Review

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# Current trends in the management of Richter's syndrome

John N Allan*,[1] & Richard R Furman[1]

[1]Department of Medicine, Division of Hematology & Medical Oncology, New York-Presbyterian Hospital, Weill Cornell Medicine, 525 East 68th Street, New York, NY 10065, USA

*Author for correspondence: joa9069@med.cornell.edu

## Practice points

- Richter's transformation is a complication of chronic lymphocytic leukemia (CLL) affecting up to approximately 10–15% of patients with CLL.
- Most cases represent transformation to diffuse large B-cell lymphoma (DLBCL) and are historically chemorefractory.
- Prognostic scoring systems are in place to guide expectations for outcomes.
- Richter's transformation represents a unique biological entity with defined mutational events that are both present in preceding CLL clone (*NOTCH1*, 17p/*TP53*) or are acquired at time of transformation (*CDKN2A/B* loss, *MYC* activation).
- Current monotherapy approaches with novel agents have done little to impact upon outcomes.
- Preclinical models currently in development may prove useful to better define actionable pathways and molecular lesions, as well as identify rational drug combinations and more effective therapies.

Richter's syndrome (RS) is a life-threatening complication of chronic lymphocytic leukemia (CLL). While previous research has increased our knowledge on the distinct evolutionary patterns of RS and provided a deeper understanding of the risk factors and molecular events predisposing to transformation, there remain few targetable aberrations and treatment is largely ineffective. The ability to obtain deeper remissions, without selecting for deletion 17p, by using novel B-cell receptor (BCR) antagonists and bcl2 inhibition might lead to a decrease in the incidence of RS, but these agents have done little to significantly change outcomes when incorporated into treatment regimens for RS. In this review we highlight the current landscape of molecular lesions specific to RS, review the data on historical treatment options, and look to the horizon for potential opportunities in the future.

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Richter's syndrome (RS), initially described in 1928 by Maurice Richter as a 'reticular cell sarcoma', was named in his honor years later by Lortholary in 1964, upon a clear recognition of a clonal transformation process [1,2]. RS is described by the WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues as the development of an aggressive lymphoma arising in the background of chronic lymphocytic leukemia (CLL) [3]. The development of RS is characterized by the onset of B symptoms, rapid growth of lymphadenopathy, extranodal disease, significant elevations of LDH, and associated multiorgan dysfunction from invasive or obstructive processes. It remains to be seen whether the incidence of RS will change with the increasing use of B-cell receptor (BCR) antagonists and bcl2 inhibitors. There is great potential for decrease due to better disease control without the use of chemotherapy, which is known to select *TP53* disruptions, but the incidence could also potentially increase due to improved longevity of patients.

New molecular testing, including next-generation sequencing (NGS), have identified novel risk factors for the development of RS including *TP53* disruption, *NOTCH1* mutations, *CDKN2A* loss and *MYC* activation. Studies have also highlighted the significance of stereotyped BCRs in transformation biology. The poor prognosis observed for these patients is due in part to the underlying molecular changes that give rise to the RS cells, but also to the

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difficulty in delivering chemotherapy because of poor marrow reserves from disease and/or residual impact of prior chemotherapy. Currently, treatments involve the use of chemoimmunotherapy (CIT) regimens that have proven effective in *de novo* diffuse large B-cell lymphoma (DLBCL) but ignore the biological and molecular characteristics unique to this disease.

This review will discuss the current understanding of the molecular biology, the historical and current clinical management, and future interventions that might yield benefits for our patients.

## Transformation subtypes & histopathologic characteristics

While case reports have demonstrated rare forms of clonal transformation and histology [4,5], in clinical practice the vast majority of RS cases are almost exclusively restricted to either DLBCL or Hodgkin variant subtypes and will be discussed further.

### Diffuse large B-cell lymphoma

RS presents as DLBCL approximately 90% of the time [6,7]. Clinical progression of CLL is often associated with an increase in the size and proliferative capacity of the cells. Because proliferation centers in the lymph nodes, patients with CLL also demonstrate these features, and distinguishing the two from one another is important. Making a diagnosis of RS requires sheets of large cells effacing the lymph node or bone marrow architecture [3]. Several publications demonstrate that the cases with more confluent proliferation centers possess poorer prognoses and perhaps represent a continuum between CLL and RS, which is different from our current threshold to diagnose RS [8–10]. Making this distinction may be important given the use of novel agents that have efficacy in CLL, but not in RS, and has been iterated by colleagues previously [11]. Data are needed to show whether 'accelerated CLL' behaves more like CLL or RS in the setting of novel agent use.

