutation. By now, it is approved for the treatment of adult patients with previously untreated CLL and as subsequent line of therapy, for R/R mantle cell lymphoma and Waldenström’s Macroglobulinemia. For DLBCL, Ibrutinib is not registered. Preclinical investigations of DLBCL cell lines unveiled BTK as a bona fide target for genetic or pharmacological inhibition with particular vulnerability observed in ABC subtype cell lines [26]. Although detailed functional investigations are still pending, Ibrutinib demonstrated single-agent activity in R/R DLBCL patients, especially in ABC DLBCL patients [41]. Importantly, Ibrutinib was effective in CD79-mutant DLBCL, where NF- κ B signaling is constitutively activated upstream in proximity to the BCR, but failed in patients with ABC subtype-enriched mutations in *TNFAIP3/A20*, *CARD11* or *MYD88*, which operate in the NF- κ B pathway parallel or downstream of BTK [42]. These findings prompted a Phase Ib trial that combined R-CHOP with Ibrutinib for newly diagnosed and relapsed B-NHL, which produced signals of enhanced efficacy, and identified 560 mg as the recommended daily oral dose of Ibrutinib [42]. Consequently, a randomized Phase III trial was launched in the USA (‘PHOENIX’; NCT01855750) to compare R-CHOP-21 \pm

Ibrutinib restricted to non-GCB DLBCL patients (according to the IHC ‘Hans classifier’). The negative outcome of this trial was recently reported, essentially saying that the addition of Ibrutinib to R-CHOP failed to improve outcome in the overall population. However, preplanned stepwise age group analyses unveiled a significant survival advantage, namely an 11.1% gain in 3-year event-free survival and a 12.3% plus in 3-year OS in patients <60 years of age [43,44]. In the overall patient population, this substantial benefit was outweighed by older patients who experienced overt toxicity, especially in the Ibrutinib arm, that resulted in a dramatic drop in R-CHOP dose adherence to a mere 70% of the intended cumulative dose, which is likely, albeit not entirely, explained by the absence of mandatory prophylaxes for elderly patients in this trial. Remarkably, when the samples were COO-reanalyzed by the Nanostring® Lymph2Cx assay, almost a third of the lymphomas was classified as GCB subtype – of which younger patients (<60 years) presented with an equal Ibrutinib benefit as seen for the ABC subtype, thereby questioning the ABC-preferential activity of Ibrutinib in this context [43,44].

Rethinking first-line trials for DLBCL patients in the R-CHOP era

The PHOENIX trial was not the last of a series of Phase III first-line ‘R-CHOP + X’ trials reported negative in DLBCL. As previously declared for the addition of the VEGF-A inhibiting antibody bevacizumab (MAIN study) [45], Bortezomib (REMoDL-B, see above [40]) or Ibrutinib [41], now also the ROBUST trial, investigating R-CHOP ± Lenalidomide, a representative of the class of immunomodulatory drugs primarily licensed for the treatment of multiple myeloma, exclusively in the ABC subtype, turned out to be negative [46]. Likewise, related strategies to improve R-CHOP efficacy by the addition of Etoposide in a Phase III head-to-head comparison with the dose-adjusted CHOEP-R protocol (CALGB/Alliance 50303 trial) [47], or by replacing Rituximab with obinutuzumab, a different, glycoengineered anti-CD20 antibody (GOYA study) [48] also failed. As alluded to above, a critical limitation of all these trials is the inclusion of too many lower risk patients that are sufficiently treated with the standard of care, thereby diluting potential response signals from higher-risk patients. This directly prompts the important question whether all-comer trials are still justified in the heterogeneous landscape of DLBCL molecular pathologies. So far, both all-comer as well as COO-preselected settings turned out unsuccessful, the latter for the reason that COO may enrich for the presumed target but is not a target itself. Given the limited *a priori* predictability of responders in settings where not a single mutant lesion is the intended target (e.g., an EZH2 mutation to be blocked by an EZH2 inhibitor) but rather a signaling condition such as the BCR/NF-κB cascade, we like to argue that smaller piloting Phase I/II all-comer trials are needed with the prime objective to identify molecular predictors of response in an unbiased manner, including a COO-agnostic fashion, which may later help to determine the entry criteria for a confirmative Phase III trial.

