

Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common B-cell non-Hodgkin Lymphoma and remains incurable in ~40% of patients. Coding-genome sequencing efforts identified several genes/pathways altered in this disease, including new potential therapeutic targets. However, the non-coding genome of DLBCL remains unexplored. This study was aimed at identifying functionally relevant non-coding mutations targeting relevant regulatory domains such as enhancers (Es) and super-enhancers (SEs).

Methods: We integrated E/SE identification by ChIP-seq analysis of H3K27 acetylation, a histone mark that decorates active regulatory domains, with whole genome sequencing (WGS) and RNA-seq analysis in 29 DLBCL cell lines representative of the major DLBCL subtypes, along with normal germinal center (GC) B cells representing the normal counterpart of DLBCL. Ad-hoc bioinformatics analysis of WGS data from an extension panel of 93 normal/tumor DLBCL biopsies was then used to nominate recurrently mutated E/SE regions that were functionally dissected for their specific effect on gene transcription.

Results: We found that active SEs are highly and specifically hypermutated (~3 somatic mutations/Kb) in 97% (118/122) of DLBCL samples analyzed, including cell lines and primary cases, as compared to the same loci when not active as SE, and to the rest of the genome (total 135 hypermutated SEs, with at least 2/case). Such aberrant somatic hypermutation (ASHM) involves both intra-genic and inter-genic SEs, displays signatures of Activation Induced Deaminase (AID) activity, and is linked to genes encoding B cell developmental regulators and oncogenes. In order to identify functional consequences of SE ASHM, we explored the SEs linked to the BCL6 and BCL2 loci, which were among the most frequently hypermutated in our panel and have known oncogenic relevance. In both regions, we identified recurrent ASHM hotspots that were not mutated in normal GC B cells, and were shown to prevent the binding of specific transcriptional repressors. Correction of selected mutations using the CRISPR/Cas9 technology induced restoration of repressor DNA binding as well as of target gene transcriptional regulation, and led to counter-selection of the corrected alleles, indicating dependency from the ASHM mutations for tumor cell survival.

Conclusions: These data identify a highly pervasive mutational mechanism involving regulatory chromatin domains in DLBCL. These findings: i) reveal a new major set of genetic lesions deregulating gene expression, including known oncogenes, likely representing an important mechanism in DLBCL pathogenesis; ii) expand the involvement of known oncogenes in DLBCL pathogenesis and identify new deregulated gene targets that may represent candidate therapeutic targets.

Keywords: Genomics, Epigenomics, and Other -Omics, Aggressive B-cell non-Hodgkin lymphoma, Indolent non-Hodgkin lymphoma

No conflicts of interests pertinent to the abstract.

005 | EARLY POSITRON EMISSION TOMOGRAPHY RESPONSE-ADAPTED TREATMENT IN LOCALIZED DIFFUSE LARGE B-CELL LYMPHOMA (AAIPI=0) : RESULTS OF THE PHASE 3 LYSA LNH 09-1B TRIAL

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Purpose: R-CHOP is the standard treatment of diffuse large B-cell lymphoma (DLBCL). In localized disease, the FLYER trial (Poeschel *et al.*, Lancet, 2019) showed that 4 cycles of R-CHOP-21 could be as effective as 6 cycles in the younger population. Several studies have shown that response assessment by early positron emission tomography (ePET) was effective to drive treatment for patients (pts) with DLBCL and aalPI score ≥ 1 (Casasnovas *et al.*, Blood, 2017). In the LNH09-1B trial, using ePET response at central review, we assessed whether 4 cycles of R-CHOP were non-inferior to 6 cycles of R-CHOP in a limited stage DLBCL patients with favorable prognosis.

Methods: We performed a randomized trial to evaluate treatment adaptation on the basis of ePET after 2 cycles of R-CHOP-21 in previously untreated stage I-II DLBCL pts, aged 18-80 with aalPI=0. The standard arm consisted of 6 cycles of R-CHOP-21, regardless of ePET results. In the experimental arm, ePET-negative pts received 4 cycles of R-CHOP-21 only, whereas ePET-positive patients received 6 cycles, if second PET performed after 4 cycles showed a complete metabolic response (defined by a Deauville score ≤ 3). Progression-free survival (PFS) was the primary endpoint. Intention-to-treat (ITT) analysis was used.