Approximately 80% of DLBCL variants of RS are clonally related to the original CLL [12]. Clonal relatedness has a profound impact upon prognosis, with clonally related cases having a median survival of around 12 months, whereas clonally unrelated RS have a prognosis akin to that of *de novo* DLBCL with a median survival of 65 months [13]. Upon transformation, the resulting DLBCL frequently loses the classic immunophenotype associated with the precedent CLL with CD5 and CD23 expression found in 15–30% of RS samples [12]. Additionally, certain cytogenetic abnormalities have been determined to be useful in differentiating *de novo* CD5+ DLBCL from RS based on the presence of Bcl-6 translocations, which are frequently common in *de novo* CD5+ DLBCL and unlikely to be found in RS [12,14]. Interestingly, it has been recently reported that PD-1 expression, which is weak and restricted only to the paraimmunoblasts of proliferation centers, is upregulated and intensely expressed on RS-DLBCL. In addition, PD-1 expression is rarely observed in *de novo* DLBCL, possibly serving as an additional marker to distinguish clonally related RS from *de novo* DLBCL [15].

### Hodgkin variant RS

Hodgkin variant RS (HVRS) accounts for 5–10% of CLL transformations [16,17]. In HVRS, the pathologic Reed–Sternberg cells retain the classic immunophenotype seen in *de novo* Hodgkin lymphoma with Pax 5, CD30 and CD15 positivity being retained in 100, 100 and 88% of cases, respectively [18]. Pathologically HVRS is characterized by two distinct patterns, Type I and Type II. In Type I HVRS the Reed–Sternberg cells exist in a background of CLL, while in Type II the Reed–Sternberg cells exist within the inflammatory background commonly seen in classical Hodgkin lymphoma. The Type I versus Type II distinction has no bearing on outcomes or prognosis, and Type I may even be a precursor lesion which can transition to Type II over time [18]. It would be nice to hypothesize that the Type I lesions result from the CLL cells transforming into the Reed–Sternberg cells, whereas the Type II lesions are the result of a bystander, non-CLL, B cell developing into a Reed–Sternberg cell. Morphologically this rationale makes sense, with the coexistence of the Reed–Sternberg cells and CLL in the lymph nodes seen in Type I and their separation in Type II.

Published data, though limited, does not support this hypothesis. It is proposed that Reed–Sternberg cells seen in *de novo* classical Hodgkin's arise from a post germinal center B cell that has undergone immunoglobulin hypermutation [19]. Thus, it has been hypothesized that clonally related HVRS transformations would arise from CLL harboring mutated *IGHV* genes. Despite the overall small numbers of patients and studies evaluating clonality, there is mounting evidence to support this notion. Unlike in DLBCL-RS, where 80–90% of transformations are clonally related, in HVRS it appears only about 50% of HVRS are clonally related to the precedent CLL. While both mutated and unmutated *IGHV* CLL associate with HVRS at similar rates, clonally related HVRS appears

to be exclusively restricted to CLL that is either Zap-70 negative or harboring a mutated *IGHV,* suggesting that Reed–Sternberg cells arise *de novo* in cases of *IGHV*-unmutated CLL and directly from the clone in *IGHV*-mutated CLL cases [18]. While this clonal relationship has important prognostic implications on outcomes in patients with DLBCL-RS, there is limited data to suggest the clinical impact of clonality in HVRS.

In most series evaluating Epstein–Barr virus (EBV) infection and HVRS, it has been demonstrated that most cases do demonstrate infection with the virus, with 67–76% EBV+ cases being reported [18,20,21]. It remains controversial whether EBV status affects the clonal relationship between CLL and HVRS as several studies have shown opposing results [18,22,23].

## Epidemiology & risk factors associated with transformation

### Diffuse large B-cell lymphoma RS

The annual incidence rate of RS in patients with CLL has been estimated around 0.5–1%, with an overall incidence rate in approximately 5–16% of all CLL patients [6,7,24,25]. It is often mistakenly believed that RS is a late event in the disease course of patients with CLL. Observational studies have demonstrated the contrary, with median times to transformation of 1.8–1.9 years [6,7]. Most importantly, up to 47% of patients had transformed prior to receiving any therapy for their CLL. Gender does not seem to impact incidence as the 2:1 ratio of men to women seen in CLL is reflected in RS [26–29]. Despite the relatively low incidence rate overall in patients with CLL, the disease is enriched in patients harboring high risk molecular characteristics. Several studies have identified baseline clinical and biological features of CLL that have independently been associated with increased risk of developing RS. Clinical risk factors at CLL diagnosis, such as advanced Rai Stage, bulky adenopathy >3 cm, elevated β-2 microglobulin and LDH have independently been shown to associate with RS development [6,29,30]. Additionally, standard high risk biologic parameters that stratify CLL outcomes, have also been associated with RS later in the disease course, with CD38, CD49d and Zap-70 expression, unmutated *IGHV*, del11q and del17p all demonstrating an increased risk of RS development [6,7]. It remains controversial regarding the impact of prior therapy on RS risk but importantly, ~50% of patients who develop DLBCL-RS transform prior to any therapy for their CLL, suggesting that the underlying biological and genetic features are more important drivers than specific treatments [7,27].

