Richter's transformation: transforming the clinical landscape

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ABSTRACT

Richter transformation (RT) represents an aggressive histological transformation from chronic lymphocytic leukaemia, most often to a large B cell lymphoma. It is characterised by chemo-resistance and subsequent short survival. Drug development has struggled over recent years in light of the aggressive kinetics of the disease, lack of pivotal registrational trials and relative rarity of the phenomenon. In this review we will highlight the diagnostic and therapeutic challenges of managing patients with RT as well as taking a look to the future therapeutic landscape. Highly active therapies developed across B cell malignancies are starting to impact this field, with T-cell activation therapies (CAR-T, bispecific antibodies), antibody-drug conjugates, and novel small molecule inhibitor combinations (e.g. BTKi-BCL2i) being actively studied. We will highlight the data supporting these developments and look to the studies to come to provide hope for patients suffering from this devastating disease.

Keywords: Chronic lymphocytic leukaemia, Richter's transformation, Diffuse large B cell lymphoma, Hodgkin lymphoma

1.1 CLINICAL FEATURES + EPIDEMIOLOGY

Chronic lymphocytic leukaemia (CLL) exists on a spectrum, with many low-risk patients having an indolent phenotype and not requiring treatment for many years. The most aggressive clinical sequelae of CLL is undoubtedly Richter transformation (RT), which entails transformation of underlying CLL most commonly to diffuse large B cell

lymphoma (DLBCL) but also to classical Hodgkin lymphoma (CHL) and rarely to less common lymphomas (Figure 1). Shared clonality of the RT lymphoma and preceding CLL can be demonstrated by immunoglobulin heavy chain variable region (IGHV) analysis, with clonally- related and clonally- unrelated CLL demonstrating divergent clinical outcomes (1). Prognosis following RT is particularly poor for clonally- unrelated RT-DLBCL. This specific entity usually has a dismal prognosis.

Figure 1: Richter Transformation

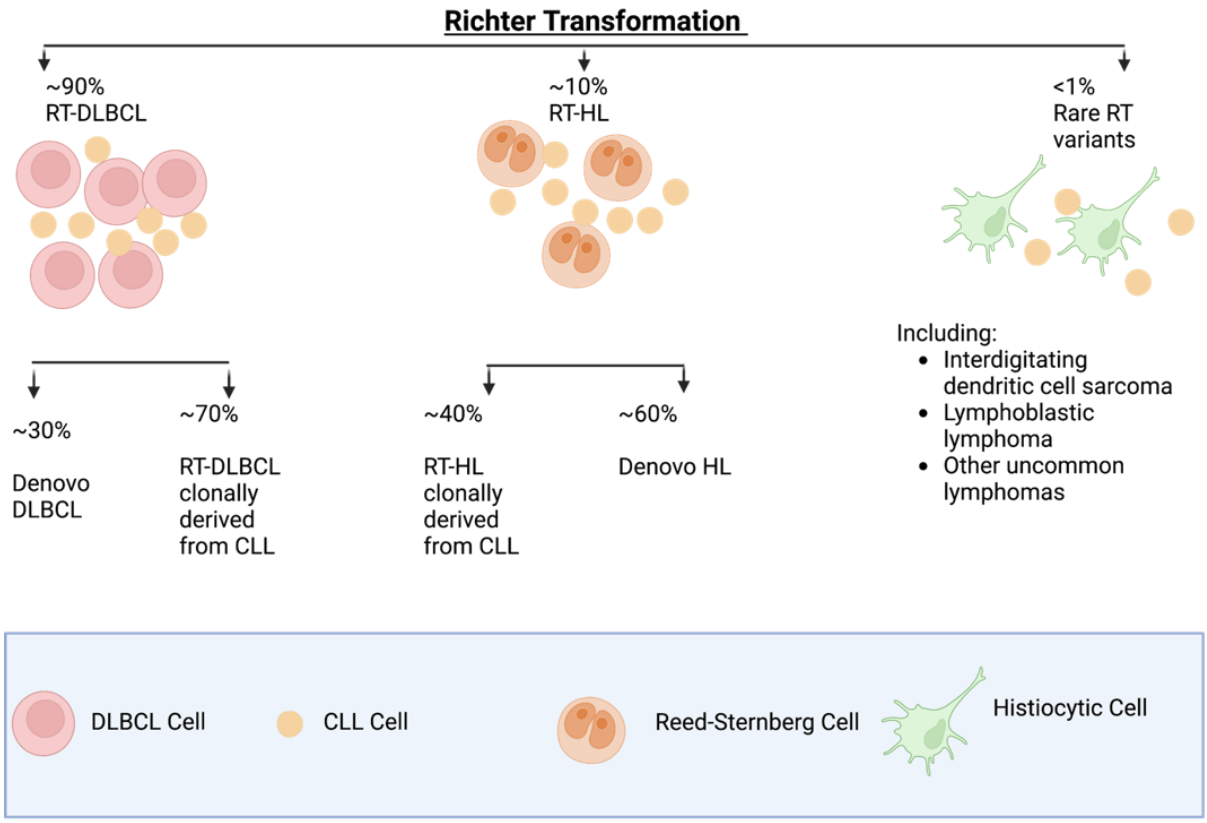

Figure 1: Richter transformation (RT) of chronic lymphocytic leukaemia (CLL). DLBCL: diffuse large B cell lymphoma. HL: Hodgkin lymphoma.

The reported prevalence of RT in CLL patients in large longitudinal analyses varies according to the cohort studied, with a rate of RT development of 0.5% seen per year per patient, which rises to 1% per year in patients requiring treatment for CLL (2). Between 70- 90% of cases share histological features with DLBCL, with the remaining cases RT-CHL (3, 4, 5, 6). There is not yet evidence for a plateau in the risk of RT over time. Time to RT-DLBCL diagnosis from CLL diagnosis is variable: it can be diagnosed concurrently with CLL as a *de novo* presentation in up to 10% of RT patients or occur many years following initial CLL diagnosis (6). The median time to transformation ranges from 23 months to 4.7 years (4, 6, 7, 8, 9). RT-CHL occurs at a median of 5.6 years from initial CLL diagnosis but can also be diagnosed at first presentation of CLL (10). Rarely other types of lymphoma such as T-cell lymphoma or plasmablastic B cell lymphoma can occur in patients with underlying CLL, although the aetiological relationship may be unclear. This manuscript will largely deal with RT-DLBCL as this

has a distinct phenotype from *de novo* DLBCL, with RT-CHL which is much more similar to its *de novo* counterpart discussed separately at the end.