Importantly, the formally negative REMoDL-B and PHOENIX Phase III studies should not be misinterpreted as proof of inefficacy of Bortezomib or Ibrutinib in the DLBCL arena. As impressively demonstrated for the PHOENIX trial, there is a large patient population that clearly benefited from the addition of Ibrutinib, if the R-CHOP-Ibrutinib regimen could be dose-administered at least nearly as planned per protocol. Moreover, there is little doubt that Bortezomib has activity in DLBCL, but predictive biomarkers capable of identifying actual responders prior to induction therapy are missing. At last, limited activity signals produced by the ‘R-CHOP + X’ Phase III trial formats do not necessarily indicate insufficient efficacy if two of those ‘X’ candidates were to be applied in combination on top of an R-CHOP backbone, especially in rational settings where those two agents reinforce target control in a potentially synergistic manner. For instance, encouraging findings in this regard were just reported at the 15th International Conference on Malignant Lymphoma (Lugano, Switzerland, 18–22 June 2019) for a lenalidomide and Ibrutinib combination in conjunction with R-CHOP for non-GCB DLBCL patients [49]. The quest, however, must be the molecular delineation of the benefiting patient population at particular need to avoid unnecessary and costly treatment of nonresponders or patients curable with R-CHOP only.

Proximal-distal double-targeting of the BCR/NF-κB cascade

A preclinical study targeting oncogenic BCR/NF-κB signaling in DLBCL and mantle cell lymphoma lines *in vitro* reported synergistic activity of Bortezomib in combination with Ibrutinib. It found efficacy to be independent of the COO status in DLBCL and even claimed Ibrutinib to serve as a re-sensitizer to Bortezomib in cell lines that previously acquired resistance to Bortezomib [50]. A large high-throughput combinatorial *in vitro* screen set up to identify drugs that cooperate with Ibrutinib in ABC DLBCL lymphoma cell killing used Bortezomib as a positive control, hence, was technically not designed to potentially retrieve a possibly synergism between these two agents, but underscored the strong cooperativity between Ibrutinib and the chemo agent Doxorubicin [51].

Based on the hypothesis that the combined addition of Ibrutinib and Bortezomib to R-CHOP might improve the outcome of, at least, a sizeable proportion of DLBCL patients, we introduce here the Ibrutinib–Bortezomib–Rituximab-CHOP combination – hereafter referred to as ‘ImbruVerCHOP’ – as an innovative regimen that augments the standard R-CHOP backbone by two presumably BCR/NF- κ B targeting nonchemo agents in first-line DLBCL treatment, thus, tackling DLBCL with a quadruple approach consisting of a signaling inhibitor (the BTK inhibitor Ibrutinib), a biological (the proteasome blocker Bortezomib), immunotherapy (the anti-CD20 antibody Rituximab) and conventional chemotherapy (the DNA-damaging and antimicrotubule agents in the CHOP regimen). Educated expectations and preliminary clinical experience with the two nonchemo drugs involved suggested that their addition might be particularly beneficial in the ABC subtype. However, since it is known that a subset of GCB lymphomas also presents with constitutive BCR/NF- κ B signaling [25] and, most importantly, other as yet unknown mechanisms of action may account for clinical efficacy of the I-B-R-CHOP protocol irrespective of the COO subtype, we decided not to *a priori* exclude any DLBCL patient based on COO status at diagnosis. It seems noteworthy to underscore that such two small compound combination on top of the immune-chemotherapy backbone may not simply be viewed as the extrapolatable sum of effects both compounds achieved as single-agent extensions of R-CHOP. While it becomes increasingly clear that monotherapy with a targeted inhibitor will invariably select for resistance across targets and entities, it is much less obvious how to combine those agents to prevent drug evasion from occurring [52]. Co-blockade of a linear signaling cascade, as, for instance, successfully demonstrated for BRAF/MEK double-inhibition in metastatic melanoma [53], as well as combinations that simultaneously shut down different pathways as currently being clinically tested in many settings, are viable concepts to avoid rapid emergence of drug resistance. However, it is an extremely demanding task to identify the optimal ‘in-patient’ dose-schedule of two agents in terms of highest efficacy and still acceptable toxicity. Therefore, the combination of Ibrutinib and Bortezomib exploited here in the further complicating R-CHOP context had to be defined based on rational considerations of the experience obtained with these agents in other clinical protocols. In essence, it is the ultimate goal of this trial to produce detailed molecular, functional and imaging information prior to and during therapy to retrieve a molecular signature that predicts clinical benefit from I-B-R-CHOP in patient subgroups being at risk to fail to a conventional R-CHOP-only induction protocol.