### Hodgkin variant RS

A transformation to Hodgkin disease can occur, though infrequently, with a reported frequency of 0.4–0.7% of patients with CLL [20,31,32]. The largest series evaluating characteristics of patients with CLL who developed HVRS have not been able to identify unique characteristics between those that transform versus those that have not [20]. Despite no significant differences in baseline characteristics, subjects that transformed had a median age ranging from 61–72 with a median interval of 4.6–7.5 years between CLL and HVRS diagnosis [20,32]. A much higher proportion of patients had prior treatment for their CLL ranging from 78–80%, which is different than the DLBCL experience. Up to 64% diagnosed with HVRS had unmutated disease. Due to small numbers and difficulty in isolating Reed–Sternberg cells, indepth molecular analysis are lacking in this rare transformation subtype and thus, all further discussion regarding the molecular risk factors and evolution of transformation will be related to DLBCL-RS.

### Genetic & molecular risk factors

Over the past decade, molecular profiling of CLL has become increasingly important to identify high risk CLL and CLL at risk of transformation. During this time, the field has identified important molecular associations with transformation risk. Not surprisingly, unmutated *IGHV*, presence of high risk cytogenetics identified by FISH (deletion11q22 or deletion 17p13), expression of Zap-70, CD49d or CD38, telomere length, and genetic polymorphisms in *CD38* and *LRP4* have all been found to be independent risk factors for developing RS in a patient's lifetime [7,33]. The independent significance of commonly mutated genes in CLL, such as *TP53*, *NOTCH1*, *SF3B1,* on the risk of transformation is still unclear though associations of increased risk have been observed implicating specifically *NOTCH1* and *TP53* as lesions having the highest risk of transformation, with *NOTCH1*-mutated patients having a RS incidence of 45% at 15 years [34]. Fabbri *et al.* reported a study evaluating 15 RS samples with paired CLL diagnosis samples. In this study nearly 69% of the paired diagnosis CLL samples harbored the same mutation at either the clonal or subclonal level highlighting this is frequently present at diagnosis and that the Notch signaling pathway is critical for transformation [35].

### Stereotyped B-cell antigen receptors

BCR stereotype provides some of the strongest evidence for antigen selection in CLL ontogeny [36]. Since the 1990s and early 2000s we have recognized the impact of mutational status of the immunoglobulin heavy chain variable region and noted a nonrandom over-representation of complementary determining region 3 (CDR3) heavy chain gene rearrangements in CLL [37,38]. These nonrandom, over-represented, near identical CDR3 sequences, termed stereotyped BCRs, highlight the importance of specific antigens in selection of the CLL clone and suggest their development from a progenitor pool of B cells with features of both innate and adaptive immunity [39]. While CLL clones expressing certain stereotyped receptors can display an indolent course, others are associated with an aggressive phenotype [40].

There is growing evidence that stereotyped BCR configuration is associated with specific immune signaling profiles as well as underlying mutational and gene expression profiles [41–43]. The association of stereotyped BCRs and transformation risk has been investigated, demonstrating an independent increased risk of transformation with a hazard ratio of 3.3 in patients with a stereotyped BCR. This study observed subset #8, which uses a V4-39 variable gene and frequently associates with *NOTCH1*, has a transformation risk approaching approximately 70% at 5 years [44]. It has been shown that subset #8 demonstrates polyreactivity to a wide range of antigens which is in stark contrast to other aggressive stereotyped subsets with a restricted antigen affinity, suggesting that the unlimited ability to bind to microenvironment antigens may lead to unabated stimulation and progressive selection of aggressive clones [36,45]. There are many stereotyped subsets observed in CLL, each with unique antigen affinities and associations with underlying genetic mutations. While there is strong evidence and leading hypotheses to explain the predilection for transformation in CLL displaying subset #8 stereotyped BCRs, further work is still required to identify other high risk stereotyped subsets receptors, as not all associate with aggressive disease.

## Evolutionary patterns of DLBCL-RS

Throughout the 1980s, investigators began to identify the cell of origin that gives rise to the Richter's transformation. Many of the case reports and case series revealed similar surface immunoglobulin usage and gene rearrangements as those present in the initial CLL clone, providing strong evidence of clonal evolution leading to transformation into an aggressive large cell lymphoma [46,47]. Throughout the 1990s, further reports substantiated both clonal and nonclonal relationships using cytogenetics and known genomic lesions common in CLL, as well as identifying cursory genetic differences between *de novo* DLBCL and RS samples [14,48,49]. Over the past few years we have gained a deeper appreciation for the underlying defects and evolutionary patterns that give rise to RS. Using next-generation whole genome sequencing techniques, investigators have been able to tease out the molecular evolutionary patterns and differences of clonally related RS and *de novo* DLBCL.

Scandurra *et al*. provided the first insight in 2010 when the group performed genome-wide DNA profiling in 13 RS samples with eight clonally related CLL samples and also compared the 13 RS samples with 48 *de novo* DLBCL cases [50]. Their results revealed a genetically heterogeneous group of genomic lesions with no unique lesion occurring in greater than 50% of the RS cases, though several different genetic alterations impacted regulation of the Myc pathway, suggesting its potential critical role in transformation.