1.1.1 Risk Factors for RT development

RT is more common in men and the median age at diagnosis is 61-72 years (11, 12). One-third to one-half of patients have not received treatment for CLL prior to RT diagnosis (2, 8). In one contemporaneous study, the number of prior therapies for CLL before RT ranged from 0 to 7, with a median of 1 prior therapy before the occurrence of RT (7). RT can occur in CLL patients treated with novel therapies as well as with traditional chemo-/immunotherapy approaches (13). RT is also recognised in the context of complete remission of CLL after treatment (14).

Numerous studies have attempted to model risk of RT in CLL patients. Advanced Rai/ Binet stage at diagnosis, lymph node size >3 cm, involvement of ≥ 3 nodal areas and diffuse bone marrow infiltration have been shown to predict for RT (2, 6), similarly to factors that make CLL itself high-risk. The requirement for CLL treatment also increases risk, as does lack of response to first-line treatment (5). In previously-treated CLL patients, RT is more likely to develop in those requiring therapy early in the course of CLL, with a median time to first treatment (TTFT) of CLL of 12.4 months in patients who later developed RT versus 90.2 months in those who did not illustrated in the German CLL Study Group (GCLLSG) trial (3). Molecular and genetic factors such as unmutated IGHV, NOTCH1, del(17p) and increased ZAP70 and CD38 expression also correlate with RT development (2, 3).

In summary, we believe those CLL patients at highest risk of RT to be those requiring treatment for CLL and those with biologically aggressive features of disease at diagnosis including *TP53* disruption.

1.1.2 Clinical Features

RT typically presents with rapidly enlarging lymphadenopathy in three-quarters of patients (4) with B-symptoms (weight loss, fevers, night sweats and extreme fatigue) seen in up to half of patients (14). Extra-nodal presentations can occur in a third of patients, with common sites including the gastrointestinal (GI) tract, bone marrow, pleura or central nervous system (CNS) (11, 14). Most cases of RT-DLBCL present with advanced-stage disease with high serum lactate dehydrogenase (LDH) (7); similarly most cases of RT-CHL have advanced stage disease with an international prognostic score (IPS) of at least 4 (10). Isolated cutaneous presentations of RT are recognised, with most subsequently progressing to systemic disease (15). New onset of hypercalcaemia may also indicate RT and should be investigated as such (16).

1.1.3 Association with EBV

Specific causation of RT in CLL patients is still unclear. Numerous studies have evaluated the association of Epstein-Barr virus (EBV) infection with RT, which is optimally ascertained by direct evaluation of lymphoma biopsies with highly sensitive *in situ* hybridisation to ascertain the presence of EBV non-coding viral RNA (EBER) (17). Although reported data are variable, thirty percent of cases of RT were

considered to be associated with EBV in one institutional series (18), with a higher rate seen in RT-CHL (19).

1.2 DIAGNOSIS + STAGING

1.2.1 Histological Features of RT- DLBCL

Histological confirmation of RT by expert haematopathology review remains the gold standard for diagnosis. Every effort should be made to obtain a diagnostic biopsy as this is critical for clinical management and inclusion on a clinical trial if available.

RT-DLBCL is considered where histological samples show infiltration somewhat akin to *de novo* DLBCL with large cells with centroblastic, immunoblastic or anaplastic morphology, which are typically positive for CD20 and CD79a with variable expression of BCL2, BCL6 and CD10, and often negative for CD5 and CD23 (20). Additional analyses include immunohistochemistry to determine the cell-of-origin by the Hans algorithm; including CD10, MUM1 and BCL6 staining (21). In one study, 87% of RT-DLBCL cases were of activated B cell (ABC) phenotype by Hans criteria (7). Fluorescence *in situ* hybridisation (FISH) for *c-myc* re-arrangement and the immunoglobulin partner gene, with subsequent *BCL2* re-arrangement testing if this is positive, should be considered to determine the presence of a so-called “double-hit lymphoma”, which is associated with poorer prognosis in *de novo* DLBCL cases. In one study, 12.1% of all RT-DLBCL cases were double-hit by FISH (*BCL6* re-arrangement also included, which is no longer classified as double-hit in the most recent World Health Organisation (WHO) update of haematological malignancies). However, studies have thus far have failed to demonstrate that this has a significant negative effect on survival in the context of RT, likely due to small sample sizes (9).

1.2.1.1 Differential Diagnoses

A high proliferation index alone without the present of diffuse infiltration with large B cells is not diagnostic of RT in CLL patients and care must be taken to differentiate “accelerated” CLL, where there are clinical features suspicious for high-grade transformation with biopsy demonstrating expanded proliferation centres and high mitotic rate but without infiltration of large B cells. These patients have a poorer outcome than non-accelerated CLL but an improved survival relative to RT-DLBCL and CLL-directed therapies such as Bruton tyrosine kinase inhibitors (BTKi) or BCL2 inhibitors may still have good effect (21, 22).

Care must be taken in patients receiving BTKi for CLL, where early interruption of therapy can lead to rapid lymph node growth or ‘flare’ which can mimic RT. Biopsies from lymph nodes of five patients with small lymphocytic leukaemia (SLL) who presented with increasing adenopathy following ibrutinib interruption for surgery or infection revealed infiltration with large B cells raising the suspicion of RT transformation; in all cases lymphadenopathy resolved with resumption of ibrutinib (with or without concurrent obinutuzumab administration) with subsequent biopsies showing only SLL (23). A similar phenomenon can occur with loss of response to ibrutinib manifesting as dramatic lymph node growth. Herpes simplex virus (HSV) infection in CLL patients can cause necrotic lymphadenitis which can also masquerade clinically and radiologically as RT but has characteristic histological findings (24).

1.2.2 Radiological Imaging in RT

Appropriate radiological imaging of RT patients is key for both initial staging of disease and response assessment, and positron emission tomography-CT (PET-CT) remains the gold standard for analysis of RT-DLBCL and RT-CHL. PET-CT has an important role in determining extra-nodal sites of disease as well as bone marrow involvement. Assessment of response by the Deauville scale is key for adequate assessment of remission status at the end of treatment. PET is specifically useful in suspected RT to guide an appropriate site of biopsy.