Results: From December 2010 to May 2017, we enrolled 650 pts, 331 and 319 in the standard and experimental arms respectively, in 74 centers in France and Belgium. Forty-four percent of pts were older than 59 yrs, 4% had a bulky disease (>10 cm), 53% had extranodal disease (head and neck: 51%, digestive tract: 17%, testis:

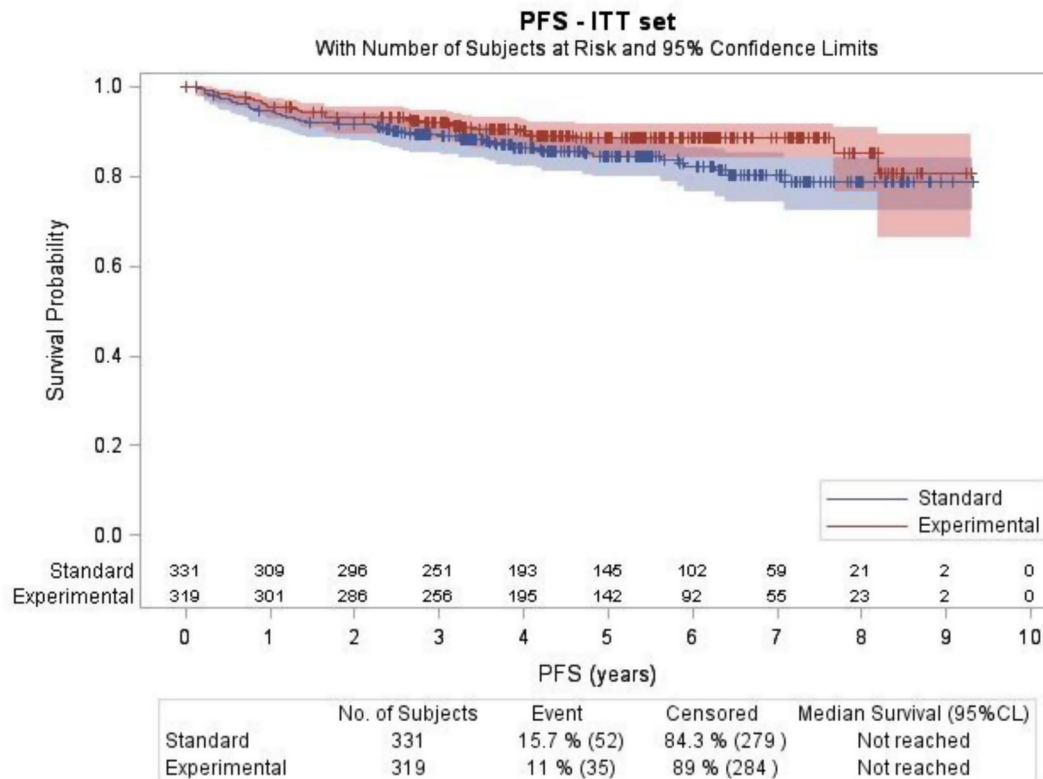


FIGURE 1 Progression free survival in the ITT population

7%, others: 25%). A central histological review was performed for 90.5% of the cases.

With a median follow-up of 5.1 years (IQR: 3.5-6.5), 87 patients (13.4%) had a PFS event. The 3-yr PFS was 89.2% (95% CI 85.3-92.2) in the standard arm and 92.0% (95% CI 88.3-94.5) in the experimental arm. The non-inferiority of the experimental arm versus the standard arm was demonstrated (hazard ratio 0.724, 90% CI 0.504-1.040, p value from Com-Nougue test <0.0001).

Superiority of experimental arm was also tested but was not observed (one-sided stratified log-rank p value=0.0702).

Toxic deaths were very rare, 0 and 1 in the experimental and standard arms, respectively.

Sixty-nine patients relapsed with median time of 25.9 months (range, 4.8 to 75.7), suggesting that late relapses may occur.

Conclusion: This study demonstrates a non-inferiority of 4 cycles of R-CHOP versus 6 R-CHOP for early good responders, confirming that 4 R-CHOP could be the new standard of care of the large majority of limited stage DLBCL patients. Occurrence of late relapses shows the need for long-term follow-up for all pts, even if outcome is very good in this population.

Keywords: Aggressive B-cell non-Hodgkin lymphoma, Chemotherapy

No conflicts of interests pertinent to the abstract.

006 | DETERMINANTS OF RESISTANCE TO ENGINEERED T-CELL THERAPIES TARGETING CD19 IN LYMPHOMA

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Introduction: Anti-CD19 chimeric antigen receptor (CAR19) T-cells have activity in patients with relapsed/refractory large B-cell lymphoma (rRLBCL), but over half of patients ultimately relapse. We applied cell-free DNA (cfDNA) analysis to patients receiving Axicabtagene Ciloleucef (axi-cel) to identify determinants of resistance and characterize molecular thresholds predictive of treatment failure.

Methods: We developed a novel hybrid-capture approach allowing evaluation of both circulating tumor-derived DNA (ctDNA) and CAR19-derived cfDNA (Fig 1A). We applied this to 381 plasma,