Rossi *et al.* followed suit in 2011, using a targeted gene sequencing panel in order to identify mutational changes occurring in RS samples and demonstrating the mutational heterogeneity of RS tumors and the emphasizing impact of *TP53* disruption in survival [13]. They found nearly 50% of the 86 RS cases had disruption of *TP53,* either in the form of mutations or deletion of 17p13. In the assessable, clonally related RS cases, *TP53* disruption was acquired at the time of transformation in 55% of the CLL/RS matched pairs. Similarly, *c-MYC* disruption occurred in 26.2% of the RS cases and was associated with *TP53* disruption 50% of the time. Interestingly, three out of four CLL/RS cases demonstrated acquisition of *c-MYC* alterations at the time of transformation. Corroborating previous studies, the group also demonstrated that RS transformations lacked many of the genetic defects that define *de novo* DLBCL. The RS samples lacked mutations in genes affecting NF-B regulation such as *TNFAIP, CD79a* and *CD79b.* Disruption of *BCL-6* was rare, consistent with previous reports [14] and the tumors lacked translocations or mutations in *BCL-2*, *PRDM1* and *EZH2.* The group demonstrated that the majority of the cases were clonally related nearly 80% of the time with clonally unrelated cases demonstrating improved outcomes similar to *de novo* DLBCL with median survivals of 62.5 months versus 14 months for clonally related RS.

In 2013, Fabbri *et al*. published results from 63 clonally related RS samples. Using whole exome sequencing and copy number analysis they revealed distinct evolutionary patterns and acquisition of novel genetic lesions [51]. They found that the majority of RS transformations arise from a predominant CLL clone. Three out of 15 cases

had a variable number of unique genetic mutations not seen in the paired CLL samples, supporting a branched evolution pattern arising from a precursor cell with selective pressures driving it towards CLL or RS through independent genetic events. RS genomes had on average 22 genomic lesions, of which there is an average of 12.5 copy number alterations with the majority of them being losses. The most common genetic chromosomal alteration was a deletion of 17p, shown in 40% of cases. The second most frequent chromosomal alteration was a deletion of 9p21 harboring the *CDKN2a/b* locus. This gene deletion was frequently associated with *TP53* disruption and *Myc* activating events and mutually exclusive of trisomy 12, which was also commonly observed in 30% of RS cases. Interestingly, there were low numbers of deletion 11q and deletion 13q abnormalities in RS samples (7 and 26%, respectively) [6,52]. The group investigated 481 targeted genes in RS samples and identified 23 that had a frequency >10%. 20 genes were enriched in RS samples and implicated in a wide array of biological functions including DNA repair, intracellular signaling or splicing regulation. The most common genes with point mutations found were *TP53,* with a frequency of approximately 50%, and *NOTCH1,* with a frequency approaching 40%. Together, with *CDKN2a* and *MYC* alterations, one or more of these four genes were found in >90% of RS samples. We have observed a similar finding in our case series of 16 transformations evaluated at our center dating back to 2015 (Unpublished Data).

Most of these earlier studies were performed from samples derived from patients treated in the era of CIT; therefore, the effect of novel agents on RS development and clonal evolution driving transformation are unclear. However, there are an increasing number of reports providing data in regards to this question. Recently, Kadri *et al.* reported on six RS cases who transformed on ibrutinib. They noted that transformed cells at sites of transformation are clonal descendants of the circulating CLL cells but that they undergo continued and isolated evolution within the lymph node microenvironment. They noted similar findings of *MYC* activating events, *CDKN2a* loss, *TP53* disruption and *NOTCH1* mutations but also demonstrated the RS tissue commonly displayed C481S Bruton's tyrosine kinase (BTK) mutations and BTK mutations within subclones not identified in the circulating CLL. Ultimately, they concluded that evolution of CLL to RS in the setting of ibrutinib relapse is not markedly different from RS arising unrelated to ibrutinib treatment [53].

In similar regards, we have recently reported on the development of two patient derived xenograft (PDX) models in patients who developed RS in the setting of novel agents [54]. We found similar results as Kadri *et al.*, demonstrating frequent associations of *MYC*, *CDKN2a*, *TP53* disruption and *NOTCH* pathway activation in these patients, which does not appear different than the common abnormalities observed in patients with RS treated solely in the era of CIT. Similarly, we identified both classic and subclonal novel *BTK* mutations in these specimens. Our study was limited by the fact we did not have preceding CLL samples, though we were able to demonstrate relatively stable genomic and transcriptomic alterations in multiple passages of the PDX in mice as compared with the primary tumor. Our initial characterization demonstrated constitutive activation of pathways such as BCR, Notch1 and Nf-β. We identified potential drivers of transformation in genes, such as *EGR2*, *KRAS*, *SETD2* and *MED12,* and documented the presence of other mutations in selected genes involved in cell-cycle control, proliferation, chromatin remodeling and epigenetic modification, suggesting their potential role in transformation. Importantly, the stable genomic and transcriptomic architecture within the PDXs as compared with the primary tumor will be utilized to further understand the pathogenesis of the disease and serve as a foundation for future drug development in this specific disease entity.