The role of PET in prediction of RT in CLL patients remains an area of clinical equipoise. Many studies have examined whether increased tumour metabolic activity as manifested radiologically by increased fluorodeoxyglucose (FDG) avidity of lesions can predict for RT. RT lesions on PET are typically significantly more FDG- avid than CLL lesions (25). One study found that FDG-avidity as expressed by a maximum standardised uptake value (SUVmax) threshold of 10 discerned between RT and CLL, with a sensitivity of 91% and specificity of 95% for diagnosis of RT (26). This may be less reliable in the era of novel agents, where an SUVmax threshold of 10 in patients treated with a BTKi or idelalisib led to 71% sensitivity and 50% specificity for diagnosis of RT (27). False positives of increased FDG avidity on PET include second primary malignancies and acute infection (28). Histological confirmation of RT diagnosis remains critically important but can be supported by radiological findings- one study demonstrated that, where RT was suspected clinically, histological and radiological findings were concordant in 32 of 34 patients leading to a negative predictive value (NPV) of 90% for radiological investigations in the work up of a patient with RT (20).

PET may also have a prognostic value in RT. A higher disease burden as determined by increased total metabolic tumour volume (TMTV) on PET appears to be predictive of inferior outcomes in RT patients (29).

We consider that PET should be used to identify an appropriate site of biopsy based on SUV max in patients who exhibit clinical features of RT, but that PET alone should not be considered diagnostic for RT. Patients with an SUV max of ≤5 effectively excludes the possibly of RT in the vast majority of patients and is a reassuring radiological feature.

1.3 MOLECULAR FEATURES

RT-DLBCL and *de novo* DLBCL have distinct molecular profiles (30, 31). Older studies report an association between specific IGHV rearrangements and risk of RT, with #subset 8 and IGHV3-49 conferring an increased risk of transformation (32). Comparison of the IGHV locus between RT and the preceding CLL revealed two patterns of shared (clonally-related) and distinct (clonally-unrelated) with divergent clinical outcomes with clonally-unrelated RT faring better, pointing to two distinct pathways to transformation (1, 33). Analysis by this method shows a clonal association of roughly three-quarters of RT with preceding CLL (7, 33), which may be an under-representation of true clonal association.

Ninety percent of RT cases share molecular lesions with the founding CLL-related clone, with mutations in *TP53* (43.3% to 80%), *CDKN2A* (30%), *MYC* (13.4%-30%), *NOTCH1* (30%-39%), or *MGA* (7%-11%) most commonly identified (1, 12, 34, 35, 36, 37). Whole genome sequencing (WGS) on synchronous circulating lymphocytes and tissue DNA from RT patients described variable relationships at transformation, including clonal expansion of subclones with mutations in DNA damage response genes and the mitogen activating protein kinase (MAPK) pathway (36). Transcriptomic evidence reports RT is less dependent on B-cell receptor signalling than CLL (30, 31), offering an explanation for the modest clinical responses seen with BTKi therapies.

Through integration of genomic and transcriptomic data from sequential CLL and RT samples, high mutational burden and complex chromosomal changes are seen including whole genome duplication and chromothripsis (30, 31). Mutational signature analysis of whole genome and whole exome datasets revealed recurrent patterns of somatic mutations including signatures associated with oxidative phosphorylation with prior therapies and reported RT-DLBCL with loss of *TP53* function (30, 31). Intriguingly, sensitive sequencing analysis of circulating neoplastic lymphocytes and plasma circulating tumour DNA (ctDNA) samples taken years prior to RT diagnosis identified small populations carrying driver mutations and transcriptional patterns of the subsequent tumour suggesting that it may be possible to identify in advance CLL patients at risk of RT (31).

Elucidating the genomic and transcriptomic events necessary for histological transformation is a rich area for future study which may improve pathways to integrated clinical, histological and molecular diagnosis and reveal therapeutic vulnerabilities.

1.4 PROGNOSTICATION

Patients with RT have a substantially inferior overall survival (OS) than CLL patients without RT, with many patients surviving only 6-12 months following the diagnosis of RT (3). The importance of enrolment on clinical trials where possible cannot be overstated. One multi-institutional series including trial- ineligible RT patients reported over half of RT patients died within three months of diagnosis (30).

A key factor determining survival likelihood in RT-DLBCL is receipt of previous CLL treatment, with improved outcomes seen in those who are treatment-naïve versus who previously required treatment of the antecedent CLL. In those who are pre-treated with traditional chemo-immunotherapy for CLL, median OS is as poor as 8-14 months with OS of 46.3-77 months if no previous treatment for CLL has been given (3, 8, 9, 38). In those with RT-DLBCL diagnosed contemporaneously with CLL, median OS may be as long as 66.9 months (9).

Clonal relatedness of RT to underlying CLL is also highly relevant. Clonally- related RT has significantly shorter OS than RT of a different clonal origin as demonstrated by IGHV analysis (median OS 14.2 versus 62.5 months, p=0.017 (1); median OS 5.4 versus 74.8 months, p=0.05 (7). Patients with clonally- unrelated RT are more likely to respond to first-line therapy for RT than those with clonally- related RT (1).

Multivariable modelling from the CLL chemo-immunotherapy era demonstrated that thrombocytopenia (<100 x10^9/L), and *TP53* aberrations, together with prior therapy for CLL, were prognostic in RT patients, with a median OS of 75.3 months if none of these factors were present (7). *TP53* disruption by mutation, or deletion of 17p is highly relevant (OS 9.4 months versus 47.1 months if *TP53* intact, p<0.001) (7); in this study a subset of patients who were *TP53* intact, had a performance score of </=1 and achieved CR to initial RT treatment had improved outcomes, with 5- year OS at 70%. Other studies in the chemo-immunotherapy era of CLL have shown that trisomy 12 and haemoglobin <10 x10^9/L were associated with poorer outcome in RT (4), as is unmutated IGHV status (39) and complex karyotype, albeit that the latter is rarely performed in a standard clinical setting (40).

Risk of RT-DLBCL or RT-CHL development appears to be neither abrogated nor precipitated by use of novel therapies such as ibrutinib, idelalisib, obinutuzumab or venetoclax (41, 42, 43, 44). Development of RT whilst undergoing treatment with the BTKi ibrutinib either in the first-line or R/R setting for CLL portends a particularly poor prognosis, with 27 patients across two series having a median OS of just 2- 3.5 months following RT diagnosis (45, 46). A small number of these patients underwent sequencing of the BTK gene, with most not having mutations conferring resistance, suggesting that RT may not occur via this mechanism in BTKi-treated patients. Factors associated with the development of RT in patients on ibrutinib include increased LDH, increased lymphadenopathy without lymphocytosis and progression as per the International Workshop on CLL (iwCLL) criteria while on treatment (47), as well as complex karyotype at time of CLL diagnosis (41).

