A bioinformatics perspective on molecular classification of diffuse large B-cell lymphoma

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INTRODUCTION

The wide range in presentation, treatment response and outcome of diffuse large B-cell lymphoma (DLBCL) reflects a large underlying biological heterogeneity [1]. Various molecular DNA-, RNA- and protein-based subtyping approaches have been proposed over time, but failed to sufficiently capture its biological heterogeneity in a clinically sufficient manner, precluding major clinical consequences [2–5]. The most recent DNA-based subtyping studies as independently proposed by the Dana Farber Cancer Institute (DFCI) and the National Cancer Institute (NCI) are a major step forward [6, 7]. These subtypes are based on DNA-mutation, genome-wide copy number aberration (CNAs), and translocation information. Despite different bioinformatic approaches, the resulting 5- to 7 subtypes largely recognize similar DLBCL pathogenics and starts to offer a clinically impactful refinement at a level sufficient to serve as a basis for exploration of personalized and targeted treatment in the coming years. Its clinical potential already paid off with the recent finding that benefit from the BTK inhibitor ibrutinib plus R-CHOP is highly specifically associated with two of the genetic subtypes [8]. To enable consistent trial designs and meaningful comparisons between studies, we consider it pivotal to harmonize the currently available DNA-subtyping knowledge into a single classification, preferably widely applicable in diagnostic routine. In this perspective we investigate harmonization opportunities and suggest potential avenues from a bioinformatics point of view.

BIOINFORMATICS APPROACHES FOR THE CURRENT DNA-BASED DLBCL SUBTYPING

The DFCI and NCI DLBCL subtyping studies are both based on whole genome sequencing data but differ essentially in a priori concepts and bioinformatic strategies. In brief, the DFCI group used unsupervised clustering combined with alteration-centric features. Driver alterations were discriminated from passengers, reducing the genetic dataset to 158 features. Next, unsupervised clustering by means of non-negative matrix factorization (NMF) identified patterns of co-occurring features to define clusters and assign each included patient sample. The NMF algorithm uncovered the optimal stability of subtype clusters to be represented by five groups of similar sizes, which the authors labeled as C1 to C5. The NCI group used semi-supervised clustering combined with gene-centered features. Prior knowledge was used to define four classes with 1 or 2 DNA “seed” features, the a priori assumption. The algorithm subsequently selected additional features with the strongest association to those seeds unsupervised by iteration. All patient samples were included for this 4-class algorithm, but only 46% of cases could be assigned [9]. Recurrent alterations in unassigned cases prompted an extension with two classes. The “seed” features for one of these additional classes were “TP53 inactivation” and “high CNA load”, in analogy with DFCIs C2 subtype with p53 mutation and deletion (17p) as its top features and a multiplicity of CNAs. This was a first step toward harmonization. The resulting Bayesian-based probability score, named Lymphgen classifier, assigned 63% of cases [7]. Despite the very different designs, most subtypes are remarkably similar with similar underlying biology [1, 7], though some are more similar than others and some are only recognized by one of the algorithms.

CRITICAL EVALUATION OF THE CURRENT SUBTYPING SYSTEMS

Prior to applying their subtyping algorithms, the DFCI and NCI groups used different ways to convert the detected DNA-alterations into features. DFCIs alteration-centric approach regards each DNA alteration type separately be it mutation, translocation, or CNA. Hence, a point-mutation of CDKN2A, a deletion at the CDKN2A-locus 9p.21 or the entire chromosome 9 arm would each be regarded as separate features. NCI’s gene-centric approach combines any DNA-alterations that impact the same gene into a single feature. Hence, any alteration detected that affects CDKN2A, would be reduced into a single feature. These two different ways of handling biological features leads to discrepancy in their contribution to subtype assignment that determine biological deregulation and clinical impact. For harmonization we argue that focal chromosomal CNAs which encompass only one or few genes [10] can be readily combined with point-mutations in a gene-centric fashion as these can be assumed to lead to the same overall biological effect [11]. The choice is less obvious for large-scale chromosomal CNAs since these harbor hundreds of genes such that biological insights remain elusive [12] and may be resolved mathematically by calculating an optimal biological characterization of the classes with either feature choice. Supervised- and unsupervised (machine learning) algorithms may be chosen for subtyping [13]. A supervised approach uses predefined
Cluster adherence was determined using NMF clustering with the 304 DLBCL samples from the DFCI cohort, recapitulating the results from the original study, including five subtypes (C1, C2, C3, C4 and C5) and identical subtype assignment for all samples (left panel). Consensus clustering by resampling [21] illustrates the unstable character of NMF clustering, core patients (solid color, middle panel) and non-core patients (dashed color, right panel). To make this distinction, a stability score was determined by examining co-occurring sample pairs in the same subtype through 1000 iterations of NMF clustering. The heatmaps show patients by column and genomic features by row. Genomic feature colors in the heatmap indicate mutations (green), copy number losses (blue), copy number gains (red) and translocations (purple). DLBCL samples are clustered by subtype. The subtype bars on top indicate core DLBCL samples (colored bars) and non-core DLBCL samples (gray and dashed bars). Lymphgen annotation of the DFCI samples were taken from Wright et al. [7]. Left panel: heatmap of all 304 DLBCL samples from the DFCI cohort. Middle panel: heatmap of the 70% core samples with a high stability score and robust molecular subtypes. Right panel: heatmap of the 30% non-core samples with inconsistent subtype assignment throughout the clustering iterations.

CONCLUDING REMARKS
Classification for a biologically heterogeneous disease like DLBCL is required for clinical trial inclusion to come to bespoke treatment. To achieve any meaningful classification, there may be well-defined quantitative criteria by which classification schemas can be objectively assessed, but these are inevitably balanced by more subjective choices. We describe here that consensus classification depends on choices concerning the incentive to recognize rare DLBCL subtypes or recognition that not all DLBCLs may have sufficiently specific DNA characteristics to be classified at all. Also, technical choices are to be made such as on the nature and weight of DNA-features, and on mathematics with their pros and cons. Most important is the choice if a consensus classification and a common classifier algorithm is timely and needed. Thereby, we feel that the added value of the achievements of the DFCI and NCI classifications should be exploited.
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AUTHOR CONTRIBUTIONS
JJ, YK and EVo re-analyzed the data to draft the figure, edited and approved the final version of the manuscript. MM wrote a first version of the manuscript, edited and approved the final version of the manuscript. DdJ designed the manuscript format and wrote the manuscript. BY initiated writing, designed the manuscript format with figure and wrote the manuscript.

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COMPETING INTERESTS
The authors declare no competing interests.

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