These major works have identified that RS transformation to DLBCL is commonly a linear evolutionary phenomenon arising from a dominant CLL clone. Specific genetic lesions or lack thereof define the disease and are different from *de novo* DLBCL highlighting the unique biology of RS. Early evidence suggests that major genetic events seen in RS occurring in the setting of novel agents like ibrutinib are not significantly different to transformations not exposed to ibrutinib and that *BTK* mutations are relatively common in RS occurring in the setting of ibrutinib, though the relevance of these mutations in the actual transformation event are still unknown and requires further investigation.

## Diagnosis

The presence of B-symptoms, rapidly enlarging lymph nodes, and markedly elevated lactate dehydrogenase clinically indicates potential transformation and should prompt further evaluation. Extranodal disease can be seen occasionally and may involve the gut, central nervous system, lungs, kidneys and skin among other sites [55]. Biopsy is ultimately required for diagnosis as these signs and symptoms may also be seen in aggressive variants of CLL.

Table 1. Published studies reporting outcomes in Richter's syndrome diffuse large B-cell lymphoma specific cohorts.

| Study agents | Year reported | Phase | Studied patients | Total no. enrolled (no. RS) | ORR (RS specific) | CR (RS specific) | PFS in months (median) | OS in months (median) |
|---|---|---|---|---|---|---|---|---|
| HyperCVXD 59 | 2001 | II | RS | 29 (29) | 41% | 38% | NR | 10 |
| FACPGM 60 | 2002 | II | RS, PLL, NHL | 22 (15) | 5% | 5% | NR | 2.2 |
| OFAR1 61 | 2008 | I–II | FR-CLL, RS | 50 (20) | 50% | 20% | NR | 6 month OS: 59% |
| OFAR2 51 | 2013 | I–II | R/R-CLL, RS | 102 (35) | 39% | 6.50% | NR | 6.6 |
| RCHOP 62 | 2014 | II | R/R CLL, RS | 60 (15) | 67% | 7% | 10 | 21 |
| OCHOP 18 | 2016 | II | RS | 37 (37) | 46% | 27% | 6.2 | 11.4 |
| REPOCH 63 | 2018 | Retrospective | RS | 46 (46) | 39% | NR | 3.5 | 5.9 |
| Selinexor 64 | 2017 | II | NHL, RS | 79 (8) | 40% | 0% | NR | NR |
| Pembrolizumab 65 | 2017 | II | R/R CLL, RS | 25 (9) | 44% | 11% | NR | 10.7 |
| CD19 CAR-T 66 67Cells | 2017 | II | R/R CLL, NHL, RS | 24 (5) | 66% | 33% | NR | NR |

CAR-T: Chimeric antigen receptor T cells; CR: Complete remission; FACPGM: Fludarabine, cytarabine, cyclophosphamide, cisplatin, GM-CSF; HyperCVXD: Cyclophosphamide, vincristine, liposomal daunorubicin, dexamethasone; NHL: Non-Hodgkin lymphoma; OCHOP: Ofatumumab, cyclophosphamide, doxorubicin, prednisone; OFAR: Oxaliplatin, fludarabine, cytarabine, rituximab; ORR: Overall response rate; OS: Overall survival. PFS: Progression-free survival; PLL: Prolymphocytic leukemia; R/R: Relpased/refractory; RCHOP: Rituximab, cyclophosphamide, doxorubicin, prednisone; RS: Richter syndrome; NR: Not reported.

### Role of PET/CT

The role of positron emission tomography/computed tomography (PET/CT) in aiding diagnosis of RS is well established. When evaluating patients with a standard uptake values (SUV) $\geq$5, studies have reported sensitives of 88–91%, specificities of 71–80% with positive and negative predictive values of 51–53% and 94–97% respectively, for transformation using that SUV cutoff [21,56]. Recent reports have demonstrated similar receiver operating characteristics when using an SUV cutoff of 10, with sensitivity and specificity of 91 and 95%, respectively. Positive and negative predictive values were reported of 60.6 and 99.2%, respectively. Importantly, using a cutoff value of 10 compared with 5 provided a more accurate proportion of correctly classified patients with RS at 94.6 versus 73.5%. Lastly, using a cutoff of $\geq$10 compared with $\leq$10 strongly correlated with mortality with significant differences in overall survival (OS) median 6.9 versus 56.9 months, respectively [57].