Design

Study objectives, design & treatment

We plan to include 60 patients in this multicenter, single-arm, open label Phase I/II study over 3 years, with about 9–12 German (and potentially other European) university centers participating. The study treatment includes a prephase therapy with Prednisone and six 21-day cycle of a combined immune-chemotherapy with the anti-CD20 antibody Rituximab together with chemotherapy consisting of Cyclophosphamide, Doxorubicin, Vincristine and Prednisone plus Bortezomib (1,3 mg/m² s.c., d1 and 8 in C1-6) and Ibrutinib (420 mg daily from C1-6) followed by two additional 3-week cycles of Rituximab, similar to the 6x R-CHOP plus 2x Rituximab backbone administered in the RICOVER-60 trial for this age group [51]. Throughout the course of the treatment, we recommend a quadruple prophylaxis with Ciprofloxacin, Sulfamethoxazol/Trimethoprim, Aciclovir and G-CSF.

The trial comprises a safety run-in phase, in other words, the Phase I part of the study, to uncover unexpected toxicities that may arise in the context of Ibrutinib and Bortezomib co-administered with the R-CHOP backbone. As soon as the first six to ten patients have been enrolled in the study, toxicity data will be carefully reviewed by the Data and Safety Monitoring Board, potentially (based on severity and frequency of toxicities observed so far) concluding dose reductions of Ibrutinib or Bortezomib. Upon clearance by the Data and Safety Monitoring Board to continue, the study enters its Phase II segment. Initially planned and therefore administered at the beginning of recruitment, patients received 560 mg Ibrutinib per day. In 2018, the dose was adjusted to 420 mg daily due to concerns and as a precautionary measure based on current toxicity findings (for elderly patients age 65 and up) randomized to Ibrutinib plus R-CHOP in the PHOENIX trial [43,44]. Our safety monitoring board supported this dose adaptation solely for Ibrutinib, which is in effect for the further course of the study.

The planned study period spans 3 years of recruitment and a 30-month follow-up.

Study end points

The primary end point is, in addition to safety and feasibility, the 2-year PFS. Based on previous experience with R-CHOP in such a patient population [38], the trial will be considered negative if $\leq 60\%$ (i.e., ≤ 36 of the 60 patients planned) will be progression-free after 2 years. Secondary end points are complete remission rate, overall response

Table 1. Inclusion and exclusion criteria.

ImbruVerCHOP inclusion and exclusion criteria	
Inclusion criteria	Exclusion criteria
<ul style="list-style-type: none"> – Age, ≥ 61 years and ≤ 80 years – CD20-positive DLBCL; including T-cell-rich large B-cell lymphoma, anaplastic large B-cell lymphoma, plasmablastic lymphoma (if CD20⁺); follicular lymphoma grade 3b or primary transformed follicular lymphoma at initial diagnosis – Unfavorable risk profile, IPI ≥ 2 – Performance status (ECOG) 0–2 – Cardiac ejection fraction ≥ 50 % – Hemoglobin ≥ 9 g/dl, absolute neutrophil count $\geq 100/\mu\text{l}$ and platelet count $\geq 100,000/\mu\text{l}$ – Adequate renal function as documented by serum creatinine level $< 2 \times$ ULN or estimated GFR ≥ 40 ml/min/1.73 m² – Adequate hepatic function (total bilirubin $\leq 15 \times$ ULN unless bilirubin rise is due to Gilbert's syndrome or of nonhepatic origin, ALT and AST $\leq 3 \times$ ULN) 	<ul style="list-style-type: none"> – Secondary transformed B-NHL or types of NHL other than DLBCL and its subtypes according to WHO classification – Prior therapy for DLBCL – Known central nervous system lymphoma – Central nervous system involvement by lymphoma – Major surgery within 4 weeks prior to start of induction therapy – History of stroke or intracranial hemorrhage within 6 months prior to study entrance – Anticoagulation with vitamin K antagonists – Clinically significant cardiovascular disease such as uncontrolled or symptomatic arrhythmias, congestive heart failure or myocardial infarction within 6 months of screening or any NYHA stage 3 or 4 – HIV or active Hepatitis C virus or active Hepatitis B virus infection – Prior malignancy (except adequately treated basal cell carcinoma and squamous cell carcinoma of the skin, cervical cancer <i>in situ</i>, or any other cancer for which the patient has been in remission for at least 5 years) – Pre-existing polyneuropathy of any kind $>$ grade I – Severe chronic obstructive pulmonary disease with hypoxemia

