

AG02.16 - Cell-of-origin classification of diffuse large B-cell lymphoma – comparison of molecular tests and extension to the PETAL trial

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Background

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous and most common lymphoma entity in Western. The distinction between ABC/GCB COO-subtypes might become more relevant since many drugs currently under development target predominantly one but not the other COO-subtype. However, areas of uncertainty need to be resolved and the performance of different assays on the same specimen has not yet been tested in detail. We aimed to compare molecular assays for the application in FFPE tissue with the widely used immunohistochemical Hans-classifier and performed COO-classification of the PETAL clinical trial, which was designed to elucidate the effect of an intensified treatment based on the interim PET screening after two cycles R-CHOP treatment.

Methods

To this end, we applied a newly available RNA-sequencing based (HTG Molecular Diagnostics, Inc.), our self-developed NanoString gene-expression (Masque-Solér et al., Blood 2013) and a newly established mass spectrometry based protein-expression classifier (Oefner et al., in preparation) as well as the Hans-classifier to a cohort of 73 DLBCL. Furthermore, COO profiling of 345 PETAL patients was done using the HTG COO-classifier. A new platform for performing extraction free targeted RNA re-sequencing using e.g. single FFPE slides.

Results

HTG analysis was successful on all samples with at least 5mm²/5µm available FFPE tumor material, resulting in an extremely low dropout rate. We used our NanoString classifier as the reference and defined major misclassifies as samples with a GCB vs. ABC/non-GCB classifier result and vice versa. All three molecular COO-classifier showed a high concordance with few major misclassifies (<5%). However, the extent of the “unclassified lymphomas” varied substantially between the molecular tests (max 25 %). The NanoString results were compared to the Hans-classifier and identified a higher proportion of major misclassifies which were mostly GCB samples by NanoString but non-GCB using Hans.

Conclusion

Our results illustrate that the molecular methods for COO-classification show a high agreement in the identification of ABC/GCB, but differ substantially in the extent of the unclassified lymphomas. Using the HTG platform with the low input material we were able to reduce potential side effects introduced in COO-analysis of clinical trials based on the availability of sufficient FFPE material.