## Historical outcomes & current trends

Upon diagnosis several scoring systems have been suggested as ways to stratify patients, help guide therapeutic strategies, and set patient expectations. In 2006, MD Anderson established the Richter score and identified five variables that when calculated at time of diagnosis could identify patients at highest risks of poor outcomes [25]. Expanding on this, Rossi *et al.* have developed a scoring system which may better stratify patients and uses performance status, *TP53* status, and response to induction therapy. With this scoring system, median OS were 8 and 24 months for high and intermediate risk patients and a 70% 5-year survival rate for low risk patients [13]. While demonstrating improvement in predicting outcomes over the MD Anderson system, it must be realized that the Rossi scoring system incorporates response to induction therapy as a variable, and thus its utility to predict outcomes at time of diagnosis is relatively limited in that respect.

Additionally, the differences in outcomes based on clonal relationship to the precedent CLL are established [13]. Those with unrelated RS to DLBCL have survivals similar to *de novo* DLBCL and seem to be able to safely be treated with standard anthracycline-based regimens combined with anti-CD20 therapy; still, the role of consolidative therapy with stem cell transplant in this population remains unclear. However, the clonally related RS patients have repeatedly demonstrated inferior responses to treatment and continue to have poor outcomes and require advanced therapeutic strategies such as allogeneic transplant to achieve long-term remissions.

### Role of chemotherapy

Anthracycline- or platinum-based chemotherapy appear to have similar response rates with overall response rates (ORRs) of approximately 40% with only 5–15% complete remission (CR) rates (Table 1) [25,58]. Recent single arm studies incorporating novel anti-CD20 agents (ofatumumab + CHOP) and large retrospective studies of RS patients uniformly treated with R-EPOCH have demonstrated no apparent significant or incremental improvement in outcomes with median progression-free survival (PFS) and OS of 6.2 and 11.4 for ofatumumab + CHOP

(OCHOP) and 3.5 and 5.9 months for R-EPOCH. This reinforces the need to identify new therapeutic strategies, targeting the unique biology that drives transformation as many patients who have RS are *TP53*-disrupted and or demonstrate genomic instability which are historically chemotherapy refractory.

In regards to HVRS, the most common regimen used remains adriamycin, bleomycin, vinblastine, decarbazine (ABVD). Despite this, HVRS has inferior outcomes compared with *de novo* Hodgkin lymphoma [68,69]. One of the largest retrospective studies to date, by Mauro *et al.*, has characterized outcomes of HVRS which included 33 patients [69]. 28 were treated with ABVD or R+ABVD demonstrating an ORR of 77.3% with a CR rate of 68% which translated into a median survival of 37.8 months. There was a significantly lower CR rate in patients with an international prognostic score (IPS) ≥4 and a median OS of 37.8 months. With the advent of and US FDA approval of anti-CD30-targeted agent brentuximab vedotin for frontline-advanced stage Hodgkin lymphoma, it remains unclear if this inferior outcome can be overcome as the reported cohorts of patients were not treated with this agent. Recent data suggests, however, that there is a modest benefit of incorporating brentuximab into frontline therapy for advanced stage *de novo* Hodgkin lymphoma, though how this translates to HVRS remains unclear and its use should be considered in order to improve outcomes, especially in patients with HVRS and an IPS ≥4 until further data becomes available [70].

## Role of transplant

In patients with RS who attain a response to induction therapy, consolidation with transplant appears to confer benefit and improve survival. Unfortunately, only a minority of patients are fit enough or achieve a deep enough response to move on to transplantation [25,64]. Which type of transplant, autologous (auto-) versus allogeneic (allo-), can be debated, but the majority of data suggest that allotransplant likely provides the best opportunity for durable remissions should a patient be able to achieve a deep remission with induction chemotherapy. The impact of transplant on RS has been best summarized by three recent retrospective studies [25,64,65]. In 2006, Tsimberidou reported 20 patients who underwent stem cell transplantation (SCT). The patients who underwent alloSCT had longer survival than those who achieved remission and received no additional therapy or those who underwent auto or alloSCT as a salvage therapy. The European Bone Marrow Registry study by Cynarski *et al.* was the largest study to date characterizing outcomes with different transplant strategies. In total 59 patients were included, 34 underwent autoSCT and 25 received alloSCT. The observed 3-year OS and relapse free survival for each strategy was 59% and 45% for autoSCT and 36% and 27% for alloSCT. This study did not investigate the clonal relationship or genetic makeup of the individuals and, while outcomes appeared better with autoSCT, this group was enriched with patients that had chemosensitive disease, with higher rates of patients achieving CR – 34% versus 4% for alloSCT. Additionally, in the alloSCT group 92% of patients had a PR or resistant disease going into alloSCT salvage.

It is our practice to consolidate with alloSCT if a remission is achieved and there is an available donor as we feel the results for long-term remission favor this consolidative strategy.