DLBCL: Diffuse large B-cell lymphoma; ECOG: The Eastern Cooperative Oncology Group; GFR: Glomerular filtration rate; IPI: International Prognostic Index; NHL: Non-Hodgkin's lymphoma; NYHA: The New York Heart Association; ULN: Upper limit normal.

rate, disease-free survival, 1-year PFS and OS in all patients. With consideration of the predictive power of subtypes (such as GCB vs ABC COO designation), further minimal residual disease (MRD) monitoring over time, frequency of secondary CNS manifestations and generation of biomarkers via bioinformatical processing of molecular profiles (lymphoma and liquid biopsies) obtained prior to and during the course of treatment (e.g., mutation patterns, gene signatures or protein expression by immunostaining) to identify patients who benefit from this extended R-CHOP protocol. Notably, individual clinico-molecular data, generated in a technically ImbruVerCHOP-matching approach (including re-biopsies under treatment, whenever feasible), from an additional, age-comparable cohort treated with standard R-CHOP outside this trial will further help to distill the most meaningful predictive information regarding the ImbruVerCHOP regimen.

Key eligibility criteria

All patients to be included in the study must provide written informed consent. For further inclusion and exclusion criteria, please refer to [Table 1](#).

Study status

The enrollment started in March 2017 and will continue until the end of 2020.

Translational research

To dissect mechanisms of drug action and potential insensitivity, transcriptome-wide GEP data will be obtained from lymphoma material at diagnosis, but also in up to two additional tumor biopsies taken acutely under initial exposure to R-CHOP in the first course of induction therapy and, if there is still a remaining lesion, during the third cycle of therapy – whenever technically feasible and without considerable risk for the patient. GEP are analyzed regarding potential outcome-discriminating transcriptional changes directly or indirectly produced by the combined action of R-CHOP, hence covering molecular responses evoked by the R-CHOP standard backbone. This transcriptome-based analysis (i.e., Affymetrix Gene Chips or RNA-Seq) will be accompanied, if material permits, by serum and lymphoma proteomics and metabolomics, as well as ‘targeted re-sequencing’ of lymphoma DNA and RNA at diagnosis to test the mutational status of approx. Eighty candidate genes (related to lymphoma biology, known cancer drivers and possible modifiers of I-B-R-CHOP drug action) and regarding biological effector programs such as apoptosis, cellular senescence and autophagy [54,55]. Moreover, it is planned to identify genomic MRD markers (e.g., IgH rearrangements or IgH translocations, potentially also driver mutations with high allelic frequency) from the primary lymphoma sample (or peripheral blood or bone marrow, if lymphoma cells are detected in these compartments), and monitor MRD levels in blood (as cell-free ‘liquid biopsy’) and bone marrow over time. In addition, we seek to establish every patient’s primary lymphoma in strongly immunocompromised mice (i.e., NOD/SCID/gamma-c [NSG] mice or related strains) to ultimately test responsiveness and long-term

outcome to components of the I-B-R-CHOP regimen in patient-derived xenograft models in a ‘co-clinical trial’-like format. Since we demonstrated in the past that E μ -*myc* transgenic mice, which develop aggressive B-cell lymphoma, serve as faithful models to predict treatment responses in DLBCL patients [30], we will add a preclinical mouse cohort harboring E μ -*Myc* lymphoma with and without defined genetic lesions in the BCR/NF- κ B pathway to obtain pharmacogenomic, mechanism-based clinical response data flanking the patient-centered scientific program of the ImbruVerCHOP trial.