In summary, we consider those with poorest prognosis following development of RT to be those with RT-DLBCL, with multiply pre-treated CLL prior to RT development, with clonally- related RT, those with *TP53* disruption and those with RT development on newer CLL- directed therapies.

1.5 STANDARD OF CARE TREATMENTS IN RT-DLBCL

1.5.1 First- Line Treatment

Outside the context of a clinical trial, treatment for RT-DLBCL generally involves chemo-immunotherapy in those sufficiently fit for such an approach. As noted above, outcomes following these therapies are generally improved in patients with clonally-unrelated RT in comparison to those with RT of the same clonal origin. Rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone (RCHOP) is a standard treatment approach. In retrospective analyses, RCHOP- like treatments led to a CR rate of 30-45% (4, 7, 48) but this rarely translates to prolonged survival. Overall response rates (ORR) are better with this approach in patients previously untreated for CLL, with rates of 71% in this group versus 40% with pre-treated patients (48). Response rates with intensive second line therapies after failure to respond to initial therapy are dismal, with just 8% achieving a complete response (CR) in this setting.

RCHOP has been assessed in a prospective clinical trial in RT-DLBCL (49). The median OS was 21 months in the RT cohort (n=15, including many with multiple prior lines of therapy for CLL) with a median progression-free survival (PFS) of 10 months.

Improved response rates were seen in patients with a longer time from CLL diagnosis to RT.

Addition of etoposide to RCHOP (R-EPOCH) was not demonstrably superior to RCHOP, with a median PFS of just 3.5 months, a high non-relapse mortality and a median OS of 5.9 months in a cohort of 46 patients (40). A complex CLL karyotype was significantly associated with poorer outcomes for these patients.

A phase II study investigated the efficacy and safety of ofatumumab, an alternate anti-CD20 antibody, combined with standard CHOP followed by 12 months of ofatumumab maintenance (50). Thirty-seven patients, of whom greater than 50% had previously received fludarabine and cyclophosphamide-based treatment, had an ORR of 46% with a CR rate of 27%. The median PFS was 6.2 months with a median OS of 11.4 months. Patients with treatment- naïve CLL had improved outcomes. Overall this approach was not deemed to be superior to historical outcomes observed with RCHOP.

In summary, where enrolment in a clinical trial is not possible, RCHOP is a commonly chosen and tolerable first-line regimen for management of RT-DLBCL which delivers some efficacy, especially patients with *TP53*-wild type (wt) RT which is clonally-unrelated to the underlying CLL.

Platinum agent and cytarabine- based regimens such as R-DHAP/ R-ESHAP used in the relapsed or refractory (R/R) setting in RT are associated with poor outcomes (51).

1.5.2 Transplantation

Consolidation with an autologous or allogeneic SCT is often considered for RT patients to increase the likelihood of long- term disease control. In one study, the median survival of all patients undergoing autologous or allogeneic SCT for RT was 55.4 months (9). Allogeneic SCT has been shown to be significantly associated with improved survival following RT-DLBCL diagnosis in multivariate analysis (p=0.027) (1). However, given the inherent chemoresistance of RT, only a small minority of patients proceed to transplant. One study reported 14% of patients successfully proceeded to SCT after first-line therapy, due to failure to achieve response to therapy in 31% of patients and comorbidities precluding SCT for the same proportion of patients (2).

A European Society for Bone Marrow Transplantation (EBMT) retrospective analysis of patients undergoing SCT from 2012 revealed that autologous SCTs were most often performed for patients achieving a CR or partial remission (PR), with allogeneic SCT performed for patients in PR or progressive disease (PD) (52). Transplant-related mortality (TRM) was increased in the allogeneic SCT group at 26% after three years versus 12% in the autologous SCT group. Improved OS was also seen after autologous SCT (59% at 3 years versus 36% with allogeneic SCT), although this is likely impacted by the disease status at time of transplantation. Adequate disease control at time of transplantation is clearly preferred, with one study reporting a significant difference in survival in patients undergoing allogeneic SCT after CR/ PR (75% OS at 3 years) versus R/R RT patients undergoing salvage allogeneic SCT (21% OS at 3 years) (12).

More recently a large retrospective analysis described similar outcomes, with a 3-year PFS of 48% with OS of 57% following autologous SCT but an improved 3-year PFS of 43% and OS of 52% seen following allogeneic SCT for RT (53). In line with the earlier study, a greater proportion of autologous SCT recipients were in CR and fewer had 17p deletion, highlighting that allogeneic SCT is often reserved for patients with a higher risk profile. Greater than or equal to three prior lines of therapy, increased age and increased comorbidities as defined by a haematopoietic SCT (HSCT) comorbidity index of >1 have also been shown to correlate with poorer outcomes following allogeneic SCT (54).

The question of for whom autologous versus allogeneic SCT is indicated remains unanswered. Based on multivariate analysis, patients were stratified into low, intermediate or high risk for death based on *TP53* disruption, response to induction treatment and performance score (1), with all patients with *TP53*wt status and a good performance score with response to first-line treatment still alive at 24 months following diagnosis. Survival significantly declines if any of these adverse features are present. British Society for Haematology (BSH) guidance suggests that SCT can be deferred in patients who are *TP53* intact with complete metabolic response (CMR) to first- line RT treatment (55). We recommend that consolidation with autologous or allogeneic SCT in first response should be considered in patients with *TP53* disruption or RT exhibiting clonal relatedness to underlying CLL. Allogeneic SCT is likely to offer the best chance of cure in those sufficiently fit for such an approach with a suitable donor.

Relapse is unfortunately still common even after allogeneic SCT for RT (56). Long term survival expectations following relapse at this point are very low. One study identified 20 patients with relapse post-allogeneic SCT- two-thirds of patients with eventual relapse had experienced initial disease response (57). Treatment approaches following disease progression were heterogenous- 25% of patients received rituximab- containing regimens, 25% received immunomodulatory agents (lenalidomide or thalidomide), half received donor lymphocyte infusions (DLI) and almost all received conventional chemotherapy the most common regimen of which was R-hyperCVAD (rituximab, cyclophosphamide, dexamethasone, doxorubicin, vincristine and cytarabine). The 2-year OS rate was 36% and no patients survived beyond 5 years, including 2 who received a second allogeneic SCT. This highlights the dismal prognosis experienced following relapse post-allogeneic SCT for RT patients, for whom new treatment approaches are urgently required.