## Role of novel agents

The role of novel agents such as ibrutinib, acalabrutinib, venetoclax, or idelalisib in RS treatment is unclear and experience regarding its effectiveness in RS is limited with only acalabrutinib studied in a large cohort of RS patients. Most of what is published in regards to BTK inhibitors is restricted to a few case reports, mostly observing ibrutinib use [59–61,71]. In many of these reports where ibrutinib was found to be effective, all patients who obtained a response were ibrutinib-naive and many did not report on clonality between the RS sample and the CLL. Importantly, duration of responses were measured in months and responses were mostly partial, thus the ability to control disease long-term and achieve deep remissions is lacking. This is further demonstrated by recent abstract of acalabrutinib in patients with RS [62]. In the 21 evaluable patients, ORR was only 38% with a CR rate of 14%. Six of 21 evaluable patients had previously been exposed to ibrutinib and in that population, three patients demonstrated a response of PR or better. While there was some efficacy in very refractory patients these reported responses did not translate into durable remissions as the duration of response was only 5.7 months in responders.

While novel agents do not appear to increase the rate of RS, transformation still represents a significant unmet need as reported studies using novel agents demonstrate transformation rates of 5–16% in high risk, heavily pretreated patients (Table 2). Several reports have demonstrated that transformation on novel agents is frequently an early event and is associated with poor outcomes; the vast majority of transformations occurring within the first 18 months of treatment and associating with a median OS of approximately 6 months after transformation [63]. It

Table 2. Studies reporting Richter's syndrome transformation rates in patients treated with novel agents.

| Author | Journal | Clinical trial | Year published | Phase | Agents | Number of subjects | Population (no.) | Comparator arm | 17p/*TP53* deleted/disrupted (%) | Median follow-up | Transformation rate (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Byrd | *NEJM* | PCYC-1102 | 2013 | Ib–II | Ibrutinib | 85 | R/R | None | 34 | 20.9 months | 8.2 |
| O'Brien | *Lancet oncology* | PCYC 1102 | 2014 | Ib–II | Ibrutinib | 31 | TN | None | 6 | 22.1 months | 3.2 |
| Byrd | *NEJM* | RESONATE | 2017 | III | Ibrutinib | 391 | R/R | Ofatumumab | 33 | 19 months | Ibrutinib: 4 |
| | | | | | | | | | | | Ofatumumab: 2.5 |
| Burger | *Lancet oncology* | NCT01520519 | 2015 | II | Ibrutinib + rituximab | 40 | TN (4) | None | 50 | 47 months | |
| | | | | | | | R/R (36) | | | | R/R: 5 |
| Farooqui | *Lancet oncology* | NCT01500733 | 2015 | II | Ibrutinib | 51 | TN (35) | None | 100 | 24 months | TN: 5 |
| | | | | | | | R/R (16) | | | | R/R: 6.25 |
| Burger | *NEJM* | RESONATE-2 | 2015 | III | Ibrutinib | 269 | TN | Chlorambucil | 0 | 18.4 months | Ibrutinib: 0 |
| | | | | | | | | | | | Chlorambucil: 1 |
| O'Brien | *Lancet oncology* | RESONATE-17 | 2016 | II | Ibrutinib | 145 | R/R | None | 100 | 27.6 | 12% |
| Byrd | *NEJM* | NCT02029443 | 2016 | I–II | Acalabrutinib | 61 | R/R | None | 31 | 14.3 months | 0% |
| Chanan-Khan | *Lancet oncology* | HELIOS | 2016 | III | Ibrutinib + BR | 578 | R/R | BR | 0 | 17 months | I + BR: 0 |
| | | | | | | | | | | | BR: 1 |
| Roberts | *NEJM* | NCT01328626 | 2016 | I | Venetoclax | 116 | R/R | None | 30 | 17 months | 16 |
| Stilgenbauer | *Lancet oncology* | NCT01889186 | 2016 | II | Venetoclax | 107 | R/R | None | 100 | 12.1 months | 10 |
| Seymour | *Lancet oncology* | NCT01682616 | 2017 | Ib–II | Venetoclax and rituximab | 49 | R/R | None | 19 | 28 months | 10 |
| Jones | *Lancet oncology* | | 2018 | II | Venetoclax | 91 | R/R to ibrutinib | None | 47 | 14 months | 5.5 |
| Seymour | *NEJM* | Murano | 2018 | III | Venetoclax and rituximab | 389 | R/R | BR | 37.5 | 23.8 months | VR: 3.1 |
| | | | | | | | | | | | BR: 2.7 |

BR: Bendamustine rituximab; NEJM: *New England Journal of Medicine;* VR: Venetoclax rituximab; R/R: Relapsed/refractory; TN: Treatment-naive.