As the overarching goal, we will bioinformatically integrate clinical courses, genomic mutational information (identified via targeted re-sequencing of a larger panel of candidate genes at diagnosis) and GEP data (and, if possible, proteomics and metabolomics data) with MRD levels measured at diagnosis, at cycle-3 and after completion of induction therapy in blood and bone marrow aspirates based on initially identified molecular markers (e.g., IgH rearrangements or distinct driver mutations in the primary lymphoma biopsy) [56]. Collectively, these analyzes – consisting of mutation detection, GEP, IHC, functional *in situ* assays (to detect apoptosis or senescence, for instance), proteomics/metabolomics, MRD quantification and, planned as an amendment, FDG-/FLT-PET imaging – will provide deeper insights into molecular effector programs evoked by the I-B-R-CHOP regimen and, in turn, into mechanisms of resistance that were already present at diagnosis (so called *de novo* resistance) or acquired/selected for during therapy. The data will be further exploited to produce a gene signature capable of identifying a patient subcohort (before induction therapy is actually started) that is in clinical need and likely to achieve a lasting benefit from I-B-R-CHOP induction therapy, and which may or may not overlap with established COO signatures. Importantly, a comparable R-CHOP standard-of-care-exposed cohort of participating DLBCL patients is simultaneously undergoing an identical schedule of sample collection and clinical follow-up in our department. Moreover, we have access to additional GEP data generated from lymphoma biopsies of DLBCL patients subjected to standard R-CHOP [15,30], and will also be able to interpret the results on a matched pair-based analysis with data from the DSHNHL RICOVER-60 trial, in which a large subcohort of patients 61–80 years of age received R-CHOP therapy [5], thus allowing us to extract and robustly compare the actual outcomes of a gene signature-identified patient subcohort to R-CHOP versus I-B-R-CHOP. Using the bioinformatically obtained and potentially quite complex gene signatures to identify responders and, in future trials, to preselect eligible patients, we also aim to convert these genetic stratifiers into simplified biomarkers of response that might be more easily applicable in clinico-pathological routine diagnostics.

In essence, the outlined array of directly patient material-originated information prior to and in the course of therapy (Figure 1), interpreted in conjunction with co-clinical patient-derived xenograft and preclinical transgenic mouse lymphoma model-derived data, will open new avenues to functionally dissect the outcome-relevant genetic networks underlying the complex action of the ImbruVerCHOP protocol as a rationally extended ‘R-CHOP plus’ regimen, and is likely to stimulate research efforts to ultimately overcome treatment resistance in DLBCL (and possibly conceptually beyond this entity).

Conclusion

In order to improve the current treatment standard for patients with unfavorable risk profile, it is key to select those likely to benefit from an extended ‘R-CHOP plus’ regimen. ImbruVerCHOP takes on this pivotal question with an innovative therapeutic and response-monitoring concept for elderly patients (age 61–80) with CD20⁺ DLBCL and a higher risk profile (IPI ≥ 2). As a first-line treatment, patients receive standard chemotherapy (CHOP) as well as immunotherapy (Rituximab), a biological (the proteasome inhibitor Bortezomib) and a signaling inhibitor (the BTK inhibitor Ibrutinib). Molecular lymphoma characterization not only prior to but also acutely under (cycle 1) and, if lesions persist, in the later course of therapy (cycle 3), are key features of this study. Using an all-comers approach, the ImbruVerCHOP trial aims at the unbiased identification of a molecular stratifier that marks DLBCL patients at risk and likely to benefit from the ImbruVerCHOP protocol as a double, proximal and distal BCR/NF- κ B-targeting extension of the current R-CHOP standard treatment.