A proposed algorithm for management of patients with RT DLBCL is outlined in figure 2.

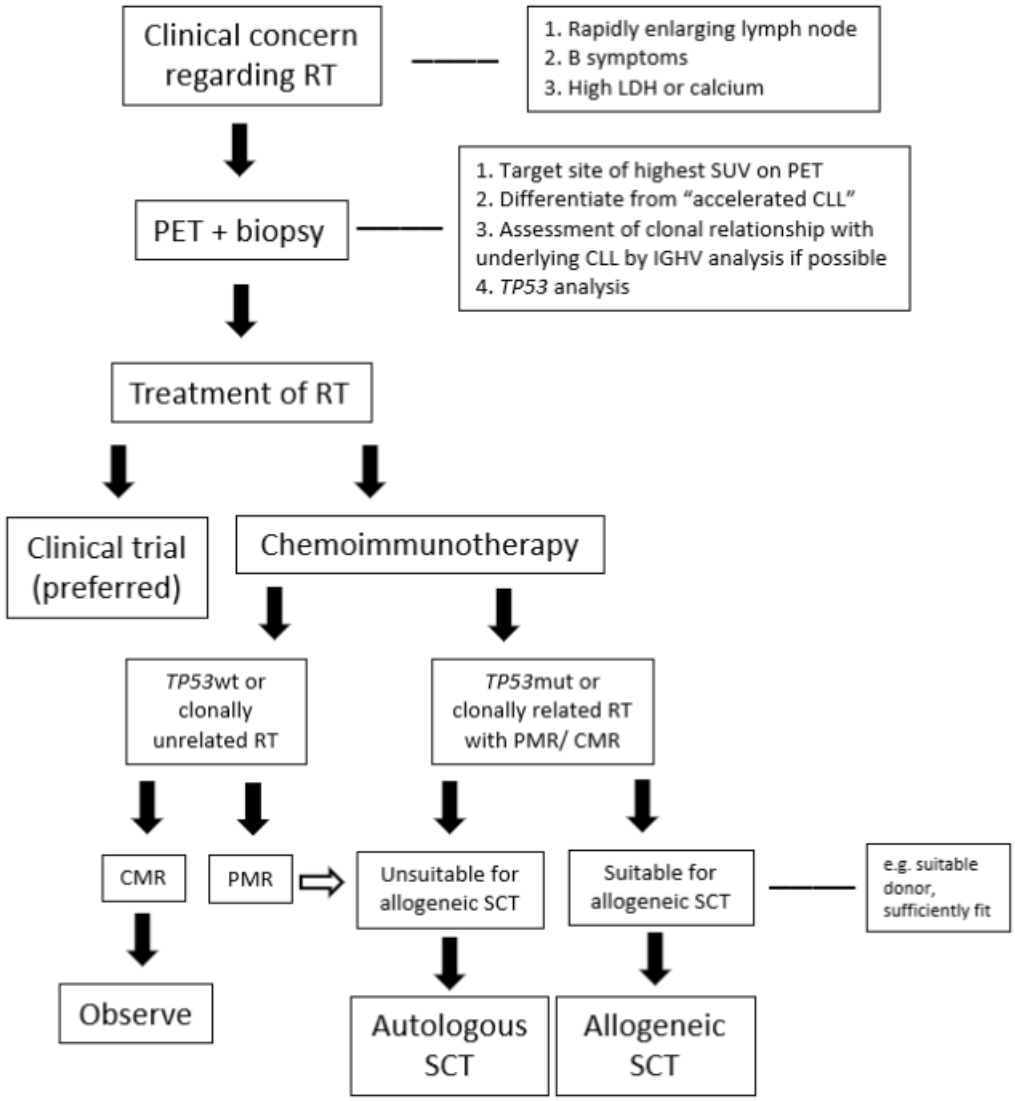

Figure 2: Algorithm for diagnosis and front-line treatment of suspected RT-DLBCL.

1.6 NEW APPROACHES

1.6.1 Chimeric Antigen Receptor Therapy (CAR-T)

The success of anti-CD19 CAR-T cell therapies is well established in *de novo* DLBCL and other (R/R) B cell lymphomas but very few data are available in the context of RT. A retrospective analysis from the DESCAR-T registry reported 12 patients infused with either Axi-cel (5 patients) or Tisa-cel (7 patients) (58). The ORR was 50% with a CR rate of 42% but with higher frequency of CAR-T cell- related toxicity as compared to *de novo* DLBCL. In a single centre study, 8 of 9 RT patients treated with Axi-cel achieved a response, with a CR rate of 55% but the median follow-up was only 6 months (59). Similar results were achieved with anti-CD19 ARI-0001 CAR-T cells, with an ORR and CR rate of 60% in 6 RT patients (60). Another case series reported 5 RT patients treated with JCAR017 CAR-T cells with a CR rate of 40% and ORR of 60% (61). Median follow-up of these studies ranges from 1.6 to 6 months, and therefore it

remains unclear if CAR-T represents an opportunity for long-term remission similarly to *de novo* DLBCL. Combination of CAR-T with other novel agents may contribute to more durable responses and is in the author's opinion the most exciting line of future research in RT-DLBCL, as discussed below.

1.6.2 Targeted Therapies in RT-DLBCL

Targeted therapies are firmly established as the mainstay of CLL therapy but have been adopted more variably across the spectrum of other B-cell malignancies according to disease-specific efficacy. The key unmet need for patients diagnosed with RT are the poor survival outcomes with standard chemoimmunotherapy approaches principally due to R/R disease. This is likely due at least in part to the genomic complexity of the disease resulting in chemotherapy resistance and rapid clonal evolution. Lastly, the disease rarity and clinical aggressiveness has greatly limited the delivery of prospective studies of novel therapies and lack of randomised control trials. Well-designed, flexible, collaborative study protocols are urgently needed to address these challenges.

An outline of outcomes from prospective trials of novel therapies in RT-DLBCL is shown in Table 1. Numbers of patients enrolled in most studies is small, highlighting the difficulty of provision of clinical trials in this rare disease. Recruitment is optimal where there are high numbers of participating centres, such as in the BRUIN trial (62) which had 56 international sites.