Table 3. Sampling of current studies incorporating novel agents in Richter's syndrome specific cohorts.

| Study agents | Phase | Study population | NCT# |
|---|---|---|---|
| Venetoclax + REPOCH | II | RS | NCT03054896 |
| Ibrutinib + Obinutuzumab + CHOP | II | RS | NCT03145480 |
| Obinutuzumab + HDMP + Lenalidomide | II | RS | NCT03113695 |
| Blinatumumab | II | RS | NCT03121534 |
| Selinexor + RICE | II | R/R BNHL, RS cohort | NCT02471911 |
| Ublituximab + Umbralisib + Pembrolizumab | I/II | R/R CLL or RS | NCT02535286 |
| Nivolumab and Ibrutinib | II | R/R CLL or RS | NCT02420912 |
| Acalabrutinib and Vistursertib | II | R/R DLBCL or RS | NCT03205046 |

CHOP: Cyclophosphamide, doxorubicin, prednisone; CLL: Chronic lymphocytic leukemia; DLBCL: Diffuse large B-cell lymphoma; HDMP: High-dose methylprednisone; R/R: Relpased/refractory; REPOCH: Rituximab, etoposide phosphate, prednisone, vincristine sulphate, cyclophosphamide, doxorubicin hydrochloride; RICE: Rituximab, ifosfamide, carboplatin, etoposide; RS: Richter's syndrome.

would be logical to assume that patients who transform on ibrutinib or other novel therapy would be unlikely to benefit from BCR or BCL2 pathway antagonists and will require novel treatment strategies due to the refractory nature of this disease. Investigation into the role of *BTK* mutations in promoting RS and other bypass pathways are needed as the use of these novel agents continues to grow.

The experience of other novel agents or investigational compounds in RS patients is limited and remains anecdotal at best; to date there are no randomized head-to-head trials with CIT approaches versus those incorporating novel agents. Though recently some encouraging early results have been published with selinexor, pembrolizumab and CD19 CAR-T cells (Table 1) [66,67,72]. In regards to pembrolizumab, a recently published single center report demonstrates the efficacy of single agent pembrolizumab may be less than that initially reported by the Mayo group showing limited, if any, durable effect of the drug in patients with RS [73]. Additionally, in expansion studies studying RS patients, both selinexor and pembrolizumab industry sponsored studies were stopped early due to poor accrual and/or lack of efficacy and thus the impact these drugs will have on the future of RS remains unclear. Regardless, there are additional studies incorporating novel agents currently accruing RS specific cohorts (Table 3). There remains an unmet need for collaboration to design rational studies for this rare subset of patients with CLL who develop RS. New models, such as that by Vaisitti *et al.* [54], hold promise to study rational drug combinations in preclinical models that mimic human disease. Additionally, strategies of early intervention in high risk patients with novel agents are currently being explored and are in development with hopes these can impact and or eliminate transformation all together.

## Conclusion

Over the past several decades we have identified underlying risk factors for RS and have unraveled the molecular basis and evolutionary processes that lead to its development. We now understand the disease as a complex entity with both clonal and nonclonal evolutionary patterns that impact upon outcomes. There are many molecular events which are unique to RS-DLBCL with a different genomic landscape as compared with *de novo* DLBCL. The molecular and genetic differences in HVRS compared with *de novo* Hodgkin disease remain largely unknown but there remains notable differences in outcomes. The majority of transformations will lead to clonally related DLBCL and have an aggressive course with a chemorefractory nature. Both routine and novel combination chemo immunotherapies have done little to improve upon outcomes, leaving patients with few effective options. Relapsed/refractory CLL patients on ibrutinib are still at risk for RS and identify a group of patients who face grave prognosis after transformation. It remains unclear the rates of RS in treatment-naive patients on BCR antagonists or whether upfront treatment with these agents can abrogate the development of this devastating disease. Continued investigation and understanding of the molecular differences between transformed CLL and its *de novo* counterparts will hopefully provide rational targeted therapies that may eventually improve outcomes in this difficult to treat population.

## Future perspective

One of the single most important ways to impact upon the poor outcomes seen with RS is to prevent its occurrence. In the next several years we will begin to see clinical trials centered around early intervention, in other

words, treatment at diagnosis, with novel agents in patients at highest risk of transformation with the belief that early control of the CLL clone at low tumor burden levels will limit subclonal heterogeneity, a risk factor for progression on novel agents. We will continue to see the trend towards increased and earlier use of novel agents accompanied by avoidance of repeated chemotherapy cycles which in turn should impact upon the incidence rate of RS; preventing both the selection of high risk chemoresistant clones and the continuous accumulation of genomic insults stemming from chemotherapeutic genomic damage or genomic instability. It is unlikely that RS will become nonexistent and it is possible that the natural history for all CLL patients in the future will be to transform as they continue to live longer. Because of this potential, continued coordination of efforts is required to build biobanks and databases to understand the underlying risk factors and inherent biology necessary for transformation in order to rationally devise therapeutic interventions and improve outcomes upon diagnosis. Development of preclinical models will be imperative and coordinated efforts between academic centers to devise RS-specific protocols will be needed to improve outcomes for patients with RS.

### Financial & competing interests disclosure

JN Allan is a member of the Advisory Board for Pharmacyclics, Acerta, Abbvie, Sunesis, Verastem, Genentech and Bayer. RR Furman is a member of the Advisory Board for Pharmacyclics, Acerta, Abbvie, Sunesis, Verastem, Genentech and Janssen. Consultancy: Acerta, Verastem, TG Therapeutics and Janssen. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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### Author contributions

JN Allan and RR Furman wrote the manuscript.



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