Future perspective

Apparently, a series of failing Phase III trials, especially in DLBCL induction therapy, underscored awareness in the community that universal treatment concepts based, at least in part, on molecularly targeted therapeutics are unlikely to benefit large proportions of patients in unselected settings and, hence, produce toxicity but no clinical gain in many patients. Likewise, molecular markers determined in tumor material prior to any therapy may not be suited to predict responsiveness to a future drug encounter. Hence, smaller but molecularly more informative piloting

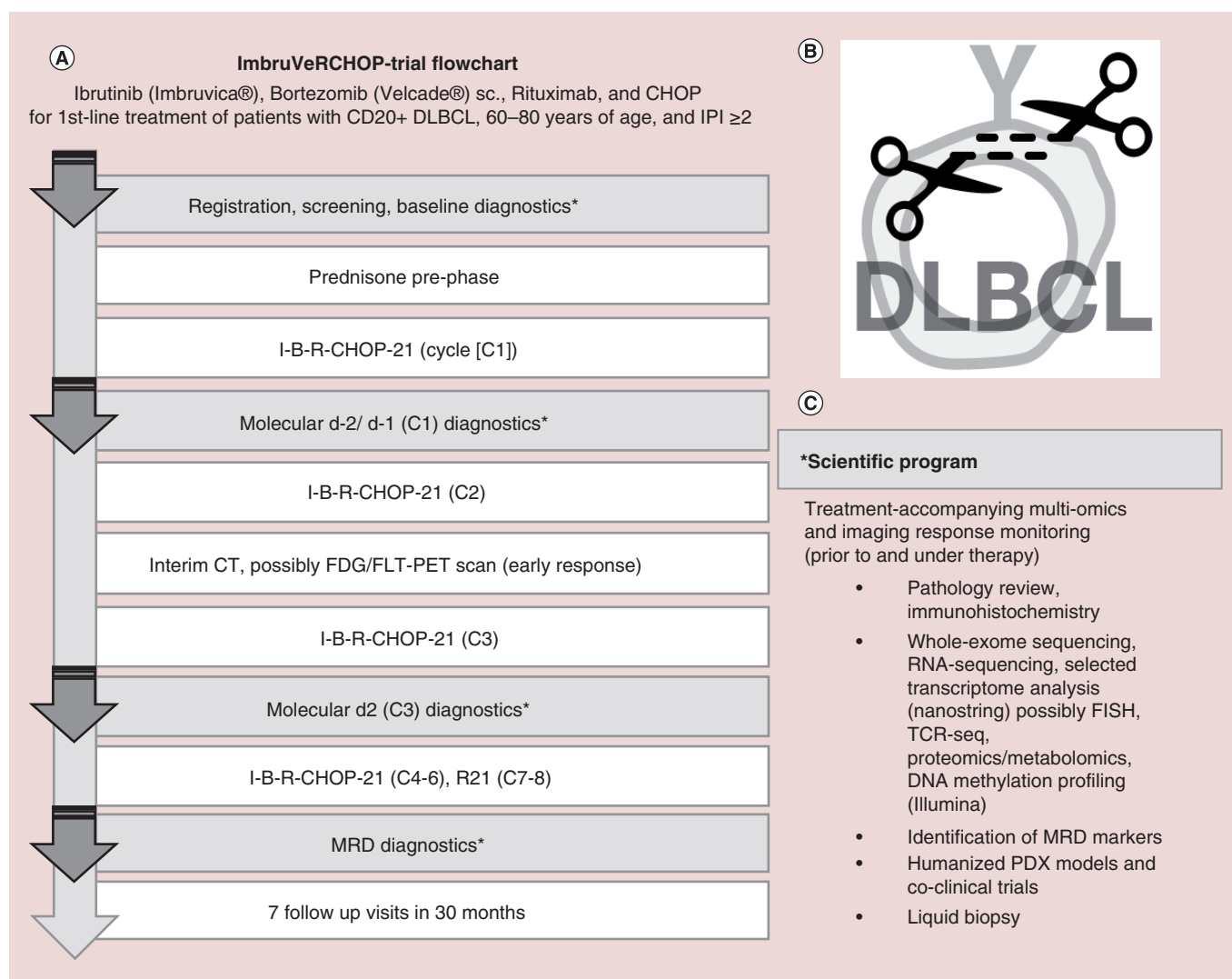


Figure 1. Flow chart of the trial sequence and time points of scientific program. (A) Flow chart showing the course of the study and when material for molecular analysis will be collected. **(B)** Study logo with double scissors for twofold target in B-cell receptor signaling. **(C)** Listing of molecular analyses.