Trial	Investigational Agent	Number of patients enrolled	Outcomes
Single-Agent BTKi, BCL2i and CPI			
ACE-CL-001 (63)	Acalabrutinib	25	ORR 40% PFS 3.2 months
BGB-3111-AU-003 (64)	Zanubrutinib	13	CR 61.5% PFS 17.3 months
BRUIN (62)	Pirtobrutinib	57	ORR 54% OS 13.1 months
BELLWAVE (65)	Nemtabrutinib	6	PR 16.7%
Davids et al (66)	Venetoclax	7	PR 43%
KEYNOTE-170 (67) (R/R)	Pembrolizumab	23	ORR 4.8%
Bispecific Antibodies and Antibody-Drug Conjugates			
BLINART (68)	Blinatumomab	41	ORR 36%
Hutchings et al (69) (R/R)	Glofitamab	12	ORR 50%
EPCORE CLL-1 (70)	Epcoritamab	10	CR 50%
WAVELINE-001 (71)	Zilovertamab vedotin	7	ORR 57% OS 24 months
Combination Therapies			
Davids et al (72) (first- line)	Venetoclax + DA-R-EPOCH	26	CR 50% OS 19.6 months

Lopez et al (73) (first- line)	Ibrutinib + Obinutuzumab + CHOP	3	CR 33.3% OS 29.1 months
Ryan et al (74)	Duvelisib + Venetoclax	8	CR 37.5%
Younes et al (75) (R/R)	Ibrutinib + Nivolumab	20	ORR 65%
Jain et al (76)	Ibrutinib + Nivolumab	24	ORR 42% OS 13 months
BGB-3111-A317-001 (64)	Zanubrutinib + tislelizumab	7	ORR 42.9%
Shouse et al (77) (R/R)	Copanlisib + Nivolumab	13	ORR 27%
MOLTO (78) (first-line)	Atezolizumab + Venetoclax + Obinutuzumab	28	CR 28.6% OS 31.6 months
Jain et al (79)	Atezolizumab + Venetoclax + Obinutuzumab	8	ORR 87.5%
Heyman et al (80)	Obinutuzumab + Lenalidomide + Methylprednisolone	7	ORR 43%

Table 1: Prospective data regarding novel therapies in RT-DLBCL. Trials conducted in mixed treatment-naïve and R/R RT patients unless otherwise specified. PFS/ OS reported as median values.

BTKi: Bruton tyrosine kinase inhibitors. BCL2i: BCL2 inhibitors. CPI: checkpoint inhibitors. R/R: relapsed/ refractory. ORR: overall response rate. PFS: progression-free survival. CR: complete response. PR: partial response. OS: overall survival.

1.6.2.1 Single Agent BTK and BCL-2 Inhibitors

Although BTK inhibitors have demonstrable activity in treating RT, their efficacy is limited by lack of response durability. Early evidence of their activity and tolerability in RT was reported in a small retrospective case series of the first-in-class covalent BTKi ibrutinib with three of four patients benefitting, but the median response duration was only 6.1 months (81). Subsequently, 25 patients enrolled within a RT cohort of the ACE-001 protocol showed an encouraging ORR of 40% but a short median PFS at 3.2 months (63). More recently, a phase 1/2 study reported data on 13 patients (both treatment-naïve and R/R) treated with the 2nd-generation BTKi, zanubrutinib, for RT with an ORR of 62% and encouraging median PFS and OS at 17.3 and 29.3 months, respectively (64). Emerging data on the non-covalent BTKi molecules - pirtobrutinib and nemtabrutinib - have been recently reported. In the phase I/II BRUIN study, 57 patients in the RT cohort were treated with pirtobrutinib conferring an ORR of 52% and median PFS and OS of 3.7 months and 13.1 months respectively (62). In the BELLWAVE study, nemtabrutinib achieved an ORR of 50% in a small number of patients with RT but no data are yet available on response durability (65).

Available data on single-agent BCL2 inhibition in RT are very limited but - similar to the BTKi monotherapy outcomes - venetoclax, a first in class BCL-2 inhibitor, appears to confer short lived activity as single agent in RT (ORR 42%) (66).

1.6.2.2 Checkpoint Inhibitors

Programmed death (PD)-1 receptor blockade with the monoclonal antibody pembrolizumab has been investigated in RT patients, informed by high expression levels of the PD1 receptor on the RT cells. The activity of pembrolizumab in RT-DLBCL and RT-CHL was disappointing in the phase 2 KEYNOTE-170 study with an ORR of 23% and median PFS and OS of 1.6 months and 3.8 months respectively (67). Given the lack of significant activity of CPI in *de novo* DLBCL, together with the additional consideration of immune dysfunction in CLL, the role of CPI in RT is likely to be limited but combination approaches are currently being assessed in clinical trials.

1.6.2.3 Bispecific Antibodies and Antibody Drug Conjugates

Bispecific T-cell-engaging molecules have been investigated in the treatment of RT. Blinatumomab is a short peptide with CD19/CD3 binding domains, established in acute lymphoblastic leukaemia (ALL) therapy, investigated in the phase II BLINART study where blinatumomab was initiated in 25 patients with RT who failed to achieve CR after two cycles of R-CHOP (68). The ORR was 36% with a CR rate of 20% in evaluable patients but PFS and OS were not specifically reported for the blinatumomab cohort. Single- agent blinatumomab was assessed in a separate phase II study with an ORR of 22% and a median OS of 10.3 months (82). Glofitamab is a full length IgG CD20:CD3 bispecific antibody (BsAb) investigated in a first in human, phase 1 study including 10 RT patients, of which 3 of 6 evaluable patients manifested a response (ORR 50%) (69). Epcoritamab is another CD20/CD3 BsAb which has been assessed in a phase 1b/II EPCORE-1 study (70). Of 10 RT patients treated, 6 responded including 5 who attained a CR with treatment. To date, the reported follow-up is short, but the preliminary data on the CD20/CD3 BsAbs is promising and warrants further study in RT.

Zilovertamab vedotin (VLS-101) is an antibody-drug conjugate (ADC) with a monomethyl auristatin E (MMAE)-payload, that targets receptor tyrosine kinase-like orphan receptor (ROR)-1 which is expressed on CLL and RT cells but not normal cells. The WAVELINE-001 study reported seven patients with RT treated with VLS-101, with an ORR of 57% and median DOR of 2.8 months (71).

1.6.2.4 Novel Combinations

Evaluation of single-agent therapies clearly suggest that durability of response in RT is difficult to achieve. Novel combination studies are clearly warranted in RT. Combination trials currently recruiting are shown in Table 1.

<u>1.6.2.4.1 Chemoimmunotherapy in combination with novel therapies:</u>

This approach has been assessed in number of clinical trials. Venetoclax in combination with R-EPOCH has been studied in 26 patients of whom only 20 were able to receive the combination (72). The CR rate was 65% with a median PFS and OS of 10.1 months and 19.6 months respectively. Nine patients subsequently received

cellular therapy and 11 patients received maintenance venetoclax. The protocol has subsequently been modified to use R-CHOP as the chemotherapy backbone in view of the infection-related toxicity observed. The STELLAR trial is a randomised controlled phase II study comparing combination of acalabrutinib and R-CHOP vs R-CHOP alone; this study is still recruiting (83). A small study (N=3) has reported combination of CHOP with obinutuzumab and ibrutinib followed by allogeneic SCT in 2 patients (73). This approach is likely most useful as bridging therapy to allogeneic SCT. A study using the CD79b-targeted ADC polatuzumab vedotin in combination with infusional R-EPOCH is currently recruiting (ClinicalTrials.gov identifier; NCT04679012).

1.6.2.4.2 Combination of small molecule inhibitors and monoclonal antibodies:

Various combinations of targeted drugs and monoclonal antibodies are under investigation for RT. Duvelisib (PI3Kγ/δ inhibitor) in combination with venetoclax has been assessed in a phase I/II study; 4/8 patients responded with 2 patients achieving CR and further receiving cellular therapy (74).

1.6.2.4.3 Combination of targeted drugs with CPI/ immunomodulatory drugs:

This approach has gained interest due to enhanced anti-tumor activity reported in the pre-clinical models. Ibrutinib in combination with the PD-1 inhibitor nivolumab was tested in 20 R/R RT patients resulting in ORR of 65% and median PFS of only 5 months (75). This combination was tested in a phase II study incorporating both treatment-naïve and R/R RT patients. Median OS was 13 months but the median OS for 14 treatment-naïve patients as 24.1 months as compared to 9.1 months for R/R patients (76). Zanubrutinib with the PD-1 inhibitor tislelizumab was assessed in 7 R/R RT patients with an ORR of 42.3% (64). A trial of the pan-phosphoinositide-3-kinase (PI3K) inhibitor copanlisib in combination with nivolumab recruited 13 RT patients reporting an ORR of 27% (77). The MOLTO study explored the combination of the PD-L1 inhibitor atezolizumab with obinutuzumab and venetoclax in a multicentre phase 2 study for treatment-naïve RT patients (78). The median duration of response (DOR), PFS and OS were 11.7, 16.2 and 31.6 months respectively. A third of responders were still in remission after 2 years. Another small phase 2 study of 8 patients using a similar combination reported a median PFS of 13 months (79). A trial using the combination of lenalidomide with obinutuzumab and high dose methylprednisolone reported outcomes in 7 patients (80). The ORR was 43% and 3 patients proceeded to receive stem cell transplantation.

Several new classes of drugs including BTK degraders, CDK9 inhibitors, novel BCL-2 inhibitors, triplet combinations, novel bispecific antibodies and CAR-T cell constructs are being assessed in clinical trials (Table 2).

Treatment	**Target**	**Reference**
Acalabrutinib+R-CHOP	BTKi, CIT	NCT03899337
Ibrutinib+Obinutuzumab+ Venetoclax	BTKi, CD20mAb, BCL-2i	NCT04939363

Pirtobrutinib+Obinutuzumab+Venetoclax	BTKi, CD20mAb, BCL-2i	NCT05536349
Zanubrutinib+Tislelizumab	BTKi, PD-1i	NCT04271956
ALX148+Rituximab+ Lenalidomide	CD47mAb, CD20mAb, IMD	NCT05025800
Durvalumab+Acalabrutinib+Venetoclax	PD-L1i, BTKi, BCL-2i	NCT05388006
Lisocel+Nivolumab+Ibrutinib	CD19 CAR-T cells, PD-1i, BTKi	NCT05672173
Lisocel+Zanubrutinib	CD19 CAR-T cells, BTKi	NCT05873712
TG1801+Ublituximab	CD47/CD19, CD20 mAb	NCT04806035
VIP152	CDK9i	NCT04978779
BGB-16673	BTK degrader	NCT 05006716
NX-5948	BTK degrader	NCT05131022
PRT2527	CDK9i	NCT05665530
LP118	BCL-2i, BCL-XLi	NCT04771572
DTRM-555	BTKi, mTORi, IMD	NCT04305444
Plamotamab	Anti-CD3/Anti-CD20 BsAb	NCT02924402
Brexu-cel	CD19 CAR-T cells	NCT05537766
DZD8586	BTKi, LYNi	NCT05824585

Table 2: Ongoing clinical trials for Richter's transformation. RCHOP: rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone.

1.6.3 New Approaches to Molecular Profiling

Current genomic tools rely on high quality DNA obtained from fresh frozen tissue and present a barrier to dissecting heterogeneity and evaluating precision therapy for high-grade B-cell lymphoma (84). Highly sensitive sequencing of ctDNA promises to allow representative detection of genomic features across nodal disease. Quantification of ctDNA burden and dynamic changes in ctDNA is an emerging biomarker in lymphoma (85) but few studies have examined ctDNA in RT.

Radiological staging of RT is typically based on the identification of nodal disease and may underestimate the disease burden of marrow and circulating neoplastic cells. In this context, the ctDNA which captures neoplastic mutations from all disease compartments might provide a more comprehensive assessment of disease burden.

Increasing baseline ctDNA burden, measured in haploid human genome equivalents per millilitre (HGE/ml) correlates with clinical stage, International Prognostic Index (IPI) and TMTV by PET-CT in DLBCL (86, 87) and IPI in other nodal lymphomas (88, 89, 90). Quantification of ctDNA using droplet digital polymerase chain reaction (PCR) has been shown to correlate with TMTV (91). In DLBCL, pre-treatment ctDNA burden (HGE/ml) predicts clinical response to first- line chemoimmunotherapy (86, 87, 92).