*Scientific program.

CT: Computed tomography; DLBCL: Diffuse large B-cell lymphoma; FISH: Fluorescence *in situ* hybridization; MRD: Minimal residual disease; PDX: Patient-derived xenograft; PET: Positron emission tomography; sc.: Subcutaneous; TCR-seq: T cell receptor sequencing.

trials are needed to deeply scrutinize the molecular dynamics in an all-comer patient population now receiving an innovative, ‘Achilles heel’-targeting extension of the standard regimen, because they are at an increased risk to fail. Furthermore, flanking co-clinical patient-derived xenograft lymphoma studies in mice follow a comprehensive approach linking individual molecular profiles to efficacy in response to single-agents and the combination therapy. Such innovative multimodality immune-signaling-biological-chemo regimen dynamically molecularly monitored over time might represent a novel template to design future Phase I/II pilot trials that serve to uncover molecular classifiers subsequently applied to stratify patients in larger randomized confirmatory Phase III clinical studies.

Author contributions

The authors were involved in all content and editorial matters and were engaged in all phases of manuscript preparation and have accepted the final version. In addition to the conditions a-d, the authors have made the following contributions: S Denker: coordinating investigator, preparation and realization of the trial, training of the participating study centers, protocol writing, submission to authorities, contract work, analysis of the trial results and the accompanying translational program, writing of the

Executive summary

- ImbruVerCHOP is a single-arm, open label Phase I/II study offering an innovative therapy concept for elderly patients with CD20⁺ diffuse large B-cell lymphoma and a higher risk profile.
- The primary end point is the 2-year progression free survival. Secondary end points are complete remission and overall response rates, disease free survival and overall survival, in all patients and with respect to the predictive power of subtypes (such as germinal center B/activated B-cell-'cell-of-origin'), evidence for secondary central nervous system manifestation, markers of minimal residual disease over time and markers determined at the end of the study (e.g. gene signatures) to identify patients who benefit from this treatment addition.
- Participants receive a prephase therapy with Prednisone and six cycles of a combined immune-chemotherapy with the anti-CD20 antibody Rituximab and chemotherapy consisting of Cyclophosphamide, Doxorubicin, Vincristine and Prednisone (R-CHOP-21) plus Bortezomib (1,3 mg/m² sc., d1 and 8 in C1–6) and Ibrutinib (420 mg daily from C1–6) followed by two additional 3-week cycles of Rituximab.
- Nine to twelve German university centers participate in the trial, recruiting a total of approximately 60 patients.
- The planned study period consists of 3 years of recruitment and a 30-month follow-up.
- Enrollment started in March 2017 and will continue until end of 2020.
- Molecular lymphoma characterization is done not only prior to but also acutely under (cycle 1), and, if lesions persist, in the later course of therapy (cycle 3). Material is collected for pathology, immunohistochemistry, whole-exome sequencing, RNA-seq and, if the amount technically permits, FISH, TCR-seq, proteomics/metabolomics, DNA methylation profiling, as well as the identification of minimal residual disease markers. Pretreatment and under-therapy peripheral blood samples will be subjected to targeted re-seq analysis, minimal residual disease marker quantification and proteomics.
- All-comers study to identify molecular markers that indicate who actually benefits from an intensified therapy.

manuscript. A Bittner: preparation of the trial, submission to authorities. IK Na: deputy principal investigator, support in project preparation and realisation, contribution of expertise. J Kase: protocol writing, preparation of the trial. M Frick: preparation of the trial and submission to authorities. I Anagnostopoulos: contribution to planning and analysis of the accompanying translational program. M Hummel: biobanking, contribution to planning and analysis of the accompanying translational program. CA Schmitt: representative of the sponsor, principal investigator/LKP, national coordinator, preparation and supervision of the trial, submission to authorities, protocol writing, analysis of the trial results and the accompanying translational program, writing of the manuscript.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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