Mutations and copy number alterations (CNA) found in the RT tumour have been detected in plasma ctDNA. In individual patients, the ctDNA changes anticipated the

histological diagnosis of RT (30, 93). Using a seven-gene targeted panel, Yeh *et al* showed ctDNA detects additional mutations compared to circulating leukocytes in R/R CLL and detection of new mutations in ctDNA anticipates high-grade transformation (93). RT copy number gains and losses are detectable in the ctDNA by shallow WGS (30, 93).

Fifteen RT patients were treated with ibrutinib and nivolumab on the LYM1002 trial. RT patients with both high and low baseline ctDNA did poorly (median PFS 3.1 vs 8.8 months, p = 0.235) and the numerical difference did not reach significance (3.3 vs 8 months, p 0.07) (94). Mutations in ctDNA affecting *TP53* were detected in 47% of RT and persisted throughout treatment. In the same study, follicular lymphoma (FL) and DLBCL with *TP53* mutations in ctDNA experienced shorter PFS, emphasising that mutations detectable in ctDNA only are clinically important.

Small studies have explored the detection of *BTK* and *PLCG2* treatment resistance mutations in ctDNA and yielded conflicting results. Plasma ctDNA detected additional mutations compared to PB-CLL in one study (95) but failed to detect *BTK* mutations in another (96).

Studying sequential ctDNA samples using a seven gene panel, Yeh *et al* reported that ctDNA, quantified as mutant allele fractions correlated with the extent of nodal disease, not lymphocytosis in CLL (93). An increased lymphocyte count is observed in the early weeks of Bruton tyrosine kinase inhibitor (BTKi) treatment, reflecting redistribution of neoplastic B-cells from the lymph nodes and BM into the circulation. Despite increasing lymphocytosis, ctDNA levels declined in ibrutinib-treated CLL, suggesting that dynamic changes in ctDNA might be suitable tool for monitoring in patients treated with a BTKi.

The potential impact of baseline ctDNA burden on CLL clinical outcomes has not been reported, although is anticipated for the CLL2-BAAG trial (97).

While sequencing of ctDNA has generated exciting results in the context of translational research and clinical trials, further work is needed to establish clinical utility of ctDNA analysis in routine practice. International efforts to standardise laboratory assays, harmonise bioinformatics pipelines and develop a roadmap for clinical applications have commenced with the publication of consensus guidelines for use of ctDNA in lymphoma (98). Although it is exciting to speculate on the potential role of ctDNA profiles in establishing a diagnosis or RT and in distinguishing RT from accelerated CLL, the sensitivity and specificity of this approach requires evaluation, ideally in the context of a prospective clinical trial.

1.7 RT-CHL

RT-CHL is diagnosed with pathognomonic Hodgkin Reed/ Sternberg (HRS) cells seen in an inflammatory infiltrate; often this is seen on an excised lymph node where CLL is also present (19). HRS cells are positive for CD15, CD30 and PAX5, with variable positivity for CD20 exhibited. Outcomes with RT-CHL are generally better than in RT-DLBCL, with a median OS between 2.5 and 3.3 years demonstrated in analyses (7, 99).

Data regarding specifically RT-CHL has been reported in CLL patients on ibrutinib affecting 3 of 165 (1.8%) ibrutinib-treated patients in one series, with one of these three having sustained survival following chemotherapy induction and allogeneic stem cell transplant (SCT) consolidation (100).

1.7.1 Treatment of RT-CHL

Treatment of RT-CHL typically follows the same treatment algorithms as for *de novo* CHL. ABVD (doxorubicin, vinblastine, dacarbazine and bleomycin) is often employed and follows the same treatment paradigms as per *de novo* CHL, with PET at interim enabling escalation or de-escalation of treatment as appropriate, albeit with a lack of evidence to support this approach in RT-CHL specifically. CR rates following ABVD are 67-71% (4, 7) and OS following ABVD may be as long as 13.2 years (44). Following treatment with a variety of regimens including ABVD, first-line brentuximab vedotin (BV) and RCHOP, 50% of RT-CHL patients had no PFS event at 2 years following diagnosis (44). The observed discrepancy in outcomes from *de novo* CHL may be explained by the higher age of RT-CHL patients, by comparison to the bimodal age distribution of *de novo* CHL, with advanced age predicting for poor survival in studies (19). Low haemoglobin, increased LDH, IPS of ≥4 and a performance score of ≥3 are also predictive for poor survival in univariate analysis (10). R/R disease is treated with salvage chemotherapy and consolidation with an autologous SCT, with newer therapies such as BV and checkpoint inhibitors (CPI) used where available; data supporting this approach in RT-CHL specifically are sparse (101, 102).

1.8 CONCLUSION

In conclusion, the recent rapid development across haemato-oncology has started to impact RT management. Promising targets and combination studies have been described. Many of these agents are very well tolerated and lend themselves to rationale combination studies. Precision medicine, ongoing clinical and pre-planned correlative biological embedded studies, and engagement of the pharmaceutical industry leading to registrational trials are necessary to give hope to our patients with RT.

PRACTICE POINTS

- RT- DLBCL has a dismal prognosis, albeit slightly improved in treatment- naïve CLL patients.
- 50-80% of RT is clonally related to underlying CLL, with lack of clonal relationship predicting for improved clinical outcome.
- PET-CT is useful to determine a site of biopsy but is neither sufficiently sensitive nor sufficiently specific to diagnose RT in the immunotherapy treatment era of CLL.
- Standard chemo-immunotherapy approaches lead to poor survival in RT-DLBCL and transplant should be considered in eligible patients. The role of CAR-T in RT-DLBCL is still to be elucidated.
- Novel therapeutic approaches in RT-DLBCL include BTK and BCL-2 inhibitors, bispecific antibodies and antibody- drug conjugates with combination therapy approaches hoped to improve future outcomes for this aggressive disease.

RESEARCH AGENDA

- Investigation of non- chemotherapy-based novel agent studies in first-line management of RT.
- Use of ctDNA to prognosticate and allow for treatment- adapted approaches in RT.
- Assessment of biological differences between RT in the chemo-immunotherapy and novel therapy eras of CLL.

FUTURE CONSIDERATIONS

- Registration- quality clinical trials with access to novel agents with involvement of multiple centres to optimise patient recruitment.
- Exploratory analyses of novel prognostic and predictive biomarkers.

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Author Contributions

AB, NA, HD, CPF, TM and TAE contributed to writing the original draft.

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TAE conceptualised the manuscript.

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